Population structure of *Phytophthora infestans* collected from potatoes in Ecuador, Colombia, Peru, Bolivia and Uruguay

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Abstract

Late blight, a destructive disease affecting potatoes, is caused by the oomycete *Phytophthora infestans* and remains a critical threat worldwide. The potential for sexual reproduction in production areas near the Peru–Bolivia border remains high due to the historical presence of mating type A2 in Bolivia and the current presence of mating type A1 in Peru. In Ecuador, there has been a past coexistence of A1 (*P. infestans*) and A2 (*P. andina*), while in Colombia, *P. infestans* has mainly exhibited mating type A1. These neighboring countries were compared with strains from Uruguay, which historically had A2 but now shows A1.

We examined the genetic structure of South American *P. infestans* populations using 182 isolates: 97 from Bolivia and southern Peru, 14 from Colombia, 57 from Ecuador (1993–2022), and 14 from Uruguay. The isolates were characterized by clonal lineage, mitochondrial haplotype, and mating type. In Bolivia, only the lineage/haplotype 2A1/Ia was identified, whereas Peru exhibited both 2A1/Ia and EC1/IIa, all of which were mating type A1. Puno was the sole department where both lineages were present. In older Ecuadorian populations, we found US1/Ib and EC1/IIa (*P. infestans*), as well as EC2/Ic and EC3/Ia (*P. andina*), while recent populations showed only US1. In Colombia, EC1/IIa and a new lineage, CO4 (Ia), were discovered. In Uruguay, 2A1/Ia was predominant.

These results offer updated insights into the genetic diversity and geographic distribution of *P. infestans* across South America and aid in the development of enhanced late blight management strategies.

Keywords: Phytophthora infestans, clonal lineage, South America, late blight

Introduction

Phytopthora infestans, the causal agent of potato late blight, has a complex population structure and history in South America. The global migration history of *P. infestans* has been a subject of extensive research and debate. There are various hypotheses regarding the origin of *P. infestans*, including Mexican, Andean, and hybrid origin theories. Goss et al. (2014) suggested that Mexico was the center of origin where it migrated to South America, then to the United States, and finally to Europe (Andrivon, 1996). A study by Gómez-Alpizar et al. (2007) found that Andean populations harbored greater nucleotide and haplotype diversity than Mexican populations, and the oldest mutations in *P. infestans* originated in South America, supporting the Andean origin theory. Currently, Coomber et al. (2025) reaffirmed that the Andes is the origin center, and Patarroyo et al. (2024) suggested a Peruvian origin with a

probability of 0.631. The migration of *P. infestans* genotypes plays a crucial role in shaping population structures and driving the emergence of epidemics worldwide, including through the introduction of new genotypes (Guha Roy et al., 2021).

The study of the population structure of *P. infestans* is complex and dynamic, and has been extensively analyzed across various regions. It is influenced by factors, such as migration natural or migration related to the movement of potato seed tubers, sexual reproduction, geographical isolation. Additionally, *P. infestans* can rapidly evolve and adapt, posing a challenge for disease management (Cooke & Lees, 2004; Dey *et al.*, 2018; Ludwiczewska *et al.*, 2025). Microsatellite markers, also known as simple sequence repeats (SSRs), are co-dominant nuclear markers that are highly polymorphic, providing valuable insights into the genetic variation and population dynamics of this devastating pathogen (Lees *et al.*, 2006).

P. infestans is able to reproduce sexually when both mating types are present in the same population. Until the 1980s, only mating type A1 was known to occur in Europe, which restricted the fungus from being reproduced asexually. The appearance of A2, first detected in Switzerland, likely facilitated by potato imports from Mexico (Rhouma *et al.*, 2024b), changed the disease dynamics (Drenth et al., 1993; Shattock, 2002). The presence of both A1 and A2 mating types allows sexual reproduction, potentially leading to increased genetic diversity and the formation of oospores as alternative inoculum sources (Drenth et al., 1993; Shattock, 2002). Oospores can persist in the soil and act as alternative sources of inoculum (Yuen & Andersson, 2013). This supplementary source of inoculum, combined with increased genetic diversity, enhances the pathogen's capacity to adapt and persist outside the host, potentially complicating traditional disease management strategies (Drenth et al., 1993, 1994).

In Peru, the population structure of *P. infestans* exhibits clonal lineages with varying dominance across regions and over time. In the southern region, isolates collected between 1994 and 1999 were characterized by clonal lineages and mitochondrial haplotypes. In Cusco, the identified lineages were US1/Ib, EC-1/IIa, PE3/Ia, PE5/IIa, and PE6/IIa. In Puno, US1/Ib, PE3/Ia, and EC1/IIa were found, whereas in Apurímac, only US1.1 was reported as a reference (Perez *et al.*, 2001). A subsequent study analyzing isolates collected between 1997 and 2013 reported the presence of PE3/Ia and EC1/IIa in Cusco, US1/Ib and PE3/Ia in Puno, and EC1/IIa in Apurímac (Lindqvist□Kreuze *et al.*, 2020). More recently, isolates from 2017 were analyzed by Lindqvist□Kreuze et al. (2020), revealing EC1/IIa in Cusco and both EC1/IIa and PE7.1/Ia (a variant of PE7) in Puno, all of which belonged to the A1 mating type. *P. infestans* populations in Bolivia were studied by determining the clonal lineage/mitochondrial haplotype from herbarium specimens, which were found to be US-1/Ib, and from modern isolates, which identified BR-1/IIa (Saville & Ristaino, 2021). Gabriel et al. (2018) identified the A1 mating type, in contrast to Goodwin, S.B. et al. (1994), who identified mating type A2. The population structure of *P. infestans* in Colombia has been reported to be dominated by the clonal lineage EC-1/IIa, whereas CO-1/IIa and CO-2/IIa have also been identified on potato (Vargas *et al.*, 2009). In contrast, US1/Ib and CO3/Ib were identified on tomato hosts (Céspedes et al., 2013; Gilchrist Ramelli et al., 2009; Vargas et al., 2009; Olave-Achury et al., 2022). In Uruguay, the presence of BR-1/IIa was previously reported (Deahl *et al.*, 2003), but more recent studies have revealed the presence of 2A1/Ia (F. Lucca et al., 2023). In Ecuador, the presence of distinct clonal lineages and mitochondrial haplotypes has been studied over the years, with EC1/IIa being dominant on potato hosts and US1/Ib on tomato hosts (Forbes *et al.*, 1997; Oyarzun Pj *et al.*, 1998). Additionally, other lineages such as EC-2 and EC-3 have been identified in *P. andina* on different Solanum species (Gómez-Alpizar *et al.*, 2008).

The importance of studying South American *P. infestans* populations lies in their considerable diversity (Coomber *et al.*, 2025), which has implications for disease management and breeding resistance. Clonal lineage 2A1, a genotype reported in Europe since the early 80s (Cooke *et al.*, 2012), was dominant on potatoes before 2006 (Mariette *et al.*, 2016). The 2_A1 lineage has also shown significant expansion beyond Europe including the eastern-Africa region (EAR), comprising Kenya, Uganda, Tanzania, Burundi, and Rwanda (Njoroge *et al.*, 2019). Its presence in South America has been associated to the introduction of infected potato tubers. This lineage is now regarded as the dominant one in Argentina, Chile, Brazil, and Uruguay (Acuña et al., 2019; F. Lucca et al., 2023)

We propose that the genetic variation and population dynamics of *Phytophthora infestans* in South America are influenced by both geographic and agroecological elements, leading to distinct regional patterns. In particular, we anticipate that populations in ecologically linked or neighboring regions (such as the central Andes—Colombia, Ecuador, Peru and Bolivia) will exhibit higher genetic similarity, while those geographically distant from this region

(such as southern Uruguay) will show more pronounced genetic differences by importation of seed potatoes from Northern Hemisphere countries.

Materials and methods

Sampling

In 2017-2019, a total of 97 leaf samples with late blight-like symptoms were collected, comprising 67 samples from Bolivia and 30 from southern Peru. Samples from Bolivia originated from the department of Cochabamba at an altitude of 2974-3922 meters above sea level (masl). In Peru, the studied departments were Cusco, Apurimac, Ayacucho, and Puno, with an altitude range of 2732-4068 masl. In Colombia, 14 samples were collected in Santander and the North of Santander, with an altitude range of 2129-2842 masl in 2022. In Ecuador, 57 samples were collected from Carchi, Pichincha, Imbabura, Tungurahua, Chimborazo, Cotopaxi, Napo, Morona Santiago, Pastaza, and Bolivar between 1993-2022. Isolates were obtained from Ecuadorian CIP *Phytophthora* collection, which was maintained in liquid nitrogen. In Uruguay, fourteen samples were collected from Canelones and San Jose between 2023-2025.

