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ORIGINAL ARTICLE

Characterization and pathogenicity of fungal species associated with hazelnut trunk diseases in North-western Italy

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Abstract

Italy is the second largest hazelnut producer worldwide and Piedmont is one of the most productive regions in the country. The changing climatic condition and fungal trunk diseases (FTD) can have a severe impact on this crop. Particularly, the considerable spread of Cytospora cankers ('Mal dello stacco') and dieback represent a serious concern for producers. Thus, considering the limited studies on the causal agents, different surveys were conducted in seven hazelnut orchards during 2021 and 2022. Eight fungal species were identified: *Anthostoma decipiens, Botryosphaeria dothidea, Diaporthe eres, Dia. rudis, Diplodia seriata, Dip. subglobosa, Dothiorella parva* and *Nothophoma brennandiae*. Species identification was achieved through multilocus phylogeny and morphology assessment. All the fungal species were pathogenic on healthy hazelnut plants (cultivar Tonda Gentile) and *A. decipiens* and *Dia. eres* were the most aggressive. The present study is the first report of *B. dothidea* and *Dia. eres* as causal agents of FTD on hazelnut in Italy and of *Dia. rudis, Dip. subglobosa* and *N. brennandiae* worldwide. Moreover, the study provides clarification of the fungal pathogens associated with FTD on this crop in Piedmont, thus laying the base for further studies on epidemiology, ecology and management strategies.

Keywords FTD · *Corylus avellana* · *Anthostoma* · Botryosphaeriaceae · *Diaporthe* · *Nothophoma*

Introduction

Hazelnut (*Corylus avellana* L.) is a perennial bushy plant belonging to Betulaceae native to Europe and western Asia. It represents one of the most economically important nut crops worldwide. The Mediterranean and Black sea areas are historical sites of hazelnut cultivation with Turkey covering more than 60% of the world's production, followed by Italy, USA, Azerbaijan, Georgia, Chile, China, Iran, France and Spain (FAOSTAT 2023). In Italy, 84,526 ha are cultivated with hazelnut and 98,666 tonnes of fruit were harvested in 2022 (ISTAT 2023). Italian hazelnut production has always been present in few geographical areas that are specialized for their environmental and climatic conditions, for the developed technical knowledge and socio-economic context and for the adoption of high quality cultivars (Botta and Valentini 2018). The regions with the highest production are Piedmont (30.5%), Latium (28.7%), Campania (22.5%) and Sicily (12.8%). In Piedmont, hazelnut cultivation covers more than 27,000 ha and the majority (16,558 ha) is located in the Cuneo province (ISTAT 2023). In this region, hazelnut production is mainly based on the cultivar Tonda Gentile (synonyms: 'Tonda Gentile delle Langhe', 'Tonda trilobata'). This cultivar is recognized as one of the best hazelnut cultivar worldwide for its taste and aroma appreciated for fresh consumption and, in particular, for the industry, due to the presence of major international companies specialized in confectionery production (Silvestri et al. 2021). During the last decade, a general expansion in terms of acreage (from 14,375 in 2013 to 24,701 ha in 2022) and yield (from 23,797 in 2013 to 30,180 tons in 2022) in hazelnut cultivation was observed in this region (ISTAT 2023).

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However, the trend in nut production is highly influenced by climatic conditions (An et al. 2020). In the last 2 years, a slight decrease was observed probably due to water deficiency, late-spring frosts and hot summer temperatures. Moreover, a considerable spread of cankers and twig dieback symptoms was observed in hazelnut orchards in Piedmont increasing the concern of the producers.

Cankers, blight, dieback and decay affecting trunk, branches, twigs and, in severe cases, the entire plant, represent a serious threat for hazelnut worldwide (Teviotdale et al. 2002). In the present study, we will include under the general name of fungal trunk diseases (FTD) the diseases caused by fungal pathogens characterized by the mentioned symptoms and that were reported in the main hazelnut growing countries across the world. In the USA, the most important hazelnut growing area is the Willamette valley in Oregon, where the eastern filbert blight (EFB) caused by *Anisogramma anomala* represented a severe problem (Pinkerton 1992). The adoption of resistant cultivars provided an effective strategy to control EFB disease (Johnson et al. 1996). However, the emergence of previously unknown hazelnut disease is observed and previously unassociated fungal pathogens, such as *Diplodia mutila, Diaporthe eres, Dothiorella omnivora* and *Valsa* cf. *eucalypti* were isolated from cankers on trunks and branches (Wiman et al. 2019). In Chile, *Diplodia coryli, Dip. mutila* and *Diaporthe australafricana* were reported as causal agent of hazelnut dieback and cankers (Guerrero and Pérez 2013a, b; Guerrero et al. 2014). *Dothiorella parva* was isolated from *Corylus avellana* in Iran and described as a new species (Abdollahzadeh et al. 2014). Studies conducted in the Guilan province, the main cultivation area of Iran, reported *Botryosphaeria dothidea* and *Diplodia theobromae* as pathogen on hazelnut plants and *Dia. amygdali* as causal agent of hazelnut trees decline (Mir Hosseini Moghaddam and Taherzadeh 2007; Mohammadi and Jabbari Firoozjah 2019; Ghasemi-Doodaran and Davari 2020). Recently, *Cytospora* sp., *Phomopsis* sp., *Lasiodiplodia* sp. and *Pestalotiopsis* sp. were found in association with dieback of hazelnut in the same region (Houshyarfard 2020). In Turkey, *Botryosphaeria dothidea* was reported as pathogen on hazelnut plants (Polat et al. 2022). Moreover, recent studies conducted on *Corylus heterophylla* in China found *Dia. eres, Dia. corylicola, Dia. donglingensis* and *Dia. huairouensis* as FTD causal agents (Gao et al. 2021; Bai et al. 2022). In Italy, different studies were carried out in the main hazelnut growing areas across the peninsula. The presence of Cytospora canker, caused by *Cytospora corylicola* and known as 'Mal dello stacco', was reported throughout the most relevant production regions with a significant yield decrease in Campania (Botta and Valentini 2018). Although the spread of *C. corylicola*, there is a lack of information about the biology and phylogeny of this species and its pathogenicity was long debated (Scortichini 2006; Lamichhane et al. 2014). In Piedmont, sporadic branch cankers were observed in hazelnut orchards and the causal agent were identified as belonging to the genera *Phomopsis* and *Sphaeropsis* (Botta and Valentini 2018). A recent study was conducted in Sardinia on twigs and branches of hazelnut trees with exudates and cankers and different fungal species were isolated form symptomatic plant materials and identified: *Dip. sapinea, Dip. seriata, Dothiorella iberica, Do. omnivora, Do. parva, Do. symphoricarposicola, Diaporthella cryptica, Gnomoniopsis smithogilvyi* and *Anthostoma decipiens* (Linaldeddu et al. 2016). The same study provided a clarification on the etiology of Cytospora canker on hazelnut caused by *A. decipiens* (Linaldeddu et al. 2016).

In Piedmont, the knowledge on FTD of hazelnut is still limited and no studies were recently conducted to investigate the etiology and epidemiology of this disease. Thus, considering the diversity of the symptoms observed in the field and the relevant economic value of this crop, this study was conducted with the aim to investigate the etiology of hazelnut FTD in this region, in detail: (i) to identify the fungal species in association with FTD of hazelnut trees using molecular tools and phylogeny, (ii) to assess the morphological features of the identified species and (iii) to test the pathogenicity of the species found and to fulfil Koch's postulates.

Materials and methods

Field survey and fungal isolation

Field surveys were conducted from March 2021 to September 2022 in seven hazelnut orchards in Piedmont (Table 1). Samples of trunks, branches and twigs were collected from symptomatic plants of 'Tonda Gentile' showing Cytospora cankers ('Mal dello stacco') and dieback symptoms. Wood samples (5–10 mm) were surface sterilised in 1% sodium hypochlorite for 1 min, rinsed in sterile distilled water (SDW) for 1 min and dried on sterile absorbent paper. Small fragments (2–3 mm) were cut from the edge between healthy and necrotic tissues and plated on potato dextrose agar (PDA, VWR Chemicals, Leuven, Belgium) amended with 25 mg l^{-1} of streptomycin sulphate (PDA-S, AppliChem GmbH, Darmstadt, Germany). The plates were incubated at 25 ± 1 °C under a 12 h photoperiod. Following 48 to 72 h of incubation, mycelial plugs were taken from the margin of developing colony and placed on new PDA-S and water agar (WA, Microbiol Diagnostici, Cagliari, Italy) plates. After 4 to 5 days, pure cultures were established by transferring single hyphal tips

Table 1 Coordinates and locations of the orchards surveyed in this study

Orchard n°	GPS coordinates	Location	Sampling year	Fungal isolates
1	44° 35′ 31.7″ N 8°03′13.8″E	Albaretto della Torre (CN)	2021	*Anthostoma decipiens* and *Nothophoma brennandiae*
2	44°38′43.5″N 7°59′23.3″E	Diano d'Alba (CN)	2021	*Anthostoma decipiens, Diaporthe eres* and *Diplodia subglobosa.*
3	44°38′40.3″N 7°59′21.5″E	Diano d'Alba (CN)	2022	*Anthostoma decipiens*
4	44°32′32.0″N 8°06′08.7″E	Feisoglio (CN)	2021	*Diplodia seriata*
5	44°32′44.0″N 8°06′32.1″E	Feisoglio (CN)	2021	*Dothiorella parva* and *Nothophoma brennandiae*
6	44°47′27.0″N 7°53′49.8″E	Monteu Roero (CN)	2022	*Anthostoma decipiens* and *Botryosphaeria dothidea.*
7	44°47′12.6″N 7°54′29.6″E	Monteu Roero (CN)	2021	*Diaporthe eres, Dia. rudis* and *Diplodia seriata*

on new PDA-S plates. A total of 35 isolates were obtained and used for characterization. Stock cultures of these isolates are kept at −80 °C in the culture collection of the University of Torino, Italy.

