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

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Abstract: In 2016 and 2017, surveys of *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 *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: *P. capensis*, *P. citricola* VII, VIII, IX, X and XI, *P. sp. botryosa*-like 2, *P. sp. meadii*-like 1 and 2, *P. sp. tropicalis*-like 2 and *P. sp. multivesiculata*-like 1 from *Phytophthora* major phylogenetic Clade 2; *P. castaneae* and *P. heveae* from Clade 5; *P. chlamydospora*, *P. gregata*, *P. sp. bitahaiensis*-like and *P. sp. sylvatica*-like 1, 2 and 3 from Clade 6; *P. cinnamomi* (*Pc*), *P. parvispora*, *P. attenuata*, *P. sp. attenuata*-like 1, 2 and 3 and *P. xheterohybrida* from Clade 7; *P. drechleri*, *P. pseudocryptogea*, *P. ramorum* (*Pr*) and *P. sp. kelmania* from Clade 8, *P. macrochlamydospora*, *P. sp. xinsolita*-like, *P. sp. xkunnunara*-like, *P. sp. xvirginiana*-like s.l. and three new taxa, *P. sp. quininea*-like, *P. sp. xGrenada 3*-like and *P. sp. xPeru 4*-like, from Clade 9; and *P. sp. gallica*-like 1 and 2 from Clade 10. The A1 and A2 mating types of both *Pc* and *Pr* co-occurred. The A2 mating type of *Pc* was associated with severe dieback of montane forests in northern Vietnam. Most other *Phytophthora* species, including *Pr*, were not associated with obvious disease symptoms. It is concluded that (1) Vietnam is within the center of origin of most *Phytophthora* taxa found including *Pc* and *Pr*, and (2) *Phytophthora* clades 2, 5, 6, 7, 8, 9, and 10 are native to Indochina.

Keywords: biosecurity; breeding systems; hybridization; *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 Bù Gia Mập NP, U Minh Hạ 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 bagiangensis*, *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 pimarinic acid, 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.

