

Current classification of the clinically relevant *Aspergillus* species complexes

Cornelia Lass-Flörl

Division of Hygiene und Medical Microbiology
Innsbruck Medical University

6th Advances Against Aspergillosis

Pre-conference workshop “*Aspergillus* Speciation in the 21st Century – Implications for Laboratory and Clinical Practice”

Madrid/Spain 27 February – 1 March 2014

Current classification of the clinically relevant *Aspergillus* species complexes

Cornelia Lass-Flörl

Division of Hygiene und Medical Microbiology
Innsbruck Medical University

Ana Alastruey-Izquierdo

Mycology Reference Lab
National Centre for Microbiology, ISCIII
Madrid, Spain

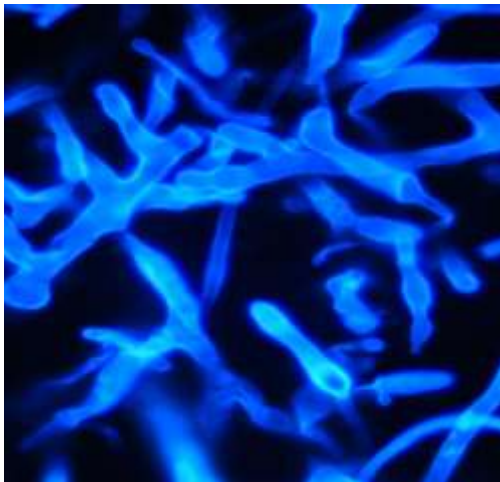
6th Advances Against Aspergillosis

Pre-conference workshop “*Aspergillus* Speciation in the 21st Century – Implications for Laboratory and Clinical Practice”

Madrid/Spain 27 February – 1 March 2014

2008-2012

1. Spectrum of opportunistic fungal pathogens is increasing
2. Aspergillus-PCR is not that helpful anymore
3. Decrease in aspergillosis, replacement by other fungal pathogens in specific patient population



Aspergillosis story began with ...

Johann Heinrich Friedrich Link (1767-1851)

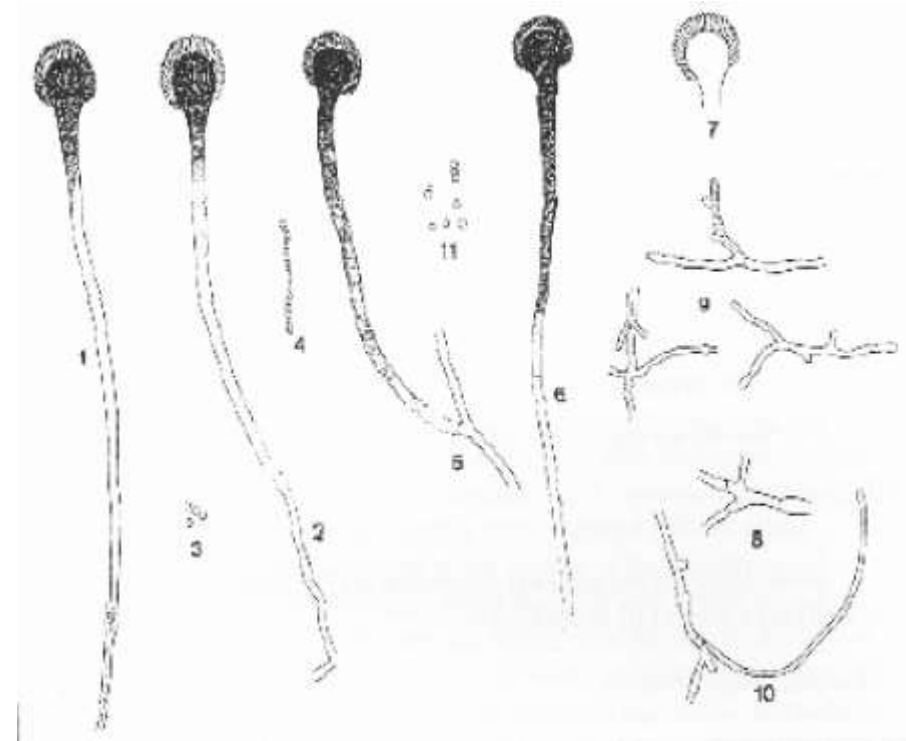
Description of *Aspergillus* species:
Aspergillus candidus, *Aspergillus flavus* and others in 1809

(Gesellschaft Naturforschender Freunde zu Berlin (1809) Magazin 3: 16).

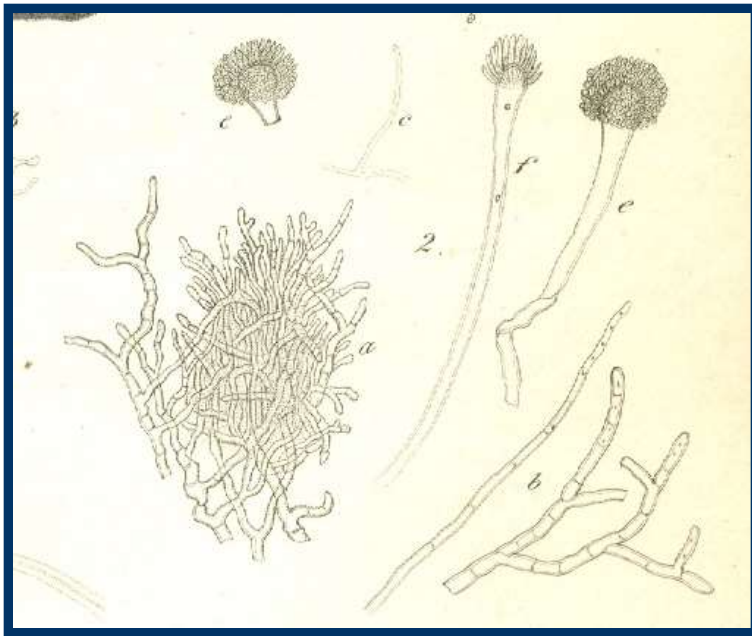
J.B. Georg W. Fresenius (1808 -1866)

First description of *Aspergillus fumigatus*

(Beiträge zur Mykologie (1863), pp. 81-82).

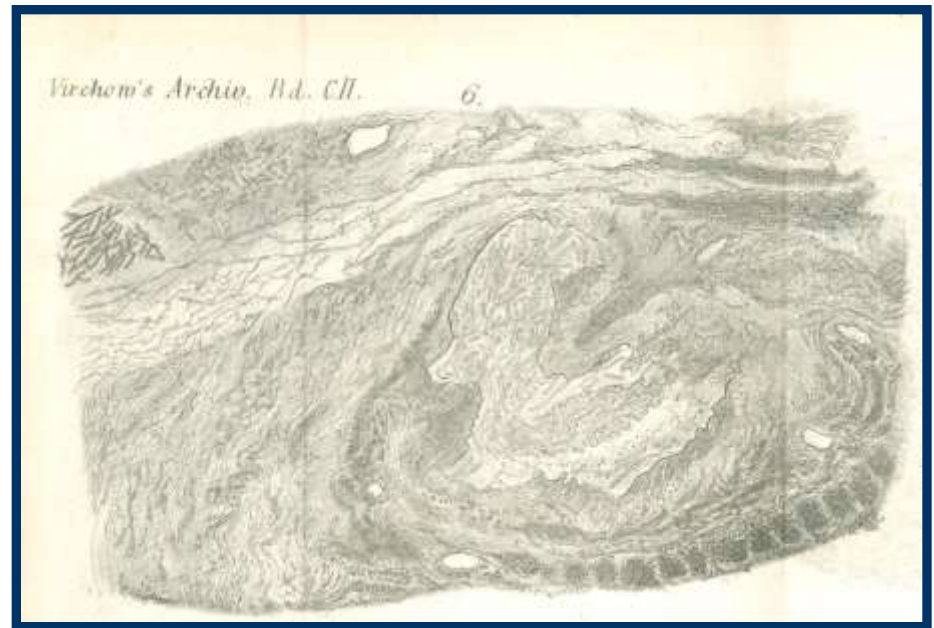


Tissue Culture



Virchow R.
Archiv f. pathol. Anat. 1856; 9 (4): 557-593.

Histopathology



Paltauf A.
Archiv f. pathol. Anat. 1885; 102 (25): 543-564.

Case of pulmonary aspergillosis (Arch. Path. Anat. (1856), 9: 557-593)

Children's Memorial Hospital
Chicago

Archiv für pathologische Anatomie und Physiologie

und für
klinische Medizin.

Herausgegeben
von
R. Virchow.

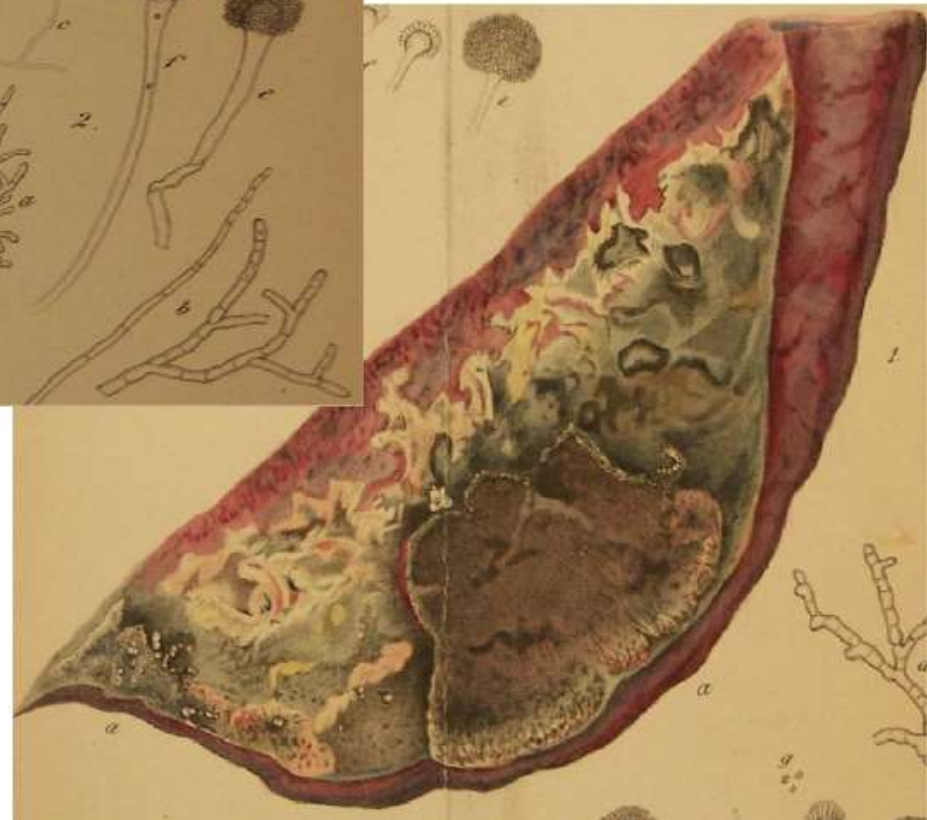
LIBRARY

Neunter Band.

Mit 4 Kupfertafeln.

27066 10 Dec 1951

Berlin,
Druck und Verlag von Georg Reimer.
1856.







Luftröhrenbefall mit Schimmelpilz „Aspergillus fumigatus“



S.E., 58 year old man

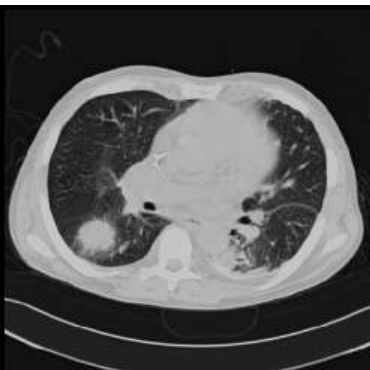
Osteomyelofibrosis, ED 1998 → Litalir

Developed chemotherapy-refractory acute leukemia 2002

>> Bone Marrow Transplantation

CT prä BMT

6-JAN-2003
11:24:36
UNIV.KL.INNSBRUCK RA
Scan: 30



Liste

^ Variable Anzeige

v Film-Modus

^ Band-Modus

Layout davor danach

Messungen...

Rekonstruktion...

Roi

Fensterung Originalbild

Zoomlupe Sequenz

Rollzoom Für alle

Studien-Optionen...

