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Short Notes

Gnomoniopsis paraclavulata, a previously unrecorded causal agent of oak decline in Italy

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Summary. Oak trees (*Quercus pubescens*) showing symptoms of twig and branch dieback, internal wood necroses, and decline, were surveyed in a public park located in Catania province (eastern Sicily, Italy). *Gnomoniopsis*-like fungi were consistently isolated from symptomatic wood tissues. Based on morphology and phylogenetic analyses of ITS, *tef1* and *tub2* loci, the fungi were identified as *Gnomoniopsis paraclavulata*. A pathogenicity test was conducted by inoculating stems of 1-year-old oak seedlings with mycelium plugs of a representative *G. paraclavulata* isolate. Three months after inoculation, internal necrosis around inoculation points and twig dieback were observed. Colonies of *G. paraclavulata* were reisolated from necrotic tissues of inoculated plants, fulfilling Koch's postulates. This is the first report of *G. paraclavulata* causing dieback and decline on *Q. pubescens* trees.

Keywords. Fungal disease, *Quercus pubescens*, dieback, wood necrosis.

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INTRODUCTION

Quercus L., (*Fagaceae*), is an important plant genus that is widespread in forest ecosystems (Tantray *et al*., 2017). Oak forests are abundant in the southern regions of Italy, particularly around Mount Etna in Sicily, making these forests significant areas for biodiversity (La Mantia *et al*., 2003; Gianguzzi *et al.,* 2015). In 2022 and 2023, symptoms of defoliation and dieback of twigs and branches were observed on *Q. pubescens* trees in a public park in Catania (37°32'22.5"N 15°01'46.6"E). These oak trees have considerable social and ecological importance, as they commemorate an historical lava flow from Mount Etna in 1669 that caused significant damage to the area (Bottari *et al*., 2022). In literature oak decline is reported as a serious ecologically important disease that is spreading in many Italian regions, being a disease syndrome that is affected by climate change, drought, wildfire, and forest mismanagement (Linaldeddu *et al*., 2014, 2017; Scortichini, 2025). Since the incidence of the declined oaks was very high in our surveys, the aim of this study was to characterize the causal agent of *Q. pubescens* decline in Catania province, providing morphological and molecular characterizations of the pathogen.

MATERIALS AND METHODS

Diseased samples were collected from 20 plants and transferred to the laboratory of the Department of Agriculture, Food and Environment, University of Catania. Wood fragments between symptomatic and healthy tissues were used for isolations onto potato dextrose agar (PDA) amended with streptomycin sulfate (100 mg L^{-1}). After incubation at 25 ± 1 °C in the dark for 7 d, *Gnomoniopsis*-like colonies were consistently isolated, with an isolation frequency of 70%. A total of eight colonies were selected to obtain single spore isolates, and these were stored in the fungal collection of the Plant Pathology Section, Department of Agriculture, Food and Environment, University of Catania.

A representative isolate (Q9) was used to study culture characteristics morphology (colony and conidium morphology). Conidial masses were placed on microscope slides with drops of 100% lactic acid, and covered with a coverslip, to measure conidium dimensions at 100× magnification, with a Zeiss Axiolab 5 microscope and Zeiss Axiocam 208 colour camera, using the software Zen Core (v.35.96.03000). Five of the collected isolates (Q3, Q4, Q7, Q9 and Q17) were grown on PDA for 14 d, and genomic DNA was extracted from each isolate after scraping the mycelium with a sterile scalpel, and using the Wizard Genomic DNA Purification Kit (Promega Corporation). The extracted DNA was stored at 4°C for further analyses. The polymerase chain reaction (PCR) was carried out in a total volume of 25 µL, using One Taq® 2× Master Mix with Standard Buffer (BioLabs), according to the manufacturer's instructions. The following loci were amplified and sequenced: the complete internally transcribed spacer region (ITS1-5.8S-ITS2) rDNA gene with the primers ITS5 and ITS4 (White *et al*., 1990), an approx. 0.7 kb fragment of the translation elongation factor 1 alpha (*tef1*) with primers EF1-688F (Alves *et al*., 2008) and EF-2 (O'Donnell *et al*., 1998), and an approx.0.5 kb fragment of the partial beta tubulin gene (*tub2*) with the primer Bt2a and Bt2b (Glass and Donaldson, 1995). PCR conditions were set as follows: 30 s at 94°C; 35 cycles each of 30 s at 94°C; 1 min at 52°C (ITS) or 54°C (*tef1* and *tub2*); 1 min at 68°C; and a final cycle for 5 min at 68°C. PCR products were visualized on 1% agarose gels (90 V for 40 min), that were stained with GelRed® Nucleic Acid GelStain (Biotium) to confirm the presence and size of PCR products. Amplicons were purified and sequenced in both direction by Macrogen Inc., Seoul, South Korea. The DNA sequences generated were assembled with Lasergene SeqMan Pro (DNASTAR), and were deposited in GenBank (https://www.ncbi.nlm.nih.gov/) (Table 1).

