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Fungi associated with table grape propagation material, with emphasis on *Neoscytalidium dimidiatum* and *Quambalaria cyanescens* in Italy

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Summary. Italy is the leading producer and the main exporting country of table grapes in the European Union. However, table grape production is affected by Grapevine Trunk Diseases (GTDs) which cause serious economic losses to grape growers. Aetiology of GTDs is crucial for application of effective management strategies, particularly regarding the quality of the grapevine propagation material. During 2022-23, four nurseries in Eastern Sicily, Southern Italy, were surveyed, and high incidence of propagation material with GTDs symptoms was found. Over 100 fungal isolates were collected from 80 symptomatic cuttings of 'Italia' and 'Victoria' cultivars grafted on rootstock 140RU. Of these isolates, 82 were molecularly analysed, and were found to belong to 22 genera. Isolation results highlighted the presence of well-known GTDs-related pathogens, including species within the *Botryosphaeriaceae*, and *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum,* and *Cylindrocarpon*-like species. Less common fungi, including *Neoscytalydium dimidiatum* and *Quambalaria cyanescens*, were also isolated and characterized by molecular, morphological and phylogenetic analyses, and Koch's postulates were fulfilled for these two species. This is the first study to associate *N. dimidiatum* and *Q. cyanescens* with table grape propagation material in Europe.

Keywords. Grapevine Trunk Diseases, nursery material, isolate characterization, pathogenicity.

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INTRODUCTION

Table grape (*Vitis vinifera* L.) is an important and widely cultivated crop plant, showing positive production trends in the last 20 years. Italy is the leading producer and the main exporting country of table grapes in the European Union, with annual production of 925.472 t produced from 40.705 ha. Most (94.4%) of this production is from southern Apulia (610.555 t from 25.285 ha) and Sicily (262.846 t from 12.075 ha) (Istat 2025). Italy occupies a

prominent commercial position with a long production season from late May to December (> 7 months). 'Italia' and 'Victoria' are the main cultivars produced, (respective proportions of production of approx. 40% and 15%), followed by 'Red Globe', 'Black Magic', and an increasing number of seedless cultivars, including 'Sugraone', 'Crimson Seedless', and 'Regal Seedless' (Pisciotta *et al*., 2022). The major rootstocks for these cultivars are 140RU, 1103 Paulsen, and 775 P.

As a highly profitable crop, it is important to incorporate effective vine health practices throughout grape production to prolong longevity and productive lifespans of vineyards. Grapevine trunk diseases (GTDs) are a disease aggregate of fungal diseases that are the most destructive biotic factor of grapevines (Guerin-Dubrana *et al.,* 2019; Azevedo-Nogueira *et al.,* 2022). Multifaceted adverse effects due to GTDs include reduced plant longevity, cumulative yield losses, increased costs due to required disease management practices, and premature replanting of severely affected vineyards (Gramaje *et al*., 2018). According to their aetiology and symptomatology, GTDs can be grouped in different syndromes: Black Foot (BF), Eutypa, Botryosphaeria and Phomopsis dieback, and the Esca and Petri disease (PD) complexes.

The first report of BF of grapevines in Italy was by Grasso and di San Lio (1975), and Grasso (1984) associated this disease with death of young grapevines in Sicily. Carlucci *et al.,* (2017) studied BF occurrence on young grapevines and nursery material, reporting *Dactylonectria torresensis* to be the most prevalent pathogenic fungus associated with GTDs in Italy. Therefore, these diseases have caused problems in Italian grapevine production for many years, especially in young plants, originating from nurseries. However, since those publications, no further studies on GTDs in young grapevine plants in Italy have been reported.

Eutypa dieback was first reported on grapevines in Italy in 1983 (Bisiach and Minervini, 1985). Sexual structures of *Eutypa lata*, the most common pathogen associated with this disease, were reported by Cortesi and Milgroom (2001). This pathogen is widespread in all regions of Italy except Sicily, probably due to the low amount of rainfall on the island, which plays a key role in the dispersal of *E. lata* inoculum. Damage caused by this pathogen is limited (Guerin-Dubrana *et al*., 2019).

Phomopsis dieback, also known as cane blight and leaf spot, was first reported in Italy with the description of the teleomorph *Diaporthe silvestris* on grapevines by Saccardo and Berlese (1885). *Phomopsis viticola*, the *D. silvestris* anamorph (originally described as *P. cordifolia*), was first reported by Uecker and Johnson (1991). *Diaporthe eres* is one of the most detected species in Italy, isolated for the first time from 1-year-old canes of grapevines in Tuscany by Cinelli *et al*., (2016). Phomopsis cane and leaf spot have been reported from all Italian regions, but is widespread in Apulia, Veneto, and Piedmont (Guerin-Dubrana *et al*., 2019).

Botryosphaeria dieback has been a significant problem in Italian viticulture since the end of the 1970s, with the first tentative association of bark cankers, dieback, and leaf chlorosis on grapevines with *Botryosphaeriaceae* fungi by Cristinzio (1978). Many studies have since been reported, and to date 16 species in *Botryosphaeriaceae* have been reported in association with grapevines in Italy (Rovesti and Montermini, 1987; Burruano *et al.,* 2008; Carlucci *et al.,* 2009; Mondello *et al.,* 2013; Carlucci *et al.,* 2015b). Nowadays, the most common and abundantly isolated species is *Diplodia seriata,* although it appears that this fungus is among the least virulent of the dieback pathogens (Carlucci *et al.,* 2015b; Aiello *et al*., 2023).

Esca and the PD complex are probably as old as grapevine cultivation, but studies on their aetiology have intensified since the 1990s (Mugnai *et al.,* 1999). Lionel Petri was the first to fulfil Koch's postulates in 1912, demonstrating that *Cephalosporium* and *Acremonium* spp. were responsible for the vascular necroses in young vineyards and nurseries, in the Sicilian provinces of Palermo, Messina and Trapani (Petri, 1912). Since then, many studies have been carried out and the main associated species are *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., and *Fomitiporia mediterranea* (Bertelli *et al.,*1998; Mugnai *et al.,* 1999; Cortesi *et al.,* 2000; Tegli *et al.,* 2000; Ciccarone *et al.,* 2004; Essakhi *et al.,* 2008; Raimondo *et al.,* 2014; Carlucci *et al.,* 2015a; Carlucci *et al.,* 2017).

Although GTDs have been extensively studied on wine grape plants, the causal agents associated with GTDs on table grape plants remain less studied in Italy. Graniti (1960) already knew about Esca in Apulia, and reported a diseased young vineyard of cultivar 'Regina' at two years after grafting. During 1995, young plants of cultivar 'Italia' in the areas of Canicattì and Mazzarrone (eastern Sicily) exhibited Esca symptoms with incidences of 9% in Canicatti and 17% in Mazzarrone, while 42% of the affected plants died (Schiliro *et al*., 1996). Also in Sicily, Sidoti *et al.* (2000) reported symptoms of decline on young vines of cultivar 'Victoria', with high mortality in the first year after planting. During the same year in Apulia, Pollastro *et al.* (2000) reported severe infections of 18-year-old cultivar 'Italia' vines, with 84% incidence of diseased wood, and 17% incidence of esca symptoms on the leaves or bunches. Sparapano *et al.* (2000a; 2000b; 2001) reported *Fomitiporia punctata* as the pri-

mary pathogen causing white rot of wood, and 'Italia' as the most susceptible cultivar. Since then, increased GTD incidence and severity have been seen in different vineyards (Pichierri *et al*., 2009; Guerin-Dubrana *et al*., 2019). This is commonly attributed to factors including the expanded planted area, increased vineyard productivity, changes in cultural practices, following of market requirements, and the poor quality of table grape propagation material produced in nurseries (Surico *et al*., 2004; Pichierri *et al*., 2009).

Knowledge of disease aetiology and epidemiology is important for developing effective control strategies that aim to minimize the economic impact of fungal pathogens in young vines, especially originating from nursery material. Effective control of the diseases is important for the future of vineyards.

To date, the quality of propagation material destined for table grape production has been little studied in Italy. To document GTDs in Sicilian cuttings of table grapes, nurseries in Comiso and Mazzarrone were surveyed from May 2022 to September 2023. The objectives in the present study were to: (a) identify the causal agents associated with GTDs on propagation material coming from Sicilian nurseries, using molecular analyses; (b) calculate isolation frequencies of these pathogens, depending on isolation points; and (c) characterize the species associated for the first time with GTDs on table grape plants in Italy, using morphology and multi-locus phylogenetic analyses, and determine their pathogenicity.

MATERIALS AND METHODS

Field surveys, sampling, and fungus isolations

Surveys were conducted in 2022 and 2023 in four nurseries in Comiso (36°57′N, 14°36′E) and Mazzarrone (37°05′N, 14°34′E), located in the Ragusa and Catania provinces of eastern Sicily, Italy, respectively. A total of 80 5- to 7-month-old cuttings of 'Italia' and 'Victoria' cultivars grafted onto 140RU rootstock (ten samples for each cultivar from each nursery) were collected and brought to the Plant Pathology laboratory at the Department of Agriculture, Food and Environment, University of Catania, for isolation and further analyses. Fungal strains were isolated from symptomatic wood tissue from different parts of the cuttings, including: (a) the graft union, (b) 15 cm from the base, and (c) the base of the rootstock. From each part, a wood segment was excised, and then fragmented in five to six pieces (each 5 mm thick). These pieces were then surface-sterilized in a 1.5% sodium hypochlorite (NaClO) solution for 1 min, rinsed in sterile water, dried on sterilized absorbent paper, and then placed onto potato dextrose agar (PDA; Lickson) in Petri plates, that was amended with 100 mg L^{-1} of streptomycin sulphate (Sigma-Aldrich) to prevent bacterial growth. The plates were then incubated in the dark at 25 ± 1°C for 7 to 14 d until fungal colonies grew sufficiently to be examined. Representative colonies were then transferred onto fresh PDA plates, and subsequently, single hypha isolates were obtained from pure cultures. These isolates were then stored as mycelial plugs in sterile water in the collection of the Plant Pathology laboratory.

