ELSEVIER

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/mycres

Phytophthora gallica sp. nov., a new species from rhizosphere soil of declining oak and reed stands in France and Germany

Thomas JUNG[a,*], *Jan NECHWATAL*[b]

[a]*Phytophthora Research and Consultancy, Thomastrasse 75, D-83098 Brannenburg, Germany*
[b]*Universität Konstanz, Phytopathology, D-78457 Konstanz, Germany*

ARTICLE INFO

Article history:
Received 18 October 2007
Received in revised form
23 March 2008
Accepted 1 April 2008
Corresponding Editor:
David E. L. Cooke

Keywords:
Fagus sylvatica
Oomycota
Phragmites australis
Phytophthora taxon 'G'
Quercus robur

ABSTRACT

A non-papillate, slow-growing *Phytophthora* species, which could not be assigned to any existing taxon, was isolated from rhizosphere soil of a declining oak in Northeast France, and from the rhizosphere of *Phragmites australis* at Lake Constance in south-west Germany in 1998 and 2004, respectively. We describe this species, previously informally designated *Phytophthora* taxon 'G', as *Phytophthora gallica* sp. nov. Morphology, growth rates, and pathogenicity against cuttings of riparian tree species and leaves of reed are described and compared with those of morphologically and phylogenetically similar *Phytophthora* species. *P. gallica* produces colonies with limited aerial mycelium and variable growth patterns. Gametangia are not formed in single or mixed cultures with tester strains of known mating types. *P. gallica* produces globose and elongated irregular chlamydospores, of which a high proportion is abortive. In water culture irregular hyphal swellings and non-papillate persistent sporangia are formed abundantly. *P. gallica* is moderately aggressive to *Alnus glutinosa* and *Fagus sylvatica*, weakly aggressive to *Quercus robur* and *Salix alba* and non-pathogenic to *Fraxinus excelsior* and *Phragmites australis*. According to ITS and mtDNA sequence data *P. gallica* belongs to a distinct *Phytophthora* clade, with *P. boehmeriae* and *P. kernoviae* being the closest relatives. The origin of *P. gallica* and its ecological role in wet ecosystems remain unclear.

Introduction

Phytophthora is a major genus of plant pathogens within the *Oomycota*, kingdom *Straminipila*. *Phytophthora* species are responsible for some of the most devastating diseases of tree species (Erwin & Ribeiro 1996). Since the early 1990s several projects in Europe were funded by the European Commission and national governments and research councils, investigating the involvement of *Phytophthora* species in the declines and diebacks of oaks, beech, chestnut, and alders (Brasier & Jung 2003, 2006; Jung *et al.* 2000, 2005; Gibbs *et al.* 2003; Vettraino *et al.* 2005; Jung & Blaschke 2004). Systematic searches for *Phytophthora* species were performed in more than a thousand stands in natural and semi-natural ecosystems, and 18 unknown *Phytophthora* taxa were detected of which 12 have been formally described as new species (Brasier & Jung 2003, 2006; Brasier *et al.* 2003a,b, 2004, 2005; Jung *et al.* 1999, 2002, 2003).

In 1998, and 2004, respectively, two isolates of a yet undescribed taxon that was previously informally named

* Corresponding author. Tel.: +49 8034 708386.
E-mail address: competence@tree-diseases.com
0953-7562/$ – see front matter
doi:10.1016/j.mycres.2008.04.007

Phytophthora taxon G (Brasier & Jung 2003, 2006) were isolated from a declining oak stand in France, and from a reed (*Phragmites australis*) stand in south-western Germany that has been reported to be in decline for about five decades (Nechwatal *et al.* 2005). Based on its unique combination of cultural and morphological characters, temperature–growth relationships, and ITS DNA and mtDNA sequence data, we formally describe this taxon as *P. gallica* sp. nov.

Materials and methods

Isolation methods

Soil samples (approximately 1 l) from the rhizosphere of mature declining oak trees in the forest stand, Forêt d'Illwald, in Northeast France (48°13′36″ N, 7°27′22″ E) and from common reed growing in the littoral of Lake Constance in south-western Germany (47°41′48″ N, 9°11′20″ E) were removed to the laboratory, carefully mixed, and isolation tests for *Phytophthora* spp. carried out according to Jung *et al.* (1999, 2000) using *Quercus robur* leaflets as baits.

Voucher material of the isolates of *Phytophthora gallica* sp. nov. has been permanently preserved at the living strain culture collection of the Centraalbureau voor Schimmelcultures (CBS) Utrecht, under accession numbers CBS 111474 and CBS 111475. Additional strains are held at the culture collection (PRC) of the Phytopathology Department of the University of Konstanz, Germany.

Morphology and physiology

Isolates were grown at 20 °C in the dark on carrot agar (CA; 16 g agar, 3 g $CaCO_3$, 100 ml carrot juice, 900 ml distilled water), V8-agar (V8A; 16 g agar, 3 g $CaCO_3$, 100 ml V8 juice, 900 ml distilled water), Sigma, Sigma-Aldrich GmbH, Deisenhofen, Germany, malt-extract agar (MEA) and Sigma corn meal agar (CMA) in 90 mm Petri dishes, and colony morphology recorded after 10 d.

For temperature–growth relationships, four replicate CA plates of two isolates of each *Phytophthora gallica* and *P.* taxon 'PgChlamydo', and one isolate of each *P. gonapodyides* and *P.* taxon 'salixsoil' were incubated for 24 h at 20 °C to stimulate onset of growth, and then transferred to 5, 10, 15, 20, 25, 30, 33, and 35 °C. The growth rate was recorded 5–7 d after the onset of linear growth along two lines intersecting the centre of the inoculum at right angles (Jung *et al.* 1999).

Characterisation and measurements of the morphological structures of the two isolates of *P. gallica* and comparisons with known species (Table 1) were made under the light microscope on CA at ×320 according to Jung *et al.* (2002, 2003). For each isolate dimensions and characteristic features of 50 fully-mature sporangia, the diameters of 50 globose chlamydospores and length and breadth of 50 elongated chlamydospores, and diameters of 25 primary hyphae, chosen at random, were measured.

Sexual compatibility type was tested using 'direct' pairing tests on 9 cm CA plates. Both isolates of *P. gallica* were paired with each other and with A1 and A2 tester isolates (Table 1).

Table 1 – Species and isolates of *Phytophthora* spp. examined

Phytophthora spp.	ITS clade[a]	PRC no.[b]	Other references[b]	Geographical location, year	Isolated from	Source[b]
P. gallica	10	GAL 1	CBS 111474	NE France, 1998	*Quercus robur*[c]	PRC
	10	GAL 2	CBS 111475	Konstanz, Germany, 2004	*Phragmites australis*[c]	UKN
P. gonapodyides	6	GON 3		Freising, Germany, 1994	*Q. robur*[d]	PRC
	6	GON 18		Bad Aibling, Germany, 1998	*Alnus glutinosa*[d]	PRC
P. taxon 'PgChlamydo'[e]	6	CHLA 5		Nursery, Germany, 2001	*A. glutinosa*[c]	PRC
	6	CHLA 7		Nursery, Germany, 2001	*A. glutinosa*[c]	PRC
P. taxon 'salixsoil'[e]	6	SAL 1[f]	UKN 1	Konstanz, Germany, 2003	*P. australis*[c]	UKN
P. cambivora	7	CAM 109		Freising, Germany, 2004	*Fagus sylvatica*[d]	PRC
P. citricola	2	CIT 55[f]	BU 137	Garmisch, Germany, 1997	*F. sylvatica*[c]	UKN
	2	CIT 135		Nursery, Germany, 2002	*F. sylvatica*[d]	PRC
P. alni ssp. *alni*	7	ALN 377		Bamberg, Germany, 2002	*A. glutinosa*[d]	PRC
P. cambivora (A1)	7	CAM A1	CBS 356.78	Belgium, 1978	*Chamaecyparis* sp.	Kamoen O.
P. cambivora (A2)	7	CAM 1[f]		Freising, Germany, 1995	*F. sylvatica*[d]	PRC
P. cinnamomi (A1)	7	CIN A1	CBS 341.72	California, 1972	*Camelia japonica*	Zentmyer G.A.
P. cinnamomi (A2)	7	CIN 8		Dominican Republic, 2002	*Pinus occidentalis*[c]	PRC
P. drechsleri (A2)	8	DRE 3460	TUM 3460	Germany	NK	Zinker-nagel V.
P. cryptogea (A2)	8	CRY 1		Nursery, Germany, 1999	*Q. robur*[c]	PRC