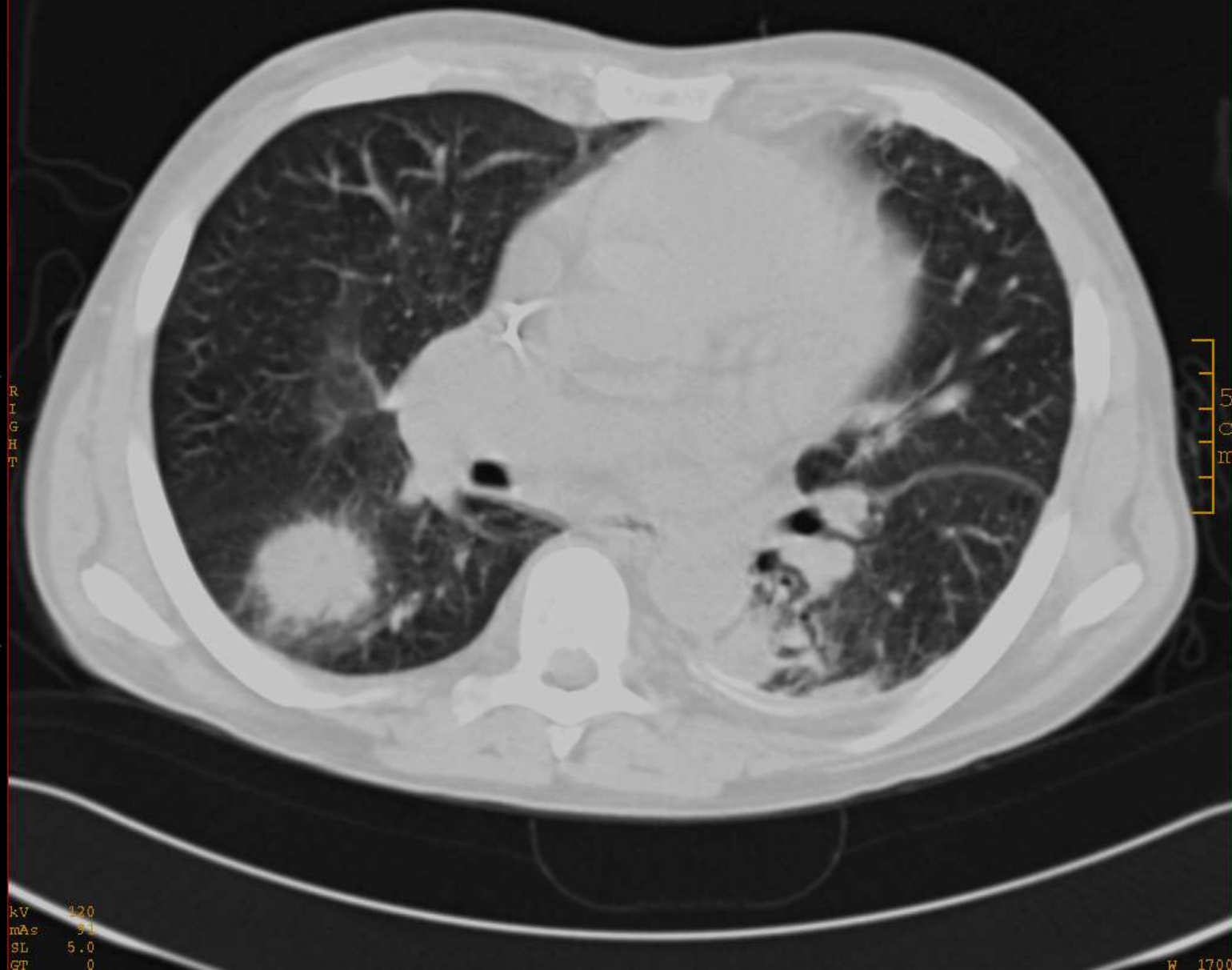
Bildbearbeitung...

Visualisierung...

System...



Programmwechsel



RIGHT

5 cm

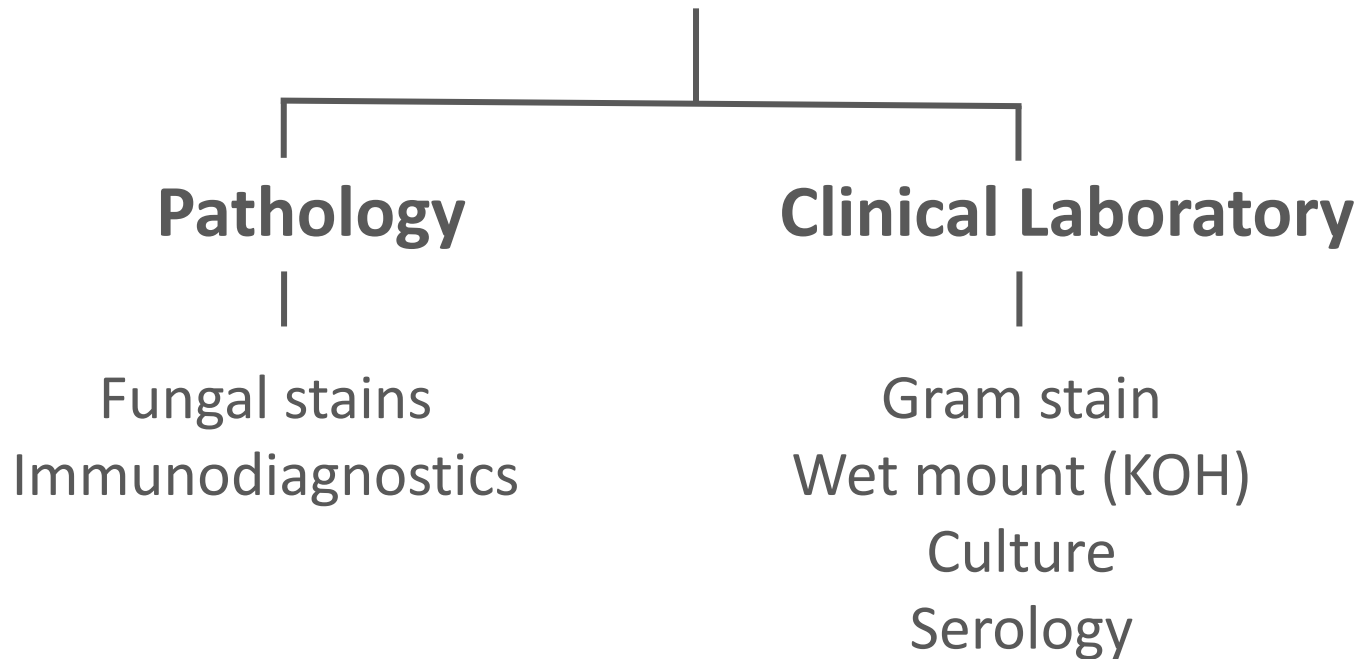
kV 120
mAs 9.1
SL 5.0
GT 0

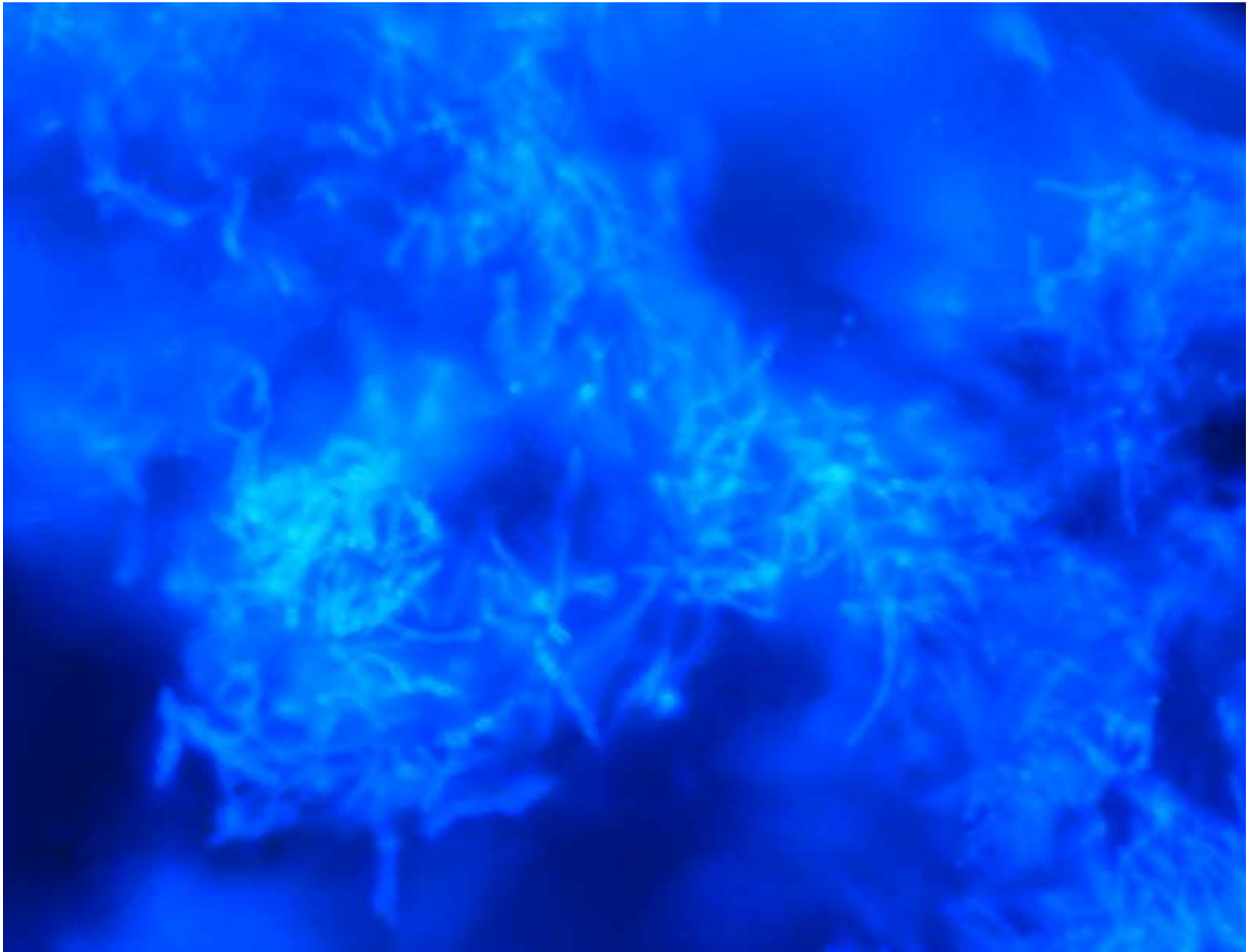
W 170

What to do, when and how – the major principles!