Isolation frequencies (%) were estimated for the main fungal morphotypes recovered from each isolation point on the symptomatic cuttings. Each value was calculated as the average obtained from the four nurseries investigated, and a single value from each nursery was calculated as the number of isolation positive tissue pieces (from which each morphotype was isolated) divided by the total number of analyzed tissue pieces (Šišić *et al*., 2018; Dastogeer *et al*., 2020).

DNA extractions and PCR

Eighty-two of the collected isolates were grown on Malt Extract Agar (MEA) plates incubated at room temperature (20°C) for 7–15 d. Mycelium from each isolate was then collected in a 1.5 mL sterile Eppendorf tube using a sterile scalpel blade. Genomic DNA was extracted from these samples using the Wizard® Genomic DNA Purification Kit (Promega Corporation), following the manufacturer's protocol. DNA amplification and sequencing of partial regions of various genetic loci were carried out for identification purposes. Specifically, the universal oligonucleotide primers ITS4 and ITS5 (White *et al*., 1990) were used to amplify the ITS1-5.8S-ITS2 region of the rDNA for each isolate (Supplementary Tables S1), while a partial region of the translation elongation factor 1-alpha (*tef1-a*) gene was also amplified for representative isolates of the collection (Supplementary Table S1). Furthermore, for ten *Quambalaria* isolates, fragments of the large subunit (LSU) of the rDNA were amplified using the primer sets NL1 and NL4 (Boekhout *et al*., 1995), and the second largest subunit of RNA polymerase II (*rpb2*) was amplified using the primer sets bRPB2-6F and bRPB2-7R (Matheny, 2005). Similarly, for five *Neoscytalidium* isolates, the *tef1-α* gene was amplified using the primer sets EF1-728F and EF1-986R (Carbone and Kohn, 1999), and the beta-tubulin (*β-tub*) gene was amplified using T1 and Bt-2b (Glass and Donaldson, 1995). All PCR reactions were each carried out in a final volume of 20 µL, containing: 1 µL of each primer (10 µM), 4 µL of the

appropriate buffer, 2 µL $MgCl_2$, 0.4 µL dNTPs, 0.2 µL Taq polymerase (5 U μL^{-1}; KAPA Taq 500 U), 10.4 µL sterile water, and 1 µL DNA template (5 µg µL). The amplifications were performed using the following programme: an initial denaturation at 94°C for 5 min; followed by 35 cycles each of denaturation at 94°C for 30 sec, primer annealing for 1 min at 52°C for ITS, 51°C for *rpb2,* 56°C for LSU, or 30 sec at 60°C for *tef1-a* and *β-tub*; extension at 72°C for 1 min; and a final extension at 72°C for 8 min. PCR products were resolved on 1.5% agarose gels in Tris-acetate-EDTA buffer, stained with SYBR™ Safe DNA gel stain (Invitrogen), and were visualized under UV light. After confirmation by agarose gel electrophoresis, the PCR products were sequenced in both directions using the same primer pairs used for amplification, by Macrogen Inc. (Seoul, South Korea). The retrieved nucleotide sequences were assembled and edited with MEGA X (Kumar *et al.,* 2018).

Morphological descriptions of Quambalaria cyanescens *and* Neoscylalidium dimidiatum *isolates*

Two representative isolates of *Q. cyanescens* (GP9 and GP15) and of *Neos. dimidiatum* (GP33 and GP40), were selected for morphological characterization. Mycelium plugs (4 mm in diam.) were placed into 85 mm-diam. Petri dishes containing PDA, and were incubated at 25°C in the dark for 1–3 weeks. Actively growing colonies of *Neos. dimidiatum* were transferred to plates containing water agar (WA) supplemented with sterile pine needles to allow pycnidium formation (Smith *et al*., 1996). The inoculated Petri dishes were then incubated at room temperature (24 ± 2°C) under a 12 h/12 h fluorescent light/dark regime for 3–4 weeks. For microscopic characterization, pycnidia, pycnidiospores and arthroconidia produced by the hyphomycetous and coelomycetous morphs of *Neos. dimidiatum*, and conidiophores and conidia of *Q. cyanescens*, were mounted in sterile lactic acid. Morphology of all reproductive structures was determined at appropriate magnifications using an Olympus BZX16 dissecting microscope and Olympus ColorView I camera, or a Zeiss AX10 compound microscope and Zeiss AxionCam MRc 5 camera. Mean, maximum, and minimum dimensions (± standard deviations) of *Neos. dimidiatum* and *Q. cyanescens* reproductive structures were calculated, as well as the conidium length-to-width ratios (L/W). Colony morphologies of the respective isolates were described on PDA, malt extract agar (MEA; Sigma-Aldrich), oatmeal agar (OA; Sigma-Aldrich), and corn meal agar (CMA; Sigma-Aldrich), while colony colours were also determined for each medium, based on Rayner's (1970) charts.

Effects of temperature on mycelium growth

Optimum temperatures for mycelium growth of *Q. cyanescens* (isolates GP9 and GP15) and *Neos. dimidiatum* (isolates GP33 and GP40) were determined. Mycelium plugs (each 4 mm diam.) from the margins of actively growing cultures were transferred into the centre of Petri dishes containing PDA, and were incubated in the darkness at constant temperatures from 5 to 35°C (5°C intervals). Two perpendicular diameters of resulting colonies were recorded daily for *Neos. dimidiatum* over 2 d, and for *Q. cyanescens* over 14 d. Three replicates were prepared per isolate and the experiment was repeated once.

Regression curves were fitted for each isolate at the different temperatures and the data were analyzed using the Kruskal-Wallis test (non-parametric). The optimum growth temperature and the mycelium growth rate (mm d^{-1}) were calculated for each isolate, and means per fungus species were compared using Dunn's test for multiple comparisons ($P \leq 0.05$). Statistical analyses of data were carried out using SPSS (v. 25, IBM Corporation,) and graphically presented with GraphPad Prism (v. 10.1.0, GraphPad Software).

Phylogenetic analyses of Quambalaria *and* Neoscytalidium *isolates*

Raw sequence chromatograms of each locus (forward and reverse) generated for *Quambalaria* and *Neoscytalidium* isolates were retrieved, and their quality was evaluated using the FinchTV software (version 1.4.0) (Geospiza Inc.). Consensus sequences were assembled using MEGA software (version 7.0.26) (Kumar *et al*., 2018) with ClustalW (Thompson *et al*., 1994). All the sequences generated in this study, along with reference sequences from NCBI (Tables 3 and 4) were aligned with MAFFT v. 7.110 (Katoh *et al*., 2019), using the default parameters. Manual adjustments were made, when necessary, using MEGA software (version 7.0.26) (Kumar *et al*., 2018). The alignments of each locus were concatenated in Sequence Matrix v.1.8 software (Vaidya *et al*. 2011). The concatenated sequence alignments were analyzed using Maximum Likelihood (ML) in IQ-TREE software (version 2.3.4) (Minh *et al*., 2020), with the best evolutionary model selected using ModelFinder (Kalyaanamoorthy *et al*., 2017). Branch support was estimated using 1000 replicates of the ultrafast approximation (UFBoot2) (Hoang *et al*., 2018). Bayesian inference (BI) was carried out using MrBayes v3.2.7 (Ronquist *et al*., 2012). Two independent Markov Chain Monte Carlo (MCMC) runs (each with one cold and three heated chains) were conducted for 1,000,000 generations, and

trees and parameters were sampled every 100 generations. Convergence was monitored using the average standard deviations of split frequencies, with a target value of <0.01, assessed every 1,000 generations. The first 25% of samples were discarded as burn-in, and a 50% majority-rule consensus tree was generated from the remaining trees, with posterior probabilities (PP) used as nodal support values. For *Quambalaria* species, no *rpb2* sequence was available for their type strains.

Pathogenicity tests

To determine abilities to infect and induce symptoms on host plants, pathogenicity tests were carried out using isolate GP9 of *Q. cyanescens* and isolate GP40 of *Neos. dimidiatum*. Inoculations were carried out *in vivo* on asymptomatic cuttings of 'Italia' grafted onto 140RU rootstock. Each isolate was inoculated onto 12 green and 12 woody shoots of scion and onto six rootstocks, using a mycelial plug in each case. Before inoculations, the shoots and rootstocks were surface-disinfected with a 70% aqueous solution of ethanol. For each inoculation, the bark was gently scraped using a sterile blade, and an agar plug (5 mm diam.) from a 20-d-old fungal culture grown on PDA supplemented with lactic acid (2.5 mL of 25% [v:v] per L; APDA) at 25 ± 1 °C was inserted into each wound. The wounds were then sealed with Parafilm® (Pechney Plastic Packaging Inc.) to prevent contamination and dehydration. Controls consisted of 12 plants each inoculated with a sterile APDA plug. All the plants were then moved to a growth chamber set with a 12 h light 12 h dark daily cycle, and maintained at 25°C. The plants were regularly watered and monitored weekly for development of symptoms. Symptom evaluation on the scions was carried out after 1 month for half of the inoculated shoots, and at 3 months for the remaining shoots. For the rootstock, symptom evaluation was performed at 3 months post-inoculation. Mean lengths of necrotic lesions (external or internal) extending both upward and downward from each inoculation site were determined. The experiment was carried out twice. Isolations of fungal species from diseased plant tissues were carried out to assess fulfilment of Koch's postulates.

RESULTS

Field surveys, sampling and fungus isolations

In the four surveyed nurseries, 95% of the sampled rooted and grafted table grape cuttings were symptomatic (76 of 80 cuttings examined). The symptoms included necroses and discolourations at the graft points extending upward the scions, wood necroses and black streaking at the rootstock bases, as well as pith necroses and black streaking on vascular tissues (Figure 1). More than 100 isolates were collected from symptomatic cuttings, and 82 representative strains from different nurseries and plant parts were characterized using molecular analyses.