The phylogenetic analyses were performed using Maximum Likelihood (ML) and Maximum Parsimony (MP) methods. Thirty-seven reference sequences were retrieved according to the studies of the *Gnomoniopsis* by Jiang *et al*. (2021), Bashiri and Abdollahzadeh (2024), and Li *et al*. (2025), including *Apiognomonia errabunda* AR 2813 used as outgroup, and the five representative isolates from the present study were included. Sequence alignments for phylogenetic analyses were produced with the server version of MAFFT (https://mafft.cbrc.jp/alignment/server/), and were checked and refined using BioEdit Sequence alignment Editor 7.7.1.0 (Hall, 1999). Three loci (ITS, *tef1*, *tub2*) were concatenated to a combined matrix using Phyutility v. 2.2 (Smith and Dunn, 2008). Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis, 2006), as implemented in raxmlGUI 2.0 (Silvestro and Michalak, 2012), using the ML + rapid bootstrap setting and the GTRGAMMA+I substitution model. Bootstrap analyses were carried out with 1,000 bootstrap replicates. Maximum parsimony (MP) bootstrap analyses were performed with Phylogenetic Analyses Using Parsimony (PAUP) v. 4.0a169 (Swofford, 2002). A total of 1,000 bootstrap replicates were implemented using five rounds of heuristic search with random sequence addition, followed by tree-bisection-reconnection (TBR) branch swapping. The MULTREES option was enabled, the steepest descent option was disabled, the COLLAPSE command was set to MINBRLEN, and each replicate was limited to 1 million rearrangements. All molecular characters were treated as unordered and assigned equal weight, with gaps considered as missing data. For evaluation and interpretation of bootstrap support, values between 70% and 90% were considered moderate, above 90% as high, and 100% as the maximum.

Pathogenicity tests were conducted on 12 one-year-old potted healthy *Q. pubescens* plants. Mycelial plugs (5 mm diam.), from colonies of isolate Q9 grown for 7 d at 25 ± 1 °C, were inoculated onto the plant stems that had been previously surfaced sterilized with 70% ethanol solution and wounded with a sterile cork borer (6 mm diam.). Inoculation controls were similarly treated with sterile PDA. After inoculation, the plant wounds were sealed with Parafilm®, and the plants were then maintained at 25 ± 1°C in growth chamber. Symptoms were evaluated after three months, and re-isolation from symptomatic lesions was performed. Thus, single colonies were transferred onto MEA plates, and conidia were observed under light microscope after 25 d.

Table 1. Information on fungal isolates deposited in GenBank and used in the phylogenetic analyses in the present study.

