ABSTRACT

LASSITER, ERICA LYNN SUEKO. Inferring Evolutionary Relationships of the *Phytophthora* Ic Clade Using Nuclear and Mitochondrial genes. (Under the direction of Jeffrey L. Thorne).

The genus *Phytophthora* contains many plant pathogens that cause billions of dollars worth of damage to ornamental and agricultural plants. Large scale analyses of *Phytophthora* have been carried out in an effort to better classify members of this genus. These analyses have formed the basis of the *Phytophthora* phylogeny, placing *P. infestans*, the causative agent of late blight, in the Ic clade. The first objective of this study was to sequence and annotate the mitochondrial genomes of the other species of the Ic clade, including *P. mirabilis*, *P. andina*, *P. ipomoeae*, and *P. phaseoli*, and use those genomes to study the evolutionary relationships of the species in this clade. We sequenced the mitochondrial genomes of the sister species of *P. infestans,* using the whole genome sequences and coalescent analysis to resolve the evolutionary histories of the Ic clade. We found that gene order and content were conserved among the Ic clade species. The Ic mitochondrial haplotype of *P. andina* had a unique open reading frame (ORF79), marked by two indels. *P. phaseoli* diverged earliest from the rest of the species of the Ic clade. *P. infestans* and *P. andina* then diverged from a common ancestor. *P. mirabilis* and *P. ipomoeae*, two species reported to date only in Mexico, diverged most recently from a *P. andina* lineage. Our data suggest that the Andean region is more likely than Mexico to be the center of evolutionary origin for the clade. Although some species are found in Mexico, *P. andina* has only been reported in Ecuador. The center of origin for the host species for all Ic clade species is the Andes, leading to the conclusion that this is the center of origin for the clade. We cannot rule out that *P. andina* may exist in

Mexico, or alternatively, the possibility of the occurrence of *P. mirabilis* and *P. ipomoeae* in the Andes.

The second objective of this study was to use nuclear and mitochondrial genes from several isolates of Ic clade species and conduct a phylogenetic analysis to compare to the currently accepted phylogeny of the genus *Phytophthora*. From this, we sought to establish the history of *P. andina*, determining whether or not it was a hybrid of *P. infestans* and *P. mirabilis*. We sequenced at least 5 isolates of each of the species in the Ic clade using molecular markers for both nuclear and mitochondrial genes (*Elongation factor 1α*, *β tubulin*, *Ras*, intron 1 of *ras*, and P4 (*coxI* and *atp9*)), corresponding to over 2500 nucleotide sites. These sequences were trimmed and Bayesian analysis was used to infer gene trees for the Ic clade. Confirming the widely accepted *Phytophthora* phylogeny, *P. phaseoli* is the most basal member of the clade, with *P. ipomoeae*, *P. mirabilis*, and *P. andina* being more closely related to *P. infestans*. *P. andina* often clusters with *P. infestans*, while the locations of *P. mirabilis* and *P. ipomoeae* in the tree change depending on the gene of interest. Several nuclear heterozygous sites were found in both the Ic and Ia mitochondrial haplotypes of *P. andina*, corresponding to a nucleotide found in *P. infestans* and one found in *P. mirabilis*. There is potential for *P. infestans* and *P. mirabilis* to have hybridized at some time in the past, however, host specialization makes this scenario unlikely. Other evidence, such as the existence of a potential bridging host, points towards *P. andina* and *P. infestans* mating to form *P. mirabilis*.

Inferring Evolutionary Relationships of the *Phytophthora* Ic Clade Using Nuclear and Mitochondrial Genes

by
Erica Lynn S. Lassiter

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Genetics

Raleigh, North Carolina

2010

APPROVED BY:

Dr. Jean B. Ristaino

Dr. Eric A. Stone

Dr. Jeffrey L. Thorne
Head of Advisory Committee

DEDICATION

In Hawaiian, the word “ohana” means family. Family are the people who are always by your side in life, no matter what happens. Throughout these last few years, there have been trying times and many stresses, but it was my ohana that got me through it all, so this work is dedicated to them. A'ohe hana nui ka alu'ia (No task is too big when done together).

BIOGRAPHY

Erica Lynn Sueko Beals was born on October 26, 1985 in a pink Army hospital in Honolulu, Hawaii to Daniel W. and Susan N. Beals. At the age of three, the family moved from Hawaii to Tampa, Florida where Erica started kindergarten at Chiaramonte Elementary School. After that, it was on northward to North Carolina, where her father had grown up. It was here, in third grade, that she found a love for science. Projects like building simple machines and creating salt crystals enthralled the young girl, and she knew from then on that whatever she ended up doing in life, it would have to do with science. She graduated from Northeastern High School as Valedictorian and captain of the varsity women's soccer team. In August of 2004, she began her undergraduate career at Meredith College in Raleigh, North Carolina. Here, she received a Bachelor of Science degree in Molecular Biology with minors in Psychology and Latin. Upon graduation, she enrolled in the Master of Science program in Genetics at North Carolina State University, graduating from there in December of 2010.

ACKNOWLEDGEMENTS

There are many people without whom I could not have finished this thesis. First, my advisory committee: Dr. Jeffrey L. Thorne, Dr. Jean Ristaino, and Dr. William Atchley. I would also like to thank Dr. Eric Stone for substituting for Dr. Atchley in his illness. The Ristaino lab has been particularly helpful in completing the experiments and analyses for this project. In particular, Dr. Julia Hu, whose experience and thoughtfulness were invaluable throughout this project. Also, the Thorne lab, including Dr. Ben Redelings, Dr. Reed Cartwright, and Kristin Lamm for all their insight into troubleshooting any analytical or computer problems that may have arisen during the course fo this work. Members of Dr. Ignazio Carbone's lab, particularly Dr. Geromy Moore, were particularly helpful when running coalescent analysis simulations.

I would also like to thank my wonderful family, that has been so supportive of me when I decided to pursue my Master's degree. To my gracious husband who pushed me to finish work when I really wanted to go outside and kick a soccer ball, thank you for being patient, understanding, and more than anything, motivating. For my parents, who always taught me that I could do anything that I wanted to, thank you for letting me be the person that I wanted to be and no one else.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

CHAPTER 2

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 1

MITOCHONDRIAL GENOME SEQUENCES AND EVOLUTIONARY HISTORY OF THE *PHYTOPHTOHRA* IC CLADE SPECIES

ABSTRACT

The *Phytophthora* Ic clade, consisting of *P. infestans*, *P. phaseoli*, *P. ipomoeae*, *P. mirabilis*, and *P. andina*, contains some of the world's most destructive plant pathogens, yet little attention has been give to the clade as a whole. The mitochondrial genome has proved to be an invaluable tool for molecular research and can be used as a method for identification and evolutionary studies. We sequenced the mitochondrial genomes of the sister species of *P. infestans* in the Ic clade including *P. phaseoli, P andina, P. mirabilis* and *P. ipomoeae* and used the whole genome sequences and coalescent analysis to resolve the evolutionary histories of members of the Ic clade. Both gene order and content were conserved among the Ic clade *Phytophthora* species, with the only difference in coding genes occurring in ORF79 of *P. andina,* where there is a 12 amino acid deletion. The rooted tree inferred documents the earliest split separating *P. phaseoli* from the rest of the Ic clade species. Next, the lineage ending at *P. andina* diverged from the lineage leading to *P. infestans*. *P. mirabilis* and *P. ipomoeae* diverged most recently from a *P. andina* ancestor. The inferred tree has *P. mirabilis* and *P. ipomoeae* being more closely related to each other than either is to *P. infestans*. Our data are consistent with the hypothesis that the Andean region is the center of evolutionary origin for all the species in the clade since *P. andina* and *P. infestans* coexist

there. Alternatively, we cannot rule out a Mexican origin for the Ic clade or the chance that *P. andina* may exist in Mexico. Further surveys are needed to test this possibility and the alternative possibility of the occurrence of *P. mirabilis* and *P. ipomoeae* in the Andean region.

INTRODUCTION

The mitochondrial genome has been used to study the evolutionary histories of many types of organisms (Avila-Adame et al. 2006; Chesnick et al. 2000; Paquin et al. 1997). Population geneticists have employed the mitochondrial genome as a tool for determining evolutionary relationships among species and the mitochondrial *cox* I gene has become a standard tool for taxonomic studies and identification of many organisms (Advise et al. 1987; Moritz et al. 1987; Ratnasingham and Herbert 2007). Reasons for utilizing mitochondrial DNA range from practical to fundamental. The mitochondrial genome is small compared to the nuclear genome and its genes are relatively easy to amplify. Fundamentally, mitochondria have become a favorable tool in molecular studies because of their mutation rate, uniparential inheritance with little recombination, and evolutionary rates. Although the use of mitochondrial genomes as molecular markers has recently come under scrutiny for actually violating these assumptions (Galtier et al. 2009), they still remain valuable for determining evolutionary histories. Hundreds of mitochondrial genomes have been

sequenced from animals, plants, fungi, and protists (Chesnick et al. 2000), but relatively few have been sequenced from plant pathogens.

Phytophthora infestans (Montagne) de Bary is the pathogen responsible for the Irish potato famine of the mid-1800's (Ristaino 1998), and is still a widespread and destructive pathogen on both potato and tomato (Gómez-Alpizar et al. 2007; Moskin 2009; Vargas et al. 2009). Although originally placed in the Kingdom Fungi, analysis of several genes in the mitochondrial genome of the pathogen shed new light onto the correct phylogenetic classification of this organism. *P. infestans* and other oomycetes were shown to be more closely related to members of the Kingdom Stramenopila than the Kingdom Fungi following analysis of the *nad*4L subunit of the NADH dehydrogenase complex (Chesnick et al. 1996) and the nuclear small subunit rRNA (Gunderson et al. 1987; Föster et al. 1990).

Mitochondrial haplotypes have been designated in *P. infestans* including: type Ia, Ib, IIa and IIb using PCR and RFLP analysis of mitochondrial DNA (Carter et al. 1990; Griffith and Shaw 1998). The entire mtDNA genome of the Ib haplotype (US-1 genotype) of *P. infestans* was previously sequenced (Lang and Forget, 1993). The remaining three extant mtDNA haplotypes of *P. infestans* were sequenced by our group, and mutations leading to the evolution of both type I and type II mitochondrial lineages were identified (Avila-Adame et al. 2006). The two lineages clearly evolved independently from a shared common ancestor and the type II lineages did not diverge from the type I lineages as was previously proposed (Avila-Adame 2006; Carter et al. 1990; Gavino and Fry 2002).

P. infestans is grouped in Waterhouse's morphological group IV, which includes both heterothallic (outcrossing) and homothallic (inbreeding) species (Waterhouse 1963). In 2000, Cooke et al. reported a phylogenetic analysis of 50 *Phytophthora* species based on the internal transcribed spacer regions (ITS1 and ITS2) of the rRNA genes, and this phylogeny is considered the most comprehensive to date. Subsequent studies with several species by Kroon et al. (2004) and Blair et al. (2008) support the phylogeny reported by Cooke. These studies utilized several markers, both nuclear and mitochondrial, other than those used by Cooke. These phylogenies divide the genus *Phytophthora* into eight main clades, designated 1-8. Clade I is further subdivided into three smaller clades: Ia, Ib, and Ic. *P. infestans* clusters into the Ic clade along with *P. phaseoli* and *P. mirabilis*. Kroon and Blair expanded upon Cooke's Ic clade by also including *P. ipomoeae* and *P.* sp. "*andina*," a novel term Kroon ascribed to an Ecuadoran isolate of *P. infestans sensu lato*. However, the evolutionary relationships between the five species remained unresolved (Cooke et al. 2008).

In 2002, Flier et al. described *P. ipomoeae*, a novel *Phytophthora* species that causes leaf blight on *Ipomoea longipedunculata* in central Mexico. Phylogenetic analysis of the ITS sequence as well as other genes placed *P. ipomoeae* in the Ic clade along with the three other species. Analyses of morphology, host specificity, allozyme patterns, and RFLPs confirmed that *P. ipomoeae* was indeed a new species, and it was subsequently added to the Ic clade. The Toluca Valley in central Mexico has been proposed as the center of origin of *P. infestans*, *P. mirabilis*, *P. phaseoli*, and *P. ipomoeae* (Brasier and Hansen 1992; Flier et al. 2002; Flier et al.2003; Goodwin et al 1992; Grunwald and Flier 2005; Niederhauser 1991).

Flier and others cite the shared morphological characteristics and lack of ITS diversity among these species as evidence that the highlands of central Mexico are a speciation "hot-spot" for the divergence of species in this clade. They hypothesized that hybridization and host specialization were the main factors that drove the speciation of the clade species, but also mention that is would be difficult for them to speculate on the exact evolutionary origin of *P. ipomoeae*.

P. infestans is currently evolving on *Solanum* hosts in the Andean region (Adler et al. 2004; Flier et al. 2003; Gómez-Alpizar et al. 2008; Oliva et al. 2007; Oliva et al. 2010). Three clonal lineages of *P. infestans* (US-1, EC-1, EC-3) and one heterogeneous group named *Phytophthora infestans* sensu lato were found in association with different wild species in the genus *Solanum* from the section *Anarrhichomenun* in Ecuador (Adler et al. 2004). One lineage within the EC-2 group is characterized by the A2 mating type, the Ic mitochondrial haplotype, and allozyme genotypes for glucose phosphate isomerase (*Gpi*) 100/100 and peptidase (*Pep*) 76/100, known as the EC-2 Ic haplotype; the other lineage is characterized by the A1 mating type, Ia mtDNA haplotype, *Gpi* 100/100 and *Pep* 76/100 (EC-2, Ia haplotype) (Ordonez et al. 2000; Adler et al. 2004). The EC-1 clonal lineage of *P. infestans* sensu lato was confirmed to be *P. infestans* based on sequences of the mitochondrial cytochrome oxidase I (*cox I*) gene and intron 1 of the *ras* gene (Gómez-Alpizar et al. 2008). However, the EC-2 (1c) isolates formed a distinct branch in the same clade with *P. infestans, P. mirabilis, P. phaseoli and P. ipomoeae,* for both *cox I* and *ras* intron 1, and were identified as the newly described species *P. andina* (Gómez-Alpizar et al. 2008; Kroon et al. 2004). Kroon et al. (2004) sequenced several nuclear and

mitochondrial genes from isolates of the EC 2 lineage (Ic haplotype) and placed them in the *Phytophthora* Ic clade using the name "*P. andina*" for the first time. In their later study, Blair et al. (2008) referred to the species as "*P*. sp. '*andina*.'" Oliva et al. (2010) have recently published a species description of *P. andina* and also suggest that in addition to the EC-2 (1c) strains, the EC-3 lineages are also *P. andina*.

Ras intron 1 sequence data suggest that *P. andina* may have arisen via hybridization between *P. infestans* and *P. mirabilis* (Gómez-Alpizar et al. 2008). *P. mirabilis* is closely related to *P. infestans* and was first described on *Mirabilis jalapa* also known as 4'o clock or the "Flower of Peru" in Mexico (Galindo and Hohl 1985; Goodwin and Fry 1994). This high degree of relatedness has several implications for the possibility of gene flow between the members of the Ic clade. Studies have shown that *P. infestans* and *P. mirabilis* are reproductively isolated (Goodwin et al. 1999); however, it is possible that at some time in the past, the species could have interbred and produced viable offspring that may have included *P. andina* (Gómez-Alpizar et al. 2008). However, *P. andina* is native to the Andean region of South America in Ecuador, and presently has not been confirmed to occur anywhere else including Mexico where *P. infestans* and *P. mirabilis* coexist. Thus, the hybridization would have had to occur in the Andes, supporting a South America origin of this species as has been proposed for *P. infestans* (Gómez-Alpizar et al. 2007). Conversely, *P. mirabilis* does not appear to be conspecific with *P infestans* anywhere except Mexico. However, *Mirabilis jalapa* occurs widely in South America, but has not been surveyed for infection by *P. mirabilis*, although the host is present in Peru and elsewhere in the Andean region as well as in the U.S. *P.*

mirabilis has recently been found in Colorado (Adams unpublished). Several plants including pear melon (*Solanum muricatum*) and tree tomato (*S. betaceum*) are hosts to both *P. infestans* and *P. andina*.

The objectives of our work were: 1) to sequence and annotate the complete mitochondrial genomes of the remaining four members of the Ic clade of *Phytophthora* including: *P. mirabilis*, *P. ipomoeae*, *P. phaseoli*, and *P. andina*; 2) compare the genomes to the complete mitochondrial genome of the Ib mt haplotype of *P. infestans*; and 3) document the evolutionary history of species in the clade using whole mitochondrial genomes. A preliminary abstract of a portion of this research has been published (Lassiter et al. 2010).

MATERIALS AND METHODS

Isolates

Four isolates of *Phytophthora* were used for this study including: *P. mirabilis*, *P. ipomoeae*, *P. phaseoli*, and *P. andina* (haplotype Ic). The mitochondrial genome sequence of the *P. infestans* Ib mt haplotype (NC 002387), previously sequenced, was used for comparison (Table 1.1). All isolates were maintained on rye-V8 or lima bean agar at 18°C. Two to three agar plugs containing mycelia were transferred into Petri dishes containing pea broth with 0.5 g/L sucrose for 2-3 weeks. Mycelia from approximately 40 Petri dishes was harvested by filtration with Whatman #1 membrane filter paper, and then frozen in liquid

nitrogen. Mycelia was stored at -20°C until further use. Approximately 10g of mycelia was used for DNA extraction.

DNA Preparation

Previously collected mycelia were frozen in liquid nitrogen and then ground into a fine powder using a mortar and pestle. DNA extraction was performed using the Qiagen DNeasy Plant Maxi® kit (Qiagen Corporation, Maryland, USA). The mtDNA was purified in a cesium chloride (CsCl) density gradient (1.1g CsCl/mL DNA-AE solution) with the addition of bisbenzimide (final concentration of 10 mg/mL) as described previously (Avila-Adame et al. 2006; Carter et al. 1990; Garber and Yoder 1983; Klimczak and Prell 1984). The DNA was purified by two consecutive centrifugations in a Beckman ultracentrifuge with a 70.1 Ti rotor at 55K for 42-48 hours at 20°C. The bisbenzimide and CsCl were removed with the use of isopropanol and dialysis in TE buffer (at 4°C for 16 hours including three changes of buffer), respectively. The mtDNA was concentrated by ethanol precipitation in the presence of 1/10 3.0 M sodium acetate (pH 5.2), and resuspended by dissolving in AE buffer provided in the Qiagen kit.

Sequencing

The mitochondrial genomes were sequenced at the BROAD Institute of MIT with a minimum of 100-fold sequence coverage with standard 454 technology fragment reads using methods described previously (Lennon et al. 2010). The mtDNA was sheared and size

selected to generate fragments from 400 to 800 bp in length. Fragments were blunt ended, ligated on both ends with 454 adapter sequences, and the resulting library was subjected to emulsion PCR. The DNA was then sequenced using the 454 Titanium sequencing platform.

Annotation

The 454 reads were reassembled using Newbler (Roche). The identification of genes, open reading frames, and other features in the mitochondrial genomes was based on the previously published mitochondrial genome sequence of *P. infestans* (mtDNA haplotype Ib available in GenBank accession no. NC002387). Portions of the four genomes were aligned with the *P. infestans* genome using BioEdit 7.0.5.3 (Hall 1999). Each gene within the *P. infestans* genome was locally aligned with the entire genome of each of the remaining species. The mitochondrial genomes of each species were analyzed and mapped using the software program Vector NTI suite 9.0. Annotated sequence data was submitted to GenBank and accession numbers are HM590421 (*P. mirabilis*), HM590419 (*P. andina*), HM590418 (*P. phaseoli*), and HM590420 (*P. ipomoeae*) (Table 1.1).

Evolutionary Analysis

Sequences of the mtDNA were aligned using BioEdit version 7.0.5.3 (Hall 1999). Relationships among the species were determined by using Bayesian inference to construct gene trees in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) (Appendix 1.4). Coalescence-based gene genealogies were constructed using Genetree in SNAP Workbench

(Carbone et al. 2004; Griffiths and Tavaré 1994; Price and Carbone 2005). The rooted haplotype tree and the mapping of nucleotide substitutions to branches on the tree were estimated with coalescent-based techniques (Carbone et al. 2004). Although the Ic clade does not match all assumptions of these coalescent-based techniques (*e.g.*, the assumption of a single randomly mating population with no subdivision), we do not expect the violations of assumptions to cause incorrect rooting of the haplotype tree or the incorrect mapping of substitutions to branches on the tree.

RESULTS

We completed the mitochondrial genome sequencing of *P. andina*, *P. mirabilis*, *P. ipomoeae*, and *P. phaseoli*. The genome sizes are presented in Table 1.2. The mitochondrial genome sequence for the Ib haplotype of *P. infestans*, published previously by B. Franz Lang at the University of Montreal as part of the Fungal Mitochondrial Genome Project, was used for comparison (Lang and Forget 1993; Paquin et al. 1997). *P. mirabilis* had the smallest mitochondrial genome and *P. infestans* had the largest. The genome sizes for *P. infestans*, *P. phaseoli*, *P. andina*, *P. ipomoeae*, and *P. mirabilis* were 37,914, 37,957, 37,874, 37,872 and 37,778 respectively (Table 1.2).

The mitochondrial genomes of *P. andina, P. ipomoeae*, and *P. phaseoli* were 83, 85, and 43 bp smaller than the mitochondrial genome of the Ib haplotype of *P. infestans*. The

largest differences in mitochondrial genome size were observed between *P. infestans* and *P. mirabilis*. The mitochondrial genome of *P. mirabilis* was 179 bp smaller than that of the Ib haplotype of *P infestans* (Table 1.2).

Coding sequence identity between each of the Ic clade species and *P. infestans* Ib mtDNA haplotype was greater than 99% (Table 1.2). All five genomes were adenine and thymine rich and G-C content was less than 23% in each of the species (Table 1.2). The coding regions in all of the genomes accounted for more than 90% of the mt genomes (Table 1.2). A total of 61 genes with known function were identified including: 18 genes involved in electron transport, 2 ribosomal RNA genes, 16 ribosomal protein genes, and 25 transfer RNA genes (Table 1.3). Six ORFs with unknown function were also identified. Genes were coded in both strands of the DNA, and gene order was identical among all five Ic clade species (Figures 1.1A-D, Appendix 1.1). None of the ORFs identified in the type II mitochondrial haplotypes of *P. infestans* (Avila-Adame et al. 2006) were found among the four sister species. The genes coding for the ribosomal proteins *rps*12 and *rps*7 overlapped and share 71 base pairs in all species sequenced. Similarly, there was an observed overlap between the genes coding for the NADH dehydrogenase subunits 1 (*nad*1) and 11 (*nad*11); these genes share 4 base pairs. Two indels weree detected in the *P. andina* ORF79 corresponding to a 12 nucleotide deletion in the region (Figure 1.1E). These indels were not found in any of the other genomes and do not correspond to any of the known restriction enzyme sites used to differentiate *P. andina* Ic mt haplotype from *P. infestans* (Ordonez et al. 2000).

A total of 594 polymorphic sites exist among the five species in the clade (Table 1.4, Appendix 1.5). Among these polymorphic sites were 176 transversions and 418 transitions (Appendix 1.5). Several single nucleotide substitutions produced 129 amino acid changes in the coding regions of the mitochondrial genomes (Appendix 1.3). None of these polymorphic sites produced frameshifts, with the exception of the 12 nucleotide (4 amino acid) deletion in *P. andina* ORF79 (Figure 1.1E).

Coalescent analysis was used to infer the mutational history, time scale, and evolution of the polymorphism among the species. The genome sequences were collapsed into five unique haplotypes, after removing indels and excluding infinite-sites violations using SNAP workbench (Carbone et al. 2004; Price and Carbone 2004). Only regions corresponding to coding genes, tRNAs, or rRNAs were used for coalescent simulations. The five haplotypes were distinguished by the 594 different substitutions/variable sites (Appendix 1.5, 1.6).

According to the coalescent analysis, the earliest split on the haplotype tree separates *P. phaseoli* from the other four haplotypes (Figure 1.2). Next, the *P. infestans* lineage diverged from the lineages leading to the remaining three haplotypes; *P. andina* diverged next. *P. mirabilis* and *P. ipomoeae,* two species that are known only in Mexico, are inferred to be the most closely related according to the haplotype tree and diverged more recently from a common ancestor of *P. andina* (Figure 1.2). Each branch on the haplotype tree indicated multiple substitutions in the mitochondrial genome. *P. phaseoli* had the greatest number of unique substitutions (175), more than any other member of the Ic clade. *P. ipomoeae* had the second highest number (113), followed by *P. andina* (95), *P. infestans*

(79), and *P. mirabilis* (69). The lineage that led to the separation of the other four members of the clade from *P. phaseoli* included 45 substitutions. There were 14 substitutions inferred on the branch leading to *P. andina*, *P. ipomoeae*, and *P. mirabilis,* and 4 substitutions separate *P. ipomoeae* and *P. mirabilis* from *P. andina* (Appendix 1.6).

DISCUSSION

The use of mitochondria for the inference of evolutionary history is particularly valuable. Analysis of mitochondrial genomes of *Phytophthora* species, including *P. ramorum* and *P. sojae*, has shown that there is a high degree of gene conservation in the genus (Martin et al. 2007). This degree of gene conservation was also observed among the *Phytophthora* species in the Ic clade. Both gene content and order were identical amongst the five species in the clade. One hypothesis for this level of similarity could be that the mitochondrial genome is a small organelle and there are many important coding genes contained within it (Martin et al. 2007). Neither unique ORFs nor insertions were found amongst the Ic clade species of *Phytophthora*, with the exception of *P. andina* ORF79, which appears to have a 4 amino acid deletion of phenylalanine, leucine, serine, and isoleucine. The high degree of similarity in gene order and function is needed for coding sequences for structural and functional genes. Unique genes that distinquish species within the clade most

likely comes from nuclear genes that encode for host specialization, virulence, or pathogenicity.

The mitochondrial genomes of *Phytophthora* species in the Ic clade were highly similar, differing only by 594 polymorphisms in the coding regions, indicating that the species themselves are closely related. The gene content and order were both conserved among all five species of the Ic clade, indicating that the mitochondrial genomes of the clade are not highly divergent. Comparison of our data with the type II mitochondrial haplotypes of *P. infestans* showed that *Phytophthora* species in the Ic clade more closely resemble the type I mitochondrial haplotype of *P. infestans*. The four remaining Ic clade species show none of the unique ORFs found among the type II haplotypes and also these species lack the 2 kb insertion sequence that is characterisitic of type II haplotypes of *P. infestans* (Avila-Adame et al. 2006 Carter et al. 1990; Gavino and Fry 2002). It had previously been proposed that the Ib haplotype was the ancestral lineage of *P. infestans* and that the type II's evolved from the type I's (Goodwin et al. 1994). However, Avila-Adame et al. (2006) found that the type II haplotypes of *P. infestans* diverged earlier from a common ancestor than the type I haplotypes. Based on our data, the remaining members of the *Phytophthora* Ic clade species may have diverged from the type I mitochondrial haplotype of *P. infestans* after their divergence from the type II's haplotypes of *P. infestans*. This hypothesis is currently under investigation by running coalscent simulations using the Ic clade sister species as well as all four mitochondrial haplotypes (Ia, Ib, IIa, IIb) of *P. infestans*.

Comparisons of the genomes' amino acid replacements suggest that each member of the Ic clade, including *P. andina*, is a distinct species and not a subspecies of another (Oliva et al. 2010). A particularly distinguishing feature of the *P. andina* genome is the presence of two indels in ORF79, a feature that can be used to distinguish *P. andina* from the other species that have a longer ORF79. This site may be used with other known regions to differentiate *P. andina* and *P. infestans* from one another. Creating a PCR primer specific to the *P. andina* ORF79 could enable a quick diagnostic tool for identification of this particular species in an infected plant. It is not clear whether this indel is also present in the Ia mt haplotypes of *P. andina*. Therefore, the primer would need to be tested on many isolates, including Ia haplotypes, to determine specificity.

It has been proposed that the Toluca Valley in Mexico is the center of origin for *P. infestans* and its close relatives *P. ipomoeae* and *P. mirabilis* (Gavino and Fry 2002; Grünwald and Flier 2005; Flier et al. 2003). However, alternative data suggests the Andes of South America as the center of origin of *P. infestans* (Gómez-Alpizar et al. 2004). The evolutionary history of the Ic clade inferred from the coalescent analyses presented here supports the latter hypothesis. *P. infestans*, *P. mirabilis*, *P. andina*, and *P. ipomoeae* coalesced into the same group, indicating that these four species share a common ancestor that may have been present in either Mexico or South America. To date, *P. andina* has only been reported in the Andean region of South America. This suggests that the divergence of *P. andina* from *P. infestans* occurred in the Andean region. *P. andina* diverged from the same mitochindrial lineage that gave rise to the type Ib *P. infestans*, a haplotype that is not

known to occur in Mexico. Subsequently, *P. ipomoeae* and *P. mirabilis* evolved more recently from a common ancestor of *P. andina*. These data suggest that *P. mirabilis* and *P. ipomoeae*, two species currently reported only in Mexico, are more recently diverged from a *P. andina* lineage.