Figure 1. Symptoms observed on table grape propagation material in nurseries. **A** and **B**, views of two of the table grape nurseries investigated. **C** and **D**, vertical sections of symptomatic cuttings. **E**, **F** and **G**, necroses and discolouration at graft unions. **H**, vascular discolouration. **I**, pith necrosis. **J**, black streaking and wood necrosis at bases of rootstock plants.

Fungus identifications

Initial molecular identification of the 82 representative isolates was based on their ITS sequences. These identifications were then supported by the *tef1-a* sequences for 28 representative isolates of the main species recovered from the propagation material. BLASTn analysis identified 22 genera: *Acremonium*, *Alternaria*, *Arthrinium*, *Aspergillus*, *Botryosphaeria*, *Cadophora*, *Cladosporium*, *Clonostachys*, *Dactylonectria*, *Diaporthe*, *Diplodia*, *Entoleuca*, *Fusarium*, *Idriella*, *Ilyonectria*, *Neofusicoccum*, *Neoscytalidium*, *Phaeoacremonium*, *Phaeomoniella*, *Paraphoma*, *Quambalaria* and *Trichoderma* (Supplementary Table S1). Fungi recovered at low frequency in the present survey and/or generally considered as saprophytes or antagonists (*Acremonium* sp., *Alternaria* sp., *Aspergillus* spp., *Cladosporium* spp., *Entoleuca* spp., *Idriella* sp., *Paraphoma* sp., *Clonostachys* sp. and *Trichoderma* spp.) were not examined further. Sequences of *tef1-α* confirmed the identity of 16 species. Some of these are well-known GTDs pathogens, including *Botryosphaeriaceae* (*N. parvum*, *D. seriata*, *N. australe*, *N. luteum, B. dothidea*), *Ph. chlamydospora*, *P. minimum*, *Cadophora luteo-olivacea*, and *Cylindrocarpon*-like species (*I. liriodendri*, *I. destructans*, *D. macrodidyma*, *D. torresensis*). Other usually less common fungi (*F. proliferatum*, *F. oxysporum*, *Neos. dimidiatum*, *Q. cyanescens*) were also identified. The ITS and *tef1-a* sequences generated in this study were deposited in GenBank (Supplementary Table S1).

For isolation frequencies, fungal colonies obtained were classified into seven morphotypes, based on colony morphology, *Botryosphaeriaceae*, *Fusarium* spp., *Cylindrocarpon*-like spp., *Q. cyanescens*, *Neos. dimidiatum*, *Ph. chlamydospora* and *P. minimum*. Morphological identifications of fungal morphotypes were confirmed from molecular analyses (Table 1).

Isolation results indicated that fungus incidence varied between the different parts of the plant cuttings. Isolation frequency (%) at the graft unions showed a prevalence of *Q. cyanescens*, followed by *Neos. dimidiatum*, other *Botryosphaeriaceae* and *Fusarium* spp. Isolations at 15 cm from rootstock base showed prevalence of *Ph. chlamydospora* followed by *Q. cyanescens* and *Neos. dimidiatum*, while from the rootstock base, the dominant species were similar to those from the graft unions (Table 1).

Morphological analyses of Neoscytalidium *and* Quambalaria *isolates*

Colonies of *Q. cyanescens* (isolates GP9 and GP15) were white, flat, with smooth margins and slow growth on PDA, MEA, CMA, and OA media, after 7 d incubation at 25°C in darkness. On OA, pale vinaceous grey-purple haloes developed around the colonies (Figure 2). Conidiogenous cells for both isolates developed at the ends along the sides of conidiophores, which were indistinguishable from the vegetative hyphae. Primary conidia of the *Q. cyanescens* isolates were ellipsoidal to subcylindrical, hyaline, often guttulate, and aseptate; secondary conidia were obovoid to guttiform, hyaline, often guttulate, and aseptate (Figure 3). Characteristics of conidium and conidiogenous cells are reported in the Supplementary Table S2. Overall, their morphologies were in line with the original description of *Q. cyanescens* by de Hoog and de Vries (1973) and earlier descriptions of the species. No sexual morphs were observed.

Both isolates of *Neos. dimidiatum* (GP33 and GP40) had characteristics consistent with the description of the type-strain of this species described by Campbell and Mulder (1977) (Supplementary Table S3). The two isolates grew rapidly at 25°C, with mycelium covering the surfaces of 85 mm diam. plates of PDA, MEA, and OA in less than 72 h, and after 4 d on CMA. The colonies were initially hyaline to white with smooth margins on the four media, and with aerial mycelium on OA and MEA. With time, colonies on PDA turned smoke grey and grey olivaceous to black, beginning from their centres, while on MEA they developed olivaceous grey to pale greyish colours, both with powdery texture. On OA, the colonies turned grey olivaceous to olivaceous black and cottony, whereas on CMA, they

Table 1. Isolation frequencies (%) of the main fungi recovered from different parts of symptomatic table grape propagation material.

Fungal species/taxon	Isolation frequency (%) per plant part [a]		
	Graft point	15 cm from the base	Rootstock base
Quambalaria cyanescens	20.5	11.1	18.3
Neoscytalidium dimidiatum	13.1	11	15.1
Other *Botryosphaeriaceae*	10.1	3.5	12.1
Fusarium spp.	6.4	5.1	1.3
Phaeomoniella chlamydospora	0.6	16.8	8.2
Phaeoacremonium minimum	1.7	2.1	0.6
Cylindrocarpon-like species	-	-	2.0
other	1.1	2.9	1.7
Total	53.5	52.5	59.3

[a] Each isolation frequency is the average of frequencies obtained from four table grape nurseries. The frequency from each nursery was calculated as the number of positive tissue pieces from which each morphotype was isolated, divided by the total number of analyzed tissue pieces.

were flat and progressed from hyaline to white to grey olivaceous (Figure 2). Pycnidia were irregular dark brown to black, and pycnidiospores were also irregular in shape, and dark brown to black. Arthroconidia were produced by hyphal disarticulation, and were hyaline to pale brown, 0 to 1 (rarely 2) septate and cylindrical, and were produced singly or in arthric chains (Figure 3). No sexual morphs were observed.

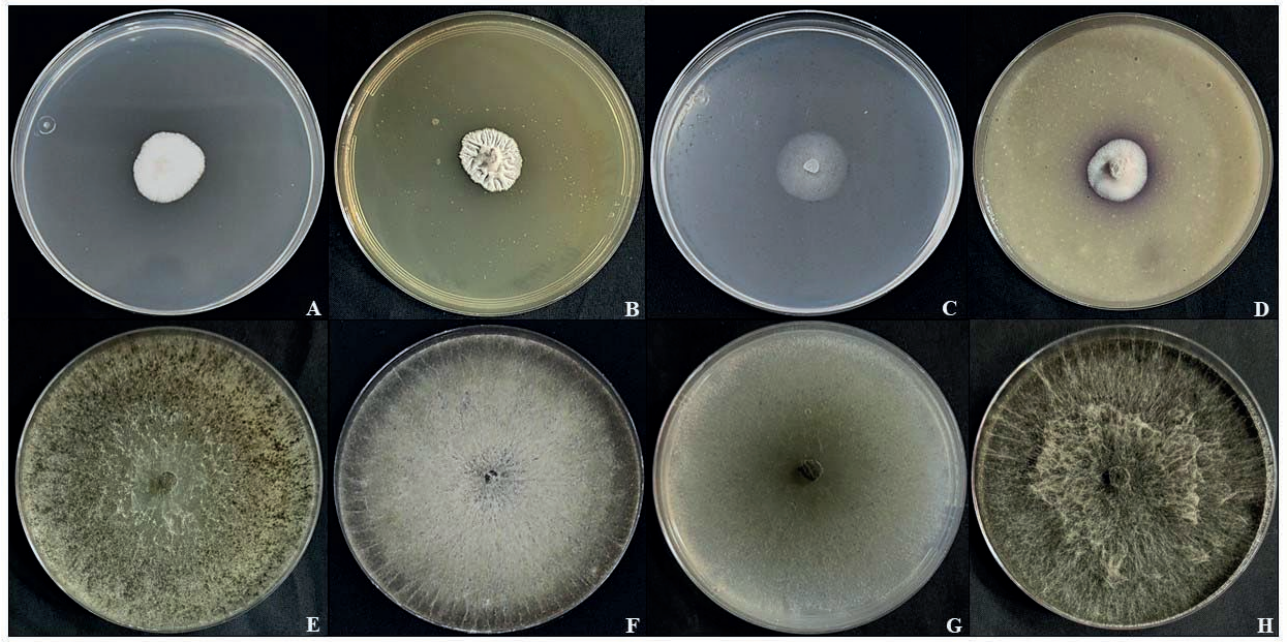

Figure 2. A to **D**, colonies of *Quambalaria cyanescens* (isolate GP9). **E to H**, colonies of *Neoscytalidium dimidiadum* (isolate GP40). Both isolates were grown for 7 d at 25°C in darkness on PDA (**A** and **E**), MEA (**B** and **F**), CMA (**C** and **G**), and OA (**D** and **H**).

