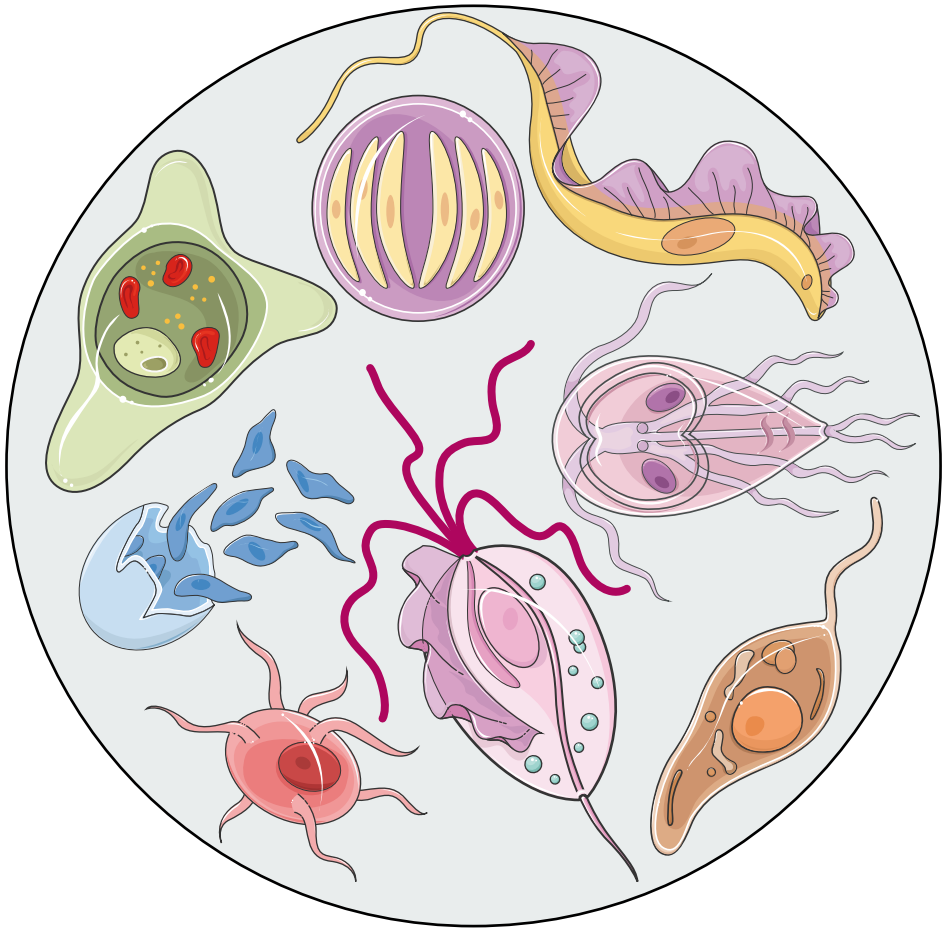
Biology of parasitic protozoa

VIII. Pneumocystis (Opisthokonta, Fungi)



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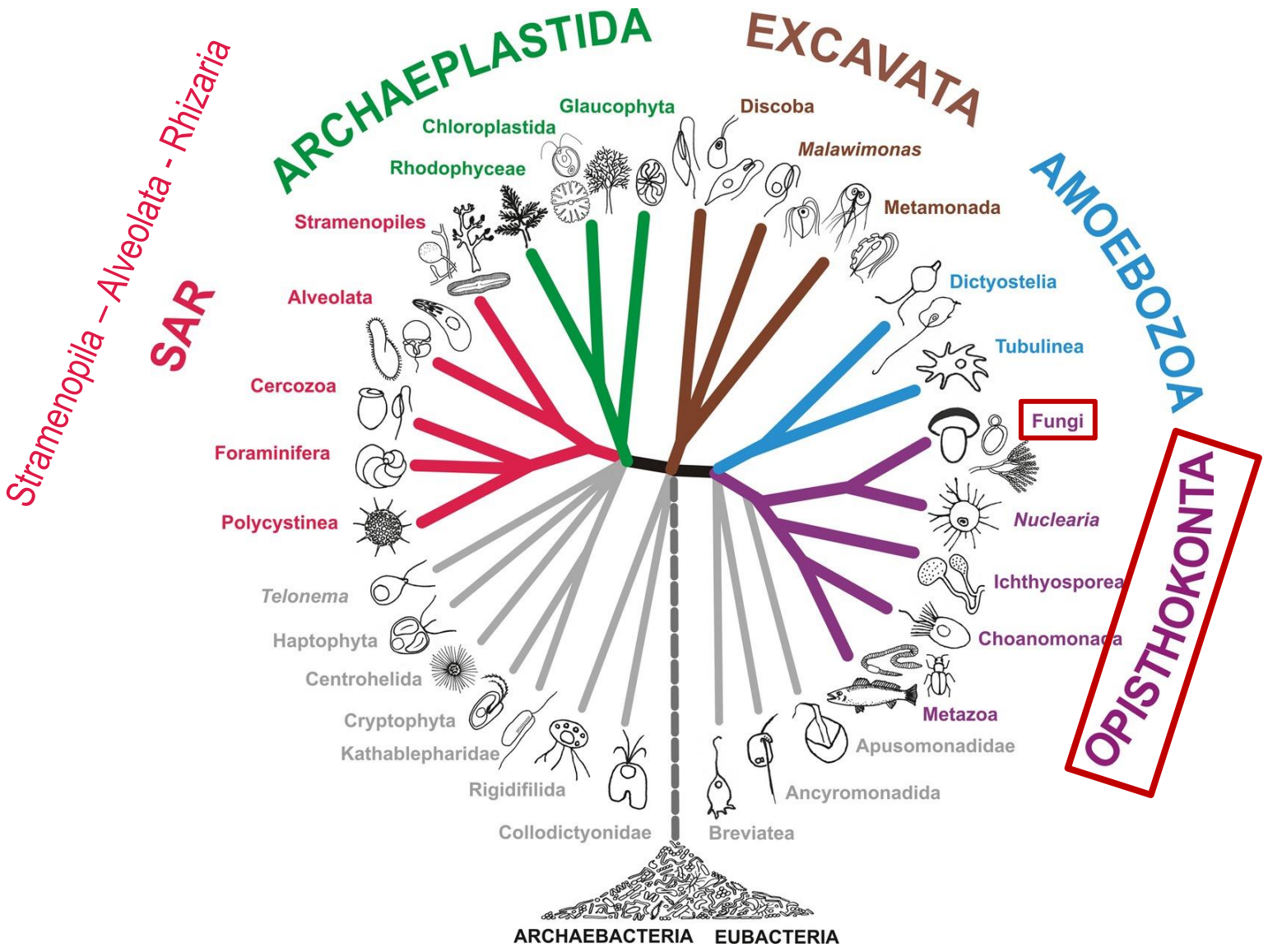
5 supergroups = megagroups



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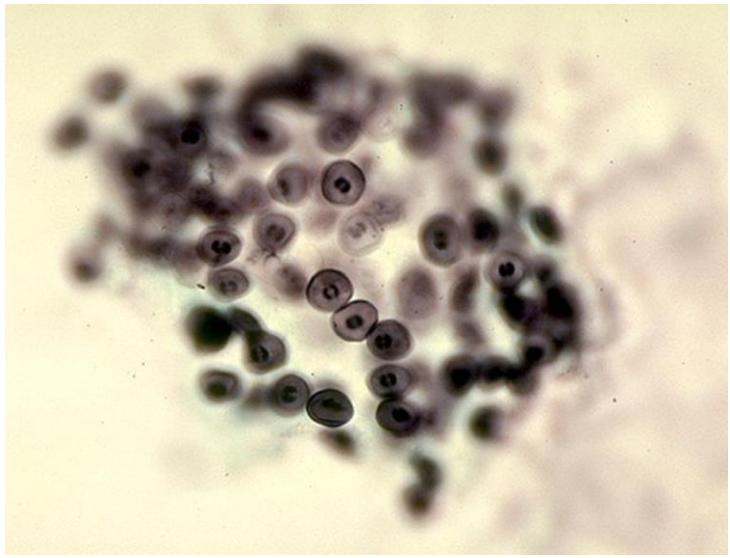
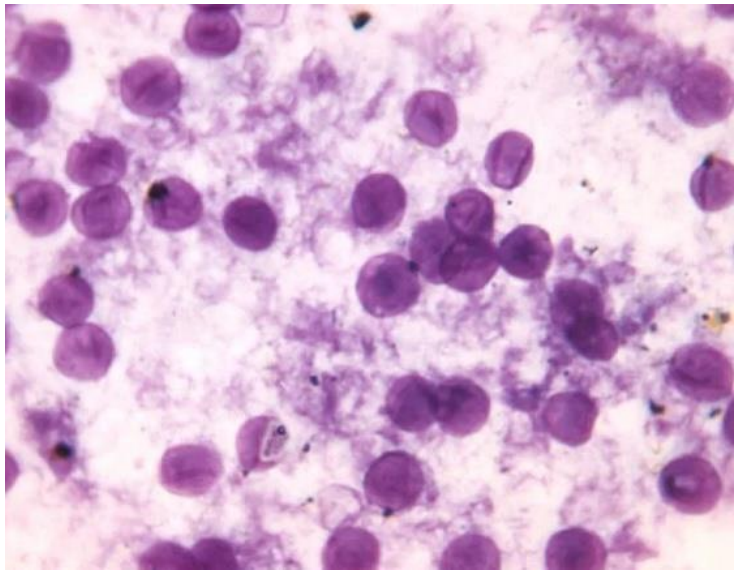
Pneumocystis and pneumocystosis

