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Phytophthora andina sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands

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A blight disease on fruits and foliage of wild and cultivated *Solanum* spp. was found to be associated with a new species of *Phytophthora*. The proposed novel species is named *Phytophthora andina* Adler & Flier, sp. nov. based on morphological characteristics, pathogenicity assays, mitochondrial DNA haplotyping, AFLP fingerprinting and nuclear and mitochondrial DNA sequence analyses. Isolates of *P. andina* (*n* = 48) from the Andean highland tropics of Ecuador were collected from 1995 to 2006. *Phytophothora andina* is closely related to *P. infestans* and has semipapillate, ellipsoidal sporangia borne on sympodially branched sporangiophores. It is heterothallic and produces amphigynous antheridia. The species consists of several clonal lineages, including the EC-2 and EC-3 RFLP lineages, which were described previously as *P. infestans*. Approximately 75% of isolates react as compatibility type A2 when paired with an A1 compatibility type isolate of *P. infestans*. However, when A2 isolates from the Anarrhichomenum section of *Solanum* were paired in all combinations, viable oospores were obtained in several crosses, suggesting that there is a unique compatibility interaction in *P. andina* that is complementary to that described in *P. infestans*. Nuclear and mitochondrial sequence analysis supported the species designation of *P. andina*. This newly identified heterothallic pathogen shares a common ancestor with *P. infestans* and may have arisen from hybridization events with sister taxa in the Andes.

Keywords: pepino, *Phytophthora infestans,* potato, *Solanum* spp., tomato, tree tomato

Introduction

The Andean highlands are considered a centre of origin and diversity for the plant genus *Solanum* (Hawkes, 1990). In addition to potato (*Solanum tuberosum*) and tomato (*S. lycopersicum*), several solanaceous crops have been domesticated there, including *S. betaceum*, known as tomate de árbol or tree tomato, and as tamarillo outside the Andes, *S. muricatum*, known as pepino dulce, pepino or pear melon, and *S. quitoense*, known as naranjilla (Ecuador) or lulo (Colombia). The first two are globally important crops, whilst the latter are primarily restricted to the Andes (Popenoe *et al.*, 1989). Cultivated *Solanum* crops are often grown in close proximity to wild *Solanum* spp. including *S. caripense, S. hispidum* and *S. ochranthum* and the poorly defined species in the Anarrhichomenum section (e.g. *S. tetrapetalum, S. sodiroi*, *S. siphonobasis* and *S. brevifolium*). Although the taxonomy of tuber-bearing *Solanum* spp. and solanaceous fruit crops remains complex, most species have been described and classified (Spooner *et al.*, 2003). Much less is known, however, about the phylogenetic relationships and taxonomy of non-tuber-bearing wild *Solanum* spp.

Phytophthora populations associated with *Solanum* hosts in the Andes appear to be more complex than was originally believed. Until 1994, genetic diversity studies in Ecuador revealed only limited variation in the local populations of *P. infestans* on cultivated species. Late-blight epidemics on potato and tomato were exclusively caused by the EC-1 and US-1 clonal lineages of *P. infestans*, respectively (Forbes *et al.*, 1997; Oyarzun *et al.*, 1998). Both lineages have the A1 mating type and reproduce clonally. This simple appraisal of the genetic structure of the pathogen population began to change in 1995 when four *Phytophthora* isolates were collected from

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blighted leaves of a plant in Ecuador identified as *S. brevifolium*. The isolates from *S. brevifolium* were the A2 mating type when crossed with A1 tester isolates of *P. infestans* and were initially identified as *P. infestans*, although they were recognized as novel and characterized by a previously unknown mtDNA haplotype (Ic) and a new RFLP fingerprint (EC-2) (Ordoñez *et al.*, 2000). A total of 53 isolates were collected between 1996 and 1999 from hosts identified at the time as *S. brevifolium* and *S. tetrapetalum*. Based on a unique RFLP fingerprint with limited polymorphism, a new mtDNA haplotype and A2 mating type, these isolates were classified in a new clonal lineage of *P. infestans* designated EC-2 (Ordoñez *et al.*, 2000). Further study of the hosts revealed that the original 53 isolates came from one or more *Solanum* species in the Anarrhichomenum section of *Solanum* (Spooner *et al.*, 2003). Ordoñez and co-workers did not rule out the possibility that the EC-2 lineage was indigenous to Ecuador. The identification of the clonal lineage as *P. infestans* remained unsatisfactory, so the isolates were classified as *Phytophthora infestans sensu lato* (Ordoñez *et al.*, 2000).

In a subsequent study which included a systematic sampling of foliar *Phytophthora* pathogens from various wild and cultivated solanaceous hosts, Adler *et al.* (2004) reported the presence of another clonal lineage (EC-3) on *S. betaceum*. These authors also found numerous isolates that were not of the EC-2 lineage attacking plants in the Anarrhichomenum complex. These isolates were the A1 mating type and Ia mtDNA haplotype. In the study by Adler *et al.* (2004), amplified fragment length polymorphism (AFLP) fingerprinting confirmed the presence of host-specific groups of isolates, but the taxonomic status of these *Phytophthora* isolates attacking several non-tuber-bearing *Solanum* spp. remained unresolved (Adler *et al.*, 2004).

The phylogeny of the genus *Phytophthora* was the subject of several studies in which molecular techniques were used to supplement morphological classification (Waterhouse, 1963) and facilitate phylogenetic inference (Cooke *et al.*, 2000; Martin & Tooley, 2003; Kroon *et al.*, 2004). Sequence variation for the ITS region of *Phytophthora* species was shown to be useful for resolving the general relationships within the genus (Cooke *et al.*, 2000), but proved insufficient to discriminate among *P. infestans*, *P. mirabilis*, *P. phaseoli* and *P. ipomoeae*, which are all placed in group IV (*sensu* Waterhouse) and clade Ic (*sensu* Cooke) (Flier *et al.*, 2002). This lack of resolution of the clade Ic species using ITS DNA has led to other studies with more variable DNA regions. Martin & Tooley (2003) sequenced part of the mitochondrial *Cox I* gene, whilst Kroon *et al.* (2004) used parts of the *β-tubulin*, translation elongation factor 1-alpha, NADH-4 and *Cox I* genes to construct a high-resolution molecular phylogeny for the genus *Phytophthora*. The latter study demonstrated that the EC-2 clonal lineage of *P. infestans sensu lato* was closely related to *P. infestans, P. ipomoeae* and *P. mirabilis*, yet not identical to them, thus stimulating discussion on the origin of the *Phytophthora* spp. in Ecuador. Although a new species had not been formally described, Kroon *et al.* (2004) referred to the one isolate from Ecuador they had examined as *P. andina*. That isolate had come from an Anarrhichomenum section host and corresponded to the EC-2 lineage of Ordoñez *et al.* (2000), and this was apparently the first reported use of the name. Wattier *et al.* (2003) also concluded that isolates from hosts of the Anarrhichomenum complex were distinct from *P. infestans* based on mitochondrial inter-gene sequences, although the species name *P. andina* was not used in their report. Four isolates of the EC-2 lineage from Anarrhichomenum section hosts were included in a coalescent analysis in which a South American origin was inferred for *P. infestans*, and these four isolates were referred to as *P. andina* (Gomez-Alpizar *et al.*, 2007). The four isolates used in that study shared a common ancestor with *P. infestans* but were derived from a distinct mitochondrial lineage (Ic) that had undergone many mutations.

