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Diversity of *Phytophthora* species in natural ecosystems of Taiwan and association with disease symptoms

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In 2013 a survey of *Phytophthora* diversity was performed in 25 natural and semi-natural forest stands and 25 rivers in temperate montane and subtropical lowland regions of Taiwan. Using baiting assays, ten described species and 17 previously unknown taxa of *Phytophthora* were isolated from 71.5% of the 144 rhizosphere soil samples from 33 of 40 tree species sampled in 24 forest stands ,and from 19 rivers: *P. capensis, P. citrophthora, P. plurivora*, *P. tropicalis, P. citricola* VII, *P.* sp. x botryosa-like, *P.* sp. x meadii-like and *P.* sp. occultans-like from Clade 2; *P. palmivora* from Clade 4; *P. castaneae* and *P. heveae* from Clade 5; *P. chlamydospora* and *P.* sp. forestsoil-like from Clade 6; *P. cinnamomi* (*Pc*), *P. parvispora*, *P. attenuata* nom. prov., *P. flexuosa* nom. prov., *P. formosa* nom. prov., *P. intricata* nom. prov., *P.* x *incrassata* nom. prov. and *P.* x *heterohybrida* nom. prov. from Clade 7; *P.* sp. *palustris* and five new hybrid species from Clade 9. The A1 mating type of *Pc* was widespread in both montane and lowland forests and rarely associated with disease, whereas the A2 mating type was limited to lowland forests and in some cases causing severe dieback. Most other *Phytophthora* species were not associated with obvious disease symptoms. It is concluded that (1) Taiwan is within the center of origin of most *Phytophthora* taxa found, (2) *Pc*A2 is an introduced invasive pathogen, and (3) interspecific hybridisations play a major role in speciation and species radiations in diverse natural ecosystems.

Key words: biosecurity, breeding systems, hybridisation, forest survey, river survey, centre of origin

Introduction

Many devastating declines of trees and natural ecosystems are driven by non-native *Phytophthora* species which remain unnoticed in their native environment and after their introduction to other continents became invasive, threatening a non-adapted flora which due to a lack of co-evolution contained a high number of susceptible species. Well documented examples include *P. cinnamomi* (Erwin & Ribeiro 1996; Hardham 2005), *P. lateralis* (Erwin & Ribeiro 1996), *P. plurivora* (Jung & Burgess 2009; Jung *et al.* 2013) and *P. ramorum* (Rizzo *et al.* 2002).

Due to its diversity of geology, geomorphology, macroclimate and orographic climates, the complex palaeoclimatic history, the repeated immigration of plant species from northern latitudes, the temporary connection of a multitude of islands to the mainland during glacial periods in the pleistocene followed by separation during interglacials, Southeast Asia is a hotspot of plant diversity (Gower *et al.* 2012). There is an accumulating body of evidence that *P. cinnamomi*, *P. lateralis*, *P. plurivora* and other important *Phytophthora* pathogens with global distribution are native to Southeast Asia suggesting this region might be one center of origin of the genus *Phytophthora*: the widespread occurrence of devastating Phytophthora diseases of horticultural trees and crops like *Hevea brasiliensis* (*Phytophthora botryosa*, *P. capsici*, *P. citrophthora*, *P. heveae*, *P. meadii*, *P. palmivora*), *Theobroma cacao* (*P. cactorum*, *P. heveae*, *P. meadii*, *P. palmivora*), *Citrus* spp. (*P. citrophthora*), *Colocasia esculenta* (*P. colocasiae*), *Durio zibethinus* (*P. nicotianae*, *P. palmivora*), *Cinnamomum burmannii* and *Cinnamomum osmophloeum* (*P. cinnamomi*), *Piper nigrum* (*P. capsici*, *P. cinnamomi*, *P. nicotianae*) and various horticultural herbs (*P. citrophthora*, *P. nicotianae*, *P. palmivora*, *P. tropicalis*) (Chang *et al.* 1996; Erwin & Ribeiro 1996; Ho and Lu 1997; Drenth and Guest 2004; Zeng *et al.* 2009; Ann *et al.* 2010); occurrence of both mating types of several heterothallic *Phytophthora* species including *P. botryosa*, *P. capsici*, *P. cinnamomi*, *P. colocasiae*, *P. meadii*, *P. nicotianae* and *P. palmivora* (Ko *et al.* 1978, 2006; Arentz & Simpson 1986; Ho 1990; Erwin & Ribeiro 1996); and apparent absence of Phytophthora epidemics in natural ecosystems despite of presence of several *Phytophthora* species (Ko *et al.* 1978, 2006; Arentz & Simpson 1986; Ho & Lu 1997; Zeng *et al.* 2009; Brasier *et al.* 2010; Vettraino *et al.*, 2011; Huai. *et al.*, 2013). *Phytophthora cinnamomi*, arguably the world' s most notorious and widespread plant pathogen, was first described causing stripe cankers of cinnamon trees in Sumatra and it was suspected that the pathogen jumped from healthy surrounding rainforests onto the plantation trees (Rands 1922). In Northwestern Yunnan, *P. chlamydospora*, *P. cryptogea*, *P. gonapodyides*, *P. gregata*, *P. lacustris*, *P. plurivora* and two previously unknown *Phytophthora* species were isolated from soil samples of healthy undisturbed oak forests and from forest streams (Huai *et al.* 2013). In a remote subtropical to

temperate forest area in Nepal, *P. plurivora* (previously named *P. citricola*), *P. nicotianae* and *P. himalsilva* were common in the rhizosphere of a range of broadleaved tree species without causing obvious disease symptoms (Vettraino *et al.* 2011). *Phytophthora castaneae* (previously named *P. katsurae*), *P. cinnamomi*, *P. heveae*, *P. insolita*, *P. nicotianae* and *P. plurivora* were widespread in soils and streams of protected, healthy montane forests in the tropical island of Hainan (Zeng *et al.* 2009). The best studied region of Southeast Asia regarding *Phytophthora* diversity is the island of Taiwan. Re-examining the original *Phytophthora* cultures collected by Sawada between 1911 and 1943 and reviewing more recent literature, Ho (1990) lists 21 valid *Phytophthora* species of which *P. castaneae*, *P. cinnamomi* and *P. heveae* were recovered from soils of natural forests without causing disease (Ko *et al.* 1978, 2006). More recently, Brasier *et al.* (2010) detected *P. bisheria*, *P. cinnamomi* and *P. lateralis* in healthy, undisturbed montane forest of *Chamaecyparis obtusa*.

Taiwan is located 130 km from mainland China between the Taiwan Strait and the Pacific around the tropic of Cancer and, despite its small size of 35.800 km^2, harbours more than 4000 indigenous plant species of which more than 1000 are endemic (Chang-Fu *et al.* 1994). This high diversity and degree of endemism is related to Taiwan' s complex geology and geomorphology, the latter causing highly diverse precipitation patterns during winterly Northeast monsoons, and repeated temporary connections of the island to the Eastern Asiatic and Malesian Floristic Regions during glacial periods alternating with periods of isolation during the interglacials (Chang-Fu & Chung-Fu 1994; Chang-Fu *et al.* 1994; Chung-Fu 1994). The Taiwanese flora contains 90 and 40 native species in 14 and 7 genera of the Lauraceae and Fagaceae, respectively, (http://www.efloras.org) both families known for the high susceptibility of many of their European and North American members to *Phytophthora* species (Rizzo *et al.* 2002; Jung 2009; Jung & Burgess 2009; Hansen *et al.* 2012; Jung *et al.* 2013). Consequently, a high diversity of unknown *Phytophthora* species, posing a potential threat to European forests and natural ecosystems as a result of their co-evolution with tree species from genera also present in Europe, might be expected.

In March and August 2013, in the frame of a collaborative research project between the University of Algarve and the Taiwanese Forestry Research Centre, a survey of *Phytophthora* diversity was performed in a diverse range of natural forest types and river systems across Taiwan. This paper reports on the results of this *Phytophthora* survey and the association of *Phytophthora* spp. with disease symptoms of forest trees in Taiwan, and discusses the potential threat posed by previously unknown *Phytophthora* spp. to European and North American forests.

Material and methods

Sampling and Phytophthora isolation

Twenty-five forest sites covering a wide range of tree species, climates and landscapes across Taiwan were selected for sampling (Figs. 1 and 2a-f). In addition, 25 rivers and streams were randomly selected during three trips around Taiwan for sampling soils from the forest sites (Fig. 1). Soil sampling and isolation methodology was according to Jung (2009). In total, 144 rhizosphere soil samples were taken from mature trees of 38 species in 24 natural forest stands and from young trees of 6 *Quercus* spp. and *Rhododendron* spp. in an arboretum (site F02) established ca 20 years prior in a natural subtropical *Castanopsis* - *Machilus* forest (F01; Table 1). Three soil monoliths with a size of 20x30 cm 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 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 a walk-in growth chamber with 12 hrs natural daylight using 3- to 10-day-old leaflets of native species, mainly *Quercus variabilis* and *Castanopsis indica*, as baits floated over flooded soil. Brownish leaflets were examined at x80 under a light microscope for presence of *Phytophthora* sporangia. Infected leaflets were blotted dry, 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). Petri dishes were incubated at 20°C in the dark and examined for *Phytophthora*-like hyphae after 24-48 hours. In cases where no *Phytophthora* grew from leaves with typical *Phytophthora* sporangia on PARPNH, additional leaves were plated onto non-selective potato-dextrose agar (PDA; Oxoid Ltd., UK).

