# New Notions on *Pneumocystis* Natural History

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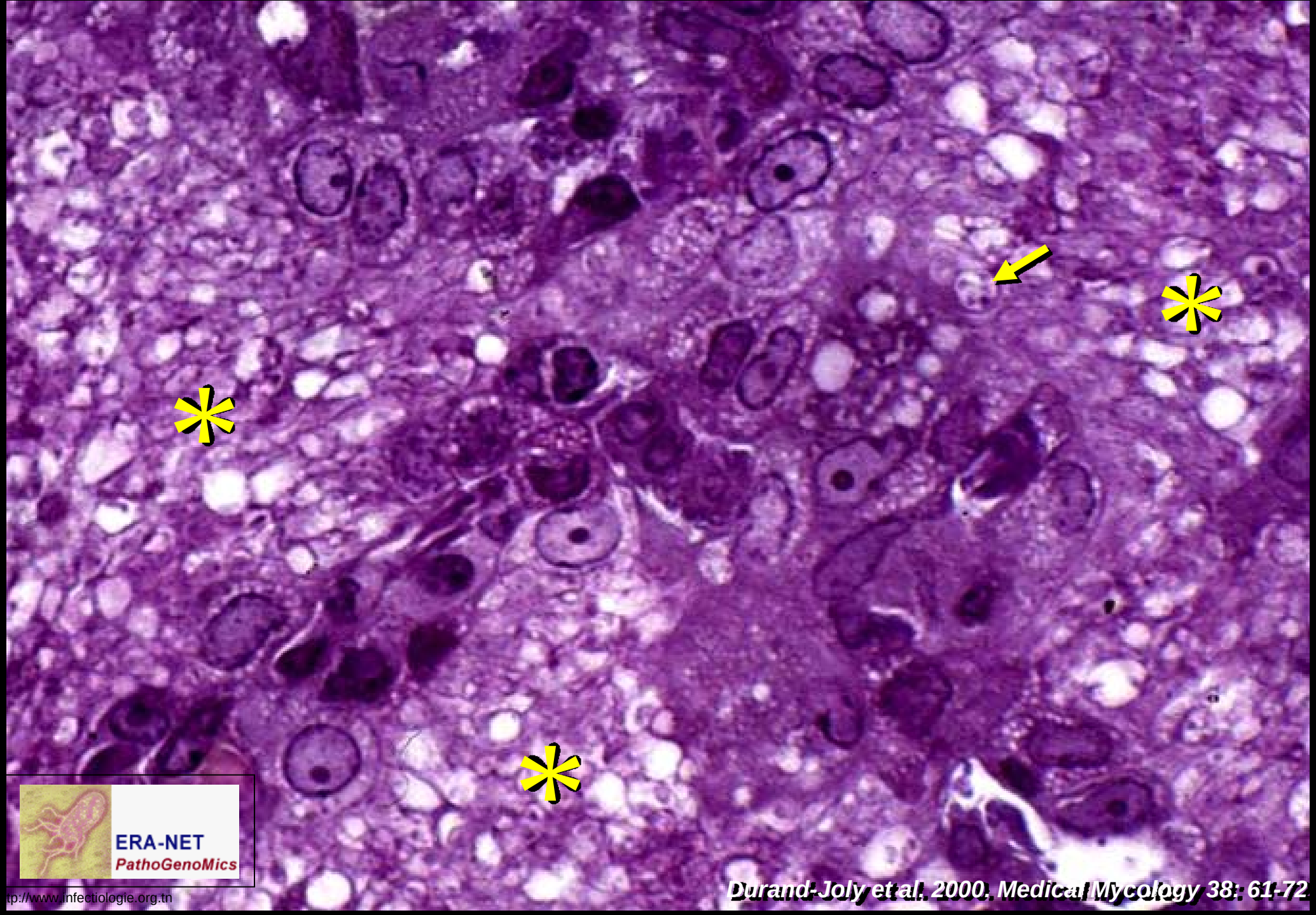
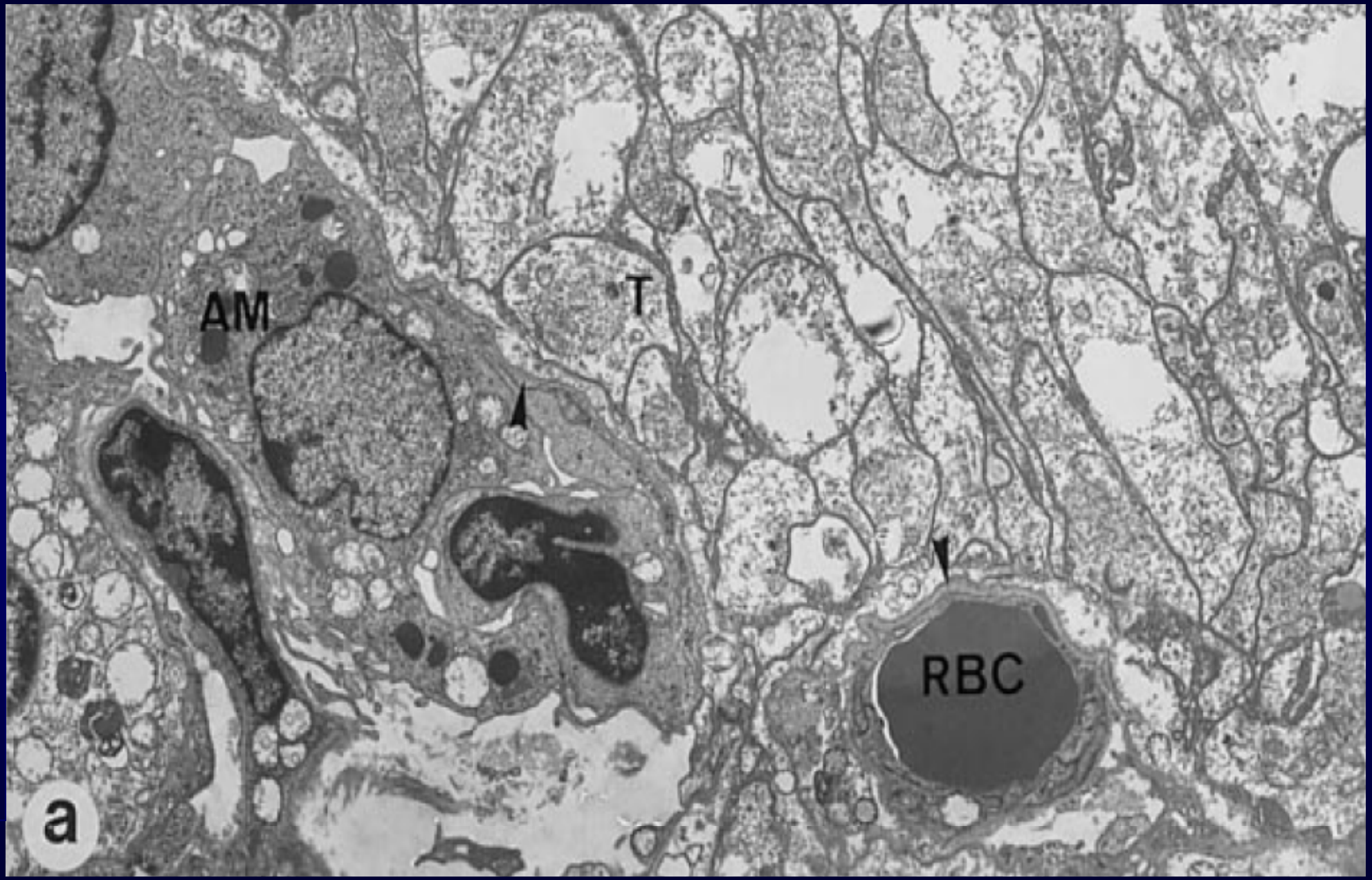
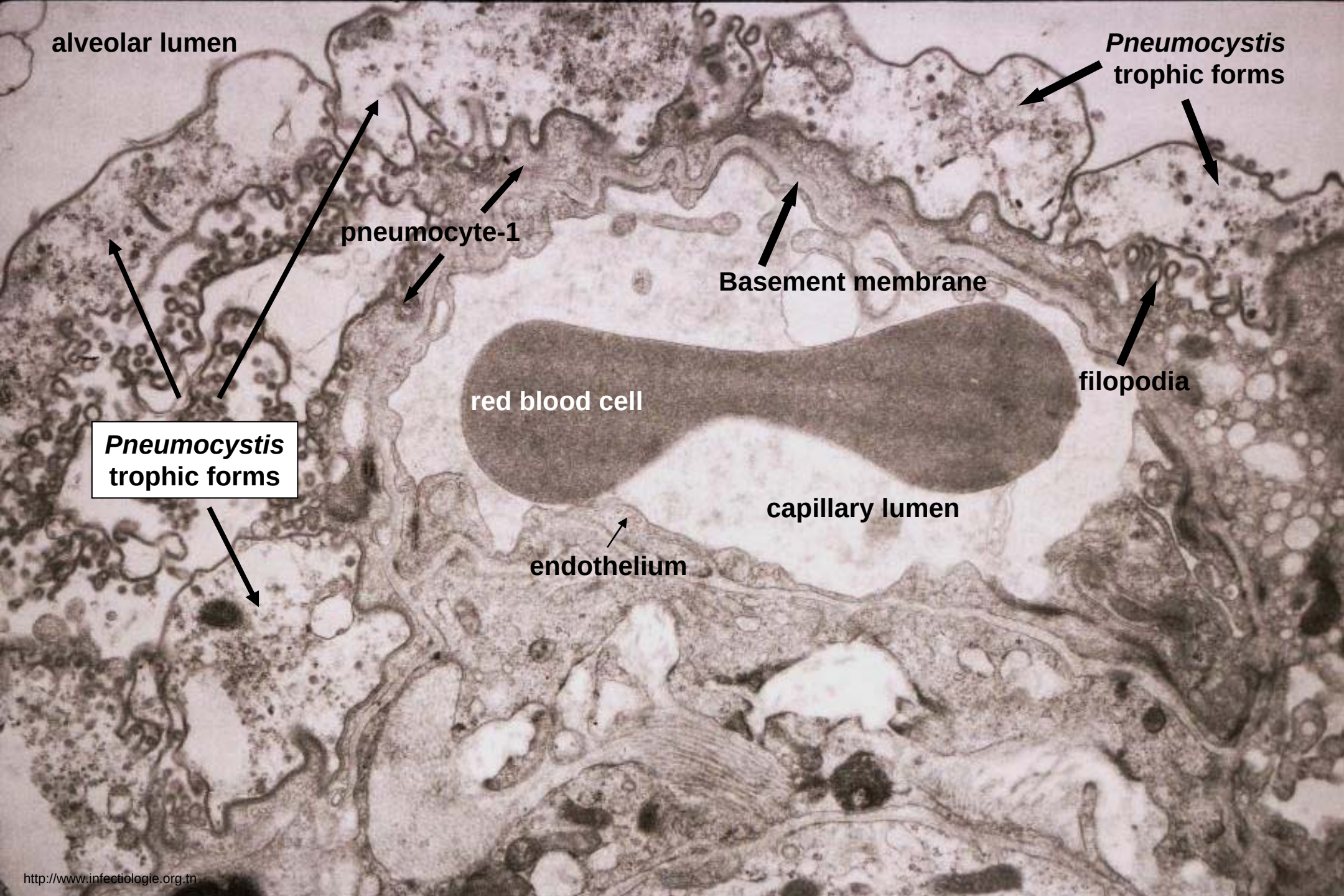
# *Pneumocystis*: what is it?