The host of *P. mirabilis, Mirabilis jalapa* and the host plant for *P. ipomoeae*, *Ipomoeae longipedunculata,* both originated from the Andean region of South America, and were transported to Mexico hundreds of years ago; neither are native to Mexico. One potential scenario includes the possibility that these *Phytophthora* species jumped hosts in Mexico from a wild *Solanum* host. An alternative possibility is that the entire Ic clade species evolved in South America on the hosts that were native there and were then moved to Mexico on infected plants. The later hypothesis is more likely since since *P. andina* and *P. infestans* are sympatric in the Andean region and it is clear that *P. mirabilis* and *P. ipomoeae* diverged more recently from a *P. andina* common ancestor.

However, we cannot rule out the alternative hypothesis of the entire Ic clade evolving in Mexico. *P. andina* has not been found in Mexico to date, thus requiring further surveys. Although other members of the Ic clade have been found in Mexico, the hosts that they infect were brought to the region from South America. We cannot rule out that *P. andina* is present in Mexico, or has evolved there and then diverged to form *P. mirabilis* and *P. ipomoeae,* but there are currently no data to support this hypothesis since *P. andina* has not been found in this region. Further surveys for *P. andina* in Mexico and elsewhere in Central and South America are warranted in addition to reciprocal surveys for *P. mirabilis* and *P. ipomoeae* in

the Andean region. South America is also considered as the center of origin for the entire genus *Solanum*, a major host of *Phytophthora* species in the Ic clade (Hawkes 1990). Host speciation may have led to the evolution of the unique mitochondrial lineages of the Ic clade species of *Phytophthora*.

Further research must be conducted, using the use of whole genome sequences of these species, in order to fully understand the evolutionary history of the *Phytophthora* Ic clade and implications for future *Phytophthora* diseases on their respective hosts. Also, surveys conducted in both Mexico, central and South America are needed to resolve the center of origin debate for the clade.

ACKNOWLEDGMENTS

The work by E. Lassiter and J.L. Thorne was partially supported by NIH grant GM070806. Sequencing of the mitochondrial genomes was performed at the Broad Institute in Cambridge MA. The technical support of Dr. Julia Hu and Dr. Monica blanco Meneses is appreciated. Dr. Ignazio Carbone, Dr. Geromy Moore, Dr. Ben Redelings, Dr. Reed Cartwright, and Kristin Lamm helped troubleshoot analytical methods and simulations.

REFERENCES

Adler NE, Chacón G, Forbes G, Flier W (2002) *Phytophthora infestans* sensu lato in South America population substructuring through host-specificity. In Lizárrega C (ed.) Proceedings of the Global Initiative on Late Blight Conference, International Potato Center, Lima, Peru, pp 13-17

Adler NE, Erselius LJ, Chacon MG, Flier WG, Ordonez ME, Kroon LPNM, Forbes GA (2004) Genetic diversity of *Phytophthora infestans* sensu lato in Ecuador provides new insight into the origin of this important plant pathogen. Phytopathology 94: 154-162

Advise JC, Arnold J, Ball RM, et al. (1987) Intraspecific phylogeography- the mitochondrial-DNA bridge between population genetics and systematics. Ann Rev of Eco Syst 18: 489-522

Avila-Adame C, Gómez-Alpizar L, Zisman V, Jones K, Buell CR, Ristaino JB (2006) Mitochondrial genome sequences and molecular evolution of the Irish potato famine pathogen, *Phytophthora infestans*. Curr Genet 49: 39-46

Blair JE, Coffey MD, Park S-Y, Geiser DM, Kang S (2008) A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. Fungal Genetics and Biology 45: 266-277

Brasier CM and Hansen EM (1992) Evolutionary biology of *Phytophthora*. Part II: phylogeny, speciation, and population structure. Ann Rev Phytopathol 30: 173-200

Carbone I, Liu Y-C, Hillman BI, Milgroom MG (2004) Recombination and migration of *Cryphonectria* hypovirus I as inferred from gene geneologies and the coalescent analysis. Genetics 166: 1611-1629

Carter DA, Archer SA, Buck KW (1990) Restriction fragment length polymorphism of mitochondrial DNA of *Phytophthora infestans*. Mycol Res 8: 1123-1128

Chesnick JM, Goff M, Graham J, Ocampo C, Lang BF, Seif E, Burger G (2000) The mitochondrial genome of the stramenopile alga *Chrysodidymus synuroideus*. Complete sequence, gene content and genome organization. Nucleic Acids Res 28: 2512-2518

Chesnick JM, Tuxbury K, Coleman A, Lang BF (1996) Utility of the mitochondrial *nad4L* gene for algal and protistan phylogenetic analysis. J Phycol 32: 452-456.

Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM (2000) A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Gen Biol 30: 17-32

Flier WG, Grünwald NJ, Kroon LPNM, Sturbaum AK, van den Bosch TBM, Garay-Serrano E, Lozoya-Saldana H, Fry WE, Turkensteen LJ (2003) The population structure of *Phytophthora infestans* from the Toluca Valley of Central Mexico suggests genetic differentiation between populations of cultivated potato and wild *Solanum* spp. Phytopathology. 93: 382-390

Flier WG, Grünwald NJ, Kroon LPNM, van den Bosch TBM, Garay-Serrano E, Lozoya-Saldana, Bonants PJM, Turkensteen LJ (2002) *Phytophthora ipomoeae* sp. nov., a new homothallic species causing leaf blight on *Ipomoea longipedunculata* in the Toluca Valley of central Mexico. Mycol Res 106: 848-856

Föster H, Coffey MD, Elwood H, Sogin ML (1990) Sequence analysis of the small-subunit ribosomal rRNA's of three zoosporic fungi and implications for fungal evolution. Mycologia 82: 306-312

Galindo J and Hohl HR (1985) *Phytophthora mirabilis*, a new species of *Phytophthora*. Sydowia 38: 87-96

Galtier N, Nabholz B, Glémin S, Hurst GDD (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Mol Eco 18: 4541-4550

Garber RC, Yoder OC (1983) Isolation of DNA from filamentous fungi and separation into nuclear, mitochondrial, ribosomal, and plasmid components. Anal Biochem 135: 416-422

Gavino PD, Fry WE (2002) Diversity in and evidence for selection of the mitochondrial genome of *Phytophthora infestans*. Mycologia. 94: 781-793

Grünwald NJ and Flier WG. 2005. The biology of *Phytophthora infestans* at its center of origin. Annu Rev Phytopathol 43: 171-190.

Gómez-Alpizar L, Carbone I, Ristaino JB (2007) An Andean origin of *Phytophthora infestans* inferred from mitochondrial and nuclear gene geneologies. Poc Natl Acad Sci 104: 3306-3311

Gómez-Alpizar L, Hu CH, Oliva RF, Forbes GA, Ristaino JB (2008) Phylogenetic relationships of *Phytophthora andina*, a new species from the highlands of Ecuador that is closely related to the Irish potato famine pathogen *Phytophthora infestans*. Mycologia 100: 590-602

Goodwin SB, Cohen BA, Fry WE (1994) Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. Proc Natl Acad Sci 91: 11591-11595

Goodwin SB and Fry WE (1994) Genetic analysis of interspecific hybrids between *Phytophthora infestans* and *Phytophthora mirabilis*. Experimental Mycology 18: 20-32

Goodwin SB, Legard DE, Smart CD, Levy M, Fry WE (1999) Gene flow analysis of molecular markers confirms that *Phytophthora mirabilis* and *P. infestans* are separate species. Mycologia 91: 796-810

Goodwin SB, Spielman LJ, Matuszak JM, Bergeron SN, Fry WE (1992) Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopath 84: 1224-1227

Griffith GW, Saw DS (1998) Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. Appl Environ Microbiol 64: 4007-4014

Griffiths RC, Tavaré S (1994) Ancestral inference in population genetics. Stat Sci 9: 307-319

Grünwald NJ, Flier WG (2005) The biology of *Phytophthora infestans* at its center of origin. Annual Rev Phytopathol. 43: 171-190

Gunderson JH, Elwood H, Ingold A, Kindle K, Soin ML (1987) Phylogenetic relationships between chlorophytes, chrysophytes, and oomycetes. Proc Natl Acad Sci 84: 5823-5827

Hall, TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser. 41: 95-98

Hawkes JG (1990) The potato: evolution, biodiversity and genetics resources. London: Belhaven Press

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754-755

Klimczak LJ, Prell HH (1984) Isolation and characterization of mitochondrial DNA of the oomycete fungus *Phytophthora infestans*. Curr Genet 8: 323-326

Kroon LPNM, Bakker FT, van den Bosch BGM, Bonants PJM, Flier WG (2004) Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. Fungal Genetics and Biology 41: 766-782

Lang BF, Forget L (1993) The mitochondrial genome of *Phytophthora infestans*. In O'Brien SJ (ed) Genetic maps: locus maps of complex genomes. Cold Spring Harbor Laboratory Press, Plan View, pp 3.133-3.135

Lassiter ES, Russ C, Nusbaum C, Zeng Q, Hu CH, Thorne JL, and Ristaino JB (2010) Inferring evolutionary relationships of species in the *Phytophthora* Ic clade using nuclear and mitochondrial genes. Phytopathology S 68.

Lennon NJ et al. (2010) A scalable, fully automated process for construction of sequence-ready barcoded libraries for 454. Genome Biology 11: R15.

Martin FN, Benasson D, Tyler BM, Boore JL (2007) Mitochondrial genome sequences and comparative genomics of *Phytophthora ramorum* and *P. sojae*. Curr Genet 51: 285-296

Mortiz C, Dowlin TE, Brown WM (1987) Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Ann Rev Ecol and Syst 18: 269-292

Moskin J (2009) Outbreak of fungus threatens tomato crop. New York Times July 17, 2009, morning ed.:A16

Niederhauser JS (1991) *Phytophthora infestans*: the Mexican connection. In Lucas JA, Shattock RC, Shaw DS, Cooke LR (eds) *Phytophthora*: an international symposium, Cambridge University Press, Cambridge, UK, pp 25-45

Oliva RF, Chacón MG, Cooke DEL, Lees AK, Forbes GA (2007) Is *Phytophthora infestans* a good taxonomist? Host recognition and co-evolution in the *Phytophthora*/*Solanum* interaction. Acta Horticulturae 745: 465-471

Oliva RF, Kroon LPNM, Chacón G, Flier WG, Ristaino JB, Forbes GA (2010) *Phytophthora andina* sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands. Plant Path 100(4): 590-602

Ordonez ME, Hohl HR, Velasco JA, Ramon MP, Oyarzun PJ, Smart CD, Fry WE, Forbes GA, Erselius LJ (2000) A novel population of *Phytophthora*, similar to *P. infestans*, attacks wild solanum species in Ecuador. Ecology and Population Biology 90 (2): 197-202

Paquin B, Laforest M-J, Forget L, Roewer I, Wang Z, Longcore J, Lang BF (1997) The fungal mitochondrial genome project: evolution of fungal mitochondrial genomes and their gene expression. Curr Genet 31: 380-395

Price EW, Carbone I (2005) SNAP: workbench management tool for evolutionary population genetic analysis. Bioinformatics 21: 402-404

Ratnasingham S, Herbert PD (2007) bold: The Barcode of Life Data System (http://www.barcodinglife.org) Molecular Ecology Notes 7: 355-364

Ristaino JB (1998) The importance of archival and herbarium materials in understanding the role of oospores in late blight epidemics of the past. Phytopathology 88: 1120-1130

Vargas AM et al. (2009) Characterization of *Phytophthora infestans* populations in Columbia: first report of the A2 mating type. Phytopathology 99: 82-88

Waterhouse GM (1963) Key to the species of *Phytophthora* de Bary. Mycol Paper 162: 1-22

Table 1.1 Isolates and GenBank accession numbers of *Phytophthora* used for mitochondrial genome sequencing.

Species	Isolate	Collection	Origin	GenBank accession number
P. infestans	West Virginia 4	ATCC[a] 16981	ATCC	NC002387
P. phaseoli	Phy P18		Delaware	HM590418
P. andina	EC 3425	CBS[b] 122202[c]	Ecuador	HM590419
P. ipomoeae	Pic 99167	CBS 122203	Mexico	HM590420
P. mirabilis	Pic 99114	CBS 122204	Mexico	HM590421

[a]ATCC- American Type Culture Collection, Manasas, Virginia
[b]CBS- Fungal Diversity Centre, Netherlands
[c]Listed as an isolate of *P. infestans*

Table 1.2 Mitochondrial genome content of *Phytophthora* species in Ic clade.

Species	Genome size (bp)	% Identity[a]	Coding		Non-coding		G + C
			bp	%	bp	%	Content (%)
P. infestans (Ib)	37957	-	34209	90.1	3748	9.9	22.3
P. phaseoli	37914	99.0	34284	90.4	3630	9.6	22.4
P. andina	37874	99.3	34272	90.4	3602	9.5	22.1
P. ipomoeae	37872	99.3	34284	90.5	3670	9.7	22.4
P. mirabilis	37778	99.4	34284	90.7	3494	9.2	22.4

[a]% identity is calculated from alignment of only coding regions and compared to *P. infestans*.

Table 1.3 Gene content of the mitochondrial genomes of five species of the *Phytophthora* Ic clade[a]. Genes were defined according to Paquin et al. (1997).

	Species				
	P. infestans	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*
Genes involved in electron transport and coupled oxidative phosphorylation	18	18	18	18	18
RNA-encoding genes	2	2	2	2	2
Ribosomal protein genes	16	16	16	16	16
Conserved ORFs[b]	6	6	6	6	6
Unique ORFs	0	0	0	0	0
Transfer RNA genes	25	25	25	25	25

[a]Fig. 1.1 shows the distribution of genes in the genome.
[b] The conserved ORFs include ymf16, which codes for secY-independent transporter, ORFs 32, 79, 100, 142, and 217.

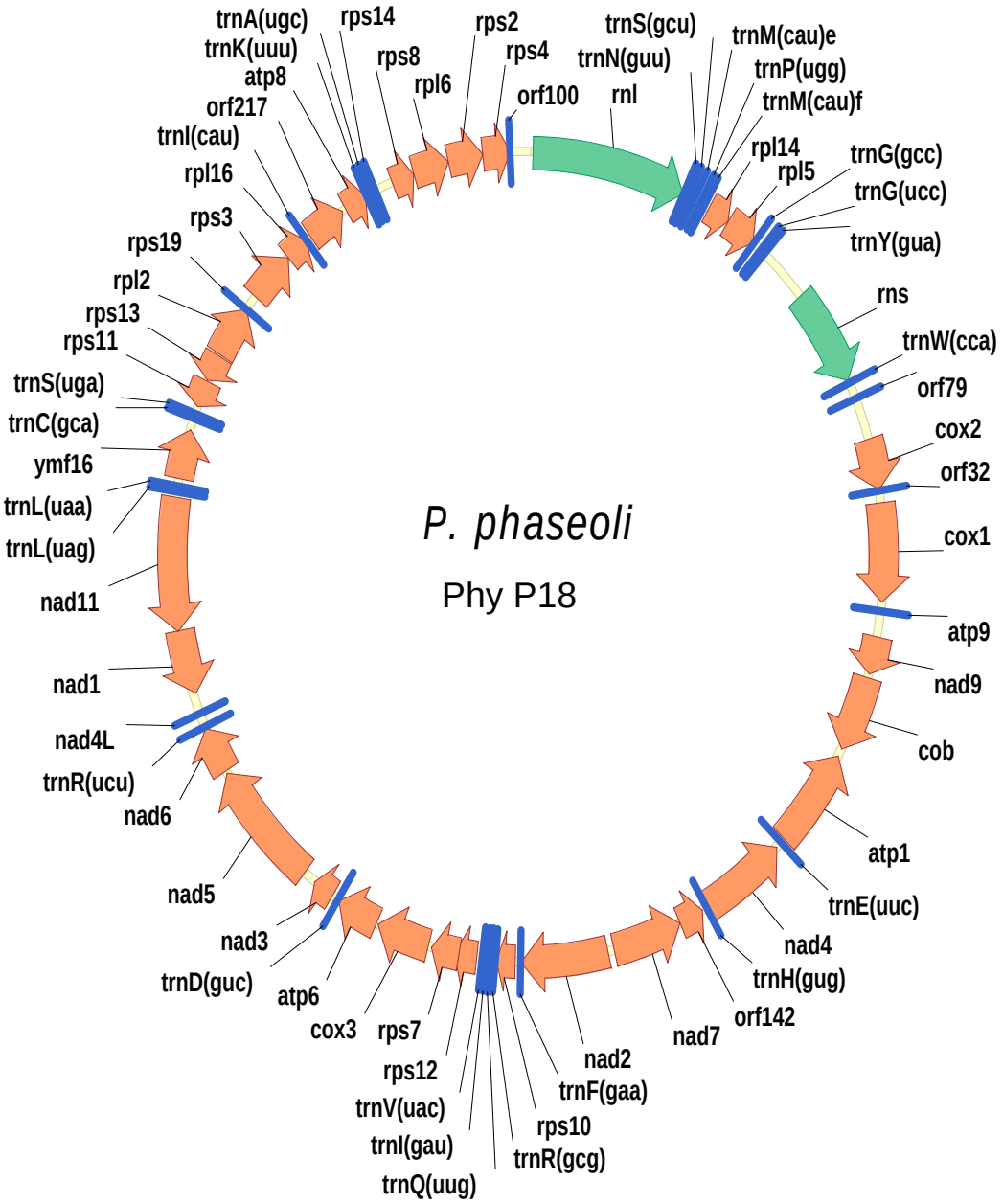
Table 1.4 Summary of polymorphisms[a] found among the *Phytophthora* Ic clade species.

Species	*P. infestans*	*P. phaseoli*	*P. andina*	*P. ipomoeae*	*P. mirabilis*
P. infestans	0	337	225	236	189
P. phaseoli	-	0	364	380	344
P. andina	-	-	0	241	199
P. ipomoeae	-	-	-	0	213
P. mirabilis	-	-	-	-	0

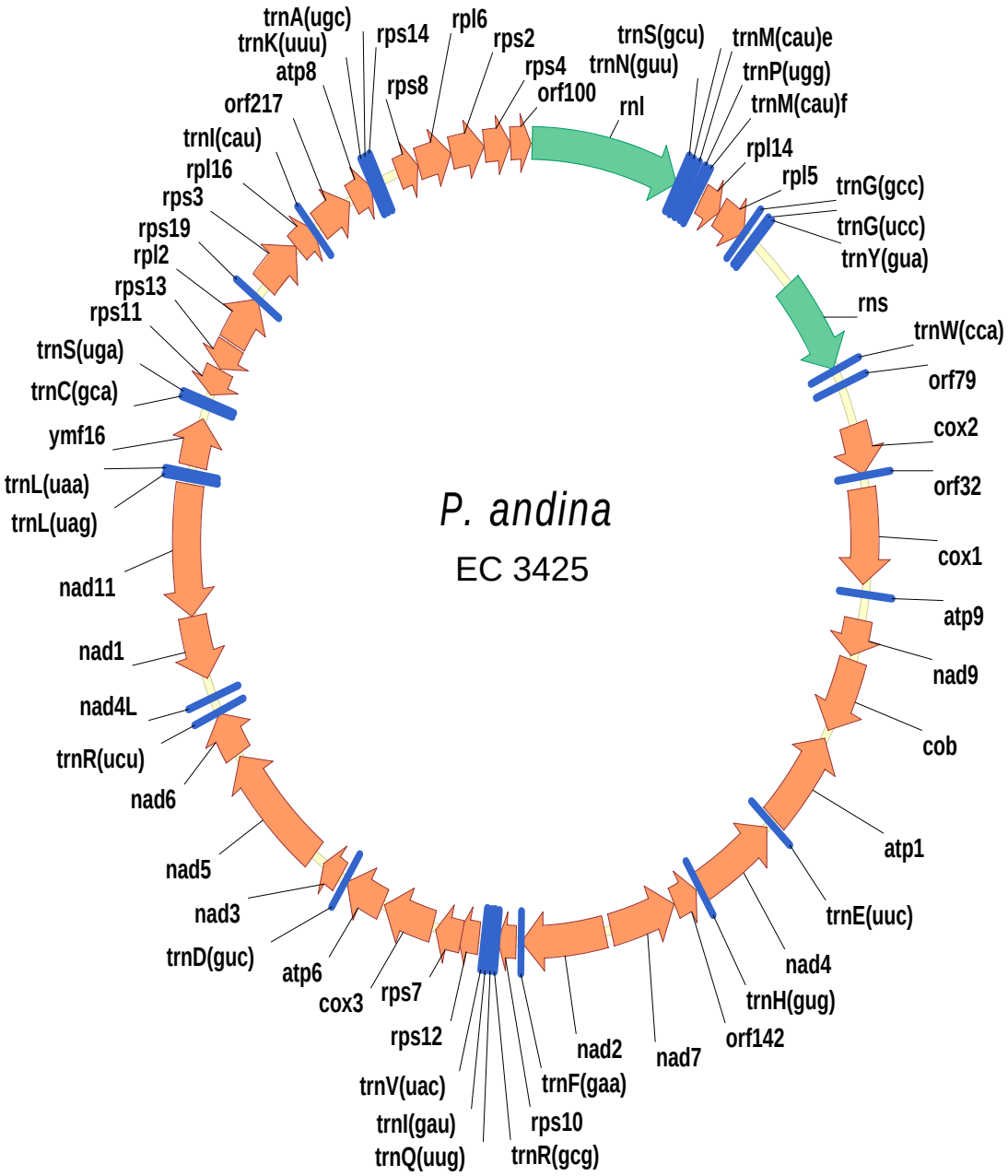
[a]Polymorphic sites include single base pair substitutions within coding sites (594); these sites do not include indels greater than one bp.

Figure 1.1 Mitochondrial genome maps of the *Phytophthora* Ic clade (A) *P. phaseoli* 37,914 bp; (B) *P. andina* with a size of 37,874 bp; (C) *P. mirabilis* 37,778 bp; and (D) *P. ipomoeae* 37,872 bp. Gene order and size are both conserved among the species; genome size is also highly similar. (E) Schematic of *P. infestans* ORF79 and *P. andina* ORF 79 showing a 12 nucleotide indel present only in *P. andina*.

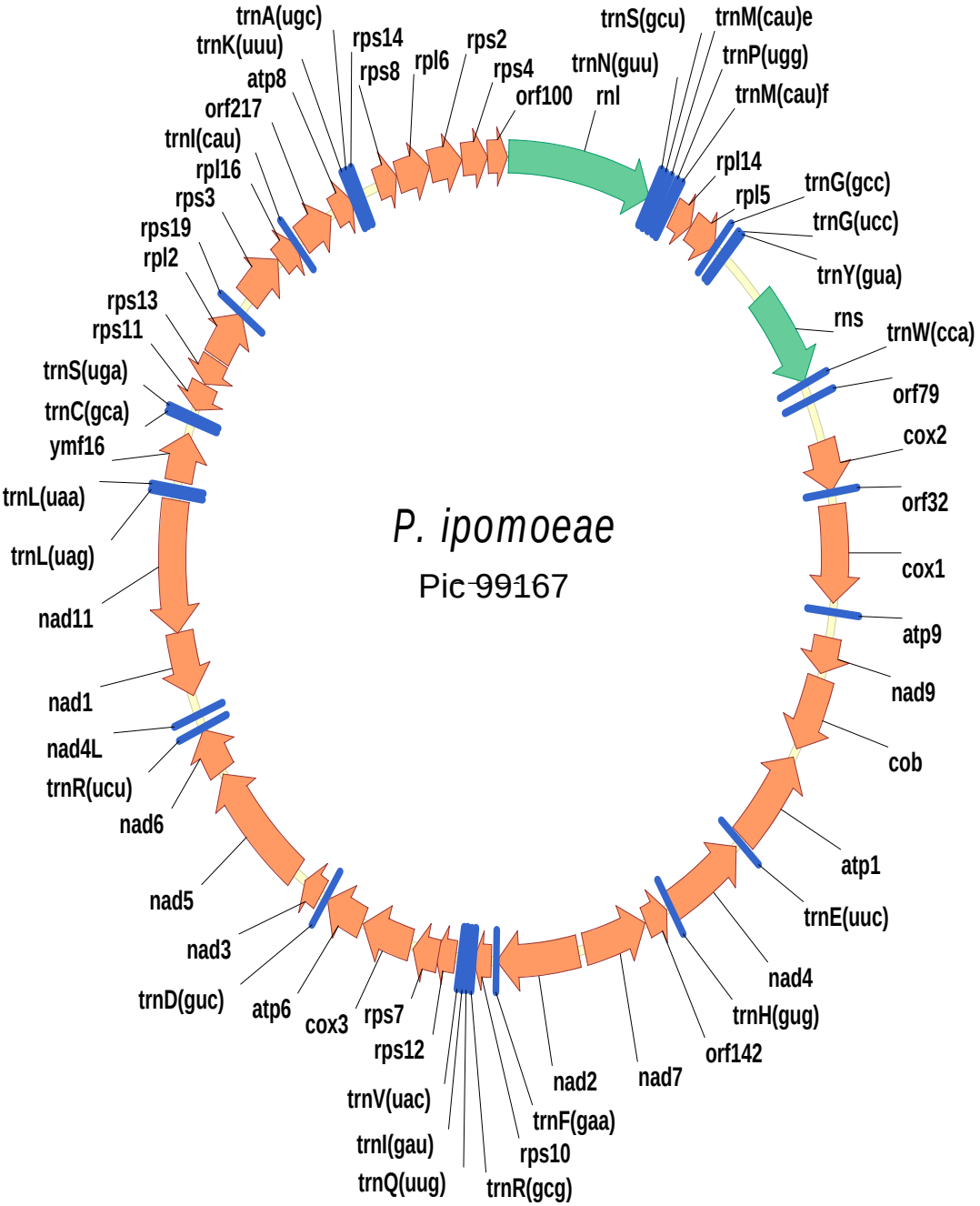
A


P. phaseoli
Phy P18
trnA(ugc)
trnK(uuu)
atp8
orf217
trnI(cau)
rpl16
rps3
rps19
rpl2
rps13
rps11
trnS(uga)
trnC(gca)
ymf16
trnL(uaa)
trnL(uag)
nad11
nad1
nad4L
trnR(ucu)
nad6
nad5
nad3
trnD(guc)
atp6
cox3
rps7
rps12
trnV(uac)
trnI(gau)
trnQ(uug)
rps14
rps8
rpl6
rps2
rps4
orf100
trnS(gcu)
trnN(guu)
rnl
trnM(cau)e
trnP(ugg)
trnM(cau)f
rpl14
rpl5
trnG(gcc)
trnG(ucc)
trnY(gua)
rns
trnW(cca)
orf79
cox2
orf32
cox1
atp9
nad9
cob
atp1
trnE(uuc)
nad4
trnH(gug)
orf142
nad7
nad2
trnF(gaa)
rps10
trnR(gcg)

