Phytophthora polonica, a new species isolated from declining *Alnus glutinosa* stands in Poland

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Abstract

In a survey of *Phytophthora* associated with alder decline in Poland, several isolates of a homothallic *Phytophthora* sp., which could not be assigned to other taxa including *Phytophthora alni* subspecies, were consistently recovered from rhizosphere soil samples. Their morphology and pathogenicity, as well as sequence data for three nuclear regions (internal transcribed spacer rDNA, elongation factor-1α and β-tubulin) and a coding mitochondrial DNA region (*nadh1*), were examined. The new *Phytophthora* species is characterized by the moderate to slow growth rate of its colony in carrot agar at 20 °C, high optimal (*c.* 30 °C) and maximum (*c.* 38 °C) growth temperatures, formation of catenulate, often lateral, hyphal swellings, large chlamydospores in agar media and in soil extract, persistent, ovoid to ellipsoid nonpapillate sporangia and large oogonia with paragynous and sometimes amphigynous antheridia. *Phytophthora polonica* was slightly pathogenic to alder twigs and not pathogenic to trunks of several tree species. In a phylogenetic analysis using either Bayesian inference or maximum likelihood methods, *P. polonica* falls in clade 8 'sensu Kroon *et al.* (2004)' of *Phytophthora*.

Introduction

Species within the genus *Phytophthora* are well-known plant pathogens causing important diseases in agriculture, arboriculture and natural ecosystems (Erwin & Ribeiro, 1996). Although traditionally studied by mycologists, *Phytophthora* spp. are unrelated to the true fungi (*Eumycota*), and nowadays are classified in a distant phylogenetic position within the diploid, algae-like Oomycetes in the *Straminipila* lineage of the *Eukaryota* domain (Cavalier-Smith, 1986; Hawksworth *et al.*, 1995). The genus represents over 70 species, most of the discoveries in the past 10 years including notable disease agents of ornamentals (e.g. Cacciola *et al.*, 1996; Ilieva *et al.*, 1998; Werres *et al.*, 2001; De Cock & Lévesque, 2004) and forest trees (e.g. Jung *et al.*, 1999, 2003; Werres *et al.*, 2001; Brasier *et al.*, 2003, 2004, 2005; Hansen *et al.*, 2003). Interest in surveying for *Phytophthora* in natural ecosystems has increased after awareness of the implication of several *Phytophthora* spp. in some extensive cases of forest decline and tree mortality in Europe (Brasier *et al.*, 1993, 2004; Jung *et al.*, 2000), sudden oak death in America (Rizzo *et al.*, 2002) and 'Jarrah dieback' in Australia (Weste & Marks, 1987).

Recent molecular analysis (Crawford *et al.*, 1996; Cooke *et al.*, 2000; Martin & Tooley, 2003) has largely improved our understanding of the phylogenetic relationships between *Phytophthora* species. These studies have been, however, hampered by the use of sequence information on single DNA regions and by the limited subset of *Phytophthora* species covered. Cooke *et al.* (2000), on the basis of neighbour-joining analysis of internal transcribed spacer (ITS) data, proposed that *Phytophthora* is paraphyletic, with the main cluster consisting of eight clades and two additional closely related ones comprising *Phytophthora macrochlamydospora*, *Phytophthora richardiae* ('clade 9') and *Phytophthora insolita* ('clade 10'). Such a tree topology has also been noticed by Kroon *et al.* (2004) in a separate analysis of mitochondrial DNA. However, in the same study, the nuclear DNA and the combined nuclear and mitochondrial data set analysis showed that *P. insolita* and *P. richardiae* were situated within the main *Phytophthora* clade. The use of a significantly larger data set has resulted in a robust phylogeny, largely supporting those of Cooke *et al.* (2000) and Martin & Tooley (2003).

Since 1993, a new disease of alder trees (*Alnus glutinosa*) has been spreading across Europe (Brasier *et al.*, 2004). The causal agent is an incipient species probably resulting from hybridization between *Phytophthora cambivora* and an unknown *Phytophthora* closely related to *Phytophthora fragariae* (Brasier *et al.*, 2004). Its taxonomic status has been resolved by erecting the species *Phytophthora alni*, with several subspecies differing mainly in their karyotype, gametangial morphology and aggressiveness towards alder (Brasier *et al.*, 2004).

In the framework of the present research on *Phytophthora* spp. associated with declining alder stands in Poland, an unidentified *Phytophthora*, superficially resembling *P. insolita*, was isolated from the soil samples of several investigated stands. This paper describes this species as *Phytophthora polonica* sp. nov. and provides details of its morphology, physiology and pathogenicity towards alder and *Quercus* spp. Molecular evidence is used to sustain its specific status. A full analysis of several nuclear and mitochondrial genetic markers elucidates its phylogenetic position.

