Workshop, Annual APS Meeting Minneapolis

Phytophthora: Culturing, storage and a DNA Bank

Michael David Coffey

UNIVERSITY of CALIFORNIA Riverside

PENNSTATE

Saturday, July 26, 2008

WPC

The beginnings 1966

Phytopathology News

Volume 1 February 1967 Number 1

Annual meeting tops for attendance and papers

New and old members of the APS Council. Photo taken at the Denver meeting, August 23, 1966. Left to right: George Gries, H. H. Flor, Thor Kommedahl, Arthur Kelman, J. E. Mitchell, George Zentmyer, J. P. Fulton, A. W. Dimock, W. J. Zaumeyer, David Marsden, and Ralph Caldwell.

COUNCIL LAUNCHES NEWSLETTER PROGRAM

See "The Council's Corner," page 2, and "The Editor's View," page 4, for details of Phytopathology News, latest publication of The American Phytopathological Society.

President Zentmyer reports progress

Some 740 members and 192 wives were on hand when President G. A. Zentmyer cracked the gavel to open the 58th Annual Meeting of The American Phytopathological Society.

Program highlights included:

- A conference on undergraduate education in plant pathology co-sponsored by the Society and the Commission on Education in Agriculture and Natural Resources.
- A special session on the impact of the International Biological Program on plant pathology.
- Two symposia—Population Dynamics of Plant Parasitic Nematodes, and Subcellular Sites of Virus Synthesis.
- Fourteen discussion sessions and 279 contributed papers.

15 Society Fellows Named

Fellow of the Society awards went to: C. W. Bennett, L. M. Black, V. W. Cochrane, A. E. Dimond, Charles Drechsler, David Gottlieb, G. H. Hepting, F. O. Holmes, Thorvaldur Johnson, R. A. Ludwig, S. E. A. McCallan, K. O. Müller, W. C. Snyder, E. E. Wilson, and R. A. Young.

The first Ruth Allen award was presented to H. H. Flor.

Officers Report Highlights

PRESIDENT'S REPORT—Strong publishing activity during the year; special items include three / *to page 3*

Service Library Planned for American Tropics

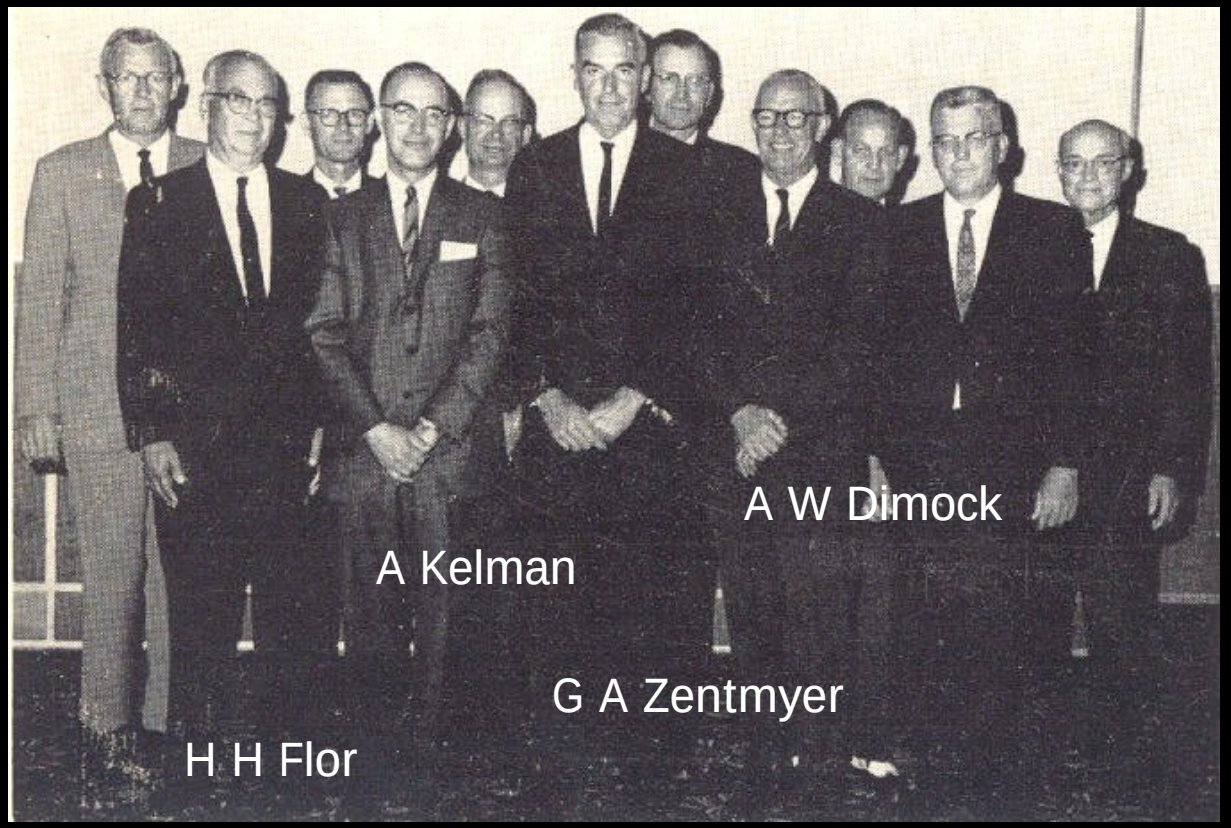
A service library to supplement and furnish research literature and aid in training and research teaching is planned for the American tropics.

According to F. L. Wellman, G. B. Lucas, and Carmen de Mendoza Marín, North Carolina State University, it is to be a computer-run, modern service institution, storing plant disease information on tape for instant electronic recovery. For the first years it will be located at NCSU.