In an effort to clarify the taxonomy of this group of *Phytophthora* isolates from solanceous hosts in Ecuador, phylogenetic analysis of 11 EC-2 and eight EC-3 isolates was conducted using sequences of the *Cox I* gene and intron 1 of the *Ras* gene (Gomez-Alpizar *et al.*, 2008). In that study EC-2 isolates with the Ic mtDNA haplotype were considered distinct and were designated *P. andina*, but no clear description of the new species was given (Gomez-Alpizar *et al.*, 2008). Furthermore, the taxonomy of the EC-3 group of isolates from *S. betaceum* remained unclear since few EC-3 isolates were examined and some of these grouped more closely to *P. infestans* than *P. andina* in the mitochondrial genealogy. Using microsatellite (SSR) markers, Oliva *et al.* (2007) found that EC-2 and EC-3 lineages were clearly separated from the US-1 and EC-1 lineages of *P. infestans*. However, the authors referred to the whole population as *P. infestans*, only mentioning *P. andina* in reference to earlier papers. In a recent and comprehensive multilocus phylogeny of the genus *Phytophthora*, two Ecuadorian isolates corresponding to the EC-2 lineage were considered closely related to, but distinct from *P. infestans* (Blair *et al.*, 2008). In reference to an uncertain taxonomic designation for the isolates, the authors referred to the taxon as *P.* sp "*andina*".

All the previous studies examining the genetic relatedness among clade-Ic species of *Phytophthora* species and isolates from the putative *P. andina,* i.e. those originally described as EC-2 and EC-3 lineages of *P. infestans* (*sensu lato*), have suggested a close relationship between this novel species, *P. infestans* and *P. mirabilis* (Wattier *et al.*, 2003; Kroon *et al.*, 2004; Blair *et al.*, 2008; Gomez-Alpizar *et al.*, 2008), but a species description has not been given. Thus, as recognized by Blair *et al.* (2008) with their use of quotation marks, the taxon sometimes referred to as *P. andina* has not been defined. *Phytophthora* species attacking solanaceous hosts in the Andes cause severe damage on several regionally important crops and provide a very interesting model of host/

pathogen coevolution (Oliva *et al.*, 2007). Further studies of these pathogens are to be expected and taxonomic uncertainty of the species could hinder progress.

This papers formally describes the new species *P. andina* and clarifies its taxonomic status and relatedness to *P. infestans*. To achieve this, a representative sample of blight-causing *Phytophthora* isolates collected between 1995 and 2006 from the highlands of Ecuador were investigated. The new species is described on the basis of morphological characteristics, host range, mating behavior, allozyme genotype, RLFP genotype and mitochondrial DNA haplotype. Genetic relatedness to *P. infestans* is based on one mitochondrial gene (*Cox II*), ITS sequences and a portion of a single-copy nuclear gene (*Ras*). AFLP fingerprinting was used to explore genetic similarities between host- specific groups of *P. andina* and their relatedness to other *Phytophthora* species in the Ic clade that attack *Solanum*, *Ipomoea* and *Mirabilis* spp. Results are discussed in relation to previous studies, particularly for relatedness to *P. infestans* (Oliva *et al.*, 2007) and for host range (Chacón, 2007).

Materials and methods

Pathogen collection and maintenance

Isolates of *Phytophthora* species used in this study were obtained from diseased host plants collected during sampling trips throughout the Ecuadorian Andes (Fig. 1, Table 1). Isolates were collected in almost all provinces of Ecuador between 1995 and 2006, ranging from 800 to 3800 m a.s.l. A map describing the main collection areas was published by Adler *et al.* (2004). Individual 1-cm^2 pieces of diseased leaves and fruits were transferred to selective Rye B (RB) agar (Caten & Jinks, 1968) amended with vancomycin (100 mg), polymyxin-B (500 mg), ampicillin (200 mg), rifampicin (20 mg), pentachloronitrobenzene (67 mg) and benomyl (100 mg) in 1000 mL distilled water. Colonies growing on selective media were transferred to RB agar for initial identification as *Phytophthora* species. All isolates were maintained for short periods on Rye A (RA) or RB agar at 18°C in the dark (Ordoñez *et al.*, 2000). Isolates were stored for longer periods on RA slants at 15°C with a 12-h photoperiod in Quito, Ecuador and in liquid nitrogen at Plant Research International in Wageningen, the Netherlands. Isolates were compared with reference isolates of *P. infestans*, *P. mirabilis*, *P. ipomoeae* and *P. phaseoli* from the *Phytophthora* culture collection at Plant Research International (Table 1).

Morphology

Sixteen isolates were examined in morphological studies (Table 1). Sporangia were observed in an aqueous suspension prepared by flooding a 10-day-old culture grown on pea agar (PA), prepared by autoclaving 120 g frozen peas in 1 L water for 20 min at 121°C. The broth was filtered through cheesecloth to remove the peas and then autoclaved again with 17 g agar L^{-1}. Measurements of 40 randomly selected sporangia for each isolate were made at ×400 magnification. Oospores of *P. andina* were measured from four *in vitro* crosses: EC3189 × EC3399, EC3233 × EC3414, EC3658 × EC3358 and EC3818 × EC3261. Diameters of 40 randomly selected oospores for each cross were measured.

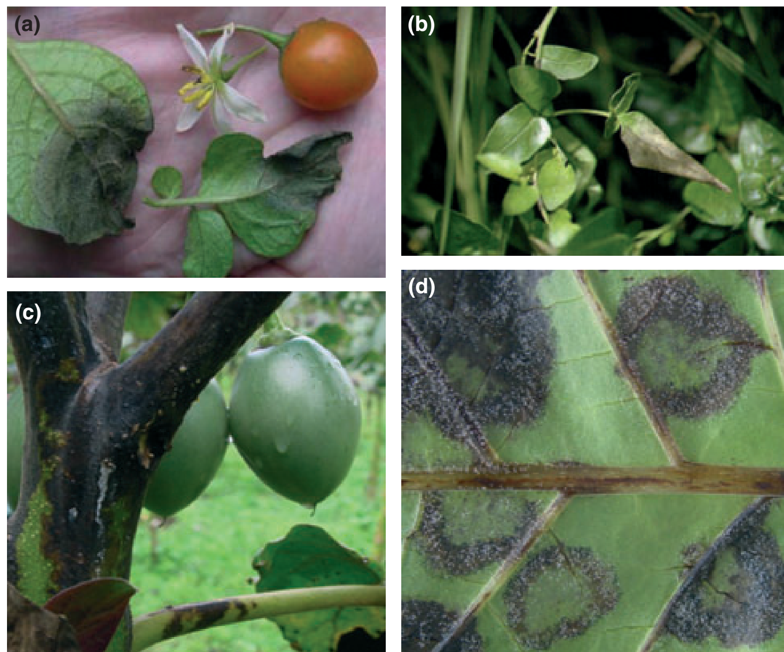

Figure 1 Blight symptoms caused by *Phytophthora andina* on *Solanum* species belonging to the Anarrhichomenum complex (a, b) and on *S. betaceum* (tree tomato) (c, d).