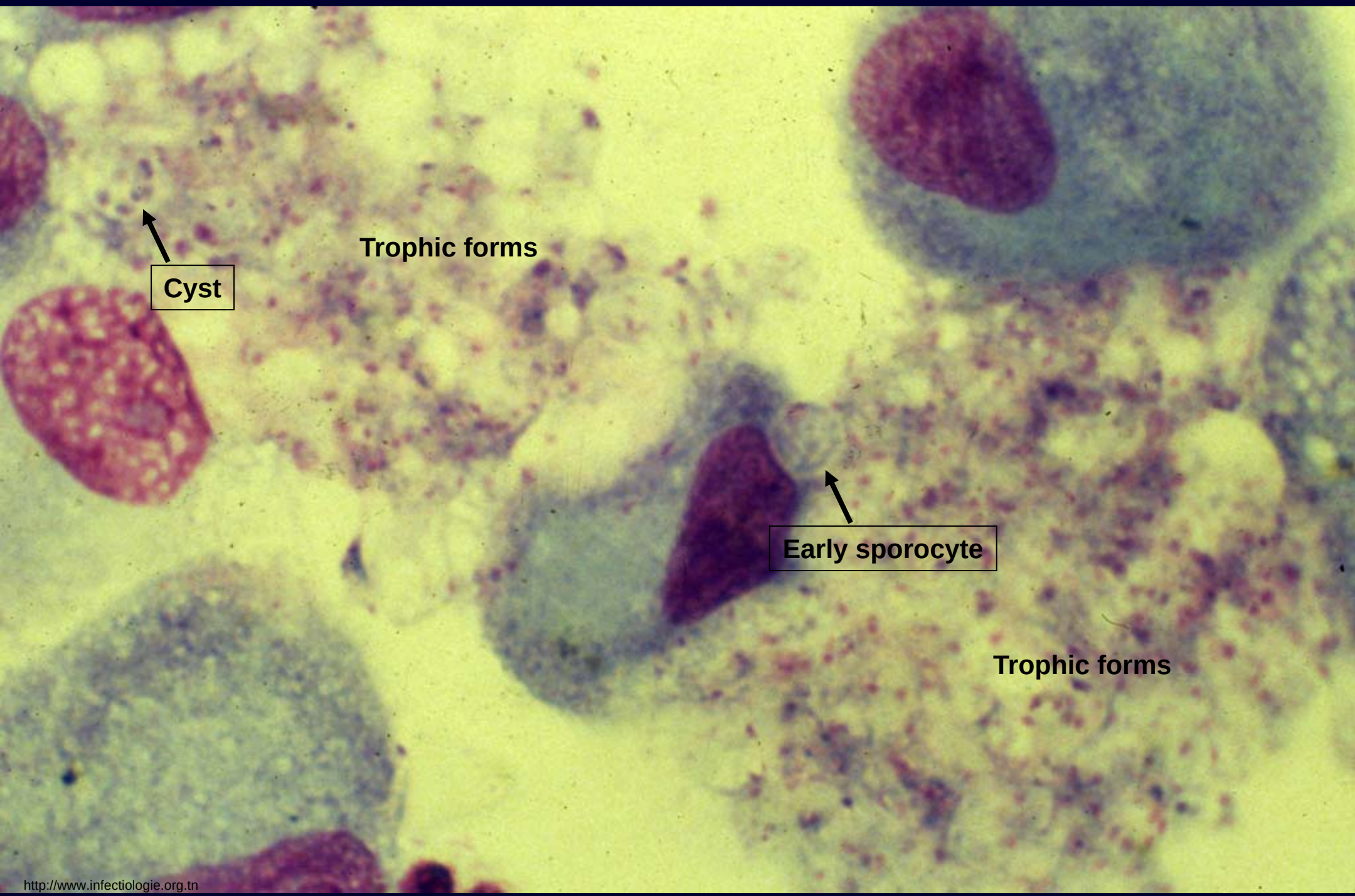


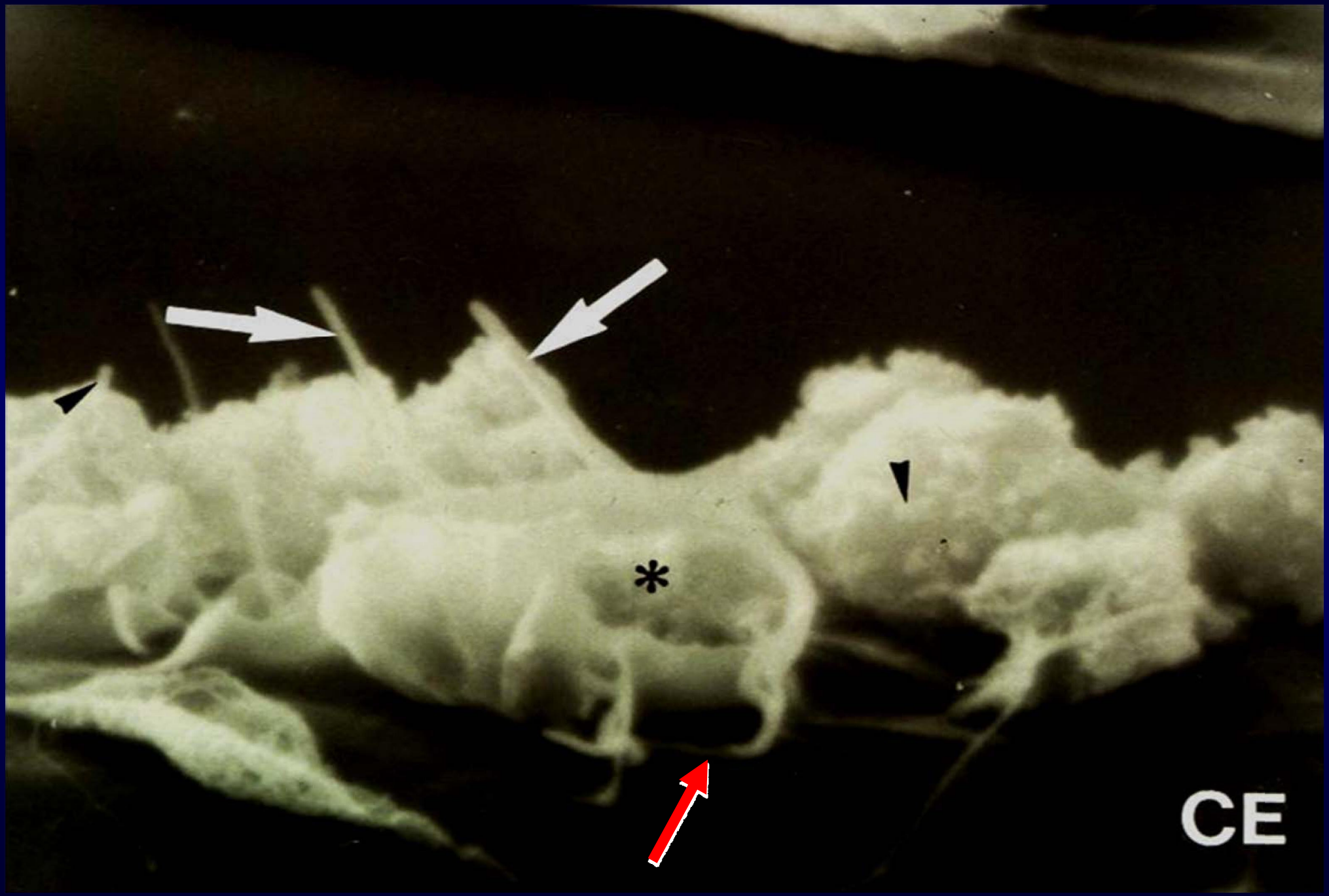

Durand-Joly et al. 2000. Medical Mycology 38: 61-72




*Durand-Joly et al, 2000, Med Mycol 38: 61-72*

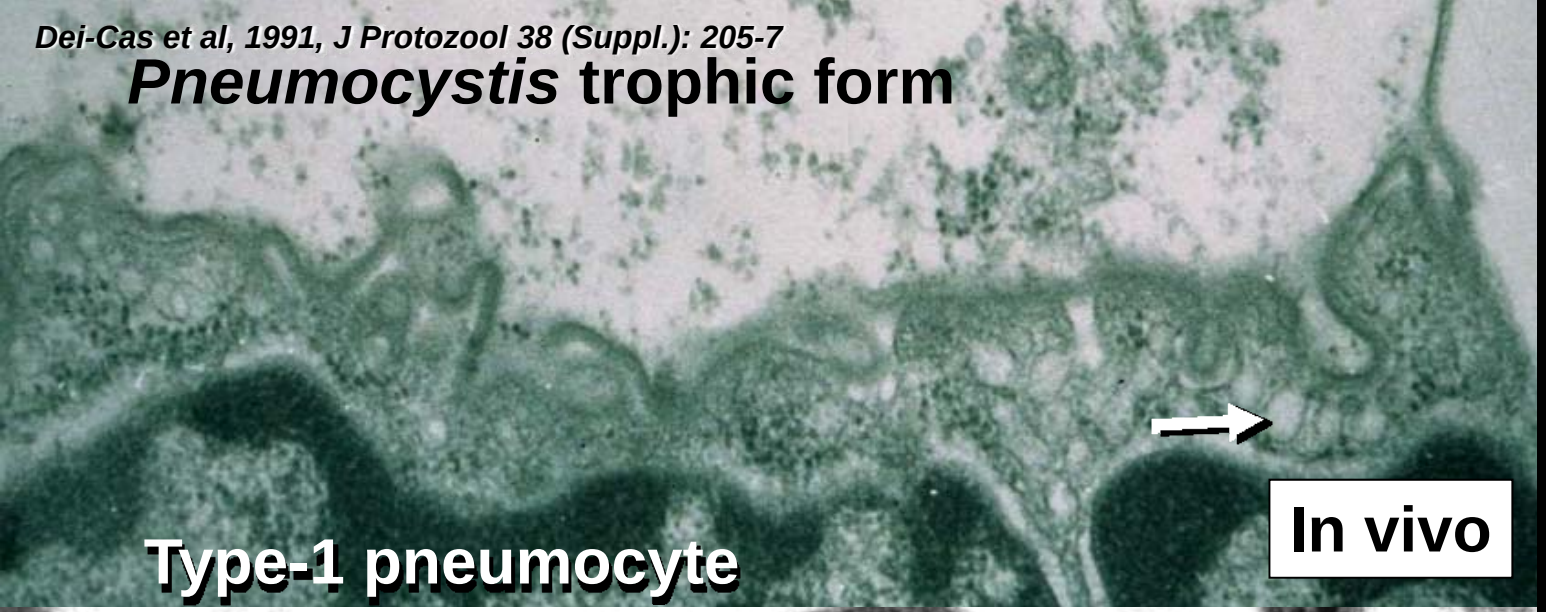






Dei-Cas et al, 1991, J Protozool 38 (Suppl.): 205-7

# *Pneumocystis* trophic form



Type-1 pneumocyte

In vivo

Aliouat EM (original micrograph)


