TEM Investigation of FD61 (*Paraphysoderma sedebokerense,* Blastocladiomycota), an epibiotic parasite of *Scenedesmus dimorphus*

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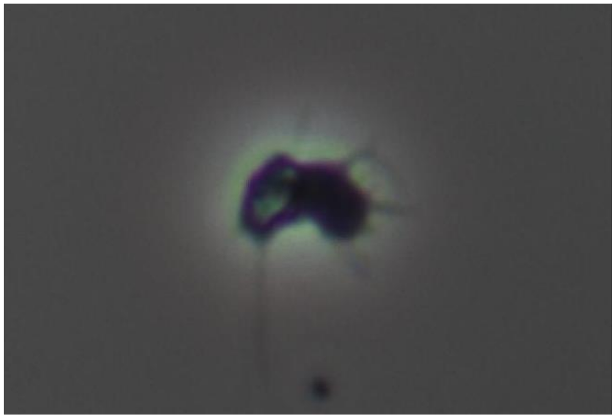
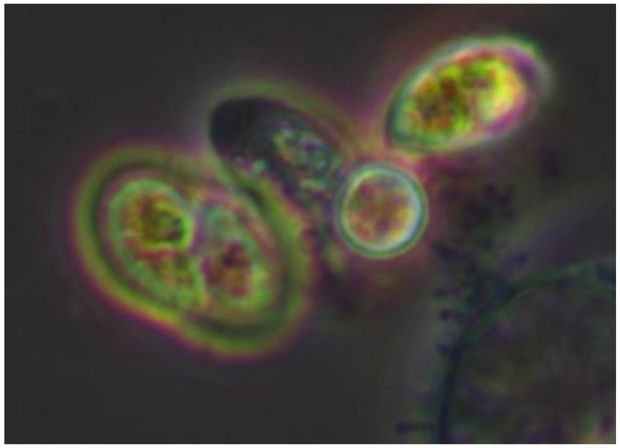
The University of Alabama

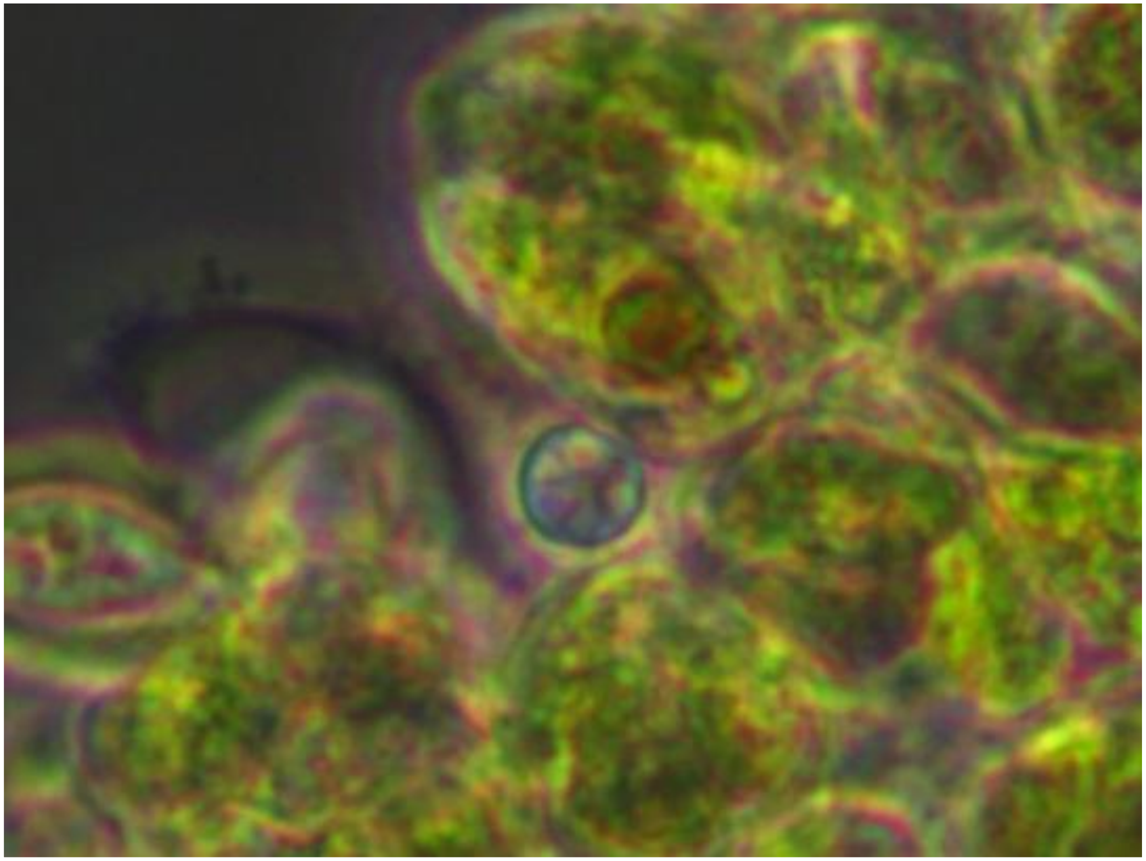
FD 61 light microscopy, courtesy Sal Lopez

pseudopodiate amoeba





amoeba “docking” with
algal cell



parasite sporangium

Historical

(1) An amoeboid, aplanosporic parasite of *Haematococcus* morphologically characterized but not named:

Hoffman Y, Aflalo C, Zarka A, Gutman J, James TY, Boussiba S. 2008. Isolation and characterization of a novel chytrid species (phylum Blastocladiomycota), parasitic on the green alga *Haematococcus*. Mycological Research 112:70-81.

(2) A phylogeny of Blastocladiomycota, placing unnamed isolate from 2008 study as sister to *Physoderma* and *Urophlyctis*, and designating it *Paraphysoderma sedebokerense* nom. prov.:

Porter TM, Martin W, James TY, Longcore JE, Gleason FG, Adler PH, Letcher PM, Vilgalys R. 2011. Molecular phylogeny of the Blastocladiomycota (Fungi) based on nuclear ribosomal DNA. Fungal Biology 115:381-392.

(3) As provisional names ("nom. prov.") are not taxonomically valid, proper taxonomic naming of *Paraphysoderma sedebokerense* as a new genus and species:

James TY, Hoffman Y, Zarka A, Boussiba S. 2011. *Paraphysoderma sedebokerense,* gen. et sp. nov., an aplanosporic relative of *Physoderma* (Blastocladiomycota). Mycotaxon 118:177-180.

rDNA sequence similarity

18S: Tim James' Blastocladiales sp. EF565163 (*P. sedebokerense*) 18S is about 1700 bp, 5'→3';

The 1st 600 bases of Sapphire FD61 18S align with the last (3' end) 600 bases of EF565163, 99% similarity;

The next ~2200 bases of the FD61 sequence aligns with *Physoderma maydis* 28S, ~93% similarity

The 18S similarity indicates EF565163 *P. sedebokerense* and FD61 to be the same organism; alternatively, it may be a result of sequence similarity in a conserved region of 18S. We need sequencing of entire 18S to ascertain identity of FD61; molecular protocols and primers in: Porter TM, Martin W, James TY, Longcore JE, Gleason FG, Adler PH, Letcher PM, Vilgalys R. 2011. Molecular phylogeny of the Blastocladiomycota (Fungi) based on nuclear ribosomal DNA. Fungal Biology 115:381-392.

Life cycle of FD61

Day 1: healthy algal cells

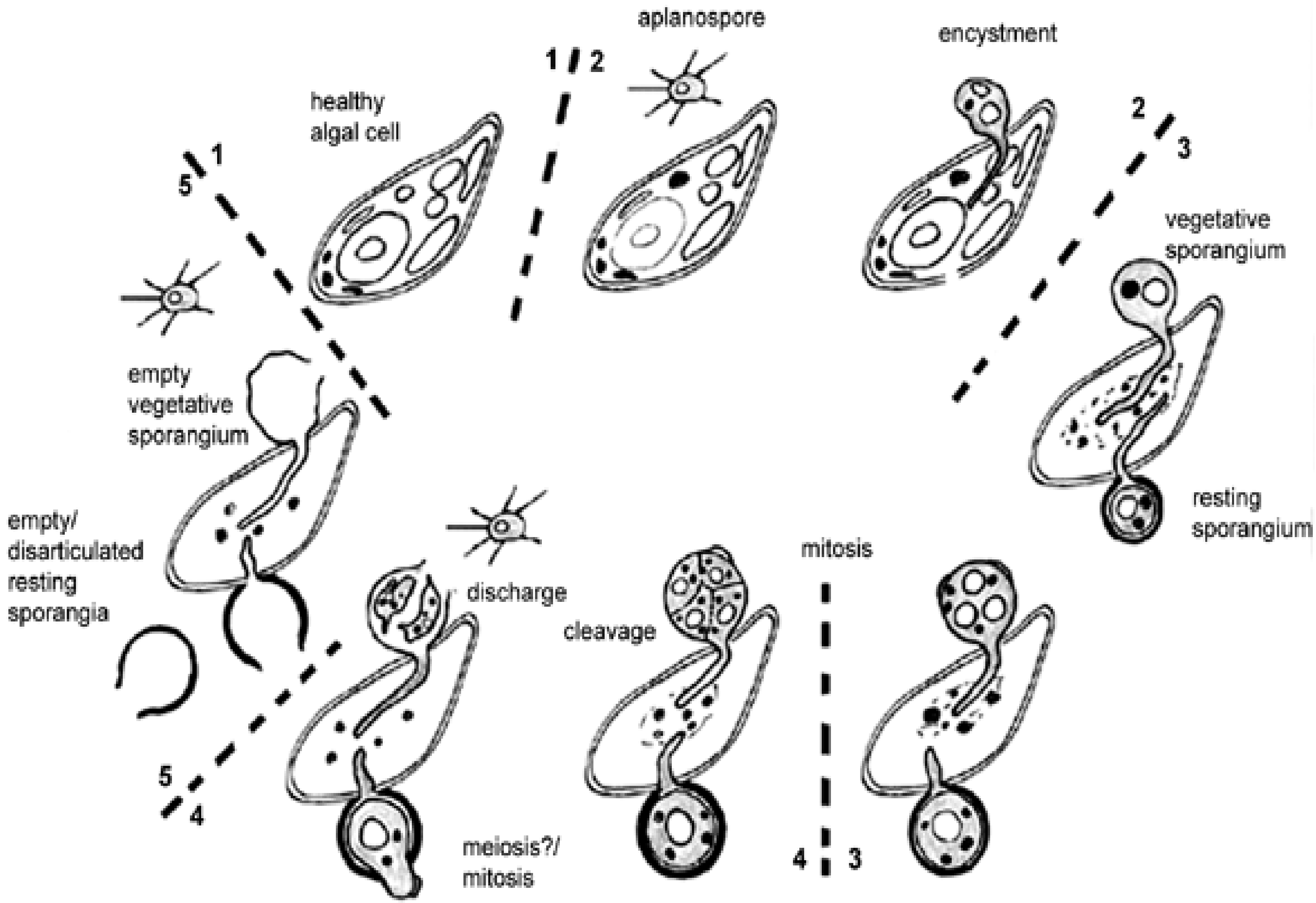
Day 2: abundant amoeboid aplanospores, aplanospores docking with algal cells, and early infection via thin-walled vegetative sporangia.

Day 3: full infection, host contents fully degraded; appearance of thick-walled resting sporangia; mitosis; early cleavage.