- *Pneumocystis jirovecii* (previously *Pneumocystis carinii*) was previously classified as a protozoan
- traditionally studied by protozoologists
- **fungus** based on molecular and biochemical analysis
- cause of pneumonia = ***Pneumocystis* pneumonia** (**PCP**) in people with compromised immune system and in premature, malnourished infants
- source of opportunistic infection - especially in people with cancer undergoing chemotherapy, HIV/AIDS, and the use of medications (e.g. corticosteroids) that suppress the immune system





Pneumocystis: history of the discovery

Carlos Chagas in **1909** inoculated human blood infected with *Trypanosoma cruzi* into guinea pigs:

- detected cystic, multinucleated bodies lungs of these experimental animals but confused it with part of the lifecycle of *T. cruzi*
- later named both organisms *Schizotrypanum cruzi*

Subsequent observations rule out the association with *T. cruzi*:

- **Carini** in **1910** described *Pneumocystis* in lungs of rats without *T. cruzi*
- **Delanoe** and **Delanoe** in **1912** performed study of rats in Paris and described *Pneumocystis carinii*
- in **1938** German pathologists described a special disease of malnourished infants - interstitial plasma cell pneumonia (IPP) (but the causative agent remained unknown)
- in **1942** Dutch pathologists described the finding of *Pneumocystis* in human lungs (discovery did not elicit any scientific response)
- in **1951-1952** Czech researchers **Vaněk** and **Jírovec** identified *Pneumocystis* as the etiological agent of IPP in infants

genus *Pneumocystis*

- extracellular fungus
- worldwide, in humans and animals
- found in the lungs of mammals where it resides without causing overt infection until the host's immune system becomes debilitated, which can lead to fatal pneumonia
- complete life cycle of any of the *Pneumocystis* species not known
- life cycle of *P. jirovecii* is thought to include both asexual and sexual phases
- both stages known so far (= morphologically distinct), i.e. amoeboid **trophozoites** and globular **cysts**, can be found in the lungs and cannot be cultured *ex vivo*
- 8 spores form within cyst, these are released by rupture of the cyst wall
- terminology follows zoological terms, rather than mycological ones

P. carinii
P. wakefieldiae
- rats

P. oryctolagi
- rabbitts

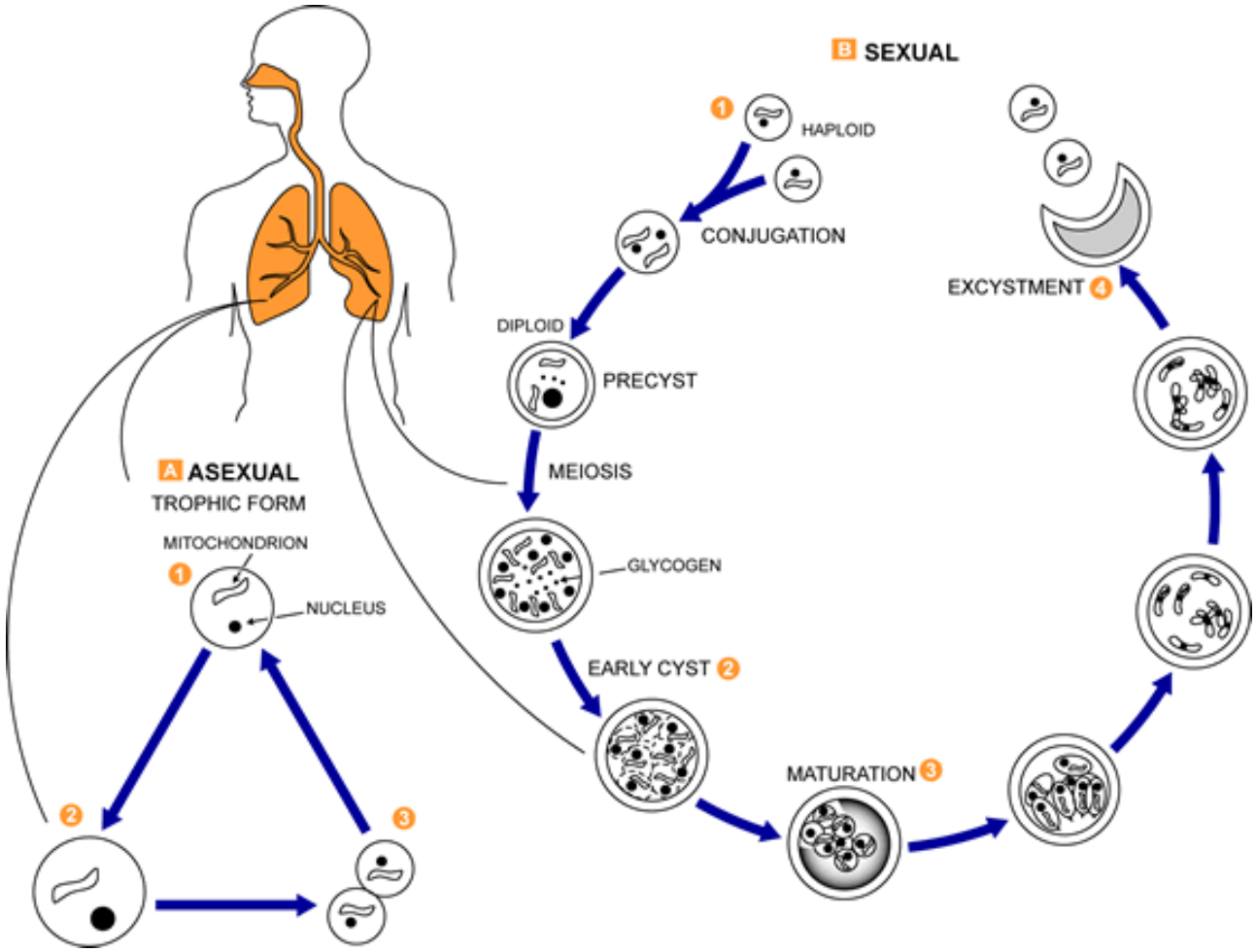
P. murina
- laboratory mice

Pneumocystis jirovecii

- the only species found in humans
- number of genotypes
- spreading from person to person through the air
- **PCP** is extremely rare in healthy people, but the fungus causing it can live in their lungs without causing symptoms
- serologic evidence (70-80% positivity) indicates that most healthy children have been exposed by age 3 to 4
- up to 20% of adults might carry this fungus at any given time and their immune system removes it after several months
- host immune status of the determines whether the host clears *Pneumocystis* or becomes a permanent asymptomatic carrier or becomes ill
- e.g. young rabbits and piglets commonly develop pneumocystis pneumonia infection after weaning, which resolves after 2-3 weeks
- AIDS, transplantation, malignancy patients - necessary prevention or treatment (pentamidine, trimethoprim + co-trimoxazole

https://www.youtube.com/watch?v=cuZb539SaaY

Generalised life cycle proposed for *Pneumocystis* spp.



Asexual phase: Trophic forms (1) replicate by mitosis (2) to (3). **Sexual phase:** Haploid trophic forms conjugate (1) and produce a zygote or sporocyte (early cyst) (2). The zygote undergoes meiosis and subsequent mitosis to produce 8 haploid nuclei (late phase cyst) (3). Spores exhibit different shapes (such as, spherical and elongated forms). It is postulated that elongation of the spores precedes release from the spore case. It is believed that the release occurs through a rent in the cell wall. After release, the empty spore case usually collapses, but retains some residual cytoplasm (4). A trophic stage, where the organisms probably multiply by binary fission is also recognised to exist.

