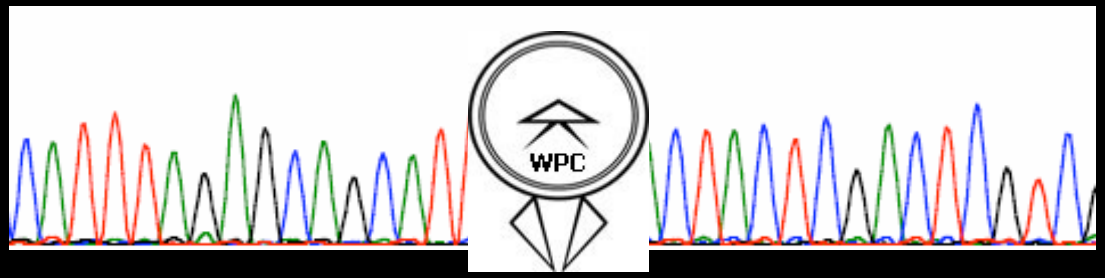
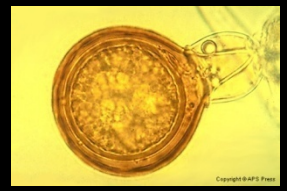
The World Oomycetes Genetic Resource Collection (WOGRC)
formerly World Phytophthora Collection (WPC):
The history, mission, goals and projections for the future





Michael D. Coffey
University of California, Riverside

Genus *Phytophthora*

Phytophthora infestans
Late Blight of Potato
(Solanaceous plants)


Scott Bauer/ARS


http://www.msu.edu/user/douchesd/research/


http://vassun.vassar.edu/~sttaylor/FAMINE/ILN/

1840s – Irish potato famine
DeBary (1861)

Phytophthora ramorum
Sudden Oak Death
(Ramorum Blight)


COMTF

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Europe
Middle East
South Asia
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Mystery oak killer hits UK trees

By Alex Kirby
BBC News Online environment correspondent

A disease which has destroyed many thousands of Californian oaks has been found for the first time in several well-loved British tree species.

The fungus strikes: Death is inevitable *(Image: Forestry Commission)*

It is a fungus called sudden oak death, and till now it had been found only in UK shrubs and a tree native to the US.

But the Forestry Commission says the disease has now str
beech, horse chestnuts and holm oaks in Cornwall.

There is no known cure for the disease, which kills t
bark and is thought likely to affect othe

The fungus, known as *Phytophthora r*
of one oak species in the western U

It was discovered last year i
garden centres, and there ha
subsequent outbreaks in plan
rhododen

UC DAVIS MAGAZINE ONLINE

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This Issue | Past Issues | Magazine Home | Search Class Notes | Send a Letter

Features: Where the Wild Things Are | An African Son | 'Do Tell | The Mighty Are Falling
Departments: Campus Views | Letters | News & Notes | Class Notes | Aggies Remember | End Notes

The Mighty Are Falling

By Jeff Hudson

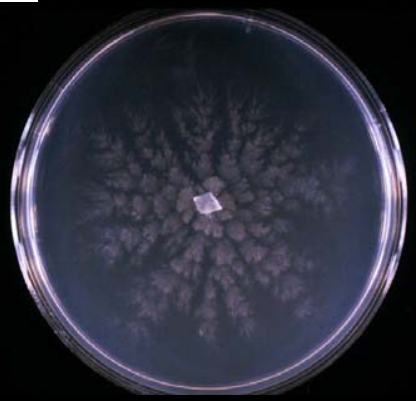
A species of the organism that caused the devastating Irish potato famine is now killing the California oaks.

Something is killing the oak trees—the handsome, long-lived, slope-dwelling trees that are the visual signature on many of Marin County's beautiful and expensive hillsides.

Arborists started noticing dying tanoaks in 1994. By 1996, people were noticing that coast live oaks were dying as well. And so were the black oaks.

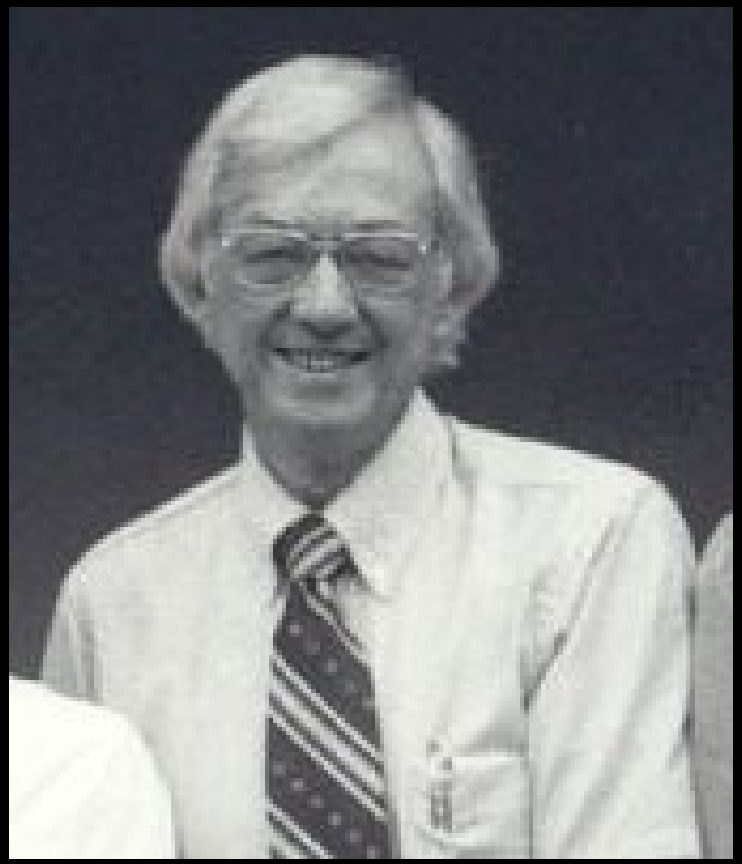
The foliage would turn brown, and sap would ooze from the bark on the trunk. Seemingly he
trees could become dead trees within a matter of weeks

The newspapers soon gave the phenomenon a name
what was causing the tre e, though a number of
bug might be found at th of it all. Others though
even a symptom of



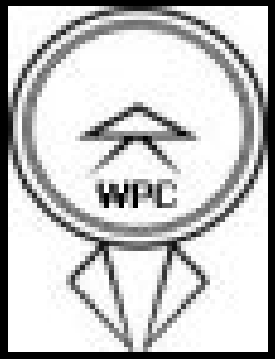
WPC founders

D.C. Erwin



G.A. Zentmyer

Source: Michael D Coffey



World Phytophthora Genetic Resource Collection (WPC)

The origins of this important collection were in the research works of Professor Erwin and Professor Zentmyer at the University of California, Riverside

Erwin collected mainly isolates from alfalfa (lucerne)

Zentmyer isolates of *P. cinnamomi* and *Phytophthora* species from cacao

WPC founders

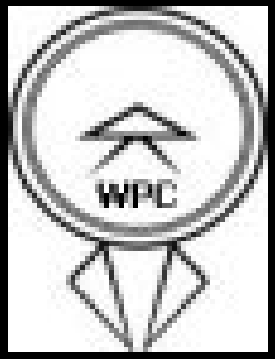
D.C. Erwin

1962

G.A. Zentmyer

1965

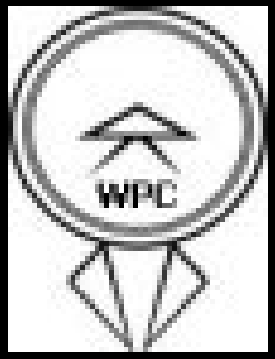
Source: Michael D Coffey



World Phytophthora Genetic Resource Collection (WPC)

In 1962, the first accessions of the World Phytophthora Genetic Resource Collection (WPC) were placed in glass culture tubes and a great adventure began.

The oldest deposition of the existing cultures is P0127, an isolate of *Phytophthora medicaginis* from Australia



World Phytophthora Genetic Resource Collection (WPC)

There was also a limited attempt to accumulate representative species of the genus.
With Zentmyer's retirement in 1979 some of the accessions were sent to ATCC which provided them with a core collection

Many cultures were lost at this point due to the difficulty of maintaining them using traditional methods such as preservation under mineral oil

CRYOPRESERVATION
of the World Phytophthora Collection (WPC)
1981-2008

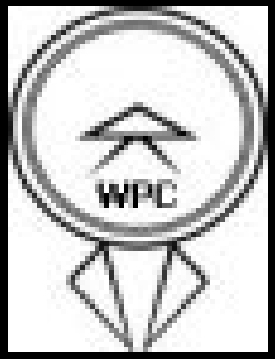
In 1986 a major development was the provision of funds by the

UC Genetic Resources Conservation Program

(UC GRCP) for Imperiled Microbial Collections to allow the WPC to be stored under liquid nitrogen using

cryogenic techniques

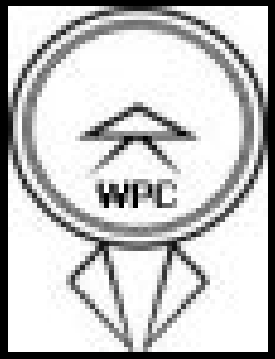
UC GRCP was terminated in June 2008



World Phytophthora Genetic Resource Collection (WPC)
A part of the WOGRC

The WPC has grown in stature over the last 25 years increasing in size from 600 to over 9500 accessions of *Phytophthora* (August 2008) of the more than 95 species which represent our current taxonomic information on this most important oomycete genus

Many of the accessions have been intensively studied over the years and thus the WPC is not only unique in size but also in terms of its importance as a genetic resource



Pythium species Genetic Resource Collection
A part of the WOGRC

This collection has grown in stature over the last 20 years increasing in size from 30 to over 900 accessions of *Pythium* (August 2008) representing more than 97 species.