Fungal species	Isolate ID[a]	Host	Location	GenBank accession numbers[b]		
				ITS	*tef1*	*tub2*
Apiognomonia errabunda	AR 2813	*Fagus sylvatica*	Switzerland	DQ313525	-	EU219134
Gnomoniopsis agrimoniae	MFLUCC 14–0844 T	*Agrimonia eupatoria*	Italy	-	MF377585	-
Gnomoniopsis alderdunensis	CBS 125680 T	*Rubus parviflorus*	USA	GU320825	GU320801	GU320787
Gnomoniopsis angolensis	CBS 145057 T	unknown	Angola	MK047428	-	-
Gnomoniopsis chamaemori	CBS 804.79	*Rubus chamaemorus*	Finland	GU320817	GU320809	GU320777
Gnomoniopsis chinensis	CFCC 52286 T	*Castanea mollissima*	China	MG866032	MH545370	MH545366
Gnomoniopsis clavulata	CBS 121255	*Quercus falcata*	USA	EU254818	GU320807	EU219211
Gnomoniopsis castanopsidis	CFCC 54437 T	*Castanopsis hystrix*	China	MZ902909	MZ936385	-
Gnomoniopsis comari	CBS 806.79	*Comarum palustre*	Finland	EU254821	GU320810	EU219156
Gnomoniopsis daii	CFCC 54043 T	*Castanea mollissima*	China	MN598671	MN605519	MN605517
Gnomoniopsis diaoluoshanensis	SAUCC DL0963 T	*Castanopsis chinensis*	China	ON753744	ON759769	ON759777
Gnomoniopsis fagacearum	CFCC 54316 T	*Lithocarpus glaber*	China	MZ902916	MZ936392	MZ936408
Gnomoniopsis flava	CFCC 71563 T	*Castanopsis carlesii*	China	PV257808	PV268106	PV339811
Gnomoniopsis fragariae	CBS 121226	*Fragaria vesca*	USA	EU254824	GU320792	EU219144
Gnomoniopsis guangdongensis	CFCC 54443 T	*Castanopsis fargesii*	China	MZ902918	MZ936394	MZ936410
Gnomoniopsis guttulata	MS 0312	*Agrimonia eupatoria*	Bulgaria	EU254812	-	-
Gnomoniopsis hainanensis	CFCC 54376 T	*Castanopsis hainanensis*	China	MZ902921	MZ936397	MZ936413
Gnomoniopsis idaeicola	CBS 125672	*Rubus* sp.	USA	GU320823	GU320797	GU320781
Gnomoniopsis lithocarpi	SAUCC YN0743 T	*Lithocarpus fohaiensis*	China	ON753749	ON759765	ON759783
Gnomoniopsis macounii	CBS 121468	*Spiraea* sp.	USA	EU254762	GU320804	EU219126
Gnomoniopsis mengyinensis	SAUCC MY0293 T	*Castanea mollissima*	China	ON753741	ON759766	ON759774
Gnomoniopsis occulta	CBS 125677	*Potentilla* sp.	USA	GU320828	GU320812	GU320785
Gnomoniopsis paraclavulata	CBS 123202	*Quercus alba*	USA	GU320830	GU320815	GU320775
Gnomoniopsis paraclavulata	CBS:121912 T	*Quercus alba*	USA	MH863162	-	-
Gnomoniopsis paraclavulata	66G	*Quercus robur*	Poland	MZ078654	MZ078875	MZ078820
Gnomoniopsis paraclavulata	CBS 115312	*Quercus* sp.	Netherlands	EU254840	-	EU219236
Gnomoniopsis paraclavulata	**Q3**	*Quercus pubescens*	Italy	**PV628520**	**PV646569**	**PV646574**
Gnomoniopsis paraclavulata	**Q4**	*Quercus pubescens*	Italy	**PV628521**	**PV646570**	**PV646575**
Gnomoniopsis paraclavulata	**Q7**	*Quercus pubescens*	Italy	**PV628522**	**PV646571**	**PV646576**
Gnomoniopsis paraclavulata	**Q9**	*Quercus pubescens*	Italy	**PV628523**	**PV646572**	**PV646577**
Gnomoniopsis paraclavulata	**Q17**	*Quercus pubescens*	Italy	**PV628524**	**PV646573**	**PV646578**
Gnomoniopsis quercicola	IRAN 4313C T	*Quercus brantii*	Iran	OR540614	OR561996	OR561907
Gnomoniopsis racemula	CBS 121469 T	*Chamerion angustifolium*	USA	EU254841	GU320803	EU219125
Gnomoniopsis rosae	CBS 145 085 T	*Rosa* sp.	New Zealand	MK047451	-	-
Gnomoniopsis rosae	TMR4	unknown	unknown	OR095582	-	OR094914
Gnomoniopsis rossmaniae	CFCC 54307 T	*Castanopsis hainanensis*	China	MZ902923	MZ936399	MZ936415
Gnomoniopsis sanguisorbae	CBS 858.79	*Sanguisorba minor*	Switzerland	GU320818	GU320805	GU320790
Gnomoniopsis silvicola	CFCC 54418 T	*Quercus serrata*	China	MZ902926	MZ936402	MZ936418
Gnomoniopsis smithogilvyi	CBS 130190 T	*Castanea* sp.	Australia	JQ910642	KR072534	JQ910639
Gnomoniopsis tormentillae	CBS 904.79	*Potentilla* sp.	Switzerland	EU254856	GU320795	EU219165
Gnomoniopsis xunwuensis	CFCC 53115 T	*Castanopsis fissa*	China	MK432667	MK578141	MK578067
Gnomoniopsis yunnanensis	SAUCC YN1659 T	*Castanea mollissima*	China	ON753746	ON759771	ON759779

