

1 **ABSTRACT**

2 The complete sequences of the mitochondrial genomes of the oomycetes of *Phytophthora*
3 *ramorum* and *P. sojae* were determined during the course of their complete nuclear
4 genome sequencing (Tyler et al. 2006). Both are circular, with sizes of 39,314 bp for *P.*
5 *ramorum* and 42,975 bp for *P. sojae*. Each contains a total of 37 identifiable protein-
6 encoding genes, 25 or 26 tRNAs (*P. sojae* and *P. ramorum*, respectively) specifying 19
7 amino acids, and a variable number of ORFs (7 for *P. ramorum* and 12 for *P. sojae*)
8 which are potentially additional functional genes. Non-coding regions comprise
9 approximately 11.5% and 18.4% of the genomes of *P. ramorum* and *P. sojae*,
10 respectively. Relative to *P. sojae*, there is an inverted repeat of 1,150 bp in *P. ramorum*
11 that includes an unassigned unique ORF, a tRNA gene, and adjacent non-coding
12 sequences, but otherwise the gene order in both species is identical. Comparisons of
13 these genomes with published sequences of the *P. infestans* mitochondrial genome
14 reveals a number of similarities, but the gene order in *P. infestans* differs in two adjacent
15 locations due to inversions. Sequence alignments of the three genomes indicated
16 sequence conservation ranging from 75 to 85% and that specific regions were more
17 variable than others.

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19 Keywords: inverted repeat, *Phytophthora infestans*

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INTRODUCTION

1
2 The genus *Phytophthora* has a wide geographic distribution throughout the world and
3 contains more than 70 species, many of which cause important plant diseases (Erwin and
4 Ribiero 1996). While members of the genus, along with other oomycetes, share
5 morphological similarities with eumycotian fungi, these features have arisen
6 independently, because oomycetes are more closely related phylogenetically to
7 chromophyte algae within the larger group Stramenopiles (Förster et al. 1990a; Knoll
8 1992; Baldauf and Palmer 1993; Wainright et al. 1993; Bhattacharya and Stickel 1994;
9 Weerakoon et al. 1998; Dick 2001). Members of this genus differ from eumycotan fungi
10 in features such as being diploid throughout their life cycle and formation of motile,
11 biflagellate spores called zoospores that are capable of swimming in water.

12
13 The mitochondrial genomes in the genus have been reported to be circular and to range in
14 size from approximately 37.0 to 45.3 kb (Paquin et al. 1997, Avila-Adame et al. 2005;
15 McNabb and Klassen 1988, Förster et al. 1987, Shumard-Hudspeth and Hudspeth 1990).
16 These have been commonly used in RFLP studies for identification of isolates and to help
17 clarify the taxonomic placement of particular species (reviewed in Erwin and Ribeiro
18 1996). Mitochondrial gene sequences also have been used to infer phylogenetic
19 relationships among species of the genus as well (Martin and Tooley 2003, 2004; Kroon
20 et al. 2004). The only species of *Phytophthora* for which we have a complete
21 mitochondrial genome sequence is *P. infestans*, the causal agent of potato late blight.
22 This has been determined for four separate haplotypes, which range in size from 37,957
23 bp for the Ia haplotype (Paquin et al. 1997) and from 37,992 to 39,870 bp for the Ia, IIa,

1 and Iib haplotypes (Avila-Adame et al. 2005). A total of 67 shared coding regions were
2 identified in these genomes encoding for mitochondrial respiratory chain proteins,
3 subunits of the mitoribosome, ribosomal RNAs, tRNAs, and unassigned ORFs. This
4 same set of coding regions were also found in the related Peronosporomycete
5 *Saprolegnia ferax* (Grayburn et al. 2004). Comparisons among the different *P. infestans*
6 haplotypes indicate that intraspecific variation is due to changes in nucleotide sequences
7 dispersed throughout the genome as well as length mutations caused by
8 insertions/deletions that occurred primarily in two locations. It is unclear how this
9 intraspecific variation relates to interspecific variability and genome divergence among
10 species.

11

12 Interest in the genus *Phytophthora* has increased lately due to the serious impact several
13 species are having as plant pathogens. *Phytophthora ramorum* is a recently described
14 species that initially was found to be responsible for diseases of nursery crops in
15 Germany and the Netherlands (Werres et al. 2001). It has subsequently spread to other
16 European countries and more recently has become a problem in field ecosystems (Brasier
17 et al. 2005). While this species also is a problem in some nursery production crops in
18 North America, a far bigger impact has been its role as the cause of sudden oak death, a
19 disease that has killed large numbers of trees and shrubs in natural ecosystems in central
20 coastal California (Rizzo et al. 2002, Davidson et al. 2003). This is a highly regulated
21 pathogen with stringent quarantine restrictions in place in North America and Europe in
22 an effort to halt its spread. *Phytophthora sojae* is widely spread in soybean (*Glycine*
23 *max*) production areas of North America and Australia and causes serious crop

1 production losses due to root and stem rot (Erwin and Ribiero 1996). There is a
2 continuing effort in soybean breeding programs to develop resistant germplasm as a
3 means for controlling the disease.

4

5 Complete draft sequences for the nuclear genomes of *P. ramorum* and *P. sojae* have been
6 recently determined (Tyler et al. 2006). As part of this sequencing project, the complete
7 mitochondrial genomes were also assembled. The objective of this submission is to
8 annotate and describe these mitochondrial genomes and compare them to the
9 mitochondrial genomes of *P. infestans* and other Peronosporomycetes.