In vitro

# *Pneumocystis* Attachment

No apparent direct alteration of the host cell


Settnes OP, Nielsen MJ, 1991, J Protozool 38 (Suppl): 174-6

Dei-Cas et al, 1991, J Protozool 38 (Suppl.): 205-7

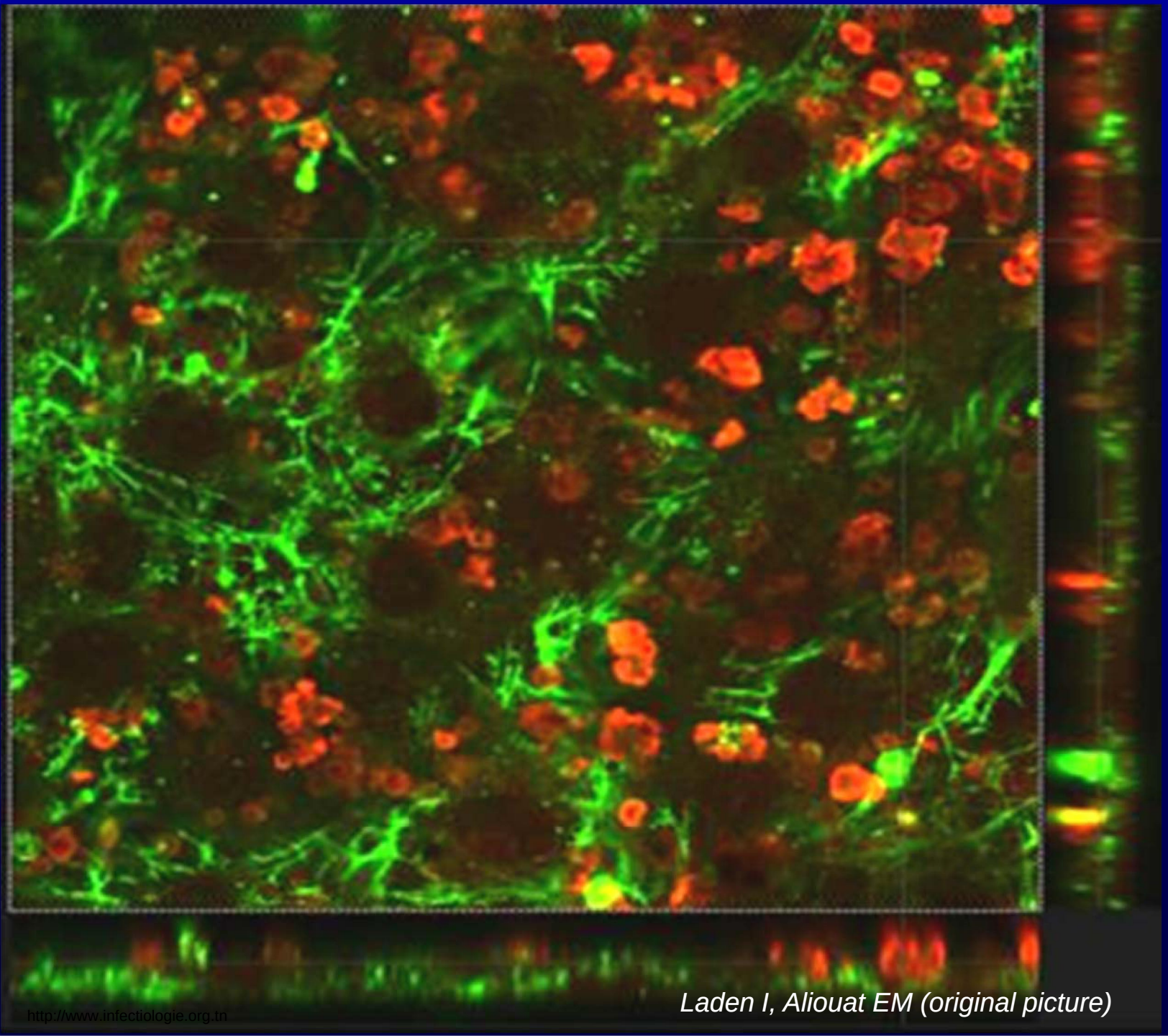
Aliouat et al, 1992, Biol Cell 77: 209-17

Beck et al, 1998, Am J Physiol 275: L118-25

Dei-Cas et al, 1998, J Mycol Méd 8: 1-6

Dei-Cas E. 2000, Med Mycol 38 Suppl 1: 23-32

Dei-Cas et al, 2005, Cellular Structure, In : 'Pneumocystis carinii Pneumonia' Marcel Dekker, Inc., New York, p 61-94



*Laden I, Aliouat EM (original picture)*



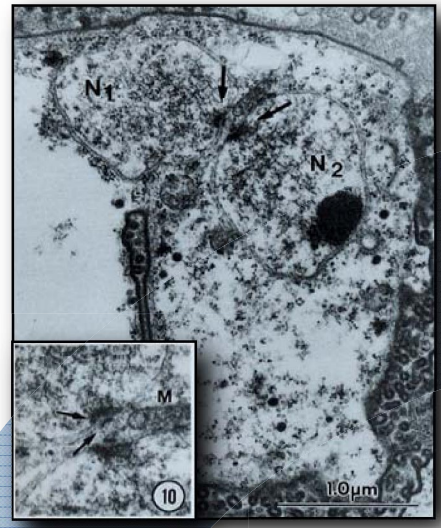
# *P. carinii* organisms co-cultured with L2 cells

*P. carinii* did not change:

- fibronectin matrix organisation
- actin network
- distribution of endosomal vesicles

## ✓ Main morphological changes:

- Cell wall thickening and rarefaction of filopodia
- Synaptonemal complexes
- Multiple nuclear division
- Unclear evidence of binary fission
- Morphogenesis of spores
- Evidence of conjugation



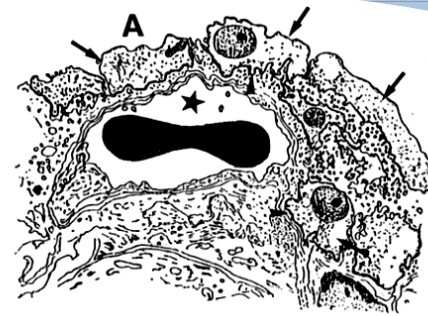

Itatani CA, 1996 J Parasitol 82:163-71

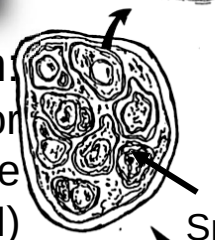
n + n

2 n


Matsumoto & Yoshida, 1984
J Protozool 31:420-8

Trophic forms



Cyst

Spores

Late sporocyte

Intermediate sporocyte

Early sporocyte

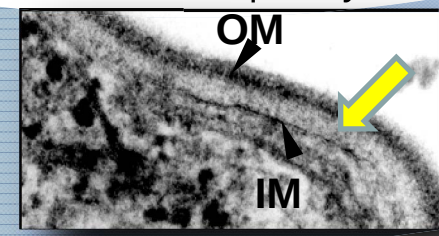
n

### ✓Conjugation:

- *Ste3* pheromone receptor
- *Ste2*-like homologue
- *P. carinii* MAPK (PCM)
- PC homol *Ste12/Ste20*
- PCST20


Aliouat et al,1999 J Eukaryot Microbiol 46 (Suppl): 116-7
Aliouat-Denis et al, 2009 Mem Inst O Cruz 104: 419-26
Burgess et al, 2008 Infect Immun 76: 417-25
Chabé et al, 2004 EurJ Clin Microbiol Infec Dis 23: 89-97
Cushion MT 2004 Trends Microbiol 12: 223-9
Cushion MT et al, 2007 PLoS ONE 2(5): e423. doi:10.137.
Kottom et al, 2003 Infect Immun 71: 6463-71
Kottom et al, 2000 Am J Respir Cell Mol Biol. 22:722-31
Schmatz et al, 1990 Proc Natl Acad Sci USA 87: 5950-4
Schmatz et al, 1991 J Protozool 38 (Suppl.): 151-3
Smulian et al, 2001 Genetics 157: 991-02
Stringer & Cushion, 1998 FEMS Immunol Med Microbiol 22: 15-26
The Pneumocystis Genome Project - http://pgp.cchmc.org/
Thomas et al, 1998 Am J Physiol 275: L193-9
Villegas et al, 2009 Am J Respir Cell Mol Biol (E-pub)
Vohra et al, 2003 Gene 312: 173-9
Vohra et al, 2003 FEBS Lett 551: 139-46
Vohra et al, 2004 Biochem Biophys Res Commun 319: 193-9
Wyder et al, 1998 J Eukaryot Microbiol 45: 233-9


n



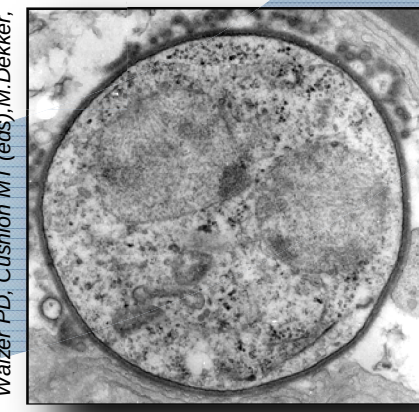

Palluault et al, 1992 Parasitol Res 78: 437-44

### ✓Meiosis:

- PC*Mei2*
- PC*Ran1*

### ✓Mitosis:

- PCCdc2
- PM*Cdc2*
- Cdc13
- Cdc25



Dei-Cas et al, 2004, Cellular Structure. In : Walzer PD, Cushion MT (eds),M.Dekker,

# *Pneumocystis* Diversity

*Aliouat-Denis et al, 2008.* ***Infect Genet Evol*** *8: 708-26.*

*Dei-Cas et al, 2006.* ***FEMS Microbiology Reviews*** *30: 853-71.*

*Redhead et al, 2006.* ***J Eukaryot Microbiol*** *53: 2–11.*

# Taxonomy of *Pneumocystis*

## MAIN REASONS WHY *PNEUMOCYSTIS* IS A FUNGUS

1- Numerous fungal ortholog genes

2- Presence of Elongation Factor 3 gene

3- DHFR and TS activities supported by 2 different proteins

4- Other reasons:

- microscopic stain affinity
- chitin in cell wall
- susceptibility to sordarins
- susceptibility to echinocandins

http://www.infectiologie.org.tn

## Bats

***Pneumocystis* spp. from numerous bat species**

Tropical Rainforest

## Marsupials

***Pneumocystis* sp. from 1 species**

Tropical Rainforest

## Cetaceans

***Pneumocystis* sp. from 1 species (needs confirmation)**

Marine biome

Table 3. Pairwise distances (%) of eight *Pneumocystis* genes

| | SODA | | TS | | HSP70 | | DHPS | | DHFR | | AROM | | mtrRNA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nt | AA | Nt | AA | Nt | AA | Nt | AA | Nt | AA | Nt | AA | LSU | SSU |
| *P. oryctolagi*/*P. jirovecii* | 15.0 | 14.6 | 12.5 | 9.1 | 34.4 | 33.1 | 15.0 | 16.3 | 24.0 | 25.5 | 17.3 | 16.7 | 18.0 | 17.0 |
| *P. oryctolagi*/*P. carinii* | 24.4 | 29.7 | 16.5 | 14.2 | 35.7 | 35.3 | 17.6 | 18.9 | 33.4 | 39.1 | 21.9 | 25.0 | 26.4 | 17.0 |
| *P. oryctolagi*/*P. wakefieldiae* | NA | NA | NA | NA | 16.4 | 36.1 | NA | NA | NA | NA | NA | NA | 22.2 | 18.1 |
| *P. oryctolagi*/*Pc* f.sp. *mustelae* | NA | NA | NA | NA | NA | NA | 17.8 | 23.1 | 32.8 | 32.8 | 18.8 | 26.0 | 18.0 | 17.0 |
| *P. oryctolagi*/*P. murina* | 24.0 | 29.1 | 15.9 | 15.2 | 23.5 | 16.2 | 17.2 | 19.3 | 33.7 | 35.5 | 21.0 | 25.0 | 21.6 | 16.0 |
| *P. oryctolagi*/*Pc* f.sp. *macaca* | 15.6 | 13.4 | NA | NA | NA | NA | 15.2 | 17.4 | 25.2 | 28.2 | NA | NA | 19.8 | 14.5 |
| *P. jirovecii*/*P. carinii* | 23.0 | 25.5 | 16.5 | 11.2 | 17.1 | 14.0 | 16.5 | 17.4 | 33.7 | 40.0 | 18.2 | 20.4 | 24.0 | 19.5 |
| *P. jirovecii*/*P. wakefieldiae* | NA | NA | NA | NA | 19.1 | 14.0 | NA | NA | NA | NA | NA | NA | 23.4 | 20.5 |
| *P. jirovecii*/*Pc* f.sp. *mustelae* | NA | NA | NA | NA | NA | NA | 18.5 | 22.3 | 18.2 | 28.2 | 17.3 | 19.5 | 19.8 | 18.1 |
| *P. jirovecii*/*P. murina* | 21.8 | 24.3 | 16.2 | 13.2 | 33.5 | 30.2 | 15.1 | 17.4 | 32.5 | 33.7 | 17.3 | 19.5 | 21.6 | 18.4 |
| *P. jirovecii*/*Pc* f.sp. *macaca* | 7.1 | 1.9 | NA | NA | NA | NA | 9.9 | 11.7 | 18.8 | 21.9 | NA | NA | 12.5 | 10.3 |
| *P. carinii*/*P. wakefieldiae* | NA | NA | NA | NA | 14.9 | 10.3 | NA | NA | NA | NA | NA | NA | 9.6 | 8.9 |
| *P. carinii*/*Pc* f.sp. *mustelae* | NA | NA | NA | NA | NA | NA | 18.0 | 23.4 | 28.8 | 33.7 | 18.5 | 23.2 | 21.6 | 16.0 |
| *P. carinii*/*P. murina* | 5.1 | 3.7 | 14.8 | 3.1 | 31.5 | 31.7 | 5.9 | 6.5 | 16.1 | 17.3 | 6.8 | 7.5 | 9.0 | 7.5 |
| *P. carinii*/*Pc* f.sp. *macaca* | 22.6 | 25.5 | NA | NA | NA | NA | 15.5 | 18.5 | 34.3 | 40.0 | NA | NA | 28.3 | 14.9 |
| *P. wakefieldiae*/*Pc* f.sp. *mustelae* | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 19.2 | 15.2 |
| *P. wakefieldiae*/*P. murina* | NA | NA | NA | NA | 33.0 | 33.1 | NA | NA | NA | NA | NA | NA | 9.6 | 7.8 |
| *P. wakefieldiae*/*Pc* f.sp. *macaca* | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 26.4 | 17.4 |
| *Pc* f.sp. *mustelae*/*P. murina* | NA | NA | NA | NA | NA | NA | 16.8 | 21.9 | 27.3 | 26.4 | 17.6 | 21.3 | 18.6 | 15.6 |
| *Pc* f.sp. *mustelae*/*Pc* f.sp. *macaca* | NA | NA | NA | NA | NA | NA | 18.3 | 23.8 | 30.0 | 31.9 | NA | NA | 23.7 | 14.9 |
| *P. murina*/*Pc* f.sp. *macaca* | 21.6 | 24.3 | NA | NA | NA | NA | 15.3 | 18.5 | 31.3 | 34.6 | NA | NA | 27.0 | 13.5 |