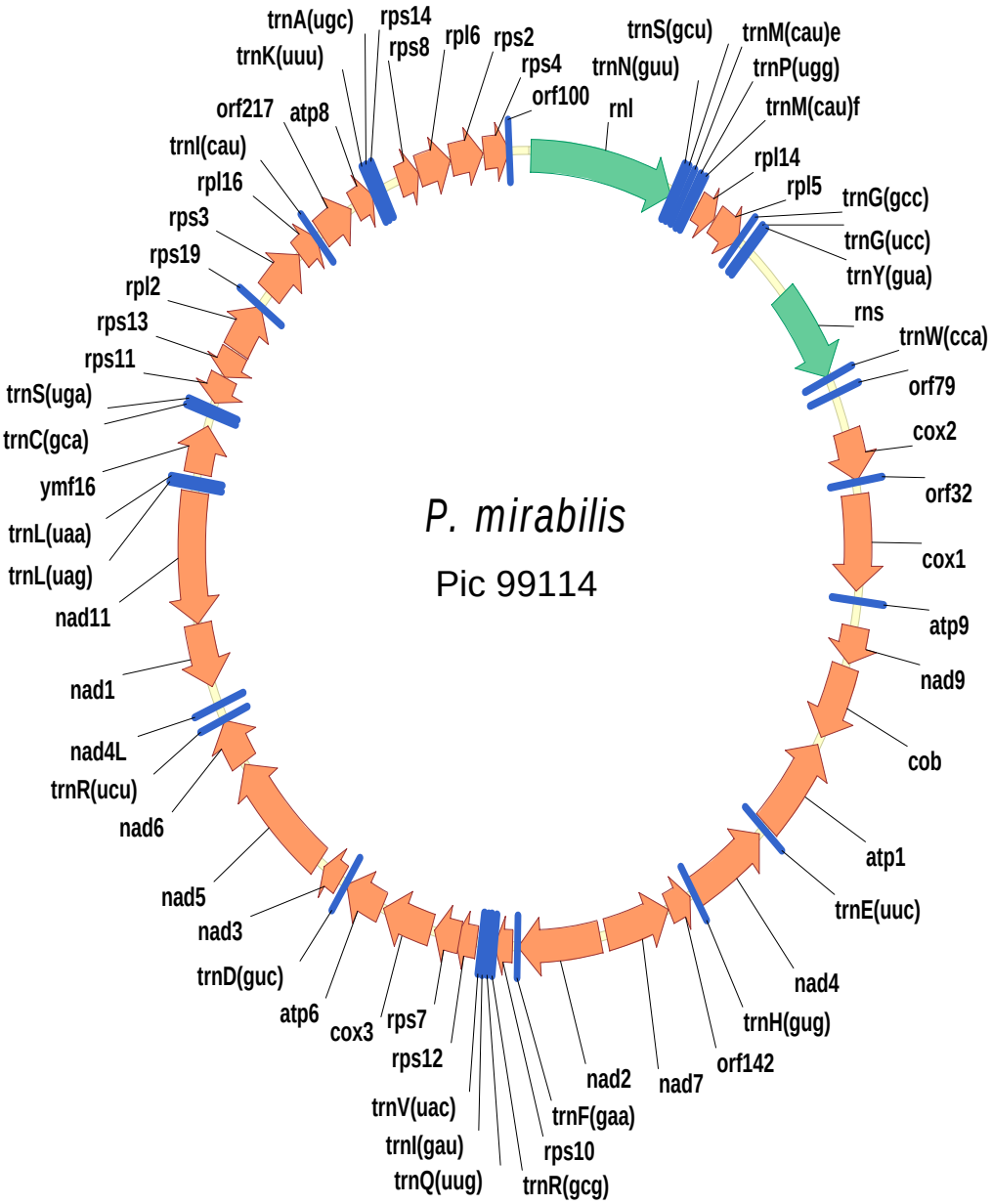
B


P. andina
EC 3425
trnA(ugc)
trnK(uuu)
atp8
orf217
trnI(cau)
rpl16
rps3
rps19
rpl2
rps13
rps11
trnS(uga)
trnC(gca)
ymf16
trnL(uaa)
trnL(uag)
nad11
nad1
nad4L
trnR(ucu)
nad6
nad5
nad3
trnD(guc)
atp6
cox3
rps7
rps12
trnV(uac)
trnI(gau)
trnQ(uug)
trnR(gcg)
rps10
trnF(gaa)
nad2
nad7
orf142
trnH(gug)
nad4
trnE(uuc)
atp1
cob
nad9
atp9
cox1
orf32
cox2
orf79
trnW(cca)
rns
trnY(gua)
trnG(ucc)
trnG(gcc)
rpl5
rpl14
trnM(cau)f
trnP(ugg)
trnM(cau)e
trnN(guu)
trnS(gcu)
rnl
orf100
rps4
rps2
rpl6
rps14
rps8

C


P. ipomoeae
Pic-99167
trnA(ugc)
trnK(uuu)
atp8
orf217
trnI(cau)
rpl16
rps3
rps19
rpl2
rps13
rps11
trnS(uga)
trnC(gca)
ymf16
trnL(uaa)
trnL(uag)
nad11
nad1
nad4L
trnR(ucu)
nad6
nad5
nad3
trnD(guc)
atp6
cox3
rps7
rps12
trnV(uac)
trnI(gau)
trnQ(uug)
trnR(gcg)
rps10
trnF(gaa)
nad2
nad7
orf142
trnH(gug)
nad4
trnE(uuc)
atp1
cob
nad9
atp9
cox1
orf32
cox2
orf79
trnW(cca)
rns
trnY(gua)
trnG(ucc)
trnG(gcc)
rpl5
rpl14
trnM(cau)f
trnP(ugg)
trnM(cau)e
trnS(gcu)
rnl
trnN(guu)
orf100
rps4
rps2
rpl6
rps8
rps14

D


P. mirabilis
Pic 99114
trnA(ugc)
rps14
rps8
rpl6
rps2
rps4
orf100
trnK(uuu)
orf217
atp8
trnI(cau)
rpl16
rps3
rps19
rpl2
rps13
rps11
trnS(uga)
trnC(gca)
ymf16
trnL(uaa)
trnL(uag)
nad11
nad1
nad4L
trnR(ucu)
nad6
nad5
nad3
trnD(guc)
atp6
cox3
rps7
rps12
trnV(uac)
trnI(gau)
trnQ(uug)
trnR(gcg)
rps10
trnF(gaa)
nad2
nad7
orf142
trnH(gug)
nad4
trnE(uuc)
atp1
cob
nad9
atp9
cox1
orf32
cox2
orf79
trnW(cca)
rns
trnY(gua)
trnG(ucc)
trnG(gcc)
rpl5
rpl14
trnM(cau)f
trnP(ugg)
trnM(cau)e
trnS(gcu)
trnN(guu)
rnl

E

```
                    10        20        30        40        50        60        70        80        90       100
P. infestans orf79  atgcaaaaaaaattaaaaattttattcttattttattttaagtataagcataagcattcttatcttatacttacataacgttttaccttatattaatttaaaaat
P. andina orf 79    atgcaaaaaaaattaaaaattttatt------tttattcttaagtataagcat------ttttatcttatacttacataacgttttaccttatattaatttaaaaat
```

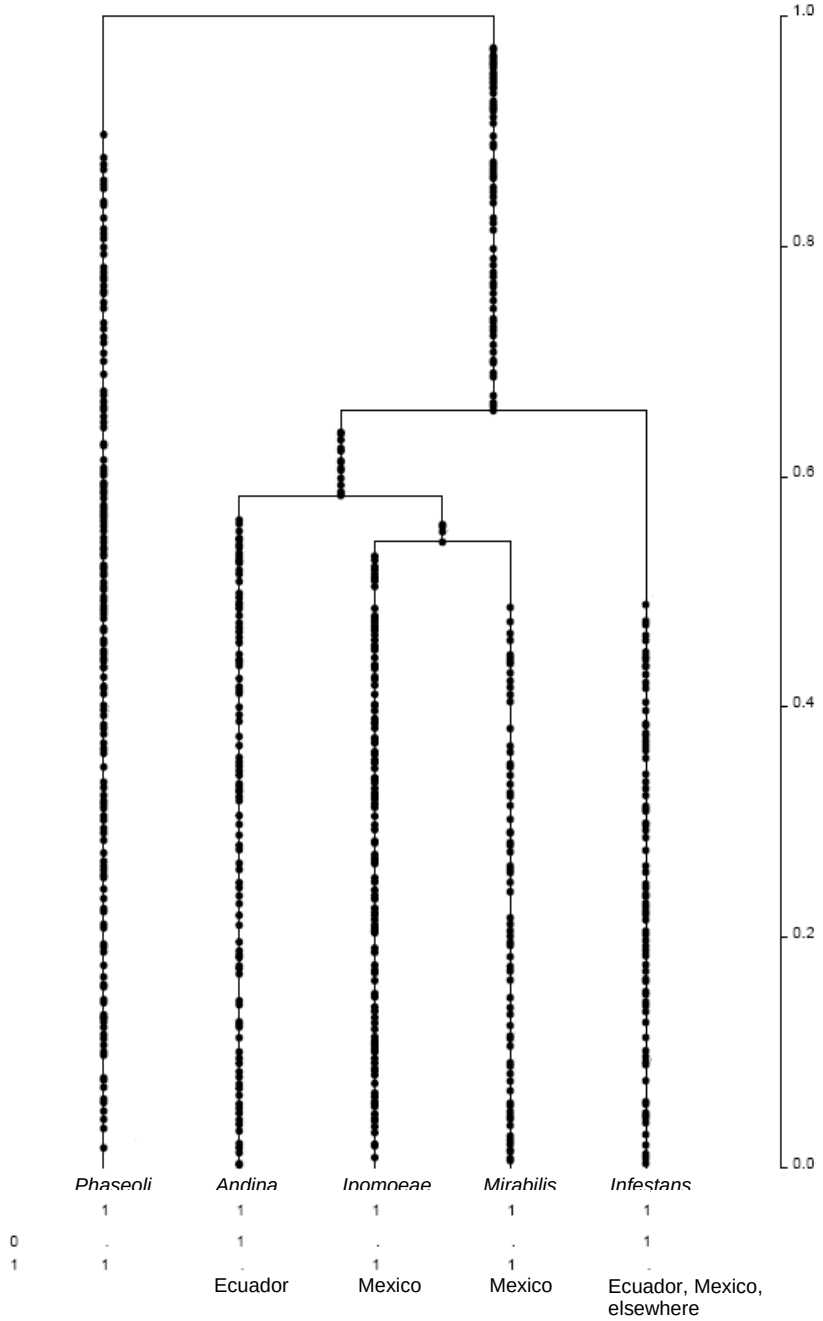


Figure 1.2 Coalescent analysis of *Phytophthora* Ic clade species. The analyses were run a total of 5,000,000 simulations three separate times. *P. phaseoli* diverged earliest from all other species, while *P. ipomoeae* and *P. mirabilis* diverged most recently.

CHAPTER 2

PHYLOGENETIC ANALYSIS OF THE PHYTOPTHORA IC CLADE SPECIES USING NUCLEAR AND MITOCHONDRIAL DNA SEQUENCES

ABSTRACT

The genus *Phytophthora* contains many plant pathogens that cause billions of dollars worth of damage to agricultural and ornamental plants. One particular *Phytophthora*, *P. infestans*, is most famous for causing the Irish potato famine of the 1840's, as well as more recent outbreaks of late blight across the United States. Large scale analyses of *Phytophthora* have been carried out in an effort to better classify members of this genus. These analyses have formed the basis of the *Phytophthora* phylogeny, placing *P. infestans* in the Ic clade. Much research has been dedicated to studying and characterizing this particular pathogen, yet little attention is given to its sister species in the Ic clade, *P. mirabilis*, *P. andina*, *P. ipomoeae*, and *P. phaseoli*. These pathogens are just as destructive as their more infamous cousin. To date, no one has focused a phylogenetic study on the Ic clade alone. We sequenced between 5 and 13 isolates of members of the Ic clade using a variety of molecular markers for both nuclear and mitochondrial genes (*Elongation factor 1α*, *β tubulin*, *Ras*, intron 1 of *ras*, and P4 (part of *coxI* and *atp9*)) corresponding to over 2500 nucleotide sites. Bayesian analysis was used to infer gene trees for the Ic clade. Confirming the widely accepted *Phytophthora* phylogeny, *P. phaseoli* is the most basal member of the clade, with *P. ipomoeae*, *P. mirabilis*, and *P. andina* being more closely related to *P. infestans*. There has

been speculation that *P. andina* is a hybrid species of *P. infestans* and another member of the Ic clade, most likely *P. mirabilis*. Our data are inconclusive with respect to this issue, however other evidence points towards the altnerative hypothesis of *P. infestans* and *P. andina* mating to form *P. mirabilis*. The existence of a potential bridging host (the pear melon) as well as analysis of the mitochondrial genomes of the Phytopthora Ic clade points more towards the latter hypothesis.

INTRODUCTION

Phytophthora species are particularly devastating plant pathogens that cause billions of dollars worth of damage to both agricultural and ornamental and native plants. These pathogens belong to the Kingdom Stramenopila within the class Oomycota. The Oomycetes, or water molds, are diploid (during most of their life cycle), fungus-like eukaryotic organisms that reproduce both sexually and asexually (Margulis and Schwartz 2000). Oomycetes occupy a wide range of niches, from the deserts of Iran (Mirzaee et al. 2009) to the frigid regions of Antarctica (Bridge et al. 2008, Hughes et al. 2003). However, most members of the oomycetes have adapted to a pathogenic lifestyle within various plant species. Plant pathogenicity has arisen twice within the *Peronosporales* lineage, which includes *Phytophthora* species (Thines and Kamoun 2010). The genus *Phytophthora* itself is composed of over 100 species of pathogens that infect a wide range of plant species (Erwin

and Ribeiro 1996). The genus has been divided into six groups based on morphology and reproductive characteristics (Waterhouse 1963); however, these groupings do not correspond to phylogenetic groups (Cooke et al. 2000). Many phylogenetic studies have been conducted on the genus *Phytophthora* (Cooke et al. 2000, Kroon et al. 2004, Blair et al. 2008), but little attention has been given detailed analysis of specific clades within the genus.

P. infestans, the causative agent of the Irish potato famine of the mid-1800's, is a devastating plant pathogen and is a threat to food security (Gómez-Alpizar et al. 2007, Moskin 2009, Vargas et al. 2009). *P. infestans* belongs to the Ic clade of *Phytopthora* along with its closely related sister species, *P. andina*, *P. mirabilis, P. ipomoeae*, and *P. phaseoli* (Blair et al. 2008). Traditionally, species in the Ic clade have been differentiated based on morphology and host specificity. Some members within the Ic clade have very specific hosts which they infect (Table 2.1). *P. mirabilis* infects 4 o'clock (*Mirabilis jalapa*) and *P. ipomoeae* infects members of the morning glory family (*Ipomoeae purpurea, I. longipedunculata*) respectively. Others can infect a range of hosts including *P. infestans*, which attacks both tomato and potato as well as a number of wild *Solanaceous* species (causing late blight), and *P. phaseoli* which attacks various members of the bean family. *P. andina* is known to infect non-tuber bearing *Solanum* species belonging to the Anarrhichomenum complex as well as *Solanum betaceum, Solanum muricatum* (Adler et al. 2004, Ordonez et al. 2000, Oliva et al. 2002).

P. mirabilis and *P. ipomoeae* were first described in the Toluca Valley of central Mexico, one putative center of origin for the entire Ic clade (Brasier and Hansen 1992, Flier

et al. 2002, Flier et al. 2003, Goodwin et al 1992, Grünwald and Flier 2005, Niederhauser 1991). Although both species were first described in Mexico, the host plants, *M. jalapa* and *I. purpurea*, originated in the Andean region of South America. To date, *P. andina* has only been found in the Andean highlands of Ecuador (Oliva et al. 2010). This raises a problem with the Mexican center of origin hypothesis for the Ic clade. In order for Mexico to be the center of origin, *P. andina* should also be found in the region. On the other hand, all the host plants of the Ic clade *Phytophthora* species originated in the Andean region of South America (Avila-Adame et al. 2006), supporting the hypothesis of a South American rather than Mexican origin of the entire clade.

The evolutionary history of the clade has yet to be fully resolved. There is speculation that at some point in time, *P. mirabilis* and *P. infestans* may have interbred and produced offspring (Goodwin et al. 1999). In 2007, using nuclear and mitochondrial sequences, Gómez-Alpizar et al. showed that isolates of *P. infestans sensu lato* of the EC-2 lineage were a distinct clade, but shared a common ancestor with *P. infestans*. This led to the proposal of a novel species, *P. andina*. Subsequent analyses (Gómez-Alpizar et al. 2008, Oliva et al. 2010) confirmed *P. andina* as a distinct member of the Ic clade that is closely related to *P. infestans*

Phylogenies, or evolutionary trees, are used to show evolutionary relationships between species and serve as a tool for statistical analysis of those relationships (Felsenstein 2004). Molecular studies of *Phytophthora* have covered a wide range of genes including the ITS genes and the mitochondrial cox I and II regions (Cooke et al. 2000, Martin and Tooley

2003). In 2008 Blair et al. conducted a phylogenetic study using several different gene regions including *enolase*, *elongation factor 1α*, and several other nuclear and mitochondrial genes. Their study focused on the genus as a whole, but little emphasis was placed on the relationships within the Ic clade because the topology agreed with that previously found by Kroon et al. (2004). Kroon et al. (2004) showed a phylogeny in which *P. phaseoli* is the most distantly related species to *P. infestans*, and that *P. ipomoeae* and *P. andina* were closely related to *P. infestans*. However, in both studies, few isolates of each species in the Ic clade were included. Kroon's phylogeny only used several nuclear and mitochondrial genes, and placed *P. andina* as an intermediary between *P. infestans* and *P. mirabilis*. This was particularly interesting because *P. andina* is known to only occur in Ecuador while *P. mirabilis* is known only in Mexico. It was proposed in that study that *P. andina* could be a hybrid species between *P. infestans* and another member of the clade (Kroon et al. 2004).

Since the analyses of Kroon and Blair, *P. andina* has been described as a novel species in the clade (Oliva et al. 2010). In Ecuador, three clonal lineages of *P. infestans* (US-1, EC-1, EC-3) and one heterogeneous group named *Phytophthora infestans sensu lato* (EC-2 (1c) EC-2 (1a)) were found in association with different wild, non-tuber bearing species in the genus *Solanum* from the section *Anarrhichomenun* (Adler et al. 2004). One lineage within the EC-2 group is characterized by the A2 mating type (Ic mitochondrial haplotype), and the other lineage is characterized by the A1 mating type (Ia mtDNA haplotype) (Adler et al. 2004, Ordonez et al. 2000). The EC-1 clonal lineage of *P. infestans* sensu lato was confirmed to be *P. infestans* based on sequences of the mitochondrial cytochrome oxidase I (*cox I*) gene and intron 1

of the *ras* gene (Gómez-Alpizar et al. 2008). However, the EC-2 (1c) isolates formed a distinct branch in the same clade with *P. infestans, P. mirabilis, P. phaseoli and P. ipomoeae,* for both *cox I* and *ras* intron 1, and were identified as the newly described species *P. andina* (Gómez-Alpizar et al. 2008; Kroon et al. 2004). Gómez-Alpizar et al. used *Ras* intron 1 sequence data and suggested *P. andina* may have arisen via hybridization between *P. infestans* and *P. mirabilis* (Gómez-Alpizar et al. 2008). Determining a robust and well-supported phylogeny of this particular clade could help in improving our understanding of the evolutionary history of the clade, improve current diagnostic tools for identification, and also help us understand the origin of the newest species in the clade, *P. andina*.

The objectives of this study were 1) to utilize several nuclear and mitochondrial genes from many isolates of Ic clade *Phytophthora* species in order to evaluate the true phylogeny of the group and 2) to test the hypothesis that *P. andina* originated as a hybrid species of *P. infestans* and *P. mirabilis*.

MATERIALS AND METHODS

Isolates and culturing

Isolates of *Phytophthora* from each of the species of the *Phytophthora* Ic clade were chosen based on availability of both culture and sequence (Table 2.2, Appendix 2.1). All cultures were maintained on rye-V8 or lima bean agar at 18°C. Two to three hyphal plugs

were transferred to Petri dishes containing pea broth with 0.5g/L sucrose for 2-3 weeks. Mycelium was harvested by filtration with Whatman Numeber 1 membrane, and frozen in liquid nitrogen then stored at -20°C until further use.

DNA extraction

DNA extractions were performed using either a Qiagen® DNeasy Plant Mini kit or extracted using a CTAB extraction method (Ristaino et al. 2001). DNA was suspended in 30µL elution buffer. DNA was diluted 1:10 or 1:100 and stored at -20°C until use.

DNA amplification and sequencing

A set of five gene regions were chosen based on previous work and the presence of phylogenetically informative sites. The regions chosen included the first intron of the *ras*-related protein, the *ras*-related protein, *elongation factor 1α*, *beta tubulin*, and *cytochrome oxidase I*. Each gene was amplified using specific primers in Table 2.3 (Appendix 2.2). PCR was performed in a 50µL reaction with 1 µL of genomic DNA (1:10 dilution). Each reaction contained 35.25µL ddH20, 5 µL 10X PCR buffer, 2.5 µL dNTPs (2mM each), 2 µL of each forward and reverse primer (10µM), 1.8 µL MgCl2 (50 mM), 0.25 µL BSA (20 mg/mL), and 0.2 µL Taq polymerase (5U/µL). The reaction was performed in a PTC-100™ Peltier Thermal Cycler for an initial denaturation for 2 minutes at 96°C, 35 cycles of denaturation at 96°C for 1 minute, annealing for 1 minute (temperatures varied with specific primers, Table 2.3), extension at 72°C for 2 minutes, and a final extension for 10 minutes at

72°C. Approximately 8 µL of the PCR product was run in a 1.6% agarose gel in 0.5X TBE buffer. The presence of a single band for each sample was checked for successful amplification. The PCR product was cleaned by adding 5 µL ExoSAP-IT to 12.5 µL product, then incubated at 37ºC for 15 minutes, then 80ºC for 15 min to inactivate ExoSAP-IT. The cleaned products were sequenced by GeneWiz (Germantown, MD). Several isolates were also sent to Eton Biosciences (Durham, NC) for sequencing.

Phylogenetic analysis

Sequence data for the five DNA regions (part of *cox1*, *ras*, *intron ras*, *beta tubulin*, *elongation factor*) (Appendix 2.2) were compared with several Genbank accessions. Sequences that were not available through Genbank were generated through PCR from DNA provided by other researchers (Table 2.2). Isolates from *P. infestans* were from both Mexico and Ecuador. Sequences were aligned using BioEdit 7.0.5.3 (Hall 1999) and then converted into a Nexus format using Mesquite (Madison and Madison 2010).

A Bayesian phylogenetic analysis was conducted for each gene in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) using *P. ilicis* sequences as outgroups. DNA sequences from each gene region were concatenated into a single dataset and another phylogenetic analysis was run. The analyses were run for 1,000,000 and 5,000,000 generations respectively, following with a burn-in of 1000 generations. The majority rule consensus tree topologies were identical from the two analyses. The effective sample size for each tree was greater than 2000 for all parameters according to Tracer v1.5 (Rambaut and Drummond

2007). Trees were visualized using TreeView 1.6.6 (Page 1996). Maximum parsimony and neighbor joining methods were also used to construct phylogenetic trees from the data. Maximum parsimony and neighbor joining were conducted with MEGA software version 4 (Tamura et al. 2007). The close neighbor interchange (CNI) with search level 1 was selected for MP analysis and the Kimura 2-K parameter method was used for the NJ tree (Kimura 1980).

RESULTS

DNA was sequenced or obtained from Genbank for 57, 53, 48, and 54 isolates of the nuclear genes *Elongation factor 1α*, *β-tubulin*, *Ras*, and intron 1 of *ras* respectively (Table 2.2). The concatenated nuclear DNA data set included 33 isolates of *Phytophthora* including *P. ilicis* (used as an outgroup) (Table 2.2). The total DNA set for the mitochondrial region P4, corresponding to a portion of the *cox 1* and *atp 9* genes (Appendix 2.2) and included 49 isolates.

Sequences for the species in the Ic clade were over 93% identical for each of the gene sets. The greatest percent difference occurred in the ras gene sequence. *P. mirabilis* differed from the other members by 7-8% (Table 2.4). Percent identity of most of the species with *P. ilicis* varied from 30% to 92% depending on the gene. *P. andina*, *P. infestans*, and *P. mirabilis* contained a series of heterozygous sites in several nuclear genes (Table 2.5). These

sites correspond to the *ras* and intron *ras* heterozygous sites previously reported by Gómez-Alpizar et al. (2008). In many cases, heterozygous sites found in *P. andina* were associated with one nucleotide change from *P. infestans* and one nucleotide from *P. mirabilis*. The majority of these sites are found in the *β tubulin gene* (Table 2.5). These heterozygous sites are treated as uncertainty in the MrBayes program (Huelsenbeck and Ronquist 2001).

Bayesian analysis of the data set with 33 isolates in MrBayes yielded trees with highly similar topologies (Figures 2.1-5). These trees agreed with the topologies that resulted from analyses with all available sequences (Appendices 2.3-7)). These trees were also in consensus with the Bayesian analysis trees and are not shown here. In most cases, each species in the Ic clade formed its own distinct clade with estimated posterior probabilities greater than 0.5. *P. andina* and *P. infestans* clustered together in a single clade for *ras*, *elongation factor 1α*, and *β tubulin*. However, when *P. infestans* and *P. andina* formed distinct clades, isolates of *P. andina* with the Ia mitochondrial haploytpe (EC-3) tended to cluster with *P. infestans* isolates rather than with the isolates of *P. andina* with Ic mt haplotype. This was true for all genes except for the gene tree for the intron 1 of *ras*, where all *P. andina* isolates clustered together. Analysis of the mitochondrial region conferred by the P4 primer resulted in a tree that had similar topology to those of the nuclear genes (Figure 2.5). *P. phaseoli* and *P. ipomoeae* were the most basal members of the Ic clade in all topologies, indicating that they are the most distantly related species in the Ic clade. According to most gene topologies, *P. mirabilis* is most closely related to *P. infestans*

and *P. andina*. In all tree topologies, *P. infestans* and *P. andina* are the most closely related species in the clade.

Concatenated gene trees resulted in topologies where each species formed a distinct clade (Figure 2.6). Estimated posterior probabilities for the concatenated gene trees were greater than those of individual gene trees. When all gene regions were used in the analysis, *P. phaseoli* was the most basal member of the ingroup; this changes when only nuclear genes were analyzed, and *P. mirabilis* became the most basal. In both cases, *P. andina* and *P. infestans* are closely related and cluster together. Using all gene regions yields a tree in which *P. mirabilis* is also closely related to *P. andina* and *P. infestans*, however, *P. mirabilis* is more basal to *P. infestans* and *P. andina* than *P. ipomoeae* (Figure 2.6).

DISCUSSION

Host specificity is thought to be the main barrier to sexual reproduction between the three heterothallic species in the *Phytophthora* Ic clade: *P. infestans*, *P. mirabilis*, and *P. andina* (Goodwin et al. 1999). However, it has been reported that both A1 and A2 mating types of *P. infestans* infect the pear melon (*S. muritcatum*) in Ecuador. At the time of that report, *P. andina* was not formally recognized as a separate species, and was referred to as *P. infestans sensu lato* (Adler et al. 2002). The A2 mating types of *P. infestans* on the pear melon were found to be *P. andina* (Oliva et al. 2002, Oliva et al. 2010). The breakdown of

the reproductive isolation of these species of *Phytophthora* may identify the pear melon as a potential bridging host between heterothallic *Phytophthora*.

There are several molecular similarities between *P. infestans* and *P. mirabilis*. Restriction enzyme digestion of the ITS2 region has failed to differentiate the two because they are identical (Tooley et al. 1996). Restriction enzyme digestion of the two species also yields similar patterns (Goodwin et al. 1992, Moller et al. 1993, Judelson and Randall 1998). Taken together, these results indicate that *P. mirabilis* and *P. infestans*, although distinct species, have speciated relatively recently. This recent speciation may indicate that at one point in time, the two species could have interbred to form *P. andina*. The heterozygous sites in *P. andina* warrant further study through clonal analysis in order to determine whether the two phases are representative of allele from *P. infestans* and *P. mirabilis*.

Heterozygous sites are consistently found in the nuclear genes of *P. andina*, and many of these sites correspond to a nucleotide type found in *P. infestans* and a nucleotide type found in *P. mirabilis*, leading to the speculation that these two members of the clade are the parent species to *P. andina*. This is further supported by examining the indel in the intron 1 of the *ras* gene, where *P. andina* and *P. mirabilis* both have an insertion relative to *P. infestans*. It has been established that *P. mirabilis* and *P. infestans* are separate species and that gene flow between the two is not currently occurring (Goodwin et al. 1999). However, this does not mean that gene flow could not have occurred at some time in the past. Reproductive isolation is a driving force for speciation, and since the two species occur in the

same region, host specificity is the most likely cause for isolation of these species (Galindo and Hohl 1985, Goodwin and Fry 1994).

Despite the inability of these two species to form natural viable hybrids, sexual crosses of the two species have been induced in a laboratory setting (Goodwin and Fry 1994). In 2002, Oliva et al. performed a similar study to that of Goodwin and Fry (1994). However, in their study, the parental *Phytophthora* species were several isolates from the Ecuadorian population of *P. infestans sensu lato* (*P. infestans and P. andina*). They were also able to induce sexual structures from their matings (Oliva et al. 2002), leading to the alternative hypothesis of the *P. infestans*, *P. mirabilis* hybrid theory in which *P. andina* and *P. infestans* may be the parental species and *P. mirabilis* the progeny. Coalescent analysis of the mitochondrial genomes of the Ic *Phytophthora* species suggests that *P. andina* is an older mitochondrial lineage than that of *P. mirabilis* (Lassiter et al. unpublished), furthering the speculation that it may be one of the parental species to *P. mirabilis*.

Phytophthora hybrids have been known to occur naturally. *P. alni* (Brasier et al. 2004), *Phytophthora* × *pelgrandis* (Nirenberg et al. 2009), and crosses between *P. nicotianae* and *P. cactorum* (Hurtado-Gonzales et al. 2009, Man in't Veld et al. 1998) are all naturally occurring hybrids of *Phytophthora*. In several cases, particularly the hybrids of *P. nicotianae* and *P. cactorum*, heterozygous sites, similar to those we found in *P. andina*, are found within the genes sequenced. The heterozygous sites found within *P. andina* correspond to regions where *P. infestans* and *P. mirabilis* are different (Table 2.5), suggesting that these two species are parental to *P. andina*. However, the same cannot be said for the nuclear

sequences. Analysis of the P4 primer region of the *cox I* gene shows that *P. andina* has its own unique mitochondrial sequence. This was also found to be true among the majority of mitochondrial genes. Coalescent analysis of the mitochondrial genomes of the Ic clade indicate that *P. mirabilis* descended from a *P. andina* lineage, not the other way around (Lassiter et al. unpublished). However, the coding regions of mitochondrial genomes of all three species are 99% identical. This may be explained by the uniparental inheritance of the mitochondrial genome from a single parent (Forster and Coffey 1991, Whittaker et al. 1994), with subsequent mutation in the *P. andina* genome.