Information will be disseminated by cable, telephone, Telstar, or other modern means. A corps of the most respected, semi-retired pathologists from all parts of the world will work at a central institution to review, abstract, and synthesize information. In close association, / *to page 3*

WPC

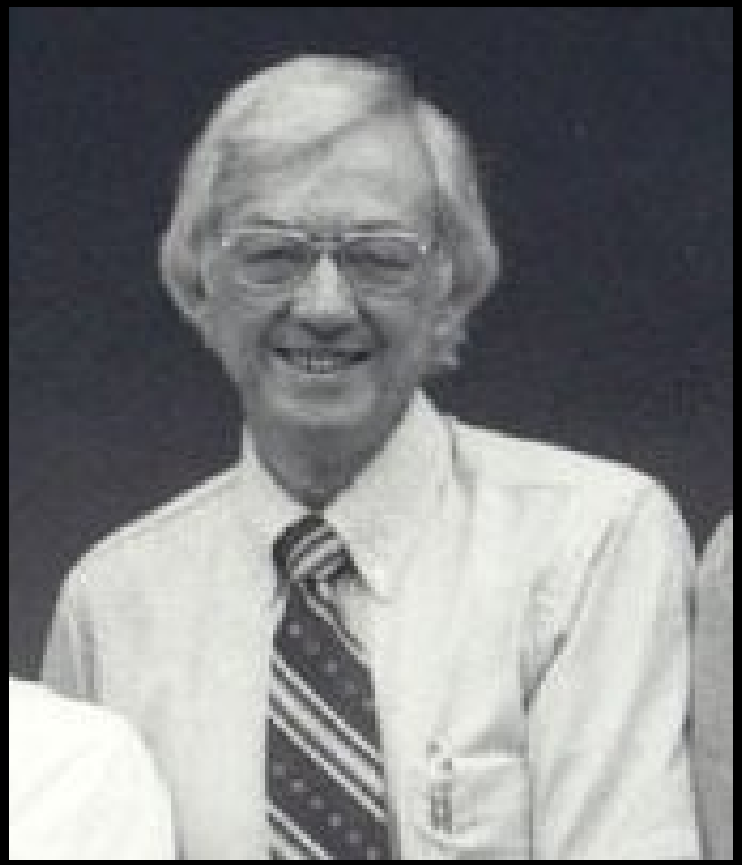
Denver
August 23, 1966



APS Council 1966

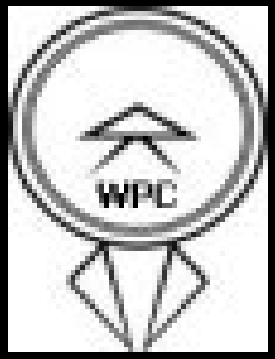
WPC founders

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 work of Professor Erwin and Professor Zentmyer at the University of California, Riverside

Erwin collected mainly isolates from alfalfa and Zentmyer isolates of *P. cinnamomi* and *P. palmivora* from cacao

WPC

WPC founders

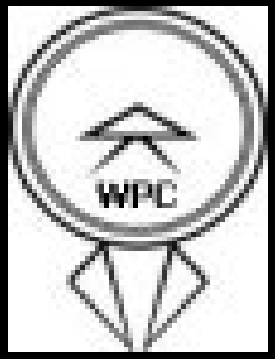
D.C. Erwin

1962

G.A. Zentmyer

1965

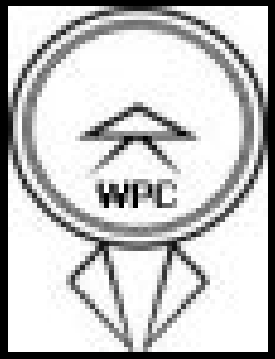
Source: Michael D Coffey



World Phytophthora Genetic Resource Collection (WPC)

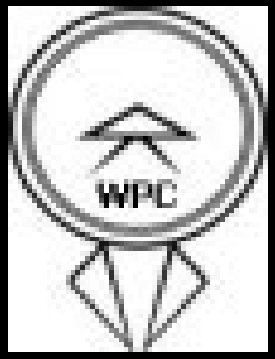
In 1962, the first accessions of the World Phytophthora Genetic Resource Collection (WPC) were placed in glass culture tubes and a great adventure began.

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 some of the accessions were sent to ATCC and 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 mineral oil preservation



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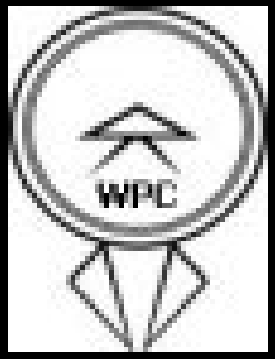
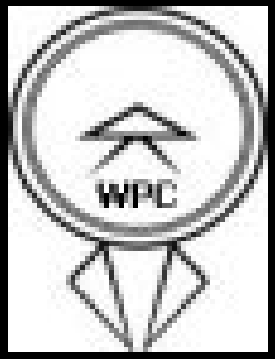


CRYOPRESERVATION of the World Phytophthora Genetic Resource Collection (WPC) 1986-2008

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)

The WPC has grown in stature over the last 25 years increasing in size from 600 to over 9500 accessions of *Phytophthora* (July 2008) of the more than 90 species or taxa which represent this most important plant pathogen.

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

Phytophthora Species

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

Multilocus Phylogeny – a population genetics approach

- type species v. global population

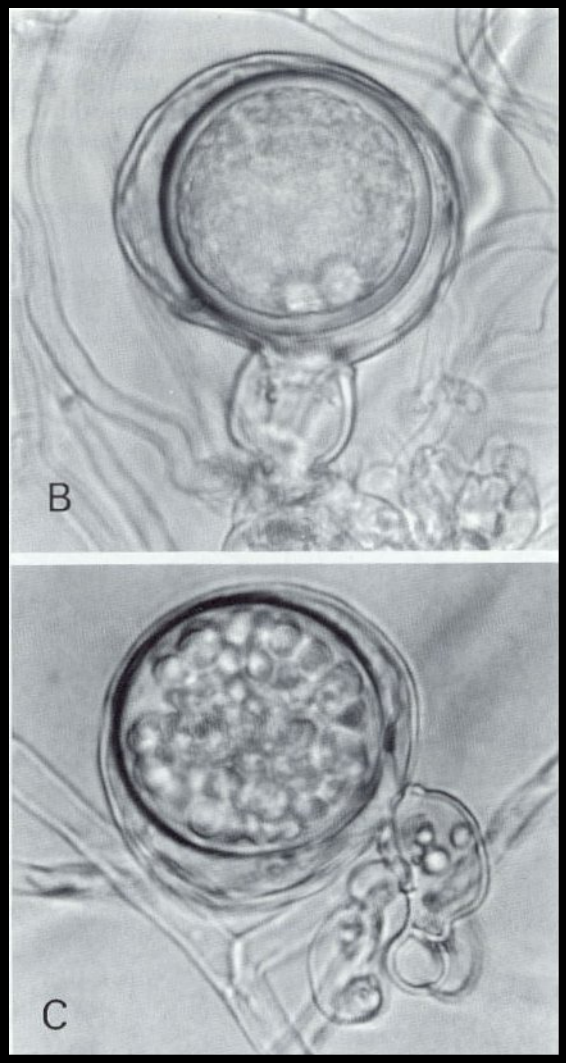
- World Phytophthora Genetic Resource Collection (WPC at UCR)