10

11

MATERIALS AND METHODS

12 **Strains sequenced** – Mitochondrial sequences were obtained from *P. ramorum* strain Pr-
13 102 (isolated from California) and *P. sojae* strain P6497 (isolated from Mississippi).

14 Sequences for the Ia haplotype mitochondrial genome of *P. infestans* (Paquin et al. 1997)
15 were obtained from GenBank (NC002387), as were those recently available for
16 haplogypes Ib, IIa, and IIb (AY894835, AY898627, AY898628; Avila-Adame et al.
17 2005) and the peronosporomycete *S. ferox* (AY534144; Grayburn et al. 2004)

18

19 **Sequencing and contig assembly**– For each of these two species, total DNA
20 preparations were randomly sheared using a Hydroshear device (Gene Machines, **location**
21 **needed**), in separate aliquots, to fragments averaging either about 3 kb or about 8 kb.

22 These were gel purified and enzymatically repaired to blunt ends, then cloned into
23 plasmids to generate two genomic libraries. An additional library was created in a

1 fosmid vector. End sequences were determined for a large number of randomly selected
2 clones from each of these libraries, then assembled using JAZZ (**REF**) to form a
3 complete draft whole-genome shotgun assembly (**REF**) of these nuclear genomes.

4 Detailed protocols are available at <**WEBSITE**> and this process and the results will be
5 further described in a manuscript reporting the complete nuclear genome sequences.

6 Although no effort was expended to target the mitochondrial genomes, even a small
7 contamination by mtDNA in these preparations, coupled with the high molarity of these
8 sequences compared to any portion of the nuclear genomes, guarantees that any whole-
9 genome shotgun sequencing projects will include many sequencing reads from clones of
10 mtDNA. **ADD STATISTICS SPECIFIC FOR THE MTDNAS ON THE NUMBER**
11 **OF READS FROM EACH LIBRARY, THE DEPTH OF COVERAGE, AND THE**
12 **OVERALL QUALITY OF THE ASSEMBLY AND OF THE CONSENSUS**
13 **SEQUENCE.**

14

15 **Annotation and comparative genomics** - Annotation of coding regions was done using
16 DS Gene v1.5 (Accelrys, San Diego, CA). Identification of protein- and rRNA-encoding
17 genes was done by comparison with sequences reported for *P. infestans* (Paquin et al.
18 1997; NC002387) and BLAST analysis to other sequences in GenBank. Genes for
19 tRNAs were found using tRNAscan SE v1.1 (Lowe and Eddy 1997;
20 <http://www.genetics.wustl.edu/eddy/tRNAscan-SE/>). Comparisons among genomes was
21 done using mVISTA (Mayor et al. 2000, Frazer et al. 2004;
22 <http://genome.lbl.gov/vista/servers.shtml>). Sequence alignments within mVISTA were
23 done using LAGAN (Brudno et al. 2003).

1

2

RESULTS AND DISCUSSION

3 **Genome size and organization**

4 The mitochondrial genomes for both species are circular and range in size from 39,314
5 bp for *P. ramorum* (Fig. 1; GenBank XXX) to 42,975 bp for *P. sojae* (Fig. 2; GenBank
6 XXX) with a %GC content of 22.0% and 21.7%, respectively. This compares to 37,957
7 bp for the Ia haplotype (Paquin et al 1997) and 37,992, 39,870 and 39,840 bp for the Ia,
8 IIa, and IIb haplotypes of *P. infestans*, respectively (Avila-Adame et al. 2005). The 37
9 protein- and rRNA-encoding genes found in *P. infestans* mtDNA (Paquin et al. 1997) are
10 also present in each of these two other species and similar to *P. infestans*, none contain
11 introns. This set comprises 18 respiratory chain proteins, 16 ribosomal proteins, the
12 rRNAs for the large and small ribosomal subunits, and an import protein (*secY*) (Fig. 3).
13 ATG was the start codon for all genes and with the exception of *nad11* (TGA), the
14 termination codon for all assigned genes is TAA. This gene had the same termination
15 codon in *P. infestans* (Paquin et al. 1997), but in *S. ferax* it was TAA (Grayburn et al.
16 2005).

17

18 There are a total of 19 amino acids encoded with the same 25 tRNAs as reported for *P.*
19 *infestans* (Paquin et al. 1997) including multiple tRNAs for *trnG* (GCC, UCC), *trnL*
20 (UAA, UAG), *trnR* (UCU, GCG), *trnS* (GCU, UGA) and three copies of *trnM* (CAU)
21 identified by tRNA Scan. But one of these *trnM_{CAU}* is identical to what was classified as
22 *trnI_{CAU}* in *P. infestans* and *S. ferax* (Paquin et al. 1997, Grayburn et al. 2004). For both
23 these species it was determined that in view of the conclusions of Gray et al. (1998) that

- 1 this tRNA is actually a *trnI_{CAU}* due to post transcriptional modification to lysidine to
- 2 allow translation of the AUA codon for isoleucine. The other two copies of *trnM_{CAU}*
- 3 function in initiator and elongator roles. *TrnT* is not encoded in these genomes.
- 4 *Phytophthora ramorum* has an additional copy of *trnR_{UCU}* adjacent to *cob* relative to *P. sojae* due to this tRNA being encoded in the inverted repeat (

