A Survey in Natural Forest Ecosystems of Vietnam Reveals High Diversity of both New and Described Phytophthora Taxa including \textit{P. ramorum}

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**Abstract**: In 2016 and 2017, surveys of \textit{Phytophthora} diversity were performed in 25 natural and semi-natural forest stands and 16 rivers in temperate and subtropical montane and tropical lowland regions of Vietnam. Using baiting assays from soil samples and rivers and direct isolations from naturally fallen leaves, 13 described species, five informally designated taxa and 21 previously unknown taxa of \textit{Phytophthora} were isolated from 58 of the 91 soil samples (63.7%) taken from the rhizosphere of 52 of the 64 woody plant species sampled (81.3%) in 20 forest stands (83.7%), and from all rivers: \textit{P. capensis}, \textit{P. citricola} VII, VIII, IX, X and XI, \textit{P. sp. botryosa}-like 2, \textit{P. sp. meadii}-like 1 and 2, \textit{P. sp. tropicalis}-like 2 and \textit{P. sp. multivesiculata}-like 1 from \textit{Phytophthora} major phylogenetic Clade 2; \textit{P. castaneae} and \textit{P. heveae} from Clade 5; \textit{P. chlamydospora}, \textit{P. gregata}, \textit{P. bitahaiensis}-like and \textit{P. sp. sylvatica}-like 1, 2 and 3 from Clade 6; \textit{P. cinnamomi (Pc)}, \textit{P. parvispora}, \textit{P. attenuata}, \textit{P. sp. attenuata-like 1}, \textit{P. sp. heterophybrida} from Clade 7; \textit{P. drechsleri}, \textit{P. pseudocryptogea}, \textit{P. ramorum (Pr)} and \textit{P. sp. kelmania} from Clade 8, \textit{P. macrochlamydospora}, \textit{P. sp. xinsolita-like}, \textit{P. sp. xkunnunara-like}, \textit{P. sp. xvirginiana-like s.l.} and three new taxa, \textit{P. sp. quininea-like}, \textit{P. sp. xGrenada 3-like} and \textit{P. sp. xPeru 4-like}, from Clade 9; and \textit{P. sp. gallica-like 1} and 2 from Clade 10. The A1 and A2 mating types of both \textit{Pc} and \textit{Pr} co-occurred. The A2 mating type of \textit{Pr} was associated with severe dieback of montane forests in northern Vietnam. Most other \textit{Phytophthora} species, including \textit{Pr}, were not associated with obvious disease symptoms. It is concluded that (1) Vietnam is within the center of origin of most \textit{Phytophthora} taxa found including \textit{Pc} and \textit{Pr}, and (2) \textit{Phytophthora} clades 2, 5, 6, 7, 8, 9, and 10 are native to Indochina.

**Keywords**: biosecurity; breeding systems; hybridization; \textit{Phytophthora cinnamomi}; biogeography; center of origin
1. Introduction

The number of devastating declines of trees and other woody plants driven by introduced invasive Phytophthora species in natural ecosystems in Australia, Europe, and North America has increased exponentially since the 1960s [1–9]. Therefore, numerous surveys in natural and semi-natural ecosystems have been performed in the past two decades to assess Phytophthora diversity in these continents and in Africa, Asia, and South America [4,5,10–19]. As a result of these surveys and molecular re-evaluations of culture collections and several species complexes, the number of described species and informally designated taxa of Phytophthora has tripled since 1999 [2,18,20–28]. A conservative estimate predicted the existence of 200–600 unknown Phytophthora species in natural ecosystems of as yet unsurveyed regions of the world [26]. These are distributed among 12 major phylogenetic clades [23,28,29].

Accumulating circumstantial evidence suggests that Southeast and East Asia might be one center of origin of the genus. This included the common occurrence of both mating types of several heterothallic Phytophthora species, the occurrence of many Phytophthora diseases on mainly non-native horticultural trees and crops, and the apparent absence of Phytophthora diseases in natural ecosystems, despite the presence of species which cause severe forest dieback elsewhere [2,10,12,13,15,16,30–35]. In 2013, a survey in natural forests and streams of Taiwan demonstrated remarkably high diversity including ten described species and 17 previously unknown taxa of which nine were of hybrid origin. The results suggested that most of these taxa including the A1 mating type of P. cinnamomi were indigenous to Taiwan, whereas the A2 mating type of P. cinnamomi is introduced; that major Phytophthora phylogenetic clades 2, 5, 6, 7 and 9 are native to Southeast and Eastern Asia; and that interspecific hybridisation may have a major role in speciation and radiations in diverse natural ecosystems [10,22].

The high Phytophthora diversity in Taiwan probably reflects both the high floristic, geological, and climatic diversity of this island and repeated immigration of Phytophthora species from mainland Asia via temporary landbridges during glacial periods in the pleistocene followed by periods of separation and speciation during interglacials [10,22,36–39]. Similarly, due to its complex geology, geomorphology, and orographic climates and the repeated immigration of plant species from both northern latitudes and the numerous islands of Sundaland during glacial periods, Indochina is also a biodiversity hotspot, harbouring 20%–25% of the world’s plant species [39–41]. With a north–south extension of 1650 km and a west-east extension ranging between 50 and 600 km, Vietnam is located between 8°30’ and 23°30’ northern latitude and 102°10’ and 109°27’ eastern longitude in eastern Indochina along the South China Sea, covering approximately 330,000 km². In Vietnam, seven climatic regions are distinguished. In simple terms, northern Vietnam has a humid subtropical monsoon climate with cool winters and hot rainy summers in lowland areas and cold misty winters and warm rainy summers in montane regions. Southern Vietnam has a tropical monsoon climate with warm winters and hot summers and a pronounced rainy period between May and October due to the East Asian monsoon. However, regionally, temperature and precipitation patterns can vary considerably due to orographic influences. The geology and geomorphology of Vietnam are also highly complex. Due to this environmental heterogeneity, the flora of Vietnam is remarkably diverse, comprising more than 10,350 species and 2256 genera of vascular plants, of which 10% and 3%, respectively, are endemic [40]. This includes 245 and 211 native species of the Lauraceae and Fagaceae respectively, families known for the high susceptibility of their European and North American members to introduced Phytophthora species [5–7,42–44]. Therefore, as in Taiwan, a high diversity of unknown Phytophthora species might be expected in Vietnam. Further, due to their co-evolution with Vietnamese tree genera also present in Europe and North America, some of these might pose a threat to forests and natural ecosystems in the latter two continents.
In spring 2016 and 2017, in the frame of a collaborative research project between the Mendel University in Brno, Forest Research and the University of Sassari, a survey of *Phytophthora* diversity was performed in a diverse range of natural forest types and river systems across Vietnam. This paper reports on the results of this *Phytophthora* survey and the association of *Phytophthora* spp. with disease symptoms of forest trees in Vietnam, and discusses the potential threat posed by previously unknown *Phytophthora* spp. to European and North American forests.

2. Material and Methods

2.1. Sampling and *Phytophthora* Isolation

Twenty-five natural forest stands covering a wide range of tree species, climates, and landscapes across Vietnam were selected for sampling (Figures 1 and 2). The forest stands were located in northern Vietnam in Hoàng Liên National Park (NP) (12 stands) and on two neighboring mountains (two stands), in Ba Vì NP (three stands) and in Cuc Phuong NP (five stands), and in southern Vietnam in Bử Giáp NP, U Minh Hǎ NP and Côn Đảo NP on Côn Đảo island (each one stand). In addition, 16 rivers and streams were sampled in northern Vietnam (Figures 1 and 2c). Soil sampling and isolation methodology were according to [4,10]. In total, 91 rhizosphere soil samples were taken from 142 mature specimens of 64 native tree and shrub species. Three 20 × 30 × 20 cm soil monoliths were taken around each tree, at a distance of 30–150 cm from the stem base and at a soil depth of 10–30 cm. Aliquots of ca. 2 litres of rhizosphere soil together with roots (diameter ≤ 5 mm) from all monoliths were bulked, and subsamples of ca. 200 mL were used for isolation tests. Isolations from soil samples were carried out at 18–20 °C in an airconditioned laboratory at natural light using 3- to 10-day-old leaflets of native tree species, mainly *Lithocarpus bacangensis*, *L. corneus*, *Quercus glauca*, *Q. chapaensis*, *Q. gilva*, *Castanopsis indica* and *Chamaecyparis hodginsii*, and the introduced *Acacia mangium* as baits floated over flooded soil. Brownish leaflets were examined at ×80 under a light microscope for presence of *Phytophthora* sporangia. Infected leaflets were blotted dry, necrotic lesions cut into small segments and plated onto selective PARPNH agar (V8-juice agar (V8A) amended with 10 µg/mL pimaricin, 200 µg/mL ampicillin, 10 µg/mL rifampicin, 25 µg/mL pentachloronitrobenzene (PCNB), 50 µg/mL nystatin and 50 µg/mL hymexazol).

In forest stand F07, the isolation of *Phytophthora* was also attempted from a bleeding bark lesion on a surface root of a mature *Castanopsis acuminatissima* (Figure 3e). Necrotic bark pieces were transported in distilled water to the lab and blotted dry on filter paper. Then, ca. 2 mm pieces were cut from the lesion margins and plated onto PARPNH agar.

In forest stand F11, freshly fallen leaves of a mature *Rhododendron arboreum* with necrotic lesions were collected from the forest floor close to forest stream R05 ca. 1 m above the waterline. The isolation of *Phytophthora* from these leaves was carried out as described below for leaves collected from rivers.
Figure 1. Location of the 25 forest sites (F01–F25; green triangles) and the 16 riparian sites (R01–R16; blue dots) included in the *Phytophthora* survey in Vietnam; blue triangles represent sites included in both the riparian and forest survey. For geographical coordinates and details of sites see Tables 1 and 2.
Figure 2. Representative forest stands and streams sampled in Vietnam; (a) Hoàng Liên National Park around the Fansipan mountain with diverse montane evergreen cloud forests and montane evergreen broadleaved forests; (b) diverse montane evergreen cloud forest F04 in Hoàng Liên National Park dominated by Fagaceae and Lauraceae species; (c) Cat Cat River (R10) running through a diverse montane evergreen forest in Hoàng Liên National Park; (d) montane Chamaecyparis hodginsii—Quercus forest on Sau Chua mountain; (e) montane Alnus nepalensis stand on Xin Chài mountain; (f) diverse, suprtropical, humid evergreen forest F15 in Ba Vì National Park; (g) Cuc Phuong National Park with diverse, tropical, evergreen lowland rainforests growing on limestone; (h) diverse, tropical, evergreen lowland rainforest stand F20. For GPS coordinates see Tables 1 and 2; for location of sites see Figure 1.
National Park dominated by Fagaceae and Lauraceae species; (c) Cat Cat River (R10) running through a diverse montane evergreen forest in Hoàng Liên National Park; (d) montane Chamaecyparis hodginsii—Quercus forest on Sau Chua mountain; (e) montane Alnus nepalensis stand on Xin Chài mountain; (f) diverse, suptropical, humid evergreen forest F15 in Ba Vì National Park; (g) Cuc Phuong National Park with diverse, tropical, evergreen lowland rainforests growing on limestone; (h) diverse, tropical, evergreen lowland rainforest stand F20. For GPS coordinates see Tables 1 and 2; for location of sites see Figure 1.

Figure 3. Disease symptoms of mature native trees in natural forest stands in Vietnam associated with presence of *Phytophthora* species in the rhizosphere; (a–f) montane evergreen cloud forests in Hoàng Liên National Park; (a) crown thinning and dieback of *Quercus glauca* in forest stand F03 (2337 m a.s.l.; *P. cinnamomi* A2); (b) crown dieback and mortality of *Castanopsis acuminatissima* and *Neolitsea poilanei* in forest stand F05 (2249 m a.s.l.; *P. attenuata*, *P. castaneae*, *P. cinnamomi* A2); (c,d,f) severe crown dieback and mortality of *C. acuminatissima* in a swampy depression of forest stand F06 close to stream R01 (2083 m a.s.l.; *P. castaneae*, *P. cinnamomi* A2, *P. gregata*); (f) the white flowers and young leaves in the crowns of *C. acuminatissima* belong to the epiphytic *Rhododendron leptocladus*; (e) bark lesion with staining of the underlying cambium caused by *P. cinnamomi* A2 on a surface root of *C. acuminatissima* in forest stand F07; (g) mortality of *Dysoxylum juglandis* in suptropical humid evergreen forest stand F15 in Ba Vi National Park (1108 m a.s.l.; *P. sp. attenuata-like 3*).

For the isolation of *Phytophthora* spp. from the 16 rivers and streams, an in-situ baiting technique was used [10,11]. Twelve of the 16 riparian baiting sites were located inside or downstream of natural forests (Figure 2c). At each site, 15–20 non-wounded young leaves of the native *C. indica*, *Citrus sinensis*, *L. bacgangensis*, *Q. glauca*, and, in some cases, *Carpinus sp.*, *C. hodginsii*, *Cinnamomum iners*, *Dipterocarpus alatus*, *Prunus sp.*, *Q. gilva* and *A. mangium* were placed as baits in a 25 × 30 cm raft, prepared using
fly mesh and styrofoam, and the raft put to float at a place where water flow was calm. The rafts were collected after 2–3 days. In addition, in 2017 freshly fallen leaves of different tree species and flowers of *Rhododendron arboreum* and *R. leptocladus* were collected from forest streams R01, R02, R10 and R11. Baiting leaves and the collected fallen leaves and flowers were washed in distilled water and blotted dry on filter paper. Five to ten pieces (approximately 2 × 2 mm) were cut from the margins of each watersoaked or necrotic lesion of each leaf or flower, blotted on filter paper and plated onto PARPNH agar.