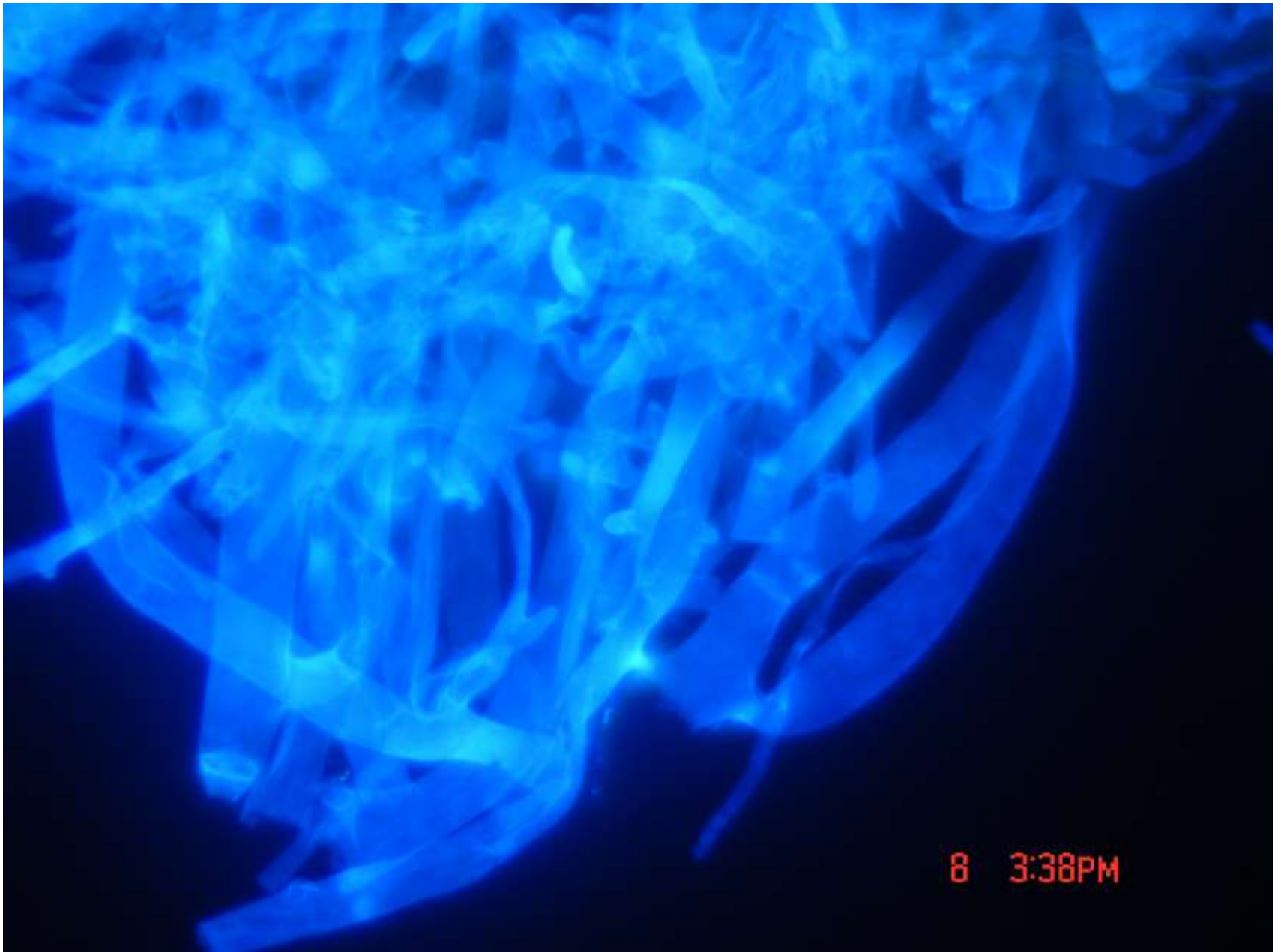


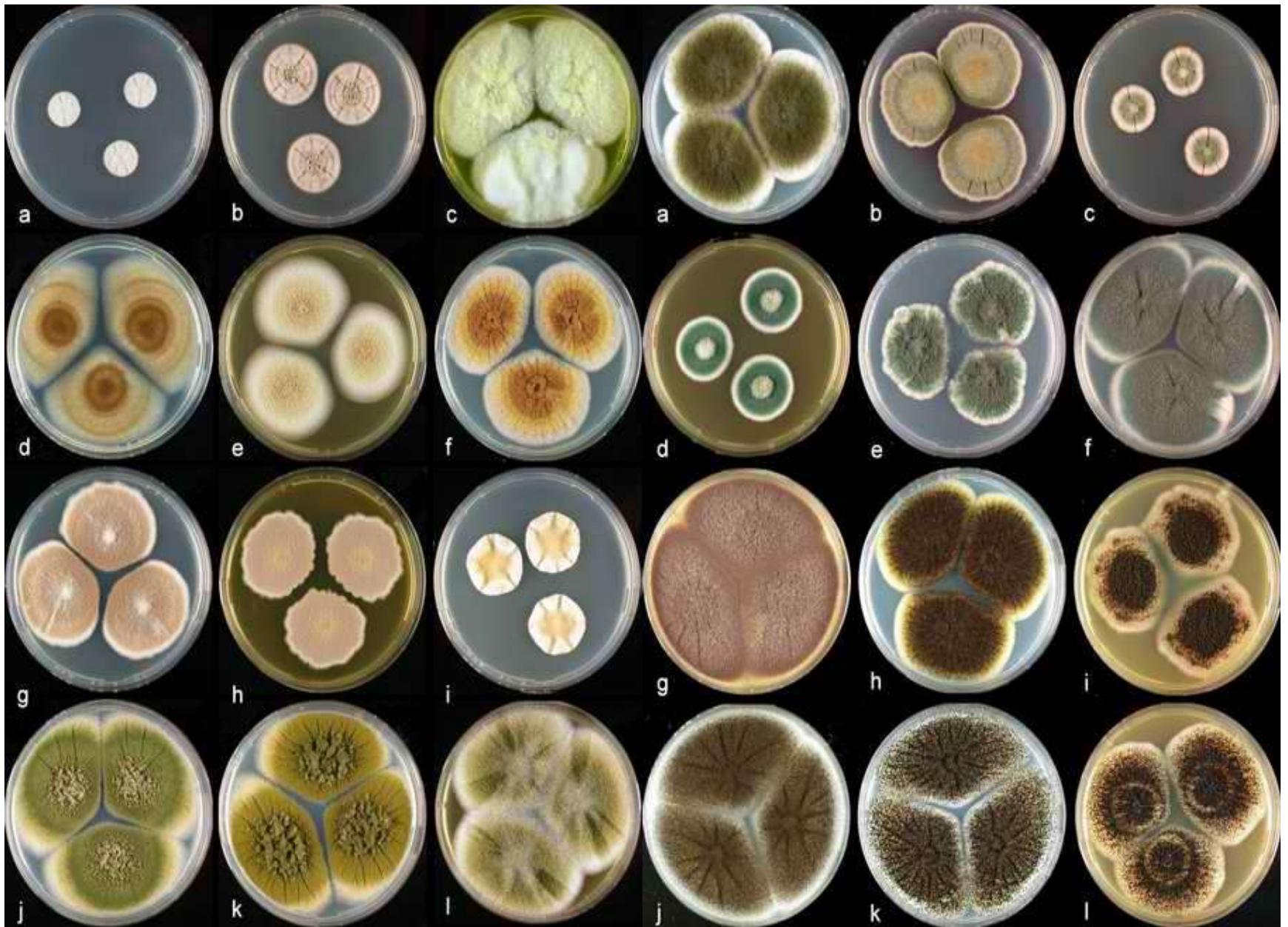
Diagnostic specimen for fungus











Klich MA. Identification of common *Aspergillus* species (2002). CBS.

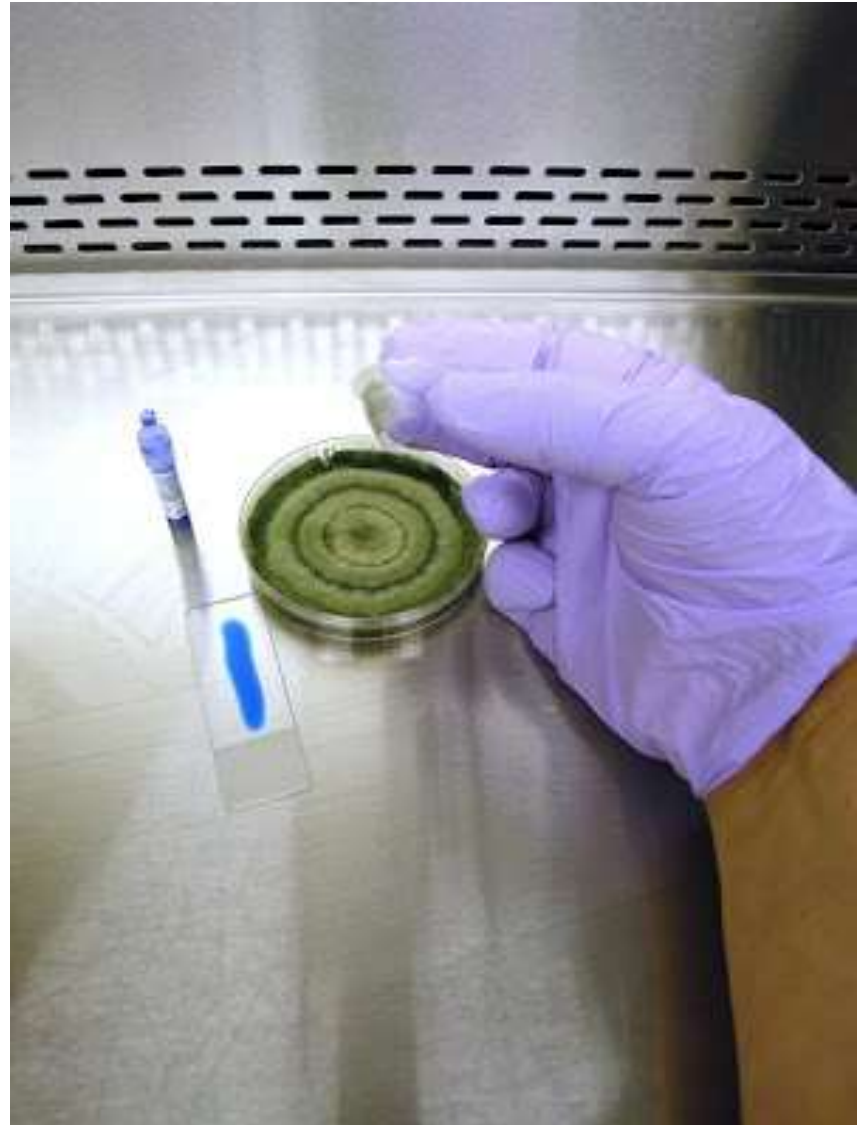
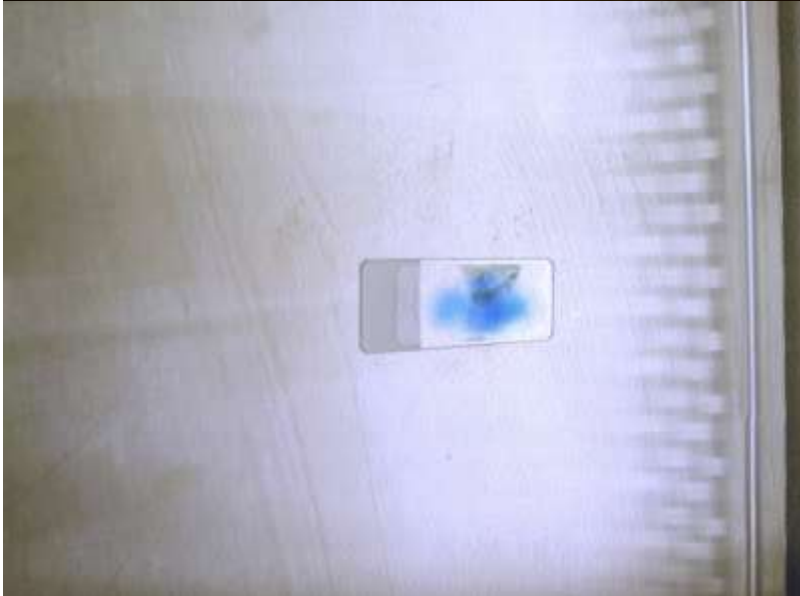
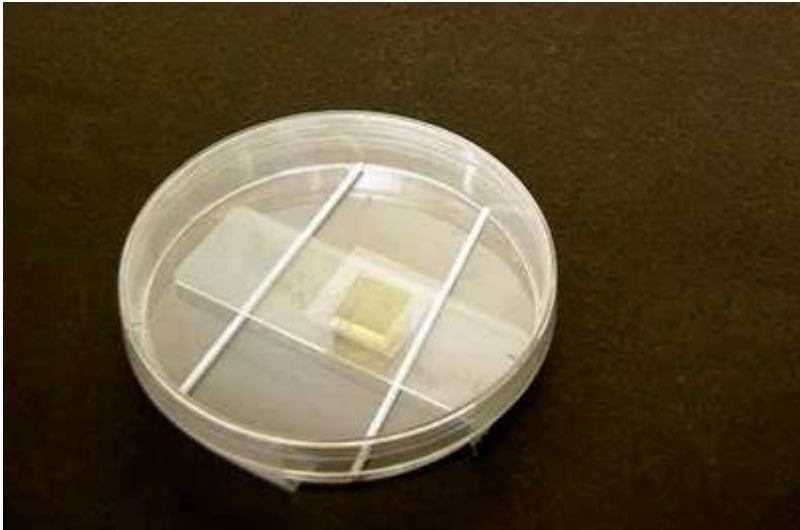
Tissue biopsies

