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Phytophthora ipomoeae sp. nov., a new homothallic species causing leaf blight on *Ipomoea longipedunculata* in the Toluca Valley of central Mexico

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A *Phytophthora* species was found on blighted foliage of *Ipomoea longipedunculata*, a morning glory native to the highlands of central Mexico. Based on host range, morphology, allozymes, mitochondrial DNA haplotype and rDNA sequences it is concluded that a new *Phytophthora* species, *P. ipomoeae* sp. nov., is the causal agent of leaf blight disease on *I. longipedunculata*.

INTRODUCTION

The central highlands of Mexico, which include the Toluca Valley, are thought to be the centre of origin and diversity of both *Phytophthora infestans*, causal organism of potato late blight (Niederhauser 1991, Goodwin *et al.* 1992) found on wild and cultivated *Solanum* species, and the closely related *P. mirabilis*. *P. mirabilis* causes leaf blight on *Mirabilis jalapa*, which is commonly known as 'four o'clock' (Galindo & Hohl 1985). *Mirabilis* is in the family *Nyctaginaceae*, which is not closely related to the *Solanaceae*.

Several lines of evidence suggest that *P. mirabilis* and *P. infestans* evolved from one common ancestor. Based on morphology alone, these two species cannot be distinguished, except for the fact that they are host specific. Isozyme and RFLP analysis have shown these two species to be of unique and distinguishable genotypes (Goodwin *et al.* 1999). Goodwin (1996) postulated that both these species evolved sympatrically in the central highlands of Mexico, as both host plants co-occur in close proximity. It is thought that reproductive isolation and host plant specificity resulted in sympatric speciation (Goodwin *et al.* 1999).

Throughout the summer of 1999, *P. infestans* and *P. mirabilis* isolates were obtained from both native and cultivated host plant species, and amongst these were several isolates collected from *Ipomoea longipedunculata* (Sánchez Sánchez 1968) showing symptoms of blighted leaves and stems. *I. longipedunculata* is a native of

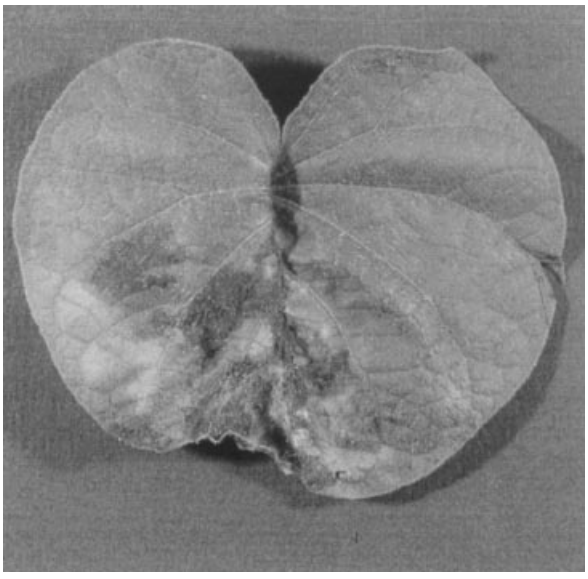

Fig. 1. Symptoms of *Phytophthora ipomoeae* on *Ipomoea longipedunculata* leaves.

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central Mexico and is occasionally found climbing shrubs and deciduous trees in shrub/woodland areas. The disease symptoms on *I. longipedunculata* were marked by purple black or brownish lesions in the centre of the leaf (Fig. 1), and the upper part of the stem. Under conditions of high relative humidity, sporangiophores bearing numerous sporangia appeared on the underside of the leaf.

The purpose of this paper was to test the hypothesis that the isolates collected from *I. longipedunculata* belong to a new *Phytophthora* species, closely related to *P. infestans* and *P. mirabilis* (Waterhouse 1963). Morphology, host specificity, allozyme patterns and rDNA sequences were studied to test the hypothesis. For convenience, we refer to the *Phytophthora* isolates obtained from *I. longipedunculata* as *P. ipomoeae* throughout this paper.

MATERIALS AND METHODS

Isolation

Isolates were obtained from single lesions on leaves of *Ipomoea longipedunculata* (Fig. 1) growing on a hill at Metepec in the Valley of Toluca. Pieces of tissue adjacent to the sporulating region of the lesion were cut out and surface sterilised by soaking them in 80% ethanol for 10 s and 0.5% sodium hypochlorite for 3 min followed by a rinse in sterile tap water for 1 min. Sterilised pieces were plated on Rye A agar (Caten & Jinks 1968) supplemented with ampicillin (200 mg l^{-1}), Benlate (50% WP, 100 mg l^{-1}), PCNB (75% WP, 67 mg l^{-1}), polymixin B (50 mg l^{-1}) and rifampicin (20 mg l^{-1}) (Forbes 1997). The six isolates obtained were maintained on Rye A agar at room temperature (20 ± 1 °) with transfers every three to four months.

In planta *formation of oospores*

Infected leaflets were examined for the presence of oospores. Leaflets with single lesions were incubated for 2 d at room temperature in water agar (10 g l^{-1}) Petri dishes. Leaflets were then clarified in boiling ethanol (96% v/v) for 5 min, bleached in 1% sodium hypochlorite for at least 6 h and mounted on microscope slides (Flier *et al*. 2001). Clarified leaflets were examined for the presence of oospores using a bright-field microscope at a magnification of $\times 100$.