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Total genomic DNA was extracted from 0.1 g of mycelium grown on PDA-S, using the E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-Tek), following the manufacturer's instructions. Four different genomic loci were amplified and sequenced for species identification. The nuclear ribosomal internal transcribed spacer (ITS) region of each isolate was amplified using the universal primers ITS1 and ITS4 (White et al. 1990). The primers EF1-728F and EF1-986R (Carbone and Kohn 1999) were used to amplify partial region of translation elongation factor-1α (*tef1*) in isolates of Botryosphaeriaceae and *Diaporthe* species (Guarnaccia et al. 2020, 2022a; Aiello et al. 2022). The partial β-tubulin (*tub2*) gene was amplified with primers T1–Bt2b (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997) for isolates belonging to Botryosphaeriaceae family and *Diaporthe* genus (Guarnaccia et al. 2020, 2022a), whilst primers Tub2fd–Tub4fd (Woudenberg et al. 2009) were used to amplify the same region in isolates of the genus *Nothophoma* (Chen et al. 2015). The partial RNA polymerase second largest subunit (*rpb2*) gene was amplified with the primers: Rpb2-5f2–Rpb2-7cr (Liu et al. 1999; Reeb et al. 2004) for isolates identified as *Nothophoma* sp. (Chen et al. 2015). PCR mixtures and conditions were followed as described in the above-cited references. PCR amplification was examined by electrophoresis on 1% agarose (VWR Life Science AMRESCO® biochemicals) gels stained with GelRed™. Eurofins Genomics Service (Cologne, Germany) sequenced PCR products in both directions. The DNA sequences generated were analysed and consensus sequences were computed using the program Geneious v. 11.1.5 (Auckland, New Zealand).

Phylogenetic analyses

The sequences obtained were blasted against the NCBI's GenBank nucleotide database to determine the closest relatives for a taxonomic framework of the studied isolates. Alignments of different genomic regions, including sequences obtained from this study and sequences downloaded from GenBank, were initially performed with the MAFFT v. 7 online server (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh and Standley 2013), and then manually adjusted in MEGA v. 7 (Kumar et al. 2016). Analyses were conducted individually for each locus (data not shown) and as multilocus sequence analyses using the following loci combinations: ITS, *tub2* and *tef1* for members of Botryosphaeriaceae and *Diaporthe* spp. (Guarnaccia et al. 2020; Zhang et al. 2021) and ITS, *tub2* and *rpb2* for *Nothophoma* spp. (Chen et al. 2015). For isolates belonging to *Anthostoma* a single locus analysis was performed on ITS region (Linaldeddu et al. 2016). *Lasiodiplodia theobromae* (CBS 164.96) (Zhang et al. 2021) was used as outgroup for species belonging to Botryosphaeriaceae. *Diaporthella corylina* (CBS 121124) (Guarnaccia et al. 2020) was selected as outgroup for *Diaporthe* spp. *Allophoma minor* (CBS 325.82) (Chen et al. 2015) was used as outgroup for *Nothophoma* spp. and *Cryptovalsa ampelina* (STEU 8113) (Moyo et al. 2018) was selected as outgroup for *Anthostoma* sp. Phylogenies were based on Bayesian Inference (BI) and Maximum Parsimony (MP) analyses. Regarding BI, the best evolutionary model for each partition was determined with MrModeltest v. 2.3 (Nylander 2004) and incorporated in the analyses. MrBayes v. 3.2.5 (Ronquist et al. 2012) was used to generate phylogenetic trees under optimal criteria per partition. The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. The heating parameter was set with the value of 0.2 and trees were sampled every 1000 generations. Analyses stopped at the moment which the average standard deviation of split frequencies was below 0.01. The MP analyses were performed using PAUP

(Phylogenetic Analysis Using Parsimony, v. 4.0b10) (Swofford 2003). Phylogenetic relationships were estimated by heuristic searches with 100 random addition sequences. Tree bisection-reconnection was used, with the branch swapping option set on 'best trees' only with all characters weighted equally and alignment gaps treated as fifth state. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC) were calculated for parsimony and the bootstrap analyses (Hillis and Bull 1993) were based on 1000 replications.

Morphology

Based on molecular characterization, representative isolates were selected to assess their morphological features. Agar plugs (5-mm-diam) were taken from the edge of actively growing cultures and transferred to the center of 9-cm-diam Petri dishes containing PDA-S. Isolates belonging to Botryosphaeriaceae were transferred onto the centre of 9 cm diam Petri dishes containing 2% water agar supplemented with sterile pine needles (PNA) (Smith et al. 1996) to induce sporulation. Isolates belonging to *Diaporthe* were placed both on PNA and on malt extract agar (MEA; Merck, Darmstadt, Germany) to induce sporulation. Plates were then incubated at 25 ± 1 °C under a 12 h photoperiod. Colony characters were observed after 7 days and culture colours were determined according to Rayner (1970). Cultures were examined periodically for the development of conidiomata. Conidia characteristics were examined by mounting fungal structures in SDW and the length and width of 30 conidia were measured for each isolate using an optic microscope at ×40 magnification. The average and standard deviation were calculated.

Pathogenicity

The pathogenicity of representative isolates of the identified species was determined to fulfil Koch's postulates. Two representative isolates of each species were used to inoculate 1-year-old healthy hazelnut plants of 'Tonda Gentile'. Seven plants per fungal isolate were inoculated. For each plant, one inoculation point was considered. The inoculation area was surface disinfected with 70% ethanol solution. A sterile scalpel was used to remove the outer bark to expose the vascular tissues. Mycelium plugs (4 mm diam.) were taken from 7-days-old cultures on PDA-S and placed with the mycelium in contact with the internal plant tissues. The same number of plants were treated with sterile PDA-S plugs as controls. The inoculation points were sealed with Parafilm®. All the plants were placed in a completely randomized design under a shade canopy for 3 months. After this period, the bark was removed, and the internal lesion length was measured. Small wood pieces (2–3 mm) of symptomatic tissue from the margin of the lesions were surface disinfected and placed on PDA-S to re-isolate the inoculated fungal species to fulfill Koch's postulate. The identification of the pathogens was confirmed by colony characteristics. The data obtained were tested for normality, homogeneity of variances, and residual patterns. Logarithmic transformation of the data was performed. ANOVA was conducted with lesion length as dependent variable and fungal isolates as independent variable. Treatment means of lesion length were compared according to Tukey's HSD test at $\alpha = 0.05$. The data analysis was conducted using SPSS software 26 (IBM Corporate).

Results

Field survey and fungal isolation

In all the surveyed orchards (Table 1), with the exception of orchard 3, dieback symptoms, observed with a variable disease incidence from 30 to 40%, seriously compromised entire portions of the tree canopy, mainly in the higher part (Fig. 1a-b). Longitudinal streaks were observed on the bark (Fig. 1c-d) and internal dark brown vascular necrosis were found looking at transversal section (Fig. 1e) and, after bark removal, at longitudinal section (Fig. 1f-g). In four out of seven surveyed orchards (orchard 1, 2, 3, and 6), symptoms of Cytospora canker, known as 'Mal dello stacco', were observed with a disease incidence from 10 to 20%. Typical reddish-brown irregular spots and elongated cankers on the bark were found leading, in severe cases, to the break of branches (Fig. 1h-i). Oozing conidia in a reddish-brown matrix were observed on the outer surface of the trunk in late summer (Fig. 1j). Tissues under the bark showed necrotic dark brown vascular discoloration (Fig. 1k). Both the described symptoms were observed more frequently on mature plants (>10 year-old). Fungal isolates obtained from symptomatic plant samples were grouped as Botryosphaeriaceae, Diaporthe, Cytospora-like and Phoma-like species according to their culture characteristics. The species recovered from each orchard are reported in Table 1.

Phylogenetic analyses

Sequences generated in this study are deposited in GenBank (Table 2). The combined-locus phylogeny of Botryosphaeriaceae consisted of 79 sequences, including the outgroup, and a total of 1444 characters (ITS: 1–549, *tef1*: 556–958 and *tub2*: 965–1444) was included. A total of 31 sequences, including the outgroup, was included in the *Diaporthe* phylogenetic analyses that consisted of 1690 characters (ITS: 1–612, *tef1*: 619–1036 and *tub2*: 1043–1690). The analyses conducted on the *Nothophoma* group

Fig. 1 Symptoms of FTD of hazelnut trees observed in the field. **a, b** Dieback symptoms affecting the higher part of the tree canopy. **c, d** Longitudinal streaks on the bark. **e**–**g** Internal dark brown vascular necrosis. **h, i** Cytospora canker ('Mal dello stacco') symptoms causing the break of branches. **j** Oozing conidia in a reddish-brown matrix. **k** Necrotic dark brown internal vascular discoloration

Table 2 Information on fungal isolates used in the phylogenetic analyses and their corresponding GenBank accession numbers