The pairwise distances (%) presented in the matrix were calculated for each pair of aligned sequences as follows: 100 – % identity.

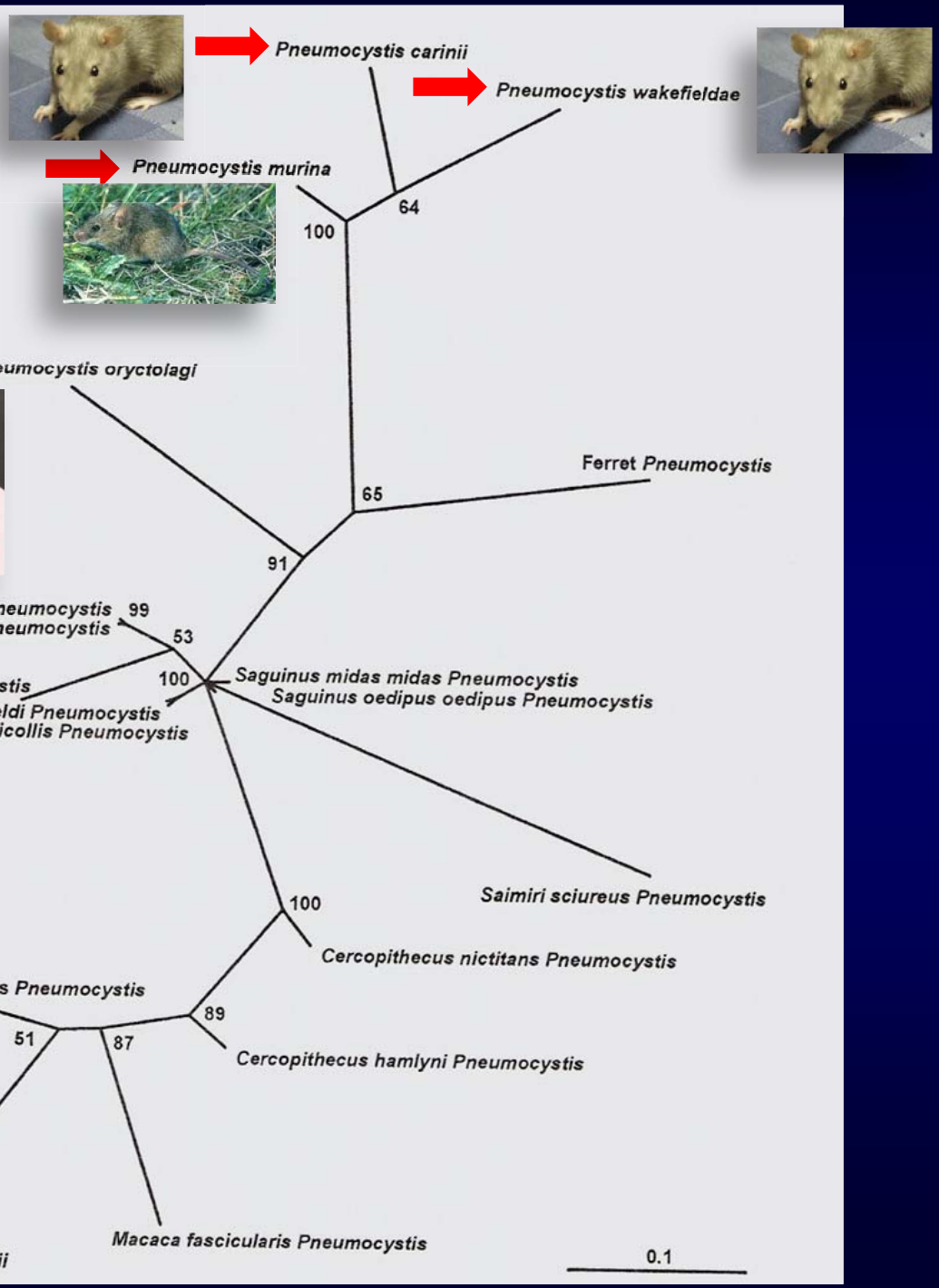
Common positions of the DNA alignments and common residues of the protein alignments:

SODA: nt: 496 AA: 165. TS: nt: 297 AA: 99. HSP70: nt: 410 AA: 136. DHPS: nt 798 AA: 265. DHFR: nt: 330 AA: 110. AROM: nt 325 AA: 108. mtLSU-rRNA: 167. mtSSU-rRNA: 283. NA, nonaligned.