Sexual compatibility and mating system

The *P. infestans* mating system is known to comprise two compatibility types, A1 and A2, and has been used as a phenotypic marker and for genetic studies for decades (Gallegly & Galindo, 1958). The following experiment was designed to learn more of the *P. andina* mating system. Ten isolates from *P. andina* (Anarrhicomenum complex; mtDNA Ic) were used for oospore production studies (Tables 1 and 2). These isolates, which reacted as A2 compatibility type when paired with A1 isolates of *P. infestans,* were mated in all possible combinations (Table 2). Parental isolates were transferred to RA plates and cultured for 10 days at 20°C. Agar discs (5-mm diameter) taken from margins of fast growing colonies of the parental isolates (one disc for each parent) were placed 30 mm apart in a plate (9-cm diameter) containing 10 mL RA, amended with 0·05 g β-sitosterol L^{-1} to stimulate oospore production (Flier *et al.*, 2001). Two plates were prepared for each parental combination. Plates were incubated for 15 days at 20°C in the dark. The presence and density of oospores were determined microscopically at ×100 magnification.

Allozyme analysis

Electrophoretic banding patterns for the allozymes glucose-6-phosphate isomerase (*Gpi*, EC 5.3.1.9) and peptidase (*Pep*, EC 3.4.3.1) were assessed on polyacrylamide gels following a published method (Ordoñez *et al.*, 2000). Polyacrylamide gel electrophoresis (PAGE) was performed using 1-mm-thick 7·5% gels with 25 mM Tris, 0·19 M glycine, pH 8·8 as buffer. Bands were clearer when a 1-cm stacking gel (2·5% acrylamide, 0·06 M Tris-HCl, pH 6·7) was used. PAGE gels were run with a constant current of 5 mA for 1 h, and then increased to 10 mA. Voltage rose continuously throughout, from approximately 50 to 280 V. Allozyme genotypes (inferred from banding pattern phenotypes) were scored as described by Spielman *et al.* (1990).

DNA extraction

Isolates were grown for 10–14 days at 20°C in pea broth. The mycelium grown on pea broth was harvested, lyophilized and stored at −80°C. Lyophilized mycelium (10–20 mg) was ground in microcentrifuge tubes with a pestle and sterile sand. Total DNA was extracted using the Puregene kit (Gentra/Biozym) according to the manufacturer's instructions. DNA was dissolved in 100 μL TE (10 mM Tris-HCl pH 8·0 and 1 mM EDTA pH 8·0) and stored at −20°C.

MtDNA haplotypes

Mitochondrial haplotypes were determined by amplification of DNA of each isolate using primers designed for four specific regions of the mitochondrial genome of *P. infestans* (Griffith & Shaw, 1998). PCR reactions were performed in a PTC200 thermocycler (MJ Research). Digestion of the amplified regions with restriction enzymes *Cfo*I, *Msp*I and *Eco*RI yielded banding patterns used for classification into mtDNA haplotype: Ia, Ib, IIa IIb (Griffith & Shaw, 1998) and Ic (Oliva *et al.*, 2002).

RFLP fingerprinting

RFLP fingerprints were generated according to Ordoñez *et al.* (2000) using the moderately repetitive clone RG57 (Goodwin *et al.*, 1992). Restriction fragments were detected using the ECL non-radioactive kit (Amersham) according to the manufacturer's instructions.

AFLP fingerprinting

All *Phytophthora* isolates from Ecuador and described in a previous study (Adler *et al.*, 2004) were analysed with isolates of *P. ipomoeae*, *P. mirabilis* and *P. phaseoli*. AFLP fingerprinting was performed according to Flier *et al.* (2003). Selective PCR was performed using Cy5-labelled fluorescent Eco21 primer (5′-CTCGTAGA CTGCGTACC-3′), and Mse16 primer (5′-GATGAGTC CTGAGTAACC-3′). A total of 172 distinct and reproducible AFLP bands were identified in 29 isolates. Bands were treated as putative single AFLP loci and a binary matrix for presence (1) or absence (0) of these reproducible bands was constructed and used for further analysis. A similarity matrix of AFLP genotypes was constructed using Rogers' modified distance method in the statistical software package GENSTAT version 6.1 (Lawes Agricultural Trust). Trees were constructed from the distance matrix using the neighbour-joining algorithm and visualized with TREECON version 1.3b software (van de Peer & de Wachter, 1997).

Mitochondrial and nuclear sequences

The mitochondrial *CoxII* (cytochrome c oxidase subunit II) gene, the ITS regions and part of the single copy *Ras* gene (Chen & Roxby, 1996) were amplified and sequenced for a subset of 22 *Phytophthora* isolates collected in Ecuador (Table 1). The *CoxII* gene was amplified by primers FM35 (5′-CAGAACCTTGGCA ATTAGG) and FMPh-10b (5′-GCAAAAGCACTA AAAATTAAATATAA) (Martin & Tooley, 2003; Martin *et al.*, 2004). ITS DNA was amplified using ITS primers 4 and 6 (Cooke *et al.*, 2000). Primers Iras F (5′-TTGC AGCACAACCCAAGACG) and Iras R (5′-TGCACGTA CTATTCGGGGTTC) were used to amplify a 223-bp fragment of the intron 1 of the *Ras* gene (Gomez-Alpizar *et al.*, 2007). Reactions were carried out in a 50-μL volume according to the authors' description. DNA was sequenced in the forward and reverse directions using a Macrogen system (Macrogen Sequencing System). Sequences were aligned and chromatograms checked using BIOEDIT (Hall, 1999) before alignment with other *Phytophthora* species from GenBank. Phylogenetic relationships were inferred using neighbour-joining analysis

Table 1 Characteristics of isolates of *Phytophthora andina, P. infestans*, *P. mirabilis*, *P. ipomoeae* and *P. phaseoli*: morphology, sexual compatibility, AFLP fingerprinting and analysis of nuclear and mitochondrial DNA sequences