DNA from many of the accessions has been extracted and retained in the WOGRC DNA Bank and thus the Pythium Collection is not only unique in size but also in terms of its importance as a genetic resource

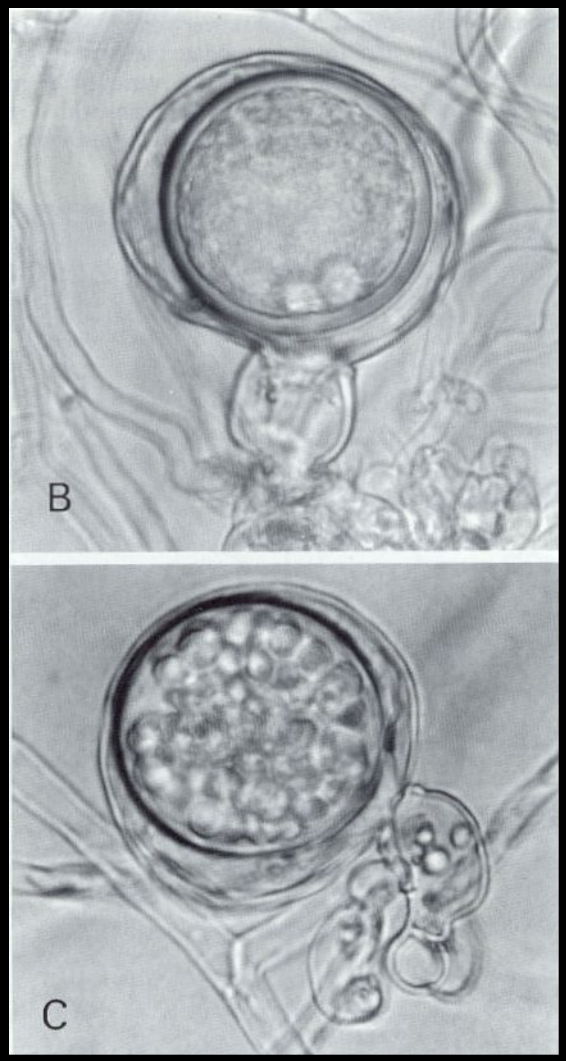
Phytophthora Species

- 95 plus species described
- variability in morphological traits
- limited morphological traits
- atypical isolates
- isozymes, mtDNA RFLP
- ITS sequences
- genomics, multilocus analysis and phylogenetic species

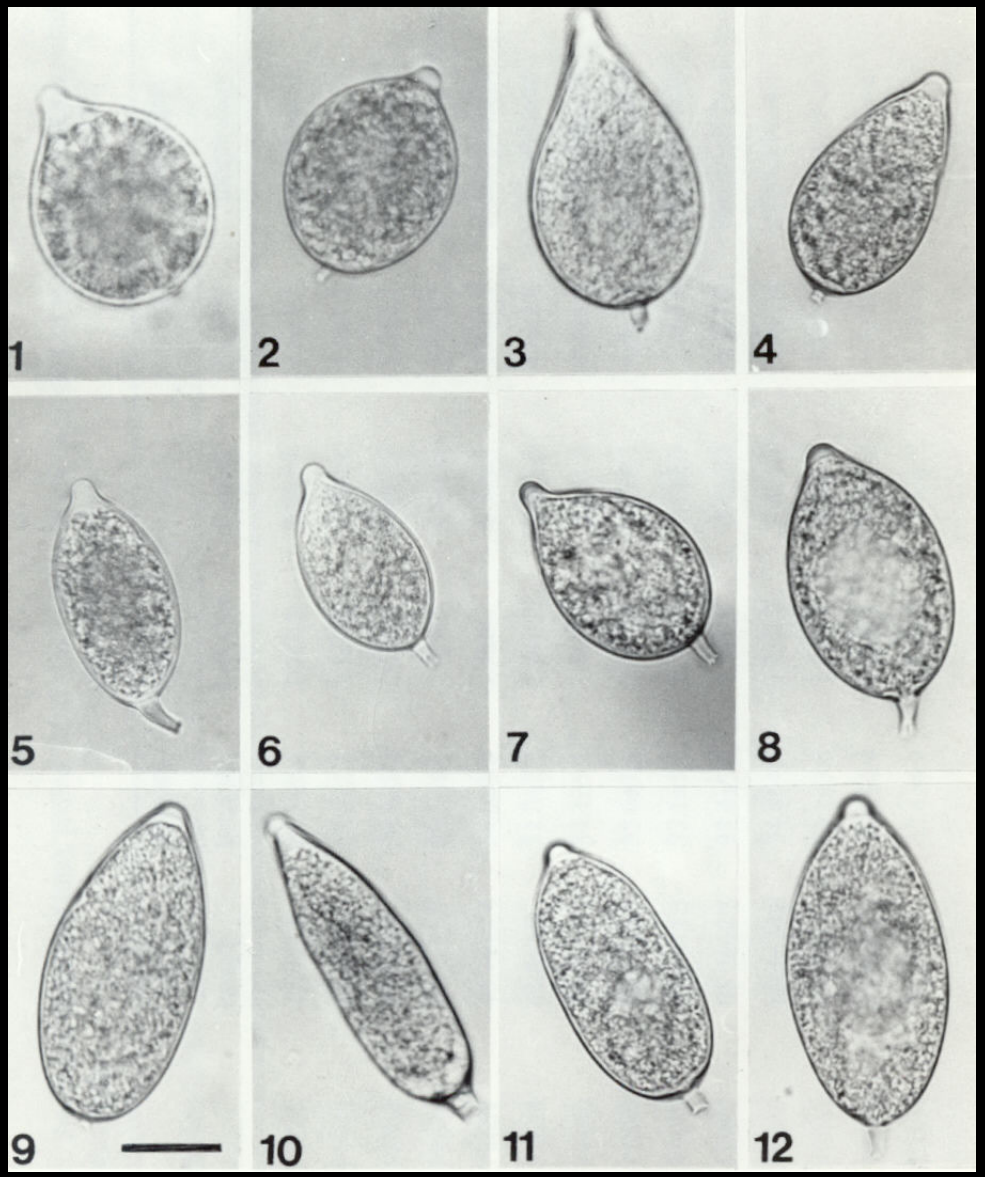
Waterhouse Groups

- papillate
  - paragynous I
  - amphigynous II
- semipapillate
  - paragynous III
  - ampigynous IV
- nonpapillate
  - paragynous V
  - amphigynous VI