1 10.8 to 25% at an amino acid level. This compares to a sequence divergence for the *cox*
2 2 mitochondrially encoded gene of 5.9 to 6.0% at a nucleotide level and 1.6 to 3.5% at an
3 amino acid level. It is questionable if *orf79* represents a functional gene since it has
4 limited sequence conservation among *P. infestans*, *P. ramorum* and *P. sojae* at either a
5 nucleotide or amino acid level (Table 2). There were six and one additional putative
6 ORFs greater than 100 bp in *P. sojae* and *P. ramorum*, respectively, ranging in size from
7 294 to 621 bp that were not present in the other species. The six additional ORFs in *P.*
8 *sojae* are present in two locations; *orf206* is between *trnY_{GUA}* and the *rrnS* while the
9 remaining five (*orf115*, *orf97*, *orf111_a*, *orf111_b*, and *orf100_b*) are clustered together
10 between *nad6* and *nad4L* (Fig. 2). The 3' end of *orf115* overlaps the 5' end of *orf97* by
11 20 bp and the 3' end of *orf97* overlaps *orf111_a* by 17 bp. There is only one unique ORF
12 (*orf175*) longer than 100 bp in *P. ramorum* mtDNA, and this is part of the inverted repeat
13 and is present in two copies in opposite orientation (Fig. 1 and discussed below). The
14 termination codon for all of these unassigned ORFs is TAA with the exception of *orf97*
15 and *orf111_b* (TAG) and *orf111_a* (TGA). Both these termination codons are used in
16 different ORFs in haplotype IIa and IIb of *P. infestans* (Avila-Adame et al. 2005).
17 BLAST analysis of sequences in GenBank did not identify any sequence homology
18 among these ORFs or potential homologs for these putative coding regions.

19

20 The coding regions are closely packed in the genome with 70% of the spacer regions less
21 than 30 bp long (Table 3). Overall the spacer regions represent a relatively small
22 percentage of the genome compared to coding regions (11.5% and 18.4% for *P. ramorum*
23 and *P. sojae*, respectively). Genes are divided between the two strands and in general are

- 1 clustered into five non-overlapping groups alternating between strands (Fig. 1 and 2).
- 2 Starting at base 1 the first group is *rrnL* through *cox1* followed by *trnR* through *nad 2*,
- 3 *nad7* through *nad6*, *nad4L* through *trnL*, and *rpl2* to *orf100*. There are some exceptions
- 4 to these groupings, most notably transcription of *orf206* in *P. sojae* and *secY* through
rps13 ¹ e c i ¹ c q 0.24 0 0 0.24d

1 **Inverted repeat**

2 One unique feature of the *P. ramorum* genome is that it contains an inverted repeat (IR)
3 of 1,150 bp located in one case between *cox1* and *cob* (bases 9,540 to 10,689) and again
4 in opposite orientation between *nad6* and *nad4L* (bases 26,173 to 27,322). This inverted
5 repeat contains *orf175*, which is unique to this species, as well as *trnR_{UCU}*. The first
6 copy of the IR starts eight bp after the termination codon of *cox1* and the opposite end
7 includes 13 bp of the 3' end of *cob*. The second copy starts with 74 bp of the 3' end of
8 the *nad6* gene and has 38 bp of the 3' end of *nad4L*. This copy of the IR is in the same
9 position of the genome as the clustered five unique ORFs of *P. sojae*. Comparisons of
10 sequences between *cox1* and *cob* and *nad6* and *nad4L* for *P. ramorum* and *P. sojae* (the
11 region where the IR is found in *P. ramorum*) revealed limited sequence similarity
12 between these two species. Likewise, there was limited sequence similarity in
13 comparison of region between *nad6* and *nad4L* from both these species and *P. infestans*.
14 Without further analysis of a greater number of mitochondrial genomes in the genus it is
15 unclear if the IR arose from duplication of a specific region of the genome or if its
16 presence in a reduced state reflects a deletion of the large IR found in other genera in the
17 Peronosporomycetes (discussed more below). However, given that the single copy of
18 *trnR_{UCU}* in *P. infestans* and *P. sojae* is present adjacent to the *nad6* gene, and one of the
19 copies of this tRNA gene is in the same position for *P. ramorum*, it is possible that this
20 location would reflect the ancestral position and the other arm of the IR between *cox1*
21 and *cob* being the secondarily duplicated copy.

22

1 The only other example of a completely sequenced mitochondrial genome containing an
2 IR is *S. ferax*, which has an IR of 8,618 bp, representing 37% of the genome size and
3 encoding four proteins, five tRNA genes, and both rRNAs (Grayburn et al. 2004). These
4 coding regions are transcribed from both strands of the mitochondrial genome, which is
5 different from the two coding regions in the IR of *P. ramorum* transcribed in the same
6 direction. One similarity between these genomes is that in *S. ferax*, one arm of both
7 copies of the IR terminates with a partial sequence of a coding region (the 3' end of
8 *nad5*), whereas in *P. ramorum*, three of the four termini end in a coding region. One end
9 of the IR encodes the terminal 13 bp of the 3' end of *cob* and the same end of the second
10 copy has 74 bp of the 3' end of *nad6*. The opposite end either terminates within the
11 spacer region before the *cox1* gene in one copy or has 38 bp of the 3' end of *nad4L* in the
12 other.

