Phytophthora species identification using ITS rDNA and COI genes from DNA of culture samples and the *IDphy* website for BLAST and phylogenetic tree analysis

In this protocol, we present strategies for BLAST and draft phylogenetic analysis of sequences of the internal transcribed spacer rDNA (ITS rDNA) and cytochrome c oxidase subunit 1 (COI) generated from DNA extracted from cultures of *Phytophthora* suspect samples. This SOP, along with the steps for analyzing your sequence, can be found on the Molecular page of *IDphy: Molecular and Morphological Identification of Phytophthora based on the types*.

Topic	Table of contents	Page
I	Related SOPs	1
II	Introduction	1
III	Equipment, materials and reagents	2
IV	Primer preparation for amplification and sequencing	2
V	Sample preparation for PCR assays	3
VI	Master mix preparation for PCR assays	4
VII	Addition of sample or control DNA	5
VIII	Thermocycler parameters	5
IX	Gel electrophoresis, submission of samples to sequencing facility	5
X	Using the *IDphy* website to identify *Phytophthora* unknowns based on the ex-types and well-authenticated specimens	6
	References	18
	Citation	18
	Revisions	19
	APPENDIX	
	ITS and COI accession numbers – species in Clade 1	20
	ITS and COI accession numbers – species in Clade 2	21
	ITS and COI accession numbers – species in Clade 3, 4, 5 and no clade	22
	ITS and COI accession numbers – species in Clade 6	23
	ITS and COI accession numbers – species in Clade 7	24
	ITS and COI accession numbers – species in Clade 8	25
	ITS and COI accession numbers – species in Clade 9, 10	26
	Phytophthora not included in ITS rDNA tree and voucher sequences	27
	Phytophthora species of concern	28
	Phytophthora ITS rDNA phylogenetic tree for 161 species	29

I. Related SOPs

SOP-PID-01 Molecular: Equipment, materials, and reagents for DNA extraction and PCR
SOP-PID-02 Molecular: DNA extraction for *Phytophthora* species using Qiagen kit
SOP-PID-03 Molecular: Primer dilutions for PCR amplifications
SOP-PID-04 Molecular: Gels, electrophoresis, and photos

II. Introduction

The genus *Phytophthora*, with 182 species described as of May 2018, contains members that cause significant economic impact to crops, ornamentals, and forest ecosystems around the world. The genus contains 162 culturable species, one described with an assembled mitochondrial genome (*P.*

betacei, Mideros et al. 2018), eight unculturable species, five hybrids, three lost species, and three invalid species (see Appendix, page 26). Many species are of quarantine importance for the USA, including 29 species of concern listed by Plant Epidemiology and Risk Analysis Laboratory (Schwartzburg et al, 2009) (page 27), Offshore Pest Information System (OPIS), or Cooperative Agriculture Pest Survey (CAPS). In order to facilitate molecular identification for the 161 culturable *Phytophthora* species, we are providing in this SOP the voucher sequences of ITS rDNA and COI and an ITS rDNA draft phylogenetic tree system based on **161 species, including 139 ex-types and 22 well-authenticated specimens** (selected by Dr. Gloria Abad to be good candidates for epitypes for lost specimens). The ITS rDNA phylogenetic tree is the most updated for the genus *Phytophthora* since Cooke et al. (2000) published the ITS rDNA phylogeny including 49 species and 9 ex-types. This SOP is part of *IDphy: Molecular and Morphological identification of Phytophthora based on the types* (https://idtools.org/id/phytophthora/), which includes a Lucid key, a tabular key, and sequencing analysis.

III. Equipment, materials, and reagents

See SOP-PID-01 Molecular: Equipment, Materials, and Reagents for DNA extraction and PCR

- 1.7 mL microcentrifuge tubes
- 0.2 mL PCR 8-tube strips with attached flat caps
- Sterile filter (barrier) pipette tips for the corresponding pipets
- Gloves
- Molecular grade (MG)/DNase free water (any vendor)
- Primers and reagents for PCR amplification

IV. Primer preparation for amplification and sequencing

See SOP-PID-03 Molecular: Primers dilutions for PCR amplifications.

Note: Primer stock and working concentrations do not need to be prepared each time the PCR assay is conducted. Primer solutions are stored in small aliquots at the working concentration in a -20°C freezer until needed to avoid detrimental effects caused by repeated freeze/thaw of reagents. Primers should not be used longer than 12 months from date of preparation. It is recommended that new reagents be tested (using controls) as a measure of quality control prior to testing samples.

Order primers with purification: standard desalting.

Table 1. Primers for ITS rDNA amplification and sequencing

Primer	Sequence 5'-3'	Working concentration
ITS rDNA universal primers, White et al 1990		
ITS5	5' – GGAAGTAAAAGTCGTAACAAGG -3'	10 µM for amplification 5µM for sequencing
ITS4	5' – TCCTCCGCTTATTGATATGC – 3'	10 µM for amplification 5µM for sequencing

Table 2. Primers for COI mtDNA amplification and sequencing

Primer	Sequence 5'-3'	Working Concentration
COI primers, Robideau et al 2011		
COIF-1	5' – TCAWCWMGATGGCTTTTTTCAAC – 3'	10 µM for amplification 5µM for sequencing
COIR-1	5' – RRHWACKTGACTDATRATACCAAA – 3'	10 µM for amplification 5µM for sequencing

The following steps must be done in a decontaminated PCR hood/enclosure on a new disposable paper mat.

Concentrated freezer stock solutions (100 µM) of primers

- Centrifuge tubes with lyophilized primers and probes briefly, 20-30 seconds at ≥ 10,000 rpm (6,708 × g), before opening to ensure that the lyophilized material is at the bottom of the tube.
- Rehydrate primers to **100 µM** in molecular grade (MG) water. The amount of MG water is determined by multiplying the nmoles provided by the vendor by 10 in µL. Example: for 47.54 nmoles of primer add 475.40 µL of MG water for a concentration of 100 µM.
- Vortex, centrifuge briefly, and chill on ice for approximately 30 min. Keep primers on ice during process of preparing working solutions.
- Store the 100 µM stock solution of primers at -20°C.

Working solutions of primers for PCR amplifications (10 µM)

- UV all necessary 1.5 mL microcentrifuge tubes for 10-15 min or use Stratlinker UV Crosslinker.
- For each primer, make 1 mL of a **10 µM** solution: add 100 µL of the 100 µM stock solution to 900 µL of sterile MG water in a 1.5 mL microcentrifuge tube.
- Vortex for 5-10 seconds, then centrifuge briefly (10-20 seconds) at 10,000 rpm (6,708 × g).
- Aliquot 100-200 µL of the primer in UV-treated microcentrifuge tubes.
- Store tubes in -20°C freezer.

Solutions of primers to submit for sequencing (5 µM)

- For each primer, make 1 mL of a **5 µM** solution: add 50 µL of the 100 µM primer stock to 950 µL of sterile MG water in a 1.5 mL microcentrifuge tube. Vortex for 5-10 seconds and centrifuge briefly (10-20 seconds) at 10,000 rpm (6,708 × g). Dispense 50 µL aliquots in 0.2 mL PCR 8-tube strips.
- Label tubes for each primer (suggestion: use different color markers).
- Submit one 0.2 mL tube with **50 µL of each primer** for the amplification of **10 samples**. You will need **5 µl for each sequencing reaction.** If more than 10 samples are submitted, include 2 or more tubes.
- Store the stock solution and working solution at -20°C for future use or on ice for same-day use.

V. Sample preparation for PCR assays

- Extract DNA from the sample cultures following the protocol outlined in **SOP-PID-02 Molecular: DNA extraction for *Phytophthora* species using Qiagen kit**

- Determine concentration of DNA in a Qubit or NanoDrop, and adjust concentration to 500 pg/µl (if possible). If not possible, work with the undiluted DNA and 1:10 dilutions as too highly concentrated DNA could inhibit PCR amplification.
- On a clean (decontaminated and new lab mat) work surface, label one 1.5 mL microcentrifuge tube for each sample. UV the tubes containing only MG water for 15 minutes (i.e. 1:10 dilution using 45 µL of MG water).
- If samples are frozen, thaw briefly for about 5-10 min at room temperature until completely thawed. Vortex 5-10 seconds and centrifuge briefly, 20-30 seconds at 10,000 rpm (6,708 × g) to settle the liquid at the bottom of the tubes. Place DNA extracts in ice until needed.

VI. Master mix preparation for PCR assays

Master mix preparation and aliquoting must be done in a decontaminated PCR workstation/enclosure.

- Thaw the primer mixes and all other reagents, except Taq polymerase, at room temperature. Vortex reagents for 5-10 seconds, then centrifuge 20-30 seconds at 10,000 rpm (6,708 × g) to settle the liquid to the bottom of the tube. Place back in ice.
- Taq polymerase should be kept on ice after removal from the freezer. Mix Taq polymerase by flicking the tube several times and place back on ice.
- Calculate the volume of each reagent needed to prepare the master mix for the total number of reactions (Table 3). The total number of reactions should include the number of DNA samples, a positive control, and a non-template water control (NTC). Prepare one or two extra reactions for every 10 reactions needed to ensure sufficient volume for all samples.
- In a decontaminated PCR workstation, prepare the master mixes for ITS rDNA and COI PCR (Table 3).
- Ensure that the master mix is thoroughly mixed by slowly pipetting up and down several times. Keep the master mix for on ice until dispensed into 0.2 mL reaction tubes.