Species	Culture No. *	GenBank accession number			
		ITS	*tef1*	*tub2*	*rpb2*
Allophoma minor	CBS 325.82	MH861501	–	GU237632	KT389553
Anthostoma decipiens	IPV-FW349	AM399021	–	–	–
Anthostoma decipiens	JL567	JN975370	–	–	–
Anthostoma decipiens	CD	KC774565	–	–	–
Anthostoma decipiens	**CVG1374**	**OR244431**	–	–	–
Anthostoma decipiens	**CVG1380**	**OR244432**	–	–	–
Anthostoma decipiens	**CVG1381**	**OR244433**	–	–	–
Anthostoma decipiens	**CVG1382**	**OR244434**	–	–	–
Anthostoma decipiens	**CVG2197**	**OR244435**	–	–	–
Anthostoma decipiens	**CVG2198**	**OR244436**	–	–	–
Anthostoma decipiens	**CVG2202**	**OR244437**	–	–	–
Anthostoma decipiens	**CVG2203**	**OR244438**	–	–	–
Anthostoma decipiens	**CVG2211**	**OR244439**	–	–	–
Anthostoma decipiens	**CVG2213**	**OR244440**	–	–	–
Anthostoma decipiens	**CVG2216**	**OR244441**	–	–	–
Anthostoma decipiens	**CVG2221**	**OR244442**	–	–	–
Anthostoma decipiens	**CVG2222**	**OR244443**	–	–	–
Botryosphaeria agaves	CBS 133992 t	JX646791	JX646856	JX646841	–
Botryosphaeria agaves	MFLUCC 10-0051	JX646790	JX646855	JX646840	–
Botryosphaeria corticis	ATCC 22927	DQ299247	EU673291	EU673108	–
Botryosphaeria corticis	CBS 119047 t	DQ299245	EU017539	–	–
Botryosphaeria dothidea	CBS 110302	AY259092	AY573218	EU673106	–
Botryosphaeria dothidea	CBS 115476 = CMW 8000 t	AY236949	AY236898	AY236927	–
Botryosphaeria dothidea	**CVG2218**	**OR244444**	**OR257745**	**OR257761**	–
Botryosphaeria dothidea	**CVG2219**	**OR244445**	**OR257746**	**OR257762**	–
Botryosphaeria fabicerciana	CBS 127194 = CMW 27094 t	HQ332197	HQ332213	KF779068	–
Botryosphaeria fabicerciana	CERC 2948	KX277983	KX278088	KX278193	–
Botryosphaeria kuwatsukai	CBS 135219 = PG2 t	KJ433388	KJ433410	–	–
Botryosphaeria kuwatsukai	LSP5	KJ433395	KJ433417	–	–
Botryosphaeria qingyuanensis	CERC 2946 = CGMCC 3.18742 t	KX278000	KX278105	KX278209	–
Botryosphaeria ramosa	CBS 122069 = CMW 26167 t	EU144055	EU144070	KF766132	–
Botryosphaeria scharifii	CBS 124702 = IRAN1543C	JQ772019	JQ772056	–	–
Botryosphaeria scharifii	CBS 124703 = IRAN1529C t	JQ772020	JQ772057	–	–
Cryptosphaeria pullmanensis	ATCC52655	KT425235	–	–	–
Cryptovalsa ampelina	STEU 8113	KY111659	–	–	–
Diaporthe acaciigena	CBS 129521	KC343005	KC343731	KC343973	–
Diaporthe ampelina	CBS 114016 t	AF230751	GQ250351	JX275452	–
Diaporthe amygdali	CBS 126679 t	KC343022	KC343748	KC343990	–
Diaporthe arecae	CBS 535.75	KC343032	KC343758	KC344000	–
Diaporthe australafricana	CBS 111886	KC343038	KC343764	KC344006	–
Diaporthe baccae	CBS 136971	KJ160564	KJ160596	–	–
Diaporthe baccae	CBS 136972 t	KJ160565	KJ160597	MF418509	–
Diaporthe carpini	CBS 114437	KC343044	KC343770	KC344012	–
Diaporthe citri	CBS 135422	KC843311	KC843071	KC843187	–
Diaporthe eres	CBS 109767	KC343075	KC343801	KC344043	–
Diaporthe eres	CBS 116953	KC343147	KC343873	KC344115	–
Diaporthe eres	CBS 134739	KJ160570	KJ160602	–	–
Diaporthe eres	CBS 138594	KJ210529	KJ210550	KJ420799	–
Diaporthe eres	**CVG1334**	**OR244446**	**OR257747**	**OR257763**	–

Table 2 (continued)

Species	Culture No. *	GenBank accession number			
		ITS	*tef1*	*tub2*	*rpb2*
Diaporthe eres	**CVG1338**	**OR244447**	**OR257748**	**OR257764**	–
Diaporthe eres	**CVG1363**	**OR244448**	**OR257749**	**OR257765**	–
Diaporthe eres	**CVG1365**	**OR244449**	**OR257750**	**OR257766**	–
Diaporthe loniceranae	MFLUCC 17-0963	KY964190	KY964146	KY964073	–
Diaporthe malorum	CAA734	KY435638	KY435627	KY435668	–
Diaporthe notophagi	BRIP 54801 t	JX862530	JX862536	KF170922	–
Diaporthe perjuncta	CBS 109745 t	KC343172	KC343898	KC344140	–
Diaporthe rudis	CBS 114011	KC343235	KC343961	KC344203	–
Diaporthe rudis	CBS 114436	KC343236	KC343962	KC344204	–
Diaporthe rudis	CBS 266.85	KC343237	KC343963	KC344205	–
Diaporthe rudis	CPC 23800	KJ160590	KJ160622	KJ160538	–
Diaporthe rudis	CPC 23801	KJ160591	KJ160623	KJ160539	–
Diaporthe rudis	**CVG1332**	**OR244450**	**OR257751**	**OR257767**	–
Diaporthe rudis	**CVG1333**	**OR244451**	**OR257752**	**OR257768**	–
Diaporthe sterilis	CBS 136969 t	KJ160579	KJ160611	KJ160528	–
Diaporthe toxica	CBS 594.93 t	KC343220	KC343946	KC344188	–
Diaporthella corylina	CBS 121124 t	KC343004	KC343730	KC343972	–
Diatrype stigma	UCD DCash200	DQ006945	–	–	–
Diatrype whitmanensis	DCA800	GQ293953	–	–	–
Diplodia africana	CBS 120835 = CPC 5908 t	EF445343	EF445382	KF766129	–
Diplodia afrocarpi	CBS 131681	MT587333	MT592035	MT592471	–
Diplodia agrifolia	CBS 124.30	KX464087	KX464557	KX464783	–
Diplodia allocellula	CBS 130408 = CMW 36468 t	JQ239397	JQ239384	JQ239378	–
Diplodia bulgarica	CBS 124135	GQ923852	GQ923820	–	–
Diplodia bulgarica	CBS 124254 = CAP332 t	GQ923853	GQ923821	–	–
Diplodia citricarpa	CBS 124715 = CJA 131 = IRAN 1578C t	KF890207	KF890189	KX464784	–
Diplodia corticola	CBS 112546	AY259090	EU673310	EU673117	–
Diplodia corticola	CBS 112549 = CAP 134 t	AY259100	AY573227	DQ458853	–
Diplodia cupressi	CBS 168.87 t	DQ458893	DQ458878	DQ458861	–
Diplodia cupressi	CBS 261.85	DQ458894	DQ458879	DQ458862	–
Diplodia eriobotryicola	CBS 140851 = BN-21 t	KT240355	KT240193	MG015806	–
Diplodia estuarina	CMW 41231	KP860831	KP860676	KP860754	–
Diplodia estuarina	CMW 41363	KP860829	KP860674	KP860752	–
Diplodia fraxini	CBS 136010 = CAD001 t	KF307700	KF318747	MG015807	–
Diplodia gallae	CBS 212.25	KX464091	KX464565	KX464796	–
Diplodia malorum	CBS 124130 = CAP271 t	GQ923865	GQ923833	–	–
Diplodia mutila	CBS 112553 t	AY259093	AY573219	KY554743	–
Diplodia mutila	CBS 121862 = PD 03708099	KX464093	KX464567	KX464799	–
Diplodia neojuniperi	CPC 22753 = B0031 t	KM006431	KM006462	–	–
Diplodia neojuniperi	CPC 22754	KM006432	KM006463	–	–
Diplodia olivarum	CBS 121887 = CAP 254 t	EU392302	EU392279	HQ660079	–
Diplodia olivarum	IMI 390972	HM028640	HQ660078	HQ660080	–
Diplodia pseudoseriata	CBS 124906	EU080927	EU863181	MG015820	–
Diplodia quercivora	CBS 133852 = BL8 t	JX894205	JX894229	MG015821	–
Diplodia rosulata	CBS 116470 t	EU430265	EU430267	EU673132	–
Diplodia rosulata	CBS 116472	EU430266	EU430268	EU673131	–
Diplodia sapinea	CBS 124462 = CAP273	GQ923858	GQ923826	–	–
Diplodia sapinea	CBS 393.84 t	DQ458895	DQ458880	DQ458863	–
Diplodia scrobiculata	CBS 118110 = CMW 189 = BOT 1195 t	AY253292	AY624253	AY624258	–

Table 2 (continued)