[a] Isolates and sequences generated in the present study are shown in bold font; T = Isolates linked to type specimens.
[b] ITS, internal transcribed spacer; *tef1*, translation elongation factor 1-α; *tub2*, beta-tubulin.

RESULTS AND DISCUSSION

Incidence of the disease was estimated at approx. 20% on about 1,000 plants, with a mortality rate of 5%. Cross sections of stems and branches of affected trees revealed internal necroses, and in the severe cases irregular necroses extending to the external wood (Figure 1). Isolated colonies on PDA were 70 mm diam. after 15 d at 25°C, with sparse aerial mycelium and irregular margins forming a concentric ring, and developing a lobed rosette-like appearance. Colonies on malt extract agar (MEA) were >90 mm diam. after 23 d at 25°C, and were brownish, forming solitary, erumpent, pulvinate, conidiomata exuding pale creamy conidial masses (Figure 2, a and b). Conidia were oval to oblong, straight or slightly curved, aseptate, and hyaline, with dimensions of (min, average – SD, average + SD max; length/width ratio) (4.5–) 6.6–8.3 (–9.4) × (2.3–)3.1–4.3(–5.2) µm, l/w = (1.3–)1.7–2.4(–3) (n = 100) (Figure 2c).

BLASTn searches of ITS, *tef1* and *tub2* sequences showed 100% identity with those of *Gnomoniopsis paraclavulata* Sogonov (*Gnomoniaceae, Diaporthales*) isolate 477E (GenBank accession No. MZ078819.1). According to *Index Fungorum* (https://www.indexfungorum.org/Names/Names.asp; Accessed May 4, 2025), a total of 52 *Gnomoniopsis* species have been previously described, with sequence data available only for 32 of these taxa. Of the 1735 characters (550 from ITS, 700 from *tef1*, and 485 from *tub2*) of the combined matrix used for phylogenetic analyses, 633 were parsimony informative (105 from ITS, 332 from *tef1*, and 196 from *tub2*), 223 were parsimony-uninformative and 879 were constant. The ML tree (-lnL = 15318.023949) obtained by RAxML is shown in Figure 3. ML analysis resulted in a tree topology similar to that revealed by MP analysis. The present study isolates were placed within the clade of *G. paraclavulata*, with maximum and medium support (100% ML, 83% MP).

Gnomoniopsis paraclavulata is phylogenetically close to the recently described *G. quercicola*, for which sequence data are available only for one isolate (IRAN 4313C). In accordance with Bashiri and Abdollahzadeh (2024), some nucleotide differences were observed between the present study *G. paraclavulata* isolates and that of *G. quercicola* in all three loci ITS (four substitutions, one deletions/insertions), *tef1* (38 substitutions, four deletions/insertions), and *tub2* (36 substitutions). However, the present study isolates differed from those of *G. paraclavulata* included in the analyses for nucleotide substitutions and insertion/deletions within the *tef1* introns (four substitutions and three deletions/insertions with *G. paraclavulata* 66G, and 18 substitutions and three deletions/insertions with *G. paraclavulata* CBS 123202).

