The state of *Phytophthora* diagnostics

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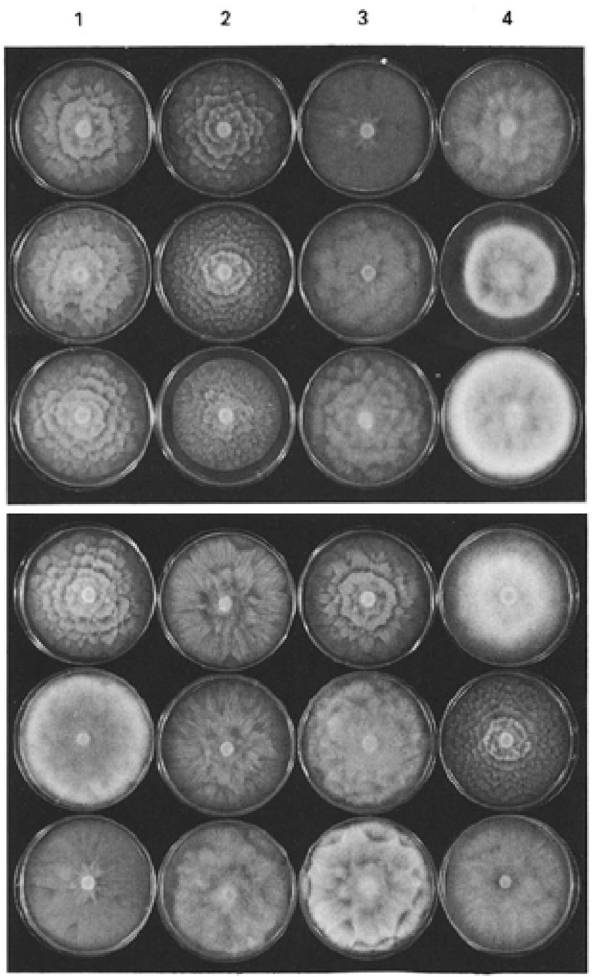
May 18, 2017



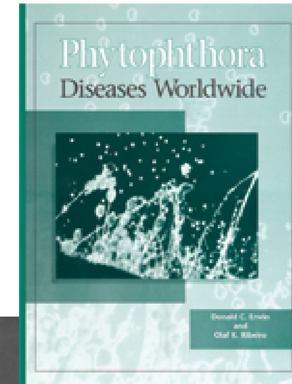
Outline

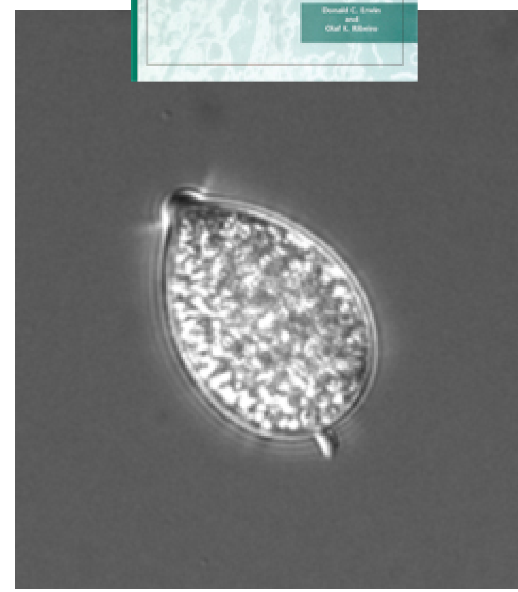
- *Phytophthora* diagnostics
  - The growing pains of moving to a DNA-based classification system
  - Defining *Phytophthora* species
- Case study: UC Davis campus oaks
- Case study: *Phytophthora drechsleri-cryptogea* species complex

Omnivorous *P. cactorum*

From **Erwin & Ribeiro (1996)**:

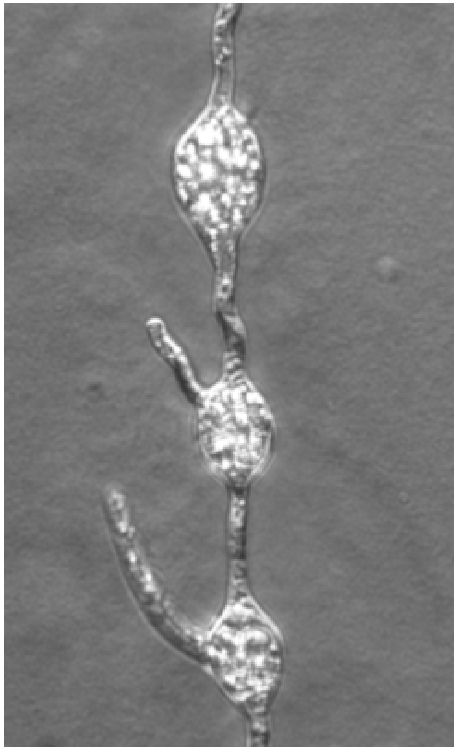
P. cactorum, first reported from rotting cacti in Czechoslovakia by Lebert & Cohn (1870) **occurs worldwide** but is most commonly found in temperate regions. It causes root and collar rots, fruit rots, cankers, leaf blights, wilts and seedling blights. **It parasitizes more than 200 plant species** in 150 genera representing 60 plant families.





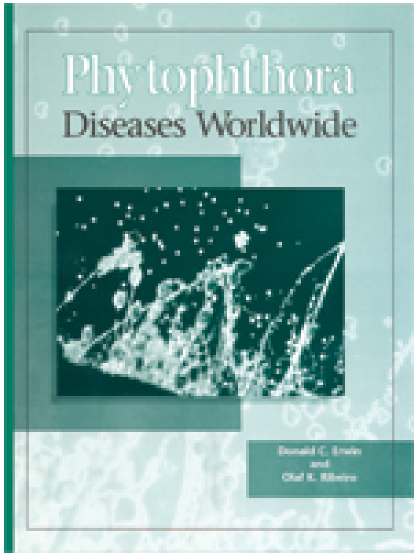
P. cactorum has greatly varying interactions with different plants

Isolates of *P. cactorum* **are generally not host specific**; for instance, an isolate of *P. cactorum* from apple infected a large number of weed species, and isolates from 23 host genera were pathogenic to pear trees. **Marked differences occur in degree of pathogenicity to different hosts**, however. Although specialization to a single host is rare, forest soil isolates of *P. cactorum* did not infect ginseng.



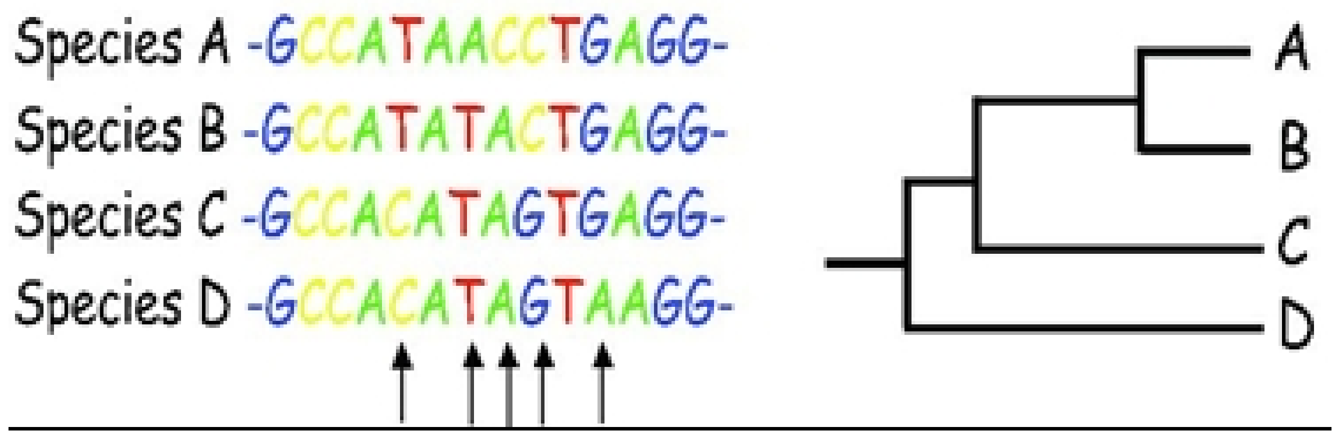
DNA sequences have revolutionized the study of *Phytophthora*

- Yet because we **know so much more**, we **understand so much less** about *Phytophthora* species now than we did in 1996
- Why?