NK, not known.
a ITS clades according to Cooke *et al.* (2000) and http://www.phytophthoradb.org.
b PRC, Phytophthora Research and Consultancy, Thomas Jung, Germany; TUM, Technische Universität München, Germany; UKN, University of Konstanz, Jan Nechwatal, Germany; CBS, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.
c Soil.
d Bark.
e Taxon not yet formally described in the literature; according to ITS sequence analysis related to *P. gonapodyides* (Brasier *et al.* 2003b).
f Isolate also used in the underbark inoculation test.

The plates were incubated at 20 °C in darkness and scored for oogonial formation 21 d after the two colonies had met.

Sequence analysis

In order to determine the phylogenetic position of the new *Phytophthora* species within the genus, sequence analyses of the ITS regions of the rDNA repeats and the cytochrome oxidase II (*cox* II) gene were performed and sequences compared with those of related species obtained from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html). DNA isolation, PCR amplification of ITS1, 5.8S and ITS2, and *cox* II gene regions and sequence editing and aligning were carried out as described previously (Cooke & Duncan 1997; Martin & Tooley 2003; Nechwatal *et al.* 2005). Sequence data were analysed and NJ phylogenetic analyses conducted using the programs DNADIST and NEIGHBOR from the PHYLIP package (v. 3.5, Felsenstein 1993), as described in Cooke *et al.* (2000). A sequence of *Pythium aphanidermatum* (AY598622) and *P. irregulare* (AF196601) were used as outgroup taxa for the ITS and *cox* II analyses, respectively. Tree topology was tested with 1 K BS trials using SEQBOOT and CONSENSE (Felsenstein 1993). Trees were drawn using TreeView (Page 1996).

Pathogenicity toward shoots of tree species

Pathogenicity of *Phytophthora gallica* was tested against abscised shoots of a selection of tree species. Species selected were European beech (*Fagus sylvatica*, host of the closest relative *P. kernoviae*; Brasier *et al.* 2005), pedunculate oak (*Quercus robur*, main tree species at isolation site of strain GAL 1), white willow (*Salix alba*, species abundantly found at isolation site of strain GAL 2), and two other widespread riparian tree species, common alder (*Alnus glutinosa*) and common ash (*Fraxinus excelsior*). Two different inoculation methods were used on abscised shoots of tree species.

Zoospore inoculation. In January, one-year-old dormant shoots (length *ca* 25 cm) were cut from six-year-old nursery-grown plants of *Alnus glutinosa*, and mature trees of *Fraxinus excelsior* and *Salix alba*, sealed in a wet plastic bag, and incubated for 24 h at 10 °C. Agar discs (30 × 20 mm), cut from the growing edge of 5–7-d-old cultures of each of the two isolates of *Phytophthora gallica* and other non-papillate *Phytophthora* species occurring in these wet habitats (namely *P. alni* ssp. *alni*, *P. cambivora*, *P. gonapodyides*, *P.* taxon 'PgChlamydo', and *P.* taxon 'salixsoil', and *P. citricola*) grown on CA at 20 °C in the dark. The discs were flooded with demineralised water and after 24 h placed in 1000 ml Erlenmeyer flasks containing 800 ml demineralised water (one flask per isolate, two discs per flask). After being sealed with parafilm, each flask received 20 cuttings per tree species inserted through a hole in the parafilm and fixed so that the distance between the agar discs at the bottom of the flask and the submerged freshly cut ends of the cuttings was *ca* 2 cm. Sterile agar discs were used as control. After 4 d at 18 °C in natural light the cuttings were removed, and formation of sporangia and release of zoospores on the discs was recorded under a light microscope. The cuttings were inserted with the cut ends in moist autoclaved peat mixture and incubated under plastic bags at 18 °C and natural light. Three weeks later the cuttings were examined for the presence of bark necrosis. The longitudinal extension of necroses from the cutting base was measured, and re-isolations carried out from necrotic tissue using PARPNH-agar. There were three replicates (each of 20 cuttings) per isolate and tree species combination.

Underbark inoculation. Tree species selected for this test were *Quercus robur*, *Salix alba,* and *Fagus sylvatica.* An isolate of each *P. citricola*, *P. cambivora,* and *P.* taxon 'salixsoil' were included in the tests for comparison with the two isolates of *P. gallica.* One-year-old twigs (*ca* 3–6 mm diam) were collected in the field from single mature trees in May, shortly after bud burst. Leaves were removed, twigs were cut to about 10–15 cm length, and inoculated by placing an *ca* 5 × 5 mm piece from actively growing V8A cultures of the isolates tested on an equally sized area of bark removed to the cambial surface. Ten twigs per isolate were placed in glass Petri dishes containing two layers of moist filter paper. Controls received plain V8A pieces. Plates were sealed with parafilm and incubated for one week at 22 °C in the dark. The length of any necroses was recorded and random re-isolations made from necrotic tissue on PARPNH. There were three replicates (each of ten twigs) per isolate and tree species combination.

In both abscised shoots tests, data were analysed using the Tukey–Kramer multiple comparisons test (program Prism 3, GraphPad, San Diego, CA).

Pathogenicity toward leaves of common reed

For the assessment of the pathogenicity of *Phytophthora gallica* towards common reed (*Phragmites australis*), the dominating plant species at the isolation site of strain GAL 2, leaves of six-month-old greenhouse-grown reed were inoculated as described by Nechwatal *et al.* (2005). The leaves were incubated at 20 °C in the dark for up to two weeks and checked regularly for the occurrence of necroses.

Results

The main morphological and physiological characters of *Phytophthora gallica* and those of phylogenetically related and morphologically similar species are listed in Table 2. Colony growth patterns and temperature–growth relationships of *P. gallica* and related species are shown in Figs 1 and 2, respectively.

