



Identification of an A2 population of *Phytophthora andina* attacking tree tomato in Peru indicates a risk of sexual reproduction in this pathosystem

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Tree tomato, *Solanum betaceum*, is an Andean fruit crop previously shown to be attacked by *Phytophthora andina* in Ecuador and Colombia. Blight-like symptoms were discovered on tree tomato plants in the central highlands of Peru in 2003 and shown to be caused by *P. andina*. Isolates of *P. andina*, collected from three different plantations in Peru over a 6-year time span (2003–2008), were compared genetically with *P. andina* isolates from Colombia and Ecuador to test whether the pathogen population is geographically structured in the Andes. Restriction fragment length polymorphism (RFLP), mitochondrial DNA and simple sequence repeat (SSR) genetic markers, and mating type behaviour indicated that the Peruvian *P. andina* population from tree tomato is genetically distinct from populations infecting tree tomato in Colombia (CO-1) and Ecuador (EC-3, Ia, A1), but is more similar to the population infecting solanaceous hosts of the *Anarrhichomenum* complex (EC-2, Ic, A2). Such geographic substructuring within this pathogen species could result from spatial isolation. Most strikingly, in contrast to the Ecuadorian and Colombian *P. andina* isolates from tree tomato, the Peruvian isolates have the A2 mating type. The presence of both mating types in the Andean population of *P. andina* attacking tree tomato indicates a risk of sexual reproduction and the presence of long-lasting oospores in this pathosystem.

Keywords: *Phytophthora andina*, population structure, *Solanum betaceum*, tree tomato

Introduction

Phytophthora andina is an increasingly common pathogen of the Andean crops *Solanum betaceum* (tree tomato, *tomate de arbol*, *sachatomate*), *S. muricatum* (*pepino dulce*) and *S. quitoense* (*lulo*, *naranjillo*). In addition, it is found on wild species of the *Solanum* sect. *Anarrhichomenum* Bitter. *Phytophthora andina* is thought to originate from the Andes, because it has not been found elsewhere, and to have emerged as a result of hybridization between *Phytophthora infestans* and an unknown *Phytophthora* species (Goss *et al.*, 2011; Blair *et al.*, 2012). *Phytophthora andina* is a sister lineage of *P. infestans* and shares a South American ancestry (Gómez-Alpizar *et al.*, 2007). *Phytophthora infestans* and *P. andina* are morphologically indistinguishable, but genetically different. Distinct lineages of *P. andina* have been documented using multilocus genotyping of mitochondrial and nuclear genes (Gómez-Alpizar *et al.*, 2008). Based on allozyme genotype, mitochondrial DNA (mtDNA) haplotype, RG57 restriction fragment length polymorphism (RFLP) pattern, and mating type, the cur-

rently known diversity of *P. andina* consists of three distinct clonal lineages described from Ecuador (Oliva *et al.*, 2010). Two clonal lineages that differ in mating type (A1 versus A2) and mtDNA haplotype (Ia versus Ic), but have the same RFLP fingerprint (EC-2), were discovered from wild species of *Solanum* section *Anarrhichomenum* (Ordoñez *et al.*, 2000; Oliva *et al.*, 2010). A third lineage, with EC-3 RFLP fingerprint, A1 mating type and Ia mtDNA haplotype, was found on tree tomato (Adler *et al.*, 2004; Oliva *et al.*, 2010). EC-3 isolates of *P. andina* (*P. infestans sensu lato*) attacking tree tomato have been described from southwestern Colombia (Revelo *et al.*, 2011). Also, other isolates from Colombia classified as *P. infestans*, with Ia mtDNA haplotype (Cárdenas *et al.*, 2011) and A2 mating type (Vargas *et al.*, 2009), are probably *P. andina*. The question of whether *P. andina* is a true species was addressed by Forbes *et al.* (2012). Exact comparisons between *P. andina* populations in Colombia and Ecuador using existing published data are difficult because different marker systems have been used in these studies. The mitochondrial genomes of *P. andina* and its sister Ic clade species have been sequenced (Lassiter *et al.*, 2015). The Ic mitochondrial lineage of *P. andina* forms a sister lineage with *Phytophthora ipomoeae* and *Phytophthora mirabilis* and diverged from a common ancestor, while the Ia mitochondrial lineage of *P. andina* is sister to *P. infestans*

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and diverged later. The current Ecuadorian population of *P. infestans* consists of two main clonal lineages: US-1 on tomato, *Solanum caripense* and *Solanum ocbrautum*, and EC-1 on potato, *Solanum andreamum*, *Solanum colombianum*, and other species (Oliva *et al.*, 2007, 2010; Delgado *et al.*, 2013). In Colombia, the *P. infestans* population found on potato is EC-1 lineage (Vargas *et al.*, 2009), while other lineages have been found on *Physallis peruviana*. In Peru, three different clonal lineages of *P. infestans* have been described: EC-1 mainly infects cultivated potato, while PE-3 and PE-7 are found on native potato landraces (Perez *et al.*, 2001) or wild tuber-bearing species (Garry *et al.*, 2005).

Phytophthora andina is heterothallic and does not form oospores in single cultures; however, it may have a mating system different from *P. infestans*, because the isolates that behave as A2 when tested with an A1 isolate of *P. infestans* also produce oospores when crossed with isolates of *P. infestans* of the same mating type (Oliva *et al.*, 2010).

