# TEM analysis of FD 105, a potential new species of *Amoeboaphelidium*

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# FD 105

FD 105 is a potential new species in the genus *Amoeboaphelidium*, the same genus as FD 01 *A. protococcarum* and FD 95 *A. occidentale* n. sp.

Morphological divergence:

smaller aplanospores

aplanospore cyst is not persistent

Molecular divergence: need sequences for comparison

Ultrastructural divergence:

no ER backing the nucleus

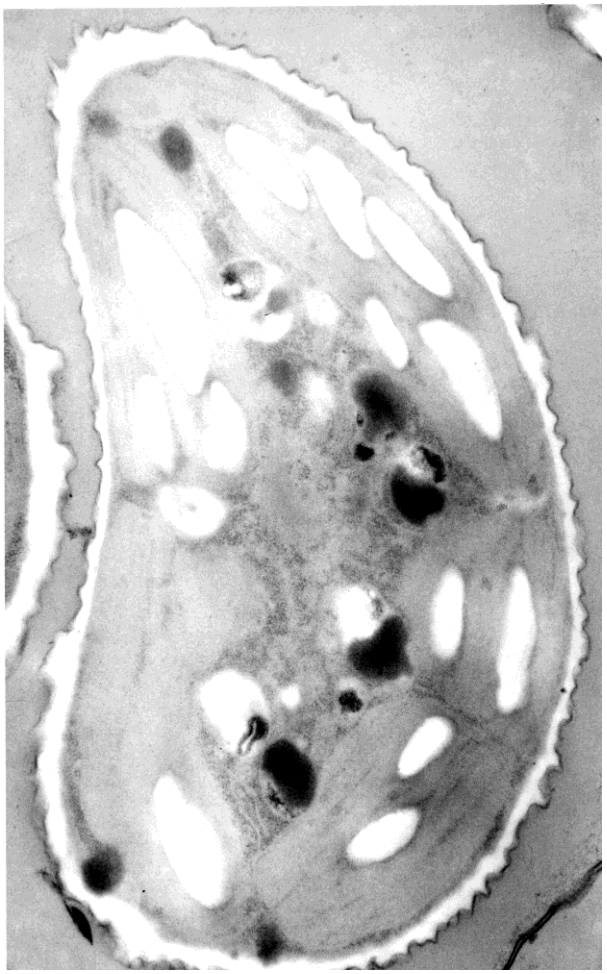
# The algal cell

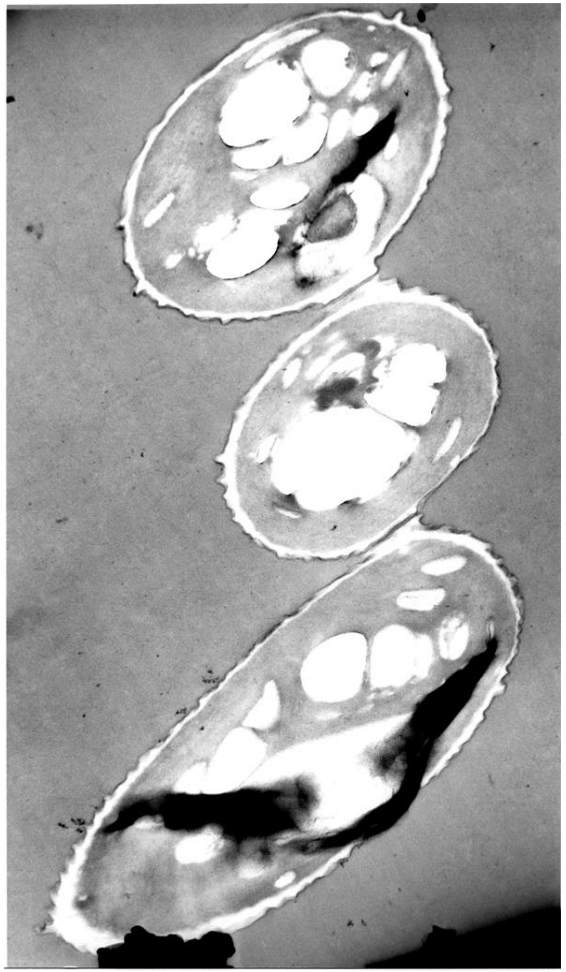
The next slide is composed of three images of uninfected algal cells (L- x15,000; C- x8,000; R- x3000).

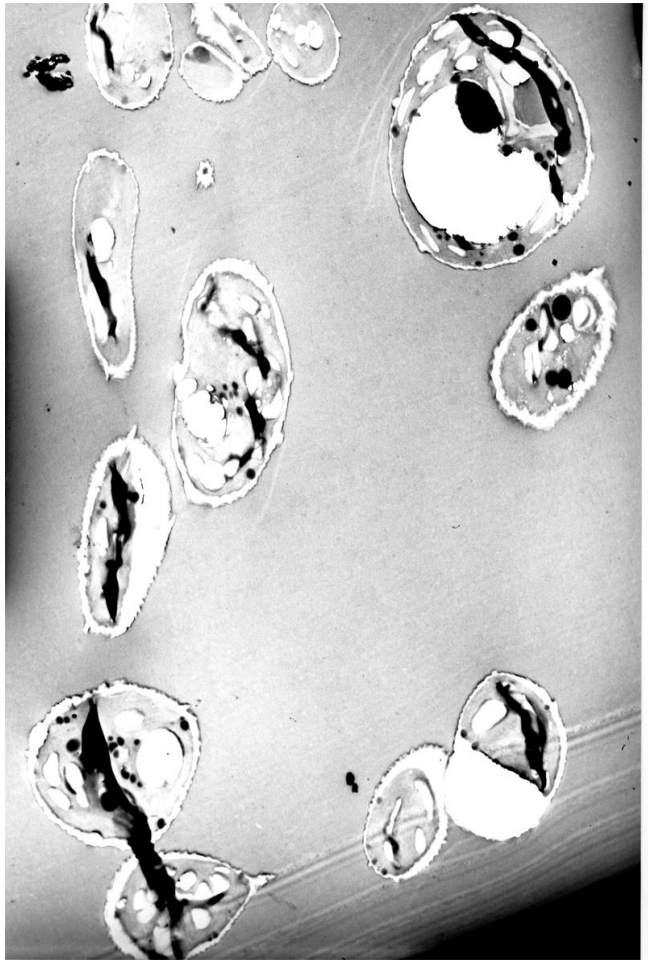
There is no evidence of hypertrophy of the algal cell wall in any of the cells here.

However, TEM images are two-dimensional, and hypertrophy of the cell wall may be present in another plane.