Species	Culture No. *	GenBank accession number			
		ITS	*tef1*	*tub2*	*rpb2*
Diplodia seriata	CBS 112555 = HAP 052 = CAP 063 t	AY259094	AY573220	DQ458856	–
Diplodia seriata	CBS 119049	DQ458889	DQ458874	DQ458857	–
Diplodia seriata	CBS 171.82	KX464108	KX464598	KX464834	–
Diplodia seriata	CPC 28088	MW413849	MW419167	MW419230	–
Diplodia seriata	CPC 28101	MW413856	MW419174	MW419237	–
Diplodia seriata	**CVG1344**	**OR244452**	**OR257753**	**OR257769**	–
Diplodia seriata	**CVG1346**	**OR244453**	**OR257754**	**OR257770**	**–**
Diplodia seriata	**CVG1405**	**OR244454**	**OR257755**	**OR257771**	–
Diplodia seriata	**CVG1406**	**OR244455**	**OR257756**	**OR257772**	–
Diplodia subglobosa	CBS 124132 = JL375	DQ458887	DQ458871	DQ458852	–
Diplodia subglobosa	CBS 124133 = JL453 t	GQ923856	GQ923824	MT592576	–
Diplodia subglobosa	**CVG1367**	**OR244456**	**OR257757**	**OR257773**	–
Diplodia subglobosa	**CVG1368**	**OR244457**	**OR257758**	**OR257774**	–
Dothiorella americana	CBS 128309 t	HQ288218	HQ288262	HQ288297	–
Dothiorella iberica	CBS 115041 = CAP 145 t	AY573202	AY573222	EU673096	–
Dothiorella iberica	CBS 113189 = DE-14	AY573199	AY573230	KX464855	–
Dothiorella ominivora	CBS 124716 = CJA 241 = IRAN 1573C	KC898232	KC898215	KX464864	–
Dothiorella ominivora	CBS 124717 = CJA 214 = IRAN 1570C	KC898233	KC898216	KX464865	–
Dothiorella ominivora	CBS 188.87	EU673316	EU673283	EU673119	–
Dothiorella ominivora	CBS 242.51	EU673317	EU673284	EU673105	–
Dothiorella ominivora	CBS 392.80	KX464133	KX464626	KX464897	–
Dothiorella parva	CBS 124720 = CJA 27 = IRAN 1579C t	KC898234	KC898217	KX464866	–
Dothiorella parva	CBS 124721 = CJA 35	KX464123	KX464615	KX464867	–
Dothiorella parva	CBS 125580	KX464124	KX464616	KX464868	–
Dothiorella parva	**CVG1414**	**OR244458**	**OR257759**	**OR257775**	–
Dothiorella parva	**CVG1415**	**OR244459**	**OR257760**	**OR257776**	–
Dothiorella sarmentorum	IMI 63581b	AY573212	AY573235	EU673102	–
Dothiorella sempervirentis	IRAN 1583C = CBS 124718 = CJA 264 t	KC898236	KC898219	KX464884	–
Dothiorella vidmadera	CBS 621.74	KX464129	KX464621	KX464887	–
Dothiorella vidmadera	CBS 725.79	KX464130	KX464622	KX464888	–
Dothiorella viticola	CBS 117009 t	AY905554	AY905559	EU673104	–
Etypella vitis	MSU ELM13	DQ006943	–	–	–
Eutypa lata	CBS 121430	KY752766	–	–	–
Eutypa lata	CBS 289.87	DQ006928	–	–	–
Eutypella cerviculata	CBS 221.87	JF340269	–	–	–
Eutypella citricola	STEU 8098	KY111634	–	–	–
Eutypella semicircularis	MP4669 t	JQ517314	–	–	–
Lasiodiplodia thobromae	CBS 164.96	DQ458890	DQ458875	DQ458858	–
Neofusicoccum parvum	CMW 9081 t	AY236943	AY236888	AY236917	–
Nothophoma acaciae	CBS 143404 t	MG386056	–	MG386167	MG386144
Nothophoma anigozanthi	CBS 381.91 t	GU237852	–	GU237580	KT389655
Nothophoma arachis-hypogaeae	CBS 125.93 t	GU237771	–	GU237583	KT389656
Nothophoma brennandiae	CBS 145912	MN823579	–	MN824753	MN824604
Nothophoma brennandiae	MFLUCC 16-1392	KY053896	–	KY053899	KY053898
Nothophoma brennandiae	JW 1066	MN823578	–	MN824752	MN824603
Nothophoma brennandiae	**CVG1361**	**OR244460**	–	**OR257777**	**OR257739**
Nothophoma brennandiae	**CVG1376**	**OR244461**	–	**OR257778**	**OR257740**
Nothophoma brennandiae	**CVG1377**	**OR244462**	–	**OR257779**	**OR257741**
Nothophoma brennandiae	**CVG1378**	**OR244463**	–	**OR257780**	**OR257742**

Table 2 (continued)

Species	Culture No. *	GenBank accession number			
		ITS	*tef1*	*tub2*	*rpb2*
Nothophoma brennandiae	**CVG1431**	**OR244464**	–	**OR257781**	**OR257743**
Nothophoma brennandiae	**CVG1432**	**OR244465**	–	**OR257782**	**OR257744**
Nothophoma eucalyptigena	CBS 142535	KY979771	–	KY979935	KY979852
Nothophoma gossypiicola	CBS 377.67	GU237845	–	GU237611	KT389658
Nothophoma infossa	CBS 123395 t	FJ427025	–	FJ427135	KT389659
Nothophoma infuscata	CBS 121931	MN973559	–	MT005662	MT018203
Nothophoma macrospora	CBS 140674 t	LN880536	–	LN880539	LT593073
Nothophoma prosopidis	CBS 136415 t	KF777149	–	–	–
Nothophoma pruni	MFLUCC 18-1601	MH827005	–	MH853669	MH853662
Nothophoma quercina	CBS 633.92	GU237900	–	GU237609	KT389657
Nothophoma quercina	MFLUCC 18-1588	MH827008	–	MH853672	MH853665
Nothophoma quercina	UTHSC:DI16-270	LT592929	–	LT592998	LT593067
Nothophoma rai	MCC 1082 t	MF664467	–	MF664468	–
Nothophoma variabilis	UTHSC:DI16-285 t	LT592939	–	LT593008	LT593078
Peroneutypa scoparia	CBS 242.87	AJ302465	–	–	–

Isolates from this study are indicated in bold type
* *t* type specimen

consisted of 25 sequences, including the outgroup, with a total of 1437 characters (ITS: 1–493, *tub2*: 500–835 and *rpb2*: 842–1437). The single-locus phylogeny of *Anthostoma* based on ITS consisted of 28 taxa, including the outgroup, and it consisted of 573 characters. A maximum of 1000 equally most parsimonious trees were saved and characteristics of the combined gene partitions used for each phylogenetic analysis are reported in Table 3. Bootstrap support values from the Maximum Parsimony (MP) analysis were plotted on the Bayesian Inference (BI) phylogenies presented in Figs. 2 to 4. For the BI analyses, the models recommended by MrModeltest are reported in Table 4. Unique site patterns for each partition and all the parameters of the Bayesian analyses are reported in Table 3. In the Botryosphaeriaceae analyses, two isolates clustered with the epitype and one reference isolate of *Botryosphaeria. dothidea*, four isolates clustered with the epitype and four reference isolates of *Diplodia seriata*, two isolates clustered with the epitype and one reference isolate of *Dip. subglobosa* and two isolates clustered with the ex-type and two reference isolates of *Dothiorella parva* (Fig. 2). Regarding phylogenies of *Diaporthe*, two isolates clustered with five reference isolates of *Dia. rudis*, whilst four isolates clustered with four reference isolates of *Dia. eres* (Fig. 3). The final tree generated for *Nothophoma* showed that six isolated clustered with the ex-type and two reference isolates of *N. brennandiae* (Fig. 4). Thirteen isolates clustered with three representative sequences of *A. decipiens* (Fig. 5).

Table 3 Parsimony and Bayesian analyses characteristics in this study

		Botryosphaeriaceae	*Diaporthe*	*Nothophoma*	*Anthostoma*
	Locus(i)	ITS + *tef1* + *tub2*	ITS + *tef1* + *tub2*	ITS + *tub2* + *rpb2*	ITS
Parsimony analysis	Total sites	1432	1678	1461	573
	Constant sites	781	756	1134	314
	Variable sites	138	391	137	68
	Parsimony informative sites	513	531	190	191
	Tree length	1466	2298	550	525
	Consistency index	0.679	0.651	0.711	0.739
	Retention index	0.947	0.782	0.771	0.840
	Rescaled consistence index	0.643	0.509	0.548	0.621
Bayesian analysis	Unique site patterns of ITS	191	181	84	246
	Unique site patterns of *tef1*	252	292	–	–
	Unique site patterns of *tub2*	150	318	87	–
	Unique site patterns of *rpb2*	–	–	150	–
	Generation ran	1,730,000	500,000	960,000	865,000
	Generated trees	3462	1002	1922	1732
	Sampled trees	1731	376	1442	1300

