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ORIGINAL ARTICLE

Fishing for *Phytophthora* in watercourses of the highly urbanized Swiss Plateau

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Abstract

Phytophthora species are a cause for concern due to their invasive potential and the damage they can cause in agriculture, forestry, and natural ecosystems worldwide. Since water plays a crucial role in their dispersal, stream and river baiting is commonly used to survey risk areas for the presence of quarantine *Phytophthora* species. However, our understanding of the distribution and diversity of *Phytophthora* species in European watercourses remains incomplete. This study investigated the presence and diversity of *Phytophthora* species in Swiss watercourses, with a focus on the highly urbanized Swiss Plateau. Over the period 2012–2016, we sampled 32 watercourses, including major rivers and smaller streams. We isolated *Phytophthora* on selective media and sequenced the internal transcribed spacer region to identify the species. We recovered 241 *Phytophthora* isolates, representing 11 species from five major clades. *Phytophthora* clade 6 prevailed, with *P. lacustris* being the most common, found in 94.7% of the watercourses. The number of *Phytophthora* species per watercourse ranged from one to five, with no correlation to watercourse complexity. Our study reveals the presence of six previously unreported species in Switzerland, while known invasive species were not found. Watercourses appear less suited to detect invasive pathogenic *Phytophthora* species with a still limited distribution in the environment.

Keywords Oomycetes · Aquatic microorganisms · ITS clade 6 *Phytophthora* · Monitoring · Invasive species

Introduction

Phytophthora is a genus of fungal-like oomycetes whose number of species has nearly doubled in the last decade (Brasier et al. 2022). *Phytophthora* species are currently classified into twelve major phylogenetic clades representing a highly versatile group of plant pathogens and saprophytes (Jung et al. 2017; Brasier et al. 2022). The genus includes several important plant pathogenic species that cause economic losses of cash crops in both agriculture and nursery settings. When introduced into natural ecosystems, Phytophthoras may become high-risk pathogens and cause severe damage. For instance, *P. cinnamomi* is listed as one of the 100 worst invasive alien species (Lowe et al. 2000) and is found in nurseries, parks, gardens, and forests around the globe. In New Zealand, *P. agathidicida* causes a lethal root rot on Kauri (*Agathis australis*), which is one of the world's largest and longest-living conifer species (Bradshaw et al. 2020). Sudden Oak Death in the Western United States (Rizzo et al. 2002) and Sudden Larch Death in Great Britain (Brasier and Webber 2010; Harris and Webber 2016) resulting from the accidental introduction of *P. ramorum* possibly via plant trade challenge the local commercial timber production. The ever-increasing reports of *Phytophthora*-associated tree declines and emerging diseases indicate that *Phytophthora* species will continue to threaten biodiversity and the sustainability of forest ecosystems worldwide (Jung et al. 2017; Brasier et al. 2022).

The devastating impact of some *Phytophthora* species in natural ecosystems resulted in several large-scale surveys to assess their occurrence. In this context, leaf baiting is commonly used to survey risk areas for the presence of quarantine *Phytophthora* species (Sutton et al. 2009; O'Hanlon et al. 2018). Indeed, water plays a crucial role in facilitating the dispersal of asexual and short-lived zoospores, which serve as the

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primary propagules responsible for both dispersion and infection in oomycetes. At least 85 *Phytophthora* species from most clades are found to disperse in aquatic environments disassociated from their plant host, and 41 species from five clades have adapted to a primarily aquatic lifestyle (Brasier et al. 2022). These species are considered mostly benign or weak pathogens and derive their energy and nutrition from decaying organic matter (Marano et al. 2016; Brasier et al. 2022). *Phytophthora* clade 6, which complete their life cycle in water (Hansen et al. 2012; Marano et al. 2016), dominate the aquatic environment, but their ecological function is still unclear. Monitoring efforts have led to the discovery of many *Phytophthora* species in new habitats and the description of new species and previously uncharacterized hybrids (e.g., Scott et al. 2013; Hansen 2015; Jung et al. 2017; Van Poucke et al. 2021).