# Day 0- healthy algal cells- no





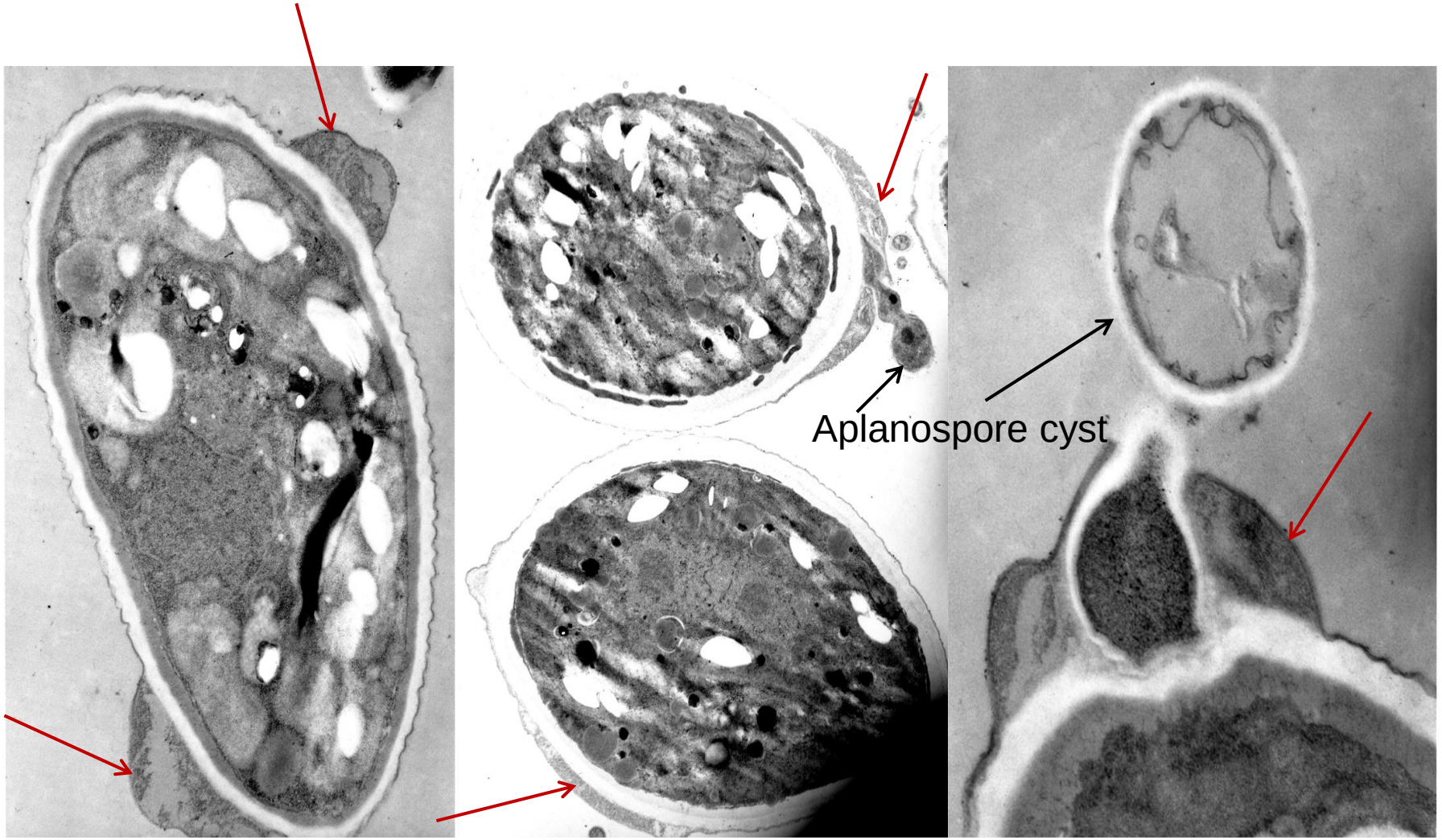


# Hypertrophy of the cell wall at the point of infection

Hypertrophy of the algal cell wall at the site of infection by FD 105 is readily apparent (arrows on images in the next slide).

Whether this represents an algal reaction to the infection, or whether the parasite attacks a specific portion of the wall that is normally thickened in healthy algal cells is unresolved by TEM.

# Hypertrophy (red arrows) of the cell wall at the point of infection by the aplanospore

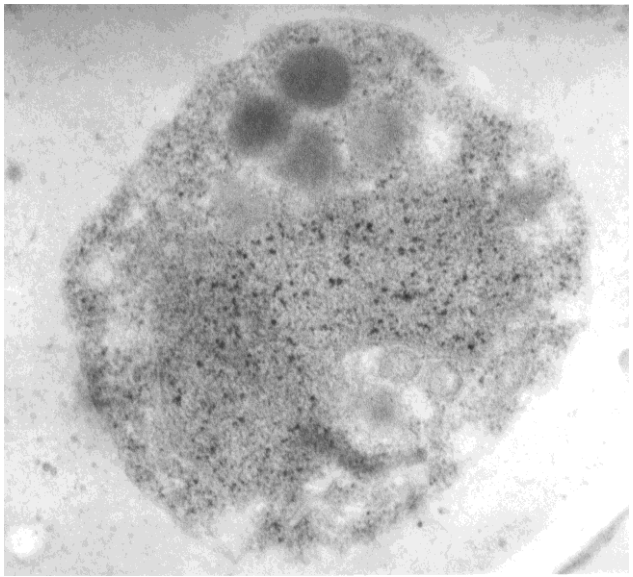


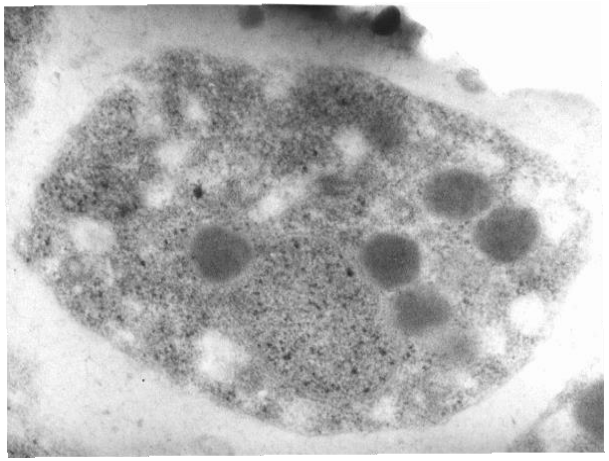
# Host wall hypertrophy

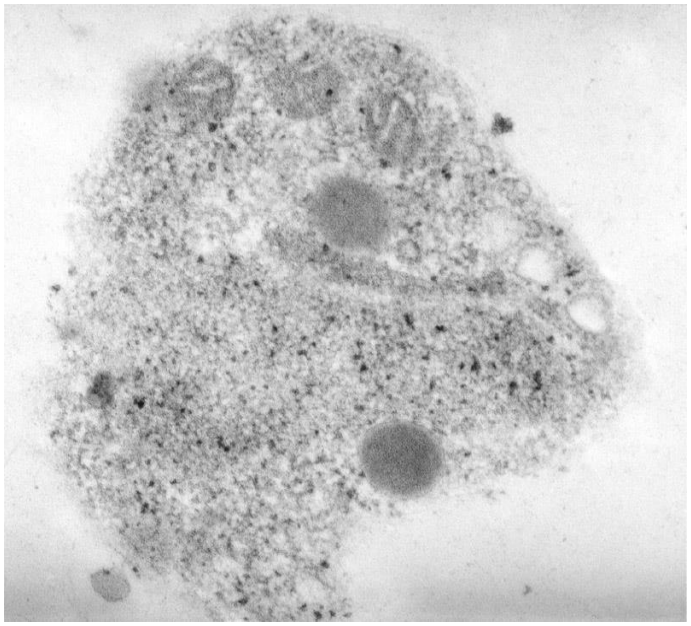
The difference in the cell wall of a healthy algal cell and an infected algal cell is readily apparent from multiple and numerous TEM observations.

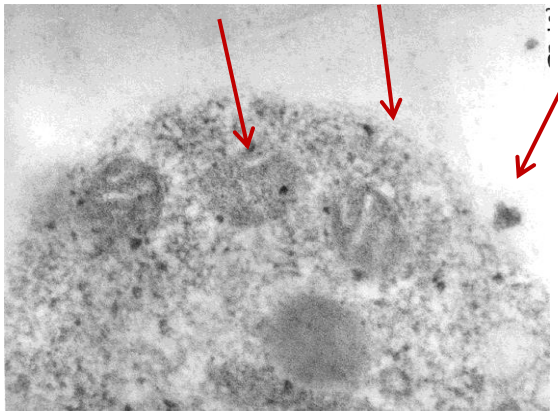
It is my opinion that the hypertrophy is a host response to the parasite infection and is a response that I have not seen with FD 01 *A. protococcarum* or FD 95 *A. occidentale* n. sp., which are parasites on *Scenedesmus dimorphus*.

