LE INFEZIONI FUNGINE: UN PROBLEMA EMERGENTE DI SANITÀ PUBBLICA, DALLA EZIOLOGIA ALLA TERAPIA

SHORT MASTER

Prof. Gustavo Giusiano

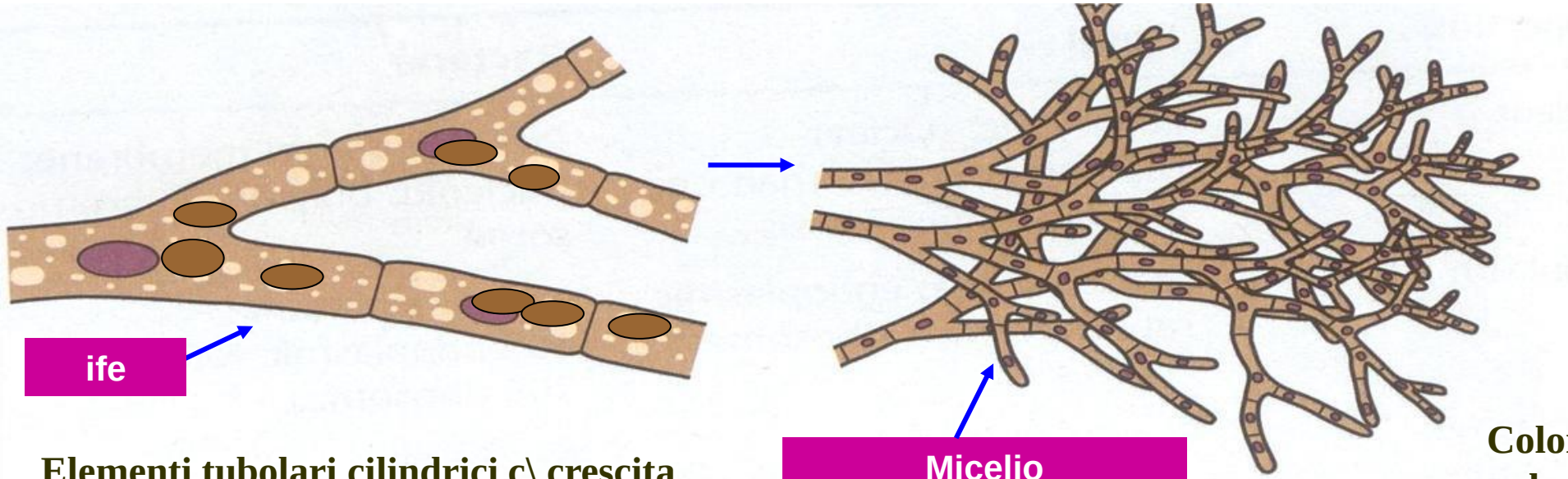
Direttore Dpto. Micologia - Instituto de Medicina Regional - CONICET
Microbiología e Inmunología. Facultad de Medicina
Universidad Nacional del Nordeste – Argentina
gustavogiusiano@yahoo.com.ar – giusianogustavo@gmail.com

venerdi 05 Maggio 2023

I funghi dimorfi e dematiacei in laboratorio:

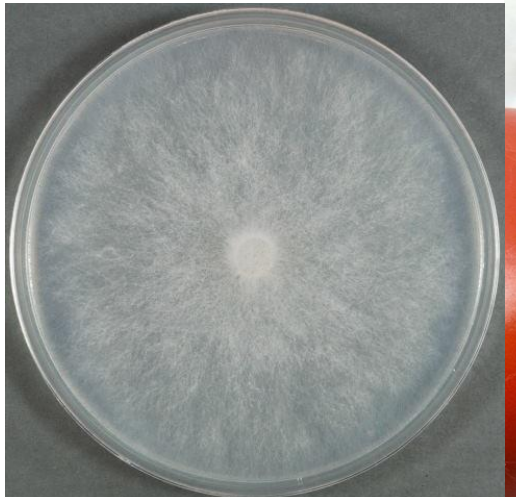
riconoscimento a livello di genere

Strutture morfologiche



Elementi tubolari cilindrici c\ crescita apicale

Colonia velutata a lanosa/cotonosa



Funghi multinucleati (filamentoso – muffa)

Funghi multinucleati (filamentoso – muffa)

Ife

settata

2-5 micron

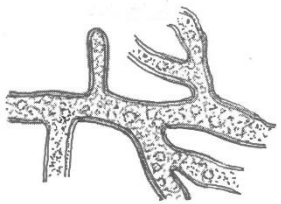
non settata

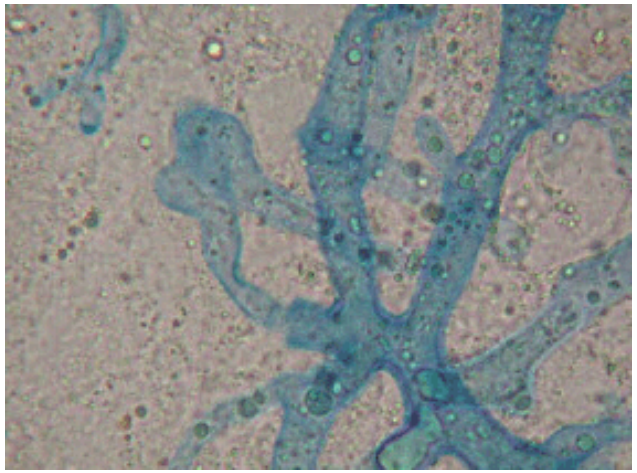
10-15 micron

Micelio continuo

o

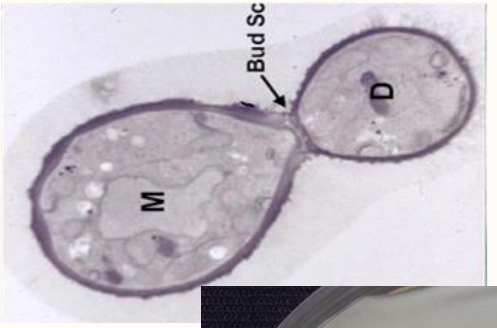
cenocitico





Strutture morfologiche

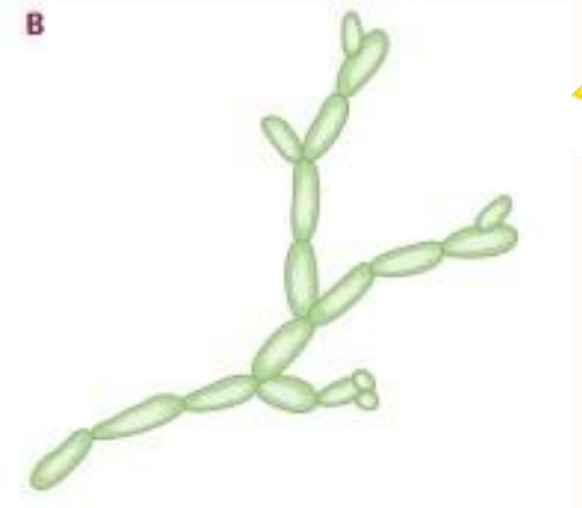
Lieviti

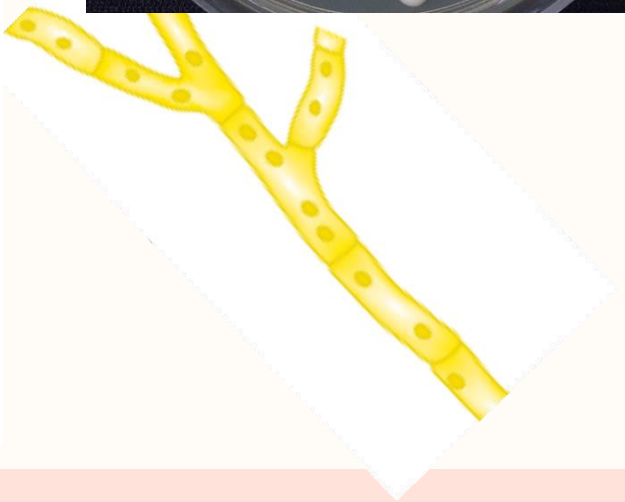


- Funghi unicellulari
- Forma sferica/ovale
- Riproduzione asessuata per gemmazione
  - Cellula figlia = blastoconidio
- Colonie umide, consistenza liscia e uniforme

Pseudoifa

Gemmazione che non si stacca dalla cellula madre e, qualche tempo dopo, subisce allungamenti per dare origine ad una struttura simile alle ife





Funghi dimorfi
"dimorphic switching"

dal greco **dís** (due) - **morphé** (forma)

Dimorfismo

Capacità di modificare la sua morfologia in base alle condizioni ambientali

Lievito/sferule ⟷ Filamentoso

Reversibile

35-37°

forma parassitaria

Fattori ambientali

- Temperatura
- Nutrienti
- CO_2, fonti di azoto, cisteina, estradiolo, O_2, L-DOPA, feromoni, lipossigenasi, cicloossigenasi, ecc.

25-28°c

forma saprofita

Funghi dimorfi

- **Dimorfi termici**
- **Dimorfi non termici**

Il cambiamento morfologico è essenziale per la patogenesi del fungo dimorfo

Gauthier GM. 2015 PLoS Pathog. PMID: 25675433

Table 1. Dimorphic fungal pathogens. *

Fungus	Phylum	Disease	Major stimuli for the morphologic switch
Mammalian pathogens			
Blastomyces dermatitidis†, ‡	Ascomycota	Blastomycosis	Temperature
Coccidioides immitis† & *posadasii*†	Ascomycota	Coccidioidomycosis	Temperature
Histoplasma capsulatum†	Ascomycota	Histoplasmosis	Temperature
Emmonsia spp. †	Ascomycota	Emmonsiosis	Temperature
Lacazia loboi†, §	Ascomycota	Lacaziosis	Temperature
Penicillium marneffei†, ¶	Ascomycota	Penicilliosis	Temperature
Paracoccidioides brasiliensis† & *lutzii*†	Ascomycota	Paracoccidioidomycosis	Temperature
Sporothrix schenckii†	Ascomycota	Sporotrichosis	Temperature
Hortaea werneckii	Ascomycota	Tinea nigra	Temperature, nutrition, inoculum size
Malassezia furfur	Basidiomycota	Pityriasis versicolor	L-DOPA
Mucor spp.	Mucoromycotina#	Mucormycosis	O_2 & CO_2 tension
Cokeromyces recurvatus	Mucoromycotina#	Mucormycosis	Temperature, Nutrients, O_2 & CO_2 tension
Plant pathogens			
Ophiostoma ulmi & *O. novo-ulmi*	Ascomycota	Dutch elm disease	Nitrogen source, quorum sensing
Holleya sinecauda	Ascomycota	Mustard seed rot	Unknown
Taphrina deformans	Ascomycota	Peach & almond leaf curl	Unknown
Ustilago maydis	Basidiomycota	Corn smut	Pheromones, plant lipids, plant hydrophobicity, pH, nitrogen
Insect Pathogens			
Beauveria bassinia	Ascomycota	White muscardinePlant endophyte	Insect hemolymph
Metarhizium spp.	Ascomycota	Green muscardinePlant endophyte	Insect hemolymph
Ophiocordyceps unilateralis	Ascomycota	"Zombie ant"	Insect hemolymph

*This table includes the most common dimorphic fungal pathogens of mammals, plants, and insects; thus, it is not all-inclusive. *Saccharomyces cerevisiae*, which rarely causes human infection, is not included because it converts between yeast and pseudohyphae. Although *Cryptococcus neoformans* converts to a filamentous form during mating, is not traditionally considered dimorphic fungus. *Candida albicans* can be considered a polymorphic fungus because it grows as yeast, pseudohyphae, and hyphae.

† Thermally dimorphic fungi.

‡ Phylogenetic analysis suggests that *Blastomyces* may include two species, *B. dermatitidis* and *B. gilchristii* sp. nov.

§ Although *Lacazia loboi* cannot be grown in vitro, it is phylogenetically related to *P. brasiliensis* and other thermally dimorphic fungi.

¶ *Penicillium marneffei* has been renamed *Talaromyces marneffei*.

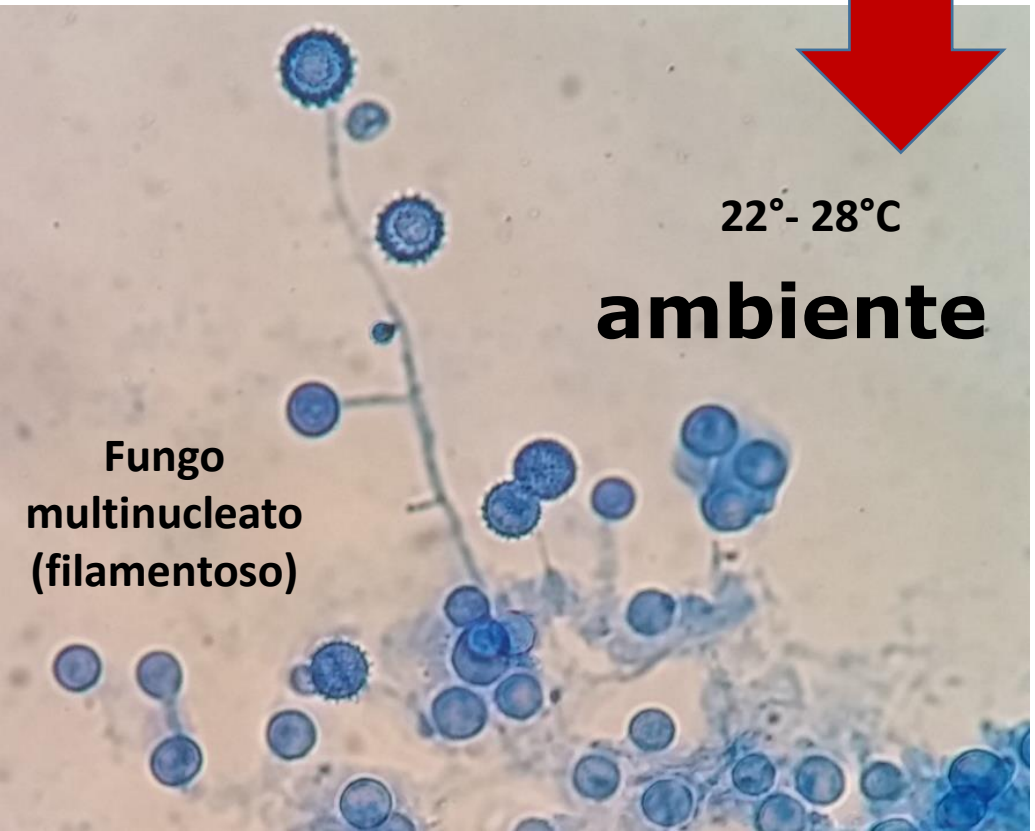
The taxonomic location of the subphylum mucoromycotina is uncertain (the zygomycota phylum is obsolete).

Funghi dimorfi

Histoplasma

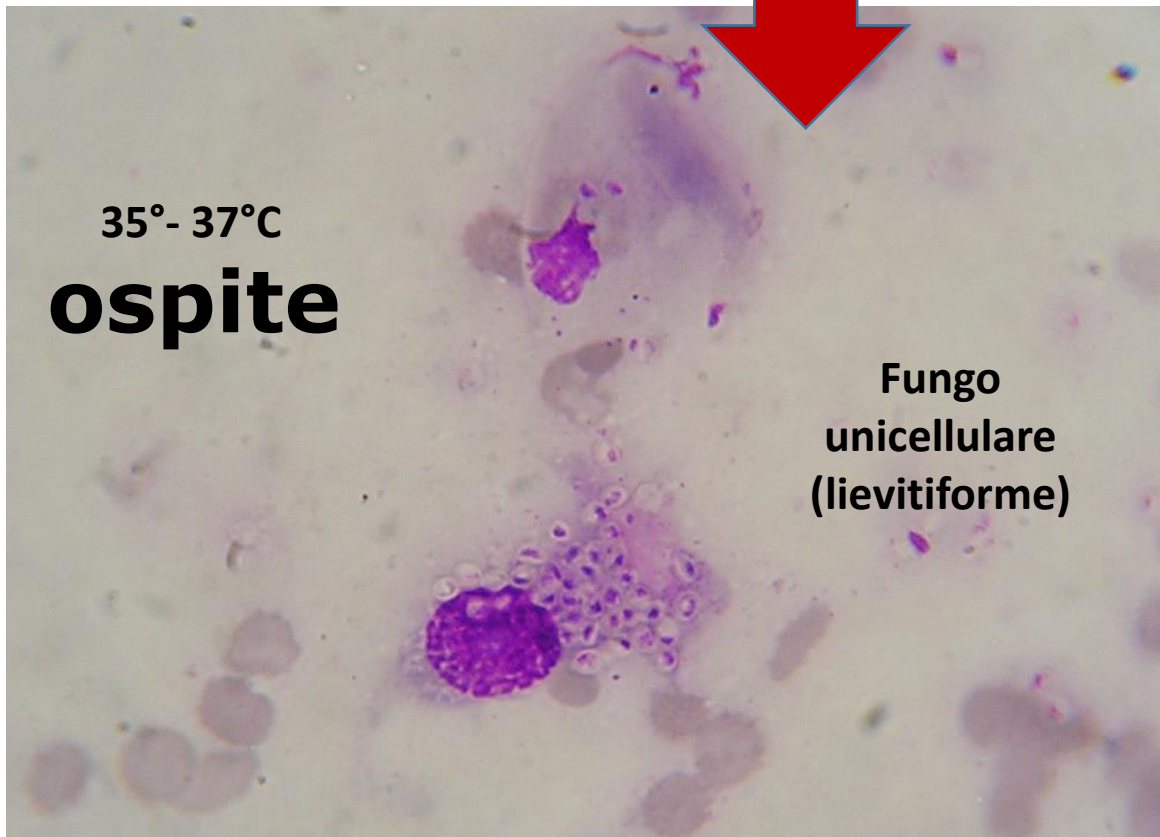
Fase saprofita

saprobio nel suolo
Resistente a pH, humiditá, Temp

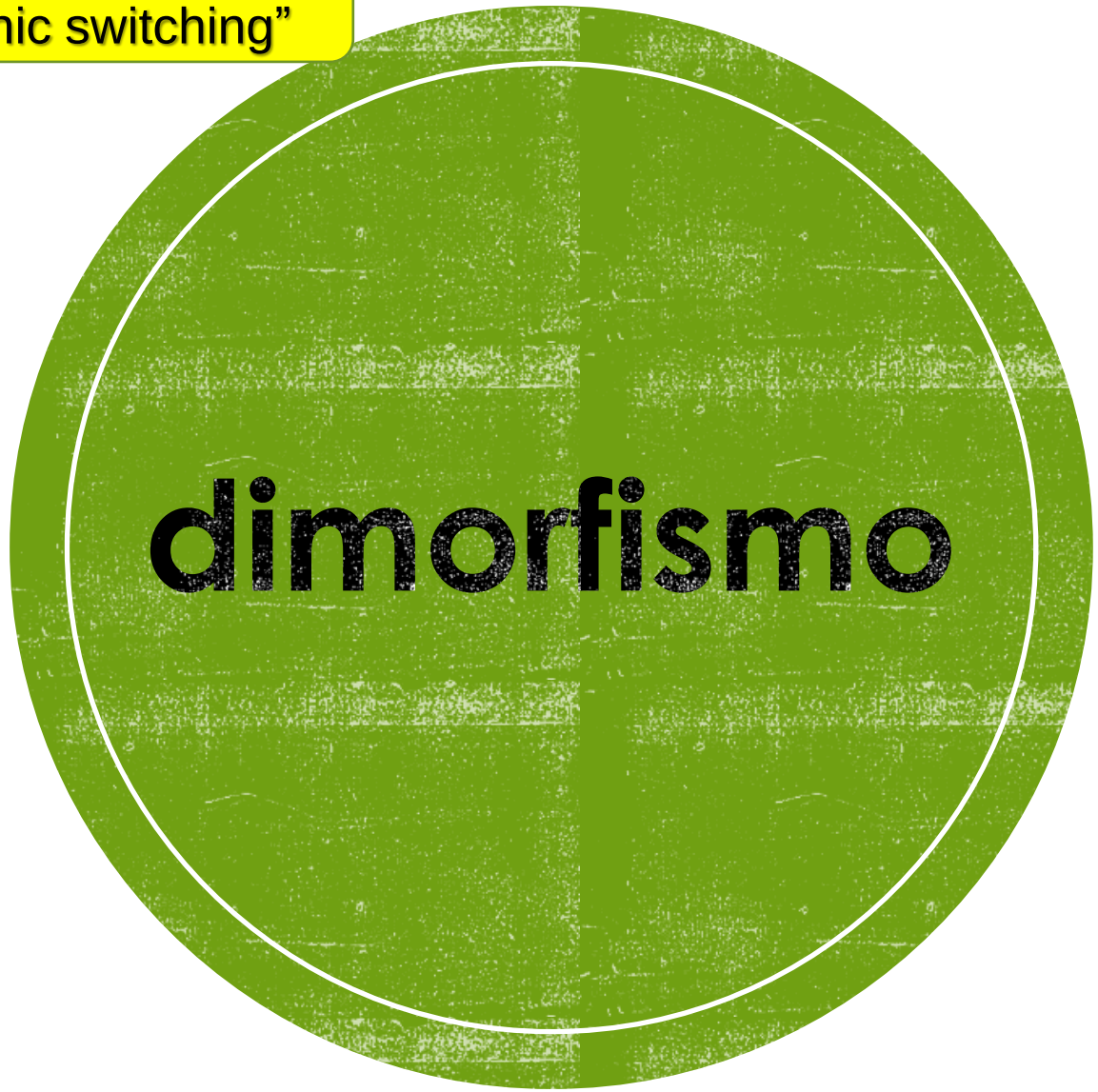


Fase parassitaria

nei tessuti
Capacitá invasora



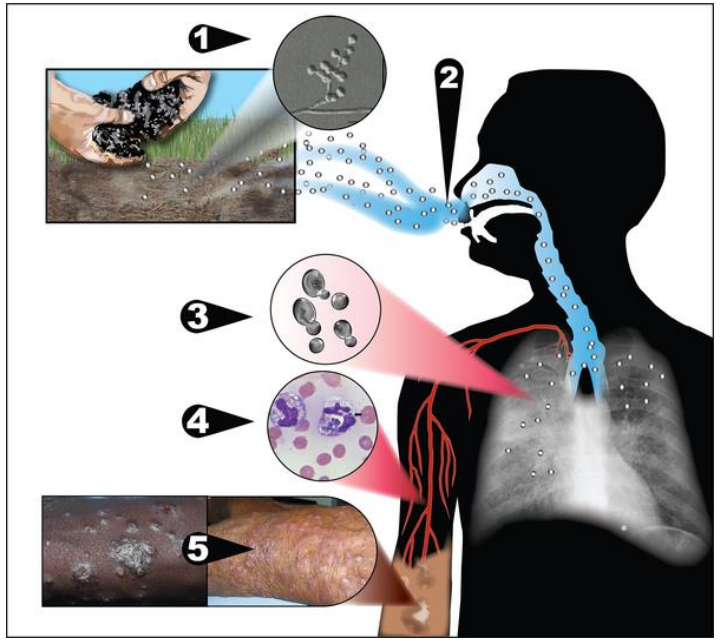
Funghi dimorfi
"dimorphic switching"



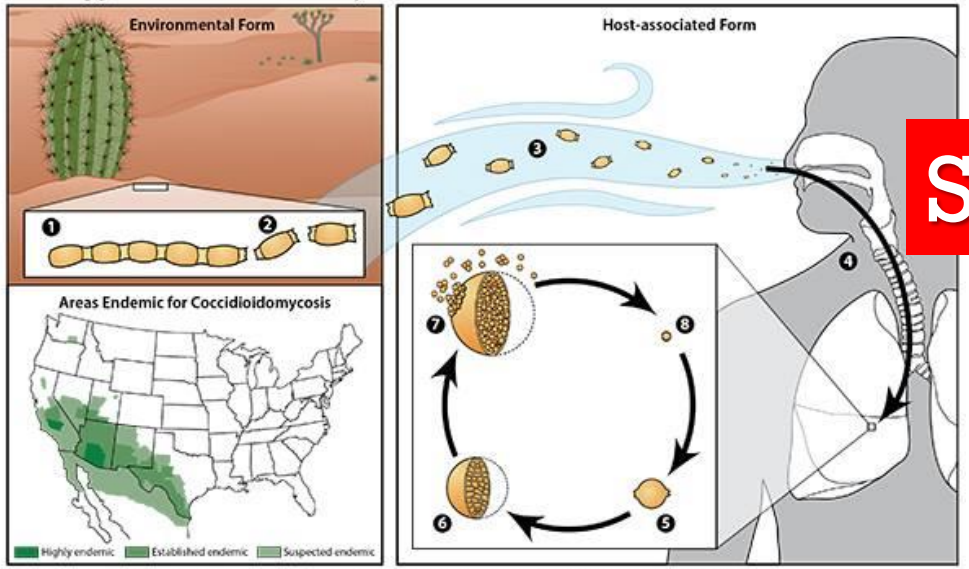
Considerato un importante fattore di virulenza.

La capacità di sviluppare diversi tipi di crescita favorisce **l'adattamento** all'ospite e facilita **l'evasione** di meccanismi difensivi come la fagocitosi e la risposta agli anticorpi.

Biology of Emergomycosis



Biology of Coccidioidomycosis

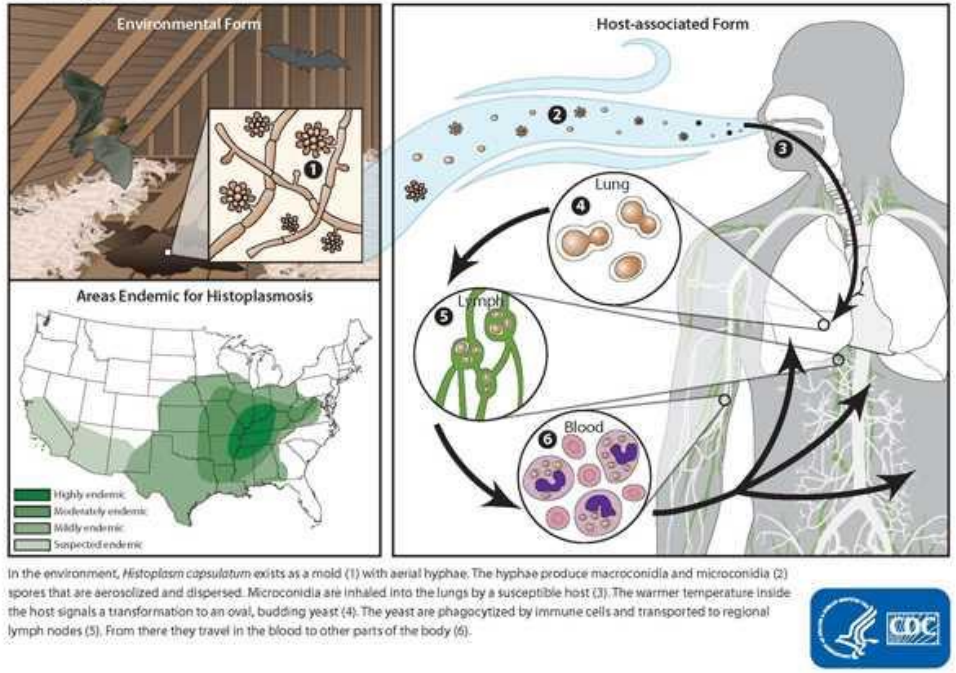


In the environment, *Coccidioides spp.* exists as a mold (1) with septate hyphae. The hyphae fragment into arthroconidia (2), which measure only 2-4 µm in diameter and are easily aerosolized when disturbed (3). Arthroconidia are inhaled by a susceptible host (4) and settle into the lungs. The new environment signals a morphologic change, and the arthroconidia become spherules (5). Spherules divide internally until they are filled with endospores (6). When a spherule ruptures (7) the endospores are released and disseminate within surrounding tissue. Endospores are then able to develop into new spherules (6) and repeat the cycle.

Sapronosi

Via di infezione

Biology of Histoplasmosis



In the environment, *Histoplasm capsulatum* exists as a mold (1) with aerial hyphae. The hyphae produce macroconidia and microconidia (2) spores that are aerosolized and dispersed. Microconidia are inhaled into the lungs by a susceptible host (3). The warmer temperature inside the host signals a transformation to an oval, budding yeast (4). The yeast are phagocytized by immune cells and transported to regional lymph nodes (5). From there they travel in the blood to other parts of the body (6).

The fungus enters the lung by inhalation of conidia or hyphal fragments, which can give rise to a subclinical condition that may go asintomatic or to an overt disease of variable intensity, depending on the age and immunological condition of the host.

Dissemination occurs via lymphatic or hematic routes

frontiers in Cellular and Infection Microbiology

MINI REVIEW
published: 11 February 2021
doi: 10.3389/fcimb.2020.605679

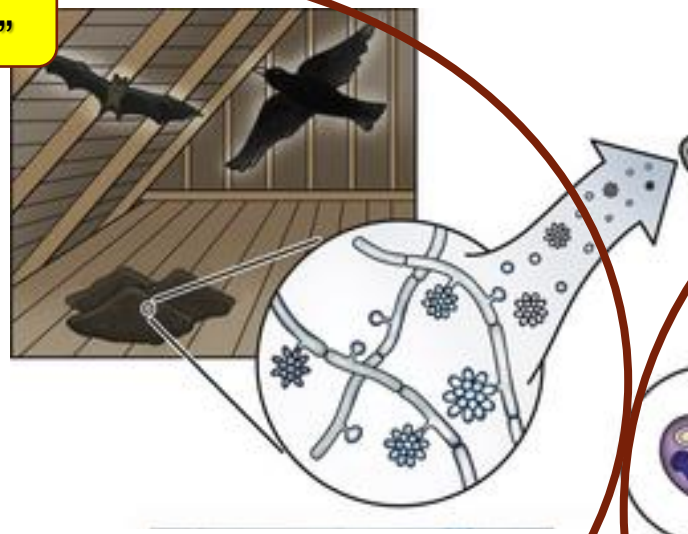
The Trojan Horse Model in *Paracoccidioides*: A Fantastic Pathway to Survive Infecting Human Cells

Gustavo Giusiano *

Mycology Department, Instituto de Medicina Regional, Universidad Nacional del Nordeste, Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET), Resistencia, Argentina

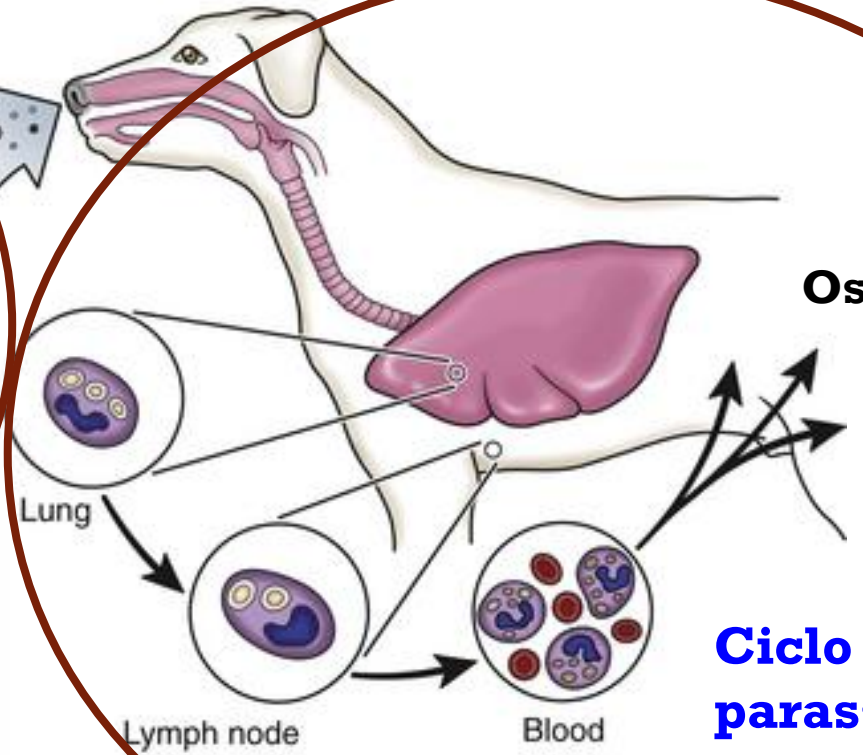
Funghi dimorfi
"dimorphic switching"

Ambiente
22°- 28°C

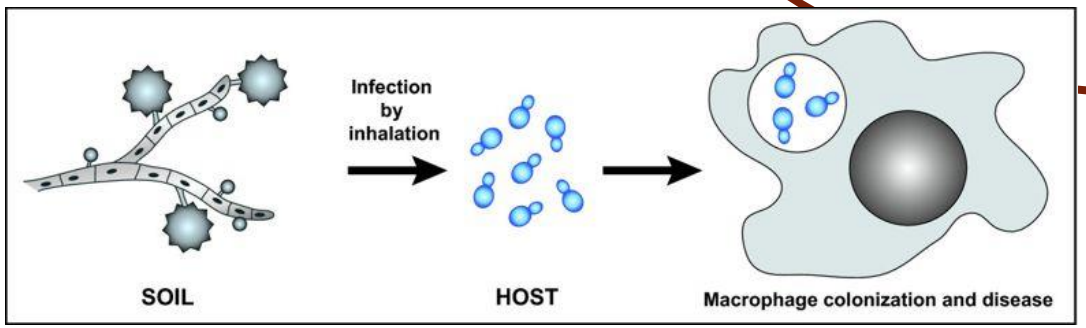


Saprofita o
ciclo di vita ambientale

Ospite
37°C

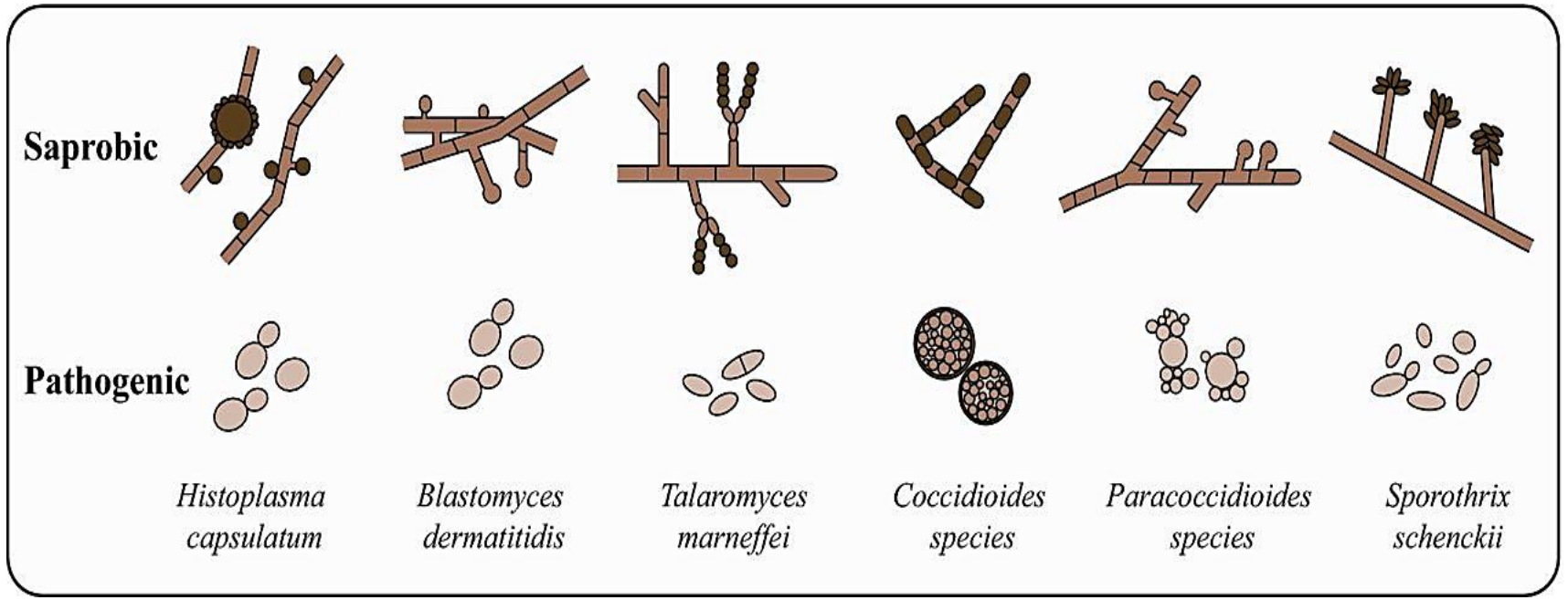


Ciclo di vita
parassitario



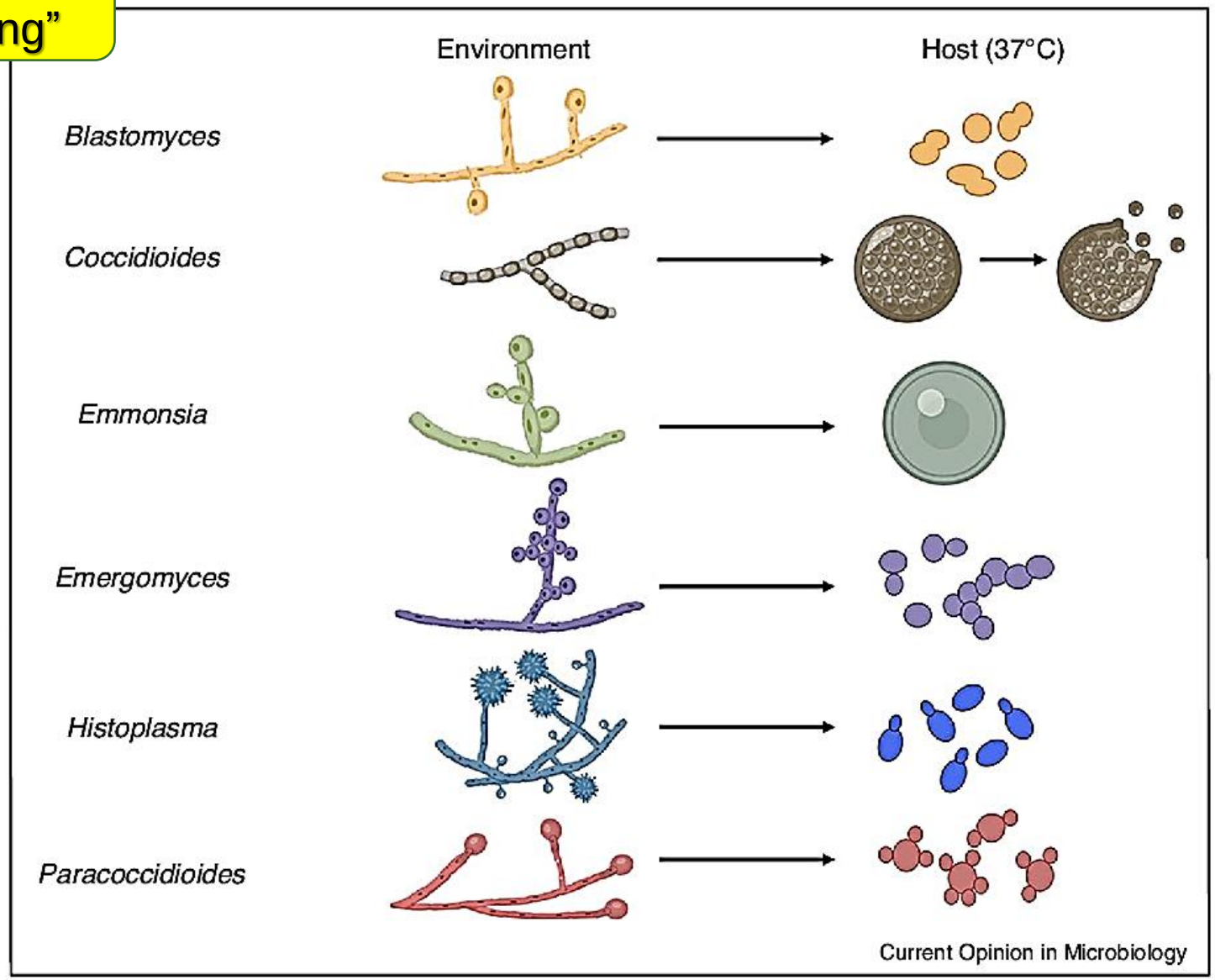
Histoplasma

FIGURE 2. Cellular morphologies of dimorphic human fungal pathogens.
The growth morphologies of the dimorphic ascomycetes *histoplasma capsulatum (ajellomyces capsulatum), blastomyces dermatitidis (ajellomyces dermatitidis), talaromyces marneffei (penicillium marneffei)*, *coccidioides immitis, paracoccidioides brasiliensis* and *sporothrix schenckii* (*ophiostoma schenckii*).
In general, these fungi grow in a multicellular hyphal form at 25°c and switch to producing unicellular yeast growth forms at 37°c, with the exception of *coccidioides* species that produce spherules. for a number of these fungi, the yeast form serves to accommodate intracellular growth within host phagocytes.



