MMTN
MEDICAL MYCOLOGY
TRAINING NETWORK

Mucormycosis & pythiosis – new insights

Dr Ariya Chindamporn
Associate Professor
Department of Microbiology
Faculty of Medicine, Chulalongkorn University
Bangkok, Thailand

ASIA FUNGAL WORKING GROUP
an ISHAM working group

ISHAM
INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Presented at MMTN Vietnam Conference
1–3 December 2017, Ho Chi Minh City, Vietnam

MMTN
MEDICAL MYCOLOGY
TRAINING NETWORK

Hot topics in Asian medical mycology

Mucormycosis & Pythiosis – new insight

Ariya Chindamporn
Department of Microbiology, Faculty of Medicine,
Chulalongkorn University
Bangkok, Thailand

ASIA FUNGAL WORKING GROUP
an ISHAM working group

ISHAM

Outline

- Introduction
- Recent Taxonomy
- Trend of incidence
- Epidemiology between developed and developing countries
- Pathogenesis : role of CotH receptor agents –agents causing infection
- Treatment

Taxonomy of Fungi Causing Mucormycosis and Entomophthoramycosis (Zygomycosis) and Nomenclature of the Disease: Molecular Mycologic Perspectives

Kyung J. Kwon-Chung

Molecular Microbiology Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Figure 1. Old (*A*) and a proposed new (*B*) classification schemes of the kingdom Fungi.

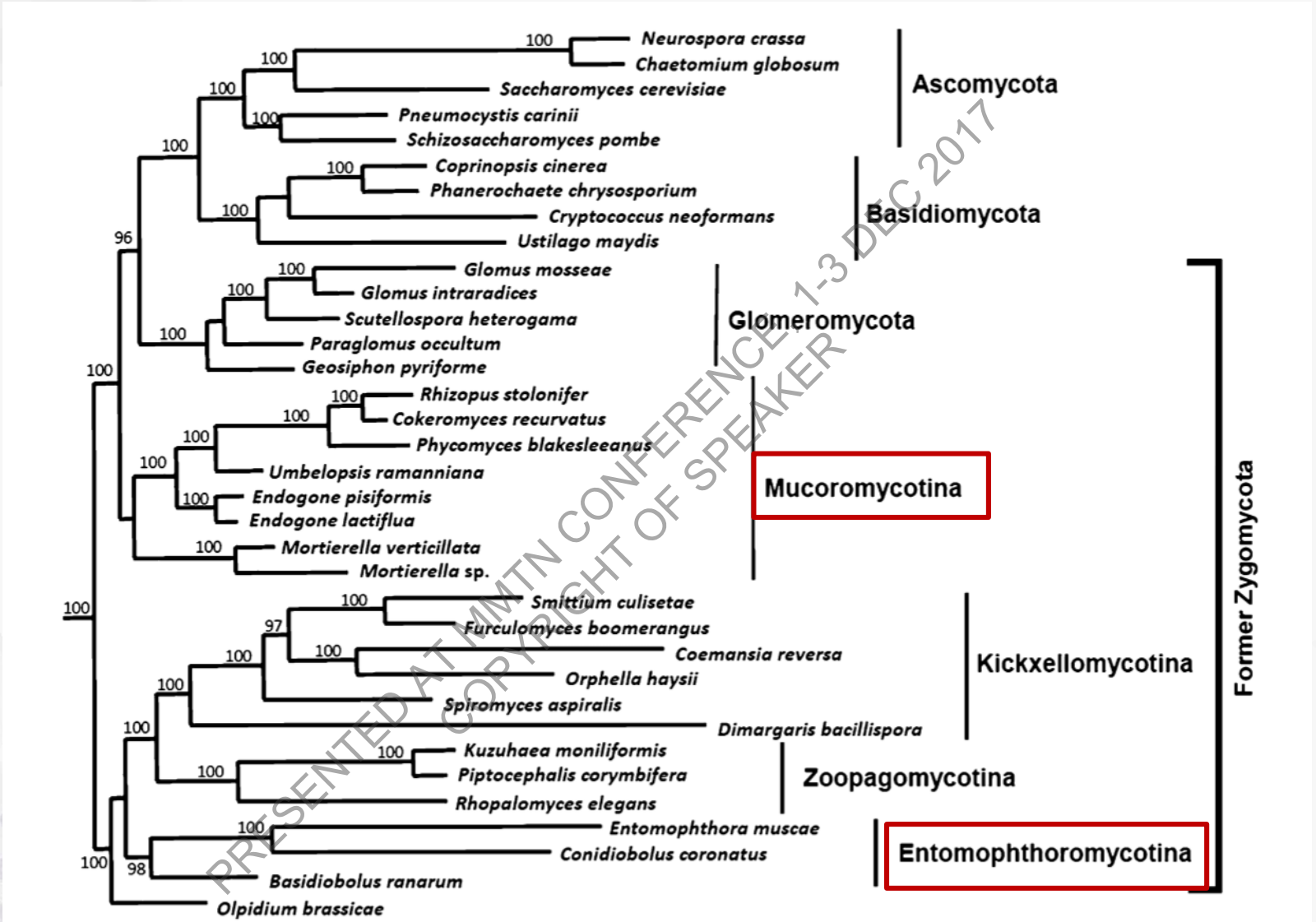


Figure 3. Phylogenetic tree provided by T. Y. James at the University of Michigan, Ann Arbor, which was based on unpublished results from the AFTOL (Assembling the Fungal Tree of Life) project [10]. Results are similar to those reported by James et al [5], but additional basal taxa are included.

Mucormycosis *VS* Entomophthoromycosis

	Mucormycosis	Entomophthoromycosis
Synonym	Phycomycosis, Zygomycosis	--
Infection **- Host, mostly**	Immunocompromised: HM, HSCT, SOT, Diabetic ketoacidosis	Immunocompetent
Clin. Manifestation	Sinus, Pulmonary, Cutaneous, GI, Acute thrombosis	Chronic & Subcutaneous
Treatment	AmphotericinB, posaconazole	Itraconazole
Route of infection	Inhalation, ingestion, or through direct inoculation via abraded skin	Abrasion
Pathogenic form	Aseptate hyphae 3-25 um, thin wall, non dichotomous branching	Aseptate hyphae surround by thick eosinophilic sleeves
Habitat	Decaying organic substrate	Amphibians, GI of Lizard, decayed plant
Distribution	World wide	Tropical & Subtropical
Pathogen	Subphylum Mucoromycotina: Order Mucorales: *Rhizopus, Mucor, Lichtheimia (Absidia), etc.*	Subphylum Entomophthoromycotina: Order Entomophthorales: *Basidiobolus, Conidiobolus*

Modified Kwon-Chung K., CID 2012:54 (Suppl 1) & Famakiotis & Kontoyiannia. Infect Dis Clin N Am 30 (2016) 143-63

Subcutaneous *Saksenaea vasiformis* infection presenting as disfiguring facial plagues

- 51 yr –old labourer
- 2 mo. painless mass, Normal nasal cavity & nasopharynxgeal mucosa
- History :
  - 4 mo. before: shallow abrasion
  - Abt & debridement – mass enlarged
  - No systemic symptom

AmB (1.2 mg/kg/d) & Itra (600 mg/d) 40 d.

Diffuse erythematous infil. w skin thickening over forehead, both eyelids & nose

After AmB & Itra

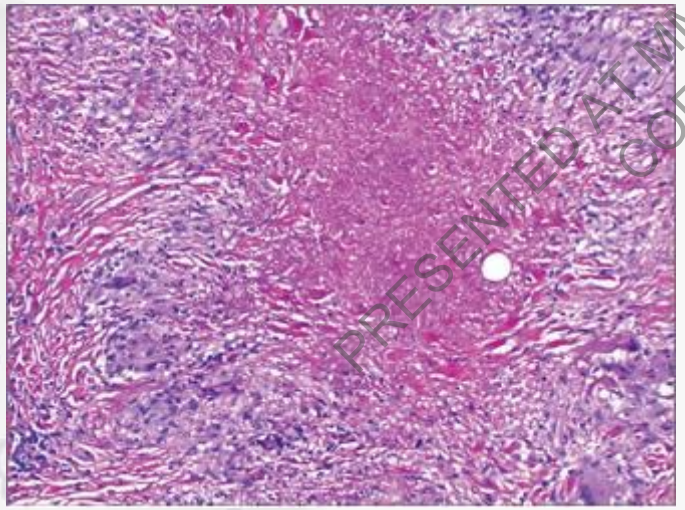


Fig. Diffuse granulomas in the reticular dermis & subcu. tissue w necrobiotic collagen. H&E, x200

ITS1-2 region Iden.