Distribution, ecology, pathogenicity and phylogeny

Distribution and ecology

Phytophthora gallica could only be isolated twice. One isolate was recovered from rhizosphere soil sampled below a mature declining *Quercus robur* tree in the riparian forest, Forêt d'Illwald, in northeastern France, the other isolate was recovered in the littoral zone of Lake Constance in south-western Germany. The distance between both sites was *ca* 240 km. The soils from which the *P. gallica* isolates were recovered were seasonally wet and had pH ($CaCl_2$) values of 6.4 (oak stand) and 7 (reed stand). In the oak stand *P. syringae* and *Pythium anandrum* were isolated from the same soil sample as *Phytophthora gallica*, while *P. citricola* and *P. megasperma*

Table 2 – Morphological and physiological characteristics of *Phytophthora gallica*, other ITS clade 10 species, and other non-papillate *Phytophthora* species commonly occurring in wet habitats

	Phytophthora gallica	*P. kernoviae*	*P. boehmeriae*	*P. gonapodyides*	*P. inundata*	*P.* taxon 'PgChlamydo'	*P.* taxon 'salixsoil'	*P. alni* ssp. *alni*
No. of isolates investigated and/or literature source	2	0 Brasier *et al.* (2005)	0 Erwin & Ribeiro (1996)	1 Brasier *et al.* (1993, 2003b)	0 Brasier *et al.* (2003a)	2 Brasier *et al.* (2003b)	1 Nechwatal & Mendgen (2006)	Brasier *et al.* (1995, 2004)
Waterhouse Group	VI	II	II	VI	VI	VI	VI	V
ITS clade[a]	10	10	10	6	6	6	6	7
Sporangia	Non-papillate, obpyriform, ovoid, peanut, limoniform,	Papillate, ovoid, limoni-form,	Papillate, spherical, broad ovoid	Non-papillate, obpyriform, ovoid	Non-papillate, ovoid, obpyriform	Non-papillate, obpyriform, ovoid, limonif.	Non-papillate, obpyriform, ovoid, limonif.	Non-papillate, ovoid, obpyriform
Most common shapes								
Distorted shapes	+	+ (mouseshaped)	+ (bipapillate)	-	-	-	(+)	-
Caducous	−	+	+	-	-	-	-	-
l × b mean (µm)	52 × 27	n.k.	44–52 × 32–40[b]	54 × 34[b]	65 × 48	49 × 29	44 × 31[b]	50 × 35[b]
Range (µm)	30–100 × 19–48	34–52 × 19–31[b]	27–72 × 20–46[b]	37–64 × 25–40[b]	n.k.	33–66 × 20–40	36–51 × 27–39[b]	35–70 × 28–50[b]
Isolate means (µm)	51–54 × 25–29	39–46 × 23–27[b]	n.k.	52–67 × 35–41[b]	55–86 × 40–67	49–51 × 29	n.k.	48–60 × 31–43[b]
L:b isolate means	1.95–2.06	1.5[b]	1.25–1.4[b]	1.48–2.06[b]	1.2–1.5	1.72–1.79	1.34–1.68[b]	1.32–1.62[b]
Irregular hyphal swellings in water	+	-	-	-	-	+	-	-
Globose chlamydospores		-		-	-		-	-
Mean diam (µm)	47 ± 6.7		30–41			31 ± 5.6		
Diam range (µm)	25–63		17–51			20–50		
Isolate means (µm)	47–48		n.k.			29–32		
Elongated irregular chlamydospores		-	-	-	-		-	-
l × b mean (µm)	72 ± 19 × 34 ± 7					43 ± 11 × 27 ± 8		
Range (µm)	40–125 × 15–50					25–76 × 14–46		
Isolate means (µm)	61 × 35–78 × 34					37–49 × 25–29		
Sex	Sterile	Homothallic; amphigynous	Homothallic; Amphigynous	Sterile; silent A1	Heterothallic, amphigynous	Sterile; silent A1	Sterile; silent A1	Homothallic; oospore abortion
Colony appearance								
CA	Appressed with submerged margins and dendroid sectors of limited aerial mycelium	Largely submerged without pattern.	Uniform colonies with dense aerial mycelium and well-defined margins	Appressed, with limited aerial central boss; faintly petaloid	Irregular, stellate to broad-lobed with limited aerial mycelium	Limited aerial; faintly petaloid	Limited aerial; petaloid	Appressed-felty; no or sparse aerial mycelium; uniform
MEA	Rosaceous pattern, largely appressed to submerged with very limited aerial mycelium.	n.k.		Limited aerial; chrysanthemum	n.k.	Limited aerial; petaloid	Limited aerial; petaloid	Appressed-felty; no or sparse aerial mycelium; uniform
CMA	Largely submerged; Faintly stellate with narrow-lobed to chrysanthemum margin	n.k.		Largely submerged stellate	n.k.	Largely submerged chrysanthemum to broad-lobed	Appressed to submerged; faintly stellate	n.k.

Temperature–growth relations on CA									
Maximum (°C)	30–32	26[b]	32.5[b]	<35 (34–36[b])	35–37°	<35 (36–37[b])	38[b]	c. 29[b]	
Optimum (°C)	20	18[b]	25[b]	25 (25–28[b])	28–30°	20–25 (25–30[b])	33[b]	23–25[b]	
Radial growth (mm d^{-1}) at optimum	2.1 (1.9–2.3)	n.k.	n.k.	3.8 (2.8–4.6[b])	6–8	4 (3.2–4.1[b])	n.k.	5.9 (4.1–7.5[b])	
At 20 °C	2.1 (1.9–2.3)	4.2 (3.8–4.6)[b]	n.k.	3.5	n.k.	4	3.1	n.k.	

l × b, Length × breadth; l:b, length to breadth ratio; n.k., not known; +, feature occurring frequently; (+), feature occurring infrequently; –, feature not observed; CA, carrot agar; MEA, malt-extract agar; CMA, corn meal agar.
a ITS clades according to Cooke *et al.* (2000) and http://www.phytophthoradb.org.
b Data from literature.

were recovered from several oak trees growing nearby. In the reed zone *Phytophthora* taxon 'salixsoil' and various *Pythium* spp. were ubiquitous.

Pathogenicity toward abscised shoots of trees

Zoospore inoculation (Table 4). The microscopic investigation of the flooded agar discs after 4 d incubation of the cuttings in the Erlenmeyer flasks showed an abundant production of viable sporangia and release of zoospores for all isolates and all flasks. After another three weeks of incubation in peat, both isolates of *Phytophthora gallica* were moderately aggressive to *Alnus glutinosa* (mean lesion length of 4.2 ± 2.2 cm and 1.7 ± 1.7 cm) and non-pathogenic to *Fraxinus excelsior* and *Salix alba*. Except for *P. gonapodyides* the other five *Phytophthora* species tested were all more aggressive to *A. glutinosa* than *P. gallica*. *P.* taxon 'salixsoil', and *P. cambivora* were equally aggressive to *A. glutinosa* as was *P. alni* ssp. *alni*. *P. citricola* caused even longer lesions in *Alnus* cuttings than *P. alni* ssp. *alni*. *S. alba* was resistant to zoospore infections by all seven *Phytophthora* species tested, whereas *Fraxinus excelsior* was susceptible to three of the seven *Phytophthora* species tested, i.e. *P. cambivora*, *P. citricola* and *P.* taxon 'PgChlamydo'.

Underbark inoculation (Table 4). Both isolates of *Phytophthora gallica* were moderately aggressive towards all three tree species tested. In *Fagus sylvatica*, lesions caused by GAL 1 and GAL 2 were substantial (4.6 or 4.2 cm, respectively), but still significantly smaller than those caused by *P. cambivora*, *P. citricola*, and *P.* taxon 'salixsoil'. In *Salix alba*, necroses caused by *P. gallica* were small, and not significantly different from those caused by *P. citricola*. *P. cambivora* did not infect *S. alba*, while *P.* taxon 'salixsoil' proved to be relatively aggressive on this species. In *Quercus robur*, lesions caused by *P. gallica* were small, as were those caused by *P. cambivora*. *P. citricola* and *P.* taxon 'salixsoil' caused significantly larger lesions.