Isolate	Species	Origin	Host	Allozyme[a] genotype		MtDNA[b] haplotype	RG57 RFLP[c]	Mating type[e]	Used in[d]
				Pep	*Gpi*				
EC1836	*P. andina*	Ecuador	Anarrhichomenum[f]	76/100	100/100	Ic	EC-2	A2	a, b
EC3163	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	d
EC3186	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3189	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3190	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3229	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3231	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3232	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3233	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3261	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3262	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3323	*P. andina*	Ecuador	*Solanum quitoense*	76/100	86/100	Ia	nd	A1	d
EC3358	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3363	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	c
EC3364	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	c
EC3365	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	c
EC3369	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b,e
EC3370	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ia	EC-2	A1	c
EC3371	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ia	EC-2	A1	e
EC3375	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ia	EC-2	A1	e
EC3380	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	c, e
EC3392	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ia	EC-2	A1	e
EC3394	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	c
EC3395	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	e
EC3396	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	c
EC3399	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ia	EC-2	A1	a, b
EC3400	*P. andina*	Ecuador	*Brugmansia* spp.	76/100	100/100	Ia	EC-2	A1	e
EC3402	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	e
EC3414	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3417	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ia	EC-2	A1	c
EC3421	*P. andina*	Ecuador	*S. muricatum*	76/100	100/100	Ic	EC-2	A2	c, e
EC3423	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	e
EC3424	*P. andina*	Ecuador	*S. muricatum*	76/100	100/100	Ic	EC-2	A2	c
EC3514	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	d
EC3540	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	e
EC3542	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	e
EC3561	*P. andina*	Ecuador	*S. quitoense*	76/100	86/100	Ia	nd	A1	d
EC3644	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	d
EC3650	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ia	EC-2	A1	d
EC3658	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	a, b
EC3674	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	d
EC3783	*P. andina*	Ecuador	*S. hispidum*	76/100	100/100	Ic	EC-2	A2	d
EC3816	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ic	EC-2	A2	d
EC3818	*P. andina*	Ecuador	Anarrhichomenum	76/100	100/100	Ia	EC-2	A1	a, d
EC3830	*P. andina*	Ecuador	*S. hispidum*	76/100	100/100	Ic	EC-2	A2	d
EC3860	*P. andina*	Ecuador	*Solanum* sp.	76/100	100/100	Ic	nd	A2	d
EC3875	*P. andina*	Ecuador	*S. betaceum*	76/100	86/100	Ia	EC-3	A1	d
EC3120	*P. infestans*	Ecuador	*S. ochranthum*	96/100	90/100	IIa	nd	A1	e
EC3241	*P. infestans*	Ecuador	*S. ochranthum*	92/100	86/100	Ib	US-1	A1	d, e
EC3322	*P. infestans*	Ecuador	*S. quitoense*	76/100	86/100	Ib	nd	A1	d
EC3338	*P. infestans*	Ecuador	*S. paucijugum*	96/100	90/100	IIa	EC-1	A1	e
EC3351	*P. infestans*	Ecuador	*S. tuberosum*	96/100	90/100	IIa	EC-1	A1	c
EC3355	*P. infestans*	Ecuador	*S. tuberosum*	96/100	90/100	IIa	EC-1	A1	c
EC3361	*P. infestans*	Ecuador	*S. tuberosum*	96/100	90/100	IIa	EC-1	A1	e
EC3378	*P. infestans*	Ecuador	*S. lycopersicum*	92/100	86/100	Ib	US-1	A1	c, e
EC3379	*P. infestans*	Ecuador	*S. minutifoliolum*	96/100	90/100	IIa	EC-1	A1	e
EC3381	*P. infestans*	Ecuador	*S. lycopersicum*	92/100	86/100	Ib	US-1	A1	c, d, e
EC3382	*P. infestans*	Ecuador	*S. tuberosum*	96/100	90/100	IIa	EC-1	A1	e

Table 1 *Continued.*

Isolate	Species	Origin	Host	Allozyme[a] genotype		MtDNA[b] haplotype	RG57 RFLP[c]	Mating type[e]	Used in[d]
				Pep	*Gpi*				
EC3383	*P. infestans*	Ecuador	*S. phureja*	96/100	90/100	IIa	EC-1	A1	c
EC3389	*P. infestans*	Ecuador	*S. lycopersicum*	92/100	86/100	Ib	US-1	A1	c, d
EC3390	*P. infestans*	Ecuador	*S. solisii*	96/100	90/100	IIa	EC-1	A1	e
EC3404	*P. infestans*	Ecuador	*S. caripense*	92/100	86/100	Ib	US-1	A1	c, e
EC3413	*P. infestans*	Ecuador	*S. paucijugum*	96/100	90/100	IIa	EC-1	A1	e
EC3415	*P. infestans*	Ecuador	*S. minutifoliolum*	96/100	90/100	IIa	EC-1	A1	e
EC3435	*P. infestans*	Ecuador	*S. colombianum*	96/100	90/100	IIa	EC-1	A1	e
EC3445	*P. infestans*	Ecuador	*S. colombianum*	96/100	90/100	IIa	EC-1	A1	e
EC3447	*P. infestans*	Ecuador	*S. phureja*	96/100	90/100	IIa	EC-1	A1	c
EC3528	*P. infestans*	Ecuador	*S. andreanum*	96/100	90/100	IIa	EC-1	A1	e
EC3529	*P. infestans*	Ecuador	*S. tuberosum*	96/100	90/100	IIa	EC-1	A1	d
EC3531	*P. infestans*	Ecuador	*S. tuberosum*	96/100	90/100	IIa	EC-1	A1	e
EC3532	*P. infestans*	Ecuador	*S. tuberosum*	96/100	90/100	IIa	EC-1	A1	e
EC3534	*P. infestans*	Ecuador	*S. andreanum*	96/100	90/100	IIa	EC-1	A1	e
EC3620	*P. infestans*	Ecuador	*S. ochranthum*	92/100	90/100	Ib	EC-1	A1	d
EC3774	*P. infestans*	Ecuador	*S. ochranthum*	96/100	86/100	Ib	US-1	A1	d
EC3808	*P. infestans*	Ecuador	*S. muricatum*	92/100	86/100	Ib	US-1	A1	d
EC3842	*P. infestans*	Ecuador	*S. habrochaites*	92/100	86/100	Ib	US-1	A1	d
PICWF021	*P. mirabilis*	Mexico	*M. jalapa*[f]	96/96	100/100	*HaPmir*I	nd	nd	c
PICWF031	*P. mirabilis*	Mexico	*M. jalapa*	96/96	100/111	*HaPmir*I	nd	nd	c
PICWF005	*P. mirabilis*	Mexico	*M. jalapa*	96/96	90/111	*HaPmir*I	nd	nd	c
PICWF053	*P. mirabilis*	Mexico	*M. jalapa*	96/96	100/111	*HaPmir*I	nd	nd	c
PIC991-2	*P. ipomoeae*	Mexico	*I. longipedunculata*[f]	78/78	108/108	*HaPipo*I	nd	sf	c
PIC991-1	*P. ipomoeae*	Mexico	*I. longipedunculata*	78/78	108/108	*HaPipo*I	nd	sf	c
PIC993-3	*P. ipomoeae*	Mexico	*I. longipedunculata*	78/78	108/108	*HaPipo*I	nd	sf	c
PIC993-4	*P. ipomoeae*	Mexico	*I. longipedunculata*	78/78	108/108	*HaPipo*I	nd	sf	c
CBS556.88	*P. phaseoli*	nd	*Phaseolus* sp.	nd	nd	*HaPpha*I	nd	sf	c
PiIa[g]	*P. infestans*	AY894835	*S. tuberosum*	nd	nd	Ia	nd	nd	d
PiIIa	*P. infestans*	AY898627	*S. tuberosum*	nd	nd	IIa	nd	nd	d
PiIIb	*P. infestans*	AY898628	*S. tuberosum*	nd	nd	IIb	nd	nd	d
Pmi	*P. mirabilis*	AY129214	*M. jalapa*	nd	nd	nd	nd	nd	d
Pphas	*P. phaseoli*	AY129221	*P. lunatus*	nd	nd	nd	nd	nd	d

[a]Allozyme alleles scored at the putative peptidase and glucose-6-phosphate isomerase loci.
[b]Haplotype nomenclature according to Griffith & Shaw (1998) and Oliva *et al.* (2002).
[c]Previously published RFLP fingerprints (Adler *et al.*, 2004; Forbes *et al.*, 1997; Ordoñez *et al.*, 2000). Both the Ia and Ic mtDNA groups of isolates from Anarrhichomenum section hosts can have either the EC-2 or EC-2.1 fingerprints.
[d]Isolates used in a = morphology, b = sexual compatibility, c = AFLP analysis, d = sequence studies, and e = pathological test.
[e]Mating type determined after crosses with tester isolates A1 (*P. infestans*) and A2 (*P. andina*) isolates.
[f]Section Anarrhichomenum species; *M. jalapa* = *Mirabilis jalapa*; *I. longipedunculata* = *Ipomoea longipedunculata*.
[g]PiIa correspond to GenBank Accession No. AY894835.
nd, not determined; sf, self fertile.

based on the Kimura 2-parameter distance method (Kimura, 1980) under MEGA 3 (Kumar *et al.*, 2004). In addition, the maximum likelihood (ML) method described in the RAxML web interface (Stamatakis *et al.*, 2005) was also performed.