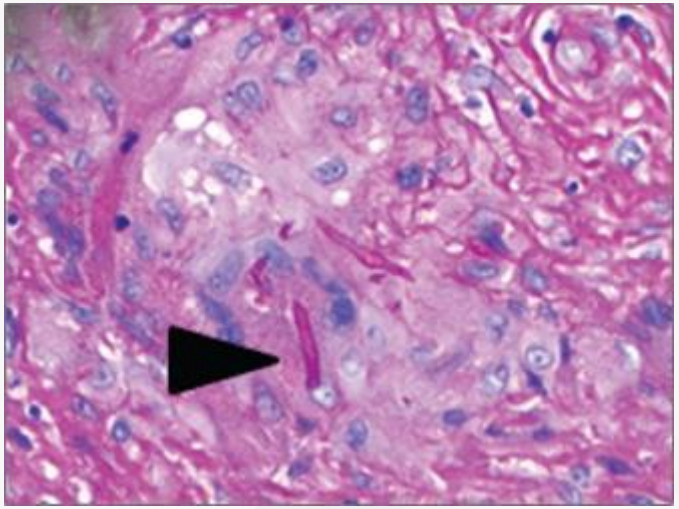


Fig. Aseptate in multinucleated giant cells, PASx200

Anamorph & Teleomorph Characters in Mucorales *VS* Entomophthorales

Order Entomopthorales

Order Mucorales

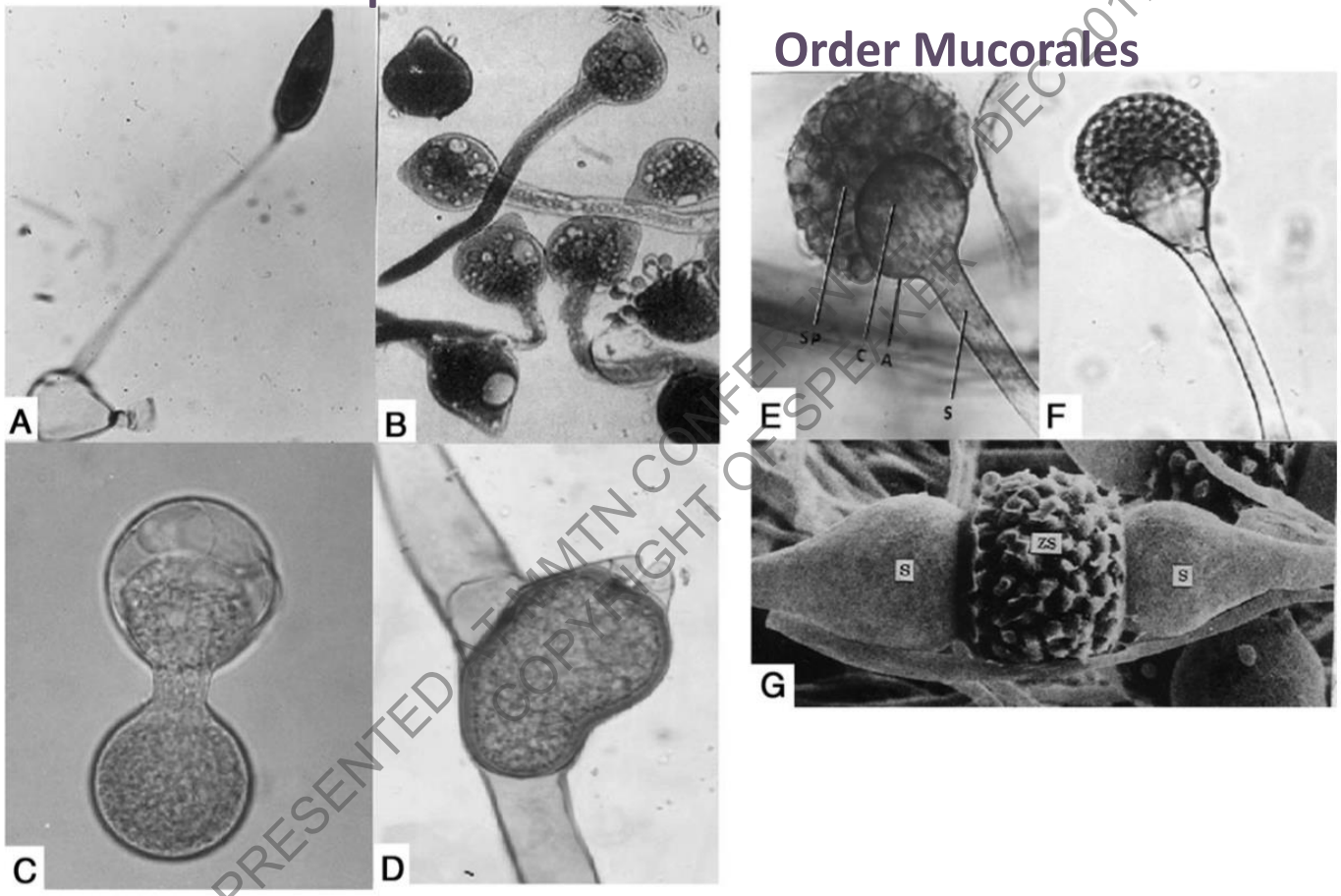
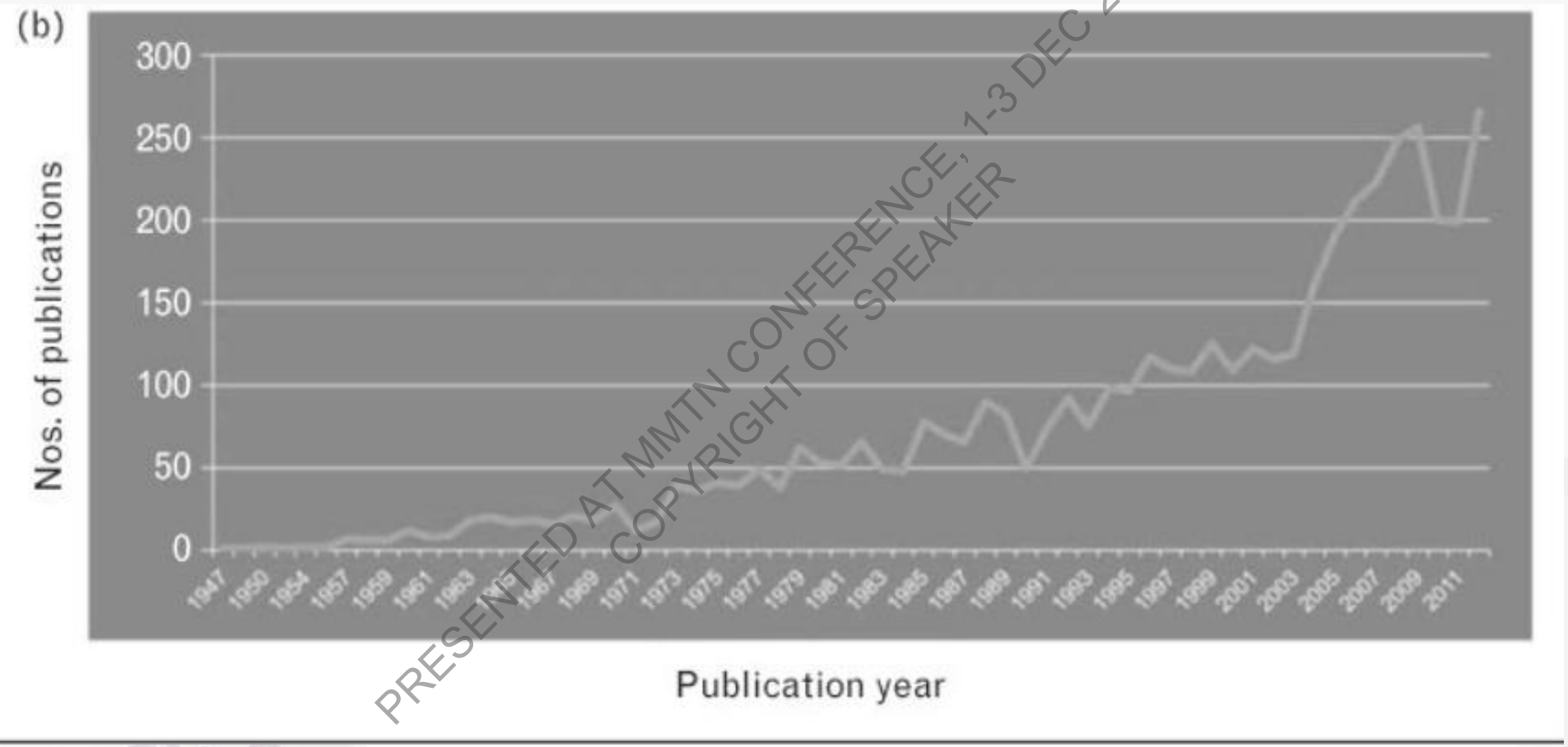


Figure 4. Morphology of conidia and zygospores (scale bar, 20 μm). *A*, Reproduction of a conidium in *Basidiobolus ranarum*. A conidium discharged onto a Petri dish cover germinated and produced a conidiophore bearing a single conidium. *B*, Primary conidia of *Conidiobolus incongruus* germinated to produce long hyphae bearing subglobose conidia [38]. *C*, Secondary conidium formation by replication in *Conidiobolus coronatus*. *D*, Zygospore of *Basidiobolus ranarum*, with a characteristic beak, is produced by the fusion of 2 adjacent hyphal cells. *E*, Sporangial structure of *Rhizopus* species showing the sporangiophore (*S*), apophysis (*A*), columella (*C*), and sporangiospores (*SP*). *F*, Sporangium of *Lichtheimia* (*Absidia*) *corymbifera*. *G*, Electron microscopy of Zygospore (*ZS*) of *Rhizopus* species produced between 2 suspensors (*S*) originating from hyphae of 2 sexually compatible strains (courtesy of Dr S. L. Flegler). Images in *A* and *C–F* are from Kwon-Chung and Bennett [33].

Kwon-Chung K., CID 2012:54 (Suppl 1)

Annual number of published articles on mucormycosis since 1975 (SCOPUS, accessed July, 2013)



Ibrahim AS.et al. Curr Opin Infect Dis. 2013 December ; 26(6): 508–15

Underlying Conditions in Mucormycosis patients in various studies

Location	Period	Cases No.	Underlying conditions % of cases							Ref.
			DM	HM	SOM/ SOT	DFO	HIV	AutoIm/ Cortico	Trauma / no	
Global	1885-2004	929	36.0	21.0	7.0	6.0	2.0	1.0	19.0	*Roden et al. 2005*
France	1997-2006	53	16.2	17.3	7.1	---	4.9	---	54.4	*Bitar etal. 2009*
Italy	2004-2007	60	18	61.7	1.7	---	1.7	3.3	40.0	*Pagano et al.2009*
Belgium	2000-2009	31	6.4	77.0	13.0	---	3.0	---	13.0	*Saegeman et al.2010*
Global	2006-2009	41	17.1	63.4	9.8	---	---	---	---	*Ruping et al. 2009*
Europe	2005-2007	230(>1-8%)	17.0	55.0	9.0	1.0	2.0	7.0	20.0	*Skiada et al., 2011*
India	2006-2007	178	73.6	1.1	0.6	---	---	---	19.1	*Chakarbarti et al. 2006*
Spain	2007-2015	19	0	52.6	---	---	---	---	52.6	*Guinea et al.2017*
Mexico	1982-2016	418	72	18(5/77DM) 93 (HM)	---	---	---	---	9.3	*Corzo-Leon et al. 2017*

Modified Petrikkos G., et al. CID.2012:54 (Suppl1)

Epidemiology in Mexico. 1982-2015

414 cases: Mucormycosis, 4 cases: Entomopthoromycosis

Table 1. Demographic and clinical characteristics: differences between populations with diabetes and malignancy.

Characteristic	Diabetes N = 302/418 (72%)	Malignancy* [77/418 (18%) Hematological 72/77 (93%)[	Total population** N = 418 (%)
Age, years (median, IIR)	50 (38–60)	26 (18–43)	42 (0–80)
Sex (Male)	96/187 (51)	11/27 (41)	225 (54)
Mortality rate	101/192 (53)	11/25 (44)	127/246 (52)
N = 246 (%)[a]		11/23 (48)	
Type of infection[b]	N = 181 (%)	N = 31 (%)	N = 418 (%)
Sinus (overall)	159 (88)	11 (35)	315 (75)
Palatine infection	39/159 (24)	2/11 (18)	45/315 (14)
Sinocerebral/cerebral	85/159 (53)	3/11 (27)	210/315 (66)
Pulmonary	8 (4)	11 (35)	26 (6)
Cutaneous	9 (5)	2 (6)	28 (6.5)
Disseminated[+]	2 (1)	4 (13)	23 (5.5)
Unspecified***	1 (0.5)	0	19 (4.5)
Abdominal[++]	1 (0.5)	3 (10)	5 (1)
Cerebral [&]	1 (0.5)	0	2 (0.5)

IIR, Interquartile interval range.

[a]Mortality rate estimated with the available information of 246 individuals.

[b]Type of infection was estimated depending on the number of individuals with available information: 181 with diabetes mellitus and 31 with malignancy. Although an overall estimation was possible for some variables among the 418 cases, in some reports only the site of infection was reported without mention of the underlying disease.