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

Waterhouse Groups

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

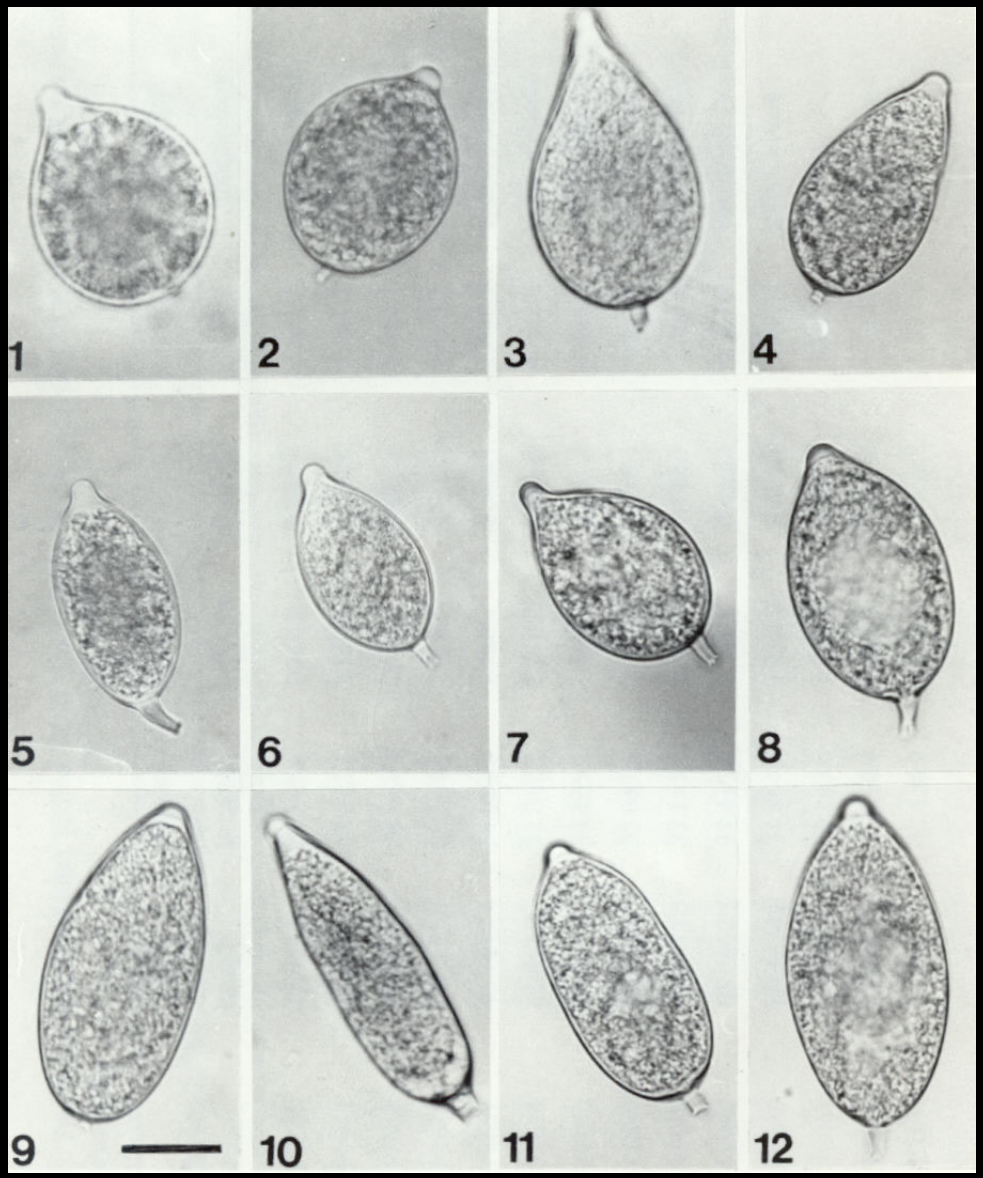
Amphigynous versus Paragynous Antheridium