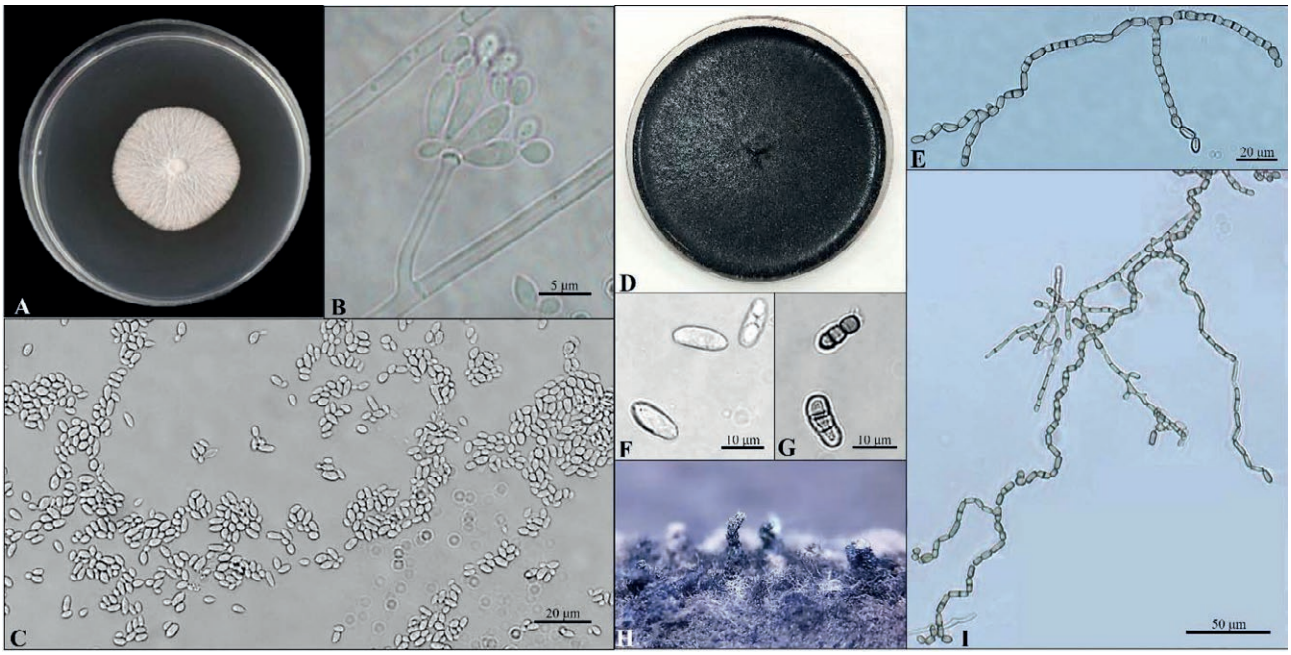

Figure 3. Morphological characteristics of *Quambalaria cyanescens* and *Neoscytalidium dimidiatum*. **A**, colony of *Q. cyanescens* (isolate GP9; 2 weeks old). **B**, conidiophore with primary and secondary conidia. **C**, general view of conidia. **D**, colony of *Neos. dimidiatum* (isolate GP40; 3 weeks old). **E** and **I**, chains of arthroconidia. **F** and **G**, pycnidiospores without (F) and with one or two septa (G). **H**, pycnidia.

Effects of temperature on mycelium growth

Analysis of variance showed no differences ($P < 0.05$) in mycelium growth among experiments, allowing data to be pooled. Relationships between temperature and growth were modelled using a cubic response model ($y = aT^3 + bT^2 + cT + d$), with R^2 values ranging from 0.95 to 0.98, indicating excellent fit (Table 2).

Maximum mycelium growth was recorded after 2d for the two *Neos. dimidiatum* isolates and 14 d for the two *Q. cyanescens* isolates. The *Neos. dimidiatum* isolates had optimum growth temperatures between 30 and 35°C, with no growth observed at 5 and 10°C after 14 d. Although no significant differences were found for optimum growth temperature between the two isolates (GP33, 31.93°C; GP40, 32.86°C), maximum growth rates (approx. 41 mm d^{-1}) did not differ ($P > 0.05$). Colony diameters after 48 h ranged from 8.6 mm at 15°C to 64.6 mm at 25°C.

The two *Q. cyanescens* isolates had optimum growth at 25.17°C and 27.06°C, with maximum daily growth rates of 2.56 to 3.42 mm, and no differences ($P > 0.05$) between the isolates. After 14 d, mean colony diameters were from 5.3 mm at 5°C to 41.5 mm at 30°C (Figure 4).

Phylogenetic analyses of Quambalaria *and* Neoscytalidium

Sequence alignment of the three genetic loci (ITS, LSU, and *rpb2*) prepared for *Quambalaria* isolates consisted of a 2020 character dataset, of which 1389 were constant, 442 were parsimony-informative, and 189

Table 2. Temperature-mycelium growth relationships for *Neoscytalidium dimidiatum* and *Quambalaria cyanescens* isolates obtained from table grape propagation material w.

Species	Isolate	Adjusted model[x]					Optimum temperature (°C)[y]	Growth rate (mm/day)[z]
		R^2	*a*	*b*	*c*	*d*		
Neos. dimidiatum	GP33	0.98	-0.0071	0.434	-59.99	21.10	31.93 a	40.89 a
Neos. dimidiatum	GP40	0.98	-0.0066	0.415	-59.15	21.30	32.86 a	41.18 a
Q. cyanescens	GP9	0.95	-0.0007	0.034	-0.361	15.29	25.17 b	2.56 b
Q. cyanescens	GP15	0.96	-0.0007	0.035	-0.371	15.79	27.06 b	3.42 b

[w] Data are means of six replicates per isolate. Means in each column accompanied by the same letter, are not different ($P = 0.05$), according to Kruskal-Wallis and Dunn's test for multiple comparisons.
[x] Mycelium growth of PDA at 5 to 35°C (5°C increments) was adjusted to a quadratic model of $y = aT^3 + bT^2 + cT + d$: where y = mycelium growth (mm d^{-1}); a, b, c, d = regression coefficients; and R^2 = coefficient of determination.
[y] Optimum temperatures for each isolate were estimated using the adjusted model.
[z] Maximum growth rate per isolate estimated using the adjusted model.

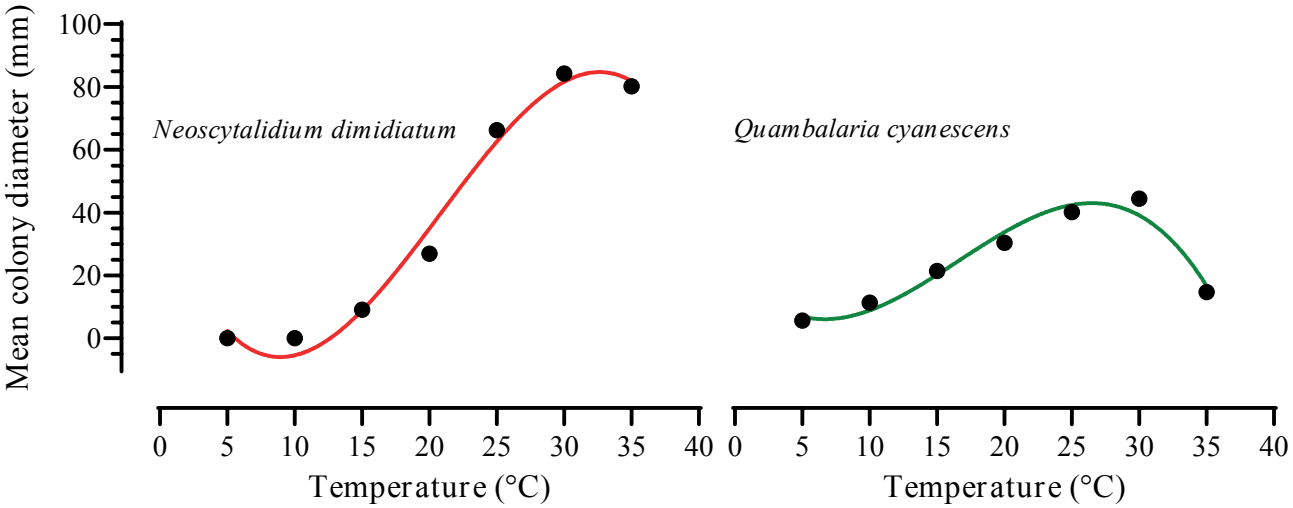

Figure 4. Mean colony diameters (mm) at seven temperatures (5 to 35°C) for two isolates each of *Neoscytalidium dimidiatum* (after 2 d) and *Quambalaria cyanescens* (after 14 d). The isolates were obtained from table grape propagation material in Catania, Italy.