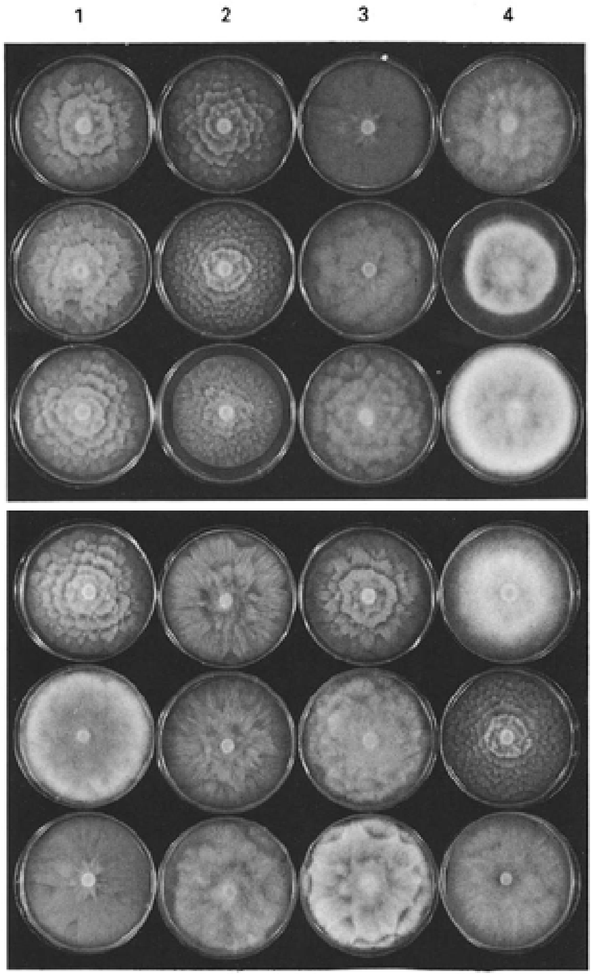


Growing pains

- We are in the middle of moving from a morphology-based system to a DNA-based system for classification and identification



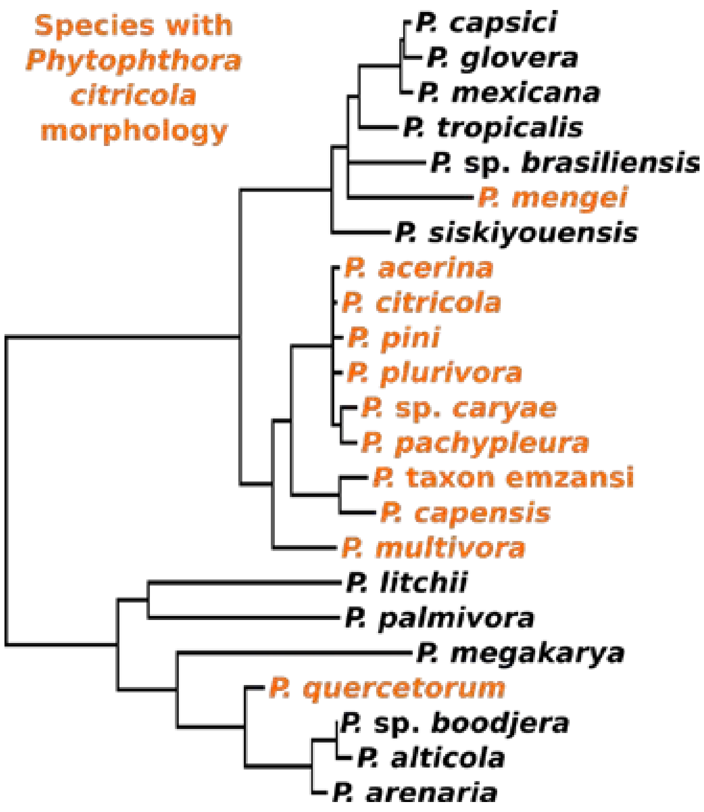
We know a lot more about what we don't know



- The number of *Phytophthora* species that have been described since 2000 surprised almost everyone
  - Now, we think we've only discovered 1/3 of the species that exist, at most

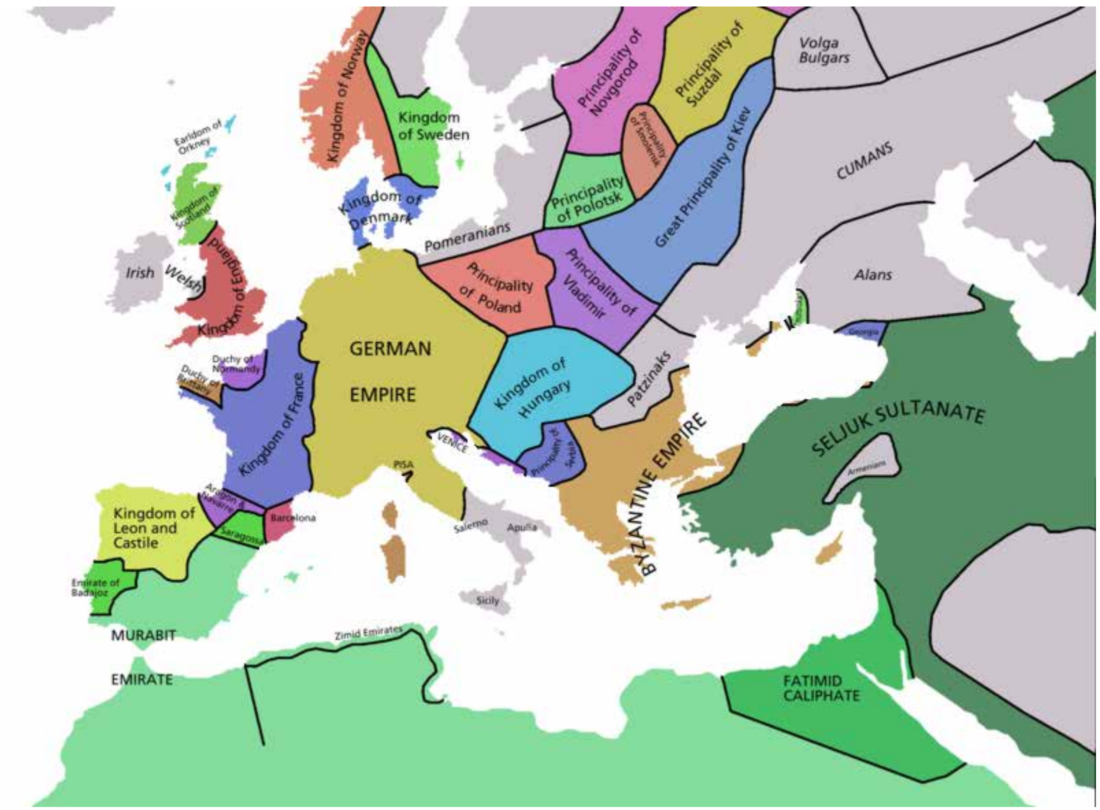
Cryptic species

- Many of the recent species were “cryptic species”
  - They morphologically resemble other species but can now be distinguished using DNA
  - In the past they all would have been identified as a single species



Taxonomic changes render old knowledge inapplicable

- Much of what we know about the old species is now very difficult to interpret



So, why switch at all to sequence-based identification at all? (Cons)

- Breaks compatibility with old system, creates ambiguity
- Generating sequences requires additional laboratory resources and training not available to all researchers
  - As of 2017, more expensive per strain than morphology-based identification methods

Benefits of switching to sequence-based identification? (Pros)

- Precision
  - Already greater than morphology and increasing
  - Allows us to see many cryptic species for the first time
  - New tools and databases to discern if a species is native or exotic
- Easier to characterize hybrids
- Consistency
  - Eventually…

Biggest (future) benefit of switching

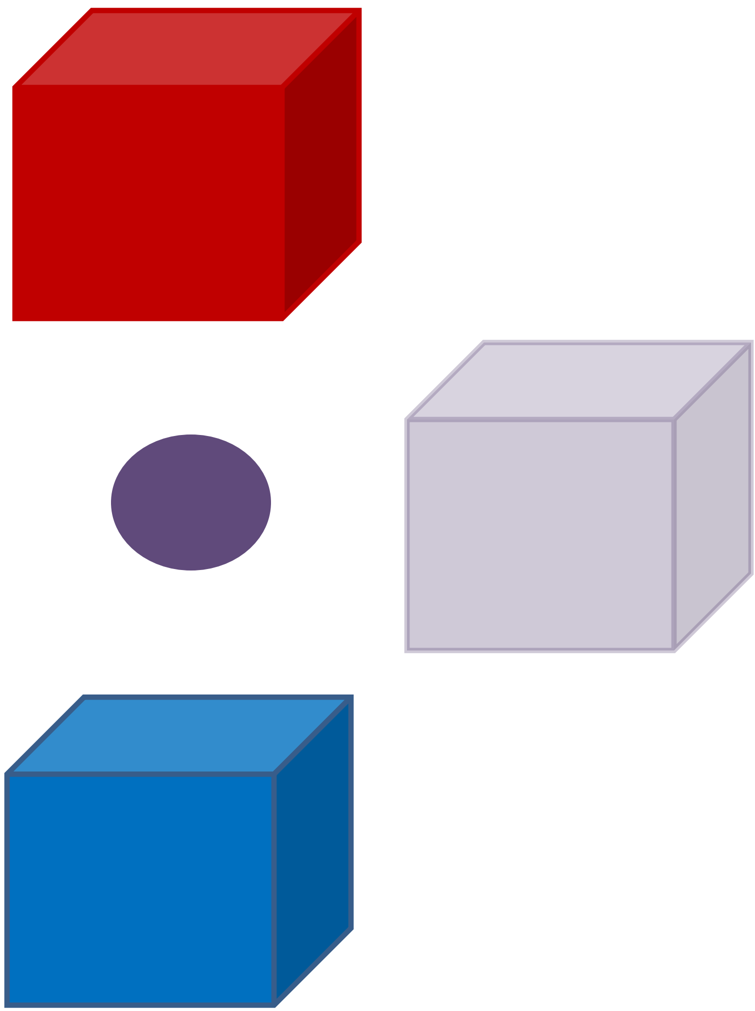
- Environmental PCR (metabarcoding)
  - DNA is extracted directly from substrate (soil, filtered water) and then massively sequenced, so that we can infer what was there
  - Cheap (given lab resources exist) and straightforward; no need to bait, no need to culture and incredibly sensitive
  - But, requires sophisticated understanding and automation of how sequences relate to species

Phytophthora quercina and the need for metabarcoding

- *P. quercina*, a species of great regulatory importance, specializes on oak roots and can only be reliably baited with *Quercus* leaves
  - However, baiting projects typically use *Rhododendron* leaves and/or pear fruit
  - *P. quercina* found on four outplantings of *Q. lobata* in CA
- How many other species are hiding in plain sight, simply because we're not using the right bait?