All Petri dishes with plated leaf, flower or bark pieces were incubated at 20 °C in the dark and repeatedly examined under the stereo microscope at ×20 for *Phytophthora*-like hyphae after 12–48 h. Pure cultures were obtained by transferring single hyphal tips from the edge of the colonies onto V8A. Stock cultures were maintained on carrot agar (CA) [45] at 10 °C in the dark.

2.2. Molecular Identification of Isolates

For all *Phytophthora* isolates obtained in this study mycelial DNA was extracted from one-week old V8A cultures. Total DNA was extracted using the Phire Plant Direct PCR Kit (Thermo Fisher Scientific Inc., Waltham, MA USA) following the manufacturer’s instructions. DNA was stored at −20 °C until further use. For all isolates the region spanning the internal transcribed spacer (ITS1-5.8S-ITS2) region of the ribosomal DNA was amplified using primer-pairs ITS1/ITS4 or ITS5/ITS4 [29,46]. For representative isolates of several known and all putative new species the mitochondrial **cox1** gene was amplified with both primer-pairs COXF4N/COXR4N and FM84/FM83 [47,48]. The PCR reaction mixture and the amplification conditions for ITS and **cox1** were according to [29,47,48]. PCR consumables were provided by Thermo Fisher Scientific. PCR products were purified and sequenced by GATC Biotech (Konstanz, Germany) and by Source Bioscience (Nottingham, UK) in both directions with the primers used for PCR amplification.

Sequences were edited using Geneious (Version 11.1.2, Biomatters Ltd., Auckland, New Zealand). Heterozygous sites observed were labelled according to the IUPAC coding system. Consensus sequences were aligned using the CLUSTAL W algorithm. The consensus sequences were subjected to an NCBI BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/) and to a blast search in a local database containing sequences of ex-type isolates or key isolates from published studies to identify the closest related sequences. Isolates were assigned to a species when sequence identities were above a 99% cut-off in respect to those of ex-type isolates or key isolates. ITS and **cox1** sequences from representative isolates of all known and all putative new *Phytophthora* species obtained in this study were deposited at GenBank and accession numbers are given in Supplementary Table S1.

2.3. Classical Identification of Isolates

Colony growth patterns of 7-d-old cultures grown at 20 °C in the dark on V8A, malt-extract agar (MEA; Oxoid Ltd., Basingstoke, UK) and PDA [21] and morphological characters of sporangia, oogonia, antheridia, chlamydospores, hyphal swellings, and aggregations were compared with isolates from known species and with species descriptions in the literature.

Sporangia production and microscopic examinations and measurements of morphological structures at ×400 were according to [21,22] using a compound microscope (Zeiss Axioimager.Z2, Carl Zeiss AG, Oberkochen, Germany), a digital camera (Zeiss Axioicam ICc5) and a biometric software (Zeiss ZEN). Self-sterile isolates were paired on both V8A and CA with known A1 and A2 mating type tester strains of *P. cinnamomi*, *P. ×cambivora* and *P. ×heterohybrida* (isolates with non-papillate sporangia) or *P. botryosa*, *P. colocasiae* and *P. meadii* (isolates with papillate sporangia). All pairings were examined after 4–6 weeks incubation at 20 °C in the dark in order to determine whether self-sterile isolates are heterothallic or sterile and to which mating type heterothallic isolates belong [21]. All isolates are preserved in the culture collections maintained at Mendel University and Forest Research.
3. Results

In total, 943 oomycete isolates, including 652 Phytophthora isolates and 291 isolates from other oomycete genera, were obtained from forest stands (Table 1) and river systems (Table 2) in Vietnam. The Phytophthora isolates belonged to 13 described species, five informally designated taxa and 21 previously unknown taxa. From the other oomycete genera, 122 isolates were identified to species level. They could be assigned to the recently described Nothophytophthora vietnamensis (26 isolates), Phytophthora vexans sensu lato (63 isolates from 14 partly highly different haplotypes), four other known species and three novel taxa of Phytophthora (16 isolates), two described species and six novel taxa of Pythium (17 isolates) and one novel taxon of Elongisporangium. The remaining 169 isolates, which were not identified to species level, belonged to Phytophthora (161 isolates), Pythium (7 isolates) and Saprolegnia (1 isolate), respectively. GenBank accession numbers of ITS sequences of representative isolates of all oomycete taxa and of cox1 sequences of representative isolates of most Phytophthora taxa are given in Supplementary Table S1. Detailed descriptions of morphological characteristics, morphometric and temperature-growth data, and multigene phylogenies for all new Phytophthora species will be presented in separate publications.

3.1. Phytophthora Diversity in Natural and Semi-Natural Forest Stands

In 20 forest stands (80%), 20 Phytophthora taxa were isolated from 58 of the 91 soil samples (63.7%) taken from the rhizosphere of 52 of the 64 woody plant species sampled (81.3%); from the root lesion of C. acuminatissima in stand F07; and from all four freshly fallen Rhododendron leaves collected from the ground in stand F11: P. attenuata, P. castaneae, P. chlamydospora, P. cinnamomi, P. gregata, P. heveae, P. parvispora, P. ramorum, P. citricola VII, three new species related to P. attenuata, three new species from the ‘Phytophthora citricola complex’, three new species related to P. botryosa and P. meadii, and one new species related to P. multivesiculata and another to P. tropicalis, respectively (Table 1). From 29 of the 35 Phytophthora-negative soil samples, several known and previously unknown Phytophthora or Pythium spp. were isolated (Table 1). The only forest site from which no oomycete isolates could be obtained was subalpine Rhododendron scrub at 2903 m altitude near the Fansipan peak (F01).

Phytophthora cinnamomi, Clade 7c, was isolated from 26 of 66 rhizosphere soil samples (39.4%) collected from 27 of the 50 tree and shrub species (54%) in 13 of the 17 mountainous forest stands sampled (76.5%), making it the most widespread and common Phytophthora species above 700 m altitude. The A2 mating type of P. cinnamomi was present in 11 forest stands with an altitudinal amplitude ranging from 713 to 2337 m above sea level (a.s.l.). In contrast, the A1 mating type was only found in four forest stands located between 1108 and 2636 m a.s.l. (Figure 1; Table 1). Both mating types co-occurred in one stand in Hoàng Liên NP and another in Ba Vì NP. Interestingly, in Hoàng Liên NP, the A1 mating type was present in the upper montane Rhododendron forest F02 at 2636 m a.s.l. and in the lower montane stands F11 and F12 at 1900 m a.s.l., but was not detected in the eight forest stands (F03–F10) sampled between 2337 and 2022 m a.s.l., all highly infested by the A2 mating type. The latter was also isolated from a bark lesion on a surface root of C. acuminatissima of stand F11; and from all four freshly fallen Rhododendron leaves collected from the ground in stand F11: P. attenuata, P. castaneae, P. chlamydospora, P. cinnamomi, P. gregata, P. heveae, P. parvispora, P. ramorum, P. citricola VII, three new species related to P. attenuata, three new species from the ‘Phytophthora citricola complex’, three new species related to P. botryosa and P. meadii, and one new species related to P. multivesiculata and another to P. tropicalis, respectively (Table 1). From 29 of the 35 Phytophthora-negative soil samples, several known and previously unknown Phytophthora or Pythium spp. were isolated (Table 1). The only forest site from which no oomycete isolates could be obtained was subalpine Rhododendron scrub at 2903 m altitude near the Fansipan peak (F01).
Phytophthora parvispora was exclusively found in stand F15 in Ba Vi NP where it co-occurred with both mating types of its closest relative P. cinnamomii (Table 1). Compared to the ex-type of P. parvispora (CBS 132772; KC478667), the three isolates had identical cox1 sequences and differed in ITS by one heterozygous site at position 73 (Y instead of T) (Supplementary Table S1). In mating tests with A1 and A2 tester strains of P. cinnamomii, all isolates were sterile.

Phytophthora attenuata from Clade 7a and three previously unknown taxa closely related to P. attenuata were recovered from five forest stands in Hoà Linh NP and Ba Vi NP (Table 1). The individual taxa from this ‘P. attenuata complex’ differed in their altitudinal amplitude and geographical distribution (Figure 1; Table 1). Phytophthora attenuata, P. sp. attenuata-like 1 and P. sp. attenuata-like 2 were only found in Hoà Linh NP. Most widespread was P. sp. attenuata-like 1 which was isolated from the rhizosphere of five tree species in three stands located between 2249 and 2636 m a.s.l., followed by P. attenuata (three tree species in two stands; 1910–2249 m a.s.l.) and P. sp. attenuata-like 2 (2 tree species in 1 stand; 1910 m a.s.l.). In contrast, P. sp. attenuata-like 3 was exclusively found between 713 and 1108 m altitude in two of the three forest stands sampled in Ba Vi NP where it was associated with six tree species (Table 1). The ITS and cox1 sequences of P. attenuata isolates from Vietnam differed from the ex-type isolate (CBS 141199; GenBank nos. KU517154 and KU517148) and other isolates of P. attenuata from Taiwan at 0–1 and 0–5 positions. Phytophthora sp. attenuata-like 1, P. sp. attenuata-like 2 and P. sp. attenuata-like 3 showed differences to P. attenuata in ITS at 0–1, 1–2 and 2–3 positions, respectively, and in cox1 at 6–8, 9–11 and 6–8 positions, respectively. The cox1 sequences of the three new taxa differed from each other at 8–17 positions. Heterozygous sites were present in the ITS sequences of all isolates of P. sp. attenuata-like 2 (R at position 184) and most isolates of P. sp. attenuata-like 3 (Y in position 54; K in position 152). The ITS sequence of one isolate of P. sp. attenuata-like 1 from stand F05 contained seven heterozygous sites possibly suggesting hybrid origin.

Phytophthora castaneae from Clade 5 showed a similar altitudinal (1108–2242 m a.s.l.) and geographical distribution to P. cinnamomii (Figure 1; Table 1). It was isolated from the rhizosphere of 13 tree species from the genera Castanopsis, Lithocarpus, Neolitsea, Meliosma, Illicium and Rhododendron in seven stands in Hoà Linh NP and Ba Vi NP, and C. hodginsii in stand F14 on Sau Chua mountain where it was the only Phytophthora species recovered (Table 1). The ITS sequences of all isolates from Hoà Linh NP and several isolates from Ba Vi NP matched the ex-type of P. castaneae (ICMP 19434; GenBank no. KP295319). However, several isolates from Ba Vi NP had a unique polymorphism at position 54 (A or R instead of G) while all isolates from Sau Chua mountain were characterised by having a unique polymorphism at position 590 (A instead of G). The cox1 sequences of 15 isolates from the seven stands constituted six haplotypes which differed from the ex-type isolate (KP295234) by 0–1 bp. Interestingly, all four tested isolates from Sau Chua mountain had a unique polymorphism at position 421 (A instead of G). Five of the six tested P. castaneae isolates from stand F15 in Ba Vi NP shared a T at position 369 with P. heveae isolates from the same stand and with the P. heveae ex-type (CBS296.29; GenBank nos. HQ643238 and KP295326) whereas P. castaneae isolates from the other stands and the P. castaneae ex-type have a C at this position. Compared to P. castaneae, the other Clade 5 species found in this survey, P. heveae, had a lower altitudinal amplitude. Phytophthora heveae was isolated from the rhizosphere of 10 tree species in the subtropical lower montane stands F15 and F16 in Ba Vi NP and in four tropical lowland rainforest stands in Cuc Phuong NP, Bù Gia Mập NP and Côn Đảo NP (Figure 1; Table 1). Both Clade 5 species only co-occurred in stand F15. The ITS sequences of all P. heveae isolates (Table S1) matched the ex-type of P. heveae. The cox1 sequences of all isolates differed from the ex-type (GenBank no. KP295239) at position 536 (T instead of C). Isolates from Cuc Phuong NP and Bù Gia Mập NP had unique polymorphisms at positions 30 (C instead of A) and 390 (A instead of T), respectively. The morphology of all isolates of P. castaneae and P. heveae was in accordance with the original descriptions [2].
Table 1. Location, altitude, geological substrate and vegetation of 25 forest sites sampled in spring 2016 and 2017 in Vietnam, sampled tree species and *Phytophthora* and other oomycete taxa isolated.