# Day 1: FD 105 aplanospores



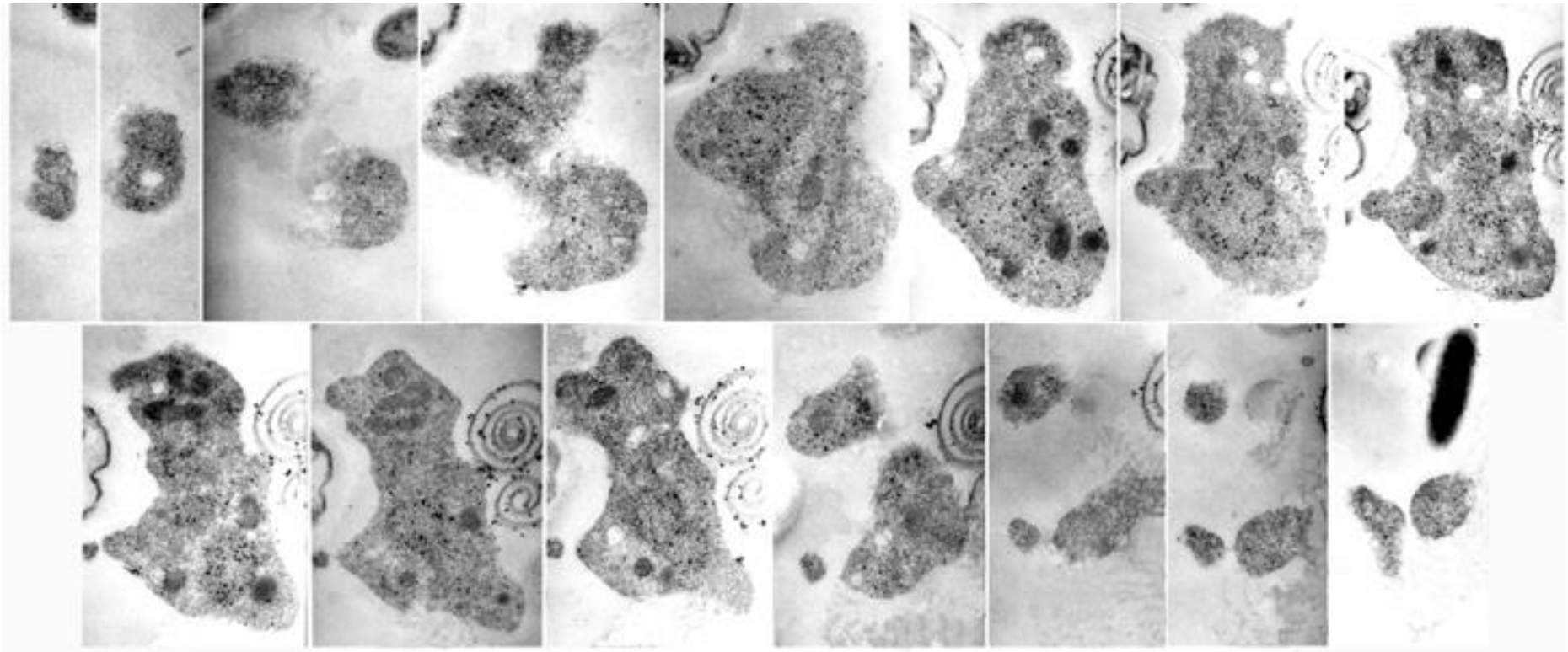






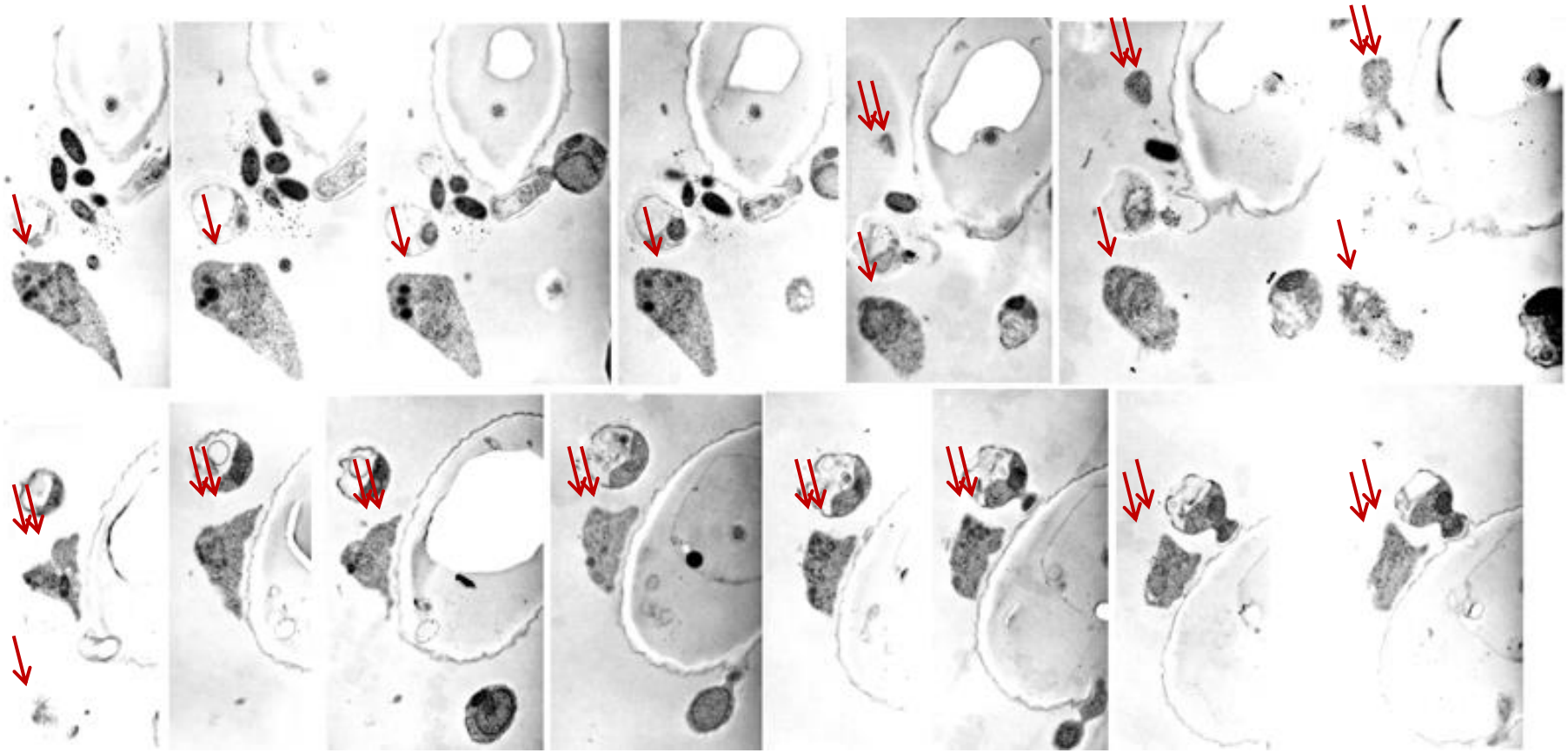
Mitochondria with flat cristae (arrows)

# Aplanospore serial sections (1)



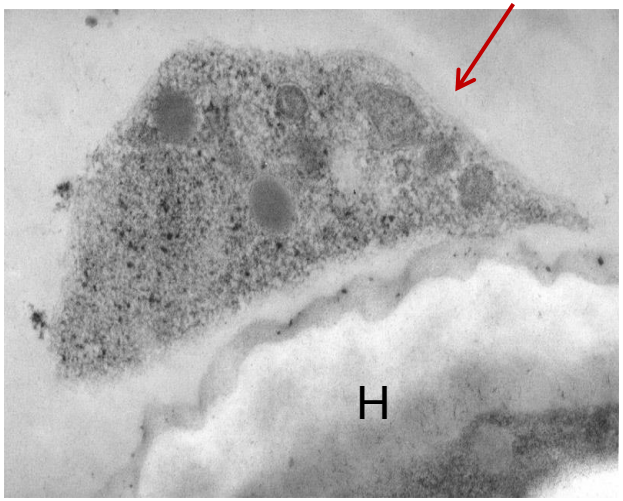
15 serial sections through a single aplanospore; each section 100 nm thickness; aplanospore ~1.5 microns