FEMS Microbiol Rev, Volume 39, Issue 6, November 2015, Pages 797–811, https://doi.org/10.1093/femsre/fuv035

Funghi dimorfi
"dimorphic switching"



WHO fungal priority pathogens list (WHO FPPL)

Il processo di prioritizzazione si è concentrato sui patogeni fungini che possono causare infezioni fungine sistemiche acute e subacute invasive, per i quali esiste una resistenza ai farmaci o altre sfide di trattamento e gestione.

https://www.who.int/publications/i/item/9789240060241

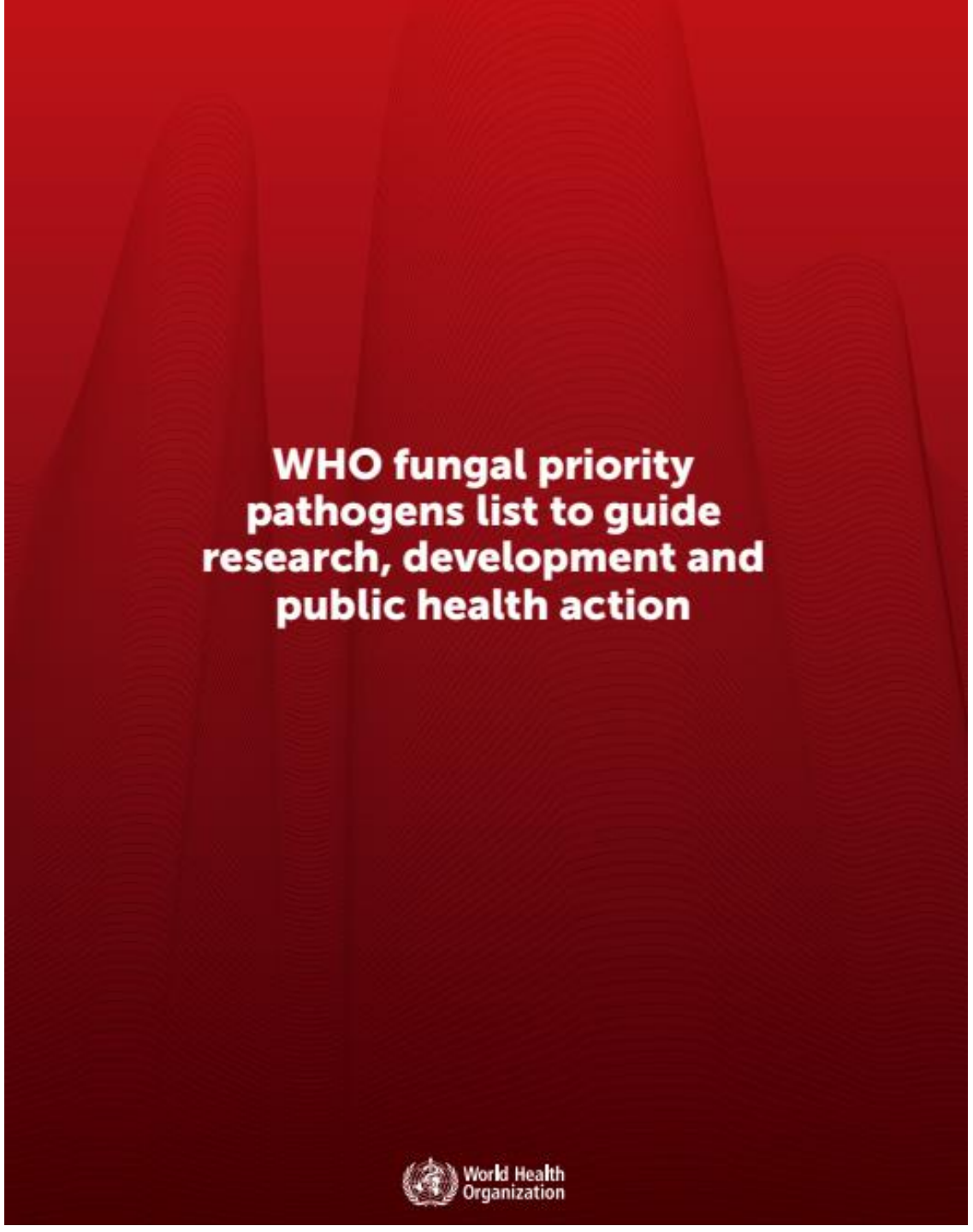


Table 3. WHO fungal priority pathogens list

Critical group	High group	Medium group
 *Cryptococcus neoformans*	 *Nakaseomyces glabrata (Candida glabrata)*	 *Scedosporium* spp.
 *Candida auris*	 *Histoplasma* spp.	 *Lomentospora prolificans*
 *Aspergillus fumigatus*	 Eumycetoma causative agents	 *Coccidioides* spp.
 *Candida albicans*	 Mucorales	 *Pichia kudriavzeveii (Candida krusei)*
	 *Fusarium* spp.	 *Cryptococcus gattii*
	 *Candida tropicalis*	 *Talaromyces marneffei*
	 *Candida parapsilosis*	 *Pneumocystis jirovecii*
		 *Paracoccidioides* spp.

Fungal Diversity (2018) 90:245–291

La famiglia Ajellomycetaceae è **adattata agli ospiti vertebrati**, una caratteristica condivisa da tutti i membri di questa famiglia.

Division Ascomycota
Order Onygenales
Family Ajellomycetaceae

Questa famiglia è unica nel numero di generi che mostrano **caratteristiche invasive** per adattarsi, sopravvivere e replicarsi all'interno degli ospiti dei mammiferi, e quasi tutti condividono caratteristiche morfologiche ed ecologiche del **dimorfismo termico**.

Fungal Diversity (2018) 90:245–291

Division Ascomycota
Order Onygenales
Family Ajellomycetaceae

Ajellomycetaceae - una famiglia di patogeni associati ai mammiferi all'interno dell'ordine Onygenales, che comprende generi patogeni ben noti come:

Histoplasma ***Paracoccidioides***

Blastomyces

Emmonsia

Emergomyces

Emmonsiellopsis Descritto di recente

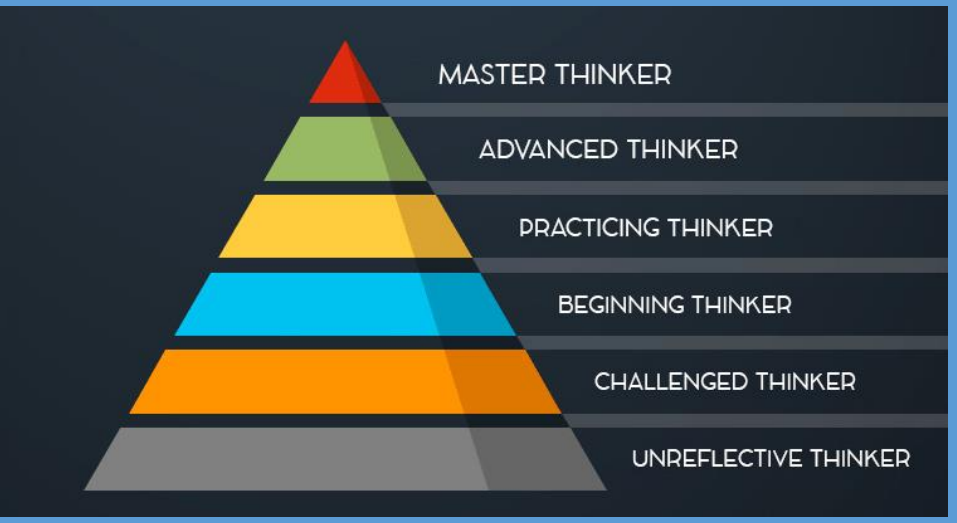
Lacazia loboi *was also included in this group, as a Paracoccidioides sister species*

Fungal Diversity (2018) 90:245–291

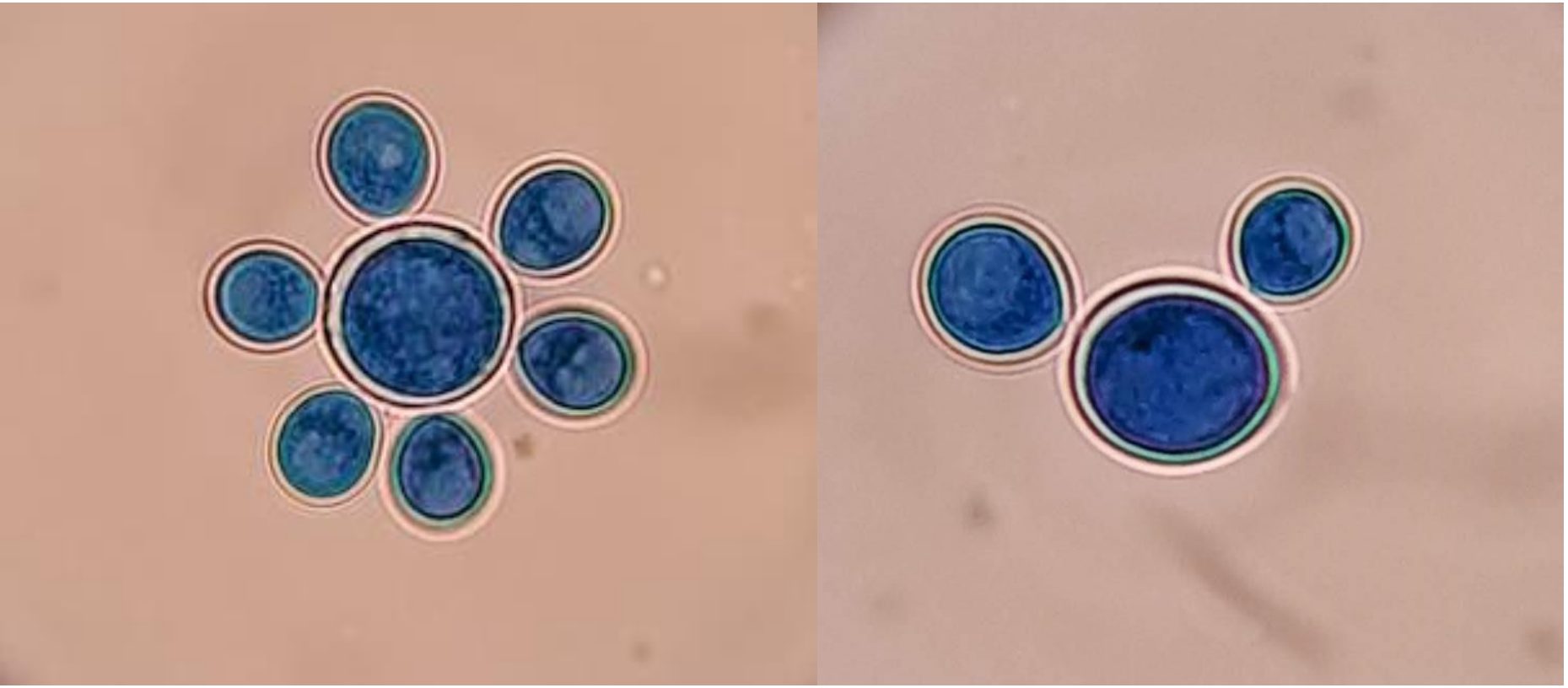
División Ascomycota
Order Onygenales
Family Onygenaceae

Coccidioides

considerando il genere Coccidioides come un outgroup

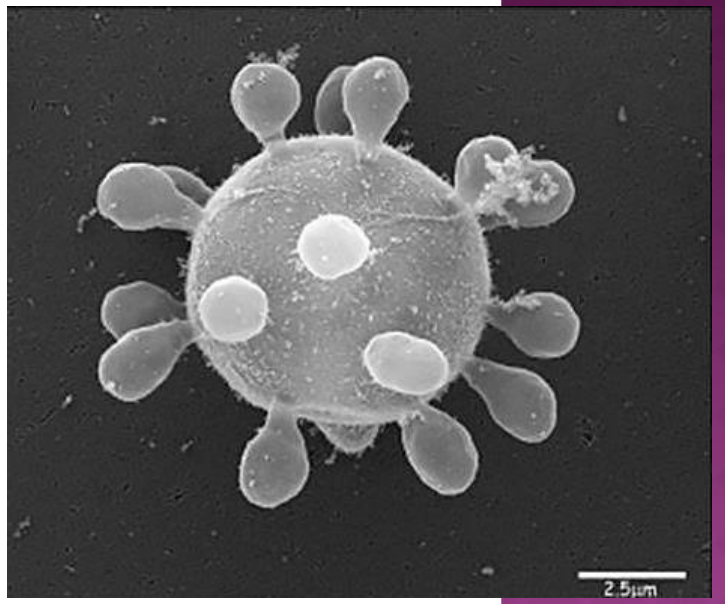

MASTER THINKER
ADVANCED THINKER
PRACTICING THINKER
BEGINNING THINKER
CHALLENGED THINKER
UNREFLECTIVE THINKER


CRITICAL THINKING
Problem
Thinking
Reasoning
Analyzing
Evaluating
Problem Solving
Decision Making
Solution
shutterstock.com · 1637370907



Paracoccidioides

Paracoccidioides



***Paracoccidioides brasiliensis* (S1a / S1b)**
***Paracoccidioides americana* (PS2)**
***Paracoccidioides restrepiensis* (PS3)**
***Paracoccidioides venezuelensis* (PS4)**

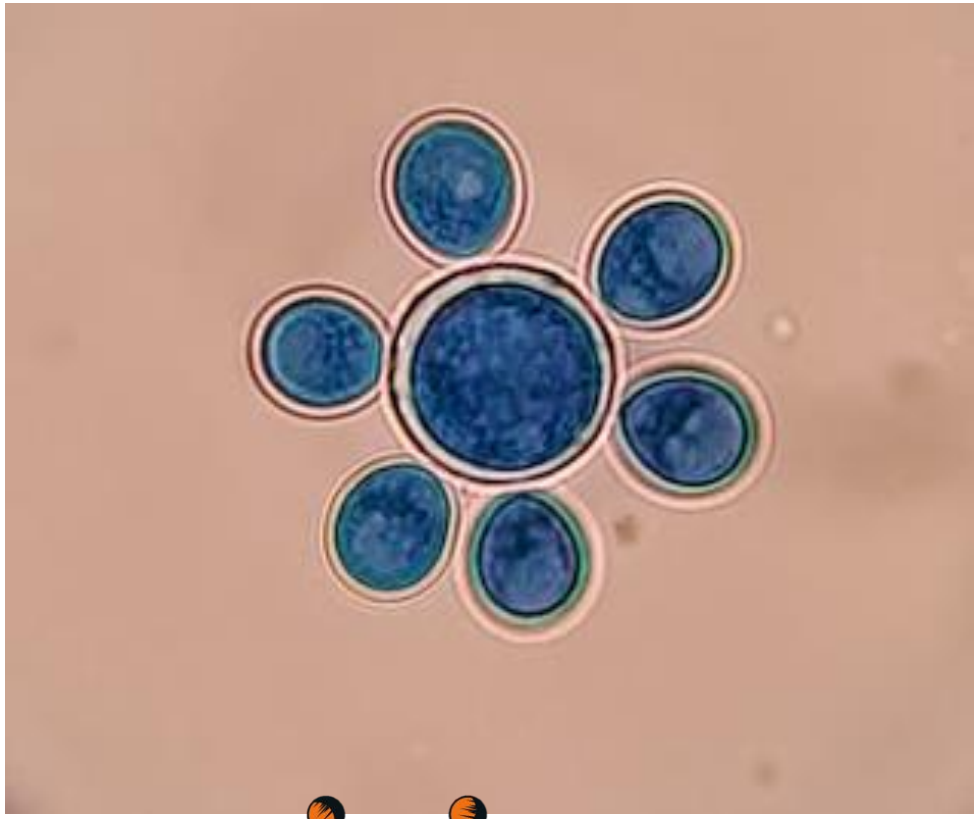
Paracoccidioides lutzii

Fungal Genetics and Biology 2017; 106: 9–25

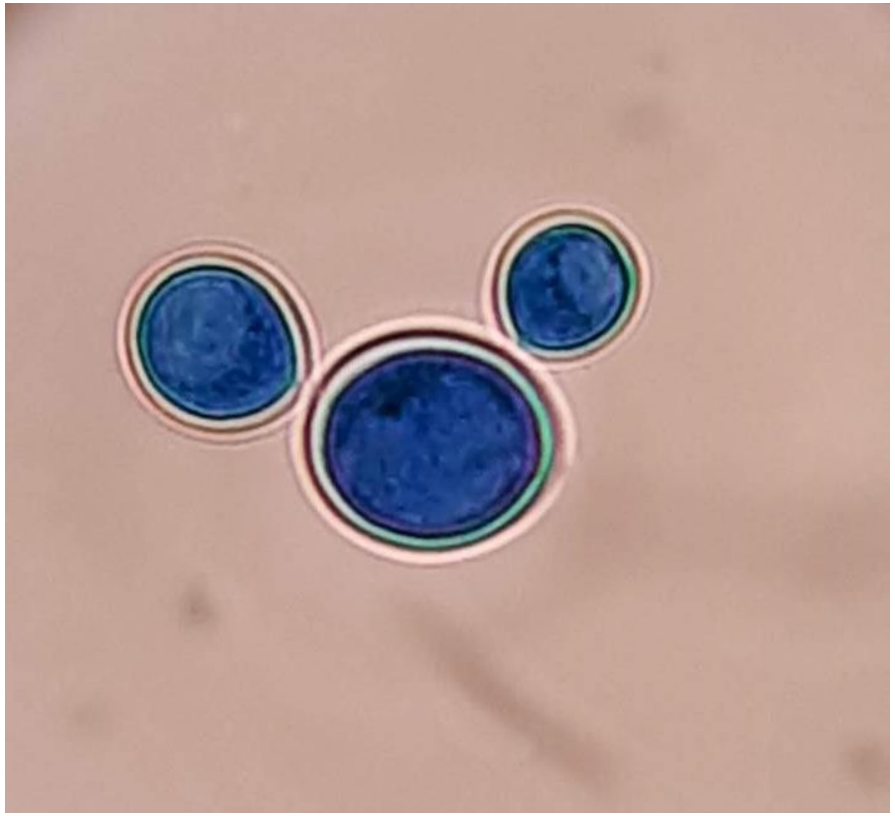
Riconoscimento

campione clinico

Paracoccidioides

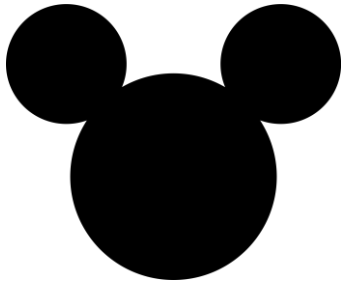


400X



ship's rudder wheel

10 – 40 microns



campione clinico

Esame diretto

Lattofenolo - cotton blue

10 – 40 micron

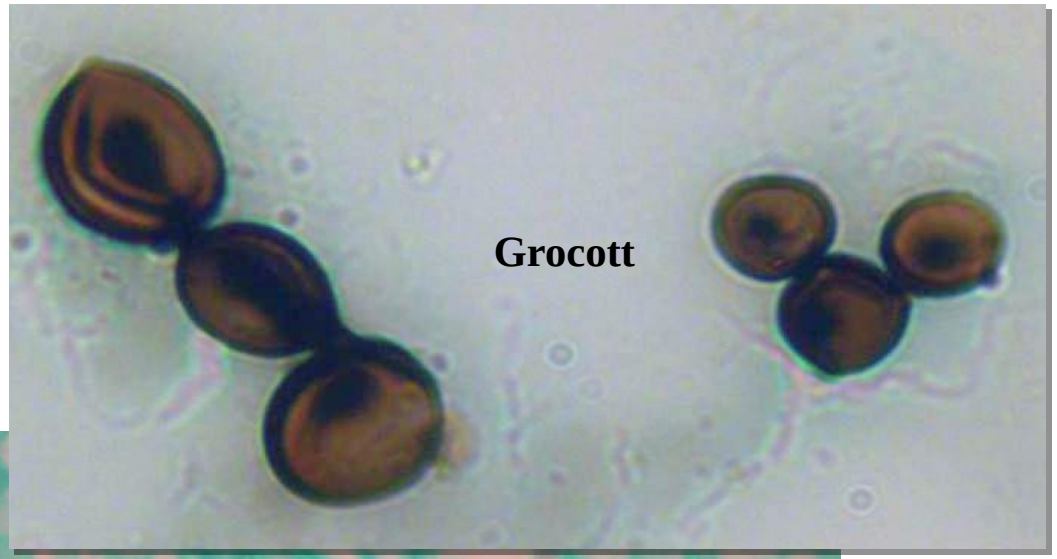
Lievito con gemme multiple

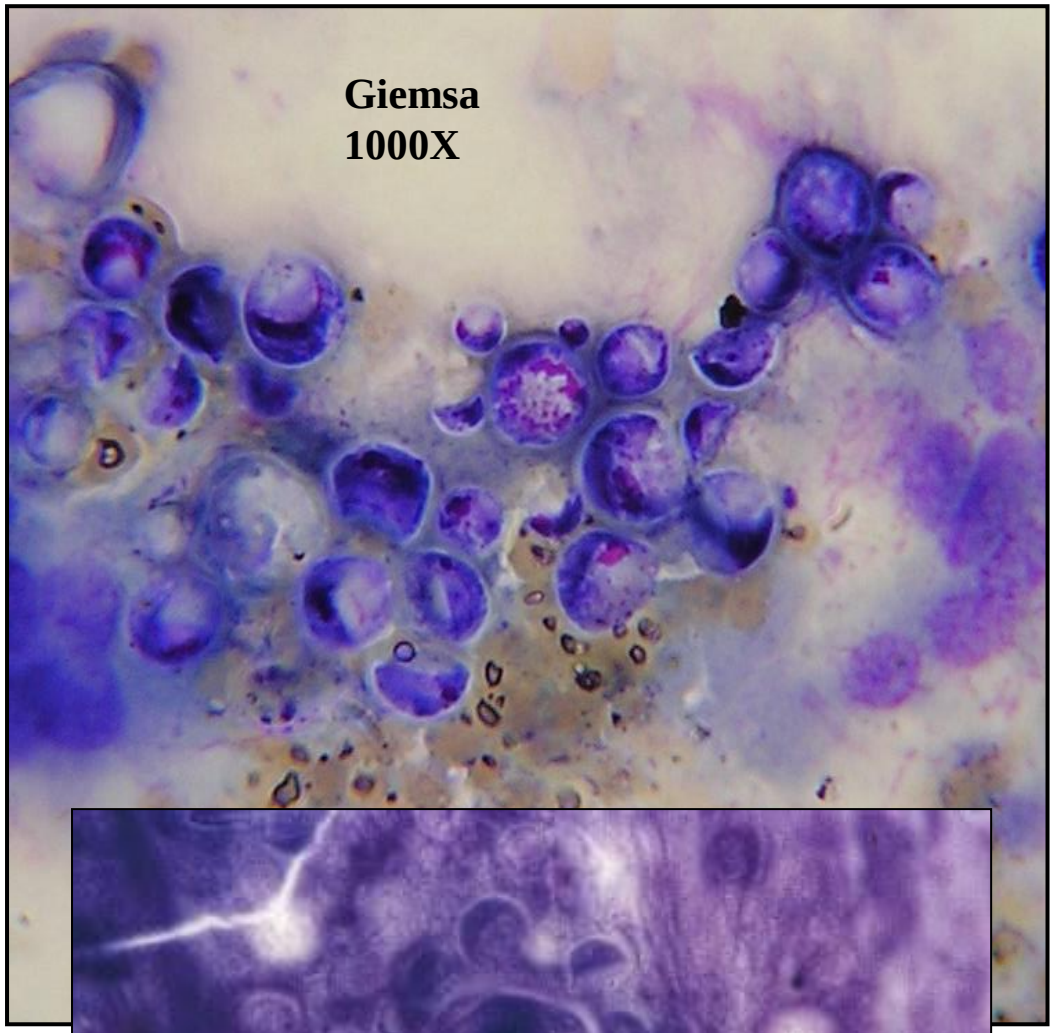
Dopia parete, spessa, rifrangente

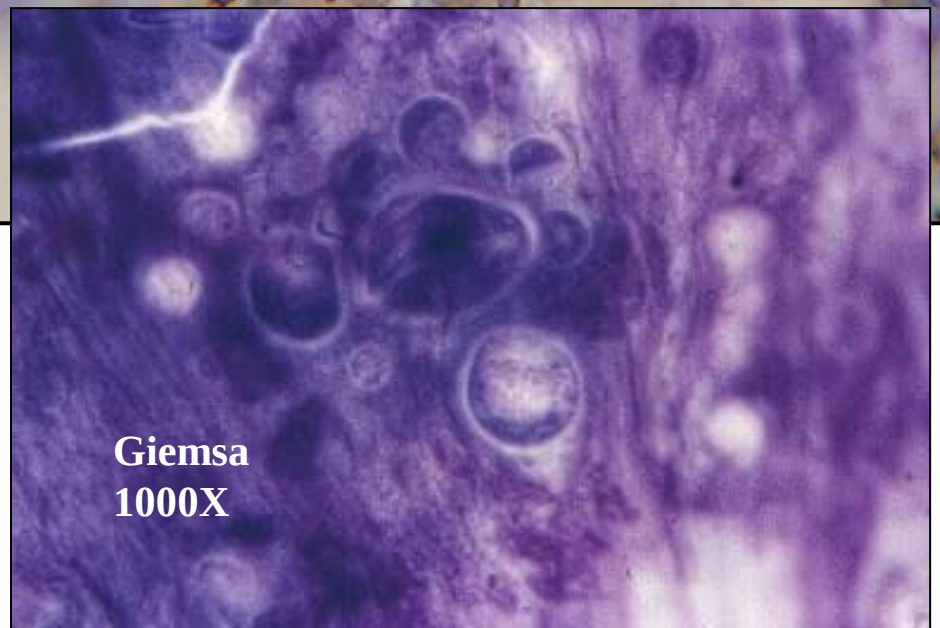
campione clinico

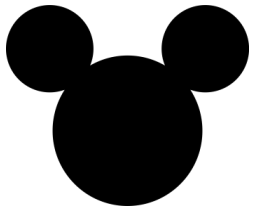
Lievito con gemme multiple

10 – 40 micron









Riconoscimento

dalla cultura

Coltivazione

Sempre con antibiotici !!!

cloramfenicolo, gentamicina, penicillina, streptomicina.

- **Sabouraud** — **Da 2 a 3 tubi**

Terreno acido-povero: glucosio, peptone, agar-agar.

- **Brain Heart Infusion (BHI)** — **Da 2 a 3 tubi**

- **Terreni selettivi:**
  - **Mycosel (BBL, Becton Dickinson)**
  - **Mycobiotic (Thermo Fisher Scientific, etc.)**

Da 2 a 3 tubi

Cicloheximide

Inibisce specie di *Cryptococcus, Scopulariopsis, Fusarium, Trichosporon, Aspergillus, Mucorales*

TERMODIMORFICO

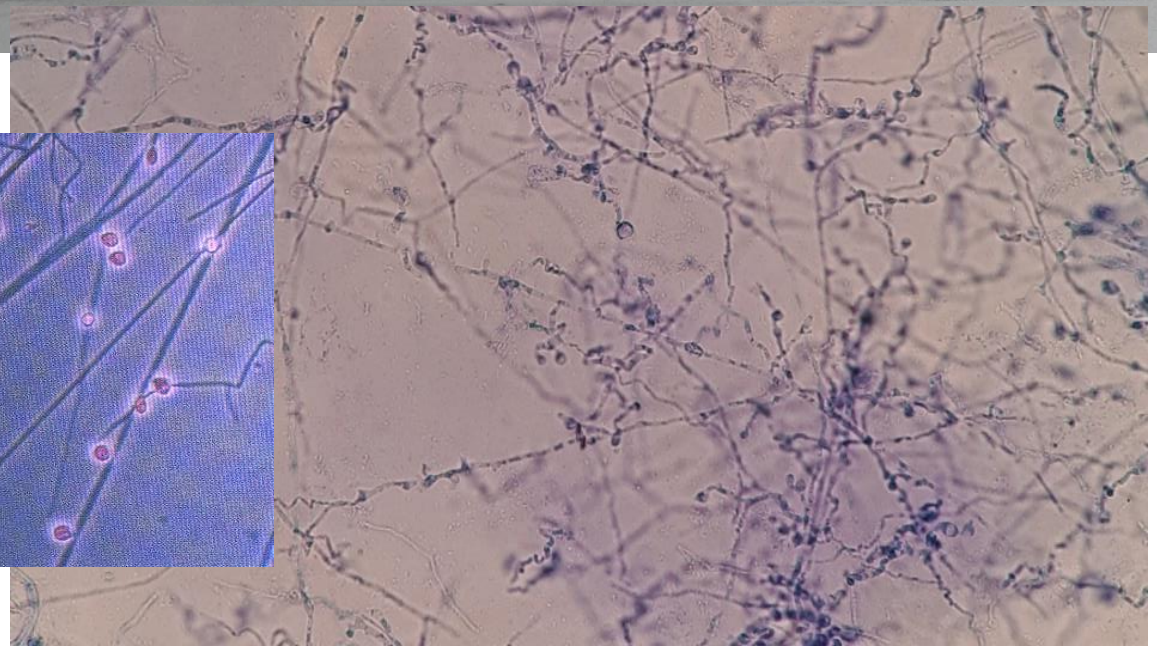
Tº : 25-28ºC

Tº: 35-37ºC

3 a 4 settimane

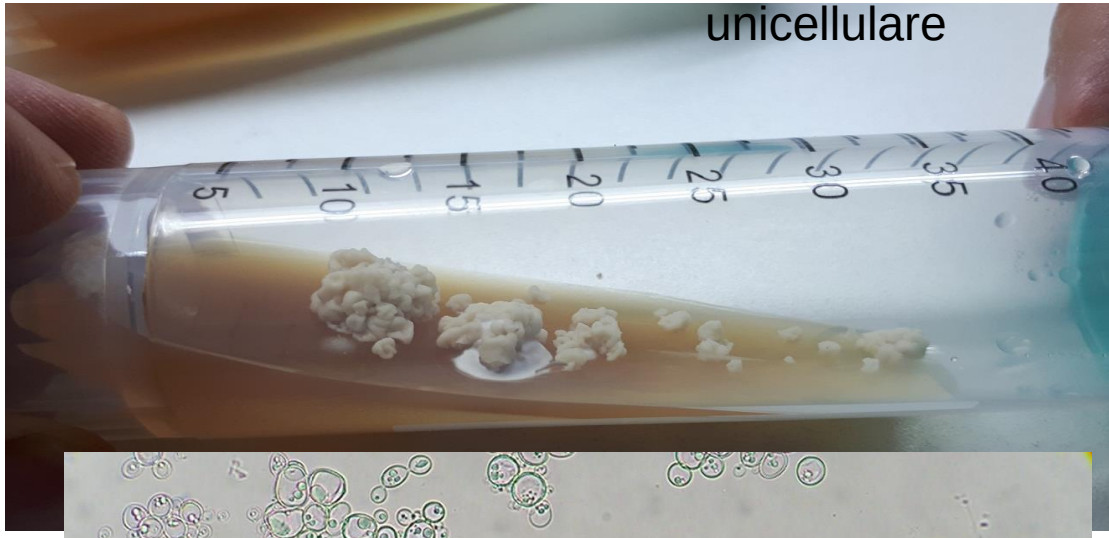
Fase filamentosa (saprofita)