Phytophthora isolations from the 25 rivers and streams were performed using a modified *in situ* baiting technique (Reeser *et al.* 2011; Hüberli *et al.* 2013). The baiting sites were mainly located inside or downstream of natural forests (Fig. 2g-h). Fly mesh and styrofoam were used to prepare 25x30cm bait bags rigged to float on the water surface (in the following called rafts; Fig. 2h). At each site, 10-15 non-wounded young leaves of *Q. variabilis* and other native *Quercus* spp., *C. indica*, *Rhododendron* spp., *Citrus sinensis* and in some cases *Chamaecyparis obtusa*, *Cinnamomum camphora*, *Cinnamomum iners*, *Coffea arabica*, *Podocarpus nakaii* and *Zelkova serrata*, were placed as baits in a raft, and the raft put to float at a place where water flow was calm. The rafts were collected after 2-3 days. Baiting leaves were washed in distilled water and blotted dry on filter paper. Five to ten pieces (approximately 2x2 mm) were cut from the margins of each watersoaked or necrotic lesion of each leaf, blotted again on filter paper and plated onto PARPNH agar.

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; Scanu *et al.* 2014) at 10°C in the dark.

Molecular identification of isolates

For all *Phytophthora* isolates obtained in this study mycelial DNA was extracted from pure cultures grown in V8-juice medium. Total DNA was extracted using the DNeasy Plant Mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions and checked for quality (gel electrophoresis on 0.7% agarose gels stained with ethidium bromide) and quantity (spectrophotometry, absorbance at 260nm). 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 the forward primers ITS1 (White *et al.* 1990) or ITS6 (Cooke *et al.* 2000) and the reverse primer ITS4 (White *et al.* 1990). For representative isolates of several known and all putative new species the mitochondrial *cox1* gene was amplified with primer-pairs COXF4N/COXR4N and FM84/FM83 as described by Kroon *et al.* (2004) and Martin & Tooley (2003), respectively. The PCR reaction mixture and the amplification conditions for ITS and *cox1* were as described by Cooke *et al.* (2000), Nagy *et al.* (2003), Martin & Tooley (2003) and Kroon *et al.* (2004), respectively. PCR consumables were provided by Thermo Fisher Scientific Inc. (Waltham, MA USA). Thermocycles were carried out using Bio-Rad C1000™ or Applied Biosystems® 2720 Thermal Cyclers. PCR products were purified and sequenced by Macrogen Europe (Amsterdam, the Netherlands) in both directions with the primers used for PCR amplification.

Sequences were edited using the Sequencher software (Version 4.7, Gene Codes Corporation). Heterozygous sites observed were labelled according to the IUPAC coding system. Consensus sequences were aligned using the CLUSTAL W program. 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 sequences from representative isolates of all *Phytophthora* species and *cox1* sequences of representative isolates of several known species and all putative new species obtained in this study were deposited at GenBank and accession numbers are given in Supplementary Table 1S.

Classical identification of isolates

Colony growth patterns of 7-d-old cultures grown at 20°C in the dark on V8 agar (V8A), malt-extract agar (MEA; Oxoid Ltd., UK) and PDA (Jung *et al.* 2011) and morphological features of sporangia, oogonia, antheridia, chlamydospores, hyphal swellings and aggregations were compared with known isolates and with species descriptions in literature.

Induction of sporangia formation and microscopic examinations and measurements of morphological structures at x200 and x400 were according to Jung *et al.* (2011) using a compound microscope (Zeiss Imager.Z2), a digital camera (Zeiss Axiocam ICc3) and a biometric software (Zeiss AxioVision). Self-sterile isolates were paired on V8A and CA with known A1 and A2 mating type tester strains of *P. cinnamomi* and *P. cambivora* (isolates with non-papillate sporangia) or *P. botryosa*, *P. colocasiae* and *P. meadii* (isolates with papillate sporangia), and examined after 4–6 weeks incubation at 20°C in order to determine whether they are heterothallic or sterile (Jung *et al.* 2011). Selected isolates from putative new species were also mated with tester strains using the polycarbonate membrane method to produce selfed sexual structures (Yang & Hong 2013). All isolates are preserved in the culture collection maintained at the University of Algarve.

Results

In total 401 isolates of 10 described species and 17 previously unknown taxa of *Phytophthora* were obtained from forest stands (Table 1) and river systems (Table 2) in Taiwan. GenBank accession numbers of ITS sequences of representative isolates of all 27 *Phytophthora* taxa and different haplotypes of *P. cinnamomi*, and of *cox1* sequences of representative isolates of several described species and all new *Phytophthora* taxa are given in Supplementary Table 1S. Polymorphisms in ITS sequences differentiating Taiwanese haplotypes and the ex-type of *P. cinnamomi* are presented in Supplementary Table 2S. Polymorphisms in ITS and *cox1* sequences separating most of the new *Phytophthora* species from Clades 2, 7 and 9 from closely related

known species are shown in Supplementary Tables 3S-8S. Detailed descriptions of morphological characteristics, morphometric data, temperature-growth data and multigene phylogenies for all new *Phytophthora* species will be presented in separate publications.

***Phytophthora* diversity in natural and semi-natural forest stands of Taiwan**

In 24 forest stands (96%) 17 *Phytophthora* taxa were isolated from 103 of the 144 rhizosphere soil samples (71.5%) of 33 of the 40 tree species sampled (82.5%): *P. castaneae, P. chlamydospora*, *P. cinnamomi*, *P. citrophthora, P. heveae, P. palmivora, P. parvispora, P. plurivora* and a closely related new species from the '*P. citricola* complex', two new species related to *P. botryosa* and *P. meadii*, four new species from Clade 7a and two new species from Clade 9 (Table 1). The only forest site from which no *Phytophthora* isolates could be obtained was the montane, warm-temperate *Quercus glauca* - *Q. variabilis* forest on the dry rocky peak of Huagang Mountain (F13). Here, however, an unknown *Phytophthora* species was abundantly forming papillate and bi-papillate, persistent sporangia of variable shapes on many *Q. variabilis* baiting leaves but could not be cultured after plating them onto both selective PARPNH agar and PDA despite repeated attempts.

Phytophthora cinnamomi was isolated from 59 rhizosphere soil samples (41%) of 23 tree species (57.5%) in 15 of the 25 forest stands sampled (60%) making it by far the most widespread and common species. The A1 mating type of *P. cinnamomi* was present in 14 montane and lowland forests with an altitudinal amplitude ranging from 365 to 2287 m above sea level (asl). In contrast, the A2 mating type was only found in seven lowland forests located between 365 and 733 m asl (Table 1; Fig. 1). Both mating types co-occurred in six lowland forests. Interestingly, in three stands A1, A2 and homothallic A2 isolates, the latter stimulating oogonia formation in A1 isolates and readily producing oogonia in single culture, were found whereas none of the 52 A2 isolates from the *Castanopsis-Machilus* forest in Lenhuachih were able to produce oogonia in single culture (Table 1). Over all stands, the A1:A2 mating type ratio of the 183 isolates was 53.6 : 46.4 whereas in the six lowland stands with co-occurrence of both

mating types the A1:A2 ratio of the 134 isolates was 41.8 : 58.2. In the *Castanopsis-Machilus* forest in Lenhuachih the A1:A2 mating type ratio of the 68 isolates was 33.5 : 76.5. The alignment of the ITS sequences including a short part of the 18S region of the 183 isolates from this study and the ex-type isolate (CBS 144.22; GenBank no. HQ643189) from Sumatra revealed eight polymorphic sites, six indels and one deletion. The Taiwanese isolates belonged to eight haplotypes that differed from each other and from the ex-type isolate by 1-7 basepairs (bp) (Tables 1S, 2S). Four of the eight haplotypes comprised isolates from both mating types. Each of the other four haplotypes was only represented by one isolate, two of which were A1 and two were A2 (Table 2S). Single isolate haplotypes 1 and 8 had two and one heterozygous positions, respectively (Table 2S). The dominant haplotype 4 which was present in 13 stands comprised 72 A1, 54 A2 and 5 homothallic A2 isolates. With the exception of the montane coniferous forest in Chilan, where A1 isolates from haplotypes 4 and 6 were found, the seven montane forest stands infested by *P. cinnamomi* exclusively contained A1 isolates from haplotype 4. In contrast, in the seven lowland forests with presence of both mating types between two and four different haplotypes per stand were found. With each four haplotypes the highest diversity was found in the *Castanopsis-Machilus* forest F02 in Fushan (Fig. 2d) and in the evergreen *Quercus* forest F24 on the Hengchun Peninsula.

Although having been less frequently isolated than *P. cinnamomi*, *P. castaneae* (previously *P. katsurae*) from Clade 5 showed a similar altitudinal amplitude (440 - 2182 m asl) and geographical distribution as the A1 mating type of *P. cinnamomi* (Fig. 1). *Phytophthora castaneae* was isolated from *Castanopsis*, *Lithocarpus*, *Quercus* and *Machilus* spp. in four subtropical lowland stands, including the arboretum F01 in Fushan, the *Castanopsis-Machilus* forests F02 (Fig. 2d) and F15 in Fushan and Lenhuachih, and the broadleaved evergreen forest F20 on the southernmost Hengchun peninsula (Fig. 2f). In addition, it was recovered from *Fagus hayatae* and *Chamaecyparis formosensis* in the cool-temperate montane forests F04 (Fig. 2b) and F08 (Table 1; Fig. 1). All isolates matched the ITS sequence of the ex-type of *P. castaneae* (ICMP 19434; GenBank no. KP295319) except for one isolate from Fushan with two polymorphisms

and two indels of 3 bp and 1 bp, respectively, and two isolates from Hengchun Peninsula with another polymorphism (Table 1S). *Phytophthora heveae* from Clade 5 showed a more limited altitudinal distribution than its relative *P. castaneae* and was only found in four subtropical broadleaved forests below 365 m asl (Table 1; Figs. 1, 2e). The ITS and *cox1* sequences (Table 1S) matched the ex-type of *P. heveae* (CBS296.29; GenBank nos. HQ643238 and HQ643238). The morphology of all isolates of *P. castaneae* and *P. heveae* was congruent with the original descriptions (Erwin & Ribeiro 1996).