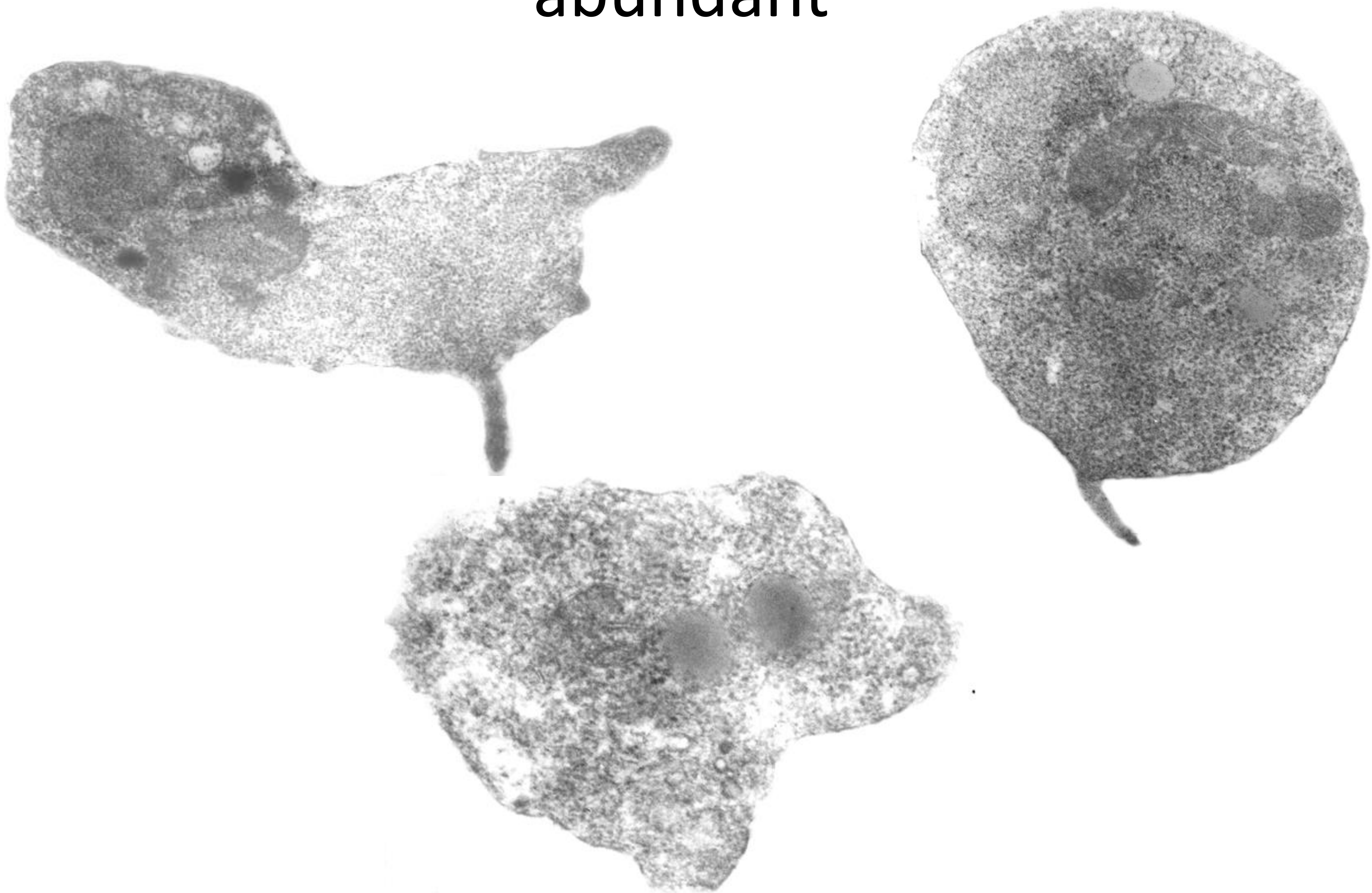
Day 4: continued mitosis; full cleavage; aplanospore discharge; motile aplanospores.

Day 5: motile aplanospores; thin-walled and thick-walled sporangia empty.

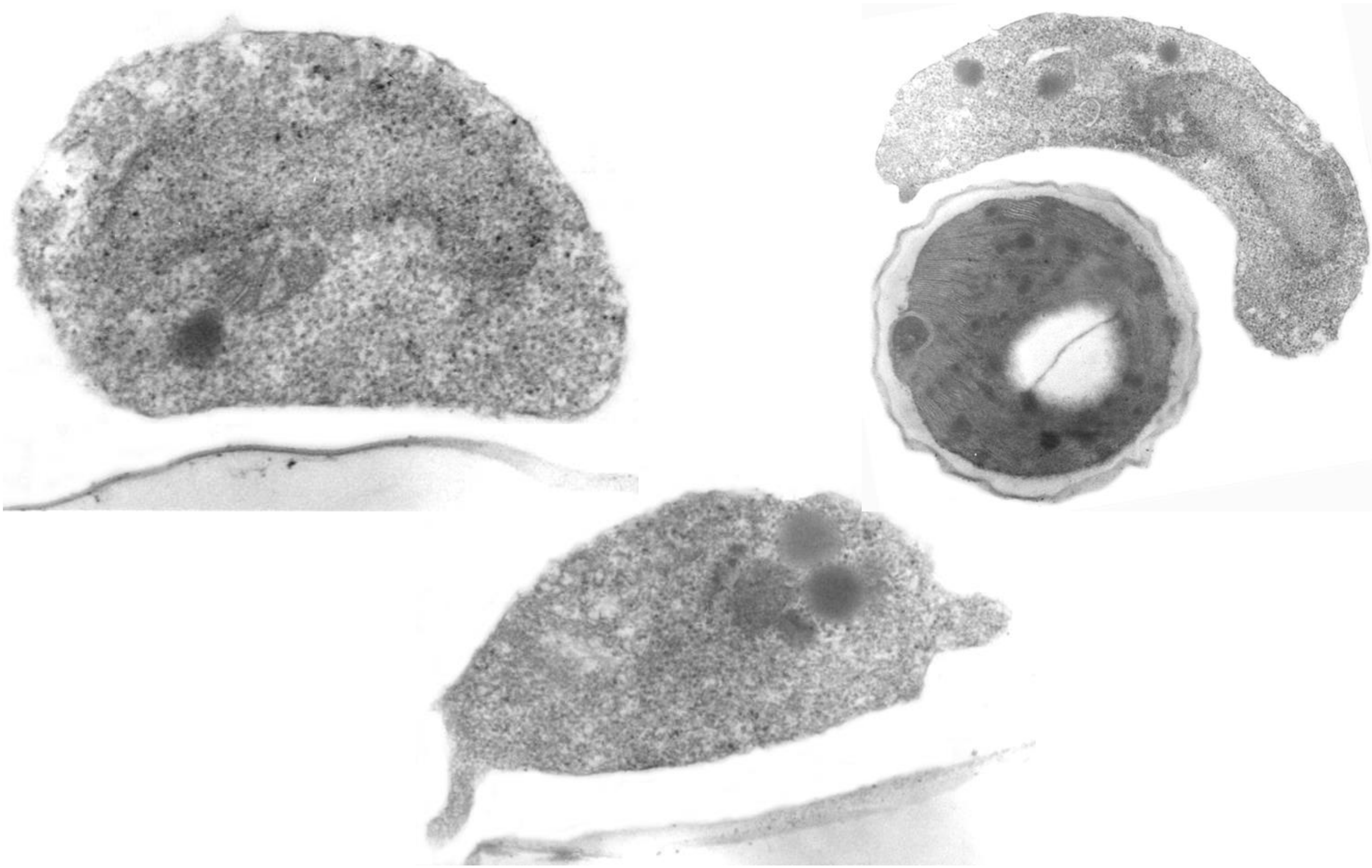
Life cycle schematic



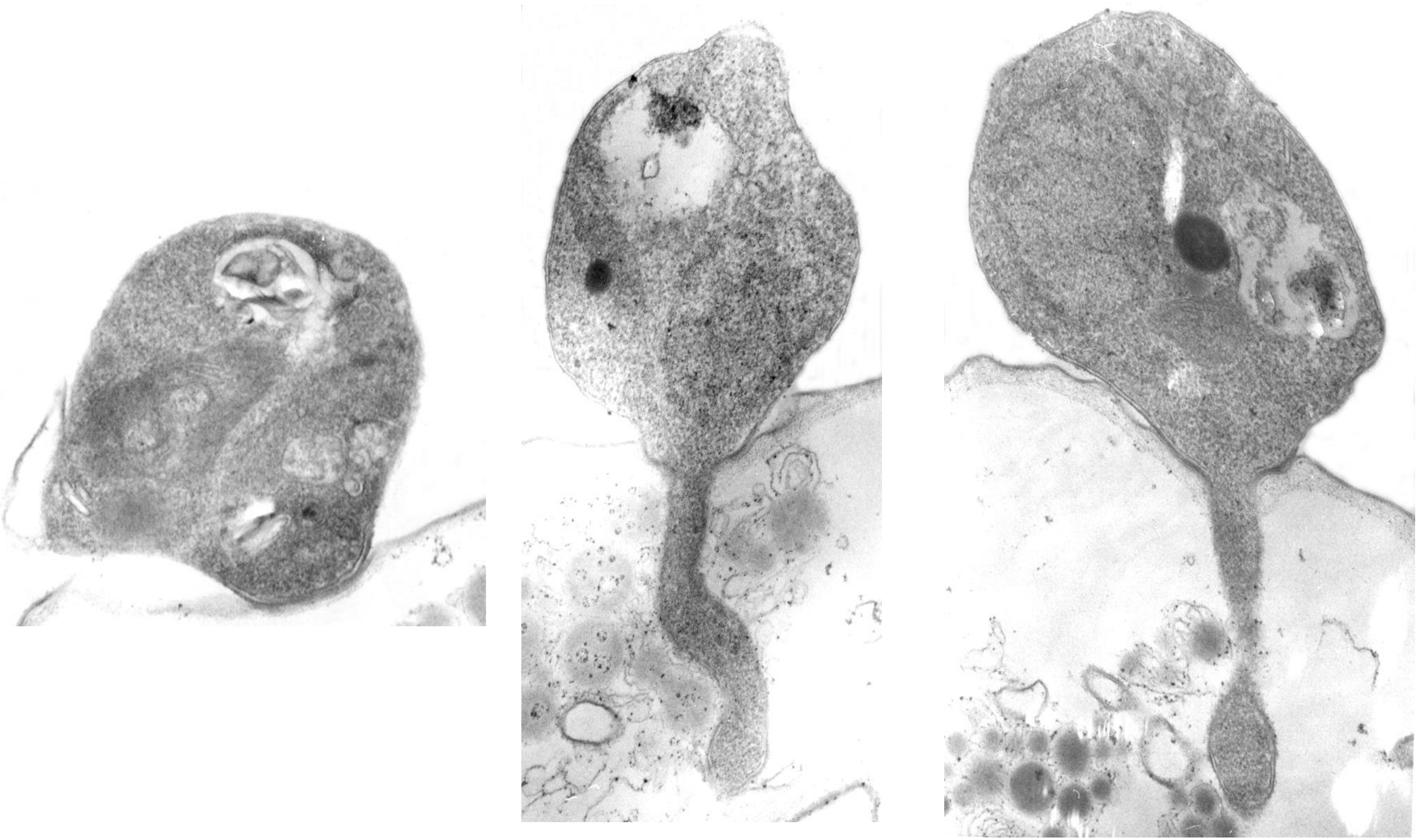
Day 2: motile pseudopodiate aplanospores abundant



Day 3: aplanospores docking with host

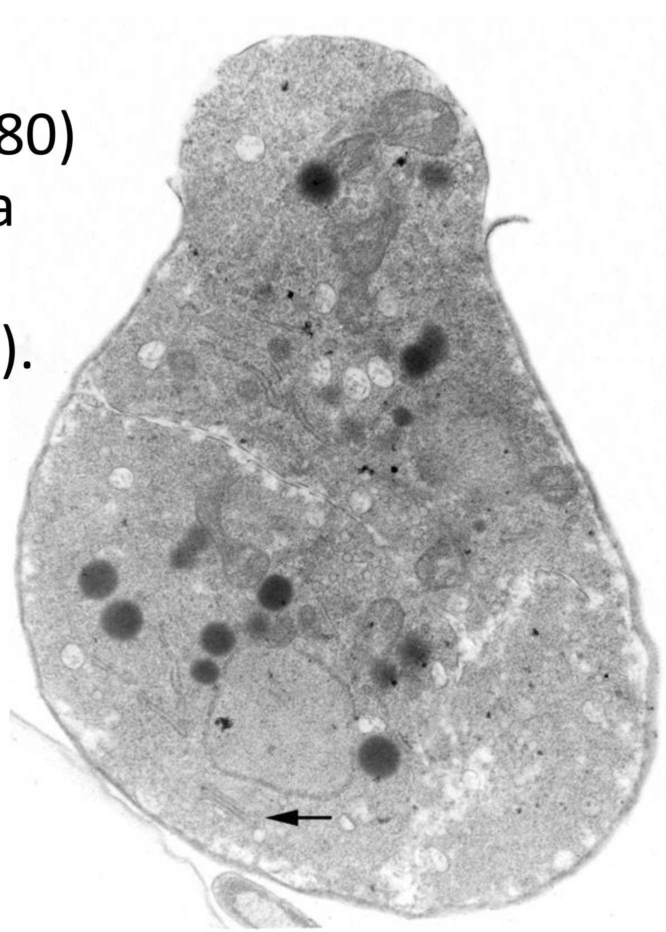


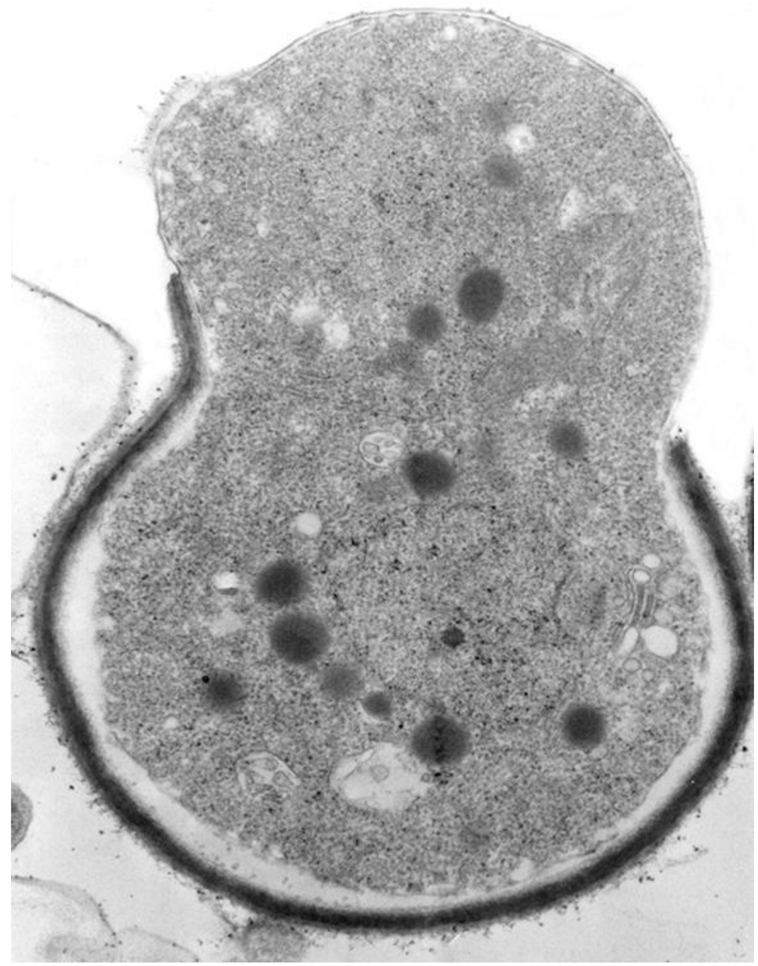
Day 3: aplanospores encysting (L), and penetrating host (C, R) via a tubular haustorium



Day 3

As well as thin-walled vegetative sporangia (L), there are thick-walled resting sporangia (R) similar to those of Physoderma maydis (Lange and Olson 1980) and Paraphysoderma sedebokerense (Hoffman et al. 2008).