13

14 The presence of an IR in *P. ramorum* is unusual for the genus *Phytophthora*. The only
15 other example where this has been observed is in *P. megasperma* (Schumrd-Hudspeth
16 and Hudspeth 1990). Based on restriction mapping and Southern analysis a short
17 inverted repeat of 0.5 to 0.9 kb in size was identified with one copy adjacent to *cox2* and
18 the other adjacent to the *cob/atp9* genes (this later position is similar to what was
19 observed for one arm of the IR in *P. ramorum*). Inverted repeats in the mitochondrial
20 genome are common in the closely related genus *Pythium* (McNabb et al. 1987, McNabb
21 and Klassen 1988, Martin 1991, Martin 2000) and have also been found in other
22 oomycetes such as *Achyla* spp. (Hudspeth et al. 1983, Boyd et al. 1984, Schumard et al.
23 1986), *Aplanopsis terrestris*, *Leptolegnia caudate* and *Sapromyces elongates* (McNabb

1 and Klassen 1988) and *Saprolegnia ferax* (Grayburn et al 2004). An IR also has been
2 reported in the chytrid *Hypochytrium catenoides* (McNabb et al. 1988). In cases where
3 an IR has been described this region represents reflect a larger proportion of the genome
4 size (greater than 37% overall, but more than 71% for *Pythium* spp.) and contains the
5 large and small ribosomal RNA coding regions, which was not the case for the IR
6 observed with *P. ramorum*.

7

8 **Genome comparisons**

9 With the exception of the IR in *P. ramorum* the gene order in *P. sojae* was the
10 same, however, with *P. infestans* there are two inversions relative to *P. ramorum* and *P.*
11 *sojae* that have reversed the gene order in adjacent regions (Fig. 3). One inversion
12 includes *cob*, *nad9* and *atp9* while the other is immediately adjacent and includes a total
13 of 18 coding regions spanning from *nad3* to *atp1* (Fig. 3). The gene order in several
14 regions also were found to be conserved with *S. ferax* (Fig. 3), including the linkage of
15 *rps8*, *rpl6*, *rps2*, and *rps4* that Grayburn et al. (2004) noted was also conserved in the
16 stramenopile *Chrysodidymus synuroides* (Chesnick et al. 2000). Although the gene order
17 of more *Phytophthora* species needs to be examined to confirm this, the conservation of
18 gene order in *P. ramorum* and *P. sojae* relative to *P. infestans* may be reflective of the
19 evolutionary relationship among these species. In phylogenetic analysis using *cox2* and
20 rDNA ITS data, *P. ramorum* and *P. sojae* were more closely grouped, with *P. infestans*
21 being less so (Martin and Tooley 2004). A similar relationship was observed for analyses
22 done with sequence data from the ITS region (Cooke et al. 2000) and data from β -tubulin,
23 elongation factor 1- α , *cox1*, and *nadh1* (Kroon et al. 2004).

1

2 Whole genome sequence alignments between *P. ramorum* and *P. sojae* revealed a
3 sequence conservation of 83% and 76% identity, respectively (Table 4) with the lower
4 value for *P. sojae* due to the larger genome size. Sequence alignments were also done
5 with the type 1b mitochondrial haplotype of *P. infestans*, which is smaller than the other
6 genomes at 37,957 bp (Paquin et al. 1997). The Ia haplotype of *P. infestans* has 85%
7 sequence identity with the other two species whereas *P. ramorum* and *P. sojae* have 82%
8 and 75.4% identity with *P. infestans*, respectively.

9

10 Genome comparisons using mVISTA provides a graphic representation of the variation
11 among genomes. Using *P. ramorum* as the base sequence, comparison with *P. sojae*
12 reveals a low level of sequence variation throughout the genome, but specific regions
13 were more variable than others (Fig. 4). The most extensive variation was found in the
14 spacer regions and in general, the larger the spacer region, the greater the sequence
15 variability between species. For example, the spacer region between *orf79* and *cox2* was
16 446 bp long for *P. ramorum* (327 bp for *P. sojae*) and exhibited a low level of sequence
17 conservation with *P. sojae* (Table 3, Fig. 4). Likewise, the regions between *trnY* and
18 *rrnS*, *nad9* to *atp9*, *atp9* to *nad3*, and *atp1* to *nad5* have similar low levels of sequence
19 conservation between species, as did the regions represented by the IR in *P. ramorum*.
20 However, this association between spacer length and sequence variation was not always
21 observed. The spacer region between *nad7* and *orf142* is 42 bp for *P. ramorum* (303 bp
22 for *P. sojae*) and has minimal sequence conservation while the spacer between *trnL* and
23 *nad11* is 144 bp for *P. ramorum* (149 bp for *P. sojae*) and has a sequence conservation of

1 79%. Likewise, the spacer between *nad2* and *nad7* is 116 bp for *P. ramorum* (113 bp for
2 *P. sojae*) and has a sequence conservation of 84.4%. Vista comparisons including the
3 mitochondrial genome of *P. infestans* (with specific regions reverse complemented to
4 account for the inversions) gave results that did not differ appreciably from those
5 observed in Fig. 4 (data not shown). In intraspecific comparisons of the four
6 mitochondrial haplotypes of *P. infestans*, the majority of the variation was observed
7 between *trnY* and *rrnS* as well as downstream of *orf79* (the regions where
8 insertions/deletions were observed; Avila-Adame et al. 2005), however, there is also a
9 region of 25 bp in the spacer between *nad3* and *nad5* where there is only 32% sequence
10 conservation between the IIb haplotype and the others (data not shown).