In Switzerland, the first *Phytophthora* species officially reported was *P. infestans*, the causal agent of potato late blight, in 1845 (Dufour 1889). Until 1995, only 15 species had been identified, most of which in orchards and agricultural crops: *P. cactorum* (first report 1904), *P. erytroseptica* (1912), *P.* × *cambivora* (1942), *P. porri* (1964), *P. syringae* (1975), *P. megasperma* (1976), *P. cryptogea* (1976), *P. citricola* (1976), *P. drechsleri* (1977), *P. nicotianae* (1980), *P. fragariae* var. *rubi* (1980), *P. fragariae* (1981), *P. cinnamomi* (1981), and *P. citrophthora* (1981) (Bolay and Schwinn 1986). In 2003, *P. ramorum* was officially detected on *Viburnum* × *bodnantense* in an ornamental nursery (Heiniger and Stadler 2003). As a result, all nurseries that import or produce putative host plants of this quarantine organism are inspected annually (Prospero et al. 2013). These inspections resulted in the detection of *P. ramorum* in several nurseries, mostly on imported nursery stocks, and in a few cases on outplanted host plants in urban areas (Prospero et al. 2013). These findings also led to surveys in forests, rivers, and streams, which made it possible to gather valuable information on the distribution of *Phytophthora* species in the country. Here, we present the results of a nationwide *Phytophthora* monitoring that investigates *Phytophthora* community composition in Swiss watercourses. Given that in Switzerland, all nurseries tested positive for *P. ramorum*, and most human infrastructure and activity are concentrated in the Plateau region (i.e., the densely populated area between the Jura Mountains and the Swiss Alps stretching from Geneva to St. Gallen); we focused on watercourses flowing through this area to increase chances of also detecting exotic *Phytophthora* species.

Materials and methods

Sampled watercourses

From 2012 to 2016, a total of 32 watercourses (rivers, streams, and creeks) flowing through the highly urbanized Swiss plateau were sampled (Fig. 1, Table 1). These included the three major Swiss rivers Aare (tributary of the Rhine, rises and ends in Switzerland; drainage area of 17779 km^2), Rhine (discharging into the North Sea; drainage area of 36472 km^2 in Switzerland), and Rhone (discharging into the Mediterranean Sea; drainage area of 8000 km^2 in Switzerland), whose sources are in the Swiss Alps, as well as minor streams (Supplementary Figure 1). Watercourse complexity and size were characterized using the Strahler stream classification system (Strahler 1957). According to this system, watercourses of the first order are the outermost tributaries. If two watercourses of the same order merge, the resulting watercourse is given a number that is one higher. If two watercourses with different orders merge, the resulting watercourse is given the higher of the two numbers. The sampled watercourses resulted in a Strahler order from three to nine (https://t.ly/OGJHQ; Table 1). The Person's correlation coefficient and the corresponding *p*-value were calculated on https://www.socscistatistics.com/tests/pearson. Each watercourse was also attributed to one of the following three river basins: Aare, Rhine, and Rhone (Supplementary Figure 1 and Supplementary Table 1).

Phytophthora baiting

For *Phytophthora* baiting, a protocol using rhododendron (*Rhododendron catawbiense*) leaves as baits was adopted (Oak et al. 2008). The leaves were at least 1 year old and featured a well-developed cuticula without signs of injury or fungal infection. Sampling sites were chosen in sectors of a watercourse with constantly flowing water and adjacent riparian vegetation. For the river Rhone, the sampling site was located close to the river estuary into a lake (Fig. 1).

At each site (52 sites in total; Supplementary Table 1), at least four leaves (individually or in pairs per bait net) were placed on the water surface in a quiet spot between August and October for every year between 2012 and 2016. To allow them to float, the nets were tied to adjacent riparian vegetation with a nylon thread. After approximately 1 week (6–8 days), the nets were retrieved from the water and immediately brought to the laboratory for further analysis.

Phytophthora isolation

All rhododendron leaves were rinsed with demineralised water, dried with paper towels, and subsequently inspected for signs of infection, such as brown and/or transparent lesions on the surface. Such areas were cut into pieces of approximately 3 × 3 cm in size. If there was no sign of infection, parts of the leaf edges and the middle vein were taken. The pieces were then surface sterilized for 1 min in sodium hypochlorite solution (0.5% active chlorine), rinsed