Dei-Cas et al, 2006, FEMS Microbiol Rev 30: 853-71

*Dei-Cas et al, 2006, FEMS Microbiol Rev 30: 853-71 (modifié)*




http://rulai.cshl.edu/gbrowse/images/house_mouse_picture.jpg

*Dei-Cas et al, 2006, FEMS Microbiol Rev 30: 853-71 (Modifié)*

http://rulai.cshl.edu/gbrowse/images/house_mouse_picture.jpg

*Pneumocystis carinii*

*Pneumocystis wakefieldae*

*Pneumocystis murina*

64

100

*Pneumocystis oryctolagi*

Ferret *Pneumocystis*

65

91

*Callithrix jacchus Pneumocystis*
*Callithrix geoffroyi Pneumocystis*

99

53

100

*Pithecia pithecia Pneumocystis*

*Callimico goeldi Pneumocystis*
*Saguinus fuscicollis Pneumocystis*

*Saguinus midas midas Pneumocystis*
*Saguinus oedipus oedipus Pneumocystis*

*Saimiri sciureus Pneumocystis*

100

*Cercopithecus nictitans Pneumocystis*

Indian macaca rhesus *Pneumocystis*

89

51

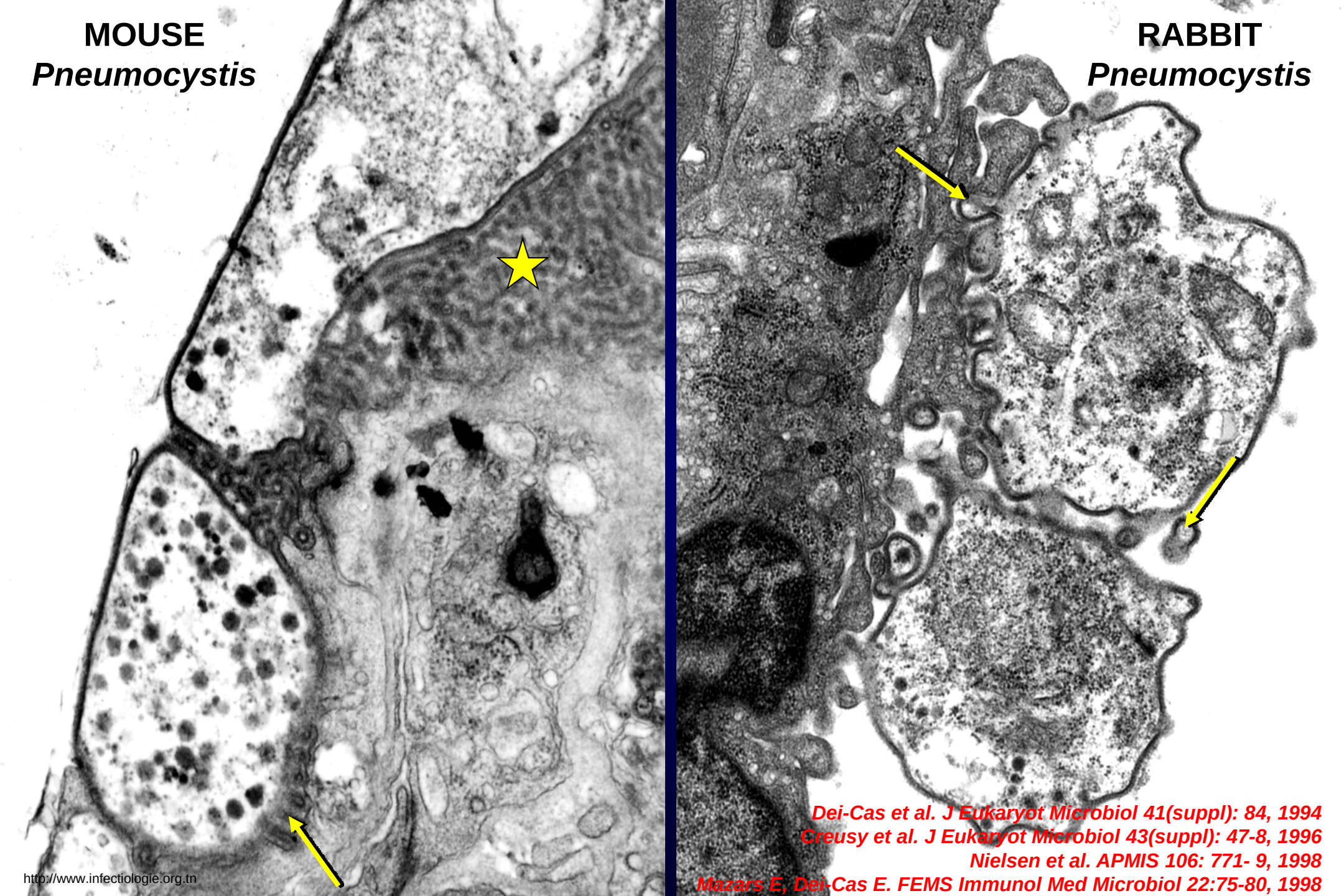
87

*Cercopithecus hamlyni Pneumocystis*

*Macaca fascicularis Pneumocystis*

*Pneumocystis jirovecii*

0.1

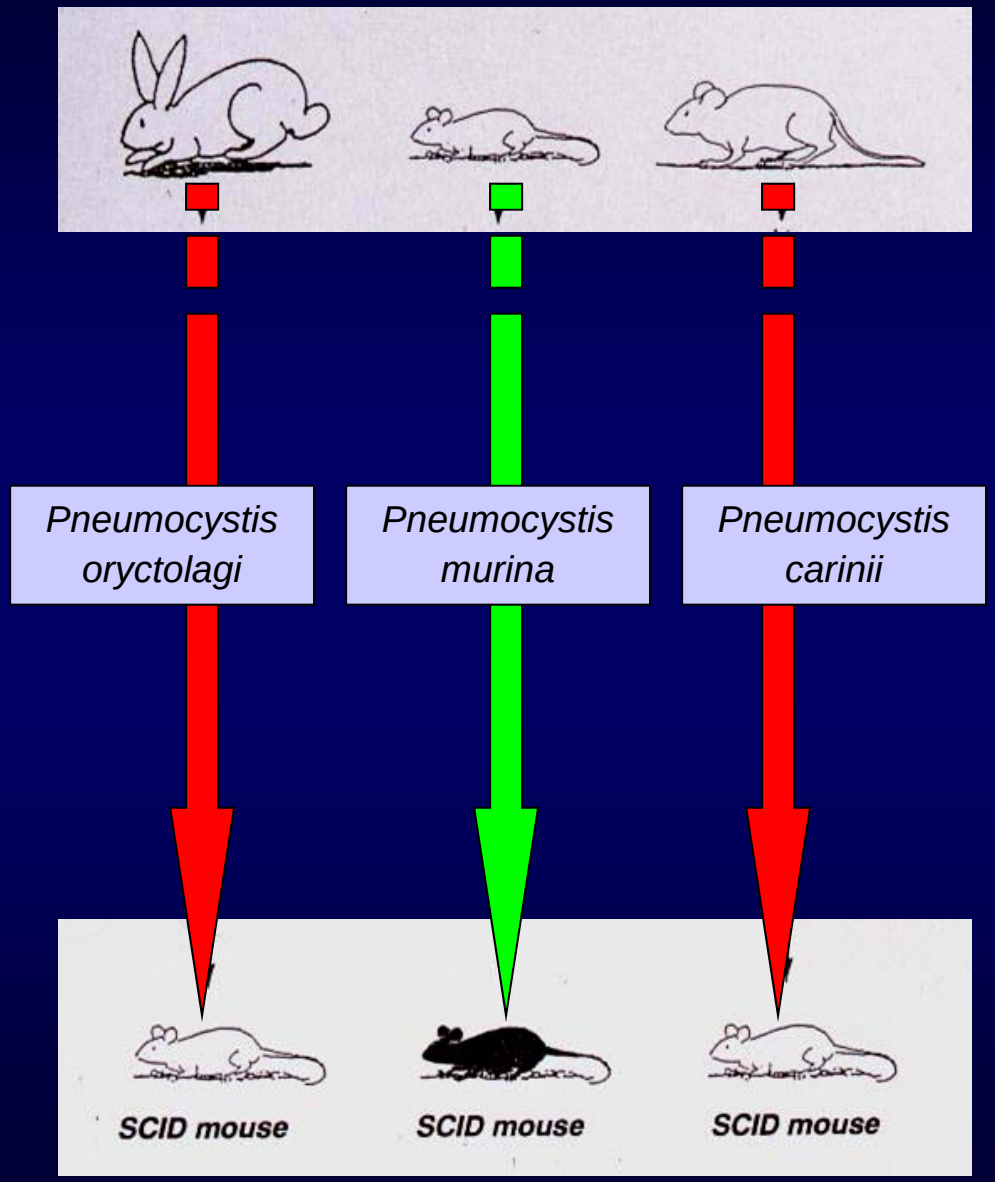

MOUSE
Pneumocystis
RABBIT
Pneumocystis
Dei-Cas et al. J Eukaryot Microbiol 41(suppl): 84, 1994
Creusy et al. J Eukaryot Microbiol 43(suppl): 47-8, 1996
Nielsen et al. APMIS 106: 771- 9, 1998
Mazars E, Dei-Cas E. FEMS Immunol Med Microbiol 22:75-80, 1998
http://www.infectiologie.org.tn

# *Pneumocystis* spp : different growth rate in terms of the host species

| Host species | Doubling time (days) *In vivo* | *In vitro* |
|---|---|---|
| Rat | 4.5 | 4.1 (co-culture)* |
| Mouse | 10.3 | ND |
| Rabbit | 1.5 | ND |

* 1.7 - 2.2 in axenic cultures.

*(Aliouat et al, 1999, J Eukaryot Microbiol 46 Suppl:116 - 117)*



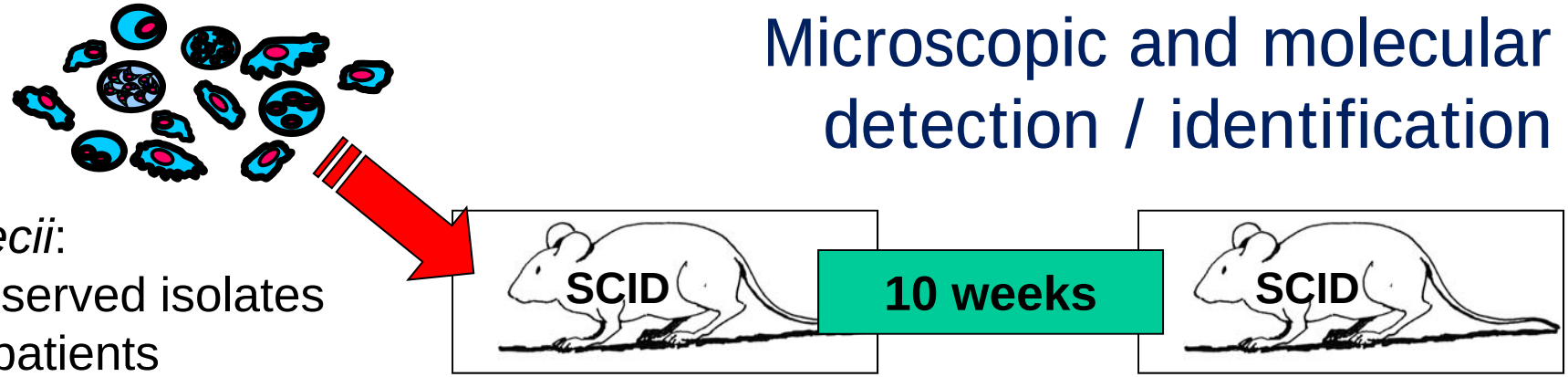

# Cross Infection Experiments :

## close host specificity

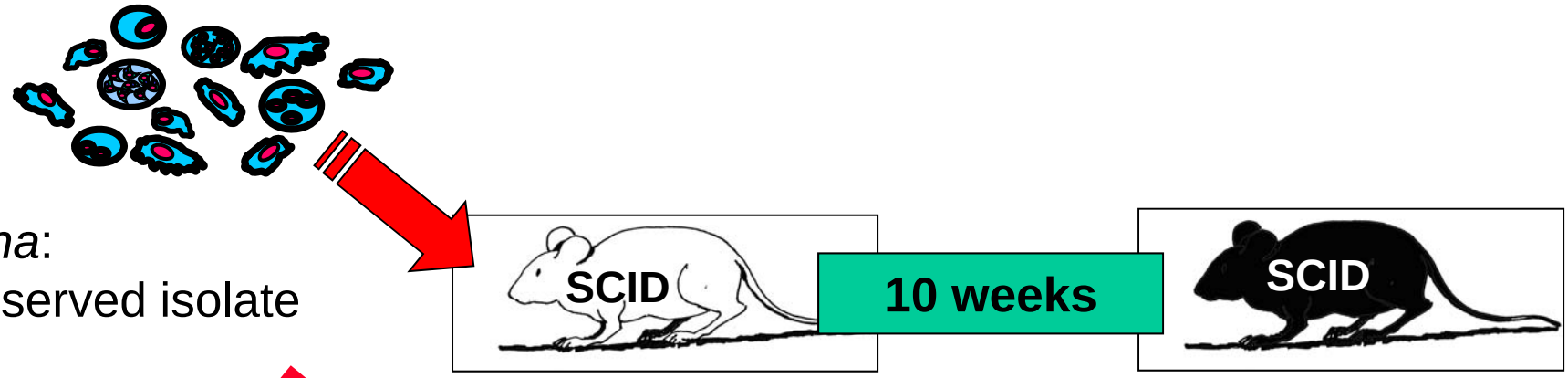
*Aliouat et al, 1993, J. Protozool. Res. 3: 94–8*
*Aliouat et al, 1994, J. Eukaryot. Microbiol. 41(Suppl.): 71*
*Atzori et al, 1999, J. Eukaryot. Microbiol. 46(Suppl.): 112*
*Durand-Joly et al. 2002, J Clin Microbiol 40: 1862-5*
*Furuta et al, 1987, Jpn. J. Exp. Med. 57: 11–7*
*Furuta et al, 1993, Parasitol. Res. 79: 624–8*
*Gigliotti et al, 1993, Infect. Immun. 61:2886–90*

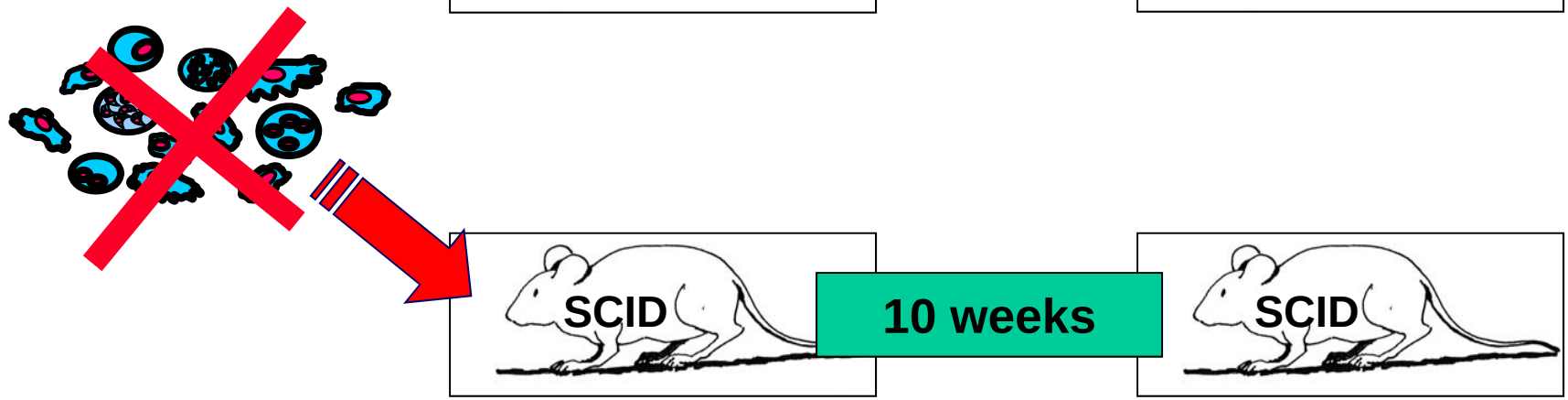
# Microscopic and molecular detection / identification