Phytophthora plurivora from Clade 2c was isolated from *Alnus formosensis* and *Liquidambar formosensis* at 1848 m asl in the montane, temperate and seasonally dry deciduous forest F12 in a remote area of the Central Mountain Ridge near Tunyuan (Table 1; Figs. 1, 2c). The morphology of the isolates and their ITS and *cox1* sequences (Table 1S) conformed to the original description (ex-type CBS 124093; GenBank nos. FJ665225 and KC855435; Jung & Burgess 2009). In a neighbouring montane forest (F11) at 2023 m altitude a new species from the '*P. citricola* complex', designated as *P. citricola* VII, was isolated from the rhizosphere of *Q. variabilis* trees (Table 1; Fig. 1). It differed from its closest relatives *P. citricola* III (isolate 1E1; GenBank nos. of ITS and cox1 FJ392326 and GU071239), *P. citricola* sensu stricto (IMI021173; FJ237526 and FJ237512), *P. pini* (CBS181.25; FJ392322 and GQ247650) and *P. plurivora* (CBS 124093; FJ665225 and KC855435) in ITS by 2, 3, 5 and 5 bp, respectively, and in *cox1* by 7, 13, 5 and 8 bp, respectively. Similar to all known species from the '*P. citricola* complex', its morphology was characterised by a homothallic breeding system, paragynous antheridia and variable semipapillate sporangia.

A1 mating type isolates of *P. parvispora* which differed in ITS (Table 1S) from the ex-type of *P. parvispora* (CBS 132772; KC478667) by one heterozygous site were isolated from *Glochidion rubrum* in a subtropical lowland swamp forest on the Hengchun peninsula together with *P. cinnamomi* A1 (Tables 1, 1S; Fig. 1). Three other known *Phytophthora* species were also only sporadically isolated: *P. chlamydospora* from wet soil of ephemeral streambeds in two

Chamaecyparis obtusa forests at Taiping Mountain and in Chilan above 1900 m altitude; *P. citrophthora* from *Q. tarokoensis* in Fushan arboretum and *P. palmivora* A1 from *Zelkova serrata* in a subtropical, evergreen lowland forest on the Hengchun Peninsula (Tables 1, 1S; Fig. 1).

Four new homothallic *Phytophthora* species from Clade 7a were detected in four natural forest stands and in the arboretum in Fushan (Tables 1, 1S; Fig. 1). They were clearly separated from each other and from their closest relatives by morphological characters, colony growth patterns, temperature-growth rates and multigene sequence differences which will be presented in detail elsewhere. *Phytophthora attenuata* nom. prov., which differed from its closest relatives *P. fragariae* and *P. rubi* in ITS by 6-7 bp and in *cox1* by 14 bp and 10 bp, respectively, (Tables 3S, 4S) and in morphology by the production of ornamented oogonia with tapering bases, was recovered from *C. formosensis* and *Castanopsis carlesii* in two cool-temperate montane forests (F08, F10) growing above 2170 m altitude in Sheipa National Park (Figs. 1, 2a). *Phytophthora flexuosa* nom. prov., the closest relative of *P. europaea* (differences in ITS and *cox1* of 1-2 bp and 9 bp, respectively; Tables 3S, 4S), was isolated alongside *P. castaneae*, *P. cinnamomi* A1 and *Elongisporangium undulatum* from *Fagus hayatae* in a cool-temperate montane forest on a remote mountain ridge in Taipingshan (F04; Figs. 1, 2b). Morphologically, it differed from *P. europaea* mainly by the production of ornamented, irregular-flexuose oogonia. *Phytophthora formosa* nom. prov., which is separated from *P. attenuata* nom. prov. in ITS and *cox1* by 6 bp and 8-9 bp, respectively, (Tables 3S, 4S) was associated with *Araucaria cunninghamii* in the subtropical *Castanopsis-Machilus* forest in Lenhuachih (F15) and with *Quercus glandulifera* in the arboretum in Fushan (F01) (Fig. 1). *Phytophthora intricata* nom. prov., the closest relative of *P. formosa* nom. prov. (differences in ITS and *cox1* of 5 bp and 6-7 bp, respectively; Tables 3S, 4S) was exclusively found in the rhizosphere of *Quercus tarokoensis* trees in the arboretum in Fushan (F01). Morphologically, this species is characterised by twisted, intermingling oogonial and antheridial stalks and thickwalled oospores.

Two new species from Clade 2a, informally designated as *P.* sp. x botryosa-like and *P.* sp. x meadii-like, were recovered from the rhizosphere of *C. camphora*, *Styrax suberifolia*, *Glochidion philippicum* and *Trema australis* trees growing in three subtropical, lowland monsoon forests (F17-F19) along the Pacific coast (Tables 1, 1S; Figs. 1, 2e). *Phytophthora* sp. x botryosa-like was separated from its closest relative *P. botryosa* by sequence differences of 7-13 and 16-19 bp in ITS and *cox1*, respectively, and from *P. meadii* by 11-15 and 5-12 bp in ITS and *cox1* (Tables 5S, 6S). *Phytophthora* sp. x meadii-like differed in ITS and *cox1* from its closest relative *P. meadii* by sequence differences of 5-13 and 5-12 bp, respectively, and from *P. botryosa* by 7-14 and 16-18 bp, respectively (Tables 5S, 6S). The occurrence of 10 heterozygous sites in their ITS sequences with 0-5 and 3-7 heterozygous sites per isolate, respectively, suggests a hybrid origin for both *P.* sp. x botryosa-like and *P.* sp. x meadii-like (Tables 5S, 6S). All isolates of both species belong to the A1 mating type, forming oogonia when mated with A2 tester strains of both *P. botryosa* and *P. meadii*, and produce papillate, bi-papillate or tri-papillate, variable and often caducous sporangia with short to medium-length pedicels suggesting an aerial lifestyle. In one of these lowland monsoon forests (F17) also a new species from Clade 9, *P.* sp. x Kunnunara-like, was isolated which is related to *P.* sp. Kunnunara (Tables 1, 1S, 7S, 8S; Fig. 1). A new sterile *Phytophthora* species was detected in the subtropical lowland swamp forest F20 on the Hengchun peninsula and is informally designated as *P.* sp. palustris (Tables 1, 1S; Fig. 1). This taxon was only distantly related to its nearest relatives in Clade 9, *P.* sp. x Hennops from river systems in South Africa (61 and 17 bp sequence differences in ITS and *cox1*, respectively, to isolate CMW33363; GenBank nos. GU799663 and GU799640), *P. virginiana* (63 and 14 bp sequence differences in ITS and *cox1*, respectively, to the ex-type isolate 46A2; GenBank nos. KC295544 and KC295546) and *P.* sp. lagoariana (66 and 12 bp sequence differences in ITS and *cox1*, respectively, to isolate P8220, GenBank nos. HQ261695 and HQ261442).

Besides many *Pythium* isolates which were not identified to species level, *Phytopythium vexans* was recovered from seven subtropical forests (F02 and F15-F19, F21), whereas *Elongisporangium undulatum* and *Elongisporangium anandrum* were detected in two (F04, F06) and one (F08) montane temperate forest, respectively (Table 1).

Altitude had a strong influence on the composition of the *Phytophthora* populations in Taiwanese forest stands. *Phytophthora attenuata* nom. prov., *P. flexuosa* nom. prov., *P. chlamydospora*, *P. plurivora* and *P. citricola* VII only occurred in montane stands. In contrast, *P. cinnamomi* A2, *P. citrophthora*, *P. heveae*, *P. intricata* nom. prov., *P. palmivora*, *P. parvispora*, *Phytophthora* sp. x botryosa-like, *P.* sp. x meadii-like, *P.* sp. x Kunnunara-like, *P.* sp. palustris and *P. formosa*, the closest relative of the montane species *P. attenuata* nom. prov., were exclusively found in lowland forests. The only species with a wide altitudinal amplitude were *P. cinnamomi* A1 and *P. castaneae*.

Phytophthora diversity in natural rivers of Taiwan

Using rafts with leaves of *Q. variabilis*, *C. sinensis*, *C. indica*, *Rhododendron* spp. and in some cases *C. camphora*, *C. iners*, *Z. serrata* and other tree species as *in situ* baits, four known and 12 previously unknown *Phytophthora* species were isolated from 19 of the 25 rivers tested (76%). Due to a typhoon with more than 500 ml of precipitation within a few hours and the steep terrain, all tested rivers running from the Central Mountain Ridge to the Pacific coast including three of the six *Phytophthora*-negative rivers, Lee-wu River, Ma-tai-an River and La-ku-la-ku River, experienced severe floodings with extremely fast currents during the baiting period in August 2013. Four rivers that were originally included, Bei-gang River, North Qing-shui River, Wan-lee-qiao River and Fang-ping River, had to be discarded since the baiting rafts were found hanging dry in the canopy of riparian shrubs or buried underneath rocks.