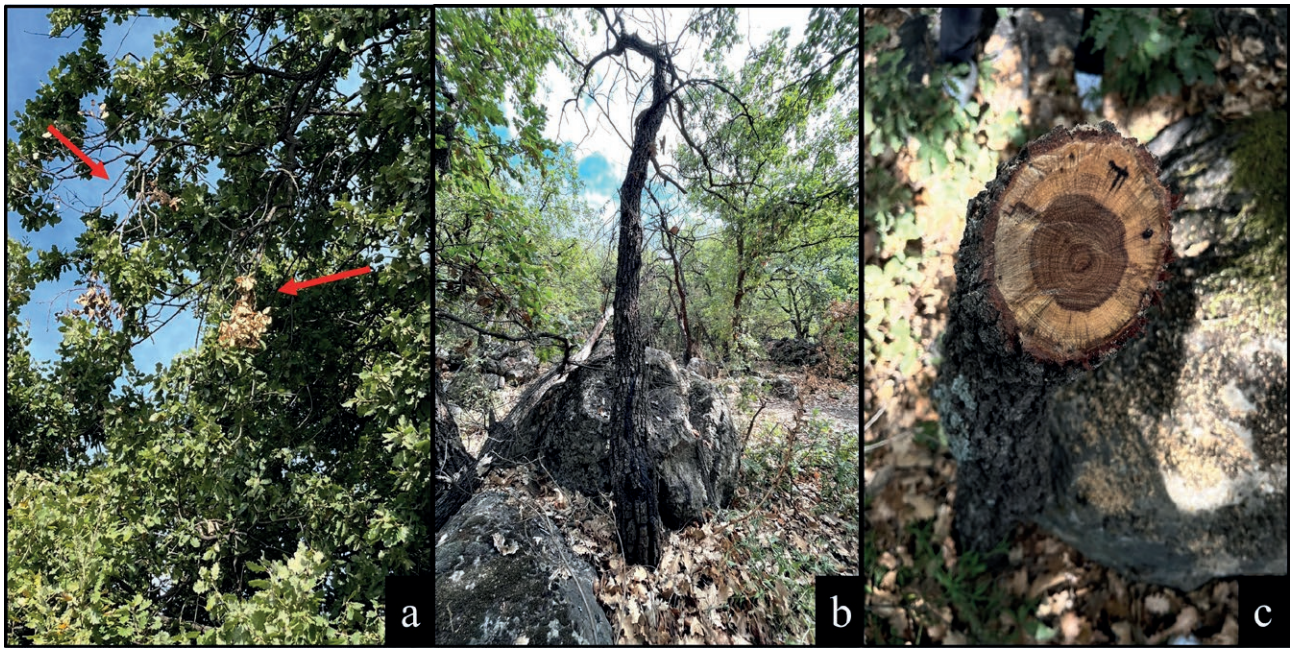

Figure 1. Decline of pubescent oak trees caused by *Gnomoniopsis paraclavulata* in a public park located in Catania, Italy. (a**)** Dieback of twigs showing defoliation. (b) Severe dieback of oak tree. (c) Cross-section of the trunk of a declining tree, with regular brown necrosis and irregular black streaking in the inner wood tissues.