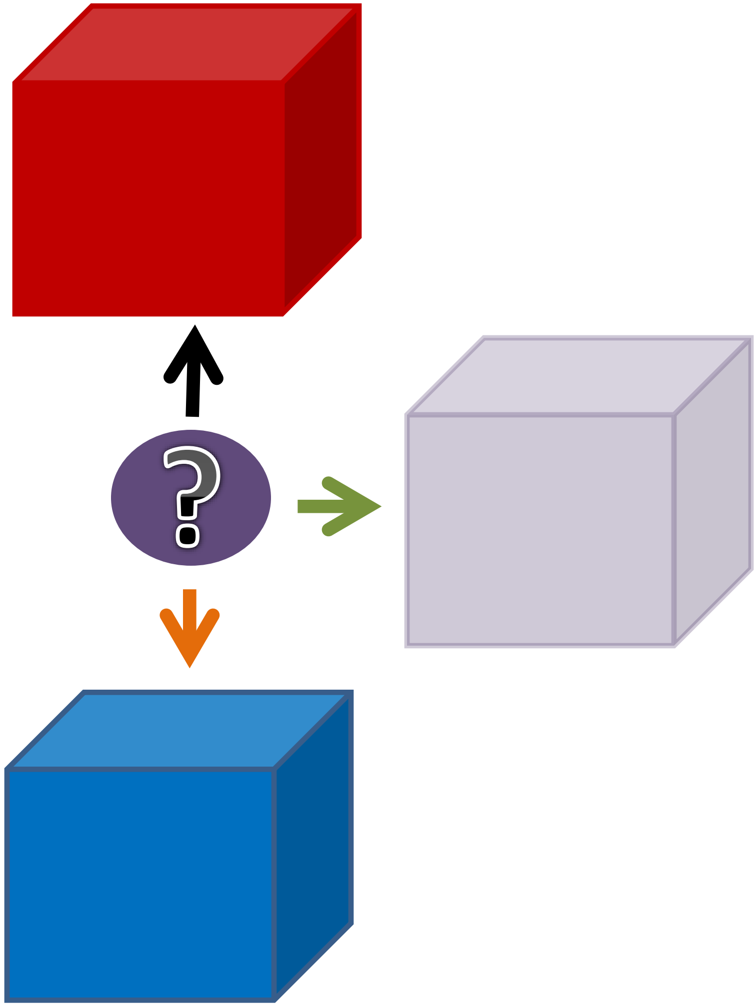
Reconciling DNA & species names: It's difficult to establish boundaries

- A strain was isolated and produced a sequence that appears ambiguous
  - DNA sequence is slightly different from all other known sequences
- What should it be called?
- How different does it have to be from known species before it's considered a new species?



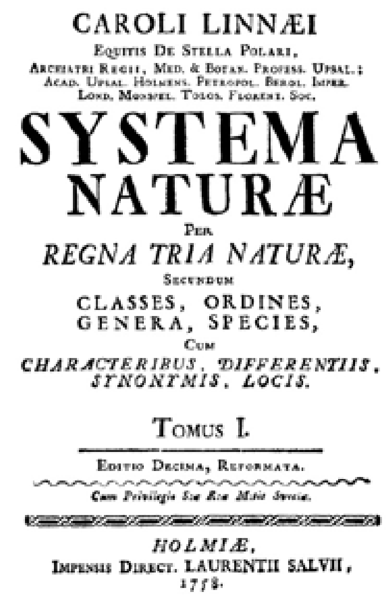
Ambiguous species boundaries

- This is a major reason that given the **same set of *Phytophthora* strains**, different Phytophthorologists will come up with slightly (or significantly) **different lists of species**
- We haven't yet developed a set of rules about how to do this
  - Phytophthora-ID.org & PhytophthoraDB.org



What are species, really?

- When Linnaeus developed his system of classification (1735), species were not “real”
  - Darwinian evolution hadn’t been discovered yet
  - Species were just the smallest boxes
- Even after Darwin (1859), it took nearly a hundred years to reconcile species with ecology and evolution
  - Part of biology’s “modern synthesis”



CAROLI LINNÆI
EQUITIS DE STELLA POLARI,
ARCHIATRI REGII, MED. & BOTAN. PROFESS. UPSAL.;
ACAD. UPSAL. HOLMENS. PETROPOL. BEROL. IMPER.
LOND. MONSPEL. TOLOS. FLORENT. SOC.

SYSTEMA
NATURÆ
PER
REGNA TRIA NATURÆ,
SECUNDUM
CLASSES, ORDINES,
GENERA, SPECIES,
CUM
CHARACTERIBUS, DIFFERENTIIS,
SYNONYMIS, LOCIS.

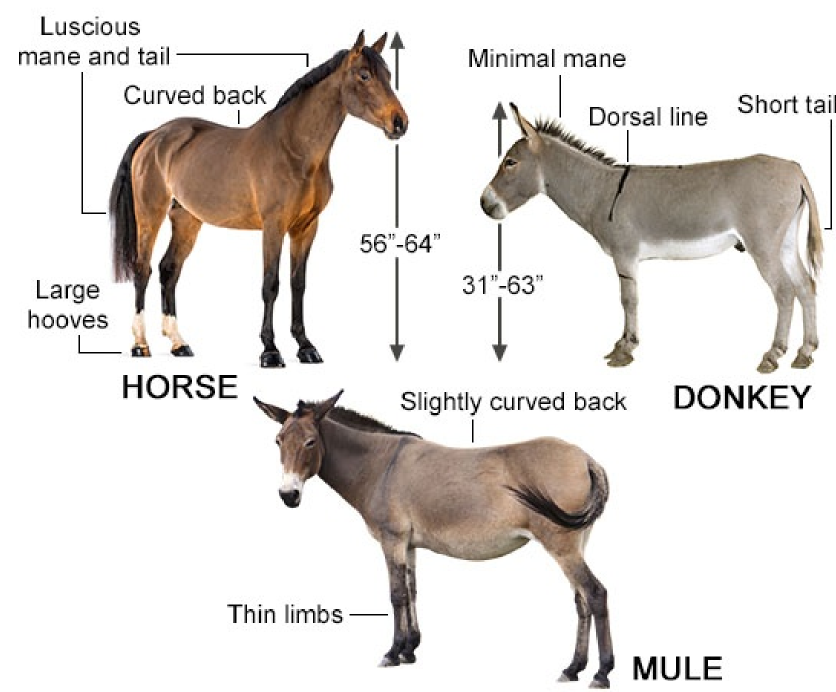
TOMUS I.

EDITIO DECIMA, REFORMATA.

HOLMIÆ,
IMPENSIS DIRECT. LAURENTII SALVII,
1758.

Species are real and can be measured

- "Biological species concept" proposed by Mayr (1942) provides a straightforward criterion for delimiting species
  - Sterility of mules proves that horses and donkeys are distinct species (reproductively isolated)

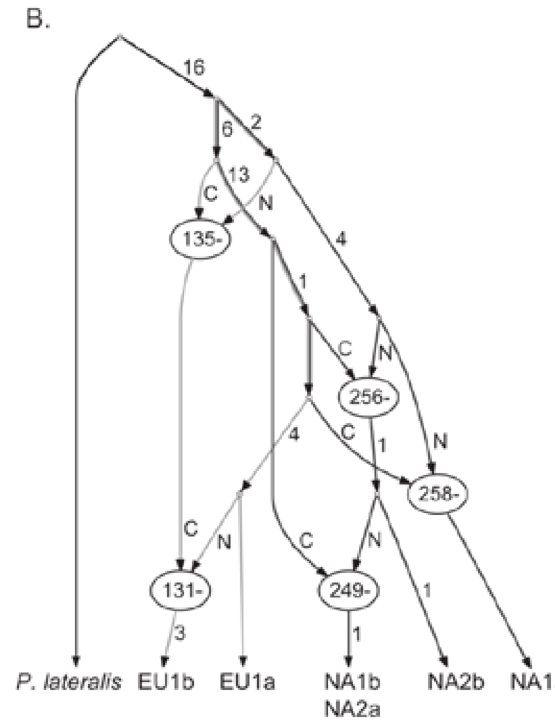


Many plants (and microbes) have more complicated species

- Stebbins (1950) and other botanists proposed additional species concepts to accommodate more complex species that may produce fertile hybrids or propagate clonally

Phytophthora species are real

- Like most modern botanists and zoologists, most Phytophthorologists now believe that species exist as "real," measurable evolutionary entities
  - But we are still learning how to measure them



P. ramorum

Species concepts in *Phytophthora*

- 1870s-1930s: Species concepts not yet well developed for many organisms
- *Phytophthora* was presumed to be host-specific by many researchers
  - Every time *Phytophthora* was found on a new host, it was described as a new species
- Unfortunately, some species have extremely wide host ranges

