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The morphology, behaviour and molecular phylogeny of *Phytophthora* taxon Salixsoil and its redesignation as *Phytophthora lacustris* sp. nov.

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Since its first isolation from *Salix* roots in 1972, isolates of a sexually sterile *Phytophthora* species have been obtained frequently from wet or riparian habitats worldwide and have also been isolated from roots of *Alnus* and *Prunus* spp. Although originally assigned to *Phytophthora gonapodyides* on morphological grounds, it was recognized that these isolates, informally named *P.* taxon Salixsoil, might represent a separate lineage within ITS Clade 6. Based on phylogenetic analyses and comparisons of morphology, growth-temperature relationships and pathogenicity, this taxon is formally described here as *Phytophthora lacustris* sp. nov. Isolates of *P. lacustris* form a clearly resolved cluster in both ITS and mitochondrial *cox1* phylogenies, basal to most other Clade 6 taxa. *Phytophthora lacustris* shares several unusual behavioural properties with other aquatic Clade 6 species, such as sexual sterility and tolerance of high temperatures, that have been suggested as adaptations to riparian conditions. It appears to be widespread in Europe and has also been detected in Australia, New Zealand and the USA. It was shown to be weakly or moderately aggressive on inoculation to *Alnus*, *Prunus* and *Salix*. The extent of *P. lacustris*' activity as a saprotroph in plant debris in water and as an opportunistic pathogen in riparian habitats needs further investigation. Its pathogenic potential to cultivated fruit trees also deserves attention because *P. lacustris* has apparently been introduced into the nursery trade.

Keywords: *Alnus*, phylogeny, *Phytophthora* ITS Clade 6, *Prunus*, riparian habitat, tree pathogen

Introduction

In June 1972 an isolate of a sexually sterile, slow-growing *Phytophthora* was obtained from diseased roots of the ornamental *Salix matsudana* after a stream flooding episode in southern England (C.M. Brasier & R.G. Strouts, Forest Research, Farnham, UK, unpublished data). The isolate, P245 (=IMI389725), was assigned to *P. gonapodyides* on morphological grounds (Brasier *et al.*, 1993).

Later, P245 was included in a study of colony characters, sexual behaviour and protein polymorphisms in 22 *P. gonapodyides*-like isolates from the UK and western North America (Brasier *et al.*, 1993). In this study, P245 was confirmed as having a similar colony pattern to *P. gonapodyides* and, also like *P. gonapodyides*, to be self-sterile but having a 'silent A1 mating type', inducing gametangial formation in A2 isolates of several heterothallic *Phytophthora* species both in direct and in membrane pairings. In its protein electrophoretic patterns, though similar to *P. gonapodyides* isolates, P245 lacked a dense, highly characteristic 'PG-band' and was unusual in being able to grow at 37°C (Brasier *et al.*, 1993).

During the 1990s many isolates of uncertain affinity collected during studies of phytophthoras from trees and riparian ecosystems in Europe that had ITS (internal transcribed spacer region of the rDNA repeats) sequences placing them within major ITS Clade 6 of Cooke *et al.* (2000) were compared for morphological and behavioural criteria and for their phylogeny (Brasier *et al.*, 2003b). On this basis, unknown isolates were assigned to species groups which included multiple previously unknown taxa having unique ITS lineages. Seven of these groups

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were newly designated as informal taxa, and one comprised isolate P245 and a similar isolate (P878), obtained from *Alnus* debris in a pond in Odense, Denmark in 1995. In this study P245 and P878 were culturally very similar to *P. gonapodyides*, but both isolates were again shown to have a higher maximum temperature for growth. It was concluded that P245 and P878 probably represented an independent evolutionary lineage and that they resembled *P. gonapodyides* in morphology and behaviour as a result of convergent evolution in similar riparian habitats such as ponds and rivers. The two isolates were informally designated as *Phytophthora* taxon Salixsoil (Brasier *et al.*, 2003b). *Phytophthora* isolates obtained in 1999 from young declining peach trees in Calabria and Emilia Romagna, Italy, had an identical ITS sequence to *P.* t. Salixsoil and were suggested to be the same taxon (Cacciola *et al.*, 2001; Brasier *et al.*, 2003b).

Since its original isolation from *S. matsudana* in 1972 and its informal designation in 2003, *P.* t. Salixsoil has been recorded increasingly from aquatic habitats, wetlands and also from nurseries. Examples of the distribution of *P.* t. Salixsoil include its occurrence in riparian sediments in the littoral zone of Lake Constance, Germany, in declining *Fraxinus excelsior* in Poland, in rivers in Alaska and western Oregon, USA, in a drainage sump in Western Australia and in nurseries in the USA and Europe (Nechwatal & Mendgen, 2006; Jung *et al.*, 2011a; Orlikowski *et al.*, 2011; Reeser *et al.*, 2011).

In the course of the present study, a variety of isolates was collected from locations worldwide and examined in detail, mostly in comparison with *P. gonapodyides*. In the present paper, the results of these studies are summarized and *P.* t. Salixsoil is designated as *Phytophthora lacustris* sp. nov.

Materials and methods

Culture media, isolation and isolate maintenance

Carrot agar (CA), clarified V8 juice agar (cV8A), V8A and PARPBH selective medium were prepared as described by Erwin & Ribeiro (1996). Stock cultures on CA or V8A plates were kept at 25°C and subcultured at 3–4 week intervals. Soil and root samples for the isolation of *Phytophthora* spp. were collected at different locations in Hungary, Germany and Italy.

Hungary
Rhizosphere soil samples from the upper 5–25 cm layer around declining alder (*Alnus glutinosa*) trees were collected in two permanently and three periodically flooded alder stands at five locations in 2002–2003 (Table 1). Isolations were carried out both from necrotic alder roots and by baiting from soil.

Germany
Samples from reed (*Phragmites australis*) rhizosphere soil were taken between summer 2003 and winter 2005/06 from permanently or periodically flooded habitats on several sites at the littoral of Lake Constance, southwest Germany (Nechwatal & Mendgen, 2006). Additional reed soil samples were taken from two lakes in southern Germany (Table 1).

Italy
Root samples from 10 young peach trees grafted on GF677 (*Prunus persica* × *Pr. dulcis*) rootstock showing symptoms of leaf chlorosis and defoliation, as well as root and crown rot, were taken in June 1999 in a commercial peach orchard that had been established by planting of nursery stock in 1996 in Calabria, southern Italy (Cacciola *et al.*, 2001). The orchard had experienced repeated soil waterlogging following heavy rains in winter. Additional isolates were obtained from necrotic fine roots of a sample taken in a fruit tree nursery located in northern Italy (Emilia Romagna region).

Isolations from soil were carried out by baiting using common cherry laurel or young oak leaflets as baits. Baits showing necrosis after a few days were dried on paper towels, the lesions excised and plated onto selective PARPBH agar plates. Isolations from roots were carried out by plating of necrotic rootlets or root pieces, after washing in tap water, onto PARPBH. Plates were inspected daily for the occurrence of *Phytophthora* hyphae. Hyphal tip subcultures were made on CA or V8A from every *Phytophthora* culture growing on the medium and used for further studies.

Additional isolates of *P. lacustris* came from the UK where the species was first detected (Brasier *et al.*, 2003b), as well as from Denmark, Australia, New Zealand and the USA. A total of 37 isolates of *P. lacustris* were analysed in detail in the present study. The isolates examined and their sources are listed in Table 1, including reference isolates of the closely related and morphologically similar *P. gonapodyides* used for comparisons.

DNA isolation, amplification and sequencing

Strains were grown in pea broth medium (Erwin & Ribeiro, 1996) for 7–10 days at 25°C without shaking. Mycelium was harvested by filtration through filter paper, washed with sterile deionized water, freeze-dried, and ground in liquid nitrogen. DNA was extracted by phenol/chloroform extraction as described by Bakonyi & Justesen (2007). Alternatively, DNA from various *Phytophthora* cultures analysed during this study was isolated by applying a quickprep method using Chelex resin (Bio-Rad) with mycelium scraped off from fully colonized agar plates (Wirsel *et al.*, 2002).

For species identification and discrimination of *P. lacustris* from *P. gonapodyides*, sequences of the ITS regions were used for most isolates analysed or obtained during this study. For some isolates, DNA extracts were subjected to *P. lacustris* specific PCR as described earlier (Nechwatal & Mendgen, 2006), to confirm species assignment.

