

Article

Phytophthora mediterranea sp. nov., a New Species Closely Related to *Phytophthora cinnamomi* from Nursery Plants of *Myrtus communis* in Italy

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Abstract: Monitoring surveys of *Phytophthora* related diseases in four forest nurseries in Italy revealed the occurrence of fourteen *Phytophthora* species to be associated with collar and root rot on fourteen plants typical of Mediterranean and alpine regions. In addition, a multilocus phylogeny analysis based on nuclear ITS and β -tubulin and mitochondrial *cox1* sequences, as well as micromorphological features, supported the description of a new species belonging to the phylogenetic clade 7c, *Phytophthora mediterranea* sp. nov. *Phytophthora mediterranea* was shown to be associated with collar and root rot symptoms on myrtle seedlings. Phylogenetically, *P. mediterranea* is closely related to *P. cinnamomi* but the two species differ in 87 nucleotides in the three studied DNA regions. Morphologically *P. mediterranea* can be easily distinguished from *P. cinnamomi* on the basis of its smaller sporangia, colony growth pattern and higher optimum and maximum temperature values. Data from the pathogenicity test showed that *P. mediterranea* has the potential to threaten the native Mediterranean maquis vegetation. Finally, the discovery of *P. cinnamomi* in alpine nurseries, confirms the progressive expansion of this species towards cold environments, probably driven by climate change.

Keywords: cryptic species; emerging diseases; global trade; biosecurity



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1. Introduction

Over the last few years, the horticultural industry has had a progressive expansion worldwide, which resulted in an increase in the trade of plants and commodities among continents [1,2]. In Italy, the horticulture industry is an important component of the agricultural sector; its production covers over 30,000 hectares with 100,000 employees and has a sellable production of about 2.5 billion euros per year [3].

Over the last few decades, the exponential increase in plant materials and commodities traded, together with limited and often ineffective diagnostic measures to detect pests and pathogens at the borders have contributed to a rapid diffusion of exotic and invasive species worldwide [4,5]. Among the most destructive pathogens that are introduced annually through trade and transport of plant material, many belong to the genus *Phytophthora* [6,7].

The genus *Phytophthora* encompasses a range of morphologically and ecologically diverse taxa grouped into 12 well-defined phylogenetic clades [8–10]. Some species have a saprophytic or opportunistic lifestyle, whereas others are aggressive pathogens that cause root and collar rot, bleeding canker and leaf blight symptoms in a huge number of plant species [11,12]. In particular, *Phytophthora* spp. are responsible for severe outbreaks with reduced vitality and quality of nursery plants [13]. In addition to the direct economic losses caused to the horticulture industry, *Phytophthora* spp. pose a threat to biodiversity of natural

ecosystems as they are often introduced through the seedlings used for reforestation and restoration programmes [14–16]. In this regard, the *P. ramorum* and *P. lateralis* outbreaks in North America and *P. cinnamomi* in Australia are emblematic, having caused the devastation of extensive ecosystems [17–20].

Due to intensive cultivation techniques and the occurrence of different plant species in an often limited space, nurseries are an ideal place for many *Phytophthora* species [13]. Moreover, the proximity of many plant species and possible encounters between genetically related species can easily give rise to new host–pathogen associations as well as hybridization phenomena [21]. High-humidity conditions and recurrent water irrigation can also favour the reproduction and dissemination of these pathogens [22–25]. Many *Phytophthora* species, in fact, need water to complete their biological cycle [13,26]. Furthermore, many agronomic practices such as recycling of soil and pots could be pervasive to increase *Phytophthora* inoculum inside nurseries [13,27–29]. At the same time, the intensive use of chemicals can promote the formation of fungicide-resistance in pathogen populations [30–32].

Phytophthora occurrence in North American, Australian and European nurseries is well documented [33–39]. The increased attention paid to *Phytophthora* species in the last decade has allowed the knowledge about biology and ecology of many species to be expanded, as well as the discovery of about 30 new species in nurseries [10]. Some of these new *taxa* are related to the aquatic environment and characterized by a saprophytic lifestyle [40–44]. In contrast, others such as *P. niederhauserii* and *P. kernoviae* are polyphagous and potentially invasive [45,46].

With the expansion of ornamental horticulture, *Phytophthora* related diseases are also becoming a serious problem in Italy. Recent studies have revealed a very high diversity of *Phytophthora* species in ornamental nurseries [47,48]. This is linked both to the widespread presence of invasive and ubiquitous species such as *P. nicotianae* and *P. palmivora*, but also of new species, such as *P. parvispora*, a cryptic species closely related to *P. cinnamomi* [48,49]. At the same time, the discovery of rare species on nursery plants, such as *Phytophthora pistaciae*, characterized by a limited geographic distribution points out the risks posed by nursery material to the conservation and integrity of natural ecosystems in the new areas [50].

Therefore, given the constant discovery of new or rare *Phytophthora* species in Italian nurseries, a thorough study was conducted to isolate, identify and characterize the main *Phytophthora* species associated with symptomatic plants in four nurseries spanning from the Mediterranean to the alpine climate region.

2. Materials and Methods

2.1. Surveys and Sampling Procedure

Field surveys were conducted from spring 2019 to autumn 2020 in four Italian forest nurseries located in Sardinia (N1 and N2), Veneto (N3) and Friuli Venezia Giulia (N4) regions (Table 1). In each nursery, all potting plants between 6 months and 5 years were visually checked for the presence of *Phytophthora* related disease symptoms such as chlorosis, defoliation, shoot blight, sudden death, as well as collar and root rot. Among the monitored plants, 76 were randomly selected for diagnostic analyses. The sampled species included *Abies alba*, *Arbutus unedo*, *Alnus incana*, *Castanea sativa*, *Fagus sylvatica*, *Helichrysum italicum*, *Ilex aquifolium*, *Juglans regia*, *Laurus nobilis*, *Lavandula officinalis*, *Myrtus communis*, *Phyllirea latifolia*, *Pistacia lentiscus* and *Quercus ilex* (Table 1). The plant samples were sealed in plastic bags, labelled and used for *Phytophthora* isolations within 24–48 h.

Table 1. Nurseries information and plant species monitored for *Phytophthora*.

Nursery	Elevation (m. a.s.l.)	Geographic Coordinates		Plant Species *
N1	860	40°25'54'' N	8°58'43'' E	Cs (12), Ia (6), Jr (12), Ln (6), Phl (6)
N2	16	39°57'43'' N	8°36'02'' E	Au (4), Hi (4), Lo (2), Mc (3), Pl (7), Qi (6)
N3	1077	46°06'54'' N	12°26'05'' E	Aa (2), Fs (2)
N4	191	46°11'51'' N	13°14'13'' E	Ai (4)

* In brackets the number of plants collected: *Abies alba* (Aa), *Arbutus unedo* (Au), *Alnus incana* (Ai), *Castanea sativa* (Cs), *Fagus sylvatica* (Fs), *Helichrysum italicum* (Hi), *Ilex aquifolium* (Ia), *Juglans regia* (Jr), *Laurus nobilis* (Ln), *Lavandula officinalis* (Lo), *Myrtus communis* (Mc), *Phyllirea latifolia* (Phl), *Pistacia lentiscus* (Pl) and *Quercus ilex* (Qi).

2.2. Isolation of *Phytophthora* Species

In the laboratory, plant samples were initially checked for collar and root symptoms and then used for *Phytophthora* isolation. From each sample about 300 g of rhizosphere soil and roots were positioned inside plastic containers with 2 L of distilled water. After 24 h the water surface was cleaned and young *Quercus suber* and *Sambucus nigra* leaves placed on the surface as bait [51]. Containers were kept at 20 °C under natural daylight for 3–7 days. Leaves showing necrotic spots were cut in small pieces (5 mm²) and placed on petri dishes containing potato dextrose agar (PDA 39 g/L, Oxoid Ltd., Basingstoke, UK) supplemented with 100 mL/L of carrot juice, 0.013 g/L of pimarinol and 0.05 g/L of hymexazol (PDA+) [50]. Hyphal tips typical of *Phytophthora* from the emerging colonies were sub-cultured on PDA and carrot agar (CA) [11] and incubated at 20 °C in the dark.

Isolations of *Phytophthora* species were also performed from collar and root lesions. After removing the outer bark, inner bark fragments were aseptically cut from the margin of necrotic lesions with a sterile scalpel and placed onto 90 mm Petri dishes containing PDA+. The dishes were incubated in the dark at 20 °C and examined every 6–12 h. Pure colonies were obtained as described above.