Anton de Bary

Species concepts in *Phytophthora*

- 1930s-1990s: The old system was gradually replaced by a morphological classification: *Phytophthora* species each possessed a unique combination of sexual and asexual spores
  - Worldwide collections of living strains established to be directly compared

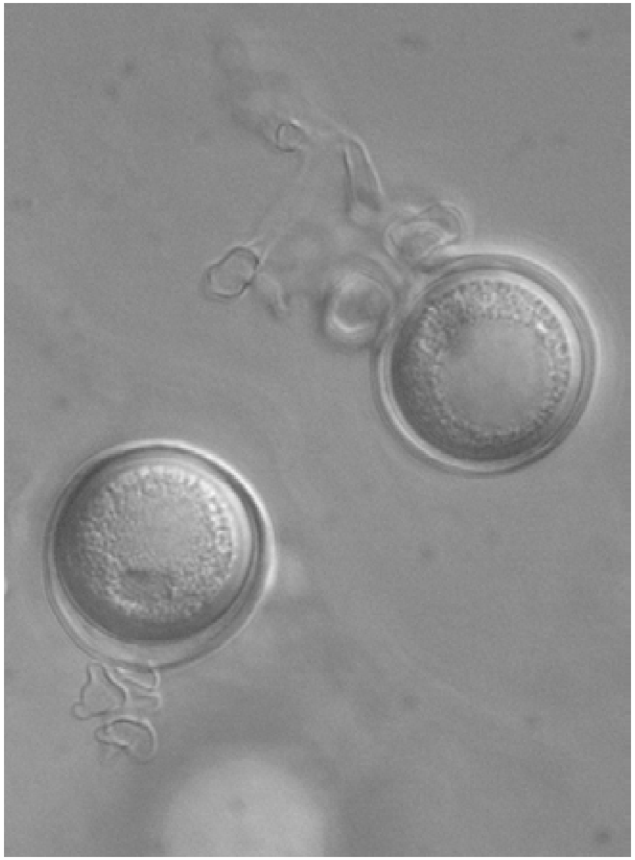
Grace Waterhouse

Species concepts in *Phytophthora*

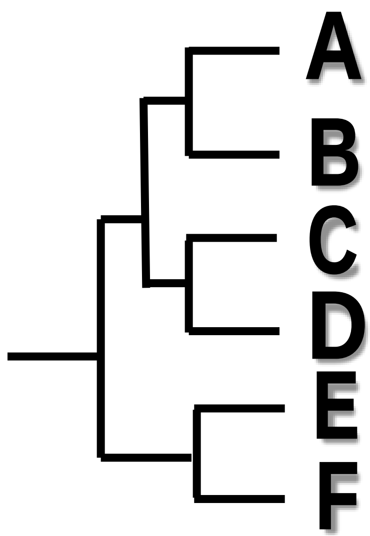
- 2000s-present: DNA provides new tools, but it still isn't clear how to reconcile sequence-based results with species names
  - We don't have a perfect or reproducible DNA:species translator
  - Phytophthora-ID.org & PhytophthoraDB.org

The biological species concept can't be applied to most *Phytophthora* species

- Sexual proclivities of most *Phytophthora* species make mating tests impractical or meaningless
- Discerning each closely related pair or group of species (species complex) then becomes a complicated population genetics study
  - Difficult to establish species boundaries

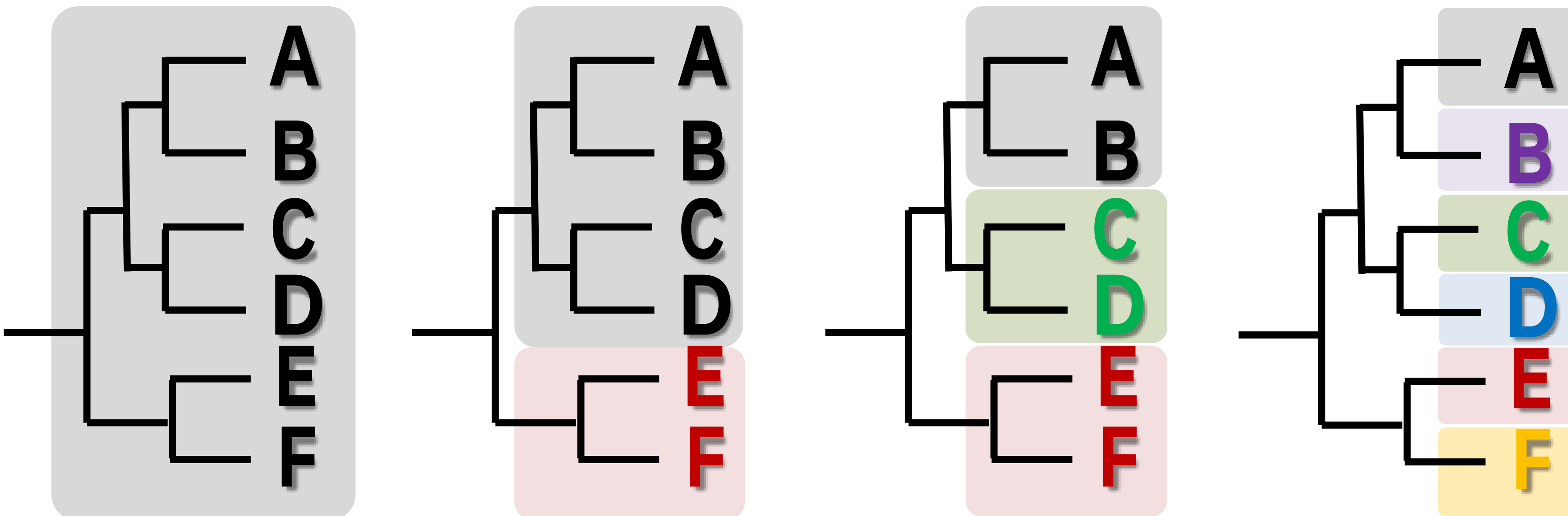


A practical species concept



- For the time being, a *de facto* “phylogenetic species concept” is in place
  - requires novel *Phytophthora* species to have **ITS and COX1 DNA sequences that are both unique**
- This provides something to go on, but it is artificial

This phylogenetic species concept still doesn't tell us where to draw species boundaries, and tends to favor "splitting"



1 species? 2 species? 3 species? 6 species

Provisional taxa

- This uncertainty has led many Phytophthorologists to describe seemingly novel species they encounter as “provisional” or “placeholders” until consensus is reached about how *Phytophthora* species work, and how best to study them
  - *Phytophthora* taxon oaksoil
  - *Phytophthora* sp. *kelmania*

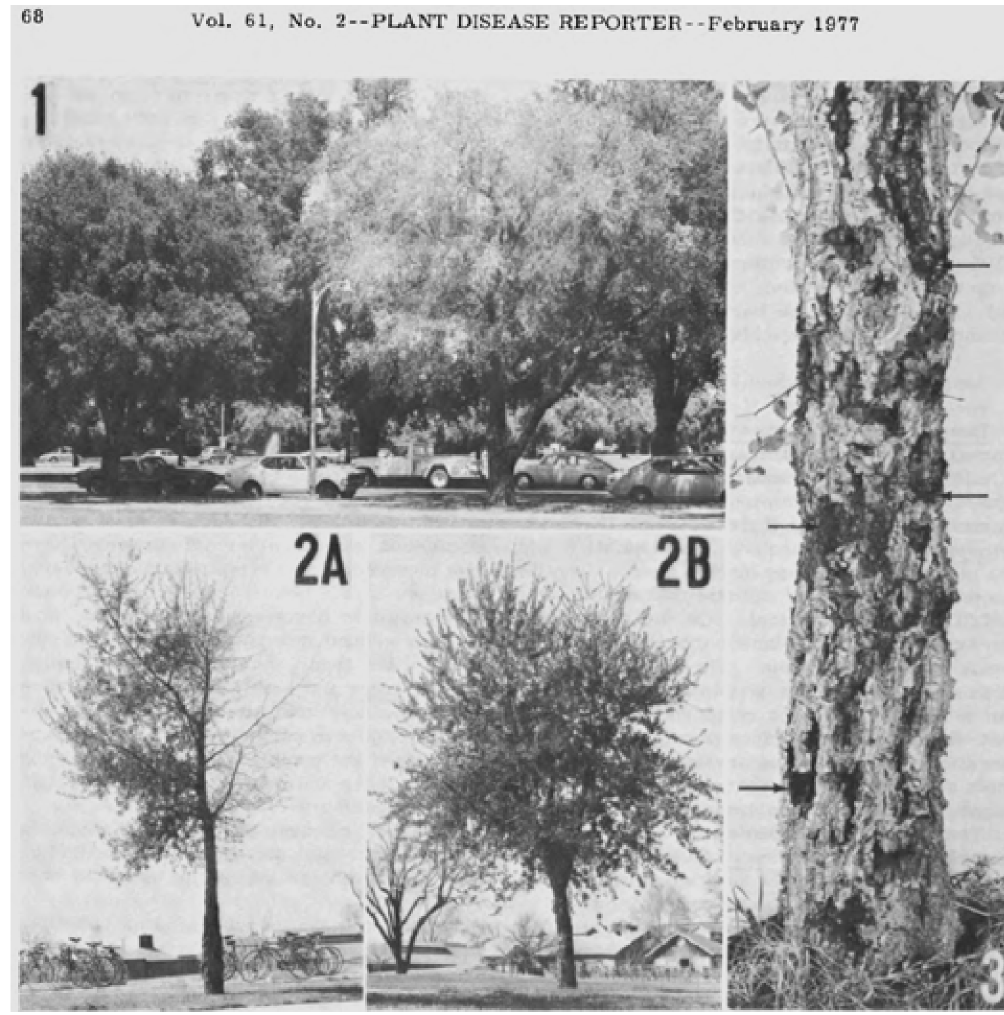
Case study
Phytophthora on UC Davis campus oaks