Phylogenetic trees from this study confirmed previous findings of Blair et al. (2008) and Kroon et al (2004), where *P. andina* is placed as an intermediary between *P. infestans* and *P. mirabilis*. In each case, *P. andina* forms its own distinct clade separate from *P. infestans* and *P. mirabilis*. Sequence data shows that *P. andina* is more similar to *P. mirabilis* and *P. infestans* than any other member of the Ic clade, leading to the proposal of its hybrid origin. However, a high degree of sequence similarity exists among the species of the Ic clade, and a wider range of gene regions may be needed in order to fully resolve the evolutionary relationships of the group. Clonal analysis of *P. andina* isolates may be more useful in fully determining the origin of the species, but it is clear that the nuclear and mitochondrial histories are different in this newest member of the clade.

We analyzed DNA sequences from several nuclear and mitochondrial genes of species in the Ic clade. Our data yielded a gene tree which was consistent with Blair et al. (2008) and indicated that Ia mt haplotypes of *P. andina* clustered with *P. infestans* a majority

of the time, with the exception of the intron 1 of *ras*. This is most likely due to a 3 nucleotide indel in this gene region in all *P. andina* isolates, both Ic and Ia, that is shared with all members of the Ic clade except for *P. infestans* (Figure 2.8). This indel is present in all isolates of *P. andina* examined in this data set, regardless of mitochondrial haplotype, and may be used as a marker to distinguish *P. andina* from *P. infestans.* The ITS regions, generally used for species identification, of both of these species are highly similar and therefore are not useful for species determination (Tooley et al. 1996). *P. mirabilis* also has two distinguishing single base pairs indel in the *ras* gene (Figure 2.9) that is not exhibited by the other members of the clade.

Phylogenetic analysis of the *Phytophthora* Ic clade has shown that the species are closely related, with *P. phaseoli* and *P. ipomoeae* being the most basal. The remaining species, *P. infestans*, *P. mirabilis*, and *P. andina* are closely related, sharing several morphological characteristics. These three species are all heterothallic, meaning that they need a different mating strain to produce sexual oospores. However, the species cannot interbreed in nature due to reproductive isolation based on host specificity and location. Crosses of both *P. infestans* and *P. mirabilis* as well as *P. infestans* and *P. andina* have been shown to produce oospores in the lab (Goodwin and Fry 1994, Oliva et al. 2002). Heterozygous sites consistently found within the nuclear genes of *P. andina* point to the hypothesis of *P. infestans* and *P. mirabilis* being parental species to a *P. andina* hybrid, but to date, there is no known host that these two species can both infect, thus preventing any type of mating between the species. Alternatively, *P. infestans* and *P. andina* have both been

found on the pear melon, creating a potential bridging host for these two species to interbreed on. Coalescent analysis of the mitochondrial genomes also shows *P. mirabilis* diverging from a *P. andina* lineage (Lassiter et al. unpublished), giving more evidence to the *P. infestans*, *P. andina* parental hypothesis. In order to further substantiate either hypothesis, further surveys of Ic *Phytopthora* species must be conducted to find potential bridging hosts in the wild as well as more molecular studies to determine if the heterozygous sites found in *P. andina* confer a hybrid.

ACKNOWLEDGMENTS

Some isolates of *P. phaseoli*, *P. ipomoeae*, and *P. mirabilis* were provided by P. Bonants at Stichting Dienst Landbouwkundig Onderzoek (DLO). Sequencing of PCR products were performed by GeneWiz or Eton Biosciences.

LITERATURE CITED

Adler NE, Chacón G, Flier WG, Forbes GA. 2002. The Andean fruit crop, pear melon (*Solanum muricatum*) is a common host for A1 and A2 strains of *Phytophthora infestans* in Ecuador. Plant Pathology 51: 802.

Adler NE, Erselius LJ, Chacon MG, Flier WG, Ordonez ME, Kroon LPNM, Forbes GA. 2004. Genetic diversity of *Phytophthora infestans* sensu lato in Ecuador provides new insight into the origin of this important plant pathogen. Phytopathology 94: 154-162.

Avila-Adame C, Gómez-Alpizar L, Zismann V, Jones KM, Buell CR, Ristaino JB. 2006. Mitochondrial genome sequences and molecular evolution of the Irish potato famine pathogen, *Phytophthora infestans*. Curr Genet 49: 39-46.

Blair JE, Coffey MD, Park S-Y, Geiser DM, Kang S. 2008. A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. Fungal Genet. Biol. 45: 266-277.

Brasier CM and Hansen EM. 1992. Evolutionary biology of *Phytophthora*. Part II: phylogeny, speciation, and population structure. Ann Rev Phytopathol 30: 173-200.

Brasier CM, Kirk SA, Delcan J, Cooke DEL, Jung T, Man in't Veld. 2004. *Phytophthora alni* sp. nov. and its variants: Designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycol Res 108: 1172-1184.

Bridge PD, Newsham KK, Denton GJ. 2008. Snow mould cause by a *Pythium* sp.: a potential vascular plant pathogen in the maritime Antarctic. Plant Pathol 57: 1066-1072.

Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM. 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Genet. Biol. 30: 17-32.

Erwin DC, Ribeiro OK. 1996. *Phytophthora* Diseases Worldwide. APS Press, St. Paul, MN.

Felsenstein, J. 2004. Inferring Phylogenies. Sinauer Associates, Inc: Sunderland, MA.

Flier WG, Grünwald NJ, Kroon LPNM, Sturbaum AK, van den Bosch TBM, Garay-Serrano E, Lozoya-Saldana H, Fry WE, Turkensteen LJ. 2003. The population structure of *Phytophthora infestans* from the Toluca Valley of Central Mexico suggests genetic differentiation between populations of cultivated potato and wild *Solanum* spp. Phytopathology. 93: 382-390.

Flier WG, Grünwald NJ, Kroon LPNM, van den Bosch TBM, Garay-Serrano E, Lozoya-Saldana, Bonants PJM, Turkensteen LJ. 2002. *Phytophthora ipomoeae* sp. nov., a new homothallic species causing leaf blight on *Ipomoea longipedunculata* in the Toluca Valley of central Mexico. Mycol Res 106: 848-856.

Forbes GA, Escobar XC, Ayala CC, Revelo J, Ordonez ME, Fry BA, Doucett K, Fry WE. 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. Phytopathology 87: 375-380.

Forster H, Coffey MD. 1991. Approached to the taxonomy of *Phytophthora* using polymorphisms in mitochondrial and nuclear DNA. In Lucas JA, Shattock RC, Shaw DS, Cooke LR (eds.), *Phytophthora* (pp. 164-183). Cambridge: Cambridge University Press.

Galindo J, Hohl HR. 1985. *Phytophthora mirabilis*, a new species of Phytophthora. Sydowia 38: 87-96.

Gómez-Alpizar L, Carbone I, Ristaino JB. 2007. An Andean origin of *Phytophthora infestans* inferred from mitochondrial and nuclear gene geneologies. Proc. Nat'l Acad. Sci 104: 3306-3311.

Gómez-Alpizar L, Hu CH, Oliva R, Forbes G, Ristaino JB. 2008. Phylogenetic relationships of *Phytophthora andina*, a new species from the highlands of Ecuador that is closely related to the Irish potato famine pathogen *P. infestans*. Mycologia 100(4): 590-602.

Goodwin SB, Drenth A, Fry WE. 1992. Cloning and genetic analysis of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. Curr Genet 22: 107-115.

Goodwin SB, Fry WE. 1994. Genetic analyses of interspecific hybrids between *Phytophthora infestans* and *Phytophthora mirabilis*. Exp Mycol 18: 20-32.

Goodwin SB, Spielman LJ, Matuszak JM, Bergeron SN, Fry WE. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopath 84: 1224-1227.

Goodwin SB, Legard DE, Smart CD, Levy M, Fry WE. 1999. Gene flow analysis of molecular markers confirms that *Phytophthora mirabilis* and *P. infestans* are separate species. Mycologia 91: 796-810.

Griffith GW, Shaw DS. 1998. Polymorphisms in Phytophthora infestans: four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. Appl Envir Micro 64: 4007-4014.

Grünwald NJ, Flier WG. 2005. The biology of *Phytophthora infestans* at its center of origin. Annu Rev Phytopahtol 43: 171-190.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41: 95-98.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754-755.

Hughes KA, Lawley B, Newsham KK. 2003. Solar UV-B radiation inhibits the growth of Antarctic terrestrial fungi. Appl Environ Microbiol 69: 1488-1491.

Hurtado-Gonzales OP, Aragon-Caballero LM, Flores-Torres JG, Man in't Veld WA, Lamour KH. 2009. Molecular comparison of natural hybrids of *Phytophthora nicotianae* and *P. cactorum* infecting loquat trees in Peru and Taiwan. Mycologia 101: 496-502.

Judelson HS, Randall TA. 1998. Families of repeated DNA in the oomycete *Phytophthora infestans* and their distribution within the genus. Genome 41: 605-615.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111-120.

Kroon LPNM, Bakker FT, van den Bosch GBM, Bonants PJM, Flier WG. 2004. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. Fungal Genet. Biol. 41: 766-782.

Maddison WP, Maddison DR. 2010. Mesquite: a modular system for evolutionary analysis. Version 2.73 http://mesquite project.org

Man in't Veld WA, Veenbaas-Rijks WJ, Ilieva E, de Cock AWAM, Bonants PJM, Pieters R. 1998. Natural hybrids of *Phytophthora nicotianae* and *Phytophthora cactorum* demonstrated by isozyme analysis and random amplified polymorphic DNA. Phytopathology 88: 922-929.

Margulis L, Schwartz KV. 2000. Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth. W.H. Freeman and Co., New York.

Martin FN and Tooley PW. 2003. Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. Mycologia 95: 269-284.

Mirzaee, Abassi M, Mohammadi M. 2009. *Albugo candida* causing white rust on *Erysimum crassicaule* in Iran. Australa Plant Dis Notes 4: 124-125.

Moller EM, de Cock AWAM, Prell HH. 1993. Mitochondrial and nuclear DNA restriction enzyme analysis of the closely related *Phytophthora* species, *P. infestans*, *P. mirabilis*, and *P. phaseoli*. J Phytopathol 139: 309-321.

Moskin J. 2009. Outbreak of fungus threatens tomato crop. New York Times July 17, 2009, morning ed.: A16.

Niederhauser JS. 1991. *Phytophthora infestans*: the Mexican connection. In Lucas JA, Shattock RC, Shaw DS, Cooke LR (eds) *Phytophthora*: an international symposium, Cambridge University Press, Cambridge, UK, pp 25-45.

Nirenberg HI, Gerlach WF, Grafenhan T. 2009. *Phytophthora ×pelgrandis*, a new natural hybrid pathogenic to *Pelargonium grandiflorum* hort. Mycologia 101: 220-231.

Oliva RF, Erselius LJ, Adler N E, Forbes GA. 2002. Potential of sexual reproduction among host-adapted populations of *Phytophthora infestans sensu lato* in Ecuador. Plant Pathol. 51:710-719.

Oliva RF, Kroon LPNM, Chacón G, Flier WG, Ristaino JB, Forbes GA. 2010. *Phytophthora andina* sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands. Plant Pathology 59(4): 613-625.

Ordonez ME, Hohl HR, Velasco JA, Ramon MP, Oyarzun PJ, Smart CD, Fry WE, Forbes GA, Erselius LJ. 2000. A novel population of *Phytophthora*, similar to *P. infestans*, attacks wild solanum species in Ecuador. Ecology and Population Biology 90 (2): 197-202.

Page RDM. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357:358.

Rambaut A, Drummond AJ. 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer

Ristaino JB, Groves CT, Parra GR. 2001. PCR amplification of the Irish potato famine pathogen from historic specimens. Nature 411: 695-697.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: molecular evolutionary genetics análisis (MEGA) software version 4.0. Mol Biol Evol 24: 1596-1599.

Thines M, Kamoun S. 2010. Oomycete-plant coevolution: recent advances and future prospects. Curr Opinion in Plant Biology 13: 1-7.

Tooley PW, Carras MM, Falenstein KF. 1996. Relationships among group IV Phytophthora species inferred by restriction enzyme analysis of the ITS2 region. J Phytopathol 144: 363-369.

Vargas AM et al. 2009. Characterization of *Phytophthora infestans* populations in Columbia: first report of the A2 mating type. Phytopathology 99: 82-88.

Waterhouse GM. 1963. Key to the species of *Phytophthora* de Bary. Mycol. Papers No. 92.

Whittaker SL, Assinder SJ, Shaw DS. 1994. Inheritance of mitochondrial DNA in *Phytophthora infestans*. Mycol Res 98: 569-575.

Table 2.1. Species of *Phytophthora* in Ic clade and their respective host plants.

Pathogen	Host
P. infestans	tomato (*Lycopersicon esculentum*), potato (*Solanum* spp.), *Solanum muricatum*, morning glory
P. andina	*Brugmansia sanguinea, Solanum* species belonging to the Anarrhichomenum complex, *Solanum betaceum, Solanum muricatum*, *Solanum tetrapetalum*, *Solanum brevifolium*
P. phaseoli	lima bean (*Phaseolus lunatus*), other common beans, tomato
P. ipomoeae	morning glory (*Ipomoea purpurea*), *Ipomoea longipedunculata*
P. mirabilis	4 o'clock (*Mirabilis jalapa*)

Table 2.2. Isolates of *Phytophthora* used in this study. Genbank accessions are included.

Species	Isolate	mt haplotype[a]	RG-57 lineage[b]	Mating type	Allozyme Genotype[c]		Country of origin	Source[d]
					Pep	*Gpi*		
P. andina	EC3163	Ic	EC-2	A2	76/100	100/100	Ecuador	G. Forbes
	EC3164	Ic	EC-2	A2	[e]		Ecuador	G. Forbes
	EC3165	Ic	EC-2	A2			Ecuador	G. Forbes
	EC3167	Ic	EC-2	A2			Ecuador	G. Forbes
	EC3189	Ic	EC-2	A2	76/100	100/100	Ecuador	G. Forbes
	EC3190	Ic	EC-2	A2	76/100	100/100	Ecuador	G. Forbes
	EC3417	Ic	EC-2	A2	76/100	100/100	Ecuador	W. Flier
	EC3421	Ic	EC-2	A2			Ecuador	W. Flier
	EC3425	Ic	EC-2	A2			Ecuador	W. Flier
	P13365						Ecuador	N. Adler
	P13400						Ecuador	N. Adler
	EC3237	Ia	EC-3	A1			Ecuador	G. Forbes
	EC3238	Ia	EC-3	A1			Ecuador	G. Forbes
	EC3394	Ia	EC-3	A1	76/100	86/100	Ecuador	G. Forbes
	EC3414	Ia	EC-2	A1	76/100	100/100	Ecuador	W. Flier
P. infestans	EC3099	IIa	EC-1	A1			Ecuador	W. Flier
	EC3300	IIa	EC-1	A1			Ecuador	G. Forbes
	EC3336	IIa	EC-1	A1			Ecuador	G. Forbes
	EC3301						Ecuador	G. Forbes
	PCZ033	IIa	EC-1.1	A1	96/100	90/100	Peru	G. Forbes
	PCZ098	IIa	EC-1.2	A1	96/100	90/100	Peru	G. Forbes
	PHU006	IIa	EC-1	A1	96/100	90/100	Peru	G. Forbes
	POX004	IIa	EC-1	A1	96/100	90/100	Peru	G. Forbes
	PCO038	IIa	EC-1	A1	96/100	90/100	Peru	G. Forbes
	PCZ118	IIa	EC-1.2	A1	96/100	90/100	Peru	G. Forbes
	Pic 97180	Ia	ND	A1			Mexico	N. Grunwald

Table 2.2 cont.

Species	Isolate	mt haplotype	RG-57 lineage	Mating type	Allozyme Genotype		Country of origin	Source
					Pep	*Gpi*		
	Pic 97207	Ia	ND	A1			Mexico	N. Grunwald
	Pic 97224	Ia	ND	A2			Mexico	N. Grunwald
	Pic 97322	Ia	ND	A1			Mexico	N. Grunwald
	Pic97370	Ia	ND	A2			Mexico	N. Grunwald
	Pic 97388	Ia	ND	A2			Mexico	N. Grunwald
	Pic 97605	Ia	ND	A1			Mexico	N. Grunwald
	Pic 97620	Ia	ND	A1			Mexico	N. Grunwald
	Pic 97630	Ia	ND	A2			Mexico	N. Grunwald
	Pic 97652	Ia	ND	A2			Mexico	N. Grunwald
	Pic 98301	Ia	ND	A2			Mexico	N. Grunwald
	Pic 98305	Ia	ND	A1			Mexico	N. Grunwald
	Pic 98366	Ia	ND	A1			Mexico	N. Grunwald
	Pic 98369	Ia	ND	A1			Mexico	N. Grunwald
	Pic 98372	Ia	ND	A2			Mexico	N. Grunwald
	Pic 98388	Ia	ND	A1			Mexico	N. Grunwald
	Pic 98392	Ia	ND	A2			Mexico	N. Grunwald
	P10650						Mexico	M. Coffey
P. mirabilis	Pic 99114						Mexico	W. Flier
	G9-5						Mexico	W. Fry
	G4-4						Mexico	W. Fry
	P3007						Mexico	M. Coffey
	P3005						Mexico	M. Coffey
	P3001						Mexico	P. Bonants
	Pic 99145						Mexico	P. Bonants
	G15-4						Mexico	P. Bonants
	Pic 99129						Mexico	P. Bonants

Table 2.2 cont.

Species	Isolate	mt haplotype	RG-57 lineage	Mating type	Allozyme Genotype		Country of origin	Source
					Pep	*Gpi*		
P. ipomoeae	Pic 99167						Mexico	W. Flier
	Pic 99139						Mexico	P. Bonants
	P10227						Mexico	M. Coffey
	Pic 99165						Mexico	L. Kroon
	P10226						Mexico	M. Coffey
P. phaseoli	Phy P18						US (De)	T. Evans
	P22							T. Evans
	CBS 556.88	HaPphaI	ND	SF	ND	ND		P. Bonants
	P10150						US (De)	M. Coffey
	P6609						US (Md)	M. Coffey
	P10145						US (De)	M. Coffey
	P10146						US (De)	M. Coffey
P. ilicis	384							W. Flier
	PD 91/595							L. Kroon
	P6860							M. Coffey
	SCRP379							D. Cooke

[a]Mitochondrial haplotype determined by the methods of Griffith and Shaw (1998).
[b]Previously published RFLP fingerprints (Forbes et al. 1997, Ordonez et al. 2000, Adler et al. 2004).
[c]Allozyme alleles scored at the putative Peptidase and Glucose-6-phosphate isomerase loci.
[d]Indicates the original curator of the isolate.
[e]Isolate was not genotyped using allozyme genotyping.

Table 2.3. PCR[a] primers used to amplify nuclear and mitochondrial genes.

Gene	Name	Primer Sequence	location[b]	Tm[c]
Ras	**RASF**	CGTGTCTGCTTCTCCGTTTCG	916	54
Ras	**RASR**	CCAGGCTTTCGGCAAATTCC	1515	54
β Tubulin	**TUBUF2**	CGGTAACAACTGGGCCAAGG	194	60
β Tubulin	**TUBUR1**	CCTGGTACTGCTGGTACTCAG	790	60
β Tubulin	**TUBUF1**	GCCAAGTTCTGGGAGGTCATC	420	60
β Tubulin	**TUBUR2**	GATCCACTCAACGAAGTACG	939	60
Elongation factor 1α	**ELONGF1**	TCACGATCGACATTGCCCTG	212	60
Elongation factor 1α	**ELONGR1**	ACGGCTCGAGGATGACCATG	1183	60
CoxI, atp9	**P4F[d]**	TGGTCATCCAGAGGTTTATGTT	741	53
CoxI, atp9	**P4R**	CCGATACCGATACCAGCACCAA	1704	53
Intron 1 of *ras*	**IRF**	TTGCAGCACAACCCAAGACG	442	54
Intron 1 of *ras*	**IRR**	TGCACGTACTATTCGGGGTTC	789	54

[a]Cycling parameters are the same for each primer and are described in the materials and methods
[b]Location is relative to the length of the gene from *P. infestans*
[c]Tm is changed during each PCR reaction in order to amply the specific DNA region
[d]P4 primer is designed to amplify the 3' region of the *coxI* gene and the 5' end of *atp9*

Table 2.4. Percent identity among species of *Phytophthora* in the Ic clade according to each gene used.

Elongation Factor 1α					
	Species				
	P. infestans	*P. andina*	*P. mirabilis*	*P. ipomoeae*	*P. phaseoli*
P. infestans	99.6	97.8	98.1	97.2	97.5
P. andina		98.2	96.6	96.8	96.6
P. mirabilis			99.9	96.8	97
P. ipomoeae				99.2	96.6
P. phaseoli					99.9

Beta Tubulin					
	Species				
	P. infestans	*P. andina*	*P. mirabilis*	*P. ipomoeae*	*P. phaseoli*
P. infestans	100	98.7	99.6	99.6	98.7
P. andina		100	98.9	98.7	98.3
P. mirabilis			100	99.6	99.2
P. ipomoeae				100	98.7
P. phaseoli					100

RAS					
	Species				
	P. infestans	*P. andina*	*P. mirabilis*	*P. ipomoeae*	*P. phaseoli*
P. infestans	100	98.2	93.4	97.9	96.3
P. andina		100	93.4	96.9	95.1
P. mirabilis			99.6	93.6	92
P. ipomoeae					96.7
P. phaseoli					100

Intron 1 of *RAS*					
	Species				
	P. infestans	*P. andina*	*P. mirabilis*	*P. ipomoeae*	*P. phaseoli*
P. infestans	100	94.7	95.2	94.7	95.2
P. andina		100	94.7	95.2	94.7
P. mirabilis			99.1	97.8	97.8
P. ipomoeae				100	97.4
P. phaseoli					100

CoxI* and *atp9					
	Species				
	P. infestans	*P. andina*	*P. mirabilis*	*P. ipomoeae*	*P. phaseoli*
P. infestans	100	99.2	99.2	98.6	98.6
P. andina		100	98.2	99	98.6
P. mirabilis			99.8	98.6	98.1
P. ipomoeae				100	98
P. phaseoli					99.6

Table 2.5. Nuclear gene heterozygous sites found in species of *Phytophthora* from the Ic clade.

beta tubulin	location[a]										
Species	110	237	306	363	432	546	655	720	774	846	849
P. infestans	T	T/C	T/C	T/C	A		C	T	G	A	T
P. andina	T/C	T/C	T/C	T/C	G/A	T/C	T/C	T/C	G/A	A/C	G/T
P. mirabilis	C	T/C	C	C	G	C	C	T	G	C	T
P. ipomoeae	T	T	C	C	G	C	C	T	G	A	T
P. phaseoli	T	T	C	C	G	C	C	T	G	C	G

Elongation factor 1 α	location									
Species	88	233	244	322	364	370	433	478	625	805
P. infestans	G	G/A	T/C	G	T/C	C	C	G/T	T/C	T/C
P. andina	G/A	G/A	T/C	G/A	T/C	T/C	T/C	G/T	T/C	T/C
P. mirabilis	G	G/A	T/C	G	T/C	T/C	C	G/T	C	T/C
P. ipomoeae	G	A	T/C	G	C	C	C	G	C	C
P. phaseoli	G	A	C	G	C	C	C	T	T/C	C

ras	location								
Species	67	74	112	134	135	363	423	424	433
P. infestans	A	A	C	T	T	T	C	C	A
P. andina	G/A	G/A	G/C	T/C	T/C	T/C	T/C	T/C	A/T
P. mirabilis	G	G	G	C	C	C	T	T	T
P. ipomoeae	A	A	G	C	C	C	C	C	A
P. phaseoli	A	A	G	C	C	C	C	C	A

intron ras	location								
Species	80	150	154	159	164	171	242	243	270
P. infestans	G	C	A	A	A	A	gap	gap	G
P. andina	G/A	A/C	G/A	A/C	A/C	G/A	T/C	T/C	G/A
P. mirabilis	G	A	G	C	C	G	T	C	G
P. ipomoeae	G	A	G	C	C	G	T	C	G
P. phaseoli	G	A	G	C	C	C	T	C	G

[a]Location is relative to where the genes were trimmed for this study, they are not based on the Genbank accession locations.

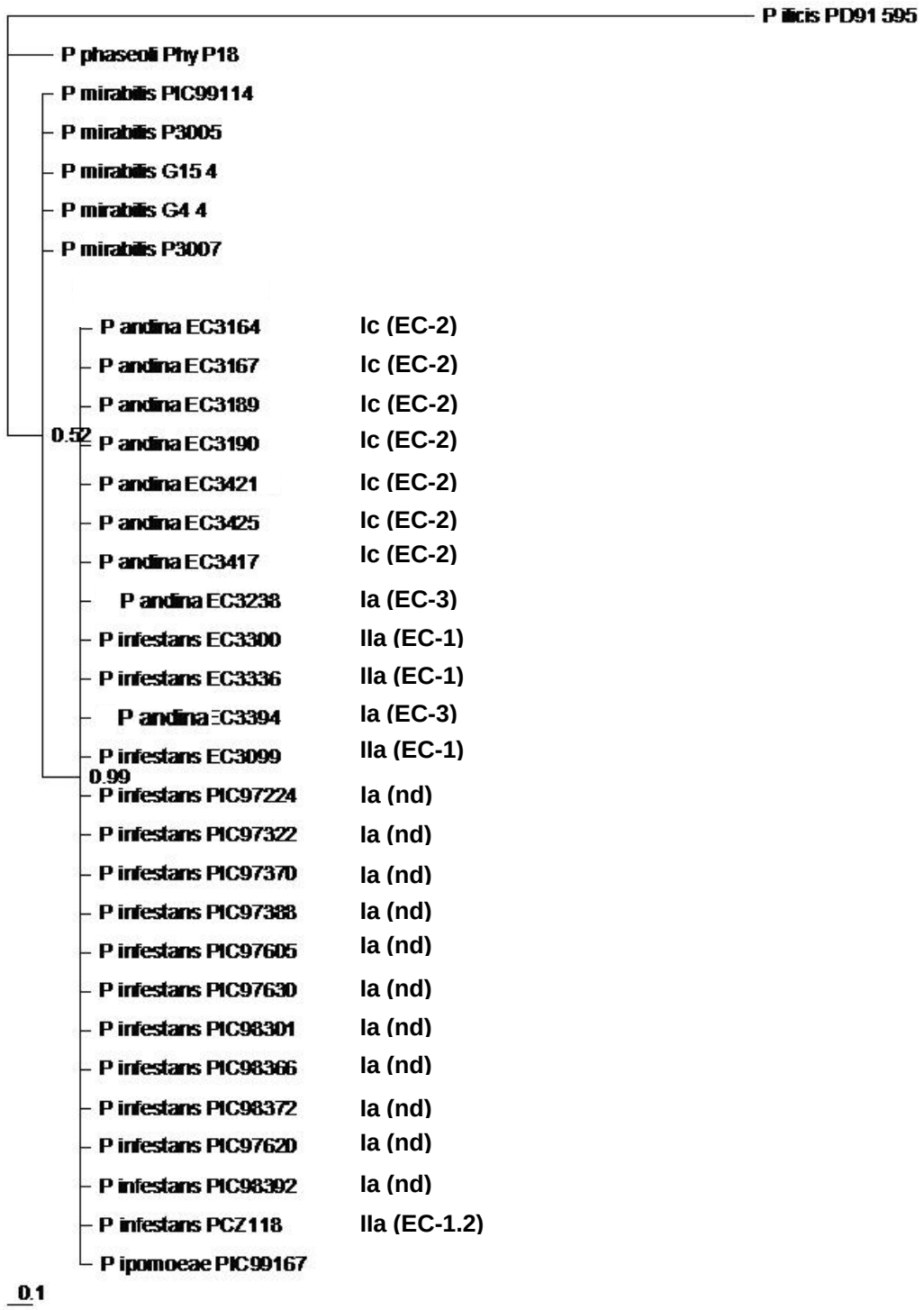


Figure 2.1. Phylogenetic tree of species in the *Phytophthora* Ic clade using *elongation factor 1α*.