| Site no. | GPS Coordinates | Altitude (m a.s.l) | Location | Geological Substrate | Vegetation               | Sampled Tree Species (no. of *Phytophthora*-Positive/ Sampled Trees) | Phytophthora and Nothophytophthora spp. (no. of Positive Samples) |
|----------|-----------------|--------------------|----------|----------------------|--------------------------|---------------------------------------------------------------------|------------------------------------------------------------------|
| F01      | N22 18.466      | 2903               | Fansipan, Hoàng Liên National Park (NP) | Triassic schists and sandstones | Subalpine Rhododendron scrub | Rhododendron spp. (0/3)                                             | -                                                                |
| F02      | N22 19.194      | 2636               | Fansipan, Hoàng Liên National Park (NP) | Triassic schists and sandstones | Upper montane Rhododendron ('Elfin') cloud forest | Rhododendron arboresum, mix from 3 trees with dieback (DB) (1/1) | ATT1 (1), CIN A1 (1)                                           |
| F03      | N22 19.563      | 2337               | Hoàng Liên NP | Triassic schists and sandstones | Montane evergreen cloud forest | Quercus glauca, DB (2/2)                                          | CIN A2 (2)                                                        |
| F04      | N22 19.670      | 2242               | Hoàng Liên NP | Triassic schists and sandstones | Montane evergreen cloud forest | Meliosma henryi (1/1) Betula alnoides & Elaeocarpus japonicus (1/1) Castanopsis acuminatissima, mix from 2 trees, DB (1/1) C. acuminatissima with DB & Acer campbellii (1/1) | ATT1 (1), CIN A2 (1) e ATT1 (1), CIN A2 (1) |
| F05      | N22 19.786      | 2249               | Hoàng Liên NP | Triassic schists and sandstones | Montane evergreen cloud forest | Neolitsea polani, DB (3/3) C. acuminatissima mix from 3 trees, DB (1/1) Illicium griffithii & C. acuminatissima, DB (1/1) | ATT1 (3), CIN A2 (3) ATT1 (1), CIN A2 (1) CAS (1) |
| F06      | N22 20.127      | 2083               | Hoàng Liên NP | Triassic schists and sandstones | Montane evergreen cloud forest | C. acuminatissima, DB (2/2) M. henryi & A. campbellii (1/1) M. henryi & Neolitsea merilliana (1/1) | CIN A2 (2), GRE (1), CAS (1) d GRE (1) CIN A2 (1), MuV1 (1) |
| F07      | N22 20.430      | 2010               | Hoàng Liên NP | Triassic schists and sandstones | Montane evergreen cloud forest | Illicium taiti & Rhododendron sinofalcomeri (1/1) C. acuminatissima, DB, necrotic root lesion (1/1) | CAS (1) CIN A2 (1) |
| F08      | N22 20.331      | 2006               | Hoàng Liên NP | Triassic schists and sandstones | Montane evergreen cloud forest | Casuaria annamensis (1/1) Acer oblongum, mix from 2 trees (0/1) | CIN A2 (1) - e                                               |
| F09      | N22 20.565      | 2010               | Hoàng Liên NP | Triassic schists and sandstones | Montane evergreen cloud forest | C. acuminatissima (0/1) Q. glauca (0/2) | - e                                                            |
| F10      | N22 20.632      | 2022               | Hoàng Liên NP | Triassic schists and sandstones | Montane, evergreen cloud forest | Neolitsea polycarpa, mix from 3 trees, DB (1/1) N. polycarpa, Symplocos pseudobarberina & Beilschmiedia roxburghiana (1/1) | CIN A2 (1) |
| F11      | N22 21.026      | 1910               | Hoàng Liên NP | Triassic schists and sandstones | Montane, evergreen broadleaved forest | A. oblongum & Symplocos dryophila (2/2) C. acuminatissima DB, Ilex lesseneri & Eurya annamensis (1/1) R. arboresum (1/1) | ATT (1), ATT2 (1), CIN A1 (2), CIN A2 (1), CIN A2ho (1) e CIN A2 (1), CAS (1) CHL (1), RAM A1 (1) |
| Site no. | GPS Coordinates | Altitude (m a.s.l) | Location | Geological Substrate | Vegetation | Sampled Tree Species (no. of Phytophthora-Positive/Sampled Trees) | Phytophthora and Nothophytophthora spp. (no. of Positive Samples) |
|---------|-----------------|--------------------|----------|----------------------|------------|---------------------------------------------------------------|---------------------------------------------------------------|
| F12     | N22 20.909      | 1895               | Hoang Liên NP | Triassic schists and sandstones | Montane, evergreen broadleaved forest | *Acer oliverianum*, *Erythroxyca caulerici* & *Symplacos quillimini* (1/1) | CIN A1 (1) |
|         | E103 46.199     |                    |          |                      |            | *Quercus glauca* (1/1)                                       | CIN A1 (1) |
| F13     | N22 21.090      | 1717               | Xin Chai mountain | Triassic schists and sandstones | Montane *Alnus* forest on steep loamy slope | *Alnus nepalensis* (2/3)                                    | CIT VII (1), MEA1 (1), xTRO2 (1), VIE |
| F14     | N22 22.168      | 1367               | Sau Chua mountain | Triassic schists and sandstones | Montane *Chamaecyparis-Quercus* forest | *Chamaecyparis hodginsii* (7/9)                              | CAS (7) |
| F15     | N21 3.699       | 1108               | Ba Vi National Park (NP) | Triassic schists and sandstones and porphyrites | Suptropical humid evergreen forest | *Castanopsis chinensis* (2/2) *C. chinensis* & *Beilschmiedia fordii* (1/1) *Dysoxylum jaglans* *DB* (1/1) *Eberhardtia tonkinesis* *Antidesma* sp. & *Jasminum* sp. (0/1) *Eurya japonica* & *Nepheleium lappaceum* (1/1) *Lithocarpus bucgangensis* (1/1) *Lithocarpus pseudosandaicus* (0/1) *Machilus bonii* (1/1) *Magnolia annamensis* (1/1) *Q. glauca*, mix from 3 trees (1/1) *Vernicia montana* & *Antidesma* sp. (1/1) | ATT3 (1), CAS (1), CIN A1 (1) |
|         | E105 21.733     |                    |          |                      |            |                                                              | CAS (1), HEV (1), PAR (1) |
| F16     | N21 04.455      | 807                | Ba Vi NP | Triassic schists and sandstones and porphyrites | Suptropical humid evergreen forest | *Caryodaphnosia baviensis* (0/2) *Lithocarpus bucgangensis* (1/1) *Melorea arnottiana* (1/1) *Phoebe petetotii*, *Machilus thumbergii* & *Claxylon indicum* (1/1) | ATT3 (1), CAS (1), CIN A1 (1) |
|         | E105 21.810     |                    |          |                      |            |                                                              | HEV (1) |
| F17     | N21 04.587      | 713                | Ba Vi NP | Triassic schists and sandstones and porphyrites | Suptropical humid evergreen forest | *Alsodeaphne velutina* & *Litsea brevipetiola* (1/1) *Bischofia javanica* & *Litsea monophylla* (0/1) *C. chinensis* (1/1) *Castanopsis tonkinensis* (1/1) *Q. glauca* (0/1) | ATT3 (1), CIN A2 (1) |
|         | E105 22.016     |                    |          |                      |            |                                                              | CIN A1 (1) |
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**Table 1. Cont.**

| Site no. | GPS Coordinates | Altitude (m a.s.l.) | Location | Geological Substrate | Vegetation | Sampled Tree Species (no. of Phytophthora/Positive/ Sampled Trees) | Phytophthora and Nothophytophthora spp. (no. of Positive Samples) |
|----------|-----------------|--------------------|----------|----------------------|------------|---------------------------------|---------------------------------|
| F18      | N20 20.876 E105 35.793 | 392 | Cuc Phuong National Park (NP) | Triassic limestones | Tropical evergreen lowland rainforest | C. javensis & Litsea robusta (0/1) | - e |
|          |                 |     |                       |                     |            | Dracantonium dupperanum, mix from 2 trees (0/1) | - e |
|          |                 |     |                       |                     |            | Saraca dives, mix from 2 trees (1/1) | HEV (1) e |
| F19      | N20 20.779 E105 36.099 | 356 | Cuc Phuong NP | Triassic limestones | Tropical evergreen lowland rainforest | Allophylus cobbe, mix from 2 trees (0/1) | - e |
|          |                 |     |                       |                     |            | D. duperanum & S. dives (0/2) | - e |
| F20      | N20 20.366 E105 36.452 | 318 | Cuc Phuong NP | Triassic limestones | Tropical evergreen lowland rainforest | A. coxei, Ficus sp., Merremia boissiana & Homalium sp. (1/1) | MEA2 (1) e |
|          |                 |     |                       |                     |            | S. dives (0/2) | - e |
| F21      | N20 19.755 E105 36.979 | 267 | Cuc Phuong NP | Triassic limestones | Tropical evergreen lowland rainforest | Anogeissus acuminata (0/1) | CIT X (1) e |
|          |                 |     |                       |                     |            | A. acuminata & Tactrostiphis macrophylla (1/2) | - |
| F22      | N20 18.963 E105 38.101 | 264 | Cuc Phuong NP | Triassic limestones | Tropical evergreen lowland rainforest | C. javensis (0/1) | - e |
|          |                 |     |                       |                     |            | C. javensis & S. dives (0/1) | - e |
|          |                 |     |                       |                     |            | S. dives, mix from 2 trees (0/1) | - e |
| F23      | N12 06.326 E107 09.396 | 417 | Bù Giá Mỹ National Park | Quaternary alluvial sediments | Tropical evergreen lowland rainforest | Dypterocarpus alatus, Alphansus tripheya, Hopea odorata & Dalbergia oliveri (1/1) | HEV (1) e |
| F24      | N9 13.645 E105 57.330 | 4 | U Minh Ha National Park | Quaternary peat | Tropical lowland peat forest | Melaleuca cafuati (0/3) | - k |
| F25      | N8 40.621 E106 34.836 | 55 | Cồn Đảo National Park, Cồn Lôn island | Rhyolite and diorite | Tropical evergreen lowland rainforest | Chukrasia tabularis (0/1) | - e |
|          |                 |     |                       |                     |            | A. tripheya, C. tabularis (1/1) | CIT XI e |
|          |                 |     |                       |                     |            | Leucaena leucophylla, Canarium album & Hopea odorata (1/1) | BOT2 e |
|          |                 |     |                       |                     |            | H. odorata, C. album, D. alatus (1/1) | HEV L |

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* ATT = P. attenuata, ATT 1 = P. attenuata-like 1, ATT 2 = P. attenuata-like 2, ATT 3 = P. attenuata-like 3, BOT2 = P. sp. botryosa-like 2, CAS = P. castaneae, CHL = P. chlamydothora, CIN = P. cinnamoni, CIT VII = P. citricola VII, CIT IX = P. citricola IX, CIT X = P. citricola X, CIT XI = P. citricola XI, CRE = P. gegeat, HEV = P. heveae, MEA1 = P. sp. meadii-like 1, MEA2 = P. sp. meadii-like 2, MUV1 = P. sp. multivesiculata-like 1, PAR = P. parvispora, RAM = P. ramarum; TRO2 = P. sp. tropicalis-like 2, VIE = Nothophytophthora vietnamensis. b Mating types: A1 = forming oogonia only in dual cultures with A2 tester strains; A2 = forming oogonia only in dual cultures with A1 tester strains; A2ho = forming oogonia in dual cultures with A1 tester strains and in ageing single cultures. c Pythium senticosum also isolated. d Pythomyctrium sp. also isolated. e Pythomyctrium vexans s.l. also isolated. f Pythomyctrium sp. 1 PB-2013 also isolated. g Fallen leaves collected from the ground. h Pythium intermedium also isolated. i Pythium sp. conidiophorum-like also isolated. j Pythomyctrium chaumagrophus also isolated. k Pythomyctrium cucurbitacearum also isolated. l Pythomyctrium vexans also isolated. m Pythomyctrium sp. Cồn Đảo also isolated.
Table 2. Location and altitude of the 16 riparian sites sampled in spring 2016 and 2017 in Vietnam and *Phytophthora* and other oomycete taxa isolated.

| Site no. | GPS Coordinates       | Altitude (m a.s.l) | River; Province                  | Location of Catchment and Vegetation                                                                 | Sampling Method a          | *Phytophthora* and *Nothophytophthora* spp. b,c |
|----------|-----------------------|--------------------|----------------------------------|------------------------------------------------------------------------------------------------------|-----------------------------|-----------------------------------------------|
| R01      | N22 20.127 E103 46.782 | 2083               | Forest stream 1; Lào Cai         | Hoàng Liên NP; subalpine and montane Rhododendron scrub and forests, montane broadleaved forests        | Baiting raft               | CAP, xHET A1, xHET A1ho, MUV1, RAM A1 i,e    |
|          |                       |                    |                                  |                                                        | Fallen leaves/flowers       | CIT VII, RAM A1, SYL2, VIE                  |
| R02      | N22 20.440 E103 46.576 | 2007               | Forest stream 2; Lào Cai         | Hoàng Liên NP; subalpine and montane Rhododendron scrub and forests, montane broadleaved forests        | Baiting raft               | RAM A1, SYL2                                |
|          |                       |                    |                                  |                                                        | Fallen leaves/flowers       | GAL1, GAL2, MUV1, RAM A1, SYL2, VIE e,g,h   |
| R03      | N22 21.046 E103 46.273 | 1913               | Forest stream 3, tributary of forest stream 5; Lào Cai | Hoàng Liên NP; montane broadleaved forests                                                             | Baiting raft               | CHL, CIT VII, RAM A1, RAM A2, SYL2 e          |
| R04      | N22 21.029 E103 46.317 | 1904               | Forest stream 4, tributary of forest stream 5; Lào Cai | Hoàng Liên NP; montane broadleaved forests                                                             | Baiting raft               | CHL, xHET A1, RAM A1, RAM A2, SYL2, SYL3 l   |
| R05      | N22 20.906 E103 46.197 | 1895               | Forest stream 5, Gold river, downstream of R03, R04, R06-R08; Lào Cai | Hoàng Liên NP; montane broadleaved forests                                                             | Baiting raft               | CHL, CIT VII, xHET A1, RAM A1, SYL2 e         |
| R06      | N22 20.911 E103 46.199 | 1896               | Forest stream 6, tributary of forest stream 5; Lào Cai | Hoàng Liên NP; montane broadleaved forests                                                             | Baiting raft               | CHL, xHET A1 h,i                            |
| R07      | N22 20.902 E103 46.261 | 1912               | Forest stream 7, tributary of the Gold river; Lào Cai | Hoàng Liên NP; montane broadleaved forests                                                             | Baiting raft               | xHET A1, RAM A1                             |
| R08      | N22 20.904 E103 46.259 | 1911               | Forest stream 5, Gold river; Lào Cai | Hoàng Liên NP; montane broadleaved forests                                                             | Baiting raft               | CIT VII, SYL2, SYL3                         |
| R09      | N22 18.597 E103 52.426 | 1013               | Muong Hao River; Lào Cai         | Hoàng Liên NP; subalpine and montane Rhododendron scrub and forests, montane broadleaved forests, rice fields | Baiting raft               | KEL, PSC, xKUN                              |
| R10      | N22 19.372 E103 49.780 | 1193               | Forest stream 9, Cat Cat River; Lào Cai | Hoàng Liên NP; montane broadleaved forests                                                             | Baiting raft               | CAP, CIT VII, CIT VIII, SYL1, SYL3 l         |
|          |                       |                    |                                  |                                                        | Fallen leaves/flowers       | CHL, PSC, QUI, SYL 1, SYL3, RAM A1, VIE l,j,k|
| R11      | N22 22.230 E103 52.615 | 1308               | Forest stream 8; tributary of Ngòi Đuội River; Lào Cai | Sau Chua mountain; *Chamaecyparis hodginsii* forest F24; broadleaved mountain forests and *Cunninghamia lanceolata* plantations | Baiting raft               | BIT, CIT VII, CIT IX, MAC, QUI, SYL3         |
|          |                       |                    |                                  |                                                        | Fallen leaves/flowers       | CIT IX, KEL, RAM A1 l                       |
| Site no. | GPS Coordinates | Altitude (m a.s.l) | River; Province | Location of Catchment and Vegetation | Sampling Method | Phytophthora and Nothophytophthora spp. b,e |
|---------|-----------------|-------------------|----------------|--------------------------------------|---------------|------------------------------------------|
| R12     | N22 16.787      | 63                | Red River (Sông Hồng); Lào Cai | Large catchment in N-Vietnam and Yunnan; subalpine and montane Rhododendron scrub and forests, montane broadleaved forests, forest plantations, rice fields, horticulture | Baiting raft  | ×KUN, ×PER4, ×VIR                       |
| R13     | N21 03.275      | 59                | Stream 9; Hanoi | Ba Vì NP; subtropical evergreen forests, rice fields | Baiting raft  | ×INS, ×GRE3, ×KUN, ×PER4, ×VIR e        |
| R14     | N21 3.261       | 60                | Stream 10, tributary of stream 9; Hanoi | Ba Vì NP; subtropical evergreen forests, rice fields | Baiting raft  | ×KUN, ×PER 4 i                          |
| R15     | N21 06.177      | 26                | Black River (Sông Đà); Hanoi | Large catchment in N-Vietnam and Yunnan; subalpine and montane Rhododendron scrub and forests, montane broadleaved forests, subtropical evergreen forests, forest plantations, rice fields, horticulture | Baiting raft  | DRE A1, ×VIR l,m                        |
| R16     | N21 01.576      | 26                | Stream 11; Hanoi | Forest plantations, rice fields, horticulture | Baiting raft  | ×PER4, ×VIR n                          |