- In the 1970s many oaks around the UC Davis campus were in decline, sometimes with bleeding cankers
- *Phytophthora* incidence was studied on coast live oak (*Q. agrifolia,* QUAG) and cork oak (*Q. suber*)

First study of *Phytophthora* on oaks

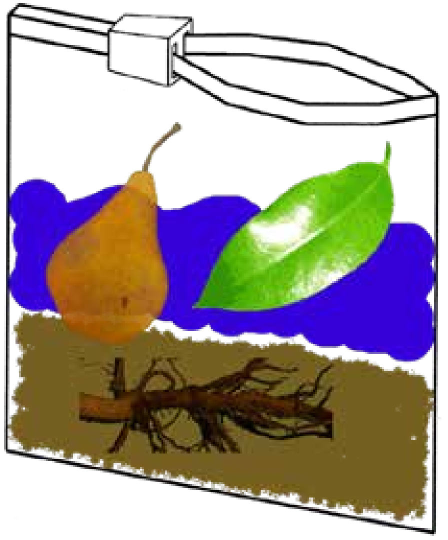
- *P. cinnamomi* was isolated from the cork oaks, while ***P. cactorum* and *P. citricola* were isolated from the QUAGs**
- Most of the oaks recovered after irrigation systems were changed

Mircetich, Campbell & Matheron (1977)



Rizzo lab campus oak baiting

- Since 2012, Rizzo lab has periodically baited soil from beneath a QUAG in front of Haring Hall, keeping a list of species:
  - *P. cactorum*
  - *P. acerina*
  - *P. multivora*
  - *P. quercetorum*



Dead trees

During the summer of 2016, another campus QUAG (in front of **Storer Hall**) rapidly declined with some bleeding lesions, and was removed.

- *P. acerina* was baited from the soil of the dead tree

Dead trees

The Haring Hall QUAG blew over during a windstorm Jan 2017, the victim of butt rot in addition to *Phytophthora* root rot

Can we reconcile our modern results with the study from the 1970s?

1970s QUAGs

(using morphology)

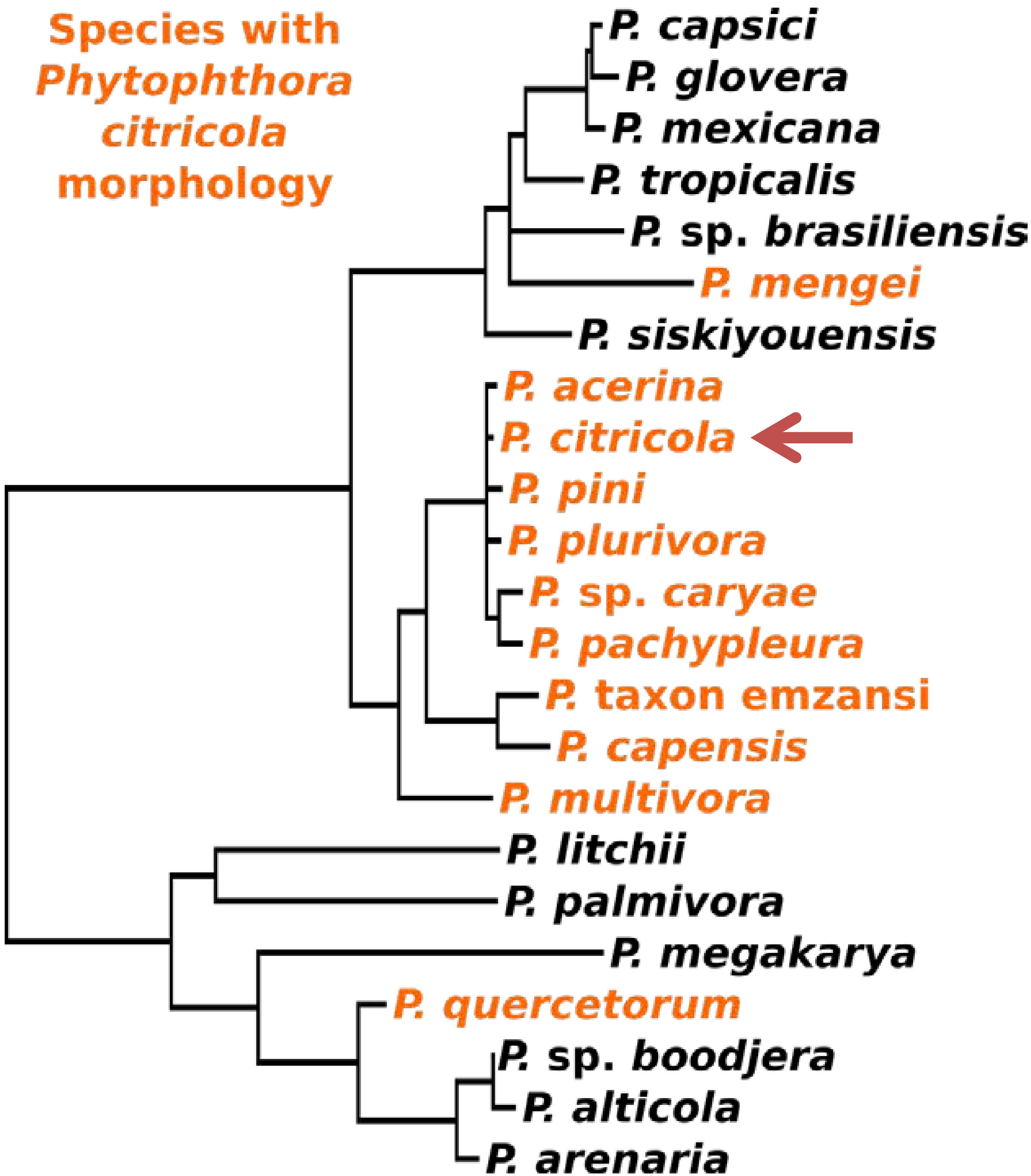
- *P. cactorum*
- *P. citricola*

2010s QUAGs

(using DNA sequences)

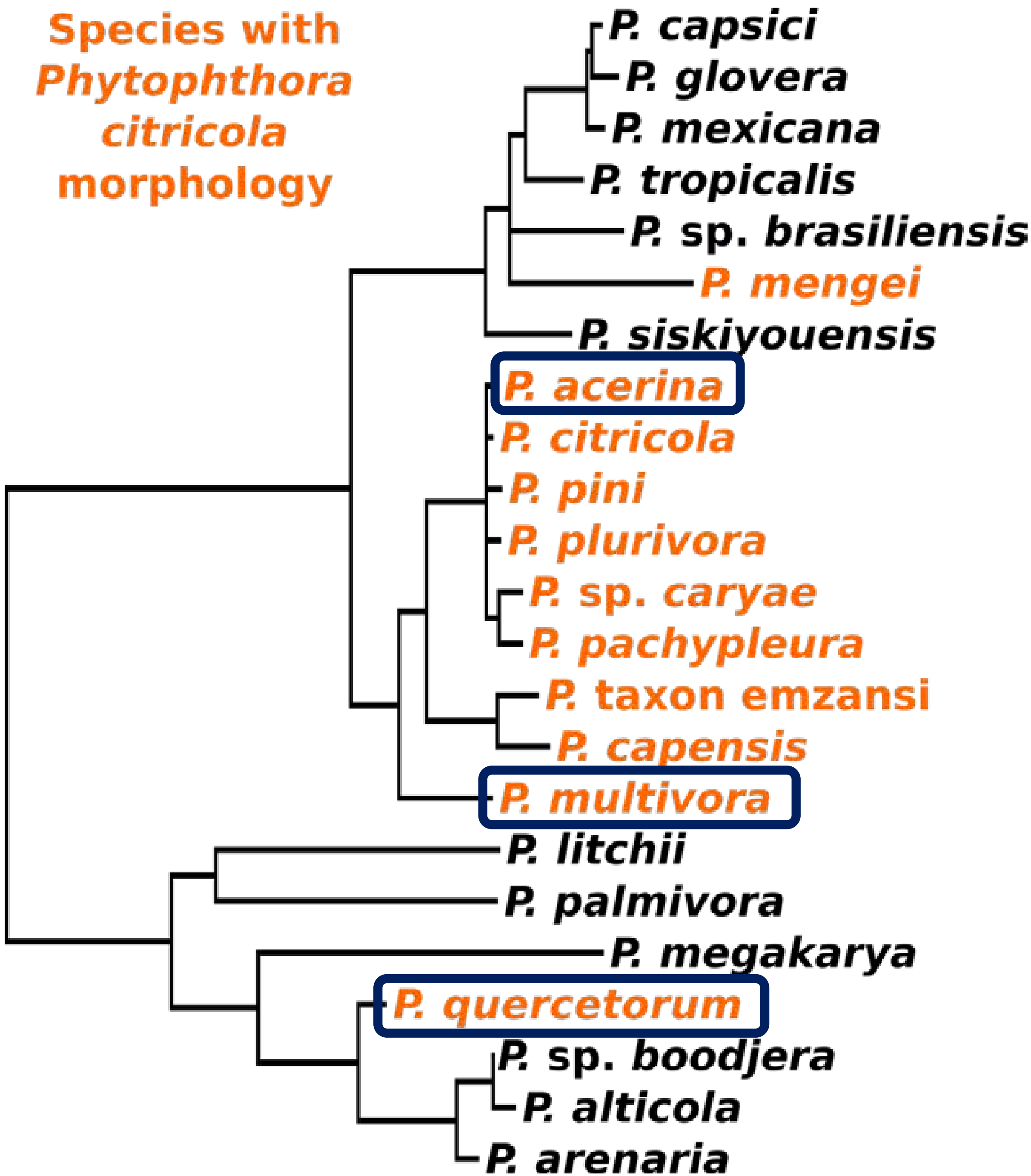
- *P. cactorum*
- ***P. acerina* (under both trees)**
- ***P. multivora* (all three years)**
- *P. quercetorum*

- What happened to *P. citricola*? Where did *P. acerina, P. multivora* and *P. quercetorum* come from?