Table 3. ITS rDNA and COI master mix.

Master Mix	ITS rDNA 1 Reaction (µL)	COI 1 Reaction (µL)
MG water	14.50	14.50
PCR 10X Reaction Buffer (provided with Taq polymerase)	2.50	2.50
50 mM MgCl2 (provided with Taq polymerase)	1.25	1.25
Primer F (10µM)	2.50	2.50
Primer R (10µM)	2.50	2.50
10 mM mixture dNTPs	0.50	0.50
5u/µL Platinum Taq Polymerase (Invitrogen)	0.25	0.25
DNA	1.00	1.00
Total Vol. (µl)	25.00	25.00

- Mix Master Mix well by pipetting up and down several times, then pipet 24.0 µL into the 0.2 mL tubes. Close the caps on the tubes to prevent contamination during transportation to sample addition area.

VII. Addition of sample or control DNA

Note: Do not add controls or samples DNA while working inside the PCR workstation.

Working on a new disposable lab mat to prevent contaminating the area with target DNA, add 1 µL of each sample DNA dilutions and controls into each of the designated tubes (total volume 25 µL).

VIII. Thermocycler parameters

- PCR cycling conditions for ITS rDNA and COI are presented in Table 4.
- Turn on the thermocycler and allow the machine to run through its self-testing procedures. For more information on instrument set-up see the user's manual for the thermocycler to be used.
- Place the PCR tubes into the thermocycler and check to make sure the cycling parameters (below) are correctly programmed.
- Start the run.

Table 4. PCR cycling conditions for ITS rDNA and COI.

PCR Cycling Conditions for ITS rDNA
a. Denaturation step of 1 min, 25 sec at 95°C
b. Followed by 34 cycles each consisting of:
i. 35 sec denaturing at 92°C
ii. 55 sec annealing at 62°C
iii. 50 sec elongation at 72°C
c. 10 min extension at 72°C
d. Followed by a 4°C incubation

from protocol described by White et al. 1990

PCR Cycling Conditions for COI
a. Denaturation step of 2 min at 95°C
b. Followed by 35 cycles each consisting of:
i. 30 sec denaturing at 95°C
ii. 30 sec annealing at 58°C
iii. 30 sec elongation at 72°C
c. 5 min extension at 72°C
d. Followed by a 4°C incubation

from protocol described by Robideau et al. 2011

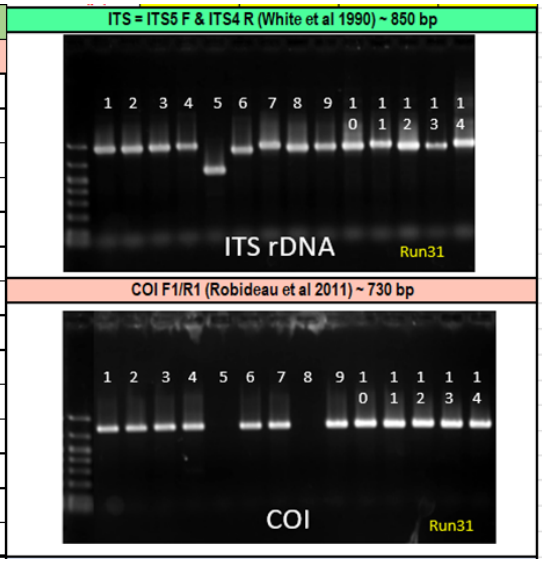
IX. Gel electrophoresis and submission of samples to sequencing facility

see SOP-PID-04 Molecular: Gels, electrophoresis, and photos (ITS rDNA and COI).

Agarose gel electrophoresis of PCR products with ITS rDNA and COI markers with some *Phytophthora* isolates is presented in Table 5.

Table 5. Example of *Phytophthora* specimens with amplified products using ITS rDNA and COI.

#	Sample	Results	
		ITS	COI
1	P_austrocedri_ET_P16040_5	Pos	Pos
2	P_inundata_EA_P8479_20	Pos	Pos
3	P_pinifolia_ET_P16100_49	Pos	Pos
4	P_rubi_ET_P16899_54	Pos	Pos
5	P_cactorum__P1430_135 (contamination)	PosS	Neg
6	P_capsici__P1314_136	Pos	Pos
7	P_cinnamomi__P2121_137	Pos	Pos
8	P_colocasiae__P8604_138	Pos	Neg
9	P_cryptogae__P1380_139	Pos	Pos
10	P_dreschleri__P1691_140	Pos	Pos
11	P_fragariaefolia__P19990_141	Pos	Pos
12	P_infestans__P1381_142	Pos	Pos
13	P_infestans__P19941_143	Pos	Pos
14	P_insolita_ET_P6195_144	Pos	Pos

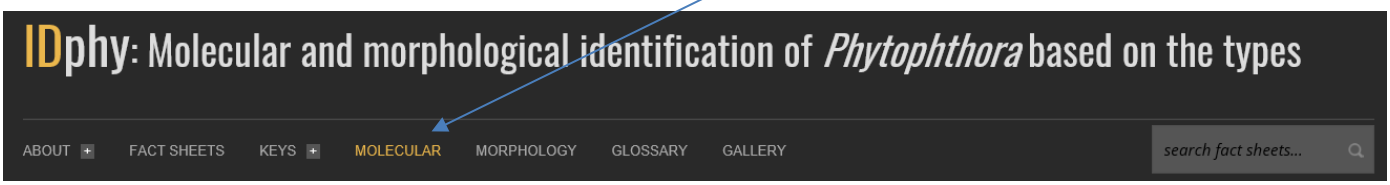


Note: Typically, the PCR product sequencing samples and sequencing primers are submitted in separate 8-strip PCR tubes. On average, 15 µL per tube of post-electrophoresis PCR product is remaining for each sample. Generally, 5 µL of **each** 5 µM sequencing primer is needed per sample. Clearly label each tube following the order receipt. If you are submitting samples with faint bands, it is recommended you provide the gel image to the sequencing facility to alert personnel for proper processing of difficult samples.

- PCR clean-up is required when submitting PCR product. Either request this from the sequencing facility, or use a PCR clean-up kit such as Qiagen's QIAquick PCR Purification Kit.
- Using an appropriate sequencing software (Geneious, Bioedit, etc) to obtain FASTA sequences for your samples.
- Import the ABI files for the sequences amplified with ITS rDNA or COI forward and reverse primers, perform the assembly, generate the consensus sequence, and obtain the FASTA sequence.
- Copy name and sequence and paste into a document for quick reference (add > in front of the sequence name; the recommended font is Courier New, size 8).

X. Identifying *Phytophthora* unknowns – sequence analysis

Follow along with these steps from the *IDphy* website molecular section.
http://idtools.org/id/phytophthora/molecular.php



For first time molecular identifiers, use the following FASTA sequences for unknown examples 1 and 2 to practice the steps. Once you understand the process, you can use it to identify your own unknown sequences.

Copy sequence using Control-C on a PC or Command-C on a Mac. Paste sequence using Control-Shift-V on a PC or Command-Shift-V on a Mac.