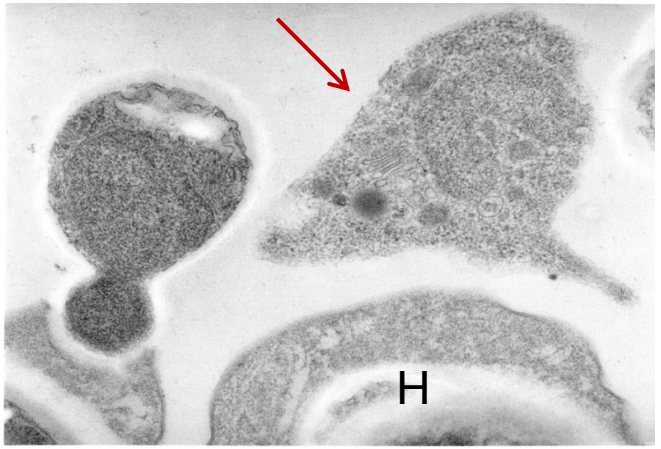
# Aplanospore serial sections (2)



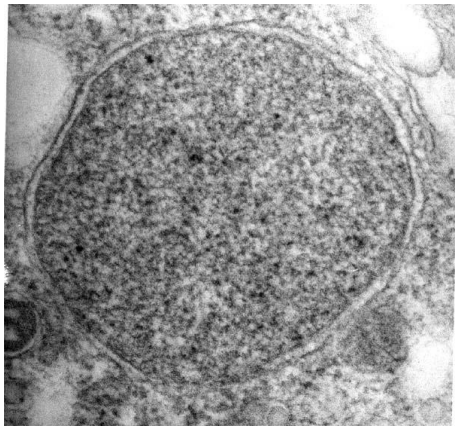
Sections through 2 aplanospores (single and double red arrows)

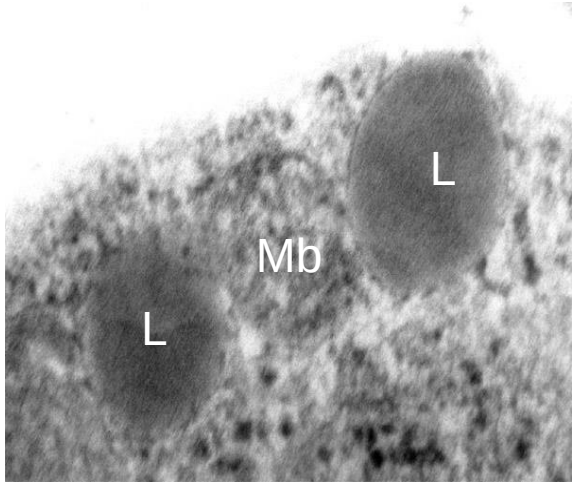
# Day 2: FD 105 pseudopodiate aplanospores (arrows) “docking” with algal cells (H)

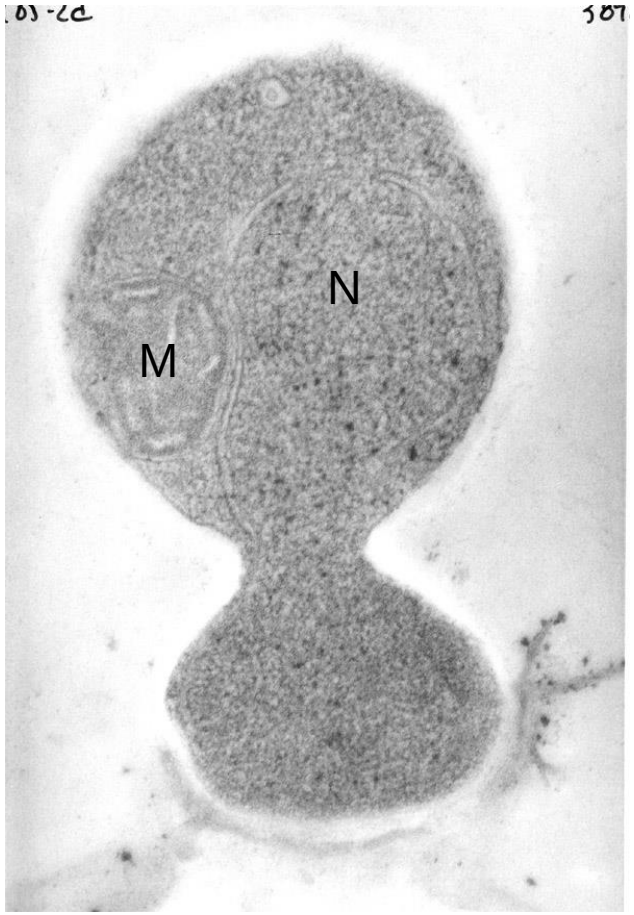

Nucleus






Lipid globules (L) and a microbody (Mb)