Amphigynous versus Paragynous Antheridium

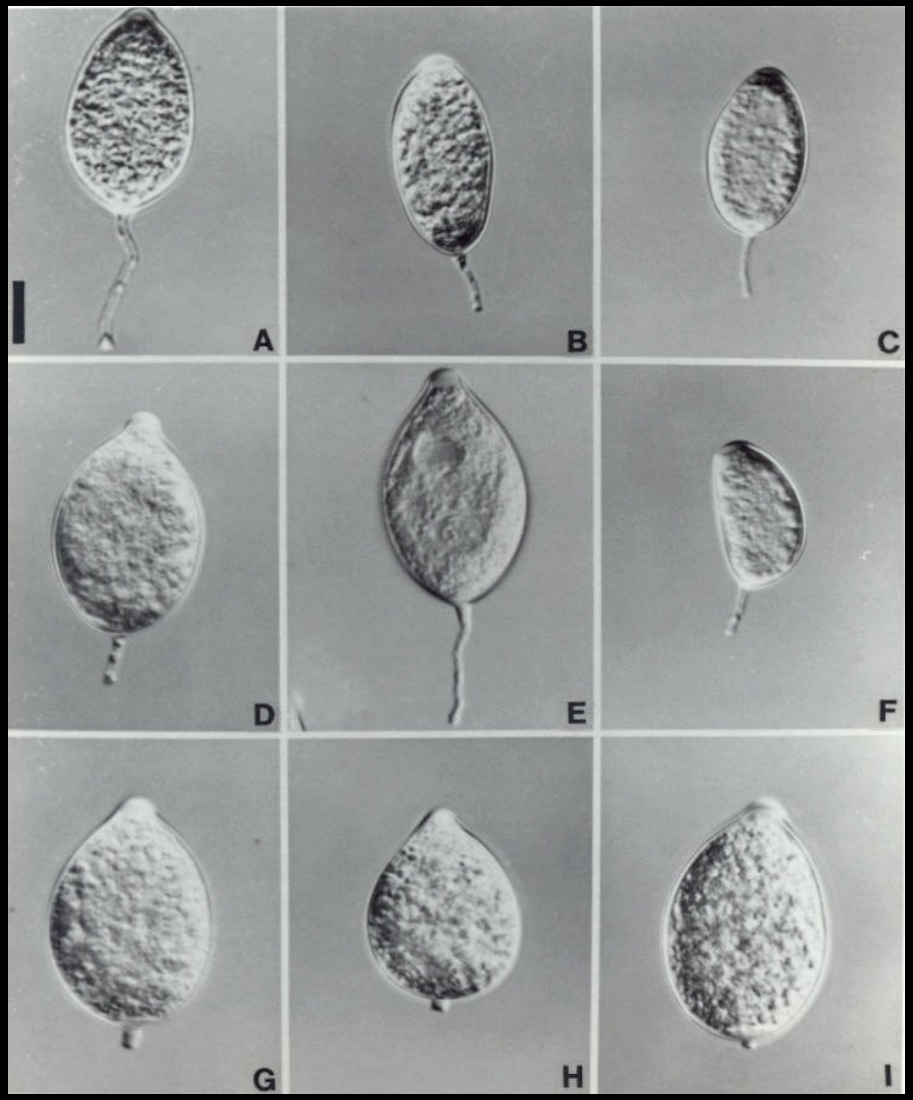


Sporangial Morphology



palmivora

Pedicel Length and Sporangial Dimensions



Multilocus Phylogeny – a population genetics approach

- type species v. global population

- World Phytophthora Genetic Resource Collection (WPC at UCR)

- emphasis on the use of genetic traits
- how many isolates need to be studied to characterize a species?
- what methods need to be used?

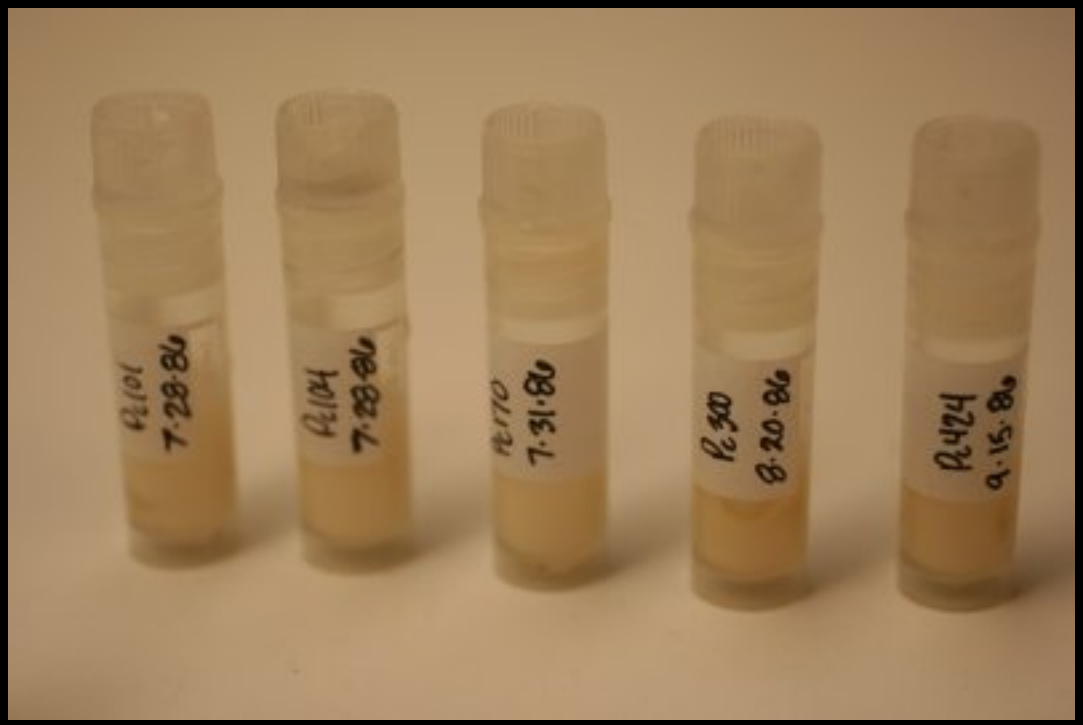
A population genetics approach

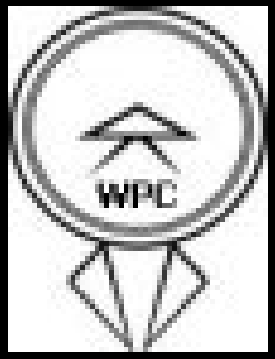
Type species versus global population

World Phytophthora Genetic Resource Collection

THE FREEZING PROCESS

Cryostorage of isolates at the WPC





World Phytophthora Genetic Resource Collection (WPC)

For long-term storage of Phytophthora cryogenic temperatures are essential

temperatures below the glass transition temperature of water

This is the temperature at which all biological activity ceases, and is generally accepted as -130°C

Biological and chemical activity can persist as long as water activity exists, however below -130°C all activity ceases

The basic process involves the following steps:

Equilibration of the sample with a cryoprotectant (DMSO) at room temperature to permit uptake of the solution

Cooling of the samples at ~ 1 to 2C per min to 0C, then 10 min at OC, followed by ~1C per min down to –10C

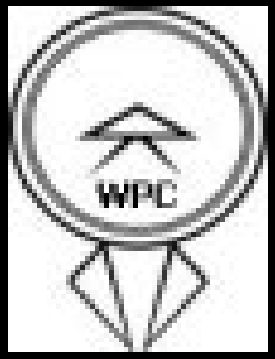
Following thermal equilibration of the samples prior to ice crystal growth, the temperature is then dropped further to -44 C

Finally, the samples are cooled very rapidly from -44 C to – 120 C in ~10 min

Taylor
Wharton
8K

Liquid or
Vapor-phase

THE DNA BANK



Phytophthora DNA Bank

ARCHIVAL DNA SOURCES stored at -86C in ultrapure water

DNA ‘dilution’ tubes (~10ng/uL) prepared from the ARCHIVAL DNA SOURCE
These are stored at -20C in low TE

Freeze dried material stored at -70C as the
Frozen Mycelium Inventory
from which to make fresh DNA extractions

Current Activities at the WPC

- Live cultures (~9500)
- DNA Bank (~6000)
- Frozen Mycelium (~6000)
- Databases (ITS ~2000)

- Molecular and Integrated Phylogeny
- Diagnostics Research
- Workshops and Training

MOLECULAR PHYLOGENY AND DIAGNOSICS

Multilocus Phylogeny – a population genetics approach

What methods need to be used?

ITS 1 and 2
B-TUBULIN
LARGE SUBUNIT (LSU, 28S)
Cox1, Cox2

Multilocus Phylogeny

A genus-wide phylogeny for *Phytophthora*utilizing complete genome sequences

6 November 2007 Disk Used

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Fungal Genetics and Biology xxx (2007) xxx–xxx

FUNGAL GENETICS AND BIOLOGY

www.elsevier.com/locate/yfgbi

A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences

Jaime E. Blair [a,*], Michael D. Coffey [b], Sook-Young Park [a], David M. Geiser [a], Seogchan Kang [a]

[a] *Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA*
[b] *Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA*

Received 23 April 2007; accepted 15 October 2007

Abstract

Phytophthora species are devastating plant pathogens in both agricultural and natural environments. Due to their significant economic and environmental impact, there has been increasing interest in *Phytophthora* genetics and genomics, culminating in the recent release of three complete genome sequences (*P. ramorum*, *P. sojae*, and *P. infestans*). In this study, genome and other large sequence databases were used to identify over 225 potential genetic markers for phylogenetic analyses. Here, we present a genus-wide phylogeny

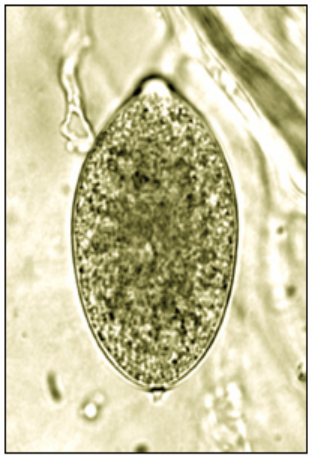
Genome Resources…

Evolutionary Relationships

JGI

Phytophthora sojae v1.0

Search | BLAST | Browse | GO | KEGG | KOG | AdvancedSearch | Download | Info | Home | HELP!



Phytophthora ramorum, photo courtesy of Matteo Garbelotto, UC Berkeley

Phytophthora is a genus of the Oomycetes (water molds) which, through convergent evolution, have similarities to fungi. However, oomycetes are not fungi (as had been earlier thought), but are part of Stramenopiles, a kingdom distinct from plants, fungi, and animals that also includes diatoms and golden-brown and brown algae, such as kelp.