Example 1: NCBI BLAST ANALYSIS

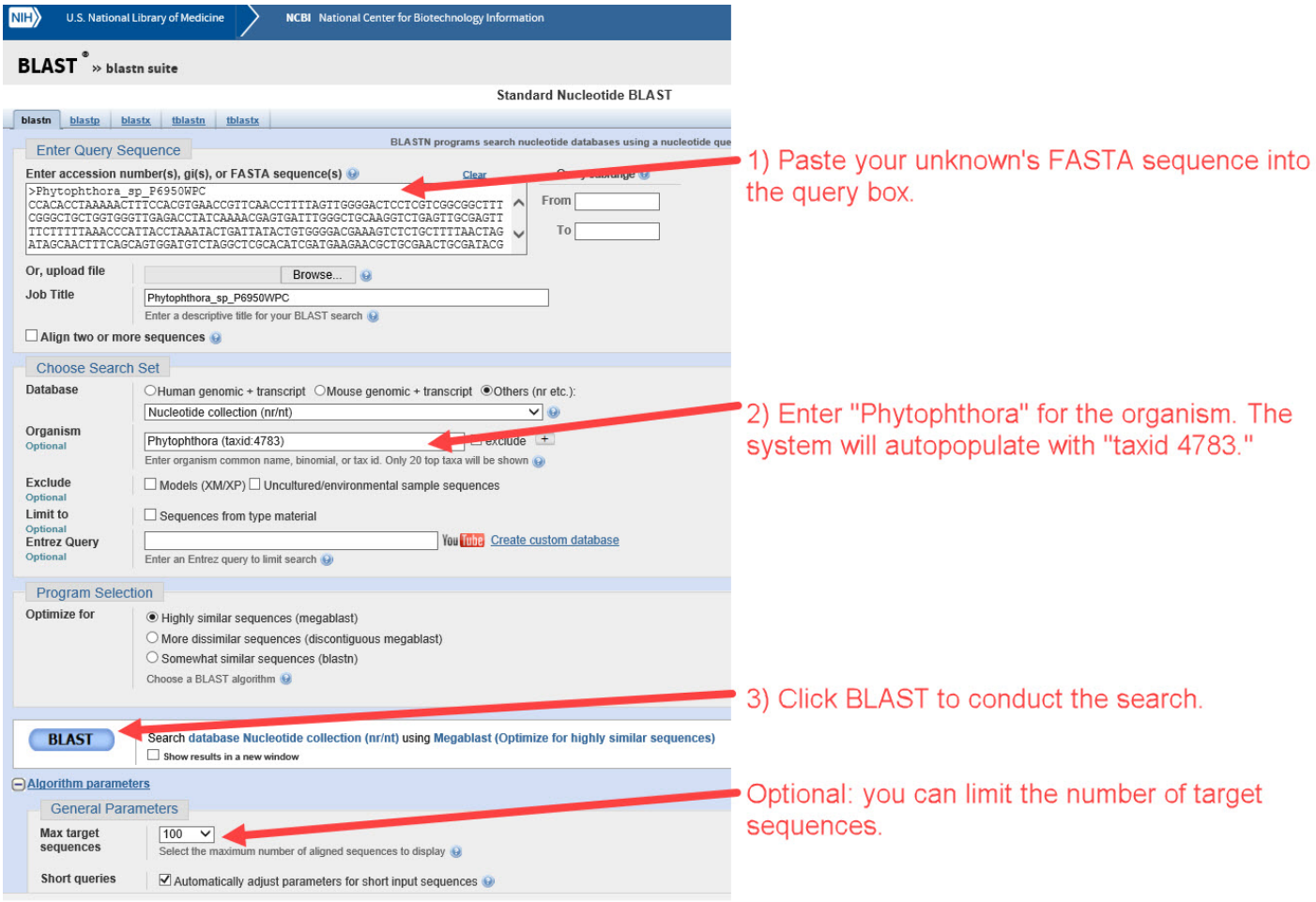
>Phytophthora_sp_P6950WPC_ITS
CCACACCTAAAAACTTTCCACGTGAACCGTTCAACCTTTTAGTTGGGGACTCCTCGTCGGCGGC
TTTCGGGCTGCTGGTGGGTTGAGACCTATCAAAACGAGTGATTTGGGCTGCAAGGTCTGAGTTG
CGAGTTTTCTTTTTAAACCCATTACCTAAATACTGATTATACTGTGGGGACGAAAGTCTCTGCT
TTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCG
AACTGCGATACGTAATGCGAATTGCAGGATTCAGTGAGTCATCGAAATTTTGAACGCATATTGC
ACTTCCGGGTTATGCCTGGGAGTATGCCTGTATCAGTGTCCGTACATCAACCTTGGCTTCCTTC
CTTCCGTGTAGTCGGTGGATGGGAATGCCAGACGTGAAGTGTCTTGCTGTAGCGGTCTTCGGAC
TGCGGCGGCGAGTCCTTTGAAATGTAGATACTGTTCTTCTCTTTGCTCGAAAAGCTGTGCAGAA
TGTATGTTGTGGAGGCTGCCATCACGGCATGTCGGCGACTGATTTCGTGCTGAGGCGTTTTGGA
GAGGGGTTCGATTCGCGGTAGTGTTGGTTTCGGCCGAACAATCTGCTTATTGGGTTCTTTTCCA
GGCTTTGGCGTTTGCGGAGTCGGTGTACCGTAGCTGTGCGTGGCTTGGCCTTTGAACGCTCGTT
TTGTGTGGCGAAGTAGAGTGGCGAAGTGTTTGTTTTCGGACGAATGCGGCTGCCGAGTGGACGA
AAATATTTTGGGAAAATTTAGTGTGCGCTTTGCCTTCGGGTATTGTGTGCATCTCAA

Example 2: NCBI BLAST AND DISTANCE TREE ANALYSIS

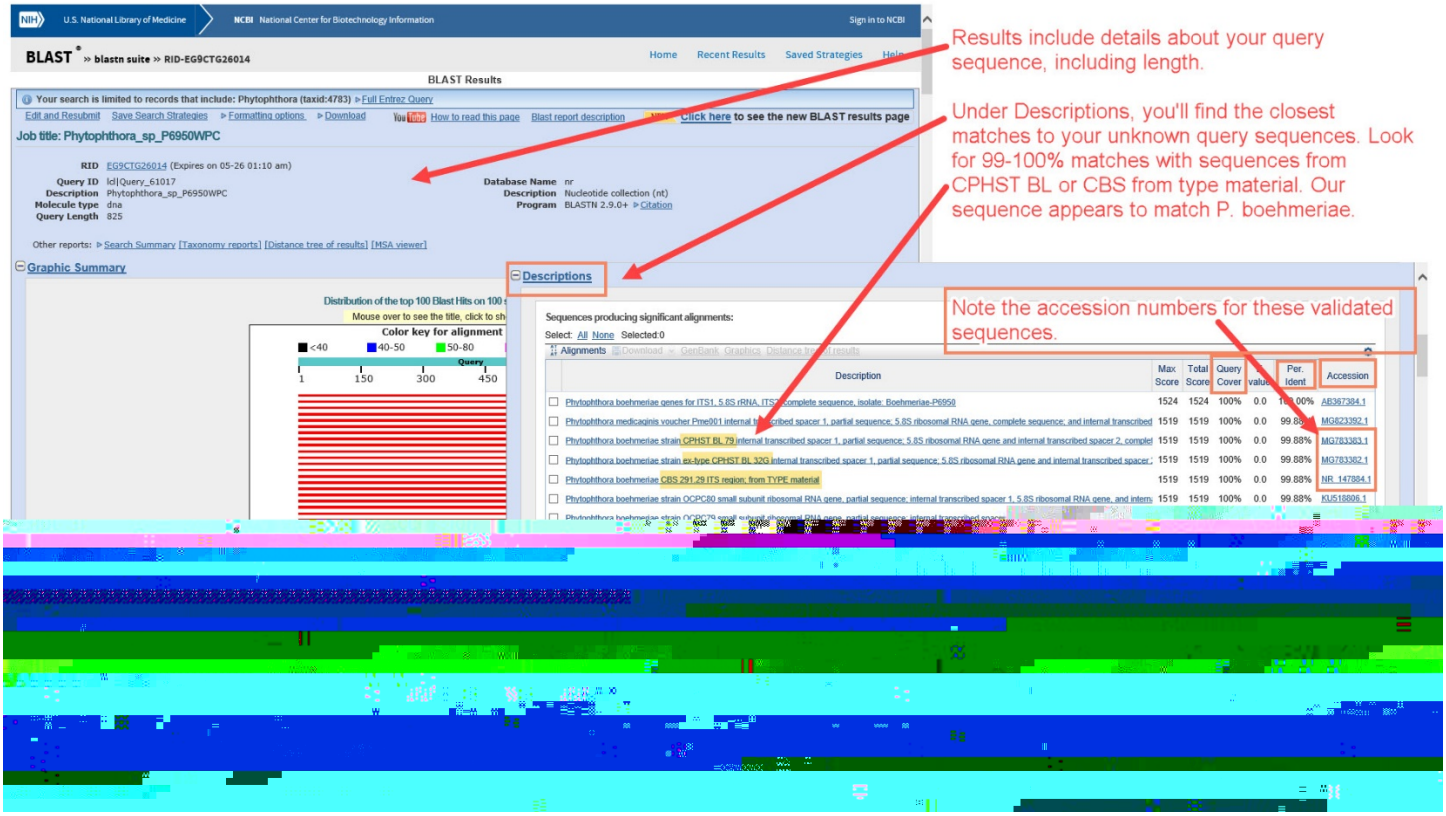
>Phytophthora_sp_Ph3450_ITS
GGTTGAGGCCTATCAAAGCGAGTGATTTGGGCCGCAAGGTCTGAGTTGCGAGTTTTCCTTTTAA
ACCCATTACTTAAATACTGATTATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAA
CTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTAAT
GCGAATTGCAGGATTCAGTGAGTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTATGCC
TGGGAGTATGCCTGTATCAGTGTCCGTACATCAACCTTGGCTTCCTTCCTTCCGTGTAGTCGGT
GGATGGGAATGCCAGACGTGAAGTGTCTTGCTGTAGCGGTTTTCGGACTGCGGCGGCGAGTCCT
TTGAAATGTAGATACTGTTCTTCTCTTTGCTCGAAAAGCTGTGCAGAATGTATGTTGTGGAGGC
TGCCATCATGGCATGTCGGCGACTGATTTCGTGCTGAGGCGTTTTGGAGAGGGGTTCGATTCGC
GGTAGTGTTGGTTTCGGCCGAACAATCTGCTTATTGGGTTCTTTTCCAGGCTTTGGCGTATGCG
GAGTCGGTGTACCGTAGTTGTGCGTGGCTTGGCCTTTGAACGCTCGTTTTGCGTGGCGAAGTAG
AGTGGCGAAGTGTTTGTTTTCGGACGAATGCGGCTGCCGAGGAGGCGATAAATATTTTGGGAAA
ATTTAGTGTGCGCTT

First, identify *Phytophthora* unknown species using BLAST analysis with vouchers of the ITS rDNA gene of 139 ex-types and 22 well-authenticated species (steps 1-7).

1) Go to NCBI BLAST blastn suite and perform a nucleotide BLAST analysis of the *Phytophthora* unknown FASTA sequence.



2) In the "Description," look for the names of species that have the closet alignment with your query (unknown) sequence.