Most common were five new species from Clade 9 which were recovered from 12 watercourses located around the island (Table 1; Fig. 1). ITS and *cox1* sequences of *P.* sp. x insolita-like (Table 1S) differed from its closest relative *P. insolita* (ex-type IMI288805; AF271222 and GU945482) by 6-12 bp and 4-9 bp, respectively. The ITS sequences of eight isolates from five rivers had in total 13 heterozygous sites with 2-8 heterozygous sites per isolate. There were no heterozygous sites in the *cox1* sequences indicating that this taxon originated from sexual interspecific hybridisation. Similar to *P. insolita*, *P.* sp. x insolita-like produces oogonia without antheridia in single culture, non-papillate, internally proliferating sporangia, irregular hyphal swellings and thin-walled globose chlamydospores. The four new species *P.* sp. x virginiana-like 1 (5 rivers), *P.* sp. x virginiana-like 2 (7 rivers), *P.* sp. x virginiana-like 3 (5 rivers) and *P.* sp. x Kunnunara-like (9 rivers) were separated from their closest relatives *P. virginiana*, *P.* sp. lagoariana and *P.* sp. Kunnunara by ITS sequence differences of 1-12 bp (Tables 1S, 7S). The occurrence of in total 14, 24, 18 and 20 heterozygous sites, respectively, with 1-14 heterozygous sites per isolate, strongly suggests interspecific hybridisation (Table 7S). *Cox1* sequences suggest as maternal parents *P.* sp. lagoariana for *P.* sp. x virginiana-like 1 and *P.* sp. x virginiana-like 3, *P. virginiana* or *P.* sp. x Hennops for *P.* sp. x virginiana-like 2 and *P.* sp. Kunnunara for *P.* sp. x Kunnunara-like (Tables 1S, 8S). All four taxa are of silent A1 mating type (not forming oogonia but stimulating oogonia formation in A2 tester strains of *P. cinnamomi* in polycarbonate membrane mating tests) and produce globose, club-shaped and irregular swellings and globose thin-walled chlamydospores, typical features of aquatic Clade 9 species including *P. virginiana* (Yang & Hong 2013).

Phytophthora capensis from the '*P. citricola* complex' in Clade 2c was isolated from a tributary of Hapen River in Fushan and a tributary of Bei-shih river neir Pingling Township, both located in the northeast part of Taiwan (Table 2; Fig. 1). The ITS and *cox1* sequences (Table 1S) and the morphology of all isolates were congruent with the original description (Bezuidenhout *et al.* 2010). From Cu-keng River in Fushan and from the tributaries of Hapeng River and Bei-shih River, a *Phytophthora* species from Clade 2a was recovered which in ITS shows 100% identity to

P. occultans isolates from Europe and Oregon (Man In' t Veld *et al.* 2015; Reeser *et al.* 2015) and differences of 2 bp to *P. himalsilva*, 3 bp to *P. terminalis*, and 3-5 bp to *P. citrophthora* (Vettraino *et al.* 2011; Tables 2, 1S, 5S). However, in *cox1* the isolates from Taiwan were much closer to *P. himalsilva* from Nepal (7 bp difference to the ex-type isolate) than to *P. occultans* (28 bp difference) and *P. citrophthora* (28-30 bp) (Tables 1S, 6S). This new taxon which is informally designated as *P.* sp. occultans-like shares with both *P. occultans* and *P. himalsilva* the homothallic breeding system and the production of papillate, highly variable caducous sporangia. The other two new species from Clade 2a, *P.* sp. x botryosa-like and *P.* sp. x meadii-like, were both recovered from Xiao-Qingshui Creek which is flowing from Taroko National Park through a subtropical evergreen monsoon forest (F16) to the Pacific coast (Tables 2, 1S; Fig. 1). The Clade 2b species *P. tropicalis* was detected in two tributaries of Da-jia River (Table 2; Fig. 1) which are surrounded by both evergreen broadleaved forests and plantations of betel nut (*Areca catechu*). *Phytophthora parvispora* from Clade 7 was also isolated from one of these streams and from the Ma-zhu-kung and Shui-she-shui-wei Rivers (Table 2), all located in the Western foothills of Taiwan (Fig. 1). All isolates of *P. tropicalis* and *P. parvispora* belonged to the A1 mating type and their morphology and ITS sequences (Table 1S) matched the original descriptions (Aragaki & Uchida 2001; Scanu *et al.* 2014).

Phytophthora formosa nom. prov. and two other new species from Clade 7a were isolated from the tributary of Hapen River upstream of the arboretum in Fushan, and from Hapen River and Cu-keng River flowing through the subtropical *Castanopsis-Machilus* forests in Fushan (Table 2; Fig. 1): *Phytophthora* x *heterohybrida* nom. prov. and *P.* x *incrassata* nom. prov. share main morphological characters with their closest relative *P. cambivora* such as fluffy colonies on all agar media used, a heterothallic breeding system, production of large non-papillate sporangia and ornamented oogonia with amphigynous antheridia and absence of chlamydospores. The ITS sequences of *P.* x *heterohybrida* nom. prov. and *P.* x *incrassata* nom. prov. show 11-19 bp and 7-14 bp differences to *P. cambivora* and have four and five heterozygous sites, respectively, indicating interspecific hybridization (Tables 1S, 3S). The *cox1* sequences of *P.* x *heterohybrida*

nom. prov. and *P.* x *incrassata* nom. prov. differed from *P. cambivora* by 7 bp and 13 bp, respectively (Tables 1S, 4S). All isolates of *P.* x *incrassata* nom. prov. belonged to the A2 mating type while for *P.* x *heterohybrida* nom. prov. isolates from both mating types plus A1/A2 isolates, which formed oogonia in mating tests with both A1 and A2 strains, were obtained.

Phytophthora chlamydospora from Clade 6 was isolated from Cu-keng River in Fushan while *P.* sp. forestsoil-like, a new Clade 6 species with 10-15 bp and 34-36 bp differences in ITS and *cox1*, respectively, (Table 1S) to its closest relatives *P.* taxon 'forestsoil' (P1054; AF541908 and JN935966) and *P.* taxon 'hungarica' (isolate H-8/02; EF522141 and JN935964) was recovered from Cu-keng River and the tributary of Hapen River in Fushan (Table 2; Fig. 1).

With six *Phytophthora* species and three different mating types (A1, A2, A1/A2) of *P.* x *heterohybrida* nom. prov. the tributary of Hapen River in Fushan showed the highest *Phytophthora* diversity followed by Da-an River in Taichung County and San-zhan River in Hualien County with each five *Phytophthora* species (Table 2). In March predominantly species from Clade 7a, and *P. capensis*, *P.* sp. occultans-like and *P.* sp. forestsoil-like were found whereas in August the five new taxa from a high-temperature tolerant cluster of Clade 9 and the high temperature species *P. parvispora* and *P. tropicalis* prevailed.

Association between Phytophthora presence in the rhizosphere and disease symptoms

In the majority of sampled forests (Fig. 2a-f) most of the 33 tree species from which *Phytophthora* species were recovered, including *Alnus formosana*, *Araucaria cunninghamii*, *Castanopsis faberi*, *C. indica*, *Chamaecyparis formosensis*, *C. obtusa*, *Cinnamomum camphora*, *Fagus hayatae*, *Glochidion philippicum*, *G. rubrum*, *Liquidambar formosana*, *Lithocarpus hancei*, *L. shinsuiensis*, *Machilus kusanoi*, *M. thunbergii*, *Quercus aliena*, *Q. glauca*, *Q. variabilis*, *Styrax suberifolia* and *Zelkova serrata*, appeared generally healthy. Symptoms indicative of Phytophthora diseases were only found in five of the 25 forest stands sampled.

In the subtropical *Castanopsis-Machilus* forest in Lenhuachih (F15) dieback of the upper crowns was observed in individual trees of *Castanopsis kawakami* and *C. uraiana* with presence of *P. cinnamomi* A1 and A2 or *P. castaneae* in their rhizosphere (Fig. 3a). All trees of *M. kusanoi* and *M. thunbergii* appeared healthy. In the subtropical *Castanopsis-Machilus* forest F02 located in the hills around the arboretum in Fushan (F01) patches of trees of *C. carlesii*, *C. micranthum* and *Diospyros morrisiana* showed severe thinning and dieback of crowns (Fig. 3b-e) and mortality. In addition, several *C. carlesii* trees were suffering from bleeding cankers at the stembase (Fig. 3f). The visual examination of root samples from five declining *C. carlesii* trees and three declining trees each of *C. micranthum* and *D. morrisiana*, all infested by *P. cinnamomi* and/or *P. castaneae*, demonstrated in all three tree species severe losses of lateral roots and fine roots and dieback of small woody roots (Fig. 4a-d). No symptoms of decline were found in *M. kusanoi*, *M. thunbergii* and *L. hancei*. At a recently disturbed site (construction of a trekking path) in the upper montane evergreen broadleaved forest F09 in Sheipa National Park, trees of *Q. morii* and *Q. sessilifolia* growing in a loamy soil infested by *P. cinnamomi* A1 showed severe thinning and dieback of crowns (Fig. 3g), mortality and excessive losses of lateral roots and fine roots (Fig. 4e). In the subtropical evergreen broadleaved forest F24 on Hengchun Peninsula, severe crown dieback and high mortality was observed in *Quercus championii* trees with presence of *P. cinnamomi* A1 and A2 in their rhizosphere while *Quercus longinux* trees appeared healthy. Unfortunately, crown symptoms could not be documented since the sampling was performed during a typhoon. Crown dieback and mortality was also found in *Trema orientalis* trees in the subtropical evergreen monsoon forest F19 which was infested by *P.* sp. meadii-like.