Samples were collected on FTA cards (Whatman FTA Classic Card, catalog number WB120055; GE Healthcare UK Ltd.) according to the manufacturer's guidelines (Cooke, 2020). Each sample was placed on FTA paper with the sporulating side down and individually crushed in the field (Gamboa *et al.*, 2019). The FTA cards were then stored in zip-lock bags and mailed to the pathology laboratory at the International Potato Center (CIP) in Lima, Peru, for processing.

Genotypic Analysis

DNA extraction from FTA cards (Qiagen®) was performed following the manufacturer's instructions with some modifications using 2 discs of 3 mm, two rinses with modified Tris (hydroxymethyl) aminomethane-ethylenediaminetetraacetic acid TE^{-1} buffer (10 mM Tris and 0.1 mM EDTA), and the discs were resuspended in 50 µl TE buffer (10mM Tris and 1mM EDTA) and incubated for 5 min at 95 °C as the last step before being stored at -20 °C until use.

Mating Type

W16 CAPS assay

To identify the A1 and A2 mating types of *P. infestans* isolates, we used PCR primers W16-1 (5'-AAC ACG CAC AGG CAT ATA AAT GTA-3') and W16-2 (5'-GCG TAA TGT AGC GTA ACA GCT CTC-3'), according to the method described by Judelson et al. (1995). The PCR reaction was conducted in a 20 µL solution, comprising 4 µL of 5x buffer (Gotaq, Promega), 6 µL of $MgCl_2$ (25 mM), 0.2 µL of dNTPs (100 µM), 0.4 µM of each primer, 1 U of Taq DNA polymerase (Gotaq®, Promega), and 10 ng of template DNA. The thermal cycling protocol included an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 53 °C for 1 min, and elongation at 72 °C for 1 min. The PCR process ended with a final elongation step at 72 °C for 10 minutes. Afterwards, the PCR products underwent a three-hour digestion at 37 °C using the restriction enzyme Hae III (recognition site 5′-GG/CC-3′, New England Biolabs (NEB) #R0108 (https://www.neb.com). Each digestion mixture contained 7.7 µL of H2O, 2 µL of CutSmart Buffer 10X, 0.3 µL of Hae III , and 10 µL of PCR product (Brylińska *et al.*, 2018).

Culture

In the case of the Ecuadorian samples, mating type was determined by pairing them with tester strains A1 (*P. infestans*) and A2 (*P. andina*) isolates. To achieve this, a dual-culture method was employed using 10% clarified V8 agar. The plates were incubated at 15 °C in the dark, and after four weeks, they were checked for oospore formation. A small section from an agar culture of the test isolate was placed approximately 2 cm from a similar section of the reference A1 or A2 isolates. After incubating for two weeks at 16–18 °C in the dark, the plates were inspected microscopically for oospore formation at the hyphal interfaces within the contact zone. Isolates that generated sexual oospores with reference to A1 and A2 mating-type isolates were classified as A2 and A1, respectively. This was done during the sampling year.

Mitochondrial haplotype

The haplotypes of mitochondrial DNA (mt DNA) were identified by comparing the DNA profiles with those of reference isolates that exhibited Ia, Ib, IIa, and Ic mitochondrial genome profiles (Griffith & Shaw, 1998). DNA profiles were determined by amplification of specific mitochondrial regions using the P2 primer, followed by digestion of the amplified products with the restriction enzyme MspI (Griffith & Shaw, 1998). The resulting restriction fragment length polymorphisms (RFLPs) were separated by gel electrophoresis and compared to reference profiles to accurately identify haplotypes.

Molecular Markers

Genomic DNA extracted from *P. infestans* isolates was amplified using a specific set of microsatellite markers, following a modified version of the 12-plex protocol described by Li et al. (2013). The PCR reactions used the Type-It Microsatellite PCR kit from QIAGEN (https://www.qiagen.com), following a modified protocol by Saville et al. (2016). The resulting PCR amplicons were sent to Arizona State University for post-PCR processing in 96-well plates. For fragment analysis on the 3730 instrument (Applied Biosystems), 3 μL of the LIZ500 size standard ladder was mixed with 1 mL of formamide in a 96-sample plate. Each well contained 9 μL of the ladder/formamide mixture and 2 μL of a 1:50 diluted sample, resulting in a total sample volume of 11 μL per well when loaded onto 3730 instruments for fragment analysis. SSR alleles were sized using the GENEMARKER v. 1.9 software from SoftGenetics (https://softgenetics.com/), applying the allele sizing parameters specified by Li et al. (2013) along with a reference dataset of *P. infestans* isolates from the United Kingdom and Argentina (2A1), previously genotyped by David Cooke's laboratory (https://thz.lab.mcgill.ca/) at The James Hutton Institute (JHI) in the UK, as well as the Peruvian EC1 clonal lineage. Moreover, additional clonal lineages were included from Peru, specifically PE3, PE7, PE7.1(*P.infestans*), PE8 (*P.andina*), and Bolivia, namely, BR1(Saville & Ristaino, 2021) and US8 from USA, and which was reported in Colombia (Vargas *et al.*, 2009). The variant PE7.1, named to indicate its derivation from PE7 (Lindqvist□Kreuze *et al.*, 2020), was used in this study to ascertain whether it belonged to any other recognized clonal lineage. For Ecuadorian strains, the references were EC3 and US1 (previously genotyped by JHI), and one EC2 (previously genotyped by RFLP (Adler *et al.*, 2004; Oliva *et al.*, 2010)) was added to our analysis as a sample.

Data Analysis

Statistical analyses were conducted using the R statistical software, version 4.3.1 (R Core Team, 2019). For locus and population statistics, as well as for calculating the number of multilocus genotypes (MLGs), the R package poppr was utilized (Kamvar *et al.*, 2014). Clonality was assessed using the index of association (IA and r□d), with statistical significance evaluated through 999 permutations for each data set. Population clustering was examined using principal component analysis (PCA) based on the genetic distance metric proposed by Bruvo et al. (2004) and analyzed using Polysat (Clark & Jasieniuk, 2011). Also, a phylogenetic tree was developed by considering the Bruvo distance and was created using the unweighted pair group method with arithmetic mean (UPGMA) for hierarchical clustering. Genotypic relationships were examined through a minimum spanning network (MSN) which was constructed and visualized using the□R□package□poppr (Kamvar *et al.*, 2014) and which was used to infer population structure. Control isolates were included across PCA, UPGMA, and MSN analyses to guide and validate cluster assignment, under the premise that these reference genotypes should consistently form distinct groups representing known clonal lineages.

Diversity statistics were computed for each population, including the number of multilocus genotypes (MLG), number of expected multilocus genotypes (eMLG), Shannon-Wiener Index(H), Simpson's complement (λ), evenness (E.5), and expected heterozygosity (Hexp). Additional locus-level statistics included allelic richness, Simpson's 1–D diversity, heterozygosity (Hexp), and evenness (Shannon, 1948, 2001; Simpson, 1949; Hedrick, 1999).

To avoid artificially increased population differentiation due to repeated sampling of identical genotypes, the population was tested for clone-corrected data using analysis of molecular variance (AMOVA), and the null hypothesis of no genetic difference was rejected at $p < 0.05$.

Results

Samples collected

In Bolivia, 67 samples were collected from the Cochabamba Department, spanning 10 provinces situated approximately 570 km south of Puno, Peru. In Peru, samples were obtained from the departments of Cusco (1), Apurimac (4), Ayacucho (6), and Puno (19). In Colombia, 14 samples were collected from two departments: North of Santander (6) and Santander (8). In Ecuador, a total of 58 samples were collected from 10 different departments: Carchi (11), Bolivar (2), Chimborazo (2), Cotopaxi (5), Imbabura (3), Morona Santiago (1), Napo (9), Pastaza (2), Tungurahua (8), and Pichincha (14). In 2022, two isolates were obtained from the latter department. In Uruguay, fourteen samples were collected from two departments, Canelones (10) and San Jose (4) (Tables 1 and S1).