Fifty-nine species of *Phytophthora* are recognized. They attack hundreds of different plant species, including many crops, costing tens of billions of dollars in damage per year. Genome sequencing efforts at JGI have focused on two species, *Phytophthora sojae* and *P. ramorum*. *P. sojae* has been developed as a model species for the genus, having in place excellent genetic and genomics resources (including genetic maps, BAC libraries, and EST sequences), as well as having a well organized community of researchers. The particularly virulent *P. ramorum* is now destroying coastal oaks in California (causing "Sudden Oak Death"), attacks black oak, shreve oak, and tan oak, as well as a variety of shrubs that inhabit the oak ecosystems, and threatens the oak forests in the Sierra Nevada and, potentially, the red oak forests of the east coast

Oomycete Genomics Database

OGD

Phytophthora Functional Genomics Database

Phytophthora ramorum

Phytophthora sojae

Phytophthora infestans

Phytophthora capsici

Other Oomycetes (ESTs):

Saprolegnia parasitica

Plasmopara halstedii

Aphanomyces cochlioides

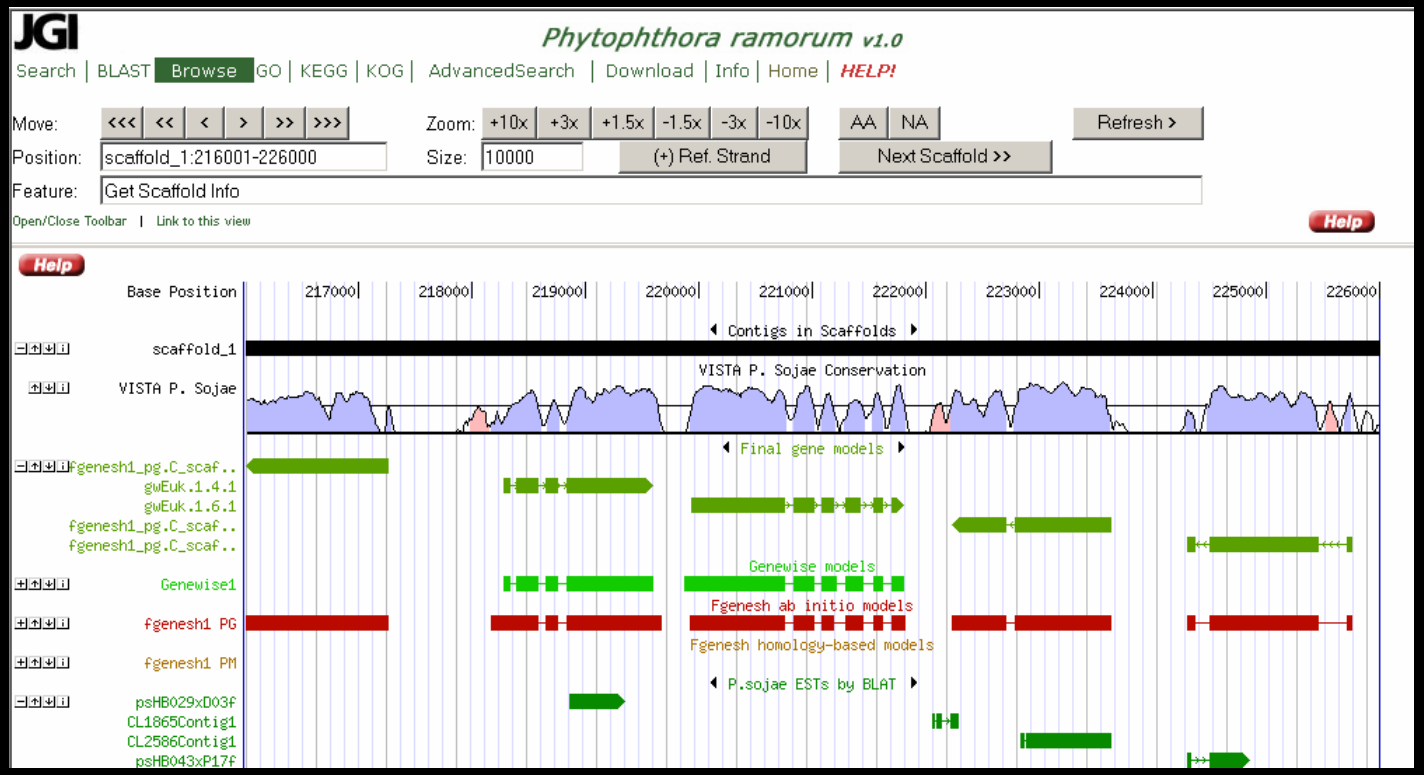
Hyaloperonospora parasitica

Source: Jaime Blair

Evolutionary Relationships Among *Phytophthora*

Source: Jaime Blair

Genome Resources…



Utilize Complete Genomes to Design Markers for Genus-wide Phylogeny

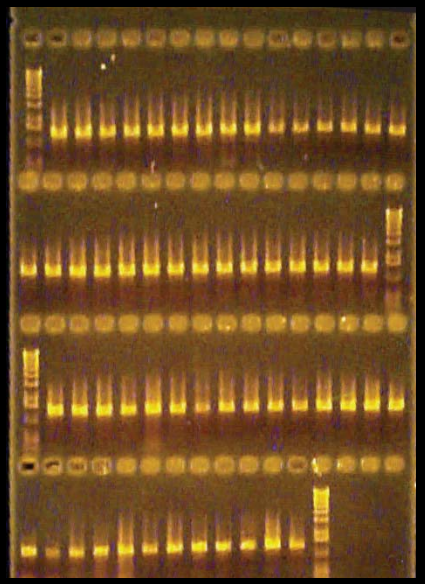
~82 + species, 200+ isolates mainly at the WPC at UC Riverside

Phytophthora Phylogeny

Marker Selection

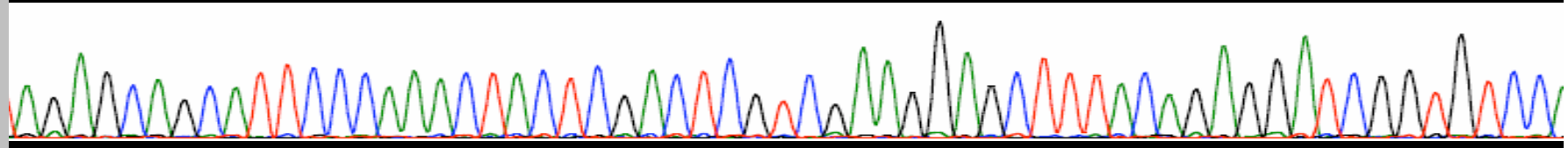
- ~225 loci identified as potentially informative
- Primers designed for 27 potential markers
- 16 produced PCR products, sequence data

