ORIGINAL ARTICLE

High diversity of *Phytophthora* species in natural ecosystems and nurseries of Portugal: Detrimental side effect of plant introductions from the age of discovery to modern globalization

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Abstract

Between 2010 and 2015, an extensive survey of *Phytophthora* diversity was performed across Portugal. Baiting and direct isolation tests obtained 48 *Phytophthora* taxa in 62 of 70 forest stands (89%) from 148 of 302 rhizosphere soil samples (49%) and 25 of 29 tree species (86%), and from bark cankers of *Alnus lusitanica* and *Fagus sylvatica*; from 33 of 34 rivers and forest streams (97%); and in 12 of 13 forest nurseries (92%) from 54 of 87 root ball samples (61%) and 16 of 21 tree species (76%). The 799 *Phytophthora* isolates belonged to 28 known and three recently described species; one designated and two new non-hybrid taxa; and five designated and nine new hybrid taxa. The majority, 34 *Phytophthora* taxa (71%), are introduced aliens in Europe while nine (19%) and five (10%) taxa are of cryptic and native origin, respectively. *Phytophthora cinnamomi* was the most common species in forests (56%) and forest nurseries (61%). Multiple first records for Portugal and partly Europe of both *Phytophthora* taxa and tree declines and new host–*Phytophthora* associations were found. Isolation rates from forest soil differed considerably between declining (65%)

and healthy trees (10%), regardless of the tree species. The widespread infestations of nurseries with aggressive wide-host range pathogens like *P. cinnamomi, P. pseudocryptogea, P. ×cambivora, P. multivora* and *P. plurivora,* and host-specific pathogens like *P. alticola, P. quercetorum* and *P. quercina,* will inevitably cause their further spread to the wider environment, exacerbating pathogen threats to forest ecosystems and thus weakening their resilience to climatic extremes.

1 | INTRODUCTION

The genus *Phytophthora* (Oomycota, Stramenopiles and Peronosporaceae) currently comprises more than 260 described species and numerous informally designated taxa organized in 14 phylogenetic clades with high biological cohesion (Abad et al., 2023; Brasier et al., 2022; Chen et al., 2022; Jung et al., 2022, 2024). Many devastating declines of horticultural crops, forest trees and natural ecosystems are driven by exotic *Phytophthora* species which, due to coevolution with their native host plants, remain unnoticed in their native habitats. However, following their introduction to other biogeographic regions, they often become invasive, threatening a non-coevolved flora containing a high number of susceptible species (Chen et al., 2022; Erwin & Ribeiro, 1996; Jung, Pérez-Sierra, et al., 2018; Lamour, 2013). Since the 1960s, the number of important Phytophthora diseases of forest trees and natural ecosystems has increased exponentially from five to currently 41 (Brasier et al., 2022). Several disease syndromes of forests and other natural ecosystems, namely dieback of eucalypt forests and *Banksia* heathlands in Australia, oak declines in Europe and the United States, beech decline in Europe, and decline and dieback of Mediterranean maquis vegetation in Italy and California, involve pathogens from multiple (up to nine) *Phytophthora* clades (Bourret et al., 2023; Brasier et al., 2022; Frankel et al., 2020; Jung, Pérez-Sierra, et al., 2018; Scanu et al., 2015). An array of *Phytophthora* species stand out due to their high invasiveness and their aggressiveness to either a wide range of host species (*P. cactorum, P. cinnamomi, P. palmivora, P. multivora, P. plurivora, P. ramorum* and *P. ×cambivora*) or to specific hosts (*P. austrocedri, P. lateralis* and *P. ×alni*) (Brasier et al., 2004, 2022; Brasier & Webber, 2010; Drenth & Guest, 2004; Erwin & Ribeiro, 1996; Greslebin et al., 2007; Grünwald et al., 2012; Hansen et al., 2000; Hardham & Blackman, 2018; Jung & Burgess, 2009; Jung, Pérez-Sierra, et al., 2018; Scott et al., 2009). Recently, worldwide surveys in natural ecosystems and molecular population analyses have unveiled the geographic origins of several notorious *Phytophthora* species: *P. cactorum* in North America; *P. cinnamomi* in Southeast Asia; *P. kernoviae* in Chile; *P. lateralis* in Taiwan; *P. multivora* in South Africa; *P. plurivora, P. ramorum* and *P. ×cambivora* in East Asia; and *P. pseudosyringae* in Europe (Bourret et al., 2022; Brasier et al., 2012; Jung et al., 2021, 2022, 2024; Jung,

Durán, et al., 2018; Mullett et al., 2023, 2024; Shakya et al., 2021; Tsykun et al., 2022).

The intercontinental trade in living plants has been identified as the main pathway for accidental introductions of plant pathogens, including Phytophthoras, from other continents (Brasier, 2008; Jung et al., 2016; Liebhold et al., 2012; Santini et al., 2013). During the past two decades, several studies demonstrated almost ubiquitous infestations of nurseries with a wide range of *Phytophthora* pathogens and presented strong evidence for the planting of infested nursery stock constituting the major pathway of invasive harmful *Phytophthora* pathogens to the wider environment (Bačova et al., 2024; Bienapfl & Balci, 2014; Frankel et al., 2020; Garbelotto et al., 2018; Jung et al., 2016; Leonberger et al., 2013; Migliorini et al., 2015; Moralejo et al., 2009; Mora-Sala et al., 2022; Prigigallo et al., 2015, 2016; Rooney-Latham et al., 2019; Sims & Garbelotto, 2021).

Situated along the Atlantic in the southwesternmost corner of Europe, mainland Portugal has a long history of maritime exploration and overseas trade going back to the early 15th Century. Following the establishment of colonies and trading posts during the 16th Century in Brazil, along the West African coast (Cape Verde, Guinea Bissau, São Tomé and Principe, Angola) and the East African Swahili coast (e.g., Mozambique, Mombasa, Zanzibar), in India (Goa, Mumbai), the Malay archipelago (Malacca, the Moluccas, Timor), China (Macau), Taiwan and Japan (Nagasaki) (Birmingham, 2000; Disney, 2009; Page, 2013), numerous ornamental and horticultural plants were introduced to Portugal over centuries. With 667 plant taxa, 17.9% of the total Portuguese flora, originating from other biogeographic regions and more than 300 alien plant species first recorded for Europe, Portugal is a major gateway country for alien plant introductions, ranking third behind the UK and Belgium (Arianoutsou et al., 2021; Domingues de Almeida & Freitas, 2012). Consequently, it can be assumed that a rich diversity of non-native *Phytophthora* species has been introduced as hitchhikers of the intense plant trade to Portugal. Indeed, unambiguous symptoms of ink disease of sweet chestnut (*Castanea sativa*) were first reported in Portugal in 1838 (Crandall et al., 1945), suggesting the causal agents *P. cinnamomi* and *P. ×cambivora* were introduced and established long before the genus *Phytophthora* had been erected in 1876 by de Bary (Chen et al., 2022). Presence of both pathogens in Portuguese

chestnut and cork oak (*Quercus suber*) stands was unveiled in the 1940s (Lopes-Pimentel, 1946, 1947). Fifty years later, Brasier et al. (1993) demonstrated the involvement of *P. cinnamomi* in the widespread oak decline in Portugal and Spain, which was later confirmed by other studies (e.g., Gallego et al., 1999; Jung et al., 2016; Moreira & Martins, 2005).

Worldwide, oomycete surveys of waterbodies and riparian ecosystems have revealed an astonishing diversity of *Phytophthora* species, including—in addition to numerous primarily aquatic species—many soilborne and airborne primary pathogens (Corcobado et al., 2023; Hüberli et al., 2013; Jung et al., 2019, 2020, 2022, 2024; Jung, Chang, Bakonyi, et al., 2017; Jung, Durán, et al., 2018; Oh et al., 2013; Reeser et al., 2011; Shrestha et al., 2013). In Portugal, *P. ×alni*, the main causal agent of root and collar rot and mortality of alders, was first reported in 2016 (Jung et al., 2016; Kanoun-Boulé et al., 2016) while a small-scale survey in five riparian stands demonstrated the presence of 11 *Phytophthora* species, including *P. multivora*, *P. plurivora* and *P. rosacearum* (Bregant et al., 2023a).

This paper reports on the results of a survey of *Phytophthora* diversity performed between May 2010 and October 2015 in a diverse range of natural and planted forests, river systems and forest nurseries across Portugal, and on the association of *Phytophthora* spp. with disease symptoms of forest trees. Many nursery results were previously included in the extensive dataset from a Europe-wide survey of almost 2000 nursery stands (Jung et al., 2016) but updated molecular species identifications and DNA sequences, detailed isolation methods, infestation rates, host plants and disease symptoms are given here for the first time.

2 | MATERIALS AND METHODS

2.1 | Sampling and *Phytophthora* isolation

Seventy forest sites covering a wide range of forest types and tree species, 39 riparian sites along 34 rivers and forest streams and 13 forest nurseries across Portugal were selected for sampling. The locations of the forest and riparian sites are shown in Figure 1 and their GPS coordinates are given in Tables 1 and 2. Because anonymity was a precondition for all participating nursery owners, no precise locations and names of nurseries are provided. A selection of forest types sampled is shown in Figure 2.

With the forest sites, soil sampling and isolation tests were performed according to Jung (2009) and Pérez-Sierra et al. (2013, 2022). In total, 302 rhizosphere soil samples were taken from mature trees of 29 species in 63 natural and semi-natural forest stands, two stands with a mixture of mature cork oaks and planted younger cork oaks (F45) or *Arbutus unedo* (F67), and five agroforestry plantations of *C. sativa*, *Juglans regia*, *Pinus pinea* and *Q. suber* (F12, F18, F42, F57 and F68) (Figure 1; Table 1). After removal of the organic surface layer, three soil monoliths with a size of 20×20×30 cm were taken from the rhizosphere of each tree, at a distance of 30–150 cm from the stem base. Aliquots of soil together with fine and coarse roots

(diameter ≤5 mm) from all monoliths were bulked, and subsamples of approximately 500–750 mL were spread in a plastic container or tub (c. 18×25–27×13–14 cm) and flooded with distilled water so that the waterline was around 2–3 cm above the soil surface. After removal of floating debris with tweezers and tissue paper, 3- to 10-day-old leaflets of *Q. suber*, *Q. pyrenaica*, *Q. robur* and *Ceratonia siliqua* were floated as baits on the water covering the whole surface. Isolations were carried out at 18–20°C in an air-conditioned room in natural daylight. Brownish leaflets were examined at 80× under a light microscope for the 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; Jung, 2009; Pérez-Sierra et al., 2022).

Bleeding bark cankers were sampled from six *Alnus lusitanica* trees growing in four riparian forests (F22, F23, F28 and F41) on the riverbanks of the Tâmega, Fervença, Alva and Côa Rivers, and from one *F. sylvatica* tree in a montane forest (F37) in the Parque Nacional da Serra da Estrela (Figures 1 and 3). Samples were taken from the upper 20 cm of active lesions with orange-brown discolouration of the inner bark, placed in distilled water and transported to the laboratory where the water was replaced four times per day over 2–3 days in order to remove excess polyphenols. Small pieces (c. 8×3×3 mm) were cut from the lesion, blotted on filter paper and plated onto selective PARPNH agar.

Phytophthora isolations from the 34 rivers and forest streams were performed using an in-situ baiting technique (Jung et al., 2019; Jung, Chang, Bakonyi, et al., 2017; Jung, Durán, et al., 2018). At each of the 39 sites, 15–20 non-wounded young leaves of *Q. suber*, *C. siliqua* and *Citrus sinensis* were placed as baits in a 25 ×30 cm raft, prepared using nylon fly mesh and styrofoam, and the raft put to float at a place with calm water flow (Figure 2h). The rafts were collected after 3–4 days. Baiting leaves were washed in distilled water and blotted on filter paper. Five to 10 pieces (c. 2×2 mm) were cut from each necrotic lesion of each leaf, blotted again on filter paper and plated onto PARPNH agar.

In the 13 forest nurseries, 87 bulked samples (1–13 bulked samples per nursery), each consisting of the root balls (= root systems and adhering soil) of three to six 1- to 3-year-old container plants from the same species, were harvested from 21 tree species. If present, seedlings with symptoms such as wilting, dieback, chlorosis and root and collar rot were sampled preferentially. In the laboratory, all root systems of a bulked sample were opened up and spread in a plastic container or tub (c. 18 × 25–27 × 13–14 cm), flooded with distilled water and baited in the same way as the forest soil samples. In addition, in one nursery a *C. sativa* seedling with a fresh collar rot was sampled and the necrotic bark tissue plated onto PARPNH agar as described before.

All PARPNH Petri dishes with plated leaf or bark segments were incubated at 20°C in the dark and examined for *Phytophthora*-like hyphae after 24–48 h. Pure cultures were obtained by transferring single hyphal tips from the edge of the colonies onto V8A. Stock
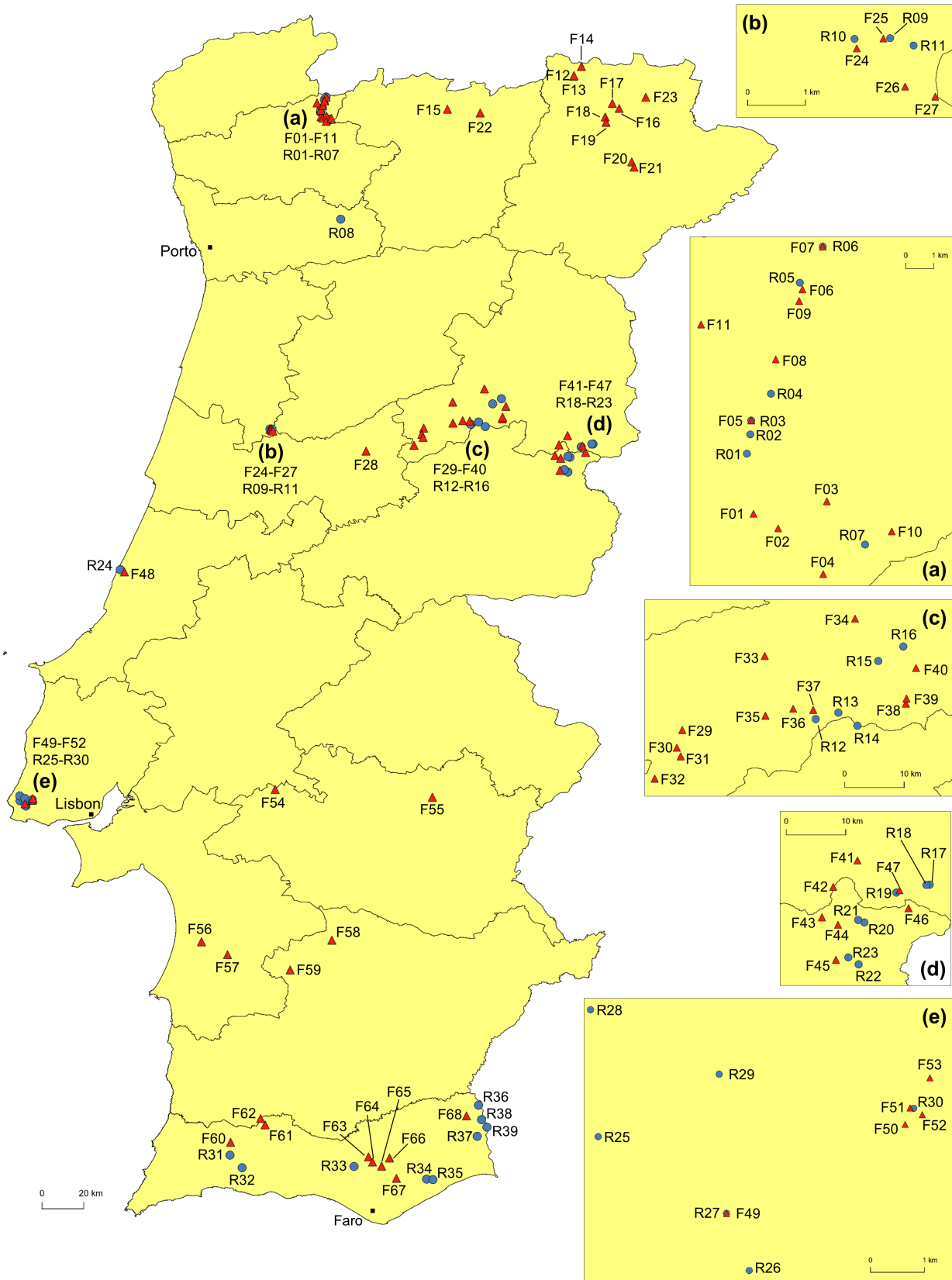
FIGURE 1 Location of 68 forest stands (F01–F68; red triangles) and 39 riparian sites (R01–R39; blue circles) along 34 rivers and streams included in the *Phytophthora* survey in mainland Portugal. Forest stands F69 (São Miguel Island) and F70 (Madeira Island) are not shown. Small maps provide detailed locations of the sites in (a) Parque Nacional da Peneda-Gerês; (b) Mata Nacional do Buçaco; (c) Parque Nacional da Serra da Estrela; (d) Reserva Natural da Serra da Malcata; and (e) Parque Natural Sintra-Cascais. For GPS coordinates see Tables 1 and 2.

TABLE 1 Location, altitude, geological substrate and vegetation of 70 forest sites sampled in Portugal, sampled tree species and their health condition, and *Phytophthora* and other oomycete taxa isolated.