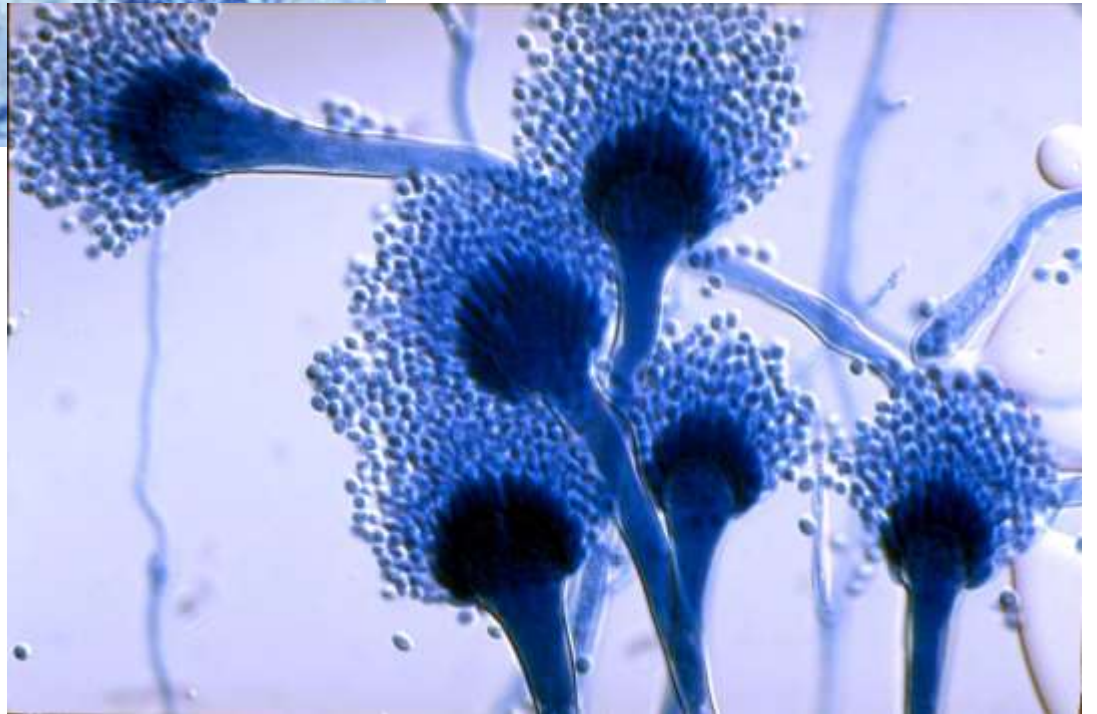
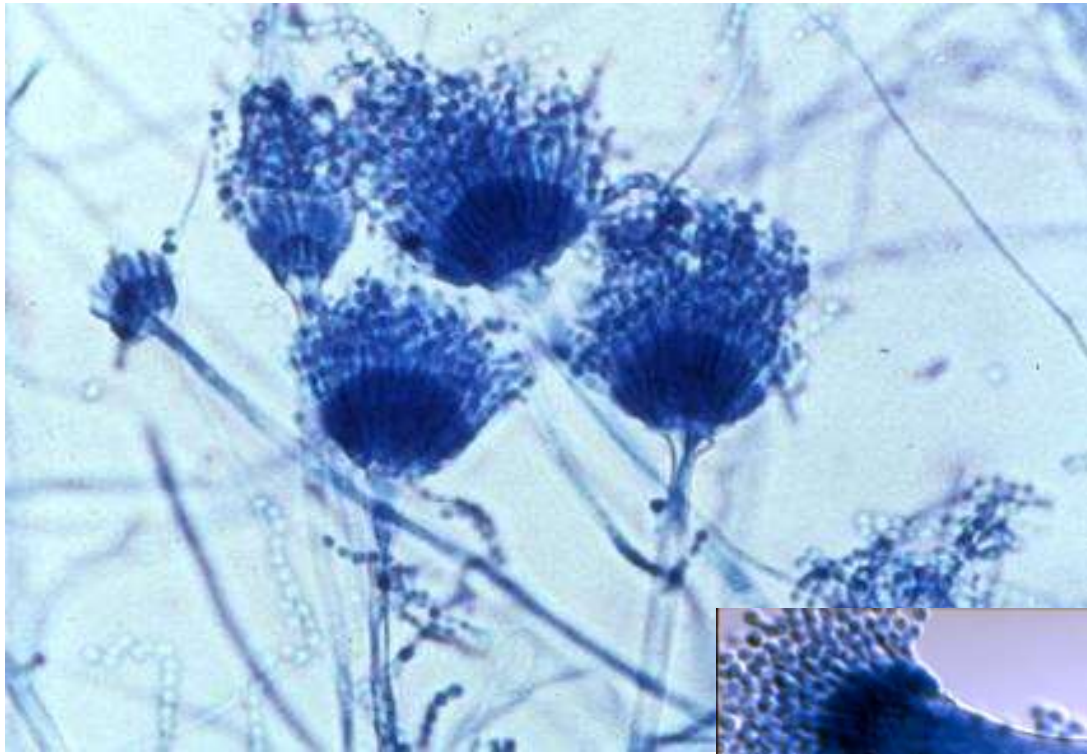