Materials and methods

Isolation

A wide survey and identification, by ITS sequencing, of numerous *Phytophthora* and *Pythium* isolates from different regions in Poland resulted in the identification of an undetermined *Phytophthora* (L. Belbahri, T. Oszako & F. Lefort, unpublished data). Fourteen isolates of this *Phytophthora* species were collected from soil samples associated with declining alder stands in the towns of Kolo, Adamowizna and Siestrzen by plating pieces of rhododendron leaves used as baits on PARP or PARPNH, selective media for *Phytophthora* (Erwin & Ribeiro, 1996). Occasionally, oak (*Quercus robur*) decline symptoms were also observed in the vicinity of the alder stands. Stock cultures were maintained in carrot agar (CA; Brasier, 1967). The isolates UASWS0197 and UASWS0198 have been deposited at the CBS (Centraalbureau voor Schimmelcultures). All others (UASWS0199, UASWS0205, UASWS0207, UASWS0209, UASWS0210, UASWS0211, UASWS0230, UASWS0231, UASWS0232, UASWS0233, UASWS0234 and UASWS0235) are maintained in the authors' culture collection at the University of Applied Sciences of Western Switzerland. Only the isolates from Kolo are described here because this undetermined *Phytophthora* sp. was particularly represented.

Morphology and physiology

Stock cultures were initiated from single zoospores. Isolates were grown at 20 °C in the dark in 90 mm diameter Petri dishes on cornmeal agar (CMA), CA, malt extract agar (MEA) and potato dextrose agar (PDA), and colony morphologies were examined after 7 days. Colony radial growth rates in 90 mm Petri dishes on CA were measured at 5 °C intervals between 5 and 40 °C, with two replicates for each temperature and isolate combination. Two points on the colony margin, sited at right angles from the centre of a colony grown for 4 days at 20 °C, were marked below the Petri dish with a grease pencil. Radial growth was measured after 48 h exposure at each temperature tested, and the daily radial-growth rate was then calculated.

The presence of sporangia, hyphal swellings and chlamydospores was checked in all media during 2 weeks. In a failed attempt to induce sporangial formation, three 12 mm diameter mycelial plugs taken from the edge of a 5-day-old colony grown on CA at 20 °C were placed into a 60 mm diameter Petri dish previously flooded with 5 mL of a soil extract (Moralejo *et al.*, 2005). The dishes were kept for 48–72 h at 20 °C either under continuous white light or in darkness. Abundant hyphal swellings and chlamydospores were formed, but no sporangia. In a second attempt we assessed the requirement of nutrients (e.g. amino acids) in solution for the development of sporangia. A sparse sporangial crop was obtained by flooding mycelial plugs with 5 mL of a gelatine solution (10 g of commercial gelatine dissolved in 1 L of distilled water and autoclaved at 121 °C for 15 min). The plates were incubated for 24 h at 20 °C. Subsequently, the gelatine solution was decanted and replaced with tap water, and the dishes were incubated at 25 °C for 48 h in darkness. Zoospore suspensions were obtained by chilling the plates at 7 °C for 1 h, and then incubating at room temperature for 30 min. For the production of single zoospore isolates, 0.1 mL of the zoospore suspension was evenly spread on water agar in a 90 mm diameter Petri dish. The plates were checked for zoospore cyst germination under a dissecting microscope. After 24 h at 20 °C in darkness, single germinated cysts were lifted with a sterile needle and plated individually on CA.

The development of oogonia, antheridia and oospores on CA at 20 °C was examined during 10 days. The above structures as well as chlamydospores and hyphal swellings were lifted with a needle from cultures grown on CA or soil extract, mounted in distilled water and examined at × 400 magnification. About 20 samples of each fungal structure were chosen at random and measured using a calibrated eyepiece under an Olympus BX50F-3 compound microscope equipped with differential interference contrast (DIC) optics. An Olympus DP 12 digital camera adapted to the microscope was used for photography.

DNA extraction, PCR amplification, PCR products purification and sequencing

Mycelial DNA was purified from pure cultures grown in pea broth (Kroon *et al.*, 2004) and checked for quality with a NanoDrop NT-100 UV spectrophotometer. DNA

amplifications were performed for three nuclear and one mitochondrial loci. Ribosomal DNA ITS amplifications were carried out using the previously described universal primers ITS4 and ITS6 that target conserved regions in the 18S and 28S rDNA genes (White *et al.*, 1990; Cooke *et al.*, 2000). Amplifications for the translation elongation factor 1α gene (*EF*-1α), the β-tubulin (β-*tub*) gene and the NADH dehydrogenase subunit 1 gene (*nadh*1) were performed according to Kroon *et al.* (2004) using primers ELONGF1 and ELONGR1 for the *EF*-1α gene, TUBUF2 and TUBUR1 for the β-*tub* gene, and NADHF1 and NADHR1 for the *nadh*1 gene. PCR products were purified with a Minelute PCR Purification Kit (Qiagen, Switzerland) and quantity and quality were checked as reported above. Amplicons were sequenced directly in both sense and antisense directions. ITS amplicons from 14 isolates were sequenced twice and a consensus sequence was created from the duplicates. ITS sequences were registered in GenBank under accession numbers DQ396409 (UASWS0197), DQ396410 (UASWS0198), DQ396411 (UASWS0199), DQ396417 (UASWS0205), DQ396419 (UASWS0207), DQ396421 (UASWS0209), DQ396422 (UASWS0210), DQ396423 (UASWS0211), DQ500127 (UASWS0230), DQ500128 (UASWS0231), DQ500129 (UASWS0232), DQ500130 (UASWS0233), DQ500131 (UASWS0234) and DQ500132 (UASWS0235). The sequences for the genes β-*tub*, *nadh*1*b* and *EF*-1α were obtained for the isolates UASWS0197 and UASWS0198 and are registered under accession numbers DQ399843, DQ399844, DQ399845, DQ399846, DQ399847, DQ399848, DQ399849, DQ399850 and DQ399851, respectively.