Sporangial Morphology

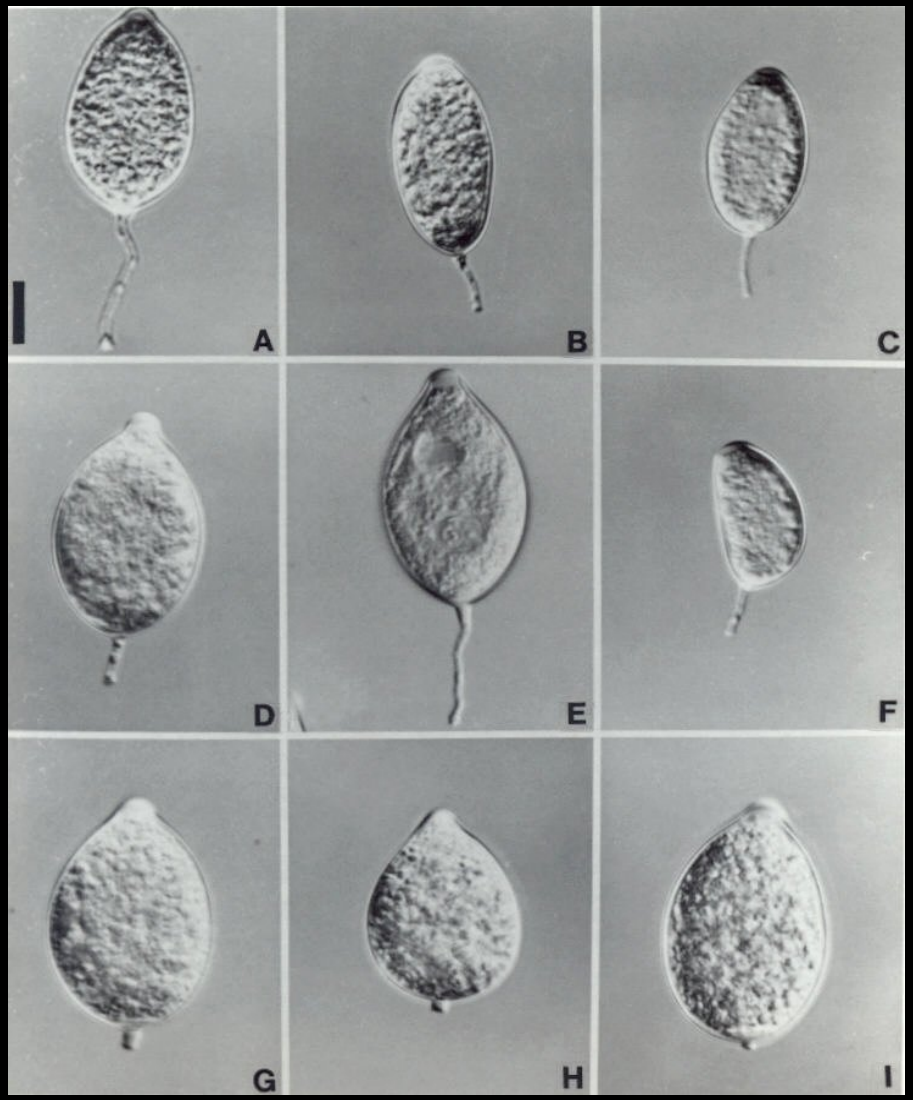
citrophthora

Sporangial Morphology

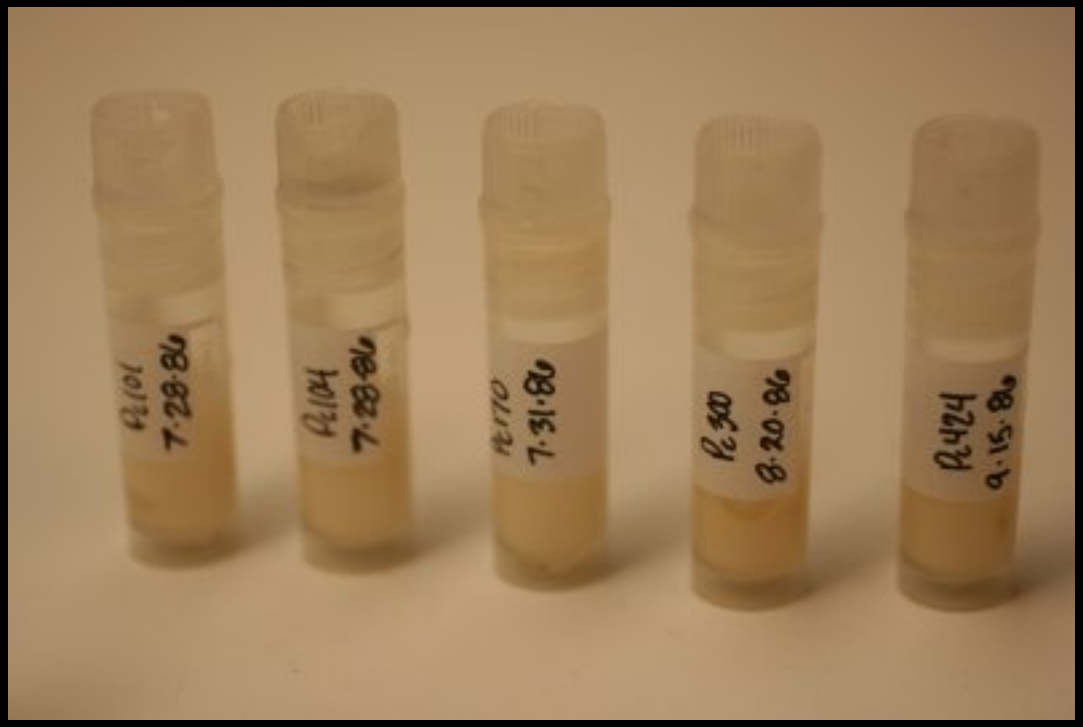


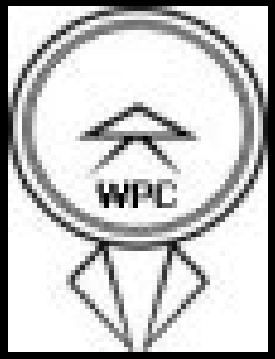
palmivora

Pedicel Length and Sporangial Dimensions



Storage 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

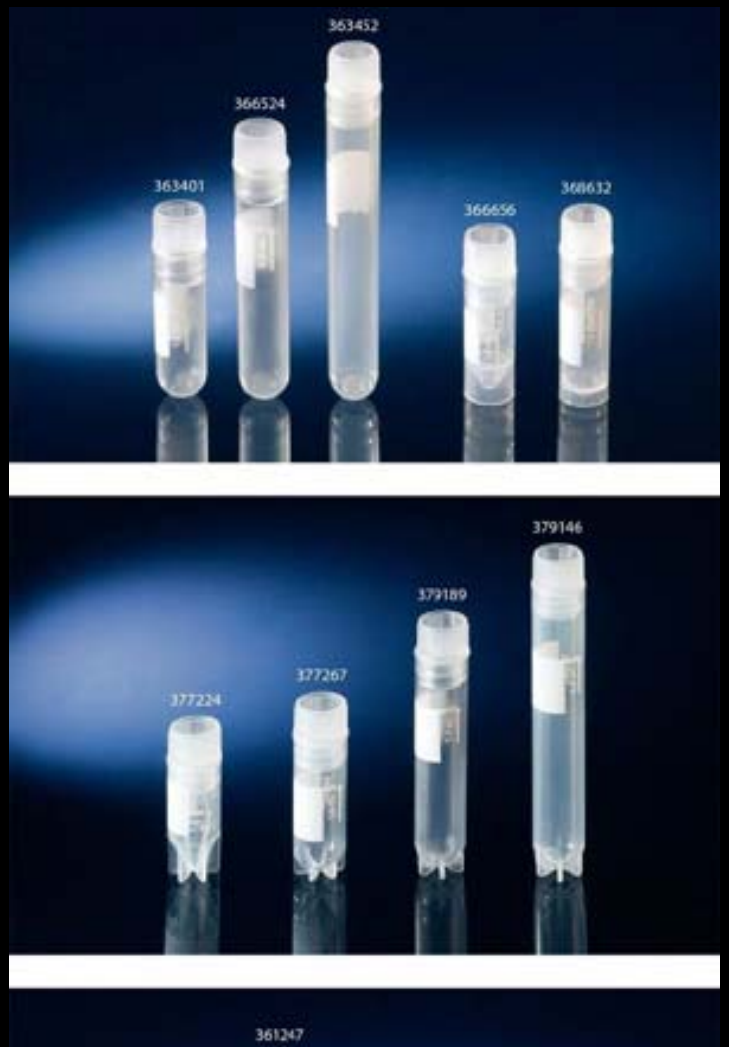
HAZARDS

Over-pressurization and explosion due to LN_2 vaporizing to nitrogen gas (700x expansion ratio) in unvented containers (e.g. cryovials) and equipment