11

12 Some of the regions where high levels of sequence variability were observed in
13 comparisons between *P. ramorum* and *P. sojae* corresponded to the location of
14 differences in genomic organization among species. For example, the terminal regions of
15 the genomic inversions observed with *P. infestans* corresponded to regions of low
16 sequence similarity between *P. ramorum* and *P. sojae* (Fig. 4, between *cox1* - *cob*, *atp9* -
17 *nad3*, and *atp1* - *nad5*). One of these regions, the area between *cox1* and *cob*, is where
18 one arm of the inverted repeat is located in *P. ramorum*. The other arm is located
19 between *nad6* and *nad4L*, which also was where the five unique ORFs in *P. sojae* are
20 located. Furthermore, *orf206* in *P. sojae* is located between *trnY* and *rrnS*, which was the
21 same location in the genome of *P. infestans* where length mutations associated with the
22 major intraspecific differences in genomic sequences were observed (Avila-Adame et al.
23 2005). Another region of the *P. infestans* mitochondrial genome where smaller length

1 mutations were observed is downstream of *orf79* (34 and 36 bp in length), which is also a
2 region of sequence variation in comparisons between *P. ramorum* and *P. sojae*.

3 Interestingly, the spacer region in *P. infestans* between *nad3* and *nad5*, which is one
4 juncture of the inversion relative to *P. ramorum* and *P. sojae*, is also variable in
5 haplotype IIb relative to the other three haplotypes. From comparing the gene maps for
6 these three species it is interesting to note that two of the regions variable in interspecific
7 comparisons (between *cox1* and *cob* and *nad6* and *nad4L*) correspond to the head-to-head
8 juncture of two clusters of genes transcribed from opposite directions.

9

10 The results thus far suggest a high degree on gene order conservation in the genus
11 *Phytophthora* with the differences observed explained by two inversions. One reason for
12 this may be the large percentage of the genome represented by coding regions and the
13 small sizes of the intervening spacer regions conferring some level of genome stability.
14 When interspecific variation is observed (rearrangements, unique ORFs, IR) these tend to
15 be found in specific locations in the genome where there are low levels of interspecific
16 sequence conservation in the spacer regions. However, before firm conclusions about
17 genome stability can be drawn additional comparisons among more species is needed to
18 clarify this. This would also clarify the relationship between changes in genome
19 organization and phylogeny in the genus.

20

21

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11

1 Table 1. Differences in size (in bp) of specific genes among *Phytophthora infestans*, *P.*
 2 *ramorum*, and *P. sojae*.

3

4

<u>Gene</u>	<u><i>P. infestans</i>^a</u>	<u><i>P. ramorum</i></u>	<u><i>P. sojae</i></u>
<i>cob</i>	1,152	1,161	1,161
<i>rpl5</i>	534	528	534
<i>rps3</i>	804	816	831
<i>rps7</i>	474	432	432
<i>rps11</i>	417	420	417
<i>rps13</i>	414	417	414
<i>rps19</i>	234	237	237
<i>secY</i>	747	744	744

5

6 ^a Data from Paquin et al. 1997

1 Table 2. Sizes and % sequence divergence of open reading frames (ORF) shared among
 2 *Phytophthora infestans*, *P. ramorum*, *P. sojae*, and *Saprolegnia ferax*.

3
 4
 5

		bp	<i>P. ramorum</i>		<i>P. sojae</i>		<i>S. ferax</i> ^a	
			DNA	AA	DNA	AA	DNA	AA
Orf32	<i>P. infestans</i> ^b	99	15.2%	25%	11.1%	12.5%	-	-
	<i>P. ramorum</i>	99	-	-	8.1%	15.6%	-	-
	<i>P. sojae</i>	99	-	-	-	-	-	-
Orf64	<i>P. infestans</i>	204	7.8%	16.9%	5.9%	10.8%	30.9%	58.7%
	<i>P. ramorum</i>	198	-	-	6.1%	12.3%	28.8%	58.7%
	<i>P. sojae</i>	198	-	-	-	-	28.8%	58.7%
	<i>S. ferax</i>	195	-	-	-	-	-	-
Orf79	<i>P. infestans</i>	240	35%	53.2%	35.4%	61.04%	-	-
	<i>P. ramorum</i>	243	-	-	41.2%	55.1%	-	-
	<i>P. sojae</i>	243	-	-	-	-	-	-
Orf100	<i>P. infestans</i>	303	11.9%	18.0%	10.2%	16.0%	-	-
	<i>P. ramorum</i>	309	-	-	9.7%	17%	-	-
	<i>P. sojae</i>	303	-	-	-	-	-	-
Orf142	<i>P. infestans</i>	429	10.5%	20.1%	9.8%	16.2%	28.4%	53.24%
	<i>P. ramorum</i>	420	-	-	10.7%	18.7%	26.0%	49.6%
	<i>P. sojae</i>	429	-	-	-	-	27.3%	53.2%
	<i>S. ferax</i>	432	-	-	-	-	-	-
Orf217	<i>P. infestans</i>	654	11.8%	19.0%	8.9%	13.8%	-	-
	<i>P. ramorum</i>	660	-	-	9.7%	16.0%	-	-
	<i>P. sojae</i>	678	-	-	-	-	-	-
Cox 2	<i>P. infestans</i>	774	5.9%	1.6%	6.6%	2.7%	24.3%	26.2%
	<i>P. ramorum</i>	777	-	-	5.9%	3.5%	25.0%	27.0%
	<i>P. sojae</i>	777	-	-	-	-	25.1%	25.8%
	<i>S. ferax</i>	759	-	-	-	-	-	-

6

7 ^a Data from Grayburn et al. 2004

8

9 ^b Data from Paquin et al. 1997

1 Table 3. The sizes of spacer regions (bp) between coding regions and the percent
 2 sequence conservation in comparisons between *Phytophthora ramorum* and *P. sojae*.
 3