Mating type test

The primer pair W16–1 and W16–2 produced a 557-bp fragment. When digested with the HaeIII enzyme, the A1 mating-type isolates showed two fragments, measuring 557 bp and 457 bp, whereas the A2 mating-type isolates of *P. infestans* resulted in a single 457 bp fragment. All isolates from Bolivia, Peru, Colombia, and Uruguay were classified as A1 (Figure S1, TableS1). In the case of Ecuador, most samples were A1 and only one (Ec-3365) from the wild species *Solanum brevifolium* was A2 (*P.andina*) (Table S1).

Genotypic characterization

Analysis of the mitochondrial haplotypes revealed that isolates from Bolivia (67), Uruguay (14), Peru (8), Colombia (10), and Ecuador (2) were classified under the Ia haplotype. Twenty-three isolates from Peru, 3 from Colombia, and 38 from Ecuador corresponded to the IIa haplotype. Sixteen isolates from Ecuador corresponded to Ib, two of which were sampled in 2022. A single isolate (Ec-3365) from the wild species *Solanum brevifolium* in Ecuador corresponded to haplotype Ic (Figure S2, Table S1).

PCA was performed to identify related clusters, which took into account isolates from 19 departments in 5 countries and nine controls, revealing that group PE7.1 clustered with the well-known 2A1 clonal lineage (Figure1).

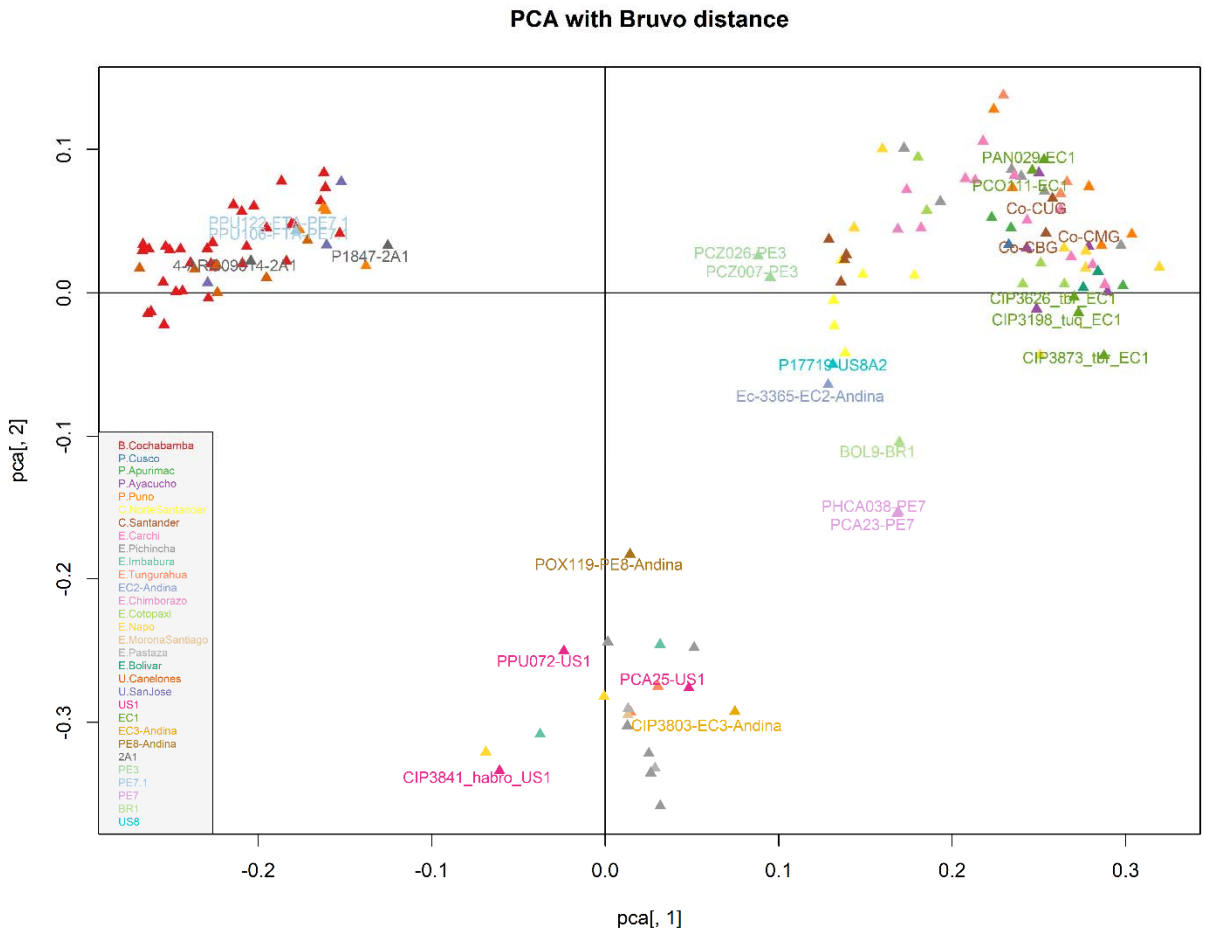
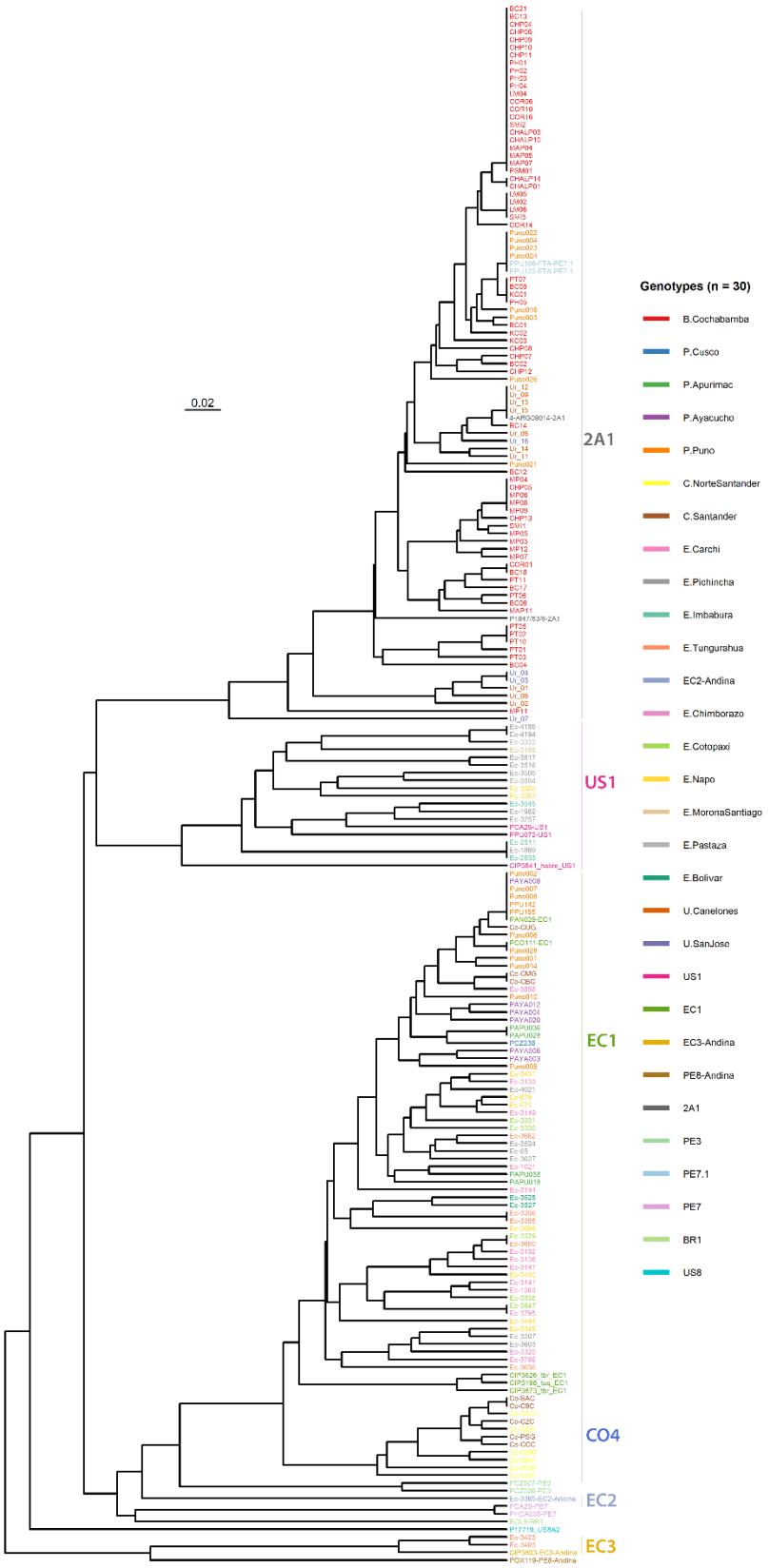