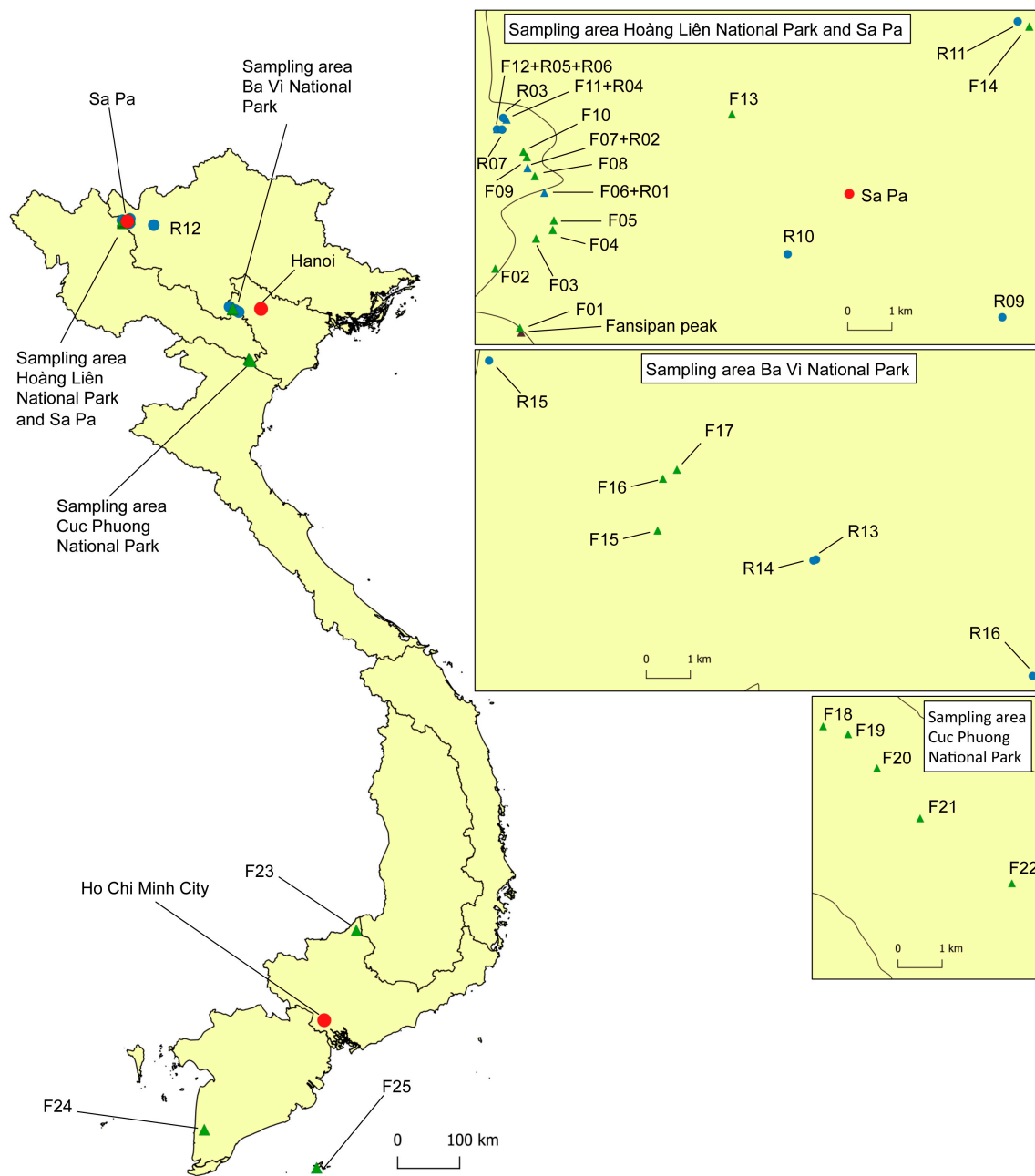


Figure 1. Location of the 25 forest sites (F01–F25; green triangles) and the 16 riparian sites (R01–R16; blue dots) included in the *Phytophthora* survey in Vietnam; blue triangles represent sites included in both the riparian and forest survey. For geographical coordinates and details of sites see Tables 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, subtropical, humid evergreen forest F15 in Ba Vi 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 juglans* in subtropical 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 ITS6/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 (Nottinham, 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, chlamydo spores, 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 AxioCam 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), *Phytophythium vexans* sensu lato (63 isolates from 14 partly highly different haplotypes), four other known species and three novel taxa of *Phytophythium* (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 *Phytophythium* (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 *Phytophythium* 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* in stand F07. Two A2 isolates from stand F11 were able to produce oogonia in single culture on V8A (Table 1). Over all stands, the A1:A2 mating type ratio of the 151 *P. cinnamomi* isolates was 30.5:69.5, whereas in the two stands with co-occurrence of both mating types the A1:A2 ratio of the 44 isolates was 59.1:40.9. Among the 39 *P. cinnamomi* isolates for which ITS sequences were produced, 32 isolates belonged to the same haplotype as the ex-type isolate from Sumatra (CBS 142.22; GenBank accession no. KU899160) (Table S1). Six isolates, representing both mating types, from stands F02 and F05 in Hoàng Liên NP and stand F17 in Ba Vì NP were heterozygous at position 767 (K instead of G) (Table 1S) and shared the same haplotype with an isolate from a subtropical *Quercus* forest in Taiwan (TW213; GenBank accession no. KU682570). Another isolate from stand F17 shared the heterozygous position 767 and was also heterozygous at position 89 (Y instead of C) (Supplementary Table S1). The 15 isolates for which the *cox1* gene was sequenced belonged to eight different haplotype which differed over a 712 bp alignment from the ex-type isolate (KU899315) at 1–4 positions.