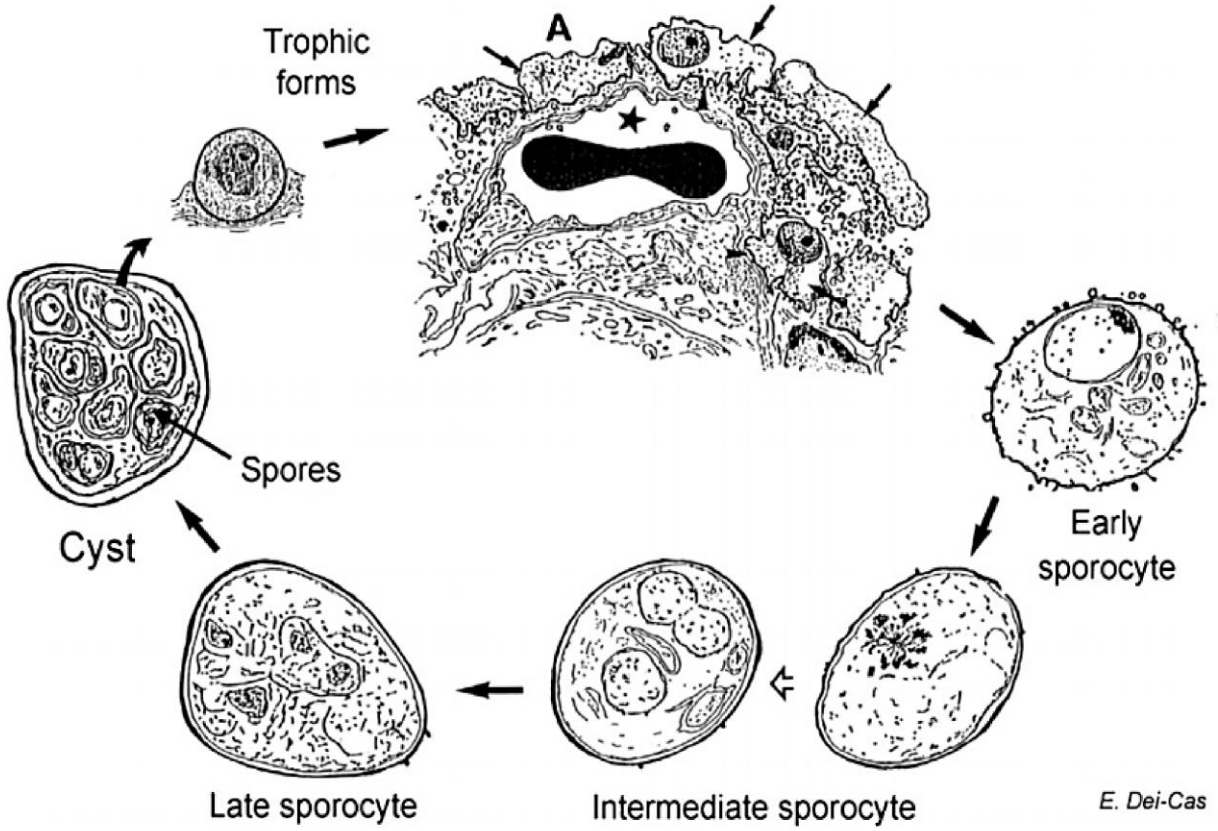
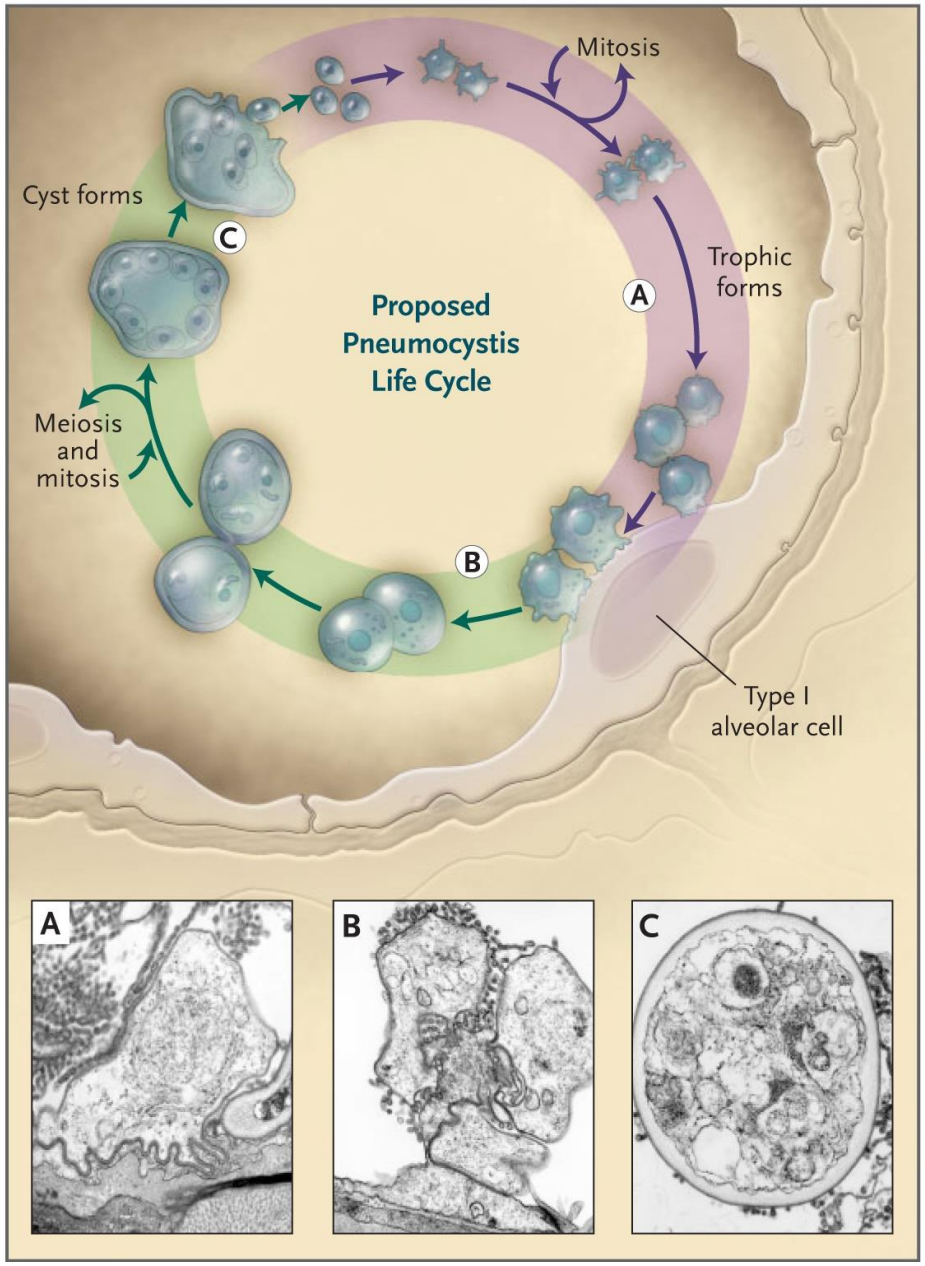


Fig. 1. A hypothetical life-cycle of *Pneumocystis* species. Parasites are represented as observed in the lung using transmission electron microscopy. Pleomorphic, thin-walled mononuclear trophic forms are shown attached to type-I epithelial alveolar cells close to an alveolar capillary vessel (star). Trophic forms (small arrows) evolve into thick-walled sporocytic and cystic stages, in which a multiple nuclear division leads to the formation of eight spores. These forms are able to leave the cyst and to attach specifically to type-I epithelial alveolar cells (modified from Dei-Cas et al., 2004).



Proposed *Pneumocystis* life cycle

The life cycle of pneumocystis is complex, and several forms are seen during infection. Electron micrograph in **A** shows a trophic form that is tightly adherent to the alveolar epithelium by apposition of its cell membrane with that of the host lung cell membrane. During infection, trophic forms are more abundant than cysts (approximately 9:1), and the majority of the trophic forms are believed to be haploid during normal growth, with a smaller fraction that are diploid. Trophic forms attach to one another, as shown in the electron micrograph in **B**, and clusters of clumped trophic forms can be seen during infection. The events that lead to the formation of the cyst, shown in the electron micrograph in **C**, are unclear, but we hypothesize that the trophic forms conjugate and mature into cysts, which contain 2, 4, or 8 nuclei as they mature.

