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Molecular-Based Reappraisal of a Historical Record of Dothistroma Needle Blight in the Centre of the Mediterranean Region

Chiara Aglietti [1], Alessandra Benigno [1], Edoardo Scali [1], Paolo Capretti [1], Luisa Ghelardini [1,2,*] and Salvatore Moricca [1]

[1] Department of Agricultural, Food, Environmental and Forest Sciences and Technologies, University of Florence, Piazzale delle Cascine 18, 50144 Firenze, Italy; chiara.aglietti@unifi.it (C.A.); alessandra.benigno@unifi.it (A.B.); edoardo.scali@unifi.it (E.S.); paolo.capretti@unifi.it (P.C.); salvatore.moricca@unifi.it (S.M.)

[2] National Research Council (CNR), Institute for Sustainable Plant Protection, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy

* Correspondence: luisa.ghelardini@unifi.it

Abstract: In this work, we rechecked, using species-specific Loop mediated isothermal AMPlification (LAMP) diagnostic assays followed by sequencing of fungal isolates at the beta-2-tubulin (tub2) gene region, a historical and never confirmed report of Dothistroma needle blight (DNB) in the introduced Monterey pine (*Pinus radiata* D. Don) in the mountains in the extreme tip of southern Italy. The report dates back to the mid-1970s, and predates the molecular-based taxonomic revision of the genus *Dothistroma* that defined the species accepted today. In the fall of 2019, symptomatic needles of Monterey pine and Corsican pine (*Pinus nigra* subsp. *laricio* (Poir.) Palib. ex Maire) were sampled in the area of the first finding. The applied diagnostic methods revealed the presence of *Dothistroma septosporum* (Dorogin) M. Morelet on both pine species. In this way, we: (i) confirmed the presence of the disease; (ii) clarified the taxonomic identity of the causal agent now occurring at that site; (iii) validated the species-specific LAMP diagnostic protocol we recently developed for *Dothistroma* for use on a portable field instrument, and (iv) showed that the pathogen now also attacks the native *P. nigra* subsp. *laricio*, a species particularly susceptible to the disease, indigenous to the mountains of Calabria, which is one of the very few areas where the species' genetic resources are conserved. Comparative genetic analysis of the rare populations of *D. septosporum* found in the central Mediterranean region and in the native range of *P. nigra* subsp. *laricio* could help to clarify the history of the spread of the pathogen in southern Europe and better evaluate the risk it poses to the conservation of native pine species.

Keywords: *Dothistroma septosporum*; *Mycosphaerella pini*; loop-mediated isothermal amplification; molecular diagnostics; field-portable diagnostics; *Pinus nigra* subsp. *laricio*; forest health protection; forest conservation

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1. Introduction

Dothistroma needle blight (DNB) is a serious foliar disease of pines with a worldwide distribution [1,2]. Causal agents of the disease are two closely related ascomycete fungi, *Dothistroma septosporum* (Dorog.) Morelet (syn. Scirrhia pini Funk & Parker, teleomorph: *Mycosphaerella pini* Rostr.), and *Dothistroma pini* Hullbary (teleomorph:unknown) [3]. These pathogens induce quite similar symptoms consisting initially of water-soaked lesions that later develop into small brown-to-reddish spots. Within these spots, black conidiomata differentiate under favorable conditions. Spots later expand to the whole needle circumference, resulting in transverse bands, hence the alternative name "red band needle blight". Infected needles gradually necrotize and fall [3,4].

Moisture, rainfall and temperature are primary factors in DNB incidence and severity, both in plantations and in nurseries [5]. Stagnation of air and humidity are the most significant factors contributing to the disease at the population scale. The impact of the disease is higher on lower pine branches, especially on those that are shaded and closer to the ground. Pines growing in dense stands are more susceptible than pines growing in more open stands; young pines in open stands, where canopy conditions promote the development of thick understory vegetation, are particularly susceptible; and pines growing inside the stands are always more susceptible than those growing on the margin [6]. On a single tree, infection spreads from the lower branches to the upper parts of the canopy. Under favorable climatic conditions (the optimum is high relative humidity and temperatures around 16–18 °C), the disease can be severe and cause a reduction in photosynthesis, extensive defoliation, stunted growth and mortality. The wide distribution of the disease in large areas of the northern and the southern hemispheres, under varied climatic and ecological conditions, proves the high adaptability of DNB pathogens. This is confirmed by climate models forecasting for the agents of DNB the ability to persist under a range of climates, from sub-arctic to temperate, continental, subtropical and dry tropical regions [7].