2.3. Morphological Identification and Characterization of Isolates

All *Phytophthora* isolates were grouped into morphotypes on the basis of colony growth patterns including surface and reverse colony appearance observed after 7 days of incubation on PDA and CA at 20 °C in the dark and morpho-biometric data of oogonia and sporangia. To enhance sporangia production, CA plugs (5 mm diameter) of each isolate, taken from 4-day-old colonies, were positioned inside petri dishes containing unsterile pond water and three cork oak fine roots (1 cm long). Petri dishes were kept at 20 °C in the dark and sporangia production was assessed every 12 h for 4 days.

Colony growth patterns of the new species were evaluated on 7-day-old cultures incubated at 25 °C in the dark on CA, PDA and malt extract agar (MEA, 20 g/L, Oxoid Ltd., Basingstoke, UK). In addition, size and shape of fifty chlamydospores, hyphal swellings and sporangia were recorded. Cardinal temperatures for growth were evaluated on 90 mm CA plates incubated at 5, 10, 15, 20, 25, 30, 32, 35, 37 and 40 °C (± 0.5 °C) in the dark for 96 h. Five replicate plates for each isolate were made. An isolate of both *P. parvispora* (CB86) and *P. cinnamomi* (CB21) obtained in the study were also included in the growth bioassay for comparison.

Measurements and photos of the main morphological structures were taken at 400 \times and 600 \times magnification and recorded using the software Motic Images Plus 3.0 paired with a Moticam 10+ camera connected to a Motic BA410E microscope. Sporangia dimensions are presented as mean values \pm standard deviation.

Representative isolates of each species were stored on PDA and CA slants under oil in the culture collection of the Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova. Ex-type culture of the new species was deposited at the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, and nomenclatural data in MycoBank (www.Mycobank.org, accessed on 4 May 2021). The holotype was lodged with the herbarium of Westerdijk Fungal Biodiversity Institute as a dried culture on CA.

2.4. DNA Extraction, Polymerase Chain Reaction (PCR) Amplification and Sequencing

For all isolates genomic DNA was extracted from mycelium of 5-day-old colonies grown on PDA at 20 °C using Instagene Matrix (BioRad Laboratories, Hercules, CA, USA). The entire internal transcribed spacer (ITS) region of the ribosomal DNA, including the 5.8S rRNA gene, was amplified and sequenced using primers ITS1 and ITS4 [52]. ITS sequences were used to confirm the identification at species level. For four isolates belonging to the clade 7 [8] including those of the new species, other two DNA regions namely β -tubulin (Btub) and cytochrome c oxidase subunit I (*cox1*), were amplified and sequenced using the primer-pairs TUBUF2/TUBUR1 and FM84/FM83 [53,54], respectively. Polymerase chain reactions (PCR) were performed as described in Bregant et al. [51].

PCR products were purified using a EUROGOLD gel extraction kit according to the manufacturer's instructions (EuroClone S.p.A., Pero, Italy). Both strands were sequenced by BMR Genomics DNA sequencing service (www.bmr-genomics.it, access on 4 May 2021). Sequences were edited with FinchTV v1.4.0 (Geospiza, Inc., <http://www.geospiza.com/finchtv>, accessed on 4 May 2021) and compared with sequences of ex-type culture available in GenBank using the BLASTn algorithm (<http://blast.ncbi.nlm.nih.gov>, access on 4 May 2021). New sequences were deposited in GenBank (Tables 2 and 3). Alignments and trees are available in TreeBase (study ID 28180).

Table 2. *Phytophthora* isolates used in the phylogenetic analysis. Ex-type cultures are reported in bold and newly generated sequences are indicated in italics.

Species	Code	Host	GenBank Accession Number		
			ITS	Btub	Cox1
<i>P. asiatica</i>	CBS 133347	<i>Pueraria lobata</i>	AB688422	AB539560	AB740169
<i>P. attenuata</i>	CBS 141199	<i>Castanopsis carlesii</i>	KU517154	KU899277	LC595899
<i>P. cajani</i>	P3105	<i>Cajanus cajan</i>	MG783386	MH493912	MH136859
<i>P. cinnamomi</i>	CBS 144.22	<i>Cinnamomum</i> sp.	MG865473	MH493920	MH136869
<i>P. cinnamomi</i>	CB21	<i>Abies alba</i>	MW892397	MW900442	MW900446
<i>P. cambivora</i>	P19997	<i>Castanea sativa</i>	MG783387	MH493913	MH136860
<i>P. europaea</i>	CBS 109049	<i>Quercus robur</i>	MG865488	MH493935	MH136884
<i>P. flexuosa</i>	CBS 141201	<i>Fagus hayatae</i>	KU517152	KU899302	LC595910
<i>P. formosa</i>	CBS 141203	<i>Araucaria</i> sp.	KU517153	KU899270	LC595912
<i>P. fragariae</i>	CBS 209.46	<i>Fragaria ×ananassa</i>	MG865494	MH493938	MH136890
<i>P. fragariaefolia</i>	CBS 135747	<i>Fragaria ×ananassa</i>	MG865495	MH493939	MH136891
<i>P. intricata</i>	CBS 141211	<i>Quercus tarokoensis</i>	KU517155	KU899284	LC595921
<i>P. niederhauserii</i>	P10616	<i>Hedera helix</i>	MG865552	MH493988	MH136944
<i>P. melonis</i>	CBS 582.69	<i>Cucumis sativus</i>	MG865536	MH493974	MH136931
<i>P. mediterranea</i>	CB84	<i>Myrtus communis</i>	MW892398	MW900443	MW900447
<i>P. mediterranea</i>	CB85	<i>M. communis</i>	MW892399	MW900444	MW900448
<i>P. nagaii</i>	CBS 133248	<i>Rosa</i> sp.	MG865547	MN207274	MH136940
<i>P. parvispora</i>	CBS 132772	<i>Arbutus unedo</i>	KC478667	KC609402	KC609413
<i>P. parvispora</i>	CB86	<i>A. unedo</i>	MW892401	MW900445	MW900449
<i>P. pisi</i>	CBS 130350	<i>Pisum sativus</i>	MG865567	MH494000	MH477754
<i>P. pistaciae</i>	P19883	<i>Pistacia vera</i>	KT183043	KX251749	LC595934
<i>P. rubi</i>	CBS 967.95	<i>Rubus idaeus</i>	MG865584	MH494011	MH136976
<i>P. sojiae</i>	CBS 382.61	<i>Glycine max</i>	MG865587	MN207279	MH136979
<i>P. tyrrhenica</i>	CBS 142301	<i>Quercus ilex</i>	KU899188	KU899265	LC595950
<i>P. uliginosa</i>	CBS 109054	<i>Q. robur</i>	MG865597	MH494023	MH136988
<i>P. uniformis</i>	P16206	<i>Alnus glutinosa</i>	MK496514	MH493905	MH136992
<i>P. vignae</i>	P3019	<i>Vigna sinensis</i>	MG865598	MH494024	MH136989
<i>P. vulcanica</i>	CBS 141216	<i>Fagus sylvatica</i>	MF036209	MF036235	LC595951
<i>P. ×alni</i>	IMI 392314	<i>A. glutinosa</i>	MK496513	MH493903	MH136991
<i>P. ×heterohybrida</i>	CBS 141207	Water	KU517151	KU899290	LC595953
<i>P. ×incrassata</i>	CBS 141209	Water	KU517156	KU899286	LC595954
<i>P. ×multiformis</i>	P16202	<i>A. glutinosa</i>	MG783372	MH493904	MK493472

Table 3. Number of isolates of *Phytophthora* species obtained from monitored plants in the investigated nurseries.

Species	Accession Number	ITS Clade	Plant Species *	Nursery
<i>P. acerina</i>	MW892395	2	Ai (1)	N4
<i>P. bilorbang</i>	MW959911	6	Phl (2)	N1
<i>P. cactorum</i>	MW892396	1	Cs (1)	N1
<i>P. cinnamomi</i>	MW892397	7	Cs (8), Fs (2), Aa (2), Jr (8), Qi (6)	N1-2-3
<i>P. citrophthora</i>	MW959916	2	Ln (1)	N1
<i>P. mediterranea</i>	MW892398	7	Mc (2)	N2
<i>P. megasperma</i>	MW959913	6	Ln (3)	N1
<i>P. nicotianae</i>	MW892400	1	Lo (1), Pl (2), Hi (4), Mc (2)	N2
<i>P. palmivora</i>	MW959917	4	Phl (5)	N1
<i>P. parvispora</i>	MW892401	7	Au (3)	N2
<i>P. pistaciae</i>	MW892402	7	Pl (2)	N2
<i>P. plurivora</i>	MW892403	2	Ai (3)	N4
<i>P. pseudocryptogea</i>	MW959912	8	Ln (4)	N1
<i>P. pseudosyringae</i>	MW959914	3	Ia (3)	N1
<i>P. psychrophila</i>	MW959915	3	Ia (1)	N1

* In brackets the number of *Phytophthora* isolates on: *Abies alba* (Aa), *Arbutus unedo* (Au), *Alnus incana* (Ai), *Castanea sativa* (Cs), *Fagus sylvatica* (Fs), *Helichrysum italicum* (Hi), *Ilex aquifolium* (Ia), *Juglans regia* (Jr), *Laurus nobilis* (Ln), *Lavandula officinalis* (Lo), *Myrtus communis* (Mc), *Phyllirea latifolia* (Phl), *Pistacia lentiscus* (Pl) and *Quercus ilex* (Qi).