Figure 1. Principal component analysis (PCA) plot of *Phytophthora* isolates from B: Bolivia: Cochabamba (red), P: Peru: Cusco (Blue), Apurimac (Green), Ayacucho (Purple), Puno (Orange), C: Colombia: Norte de Santander (Bright Yellow), Santander (Brown), E: Ecuador: Carchi (Pink), Pichincha (Gray), Imbabura (Teal), Tungurahua (Coral), Chimborazo (Light fuchsia), Cotopaxi (Lime Green), Napo (Soft yellow), MoronaSantiago (Sandy Beige), Pastaza (Light Gray), Bolivar (Forest Green), U: Uruguay: Canelones (Burnt Orange), San José (Indigo). US1(Fuchsia), EC1(Olive green), 2A1 (Dark Gray), PE3 (Mint Green), PE7.1 (Sky Blue), PE7 (Lavender) and BR1(Pale Green), US8(Turquoise) [*P. infestans*], EC2 (Dusty Blue), EC3(Mustard), PE8 (Bronze) [*P. Andina*] were used as the clonal lineage controls.

The phylogenetic tree based on the Bruvo distance, with a maximum distance of 0.02 confirms six main groups (2A1, US1, EC1, EC2, EC3, and a new clonal lineage named CO4-found in Colombia) (Figure2). In Bolivia (67) and Uruguay (14), all of their strains were identified as 2A1; in the case of Puno-Peru, 11 isolates were EC1, and 8 isolates were 2A1. The other departments of southern Peru [Cusco (1), Apurimac (4), and Ayacucho (6)] were 100% EC1, which showed that EC1 was the predominant clonal lineage (73.3%), followed by 2A1 (26.6%). In Ecuador, US-1 was not found in cultivated potato, but was detected in *S. lycopersicum* (18.8%), *S. muricatum* (12.5%), *S. andreanum* (6.2%), *S. quitoense* (56.3%) and *S. caripense* (6.2%). In contrast, EC1 was found in cultivated potatoes (39.5%), and in wild species such as *S. colombianum* (18.4%), *S. ochrantum* (23.7%), *S. paucijugum* (10.5%), and *S. andreanum* (7.9%). Within the Ecuadorian population, EC1 accounted for 66.7%, US1 for 28.1% and EC3 for 3.5% all of which were found in *S. betaceum.* Additionally, EC2 represented 1.8% of the population and was found in *S. brevifolium.* The largest number of isolates were collected from the departments of Carchi and Pichincha, with Carchi having 100% EC1, while Pichincha had 42.9% EC1 and 57.1% US1. The strains collected in 2022, representing 14.3% of Pichincha's isolates, belonged to the US1 genotype. Additionally, the department of Tungurahua exhibited three clonal lineages: EC1 at 62.5%, EC2 at 12.5%, and EC3 at 25%. In Colombia, there were two well-differentiated clonal lineages, with 78.6% corresponding to CO4, which was present in both studied departments and 21.4% EC1, found only in Santander (Figure 2, 3 A, B).

SSR Analysis of Phytophthora infestans

Bruvo distance



260

Figure 2. UPGMA dendrogram based on Bruvo distance for *Phytophthora* isolates from Bolivia, Peru, Colombia, Ecuador, and Uruguay. US1, EC1, 2A1, PE3, PE7.1, PE7, BR1, US8 (*P.infestans)*, EC2, EC3, and PE8 (*P.andina*) were used as the clonal lineage controls.

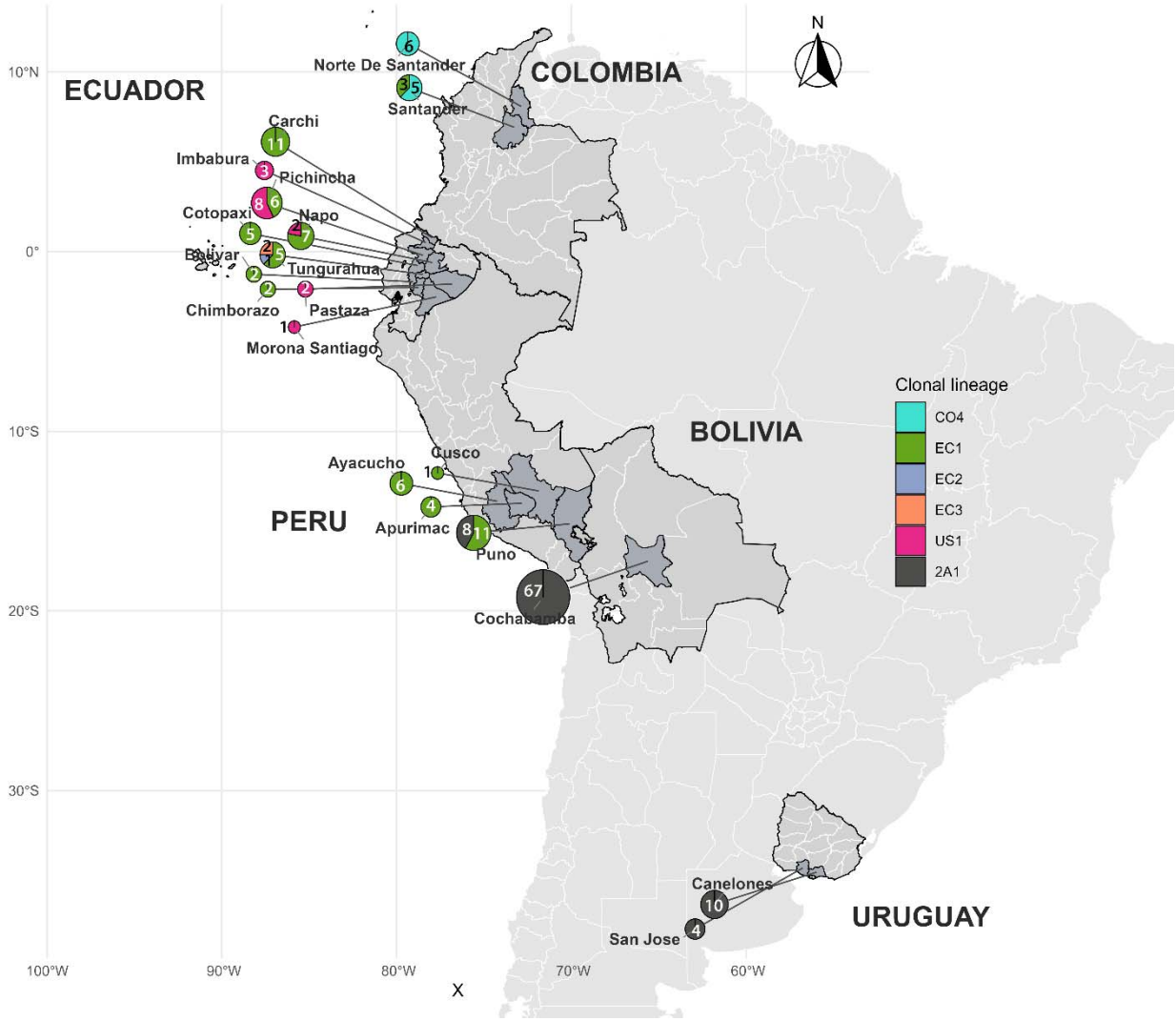


Figure 3. Geographical distribution of *Phytophthora* isolates (n = 173) collected in Bolivia and Peru (2017–2019), Ecuador (1993–2022), Colombia (2022), and Uruguay (2023–2025). Departments highlighted in gray indicate sampled locations, and pie charts show the relative composition of clonal lineages 2A1, EC1, CO4, and US1 (*P. infestans*), and EC2 and EC3 (*P. andina*). Pie chart size is proportional to the total number of isolates per department, and numerical values indicate the number of isolates per clonal lineage.