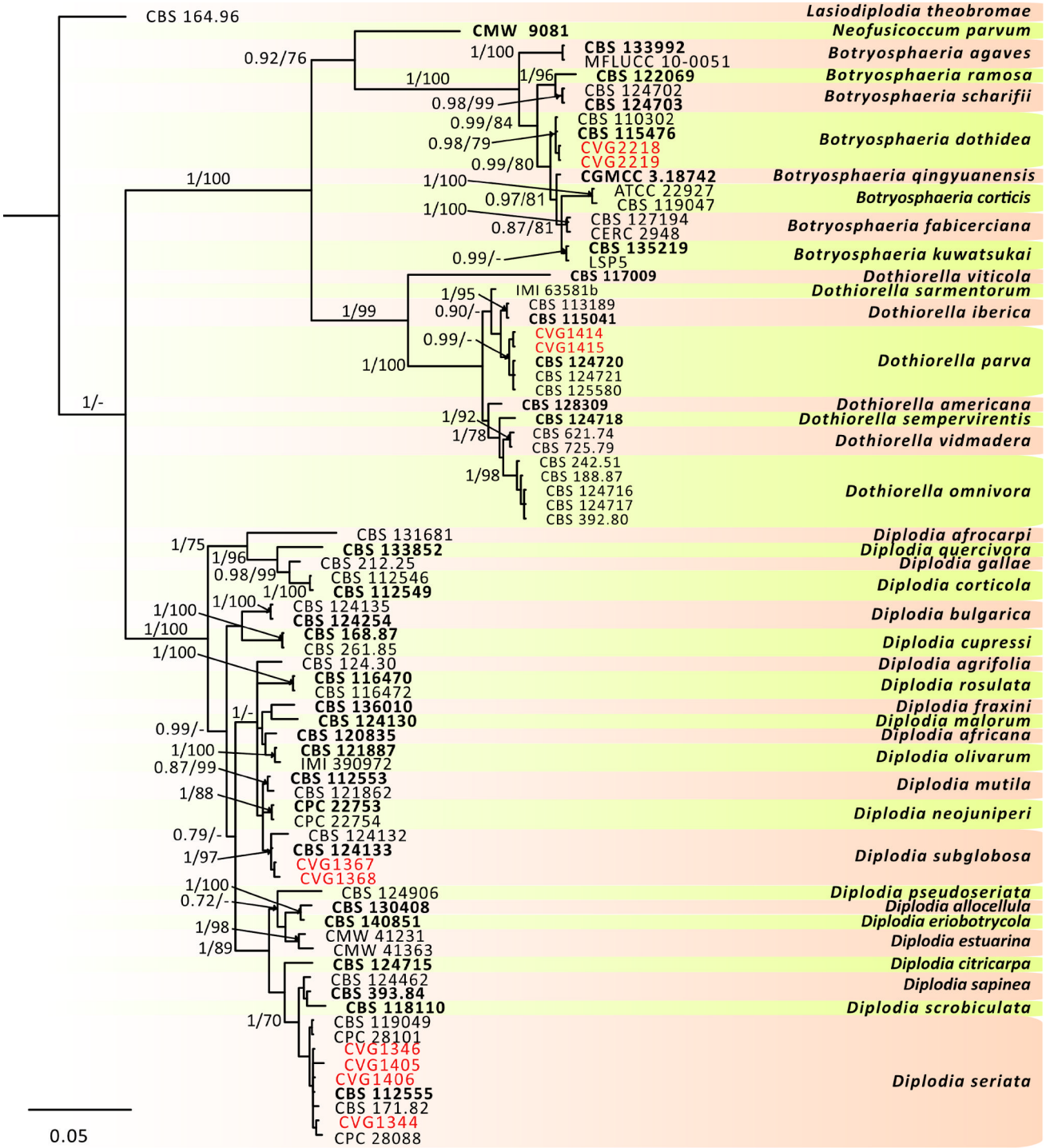

Fig. 2 Consensus phylogram of 3462 trees resulting from a Bayesian analysis of the combined ITS, *tef1* and *tub2* sequences of Botryosphaeriaceae isolates. Bayesian posterior probability values and bootstrap support values are indicated at the nodes. The tree was rooted to *Lasiodiplodia theobromae* (CBS 164.96). Isolates from the current study are indicated in red

Morphology

Morphological features, supported by phylogenetic inference, were observed and used to describe the eight known species (Fig. 6). Colony characters and colours were observed on plates of PDA-S. Botryosphaeriaceae isolates were characterized by the presence of abundant fast-growing aerial mycelium that covered the entire PDA-S petri dishes after 7 days. *Botryosphaeria dothidea* colonies were white to pale grey. Colony reverse color was pale

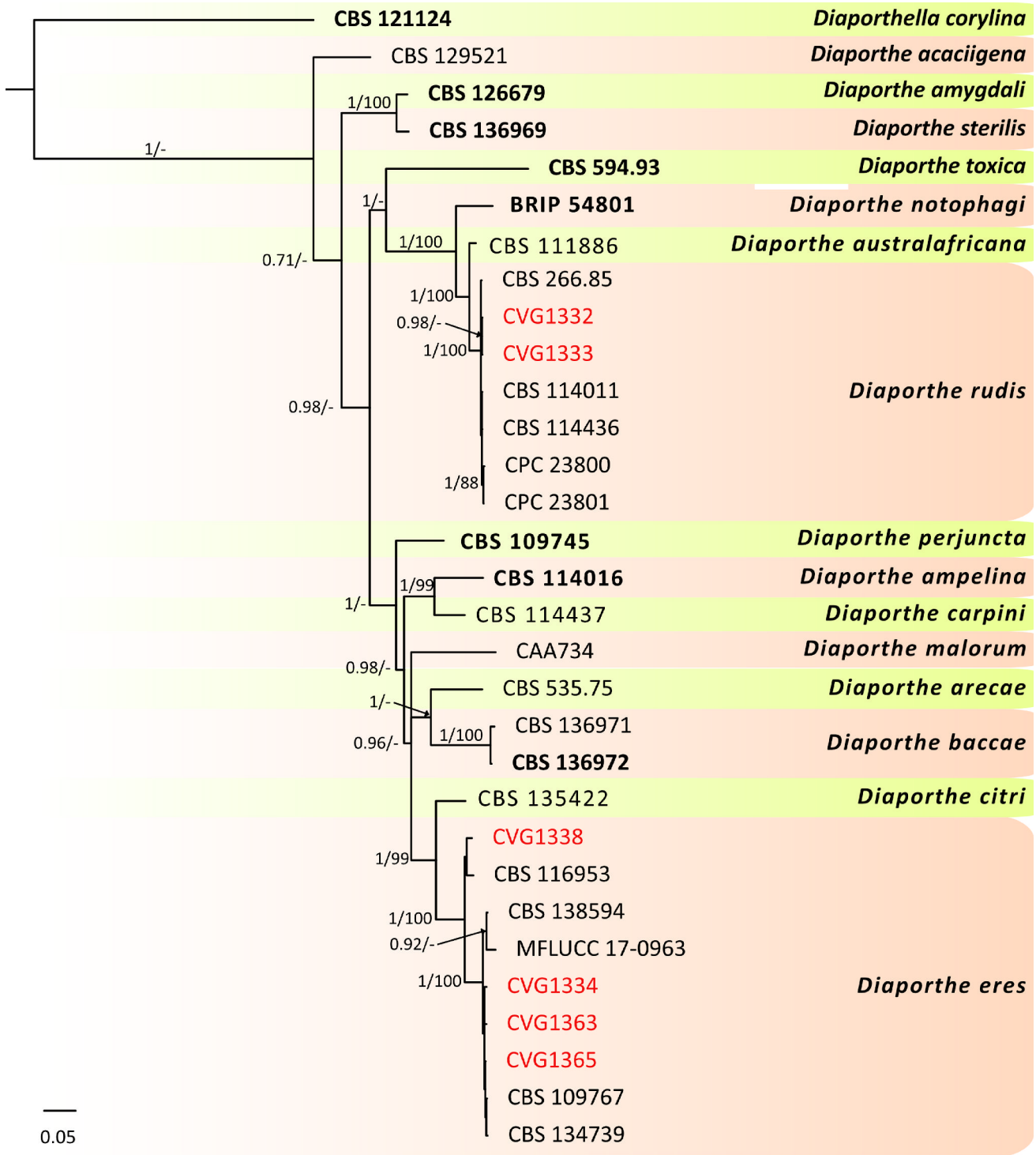

Fig. 3 Consensus phylogram of 1002 trees resulting from a Bayesian analysis of the combined ITS, *tef1* and *tub2* sequences of *Diaporthe* isolates. Bayesian posterior probability values and bootstrap support values are indicated at the nodes. The tree was rooted to *Diaporthella corylina* (CBS 121124). Isolates from the current study are indicated in red

grey. Conidia were hyaline, aseptate, thin-walled, fusiform to subclavate, with dimensions of 20.7–28.9 × 4.6–6.7 µm, mean ± SD = 24.8 ± 2 × 5.6 ± 0.6 µm. Colonies of *Dip. seriata* were light grey on the front side and light grey turning dark in the centre on the reverse side. Conidia were light brown, ovoid with truncated or rounded base and obtuse apex, aseptate, with dimensions of 20.2–26.8 × 8.2–11.2 µm, mean ± SD = 23 ± 1.6 × 9.5 ± 0.8 µm. Colonies of