a Baiting rafts were collected in March–April 2016; fallen leaves were collected in March 2017. b BIT = P. bitahaiensis-like, CAP = P. capensis, CHL = P. chlamydospora, CIT VII = P. citricola VII, CIT VIII = P. citricola VIII, CIT IX = P. citricola IX, DRE = P. drechsleri, GAL1 = P. gallica-like 1, GAL2 = P. gallica-like 2, KEL = P. sp. kelmania, MAC = P. macrochlamydospora, MUV1 = P. sp. multivesiculata-like 1, PSC = P. pseudocryptogea, QUI = P. sp. quininea-like, RAM = P. ramorum, SYL1 = P. sp. sylvatica-like 1, SYL2 = P. sp. sylvatica-like 2, SYL3 = P. sp. sylvatica-like 3, ×GRE3 = P. sp. ×Grenada 3-like, ×HET = P. ×heterozygilda, ×INS = P. sp. ×insolita-like, ×KUN = P. sp. ×kunnunara-like, ×PER4 = P. sp. ×Peru 4-like, ×VIR = P. sp. ×virginiana-like s.l., VIE = Nothophytophthora vietnamensis. c Mating types: A1, A2, A1ho (homothallic and stimulating oogonia formation in A2 tester strains). d Elongisporangium sp. Hoa Lien also isolated. e Unidentified Pythium sp. also isolated. f Pythophysium vexans aff. also isolated. g Pythium senticosum also isolated. h Pythium sp. ×ZSF0056-like also isolated. i Pythophysium sp. 1 PB-2013 also isolated. j Pythophysium litorale also isolated. k Pythium sp. CAL_2011f also isolated. l Pythium sp. 1_MNS-2013 also isolated. m Pythium sp. 2_ROH-2015 also isolated. n Pythophysium palingenes also isolated.
In total, nine previously unknown *Phytophthora* species from four of the five subclades within Clade 2 were detected in forest stands. From Clade 2a, *P. sp. meadii-like 1* was isolated from the montane *A. nepalensis* stand F13 at 1717 m a.s.l. on Xin Chai mountain, while *P. sp. meadii-like 2* was found in the tropical lowland rainforest stand F20 in Cuc Phuong NP (Figure 1; Table 1). The ITS sequences of all isolates of *P. sp. meadii-like 1* were identical except for one isolate with an extra T in position 11 and an A instead of a T in position 12 (Supplementary Table S1). This new taxon differed in the ITS from *P. meadii* (isolate P75; GenBank no. GU993903) at positions 137 and 632 which were shared with the ex-type isolate of *P. botryosa* (CBS586.69; GenBank no. HQ643151), and from *P. botryosa* at five positions (72, 152, 444, 460, 773) which were identical with *P. meadii*. In addition, all isolates of *P. sp. meadii-like 1* had a unique deletion at position 146. The ITS sequences of *P. sp. meadii-like 2* showed intraspecific variability at positions 11, 13, 22. Most isolates differed from *P. sp. meadii-like 1*, *P. meadii* isolate P75 and the ex-type isolate of *P. botryosa* by having four unique heterozygous positions (161, 444, 502, 713). In addition, *P. sp. meadii-like 2* showed in the ITS the same differences to *P. meadii* and *P. botryosa* as *P. sp. meadii-like 1*. The ITS sequences of both new taxa showed differences to the ex-type isolate of the recently described *P. mekongensis* from southern Vietnam (CBS135136; GenBank no. KC875838) at eight positions (152, 155, 163, 165, 166, 175, 179, 750). A third new taxon from Clade 2a, *P. sp. botryosa-like 2*, was exclusively isolated from the tropical lowland rainforest stand F25 on Côn Đảo island (Figure 1; Table 1). The ITS sequences of all isolates were identical to each other and differed from *P. botryosa* and *P. meadii* at four (72, 137, 161, 460) and five positions (152, 161, 444, 632, 773), respectively. In a 610 bp alignment of *cox1*, *P. sp. meadii-like 1*, *P. sp. meadii-like 2* and *P. sp. botryosa-like 2* differed from *P. meadii* (isolate p75; GU945489) at 14, 13 and 12 positions, respectively, and from *P. botryosa* (HQ261256) at 10, 9, and 8 positions, respectively. According to sequence analyses, the closest relatives of *P. sp. botryosa-like 2* were an isolate obtained in 1930 from *Cocos nucifera* in Sulavesi (CBS235.30) which differed in ITS (HQ643140) by five heterozygous positions and in *cox1* (HQ708214) at five positions, and an isolate of unknown origin which was obtained from a vanilla plant in 1928 (CBS238.28) and showed differences at five positions in both ITS (HQ643139) and *cox1* (HQ708213) of which three were heterozygous in ITS. All isolates of *P. sp. botryosa-like 2*, *P. sp. meadii-like 1* and *P. sp. meadii-like 2* produce caducous papillate sporangia with variable shapes and are heterothallic, exclusively belonging to mating type A1. Oospore abortion rates in mating tests with A2 tester strains of *P. meadii* and *P. botryosa* exceeded 95%.

From the montane *A. nepalensis* stand F13 a new *Phytophthora* species from Clade 2b was isolated which differed from the ex-type isolate of *P. tropicalis* (CBS434.91) in ITS (HQ643369) and *cox1* (HQ708417) at 5 and 7 positions, respectively, and is hence informally designated as *P. sp. tropicalis-like 2*. Similar to *P. tropicalis*, all isolates produce thickwalled chlamydospores and papillate sporangia. *Phytophthora* sp. tropicalis-like 2 differs from *P. tropicalis* [49] by producing sporangia which are only partially caducous and have shorter pedicels (24.7 ± 16.8 µm vs. > 50 µm) and shorter length/breadth (l/b) ratio (1.8 ± 0.3 vs. 1.8–2.4).

*Phytophthora citricola* VII, informally designated from a mountain forest in Taiwan [10], and another three new taxa from the ‘*P. citricola* complex’ in Clade 2c, informally designated here as *P. citricola* IX, *P. citricola* X and *P. citricola* XI, were isolated from the montane *A. nepalensis* stand F13, the subtropical evergreen forest stand F16 and the tropical lowland rainforest stands F21 and F25, respectively (Figure 1; Table 1). *Phytophthora citricola* VII, IX, X and XI differ from the authentic type of *P. citricola* s.s. (CBS295.29; ITS–FJ560913; *cox1*—KC855432) in the ITS (771 bp alignment) at 3, 3, 12 and 11 positions, and in *cox1* (1231 bp alignment) at 23, 19, 29, and 15 positions, respectively. Like other members of the *P. citricola* complex, the four new species are homothallic forming smooth-walled oogonia with paragynous antheridia. The sporangia of *P. citricola* VII and IX resemble those produced by other species from Clade 2c in being semipapillate, persistent and with exclusively external proliferation. In contrast, *P. citricola* X and XI produce mainly papillate sporangia with both external and, infrequently, also internal extended and nested proliferation. In addition, *P. citricola* X is distinguished from all known
related species by forming abundant catenulate hyphal swellings in water. *Phytophthora* citricola VII produces a high proportion of zoospores with a ring-like to oval coiling of both flagella ends.

From a swampy depression in the montane evergreen cloud forest F06 in Hoàng Liên NP, a previously unknown *Phytophthora* species from Clade 2e was isolated which is provisionally named as *P.* sp. multivesiculata-like 1. Its ITS and *cox1* sequences differ from the ex-type isolate of *P. multivesiculata* (CBS5345.96; HQ643288 and HQ708340) at eight and 38 positions, respectively. The ITS sequences of two yet undescribed species, *Phytophthora* sp. aquatilis (GenBank no. FJ666126) and *Phytophthora* sp. Costa Rica 5 (KC479200), show differences to *P.* sp. multivesiculata-like 1 at 8 and 6 positions, respectively. Like *P. multivesiculata*, *P.* sp. multivesiculata-like 1 is homothallic with aplerotic oospores and produces in water numerous catenulate hyphal swellings and both nonpapillate and semipapillate sporangia with external and internal proliferation. However, it can easily be distinguished from *P. multivesiculata* [50] by forming considerably larger sporangia (on av. 57.2 × 32.8 vs. 45 × 33 μm), larger oogonia (45 vs. 41 μm) with highly variable shapes ranging from globose, excentric or elongated with long tapering bases to comma-shaped, and exclusively amphiogenous antheridia.

Also in Hoàng Liên NP, *P. gregata* from Clade 6b was recovered from the rhizosphere of *Meliosma henryi* and *Neolitsea merilliana* in the montane evergreen cloud forest F06 while the other Clade 6b species *P. chlamydospora* and *P. ramorum* from Clade 8c were isolated from fallen leaves of *R. arboreum* collected from the forest ground in the montane, evergreen broadleaved forest F11 (Table 1).

Besides the recently described *Nothophytophthora vietnamensis* [51] which was isolated from the rhizosphere of *C. acuminitissima* and *Acer campbellii* in the montane evergreen cloud forest F04 and *A. nepalensis* in stand F13 on Xin Chải mountain, a range of *Pythium* and *Phytophthysium* species including *Py. intermedium*, *Py. senticosum*, *Ph. chamaehyphon*, *Ph. cucurbitacearum*, *Ph. sp.* 1 PB-2013, 14 haplotypes from the *Ph. vexans* complex and two previously unknown taxa, informally designated as *Py.* sp. conidiophorum-like and *Ph. sp.* Côn Đảo, were obtained from 15 forest stands (Table 1 and Supplementary Table S1).

### 3.2. *Phytophthora* Diversity in Natural Forest Streams and Rivers

Using rafts with leaves of *C. indica*, *C. sinensis*, *L. bacgangensis*, *Q. glauca*, and, less frequently, *Carpinus* sp., *C. hodginsii*, *Cinnamomum iners*, *Dipterocarpus alatus*, *Prunus* sp., *Q. ilicifolia* and *A. mangium* as in situ baits in all 16 rivers and streams tested, and freshly fallen leaves of different tree species and flowers of *R. arboreum* and *R. leptocladus* in four forest streams, seven known species (*P.* capensis, *P. chlamydospora*, *P. drechleri*, *P. macrochlamydospora*, *P. pseudocyptogea*, *P. ramorum*, *P. xheterohybrida*), five informally designated taxa (*P. citricola* VII, *P.* sp. kelmania, *P.* sp. ×insolita-like, *P.* sp. ×kununara-like, *P.* sp. ×virginiana-like s.l.) and 12 previously unknown taxa of *Phytophthora* were isolated (Table 2). The latter included *P.* sp. multivesiculata-like 1, two new species from the ‘*P. citricola complex’*, three and one new species related to the Clade 6 taxa *P.* sp. sylvatica and *P.* sp. bitahaiensis, respectively, three new species from Clade 9 and two new species related to *P. gallica* from Clade 10.

The *Phytophthora* communities in the 11 montane streams above 1000 m a.s.l. with a temperate climate were dominated by species belonging to Clades 2, 6, 7, and 8 whereas from the five lowland rivers with subtropical to tropical climate almost exclusively *Phytophthora* species from Clade 9 were obtained (Figure 1; Table 2).