Reference isolates

The six *Phytophthora* isolates obtained from *Ipomoea longipedunculata* were compared with six of *P. infestans* collected at the same time from cultivated potato (*Solanum tuberosum*) and the wild *S. stoloniferum*, and six of *P. mirabilis* from *Mirabilis jalapa*. These isolates were also collected in the Toluca valley within an area of approx. 2 km^2 around Metepec. In addition, a single isolate of *P. phaseoli* was obtained from the Centraal bureau voor Schimmelcultures (CBS), Utrecht. Characteristics of the strains used in this study are listed in Table 1. All reference isolates were maintained on rye A agar and stored under liquid nitrogen (Flier & Turkensteen 1999).

Morphology

Colony morphology and growth rate were compared on Rye A agar (RA), a minimal medium (MM) (Kamoun *et al*. 1994) and four reference media: Cherry decoction agar (CA), Potato Dextrose Agar (PDA), V-8 Agar, and Oatmeal Agar (OA). Agar plugs (5 mm diam) from the margins of actively growing 10-d-old

Table 1. Characteristics of the 19 *Phytophthora* isolates used in the morphology and allozyme comparisons.

Strain	Source	*Phytophthora* species	Host	Origin	Mating type
PIC 99010	PICTIPAPA[a]	*P. infestans*	*Solanum tubersosum*	Metepec, México	A2
PIC 99012	PICTIPAPA	*P. infestans*	*S. tubersosum*	Metepec, México	A1
PIC 99050	PICTIPAPA	*P. infestans*	*S. tubersosum*	Metepec, México	A1
PIC 99180	PICTIPAPA	*P. infestans*	*S. stoloniferum*	Metepec, México	A1
PIC 99181	PICTIPAPA	*P. infestans*	*S. stoloniferum*	Metepec, México	A1
PIC 99182	PICTIPAPA	*P. infestans*	*S. stoloniferum*	Metepec, México	A1
PIC 99105	PICTIPAPA	*P. mirabilis*	*Mirabilis jalapa*	Metepec, México	A2
PIC 99111	PICTIPAPA	*P. mirabilis*	*M. jalapa*	Metepec, México	A2
PIC 99121	PICTIPAPA	*P. mirabilis*	*M. jalapa*	Metepec, México	A2
PIC 99128	PICTIPAPA	*P. mirabilis*	*M. jalapa*	Metepec, México	n.a.
PIC 99131	PICTIPAPA	*P. mirabilis*	*M. jalapa*	Metepec, México	A2
PIC 99153	PICTIPAPA	*P. mirabilis*	*M. jalapa*	Metepec, México	A2
CBS 556.88	CBS[b]	*P. phaseoli*	Unknown	Unknown	S.F.[c]
PIC 99193	PICTIPAPA	*P. ipomoeae*	*Ipomoea longipedunculata*	Metepec, México	S.F.[c]
PIC 99194	PICTIPAPA	*P. ipomoeae*	*I. longipedunculata*	Metepec, México	S.F.[c]
PIC 99164	PICTIPAPA	*P. ipomoeae*	*I. longipedunculata*	Metepec, México	S.F.[c]
PIC 99165	PICTIPAPA	*P. ipomoeae*	*I. longipedunculata*	Metepec, México	S.F.[c]
PIC 99167	PICTIPAPA	*P. ipomoeae*	*I. longipedunculata*	Metepec, México	S.F.[c]
PIC 99169	PICTIPAPA	*P. ipomoeae*	*I. longipedunculata*	Metepec, México	S.F.[c]

[a] From the PICTIPAPA culture collection.
[b] From CBS.
[c] Oospores observed on agar media.

colonies were placed in the centre of Petri dishes (9 cm diam) and incubated at 20 °C. Four replicate plates were used. Radial growth was measured after 7 d in two perpendicular directions per plate with an electronic marking gauge (Mitutoyo Absolute digimaster, Veenendaal. Growth rates were corrected for plug diameter. Dimensions of sporangia for all four *Phytophthora* species and oospores of the homothallic species *P. phaseoli* and *P. ipomoeae* were measured for cultures grown on RA. All data are based on at least 30 measurements for each isolate. Oospore dimensions for *P. infestans* and *P. mirabilis* were measured from a mating of PIC 99010 × PIC 99012 and PIC 99111 × PIC 99124 on RA, respectively (Table 1).

Host specificity

Host specificity of a subset of *Phytophthora* isolates was tested by inoculating detached leaves of potato cv. 'Bintje', tomato cv. 'Moneymaker', *M. jalapa*, and *I. longipedunculata* as well as tuber slices of potato cv. 'Bintje' and sweet potato cv. 'A26/7' (*Ipomoea batata*). Detached leaflets, inoculated with six 10 µl droplets of sporangial inoculum (1×10^4 sporangia ml^{-1}) on the lower side of each leaf, were placed in Petri dishes (15 cm diam) filled with 15 ml of 2% water agar. Inoculum was prepared from 2-wk-old cultures grown on RA. Petri plates with the inoculated leaves were wrapped in transparent polythene bags and incubated for 14 d at 15 ° at a light intensity of 12 Wm^{-2}, 16 h light. Disease symptoms were assessed on a 0–3 scale (0, no symptoms; 1, small confined necrotic spots HR reaction; 2, expanding lesions with few sporangia; 3, abundant sporulation present). Disease symptoms were recorded at day 7, 10 and 14. The experiment was repeated twice. Tubers of potato cv. 'Bintje' and sweet potato were surface sterilized in 5% sodium hypochlorite for 5 min, rinsed in tap water and wiped dry with Kleenex tissue paper. Slices of approximately 8 mm thick were cut and placed in Petri dishes (9 cm diam); one slice in each Petri dish. A total of five slices were inoculated for each isolate. Slices were inoculated with a single 10 µl droplet of a sporangial inoculum (1×10^4 sporangia ml^{-1}), incubated for 10 d in closed Petri dishes in an incubator at 15 ° and 85% RH in the dark and evaluated for the presence of mycelium on the tuber slice surface. Presence of mycelium was considered as an indicator for the level of compatibility between the host and the pathogen. Disease symptoms were assessed on a 0–3 scale (0, no symptoms; 3, abundant mycelium present).