Tree tomato, *S. betaceum*, syn. *Cyromandra betacea*, which is indigenous to the Andes in Bolivia, Chile, Ecuador, Argentina and Peru, has been virtually globalized and can be seen growing in most tropical and subtropical parts of Africa and Asia. Cultivar groups are recognized by different morphological characteristics and named after the fruit colour and shape (Acosta-Quezada *et al.*, 2012). The species is mostly known from cultivation, but was apparently found in the wild in southern Bolivia and northwestern Argentina and it is thought to have diverged from wild relatives relatively recently (Bohs, 2007). It appears that tree tomato was already domesticated at least during the late pre-Columbian era, as pottery from that era depicting the tree tomato plant has been found in Peru (Towle, 1961).

In Peru, tree tomato is an underexploited crop and is cultivated on a small scale in home gardens in the central and northern highlands (Tapia, 1997; Anonymous, 2004; Medina & Roldan, 2007), particularly in the regions known as Yungay and Quechua Baja (Pulgar Vidal, 1996). Little or no information is available about diseases affecting this crop in Peru and no published report exists that describes *P. andina* from Peru. Blight symptoms were observed on tree tomato in Oxapampa in 2003 and the first samples were collected from localities around this region in 2003, 2005, 2007 and 2008. The

aim of this study was to determine the species identity of blight on tree tomato in Peru and to compare this population to other populations of *P. andina* from Ecuador and Colombia to determine whether the pathogen population in the Andes is geographically structured.

Materials and methods

Phytophthora andina isolates

Eighteen isolates (Tables 1 & S1) were collected from tree tomato foliage with symptoms from gardens and small fields located in Oxapampa (10°31'23"S, 75°26'21"W, 1797 m a.s.l.) in the central rainforest mountains of Peru. In 2003 five isolates were collected from individual plants from one field located in the village of Quillazu. In 2005 and 2007 five and four more isolates, respectively, were collected from two different fields in the same village. In 2008 four isolates were collected from one field located in the village of Grapanazu. Fields were generally separated by a distance of 1 km and, overall, the samples represented an area of about 10 km². Each isolate originated from a single plant. Small pieces of infected leaves (0.5 cm²) were washed with running water, dried with a sterilized paper towel and then placed on V8 juice agar (V8A) culture media (Forbes, 2007) at 18 °C in darkness. After 5 days, cultures were transferred to fresh V8A plates for further studies. Some infected leaves were placed in a moist chamber at 18 °C with a photoperiod of 12 h to promote sporulation. With the help of a stereomicroscope, a small number of sporangiophores with attached sporangia were captured by touching them with small plugs of V8A, which were then placed on the surface of sterile V8A plates and incubated at 18 °C for 5 days. Isolates were stored for short periods in V8A at 18 °C in the dark and for longer periods in liquid nitrogen (Dahmen, 1983).

For genotyping, lyophilized mycelium of 17 *P. andina* and *P. infestans* isolates were obtained from Ecuador and 15 *P. andina* isolates from south Colombia. The host species, location and year of collection of all isolates are detailed in Table S1.

Pathogenicity, host range and sporangial morphology

Plants of tree tomato, *S. quitoense*, *P. peruviana*, *S. lycopersicum* 'Rutgers' and *S. tuberosum* 'Yungay' were propagated in the greenhouse and inoculated with seven isolates (POX102, POX103, POX104, POX117, POX118, POX119 and POX120) using 3×10^4 sporangia mL⁻¹. The inoculated plants were kept in a moist chamber at 18 ± 2 °C, relative humidity >80%, and photoperiod of 12 h. Host–pathogen interactions were assessed at 12 days after inoculation. Sporangial sizes were measured

Table 1 Summary of the *Phytophthora andina* isolates and their characteristics based on RG57 restriction fragment length polymorphism (RFLP) patterns, mitochondrial DNA (mtDNA) haplotype and mating type

Host	Country	Isolation year	No. of isolates	mtDNA haplotype	RFLP fingerprint	Mating type	Reference
<i>Solanum betaceum</i>	Peru	2003–2008	18	lc (14)	PE-8 (18)	A2 (15)	This study
<i>S. betaceum</i>	Ecuador	1998–2005	8	la (6)	EC-3 (8)	A1 (8)	This study
<i>S. betaceum</i>	Colombia	2009	15	n.a.	CO-1 (2)	n.a.	This study
<i>S. brevifolium</i>	Ecuador	1997–1998	2	lc (2)	EC-2.1 (2)	A2 (2)	Oliva <i>et al.</i> (2010)
<i>S. tetrapetalum</i>	Ecuador	1997–1998	2	lc (2)	EC-2 (2)	A2 (2)	Oliva <i>et al.</i> (2010)

The number of isolates analysed by each method is shown in brackets. n.a.: not analysed.

from pieces of V8A incubated at 20 °C in darkness for 12 days. One hundred sporangia were measured for each isolate.

Mating type

Isolates were paired with two known A1 *P. infestans* isolates (PE84006 and POX67; Perez *et al.*, 2001; Villamon *et al.*, 2005) on 10% clarified V8 agar (Forbes, 2007). The presence or absence of oospores was observed after 4 weeks using a microscope ($\times 400$).

DNA extraction and molecular markers

Total DNA was extracted from lyophilized mycelium grown on pea broth as described by Perez *et al.* (2001). Mitochondrial haplotypes were determined using primers designed by Griffith & Shaw (1998) followed by the digestion of the amplified regions with restriction enzymes *CfoI*, *MspI* and *EcoRI*. RG57 RFLP fingerprints were generated as described by Perez *et al.* (2001).