2.5. Phylogenetic Analysis

ITS, *Btub* and *cox1* sequences of four isolates obtained in this survey were compiled in a dataset together with 78 sequences from 26 *Phytophthora* species representative of described species in the clade 7 (including ex-type culture) for which molecular data are available in GenBank (Table 2).

Sequence alignments were performed with ClustalX v. 1.83 [55], using the parameters reported in Bregant et al. [51]. Alignments were checked and edited with BioEdit Alignment Editor v. 7.2.5 [56]. Phylogenetic analyses were completed with MEGA-X 10.1.8 [57]. All gaps were included in the analyses. The best model of DNA sequence evolution was determined automatically by the software. Maximum likelihood (ML) analysis was performed with a neighbour-joining (NJ) starting tree generated by the software. A bootstrap analysis (1000 replicates) was used to estimate the robustness of nodes.

2.6. Pathogenicity Test

Pathogenicity of the new *Phytophthora* species was tested on 3-year-old myrtle (*Myrtus communis*) and lentisk (*Pistacia lentiscus*) seedlings grown in plastic pots (20 cm diameter, 2 L volume). Eight seedlings of each plant species were inoculated with the isolate CB84 (ex-type culture). In addition, the same number of each plant species were inoculated with the isolate CB21 of *P. cinnamomi* for comparison. Finally, the same number of seedlings were used as control. Inoculation was performed at the collar. The inoculated point was surface-disinfected with 70% ethanol and a small piece of outer bark (5 mm diameter) was removed with a flame-sterilised cork borer and replaced with an agar-mycelium plug of the same size, taken from the margin of an actively growing colony on CA. The inoculation point was covered with cotton wool soaked in sterile water and wrapped with an aluminium foil to retain moisture. Controls were inoculated with a sterile CA plug.

All inoculated seedlings were kept in a climatic chamber at 25 °C and watered every two days until the end of the experimental period. After 20 days, all plants were checked for the presence of disease symptoms and the length of necrotic lesion surrounding the inoculation site was measured after removing the outer bark with a sterile scalpel.

Re-isolation was performed by transferring 10 pieces of inner bark taken around the margin of the necrotic lesions onto PDA+. Growing colonies were sub-cultured onto CA

and PDA, incubated in the dark at 20 °C and identified by morphological and molecular analysis (ITS region) to confirm Koch's postulates.

2.7. Data Analysis

Data from the pathogenicity assay were first checked for normality and then subjected to analysis of variance (ANOVA). Significant differences among mean values were determined using Fisher's least significant differences multiple range test ($p = 0.05$) after one-way ANOVA using XLSTAT 2008 software (Addinsoft).

3. Results

3.1. Symptomatology

Phytophthora related diseases were observed in all investigated nurseries. Regardless of the species, potted plants showed wilting foliage, chlorosis, stunted growth and sudden death symptoms (Figure 1). Sudden death was very common in plants of the alpine nursery (N3) and, in particular, of silver fir and beech seedlings.

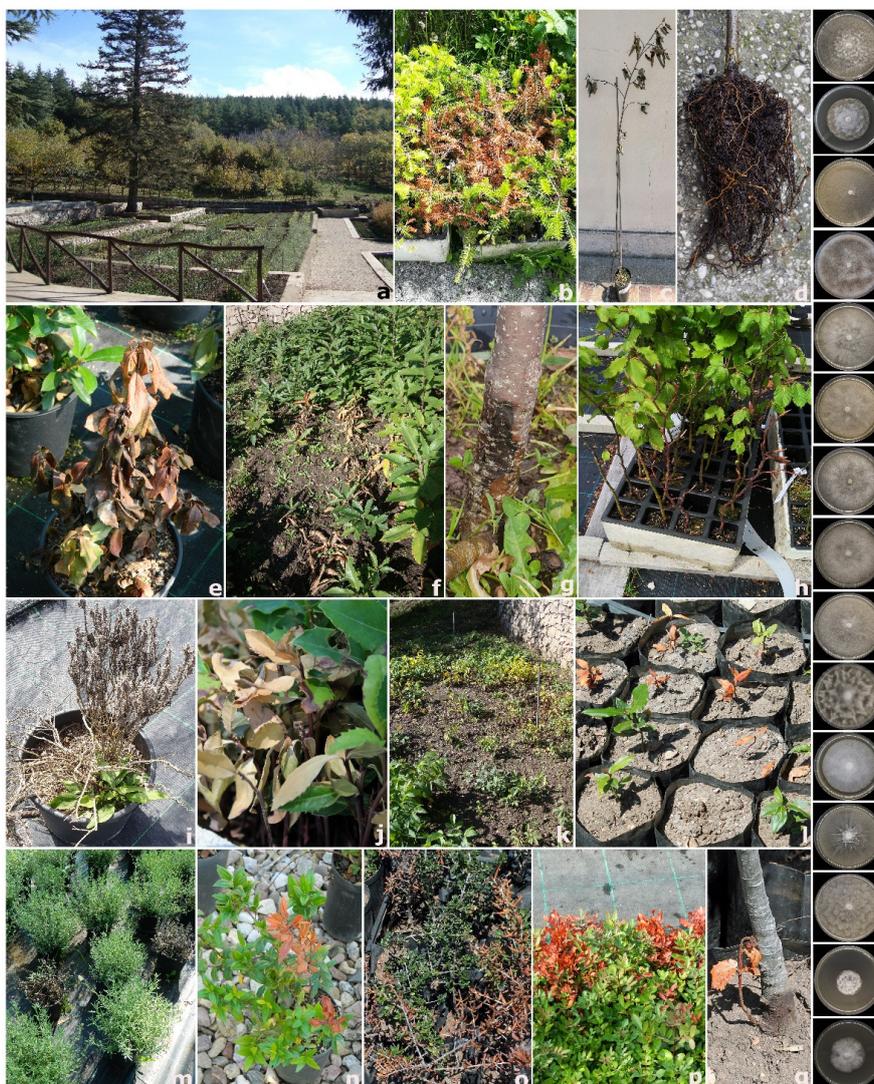


Figure 1. Overview of the forest nursery N1 (a), symptoms of wilting, sudden death and collar and root rot observed on: *Abies alba* (b), *Alnus incana* (c,d), *Arbutus unedo* (e), *Castanea sativa* (f,g), *Fagus sylvatica* (h), *Helichrysum italicum* (i), *Ilex aquifolium* (j), *Juglans regia* (k), *Laurus nobilis* (l), *Lavandula officinalis* (m), *Myrtus communis* (n), *Phyllirea latifolia* (o), *Pistacia lentiscus* (p) and *Quercus ilex* (q) seedlings. On the right from the top, colony morphology of: *Phytophthora acerina*, *P. bilorbang*, *P. cactorum*, *P. cinnamomi*, *P. citrophthora*, *P. mediterranea*, *P. megasperma*, *P. nicotianae*, *P. palmivora*, *P. parvispora*, *P. pistaciae*, *P. plurivora*, *P. pseudocryptogea*, *P. pseudosyringae* and *P. psychrophila* after 7 days growth at 25 °C on CA in the dark.

All canopy symptoms described above were associated with collar and root rot symptoms. In most cases, the root system was completely compromised and fine roots absent (Figure 1). The mortality rates, especially in one-year-old seedlings, were very high with losses close to 60–70%.

3.2. *Phytophthora* Species Associated with Nursery Plants

Isolations performed on 76 symptomatic plants yielded a total of 66 *Phytophthora* isolates. The largest number of isolates (36) was obtained from site N1, followed by site N2 (22), whereas a total of 8 isolates were obtained from sites N3 and N4.

On the basis of micromorphological features and ITS sequence data, fourteen *Phytophthora* species belonging to seven phylogenetic clades were identified: *P. cinnamomi* (26 isolates), *P. nicotianae* (9), *P. palmivora* (5), *P. pseudocryptogea* (4), *P. megasperma* (3), *P. plurivora* (3), *P. parvispora* (3), *P. pseudosyringae* (3), *P. pistaciae* (2), *P. bilorbang* (2), *P. acerina* (1), *P. cactorum* (1), *P. citrophthora* (1) and *P. psychrophila* (1). For each species BLAST searches against GenBank showed 100% identity to reference sequences of representative strains including those of ex-type culture (Table 3). Two isolates obtained from declining myrtle seedlings could not be assigned on the basis of morphological features and ITS sequence data to any formally described species or informally designated *Phytophthora* and were therefore considered a new *taxon* unknown to science.