Table 1 Identity, host, location, isolation information and GenBank accession numbers for *Phytophthora* isolates used in this study

Isolate code	Other collection no.	Substrate	Host	Location	Isolated by, method[a]	Date	Tests performed[b]	Sequence analysed		GenBank accession	
								ITS	*cox1*	ITS	*cox1*
P. taxon Salixsoil											
H-10/02	CBS 117384	Root	*Alnus glutinosa*	Jánossomorja, HU	JB, ZN, d	Jun 2002	1, 2a, b, c, 3	+	+	JF714489	
H-11/02	CBS 117385	Soil	*A. glutinosa*	Földsziget, HU	JB, ZN, b	Jun 2002	1, 2a, c, 3, 4a, c, d	+	+		JF896560
H-12/02		Soil	*A. glutinosa*	Földsziget, HU	JB, ZN, b	Jun 2002	1, 2c, 3	+[c]	+		
H-13/02	CBS 117383	Root	*A. glutinosa*	Ócsa, HU	JB, ZN, d	May 2002	1, 2a, b, c, 3, 4c	+[c]	+		
H-15/02		Soil	*A. glutinosa*	Ócsa, HU	JB, ZN, b	May 2002	1, 2a, b, c, 3	+[c]			
H-16/02		Soil	*A. glutinosa*	Teskánd, HU	JB, b	Oct 2002	1, 2a, b, c, 3, 4c, d	+	+		JF896559
P1/03		Root	*A. glutinosa*	Bokod, HU	JB, d	Mar 2003	1, 2b, c, 4c	+[c]			
UKN-Ph1		Soil	*Phragmites australis*	Konstanz, DE	JN, b	Apr 2003	1, 2a, b, c, 3, 4d	+		AY762973	
UKN-Ph2		Soil	*Ph. australis*	Konstanz, DE	JN, b	May 2003	1, 2c	+			
UKN-Ph3		Soil	*Ph. australis*	Konstanz, DE	JN, b	May 2003	1, 2a, b, c, 3	+			
UKN-Ph4		Soil	*Ph. australis*	Konstanz, DE	JN, b	May 2003	1, 2a, b, c, 3, 4d	+			
UKN-Ph5		Soil	*Ph. australis*	Konstanz, DE	JN, b	May 2003	1, 2c, 3	+			
UKN-Ph6		Soil	*Ph. australis*	Konstanz, DE	JN, b	Apr 2003	1, 2a, b, c, 3	+			
UKN-Ph7		Soil	*Ph. australis*	Konstanz, DE	JN, b	Apr 2003	1, 2c, 3	+			
UKN-Ph8		Soil	*Ph. australis*	Konstanz, DE	JN, b	Apr 2003	1, 2c, 3	+			
UKN-Ph9		Soil	*Ph. australis*	Konstanz, DE	JN, b	Apr 2003	1, 2c, 3	+			
UKN-Ph14		Soil	*Ph. australis*	Konstanz, DE	JN, b	Jun 2003	1, 2a, b, c, 3	+			
UKN-Ph15		Soil	*Ph. australis*	Konstanz, DE	JN, b	Jun 2003	1, 2c, 3	+			
UKN-V3b		Soil	*Ph. australis*	Konstanz, DE	JN, b	Nov 2005	3, 4d	+			
UKN-V4c		Soil	*Ph. australis*	Konstanz, DE	JN, b	Nov 2005	3, 4d	+			
UKN-V8a		Soil	*Ph. australis*	Konstanz, DE	JN, b	Nov 2005	3, 4d	+			
UKN-V15a		Soil	*Ph. australis*	Iffeldorf, DE	JN, b	Dec 2005	3, 4d	+			
UKN-V19c		Soil	*Ph. australis*	Augsburg, DE	JN, b	Dec 2005	3, 4d	+			
UKN-V20b		Soil	*Ph. australis*	Konstanz, DE	JN, b	Jan 2006	3, 4d	+			
UKN-V21b		Soil	*Ph. australis*	Konstanz, DE	JN, b	Jan 2006	3, 4d	+			
UKN-V22c		Soil	*Ph. australis*	Eriskirch, DE	JN, b	Jan 2006	3, 4d	+			
UKN-V24b		Soil	*Ph. australis*	Lindau, DE	JN, b	Jan 2006	3, 4d	+			
PESCO 1		Root	*Prunus* hybrid	Calabria, IT	SC, d	Jun 1999	1, 2a, b, c, 3, 4a, d	+			
PESCO 5		Root	*Prunus* hybrid	Calabria, IT	SC, d	Jun 1999	1, 2c, 3, 4d	+			
PESCO 10		Root	*Prunus* hybrid	Calabria, IT	SC, d	Jun 1999	1, 2a, c, 3	+			
PESCO 11		Root	*Prunus* hybrid	Emilia Romagna, IT	SC, d	Jun 1999	1, 2a, c, 3	+			
PESCO RC		Root	*Prunus* hybrid	Calabria, IT	SC, d	Jun 1999	1, 2c, 3, 4b	+	+	JF804802	JF896562
HSA 1959		Sump soil	-	Welshpool, AUS	R. Hart, b	1994	3	+	+	HQ012956	HQ012880
P14	SCRP989	River soil	-	Allegan County, Michigan, USA	D. Fulbright, b	2000	1, 2a, b, c, 3	+	+	JF907579	JF896563
ICMP 16270	SCRP993, CBS113346	Storage water	-	Auckland, NZ	C.F. Hill, b	May 2003	1, 2a, b, c	+		JF804803	

Table 1 (Continued)

Isolate code	Other collection no.	Substrate	Host	Location	Isolated by, method[a]	Date	Tests performed[b]	Sequence analysed		GenBank accession	
								ITS	*cox1*	ITS	*cox1*
P245	IMI 389725, WPC-P10337	Root	*Salix matsudana*	Bexley Heath, UK	CB, d	Jun 1972	1, 2a, b, c, 3, 4a, d	+	+	AF266793	JF896561
P878		Debris	*Alnus* sp.	Odense, DK	K. Thinggaard, d	1995	3, 4a	+		AF541909	
P. gonapodyides											
H-4/02		Soil	*A. glutinosa*	Söjtör, HU	JB, b	Oct 2002	1, 2a, b, c, 3, 4a, c, d	+		JN416847	
H-14/02		Root	*A. glutinosa*	Ócsa, HU	JB, d	May 2002	1, 2b, c, 4a, c	+	+	JF912516	JF742604
ICMP 14157		Root	*Castanea sativa*	South Canterbury, NZ	M. Braithwaite, d	Jan 2000	1, 2a, b, c	+		JN416848	
IMI 68755	WPC-P7050, ATCC 46726	Debris	-	UK	E. Perrott	unknown	1, 2a, b, 3	+		GU258909	
IMI 340619	WPC-P7186	Water	-	UK	G. Hall, b	1989	1, 2b, c, 3, 4d	+		GU258911	
UKN-BuKN 1a		Soil	*Fagus sylvatica*	Konstanz, DE	JN, b	May 2003	3, 4d	+			
UKN-BuKN 1b		Soil	*F. sylvatica*	Konstanz, DE	JN, b	May 2003	1, 2a, b, c, 3	+	+	JF912517	JF742605
UKN-BuKN 2a		Soil	*F. sylvatica*	Konstanz, DE	JN, b	May 2003	3	+			
UKN-BuKN 2b		Soil	*F. sylvatica*	Konstanz, DE	JN, b	May 2003	3	+			
UKN-MÜ 04a		Soil	*Ph. australis*	Konstanz, DE	JN, b	Apr 2005	3	+			
IFB-GON2		Soil	*Quercus robur*	Freising, DE	TJ, b	Sep 1994	3	+			
IFB-GON3		Root	*Q. robur*	Freising, DE	TJ, b	Sep 1994	3	+		AJ007368	
IFB-GON8		Soil	*Q. robur*	Bienwald, DE	TJ, d	Sep 1995	3	+			
UKN-V1b		Soil	*Ph. australis*	Konstanz, DE	JN, b	Nov 2005	3, 4d	+			
UKN-V12b		Soil	*Ph. australis*	Konstanz, DE	JN, b	Nov 2005	3, 4d	+			
UKN-V23a		Soil	*Ph. australis*	Immenstaad, DE	JN, b	Jan 2006	4d	+			

ATCC: American Type Culture Collection, USA; CBS: Centraalbureau voor Schimmelcultures, the Netherlands; ICMP: International Collection of Mircoorganisms from Plants, New Zealand; IFB: Institute of Forest Botany, TU München, Germany; IMI: CABI Genetic Resource Collection, UK; SCRP: Scottish Crop Research Institute, UK; UKN: University of Konstanz, Germany; WPC: World Phytophthora Collection, USA.