Until the 1970s, the disease was known primarily for the severe damage it caused to Monterey pine (*Pinus radiata* D. Don), a fast-growing species that was widely used in southern hemisphere plantations, especially in Tanzania and Kenya, and the causal agent was described as *D. pini* Hulbary [4]. The continued increase in demand for woody material that has driven the growth of *P. radiata* plantations worldwide led to a rapid epidemic and sometimes devastating spread of DNB, which appeared in Chile, New Zealand, and central and southern Africa. At the time, the disease, although present in the northern hemisphere, for instance in British Columbia, California and Oregon, had not had particularly significant epidemic manifestations [4]. However, DNB also appeared in Central France and Southern Europe (Spain, ex-Yugoslavia, Romania), where *Dothistroma* was reported in *P. radiata* and black pine (*Pinus nigra* J.F.Arnold) plantations [8–10].

Additionally, in Italy, in the post-war period, there was an increase in the number of plantations of fast-growing species for wood production, and in particular of *P. radiata* plantations in the central-southern regions. Magnani [11], supported by the opinion of the mycologists de Ana Magan in Spain and Morelet in France, reported the presence of *D. pini* Hulbary in the south of the peninsula. The report was again from an artificial plantation of the non-native *P. radiata*.

This first record of DNB in Italy was in the center of the Mediterranean region, in the southern end of the Apennine mountains (Calabria, southern Italy). The outbreak site, San Pietro di Caridà, is close to the mountain ridge, in an area with a microclimate characterized by high moisture and high annual rainfall [12]. Here, the temperate and humid air masses rising from the Ionian sea and the Tyrrhenian sea, the two seas separated by the small strip of land that is southern Calabria, meet on the ridge, giving rise to turbulence and frequent precipitation.

Because the taxonomy of the genus *Dothistroma* has been decisively revised since the disease was first reported in southern Italy more than forty years ago, and because DNB is now an important foliar disease of pines considered to be emerging globally, in the present study, a survey was carried out in this historical outbreak site in order to: (1) ascertain if DNB was still occurring in the area; and, if this was the case, (2) identify unequivocally and conclusively the causal agent; and finally (3) assess the current host species of the pathogen. A proper identification of the causal agent had both taxonomic and epidemiological relevance, since the disease was originally ascribed to *D. pini* on the basis of micromorphological examination alone [11]. However, later research demonstrated an accurate identification of the agent(s) of DNB to be possible only by means of molecular methods [13,14]. Recently, the differential diagnosis of the pine pathogens *D. pini*, *D. septosporum*, and *Lecanosticta acicola* can be performed more quickly and yet sensitively using specific assays based on loop-mediated isothermal amplification (LAMP) [15]. Considering

the widespread presence of *D. septosporum* in Calabria on *Pinus nigra* subsp. *laricio* in the Sila National Park, located approximately 150 km to the north [16,17], the last objective of the study was related to the concern that, also in this area, the pathogen might have made a host jump from the introduced *P. radiata* to other pine species. In fact, the area falls within the native range of Corsican pine (*Pinus nigra* subsp. *laricio* (Poir.) Maire), which is represented by a few populations distributed between Corsica and Southern Italy [18], in which the genetic resources of the species are conserved [19,20]. *P. nigra* subsp. *laricio* has been found to be particularly susceptible to DNB both in the Sila National Park [17] and in various other localities outside its native range of distribution, for instance in continental France, in the United Kingdom, and in New Zealand, where the species is grown in artificial plantations [21–26].