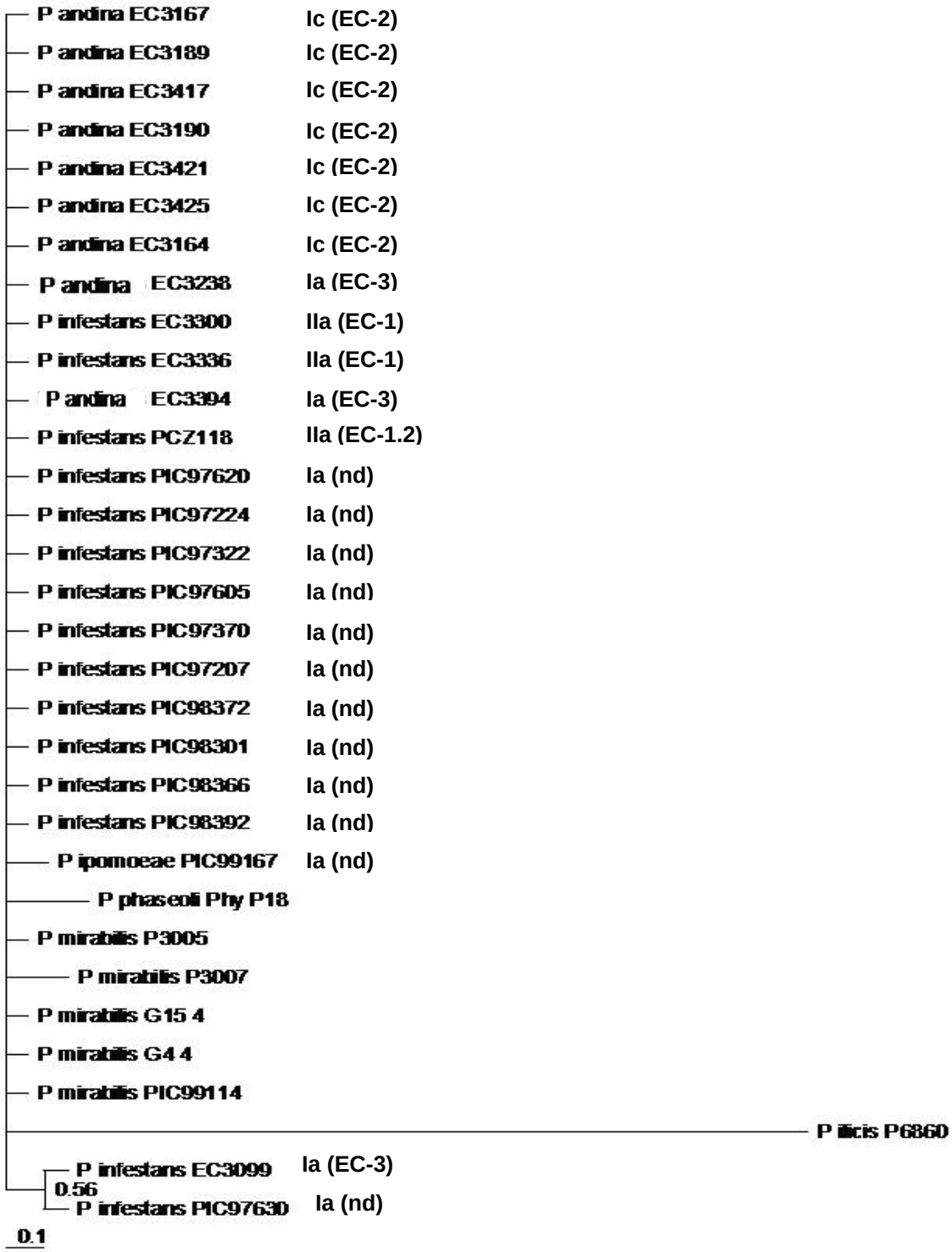


Figure 2.2. Phylogenetic tree of species in the *Phytophthora* Ic clade using *β tubulin*.

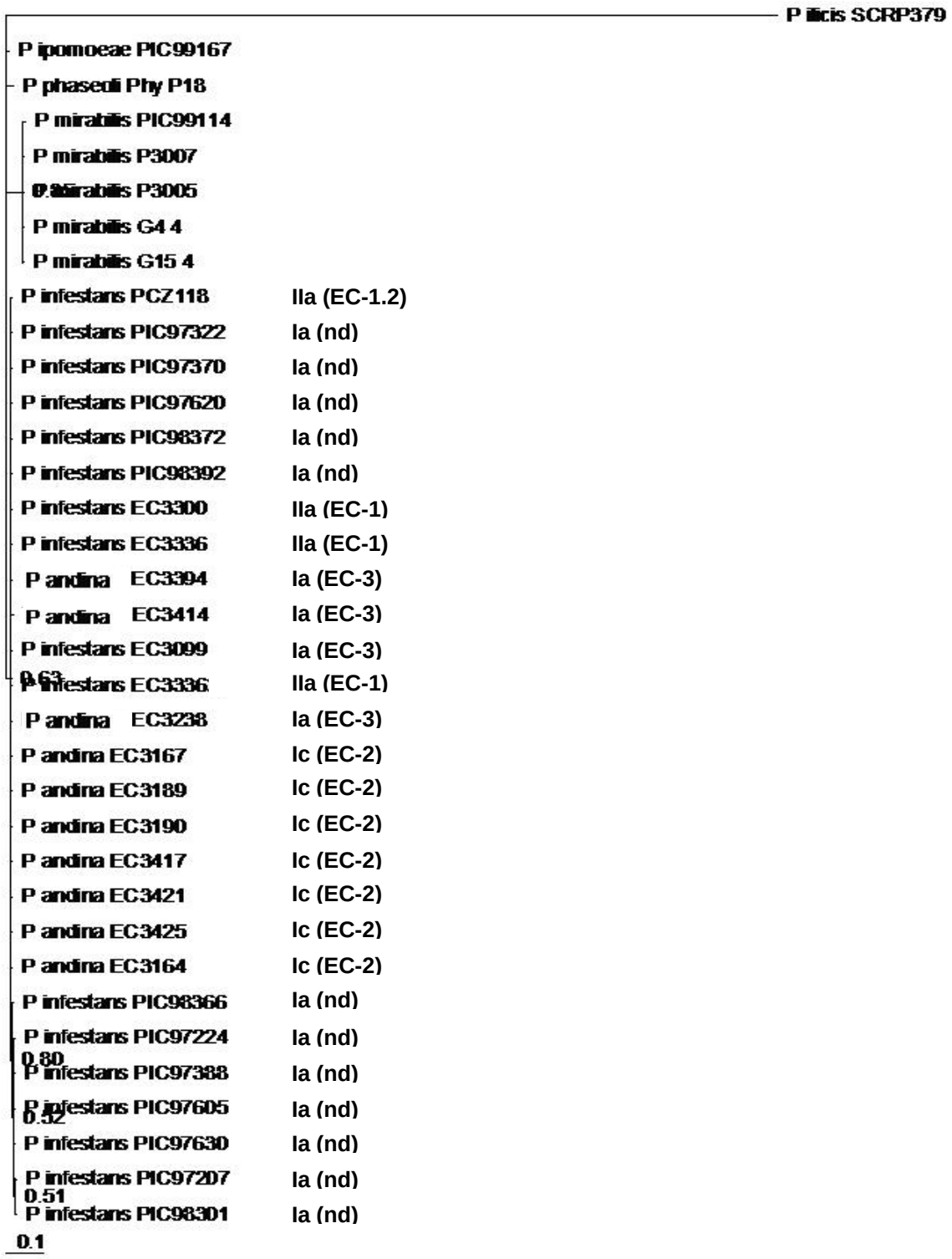


Figure 2.3. Phylogenetic tree of species in the *Phytophthora* Ic clade using *ras*.

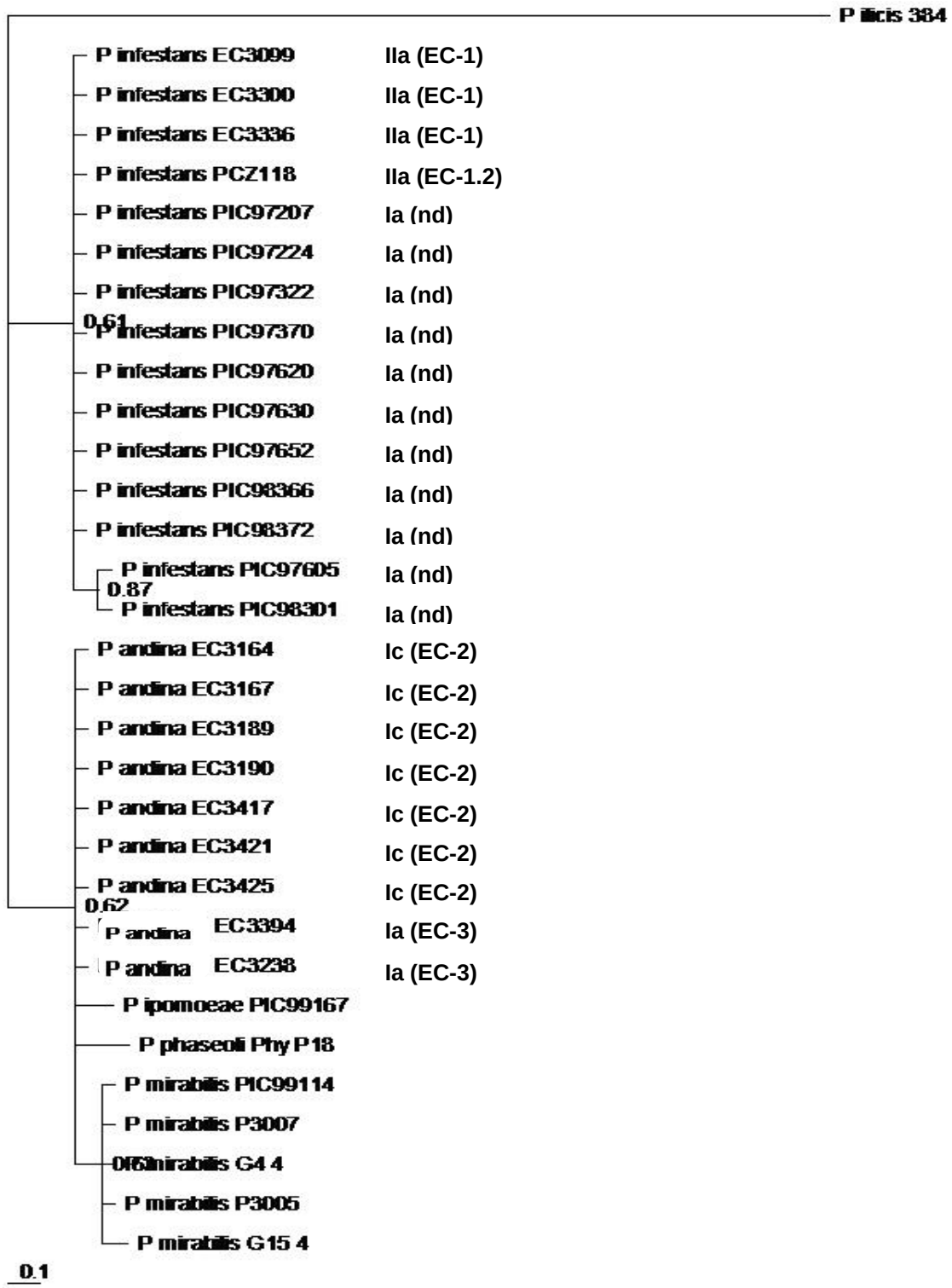


Figure 2.4. Phylogenetic tree of species in the *Phytophthora* Ic clade using intron 1 of *ras*.

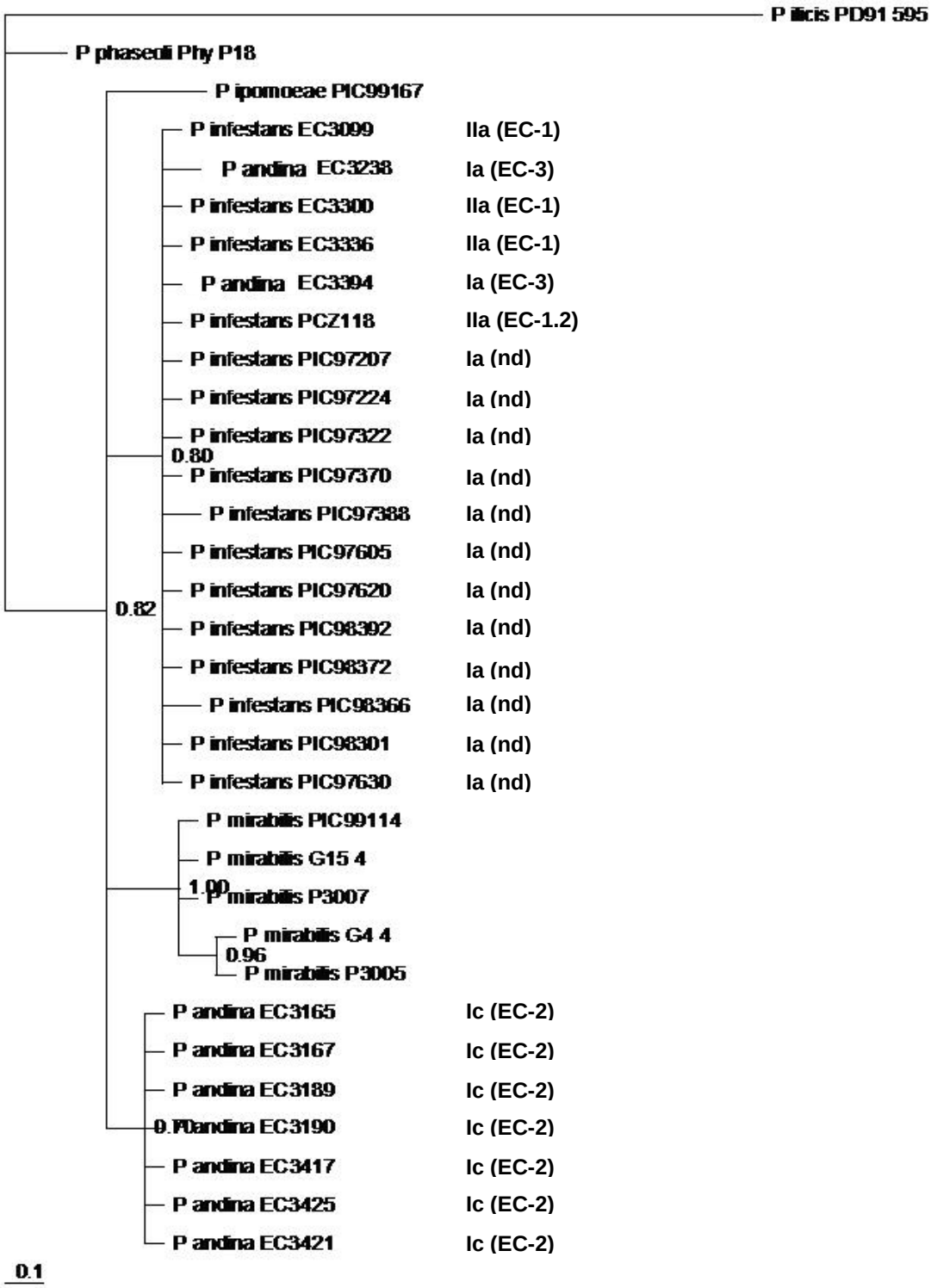


Figure 2.5. Phylogenetic tree of species in the *Phytophthora* Ic clade using P4 (*Cox 1* and *atp9*)

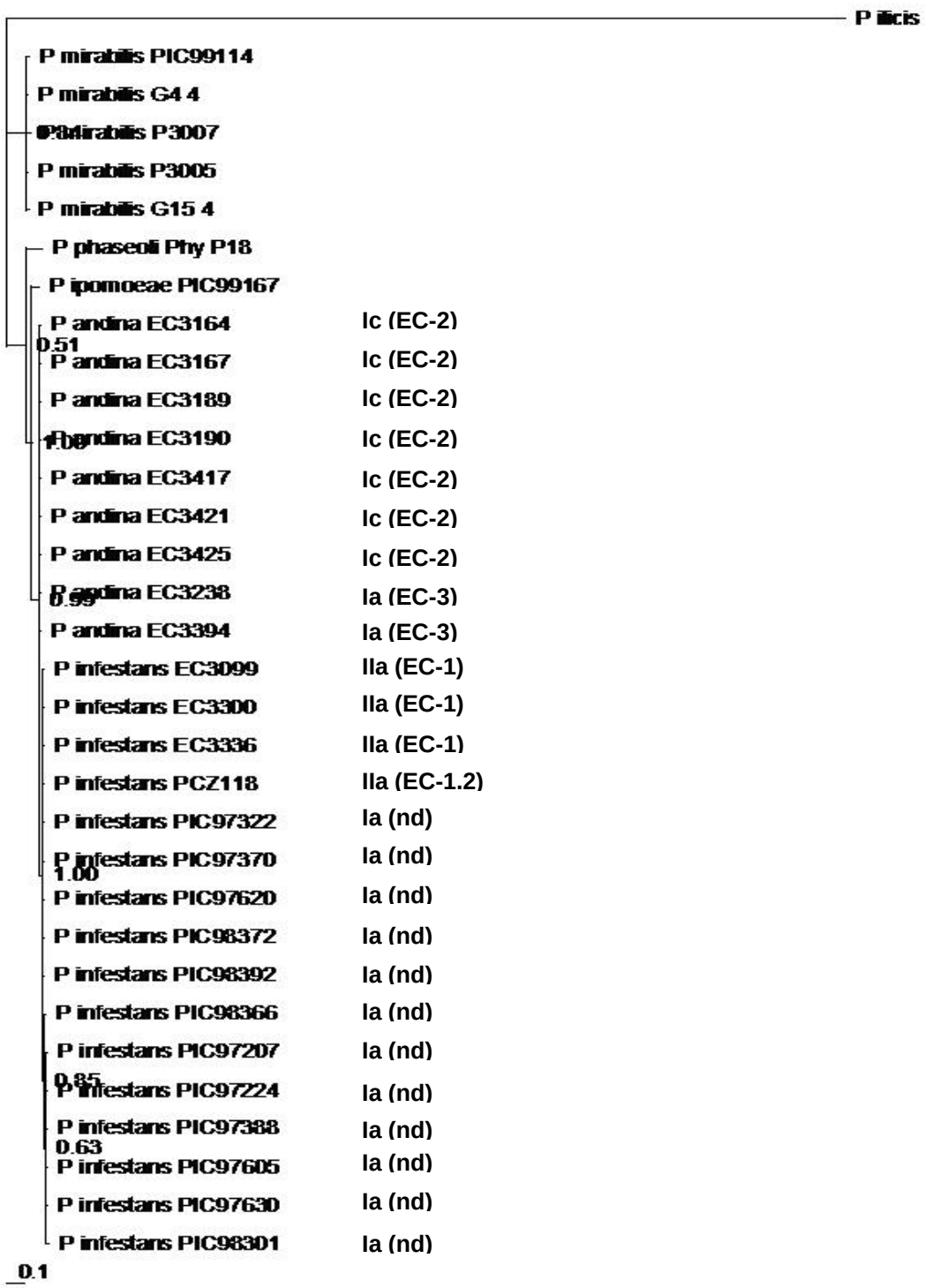


Figure 2.6. Phylogenetic tree of species in the *Phytophthora* Ic clade using concatenated nuclear genes

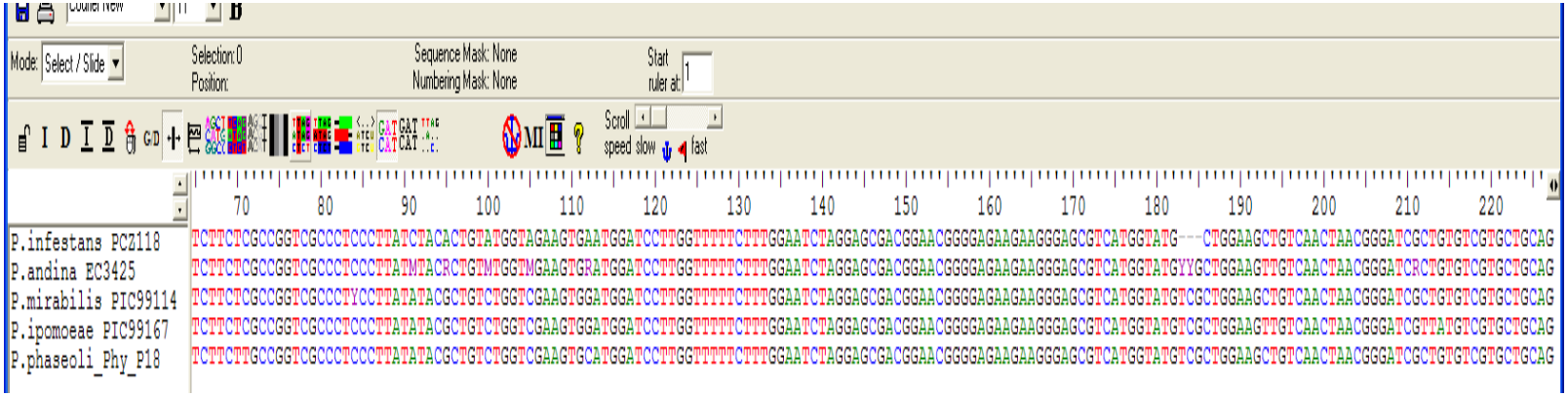


Figure 2.7. Sequences of the intron I of *ras*. *P. infestans* is missing 3 nucleotides that are present in all other Ic clade species.

```
P.andina EC3190       AAATTGTACGCCGCTAAAAAAACATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAACAGTGGG
P.andina EC3417       AAATTGTACGCCGCTAAAAAAACATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAACAGTGGG
P.andina EC3421       AAATTGTACGCCGCTAAAAAAACATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAACAGTGGG
P.andina EC3425       AAATTGTACGCCGCTAAAAAAACATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAACAGTGGG
P.andina EC3164       AAATTGTACGCCGCTAAAAAAACATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAACAGTGGG
P.mirabilis PIC99114  AAATTGTACGTCGCTAAAAAA-CATTTGTCCCCGCGTG-TTCCTATTTAATTAACGGTACTCTTTTTTTCTACAGTGGG
P.mirabilis P3007     AAATTGTACGTCGCTAAAAAA-CATTTGTCCCTGCGTG-TTCCTATTTAACTAACGGTACTCTTTTTTTCTACAGTGGG
P.mirabilis P3005     AAATTGTACGTCGCTAAAAAA-CATTTGTCCCTGCGTG-TTCCTATTTAACTAACGGTACTCTTTTTTTCTACAGTGGG
P.mirabilis G4-4      AAATTGTACGTCGCTAAAAAA-CATTTGTCCCTGCGTG-TTCCTATTTAACTAACGGTACTCTTTTTTTCTACAGTGGG
P.mirabilis G15-4     AAATTGTACGTCGCTAAAAAA-CATTTGTCCCTGCGTG-TTCCTATTTAACTAACGGTACTCTTTTTTTCTACAGTGGG
P.infestans EC3414    AAATTGTACGCCGCTAAAAAAACATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAACAGTGGG
P.infestans EC3099    AAATTGTACGCCGCTAAAAAAACATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAACAGTGGG
P.infestans EC3336    AAATTGTACGCCGCTAAAAAAACATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAACAGTGGG
P.infestans_EC3238    AAATTGTACGCCGCTAAAAAAACATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAACAGTGGG
```

Figure 2.8. Sequences from the *ras* gene. Isolates of *P. mirabilis* have two indels within the gene region which are unique to that species among the Ic clade *Phytophthoras*.

APPENDICES

Appendix 1.1 Annotation of mitochondrial genes of the Ic clade species of *Phytophthora*

	Species					
	P. infestans	*P. phaseoli*	*P. andina*	*P. ipomoeae*	*P. mirabilis*	Location
Start	26	26	26	26	24	rnl
End	2679	2679	2679	2679	2677	rnl
Start	2686	2686	2686	2686	2684	trnN
End	2757	2757	2757	2757	2755	trnN
Start	2770	2770	2770	2770	2768	trnS (gcu)
End	2858	2858	2858	2858	2856	trnS (gcu)
Start	2879	2879	2879	2879	2877	trnM (cau)
End	2950	2950	2950	2950	2948	trnM (cau)
Start	2994	2994	2994	2994	2992	trnP
End	3068	3068	3068	3068	3066	trnP
Start	3082	3082	3082	3082	3080	trnM
End	3153	3153	3153	3153	3151	trnM
Start	3171	3171	3171	3171	3169	rpl14
End	3542	3542	3542	3542	3540	rpl14
Start	3549	3549	3549	3549	4547	rpl5
End	4082	4082	4082	4082	4080	rpl5
Start	4090	4090	4090	4090	1088	trnG (gcc)
End	4161	4616	4616	4616	4159	trnG (gcc)
Start	4253	4253	4253	4253	4251	trnG (ucc)
End	4324	4324	4324	4324	4322	trnG (ucc)
Start	4330	4330	4330	4330	4328	trnY
End	4413	4413	4413	4413	4411	trnY
Start	5199	5210	5194	5187	5209	rns
End	6701	6712	6696	6689	6711	rns
Start	6736	6747	6731	6724	6746	trnW
End	6807	6818	6802	6795	6817	trnW
Start	7002	7013	6983	6988	7000	orf79
End	7241	7252	7210	7227	7239	orf79

Appendix 1.1 cont.

	Species					
	P. infestans	*P. phaseoli*	*P. andina*	*P. ipomoeae*	*P. mirabilis*	Location
Start	7661	7651	7617	7631	7596	cox2
End	8437	8427	8393	8407	8372	cox2
Start	8453	8443	8409	8423	8388	orf32
End	8551	8541	8507	8521	8486	orf32
Start	8639	8624	8591	8605	8572	cox1
End	10117	10102	10069	10083	10050	cox1
Start	10266	10248	10215	10230	10198	atp9
End	10493	10475	10442	10457	10425	atp9
Start	10655	10633	10601	10615	10586	nad9
End	11221	11199	11167	11181	11152	nad9
Start	11282	11260	11228	11242	11213	cob
End	12433	12411	12379	12393	12364	cob
Start	12572	12542	12506	12528	12496	atp1
End	14101	14071	14035	14057	14025	atp1
Start	14182	14152	14166	14137	14106	trnE
End	14253	14223	14187	14208	14177	trnE
Start	14283	14253	14217	14238	14207	nad4
End	15758	15728	15692	15713	15682	nad4
Start	15781	15751	15715	15736	15705	trnH
End	15853	15823	15787	15808	15777	trnH
Start	15857	15827	15791	15812	15781	orf142
End	16285	16255	16219	16240	16209	orf142
Start	16295	16265	16229	16250	16219	nad7
End	17473	17443	17407	17428	17397	nad7
Start	17591	17559	17523	17544	17515	nad2
End	19084	19052	19016	19037	19008	nad2
Start	19091	19059	19023	19044	19015	trnF
End	19164	19132	19096	19117	19088	trnF

Appendix 1.1 cont.

	Species					
	P. infestans	*P. phaseoli*	*P. andina*	*P. ipomoeae*	*P. mirabilis*	Location
Start	19183	19151	19115	19136	19107	rps10
End	19509	19477	19441	19462	19433	rps10
Start	19514	19482	19446	19467	19438	trnR (gcg)
End	19587	19555	19519	19540	19511	trnR (gcg)
Start	19598	19566	19530	19551	19522	trnQ
End	19669	19637	19601	19622	19593	trnQ
Start	19671	19639	19603	19627	19595	trnI (gau)
End	19744	19712	19676	19697	19668	trnI (gau)
Start	19747	19715	19679	19700	19671	trnV
End	19819	19787	19751	19772	19743	trnV
Start	19836	19804	19768	19789	19760	rps12
End	20216	20184	20148	20169	20140	rps12
Start	20146	20114	20078	20099	20070	rps7
End	20619	20587	20551	20572	20543	rps7
Start	20675	20643	20607	20628	20599	cox3
End	21592	21560	21523	21545	21516	cox3
Start	21620	21588	21552	21573	21544	atp6
End	22339	22307	22271	22292	22263	atp6
Start	22370	22338	22302	22322	22294	trnD
End	22443	22411	22375	22395	22367	trnD
Start	22480	22448	22412	22432	22404	nad3
End	22833	22801	22765	22785	22757	nad3
Start	23053	23031	22983	22995	22877	nad5
End	25047	25025	24977	24989	24871	nad5
Start	25091	25069	25021	25033	24915	nad6
End	25801	25779	27571	25743	25625	nad6
Start	25827	25804	25747	25769	25651	trnR (ucu)
End	25899	25876	25819	25841	25723	trnR (ucu)

Appendix 1.1 cont.

	Species					
	P. infestans	*P. phaseoli*	*P. andina*	*P. ipomoeae*	*P. mirabilis*	Location
Start	26064	36025	25985	25985	25887	nad4L
End	26367	26327	26287	26287	26189	nad4L
Start	26370	26330	26290	26290	26192	nad1
End	27350	27310	27270	27270	27172	nad1
Start	27347	273007	27267	27267	27169	nad11
End	29353	29313	29273	29273	29175	nad11
Start	29438	29396	29356	29356	29260	trnL (uag)
End	29520	29478	29438	29438	29342	trnL (uag)
Start	29530	29488	29448	29448	29352	trnL (uaa)
End	29613	29571	29531	29531	29435	trnL (uaa)
Start	29633	29591	29551	29551	29455	ymf16
End	30379	30337	30297	30297	30201	ymf16
Start	30570	30526	30486	30486	30390	trnC
End	30640	30596	30556	30556	30460	trnC
Start	30652	30608	30568	30568	30472	trnS (uga)
End	30736	30692	30652	30652	30556	trnS (uga)
Start	30758	30712	30673	30673	30577	rps11
End	31174	31128	31089	31089	30991	rps11
Start	31187	31141	31102	31102	31006	rps13
End	31600	31554	31515	31515	31419	rps13
Start	31629	31583	31544	31544	31448	rpl2
End	32435	32389	32350	32350	32254	rpl2
Start	32439	32393	32354	32354	32258	rps19
End	32672	32626	32587	32587	32491	rps19
Start	32676	32630	32591	32591	32495	rps3
End	33479	33433	33394	33394	33298	rps3
Start	33482	33436	33397	33397	33301	rpl16
End	33886	3384	33801	33801	33705	rpl16

Appendix 1.1 cont.