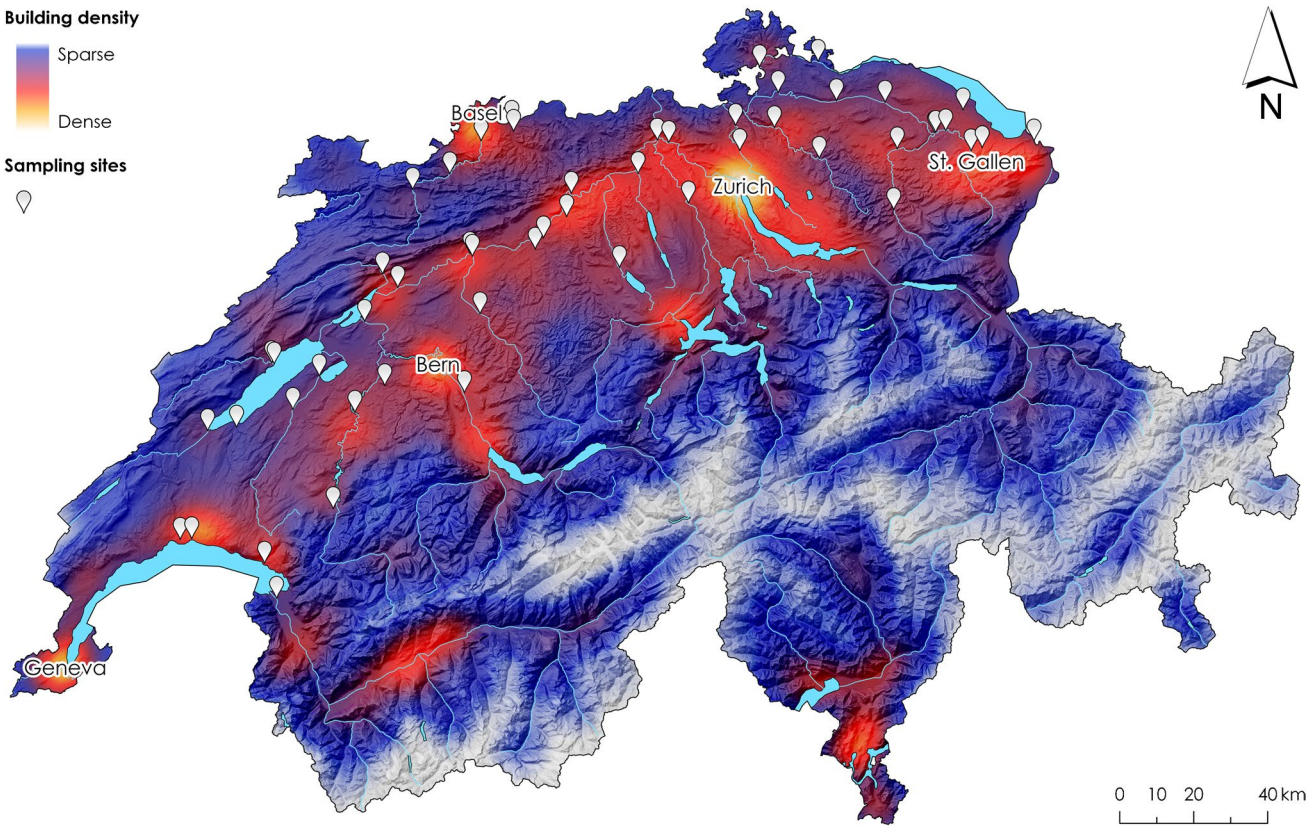

Fig. 1 The spatial arrangement of 52 *Phytophthora* baiting sites, represented by white symbols, spans across 32 watercourses examined for the presence of *Phytophthora* species in the densely populated Swiss Plateau. Additional details about the baiting sites can be found in Supplementary Figure 1 and Supplementary Table 1

twice for 1 min in sterile water, and dried with paper towels. The surface sterilized pieces were cut into six small fragments of approximately 5 × 5 mm and placed on a selective CARP medium containing 17 g cornmeal agar, 10 mg pimaricin, 200 mg ampicillin, 10 mg rifampicin, 15 mg benomyl, and 25 mg hymexazol per 1 L of distilled water (Hansen and Hamm 1996). The CARP plates were incubated at room temperature (20 °C) and checked after 24 h under a microscope. This allowed differentiation between fast- and sparsely-growing mycelium, which is typical for some species of the genus *Pythium*, and slow- and densely growing *Phytophthora* mycelium. From the colonies with the latter pattern, an agar plug was transferred to Petri dishes containing Potato Dextrose Agar (39 g/L PDA; Difco, Voight Global Distribution, Lawrence, MD, USA) and incubated at room temperature.

Phytophthora identification

From the putative *Phytophthora* cultures, an agar plug was either transferred to a Petri dish containing 15 ml V8 liquid medium or directly used for DNA extraction. The V8 medium was prepared by mixing 330 ml of V8 juice with 5 g of calcium carbonate in a sterile flask, stirring for 15–20 min. The mixture was transferred to 50-ml Falcon tubes and centrifuged at 6500 rpm for 5 min to separate solids from liquids. Distilled water was added to the filtered supernatant to reach 600 ml. A total of 200 ml V8 concentrate per 1 L of distilled water is used for the final medium (Miller 1955). After approximately 1 week, the growing mycelium in the V8 liquid medium was harvested using vacuum filtration. About 10 mg of fresh mycelium was put into a sterile Eppendorf tube. DNA extraction was performed using Qiagen DNeasy 96 Plant Kit following the manufacturer's instructions. From the agar plug, DNA was extracted using LGC Genomics reagents on Kingfisher 96 Flex (Thermofisher) according to the manufacturer's protocol. DNA concentrations were measured with an Eppendorf Bio Photometer using 54 µl of distilled H_2O and 6 µl of pure DNA. Before PCR, DNA samples were tenfold diluted to final concentrations of 1–5 µg/ml. The region spanning the internal transcribed spacer (ITS1-5.81-ITS2) of the ribosomal DNA was PCR amplified and sequenced using the primers ITS6 (5′-GAAGGTGAAGTCGTAACAAGG-3′; Cooke et al. 2000) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′; White et al. 1990). The PCR mix consisted of 10.4 µl distilled and sterile