Molecular phylogeny

Sequence data for three nuclear regions (ITS rDNA, EF-1α and β-tub) and a coding mtDNA region (*nadh*1) were compared with those of *Phytophthora* spp. listed in Kroon *et al.* (2004) and for *P. polonica* isolates UASWS0197 and UASWS0198. We conducted separate phylogenetic analyses for individual genes, for combined nDNA sequences and for combined mtDNA+nDNA sequences after a method already reported (Belbahri *et al.*, 2005; Paul *et al.*, 2006). Sequences were aligned manually using Seaview (Galtier *et al.*, 1996). The maximum likelihood (ML) trees were obtained using the PhyML program (Guindon & Gascuel, 2003), with the HKY (Hasegawa *et al.*, 1985) model allowing transitions and transversions to have potentially different rates and the general time reversible (GTR) model allowing all rates to be different (Lanave *et al.*, 1984; Rodriguez *et al.*, 1990). In order to correct the among-site rate variations, the proportion of invariable sites (*I*) and the parameter of *g* distribution (*G*), with eight rate categories, were estimated by the program and taken into account in all analyses.

Nonparametric ML bootstraps (BSs) (with 100 replicates) were calculated using PhyML. Bayesian inferences (BIs) were obtained with MrBayes v.3.0 (Huelsenbeck & Ronquist, 2001) using the same models of DNA evolution as for the ML analyses. The program was run for 2 000 000 generations, sampled every 100 generations, with four simultaneous chains. The trees, sampled before the chains reached stationarity, were discarded. Neighbour-joining plot and Treeview were used to view ML and Bayesian trees, respectively.

Pathogenicity

In a first trial, the pathogenicity of UASWS0197 and UASWS0198 was determined by wound-inoculating twigs of *A. glutinosa c.* 10 cm below the apex. A 4 mm long sliver of bark was lifted with a scalpel and a mycelial plug *c.* 4 mm^2 from a 7-day-old colony was inserted beneath. For controls, a sterile CA plug was used. A moist cotton plug was placed on the wound and sealed with parafilm. Five twigs were used for each isolate, one of them as control. They were placed in sets of five in 250 mL Erlenmeyer flasks filled with 200 mL DW, closed with cotton plugs and incubated at 20 °C for 10 days. The full length of the discoloured tissue was measured in millimetres. A small piece of discoloured tissue was plated on PARP medium for reisolation of the fungus. The identity of the emerged colonies was morphologically examined.

In the second pathogenicity test carried out in winter, we assessed the capacity of UASWS0198 for infecting trunks of other tree species. Taking advantage of material available at one of the labs in Spain (IMEDEA), the inner bark of logs of four tree species from NE Spain, i.e. *Quercus canariensis*, *Quercus faginea*, *Quercus suber* and *Fraxinus angustifolia*, was wound inoculated using the log inoculation method of Brasier & Kirk (2001). As positive controls, we used isolates of *Phytophthora ramorum* and one of *Phytophthora cinnamomi*, two well-known pathogens of oak. Eight freshly cut logs (1 m length × 20 cm diameter) of each species were inoculated with mycelial plugs. Plain agar plugs were inserted as controls. The logs were sealed with plastic sheet and incubated at 20 °C. Lesion formation was examined by carefully shaving the outer bark 40 days after inoculating. The outline of the necrotic lesions was traced on a transparent paper with a pen. The image was scanned and the area was calculated using Olympus 12P software. Differences in aggressiveness among isolates were analysed with Fisher's least significant difference test of a one-way analysis of variance (ANOVA) design using the GLM, with isolates as categorical predictors. Lesion area data were log transformed to meet the ANOVA assumption of homogeneity of variance (Levene's test).

Results

Taxonomy

Phytophthora polonica Belbahri L, Moralejo E & Lefort F. sp. nov.

Etymology

'*polonica*' refers to the country where it was isolated.

Description

Coloniae in agaro Dauci carotae 'carrot agar' moderate crescentes, leviter rosaceae, adpressae. Hyphae principales 6–8 μm latae. Hyphae fumescentes in agaro et in extracto aquatico ex humo, hyphae inflatae irregulares, laterales, saepe catenulatae, interdum aggregatae. Chlamydosporae laterales vel terminales abundantes supra bracchiis curtis, 48.4 μm diam, subglobosae vel globosae, levies. Sporangia absentia in agaro et in extracto aquatico ex humo; in medio gelatinoso diluto, sed praesentes, non-papillosa, ovoidea vel ellipsoidea, 52–67 × 32–44 μm, porus plus quam 10 μm latus. Homothallica. Oogonia globosa, levia, 41.8 μm diam. Oosporae typice apleroticae, 38.1 μm latae, parietes moderate crassae. Antheridia solitaria, clavata vel doliiforma vel globosa, plerumque diclina et paragyna, aliquando amphygina, interdum cum productionis hyphalis.