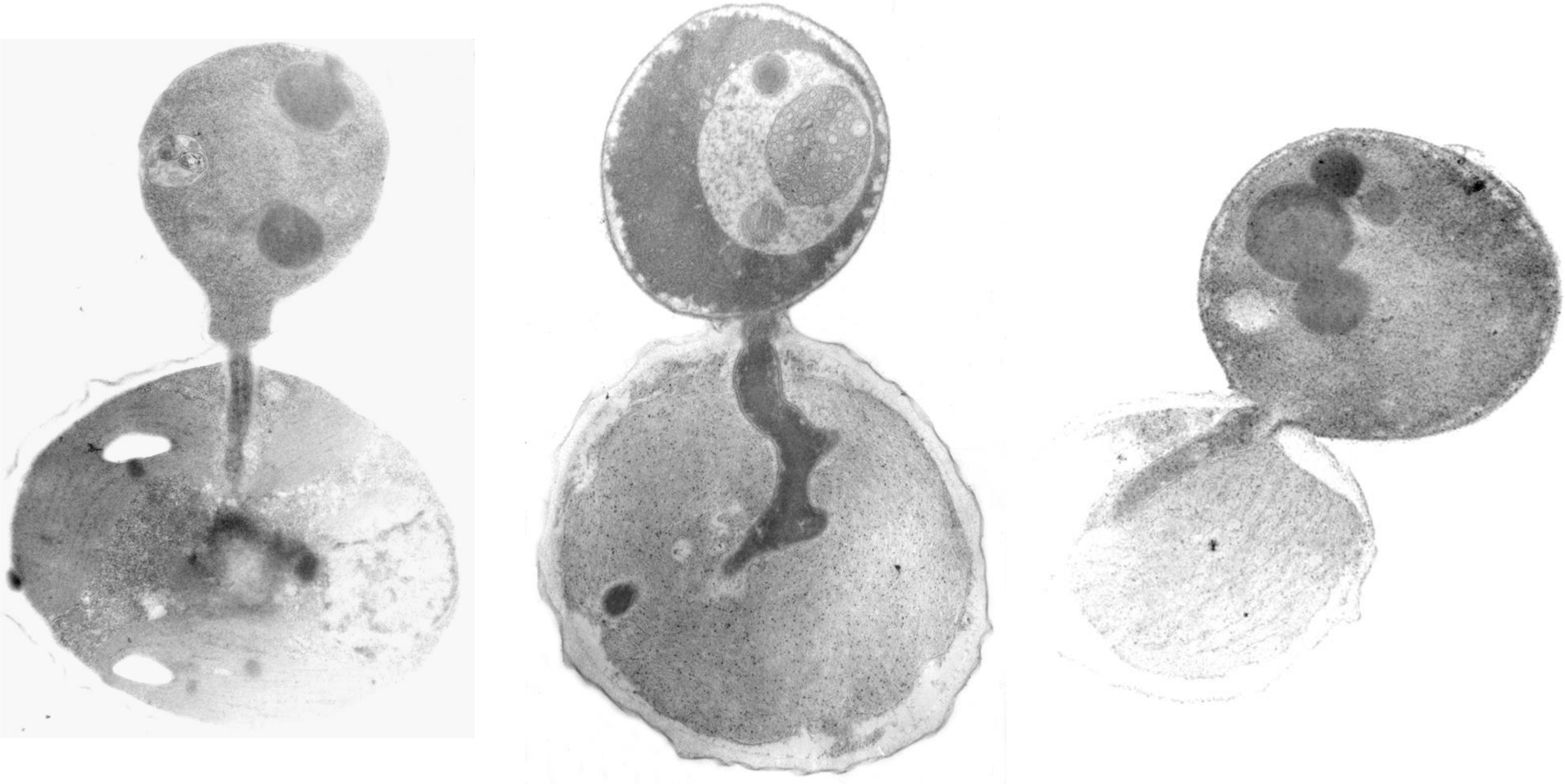
Day 3: I will illustrate development of thin-walled vegetative sporangia first (slides 13-19), then development of thick-walled resting sporangia (slides 22-27).

It appears that an aplanospore can develop either way, but it is difficult to determine initially, until the wall further develops, which route a spore is taking (slide 12).

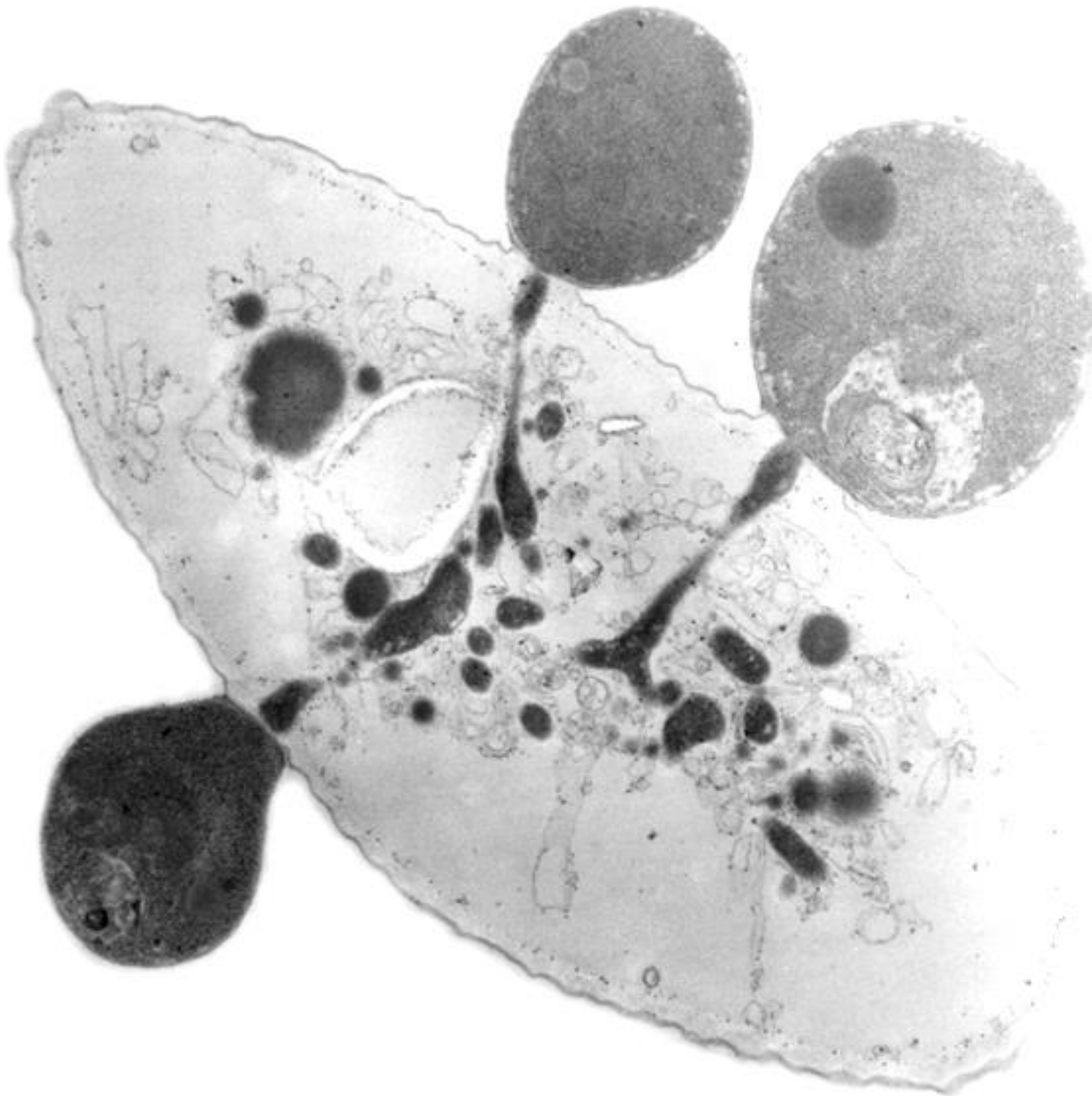
Vegetative sporangia are asexual, undergoing only mitosis.

Resting sporangia may possibly be sexual, the content of the sporangium being the product of fusion of haploid gametes (1N aplanospores) to produce a diploid 2N zygote, which then must undergo meiosis (sexual) to produce haploid nuclei, and perhaps subsequent cycles of mitosis to produce additional haploid gametes.