Spacer region	Spacer length <i>P. ramorum</i>	Spacer region	Spacer length <i>P. sojae</i>	% Identity
<i>rrnI</i> to <i>trnN_{GUU}</i>	6		6	100%
<i>trnN_{GUU}</i> to <i>trnS_{GCU}</i>	11		12	83.0%
<i>trnS_{GCU}</i> to <i>trnM_{CAU}</i>	13		20	60.0%
<i>trnM_{CAU}</i> to <i>trnP_{UGG}</i>	26		44	39.6%
<i>trnP_{UGG}</i> to <i>trnM_{CAU}</i>	13		13	92.3%
<i>trnM_{CAU}</i> to <i>rpl14</i>	18		20	80.0%
<i>rpl14</i> to <i>rpl5</i>	6		6	100%
<i>rpl5</i> to <i>trnG_{GCC}</i>	7		7	100%
<i>trnG_{GCC}</i> to <i>trnG_{UCC}</i>	100		96	83.0%
<i>trnG_{UCC}</i> to <i>trnY_{GUA}</i>	14		26	53.8%
<i>trnY_{GUA}</i> to <i>rrns</i>	234	<i>trnY_{GUA}</i> to <i>orfB^a</i>	53	47.2%
		<i>orfB</i> to <i>rns</i>	159	65.0%
<i>rrns</i> to <i>trnW_{CCA}</i>	33		48	58.3%
<i>trnW_{CCA}</i> to <i>orf79</i>	157		167	87.4%
<i>orf79</i> to <i>cox2</i>	446		327	65.0%
<i>cox2</i> to <i>orf32</i>	13		11	84.6%
<i>orf32</i> to <i>cox1</i>	103		110	82.8%
<i>cox1</i> to <i>cob</i>	1,145 ^b		1,181	64.4%
<i>cob</i> to <i>nad9</i>	45		46	82.6%
<i>nad9</i> to <i>atp9</i>	141		163	63.4%
<i>atp9</i> to <i>nad3</i>	186		389	40.4%
<i>nad3</i> to <i>trnD_{GUC}</i>	36		35	51.2%
<i>trnD_{GUC}</i> to <i>atp6</i>	50		47	64.7%
<i>atp6</i> to <i>cox3</i>	25		27	70.4
<i>cox3</i> to <i>rps7</i>	69		60	79.7%
<i>rps7</i> to <i>rps12</i>	-26		-26	overlap
<i>rps12</i> to <i>trnV_{UAC}</i>	20		19	75.0%
<i>trnV_{UAC}</i> to <i>trnI_{GAU}</i>	3		3	33.3%
<i>trnI_{GAU}</i> to <i>trnQ_{UUG}</i>	1		1	100%
<i>trnQ_{UUG}</i> to <i>trnR_{GCG}</i>	14		14	85.7%
<i>trnR_{GCG}</i> to <i>rps10</i>	4		4	75.0%
<i>rps10</i> to <i>trnF_{gaa}</i>	18		17	88.9%
<i>trnF_{GAA}</i> to <i>nad2</i>	6		6	100%
<i>nad2</i> to <i>nad7</i>	116		113	84.4%
<i>nad7</i> to <i>orf142</i>	48		303	12.5%
<i>orf142</i> to <i>trnH_{GUG}</i>	3		4	75.0%
<i>trnH_{GUG}</i> to <i>nad4</i>	26		30	66.6%
<i>nad4</i> to <i>trnE_{UUC}</i>	21		31	54.8%
<i>trnE_{UUC}</i> to <i>atp1</i>	75		81	81.4%
<i>atp1</i> to <i>nad5</i>	151		1,052	10.6%
<i>nad5</i> to <i>nad6</i>	68		62	70.4%
<i>nad6</i> to <i>trnR_{UCU}</i>	15		26	46.2%
<i>trnR_{UCU}</i> to <i>nad4L^c</i>	950		2,582	18.2%
<i>nad4L</i> to <i>nad1</i>	2		5	40.0%
<i>nad1</i> to <i>nad11</i>	-4		-4	overlap
<i>nad11</i> to <i>trnL_{UAG}</i>	143		149	79.0%
<i>trnL_{UAG}</i> to <i>trnL_{UAA}</i>	9		9	66.6%
<i>trnL_{UAA}</i> to <i>SecY</i>	17		20	60.0%
<i>SecY</i> to <i>orf64</i>	4		4	75.0%
<i>orf64</i> to <i>trnC_{GCA}</i>	11		27	37.0

<i>trnC_{GCA}</i> to <i>trnS_{UGA}</i>	4	5	80%
<i>trnS_{UGA}</i> to <i>rps11</i>	10	10	100%
<i>rps11</i> to <i>rps13</i>	12	12	91.7
<i>rps13</i> to <i>rpl2</i>	28	28	100%
<i>rpl2</i> to <i>rps19</i>	2	3	66.6%
<i>rps19</i> to <i>rps3</i>	3	3	66.6%
<i>rps3</i> to <i>rpl16</i>	2	2	100%
<i>rpl16</i> to <i>trnM_{CAU}</i>	3	7	42.9%
<i>trnM_{CAU}</i> to <i>orf217</i>	17	31	45.2%
<i>orf217</i> to <i>atp8</i>	65	61	82.0%
<i>atp8</i> to <i>trnK_{UUU}</i>	25	28	82.1%
<i>trnK_{UUU}</i> to <i>trnA_{UGC}</i>	2	2	100%
<i>trnA_{UGC}</i> to <i>rps14</i>	29	18	44.8%
<i>rps14</i> to <i>rps8</i>	7	7	100%
<i>rps8</i> to <i>rpl6</i>	5	8	50.0%
<i>rpl6</i> to <i>rps2</i>	6	8	75.0%
<i>rps2</i> to <i>rps4</i>	8	8	87.5%
<i>rps4</i> to <i>orf100</i>	2	7	28.6%
<i>orf100</i> to <i>rrn1</i>	280	27	9.5%

1

2

3 ^a In *P. sojae* there is a unique ORF (*orf206*) that splits this spacer region in two but it can
4 still be aligned with the spacer region in *P. ramorum*. There is virtually no sequence
5 homology between this spacer region in *P. ramorum* and *orf206* in *P. sojae*.