2. Materials and Methods

2.1. Study Area and Plant Material

Sampling was performed in the fall of 2019 in the Prateria district, in the Municipality of San Pietro di Caridà, Province of Reggio Calabria (Calabria, southern Italy), in which the samples observed by Magnani [11] had also been collected in 1977. The Prateria district, in the Calabrian Apennine, extends between 800 and 1100 m above sea level, in a mountainous area of transition between the southern side of the Serre mountains and the northern side of the Aspromonte massif. Monitoring in search of the old DNB outbreak site focused on the south-facing slopes that drain into the Metramo lake (38°28′ N, 16°13′ E, about 900 m above sea level), an artificial water reserve built at the beginning of this century for irrigation purposes (Figure 1). The vegetation, compared to the descriptions from the 1970s, when there were in the area extensive plantations of *P. radiata* and *P. nigra* subsp. *laricio* cultivated since the middle of the twentieth century for the production of wood, is now very different. Broad-leaved trees prevail, mainly beech (*Fagus sylvatica* L.), which grows in extensive pure high forests and to a lesser extent in coppice forests, covering all the mountains around. There are, however, scattered in the beech forest, nuclei of *P. nigra* subsp. *laricio* and still some remnants of the old plantations of *P. radiata* that have been cut down over time.

Needle samples were collected from adult plants of *P. radiata* and *P. nigra* subsp. *laricio* that showed typical DNB symptoms such as defoliation, reduced growth, and characteristic red bands on the needles, with or without the presence of fruiting bodies attributable to *Dothistroma* on the basis of simple visual inspection with the naked eye. Samples were taken from six plant groups (4 groups of *P. nigra* subsp. *laricio* and 2 groups of *P. radiata*) at least 250 m apart in an area of approximately 4 square kilometers (Figure 1c). Needle samples, bagged in sealed envelopes (two separate envelopes for each pine group and each containing several dozen needles from different pine plants of the same species), were transported to the laboratory and kept refrigerated until further handling.

2.2. Isolation of Fungi

In the laboratory, symptomatic needles that clearly showed deep red bands, orange-red-brown needle apices, and green bases were examined under the stereomicroscope (Optica Lab, Geass, Torino, Italy). In order for fungal fruiting bodies to mature, needles were incubated at room temperature for 5 days in Petri dishes containing moist blotting paper. Conidia were mounted in lactophenol cotton blue and examined under a light microscope (Axiophot, Carl Zeiss) at up to ×40 magnification.