* Five individuals with malignancy had diabetes mellitus as comorbidity

** Includes cases without underlying condition or without diabetes and without malignancy (N = 39, 9.3%). This group had mortality rate in 52% (15/29). Ten individuals with no underlying condition (19/39, 49%) did not have information available and eight were reported as previously healthy. Prior trauma was present in eight of 39 (20%), of these five individuals had no other associated condition. Five individuals had autoimmune disease (5/39, 13%), three with HIV (human immunodeficiency virus) infection (3/39, 8%), other prior conditions were drug toxicity (2/39, 5%), post-surgery (2/39, 5%). Drug toxicities consisted in agranulocytosis or neutropenia due to drugs. Numbers reported in populations with diabetes and malignancy vary due to the availability of the information.

*** Unspecified: refers to information of the site/type of infection was unavailable

[+]2 or more sites affected.

[++]Only abdominal infection. These were gastric, renal, hepatic, splenic, and intestinal presentation.

[&]Only due to trauma and postsurgical process, no sinus infection.

Literature review in Mexico 1982-2015

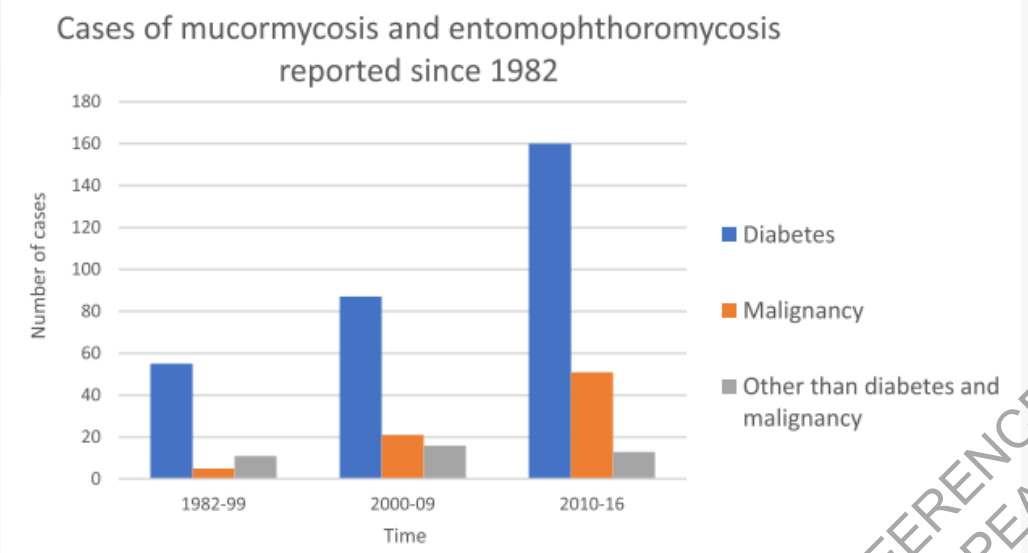
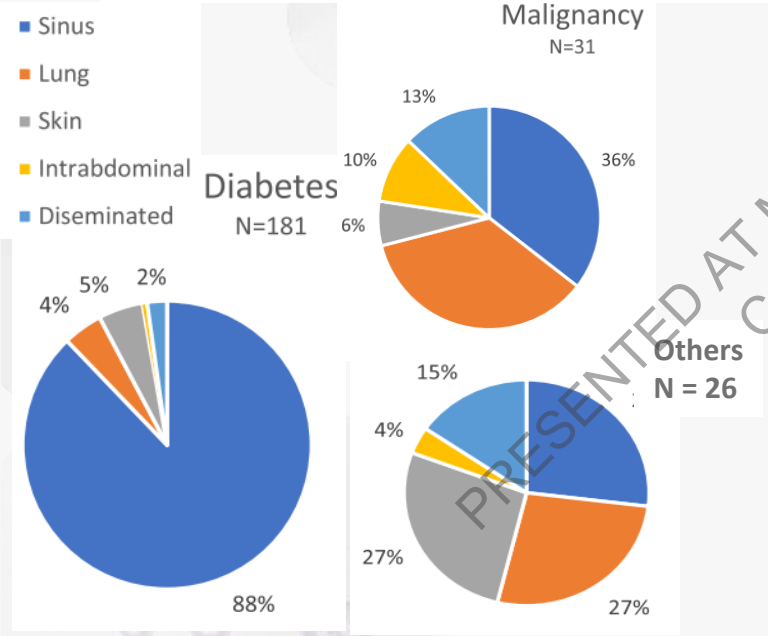


Table 6. Clinical isolates reported from 250 patients.

Organism isolated	Total population n = 250 (%)
Rhizopus species	148 (59)
Rhizopus oryzae/R. arrhizus	108/148 (73)
Rhizopus sp.	34/148 (23)
Rhizopus rhizopodiformis	2/148 (1.3)
Rhizopus microsporus	2/148 (1.3)
Rhizopus azygosporus	1/148 (0.6)
Rhizopus pusillus	1/148 (0.6)
Mucor species	71 (28)
Mucor sp.	66/71 (93)
Mucor circinelloides	5/71 (7)
Rhizomucor sp.	10 (4)
Lichtheimia corymbifera	8 (3)
Cunninghamella sp.	4 (1.5)
Syncephalastrum racemosum	3 (1)
Basidiobolus sp.	3 (1)
Conidiobolus sp.	1 (0.5)
Apophysomyces mexicanus	1 (0.5)
Absidia sp.	1 (0.5)



Performance of Diagnostic Testing

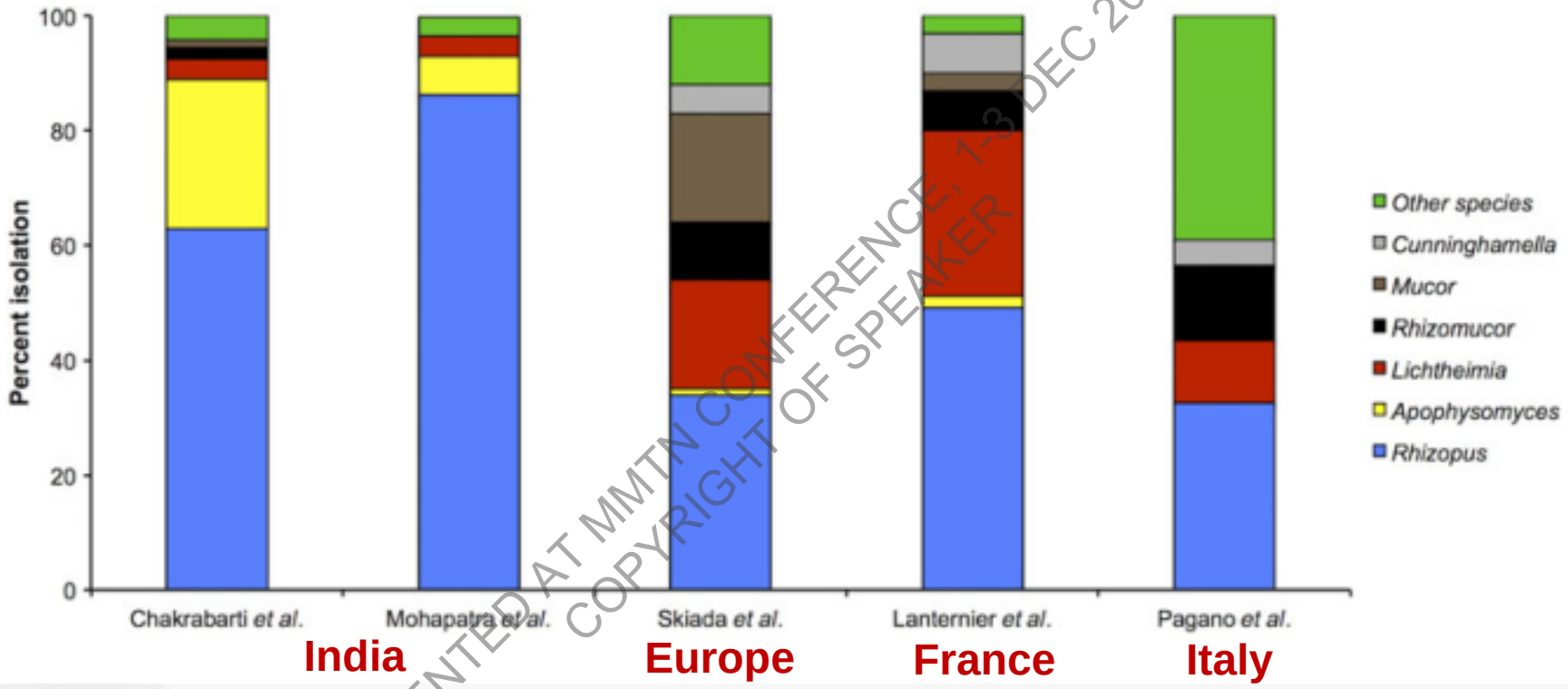
Diag tool	nonspecialized center	specialized center	Total
Pos. Direct smear/cytology	73/76 (95%)	158/158 (100%)	231/234 (98%)
Pos. Culture	120/211 (57%)	142/158 (90%)	262/369 (71%)

- **158/369 (41%) cases were reported by a specialized center,**
- **211 cases by non specialized center**

Figure 3. Clinical presentations of mucormycosis and entomophthoromycosis in Mexico. Pie charts showing the clinical presentations by underlying condition using proportions

Corzo-Leon DE., et al. Med Mycol.2017, 0, 16

Etiologic agents of Zygomycosis (Mucormycosis)



The data shown are from studies by Chakrabarti et al. , Mohapatra (India)., Skiada (Europe), Lanterniaer et al. (France), and Pagano et al. (Italy)

