Chytrid Biology 101

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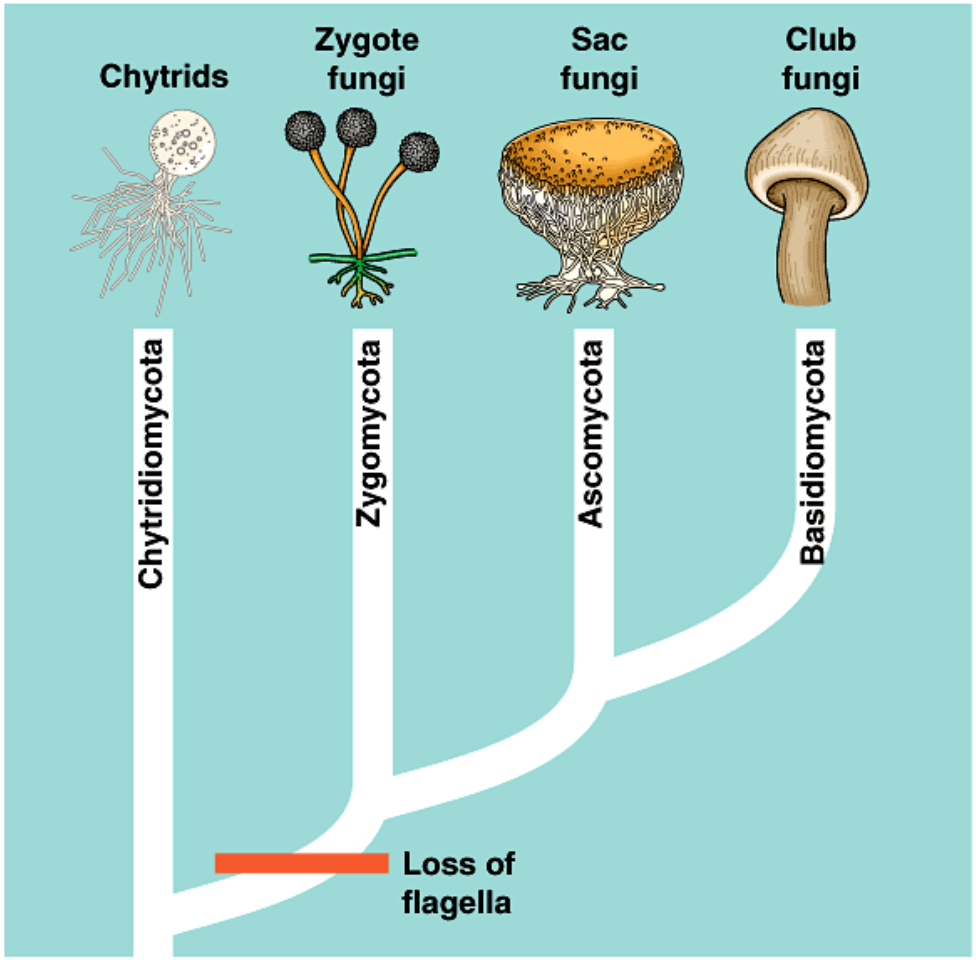
Chytrids began causing pond interruption during Sapphire's first growing season

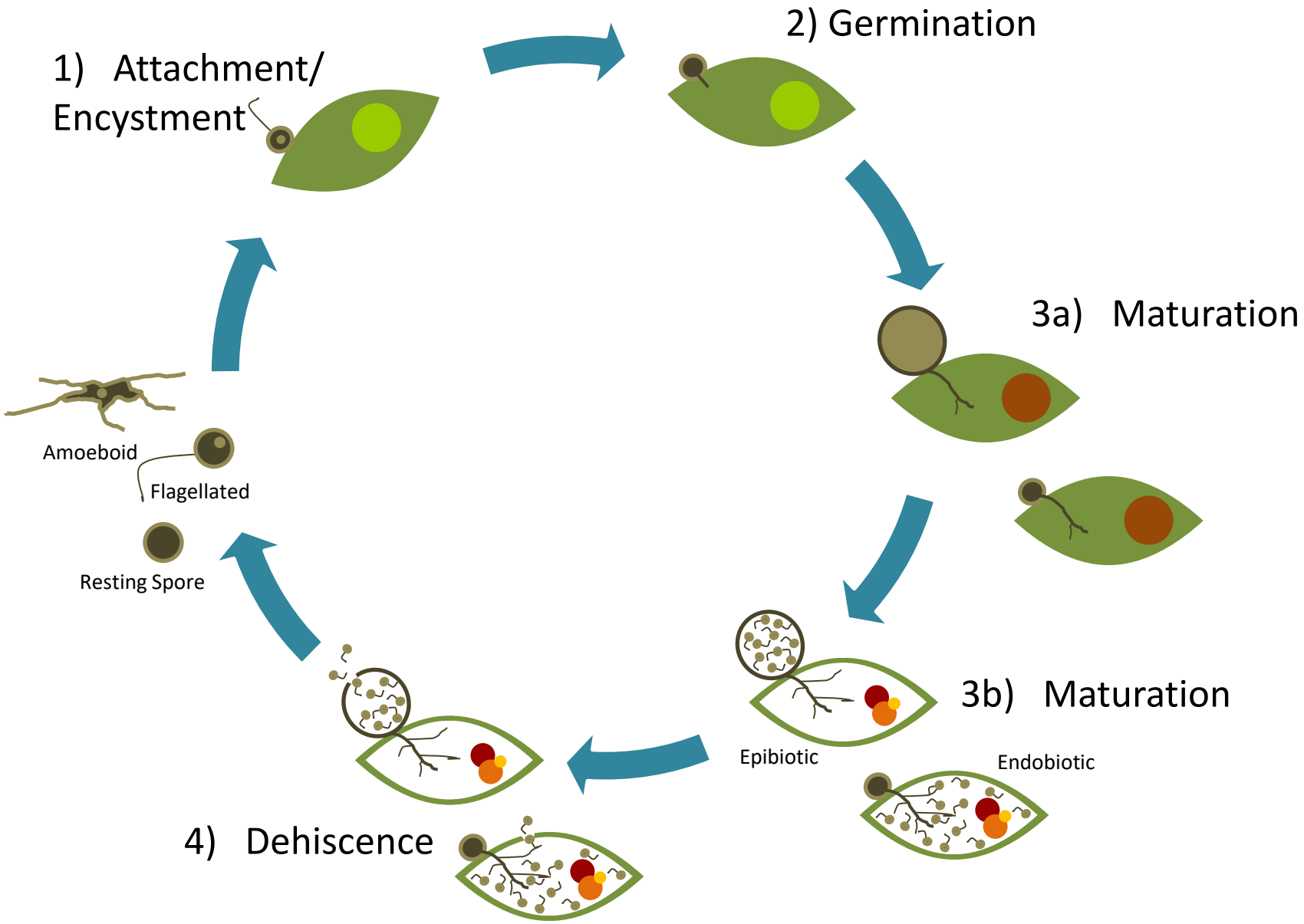
- First raceways were inoculated Winter 2008/2009
- First chytrids were observed and captured Summer 2009
- Desmid ponds were crashing
  48-72 hours after inoculation