Typus

Poland: Kolo, isol. ex solo et rhizosphaera *Alni glutinosae*, July 2004, *T. Oszako*, (*cultura sicca in agaro Dauci carotae in herbario Universitatis Helvaticae Occidentalis conservatus*) – holotypus; UASWS0198 = CBS 119650 ex type-culture.

Ecology

Phytophthora polonica was recovered from the soil of an alder stand in Poland during the summer of 2004.

Morphological description

Main hyphae up to 8 μm wide. *Colony pattern*: aerial mycelium on CA appressed to limited, slightly stellate to rosaceous; concentric growth rings somewhat noticeable on the underside of the Petri dish, colony on CMA submerged and somewhat radiate, on MEA aerial mycelium appressed, fairly felty, markedly rosaceous, on PDA felty and broadly lobed, rosaceous.

Hyphal swellings

Readily and abundantly formed, usually large (up to *c.* 50 μm long), single or more frequently catenulate, intercalary or lateral, or aggregated, inflated, toruloid, irregularly shaped to globose (Figs 1a and b). Found either in agar or in soil water extract.

Chlamydospores

Abundant within 10 days on CA, CMA and in soil extract; spherical to subglobose or pyriform, average diameter 48.4 μm (ranging from 16 to 69 μm), moderately thin walled (1–2 μm), intercalary, lateral or terminal on short branches. In water, forming extensive networks of geniculate hyphae with lateral chlamydospores at the joints (Figs 1c and d).

Sporangia

Not observed on any culture media. None formed in water soil extract and only a few in gelatine solution. Borne on long nonbranching sporangiophores, mostly ovoid to ellipsoid, *c.* 52–67 × 32–44 μm, noncaducous, nonpapillate, proliferating internally, often nested or catenulate. Zoospores discharged through an exit pore 10–18 μm wide (Figs 1e and f).

Oogonia

Abundant in single-zoospore isolates on CA after 1 week. Mostly borne on stalked branches, spherical to subglobose, smooth- and thin-walled, 41.8 ± 2.8 SD μm diameter (Figs 2a–d).

Oospores

From aplerotic to nearly filling the oogonia, 38.1 ± 2.5 μm diameter, moderately thick walled (average 2.9 ± 0.8 μm) (Figs 2c and d). A high proportion of aborted oogonia were seen.

Antheridia

Mostly clavate to irregularly shaped, less frequently spherical to barrel shaped (Fig. 2a), 16.2 ± 2.8 μm long × 13 ± 2.1 μm wide; borne on long stalks, mostly attached near the oogonial base (Figs 2b–d), occasionally with hyphal extensions (Fig. 2d). Predominantly paragynous (Figs 2b–d) but sometimes amphigynous (Fig. 2a).

Colony growth rates

Moderately slow (Fig. 3) on CA and CMA at 20 °C and slow on PDA and MEA. Optimum temperature *c.* 30 °C, minimum *c.* 5 °C and maximum *c.* 38 °C. In cultures on CA at 20 °C contaminated by bacteria, growth was stimulated on the side facing the bacterial colonies.

Sequence analysis and phylogenetic position of *P. polonica*

The rRNA gene ITS sequences of eight *P. polonica* isolates had 100% identity and only 90% identity with their closest match *P. insolita* over an 824 bp sequence run. On the basis of the ITS sequence, *P. polonica* falls within 'clade 10' of Cooke *et al.* (2000), together with *P. insolita*. Based on nuclear DNA analysis, as well as by combining mitochondrial and nuclear data sets, *P. insolita*, *P. richardiae* and