Site no.	GPS coordinates	Altitude (ma.s.l.)	Location; parish, municipality, district	Geological substrate	Vegetation	Sampled tree species and health condition (no. of Phytophthora-positive/sampled trees)[a]	Phytophthora spp. (no. of positive samples)[b–e]
F01	41°43.110′ N, 08°09.177′ W	711	Parque Nacional da Peneda-Gerés (PNGP); Vilar da Veiga, Terras de Bouro, Braga	Granite	Submontane broadleaved forest	Acer pseudoplatanus DB (1/1)	×CAM-DAPC9 (1)
						A. pseudoplatanus DC (0/1)	–
						Arbutus unedo DB (1/2)	SYR (1)
						Betula celtiberica DB (1/2)	×CAM-DAPC9 (1)
						Quercus robur (0/1)	–
						Salix caprea (1/1)	×CAM-DAPC9 (1)
F02	41°42.828′ N, 08°08.703′ W	775	PNGP; Vilar da Veiga, Terras de Bouro, Braga	Granite	Submontane birch forest	B. celtiberica (0/5)	–
F03	41°43.350′ N, 08°07.765′ W	726	PNGP; Vilar da Veiga, Terras de Bouro, Braga	Granite	Submontane broadleaved forest	Quercus rubra DB & bleeding bark canker (1/2)	CIN-PcG1 (1)
F04	41°41.948′ N, 08°07.835′ W	529	PNGP; Vilar da Veiga, Terras de Bouro, Braga	Granite	Submontane broadleaved forest	Quercus suber DB (1/1)	CIN-PcG2 (1)
F05	41°44.908′ N, 08°09.221′ W	597	PNGP; Vilar da Veiga, Terras de Bouro, Braga	Granite	Submontane broadleaved forest	Castanea sativa DC (1/2)	CIN-PcG1 (1)
						C. sativa (0/2)	–
						Q. robur (0/1)	–
F06	41°47.434′ N, 08°08.235′ W	699	PNGP; Campo do Gerês, Terras de Bouro, Braga	Granite	Submontane broadleaved forest	Q. robur DB (1/1)	QUE (1)
F07	41°48.253′ N, 08°07.843′ W	748	PNGP; Campo do Gerês, Terras de Bouro, Braga	Granite	Submontane mixed broadleaved–pine forest	Quercus pyrenaica (0/2)	–
						Q. robur (0/5)	–
						Q. robur DC & Q. pyrenaica DB (1/2)	CIN (1)
						Q. pyrenaica DB & Pinus pinaster DB (1/1)	CIN (1), ×CAM-DAPC9 (1)
F08	41°46.083′ N, 08°08.749′ W	849	PNGP; Campo do Gerês, Terras de Bouro, Braga	Granite	Lower montane broadleaved forest	Fagus sylvatica DB (1/1)	CIN (1)
F09	41°47.208′ N, 08°08.298′ W	744	PNGP; Campo do Gerês, Terras de Bouro, Braga	Granite	Submontane broadleaved forest	F. sylvatica (0/4)	–
F10	41°42.771′ N, 08°06.513′ W	689	PNGP; Vilar da Veiga, Terras de Bouro, Braga	Granite	Submontane broadleaved forest	Q. suber DB (2/4)	CIN (2)
F11	41°46.756′ N, 08°10.188′ W	552	PNGP; Campo do Gerês, Terras de Bouro, Braga	Granite	Submontane broadleaved forest	Q. robur (0/5)	–
F12	41°53.620′ N, 07°04.070′ W	823	Perímetro Florestal Serra da Coroa (PFSC)/Parque Natural Montesinho (PNM); Tuizelo, Vinhais, Bragança	Phyllites, quartzites, vulcanites	Chestnut–walnut plantation	C. sativa DB & bleeding bark cankers (2/4)	×CAM-DAPC9 (2)
						Juglans regia DC (0/1)	–

TABLE 1 (Continued)

Site no.	GPS coordinates	Altitude (ma.s.l.)	Location; parish, municipality, district	Geological substrate	Vegetation	Sampled tree species and health condition (no. of Phytophthora-positive/sampled trees)[a]	Phytophthora spp. (no. of positive samples)[b–e]
F13	41°53.783′ N, 07°03.997′ W	857	PFSC/PNM; Tuizelo, Vinhais, Bragança	Phyllites, quartzites, vulcanites	Lower montane broadleaved forest	Q. pyrenaica DC (1/2) Q. pyrenaica (0/3)	xCAM-DAPC9 (1) –
F14	41°56.110′ N, 07°02.070′ W	826	PFSC/PNM; União de Freguesias (UF) Moimenta e Montouto, Vinhais, Bragança	Phyllites, quartzites, vulcanites	Lower montane broadleaved forest	C. sativa DB (2/3) C. sativa (0/2) Q. pyrenaica DC (1/2)	xCAM-DAPC9 (2) – xCAM-DAPC9 (1)
F15	41°45.106′ N, 07°36.599′ W	561	UF Ardãos e Bobadela, Boticas, Vila Real	Granite	Submontane broadleaved forest	Q. pyrenaica DB (1/1) Q. pyrenaica (0/5)	xCAM-DAPC9 (1), QUE (1) –
F16	41°45.282′ N, 06°52.352′ W	1043	UF Castrelos e Carrazedo, Bragança, Bragança	Metagabbro and schists	Montane broadleaved forest	B. celtiberica DB (4/4) Prunus avium (1/2) Streambed mud (1/1)	GON (2), PSE-DAPC1 (2) PSE-DAPC1 (1) GON (1)
F17	41°46.604′ N, 06°54.116′ W	890	UF Castrelos e Carrazedo, Bragança, Bragança	Metagabbro and schists	Lower montane broadleaved forest	Quercus ilex DB (1/1) Q. ilex (0/2) Q. ilex DC & Q. pyrenaica DC (0/1) Q. pyrenaica DB (1/1)	xCAM-DAPC9 (1) – – PSE-DAPC1 (1)
F18	41°43.096′ N, 06°55.949′ W	871	Celas, Vinhais, Bragança	Schists, peralkaline vulcanites, phyllites, quartzites	Chestnut plantation	C. sativa DB & bleeding bark cankers (3/5)	CIN-PcG2 (1), xCAM-DAPC9 (2)
F19	41°41.702′ N, 06°55.734′ W	798	Celas, Vinhais, Bragança	Schists, peralkaline vulcanites, phyllites, quartzites	Submontane broadleaved forest and riparian gallery forest downhill of a chestnut plantation	Q. pyrenaica DB (1/4) Alnus lusitanica DC (1/1)	QUE (1)[f] CIN (1)
F20	41°31.530′ N, 06°49.135′ W	640	Vinhas, Macedo de Cavaleiros, Bragança	Metagabbro and schists	Submontane open oak woodland	Q. ilex (0/4) Q. suber (0/3)	– –
F21	41°30.161′ N, 06°48.512′ W	713	Morais, Macedo de Cavaleiros, Bragança	Metagabbro and schists	Submontane open oak woodland	Q. suber DB & bleeding bark cankers (2/5)	CIN-PcG2 (2)
F22	41°43.682′ N, W07°28.620	350	Rio Tâmega; UF Madalena e Samaiões, Chaves, Vila Real	Quaternary alluvial sediments	Riparian forest	A. lusitanica bleeding bark canker (1/1)	xALN (1)
F23	41°48.201′ N, 06°45.500′ W	662	Rio Fervença; UF Santa Maria e Meixedo, Bragança, Bragança	Quaternary alluvial sediments	Riparian forest	A. lusitanica bleeding bark cankers (2/2)	xALN (2)

TABLE 1 (Continued)

Site no.	GPS coordinates	Altitude (ma.s.l.)	Location; parish, municipality, district	Geological substrate	Vegetation	Sampled tree species and health condition (no. of Phytophthora-positive/sampled trees)[a]	Phytophthora spp. (no. of positive samples)[b–e]
F24	40°22.596′ N, 08°22.303′ W	315	Mata Nacional do Buçaco (MNB); Luso, Mealhada, Aveiro	Schists, greywackes, quartzites, conglomerates	Colline broadleaved forest	Phillyrea latifolia DB (1/2) P. latifolia DC & Ilex aquifolium DC (1/1) Q. robur DB (2/4) C. sativa DB (1/2)	CIN (1) QUE (1) CIN (2) CIN-PcG2 (1)
F25	40°22.687′ N, 08°22.055′ W	339	MNB; Luso, Mealhada, Aveiro	Schists, greywackes, quartzites, conglomerates	Colline broadleaved forest	Fraxinus angustifolia (0/1)	–[g]
F26	40°22.240′ N, 08°21.853′ W	557	MNB; Luso, Mealhada, Aveiro	Schists, greywackes, quartzites, conglomerates	Colline to submontane broadleaved forest	A. unedo (0/1) F. sylvatica (0/1) P. latifolia DB (1/2) Q. pyrenaica (0/1) Q. robur DC (0/3) Q. suber (0/1)	– – CIN (1) – – –
F27	40°22.146′ N, 08°21.572′ W	524	MNB; Luso, Mealhada, Aveiro	Schists, greywackes, quartzites, conglomerates	Colline to submontane broadleaved forest	Abies alba (0/2) Q. robur DB (1/1)	– CIN-PcG2 (1)
F28	40°17.006′ N, 07°57.554′ W	192	Rio Alva; UF Vila Cova de Alva e Anseriz, Arganil, Coimbra	Quaternary alluvial sediments	Riparian gallery forest	A. lusitanica bleeding bark cankers (2/2)	xALN (2)
F29	40°22.914′ N, 07°42.716′ W	553	Parque Nacional da Serra da Estrela (PNSE); UF Seia, S. Romão e Lapa dos Dinheiros, Seia, Guarda	Biotide granitoid	Submontane broadleaved forest	C. sativa DC (0/1) C. sativa (0/2) F. angustifolia (0/1) Q. robur DC (1/2)	– – – CIN-PcG1 (1)
F30	40°21.300′ N, 07°43.206′ W	749	PNSE; Valezim, Seia, Guarda	Biotide granitoid	Submontane broadleaved forest	C. sativa DB (6/6)	CIN (5), QUE (3)
F31	40°20.514′ N, 07°42.856′ W	966	PNSE; Valezim, Seia, Guarda	Biotide granitoid	Lower montane broadleaved forest	Q. rubra (0/1)	–
F32	40°18.506′ N, 07°45.206′ W	464	PNSE, Rio Lima; UF Vide e Cabeça, Seia, Guarda	Biotide granitoid	Submontane broadleaved forest and riparian gallery forest	A. lusitanica DB (1/1) Prunus lusitanica DC (1/1) S. caprea & Olea oleaster DC (1/1) Streamed soil (0/1)	CIN (1) PLU (1)[f] PSC×KEL (1)[g] –

(Continues)

TABLE 1 (Continued)

Site no.	GPS coordinates	Altitude (m a.s.l.)	Location; parish, municipality, district	Geological substrate	Vegetation	Sampled tree species and health condition (no. of Phytophthora-positive/sampled trees)[a]	Phytophthora spp. (no. of positive samples)[b–e]
F33	40°29.619' N, 07°35.238' W	769	PNSE; UF S. Pedro e S. Julião, Gouveia, Guarda	Biotide granitoid	Submontane broadleaved forest	C. sativa DB (3/3)	CIN (1), QUE (2)
						C. sativa DC & Q. robur DC (1/1)	QUE (1)
						Q. robur DB (3/3)	CIN (1), QUE (2)
						Q. robur × pyrenaica (0/1)	–
F34	40°33.005' N, 07°27.059' W	712	PNSE; Ribeira de Linhares; Linhares, Celorico da Beira, Guarda	Biotide granitoid	Submontane broadleaved forest and riparian gallery forest	A. lusitanica (1/1)	GON (1), C6PT1 (1)
						Q. pyrenaica DC (4/6)	CIN (1), QUE (3)[h]
F35	40°24.209' N, 07°35.178' W	1428	PNSE; UF Aldeias e Mangualde da Serra, Gouveia, Guarda	Schists, greywackes, quartzites, conglomerates	Montane broadleaved forest	B. celtiberica (0/4)	–
						Sorbus aucuparia (0/1)	–
F36	40°24.831' N, 07°32.663' W	1194	PNSE; Santa Maria, Manteigas, Guarda	Schists, greywackes, quartzites, conglomerates	Montane broadleaved forest	C. sativa DB (3/5)	CIN-PcG2 (3)
F37	40°24.738' N, 07°30.846' W	1083	PNSE; Santa Maria, Manteigas, Guarda	Schists, greywackes, quartzites, conglomerates	Montane broadleaved forest	C. sativa DB & bleeding bark cankers (2/4)	×CAM-DAPC9 (2)
						F. sylvatica DB & bleeding bark canker (1/1)	×CAM-DAPC9 (1)
						F. sylvatica DB (1/2)	CIN (1), ×CAM-DAPC9 (1)
						Q. rubra (0/1)	–
F38	40°25.303' N, 07°22.451' W	901	PNSE; Famalicão, Guarda, Guarda	Biotide granitoid	Lower montane broadleaved forest	C. sativa DB (2/2)	CIN-PcG2 (2)
F39	40°25.489' N, 07°22.523' W	892	PNSE; Famalicão, Guarda, Guarda	Biotide granitoid	Lower montane broadleaved forest	C. sativa DB & bleeding bark cankers (5/9)	CAS (4), CIN-PcG2 (3), PLU (1), ×CAM-DAPC9 (1)
F40	40°28.519' N, 07°21.529' W	909	PNSE; Fernão Joanes, Guarda, Guarda	Biotide granitoid	Lower montane broadleaved forest	C. sativa DB (0/2)	–
F41	40°20.991' N, 07°05.656' W	745	Rio Côa; UF do Sabugal e Aldeia de Santo António, Sabugal, Guarda	Quaternary alluvial sediments	Riparian gallery forest	A. lusitanica bleeding bark canker (1/1)	×ALN (1)
F42	40°18.596' N, 07°07.858' W	890	Reserva Natural da Serra da Malcata (RNSM); Meimão, Penamacor, Castelo Branco	Schists, greywackes, quartzites, conglomerates	Chestnut plantation	C. sativa DB (2/5)	CIN (2)

WILEY | 9

TABLE 1 (Continued)

Site no.	GPS coordinates	Altitude (m a.s.l.)	Location; parish, municipality, district	Geological substrate	Vegetation	Sampled tree species and health condition (no. of Phytophthora-positive/sampled trees)[a]	Phytophthora spp. (no. of positive samples)[b-e]
F43	40°15.846′ N, 07°08.883′ W	660	RNSM; Meimão, Penamacor, Castelo Branco	Schists, greywackes, quartzites, conglomerates	Submontane broadleaved forest	Q. ilex DC (2/2) Q. ilex (1/1)	QUE (2) QUE (1)
F44	40°15.166′ N, 07°07.423′ W	675	RNSM; Meimão, Penamacor, Castelo Branco	Schists, greywackes, quartzites, conglomerates	Submontane broadleaved forest	A. unedo (2/4) Q. pyrenaica DC (2/3)	QUE (2) QUE (2)
F45	40°11.999′ N, 07°07.611′ W	534	RNSM; Penamacor, Penamacor, Castelo Branco	Schists, greywackes, quartzites, conglomerates	Submontane cork oak plantation and mature forest	Q. suber DB & bleeding bark cankers (7/7)	CIN (6), QUE (1)
F46	40°16.669′ N, 07°01.017′ W	972	Reserva Natural da RNSM; Penamacor, Penamacor, Castelo Branco	Biotide granitoid	Lower montane broadleaved forest; in vicinity eucalypt plantations	C. sativa DB (3/5)	CIN (3)
F47	40°18.291′ N, 07°01.836′ W	824	RNSM; Quadrazais, Sabugal, Guarda	Biotide granitoid	Lower montane broadleaved forest	Q. pyrenaica DB (4/5)	QUE (3), xCAM (1)
F48	39°45.840′ N, 08°59.792′ W	71	Mata Nacional da Leiria (MNL); Marinha Grande, Marinha Grande, Leiria	Sand, gravels	Lowland mixed oak-pine forest and riparian gallery forest	A. lusitanica (0/1) P. pinaster (0/2) Q. robur (0/1) Streambed soil (0/1)	– – – –
F49	38°46.078′ N, 09°25.405′ W	263	Parque Natural Sintra-Cascais (PNSC); UF Santa Maria, São Miguel e São Pedro de Penaferrim, Sintra, Lisboa	Sienite	Colline, Atlantic mixed broadleaf-conifer forest	Acacia dealbata (1/1)	MUL (1), xPSC (1)
F50	38°46.965′ N, 09°23.624′ W	454	PNSC; UF S. Maria, S. Miguel e S. Pedro de Penaferrim, Sintra, Lisboa	Biotide granite	Colline, Atlantic mixed broadleaf-conifer forest	A. alba (0/1) A. alba DC & C. sativa DB (1/1) C. sativa DB (1/1) F. sylvatica (0/1) Q. robur (0/2)	– CIN (1) CIN (1) – –
F51	38°47.130′ N, 09°23.574′ W	425	PNSC; UF S. Maria, S. Miguel e S. Pedro de Penaferrim, Sintra, Lisboa	Biotide granite	Colline, Atlantic mixed broadleaf-conifer forest	Q. robur DB (2/3) C. sativa DB & A. alba DC (1/1) C. sativa DC & F. sylvatica DC (1/1)	GON (1), PLU (1) CIN-PcG1 (1) AMN (1), CHL (1), PLU (1)

(Continues)

TABLE 1 (Continued)