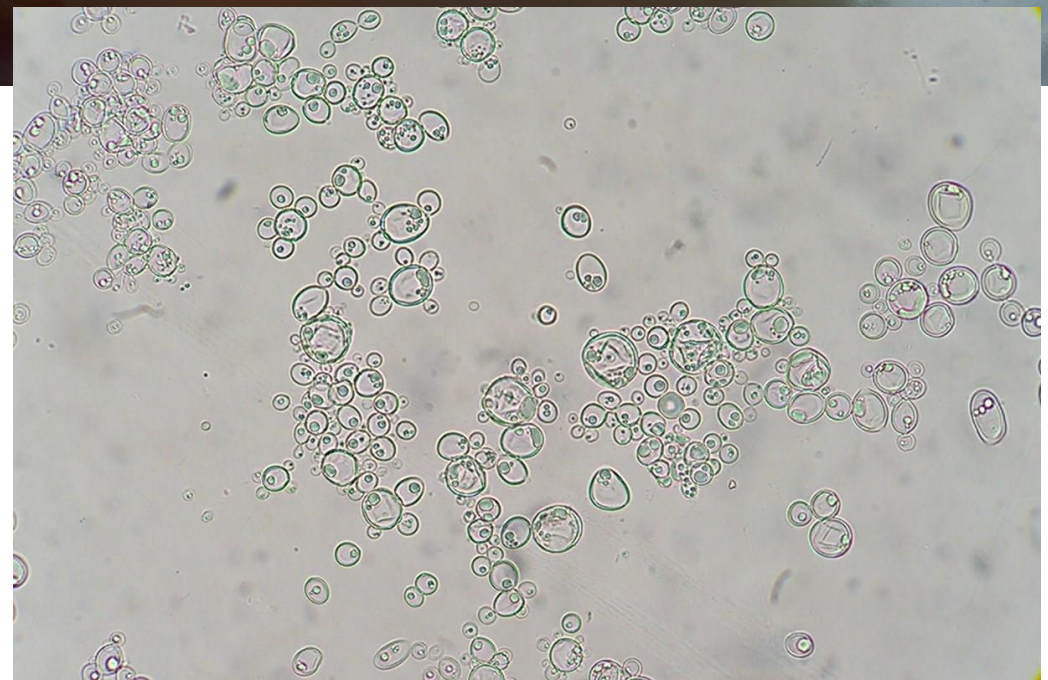
pluricellulare



Fase di lievito (parassitaria)

unicellulare





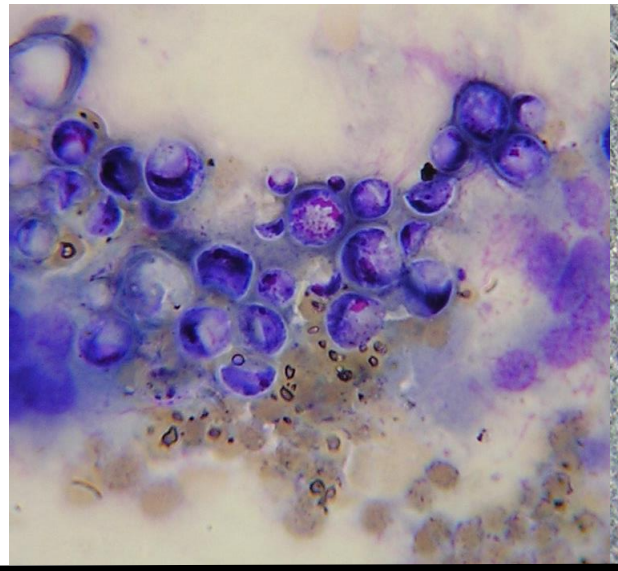
Paracoccidioides – fungo dimorfo

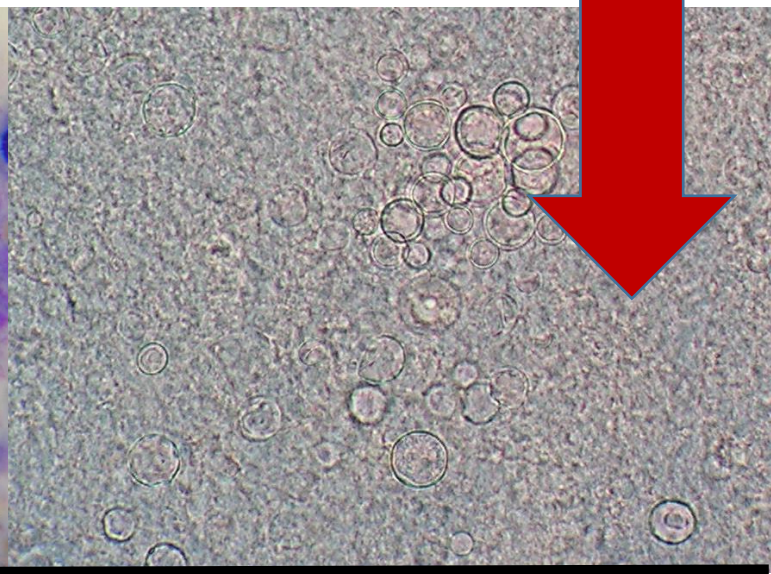
Fase saprofita **Tº : 25-28ºC**

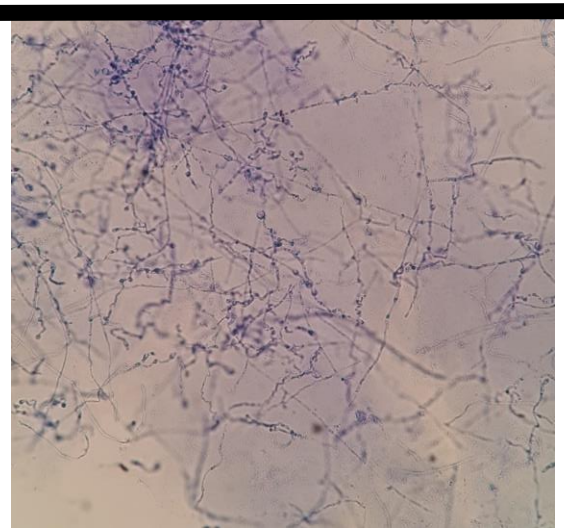
Tº: 35-37ºC **Fase parassitaria**

ambiente

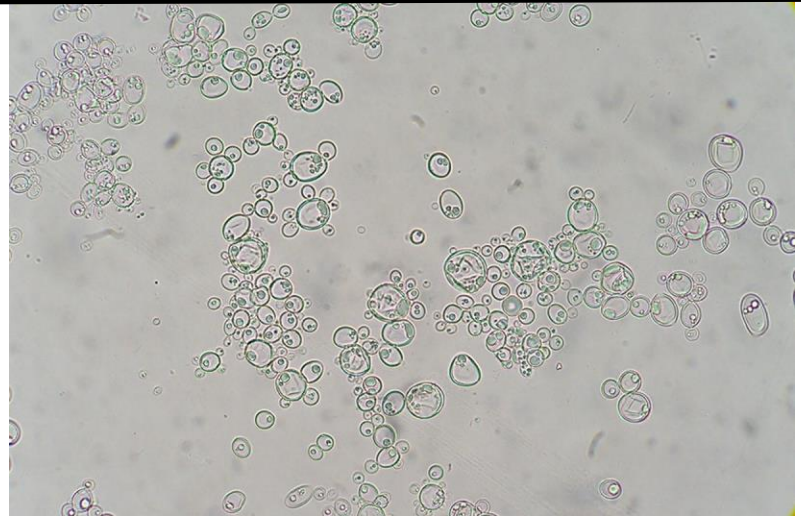
campione clinico







coltura



Fungal Genetics and Biology 140 (2020) 103395

Contents lists available at ScienceDirect

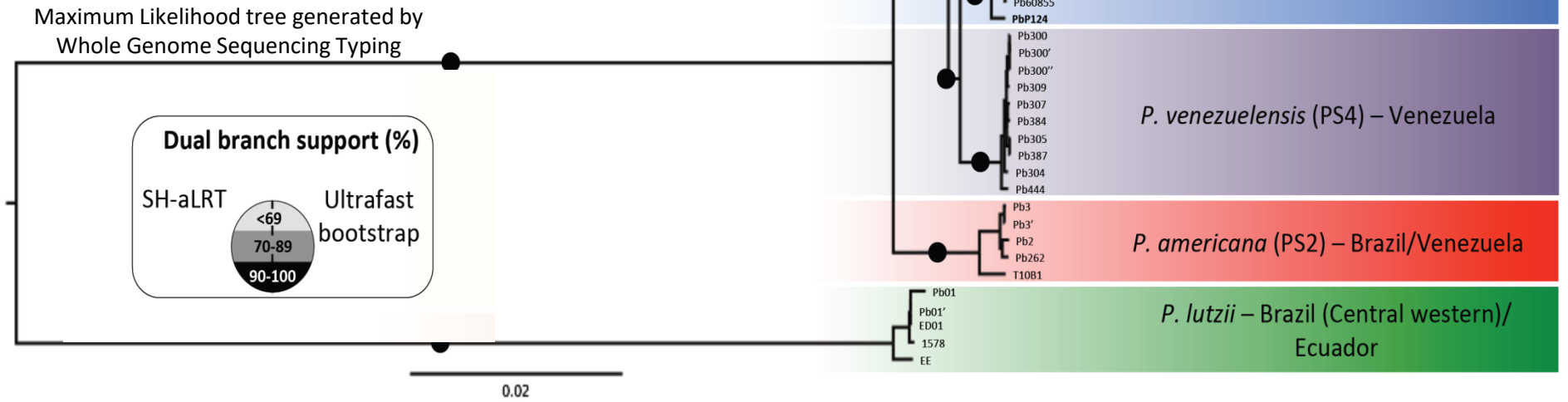
Fungal Genetics and Biology

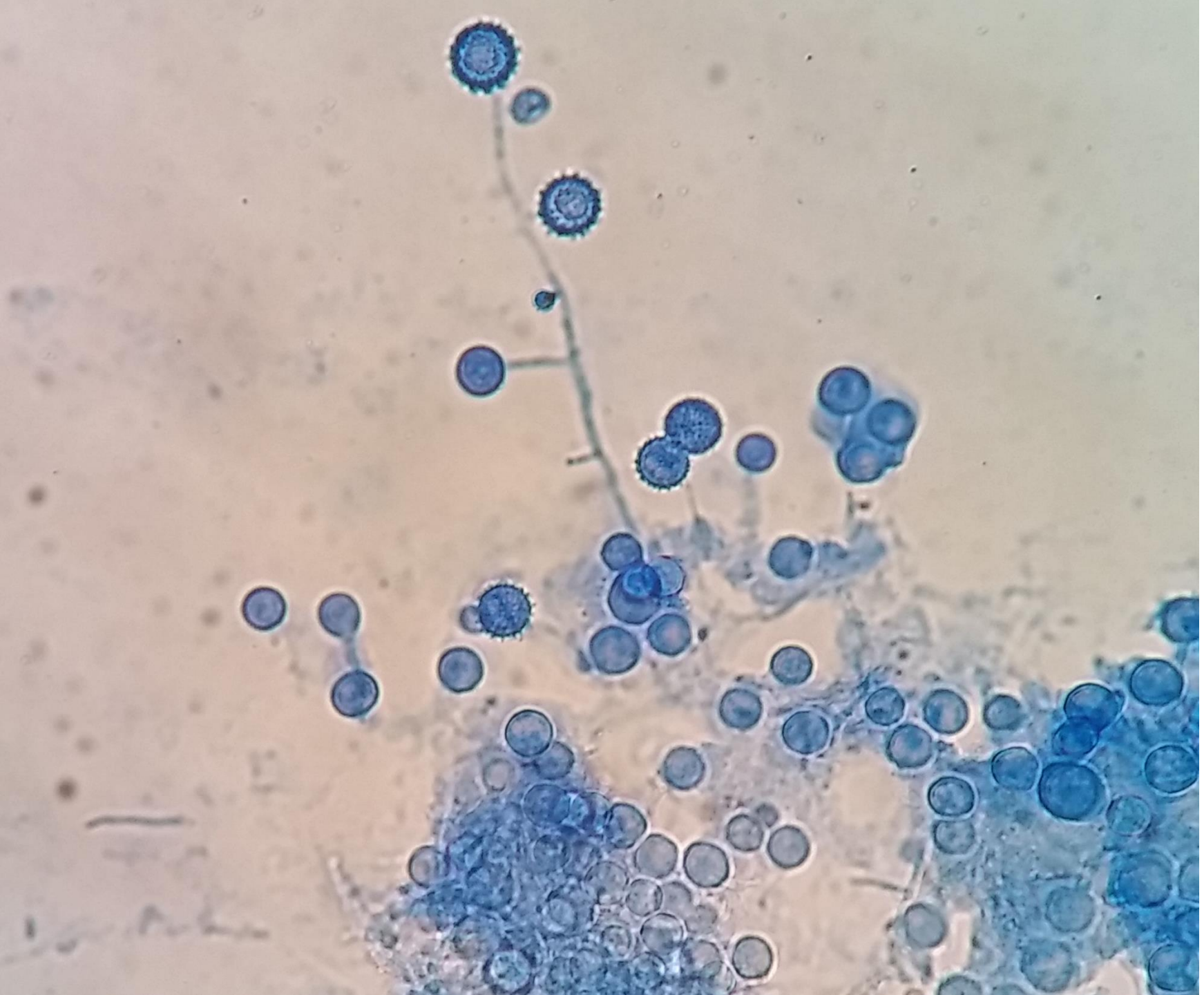
journal homepage: www.elsevier.com/locate/yfgbi

Genomic diversity of the human pathogen *Paracoccidioides* across the South American continent

Marcus de Melo Teixeira[a,b,*,1], Maria Emilia Cattana[a,d,1], Daniel R. Matute[e], José F. Muñoz[f], Alicia Arechavala[g], Kristin Isbell[e], Rafael Schipper[b], Gabriela Santiso[g], Fernanda Tracogna[d], María de los Ángeles Sosa[h], Norma Cech[i], Primavera Alvarado[j], Laura Barreto[k], Yone Chacón[l], Juana Ortellado[m], Cleoni Mendes de Lima[n], Marilene Rodrigues Chang[o], Gustavo Niño-Vega[p], Maria Aparecida Shikanai Yasuda[q], Maria Sueli Soares Felipe[r], Ricardo Negroni[g], Christina A. Cuomo[f], Bridget Barker[a,2], Gustavo Giusiano[c,*,2]

[a] Northern Arizona University, Flagstaff, AZ, USA
[b] Universidade de Brasília, Brasilia, Brazil
[c] Universidad Nacional del Nordeste, Resistencia, Chaco, Argentina
[d] Hospital Dr. Julio C. Perrando, Resistencia, Chaco, Argentina
[e] Biology Department, University of North Carolina, Chapel Hill, NC, USA
[f] Broad Institute of MIT and Harvard, Cambridge, USA
[g] Hospital Muñiz, Buenos Aires, Argentina
[h] Laboratorio Central De Redes y Programas, Corrientes, Argentina
[i] Hospital 4 de Junio, Chaco, Argentina
[j] Servicio Autónomo Instituto de Biomedicina Dr. Jacinto Convit, Caracas, Venezuela
[k] Instituto Superior de Formación Docente Salome Ureña, Santo Domingo, Dominican Republic
[l] Hospital Señor del Milagro, Salta, Argentina
[m] Universidad Nacional de Asunción, Asuncion, Paraguay
[n] Universidade Federal de Rondônia, Porto Velho, Rondônia, Brazil
[o] Universidade Federal do Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil
[p] Universidad de Guanajuato, Guanajuato, Mexico
[q] Universidade de São Paulo, São Paulo, Brazil
[r] Universidade Católica de Brasília, Brasília, Distrito Federal, Brazil





Histoplasma

Istoplasmosi capsulatum

Istoplasmosi: agenti eziologici

- ***H. capsulatum var capsulatum***

  Istoplasmosi americana o istoplasmosi capsulati.

Distribuzione globale che causa infezioni polmonari e sistemiche in una varietà di mammiferi, compresi gli esseri umani - responsabili dell'istoplasmosi classica

- ***H. capsulatum var duboisii***

  Istoplasmosi africana duboisii istoplasmosi

Confinato in Africa centrale e occidentale – causa linfoadenopatia e diffusione alla pelle e alle ossa, principalmente negli esseri umani e in altri primati

- ***H. capsulatum var farciminosum***

  Agente di linfangite epizootica di cavalli in varie parti del mondo

Colpisce la pelle e il sistema linfatico sottocutaneo negli equidi (cavalli, asini e muli) ma è stato recuperato anche da esseri umani, cani, gatti e tassi.

Fungal infections in animals: a patchwork of different situations. Medical Mycology, 2018, 56, S165–S187.

Riconoscimento

campione clinico

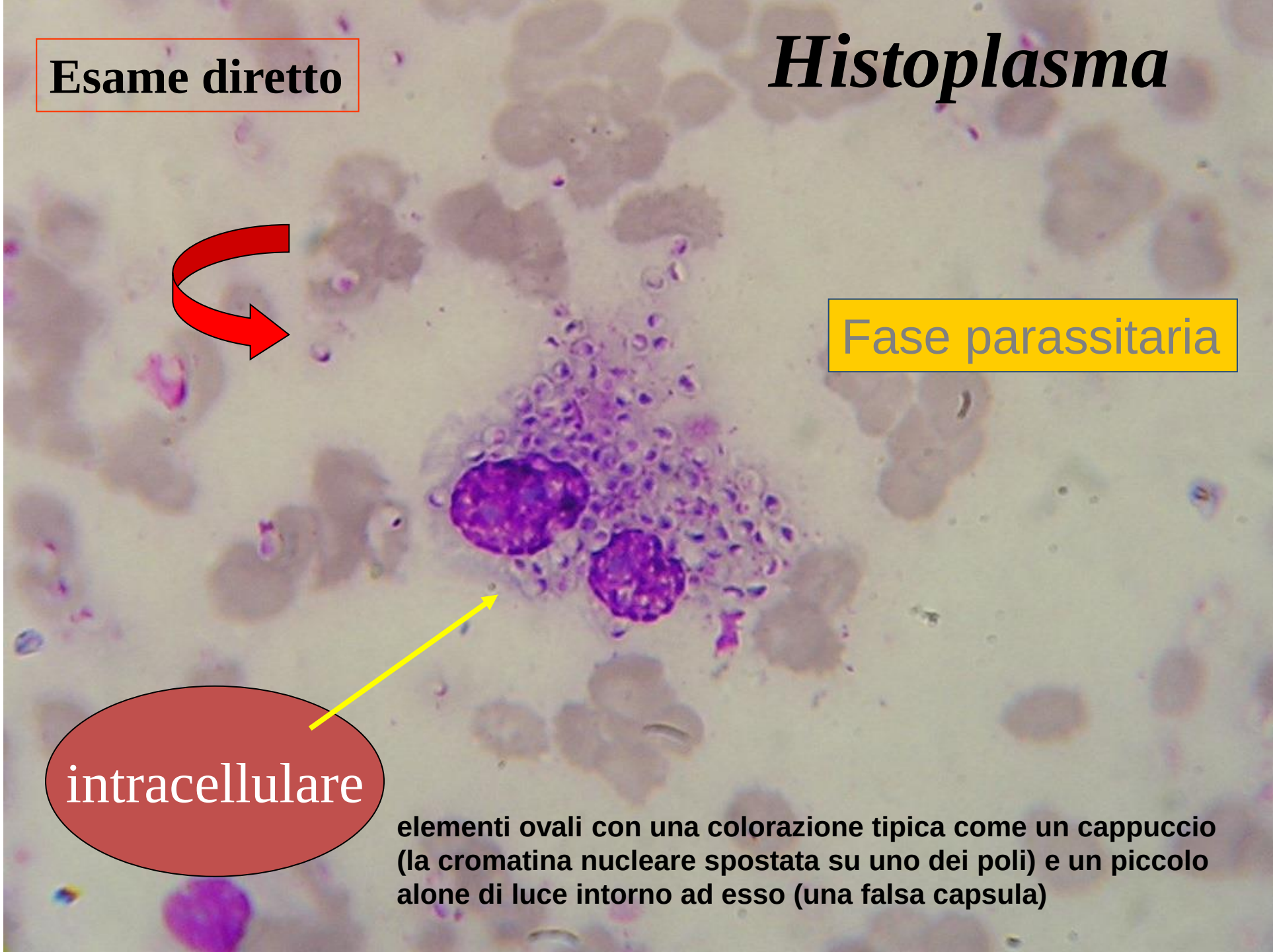
Histoplasma

Esame diretto

Giemsa

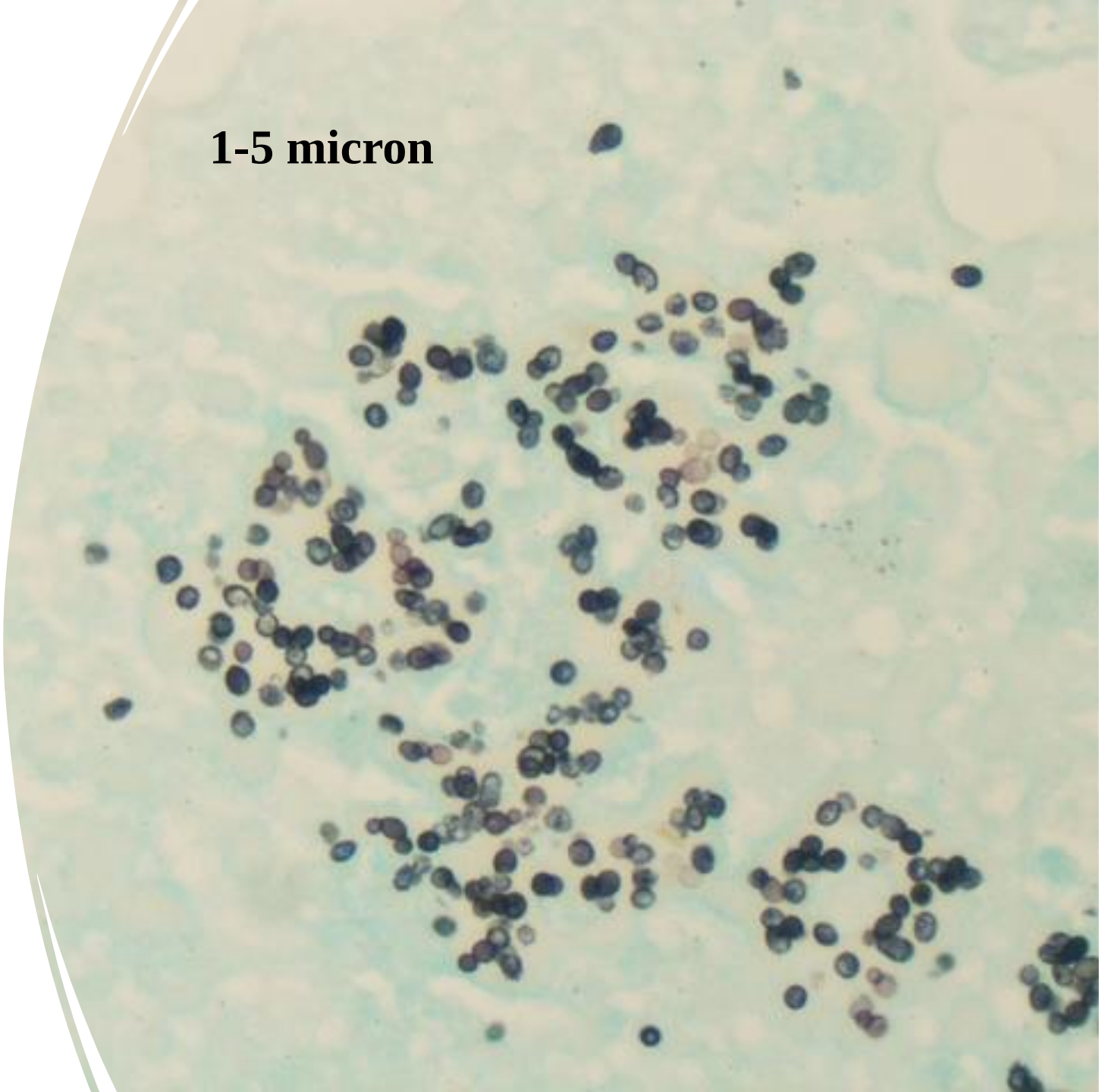
1-5 micron

La sensibilità dell'esame varia a seconda del carico della malattia e dipende in gran parte dal grado di immunosoppressione dell'ospite



elementi ovali con una colorazione tipica come un cappuccio (la cromatina nucleare spostata su uno dei poli) e un piccolo alone di luce intorno ad esso (una falsa capsula)

- **Grocott**



Riconoscimento

dalla cultura

TERMODIMORFICO

Tº : 25-28ºC ⇄ 3 a 4 settimane ⇄ **Tº: 35-37ºC**

2 o 3 tubi ad ogni temperatura

Fase filamentosa (saprofita)

pluricellulare

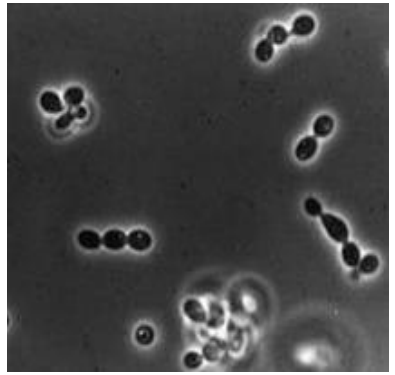
Colonie a crescita lenta
(2 o 4 settimane)

Colonie cotonate
Biancastro poi marrone

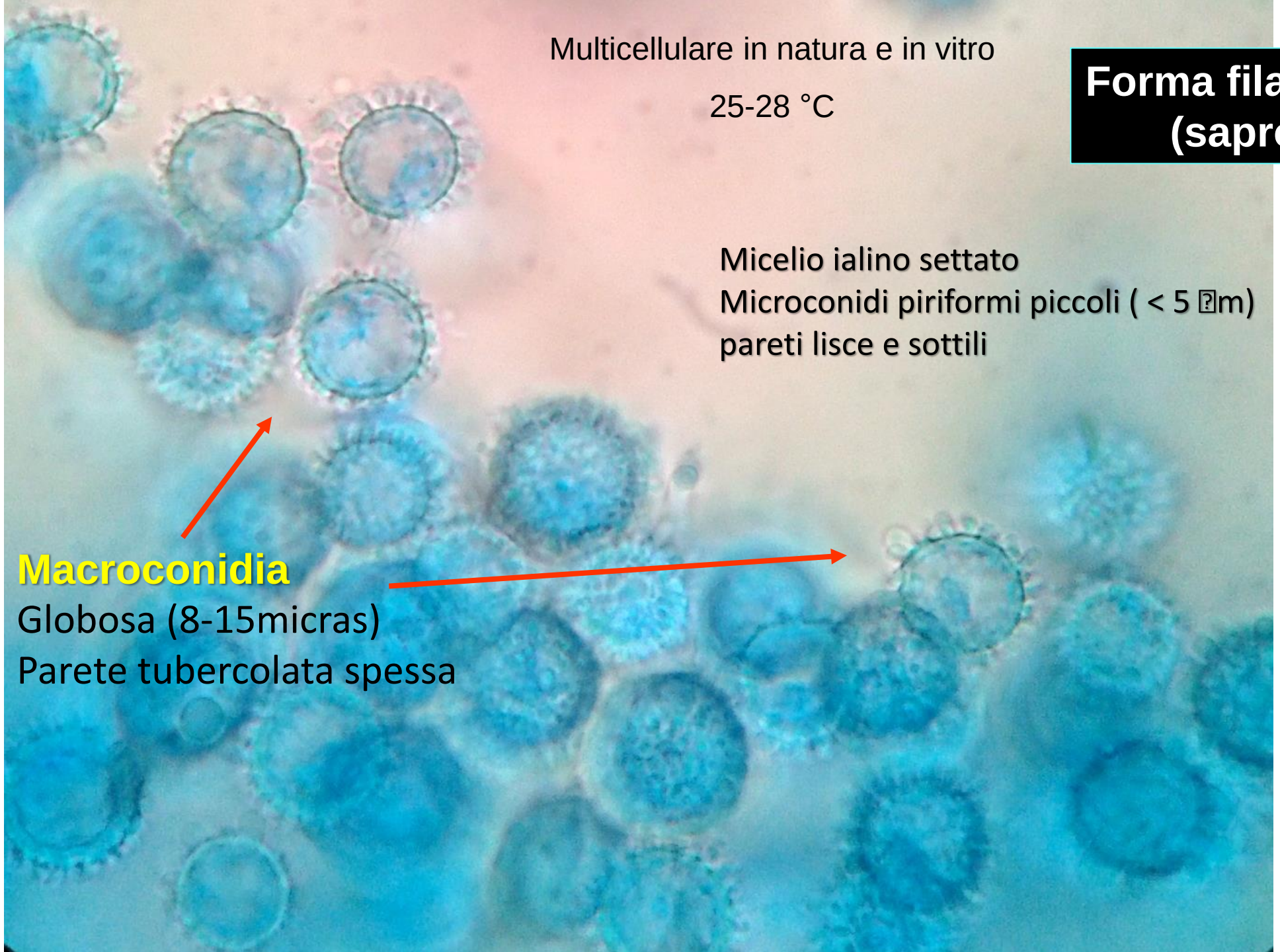
microscopia

Fase di lievito (parassitaria)

unicellulare



Lieviti (1-5 micron) (unicellulare) ovoidale con una singola gemma



Multicellulare in natura e in vitro

25-28 °C

Forma filamentosa (saprofita)

Micelio ialino settato
Microconidi piriformi piccoli (< 5 □m)
pareti lisce e sottili

Macroconidia
Globosa (8-15micras)
Parete tubercolata spessa

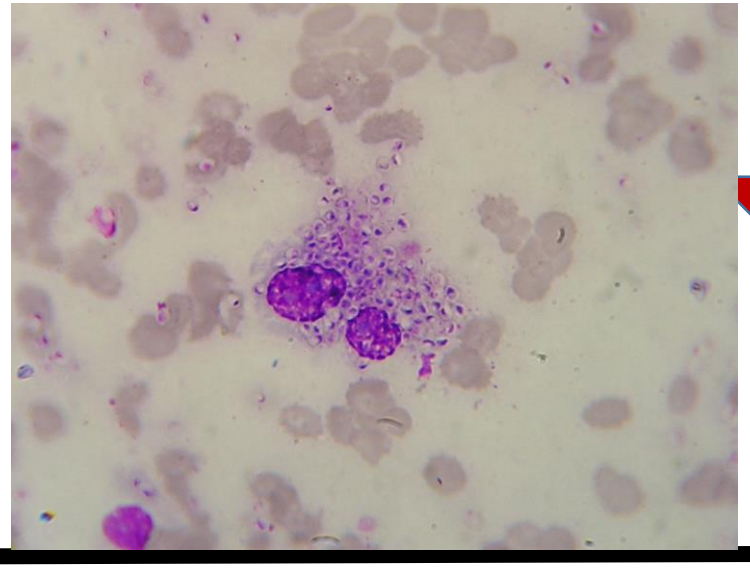
Histoplama – fungo dimorfo

Fase saprofita **Tº : 25-28ºC**

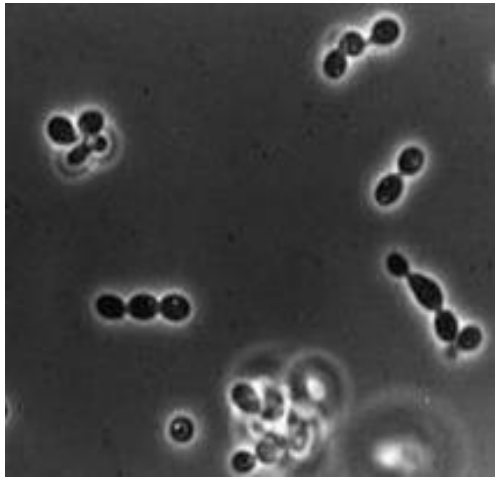
Tº: 35-37ºC **Fase parassitaria**

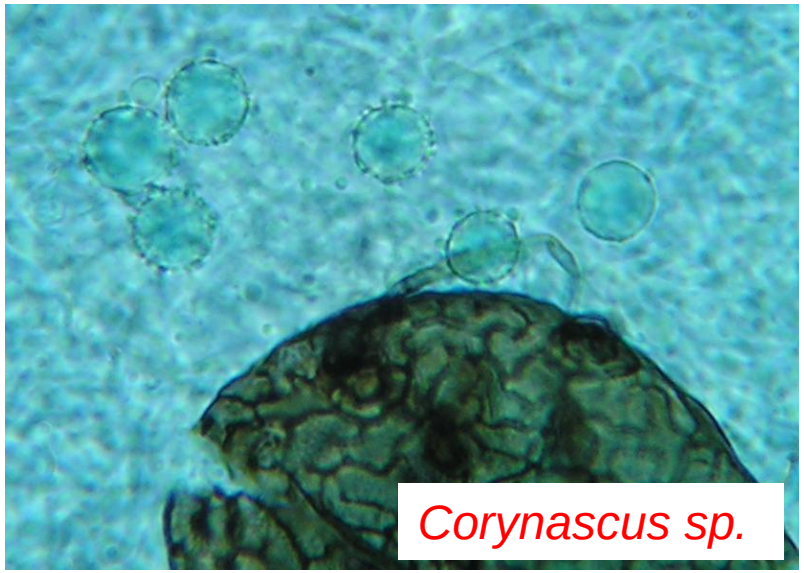
ambiente

campione clinico

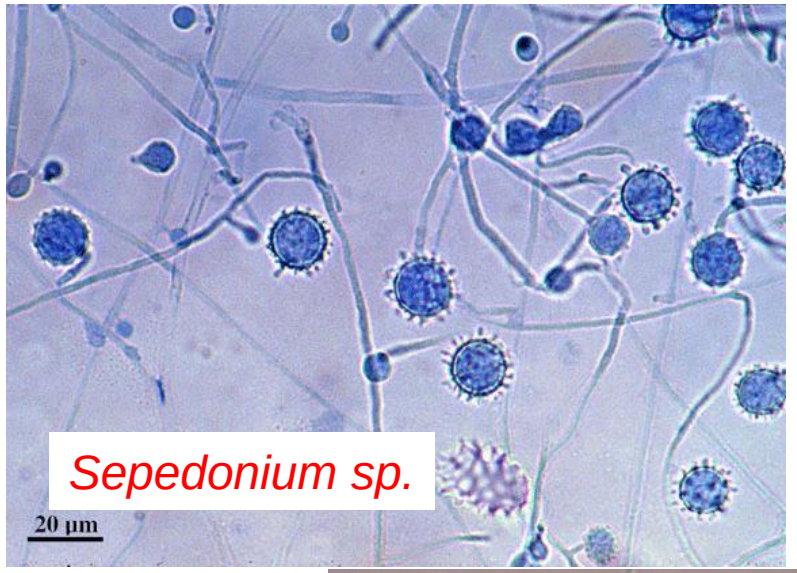


coltura



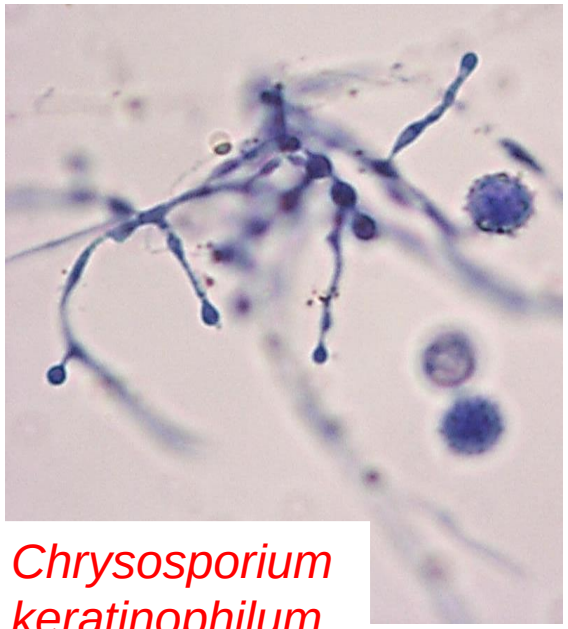


Corynascus sp.



Sepedonium sp.

Hongos ambientales



Chrysosporium keratinophilum

Aphanoascus fulvescens

Histoplasma capsulatum

Renispora sp.