[a]Isolation method: b: baiting; d: direct plating.

[b]Tests performed: 1: growth rate and culture morphology at 25°C; 2a: minimum temperature for growth; 2b: optimum temperature for growth; 2c: maximum temperature for growth; 3: sporangium size; 4a: pathogenicity soil infestation *Alnus*/*Prunus*; 4b: pathogenicity stem *Prunus*; 4c: pathogenicity stem *Alnus*; 4d: pathogenicity abscised twigs *Alnus*/*Salix*.

[c]Isolates confirmed by specific PCR only (after Nechwatal & Mendgen, 2006).

Phylogenetic analysis

To determine the phylogenetic relationship of the new species, sequencing of the rDNA ITS regions and the mitochondrial cytochrome oxidase I (*cox1*) gene was performed and data compared to those of related *Phytophthora* Clade 6 species. PCR amplification of the ITS regions and a 970 bp fragment of the *cox1* gene was generated with primers ITS6/ITS4 (White *et al.*, 1990; Cooke *et al.*, 2000) and COXF4N/COXR4N (Kroon *et al.*, 2004), respectively. Sequencing was initiated using the same primers with dye-terminator cycle sequencing (FS sequencing kit, Applied Biosystems) on an ABI 3730 capillary sequencer. PCR fragments were sequenced in both forward and reverse orientations and edited using SEQUENCHER (GeneCodes). In the case of isolates P31 and P32 with heterozygous bases (see results), the ITS PCR products were cloned using standard methods into pGEM T-Easy (Promega) and 10 randomly picked clones of each isolate sequenced. Additional sequences from published phylogenetic analyses were obtained from GenBank via BLAST analysis. CLUSTALW (Thompson *et al.*, 1994) was used for sequence alignment. To minimize any disruption of the accurate Clade 6 taxa alignment, a prior alignment of only Clade 6 taxa sequences was generated and the CLUSTALW 'Profile' option used to subsequently align this to the sequences of the outgroup taxa. Bayesian phylogenetic analysis was conducted using a stand-alone version of MRBAYES v. 3.1.1 (Ronquist & Huelsenbeck, 2003). In addition, TOPALI v. 2.5 (Milne *et al.*, 2008) was used to run MRBAYES maximum likelihood (PHYML) and neighbour-joining (NJ) methods to examine the resolution of heterozygous bases in the ITS tree. Two duplicate MRBAYES analyses were run simultaneously for 2 million generations with a sampling frequency of every 10th generation and the first 20% of samples discarded as burn-in. The general time reversible model with gamma-distributed rate variation and a proportion of invariable sites was selected on the basis of the model selection in TOPALI. The default settings of a flat Dirichlet prior using the six rate parameter of the GTR substitution model with three heated chains (temperature = 0·2) and one cold chain were used. A 4by4 model of nucleotide evolution was used for the ITS analysis, and for the *cox1* gene data a codon position model was applied. At the end of each run the convergence diagnostic potential scale reduction factor (PSRF) values for the taxon bipartitions were examined to ensure they were at or approaching a value of one. In addition, the output files were examined in TRACER v. 1.5 (Rambaut & Drummond, 2007) to assess the levels of convergence and mixing after the burn-in period.

Morphology and growth-temperature relationships

Colony morphology, radial growth rate and cardinal temperatures for growth were assessed following the method of Brasier *et al.* (2003b). Two 90 mm Petri dishes of CA, cV8A and potato dextrose agar (PDA; Oxoid) (20 mL each) were inoculated centrally with mycelial agar plugs taken from an actively growing colony kept at 25°C. Inoculated plates were incubated in the dark for 2 weeks at different temperatures. Optimum, maximum and minimum temperature for growth were tested between 23 and 38°C or at 2–5°C, respectively, with 1 ± 0·2°C increments in at least two independent experiments. Daily radial growth rates were calculated from the 7-day diameter data of cultures grown at 25 ± 0·2°C on all three media. In maximum and minimum temperature limit tests, CA plates inoculated with three 4 mm plugs were immediately transferred to the test temperatures and the temperature with at least trace radial growth after 3 days of incubation in darkness was recorded. Plates were incubated at 25°C for three additional days to check regrowth. Colony morphologies were assessed from 7-day-old cultures grown at 25°C.

Production of sporangia, hyphal swellings, chlamydospores and gametangia were examined after 2 weeks in all agar media used for the growth tests. Isolates of *P. lacustris* were tested for heterothallism and their ability to induce gametangial formation by pairing isolates of the same taxon together, and by pairing single isolates with both A1 and A2 sexual compatibility type testers of *P. cambivora*, *P. cryptogea* and *P. drechsleri* as described by Brasier *et al.* (2003a). Pieces from the growing margins of fresh V8A cultures were overlaid with non-sterile soil filtrate (Jung *et al.*, 1996) to induce sporangium formation. Dimensions of 30–50 mature sporangia, chosen at random, and other characteristic features were analysed using a light microscope at ×400 magnification.

Pathogenicity

The isolates used in the following pathogenicity tests are indicated in Table 1. In central Italy, pathogenicity of *P. lacustris* and *P. gonapodyides* towards roots of 14–18-week-old seedlings of *Pr. persica* and *A. glutinosa* was tested in a soil infestation test at 20°C as described by Jung *et al.* (1996). There were five replicates per isolate and plants were exposed to two 24 h flooding periods, at days 5 and 15. An isolate of *P. megasperma* was used as a pathogen control. After 65 days, necrotic and healthy roots were separated (based on macroscopic criteria, resilience, brittleness, bark integrity and colour of the stele) and dried and weighed as described by Vettraino *et al.* (2003). Additional tests analysed the potential virulence of *P. lacustris* towards stems and twigs of several important woody hosts by wound inoculations. In Sicily, southern Italy, stem inoculations using a modified procedure of Scott *et al.* (1992) were performed in a greenhouse on 2-year-old seedlings of five species and hybrids of *Prunus* (Table S1). *Phytophthora inundata* (two isolates), *P. cambivora* (one) and *P. megasperma* (one) were included for comparison. There were 12 replicate seedlings per isolate. Lengths of cambial lesions were measured after 2 months. In Hungary, pathogenicity of *P. lacustris* to alder was tested by stem inoculation on 8–10-year-old *A. glutinosa* in a natural field stand, using

the method of Jung *et al.* (1996). Two isolates of *P. gonapodyides* and one of *P. alni* ssp. *alni* were included for comparison (Table S2). There were three replicate trees per isolate. After 7 months (May to December 2005), lesion sizes were measured and lesion areas calculated from the weight of tracings on paper. In Germany, 1-year-old, 15 cm long woody twigs of field grown *A. glutinosa* and *Salix alba* were inoculated *in vitro* with *P. lacustris* or *P. gonapodyides* (16 and six isolates, respectively), as described by Jung & Nechwatal (2008), with five replicates per isolate. Lengths of the necroses were recorded after 1 week. Three separate tests were performed and the data combined. Mock-inoculated controls were included in all tests, and re-isolations attempted by plating necrotic tissues on PARPBH medium.

Results

Isolation

In Hungary, seven isolates of *P. lacustris* were obtained from five out of 22 soil samples collected at five different locations. Three isolates were recovered from necrotic alder roots, and four isolates from soil. In total, *P. lacustris* was obtained from five out of 20 alder stands. In Germany, the species was regularly found in littoral soil samples from reed stands of three freshwater lakes. In total, 20 isolates were obtained, with *P. lacustris* being isolated from 16 out of 40 sites sampled. In southern Italy, *P. lacustris* was consistently isolated from necrotic *Prunus* roots and stem bases in a waterlogged commercial peach orchard. Furthermore, *P. lacustris* isolates were obtained from necrotic roots of a peach tree in a commercial nursery in Emilia Romagna (northern Italy). Five isolates out of a total of 35 (four from southern, one from northern Italy) were arbitrarily selected for this study (Table 1).