Site no.	GPS coordinates	Altitude (m a.s.l.)	Location; parish, municipality, district	Geological substrate	Vegetation	Sampled tree species and health condition (no. of Phytophthora-positive/sampled trees)[a]	Phytophthora spp. (no. of positive samples)[b-e]
F52	38°47.063′ N, 09°23.452′ W	475	PNSC; UF S. Maria, S. Miguel e S. Pedro de Penaferrim, Sintra, Lisboa	Biotide granite	Colline, Atlantic mixed broadleaved forest	A. pseudoplatanus DC (2/2) Laurus nobilis (0/1) Q. pyrenaica DB (1/2) Q. pyrenaica (0/1)	CHL (2), MUL (2), PLU (1) – PLU (1) –
F53	38°47.429′ N, 09°23.376′ W	402	PNSC; UF S. Maria, S. Miguel e S. Pedro de Penaferrim, Sintra, Lisboa	Biotide granite	Colline, Atlantic mixed broadleaved forest	A. pseudoplatanus DC (2/2) A. pseudoplatanus DC & F. angustifolia DC (1/1) A. pseudoplatanus DC & Q. robur DC (0/1) A. pseudoplatanus DC & Tilia cordata DC (0/1) Q. robur DC (0/1)	MUL (1), PSE-DAPC5 (1) PLU (1)[i] – – –
F54	38°49.777′ N, 08°20.978′ W	125	Herdade das Antas; UF Cortiçadas de Lavre e Lavre, Montemor-o-Novo, Évora	Sandstones, sand, gravels	Lowland open oak woodland (Montado)	Q. suber DB (5/6)	CIN (5)
F55	38°47.782′ N, 07°40.463′ W	225	Herdade da Junceira; Évora Monte (Santa Maria), Estremoz, Évora	Biotide granitoid	Lowland open oak woodland (Montado)	Q. ilex DB (1/4) Q. ilex & Q. suber DB (2/2) Q. suber DB (1/1)	CIN (1) CIN (2) CIN (1)
F56	38°10.532′ N, 08°39.951′ W	200	Melides, Grandola, Setúbal	Slates, greywackes, flysch	Lowland open oak woodland (Montado)	Q. suber DB (2/5)	CIN-PcG2 (2)
F57	38°7.218′ N, 08°33.260′ W	130	UF Grândola e S. Margarida da Serra, Grândola, Setúbal	Slates, greywackes, flysch	Cork oak plantation	Q. suber DB (2/5) Q. ilex (0/1)	CIN-PcG2 (2) –
F58	38°10.935′ N, 08°06.368′ W	123	Alvito, Alvito, Beja	Shales, sandstones, conglomerates	Lowland open oak woodland (Montado)	Q. ilex DC (3/3) Q. suber DB (1/2)	QUE (2), PSY (2) CIN (1)[i]
F59	38°03.261′ N, 08°17.110′ W	75	Herdade do Outeiro; Figueira dos Cavaleiros, Ferreira do Alentejo, Beja	Shales, sandstones, conglomerates	Lowland open oak woodland (Montado)	Q. suber DC (2/2)	QUE (1), ULI (1)
F60	37°18.851′ N, 08°32.455′ W	496	Serra Algarvia (Barlavento); Monchique, Monchique, Faro	Sienite	Colline broadleaved forest	C. sativa (1/3) C. sativa DC (1/1) C. sativa DB (1/1)	CAS (1) CAS (1) CIN-PcG1 (1)
F61	37°23.342′ N, 08°23.561′ W	152	Serra Algarvia (Barlavento), Cerro do Malhão; S. Marcos da Serra, Silves, Faro	Slates, greywackes, flysch	Lowland open oak woodland (Montado)	Q. suber DB (1/1)	CIN (1)

TABLE 1 (Continued)

Site no.	GPS coordinates	Altitude (ma.s.l.)	Location; parish, municipality, district	Geological substrate	Vegetation	Sampled tree species and health condition (no. of Phytophthora-positive/sampled trees)[a]	Phytophthora spp. (no. of positive samples)[b-e]
F62	37°24.968′ N, 08°24.721′ W	210	Serra Algarvia (Barlavento), Foz do Grou; S. Marcos da Serra, Silves, Faro	Slates, greywackes, flysch	Lowland open oak woodland (Montado)	Q. suber DB (1/1)	CIN-PcG1 (1)
F63	37°15.088′ N, 07°56.935′ W	485	Serra Algarvia (Sotavento); Salir, Loulé, Faro	Slates, greywackes, flysch	Colline open oak woodland (Montado)	Q. suber DB (1/1)	CIN (1)
F64	37°13.737′ N, 07°55.894′ W	500	Serra Algarvia (Sotavento), Barranco do Velho; Salir, Loulé, Faro	Slates, greywackes, flysch	Colline open oak woodland (Montado)	Q. suber DB (4/6)	CIN-PcG2 (4), ×PSC (1)
F65	37°12.709′ N, 07°53.544′ W	472	Serra Algarvia (Sotavento); S. Brás de Alportel, S.Brás de Alportel, Faro	Slates, greywackes, flysch	Colline open oak woodland (Montado)	Q. suber DB (1/1)	CIN (1)
F66	37°14.805′ N, 07°51.500′ W	423	Serra Algarvia (Sotavento); S. Brás de Alportel, S.Brás de Alportel, Faro	Slates, greywackes, flysch	Colline open oak woodland (Montado)	Q. suber DB (1/2)	CIN (1)
F67	37°09.548′ N, 07°49.746′ W	199	Santa Catarina da Fonte do Bispo, Tavira, Faro	Slates, greywackes, flysch	Arbutus plantation and open oak woodland (Montado)	A. unedo DB (1/1) Q. suber DB (1/1)	CIN (1) CIN (1), QUE (1)
F68	37°25.649′ N, 07°31.695′ W	223	Serra Algarvia (Sotavento); UF Alcoutim e Pereiro, Alcoutim, Faro	Slates, greywackes, flysch	Pine agroforestry plantation	Pinus pinea DC (1/1)	×PSC (1)
F69	37°51.300′ N, 25°47.073′ W	273	Lagoa das Sete Cidades; Sete Cidades, Ponta Delgada, São Miguel (Azores)	Basalt, trachyte	Colline broadleaved forest	Q. rubra DC (1/2)	MUL (1)
F70	32°48.569′ N, 17°08.460′ W	1161	Posto Forestal do Fanal; Ribeira da Janela, Porto Moniz, Funchal (Madeira)	Basalt	Atlantic montane laurisilva forest	Ocotea foetens & Persea indica (1/1)	CHL (1)

[a]DB=crown dieback, DC=crown decline (increased transparency). No mentioning of disease symptoms denotes visually healthy trees.

[b]AMN=P. amnicola, CAS=P. castanetorum, CHL=P. chlamydospora, CIN=P. cinnamomi, GON=P. gonapodyides, MUL=P. multivora, PLU=P. plurivora, PSE=P. pseudosyringae, PSY=P. psychrophila, QUE=P. quercina, SYR=P. syringae, ULI=P. uliginosa, C6PT1=P. taxon Clade06b_PT1, PSC×KEL=P. taxon pseudocryptogea × kelmanii, ×ALN=P. ×alni, ×CAM=P. ×cambivora, ×PSC=P. taxon ×pseudocryptogea-like.

[c]DAPC groups within P. ×cambivora according to the discriminant analysis of principal components (DAPC) of the population genomic study of Mullett et al. (2023). DAPC9 isolates belong to the A2 mating type.

[d]Phytophthora cinnamomi panglobal clonal lineages PcG1 and PcG2, according to the population genomic study of Shakya et al. (2021). All isolates of PcG1 and PcG2 belong to the A2 mating type.

[e]DAPC groups within P. pseudosyringae according to the DAPC of the population genomic study of Mullett et al. (2024).

[f]Elongisporangium taxon anandrum-like also isolated.

[g]Phytopythium taxon REB326-70 also isolated.

[h]Phytopythium citrinum also isolated.

[i]Phytopythium taxon UZ612 also isolated.

[j]Elongisporangium sp. also isolated.

TABLE 2 Location and altitude of the 39 riparian sites sampled at 34 rivers and streams in Portugal and *Phytophthora* and other oomycete taxa isolated.

Site no.	GPS coordinates	Altitude (m a.s.l.)	Location; parish, municipality, district	Location of catchment and vegetation	*Phytophthora* and *Nothophytophthora* spp.[a–d]
R01	41°44.266′ N, 08°09.304′ W	497	Ribeira da Quelha Verde; Vilar da Veiga, Terras de Bouro, Braga	Parque Nacional da Peneda-Gerês (PNPG); sub-/montane broadleaved forests, heath- & grasslands	CHL, GON, PLU, PSE-DAPC5
R02	41°44.637′ N, 08°09.239′ W	542	Ribeiro da Figueira; Vilar da Veiga, Terras de Bouro, Braga	PNPG; sub-/montane broadleaved forests, heath- & grasslands	–
R03	41°44.908′ N, 08°09.221′ W	597	Forest stream, Secelo; Vilar da Veiga, Terras de Bouro, Braga	PNPG; sub-/montane broadleaved forests, heath- & grasslands	GON, PLU, ×CAM, ×PSC
R04	41°45.421′ N, 08°08.841′ W	779	Ribeira da Cantina; Vilar da Veiga, Terras de Bouro, Braga	PNPG; sub-/montane broadleaved forests, heath- & grasslands	GON
R05	41°47.559′ N, 08°08.285′ W	673	Forest stream, Lagos (Albergaria); Campo do Gerês, Terras de Bouro, Braga	PNPG; sub-/montane broadleaved forests, heath- & grasslands	GON
R06	41°48.219′ N, 08°07.925′ W	725	Forest stream; Curral de S. Miguel; Campo do Gerês, Terras de Bouro, Braga	PNPG; sub-/montane broadleaved forests, heath- & grasslands	GON, ×CAM-DAPC5, ×CAM-DACP9
R07	41°42.516′ N, 08°07.028′ W	361	Rio Arado, (Ponte das Cervas); Vilar da Veiga, Terras de Bouro, Braga	PNPG; sub-/montane broadleaved forests, heath- & grasslands; horticulture	GON, ×CAM-DAPC9
R08	41°16.747′ N, 08°04.090′ W	75	Rio Tâmega, Lufrei, Amarante, Porto	Lowland to submontane broadleaved forests; horticulture	LAC, ×C9PT1, ×P16855
R09	40°22.690′ N, 08°21.992′ W	342	Forest stream, Vale dos Fetos (Buçaco); Luso, Mealhada, Aveiro	Mata Nacional do Buçaco (MNB); colline broadleaved forest and a castle park	PLU
R10	40°22.684′ N, 08°22.325′ W	283	Forest stream, Fonte Fria (Buçaco); Luso, Mealhada, Aveiro	MNB; colline broadleaved forest and a castle park	CHL, PCIP, PLU, P16855[e]
R11	40°22.622′ N, 08°21.774′ W	373	Forest stream, Vale dos Abetos (Buçaco); Luso, Mealhada, Aveiro	MNB; colline broadleaved forest and a castle park	CHL, PLU, ×PSC
R12	40°23.896′ N, 07°30.625′ W	682	Rio Zêzere; Santa Maria, Manteigas, Guarda	Parque Natural da Serra da Estrela (PNSE); sub-/ montane broadleaved forests, heath- & grasslands; horticulture	CHL, GON, LAC, PLU, PSC×KEL, ×CAM-DAPC1
R13	40°24.476′ N, 07°28.570′ W	595	Rio Zêzere; Sameiro, Manteigas, Guarda	PNSE; sub-/ montane broadleaved forests, heath- & grasslands; horticulture	CHL, GON, LAC, PLU[f]
R14	40°23.293′ N, 07°26.841′ W	545	Rio Beijames; Vale de Amoreira, Manteigas, Guarda	PNSE; sub-/ montane broadleaved forests, heath- & grasslands; horticulture	AMN, CHL, LAC, PLU, PSC×KEL, ×STA[g]
R15	40°29.161′ N, 07°24.953′ W	816	Rio Mondego; União de Freguesias (UF) Corujeira e Trinta, Guarda, Guarda	PNSE; montane broadleaved forests, heath- & grasslands; horticulture	BIL, GON, LAC, ×CAM-DAPC5
R16	40°30.478′ N, 07°22.686′ W	763	Rio Mondego; Videmonte, Guarda, Guarda	PNSE; sub-/montane broadleaved forests, heath- & grasslands; horticulture	PLU, ×CAM-DAPC9[h,i]
R17	40°18.802′ N, 06°59.097′ W	818	Rio Côa; Quadrazais, Sabugal, Guarda	Reserva Natural da Serra da Malcata (RNSM); montane broadleaved forests, heath- & grasslands; horticulture	CHL, GON, LAC, ×HEN, ×LAC
R18	40°18.780′ N, 06°59.390′ W	817	Rio Côa; Quadrazais, Sabugal, Guarda	RNSM; montane broadleaved forests, heath- & grasslands; horticulture	LAC, PSC, ×HEN, ×LAC
R19	40°18.085′ N, 07°02.128′ W	796	Ribeira da Barroca da Amieira; Quadrazais, Sabugal, Guarda	RNSM; montane broadleaved forests, heath- & grasslands; horticulture	GON
R20	40°15.378′ N, 07°05.031′ W	604	Ribeira da Meimoa; Penamacor, Penamacor, Castelo Branco	RNSM; sub-/montane broadleaved forests, heath- & grasslands	PLU, PSC, ×PSC[e]
R21	40°15.602′ N, 07°05.597′ W	590	Ribeira da Meimoa; Meimão, Penamacor, Castelo Branco	RNSM; sub-/montane broadleaved forests, heath- & grasslands	BIL, LAC, PSC, THE×AMN, ×AMN, ×WS
R22	40°11.578′ N, 07°05.549′ W	442	Ribeiro da Mouca; Penamacor, Penamacor, Castelo Branco	RNSM; sub-/montane broadleaved forests, heath- & grasslands	BIL, KEL, LAC, PSC×KEL
R23	40°12.206′ N, 07°06.483′ W	530	Ribeira do Salgueirinho; Penamacor, Penamacor, Castelo Branco	RNSM; sub-/montane broadleaved forests, heath- & grasslands	PLU, PSC, PSC×KEL
R24	39°46.436′ N, 09°00.880′ W	37	Forest stream; Marinha Grande, Marinha Grande, Leiria	Mata Nacional de Leiria; Mediterranean mixed pine-oak forests and Machia vegetation	AMN, GON, MUL, AMN×CHL, THE×AMN

TABLE 2 (Continued)

Site no.	GPS coordinates	Altitude (m a.s.l.)	Location; parish, municipality, district	Location of catchment and vegetation	*Phytophthora* and *Nothophytophthora* spp.[a–d]
R25	38°46.843′ N, 09°26.686′ W	360	Fonte das 2 Bicas; Colares, Sintra, Lisboa	Parque Natural Sintra-Cascais (PNSC); Atlantic colline broadleaved and pine forests	PSE-DAPC3, THE, ×AMN
R26	38°45.508′ N, 09°25.179′ W	113	Forest stream, Quinta do Pisão; Alcabideche, Cascais, Lisboa	PNSC; Atlantic lowland to colline broadleaved and pine forests; horticulture	AMN, AMN×CHL, MUL, THE×AMN
R27	38°46.078′ N, 09°25.405′ W	166	Tributary of Barragem do Rio da Mula; UF Santa Maria, São Miguel e São Pedro de Penaferrim, Sintra, Lisboa	PNSC; Atlantic colline to colline broadleaved and pine forests	AMN
R28	38°48.109′ N, 09°26.763′ W	18	Ribeira de Colares; Colares, Sintra, Lisboa	PNSC; Atlantic lowland to colline broadleaved and pine forests; horticulture	AMN, BIL, CHL, LAC, PCIP
R29	38°47.466′ N, 09°25.479′ W	193	Forest stream, Monserrate; UF S. Maria, S. Miguel e S. Pedro de Penaferrim, Sintra, Lisboa	PNSC; Atlantic colline broadleaved and pine forests & heathlands	AMN, AMP, CHL
R30	38°47.126′ N, 09°23.538′ W	427	Small stream, Vale dos Fetos, Pena; UF S. Maria, S. Miguel e S. Pedro de Penaferrim, Sintra, Lisboa	PNSC; Atlantic colline broadleaved and pine forest; Botanical Garden with exotic plants	AMN, AMN×CHL GON, RAM[j]
R31	37°15.530′ N, 08°32.607′ W	75	Ribeira de Vale do Boi; Monchique, Monchique, Faro	Mediterranean, lowland to colline broadleaved and pine forests; heathlands; acacias and eucalypts; horticulture	AMN, GON, LAC, THE, THE×AMN, THE×GON, ×P16855
R32	37°13.114′ N, 08°30.464′ W	25	Ribeira de Odelouca; Silves, Silves, Faro	Mediterranean, lowland to colline broadleaved and pine forests; heathlands; acacias and eucalypts; horticulture	×P16855, HON, ×HEN1, ZEN×PER
R33	37°12.607′ N, 08°00.660′ W	137	Ribeira da Menalva (Fonte Benémola); UF Querença, Tôr e Benafim, Loulé, Faro	Mediterranean, lowland to colline broadleaved and pine forests & heathlands	LAC, ×P16855, ZEN×PER
R34	37°09.339′ N, 07°41.966′ W	33	Ribeira da Assêca, Pêgo do Inferno; UF Luz de Tavira e Santo Estevão, Tavira, Faro	Mediterranean, lowland broadleaved forests & heathlands; horticulture	HON
R35	37°09.214′ N, 07°40.343′ W	10	Rio Séqua; UF Santa Maria e Santiago, Tavira, Faro	Mediterranean, lowland to colline oak and pine forests; heathlands; horticulture incl. *Citrus* plantations	CIP, HON, INU, LAC, PCIP, SYL, ×LUS, ZEN×PER
R36	37°28.390′ N, 07°28.592′ W	25	Ribeira de Cadavais; UF Alcoutim e Pereiro, Alcoutim, Faro	Mediterranean, lowland broadleaved and pine woodlands; heathlands; horticulture	HON
R37	37°20.384′ N, 07°28.933′ W	25	Ribeira de Odeleite; Odeleite, Castro Marim, Faro	Mediterranean, lowland broadleaved and pine woodlands; heathlands; horticulture	LAC
R38	37°24.666′ N, 07°27.810′ W	17	Rio Guadiana; UF Alcoutim e Pereiro, Alcoutim, Faro	Huge catchment; Mediterranean, lowland to submontane broadleaved and pine forests; heathlands; horticulture	HON, THE
R39	37°22.723′ N, 07°26.423′ W	5	Rio Guadiana, Álamo; UF Alcoutim e Pereiro, Alcoutim, Faro	Huge catchment; Mediterranean, lowland to submontane broadleaved and pine forests; heathlands; horticulture	HON

[a]Non-hybrid taxa: AMN = *P. amnicola*, AMP = *N. amphigynosa*, BIL = *P. bilorbang*, CHL = *P. chlamydospora*, CIP = *P. citrophthora*, GON = *P. gonapodyides*, HON = *P. honggalleglyana*, INU = *P. inundata*, KEL = *P. kelmanii*, LAC = *P. lacustris*, MUL = *P. multivora*, PLU = *P. plurivora*, PCIP = *P. pseudocitrophthora*, PSC = *P. pseudocryptogea*, PSE = *P. pseudosyringae*, RAM = *P. ramorum*, SYL = *P. taxon sylvatica-like 1*, P16855 = *P. taxon P16855*, THE = *P. thermophila*.