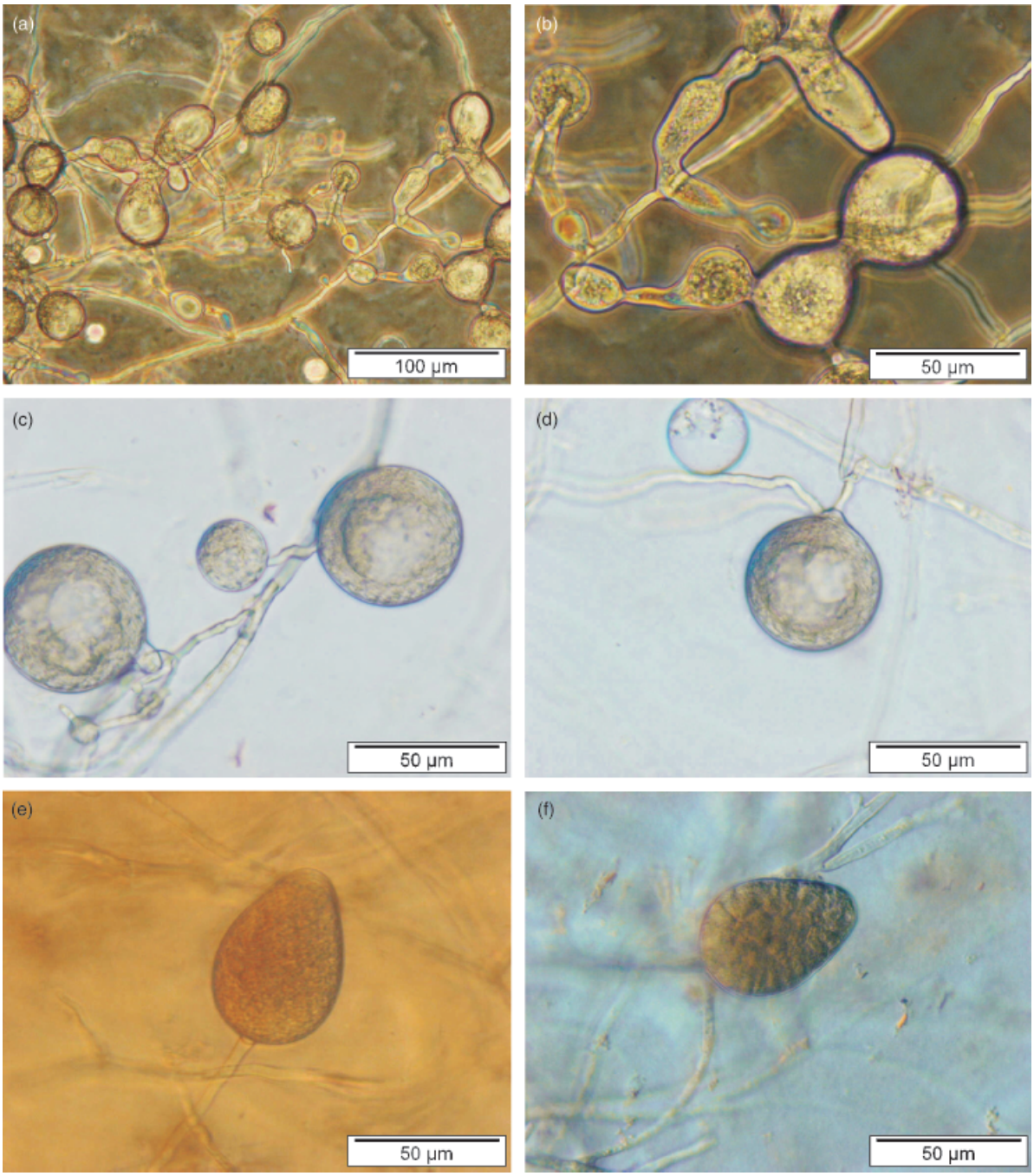

Fig. 1. Asexual structures of *Phytophthora polonica* formed on CA. (a and b) Hyphal swellings (UASWS0198). (c and d) Thin-walled chlamydospores on CMA (UASWS0197). (e and f) Ovoid to ellipsoid sporangia (UASWS0197).

P. polonica are located within the main *Phytophthora* clade (Figs 4 and 5). *Phytophthora polonica* shows a phylogenetic position close to or lying between *Phytophthora quininea*/*P. richardiae* and *P. insolita* within clade 8, as described by Kroon *et al.* (2004).

Pathogenicity

Phytophthora polonica isolates were slightly pathogenic to alder twigs, tissue discolouration progressing only a few millimetres beyond the inoculation wound after 10 days.