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. cinnamomi* (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. cinnamomi*, 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 Hoàng Liên 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 Hoàng Liên 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. cinnamomi* (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 Hoàng Liên 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 Hoàng Liên 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 <i>Phytophthora</i> -Positive/ Sampled Trees)	<i>Phytophthora</i> and <i>Nothophytophthora</i> spp. (no. of Positive Samples) ^{a,b}
F01	N22 18.466 E103 46.480	2903	Fansipan, Hoàng Liên National Park (NP)	Triassic schists and sandstones	Subalpine <i>Rhododendron</i> scrub	<i>Rhododendron</i> spp. (0/3)	-
F02	N22 19.194 E103 46.177	2636	Fansipan, Hoàng Liên NP	Triassic schists and sandstones	Upper montane <i>Rhododendron</i> ('Elfin') cloud forest	<i>Rhododendron arboreum</i> , mix from 3 trees with dieback (DB) (1/1)	ATT1 (1), CIN A1 (1)
F03	N22 19.563 E103 46.679	2337	Hoàng Liên NP	Triassic schists and sandstones	Montane evergreen cloud forest	<i>Quercus glauca</i> , DB (2/2)	CIN A2 (2)
F04	N22 19.670 E103 46.885	2242	Hoàng Liên NP	Triassic schists and sandstones	Montane evergreen cloud forest	<i>Meliosma henryi</i> (1/1) <i>Betula alnoides</i> & <i>Elaeocarpus japonicus</i> (1/1) <i>Castanopsis acuminatissima</i> , mix from 2 trees, DB (1/1) <i>C. acuminatissima</i> with DB & <i>Acer campbellii</i> (1/1)	CAS (1) ATT1 (1), CIN A2 (1) ^c ATT1 (1), CIN A2 (1) VIE (1)
F05	N22 19.786 E103 46.899	2249	Hoàng Liên NP	Triassic schists and sandstones	Montane evergreen cloud forest	<i>Neolitsea poilanei</i> , DB (3/3) <i>C. acuminatissima</i> mix from 3 trees, DB (1/1) <i>Illicium griffithii</i> & <i>C. acuminatissima</i> , DB (1/1)	ATT1 (3), CAS (1), CIN A2 (3) ATT (1), CIN A2 (1) CAS (1)
F06	N22 20.127 E103 46.782	2083	Hoàng Liên NP	Triassic schists and sandstones	Montane evergreen cloud forest	<i>C. acuminatissima</i> , DB (2/2) <i>M. henryi</i> & <i>A. campbellii</i> (1/1) <i>M. henryi</i> & <i>Neolitsea merilliana</i> (1/1)	CIN A2 (2), GRE (1), CAS (1) ^d GRE (1) CIN A2 (1), MUV1 (1)
F07	N22 20.430 E103 46.574	2010	Hoàng Liên NP	Triassic schists and sandstones	Montane evergreen cloud forest	<i>Illicium tsaii</i> & <i>Rhododendron sinofalconeri</i> (1/1) <i>C. acuminatissima</i> , DB, necrotic root lesion (1/1)	CAS (1) CIN A2 (1)
F08	N22 20.331 E103 46.664	2066	Hoàng Liên NP	Triassic schists and sandstones	Montane evergreen cloud forest	<i>Casearia annamensis</i> (1/1) <i>Acer oblongum</i> , mix from 2 trees (0/1)	CIN A2 (1) - ^e
F09	N22 20.565 E103 46.565	2010	Hoàng Liên NP	Triassic schists and sandstones	Montane evergreen cloud forest	<i>C. acuminatissima</i> (0/1) <i>Q. glauca</i> (0/2)	- ^e -
F10	N22 20.632 E103 46.523	2022	Hoàng Liên NP	Triassic schists and sandstones	Montane, evergreen cloud forest	<i>Neolitsea polycarpa</i> , mix from 3 trees, DB (1/1) <i>N. polycarpa</i> , <i>Symplocos pseudobarberina</i> & <i>Beilschmiedia roxburghiana</i> (1/1)	CIN A2 (1) CIN A2 (1)
F11	N22 21.026 E103 46.315	1910	Hoàng Liên NP	Triassic schists and sandstones	Montane, evergreen broadleaved forest	<i>A. oblongum</i> & <i>Symplocos dryophila</i> (2/2) <i>C. acuminatissima</i> DB, <i>Ilex leseeneri</i> & <i>Eurya annamensis</i> (1/1) <i>R. arboreum</i> (1/1) ^g	ATT (1), ATT2 (1), CIN A1 (2), CIN A2 (1), CIN A2ho (1) ^{e,f} CIN A2 (1), CAS (1) CHL (1), RAM A1 (1)