In both abscised shoots tests all pathogens could be reisolated from necrotic tissues. All control cuttings remained healthy and developed only limited discolourations around the inoculation sites.

Pathogenicity toward common reed leaves

Phytophthora gallica was non-pathogenic towards *Phragmites australis* leaves. Neither isolate caused any lesions, even when inoculated onto artificially wounded leaf blades (data not shown).

Phylogeny

Both isolates of *Phytophthora gallica* had identical ITS sequences with the length of the complete ITS1, 5.8S and ITS2 being 818 bp. The sequence has been submitted to GenBank (DQ286726). BLAST searches indicated the species' relatedness to *P. boehmeriae* and the newly described *P. kernoviae* (Brasier *et al.* 2005). However, based on pairwise alignments the sequence was only 84 or 85 % identical to GenBank database entries for these species (AF228076 and AY940661), corresponding to a difference of 133 or 121 positions, respectively. NJ phylogenetic analysis of the ITS sequence data confirmed the position of the new species outside the main *Phytophthora* cluster (Fig 3), within ITS clade 10 of Cooke *et al.* (2000), previously consisting of *P. kernoviae* and

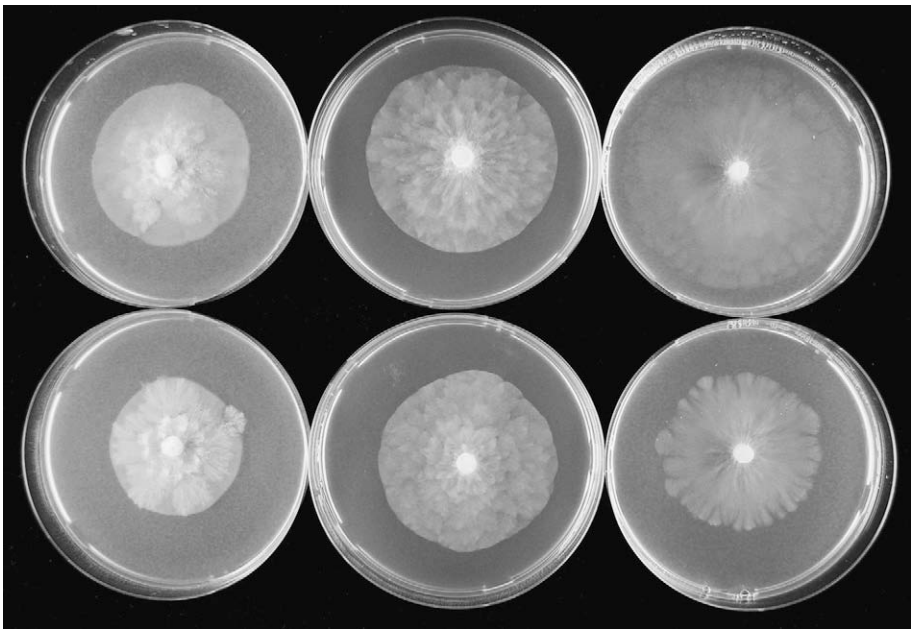

Fig 1 – Colony morphology of *Phytophthora gallica* isolates GAL 1 (top row) and GAL 2 (bottom row) in darkness at 20 °C on CA, MEA, and CMA after 10 d (from left to right).

P. boehmeriae (http://www.phytophthoradb.org), with high BS values.

There was only one base pair sequence difference between the two *P. gallica* isolates within the 568 bp subsequence of the *cox* II. Both sequences have been submitted to GenBank (EF192238 and EF192239). Again, *P. boehmeriae* proved to be among the closest relatives of the new species after BLAST searches of the GenBank database. Sequence identities within the *cox* II subsequence investigated were 95 % to *P. boehmeriae* and 94 % to two other species from ITS clades 9 and 10 (*P. insolita*, *P. richardiae*). In a phylogenetic analysis of the *cox* II sequence, *P. gallica* clustered with these species within the main *Phytophthora* cluster, i.e. within clade 8e and 8f *sensu* Kroon *et al.* (2004). BS support for these particular subclades, however, was low (data not shown).

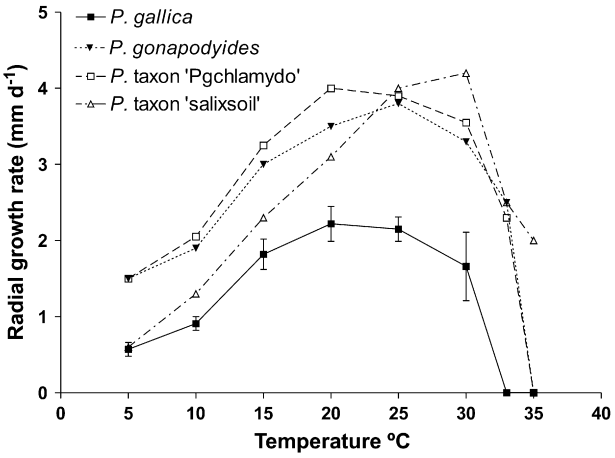

Fig 2 – Comparison of temperature–growth relationships of *Phytophthora gallica*, *P. gonapodyides*, P. taxon 'PgChlamydo', and P. taxon 'salixsoil' on CA. For better clarity standard deviations are given for *P. gallica* only.

Taxonomy

Phytophthora gallica T. Jung & J. Nechwatal, **sp. nov.**
MycoBank no.: MB497405

Etym.: '*gallica*' refers to the origin of isolate GAL 1 in France.

Coloniae lente crescentes (3 mm ad 20 °C in agaro 'V8A'); culturae steriliae, sporangia in cultura liquida nonpapillata, persistentia, terminalia,, in medio 54 × 29 μm; chlamydosporae globosae (in medio 48 μm) vel irregularia (in medio 78 × 34 μm), saepe abortivae; inflationes hypharum abundantia in cultura liquida, globosae aut irregulares; regiones 'rDNA ITS' et 'coxII' cum unica sequentia (GenBank DQ286726, EF192238).

Typus: **France**: Forêt d' Illwald, isol. ex solo rhizosphaerae arboris, *Quercus robur*, Apr 1998, *T. Jung* (PRC-Ill 5/1 —holotypus; CBS 111474 et PRC-GAL 1—culturae vivae).

Previously known informally as *Phytophthora* taxon G.

Colonies generally appressed with submerged margins and dendroid sectors of aerial mycelium on CA, largely submerged with a rosaceous pattern on MEA, and faintly stellate with narrow-lobed to chrysanthemum margins on CMA (Fig 1). Primary hyphae on CA 2.5–7.5 μm wide (av. 4.5 ± 1.5 μm). At 20 °C *Phytophthora gallica* colonies grew slowly on CA, V8A, MEA and CMA with 2.1–2.9 mm d^{-1} (Table 3). Growth on CA occurred from 5–30 °C with an optimum at 20 °C (radial growth rate 2.2 ± 0.2 mm d^{-1}; Fig 2). No growth at 33 and 35 °C. In contrast to *P. gonapodyides* and *P.* taxon 'PgChlamydo' both isolates of *P. gallica* showed no re-growth after 7 d at 35 °C when returned to 20 °C.