Among the detected species, *P. cinnamomi* was the dominant one, being isolated from five different hosts in three nurseries (Table 3). *Phytophthora nicotianae* was the most abundant species in the nursery N2. The other species were isolated only from a single host. Interestingly, some of the monitored plant species were positive for two or three *Phytophthora* species.

3.3. Phylogenetic Analysis

Phylogenetic relationships among the *Phytophthora* species belonging to clade 7 and four representative isolates obtained in this study were elucidated using a multilocus analysis based on the sequences of ITS, *Btub* and *cox1* regions.

Fragments of approximately 800, 920 and 1020 bp were obtained for ITS, *Btub* and *cox1* regions, respectively. Individual gene phylogenies revealed similar tree topologies, indicating that the three loci could be combined (data not shown). The ML evolutionary reconstruction allowed for the differentiation of 27 distinct lineages within clade 7, corresponding to 27 species and hybrids (Figure 2). The four isolates obtained in this study were distributed into three sub-clades in clade 7c (Figure 2). In particular, isolate (CB21) clustered with ex-culture type of *P. cinnamomi* and isolate (CB86) with ex-culture type of *P. parvispora*. The remaining two isolates clustered together in a well-supported terminal clade (ML bootstrap = 100%) and were considered to represent a new species described here as *Phytophthora mediterranea* sp. nov. (Figure 2).

Phylogenetically, the new *Phytophthora* species is closely related to *P. cinnamomi*, from which it can be distinguished on the basis of 15, 23 and 49 bp in ITS, *Btub* and *cox1* loci, respectively. The other evolutionarily closest species, *P. parvispora* differs by a total of 105 nucleotides in the investigated DNA regions.

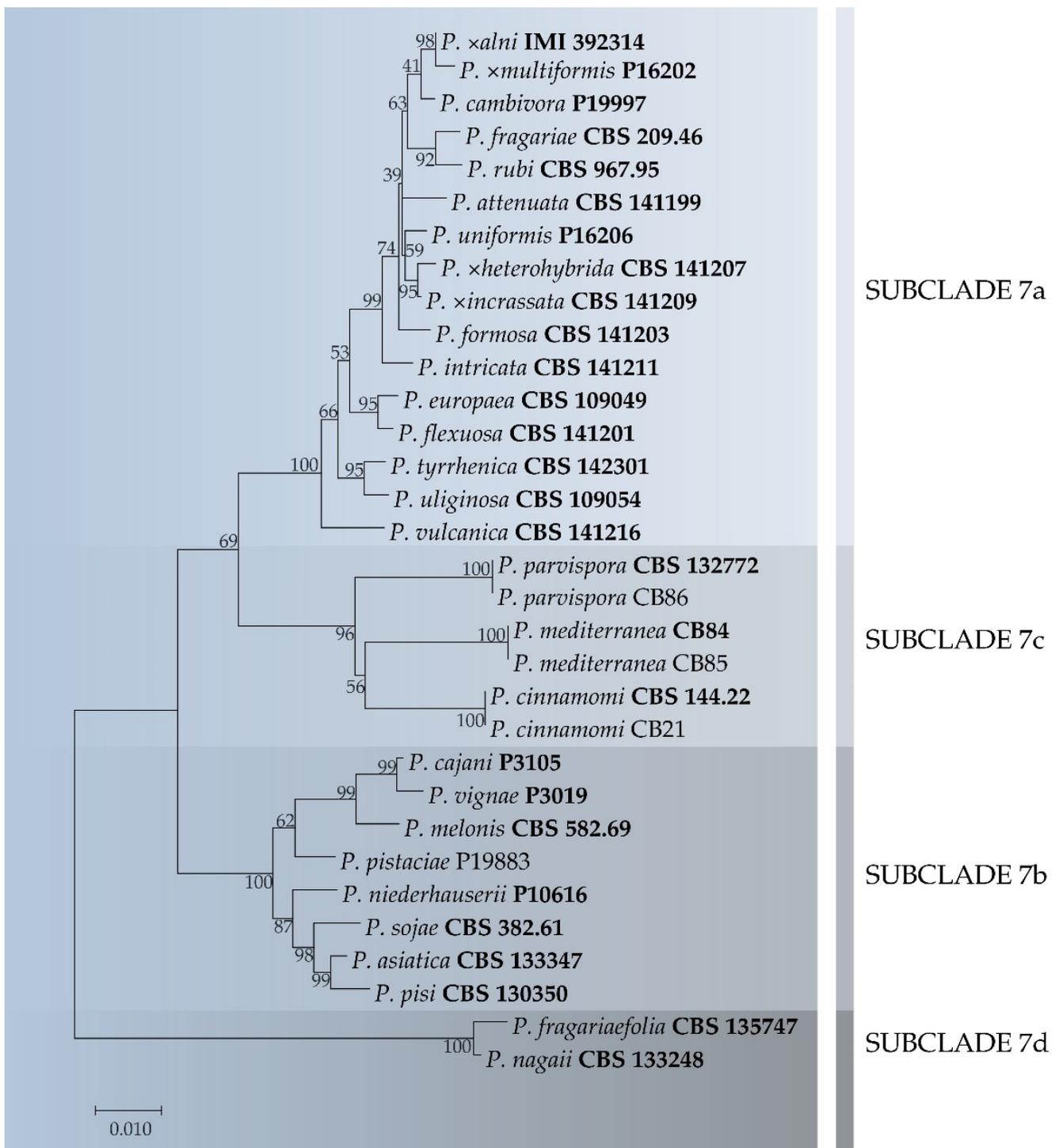


Figure 2. Maximum likelihood tree obtained from combined ITS, Btub and *cox1* sequences of *Phytophthora* species belonging to clade 7. Data are based on the general time reversible model. A discrete gamma distribution was used to model evolutionary rate differences among sites. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap support values in percentage (1000 replicates) are given at the nodes. Ex-type cultures are reported in bold.

3.4. Taxonomy

Phytophthora mediterranea Bregant, Mulas and Linaldeddu sp. nov. (Figure 3).

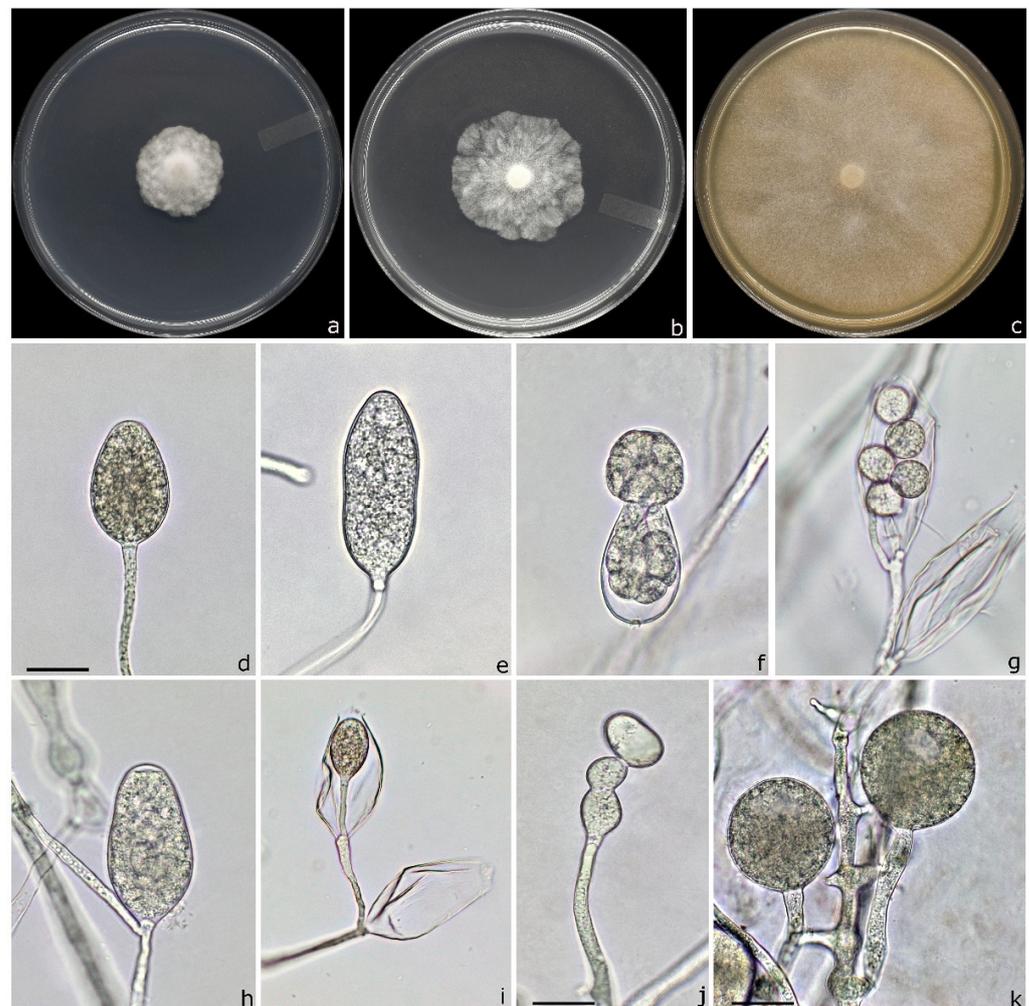


Figure 3. Colony morphology of *Phytophthora mediterranea* (CB84) after 7 days growth at 25 °C on potato dextrose agar (PDA) (a), malt extract agar (MEA) (b) and carrot agar (CA) (c). Sporangia observed in pond water: non-papillate, persistent and ovoid (d), elongated (e), releasing zoospores (f,g). External (h) and internal proliferations (i), hyphal swellings (j) and terminal chlamydospores (k). Scale bars = 20 µm.