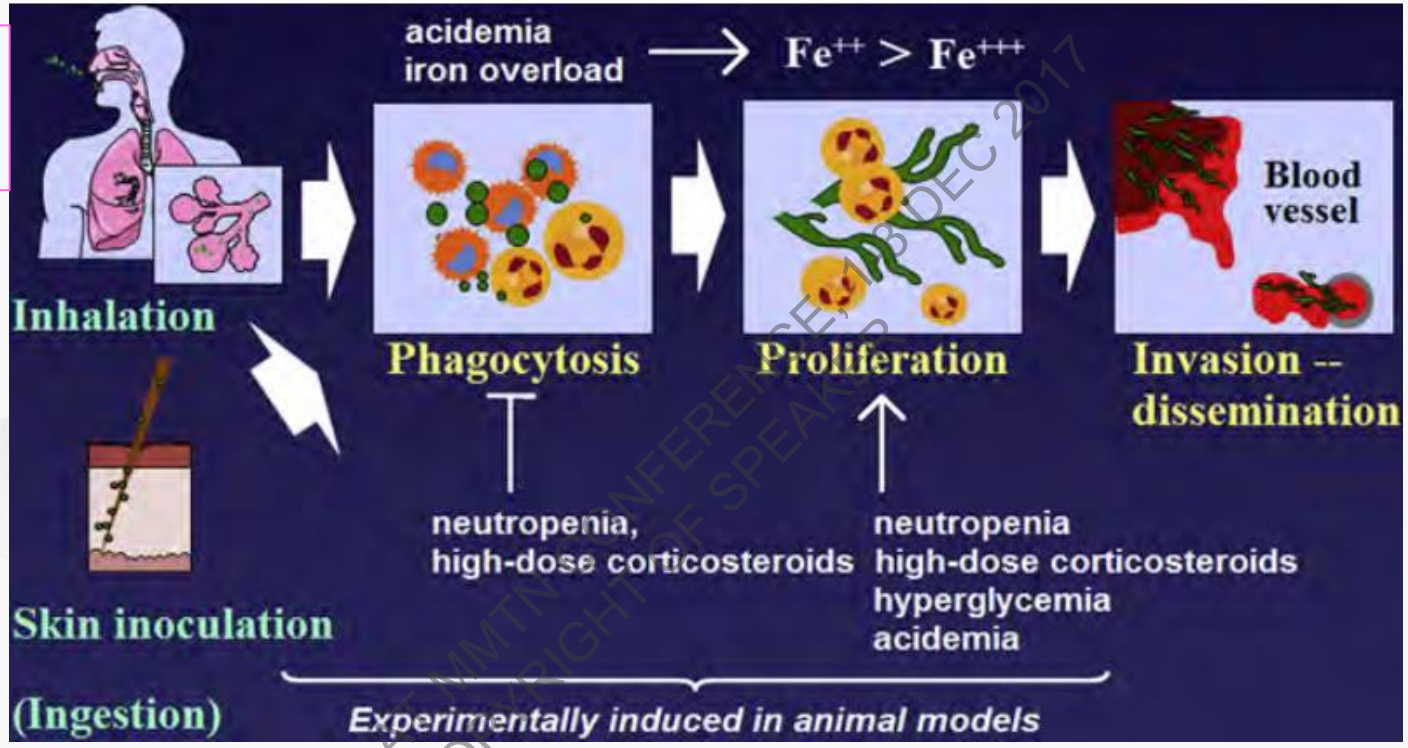
Chakrabarti A. etal. Mycoses, 2014, 57 (Suppl. 3), 85–90

Laboratory Diagnosis

- Collect specimens : pus, bloody tissue, debris
- Transportation : Not on ice
- Processing :
  - Cut into small pieces in sterile plate
  - Direct examination : KOH preparation, KOH calcofluor stain - REPORT
  - Other common stain in Microbiol lab. : Gram stain, AFB stain - REPORT
  - Histopathology : tissue reaction (H&E, PAS); shape (GMS)
  - Culture : SDA, SDA+abt, SDB, Blood agar 2-3 days, 30&35C
  - Identification: classical (colony & sporulation)/ PCR/ MALDI-TOF
- Serology : Negative GM & BG

Pathogenesis of Mucormycosis

Sizes
- 3-11um
- >10 um



- IR: **Steriod:** impair ϕ migration, ingestion. Phagolysosome fusion
- **Hemat. Malignacies: Neutropenia**: impair chemotaxis & diminish fungicidal mechanisms
- **DKA**: weak neutrophil, low pH, higher glucose level, free iron
- Mucorales is **able to extract iron** from desferrioxamine.

Farmakiotis &Kontoyiannis. Infect Dis Clin N Am 30(2016)143-63

Isavuconazole :

- Broad spectrum 2nd gen. triazole
- Inhibit CYP enzyme laosterol 14-alpha-demethylase (CYP51) –blocking synthesis of ergosterol
- Metabolites via CYP3A4 & CYP3A5 which may alter the plasma concentrations
- a moderate inhibitor of CYP3A4, and a mild inhibitor of P-glycoprotein (P-gp), and organic cation transporter 2 (OCT2)

Table 1 In vitro activity of isavuconazole against clinically important species of *Aspergillus* and Mucorales (adapted from Ref. [15])

Organism	No. of isolates	MIC range[a] (μg/mL)	MIC_{90} range[a] (μg/mL)	MFC range[a] (μg/mL)
Aspergillus species				
A. flavus	97	0.25–16	1–16	0.5–4
A. fumigatus	939	0.06–4	0.5–2	0.125–4
A. nidulans	70	0.06–2	1	NA
A. niger	84	0.125 to >16	2–4	0.25 to >8
A. terreus	222	0.125 to >16	0.5–4	0.25–2
Mucorales				
Cunninghamella spp.	25	0.12 to >8	>8	2 to >16
Lichtheimia spp.	111	0.03 to >8	1 to >8	4 to >16
Mucor circinelloides	16	2–8	8	NA
Mucor spp.	107	<0.015 to >8	2 to >8	2 to >16
Rhizomucor spp.	38	<0.015 to >8	>8	2 to >8
Rhizopus spp.	189	0.12 to >8	1 to >8	1 to >16
Syncephalastrum spp.	2	0.125–4	NA	1–16

NA not available, *MFC* minimum fungicidal concentration, *MIC* minimum inhibitory concentration, MIC_{90} MIC at which 90 % of isolates are inhibited

[a] Across individual studies reviewed in Ref. [15]

- Isavuconazole: good *in vitro* activity against *Rhizopus & Rhizomucor*
- However, its activity is limited for other Mucolares especially *Mucor circinelloides*
- Overall, MIC of Mucorales higher than *Aspergillus* group

Shirley et al. 2016

- A 57 year-old Thai man with β-thalassemia disease was admitted (day 0) due to low grade fever and swelling Rt arm & forearm (deep and superficial soft tissue) with rapid progression lesions for −3 months.
- History of planting in a swampy area before lesion occurred.
- **KOH & PAS & GMS** : Non-septate hyphae

Yellow tissue covered with pus, measuring 6x11 cm.

What?? and How should we do next ??

- **CTA:** Rt upper extremity: Occluded distal Rt. Brachial artery above the bifurcation.
- ***Pythium insidiosum*** **antibody** by ELISA: Pos

- **Dx:** Pythiosis
- **Tx:** Debridement + oral terbinafine & itraconazole + Immunotherapy

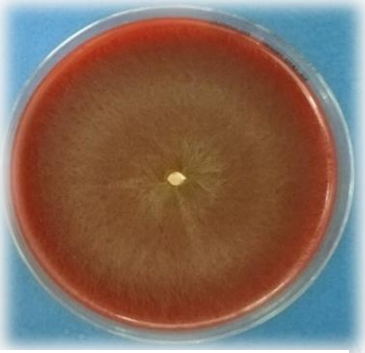
After 1st debridement (+6 days)

After 2nd debridement (+15 days)

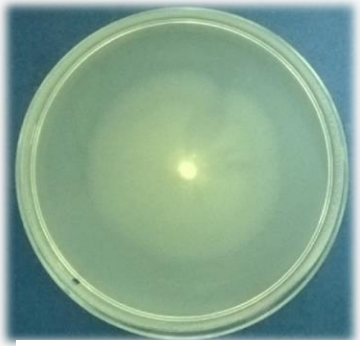
Before discharge (+24 days)

Human pythiosis: *Pythium insidiosum* (fungus-like organism)

Only one case reported in 2011 ….. An American soldier acquired traumatic wound infection by *Pythium aphanidermatum* in Afghanistan *(Farmer A et al. J clin Microbiol, 2011)*



Blood agar

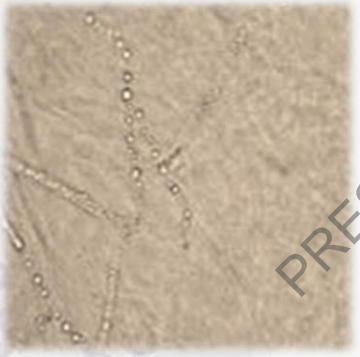


Corn meal agar

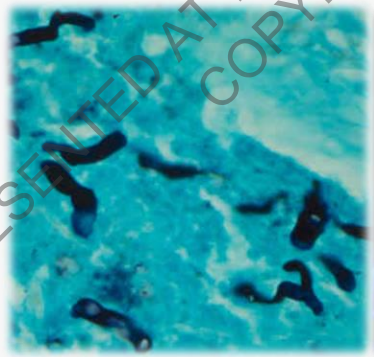
Sabouraud dextrose agar

Sabouraud dextrose broth

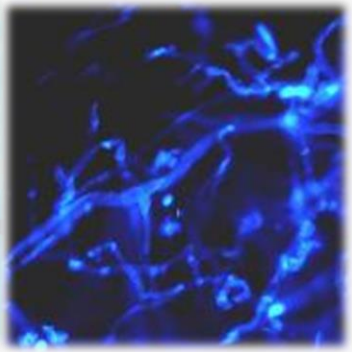
Macroscopic: Mycelium like fungi, rapid growing, submerged, white to colorless colony, 35C, 24h