Chytrids are a basal fungi that produce motile (flagellated or amoeboid) zoospores

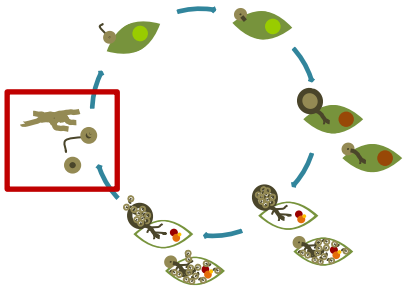


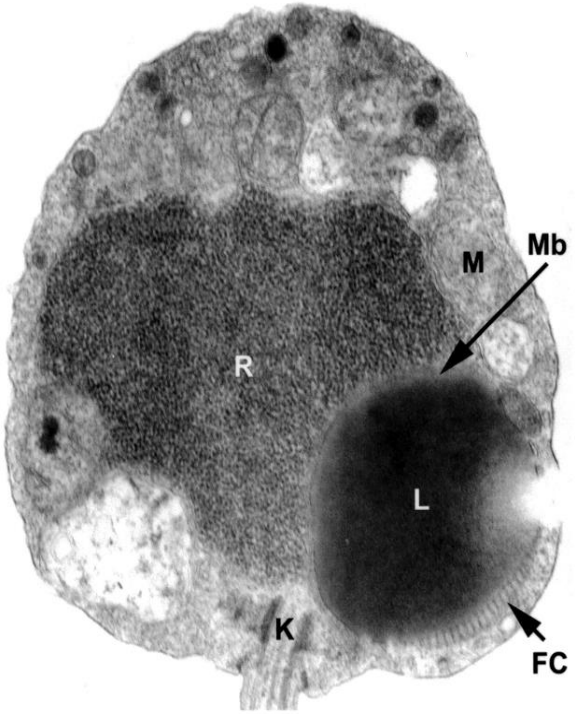
Phylum	Key Reproductive Feature
Chytridiomycota (chytrids)	Motile spores with flagella
Zygomycota (zygote fungi)	Resistant zygosporangium as sexual stage
Ascomycota (sac fungi)	Sexual spores borne internally in sacs called asci
Basidiomycota (club fungi)	Sexual spores borne externally on club-shaped structures called basidia

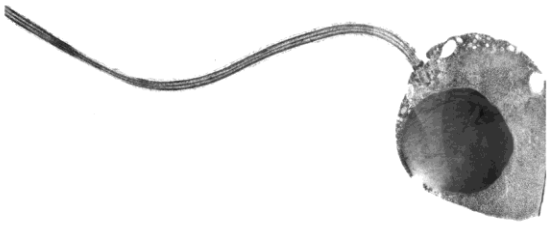
Chytrids have distinct life cycle stages



Chytrids can be characterized through zoospore ultrastructure







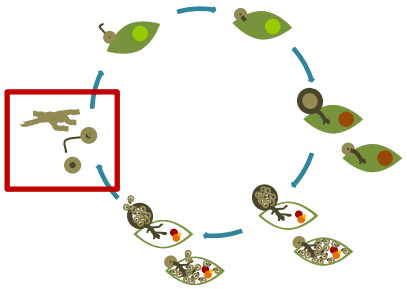
- Initially, chytrids were characterized through sporangia morphology.
- Sporangia morphology is not conserved amongst species.
- Zoospore ultrastructure is static amongst species.

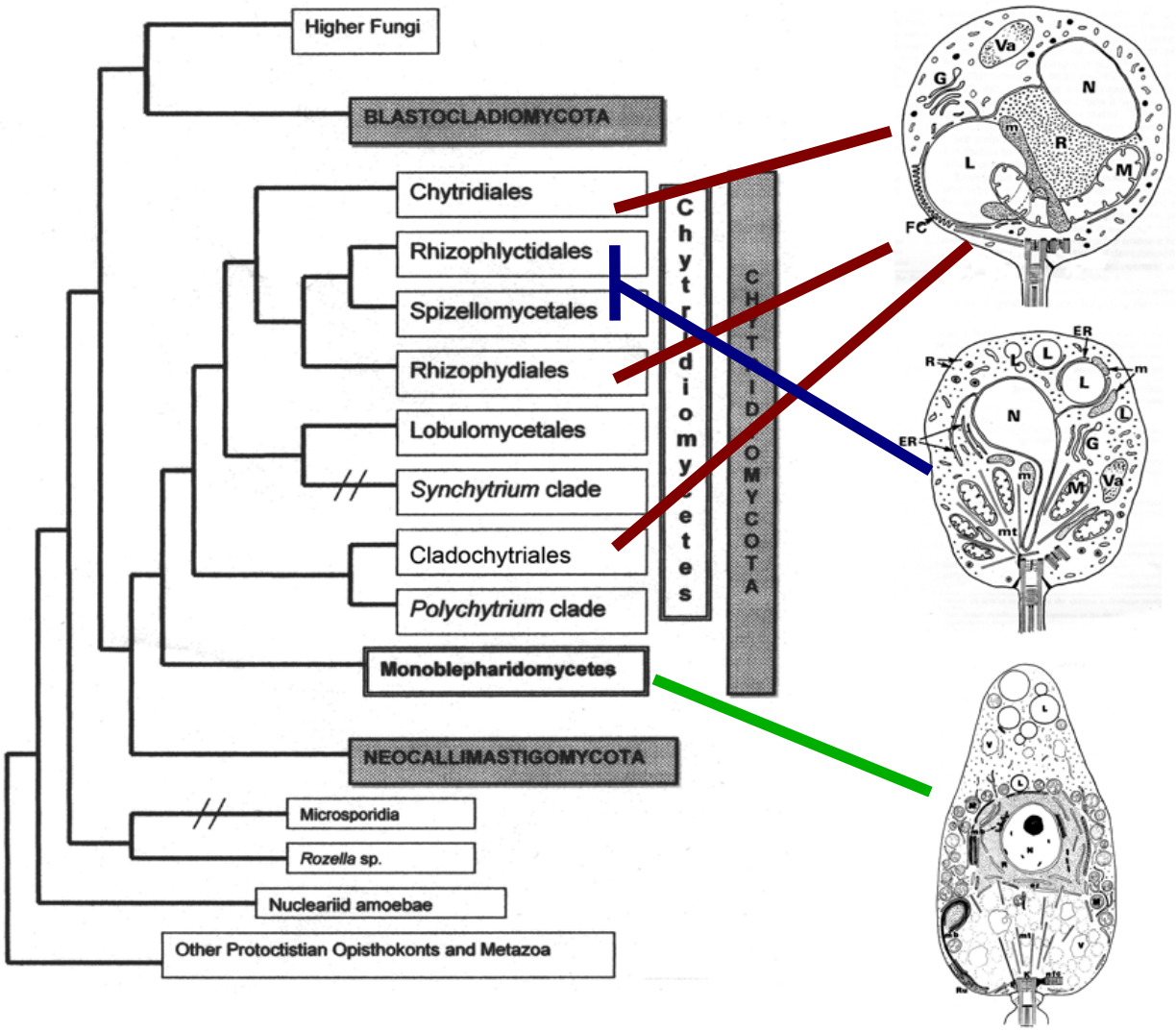
L: Lipid globule
M: Mitochondria
R: Ribosomes
FC: Fenestrated cisterna