In montane streams, the most widespread species was *P. ramorum* which could be recovered from seven of the eight forest streams above 1890 m altitude in the Fansipan area and in 8–12 km distance to these sites from stream R11 originating from the *C. hodginsii* forest F24 at Sau Chua mountain in 1300 m altitude. Both mating types were obtained with the A1 mating type occurring in eight streams and the A2 in two streams. In the latter (streams R03, R04) both mating types co-occurred (Table 2). In the two streams (R01, R02) sampled in both 2016 and 2017 only mating type A1 was isolated. The 65 *P. ramorum* isolates exhibited five slightly different ITS genotypes. Eight isolates from four streams (R02, R04, R05, R10) were identical to the ex-type isolate from Germany, which belongs to the EU1 lineage (CBS101553; HQ643339). The most common genotype (46 isolates) differed from the ex-type by
having a T instead of a Y at position 616, while three isolates from three streams (R01, R02, R10) had a C at this position. Four *P. ramorum* isolates from streams R02 and R03 were distinguished from the ex-type by being heterozygous at position 682 (R instead of G) while in one isolate from stream R03 both heterozygous positions occurred. For 43 isolates, representative for all eight streams, forest site F11 and both mating types, *cox1* was sequenced and compared to representative isolates of the four known *P. ramorum* lineages EU1, EU2, NA1, and NA2. In a 1240 bp long *cox1* alignment all but one of the Vietnamese *P. ramorum* isolates were identical and differed from four representative isolates of the North American NA2 lineage only at position 123. They differed from EU1 by 5 bp (positions 123, 808, 1141, 1156, 1202), EU2 by 5 bp (624, 966, 1035, 1156, 1240) and NA1 by 4 bp (123, 808, 1156, 1202), respectively. Isolate VN88 differed from the other 42 isolates by having a unique polymorphism at position 1228 (C instead of A). The morphological structures of all isolates were congruent with the original description of *P. ramorum* [52].

From Clade 2c, *P. capensis*, *P. citricola* VII, *P. citricola* VIII, and *P. citricola* IX were isolated from two, one, five, and one montane forest streams, respectively (Table 2). From stream R11, *P. capensis*, and *P. citricola* VII and VIII were obtained while in another two streams (R01, R10) two different species from the ‘*P. citricola* complex’ co-occurred. Compared to the ex-type isolate of *P. capensis* (P1819; ITS—GU191232; *cox1*—GU191275) from South Africa, the ITS sequences of the Vietnamese isolates were identical but their *cox1* sequences were separated in a 598 bp alignment by 9 bp (1.5%). *Phytophthora citricola* VIII differed from the authentic type isolate of *P. citricola* s.s. (CBS295.29; ITS—FJ560913; *cox1*—KC855432) and from *P. citricola* VII, IX, X and XI in ITS (771 bp alignment) at 2, 2–3, 4, 12 and 11 positions, and in *cox1* (1231 bp alignment) at 33, 26, 35, 42, and 37 positions. Being homothallic with paragynous antheridia and producing semipapillate sporangia of variable shapes, *P. citricola* VIII morphologically resembles other species from Clade 2c.

*Phytophthora* sp. multivesiculata-like 1 from Clade 2e was present in streams R01 and R02 which originate from a catchment area around forest stand F06 where this new taxon was also found.

From Clade 6b, which contains numerous predominantly aquatic *Phytophthora* species, *P. chlamydospora*, three new species informally designated as *P. sp. sylvatica*-like 1, 2 and 3, and another new species designated as *P. sp. bitahaiensis*-like were recovered from 8 of the 11 mountain streams (Table 2). Most common were *P. sp. sylvatica*-like 2 (six streams), *P. chlamydospora* (5 streams) and *P. sp. sylvatica*-like 3 (four streams). In five streams more than one Clade 6b species were found. In the ITS (844 bp alignment) and *cox1* (861 bp alignment), *P. sp. sylvatica*-like 1 differs from its closest relative *P. sp. forestsoil*-like from Taiwan (KU682574) at 2 and 7 positions, respectively, while *P. sp. sylvatica*-like 2 and 3 show differences to *P. sp. forestsoil*-like in ITS at 12–13 and 11–12 positions, respectively, and in *cox1* at 29 and 27 positions. *Phytophthora* sp. *sylvatica*-like 1 differs from *P. sp. sylvatica*-like 2 and 3 in ITS by 9–11 and 7–8 bp, respectively, and in *cox1* by 44 and 46 bp, respectively.

The latter two species can be distinguished in the ITS and *cox1* by differences at 2–4 and 11 positions, respectively. *Phytophthora* sp. *bitahaiensis*-like differs in the ITS from *P. sp. bitahaiensis* (isolate BHL1; KT183432) from a forest stream in Yunnan, China, at 4 positions. Unfortunately, for *P. sp. bitahaiensis* no *cox1* sequences are available. Similar to *P. sp. forestsoil*-like and many other aquatic Clade 6 species [10,21,53], *P. sp. sylvatica*-like 1, 2, and 3 and *P. sp. bitahaiensis*-like are sterile and form abundantly nonpapillate sporangia with internal nested and extended proliferation. All ten sequenced isolates from *P. chlamydospora* were identical in both the ITS and *cox1* and differed from the ex-type isolate of *P. chlamydospora* from the UK (P236; AF541900, MH136867) only by being in the ITS heterozygous at position 57 (R instead of G) while being identical to the ex-type in *cox1*.

From five mountain streams above 1890 m altitude, the recently described Clade 7a hybrid species *P. heterohybrida* was isolated. All 12 isolates differed in the ITS from the ex-type isolate from Taiwan (CBS141207; KU517151) at position 77 (Y instead T). In addition, three isolates from stream R01 were separated from the ex-type by being homozygous at position 428 (T instead of Y) and by having two unique heterozygous positions (656 and 748). In a 876 bp alignment of *cox1*, one isolate from stream R01 differed at three positions from the ex-type isolate (KU517145) and from all other Vietnamese and
Taiwanese isolates which were identical. Mating tests with A1 and A2 tester strains of \( P. \times \text{heterohybrid} \) from Taiwan showed that all isolates from Vietnam belonged to the A1 mating type. In addition, two isolates from stream R01 produced oogonia abundantly in single culture. The morphology of the ornamented oogonia, mostly two-celled amphigenous antheridia and nonpapillate sporangia of the Vietnamese isolates matched the original description of \( P. \times \text{heterohybrid} \) [22].

\textit{ Phytophthora pseudocryptogea } and \( P. \) sp. \( \times \) kelmania from Clade 8a were isolated from each two of the three lower montane streams R09-R11 (Table 2). The three isolates of \( P. \) pseudocryptogea from streams R09 and R10 differed in ITS (816 bp alignment) and \( \text{cox1} \) (672 bp alignment) from the Australian ex-type isolate (VHS16118; KP288376, KP288342) at three and one positions, respectively. The five isolates of \( P. \) sp. kelmania from streams R09 and R11 showed differences in ITS (841 bp alignment) and \( \text{cox1} \) (582 bp alignment) to isolate P10614 (HQ261691, HQ261438) from North America at 3, 4, and 5 positions, respectively. The morphology of all Vietnamese isolates of \( P. \) pseudocryptogea and \( P. \) sp. \( \times \) kelmania matched the descriptions in literature [54].

From forest stream R02 in 2007 m altitude two new species from Clade 10 were isolated from naturally fallen leaves. \textit{ Phytophthora } sp. \( \times \) gallica-like 1 and \( P. \) sp. \( \times \) gallica-like 2 were distantly related to \( P. \) gallica differing in a 885 bp alignment of the ITS from the ex-type isolate of the latter (CBS 111474 = GAL1; DQ286726) at 53 and 68 positions, respectively, while being separated from each other by 49 bp. Morphologically, both new species can easily be distinguished from the sterile \( P. \) gallica [55] by being homothallic forming smooth-walled oogonia with paragynous antheridia. \textit{ Phytophthora } sp. \( \times \) gallica-like 1 produces globose chlamydospores like \( P. \) gallica whereas \( P. \) sp. \( \times \) gallica-like 2 does not form chlamydospores.

From Clade 9a2, \( P. \) \( \times \) macrochlamydospora and a new species related to \( P. \) \( \times \) quininea, informally designated as \( P. \) sp. \( \times \) quininea-like, co-occurred in montane forest stream R11. Isolate VN1006 of \( P. \) \( \times \) macrochlamydospora differed in the ITS (816 bp alignment) and in \( \text{cox1} \) (670 bp alignment) from the Australian ex-type isolate (P10263; FJ801351, MH136923) at 2 and 3 positions, respectively. In accordance with the original description of \( P. \) \( \times \) macrochlamydospora [2], the Vietnamese isolate was sterile and produced large chlamydospores and semipapillate to non-papillate sporangia. The four isolates of \( P. \) sp. \( \times \) quininea-like were separated in the ITS and \( \text{cox1} \) from the ex-type isolate of \( P. \) \( \times \) quininea (CBS407.48 = P8488; HQ261660; AY564200 + HQ708386) from Peru by differences of 7 and 27 bp, respectively. Like \( P. \) \( \times \) quininea, \( P. \) sp. \( \times \) quininea-like produces non-papillate sporangia with internal and external proliferation, catenulate irregular hyphal swellings and thick-walled large chlamydospores. Interestingly, two of the four isolates were sterile while the other two isolates were homothallic like \( P. \) \( \times \) quininea [2] but can be distinguished from the latter by forming amphigenous instead of paragynous antheridia. Only one isolate of the Clade 9a1 hybrid taxon \( P. \) sp. \( \times \) kunnunara-like could be obtained from one lower montane stream (R09) in Hoàng Liên NP (Table 2).

In contrast to the montane streams, the \textit{ Phytophthora } communities in the five lowland rivers with subtropical to tropical climate were dominated by \textit{ Phytophthora } taxa from the high-temperature tolerant Clades 9a1 and 9a3. From Clade 9a1 potential hybrid isolates related to \( P. \) \( \times \) virginiana were obtained from the Red River, the Black River and two other streams (R13, R16) (Table 2). The potential hybrids differed in the ITS from the ex-type isolate of \( P. \) \( \times \) virginiana (46A2; KC295544) by having in total 10 heterozygous positions, with 1–8 heterozygous positions per isolate, which are partly not present in the three hybrid taxa \( P. \) sp. \( \times \) virginiana-like 1, 2, and 3 from Taiwan. Therefore, these Vietnamese isolates are informally designated as \( P. \) sp. \( \times \) virginiana-like sensu lato. \textit{ Phytophthora } sp. \( \times \) kunnunara-like was found in the Red River and the two streams originating from Bá Vi NP (R13, R14) (Table 2). Compared to \( P. \) sp. kunnunara from Western Australia, the ITS of the Vietnamese isolates had 10 heterozygous positions, with 1–8 heterozygous positions per isolate, which were only partly shared with Taiwanese isolates of \( P. \) sp. \( \times \) kunnunara-like (KU682602, KU682603). Another swarm of potential hybrid isolates was abundantly obtained from four of the five lowland streams. They differed in the ITS from \( P. \) sp. Peru 4 (KC479209) at 10 positions which were all heterozygous (1–6 per isolate) and are, hence, informally designated as \( P. \) sp. \( \times \) Peru 4-like. Finally, isolates of another new potential
hybrid taxon, *P. sp. ×Grenada 3-like* were recovered from stream R13 which were distinguished in the ITS from *P. sp. Grenada 3 (KC479208)* by having five instead of one heterozygous position. All isolates of *P. sp. ×Grenada 3-like, P. sp. ×kunnunara-like, P. sp. ×Peru 4-like and P. sp. ×virginiana-like* were in culture fast-growing, self-sterile and produced intercalary or laterally globose, club-shaped to irregular hyphal swellings, mostly globose thin-walled chlamydospores and non-papillate sporangia with internal nested and extended proliferation, typical features of aquatic Clade 9 species [56].

*Phytophthora* sp. ×*insolita-like* from Clade 9a3 was found in stream R13. Compared to the Taiwanese ex-type isolate of *P. insolita* (IMI288805; AF271222) the Vietnamese isolates showed differences in the ITS at eight positions of which four were heterozygous and mostly shared with Taiwanese isolates of *P. sp. ×insolita-like* (KU682601). Morphologically, all isolates from stream R13 were similar to both *P. insolita* and *P. sp. ×insolita-like* from Taiwan [2,10], producing in single culture smooth-walled oogonia without antheridia, thin-walled chlamydospores and non-papillate sporangia with internal nested and extended proliferation.

The only *Phytophthora* species recovered from a lowland stream (Black River) and not belonging to Clade 9 was *P. drechsleri* from Clade 8a. The isolates differed from the ex-type isolate of *P. drechsleri* (ATCC 46724 = 23J5; AF266798, MH620076) in the ITS at one position (136; Y instead of T) and in a 862 bp alignment of *cox1* at five positions. All three isolates belonged to the A1 mating type.

With 10, eight and six *Phytophthora* species, respectively, the montane forest streams R10, R11 and R02 harboured the highest diversity of *Phytophthora* species while the lowland rivers contained highly diverse assemblies of Clade 9 hybrids with almost all isolates being different from each other in the ITS.

*Nothophytophthora vietnemensis* was isolated from the montane forest streams R01, R02 and R10 in Hoàng Liên NP. In addition, the novel *Elongisporangium* sp. Hoàng Liên, *Phytophthium litorale, Ph. vexans* s.l., *Ph. sp. 1 PB-2013, Py. senticosum, Py. sp. CAL_2011f, Py. sp. 1_MNS-2013*, the previously unknown hybrid taxon *Py. sp. ×ZSF0056-like* and unidentified *Pythium* spp. were recovered from eight of the eleven mountain streams (Table 2 and Table S1). In four of the five lowland rivers, *Ph. palingenes, Ph. sp. 1 PB-2013, Py. sp. 1_MNS-2013, Py. sp. 2_ROH-2015* and unidentified *Pythium* spp. were found (Table 2 and Table S1).