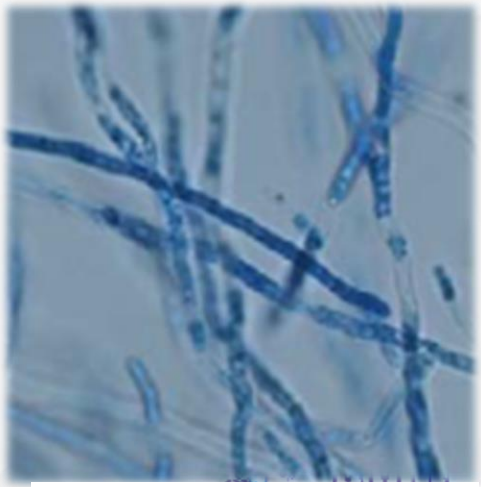
KOH preparation



GMS stain



KOH-Calcofluor white stain



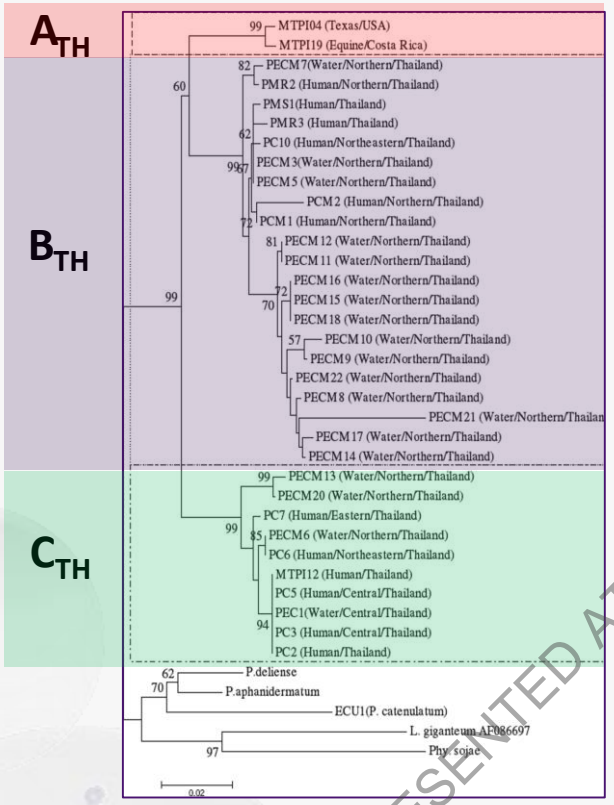
LPCB wet mouth

Microscopic: Sparsely rare septate hyphae

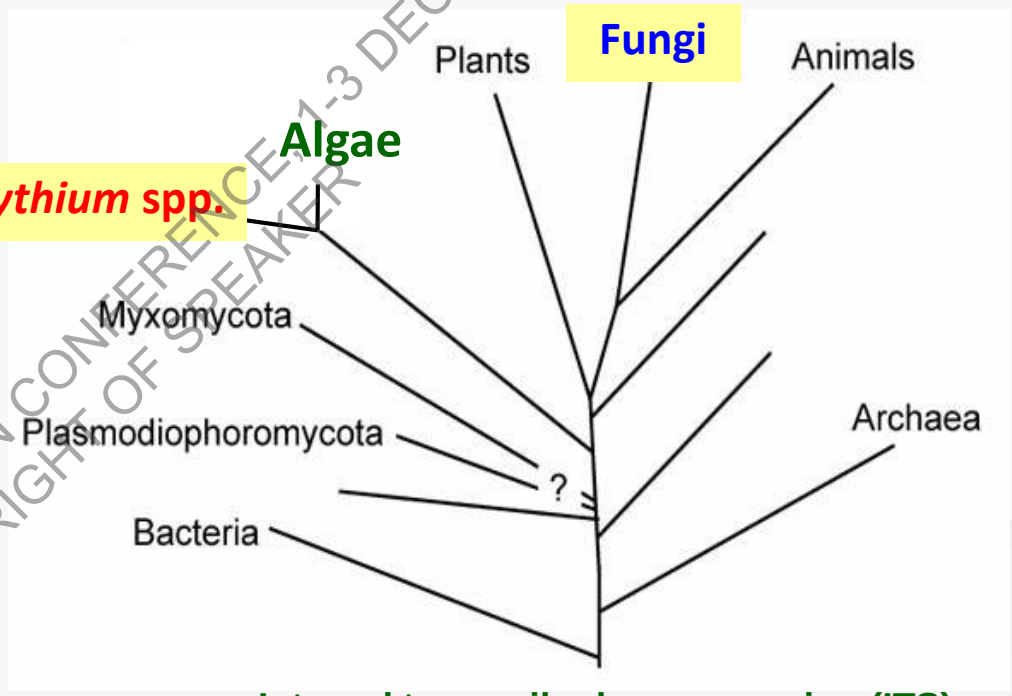
Recent Taxonomy

Kingdom **Straminipila** Class **Oomycetes** Order **Pythiales**

Family **Pythiaceae** Genus ***Pythium*** Species ***insidiosum***



Kammarnjessadakul et al., Med Mycol, 2011



Internal transcribed spacer region (ITS)

Rossman et al., Pest Management, 2006

Phylogenetic tree of *P. insidiosum based on*

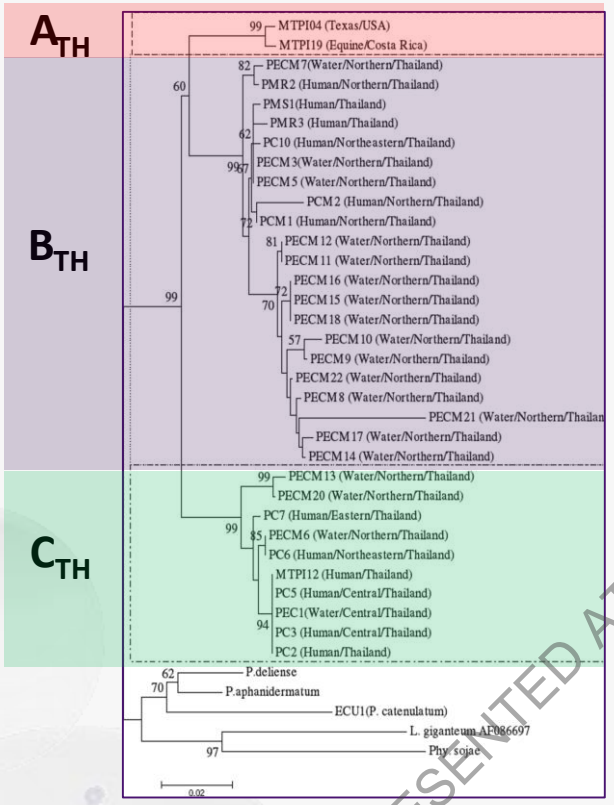
- ITS region *(Schurko et al. Mycol Res, 2003)*
- *IGS region (Frank N et al. Mycologia, 2003)*
- *Cox* 2 gene *(Kammarnjessadakul et al., Med Mycol, 2011)*
- *Exo-1,3-beta glucanase (Ribeiro TC et al. Infection, Genetics and Evolution, 2017)*

MMTN MEDICAL MYCOLOGY TRAINING NETWORK

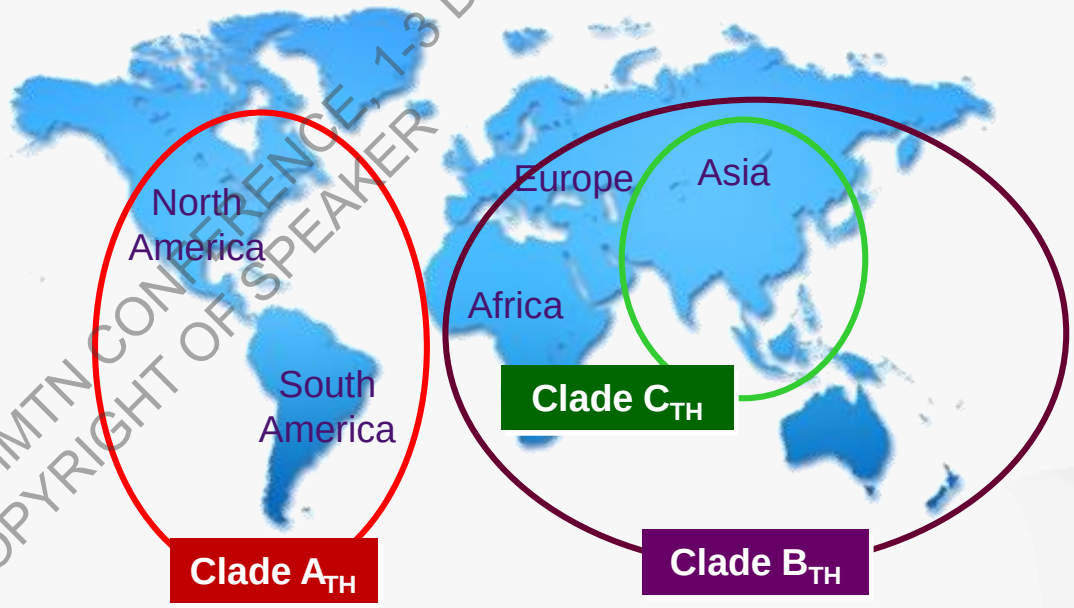
Recent Taxonomy & Epidemiology

Kingdom **Straminipila** Class **Oomycetes** Order **Pythiales**

Family **Pythiaceae** Genus ***Pythium*** Species ***insidiosum***



Kammarnjessadakul et al., Med Mycol, 2011



Schurko et al. Mycol Res, 2003.

Phylogenetic tree of *P. insidiosum based on*

- ITS region *(Schurko et al. Mycol Res, 2003)*
- *IGS region (Frank N et al. Mycologia, 2003)*
- *Cox* 2 gene *(Kammarnjessadakul et al., Med Mycol, 2011)*
- *Exo-1,3-beta glucanase (Ribeiro TC et al. Infection, Genetics and Evolution, 2017)*

MMTN MEDICAL MYCOLOGY TRAINING NETWORK

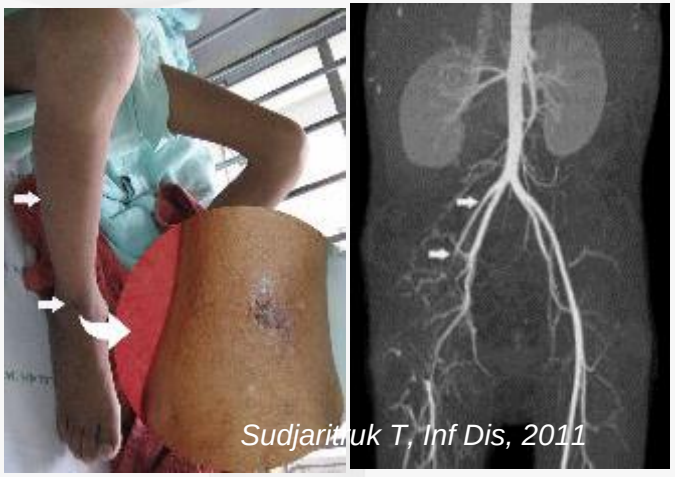
Natural habitat

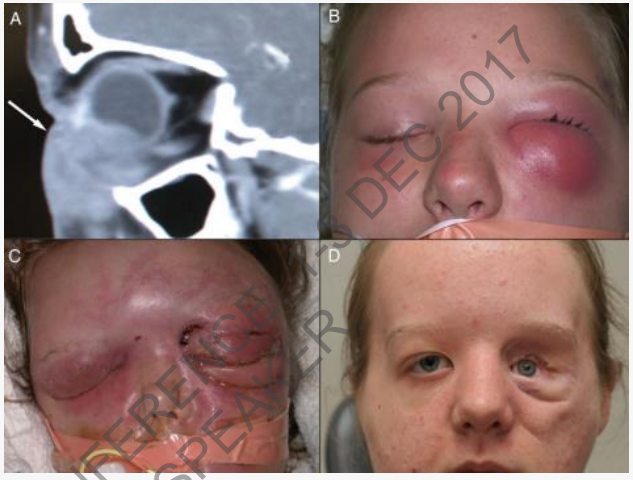
- Tropical & Sub tropical regions
- Moist soil / stagnant water *ie.* rice field
- Also has been isolated from irrigation water and reservoir in northern part of Thailand *(Supabandhu, Med Mycol, 2008)*

Environmental form	Infected Stage	Infected Host
Hyphae&zoospore form	**Zoospore form**	**Hyphae form**