Posterior, whiplash flagella

Images courtesy Dr. Peter Letcher

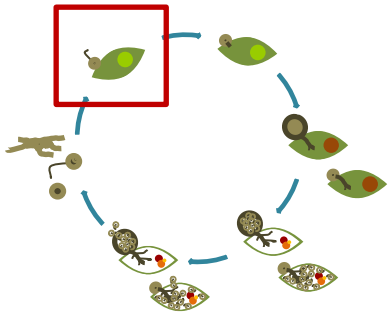
Chytrids can be characterized through zoospore ultrastructure

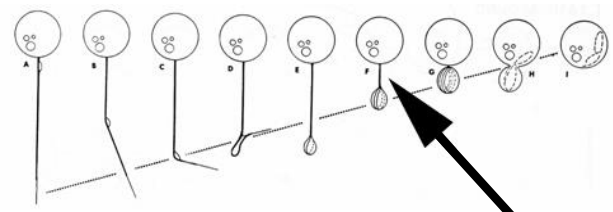


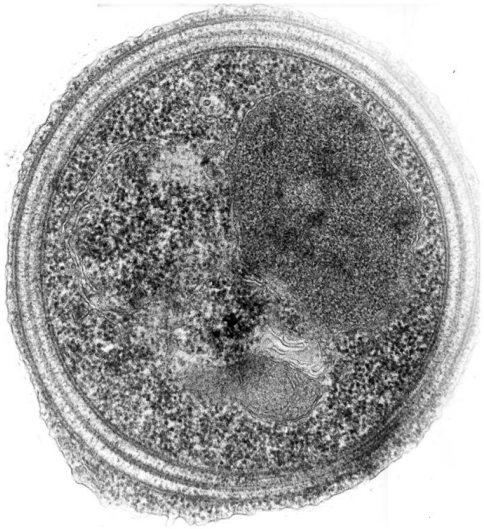


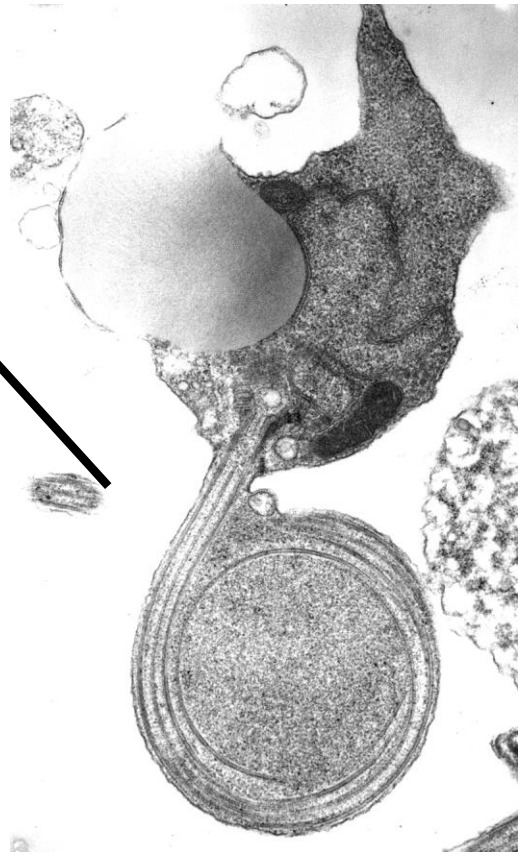
Images courtesy Dr. Peter Letcher

Chytrids begin attachment/encystment after finding suitable substrate



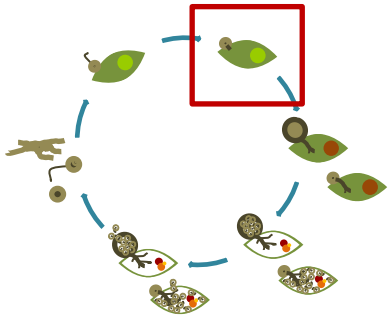


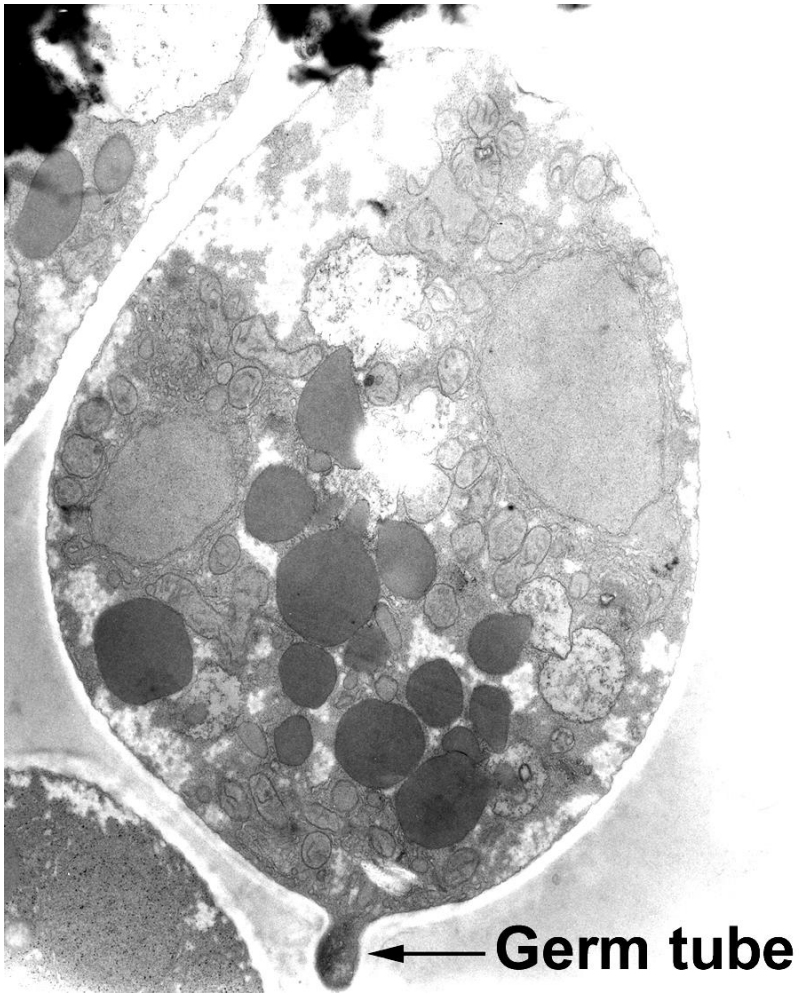




Images courtesy Dr. Peter Letcher

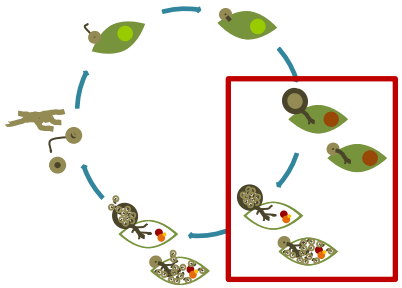
Chytrids begin germination after successful encystment