Coccidioides

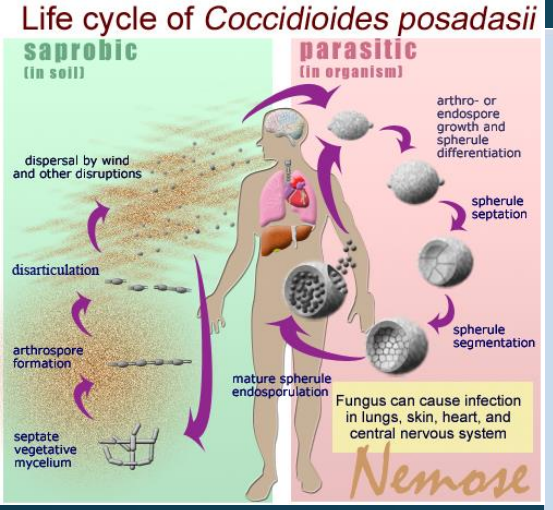
Due specie criptiche all'interno del genere *Coccidioides*

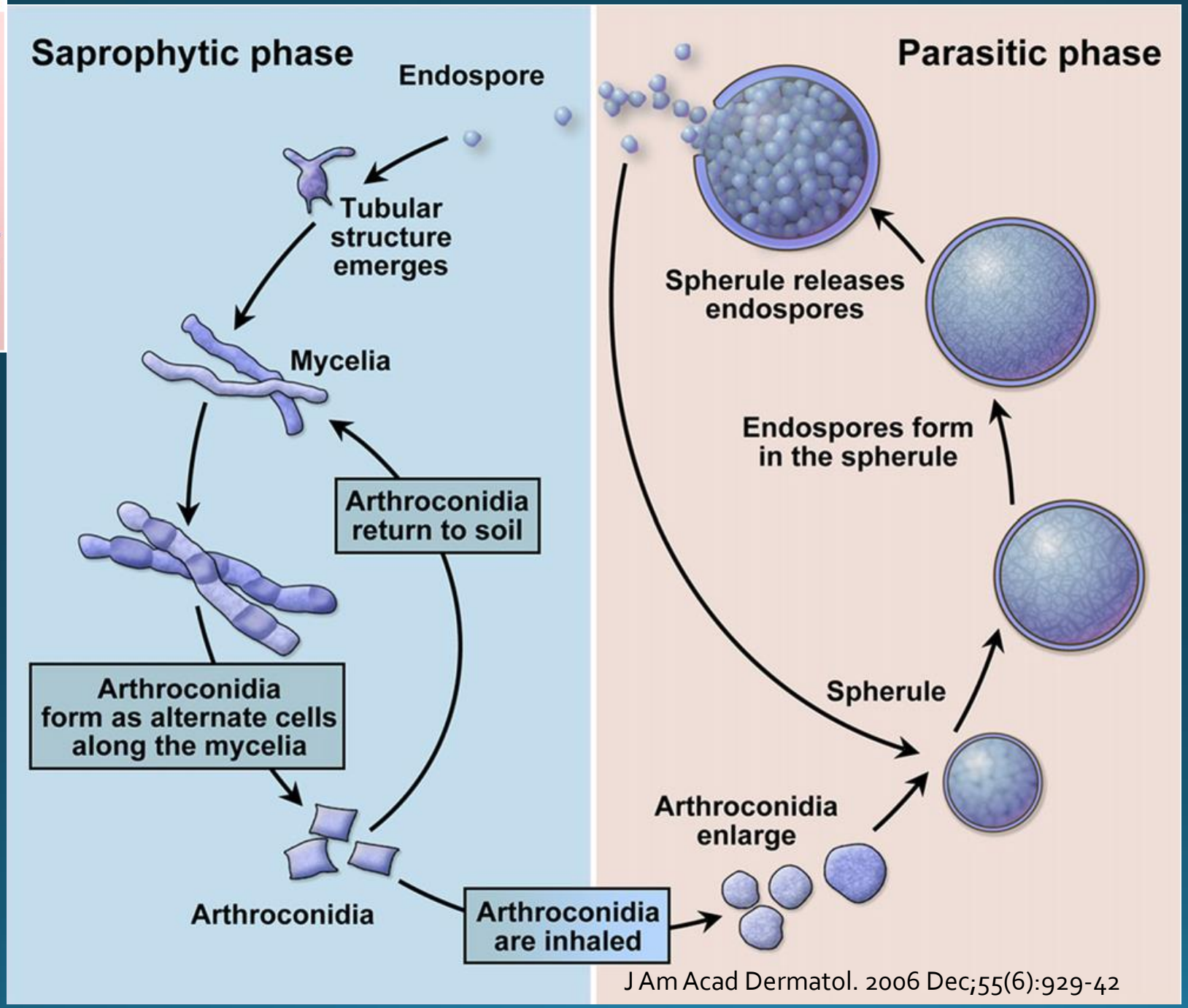
Coccidioides immitis (San Joaquin Valley)

Coccidioides posadasii (USA- Mexico- Latinamerica)

Riconoscimento

campione clinico

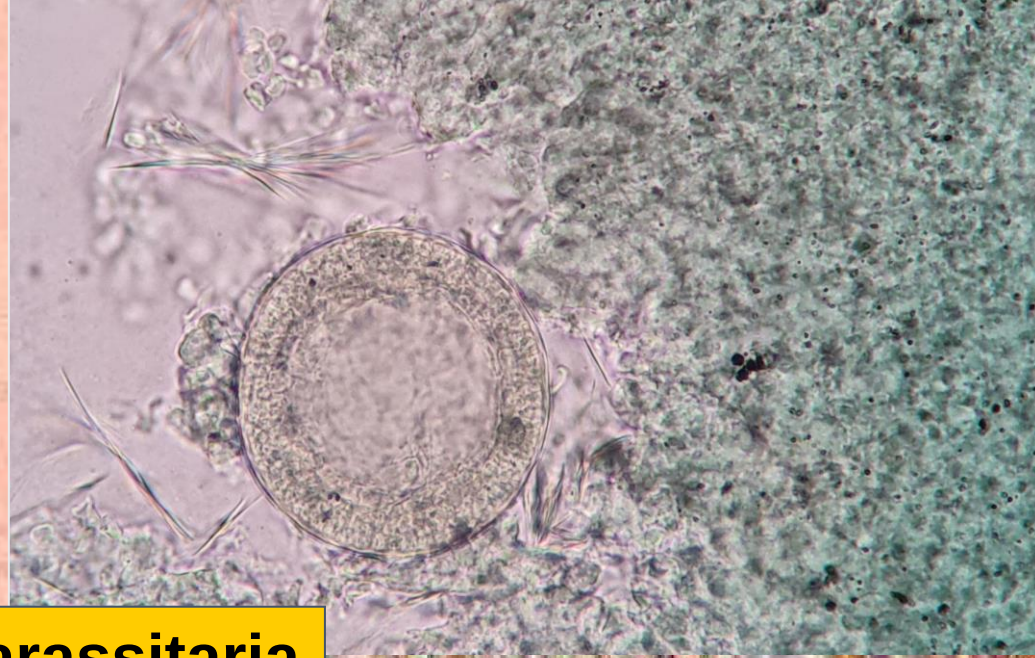




J Am Acad Dermatol. 2006 Dec;55(6):929-42

La sferula nel polmone crescerà e, alla fine, scoppierà rilasciando endospore, che si sviluppano in più sferule.

All'interno del polmone, la spora si trasforma in una struttura multicellulare più grande chiamata sferula

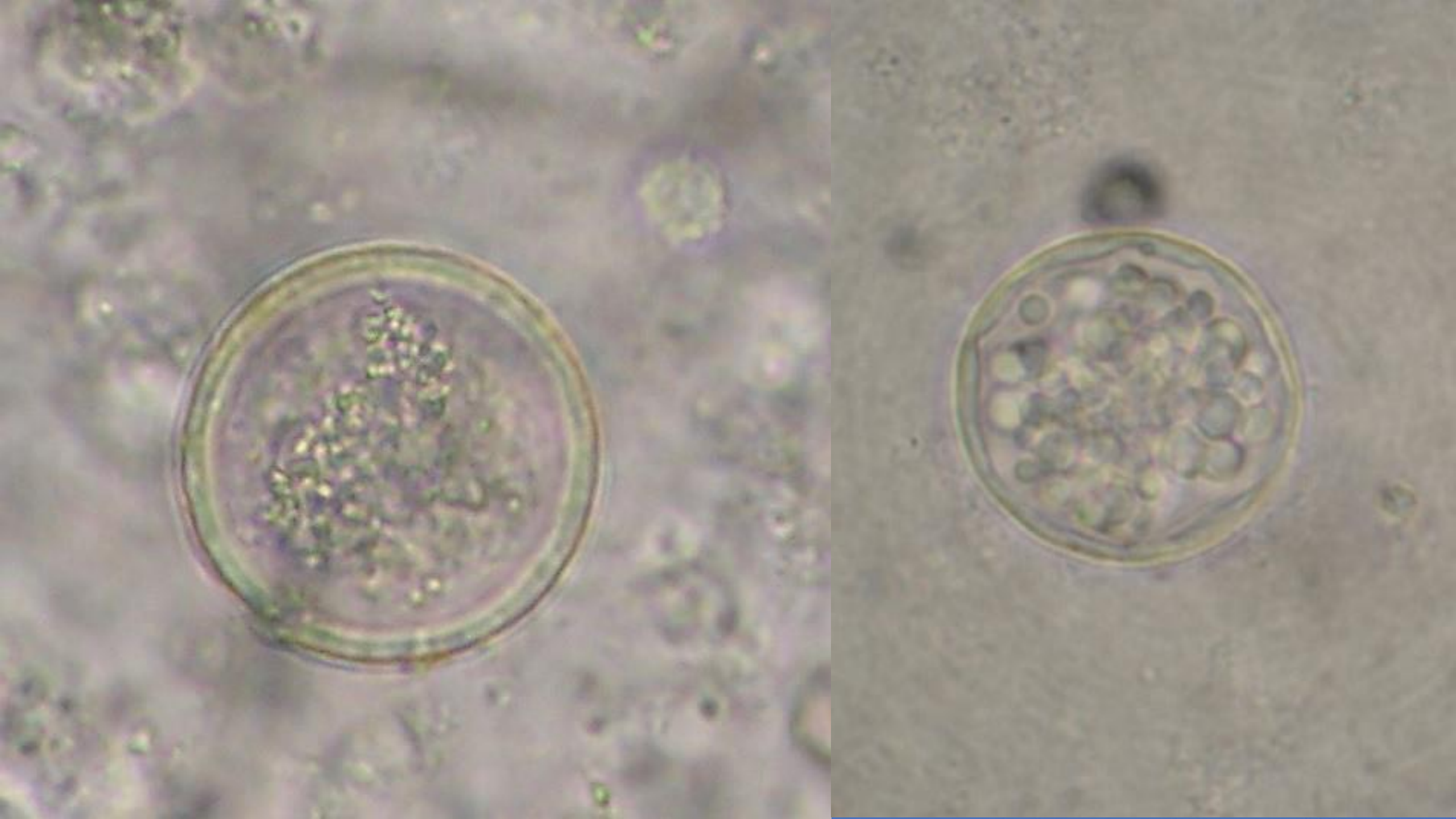


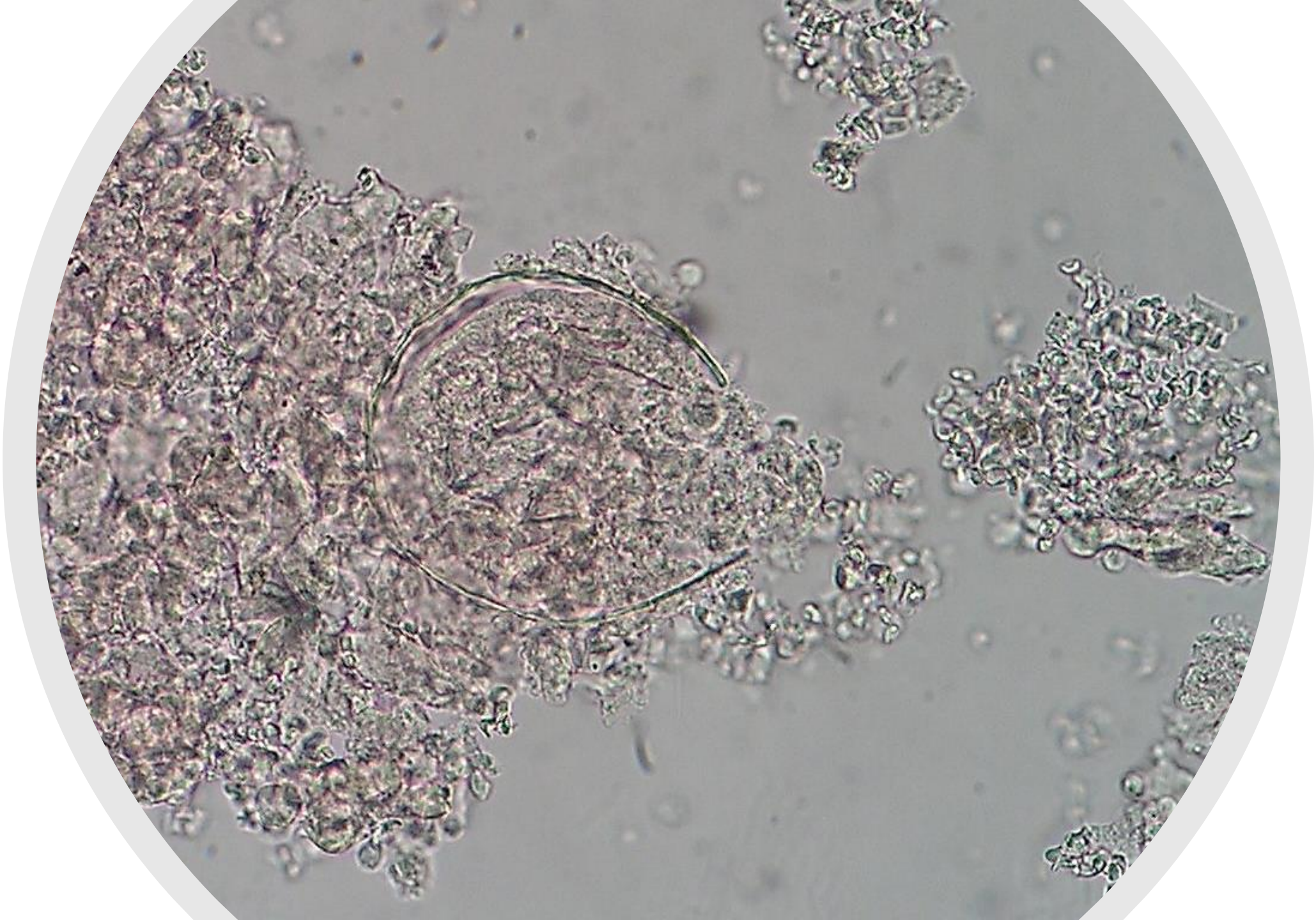
Fase parassitaria

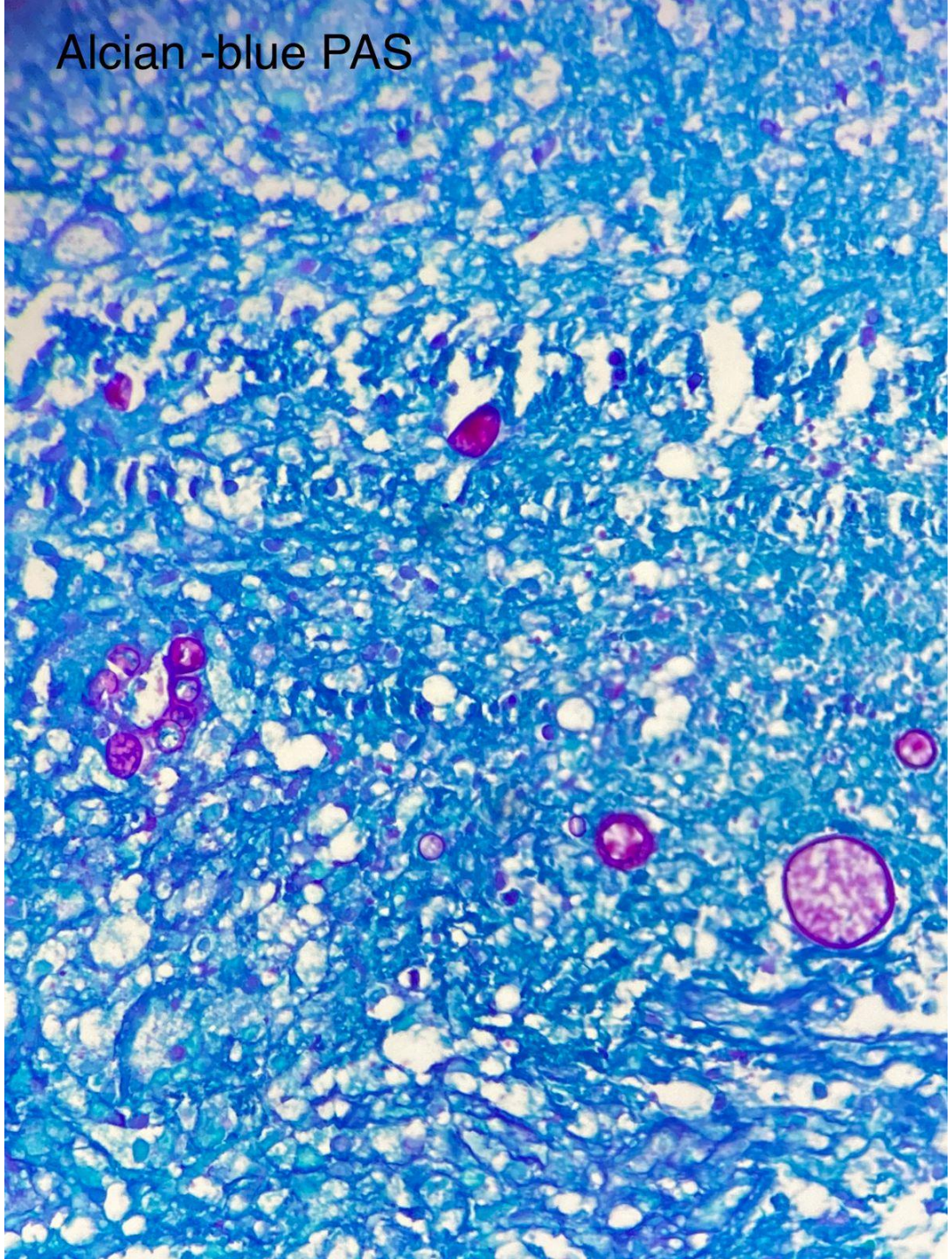
Esame diretto

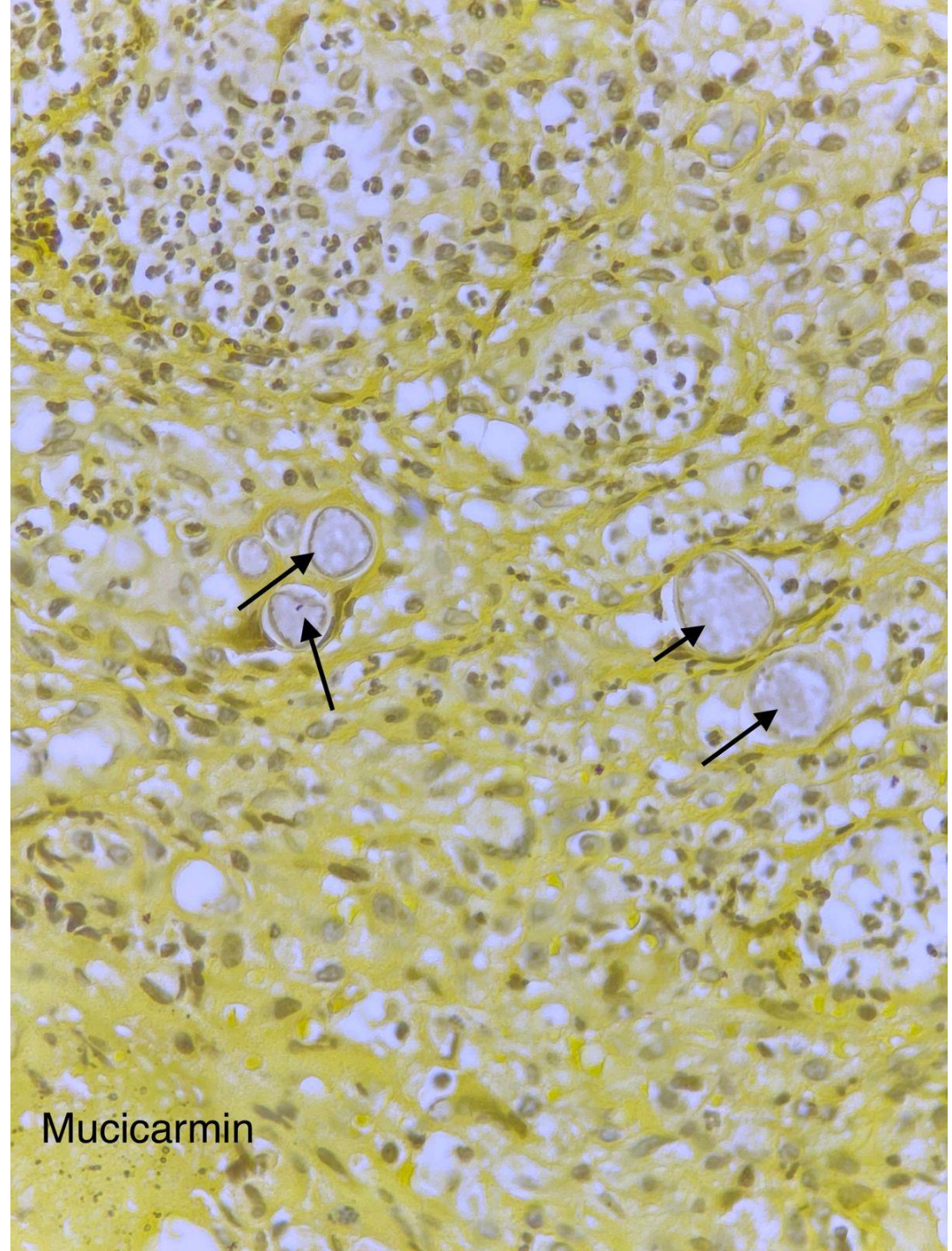
Blue di lattofenolo

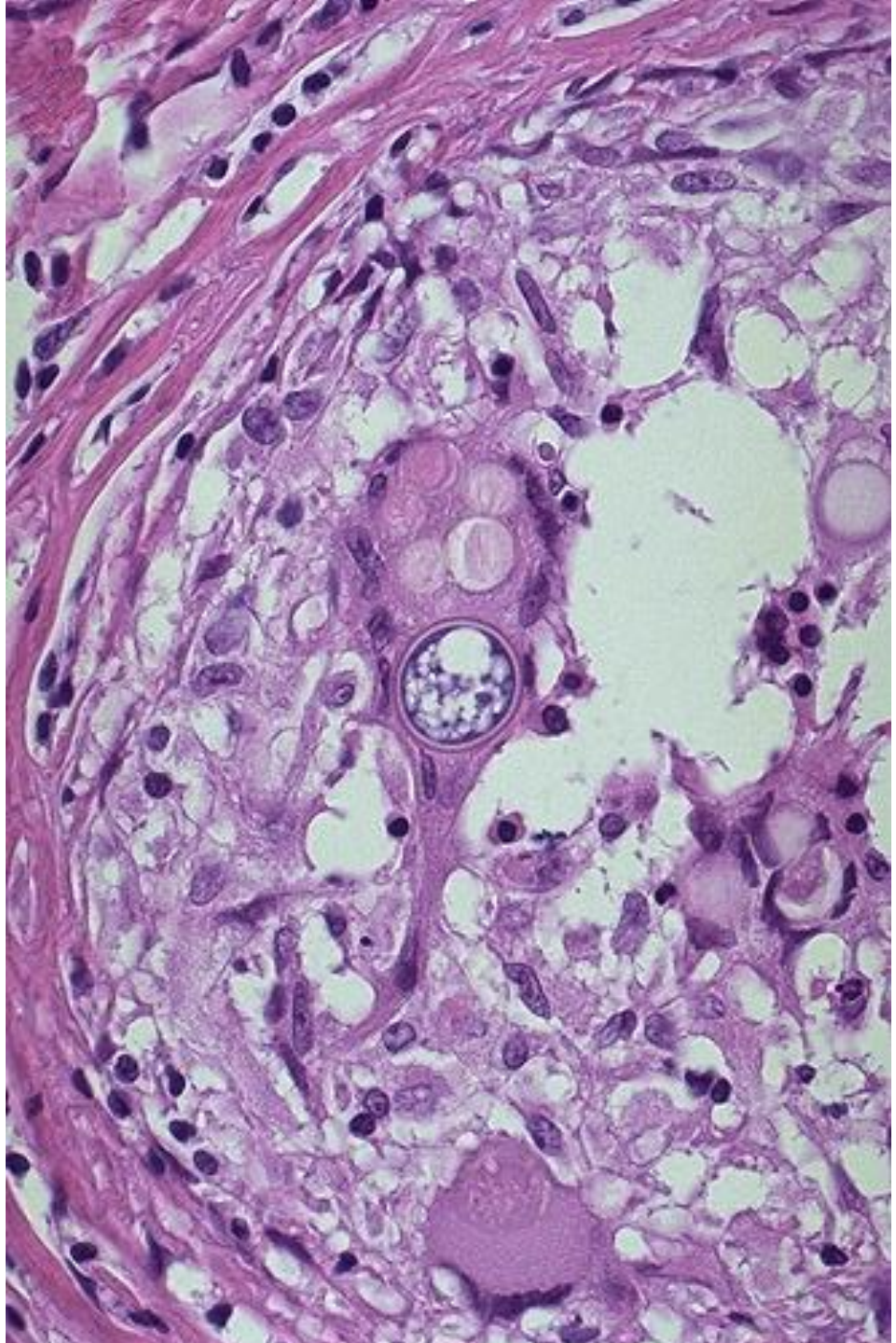
Da 20 a 80 µ di diametro. Parete spessa,
con endospore all'interno

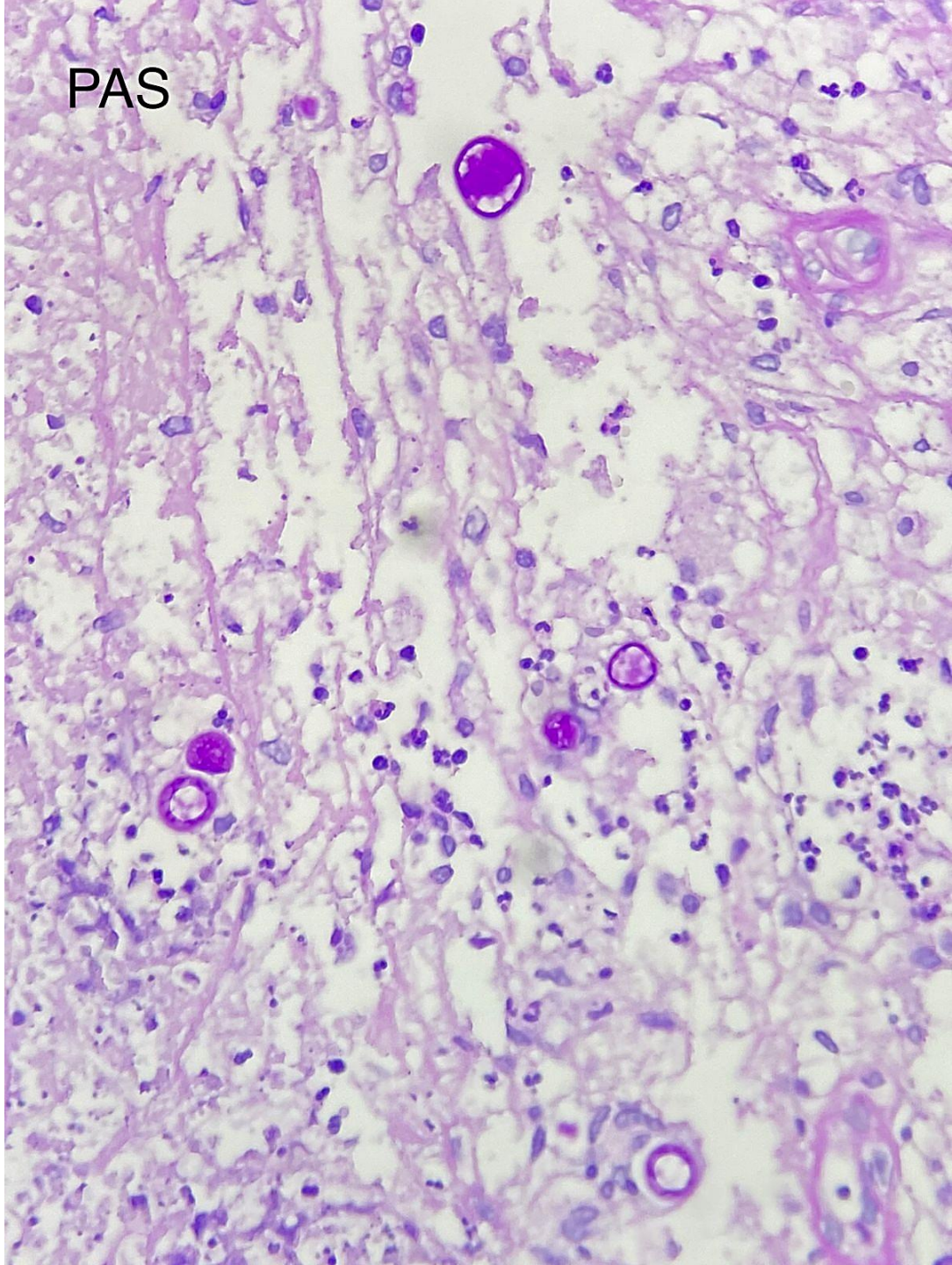


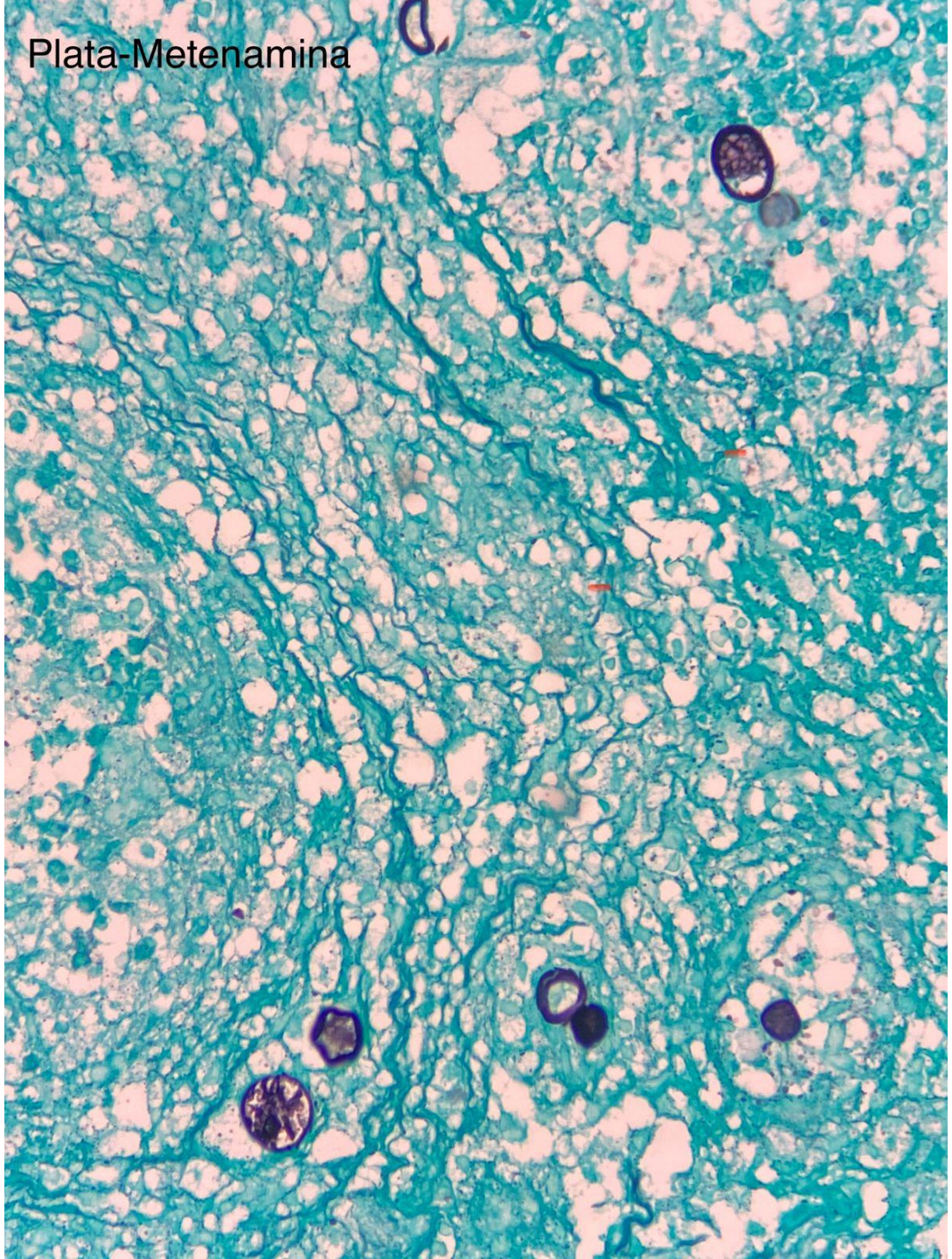


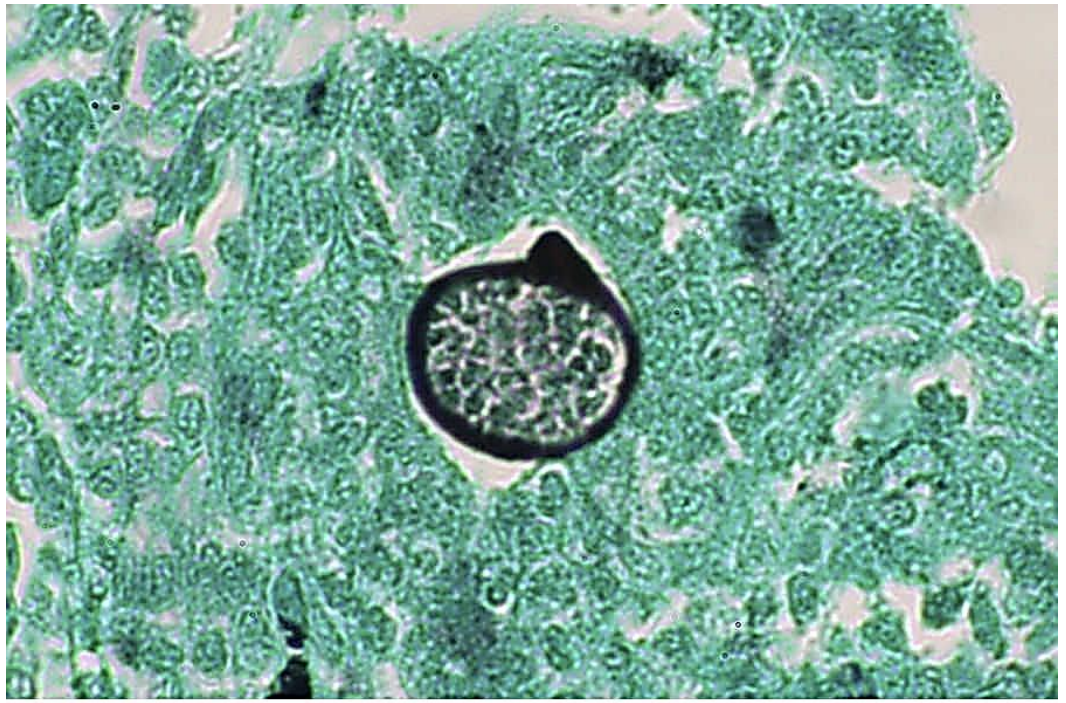

Alcian -blue PAS


Mucicarmin




PAS

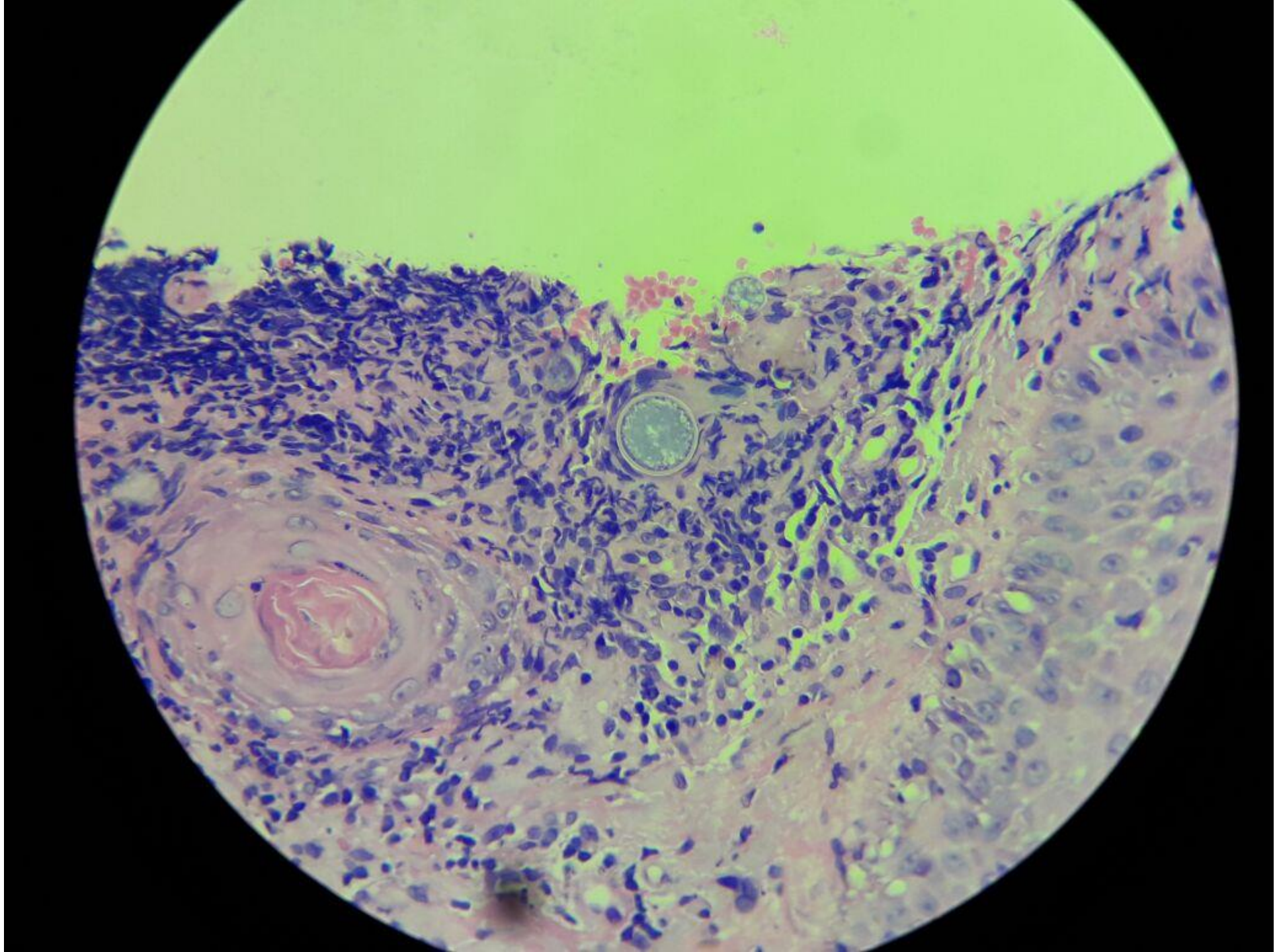




Actas Dermosifiliogr. 2014;105:5–17

Grocott

H&E



Riconoscimento

dalla coltura

NO - TERMODIMORFO

35-37ºC

25-28ºC

2 o 3 tubi ad ogni temperatura

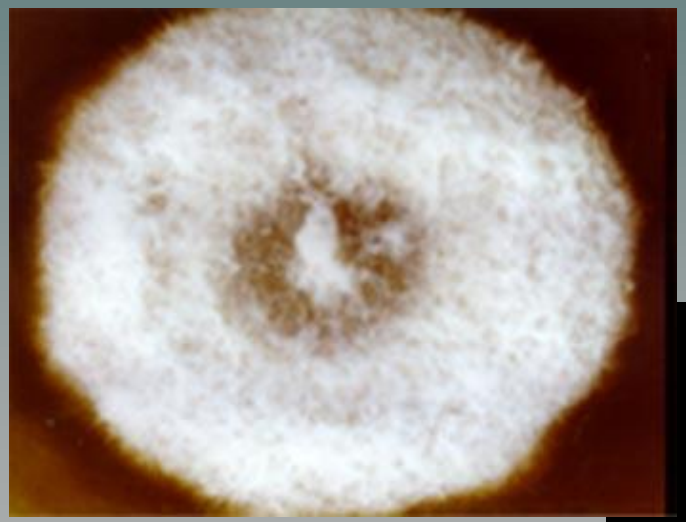
Tra 4-5 giorni a
più di 30 giorni

Fase filamentosa (saprofita)