Severe burns caused by exposure to extremely cold temperatures (-196C)

Asphyxiation due to displacement of oxygen in the air by the nitrogen gas in confined work areas

Hazards


363452
366524
363401
366656
368632
379146
379189
377267
377224
361247


DANGER
!!! EXTREME COLD !!!
SKIN BURN HAZARD
USE PROTECTIVE GEAR

THE FREEZING PROCESS

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

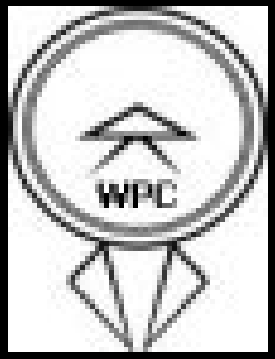
Taylor Wharton 8K

Liquid or Vapor-phase

MVE
HE series
-150C

Vapor phase

THE DNA BANK

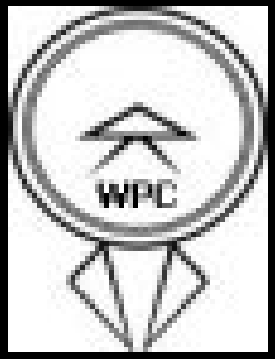


Phytophthora DNA Bank

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



Phytophthora DNA Bank

Low TE Buffer

10 mM Tris-Cl, pH 7.5
0.1 mM EDTA

Made from 1 M stock of Tris-HCl (pH 7.5) and
50 mM stock of EDTA (pH 8.0)

Current Activities

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

Molecular Phylogeny

Diagnostics Research

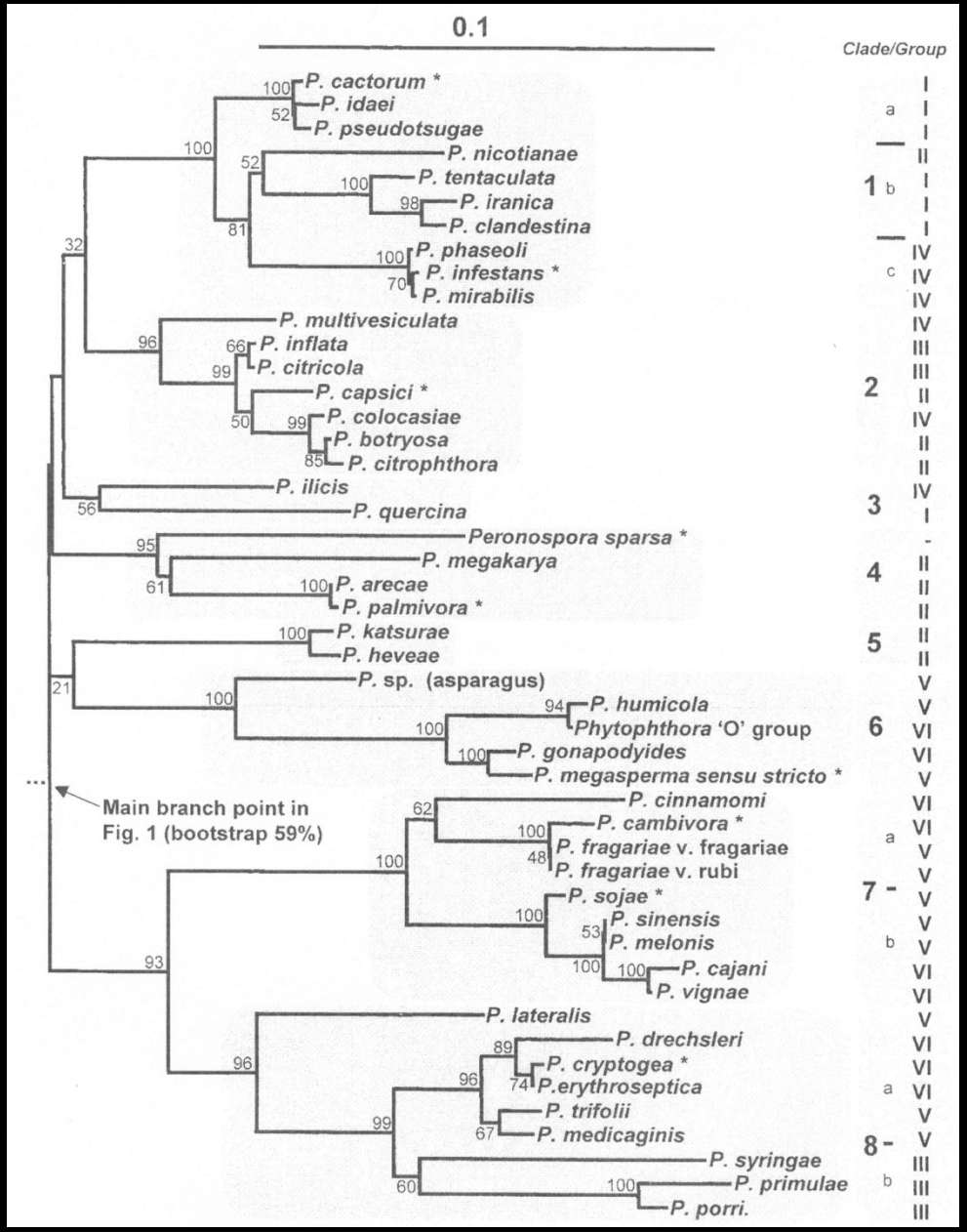
Workshops

MOLECULAR PHYLOGENY AND DIAGNOSICS

Evolutionary Relationships among *Phytophthora* species

ITS1 + 5.8S + ITS2
(889 bp, 48.5% constant)

- 10 major clades with variable support
- No resolution of backbone



from Cooke *et al.* (2000) *FG&B* 30:17

A genus-wide phylogeny for *Phytophthora* utilizing whole genome sequence data

J. E. Blair (1), M.D. Coffey (2), D. M. Geiser (1), S. Kang (1)

(1) Penn State University; (2) University of California, Riverside

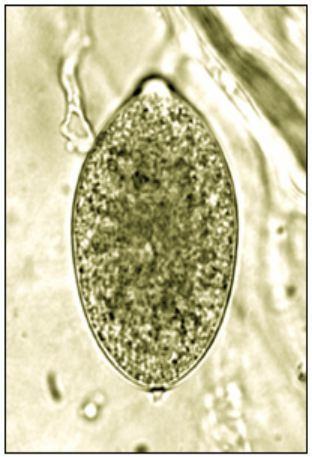
Genome Resources…

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 ramorum

Phytophthora sojae

Phytophthora infestans

Phytophthora capsici

Other Oomycetes (ESTs):