Genetic diversity of the isolates was analysed for simple sequence repeat (SSR) loci 4B, 1F, G11, Pi63 and Pi66 using primers detailed in Table 2. PCR amplifications were performed in a 10 μ L volume containing 25 ng of genomic DNA, 1 μ L 10 \times reaction buffer B (Promega), 0.01 mM each dNTP, 0.2 μ M each forward and reverse primer, and 0.7 U *Taq* DNA polymerase (Promega). PCR was performed in a Veriti cycler (Applied Biosystems) as described in Knapova & Gisi (2002) and Lees *et al.* (2006) for each marker. PCR reactions were denatured for 5 min at 94 °C and alleles were separated on a 6% denaturing acrylamide gel. Alleles were scored visually after silver staining.

Genetic analysis

The genetic similarities between 13 *P. andina* isolates from Peru, 15 *P. andina* isolates from Ecuador, 13 *P. andina* isolates from Colombia, 10 *P. infestans* isolates from Peru, and 4 *P. infestans* isolates from Ecuador were inferred from the SSR data. All alleles scored as fragment sizes (bp) were included in the analysis assuming that the isolates were tetraploids and considering null alleles as missing data. An unweighted neighbour joining tree was constructed based on 30 000 bootstraps using a simple matching coefficient and the genetic structure in the data set was determined by factorial analysis using the DARWIN package (Perrier & Jacquemoud-Collet, 2006). The genetic structure was also computed using the Bayesian clustering program STRUCTURE v. 2.2 (Pritchard *et al.*, 2000) assuming 1–10 clusters (*K*). To

determine the most probable number of clusters in the data set, six independent runs were analysed using STRUCTURE HARVESTER (Earl & von Holdt, 2012).

Results

Morphology and pathogenicity of the Peruvian *P. andina* isolates

Characteristic blight lesions were found in the leaves and fruits of tree tomato plants that were intercropped with potato and tomato in three separate fields in the Peruvian Andes near Oxapampa. Blight lesions were very similar to those produced by *P. infestans* and sporulation was usually visible only at the edges of lesions and more abundant on the abaxial side of leaves (Fig. 1). A total of 18 different isolates were obtained from the tree tomato plants. Sporangia were semipapillate and no differences in shape or dimension were observed among isolates tested. No consistent differences in length and width of sporangia were observed. Length of sporangia ranged from 33.50 to 55.30 μ m (average 44.26 μ m) and width from 13.81 to 21.65 μ m (average 16.52 μ m). Zoospores were released 2 h after the incubation of sporangia in cold water (8 °C.) Neither hyphal swellings nor chlamydozoospores were observed in culture.

No oospores were observed in single cultures, but they were produced when paired with A1 *P. infestans* isolates (PE84006 and POX067). Amphigynous antheridia (average 20.21 μ m), smooth-walled oogonia with an average diameter of 39.45 μ m and plerotic oospores with smooth-walled and tinted yellow-brown colour were observed. Diameters of the oospores ranged from 35.2 to 40.3 μ m with an average of 38.6 μ m. Thus, the Peruvian *P. andina* isolates can be considered as A2 according to the *P. infestans* mating system.

Lesions caused by *P. andina* isolates on plants of tree tomato under greenhouse conditions were similar to those observed in the plantations (Fig. 1). The symptoms appeared 5 days after inoculation and the reisolation successfully fulfilled Koch's postulates. None of the *P. andina* isolates were pathogenic on any of the other plants tested.

Table 2 Characteristics of the simple sequence repeat (SSR) markers and the PCR primers used in this study

Marker	Repeat	Primer sequence (5'–3')	Size range (bp)	Annealing temperature (°C)	
4B	(TC) ₃₄	F	AAAATAAAGCCTTTGGTTCA	205–217	58
		R	GCAAGCGAGGTTGTAGATT		
G11	(TC) ₂₇	F	TGCTATTTATCAAGCGTGGG	142–166	56
		R	ACAATCTGCAGCCGTAAGA		
1F	(TC) ₇	F	GAGAGTGAATGAGAGCGAG	94–166	59
		R	ACAATCTGCAGCCGTAAGAG		
Pi63	(GAG) ₃	F	ATGACGAAGATGAAAGTGAGG	148–160	58
		R	ATTCATTATTGGCAATGTTGG		
Pi66	(GT) ₇	F	ACCGACAGCTTCTGAAACC	153–155	58
		R	AAAATAAGAAGAGATTTCGTGCC		

