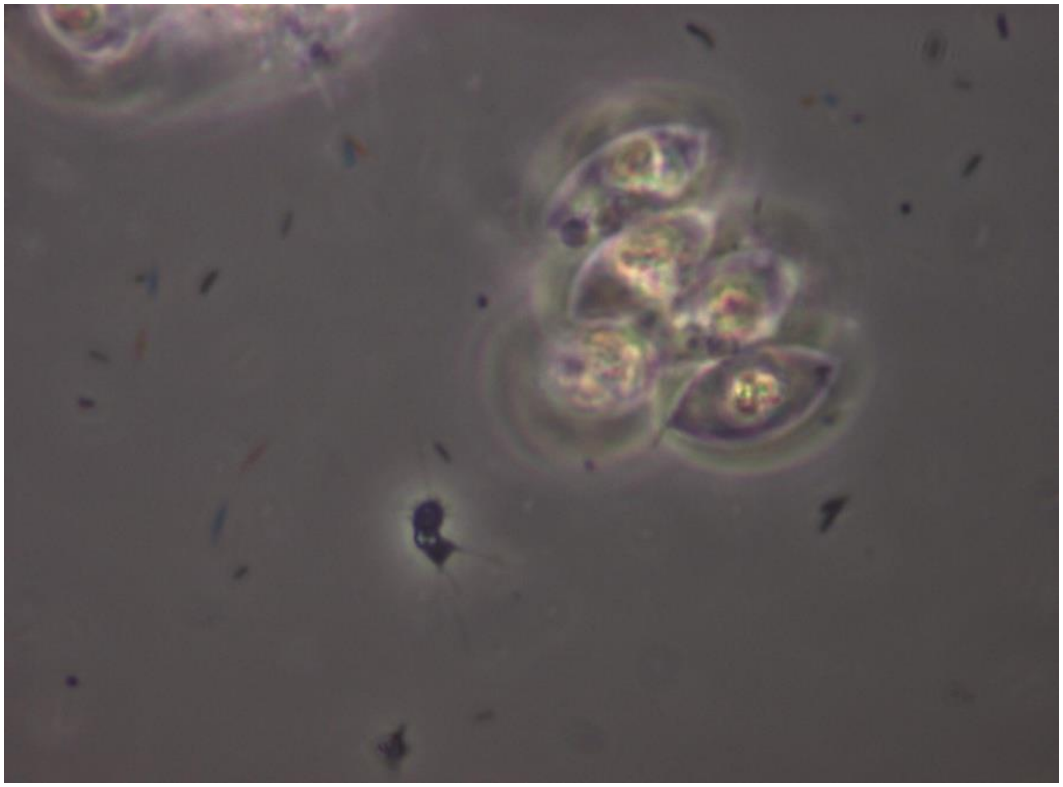
TEM Investigation of Sapphire Culture FD01 Infecting UTEX 1237, *Scenedesmus dimorphus*



Dr. Peter M. Letcher, Consultant
Biological Sciences
The University of Alabama

To investigate and better understand the morphology and life cycle of the putative algal parasite (FD01) of the green alga *Scenedesmus dimorphus* (UTEX 1237):

A pure culture of UTEX 1237 was inoculated with an aliquot of FD01 that had recently caused UTEX 1237 to crash.

1 ml portions of the inoculated culture were fixed for TEM observation at days 2, 3, 4, 5, 6, 7, 8, 14, and 26 after inoculation.

TEM: Methods:

Fixation- 2.5% glutaraldehyde in 0.1 M sym-collidine buffer;

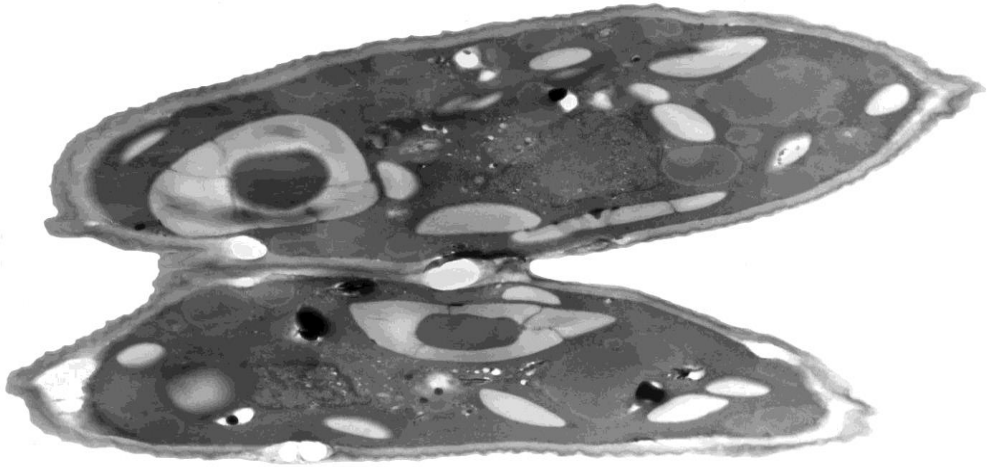
Post-fixation- 1% osmium tetroxide;

Fixed material was post-stained in saturated uranyl acetate (UrAc) and embedded in agar, then dehydrated in a graded acetone series and embedded in plastic resin;