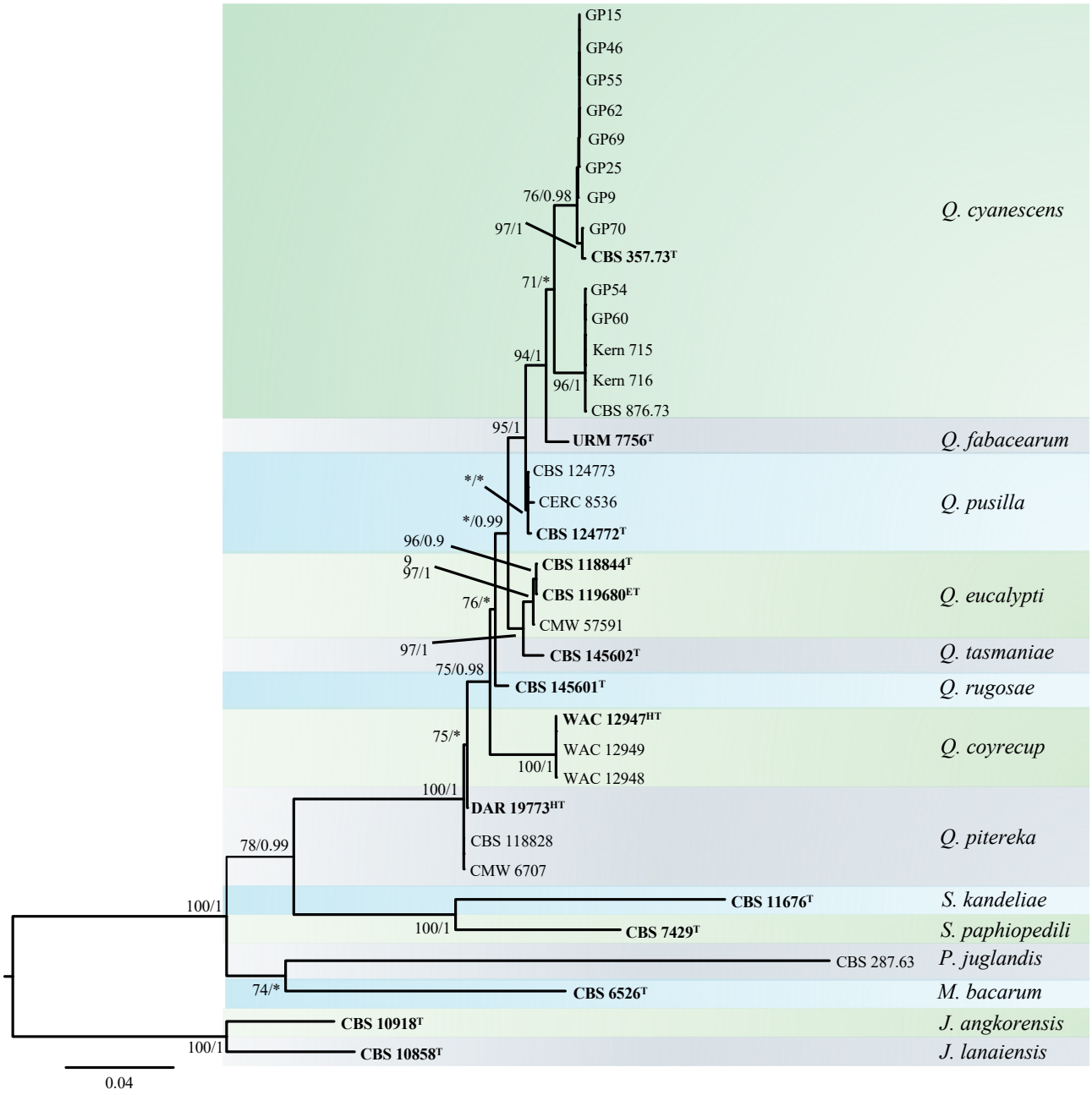

Figure 5. Phylogenetic tree inferred from Maximum Likelihood (ML) and Bayesian Inference (BI) analyses, based on aligned and concatenated ITS, LSU, and *rpb2* sequences of 35 isolates in *Quambalariaceae*. Strains CBS 10918T (*Jaminaea angkorensis*) and CBS 10858T (*Jaminaea lanaiensis*) were used as the outgroup taxa. Numbers at branches indicate support values: Ultrafast bootstrap (UFBoot2) ≥70% and Bayesian posterior probability (B-PP) ≥0.95, with asterisks (*) indicating values <70% and <0.95, respectively. Ex-type, and ex-epitype isolates are indicated in bold. The scale bar represents the expected number of changes per site.

were singleton sites, with 614 distinct patterns. For maximum likelihood (ML) analysis, the ModelFinder determined SYM + I + G4 as the best-fit model. ML and BI phylogenetic analyses with strong supports (respectively 94% and 1) clustered all the *Quambalaria* isolates obtained in the present study in the same clade with other *Q. cyanescens* isolates and *Q. fabacearum* (URM 7756) (Figure 5).

Sequence alignment prepared for the three loci (ITS, *β-tub*, and *tef1-a*) of *Neoscytalidium* isolates consisted of a dataset of 1321 characters, of which 832 were constant, 447 were parsimony-informative, and 42 were singleton

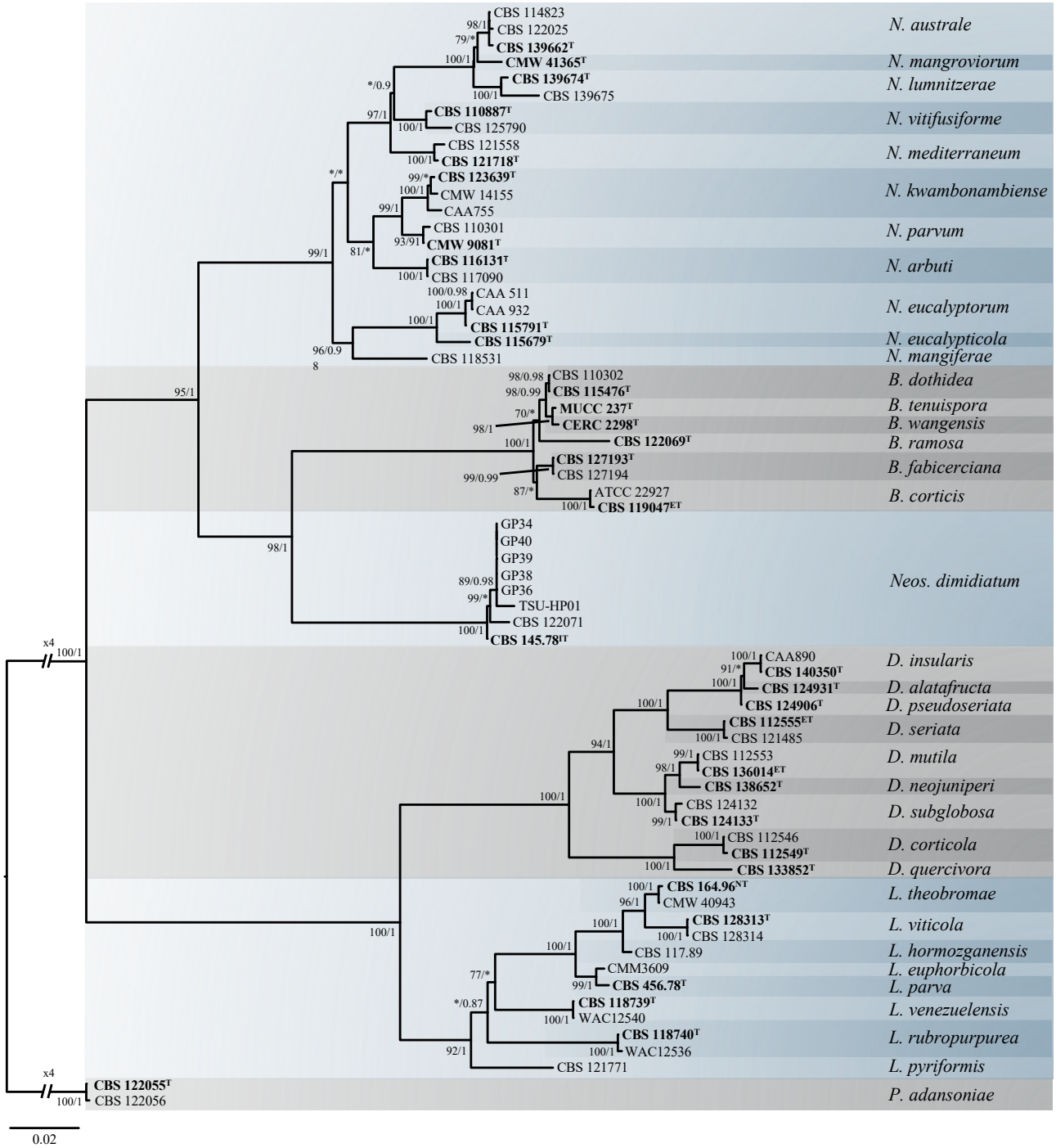

Figure 6. Phylogenetic tree inferred from Maximum Likelihood (ML) and Bayesian Inference (BI) analyses, based on aligned and concatenated ITS, *β-tub*, and *tef1-a* sequences of 67 isolates belonging to *Botryosphaeriaceae*. The strains CBS 122055T and CBS 122056 (*Pseudofusicoccum adansoniae*) were used as the outgroup taxa. Numbers at branches indicate support values: Ultrafast bootstrap (UFBoot2) ≥70% and Bayesian posterior probability (B-PP) ≥0.95; with asterisks (*) indicating values <70% and <0.95, respectively. Ex-type, ex-epitype, ex-isotype, and ex-neotype strains are indicated in bold. The scale bar represents the expected number of changes per site.

sites, with 577 distinct patterns. For maximum likelihood (ML) analysis, the ModelFinder determined TIM + F + I + G4 as the best-fit model. ML and BI phylogenetic analyses with strong support (respectively 100% and 1) clustered all the present study *Neoscytalidium* isolates in the same clade as *Neos. dimidiatum* reference isolates (Figure 6).

Table 3. Phylogenetic analysis of *Quambalaria cyanescens* isolates assessed in the present study, with details of their geographic origins, hosts, and GenBank accession numbers a.