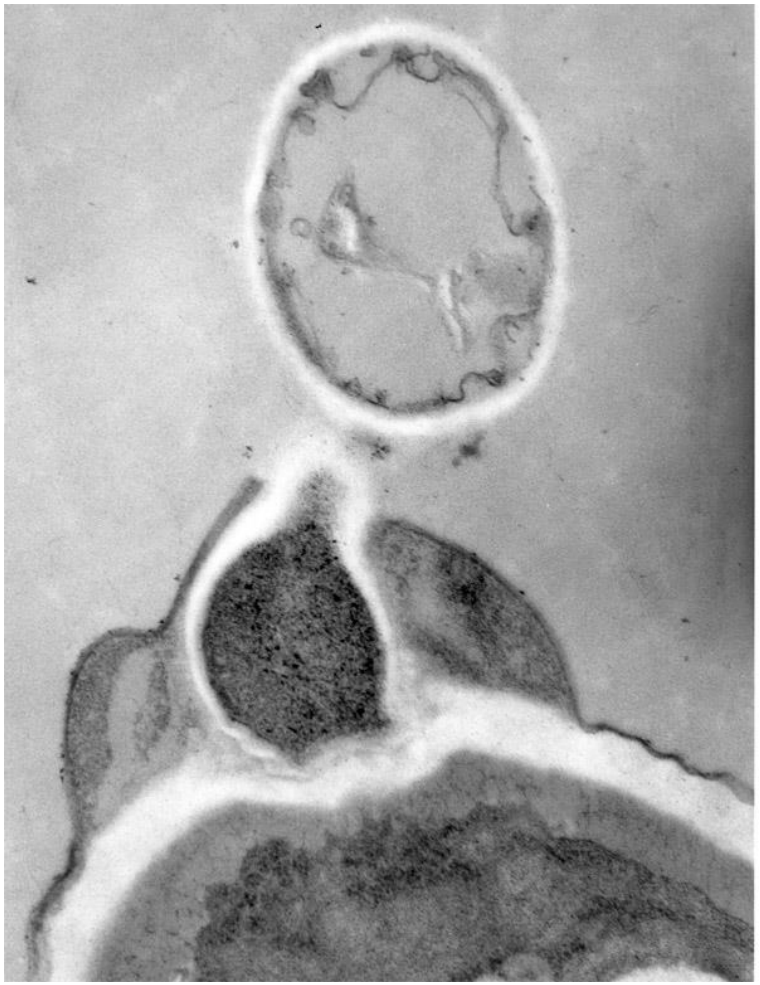
# Days 2 and #: encysted aplanospores




Parasite nucleus (arrows) being inserted into host through penetration tube

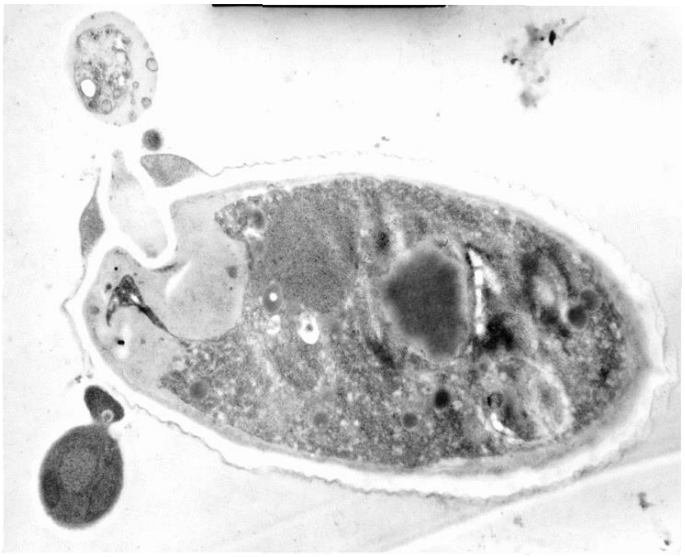
# Encysted aplanospores

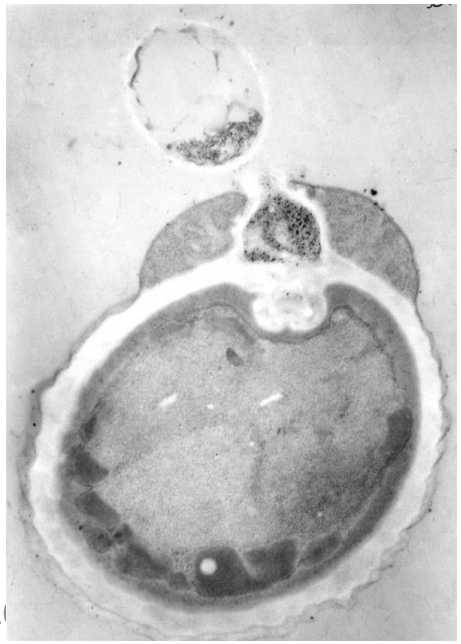
parasite nucleus going into host



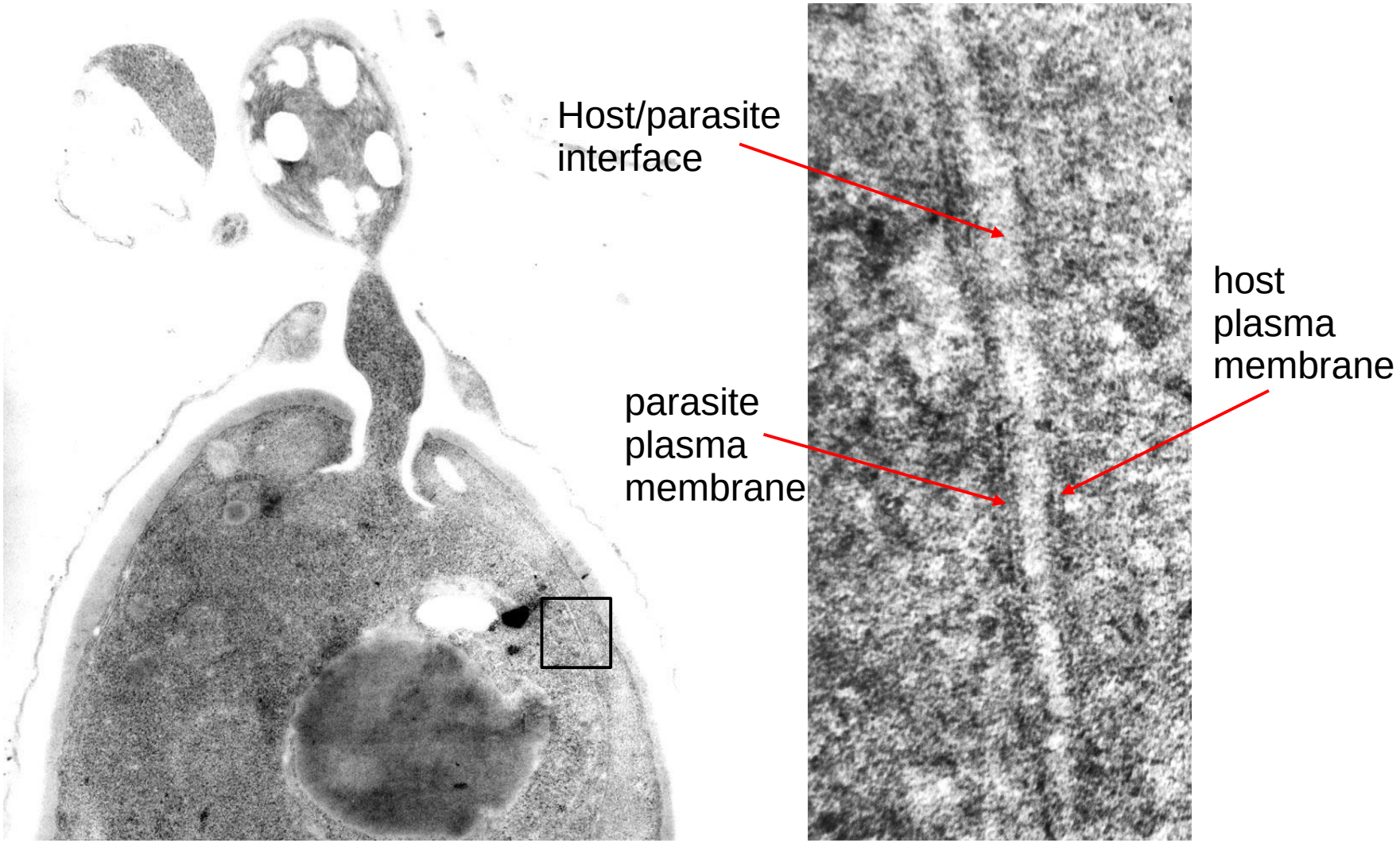
empty aplanospore cyst and host wall hypertrophy