Mycobank: MB839612.

Etymology: the epithet refers to the Mediterranean region, where the species was originally discovered.

Holotype: CBS H-24768.

Host/distribution: potted *Myrtus communis* plants with root rot symptoms in Italy.

Description: sporangia were abundantly produced in unsterile pond water after 36–48 h of incubation at 20 °C. They were non-papillate, rarely semi-papillate, persistent, from ovoid to ellipsoid and less frequently elongated or distorted (Figure 3d,e,h). Typically, sporangia were borne terminally on unbranched sporangiophores and only occasionally on compound sporangiophores. Sporangial proliferation was usually external and very rarely internal (nested and extended) (Figure 3g–i).

Sporangia size ranged from 27.2 to 64.9 µm in length (av. 42.9 ± 9.3 µm) and from 20.1 to 46.8 µm in breadth (av. 29.8 ± 5.3 µm) with a length/breadth ratio of 1.4 ± 0.1 ($n = 50$). Zoospores were abundantly produced in liquid cultures after 36–48 h (Figure 3f,g). Globose to sub-globose, irregular and catenulate hyphal swellings were abundantly produced on CA (Figure 3j). Spherical chlamydospores were mostly terminal and only occasionally lateral and intercalary, and were abundantly produced on CA and water (Figure 3k). Chlamy-

dospores ranged from 14.9 to 37.5 μm in diameter (av. $24.5 \pm 5.0 \mu\text{m}$). No gametangia were observed on pure cultures on CA suggesting a heterothallic behaviour.

Cultural characteristics: colonies were stellate on PDA and radiate on MEA whereas on CA showed a regular margin with a cottony and aerial mycelium without a distinct pattern. On PDA and MEA colony growth was slow, whereas on CA colony reached 80 mm diameter in 7 d at 25 °C.

Cardinal temperatures: *minimum* <10 °C, *maximum* >37 °C and *optimum* 32 °C. Both isolates failed to grow at 40 °C and mycelium did not resume growth when plates were moved to 20 °C.

Material examined: ITALY: Oristano, isolated from roots of a potted plant of *Myrtus communis*, 18 April 2019, isolated by Antonio Mulas, HOLOTYPE CBS H-24768, a dried culture on CA, culture ex-holotype CB84 = CBS 147720. ITALY: Oristano, isolated from rhizosphere and fine roots of a potted plant of *Myrtus communis*, 12 November 2020, isolated by Carlo Bregant (culture CB85).

Notes: *Phytophthora mediterranea* differs from the closely related species *P. cinnamomi* and *P. parvispora* through a combination of unique morphological characters and molecular data such as sporangia shape and sizes, growth pattern on different culture media, a higher *maximum* and *optimum* temperature value for growth (Figure 4) as well as a total of 87 (*P. cinnamomi*) and 105 (*P. parvispora*) fixed differences in the ITS, Btub and *cox1* sequences.

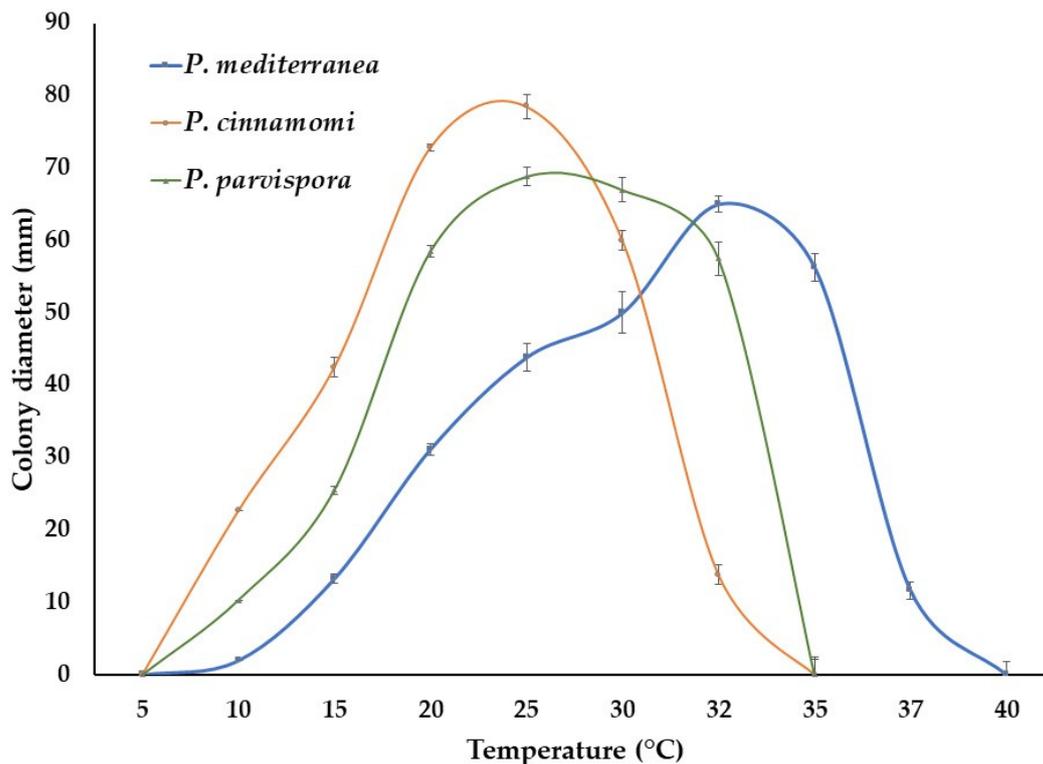


Figure 4. Mean colony diameter (\pm standard deviation) of *P. cinnamomi* (CB21), *P. mediterranea* (CB84) and *P. parvispora* (CB86) after 96 h on CA in the dark at different temperatures.

3.5. Pathogenicity Test

In artificial inoculation trials, *P. mediterranea* was shown to be pathogenic on myrtle and lentisk seedlings. After 20 days from inoculation, all plants inoculated with *P. mediterranea* displayed necrotic inner bark lesions that spread up and down from the inoculation site (Figure 5). On myrtle, the necrotic lesions caused by the isolate of *P. mediterranea* were significantly larger than those caused by *P. cinnamomi*; in contrast on lentisk, *P. cinnamomi* showed itself to be more aggressive than *P. mediterranea* (Table 4).



Figure 5. Symptoms observed on myrtle and lentisk seedlings 20 days after inoculation with *Phytophthora mediterranea* (a,d) and *P. cinnamomi* (b,e). Control seedlings (c,f).

Table 4. Mean lesion length (cm) (\pm standard deviation) caused by *Phytophthora mediterranea* (CB84) and *P. cinnamomi* (CB21) on myrtle (M) and lentisk (L) seedlings and percentage of positive re-isolations.

Species	Myrtle *	Lentisk *	Re-Isolation Frequency (%)
<i>Phytophthora mediterranea</i>	1.01 \pm 0.4a	1.9 \pm 0.6b	100 (M) and 100 (L)
<i>Phytophthora cinnamomi</i>	0.68 \pm 0.3b	2.3 \pm 0.8a	100 (M) and 100 (L)
Control	-	0.5 \pm 0.2c	-

* Values in column with the same letter do not differ significantly at $p = 0.05$, according to LSD multiple range test.

Control plants inoculated with sterile CA plugs remained symptomless, only a light brown discoloration limited to the inoculation point was observed on lentisk seedlings (Figure 5). Both *Phytophthora* species were successfully re-isolated from all inoculated plants, fulfilling Koch's postulates. No *Phytophthora* isolates were re-isolated from control plants.

4. Discussion

The results obtained allowed us to expand knowledge on the diversity of *Phytophthora* species in Italian forest nurseries. The investigated nurseries are located in sites with very different climatic conditions, ranging from the Mediterranean (N2) to alpine ones (N3), including an intermediate climate typical of temperate hilly and low mountain areas (N1 and N4). These differences were also reflected in the plant species cultivated, which were typical of the different geographic regions.