Species	Strain code[c,d]	Host	Country	GenBank Accession Number[b]		
				ITS	LSU	*rpb2*
Jaminaea angkorensis	CBS 10918^{T}=CCY 88-1-1=C5b	Decaying leaves	Cambodia	KY103614	KY107895	KP323082
Jaminaea lanaiensis	CBS 10858^{T}=BCRC 23177=LM418	Driftwood	USA	KY105576	KY109812	KP323080
Microstroma bacarum	CBS 6526^{T}=IGC4391=CGMCC2.3190	*Ribes nigrum*	UK	DQ317629	AF352055	KP323098
Pseudomicrostroma juglandis	CBS 287.63	*Juglans regia*	The Netherlands	DQ789989	AF009867	DQ789989
Quambalaria cyanescens	CBS 357.73^{T}=CMW 5583=MUCL 19329	Skin of man	The Netherlands	DQ317622	DQ317615	-
Q. cyanescens	CBS 876.73=CMW 5584	*Eucalyptus pauciflora*	Australia	DQ317623	DQ317616	-
Q. cyanescens	Kern 715	*Vitis vinifera*	USA	OP038078	OP076927	OP095256
Q. cyanescens	Kern 716	*V. vinifera*	USA	OP038079	OP076928	OP095257
Q. cyanescens [b]	**GP9**	***V. vinifera***	**Italy**	**PV440727**	**PV466206**	**PV469421**
Q. cyanescens	**GP15**	***V. vinifera***	**Italy**	**PV440730**	**PV466207**	**PV469422**
Q. cyanescens	**GP25**	***V. vinifera***	**Italy**	**PV440801**	**PV466208**	**PV469423**
Q. cyanescens	**GP46**	***V. vinifera***	**Italy**	**PV440732**	**PV466209**	**PV469424**
Q. cyanescens	**GP54**	***V. vinifera***	**Italy**	**PV440733**	**PV466210**	**PV469425**
Q. cyanescens	**GP55**	***V. vinifera***	**Italy**	**PV440734**	**PV466211**	**PV469426**
Q. cyanescens	**GP60**	***V. vinifera***	**Italy**	**PV440736**	**PV466212**	**PV469427**
Q. cyanescens	**GP62**	***V. vinifera***	**Italy**	**PV521998**	**PV466213**	**PV469428**
Q. cyanescens	**GP69**	***V. vinifera***	**Italy**	**PV440738**	**PV466214**	**PV469429**
Q. cyanescens	**GP70**	***V. vinifera***	**Italy**	**PV440803**	**PV466215**	**PV469430**
Quambalaria eucalypti	CBS 118844^{T}=CMW 1101	*Eucalyptus grandis*	South Africa	DQ317625	DQ317618	-
Q. eucalypti	CBS 119680^{ET}=CMW 11678	*E. grandis*	South Africa	DQ317626	DQ317619	-
Q. eucalypti	CMW 57591	*E. pellita*	Indonesia	OR345271	OR345280	-
Quambalaria pitereka	CBS 118828=CMW 5318	*Corymbia citriodora* subsp. *Variegata*	Australia	DQ317628	DQ317621	-
Q. pitereka	CMW 6707	*Corymbia maculata*	Australia	DQ317627	DQ317620	-
Q. pitereka	DAR 19773^{HT}	*Corymbia eximia*	Australia	DQ823423	DQ823438	-
Quambalaria pusilla	CBS 124773	*Eucalyptus* sp.	Thailand	GQ303291	GQ303322	-
Q. pusilla	CERC 8536	*E. urophylla* x *E. grandis*	China	KY615046	KY615061	-
Q. pusilla	CBS 124772^{T}=CPC 14499	*Eucalyptus tintinnans*	Australia	GQ303290	GQ303321	-
Quambalaria tasmaniae	CBS 145602^{T}=CPC 25464	*Eucalyptus* spp.	Australia	MN162015	MN162213	-
Quambalaria rugosae	CBS 145601^{T}=CPC 20162	*Eucalyptus rugosa*	Australia	MN162014	MN162212	-
Quambalaria coyrecup	WAC 12947^{HT}	*Corymbia calophylla*	Australia	DQ823431	DQ823444	-
Q. coyrecup	WAC 12948	*Corymbia calophylla*	Australia	DQ823433	DQ823446	-
Q. coyrecup	WAC 12949	*Corymbia calophylla*	Australia	DQ823432	DQ823445	-
Quambalaria fabacearum	URM 7756^{T}	*Mimosa tenuiflora*	Brazil	MG253664	MG253665	
Sympodiomycopsis kandeliae	CBS 11676^{T}=BCRC 23165=FIRDI 007	*Kandelia candel*	Taiwan	KY105575	KY109811	KP323077
Sympodiomycopsis paphiopedili	CBS 7429^{T}=IGC5543=CGMCC2.1398	*Paphiopedilum primurinum*	Japan	KY105577	AF190005	KP323099

[a] GenBank accession numbers for the sequences of four loci: internal transcribed spacer regions and intervening 5.8S rRNA gene (ITS), large subunit of the rRNA (LSU), and the second largest subunit of RNA polymerase II (*rpb2*) that were generated in the present study or from others.

[b] Sequences from isolates in our collection are highlighted in bold.

[c] **BCRC:** Bioresources Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan (same as CCRC); **CBS:** Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CGMCC:** China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; **CMW:** Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa; **CPC:** Culture collection of Pedro Crous, housed at the Westerdijk Institute; **DAR:** Plant Pathology Herbarium, Orange Agricultural Institute, Orange, New South Wales, Australia; **FIRDI:** Food Industry Research and Development Institute, Hsin-Chu, Taiwan; **MUCL:** Belgian Coordinated Collections of Microorganisms, Université Catholique de Louvain, Earth and Life Institute, Belgium; **URM:** Culture collection at the Universidade Federal de Pernambuco, Recife, Brazil; **WAC:** Western Australian Plant Pathology Reference Culture Collection, Perth, Australia.

[d] Status of the isolates = ET: ex-epitype; HT: ex-holotype; T: ex-type. Sequences in bold indicate isolates collected and characterized in the present study.

Table 4. Isolates used in this study for the phylogenetic analysis of *Neoscytalidium dimidiatum*, with details of their geographic origins, hosts, and GenBank accession numbers a.

Species	Strain[c,d]	Host	Country	GenBank Accession Numbers[b]		
				ITS	*β-tub*	*tef1-a*
Botryosphaeria corticis	CBS 119047^{ET}	*Vaccinium corymbosum*	USA	DQ299245	EU673107	EU017539
B. corticis	ATCC 22927	*Vaccinium* sp.	USA	DQ299247	EU673108	EU673291
Botryosphaeria dothidea	CBS 115476^{T}=CMW 8000	*Prunus* sp.	Switzerland	AY236949	AY236927	AY236898
B. dothidea	CBS 110302	*Vitis vinifera*	Portugal	AY259092	EU673106	AY573218
Botryosphaeria. fabicerciana	CBS 127193^{T}=CMW 27094	*Eucalyptus* sp.	China	HQ332197	KF779068	HQ332213
B. fabicerciana	CBS 127194	*Eucalyptus* sp.	China	HQ332198	KF779069	HQ332214
Botryosphaeria ramosa	CBS 122069^{T}=CMW 26167	*Eucalyptus camaldulensis*	Australia	EU144055	KF766132	EU144070
Botryosphaeria tenuispora	MUCC 237^{T}	*Leucothoe catesbaei*	Japan	LC585278	LC585174	LC585150
Botryosphaeria wangensis	CERC 2298=CGMCC3.18744^{T}	*Cedrus deodara*	China	KX278002	KX278211	KX278107
Diplodia alatafructa	CBS 124931^{T}=CMW 22627	*Pterocarpus angolensis*	South Africa	FJ888460	MG015799	FJ888444
Diplodia corticola	CBS 112549^{T}	*Quercus suber*	Portugal	AY259100	DQ458853	AY573227
D. corticola	CBS 112546	*Quercus ilex*	Spain	AY259090	EU673117	EU673310
Diplodia insularis	CBS 140350^{T}	*Pistacia lentiscus*	Italy	KX833072	MG015809	KX833073
D. insularis	CAA890	*Eucalyptus globulus*	Portugal	MK940299	MT309385	MT309406
Diplodia mutila	CBS 136014^{ET}	*Populus alba*	Portugal	KJ361837	MG015815	KJ361829
D. mutila	CBS 112553	*V. vinifera*	Portugal	AY259093	MZ073931	AY573219
Diplodia neojuniperi	CBS 138652^{T}=CPC 22753	*Juniperus chinensis*	Thailand	KM006431	MT592516	KM006462
Diplodia pseudoseriata	CBS 124906^{T}=CMW 26771	*Blepharocalyx salicifolius*	Uruguay	EU080927	MG015820	EU863181
Diplodia quercivora	CBS 133852^{T}	*Quercus canariensis*	Tunisia	JX894205	MG015821	JX894229
Diplodia seriata	CBS 112555^{ET}=HAP 052=CAP 063	*V. vinifera*	Portugal	AY259094	DQ458856	AY573220
D. seriata	CBS 121485	*V. vinifera*	Spain	EU650671	MT592556	MT592093
Diplodia subglobosa	CBS 124133^{T}	*Lonicera nigra*	Spain	GQ923856	MT592576	GQ923824
D. subglobosa	CBS 124132	*Fraxinus excelsior*	Spain	DQ458887	DQ458852	DQ458871
Lasiodiplodia euphorbicola	CMM3609	*Jatropha curcas*	Brazil	KF234543	KF254926	KF226689
Lasiodiplodia hormozganensis	CBS 117.89	*Quercus cerris*	Italy	KX464134	MT592620	KX464627
Lasiodiplodia parva	CBS 456.78^{T}	Cassava field soil	Colombia	EF622083	KU887523	EF622063
Lasiodiplodia pyriformis	CBS 121771	*Acacia mellifera*	Namibia	EU101308	KU887528	EU101353
Lasiodiplodia rubropurpurea	CBS 118740^{T}=WAC12535	*Eucalyptus grandis*	Australia	DQ103553	EU673136	DQ103571
L. rubropurpurea	WAC12536	*E. grandis*	Australia	DQ103554	KU887530	DQ103572
Lasiodiplodia theobromae	CBS 164.96^{NT}	fruit along coral reef coast	Papua New Guinea	AY640255	KU887532	AY640258
L. theobromae	CMW 40943	*Myrtales* plant	South Africa	MG367178	KP872426	MG367173
Lasiodiplodia venezuelensis	CBS 118739^{T}=WAC12539	*Acacia mangium*	Venezuela	DQ103547	KU887533	EU673305
L. venezuelensis	WAC12540	*A. mangium*	Venezuela	DQ103548	KU887534	DQ103569
Lasiodiplodia viticola	CBS 128313^{T}	*Vitis* sp.	USA	HQ288227	HQ288306	HQ288269
L. viticola	CBS 128314	*V. vinifera*	USA	HQ288228	HQ288307	HQ288270
Neofusicoccum arbuti	CBS 116131^{T}	*Arbutus menziesii*	USA	AY819720	KF531793	KF531792

(Continued)

Table 4. (Continued).