### 3.3. Association between Phytophthora Presence in the Rhizosphere and Disease Symptoms

In the 20 *Phytophthora*-inhabited forests sampled, the majority of the 52 tree species from which *Phytophthora* species were recovered appeared generally healthy (Figure 2a–f). Symptoms indicative of Phytophthora root diseases were almost exclusively found in eight montane forest stands and were mainly restricted to tree species belonging to the Ericaceae, Fagaceae and Lauraceae (Figures 3 and 4).

In Hoàng Liên NP, scattered dieback of *Rhododendron arboreum* trees with presence of *P. cinnamomi* in *A1* and *P. sp. attenuata-like* 1 in the rhizosphere was observed in the upper montane *Rhododendron* cloud forest at 2636 m altitude. In the montane evergreen cloud forest F03 at 2337 m altitude, groups of mature *Quercus glauca* trees showed severe thinning and dieback of the crowns (Figure 3a) which was associated with presence of *P. cinnamomi* A2 in the rhizosphere. In contrast, in forest stand F12 at 1895 m altitude, infested by *P. cinnamomi* A1, all *Q. glauca* trees appeared healthy. In six of the nine montane evergreen forest stands sampled between 1895 and 2242 m altitude, *C. acuminate* and sometimes also *Neolitsea poilanci* and *N. polycarpa* showed severe thinning and dieback of the crowns and mortality (Figure 3b–d,f). Disease incidence was particularly high in the swampy depression sampled in stand F06 (Figure 3c,d,f). In seven and five of the nine stands, *P. cinnamomi* A2 and *P. castaneae*, respectively, were recovered from rhizosphere soil samples while *P. cinnamomi* A1, *P. attenuata, P. gregata, P. sp. attenuata-like* 1, *P. sp. attenuata-like* 2 and *P. sp. multivesiculata-like* 1 were only infrequently found (Table 1). On a visual examination root samples from three declining trees each of *C. acuminate* and *N. polycarpa*, all infested by *P. cinnamomi* A2 and/or *P. castaneae*, exhibited severe losses of lateral and fine roots and open callussing lesions on coarse roots (Figure 4a–d). In stand F07, *P. cinnamomi* A2 was isolated from a bleeding bark lesion on a surface root of a declining *C. acuminate* tree (Figure 3e).
Figure 4. Symptoms on root systems of declining Neolitsea polycarpa trees in the montane evergreen cloud forest F10 in Hoàng Liên National Park associated with presence of *P. cinnamomi* A2 in the surrounding soil; (a,b) severe losses of lateral roots and fine roots and open callussing lesions on coarse roots (arrows); (c,d) detailed view of the open callussing lesions on the coarse roots from Figure 4a.

In contrast to the montane forests of Hoàng Liên NP, in the three submontane (700–1100 m a.s.l.) stands sampled in the subtropical, humid evergreen forests of Ba Vì NP all tree species, including several species from the Fagaceae genera *Castanopsis*, *Lithocarpus* and *Quercus* and the Lauraceae genera *Litsea*, *Machilus* and *Phoebe*, were healthy despite the occurrence of both mating types of *P. cinnamomi* and a range of five other *Phytophthora* species, including *P. castaneae*, *P. heveae*, *P. parvispora*, *P. citricola* IX and *P. sp. attenuata-like 3*, in the soil. The only exception was a small patch dieback of *Dysoxylum juglans* with presence of *P. sp. attenuata-like 3* in the rhizosphere (Figure 3g).

In the five *Phytophthora*-infested tropical lowland rainforest stands in Cuc Phuong (F18, F20, F21), Bù Gia Mập (F23), and Côn Đảo (F25) National Parks, no symptoms suggestive of *Phytophthora* diseases were observed.

4. Discussion

Vietnam harbours an extremely diverse flora probably due to its heterogeneous geology, geomorphology and climates and its transitional position between the eastern Himalayas, Yunnan, and the Indomalaysian archipelago on the Asian continental shelf [39–41]. The latter enabled repeated immigrations of plant and most likely also fungal and oomycete species during various glacial periods followed by subsequent speciations and species radiations in the interglacials. A similar scenario was proposed earlier for Taiwan [10,36–38]. We have shown here that the floristic and environmental diversity of Vietnam is reflected by the high diversity of oomycete taxa. In this survey of 25 natural forests and 16 rivers 13 described species, five informally designated taxa and 21 previously unknown taxa of *Phytophthora*, together with *N. vietnamensis* and a range of seven described and ten undescribed species of *Elongisporium*, *Pythium* and *Phytophthium* were obtained. Considering the relatively limited
number and diversity of the sampled sites and ecosystem types it may be assumed that the true *Phytophthora* diversity of Vietnam is markedly higher. The finding of 20 *Phytophthora* taxa in 98 soil and four leaf samples from the 25 forest stands and an additional 15 *Phytophthora* taxa in 11 forest streams in Vietnam indicates a much higher diversity of forest *Phytophthoras* exists in Vietnam than occurs in Europe, the eastern US or the western US. In the latter areas, 39, 7, and 21 *Phytophthora* species, respectively, were detected in numerous surveys involving many more samples collected over much larger areas and a wider range of ecosystems [5,14,23,44,57–59].

The remote location of most sampled forest stands and forest streams in Vietnam, absence of introduced crop or tree species in the catchment areas and, apart from *P. cinnamomi* A2 in higher altitudes, the lack of association of *Phytophthora* with obvious disease symptoms suggest that most of the 35 forest *Phytophthora* species obtained are native to Vietnam. In contrast, only nine of the 32 *Phytophthora* species from European forests are considered indigenous [5,23,60]. The forest *Phytophthora* populations in Vietnam and Europe share only five species, *P. chlamydospora, P. cinnamomi* A2, *P. pseudocryptogea, P. ramorum* and *P. sp. kelmania*, while Vietnamese and North American forests have only *P. chlamydospora, P. cinnamomi* A2 and *P. ramorum* in common. Recent surveys in Taiwan, where floristic diversity is comparable to Vietnam, revealed a comparable *Phytophthora* diversity, with ten described and 17 previously unknown species from 30 forest stands and 25 streams [10,13].

Further, the *Phytophthora* communities revealed in Vietnam and Taiwan shared 12 taxa: *P. attenuata, P. capensis, P. castaneae, P. chlamydospora, P. cinnamomi* A1 and A2, *P. citricola* VII, *P. heveae, P. parvispora, P. xheterohybrida, P. sp. xinsolita-like, P. sp. xxkunnunara-like and P. sp. xvirginiana-like s.l.. In three areas in northern Yunnan, a Chinese province adjacent to northern Vietnam, eight *Phytophthora* species were isolated from streams running through sclerophyllous oak forests but only two of them, *P. chlamydospora* and *P. plurivora*, were recovered from forest soil samples [16]. The only *Phytophthora* species common to Vietnam and northern Yunnan were *P. chlamydospora* and *P. gregata*. In montane forests of the tropical island Hainan, located in the South China Sea close to Vietnam, six *Phytophthora* species were found [12] of which three species, *P. castaneae, P. cinnamomi* and *P. heveae*, also occurred in Vietnam. The lower *Phytophthora* diversities in the north Yunnan and Hainan surveys compared to Vietnam were most likely due to the smaller number of sites and forest types sampled and the use of different isolation techniques.

In recent years, an impressive diversity of both known and previously unknown *Phytophthora* species has been revealed from stream surveys in several countries, including the eastern and western USA, Chile, Australia, South Africa and Taiwan [10,11,14,17,18,44,61], as discussed previously [10]. By comparison the riparian *Phytophthora* communities identified here in Vietnam are remarkably rich, with seven described species, five informally designated taxa and 12 previously unknown taxa. Several montane streams with small catchments in the forests around the Fansipan harbour an unprecedented diversity of up to ten *Phytophthora* species per stream.

Interestingly, the most common *Phytophthora* species in Vietnamese forest soils, *P. cinnamomi, P. castaneae, P. heveae* and the four species from the ‘*P. attenuata* complex’, were never isolated from streams running through or originating from infested forests. Overall, the *Phytophthora* communities found in the forest soils (20 taxa) and in the streams (24 taxa) shared only four species, *P. chlamydospora, P. citricola* VII and IX and *P. sp. multivesiculata-like 1*. Similar differences between terrestrial and aquatic *Phytophthora* populations were observed in comparable studies in Europe, Chile, Taiwan, South Africa and the USA [10,11,18,44,58]. This is consistent with previous observations that most *Phytophthora* species are adapted either to a soilborne and root-infecting or aerial foliage-infecting lifestyle, or are aquatic saprotrophs that tend to be opportunistic pathogens [6,10,11,21,53,62]. Consequently, when sampling *Phytophthora* diversity in a diverse environment both soils and streams should be analysed using optimal baiting methods for each or metagenomic approaches based on high-throughput pyrosequencing of environmental DNA with *Phytophthora*-specific primers [19,63]. Ideally, because metagenomic analyses can sometimes result in false molecular operational taxonomic units (MOTUs),
Altitude had a strong influence on *Phytophthora* distribution. The ‘*P. attenuata* complex’, *P. castaneae* and *P. cinnamomi* occurred only in soils of submontane and montane forests above 700 m a.s.l. while *P. heveae* and most taxa from Clade 2a were restricted to forests below 1100 m altitude. The altitudinal influence on aquatic *Phytophthora* was even more pronounced. While the 11 montane streams above 1000 m altitude with a subtropical to temperate climate contained mainly species belonging to Clades 2c, 2e, 6b, 7a, and 8c, the *Phytophthora* communities in the five lowland rivers with subtropical to tropical climate were dominated by species and hybrids from the high-temperature tolerant Clades 9a1 and 9a3 [28]. Clade 9 species and hybrids were also most common in lowland streams in Taiwan and South Africa [10,18].

The results of this survey offer new insights into the origin of several invasive *Phytophthora* pathogens and of clades and subclades of *Phytophthora*. Most notably, the finding of the highly invasive, wide-host range pathogen *P. ramorum* in eight forest streams around the Fansipan and Sau Chua mountains with both A1 and A2 mating types present, together with an apparent absence of overtly visible disease symptoms on potentially susceptible Ericaceae, Fagaceae, or Lauraceae, susceptible genera where *P. ramorum* is damaging and introduced in Europe and North America [6,7,64], suggests an equilibrium between the pathogen and the north Vietnamese vegetation as a consequence of long term endemism and co-evolution. This is supported by variability in the ITS and *cox1* sequences of the Vietnamese isolates and by *cox1* sequence differences between the Vietnamese isolates and the North American NA1 and NA2 and the European EU1 and EU2 lineages. Because of the implications both for the origin of the pathogen and for international biosecurity a detailed comparative phenotypic and molecular analysis of the Vietnamese *P. ramorum* isolates and the known EU1, EU2, NA1, and NA2 lineages [65] is currently ongoing to further characterise the Vietnamese population and its relationship to the known lineages. Since southern Yunnan, northern Laos, and the eastern Himalayas belong to the same biogeographic area as the Fansipan region mountain forests in these regions may also harbour endemic *P. ramorum* populations. Further surveys are needed to confirm this hypothesis.

*Phytophthora cinnamomi* was the most common soilborne *Phytophthora* species above 700 m. The A2 mating type of *P. cinnamomi* was more widespread, occurring in 11 forest stands between 713 and 2337 m, whereas the A1 occurred only in four forest stands located between 1108 and 2636 m a.s.l. In Taiwan and Papua New Guinea also the A1 mating type occurs at higher altitudes than the A2 indicating higher tolerance to low temperatures [10,66]. However, in both of these locations the altitudinal differences between the mating types are larger than in Vietnam, the A2 being confined to the lowland forests. In each one stand in Hoàng Liên NP and Ba Vi NP both mating types co-occurred. In Hoàng Liên NP the A1 was present in the upper montane *Rhododendron* forest at 2636 m and in two lower montane stands at 1900 m. However, it was not detected in the eight forest stands between 2337 and 2022 m in which not only the A2 type was present but severe dieback of Fagaceae and Lauraceae was observed (notably *C. acuminatissima*, *Q. glauca*, *N. poilanei* and *N. polycarpa*). Pathogenicity trials are required to fulfill Koch’s postulates for these host-pathogen associations and confirm that *P. cinnamomi* A2 is causing the dieback of these native Fagaceae and Lauraceae species. In contrast, no dieback was observed in the three forest stands in Ba Vi NP between 713 and 1100 m, despite the presence of *P. cinnamomi* A2. In the two stands with the co-occurrence of both mating types the A1:A2 ratio of the 44 isolates was 59.1:40.9, whereas the overall mating type ratio of the 151 isolates from 13 *P. cinnamomi* infested stands was 30.5:69.5. Collectively, these results suggest that, as a consequence of current climatic warming, the more thermophilic but frost sensitive A2 mating type may be spreading into higher altitudes in Vietnam. Such a progression of *P. cinnamomi* A2 into higher latitudes and altitudes with climate change was predicted by CLIMEX modelling [67–69]. In the newly A2 invaded high-altitude forests in Vietnam the A2 may be outcompeting and replacing the native co-evolved A1, causing dieback in the susceptible non-coevolved hosts. The widespread distribution of *P. cinnamomi* in northern Vietnam, the co-occurrence of both mating types in several stands, and the absence
of disease symptoms in lower altitudes also indicates that Vietnam lies within the origin of both mating types. Phytophthora cinnamomi is the most invasive member of the genus with a host range of almost 5000 woody plant species [2,70,71]. Two genotypes of the A2 mating type have reached a panglobal distribution causing epidemics in numerous natural and managed ecosystems while the A1 mating type has a limited distribution outside of Asia and has never been associated with epidemic disease [2,5,6–9,11,60,66,72–76].