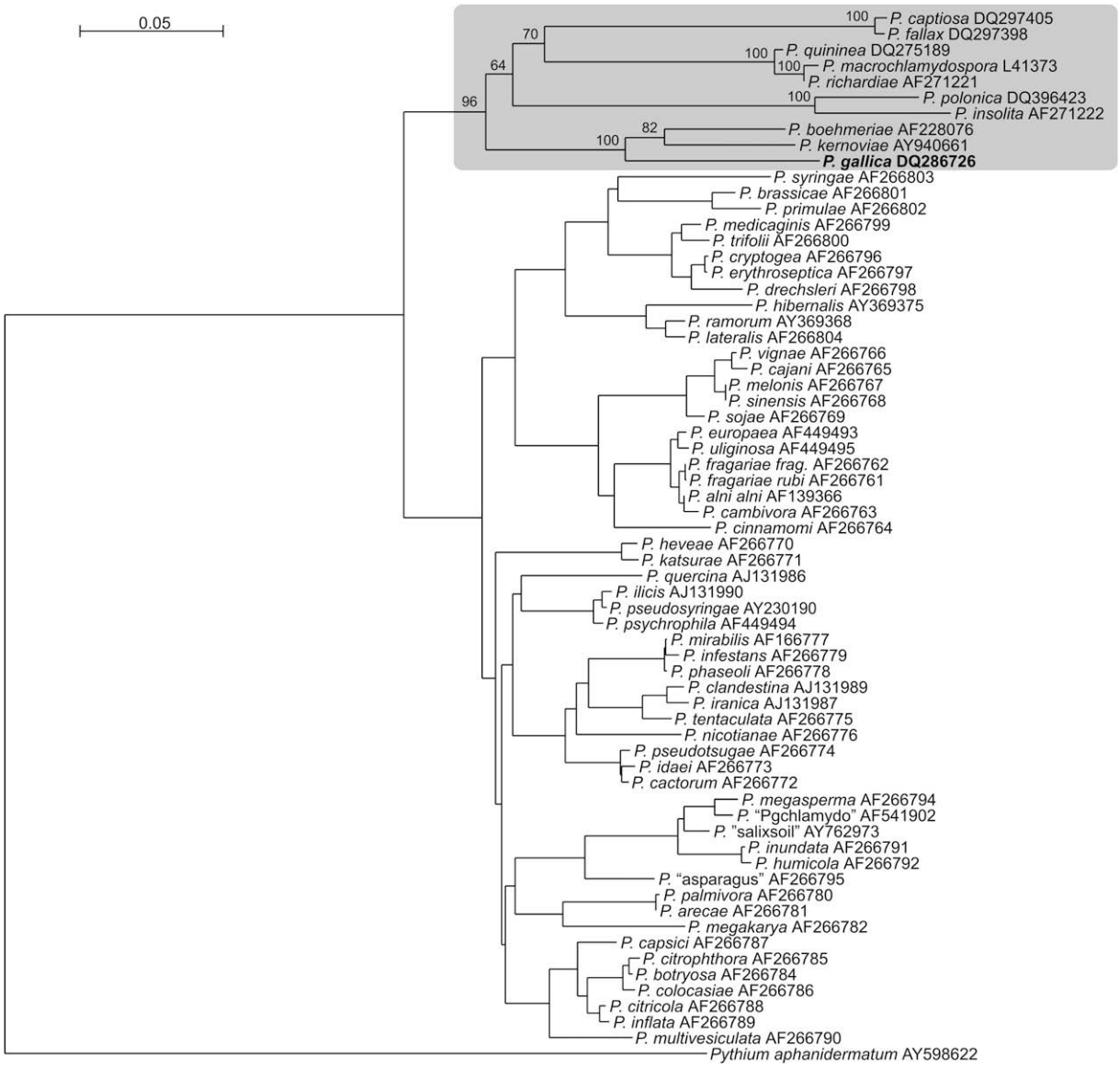

Fig 3 – Phylogenetic tree of *Phytophthora* spp., including *P. gallica*, constructed after distance-based analysis of ITS1, 5.8S, and ITS2 regions of the rDNA. *Pythium aphanidermatum* was used as an outgroup taxon. Numbers at the branch points indicate the percentage of 1 K bootstrapped datasets that recoverered this tree topology. Scale bar unit: number of nucleotide substitutions per site. Code numbers refer to GenBank accession numbers.

Sporangia produced abundantly in water culture. Borne terminally, on unbranched sporangiophores or more rarely in lax sympodia. Non-papillate, proliferating internally in an extended or more rarely in a nested way (Fig 4F–G). Sporangial shapes obpyriform (35 %, Fig 4B), ovoid (24 %, Fig 4A), peanut-shaped (17 %, Fig 4G–I) or limoniform (12 %, Fig 4C). Sporangia averaging $52.5 \pm 11 \times 27 \pm 5$ μm (range 30–100 × 19–47.5 μm) with isolate means of 51–54 × 25–29 μm, and a length:breadth ratio of 2 ± 0.5 (range of isolate means 1.9–2.1). Zoospores discharged through an exit pore 7.5–19 μm wide (av. 11.5 ± 3 μm; Fig 4E). Direct germination of sporangia common in older water cultures. *Oogonia* neither produced in single cultures nor in paired cultures of isolates GAL 1 and GAL 2 or in paired cultures with A1 and A2 tester strains of *P. cambivora* and *P. cinnamomi*, and A2 tester strains of *P. cryptogea* and *P. drechsleri*. *Chlamydospores* globose and elongated, pyriform, club-shaped and irregular (Fig 5A–H). Attached terminally (Fig 5A, E, H) or laterally (Fig 5C, F). Globose chlamydospores averaging 47.5 ± 7 μm (range 25–62.5 μm, range of isolate means 47–47.5 μm). Elongated pyriform, club-shaped and irregular chlamydospores averaging $72 \pm 19 \times 34 \pm 7$ μm (range 40–125 × 15–50 μm). Most chlamydospores starting to abort a few days after formation (Fig 5D–F, H). Spherical and irregular *hyphal swellings* (Fig 5I) produced in water culture.

Discussion

Phytophthora gallica is an apparently sterile species with non-papillate sporangia and belongs to group VI of the

Table 3 – Radial growth rates of *Phytophthora gallica* and other non-papillate *Phytophthora* spp. from wet habitats on different agar media at 20 °C

Phytophthora spp. (no. of isolates)	Radial growth rate (mm d^{-1}) on			
	CA	V8A	MEA	CMA
P. gallica (2)	2.2 ± 0.2	2.7 ± 0.4	2.6 ± 0.1	2.9 ± 0.6
P. gonapodyides (1)	3.5	4.1	3.2	3.6
P. taxon 'PgChlamydo' (2)	4	4.2 ± 0.1	2.2	4.6 ± 0.1
P. taxon 'salixsoil' (1)	3.1	4.9	3.1	4.2

CA, carrot agar; V8A, V8 agar; MEA, malt-extract agar; CMA, corn meal agar.

morphological classification system of Waterhouse (Waterhouse 1963; Erwin & Ribeiro 1996). However, it is now known that the Waterhouse Groups do not reflect natural assemblages, and DNA sequence analyses places *P. gallica* in ITS clade 10 of Cooke *et al.* (2000) and clade 8 of Kroon *et al.* (2004), and therefore, related to *P. kernoviae* and *P. boehmeriae*. Interestingly, these two related species are homothallic with caducous sporangia adapted to an aerial lifestyle, while *P. gallica* is sterile and morphologically and ecologically more similar to several non-papillate species of ITS clade 6 of Cooke *et al.* (2000), i.e. *P. gonapodyides*, P. taxon 'PgChlamydo', P. taxon 'salixsoil' and *P. inundata*, all occurring in wet habitats (Brasier *et al.* 2003b). While data of Cooke *et al.* (2000) indicated that members of ITS clade 10 clearly group outside the main *Phytophthora* clade, and therefore, might represent one or more different genera, Kroon *et al.* (2004), in a multigene analysis, showed that *Phytophthora* is most likely to be monophyletic. The authors included ITS clade 9 and 10 species within the main *Phytopthora* clade, i.e. within clade 8. This was confirmed by the phylogenetic analysis of the *cox* II subsequence performed in this study. Therefore, on present knowledge, *P. gallica* as other clade 9 and 10 species, should be retained within the genus *Phytophthora*, despite the large sequence differences to all other species of the genus observed in the ITS regions.