Over the past two decades, diseases caused by *Phytophthora* species have been reported in a wide range of economically important ornamental plants worldwide and are considered yield-limiting factors in nursery production [13,29].

Our findings demonstrated that all the investigated nurseries were severally impacted by *Phytophthora* diseases. The N1 in particular was the most affected by the problem, and basically all cultivated plant species typical of the Mediterranean region were affected by *Phytophthora* infections.

In total, 15 *Phytophthora* species were isolated in pure culture from 14 hosts and identified on the basis of morphological features and DNA sequence analysis. Nine *Phytophthora* species were isolated in N1, five in N2, two in N4 and one in N3. The species obtained are representative of seven phylogenetic clades and included 14 previously known species: *P. acerina*, *P. bilorbang*, *P. cactorum*, *P. cinnamomi*, *P. citrophthora*, *P. megasperma*, *P. nicotianae*, *P. palmivora*, *P. parvispora*, *P. pistaciae*, *P. plurivora*, *P. pseudocryptogea*, *P. pseudosyringae* and *P. psychrophila*. In addition, two isolates could not be assigned to any known species and are therefore described here as *P. mediterranea* sp. nov.

Phytophthora mediterranea was shown to be associated with collar and root rot symptoms of myrtle seedlings. Phylogenetically, *P. mediterranea* is closely related to *P. cinnamomi* but the two species differ in 87 nucleotides in the three DNA regions studied. Morphologically *P. mediterranea* can be easily distinguished from *P. cinnamomi* on the basis of its smaller sporangia, colony growth pattern and higher optimum and maximum temperature values. Data from the pathogenicity tests showed that *P. mediterranea* has the potential to threaten the native Mediterranean maquis vegetation. A study is currently in progress to evaluate the susceptibility of the main Mediterranean maquis species to this new pathogen (Linaldeddu, unpublished data). The high optimum temperature value for growth of 32 °C and the high production of long-term survival propagules (chlamydospores) suggest that *P. mediterranea* is well adapted to survive in Mediterranean environments.

Phytophthora mediterranea belongs to clade 7c together with *P. cinnamomi* and *P. parvispora*, which were also obtained in this study. *Phytophthora cinnamomi* was the dominant species as it has been detected on five different hosts confirming its well-known polyphagous nature [11,58], and in three of the four monitored nurseries. The three nurseries are characterized by different environmental conditions ranging from the Mediterranean climate of site 2 located in Sardinia to the cold habitat of site 3 in the pre-Alps. This aspect emphasizes the potential of *P. cinnamomi* to survive in very different environments including low temperature habitats, as confirmed by a recent study in which this pathogen was detected in an outdoor blueberry stand in Germany [59]. Cold regions such as alpine and sub-alpine regions were long considered *P. cinnamomi* free, due to the inactivity of this species in soil at temperatures below 10 °C [60]. Analysis on current distribution data, global change and a forecast model, have allowed a global map to be created, identifying the cold areas of the planet as those at greatest risk of introduction and spread of *P. cinnamomi* in the future [61]. The discovery of *P. cinnamomi* in mountain nurseries in Italy confirms this trend and poses the risk of diffusion of this pathogen on new susceptible hosts among the alpine species.

Phytophthora nicotianae was the second dominant species. It has been isolated from four host species confirming the results of previously studies on the role of the nursery trade as one of the main ways of spreading for this pathogen [48,62]. The other twelve species have been isolated from a single plant host and only one nursery. Differences in species diversity among nurseries are difficult to interpret. It is plausible that different agronomical

practices including sanitation, water management, origin and treatment of seeds as well as host plant diversity could have a role in the differences observed in terms of *Phytophthora* assemblages. Some species such as *P. parvispora* and *P. pistaciae* have confirmed a specificity towards some plant hosts already reported in other studies [49,50]. *Phytophthora parvispora*, long considered a variety of *P. cinnamomi*, is closely linked to *Arbutus unedo* plants in both nursery and natural areas of the Mediterranean Basin [49]. It has also recently been reported in North American nurseries associated with seedlings destined for reforestation programmes and in pomegranate orchards in Turkey [63,64]. *Phytophthora pistaciae* is a species characterized by a very limited geographic distribution and host range; it was originally described in Iran associated with *Pistacia vera* gummosis [65] and more recently in Italy on potted *Pistacia lentiscus* seedlings [50].

The other species, except *P. acerina*, have already been reported in different nurseries in Europe, Australia and North America on several plant hosts [34,39,48,63]. Nonetheless, seven new *Phytophthora*–host associations were detected in this study: *P. psychrophila*/*Ilex aquifolium*, *P. pseudosyringae*/*I. aquifolium*, *P. pseudocryptogea*/*Laurus nobilis*, *P. megasperma*/*L. nobilis*, *P. citrophthora*/*L. nobilis*, *P. bilorbang*/*Phyllirea latifolia* and *P. palmivora*/*P. latifolia*.

Phytophthora acerina was originally described in Northern Italy from declining maple trees [66] and more recently on declining olives with sudden death symptoms [67]. Its reports have increased in the last years in both natural and agricultural environments in Italy and its role in the onset of sudden death and dieback of kiwi, walnut and pomegranate orchards as well as oaks, chestnut and ash forests has been ascertained (Linaldeddu, unpublished data). In a nursery, *P. acerina* appears to be a very rare species, this is the first report on nursery plants in Europe, but it was recently reported in nursery plants in California [68]. The discovery of *P. acerina* and *P. plurivora* on seedlings potentially destined to reforestation programmes highlights the risk of further spread of these species, directly associated with the decline of grey alder on the Alps [51].

5. Conclusions

The findings obtained in this study highlight the occurrence of multiple *Phytophthora* species in Italian forest nurseries. Many of the species isolated are common in Italian nurseries, while others are rare or have never been reported. *Phytophthora pistaciae* is an example of a rare and possibly exotic species with a high risk of spread to the Mediterranean maquis. In addition, a new species closely related to *P. cinnamomi* and *P. parvispora* was isolated from potted myrtle plants. The myrtle is a typical shrub of the Mediterranean Basin, and Sardinia is recognized as one of its main local centres of diversity [69]. Its fruit and leaves exhibit antioxidant, antibacterial and antifungal properties, as well as being used for their content of essential oils and most commonly as an ingredient in a locally made liquor [70]. Nursery plants are often used for the creation of new orchards [71]. The use of infected plants could represent an important pathway for *P. mediterranea*.

Finally, the discovery of *P. cinnamomi* in alpine nurseries confirms the progressive expansion of this species towards the coldest areas of the globe, probably driven by climate change. A survey is currently in progress to map the distribution of *Phytophthora* species in alpine nurseries in Italy, with the purpose of evaluating the risk of diffusion of these pathogens through restoration and reforestation programmes.