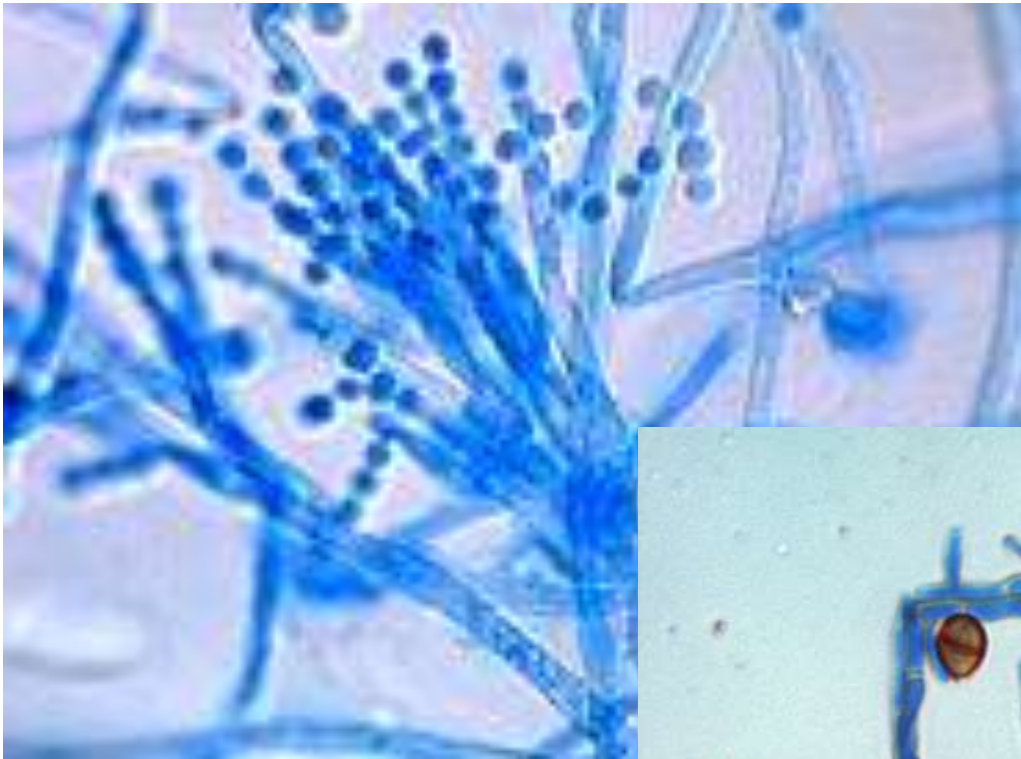
„Proof“ of IFI

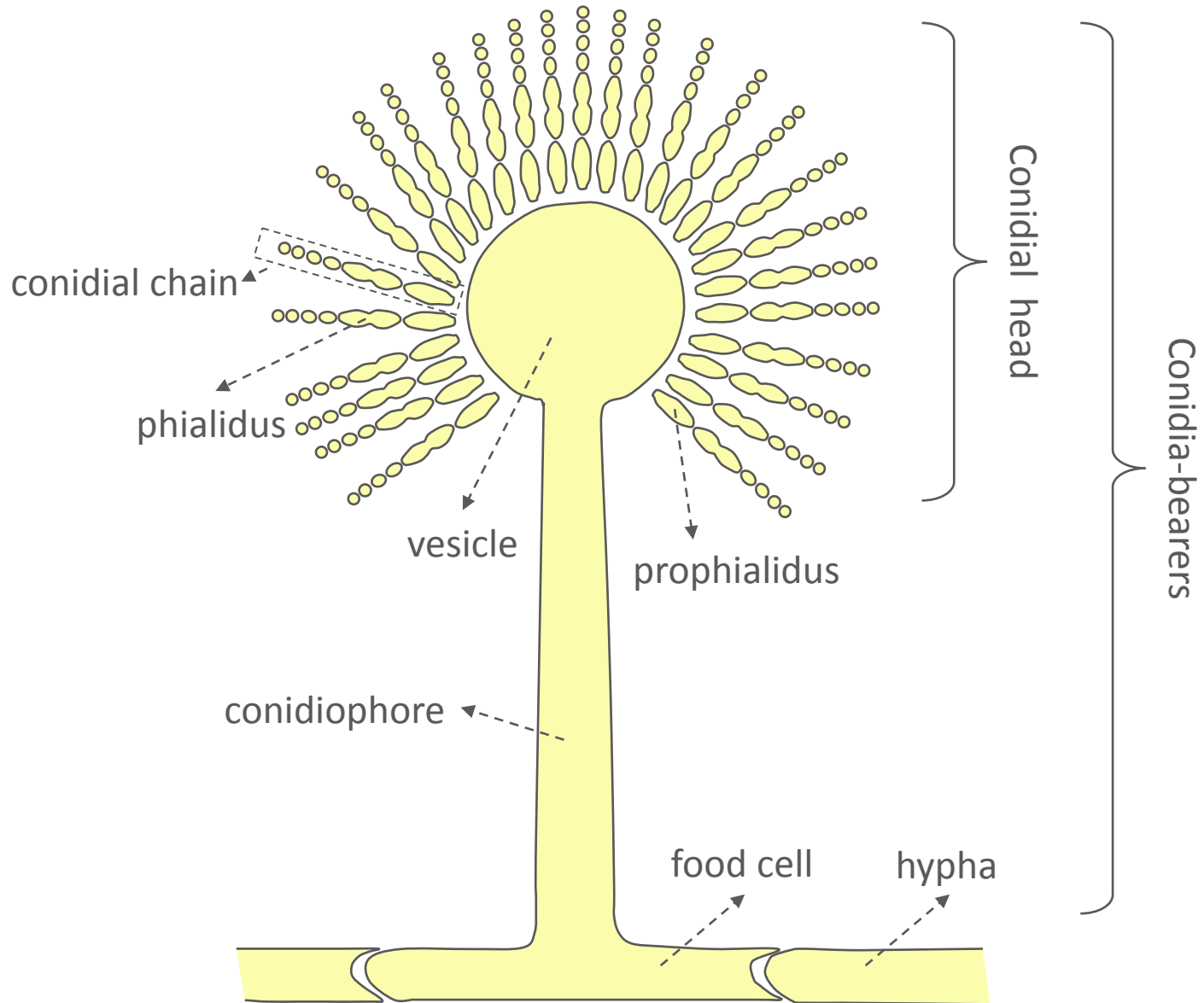


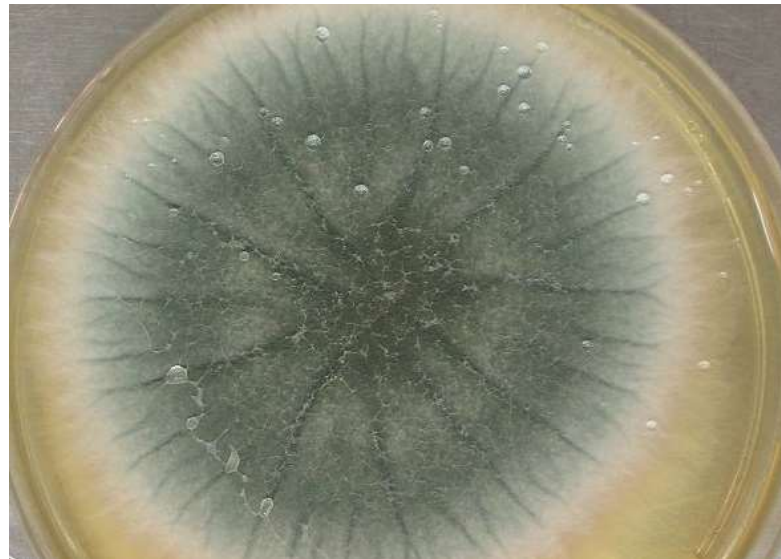
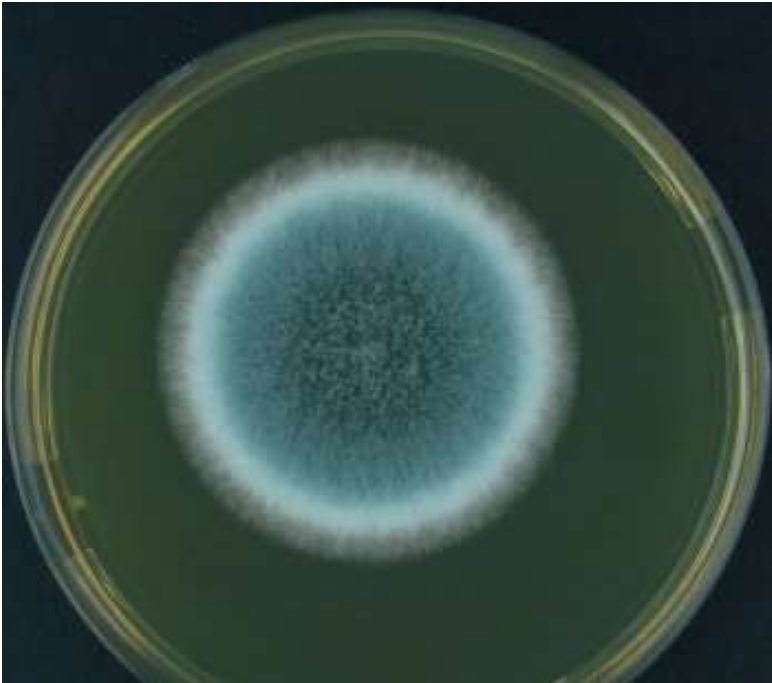
Slide Culture or Adhesive Tape Preparation











Aspergillus species

Uniseriate

Aspergillus fumigatus
Aspergillus glaucus
Aspergillus clavatus
Others

Blue/green Atypical form Turns white <i>A. fumigatus</i>	Yellow/green/ brown Ascocarp usually present <i>A. glaucus</i>
Bluish/green Smooth, hyaline & dense Felt of conidiophores Clavate vesicle <i>A. clavatus</i>	

Biseriate

Aspergillus niger
Aspergillus terreus
Aspergillus nidulans
Aspergillus versicolor
Aspergillus ustus
Others

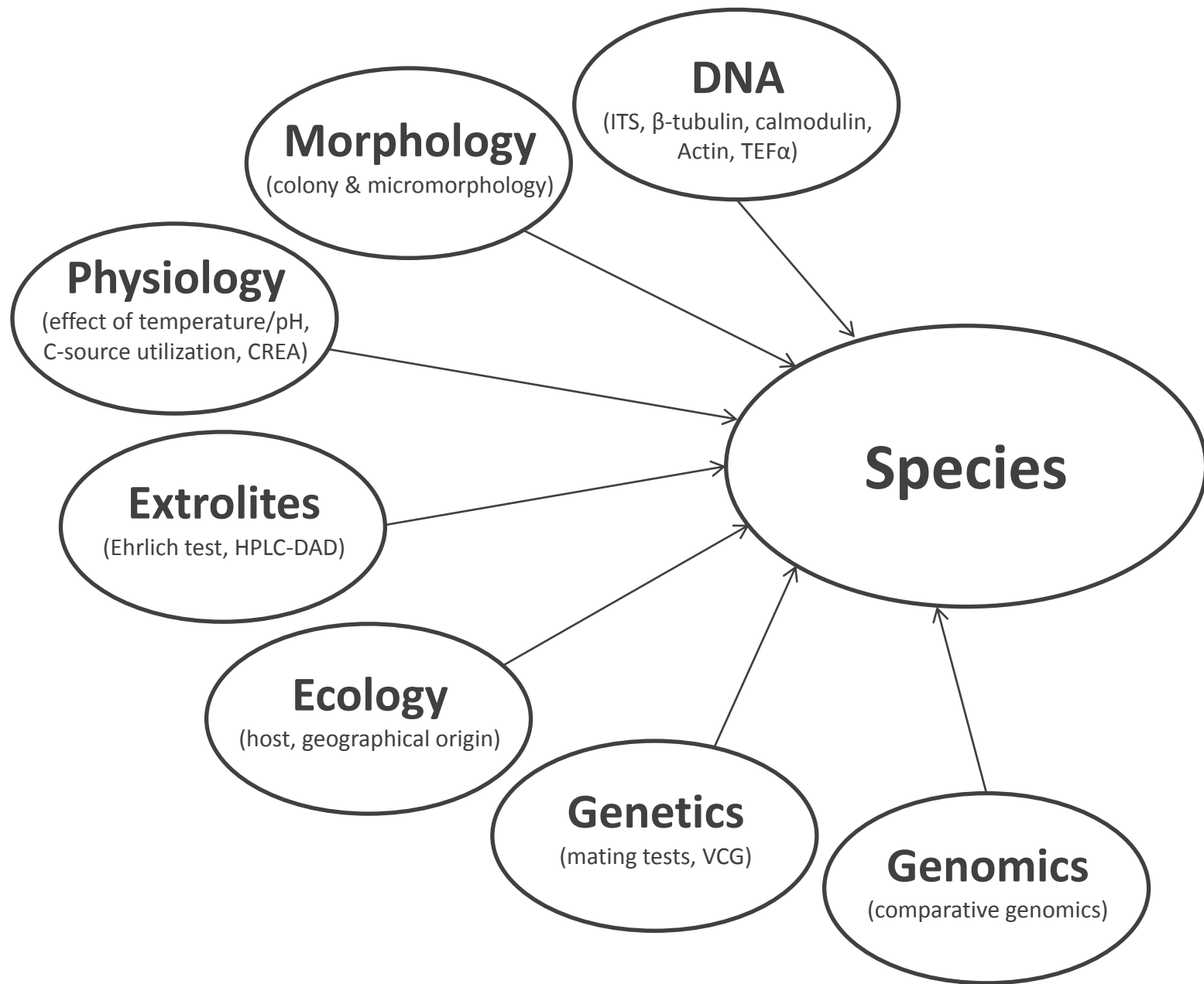
Jet black (yellow/ white) Conidiophore Brown & smooth <i>A. niger</i>	Olive to dull brown Brown shaded smooth conidiophore Irregular hulle cells <i>A. ustus</i>	Cinnamon Aleurioconidia Foot cell <i>A. terreus</i>
Green/yellow/orange/deep purple Hulle cells, Ascocarp Smooth wall & brown conidiophore <i>A. nidulans</i>		
Green orange, white/yellow Penicillium-like structure Smooth wall & hyaline conidiophore Hulle cells may be seen <i>A. versicolor</i>		

Uniseriate & Biseriate

Aspergillus flavus
Aspergillus ochraceus
Aspergillus oryzae
Others

Yellow/green or Green/yellow Rough walled Hyaline conidiophore <i>A. flavus</i>	Yellow/orange Ochraceous or buff Brownish conidiophore Lightly rough walled <i>A. ochraceus</i>
Pale greenish yellow, olive-yellow or different greenish and dull brown shades. Subspherical vesicle. Radiate hyaline conidiophore <i>A. oryzae</i>	

If a clinical laboratory identifies an *Aspergillus* isolate using conventional criteria, without molecular or extrolite data, then the term “complex” is best added after the species name to accomodate for the lack of exclusion of cryptic species, as in “*A. niger* complex”.



Scheme showing the various data sets which can be combined for a polyphasic taxonomic classification of an *Aspergillus* taxon.

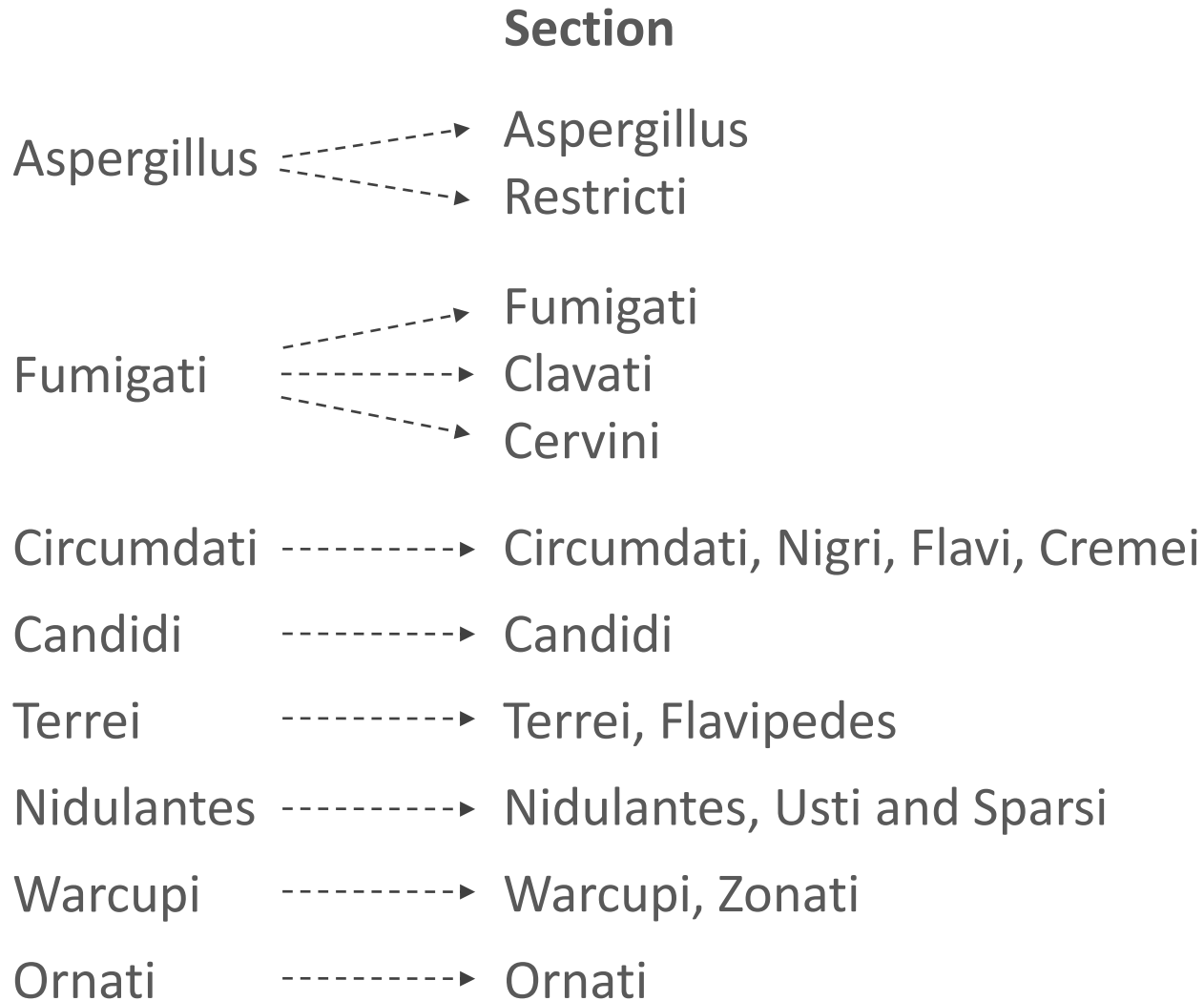
If further identification procedures are conducted to determine the species then that full name should be used as in *A. niger* or *A. awamori*. This might include Maldi-TOF or other proteomic identification, once the specificity of the technique for that group of species has been fully assessed.

The term “section” is best used in publications and by taxonomists when describing a group of closely related species, which have been fully identified at species level.