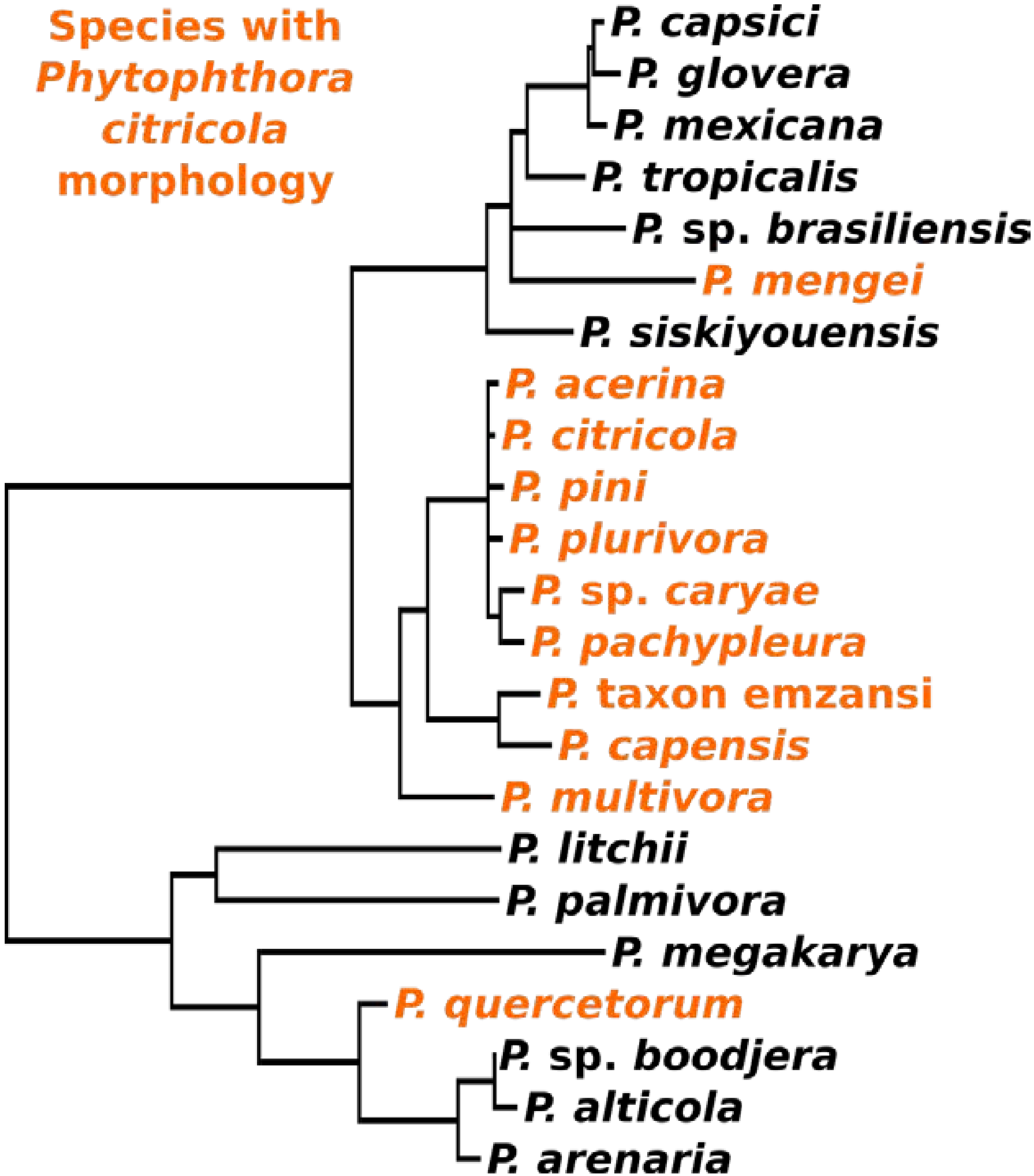
At least ten species are morphologically difficult or impossible to distinguish from *P. citricola*

Many are closely related to *P. citricola* but at least two are only distantly related



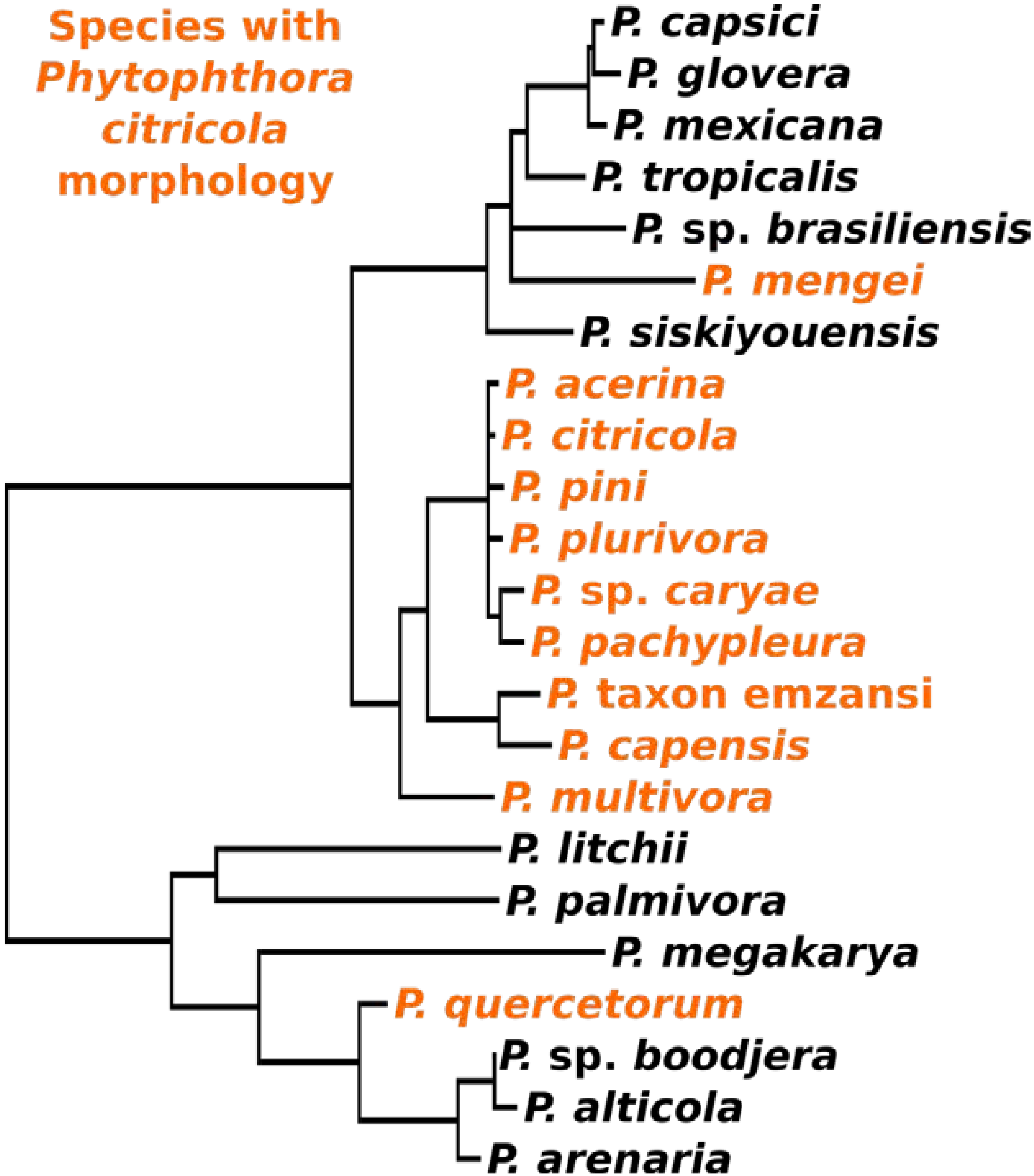
All of the campus species found in the 2010's besides *P. cactorum* resemble *P. citricola* (they are cryptic species)

The "*P. citricola*" strains found in the 1970's could have been any combination of the orange species



The subjects of nearly a century of research about *P. citricola* could have been any combination of these species!