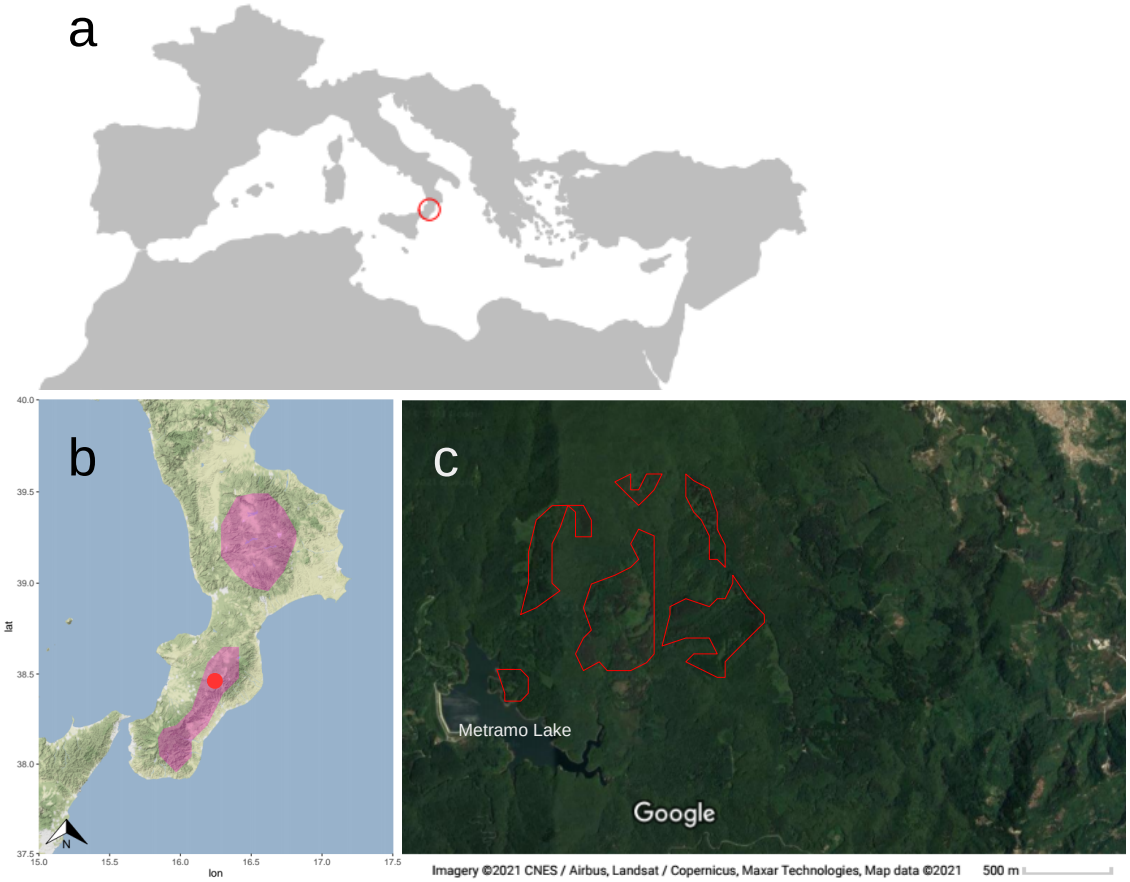

Figure 1. Map position of the study area of Prateria (red symbols) in the center of the Mediterranean region (**a**,**b**) on the south-facing slope of the Serre mountains towards the Metramo Lake (San Pietro di Caridà, Reggio Calabria, Italy) (**c**). The approximate local distribution range of *Pinus nigra* subsp. *laricio* (adapted from the European Forest Genetic Resources Programme, EUFORGEN http://www.euforgen.org/species/pinus-nigra/ (accessed on 3 July 2021)) is shown in magenta coloring (**b**). Maps were drawn using the sf [27] and ggplot2 [28] packages in R [29].

In a laminar flow cabinet, the needles were surface-sterilized by gently wiping with 70% ethanol, and conidiomata were excised from tissues with a fine-pointed scalpel under the stereomicroscope (×10 magnification) and crushed into a drop of sterile distilled water. The dispersion (10 µL) was taken with a pipette and streaked onto a 90 mm Petri dish containing 1.5% Malt Extract Agar (MEA) plus streptomycin (0.050 gL^{-1}). Three streaks (30 µL) were made on each dish. Dishes were incubated in the dark at 20 °C according to species requirements [30].

2.3. DNA Extraction from Mycelia and Pine Needles

The mycelium of axenic cultures was grown on 1.5% MEA plus cellophane into 90 mm Petri dishes and maintained in the dark at 20 °C. After 7–15 days, ca. 70 mg (fresh weight) of mycelium was scraped from the cellophane surface and put in a 2 mL Eppendorf tube. Similarly, fragments (2–5 mm long) of symptomatic *P. radiata* and *P. nigra* subsp. *laricio* pine needles (ca. 70 mg fresh weight obtained by pooling together pieces of different symptomatic needles from the envelope corresponding to a sample) were transferred into 2 mL microcentrifuge tubes (12 samples for DNA extraction in total). Needle samples were frozen at −20 °C overnight and ground for 1 min at 30 Hz with two 3 mm sterile steel beads in a MM400 mixer mill (Retsch GmbH, Haan, Germany).

DNA was extracted from fungal mycelia following the CTAB (cetyl trimethylammonium bromide) protocol described in [31], while DNA was extracted from pine needles using the Nucleospin Plant II kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. Total DNA concentrations were estimated using a NanoDrop®

ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Eluted DNA samples were kept at −20 °C until further processing.

2.4. LAMP on DNA Extracts from Needles

Each DNA extract from pine needles was tested by LAMP reaction using the species-specific assays previously optimized for *D. septosporum*, *D. pini* and *L. acicola* [15]. Each run was performed separately on a portable Genie III instrument (OptiGene Limited, Horsham, UK) according to conditions described in [15]. Each sample was tested in duplicate, including in each test, positive controls (containing external target DNA extracts from *D. septosporum*, *D. pini* or *L. acicola* cultures) and two no-template controls (NTC, in which 5 µL of pure water were used instead of DNA), to ensure effective LAMP reaction conditions and verify the absence of contamination.