Allozyme and mtDNA haplotype characterisation

Allozyme genotype for glucose-6-phosphate isomerase (*Gpi*, EC 5.3.1.9) and peptidase (*Pep*, EC 3.4.3.1) was determined using cellulose acetate plates (Goodwin, Schneider & Fry 1995).

Mitochondrial DNA (mtDNA) was amplified using four sets of primers designed to amplify specific regions (P1–P4) of the mitochondrial genome of *P. infestans* (Griffith & Shaw 1998). PCR was performed according to Ordoñez *et al.* (2000). PCR products were digested with the restriction enzymes *Cfo*I (P1), *Msp*I (P2), and *Eco*RI (P3 & P4). Ten µl of the amplified product was digested with 1 unit of the restriction enzyme for 4 h. The digested products were run on a 1.8% agarose gel in TBE buffer at 10 V cm^{-1} and visualised with ethidium bromide under UV light.

rDNA amplification and sequencing

Isolates of *Phytophthora infestans* (PIC 97757), *P. mirabilis* (G4–6), *P. phaseoli* (CBS 556.88) and *P. ipomoeae* (PIC 99169) were taken from liquid nitrogen storage, revitalised on RA plates for 2 wk and grown for 10 to 14 d at 20 ° in clear pea broth. Mycelium was harvested and lyophilised (Ordoñez *et al.* 2000). DNA was isolated using the Puregene kit (Gentra/Biozym, Landgraaf) according to the manufacturer's instructions with slight modifications. ITS–PCR was performed using primers ITS1 and ITS4 (White *et al.* 1990, Cooke *et al.* 2000). The ribosomal DNA (rDNA) internal transcribed spacer region 1 (ITS1) and 2 (ITS2) products were directly sequenced on an ABI3700 automatic sequencer (Perkin–Elmer, Nieuwerkerk a/d IJssel). ITS sequences from various *Phytophthora* species, *Pythium aphanidermatum* and *Achlia bisexualis* were obtained from GenBank, based on reports by Crawford *et al.* (1996) and Cooke *et al.* (2000) (Table 5).

All sequences were aligned to identify any dissimilar nucleotides or deletions using the ClustalW method of Dambe Version 4.0 (Data Analysis in Molecular Biology and Evolution) (Xia 2000), available on-line Xuhua Xia at the University of Hong Kong. Phylogenetic inference was based on the Neighbour-joining method in Dambe.

RESULTS

Morphology and growth characteristics

All six isolates from *Ipomoea longipedunculata* grew well with radial growth rates of approx 10 mm d^{-1} on solid agar media such as Rye A agar (RA), Cherry

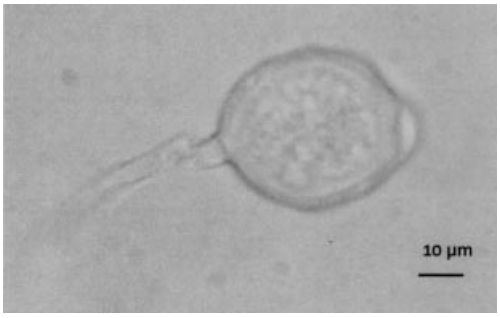

Fig. 2. *Phytophthora ipomoeae* sporangium shape on minimal medium.

Table 2. Growth rates, dimensions and allozyme alleles scored at *Pep* and *Gpi* loci for 19 isolates of *Phytophthora* species.

Isolate	*Phytophthora* species	Growth rate (mm day^{-1})		Sporangium dimensions (μm)		Oospore diam (μm)	Allozyme genotype	
		RA	MM	Length	Width		*Pep*	*Gpi*
PIC99010	*P. infestans*	5.8	0.0	35	23	–	100/100	86/122
PIC99012	*P. infestans*	3.4	0.0	30	19	–	100/100	100/122
PIC99050	*P. infestans*	9.8	1.9	32	20	–	100/100	100/100
PIC99180	*P. infestans*	9.4	3.6	30	19	–	100/100	100/100
PIC99181	*P. infestans*	6.9	4.5	29	20	–	100/100	86/100
PIC99182	*P. infestans*	6.9	2.1	33	21	–	100/100	86/100
PIC99105	*P. mirabilis*	8.1	8.7	32	17	–	96/96	83/111
PIC99111	*P. mirabilis*	3.3	5.5	34	16	–	96/96	100/100
PIC99121	*P. mirabilis*	10.9	6.4	35	19	–	96/96	90/111
PIC99128	*P. mirabilis*	10.1	3.4	31	17	–	96/96	83/108
PIC99131	*P. mirabilis*	8.2	4.8	28	14	–	96/96	100/111
PIC99153	*P. mirabilis*	10.2	7.2	30	16	–	96/96	90/100
CBS556.88	*P. phaseoli*	1.5	0.0	24	15	21	n.a.	n.a.
PIC99193	*P. ipomoeae*	12.9	6.0	36	19	29	78/96	108/108
PIC99194	*P. ipomoeae*	12.0	5.6	39	24	27	96/96	108/108
PIC99164	*P. ipomoeae*	9.9	4.0	40	21	31	78/78	108/108
PIC99165	*P. ipomoeae*	11.2	5.3	38	22	27	78/78	108/108
PIC99167	*P. ipomoeae*	10.3	5.1	44	25	29	78/78	108/108
PIC99169	*P. ipomoeae*	10.1	5.7	40	21	30	78/78	108/108