Clinical manifestations

Vascular pythiosis

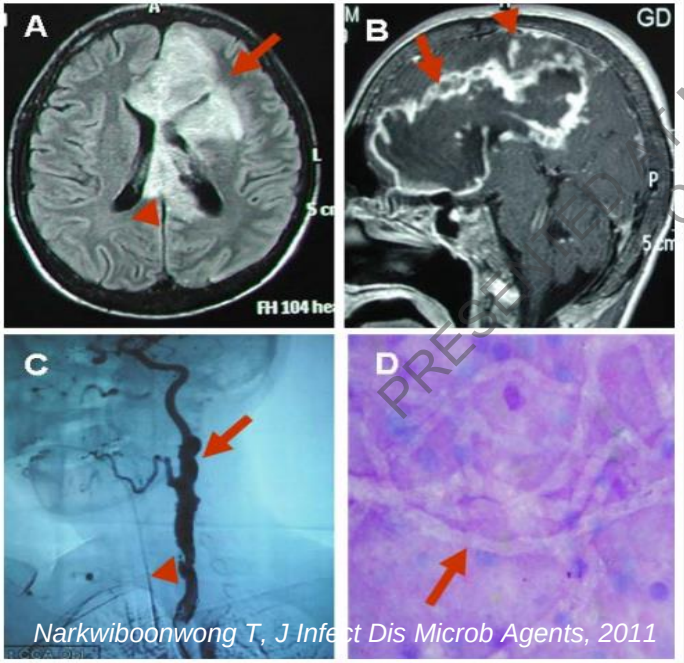




Orbital and Facial Infection

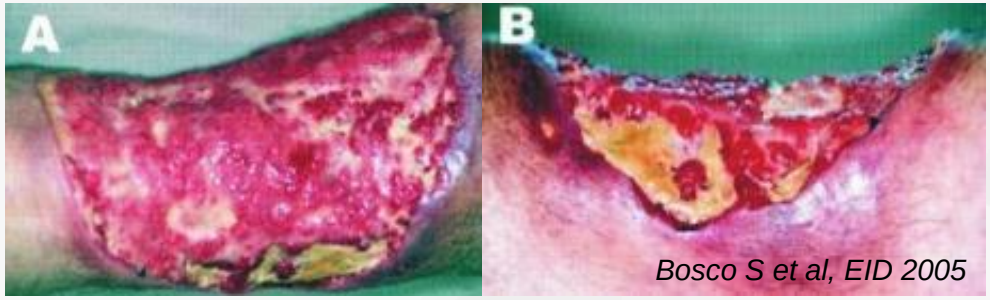
Kirzhner M, J Ped Inf Dis, 2014

Cerebral pythiosis (cerebral hemisphere)



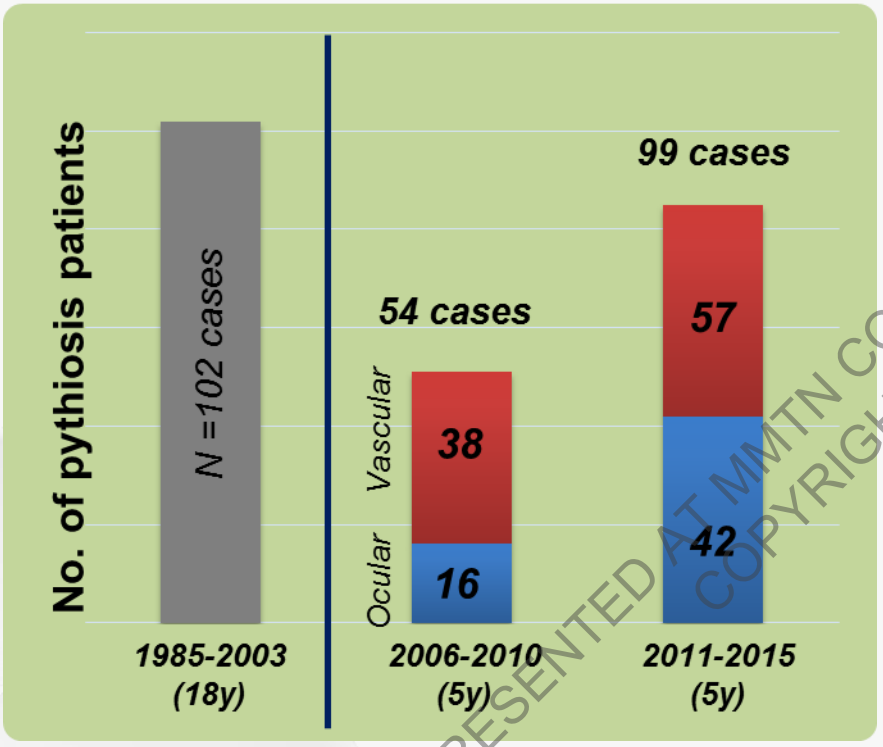
Keratitis pythiosis

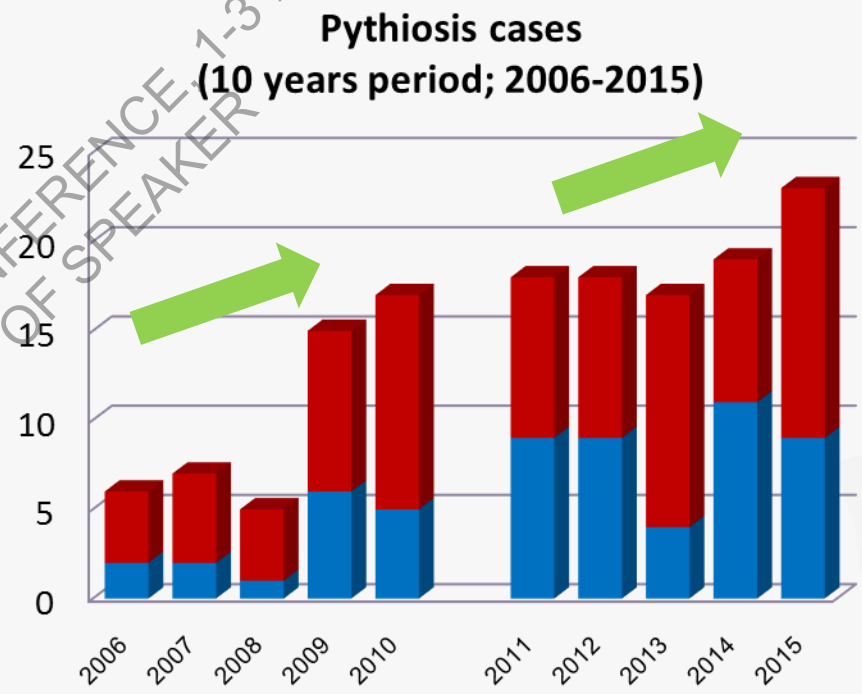
(sub) Cutaneous form



Human Pythiosis

- Human pythiosis was first described in Thailand, in 1987.
- So far Thailand has been ranged as the highest incidence of human pythiosis in the world.





- Base on the immunotherapy requested from Mycology unit, KCMH, the increasing trend was presented.

MMTN
MEDICAL MYCOLOGY TRAINING NETWORK

Human Pythiosis

Pubmed Search *(search on 29th Nov 2017)*

Keywords	Number of publications		
	Before 2006	2006-present (12 years approx.)	Total
Human pythiosis	36	87	123
***Pythium* in human**	75	120	195
Human pythiosis case report	10	22	32
Human vascular pythiosis	6	14	20
Human keratitis pythiosis	6	10	16
Human ocular pythiosis	2	12	14
Thai human pythiosis	3	11	14

Human Pythiosis

- Not only in Thailand, some human pythiosis cases were also reported from other country around the world except Europe.

Regions	Country	Pythiosis Cases	Reference
Asia	Malaysia (Kuala Lumpur)	*Pythium* keratitis disposable contact lens wear, and swimming in the Kelang River	*Badenoch et al., 2001*
	India (Telangana)	13 *Pythium* keratitis cases during 2010-2012	*Sharma S et al. 2015*
	China (Hainan)	*Pythium* keratitis in a boy who was scraped by twigs while climbing a tree	*Hong H et al. 2016*
	Israel	Contact lens-related *Pythium* keratitis	*Tanhehco TY et al. 2011*
Australia	New Zealand (Auckland)	Keratitis pythiosis in a man who played ball in hot pool	*D Murdoch et al. 1997*
	Australia (Darwin)	Keratitis pythiosis in a child who swam in public and backyard swimming pools	*Badenoch PR et al. 2009*
	Australia	2 cutaneous pythiosis cases with the history of exposure to either swampy water or horses.	*Triscott JA et al. 1993*
America	Brazil	Subcutaneous pythiosis in a police after spend his vacation with water-associated leisure activities	*Bosco M et al. 2005*
	USA (Iowa, Florida, Texas)	5 Orbital pythiosis in young children	*Mendoza L et al. 2004*

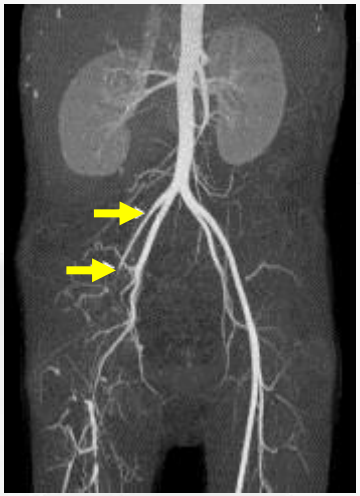
Symptoms & Diagnosis and Treatment

	Vascular form	Ocular form
Symptoms	• Presents as granulomatous cutaneous and subcutaneous lesions • Intermittent claudication • Arterial obstruction / aneurysm resulting ischemia / gangrene • Other signs of arterial insufficiency	• pain and redness • Less vision • Ulcerative keratitis which may progress to endophthalmitis
Underlying Dis. & History	• Underlying hemoglobinopathy (thalassemia, PNH) • Agriculture-related occupations ie. farmer or history of water exposure	• No • Water spilled

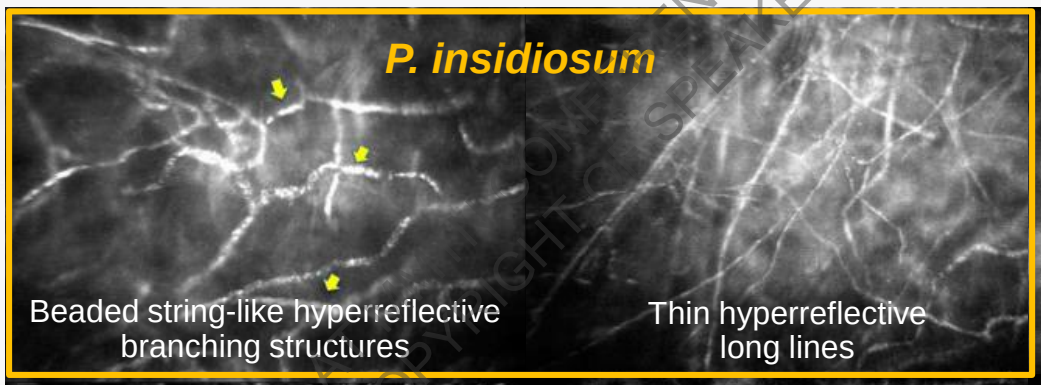
Permpalung et al., J Antimicrob Chemother, 2015