https://pubmed.ncbi.nlm.nih.gov/15190141

https://doi.org/10.1038/nrmicro1621

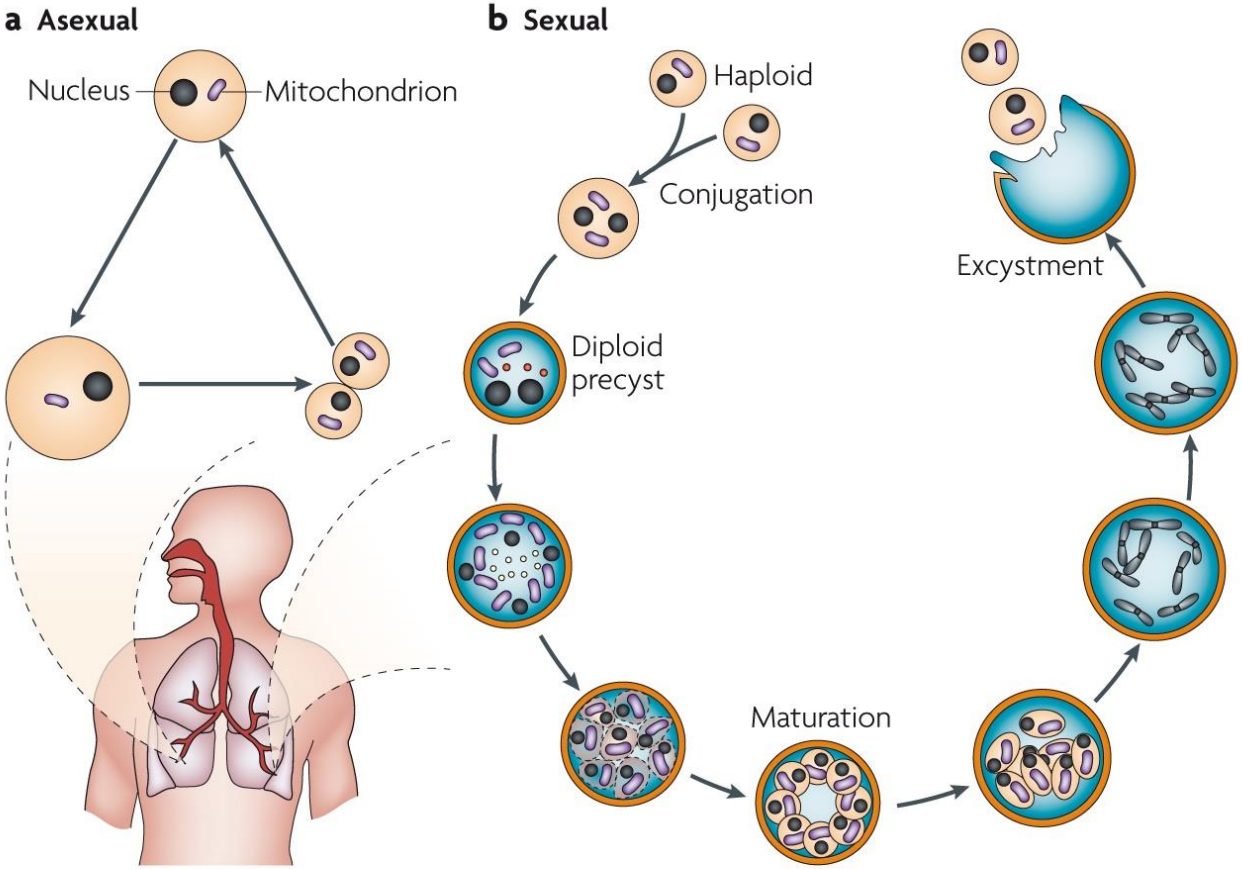
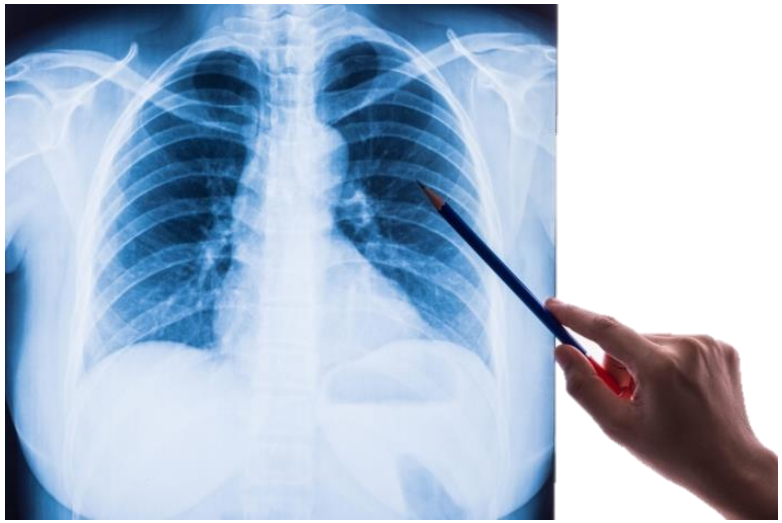


Figure 1 | ***Pneumocystis* life cycle.** There are two predominant life-cycle forms of *Pneumocystis*, the trophic form and the cyst form. It has been hypothesized that the trophic form can conjugate by binary fission and therefore undergo asexual reproduction (**a**). In addition, there is a sexual cycle (**b**). Three intermediate cyst stages have been visualized by electron microscopy, and they contain complements of 2, 4 and 8 nuclei, respectively[32]. The mature cyst contains 8 intracystic nuclei. It has been suggested that trophic forms emanate from the intracystic nuclei of the mature cyst as it ruptures, and then undergo vegetative growth or conjugate to re-form the cyst. Modified, with permission of J. Ruffalo, from REF. 155 © (1998) Arnold Publishing.

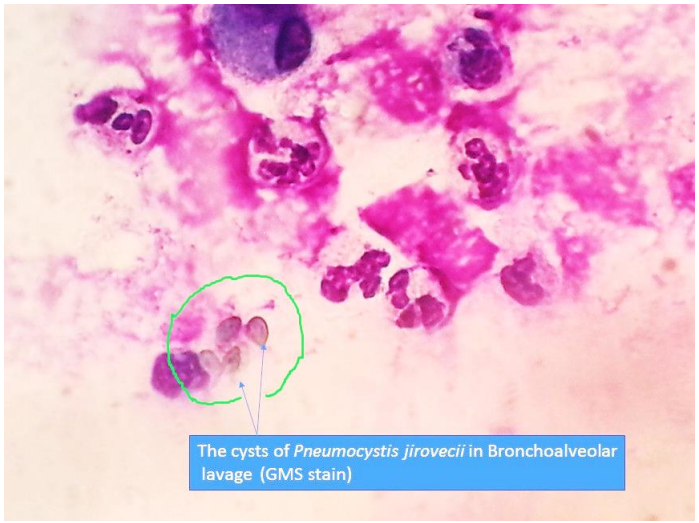
Clinical presentation of *Pneumocystis* pneumonia

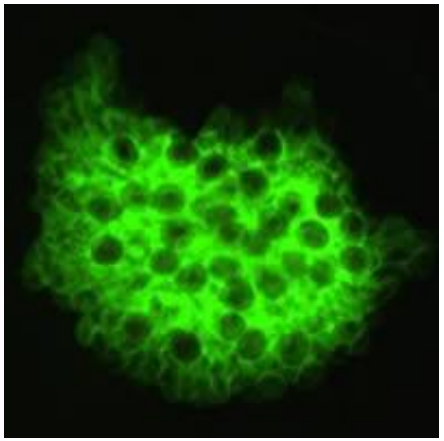
- **symptoms of PCP** include dyspnoea, non-productive cough, chest pain, chills, fatigue, fever
- chest radiography demonstrates bilateral infiltrates
- **extrapulmonary lesions** occur in a minority (<3%) of patients, involving most frequently the lymph nodes, spleen, liver, and bone marrow
- in untreated PCP increasing pulmonary involvement leads to **death**