*P. jirovecii*:
cryopreserved isolates
from 5 patients




*P. murina*:
cryopreserved isolate

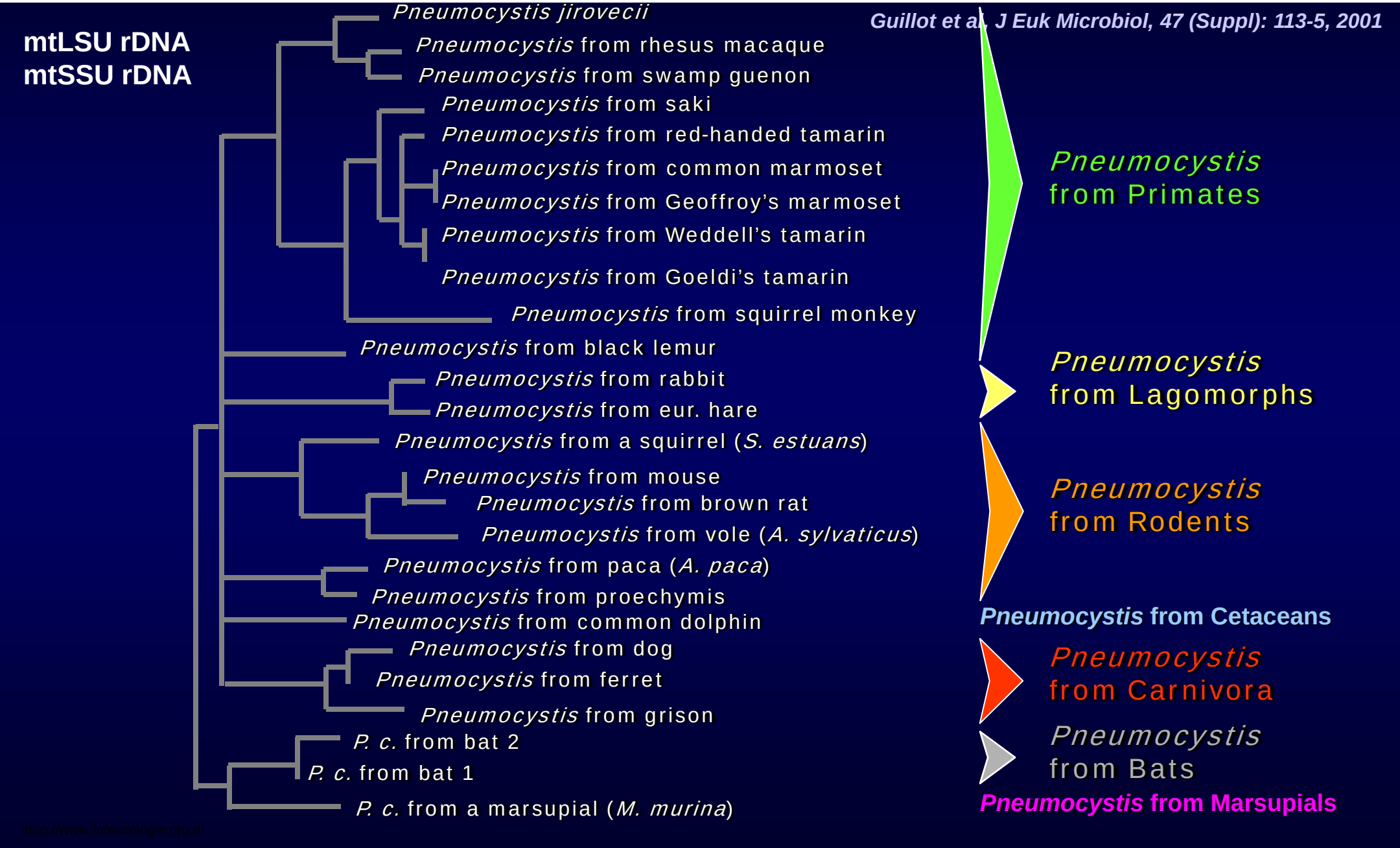


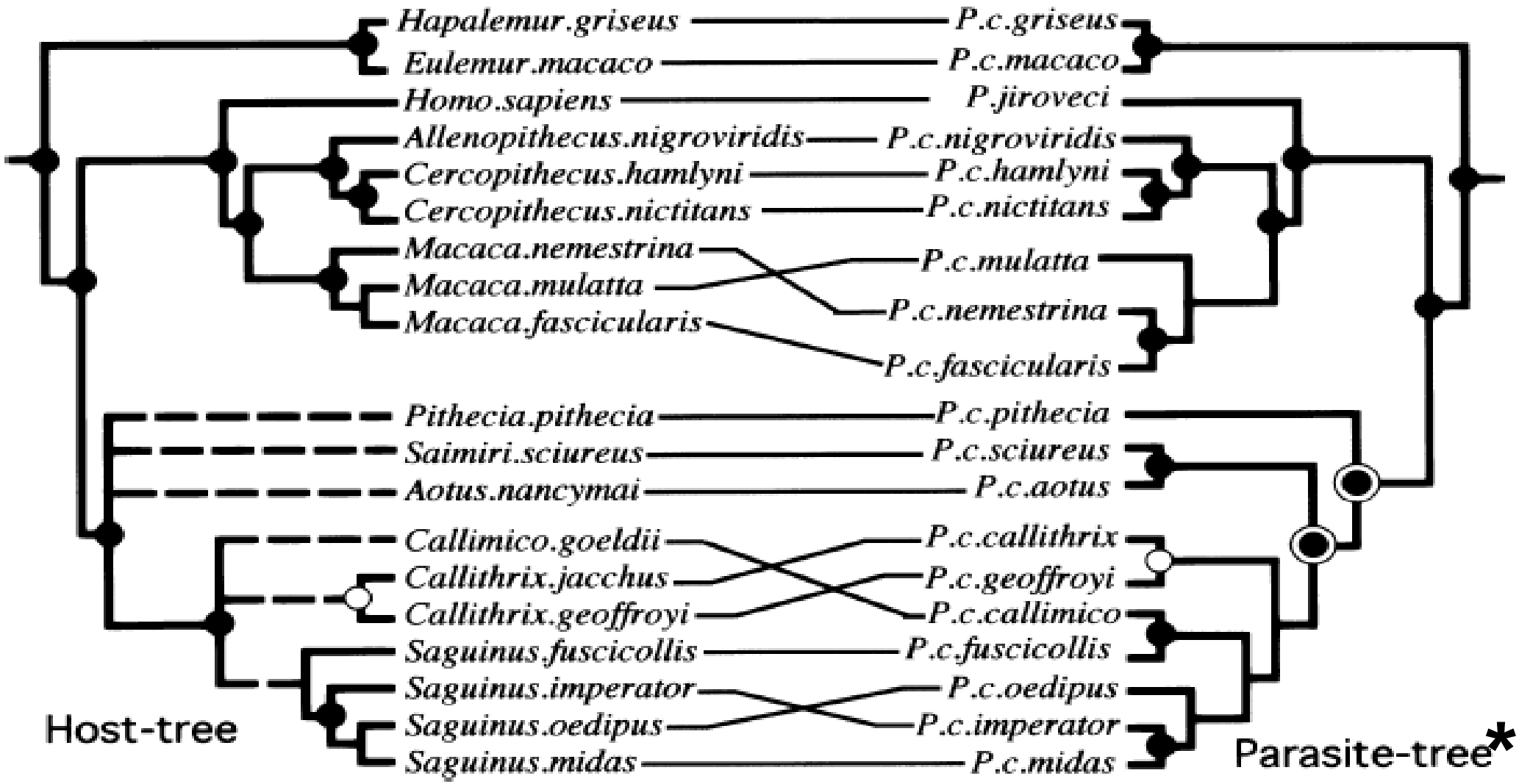






***Durand-Joly et al. 2002, J Clin Microbiol 40: 1862-5***

# Coevolution of *Pneumocystis* spp. with their hosts






* Analysis of *Pneumocystis* mtLSU rDNA, mtSSU rDNA, DHPS gene combined data

# *Pneumocystis* Transmission

*Aliouat-Denis et al, 2008.* ***Infect Genet Evol*** *8: 708-26.*

# *Pneumocystis jirovecii* circulating in human populations

Severely immunocompromised hosts

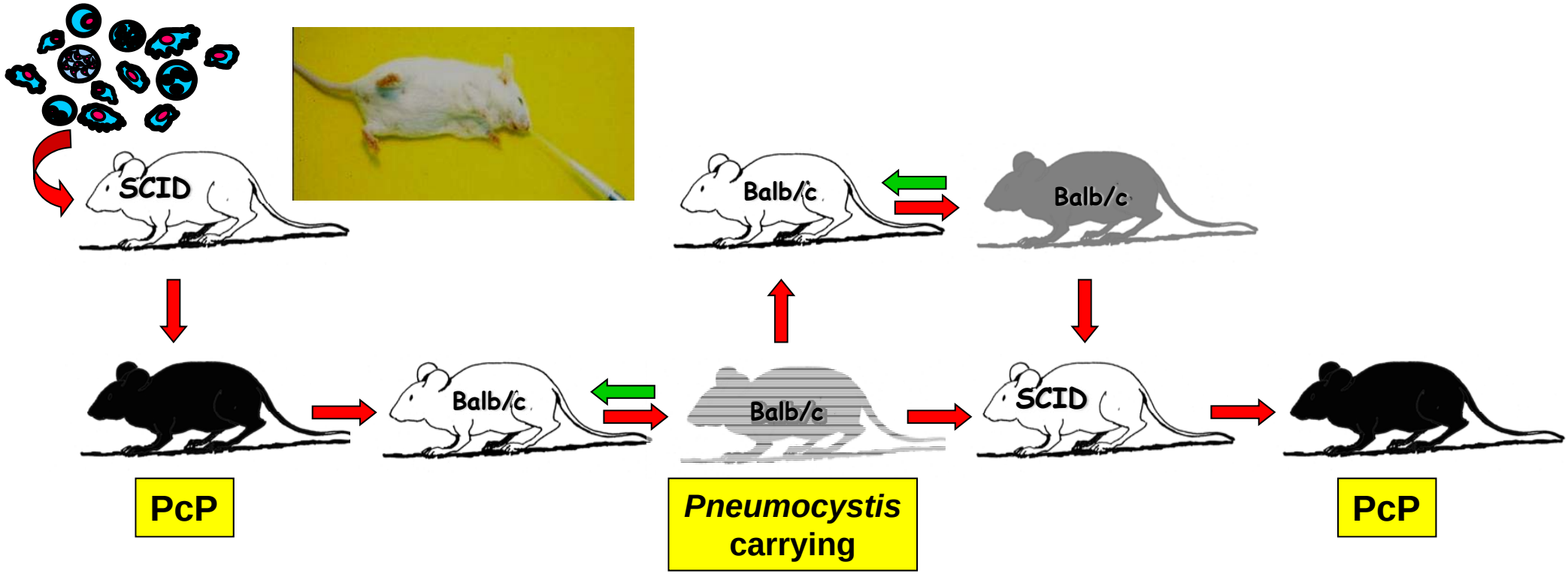
Patients with chronic respiratory diseases

Healthcare workers

Elderly persons

Pregnant women and immunologically naive infants

# *Pneumocystis* airborne circulation: the dynamic reservoir




ERA-NET PathoGenoMics

http://www.infectiologie.org.tn

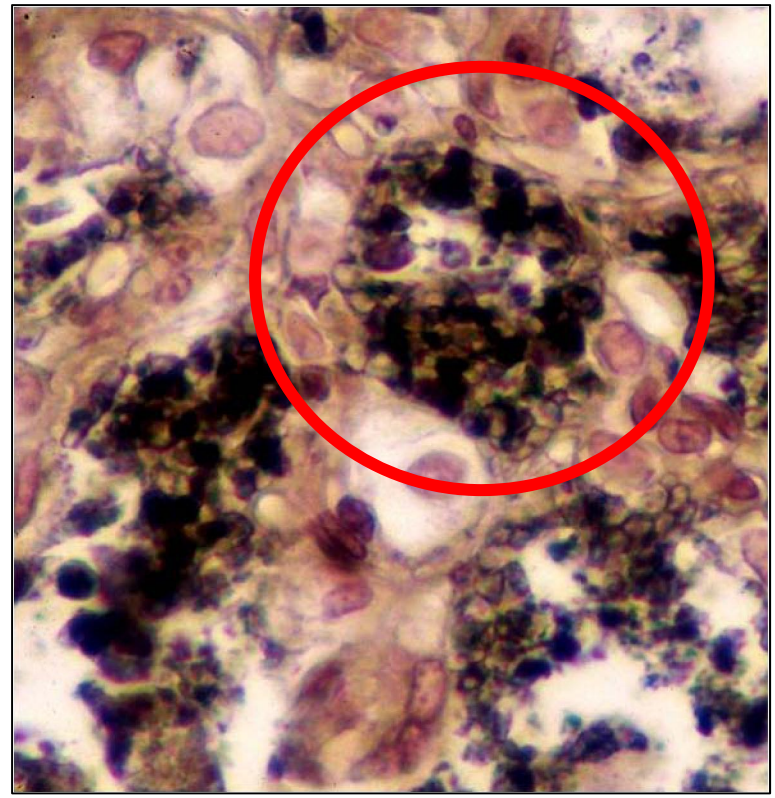
*Dumoulin et al, 2000 Eur J Clin Microbiol Infect Dis 19: 671-8*
*Chabé et al, 2004 Eur J Clin Microbiol Infect Dis 23: 89-97*