We only know the true subjects of old studies if they were saved and subsequently sequenced (rare)



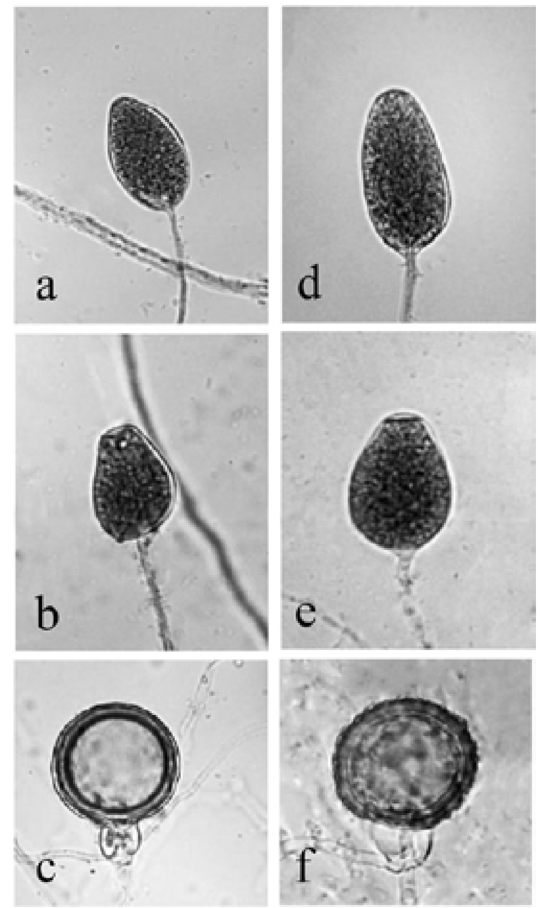
If an old study claims that *P. citricola* is resident somewhere, to which of these species are they referring?

If a shrub was determined to be *P. citricola*-resistant, is it resistant to all of these species?

Case Study

P. drechsleri-cryptogea species complex

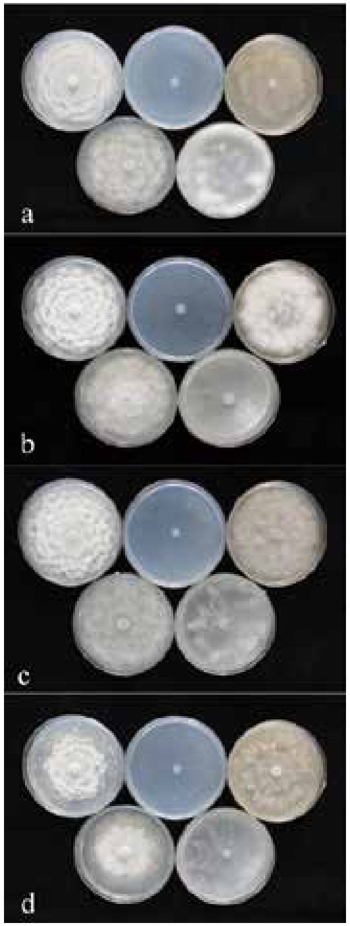
- Described in 1930 and 1919, *P. drechsleri* and *P. cryptogea* are omnivorous root-rotters
- The species are very similar, but were kept separate based on subtle differences



Case Study

P. drechsleri-cryptogea species complex

- For nearly a century, the two species have been near-impossible to reliably separate, confounding Phytophthorologists
  - Other species were also added to the complex
- Sequences did not initially solve the problem



In just one study, ambiguity is replaced with clarity

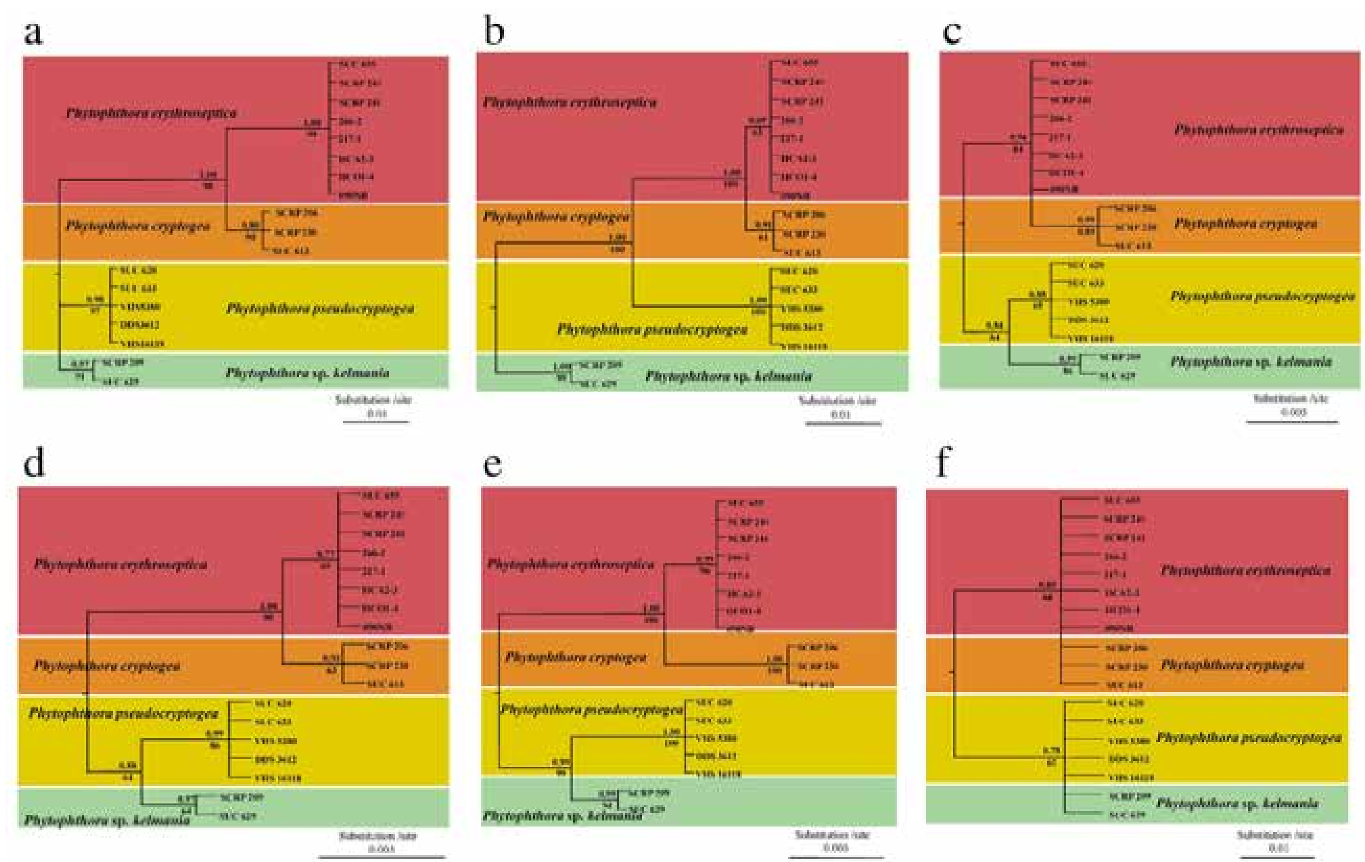
Mycol Progress (2015) 14: 108
DOI 10.1007/s11557-015-1129-9

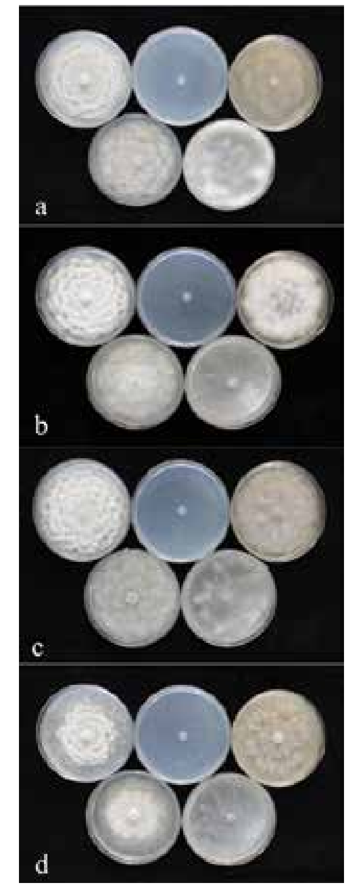
ORIGINAL ARTICLE

Re-evaluation of the *Phytophthora cryptogea* species complex and the description of a new species, *Phytophthora pseudocryptogea* sp. nov