Species	Strain[c,d]	Host	Country	GenBank Accession Numbers[b]		
				ITS	*β-tub*	*tef1-a*
N. arbuti	CBS 117090	*A. menziesii*	USA	AY819724	KF531794	KF531791
Neofusicoccum australe	CBS 139662^{T}=CMW 6837	*Acacia* sp.	Australia	AY339262	AY339254	AY339270
N. australe	CBS 122025	*Eucalyptus* sp.	Spain	KX464160	KX464949	KX464672
N. australe	CBS 114823	*Eucalyptus* sp.	South Africa	KX464159	KX464947	KX464671
Neofusicoccum eucalypticola	CBS 115679^{T}	E. grandis	Australia	AY615141	AY615125	AY615133
Neofusicoccum eucalyptorum	CBS 115791^{T}	*E. grandis*	South Africa	AF283686	AY236920	AY236891
N. eucalyptorum	CAA 932	*E. globulus*	Portugal	MK940311	MT309396	MT309422
N. eucalyptorum	CAA 511	*E. globulus*	Portugal	KX505907	KX505919	KX505896
Neofusicoccum kwambonambiense	CBS 123639^{T}	*Syzygium cordatum*	South Africa	EU821900	EU821840	EU821870
N. kwambonambiense	CAA755	*E. globulus*	Portugal	KT440946	KX505917	KT441006
N. kwambonambiense	CMW 14155	-	-	EU821923	EU821863	EU821893
Neofusicoccum lumnitzerae	CBS 139674^{T}=CMW 41469	*Lumnitzera racemosa*	South Africa	KP860881	KP860801	KP860724
N. lumnitzerae	CBS 139675=CMW 41228	*L. racemosa*	South Africa	KP860882	KP860802	KP860725
Neofusicoccum mangiferae	CBS 118531=CMW 7024	*Mangifera indica*	Australia	AY615185	AY615172	DQ093221
Neofusicoccum mangroviorum	CMW 41365^{T}	*Avicennia marina*	South Africa	KP860859	KP860779	KP860702
Neofusicoccum mediterraneum	CBS 121718^{T}	*Eucalyptus* sp.	Greece	GU251176	GU251836	GU251308
N. mediterraneum	CBS 121558	*V. vinifera*	USA	GU799463	GU799461	GU799462
Neofusicoccum parvum	ATCC 58191=CMW 9081^{T}	*Populus nigra*	New Zealand	AY236943	AY236917	AY236888
N. parvum	CBS 110301	*V. vinifera*	Portugal	AY259098	EU673095	AY573221
Neofusicoccum vitifusiforme	CBS^{T} 110887 ^{T}	*V. vinifera*	South Africa	AY343383	KX465061	AY343343
N. vitifusiforme	CBS 125790	*Acacia mearnsii*	-	MH863762	MT592749	MT592257
Neoscytalidium dimidiatum	IMI 198935 = CBS 145.78^{IT}	*Homo sapiens*	USA	KF531816	KF531796	KF531795
Neos. dimidiatum	TSU-HP01	*Hylocereus polyrhizus*	Thailand	LC590860	LC647833	LC590863
Neos. dimidiatum	CBS 122071	*Crotalaria medicaginea*	Australia	KF766207	MT592760	EF585580
Neos. dimidiatum	**GP34**	***V. vinifera***	**Italy**	**PV440740**	**PV541662**	**PV591900**
Neos. dimidiatum	**GP36**	***V. vinifera***	**Italy**	**PV440742**	**PV541663**	**PV591901**
Neos. dimidiatum	**GP38**	***V. vinifera***	**Italy**	**PV440744**	**PV541664**	**PV591902**
Neos. dimidiatum	**GP39**	***V. vinifera***	**Italy**	**PV440745**	**PV541665**	**PV591903**
Neos. dimidiatum	**GP40**	***V. vinifera***	**Italy**	**PV392803**	**PV541666**	**PV591904**
Pseudofusicoccum adansoniae	CBS 122055^{T}=CMW 26147	*Adansonia gibbosa*	Australia	EF585523	MT592771	EF585571
P. adansoniae	CBS 122056=CMW 26148	*Ficus opposita*	Australia	EF585524	MT592772	MT592279

[a] GenBank accession numbers for the sequences of four loci: internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS), β-tubulin (*β-tub*), and translation elongation factor 1-a (*tef1-a*) that were generated in this study or from others.

[b] Sequences from isolates obtained in the present study are highlighted in bold.

[c] **ATCC:** American Type Culture Collection, Manassas, Virginia, USA; **CBS:** Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CGMCC:** China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; **CMW:** Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa; **CPC:** Culture collection of Pedro Crous, housed at the Westerdijk Institute; **MUCC:** Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie Prefecture, Japan; **WAC:** Western Australian Plant Pathology Reference Culture Collection, Perth, Australia.

[d] Status of the isolates. NT = ex-neotype; ET = ex-epitype; IT = ex-isotype; T = ex-type.

All the sequences generated in the present study were deposited in GenBank (Tables 3 and 4).

Pathogenicity tests

Quambalaria cyanescens and *Neos. dimidiatum* both caused symptoms on inoculated 'Italia' cuttings. One month after inoculations with the *Q. cyanescens* isolate, the mean external lesion length on green shoots was 2.83 ± 1.33 cm, and that with the *Neos. dimidiatum* isolate was 2.63 ± 0.47 cm. The two fungi were more virulent to woody shoots, causing lesions that extended under the bark as internal wood discolourations. The mean lesion length was 3.2 ± 1.26 for *Q. cyanescens* isolates, and 3.4 ± 0.51 cm for *Neos. dimidiatum* (Figure 7). After 3 months, inoculated green and woody shoots were wilted, and the rootstocks also had internal necrotic lesions above and below the inoculation points and extending under the bark. The mean internal lesions lengths were 3.30 ± 1.43 cm for *Q. cyanescens* and 3.37 ± 0.9 cm for *Neos. dimidiatum* (Figure 7). Colonies of the respective fungi were obtained from necrotic tissues of plants inoculated with one or other of the fungi, and identified based on morphology. Control plants did not show any symptoms except those due to wound oxidation.

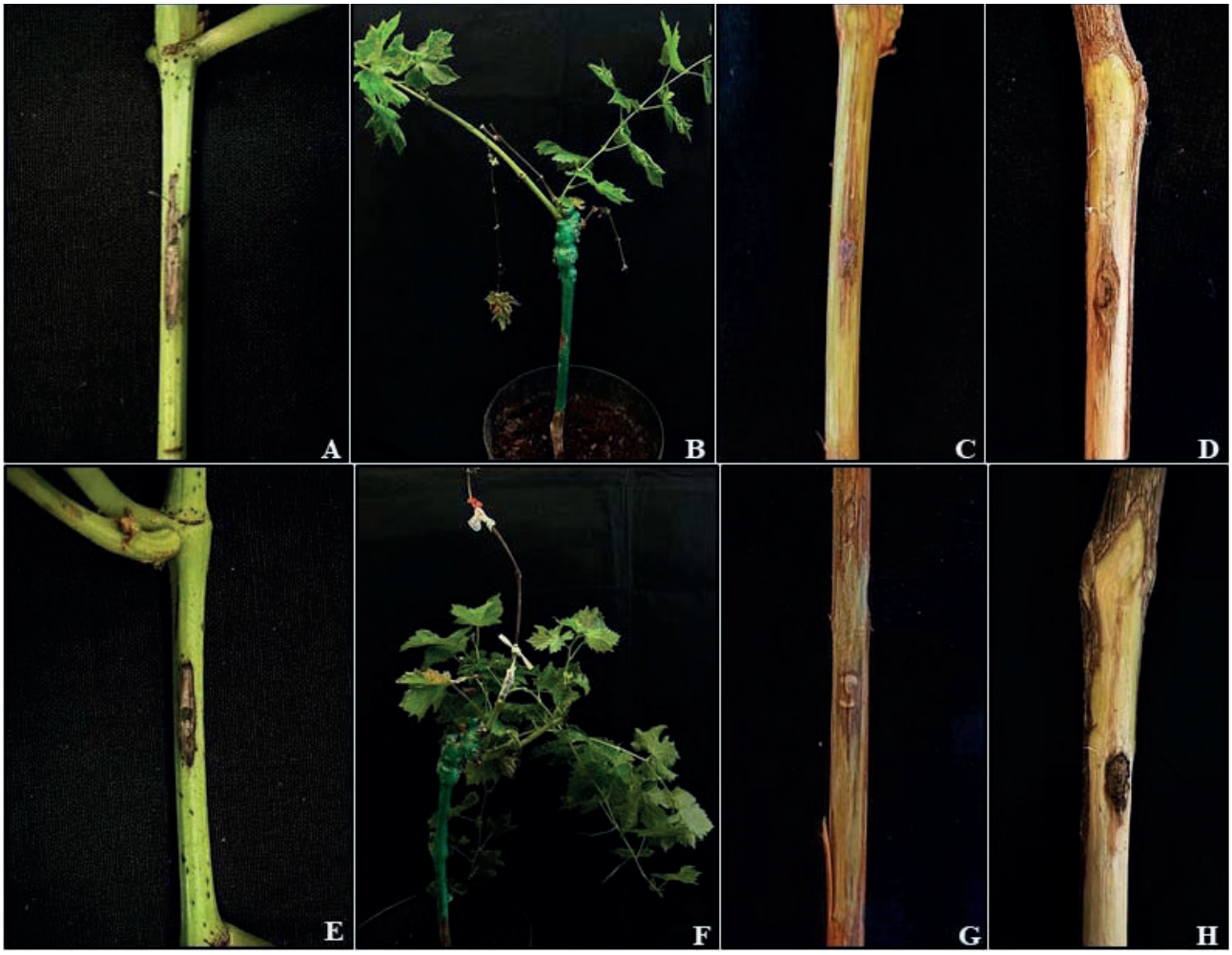

Figure 7. Results of pathogenicity tests with representative isolates of *Quambalaria cyanescens* and *Neoscytalidium dimidiatum*. **A**, external necrotic lesion caused by *Q. cyanescens* on a green shoot at 1 month after inoculation with *Q. cyanescens*. **B**, wilted green shoots caused by *Q. cyanescens* at 3 months post inoculation. **C**, internal necrotic lesion caused by *Q. cyanescens* on a woody shoot at 1 month post inoculation. **D**, internal necrotic lesion caused by *Q. cyanescens* on a rootstock plant 3 months post inoculation. **E**, external necrotic lesion caused by *Neos. dimidiatum* on a green shoot at 1 month post inoculation. **F**, a wilted green shoot caused by *Neos. dimidiatum* at 3 months post inoculation. **G**, internal necrotic lesion caused by *Neos. dimidiatum* on woody shoot at 1 month post inoculation. **H**, internal necrotic lesion caused by *Neos. dimidiatum* on a rootstock plant 3 three months post inoculation.