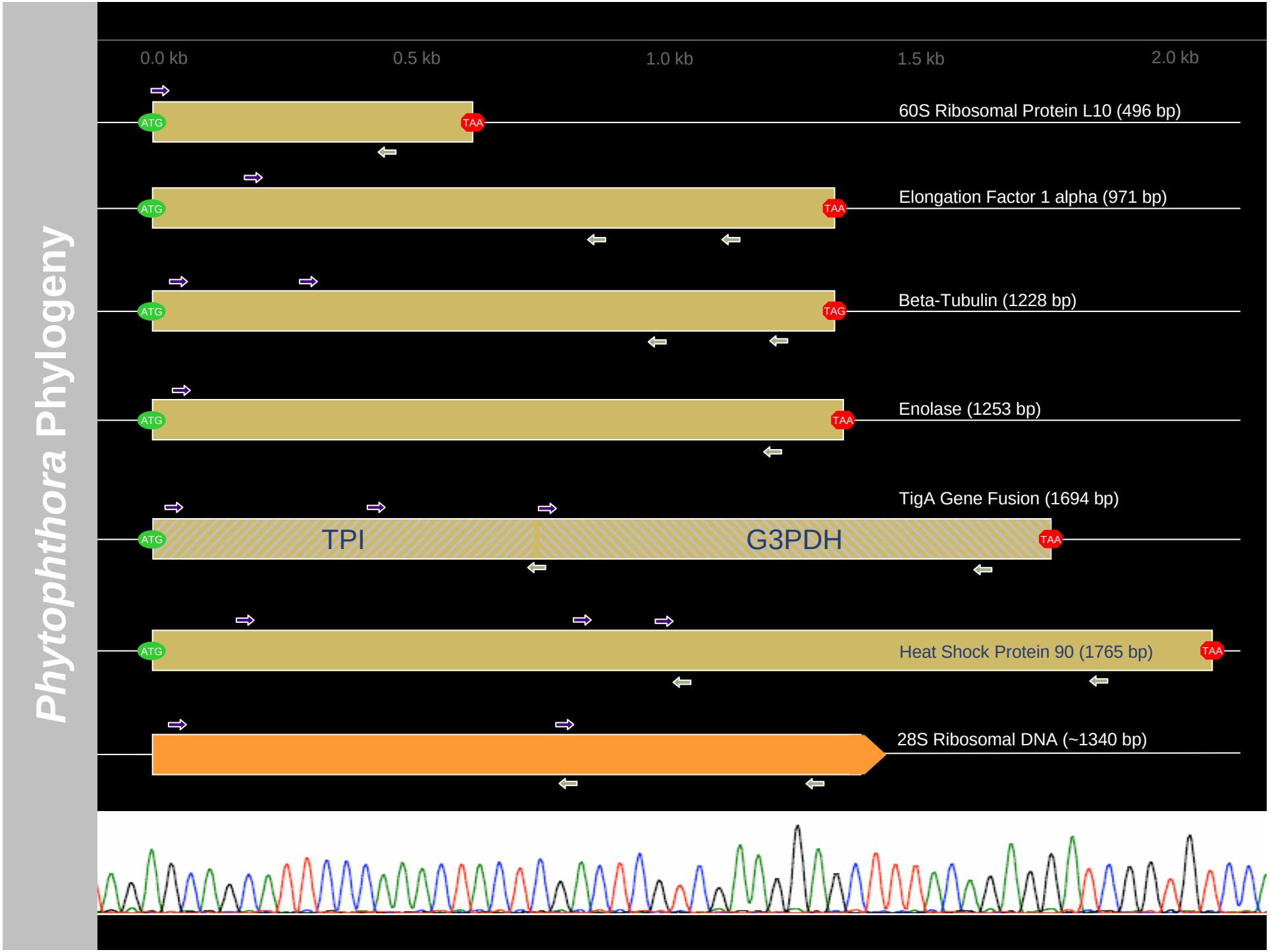
**Amplify AND Align across genus



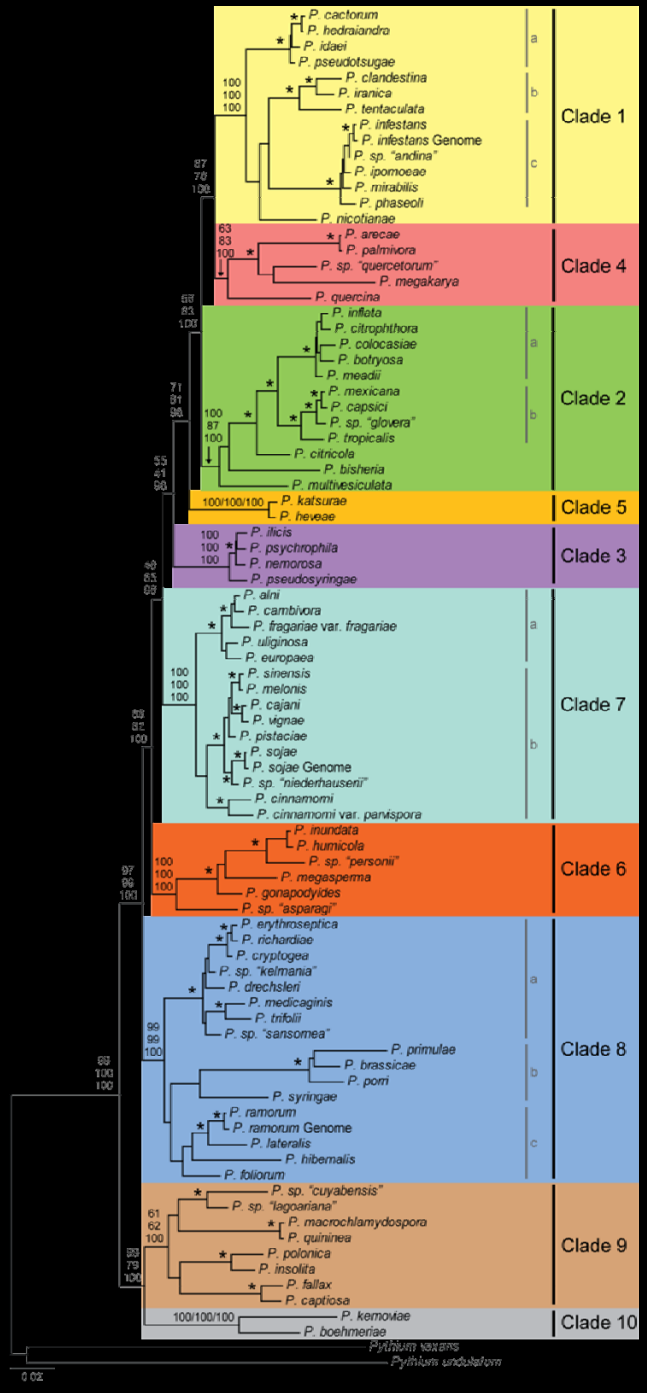
Marker Sequencing

- 1600+ sequences generated
- 234 isolates representing 84 species
- 7 markers chosen for phylogeny (~7600 bp)




Phytophthora Phylogeny
0.0 kb
0.5 kb
1.0 kb
1.5 kb
2.0 kb
ATG
TAA
60S Ribosomal Protein L10 (496 bp)
Elongation Factor 1 alpha (971 bp)
TAG
Beta-Tubulin (1228 bp)
Enolase (1253 bp)
TigA Gene Fusion (1694 bp)
TPI
G3PDH
Heat Shock Protein 90 (1765 bp)
28S Ribosomal DNA (~1340 bp)

Phytophthora Phylogeny



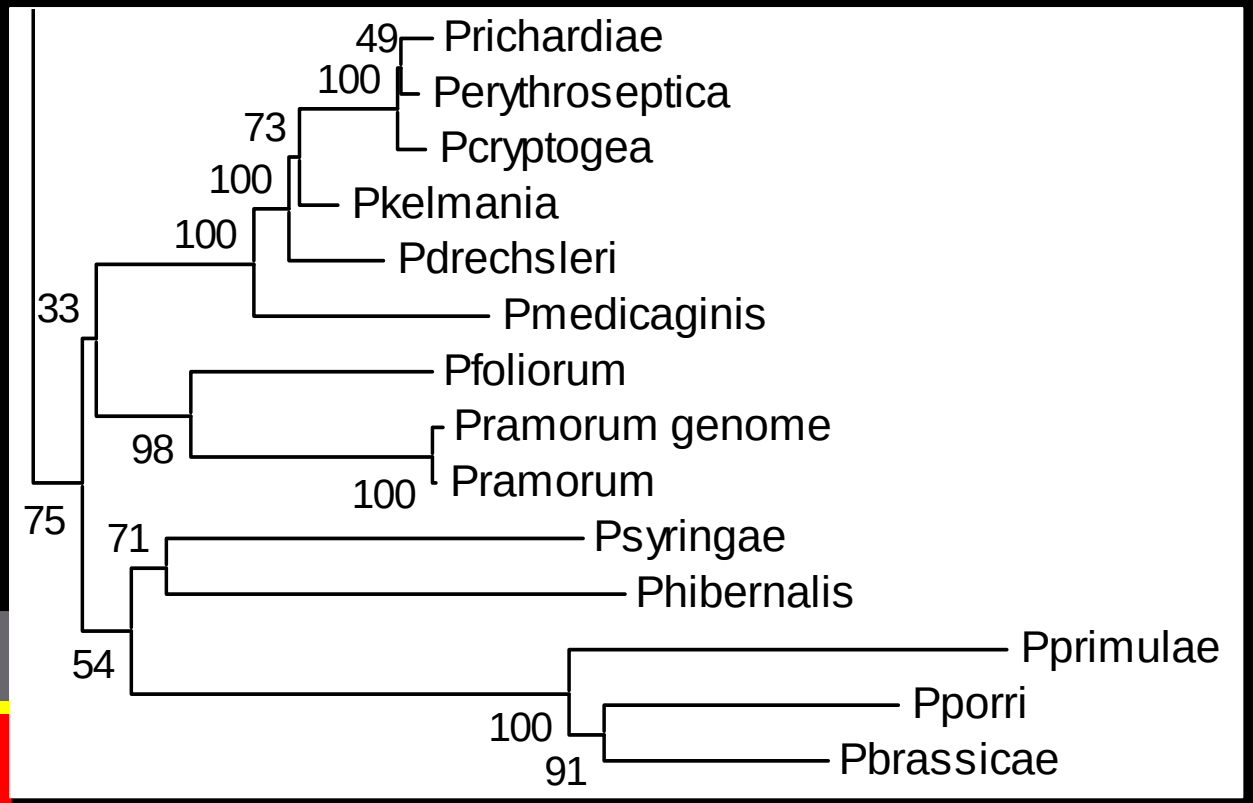
82 *Phytophthora* sp.
2 *Pythium* outgroups

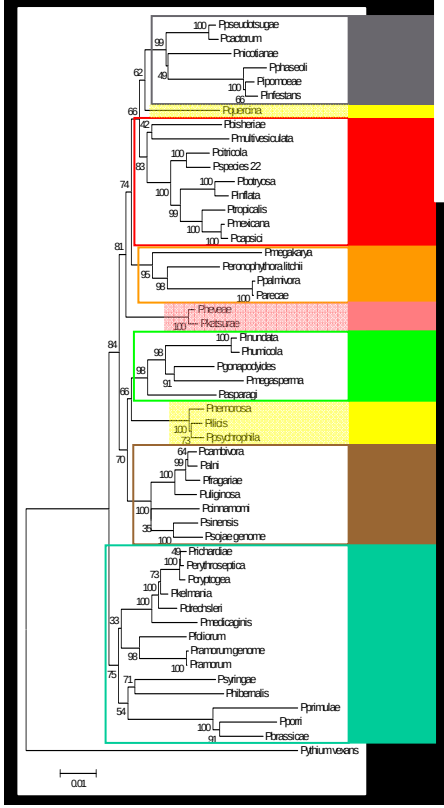
24 species not previously analyzed
(including 10 new species)

10 Well Supported Clades
Small Inter-Clade Distances

ModelTest – GTR+I+G
ML – Garli, 1000 bootstraps with ML parameters
MP – PAUP*, random addition, TBR, 1000 bootstraps
MrBayes – 2 analyses, flat priors, 2 million gen.
BEAST – uniform priors, 5 million gen.