Table 1. Cont.

Site no.	GPS Coordinates	Altitude (m a.s.l)	Location	Geological Substrate	Vegetation	Sampled Tree Species (no. of <i>Phytophthora</i> -Positive/ Sampled Trees)	<i>Phytophthora</i> and <i>Nothophytophthora</i> spp. (no. of Positive Samples) ^{a,b}
F12	N22 20.909 E103 46.199	1895	Hoàng Liên NP	Triassic schists and sandstones	Montane, evergreen broadleaved forest	<i>Acer oliverianum</i> , <i>Eryobotrya cavaleriei</i> & <i>Symplocos quillaminii</i> (1/1) <i>Q. glauca</i> (1/1)	CIN A1 (1) CIN A1 (1)
F13	N22 21.090 E103 49.092	1717	Xin Chài mountain	Triassic schists and sandstones	Montane <i>Alnus</i> forest on steep loamy slope	<i>Alnus nepalensis</i> (2/3)	CIT VII (1), MEA1 (1), ×TRO2 (1), VIE ^e
F14	N22 22.168 E103 52.758	1367	Sau Chua mountain	Triassic schists and sandstones	Montane <i>Chamaecyparis-Quercus</i> forest	<i>Chamaecyparis hodginsii</i> (7/9)	CAS (7) ^{c,e}
F15	N21 3.699 E105 21.733	1108	Ba Vi National Park (NP)	Triassic schists and sandstones and porphyrites	S subtropical humid evergreen forest	<i>Castanopsis chinensis</i> (2/2) <i>C. chinensis</i> & <i>Beilschmiedia fordii</i> (1/1) <i>Dysoxylum juglans</i> , DB (1/1) <i>Eberhardia tonkinensis</i> , <i>Antidesma</i> sp. & <i>Jasminum</i> sp. (0/1) <i>Eurya japonica</i> & <i>Nephelium lappaceum</i> (1/1) <i>Lithocarpus bacgangensis</i> (1/1) <i>Lithocarpus pseudosundaicus</i> (0/1) <i>Machilus bonii</i> (1/1) <i>Magnolia annamensis</i> (1/1) <i>Q. glauca</i> , mix from 3 trees (1/1) <i>Vernicia montana</i> & <i>Antidesma</i> sp. (1/1)	ATT3 (1), CAS (1), CIN A1 (1) ^e CAS (1), HEV (1), PAR (1) ^d ATT3 (1) ^d - ^d CAS (1) ^e CAS (1) ^d - CIN A1 (1) ^e CIN A2 (1) ^e ATT3 (1), CIN A1 (1), HEV (1), PAR (1) ^e ATT3 (1), CIN A1 (1) ^d
F16	N21 04.455 E105 21.810	807	Ba Vi NP	Triassic schists and sandstones and porphyrites	S subtropical humid evergreen forest	<i>Caryodaphnopsis baviensis</i> (0/2) <i>Lithocarpus bacgangensis</i> (1/1) <i>Meliosma arnottiana</i> (1/1) <i>Phoebe petelotii</i> , <i>Machilus thunbergii</i> & <i>Claoxylon indicum</i> (1/1)	- ^e HEV (1) ^e CIT IX (1) ^e CIN A2 (1) ^e
F17	N21 04.587 E105 22.016	713	Ba Vi NP	Triassic schists and sandstones and porphyrites	S subtropical humid evergreen forest	<i>Alsodaphne velutina</i> & <i>Litsea brevipetiolata</i> (1/1) <i>Bischofia javanica</i> & <i>Litsea monocephala</i> (0/1) <i>C. chinensis</i> (1/1) <i>Castanopsis tonkinensis</i> (1/1) <i>Q. glauca</i> (0/1)	- ^e - ^e ATT3 (1), CIN A2 (1) ^d ATT3 (1) ^e - ^e