Phytophthora attenuata, recently described from montane forests in Taiwan [22], and three closely related but previously unknown species, were found in the submontane and montane forests of northern Vietnam. Phytophthora attenuata, P. sp. attenuata-like 1 and P. sp. attenuata-like 2 were detected in the temperate, montane cloud forests around the Fansipan. However, P. sp. attenuata-like 3 was found only in the subtropical, humid submontane evergreen forests in Ba Vi NP. These four closely related species most likely result from sympatric species radiation, suggesting northern Indochina as the center of origin of the ‘P. attenuata complex’. A pathogenicity trial is required to confirm that P. sp. attenuata-like 3 is causing the dieback of Dysoxylum juglandis in Ba Vi National Park. Another Clade 7a species that was first described from Taiwan, P. ×heterohybrida, was widespread in Vietnamese montane forest streams. This allopolyploid hybrid species has a functional but peculiar sexual system. In Taiwan, all isolates were self-sterile with both mating types being common and one isolate mating with both mating types [22]. In contrast, almost all Vietnamese isolates were self-sterile and belonged to the A1 mating type while one isolate was prolifically homothallic and stimulated oogonia formation in A2 tester strains.

The results indicate that the ‘P. citricola complex’ from Clade 2c also underwent a species radiation process in Vietnam. Besides P. capensis, originally described from nursery plants in South Africa and also isolated from natural streams in Taiwan [10,77], and P. citricola VII, which was previously reported from a montane forest in Taiwan [10], four previously unknown taxa were found in this survey. Most common was P. citricola VII which occurred in mountain streams inside and outside of Hoàng Liên NP and in the rhizosphere of a montane Alnus forest, whereas the new taxa P. citricola VIII to XI had only cryptic distributions. It is notable that P. citricola X and XI were only found in tropical lowland rainforests and that they differ from all other species of the ‘P. citricola complex’ by producing mainly papillate instead of semipapillate sporangia. The occurrence of P. citricola VII to X within only ca 300 km in northern Vietnam and the co-occurrence of P. citricola VII, VIII and IX in individual streams suggest sympatric species radiation from a common ancestor. In contrast, the exclusive finding of P. citricola XI and also P. sp. botryosa-like 2 from Clade 2a on Côn Lôn island, situated on the Asian shelf 50 km off the southern Vietnamese coast, are more consistent with allopatric island speciation. The invasive wide-host range pathogen P. plurivora occurs in undisturbed, healthy, often deciduous temperate mountain forests in Taiwan, Nepal and Yunnan [10,15,16,43]. However, it was not found here in the subtropical and tropical forests of Vietnam. This suggests that P. plurivora is native to temperate mountainous regions of South and East Asia.

The detection of five new species and of P. capensis from the ‘P. citricola complex’ in Clade 2c, three new species from Clade 2a and P. sp. tropicalis-like 2 and P. sp. multivesiculata-like 1 from Clades 2b and 2e in this survey, the findings of P. bisheria, P. capensis, P. plurivora, P. citrophthora, P. tropicalis and the three new Clade 2a species P. sp. ×botryosa-like, P. sp. ×meadii-like and P. sp. occultans-like from natural ecosystems in Taiwan [10,13] together with the widespread occurrence of P. botryosa, P. citricola, P. colocasiae and P. meadii across Southeast Asia [2,12,33–35] suggest South, Southeast and East Asia as the center of origin of Phytophthora major Clade 2.

Interestingly, all known isolates from the new Clade 2a taxa P. sp. meadii-like 1 and 2 and P. sp. botryosa-like 2 from Vietnam, as well as P. sp. ×botryosa-like and P. sp. ×meadii-like from Taiwan [10] are of A1 mating type and are characterised by oospore abortion rates exceeding 95% in mating tests with tester strains of P. botryosa and P. meadii. It appears that in this complex of aerial Phytophthora species the A1 is better adapted to and, hence, more common in natural forests than the A2. It is even possible that these self-sterile taxa, like many aquatic Clade 6 species [21,53], lack the A2 mating
type and have abandoned sexual reproduction in favour of exclusive asexual reproduction, spreading via their caducous sporangia from infected to non-infected above-ground tissues. This possibility is supported by the extremely high oospore abortion rates in mating tests with tester strains of *P. botryosa* and *P. meadii*. More field surveys and laboratory tests are needed to verify this hypothesis.

*Phytophthora castaneae* and *P. heveae* from Clade 5 are also considered being native to Taiwan and Hainan [2,10,12,31]. Their widespread occurrence in Vietnamese forests and the lack of association with disease symptoms in the native vegetation indicate that Indochina also lies within the origin of both species.

As previously demonstrated in Australia, Chile, South Africa and Taiwan putative interspecific hybrids, indicated by multiple heterozygous sites in their ITS sequences, are common in watercourses and can also be found in forest soils [10,11,17,18,78,79]. As with predominantly aquatic species and hybrids from Clade 6, all Clade 9 hybrids from Vietnamese streams, with the exception of *P. sp. xinsolita-like* which produces oogonia without antheridia, are sterile and apparently adapted to rapid and continuous asexual proliferation via zoospores. Also, like many Clade 6 taxa, this may reflect adaptation to a mostly saprotrophic lifestyle as decomposers of naturally fallen leaves [21,53]. As with the Clade 6 hybrid *P. thermophila × P. amnicola* in the Valdivia River in Chile [11], no putative parents of the Clade 9 hybrids *P. sp. ×Grenada 3-like*, *P. sp. ×xinsolita-like*, *P. sp. ×kunnunara-like*, *P. sp. ×Peru 4-like* and *P. sp. ×virginiana-like* were detected in the Vietnamese rivers. Possibly the hybridisation events occurred in the Vietnamese streams and the parents were outcompeted by the better adapted hybrids. Alternatively, the hybrids could be introduced from elsewhere. Since the multicycopy ITS locus is of limited use for hybrid studies sequencing of appropriate mitochondrial and nuclear genes are needed to confirm the hybrid status and elucidate the parents of the putative hybrid taxa.

Pan-global distributed pathogens from *Phytophthora* Clades 1 (*P. cactorum, P. infestans, P. nicotianae*) and 4 (*P. palmivora*) commonly cause diseases of horticultural crops and ornamental plants in mainland China, Hainan and Taiwan [12,33,35]. However, in this survey, as in previous surveys in Taiwan, species from Clades 1 and 4 (exception for one isolate of *P. palmivora* in Taiwan) were not detected in natural forests and streams [10,13], indicating that these two clades are not native to Taiwan and Southeast Asia. The same probably applies to Clades 3, 11 and 12 [23,28].

Although the natural hosts of the putatively endemic Vietnamese forest *Phytophthoras* obtained in this study are still unknown, it is evident that many native Asian forest *Phytophthoras* have co-evolved with a variety of tree genera also present in Europe and North America, including Fagaceae, Lauraceae, Aceraceae, Oleaceae, and Pinaceae. In this case high susceptibility of many non-coevolved European and North American trees to these Asian *Phytophthora* species is possible, as already well demonstrated for *P. cinnamomi, P. plurivora, P. ×cambivora* and, more recently, for six new Clade 7a species from Taiwan [22]. An extensive host range study with *Phytophthora* species from Asia, South and Central America has been initiated and will be published separately. In one part of this study, the pathogenicity of five Asian species (*P. castaneae, P. heveae* and the three new Vietnamese species *P. citricola* X, *P. sp. multivesiculata-like 1* and *P. sp. tropicalis-like 2*) to *Castanea sativa, Quercus suber* and *Quercus robur* has been investigated and all five caused significant rot and loss of fine roots and suberised lateral roots in all three hosts, *C. sativa* being most susceptible [80].

Against this background, the annual importation of over three billion plants-for-planting into Europe [81], the large numbers of previously unknown *Phytophthora* species in natural and horticultural ecosystems being identified in Asia, South and Central America ([10,11,16,22,82], this study) and the occurrence of at least 47 exotic *Phytophthora* species in European nurseries and associated outplantings [60] represents a significant biosecurity risk for forestry, horticulture, and natural ecosystems in Europe and North America.

Many recent epidemics of trees and horticultural crops have been caused by introduced pathogens that were previously unknown to science, probably due to the organisms being co-evolved and benign in their centres of origin [6,10,83]. Although often introduced via the plants-for-planting pathway, none of them has ever been intercepted pre-emptively during routine phytosanitary controls
at the ports of entry [60,81,83,84]. Despite overwhelming scientific evidence, current sanitary and phytosanitary (SPS) protocols largely ignore the risks from unknown, benign, co-evolved and unescaped organisms [6,60,83–85]. However, preventing further introductions of potentially harmful invasive Phytophthoras is a key issue for international forest biosecurity. A series of international research projects and organisations (listed in [10]) have come to similar conclusions. The current, outdated and scientifically flawed species-by-species regulation approach based on random visual inspections for symptoms of described pests and pathogens needs to be replaced by a sophisticated pathway regulation approach using pathway risk analyses, risk-based inspection regimes and molecular high-throughput detection tools [6,60,81,83,84,86,87].

To further define areas of Phytophthora diversity, including high-risk areas for the origin of potentially harmful pathogens, more Phytophthora surveys are needed in natural ecosystems in unsurveyed areas of Asia, Africa, and South and Central America, followed by host range testing of new taxa on naive tree hosts in Europe and elsewhere. Such surveys should also contribute to a better understanding of the global diversity of Phytophthora, the ancient biogeographic radiation of the Phytophthora species and Clades, and the influence of local environmental and host factors on breeding strategies and adaptation in the genus.

5. Conclusions

A remarkable diversity of 13 described species, five informally designated taxa and 21 previously unknown taxa of Phytophthora were obtained from 25 natural and semi-natural forest stands and 16 rivers in temperate and subtropical montane and tropical lowland regions of Vietnam. It is concluded that Vietnam is within the center of origin of most Phytophthora taxa found, including P. cinnamomi and P. ramorum, and that Phytophthora clades 2, 5, 6, 7, 8, 9, and 10 are native to Indochina.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/1/93/s1, Table S1: GenBank accession numbers of ITS and partial cox1 sequences generated in this study for representative Phytophthora, Elongisporangium, Nothophytophthora, Phytophthium and Pythium isolates from Vietnamese forests and rivers and isolates from related Phytophthora species used for comparisons.

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References

1. Brasier, C.M.; Robredo, F.; Ferraz, J.F.P. Evidence for Phytophthora cinnamomi involvement in Iberian oak decline. Plant Pathol. 1993, 42, 140–145. [CrossRef]
2. Erwin, D.C.; Ribeiro, O.K. Phytophthora Diseases Worldwide; APS Press: Saint Paul, MN, USA, 1996.
3. Hansen, E.M.; Goheen, D.J.; Jules, E.S.; Ullian, B. Managing Port-Orford–Cedar and the introduced pathogen Phytophthora lateralis. Plant Dis. 2000, 84, 4–14. [CrossRef] [PubMed]
4. Jung, T.; Blaschke, H.; Osswald, W. Involvement of soilborne Phytophthora species in Central European oak decline and the effect of site factors on the disease. Plant Pathol. 2000, 49, 706–718. [CrossRef]
5. Jung, T.; Vettraino, A.M.; Cech, T.L.; Vannini, A. The impact of invasive Phytophthora species on European forests. *Phytophthora: A Global Perspective*; Lamour, K., Ed.; CABI: Wallingford, UK, 2013; pp. 146–158, ISBN 978-1-78064-093-8.

6. Jung, T.; Pérez–Sierra, A.; Durán, A.; Horta Jung, M.; Balci, Y.; Scanu, B. Canker and decline diseases caused by soil- and airborne *Phytophthora* species in forests and woodlands. *Persoonia* 2018, 40, 182–220. [CrossRef]

7. Rizzo, D.M.; Garbelotto, M.; Davidson, J.M.; Slaugter, G.W. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. *Plant Dis.* 2002, 86, 205–214. [CrossRef]

8. Hardham, A.R. *Phytophthora cinnamomi*. *Mol. Plant Pathol.* 2005, 6, 589–604. [CrossRef]

9. Cahill, D.M.; Rookes, J.E.; Wilson, B.A.; Gibson, L.; McDougall, K.L. Turner Review No. 17. *Phytophthora cinnamomi* and Australia’s biodiversity: Impacts, predictions and progress towards control. *Aust. J. Bot.* 2008, 56, 279–310. [CrossRef]

10. Jung, T.; Chang, T.T.; Bakonyi, J.; Seress, D.; Pérez–Sierra, A.; Yang, X.; Hong, C.; Scanu, B.; Fu, C.H.; Hsueh, K.-L.; et al. Diversity of *Phytophthora* species in natural ecosystems of Taiwan and association with disease symptoms. *Plant Pathol.* 2017, 66, 194–211. [CrossRef]

11. Jung, T.; Durán, A.; Sanfuentes von Stowasser, E.; Schena, L.; Mosca, S.; Fajardo, S.; González, M.; Navarro Ortega, A.D.; Bakonyi, J.; Seress, D.; et al. Diversity of *Phytophthora* species in Valdivian rainforests and association with severe dieback symptoms. *Forest Pathol.* 2018, 48, e12443. [CrossRef]

12. Zeng, H.-C.; Ho, H.-H.; Zheng, F.-C. A survey of *Phytophthora* species on Hainan Island of South China. *J. Phytopathol.* 2009, 157, 33–39. [CrossRef]

13. Brasier, C.M.; Vettraino, A.M.; Chang, T.T.; Vannini, A. *Phytophthora lateralis* discovered in an old growth *Chamaecyparis* forest in Taiwan. *Plant Pathol.* 2010, 59, 595–603. [CrossRef]

14. Reeser, P.W.; Sutton, W.; Hansen, E.M.; Remigi, P.; Adams, G.C. *Phytophthora* species in forest streams in Oregon and Alaska. *Mycologia* 2011, 103, 22–35. [CrossRef] [PubMed]

15. Vettraino, A.M.; Brasier, C.M.; Brown, A.V.; Vannini, A. *Phytophthora himalaisiva* sp. nov. An unusually phenotypically variable species from a remote forest in Nepal. *Fungal Biol.* 2011, 115, 275–287. [CrossRef] [PubMed]