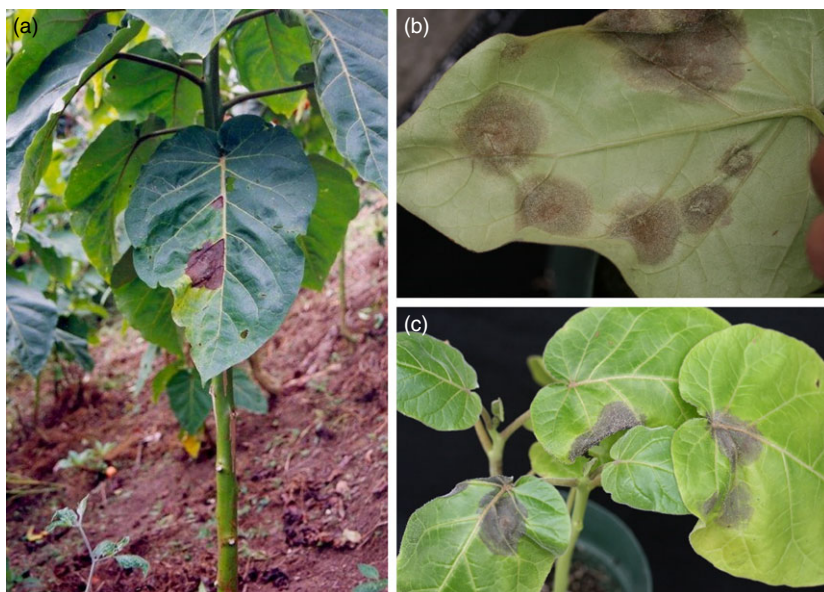


Figure 1 Blight symptoms caused by *Phytophthora andina* on naturally infected tree tomato (*Solanum betaceum*) plants (a, b) and after inoculation (c).

Genetic characterization of *P. andina* strains from Peru, Ecuador and Colombia

To test whether the *P. andina* population in the Andes is geographically structured, *Phytophthora* isolates collected from Peru were compared with those from Ecuador and southern Colombia. All Peruvian tree tomato *P. andina* isolates had the Ic mtDNA haplotype whilst those from Ecuador had the Ia haplotype (Table 1; Fig. S1). Colombian isolates were not tested for mtDNA. The RFLP patterns of Colombian and Peruvian *P. andina* isolates were distinct from the previously detected patterns of *P. andina* from Ecuador (EC2, EC2.1 and EC3), and also differed from the RFLP pattern of *P. infestans* isolates (Figs 2 & S2; Table S2). The RFLP pattern of the Peruvian *P. andina* lineage was named PE-8 and the Colombian *P. andina* lineage CO-1. The relationships between the different *P. andina* lineages could not be well resolved based on the RFLP pattern alone (Fig. 2).

The five SSR loci used in this study generated a total of 52 alleles (Table S3). The tree tomato population of *P. andina* from Ecuador had much higher allelic diversity than the populations from Colombia or Peru, even though the sample size from Ecuador was smaller (Table 3). This also led to the population in Ecuador having a higher number of multilocus genotypes (MLGs). In Peru, all isolates collected had the same MLG, while in Colombia, five MLGs were found.

The complete SSR data set, consisting of all detected alleles scored by fragment size, was used to construct a pairwise similarity matrix and an unweighted neighbour-joining tree. The isolates formed three main clusters supported by bootstrap analysis (Fig. 3). The first cluster consisted of *P. andina* isolates from Colombia, with the RFLP fingerprint CO-1 and *P. andina* isolates from Ecuador with the fingerprint EC-3. The second cluster was formed by *P. andina* isolates from Peru with the

RFLP fingerprint PE-8 and *P. andina* isolates from Ecuador with EC-2 and EC-2.1 RFLP fingerprints. The last cluster consisted of *P. infestans* isolates with three distinct RFLP patterns.

The principal component analysis (Fig. 4) and the population structure analysis (Fig. 5) separated the *Phytophthora* isolates into three clear groups. *Phytophthora andina* isolates from Peru grouped together with the *P. andina* isolates collected from *S. brevifolium* and *S. tetrapetalum* in Ecuador (EC-2 or EC2.1 lineage/Ic mtDNA haplotype), while the *P. andina* collected from tree tomato in Ecuador and Colombia formed a group of their own (EC-3 lineage/Ia mtDNA haplotype). All *P. infestans* isolates formed a separate group from all *P. andina* isolates.

Discussion

The results show that the blight symptoms found in tree tomato in central Peru were caused by *P. andina*. The isolates collected are morphologically similar to those found on tree tomato in Ecuador and Colombia and the lesions in the field also resemble those published from Ecuador (Oliva *et al.*, 2010). Reinoculation of tree tomato with these isolates was successful and caused disease symptoms identical to those seen in the field. Thus, the pathogen characterized belongs to the general population of *P. andina* attacking tree tomato in the Andes.

Nonetheless, the data also demonstrated that this Peruvian population of *P. andina* constituted a single clonal lineage that was genetically distinct from the *P. andina* population that had been earlier described as infecting tree tomato in Ecuador (Adler *et al.*, 2004; Oliva *et al.*, 2010) and that of Colombia. Interestingly, the Peruvian population was genetically more similar to the EC-2 lineage that has not previously been found infecting tree tomato. The EC-2, EC-2.1 and Peruvian lineages all have the same