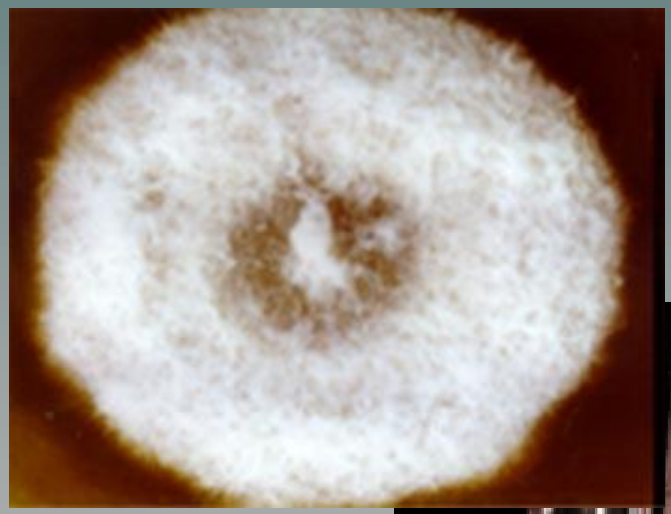
pluricellulare

Fase filamentosa (saprofita)

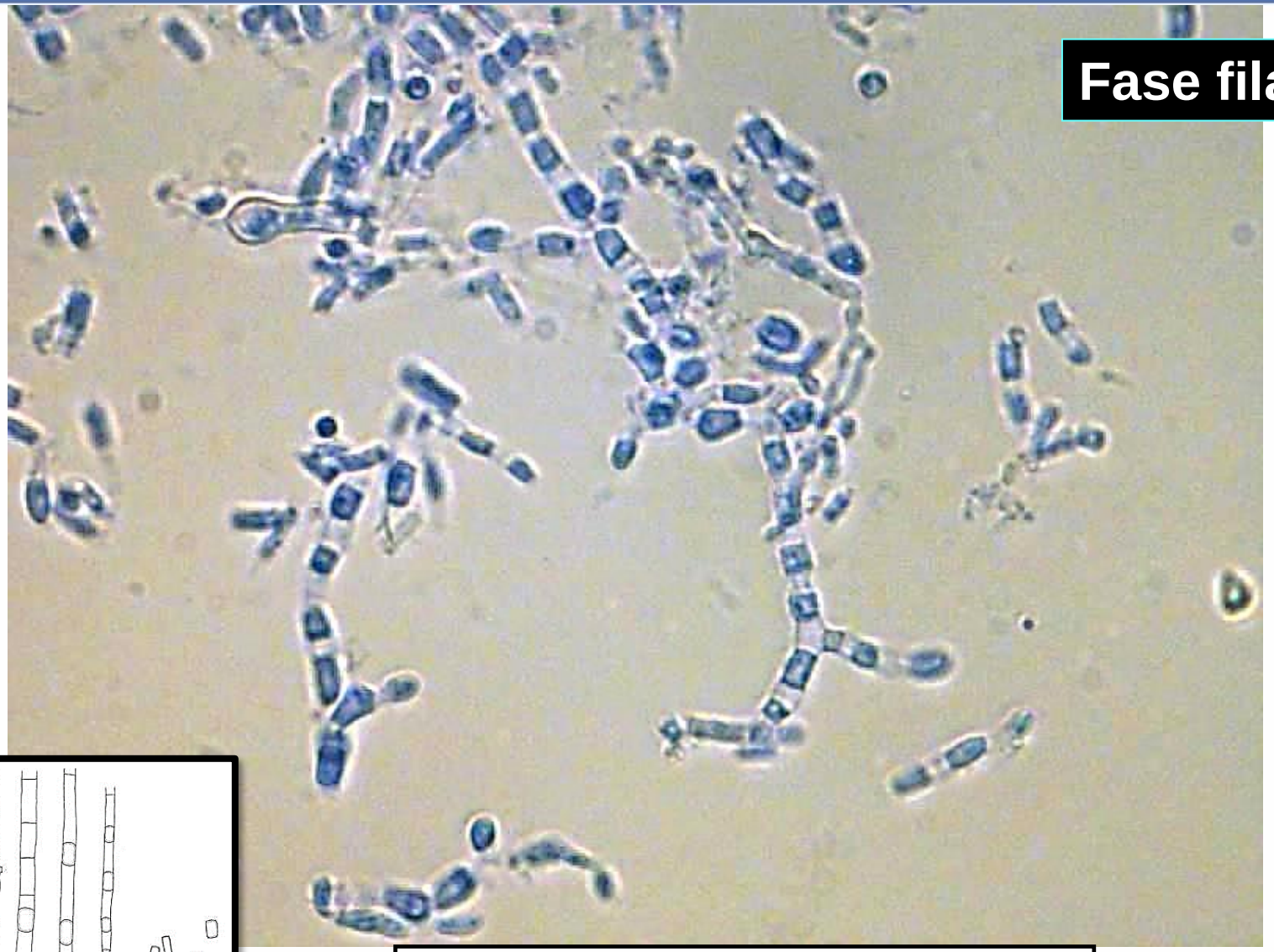
pluricellulare

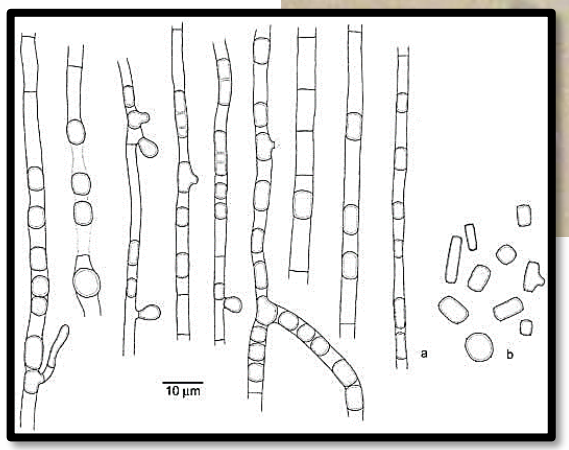


Caratteristiche:
Colonie bianco-grigie sul retro del colore crema



Fase filamentosa





Micromorfologia

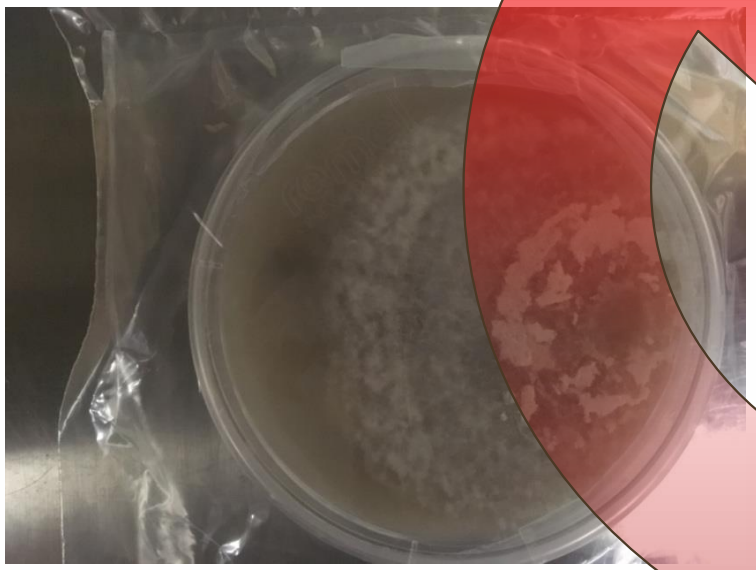
Fase saprofita:

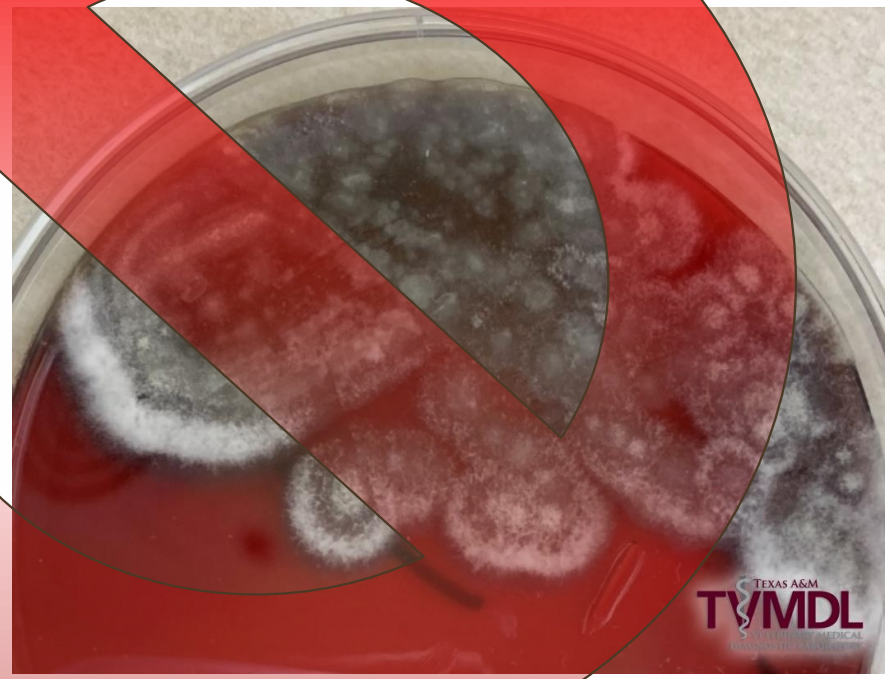
Ife fertili che crescono ad angolo retto e producono Chlamydo-arthrochoidia ialina di una cellula sotto forma di barilotto di pareti lisce e spesse che si alternano a celle interruttori automatici vuoti. (Elementi infettivi)

Tº : 25-28ºC y 35-37ºC

Fase filamentosa

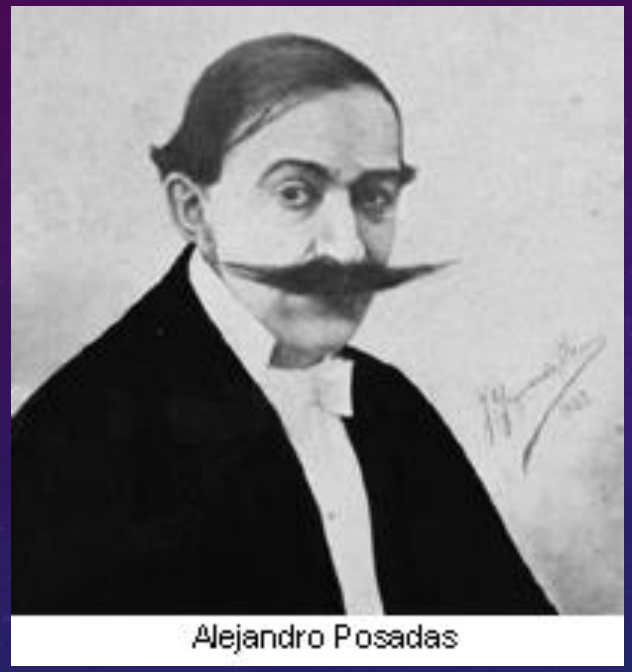
Alto rischio in laboratorio



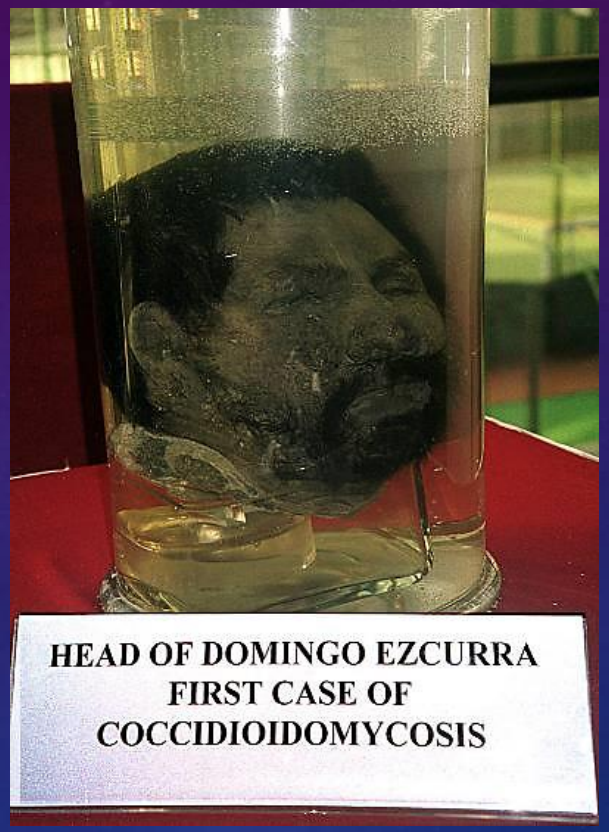


Le colture di questi funghi rappresentano un grave rischio biologico per il personale di laboratorio e devono essere maneggiate con estrema cautela in una cappa adatta alla manipolazione di agenti patogeni.

la coltura deve essere manipolata in una cappa di biosicurezza di classe II tipo B2



1892



Fino ad oggi, la testa di Domingo è conservata nel Centro di Micologia dell'Università di Buenos Aires, Facoltà di Medicina.
Argentina

Medical Mycology, 2019, 57, S3–S15

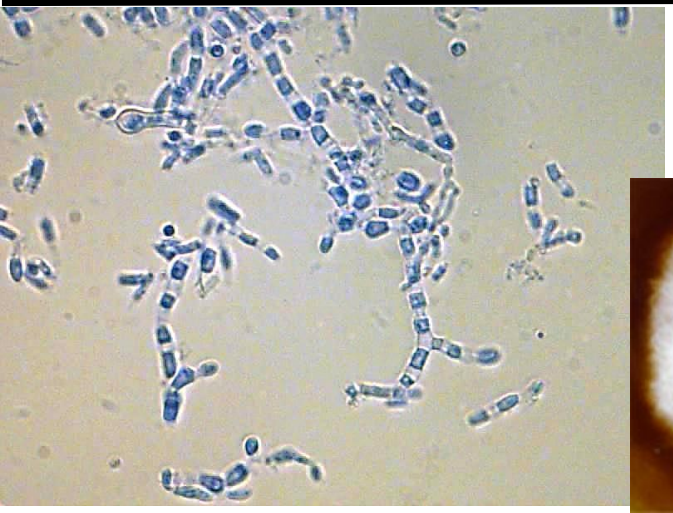
Coccidioides – fungo dimorfico

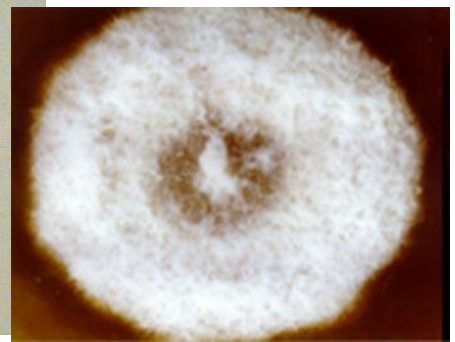
Fase saprofita **Tº : 25-28ºC**

Tº: 35-37ºC **Fase parassitaria**

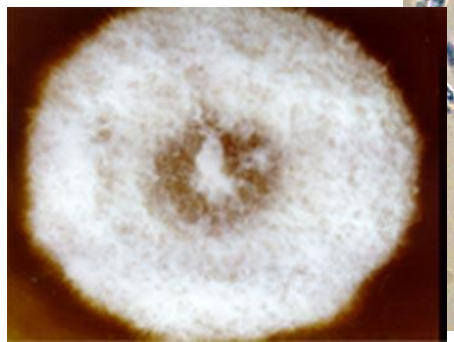
ambiente

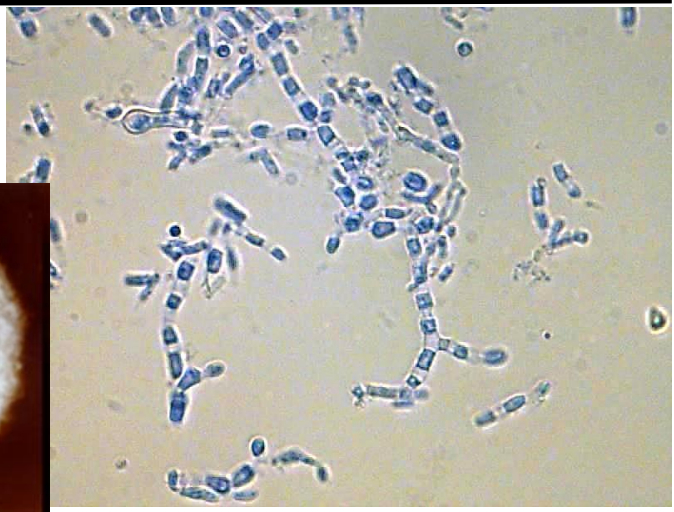
campione clinico





coltura





L'arrivo di Emergomyces

Sconosciuto fino a poco tempo fa, il genere *Emergomyces* comprende funghi dimorfici potenzialmente patogeni che sono stati precedentemente identificati come *Emmonsia*

Nel 2017, funghi simili a *Emmonsia* sono stati riclassificati nel genere di nuova formazione *Emergomyces*.

Emmonsia pasteuriana* has been reclassified as *Emergomyces pasteurianus

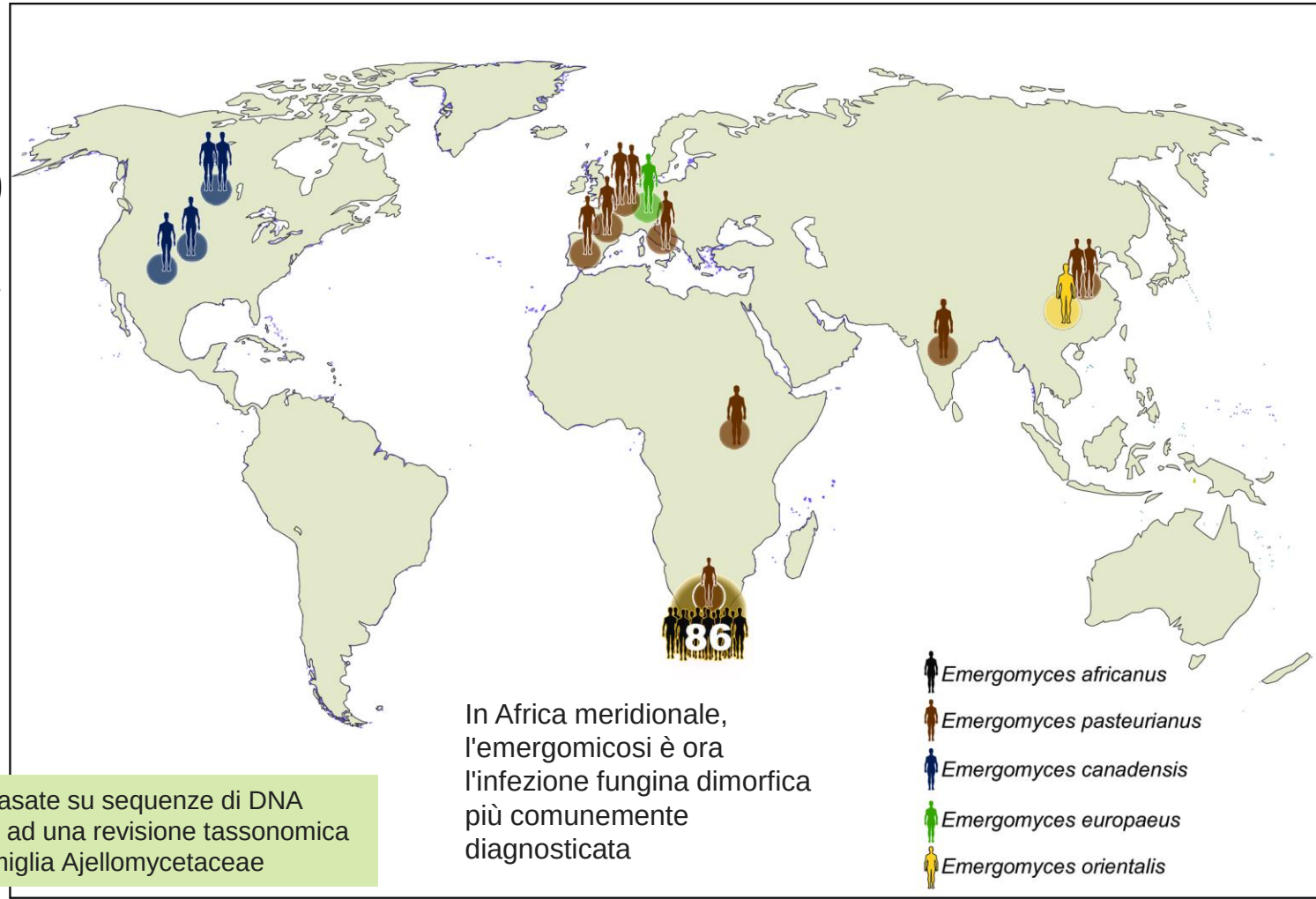
Emmonsia parva* has been reclassified as *Blastomyces parvus

1. Jiang Y, et al. Phylogeny, ecology and taxonomy of systemic pathogens and their relatives in Ajellomycetaceae (onygenales): Blastomyces, emergomyces, emmonsia, emmonsiellopsis. Fungal Divers 2018;90(1):245–91.
2. Schwartz IS, Govender NP, Sigler L, et al. Emergomyces: the global rise of new dimorphic fungal pathogens. PLoS Pathog 2019;15(9):e1007977.

Cinque specie sono ora descritte all'interno di *Emergomyces*, e casi di malattia sono stati segnalati a livello globale

- ***Es. pasteurianus*** (specie tipo)
  - Europe (Italy, Spain, France, the Netherlands)
  - Asia (China and India)
  - Africa (Uganda [ex-Rwanda] and South Africa.
- ***Es. africanus***
  - South Africa and Lesotho.
- ***Es. canadensis***
  - Canada and USA
- ***Es. orientalis***
  - China
- ***Es. europaeus***
  - Germany

PLOSPathogens | https://doi.org/10.1371/journal.ppat.1007977



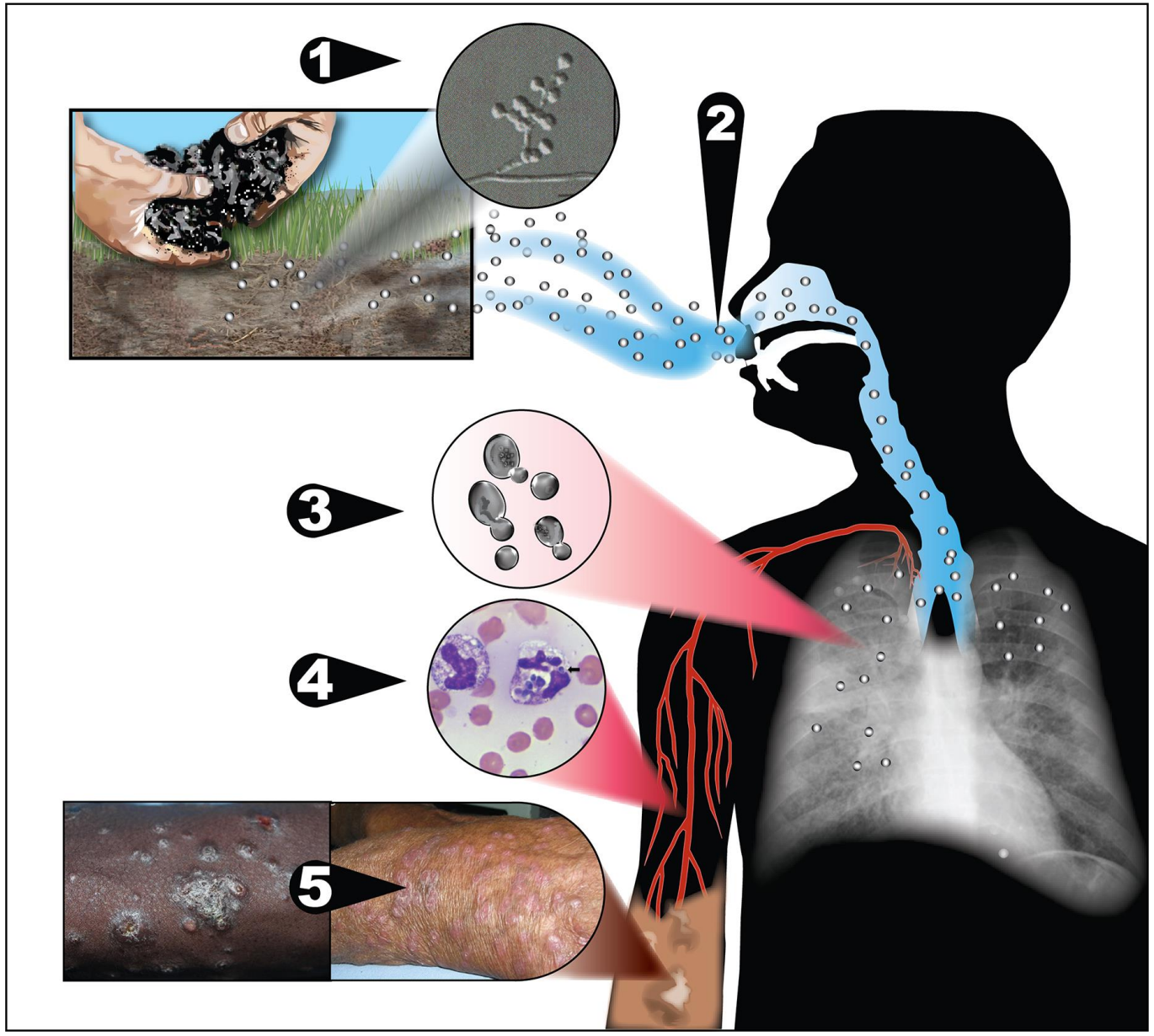
In Africa meridionale, l'emergomicosi è ora l'infezione fungina dimorfica più comunemente diagnosticata

Le analisi genetiche basate su sequenze di DNA ribosomiale hanno portato ad una revisione tassonomica all'interno della famiglia Ajellomycetaceae

Riconoscimento

campione clinico

Ecologia Fungo dimorfo termico

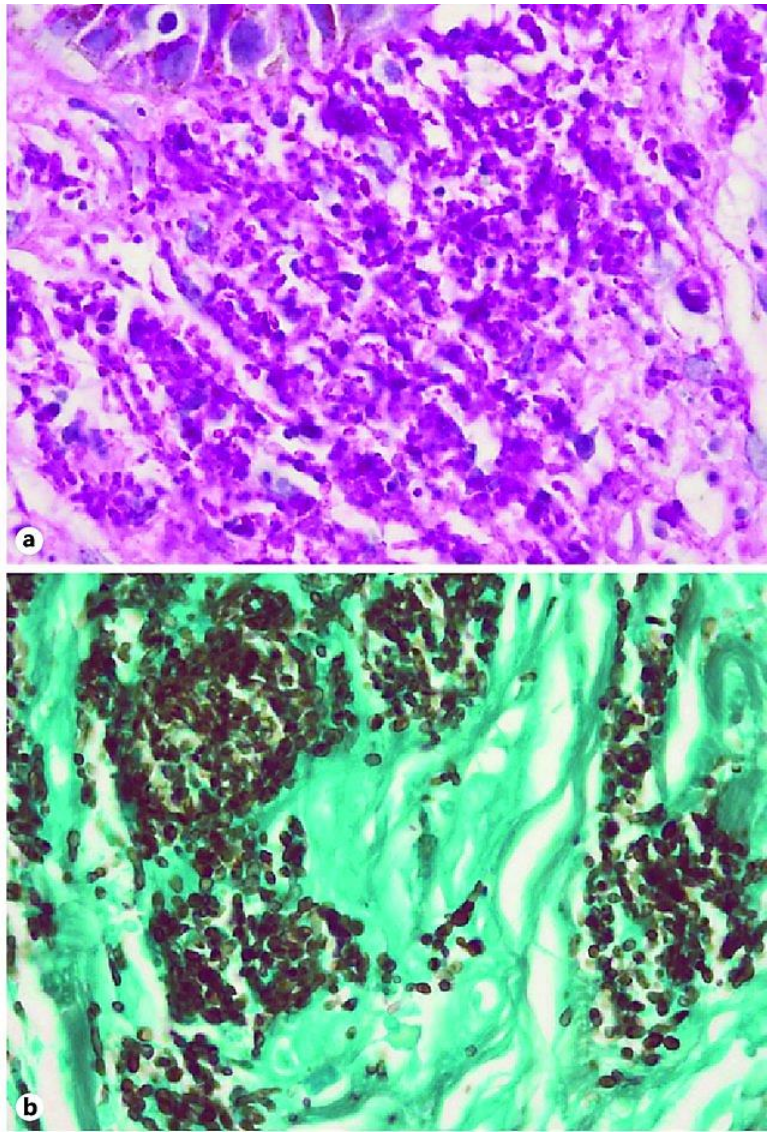


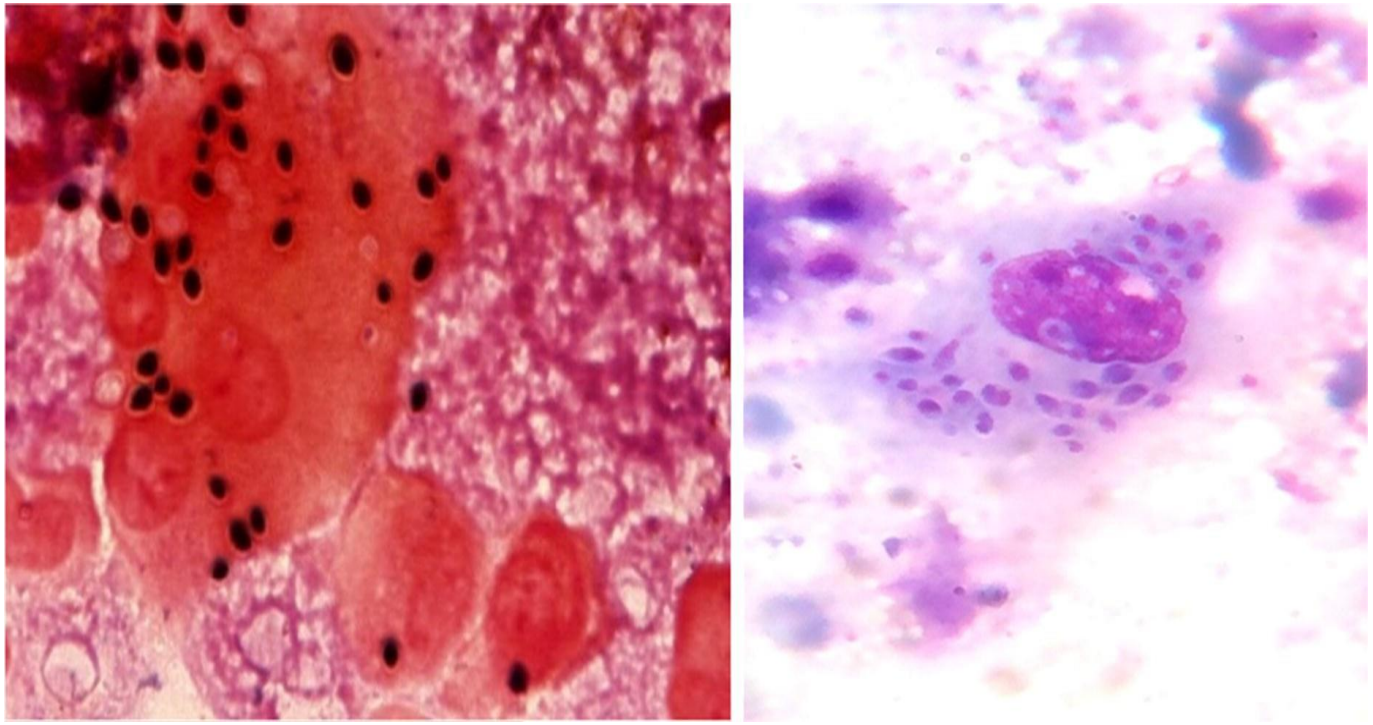
Sapronosi

1) Le specie di *Emergomyces* esistono nel suolo in fase di muffa
2) i conidi vengono rilasciati e aerosolizzati
3) Dopo l'inalazione, i conidi subiscono una trasformazione dipendente dalla temperatura nei polmoni in cellule lievitiformi e sono in grado di causare malattie polmonari in individui sensibili.
4) Lieviti si diffondono ematogenamente nei macrofagi, causando malattie extrapolmonari.
5) La malattia cutanea è più frequentemente segnalata, anche se praticamente qualsiasi sito del corpo può essere influenzato.

Emergomyces: The global rise of new dimorphic fungal pathogens

a Periodic acid-Schiff stain
b Grocott stain.