Table 1 *Phytophthora* species detected in the 32 watercourses sampled between 2012 and 2016 in the Swiss Plateau. In total, 52 *Phytophthora* baiting sites were considered

Watercourse	Nb of sampling sites	Watercourse order	*Phytophthora* species (clade)											Total isolates	Total species
			P. lacustris (6)	*P. plurivora* (2)	*P. gonapodyides* (6)	*P. hydropathica* (9)	*P. riparia* (6)	*P. chlamydospora* (6)	*P. cryptogea* (8)	*P. polonica* (9)	*P. citrophthora* (2)	*P. gallica* (10)	*P. bilorbang* (6)		
Aabach	1	4	2	0	0	0	0	0	0	0	1	0	0	3	1
Aach	1	4	18	2	1	0	0	0	0	2	0	0	0	23	4
Aare	3	9	6	0	0	0	0	0	0	0	0	0	0	6	1
Aare-Hagneck Kanal	1	9	3	0	0	0	0	0	0	0	0	0	0	3	1
L'Areuse	2	4	3	0	0	0	1	0	0	0	0	0	0	4	2
Birs	3	6	4	0	0	0	0	0	0	0	0	0	0	4	1
Emme	2	7	2	0	0	0	0	0	0	0	0	0	0	2	1
Ergolz	2	6	4	0	0	0	0	0	0	0	0	0	0	4	1
Giesse	1	5	2	0	0	0	0	0	0	0	0	0	0	2	1
Glatt	2	6	6	2	0	0	0	0	0	0	0	0	0	8	2
La Broye	2	6	2	0	1	0	0	0	0	0	0	0	0	3	2
La Chamberonne	1	4	1	0	1	0	0	0	0	0	0	0	0	2	2
La Menthue	1	5	2	0	0	0	0	0	0	0	0	0	0	2	1
La Thielle	1	4	1	0	0	0	0	0	0	0	0	0	0	1	1
La Trême	1	4	0	1	0	0	0	0	0	0	0	0	1	2	1
La Veveyse	1	5	0	1	0	0	0	0	0	0	0	0	0	1	1
Langete	1	5	1	0	1	0	0	0	0	0	0	0	0	2	2
Limmat	1	8	1	1	0	0	0	0	0	0	0	0	0	2	2
Murg	1	5	1	0	0	0	0	0	0	0	0	0	0	1	1
Reuss	2	8	33	0	2	4	2	1	0	0	0	0	0	42	5
Rhine	3	9	34	3	3	0	0	0	1	0	0	0	0	41	4
Rhone	1	7	1	0	0	0	0	0	0	0	0	0	0	1	1
Saane	1	7	1	0	0	0	0	0	0	0	0	0	0	1	1
Sense	1	7	2	1	0	0	0	0	0	0	0	0	0	3	2
Sitter	3	6	19	3	1	0	0	0	1	0	0	0	0	24	4
Steinach	1	4	2	1	0	0	0	0	0	0	0	0	0	3	2
Suhre	1	4	3	0	0	0	0	0	0	0	0	0	0	3	1
La Suze	1	3	1	0	0	0	0	0	0	0	0	0	0	1	1
Thur	6	7	21	11	1	0	0	0	0	0	0	0	0	33	3
Toess	2	6	3	4	3	0	0	0	0	0	0	1	0	11	4

Table 1 (continued)

Water-course	Nb of sampling sites	Water-course order	*Phytophthora* species (clade)											Total isolates	Total species
			P. lacustris (6)	*P. plurivora* (2)	*P. gonapodyides* (6)	*P. hydropathica* (9)	*P. riparia* (6)	*P. chlamydospora* (6)	*P. cryptogea* (8)	*P. polonica* (9)	*P. citrophthora* (2)	*P. gallica* (10)	*P. bilorbang* (6)		
Venoge	1	4	1	0	0	0	0	0	0	0	0	0	0	1	1
Wigger	1	6	2	0	0	0	0	0	0	0	0	0	0	2	1
Total isolates			182	30	14	4	3	1	2	2	1	1	1	241	-
%			75.5	12.4	5.8	1.7	1.2	0.4	0.8	0.8	0.4	0.4	0.4	-	-
Positive water-courses			30	11	9	1	2	1	2	1	1	1	1	32	-
%			93.8	34.4	28.1	3.1	6.3	3.1	6.3	3.1	3.1	3.1	3.1	-	-

H_2O, 7.6 µl of Jump Start Taq polymerase (Sigma Aldrich), 1 µl Primer-Mix ITS6 and ITS4 (12.5. pmol/µl) and 1 µl of tenfold diluted DNA. The PCR was run on Veriti 96 well thermocyclers (Applied Biosystems) with initial denaturation at 95 °C for 2 min, 35 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min, and a final extension of 10 min at 72 °C. Before sequencing, the PCR products were purified using 2 µl of ExoProStar 1-Step (GE Healthcare). For this, 5 µl of the PCR product was incubated for 15 min at 37 °C and for another 15 min at 80 °C.