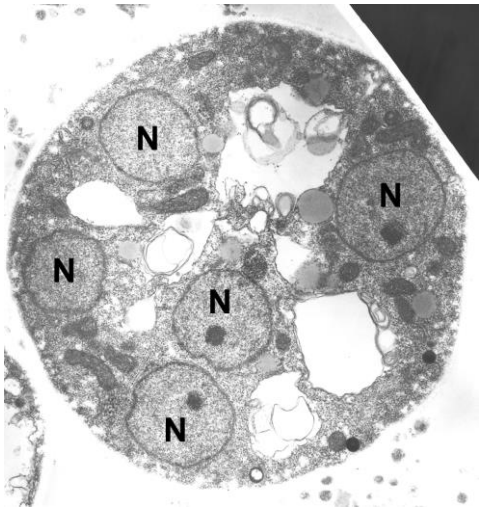




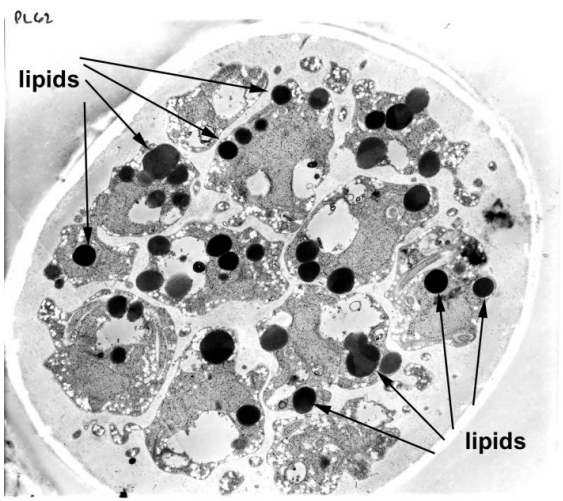
Images courtesy Dr. Peter Letcher

Chytrids zoospore development commences following germination

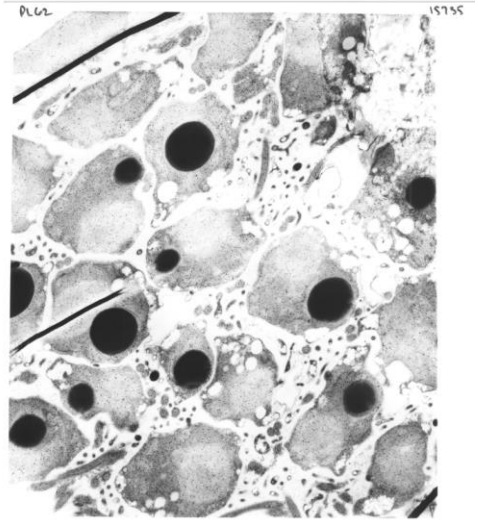




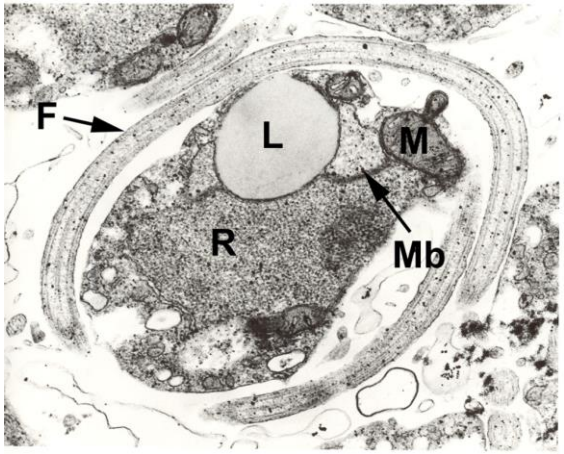
Mitosis = Multiple nuclei



Lipid globules begin coalescing



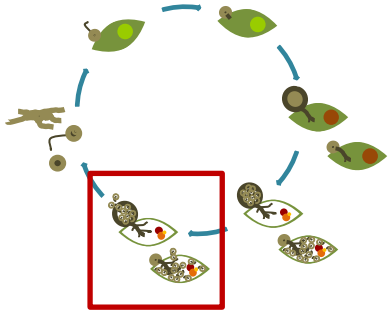
Single lipid globules

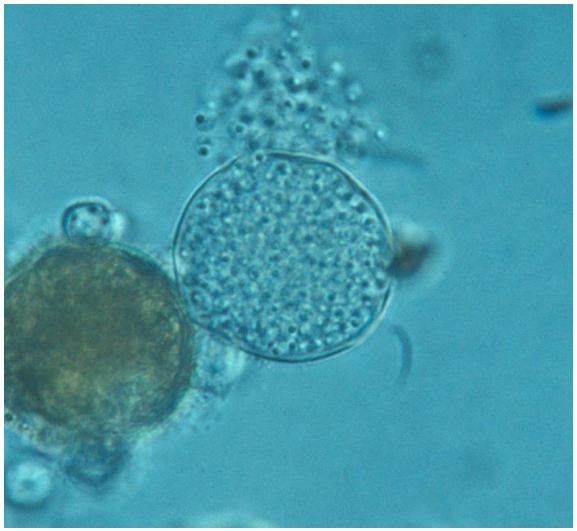


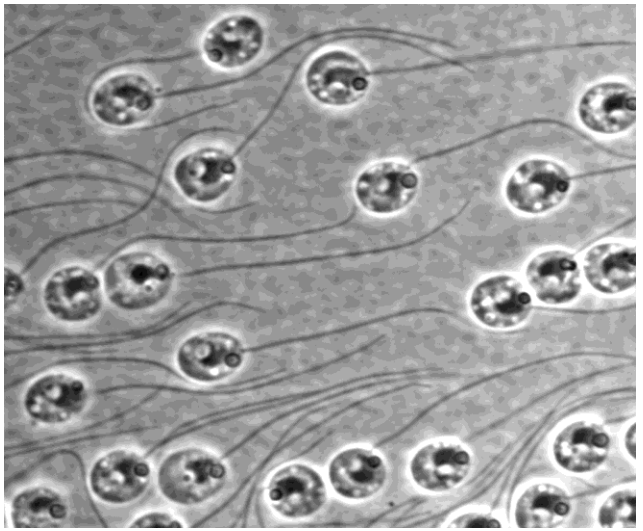
Fully cleaved zoospore

Images courtesy Dr. Peter Letcher

Chytrids dehisce after progeny has matured





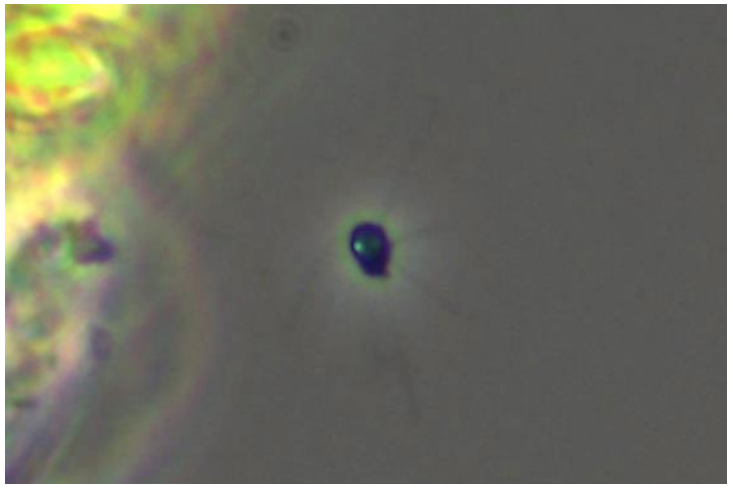


Images courtesy Dr. Peter Letcher

Chytrid-like parasites isolated at the LCTS demonstrate chytrid life cycles

FD01

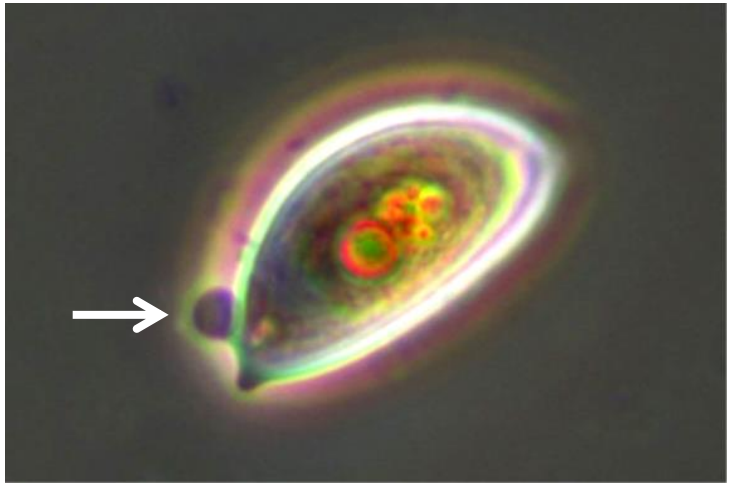