The unknown sequence appears to match most closely with *P. boehmeriae*. However, we must compare the unknown sequence with one from the ex-type to be sure.

Note the accession numbers of the three matches to CPHST BL or CBS type material specimens: MG783383, MG783382, NR_147884.

First, use the clade table to find the clade where the possible species positions. For this example, P. boehmeriae positions in clade 10b.

References

Abad Z.G., Burgess T.I., Bienapfl J.C., Redford A.J. and Srivastava, S. 2021. IDphy: molecular and morphological identification of *Phytophthora* based on the types. An International Online Resource. Plant Disease Feature Article (in progress for 2021).

Cooke D. E. L., Drenth A., Duncan J. M., Wagels G., and Brasier C. M. 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Genet. Biol. 30: 17-32.

Martin F.N., Abad Z.G., Balci Y., and Ivors K. 2012. Identification and detection of *Phytophthora*: reviewing our progress, identifying our needs. Plant Disease 96: 1080-1103.

Mideros M.F., Turissini D.A., Guayazán N., Ibarra-Avila H., Danies G., Cárdenas M., Myers K., Tabima J., Goss E.M., Bernal A., Lagos L.E., Grajales A., Gonzalez L.N., Cooke D.E.L., Fry W.E., Grünwald N., Matute D.R., Restrepo S. 2018. *Phytophthora betacei*, a new species within *Phytophthora* clade 1c causing late blight on *Solanum betaceum* in Colombia. Persoonia. 41: 39-55

Robideau G.P., De Cock A.W., Coffey M.D., Voglmayr H., Brouwer H., Bala K., Chitty D.W., Desaulniers N., Eggertson Q.A., Gachon C.M., Hu C.H., Kupper F.C., Rintoul T.L., Sarhan E., Verstappen E.C., Zhang Y., Bonants P.J., Ristaino J.B., Lévesque A.C. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Mol. Ecol. Resour. 11: 1002-1011.

Schwartzburg K., Hartzog H., Landry C., Rogers J., Randall-Schadel B. 2009. *Phytophthora* of concern to the United States February 18, 2009. USDA-APHIS-PPQ-CPHST-PERAL, Raleigh, NC.

White T.J., Bruns T., Lee S.B., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR protocols: A Guide to Methods and Applications. Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. Academic Press, San Diego, CA.

Citation

Abad, Z.G., Burgess T., Bienapfl J.C., Redford A.J., Coffey M., and Knight L. 2019. IDphy: Molecular and morphological identification of *Phytophthora* based on the types. USDA APHIS PPQ S&T Beltsville Lab, USDA APHIS PPQ S&T ITP, Centre for Phytophthora Science and Management, and World Phytophthora Collection. http://idtools.org/id/phytophthora/index.php, <date you accessed site>

Revision V2

Substantial modifications to version V1 of the SOP:

- Combine sequencing of ITS rDNA (in SOP-PID-05) with sequences from the COI region.
- Include new accession numbers for sequences of ITS rDNA to replace the old codes for the ex-types and well-authenticated specimens submitted to NCBI by the CPHST Beltsville Laboratory (through collaboration with Dr. Subodh Srivastava, bioinformatician.
- Include accession numbers for sequences of the COI region from the ex-types and well-authenticated specimens submitted to NCBI by CPHST BL (with Dr. Srivastava).
- Add molecular protocols for amplification and sequencing for the COI barcoding gene.
- Remove content regarding submissions to Genewiz sequencing facility.
- Include instructions for the BLAST and phylogenetic tree sequencing analyses
- Add new tables to the appendix for: clade 1; clade 2; clade 3, 4, 5 and X; clade 6; clade 7; clade 8; and clades 9, 10; with their respective updated phylogenetic tree using material from CPHST BL.

Document revision history

Status (Original/Revision/ Cancelled)	Document Revision Number	Effective Date	Description
Original	**SOP-PID-05.00**	1.21.2016	*Phytophthora* species identification from DNA of culture samples using ITS rDNA gene sequencing for BLAST and phylogenetic analysis.
V2	**SOP-PID-05.02 V2**	6.29.2018	*Phytophthora* species identification for ITS rDNA and COI genes using DNA of culture samples and the IDphy "online resource" for BLAST and draft phylogenetic analysis.
V3	**SOP-PID-05.03 V3**	10.04.2018	This version includes corrections to the *Phytophthora* species of concern table on page 15 of the appendix. Removed ranking of species and included information for CAPS, OPIS, and US regulated pathogens
V4	**SOP-PID-05-04 V4**	08.22.2019	Edited to update format and make corrections and clarifications based on comments from beta reviewers.
V5	**SOP-PID-05-05 V5**	03.05.2021	Added Table 3 (page 4) for Master Mixes for ITS and COI. Added Table 5 (page 6) with example of *Phytophthora* species amplified with ITS and COI. Updated citation for Abad et al. 2021. Edited to update the Table of contents.

Prepared and submitted by: Z. Gloria Abad, Ph.D., Senior Plant Pathologist, S&T Beltsville Laboratory

ZOILA ABAD
Digitally signed by ZOILA ABAD
Date: 2021.03.09 10:09:17 -05'00'

Approved by: Yazmín Rivera, Ph.D., Assistant Director, S&T Beltsville Laboratory

YAZMIN RIVERA RIVERA
Digitally signed by YAZMIN RIVERA RIVERA
Date: 2021.03.09 11:23:48 -05'00'

Approved by: Nicole O'Donahue, Quality Assurance Specialist, S&T Beltsville Laboratory

NICOLE ODONAHUE
Digitally signed by NICOLE ODONAHUE
Date: 2021.03.09 09:56:40 -05'00'

Approved by: Mark Nakhla, Ph.D., Director, S&T Beltsville Laboratory

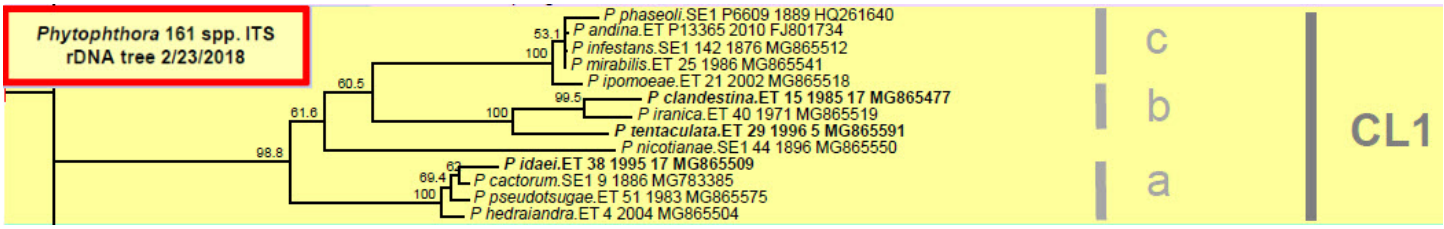
MARK NAKHLA
Digitally signed by MARK NAKHLA
Date: 2021.03.09 10:20:33 -05'00'

Appendix

ITS and COI accession numbers for *Phytophthora* species in clade 1

SPECIES NAME, YEAR	CLADE	TYPE	ISOLATE CODE	ITS rDNA	COI
P. nicotianae (1896)	1	SE1	CPHST BL 44 (P7661)	MG865550	MH136943
P. cactorum (1886)	1a	SE1	CPHST BL 9 (P0714)	MG783385	MH136858
P. hedraiandra (2004)	1a	ET	CPHST BL 4 (P19523)	MG865504	MH136898
P. idaei (1995) (17)	1a	ET	CPHST BL 38 (P6767)	MG865509	MH136903
P. pseudotsugae (1983)	1a	ET	CPHST BL 51 (P10339)	MG865575	MH136967
P. clandestina (1985) (17)	1b	ET	CPHST BL 15 (P3943)	MG865477	MH136873
P. iranica (1971)	1b	ET	CPHST BL 40 (P3882)	MG865519	MH136913
P. tentaculata (1996) (5)	1b	ET	CPHST BL 29 (P8496)	MG865591	MH136983
P. andina (2010)	1c	ET	P13365 (WPC), CPHST BL 32 (P13365)	FJ801734	MH136846
P. infestans (1876)	1c	SE1	CPHST BL 142 (P1381)	MG865512	MH136906
P. ipomoeae (2002)	1c	ET	CPHST BL 21 (P10225)	MG865518	MH136912
P. mirabilis (1986)	1c	ET	CPHST BL 25 (P3008)	MG865541	MH136934
P. phaseoli (1889)	1c	SE1	CPHST BL 28 (P10150)	MG865564	MH136956