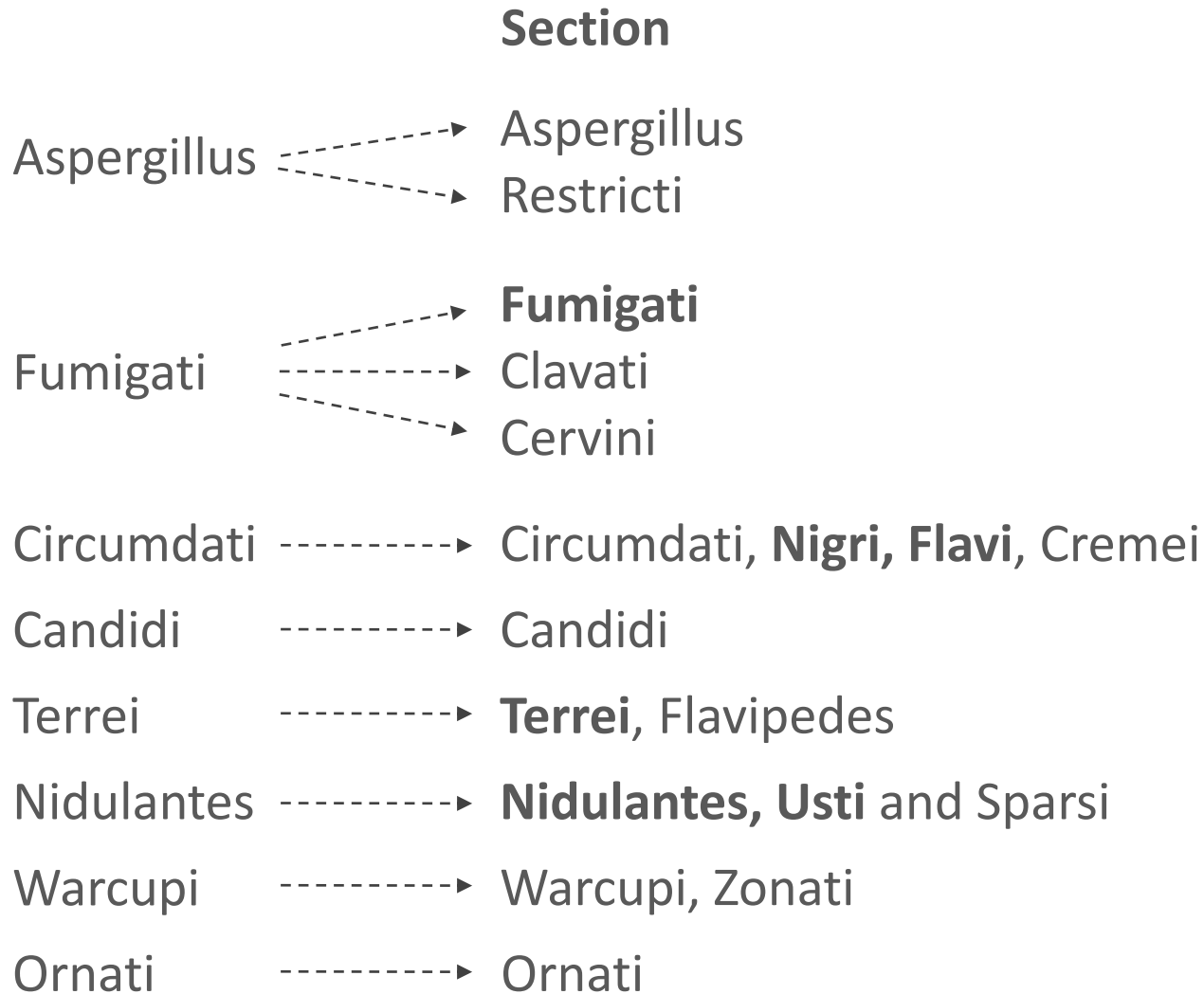
Aspergillus spp. are the most frequent molds isolated from clinical samples. The genus Aspergillus contains about 250 species divided into eight subgenera, subdivided into several sections or species complexes.

The most frequent cause of aspergillosis is *A. fumigatus*, followed by *A. flavus*, *A. terreus* and *A. niger*.

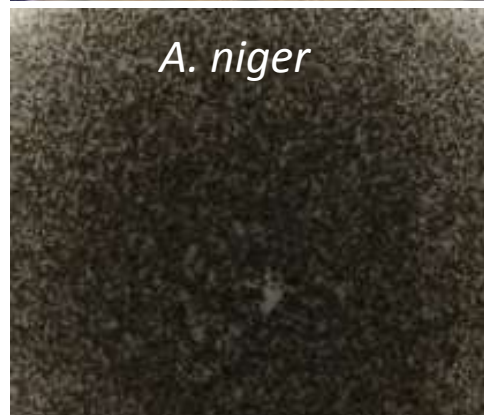
Aspergillus – 8 Subgenera (Genus)



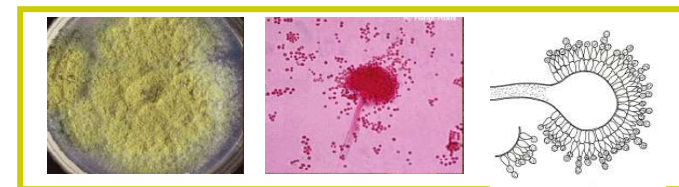
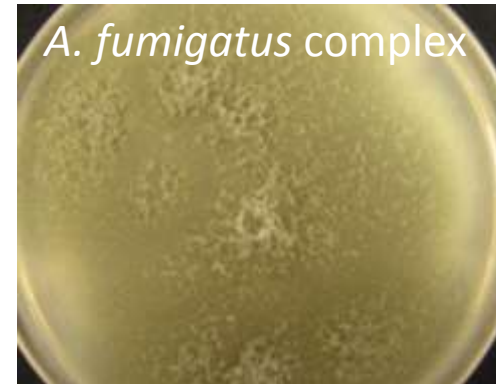
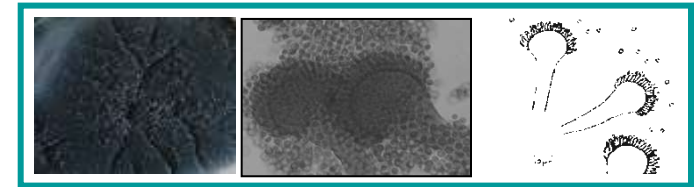
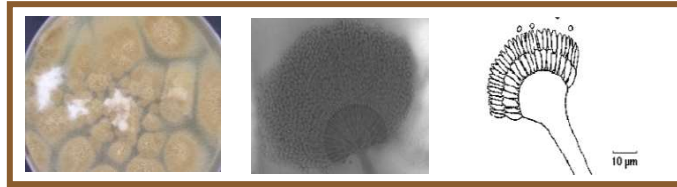
Aspergillus – 8 Subgenera (Genus)



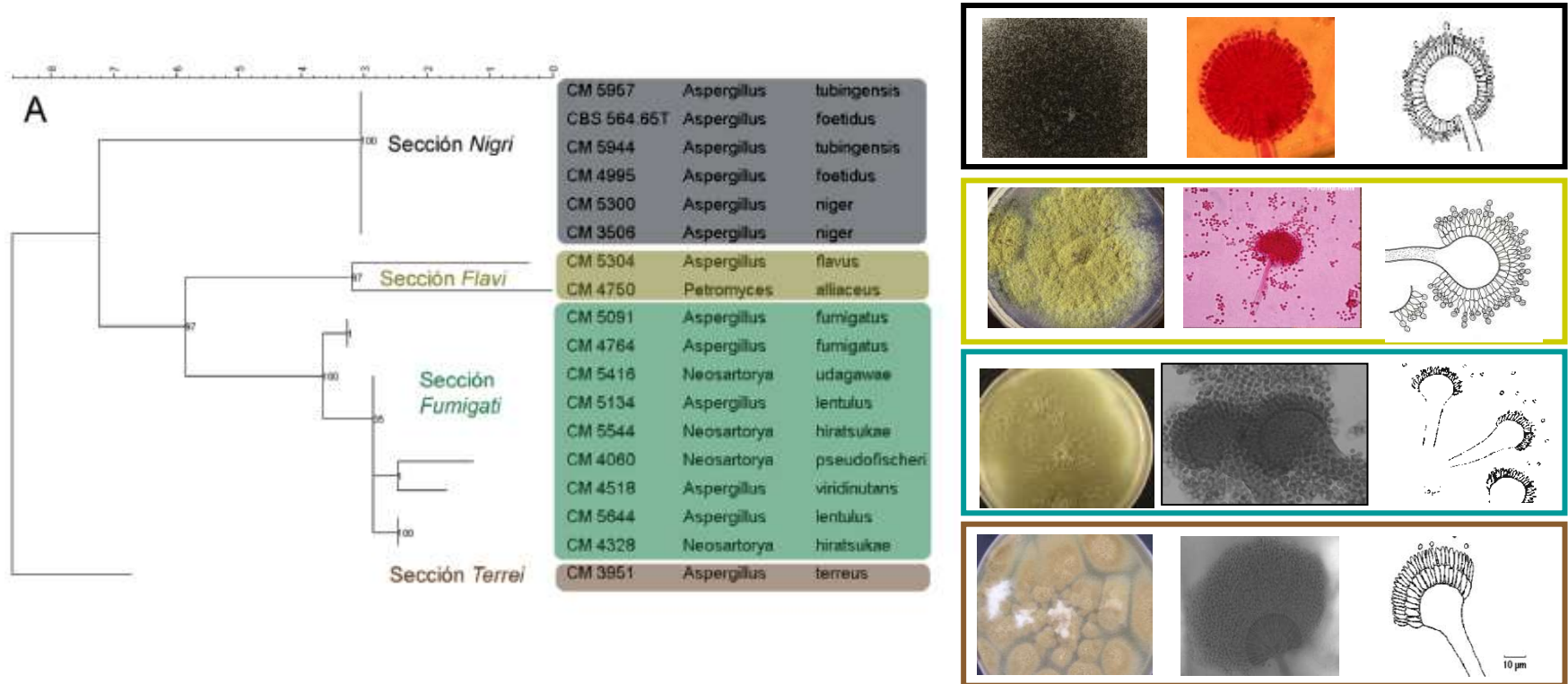
Clinically relevant *Aspergillus*



Clinically relevant *Aspergillus*



Clinically relevant *Aspergillus*



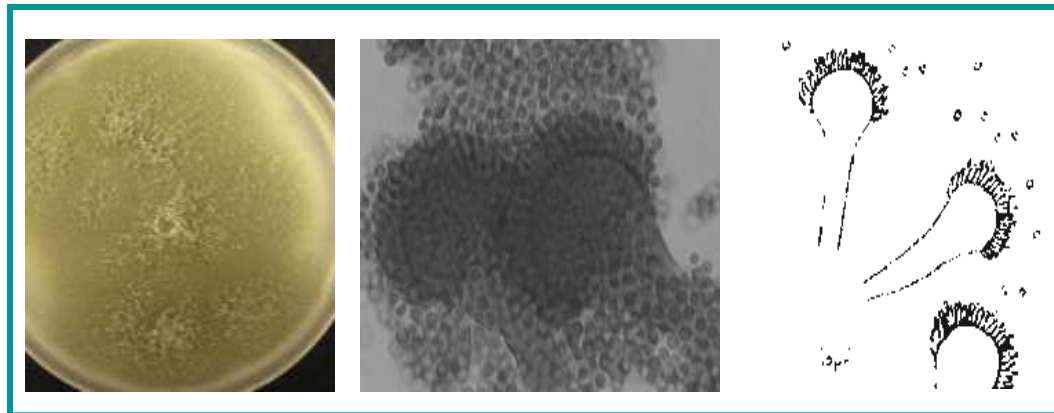
Alastruey-Izquierdo et. al 2010

Aspergillus species distribution according to epidemiological surveys from Spain and the U.S.