ITS similar to uncultured fungus clone F1210G



Zoospore
Size: 2-3 uM

Body: Irregular. Motile; amoeboid.

Center refracts light, revealing a greenish hue. Move slowly, using their extended pseudopodia. Best seen with 100X objective, phase III contrast.



Sporangia
Size: 2-4 uM

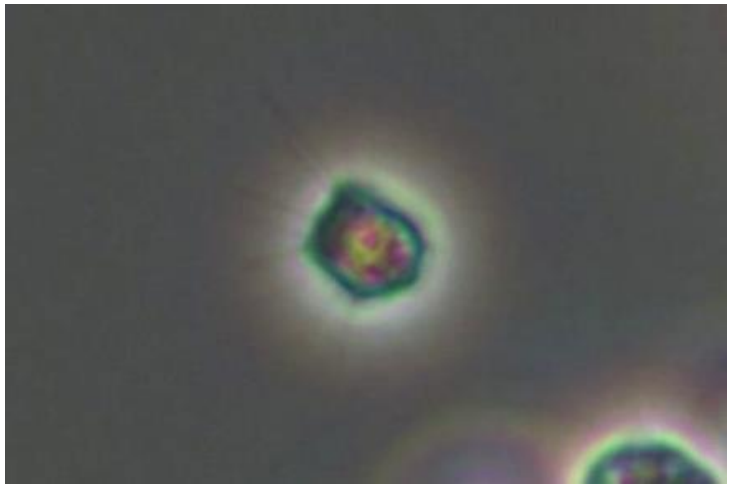
Body: Spherical. Non-motile

Opaque. Progeny don't seem to mature inside the sporangia. Best seen at 100X objective, phase III contrast.

Chytrid-like parasites isolated at the LCTS demonstrate chytrid life cycles

FD61

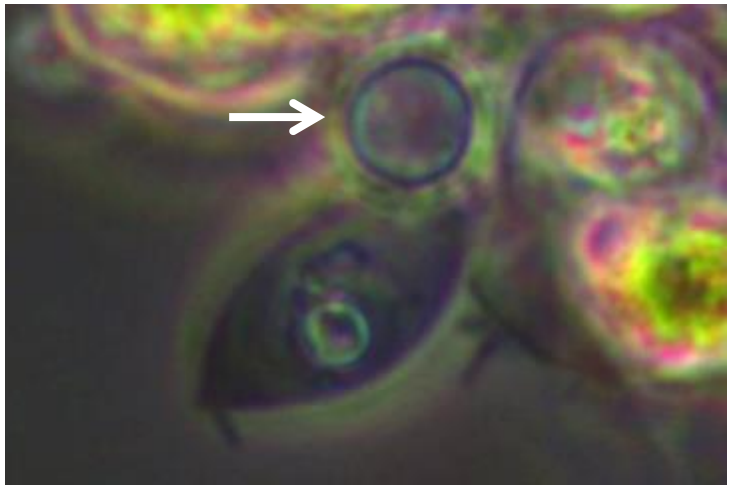
ITS similar to uncultured fungus clone IVN1-23



Zoospore
Size: 4-6 uM

Body: Irregular. Motile; amoeboid

Refract light, revealing a greenish hue. Move slowly, using their pseudopodia. Best seen with 100X objective, phase III contrast.