Pathogenicity

Plant material of a range of species used for detached-leaf assays came from different sources: host plants collected directly from their natural environments, plants derived from true potato seed (from berries collected in the field) and seed tubers and plants growing near CIP station in Quito, Ecuador. Growing condition and plant accessions were used exactly as previously described by Chacón (2007). Fourteen different inoculation assays were conducted between 2001 and 2004 based on the availability of plant material. In potato, the first pair of fully expanded lateral leaflets of the upper third of the plant was used. In *S. ochranthum*, the first pair of fully expanded lateral leaflets of mature and well developed leaves was used. In tomato, *S. caripense* and Anarrhichomenum hosts, whole leaves were employed. In the case of S. *betaceum*, pieces of the central part of the leaves were cut out and used. For the sake of simplicity, all tissues are hereafter referred to as leaves. Prior to inoculation, leaves were washed with tap water, towel-dried and stored abaxial side up in the lids of inverted Petri dishes which contained water agar (4% w/v) in the base. Petri dishes were

Table 2 Average numbers of oospores per mm^2 from *in vitro* pairings of isolates of *Phytophthora andina* collected from hosts of the section Anarrhichomenum

	Isolate number									
Isolate	1	2	3	4	5	6	7	8	9	10
EC1836(1)	0									
EC3186(2)	0	0								
EC3190(3)	0	14	0							
EC3229(4)	5	12	0	0						
EC3231(5)	1	0	19	3	0					
EC3232(6)	24	0	0	11	18	0				
EC3233(7)	19	102	0	41	155	129	0			
EC3261(8)	0	0	3	56	7	9	41	0		
EC3262(9)	0	0	63	79	25	46	84	0	0	
EC3369(10)	0	0	2	2	1	160	7	0	0	0

[a]Putative compatibility type designation is based on crosses between parental isolates of *P. andina* and are different from those found in crosses between *P. andina* and *P. infestans* (Table 1).

subsequently used as high-humidity chambers for inoculation and incubation.

Before inoculation, the isolates from tuber bearing species (such as potato and wild relatives), were multiplied on leaves of their original hosts to restore aggressiveness after cultivation on rye agar. Isolates from *S. ochranthum* and *B. sanguinea* were washed directly from pure culture plates (RA). Sporangia from either living tissue or RA medium were rinsed several times with distilled water over a 12-μm filter before use. Irregular sporulation among the isolates made it impossible to use a common inoculum concentration, and therefore concentrations ranged from $2{\cdot}0$ to $3{\cdot}0 \times 10^4$ sporangia mL^{-1}. One 10-μL^{-1} drop of sporangial suspension was placed on the abaxial side of each leaf close to the midrib.

Given the large number of host–pathogen interactions, difficulty in producing consistent inoculum and the further problem of erratic availability of tissue from such wide diversity of hosts, assays were run sequentially, as inoculum and leaf tissue became available. Overall, 79 host–pathogen interactions were assessed and the following applied to all cases. Each host-genotype × isolate interaction was assessed on six different leaves, distributed into three different Petri dishes. Petri dishes containing inoculated leaflets were incubated by replication (in blocks) at 15 ± 2°C with 14 h fluorescent light per day. Inoculated leaves were monitored 5, 6, 7 and 10 (sometimes 11) days after inoculation for disease development and were visually and qualitatively rated for the presence or absence of disease. Infection (compatible reaction) was defined as the presence of expanding, sporulating lesions. Any other type of reaction observed [no reaction at all; macroscopic hypersensitive response (nonsporulating, nonexpanding lesions); or expanding, nonsporulating lesions] was rated as incompatible. In total, 12 *P. andina* and 18 *P. infestans* isolates were tested (Tables 1 and 3).

Table 3 Pathogenicity of isolates of *Phytophthora infestans* and *P. andina* on several solanaceous hosts in Ecuador

				Inoculated host[d]								
	Host of origin[a]	RFLP[b]	N[c]	tbr1	tbr2	mur	lyc	car	och	bet	ana1	ana2
P. infestans	*S. caripense*	US-1	1	**0**/+	+	+	–	0	+	–	–	–
	S. lycopersicum	US-1	2	0/+	+	0	+	0	0/+	–	+	0
	S. ochranthum	US-1	2	0/+	+	–	0/+	0	+	0	0	0
	S. andreanum	EC-1	2	+	+	0	+	0/+	0/+	0	+	**0**/+
	S. colombianum	EC-1	2	+	+	0	–	–	+	–	–	0
	S. minutifoliolum	EC-1	2	+	+	0	–	–	+	–	–	0
	S. paucijugum	EC-1	2	+	+	–	–	0	+	–	0/+	**0**/+
	S. solisii	EC-1	1	+	+	0	+	0	0	–	0/+	–
	S. tuberosum	EC-1	4	+	+	0	+	0	+	0	0/**+**	+
P. andina	*S. betaceum*	EC-3	5	0	0	0	0/**+**	0	0	+	+	**0**/+
	B. sanguinea[e]	EC-2	1	–	–	–	–	0	0	–	+	–
	Anarrhichomenum 1[f]	EC-2	3	0	**0**/+	**0**	0/**+**	0	0	–	+	+
	Anarrhichomenum 2	EC-2	2	0	0	0	–	0	0	–	–	–
	S. muricatum	EC-2	1	0	0	0	–	–	–	–	–	–

[a]*Solanum* host species from which *P. infestans* and *P. andina* isolates were collected.
[b]Pathogen RFLP profile according to RG57 probe.
[c]Number of isolates tested.
[d]Inoculated host species: tbr1 and tbr2 = *S. tuberosum* cvs Cruza 148 and Yungay, respectively; mur = *S. muricatum*; lyc = *S. lycopersicum* cv. Roma; car = *S. caripense*; och = *S. ochranthum*; bet = *S. betaceum*; ana1 and ana2 = taxonomically undefined plants from the section Anarrhichomenum.
0 = incompatible reaction, including: no reaction at all; macroscopic hypersensitive response (nonsporulating, nonexpanding lesions); and expanding, nonsporulating lesions.
+ = compatible reaction: expanding, sporulating lesions.
– = not inoculated; in bold; reaction most commonly observed.
[e]*Brugmansia sanguinea* belongs to the Solanaceae family.
[f]Hosts from section Anarrhichomenum.

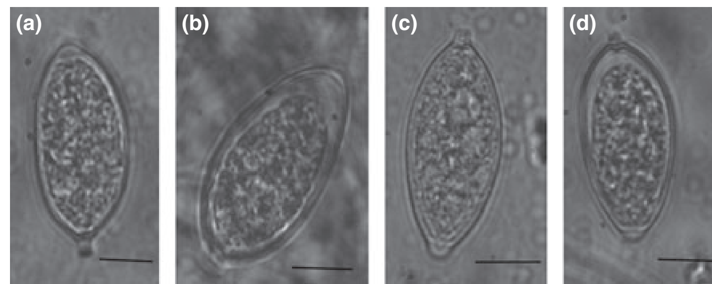

Figure 2 Sporangia of *Phytophthora andina* isolates EC3189 (a), EC3186 (b) and EC3232 (c, d), produced on pea agar. Bars represent 10 μm.