Species	Section	Transnet		Spanish study	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1
<i>A. udagawae</i>	Fumigati	3	1.4	0	0.0
<i>A. pseudofischeri</i>	Fumigati	1	0.5	1	0.4
<i>A. viridinutans</i>	Fumigati	0	0.0	1	0.4
<i>A. fumigatiafinis</i>	Fumigati	0	0.0	1	0.4
<i>A. flavus</i>	Flavi	29	13.3	27	9.7
<i>A. alliaceus</i>	Flavi	0	0.0	3	1.1
<i>A. terreus</i>	Terrei	11	5.0	26	9.4
<i>A. carneus</i>	Terrei	0	0.0	1	0.4
<i>A. tubingensis</i>	Nigri	6	2.8	22	7.9
<i>A. niger</i>	Nigri	13	6.0	21	7.6
<i>A. calidoustus</i>	Usti	6	2.8	4	1.4
<i>A. insuetus</i>	Usti	0	0.0	1	0.4
<i>A. keveii</i>	Usti	0	0.0	1	0.4
<i>A. sydowii</i>	Versicolores	2	0.9	1	0.4
<i>A. versicolor</i>	Versicolores	3	1.4	0	0.0
<i>E. quadrilineata</i>	Nidulantes	1	0.5	0	0.0
<i>A. nidulans</i>	Nidulantes	0	0.0	8	2.9
<i>A. westerdijkiae</i>	Circumdati	0	0.0	1	0.4
Total		218	100	278	100

Aspergillus species distribution according to epidemiological surveys from Spain and the U.S.

Species	Section	Transnet		Spanish study	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1
<i>A. udagawae</i>	Fumigati	3	1.4	0	0.0
<i>A. pseudofischeri</i>	Fumigati	1	0.5	1	0.4
<i>A. viridinutans</i>	Fumigati	0	0.0	1	0.4
<i>A. fumigatiafinis</i>	Fumigati	0	0.0	1	0.4
<i>A. flavus</i>	Flavi	29	13.3	27	9.7



Unusual or atypical isolates

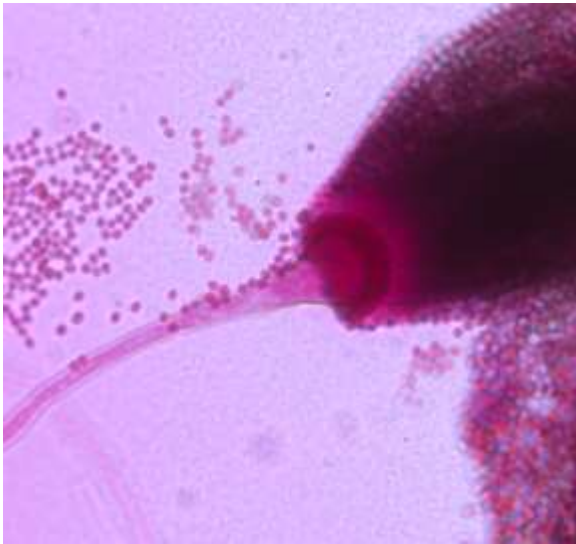


Poorly sporulating
A. fumigatus



Neosartorya fischeri with
masses of cleistothecia

Fumigati



EUKARYOTIC CELL, Mar. 2005, p. 625–632
1535-9778/05/\$08.00+0 doi:10.1128/EC.4.3.625–632.2005
Copyright © 2005, American Society for Microbiology. All Rights Reserved.

Vol. 4, No. 3

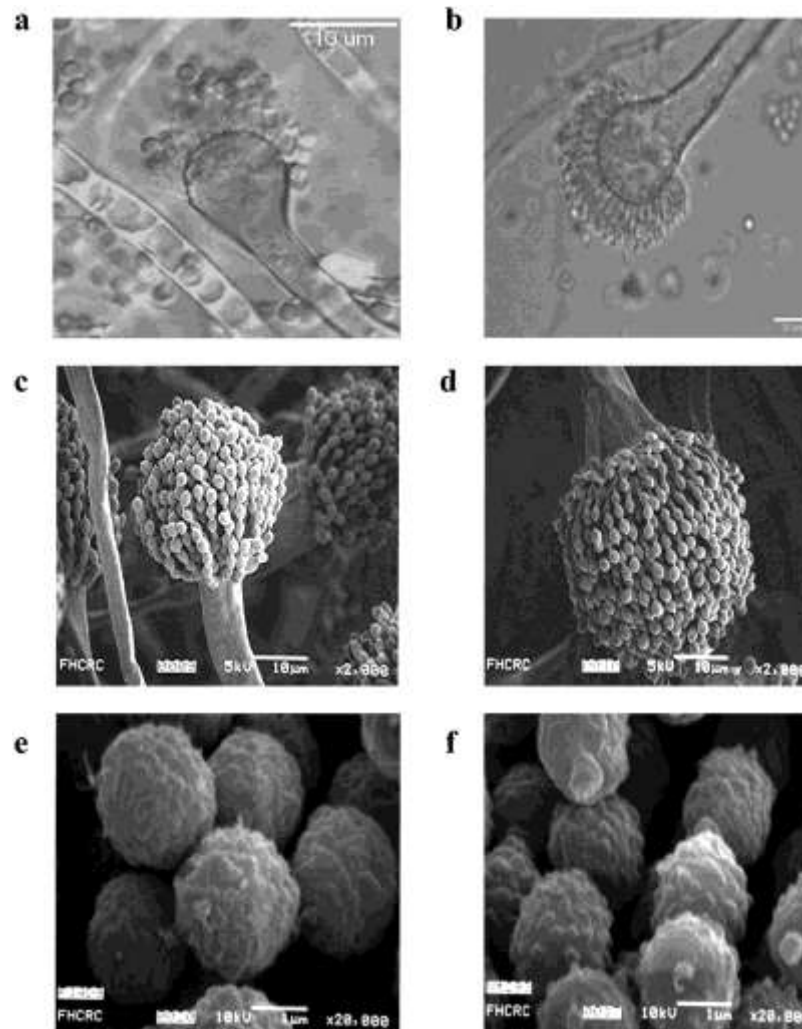
Aspergillus lentulus sp. nov., a New Sibling Species of *A. fumigatus*

S. Arunmozhi Balajee,¹ Jennifer L. Gribskov,¹ Edward Hanley,¹ David Nickle,²
and Kieren A. Marr^{1,3*}

*Program in Infectious Diseases, Fred Hutchinson Cancer Research Center,¹ and Department of Microbiology²
and Department of Medicine,³ University of Washington, Seattle, Washington*



DIC microscopy images of conidial heads of variant FH5 (a) and *A. fumigatus* Af293 (b) and SEM images of variant FH5 (c) and *A. fumigatus* Af293 (d).
e and f show higher magnification SEM images of conidia from variant FH5 and *A. fumigatus* Af293, respectively.