Sporangia
Size: 5-7 uM

Body: Spherical. Non-motile.

Semi-translucent. Progeny appear to mature inside the sporangia. Best seen at 100X objective, phase III contrast.

Chytrid-like parasites isolated at the LCTS demonstrate chytrid life cycles

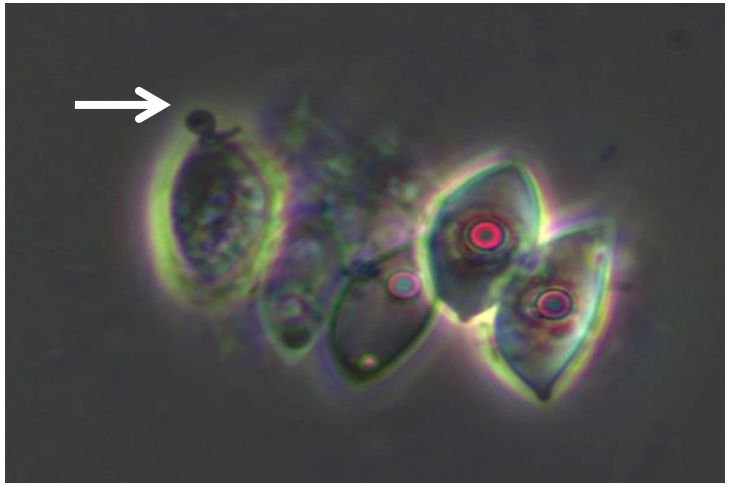
FD95

ITS similar to uncultured fungus clone 167-40

Zoospore
Size: 2-3 uM

Body: Irregular. Motile; amoeboid.

Center refracts light, revealing a greenish hue. Move slowly, using their extended pseudopodia. Best seen with 100X objective, phase III contrast.



Sporangia
Size: 2-4 uM

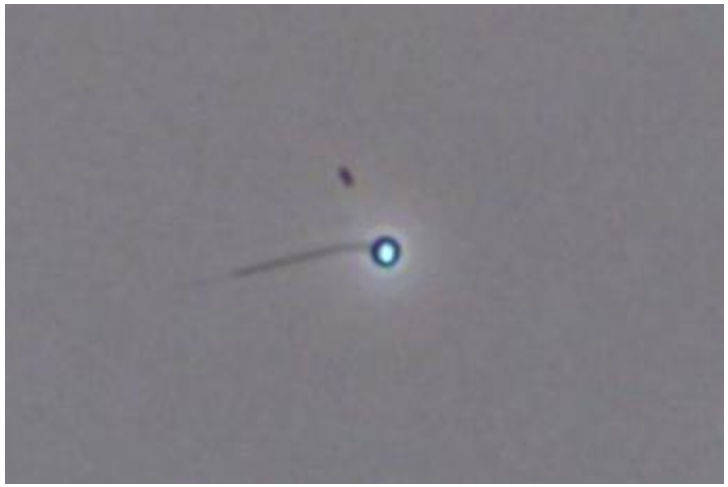
Body: Spherical. Non-motile

Opaque. Progeny don't seem to mature inside the sporangia. Best seen at 100X objective, phase III contrast.

Chytrid-like parasites isolated at the LCTS demonstrate chytrid life cycles

FD100

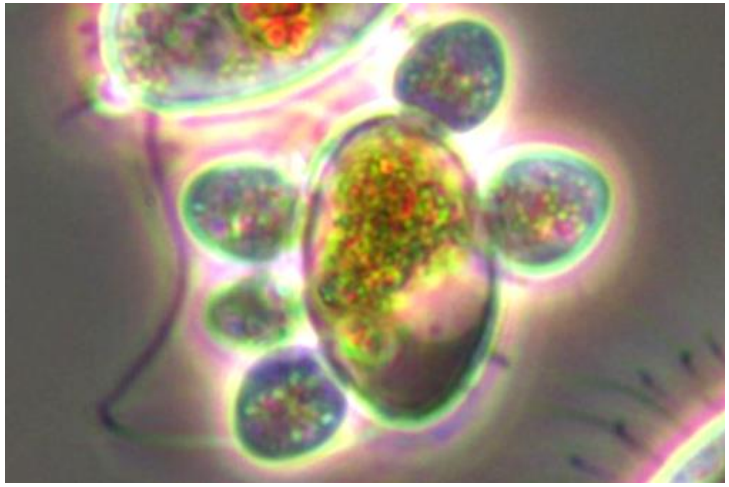
ITS similar to uncultured Chytridiomycota clone AY2009A6



Zoospore
Size: 1-2 uM

Body: Spherical. Motile; uniflagellate

Refract light, revealing a greenish hue. Move quickly in a circular path. Best seen with 100X objective, phase III contrast or with 40X objective, dark field contrast.



Sporangia
Size: 5-7 uM

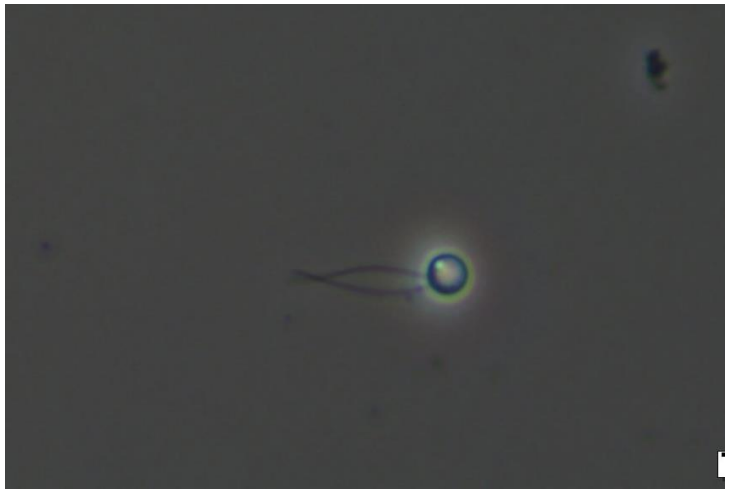
Body: Pear shaped. Non-motile.

Semi-translucent. Progeny mature inside the sporangia. Best seen at 100X objective, phase III contrast.