Molecular and phylogenetic analysis

All *P. lacustris* isolates investigated in this study were identified and confirmed using either species specific PCR or ITS sequence data, as indicated in Table 1. Representative ITS and *cox1* sequence data from this study have been submitted to GenBank (for accession numbers see Table 1).

The ITS data set had 839 characters with 146 (17·4%) that were parsimony informative and an average pairwise distance between accessions of 0·032. Seven of the nine isolates of *P. lacustris* had identical ITS sequences, with a 100% match with sequences of GenBank entries previously designated as *P.* t. Salixsoil (AF266793, AF541909; Brasier *et al.*, 2003b). Two isolates, P14 (USA) and ICMP16270 (NZ) had one and two heterozygous bases, respectively, in ITS2. Seven heterozygous bases and a 1 bp indel discriminated two isolates (P31 and P32 from an irrigation pond in the USA) of a closely related taxon. Sequencing of 10 clones each of these isolates resolved the ITS haplotypes, indicating that one type (nine clones from P31 and eight from P32) had six of the seven heterozygous bases in common with *P. lacustris*, whereas the other (one clone from P31 and two from P32) shared only two of the seven bases with *P. lacustris*. The latter type was identical to an ITS sequence of a novel taxon reported as 'new species 2' from Alaska and Oregon (Reeser *et al.*, 2011).

The Bayesian phylogenetic tree (Fig. 1a) showed the nine isolates of *P. lacustris* forming a well-supported clade, basal to all Clade 6 taxa except *P.* t. asparagi. The two representative cloned haplotypes of isolates P31 and P32 grouped with *P. lacustris* or with the 'new species 2' (Reeser *et al.*, 2011). When the ITS sequences of these two isolates with the seven heterozygous bases coded for by the respective nucleotide ambiguity codes were included in the analysis, neither Bayesian methods (MrBayes or BEAST), nor ML analysis (PHYML in TOPALi) discriminated these isolates from *P. lacustris* (data not shown). However, the NJ method implemented in TOPALi did account for these bases and placed P31 and P32 in a position intermediate between *P. lacustris* and the 'new species 2' (Reeser *et al.*, 2011; Fig. 1b).

The mtDNA *cox1* data set had 714 characters with 119 (16·7%) that were parsimony informative and an average pairwise distance between Clade 6 accessions of 0·065. Including the out-group taxa, these values were 147 (20·6%) and 0·0766, respectively. Amongst the nine sequences of *P. lacustris*, only two bases in the alignment were polymorphic. Three nucleotides (G, A or T) were observed at one of these positions and a single base change in isolate H-11/02 (Hungary) at another, which resulted in the minor subdivision within the *P. lacustris* subclade (Fig. 2). As in the ITS analysis, the most closely related taxon was represented by two isolates (P31 and P32) that differed from that of *P. lacustris* isolates at 23 bases and formed a distinct, well supported group at a basal position of the *P. lacustris* clade (Fig. 2). These nine isolates formed part of a larger clade of all the subclade II isolates. However, neither Bayesian nor ML methods (data not shown) resolved the structure of the basal roots of the three subgroups in this clade (Fig. 2).

Colony morphology

Most isolates of *P. lacustris* (e.g. P245) and all isolates of the closely related *P. gonapodyides* (e.g. H-4/02) developed very similar petaloid or chrysanthemum-like patterns with pointed lobes of different width, mostly submerged at the margins, and with appressed or little aerial mycelium towards the centre of the plate (Fig. 3). Lobes were large and loose on CA and cV8A, while cultures on PDA had small and dense, sometimes less clearly defined, lobes. On CA, two isolates of *P. lacustris*, P1/03 (Fig. 3) and H-15/02, were dome-shaped with larger amounts of aerial mycelium around the inoculum plug, whereas one isolate (P14) had an unusual radiate-stellate pattern.

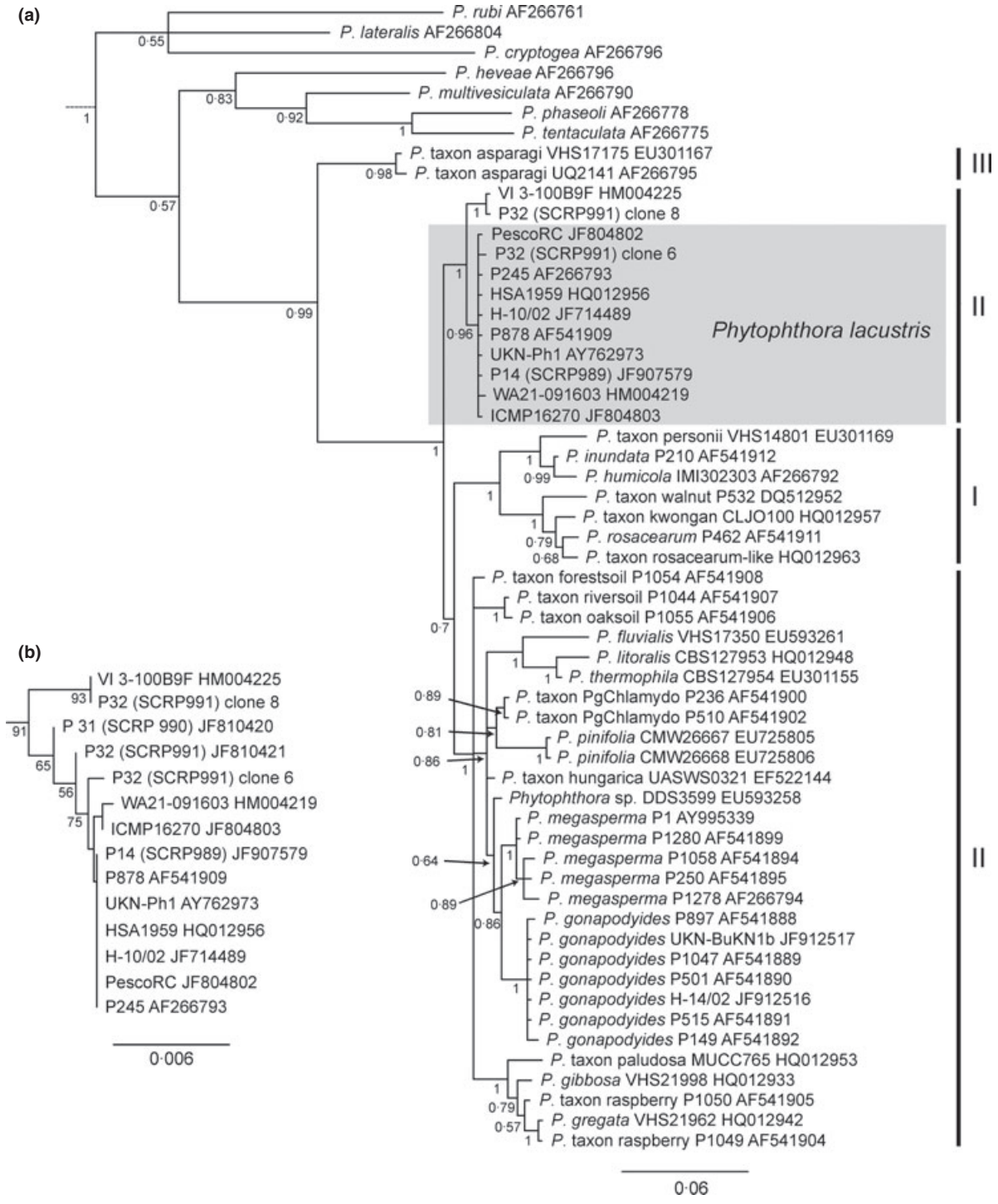

Figure 1 (a) Bayesian inference tree based on the rDNA ITS sequences of isolates of *Phytophthora* in ITS Clade 6 and a range of *Phytophthora* species as out-groups. Numbers below the branches indicate the posterior probability values. Previously defined subclades I-III (Brasier *et al.*, 2003b; Jung *et al.*, 2011a) are indicated. (b) Discrimination of *P. lacustris* isolates from pooled PCR products of two isolates of an undescribed taxon (P31 and P32) and representative cloned haplotypes according to neighbour-joining analysis. Numbers below the branches indicate the bootstrap values (n = 1000).