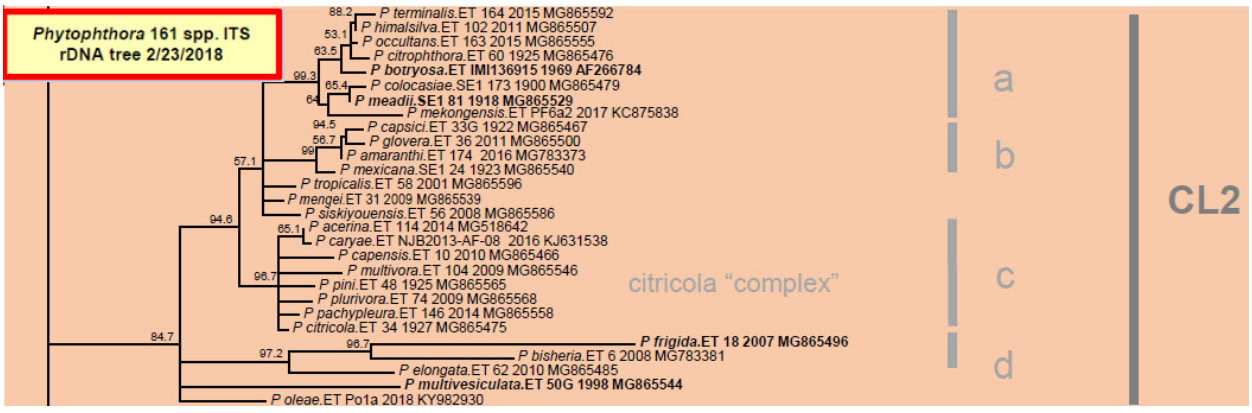
ITS rDNA	COI
MG865550	MH136943
MG783385	MH136858
MG865504	MH136898
MG865509	MH136903
MG865575	MH136967
MG865477	MH136873
MG865519	MH136913
MG865591	MH136983
FJ801734	MH136846
MG865512	MH136906
MG865518	MH136912
MG865541	MH136934
MG865564	MH136956



ITS and COI accession numbers for *Phytophthora* species in clade 2

SPECIES NAME, YEAR	ITS CLADE	TYPE	ISOLATE CODE	ITS rDNA	COI
P. mengei (2009)	2	ET	CPHST BL 31 (P16862)	MG865539	MH136932
P. multivesiculata (1998) (19)	2	ET	CPHST BL 50G (P10410)	MG865544	MH136937
P. oleae (2018)	2	ET	Po1a (CBS7669)	KY982930	MF083569
P. siskiyouensis (2008)	2	ET	CPHST BL 56 (P15122)	MG865586	MH136978
P. tropicalis (2001)	2	ET	CPHST BL 58 (P10329)	MG865596	MH136987
P. botryosa (1969) (15)	2a	ET	IMI136915, CPHST BL 132 (P3425)	AF266784	MH136855
P. citrophthora (1925)	2a	ET	CPHST BL 60 (P0479)	MG865476	MH136872
P. colocasiae (1900)	2a	SE1	CPHST BL 173 (P6317)	MG865479	MH136875
P. himalsilva (2011)	2a	ET	CPHST BL 102 (P19820)	MG865507	MH136901
P. meadii (1918) (4)	2a	SE1	CPHST BL 81 (P19007)	MG865529	MH136924
P. mekongensis (2017)	2a	ET	PF6a2 (CBS 135136)	KC875838	KT366920
P. occultans (2015)	2a	ET	CPHST BL 163 (P19955)	MG865555	MH477753
P. terminalis (2015)	2a	ET	CPHST BL 164 (P19956)	MG865592	MH136984
P. amaranthi (2016)	2b	ET	CPHST BL 174 (TARI28041)	MG783373	MH477739
P. capsici (1922)	2b	ET	CPHST BL 33G (P1091)	MG865467	MH136863
P. glovera (2011)	2b	ET	CPHST BL 36 (P11685)	MG865500	MH136895
P. mexicana (1923)	2b	SE1	CPHST BL 24 (P0646)	MG865540	MH136933
P. acerina (2014)	2c	ET	CPHST BL 114 (P19934)	MG518642	MH136845
P. capensis (2010)	2c	ET	CPHST BL 10 (P1819)	MG865466	MH136862
P. caryae (2016)	2c	ET	NJB2013-AF-08	KJ631538	N/A
P. citricola (1927)	2c	ET	CPHST BL 34 (P0716)	MG865475	MH136871
P. multivora (2009)	2c	ET	CPHST BL 104 (P19594)	MG865546	MH136939
P. pachypleura (2014)	2c	ET	CPHST BL 146 (P19987)	MG865558	MH136948
P. pini (1925)	2c	ET	CPHST BL 48 (P3274)	MG865565	MH136957
P. plurivora (2009)	2c	ET	CPHST BL 74 (P16840)	MG865568	MH136959
P. bisheria (2008)	2d	ET	CPHST BL 6 (P10117)	MG783381	MH136851
P. elongata (2010)	2d	ET	CPHST BL 62 (P19596)	MG865485	MH136881
P. frigida (2007) (18)	2d	ET	CPHST BL 39G (P16947)	MG865496	MH136892

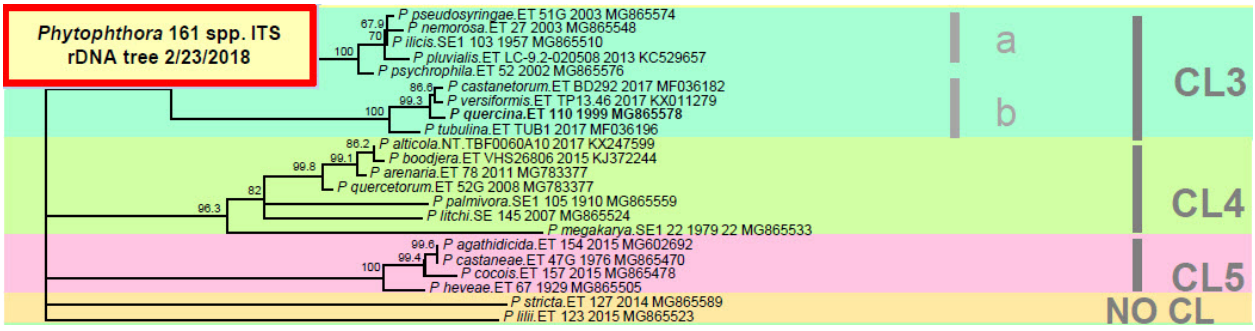
ITS rDNA	COI
MG865539	MH136932
MG865544	MH136937
KY982930	MF083569
MG865586	MH136978
MG865596	MH136987
AF266784	MH136855
MG865476	MH136872
MG865479	MH136875
MG865507	MH136901
MG865529	MH136924
KC875838	KT366920
MG865555	MH477753
MG865592	MH136984
MG783373	MH477739
MG865467	MH136863
MG865500	MH136895
MG865540	MH136933
MG518642	MH136845
MG865466	MH136862
KJ631538	MH136871
MG865475	MH136939
MG865546	MH136948
MG865558	MH136957
MG865565	MH136959
MG865568	MH136851
MG783381	MH136881
MG865485	MH136892
MG865496	



ITS and COI accession numbers for *Phytophthora* species in clade 3, 4, 5 and two in no clade

SPECIES NAME, YEAR	ITS CLADE	TYPE	ISOLATE CODE	ITS rDNA	COI
P. ilicis (1957)	3a	SE1	CPHST BL 103 (P6099)	MG865510	MH136904
P. nemorosa (2003)	3a	ET	CPHST BL 27 (P19600)	MG865548	MH136941
P. pluvialis (2013)	3a	ET	LC-9.2-020508 (MYA-4930)	KC529657	N/A
P. pseudosyringae (2003)	3a	ET	CPHST BL 51G (P10437)	MG865574	MH136966
P. psychrophila (2002)	3a	ET	CPHST BL 52 (P10433)	MG865576	MH136968
P. castanetorum (2017)	3b	ET	BD292 (CBS 142299)	MF036182	N/A
P. quercina (1999) (1)	3b	ET	CPHST BL 110 (P10439)	MG865578	MH136970
P. tubulina (2017)	3b	ET	TUB1 (CBS 141212)	MF036196	N/A
P. versiformis (2017)	3b	ET	TP13.46 (CBS 142005), CPHST BL 189 (TP13.46)	KX011279	MH477760
P. alticola (19) (2017)	4	NT	TBF0060A10 (CBS141718)	KX247599	N/A
P. arenaria (2011)	4	ET	CPHST BL 78 (P19599)	MG783377	MH136848
P. boodjera (2015)	4	ET	VHS26806, CPHST BL 181 (VH26806)	KJ372244	MH477743
P. litchii (2007)	4	SE	CPHST BL 145 (P19950)	MG865524	MH136919
P. megakarya (1979) (22)	4	SE1	CPHST BL 22 (P1664)	MG865533	MH136928
P. palmivora (1910)	4	SE1	CPHST BL 105 (P0633)	MG865559	MH136949
P. quercetorum (2008)	4	ET	CPHST BL 52G (P15555)	MG865577	MH136969
P. agathidicida (2015)	5	ET	CPHST BL 154 (P15175), ICMP 17027	MG602692	KP295222
P. castaneae (1976) (14)	5	ET	CPHST BL 47G (P10187)	MG865470	MH136866
P. cocois (2015)	5	ET	CPHST BL 157 (P19948)	MG865478	MH136874
P. heveae (1929)	5	ET	CPHST BL 67 (P3428)	MG865505	MH136899
P. stricta (2014)	X1	ET	CPHST BL 127 (P19972)	MG865589	MH136981
P. lilii (2015)	X2	ET	CPHST BL 123 (P19991)	MG865523	MH136918