# *Pneumocystis* in the lung

**Healthy host**

*Chabé et al, 2004 Eur J Clin Microbiol Infect Dis 23: 89-97*

**Host with PcP**



*Creusy et al, 1996 J Eukaryot Microbiol 43 (Suppl): 47-8*

- Transient parasite growth
- Active *Pneumocystis* multiplication
- No inflammatory changes (histology)
- Transmission to susceptible or healthy hosts

- Irreversible parasite growth
- Extensive *Pneumocystis* proliferation
- Marked inflammatory changes
- Transmission to susceptible or healthy hosts

# Molecular evidence of *Pneumocystis jirovecii* transmission from immunocompetent carriers to infant WP1, WP2, WP3

Grandmother
*P. jirovecii* G1*

Grandfather (COPD)
*P. jirovecii* G1, G3*

Healthy mother
*P. jirovecii* PCR negative*

Healthy father
*P. jirovecii* PCR negative*

Healthy brother
*P. jirovecii* PCR negative*

Six-month old infant with PcP
*P. jirovecii* G1**

Exosaprophyte
*P. jirovecii* ?

* PCR on oropharyngeal samples
** PCR on nasopharyngeal aspirate samples

Likely *P. jirovecii* transmission from immunocompetent carriers to a naive host.

Colonized persons: potential *Pneumocystis* reservoir and infection source.

*Rivero L, de la Horra C, Montes-Cano MA, Rodríguez-Herrera A, Respaldiza N, Friaza V, Morilla R, Gutiérrez S, Varela JM, Medrano FJ, Calderón EJ. Pneumocystis jirovecii transmission from immunocompetent carriers to infant. Emerg Infect Dis 2008;14:1116-8*

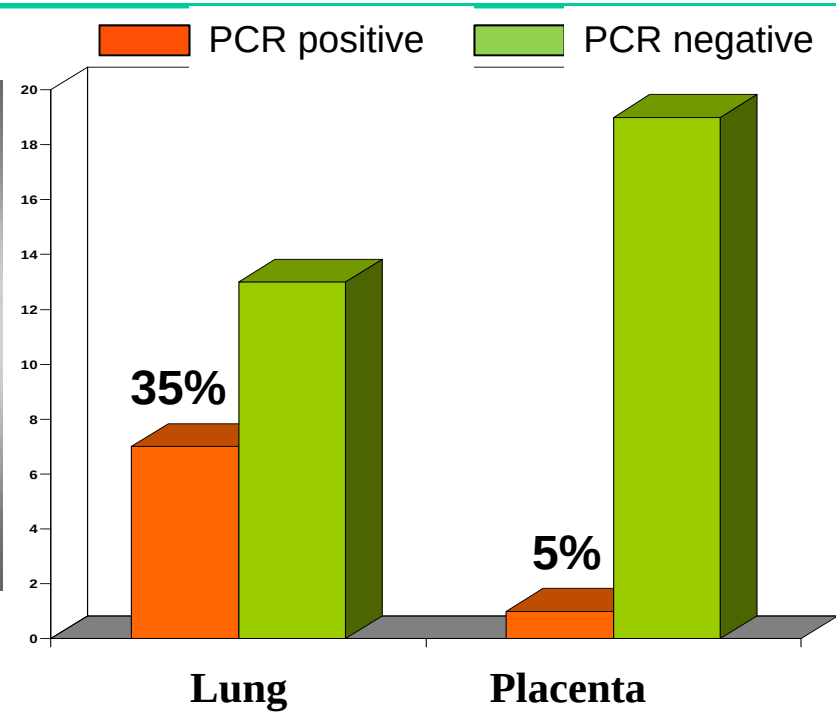
# Current evidences linking *Pneumocystis* colonization and COPD pathophysiology

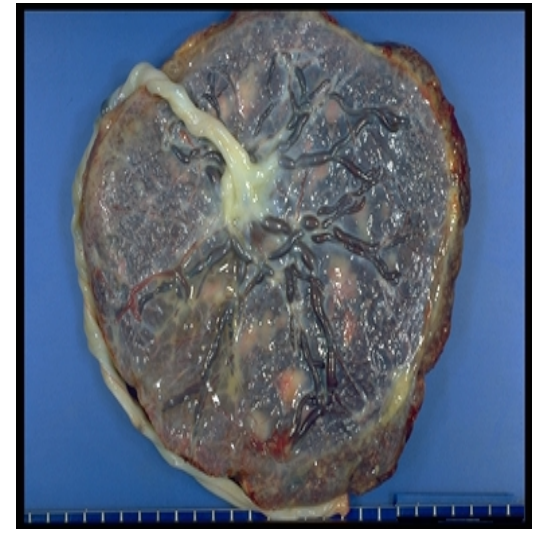
| Evidence | Host | Author, reference |
|---|---|---|
| Development of permanent COPD-like change after *Pneumocystis* pneumonia | Human | Morris AM, et al. Am J Respir Crit Care Med 2000;162: 612-6 |
| Higher peripheral lymphocyte count in colonized patients with COPD | Human | Varela JM, et al. J Eukaryot Microbiol 2003; 50 (suppl): 672-3. |
| Correlation between prevalence of colonization and COPD severity | Human | Morris A, et al. Am J Respir Crit Care Med 2004; 170: 408-13. |
| Higher systemic proinflammatory cytokine levels in colonized patients with COPD | Human | Calderon EJ, et al. Clin Infect Dis 2007; 45:e17-9. |
| Similarity of immune responses in *Pneumocystis* colonization and COPD | Simian | Patil SP, et al. J Eukaryot Microbiol 2003; 50 (suppl): 661-2. |
| Development of airway obstruction and progressive decline in pulmonary function in colonized SIV-infected monkeys | Simian | Norris KA, et al. Immunol Res 2006; 36: 175-87. |
| Accelerated emphysema in colonized hosts exposed to cigarette smoke | Mice | Christensen PJ, et al. Infect Immun 2008; 76: 3481-90. |

# Vertical transmission of *Pneumocystis jirovecii* WP1, WP2, WP3

Epidemiología y Salud Pública

**Twenty aborted foetuses from immunocompetent women who had miscarriages**
(28 ± 8 weeks of gestation)



| Gene/Tissues | Lung (7 / 20) | Placenta (1 / 20) |
|---|---|---|
| DHPS gene | Genotype1 (100%) | Genotype 1 (100%) |
| mtLSUrDNA | Genotype1 (42.8%)<br>Genotype 2 (14.4%)<br>Genotype 3 (42.8% | Genotype 1 (100%) |

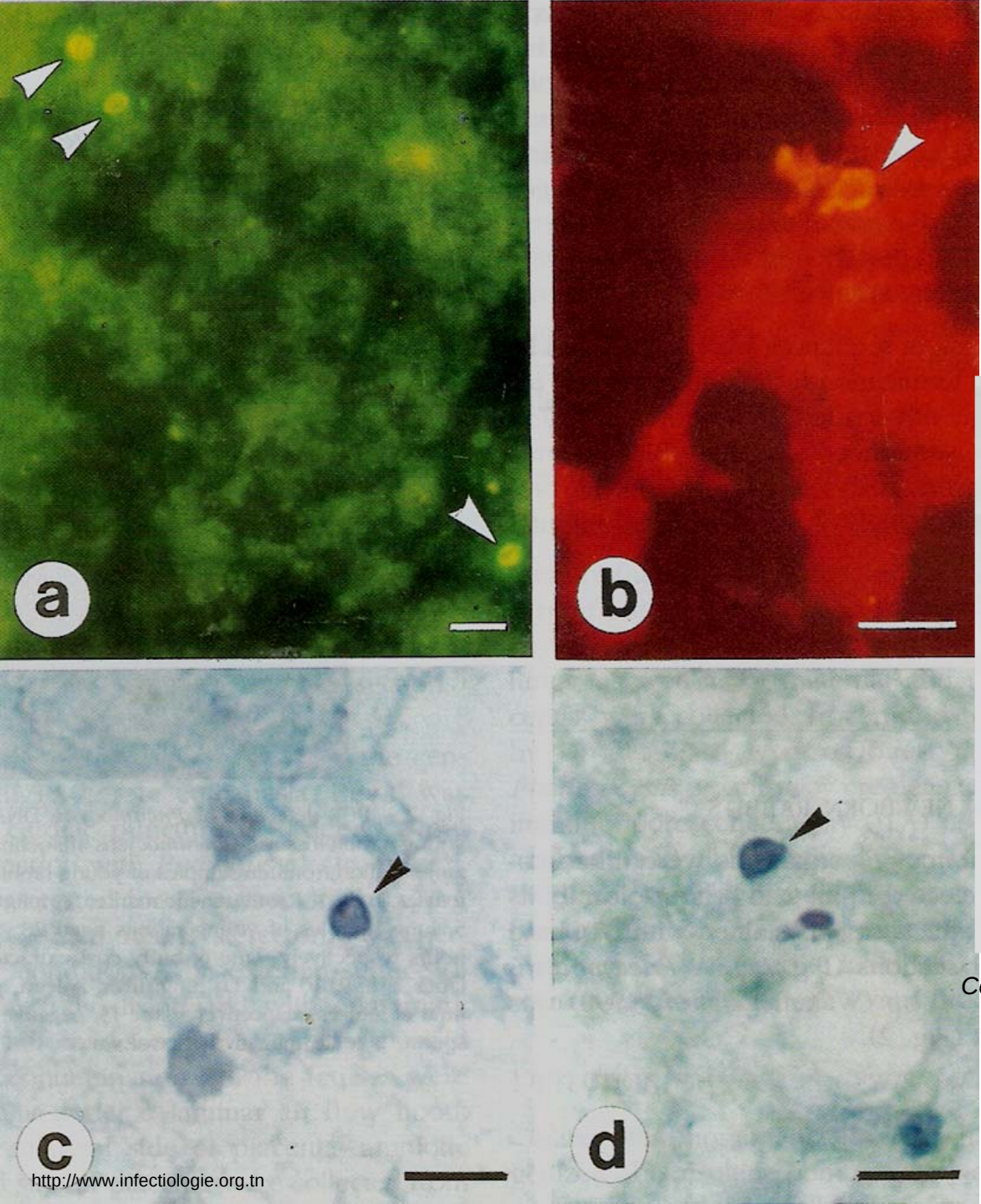
*Montes-Cano MA, Chabe M, Fontillon-Alberdi M, de la Horra C, Respaldiza N, Medrano FJ, Varela JM, Dei-Cas E, Calderon EJ. 2009. First molecular evidence of Pneumocystis jirovecii vertical transmission in Human. Emerg Infect Dis 15: 125-127*