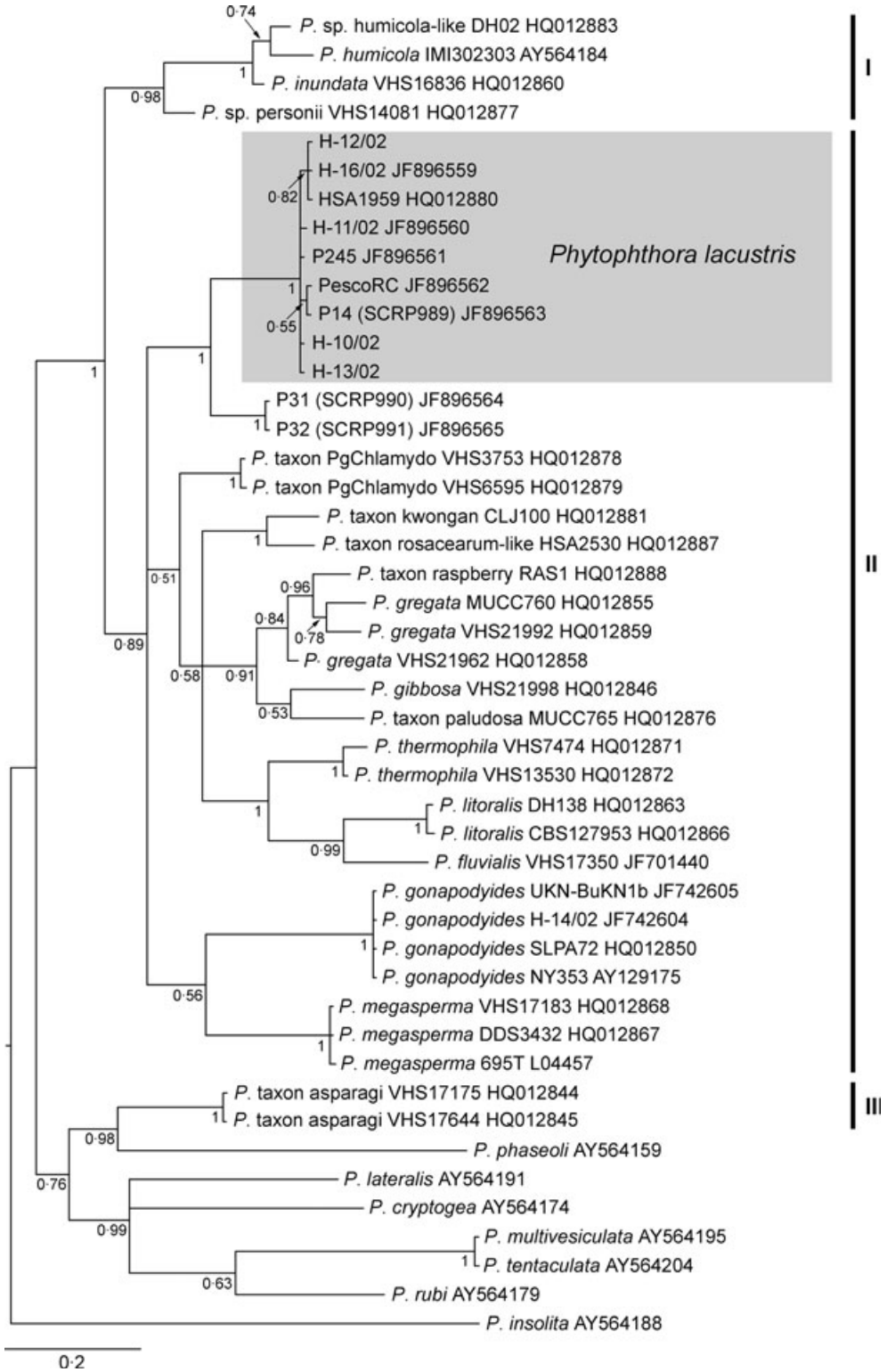

Figure 2 Bayesian inference tree based on sequences of the mitochondrial gene *cox1* of isolates of *Phytophthora* in ITS Clade 6 and a range of *Phytophthora* species as out-groups. Numbers below the branches indicate the posterior probability values. Previously defined subclades I-III (Brasier *et al.*, 2003b; Jung *et al.*, 2011a) are indicated.

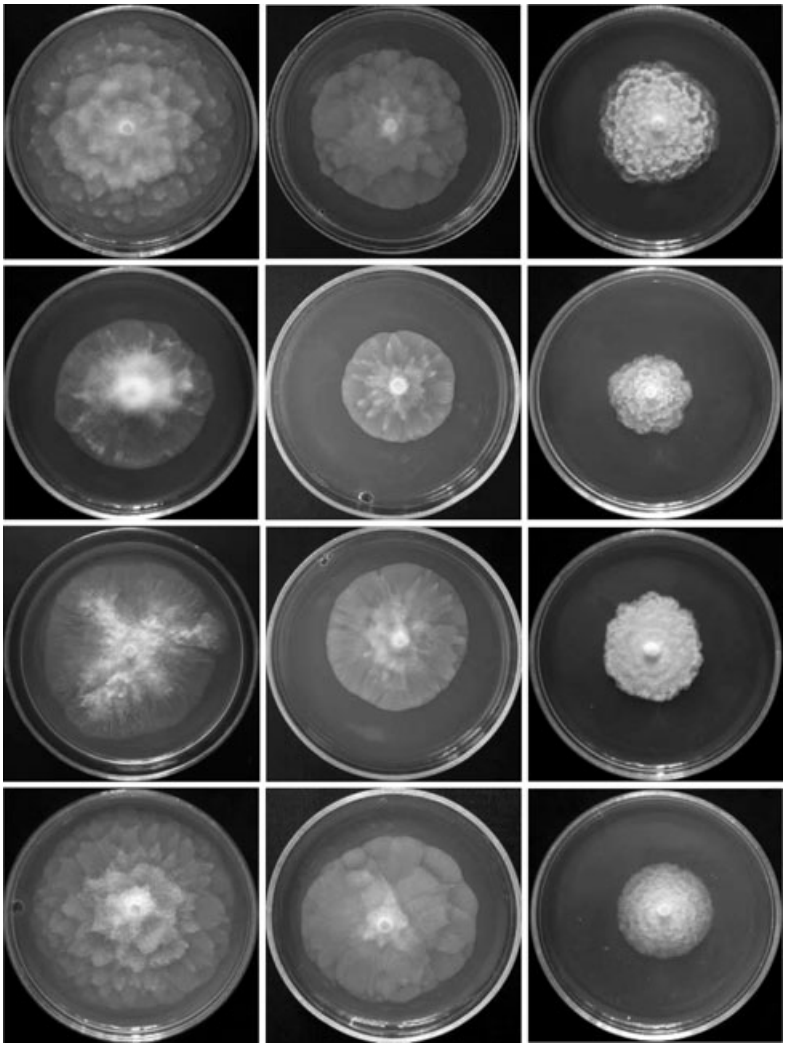

Figure 3 Colony morphology of *Phytophthora lacustris* isolates (from top to bottom) P245, P1/03 and P14, and *P. gonapodyides* isolate H-4/02, on (from left to right) carrot agar, clarified V8 juice agar and potato dextrose agar after 7 days growth at 25°C.

Morphology

Hyphal swellings, chlamydospores and sporangia were absent on solid agar media. Sporangia were produced abundantly by all *P. lacustris* isolates after 1 day when colony plugs were submerged in non-sterile soil filtrate. Non-caducous, non-papillate, mostly ovoid (Fig. 4a–h, n–o) or obpyriform (Fig. 4i,j) sporangia sometimes widening towards the base (Fig. 4k) with wide exit pores developed terminally on simple, mostly unbranched sporangiophores. Sporangia usually proliferated internally in both a nested and extended way (Fig. 4a,b,m,n,p). External proliferation was also observed, with new sporangiophores emerging just below the mature sporangium (Fig. 4l). Sporangial dimensions of the 34 *P. lacustris* isolates analysed averaged 44·8 ± 4·6 × 30·9 ± 3·5 μm (range of isolate means 35·8–54·1 × 25·7–38·8 μm). Length/breadth ratio averaged 1·44 ± 0·1 (range of isolate means 1·27–1·68). By comparison, sporangia of the closely related *P. gonapodyides* proved to be significantly larger than those of *P. lacustris* ($P \leq 0{\cdot}001$, unpaired *t*-test). Sporangial dimensions of a set of 13 isolates of this species averaged 52·6 ± 4·3 × 36·3 ± 3·8 μm (range of isolate means 46·3–59·8 × 27·2–39·7 μm). Mean length/breadth ratio of *P. gonapodyides* was 1·46 ± 0·13 (range of isolate means 1·27–1·70).