DISCUSSION

The impacts of fungal pathogens in grapevine propagation material, and within nursery management processes, have been well documented (Gramaje and Armengol, 2011; Gramaje *et al*., 2018). The present study investigated fungi associated with symptomatic table grape cuttings from four Sicilian nurseries exhibiting a high incidence of GTD symptoms. Some of these fungi have been previously associated with these symptoms on grapevines in Italy, and are known to be GTD causal agents in other countries. The isolation frequency from rootstock bases and cutting graft unions showed higher proportions of *Botryosphaeriaceae* species, than isolations from cane bases. It is probable that the large numbers of cuts made during the propagation process in nurseries expose the planting material to infections (Carlucci *et al*., 2015b; Aiello *et al*., 2020).

Sixteen species of *Botryosphaeriaceae* have been reported to be associated with grapevines in Italy (Carlucci *et al*., 2009: Linaldeddu *et al*., 2010; Carlucci *et al*., 2015b; Aiello *et al*., 2023), some of these fungi match those found in the present study (i.e. *N. parvum*, *D. seriata*, *N. australe*, *N. luteum* and *B. dothidea*). These species are important in Italy, as they are increasingly isolated from diseased wood of different plant species, including ornamentals, as reported especially for *N. parvum* (Ismail *et al*., 2013; Guarnaccia *et al*., 2016; Aiello *et al*., 2020; Gusella *et al*., 2020; Bezerra *et al*., 2021; Guselle *et al*., 2021; Aiello *et al*., 2022; Fiorenza *et al*., 2022; Gusella *et al*., 2022).

Phaeomoniella chlamydospora was present in all assessed parts of grapevine cuttings, and this was the predominant species obtained at 15 cm from the cutting bases, while *P. minimum* was isolated less frequently. Both fungi are commonly isolated from grapevine cuttings (Bertelli *et al*.,1998; Mugnai *et al*., 1999; Tegli *et al*., 2000).

Fusarium o*xysporum* and *F. proliferatum* were isolated at relatively high frequencies, mainly from grafting points of young plants, highlighting the role of these points for pathogen access to initiate plant infections (Úrbez-Torres *et al*., 2023). *Fusarium* spp. are common inhabitants in asymptomatic and symptomatic grapevines (Lorenzini and Zapparoli, 2015; Lorenzini *et al*., 2016). These fungi have been reported producing vascular discolourations in California in young grapevines and in association with vines exhibiting trunk disease symptoms California and Mexico (Bustamante *et al*., 2022; Travadon *et al*., 2022; Argüelles-Moyao *et al*., 2024). However, other reports from France and Spain have highlighted the potential role of *Fusarium* spp. as biocontrol agents, showing strain-dependent abilities of the genus (González and Tello, 2011; Bruez *et al*., 2014).

For *Cylindrocarpon*-like species causing black foot of grapevine, *I. lirioendri*, *I. destructans, D. torresensis*, and *D. macrodidyma* were only isolated from rootstock bases, and then only at low frequency (2%). These fungi have been mainly reported on mature grapevines, but in recent years they have become more commonly identified in young nursery plants in Italy (Carlucci *et al.,* 2017).

Well-recognised antagonistic fungi were infrequently isolated, including *Clonostachys rosea*, *Trichoderma* spp., as well as saprophytic and endophytic species of *Entoleuca*, *Aspergillus*, *Alternaria*, *Acremonium* and *Cladosporium*, previously found in association with grapevine (Lo Piccolo *et al*., 2015; Silva-Valderrama *et al*., 2021; Úrbez-Torres *et al*., 2020; Zhu *et al*., 2021). Some of these fungi have been reported to infect grapevine under favourable conditions (Latorre *et al*., 2011; Somma *et al*., 2012; Kizis *et al*., 2014; Bustamante *et al*., 2024; Yurchenko *et al*., 2024). Other fungi recovered at low frequency included *Cadophora* spp., which have been previously reported as grapevine pathogens (Mondello *et al*., 2020; Travadon *et al*., 2022), while *A. xenocordella* has been reported as a causal agent of fruit blight of *Pistacia vera* in Italy (Aiello *et al*., 2018).

Neoscytalidium dimidiatum and *Q. cyanescens* were the most isolated species in the present study, and were obtained from all three isolation points of the investigated cuttings. *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers, 2006 is a polyphagous and cosmopolitan plant and human-associated pathogen. This species has a wide host range and has been reported in 37 countries, associated with 126 plant species belonging to 46 families and 84 genera, including six asymptomatic hosts (Derviş and Özer, 2023). To date, *Neos. dimidiatum* has been reported in associations with grapevine in Africa, North and South America, and Asia (Al-Saadoon *et al*., 2012; Rolshausen *et al*., 2013; Correia *et al*., 2016; Kenfaoui *et al*., 2024). The only data for Italy was from Sicily in 1991, where this fungus was reported as *Natrassia toruloidera* (synonym of *Neos. dimidiatum*) on different wine grape cultivars grafted onto 140 RU rootstocks (Granata and Sidoti, 1991), where the pathogen identification was based only on morphological characters. The fungus was later isolated from symptomatic *Citrus sinensis* (sweet orange) plants showing blight, canker and gummosis symptoms, and from branch canker and dieback of *Meryta denhamii* plants, and was identified through molecular analysis (Polizzi *et al*., 2009; Gusella *et al*., 2023).

Quambalaria cyanescens (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer is a ubiquitous fun-

gus, isolated from a broad range of ecological niches. In Italy, this fungus has only been reported amongst mycobiota of withered grapes, but its role in the development of GTDs was not assessed (Lorenzini *et al*., 2016). This fungus has also been isolated from human skin and air samples (Sigler *et al*., 1990). Successively, *Q. cyanescens* and other *Quambalaria* spp., have been reported as pathogens on *Eucalyptus* and *Corymbia* plants (de Beer *et al*., 2006; Paap *et al*., 2008). In 2012, *Q. cyanescens* was found in association with GTDs in north-western Iran (Narmani and Arzanlou, 2019), and was isolated from dormant and healthy grapevine cuttings in Turkey (Görür and Akgül, 2019). More recently, Travadon *et al*., (2022) and Argüelles-Moyao *et al*., (2024) reported *Q. cyanescens* from plants exhibiting GTDs in, respectively, California and Mexico. This fungus has been reported from Russia as pollen endophyte of silver birch (*Betula pendula*), as well as in woody hosts, including in flowers of healthy pomegranate and pistachio, in Iran (Antropova *et al*., 2014; Vahedi-Darmiyan *et al*., 2017; Kari Dolatabad *et al*., 2019). However, *Q. cyanescens* has also been associated with diseased vascular tissues of declining almond trees in Iran (Baradaran Bagheri *et al*., 2015). This species was recently reported in association with larvae and pupae of the phyllophagous olive moth, the skins of healthy green frogs, and faeces and larval debris of codling moths on walnut kernels (Oliveira *et al*., 2012; Mahdizadeh *et al*., 2023; Stupar *et al*., 2023). In addition, *Q. cyanescens* has been reported for its broad-spectrum antimicrobial activity against *Aspergillus fumigatus* and *Beauveria bassiana*, pathogens of *Pistacia vera*, and *Colletotrichum acutatum* (Stodůlková *et al*., 2015; Dolatabad *et al*., 2017; Preto *et al*., 2017).

The phylogenetic analysis of the *Neoscytalydium* and *Quambalaria* isolates obtained in the present study confirmed that they belonged, respectively, to *Neos. dimidiatum* and *Q. cyanescens*. All the *Q. cyanescens* isolates were also found to be closely related to *Q. fabacearum*, as previously reported by Narmani and Arzanlou (2019). However, based on the type strains of the two species, *Q. cyanescens* has shorter conidiogenous cells than *Q. fabacearum*, and unlike *Q. fabacearum*, lacks chlamydospores (Bezerra *et al*., 2018; de Hoog and de Vries 1973). Furthermore, the molecular analyses in the present study have shown that the LSU locus is identical in these two species, while six base differences were observed at the ITS region of the *Q. fabacearum* type strain compared to the *Q. cyanescens*.

The growth temperature studies showed that *Neos. dimidiatum* and *Q. cyanescens* both had greatest growth at high temperature. These results explain the increasing spread and incidence of these pathogens in the context of climate change. *Neoscytalydium dimidiatum* was did not grow at 5 and 10°C, and had greatest mycelial approx. 33°C, and also grew very rapidly at that temperature. In contrast, *Q. cyanescens* was able to grow at all the temperatures tested, although growth was slow.

The pathogenicity assessments fulfilled Koch's postulates for isolates of *Neos. dimidiatum* and *Q. cyanescens*, confirming abilities of these fungi to cause disease symptoms on green and woody tissues, both at the grapevine scions and the rootstocks.

GTDs are an aggregate of fungal diseases that are currently considered to be the most destructive biotic factor affecting grapevines (Kanetis *et al*., 2022). Besides their adverse effects on longevity and productivity of established vineyards, GTD pathogens affect the phytosanitary status of grapevine propagation material, resulting in pathogen dissemination and costs associated with vine replanting. The present study results have shown high incidence of GTD pathogens in nursery plants and identified, for the first time, *Neos. dimidiatum* and *Q. cyanescens* as further causal agents within complex of fungi that cause GTDs in Europe, particularly in Italian nurseries. These results highlight the importance of implementing sustainable management strategies for emerging polyphagous plant pathogens that can infect an increasing number of plant species, especially in the context of climate warming. This could contribute to favourable conditions for development and spread of these pathogens in temperate regions.

Further investigations within the GTD complex are required to determine what triggers latent pathogens to transition from endophytic to a pathogenic state, and to cause symptoms on young plants. As the roles of endophytic fungi remain poorly understood, further studies are required elucidate the pathogenicity of *Fusarium* spp. and to investigate the spread of fungi such as *Q. cyanescens* and *Neos. dimidiatum* in table grape plants. The present study results, along with relevant future research, will be valuable for the early detection of fungi involved in GTDs, and for development of increasingly efficient and sustainable disease management strategies.

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DATA AVAILABILITY

Nucleotide sequences of this study are deposited with NCBI GenBank and the accession numbers are reported within this text.

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