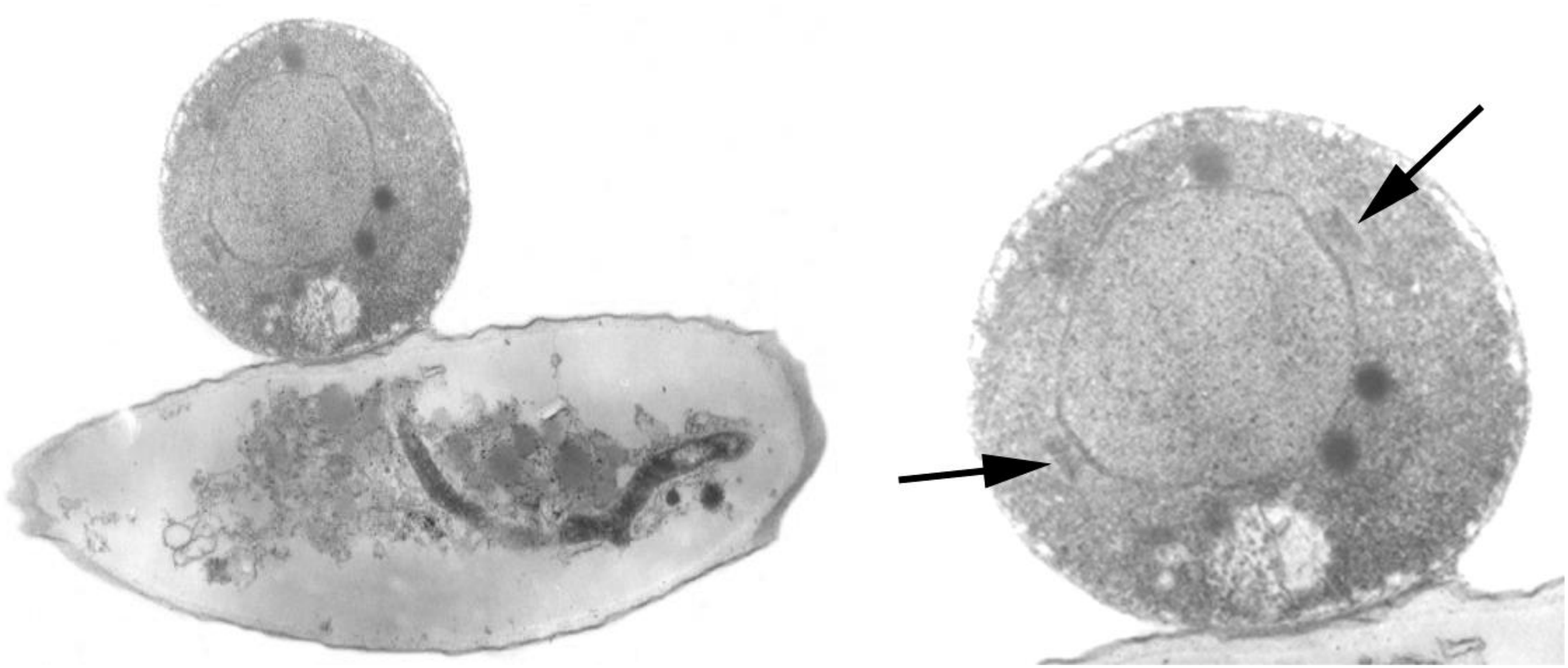
Day 3: aplanospore encystment



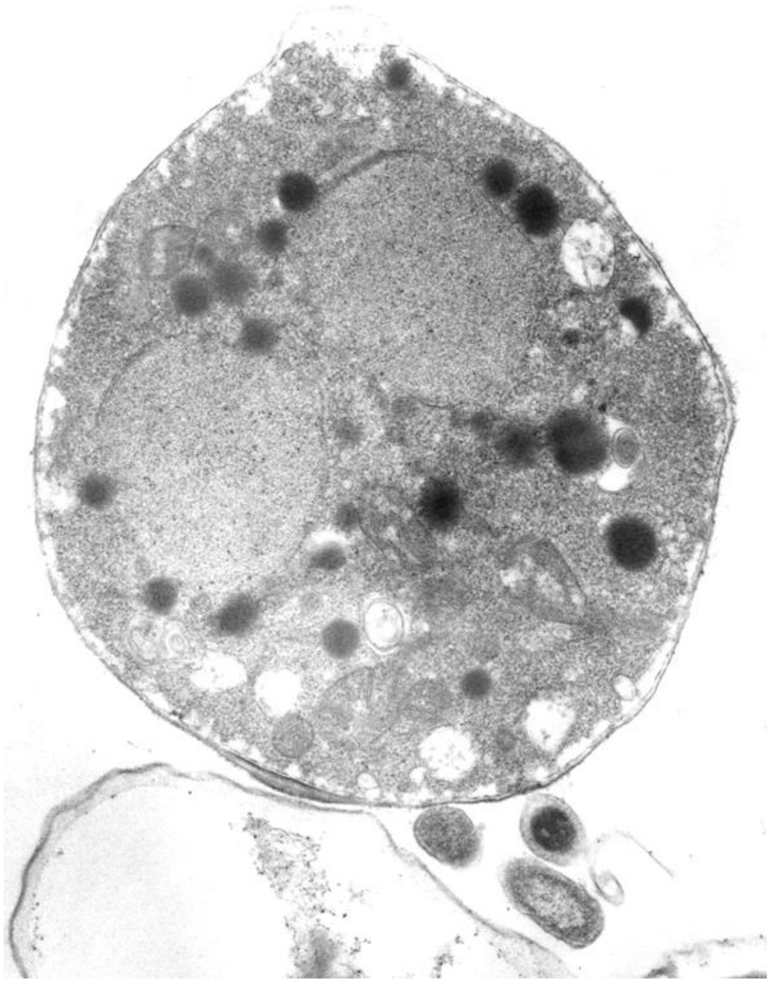
Day 3: multiple infections are frequent



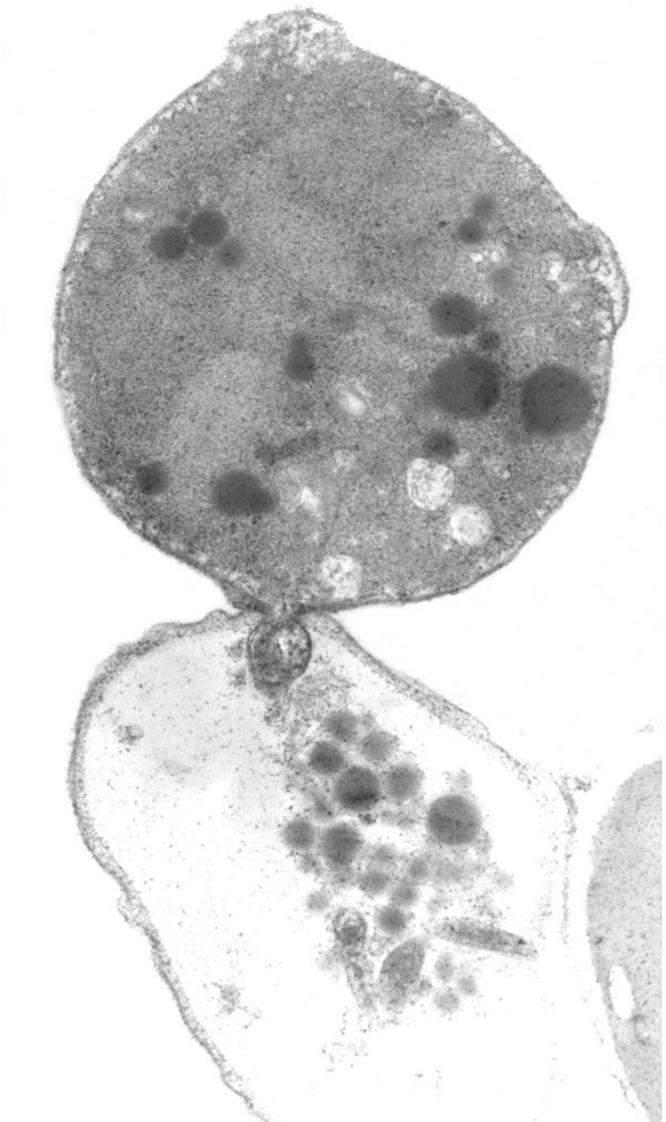
Day 3: vegetative sporangium: primary nucleus with centrioles (arrows), mitosis prophase

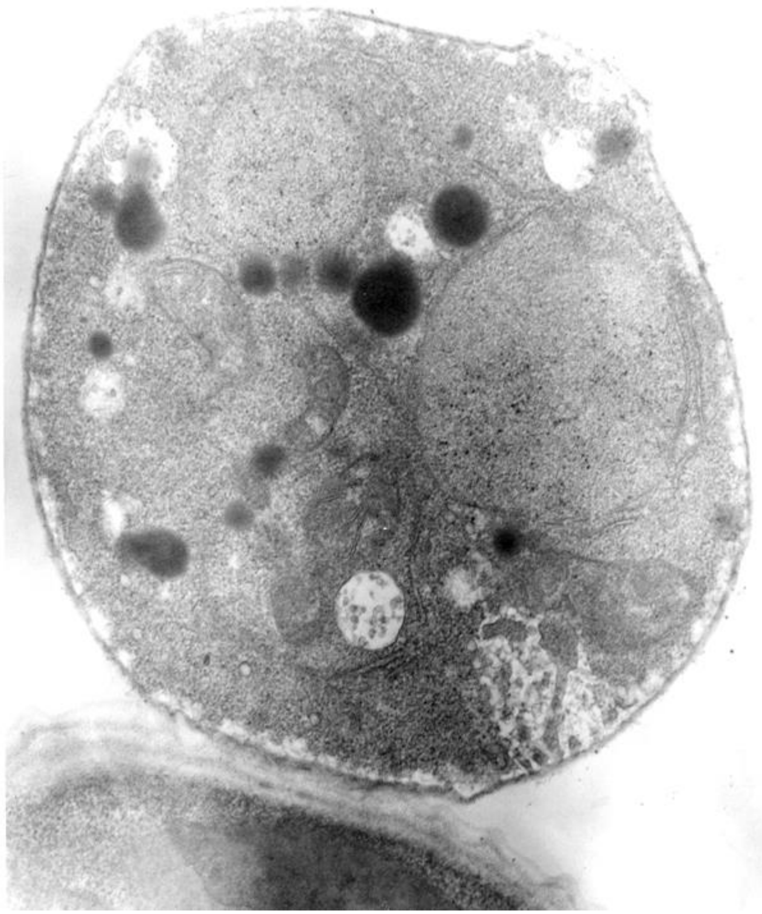


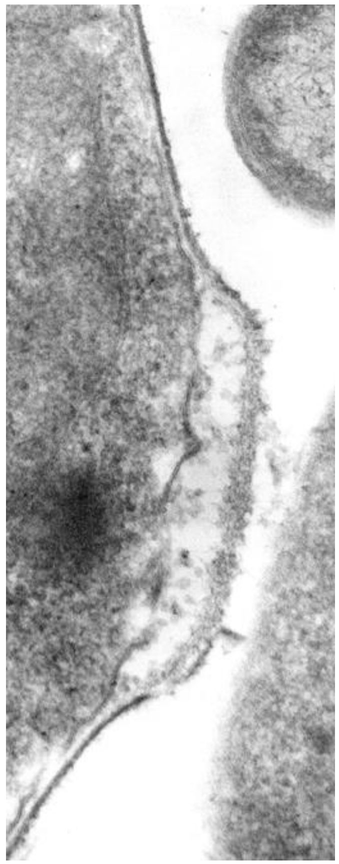
Day 3: vegetative sporangia mitosis, evidenced by multiple nuclei



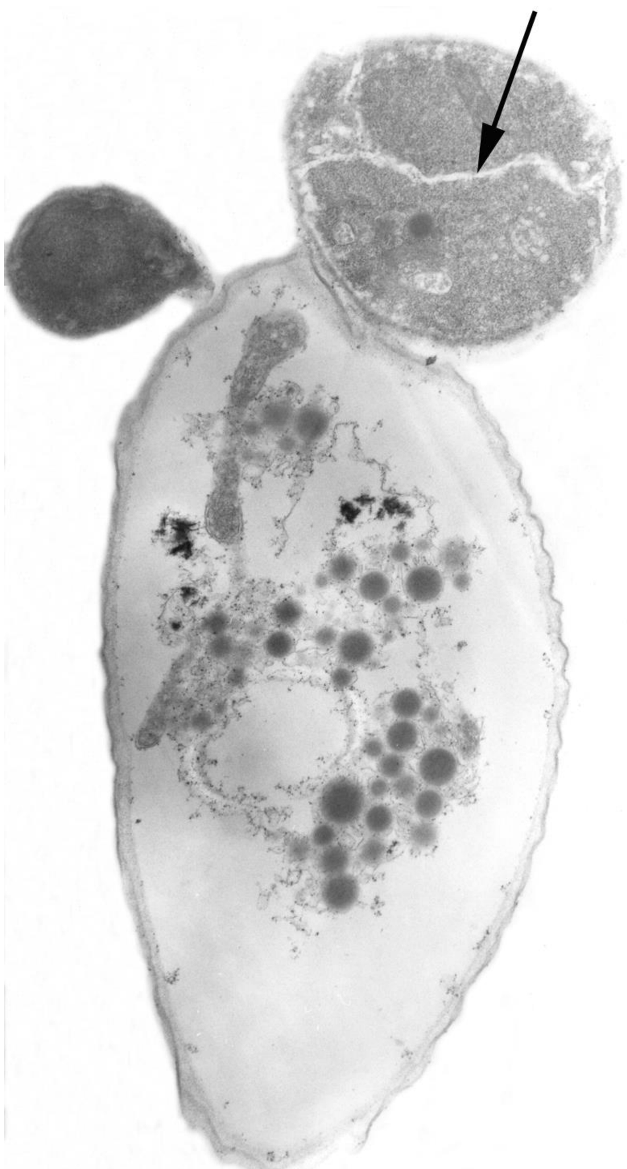
Day 3: vegetative sporangia: discharge pores forming