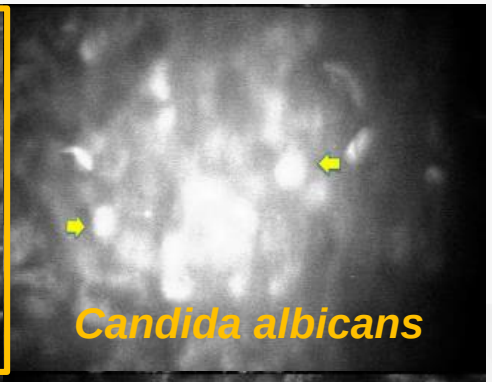
Symptoms & Diagnosis and Treatment

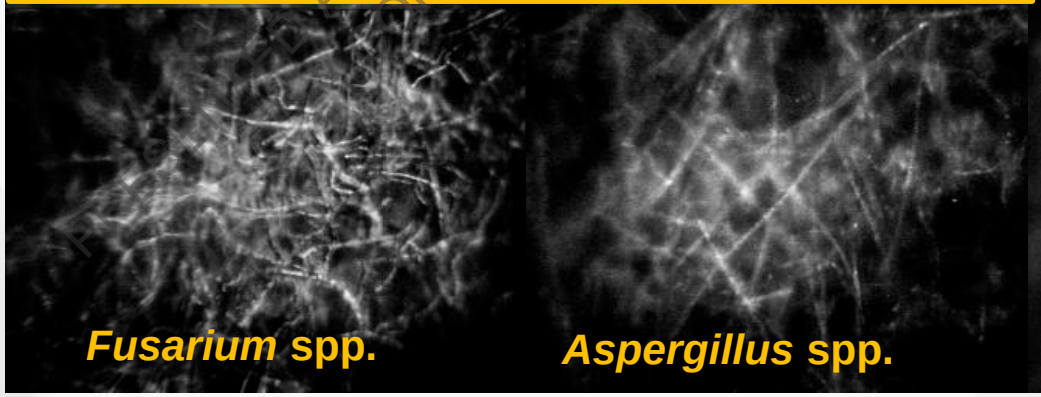
	Vascular form	Ocular form
Diagnosis	• Arterial occlusion by CT angiogram	• Confocal microscope Beaded string-like with mean branching angles at 78.6 degrees. The diameter of the hyphae varied from 1.5 to 7.5 mm. (95% sens.)

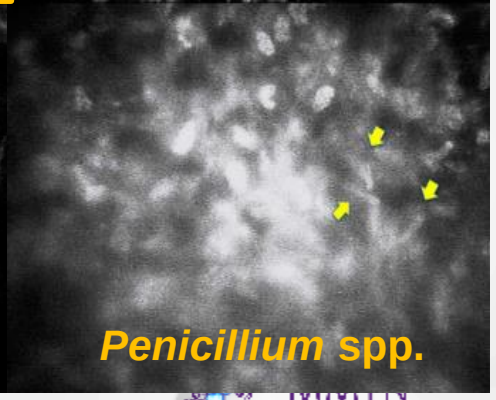


Positive Arterial occlusion by CT angiogram







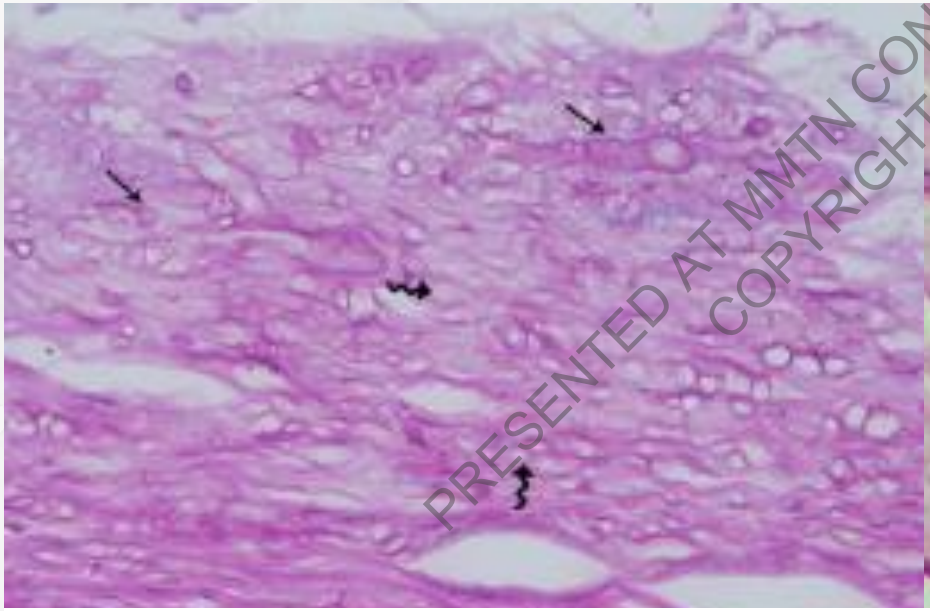


Anutarapongpan et al., Clin Sci, 2017

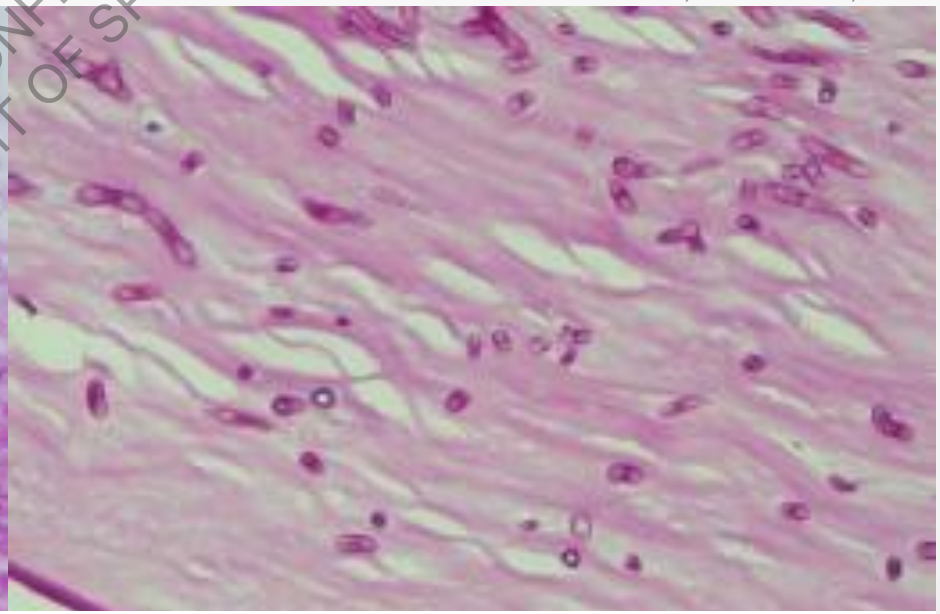
Symptoms & Diagnosis and Treatment

	Vascular form	Ocular form
Diagnosis	• Arterial occlusion by angiogram • *P. insidiosum* specific antibody (ID / ELISA / WB / Lateral flow / HA) • *P. insidiosum* isolation & zoospore production • Molecular approach for identification both culture and specimens • Histopathology	• Positive for *P. insidiosum* isolation & zoospore production • Molecular approach for identification both culture and specimens • Histopathology

Mittal et al., Basic Invest, 2017



PAS (5 mins); Pythium* spp. >> *1+ to 2+



PAS (5 mins); Other fungal infection >> 4+

Cellulose: a weaker reaction than chitin and pectin, so it needs a longer exposure time to PAS for complete oxidation to aldehydes, which then reacts with Schiff reagent to give a magenta color (purplish-red).

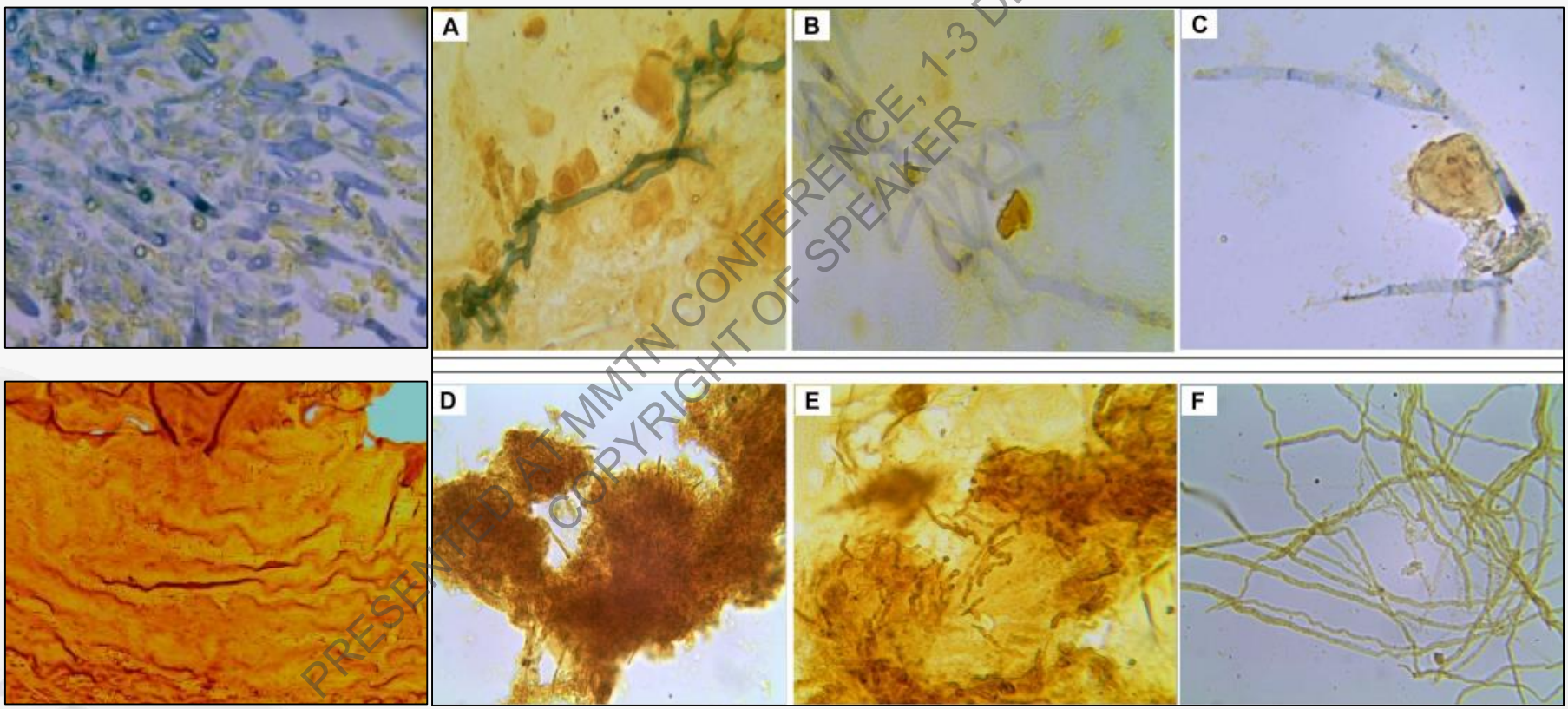
IKI-H_2SO_4 staining

I - - - - - - - Destained tissue of - - - - - - - I

Corneal tissue | **KOH** | **Gram stain** | **Pure isolate**

Pythium spp.