	Species					
	P. infestans	*P. phaseoli*	*P. andina*	*P. ipomoeae*	*P. mirabilis*	Location
Start	33889	33843	33804	33804	33708	trnI (cau)
End	33962	33916	33877	33877	33781	trnI (cau)
Start	33983	33937	33898	33898	33802	orf217
End	34636	34590	34551	34551	34455	orf217
Start	34698	34652	34613	34613	34517	atp8
End	35090	35044	35005	35005	34909	atp8
Start	35111	35066	35027	35027	34931	trnK
End	35183	35138	35099	35099	35003	trnK
Start	35187	35142	35103	35103	35007	trnA
End	35259	35214	35175	35175	35079	trnA
Start	35278	35233	35194	35194	35098	rps14
End	35577	35532	35493	35493	35397	rps14
Start	35591	35546	35509	35507	35411	rps8
End	35971	35926	35889	35887	35791	rps8
Start	35979	35934	35896	35984	35799	rpl6
End	36584	36439	36501	36499	36404	rpl6
Start	36591	36546	36508	36506	36411	rps2
End	37181	37136	37098	37096	37001	rps2
Start	37191	37146	37108	37106	37011	rps4
End	37652	37607	37569	37567	37472	rps4
Start	37655	37610	37572	37570	37475	orf100
End	37957	37912	37874	37872	37777	orf100

Appendix 1.2 Polymorphisms between Ic clade *Phytophthora* species based on *P. infestans* Ib mt genome.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
CCTCC	174	172	174	174	174	rnl
GGAGG	200	198	200	200	200	rnl
GGAGG	231	229	231	231	231	rnl
GGGGA	353	351	353	353	353	rnl
TTTCT	373	371	373	373	373	rnl
TTTTA	514	512	514	514	514	rnl
GGAGG	1002	1000	1002	1002	1002	rnl
CCTCC	1090	1088	1090	1090	1090	rnl
AAGAA	1114	1112	1114	1114	1114	rnl
CCCCT	1272	1270	1272	1272	1272	rnl
GTTTG	2022	2020	2022	2022	2022	rnl
CCCCT	2089	2087	2089	2089	2089	rnl
GGGGA	2105	2103	2105	2105	2105	rnl
AGAAA	2111	2109	2111	2111	2111	rnl
ATAAA	2572	2570	2572	2572	2572	rnl
TCCCT	2701	2699	2701	2701	2701	trnN
CAAAA	2728	2726	2728	2728	2728	trnN
GGAGG	3000	2998	3000	3000	3000	trnP
CCTCC	3203	3201	3203	3203	3203	rpl14
AAAAC	3340	3338	3340	3340	3340	rpl14
CTTTT	3365	3363	3365	3365	3365	rpl14
TAAAA	3404	3402	3404	3404	3404	rpl14
GGAGG	3594	3592	3594	3594	3594	rpl5
CCCCT	3615	3613	3615	3615	3615	rpl5
AAAGA	3791	3789	3791	3791	3791	rpl5
AAAAG	3912	3910	3912	3912	3912	rpl5
AAAAG	3913	3911	3913	3913	3913	rpl5
TTTCT	3917	3915	3917	3917	3917	rpl5
CCCCT	3874	3972	3974	3974	3974	rpl5
TTTTC	3995	3993	3995	3995	3995	rpl5
GAGGG	4397	4395	4397	4397	4397	trnY
CCCCT	5337	5347	5332	5325	5069	rns
ACCCC	5427	5437	5422	5415	5438	rns
TTGTT	5778	5788	5773	5766	5789	rns
AACAA	5853	5863	5848	5841	5864	rns
CTTTT	6086	6096	6081	6074	6097	rns
AAAAG	6146	6156	6141	6134	6157	rns
AAAAG	6248	6258	6243	6236	6259	rns

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
ATAAA	6250	6260	6245	6238	6261	rns
CCCCT	6265	6275	6260	6253	6276	rns
AAAGA	6363	6373	6358	6351	6374	rns
GGAGG	6148	6428	6413	6406	6429	rns
AAAAG	6596	6606	6591	6584	6607	rns
CCCCT	6629	6639	6624	6617	6640	rns
AAAAG	6762	6772	6757	6750	6773	trnW
TATTT	7023	7021	7004	7009	7034	orf79
CTTTC	7028	7026	7009	7014	7039	orf79
CTTTT	7080	7060	7031	7048	7073	orf79
TTTAT	7119	7099	7070	7087	7112	orf79
TCCCC	7187	7167	7138	7155	7180	orf79
TTTTC	7202	7182	7153	7170	7195	orf79
GGGGA	7235	7215	7186	7203	7228	orf79
GAAAG	7244	7224	7195	7212	7237	orf79
CCCTC	7795	7712	7733	7747	7767	cox2
GAAAA	7831	7748	7769	7783	7803	cox2
GGGAG	7871	7788	7809	7823	7843	cox2
CCCCT	7892	7809	7830	7844	7864	cox2
TCCCC	7962	7879	7900	7914	7934	cox2
CCCTC	7981	7898	7919	7933	7953	cox2
GGGGA	7984	7901	7922	7936	7956	cox2
GGGAG	8167	8084	8105	8119	8137	cox2
TTTTC	8176	8093	8114	8128	8148	cox2
GGAGG	8207	8124	8145	8159	8179	cox2
AGGGG	8224	8141	8162	8176	8196	cox2
ATAAA	8239	8156	8117	8191	8211	cox2
GAAAA	8251	8168	8189	8203	8223	cox2
TCTTT	8278	8195	8216	8230	8250	cox2
TTTCT	8284	8201	8222	8236	8256	cox2
GAGGG	8441	8358	8379	8393	8413	cox2
GAGGG	8450	8367	8388	8402	8422	cox2
TTTGT	8505	8422	8443	8457	8477	orf32
CCACC	8560	8477	8498	8512	8532	orf32
TTTTA	8677	8592	8611	8625	8644	cox1
AAAAC	8678	8593	8612	8626	8645	cox1
AAAGA	8797	8712	8731	8745	8764	cox1
CCCAC	8908	8823	8842	8856	8875	cox1
AGGGG	8390	8845	8864	8878	8897	cox1
TTTTG	8953	8868	8887	8901	8920	cox1
AAAAG	8998	8913	8932	8946	8965	cox1

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
AAAGA	9064	8979	8998	9012	9031	cox1
TTTGT	9109	9024	9043	9057	9067	cox1
TTCTT	9224	9139	9158	9172	9191	cox1
CCCTC	9287	9202	9221	9235	9254	cox1
AGAAA	9289	9205	9223	9237	9256	cox1
CCCCT	9331	9246	9265	9279	9298	cox1
TCTTT	9343	9258	9277	9291	9310	cox1
AAACA	9367	9282	9301	9315	9334	cox1
CTTTT	9383	9298	9317	9331	9350	cox1
AAAAG	9430	9345	9364	9378	9397	cox1
CTTTT	9445	9360	9379	9393	9412	cox1
ACAAA	9520	9435	9454	9468	9487	cox1
CCCCT	9527	9442	9461	9475	9494	cox1
AAACA	9535	9450	9469	9483	9502	cox1
GGAGG	9619	9534	9553	9567	9586	cox1
ACAAA	9625	9540	9559	9573	9592	cox1
GGGAG	9628	9543	9562	9576	9595	cox1
CCCTC	9733	9648	9667	9681	9700	cox1
CCCCT	9750	9665	9684	9698	9717	cox1
GTTTT	9757	9672	9691	9705	9724	cox1
AAAAG	9778	9693	9712	9726	9745	cox1
CCCCT	9790	9705	9724	9738	9757	cox1
TTTAT	9832	9747	9766	9780	9799	cox1
CCCCA	9951	9866	9885	9899	9918	cox1
CTTTC	9952	9867	9886	9900	9919	cox1
GAAAG	9982	9897	9916	9930	9949	cox1
AAAAC	9992	9907	9926	9940	9959	cox1
TTTCT	10009	9924	9943	9957	9976	cox1
TTTTC	10790	10703	10718	10732	10750	nad9
TTTTA	10801	10714	10729	10743	10761	nad9
GGAGG	10927	10840	10855	10869	10887	nad9
CCCCT	10938	10851	10866	10880	10898	nad9
AAACA	10939	10852	10867	10881	10899	nad9
GGTGG	10988	10901	10916	10930	10948	nad9
TTTTC	11164	11077	11092	11106	11124	nad9
GAGGG	11420	11333	11348	11362	11380	cob
CCTCC	11470	11383	11398	11412	11430	cob
ACAAA	11531	11444	11459	11473	11491	cob
TTTCT	11562	11475	11490	11504	11522	cob
GGGGA	11570	11483	11498	11512	11530	cob
AAACA	11581	11494	11509	11523	11541	cob

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
CCCTC	11619	11532	11547	11561	11579	cob
AGGGG	11623	11536	11551	11565	11583	cob
AAAAC	11633	11546	11561	11575	11593	cob
TTTCT	11743	11656	11671	11685	11703	cob
CCCCT	11758	11671	11686	11700	11718	cob
TTTTC	11857	11770	11785	11799	11817	cob
AAAGA	11899	11812	11827	11841	11859	cob
CCCTC	11989	11902	11917	11931	11949	cob
CTTTT	12016	11929	11944	11958	11976	cob
AAATA	12076	11989	12004	12018	12036	cob
CTTTT	12184	12097	12112	12126	12144	cob
TTTGT	12188	12101	12116	12130	12148	cob
CCCCT	12223	12136	12151	12165	12183	cob
TCTCT	12235	12148	12163	12177	12195	cob
TTTAT	12349	12262	12277	12291	12309	cob
TTCTT	12361	12274	12289	12303	12321	cob
AAAAG	12704	12610	12620	12642	12656	atp1
GGGGA	12716	12622	12632	12654	12668	atp1
CTTTT	12740	12646	12656	12678	12692	atp1
GGAGG	12741	12647	12657	12679	12693	atp1
CCCTC	12808	12714	12724	12746	12760	atp1
TTTTC	12866	12772	12782	12804	12818	atp1
CTTTT	12914	12820	12830	12852	12866	atp1
GAAAA	13085	12991	13001	13023	13037	atp1
AAACA	13121	13027	13037	13059	13073	atp1
AAAAG	13127	13033	13043	13065	13079	atp1
GGGGA	13220	13126	13136	13158	13172	atp1
TTTTC	13343	13249	13259	13281	13295	atp1
AAAGA	13346	13252	13262	13284	13298	atp1
TTTTC	13406	13312	13322	13344	13358	atp1
TCTTT	13463	13369	13379	13401	13415	atp1
AGGGG	13526	13432	13442	13464	13478	atp1
GAGGG	13542	13448	13458	13480	13494	atp1
GGGTG	13607	13513	13523	13545	13559	atp1
TAAAA	13619	13525	13535	13557	13571	atp1
AAAGA	13795	13701	13711	13733	13747	atp1
AGGGA	13853	13759	13769	13791	13805	atp1
CCTCC	13860	13766	13776	13798	13812	atp1
TTCTT	13953	13859	13869	13891	13905	atp1
GGGAG	13982	13888	13898	13920	13934	atp1
AAACA	14012	13918	13928	13950	13964	atp1

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
AAAAG	14021	13927	13937	13959	13973	atp1
GGGAG	14241	14147	14157	14178	14193	trnE
GGGGA	14322	14228	14238	14259	14274	nad4
GGGGA	14397	14303	14313	14334	14349	nad4
TTTCT	14493	14399	14409	14430	14445	nad4
CCACC	14522	14428	14438	14459	14474	nad4
GGGGA	14547	14453	14463	14484	14499	nad4
TTCTT	14601	14507	14517	14538	14553	nad4
CTTTT	14622	14528	14538	14559	14574	nad4
AAAAG	14760	14666	14676	14697	14712	nad4
TTGTT	14928	14834	14844	14865	14880	nad4
TCTTT	14972	14878	14888	14909	14924	nad4
AAAGA	15012	14918	14928	14949	14964	nad4
TTTTC	15042	14948	14958	14979	14994	nad4
AACAA	15054	14960	14970	14991	15006	nad4
CCCCA	15102	15008	15018	15039	15054	nad4
GGAGG	15168	15074	15084	15105	15120	nad4
TTCTT	15301	15207	15217	15238	15253	nad4
TTTCT	15333	15239	15249	15270	15285	nad4
GGGGA	15381	15287	15297	15318	15333	nad4
TTTGT	15425	15331	15341	15362	15377	nad4
AGGGA	15495	15401	15411	15432	15447	nad4
CCCCT	15725	15631	15641	15662	15677	nad4
CCCCT	15813	15719	15729	15750	15765	trnH
CTTTT	15914	15820	15830	15851	15866	orf142
GAAAA	16016	15922	15932	15953	15968	orf142
CCCCT	16057	15963	15973	15994	16009	orf142
AGGGG	16116	16022	16032	16053	16068	orf142
CCCCT	16160	16066	16076	16097	16112	orf142
AAAAT	16180	16086	16096	16117	16132	orf142
GAGGG	16190	16096	16106	16127	16142	orf142
CCTCC	16298	16204	16214	16235	16250	orf142
AAACA	16511	16417	16427	16448	16463	nad7
ATAAA	16547	16453	16463	16484	16499	nad7
GGGGA	16616	16522	16532	16553	16568	nad7
ACAAA	16703	16609	16619	16640	16655	nad7
TTTCT	16778	16684	16694	16715	16730	nad7
AAAGA	16811	16717	16727	16748	16763	nad7
CTTTC	16862	16768	16778	16799	16814	nad7
CCCCT	16928	16834	16844	16865	16880	nad7
TTGTT	16973	16879	16889	16910	16925	nad7

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
CCCCA	17012	16918	16928	16949	16964	nad7
TTTTC	17018	16924	16934	16955	16970	nad7
TTTTG	17033	16939	16949	16970	16985	nad7
TTTCT	17096	17002	17012	17033	17048	nad7
GGGGT	17194	17100	17110	17131	17146	nad7
AAAAC	17204	17110	17120	17141	17156	nad7
TTTTC	17210	17116	17126	17147	17162	nad7
AGAAA	17213	17119	17129	17150	17165	nad7
GGGGA	17216	17122	17132	17153	17168	nad7
AACAA	17372	17278	17288	17309	17324	nad7
TGTTT	17390	17296	17306	17327	17342	nad7
AAAAG	17404	17310	17320	17341	17356	nad7
AAGAA	17441	17347	17357	17378	17393	nad7
AGGGG	17900	17806	17814	17835	17850	nad2
GGGTG	18035	17941	17949	17970	17985	nad2
TTTCT	18100	18006	18014	18035	18050	nad2
GGGGA	18179	18085	18093	18114	18129	nad2
GGTGG	18311	18217	18225	18246	18261	nad2
GGGGA	18322	18228	18236	18257	18272	nad2
GTTTT	18397	18303	18311	18332	18347	nad2
AATAA	18449	18355	18363	18384	18399	nad2
AAAGA	18505	18411	18419	18440	18455	nad2
CCTCC	18520	18426	18434	18455	18470	nad2
GAAAA	18529	18435	18443	18464	18479	nad2
AAAAG	18538	18444	18452	18473	18488	nad2
TCTTT	18565	18471	18479	18500	18515	nad2
CCTCC	18644	18550	18558	18579	18594	nad2
AAAGA	18712	18618	18626	18647	18662	nad2
CAAAA	18841	18747	18755	18776	18791	nad2
CTTTT	18883	18789	18797	18818	18833	nad2
GGGGA	19006	18912	18920	18941	18956	nad2
TTTTC	19014	18920	18928	18949	18964	nad2
TTTTA	19021	18927	18935	18956	18971	nad2
TTTTC	19037	18943	18951	18972	18987	nad2
ACCCC	19047	18953	18961	18982	18997	nad2
GGGGA	19064	18970	18978	18999	19014	nad2
AGAAA	19079	18985	18993	19014	19029	nad2
TCCCC	19111	19017	19025	19046	19061	trnF
AAACA	19277	19183	19191	19212	19227	rps10
CCCCT	19322	19228	19236	19257	19272	rps10
AGAAA	19453	19359	19367	19388	19403	rps10

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
GAGGG	19558	19464	19472	19493	19508	trnR
GGGGA	19589	19495	19503	19524	19539	trnR
TTTTA	19865	19771	19779	19800	19815	rps12
CTTTT	19928	19834	19842	19863	19878	rps12
AACAA	19985	19891	19899	19920	19935	rps12
GGGGA	19994	19900	19908	19929	19944	rps12
TTCTT	20027	19933	19941	19962	19977	rps12
TTTTC	20028	19934	19942	19963	19978	rps12
TTTTA	20034	19940	19948	19969	19984	rps12
TCCCT	20036	19942	19950	19971	19986	rps12
GAAAA	20039	19945	19953	19974	19989	rps12
GAAAA	20045	19951	19959	19980	19995	rps12
AGAGA	20054	19960	19968	19989	20004	rps12
TTTCT	20060	19966	19974	19995	20010	rps12
GGAGG	20063	19969	19977	19998	20013	rps12
AAAAT	20072	19978	19986	20007	20022	rps12
GGGGA	20087	19993	20001	20022	20037	rps12
AGGGG	20108	20014	20022	20043	20058	rps12
CCCCA	20154	20060	20068	20089	20104	rps12
CCCCT	20189	20095	20103	20124	20139	rps12
AAAAG	20198	20104	20112	20133	20148	rps12
CCCCT	20189	20095	20103	20124	20139	rps7
AAAAG	20198	20104	20112	20133	20148	rps7
TTTTG	20385	20291	20299	20320	20335	rps7
CCCCT	20406	20312	20320	20341	20356	rps7
GGAGG	20440	20346	20354	20375	20390	rps7
GGGGA	20534	20440	20448	20469	20484	rps7
CCCAC	20729	20635	20643	20664	20679	cox3
CCCTC	20803	20709	20717	20738	20753	cox3
GGGAG	20818	20724	20732	20753	20768	cox3
TTTTG	20836	20742	20750	20771	20786	cox3
AAAAC	20896	20802	20810	20831	20846	cox3
GGGGA	20906	20812	20820	20841	20856	cox3
TCCCC	21127	21033	21041	21062	21077	cox3
CAAAA	21145	21051	21059	21080	21095	cox3
GGGGA	21187	21093	21101	21122	21137	cox3
TTTTC	21274	21180	21188	21209	21224	cox3
AAGAA	21511	21417	21425	21446	21461	cox3
CCCCT	21514	21420	21428	21449	21464	cox3
AACAA	21583	21489	21497	21518	21533	cox3
TTTCT	21649	21555	21563	21584	21599	atp6

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
GGGGA	21692	21598	21606	21627	21642	atp6
GGAGG	21725	21631	21639	21660	21675	atp6
TTTTC	21808	21714	21722	21743	21758	atp6
TTTTA	21841	21747	21755	21776	21791	atp6
CCCCT	21849	21755	21763	21784	21799	atp6
CCCCT	21928	21834	21842	21863	21878	atp6
TCTTT	21943	21849	21857	21878	21893	atp6
CCTCC	21978	21884	21892	21913	21928	atp6
AGAAA	22081	21987	21995	22016	22031	atp6
TTTCT	22111	22017	22025	22046	22061	atp6
ACCCC	22133	22039	22047	22068	22083	atp6
GGGGA	22327	22233	22241	22262	22277	atp6
GGGGA	22445	22351	22359	22379	22395	trnD
CCCCT	22514	22420	22428	22448	22464	nad3
AAAAG	22614	22520	22528	22548	22564	nad3
GGAGG	22648	22554	22562	22582	22598	nad3
CCCCT	22707	22613	22621	22641	22657	nad3
AAAAG	22713	22619	22627	22647	22663	nad3
GGGGA	22726	22632	22640	22660	22676	nad3
TTTTA	22779	22685	22693	22713	22729	nad3
AAAAG	23083	22889	22995	23007	23043	nad5
CCTCC	23084	22890	22996	23008	23044	nad5
GGTGG	23157	22963	23069	23081	23117	nad5
AAAAC	23194	23000	23106	23118	23154	nad5
ACAAA	23248	23054	23160	23172	23208	nad5
TCCCC	23310	23116	23222	23234	23270	nad5
GTTTT	23343	23149	23255	23267	23303	nad5
CCTCC	23391	23197	23303	23315	23351	nad5
AGAAA	23490	23296	23402	23414	23450	nad5
TTTCT	23532	23338	23444	23456	23492	nad5
AGAAA	23550	23355	23462	23474	23510	nad5
GGAGG	23588	23394	23500	23512	23548	nad5
CTTTC	23599	23405	23511	23523	23559	nad5
TTTTC	23655	23461	23567	23579	23615	nad5
AAAAG	23686	23492	23598	23610	23646	nad5
GAAAA	23760	23566	23672	23684	23720	nad5
CCCCT	23800	23606	23712	23724	23760	nad5
ACAAA	23850	23656	23762	23774	23810	nad5
GGGGT	23901	23707	23813	23825	23861	nad5
AAGAA	23907	23713	23819	23831	23867	nad5
AAAAG	23979	23785	23891	23903	23939	nad5

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
TTTTC	24024	23830	23936	23948	23984	nad5
AAAAG	24078	23884	23990	24002	24038	nad5
ACAAA	24190	23996	24102	24114	24150	nad5
ACCCC	24198	24004	24110	24122	24158	nad5
CCCCT	24282	24088	24194	24206	24242	nad5
ACAAA	24297	24103	24209	24221	24257	nad5
TTTCT	24631	24437	24543	24555	24591	nad5
TTTCT	24726	24532	24638	24650	24686	nad5
AAAAC	24754	24560	24666	24678	24714	nad5
AGAAA	24790	24596	24702	24714	24750	nad5
AAAGA	24811	24617	24723	24735	24771	nad5
TCCCC	24864	24670	24776	24788	24824	nad5
TTTCT	24892	24698	24804	24816	24852	nad5
TTTTC	25038	24844	24950	24962	24998	nad5
CCTCC	25203	25009	25115	25127	25163	nad6
CCTCC	25227	25033	25139	25151	25187	nad6
TATTT	25253	25059	25165	25177	25213	nad6
AAGAA	25300	25106	25212	25224	25260	nad6
CCTCC	25303	25109	25215	25227	25263	nad6
GGAGG	25349	25155	25261	25273	25309	nad6
AAGAA	25465	25271	25377	25389	25425	nad6
AAACA	25627	25433	25539	25551	25587	nad6
CCCCT	25693	25499	25605	25617	25653	nad6
GGAGG	26137	25941	26039	26039	26079	nad4L
TTTTA	26218	26022	26120	26120	26160	nad4L
AAAAG	26271	26075	26173	26173	26213	nad4L
TTTAT	26364	26168	26266	26266	26306	nad4L
AAAAG	26421	26225	26323	26323	26363	nad1
CCCCA	26502	26306	26404	26404	26444	nad1
CCCCT	26549	26353	26451	26451	26491	nad1
TTTCT	26550	26354	26452	26452	26492	nad1
TTTTA	26574	26378	26476	26476	26516	nad1
AAACA	26748	26552	26650	26650	26690	nad1
TTCTT	26877	26681	26779	26779	26819	nad1
GGGGA	26892	26696	26794	26794	26834	nad1
TTTAT	26916	26720	26818	26818	26858	nad1
AAAAG	26919	26723	26821	26821	26861	nad1
TTTGT	26973	26777	26875	26875	26915	nad1
AAAGA	27000	26804	26902	26902	26942	nad1
CCCCT	27056	26860	26958	26958	26998	nad1
AGAAA	27069	26873	26971	26971	27011	nad1

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
AAAGA	27084	26888	26986	26986	27026	nad1
CTTTT	27219	27023	27121	27121	27161	nad1
TTTGT	27333	27137	27235	27235	27275	nad1
TTTCT	27377	27181	27279	27279	27319	nad11
TTCTT	27461	27265	27363	27363	27403	nad11
AAAAG	27560	27364	27462	27462	27502	nad11
CCCCT	27603	27407	27505	27505	27545	nad11
GGGAG	27656	27460	27558	27558	27598	nad11
AAATA	27703	27507	27605	27605	27645	nad11
AAAGA	27781	27585	27683	27683	27723	nad11
GGAGG	27816	27620	27718	27718	27758	nad11
TTTGT	27828	27632	27730	27730	27770	nad11
AGGGG	27862	27666	27764	27764	27804	nad11
GTGGG	27886	27690	27788	27788	27828	nad11
AAAAG	27942	27746	27844	27844	27884	nad11
CCACC	28051	27855	27953	27953	27993	nad11
CTCCC	28181	27985	28083	28083	28123	nad11
CTCCC	28240	28044	28142	28142	28182	nad11
CCCCT	28246	28050	28148	28148	28188	nad11
TTTTC	28249	28053	28151	28151	28191	nad11
TTTTG	28280	28084	28182	28182	28222	nad11
AAAAG	28326	28130	28228	28228	28268	nad11
AAAAG	28372	28175	28273	28273	28313	nad11
TTTTG	28412	28216	28314	28314	28354	nad11
AAAAG	28436	28240	28338	28338	28378	nad11
TTCTT	28515	28319	28417	28417	28457	nad11
TTTCT	28530	28334	28432	28432	28472	nad11
CCCCT	28534	28338	28436	28436	28476	nad11
AAAAG	28550	28354	28452	28452	28492	nad11
AGAAA	28583	28387	28485	28485	28525	nad11
AGGGA	28658	28462	28560	28560	28600	nad11
AGGGA	28697	28501	28599	28599	28639	nad11
CTCCC	28766	28570	28668	28668	28708	nad11
AAAGA	28844	28648	28746	28746	28786	nad11
TGTGT	28868	28672	28770	28770	28810	nad11
GGGGA	28905	28709	28807	28807	28847	nad11
AAAGA	28928	28732	28830	28830	28870	nad11
GGGAG	28970	28774	28872	28872	28912	nad11
CCTCC	28987	28791	28889	28889	28929	nad11
AAAAG	29086	28890	28988	28988	29028	nad11
GGGGA	29101	28905	29003	29003	29043	nad11

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
GGGGA	29170	28974	29072	29072	29112	nad11
TTTTG	29198	29002	29100	29100	29140	nad11
AGGGG	29222	29026	29124	29124	29164	nad11
TTTCT	29246	29050	29148	29148	29188	nad11
TTTAT	29290	29094	29192	29192	29232	nad11
AAACA	29296	29100	29198	29198	29238	nad11
TTTGT	29298	29102	29200	29200	29240	nad11
GGAGG	29307	29111	29209	29209	29249	nad11
TCTTT	29472	29276	29372	29372	29412	trnL(uag)
TCTTT	29509	29313	29409	29409	29449	trnL(uag)
CCCCT	29512	29316	29412	29412	29452	trnL(uag)
AAAAG	29523	29327	29423	29423	29463	trnL(uag)
GTTTT	29578	29382	29478	29478	29518	trnL(uaa)
CTTTT	29678	29482	29578	29578	29618	ymf16
ACCCC	29747	29551	29647	29647	29687	ymf16
TCTTT	29854	29658	29754	29754	29794	ymf16
TATTT	29991	29795	29891	29891	29931	ymf16
TTTTC	30020	29824	29920	29920	29960	ymf16
CACCC	30030	29834	29930	29930	29970	ymf16
GAAAA	30035	29839	29935	29935	29975	ymf16
GGGGA	30053	29857	29953	29953	29993	ymf16
TAAAA	30079	29883	29979	29979	30019	ymf16
AAAAG	30085	29889	29985	29985	30025	ymf16
CCCCT	30118	29922	30018	30018	30058	ymf16
CAAAA	30160	29964	30060	30060	30100	ymf16
GAAAA	30373	31077	30273	30273	30313	ymf16
TTTTC	30393	31097	30293	30293	30333	ymf16
TTTTC	30603	30405	30501	30501	30541	trnC
CCCCT	30748	30550	30646	30646	30686	trnS
AAGAA	30851	30652	30748	30748	30787	rps11
CTTTT	30869	30670	30766	30766	30805	rps11
CCCCT	31019	30820	30916	30916	30955	rps11
GGTGG	31042	30843	30939	30939	30978	rps11
AAAAT	31121	30922	31018	31018	31057	rps11
TTTAT	31140	30941	31037	31037	31076	rps11
CTCCC	31147	30948	31044	31044	31083	rps11
GGGGA	31249	31050	31146	31146	31185	rps13
CTTTT	31262	31063	31159	31159	31198	rps13
ACCCC	31292	31093	31189	31189	31228	rps13
TGTTT	31297	31098	31194	31194	31233	rps13
TTCTT	31322	31123	31219	31219	31258	rps13