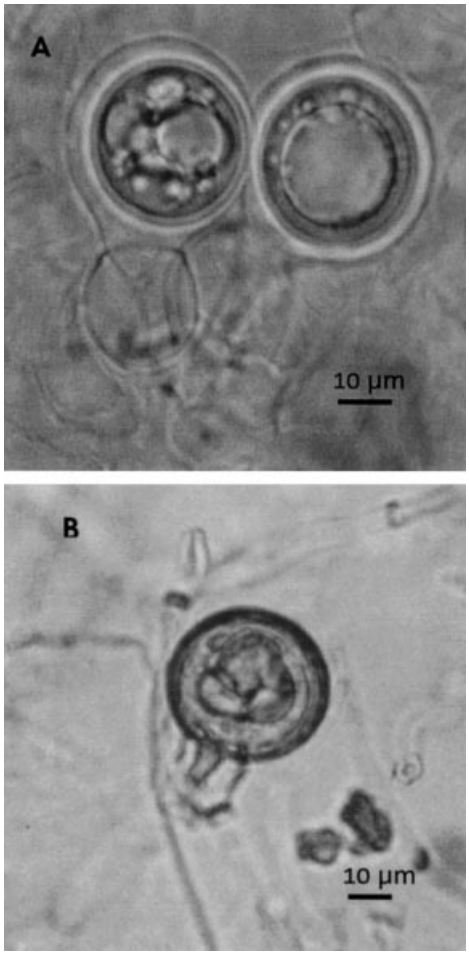

Fig. 3. *Phytophthora ipomoeae* oospores: (a) In clarified leaflets; (b) in Rye A agar medium.

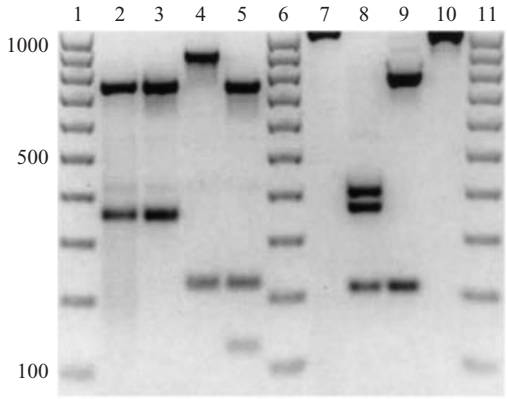

Fig. 4. Mitochondrial DNA haplotypes produced after PCR amplification and digestion of the P2 and P4 mtDNA region of *Phytophthora phaseoli*, *P. infestans*, *P. ipomoeae* and *P. mirabilis*. Lane 1, 6 and 11; DNA ladder (numbers indicate size in base pairs); lane 2 to 5, restriction fragments of amplified P2 region of *P. phaseoli*, *P. infestans* (1a haplotype), *P. ipomoeae* and *P. mirabilis*; lane 7 to 10, restriction fragments of amplified P4 region of *P. phaseoli*, *P. infestans* (1a haplotype), *P. ipomoeae* and *P. mirabilis*.

decoction agar (CA), Potato Dextrose Agar (PDA) and Oatmeal Agar (OA). Restricted radial growth of approx 5 mm d^{-1} was observed on Minimal Medium Agar (MM). Growth inhibition was observed on V-8 Agar (V8) with an average radial growth rate of 0.5 mm d^{-1} (data not shown). Colonies on MM exhibited a petaloid to rosaceous colony morphology while growth on nutrient-rich media like RA, CA, PDA and OA resulted in rather undefined fluffy aerial growth of mycelium. Hyphae were non-septate and moderately or freely branching with a hyphal diam ranging from 3.8 to 7.5 μm. Hyphal swellings on solid agar media were rarely observed.

Phytophthora ipomoeae isolates sparsely formed sporangiophores on the solid agar media tested. The long, aerial sporangiophores branched in a compound-sympodial and intermediate fashion. Occasionally,

Table 3. Pathogenicity of *Phytophthora* species on different host plant species.

Isolate	*Phytophthora* species	Disease symptoms present on different hosts					
		Solanum tuberosum cv. Bintje		*Solanum lycopersicon* cv. 'Moneymaker'	*Mirabilis jalapa*	*Ipomoea batata*	*I. longipedunculata*
		Detached leaflets	Tuber slices				
PIC 99010	*P. infestans*	3	3	3	0	0	0
PIC 99181	*P. infestans*	3	2	3	0	0	0
PIC 99111	*P. mirabilis*	1	0	0	2	0	0
PIC 99128	*P. mirabilis*	0	0	0	3	0	0
PIC 99193	*P. ipomoeae*	0	0	0	0	0	3
PIC 99167	*P. ipomoeae*	0	0	0	0	0	2
CBS 556.88	*P. phaseoli*	0	0	0	0	0	0

0, no symptoms; 1, small confined necrotic spots, HR reaction; 2, expanding lesions with few sporangia; 3, abundant sporulation.

Table 4. Mitochondrial DNA haplotypes detected after PCR amplification and restriction fragment length analysis of 4 mtDNA regions.