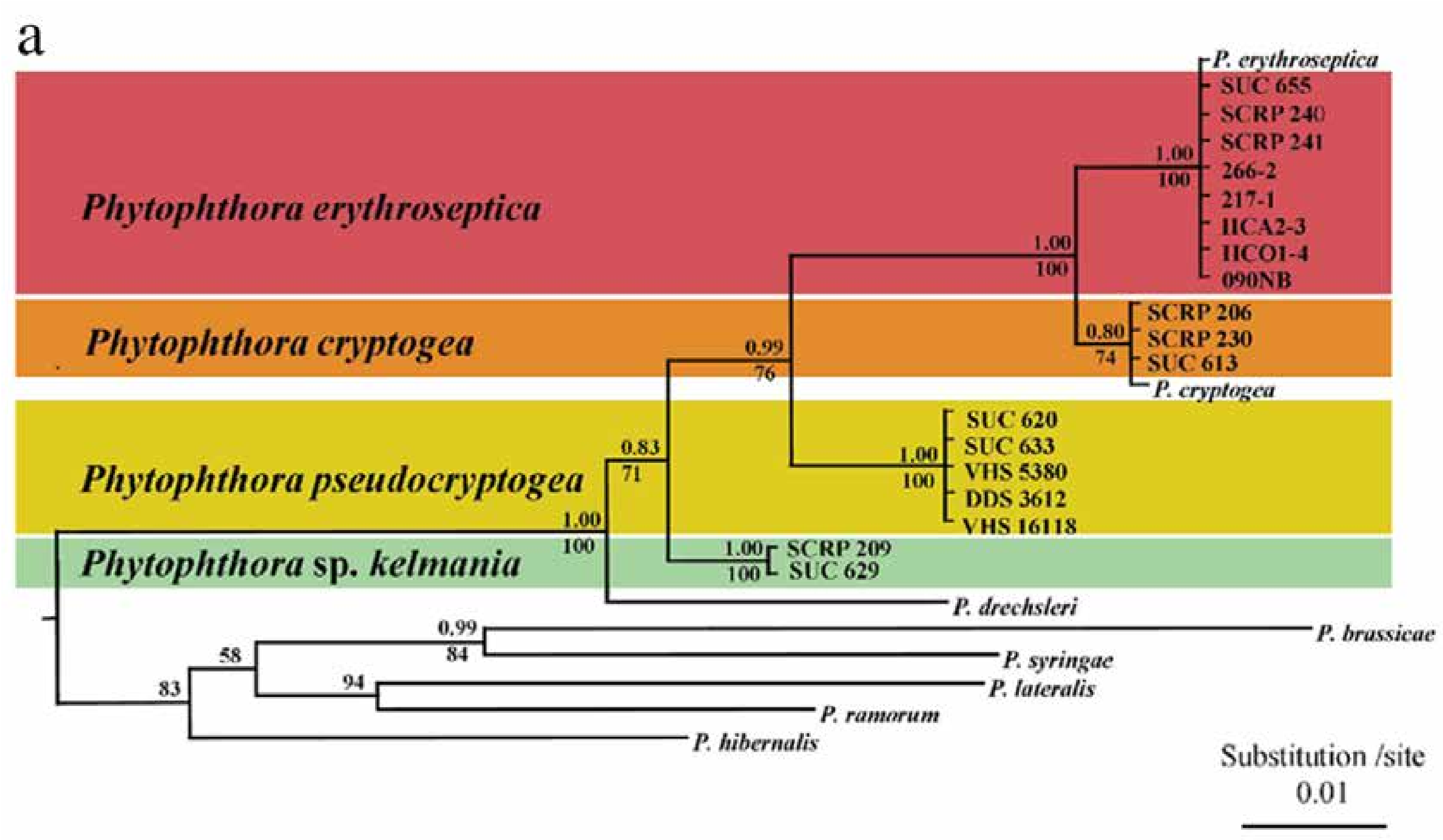
B. Safaiefarahani[1] · R. Mostowfizadeh-Ghalamfarsa[1] · G. E. St. J. Hardy[2] · T. I. Burgess[2]

They assembled a representative set of isolates and performed a study with many sources of evidence to properly set species boundaries





We can now distinguish five species:

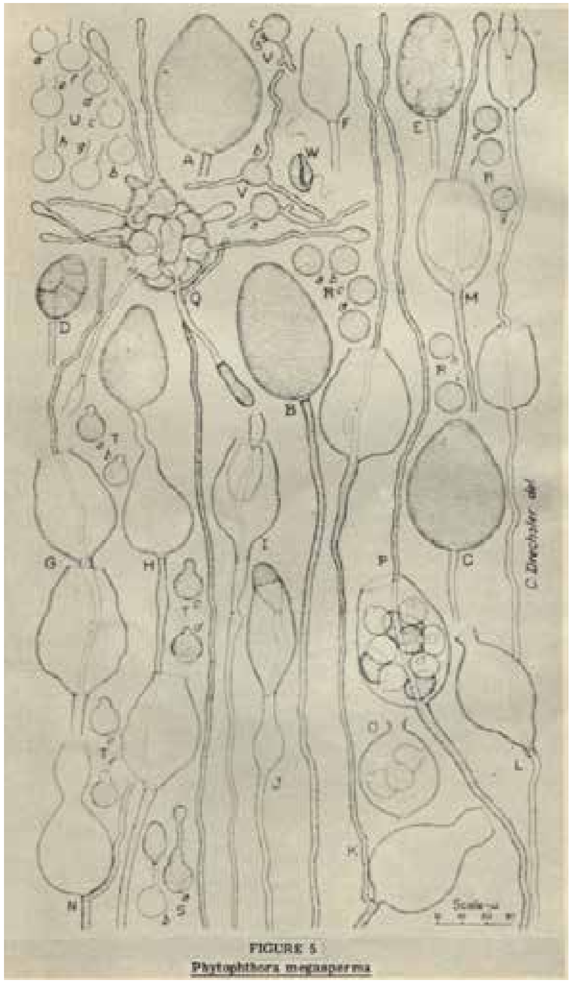


New context for Rizzo Lab results and California natural history

- The recently described *P. pseudocryptogea* was baited several times from Monterey and San Luis Obispo County streams
- *P. pseudocryptogea* was also baited several times from outplanted restoration sites in Santa Clara County
  – *P*. sp. *kelmania* baited once
- *P. cryptogea, P. drechsleri & P. erythroseptica* were not encountered

Brief history of *P. megasperma*

- Established in 1931, *megasperma* = big seed (large oospores), considered to have a broad host range
  - Unlike *P. cactorum*, many isolates appeared very host-specific
- Using the most advanced techniques available at the time, three legume-specializing strains were recognized as *P. sojae* (1958), *P. medicaginis* (1991), *P. trifolii* (1991)



Brief history of *P. megasperma*

- *P. rosacearum* (2009), specializing on fruit trees separated along with *P. sansomeana* (2009) which can infect conifers and soybeans
- *P. crassamura* (2015) emerging pathogen with a wide host range
  - *P. crassamura* only species phylogenetically close to “true” *P. megasperma*

Who really needs to know *Phytophthora* by the species?

- Regulators
  - Which species are resident, which are exotic?
- Diagnosticians
- Land owners/managers
  - What *Phytophthora* species are present on my land?
  - How did they get there?
  - How do I manage those species once I know about them?

Who really needs to know *Phytophthora* by the species?

- Some restoration ecologists
  - What's the history of the site?
  - If *Phytophthora* is already present or expected, how can effective restoration still be achieved?
  - Even if they begin *Phytophthora*-free, riparian, frequently flooded, or even over-irrigated plants are likely to encounter aquatic *Phytophthora* species after they are planted

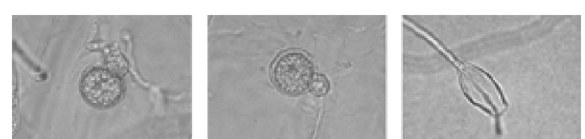
Who probably **doesn't** need to know *Phytophthora* by the species

- Many restoration ecologists
- Most nursery managers
  – Nursery sanitation practices aimed at reducing incidence and movement of *Phytophthora* are largely based around detecting the symptoms of above-ground species, or directly detecting soil-borne species; neither of these approaches actually requires identification of species
  – In agricultural settings, *Phytophthora* root rot is not typically managed on a species-by-species basis

Looking forward

- The pace of new *Phytophthora* species has only increased during the 5 years of my PhD
  - Including distinct **new taxa** discovered during SCVWD-organized survey
  - It is difficult to keep up, even for a researcher like myself
- The amount of practical knowledge about the “new” species is also rapidly increasing
  - and **the consistency of automated ID is in development**
- The more California knows about its resident *Phytophthora* species, the better the prognosis for the health of its resident plants

Phytophthora **taxon mugwort**



Phytophthora **taxon juncus**

Acknowledgments

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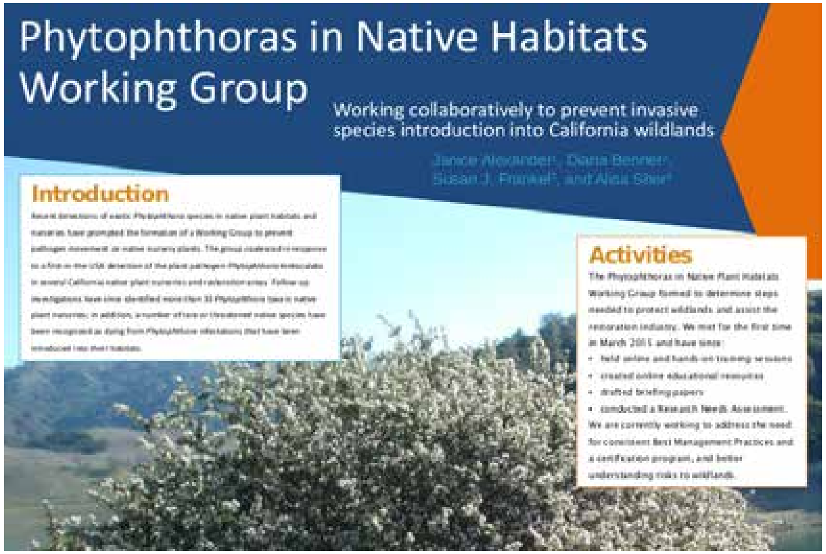
- Suzanne Latham, Cheryl Blomquist
  - CDFA



CalPhytos.org