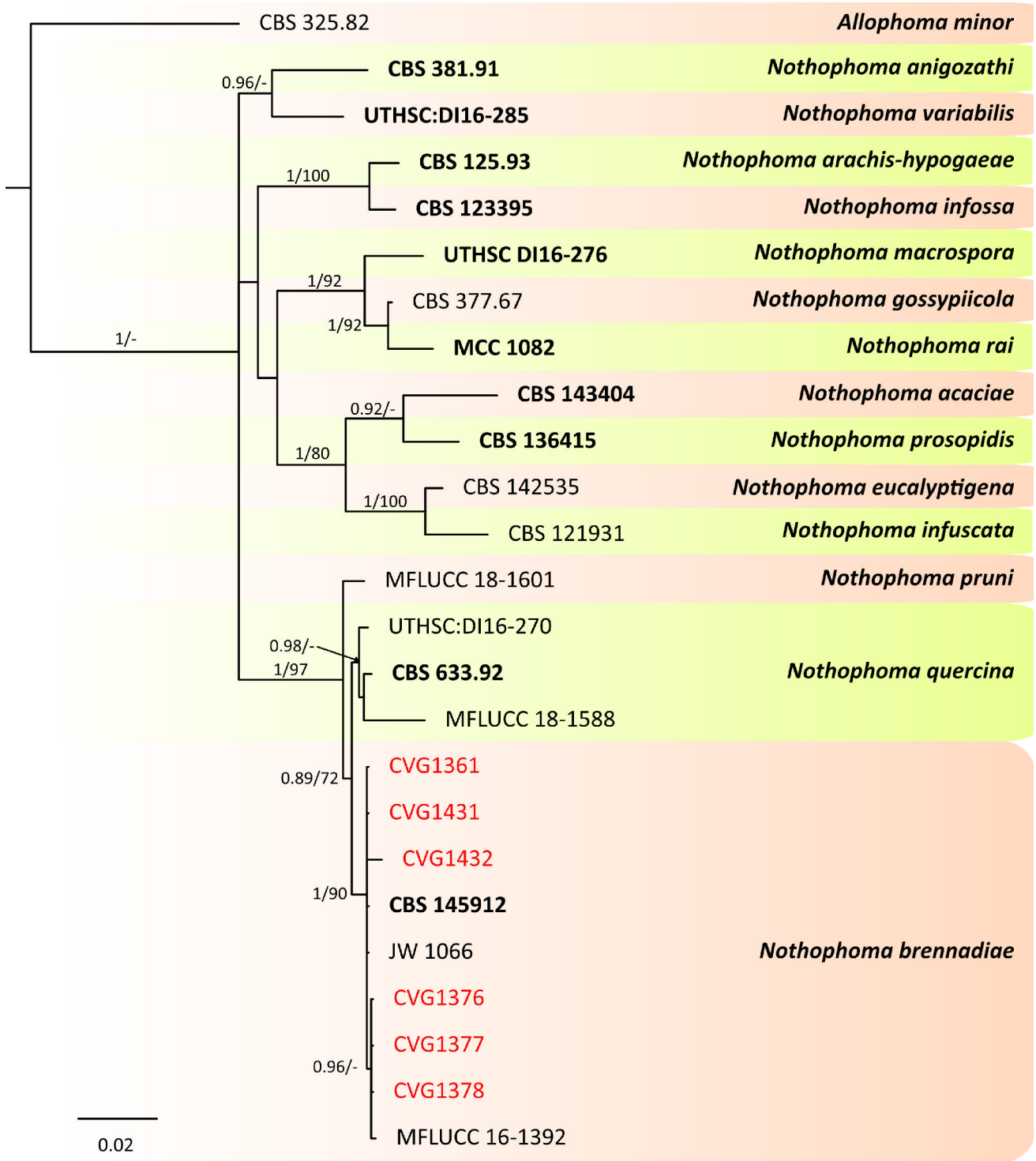

Fig. 4 Consensus phylogram of 1922 trees resulting from a Bayesian analysis of the combined ITS, *tub2* and *rpb2* sequences of *Nothophoma* isolates. Bayesian posterior probability values and bootstrap support values are indicated at the nodes. The tree was rooted to *Allophoma minor* (CBS 325.82). Isolates from the current study are indicated in red

Dip. subglobosa were light grey turning dark grey in the center both on the obverse and reverse sides. Conidia were hyaline, aseptate, smooth, thick-walled, oblong to ovoid, straight, ends broadly rounded and measured 26.9–33.2 × 13.3–17.1 µm, mean ± SD = 30.2 ± 1.3 × 15.4 ± 0.9 µm. *Dothiorella parva* colonies were pale olivaceous grey on the obverse side and dull green- to dark olivaceous-grey on the reverse side. Conidia were ellipsoid to ovoid,

Table 4 Evolutionary models as determined by MrModeltest (Nylander 2004)

Family/genus	Locus	Evolutionary model*
Botryosphaeriaceae	ITS	HKY + I + G
	tef1	HKY + G
	tub2	GTR + G
Diaporthe	ITS	SYM + I + G
	tef1	HKY + I + G
	tub2	HKY + G
Nothophoma	ITS	K80 + G
	tub2	GTR + G
	rpb2	SYM + G
Anthostoma	ITS	GTR + I

G Gamma distributed rate variation among sites, *GTR* Generalised time-reversible, *HKY* Hasegawa-Kishino-Yano, *I* Proportion of invariable sites, *K80* Kimura's two parameter model, *SYM* Symmetrical model

brown, 1-septate, moderately thick-walled, ends rounded, often with a truncate base, with dimensions of 16.3–21.5 × 7.5–10.3 µm, mean ± SD = 20.1 ± 1 × 8.9 ± 0.7 µm. Isolates of *Diaporthe* showed fluffy aerial mycelium. *Diaporthe eres* colonies were white to pale grey on the front side and pale grey turning dark in the center on the reverse side. Alpha conidia were aseptate, hyaline, smooth, ovoid to ellipsoid, guttulate and measured 5.4–7.5 × 1.5–2.9 µm, mean ± SD = 6.3 ± 0.6 × 2.2 ± 0.3 µm. Beta conidia were hyaline, aseptate, smooth, spindle shaped, slightly curved, measuring 20.4–30.3 × 1.0–1.4 µm, mean ± SD = 25.1 ± 2.5 × 1.2 ± 0.1. *Diaporthe rudis* colonies were white to beige with a brownish halo around the margin on the front side and pale beige in the margin to buff honey in the center on the reverse side. Alpha conidia were hyaline, aseptate, smooth, biguttulate and ellipsoid with subtruncate bases and measured 4.2–6.9 × 1.4–2.6 µm, mean ± SD = 5.7 ± 0.7 × 1.9 ± 0.3 µm. Beta conidia were not observed. Colonies of *N. brennandiae* were characterized by cottony moderate aerial mycelium dark brick to sepia on the obverse side and dark brick to cinnamon on the reverse. Conidia were hyaline becoming brown, ellipsoidal to oblong, straight and measured 3.9–6.2 × 2–4.3 µm, mean ± SD = 5.1 ± 0.6 × 3.2 ± 0.5 µm. *Anthostoma decipiens* colonies showed cottony scarce aerial mycelium white to pale grey on the front side and honey in the margin to buff in the center on the reverse side. Conidia were hyaline, lunate and unicellular measuring 5.9–9.7 × 1.2–2.4 µm, mean ± SD = 7.4 ± 0.8 × 1.7 ± 0.3 µm.

Pathogenicity

All the tested isolates caused symptoms similar to those found in the field (Fig. 7). The plants inoculated with the fungal isolates showed external longitudinal streaks or dark brown lesions on the bark. Plants inoculated with representative isolates of *A. decipiens* were characterized by elongate canker development at the inoculation point. Internal necrosis and vascular discolouration were observed. Isolate CVG1374 of *A. decipiens* showed the highest lesion length (76.4 ± 22.6 mm), followed by *A. decipiens* isolate CVG1380 (lesion length = 76.4 ± 22.6 mm) and *Dia. eres* isolate CVG1334 (lesion length = 30.3 ± 7.3 mm). The values of mean lesion length for nine strains ranged from 13.5 ± 4.0 to 4.8 ± 1.8 mm for *B. dothidea* strain CVG2219 and *Dia. rudis* strain CVG1333, respectively, however their aggressiveness in hazelnut plants was not significantly different among them. The four remaining strains caused lesions which showed no significant differences among them, with lesion length ranging from 4.1 ± 0.7 to 2.5 ± 0.6 mm for *Dip. subglobosa* strain CVG1367 and *Do. parva* strain CVG1415 (Fig. 8). All the inoculated species were re-isolated and the morphological characteristics (color, shape, mycelium texture and conidia) were assessed to confirm their identity. Koch's postulates were fulfilled. Weak necrosis observed on control plants (mean lesion length = 2.5 ± 0.6 mm) were restricted to the inoculation point and considered as a reaction to wounding. No fungal colonies were retrieved after re-isolation from control plants.

Discussion

The present study represents the first investigation on the fungal species diversity in association with FTD of hazelnut in Piedmont. Currently, the spread of different FTD symptoms across this area is representing an increasing concern to hazelnut producers. The surveys, conducted in seven orchards, focused on sample collection from plants showing different symptoms, thus, to clarify the identification of the fungal species associated with the main two wood diseases reported. Isolates recovered from plants showing twig and branch dieback were grouped in Botryosphaeriaceae, *Diaporthe* spp. and *Phoma*-like spp. according to their colony morphology (Phillips et al. 2013; Udayanga et al. 2014; Chen et al. 2015). Isolates from plants showing symptoms of Cytospora canker, known also as 'Mal dello stacco', were grouped in Botryosphaeriaceae and *Cytospora*-like spp., based on their culture characteristic (Phillips et al. 2013; Lawrence et al. 2018). Sequencing of molecular loci and phylogenetic analyses allowed the identification of eight different species associated with FTD on hazelnut in Piedmont: *Anthostoma decipiens, Botryosphaeria dothidea, Diaporthe eres, Dia. rudis, Diplodia seriata, Dip. subglobosa, Dothiorella parva* and *Nothophoma brennandiae*. Particularly, *Diaporthe* spp., *Diplodia* spp., *Do. parva* and *N. brennandiae* were isolated in association