Cycle sequencing was conducted using 0.75 µl of distilled and sterile H_2O, 0.75 µl of Bigdye Sequencing Buffer (5 ×) (Applied Biosystems), 1.5 µl Ready Reaction Premix (2.5 ×) (Applied Biosystems), 1.5 µl of primers ITS6 or ITS4 (3.2 pmol/µl), respectively, and 3 µl of template DNA (5ng/µl). The sequencing reaction was run on Veriti 96 well thermocyclers with 25 cycles consisting of 10 s at 96 °C, 5 s at 50 °C, and 1 min at 60 °C. The cycle sequencing product was cleaned using an XTerminator Kit (Applied Biosystems) and sequenced in both directions on an ABI prism 3130 Genetic Analyser. Sequences were aligned and edited using CLC Main Workbench 7 (www.clcbio.com). For species assignment, the edited sequences were blasted against sequences in the databases Phytophthora–ID.org (http://phytophthoradb.org), BOLD (http://www.boldsystems.org), and NCBI (http://blast.ncbi.nlm.nih.gov). Two sequences were considered to belong to the same species if they showed at least 99% similarity. To confirm species assignment, ITS sequences were analyzed using the NGphylogeny.fr webservice with the PhyML+SMS workflow (Lemoine et al. 2019). For phylogenetic comparisons, representative sequences for each species from our dataset were selected and analyzed along with reference sequences available in NCBI (https://www.ncbi.nlm.nih.gov/) for each of the five detected clades. The phylogeny is presented in Supplementary Fig. 2.

Results

Overall *Phytophthora* prevalence and diversity

A total of 241 *Phytophthora* isolates were obtained from the 32 watercourses sampled, with one (seven watercourses) to 42 (one watercourse) isolates found per watercourse (Table 1). The highest *Phytophthora* prevalence was observed in the rivers Reuss (42 isolates) and Rhine (41 isolates). Noteworthy, in the main river Rhone, which was sampled at one site in proximity to its estuary into the lake of Geneva, only one isolate of *Phytophthora* was obtained. Sequencing of the ITS region showed that the isolates belonged to 11 different *Phytophthora* species from five clades (Table 1). Clade 6 was the most represented with five species (*P. lacustris*, *P. gonapodyides*, *P. riparia*, *P.*

chlamidospora, and *P. bilorbang*), followed by clade 9 (*P. hydropathica*, *P. polonica*) and clade 2 (*P. plurivora*, *P. citrophthora*) with two species each and clade 8 (*P. cryptogea*) and clade 10 (*P. gallica*) with one species each. Overall, *P. lacustris* was the most common species, accounting for 75.5% of the isolates found, and was present in 30 of the 32 watercourses (93.8%) sampled (Table 1). The second most abundant species was *P. plurivora* (12.4% of the isolates, 11 watercourses), followed by *P. gonapodyides* (5.8% of the isolates, nine watercourses). The remaining species were each restricted to one or two watercourses, ranging from one to four isolates each. The number of *Phytophthora* species found per watercourse ranged from one to five (mean of 1.8; Table 1) and was not significantly influenced by the complexity and size of the watercourse as measured by the Strahler order number (Person's correlation coefficient $r = 0.2523$, $p = 0.164$).

Phytophthora diversity in river basins

In a single basin, three (Rhone) to eight (Aare) *Phytophthora* species were detected (Fig. 2). Three species, *P. lacustris*, *P. plurivora*, and *P. gonapodyides,* were found in all three basins, with *P. lacustris* being the predominant species everywhere, accounting for 60% (Rhone) to 80% (Aare) of the isolates. *Phytophthora plurivora* showed a similar prevalence in the basins Rhine and Rhone (17.2% and 20% of the isolates, respectively), but was considerably less frequent in the Aare basin (3.5%). On the other hand, *P. gonapodyides* was more frequent in the Rhone basin (20%) than in the other two (4.7% Aare and 6% Rhine). Private species (i.e., species specific to a single basin) were observed in the Aare (*P. hydropathica*, *P. riparia*, *P. chlamydospora*, *P. citrophthora*, *P. bilorbang*) and the Rhine (*P. cryptogea*, *P. polonica*, *P. gallica*), but not in the Rhone basin (Fig. 2). Overall, the prevalence of private species was low, ranging from 0.7 (*P. gallica*) to 4.7% (*P. hydropathica*) of all isolates.