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Data Availability Statement: Data sharing not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Hulme, P.E. Trade, transport and trouble: Managing invasive species pathways in an era of globalization. *J. Appl. Ecol.* **2009**, *46*, 10–18. [CrossRef]
- Bradley, B.A.; Blumenthal, D.M.; Regan, E.; Grosholz, E.D.; Lawler, J.J.; Miller, L.P.; Sorte, C.J.B.; D'Antonio, C.M.; Diez, J.M.; Dukes, J.S.; et al. Global Change, Global Trade, and the Next Wave of Plant Invasions. *Front. Ecol. Environ.* **2012**, *10*, 20–28. [CrossRef]
- ISTAT. *Stima Preliminare dei Conti Economici Dell'agricoltura*; Archivio, id252819; ISTAT: Rome, Italy, 2021.
- Brasier, C. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathol.* **2008**, *57*, 792–808. [CrossRef]
- Liebhold, A.M.; Brockehoff, E.G.; Garret, L.J.; Parke, J.L.; Britton, K.O. Live plant imports: The major pathway for forest insect and pathogen invasions of the US. *Front. Ecol. Environ.* **2012**, *10*, 135–143. [CrossRef]
- Liebhold, A.M.; Brockehoff, E.G.; Kalisz, S.; Nuñez, M.A.; Wardle, D.A.; Wingfield, M.J. Biological invasions in forest ecosystems. *Biol. Invasions* **2017**, *19*, 3437–3458. [CrossRef]
- Ghelardini, L.; Luchi, N.; Pecori, F.; Pepori, A.L.; Danti, R.; Rocca, G.D.; Capretti, P.; Tsopelas, P.; Santini, A. Ecology of invasive forest pathogens. *Biol. Invasions* **2017**, *19*, 3183–3200. [CrossRef]
- Yang, X.; Tyler, B.M.; Hong, C. An expanded phylogeny for the genus *Phytophthora*. *IMA Fungus* **2017**, *8*, 355–384. [CrossRef] [PubMed]
- Jung, T.; Horta Jung, M.; Cacciola, S.O.; Cech, T.; Bakonyi, J.; Seress, D.; Mosca, S.; Schena, L.; Seddaiu, S.; Pane, A.; et al. Multiple new cryptic pathogenic *Phytophthora* species from *Fagaceae* forests in Austria, Italy, and Portugal. *IMA Fungus* **2017**, *8*, 219–244. [CrossRef] [PubMed]
- Scott, P.; Bader, M.K.F.; Burgess, T.I.; Hardy, G.; Williams, N. Global biogeography and invasion risk of the plant pathogen genus *Phytophthora*. *Environ. Sci. Policy* **2019**, *101*, 175–182. [CrossRef]
- Erwin, C.D.; Ribeiro, O.K. *Phytophthora Diseases Worldwide*; APS Press: Saint Paul, MN, USA, 1996.
- Benson, D.M.; Jones, J.K. *Diseases of Woody Ornamentals and Trees in Nurseries*; APS Press: St. Paul, MN, USA, 2001.
- Parke, J.L.; Redekar, N.R.; Eberhart, J.L.; Funahashi, F. Hazard analysis for *Phytophthora* species in container nurseries: Three case studies. *HortTechnology* **2019**, *29*, 745–755. [CrossRef]
- Webber, J.F.; Mullett, M.; Brasier, C.M. Dieback and mortality of plantation Japanese larch (*Larix kaempferi*) associated with infection by *Phytophthora ramorum*. *New Dis. Rep.* **2010**, *22*, 19. [CrossRef]
- Prospero, S.; Vercauteren, A.; Heungens, K.; Belbahri, L.; Rigling, D. *Phytophthora* diversity and population structure of *Phytophthora ramorum* in Swiss ornamental nurseries. *Plant Pathol.* **2013**, *62*, 1063–1071. [CrossRef]
- Simamora, A.V.; Paap, T.; Howard, K.; Stukely, M.J.; Hardy, G.E.S.J.; Burgess, T.I. *Phytophthora* contamination in a nursery and its potential dispersal into the natural environment. *Plant Dis.* **2017**, *102*, 132–139. [CrossRef] [PubMed]
- Zentmyer, G.A. *Origin and Distribution of Phytophthora cinnamomi*; California Avocado Society: San Juan Capistrano, CA, USA, 1985; Volume 69, pp. 89–96. Available online: http://www.avocadosource.com/cas_yearbooks/cas_69_1985/cas_1985_pg_89-96.pdf (accessed on 4 May 2021).
- Hansen, E.M.; Streito, J.C.; Delatour, C. First confirmation of *Phytophthora lateralis* in Europe. *Plant Dis.* **1999**, *83*, 587. [CrossRef] [PubMed]
- Rizzo, D.M.; Garbelotto, M.; Davidson, J.M.; Slaughter, G.W.; Koike, S.T. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp and *Lithocarpus densiflorus* in California. *Plant Dis.* **2002**, *86*, 205–214. [CrossRef]
- Cobb, R.C.; Haas, S.E.; Kruskamp, N.; Dillon, W.W.; Swiecki, T.J.; Rizzo, D.M.; Frankel, S.J.; Meentemeyer, R.K. The magnitude of regional-scale tree mortality caused by the invasive pathogen *Phytophthora ramorum*. *Earth's Future* **2020**, *8*, e2020EF001500. [CrossRef]
- Man in't Veld, W.A.; De Cock, A.W.A.M.; Summerbell, R.C. Natural hybrids of residents and introduced *Phytophthora* species proliferating on multiple new hosts. *J. Plant Pathol.* **2007**, *117*, 25–33. [CrossRef]
- McDonald, J.D.; Alishtayeh, M.S.; Kabashima, J.; Stites, J. Occurrence of *Phytophthora* species in recirculated nursery irrigation effluents. *Plant Dis.* **1994**, *78*, 607–611. [CrossRef]
- Themann, K.; Werres, S.; Lüttmann, R.; Diener, H.A. Observations of *Phytophthora* spp. in water recirculation systems in commercial hardy ornamental nursery stock. *Eur. J. Plant Pathol.* **2002**, *108*, 337–343. [CrossRef]
- Hong, C.X.; Moorman, G.W. Plant pathogens in irrigation water: Challenges and opportunities. *Crit. Rev. Plant Sci.* **2005**, *24*, 189–208. [CrossRef]
- Redekar, N.R.; Eberhart, J.L.; Parke, J.L. Diversity of *Phytophthora*, *Pythium* and *Phytopythyium* species in recycled irrigation water in a container nursery. *Phytobiomes* **2019**, *3*, 31–45. [CrossRef]
- Ivors, K.L.; Moorman, G.W. Oomycete plant pathogens in irrigation water. In *Biology, Detection, and Management of Plant pathogens in Irrigation Water*; Hong, C., Moorman, G.W., Wohanka, W., Büttner, C., Eds.; APS Press: St. Paul, MN, USA, 2017; pp. 57–64.
- Shishkoff, N. Persistence of *Phytophthora ramorum* in soil mix and roots of nursery ornamentals. *Plant Dis.* **2007**, *91*, 1245–1249. [CrossRef] [PubMed]
- Crone, M.; McComb, J.A.; O'Brien, P.A.; Hardy, G.E.S.J. Survival of *Phytophthora cinnamomi* as oospores, stromata, and thick-walled chlamydospores in roots of symptomatic and asymptomatic annual and herbaceous perennial plant species. *Fungal Biol.* **2013**, *117*, 112–123. [CrossRef] [PubMed]