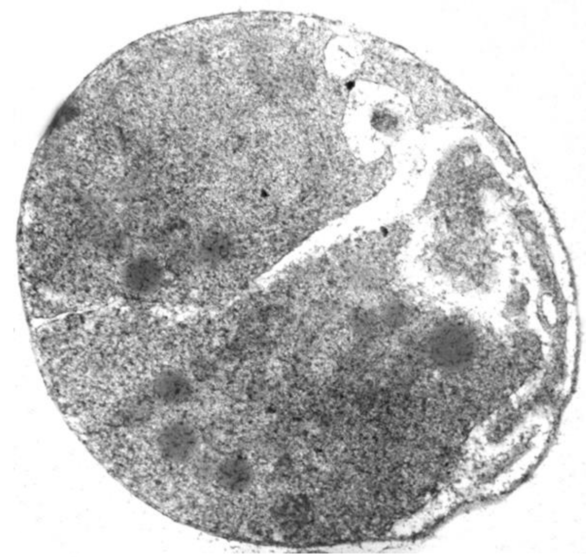


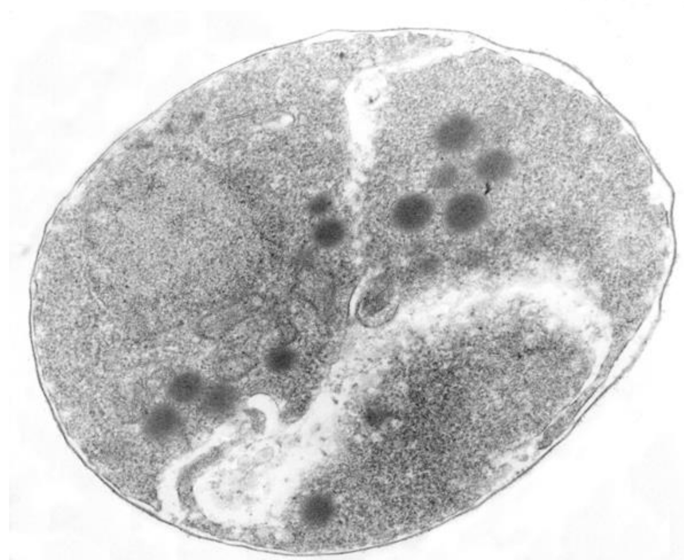




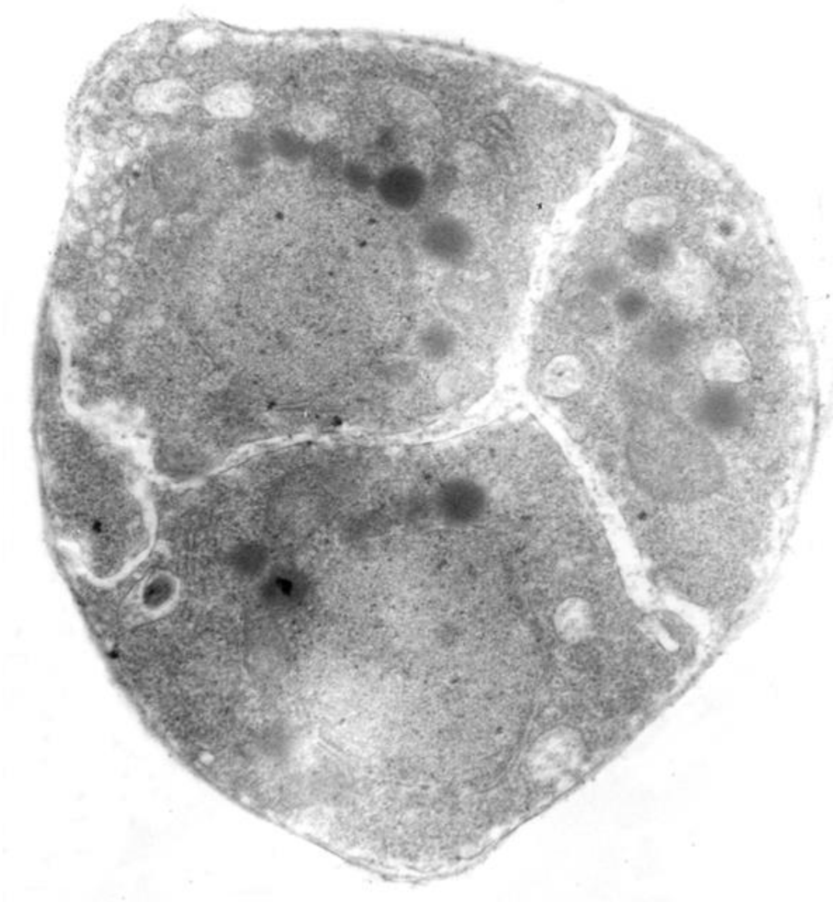
Day 3: vegetative sporangia: aplanospore cleavage, evidenced by cleavage furrows

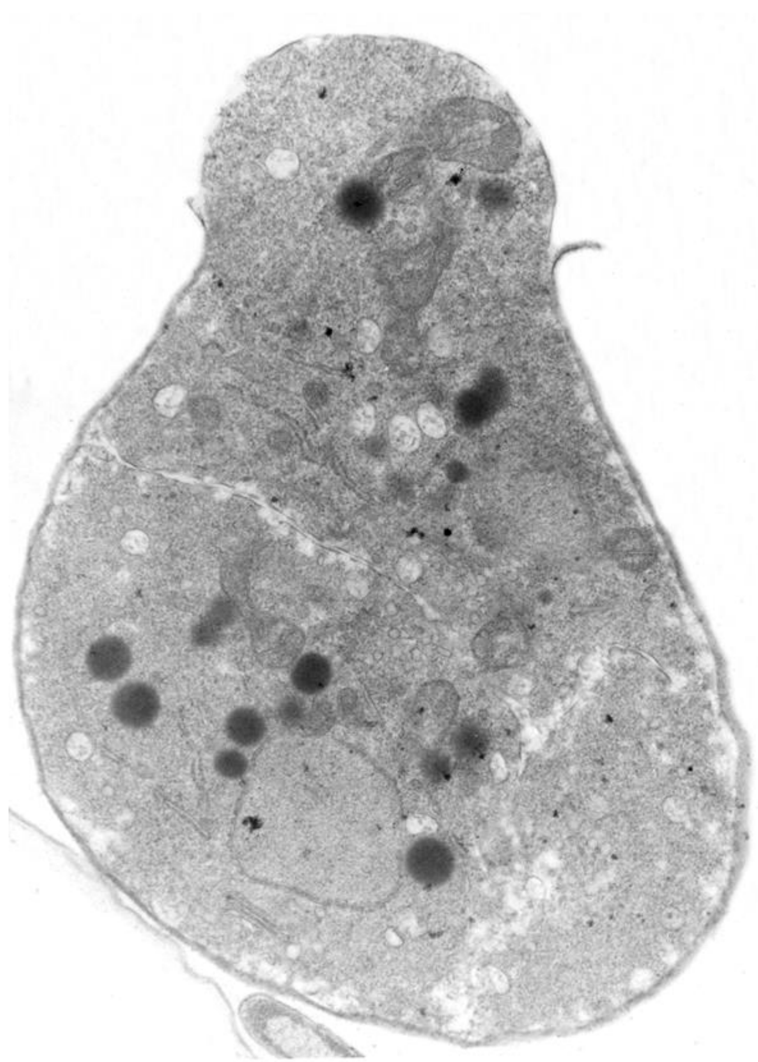




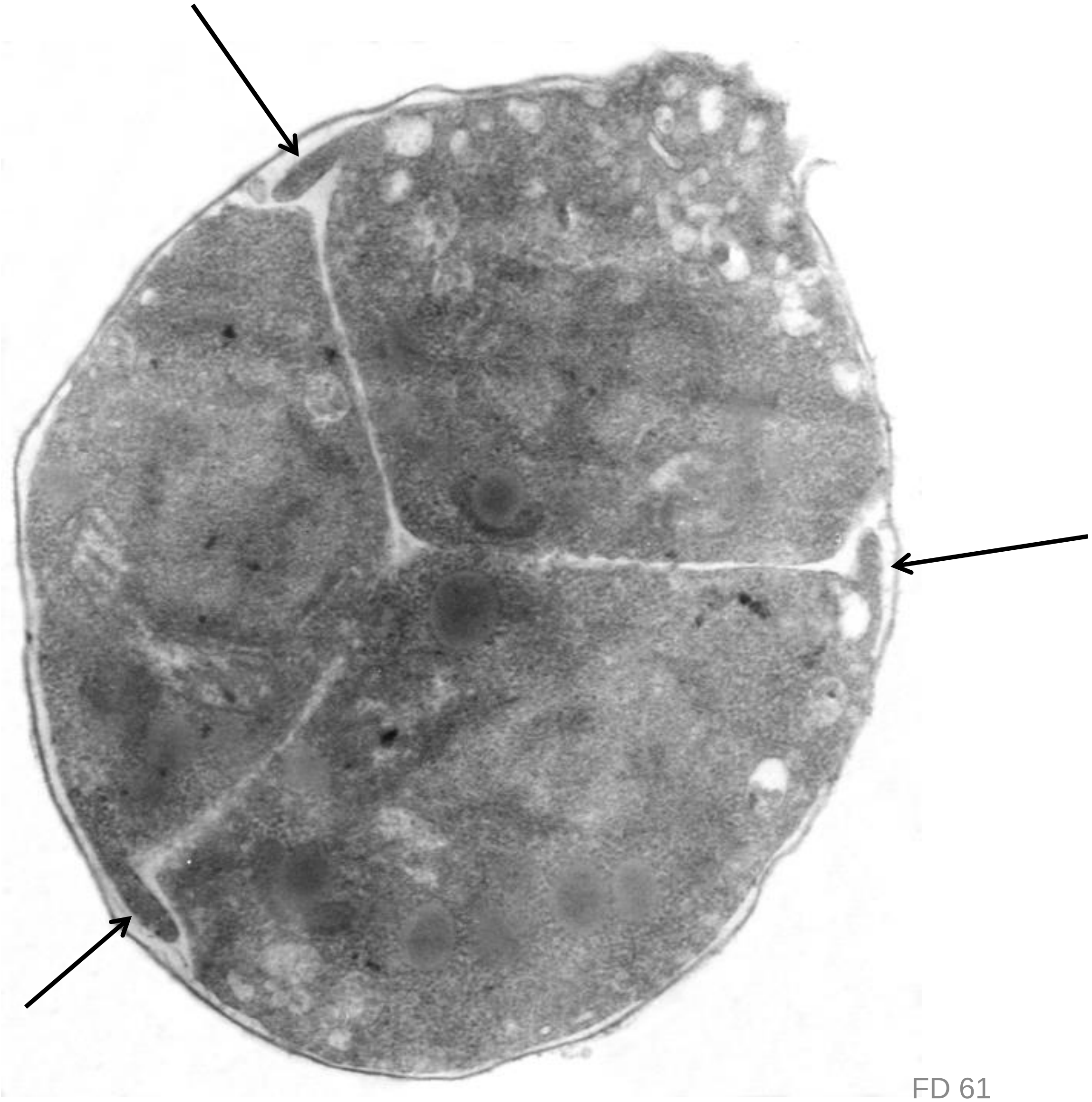


Day 3: aplanospore cleavage (L) and early discharge (R)

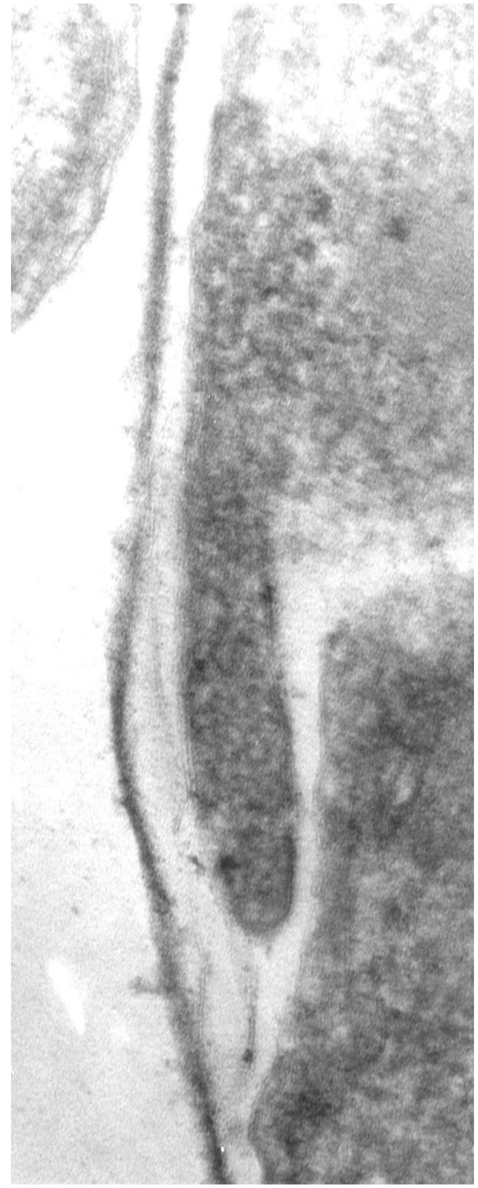




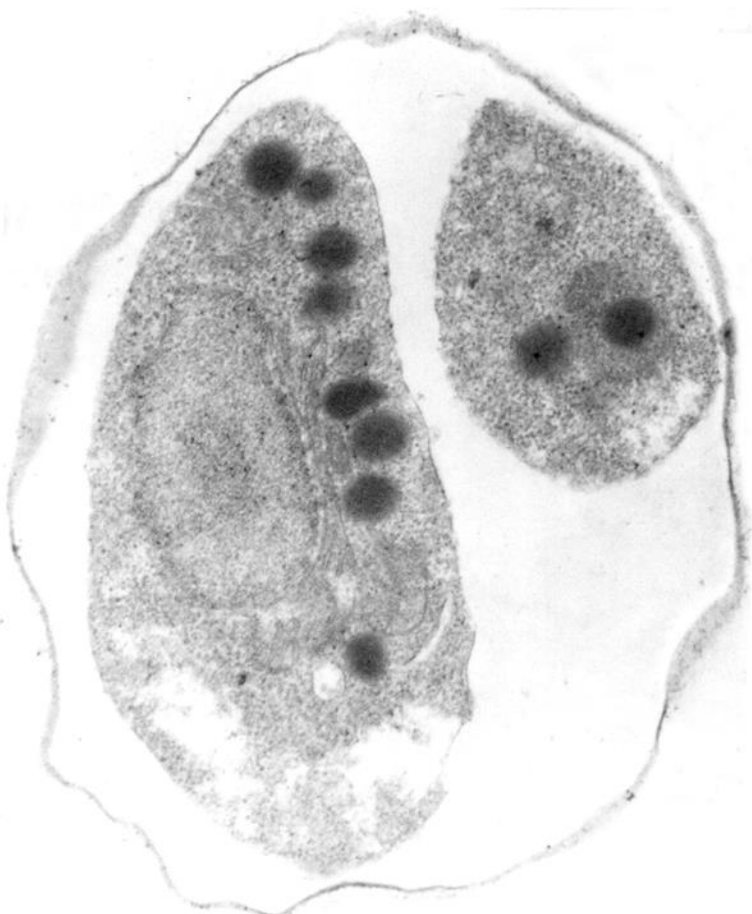
Day 3: aplanospore cleavage, aplanospores with pseudopodia (no microtubules)