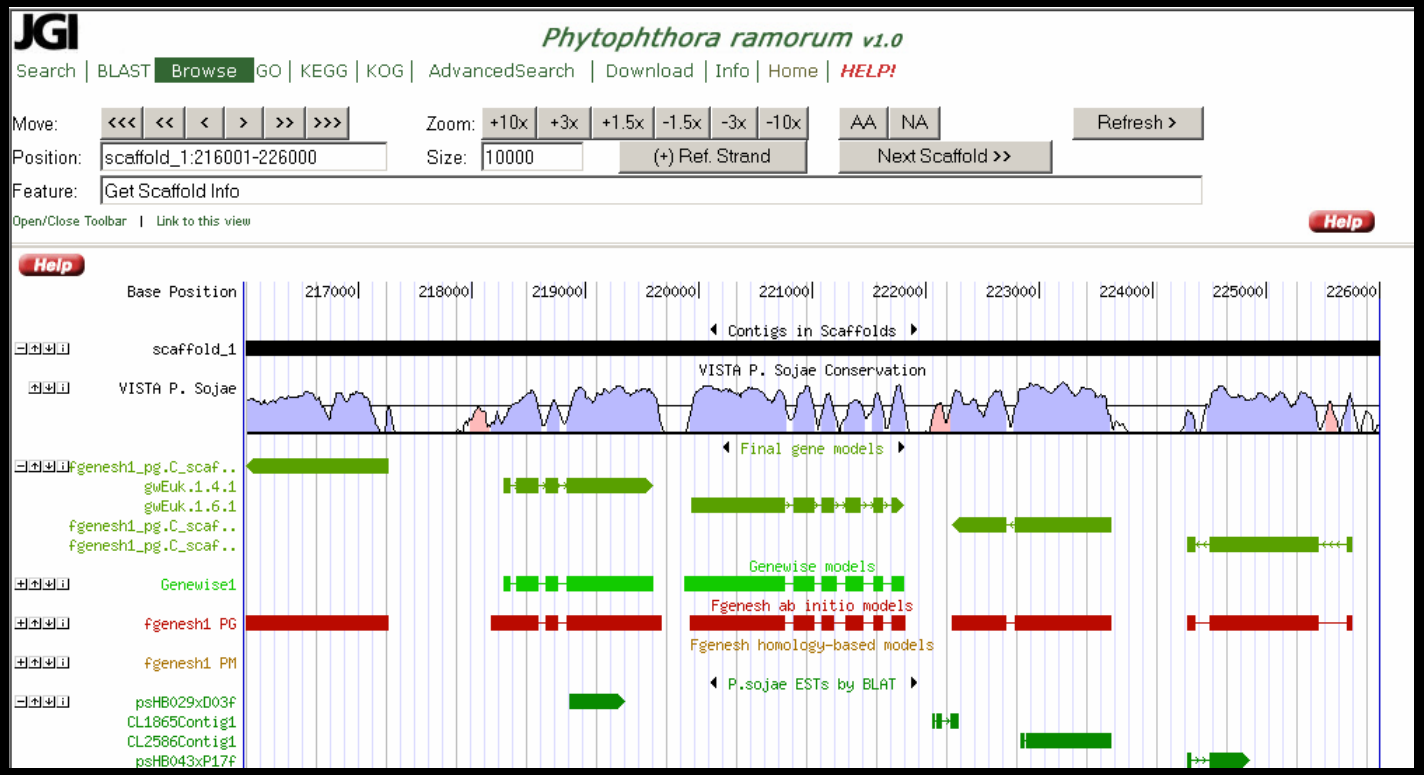
Saprolegnia parasitica
Plasmopara halstedii
Aphanomyces cochlioides
Hyaloperonospora parasitica

Source: Jaime Blair

Evolutionary Relationships Among *Phytophthora*

Source: Jaime Blair

Genome Resources…



Utilize Complete Genomes to Design Markers for Genus-wide Phylogeny

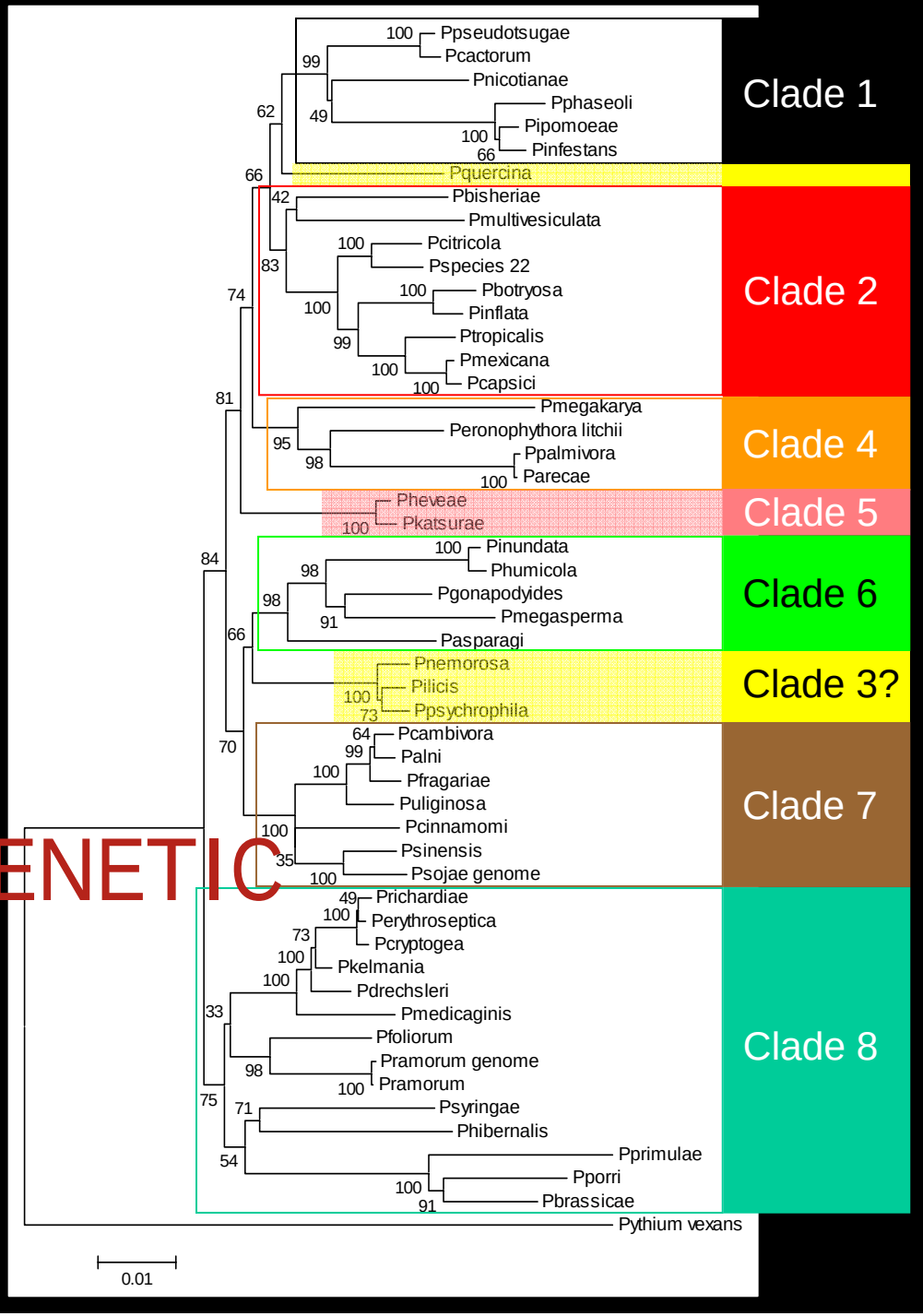
~85 + species, 200+ isolates mainly at the WPC at UC Riverside