Discussion

This study is the result of an intensive *Phytophthora* monitoring that was conducted between 2012 and 2016 in watercourses flowing through the Swiss Plateau. Using a baiting approach, we isolated *Phytophthora* in all watercourses sampled. *Phytophthora* diversity ranged from one to five species per watercourse, which is in line with the values reported in Austria and the Czech Republic (Corcobado et al. 2023) and in Australia (Hüberli et al. 2013). Since complex watercourses consisting of multiple branches can collect water from different regions, one may expect higher *Phytophthora* diversity in watercourses of higher Strahler orders. However, in our surveys, we did not observe such a correlation. For example, in the main European river Rhone, before its estuary into Lake Geneva (mean annual water discharge 1935–2019: 182 m^3/s), only one *Phytophthora* isolate was recovered. Hence, other factors may play a role in the *Phytophthora* diversity in watercourses, which may include water temperature and chemistry, and the environment

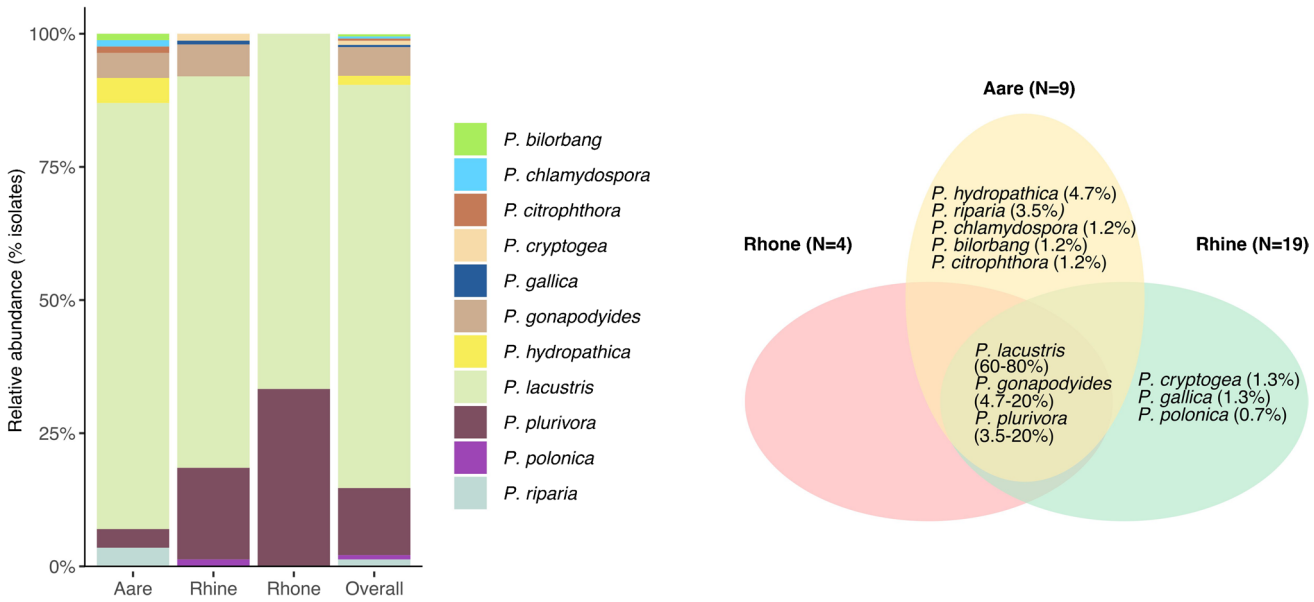

Fig. 2 The proportional representation of *Phytophthora* diversity in each basin. *Phytophthora* species were identified in the three sampled river basins within the Swiss Plateau (N denotes the number of watercourses analyzed, as specified in Supplementary Table 1). The prevalence of these species, expressed as a percentage of isolates, is provided in parentheses

drained (e.g., urban areas, forests, and agricultural land) (Redondo et al. 2018; Riolo et al. 2020).