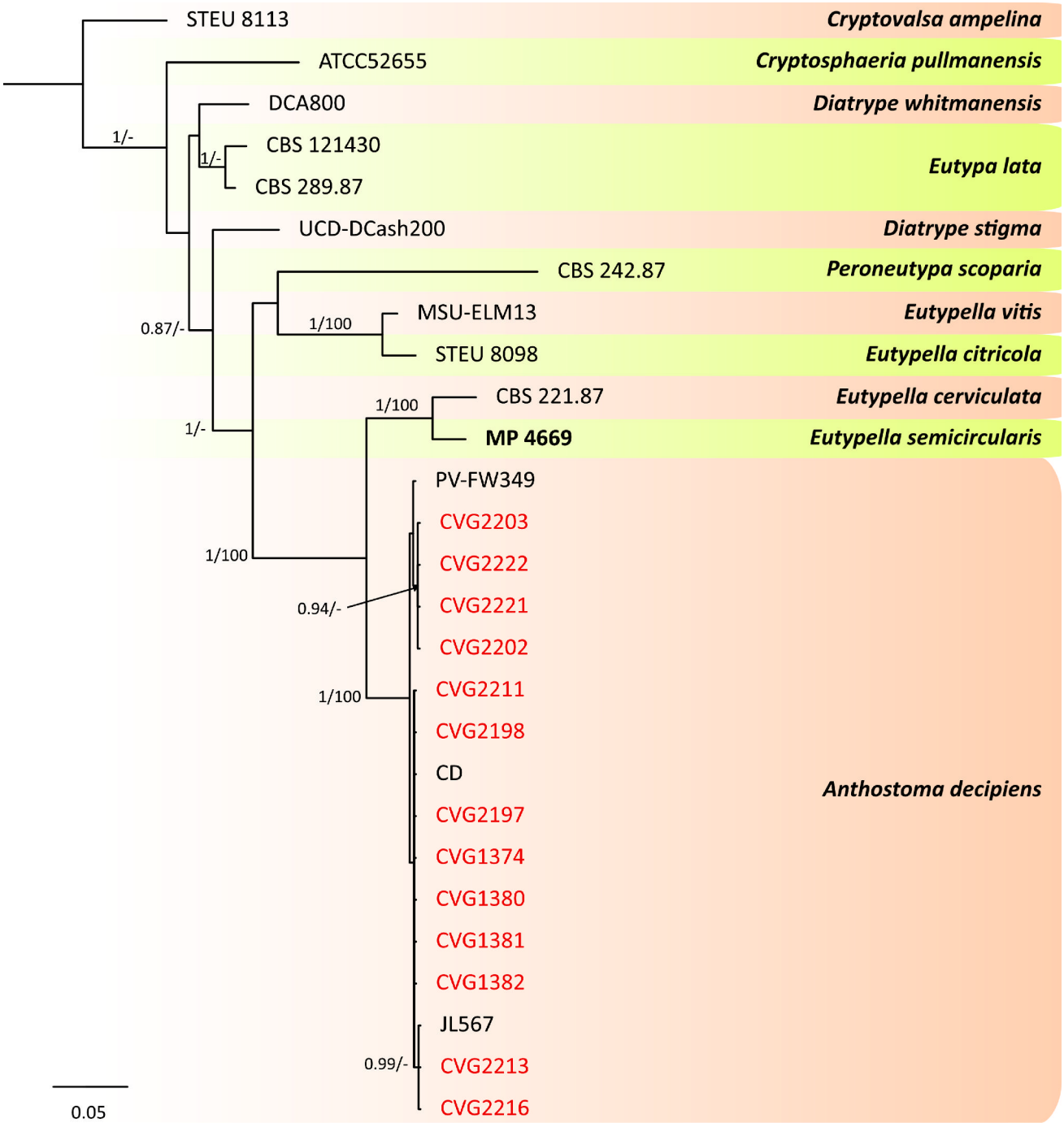

Fig. 5 Consensus phylogram of 1732 trees resulting from a Bayesian analysis of the ITS sequences of *Anthostoma* isolates. Bayesian posterior probability values and bootstrap support values are indicated at the nodes. The tree was rooted to *Cryptovalsa ampelina* (STEU 8113). Isolates from the current study are indicated in red

with twig and branch dieback, whilst *A. decipiens* and *B. dothidea* were found in association with Cytospora canker ('Mal dello stacco'). This study is also the first report of *B. dothidea* and *Dia. eres* as causal agents of FTD on hazelnut in Italy. Moreover, it is the first report of *Dia. rudis, Dip. subglobosa* and *N. brennandiae* causing twig and branch dieback on hazelnut trees worldwide. Species belonging to Botryosphaeriaceae family are well known as polyphagous pathogens for their wide distribution and virulence on multiple plant hosts (Batista et al. 2021). *Botryosphaeria dothidea* was previously reported on hazelnut trees in Iran and Turkey (Mohammadi and Jabbari Firoozjah 2019; Polat et al. 2022) and *Dip. seriata* was reported as wood pathogen on hazelnut in Sardinia

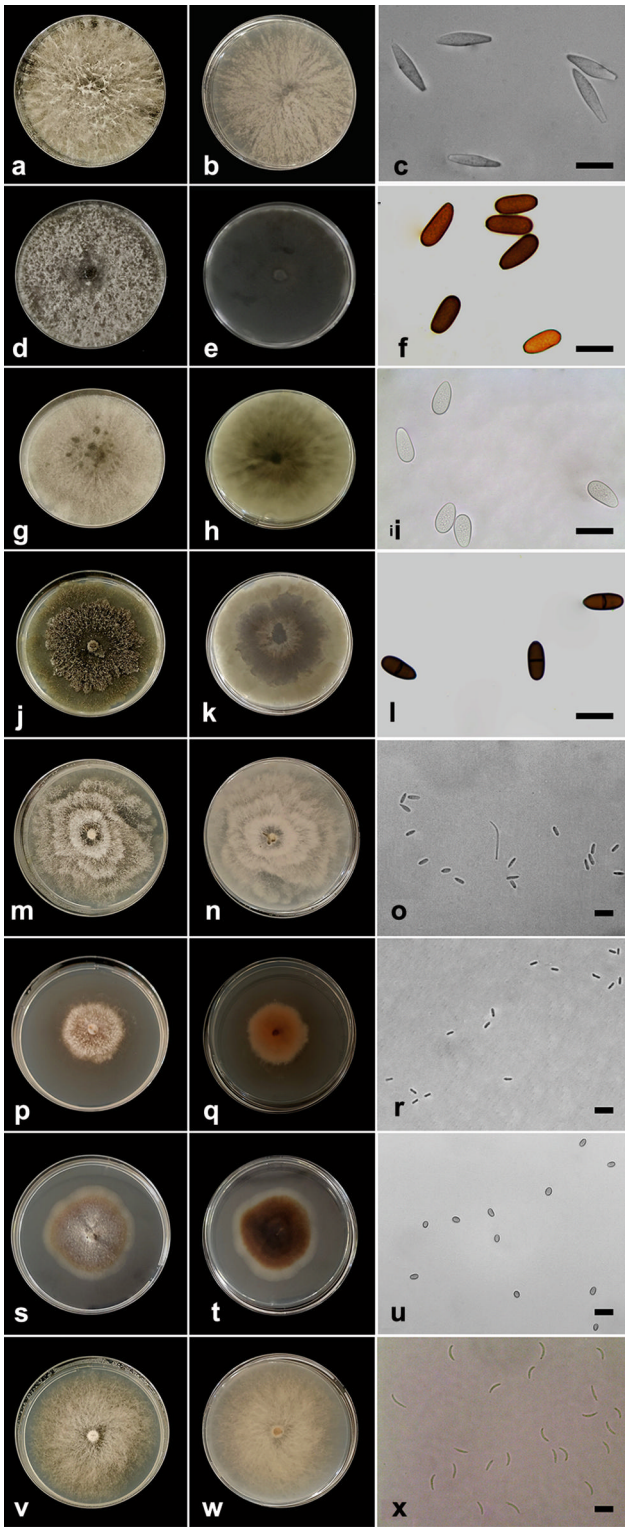

Fig. 6 Morphological characteristics of the different fungal species grown 7 days on PDA-S. **a, b, c** *Botryosphaeria dothidea*. **d, e, f** *Diplodia seriata*. **g, h, i** *Diplodia subglobosa*. **j, k, l** *Dothiorella parva*. **m, n, o** *Diaporthe eres*. **p, q, r** *Diaporthe rudis*. **s, t, u** *Nothophoma brennandiae*. **v, w, x** *Anthostoma decipiens*. Scale bar = (**c, f, i, l**) 20 µm. **o, r, u, x** 10 µm

(Linaldeddu et al. 2016). Hereby, *B. dothidea* was isolated in association with *A. decipiens* in the surveyed orchard n°6 (Monteu Roero-CN), whilst *Dip. seriata* was found in two different sites, orchard n°4 (Feisoglio-CN) and orchard n°7 (Monteu Roero-CN). *Diplodia subglobosa* was described as a new species in association with *Fraxinus* spp. and *Lonicera nigra* and it was recently reported on *Fraxinus excelsior* in Italy and Slovenia (Alves et al. 2014; Linaldeddu et al. 2020, 2022). In this study, this species was found in co-occurrence with *A. decipiens* in orchard n°2 (Diano d'Alba-CN). *Dothiorella parva* was isolated from *Corylus avellana* in Spain and reported as *Dothiorella* sp. (Phillips et al. 2008). This species was described as *sp. nov.* considering isolates from hazelnut in Iran and it was later reported as pathogen on *Ostrya carpinifolia*, a forest tree within the Betulaceae, in Slovenia and Italy (Abdollahzadeh et al. 2014; Pavlic-Zupanc et al. 2015). Recently, its name was proposed as a synonym of *Do. sarmentorum* (Zhang et al. 2021). In the present survey, it was found in co-occurrence with *N. brennandiae* in the orchard n°5 (Feisoglio-CN). All the Botryosphaeriaceae species found in the present study, *B. dothidea, Dip. seriata, Dip. subglobosa* and *Do. parva*, were pathogenic when inoculated on healthy hazelnut plants with a different virulence depending on both the tested species and isolates. Particularly, *Dip. subglobosa* showed minor symptoms with respect to the other tested isolates within Botryosphaeriaceae. The different virulence of the two isolates of *Do. parva* suggests the presence of intraspecific variability. *Diaporthe* spp. are included among the most globally relevant causal agents of FTD on different fruit crops (Lawrence et al. 2015; Guarnaccia et al. 2022b). *Diaporthe eres* was reported as wood pathogen on *Corylus avellana* in Oregon and Chile (Guerrero and Pérez 2013a; Wiman et al. 2019) and on *Corylus heterophylla* in China (Gao et al. 2021; Bai et al. 2022). *Diaporthe rudis* was reported on hazelnut only as causal agent of kernel defects, as well as *Dia. eres* (Pscheidt et al. 2019; Arciuolo et al. 2020, 2022). In this study, both species were found in co-occurrence with Botryosphaeriaceae in two out of seven orchards, particularly in orchard n°2 (Diano d'Alba-CN) and n°7 (Monteu Roero-CN). The pathogenicity test on these species showed a high virulence of one isolate of *Dia. eres*, whilst the other isolates caused minor lesions as both the isolates of *Dia. rudis*. The difference found suggests an intraspecific variability for pathogenicity in *Dia. eres* that was already reported for this species (Lawrence et al. 2015). *Nothophoma brennandiae* was originally isolated from *Ulmus* × *hollandica* in Italy and reported as *N. quercina*