Discussion

The survey performed in each of 25 natural or semi-natural forests and rivers in Taiwan revealed the presence of ten described species and 17 previously unknown taxa of *Phytophthora*. Considering Taiwan' s size of approximately 36,000 km^2, the low number of sampling sites and the fact that each site was sampled only once, such a *Phytophthora* diversity

is unrivalled. The three most common and most widespread *Phytophthora* species detected in Taiwanese forests in this and in previous studies (Ko *et al.* 1978, 2006), *P. cinnamomi*, *P. heveae* and *P. castaneae*, also occur in montane forest soils on the tropical island Hainan in the South China Sea (Zeng *et al.* 2009). The overall diversity of recovered *Phytophthora* species from forests and rivers in Taiwan was much higher than that in Hainan. This may be due to the focus of the Hainan survey on agricultural crops, from which in total 13 *Phytophthora* species had been isolated (Zeng *et al.* 2009), and the fact that the baiting methods used in the present study had been optimized for the detection of *Phytophthora* species from forest soils and river systems (Jung 2009; Jung *et al.* 2011). Including recent findings of *P. lateralis* and *P. bisheria* in *Chamaycyparis obtusa* forests in Taiwan (Brasier *et al.* 2010) ten described species and nine previously unknown taxa of *Phytophthora* have so far been detected in less than 30 Taiwanese forest stands. In comparison, in more than one thousand mixed forest and riparian stands of *F. sylvatica*, *Quercus* spp. and *Alnus* spp. sampled across Europe 24 *Phytophthora* species were isolated (summarized in Jung *et al.* 2013) of which only five are considered as indigenous (Jung *et al.* 2015). Of the 19 *Phytophthora* species present in Taiwanese forests, only *P. chlamydospora*, *P. cinnamomi*, *P. citrophthora*, *P. lateralis* and *P. plurivora*, also occur in European forests and woodlands. In North American forests, *Phytophthora* diversity appears to be lower than in Taiwan and Europe. In 125 oak forests sampled across the Eastern and North-Central US, four described and three previously unknown *Phytophthora* species were found with *P. cinnamomi* A2 being most common (Balci *et al.* 2007). Large-scale surveys with more than 10000 soil, canopy drip and tissue samples from *Quercus*, *Notholithocarpus*, *Castanopsis* and mixed conifer forests in the Western US revealed the presence of 12 *Phytophthora* species most of them considered as introduced invasive pathogens (Hansen *et al.* 2012). The *Phytophthora* populations in forests of Taiwan and North America had only three species in common, *P. chlamydospora*, *P. cinnamomi* A2 and *P. heveae*.

With four known *Phytophthora* species, *P. capensis*, *P. chlamydospora*, *P. parvispora* and *P. tropicalis*, and 14 previously unknown *Phytophthora* taxa in Clades 2, 6, 7 and 9 recovered from 19 of the 25 rivers and streams tested riparian *Phytophthora* diversity in Taiwan was also high. From a single baiting raft in the tributary of Hapen Stream running through the arboretum and subtropical forest in Fushan six *Phytophthora* species including three different mating types of the new hybrid species *P.* x *heterohybrida* nom. prov. were isolated. The few negative results were most likely caused by extremely fast waterflow during typhoon-related floodings which prevented attachment of zoospores to baiting leaves. In recent years, a series of river surveys have been conducted in several countries all of which demonstrated an impressive diversity of both known and previously unknown *Phytophthora* species. In Yunnan, six known and two new *Phytophthora* species were isolated from small forest streams with the globally distributed species *P. chlamydospora* being the only species common to Yunnan and Taiwan. In South Africa, five described species, one designated taxon and five new taxa of *Phytophthora* were recovered from eight river systems (Oh *et al.* 2013). Besides *P. chlamydospora* also *P. capensis* and *P. parvispora* occurred in rivers of both South Africa and Taiwan (Oh *et al.* 2013). Another similarity between both surveys was the dominance of previously unknown Clade 9 species in the majority of rivers. In another river survey in South Africa a range of *Phytophthora* hybrids from Clade 6 were found (Nagel *et al.* 2013). Besides multiple Clade 6 hybrid taxa eight known *Phytophthora* species and four previously unknown *Phytophthora* taxa were isolated from 48 river systems across Western Australia (Hüberli *et al.* 2013; Burgess 2015). The remarkable lack of common species between the riparian *Phytophthora* populations in Taiwan and Western Australia, with the exception of *P. parvispora*, most likely reflects the biogeographical separation between both regions and the vastly different floristic and climatic conditions. Similarly, only one *Phytophthora* species, *P. chlamydospora*, was common to the riparian *Phytophthora* populations in Taiwan and North America. In Tennessee with two baiting periods in spring and autumn, six described and six unknown species of *Phytophthora* were found in 16 rivers while a large-scale survey in 65 rivers in Oregon with 5 or 38 baiting periods over two years

demonstrated the presence of 13 described and one new *Phytophthora* species (Reeser *et al.* 2011; Shrestha *et al.* 2013). In Alaska, where like in Taiwan baitings were performed at one occasion, only two known and two new *Phytophthora* species were baited from 49 rivers and streams (Reeser *et al.* 2011). In Europe, apart from a survey in the Spanish Pyrenees using a metagenomic approach which will be discussed below, no data from surveys of riparian *Phytophthora* populations have been published. However, in an ongoing country-wide *Phytophthora* survey of natural ecosystems in Portugal 21 *Phytophthora* species have so far been obtained from 35 rivers and streams (M. Horta Jung, L. Schena, S. Mosca, C. Maia, A. Cravador and T. Jung, unpublished results). Remarkably, the new Clade 6 species *P.* sp. forestsoil-like was the only *Phytophthora* species common to river systems in Taiwan and Portugal, possibly as a result of long-standing trade relation between both countries reaching back to the 16th Century.

Only six of the 15 *Phytophthora* species recovered from forest stands, *P. chlamydospora*, *P. formosa* nom. prov., *P. parvispora*, *P.* sp. x botryosa-like, *P.* sp. x meadii-like and *P.* sp. x Kunnunara-like, were also detected in rivers. Interestingly, the two most common *Phytophthora* species in Taiwanese forests, *P. cinnamomi* and *P. castaneae*, were never isolated from rivers running through or originating from infested forests. Likewise, 10 of the 16 *Phytophthora* species obtained by stream baiting could not be isolated from forest soils. These results strongly indicate that the majority of *Phytophthora* species are adapted to a specific lifestyle, either as soil- (or airborne) pathogens or as waterborne saprotrophs and opportunistic pathogens, and underscore the necessity of doing both soil and stream baiting for getting an overview of *Phytophthora* diversity in a diverse landscape. Alternatively, novel metagenomic approaches using high-throughput pyrosequencing, *Phytophthora*-specific primers and environmental DNA from soil and water samples can be used to unravel the true *Phytophthora* diversity in natural ecosystems (Català *et al.* 2015). Applying such an approach in two forest areas in Northern Spain, the presence of 13 and 35 *Phytophthora* taxa was demonstrated in forest soils and streams, respectively (Català *et al.* 2015). Interestingly, all *Phytophthora* species from forest

soils were also detected in water samples. However, besides the risks of producing false Molecular Operational Taxonomic Units (MOTUs) due to sequencing errors and false-positives due to cross-contamination the major limitation of metagenomic approaches is the lack of isolates which are needed for the taxonomic description and host range testing of new *Phytophthora* species. Consequently, in future surveys both classical baiting methods and novel metagenomic approaches should be applied in parallel.

The high *Phytophthora* diversity in Taiwan is most likely caused by the island' s remarkable complexity of geomorphology and orographic climates, the high diversity of ecosystems and plant species with a high degree of endemism, and the repeated temporary connections to mainland Asia during glacial periods followed by periods of separation during interglacials (Chang-Fu & Chung-Fu 1994; Chang-Fu *et al.* 1994; Chung-Fu 1994) allowing immigration of *Phytophthora* species and subsequent speciations and species radiations. In this survey, 15 *Phytophthora* species from Clades 2a, 7a and 9, respectively, were detected in natural ecosystems in Taiwan often co-occurring in the same forest or river. However, none of their known closest relatives like *P. botryosa* and *P. meadii*, both widespread in Southeast Asia (Erwin & Ribeiro 1996), and *P. himalsilva*, *P. terminalis* and *P. occultans* from Clade 2a (Vettraino *et al.* 2011; Man In' t Veld *et al.* 2015), *P. rubi*, *P. europaea*, *P. uliginosa* and the globally distributed forest pathogen *P. cambivora* from Clade 7a or *P. virginiana*, *P. parsiana*, *P. hydropathica*, *P.* sp. lagoariana, *P.* sp. Kunnunara and *P.* sp. x Hennops from Clade 9 (Yang & Hong 2013; Martin *et al.* 2014) were isolated in this or previous studies (Ko *et al.* 1978, 2006; Brasier *et al.* 2010). This suggests sympatric species radiation following the introduction of either common ancestors with their closest relatives or of their closest relatives themselves which were later outcompeted by the emerging new species. Apparently, in this process interspecific hybridisations were playing an important evolutionary role since nine of the 16 new species, *P.* sp. x botryosa-like, *P.* sp. x meadii-like, *P.* x *incrassata* nom. prov., *P.* x *heterohybrida* nom. prov., *P.* sp. x insolita-like, *P.* sp. x kunnunara-like, *P.* sp. x virginiana-like 1, *P.* sp. x virginiana-like 2 and *P.* sp. x virginiana-like 3, are putative hybrids as indicated by multiple heterozygous sites in

their ITS sequences. Interestingly, 1-2 heterozygous sites were also present in the ITS sequences of several isolates of *P. cinnamomi* A2 (haplotypes 1 and 8) and *P. parvispora*. Due to its multicopy nature the ITS rDNA gene region is of limited use for hybrid studies (Nagel *et al.* 2013; Burgess 2015). Therefore, sequencing of additional mitochondrial genes and cloning of single-copy nuclear genes to confirm the hybrid status and elucidate the parental species of all putative Taiwanese hybrid taxa, and flow cytometry analysis to clarify their ploidy status are currently underway.