P. gallica can easily be distinguished from both phylogentically related and morphologically similar *Phytophthora* species by its unique combination of morphological and physiological characters, host and site relations, and DNA sequences. Based on phylogenetic analysis the recently described *P. kernoviae* and *P. boehmeriae* are the closest relative to *P. gallica*. Nevertheless, with 121 bp difference in their ITS sequences, the two species are clearly distinct. *P. kernoviae* can easily be distinguished from *P. gallica* by its production of oogonia in single culture, the absence of chlamydospores and hyphal swellings, production of papillate caducous sporangia, different colony growth patterns on CA, and different cardinal temperatures for growth. Moreover, *P. gallica* has, as yet, only been recovered from soil at wet sites, whereas *P. kernoviae* spreads aerially in ornamental gardens and beech woodlands with dense rhododendron understorey (Brasier *et al.* 2005). *P. boehmeriae* is distinguished from *P. gallica* by its homothallism, the production of papillate caducous sporangia, the absence of elongated pyriform, club-shaped and irregular chlamydospores, different colony growth patterns and higher cardinal temperatures for growth, different host ranges (Erwin & Ribeiro 1996), and different ITS and mtDNA sequences.

Whilst phylogenetically distant in both ITS and mtDNA sequence analyses, *P. gallica* morphologically most resembles species from clade 6. *P. gonapodyides* differs from *P. gallica* in the absence of chlamydospores and hyphal swellings, different colony growth patterns on CA, MEA, and CMA, higher maximum temperature for growth and higher growth rates. P. taxon 'PgChlamydo' like *P. gallica* forms both globose and irregular to pyriform chlamydospores. However, it is clearly distinguished from *P. gallica* by having markedly smaller chlamydospores, different colony growth patterns on CA, MEA, and CMA, higher maximum temperature for growth and higher growth rates. P. taxon 'salixsoil' can easily be

Table 4 – Length of necroses (cm) caused by *Phytophthora* species to bark of *Alnus glutinosa*, *Fraxinus excelsior*, and *Salix alba* cuttings in a zoospore inoculation test after three weeks, and to bark of *Fagus sylvatica*, *Quercus robur*, and *Salix alba* in an underbark inoculation test after 7 d [mean, standard deviation, level of significant difference from the control (Tukey–Kramer Multiple Comparisons Test)]

Isolate[a]	Length of necroses[b] (zoospore inoculation)			Length of necroses[b] (underbark inoculation)		
	Alnus glutinosa	*Fraxinus excelsior*	*Salix alba*	*Fagus sylvatica*	*Quercus robur*	*S. alba*
GAL 1	4 ± 2.2[b]	0[n.s.]	0[n.s.]	4.6 ± 0.5[b]	1.8 ± 0.4[b]	1.3 ± 0.7[b]
GAL 2	1.7 ± 1.7[n.s.]	0[n.s.]	0[n.s.]	4.2 ± 0.6[b]	1.7 ± 0.4[b]	1 ± 0.5[n.s.]
GON 3	3.5 ± 2.3[b]	0[n.s.]	0[n.s.]	-	-	-
CHLAM 5	8 ± 2[b]	1.2 ± 1.6[n.s.]	0[n.s.]	-	-	-
SAL 1	7.3 ± 1.8[b]	0[n.s.]	0[n.s.]	6.2 ± 0.7[b]	2.3 ± 0.5[b]	3.1 ± 1.1[b]
ALN 377	7.2 ± 1.8[b]	0[n.s.]	0[n.s.]	-	-	-
CAM 109	8 ± 3.2[b]	4.5 ± 3.9[b]	0[n.s.]	-	-	-
CAM 1	-	-	-	7.1 ± 1[b]	1.5 ± 0.7[b]	0.6 ± 0.2[n.s.]
CIT 135	12.3 ± 1.7[b]	2 ± 3.5[n.s.]	0.2 ± 0.2[n.s.]	-	-	-
CIT 55	-	-	-	7.8 ± 0.6[b]	2.4 ± 0.8[b]	1.5 ± 0.5[b]
Control	0	0	0	0.7 ± 0.2	0.6 ± 0.1	0.5

a GAL, *Phytophthora gallica* sp. nov.; GON, *P. gonapodyides*; CHLAM, P. taxon 'PgChlamydo'; SAL, P. taxon 'salixsoil'; ALN, *P. alni* ssp. *alni*; CAM, *P. cambivora*; CIT, *P. citricola*.
b n.s., Not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