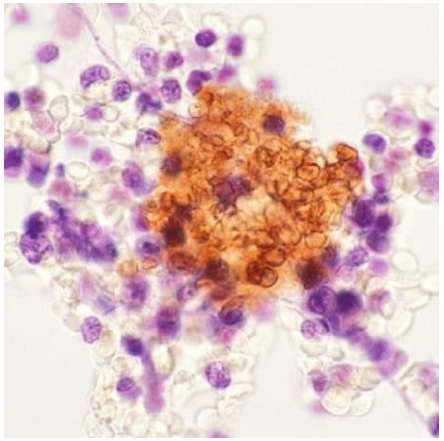


Diagnosis of PCP

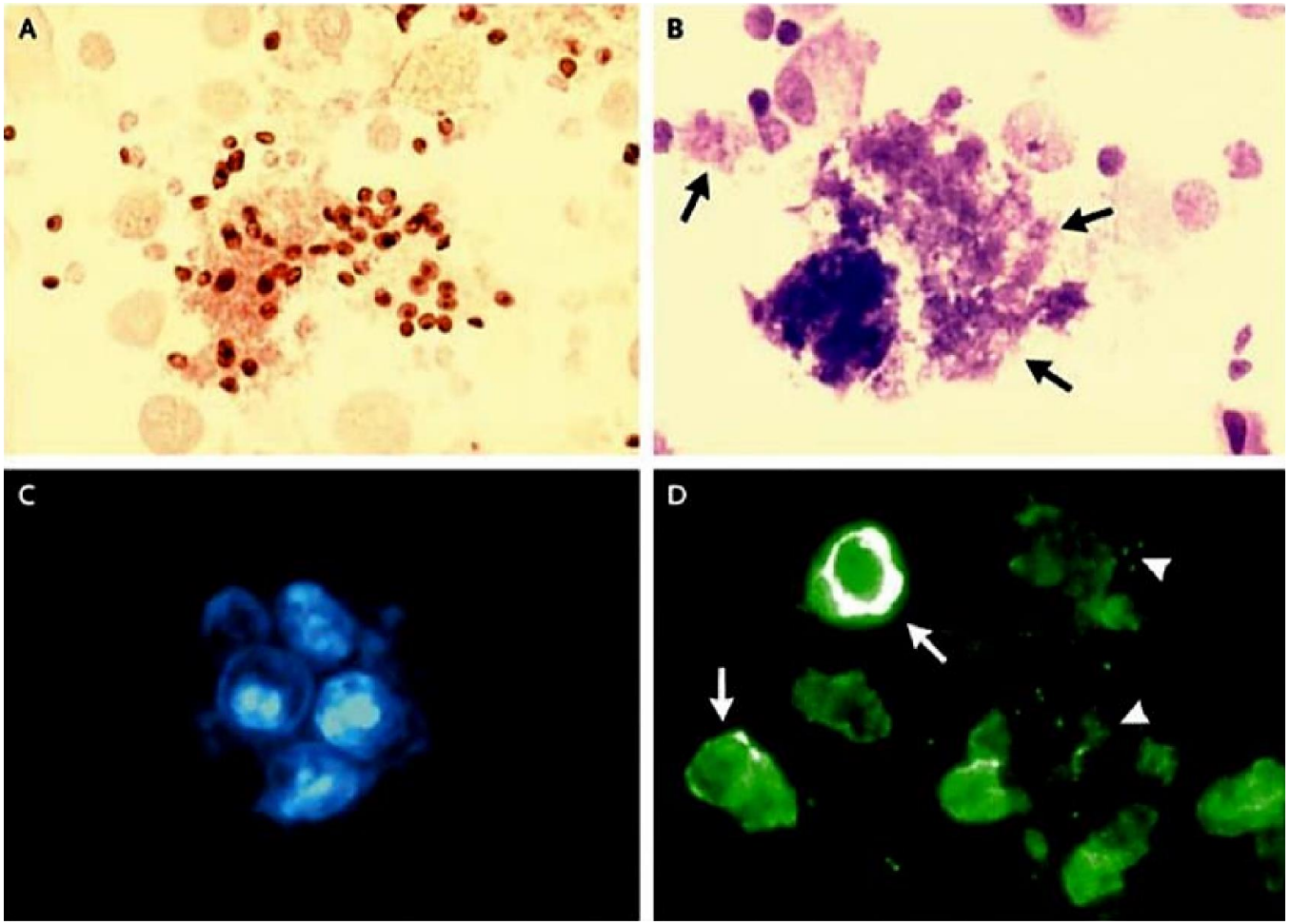
- collection of bronchioalveolar fluid with an endoscope
- identification of *P. jirovecii* in bronchopulmonary secretions obtained as induced (or noninduced) sputum or bronchoalveolar lavage (BAL)
- if the above techniques cannot be used, transbronchial or open lung biopsy may prove necessary
- Giemsa, Gram-Weigert or silver staining
- immuno(fluorescence) microscopy using monoclonal antibodies
- PCR
- serological tests: for screening only





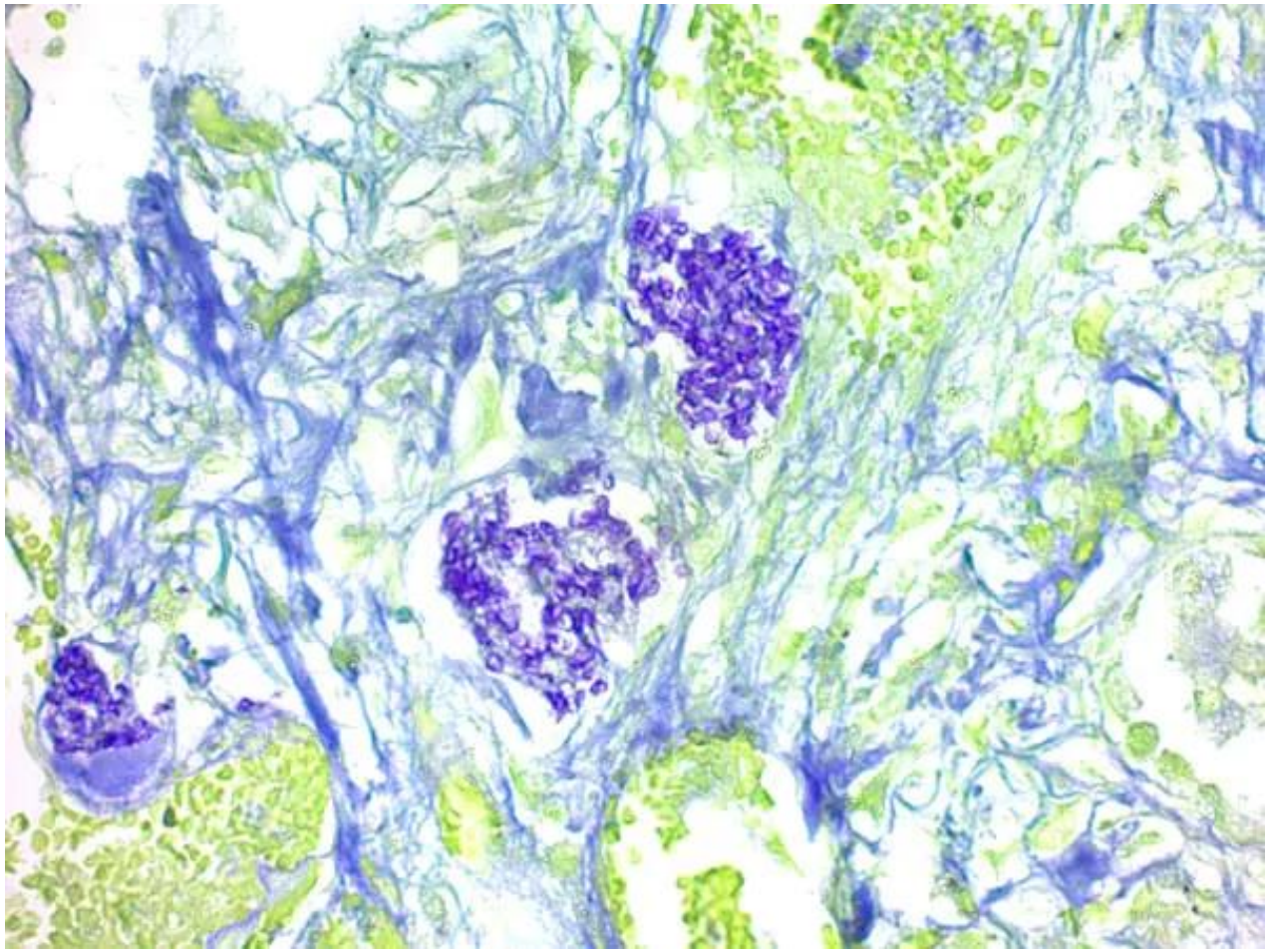


Diagnosis of PCP



Detection of *Pneumocystis* forms with the use of different stains. A) Typical pneumocystis cyst forms in a bronchoalveolar-lavage specimen stained with Gomori methenamine. Thick cyst walls and some intracystic bodies are evident. **B**) Wright–Giemsa staining can be used for rapid identification of trophic forms of the organisms within foamy exudates (arrows) in bronchoalveolar-lavage fluid or induced sputum but usually requires a high organism burden and expertise in interpretation. **C**) Calcofluor white is a fungal cyst-wall stain that can be used for rapid confirmation of the presence of cyst forms. **D**) Immunofluorescence staining can sensitively and specifically identify both *Pneumocystis* trophic forms (arrowheads) and cysts (arrows).

Diagnosis of PCP

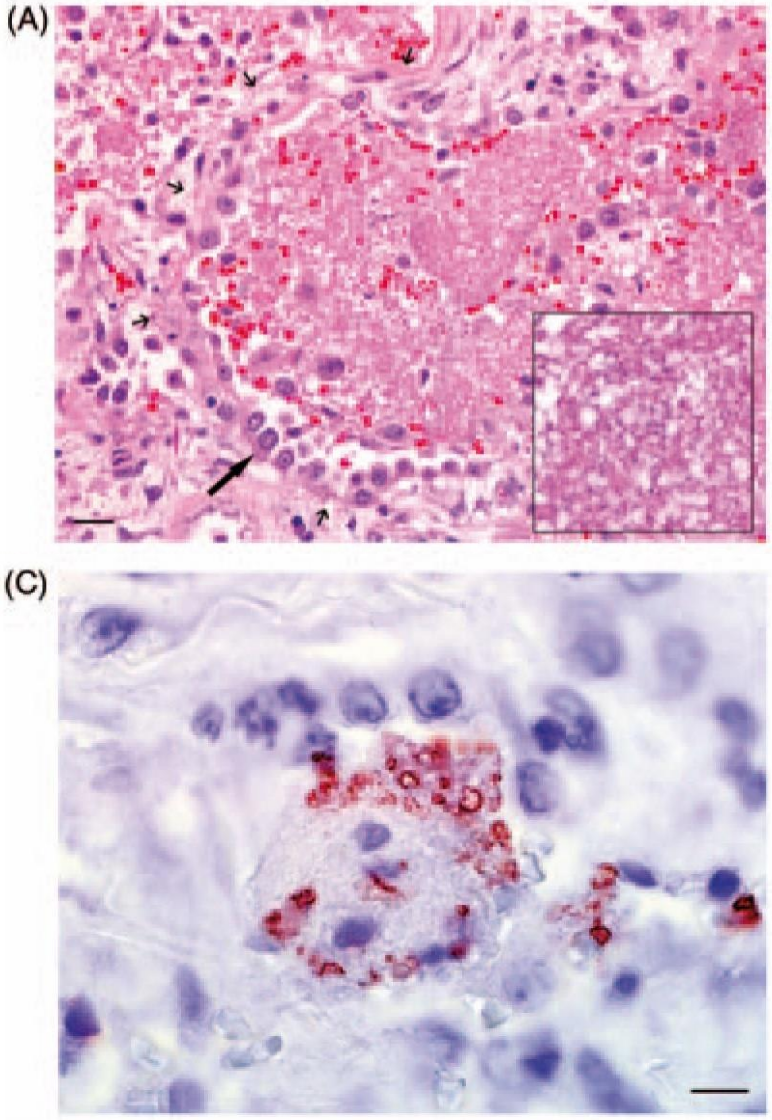


***Pneumocystis* stain kit** (formalin-fixed-paraffin embedded specimen)

Staining Interpretation

Pneumocystis carinii	**Violet/Purple**
Connective Tissue	Blue/Green
Erythrocytes	Yellow
Mucin	Rose/Purple
Cartilage	Rose/Purple