16. Huai, W.X.; Tian, G.; Hansen, E.M.; Zhao, W.X.; Goheen, E.M.; Grünwald, N.J.; Cheng, C. Identification of *Phytophthora* species baited and isolated from forest soil and streams in northwestern Yunnan province, China. *Forest Pathol.* 2013, 43, 87–103. [CrossRef]

17. Hüberli, D.; Hardy, G.E.S.T.J.; White, D.; Williams, N.; Burgess, T.I. Fishing for *Phytophthora* from Western Australia’s waterways: A distribution and diversity survey. *Australas. Plant Pathol.* 2013, 42, 251–260. [CrossRef]

18. Oh, E.; Gryzenhout, M.; Wingfield, B.D.; Wingfield, M.J.; Burgess, T.I. Surveys of soil and water reveal a goldmine of *Phytophthora* diversity in South African natural ecosystems. *IMA Fungus* 2013, 4, 123–131. [CrossRef] [PubMed]

19. Burgess, T.I.; White, D.; McDougall, K.M.; Garnas, J.; Dunstan, W.A.; Català, S.; Carnegie, A.J.; Worboys, S.; Cahill, D.; Vettraino, A.M.; et al. Distribution and diversity of *Phytophthora* across Australia. *Pac. Conserv. Biol.* 2017, 23, 1–13. [CrossRef]

20. Jung, T.; Hansen, E.M.; Winton, L.; ÓBswald, W.; Delatour, C. Three new species of *Phytophthora* from European oak forests. *Mycol. Res.* 2010, 116, 397–411. [CrossRef]

21. Jung, T.; Stukely, M.J.C.; Hardy, G.E.S.J.; White, D.; Paap, T.; Dunstan, W.A.; Burgess, T.I. Multiple new *Phytophthora* species from ITS Clade 6 associated with natural ecosystems in Australia: Evolutionary and ecological implications. *Persoonia* 2011, 26, 13–39. [CrossRef]

22. Jung, T.; Horta Jung, M.; Scanu, B.; Seress, D.; Kovács, D.M.; Maia, C.; Pérez–Sierra, A.; Chang, T.-T.; Chandelier, A.; Heungens, A.; et al. Six new *Phytophthora* species from ITS Clade 7a including two sexually functional heterothallic hybrid species detected in natural ecosystems in Taiwan. *Persoonia* 2017, 38, 100–135. [CrossRef]

23. 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]

24. Burgess, T.I.; Webster, J.L.; Ciampini, J.A.; White, D.; Hardy, G.E.S.J.; Stukely, M.J.C. Re-evaluation of *Phytophthora* species isolated during 30 years of vegetation health surveys in Western Australia using molecular techniques. *Plant Dis.* 2009, 93, 215–223. [CrossRef] [PubMed]
45. Safaiefarahani, B.; Mostowfizadeh-Ghalamfarsa, R.; Hardy, G.E.S.J.; Burgess, T.I. Re-evaluation of the
Phytophthora cinnamomi and P. cinnamomi var. parvispora are separate species. *Forest Pathol.* 2014, 44, 1–20. [CrossRef]

46. 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, ISBN 0123721806.

47. 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]

48. Martin, F.N.; Tooley, P.W. Phylogenetic relationships among *Phytophthora* species inferred from sequence
analysis of mitochondrial encoded cytochrome oxidase I and II genes. *Mycolologia* 2003, 95, 269–284. [CrossRef]

49. Aragaki, M.; Uchida, J.Y. Morphological distinctions between *Phytophthora capsici* and *P. tropicalis* sp. nov.
*Mycol. Res.* 2001, 93, 137–145. [CrossRef]

50. Ilieva, E.; Man In’t Veld, W.A.; Veenbaas-Rijks, W.; Pieters, R. *Phytophthora multivesiculata*, a new species causing rot in *Cymbidium*. *Eur. J. Plant Pathol.* 1998, 104, 677–684. [CrossRef]

51. Jung, T.; Scanu, B.; Bakonyi, J.; Kovács, G.M.; Durán, A.; Sanfuentes von Stowasser, E.; Schena, L.; Mosca, S.; Thu, P.Q.; et al. *Nothophytophthora* gen. nov., a new sister genus of *Phytophthora* from natural and semi-natural ecosystems. *Persoonia* 2017, 39, 143–174. [CrossRef]

52. Werres, S.; Marwitz, R.; Man In’t Veld, W.A.; Bonants, P.J.M.; De Weerd, M.; Themann, K.; Ilieva, E.; Baayen, R.P. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. *Mycol. Res.* 2001, 105, 1155–1165. [CrossRef]

53. Brasier, C.M.; Cooke, D.E.L.; Duncan, J.M.; Hansen, E.M. Multiple new phenotypic taxa from trees and riparian
ecosystems in *Phytophthora gonapodyides*—*P. megasperma* ITS Clade 6, which tend to be high-temperature
tolerant and either inbreeding or sterile. *Mycol. Res.* 2003, 107, 277–290. [CrossRef]

54. Safaiefarahani, B.; Mostowfizadeh-Ghalamfarsa, R.; Hardy, G.E.S.J.; Burgess, T.I. Re-evaluation of the
*Phytophthora cryptogea* species complex and the description of a new species, *Phytophthora pseudocryptogea* sp. nov. *Mycol. Prog.* 2015, 14, 108. [CrossRef]

55. Jung, T.; Nechwatal, J. *Phytophthora galliaca* sp. nov., a new species from rhizosphere soil of declining oak and
reed stands in France and Germany. *Mycol. Res.* 2008, 112, 1195–1205. [CrossRef]

56. Yang, X.; Hong, C. *Phytophthora virginiana* sp. nov., a high-temperature tolerant species from irrigation water
in Virginia. *Mycotaxon* 2013, 126, 167–176. [CrossRef]

57. Balci, Y.; Balci, S.; Eggers, J.; MacDonald, W.L.; Juzwik, J.; Long, R.P.; Gottschalk, K.W. *Phytophthora* spp.
associated with forest soils in eastern and north-central U.S. oak ecosystems. *Plant Dis.* 2007, 91, 705–710. [CrossRef]

58. Jung, T.; La Spada, F.; Pane, A.; Aloi, F.; Evoli, M.; Horta Jung, M.; Scanu, B.; Faedda, R.; Rizza, C.; Puglisi, L.; et al. Diversity and distribution of *Phytophthora* species in protected natural areas in Sicily. *Forests* 2019, 10, 259. [CrossRef]

59. Milenković, I.; Keča, N.; Karadžić, D.; Radulović, Z.; Nowakowska, J.A.; Oszako, T.; Sikora, K.; Corcobado, T.; Jung, T. Isolation and pathogenicity of *Phytophthora* species from poplar plantations in Serbia. *Forests* 2018, 9, 330. [CrossRef]

60. Jung, T.; Orlikowski, L.; Henricot, B.; Abad-Campos, P.; Aday, A.G.; Aguin Casal, O.; Bakonyi, J.; Cacciola, S.O.; Cech, T.; Chavarriaga, D.; et al. Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases. *For. Pathol.* 2016, 46, 134–163. [CrossRef]

61. Shrestha, S.K.; Zhou, Y.; Lamour, K. Oomycetes baited from streams in Tennessee 2010–2012. *Mycolologia* 2013, 105, 1516–1523. [CrossRef]

62. Brasier, C.M. Evolutionary Biology of *Phytophthora*. I. Genetic system, sexuality and variation. *Annu. Rev. Phytopathol.* 1992, 30, 153–171. [CrossRef]

63. Català, S.; Peréz-Sierra, A.; Abad-Campos, P. The use of genus-specific ampiclon pyrosequencing to assess
*Phytophthora* species diversity using eDNA from soil and water in Northern Spain. *PLoS ONE* 2015, 10, e0119311. [CrossRef]
64. Grünwald, N.J.; Garbelotto, M.; Goss, E.M.; Heungens, K.; Prospero, S. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. *Trends Microbiol.* 2012, 20, 131–138. [CrossRef]

65. Van Poucke, K.; Franceschini, S.; Webber, J.F.; Vercauteren, A.; Turner, J.A.; McCracken, A.R.; Heungens, K.; Brasier, C.M. Discovery of a fourth evolutionary lineage of *Phytophthora ramorum*: EU2. *Fungal Biol.* 2012, 116, 1178–1191. [CrossRef]

66. Arentz, F.; Simpson, J.A. Distribution of *Phytophthora cinnamomi* in Papua New Guinea and notes on its origin. *Trans. Br. Mycol. Soc.* 1986, 87, 289–295. [CrossRef]

67. Brasier, C.M.; Scott, J.K. European oak declines and global warming: A theoretical assessment with special reference to the activity of *Phytophthora cinnamomi*. *Bull. OEPP/EPPO Bull.* 1994, 24, 221–234. [CrossRef]

68. Brasier, C.M. *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. *Ann. Des. Sci. For.* 1996, 53, 347–358. [CrossRef]

69. Shearer, B.L.; Crane, C.E.; Cochrane, A. Quantification of the susceptibility of the native flora of the South-West Botanical Province, Western Australia, to *Phytophthora cinnamomi*. *Aust. J. Bot.* 2004, 52, 435–443. [CrossRef]

70. Hardham, A.R.; Blackman, L.M. *Phytophthora cinnamomi*. *Mol. Plant Pathol.* 2018, 19, 260–285. [CrossRef] [PubMed]

71. Zentmyer, G.A. *Phytophthora Cinnamomi and the Diseases it Causes*; Monograph No. 10; The American Phytopathological Society: Saint Paul, MN, USA, 1980; ISBN 0890540306.

72. Linde, C.; Drenth, A.; Kemp, G.H.J.; Wingfield, M.J.; Von Broembsen, S.L. Population structure of *Phytophthora cinnamomi* in South Africa. *Phytopathology* 1997, 87, 822–827. [CrossRef] [PubMed]

73. Dobrowolski, M.P.; Tommerup, I.C.; Blakeman, H.D.; O’Brien, P.A. Non-Mendelian inheritance revealed in a genetic analysis of sexual progeny of *Phytophthora cinnamomi* with microsatellite markers. *Fungal Genet. Biol.* 2002, 35, 197–212. [CrossRef] [PubMed]

74. Dobrowolski, M.P.; Tommerup, I.C.; Shearer, B.L.; O’Brien, P.A. Three clonal lineages of *Phytophthora cinnamomi* in Australia revealed by microsatellites. *Phytopathology* 2003, 93, 695–704. [CrossRef] [PubMed]

75. Jung, T.; Colquhoun, I.J.; Hardy, G.E.S.J. New insights into the survival strategy of the invasive soilborne pathogen *Phytophthora cinnamomi* in different natural ecosystems in Western Australia. *Forest Pathol.* 2013, 43, 266–288. [CrossRef]

76. Bezuidenhout, C.M.; Denman, S.; Kirk, S.A.; Botha, W.J.; Mostert, L.; McLeod, A. *Phytophthora* taxa associated with cultivated *Agathosma*, with emphasis on the *P. citricola* complex and *P. capensis* sp. nov. *Persoonia* 2010, 25, 32–49. [CrossRef]

77. Nagel, J.H.; Gryzenhout, M.; Slippers, B.; Wingfield, M.J.; Hardy, G.E.S.J.; Stukely, M.; Burgess, T.I. Characterization of *Phytophthora* hybrids from ITS clade 6 associated with riparian ecosystems in South Africa and Australia. *Fungal Biol.* 2013, 117, 329–347. [CrossRef]

78. Burgess, T.I. Molecular characterization of natural hybrids formed between five related indigenous Clade 6 *Phytophthora* species. *PLoS ONE* 2015, 10, e0134225. [CrossRef]

79. Jung, T.; Maia, C.; Horta Jung, M. Host range testing of known and novel *Phytophthora* species from Asia, Central and South America among major forest tree species from Europe. Unpublished work. 2020. 

80. Eschen, R.; Douma, J.C.; Grégoire, J.-C.; Mayer, F.; Rigaux, L.; Potting, R.P.J. A risk categorisation and analysis of the geographic and temporal dynamics of the European import of plants for planting. *Biol. Invasions* 2017, 19, 3243–3257. [CrossRef]

81. Rahman, M.Z.; Uematsu, S.; Takeuchi, T.; Shirai, K.; Ishiguro, Y.; Suga, H.; Kageyama, K. Two new species, *Phytophthora nagaii* sp. nov. and *P.fragariaefolia* sp. nov., causing serious diseases on rose and strawberry plants, respectively, in Japan. *J. Gen. Plant Pathol.* 2014, 80, 348–365. [CrossRef]

82. Brasier, C.M. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathol.* 2008, 57, 792–808. [CrossRef]

83. Santini, A.; Ghelardini, L.; De Pace, C.; Desprez-Loustau, M.L.; Capretti, P.; Chandelier, A.; Cech, T.; Chira, D.; Diamandis, S.; Gaitniekis, T.; et al. Biogeographic patterns and determinants of invasion by alien forest pathogens in Europe. *New Phytol.* 2013, 197, 238–250. [CrossRef] [PubMed]

84. Liebold, A.M.; Brockerhoff, E.G.; Garrett, 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]
86. Eschen, R.; Rigaux, L.; Sukovata, L.; Vettraino, A.M.; Marzano, M.; Grégoire, J.-C. Phytosanitary inspection of woody plants for planting at European Union entry points: A practical enquiry. *Biol. Invasions* **2015**, *17*, 2403–2413. [CrossRef]

87. Eschen, R.; Britton, K.; Brockerhoff, E.; Burgess, T.; Dalley, V.; Epanchin-Niell, R.; Gupta, K.; Hardy, G.; Huang, Y.; Kenis, M.; et al. International variation in phytosanitary legislation and regulations governing importation of plants for planting. *Environ. Sci. Policy* **2015**, *51*, 228–237. [CrossRef]