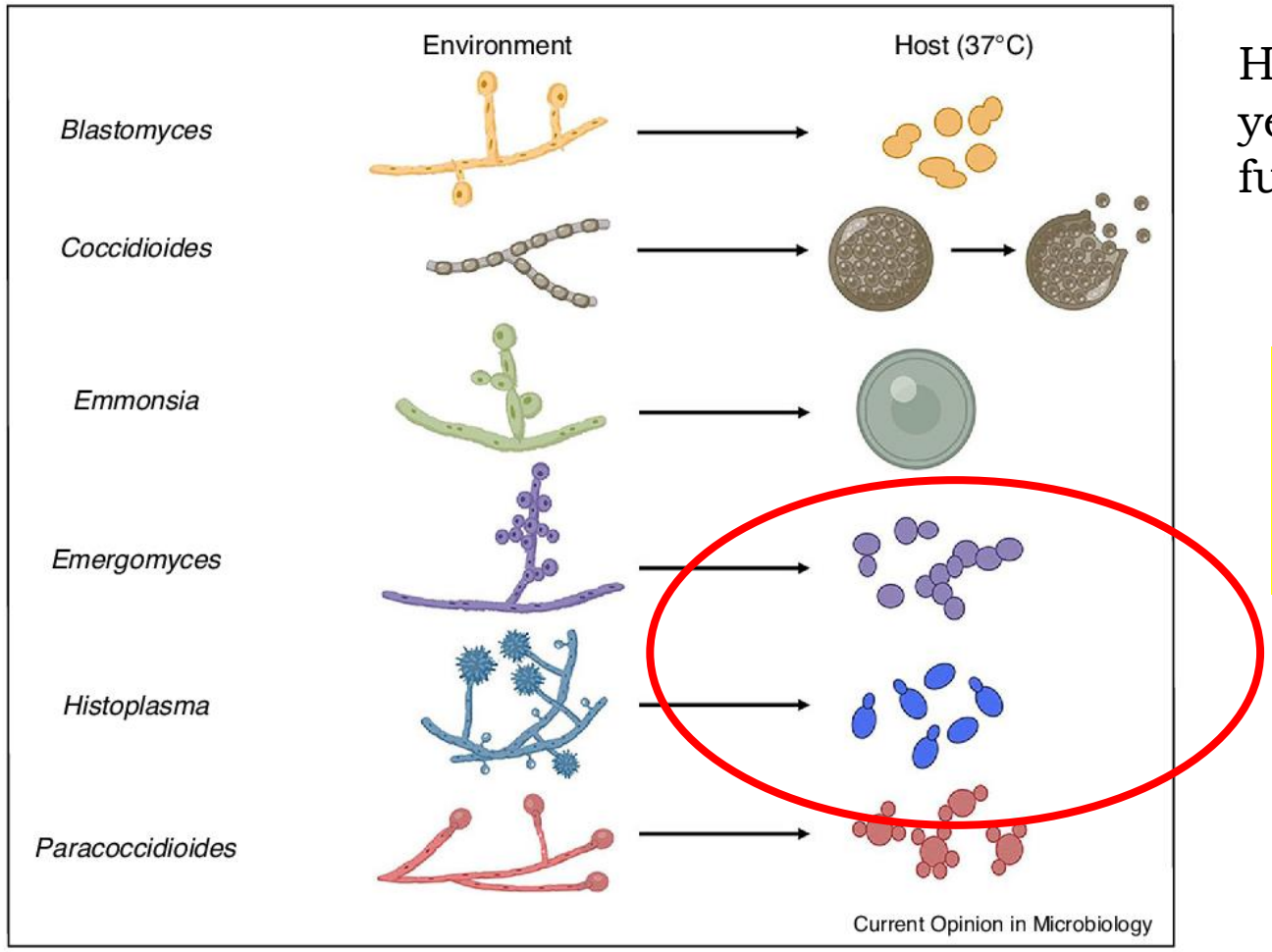




Mycopathologia, 2020, 185: 193–200

DOI: 10.1159/000495405

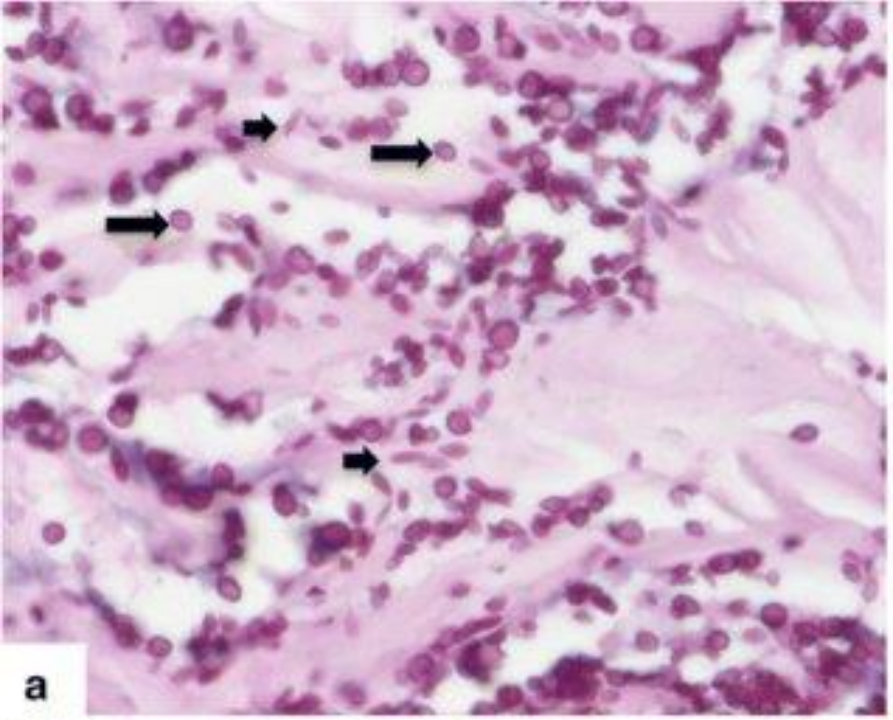
Diagnosi

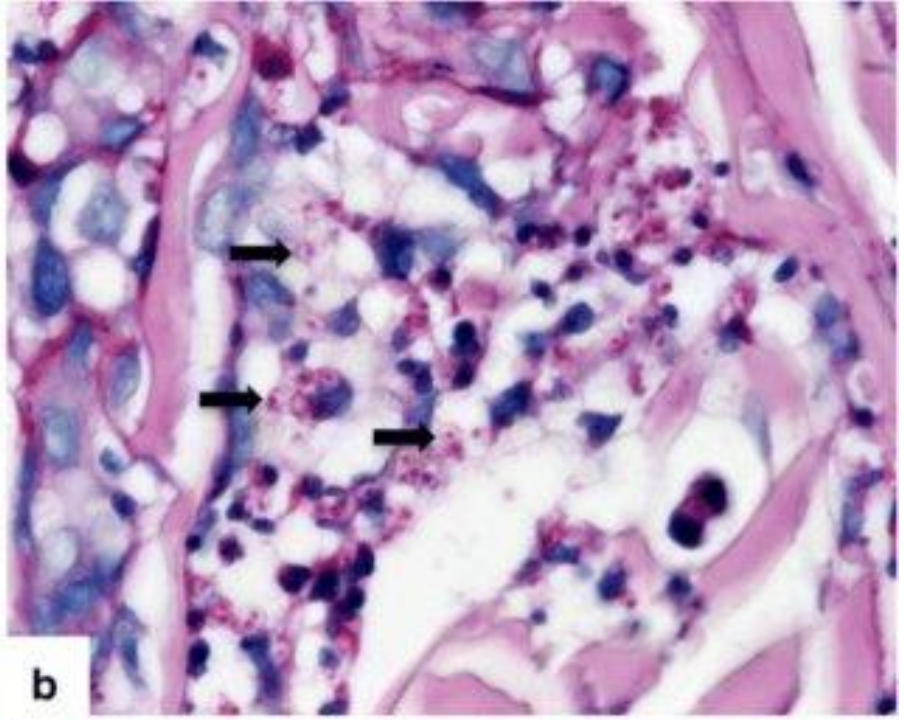


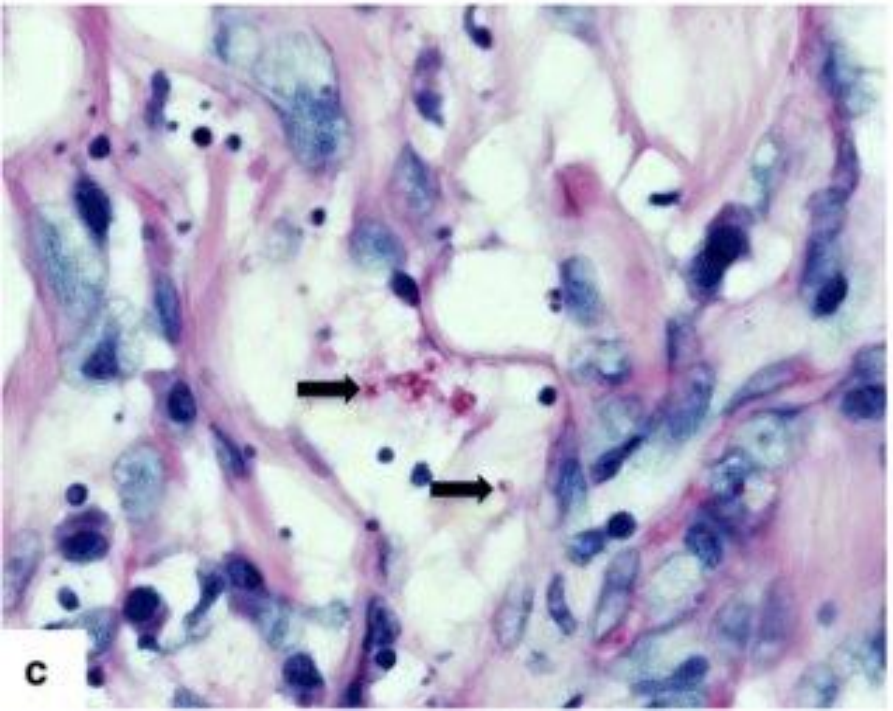
Histopathology findings include small (2–5-µm) yeasts with narrow-based budding, best seen with fungal stains

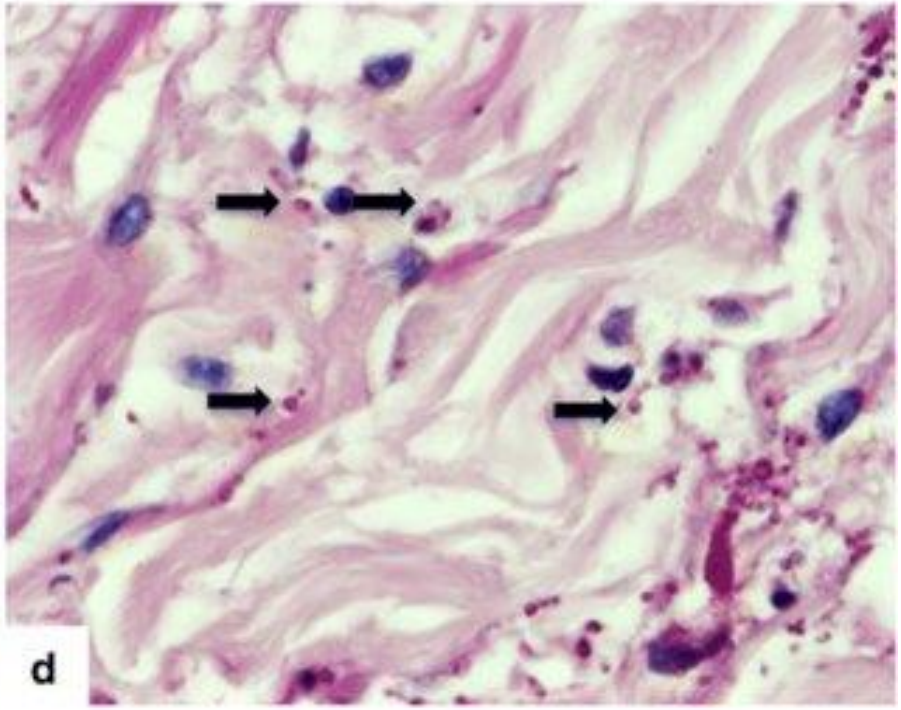
I risultati non sono sufficientemente distinti da *H. capsulatum* per consentire l'identificazione definitiva dal solo aspetto istopatologico.

PLOSPathogens | https://doi.org/10.1371/journal.ppat.1007977









Istopatologia delle biopsie cutanee di pazienti sudafricani con micosi sistemiche HIV-associate.

A) PAS. ***Sporothrix schenckii sensu stricto***. Ovoid yeast-like forms that measure 2–8 μm in size (long arrows), as well as elongated “cigar bodies” that vary in diameter from 2 to 4 and in length from 4 to 10 μm (short arrows). The larger size of the yeast-like forms and the presence of elongated forms are helpful to distinguish *S. schenkii* from *Histoplasma capsulatum* and *Emergomyces africanus*.

B) PAS. ***H. capsulatum***. Round to ovoid yeasts that vary in size from 2 to 3 to 3–5 μm with single budding nuclei and thin walls. Intra- and extracelluar organisms are present (arrows).

C,D) ***Es. a*frican*us***. Morphological features of fungal elements in tissue sections simulate the yeasts of *H. capsulatum* in particular (arrows).

Riconoscimento

dalla coltura

Diagnosi – coltura

Front Med. 2021 Apr 23;8:670731

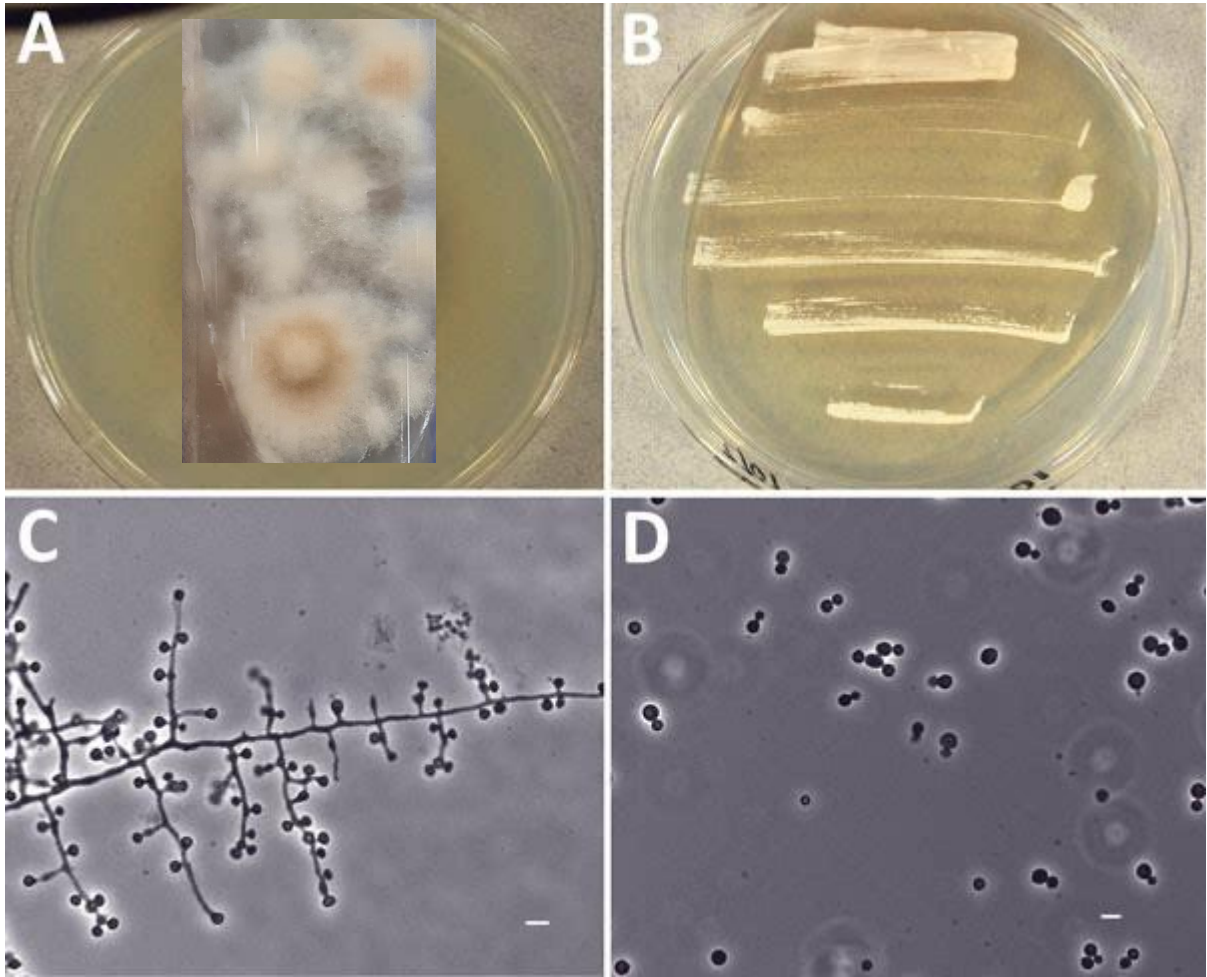
Emergomyces cresce facilmente su terreni fungini standard (es., Sabouraud agar, malt extract agar, o potato dextrose agar), Incubate a 24–30°C.

Colonies are yellowish white to tan, initially glabrous, becoming powdery, slightly raised, and furrowed, diam. 2.5-3.5 cm in 2-3 weeks.

25°C SDA medium

Yeast phase on BHI agar, showing yeast-like, pasty, cerebriform yellowish-white to tan colonies after 2–3 weeks

37°C SDA medium

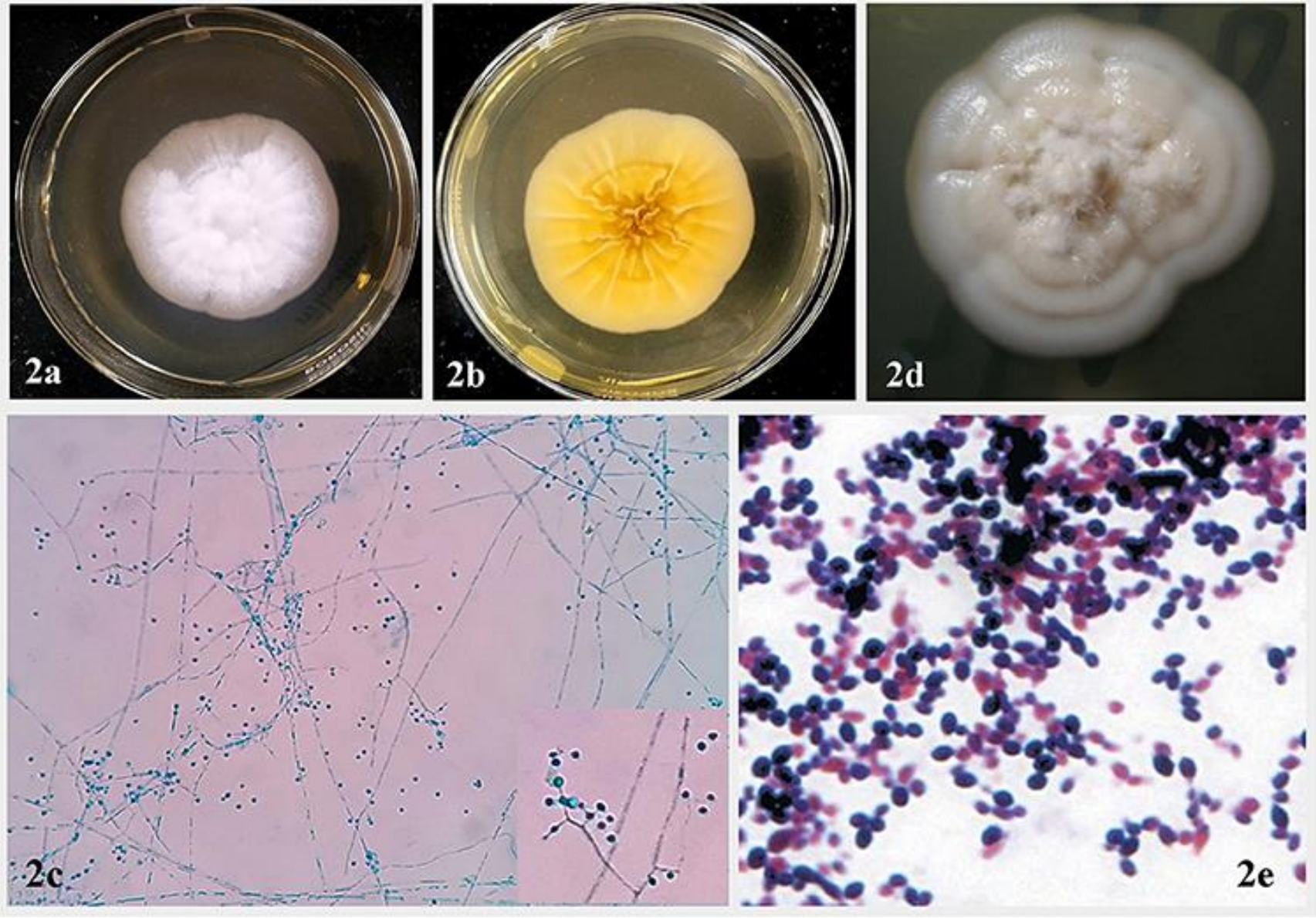


Microscopia in lattofenolo cotone blu

Mold phase: slender conidiophores that arise from hyphae at right angles and form “florets” of short secondary conidiophores bearing single small subglobose conidia.

Yeast phase: conversion from the mold to the yeast occurs readily, onto potato dextrose agar, BHI, or malt extract agar, showing yeast-like, pasty, cerebriform yellowish-white to tan colonies after 2–3 weeks of incubation.

25°C SDA medium **37°C SDA medium**



25°C SDA medium **37°C SDA medium**

Front Med. 2021 Apr 23;8:670731. doi: 10.3389/fmed.2021.670731.

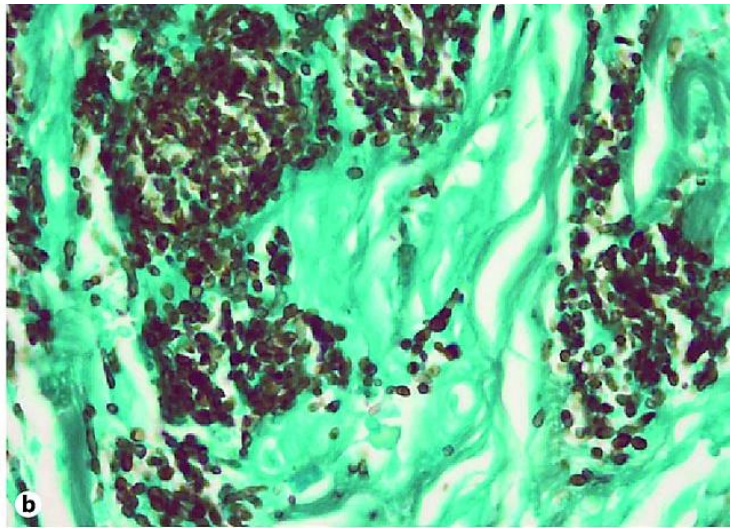
Emergomices – fungo dimorfo

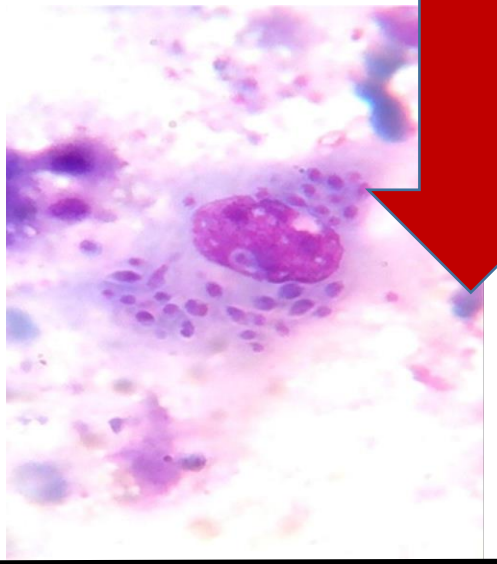
Fase saprofita **Tº : 25-28ºC**

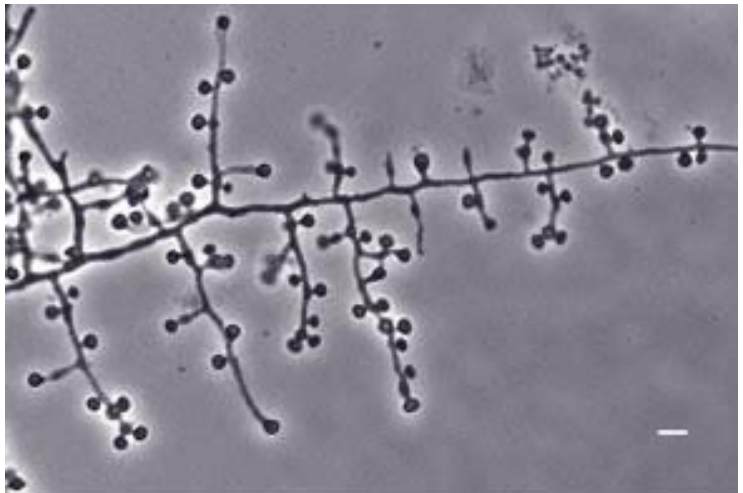
Tº: 35-37ºC **Fase parassitaria**

ambiente

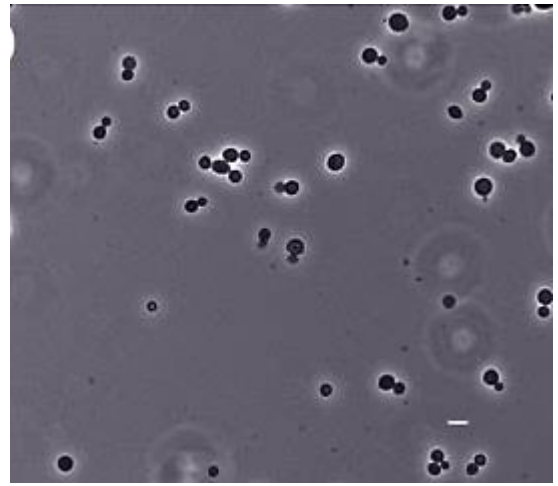
campione clinico







coltura

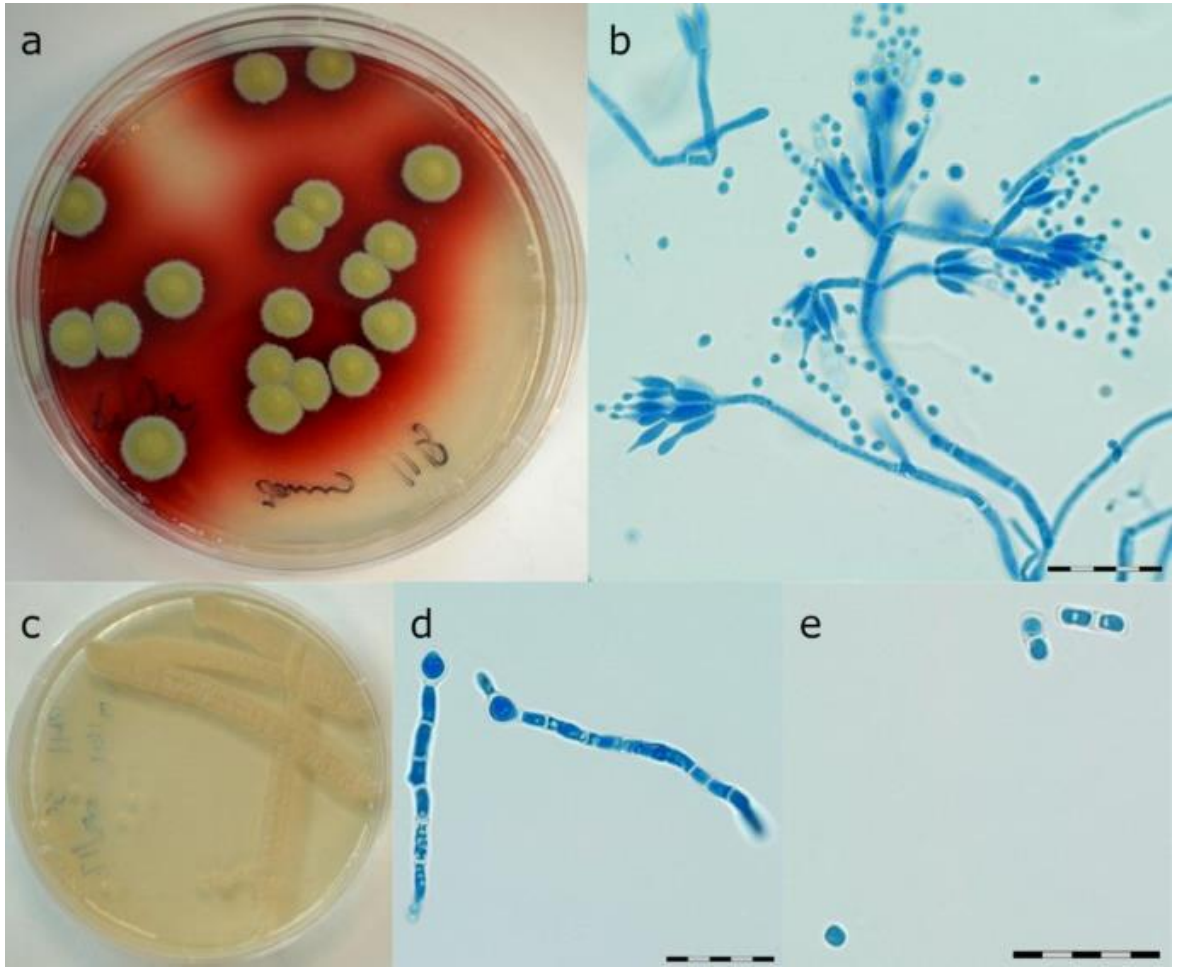


Talaromyces marneffei infection

Penicilliosi

Talaromyces marneffei

(precedentemente infezione da *Penicillium marneffei* o **peniciliosi**)



- **patogeno oportunistico emergente**

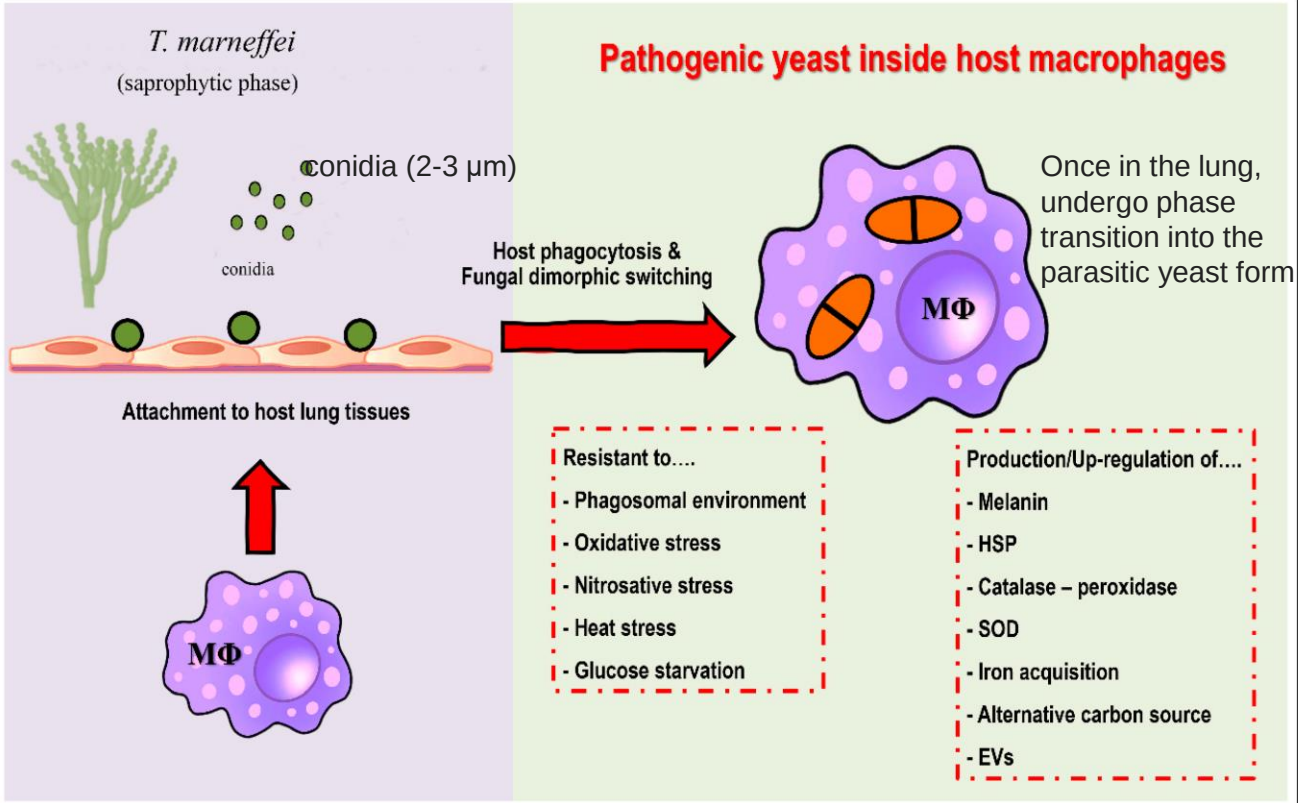
Per preservare la continuità nella letteratura, il nome della malattia rimane **peniciliosi**

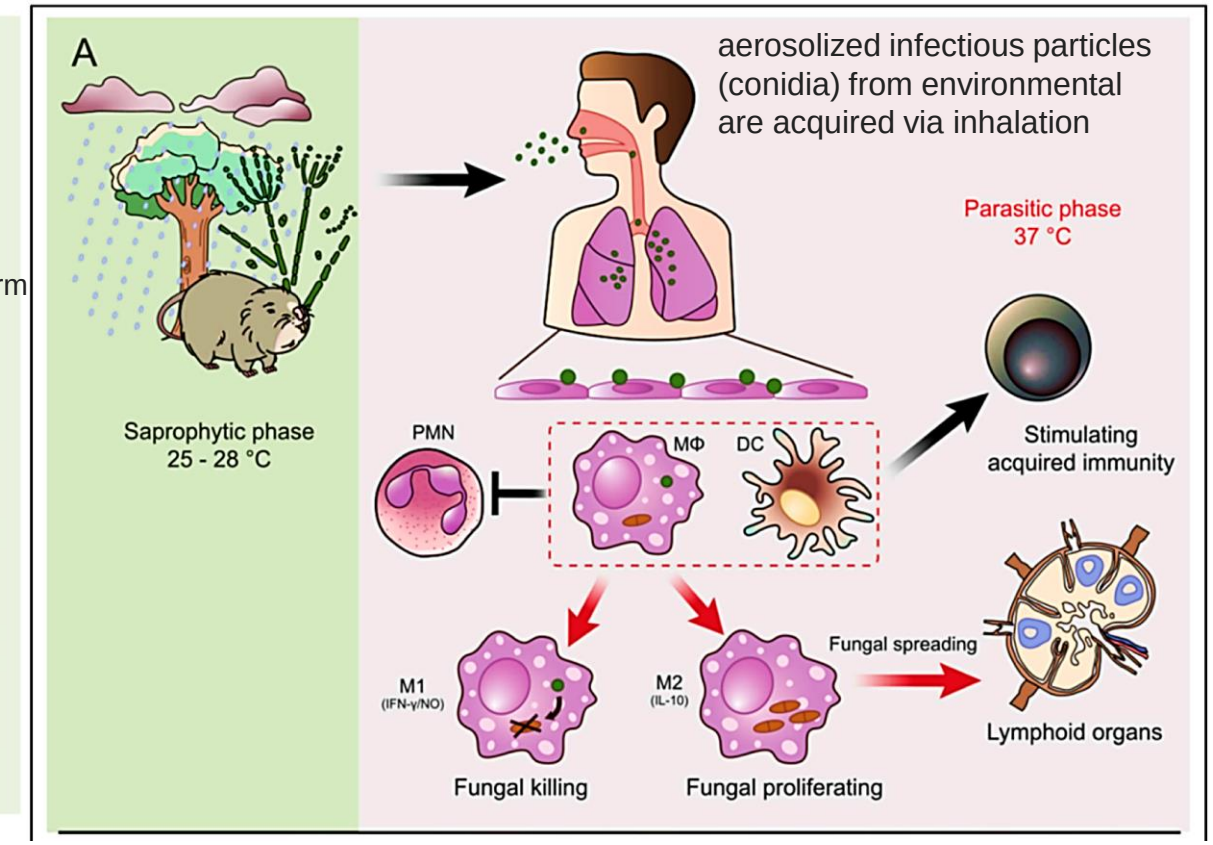
1° caso riportato nel 1984, che descrive cinque pazienti nel nord della Thailandia

Riconoscimento

campione clinico

Tra le centinaia di specie di *Talaromyces*, (*Penicillium*) **l'unica specie termo-dimorfa** noto per essere patogeno per i mammiferi, compresi gli esseri umani.





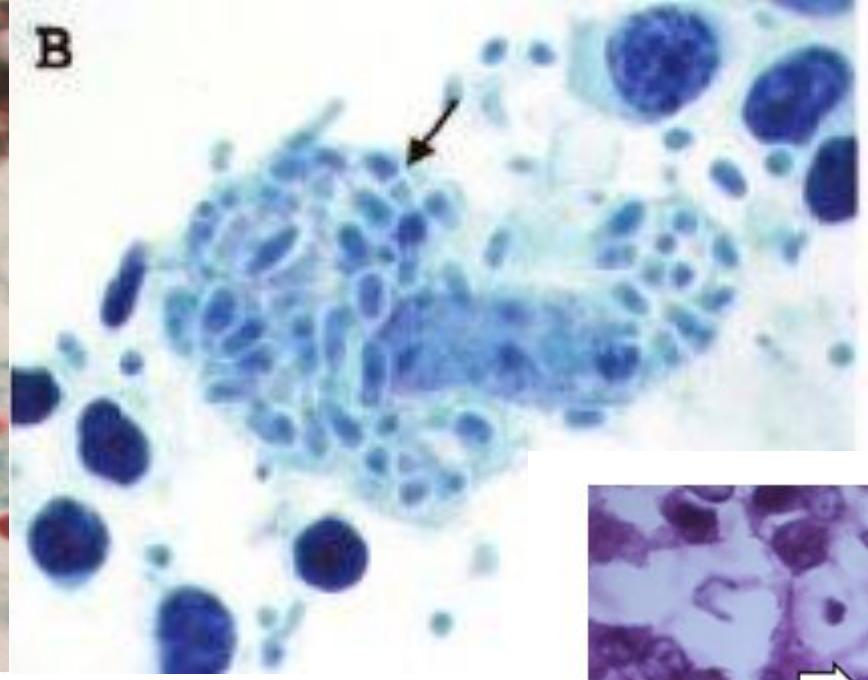
Fungo termodimorfo

T. marneffei cresce come muffa saprofita nell'ambiente, ma subisce una transizione di fase verso una cellula patogena simile al lievito a temperature fisiologiche dei mammiferi.

J. Fungi 2022, 8(2), 200; https://doi.org/10.3390/jof8020200

Diagnosi

Forma parassitaria
Fase di lievito



DOI: 10.1128/CMR.19.1.95-110.2006

Cellule yeast-like, ovali o tondeggianti, con una fissione interna, rappresentano la forma parassitaria.

Questa forma è vista nell'infezione **intracellulare** dei macrofagi e istiociti, così come **extracellulare**.