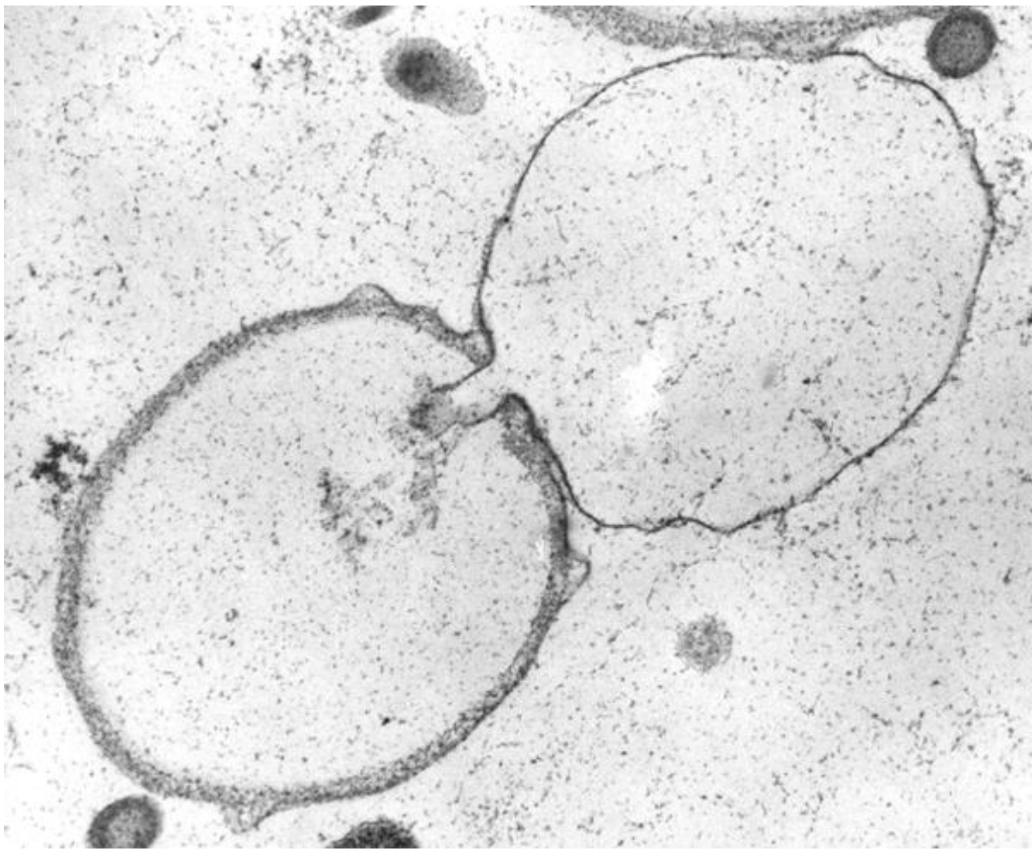
FD 61

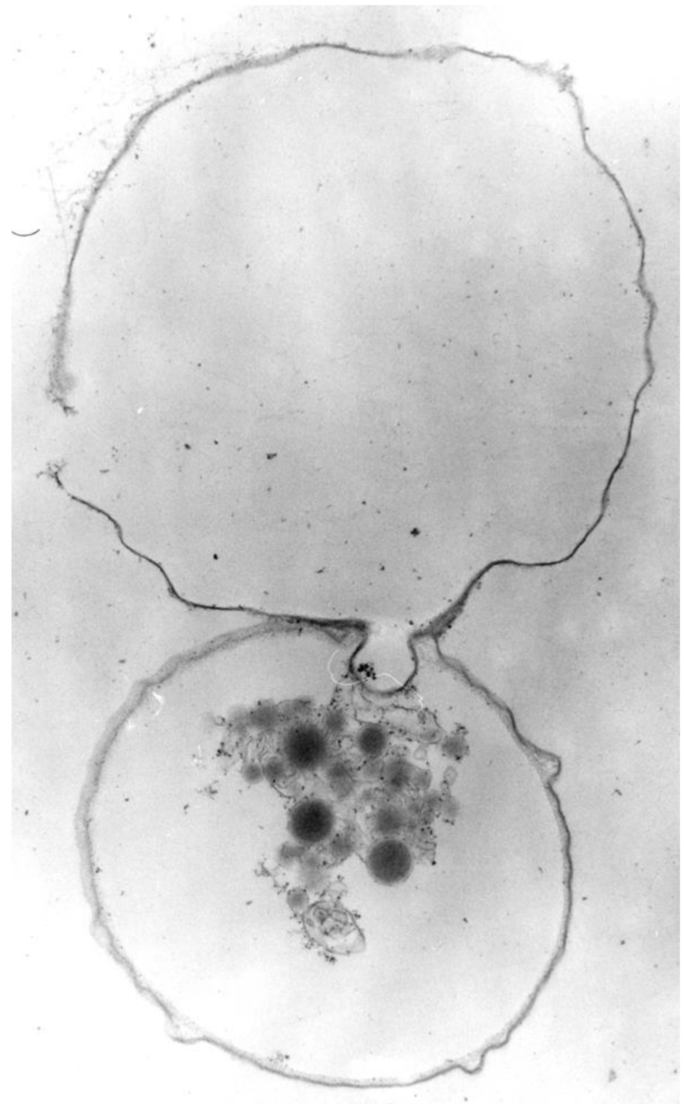


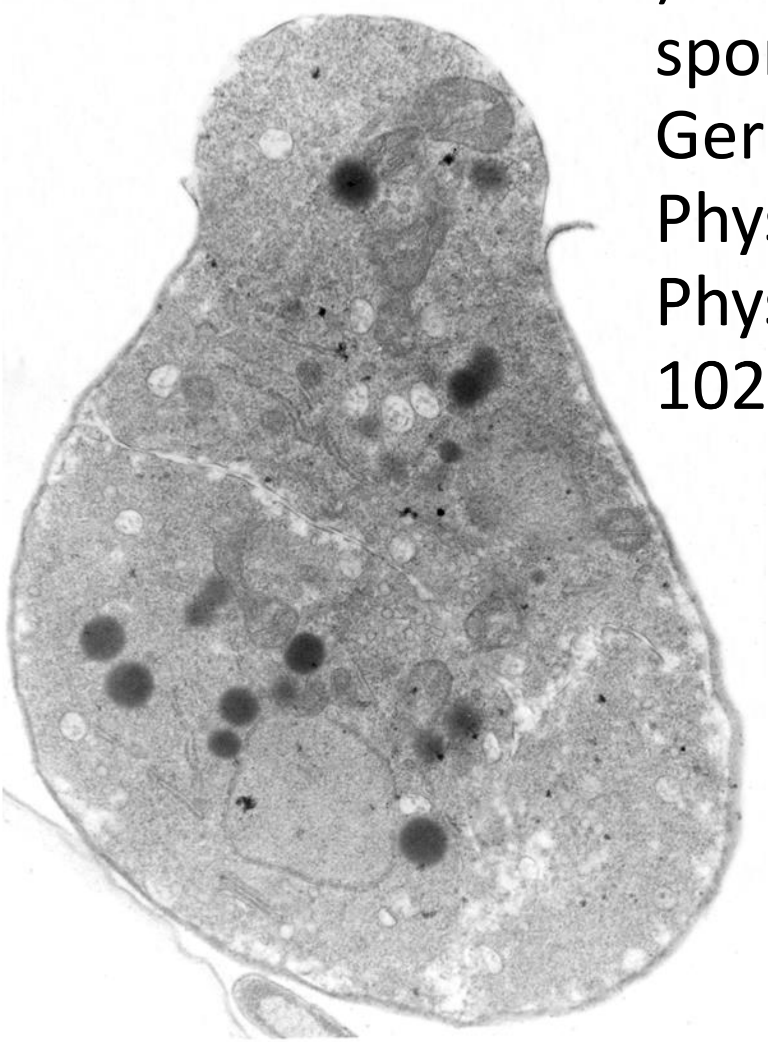
Day 3: fully formed aplanospores inside vegetative sporangium (L), and release (R)



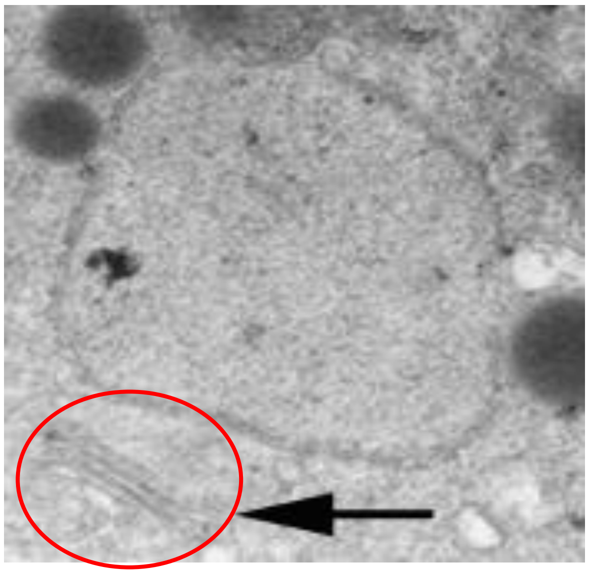
Day 4: vegetative (thin-walled) sporangia, empty



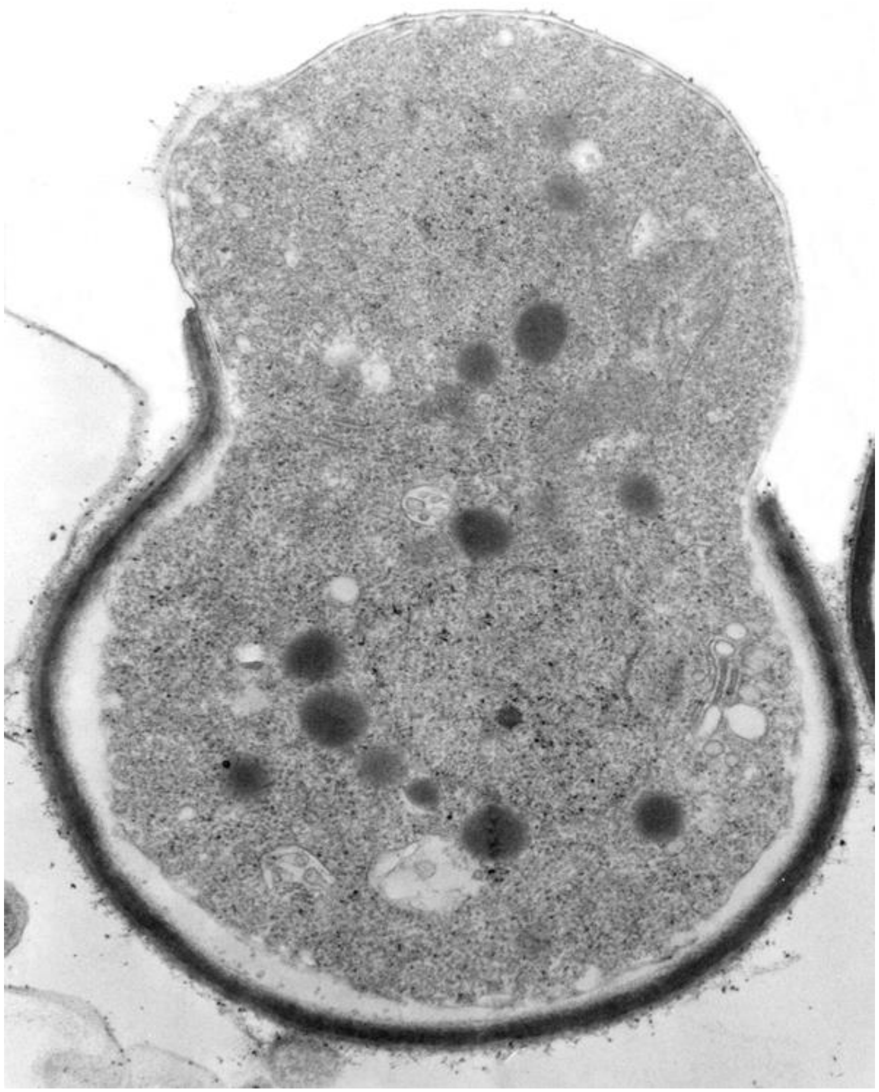


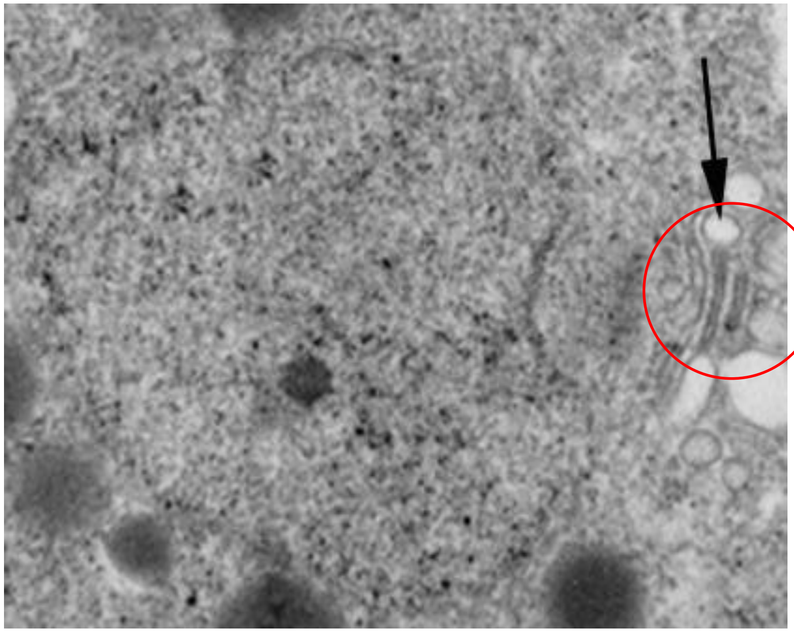


This is fascinating: the Blastocladiomycota (with the exception of Physoderma*) do not have Golgi; FD61 (AKA Paraphysoderma, and sister to Physoderma) has a Golgi (here in a vegetative sporangium): *Lange L, Olson LW. 1980. Germination of the resting spore of Physoderma maydis, the causal agent of Physoderma disease of maize. Protoplasma 102:323-342.