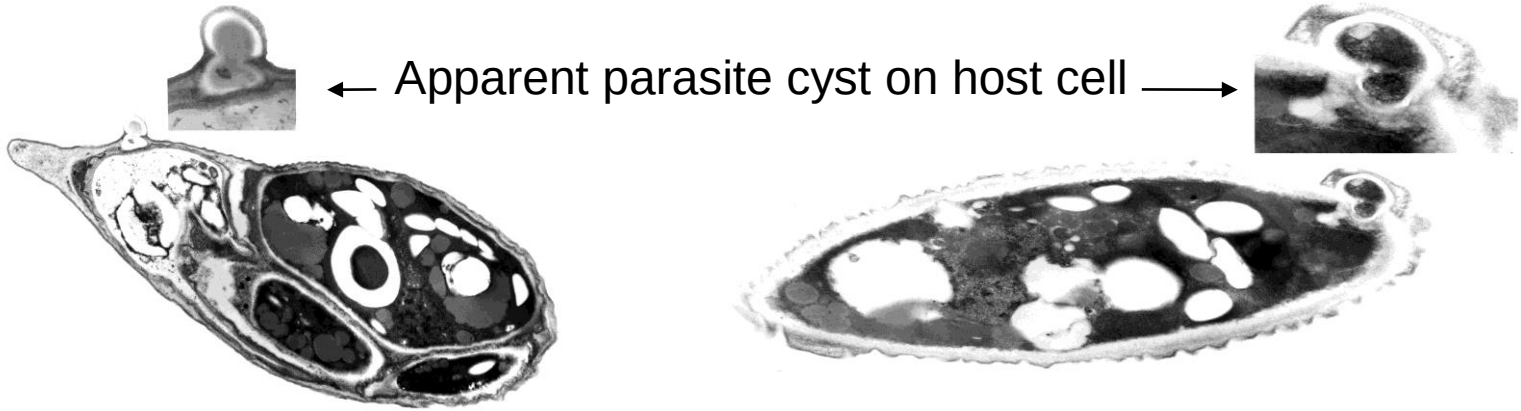
Sections were post-stained in saturated UrAc and observed on a Hitachi 7650 TEM at 60 kV.

Images were recorded on Kodak 4489 EM film, darkroom processed, and printed on Ilford multigrade RC paper.

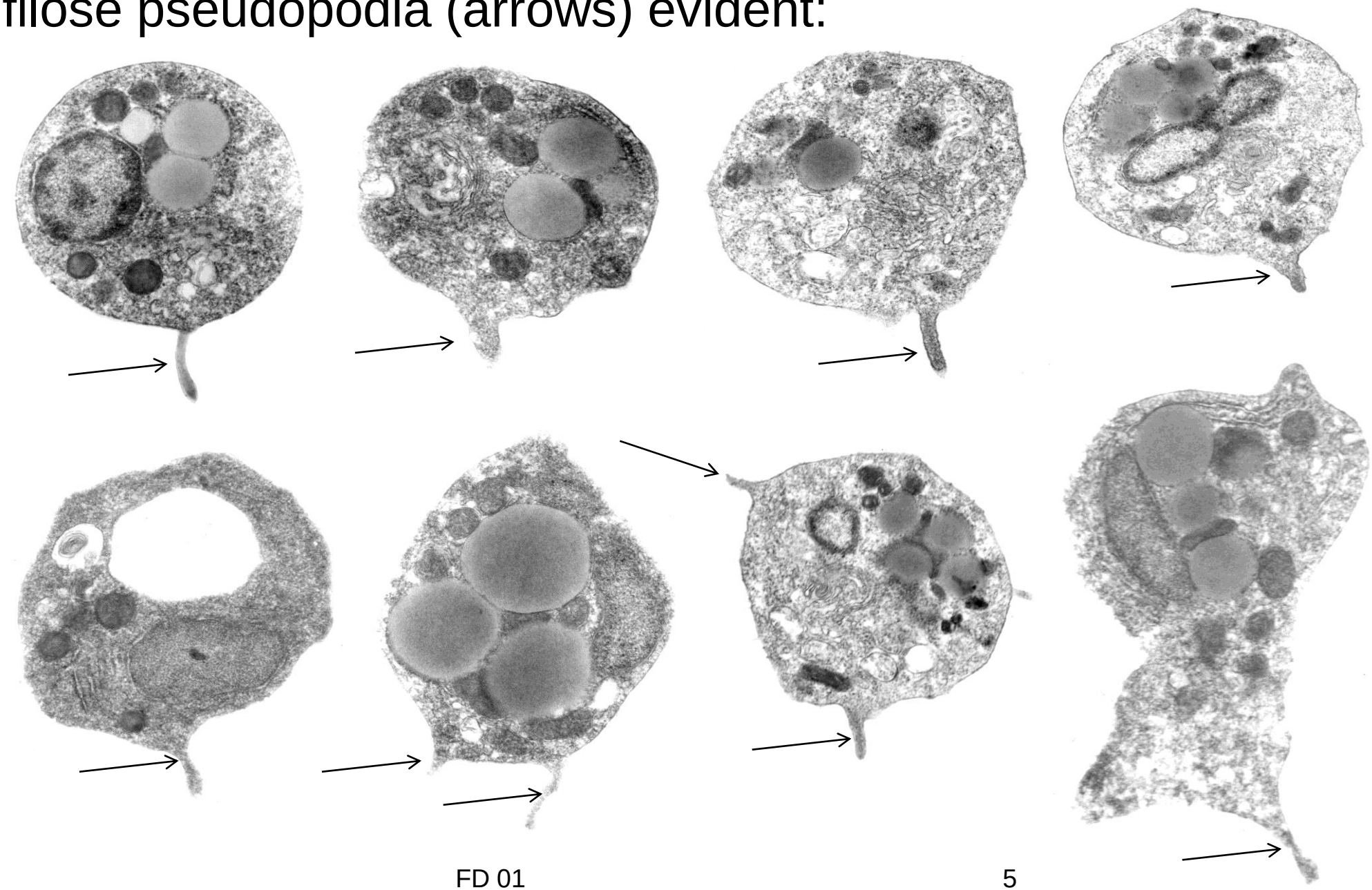
Day 2:
A majority of algal cells appeared healthy:



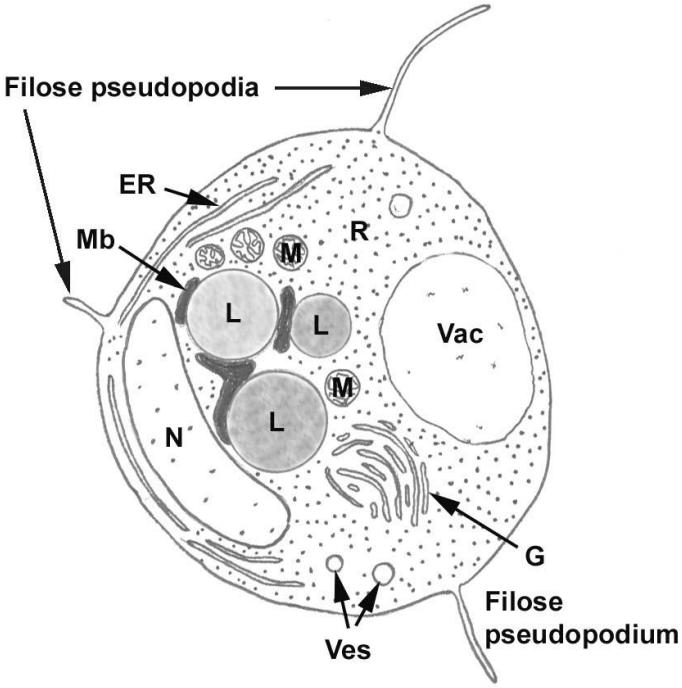
A minority of algal cells indicated early infection:

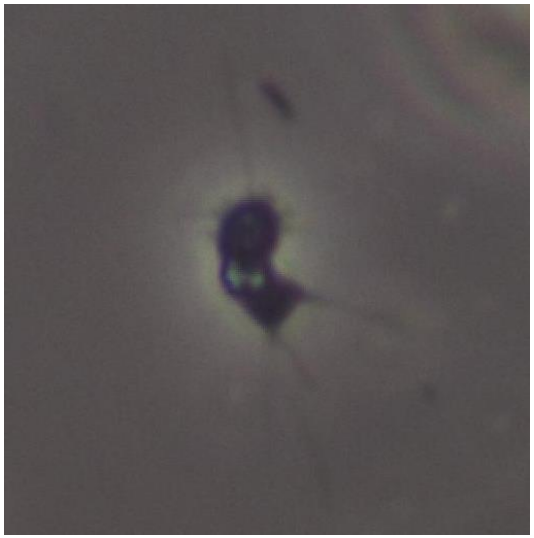


Day 3:
Abundant aplanospores (1.7-2.5 microns diameter) with filose pseudopodia (arrows) evident:



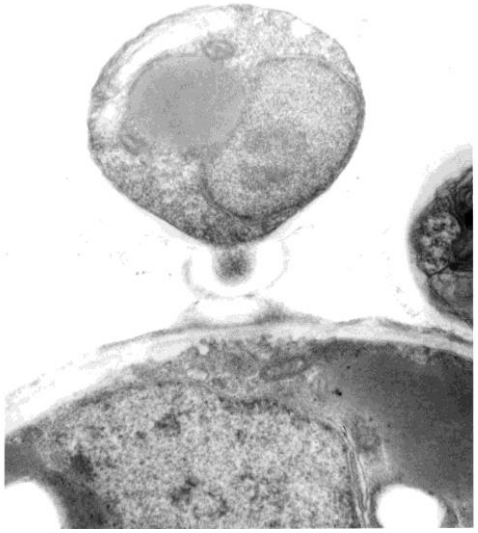
Aplanospore morphology:

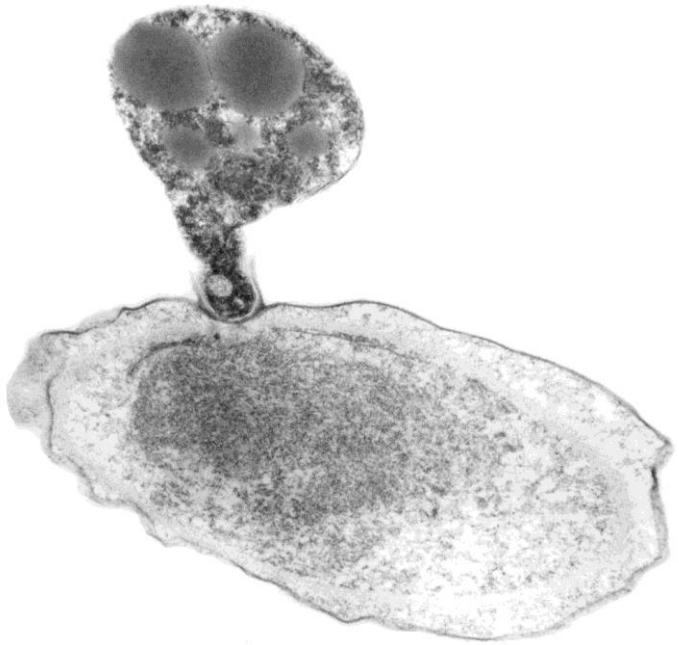


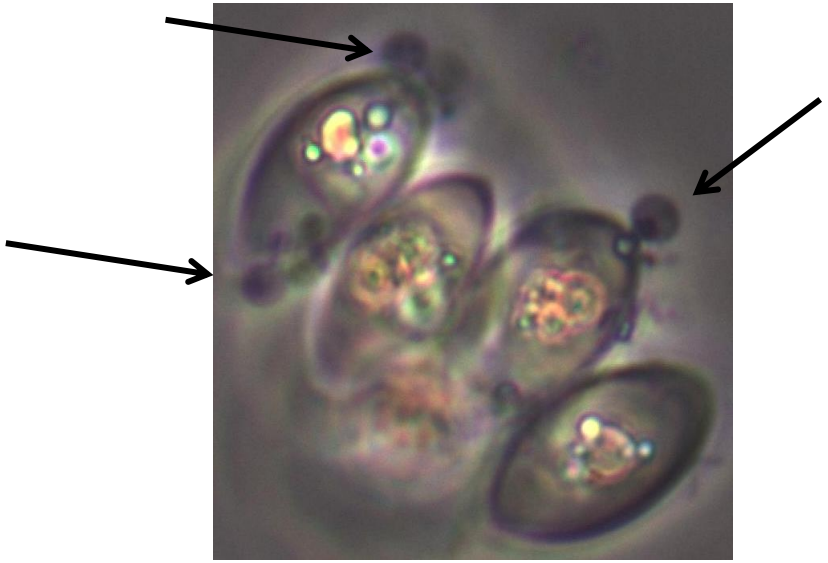


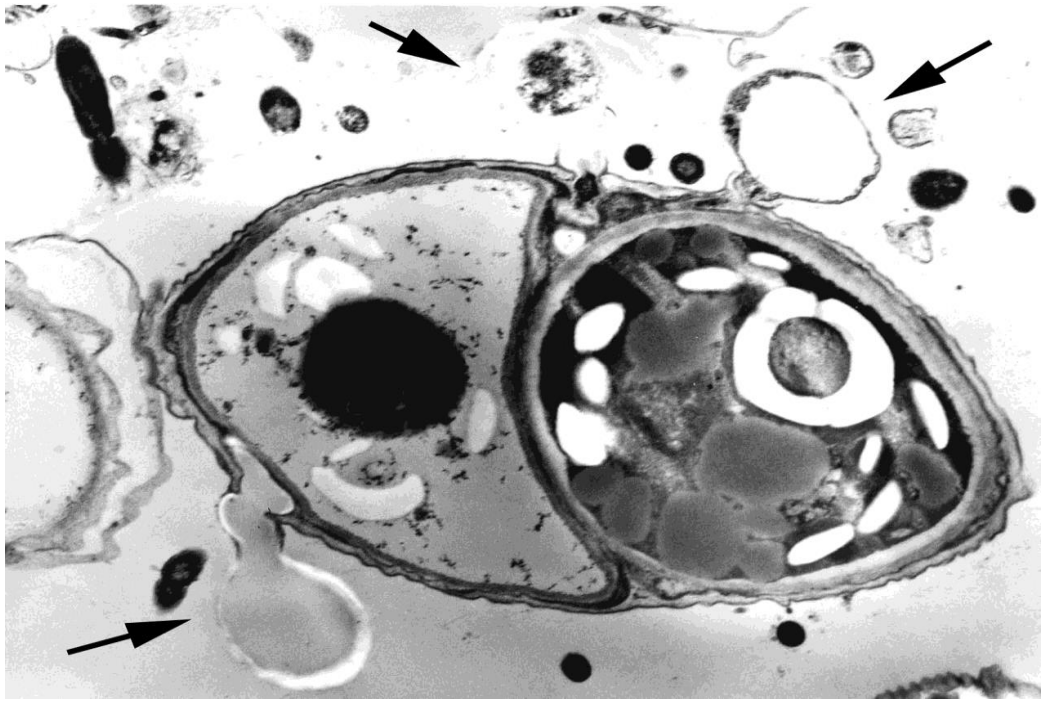
ER, endoplasmic reticulum; G, Golgi apparatus; L, lipid; M, mitochondrion; Mb, microbody; N, nucleus; R, ribosomes; Vac, vacoule; Ves, vesicles.