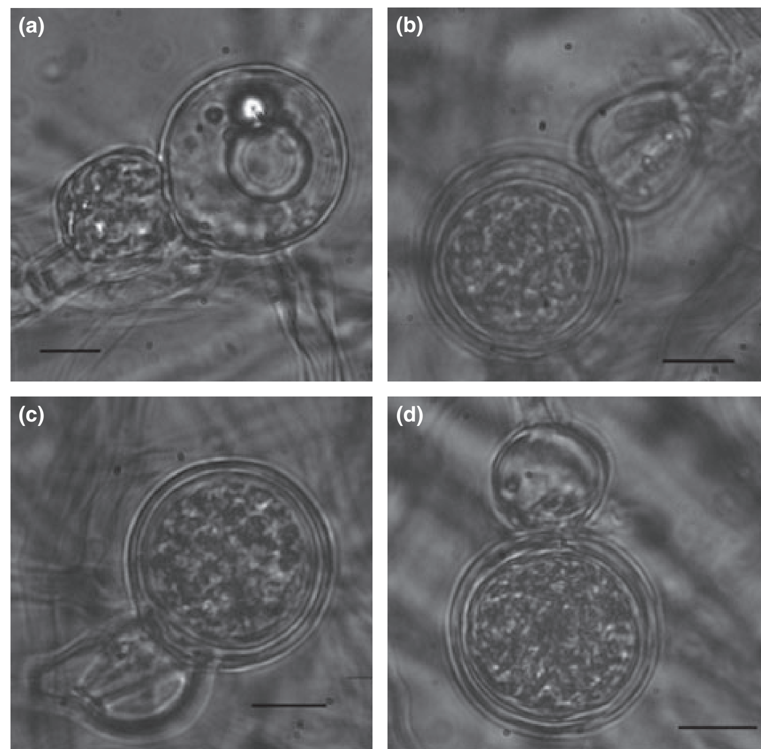

Figure 3 Amphigynous antheridia, oogonia and oospores of different crosses between *Phytophthora andina* isolates produced on pea agar. Isolate crosses: EC3233 × EC3186 (a), EC3189 × EC3399 (b, c) and EC3818 × EC3261 (d). Bars represent 10 μm.

Results

Morphology

All 16 isolates of *P. andina* (Table 1) assessed for morphology grew well on RA and PA with a radial colony expansion of 8–12 mm per day at 20°C. Neither hyphal swellings nor chlamydospores were produced in culture. Sporangia were semipapillate (Fig. 2) and although shape and dimension of sporangia varied considerably, no consistent differences among isolates were observed (Fig. 2). Length of sporangia ranged from 39·5 to 62·5 μm (average 44·6 μm) and width from 14·0 to 24·8 μm (average 17·8 μm), with a length:width ratio ranging from 2·4 to 2·7. Zoospores were released within 2 h from sporangia in water at 10°C, but zoospore release was also observed at 20°C on a few occasions. Oospores were formed following mating between compatible parental isolates on RA in Petri dishes (Fig. 3). No systematic differences in oospores size could be detected among the four crosses of *P. andina* isolates, which ranged overall in diameter from 26·5 to 37·5 μm.

Sexual compatibility in *P. andina*

Phytophthora andina is a heterothallic species. No oospores were observed in single cultures, and previous studies established that *P. andina* isolates interacted with

P. infestans and could be classified as either A1 or A2 compatibility type (Ordoñez *et al.*, 2000; Chacón, 2007). However, in the present study a complementary system was observed in *P. andina,* as oospores were produced in crosses between isolates that had been identified as A2 by a *P. infestans* tester. Oospores were observed in 29 out of 45 pairwise crosses within *P. andina* (Table 2) and oospore density at the interaction zone ranged from 1 to 160 oospores mm^{-2} with an average of 39·2 oospores mm^{-2} (Table 2). Oospores had a dark brown and thick outer wall, but appeared viable, with a large central vacuole and translucent cytoplasm (Fig. 3). Crosses of *P. mirabilis* × *P. andina* and *P. infestans* × *P. andina* also yielded oospores of similar diameter, but these were lighter in colour (not shown).

Allozyme analysis

All isolates of *P. andina* had the *Pep* allele 76 that has not been reported previously for *P. infestans*. *Phytophthora andina* alleles for *Gpi* are common in *P. infestans*, although the 86 allele has to date only been found in the US-1 lineage of *P. infestans* (Table 1). Isolates sampled from hosts from the Anarrhichomenum section and *S. hispidum* share the *Gpi* 100/100 and *Pep* 76/100 allele combinations. The combination *Gpi* 86/100 and *Pep* 76/100 is typical for EC-3 isolates collected from *S. betaceum* (Table 1) and also occurs in some isolates from *S. quitoense,* although the latter had an RFLP genotype that differed from that of EC-3 (data not shown).

MtDNA haplotypes

Two mtDNA haplotypes were found among isolates of *P. andina* in Ecuador. Haplotype Ia was associated with isolates from *S. betaceum*, some isolates from Anarrhichomenum hosts and *S. quitoense.* The haplotype Ic was associated with isolates from Anarrhichomenum hosts, *S. muricatum* and *S. hispidum* (Table 1). Haplotypes Ia, Ib and IIa were found in *P. infestans* from *S. tuberosum* and other *Solanum* hosts, which is consistent with previous reports from Ecuador (Adler *et al.*, 2004). Neither the IIb haplotype, previously described for *P. infestans*, nor the mtDNA haplotypes typical for *P. mirabilis* and *P. phaseoli* (Flier *et al.*, 2002) were found in *Phytophthora* isolates from Ecuador. However, the Ic haplotype associated with isolates from Anarrhichomenum hosts was found in isolates of *P. ipomoeae* from Mexico, possibly indicating a shared ancestry (Flier *et al.*, 2002).

RFLP fingerprinting

Phytophthora andina isolates had either EC-2 or EC-3 RFLP fingerprints. Limited polymorphism that has been described in more detail previously as EC-2.1 (Ordoñez *et al.*, 2000; Adler *et al.*, 2004) was found within the EC-2 group (data not shown). The EC-2.1 variant occurs with both Ic and Ia mtDNA haplotypes. RFLP fingerprints of *P. infestans* were either EC-1 or US-1, as previously reported (Forbes *et al.*, 1997; Oyarzun *et al.*, 1998; Adler *et al.*, 2004).

AFLP fingerprinting

Based on a neighbour-joining analysis using 172 AFLP marker loci, 12 isolates of *P. andina* formed a strongly supported clade (bootstrap support of 99%) clearly distinct from isolates of four other *Phytophthora* species included in the analysis (Fig. 4). Within the *P. andina* clade, three groups could be distinguished, one associated with the EC-3 lineage from *S. betaceum*, and two additional clusters associated with isolates of the EC-2 lineage that included both Ic and Ia mtDNA haplotypes.