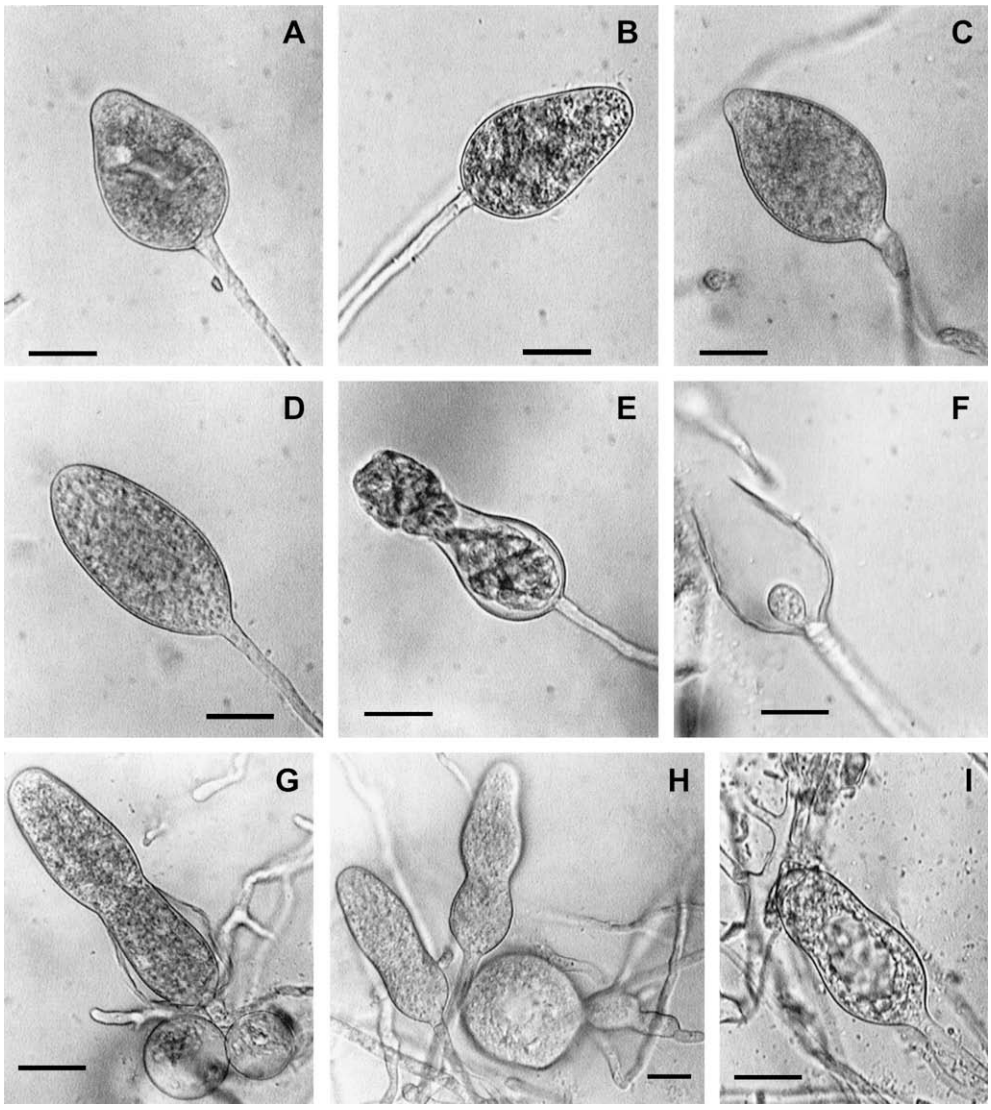

Fig 4 – Non-papillate sporangia of *Phytophthora gallica* on CA flooded with nonsterile soil filtrate after 24–48 h. (A–D) Fully mature, ovoid (A: isolate GAL 2), obpyriform (B: isolate GAL 1), limoniform (C: isolate GAL 2) and ellipsoid (D: isolate GAL 1) sporangia. (E) Release of zoospores through a wide exit pore of an obpyriform sporangium of isolate GAL 2. (F) Beginning internal proliferation through an empty ovoid sporangium of isolate GAL 2. (G) Large, peanut-shaped sporangium of isolate GAL 1 exhibiting nested proliferation through an empty sporangium and globose hyphal swellings. (H) Large, peanut-shaped sporangia and globose chlamydospore of isolate GAL 2. (I) Peanut-shaped abortive sporangium of isolate GAL 2. Bars = 20 μm.

separated from *P. gallica* by the absence of chlamydospores and hyphal swellings in water culture, different colony growth patterns on CA, MEA, and CMA, higher cardinal temperatures for growth, and higher growth rates. *P. inundata* is another widespread non-papillate *Phytophthora* species from wet sites. It differs from *P. gallica* by the heterothallic production of oogonia, the absence of chlamydospores and hyphal swellings, higher cardinal temperatures for growth, higher growth rates, and different colony growth patterns (Brasier *et al.* 2003a).

Despite systematic sampling of soil by the authors and many other research groups in more than a thousand forest stands and wet habitats across Europe, *P. gallica* has so far only been recovered from two stands in France and Germany *ca* 240 km apart. It seems that *P. gallica* may be a rare species with a fairly limited distribution in Europe. However, due to its very slow growth as compared with several other *Phytophthora* spp. it could have passed unnoticed even in isolation surveys focusing on this genus.

Although *P. gallica* was isolated from soil beneath a declining mature *Quercus robur* and from the littoral zone of a lake with *Phragmites australis* and *Salix alba* as dominants, in pathogenicity tests *Phytophthora gallica* was only weakly aggressive to *Q. robur* and *S. alba*, and non-pathogenic to *Phragmites australis*. Therefore, its ecological niche, whether as a pathogen or saprotroph, remains uncertain. However, a saprophytic lifestyle seems unlikely given the much higher growth rates of the *Pythium* species ubiquitous in these wet ecosystems (e.g. Jung *et al.* 2000; Nechwatal *et al.* 2005).

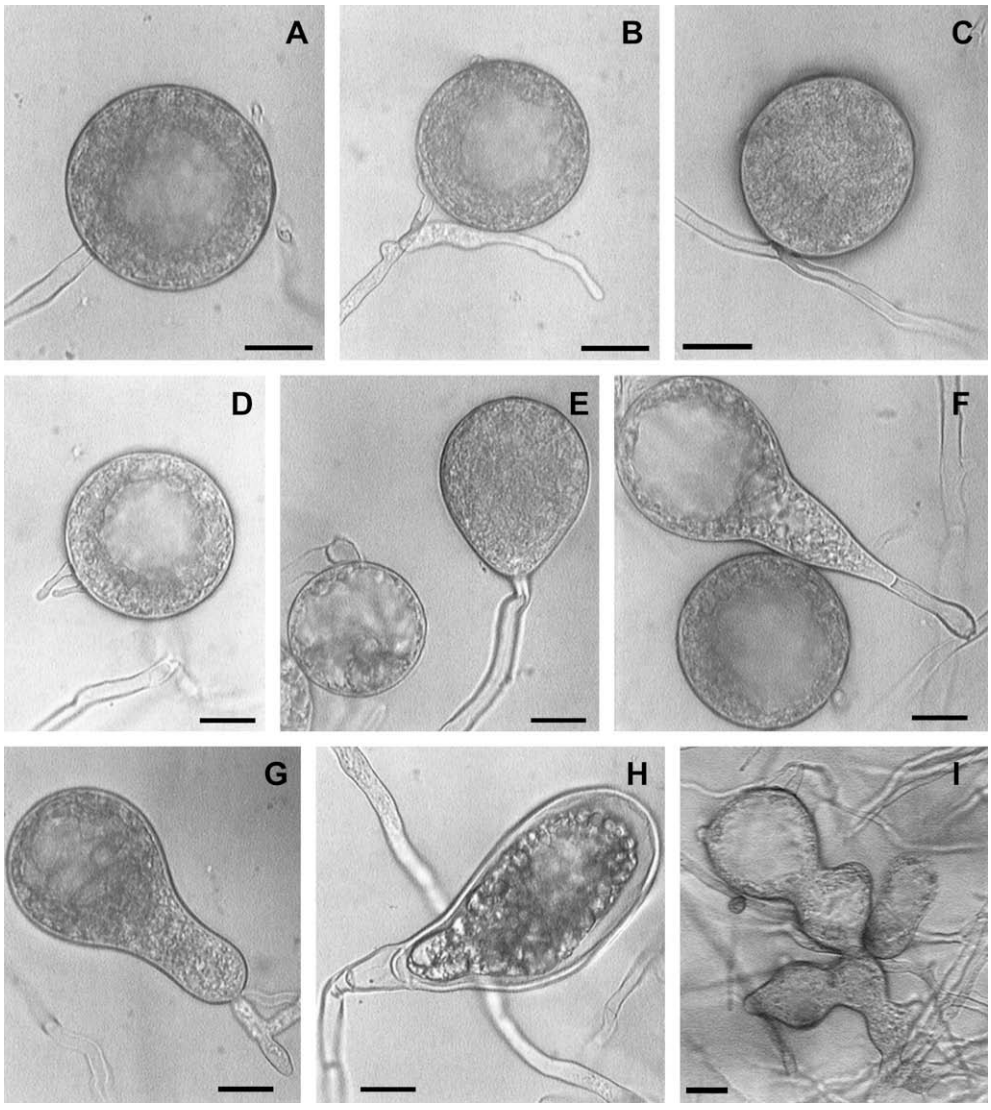

Fig 5 – Chlamydospores (isolate GAL 1) and hyphal swellings (isolate GAL 2) of *Phytophthora gallica* on CA after 14 d at 20 °C. (A–C) Globose chlamdospores, borne terminally (A) or laterally (B–C). (D) Globose chlamydospore with incipient abortion. (E) Globose aborted chlamydospore and a terminal, obovoid mature chlamydospore. (F) Globose mature chlamydospore and aborted, club-shaped chlamydospore. (G) Pyriform mature chlamydospore. (H) Terminal, club-shaped to irregular, aborted chlamydospore. (I) Inflated, irregular hyphal swellings. Bars = 20 μm.