Figure 4 illustrates the timeline of clonal lineages of *P. infestans* collected in Ecuador from 1993 to 2022. Throughout this timeframe, the EC-1 lineage emerged as the most dominant, appearing almost annually, and constituting the majority of isolates in most years of collection. Conversely, the US-1 lineage was detected sporadically, but was still found in several years, including the most recent collections, such as in 2022.

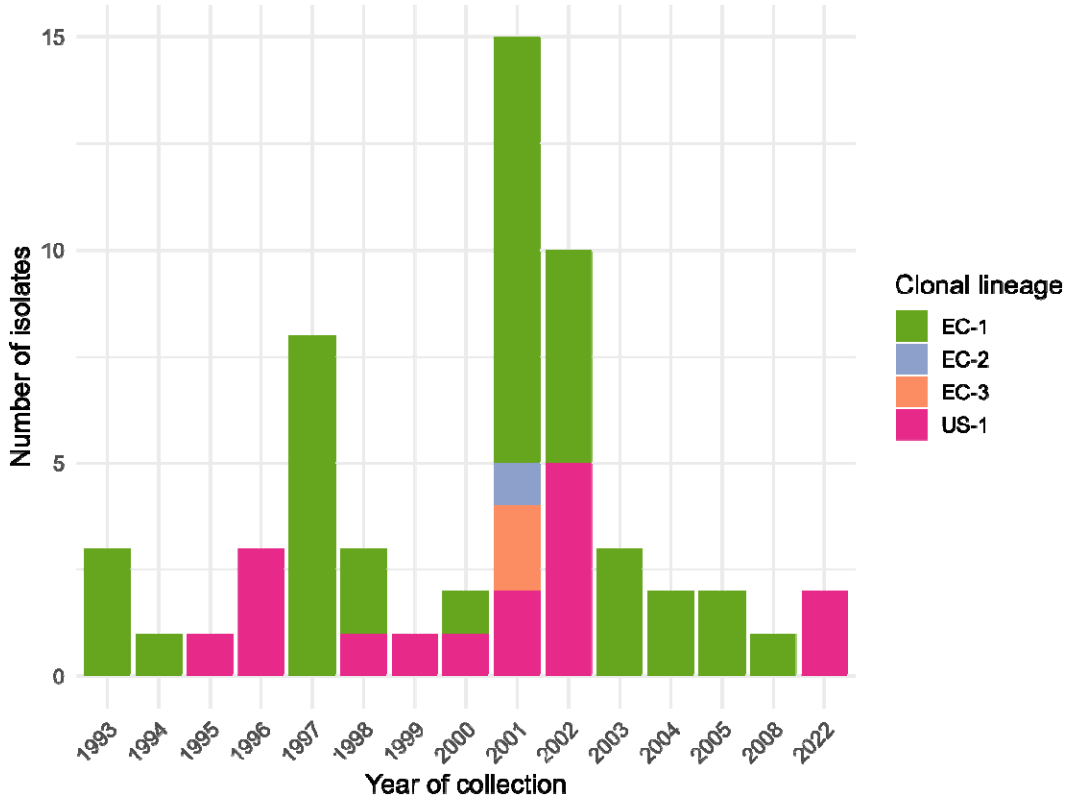


Figure 4. Clonal lineage distribution of *Phytophthora infestans* (EC-1, US-1) *and Phytophthora andina* (EC-2, EC-3) in Ecuador by year of collection (1993–2022)

Analysis of the 182 isolates yielded 126 MLGs (Table 2), and in total 141 were considered when the references were included. The three most frequent MLGs were MLG139 (n=22), MLG80 (n=7), and MLG131 (n=6) (Figure 5). MLG80 matched six isolates (one from Ayacucho and five from Puno) and one of our EC1 clonal lineage references, and MLG67 matched one isolate from Puno and the other EC1 clonal lineage reference. MLG131, which matched four isolates from Puno and our previously named PE7.1 clonal lineage reference.

D = 0.68). Similarly, in Ecuador, Tungurahua and Napo displayed high genotypic richness (MLG = 7 and 9, respectively) and elevated indices of linkage disequilibrium (Ia = 5.22 and 3.68), supporting a predominantly clonal structure. Bolivia, which had the highest number of isolates (n=67), exhibited high genotypic richness, with 32 MLGs and MLG139 belonging to the clonal lineage 2A1 (Table 2, Figure 5).

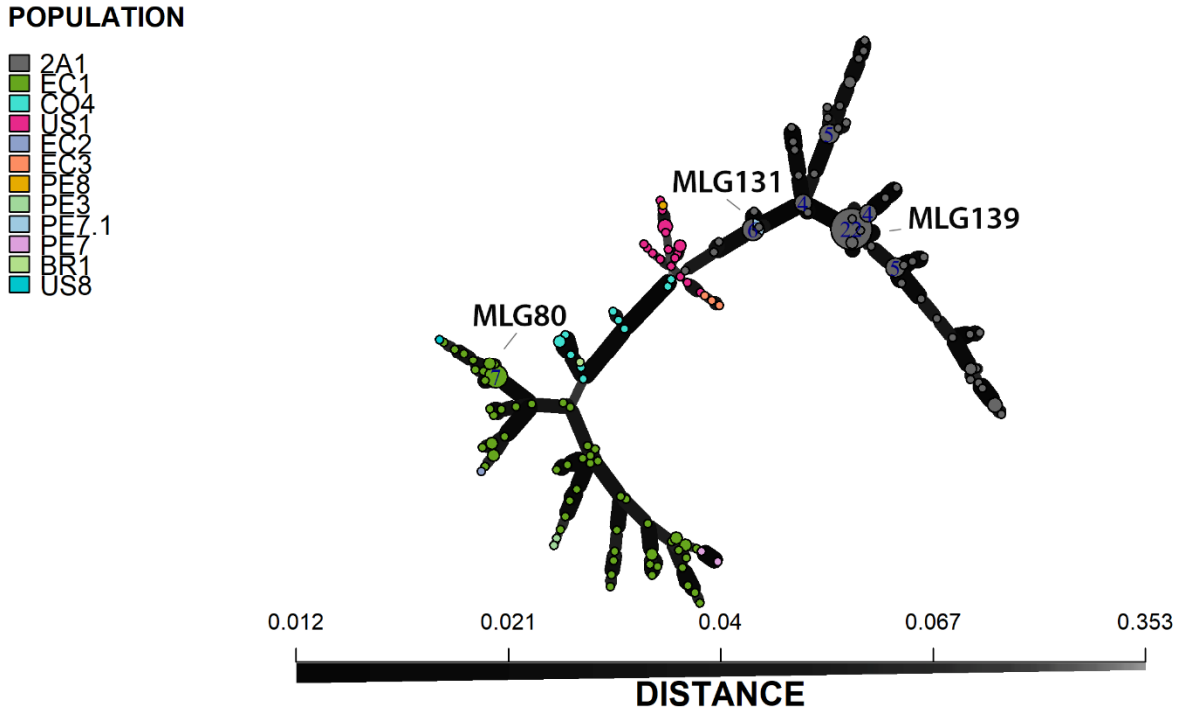


Figure 5. Minimum spanning network (MSN) of multilocus genotypes (MLGs) of *Phytophthora* clusters from Bolivia, Peru, Colombia, Ecuador, and Uruguay. Using clonal lineage controls as US1, EC1, 2A1, PE3, PE7.1, PE7, BR1, US8 (*P.infestans)*, EC2, EC3, and PE8 (*P.andina*).

The accumulation curve for multilocus genotypes (MLG) demonstrated a steady increase in the number of identified genotypes as more loci were added, yet it did not fully level off at 11 loci. This indicates that each marker plays a role in distinguishing genotypes, and that complete locus coverage is essential to fully capture the genetic diversity of *Phytophthora isolates* within this dataset (Figure 6).