Phylogenetic relationships

CoxII sequence data grouped isolates of *P. infestans* in one clade with all isolates of *P. andina* that have the Ia mtDNA haplotype. Isolates of *P. andina* with the Ic mtDNA haplotype formed a distinct branch, as did individual isolates of *P. mirabilis* and *P. phaseoli* (Fig. 5). *CoxII* sequences generated in this study were submitted to GenBank (Accession Nos GQ260982 to GQ261004). ITS sequences were identical among isolates of *P. andina* and similar to those of *P. infestans* (GenBank Accession No. AY770741). The sequence of the *Ras* intron 1 gene was identical for all isolates of *P. infestans*, including

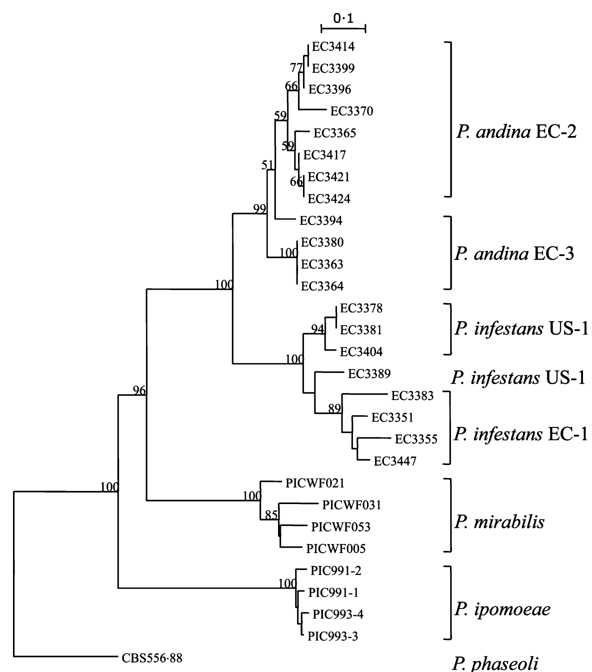

Figure 4 AFLP neighbour-joining dendrogram of isolates of *Phytophthora andina* and related *Phytophthora* species using Rogers' modified distance. Bootstrap support for tree branches is presented if value exceeds 50% based on 1000 replicates.

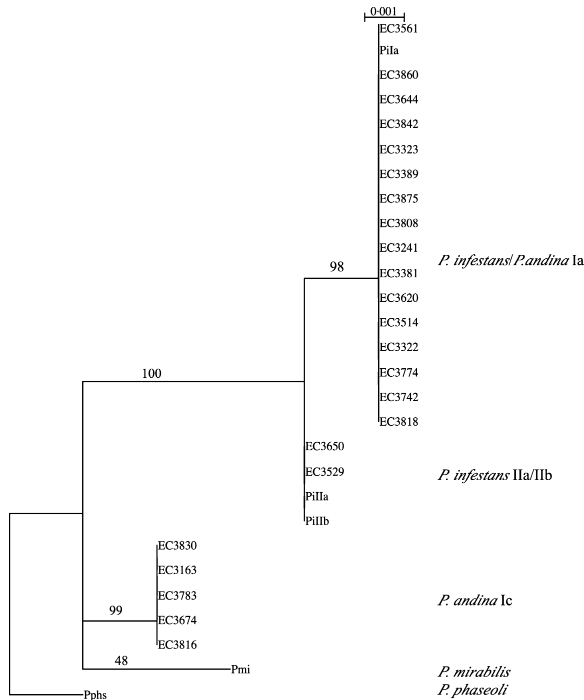

Figure 5 Phylogenetic relationship of isolates of *Phytophthora infestans* and *P. andina* using *Cox II* sequence data, based on maximum likelihood analysis. Numbers along the branches indicate bootstrap values. PiIa, PiIIa and PiIIb represent *P. infestans* accessions obtained from GenBank (AY894835, AY898627 and AY898628, respectively). Pmi and Pphs represent *P. mirabilis* (AY129214) and *P. phaseoli* (AY129221) accessions obtained from GenBank.

GenBank Accession No. U30474. All isolates of *P. andina* had identical sequences as well, but this sequence differed from that of *P infestans* by 9 bp (data not shown). In addition to the previous report made by Gomez-Alpizar *et al.* (2008), who described six heterozygous sites, this study found three more sites within the *Ras* intron 1 sequence (GenBank Accession Nos. GQ261005 to GQ261027). The additional sites corresponded to positions 551, 610 and 688, according to Accession No. U30474. Within the 223-bp fragment, heterozygous sites were found in all isolates of *P. andina*, but these were absent in *P. infestans*.

Pathogenicity

Isolates of both *P. infestans* RFLP lineages, US-1 and EC-1, caused lesions on most of the solanaceous host species examined, whilst isolates of *P. andina* had a narrower host range (Table 3). Based on qualitative assessment of pathogenicity of the 79 host–pathogen interactions, there was a tendency for isolates to more consistently infect the host they were isolated from (Table 3). None of the *P. andina* isolates were pathogenic on potato cv. Cruza 148, whilst some caused weak infections on cv. Yungay, which is more susceptible. Other solanaceous hosts, such as *S. caripense* and *S. ochranthum*, were fully resistant to the *P. andina* isolates tested (Table 3). None of the isolates were pathogenic on *S. muricatum*.

Taxonomy

Phytophthora andina Adler & Flier, sp. nov. Coloniis mycelialibus in Secal bene crescentibus. Temperies ad crescendum neccessaria. Minima temperies super 5°C, maxima temperies infra 30, optima temperies 24°C. Hyphae eseptatae et copiose ramosae, 4–8 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 44·6 μm longa (variatione 39·5–62·5 μm), ratione longitudinis/latitudinis 2·4–2·7, germinantia directe tubo germinativo vel indirecte cum zoosporis 6–8. Antheridia amphigyna, valore medio 22·0 μm longa, ratione longitudinis/latitudinis 1·4. Oogonia laevitunicata, 34–41 μm diam., basi attenuata. Oosporae laevitunicatae colore luteo, 27–37·5 μm diam.. Segregatus heterothallicis (Figs 1 and 3).

Typus: Ecuador: Province of Tungurahua: City of Baños isol. Ex *Solanum brevifolium* along roadside, July 2001, N. A. Adler, CIP EC3189, deposited in World Phytophthora Collection (University of California at Davis). Mycobank number MB514427.

Mycelial colonies grow well on RA. Minimum growth at approximately 5°C, optimum at 24°C and maximum at 30°C. Hyphae nonseptate and freely branching, hyphal diameter 4–8 μm, mostly 5·6 μm. Sporangiophore aerial, on rye agar with compound-sympodial and intermediate branches, with swellings where sporangia emerge. Sporangium semipapillate, ellipsoid or semi-ovoid, caducous with short pedicel, on average 44·6 μm long (ranging from 39·5 to 62·5 μm), with a length/width ratio of 2·4 to 2·7, germinating directly with germ tubes or indirectly with 6–8 zoospores. Antheridia amphigynous, average length 22·0 μm, ratio of length/width 1·4. Oogonia smooth-walled, average diameter 34·0–41·0 μm, with tapered base. Oospores smooth-walled, tinted yellow-brown, almost filling the oogonial cavity, average diameter 31·0 μm. Isolates heterothallic.

Discussion

This paper provides morphological and molecular evidence for the designation of *P. andina* as a distinct species attacking solanaceous plants in the Andean highlands. This designation is based on examination of a collection of isolates from different hosts made over a 10-year period. Based on AFLP, RFLP, allozyme genotypes and DNA sequence analyses, *P. andina* is closely related to *P. infestans*; however, these same markers indicate very little evidence of gene flow between the two species in nature, even though the potential for sexual reproduction has been established *in vitro* (Oliva *et al.*, 2002). Molecular data presented elsewhere also (Wattier *et al.*, 2003;

Kroon *et al.*, 2004; Oliva *et al.*, 2007; Blair *et al.*, 2008; Gomez-Alpizar *et al.*, 2008) supports a new species designation for *P. andina*.