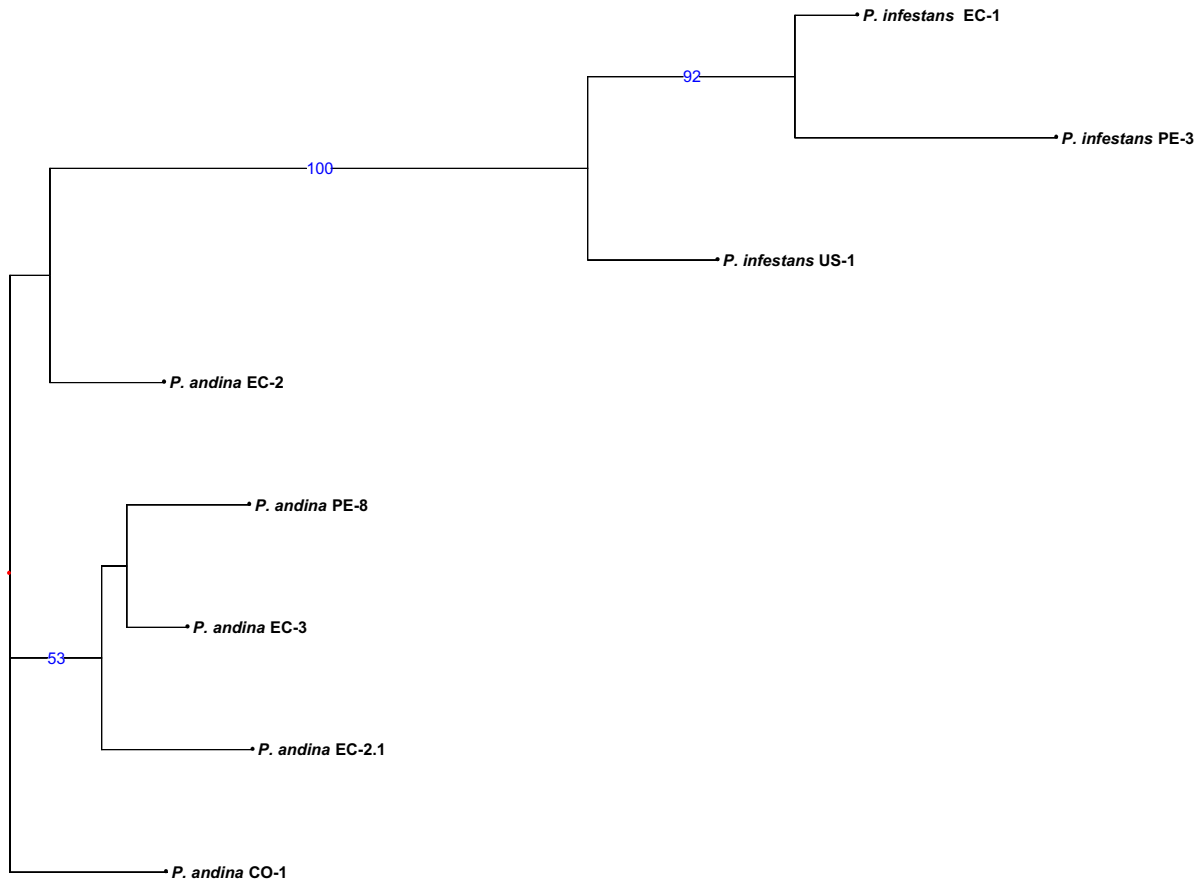


Figure 2 Unweighted neighbour-joining dendrogram of the RG57 restriction fragment length polymorphism (RFLP) band patterns of the *Phytophthora infestans* and *P. andina* isolates. Bootstrap values are based on 30 000 replicates.

Table 3 Allelic diversity of the five simple sequence repeat (SSR) loci in the subpopulations of *Phytophthora andina* and *P. infestans*

Phytophthora sp.	Host	Country of origin	No. of isolates (n = 53)	No. of alleles (n = 52)	MLG (n = 25)	Sum of alleles (average no. of alleles/isolate) for each SSR marker				
						4B (n = 20)	Pi63 (n = 3)	Pi66 (n = 6)	1F (n = 11)	G11 (n = 12)
<i>P. andina</i>	<i>Solanum betaceum</i>	Colombia	13	15	5	4 (2.9)	2 (2.0)	2 (2.0)	3 (2.0)	4 (2.2)
	<i>S. betaceum</i>	Ecuador	8	27	8	12 (3.4)	2 (2.0)	3 (1.9)	5 (2.0)	5 (2.2)
	<i>S. betaceum</i>	Peru	13	9	1	3 (3.0)	2 (2.0)	2 (2.0)	1 (1.0)	1 (1.0)
	<i>S. brevifolium</i> , <i>S. tetrapetalum</i>	Ecuador	5	19	5	5 (3.0)	2 (2.0)	3 (2.0)	4 (1.4)	5 (1.6)
<i>P. infestans</i>	<i>S. colombianum</i> , <i>S. tuberosum</i>	Ecuador	4	8	1	3 (3.0)	1 (1.0)	2 (2.0)	1 (1.0)	1 (1.0)
	<i>S. tuberosum</i> , <i>Lycopersicum</i>	Peru	10	22	5	5 (2.6)	3 (1.4)	2 (1.9)	4 (1.3)	4 (1.6)
	<i>hirsutum</i> , <i>S. caripense</i> , Solanaceae									

MLG: multilocus genotype.

mtDNA haplotype, Ic. Based on the similarity of the SSR patterns and the mtDNA haplotype it seems that the *P. andina* lineage analysed from Peru could be another variant of the EC-2 lineage. Similarly, the *P. andina* lineage from Colombia is probably a variant of the EC-3 lineage.