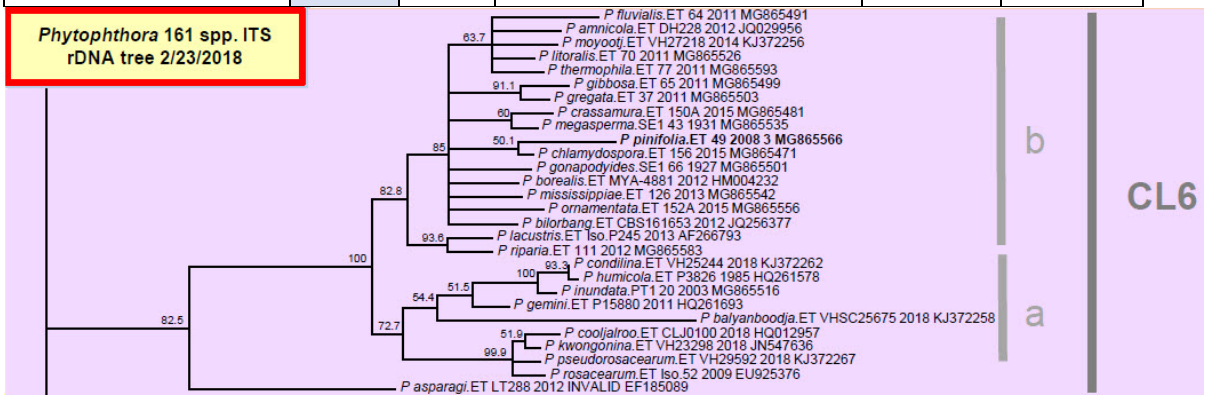
ITS rDNA	COI
MG865510	MH136904
MG865548	MH136941
KC529657	MH136966
MG865574	MH136968
MG865576	MH136970
MF036182	MH477760
MG865578	MH136848
MF036196	MH477743
KX011279	MH136919
KX247599	MH136928
MG783377	MH136949
KJ372244	MH136969
MG865524	KP295222
MG865533	MH136866
MG865559	MH136874
MG865577	MH136899
MG602692	MH136981
MG865470	MH136918
MG865478	
MG865505	
MG865589	
MG865523	



ITS and COI accession numbers for *Phytophthora* species in clade 6

SPECIES NAME, YEAR	ITS CLADE	TYPE	ISOLATE CODE	ITS rDNA	COI
P. balyanboodja (2018)	6a	ET	VHS25675 R1C , CPHST BL 186 (VHSC 25675)	KJ372258	MH477741
P. condilina (2018)	6a	ET	CBS 115029, CPHST BL 184 (VH25244)	KJ372262	MH477744
P. cooljalroo (2018)	6a	ET	CLJO100, CPHST BL 185 (CLJ0100)	HQ012957	MH477745
P. gemini (2011)	6a	ET	P15880 (WPC)	HQ261693	HQ261440
P. humicola (1985)	6a	ET	CPHST BL 42G (P3826)	HQ261578	HQ261325
P. inundata (2003)	6a	PT1	CPHST BL 20 (P8479)	MG865516	MH136910
P. kwongonina (2018)	6a	ET	VH23298, CPHST BL 187 (VH23298)	JN547636	MH477747
P. pseudorosacearum (2018)	6a	ET	VHS29592, CPHST BL 188 (VH29592)	KJ372267	MH477757
P. rosacearum (2009)	6a	ET	OSU52, CPHST BL 53 (P3112)	EU925376	MH477758
P. amnicola (2012)	6b	ET	DH228, CPHST BL 100 (P19862)	JQ029956	MH477740
P. bilorbang (2012)	6b	ET	CBS 161653 , CPHST BL 101 (P19863)	JQ256377	MH477742
P. borealis (2012)	6b	ET	AKWA58.1-0708, CPHST BL 93 (P19794)	HM004232	MH136854
P. chlamydospora (2015)	6b	ET	CPHST BL 156 (P6133)	MG865471	MH136867
P. crassamura (2015)	6b	ET	CPHST BL 150A (P20138)	MG865481	N/A
P. fluvialis (2011)	6b	ET	CPHST BL 64 (P19584)	MG865491	MH136887
P. gibbosa (2011)	6b	ET	CPHST BL 65 (P19586)	MG865499	MH136894
P. gonapodyides (1927)	6b	SE1	CPHST BL 66 (P7050)	MG865501	MH136896
P. gregata (2011)	6b	ET	CPHST BL 37 (P19588)	MG865503	MH477746
P. lacustris (2013)	6b	ET	P245, CPHST BL 69 (P10337)	AF266793	MH136916
P. litoralis (2011)	6b	ET	CPHST BL 70 (P19591)	MG865526	MH136921
P. megasperma (1931)	6b	ET	CPHST BL 43 (P6957)	MG865535	MH136930
P. mississippiae (2013)	6b	ET	CPHST BL 126 (P19994)	MG865542	MH136935
P. moyootj (2014)	6b	ET	VH27218, CPHST BL 182 (VH27218)	KJ372256	MH477750
P. ornamentata (2015)	6b	ET	CPHST BL 152B (P19906)	MG865556	MH136947
P. pinifolia (2008) (3)	6b	ET	CPHST BL 49 (P16100)	MG865566	MH136958
P. riparia (2012)	6b	ET	CPHST BL 111 (P19799)	MG865583	MH136975
P. thermophila (2011)	6b	ET	CPHST BL 77 (P19593)	MG865593	MH136985

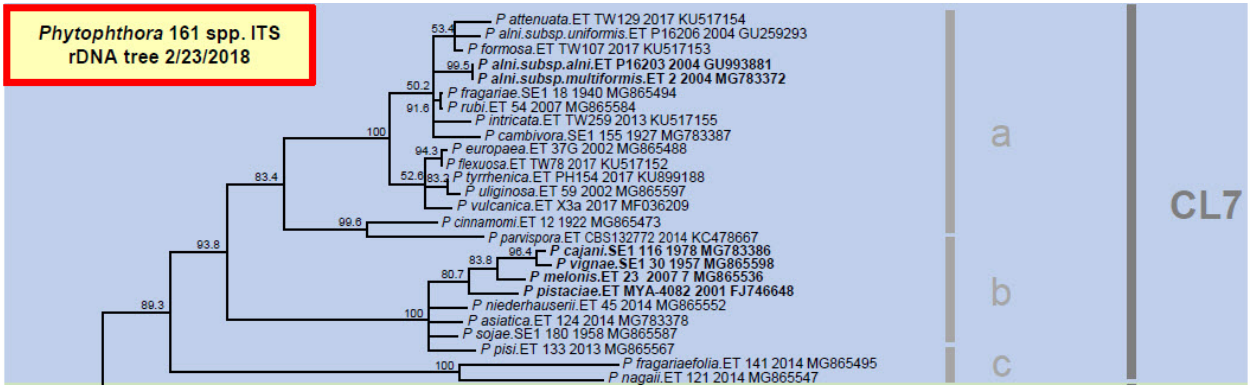
ITS rDNA	COI
KJ372258	MH477741
KJ372262	MH477744
HQ012957	MH477745
HQ261693	HQ261440
HQ261578	HQ261325
MG865516	MH136910
JN547636	MH477747
KJ372267	MH477757
EU925376	MH477758
JQ029956	MH477740
JQ256377	MH477742
HM004232	MH136854
MG865471	MH136867
MG865481	MH136887
MG865491	MH136894
MG865499	MH136896
MG865501	MH477746
MG865503	MH136916
AF266793	MH136921
MG865526	MH136930
MG865535	MH136935
MG865542	MH477750
KJ372256	MH136947
MG865556	MH136958
MG865566	MH136975
MG865583	MH136985
MG865593	



ITS and COI accession numbers for *Phytophthora* species in clade 7

SPECIES NAME, YEAR	ITS CLADE	TYPE	ISOLATE CODE	ITS rDNA	COI
P. alni (6) (2004)	7a	ET	IMI392314, CPHST BL 1 (P16203)	GU993881	MH136991
P. alni multiformis (6) (2004)	7a	ET	CPHST BL 2 (P16202), P16202	MG783372	HQ261241
P. alni uniformis (2004)	7a	ET	16206D1244 (WPC), CPHST BL 3 (P16206)	GU259293	MH136992
P. attenuata (2017)	7a	ET	TW129 (CBS 141199)	KU517154	N/A
P. cambivora (1927)	7a	SE1	CPHST BL 155 (P19997)	MG783387	MH136860
P. cinnamomi (1922)	7a	ET	CPHST BL 12 (P2110)	MG865473	MH136869
P. europaea (2002)	7a	ET	CPHST BL 37G (P10324)	MG865488	MH136884
P. flexuosa (2017)	7a	ET	TW78 (CBS 141201)	KU517152	N/A
P. formosa (2017)	7a	ET	TW107 (CBS141203)	KU517153	N/A
P. fragariae (1940)	7a	SE1	CPHST BL 18 (P19539)	MG865494	MH136890
P. intricata (2013)	7a	ET	TW259 (CBS 141211)	KU517155	N/A
P. parvispora (2014) (14)	7a	ET A1	CBS 132772 , CPHST BL 98 (P19847)	KC478667	MH136953
P. rubi (2007)	7a	ET	CPHST BL 54 (P16899)	MG865584	MH136976
P. tyrrhenica (2017)	7a	ET	PH154 (CBS 142301)	KU899188	N/A
P. uliginosa (2002)	7a	ET	CPHST BL 59 (P10413)	MG865597	MH136988
P. vulcanica (2017)	7a	ET	X3a (CBS 141216)	MF036209	N/A
P. asiatica (2014)	7b	ET	CPHST BL 124 (P19977)	MG783378	N/A
P. cajani (1978) (22)	7b	SE1	CPHST BL 116 (P3105)	MG783386	MH136859
P. melonis (2007) (7)	7b	ET	CPHST BL 23 (P6870)	MG865536	MH136931
P. niederhauserii (2014)	7b	ET	CPHST BL 45 (P10616)	MG865552	MH136944
P. pisi (2013)	7b	ET	CPHST BL 133 (P19612)	MG865567	MH477754
P. pistaciae (2001) (12)	7b	ET	ATCC MYA-4082	FJ746648	N/A
P. sojae (1958)	7b	SE1	CPHST BL 180 (P3248)	MG865587	MH136979
P. vignae (1957) (17)	7b	SE1	CPHST BL 30 (P3019)	MG865598	MH136989
P. fragariaefolia (2014)	**7c**	ET	CPHST BL 141 (P19990)	MG865495	MH136891
P. nagaii (2014)	7c	ET	CPHST BL 121 (P19989)	MG865547	MH136940