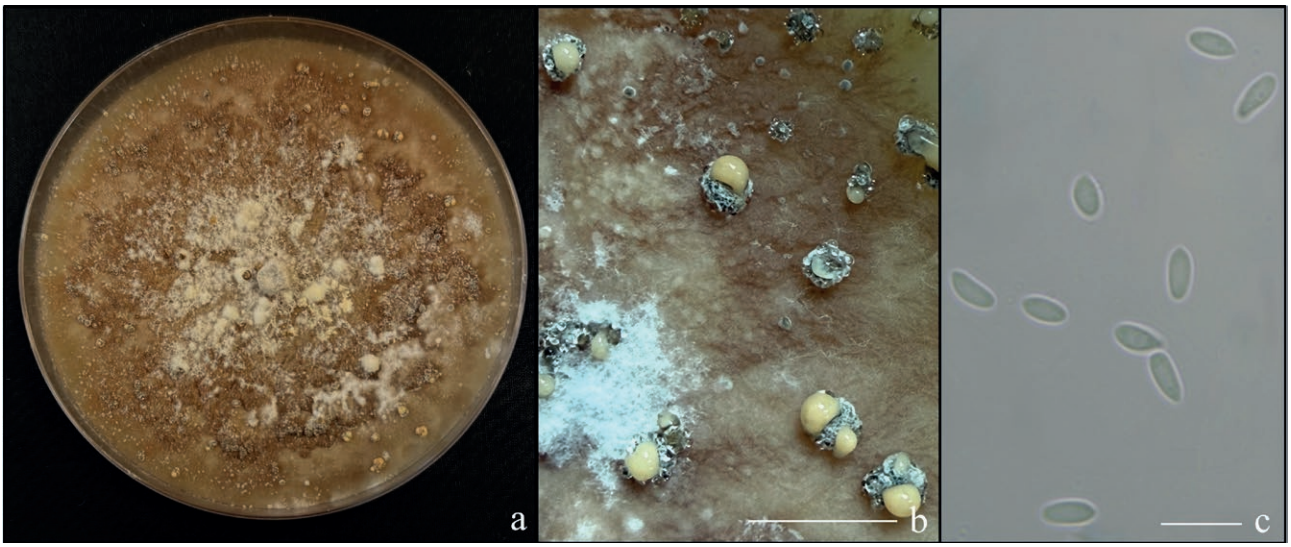

Figure 2. Morphology of *Gnomoniopsis paraclavulata* in culture. (a) Colony of isolate Q9 on MEA (after 23 d at 25 °C). (b) Conidiomata formed on MEA (after 23 d at 25°C). (c) Conidia, that are oval to oblong, straight or slightly curved, aseptate, from culture (MEA, 30 d). Scale bars: (b) = 1 mm, and (c) = 10 μm (c).

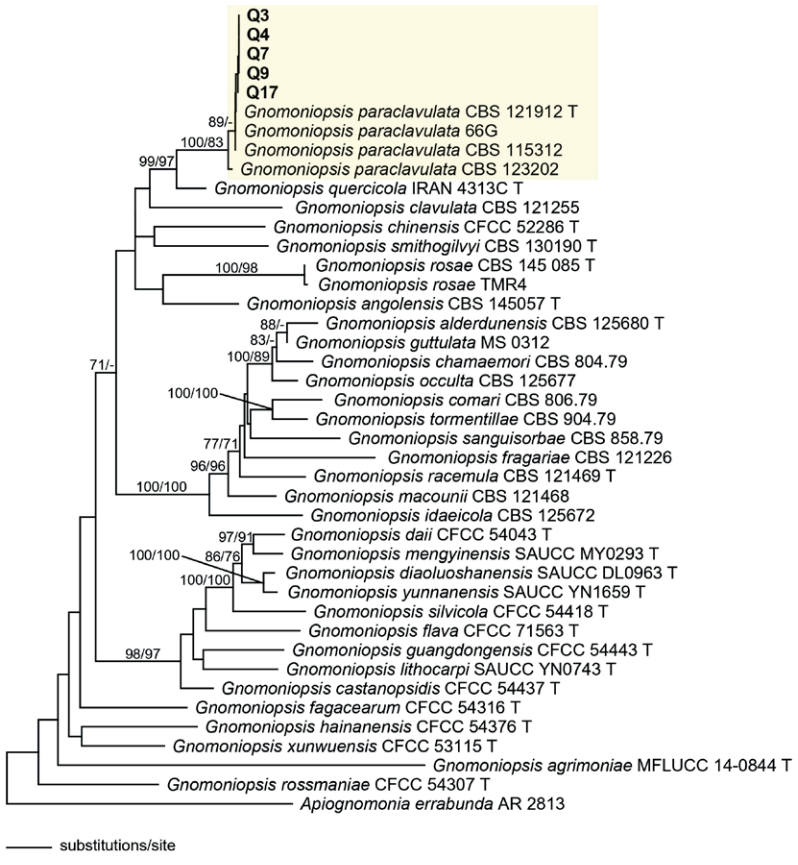

Figure 3. Phylogram of the best ML tree (-lnL = 15318.023949) revealed by RAxML from an analysis of the combined ITS-*tef1*-*tub2* matrix of *Gnomoniopsis*, showing (in bold font) the phylogenetic position of the present study isolates from diseased *Quercus pubescens*, *Apiognomonia errabunda* AR 2813 is the outgroup to root the tree. Maximum Likelihood (ML) and Maximum Parsimony (MP) bootstrap support >70% are given at first and second positions, respectively, above the tree branches. T = ex-type isolates.