[b]Hybrid taxa: AMN×CHL = *P. taxon amnicola × chlamydospora*, PSC×KEL = *P. taxon pseudocryptogea × kelmanii*, THE×AMN = *P. taxon thermophila × amnicola*, THE×GON = *P. taxon thermophila × gonapodyides*, ×AMN = *P. taxon amnicola-like*, ×CAM = *P. ×cambivora*, ×C9PT1 = *P. taxon ×Clade09a1_PT1*, ×HEN = *P. taxon ×Hennops*, ×HEN1 = *P. taxon ×Hennops-like 1*, ×LAC = *P. taxon ×lacustris-like*, ×LUS = *P. ×lusitanica*, ×PSC = *P. taxon ×pseudocryptogea-like*, ×P16855 = *P. taxon ×P16855-like*, ×STA = *P. ×stagnum*, ×WS = *P. taxon ×WS*, ZEN×PER = *P. taxon zentmyerii × Peru4-like*.

[c]DAPC groups within *P. pseudosyringae* according to the discriminant analysis of principal components (DAPC) of the population genomic study of Mullett et al. (2024).

[d]DAPC groups within *P. ×cambivora* according to the DAPC of the population genomic study of Mullett et al. (2023). DAPC1 isolates belong to the A1 mating type, DAPC5 and DAPC9 isolates to the A2 mating type.

[e]*Phytopythium litorale* also isolated.

[f]*Phytopythium* taxon PV So7 also isolated.

[g]*Pythium* taxon strain 1–9 also isolated.

[h]*Phytopythium* taxon PB-2013 also isolated.

[i]*Phytopythium* taxon KC-2014 also isolated.

[j]The isolate of *P. ramorum* belongs to the EU1 lineage according to the genomic study of Van Poucke et al. (2021).

FIGURE 2 Representative forest stands and rivers sampled in Portugal; (a) submontane mixed forest F01 in the Parque Nacional da Peneda-Gerês (PNPG); (b) submontane *Quercus suber* forest F10 in the PNPG; (c) colline to submontane, mixed forest F25 in the Mata Nacional do Buçaco; (d) submontane *Quercus pyrenaica* forest F34 in the Parque Nacional da Serra da Estrela (PNSE); (e) montane forest (F37) of *Castanea sativa*, *Fagus sylvatica* and *Quercus rubra* in the PNSE; (f) colline mixed forest F50 in the Parque Natural Sintra-Cascais; (g) open lowland woodland (Montados) F54 of *Quercus ilex* and *Q. suber* in the Alentejo region; (h) baiting raft (arrow) floating in the Rio Arado (R07) in the PNPG. For GPS coordinates see Tables 1 and 2; for location of sites see Figure 1.

cultures were maintained on both V8A and carrot agar (CA; Pérez-Sierra et al., 2022) at 10°C in the dark.

2.2 | Molecular identification of isolates

For all *Phytophthora* isolates obtained in this study, mycelial DNA was extracted from pure 1-week-old V8A cultures. Total DNA was extracted using the DNeasy Plant Mini kit (QIAGEN) or the Phire Plant Direct PCR Kit (Thermo Fisher Scientific) following the manufacturer's instructions. DNA was stored at −20°C until further use. For all isolates, the region spanning the internal transcribed spacer (ITS1-5.8S-ITS2) region of the ribosomal DNA was amplified using primer pairs ITS1/ITS4 or ITS6/ITS4 (Cooke et al., 2000; White et al., 1990). For all isolates from new taxa and a representative set of isolates from known species of *Phytophthora*, the mitochondrial *cox1* gene was amplified with both primer pairs OomCoxI-Levup/OomCoxI-Levlo and COXF4N/COXR4N (Kroon et al., 2004; Robideau et al., 2011) or FM84/FM83 (Martin & Tooley, 2003). The PCR mixture and the amplification conditions for ITS and *cox1* were as described by Cooke et al. (2000), Kroon et al. (2004), Robideau et al. (2011) and Martin and Tooley (2003). PCR consumables were purchased from Thermo Fisher Scientific. PCR was carried out in an Eppendorf Mastercycler Ep Gradient thermocycler. PCR products were purified and sequenced by Macrogen Europe (Amsterdam, Netherlands) in both directions using the amplification primers.

Electropherograms were quality-checked and forward and reverse reads were compiled using Geneious Prime v. 2024.0.2 (Biomatters Ltd). Pronounced double peaks were considered as heterozygous positions and labelled according to the International Union of Pure and Applied Chemistry (IUPAC; https://iupac.org) coding system. 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 species. 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 oomycete species and *cox1* sequences of representative isolates of several known species and putative new species obtained in this study were deposited at GenBank.

2.3 | Classical identification of isolates

Colony growth patterns of 7-day-old cultures grown at 20°C in the dark on V8A, CA and potato dextrose agar (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 the literature.

Sporangia were produced in non-sterile soil filtrate (Jung, 2009). Microscopic examinations and measurements of morphological structures at 200× and 400× were according to Jung, Horta Jung, Cacciola, et al. (2017) and Jung et al. (2022) using a compound microscope (Zeiss Imager.Z2), a digital camera (Zeiss Axiocam ICc3) and biometric software (Zeiss ZEN). Self-sterile isolates were paired on V8A and CA with known A1 and A2 mating type tester strains of *P. cinnamomi* (A1: TW12; A2: MP74; Jung, Horta Jung, Scanu, et al., 2017) and *P.* ×*cambivora* (A1: Resi75; A2: SK9; Jung, Horta Jung, Scanu, et al., 2017) (isolates with non-papillate sporangia) or *P. botryosa* (A2: MYA-4059; Jung et al., 2024), *P. colocasiae* (A1: MYA-4159; A2: 35D3; Yang et al., 2017; Jung et al., 2024) and *P. meadii* (A1: MYA-4042; A2: MYA-4043; Jung et al., 2024) (isolates with papillate sporangia), and examined after 4–6 weeks incubation at 20°C in order to determine whether they were heterothallic or sterile (Jung et al., 2022, 2024). All isolates are preserved in the culture collections maintained at the University of Algarve and Mendel University in Brno.

3 | RESULTS

Overall, 799 isolates of 48 *Phytophthora* taxa were obtained from forest stands (Table 1), river systems (Table 2) and forest nurseries (Table 3) in Portugal. Although isolates from other oomycete genera were discarded regularly during the isolation process, 25 isolates of *Elongisporangium*, *Nothophytophthora*, *Phytopythium* and *Pythium* spp. were also taken into culture. GenBank accession numbers of ITS and *cox1* sequences of representative isolates of all oomycete taxa are given in Table S1. Official species descriptions of new *Phytophthora* and other oomycete taxa including detailed descriptions of morphological characteristics, morphometric data, temperature-growth data and multigene phylogenies have been published recently (Jung et al., 2024; Jung, Horta Jung, Cacciola, et al., 2017; Jung, Scanu, Bakonyi, et al., 2017) and more will be presented in future publications. Comprehensive lists for associating sample sources (tree species, river/stream) with oomycete taxa, and oomycete taxa with sample sources and site categories are given in Tables S2–S4.

3.1 | *Phytophthora* diversity and disease symptoms in natural and semi-natural forest stands of Portugal

Most tree species in the *Phytophthora*-infested forest stands showed a range of symptoms typical of *Phytophthora* infections, such as decline (increased transparency) and dieback of crowns and losses of fine and lateral roots. Moreover, in several stands *A. lusitanica*, *C. sativa*, *F. sylvatica*, *Q. rubra* and *Q. suber* also suffered from bleeding bark cankers (Figures 3 and 4; Table 1). Declines and diebacks of tree species reported for the first time in Europe and Portugal, respectively, are listed in Table S5.

In 62 of the 70 forest stands (88.6%), 17 *Phytophthora* taxa were isolated from 148 of the 302 rhizosphere soil samples (49%) of 25 of the 29 tree species sampled (86.2%) and from all seven bark cankers of *A. lusitanica* and *F. sylvatica* sampled. The 254 *Phytophthora* isolates belonged to 13 known species from six phylogenetic clades, namely *P. multivora* and *P. plurivora* from Clade 2; *P. pseudosyringae* and *P. psychrophila* from Clade 3; *P. amnicola*, *P. chlamydospora* and *P. gonapodyides* from Clade 6; *P. cinnamomi*, *P. uliginosa*, *P.* ×*alni* and *P.* ×*cambivora* from Clade 7; and *P. syringae* and *P. quercina* from Clades 8 and 12, respectively; one new Clade 12 species, which due to its specificity to chestnut was named *P. castanetorum* (Jung, Horta Jung, Cacciola, et al., 2017); and three undescribed taxa, one new non-hybrid taxon provisionally named *P.* taxon Clade06b_PT and two Clade 8 hybrid taxa, the designated *P.* taxon *pseudocryptogea* × *kelmanii* and the new provisionally named *P.* taxon ×pseudocryptogea-like (Figure 5; Table 1). In addition, *Phytopythium citrinum*, the undescribed *Phytopythium* taxa REB326-70 and UZ612, and the undescribed *Elongisporangium* taxon anandrum-like were recovered from five soil samples (Table 1). With 39 forest sites (55.7%), an altitudinal range between 123 and 1194 m a.s.l. and 12 tree species (41.4%), *P. cinnamomi* was by far the most common species (Figure 5; Table 1). All 82 isolates belonged to the A2 mating type. A subgroup of these isolates was included in the population genomic studies of Shakya et al. (2021) that revealed the presence of both globally distributed clonal A2 mating type lineages, PcG1-A2 and PcG2-A2. *P. quercina* was isolated in 15 forest stands from the rhizosphere of four oak species, *Q. ilex*, *Q. pyrenaica*, *Q. robur* and *Q. suber*, and four tree species belonging to other genera, namely *A. unedo*, *C. sativa*, *Ilex aquifolium* and *Phyllirea latifolia* (Figure 5; Table 1). With 15 forest sites (21.4%) and eight tree species (38.1%), namely *Acer pseudoplatanus*, *Betula*
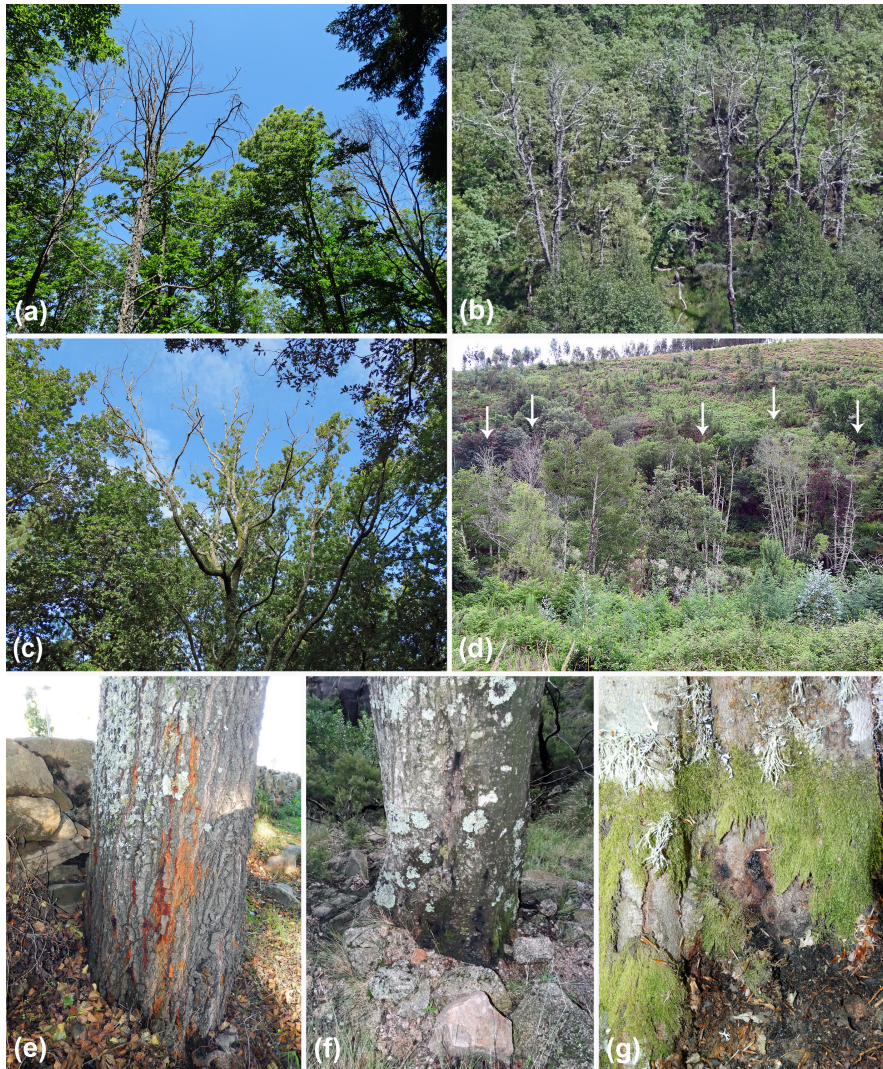
FIGURE 3 Disease symptoms of mature trees in natural and semi-natural forest stands in Portugal caused by *Phytophthora* pathogens; (a) crown dieback and mortality of *Castanea sativa* in the montane forest F37 in the Parque Nacional da Serra da Estrela (PNSE) (*P. cinnamomi* and *P. ×cambivora*); (b) severe dieback and mortality of *Quercus pyrenaica* in the submontane forest F19 in the Serra da Nogueira (*P. quercina*); (c) severe dieback of *Quercus robur* in submontane forest F06 in the Parque Nacional da Peneda-Gerês (PNPG) (*P. quercina*); (d) high mortality of *Alnus lusitanica* (arrows) along the Rio Alva (*P. ×alni*); (e) bleeding bark canker on *A. lusitanica* at the Rio Côa (*P. ×alni*); (f) bleeding bark canker on *Quercus rubra* in the submontane forest F03 in the PNPG (*P. cinnamomi*); (g) bleeding bark canker on *Fagus sylvatica* in the montane forest F37 in the PNSE (*P. ×cambivora*).

celtiberica, *C. sativa*, *F. sylvatica*, *Pinus pinaster*, *Q. ilex*, *Q. pyrenaica* and *Salix caprea*, *P. ×cambivora* was the third most frequent species (Figure 5; Table 1). This pathogen was also isolated from the bleeding collar rot lesion of a mature *F. sylvatica* tree at the montane stand F37 in the Parque Nacional da Serra da Estrela (Figure 3; Table 1). All 37 *P. ×cambivora* isolates from the forest sites were included in the population genomic study of Mullett et al. (2023) and belonged to the discriminant analysis of principal components (DAPC) group 9. Mating tests revealed all isolates were of the A2 mating type. *P. plurivora* and *P. multivora* were obtained from five (7.1%) and four (5.7%) forest stands, respectively (Figure 5; Table 1). *C. sativa* hosted the highest *Phytophthora* diversity in the rhizosphere, with seven species (*P. amnicola*, *P. castanetorum*, *P. chlamydospora*, *P. cinnamomi*, *P. plurivora*, *P. quercina* and *P. ×cambivora*, *C. sativa*); followed with five species by *Q. pyrenaica* (*P. cinnamomi*, *P. plurivora*, *P. pseudosyringae*, *P. quercina* and *P. ×cambivora*) and *Acer pseudoplatanus* (*P. chlamydospora*, *P. multivora*, *P. plurivora*, *P. pseudosyringae* and *P. ×cambivora*); and with four species by *Q. robur* and *Q. suber* (Table 1, Table S2). The *P. pseudosyringae* isolates from the forest sites included in the population genomic study of Mullett et al. (2024) belonged to two different DAPC groups, 1 and 5.

Regardless of the tree species, there was a considerable difference in the isolation rates from rhizosphere soil between visually healthy trees (nine of the 89 trees infested = 10.1%) and declining trees (139 of the 213 trees infested = 65.3%). Decline and dieback of *C. sativa* was associated with *P. cinnamomi* and *P. ×cambivora* in 12 and five stands, respectively (Table 1). *P. cinnamomi* was also associated with the decline and dieback of *Q. suber* in 16 stands (Table 1). Decline and dieback of *Q. pyrenaica* were associated with *P. ×cambivora* in four stands, with *P. cinnamomi* and *P. quercina* in each of three stands and with *P. plurivora* in one stand (Table 1). With *Q. robur*, *P. cinnamomi* and *P. quercina* were recovered from declining trees in four and two stands, respectively, and *P. plurivora* in one stand. In contrast, healthy *Q. robur* trees in six stands did not yield any *Phytophthora* species (Table 1). Decline and dieback of *Q. ilex* was found associated with *P. quercina* in two stands and with *P. cinnamomi* and *P. ×cambivora* in one stand each (Table 1). The bark cankers of riparian *A. lusitanica* trees at the Rio Alva, Rio Côa, Rio Tâmega and Rio Fervença (Figure 3) were caused by *P. ×alni* (Table 1). At three other riparian sites, *P. cinnamomi* (F19 along a forest stream, F32 along the Rio Lima) and *P. gonapodyides* (F34 along the Ribeira de Linhares) were isolated from the rhizosphere

TABLE 3 Forest nursery stock sampled in Portugal, health condition and *Phytophthora* and other oomycete taxa isolated.