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
GGGGA	31328	31129	31225	31225	31264	rps13
ACAAA	31400	31201	31297	31297	31336	rps13
GTGGG	31401	31202	31298	31298	31337	rps13
CCACC	31415	31216	31312	31312	31351	rps13
CCTCC	31538	31339	31435	31435	31474	rps13
AGAAA	31573	31374	31470	31470	31509	rps13
AAAAC	31652	31453	31549	31549	31588	rpl2
AAGAA	31712	31513	31609	31609	31648	rpl2
GGGGA	31801	31602	31698	31698	31737	rpl2
CAAAA	31943	31744	31840	31840	31879	rpl2
CCCCT	32051	31852	31948	31948	31987	rpl2
TCTTT	32072	31873	31969	31969	32008	rpl2
CCCCT	32085	31886	31982	31982	32021	rpl2
CAAAA	32114	31915	32011	32011	32050	rpl2
AAACA	32159	31960	32056	32056	32095	rpl2
CCCCA	32180	31981	32077	32077	32116	rpl2
TTTTC	32204	32005	32101	32101	32140	rpl2
CCCCT	32267	32068	32164	32164	32203	rpl2
TCTTT	32321	32122	32218	32218	32257	rpl2
GGGAG	32396	32197	32293	32293	32332	rpl2
AAAGA	32621	32422	32518	32518	32557	rps19
CCTCC	32651	32452	32548	32548	32587	rps19
TTTGT	32768	32569	32665	32665	32704	rps3
TTTTC	32774	32575	32671	32671	32710	rps3
GGGCG	32823	32624	32720	32720	32759	rps3
TTTCT	32978	32779	32875	32875	32914	rps3
TTTTC	32981	32782	32878	32878	32917	rps3
ACAAA	32986	32787	32883	32883	32922	rps3
CCTCC	32997	32798	32894	32894	32933	rps3
CTTTT	33020	32821	32917	32917	32956	rps3
CCCAC	33071	32872	32968	32968	33007	rps3
TTTTA	33083	32884	32980	32980	33019	rps3
GGGGA	33085	32886	32982	32982	33021	rps3
CCACC	33257	33058	33154	33154	33193	rps3
CCTCC	33276	33077	33173	33173	33212	tps3
ATTTT	33325	33126	33222	33222	33261	rps3
AAAAG	33359	33160	33256	33256	33295	rps3
CAAAA	33362	33163	33259	33359	33298	rps3
GGGGA	33605	33406	33502	33502	33541	rpl16
GAAAA	33682	33483	33579	33579	33618	rpl16
GAAAA	33770	33501	33597	33597	33636	rpl16

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
GGGAG	33709	33510	33606	33606	33645	rpl16
CCCCT	33714	33515	33611	33611	33650	rpl16
TTTTC	33721	33522	33618	33618	33657	rpl16
GAAAA	33754	33555	33651	33651	33690	rpl16
TTTTC	33766	33567	33663	33663	33702	rpl16
GGGGA	33779	33580	33676	33676	33715	rpl16
CCCCT	33799	33600	33696	33696	33735	rpl16
CTTTT	33806	33607	33703	33703	33742	rpl16
AAAAG	33907	33708	33804	33804	33843	trnI
GGAGG	33950	33751	33847	33847	33886	trnI
AACAA	34004	33805	33901	33901	33940	orf217
CCCAC	34019	33820	33916	33916	33955	orf217
AAAAG	34173	33974	34070	34070	34109	orf217
CTTTT	34189	33990	34086	34086	34125	orf217
AAACA	34263	34064	34160	34160	34199	orf217
CCCCT	34312	34113	34209	34209	34248	orf217
GGGGT	34330	34131	34227	34227	34266	orf217
AGAAA	34331	34132	34228	34228	34267	orf217
CCCCT	34381	34182	34278	34278	34317	orf217
GGGGA	34390	34191	34287	34287	34326	orf217
CCTCC	34410	34211	34307	34307	34346	orf217
CCTCC	34418	34219	34315	34315	34354	orf217
ATTTT	34457	34258	34354	34354	34393	orf217
ATTTT	34458	34259	34355	34355	34394	orf217
AAAGA	34539	34340	34436	34436	34475	orf217
CTCCC	34543	34344	34440	34440	34479	orf217
AAACA	34572	34373	34469	34469	34508	orf217
AAACA	34643	34444	34540	34540	34579	orf217
AAAAC	34757	34558	34654	34654	34693	atp8
AAAAG	34832	34633	34729	34729	34768	atp8
ATAAA	34864	34665	34761	34761	34800	atp8
GGAGG	34950	34751	34847	34847	34886	atp8
CCCCT	34975	34776	34872	34872	34911	atp8
TCCCT	35007	34808	34904	34904	34943	atp8
TTTAT	35030	34831	34927	34927	34966	atp8
TTTGT	35031	34832	34928	34928	34967	atp8
AAAAT	35046	34847	34943	34943	34982	atp8
TTTTA	35047	34848	34944	34944	34983	atp8
GGGGA	35053	34854	34950	34950	34989	atp8
AAAAG	35065	34866	34962	34962	35001	atp8
AAAGA	35342	35144	35240	35240	35279	rps14

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
GGGAG	35404	35206	35302	35302	35341	rps14
AAAGA	35418	35220	35316	35316	35355	rps14
CTCCC	35436	35238	35334	35334	35373	rps14
GGAGG	35506	35308	35404	35404	35443	rps14
AGGGG	35529	35331	35427	35427	35466	rps14
GGGGA	35551	35353	35449	35449	35488	rps14
TTTTA	35562	35364	35460	35460	35499	rps14
CCTCC	35662	35464	35562	35560	35599	rps8
CCCTC	35692	35494	35592	35590	35629	rps8
AAGAA	35728	35530	35628	35626	35665	rps8
TTCTT	35785	35587	35685	35683	35722	rps8
CTCCC	35820	35622	35720	35718	35757	rps8
AAAAC	35918	35720	35818	35816	35855	rps8
TTTTA	35932	35734	35832	35830	35869	rps8
GGGGA	35941	35743	35841	35839	35878	rps8
TTGTT	35962	35764	35862	35860	35899	rps8
AATAA	36011	35813	35910	35908	35948	rpl6
AAAAG	36105	35807	36004	36002	36042	rpl6
AAGAA	36109	35911	36008	36006	36046	rpl6
CAAAA	36127	35929	36026	36024	36064	rpl6
CCCTC	36136	35938	36035	36033	36073	rpl6
AAAAC	36137	35939	36036	36034	36074	rpl6
AGGGG	36237	36039	36136	36134	36174	rpl6
AAAAG	36255	36057	36154	36152	36192	rpl6
TTATT	36287	36089	36186	36184	36224	rpl6
CCCCT	36327	36129	36226	36224	36264	rpl6
AGAGA	36335	36137	36234	36232	37272	rpl6
TTTTC	36384	36186	36283	36281	36321	rpl6
TTCTT	36395	36197	36294	36292	36332	rpl6
ACAAA	36399	36201	36298	36296	36336	rpl6
AATAA	36407	36209	36306	36304	36344	rpl6
CTTTT	36419	36221	36318	36316	36356	rpl6
CTCCC	36445	36247	36344	36342	36382	rpl6
AAAGA	36542	36344	36441	36439	36479	rpl6
AAAGA	36563	36365	36462	36460	36500	rpl6
AAAAC	36615	36417	36514	36512	36552	rps2
AAAAG	36649	36451	36548	36546	36586	rps2
AAAAG	36680	36482	36579	36577	36617	rps2
GAAAA	36689	36491	36588	36586	36626	rps2
AAAAC	36716	36518	36615	36613	36653	rps2
GGGGA	36738	36540	36637	36635	36675	rps2

Appendix 1.2 cont.

Polymorphism mutation[a]	*P. infestans*	*P. mirabilis*	*P. andina*	*P. ipomoeae*	*P. phaseoli*	Gene
ACAAA	36816	36618	36715	36713	36753	rps2
AAAGA	36890	36692	36789	36787	36827	rps2
GTTTT	36952	36754	36851	36849	36889	rps2
AAAAG	37025	36827	36924	36922	36962	rps2
AGAAA	37058	36860	36957	36955	36995	rps2
TTGTT	37133	36935	37032	37030	37070	rps2
TGTTT	37169	36971	37068	37066	37106	rps2
AACAA	37184	36986	37083	37081	37121	rps2
GGGGA	37327	37129	37226	37224	37264	rps4
GGGGA	37333	37135	37232	37230	37270	rps4
AAACA	37484	37286	37383	37381	37421	rps4
TTTGT	37517	37319	37416	37414	37454	rps4
AGAAA	37588	37390	37487	37485	37525	rps4
AAATA	37595	37397	37494	37492	37532	rps4
TTGTT	37684	37486	37583	37581	37621	orf100
AAAAT	37725	37527	37624	37622	37662	orf100
CTTTT	37727	37529	37626	37624	37664	orf100
ACAAA	37800	37602	37699	37697	37737	orf100
TTTTA	37875	37677	37774	37772	37812	orf100
GAAAA	37879	37681	37778	37776	37816	orf100
CCACC	37881	37683	37780	37778	37818	orf100
TTCTT	37891	37693	37790	37788	37828	orf100
TCTTT	37924	37726	37823	37821	37861	orf100

[a]Polymorphism corresponds to the position of the nucleotide in *P. infestans*, *P. phaseoli*, *P. andina*, *P. ipomoeae*, and *P. mirabilis* respectively.

Appendix 1.3 Amino acid changes produced from transitions/transversions in the mt genomes of clade Ic *Phytophthora* species

Polymorphism[a]	*P. infestans*	*P. phaseoli*	*P. andina*	*P. ipomoeae*	*P. mirabilis*	Gene
ACAAA	Asp	Ala	Asp	Asp	Asp	rpl14
GGAGG	Asp	Asp	Asn	Asp	Asp	rpl5
ATAAA	Leu	Phe	Leu	Leu	Leu	rpl5
CAACA	Ala	Glu	Glu	Ala	Glu	rpl5
AGAAA (X2)	Lys	Gly	Lys	Lys	Lys	rpl5
TTTTA	Leu	Leu	Leu	Leu	Ile	orf79
	Phe	Phe		Phe	Phe	orf79
	Leu	Leu		Leu	Leu	orf79
	Ser	Ser		Ser	Ser	orf79
	Ile	Ile		Ile	Ile	orf79
ATTTT	Leu	Phe	Phe	Phe	Phe	orf79
TTTAT	Leu	Leu	Leu	Ile	Leu	orf79
CCTTC	Thr	Thr	Met	Met	Thr	orf79
GGGAG	Asp	Asp	Asp	Asn	Asp	cox2
CTCCC	Pro	Ser	Pro	Pro	Pro	cox2
TCCCC	Ile	Thr	Thr	Thr	Thr	cox2
GGAGG	Val	Val	Ile	Val	Val	cox2
TAAAA	Asp	Asp	Asp	Asp	Asn	cox2
GTA, TCT	Val	Val	Val	Val	Ile	cox2
TTTGT	Val	Val	Val	Gly	Val	orf32
CCTCC	Phe	Phe	Leu	Phe	Phe	orf32
TATTT	Asn	Lys	Asn	Asn	Asn	cox1
ACAAA	Lys	Gln	Lys	Lys	Lys	cox1
AGGGG	Ile	Val	Val	Val	Val	cox1
CTCCC	Ala	Val	Ala	Ala	Ala	cox1
CACCC	Thr	Asn	Thr	Thr	Thr	cox1
TATTT	Phe	Leu	Phe	Phe	Phe	nad9
CTCCC	Thr	Ile	Thr	Thr	Thr	nad9
GCT, TCA	Ala	Ala	Ser	Ala	Ala	nad9
TTTCT	Phe	Phe	Phe	Ser	Phe	cob
AGCGG	Ile	Val	Leu	Val	Val	cob
TTTGT	Ser	Ser	Ser	Ala	Ser	cob
AAGAA	Asn	Asn	Ser	Asn	Asn	atp1
GGGAG	Asp	Asp	Asp	Asn	Asp	atp1
CCTCC	Ser	Ser	Leu	Ser	Ser	atp1
TTGTT	Gly	Gly	Cys	Gly	Gly	nad4
TATTT	Ser	Thr	Ser	Ser	Ser	orf142
TTTTG	Ile	Ile	Ile	Ile	Met	nad7

Appendix 1.3 cont.

Polymorphism	*P. infestans*	*P. phaseoli*	*P. andina*	*P. ipomoeae*	*P. mirabilis*	Gene
AGGGG	Ile	Val	Val	Val	Val	nad2
GGTGG	Val	Val	Leu	Val	Val	nad2
AATAA	Asn	Asn	Tyr	Asn	Asn	nad2
CCTCC	Leu	Leu	Phe	Leu	Leu	nad2
ACCCC	Tyr	Ser	Ser	Ser	Ser	nad2
GAGGG	Val	Ile	Val	Val	Val	nad2
AGGGG	Ile	Ile	Ile	Ile	Val	nad2
AAACA	Asn	Asn	Asn	Thr	Asn	rps10
AAAAG	Asn	Asn	Asn	Asn	Asp	rps10
CTCCC	Ala	Val	Ala	Ala	Ala	rps7
GGAGG	Asp	Asp	Asn	Asp	Asp	rps7
CCCAC	Gln	Gln	Gln	Lys	Gln	cox3
GAGGG	Gly	Ser	Gly	Gly	Gly	cox3
ACCCC	Leu	Phe	Phe	Phe	Phe	cox3
GAGGG	Met	Ile	Met	Met	Met	atp6
AGAAA	Thr	Ala	Ile	Thr	Thr	nad5
AGAAA	Lys	Glu	Lys	Lys	Lys	nad5
GAGAG	Val	Ile	Val	Ile	Val	nad5
AAAAG	Ile	Ile	Ile	Ile	Val	nad5
AAAGA	Ile	Ile	Ile	Val	Ile	nad5
CCTCC	Ala	Ala	Val	Ala	Ala	nad6
TTTTC	Ser	Ser	Ser	Ser	Thr	nad6
GAAGG	Val	Ile	Ile	Val	Val	nad1
GAGGG	Gly	Ser	Gly	Gly	Gly	nad1
AAACA	Asn	Asn	Asn	Thr	Asn	nad11
TTTGT	Phe	Phe	Phe	Val	Phe	nad11
CTCCC	Pro	Ser	Pro	Pro	Pro	nad11
CTCCC	Ala	Val	Ala	Ala	Ala	nad11
GAGGG	Asp	Asn	Asp	Asp	Asp	nad11
AAAGA	Glu	Glu	Glu	Gly	Glu	nad11
AAGAA	Lys	Lys	Arg	Lys	Lys	nad11
TCTTT	Leu	Ser	Leu	Leu	Leu	nad11
AGAAA	Ile	Val	Ile	Ile	Ile	nad11
GAGGG	Gly	Ser	Gly	Gly	Gly	nad11
GGTGG	Ala	Ala	Ser	Ala	Ala	nad11
CCTCC	Ser	Ser	Phe	Ser	Ser	nad11
GAGGG	Ser	Asn	Ser	Ser	Ser	nad11
TTTTA	Phe	Phe	Phe	Phe	Tyr	ymf16
CCCCA	Thr	Thr	Thr	Thr	Lys	ymf16

Appendix 1.3 cont.

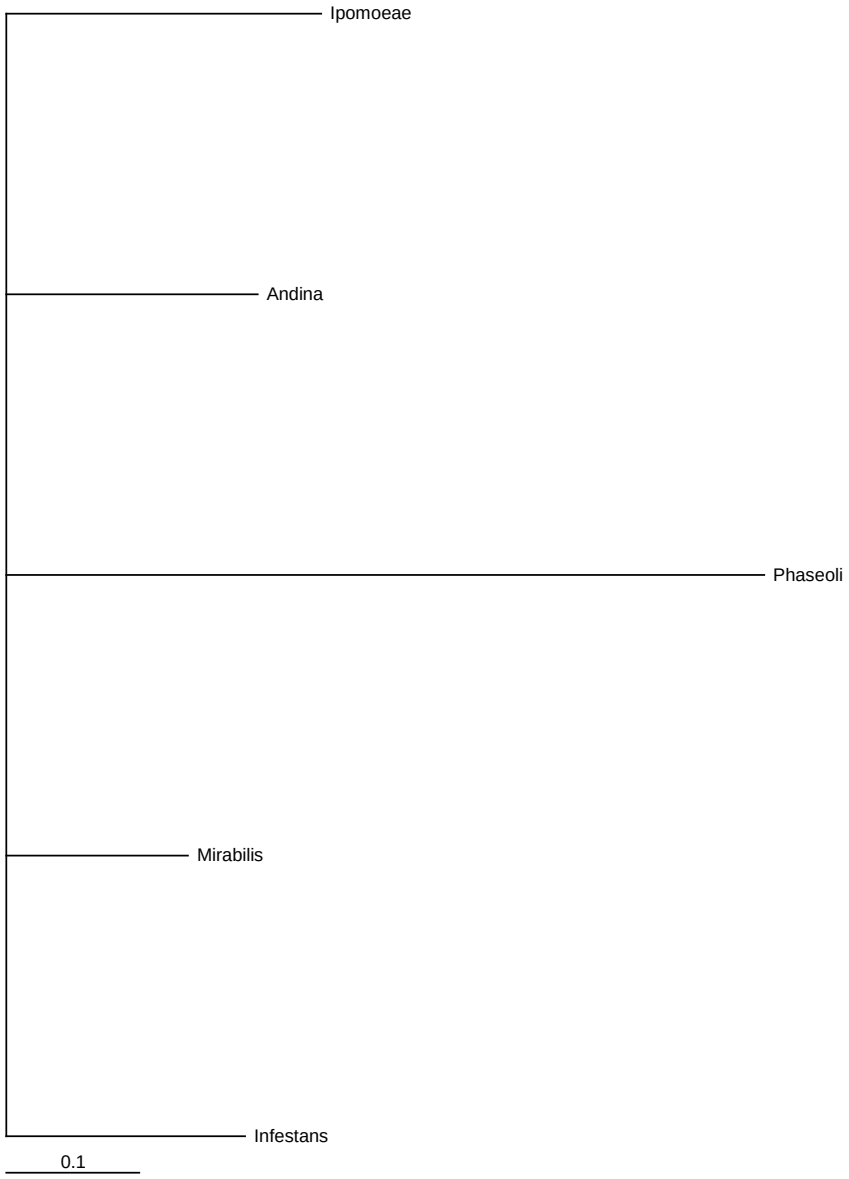
Polymorphism	*P. infestans*	*P. phaseoli*	*P. andina*	*P. ipomoeae*	*P. mirabilis*	Gene
GAAAA	Val	Ile	Ile	Ile	Ile	ymf16
GAGGG	Asp	Asn	Asp	Asp	Asp	ymf16
GGGGA	Val	Val	Val	Val	Ile	rps11
AAATA	Lys	Lys	Lys	Ile	Lys	rps11
GATAG	Val	Ile	Leu	Ile	Val	rps13
GGTGG	Gln	Gln	His	Gln	Gln	rps13
CCCCA, TTTTG	Ala	Ala	Ala	Ala	Glu	rps13
AAAAC	Lys	Lys	Lys	Lys	Gln	rps13
CTCCC	Arg	Cys	Arg	Arg	Arg	rps13
GGGCG	Glu	Glu	Glu	Gln	Glu	rps3
AAAAC	Asn	Asn	Asn	Asn	Thr	rps3
TATTT	Asn	Lys	Asn	Asn	Asn	rps3
GAGGG	Arg	Lys	Arg	Arg	Arg	rps3
CAAAA	Asp	Glu	Glu	Glu	Glu	rps3
GAGGG	Val	Ile	Val	Val	Val	rpl16
AACAA	Lys	Lys	Gln	Lys	Lys	orf217
CCCAC	Gln	Gln	Gln	Lys	Gln	orf217
AAACA	Asp	Asp	Asp	Ala	Asp	orf217
GTGGG	Glu	Asp	Glu	Glu	Glu	orf217
CCTCC	Ser	Ser	Phe	Ser	Ser	orf217
CCTCC	Pro	Pro	Ser	Pro	Pro	orf217
GTAGG	Val	Phe	Ile	Val	Val	orf217
AAAGA	Lys	Lys	Lys	Arg	Lys	orf217
AAACA	Lys	Lys	Lys	Thr	Lys	orf217
AAACA	Ser	Ser	Ser	Arg	Ser	orf217
AAAAT	Asn	Asn	Asn	Asn	Ile	atp8
GGAGG	Glu	Glu	Lys	Glu	Glu	atp8
CTCCC	Ser	Leu	Ser	Ser	Ser	atp8
TTCCC	Tyr	Tyr	His	His	His	atp8
CCTAC, TTTAT	Thr	Thr	Ile	Lys	Thr	atp8
GAGGG	Ser	Asn	Ser	Ser	Ser	atp8
GGGAG	Asp	Asp	Asp	Asn	Asp	rps14
GAGGG	Ala	Thr	Ala	Ala	Ala	rps14
AATAA	Leu	Leu	Phe	Leu	Leu	rpl6
CAAAA	Thr	Lys	Lys	Lys	Lys	rpl6
AGGGG	Asn	Asp	Asp	Asp	Asp	rpl6
AGAAA	Ile	Val	Ile	Ile	Ile	rpl6
CTCCC	Leu	Phe	Leu	Leu	Leu	rpl6
AAAAC	Asn	Asn	Asn	Asn	His	rpl6

Appendix 1.3 cont.

Polymorphism	*P. infestans*	*P. phaseoli*	*P. andina*	*P. ipomoeae*	*P. mirabilis*	Gene
ACAAA	Lys	Gln	Lys	Lys	Lys	rps2
AGAAA	Asn	Ser	Asn	Asn	Asn	rps2
AAAAC	Lys	Lys	Lys	Lys	Gln	rps2
GTTTT	Arg	Met	Met	Met	Met	rps2
TTGTT	Ile	Ile	Met	Ile	Ile	rps2
GAGGG	Arg	Lys	Arg	Arg	Arg	rps4
GAGGG	Arg	Gln	Arg	Arg	Arg	rps4
TTATT	Asn	Asn	Lys	Asn	Asn	orf100
ATAAA	His	Leu	His	His	His	orf100
CTTTT	Leu	Phe	Phe	Phe	Phe	orf100
TATTT	Ile	Lys	Ile	Ile	Ile	orf100
CCACC	Thr	Thr	Lys	Thr	Thr	orf100

[a]Polymorphism corresponds to the position of the amino acid in *P. infestans*, *P. phaseoli*, *P. andina*, *P. ipomoeae*, and *P. mirabilis* respectively.

Appendix 1.4 Phylogenetic tree of *Phytophthora* species in Ic clade constructed using MrBayes. Collapsed hapmap sequences were used to run the analysis.



Appendix 1.5 Collapsed haplotypes of *Phytophthora* Ic clade species used for coalescent analysis, including transitions (t) and transversions (v). Numbers at the top indicate the numbered mutation.

```
                         111111111122222222223333333333445555555556666666666777777777778
                 123456789012345678901234567890123456789012345678901234567890123456789 0
                 tttttvttttvtttvtvttvtvttttttttttvvvtttvtttttttvttvttttttttttttttvttttt
P. ipomoeae      CGGGCTGCACTCGAACAGCATAGCGAACCTGCCTATAAACGGACATTTACTGATAACCTGATGGAATCGG
P. andina        TAAGTTATGCTCGAACAATATAACAAATCTGCCGCTAAACAAACATTTTCTGACAGCCCGGTAGAATTGG
P. phaseoli      CGGATAGCATGTAAATAGCCTAGTAGGTTCGTCTATGGATAGGTGTCTTCCAGCAGTCCAGCGGAATTGG
P. mirabilis     CGGGTTGCACTCGGTCAGCATAGCAAATCTACCTATAATCAGACAATTTCTGACAGCCCGGTGGTACTAA
P. infestans     CGGGTTGCACGCGAATCGCACTGCAAATCTGCATACAAACAGACATCCTTTGGCGGCTCGGTGAAGTTGG

                                     1111111111111111111111111111111111111111111111111
                 888888888999999999900000000011111111112222222222333333333344444444445
                 123456789012345678901234567890123456789012345678901234567890123456789 0
                 vvvvtvtvttvtttttvtttvtvtvtttvttvvttvttvttvvtttvttvttvtttttttvtvttvttttt
P. ipomoeae      GCTAGAGTAGGTTACTCTATACCGAATCTACACTAACTTGCCGTGCACGCTGACCTGTTTTGCCATAGTG
P. andina        TATAACGTAATCCACTATATACAAAGCCTACTCTAATTTACATTGTATGACGATCTACTATTCTTCAGTA
P. phaseoli      TCACACGGGATTCATTATGTATAGAGCTTGTTACGCTCAGTAGCGCATAACGCTTCACTATTTTTTGATG
P. mirabilis     TCTAACGTAATTCGCCATATCCAGCGCCTACTCTAATTTGCAGTACCTGACGATCTACTATTCCTTAGTG
P. infestans     TCTAACATAATTCACTACACACAGAGCCGACTCCGATTTGCAGTGCATGACAATCTACCACTCTTTAGCG

                 111111111111111111111111111111111111111111111111222222222222222222222
                 555555555666666666677777777778888888888999999999900000000011111111112
                 123456789012345678901234567890123456789012345678901234567890123456789 0
                 ttttvttttttttvvttttvttttvttttvtttvvttttvttttttttvttvvtvtttttvvtvtvvtt
```

Appendix 1.5 cont.

```
P. ipomoeae     TTTACAGTGTTGGTAGGCTACAAGGCCGTTATTGTACGTCGGGCCTACGCAGCCAGACGTCTCTTCGATA
P. andina       CTTAAAGTATTGGGAAGTCGAAGGGTAGCTAGTATCCACTGTGCCTACGCAGTAAGATATCGCTTTGATA
P. phaseoli     CCTAAGACACTGGGAAACTGAGGAATCATTGTTACAAGTTATATTTATGTTGCAAAATACTTACGTTCCA
P. mirabilis    CTTAAAGTATCGAGAAGCTGAAGGGTCGTTATCATACGTTGTGCCTACGCAACATGCTATCTCTTTGATG
P. infestans    CTCGAAGTATTAGGTAACTGAAGGGTCGTCATTATACGTTGTACCCGCACAGCAAGATACCTCTTTGATA

                2222222222222222222222222222222222222222222222222222222222222222222222
                2222222223333333333444444444455555555556666666666777777777788888888889
                1234567890123456789012345678901234567890123456789012345678901234567890
                tvvtttvttvtvvttttttvtttvtvtttvtttvtttvtvtttvtttvtttttvttvttttvtttvttvvttvtt
P. ipomoeae     GATAAGTCGGGTAGCAATCGATGTTTCGACCCAGGTTAGTTTCAAGCGAGGCCACATCGGATATAGCAGT
P. andina       GCTAGGGTGTGTTATAATTAATGTTTCGACACAGGTTCGCTTCAAATAAGGCCACATCAGCCGTAGCAGT
P. phaseoli     AATGAGGTAGATAACAGTCAATACACCAACATAGAATAATCATAAATGTAGATGTGGTGACCGGCACAAC
P. mirabilis    GAGAAGGTGGGTAACAACCAATGTTTCGGCACGAGTTAGTTTCAAGTGAGGCCACATCGGCCGTAGCAGT
P. infestans    GATAAAGTGGGGAACGATCACCGTTTAGATACAGGTCAGTTTTGGATGAGACGACATCGGCCGTAGTCGT

                2222222223333333333333333333333333333333333333333333333333333333333333
                9999999990000000000111111111122222222222222222222333333333344444444445
                1234567890123456789012345678901234567890123456789012345678901234567890
                ttvttttvttttttvttttttttvttvvvtvttttttttttvvttttvvtvttvtttttttvttttvttv
P. ipomoeae     ACACGGTTCCTCACCGGCAGCAGTACGAACTCACAGTTAACAGAATAACCACCAAGCCTCCTACGACCGT
P. andina       GCCTGATTCCTTATCGGCAACAGTATTAACTTATAATTAACAGGATAACCATTAAACTTTTTGTAGACAT
P. phaseoli     ATATAGCATTTCATCAATGGTGAAGCGCACTCATAGCCGATATAGCGACTATTCAACTCCCTACGAATGA
P. mirabilis    ACATGGTTCCCCGTCGGCAGCAGTACGACCTCGTGGTTAACCGAATACCCCTTAGACTTCCAACGAACGT
P. infestans    ACATGGTTCCTCATAGGCAGCAGTACGAATGCATAGCTAGCAGAATAAACATTAAATTTCCTACGAACGT
```