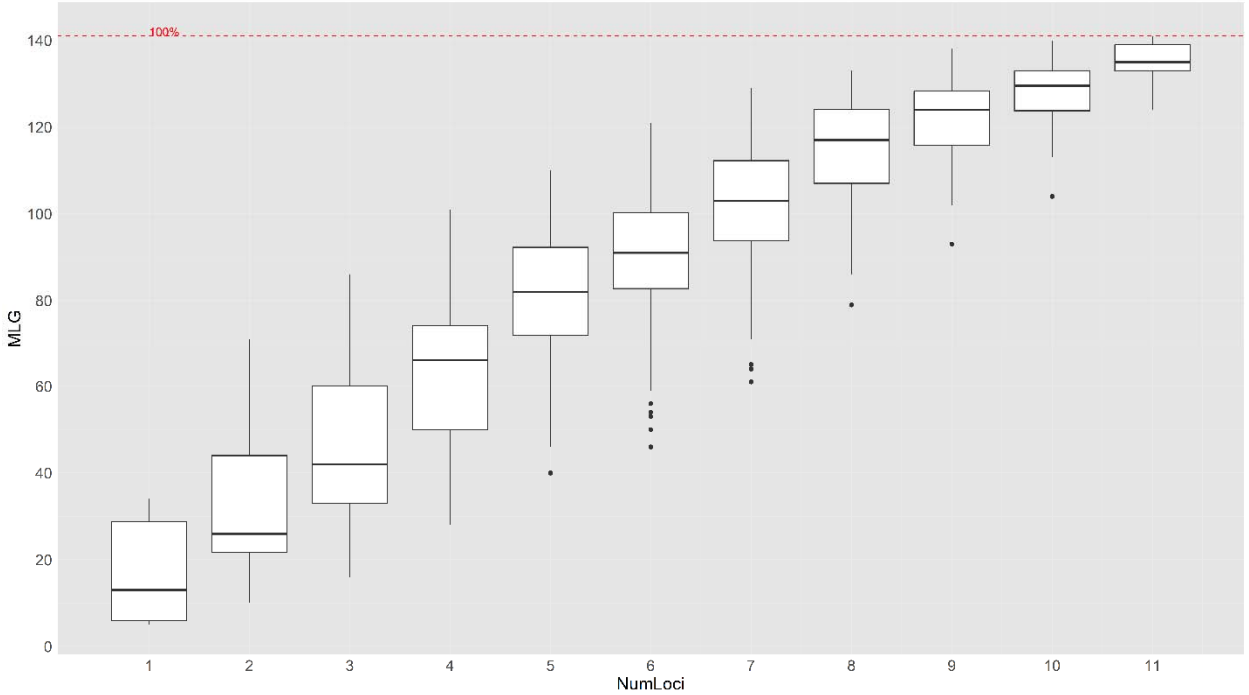


Figure 6. The genotype accumulation curve. Numloci refers to the number of SSR loci, whereas MLG denotes the number of multi-locus genotypes. The MLG resolution was depicted as 100%, as indicated by the dashed red line.

Considering the SSR markers, G11 and D13 exhibited the highest number of alleles (18 and 17, respectively). With an average of 7.92 alleles per locus, there was a moderate level of allelic diversity, with an average of seven alleles per locus. Diversity indices, including 1-D and expected heterozygosity (Hexp), range from 0.38 to 0.81, with average values of 0.60 for both indices, indicating a moderate degree of genetic diversity. The evenness values, spanning from 0.47 to 0.89 (mean=0.72), indicated a significant variation in the uniformity of allele distribution (Table 3).

AMOVA analysis of *Phytophthora* isolates indicated a notable genetic structure among clonal lineages. A significant portion of the molecular variance (57.77 %) was linked to the differences between these clonal lineages, highlighting substantial genetic differentiation at this level. Conversely, only 10.21% of the variance arose from the differences between samples within the same clonal lineage, whereas 32.02% was observed within individual samples. All hierarchical levels exhibited statistically significant differences, as verified by the Monte Carlo test (P = 0.001) (Table 4).

Discussion

The genetic composition of *Phytophthora infestans* populations is complex and constantly evolving, which has important implications for management late blight. Studies have indicated that *P. infestans* populations around the globe display significant genetic diversity and undergo rapid evolution (Cooke & Lees, 2004; Dey *et al.*, 2018). Several studies have been carried out on *P. infestans* populations in the Andean region, and Peru is considered the possible center of origin of the pathogen (Gómez-Alpizar *et al.*, 2007; Patarroyo *et al.*, 2024; Coomber *et al.*, 2025). Perez et al. (2001) studied Peruvian *P. infestans* isolates from Cusco and Puno and identified EC-1, PE-3 and US-1 as predominant clonal lineages isolated from cultivated and wild potatoes (EC-1 and US-1) and cultivated potatoes (PE-3). For the clonal lineage EC1 in the Peruvian highlands, the specificity of hosts does not seem to significantly influence the composition of the pathogen population that targets tuber-bearing Solanum species (Garry *et al.*,

2005). More recent studies in these departments identified only EC1 and a new genotype named PE7.1 (Lindqvist□Kreuze *et al.*, 2020). EC1 has been the predominant genotype in Peru, since its first report by Perez et al. (2001), contrary to US1 and PE3, which have not been identified in the more recent population studies. PE3, which was associated with native cultivated landraces or wild tuber-bearing species (Perez *et al.*, 2001; Garry *et al.*, 2005). The analysis in the current study allowed us to determine that PE7.1 belongs to the well-known 2A1 clonal lineage, which has been reported in other South American countries (Acuña et al., 2019; F. Lucca et al., 2023). US-1 was identified in Ecuador in both historical and recent collections, suggesting that the long-term survival of clonal *P. infestans* lineages likely hinges on a fine balance between their aggressiveness, adaptability to environmental conditions, and capacity to counteract disease management strategies (Mariette *et al*., 2016; Fry, 2020). Our research also corroborated previous studies on the population structure of certain strains from Ecuador, which had identified the clonal lineages EC1 and US1(*P.infestans*), EC2 and EC3 (*P.andina*) —using RFLP fingerprinting —as being associated with different host species: EC1 (cultivated and wild potatoes), EC2 (wild potato), EC3 (cultivated tree tomato), and US1 (cultivated tomato species, pepino melon, naranjilla, wild potato species, wild relative of naranjilla) (Gómez-Alpizar *et al.*, 2008; Oliva *et al.*, 2010). These findings were updated using the 12-SSR multiplex method. The identification of EC-1 and the newly discovered CO-4, in Colombia highlights a geographical influence: EC-1 has been reported in Ecuador and Venezuela (Forbes *et al.*, 1998; Garry *et al.*, 2005), while the new clonal lineage CO-4 was found only in the department of Norte de Santander, where a humid tropical ecosystem is present (Dueñas *et al.*, 2007). Norte de Santander borders Venezuela, where the Ia mitochondrial haplotype and A1 mating type have been documented, which corresponds to the profile observed in our strains from Norte de Santander (Briceño *et al.*, 2009; Cárdenas *et al.*, 2011). We did not identify the US-8 lineage, which was previously reported in Colombia and was isolated from Cape gooseberry and also possesses the Ia haplotype, but corresponded to the A2 mating type (Vargas *et al.*, 2009).

The existence of both mating types promotes gene exchange between populations, as demonstrated by the discovery of distinct genotypes in fields where both mating types are present (Goodwin, S.B. *et al.*, 1995). In 2001, Perez et al. (2001) found that the predominant mating type in Peru was A1, whereas in Bolivia, the A2 mating type was detected in the past (Goodwin, S.B. *et al.*, 1994), which belongs to the clonal lineage BR-1. The BR-1 reference was added to our SSR analysis, and we determined that it belonged to a different cluster from the isolates collected from Bolivia in our study. The risk of sexual reproduction is always present when both mating types exist. In potato production zones near the border between Peru and Bolivia, this is always a latent risk. *P. infestans* may have a broader host range than previously thought, which could have implications for disease management. In our study, the A1 mating type was detected using the W16 marker, which has been proven to be accurate in identifying mating types in clonal lineage EC1 (Brylińska *et al.*, 2018). The pairing test on V8 agar was chosen for the Ecuadorian population because the W16 marker has shown inconsistent results for the US-1 genotype and also for *P. andina*, as reported by Brylińska et al. (2018). In that study, all US-1 isolates of A1 mating type amplified the fragment typical of A2 using W16, making this marker unreliable for populations where these lineages occur.