Clade 8





Source:
Jaime E Blair
PSU

Phytophthora Database

Phytophthora DATABASE

Home | Introduction | Database | Search & Analysis

Species :: List

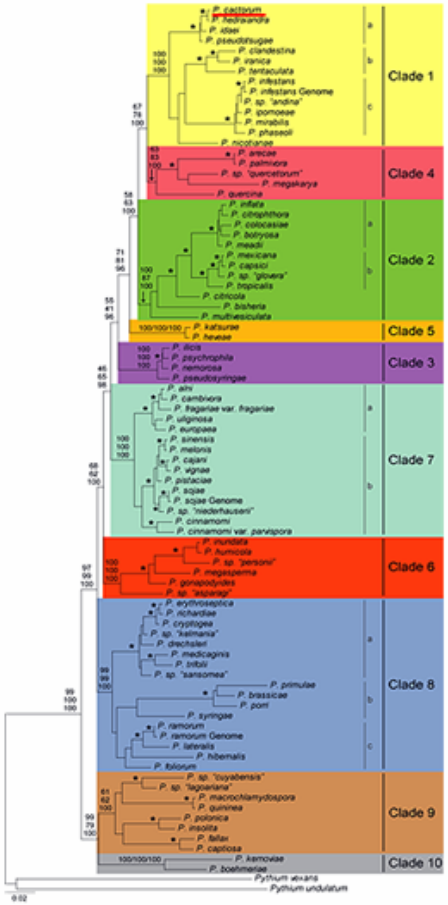
There are **84** spe

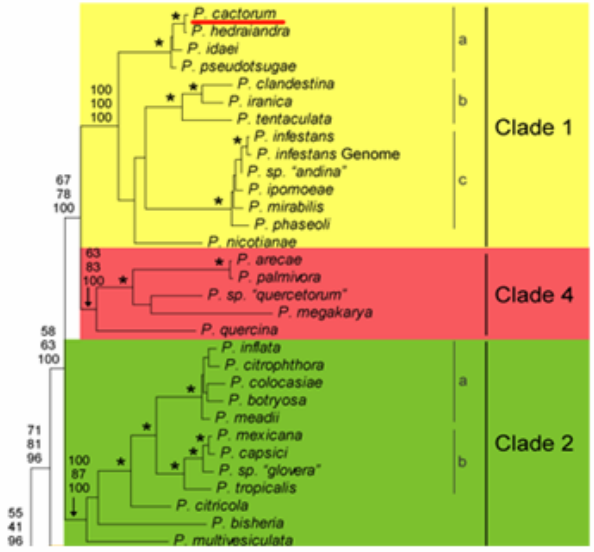
Species Name
Phytophthora alni
Phytophthora andina
Phytophthora arecae
Phytophthora asparagi
Phytophthora bisheria
Phytophthora boehmeriae
Phytophthora botryosa
Phytophthora brassicae
Phytophthora cactorum
Phytophthora cajani
Phytophthora cambivora
Phytophthora capsici
Phytophthora captiosa
Phytophthora cinnamomi
Phytophthora citricola
Phytophthora citrophthora
Phytophthora clandestina
Phytophthora colocasiae
Phytophthora cryptogea
Phytophthora cuyabensis
Phytophthora drechsleri
Phytophthora erythroseptica
Phytophthora europaea
Phytophthora fallax
Phytophthora foliorum
Phytophthora fragariae
Phytophthora glovera
Phytophthora gonapodyides
Phytophthora hedraiandra
Phytophthora heveae
Phytophthora hibernalis
Phytophthora humicola
Phytophthora idaei
Phytophthora ilicis
Phytophthora infestans
Phytophthora inflata
Phytophthora insolita
Phytophthora inundata
Phytophthora ipomoeae

Phytophthora cactorum

Phylogenetic Position within the Genus

This genus-wide phylogenetic tree contains 83 species, including *Pythium vexans* as the outgroup, and was built using sequences at seven loci (approximately 8700 nucleotides), including 60S Ribosomal Protein L10, Beta Tubulin, Enolase, Heat Shock Protein 90, Large Subunit rRNA, TigA gene fusion, and Translation Elongation Factor 1 alpha (Jaime Blair et al., unpublished data).



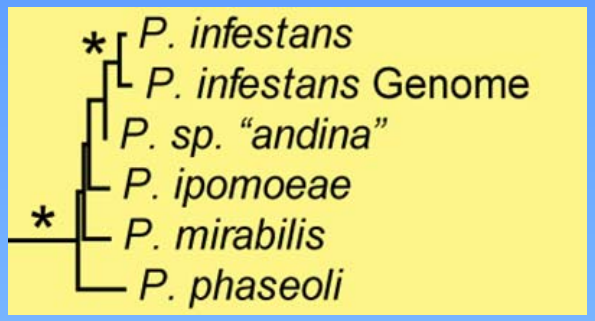


[Click the tree to enlarge it.]

CLADE 1 STUDY

- Franklin & Marshall College, Pennsylvania: Jaime E. Blair
- USDA ARS Salinas: Frank Martin