Nursery no.	Sampled tree species (no. of *Phytophthora*-positive/sampled trees)	Symptoms of sampled plants[a]	*Phytophthora* spp. (no. of positive samples)[b–d]
N01	Soil of transplanting bed (0/2)	–	–[e]
N02	*Abies alba* (1/1)	Stunted, chlorotic, small needles	CIN-PcG2 (1)
	Arbutus unedo (1/1)	Wilting	CIN-PcG1 (1)
	Chamaecyparis lawsoniana (0/1)	Healthy	–
	Cupressus sempervirens (1/1)	Some stunted & chlorotic or dead	CIN (1)
	Pinus pinea (1/2)	Some stunted & chlorotic or dead	×PSC (1)[f]
	Pinus radiata (0/1)	Some stunted & chlorotic or dead	–[g,h]
	Pinus sylvestris (1/1)	Some stunted & chlorotic or dead	PSC (1), ×PSC (1)
	Quercus pyrenaica (1/2)	Some wilting or dead	×CAM DAPC11 (1)[g]
	Quercus robur (1/1)	Some wilting or dead	×PSC (1)[i]
	Quercus suber (0/2)	Healthy	–
N03	*Castanea sativa* (1/1)	Some stunted & chlorotic or dead	CIN-PcG2 (1)
	Eucalyptus nitens (1/1)	Some stunted & chlorotic or dead	ALT (1), CIN (1)
	P. pinea (0/1)	Healthy	–
	Quercus ilex (1/1)	Some stunted & dead	CIN (1), QUT (1)
	Q. pyrenaica (0/1)	Healthy	–
	Q. robur (0/1)	Healthy	–
	Q. suber (0/1)	Healthy	–
	Quercus rubra (1/1)	Healthy	QUT (1)
N04	*C. sativa* (1/1)	Some stunted & chlorotic or dead	CIN-PcG2 (1)
	P. pinea (0/1)	Healthy	–
	Q. pyrenaica (0/1)	Healthy	–
	Q. robur (0/1)	Healthy	–
	Q. rubra (0/1)	Healthy	–
	Q. suber (1/1)	Some stunted & chlorotic	PLU (1), ×CAM DAPC11 (1)[j]
N05	*C. sativa* (1/1)	Some stunted & chlorotic or dead	CIN-PcG2 (1)
	C. sativa (1/1)	Chlorotic & collar rot, direct isolation	CIN (1)
	P. pinea (0/1)	Some stunted & chlorotic	–[k]
	Pinus pinaster (0/1)	Healthy	–[h]
	Quercus coccinea (0/1)	Healthy	–
	Q. pyrenaica (1/1)	Some stunted & chlorotic	QUT (1)
	Q. rubra (1/1)	Some stunted & chlorotic	QUT (1)
	Q. suber (0/1)	Healthy	–
N06	*C. sativa* (0/1)	Healthy	–[g]
	P. pinea (1/1)	Some stunted & chlorotic	PSC (1)
	Quercus faginea (1/1)	Healthy	QUT (1)
	Q. ilex (0/1)	Healthy	–
	Q. pyrenaica (2/2)	Some stunted & chlorotic	×CAM DAPC9 (1), QUT (1)
	Q. robur (1/1)	Healthy	×CAM DAPC9 (1)[j]
	Q. rubra (2/2)	Some stunted & chlorotic or dead	CIN (1), QUT (1)[l]
	Q. suber (0/2)	Healthy	–
N07	*P. pinea* (1/1)	Stunted & chlorotic or dead	PAR (1), PSC (1)
	P. pinaster (1/1)	Some stunted & chlorotic or dead	CIN (1)
	Q. faginea (1/1)	Some stunted & chlorotic	MUL (1), PSC (1)
	Q. ilex (1/1)	Stunted & chlorotic or dead	CIN (1)
	Q. suber (1/1)	Stunted & chlorotic or dead	CIN (1)

TABLE 3 (Continued)

Nursery no.	Sampled tree species (no. of *Phytophthora*-positive/sampled trees)	Symptoms of sampled plants[a]	*Phytophthora* spp. (no. of positive samples)[b–d]
N08	*P. pinea* (1/1)	Some stunted & chlorotic	PSC×KEL
	Q. ilex (1/1)	Dead	CAC (1), ×CAM DAPC4 (1)
	Q. ilex (1/1)	Healthy	×CAM DAPC4 (1)
	Q. suber (1/1)	Some stunted & chlorotic or dead	CIN-PcG2 (1), MUL (1)
N09	*P. pinea* (1/1)	Some stunted & chlorotic	CAC (1)
N10	*Q. ilex* (1/1)	Dead	CIN (1)
	Q. ilex (1/1)	Healthy	×CAM DAPC1 (1)
N11	*Alnus lusitanica* (1/1)	Healthy	CRA (1)
	A. unedo (1/1)	Some chlorotic or wilting	×CRA (1)
	C. sativa (2/2)	Chlorotic or dead, collar rot	CIN (1), CRA (1), ×CAM (1)
	Cupressus lusitanica (0/1)	Some stunted & chlorotic or dead	–
	Q. faginea (1/1)	Some stunted & chlorotic or dead	PLU (1)
	Q. ilex (1/1)	Stunted & chlorotic or dead	QUE (1), PSC (1)
	Q. pyrenaica (1/1)	Some stunted & chlorotic or dead	PSC (1)
	Q. robur (1/1)	Some stunted & chlorotic or dead	CRA (1), QUE (1)[g]
	Q. suber (1/1)	Stunted & chlorotic or dead	CRA (1), PSC (1)
	Taxus baccata (1/1)	Some stunted & chlorotic or dead	CRA (1)
N12	*A. lusitanica* (0/1)	Healthy	–
	A. unedo (0/1)	Mostly healthy, some wilting	–
	C. sativa (0/1)	Healthy	–
	Juglans regia (0/1)	Healthy	–
	P. pinea (0/1)	Healthy	–
	P. pinea (1/1)	Some stunted & chlorotic or dead	CAC (1)
	Q. faginea (1/1)	Some stunted & chlorotic or dead	PSC (1), QUE (1)
	Q. ilex (1/1)	Healthy	PSY (1)
	Q. pyrenaica (1/1)	Some stunted & chlorotic	QUE (1)
	Q. robur (1/1)	Healthy	GON (1)
	Q. suber (0/1)	Healthy	–
N13	*A. lusitanica* (1/1)	Healthy	PSY (1), QUE (1)
	A. unedo (0/1)	Healthy	–
	P. pinaster (1/1)	Some stunted & chlorotic or dead	MUL (1), PSC (1)
	P. pinea (0/1)	Healthy	–
	Q. faginea (0/1)	Healthy	–
	Q. ilex (0/1)	Mostly healthy, some stunted & chlorotic	–
	Q. suber (0/1)	Mostly healthy, some stunted & chlorotic	–

[a]Almost all plants with above-ground symptoms had extensive fine root losses and/or root rot.

[b]ALT = *P. alticola*, CAC = *P. cactorum*, CIN = *P. cinnamomi*, CRA = *P. crassamura*, GON = *P. gonapodyides*, MUL = *P. multivora*, PAR = *P. parvispora*, PLU = *P. plurivora*, PSC = *P. pseudocryptogea*, PSY = *P. psychrophila*, QUE = *P. quercina*, QUT = *P. quercetorum*, PSC×KEL = *P. taxon pseudocryptogea × kelmanii*, ×CAM = *P. ×cambivora*, ×CRA = *P. taxon ×crassamura-like*, ×PSC = *P. taxon ×pseudocryptogea-like*.

[c]*Phytophthora cinnamomi* panglobal clonal lineages PcG1 and PcG2, according to the population genomic study of Shakya et al. (2021). All isolates PcG1 and PcG2 belong to the A2 mating type.

[d]DAPC groups within *P. ×cambivora* according to the discriminant analysis of principal components (DAPC) of the population genomic study of Mullett et al. (2023). DAPC4 and DAPC11 isolates belong to the A1 mating type, DAPC9 isolates to the A2 mating type.

[e]*Phytopythium vexans* isolated.

[f]*Pythium diclinum* also isolated.

[g]*Phytopythium mercuriale* also isolated.

[h]*Phytopythium* taxon mercuriale-like also isolated.

[i]*Phytopythium* taxon IRAN4019C also isolated.

[j]*Phytopythium citrinum* also isolated.

[k]*Elongisporangium undulatum* also isolated.

[l]*Phytopythium litorale* also isolated.

FIGURE 4 Symptoms of cork oak (*Quercus suber*) decline and dieback in Portugal caused by *Phytophthora cinnamomi*; (a) acute wilting and mortality of a cork oak in stand F61 in the Serra Algarvia; (b) severe mortality and dieback of cork oak stand F64 in the Serra Algarvia; (c) small woody roots with extensive loss of lateral and fine roots of a declining cork oak in stand F64 in the Serra Algarvia; (d) cork oak in stand F21 at Monte Morais with aerial bleeding cankers (arrows); (e) bleeding collar rot canker of a cork oak in stand F45 in the Reserva Natural da Serra da Malcata.
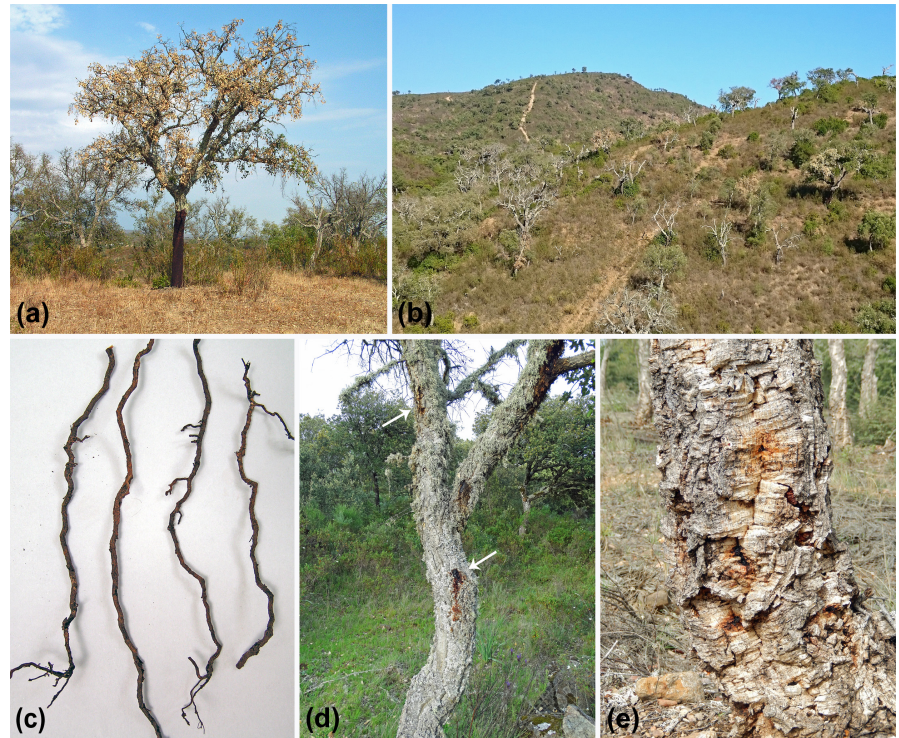
of *A. lusitanica* trees with decline and dieback symptoms (Table 1). The dieback of *B. celtiberica* in a natural montane forest (F17) of the Serra da Nogueira at 1043 m a.s.l. was associated with *P. gonapodyides* and *P. pseudosyringae* (Table 1). Conversely, no *Phytophthora* species was found in the healthy natural *B. celtiberica–Sorbus aucuparia* stand F35 at 1428 m a.s.l. in the Parque Nacional da Serra da Estrela (Table 1). On the Azores island of São Miguel, *P. multivora* was obtained from the soil sample of a declining non-native *Q. rubra* tree. On Madeira Island, the rhizosphere of *Ocotea foetens* and *Persea indica* in a natural montane laurisilva forest at 1161 m a.s.l. harboured *P. chlamydospora* (Table 1). Although conifers were sampled only occasionally, several *Phytophthora*–conifer associations were found. In two Atlantic forest stands in the Parque Natural Sintra-Cascais (F50, F51), the decline of *Abies alba* trees was associated with *P. cinnamomi* (Table 1). Both *P. cinnamomi* and *P. ×cambivora* were recovered from the rhizosphere of a declining *P. pinaster* tree in the Parque Nacional da Peneda-Gerês (F07) in the north of Portugal whereas a declining *P. pinea* tree in an agroforestry plantation (F68) in the southernmost Serra Algarvia yielded the undescribed hybrid *P.* taxon *pseudocryptogea × kelmanii*. The only tree species without *Phytophthora* records were *J. regia*, *Laurus nobilis*, *S. aucuparia* and *Tilia cordata*. However, with only one tree each, their sample size was not representative.

3.2 | *Phytophthora* diversity in rivers and forest streams of Portugal

Using an in-situ baiting approach with rafts, 34 *Phytophthora* taxa were isolated from 33 of the 34 rivers and streams tested (97.1%).

The 425 *Phytophthora* isolates belonged to 34 taxa from six phylogenetic clades: (a) 18 non-hybrid taxa including the known species *P. citrophthora*, *P. multivora* and *P. plurivora* from Clade 2; *P. pseudosyringae* from Clade 3 (DAPC groups 3 and 5; Mullett et al., 2024); *P. amnicola*, *P. bilorbang*, *P. chlamydospora*, *P. gonapodyides*, *P. inundata*, *P. lacustris* and *P. thermophila* from Clade 6; *P. kelmanii*, *P. pseudocryptogea* and *P. ramorum* from Clade 8; and *P. honggalleglyana* (previously *P. hydropathica*; Abad et al., 2023; Hong et al., 2010) from Clade 9; a new Clade 2 species recently described as *P. pseudocitrophthora* (Jung et al., 2024); and two undescribed taxa, the designated *P.* taxon P16855 from Clade 9 and the provisionally named *P.* taxon sylvatica-like 1 from Clade 6 (Figure 6; Table 2); and (b) 16 hybrid taxa including the known species *P. ×cambivora* (DAPC groups 1, 5 and 9; Mullett et al., 2023) from Clade 7 and *P. ×stagnum*, a Clade 6 hybrid between *P. chlamydospora* as its paternal parent and an unknown species closely related to *P. mississippiae* as its maternal parent (Yang et al., 2014); a new Clade 2 hybrid species with *P. citrophthora* as maternal and an unknown Clade 2 taxon as paternal parent that was recently described as *P. ×lusitanica* (Jung et al., 2024); the informally designated taxa *P.* taxon ×WS (Oh et al., 2013), *P.* taxon *amnicola × chlamydospora* and *P.* taxon *thermophila × amnicola* (Burgess, 2015; Nagel et al., 2013) from Clade 6, *P.* taxon *pseudocryptogea × kelmanii* from Clade 8 and *P.* taxon ×Hennops from Clade 9; and eight new hybrid taxa provisionally named here as *P.* taxon ×amnicola-like, *P.* taxon ×lacustris-like and *P.* taxon *thermophila × gonapodyides* from Clade 6; *P.* taxon ×pseudocryptogea from Clade 8; and *P.* taxon ×Clade09a1_PT1, *P.* taxon ×Hennops1, *P.* taxon zentmyerii × Peru4-like and *P.* taxon ×P16855-like from Clade 9 (Figure 7; Table 2). In addition, *Phytopythium litorale*, a new species from the
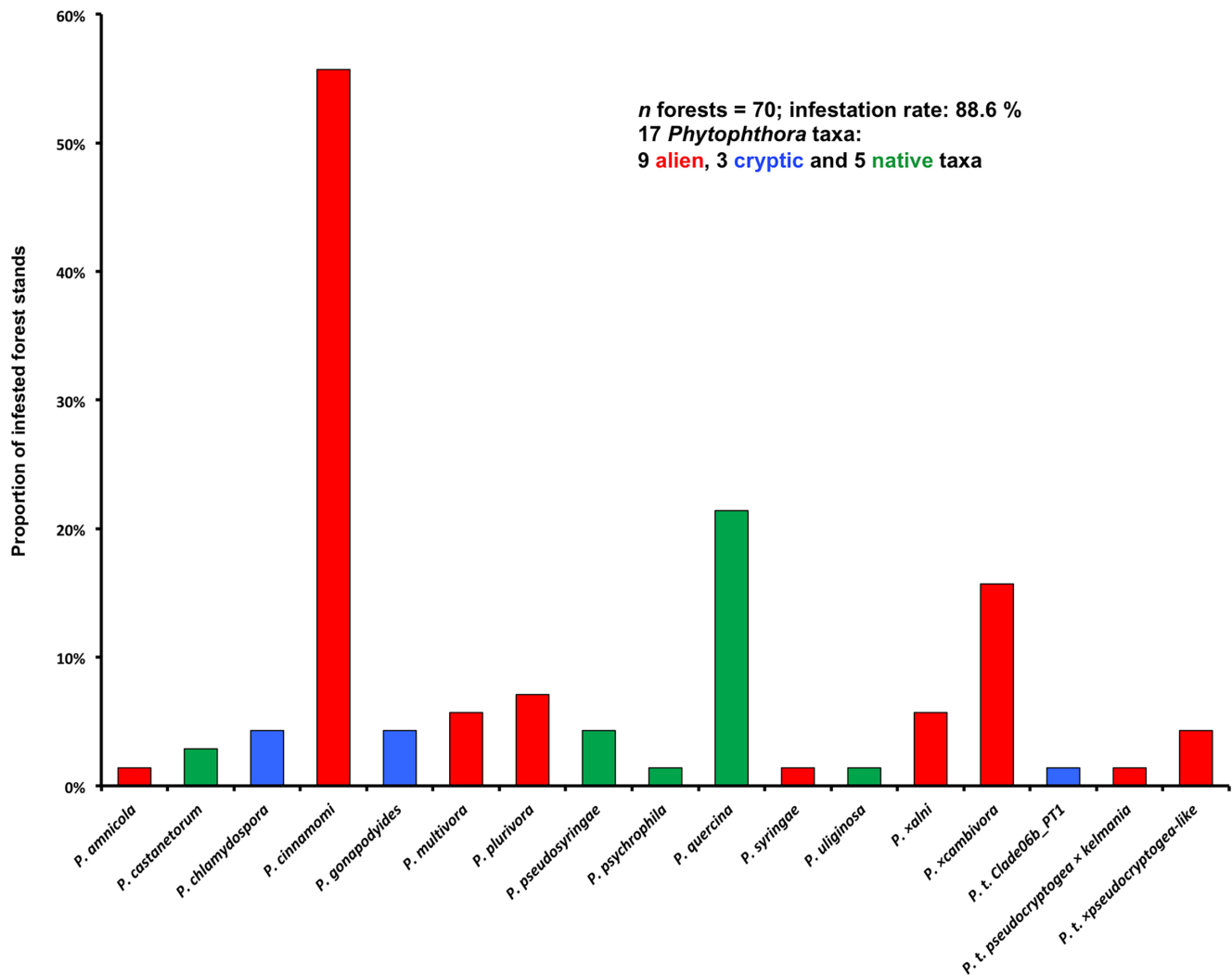
FIGURE 5 Diversity and frequency of *Phytophthora* taxa in 70 forest stands across Portugal; *P*. t. = *Phytophthora* taxon.

previously unknown oomycete genus *Nothophytophthora* recently described as *N. amphigynosa* (Jung, Scanu, Bakonyi, et al., 2017), the undescribed *Phytopythium* taxa PV So7, KC-2014 and PB-2013 and the undescribed *Pythium* taxon strain 1–9 were recovered from five waterways (Table 2).