Table 1. Cont.

Site no.	GPS Coordinates	Altitude (m a.s.l)	Location	Geological Substrate	Vegetation	Sampled Tree Species (no. of <i>Phytophthora</i> -Positive/ Sampled Trees)	<i>Phytophthora</i> and <i>Nothophytophthora</i> spp. (no. of Positive Samples) ^{a,b}
F18	N20 20.876 E105 35.793	392	Cuc Phuong National Park (NP)	Triassic limestones	Tropical evergreen lowland rainforest	<i>C. baviensis</i> & <i>Litsea robusta</i> (0/1) <i>Dracontomelum duppereanum</i> , mix from 2 trees (0/1) <i>Saraca dives</i> , mix from 2 trees (1/1)	- ^e - ^e HEV (1) ^e
F19	N20 20.779 E105 36.099	356	Cuc Phuong NP	Triassic limestones	Tropical evergreen lowland rainforest	<i>Allophylus cobbe</i> , mix from 2 trees (0/1) <i>D. duppereanum</i> & <i>S. dives</i> (0/2)	- ^e - ^{e,h}
F20	N20 20.366 E105 36.452	318	Cuc Phuong NP	Triassic limestones	Tropical evergreen lowland rainforest	<i>A. cobbe</i> , <i>Ficus</i> sp., <i>Merremia boisiana</i> & <i>Homalium</i> sp. (1/1) <i>S. dives</i> (0/2)	MEA2 (1) ^{e,i} - ^e
F21	N20 19.755 E105 36.979	267	Cuc Phuong NP	Triassic limestones	Tropical evergreen lowland rainforest	<i>Anogeissus acuminata</i> (0/1) <i>A. acuminata</i> & <i>Taxotrophis macrophylla</i> (1/2)	- ^d or ^e CIT X (1) ^{e,j}
F22	N20 18.963 E105 38.101	264	Cuc Phuong NP	Triassic limestones	Tropical evergreen lowland rainforest	<i>C. baviensis</i> (0/1) <i>C. baviensis</i> & <i>S. dives</i> (0/1) <i>S. dives</i> , mix from 2 trees (0/1)	- ^e - ^{e,j} - ^e
F23	N12 06.326 E107 09.396	417	Bù Gia Mập National Park	Quaternary alluvial sediments	Tropical evergreen lowland rainforest	<i>Dipterocarpus alatus</i> , <i>Ailanthus triphyssa</i> , <i>Hopea odorata</i> & <i>Dalbergia oliveri</i> (1/1)	HEV (1) ^e
F24	N9 13.645 E104 57.330	4	U Minh Hạ National Park	Quaternary peat	Tropical lowland peat forest	<i>Melaleuca cajuputi</i> (0/3)	- ^k
F25	N8 40.621 E106 34.836	55	Côn Đảo National Park, Côn Lôn island	Rhyolite and diorite	Tropical evergreen lowland rainforest	<i>Chukrasia tabularis</i> (0/1) <i>A. triphyssa</i> , <i>C. tabularis</i> (1/1) <i>Leucaena leucocephala</i> , <i>Canarium album</i> & <i>Hopea odorata</i> (1/1) <i>H. odorata</i> , <i>C. album</i> , <i>D. alatus</i> (1/1)	- ^{e,k} CIT XI ^{e,l} BOT2 ^{e,j} HEV ^{l,m}