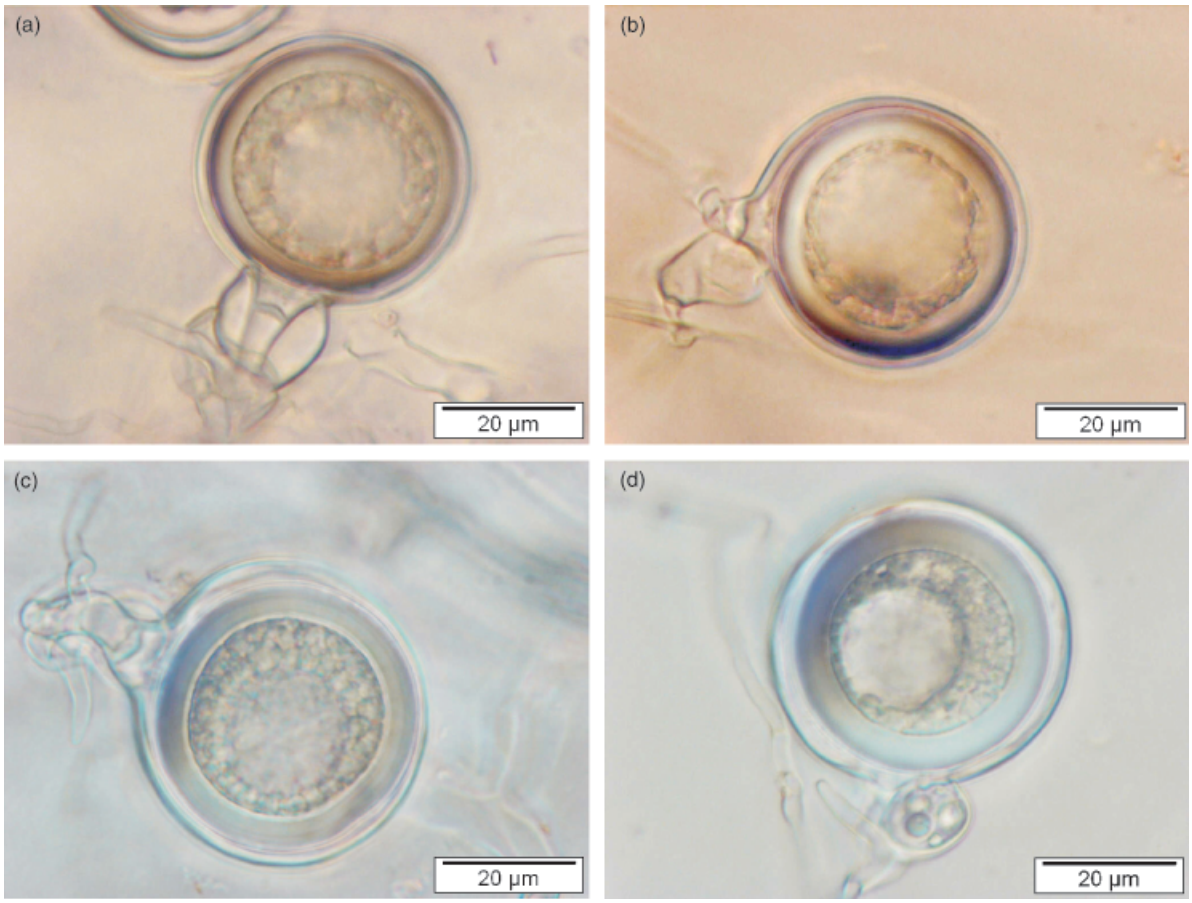

Fig. 2. Gametangia of *Phytophthora polonica* UASWS0198: (a) amphigynous antheridia; (b–d) spherical to subglobose oogonia with paragynous antheridia.

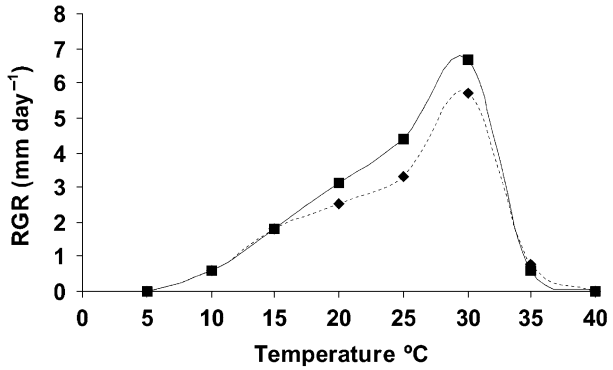

Fig. 3. Temperature and radial growth rate (RGR) relationships of two isolates of *Phytophthora polonica* grown on CA in darkness. Rectangles refer to UASWS 0197 and diamond refers to UASWS0198.

There was no significant difference in pathogenicity between both isolates, and the data are thus here combined. The mean lesion length was 8.5 mm, ranging from 6 to 11 mm. No lesion was formed on controls. The oomycete was reisolated from all inoculated twigs when plated on PARP.

Phytophthora polonica was not pathogenic to the inner bark of *F. angustifolia* (mean lesion area 1.1 ± 0.2 SD cm^2), with necrotic areas not differing ($P = 0.69$) from those of the negative controls, and not pathogenic to slightly pathogenic to the three *Quercus* species. Mostly, the lesions extended very little beyond the point of inoculation on *Q. faginea* (2.3 ± 2.1 cm^2), *Q. suber* (2.8 ± 2.2 cm^2) or *Q. canariensis* (2.8 ± 1.8 cm^2). The maximum lesion area in which the pathogen was recovered on *Q. suber* was 7.8 cm^2. Lesion areas were always less than *c.* 10% of those formed by the positive controls, *P. cinnamomi* and *P. ramorum* (data not shown).

Discussion

Phytophthora polonica sp. nov. exhibited a combination of unique morphological characters and distinctive nuclear and mitochondrial DNA sequences that easily enables distinction from other *Phytophthora* species. It belongs to group V of Waterhouse *et al.*'s (1983) morphological scheme of classification by being homothallic with paragynous antheridia, and bearing nonpapillate sporangia with internal