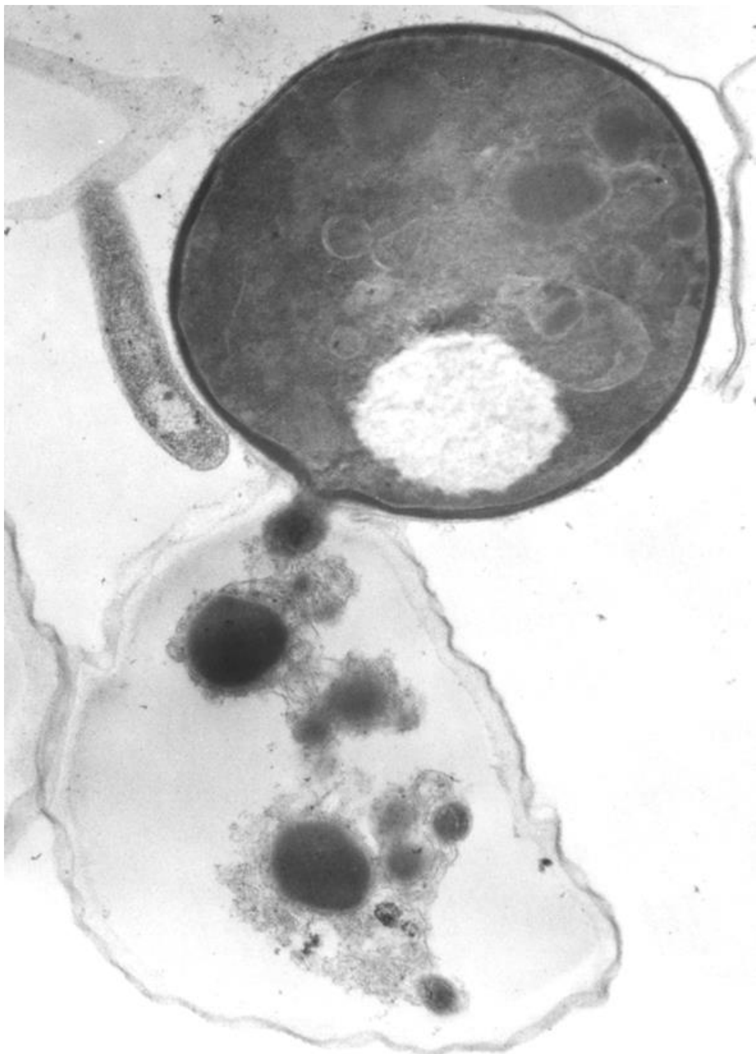


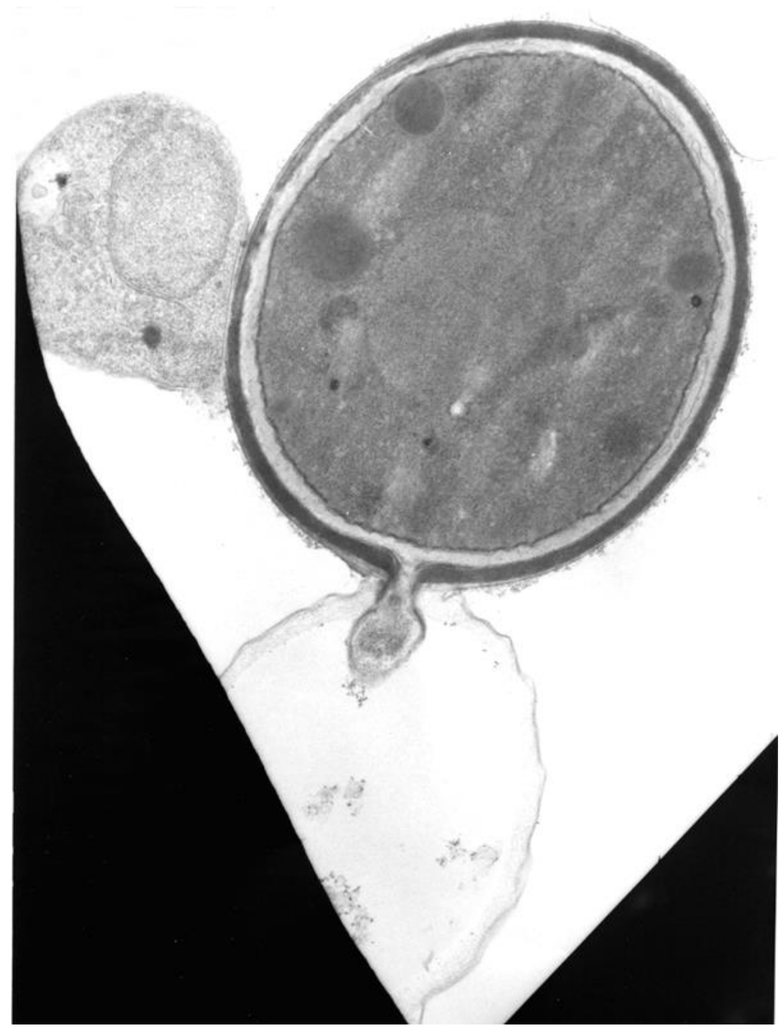
Seen not just once, but repeatedly (here in a thick-walled resting sporangium):