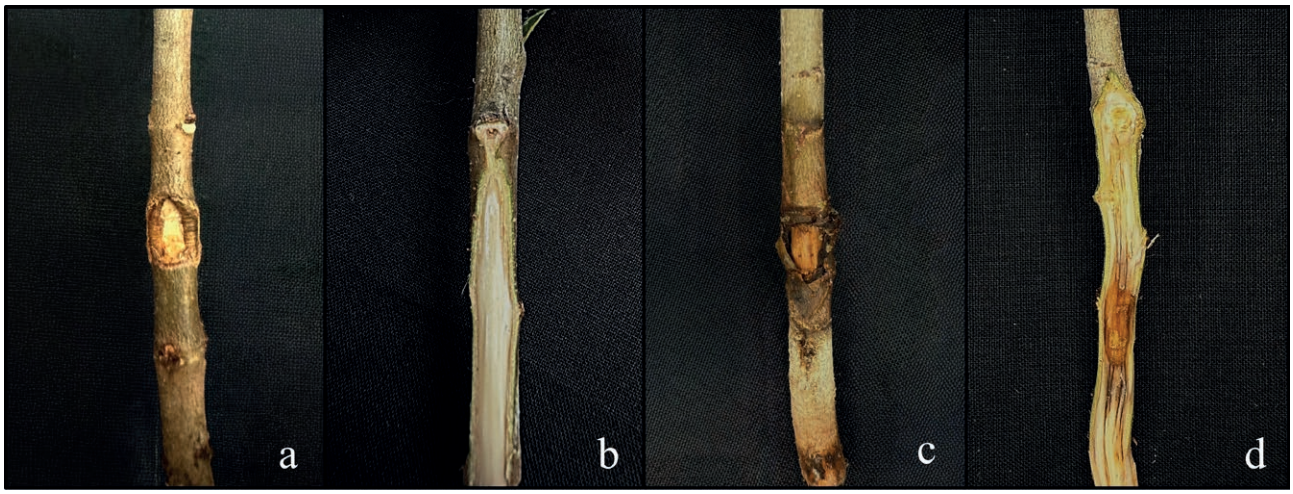

Figure 4. Pathogenicity test on pubescent oak plants. External (a) and internal (b) tissues of control stems, with no symptoms around the agar inoculation points. Stem inoculated with *Gnomoniopsis paraclavulata* isolate Q9, showing an externally brown to black lesion (c), and an internal necrotic lesion (d) with streaking extending upward and downward from the inoculation point, at 3 months after inoculation.

After three months, bark was removed from the stems of inoculated plants, and symptoms of wood discolouration and internal necroses extending upward and downward from each inoculation site were observed (Figure 4), as well as dieback of the basal twigs. Re-isolations were conducted, and these resulted (after 7 d) in colonies resembling *Gnomoniopsis paraclavulata*. Morphology and conidia of colonies isolated from the inoculated plants matched the originally inoculated isolate of *G. paraclavulata*. These results fulfil Koch's postulates for the inoculated fungus.

Gnomoniaceae (e.g., *Gnomoniopsis* spp.) have been reported among the most common pathogenic fungal genera associated with oak tree decline, causing symptoms of canker, gummosis, dieback, wilting, wood discolouration, and necroses (Moricca and Ragazzi, 2008; Sogonov *et al*., 2008; Walker *et al*., 2010; Jiang *et al*., 2021). *Gnomoniopsis paraclavulata* was first discovered on overwintered leaves of *Q. alba* in the United States of America (Sogonov *et al*., 2008), whereas in Italy, this fungus was occasionally isolated from branches with dieback symptoms from oak forests where *Q. pubescens* was also present, but without assessing its pathogenicity (Pinna *et al*. 2019). *Gnomoniopsis paraclavulata* was also isolated from the bodies of the black-banded oak borer (*Coraebus florentinus* Herbst) (Pinna *et al*., 2019), which infests oak species (Sallé *et al*., 2014; Gallardo *et al*., 2018; Cárdenas and Gallardo, 2018). Other studies have reported *G. paraclavulata* causing decline on *Q. robur* in Poland (Jankowiak *et al*., 2022), and *G. quercicola*, phylogenetically close to *G. paraclavulata*, as the most common fungus associated with decline of oak trees (*Q. brantii*, *Q. infectoria*, and *Q. libani*), along with other fungi including *Alloeutypa*, *Botryosphaeria*, *Cytospora*, *Didymella*, *Kalmusia*, and *Neoscytalidium* in Iran (Bashiri and Abdollahzadeh, 2024). The present study is the first to report *G. paraclavulata* causing dieback and internal necrosis on *Q. pubescens*.

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DATA AVAILABILITY

Nucleotide sequences of this study are deposited in NCBI GenBank and the accession number are reported in the text.

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