Diagnosis of PCP

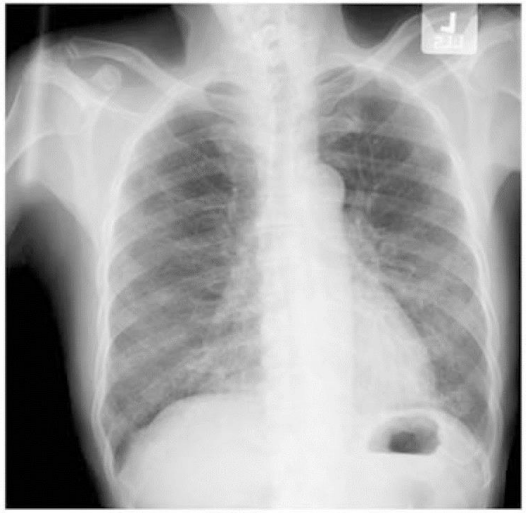


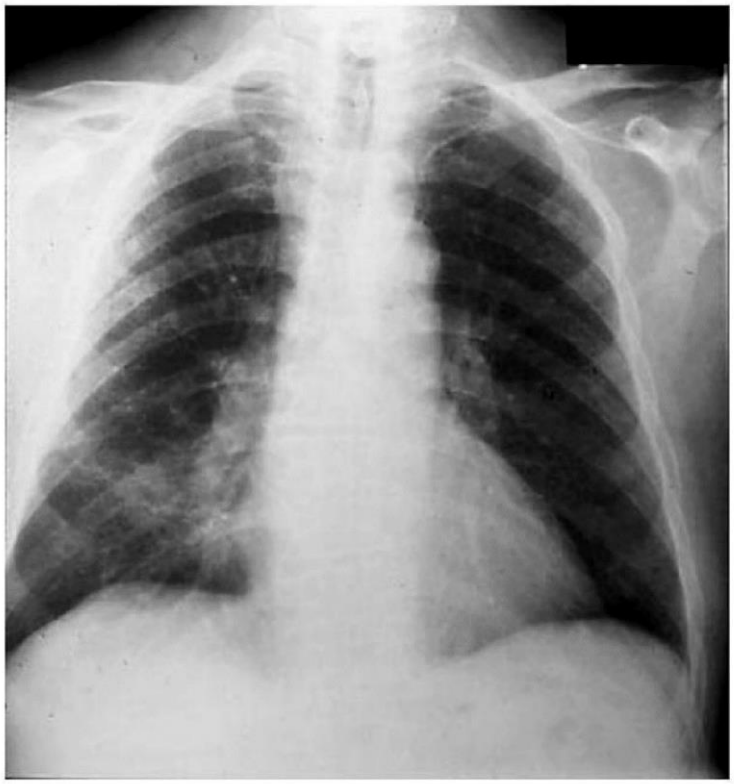
Histological features of *Pneumocystis* pneumonia. A) *Pneumocystis* pneumonia in a surgical lung biopsy specimen. The characteristic frothy exudates harbouring the organisms fills an alveolus (small arrows) lined by reactive type II cells (large arrow). Internal structures of the organisms can be discerned at high magnification (inset). HE, **B**) Silver stain of *Pneumocystis* organisms in a surgical lung biopsy. *Pneumocystis* organisms appear round or cup-shaped, some show intracystic bodies (lower right). Gomori's methenamine silver staining. **C**) Immunohistochemical staining of surgical lung biopsy for *Pneumocystis*.

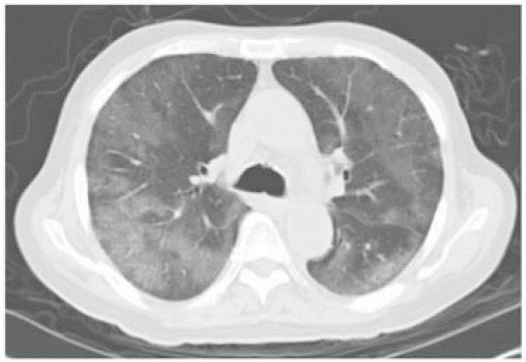
https://doi.org/10.1177/1753465810380102

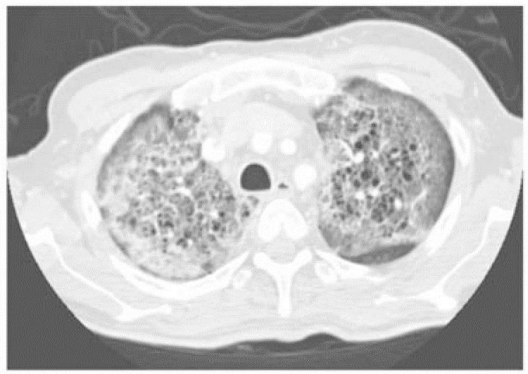
Pathology and diagnosis of PCP

A) Posteroanterior chest radiograph showing diffuse bilateral predominantly interstitial opacities in 71-year-old male with *Pneumocystis* pneumonia in the setting of diffuse large B-cell lymphoma on R-CHOP therapy. **B**) Selected non-contrast CT axial image revealing bilateral diffuse ground-glass opacities and associated interlobular and intralobular septal thickening. **C**) Contrast CT axial image revealing diffuse ground-glass pulmonary infiltrates and innumerable cystic changes predominantly in the upper lobes in a 53-year-old male with *Pneumocystis* pneumonia in the setting of recently diagnosed HIV.







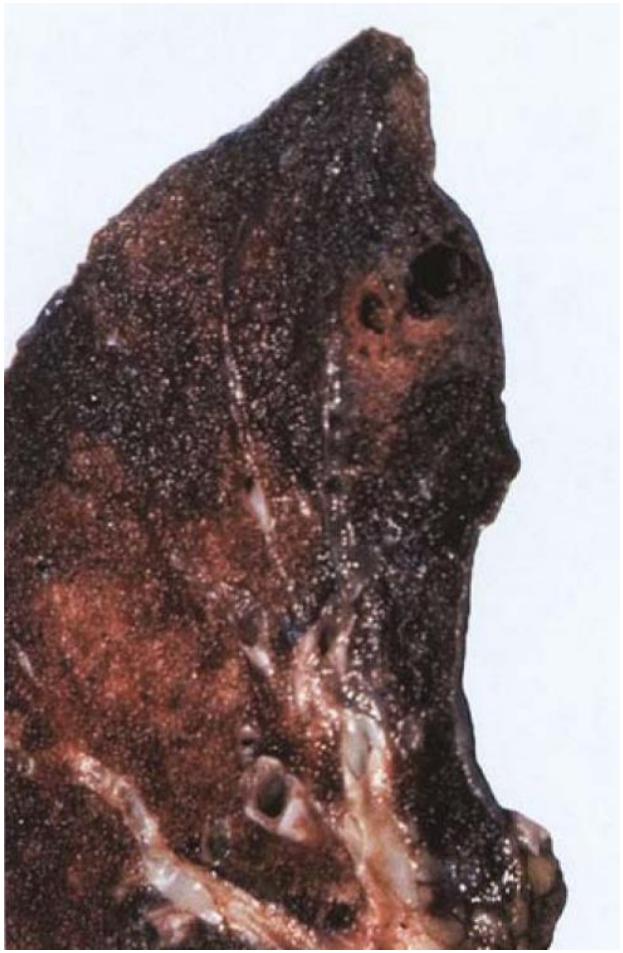


Posteroanterior chest radiograph of a 68-year-old patient with *Pneumocystis* pneumonia that developed as a consequence of long-term corticosteroid therapy for an inflammatory neuropathy.

Mixed alveolar and interstitial infiltrates are more prominent on the right side.

Pathology and diagnosis of PCP

Gross photograph of lung from a patient who died within 5 days of onset of respiratory failure. The lung weighed 1750 g and showed a tan solid cut surface, consistent with diffuse alveolar damage (DAD).