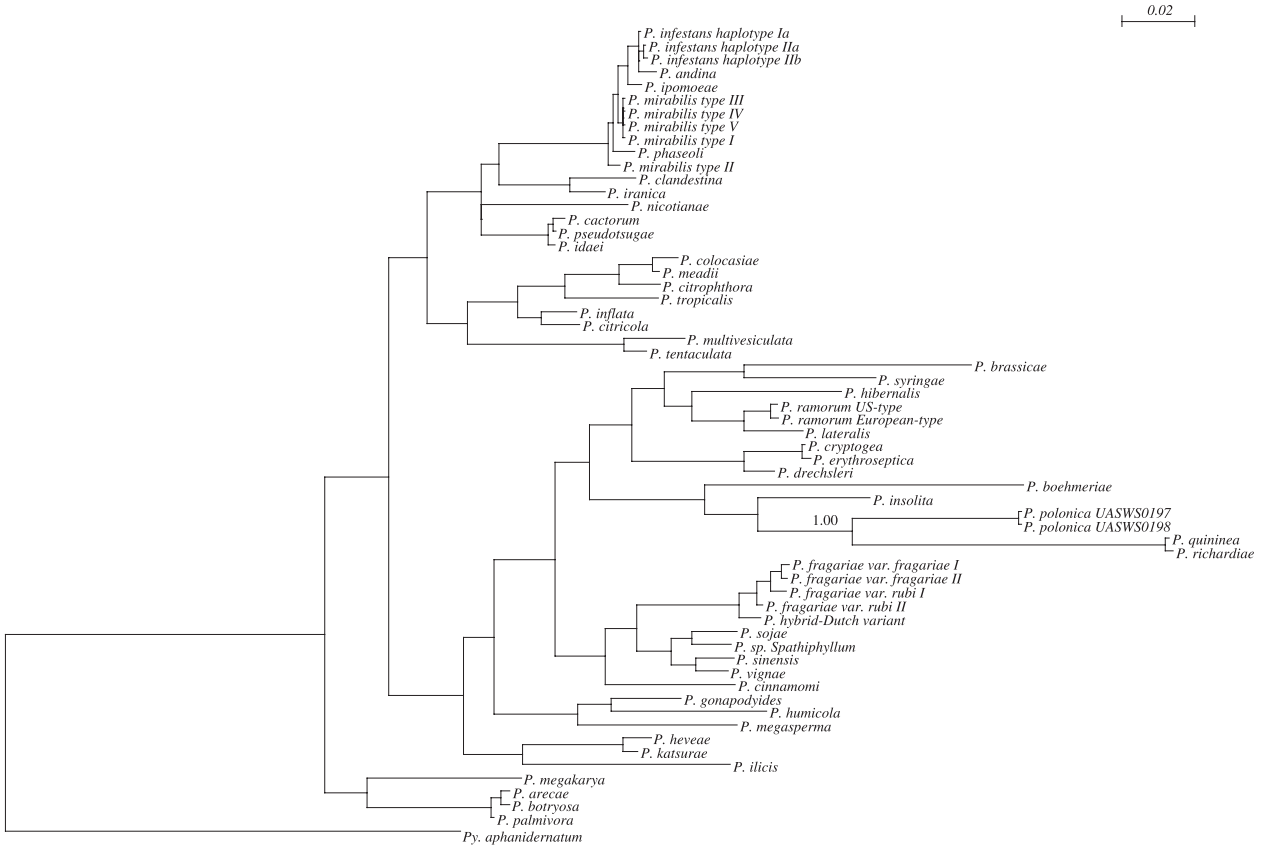

Fig. 4. Phylogenetic position of UASWS0197 and UASWS0198 inferred from nuclear and mitochondrial sequences using the BI method. The numbers at nodes are nonparametric bootstrap values higher than 80%. The length of branches is proportional to the number of substitutions per site as indicated in the scale.

proliferation. Other Phytophthoras within group V include *P. fragariae* var typ., *P. fragariae* var. *rubi*, *P. humicola*, *P. insolita*, *P. medicaginis*, *P. megasperma*, *P. quininea*, *P. sojae* and *P. trifolii*. Unlike *P. polonica*, both varieties of *P. fragariae* as well as species within the '*megasperma* complex' *sensu* Hansen *et al*. (1986) can be readily distinguished by having lower cardinal growth temperatures; *P. humicola* has unusually high optimal temperatures like *P. polonica* but does not form chlamydospores (Ko & Ann, 1985). Of those species included in clade 8 *sensu* Kroon *et al*. (2004), *P. insolita* (Ann & Ko, 1980) superficially resembles *P. polonica* in its cardinal temperatures and colony pattern and in the formation of hyphal swellings and small chlamydospores, but it is easily distinguished by its parthenogenetic oospores, i.e. without attached antheridia (Ho *et al*., 2002); *P. quininea* differs by producing larger oogonia (Crandall, 1947); *P. richardiae* is self-fertile and has a lower maximum growth temperature (Waterhouse, 1970); and *P. macrochlamydospora* does not form sexual structures (Irwin, 1991). It is noteworthy that all these species of clade 8 *sensu* Kroon *et al*. (2004) form survival structures such as hyphal swellings, chlamydospores or oospores in pure culture. By having hyphal swellings and chlamydospores, *P. cinnamomi* and *P. lateralis* could be confused with *P. polonica*; however, the first has higher colony growth rates and the second lower cardinal temperatures. Although occupying the same niche, *P. alni* and its subspecies are distinguished by their almost exclusive amphigynous antheridia and often nonsmooth oogonial walls.

The ecology and pathogenic status of *P. polonica* still remain unclear. Many of its morphological characters are shared with other typical soil-inhabiting, root-infecting as well as riparian *Phytophthora* spp. (Brasier, 1983; Brasier *et al*., 2003). In addition, like most Phytophthoras within clade 6, it exhibits unusually high cardinal temperatures and an optimal growth temperature around 28–30 °C (Brasier *et al*., 2003). This might indicate a physiological adaptation to some other ecological aspect such as litter breakdown, as suggested by Brasier *et al*. (2003). Our observation of colony growth stimulation by bacteria could be situated in this context and would require further studies. Although it has been consistently isolated from soils associated with alder decline, it is not clearly the causal agent: *P. polonica* and *P. alni* were isolated in several cases from the same soil