Evolutionary Relationships of *Phytophthora*

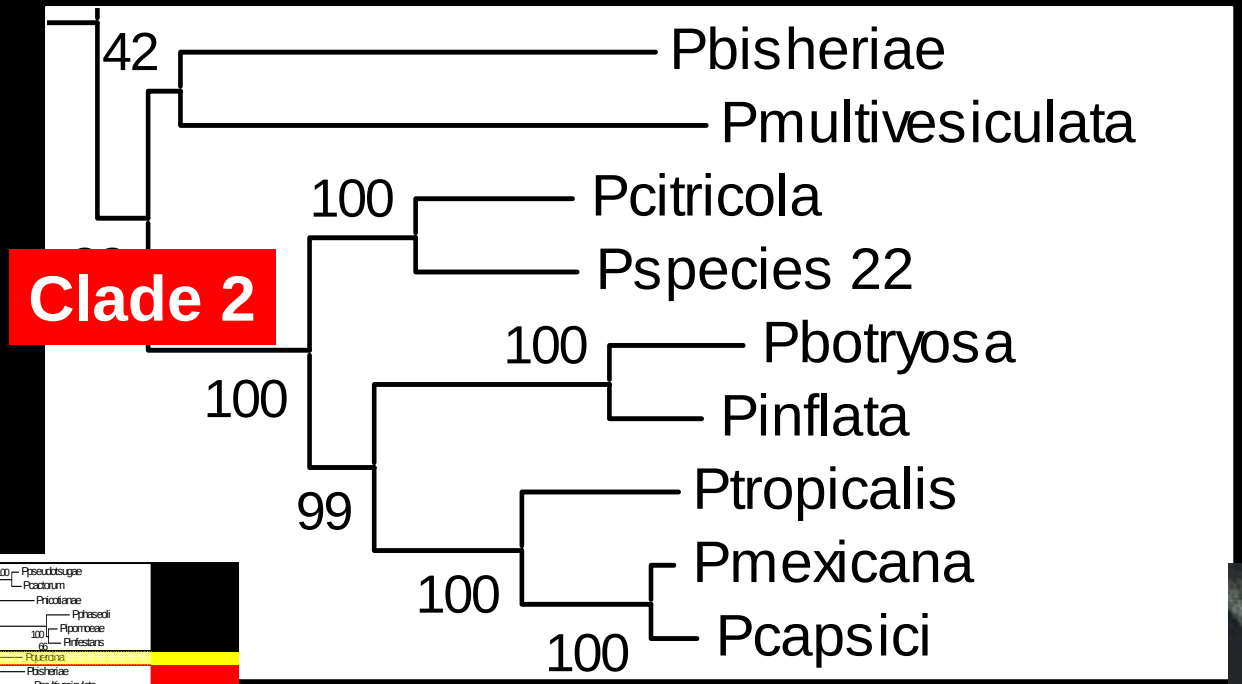
Six Protein-coding Loci:

60S Ribosomal Protein L10
Beta Tubulin
28S Ribosomal (LSU)
Enolase
Heat Shock Protein 90
Elongation Factor 1 alpha