# Day 3: infection





# Day 3: infection and host/parasite interface



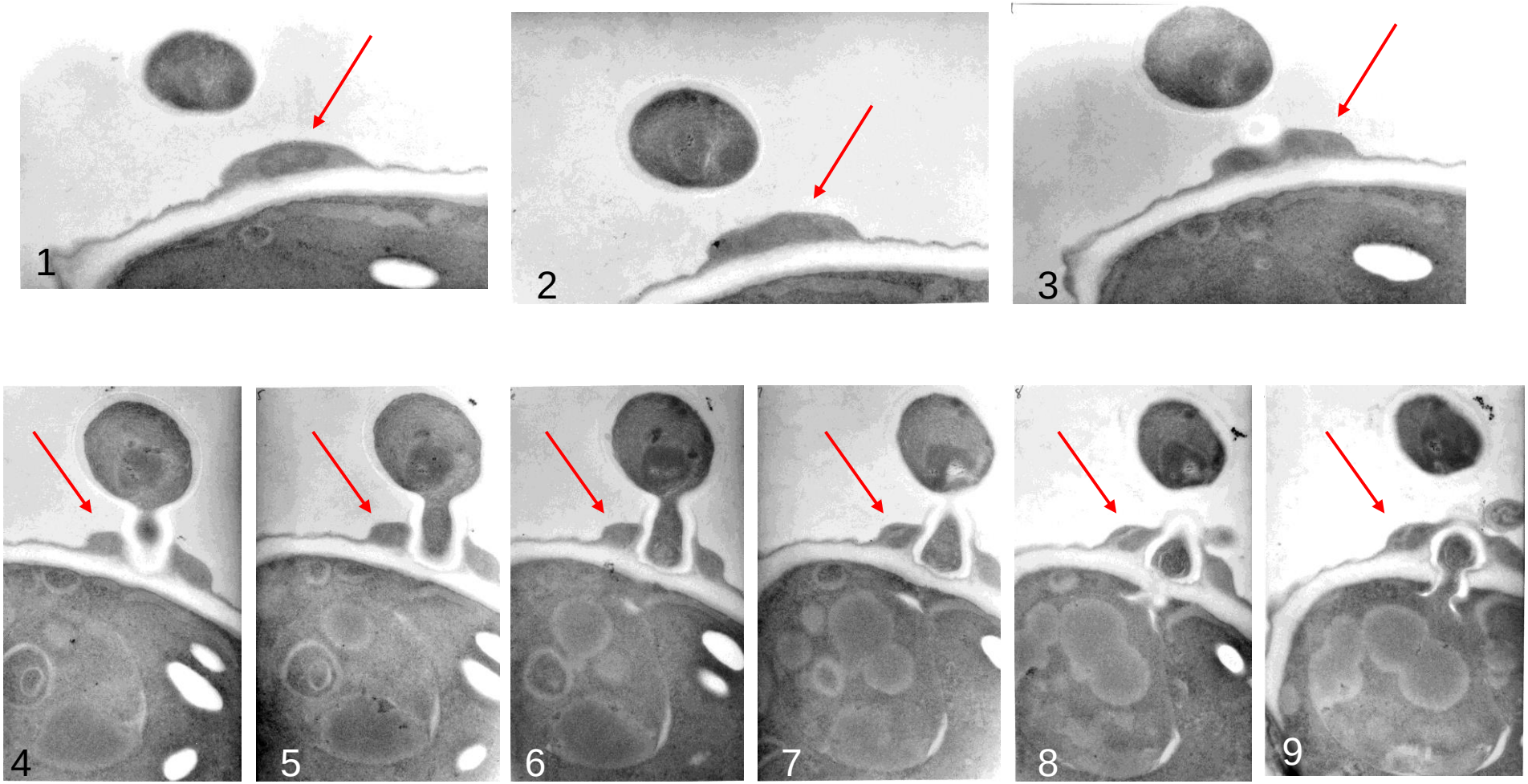
# Serial sections

- Following are 3 sets of serial sections through the longitudinal profile of the aplanospore cyst and the hypertrophied host cell wall.

- Serial sections permit 3D reconstruction from a series of 2D images.

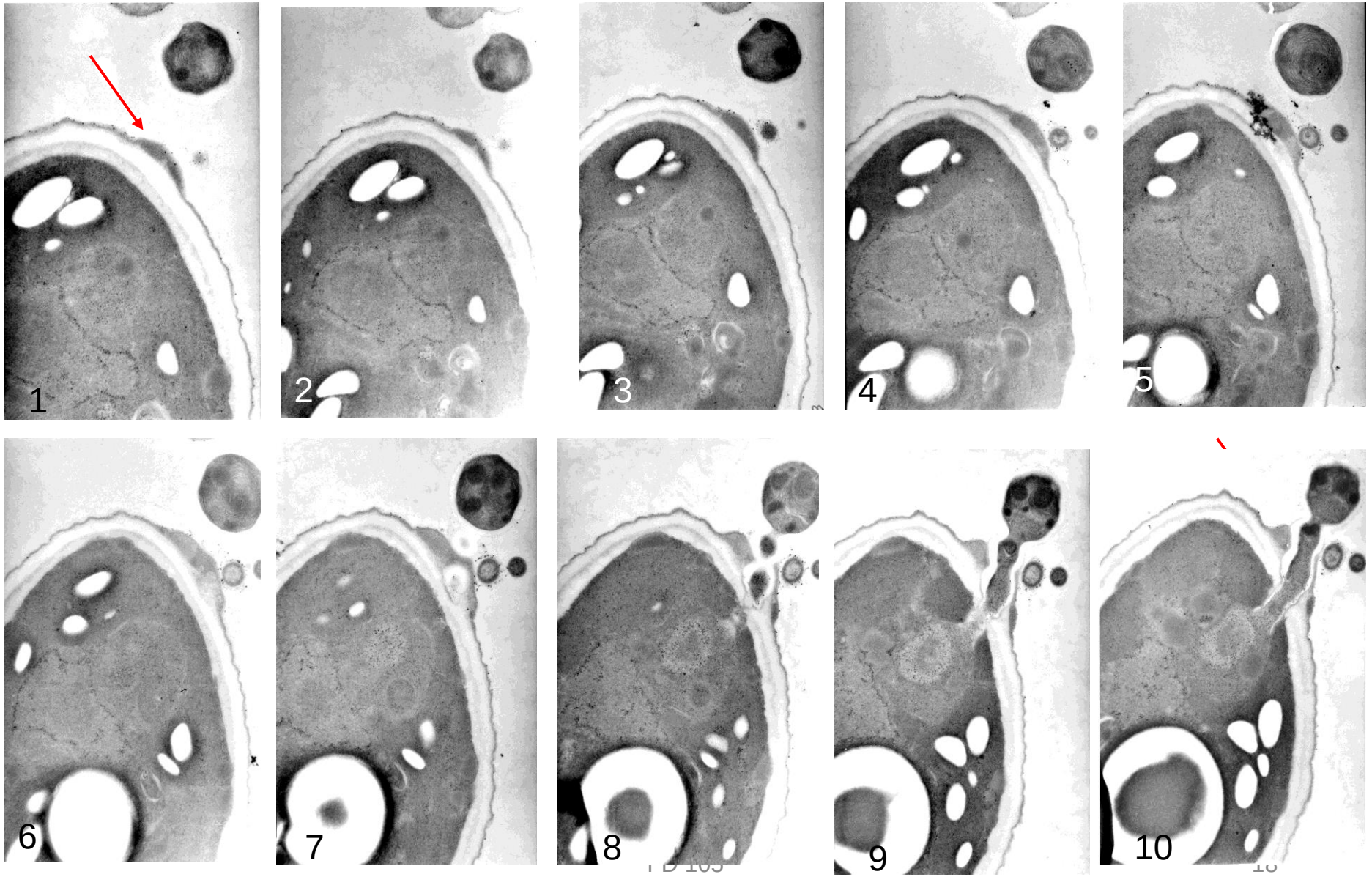
- I do not think the location of the aplanospore cyst, and the hypertrophy are co-incidental