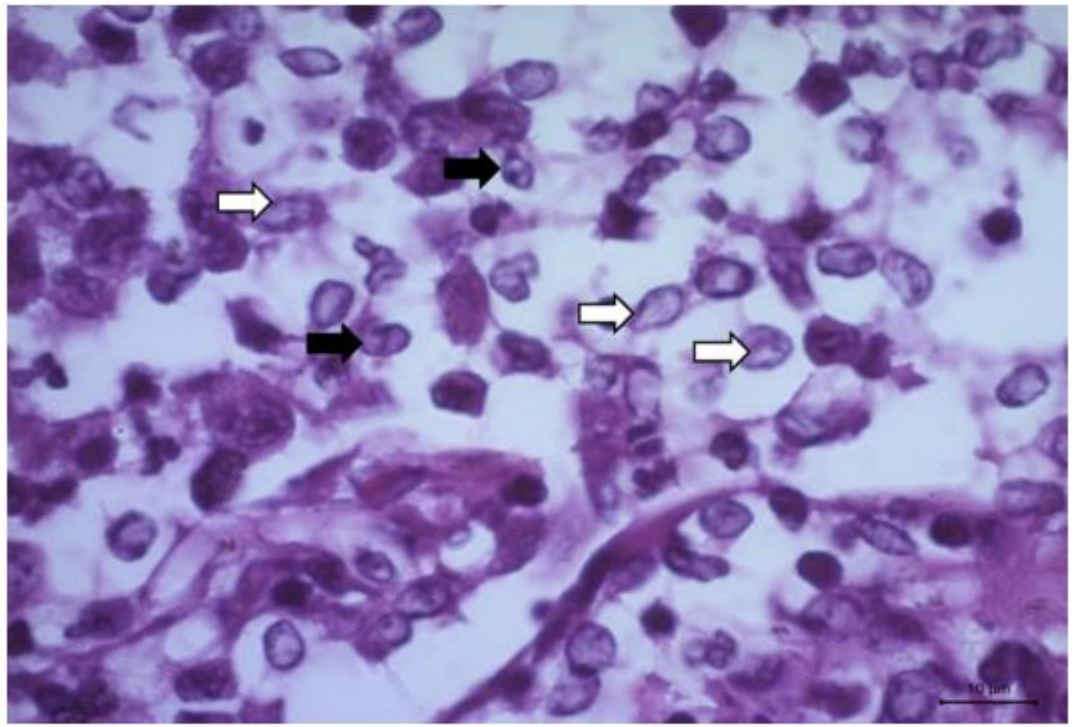
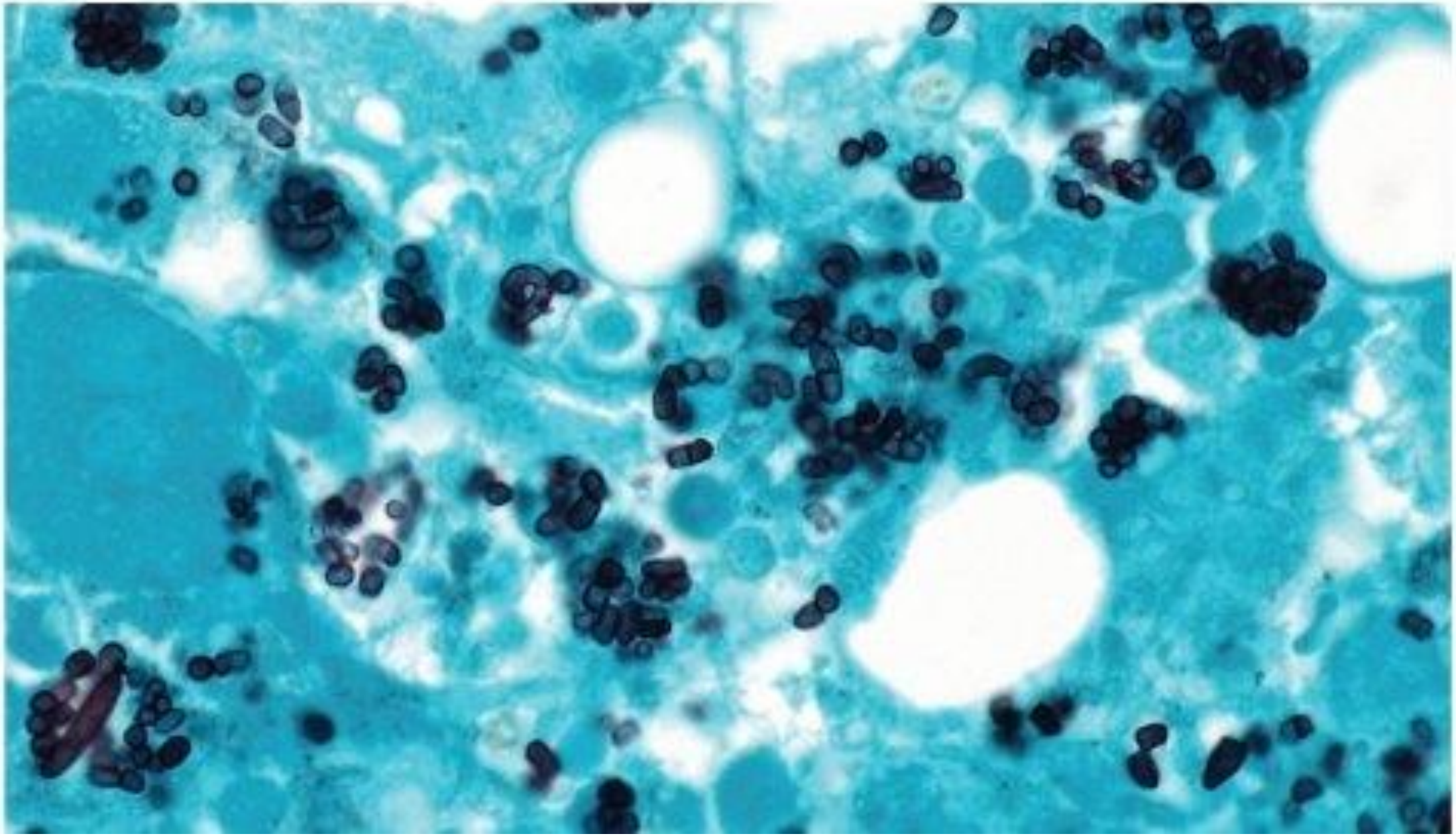


Fig. 1. Lung; several intralesional (1.5–2.5 × 4–6 μm), round to oval, fungal cells (white arrows) in the alveoli with the characteristic transverse septum of fission yeast division (black arrows) of *T. marneffei*. HE.

Diagnosis

Fase parassitaria
Forma di “lievito-like”



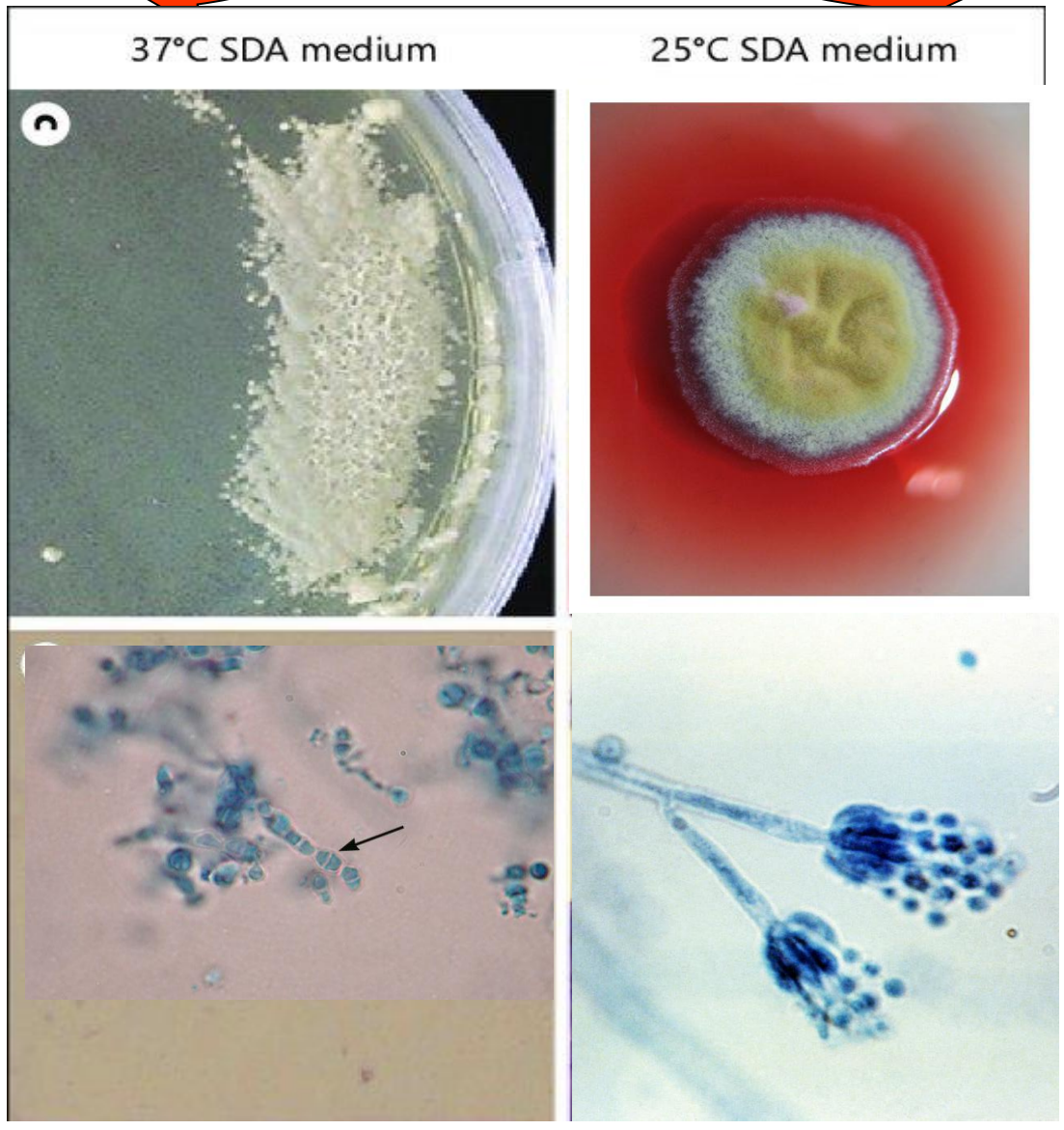
Riconoscimento

dalla coltura

TERMODIMORFICO

Fase parassitaria

unicellulare



Fase filamentosa (saprofita)

pluricellulare

Colonia verde chiaro con pigmento rosso diffuso

A temperature **inferiori a 37°C**, Il fungo cresce come un filamentoso, mostrando conidiofori e conidi tipici del genere *Penicillium*

DOI: 10.1159/000489501

https://alchetron.com/Penicillium-marneffei

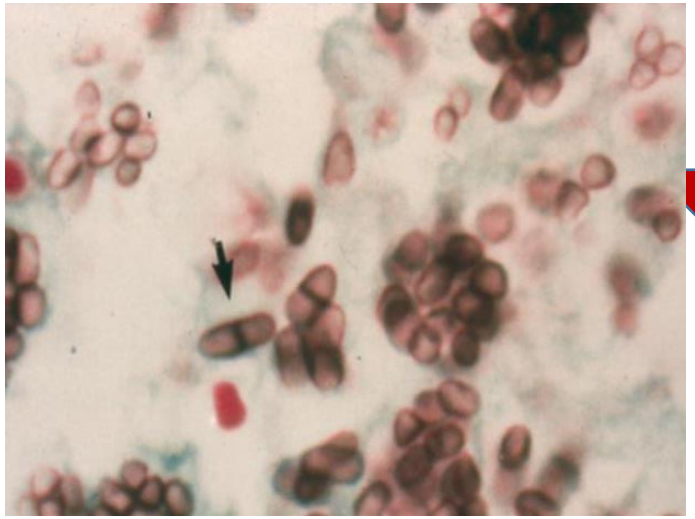
Talaromyces marneffei – fungo dimorfo

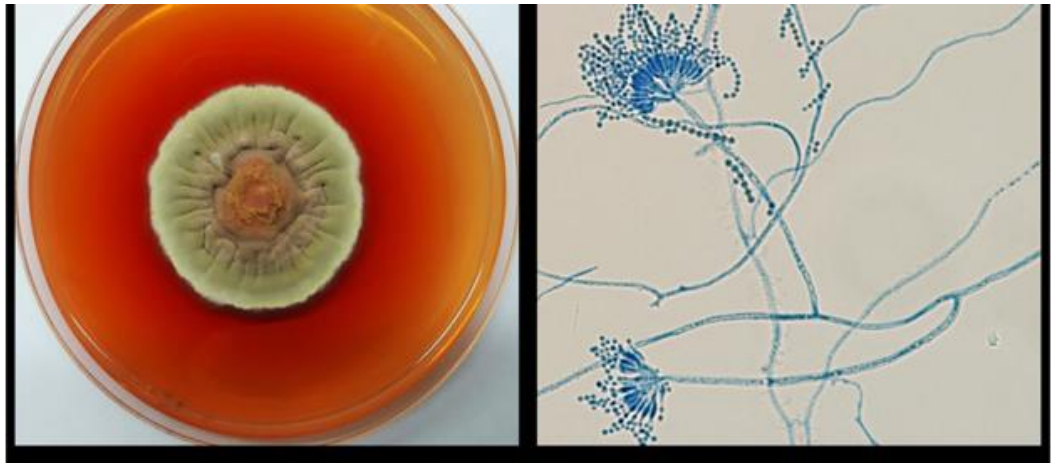
Fase saprofita **Tº : 25-28ºC**

Tº: 35-37ºC **Fase parassitaria**

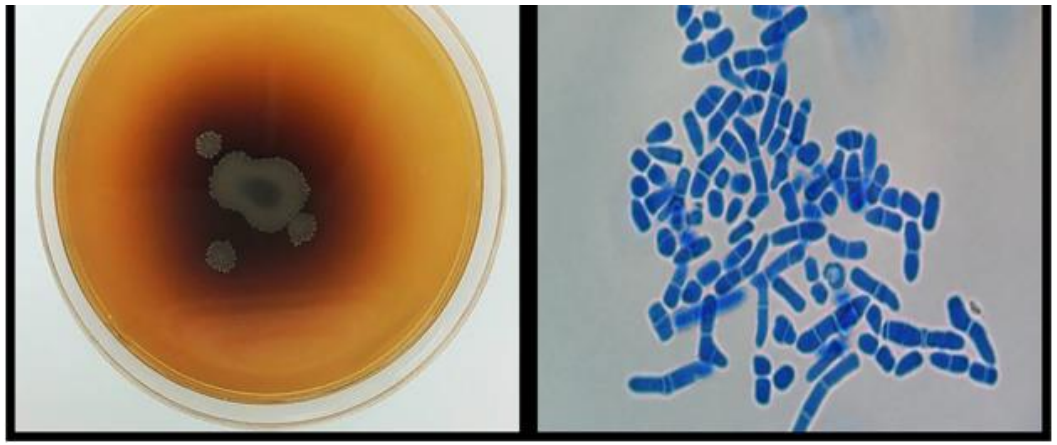
ambiente

campione clinico





coltura



Sporotricosi

malattia zoonotica emergente

Sporothrix
(ordine Ophiostomatales)

Funghi termodimorfici

fungo saprofita
Suolo, piante (piante spinose),
materia decadente, ecc.

S. schenckii sensu stricto
S. brasiliensis
S. globosa
S. mexicana
S. albicans

Cosmopolita - Predominante nelle aree tropicali e subtropicali

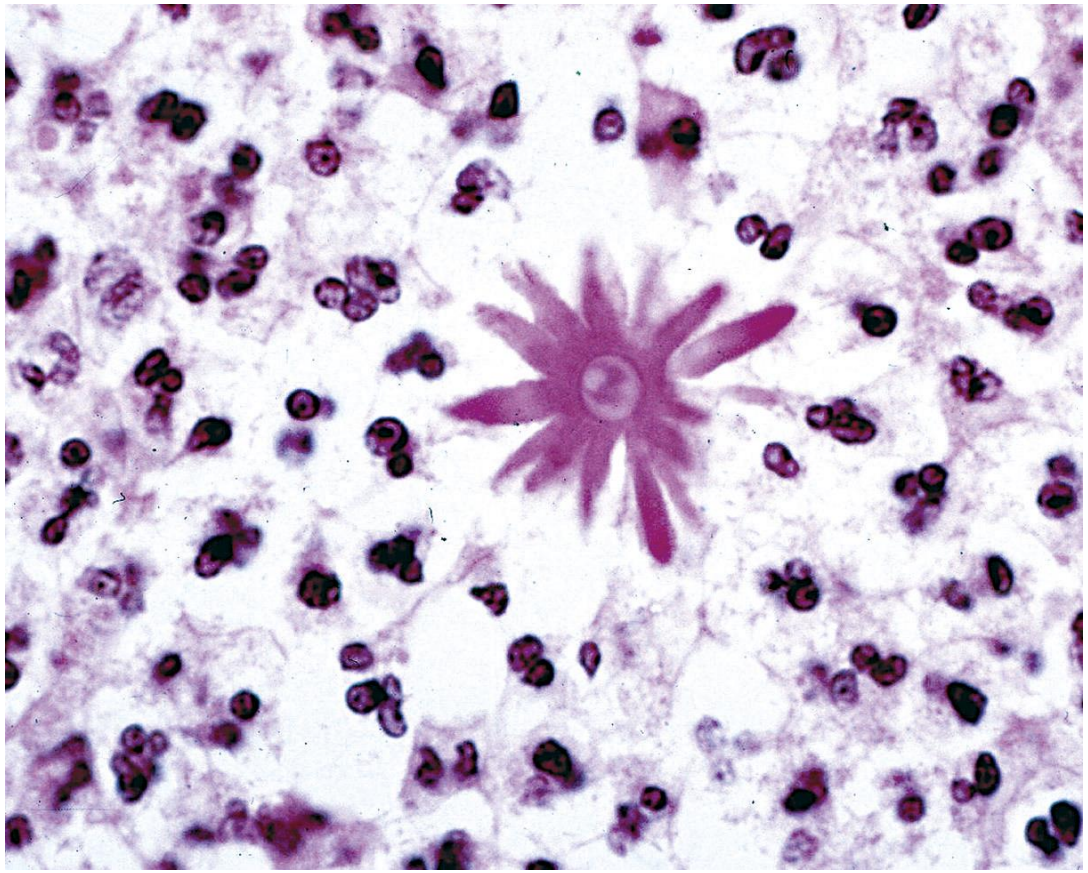
Riconoscimento

campione clinico

Esame diretto

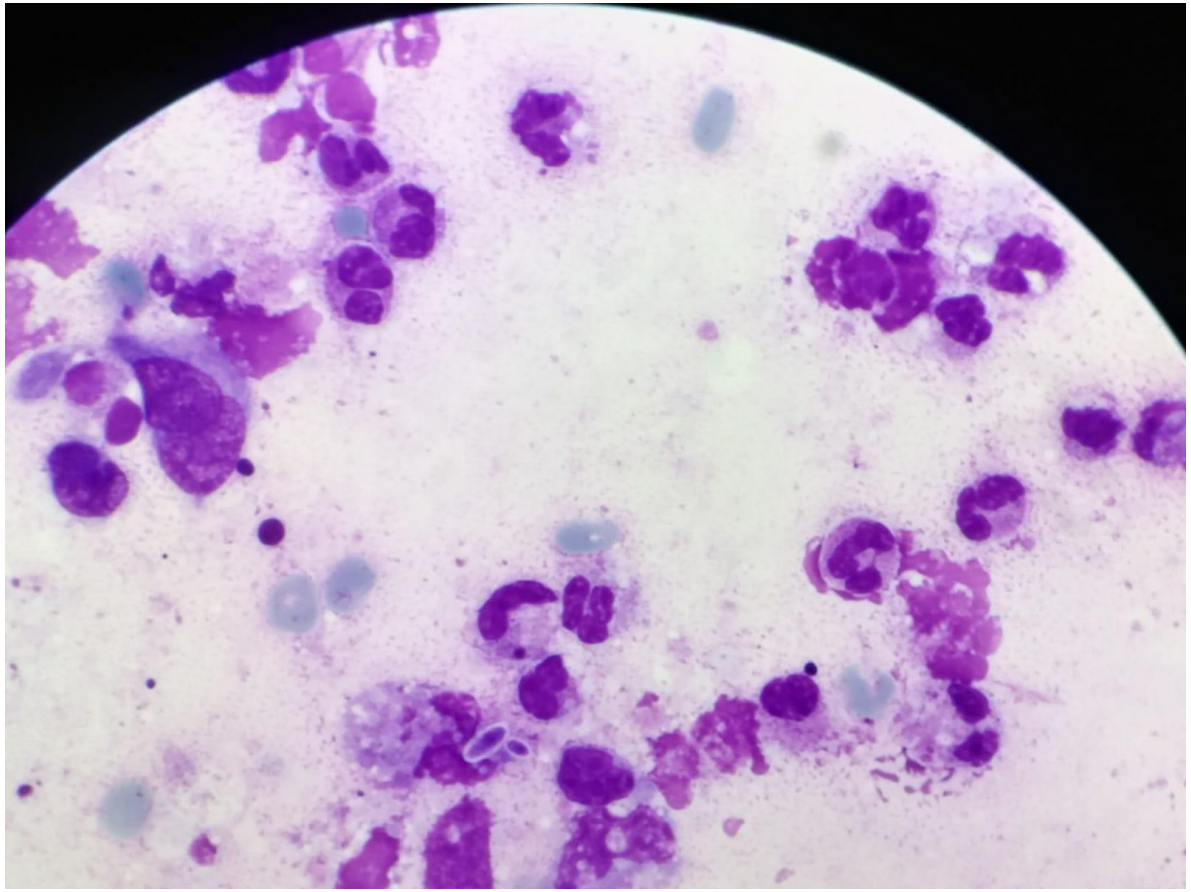
L'agente è raramente osservato all'esame microscopico diretto

Splendore-Hoeppli fenomeno (Corpuscoli asteroidi)



Splendore-Hoeppli fenomeno è la formazione in vivo di materiale intensamente eosinofilo (Configurazioni radiate, a forma di stella, asteroidi o clave) intorno al microrganismo

Il materiale di reazione Splendore-Hoeppli comprende il complesso antigene-anticorpo, i detriti tissutali e la fibrina



In generale è negativo.

Si possono osservare lieviti in gemmazione, globulari o fusiformi – a "forma di sigaro« (1 – 3 microns)

Riconoscimento

dalla coltura

TERMODIMORFICO

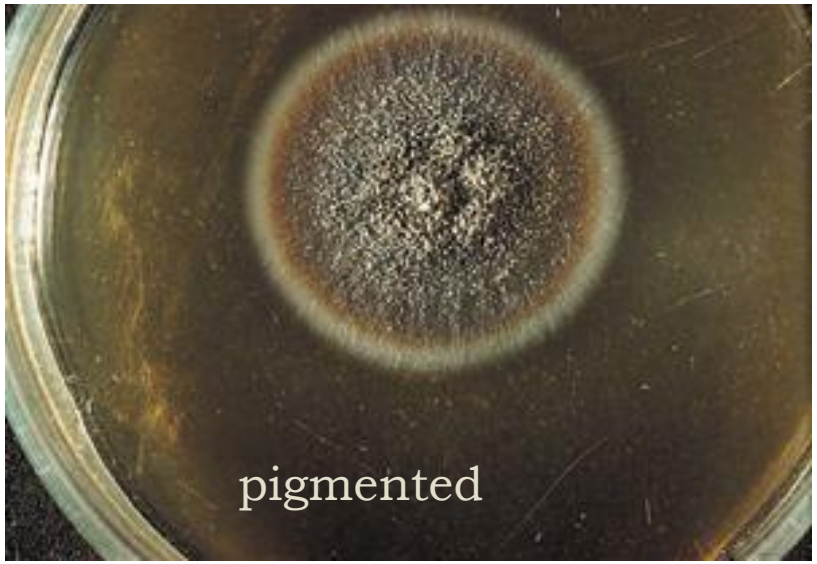
Tº : 25-28ºC

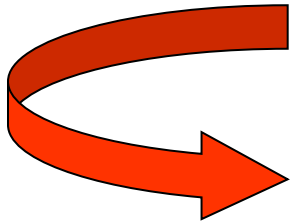
Fase parassitaria

unicellulare

- 37ºC: Colonia dall'aspetto umido, ruvida, membranosa, senza micelio aereo. Dal bianco al nero.

TERMODIMORFICO

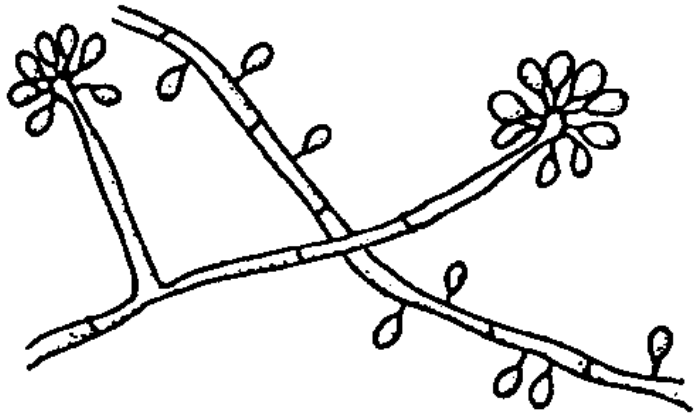




Tº: 35-37ºC

Fase filamentosa (saprofita) pluricellulare

Ife settate fini, con conidi piriformi o ovoidi (2-6 Micron) disposti a "forma di bouquet"

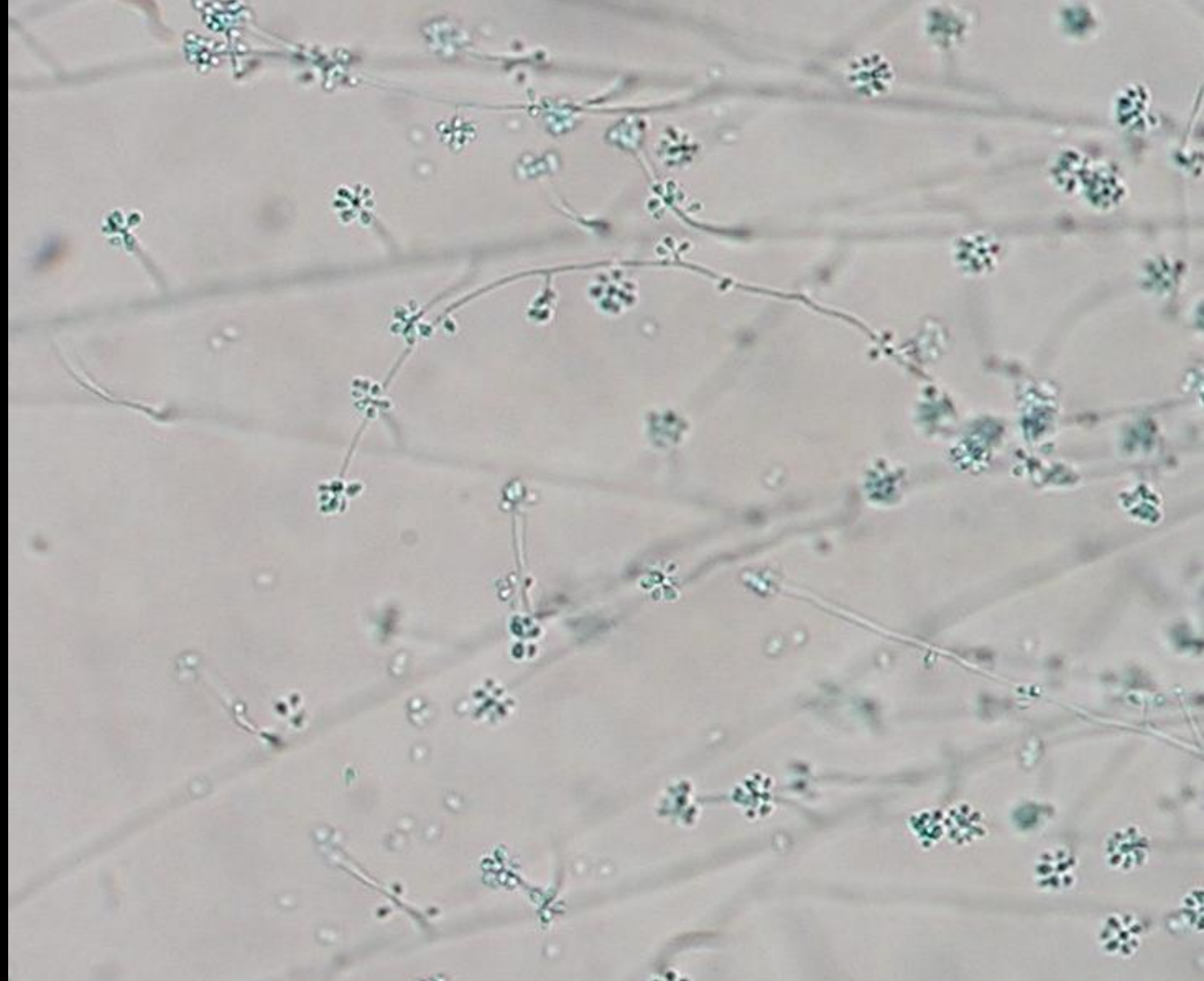


- 37ºC: Colonia dall'aspetto umido, ruvida, membranosa, senza micelio aereo. Dal bianco al nero.

❖CULTURE

25 - 28ºC

Ife settate fini, con conidi piriformi o ovoidi (2-6 Micron) disposti a "forma di bouquet"



conidi piriformi o ovoidi (2-6 Micron) disposti a "forma di bouquet"

Sporothrix – fungo dimorfo

Fase saprofita **Tº : 25-28ºC**

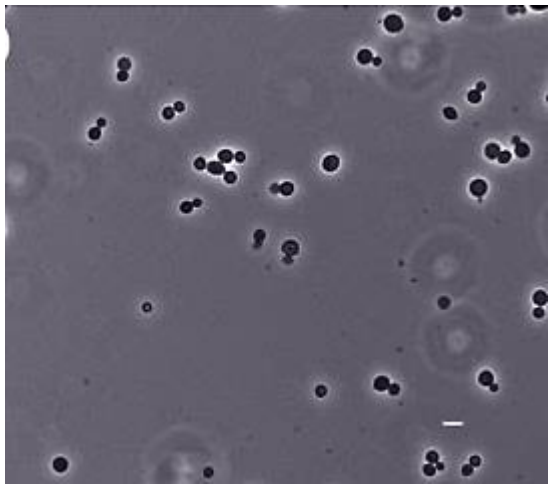
Tº: 35-37ºC **Fase parassitaria**

ambiente

campione clinico

In generale - negativo

coltura



Venerdi 05 Maggio 2023

I funghi dematiacei in laboratorio:

riconoscimento a livello di genere

Funghi multinucleati (filamentoso – muffa)

Ife

settata

2-5 micron

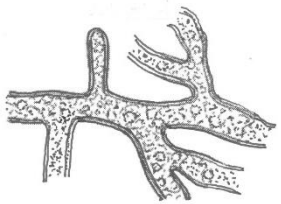
non settata

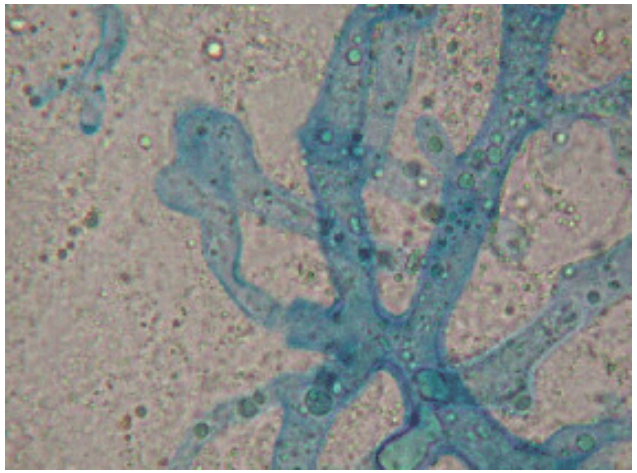
10-15 micron

Micelio continuo

o

cenocitico





Cos'è un fungo dematiaceo?