Primer	Restriction enzyme	Fragment	*Phytophthora* species						
			P. infestans						
			Haplotype Ia Ha*Pinf*Ia	Haplotype Ib[a] Ha*Pinf*Ib	Haplotype IIa[a] Ha*Pinf*IIa	Haplotype IIb[1] HaPinfIIb	*P. mirabilis* Ha*Pmir*I	*P. phaseoli* Ha*Ppha*I	*P. ipomoeae* Ha*Pipo*I
P1	*Cfol*I	211	+	+	−	−	+	+	+
		907	+	+	−	−	+	+	+
		1118	−	−	+	+	−	−	−
P2	*Msp*I	79	−	+	−	−	−	−	−
		129	−	−	−	−	+	−	−
		157	−	−	−	−	−	−	−
		193	−	−	+	−	−	−	−
		221	−	−	−	−	+	−	+
		350	+	+	−	+	−	+	−
		641	−	+	−	−	−	−	−
		720	+	−	+	+	+	+	−
		849	−	−	−	−	−	−	+
		1070	−	−	−	−	−	−	−
P3	*Eco*RI	230	+	+	−	−	+	−	+
		1078	+	+	−	−	+	−	+
		1308	−	−	+	+	−	+	−
P4	*Eco*RI	209	+	+	−	−	−	−	+
		361	+	+	+	+	−	−	−
		394	+	+	−	−	−	−	−
		603	−	−	+	+	−	−	−
		755	−	−	−	−	−	−	+
		964	−	−	−	−	+	+	−

[1] Data from Griffith & Shaw (1998).

swellings were present at the primordial sites of sporangia. Sporangia were semi-papillate, caduceus with a short pedicel, mainly ellipsoid (Fig. 2). At times, ovoid sporangia were noticed. Shape and dimension of sporangia varied considerably within and between isolates of *P. ipomoeae*, but no consistent differences between isolates were observed. Length of sporangia ranged from 35 to 47.5 μm (average 39 μm), breadth ranged from 17.5 to 265 μm (average 20.8 μm), with a length : width ratio of 1.9 (Table 2). Zoospores were readily released (within 4 h) from sporangia in a watery suspension at 10 °. On average 6.9 zoospores per sporangium were produced (range 4–8 zoospores).

Sexual structures were abundantly produced on RA, sparsely in OA, and were absent in CA, PDA V8 and MM. Distribution of sexual structures in solid agar media was under-dispersed, they were mainly found in clusters of approx. 50–100 oospores. Antheridia were amphigynous, with an average length of 19 μm (range 17.5–20 μm) and a length : width ratio of 1.3. Oogonia were spherical, smooth-walled, with an average diameter of 32.5 μm on RA. Oospores were smooth-walled, aplerotic to nearly plerotic, transparent to yellow (Fig. 3b). Oospore dimensions ranged from 25 to 32.5 μm in diam (average 28.8 μm). Oospores were readily formed in single cultures and isolates are consequently considered to be homothallic (Table 2).

In planta *formation of oospores*

Oospores were observed in 12 out of 17 leaflets of *Ipomoea longipedunculata* inoculated with single isolates. Amphigynous antheridia and aplerotic oospores

Table 5. Isolates of *Phytophthora* taxa, outgroups and groups (Stamps *et al.* 1990) used for the rDNA study.

Species	Isolate	GenBank[a] accession nos	Host	Groups
P. fragariae var. fragariae	IMI 330736	AF266762	*Fragaria × ananassa*	V
P. fragariae rubi	IMI 355974	AF266761	*Rubus idaeus*	V
P. macrochlamydospora	UQ205	–	*Glycine max*	III or IV
P. arecae	IMI 348342	AF266781	*Cocos nucifera*	II
P. cactorum	IMI 296524	AF266772	*Rubus idaeus*	I
P. cambivora	IMI 296831	AF266763	*Rubus idaeus*	VI
P. capsici	IMI 352321	AF266787	*Piper nigrum*	II
P. cinnamomi	UQ881	AF266764	*Syzygium aromaticum*	VI
P. citricola	IMI 031372	AF266788	*Rubus idaeus*	III
P. colocasiae	IMI 368918	AF266786	*Colocasia esculenta*	IV
P. cryptogea	IMI 045168	AF266796	*Lycopersicum esculentum*	VI
P. drechsleri	CBS 292.35	–	–	VI
P. erythroseptica	ATTC 36302	AF266797	*Solanum tuberosum*	VI
P. heveae	IMI 180606	AF266770	*Hevea brasiliensis*	II
P. humicola	IMI 302303	AF266793	Citrus orchard soil	V
P. ilicis	ILI1	AJ131990	*Ilex aquilifolium*	IV
P. inflata	IMI 342898	AF266789	*Syringa*	III
P. insolita	IMI 288805	AF271222	Soil	V
P. iranica	IMI 158964	AJ131987	*Solanum melongena*	I
P. katsurae	IMI 360596	AF266771	*Cocos nucifera*	II
P. lateralis	IMI 040503	AF266804	*Chamaecyparus*	VI
P. medicaginis	UQ125	AF266799	*Medicago sativa*	V
P. megasperma	IMI 133317	AF266794	*Malus sylvestris*	V
P. megakarya	IMI 337104	AF266782	*Theobroma cacao*	II
P. nicotianae	UQ848	AF266776	–	II
P. palmivora	UQ1294	AF266780	*Theobroma cacao*	II
P. phaseoli	CBS 556.88	–	–	IV
P. porri	CBS 782.97	AF266801	*Brassica chinensis*	III
P. sojae	UQ1200	AF266769	*Glycine max*	V
P. syringae	IMI 296829	AF266803	*Rubus idaeus*	III
P. tentaculata	CBS 552.96	AF266775	*Chrysanthemum leucanth.*	I
P. trifolii	UQ2143	AF266800	*Trifolium* spp.	V
P. vignae	UQ136	AF266766	*Vigna sinensis*	VI
P. infestans	PIC 97757	–	*Solanum demissum*	IV
P. mirabilis	G4-6	–	*Mirabilis jalapa*	IV
P. ipomoeae	PIC 99169	–	*Ipomoea longipedunculata*	IV
Pythium aphanidermatum	UQ2071	AF271227	Root rot	–
Achlya bisexualis	CBS 102.50	–	–	–

[a] rDNA sequences published by Cooke *et al.* (2000), with the exception of *Achlya bisexualis* (Crawford *et al.* 1996) and *P. infestans* PIC 97757, *P. mirabilis* G4–6 and *P. ipomoeae* PIC 99169 (Flier, unpubl.).

were present in clarified leaf samples (Fig. 3a). Oospores of *P. ipomoeae* were present sparsely to abundantly in leaf tissue, and showed a clumped distribution and a preference for oospore formation near the major veins of the leaves. Oospore diameters varied between oospores and leaf samples, but were in the range of 24–37 μm with an average of 28 μm.