All Peruvian *P. andina* isolates tested were A2 mating type because they produced oospores when paired with the A1 mating type tester of *P. infestans*. However, when

these isolates were paired among themselves, they did not produce oospores, as was reported in Ecuador. This then constitutes another distinct characteristic of the Peruvian population. While some *P. andina* isolates in Ecuador are A2 mating type, these come from *S. brevifolium* and *S. tetrapetalum* (and other species in the *Anarrhichomenum* complex) (Ordoñez *et al.*, 2000; Oliva *et al.*, 2002, 2010). In contrast, *P. andina* isolates

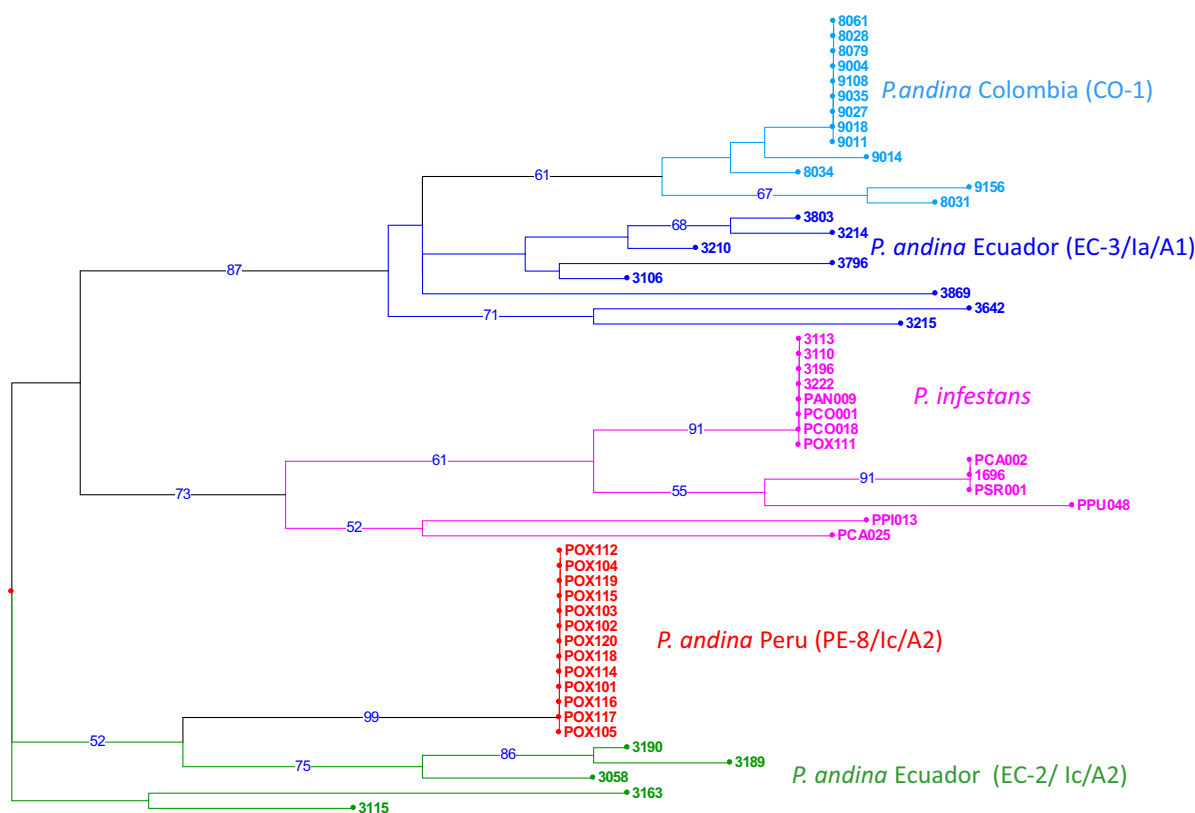


Figure 3 Unweighted neighbour-joining dendrogram of the 53 *Phytophthora infestans* and *P. andina* isolates constructed based on the presence/absence of 52 alleles from five simple sequence repeat (SSR) loci. Bootstrap values are based on 30 000 replicates.

from tree tomato in Ecuador are A1 mating type (Oliva *et al.*, 2010). Similarly, isolates from tree tomato in Colombia have been reported to be of the A1 mating type (Mideros *et al.*, 2011).

The subpopulation from Peru appeared to be genetically less diverse than in the other countries. While it is difficult to draw conclusions based on small sample sizes, the low diversity in Peru could also indicate that the population there is newer and this may reflect a migration event to Peru from Ecuador or another area. However, host range represents another reason for caution in speculation about the genetic structure of these populations in the different Andean countries. In Ecuador, the two main lineages of *P. andina* discovered to date have different host ranges (Adler *et al.*, 2004; Oliva *et al.*, 2010), while the genetically variable isolates from Colombia, and the less variable isolates from Peru all come from tree tomato. The reason for differences in host range among the subpopulations is not known, but it may reflect sampling bias. To the authors' knowledge there have been few, if any, attempts to find blight-like lesions on the species in the *Anarrhichomenum* complex of the genus *Solanum* growing in Peru and Colombia; thus there is a need for further sampling in Peru, Ecuador and Colombia on wild hosts.

Tree tomato is cultivated on a larger scale in Ecuador and Colombia than in Peru and most probably different

tree tomato varieties are grown in the different countries. In a large-scale analysis of tree tomato variation across the Andean countries, it was discovered that local cultivations harbour large diversity, consisting of many different landraces (Anonymous, 2004). These will probably contain diverse levels of resistance to the blight pathogen, as has been shown to be the case for the two Colombian varieties tested (Revelo *et al.*, 2011); thus, pathogen diversity may also reflect the host diversity. Unfortunately, no information was available on the host varieties or landraces from which the *P. andina* isolates of the current study were collected. The similarity of the tree tomato *P. andina* populations in south Colombia and Ecuador may be explained by the closer geographical proximity of the collection sites and/or exchange of planting material. EC-3 has been reported in southwest Colombia and consists of isolates with varying virulence spectra (Revelo *et al.*, 2011).