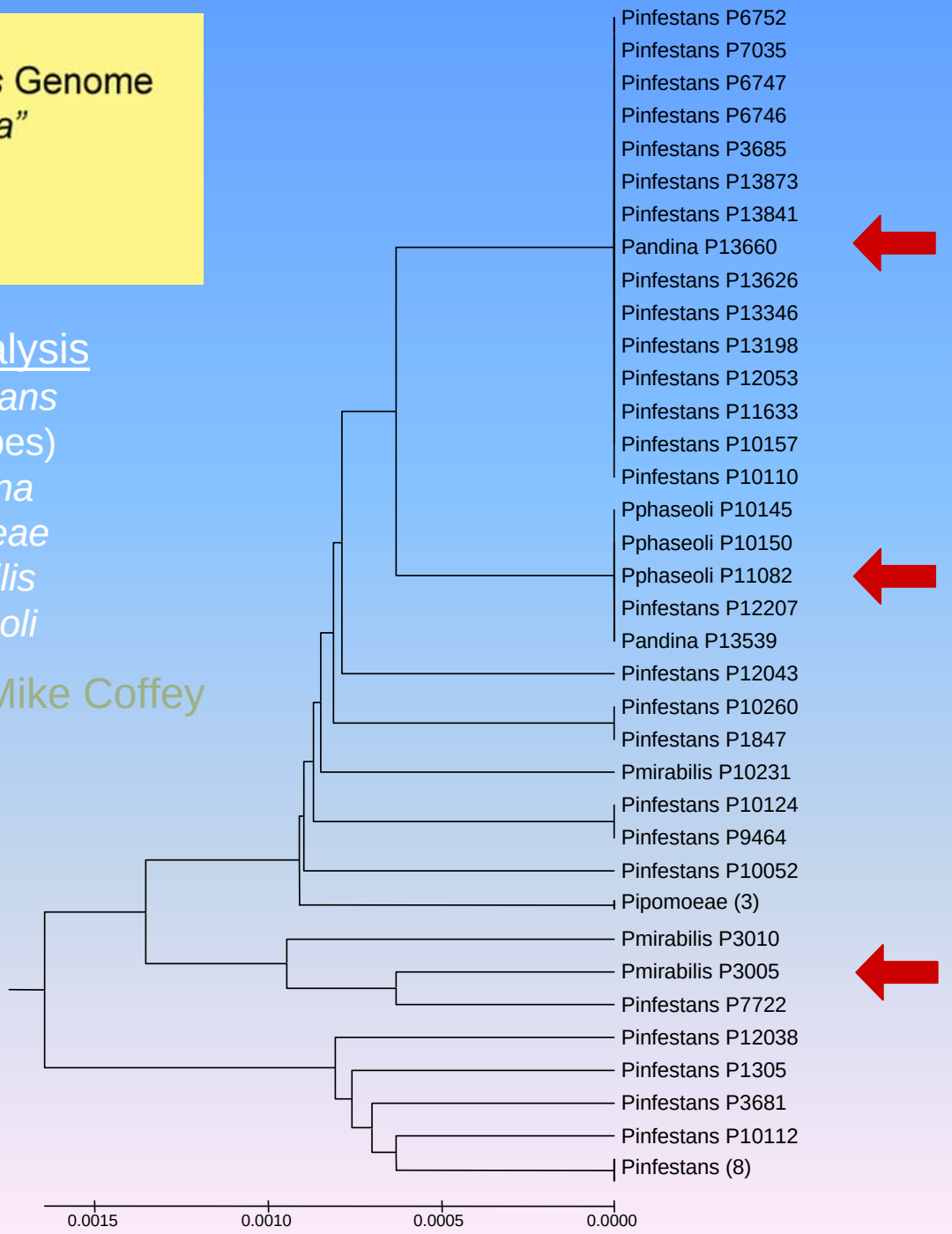
Intraspecific Diversity



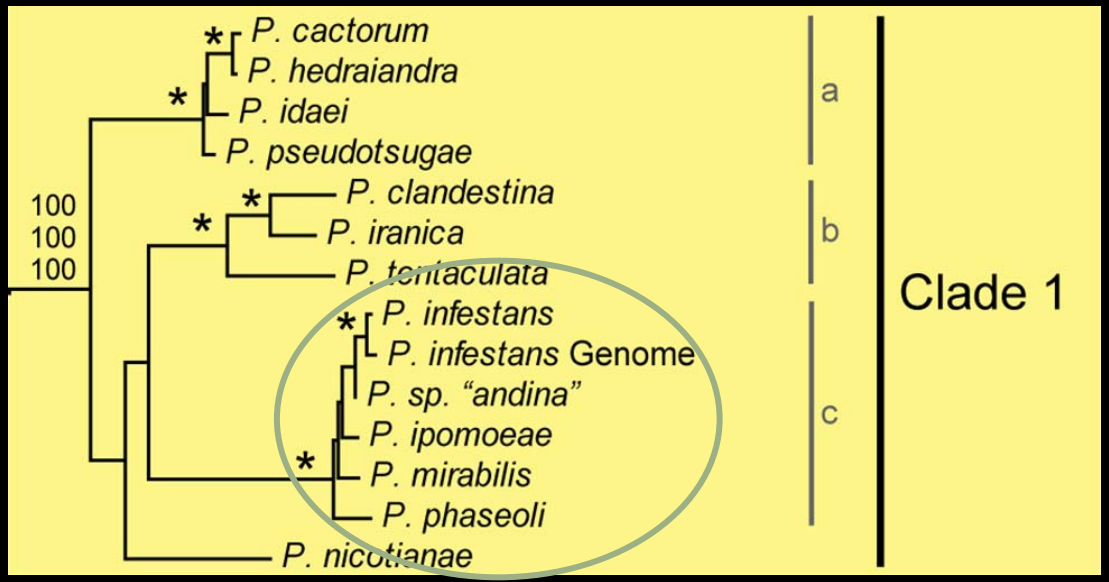
Initial ITS Analysis

- 219 *P. infestans* (66 haplotypes)
- 13 *P. andina*
- 3 *P. ipomoeae*
- 3 *P. mirabilis*
- 4 *P. phaseoli*

*Massy Peiman, Mike Coffey



Intraspecific Diversity



5 "Taxa" = 5 Species?

Initial Observations:

- *P. sp. "andina"* most likely not separate species
- "*P. infestans*" & *P. mirabilis* lineage?
- *P. ipomoeae* and *P. phaseoli* good species

Future Plans:

- Continue sequencing
- Additional isolates, recent collections

Phytophthora Database

User Authentication
(analysis “cart”)

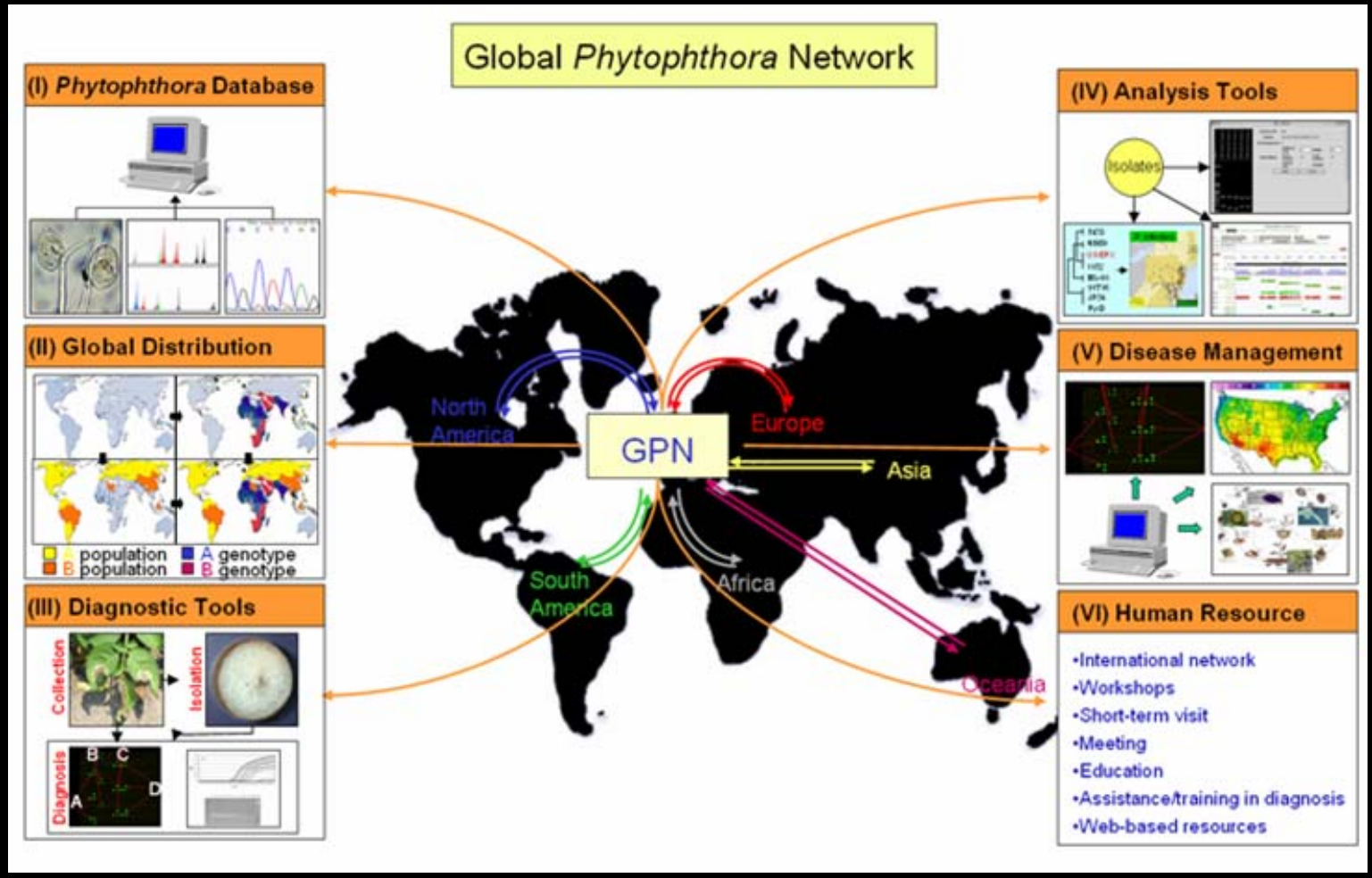
Wizard, Help Menu

Data Submission
(currently manually curated)

BLAST, ClustalW,
Phyloviewer,
Virtual RFLP

**Bongsoo Park, PSU

Phytophthora Database



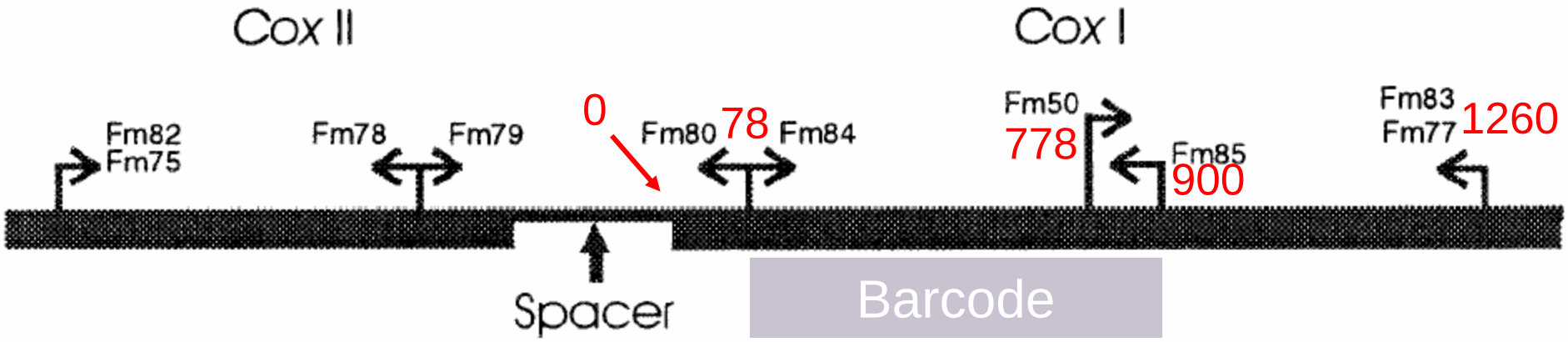
THE BARCODE PROJECT

Biodiversity Canada Agriculture: André Levesque, Gregg Robideau

Oomycete barcoding

Cytochrome Oxidase I *(COI)*
Good primers designed that amplify a 727bp region of *COI*
No introns in oomycete *COI* (as opposed to true fungi)