Host specificity

Phytophthora ipomoeae is host specific for the range of hosts tested in this study. *P. ipomoeae* isolates rarely formed small, necrotic (< 2 mm diam) spots on potato and tomato leaflets in the detached leaflet bioassays (Table 3). However, no sporangiophores were observed during the incubation period of 14 d. No disease symptoms were observed when tuber slices of potato cv. 'Bintje' or leaves of *Mirabilis jalapa* and sweet potato (*Ipomoea batata*) were inoculated. Inoculation with either of the two *P. ipomoeae* isolates on *I. longipedunculata* led to sporulating lesions (Table 3). Isolates of *P. infestans* were found pathogenic on both potato and tomato, on which abundant sporulation occurred after 5–8 d after inoculation. Both potato and *M. jalapa* leaf tissues were colonised by *P. mirabilis*, isolate PIC 99111 causing necrotic flecking and small sporulating lesions on Bintje leaflets 10 d after inoculation. No disease symptoms were observed when *P. phaseoli* was inoculated on the host plant species included.

Allozyme mtDNA and rDNA diversity

Genetic marker studies were applied to investigate the taxonomic status of *Phytophthora ipomoeae*. The *Pep* banding patterns of *P. ipomoeae* isolates consisted of the 78 and 96 alleles, with four isolates being homozygous for the *Pep* 78 allele. This allele was unique for isolates of *P. ipomoeae* while the *Pep* 96 allele was also found in *P. mirabilis*. The *Gpi* banding pattern of the *P. ipomoeae* isolates tested appeared to be 108/108 on cellulose-acetate gels, which is different from the

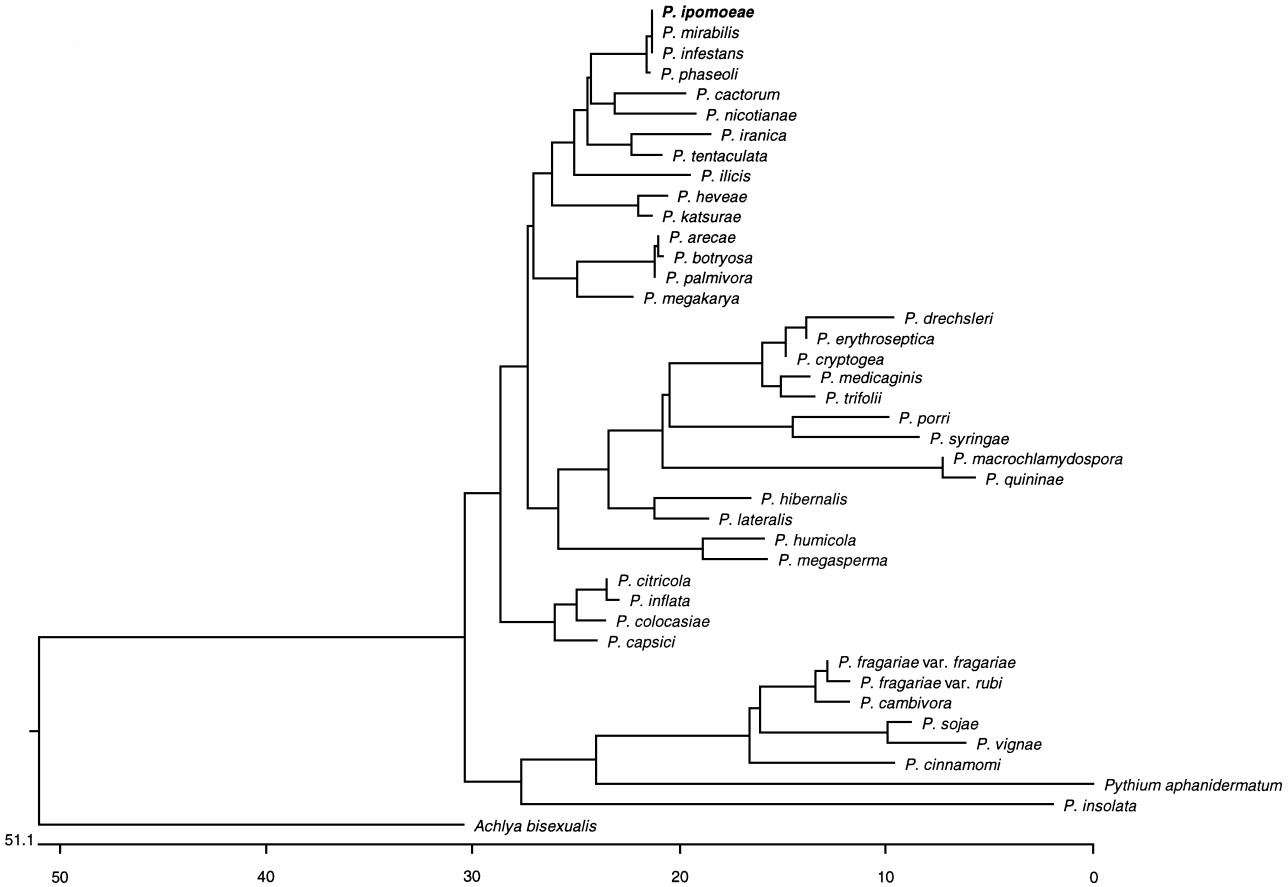

Fig. 5. Phenogram based on the ITS 1 region of the genomic ribosomal RNA tandem gene repeat for 39 *Phytophthora* taxa, one *Pythium* and one *Achlya* species. The phenogram was constructed after DNA distance-based and neighbour-joining analysis of the data.