Due to the phytosanitary regulations, which restrict the transfer of plant pathogens between regions, it was not possible to test whether the tree tomato *P. andina* isolates of opposite mating types from Ecuador and Peru are sexually compatible. Nonetheless, planting materials should be exchanged with caution between these countries to avoid the establishment of both mating types in the same plantations. If both mating types were present, this would increase the risk of sexual reproduction in the

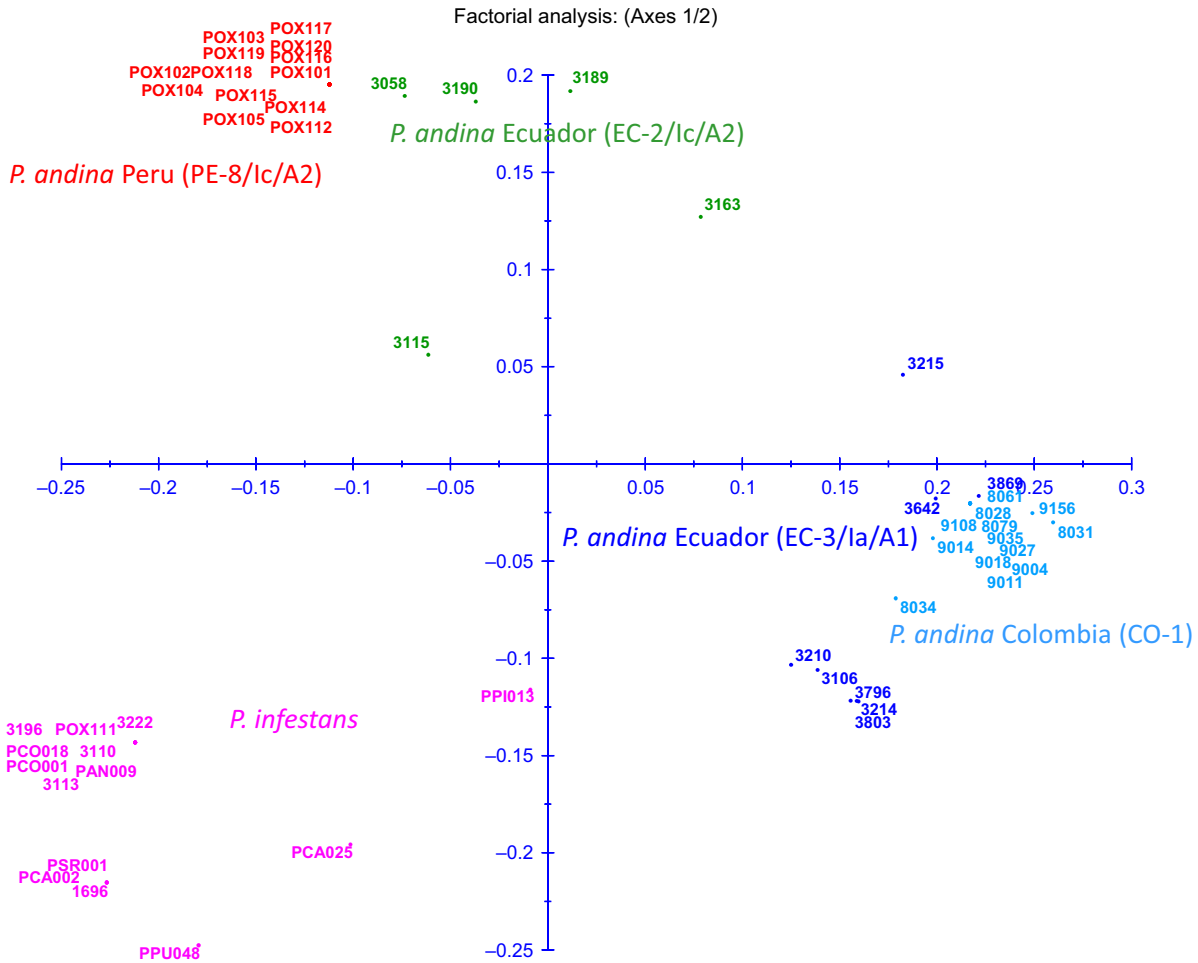


Figure 4 Principal component analysis based on the simple sequence repeat (SSR) marker data showing the substructuring of the *Phytophthora* isolates. The isolate names their subgroup names including the country of origin are shown in the same coloured font and their restriction fragment length polymorphism (RFLP) pattern, mitochondrial DNA (mtDNA) haplotype and mating type, when known, are shown in brackets.