Since most forest stands were natural and the catchments of most rivers were also covered by natural forests it is likely that Taiwan is within the center of origin of most *Phytophthora* taxa found. This is supported by the lack of disease symptoms in most sampled forest stands indicating a host-pathogen equilibrium resulting from long-term co-evolution. For *P. plurivora* from the '*P. citricola* complex' in Clade 2c, an invasive wide-host range pathogen involved in decline and dieback of *F. sylvatica*, *Quercus* spp. and other important forest tree species in Europe (Jung 2009; Jung & Burgess 2009; Jung *et al.* 2013), a Southeast Asian origin had been suggested (Jung & Burgess 2009) which is supported by the detection of *P. plurivora* in undisturbed, healthy remote mountain forests in Central Taiwan in this survey and by recent findings in healthy broadleaved forests in montainous regions of Nepal and Yunnan (Vettraino *et al.* 2011; Huai *et al.* 2013). The detection of the new species *P. citricola* VII from the '*P. citricola* complex', and the findings of *P. capensis*, which constitute the first record of this species from the '*P. citricola* complex' outside of South Africa, and of *P. plurivora*, *P. citrophthora*, *P. tropicalis* and the three new Clade 2a species *P.* sp. x botryosa-like, *P.* sp. x meadii-like and *P.* sp. occultans-like from natural ecosystems in Taiwan together with the widespread occurrence of *P. botryosa*, *P. citricola*, *P. colocasiae* and *P. meadii* across Southeast Asia (Erwin & Ribeiro 1996; Ho and Lu 1997; Drenth & Guest 2004; Zeng *et al.* 2009; Ann *et al.* 2010) suggest this region as the centre of origin of *Phytophthora* major Clade 2. Interestingly, isolates of *P.* sp. occultans-like from three rivers in Northeastern Taiwan were a 100% match in ITS to *P. occultans* isolates from Europe and Oregon (Man In' t Veld *et al.* 2015; Reeser *et al.* 2015) while their *cox1*

sequences were much closer to *P. himalsilva* from Nepal (Vettraino *et al.* 2011) and to *P. terminalis* than to *P. occultans*. This supports the hypothesis of Man In't Veld *et al.* (2015) that *P. himalsilva*, *P. occultans* and *P. terminalis* are young emerging species originating from a common ancestor after recent geographic separation. The detection of six new species from Clade 7a in natural forests and forest streams of Taiwan in this work almost duplicates the number of species in this important subclade suggesting Southeast Asia as centre of origin of Clade 7a. Also *P. heveae* and *P. castaneae* from Clade 5, *P. parvispora* (Clade 7) and *P. insolita* (Clade 9) are considered being native to Taiwan and Southeast Asia (Erwin & Ribeiro 1996; Ko *et al.* 2006; Zeng *et al.* 2009; Scanu *et al.* 2014) which is supported by results of this study. However, with the heterothallic species *P. cinnamomi* the situation is more complex and requires a separate consideration of the two mating types. *Phytophthora cinnamomi* is the most notorious wide-host range plant pathogen globally, causing epidemic dieback and mortality of more than 3000 woody species in natural ecosystems, nurseries and planted stands (Erwin & Ribeiro 1996; Hardham 2005). On a global scale, the A2 mating type is far more widespread than the A1 mating type and the success of *P. cinnamomi* is mainly based on the spread of two clonal A2 lineages (Dobrowolski *et al.* 2003). In contrast in Taiwan, using a RAPD analysis Chang *et al.* al. (1996) found high genetic variability among *P. cinnamomi* isolates of both mating types. This is confirmed by the finding of a diverse *P. cinnamomi* population with eight ITS haplotypes and both mating types in the present study. The A1 mating type is common in both lowland and montane forests and usually not causing disease symptoms whereas the A2 mating type is restricted to lowland forests where it is in several cases associated with severe decline and dieback of native tree species. Interestingly, most severe crown dieback and mortality of trees were found in the *Castanopsis-Machilus* forest in Fushan and in the evergreen *Quercus championii* forest on the Hengchun Peninsula where the *P. cinnamomi* populations showed the highest diversity with each four ITS haplotypes and co-occurrence of both mating types, suggesting an interbreeding population. Also in Papua New Guinea, the A2 mating type of *P. cinnamomi* was exclusively found in lowland stands causing disease symptoms of trees and

crops whereas the A1 mating type was widespread in natural montane forests without association to decline or dieback of trees (Arentz & Simpson 1986). The apparent differences in climatic amplitudes between both mating types indicate that they became geographically separated a long time ago, most likely during the pleistocene, and then adapted to the environmental conditions prevailing in their respective habitats. The results from this study suggest that in Taiwan the A1 mating type was introduced via a landbridge during the pleistocene and became naturalised while the A2 mating type is most likely a recently introduced invasive pathogen which is still spreading. The latter hypothesis is supported by the comparison of data obtained from the natural *Castanopsis-Machilus* forest in Lenhuachih (F15) during this study with those of Ko *et al.* (1978) from the same stand. In the 1970s this forest was healthy and the A1 : A2 mating type ratio was 84.6 : 15.4 (n=13) (Ko *et al.* 1978). Thirty years later *Castanopsis* trees showed crown dieback and the A1 : A2 mating type ratio had changed to 33.5 : 76.5 (n=68). A population-genomic study of a global collection of *P. cinnamomi* including isolates from Taiwan and other locations in Southeast Asia is currently underway to unravel the origin of both mating types of this important pathogen. *Phytophthora* species from Clades 1 (*P. cactorum*, *P. infestans*, *P. nicotianae*), 4 (*P. palmivora*), 8 (*P. cryptogea*, *P. drechsleri*, *P. porri*) and 10 (*P. boehmeriae*) are causing diseases of commercial horticultural crops and ornamental plants in Taiwan, Hainan and China (Ho and Lu 1997; Zeng *et al.* 2009; Ann *et al.* 2010). However, the complete absence of *Phytophthora* species from Clades 1, 3, 8, 10 and, with the exception of one isolate of *P. palmivora*, also Clade 4 in the present survey makes it unlikely that these five phylogenetic clades are native to Taiwan and Southeast Asia.

In a preliminary pathogenicity trial of this study using underbark inoculation pathogenicity of *P. cinnamomi* to *D. morrisiana* and *Q. morii* was demonstrated (data not shown). In the frame of a host range test of *P. cinnamomi* among native Taiwanese *Quercus* species which will be published separately, *P. cinnamomi* caused extensive root root and mortality of *Q. morii* saplings in a soil infestation test fulfilling Koch' s postulates for this pathosystem (T. Jung and M. Horta Jung, unpublished data).

The absence of decline and dieback in the majority of *Phytophthora*-infested forests in Taiwan suggests long-term co-evolution between the pathogens and a flora which contains a wide variety of tree species from genera also present in Europe and North America, including *Quercus*, *Fagus*, *Castanea*, *Abies*, *Pinus* and *Picea*. Therefore, high aggressiveness of indigenous Taiwanese *Phytophthora* species to endemic non-coevolved European and North American tree species can be expected as has already been demonstrated by *P. cinnamomi* and *P. plurivora*. For example, in a soil infestation test, the six new species from Clade 7a proved to be pathogenic to root systems of *C. sativa* and *F. sylvatica* with the two hybrid species *P.* x *incrassata* nom. prov. and *P.* x *heterohybrida* nom. prov. being highly aggressive to *C. sativa* (T. Jung and M. Horta Jung, unpublished results). These results are of great concern because more than a billion plants-for-planting are imported annually from Asia to Europe (Ludovic Rigoux, Université Libre de Bruxelles, Belgium, personal communication). Recently, a Europe-wide study demonstrated the widspread presence of 68 *Phytophthora* species in European nurseries and plantings, of which at least 47 are considered of exotic origin (Jung *et al.* 2015). Against this background, the widespread occurrence of a high number of previously unknown *Phytophthora* species in forests and rivers in Taiwan and elsewhere in Asia (eg. Huai *et al.* 2013) and in horticultural production areas in Japan (eg. Rahman *et al.* 2014) potentially poses a serious threat to forestry, horticulture, conservation and biodiversity in Europe and North America.

As a pro-active approach to plant biosecurity, further *Phytophthora* surveys in natural ecosystems in other regions of Asia and also in Africa and South America coupled with extensive host range testing of new *Phytophthora* species amongst major European tree species and agricultural crops are needed. Data generated by these surveys and trials will enable decision makers like the EU standing committee of plant health, EPPO, NAPPO, APHIS, WTO and national plant protection organisations to adapt outdated plant health laws and regulations (Brasier 2008; Jung *et al.* 2015). In addition, such *Phytophthora* surveys will give a clearer picture of natural species distribution and global diversity of the important oomycete genus *Phytophthora*

and help to develop a deeper understanding of the factors driving diversity and adaptation including the frequency and the role of interspecific hybridisations in natural ecosystems.

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Figure legends

Fig. 1. Location of the 25 forest stands (F01-F25; black dots) and 25 riparian sites (R01-R25; blue triangles) included in the *Phytophthora* survey in Taiwan. For GPS coordinates see Tables 1 and 2.

Fig. 2. Representative forest stands and rivers sampled in Taiwan; a. montane, temperate mixed coniferous forest F08 in Sheipa National Park; b. montane, temperate deciduous beech forest F04 at Taiping Mountain; c. montane, temperate, seasonally dry, deciduous broadleaved forest F12 in Tunyuan; d. subtropical evergreen *Castanopsis-Machilus* forest F02 in Fushan; e. subtropical evergreen lowland monsoon forest in the Pacific Coastal Range in Hualien County; f. subtropical evergreen broadleaved forest on Hengchun Peninsula; g. San-zhan River (R20) coming out of subtropical evergreen lowland monsoon forests and montane, temperate

evergreen broadleved forests at Taroko National Park; h. baiting raft (arrow) floating in the tributary of Hapen River (R03) in the subtropical evergreen *Castanopsis-Machilus* forest F02 in Fushan. For GPS coordinates see Table 1; for location of sites see Figure 1.