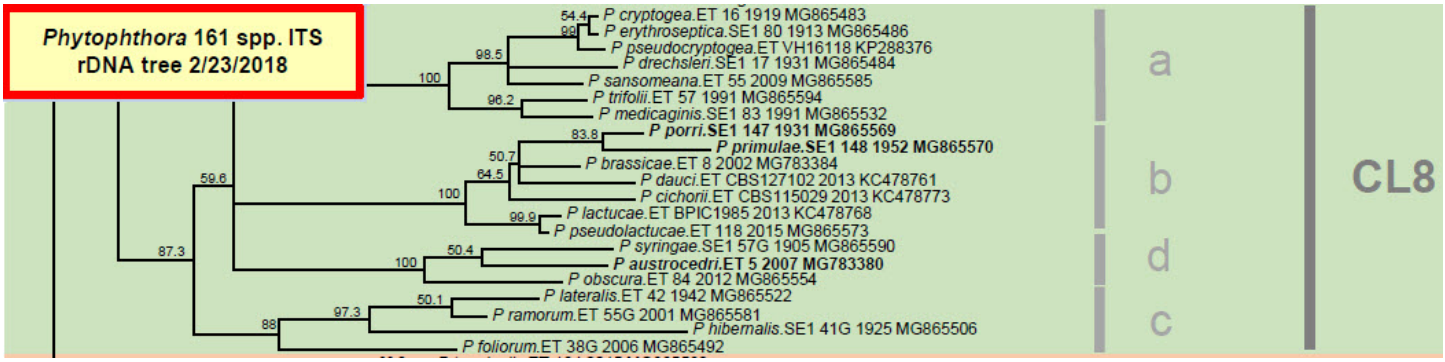
ITS rDNA	COI
GU993881	MH136991
MG783372	HQ261241
GU259293	MH136992
KU517154	MH136860
MG783387	MH136869
MG865473	MH136884
MG865488	MH136890
KU517152	MH136953
KU517153	MH136976
MG865494	MH136988
KU517155	MH136859
KC478667	MH136931
MG865584	MH136944
KU899188	MH477754
MG865597	MH136979
MF036209	MH136989
MG783378	MH136891
MG783386	MH136940
MG865536	
MG865552	
MG865567	
FJ746648	
MG865587	
MG865598	
MG865495	
MG865547	



ITS and COI accession numbers for *Phytophthora* species in clade 8

SPECIES NAME, YEAR	ITS CLADE	TYPE	ISOLATE CODE	ITS rDNA	COI
P. cryptogea (1919)	8a	ET	CPHST BL 16 (P1738)	MG865483	MH136878
P. drechsleri (1931)	8a	SE1	CPHST BL 17 (P1087)	MG865484	MH136879
P. erythroseptica (1913)	8a	SE1	CPHST BL 80 (P6180)	MG865486	MH136882
P. medicaginis (1991)	8a	ET	CPHST BL 83 (P19830)	MG865532	MH136927
P. pseudocryptogea (2015)	8a	ET	VHS16118, CPHST BL 183 (VH16118)	KP288376	MH477756
P. sansomeana (2009)	8a	ET	CPHST BL 55 (P8051)	MG865585	MH136977
P. trifolii (1991)	8a	ET	CPHST BL 57 (P6980)	MG865594	MH136986
P. brassicae (2002)	8b	ET	CPHST BL 8 (P7517)	MG783384	MH136857
P. cichorii (2013)	8b	ET	CBS 115029	KC478773	N/A
P. dauci (2013)	8b	ET	CBS 127102	KC478761	N/A
P. lactucae (2013)	8b	ET	BPIC 1985 (P19875)	KC478768	N/A
P. porri (1931) (8)	8b	SE1	CPHST BL 147 (P9186)	MG865569	MH136961
P. primulae (1952 (17)	8b	SE1	CPHST BL 148 (P10703)	MG865570	MH136962
P. pseudolactucae (2015)	8b	ET	CPHST BL 118 (P19992)	MG865573	MH136965
P. foliorum (2006)	8c	ET	CPHST BL 38G (P10974)	MG865492	MH136888
P. hibernalis (1925)	8c	SE1	CPHST BL 41G (P3822)	MG865506	MH136900
P. lateralis (1942)	8c	ET	CPHST BL 42 (P3361)	MG865522	MH136917
P. ramorum (2001)	8c	ET	CPHST BL 55G (P10103)	MG865581	MH136973
P. austrocedri (2007) (3)	8d	ET	CPHST BL 5 (P16040)	MG783380	MH136850
P. obscura (2012)	8d	ET	CPHST BL 84 (P19796)	MG865554	MH136946
P. syringae (1905)	8d	SE1	CPHST BL 57G (P10330)	MG865590	MH136982

ITS rDNA	COI
MG865483	MH136878
MG865484	MH136879
MG865486	MH136882
MG865532	MH136927
KP288376	MH477756
MG865585	MH136977
MG865594	MH136986
MG783384	MH136857
KC478773	MH136961
KC478761	MH136962
KC478768	MH136965
MG865569	MH136888
MG865570	MH136900
MG865573	MH136917
MG865492	MH136973
MG865506	MH136850
MG865522	MH136946
MG865581	MH136982
MG783380	
MG865554	
MG865590	

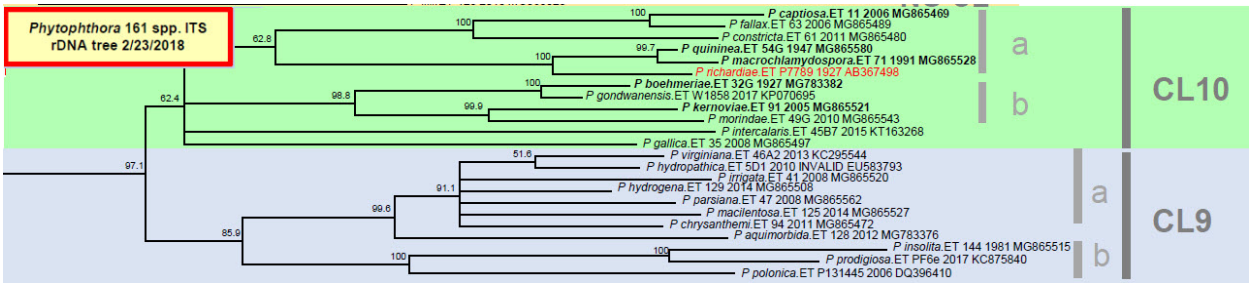


ITS and COI accession numbers for *Phytophthora* species in clades 9, 10

SPECIES NAME, YEAR	ITS CLADE	TYPE	ISOLATE CODE	ITS rDNA	COI
P. estuarina (2016)	9	ET	CCIBt 4157 (NOT INCLUDED IN TREE)	KT886034	N/A
P. rhizophorae (2016)	9	ET	CCIBt 4152 (NOT INCLUDED IN TREE)	KT886031	N/A
P. aquimorbida (2012)	9a	ET	CPHST BL 128 (P19964)	MG783376	MH136847
P. chrysanthemi (2011)	9a	ET	CPHST BL 94 (P19843)	MG865472	MH136868
P. hydrogena (2014)	9a	ET	CPHST BL 129 (P19968)	MG865508	MH136902
P. hydropathica (2010)	9a	ET	CPHST BL 68 (P19616)	EU583793	N/A
P. irrigata (2008)	9a	ET	CPHST BL 41 (P16861)	MG865520	MH136914
P. macilentosa (2014)	9a	ET	CPHST BL 125 (P19993)	MG865527	MH136922
P. parsiana (2008)	9a	ET	CPHST BL 47 (P15164)	MG865562	MH136952
P. virginiana (2013)	9a	ET	CPHST BL 165 (P19829)	KC295544	MH477761
P. insolita (1981)	9b	ET	CPHST BL 144, 45G (P6195)	MG865515	MH136909
P. polonica (2006)	9b	ET	UASWS0198, CPHST BL 109 (P19522)	DQ396410	MH136960
P. prodigiosa (2017)	9b	ET	PF6e (CBS 135138)	KC875840	KT366918.
P. captiosa (2006) (16)	10a	ET	CPHST BL 11 (P10719)	MG865469	MH136865
P. constricta (2011)	10a	ET	CPHST BL 61 (P19614)	MG865480	MH136876
P. fallax (2006)	10a	ET	CPHST BL 63 (P10722)	MG865489	MH136885
P. macrochlamydospora (1991) (11)	10a	ET	CPHST BL 71 (P10263)	MG865528	MH136923
P. quininea (1947 (13)	10a	ET	CPHST BL 54G (P8488)	MG865580	MH136972
P. richardiae (1927)	10a	SE1	RICH-P7789 , CPHST BL 76 (P7789)	AB367498	MH136974
P. boehmeriae (1927) (14)	10b	ET	CPHST BL 32G (P6950)	MG783382	MH136852
P. gallica (2008)	10b	ET	CPHST BL 35 (P16826)	MG865497	MH136893
P. gondwanensis (2017)	10b	ET	W1858 (CBS 139336)	KP070695	N/A
P. intercalaris (2015)	10b	ET	45B7 (CBS 140632)	KT163268	N/A
P. kernoviae (2005) (2)	10b	ET	CPHST BL 91 (P19827)	MG865521	MH136915
P. morindae (2010)	10b	ET	CPHST BL 49G (P19748)	MG865543	MH136936