From G.P. Robideau, A.W.A.M. de Cock, M. Peiman, K. Bala, M. D. Coffey, and C.A. Lévesque. 2008
André Levesque, Gregg Robideau

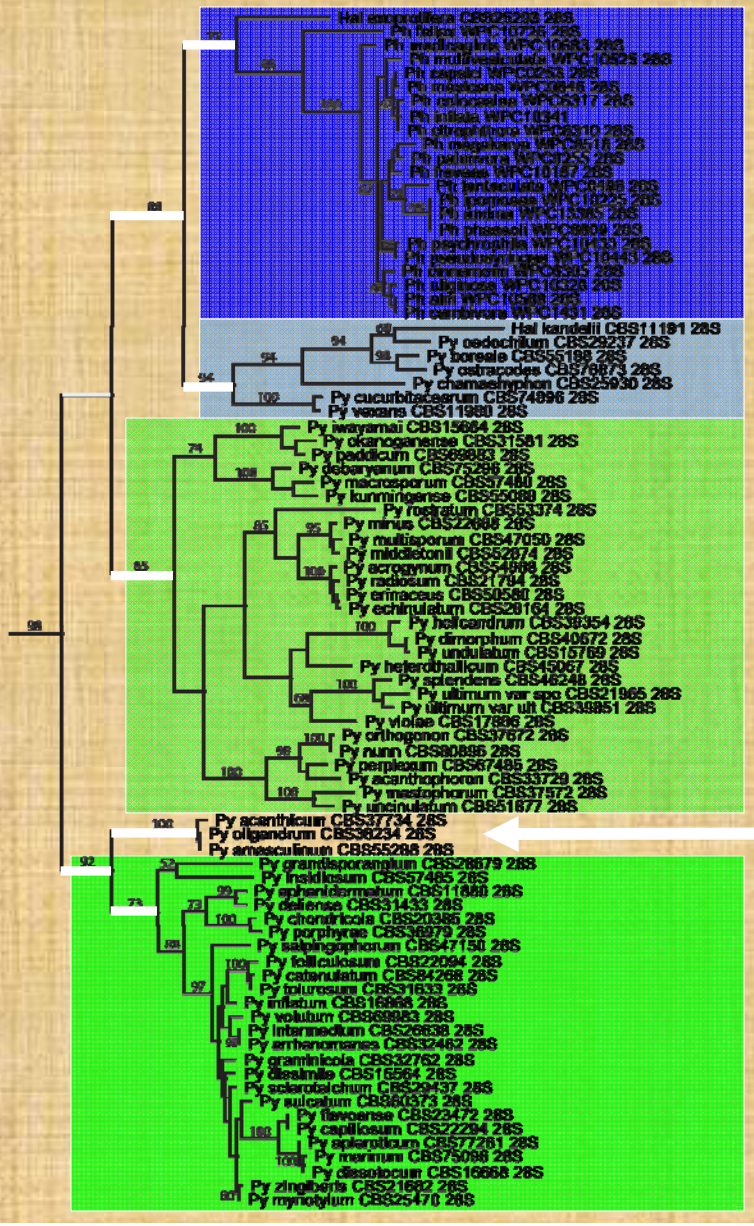


Oom-COI-Lev-up->

<-FM85-mod

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

28S Clades: Peronosporales



Phytophthora

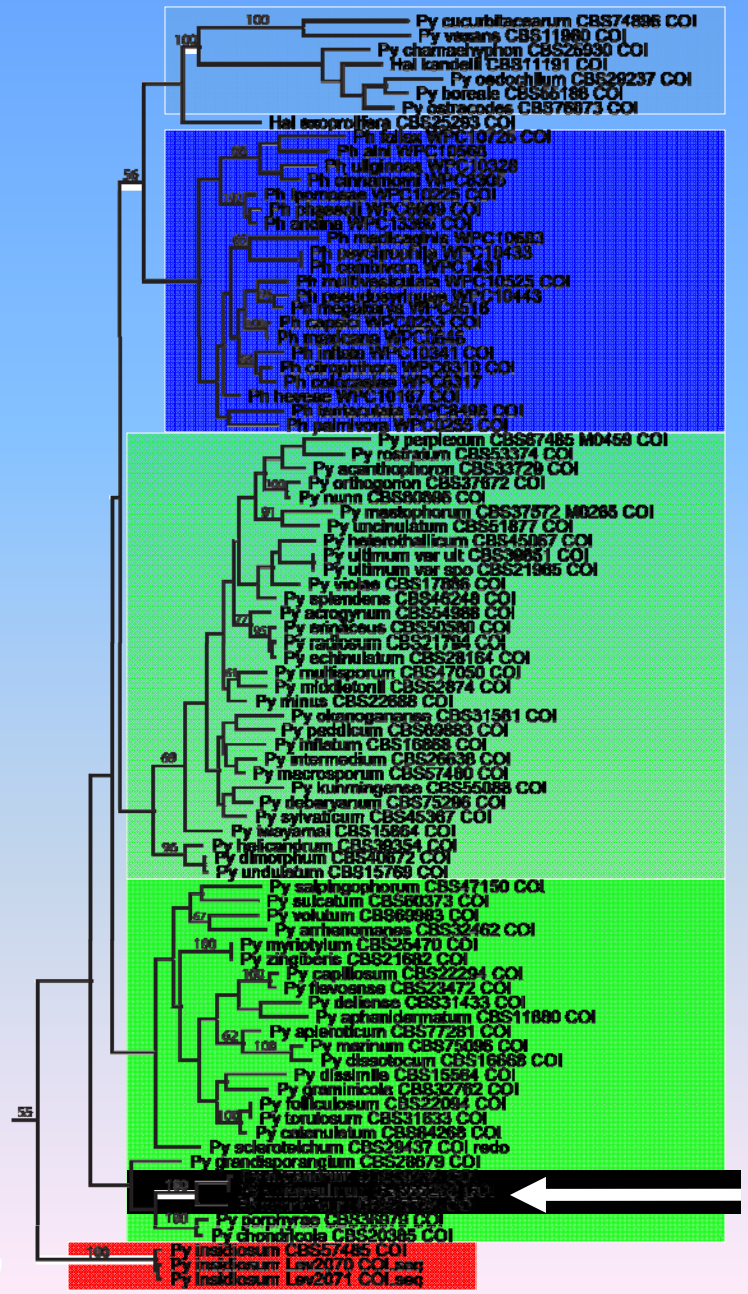
Pythium Clade K
Phytopythium

globose
Pythium

contiguous
Pythium

filamentous
Pythium

COI clades: Peronosporales

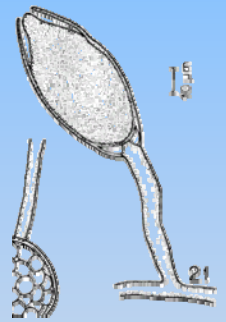


Pythium Clade K

Phytopythium gen. nov.

Phytophthora (WPC)

globose *Pythium*



filamentous *Pythium*

contiguous *Pythium*

Pythium insidiosum

WORKSHOPS

In 22 years the number of accessions in the WPC has grown to ~9500 representing over 95 species. Also represented are accessions representing the 15 described species within the marine genus *Halophytophthora.*

In the last 12 years a concerted effort has been made to add a large collection of *Pythium* species to the cryostorage inventory. In the last two years this effort has grown exponentially and there are now over 900 accessions representing 97 species of *Pythium*

An initial effort has been made to rescue the remnants of the Michael W. Dick Aquatic Phycomycetes Collection (APCC) now held at CABI.

The original mission of the **World *Phytophthora* Collection (WPC)** has been broadened with the accumulation of the phylogenetically comprehensive *Pythium* Collection and will continue with the acquisition of additional genera within the Kingdom Straminipila.

The new name for this collection will be the **World Oomycetes Genetic Resource Collection (WOGRC).**

The future goals of the WOGRC are threefold:

1) maintenance and expansion of a worldwide collection of genera within the Kingdom Straminipila

2) development of a DNA Bank to provide DNA for research

3) creation of online databases providing phenotype and genotype information on important genera.

Phytophthora and *Pythium* Workshop at NCSU

2006

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  Laura Gaggero, Pedro Martin, Ehab Sarhan

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- USDA-APHIS- CPHST: Phil Berger, Laurene Levy
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- Vietnam and Australia: Doan Nhan Ai, David Guest, Andre Drenth

THE END