predominant genotypes of both *P. infestans* (Grünwald *et al*. 2001) and *P. mirabilis* (Flier *et al*., unpubl.) in the Toluca Valley. This *Gpi* allele was also detected in one *P. mirabilis* isolate. No allozyme data are available for *P. phaseoli*.

Restriction fragment length analysis of amplified mitochondrial DNA revealed that *P. infestans* isolates from both potato and *Solanum stoloniferum* were of the haplotype Ia, but isolates of *P. mirabilis*, *P. phaseoli* and *P. ipomoeae* are all marked by mtDNA haplotypes that are distinct from those reported for *P. infestans* (Griffith & Shaw 1998) (Table 4). Amplification of mtDNA with each of the four primer sets in every case yielded a band that corresponded to published results for *P. infestans*. *P. mirabilis*, *P. phaseoli* and *P. ipomoeae* were characterised as haplotype Ia or Ib according to restriction analysis of the P1 mtDNA region. Digestion of the P3 region placed *P. mirabilis* and *P. ipomoeae* together into class I haplotypes and *P. phaseoli* into class II haplotypes. After digestion of the P2 and the P4 region, differences were detected between the described *P. infestans* haplotypes and *P. mirabilis*, *P. phaseoli* and *P. ipomoeae* haplotypes. Amplification of the P2 region and digestion with *Msp*1 produced a novel two-band pattern in *P. ipomoeae*, that included the reported 203 bp fragment characteristic for the IIa and IIb haplotype in *P. infestans* and a long novel fragment of approx 867 bp (Table 4). *P. mirabilis* and *P. phaseoli* were characterised as IIa and Ia or IIb respectively. Restriction analysis of the P4 region yielded novel haplotypes for *P. mirabilis*, *P. phaseoli* and *P. ipomoeae* not described before in *P. infestans*. In both *P. mirabilis* and *P. phaseoli*, restriction of the 964 bp P4 amplification product with *Eco*R1 failed, indicating the absence of at least two restriction sites when compared to *P. infestans*. The *P. ipomoeae* haplotype was characterised by a two-band pattern which consisted of the 209 and 755 bp fragments, recently described for Ecuadorian isolates similar to *P. infestans* but with a different host range (Ordoñez *et al*. 2000).

Phenograms based on the ITS1 region of 39 *Phytophthora* spp., *Pythium aphanidermatum* and *Achlya bisexualis* reveal that *P. ipomoeae* is closely related to group IV *Phytophthora* species (Fig. 5). Characterisation of *P. ipomoeae* rDNA sequences supported the hypothesis that this species is closely related to other *Phytophthora* species within clade Ic (Cooke *et al*. 2000) and Group IV (Waterhouse 1963). The ITS1 region in *P. ipomoeae* was identical with the sequences found in both *P. infestans* and *P. mirabilis*, and differed only one base-pair with *P. phaseoli*. One base-pair difference between *P. ipomoeae* and the other three species was also detected when the ITS2 region was characterized (data not shown).

DISCUSSION

Disease symptoms of *Phytophthora ipomoeae* on leaves of *Ipomoea longipedunculata* closely resembled those of late blight on potatoes, but the absence of pathogenicity on potato or tomato of isolates collected from *I. longipedunculata* questioned the taxonomic status of these strains. Molecular characterizations of the isolates confirm that these strains found in the same geographical area of central Mexico are very similar to the heterothallic species *P. infestans* and *P. mirabilis* and the homothallic species *P. phaseoli*. The colony growth rate of isolates collected from *Ipomoea* is somewhat higher than compared to these three species, while sporangia dimensions are notably larger than those reported for the three group IV species examined. The isolates differ from most other species in taxonomic group IV by the presence of typical basal swellings on the sympodial sporangiophores which they only share with *P. phaseoli*, *P. mirabilis* and *P. infestans*. Sex organs found in cultures of *P. ipomoeae* are similar to those of *P. infestans*, *P. mirabilis* and *P. phaseoli*, the average oospore diameter being larger as compared to *P. mirabilis* and *P. phaseoli*. The isolates share the combination of amphigyny and homothallism with *P. phaseoli*. Allozyme analysis of six *P. ipomoeae* isolates revealed limited variability at the *Pep* locus and fixation of a single homozygous genotype at the *Gpi* locus. The *Pep* 78 allele appears to be unique for *P. ipomoeae* since the *Pep* 78 allele was not detected in an extensive survey including numerous isolates of both *P. infestans* and *P. mirabilis* (Grünwald & Flier, unpubl.) collected from the Toluca Valley. The 96 *Pep* allele has been reported for *P. infestans* (Goodwin *et al*. 1999) and was also found in *P. mirabilis* (Flier, unpubl.). Isolates of *P. ipomoeae* were homozygous and monomorphic at the *Gpi* locus. The *Gpi* 108 allele present in *P. ipomoeae* was only recently reported (Goodwin *et al*. 1999) for *P. mirabilis* isolates collected in Texcoco, Mexico. In addition, the *Gpi* 108 allele has been found in *P. ilicis* (Goodwin *et al*. 1999) but does not appear to be present in *P. infestans*. In *P. phaseoli*, only the 100 *Gpi* allele has been reported (Goodwin *et al*. 1999). Allozyme data suggest only very limited gene flow between *P. ipomoeae* and the heterothallic sister-species *P. infestans* and *P. mirabilis*.