ITS rDNA	COI
KT886034	MH136847
KT886031	MH136868
MG783376	MH136902
MG865472	MH136914
MG865508	MH136922
EU583793	MH136952
MG865520	MH477761
MG865527	MH136909
MG865562	MH136960
KC295544	KT366918.
MG865515	MH136865
DQ396410	MH136876
KC875840	MH136885
MG865469	MH136923
MG865480	MH136972
MG865489	MH136974
MG865528	MH136852
MG865580	MH136893
AB367498	MH136915
MG783382	MH136936
MG865497	
KP070695	
KT163268	
MG865521	
MG865543	

NOTE: *P. estuarina* and *P. rhizophorae* position in sub-clade 9a. These species were not included in the ITS rDNA phylogenetic tree prepared on 2.23.2018. *Phytophthora estuarina* positions close to *P. macilentosa* and *P. rhizophorae* positions close to *P. virginiana*.



***Phytophthora species* not included in the ITS phylogenetic tree or voucher sequences**

Phytophthora unculturable species	*Phytophthora* hybrid species
P. cyperi (1935)	P. × heterohybrida (2017)
P. cyperi-bulbosi (1953)	P. × incrassata (2017)
P. eriugena (19xx)	P. × stagnum (2014)
P. leersiae (1992)	P. × pelegrandis (2009)
P. lepironiae (1942)	P. × serendipita (2012)
P. oryzo-bladis (2001)	
P. polygoni (1922) (22)	*Phytophthora* lost species
P. verrucosa (1940)	**P. italica (1996) (9)**
	P. japonica (1974) (10)
	P. inflata (1949)
assembled mitochondrial genome (no specimens available)	
P. betacei (2018)	

Table 1. *Phytophthora* species of concern to the United States

Phytophthora species	PERAL	CAPS 2017-2019	OPIS list updated 2015	U.S. Regulated
P. alni subsp. *alni*	2009	2017, 2018, 2019	2015 Level A	2017
P. amarnathi			2017	
P. alticola	2009			
P. amaranthi				2017
P. austrocedrae	2009	2017*, 2018*, 2019*	2015 No level asigned	
P. boehmeriae	2009			2017
P. botryosa	2009			
P. cajani	2009			
P. captiosa	2009			
P. castaneae	2009			
P. chrysanthemi				2017
P. citricola				2017
P. clandestina	2009			
P. frigida	2009			2017
P. idaei	2009			
P. kernoviae	2009	2017, 2018, 2019	2015 Level A	
P. macrochlamydospora	2009			
P. meadii	2009			2017
P. megakarya	2009			
P. melonis	2009			
P. multivesiculata	2009			
P. parvispora	2009			2017
P. pinifolia	2009			
P. pistaciae	2009			
P. porri	2009			
P. primulae	2009			
P. quercina	2009			2017
P. quininea	2009			
P. ramorum				2017
P. sojae				2017
P. tentaculata	2009			2017
P. vignae	2009			

SOURCES:

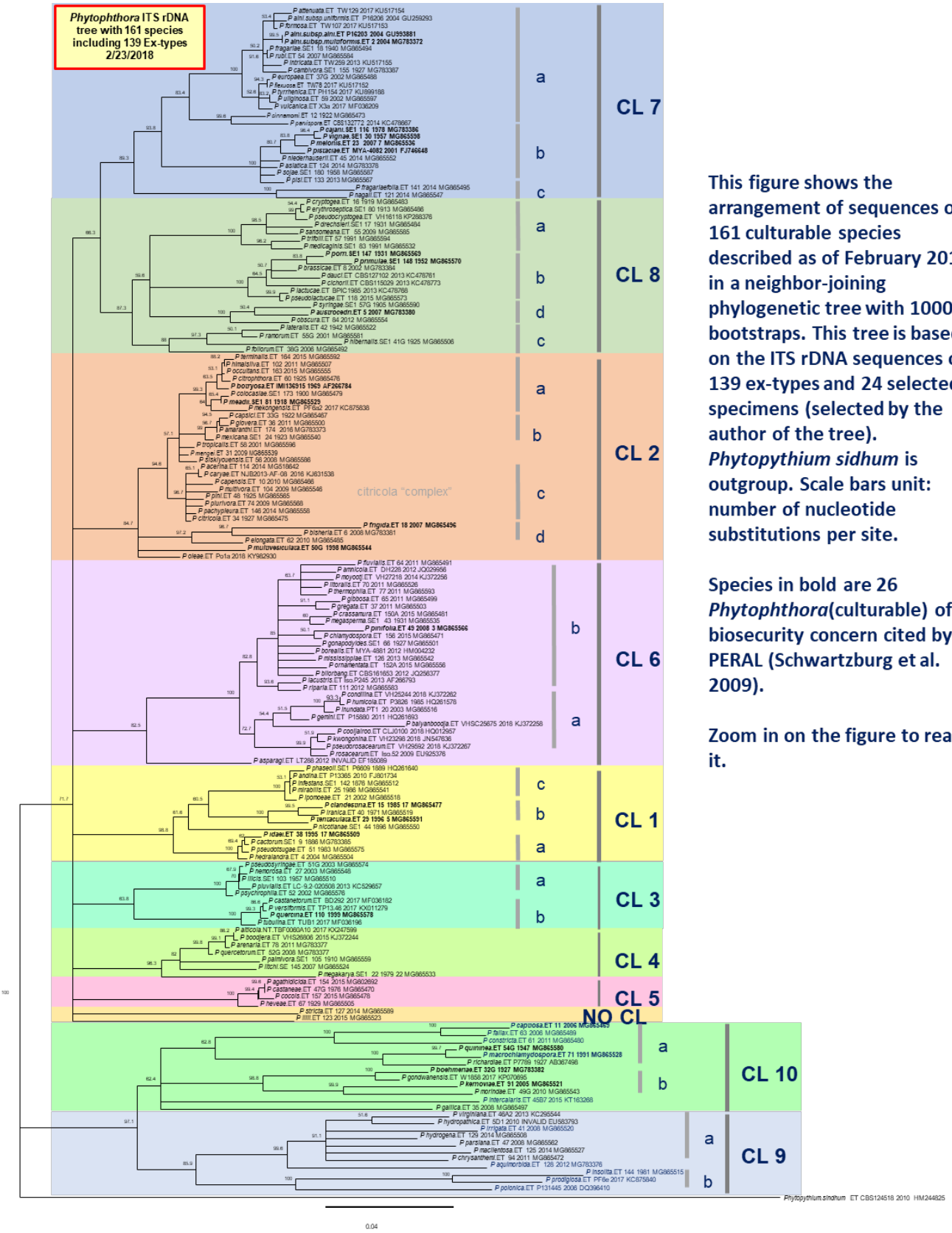
PERAL = Schwartzburg K, Hartzog H, Landry C, Rogers J, Randall-Schadel B. 2009. *Phytophthora* of Concern to the United States February 18, 2009. USDA-APHIS-PPQ-CPHST-PERAL, Raleigh, NC.

CAPS = Cooperative Agriculture Pest Survey - Pest Lists 2017-2019 https://caps.ceris.purdue.edu/pest-lists * In additional List of CAPS

OPIS = USDA- Offshore Pest Information Program Pathogens List 212 - Updated 2015

U.S. Regulated Plant Pest Table (Last modified Nov. 15, 2017).

Z. Gloria Abad, Ph.D. - USDA-APHIS CPHST-Beltsville Laboratory 9/28/2018



This figure shows the arrangement of sequences of 161 culturable species described as of February 2018 in a neighbor-joining phylogenetic tree with 1000 bootstraps. This tree is based on the ITS rDNA sequences of 139 ex-types and 24 selected specimens (selected by the author of the tree). *Phytopythium sidhum* is outgroup. Scale bars unit: number of nucleotide substitutions per site.

Species in bold are 26 *Phytophthora*(culturable) of biosecurity concern cited by PERAL (Schwartzburg et al. 2009).

Zoom in on the figure to read it.

Z. Gloria Abad, Ph.D. USDA-APHIS-PPQ-CPHST-Beltsville Laboratory 2/23/2018