Fig. 3. Disease symptoms of mature native trees in natural forest stands in Taiwan associated with presence of *Phytophthora* species in the rhizosphere; a. crown dieback of *Castanopsis kawakami* in the subtropical evergreen *Castanopsis-Machilus* forest F15 in Lenhuachih (*P. cinnamomi* A1 and A2, *P. Castaneae*); b-f. subtropical evergreen *Castanopsis-Machilus* forest F02 in Fushan (*P. cinnamomi* A1 and A2, *P. castaneae*); b and c. crown thinning and dieback of *Diospyros morissiana* (b) and *Cinnamomum micranthum* (c); d. healthy (white arrows), declining (black arrow) and dying (red arrow) trees of *Castanopsis carlesii*; e. crown thinning and dieback of *C. carlesii*; f. collar rot of *C. carlesii* with tarry spots on the outer bark; g. crown thinning and dieback of *Quercus sessilifolia* (black arrows) and *Quercus morii* (white arrow) in the montane, temperate evergreen *Quercus* forest F09 in Sheipa National Park (*P. cinnamomi* A1).

Fig. 4. a-d. Symptoms on root systems in the subtropical evergreen *Castanopsis-Machilus* forest F02 in Fushan infested by *P. cinnamomi* A1 and A2 and by *P. castaneae*; a. dieback (arrow) of woody root of *Castanopsis carlesii*; b-d. small woody roots of *C. carlesii* (b), *Cinnamomum micranthum* (c) and *Diospyros morissiana* (d) with severe losses of lateral roots and fine roots; e-f. symptomatic roots of *Quercus morii* in the montane, temperate evergreen *Quercus* forest F09 in Sheipa National Park infested by *P. cinnamomi* A1; e. small woody roots with severe losses of lateral roots and fine roots; f. coarse root with open callussing lesion.

Supplementary Tables

Table 1S. GenBank accession numbers of ITS and partial *cox1* sequences generated in this study for representative *Phytophthora* isolates from Taiwanese forests and rivers and isolates from related *Phytophthora* species used for comparisons.

Table 2S. Polymorphic nucleotides from aligned ITS sequence data showing the variation between eight haplotypes (H) of *P. cinnamomi* from Taiwanese forests and the ex-type (T) isolate of *P. cinnamomi* from Sumatra. Grey shading denotes no data available.

Table 3S. Polymorphic nucleotides from aligned ITS sequence data showing the variation between selected isolates of *P. attenuata* nom. prov., *P. flexuosa* nom. prov., *P. formosa* nom. prov., *P. intricata* nom. prov., *P.* x *heterohybrida* nom. prov. and *P.* x *incrassata* nom. prov. from Taiwan and ex-type (T) or key isolates of related *Phytophthora* species from Clade 7a.

Table 4S. Polymorphic nucleotides from aligned partial *cox1* sequence data showing the variation between selected isolates of *P. attenuata* nom. prov., *P. flexuosa* nom. prov., *P. formosa* nom. prov., *P. intricata* nom. prov., *P.* x *heterohybrida* nom. prov. and *P.* x *incrassata* nom. prov. from Taiwan and ex-type (T) or key isolates of other *Phytophthora* species from Clade 7a.

Table 5S. Polymorphic nucleotides from aligned ITS sequence data showing the variation between selected isolates of *P.* sp. x botryosa-like, *P.* sp. x meadii-like and *P.* sp. occultans-like from Taiwan and ex-type (T) or key isolates of related *Phytophthora* species from Clade 2a. Grey shading denotes no data available.

Table 6S. Polymorphic nucleotides from aligned partial *cox1* sequence data showing the variation between selected isolates of *P.* sp. x botryosa-like, *P.* sp. x meadii-like and *P.* sp. occultans-like from Taiwan and ex-type (T) or key isolates of related *Phytophthora* species from Clade 2a. Grey shading denotes no data available.

Table 7S. Polymorphic nucleotides from aligned ITS sequence data showing the variation between selected isolates of *P.* sp. x Kunnunara-like, *P.* sp. x virginiana-like 1, *P.* sp. x virginiana-like 2 and *P.* sp. x virginiana-like 3 from Taiwan and ex-type (T) or key isolates of related *Phytophthora* species from Clade 9. Grey shading denotes no data available.

Table 8S. Polymorphic nucleotides from aligned partial *cox1* sequence data showing the variation between selected isolates of *P.* sp. x Kunnunara-like, *P.* sp. x virginiana-like 1, *P.* sp. x virginiana-like 2 and *P.* sp. x virginiana-like 3 from Taiwan and ex-type (T) or key isolates of related *Phytophthora* species from Clade 9. Grey shading denotes no data available.

Table 1: Location, altitude, geological substrate and vegetation of 25 forest sites sampled in Taiwan, sampled tree species and *Phytophthora* 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* spp. (no. of positive samples) [a, b]
F01	N24 45.892 E121 35.195	653	Fushan, Yilan county	Shale	Fagaceae arboretum established in the subtropical evergreen *Castanopsis-Machilus* forest F02	*Quercus glandulifera* (2/2) *Quercus glauca* (1/1) *Quercus stenophyloides* (1/1) *Quercus tarokoensis* (4/4) *Quercus tatakaensis* (0/1) *Quercus variabilis* (0/1) *Rhododendron* sp. (0/1)	CIN A2 (1), FOR (2) CAS (1), CIN A1 (1) CIN A1 (1), CIN A2 (1) CIN A2ho (1), CIP (1), INT (4) - - -
F02	N24 45.585 E121 34.929	698	Fushan, Yilan county	Shale	Subtropical evergreen *Castanopsis-Machilus* forest	*Castanopsis carlesii* (9/13) *Cinnamomum micranthum* (3/3) *Diospyros morissiana* (4/4) *Lithocarpus hancei* (1/1) *Machilus thunbergii* (2/2)	CAS (5), CIN A1 (2), CIN A2 (3) [c] CAS (1), CIN A2 (3) CIN A1 (1), CIN A2 (3) CAS (1) CIN A1 (2/2)
F03	N24 32.000 E121 22.000	1902	Chilan mountain, Yilan county	Argillite	Montane, temperate mixed coniferous forest	*Chamaecyparis obtusa* (2/2)	CHL (2), CIN A1 (1)
F04	N24 30.348 E121 37.856	1741	Taiping mountain, Yilan county	Slate	Montane, temperate deciduous beech forest	*Fagus hayatae* (8/9)	CAS (1), CIN A1 (8), FLE (3) [d]
F05	N 24 30.240, E 121 37.346	1967	Taiping mountain, Yilan county	Slate	Montane, temperate mixed coniferous forest	*C. obtusa* (3/4)	CHL (2), CIN A1 (2)
F06	N24 29.687 E121 32.108	1973	Taiping mountain, Yilan county	Argillite and slate	Montane, temperate mixed coniferous forest	*C. obtusa* (5/5) *Rhododendron* sp. + *Juniperus* sp., mixed sample (0/1)	CIN A1 (5) [d] -
F07	N24 52.611 E120 58.255	110	Hsingfeng Township, Hsinchu County	Alluvial sediments	Subtropical, seasonally dry, partially deciduous broadleaved forest	*Quercus aliena* (0/4) *Q. variabilis* (1/4) *Cinnamomum camphora* (1/1)	- HEV (1) HEV (1)
F08	N24 30.354 E121 5.568	2182	Sheipa National Park, Hsinchu County	Shale	Montane, temperate mixed coniferous forest	*Chamaecyparis formosensis* (3/5) *Taiwania cryptomeroides* (0/1) *Rhododendron* sp. (0/2)	ATT (3), CAS (1) [e] - -

Site no.	GPS coordinates	Altitude (m a.s.l)	Location	Geological substrate	Vegetation	Sampled tree species (no. of *Phytophthora*-positive/sampled trees)	*Phytophthora* spp. (no. of positive samples) [a, b]
F09	N24 30.224 E121 5.403	2287	Sheipa National Park, Hsinchu County	Shale	Montane, temperate evergreen *Quercus* forest	*Quercus morii* (1/1) *Quercus sessilifolia* (2/2)	CIN A1 (1) CIN A1 (2)
F10	N24 30.045 E121 6.595	2174	Sheipa National Park, Hsinchu County	Shale	Montane, temperate mixed coniferous forest	*C. carlesii* (2/2)	ATT (2)
F11	N24 3.051 E121 12.925	2023	Tunyuan, Nantou County	Argillite and slate	Montane, temperate, seasonally dry, deciduous *Quercus* - *Pinus* forest	*Q. variabilis* (2/5)	CIN A1 (1), CIT (2)
F12	N24 2.511 E121 12.734	1848	Tunyuan, Nantou County	Argillite and slate	Montane, temperate, seasonally dry, deciduous broadleaved forest	*Alnus formosensis* (1/1) *Carpinus kawakami* (0/1) *Liquidambar formosensis* (1/1) *Q. variabilis* (0/2)	PLU (1) - PLU (1) -
F13	N24 1.501 E121 9.169	1343	Huagang Mountain, Nantou County	Argillite and slate	Montane, warm-temperate, seasonally dry, partially deciduous Quercus forest	*Q. glauca* (1/2) *Q. variabilis* (4/7)	non-culturable *Phytophthora* species (1) [f] non-culturable *Phytophthora* species (3) [f]
F14	N24 1.599 E121 9.382	1266	Huagang Mountain, Nantou County	Argillite and slate	Montane, warm-temperate, seasonally dry, deciduous Quercus forest	*Q. variabilis* (1/4)	CIN A1 (1)
F15	N23 55.149 E120 52.996	733	Lenhuachih, Nantou County	Sandstone and shale	Subtropical evergreen *Castanopsis-Machilus* forest	*Machilus kusanoi* (2/3) *M. thunbergii* (3/3) *Castanopsis kawakami* (6/6) *Castanopsis uraiana* (2/3) *Araucaria cunninghamii* (2/2)	CIN A1 (2), CIN A2 (1) [c] CAS (1), CIN A2 (2) CIN A1 (3), CIN A2 (5) [c] CIN A1 (1), CIN A2 (2) [c] FOR (2)
F16	N24 12.545 E121 40.244	210	Taroko National Park, Hualien County	Metamorphosed limestone	Subtropical evergreen lowland monsoon forest	*Glochidion philippicum* (1/1)	HEV (1) [c]
F17	N24 9.563 E121 36.705	108	Taroko National Park, Hualien County	Metamorphosed limestone	Subtropical evergreen lowland monsoon forest	*Cinnamomum camphora*, *Styrax suberifolia* and *G. philippicum*, mixed samples (2/2)	BOT (2), KUN (1) [c]