The 241 *Phytophthora* isolates detected belonged to 11 different species from five different major clades (Cooke et al. 2000; Yang et al. 2017). *Phytophthor*a clade 6 dominated in the watercourses of the Swiss Plateau, confirming results of previous studies from Central Europe and other continents (e.g., Corcobado et al. 2023; Jung et al. 2011; Reeser et al. 2011; Nagel et al. 2015). The most frequent species was *P. lacustris*, a ubiquitous species in riparian ecosystems throughout Europe and North America (Nechwatal and Mendgen 2006; Reeser et al. 2011; Nechwatal et al. 2013). The exact ecological function of most taxa within clade 6 is still unclear (Brasier et al. 2003). The adaptation of the nutritional strategies of most species towards a saprophytic lifestyle, lacking an obligate biotrophic phase, is thought to explain, at least partially, their dominance in water (Aram and Rizzo 2018). For instance, the facultative pathogens *P. gonapodyides* and *P. chlamydospora* although capable of causing disease symptoms (Hansen 2015; Ruffner et al. 2019) may have a competitive advantage over other colonizers with an obligate biotrophic phase. Sexual sterility and tolerance to high temperatures have also been suggested to represent an adaptation of clade 6 species to riparian conditions (Brasier et al. 2003). Although natural hybridization among clade 6 *Phytophthora* species seems to be a common phenomenon (e.g., in Western Australia, Burgess 2015; South Africa, Nagel et al. 2013; Central Europe, Corcobado et al. 2023), no hybrids were found in our study. Regarding the non-clade 6 *Phytophthora* species detected in the Swiss watercourses, all of them were already reported to occur in streams and rivers across Europe (e.g., Jung et al. 2019; Christova 2022; Corcobado et al. 2023). To mention, there is a relatively high prevalence of *P. plurivora* (12.4% of the isolates, present in 11 watercourses), a species commonly found in Europe where it is frequently associated with declining trees, especially European beech (*Fagus sylvatica*) (e.g., Ruffner et al. 2019; Corcobado et al. 2020; Jankowiak et al. 2023).

In comparison to the findings in 1996 and 2013, where 15 and nine *Phytophthora* species were respectively reported in Switzerland (Bolay and Schwinn 1996; Scott et al. 2013), our recent monitoring in Swiss watercourses identified a total of 11 species. Of these, six were reported for the first time in the country (*P. bilorbang*, *P. gallica*, *P. hydropathica*, *P. lacustris*, *P. polonica*, *P. riparia*). *Phytophthora bilorbang* (clade 6) was first described in 2012 in Western Australia as a pathogen of *Rubus anglocandicans* (Aghighi et al. 2012). Later, it was also isolated in Southern Italy from the rhizosphere and from declining plants of the Mediterranean maquis, as well as from olive trees (*Olea europaea*) with symptoms of defoliation, wilting, and root rot (Scanu et al. 2015; Santilli et al. 2020) and in Central Europe from streams and rivers (Corcobado et al. 2023). *Phytophthora gallica* (clade 10) was previously found in the rhizosphere of a declining oak in Northeastern France and in the rhizosphere of common reed (*Phragmites australis*) in Southwestern Germany (Jung and Nechwatal 2008). *Phytophthora hydropathica* (clade 9) was first described by Hong et al. (2010) from irrigation water in ornamental plant nurseries in the Eastern United States. Interestingly, both soilborne species *P. gallica* and *P. hydropathica* were also detected in watercourses in Austria and the Czech Republic (Corcobado et al. 2023), which, together with our study, confirms their occurrence in aquatic environments in Central Europe. The clade 8 species *P. polonica* has so far been reported only in Europe, mainly in association with declining alders (*Alnus* spp.) (Belbahri et al. 2006, Corcobado et al. 2023; Matsiakh et al. 2023; Tkaczyk et al. 2023). Finally, after being first identified in streams in Alaska and Oregon (Hansen et al. 2012), *P. riparia* (clade 6) has also been found in rivers and streams of other US states (Stamler 2016; Bily et al. 2022) and Central Europe (Corcobado et al. 2023), as well as in plant nurseries in California (Rooney-Latham et al. 2019).

To date, a total of 30 *Phytophthora* species have been officially confirmed in Switzerland, twice as many as were reported in 1996 (Fig. 3). In addition to species that are most likely native to Europe (e.g., *P. cactorum*, *P. gonapodyides*, *P. lacustris*, *P. polonica*), exotic species are also present, many of which have a high pathogenic potential (in particular, *P.* × *cambivora*, *P. cinnamomi*, *P.* × *alni*, *P. infestans*, *P. ramorum*). As revealed in other studies, species are not evenly distributed across environments but show typical occurrence patterns (Fig. 3). Hence, to obtain an accurate picture of the diversity and distribution of *Phytophthora* species in a given region, it is necessary to sample different types of environments. Our surveys confirm a strong preference for clade 6 species for aquatic environments, in which *P. hydropathica*, *P. polonica* (clade 9), and *P. gallica* (clade 10) have also been found so far. Invasive pathogenic species are restricted to tree nurseries (*P. multivora* and *P. ramorum*) and/or forests (*P.* × *alni*, *P.* × *cambivora*, *P. cinnamomi*). None of them could be detected in watercourses, possibly reflecting their effective absence due to successful eradication measures (e.g., *P. ramorum*) or their still relatively low prevalence in terrestrial environments (i.e., not enough inoculum to reach watercourses). On the other hand, such species may be less competitive in the aquatic environment. For example, *P. cinnamomi*, which is the main causal agent of the ink disease of sweet chestnut (*Castanea sativa*) in Southern Switzerland (Prospero et al. 2023), could not be detected in the Ticino River, into which water from infected chestnut stands flows (unpublished data). A similar situation was, for example, reported in Sardinia (Italy) in watercourses draining declining cork oak (*Quercus suber*) stands in which *P. cinnamomi* was the most common species in the