Other fungi



Mittal et al., Basic Invest, 2017

MMTN
MEDICAL MYCOLOGY TRAINING NETWORK

Symptoms & Diagnosis and Treatment

	Vascular form	Ocular form
Treatment	• AK amputation or debridement • Antifungal agent: Itraconazole + Terbinafine • Immunotherapy by PIA	• Corneal grafting or enucleation • Amphotericin B /terbinafine/Azoles • Immunotherapy by PIA

Permpalung et al., J Antimicrob Chemother, 2015

J Antimicrob Chemother. 2015;70(6):1885-92. doi: 10.1093/jac/dkv008. Epub 2015 Jan 27.

Treatment outcomes of surgery, antifungal therapy and immunotherapy in ocular and vascular human pythiosis: a retrospective study of 18 patients.

Permpalung N[1], Worasilchai N[2], Plongla R[3], Upala S[4], Sanguankeo A[4], Paitoonpong L[3], Mendoza L[5], Chindamporn A[6].

Retrospective study in 18 pythiosis cases in KCMH from 2003 to 2013

Vascular pythiosis; n=9

- 44% of vascular cases died
- Definitive surgery with adequate surgical margins

Ocular pythiosis; n=9

- 55% of ocular pythiosis underwent enucleation.
- Age might be one prognostic factor, significantly younger of non-enucleated cases than those who underwent enucleation.
- Higher non-enucleated cases (45%) was found in our center than others (12-21%), might have been due to the routine administration of PIA in our center.

RAPID and DEFINITE diagnosis + treatment !!
are significant for the patients survived (vascular) and saved globe (ocular).

Int J Low Extrem Wounds. 2015 Sep;14(3):245-50. doi: 10.1177/1534734615599652. Epub 2015 Aug 18.

Vascular Pythiosis of the Lower Extremity in Northern Thailand: Ten Years' Experience.

Reanpang T[1], Orrapin S[1], Orrapin S[1], Arworn S[1], Kattipatanapong T[2], Srisuwan T[2], Vanittanakom N[3], Lekawanvijit SP[4], Rerkasem K[5].

Retrospective study in 22 vascular cases : 10-years period (2004-2014) in Maharaj Nakorn Chiang Mai, Chiang Mai University Hospital.

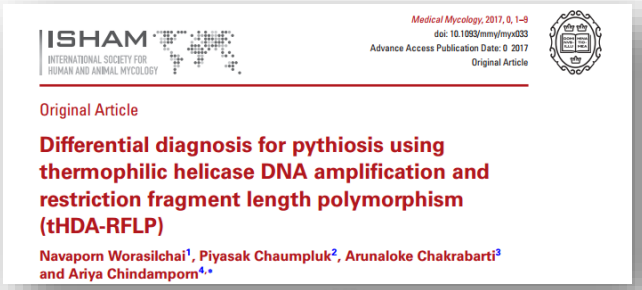
- Successful management of vascular pythiosis requires early recognition
- 4 classic clinical presentations need to be concerned:
  - (1) underlying thalassemia
  - (2) no atherosclerotic risk
  - (3) history of previous leg wound
  - (4) presentation with acute or chronic limb ischemia

Important to note that "serum antibody for *Pythium* should be tested in all suspected cases before treatment"

- Survival rate was around 63.6%.
- The only effective treatment was complete excision of the infected tissue, which was done mainly by major amputation.
- This report raises awareness of this disease, which needs preemptive diagnosis and appropriate treatment.

One more novel technology can help for pythiosis diagnosis

tHDA-RFLP



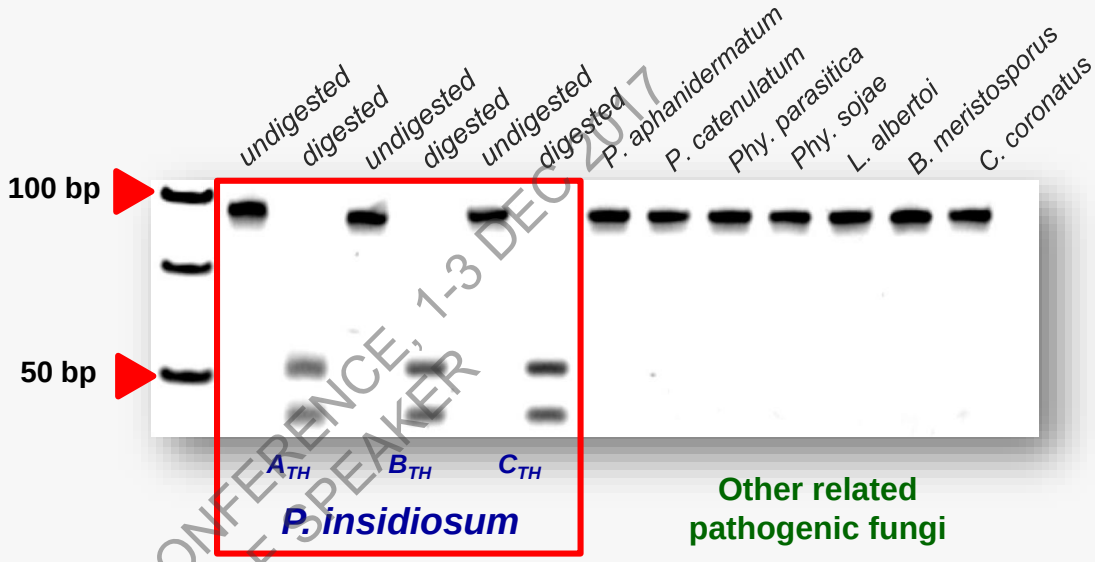
ISHAM
INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2017, 0, 1–9
doi: 10.1093/mmy/myx033
Advance Access Publication Date: 0 2017
Original Article

Original Article

Differential diagnosis for pythiosis using thermophilic helicase DNA amplification and restriction fragment length polymorphism (tHDA-RFLP)

Navaporn Worasilchai[1], Piyasak Chaumpluk[2], Arunaloke Chakrabarti[3] and Ariya Chindamporn[4,*]



- Thermophillic helicase DNA Amplification (tHDA) using *P. insidiosum* specific primer
  - Isothermal DNA amplification, no need PCR machine
  - Rapid & accurate, species-specific identification
- Can differentiate *P. insidiosum* from closely related pathogenic fungi by CviKI-1 digestion.
- Limit of Detection :
  - 100 pg (1.74×10^2 copies) for 1-step protocol
  - 100 fg (1.74×10^{-1} copies) for 2-step protocol – add denature step
- Directly amplification in clinical samples was also evaluated.

MMTN
MEDICAL MYCOLOGY TRAINING NETWORK

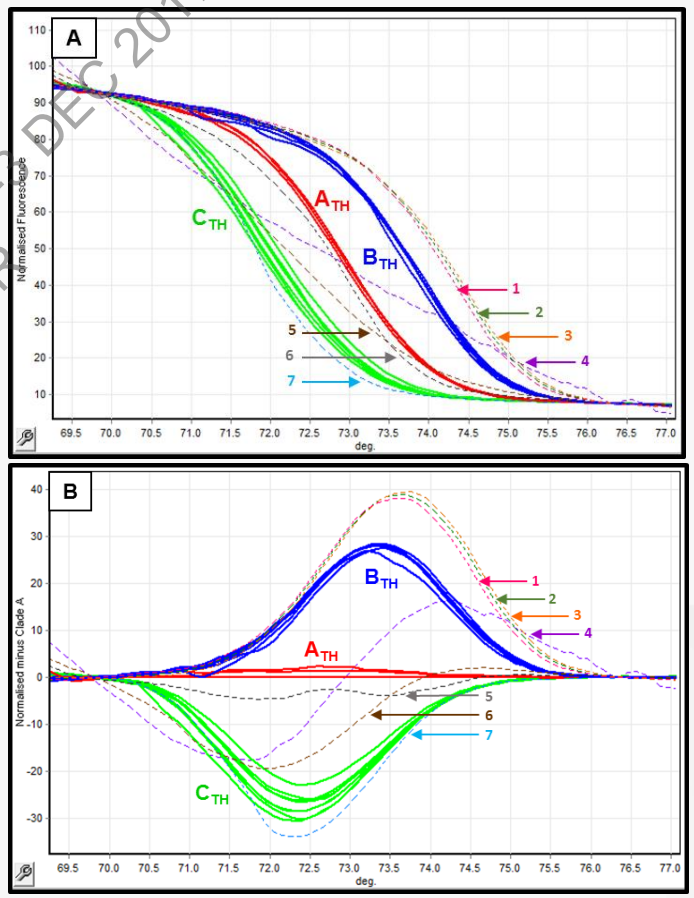
Worasilchai et al., Med Mycol, 2017

One more novel technology….for clade pythiosis diagnosis

High Resolution Melting Analysis: A Novel Approach for Clade Differentiation in *Pythium insidiosum* and Pythiosis.

Navaporn Worasilchai, Nitipong Permpalung, Ariya Chindamporn

HRM



- Real-time polymerase chain reaction (qPCR) with subsequent High Resolution Melting (HRM) using *P. insidiosum* specific primer
  - No need sequencing step
  - Rapid & accurate, clade-specific identification
- Limit of Detection :
  - 100% specificity
  - 1 pg limit of detection

Worasilchai et al., Med Mycol, 2017

Normalized graph (A) and difference graph (B): comparison among Clade A_{TH}, Clade B_{TH}, and C_{TH} *P. insidiosum* (—) and other related fungi (---) which were amplified *COX2* gene: *L. albertoi* (1); *P. aphanidermatum* (2); *P. catenulatum* (3); *C. coronatus* (4); *Phy. sojae* (5); *B. meristosporus* (6); *Phy. parasitica* (7)

…Take home messege…

Mucormycosis

- Underlying dis. in developed country : DM; developing country: HM
- Seems like a clear sky of treatment in future: 2nd triazole
- From translational research of pathogenesis : CotH member -

Guideline for pythiosis diagnosis (Thai patients, experience)

Vascular pythiosis

- Underlying hemoglobinopathy ie. thalassemia, PNH etc.
- Agricultural related occupations ie. farmer or history of water exposure
- Present acute or chronic ischemia with rapid progression
- No atherosclerotic risk & no response to any antifungal agents

Ocular pythiosis

- History of water spilled to the eye
- Present ulcerative keratitis / endophthalmitis with rapid progression
- No response to any antifungal agents

RAPID and DEFINITE diagnosis + treatment !!
are significant for the patients survived (vascular) and saved globe (ocular).

Thank you

ขอบคุณค่ะ