2.5. Conventional PCR and Sequencing on Mycelial DNA Extracts

DNA from mycelia of four isolates was amplified at the beta-2-tubulin (tub2) gene region using the Btub2Fd/Btub4Rd primer pairs [32]. The expected PCR product size was ca. 431 bp. Each PCR reaction was performed in a final volume of 25 µL using a MB series thermocycler (Cheimika, Italy). Each sample was amplified in duplicate, including in each run no template controls (NTC), in which DNA was substituted by dd-water and positive controls composed by *D. septosporum* DNA. PCR reaction cycle was as follows: a first step of 3 min at 95 °C, 40 cycles composed of (i) 30 s at 95 °C, (ii) 30 s at 54 °C, (iii) 30 s at 72 °C, a final step of 7 min at 72 °C. Each reaction mixture contained 19.25 µL of dd-water, 2.5 µL of 1× DreamTaq Green Buffer (Thermo Fisher Scientific, Waltham, USA), 0.5 µL PCR primer mixture (each at a final concentration of 50 µM), 0.5 µL of 10 mM dNTPs, 0.25 µL of 5 U* μL^{-1} DreamTaq polymerase (Thermo Fisher Scientific, Waltham, USA) and 2 µL of template DNA. For each sample, 5 µL of post-amplification products were visualized by electrophoresis runs in 1% agarose gel (Sigma-Aldrich, St. Louis, MO, USA) using 1× Tris-acetate-EDTA (TAE) as a buffer and ethidium bromide (0.5 µg mL^{-1}) for staining. DNA fragments were purified from the PCR products by using NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany), and were sent for Sanger sequencing to StarSEQ® GmbH (Mainz, Germany). Chromatograms were checked and nucleotide sequences trimmed using Unipro EUGENE [33]. Sequences were aligned using T-coffee [34] and alignments visualized with Mview [35] through the EMBL-EBI Job Dispatcher Web Services [36]. Identity searches of consensus sequences were performed with the Blastn tool in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov, accessed on 7 May 2021).

Phylogenetic analyses were performed using a qiime2 version 2021.4 pipeline [37]. De novo multiple sequence alignment was performed with MAFFT version 7 [38] with default settings. Sequences for the outgroup were downloaded from the National Center for Biotechnology Information (NCBI: https://www.ncbi.nlm.nih.gov, accessed on 3 July 2021). Three strains of *Lecanosticta* were included in the analyses (*L. variabilis*, *L. longispora* and *L. acicola*). In order to reduce alignment errors that may introduce noise and confound phylogenetic inference, alignments were masked (i.e., filtered) before phylogenetic analysis, eliminating alignment columns that were phylogenetically uninformative or misleading [39]. Max gap frequency value was set equal to 1, retaining all columns regardless of gap character frequency. The minimum conservation value was set equal to 0.4, retaining a column only if it contains at least one character that is present in at least 40% of the sequences. Phylogenetic analyses were conducted using RAxLM (randomized accelerated maximum likelihood) version 8 [40] with the addition of rapid bootstrapping support values under the gamma model [41]. The tree with maximum likelihood score was selected, setting a seed for the parsimony starting tree equal to 1993, and a seed for rapid bootstrapping equal to 2756. Alignment had 164 distinct alignment patterns, and the proportion of gaps and completely undetermined characters in this alignment corresponded to 20.00%. RAxML rapid bootstrapping and a subsequent maximum likelihood (ML) search were performed using 1 distinct models/data partitions with joint branch

length optimization, executing 1000 rapid bootstrap inferences and thereafter a thorough ML search.

3. Results

3.1. Symptoms, Signs and Conidial Micromorphology

Typical DNB symptoms were present on all needle samples taken in six different groups of pines, two of *P. radiata* and four of *P. nigra* subsp. *laricio*, on the south-facing slopes towards Lake Metramo at elevations ranging from about 900 to 1100 m a.s.l. Trees of both species were defoliated, with transparent crowns and typical lion-tailed branches. These sampling points represent, in the first case, remains of old plantations of the introduced *P. radiata*, and in the second case, naturally regenerated trees of the native *P. nigra* subsp. *laricio*.