The high proportion of genetic variation detected among clonal lineages (57.77%) is consistent with their distinct geographic and historical origins. Lineages EC1, EC2, and EC3 are predominantly associated with Andean countries, whereas lineage 2A1 has been reported mainly in Europe, while the origin of lineage US-1 remains more controversial(Forbes *et al.*, 1997; Mariette *et al.*, 2016; Martin Md *et al.*, 2016). These distinct evolutionary trajectories have likely generated strong genetic differentiation among lineages, which is reflected in the high variance component observed in the AMOVA. In a North American study of 77 isolates, Goodwin, S.B. et al. (1995) found that 63% was due to differentiation among lineages, and older lineages exhibited more pathogenic variation than recently introduced ones. The US-1 clonal lineage has reportedly been replaced in Peru, with its presence limited to wild Solanaceae species in the 2016-2017 population. This change could be attributed to the lineage's low virulence in cultivated potatoes (Lindqvist□Kreuze *et al.*, 2020). Also, EC1 was reported to be adapted to higher temperatures than US1, and to exhibit a high level of resistance to metalaxyl (Garry *et al.*, 2005), in contrast to US1, which showed over 50% sensitivity to the fungicide (Lindqvist□Kreuze *et al.*, 2020). The dynamic nature of *P. infestans populations*, with the emergence and replacement of dominant clones over time, highlights the importance of continuous monitoring and studying the genetic variation in this pathogen for effective disease management strategies (Knaus *et al.*, 2020). For example, in America, the US-23 lineage, which is currently widespread in the United States, exhibits genetic similarities with the BR-1 lineage found in Bolivia and Brazil, whereas other lineages in the U.S. are more closely related to populations in Mexico (Saville & Ristaino, 2019). In terms of management strategies, we need to consider the aggressiveness of clonal lineage EC1, as current isolates that dominate in the southern regions of Peru (Lindqvist□Kreuze *et al.*, 2020) showing different aggressive

behaviors (Chacón *et al.*, 2007). Furthermore, 2A1 shows low or moderate aggressiveness relative to the other coexisting lineages (Mariette *et al.*, 2016). Additionally, the variations in aggressiveness observed in new populations might result from better adaptation to environmental conditions like temperature or humidity (Lindqvist□Kreuze *et al.*, 2020).

P. infestans can be transmitted through infected seed potatoes, which serve as a vital initial source of inoculum (Kool & Evenhuis, 2023). The importation of infected potato seeds can play a crucial role in the spread and resurgence of late blight across different regions. Historical reports indicate that infected potato seed tubers were a likely source of late blight outbreaks in different parts of the world (Guha Roy *et al.*, 2021; Saffer *et al.*, 2024; Rhouma *et al.*, 2024a; Ryley & Drenth, 2024). Understanding the movement of potato seeds is essential for understanding changes in clonal lineages. The genetic diversity of potatoes in the Andean region is extensive, with the FAO reporting nearly 4000 cultivated varieties, 3000 of which are found in Peru (Parra-Rondinel *et al.*, 2021). Regarding the potato seed market in Peru, Peru does not import potatoes, and during 2024, Peru exported more than 9,806 tons of potatoes to destinations such as Bolivia and the United Arab Emirates (Andina Noticias, 2025). In Ecuador, seeds were brought in from Colombia, whereas in Uruguay, they were sourced from Argentina, the Netherlands, France, Germany, and Canada (World Bank, 2023a,b). Since 1949, Bolivia has imported potatoes from Europe and other American nations, although the European, Argentinian, Peruvian, and Colombian varieties have been prominent. Currently, 'Desiree' (*S. tuberosum*) and 'Huaych'a' (*S. andigena*) varieties have a greater significance in certified seed potato production (Coca-Morante, 2016). In certain Andean areas, including Colombia and Venezuela, the populations of *P. infestans* affecting potatoes exhibit low genetic diversity and mainly clonal structures. According to a previous study, part of this limited diversity is associated with the long-term self-sufficiency in potato seed tuber supply in these regions, which reduces the movement of plant material and limits the introduction of new genetic variants (Cárdenas *et al.*, 2011). In Colombia, a country known for cultivating various commercial potato varieties and maintaining a rich genetic diversity of the crop (Niño-Medina *et al.*, 2025), only 3 to 5% of the cultivated area uses certified seeds (Rodríguez *et al.*, 2025). This limited availability forces most farmers to depend on seeds produced on their own farms (Betancur Betancur *et al.*, 2023). Consequently, there is an increased risk of spreading pathogens through infected seeds, highlighting the need to study the origins and distribution pathways of the seeds used in the country, in Peru the certified seed is used less than 1% (Pinedo-Taco *et al.*, 2021). To mitigate the future impact of *P. infestans*, it will be necessary to implement enhanced phytosanitary measures (Guha Roy *et al.*, 2021) because the pathogen's ability to rapidly adapt to control strategies, including resistant cultivars, makes management challenging. The use of certified potato seeds may be a crucial tool for managing *P. infestans*, because the pathogen can rapidly adapt to control strategies (Haas *et al.*, 2009).

MLGs grouped together from the potato-growing areas of Puno EC1(MLG80) and PE7.1 (MLG131) were found approximately 40 km from the Bolivian border. The current *P. infestans* population of Puno (EC1 and 2A1) is different from that of the old population (US1, PE3 and EC1) (Perez *et al.*, 2001), suggesting that displacement of the old dominant lineages has occurred. Since 2011, a similar shift has been noted in Argentina for the oldest lineages, AR1-AR5_A2, and in Uruguay and Brazil for BR-1_A2 (Forbes *et al.*, 1998; Deahl *et al.*, 2003), and all have been replaced by EU-2_A1(Lucca *et al.*, 2019, 2023b). The most abundant MLG (MLG139) was shared between seven provinces of Cochabamba-Bolivia, with 64.2% of MLGs from Bolivia that are not unique, distribution of *P. infestans* MLGs across different localities, and the presence of clonal lineage 2A1, which is present in several South American countries, suggesting that it may be older and have had more time to spread. In the case of Peru, the unique MLGs were 56.7%, being majoritarian. The high number of unique MLGs observed in various studies suggests that *P. infestans* has a remarkable ability to generate genetic variation (Forbes *et al.*, 1998; Dey *et al.*, 2018). This difference in diversity could be attributed to the origin of the pathogen and its history of introduction (Saville *et al.*, 2016).

Our findings demonstrate that the population dynamics of *P. infestans* in South America respond to distinct regional pressures, resulting from both agricultural exchange and local adaptation, and highlight the need for transnational genetic surveillance strategies.

Author contributions

MI: coordination, sample processing, molecular laboratory work, marker scoring, analysis, interpretation of data, and drafting of the manuscript.

MC: sampling
WP: coordination, interpretation of data and revision of the manuscript
LS: sampling, sample processing
SG: sample processing.
DV: sampling
VC: coordination
BG: coordination, interpretation of data
JK: interpretation of data and revision of the manuscript.

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Data availability statement

Not applicable

Ethic declaration

Not applicable

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Conflict of Interest

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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Tables

Table 1. Summary of samples collected and analyzed in this study.

Country	Cultivated potatoes	other species	Department	Species names	Altitude (masl)	collecting year
Bolivia	45	22	Cochabamba	*S. brevicaule, x Solanum sp, S.tuberosum, S. microdontum*	2381-3922	2018-2019
Peru	1		Cusco	*S. tuberosum*	3531	2018
	4		Apurimac		3301-4068	2018
	6		Ayacucho		2732-3460	2018
	19		Puno		3440-3965	2017-2018
Ecuador	3	8	Carchi	*S.tuberosum, S.ochrantum*	2960-3351	1993-2005
	13	1	Pichincha	*S.tuberosum, S.lycopersicum, S.muricatum, S.quitoense, S.ochrantum*	1671-3300	1996-2022
	2	1	Imbabura	*S.lycopersicum, S.caripense*	2018-3117	1996-2002
	7	1	Tungurahua	*S.tuberosum, S.betaceum, S.brevifolium*	2031-2960	2001-2003
	1	1	Chimborazo	*S.tuberosum, S.andreanum*	2961-3282	2000-2004
	1	4	Cotopaxi	*S.tuberosum, S.paucijugum*	2558-3895	2001-2005
	2	7	Napo	*S.colombianum, S.quitoense*	1748-3729	1993-2002
		1	M. Santiago	*S.andreanum*	981	1998
	2		Pastaza	*S.quitoense*	1005-3020	2000-2001
		2	Bolívar	*S.andreanum*	2768-2829	2002
Colombia	6		Norte de Santander	*S.tuberosum*	2574-2842	2022
	8		Santander	*S.tuberosum*	2129-2401	2022
Uruguay	10		Canelones	*S.tuberosum*	30-52	2023-2025
	4		San José	*S.tuberosum*	28-37	2024-2025
Grand total	134	48				