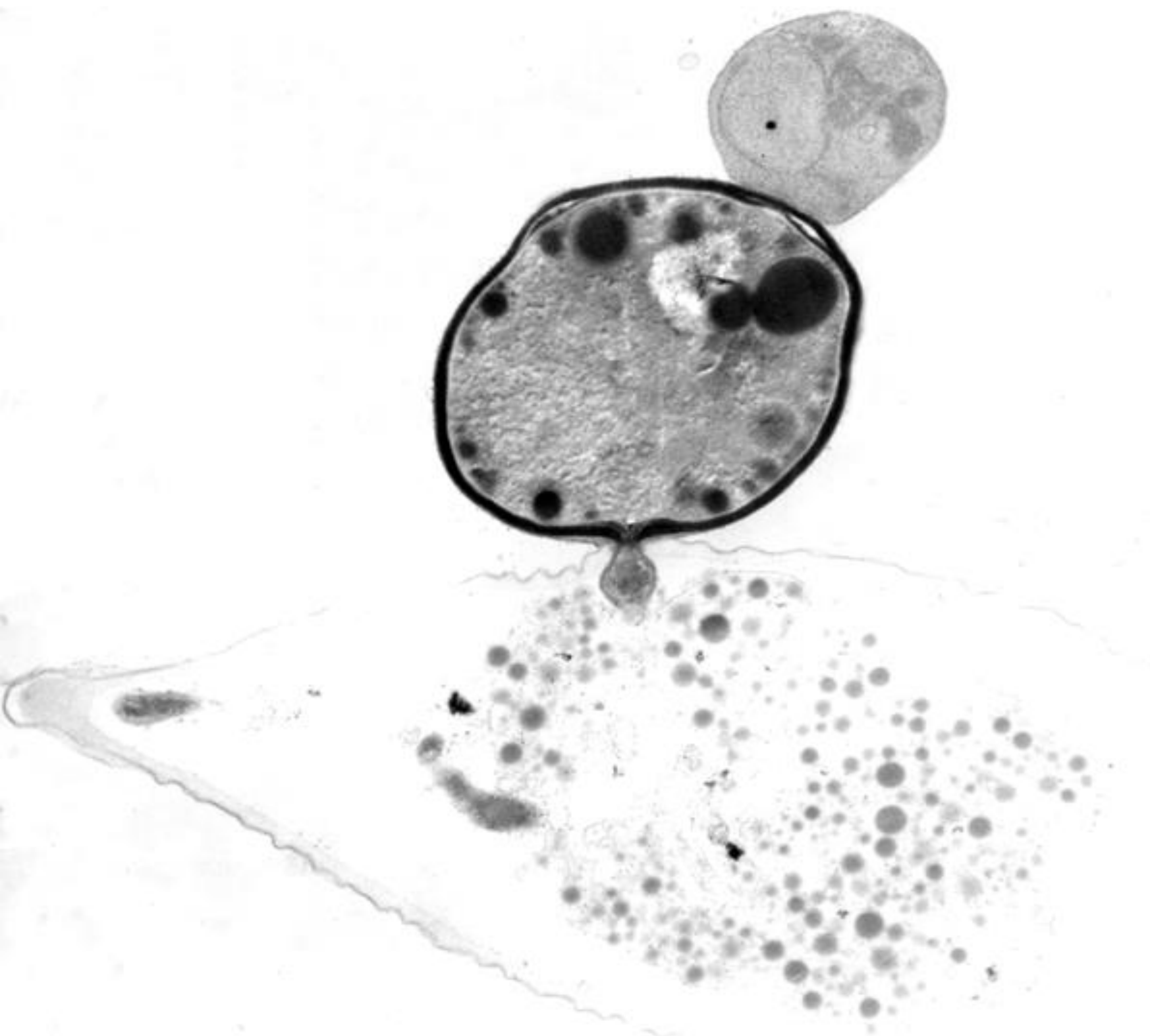


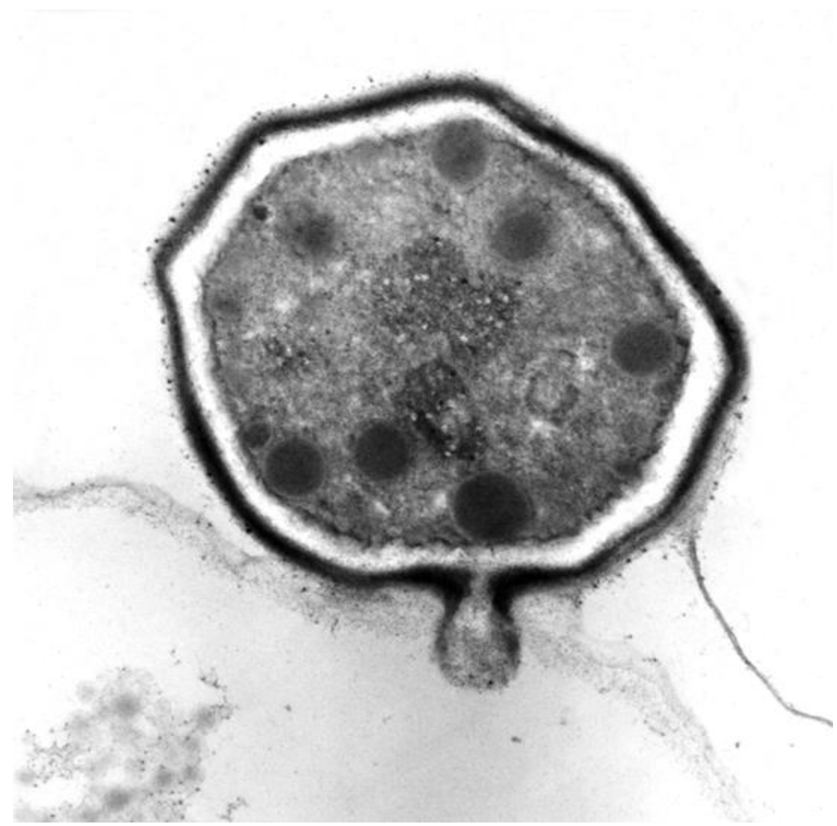
Day 3: thick-walled resting sporangia



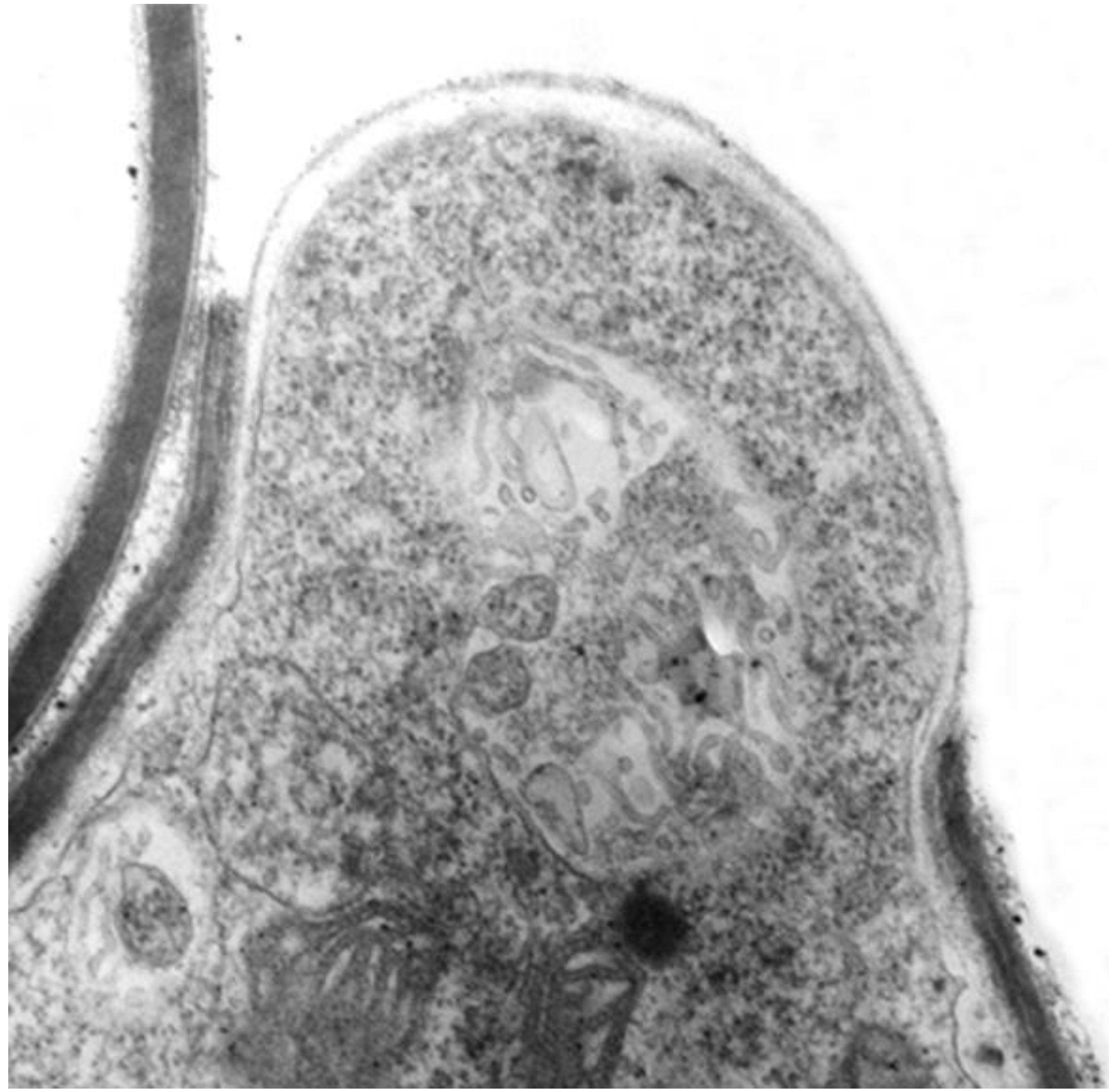


Day 4: resting sporangia; on left, hyper-parasitized by aplanospore

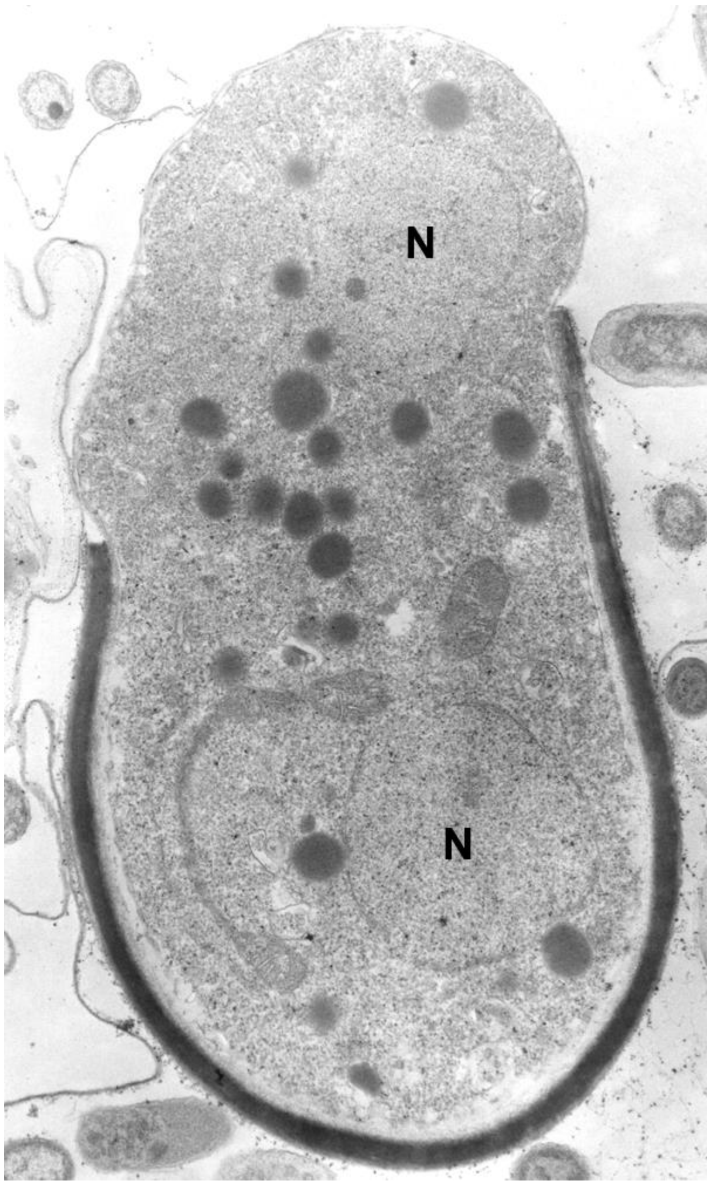




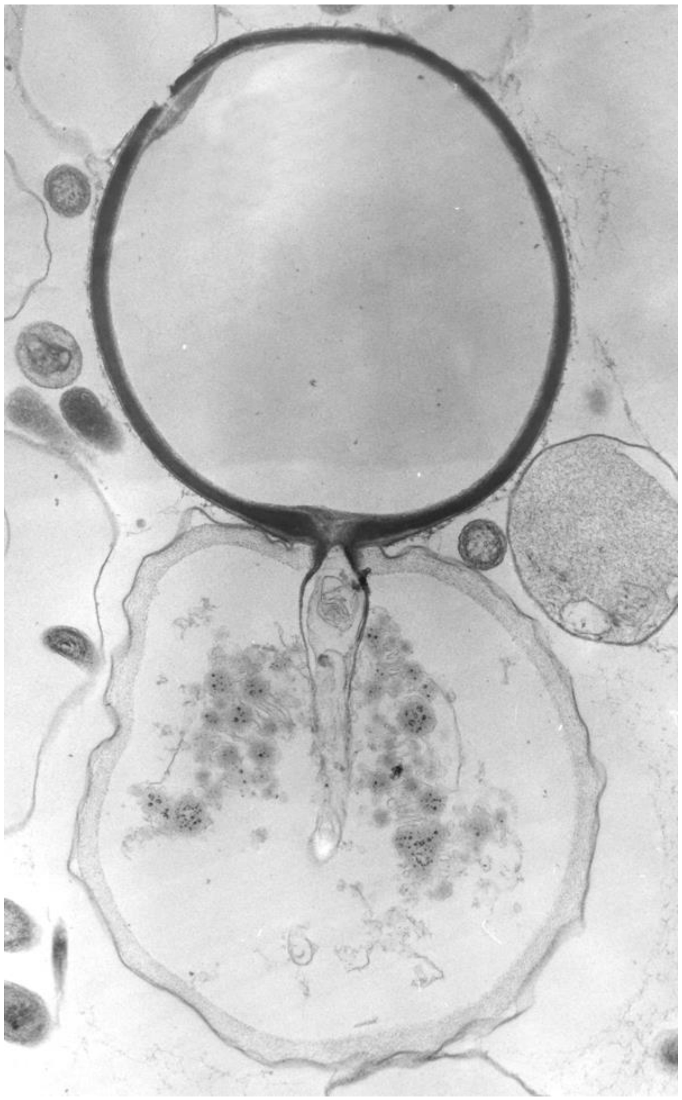
Day 4:resting sporangium extruding (walled?) protoplasm

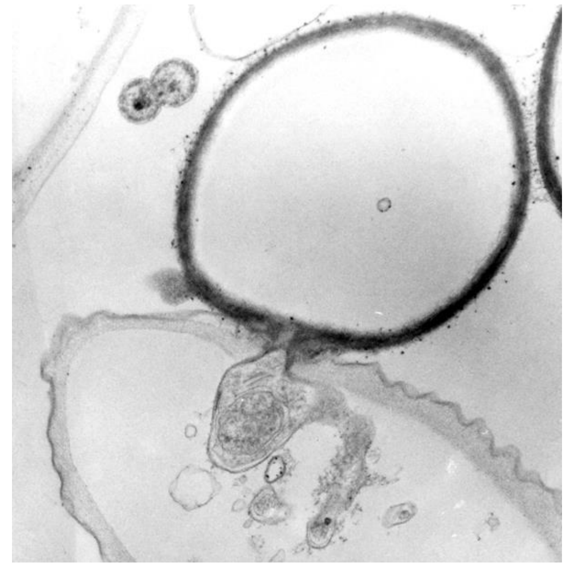


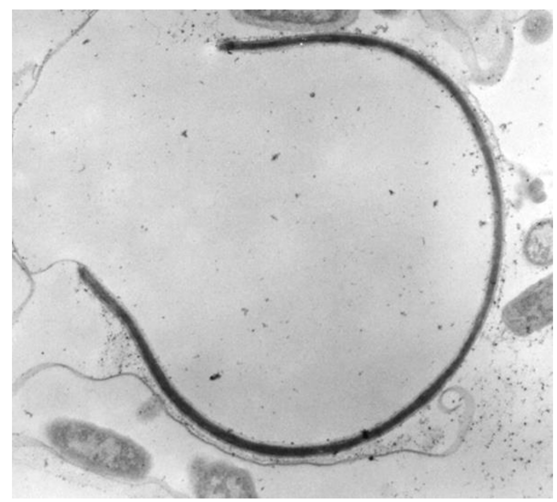
Day 4: resting sporangium multi-nucleate (L) and cleaved aplanospores (R)



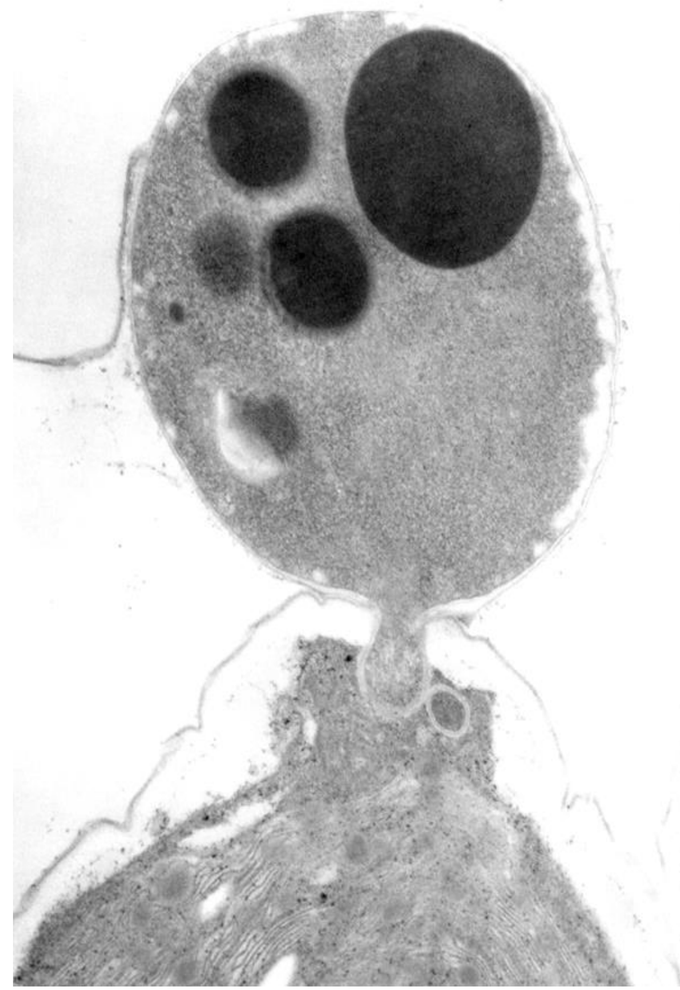
Day 4: empty resting sporangia, attached and disarticulated (lower right corner)







Day 5: life cycle completed; abundant 2nd generation motile aplanospores, docking aplanospores, and encysting aplanospores



FD 61