Necrotizing *P. jirovecii* pneumonia. Apical subpleural cavity due to necrotizing PCP in a patient with AIDS.

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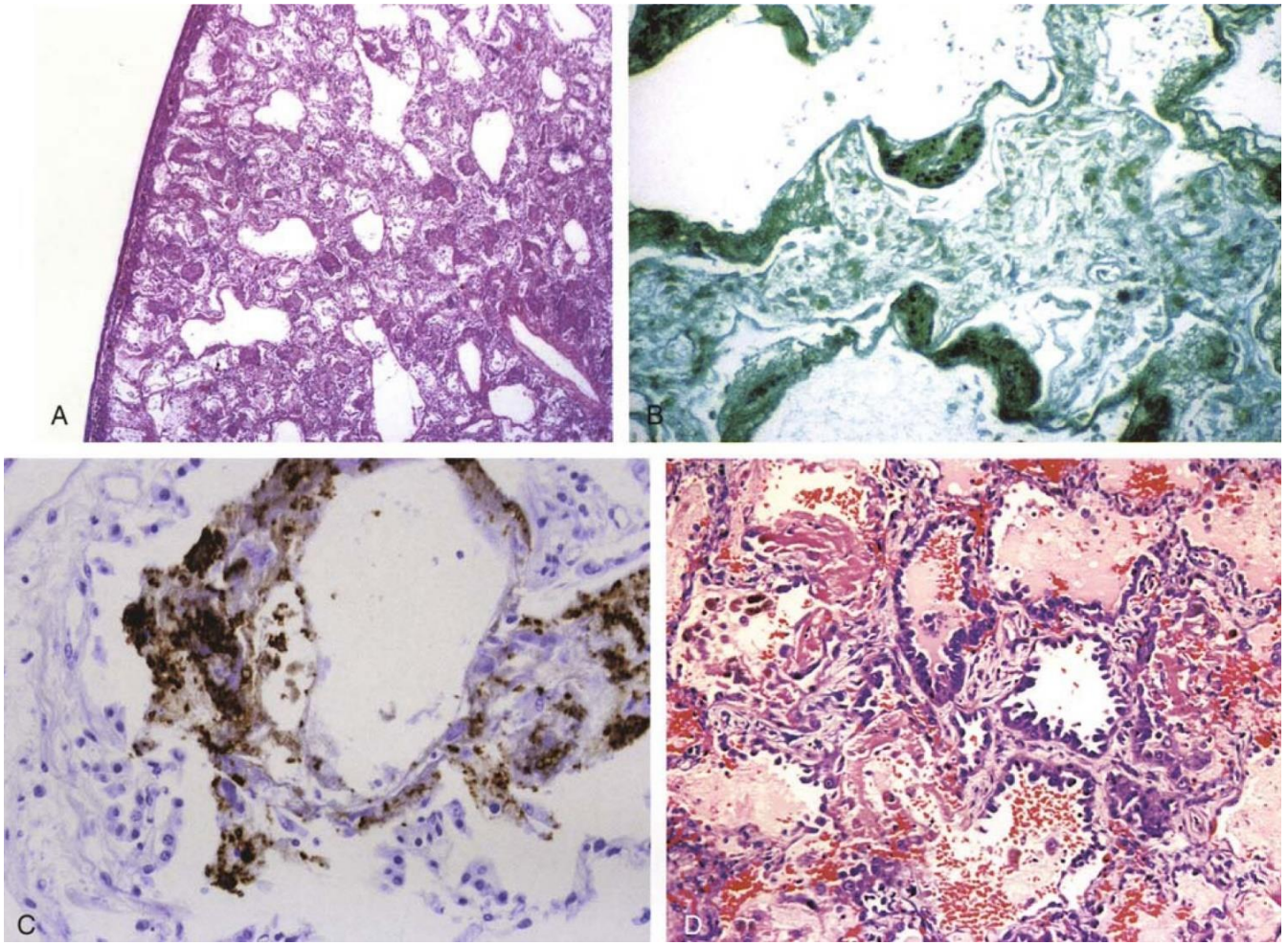
Pathology and diagnosis of PCP



A) Lung (gross photograph shown in previous slide) showing typical histologic pattern of exudative stage of DAD. Alveolar ducts are dilated and lined by hyaline membranes, and surrounded by collapsed alveoli. Intraalveolar foamy exudates of *Pneumocystis* are also visible. **B**) Higher magnification of hyaline membranes showing embedded *Pneumocystis* cysts. GMS. **C**) Immunostaining for *Pneumocystis* may also be used to demonstrate the cysts and trophozoites in the hyaline membranes. **D**) Lung from a patient with long-standing *Pneumocystis* infection shows enlarged and atypical type II pneumocytes with high nuclear cytoplasmic ratio, as seen in atypical alveolar hyperplasia.

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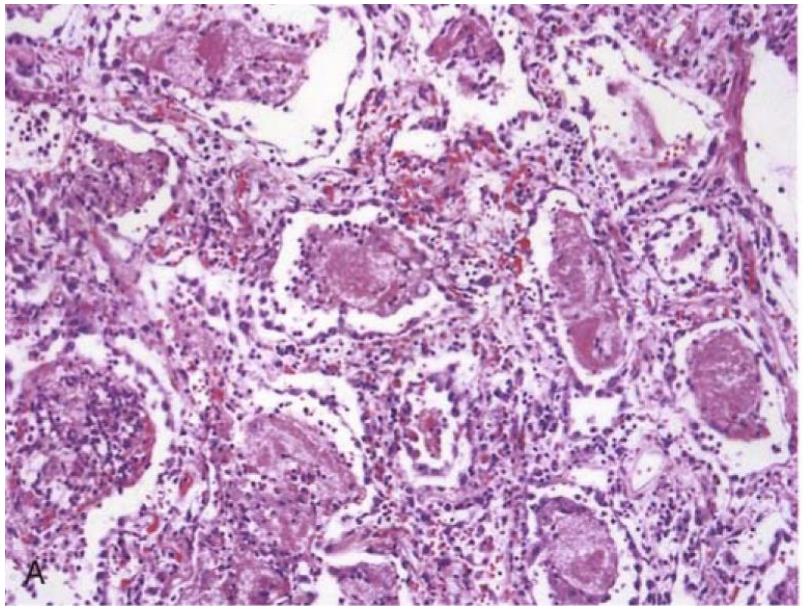
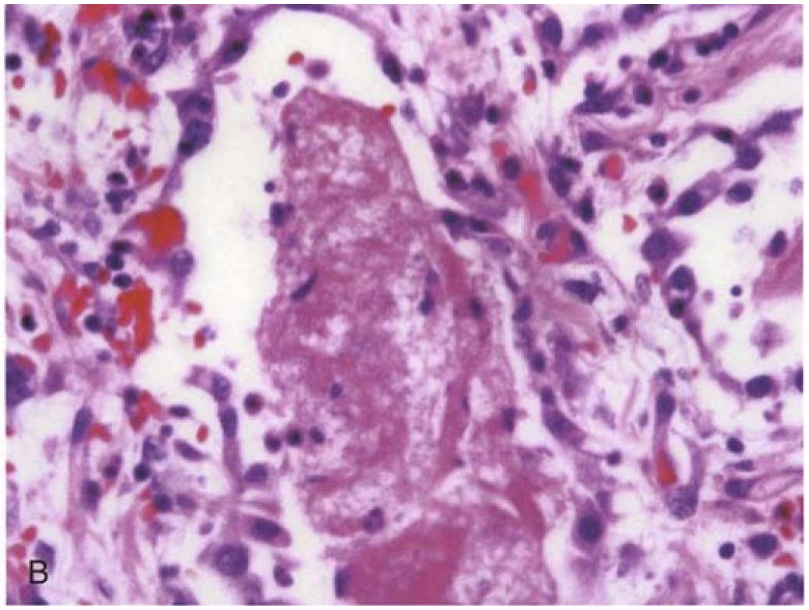
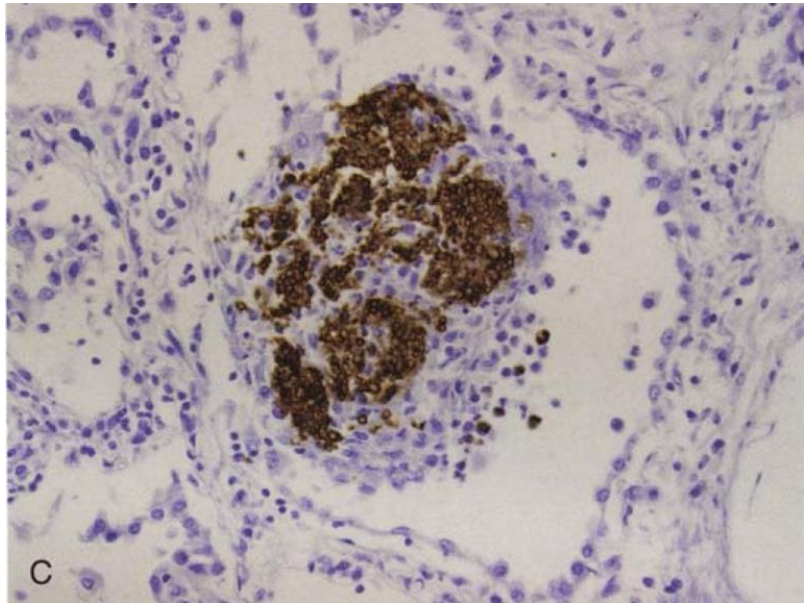
Pathology and diagnosis of PCP





A) Low-magnification photomicrograph shows thickened alveolar septa with mild interstitial inflammation and fibrosis, lined by hyperplastic type II pneumocytes. Alveoli are filled with typical foamy, eosinophilic exudate of *Pneumocystis*. **B**) Higher magnification shows an alveolus with the foamy/bubbly eosinophilic exudates and a few mononuclear cells. Basophilic dots are visible within the exudate. Alveolar septa have a few inflammatory cells and collagen, and are lined by hyperplastic type II pneumocytes. **C**) Immunostain demonstrates the cyst forms of *Pneumocystis*, surrounded by inflammatory cells. Trophozoites also stained with the immunostain; however, these are not as clearly visualized as the cyst forms.