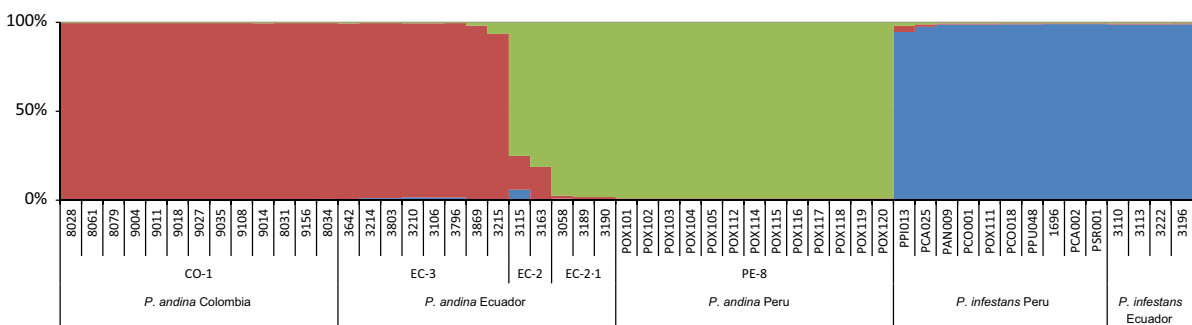


Figure 5 Population substructure of the *Phytophthora* isolates based on simple sequence repeat (SSR) markers. The x-axis shows the species designation, clonal lineage type and country of origin of the isolates. The y-axis shows the probability of the isolate belonging to one of the three subgroups indicated by the three different colours. Lineage name (host species) of the *P. andina* isolates: CO-1, EC-3, PE-8 (from *Solanum betaceum*), EC-2 (from *S. tetrapetalum*), EC-2.1 (from *S. brevifolium*).

pathogen population, which could be particularly problematic, given that tree tomato is a perennial crop.

The Peruvian *P. andina* lineage was unable to establish a compatible interaction with potato and tomato, and

similarly the local *P. infestans* isolates were not able to infect the tree tomato plants, suggesting a strong host preference. At the moment the possibility that both species can co-infect an alternative host, enabling

hybridization, cannot be discarded. In fact, both *P. infestans* and *P. andina* have been found on *S. muricatum*, *S. quitoense* and *S. ochranthum* in Ecuador (Adler *et al.*, 2004; Oliva *et al.*, 2010). *Phytophthora infestans* was isolated from potato growing nearby the tree tomato gardens in Oxapampa, which is also consistent with the hypothesis of strong host preference.

Despite the strong host preference, the tests in this study indicated that the *P. andina* PE-8 lineage and the *P. infestans* EC-1 lineage produce oospores abundantly when paired on a culture medium, as found with the EC-2 lineage and EC-1 lineage in Ecuador (Oliva *et al.*, 2002). It remains to be tested whether the offspring would be viable, and if so, what their host range would be. It would also be interesting to test whether *P. infestans* and *P. andina* can simultaneously infect one of the above mentioned solanaceous hosts, reported to be attacked by both pathogens. Interestingly, the offspring from EC-2 and EC-1 lineages in Ecuador were reported not pathogenic to the original hosts of the parents, suggesting post-mating mechanisms of reproductive isolation (Oliva *et al.*, 2002).

The results of this study raise an interesting research question relating to the origin of *P. andina*. Given that *P. andina* is a hybrid between *P. infestans* and an unknown member of clade I of the *Phytophthora* family (Goss *et al.*, 2011) the geographical substructuring and the existence of two mitochondrial lineages are an indication that the population in Peru may be a result of a separate hybridization event. This is also supported by the recent study of Lassiter *et al.* (2015) showing that the Ic mtDNA lineage of *P. andina* diverged from a common ancestor of *P. ipomeae* and *P. mirabilis* before the Ia mtDNA haplotype of *P. andina*. Separate natural hybridization events are thought to have resulted in the two distinct populations of *P. cactorum* × *P. nicotinae* infecting loquat in Peru and Taiwan (Hurtado-Gonzales *et al.*, 2009). However, more detailed molecular analysis is required to determine whether this is the case. Peru is a diversity hotspot of solanaceous species (Särkinen *et al.*, 2015), which may harbour *Phytophthora* and other pathogens with undiscovered diversity.

Many unanswered questions remain on the *P. andina* population dynamics and the potential impact of this pathogen for larger scale cultivation of tree tomato and other related host species. From the historical descriptions, as well as current experiences from various *Phytophthora*–host interactions, it is well known that large-scale monoculture, based on a few cultivars, usually results in big yield losses. Therefore, research on the virulence spectrum of the current populations, as well as the resistance spectrum of the available germplasm, would be of high importance.

Acknowledgements

The authors thank Dr Luz Estela Lagos for providing the DNA of the Colombian isolates. The authors have no conflict of interest to declare.

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Supporting Information

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

Figure S1 The four mitochondrial DNA haplotype fingerprint patterns (IIa, Ia, Ic and Ib) detected in the *Phytophthora andina* and *P. infestans* isolates of this study. The pattern IIa, here represented by isolate 3110, is typical for *P. infestans* lineage EC-1; Ia, here represented by isolate 3210, is found in lineages PE-3 of *P. infestans* and EC-3 of *P. andina*; Ic, here represented by isolate 3190, is found in lineages EC-2 and PE-8 of *P. andina*; and Ib, here represented by isolate PCA025, is typical for *P. infestans* US-1 lineage.

Figure S2 RG57 RFLP hybridization patterns of representative *Phytophthora andina* isolates from Ecuador, Peru and Colombia.

Table S1 Host plant species, country of origin, year of collection, mtDNA haplotype, RG57 RFLP pattern and mating type data of the *Phytophthora andina* and *P. infestans* isolates.

Table S2 RG57 RFLP marker patterns of *Phytophthora andina* and *P. infestans* isolates scored as presence (1) and absence (0) of the bands obtained.

Table S3 Estimated sizes of the alleles scored of the SSR markers 4B, Pi63, Pi66, 1F and G11 in the *Phytophthora andina* and *P. infestans* isolates. The marker alleles in each isolate are indicated as presence (1) or absence (0).