# Serial sections of infection (1)

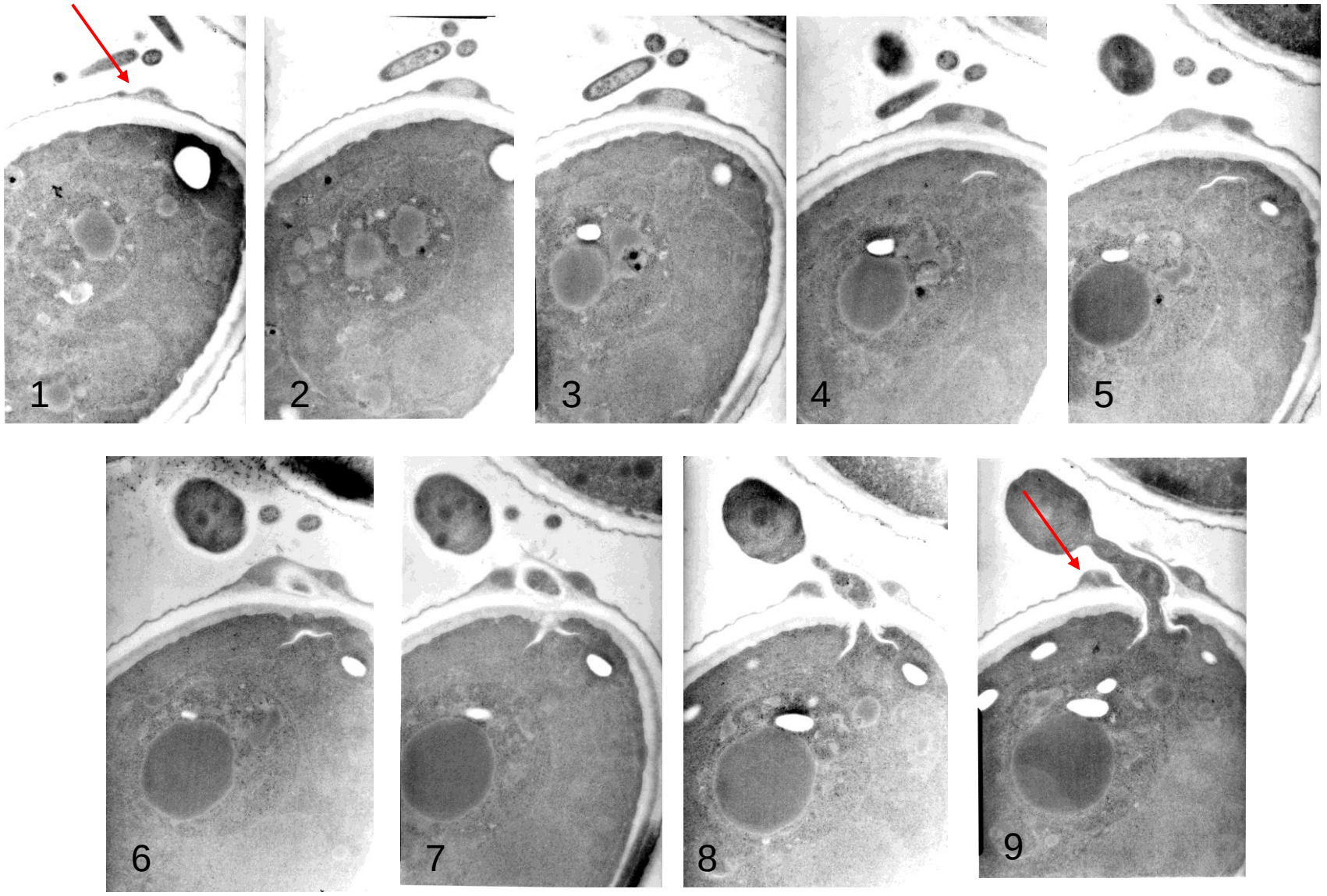



What we can see in 9 serial sections (top left to bottom right) is the aplanospore cyst penetrating a hypertrophied portion of the host cell wall (arrow). I think the hypertrophied portion of the wall is a host response to the infection.

# Serial sections of infection (2)



# Serial sections of infection (3)



# Infection

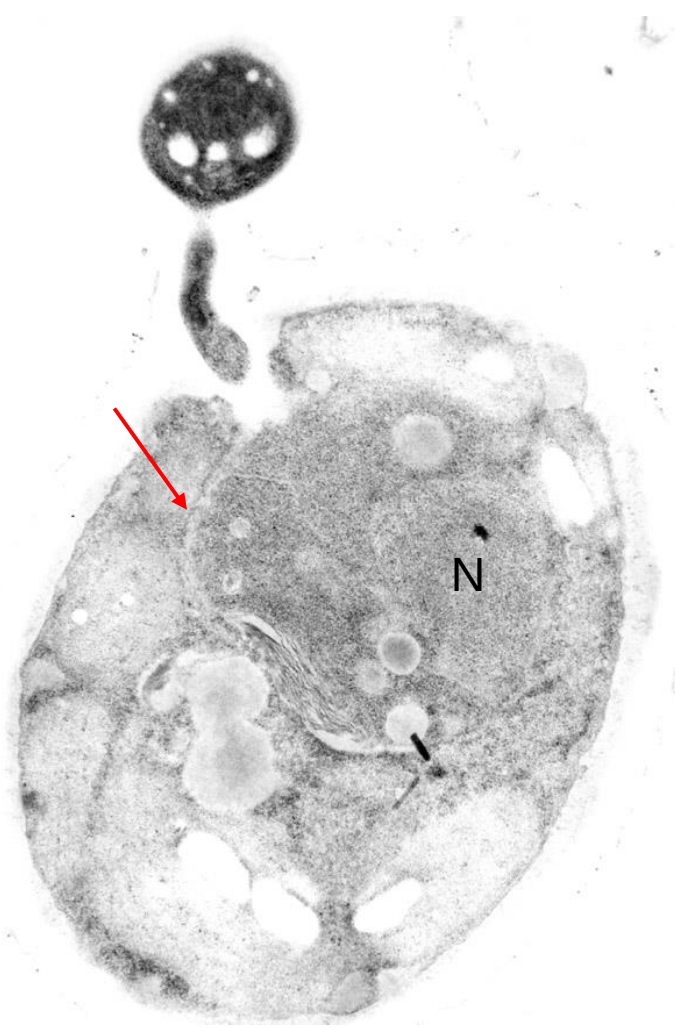
1. Either the parasite causes a hypertrophy reaction in the host cell wall at the point of infection,

2. or the parasite preferentially attacks a section of the host wall that is thickened.

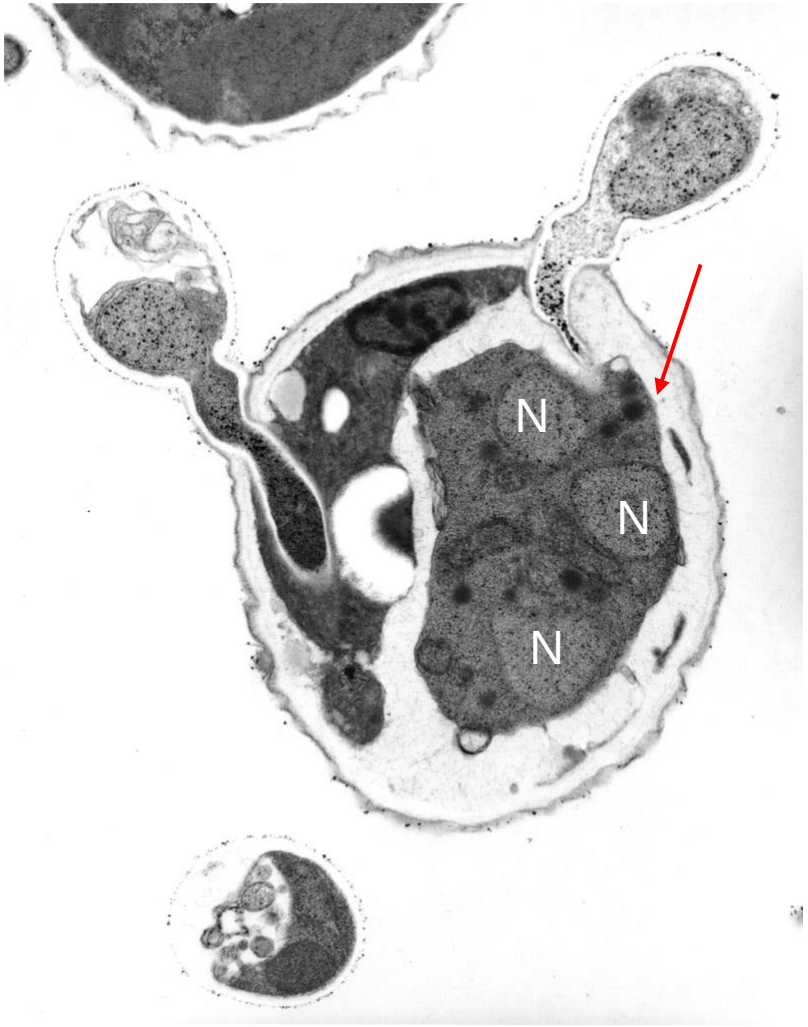
3. SEM (3D) analysis of uninfected and infected host cells may resolve this question.