Interestingly, both isolates of *Phytophthora gallica* were able to cause bark lesions in shoots of European beech, to date the major host tree along with rhododendron of the related species *P. kernoviae* in Europe (Brasier *et al.* 2005; Brown & Brasier 2007). Isolate GAL 1 also induced lesions on shoots of common alder. Therefore, although the results of an abscised shoot assay might not be directly transferable to a potential soilborne pathogen, the possibility cannot be excluded that the host range of *P. gallica* includes these two tree species. Apart from the need for more information on its overall distribution, additional pathogenicity tests are required to clarify its host range.

The geographic origin of *P. gallica* is yet unknown. The suggestion by Brasier & Jung (2006) that *P. gallica* might be ancestral to its closest relatives, *P. kernoviae* and *P. boehmeriae,* was supported by our phylogenetic analysis using ITS sequences. As *P. kernoviae* is probably native to New Zealand (RA Beever & CM Brasier, pers. comm.), and most records of *P. boehmeriae* came from Asia and Australia (Erwin & Ribeiro 1996), it is probable that *P. gallica* is not native to Europe, especially given its currently rare occurrence in a relatively limited area of Central Europe.

Up to now, *P. gallica* has only been isolated twice, but its unique combination of cultural, morphological and physiological characters, and its unique phylogenetic position within the genus justify its description as a well-defined new species for which the name *Phytophthora gallica* sp. nov. is proposed here.

Acknowledgements

We are grateful to the European Union (Project 'Pathoak' FAIR 5-CT97-3926; T.J.), and the Deutsche Forschungsgemeinschaft

(SFB 454 'Littoral Zone of Lake Constance'; J.N.) for financial support. The authors would also like to thank Johanna Lebherz for her assistance in the laboratory routines. We are also grateful to Clive Brasier (Forestry Research, Farnham), David Cooke (Scottish Crop Research Institute, Dundee), and Helmut Blaschke (Department of Ecology, Freising, Germany) for constructive discussions.

REFERENCES

Brasier CM, Jung T, 2003. Progress in understanding *Phytophthora* diseases of trees in Europe. In: McComb JA, Hardy G, Tommerup I (eds), *Phytophthora in Forests and Natural Ecosystems. Proceedings of the 2nd International IUFRO Working Party 7.02.09 Meeting, 30 September–5 October 2001, Albany, Western Australia*. Murdoch University Print, Perth, pp. 4–18.

Brasier CM, Jung T, 2006. Recent developments in *Phytophthora* diseases of trees and natural ecosystems in Europe. In: Brasier CM, Jung T, Osswald W (eds), *Progress in Research on Phytophthora Diseases of Forest Trees. Proceedings of the 3rd International IUFRO Working Party 7.02.09 Meeting,* 11–17 September 2004, Freising, Germany. Forest Research, Farnham, pp. 5–16.

Brasier CM, Sanchez-Hernandez E, Kirk S, 2003a. *Phytophthora inundata* sp. nov., a part heterothallic pathogen of trees and shrubs in wet or flooded soils. *Mycological Research* **107**: 477–484.

Brasier CM, Cooke DEL, Duncan JM, Hansen EM, 2003b. Multiple new phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides–P. megasperma* ITS Clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile. *Mycological Research* **107**: 277–290.

Brasier CM, Beales PA, Kirk SA, Denman S, Rose J, 2005. *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. *Mycological Research* **109**: 853–859.

Brasier CM, Kirk SA, Delcan J, Cooke DEL, Jung T, Man in't Veld WA, 2004. *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. *Mycological Research* **108**: 1172–1184.

Brasier CM, Robredo F, Ferraz JFP, 1993. Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. *Plant Pathology* **42**: 140–145.

Brasier CM, Rose J, Gibbs JN, 1995. An unusual *Phytophthora* associated with older mortality in Britain. *Plant Pathology* **44**: 999–1007.

Brown AV, Brasier CM, 2007. Colonization of tree xylem by *Phytophthora ramorum*, *P. kernoviae* and other *Phytophthora* species. *Plant Pathology* **56**: 227–241.

Cooke DEL, Duncan JM, 1997. Phylogenetic analysis of *Phytophthora* species based on ITS1 and ITS2 sequences of the ribosomal RNA gene repeat. *Mycological Research* **101**: 667–677.

Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM, 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* **30**: 17–32.

Erwin DC, Ribeiro OK, 1996. *Phytophthora Diseases Worldwide*. American Phytopathological Society Press, St Paul, MN.

Felsenstein J, 1993. *PHYLIP: phylogeny inference package*. Version 3.5c. Department of Genetics, University of Washington, Seattle.

Gibbs JN, van Dijk C, Webber JF (eds), 2003. *Phytophthora* disease of alder in Europe. *Forestry Commission Bulletin* **126**: 1–82.

Jung T, Blaschke M, 2004. *Phytophthora* root and collar rot of alders in Bavaria: distribution, modes of spread, and possible management strategies. *Plant Pathology* **53**: 197–208.

Jung T, Cooke DEL, Blaschke H, Duncan JM, Osswald W, 1999. *Phytophthora quercina* sp. nov., causing root rot of European oaks. *Mycological Research* **103**: 785–798.

Jung T, Blaschke H, Oßwald W, 2000. Involvement of *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. *Plant Pathology* **49**: 706–718.

Jung T, Hansen EM, Winton L, Oßwald W, Delatour C, 2002. Three new species of *Phytophthora* from European oak forests. *Mycological Research* **106**: 397–411.

Jung T, Nechwatal J, Cooke DEL, Hartmann G, Blaschke M, Oßwald W, Duncan JM, Delatour C, 2003. *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. *Mycological Research* **107**: 772–789.

Jung T, Hudler GW, Jensen-Tracy SL, Griffiths HM, Fleischmann F, Oßwald W, 2005. Involvement of *Phytophthora* species in the decline of European beech in Europe and the USA. *Mycologist* **19**: 159–166.

Kroon LPNM, Bakker FT, van den Bosch GBM, Bonants PJM, Flier WG, 2004. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology* **41**: 766–782.

Martin FN, Tooley PW, 2003. Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. *Mycologia* **95**: 269–284.

Nechwatal J, Mendgen K, 2006. Widespread detection of *Phytophthora* taxon salixsoil in the littoral zone of Lake Constance, Germany. *European Journal of Plant Pathology* **114**: 261–264.

Nechwatal J, Wielgoss A, Mendgen K, 2005. *Pythium phragmitis* sp. nov., a new species close to *P. arrhenomanes* as a pathogen of common reed (*Phragmites australis*). *Mycological Research* **109**: 1337–1346.

Page RDM, 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Application in the Biosciences* **12**: 357–358.

Vettraino AM, Morel O, Perlerou C, Robin C, Diamandis S, Vannini A, 2005. Occurrence and distribution of *Phytophthora* species in European chestnut stands, and their association with Ink Disease and crown decline. *European Journal of Plant Pathology* **111**: 169–180.

Waterhouse GM, 1963. Key to the species of *Phytophthora* de Bary. *Mycological Papers* **92**: 1–22.