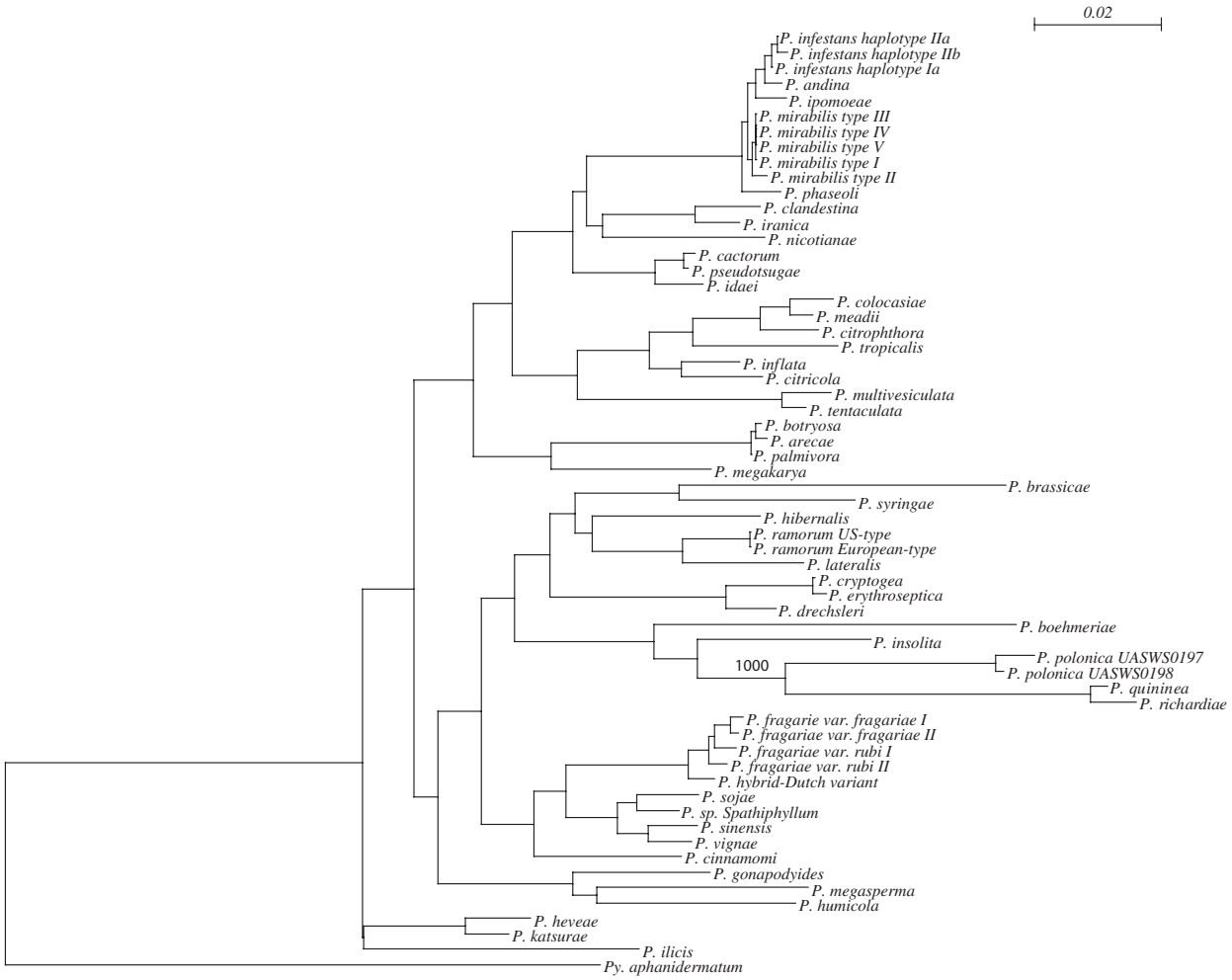

Fig. 5. Phylogenetic position of UASWS0197 and UASWS0198 inferred from nuclear and mitochondrial sequences using the ML method with the GTR+G+I model. The numbers at nodes are nonparametric bootstrap values higher than 80%. The length of branches is proportional to the number of substitutions per site as indicated in the scale.

sample. Despite being pathogenic to fruits in wound inoculations (data not shown), no reports on plant diseases attributable to *P. polonica* have appeared in Poland. On present evidence from pathogenicity tests on alder twigs and on a few Iberian trees, *P. polonica* is apparently a poor inner bark colonizer, although it is acknowledged that further pathogenicity tests on roots of riparian tree species, especially alder, and during different seasons, would be needed to solve this issue.

Phytophthora polonica clustered in a phylogenetic analysis within Cooke's clade 10 and was included in clade 8e according to the clade definition of *Phytophthora* by Kroon *et al.* (2004). It had a high ITS sequence similarity with *P. insolita*, only isolated from irrigated soils in China, Taiwan and California (Ho *et al.*, 2002). Until now, the biodiversity in clade 8 had been considerably underestimated. Recently a new *Phytophthora*, *Phytophthora kernoviae*, alien to the UK, has emerged during recent surveys of *P. ramorum* in the latter country (Brasier *et al.*, 2005). Based on the ITS sequence, it is related to *Phytophthora boehmeriae*, and clusters in Cooke's clade 10. The unusual taxon described here was only recently discovered during extensive *Phytophthora* surveys in Polish forest ecosystems. As such investigations of 'natural ecosystems' are being extended, it is expected that more taxa belonging to clade 8 will be found. We should determine if *P. polonica* exists in other parts of the world before speculating on its origin. Nevertheless, as Polish isolates were obtained from relatively undisturbed and protected forests, *P. polonica* could be indigenous to this area.

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