Subglobose to ellipsoid hyphal swellings were infrequently formed by most isolates of *P. lacustris* in non-sterile soil filtrate (Fig. 4q). None of the isolates of *P. lacustris* produced gametangia in single culture, or in direct pairings with each other. Some isolates inconsistently induced sparse and scattered formation of bullate oogonia of *P. cambivora* type when directly paired with an A2 tester strain of *P. cambivora* on the same CA plate (data not shown).

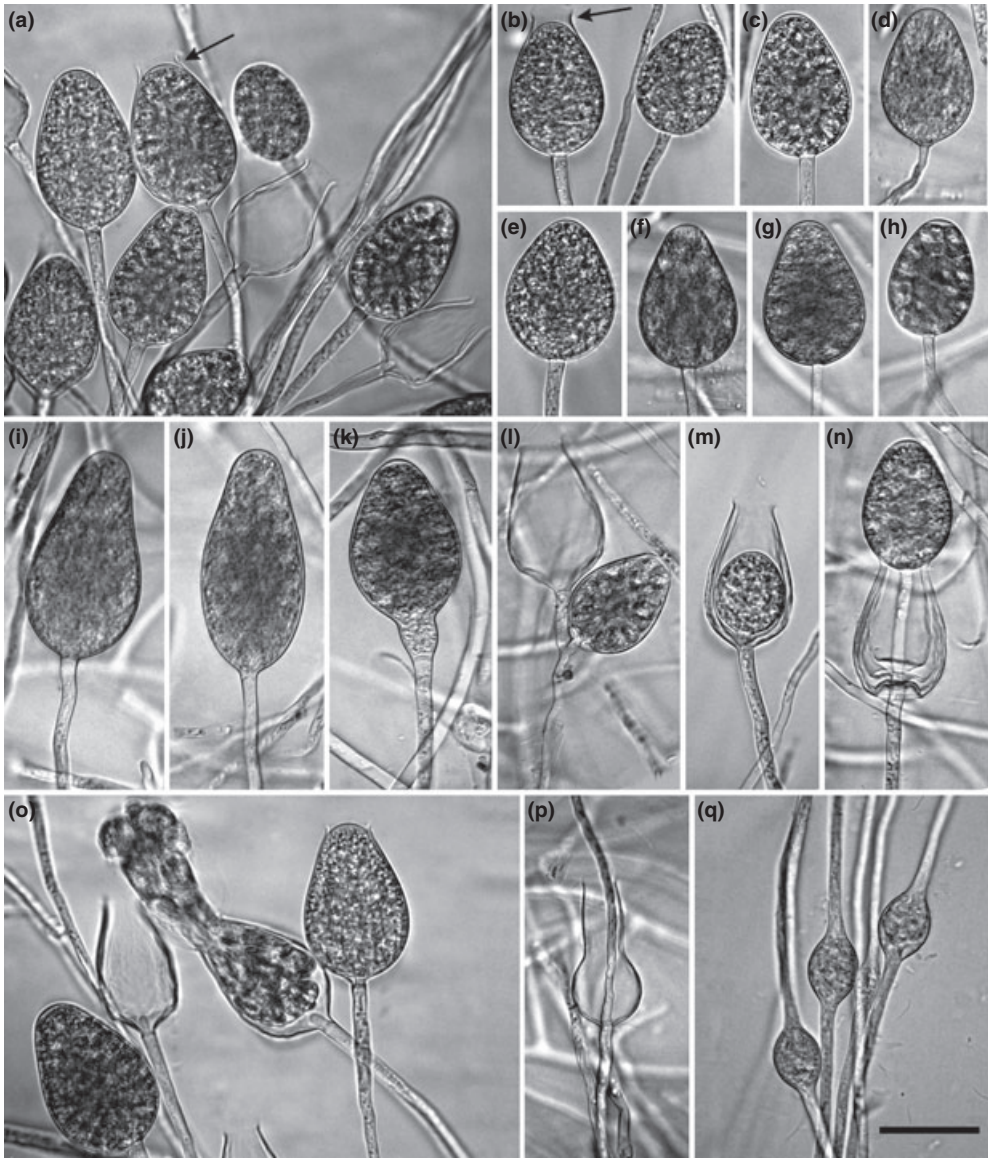

Figure 4 Morphological structures of *Phytophthora lacustris* formed on V8 agar flooded with non-sterile soil extract. (a–p), non-papillate sporangia: (a) empty and mature ovoid sporangia, one formed by nested proliferation (arrow); (b) ovoid sporangium, formed by nested proliferation (arrow); (c–g) mature ovoid sporangia; (h) mature ovoid sporangium with already differentiated zoospores; (i–j) mature elongated-obpyriform sporangia; (k) widening of sporangial base; (l) external sporangial proliferation close to the base of an empty sporangium; (m) internal nested proliferation; (n) internal nested and extended proliferation; (o) zoospore release; (p) internal extended proliferation; (q) ellipsoid hyphal swellings. Scale bar = 25 μm.

Temperature–growth relationships

The growth rates of 26 *P. lacustris* isolates on CA, cV8A and PDA were tested at 25°C. The average daily growth rate of *P. lacustris* ranged from 3·4 to 6·1 mm per day on CA, from 2·7 to 4·5 mm per day on cV8A, and from 1·8 to 2·8 mm per day on PDA. The optimum temperature for growth, tested for 14 *P. lacustris* isolates, ranged from 28 to 33°C, and the maximum temperature for growth, tested for 26 isolates on CA, ranged from 36°C to 37°C. None of the *P. lacustris* isolates grew at 38°C, but many resumed growth when returned to 25°C after 3 days of incubation at 38°C. The minimum temperature for trace growth (0·5 mm during 3 days) on CA was 2–4°C for all 16 *P. lacustris* isolates tested. Growth rates of *P. gonapodyides* at 25°C were similar to those of *P. lacustris* on two of the three agar media and significantly lower on PDA. Optimum and maximum temperatures were 24–26°C and 34–35°C, respectively, for *P. gonapodyides*, i.e. they were significantly lower. A comparison of the growth rates and cardinal temperatures of the two species is given in Table 2.

Table 2 Comparison of radial growth rates and cardinal temperatures for growth between *Phytophthora lacustris* and *P. gonapodyides*. Means within the same growth medium or within the same temperature range followed by the same letter are not significantly different ($P \leq 0{\cdot}05$, unpaired *t*-test)

	Radial growth rate at 25°C (mm/day)						Cardinal growth temperatures (°C)					
	CA		cV8A		PDA		Lower limit[a]		Optimum		Upper limit[a]	
Species (no. of isolates)	PL (26)	PG (6)	PL (26)	PG (6)	PL (26)	PG (6)	PL (16)	PG (4)	PL (14)	PG (6)	PL (26)	PG (5)
Mean (SD)	5·1 (0·7)a	4·6 (0·3)a	3·6 (0·6)a	3·7 (0·4)a	2·3 (0·3)a	2·0 (0·3)b	3·2 (0·5)a	3·0 (0)a	29·4 (1·4)a	25·0 (0·6)b	36·9 (0·3)a	34·4 (0·5)b
Min.	3·4	4·2	2·7	3·3	1·8	1·6	2	3	28	24	36	34
Max.	6·1	4·9	4·5	4·3	2·8	2·2	4	3	33	26	37	35

PG: *Phytophthora gonapodyides*, PL: *Phytophthora lacustris* (for isolates see Table 1).
[a]Indicates temperature at which at least trace (≥0·5 mm) growth occurred during 3 days of incubation.