4. Determination of this may indicate remedial strategies.

# Day 3: infection

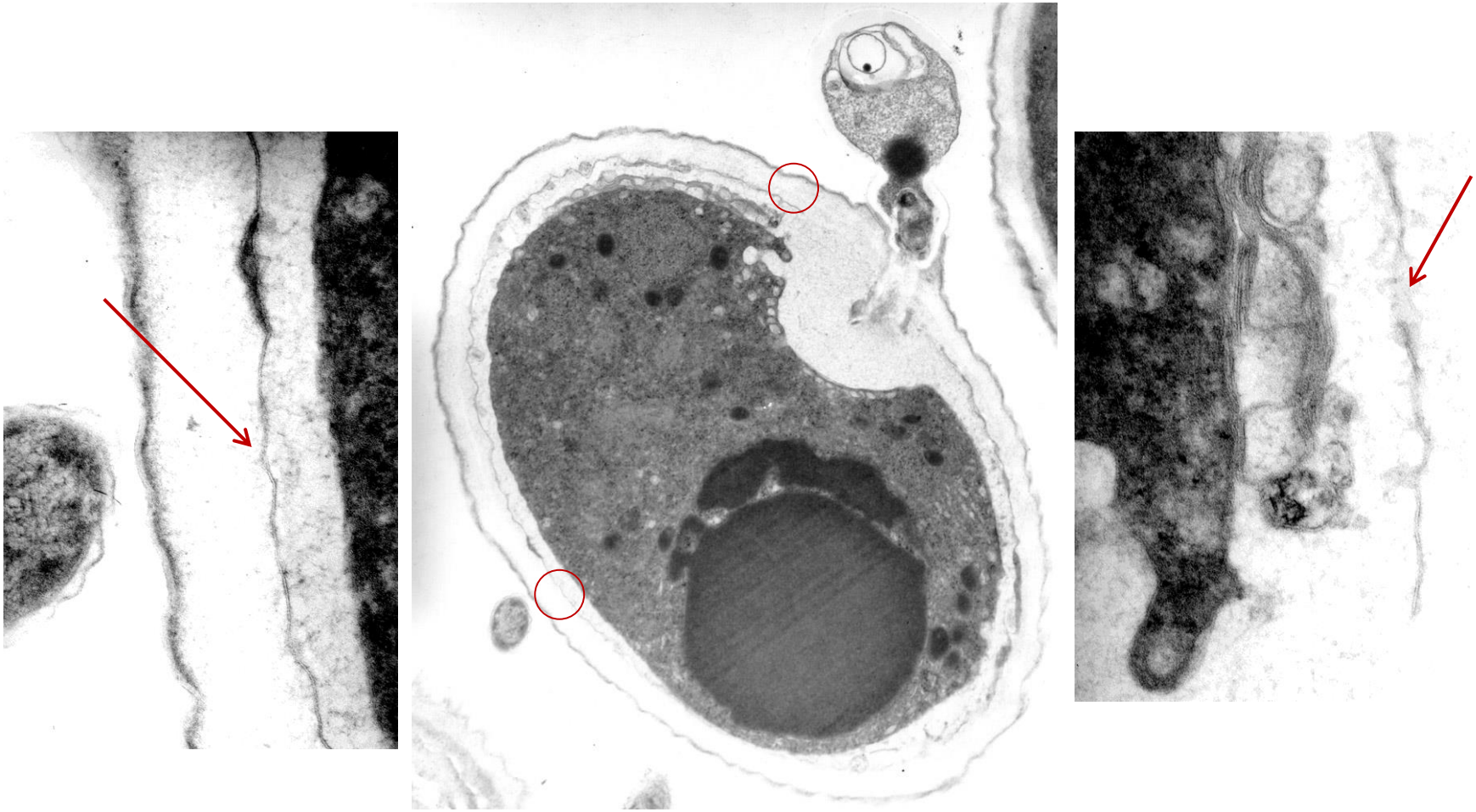



parasite protoplasm (arrow) with single nucleus



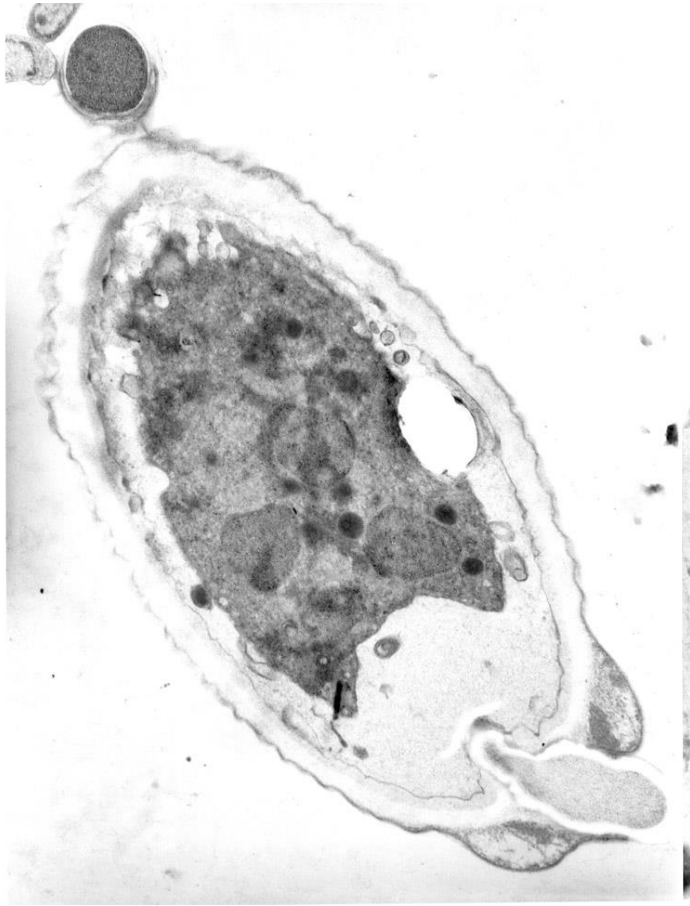

parasite protoplasm (arrow) with multiple nuclei and multiple infections

# Day 3: infection



Plasma membrane (arrows) delineating parasite protoplasm inside host cell

# Day 4: aplanospore cleavage



Multiple nuclei

7 cleaved aplanospores

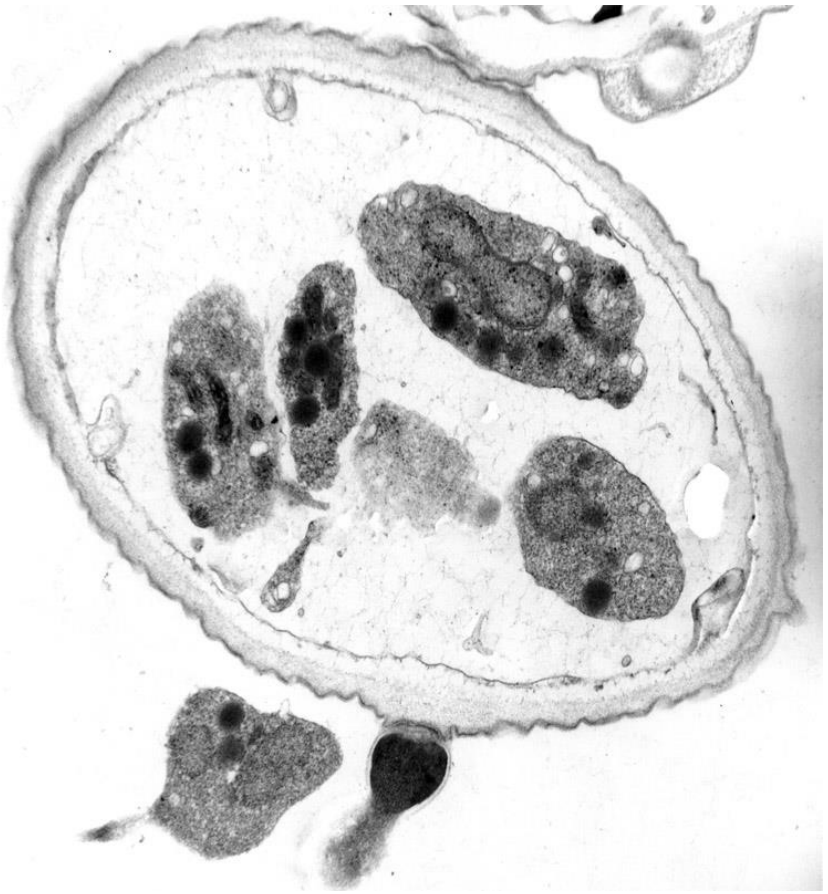
Multiple cleaved aplanospores

# Day 4: aplanospore cleavage

- Notice the sporangium on the right (previous slide), and the number of aplanospores:
- Aplanospores of FD105 are smaller and more numerous than those of FD01 or FD95:
  - FD 01: 1.7 – 2.5 µm diam
  - FD 95: 1.3 – 1.7 µm diam
  - FD 105: 1.17 – 1.50 µm diam

# Day 4: aplanospore release

Aplanospore release (arrow) through aplanospore cyst penetration tube



Aplanospores inside and outside parasite sporangium (host cell wall)

# Day 4: empty host cell

Arrow indicates remnant of aplanospore cyst penetration tube

# Conclusions

1. FD 105 exhibits characteristics of *Amoeboaphelidium*: amoeboid aplanospores, no indication of flagellation, endoparasite of alga.

2. Based on aplanospore size, host reaction, and (molecular divergence), FD 105 is an undescribed species of *Amoeboaphelidium*.