Most common were *P. gonapodyides* (13 streams = 38.2%), *P. lacustris* (12 streams = 35.3%), *P. plurivora* (10 streams = 29.4%), *P. chlamydospora* (nine streams = 26.5%) and *P. amnicola* (eight streams) followed by *P. honggalleglyana* and *P.* ×*cambivora* (five streams each = 14.7%), and *P.* taxon *thermophila* × *amnicola* and *P.* taxon ×P16855-like (four streams each) (Figure 7; Table 2). Comprising isolates from groups DAPC9 (A2 mating type), DAPC1 (A1 mating type) and DAPC5, an A1 hybrid between DAPC1 and DAPC9 (Mullett et al., 2023), the aquatic population of *P.* ×*cambivora* was genetically more diverse than the isolates recovered from forest soils and bark cankers. *P. plurivora* was widespread in upper colline and submontane rivers and streams of the Mata Nacional do Buçaco, the National Parks Peneda-Gerês and Serra da Estrela, and the Reserva Natural da Serra da Malcata between 273 and 682 m a.s.l. (Table 2). In contrast, *P. multivora* was found only in two lowland

streams (R24, R26) near the Atlantic coast at Leiria and in the Parque Natural Sintra-Cascais (Table 2). *P. ramorum* (belonging to the EU1 lineage, according to Van Poucke et al., 2021) was detected exclusively in a small stream (R30) running through a Botanical Garden with *Rhododendron* spp. and other exotic plants established within a mixed broadleaved and pine forest in the Parque Natural Sintra-Cascais (Table 2). In Portugal, this pathogen was previously found on ornamental *Viburnum* spp. (Gomes & Amaro, 2008).

With eight *Phytophthora* taxa, the highest diversity was found in the Rio Séqua (R35), which runs through lowland to colline oak and pine forests, heathlands and *Citrus* plantations in the Algarve near Tavira, and in the Ribeira da Meimoa (sites R20 and R21) with its catchment in the Reserva Natural da Serra da Malcata covered by submontane and montane broadleaved forests, heath- and grasslands (Table 2). In the Rio Séqua, all three species from the *P. citrophthora* complex—*P. citrophthora*, *P. pseudocitrophthora* and *P.* ×*lusitanica*—were present (Table 2). Seven *Phytophthora* taxa were detected in the Ribeira de Vale do Boi (R31) running through the foothills of the Algarvian mountain range of Monchique covered with broadleaved and pine forests, heathlands, introduced
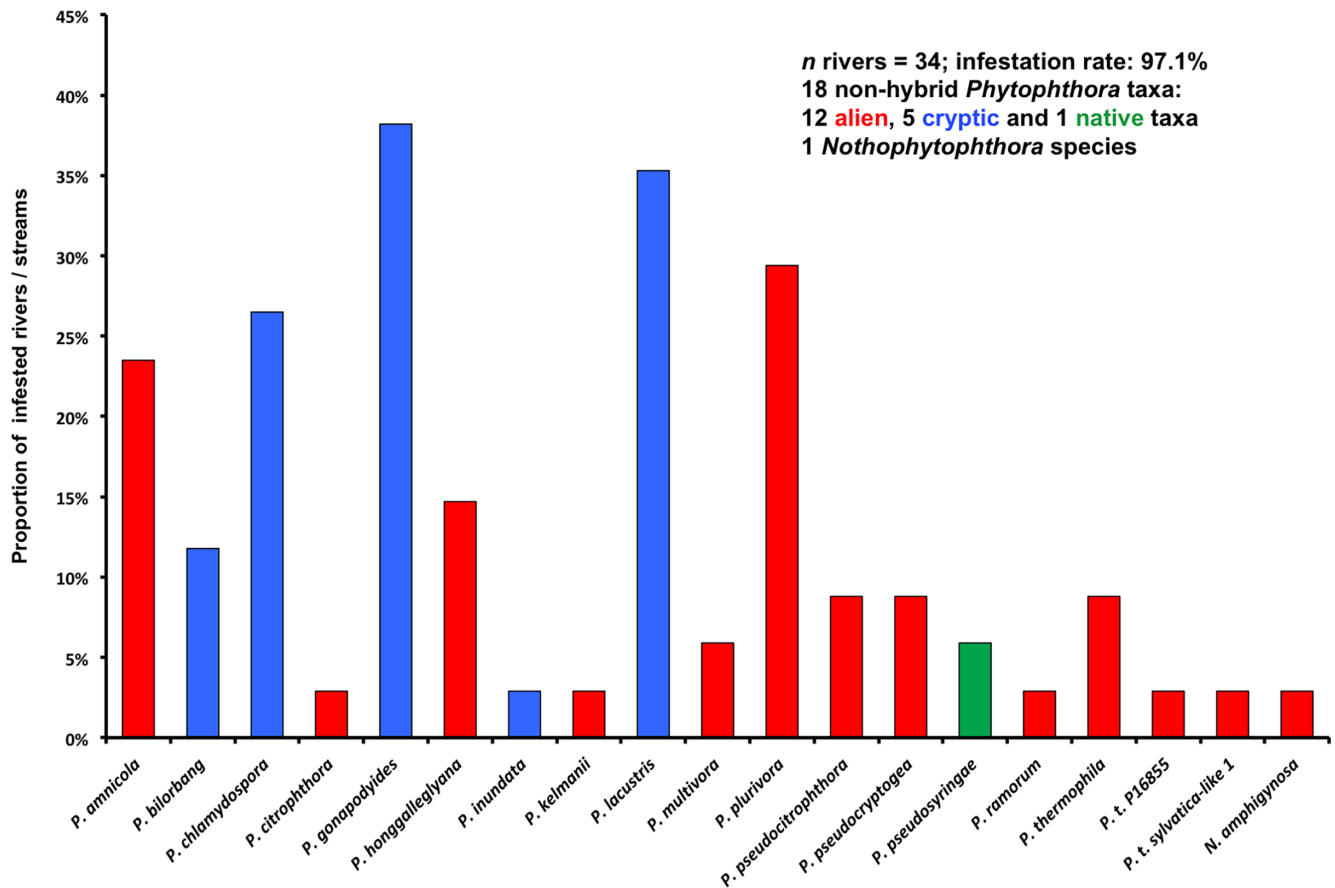
FIGURE 6 Diversity and frequency of *Nothophytophthora* and *Phytophthora* non-hybrid taxa in 34 rivers and forest streams across Portugal; *P.* t. = *Phytophthora* taxon.

acacias, eucalypt plantations and horticulture (Table 2). Six *Phytophthora* species occurred in the Rio Zêzere (R12) and the Rio Beijames (R14) in the Parque Natural da Serra da Estrela and in the Rio Côa (R17 and R18) in the Reserva Natural da Serra da Malcata, all having catchment areas with diverse submontane and montane broadleaved forests, heath- and grasslands and horticulture (Table 2). Six waterways—the Rio Mondego (R15) in the Parque Natural da Serra da Estrela, the Ribeiro da Mouca (R22) in the Reserva Natural da Serra da Malcata, the forest stream R24 in the Mata Nacional de Leiria, the Ribeira de Colares (R28) and the forest stream R26 in the Parque Natural Sintra-Cascais, and the Ribeira de Odelouca near Silves in the Algarve—hosted five *Phytophthora* taxa each (Table 2).

3.3 | *Phytophthora* diversity in forest nurseries of Portugal

In 12 of the 13 forest nurseries (92.3%), a total of 135 oomycete isolates were obtained from the collar rot of one chestnut seedling and from 54 of the 87 bulked root ball samples (61.4%) from 16 of the 21 tree species (76.2%). The only nursery without a *Phytophthora* record was nursery N01 where only two mixed soil samples from a

bare transplanting bed were tested (Table 3). The tree species without *Phytophthora* isolations were *J. regia* and the four exotic species *Chamaecyparis lawsoniana*, *Cupressus lusitanica*, *Pinus radiata* and *Quercus coccinea* (Table 3). The 122 *Phytophthora* isolates belonged to 16 taxa from eight phylogenetic clades including (a) 13 known species, namely *P. cactorum* (Clade 1), *P. multivora* and *P. plurivora* (Clade 2), *P. psychrophila* (Clade 3), *P. alticola* and *P. quercetorum* (Clade 4), *P. crassamura* and *P. gonapodyides* (Clade 6), *P. cinnamomi*, *P. parvispora* and *P.* ×*cambivora* (Clade 7), *P. pseudocryptogea* (Clade 8) and *P. quercina* (Clade 12); (b) the informally designated *P.* taxon *pseudocryptogea* × *kelmanii* (Clade 8); and (c) two new hybrid taxa provisionally named here *P.* taxon ×*crassamura*-like (Clade 6) and *P.* taxon ×*pseudocryptogea*-like (Clade 8) (Figure 8; Table 3). In addition, 13 isolates belonging to nine taxa from three other oomycete genera were also isolated: *Elongisporangium undulatum*, *Elongisporangium* sp., *Pythium diclinum*, *Phytopythium citrinum*, *Phytopythium litorale*, *Phytopythium mercuriale*, *Phytopythium vexans*, the informally designated *Phytopythium* taxon IRAN4019C, and a previously unknown taxon provisionally named here as *Phytopythium* taxon mercuriale-like (Table 3).

Most common was *P. cinnamomi*, which was isolated in eight nurseries (61.5%) from 15 bulked root ball samples (17.2%) of nine host plants (42.9%), including *A. alba*, *A. unedo*, *C. sativa*, *Cupressus*
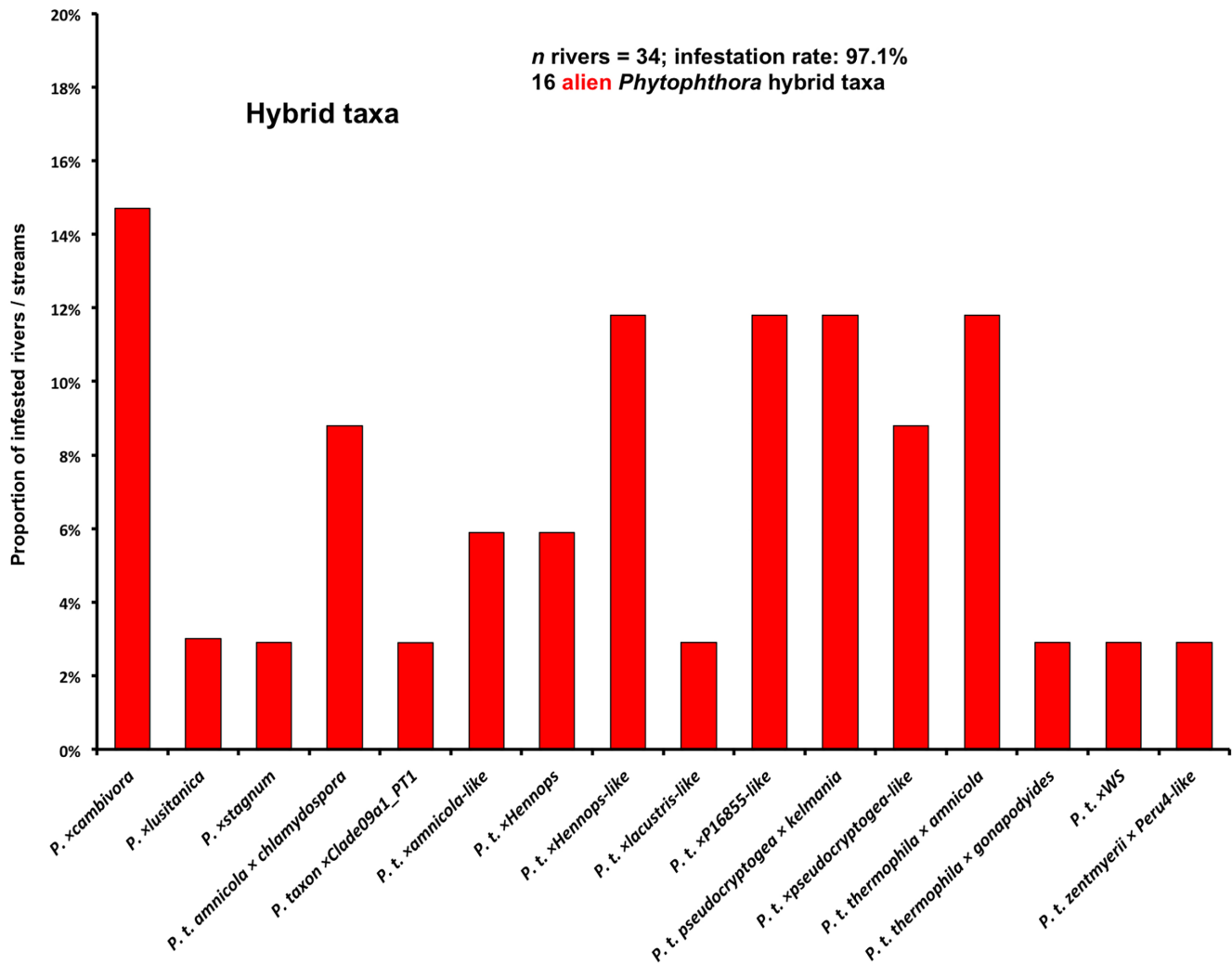
FIGURE 7 Diversity and frequency of *Phytophthora* hybrid taxa in 34 rivers and forest streams across Portugal; *P. t. = Phytophthora* taxon.

sempervirens, *Eucalyptus nitens*, *P. pinaster*, *Q. ilex*, *Q. rubra* and *Q. suber*, and from the collar rot of a *C. sativa* seedling (Figures 8 and 9; Table 3). *Phytophthora pseudocryptogea* was found in six nurseries (46.2%) in eight bulked root ball samples (9.2%) from seven host species (33.3%), all belonging to the genera *Pinus* and *Quercus* (*P. pinea*, *P. pinaster*, *P. sylvestris*, *Q. faginea*, *Q. ilex*, *Q. pyrenaica* and *Q. suber*; Table 3). Interestingly, the hybrids *P. taxon* ×pseudocryptogea-like and *P. taxon pseudocryptogea* × *kelmanii* were also obtained exclusively from oak and pine plants (Figure 8; Table 3). *Phytophthora* ×*cambivora* also occurred in six nurseries (40%) where it was isolated from eight bulked root ball samples (9.2%) of five host species (23.8%), all belonging to Fagaceae genera (*C. sativa*, *Q. ilex*, *Q. pyrenaica*, *Q. robur* and *Q. suber*; Figure 8; Table 3). The isolates belonged to groups DAPC4 and DAPC11 (both A1 mating type) and DAPC9 (A2 mating type) of the population genomic study by Mullett et al. (2023). *P. cactorum*, *P. multivora*, *P. quercetorum* and *P. quercina* were each found in three nurseries (Figure 8): *P. cactorum* in two nurseries on *P. pinea* and in one nursery on *Q. ilex*; *P. multivora* in separate nurseries on *P. pinea*, *Q. faginea* and *Q. suber*; *P.*

quercetorum on *Q. rubra* in three nurseries, *Q. pyrenaica* in two nurseries and *Q. faginea* and *Q. ilex* in one nursery each; and *P. quercina* on *A. lusitanica*, *Q. faginea*, *Q. ilex*, *Q. pyrenaica* and *Q. robur* in one nursery each (Table 3). *P. plurivora* and *P. psychrophila* were both isolated in two nurseries (Figure 8): *P. plurivora* from *Q. faginea* and *Q. suber*; and *P. psychrophila* from *A. lusitanica* and *Q. ilex* (Table 3). All remaining *Phytophthora* taxa, including *P. alticola*, which was obtained from *E. nitens*, were found in only one nursery each (Figure 8; Table 3). From five bulked root ball samples without *Phytophthora* record, *P. vexans*, *P. mercuriale*, *Phytopythium* taxon mercuriale-like and *E. undulatum* were isolated (Table 3). Nursery N11 showed the highest *Phytophthora* diversity with seven taxa detected, including *P. cinnamomi*, *P. crassamura*, *P. plurivora*, *P. pseudocryptogea*, *P. quercina*, *P.* ×*cambivora* and *P. taxon* ×crassamura-like (Table 3). Five *Phytophthora* taxa occurred in nursery N08 whereas each of four *Phytophthora* taxa were found in five nurseries (N02, N06, N07, N12 and N13; Table 3).

Although in most nurseries, the majority of plants appeared visually healthy, plants with symptoms indicative of *Phytophthora*

infections were present in all nurseries. Symptoms comprised fine root losses and root rot (found in almost all plants with above-ground symptoms), collar rot lesions (only found in *C. sativa*), stunted growth, small sized and/or chlorotic leaves/needles, wilting and mortality (Figure 9; Table 3). Overall, 44 of the 50 bulked samples taken from symptomatic or partly symptomatic plants (88%) yielded 16 *Phytophthora* taxa. In contrast, only nine of the 35 bulked samples taken from visually healthy plants (25.7%) were found infested with five *Phytophthora* species (Table 3). All bulked root ball samples from which *P. cactorum*, *P. cinnamomi*, *P. multivora*, *P. plurivora*, *P. pseudo-cryptogea* and its hybrids have been isolated came, at least partially, from symptomatic plants (Table 3). From two of the six symptomatic samples without a *Phytophthora* record, *E. undulatum*, *P. mercuriale* and *Phytopythium* taxon mercuriale-like were recovered. The latter taxon was also obtained from two samples of healthy plants (Table 3).