Pathogenicity

Soil infestation test on Alnus *and* Prunus

All *Phytophthora* isolates, including *P. lacustris,* caused a significant reduction of total root dry weight (*c.* 50%) on *A. glutinosa* seedlings compared to the controls (Table 3). Only *P. megasperma* significantly reduced the total root dry weight of *Pr. persica*. All isolates of *P. lacustris*, *P. gonapodyides* and *P. megasperma* caused significant root damage, and fine root necroses on both host species. On average, the proportion of necrotic lateral roots was higher in *A. glutinosa* than in *Pr. persica*. None of the seedlings died during the experiment.

Stem inoculation tests on Prunus

Phytophthora lacustris was moderately aggressive to almond, peach and GF677, causing lesions of *c.* 3, 6 and 7 cm in length, respectively (Table S1). Like *P. inundata* and *P. megasperma*, it was non-pathogenic to cherry and *Pr. cerasifera* cv. Mirabolan. *Phytophthora cambivora* proved to be the most virulent species; it was pathogenic to all tested rootstocks except cv. Mirabolan and was the only species that caused death of seedlings. No lesions developed on control plants.

Stem inoculation tests on Alnus

Bark lesions were small (*c.* 4–5 cm^2), similar in size for all *P. lacustris* isolates, and comparable to those caused by *P. gonapodyides* (Table S2). Statistical analysis indicated that they were not significantly different from the lesions developing on control trees (<1 cm^2). However, they were significantly smaller than those caused by the aggressive alder pathogen *P. alni* ssp. *alni*.

Abscised twig assay on Alnus *and* Salix

Bark lesions caused by *P. lacustris* and *P. gonapodyides* were generally relatively small on both species (*c.* 1–2 cm in length; Table S3). On *Salix* twigs, *P. lacustris* caused significantly larger lesions than *P. gonapodyides*. No lesions developed on any of the control twigs.

In all experiments, the *Phytophthora* spp. initially inoculated were successfully reisolated from necrotic tissues of the test plants, while no *Phytophthora* sp. could be isolated from the control plants.

Table 3 Total root dry weight (g) and percentage of necrotic lateral roots of seedlings of *Alnus glutinosa* and *Prunus persica* inoculated with *Phytophthora lacustris* and other *Phytophthora* spp. in a soil infestation test. Mean values within columns followed by the same letters are not significantly different ($P \leq 0{\cdot}05$, one-way ANOVA, Tukey's multiple comparison test)

	Host species			
	Alnus glutinosa	*Prunus persica*	*Alnus glutinosa*	*Prunus persica*
Phytophthora sp. (isolate code)	Total root dry weight (g)		Necrotic lateral roots (%)	
P. lacustris				
P245	3·4a	3·9a	78·8a	73·8a
P878	2·7a	4·2a	99·5b	83·5a
Pesco 1	2·7a	4·3a	97·6b	69·4a
H-11/02	3·5a	3·4a	92·7b	68·1a
P. gonapodyides				
H-4/02	2·7a	3·7a	97·3b	71·4a
H-14/02	3·1a	4·4a	96·4b	75·6a
P. megasperma	3·4a	2·2b	92·9b	75·0a
Control	6·3b	5·9a	0c	0b

Taxonomy

Phytophthora lacustris Brasier, Cacciola, Nechwatal, Jung & Bakonyi, sp. nov.

MycoBank no. MB564262; Fig. 4. Etymology: *lacustris* (Lat. = from lakes, lacustrine) refers to the preferred habitat of this species in wet habitats or water.

Phytophthora lacustris P. gonapodyides similis est. Coloniae crescunt in agaris 'V8A', 'CA' et 'PDA' inter 3 et 37°C, optime ad 28–30°C. Incrementum radiatum quotidianum 5·8 mm in agaro 'CA' ad 25°C. Coloniae in agaro 'CA' petaloideae cum mycelio aerio restricto. Chlamydosporae non observatae. Culturae steriliae, oosporae non observatae. Sporangia formata abundantia in cultura aqua submersa. Sporangiophora simplicia, raro ramosa in sympodiis laxis. Sporangia abundantia in cultura liquida submersa, terminalia, nonpapillata, ovoidea vel obpyriformia, in medio 41·0 × 29·5 μm (30–55 × 22·5–37·5 μm), ratio longitudo ad altitudinem in medio 1·39 (1·20–1·62). Proliferationes sporangiorum internae, niduformes et extentae, et externae. Inflationes

hypharum rarae, subglobosae aut ellipsoideae, 12–18 × 7·5–10 μm. Regiones 'rDNA ITS' et *cox1* cum unica sequentia (GenBank AF266793, AY564181).

Colonies growing between 3 and 37°C with optimum growth at 28–30°C; daily growth rate on CA at 25°C 5·8 mm. Colonies on CA with petaloid patterns, submerged at the margins, with appressed or little aerial mycelium towards the centre of the plate. *Chlamydospores* not observed. *Oogonia* and oospores not observed in single cultures, but the isolate can act as 'silent A1', remaining sterile but inducing gametangial formation in A2 isolates of other *Phytophthora* species. *Sporangia* abundantly produced in water culture, terminally on mostly unbranched sporangiophores, non-papillate, ovoid or obpyriform, mean 41·0 × 29·5 μm (range 30–55 × 22·5–37·5 μm), mean LB ratio 1·39 (range 1·20–1·62), mostly proliferating internally, nested or extended, sometimes externally. *Hyphal swellings* rarely produced, subglobose or ellipsoid, 12–18 × 7·5–10 μm in size.

Specimens examined

Typus. United Kingdom: Bexley Heath, Kent, isolated from diseased root of *Salix matsudana*, 1972, C. M. Brasier & R. G. Strouts. Holotype P245 (dried culture on CA, herbarium of the Plant Protection Institute, Hungarian Academy of Sciences, Budapest, under accession number PPIHAS-P566). Ex-type culture P245 (UK Forestry Commission Culture Collection = IMI 389725 = WPC P10337). For other specimens examined see Table 1.

Notes. In previous studies *P. lacustris* is referred to as *P.* taxon Salixsoil (e.g. Brasier *et al.*, 2003b; Nechwatal & Mendgen, 2006; Jung & Nechwatal, 2008; Burgess *et al.*, 2009; Jung *et al.*, 2011a; Orlikowski *et al.*, 2011; Reeser *et al.*, 2011). Prior to the publication of Brasier *et al.* (2003b), isolates of *P. lacustris* are referred to as *P. gonapodyides* (e.g. Brasier *et al.*, 1993; Cooke *et al.*, 2000; Cacciola *et al.*, 2001). Many accessions of unnamed *Phytophthora* isolates in the NCBI GenBank matching the sequence data of *P. lacustris* provide evidence that the new species has been isolated regularly from diverse ecosystems worldwide.

Discussion

Forming neither gametangia nor chlamydospores, *Phytophthora lacustris* sp. nov. appears to be an evolutionarily reduced *Phytophthora* with only a few characteristic morphological structures. Nevertheless, the history of *P. lacustris* nicely encapsulates the development of species concepts in *Phytophthora*.

When *P. lacustris* was first isolated in 1972, the species concept in *Phytophthora* was based mainly on the morphology of the reproductive structures in the genus, as reflected in the groups established by Waterhouse (1963). At that time the isolates of *P. lacustris* could not be distinguished morphologically from another reduced *Phytophthora*, *P. gonapodyides* and were therefore attributed to this taxon. During the 1970s and 1980s the morphological concept began to be challenged by more holistic, population-based species concepts involving behavioural characters, breeding systems, chromosome numbers and some molecular profiling in addition to the traditional criteria (Brasier, 1991). Application of a population approach, and in particular use of comparative protein patterns, led to the conclusion that isolates defined here as *P. lacustris*, although not readily morphologically distinguishable from *P. gonapodyides*, could be a separate taxon (Brasier *et al.*, 1993).

By the early 2000s the availability of molecular tools strengthened tests to establish whether population units defined by phenotype were unique evolutionary units and initiated the establishment of a natural phylogeny for the genus (Cooke *et al.*, 2000). Adoption of this approach confirmed that isolates defined here as *P. lacustris* – that fell within major Clade 6 of Cooke *et al.* (2000) – were indeed a unique ITS lineage, leading to their designation as *P.* taxon Salixsoil (Brasier *et al.*, 2003b).