Chytrid-like parasites isolated at the LCTS demonstrate chytrid life cycles

FD101

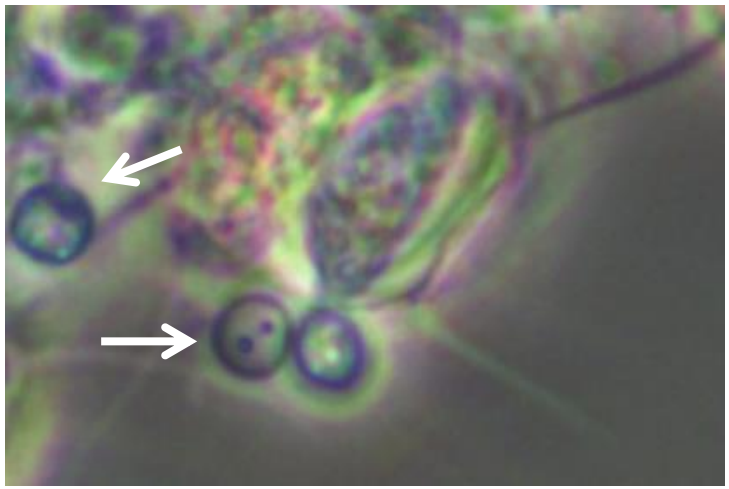
ITS similar to Rhizophydiales sp. ARG010



Zoospore
Size: 2-3 uM

Body: Spherical. Motile; biflagellate

Refract light, revealing a greenish hue. Move quickly in a circular path. Best seen with 100X objective, phase III contrast or with 40X objective, dark field contrast.