Fig. 7 Internal lesion of hazelnut branches of cv. 'Tonda Gentile' at 3 months after inoculation with mycelial plugs of the species: **a** *Anthostoma decipiens*. **b** *Botryosphaeria dothidea*. **c** *Diaporthe eres*. **d** *Dothiorella parva*. **e** *Diplodia seriata*. **f** *Nothophoma brennandiae*. **g** *Diaporthe rudis*. **h** *Diplodia subglobosa*. **i** control

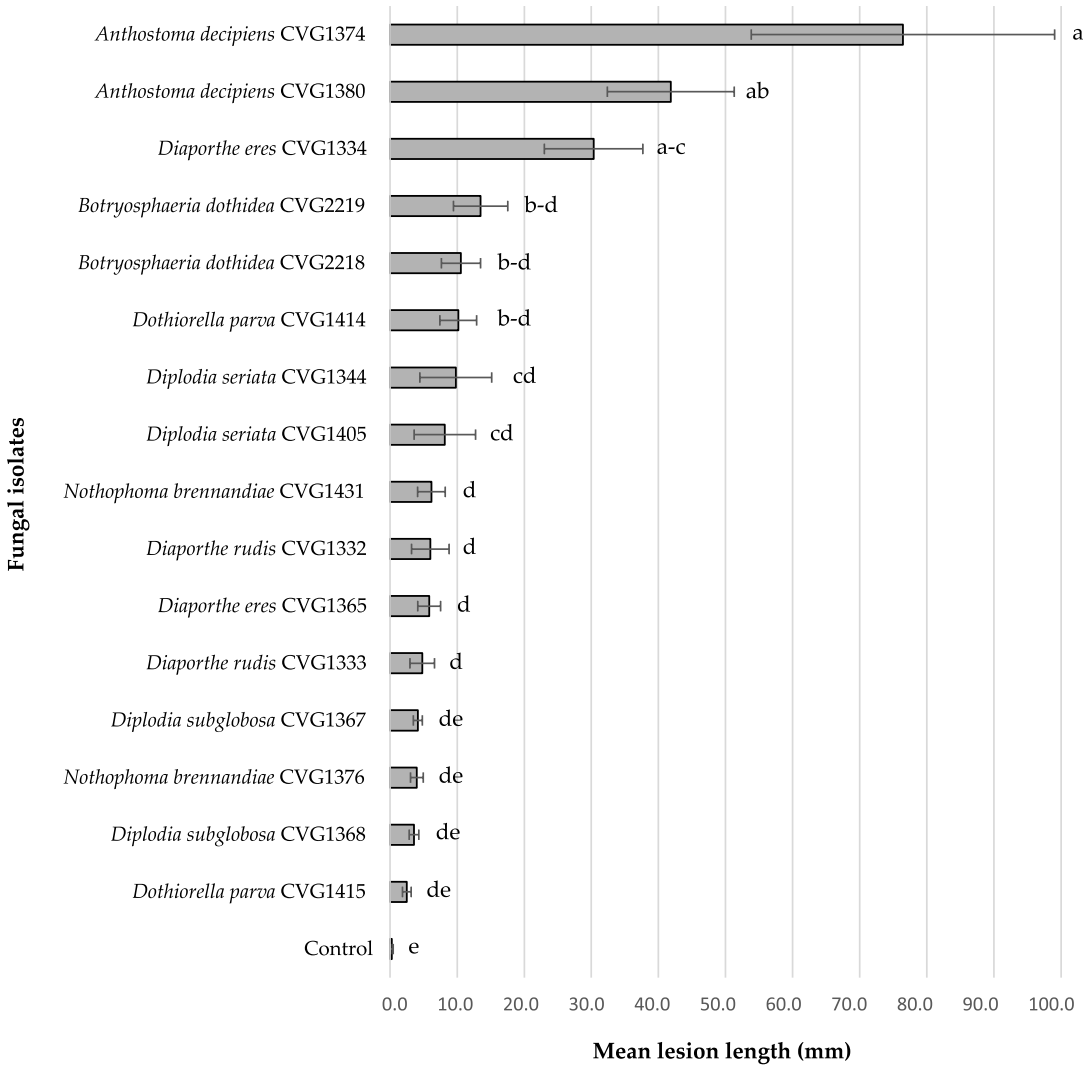

Fig. 8 Disease severity (lesion length, mm) on hazelnut plants of 'Tonda Gentile' at 3 months after inoculation of representative fungal isolates of *A. decipiens, B. dothidea, Dia.* eres, *Dia. rudis, Dip. seriata, Dip. subglobosa, Do. parva* and *N. brennandiae*. Columns represent the mean data of seven replicate plants per strain. Columns with common letters do not differ significantly according to Tukey's HSD test (P = 0.05) for lesion length. Mean comparison test was applied to log-transformed lesion length data. Horizontal lines on the columns are the standard error of the mean

and then described as a new species isolated from garden soil in The Netherlands (Hou et al. 2020). It was found in co-occurrence with *A. decipiens* and *Do. parva* in orchard n°1 (Albaretto della Torre-CN) and orchard n°5 (Feisoglio-CN), respectively. This species caused minor symptoms when inoculated on hazelnut plants. The identification of *A. decipiens* represents the clarification of the causal agent of Cytospora canker ('Mal dello stacco') in the surveyed area and it is in line with the previous report in Sardinia (Linaldeddu et al. 2016). The doubts about the fungal species causing the Cytospora canker on hazelnut was long debated in Piedmont as well as in other Italian regions (Scortichini 2006; Botta and Valentini 2018). The cultural characteristics of *A. decipiens*, which have a high similarity with *Cytospora* spp. in terms of size and shape of conidia, have so far led the attribution of Cytospora canker to *Cytospora corylicola* or *Cytospora* spp. (Linaldeddu et al. 2016). *Anthostoma decipiens* was reported as pathogen on *Carpinus betulus*, another plant of the Betulaceae, in Italy (Saracchi et al. 2008; Rocchi et al. 2010) and Iran (Mirabolfathy et al. 2018). Moreover, it was found in association with grapevine trunk disease in Spain (Luque et al. 2012) and reported as pathogen of different plant hosts such as *Alnus glutinosa, Betula pendula, Castanea sativa, Corylus avellana, Vagus sylvatica* and *Ostrya carpinifolia* (Saracchi et al. 2015). In the present study, both isolates used in the pathogenicity test showed high virulence and caused the most severe lesions with respect to the other tested species. The ability of this pathogen to cause severe internal lesion with consequent cracks in the bark represents a serious threat to hazelnut, especially in late summer when conidia evasion occurs promoting the inoculum spread. Conidia of *A. decipiens* are thought to be dispersed through wind and rains as those of Botryosphaeriaceae and *Diaporthe* spp., representing a primary source of inoculum (Phillips et al. 2013; Lawrence et al. 2015). Pruning wounds, along with natural openings, are the main pathway for the colonization (Moral et al. 2019). The use of non-contaminated tools, the protection of wounds and the removal of pruning debris must be adopted. Proper agronomic practices contribute to avoid the inoculum spread, especially considering that several pathogens hereby found were reported in the same area on other fruit crops such as apple, grapevine and blueberry (Martino et al. 2022, 2023; Dardani et al. 2023). Additional surveys in more orchards as well as pathogenicity tests are planned, to investigate the incidence of the disease in North-western Italy as well as the infection rate caused by spore suspensions of each fungal pathogen. Moreover, the influence of inoculum density on the disease development will be assessed along with the seasonal dynamic of spore production, then to provide a correct pruning timing. Further studies on co-infection of different fungal species will be addressed to provide useful information on the complexity of FTD on hazelnut. Several fungal species found in the present study caused minor symptoms on healthy hazelnut plants, suggesting that they could act as secondary pathogens. Insights on the biology and ecology of these species are needed to establish effective disease management strategies. Moreover, understanding the interaction with abiotic stress and wood microbial community could contribute to the knowledge on FTD, promoting the high value of the cultivar Tonda Gentile produced in Piedmont to which the Protected Geographical Indication (PGI) was recognized by the European Union with the name 'Nocciola Piemonte' ('Nocciola Piemonte delle Langhe', from 2019).

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

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