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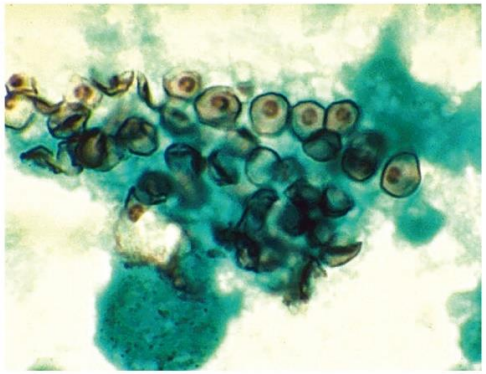
etymologia

Pneumocystis jirovecii [noo"mo-sis'tis ye"ro-vet'ze]

Ronnie Henry

A genus of unicellular fungi, *Pneumocystis* was likely originally described by Carlos Chagas in 1909 in guinea pigs, although he confused it with a trypanosome and placed it in a new genus, *Schizotrypanum*. In 1912, Delanoë and Delanoë at the Pasteur Institute published the first description of the new organism as unrelated to trypanosomes and proposed the species name *P. carinii i*n honor of Antonio Carini.

Human *Pneumocystis* infections were first reported in 1942 by van der Meer and Brug, but not until 1976 did Frenkel report different morphologic and physiologic characteristics of human and rat *Pneumocystis* isolates. He proposed the name *P. jirovecii* in honor of Czech parasitologist Otto Jírovec, who reported *Pneumocystis* as a cause of interstitial pneumonia in infants, although this name change was not accepted by researchers at the time. When *Pneumocystis* was reclassified from a protozoan to a fungus, the naming convention shifted from the International Code of Zoological Nomenclature to the International Code of Botanical Nomenclature, and the species epithet was modified from *jiroveci* to *jirovecii*.



Cysts of *Pneumocystis jirovecii* in smear from bronchoalveolar lavage. Methenamine silver stain. CDC/Dr. Russell K. Brynes

Sources

1. Frenkel JK. *Pneumocystis jiroveci* n. sp. from man: morphology, physiology, and immunology in relation to pathology. Natl Cancer Inst Monogr. 1976;43:13–30.
2. Hawksworth DL. Responsibility in naming pathogens: the case of *Pneumocystis jirovecii,* the causal agent of pneumocystis pneumonia. Lancet Infect Dis. 2007;7:3–5, discussion 5. http://dx.doi.org/10.1016/S1473-3099(06)70663-6
3. Stringer JR, Beard CB, Miller RF, Wakefield AE. A new name (*Pneumocystis jiroveci*) for *Pneumocystis* from humans and new perspectives on the host-pathogen relationship. Emerg Infect Dis. 2002;8:891–6.http://dx.doi.org/10.3201/eid0809.020096
4. Stringer JR, Beard CB, Miller RF. Spelling *Pneumocystis jirovecii.* Emerg Infect Dis. 2009;15:506. http://dx.doi.org/10.3201/eid1503.081060
5. Walzer PD, Smulian AG. *Pneumocystis* species. In: Mandell GL, Bennett, JE, Dolin R, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease. 7th edition. Philadelphia: Elsevier; 2010. p. 3377–90.

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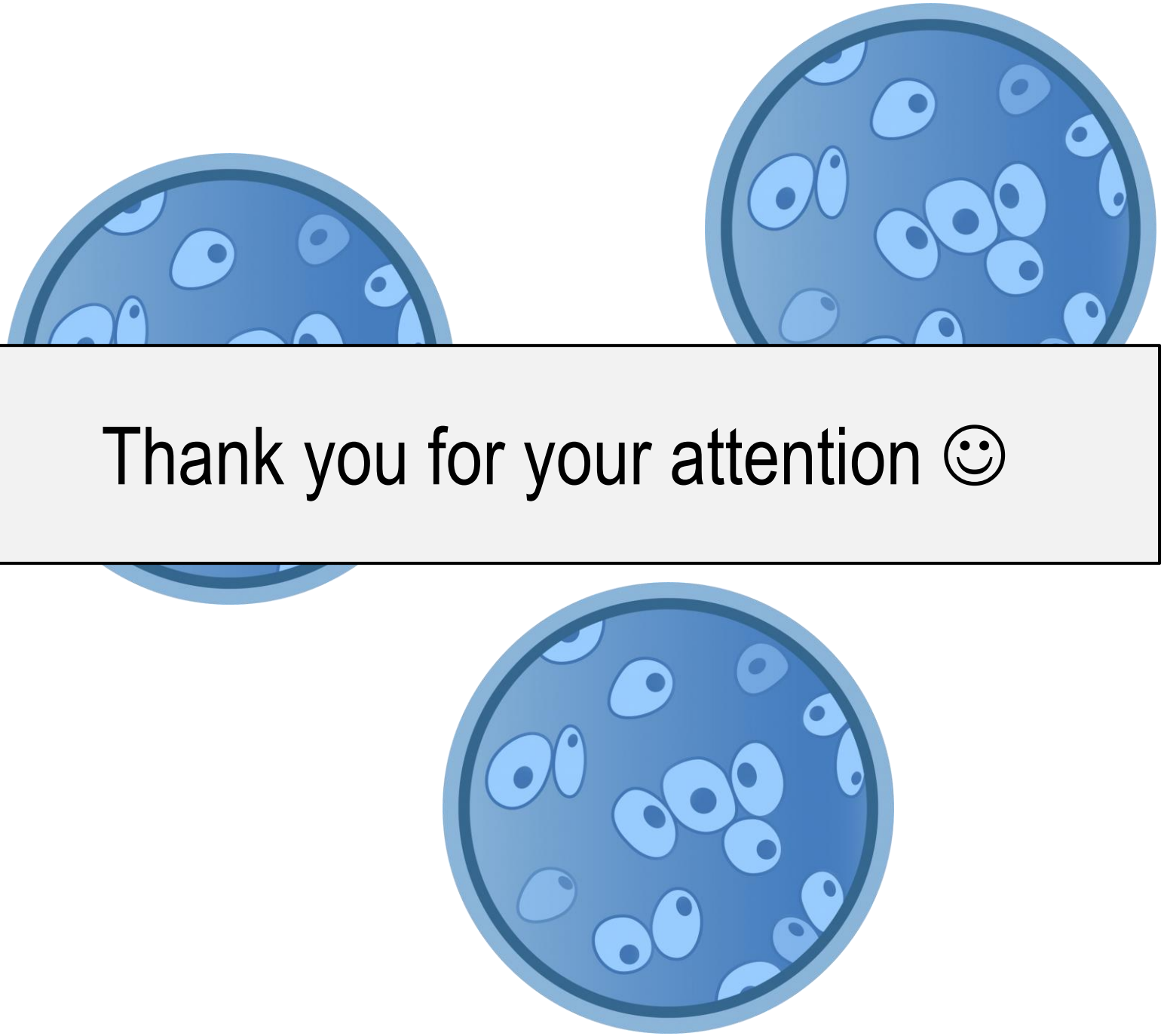

Thank you for your attention ☺

Lectures

- ✓ Introduction: BPP 2022 I
- ✓ Euglenozoa (Excavata): BPP 2022 II
- ✓ Fornicata / Preaxostyla / Parabasala (Excavata): BPP 2022 III
- ✓ Apicomplexa I (SAR): BPP 2022 IV
- ✓ Apicomplexa II (SAR): BPP 2022 V
- ✓ Amoebae (Excavata, Amoebozoa): BPP 2022 VI
- ✓ Ciliophora, Opalinata (SAR): BPP 2022 VII

- ✓ Pneumocystis (Opisthokonta, Fungi): BPP 2022 VIII
- ⇨ **Microsporidia (Opisthokonta, Fungi): BPP 2022 IX**
- Myxozoa (Opisthokonta, Animalia): BPP 2022 X