4 | DISCUSSION

This paper reports on the most extensive and diverse *Phytophthora* survey conducted in natural ecosystems and forest nurseries of a country in Europe. Across Portugal, 48 *Phytophthora* taxa from eight phylogenetic clades were obtained from 62 of 70 forest stands (88.6%), 33 of 34 rivers and forest streams (97.1%) and 12 of 13 nurseries (92.3%) tested. These comprised 28 known *Phytophthora* species; three new recently described species (*P. castanetorum*, *P. pseudocitrophthora* and *P. ×lusitanica*; Jung et al., 2024; Jung, Horta Jung, Cacciola, et al., 2017); one informally designated and two new non-hybrid taxa; five informally designated and nine new hybrid taxa. Only five *Phytophthora* species (10.4%)—*P. pseudosyringae* and *P. psychrophila* from Clade 3, *P. uliginosa* from Clade 7, and *P. castanetorum* and *P. quercina* from Clade 12—are considered native to Europe (Jung et al., 2002, 2003, 2016; Jung, Horta Jung, Cacciola, et al., 2017; Mullett et al., 2024). Another nine taxa (18.8%)—*P. bilorbang*, *P. chlamydospora*, *P. crassamura*, *P. gonapodyides*, *P. inundata*, *P. lacustris*, the new *P.* taxon Clade06b_PT1, *P.* taxon ×crassamura-like and *P.* taxon ×lacustris-like (all from Clade 6)—are of cryptic origin (Aghighi et al., 2012; Brasier, Cooke, et al., 2003; Brasier, Sanchez-Hernandez, & Kirk, 2003; Jung et al., 2016; this study). However, the remaining majority of 34 *Phytophthora* taxa (70.8%) are considered in Europe as introduced aliens. Because the international trade in living plants is considered a major pathway for the introduction of exotic *Phytophthora* pathogens (Brasier et al., 2022; Jung et al., 2016, 2024; Liebhold et al., 2012; Santini et al., 2013), the unique diversity of exotic *Phytophthora* taxa in Portugal is most probably related to the long-term import of living plants from overseas that started more than 500 years ago with the establishment of trading posts and colonies in Brazil and across Africa, Southeast and East Asia, and has intensified during the past decades with modern globalization.

A phylogeographic study of a global population of *P. cinnamomi* isolates demonstrated recently that this wide-host range pathogen originates from Taiwan, the previous Portuguese colony of Formosa, and possibly other regions in Southeast Asia (Shakya et al., 2021). Another recent phylogeographic study indicates for *P. ×cambivora* an origin in Japan and most likely also in neighbouring regions of East Asia (Mullett et al., 2023). Early reports of typical symptoms of ink disease of *C. sativa* suggest that the causal agents *P. cinnamomi* and *P. ×cambivora* were already established in Portugal in the 1830s (Crandall et al., 1945). Eventually, the association of both pathogens with declining chestnuts in Portugal was confirmed in the 1940s (Lopes-Pimentel, 1946, 1947). Numerous isolates of *P. cinnamomi* and *P. ×cambivora* were included in the population genomic studies of Shakya et al. (2021) and Mullett et al. (2023), respectively. These demonstrated comparatively high genetic variability of the Portuguese populations of *P. cinnamomi*, comprising both clonal A2 mating type lineages PcG1-A2 and PcG2-A2 that are driving the global pandemic (Shakya et al., 2021), and *P. ×cambivora*, comprising five of the 11 global DAPC groups and both mating types (Mullett et al., 2023; this study), indicating multiple introductions of both pathogens. Also for *P. parvispora*, the closest relative of *P. cinnamomi* in Clade 7c; the Clade 2 species *P. citrophthora*, *P. pseudocitrophthora*, *P. plurivora* and *P. ×lusitanica*; and the Clade 8 species *P. kelmanii* and *P. pseudocryptogea* including their interspecific hybrids, and *P. ramorum*, an origin in Southeast and/or East Asia is likely due to their occurrence in natural healthy ecosystems and/or the origin of their closest relatives in this region (Jung et al., 2020, 2021, 2024; Jung, Chang, Bakonyi, et al., 2017; Scanu, Hunter, et al., 2014). Several Clade 6 isolates from the Rio Séqua in the south of Portugal have ITS and *cox1* sequences identical to those of *P.* taxon sylvatica-like 1, a natural inhabitant of forest streams in Vietnam (Jung et al., 2020). *P. alticola* from Clade 4 was described from exotic eucalypt plantations in South Africa (Maseko et al., 2007) and was later also found widespread in *Acacia* plantations and natural forests (Bose et al., 2018, 2021). It is closely related to the eucalypt pathogens *P. arenaria* and *P. boodjera* from Western Australia (Bose et al., 2017; Rea et al., 2011; Simamora et al., 2015). From Australia are the mostly aquatic Clade 6 species *P. amnicola*, *P. thermophila* and their hybrid *P.* taxon *thermophila × amnicola* (Burgess, 2015; Crous et al., 2012; Jung et al., 2011), which were all found widespread in Portuguese rivers and streams during this study. Another aquatic Clade 6 hybrid found in Portuguese streams, *P.* taxon ×WS (= *P. amnicola × P. thermophila*), most likely originates from South Africa (Burgess, 2015; Nagel et al., 2013). However, it cannot be ruled out that the hybridizations that created *P.* taxon *thermophila × amnicola* and *P.* taxon ×WS occurred spontaneously in Portuguese waterbodies where their introduced parental species were found widespread in this survey. The emerging wide-host range pathogen *P. multivora* from Clade 2 is also native to South Africa as has been unveiled recently by a population genomic study (Tsykun et al., 2022). Another recent population genetic study presented evidence that the global wide-host range pathogen *P. cactorum* from Clade 1 most likely originates from North America (Bourret et al., 2022). Likewise, the oak-specific Clade 4 species *P. quercetorum* (Balci et al., 2008), the aquatic Clade 6 hybrid *P. ×stagnum* (Yang et al., 2014) and *P. honggalleglyana* from
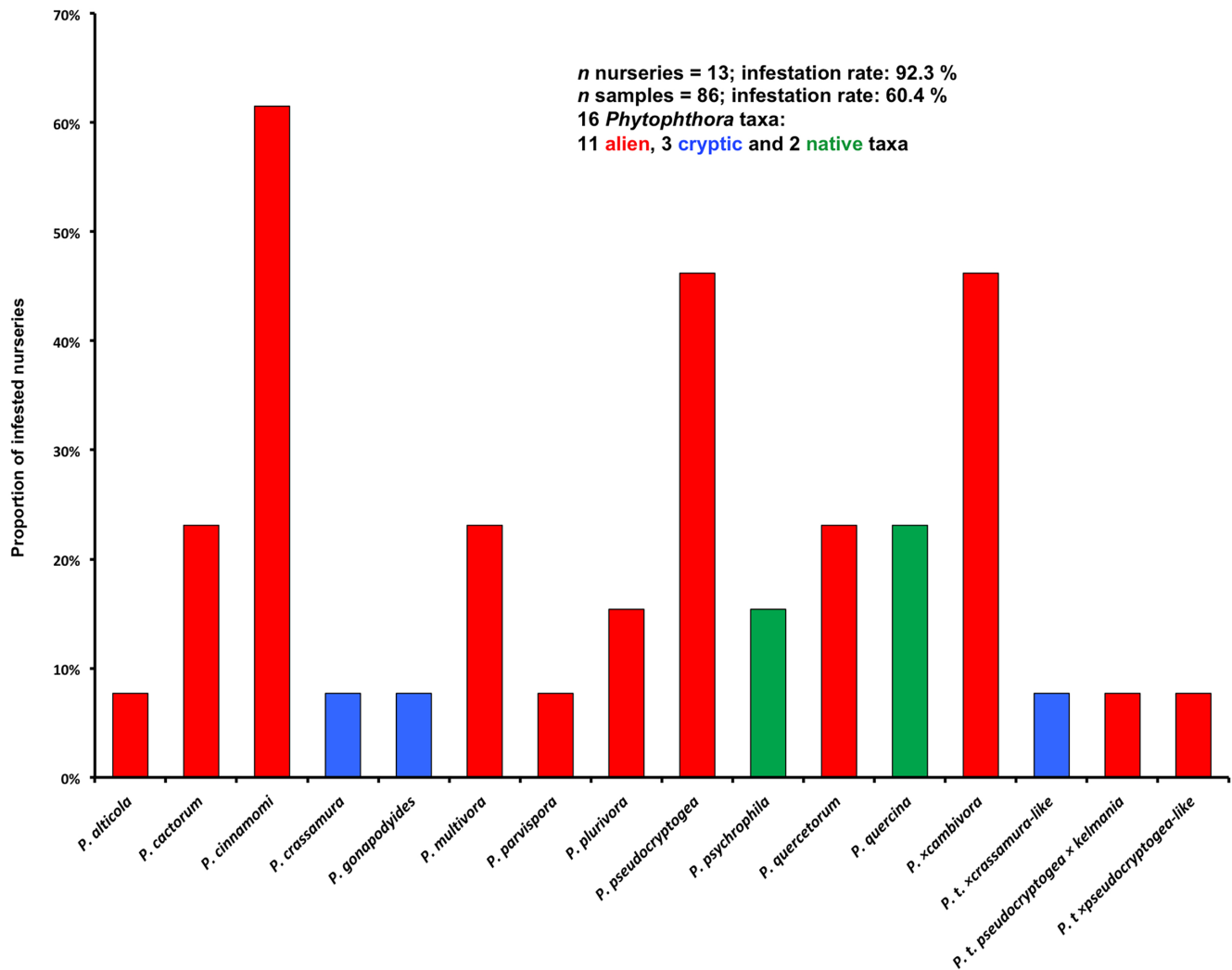
FIGURE 8 Diversity and frequency of *Phytophthora* taxa in 13 forest nurseries across Portugal; *P.* t. = *Phytophthora* taxon.

a cluster of aquatic high-temperature species within Clade 9 (Abad et al., 2023; Hong et al., 2010) are native to North America. From this Clade 9 cluster, an array of six hybrid and non-hybrid taxa were obtained from Portuguese rivers and streams which, due to their own or their closest relatives' occurrence in natural undisturbed rivers and forests, most likely have their origins in South Africa (*P.* taxon ×Hennops, *P.* taxon ×Hennops-like 1; Oh et al., 2013) and South America like *P.* taxon P16855, first found in an Amazonian rainforest in Ecuador (*cox1* GenBank accession GU594797), and three related new hybrid taxa *P.* taxon ×P16855-like, *P.* taxon ×Clade09a1_PT1 and *P.* taxon zentmyerii × Peru4-like.

Figure 10 summarizes the distribution of *Phytophthora* taxa across the three site categories surveyed. While 35 *Phytophthora* taxa were exclusively found in forests (five taxa), forest nurseries (six taxa) and river systems (24 taxa), 13 *Phytophthora* taxa occurred in more than one site category. The primarily aquatic *P. gonapodyides* with cryptic geographic origin was found at all site categories. Five introduced taxa—*P. multivora*, *P. plurivora*, *P.* ×*cambivora*, *P.* taxon *pseudocryptogea* × *kelmanii* and *P.* taxon ×pseudocryptogea-like—also occurred at all site categories, indicating their spread from

nurseries to both forests (via planting of infested nursery stock) and river systems (via nursery effluents or indirectly via infested plantings). For *P.* ×*cambivora* this is supported by the higher genetic variability of nursery populations (four DAPC groups) compared with river (three DAPC groups) and forest (one DAPC group) populations. However, due to the use of unfiltered non-sterile river water for irrigation in several nurseries included in this study, an introduction of *Phytophthora* taxa to the nurseries via infested river water cannot be ruled out. Besides the six *Phytophthora* taxa recorded from all three site categories, nurseries shared *P. pseudocryptogea* with rivers/streams and another three *Phytophthora* species, the invasive *P. cinnamomi* and the native *P. psychrophila* and *P. quercina*, with forest sites. Forest sites and river systems also had two primarily aquatic species, *P. amnicola* and *P. chlamydospora*, and the forest pathogen *P. pseudosyringae* in common.

Over all 29 tree species sampled in forest stands across Portugal, isolation rates from rhizosphere soil of declining trees exceeded those from visually healthy trees considerably (65.3% versus 10.1%), demonstrating a clear association between *Phytophthora* presence and decline and dieback of trees. Our survey showed that *P.*

FIGURE 9 Container plants in forest nurseries of Portugal; (a) *Castanea sativa* in nursery N11; healthy seedlings with yellow leaves due to autumnal senescence and dead seedlings with brown leaves due to root and collar rot caused by *Phytophthora cinnamomi* and *P.* ×*cambivora*; (b) *C. sativa* seedling in nursery N11 with a girdling collar rot lesion (arrow) caused by *P. cinnamomi*; (c) *C. sativa* seedling in nursery N05 with a girdling collar rot lesion caused by *P. cinnamomi*; note the tongue-shaped inner bark lesion (arrow); (d) high mortality of *Quercus ilex* seedlings caused by *P. cinnamomi* in nursery N08; note that the containers are standing directly on the wet ground; (e) wilting of *Arbutus unedo* seedlings in nursery N02 due to root rot caused by *P. cinnamomi*; (f) chlorosis and mortality of *Pinus sylvestris* seedlings in nursery N02 due to root rot caused by *P. pseudocryptogea* and *P.* taxon ×pseudocryptogea-like; (g) *Quercus robur* container stand in nursery N12 infested by *P. gonapodyides*; note the close distance to the ground and the debris splashed onto the containers (arrows).

cinnamomi is by far the most common *Phytophthora* species in forests and forest nurseries across Portugal, with infestation rates of 55.7% and 61.4%, respectively, and an altitudinal range of 123–1194 m a.s.l. The high *P. cinnamomi* infestation rates of forest sites and forest nurseries are most probably correlated via the planting of infested nursery stock. The invasive success of this self-sterile species in Portugal and numerous other countries with hot and dry summers like Australia, California, Italy, Spain and South Africa (Bose et al., 2018; Burgess et al., 2017; Erwin & Ribeiro, 1996; Hardham & Blackman, 2018; Jung, Pérez-Sierra, et al., 2018; Oh et al., 2013; Sanchez et al., 2002; Vettraino et al., 2005) is certainly facilitated by the ability of the A2 mating type to form oospores, highly enduring thick-walled survival structures, by selfing, in particular under environmental stress or in the presence of antagonistic fungi and plants (Brasier, 1978; Jayasekera et al., 2007; Jung et al., 2013). *P. cinnamomi* is arguably the most notorious invasive plant pathogen globally, causing root and collar rot, epidemic dieback and mortality of more than 5000 woody species in natural ecosystems, nurseries and planted stands (Brasier et al., 2022; Erwin & Ribeiro, 1996; Hardham & Blackman, 2018). In all 12 forest tree species from which it was recovered in forest stands, *P. cinnamomi* was associated with severe symptoms like fine root losses, root rot, decline, dieback and mortality, and in some stands of *C. sativa* and *Q. suber* also with bleeding

bark cankers. With eight tree species in 15.7% of forest sites and 46.2% of forest nurseries, the other wide-host range species from Clade 7, *P.* ×*cambivora*, was also common in Portugal. Like in other European countries (Day, 1938; Jung, Pérez-Sierra, et al., 2018; Vettraino et al., 2005) and Chile (Jung, Durán, et al., 2018), *P. cinnamomi* and *P.* ×*cambivora*, either separately or jointly, were responsible for ink disease and mortality of *C. sativa* in 16 of the 17 affected stands (94.1%) in this study. The severity of this epidemic in Portugal was demonstrated by Martins et al. (2007) who reported that both pathogens caused an average mortality of 58.6% in around 90 chestnut stands between 1995 and 2004. Considering their aggressiveness to *C. sativa*, the presence of *P. cinnamomi* and/or *P.* ×*cambivora* in 67% of the chestnut container stands tested in forest nurseries in this survey is concerning. *P. cinnamomi* was also recovered from *Q. suber* and *Q. ilex* trees with decline and dieback in 15 of the 19 affected stands (78.9%), reinforcing previous studies that demonstrated the involvement of *P. cinnamomi* in the devastating decline of both oak species in Portugal (Brasier et al., 1993; Moreira & Martins, 2005) and other countries (Gallego et al., 1999; Jung, Pérez-Sierra, et al., 2018; Robin et al., 1998; Sanchez et al., 2002; Seddaiu et al., 2020; Vettraino et al., 2002). In addition, *P. quercina* and in some stands also *P. pseudocryptogea*, *P. psychrophila* and *P. uliginosa* were isolated for the first time from the rhizosphere of declining
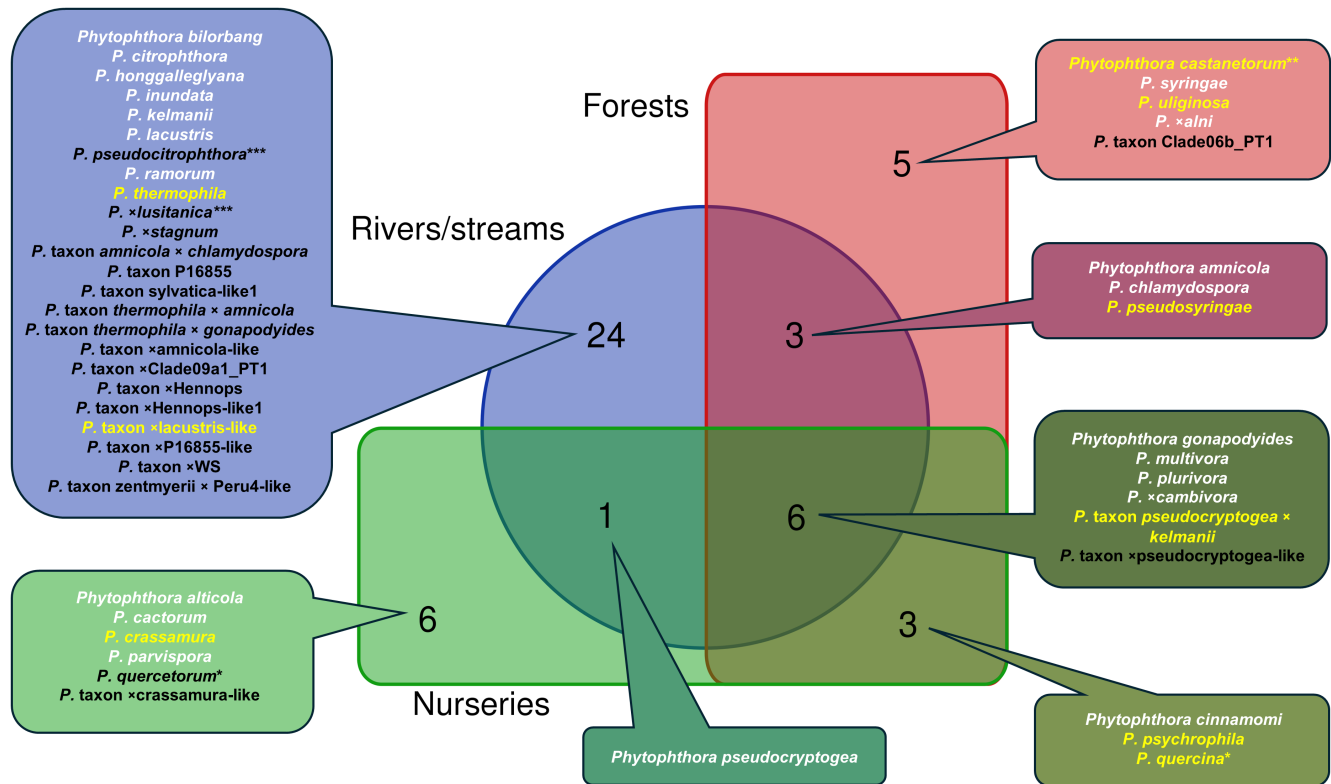
FIGURE 10 Venn diagram showing the exclusive and overlapping *Phytophthora* diversity in 70 forest stands, 34 rivers and forest streams and 13 forest nurseries across Portugal. Black and yellow taxa names indicating the first reports for Europe and Portugal, respectively. First reports from this survey previously reported: *Jung et al. (2016), **Jung, Horta Jung, Cacciola, et al. (2017), ***Jung et al. (2024).