Day 3: Algal infection evident



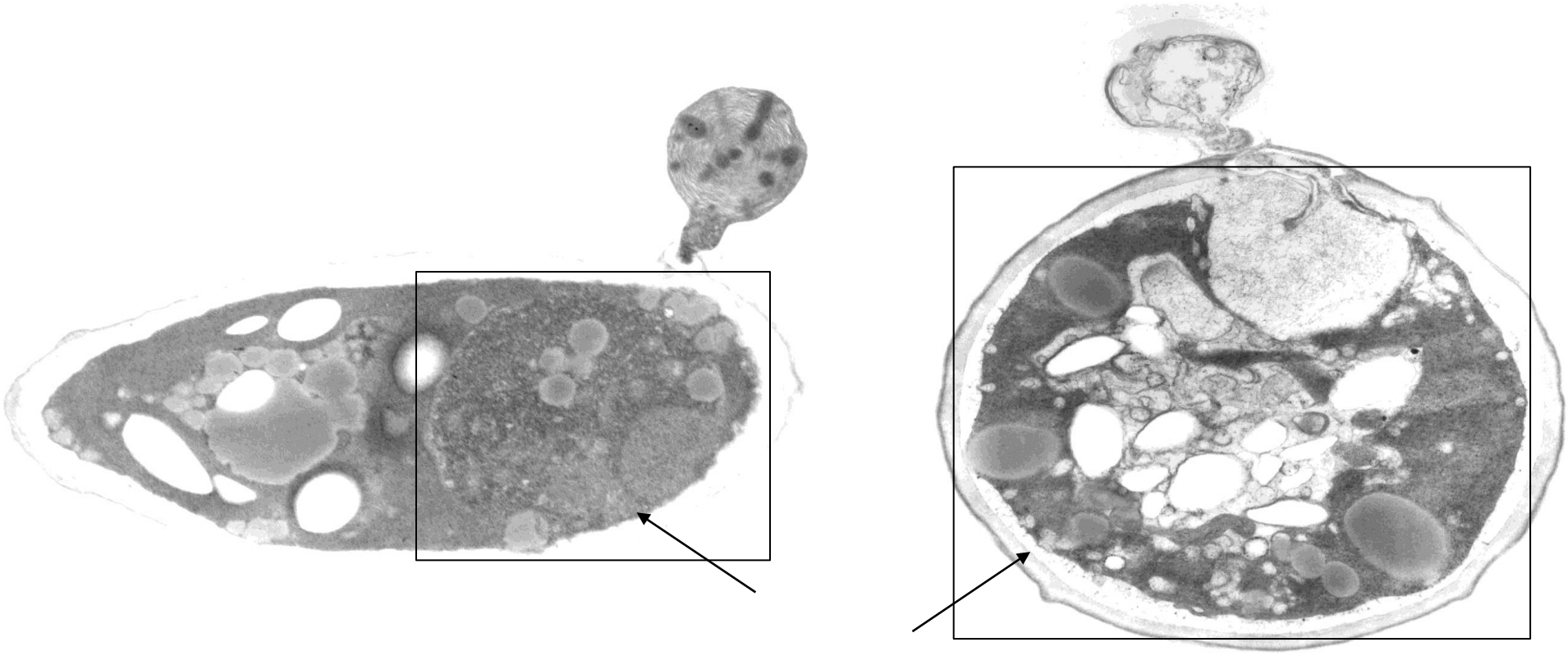






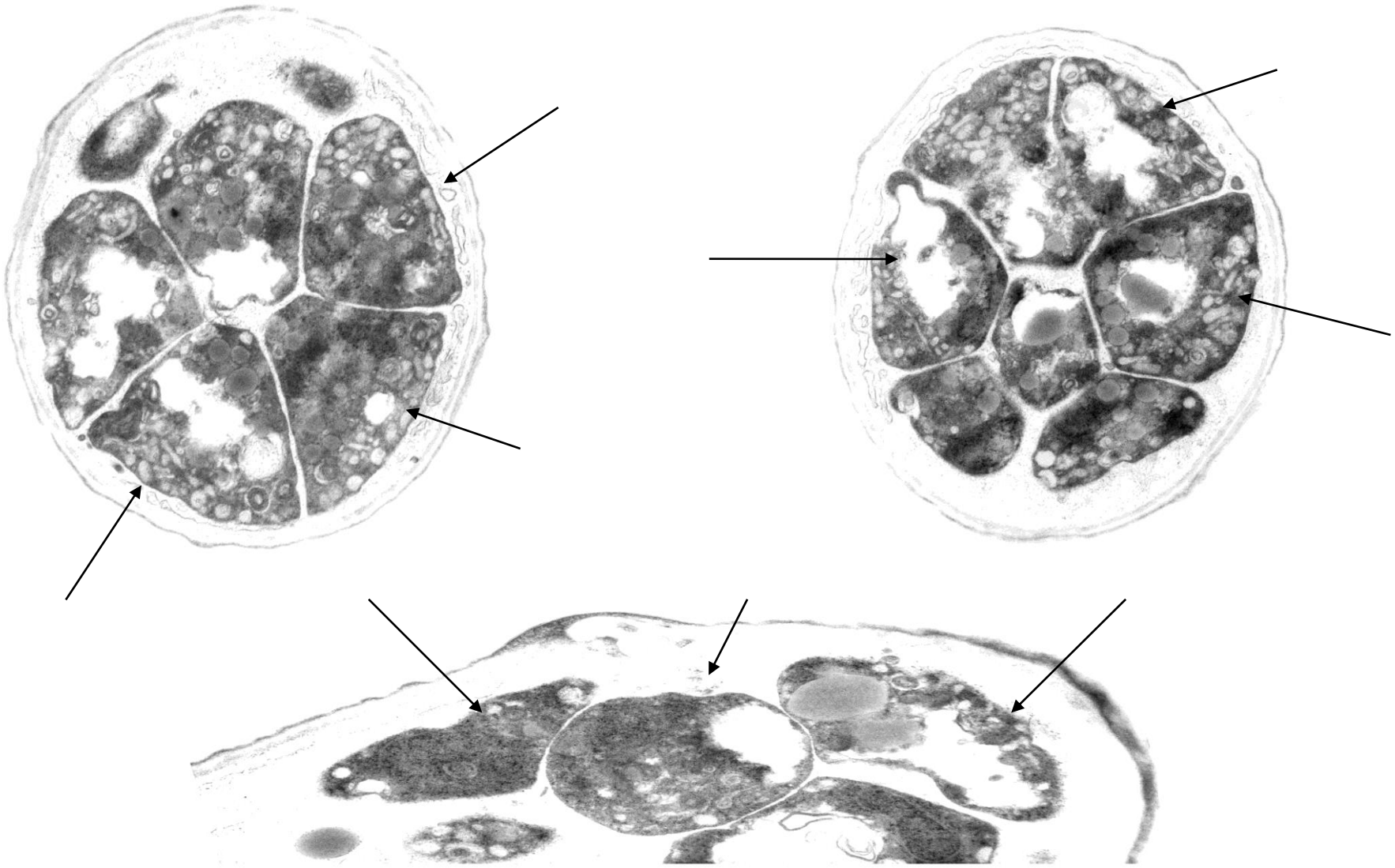
Left, upper and lower: parasite on host.
Right: three parasite infections (arrows) of a host cell.

Day 4: Continued infection: parasite protoplasm injected into host cell



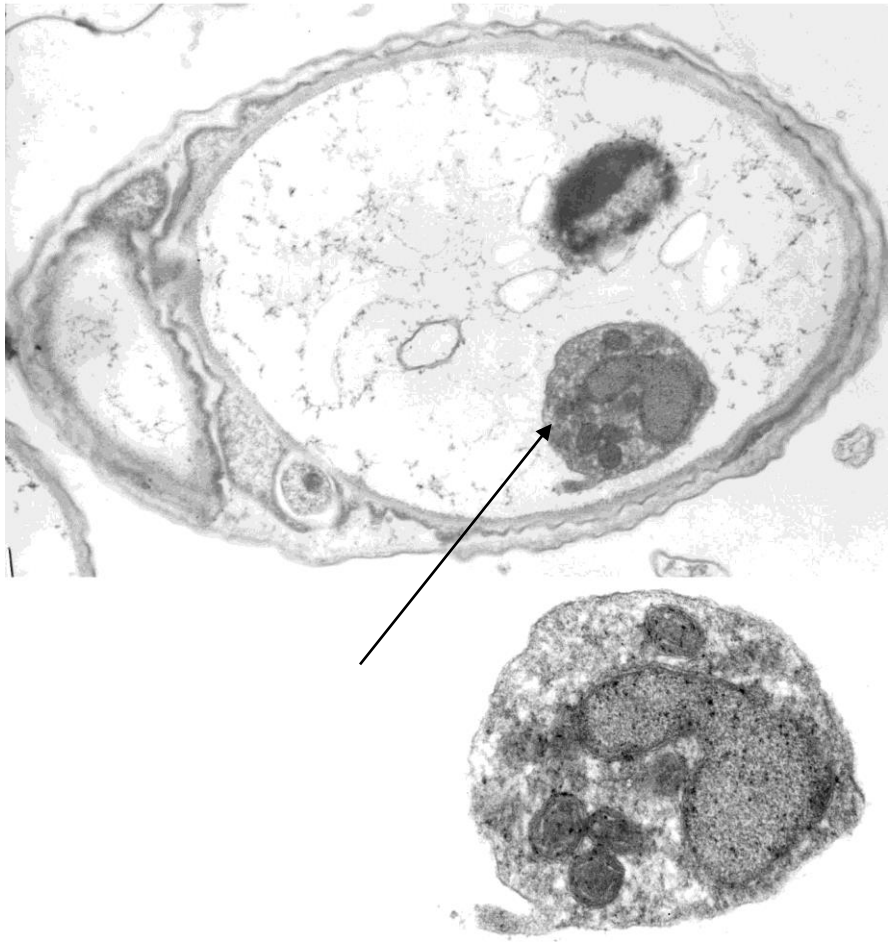
Parasite protoplasm (arrows, boxes) partially (left) or entirely (right) filling host cells