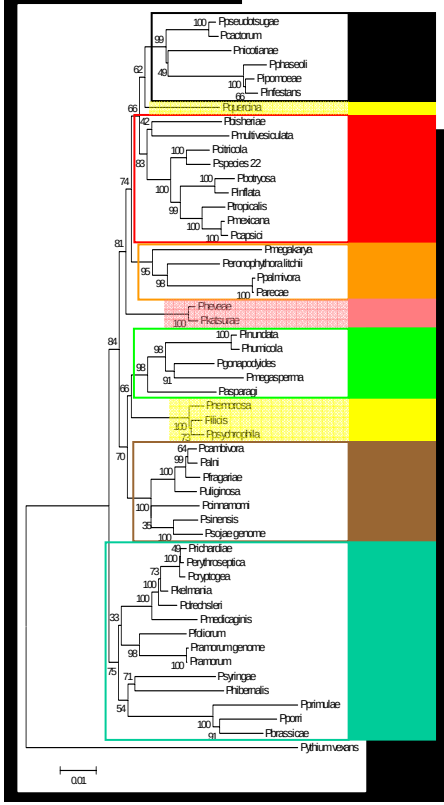
MULTIPHYLOGENETIC APPROACH



Source:
Jaime E Blair
PSU



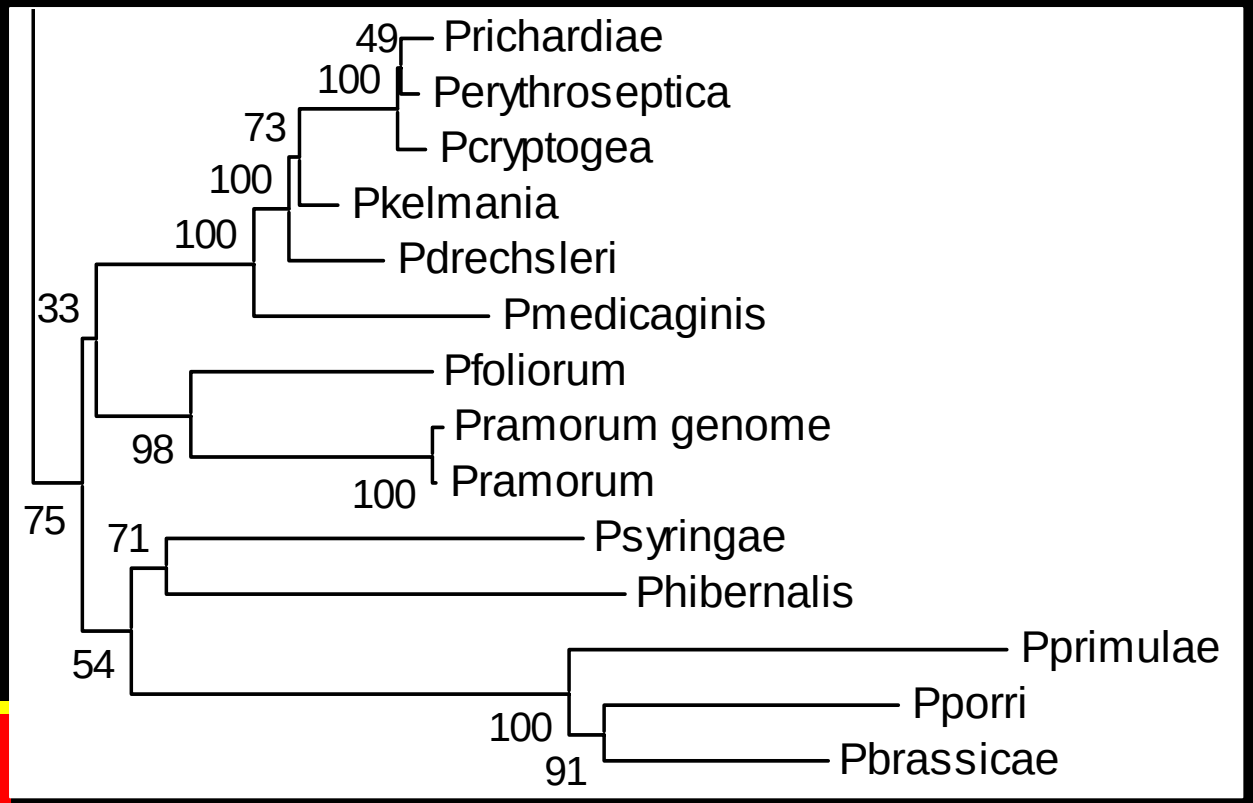
Others: *P. citrophthora*
P. colocasiae
P. meadii

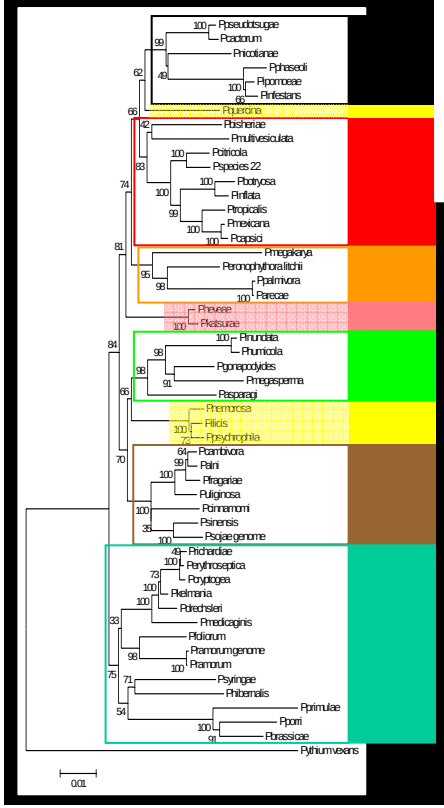


Source:
Jaime E Blair
PSU

http://www.apsnet.org/education/IllustratedGlossary/PhotosE-H/exudate.htm

Clade 8





Source:
Jaime E Blair
PSU

WORKSHOPS

Phytophthora and *Pythium* Workshop at NCSU 2006

Thank You!

- Postdoctoral scholars: Masoomeh Peiman,
  Tatiana Roubtsova, Alexei Kravtsov
- Lab Assistants: Avneet Brar, Iona Cunningham, Sandra Verdin
- Undergraduate researchers: Julie Huss, Thomas Vu,
  Bharat Sunkavally, Shirley Tu, Erik Haw, Elaine Xu
  Justin Chao, Shinly Du, Daniel Guindi, Carrie Tran,
  Peggy Ju, Giselle Vu, Linda Vu, Kacey Cao, Serena Chai,
  Eric Chan, Tien Dinh, Jorge Farias, Eric Garcia,
  Sabrina Garrovillas, Zahra Mousavi Jasemi, Victor Kieu,
  Lisa Lam, Mark Luu, Octaviano Moro, Charmi Patel,
  Joshua Smith, Virginia Tran, Jacqueline Villanueva,
  Vera Wong, Candace Woo
- Visiting scientists: Byung-Sup Kim, Grazyna Szkuta,
  Laura Gaggero, Pedro Martin, Ehab Sarhan

Collaborations!

- Penn State University: Seogchan Kang, Bongsoo Park, Sook-Young Park, Michele Mansfield, David Geiser
- Amherst College: Jaime E. Blair
- USDA ARS Salinas: Frank Martin
- USDA-APHIS-PPQ-PHP-PSPI-MDL: Gloria Abad
- USDA-APHIS- CPHST: Phil Berger, Laurene Levy
- USDA ARS Corvallis: Niklaus Grunwald
- Biodiversity Canada Agriculture: André Levesque, Gregg Robideau
- CBS Utrecht, The Netherlands: Arthur de Cock
- CSL, York, UK: Kelvin Hughes
- Ohio State University– Carla Garzon and Sophien Kamoun (Sainsbury Lab, UK)
- IFAPA Spain: Rosa M. Pérez Jiménez, Pedro Martin
- UC Riverside: Hailing Jin, Greg Douhan
- CIAT Colombia: Elizabeth Alvarez
- Universidad de los Andes Colombia: Silvia Restrepo
- CIP Peru and Ecuador: Greg Forbes
- MSU and VNIIF: Russia: Sergey Elansky, Alexei Filippov
- India NRRI: Bindu Roy C.
- Vietnam and Australia: Doan Nhan Ai, David Guest, Andre Drenth

THE END