^a ATT = *P. attenuata*, ATT 1 = *P. sp. attenuata*-like 1, ATT 2 = *P. sp. attenuata*-like 2, ATT 3 = *P. sp. attenuata*-like 3, BOT2 = *P. sp. botryosa*-like 2, CAS = *P. castaneae*, CHL = *P. chlamydospora*, CIN = *P. cinnamomi*, CIT VII = *P. citricola* VII, CIT IX = *P. citricola* IX, CIT X = *P. citricola* X, CIT XI = *P. citricola* XI, GRE = *P. gregata*, 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. ramorum*; 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 *Phytophythium* sp. also isolated. ^e *Phytophythium vexans* s.l. also isolated. ^f *Phytophythium* 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 *Phytophythium chamaelyphon* also isolated. ^k *Phytophythium cucurbitacearum* also isolated. ^l *Phytophythium vexans* also isolated. ^m *Phytophythium* sp. Côn 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	<i>Phytophthora</i> and <i>Nothophytophthora</i> 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 Fallen leaves/flowers	CAP, ×HET A1, ×HET A1ho, MUV1, RAM A1 ^d 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 Fallen leaves/flowers	RAM A1, SYL2 GAL1, GAL2, MUV1, RAM A1, SYL2, VIE ^{e,f,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, ×HET A1, RAM A1, RAM A2, SYL2, SYL3 ⁱ
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, ×HET 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, ×HET 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	×HET 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 Hoa River; Lào Cai	Hoàng Liên NP; subalpine and montane Rhododendron scrub and forests, montane broadleaved forests, rice fields	Baiting raft	KEL, PSC, ×KUN
R10	N22 19.372 E103 49.780	1193	Forest stream 9, Cat Cat River; Lào Cai	Hoàng Liên NP; montane broadleaved forests	Baiting raft Fallen leaves/flowers	CAP, CIT VII, CIT VIII, SYL1, SYL3 ^e CHL, PSC, QUI, SYL 1, SYL3, RAM A1, VIE ^{f,j,k}
R11	N22 22.230 E103 52.615	1308	Forest stream 8; tributary of Ngòi Duôi River; Lào Cai	Sau Chua mountain; <i>Chamaecyparis hodginsii</i> forest F24; broadleaved mountain forests and <i>Cunninghamia lanceolata</i> plantations	Baiting raft Fallen leaves/flowers	BIT, CIT VII, CIT IX, MAC, QUI, SYL3 CIT IX, KEL, RAM A1 ^l

Table 2. Cont.

Site no.	GPS Coordinates	Altitude (m a.s.l)	River; Province	Location of Catchment and Vegetation	Sampling Method ^a	<i>Phytophthora</i> and <i>Nothophytophthora</i> spp. ^{b,c}
R12	N22 16.787 E104 13.394	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 E105 24.050	59	Stream 9; Hanoi	Ba Vi NP; subtropical evergreen forests, rice fields	Baiting raft	×INS, ×GRE3, ×KUN, ×PER4, ×VIR ^e
R14	N21 3.261 E105 24.012	60	Stream 10, tributary of stream 9; Hanoi	Ba Vi NP; subtropical evergreen forests, rice fields	Baiting raft	×KUN, ×PER 4 ⁱ
R15	N21 06.177 E105 19.267	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 E105 27.218	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. sp.* 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. sp.* gallica-like 1, GAL2 = *P. sp.* gallica-like 2, KEL = *P. sp.* kelmaniana, 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. ×heterohybrida*, ×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. Hoàng Liên also isolated. ^e Unidentified *Pythium* sp. also isolated. ^f *Phytopythium vexans* aff. also isolated. ^g *Pythium senticosum* also isolated. ^h *Pythium* sp. ×ZSF0056-like also isolated. ⁱ *Phytopythium* sp. 1 PB-2013 also isolated. ^j *Phytopythium 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 *Phytopythium 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 Chài 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 Sulawesi (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 ($24.7 \pm 16.8 \mu\text{m}$ vs. $> 50 \mu\text{m}$) 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* (CBS545.96; 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 amphigynous 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. acuminatissima* and *Acer campbellii* in the montane evergreen cloud forest F04 and *A. nepalensis* in stand F13 on Xin Chài mountain, a range of *Pythium* and *Phytophythium* 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. gilva* 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. drechsleri*, *P. macrochlamydospora*, *P. pseudocryptogea*, *P. ramorum*, *P. xheterohybrida*), five informally designated taxa (*P. citricola* VII, *P. sp. kelmaniana*, *P. sp. xinsolita*-like, *P. sp. xkunnunara*-like, *P. sp. xvirginiana*-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. xheterohybrida* 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. ×heterohybrida* 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 amphigynous antheridia and nonpapillate sporangia of the Vietnamese isolates matched the original description of *P. ×heterohybrida* [22].