Table 2. Diversity statistics for microsatellite data across 12 loci in *Phytophthora* populations from Bolivia, Peru, Colombia, Ecuador, and Uruguay categorized by location

Pop	N	MLG	eMLG	SE	H	G	lambda	E.5	Hexp	Ia	rbarD
B.Coch	67	32	7.2	1.329	2.814	7.7	0.87	0.428	0.515	0.6031	0.0988

P.Cuz	1	1	1	0	0	1	0	NaN	0.75	NA	NA
P.Apu	4	3	3	0	1.04	2.67	0.625	0.912	0.447	1.193	0.3115
P.Aya	6	6	6	0	1.792	6	0.833	1	0.421	-0.1682	-0.0471
P.Pu	19	12	7.22	1.075	2.229	7.08	0.859	0.733	0.528	6.1668	0.6812
C.NSt	6	6	6	0	1.792	6	0.833	1	0.524	-0.0676	-0.0233
C.St	8	6	6	0	1.733	5.33	0.812	0.931	0.469	1.9952	0.4029
E.Pich	14	13	9.51	0.5	2.54	12.25	0.918	0.963	0.572	3.1127	0.3117
E.Car	11	11	10	0	2.398	11	0.909	1	0.338	0.3187	0.0489
E.Imb	3	2	2	0	0.637	1.8	0.444	0.899	0.58	5.2308	1
E.Tung	8	7	7	0	1.906	6.4	0.844	0.943	0.612	5.2196	0.4971
E.Chim	2	2	2	0	0.693	2	0.5	1	0.428	NA	NA
E.Coto	5	5	5	0	1.609	5	0.8	1	0.344	-0.7882	-0.1377
E.Nap	9	9	9	0	2.197	9	0.889	1	0.488	3.6799	0.4186
E.Mor	1	1	1	0	0	1	0	NaN	0.833	NA	NA
E.Pas	2	2	2	0	0.693	2	0.5	1	0.617	NA	NA
E.Bol	2	2	2	0	0.693	2	0.5	1	0.383	NA	NA
U.Can	10	7	7	0	1.748	4.55	0.78	0.747	0.48	0.9565	0.2092
U.SnJ	4	3	3	0	1.04	2.67	0.625	0.912	0.488	0.2264	0.1278

N number of individuals, *MLG* number of multilocus genotypes, *eMLG* expected number of MLGs, *SE* Standard error. The next columns are considering the MLG diversity: *H* Shannon- Weiner Index, *H Stoddart and Taylor's Index (G)*, *lambda* Simpson's index, *E.5* Evenness, H_{exp} Nei's unbiased gene diversity. *Ia* Index of Association, *rbarD* standardized index of association.

Table 3. Population statistics for the 12 microsatellite loci in *Phytophthora* populations from Bolivia, Peru, Colombia, Ecuador, and Uruguay

locus	**allele**	**1-D[b]**	**Hexp[b]**	**Evenness[b]**
D13	17	0.67	0.67	0.47
SSR8	5	0.64	0.64	0.84
SSR4	12	0.81	0.81	0.8
Pi04	4	0.61	0.61	0.89
Pi70	3	0.4	0.4	0.72
SSR6	7	0.54	0.54	0.77
Pi63	3	0.61	0.61	0.88
G11	18	0.7	0.7	0.53
SSR3	7	0.61	0.61	0.67
SSR11	5	0.62	0.62	0.8
SSR2	3	0.38	0.38	0.63
Pi4B	11	0.59	0.59	0.63
mean	7.92	0.6	0.6	0.72

[b] 1−D, Simpson's diversity index. *H*exp, Nei's gene diversity. Evenness indices of evenness.

Table 4. Analysis of molecular variance (AMOVA) of *Phytopthora* isolates from Bolivia, Peru, Colombia, Ecuador, and Uruguay

				Components of covariance		Monte-Carlo test for significance nrepet=999			
	Df	Sum Sq	Mean Sq	Sigma	%	Obs	Std.Obs	Alter	Pvalue
Within samples	114	69.56	0.61	0.61	32.02	0.61	-26.29	less	0.001
Between samples Within clonal lineage	23	33.51	1.46	0.19	10.21	0.19	10.00	greater	0.001
Between clonal lineage	11	129.85	11.80	1.10	57.77	1.10	9.73	greater	0.001
Total	148	232.92	1.57	1.91	100.00				

Supplementary Information

Supplementary materials are provided as separate files.

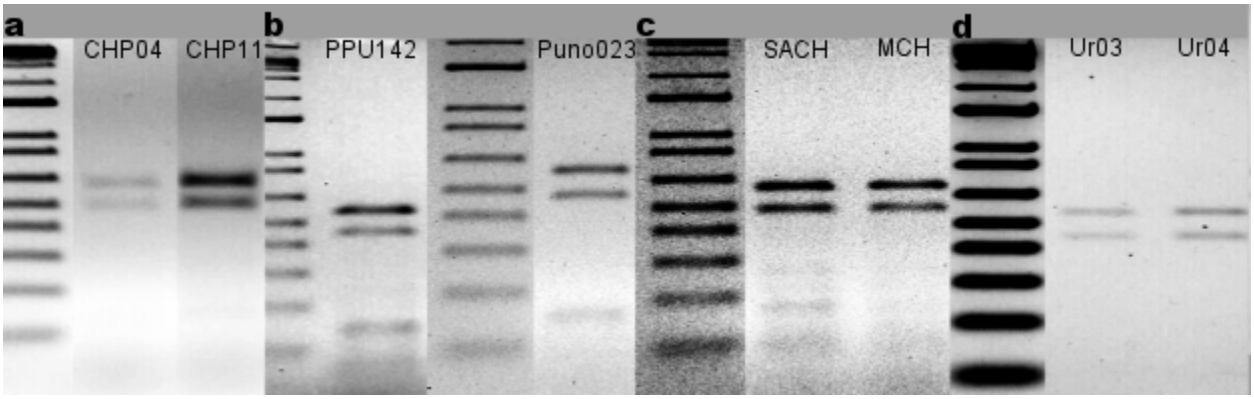

Figure S1. HaeIII Restriction Enzyme Digestion of PCR using W16 Primer Set in Selected a. Bolivian (CHP04, CHP11), b. Peruvian (PPU142, Puno023), c. Colombian (SACH, MCH), and d. Uruguayans (Ur03, Ur04) strains. Ladder 1kbplus.

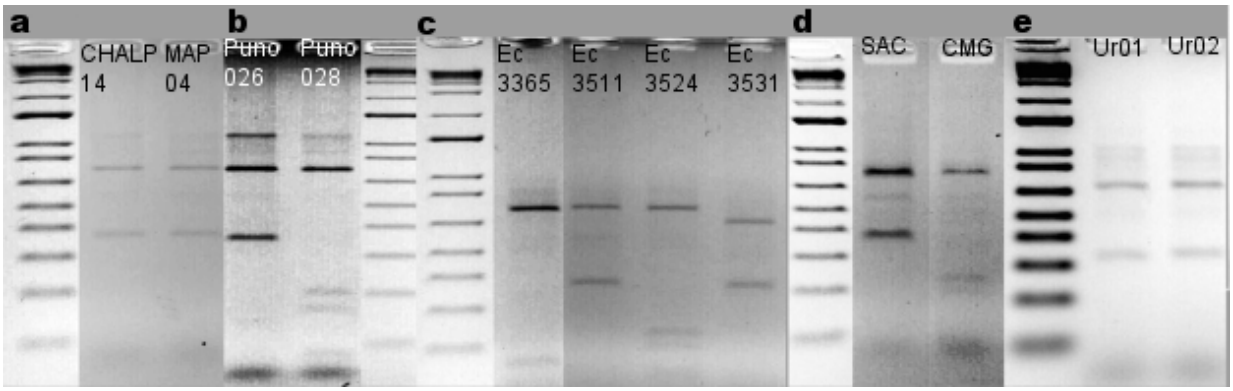

Figure S2. Results of Restriction fragments from digestion of the P2 region with enzyme *MspI* with **a**. Bolivian (CHALP14, MAP04), **b**. Peruvian (Puno026, Puno028), **c**. Ecuadorian (Ec-3365, Ec-3511, Ec-3524, Ec-3531), **d**. Colombian (SAC, CMG), and **e**. Uruguayans (Ur.1, Ur.02) strains. Ladder 1kbplus.