Day 4: Sporogenesis and spore cleavage

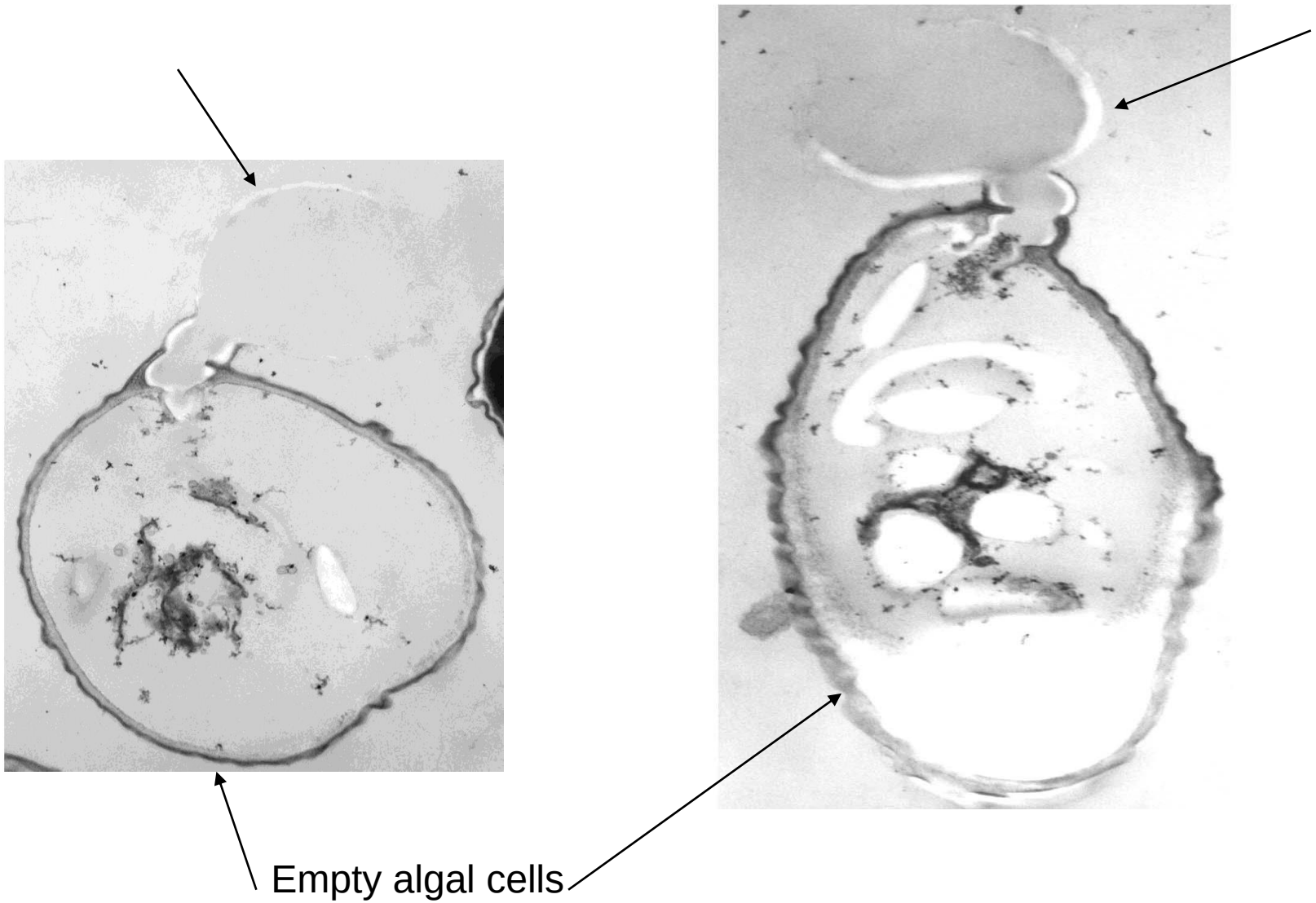


Cleaved spores (arrows) within host cells

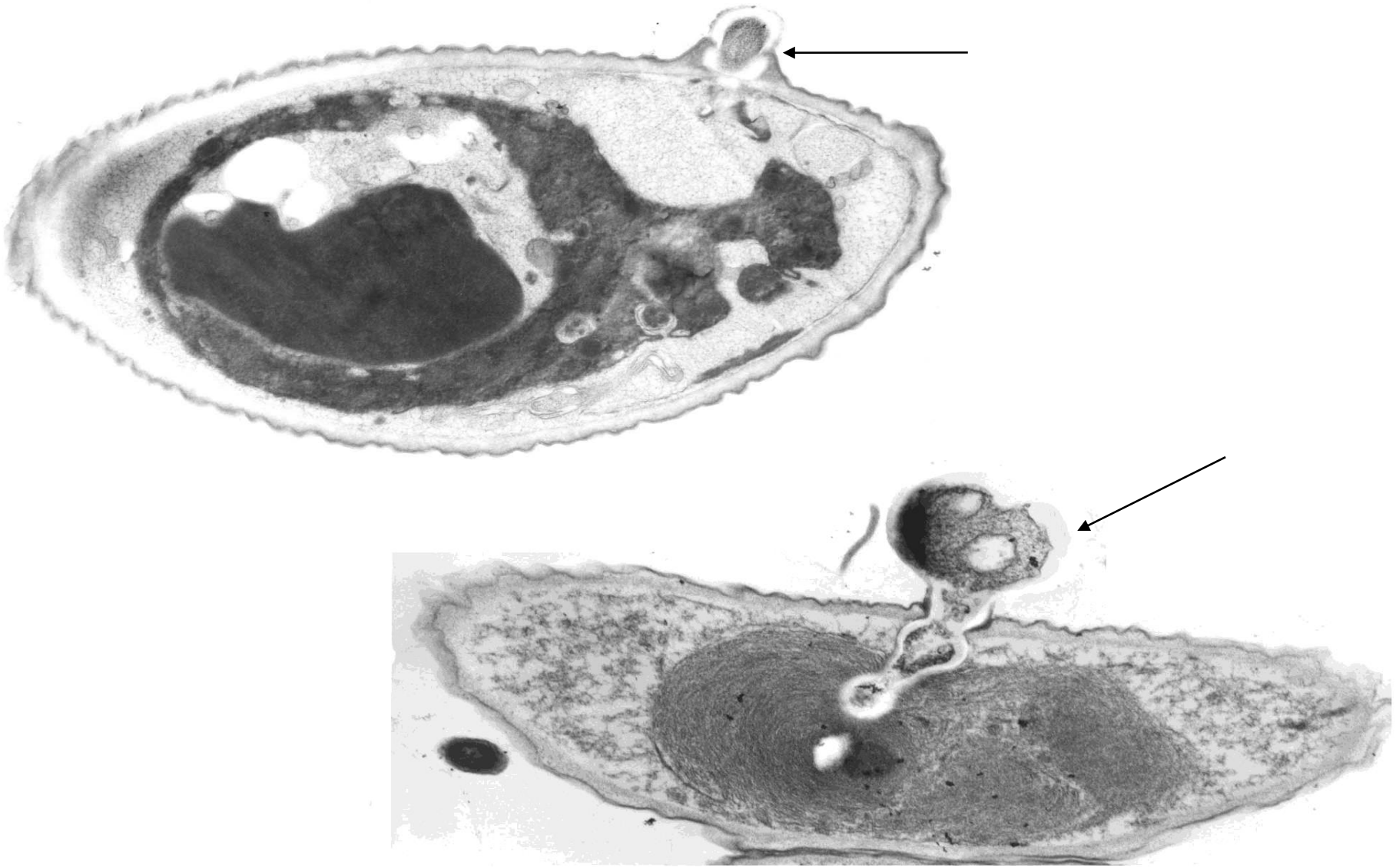
Day 4: Filose pseudopodiate spores (arrows) inside moribund algal cells



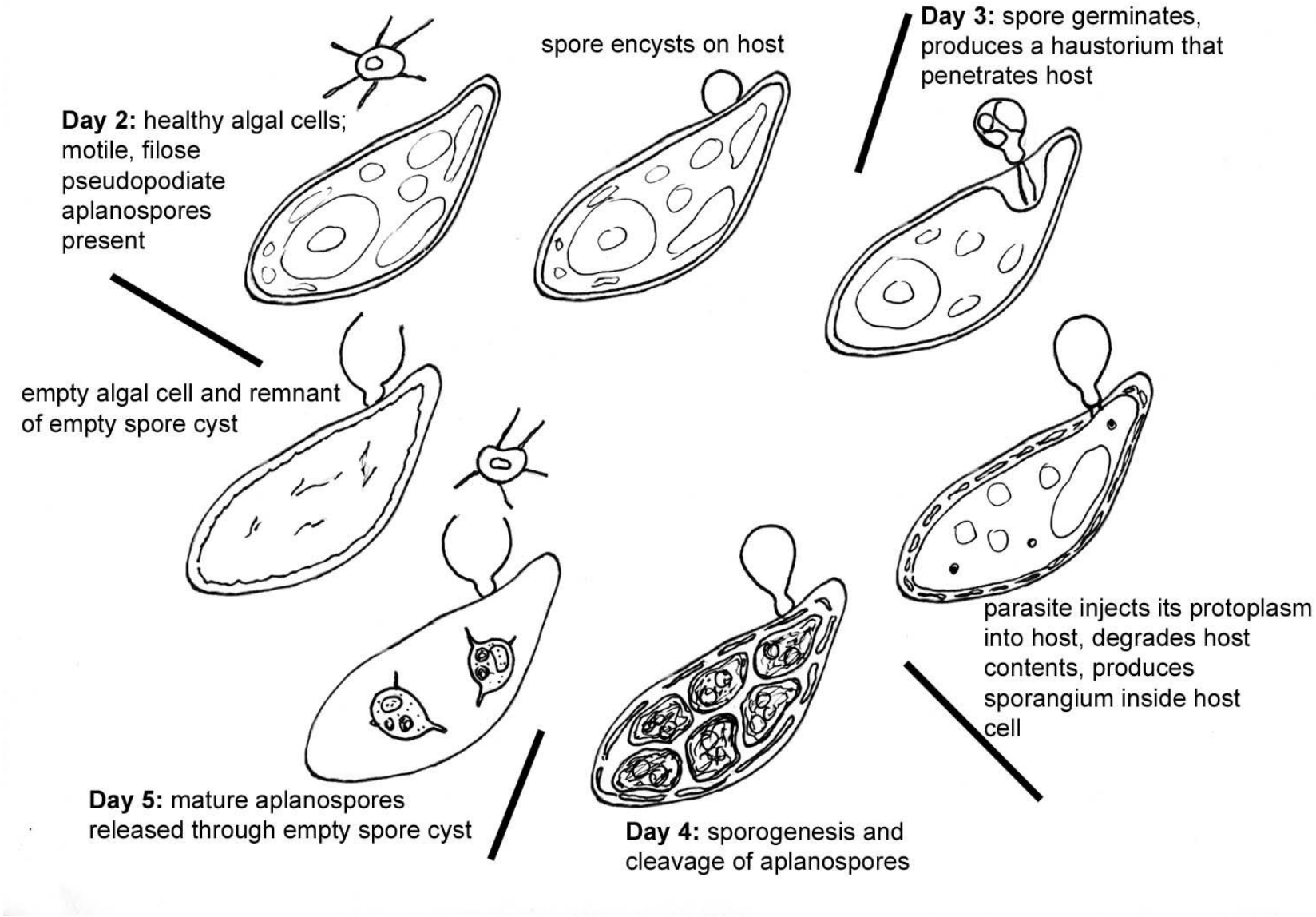
Day 5: Empty algal cells: aplanospore release/discharge presumed through cyst remnant (arrows) that appears apically eroded