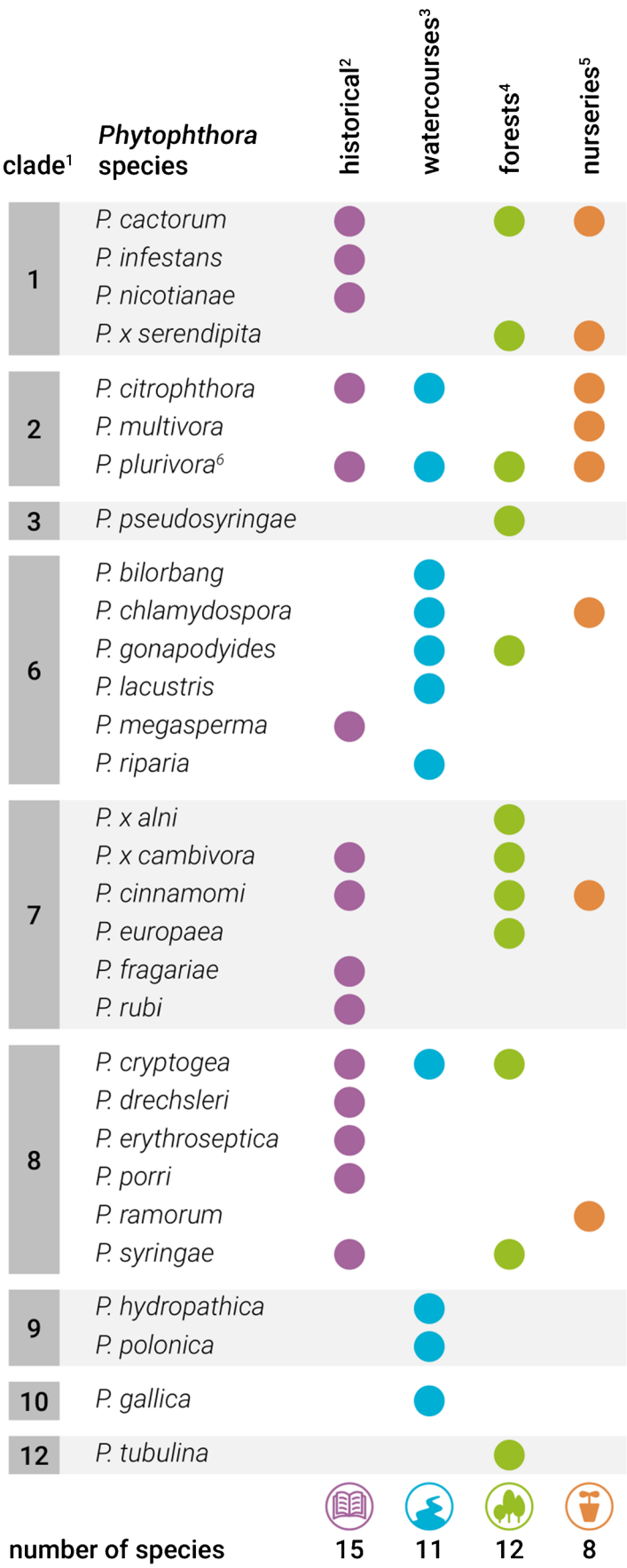

Fig. 3 *Phytophthora* species detected in Switzerland up to 2023. *Phytophthora* species were grouped into ITS Clades following Yang et al. (2017)[1]. Historial data[2]: Bolay & Schwinn (1996). Watercourse[3]: This study. Forests[4] (includes urban trees): Ruffner et al. (2019), Mizeriene et al. (2020), and Prospero et al. (2023). Nurseries[5]: Prospero et al. (2013). *P. plurivora*[6] this species identified as *P. citricola* in Bolay and Schwinn (1996)

soil (Seddaiu et al. 2020). Stream baiting has been useful to survey invasive *Phytophthora* species such as *P. ramorum*, but it might not be the method of choice to monitor other *Phytophthora* species, in particular obligate biotrophs.

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Data availability Sequences of the *Phytophthora* cultures detected in this study are available from the corresponding author upon request.

Declarations

Competing interests The authors declare no competing interests.

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