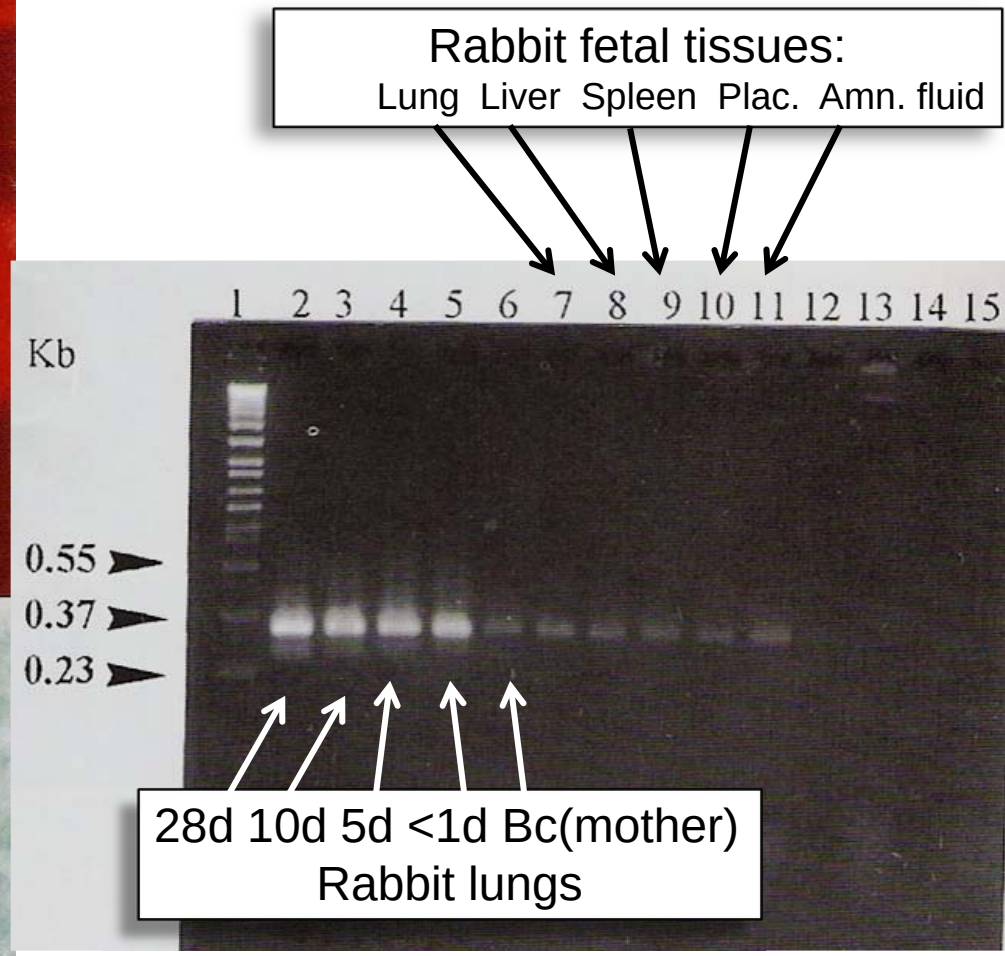
# Transplacental Transmission of *Pneumocystis* species WP1, WP3

| Host species | Placenta | *Pneumocystis* species | Molecular evidences | Morphological evidences | Authors |
|---|---|---|---|---|---|
| *Rattus norvegicus* | Hemotrichorial | *P. carinii* | NA | Controversial | Pifer et al. 1984. Diagn. Microbiol. Infect. Dis. 2: 23–36. |
| *Mus musculus* | Hemotrichorial | *P. murina* | ND | No | Ito et al. 1991. J Protozool 38 (suppl): 218-9. |
| *Homo sapiens* | Hemomonochorial | *P. jirovecii* | ND | Controversial | Mortier et al. 1995. N Engl J Med 332: 825. |
| *Oryctolagus cuniculi* | Hemodichorial | *P. oryctolagi* | **Yes** | **Yes** | Ceré et al. 1997. Parasite 4:325–30. |
| *Rattus norvegicus* | Hemotrichorial | *P. carinii* | No (ISH) | No | Hong et al. 1999. Korean J. Parasitol. 37:149–56. |
| *Rattus norvegicus* | Hemotrichorial | *P. carinii* | No | ND | Icenhour et al. 2002. Eukaryot Cell 1: 414-9. |
| *Callithrix jacchus* | Hemomonochorial | *Pneumocystis* sp | **Yes** | No | Demanche et al. 2003. Vet Rec 152: 811-3. |
| *Oryctolagus cuniculi* | Hemodichorial | *P. oryctolagi* | **Yes** | ND | Sanchez et al. 2007. Med Mycol 45: 701-7 |
| *Homo sapiens* | Hemomonochorial | *P. jirovecii* | **Yes** | ND | Montes-Cano et al. 2009. Emerg Infect Dis 15: 125-7. |



*Aliouat-Denis et al. 2008. Infect Genet Evol 8: 708-26; Chabé et al. 2009. J Mycol Med (in press)*

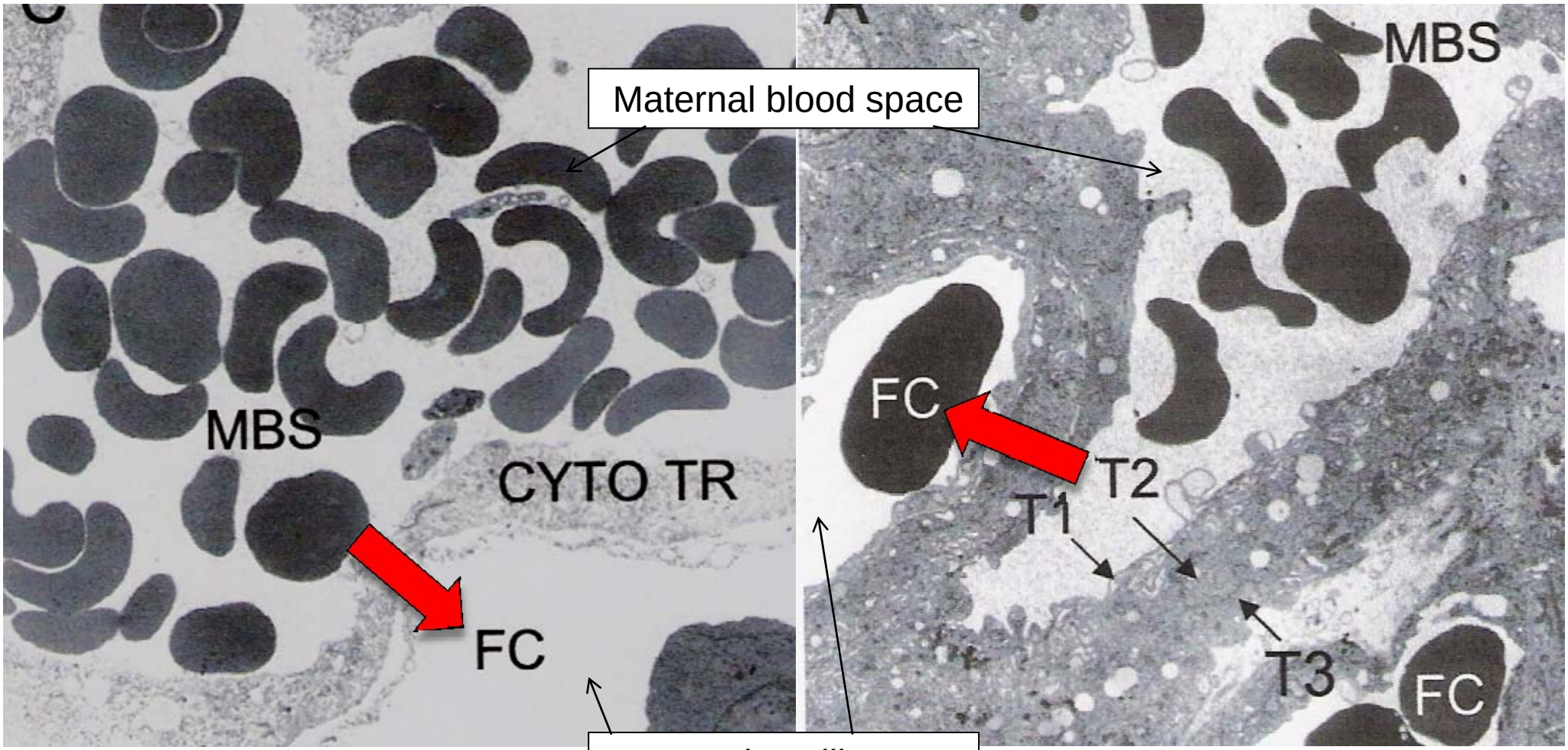



*Ceré N, Drouet-Viard F, Dei-Cas E, Chanteloup N, Coudert P. 1997. In utero Transmission of Pneumocystis carinii sp.f. oryctolagui. Parasite 4: 325-30*

## Hemomonochorial

## Hemotrichorial




*Modified from*
*Carter & Enders. 2004. Comparative aspects of trophoblast development and placentation. Reprod Biol Endocrinol 2004; 2: 46,* doi:10.1186/1477-7827-2-46.

# *Pneumocystis* Infection Today

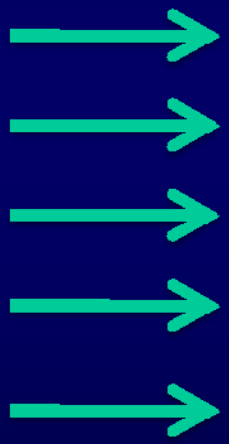
- *Pneumocystis* pneumonia (PcP):
  - high impact in AIDS patients
  - prevalence of 10%-40% was reported in immunodepressed HIV-negative patients
- *Pneumocystis* in non-immunocompromised subjects:
  - *Pneumocystis* primary infection in 95% of 2-4-year old children
  - *Pneumocystis* carriage in 8% - 20% of healthy people
  - Detected frequently in newborns, small children, pregnant women
  - Significant prevalence in COPD patients

*Pifer et al, Pediatrics 61: 35-41,1978; Peglow et al, J Infect Dis 161:296-06, 1990; Wakefield et al, Trans R Soc Trop Med Hyg 84: 800-2,1990; Calderon et al, Lancet 347: 977, 1996; Yale & Limper, Mayo Clin Proc 71: 5-13 1996; Vargas et al, Clin Infect Dis 29, 1489-93, 1999; Dei-Cas E. Med Mycol 38(suppl 1):23-32, 2000; Nevez et al, J Clin Microbiol 41, 181-6, 2003; Durand-Joly et al, J Euk Microbiol 50:614-5, 2003; Chabé et al, Microbiology, 150 : 1167-72 , 2004; Peterson & Cushion, Curr Opinion Microbiol 8: 393-8 2005.*

# PNEUMOCYSTIS : CHANGEMENT DU CADRE CONCEPTUEL

**Ancien cadre:**

- *"Pneumocystis carinii"*
- Protiste indéfini
- Une espèce unique
- Euryxène
- Terminologie zoologique (‘trophozoïte’, ‘kyste’, etc)



**Nouveau cadre:**

- *Pneumocystis* ‘species’
- Champignon (ascomycète)
- Des multiples espèces
- Espèces sténoxènes (co- évolution)
- Terminologie fongique (‘asques’, ‘ascospores’, etc.)

# New Notions on *Pneumocystis* Natural History

- *EA-3609 - Lille-2 University Faculty of Medicine & Lille Pasteur Institute, Lille, France:*
  - Anna Martinez, PhD student
  - Ludivine Odoux, PhD student
  - Cécile-Marie Aliouat, PhD
  - El Moukhtar Aliouat, PhD
  - Annie Vitse, PhD
  - Laurence Delhaes, MD, PhD
  - Nausica Gantois, Technician
  - Muriel Pottier, Technician
  - Eduardo Dei-Cas, MD, PhD
- *ERA NET 'PathoGenoMics*':
  - Enrique Calderon, MD, PhD, and coworkers from Seville University Hospital, Spain
  - Melanie Cushion, PhD, George Smulian MD, PhD, and James Stringer, PhD, from Cincinnati University, USA
- Tunisia
  - Ali Ayadi, MD, PhD, Faculty of Medicine, Sfax

ERA-NET PathoGenoMics

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