Site no.	GPS coordinates	Altitude (m a.s.l)	Location	Geological substrate	Vegetation	Sampled tree species (no. of *Phytophthora*-positive/sampled trees)	*Phytophthora* spp. (no. of positive samples) [a, b]
F18	N23 30.276 E121 26.134	102	Pacific coastal range, Hualien County	Volcaniclastic sediments	Subtropical evergreen lowland monsoon forest	*C. camphora* (3/5) *S. suberifolia* (2/2)	BOT (1), HEV (2), MEA (1) [c] MEA (2)
F19	N23 14.320 E121 19.165	671	Pacific coastal range, Taitung County	Igneous tuff	Subtropical evergreen lowland monsoon forest	*Trema orientalis* (1/1) *Morus australis* (0/1)	MEA (1) [c] -
F20	N22 14.320 E120 51.455	440	Hengchun Peninsula, Pingtun County	Sandstone and shale	Subtropical evergreen broadleaved forest	*Lithocarpus shinsuiensis* (1/1) *Engelhardia roxbhurghiana* (1/1)	CAS (1), CIN A2ho (1) CIN A2ho (1)
F21	N22 12.659 E120 51.876	361	Hengchun Peninsula, Pingtun County	Sandstone and shale	Subtropical evergreen broadleaved forest	*M. kusanoi* (0/1) *Zelkova serrata* (1/1)	- [c] PAL A1 (1)
F22	N22 9.271 E120 50.320	497	Hengchun Peninsula, Pingtun County	Sandstone and shale	Subtropical evergreen swamp forest	*Glochidion rubrum* (1/1)	CIN A1 (1), PAR A1 (1), PLS (1)
F23	N22 9.047 E120 50.504	468	Hengchun Peninsula, Pingtun County	Sandstone and shale	Subtropical evergreen broadleaved forest	*Castanopsis indica* (2/2)	CIN A1 (2), CIN A2ho (1)
F24	N22 9.024 E120 50.519	465	Hengchun Peninsula, Pingtun County	Sandstone and shale	Subtropical evergreen broadleaved forest	*Quercus championii* (3/3) *Quercus longinux* (1/1)	CIN A1 (2), CIN A2ho (2) CIN A1 (1)
F25	N22 8.435 E120 51.254	365	Hengchun Peninsula, Pingtun County	Sandstone and shale	Subtropical evergreen broadleaved forest	*Castanopsis faberi* (4/4)	CIN A1 (4), CIN A2 (1), CIN A2ho (2), HEV (1)

[a] ATT = *P. attenuata* nom. prov., BOT = *P.* sp. x botryosa-like, CAS = *P. castaneae* (previously *P. katsurae*), CHL = *P. chlamydospora*, CIN = *P. cinnamomi*, CIP = *P. citrophthora*, CIT = *P. citricola* VII, FLE = *P. flexuosa* nom. prov., FOR = *P. formosa* nom. prov., HEV = *P. heveae*, INT = *P. intricata* nom. prov., KUN = *P.* sp. x Kunnunara-like, MEA = *P.* sp. x meadii-like, PAL = *P. palmivora*, PAR = *P. parvispora*, PLS = *P.* sp. palustris, PLU = *P. plurivora*.

[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 both dual cultures with A1 tester strains and in ageing single cultures.

[c] *Phytopythium vexans* also isolated.

[d] *Elongisporangium undulatum* also isolated.

[e] *Elongisporangium anandrum* also isolated.

[f] Non-culturable *Phytophthora* species abundantly forming papillate and bi-papillate, persistent sporangia on *Q. variabilis* baiting leaves but not growing after plating them on PARPNH and PDA.

Table 2: Location and altitude of the 25 riparian sites sampled in Taiwan and *Phytophthora* taxa isolated.

Site no.	GPS coordinates	Altitude (m a.s.l)	River, county	Location of catchment	*Phytophthora* spp. (no. of positive samples) [a, b]
R01	N25 1.197 E121 30.371	6	Xin-dian River, Taipei City	Hsueshan Range, northern-most part of Western Foothills and Taipei Basin	KUN, VIR2, VIR3
R02	N24 56.828 E121 42.383	358	Tributary of Bei-shih River, Pinglin Township, New Taipei City	Northernmost part of Western Foothills	CAP, KUN, OCC
R03	N24 45.655 E121 34.933	652	Tributary of Ha-pen River, Fushan, New Taipei City [c]	Northern part of Hsueshan Range	CAP, FOR, FRS, INC A2, HET A1, HET A2, HET A1/A2, OCC
R04	N24 45.449 E121 34.781	644	Ha-pen River, Fushan, New Taipei City [d]	Northern part of Hsueshan Range	HET A1, HET A2
R05	N24 45.510 E121 37.360	523	Cu-keng River, Yilan County [e]	Northern part of Hsueshan Range	CHL, FRS, HET A1, HET A2, OCC
R06	N24 45.032 E121 39.986	101	Wu-shi Creek, Yilan County	Northern part of Hsueshan Range	-
R07	N24 22.742 E120 51.856	298	Hou-long River, Miaoli County	Western Foothills	INS, VIR1
R08	N24 20.917 E120 49.495	300	Xiao-keng River, Miaoli County	Western Foothills	INS, VIR1, VIR2
R09	N24 18.536 E120 49.120	329	Da-an River, Taichung County	Hsueshan Range (Sheipa National Park) and Western Foothills	INS, KUN, VIR1, VIR2, VIR3
R10	N24 17.263 E120 48.067	307	Sha-lian River, Taichung County	Western Foothills	*Phytophthora* sp. [f]
R11	N24 9.894 E120 50.070	483	Tributary 1 of Da-jia River, Taichung County	Western Foothills	PAR A1, TRO A1
R12	N24 9.286 E120 51.487	520	Tributary 2 of Da-jia River, Taichung County	Western Foothills	KUN, TRO A1 [g]
R13	N24 9.530 E120 51.986	537	Ma-zhu-keng River, Taichung County	Western Foothills	PAR A1
R14	N24 7.465 E120 52.577	570	Er-guei River, Taichung County	Hsueshan Range and Western Foothills	INS, KUN
R15	N24 3.055 E120 56.101	517	Tributary of Bei-gang River, Taichung County	Hsueshan Range	-
R16	N23 55.111 E120 53.062	693	Hou-xi Creek, Nantou County [h]	Hsueshan Range	-
R17	N23 53.085 E120 53.491	564	Shui-she-shui-wei River, Nantou County	Hsueshan Range	INS, KUN, PAR A1, VIR2
R18	N24 12.545 E121 40.244	210	Xiao-Qingshui Creek, Hualien County [i]	Central Mountain Ridge and Eastern Taiwan Schist Range (Taroko National Park)	BOT, MEA, VIR3
R19	N24 9.434 E121 37.079	54	Lee-wu River, Hualien County	Central Mountain Ridge and Eastern Taiwan Schist Range (Taroko National Park)	- [j]
R20	N24 6.217 E121 36.202	34	San-zhan River, Hualien County	Central Mountain Ridge and Eastern Taiwan Schist Range (Taroko National Park)	INS, KUN, VIR1, VIR2, VIR3
R21	N23 58.07 E121 29.472	147	Mu-gua River, Hualien County	Central Mountain Ridge and Eastern Taiwan Schist Range	KUN
R22	N23 41.220 E121 24.414	176	Ma-tai-an River, Hualien County	Eastern Taiwan Schist Range	- [j]
R23	N23 39.670 E121 25.157	122	Guang-fu River, Hualien County	Eastern Taiwan Schist Range	INS, VIR1, VIR2, VIR3
R24	N23 33.302 E121 22.613	136	Fu-yuan River, Hualien County	Eastern Taiwan Schist Range	KUN, VIR2
R25	N23 18.584 E121 15.312	223	La-ku-la-ku River, Hualien County	Central Mountain Ridge and Eastern Taiwan Schist Range (Yushan National Park)	- [j]

[a] BOT = *P.* sp. x botryosa-like, CAP = *P. capensis*, CHL = *P. chlamydospora*, FOR = *Phytophthora formosa* nom. prov., FRS = *P.* sp. forestsoil-like, INC = *P.* x *incrassata* nom. prov., HET = *P.* x *heterohybrida* nom. prov., INS = *P.* sp. x insolita-like, KUN = *P.* sp. x Kunnunara-like, MEA = *P.* sp. x meadii-like, OCC = *P.* sp. occultans-like, PAR = *P. parvispora*, TRO = *P. tropicalis*, VIR1 = *P.* sp. x virginiana-like 1, VIR2 = *P.* sp. x virginiana-like 2, VIR3 = *P.* sp. x virginiana-like 3.
[b] Mating types: A1, A2, A1/A2 (selfsterile, forming oogonia with both A1 and A2 tester strains).
[c] Running through Fushan Arboretum (F01).
[d] Running through *Castanopsis-Machilus* forest F02 in Fushan.
[e] running through *Castanopsis-Machilus* forest close to Fushan.
[f] A typical *Phytophthora* colony was growing from plated baiting leaves but was overgrown by a fast-growing *Pythium* species before subculturing.
[g] *Phytopythium vexans* also isolated.
[h] Downstream of *Castanopsis-Machilus* forest F15 in Lenhuachih.
[i] Running through subtropical evergreen lowland monsoon forest F16.
[j] Very fast current after a typhoon.