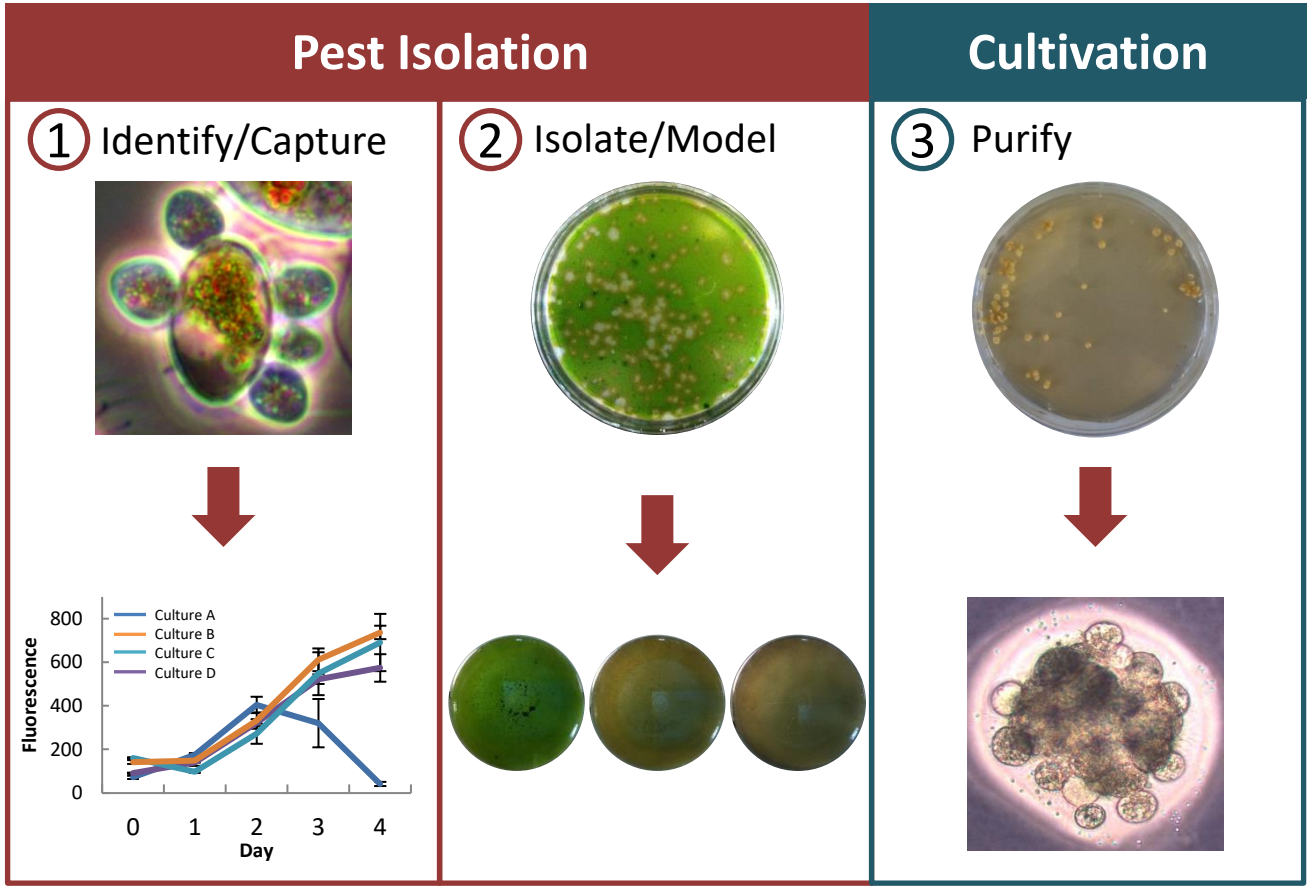


Sporangia
Size: 3-5 uM

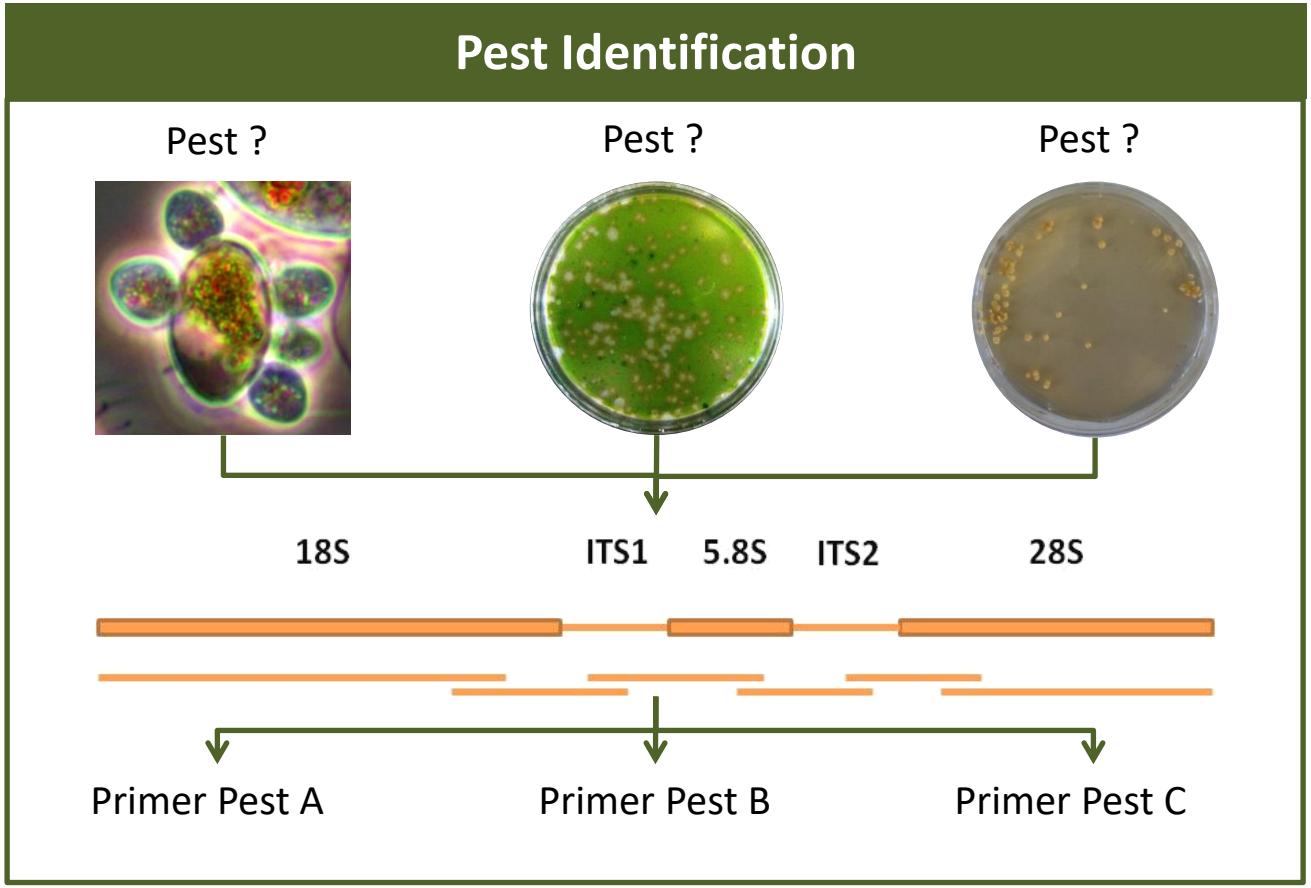
Body: Spherical. Non-motile.

Semi-translucent. Progeny appear to mature inside the sporangia. Best seen at 100X objective, phase III contrast.

Efforts were made to understand chytrid physiology in order to develop crop protections strategies



Molecular tools were developed to identify and quantitatively monitor chytrids



Multiple pesticides were screened to develop crop protection strategies for chytrids

- Various fungicides with different modes of action were screened against algae and chytrids
- Top three candidates: fluazinam, pyraclostrobin, and thiram
- EUPs were acquired for all three fungicides
- All three fungicides have been deployed in the field

Trends in CT values are used to implement crop protection strategies

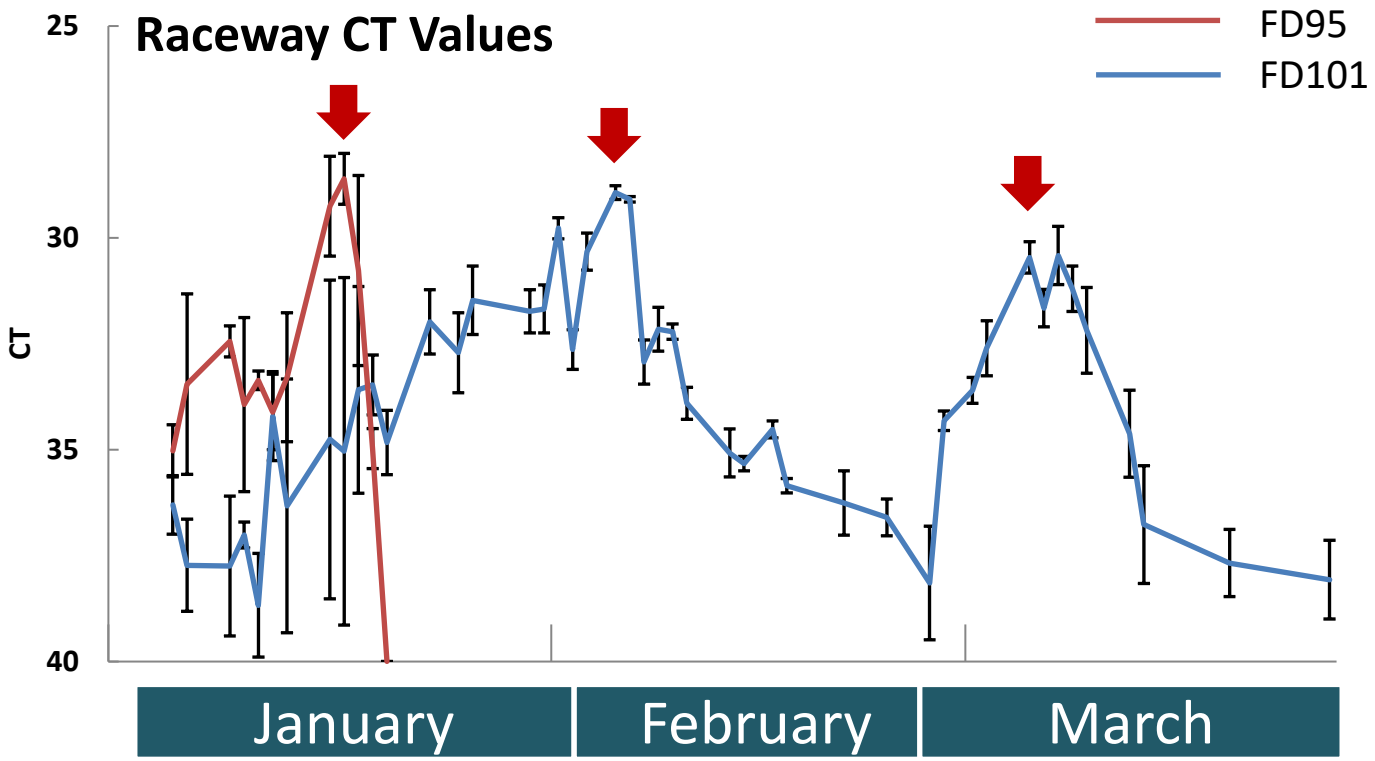


Figure 1. Two pests monitored using qPCR in two different raceways. CT values triggered crop protection strategies (red arrows) extending raceway productivity.

Our goal is to generate a pest database for evolving crop protection strategies

- One step ahead:
  - Capture/identify potential threats
  - Culture/establish lab crash models to develop monitoring tools and crop protection strategies
  - Long-term storage of pests
    - Screening of new crop protection strategies

ZERO interruption in culture productivity (due to pests)