Phytophthora pseudocryptogea and *P. sp. 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 *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 *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. 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. *Phytophthora sp. gallica-like 1* and *P. sp. 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. *Phytophthora sp. gallica-like 1* produces globose chlamydospores like *P. gallica* whereas *P. sp. gallica-like 2* does not form chlamydospores.

From Clade 9a2, *P. macrochlamydospora* and a new species related to *P. quininea*, informally designated as *P. sp. quininea-like*, co-occurred in montane forest stream R11. Isolate VN1006 of *P. macrochlamydospora* differed in the ITS (816 bp alignment) and in *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. macrochlamydospora* [2], the Vietnamese isolate was sterile and produced large chlamydospores and semipapillate to non-papillate sporangia. The four isolates of *P. sp. quininea-like* were separated in the ITS and *cox1* from the ex-type isolate of *P. quininea* (CBS407.48 = P8488; HQ261660; AY564200 + HQ708386) from Peru by differences of 7 and 27 bp, respectively. Like *P. quininea*, *P. sp. 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. quininea* [2] but can be distinguished from the latter by forming amphigynous instead of paragynous antheridia. Only one isolate of the Clade 9a1 hybrid taxon *P. sp. ×kunnunara-like* could be obtained from one lower montane stream (R09) in Hoàng Liên NP (Table 2).

In contrast to the montane streams, the *Phytophthora* communities in the five lowland rivers with subtropical to tropical climate were dominated by *Phytophthora* taxa from the high-temperature tolerant Clades 9a1 and 9a3. From Clade 9a1 potential hybrid isolates related to *P. 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. 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. ×virginiana-like 1, 2, and 3* from Taiwan. Therefore, these Vietnamese isolates are informally designated as *P. sp. ×virginiana-like sensu lato*. *Phytophthora sp. ×kunnunara-like* was found in the Red River and the two streams originating from Ba 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. ×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. ×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 nonpapillate 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 vietnamensis was isolated from the montane forest streams R01, R02 and R10 in Hoàng Liên NP. In addition, the novel *Elongisporangium sp.* Hoàng Liên, *Phytophythium 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* 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. acuminatissima* and sometimes also *Neolitsea poilanei* 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. acuminatissima* and *N. polycarpa*, all infested by *P. cinnamomi* A2 and/or *P. castaneae*, exhibited severe losses of lateral and fine roots and open callusing 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. acuminatissima* tree (Figure 3e).