6

7 ^b This region in *P. ramorum* spans the inverted repeat and includes *orf175* and one copy
8 of *trnR(ucu)* but is all spacer in *P. sojae*.

9

10 ^c In *P. ramorum* this region includes the second copy of the inverted repeat encoding
11 *orf175* while in *P. sojae* it includes 5 ORFs (*orf115*, *orf97*, *orf111_a*, *orf111_b*, and
12 *orf100_b*).

13

1 Table 4. Sequence conservation in whole mitochondrial genome comparisons with
 2 *Phytophthora infestans*, *P. ramorum* and *P. sojae*.

3	4 Genome comparisons ^a	CNS ^b	Sequence identity
5	<i>P. ramorum</i> vs <i>P. sojae</i>	89.0%	83.0% (<i>P. ramorum</i>)
6			76.0% (<i>P. sojae</i>)
7	<i>P. ramorum</i> vs <i>P. infestans</i>	88.3%	82.0% (<i>P. ramorum</i>)
8			84.4% (<i>P. infestans</i>)
9	<i>P. sojae</i> vs <i>P. infestans</i>	91.6%	75.4% (<i>P. sojae</i>)
10			85.0% (<i>P. infestans</i>)

11
 12 ^a Mitochondrial genome sizes for *P. ramorum*, *P. sojae*, and *P. infestans* were 39,314 bp,
 13 42,975 bp and 37,957 bp (NC002387, Paquin et al. 1997), respectively.

14
 15 ^b CNS = sequence conservation above 75% over a 45 bp window in mVISTA
 16

1 **Figure legends**

2 Figure 1. Mitochondrial gene map for *Phytophthora ramorum*. Arrows indicate
3 transcriptional orientation, clockwise for the outer row and counter clockwise for the
4 inner row with green representing coding regions and red putative ORFs. The position of
5 the inverted repeat is indicated on the inner ring.