It has been reported that members of *Ipomoea* serve as host plants for *Phytophthora* species. *Ipomoea hederacea* Jacq. has been reported as a host for *P. infestans* (Raj, Bhattacharyya & Sharma 1976) in India. Our limited attempt to infect a single sweet potato cultivar (*I. batatas*) with the Mexican *Phytophthora* isolates failed, but the possibility of adaptation of *P. ipomoeae* strains to sweet potato needs to be considered. So far, sweet potato production in Mexico is confined to tropical areas, while *P. ipomoeae* is known from the Toluca Valley, situated in the temperate highlands of central Mexico. However, more research is needed to elucidate the geographic distribution of both the pathogen and it's primary natural host species and this will help predict the likelihood of future transfer of *P. ipomoeae* to sweet potato.

We now hypothesize that the central highlands of Mexico are the centre of origin of group IV *Phytophthora* species. The highlands of central Mexico presumably form the centre of origin of *P. infestans*, *P. mirabilis* and *P. phaseoli* (Brasier & Hansen 1992). The shared morphological characters of the three species and *P. ipomoeae*, combined with the lack of ITS rDNA diversity, suggests a speciation 'hot-spot' in the highlands of central Mexico with a common ancestry for all four species. Host specialization and inter-specific hybridization events could both serve as possible driving forces of speciation. It is however difficult to speculate on the exact origin of *P. ipomoeae*. In *Phytophthora*, paragyny and heterothallism are considered ancestral to amphigyny and homothallism, heterothallic *Phytophthora* species being exclusively amphigynous (Brasier 1983). Homothallic amphigynous species like *P. phaseoli* and *P. ipomoeae* are thought as secondary homothallics, evolved from their heterothallic ancestors (Brasier 1983, Cooke *et al*. 2000) by speciation events followed by differential evolutionary pressures (Brasier 1983). Evidence is accumulating that inter-specific hybridization in *Phytophthora* plays an important role in the exploitation of new host plant species (Ilieva *et al*. 1998, Man in 't Veld *et al*. 1998, Brasier, Cooke & Duncan 1999). Host specificity can provide the mechanism needed for reproductive isolation and might eventually lead to sympatric speciation. The abruptness of such a hybridization event, followed by abundant asexual reproduction and oospore formation of the hybrid (homothallic) species on the new host could serve as an explanation for the accelerated speciation processes observed in *Phytophthora*.

The *Phytophthora* isolates from *Ipomoea longipedunculata* are closely related to species in the taxonomic group IV of Waterhouse (Waterhouse 1963, Stamps *et al*. 1990) and Clade 1c (Cooke *et al*. 2000). At the same time, we have shown that the *Phytophthora* isolates collected from *I. longipedunculata* do not fit any of the species descriptions based on the disparity of host range, differences in morphology, thalism, growth characteristics, allozyme patterns and mtDNA haplotypes. We therefore conclude that the *Phytophthora* strains isolated from *Ipomoea longipedunculata* belong to a new species for which we propose the name *Phytophthora ipomoeae*.

Phytophthora ipomoeae Flier & Grünwald, **sp. nov.**

Coloniis mycelialibus in Secal bene crescentibus. Cultura minima ad 11 °C, optima ad 20 ° et maxima ad 25 °. Hyphae eseptatae et copiose ramosae, 4–7.5 μm diam. Sporangiophori aerii in agaro ramis composito-sympodialibus et indeterminatis, cum tumoribus in loco sporangiis emergentes. Sporangia semipapillata, ellipsoidea, subovoidalibus, caduca cum pedicella brevi, valore medio 39 μm longa (variatione 35–47.5 μm), ratione longitudinis/latitudinis 1.9, germinantia directe

tubo germinativo vel indirecte cum zoosporis 4–8. Antheridia amphigyna, valore medio 19 μm longa, ratione longitudinis/latitudinis 1.3. Oogonia laevitunicata, valore medio 32.5 μm diam, basi attenuata. Oosporae laevitunicatae colore luteo raro aspersae, cavitatem oogonialem fere omnino impletes, valore medio 29 μm. Segregatus homothallicis.

Typus: **Mexico**: Toluca Valley, Cerro de Metepec, isol. ex *Ipomoea longipedunculata*, 17 July 1999, *W. G. Flier* (PIC 99169 – holotypus; cultura ex tip CBS 109229).

Mycelial colonies grow well on Rye A agar. Minimum growth at approx 11 °C, optimum at 20 ° and maximum at 25 °. *Hyphae* nonseptate and freely branching, 4–7.5 μm diam, mostly 5.5 μm. *Sporangiophores* aerial, sparsely formed on rye agar with compound-sympodial and intermediate branches, with swellings where sporangia emerge. *Sporangia* semipapillate, ellipsoid or semi-ovoid, caduceus with short pedicel, on average 39 μm long (range 35–47.5 μm), with a length/width ratio of 1.9, germinating directly with germ tubes or indirectly with 4–8 zoospores. *Antheridia* amphygynous, average length 19 μm, ratio of length/width 1.3. Oogonia smooth-walled, average diam 32.5 μm, with tapered base. *Oospores* smooth-walled, rarely tinted yellow, almost filling the oogonial cavity, average diam 28.8 μm. Isolates homothallic.

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