Cause comuni di feoifomicosi, micetoma e cromoblastomicosi

Riconoscimento

campione clinico

Esame diretto
Fresco e colorazioni

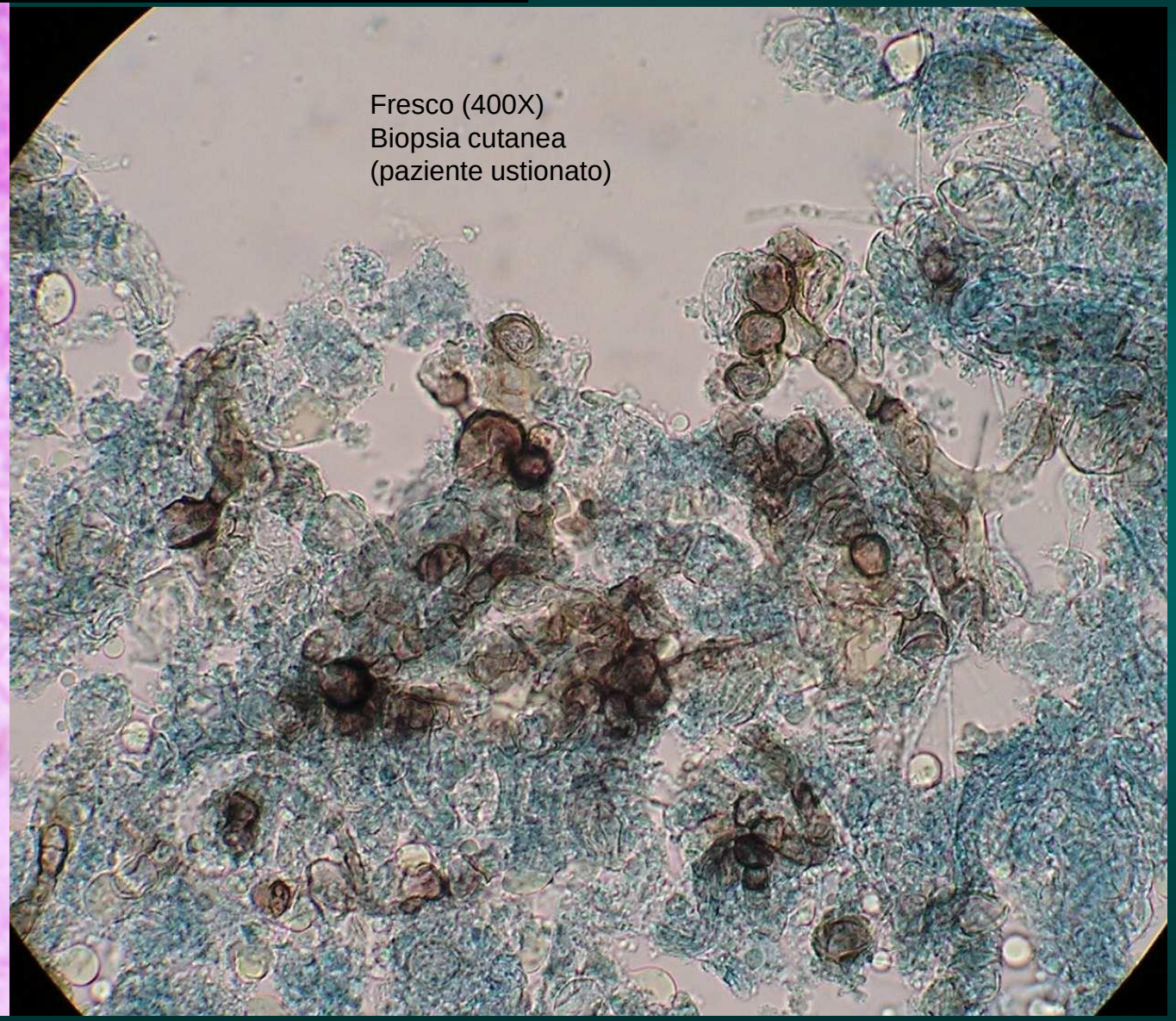
Esame diretto
unghia del piede

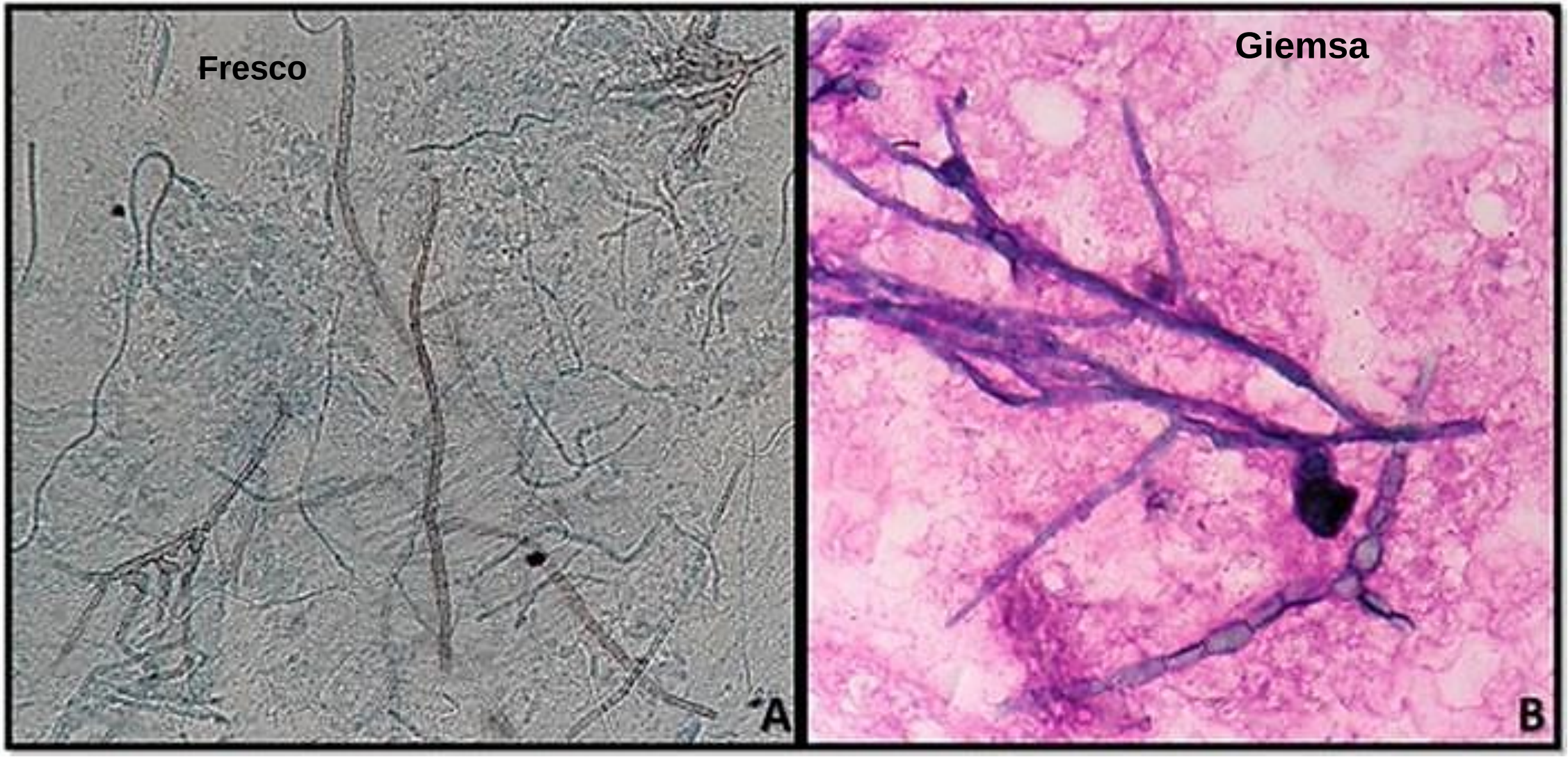
- **ife scure, settate regolari, ramificate**
- **A volte… simile a pseudoife**

Esame diretto di
materiale corneale

- **A volte… ife vescicolose, moniliformi, deformate o gonfie**
- **come elementi lievitifomi**

Biopsia del pericardio (1000X)

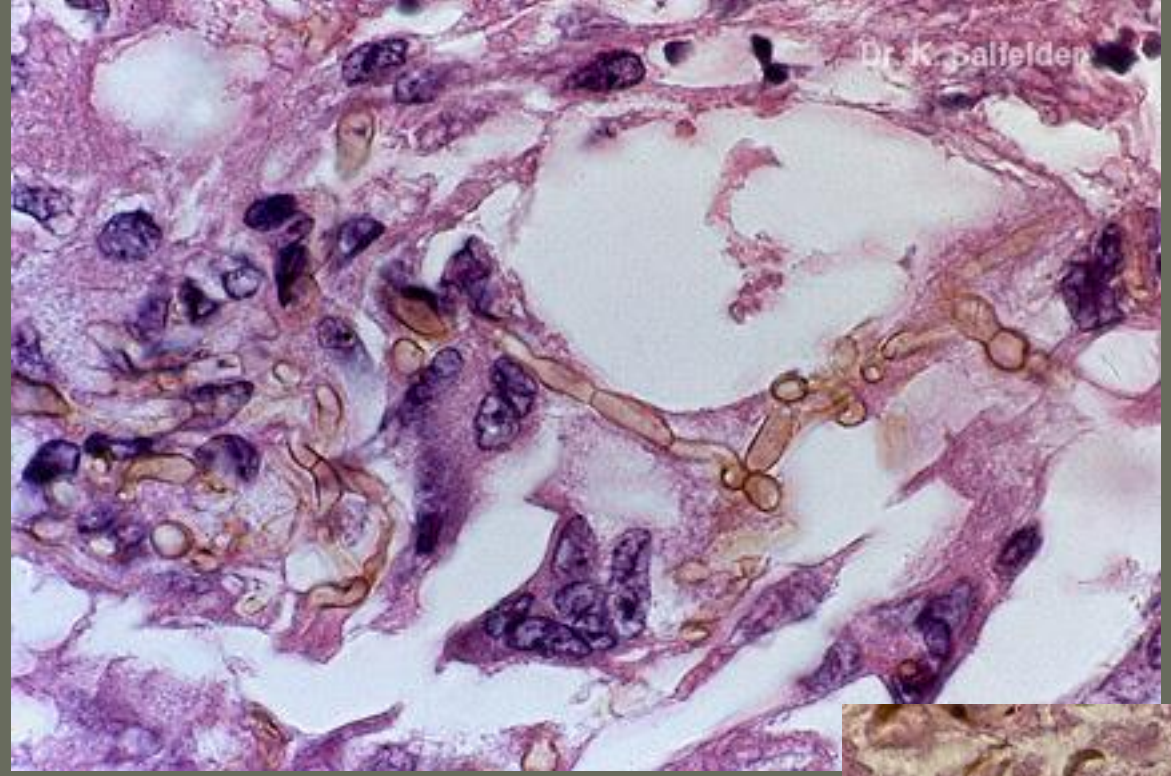


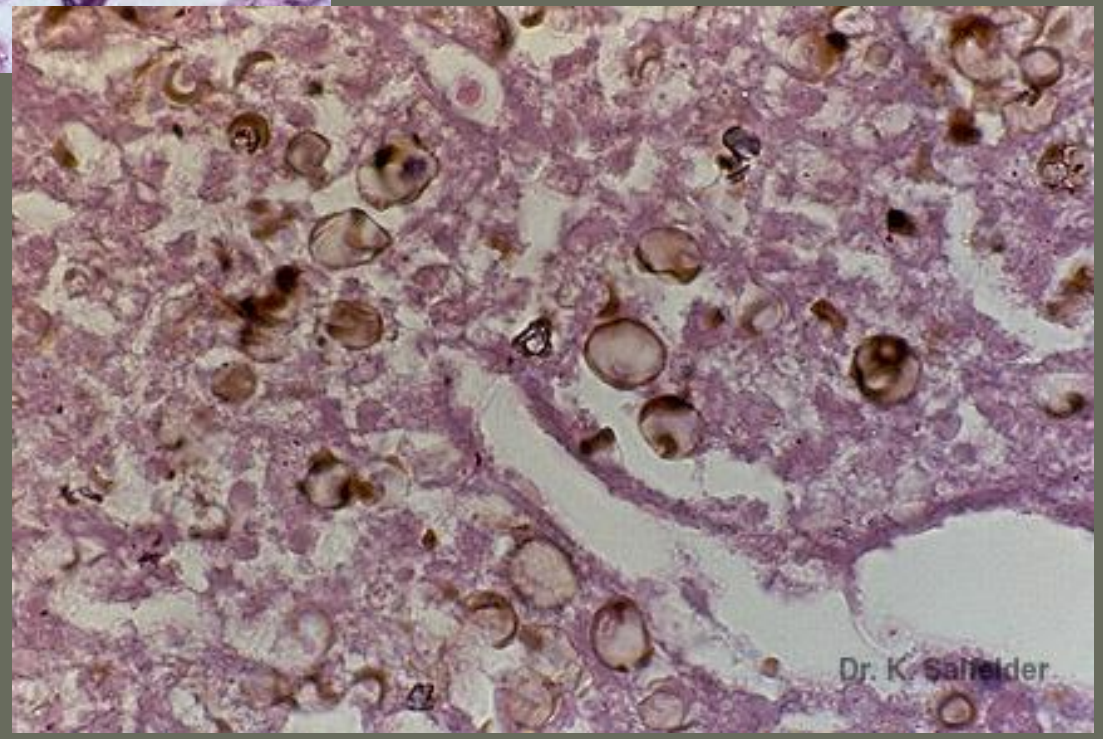


In alcuni casi la natura dematiacea degli elementi fungini NON è evidente

Il carattere dematiaceo si osserva nella coltura

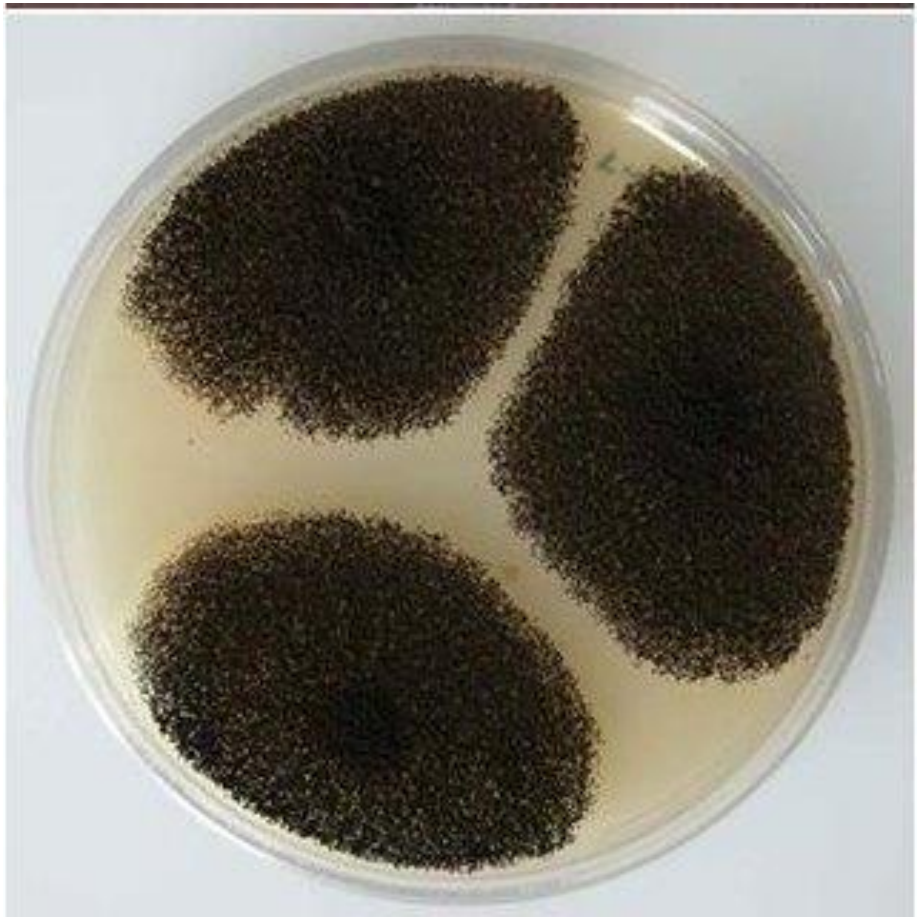
l'impregnazione della melanina nella parete cellulare può essere ritardata



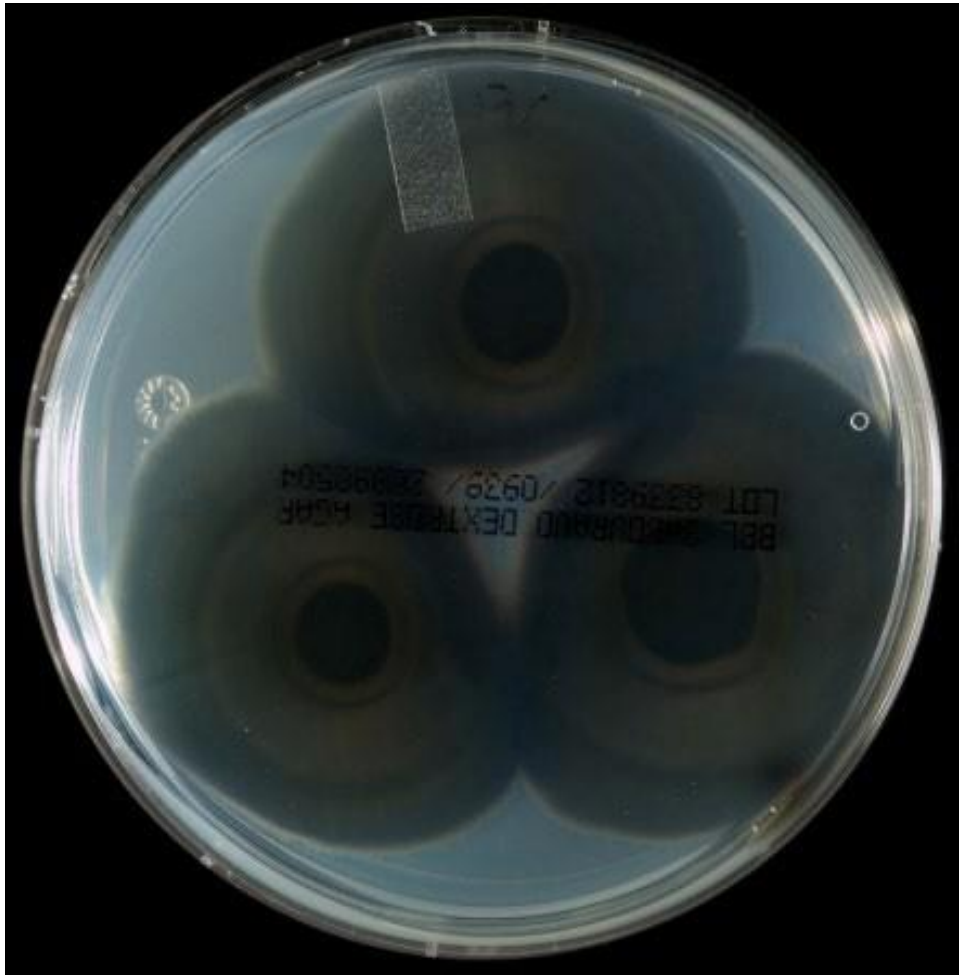


H-E

Quale dei due è un fungo dematiaceo?



Phialophora verrucosa



dematiaceo

Aspergillus niger

ialino

Il rovescio va dall’oliva al nero

Il laboratorio in Micología Médica

montaggio con lattofenolo – cotton blue

Prof. Gustavo Giusiano
Dipartimento Micología -Instituto de Medicina Regional
Facultad de Medicina
Universidad Nacional del Nordeste- Argentina
giusianogustavo@gmail.com

Bipolaris sp.

Curvularia lunata

setti trasversali e longitudinali

Alternaria alternata grupo
(Dictioconidio)

Exophiala dermatitidis

La forma del lievito è comune, alcuni isolati rimangono come lieviti e non si convertono

Colonie inizialmente cerose(lievitiforme, diventando vellutate con l'età; marrone oliva con rovescio da marrone a nero

Exophiala species

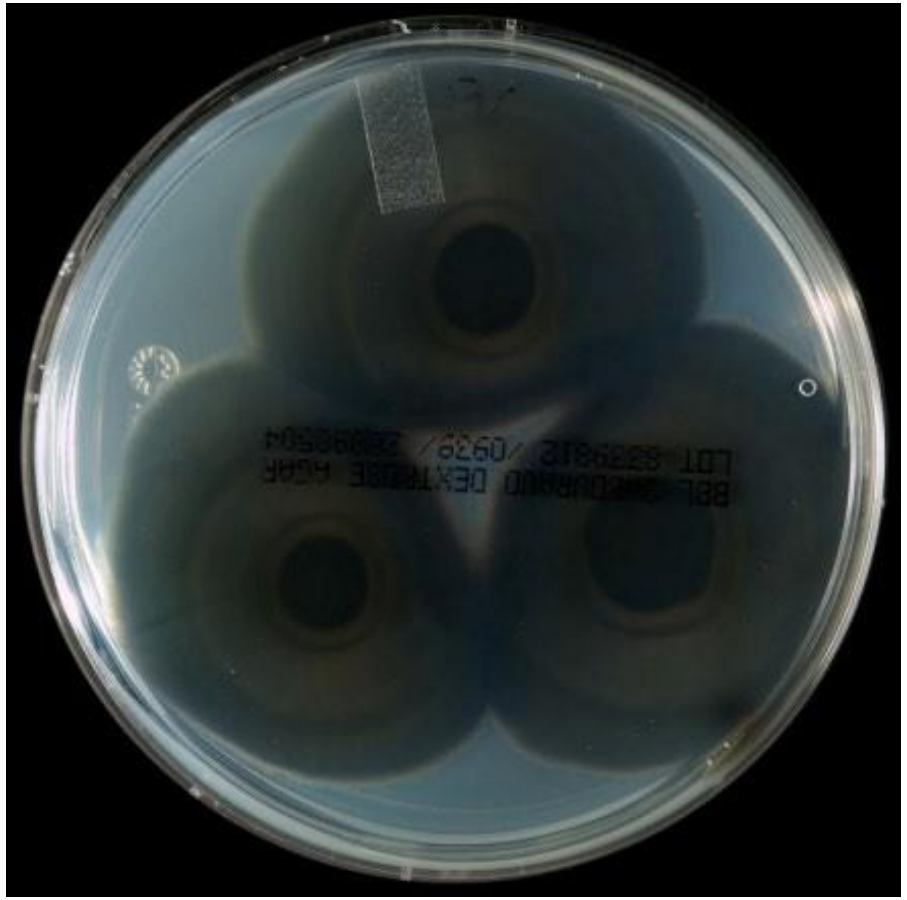
annellidic conidiogenous cell

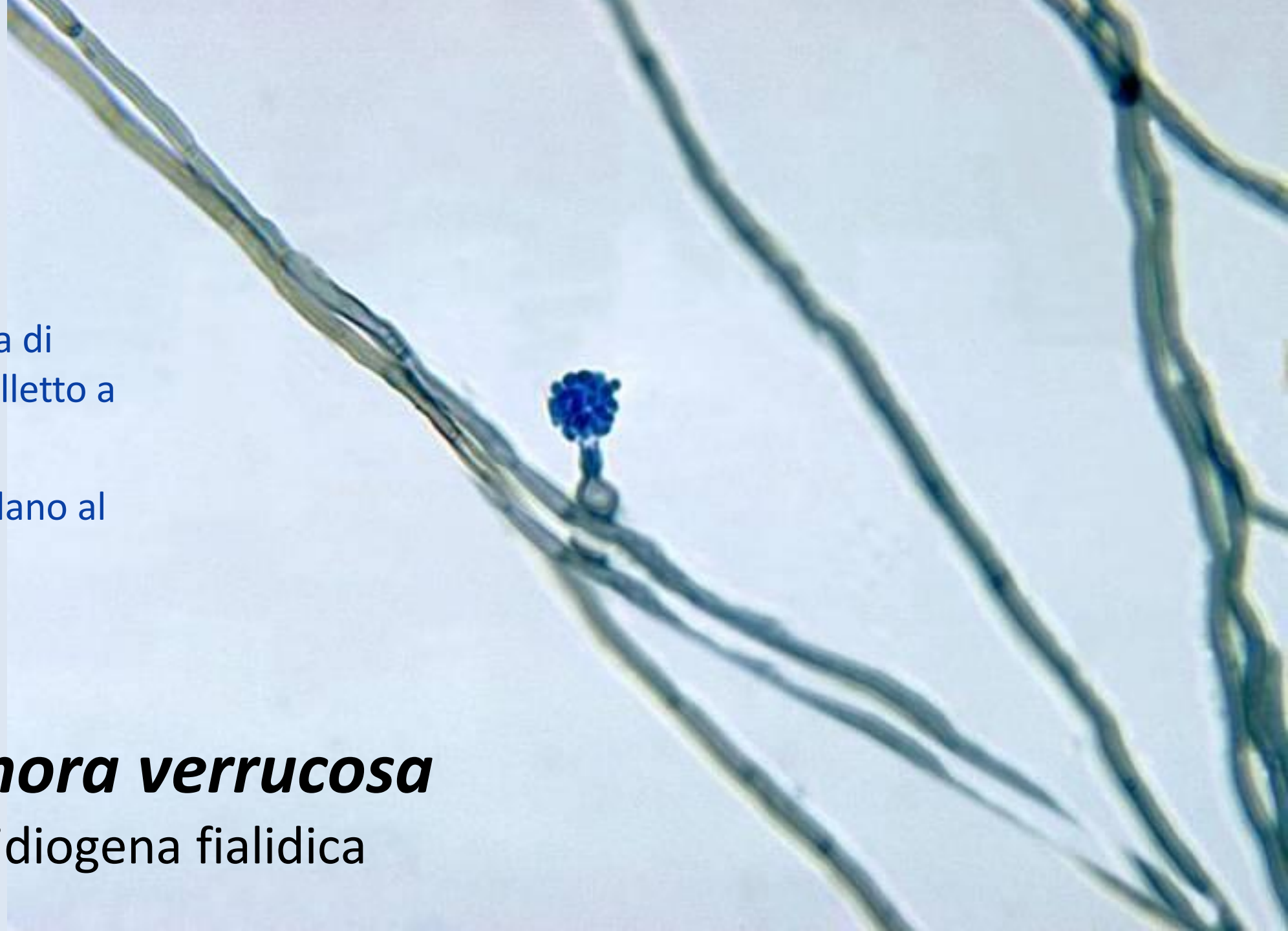
- Numerose specie, la filogenesi non è ben risolta
- Diverse specie hanno un significato clinico, E. dermatitidis, E. jeanselmei, E. Spinifera
- Cellula conidiogena spesso non evidente
- I conidi sono da rotondi a ovali e si presentano in grappoli e masse
- Contaminazioni frequenti nei frigoriferi e in altre apparecchiature mediche

Le frecce bianche indicano fiale terminali e intercalari

Le frecce nere indicano le celluleyeast-like

Phialophora verrucosa



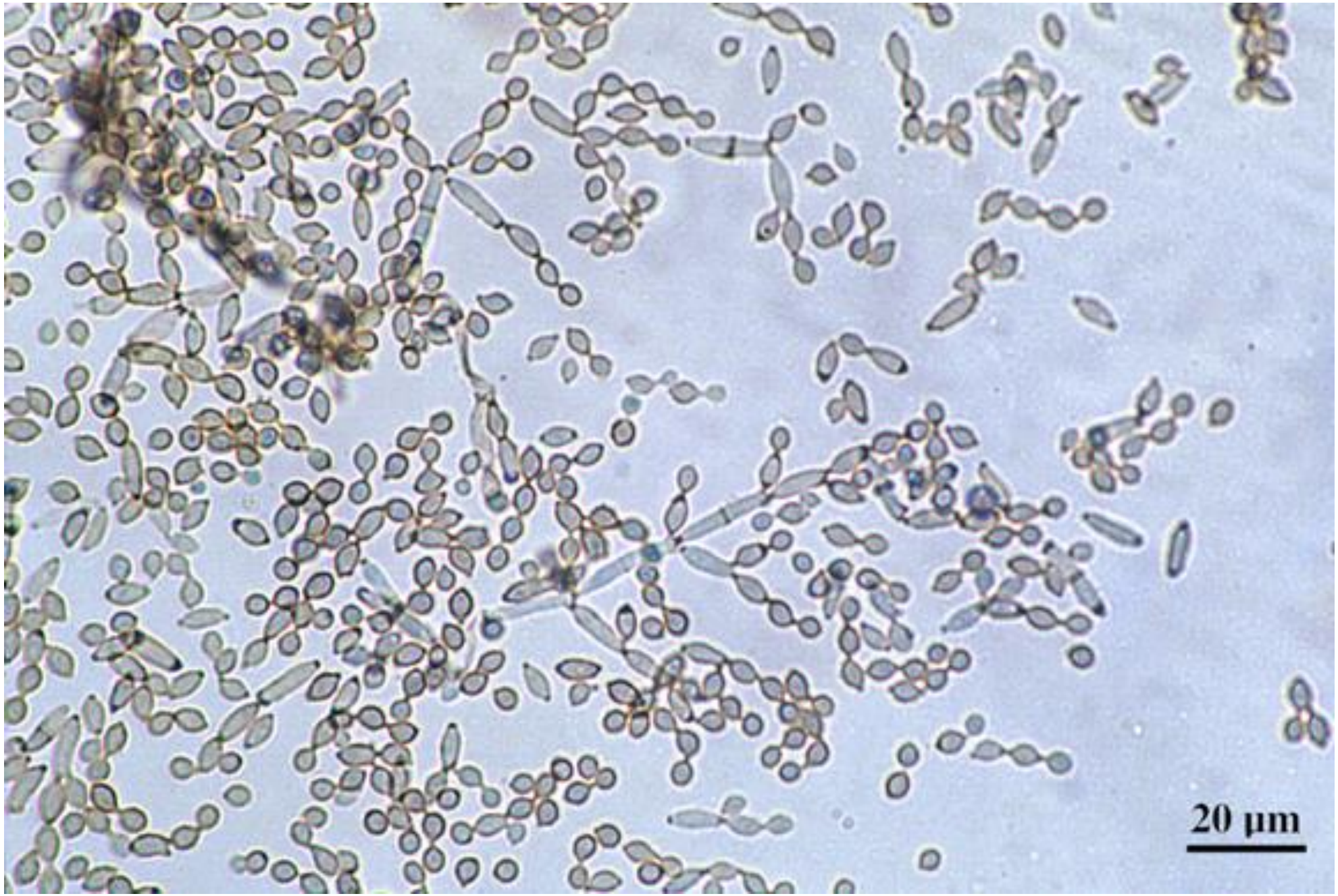


- I fialidi sono a forma di fiaschi o vasi con colletto a forma di imbuto
- Le conidi si accumulano al collarette

Phialophora verrucosa

Cellula conidiogena fialidica

Phialophora verrucosa

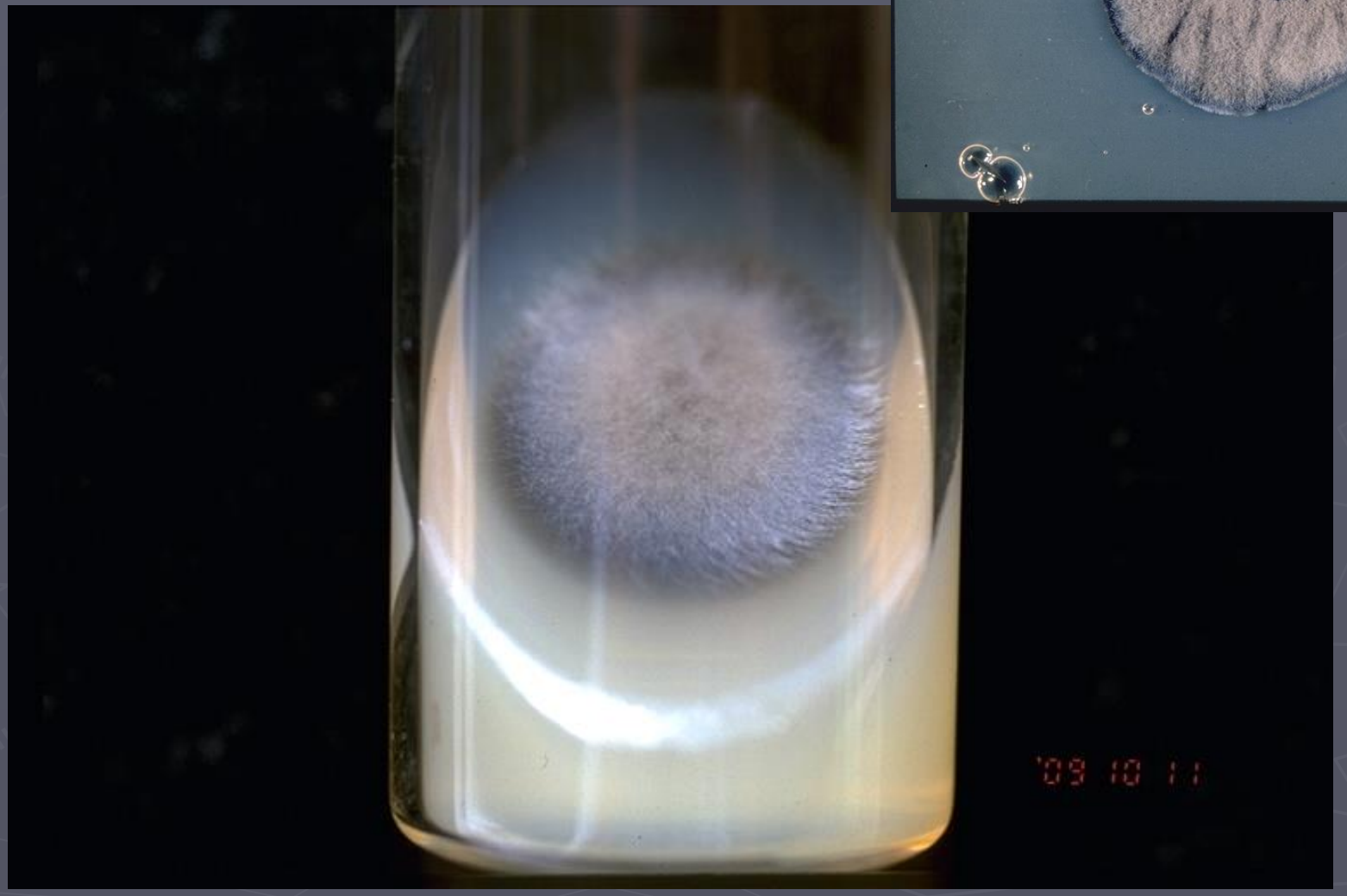


***Cladosporium* sp.**

Cladophialophora carrionii (Cladosporium carrioni)

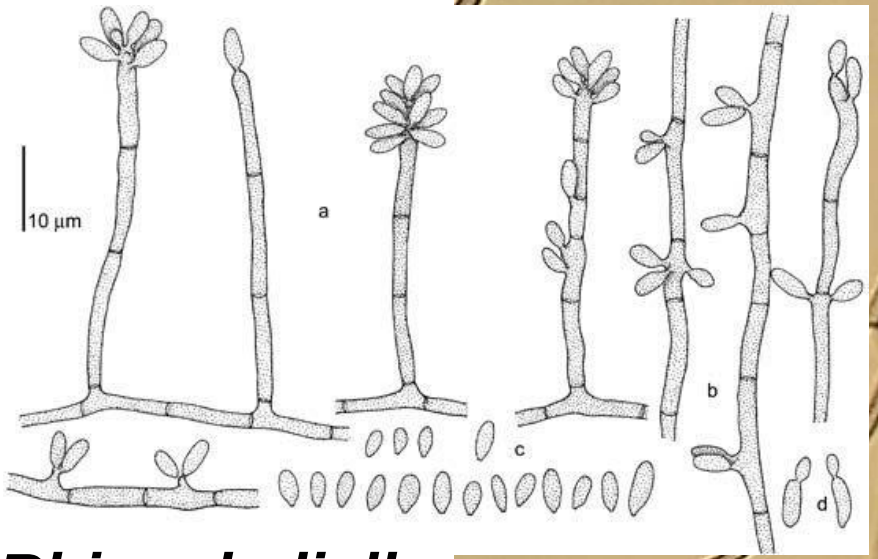
Conidi ovoidi
disposti in lunghe
catene
uni o bicelular

- ***Fonsecaea pedrosoi***



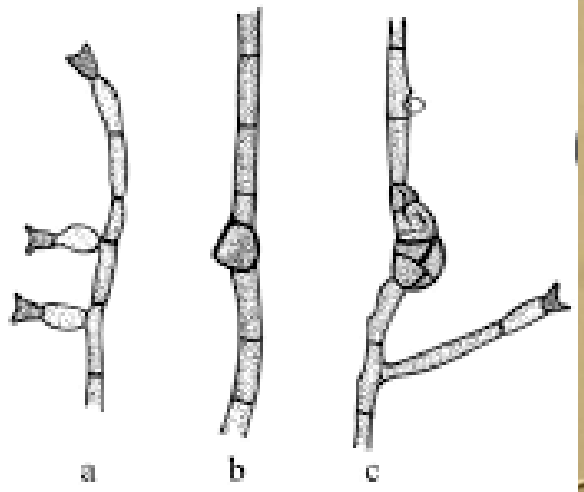
Tre tipi di fruttificazione

Fonsecaea pedrosoi



Rhinocladiella

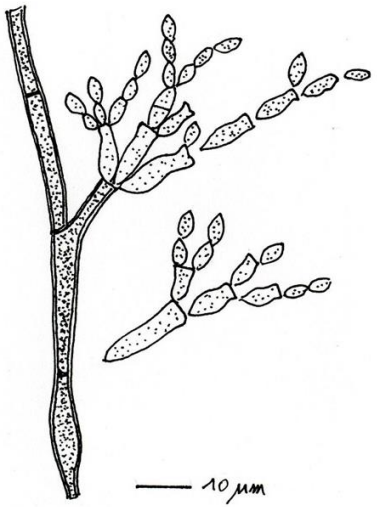
Conidi ovoidali, fusiformi, laterale, unicellulare che si formano in modo simpodiale all'estremità di Conidiofori lunghi eretto cilindrico



Phialophora

I fialidi a forma di fiaschi o vasi con colletto a forma di imbuto

Fonsecaea pedrosoi

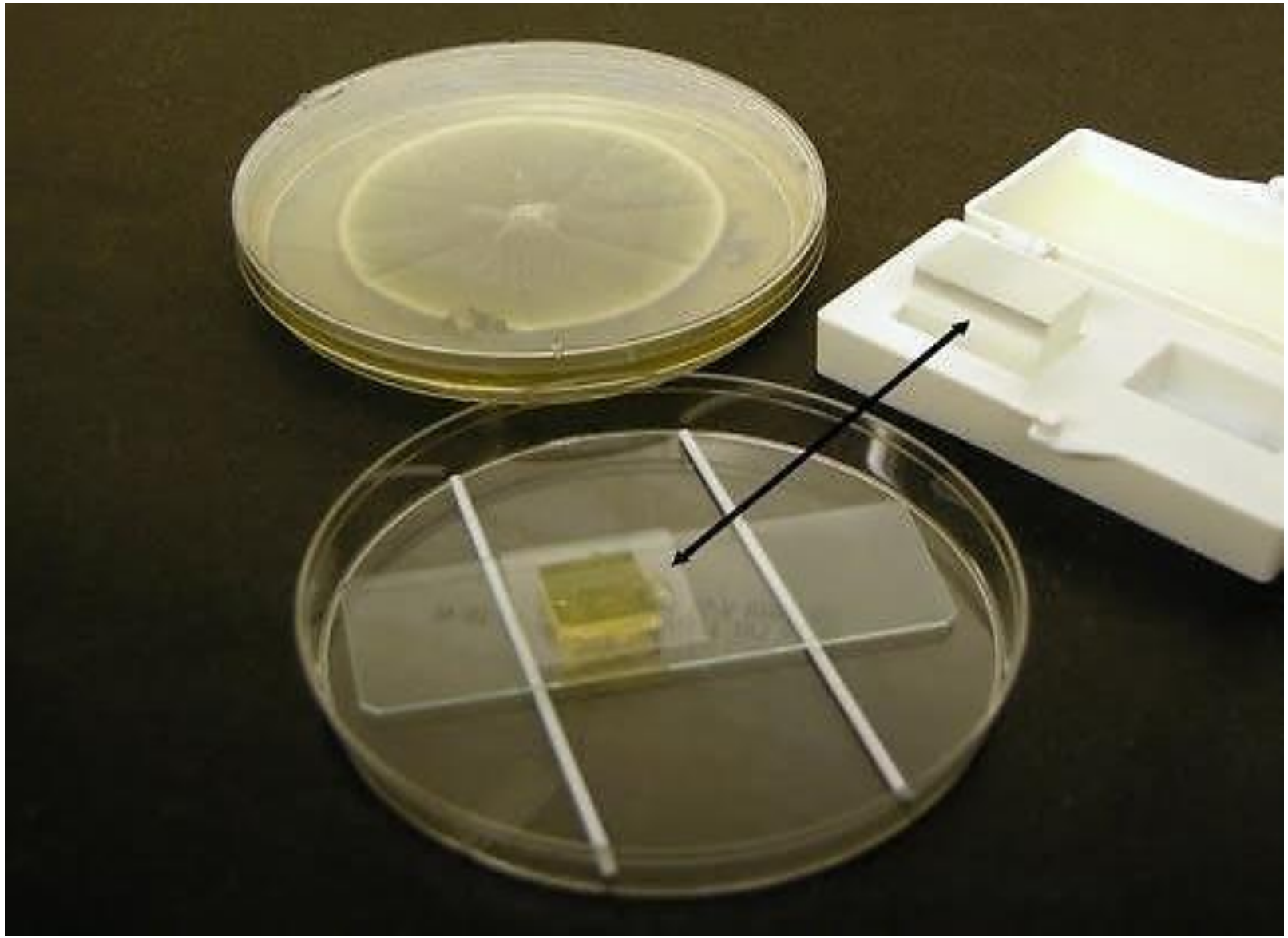


Cladosporium

Conidi ovoidi
disposti in lunghe
catene
uni o bicelular

Fonsecaea pedrosoi


www.pgodoy.com
www.pgodoy.com



MUCHAS GRACIAS!!

Gustavo Giusiano

gustavogiusiano@yahoo.com.ar
giusianogustavo@gmail.com