Under the stereoscope, necrotic brown spots and black stroma were visible beneath the needle epidermis and typical black conidiomata in the red bands and discolored areas appeared after a few days of incubation in high humidity conditions. Conidia had morphological characteristics consistent with attribution of the species to *D. septosporum* or *D. pini* [13], i.e., they were thin-walled, hyaline, smooth, fusiform to short-clavate, straight or more frequently curved, 2–3(5) septate, and measured 12–40 × 2–3 µm.

3.2. LAMP Assays

DNA extracts from pine needles with typical DNB symptoms, with or without fruiting bodies, all tested positive for *D. septosporum* and negative for both *D. pini* and *L. acicola* by applying LAMP optimized assays. Each sample that tested positive for *D. septosporum* on the portable instrument was amplified in about 20 min with amplification times (tamp), quality of amplification curves and fluorescence (F) (tamp range: 22–23 min, F range: 15,000–30,000) comparable to those obtained on the real time PCR instrument used during the optimization of the assay [15]. No amplification curves were obtained from negative samples. All positive controls were amplified (*D. septosporum* tamp 20–22 min, F 10,000–30,000; *D pini* tamp 15–22 min, F 20,000–25,000; *L. acicola* tamp 21–23 min, F 15,000–25,000), confirming the effectiveness of each run. An example of the reaction kinetics is given in Figure 2.

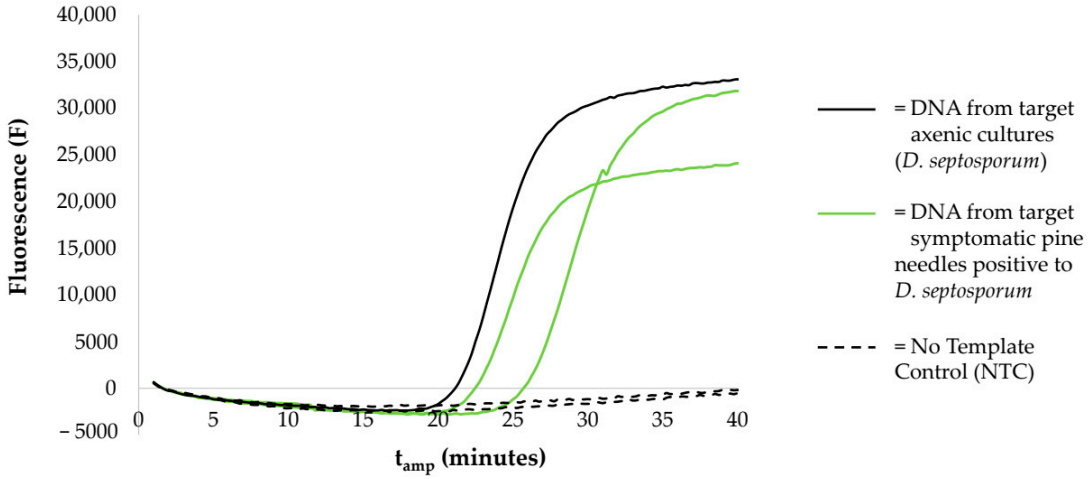

Figure 2. Selection of kinetics showing amplification results of the LAMP assay for the detection of *D. septosporum* [15] on a portable instrument (Genie III OptiGene, Limited, Horsham, UK).

3.3. Isolation of Fungi and Identification by Sequencing

Numerous dark brown or blackish colonies resembling those of *Dothistroma* species were obtained from conidia from all the groups of sampled pines. Colonies grew slowly, as is typical of *Dothistroma*, and stained the substrate red for dothistromin production after

1–2 weeks of growth. Mycelial plugs from 15 axenic colonies were cut from the agar and placed into microeppendorf tubes (1.5 mL), covered with sterile distilled water and stored at 4 °C.

Partial sequences of the tub2 gene region were obtained from four isolates from *Pinus nigra* subsp. *laricio*. Amplicon size was ca. 431 bp as expected. After quality check, trimming and alignment, sequences comprised 297–372 base pairs due to poor quality sequences requiring more trimming in some cases. Blast alignment in the GeneBank database showed that all four fungal isolates could be classified as *D. septosporum* (99.35%–100% sequence identity, best hit *D. septosporum* strain CMW44656). Sequences were deposited in GenBank under accession numbers (MZ594908-MZ594911).