The present study has shown on the basis of both nuclear ITS and mtDNA *cox1* gene sequences that isolates of *P. lacustris* form a distinct lineage in *Phytophthora* Clade 6, supporting their designation as a monophyletic taxon. Bayesian analyses of ITS data in this study (Fig. 1a) and that of Jung *et al.* (2011a) indicate a position basal to previously defined Clade 6 subclades I and II, whereas according to NJ analysis in a previous study (Brasier *et al.*, 2003b), *P. lacustris* was grouped at the base of Subclade II. Bayesian analysis of the *cox1* gene data did not resolve these basal branches within Clade 6 (Fig. 2). Phylogenetic analysis based on the *HSP*90 and the mtDNA *cox1* gene also placed *P. lacustris* in a basal position of Clade 6 (Jung *et al.*, 2011a).

Minor ITS and *cox1* sequence variation was observed amongst *P. lacustris* isolates from Europe, the United States, Australia and New Zealand but on present evidence this does not relate to geographical origin. Two isolates from the USA (P31 and P32) are closely related to *P. lacustris* but differ in colony morphology (J. Bakonyi, unpublished data) and by their ITS and *cox1* sequences (Figs 1 and 2). Within the ITS region of these two isolates, the seven base changes and the 1 bp indel were heterozygous. Discrimination of the two haplotypes by cloning indicated that one matched *P. lacustris* and the other matched the 'new species 2' from streams in Alaska and Oregon (Reeser *et al.*, 2011). Such data is consistent with a hybridization between *P. lacustris* and the other undescribed taxon. The widescale reports of *P. lacustris* in different continents suggest it is more abundant than both the intermediate form (represented only by P31 and P32) and the 'new species 2'. Further research is required to understand the potential hybridization in more detail, but this does not detract from the main findings of this study that support the description of *P. lacustris* as a novel and distinct taxon.

Apart from its unique ITS and *cox1* sequences, it is also shown here that *P. lacustris* can be distinguished from the morphologically similar *P. gonapodyides* by its smaller on average sporangia (as also shown by Nechwatal &

Mendgen, 2006) and by differences in its optimum and maximum temperatures for growth, both being significantly higher than in *P. gonapodyides* (as also shown by Brasier *et al.*, 2003b). It can be distinguished from the similar *P.* taxon Pgchlamydo (Brasier *et al.*, 2003b) and *P. litoralis* (Jung *et al.*, 2011a) by the latters' lower maximum growth temperatures and their ability to form chlamydospores; and from the similar *P. fluvialis* (Jung *et al.*, 2011b) by different colony growth patterns and by the latter's higher maximum temperature for growth (38°C).

Many Clade 6 species exhibit an unusual combination of behavioural properties compared to other *Phytophthora* clades, viz. an often riparian habitat, a tendency to tolerate high temperatures and to be either sexually sterile, partially infertile or inbreeding (Brasier *et al.*, 2003b; Jung *et al.*, 2011a). In this regard, *P. lacustris* is rather typical. It occurs naturally in lakes and rivers, has a high maximum temperature for growth, and does not produce gametangia *in vitro*. For the clade as a whole, this combination of characters has been suggested to be an adaptation to riparian conditions (Brasier *et al.*, 2003b; Jung *et al.*, 2011a). It has been proposed that, because of their frequent association with riparian detritus in lakes and rivers (Table 1), this and similar Clade 6 species such as *P. gonapodyides* may specialize in the colonization of fallen plant debris in water and perhaps also be efficient saprotrophs (Hansen & Delatour, 1999; Brasier *et al.*, 2003a,b). However, whether these species behave as true saprotrophs, able to compete efficiently with other microorganisms in non-living plant tissues in nature, has yet to be experimentally demonstrated. Their high temperature tolerance has been suggested to be associated with the ability to undertake litter breakdown (Hansen & Delatour, 1999; Brasier *et al.*, 2003b) or with the warm summer conditions at margins of water bodies (Jung *et al.*, 2011a). Possibly, the seasonal pattern in abundance found for *P. lacustris* by Reeser *et al.* (2011) in North America is also related to its high temperature tolerance. The tendency for many Clade 6 species, such as *P. lacustris,* to be sterile, inbreeding or to exhibit a breakdown of sexual reproduction has been suggested to be an adaptation to homogeneous and sometimes extreme riparian environments in which clonality is favoured over genetic heterogeneity (Brasier *et al.*, 2003b). These interpretations have recently been extended by the suggestion that, owing to its relatively high cost, sexual reproduction has been widely discarded in the clade in favour of maximizing zoospore production (Jung *et al.*, 2011a). Indeed, *P. gonapodyides* has been described as a 'weed' owing to the sheer abundance of its zoospores in water (Brasier *et al.*, 2003b). The ubiquity of *P. lacustris* in some habitats investigated in this study (e.g. reed belts, riparian alder stands) points in the same direction. Whether the ability of *P. lacustris* – like *P. gonapodyides* – to induce selfing in A2 isolates of some heterothallic species (Brasier *et al.*, 1993, 2003a) is a relict of their past sexual activity and their continued ability to produce diffusible, A1-specific chemical compounds involved in the A1/A2 compatibility reaction (Qi *et al.*, 2005), or whether it operates via another mechanism altogether remains to be shown.

In several instances *P. lacustris* and *P. gonapodyides* were found together at the same sites (Table 1, see also Reeser *et al.*, 2011). This may represent sympatric adaptive radiation, the two species occupying spatially overlapping but distinct niches. Alternatively, their apparent coexistence in some areas may be due to the recent chance introduction, establishment and spread of one or other species by man, their phenotypic differences reflecting adaptations to the conditions in their geographic origins. However, in contrast to the potential hybrid populations involving *P. lacustris* and another taxon in the USA (see above), these two closely related species have not yet been shown to form natural hybrids.

Phytophthora lacustris was first isolated from diseased roots of *S. matsudana* in the UK and has subsequently been isolated from lesions on roots of *Pr. persica* in Italy (Cacciola *et al.*, 2001), both after flooding. Recently it was isolated from root and collar rot of mature *Fraxinus excelsior* (Orlikowski *et al.*, 2011). It was shown to be pathogenic on inoculation to twigs of the riparian *S. alba* (Jung & Nechwatal, 2008), to stems of *Fr. excelsior*, *Betula pendula* and *Prunus padus* (Orlikowski *et al.*, 2011) as well as *Carpinus betulus*, *Fagus sylvatica* and *Quercus petraea* (J. Bakonyi, unpublished data). The present study has shown that *P. lacustris* causes significant fine root damage to flooded *A. glutinosa* and *Pr. persica* seedlings and that it is a weak to moderate bark pathogen on wounded stems or twigs of *Alnus*, *Prunus* and *Salix* species. Although it was less damaging in the bark tests than other well known aggressive *Phytophthora* spp., the soil infestation test showed that *P. lacustris* might be a significant invader of fine roots of trees stressed by episodes of flooding or drought. Thus, in addition to infecting plant detritus, *P. lacustris* may be an opportunistic fine root pathogen in (temporarily) wet habitats. Given the high frequency with which phytophthoras are being introduced from natural ecosystems into horticulture and forests, often causing considerable damage (Jung & Blaschke, 2004; Brasier, 2008; Moralejo *et al.*, 2008), this could be of some concern.

Evidence suggests that *P. lacustris* has entered the nursery trade in Europe and is causing root rots in commercial *Prunus* and other fruit tree plantations in Italy and central Europe (Cacciola *et al.*, 2001; T. Jung, unpublished data). In view of its tolerance of high temperatures and its ubiquitous presence in water bodies, it could be a threat to irrigated fruit tree crops in Mediterranean areas. *Phytophthora lacustris* is probably easily dispersed via latent infection and passive transport along natural water ways and irrigation systems. It also appears to be adapted to diverse climatic conditions, occurring along a broad latitudinal gradient. This could greatly facilitate contact of *P. lacustris* with susceptible plants in nurseries, plantations or natural environments, and with new potential hosts.

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Supporting Information

Additional Supporting Information may be found in the online version of the article at the publisher's web-site:

Table S1. Results of the pathogenicity test with *P. lacustris* and other *Phytophthora* spp. on stems of *Prunus* spp.

Table S2. Results of the pathogenicity test with *P. lacustris*, *P. gonapodyides* and *P. alni* ssp. *alni* on stems of *Alnus glutinosa* in the field.

Table S3. Results of the pathogenicity test with *P. lacustris* and *P. gonapodyides* on abscised *Alnus glutinosa* and *Salix alba* twigs.

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