Appendix 1.5 cont.

```
                 333333333333333333333333333333333333333333333333444444444444444444444
                 555555555666666666677777777778888888888999999999900000000011111111112
                 123456789012345678901234567890123456789012345678901234567890123456789 0
                 Tvtvttvvttvtvtttttvtttttvttvtvtvttttvttvtttttttttttvtttttttvttvvvtttttv
P. ipomoeae      AAACCCTCTGAAGGCAGTGCTACATGGGGGACCCCTTAATATCCAAGGCGGGGACAGGTGCACGGTTCAT
P. andina        ATACCTTACGTATACAATTTCACGAAATGGAACCCTTAATACTCAAGGCATGAGTAGGTGTTATATTCAT
P. phaseoli      GTGATTAATATGTATAATTTTGTGAAGTGGGCCCTCGGGGGTTTGAAACATAAGCGAAGGTTATGTTTGT
P. mirabilis     ATACCTTATGTATACGATTTTACGAAGTGTACTTCTTAATATTCAGGGTAGGAGCAGGTGTTATGCCCAT
P. infestans     ATACCTTATGTATACAACTTTACGAAGTAGACCCCTTAATATTCAAAACATGAGCAGGTATTATGTTCAG

                 444444444444444444444444444444444444444444444444444444444444444444444
                 222222222333333333344444444445555555555666666666677777777778888888889
                 123456789012345678901234567890123456789012345678901234567890123456789 0
                 tvtvtvttvttvtttttttvvvtttvvttvvvttvttvtttvvvtttttttvtvttvttvvtvtvtvtttt
P. ipomoeae      TCTTTCAGAACAATTCATCGAACGTCTTGAGCCAAAGACTCACCTCTAGCGTCCTACTATGCCTAAGAAA
P. andina        TCTTTCAGAACAATTCGTCTATCGTCTCGAGATAAGGACTCAACTCTGATTTGTTATTCTGATTAAGAAG
P. phaseoli      TCTTCCAAAGTAACCTATTGTTCATCTTAAGCCACAAATTTAAACTTGACTCGTCACTCAACCTGAAAAG
P. mirabilis     TCCATAAGAACAATTCATCGATTGTCGTGCTCCGAAGACCCAACTCCGACTTGTTCCTCTGCCTAAGAAG
P. infestans     CATTTCGGTACCGTTCACCGATCGCATTGAGCCAAAGCCTCCACTCTGACTTGTTACCCTGCCAACGGGG

                 444444444555555555555555555555555555555555555555555555555555555555555
                 999999999000000000011111111112222222222333333333344444444445555555556
                 123456789012345678901234567890123456789012345678901234567890123456789 0
                 tttttttttvvttvtvtttttvvttvvvtvtttvvvvtttttttttvtttttvvtvvttvtvttvttttv
P. ipomoeae      CTATGCTAGAAATCCGACGCCTTGCCCAAAGCCAGATGAGAGCGGGTCTATCATGTAAAATAGATCGTTA
```

Appendix 1.5 cont.

```
P. andina      CTATGCTAACCATACGACGTTTTACAAAAAACCTTATGAAGACAGGTTCGCCATGGTAGACAGAACATCA
P. phaseoli    TCACATTGGACGTATTATACCTTACAACGAGTTTTTAAGAGACGGAACCATCCAATAGAACCGGTTACTA
P. mirabilis   CTATGCTAGACATACGGCGCCTTATAAAATGCCTTATGAAGATGGGTCCATTATGTAAAACAGATCGTTC
P. infestans   CTGTGCCAGACACACGACGCCAAACAAAAAGCTTTATGAAGACGAGTCCATCATGTAAACCAAATCATTA

               555555555555555555555555555555555
               666666666777777777788888888899999
               123456789012345678901234567890123 4
               vttttvtttvtvtvttvvvttvvtvvvtvvtvtt
P. ipomoeae    ATCGGAAAAAGAGTAATTAGGCGATTATATACTT
P. andina      TTCAAAAAAAGAATAAGTCGGATAAGATATAACT
P. phaseoli    ATCAACGGACAAATGATTAAAATAATTTAAACTT
P. mirabilis   ATTAAAAAAAGCATAGTGAGGATGATATCTACTC
P. infestans   ACCAAAAAGAGAAGAATTAGGATAATACATGCTT
```

Appendix 1.6 Unique and shared mutations (site number) in the mitochondrial genomes from the coalescent analysis. Unhiglighted numbers indicate a unique mutation number. Numbers highlighted in blue are shared between *P. andina*, *P. ipomoeae*, and *P. mirabilis*. Green highlighted numbers are mutations shared between *P. infestans*, *P. andina*, *P. ipomoeae*, and *P. mirabilis*. Those highlighted in purple are shared between *P. mirabilis* and *P. ipomoeae*.

P. infestans:
591 588 574 569 562 553 550 535 513 512 503 497 493 489 488
486 484 478 462 458 446 445 438 433 432 429 427 422 421 420
410 379 368 337 329 320 311 310 295 278 277 261 255 254 247
240 237 232 231 226 222 216 189 187 186 170 155 152 144 143
139 131 129 122 99 90 88 77 66 64 58 55 50 48 36 33 22 21 17 4
6 10 12 13 20 24 26 27 29 30 32 37 38 40 43 44 45 51 52 57 60
62 73 74 78 79 85 89 92 98 100 101 103 106 108 109 111 114 119
123 125 126 133 137

P. phaseoli:
590 587 581 580 575 571 570 568 567 566 558 556 554 552 548
545 544 543 537 536 529 528 527 526 522 519 518 509 508 506
505 502 498 496 495 494 492 491 487 485 481 480 475 472 466
465 464 461 459 457 455 449 444 441 439 436 435 434 431 430
428 425 419 418 409 408 407 406 402 395 394 391 390 389 388
387 386 385 381 373 372 365 362 360 357 355 354 353 351 350
348 339 334 330 327 326 325 323 321 319 318 308 305 304 303
302 301 299 298 297 296 290 289 288 287 285 282 280 279 276
275 274 270 268 267 266 265 264 263 262 260 259 252 251 249
246 245 242 238 236 235 234 233 227 221 219 214 211 209 208
207 205 204 203 201 196 191 190 188 185 184 181 177 175 171
168 165 164 162 150 148 147 146 142 138 0

P. andina:
593 592 586 579 577 561 559 555 549 547 546 541 540 538 534
521 511 510 500 499 483 482 477 470 456 453 452 448 440 437
415 405 392 382 377 371 359 349 346 345 344 343 341 340 324
316 312 307 306 300 292 286 283 281 269 258 250 248 229 225
223 220 215 212 202 193 179 178 176 172 169 167 159 158 140
136 116 113 110 94 82 72 63 42 35 34 23 19 18 9 8 7 3 2 1 11
16 47 53 104 105 157 183 200 253 317 397 398 523
4 6 10 12 13 20 24 26 27 29 30 32 37 38 40 43 44 45 51 52 57
60 62 73 74 78 79 85 89 92 98 100 101 103 106 108 109 111 114
119 123 125 126 133 137

Appendix 1.6 cont.

P. ipomoeae:

5 25 28 41 49 54 56 59 61 68 71 75 76 80 81 83 87 93 96 97 102
107 112 118 120 121 124 127 128 130 132 135 141 145 149 154
156 160 161 163 166 174 180 182 194 198 199 206 217 218 224
230 241 257 271 272 273 284 294 314 332 333 336 338 347 352
356 358 361 363 364 367 369 370 374 375 376 378 393 400 403
404 411 412 413 414 442 463 468 469 471 473 474 479 490 501
504 514 516 517 524 525 530 531 532 539 551 564 565 573 582
583 585 134 256 401 557 11 16 47 53 104 105 157 183 200 253
317 397 398 523 4 6 10 12 13 20 24 26 27 29 30 32 37 38 40 43
44 45 51 52 57 60 62 73 74 78 79 85 89 92 98 100 101 103 106
108 109 111 114 119 123 125 126 133 137

P. mirabilis:

594 589 584 578 576 572 563 560 542 533 520 515 507 476 467
460 454 451 450 447 443 426 424 423 417 416 399 396 384 383
380 366 342 335 331 328 322 315 313 309 293 291 244 243 239
228 213 210 197 195 192 173 153 151 117 115 95 91 86 84 70 69
67 65 46 39 31 15 14 134 256 401 557 11 16 47 53 104 105 157
183 200 253 317 397 398 523 4 6 10 12 13 20 24 26 27 29 30 32
37 38 40 43 44 45 51 52 57 60 62 73 74 78 79 85 89 92 98 100
101 103 106 108 109 111 114 119 123 125 126 133 137

Appendix 2.1. Isolates of *Phytophthora* used for this study. Genbank accession numbers are indicated.

		Region[a]				
Species	**Isolate**	**Intron Ras**	**Ras**	**Elong**	**Tubu**	**P4**
P. andina	EC3163	DQ864567	X[b]	X		EF011132
	EC3164	DQ864568		X	X	EF011133
	EC3165	DQ864569		X		EF011134
	EC3167	DQ864571	X	X	X	X
	EC3189	DQ864572	X	X	X	X
	EC3190	DQ864573	X	X	X	X
	EC3417	DQ864574	X	X	X	X
	EC3421	DQ864575	X	AY564102	X	AY564160
	EC3425	DQ864584	X	X	X	X
	P13365			EU080184		
	P13400			EU080191		
	EC3237	DQ864577	X			EF011154
	EC3238	DQ864578	X	X	X	X
	EC3394	DQ864583	X	X	X	X
	EC3414	DQ864576	X	X		X
P. infestans	EC3099	DQ864589	X	X	X	X
	EC3300	DQ864593	X	X	X	X
	EC3336	DQ864596	X	X		X
	EC3301	X	X	X		
	PCZ033	EF366869	EF367025	X	X	
	PCZ098	EF366871	EF367027		X	EF366758
	PHU006	EF366875	EF367031		X	EF366760
	POX004	EF366878	EF367034		X	
	PCO038	EF366879	EF367035		X	
	PCZ118	EF366873	EF367029	X	X	EF366759
	Pic 97180	EF366901	EF367057			EF366771
	Pic 97207	EF366904	EF367060	X	X	EF366772
	Pic 97224	EF366906	EF367062	X	X	EF366773
	Pic 97322	EF366907	EF367063	X	X	EF366774
	Pic97370	EF366909	EF367065	X	X	EF366775
	Pic 97388	EF366912	EF367068	X	X	EF366776
	Pic 97605	EF366914	EF367070	X	X	EF366777
	Pic 97620	EF366915	EF367071	X	X	EF366778
	Pic 97630	EF366918	EF367074	X	X	X
	Pic 97652	EF366919	EF367075		X	EF366779
	Pic 98301	EF366922	EF367078	X	X	X
	Pic 98305	X	X	X		EF366780
	Pic 98366	EF366924	EF367080	X	X	X
	Pic 98369		X	X		
	Pic 98372	EF366925	EF367081	X	X	X

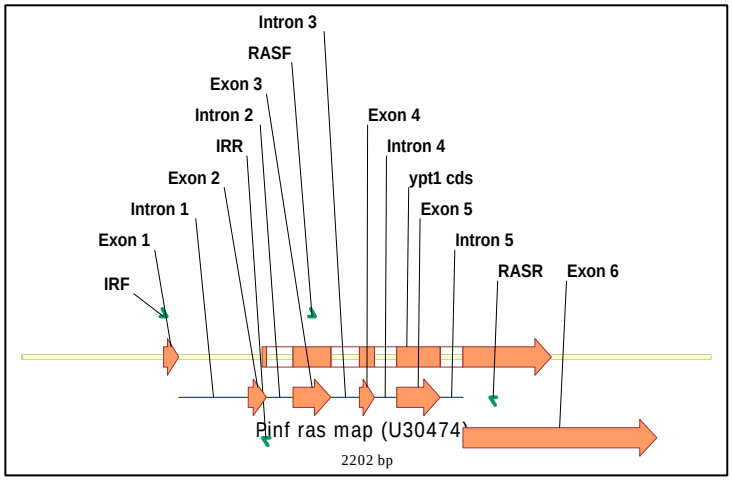
Appendix 2.1 cont.

		Region				
Species	**Isolate**	**Intron Ras**	**Ras**	**Elong**	**Tubu**	**P4**
	Pic 98388		X	X	X	
	Pic 98392		X	X	X	X
	P10650			EO079627		
P. mirabilis	Pic 99114	X	X	X	X	X
	G9-5	DQ864600				EF011140
	G4-4	DQ864599	X	AY564098	AY564041	EF011139
	P3007	DQ864601	X	X	X	X
	P3005	X	X	EU079777	EU079776	X
	P3001	X		AY564096	AY564039	AY564154
	Pic 99145	X		AY564097	AY564040	AY564155
	G15-4	X	X	AY564099	AY564042	AY564157
	Pic 99129	X		AY564095	AY564038	AY564153
P. ipomoeae	Pic 99167	X	X	X	X	X
	Pic 99139	DQ864602				EF011138
	P10227			EU080846	EU080845	
	P10225			EU080832	EU080831	
	Pic 99165	X		AY564100	AY564043	AY564158
	P10226			EU080839	EU080838	
P. phaseoli	Phy P18	X	X	X	X	X
	P22	EF012279	X	X	X	
	CBS 556.88			AY564101	AY564044	AY564159
	P10150			EU080763	EU080762	
	P6609			EU079915	EU079914	
	P10145				EU080749	
	P10146				EU080756	
P. ilicis	384	DQ864607				
	PD 91/595			AY564127		AY564186
	P6860				EU080137	
	SCRP379		DQ162963			

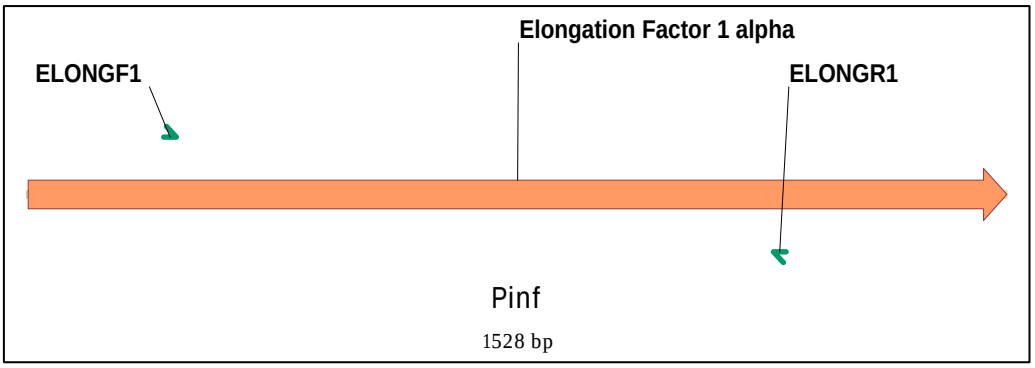
[a]Region corresponds to the primer used for amplification. Intron ras amplifies the first intron of the *ras* gene; Ras the *ras* gene; Elong corresponds to *Elongation factor 1α*; Tubu to *β tubulin*; and P4 to part of the *cox1* gene and part of the *atp9* gene.
[b]X indicates sequences obtained from direct PCR of DNA.

Appendix 2.2. Primer maps of nuclear (A-C) and mitochondrial (D) regions sequenced for this study. Size given is the total size of the gene in *P. infestans*.

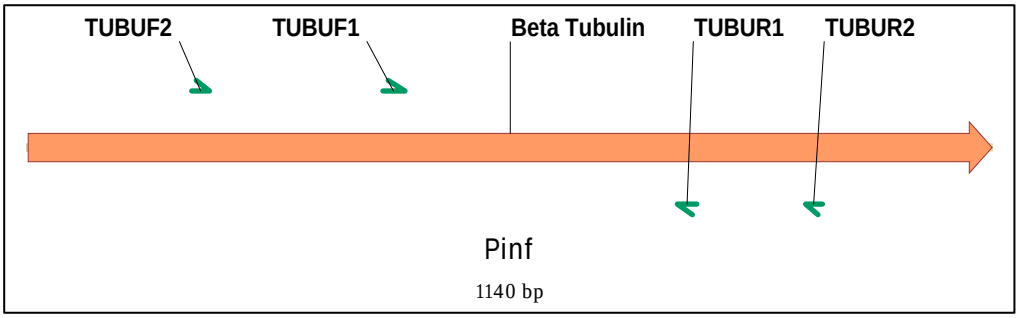
A



B

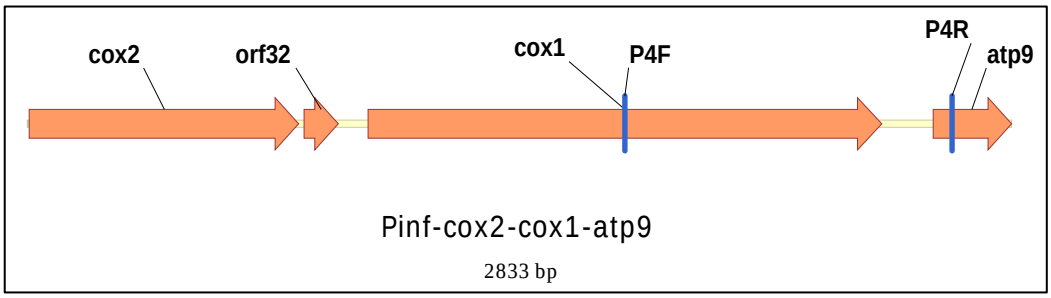


C

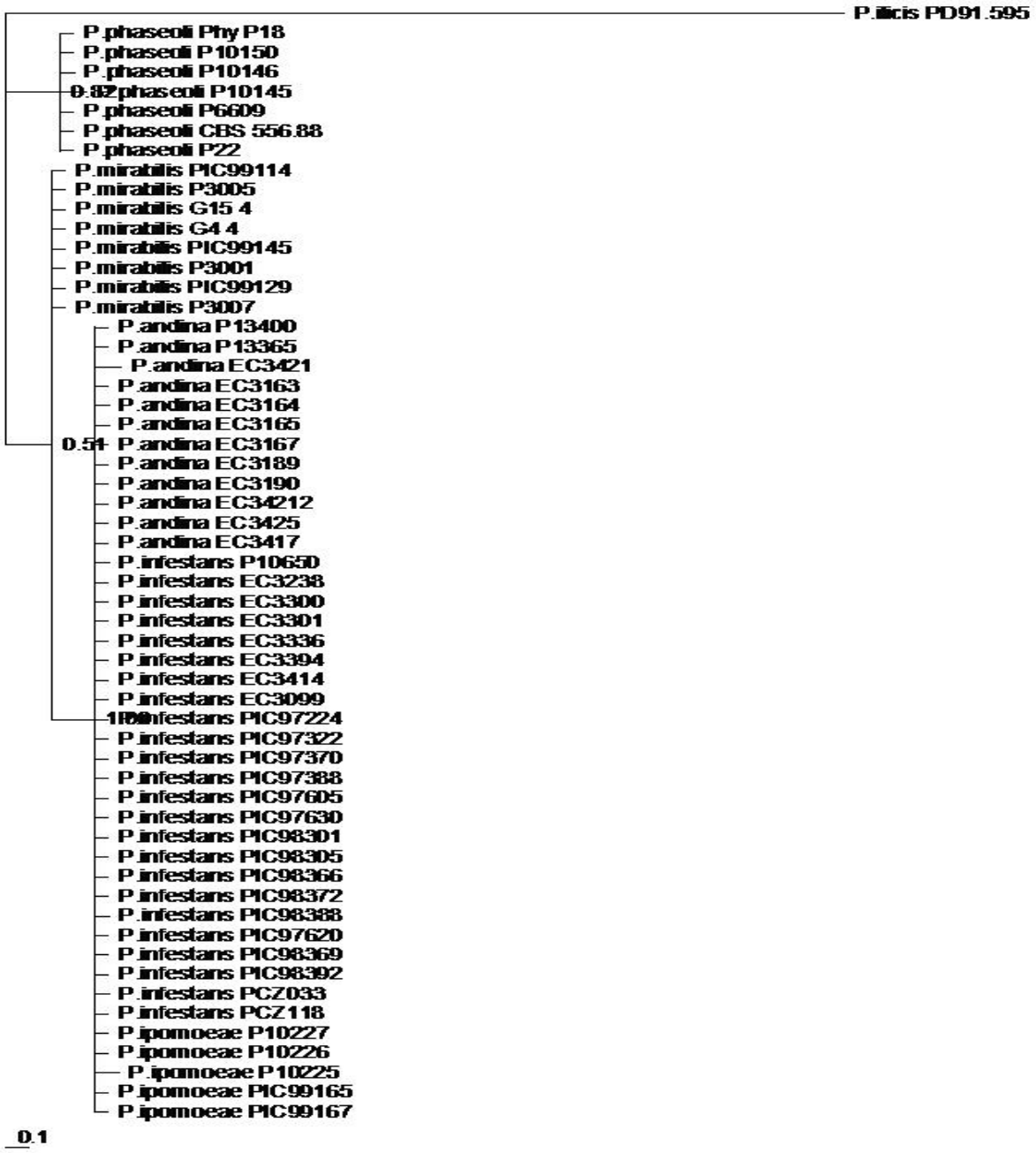


Appendix 2.2 cont.

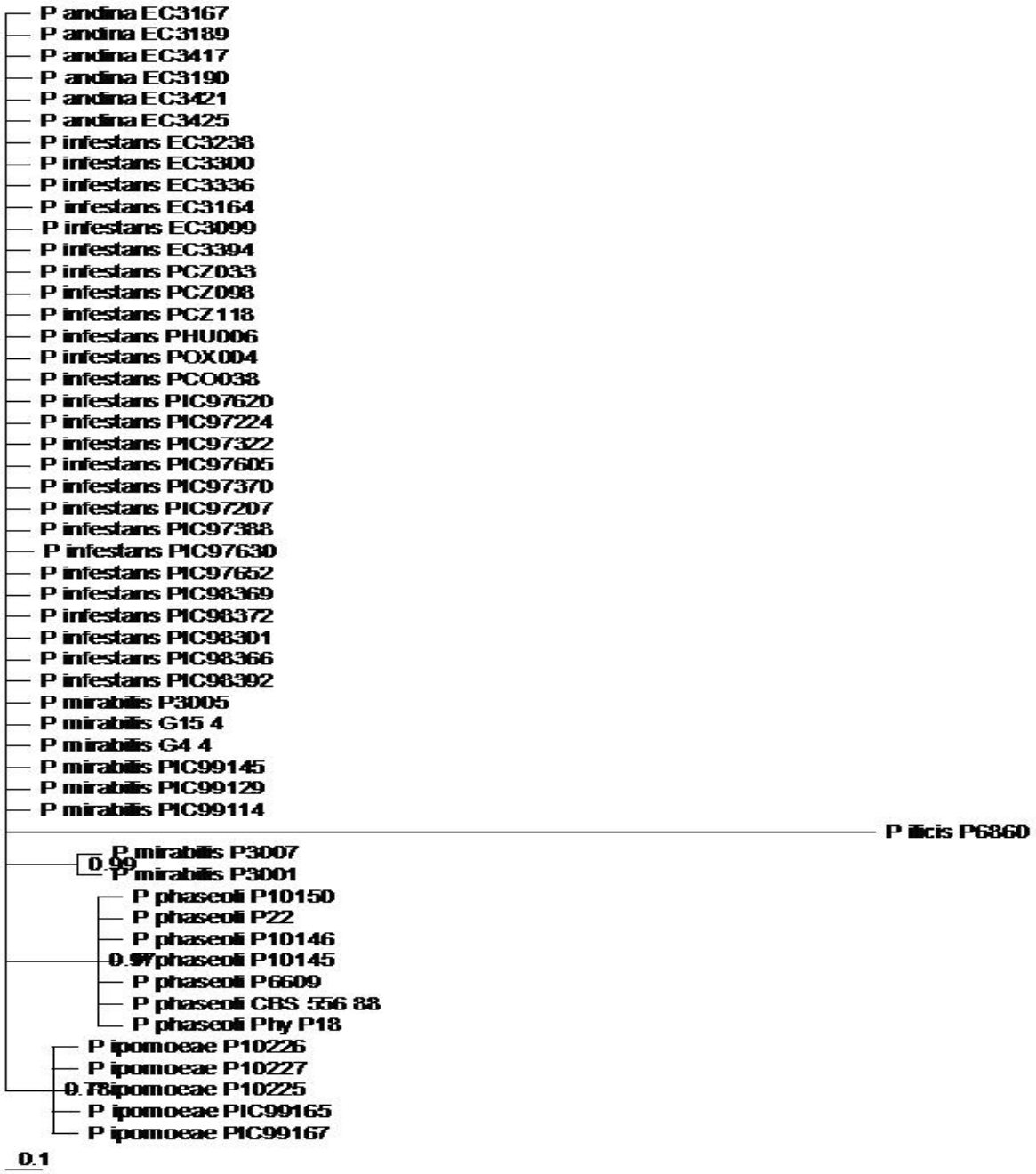
D



Appendix 2.3. Phylogenetic tree of species in the *Phytophthora* Ic clade using all gene sequences available for *Elongation factor 1α*.

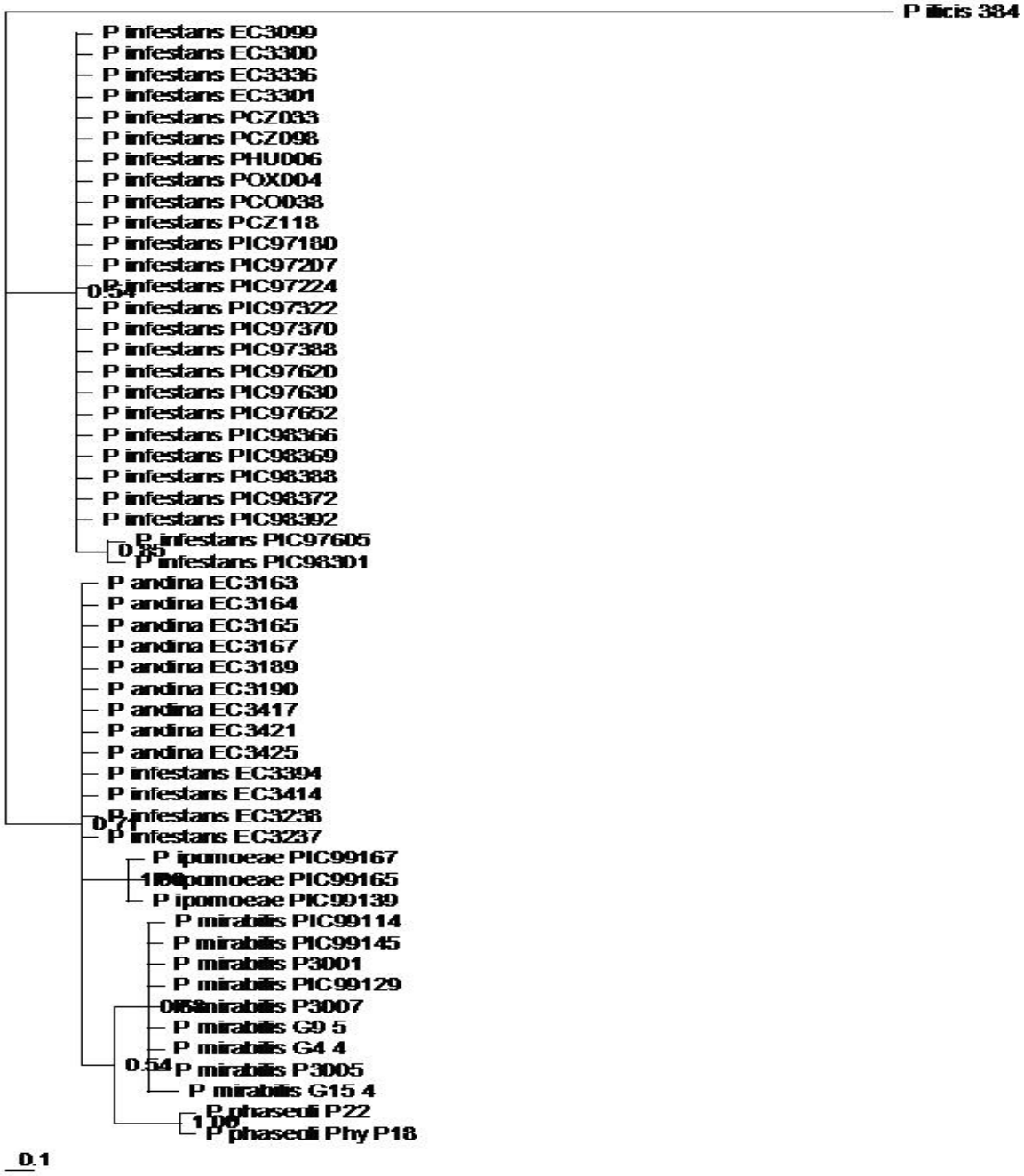


Appendix 2.4. Phylogenetic tree of species in the *Phytophthora* Ic clade using all gene sequences available for *β tubulin*.



Appendix 2.5. Phylogenetic tree of species in the *Phytophthora* Ic clade using all gene sequences available for *ras*.

Appendix 2.6. Phylogenetic tree of species in the *Phytophthora* Ic clade using all gene sequences available for intron 1 of *ras*.



Appendix 2.7. Phylogenetic tree of species in the *Phytophthora* Ic clade using all gene sequences available for P4 (*cox1* and *atp9*).