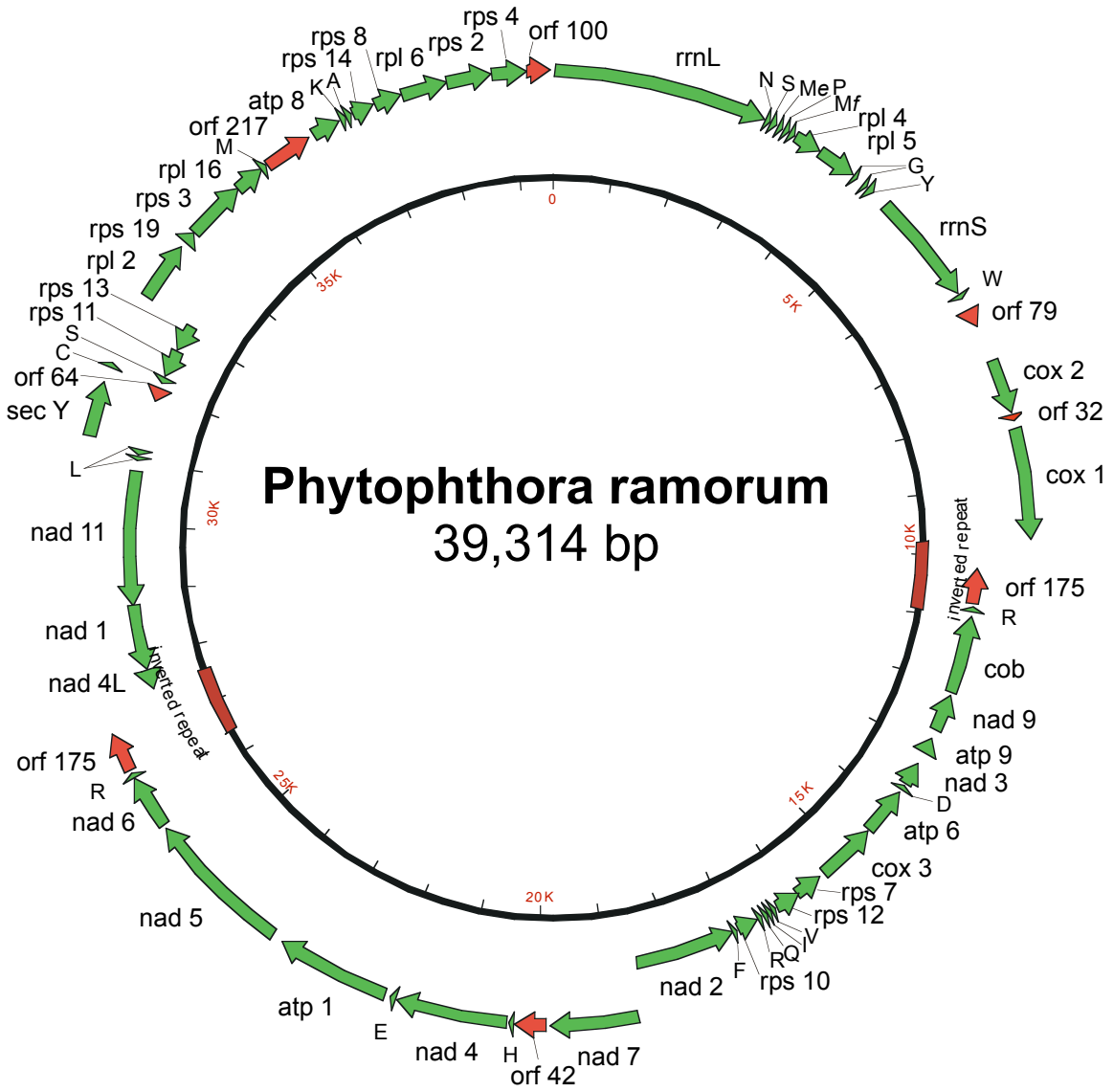
6

7 Figure 2. Mitochondrial gene map for *Phytophthora sojae*. Arrows indicate
8 transcriptional orientation, clockwise for the outer row and counter clockwise for the
9 inner row with green representing coding regions and red putative ORFs.

10

11 Figure 3. Conserved gene order in the mitochondrial genomes of *Phytophthora ramorum*
12 and *P. sojae*. Comparisons with *P. infestans* and *Saprolegnia ferax* were based on
13 Paquin et al. (1997) and Grayburn et al. (2004), respectively. The dashed line under
14 *orf217* is to indicate that in *S. ferax* this region is represented by *orf273* (which has
limited sequence conservation with *orf217*); otherwise the gene orde

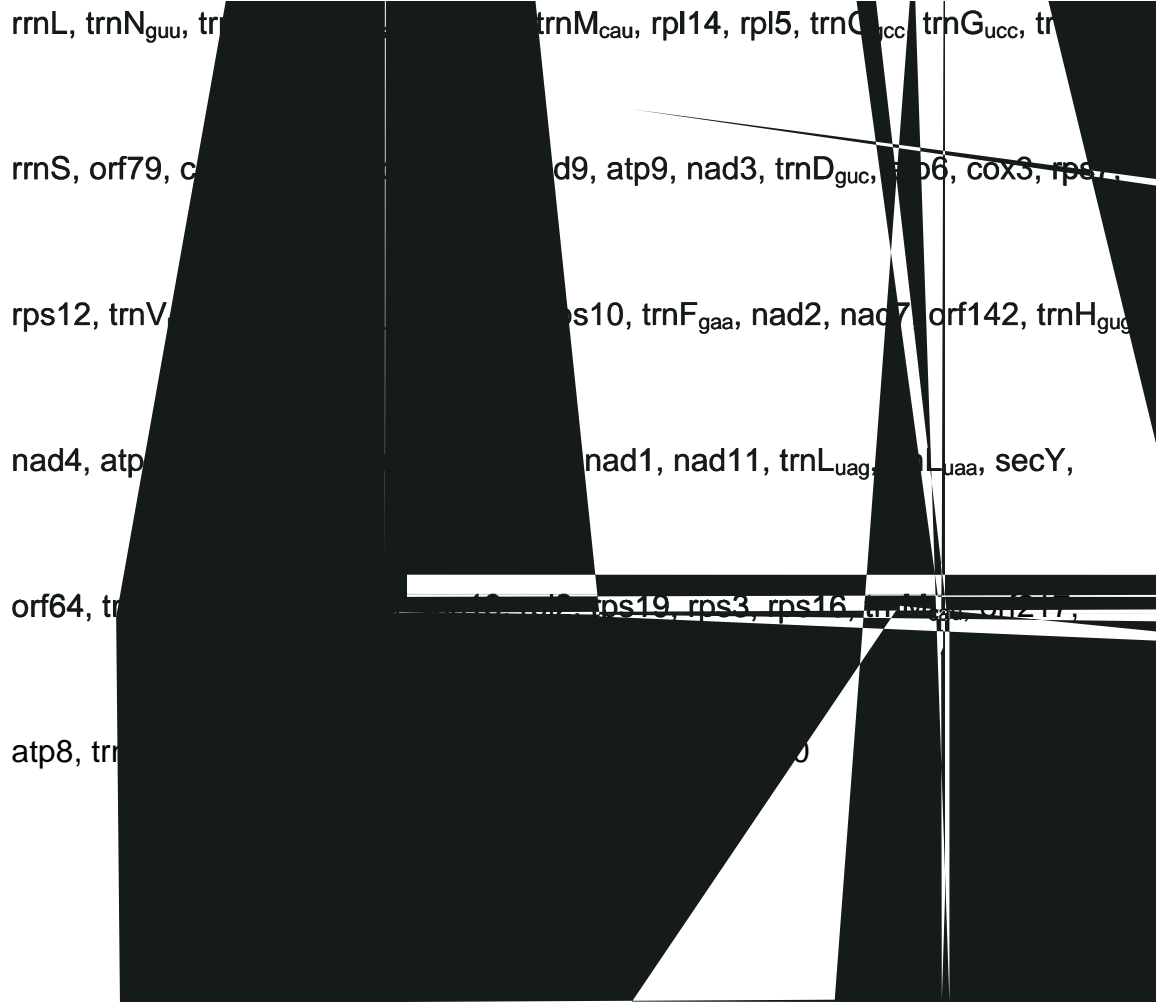
- 1 regions that were inverted in the *P. infestans* mitochondrial genome relative to *P.*
- 2 *ramorum* and *P. sojae*.
- 3



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