cork and holm oaks in Portugal. Their involvement in cork and holm oak decline was previously demonstrated in eastern Spain (Pérez-Sierra et al., 2013) and Italy (Jung et al., 1999; Seddaiu et al., 2020). Although commonly considered as specific to oaks, *P. quercina* was also recovered from the rhizosphere of tree species from other genera, namely *A. unedo*, *C. sativa*, *P. latifolia* and *I. aquifolium*, in several forest stands. Pathogenicity trials using a standardized soil infestation method are needed to clarify whether this fine root pathogen is pathogenic to these four tree species. The *Phytophthora*-mediated declines of *F. sylvatica* and *Q. robur*, found in several stands during this survey, are widespread in temperate regions of Europe and parts of Italy and the Balkans (Balci & Halmschlager, 2003; Cacciola et al., 2005; Corcobado et al., 2020; Day, 1938; Hansen & Delatour, 1999; Jönsson et al., 2003; Jung, 2009; Jung et al., 1996, 2000, 2019; Jung, Pérez-Sierra, et al., 2018; Milenković et al., 2012; Stępniewska & Dłuszyński, 2010), but were previously not known to occur in Portugal. Other first records from non-riparian forests in Portugal included (a) the decline and dieback of the Iberian *Q. pyrenaica*, which was mainly associated with *P. cinnamomi*, *P. quercina* and *P. ×cambivora*; the findings of (b) the emerging wide-host range pathogen *P. multivora* from a declining *Q. rubra* tree on the Azores island of São Miguel and from the invasive *Acacia dealbata* and declining *A. pseudoplatanus* trees in several stands of the Parque Natural Sintra-Cascais; (c) *P. cinnamomi* from declining mature silver fir (*A. alba*) trees in the same Natural Park; (d) *P. plurivora* in various forest stands of Portugal; (e) *P. ×cambivora*, *P. gonapodyides* and *P.*

pseudosyringae from declining *B. celtiberica* stands; (f) *P. ×cambivora* from *S. caprea* and *P. pinaster* with dieback; and (g) *P. chlamydospora* from *O. foetens* and *P. indica* in an Atlantic laurisilva forest on the island of Madeira. Overall, 15 and 21 new *Phytophthora*-associated decline and dieback diseases of trees were recorded for the first time in Europe and Portugal, respectively (Table S5).

Following the first reports of *P. ×alni* from bark cankers of declining planted *Alnus glutinosa* trees on the banks of the Rio Tâmega in Chaves (Jung et al., 2016) and declining mature *A. lusitanica* trees along a small stream in central Portugal (Kanoun-Boulé et al., 2016), bleeding bark cankers, decline and dieback of riparian *A. lusitanica* trees were commonly observed during this study along several rivers and streams in northern and central Portugal. At the Alva, Côa, Fervença and Tâmega Rivers where mortality rates along some river sections exceeded 50%, the disease was caused by the aggressive alder-specific hybrid pathogen *P. ×alni* in accordance with the situation in other European countries (Brasier et al., 2004; Černý & Strnadová, 2010; Corcobado et al., 2023; Gomes Marques et al., 2024; Haque et al., 2015; Jung & Blaschke, 2004; Jung, Pérez-Sierra, et al., 2018; Nagy et al., 2003; Schumacher et al., 2006; Solla et al., 2010; Thoirain et al., 2007; Trzewik et al., 2015). Our findings of *P. cinnamomi* and *P. gonapodyides* in the rhizosphere of declining *A. lusitanica* trees along several streams with scattered alder dieback confirm earlier reports of these pathogens from alders in northern Spain, Germany and Austria (Corcobado et al., 2023; Jung et al., 2016; Jung & Blaschke, 2004). In southern Portugal, *Phytophthora*-related

dieback of riparian alder forests was not observed, which is in accordance with a recent model that predicts absence of *P.* ×*alni* in this region due to summer temperatures exceeding the maximum temperature of the pathogen (Brasier et al., 2004; Gomes Marques et al., 2024; Jung, Horta Jung, Scanu, et al., 2017). In a recent survey in five riparian alder stands in northern Portugal, *P. cinnamomi, P. gonapodyides* and nine other *Phytophthora* species, including three species not found in our survey (*P. asparagi, P. polonica* and *P. rosacearum*) were found in the rhizosphere of declining alder trees whereas *P. multivora* and *P. plurivora* were isolated from bark cankers at two sites (Bregant et al., 2023a). *P. plurivora* was isolated earlier from bark cankers of *A. glutinosa* in Germany (Jung & Blaschke, 2004; Jung & Burgess, 2009) and was also found widespread in the rhizosphere of riparian alder stands in Austria, Germany, Poland, Slovakia and Turkey (Aday Kaya et al., 2018; Corcobado et al., 2023; Jung & Blaschke, 2004; Tkaczyk et al., 2023; Trzewik et al., 2015). Noteworthy, *P. uniformis*, paternal parent of *P.* ×*alni* and contributing causal agent of the Europe-wide alder epidemic (Brasier et al., 2004; Corcobado et al., 2023; Husson et al., 2015; Jung & Blaschke, 2004; Jung, Pérez-Sierra, et al., 2018; Macháčová et al., 2024), was not detected in this or any previous survey in Portugal. Its absence is most probably related to the low cardinal temperatures of *P. uniformis* as an adaptation to the temperate and sub-boreal climates in its native habitats in the Pacific Northwest and Alaska (Aguayo et al., 2013; Černý et al., 2012; Jung, Horta Jung, Scanu, et al., 2017; Jung, Pérez-Sierra, et al., 2018; Redondo et al., 2015; Schumacher et al., 2006; Sims et al., 2015), rendering the pathogen unsuited for the high summer temperatures in Portugal.

Overall, a diverse community of 20 *Phytophthora* taxa has been unveiled in rhizosphere soil and bark cankers of forest stands in Portugal by this study (17 taxa) and Bregant et al. (2023a, 2023b, nine species). Using similar or identical isolation methods, slightly lower *Phytophthora* diversities were found in various surveys in Spain (13 species; Brasier et al., 1993; Corcobado et al., 2010; Pérez-Sierra et al., 2013; Sanchez et al., 2002; Solla et al., 2010; Štraus et al., 2023; Vettraino et al., 2005), Austria (14 species; Balci & Halmschlager, 2003; Corcobado et al., 2020, 2023), France (17 taxa; Hansen & Delatour, 1999; Husson et al., 2015; Jung et al., 2002, 2003; Robin et al., 1998, 2011; Schenck et al., 2018; Vettraino et al., 2005) and Germany (17 taxa; Jung, 2009; Jung & Blaschke, 2004; Jung & Nechwatal, 2008; Jung et al., 2000, 2002, 2003; Peters et al., 2019; Schumacher et al., 2006). The European country with the highest *Phytophthora* diversity in forest stands, however, is Italy, including the islands of Sardinia and Sicily, where 38 *Phytophthora* species were isolated from rhizosphere soil, bark cankers, leaf and twig blights in numerous surveys (Bregant et al., 2020; Cacciola et al., 2005; Ginetti et al., 2014; Jung et al., 2019, 2024; Jung, Horta Jung, Cacciola, et al., 2017; Jung, Pérez-Sierra, et al., 2018; Riolo et al., 2020; Scanu et al., 2015; Scanu, Hunter, et al., 2014; Scanu, Linaldeddu, et al., 2014; Seddaiu et al., 2020; Vettraino et al., 2002, 2005).

Rivers and streams in Asia, Australia, Europe, the Americas and South Africa were shown to be inhabited by a wide range of

Phytophthora species (Caballol et al., 2024; Català et al., 2015; Corcobado et al., 2023; Dunstan et al., 2016; Huai et al., 2013; Hüberli et al., 2013; Jung et al., 2019, 2020, 2022, 2024; Jung, Chang, Bakonyi, et al., 2017; Jung, Durán, et al., 2018; Oh et al., 2013; Reeser et al., 2011; Shrestha et al., 2013). In our survey in Portugal, 34 *Phytophthora* taxa were detected in 33 of the 34 rivers and forest streams (97.1%) tested. These comprised 18 non-hybrid and 16 hybrid taxa, among them two and eight taxa, respectively, previously unknown to science. As in other river surveys listed before, *P. cinnamomi* was not recovered from any Portuguese stream, emphasizing its strictly soilborne lifestyle. Because surface water is commonly used for irrigation of nursery beds and horticultural plantations, the presence of the notorious pathogens *P. plurivora* and *P.* ×*cambivora* in 29.4% and 14.7% of the streams, the detection of *P. multivora* and *P. ramorum* in two and one stream, respectively, and the widespread occurrence of 10 new taxa with unknown host ranges are of particular concern. Bregant et al. (2023a) reported in total six *Phytophthora* species from five streams in central Portugal including *P. multivora*, four Clade 6 species and the Clade 9 species *P. polonica*, which was not found in the present study. The widespread occurrence of a diverse community of 16 hybrid taxa from Clades 2, 6, 8 and 9 in our stream survey confirms earlier studies showing that aquatic ecosystems provide ideal conditions for interspecific *Phytophthora* hybridizations and the subsequent survival of the hybrids (Burgess, 2015; Jung et al., 2020, 2024; Jung, Chang, Bakonyi, et al., 2017; Jung, Durán, et al., 2018; Nagel et al., 2013; Oh et al., 2013). It is becoming increasingly clear that in the genus *Phytophthora*, interspecific hybridizations are a common evolutionary feature facilitating the adaptation to new environmental conditions and new host species (Brasier et al., 2004; Burgess, 2015; Jung et al., 2020, 2024; Jung, Chang, Bakonyi, et al., 2017; Jung, Horta Jung, Scanu, et al., 2017; Van Poucke et al., 2021). The use of infested surface water for irrigation of nurseries and agricultural, horticultural and forest plantings poses, therefore, an inherent risk of creating new *Phytophthora*–host combinations and potentially new epidemics. The occurrence of the Clade 8 hybrids *P.* taxon *pseudocryptogea* × *kelmanii* and *P.* taxon ×*pseudocryptogea*-like in several streams and in one nursery each might suggest an introduction to the nurseries with irrigation water taken from infested rivers.

An extensive study in 23 European countries revealed the presence of (a) 49 *Phytophthora* taxa in 81% of the almost 2000 beds and container stands in 92% of the 732 nurseries tested and (b) 56 *Phytophthora* taxa in 66% of the more than 2500 planting sites tested, clearly demonstrating that the planting of infested nursery stock is the primary pathway of exotic *Phytophthora* pathogens to the wider environment (Jung et al., 2016). The efficacy and detrimental effects of *Phytophthora* spread to natural ecosystems via the nursery pathway were also demonstrated for restoration outplantings in heathlands and woodlands in California (Frankel et al., 2020; Garbelotto et al., 2018; Rooney-Latham et al., 2019; Sims & Garbelotto, 2021). Recently, using both baiting and metabarcoding approaches, 12 and 45 *Phytophthora* taxa, respectively, were reported from soil and water samples in eight Czech nurseries, among

them 15 taxa previously unknown to science (Bačová et al., 2024). In the present study, 16 *Phytophthora* taxa were detected in 12 of the 13 nurseries, which is comparable to the diversity reported for Spanish nurseries by Moralejo et al. (2009; 17 *Phytophthora* taxa) and Mora-Sala et al. (2022; 14 *Phytophthora* taxa). Besides the high infestation rates with the aggressive wide-host range pathogens *P. cinnamomi*, *P. pseudocryptogea* and *P. ×cambivora*, the findings of two other aggressive pathogens with wide-host ranges, namely *P. multivora* and *P. plurivora*, and the host-specific pathogens *P. alticola*, *P. quercetorum* and *P. quercina*, are particularly concerning. The oak-specific fine root pathogen *P. quercetorum* is involved in temperate oak decline in the eastern United States (Balci et al., 2008) and, hence, its spread with infested nursery plants to forests could exacerbate the decline of oak forests in Portugal. *P. alticola* was described from eucalypt plantations in South Africa (Maseko et al., 2007) but its status has been doubtful due to the loss of all isolates associated with the original description except one ex-paratype; discrepancies between sequence data and morphology of the single remaining isolate and the original species description; and inconclusiveness of the holotype material of *P. alticola* (Simamora et al., 2015). Based on a thorough morphological and phylogenetic study of the only existing ex-paratype isolate and a collection of new isolates from plantations and forests across South Africa, Bose et al. (2017) demonstrated that *P. alticola* and the Western Australian eucalypt pathogen *P. boodjera* (Simamora et al., 2015) are distinct, though very closely related, sister species and provided an emended description for *P. alticola*. During a survey conducted between 2012 and 2020 in numerous *Eucalyptus globulus* plantations across Portugal, crown symptoms indicative of root and collar rot were observed and, besides *P. cinnamomi*, *P. alticola* was obtained from 10 plantations (Diogo et al., 2023). Our results indicate that *P. alticola* might have been introduced to the eucalypt plantations with infested nursery plants. In another study of eucalypt plantations in Portugal, Bregant et al. (2023b) reported *P. hibernalis*, *P. multivora* and *P. niederhauserii* causing root rot and bleeding cankers on *E. globulus*. The aggressive eucalypt pathogens *P. alticola*, *P. cinnamomi*, *P. hibernalis*, *P. multivora* and *P. niederhauserii* could pose a serious threat to the Portuguese paper and pulp industry, which is primarily based on timber produced on 845,000 ha of domestic eucalypt plantations (ICNF, 2019).

In Portuguese forest nurseries, both infestation rate (88%) and *Phytophthora* diversity (16 taxa) were higher in symptomatic plants, but 26% of samples from visually healthy plants also yielded a total of five *Phytophthora* species. Furthermore, in container stands with *Phytophthora* isolations from symptomatic plants, many healthy-looking plants were almost certainly also infested with *Phytophthora*, as has been demonstrated by Jung et al. (2016) and Migliorini et al. (2015). The almost ubiquitous infestation of forest and other nurseries in Portugal and elsewhere in Europe with almost 100 known and unknown *Phytophthora* taxa will inevitably cause further spread of these pathogens to the wider environment, increasing the pathogen pressure on forest ecosystems and thus weakening their resilience to climatic extremes and decreasing the sustainability of their multiple functions to society.

This is particularly concerning as the newly approved EU regulation 2024/1991 (Nature Restoration Law; Anonymous, 2024), aiming to ensure the recovery of biodiverse and resilient nature across the EU territory, obliges the member states to perform extensive measures on nature restoration including re-establishing numerous listed habitat types in areas where those habitat types do not occur and planting of at least three billion additional trees by 2030 at Union level. Consequently, to prevent rapid spread of aggressive invasive *Phytophthora* and other plant pathogens to highly sensitive natural areas across Europe, it is paramount at the EU level to avoid *Phytophthora* infestations of nursery stock by immediately implementing an array of mandatory management measures encompassing: (a) the use of thermosterilized substrates and disinfested containers/pots and tools; (b) growing plants from seeds or, in case this is not possible, the use of non-infested plant material (confirmed by molecular tests rather than visual inspections); (c) lifting container stands at least 80 cm from the ground with well-drained surfaces underneath to avoid splash infections from the ground; and (d) irrigation with tap water and water from deep wells or, where it cannot be avoided, surface and recirculation water only after filtering with sand, lava grain or pumice filter systems or disinfestation with heat, ultrasonic, chlorination or ozone (Parke & Grünwald, 2012; Pérez-Sierra & Jung, 2013; Stewart-Wade, 2011; Ufer et al., 2008). In addition, the use of fungicides and fungistatic chemicals like potassium phosphite should be avoided as much as possible because their regular use causes the rapid evolution of resistant strains and the suppression of disease symptoms, thus resulting in the spread of visually healthy *Phytophthora*-infested plants (Jung et al., 2016; Migliorini et al., 2015; Pérez-Sierra & Jung, 2013). The Nursery Industry Accreditation Scheme Australia (NIASA; https://nurseryproductionfms.com.au/niasa-accreditation/) and the Avocado Nursery Voluntary Accreditation Scheme (ANVAS; https://avocado.org.au/our-programs/anvas/) in Australia are excellent examples of how successful accreditation schemes can be (Anonymous, 2018, 2021).

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

For all oomycete taxa found in this study, ITS and *cox1* sequences from representative isolates are available from GenBank at https://www.ncbi.nlm.nih.gov/genbank/, and accession numbers are given in Table S1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.