Dothistroma spp. included in phylogenetic analyses formed a cluster independent from *Lecanosticta* spp., which were selected as an outgroup (Figure 3). Sequences from the study site (RCDS001, RCDS003, RCDS007, RCDS009) were placed inside the *Dothistroma septosporum* clade, supporting the conclusion that these isolates belong to this species.

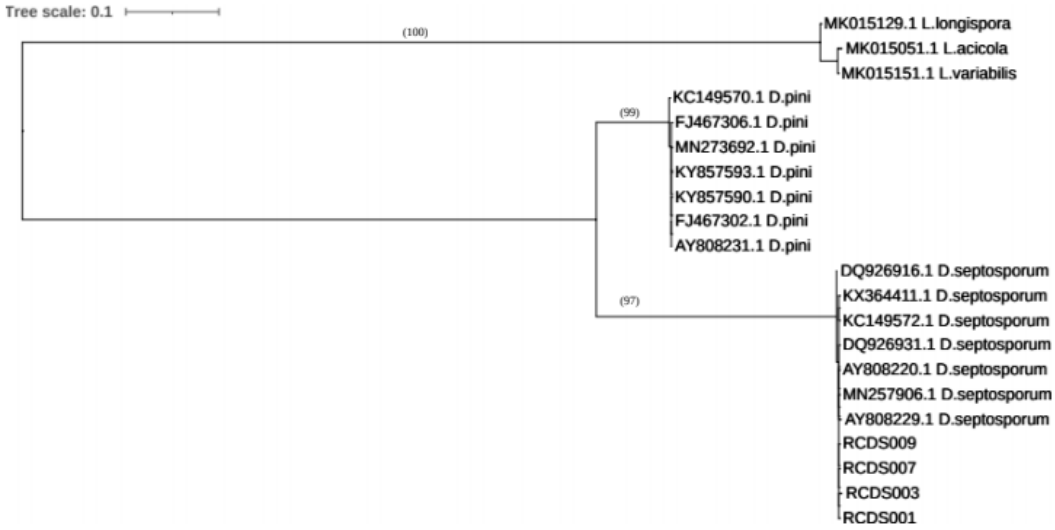

Figure 3. Phylogenetic tree with maximum likelihood score (branch support computed out of 1000 rapid bootstrapped trees) based on partial tub2 gene sequences of isolates RCDSS001, RCDS003, RCDS007, RCDS009 from *Pinus nigra* subsp. *laricio* in Prateria (San Pietro di Caridà, Reggio Calabria, Italy) and of *Dothistroma* species isolates retrieved from GenBank.

4. Discussion

Since the first report of DNB in the mountains of southern Calabria [11], the cultivation of *P. radiata* has been almost abandoned in southern Italy [42], and today, forty-four years later, in the area of that first report, only rather small nuclei of pine, especially the native *P. nigra* subsp. *laricio*, are found scattered in vast pure beech forests. Now that molecular techniques enable us to discriminate among morphologically indistinguishable species and also to recognize cryptic pathogens, we were able to test the authenticity of the record of Magnani [11], conducting our investigations at exactly the same site, and thus confirm that his observations, never verified later, were correct. In fact, the major revision of the genus *Dothistroma*, the name of the species of which changed [13], came long after the publication of Magnani's report. In this study, we can now ascertain that, about half a century after the establishment of *P. radiata* plantations, *Dothistroma* is still present in the area, although trees with clear symptoms of the disease are scattered or in small, isolated groups physically separated from each other by non-host species.

In confirming the persistence of the pathogen in the area, we demonstrated that the recently published LAMP diagnostic assays discriminating the infections by DNB agents and *L. acicola* in pine needles [15], are efficiently applicable on the portable instrument (Genie III Optigene, UK). This result encourages their prospective use directly in the field, after optimization of a suitable crude extraction protocol that is under development. Application

of LAMP diagnostics and sequencing in the tub2 gene region confirmed the pathogen found today in the study area to be the species currently identified as *D. septosporum*. Given the peculiar climatic characteristics of the area due to the orography, the position between two seas and also the additional presence of the artificial lake Metramo, which create conditions of higher atmospheric humidity than in the surrounding areas and favor the survival and spread of the pathogen, it is reasonable to assume that the population detected today likely descends from the one found in 1977, although the data do not allow us to confirm this assertion with certainty.