Analysis of genetic variation at the rRNA ITS region (Kroon & Flier, unpublished data) revealed that *P. andina* is very closely related to several other foliar pathogens classified into *Phytophthora* clade Ic (Cooke *et al.*, 2000). The rRNA ITS sequence analysis identified *P. andina* as a putative sister group to *P. infestans*, *P. mirabilis*, *P. ipomoeae* and *P. phaseoli*, a result consistent with the AFLP marker data shown here (Fig. 4) and by Adler *et al.* (2004). Additional support for the classification of *P. andina* as a sister species of *P. infestans* and *P. mirabilis* was provided by the high-resolution molecular phylogeny for the genus *Phytophthora* published by Kroon *et al.* (2004) and the phylogenies proposed by Gomez-Alpizar *et al.* (2007, 2008) and Blair *et al.* (2008).

Phytophthora andina, P. mirabilis and *P. infestans* are morphologically similar in that they have a heterothallic mating system with amphigynous antheridia and produce large semipapillate sporangia on long sporangiophores. Unique features of *P. andina* (other than host range) are the EC-2 and EC-3 RFLP and AFLP fingerprints, heterozygosity in the *ras* intron 1 sequence, the Ic mtDNA haplotype and the *Pep* 76 allele.

The origin of *P. andina* is not directly addressed in this paper, but it is possible that *P. infestans* and *P. andina* may have evolved either from a common ancestor or as a consequence of interspecific hybridization. The presence of the Ia haplotype and the *Gpi* 86 allele in both *P. infestans* and *P. andina* suggests that hybridization may have occurred. This finding is consistent with the results of Gomez-Alpizar *et al.* (2008), who found the same sequence of the *Cox I* gene among isolates comprising both *P. infestans* and isolates of *P. andina* with the Ia mtDNA haplotype. A representative sample of blight-causing *Phytophthora* genotypes with different host preferences was selected in the present study, and it was found that some *P. andina* isolates have a unique *Cox II* sequence, but others share the same sequence with *P. infestans*. The occurrence of similar sequences in both *Cox I* and *Cox II* genes is also consistent with the hypothesis of previous hybridization, in which *P. infestans* must have been involved as one of the parents. Alternatively, both species could also have descended from a common mitochondrial ancestor (Gomez-Alpizar *et al.*, 2007). The contribution of other *Phytophthora* species in the clade to the evolution of *P. andina* remains unclear and needs to be tested further using more rigorous statistical approaches.

Populations of *P. andina* appear to be clonal, with three predominant lineages. One lineage is characterized by the Ic mtDNA haplotype (Table 1) and the A2 mating type (using *P. infestans* testers), and has been isolated from hosts of the Anarrhichomenum section of *Solanum* and *S. hispidum*. The second lineage has also been isolated from hosts from the Anarrhichomenum section, but it has the Ia mtDNA haplotype and the A1 compatibility type with *P. infestans* tester isolates. Similar genotypes have also been isolated from *S. quitoense*. A third lineage is characterized by the EC-3 RFLP fingerprint and the Ia mtDNA haplotype, and to date has only been isolated from the cultivated tree tomato (*S. betaceum*) (Table 1). Two of the three lineages were distinguished in the cluster analysis of AFLP data (Fig. 4).

When 10 isolates of *P. andina* were crossed among themselves, oospores were produced in a number of the crosses. Since all 10 of these isolates react as A2 when tested against an A1 isolate of *P. infestans,* it would appear that whilst *P. andina* isolates can be classified for mating type with *P. infestans* testers, the mating system within *P. andina* is more complex. This highlights the limited utility of using mating-type testers from one species to classify isolates of another species. The incongruence of mating systems (complementary but not identical) is consistent with the designation of *P. andina* as a separate species. No attempt was made at this point to describe the mating system in *P. andina* as the sample was small (10 isolates) and not representative; all isolates came from hosts of the Anarrhichomenum section. Further work is needed to address mating behaviour in this species.

The complexity identified in the mating system of this species is not necessarily unique to *P. andina* with this *Phytophthora* clade. No mating system complementary to that of *P. infestans* has apparently been described for *P. mirabilis*. Goodwin & Fry (1994) were able to cross *P. infestans* with *P. mirabilis* and obtain hybrid oospores, but host specialization was suggested as a mechanism of reproductive isolation. In another study (R.F. Oliva, unpublished data) it was demonstrated that crosses between *P. andina* and *P. infestans* resulted in oospores that could survive up to 1 year in soil and hybrid progeny that could infect potato in the laboratory, but insufficient studies of viability of oospores resulting from crosses of *P. andina* by *P. andina* were done. Given the potential implications for genetic recombination, hybridization and disease initiation from oospores, further studies to characterize the mating systems of *P. andina* and oospore viability and epidemiology are needed.

In tree hosts (*S. betaceum, S. quitoense* and *S. hispidum*), *P. andina* causes similar but much larger leaf lesions to late blight on potato, with longer infectious periods. The pathogen also causes very serious stem infections (Fig. 1) on *S. betaceum*. Blight symptoms in general on the Andean solanaceous hosts appear to be defined more by the host than the pathogen. On the Anarrhichomenum hosts (Fig. 1), which produce vine–like growth in the underbrush, disease symptoms are very much like late blight of potato or tomato. Detached-leaf inoculations in the laboratory indicated that *P. andina* can also infect and sporulate on leaves of wild or cultivated potatoes. In nature, however, the pathogen appears to have strong host preferences, and through a decade of isolation studies *P. andina* lesions were not found on potato or tomato plants (Adler *et al.*, 2004; Oliva *et al.*, 2007). However, there are solanaceous hosts which *P. andina* and *P. infestans* may co-infect, and given the comments above on the potential for hybridization, this

could be important. On two occasions, *P. andina* was isolated from *S. muricatum*, which is generally a host of US-1 genotypes of *P. infestans* (Adler *et al.*, 2002). In each of those cases, *P. andina* appeared to cause disease of epidemic proportions; all isolates were *P. andina*. There are also other hosts from which both species have been isolated, including *S. quitoense* and *S. ochranthum* (R.F. Oliva, unpublished data).

Similar to *P. infestans* on potato or tomato, *P. andina* appears to be a pathogen of increasing and potentially great impact on native Andean fruit crops, some of which have or are being traded globally. Although epidemiological studies have not been done, a marked increase in severity of disease on *S. betaceum* can be noted. In the mid 1990s, *P. andina* infection on *S. betaceum* was difficult to find; now, for collection, one simply has to go to any of the major production areas and disease is always present. The dynamics of *P. andina* may also include *S. quitoense* in Ecuador and Colombia, which is produced in restricted regions at mid-level elevations (1000–2000 m a.s.l.) and occupies a different agroecological zone than does *S. betaceum*. Outside the genus *Solanum*, less is known about the occurrence of *P. andina* on other solanaceous crops. Late blight of Cape gooseberry (*Physalis peruviana*) was recently reported to be caused by *P. infestans* in Colombia (Vargas *et al.*, 2009), although the markers used by the authors would not have distinguished *P. infestans* from *P. andina*.

Some hosts described in this study, which are commonly attacked by *P. andina*, are no longer restricted to the Andes. Perhaps the best example is *S. betaceum*, which is widely cultivated as small orchards around the world, but also considered economically important in some non-Andean countries, particularly New Zealand. Currently, there are no reports of the pathogen outside the Andean highlands.

Analyses of the impact of this pathogen on native fruit crops in the Andean highlands and studies on the evolutionary relationships of this species with other *Phytophthora* pathogens attacking solanaceous hosts are in progress.

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