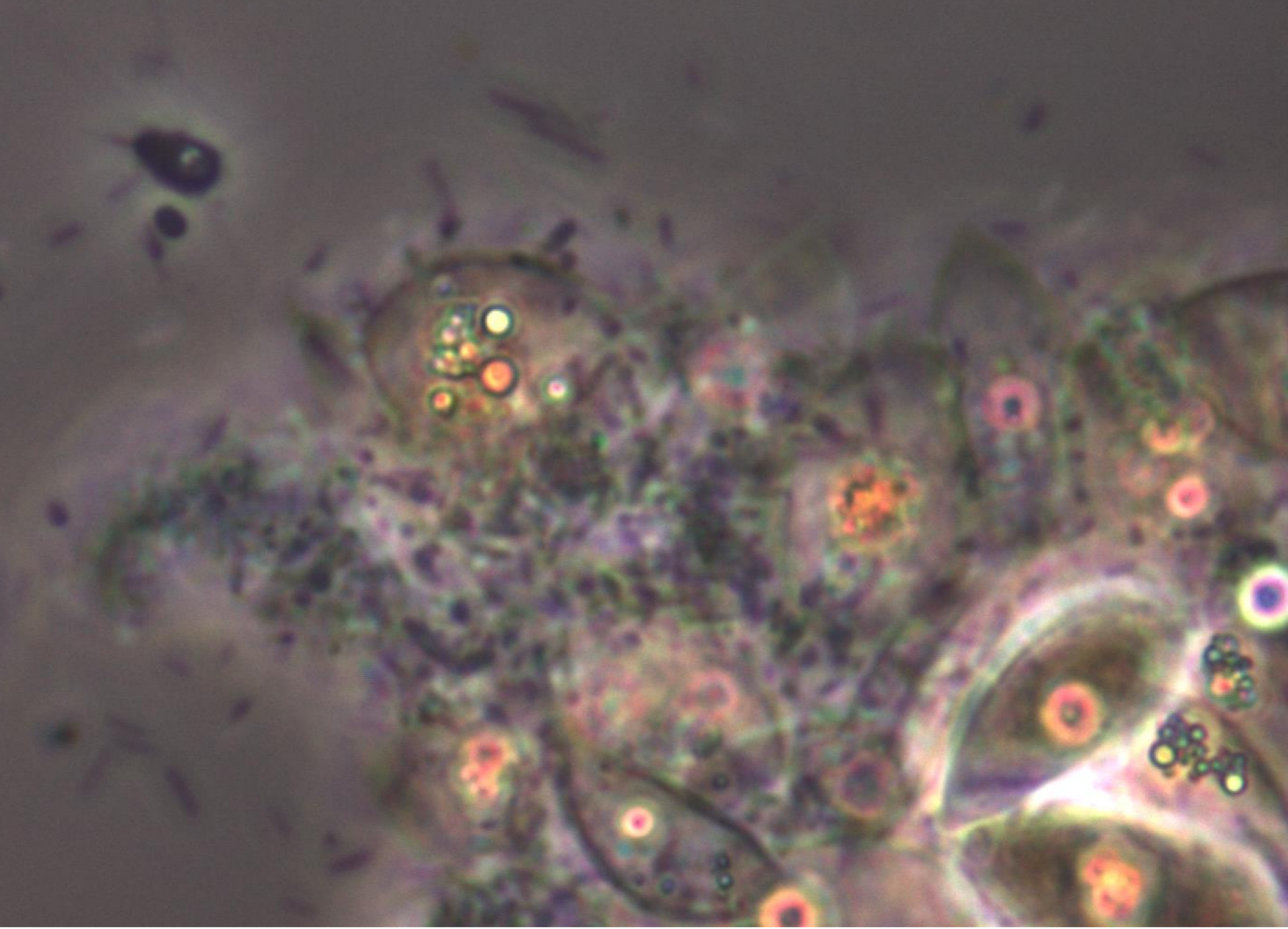


Day 5: presumed secondary infection (arrows) from released aplanospores



Hypothesized life cycle summary (see next slide, courtesy Sapphire, for motile aplanospore video):





Implications/observations:

Infection is rapid, perhaps 3 days to complete life cycle.

The aplanospore of FD01 is morphologically similar to that of *Paraphysoderma sedebokerense* (Blastocladiomycota) (James et al. 2011, Mycotaxon 118: 177-180; Hoffman et al. 2008, Mycol. Res. 112: 70-81, fig. 2D), FD61 (putatively Blastocladiomycota), and FD95 (sister to FD01).

This is the first ultrastructural investigation of a filose pseudopodiate aplanospore; thus any ultrastructural similarity to *P. sedebokerense*, FD61, or FD95 is unknown.

The life cycle of FD01 is similar to that of *Rozella* (Held 1980, CJB 58: 959-979); FD01 is sister to *Rozella* in the Sapphire molecular phylogenetic analysis.

Still need to look at later stages of fixation (8d, 14d, 28d) to assess possibility of a resting spore.