Due to changes in the politics of afforestation as well as in forest management strategies over the past 50 years, the silvicultural importance of *P. radiata* in the area seems today to be greatly reduced compared to the past, but the danger posed by *D. septosporum* to native pines remains serious. In fact, in the area included in this work, as in the Sila Massif [15] (which is also located in Calabria about 150 km north of the present finding), the pathogen was found on the native species *P. nigra* subsp. *laricio*. The few natural populations that still exist of this native species of pine are preserved today in Calabria and Sicily, in addition to the few present on the French island of Corsica. The presence of the pathogen therefore represents a threat to the genetic resources of the species.

Outside the Italian territory, reports based on molecular diagnosis of *D. septosporum* in the central southern Mediterranean area (i.e., Spain, Greece, Turkey) are isolated and sporadic and generally from pine plantations [2,43], with the exception of findings of the fungus in natural forests in Montenegro and southwestern Turkey [44–48]. The scientific debate on the geographic origin of *D. septosporum* is long and not without controversy [13,49–52], partly due to the lack of sufficient sampling of the fungus in some regions of its current range. Recently, a large body of work using microsatellite markers for genotyping an unprecedented number of samples collected from across the worldwide range of *D. septosporum* has indicated Eastern Europe (i.e., the Baltics and Western Russia) and Western Asia as the possible area of origin of the fungus, and hypothesized a possible derivation of Turkish populations from ancestral populations through an ancient separation dating prior to the colonization of Central Europe [53]. In the study by Mullet and colleagues [53], not enough isolates of the fungus from the outbreak in the Sila Massif were included to attempt reconstructing the evolutionary history of that population, while the finding of *D. septosporum* in the areas of the present study was yet to be confirmed. Whether or not *D. septosporum* is indigenous to these areas is currently unknown.

The high susceptibility of *P. nigra* subsp. *laricio* at all sites where it was planted outside its native range and came into contact with *D. septosporum* suggests a lack of coevolution with the pathogen. Similarly, the severe damage observed on this pine in its native range in the Sila Massif [17] also points to a recent introduction, although other possible explanations, e.g., an environment made more conducive to disease by climate change, cannot be excluded [54]. However, it is reasonable to assume that the populations of *D. septosporum* recently found on this pine species in its native range in Southern Italy might be recent introductions. In Corsica, to the best of our knowledge, there is only one published record of DNB, i.e., a single isolate of *D. septosporum* from *P. nigra* subsp. *laricio* included in [53], which has not been transposed yet into the EPPO database (https://gd.eppo.int/taxon/SCIRPI/distribution, accessed on 19 July 2021). According to EPPO, the pathogen was absent from Corsica at least until 2011, when a monitoring campaign was conducted, and no subsequent updates have been reported. Therefore, the finding in Corsica could also follow a recent introduction. No records exist on the occurrence of the pathogen in Sicily, where natural populations of *P. nigra* subsp. *laricio* are also present. As a prospect for a future study, a comparative genetic analysis of the Calabrian populations of the fungus could help to define in more detail the history of the pathogen's spread in Southern Europe.

5. Conclusions

DNA-based approaches are increasingly used to support and extend more classical taxonomic relationships, as well as to unravel complicated taxonomic histories and circumscript species. Molecular identification as *D. septosporum* of the fungus initially identified as *D. pini* on the basis of micromorphological characterization alone in the first reported Italian outbreak of the disease is a vivid example. The development of a rapid and highly discriminating diagnostic tool such as the LAMP technique will allow us to identify the agents of DNB in the Mediterranean area more accurately and more specifically. Further genetic analysis could also provide a more complete picture of the current distribution of the causal agents of red band needle blight in the region and insights into the origin of the disease.

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Data Availability Statement: The sequence data presented in this study were deposited in the NCBI GenBank data repository.

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