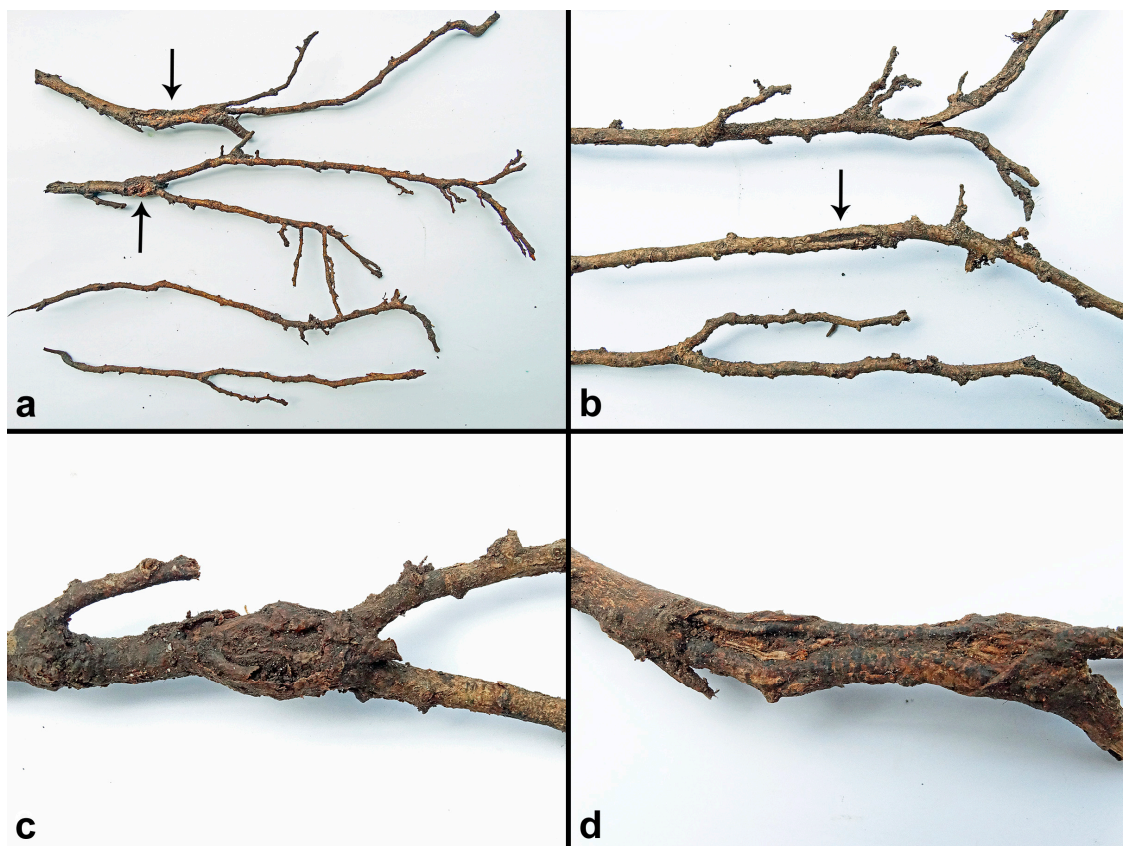


Figure 4. Symptoms on root systems of declining *Neolitsea polycarpa* trees in the montane evergreen cloud forest F10 in Hoàng Liên National Park associated with presence of *P. cinnamomi* A2 in the surrounding soil; (a,b) severe losses of lateral roots and fine roots and open callusing lesions on coarse roots (arrows); (c,d) detailed view of the open callusing 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 *Phytophythium* 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. ×heterohybrida*, *P. sp. ×insolita*-like, *P. sp. ×kunnunara*-like and *P. sp. ×virginiana*-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 harboured 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),

and because living isolates are needed for taxonomic descriptions and host range testing, baiting, and metagenomic approaches should be carried out in parallel.

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 *Phytophthoras* 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 Vì 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 Vì 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 juglans* in Ba Vi National Park. Another Clade 7a species that was first described from Taiwan, *P. xheterohybrida*, 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. xbotryosa*-like, *P. sp. xmeadii*-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. xbotryosa*-like and *P. sp. xmeadii*-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. ×insolita*-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. ×insolita*-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 multicopy 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.

Panglobally 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*, *Phytopyithium* and *Pythium* isolates from Vietnamese forests and rivers and isolates from related *Phytophthora* species used for comparisons.

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