29. Bienapfl, J.C.; Balci, Y. Movement of *Phytophthora* spp. in Maryland's nursery trade. *Plant Dis.* **2014**, *98*, 134–144. [[CrossRef](#)] [[PubMed](#)]
30. Bruin, G.C.A.; Edgington, L.V. Adaptive resistance in *Peronosporales* to metalaxyl. *Can. J. Plant Pathol.* **1981**, *3*, 201–206. [[CrossRef](#)]
31. Dobrowolski, M.; Shearer, B.; Colquhoun, I.; O'Brien, P.; Hardy, G.E.S.J. Selection for decreased sensitivity to phosphite in *Phytophthora cinnamomi* with prolonged use of fungicide. *Plant Pathol.* **2008**, *57*, 928–936. [[CrossRef](#)]
32. González-Tobón, J.; Childers, R.A.; Regnier, M.; Rodriguez, A.; Olave, C.; Fry, W.E.; Restrepo, S.; Danies, G. Is the phenomenon of mefenoxam-acquired resistance in *Phytophthora infestans* universal? *Plant Dis.* **2019**, *104*, 211–221. [[CrossRef](#)]
33. Hardy, G.E.S.J.; Sivasithamparam, K. *Phytophthora* spp. associated with container-grown plants in nurseries in Western Australia. *Plant Dis.* **1988**, *72*, 435–437. [[CrossRef](#)]
34. Davison, E.M.; Drenth, A.; Kumar, S.; Mack, S.; Mackie, A.E.; McKirdy, S. Pathogens associated with nursery plants imported into Western Australia. *Australas. Plant Pathol.* **2006**, *35*, 473–475.
35. Moralejo, E.; Pérez-Sierra, A.M.; Alvarez, L.A.; Belbahri, L.; Lefort, F.; Descals, E. Multiple alien *Phytophthora* taxa discovered on diseased ornamental plants in Spain. *Plant Pathol.* **2009**, *58*, 100–110. [[CrossRef](#)]
36. Yakabe, L.E.; Blomquist, C.L.; Thomas, S.L.; MacDonald, J.D. Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. *Plant Dis.* **2009**, *93*, 883–890. [[CrossRef](#)] [[PubMed](#)]
37. Pérez-Sierra, A.; Jung, T. *Phytophthora* in woody ornamental nurseries. In *Phytophthora: A Global Perspective*; Lamour, K., Ed.; CABI: Wallingford, UK, 2013; pp. 166–177.
38. Parke, J.L.; Knaus, B.J.; Fieland, V.J.; Lewis, C.; Grünwald, N.J. *Phytophthora* community structure analyses in Oregon nurseries inform systems approaches to disease management. *Phytopathology* **2014**, *104*, 1052–1062. [[CrossRef](#)] [[PubMed](#)]
39. Molnar, C.; Nikolaeva, E.; Kim, S.; Olson, T.; Bily, D.; Kim, J.-E.; Kang, S. *Phytophthora* diversity in Pennsylvania nurseries and greenhouses inferred from clinical samples collected over four decades. *Microorganisms* **2020**, *8*, 1056. [[CrossRef](#)] [[PubMed](#)]
40. Scott, P.; Taylor, P.; Gardner, J.; Puértolas, A.; Panda, P.; Addison, S.; Hood, I.; Burgess, T.; Horner, I.; Williams, N.; et al. *Phytophthora aleatoria* sp. nov., associated with root and collar damage on *Pinus radiata* from nurseries and plantations. *Australas. Plant Pathol.* **2019**, *48*, 313–321. [[CrossRef](#)]
41. Hong, C.X.; Gallegly, M.E.; Richardson, P.A.; Kong, P.; Moorman, G.W.; Lea-Cox, J.D.; Ross, D.S. *Phytophthora hydropathica*, a new pathogen identified from irrigation water, *Rhododendron catawbiense* and *Kalmia latifolia*. *Plant Pathol.* **2010**, *59*, 913–921. [[CrossRef](#)]
42. Yang, X.; Copes, W.E.; Hong, C.X. *Phytophthora mississippiiae* sp. nov., a new species recovered from irrigation reservoirs at a plant nursery in Mississippi. *J. Plant Pathol. Microb.* **2013**, *4*, 1–7. [[CrossRef](#)]
43. Bertier, L.; Brouwer, H.; de Cock, A.W.A.M.; Cooke, D.E.L.; Olsson, C.H.B.; Höfte, M. The expansion of *Phytophthora* clade 8b: Three new species associated with winter grown vegetable crops. *Persoonia* **2013**, *31*, 63–76. [[CrossRef](#)]
44. Yang, X.; Gallegly, M.E.; Hong, C. A high-temperature tolerant species in Clade 9 of the genus *Phytophthora*: *P. hydrogena* sp. nov. *Mycologia* **2014**, *106*, 57–65. [[CrossRef](#)]
45. Brasier, C.M.; Beales, P.A.; Kirk, S.A.; Denman, S.; Rose, J. *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. *Mycol. Res.* **2005**, *109*, 853–859. [[CrossRef](#)]
46. Abad, Z.G.; Abad, J.A.; Cacciola, S.O.; Pane, A.; Faedda, R.; Moralejo, E.; Pérez-Sierra, A.; Abad-Campos, P.; Alvarez-Bernaola, L.A.; Bakonyi, J.; et al. *Phytophthora niederhauserii* sp. nov., a polyphagous species associated with ornamentals, fruit trees and native plants in 13 countries. *Mycologia* **2014**, *106*, 431–447. [[CrossRef](#)]
47. Migliorini, D.; Ghelardini, L.; Tondini, E.; Luchi, N.; Santini, A. The potential of symptomless potted plants for carrying invasive soilborne plant pathogens. *Divers. Distrib.* **2015**, *21*, 1218–1229. [[CrossRef](#)]
48. Prigigallo, M.I.; Mosca, S.; Cacciola, S.O.; Cooke, D.E.L.; Schena, L. Molecular analysis of *Phytophthora* diversity in nursery-grown ornamental and fruit plants. *Plant Pathol.* **2015**, *64*, 1308–1319. [[CrossRef](#)]
49. Scanu, B.; Hunter, G.C.; Linaldeddu, B.T.; Franceschini, A.; Maddau, L.; Jung, T.; Denman, S. A taxonomic re-evaluation reveals that *Phytophthora cinnamomi* and *P. cinnamomi* var. *parvispora* are separate species. *For. Pathol.* **2014**, *44*, 1–20. [[CrossRef](#)]
50. Linaldeddu, B.T.; Mulas, A.A.; Bregant, C.; Piras, G.; Montecchio, L. First Report of *Phytophthora pistaciae* causing root and collar rot on nursery plants of *Pistacia lentiscus* in Italy. *Plant Dis.* **2020**, *104*, 1564. [[CrossRef](#)]
51. Bregant, C.; Sanna, G.P.; Bottos, A.; Maddau, L.; Montecchio, L.; Linaldeddu, B.T. Diversity and pathogenicity of *Phytophthora* species associated with declining alder trees in Italy and description of *Phytophthora alpina* sp. nov. *Forests* **2020**, *11*, 848. [[CrossRef](#)]
52. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols, a Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: San Diego, CA, USA, 1990; pp. 315–322.
53. Martin, F.N.; Tooley, P.W. Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. *Mycologia* **2003**, *95*, 269–284. [[CrossRef](#)]
54. Kroon, L.P.N.M.; Bakker, F.T.; Van Den Bosch, G.B.M.; Bonants, P.J.M.; Flier, W.G. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genet. Biol.* **2004**, *41*, 766–782. [[CrossRef](#)]
55. Thompson, J.D.; Gibson, T.J.; Plewniak, F.; Jeanmougin, F.; Higgins, D.G. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **1997**, *25*, 4876–4882. [[CrossRef](#)]
56. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic Acids Symposium Series*; Oxford University Press: Oxford, UK, 1999; Volume 41, pp. 95–98.

57. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [[CrossRef](#)]
58. Hardham, A.R.; Blackman, L.M. *Phytophthora cinnamomi*. *Mol. Plant Pathol.* **2018**, *19*, 260–285. [[CrossRef](#)] [[PubMed](#)]
59. Nechwatal, J.; Jung, T. A study of *Phytophthora* spp. in declining highbush blueberry in Germany reveals that *P. cinnamomi* can thrive under Central European outdoor conditions. *J. Plant. Dis. Prot.* **2021**. [[CrossRef](#)]
60. Weste, G.; Marks, G. The biology of *Phytophthora cinnamomi* in Australasian forests. *Annu. Rev. Phytopathol.* **2020**, *25*, 207–229. [[CrossRef](#)]
61. Burgess, T.I.; Scott, J.K.; McDougall, K.L.; Stukely, M.J.; Crane, C.; Dunstan, W.A.; Brigg, F.; Andjic, V.; White, D.; Rudman, T.; et al. Current and projected global distribution of *Phytophthora cinnamomi*, one of the world’s worst plant pathogens. *Glob. Chang. Biol.* **2017**, *23*, 1661–1674. [[CrossRef](#)]
62. Mammella, M.A.; Martin, F.N.; Cacciola, S.O.; Coffey, M.D.; Faedda, R.; Schena, L. Analyses of the population structure in a global collection of *Phytophthora nicotianae* isolates inferred from mitochondrial and nuclear DNA sequences. *Phytopathology* **2013**, *103*, 610–622. [[CrossRef](#)]
63. Rooney-Latham, S.; Blomquist, K.L.; Kosta, K.L.; Gou, Y.Y.; Woods, P.W. *Phytophthora* species are common on nursery stock grown for restoration and revegetation purposes in California. *Plant Dis.* **2019**, *103*, 448–455. [[CrossRef](#)]
64. Kurbetli, İ.; Karaca, G.; Aydoğdu, M. *Phytophthora* species causing root and collar rot of pomegranate in Turkey. *Eur. J. Plant. Pathol.* **2020**, *157*, 485–496. [[CrossRef](#)]
65. Mirabolfathy, M.; Cooke, D.E.L.; Duncan, J.M.; Williams, N.A.; Ershad, D.; Alizadeh, A. *Phytophthora pistaciae* sp. nov. and *P. melonis*: The principal causes of pistachio gummosis in Iran. *Mycol. Res.* **2001**, *105*, 1166–1175. [[CrossRef](#)]
66. Ginetti, B.; Moricca, S.; Squires, J.N.; Cooke, D.E.L.; Ragazzi, A.; Jung, T. *Phytophthora acerina* sp. nov., a new species causing bleeding cankers and dieback of *Acer pseudoplatanus* trees in planted forests in northern Italy. *Plant Pathol.* **2014**, *63*, 858–876. [[CrossRef](#)]
67. Linaldeddu, B.T.; Bregant, C.; Montecchio, L.; Favaron, F.; Sella, L. First report of *Phytophthora acerina*, *P. pini* and *P. plurivora* causing root rot and sudden death on olive trees in Italy. *Plant Dis.* **2020**, *104*, 996. [[CrossRef](#)]
68. Frankel, S.J.; Conforti, C.; Hillman, J.; Ingolia, M.; Shor, A.; Benner, D.; Alexander, J.M.; Bernhardt, E.; Swiecki, T.J. *Phytophthora* introductions in restoration areas: Responding to protect California native flora from human-assisted pathogen spread. *Forests* **2020**, *11*, 1291. [[CrossRef](#)]
69. Medail, F.; Quezel, P. Biodiversity hotspots in the Mediterranean basin: Setting global conservation priorities. *Conserv. Biol.* **1999**, *13*, 1510–1513. [[CrossRef](#)]
70. Melito, S.; Chessa, I.; Erre, P.; Podani, J.; Mulas, M. The genetic diversity of Sardinian myrtle (*Myrtus communis* L.) populations. *Electron. J. Biotechnol.* **2013**, *16*, 7. [[CrossRef](#)]
71. Mulas, M. Problematiche legate alla coltivazione del mirto. *Italus Hortus* **2004**, *11*, 308–312.