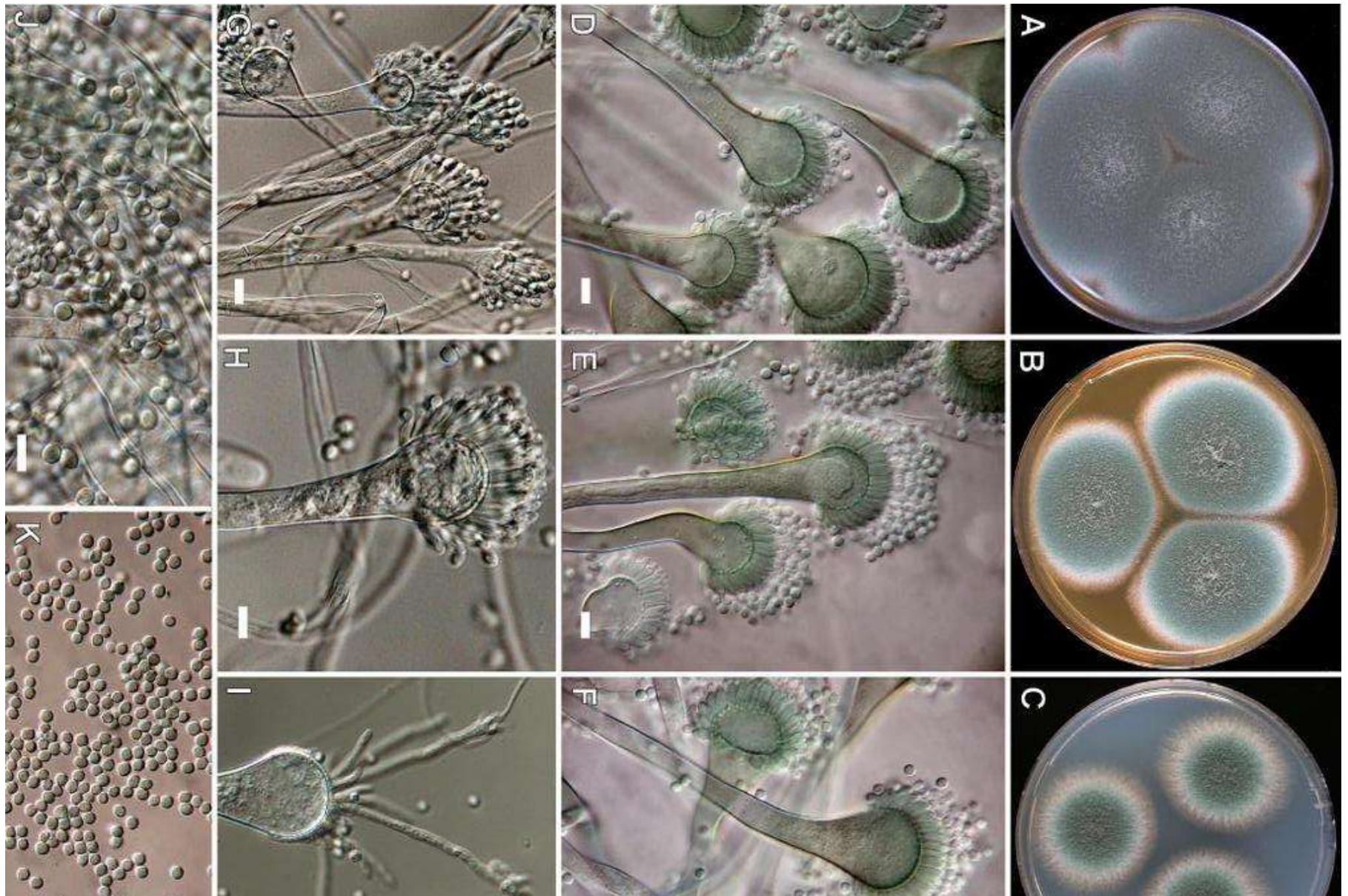


Balajee S A et al. Eukaryotic Cell 2005;4:625-632

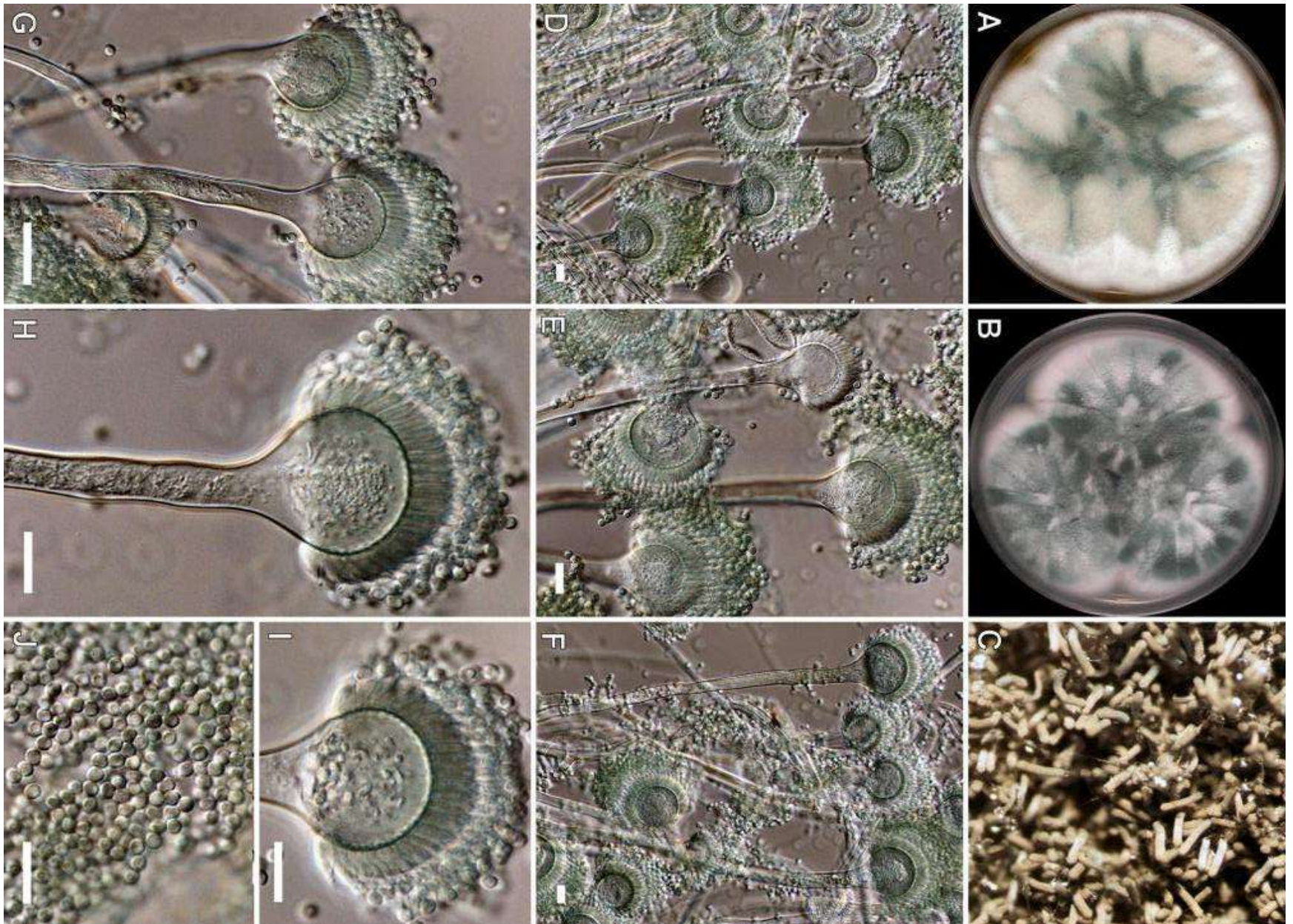
Eukaryotic Cell

Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya*

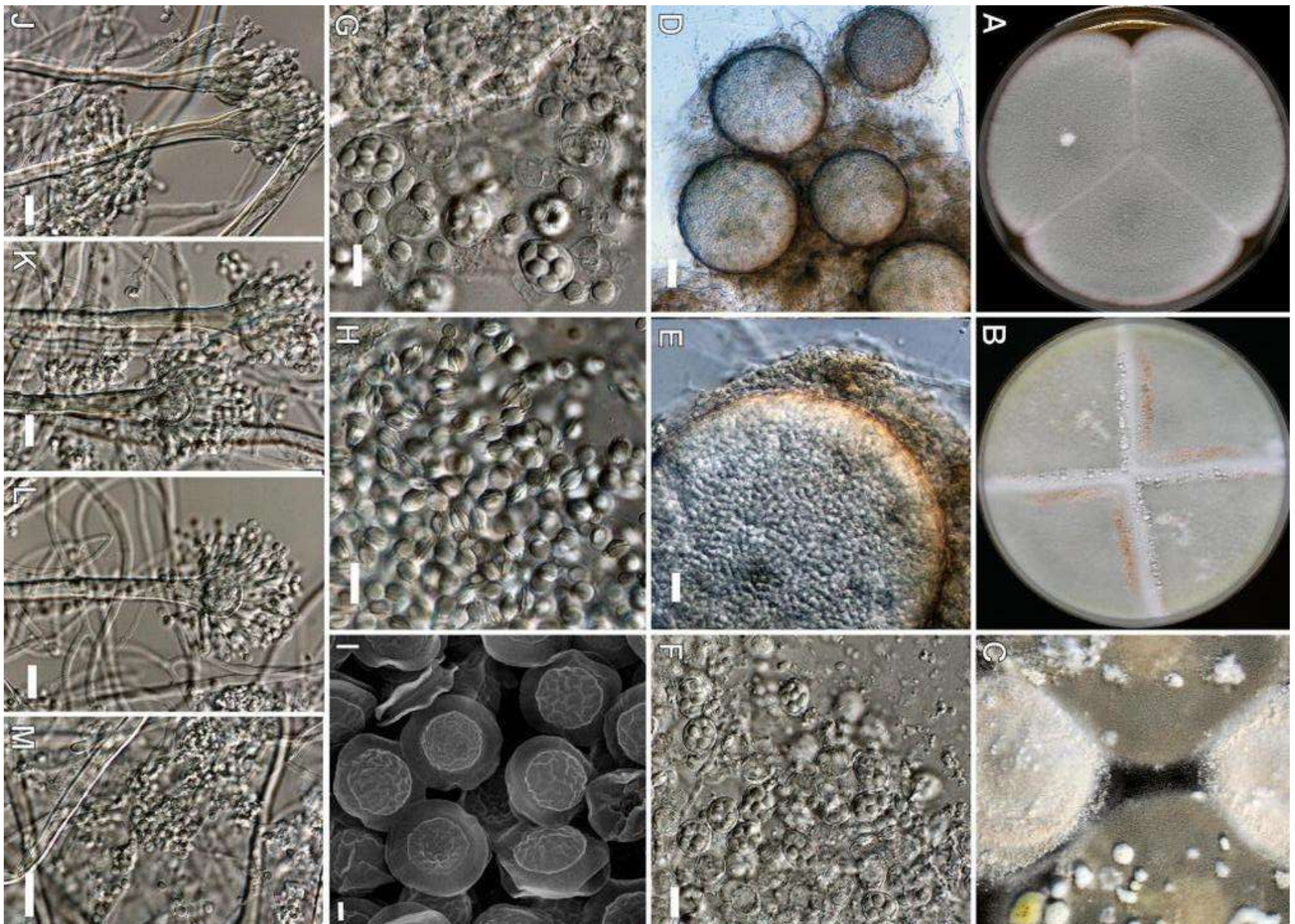
Samson, R. *Studies in Mycology* (2007), vol. 59 , 147-203.



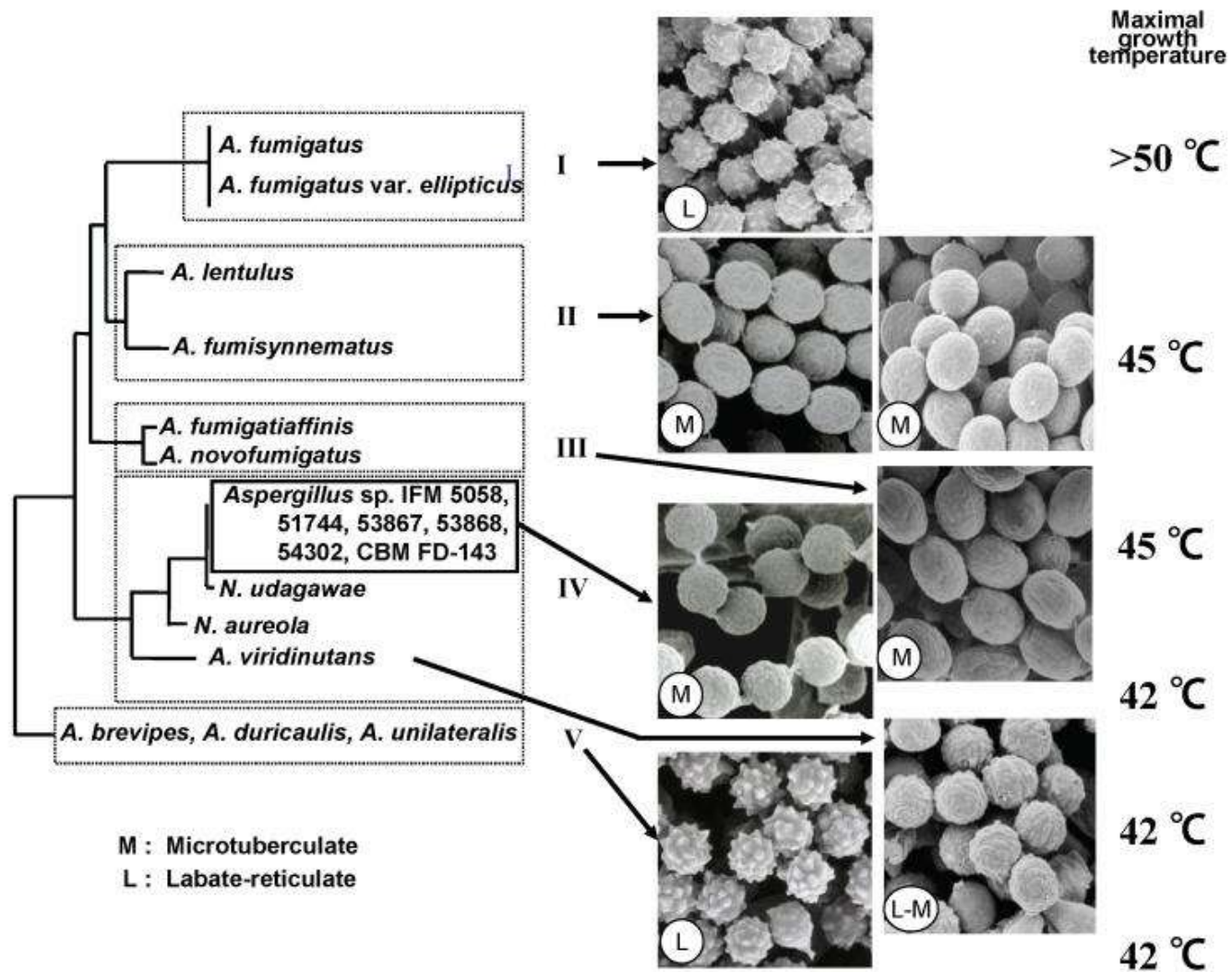
Aspergillus fumigatus. A-C. Colonies 7 d 25 °C. A. CYA. B. MEA. C. CYA 37 °C. after 3 d. D-I. Conidiophores. D-F. *A. fumigatus*. G-H. *A. fumigatus* var. *ellipticus*. I. Atypical conidiophore of CBS 133.61. J. Conidia of *A. fumigatus* var. *ellipticus*. K. Conidia of *A. fumigatus*.



Aspergillus lentulus. A-B. Colonies 7 d 25 °C. A. CYA. B. MEA. C. Macroscopic view of the columnar conidial heads. D-I. Conidiophores. J. Conidia. Scale bars = 10 μm



Neosartorya fennelliae. A-B. Colonies 14 d 25 °C. A. MEA. B-C. Crossing of mating types on MEA. D-E. Ascomata. F-G. Asci and ascospores. H. Ascospores. I. SEM of ascospores. J-L. Conidiophores. M. Conidia. Scale bars = 10 μ m, except D = 30 μ m, E = 15 μ m, I = 1 μ m

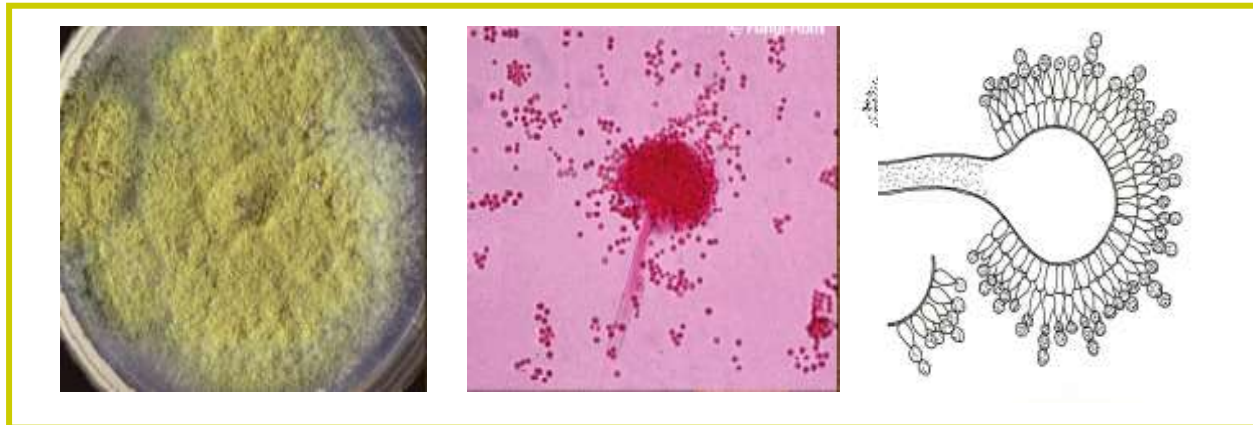


Correlation among phylogeny, detailed morphology and maximal growth temperatures on *Aspergillus* section *Fumigati*. The letters L and M refer to the conidial ornamentation as observed by scanning electron microscopy.

A. fumigatus species complex, such as *A. lentulus*, *A. viridinutans*, *N. pseudofischeri*, *A. fumigatiafinnis*, have been described to be resistant to all azoles.

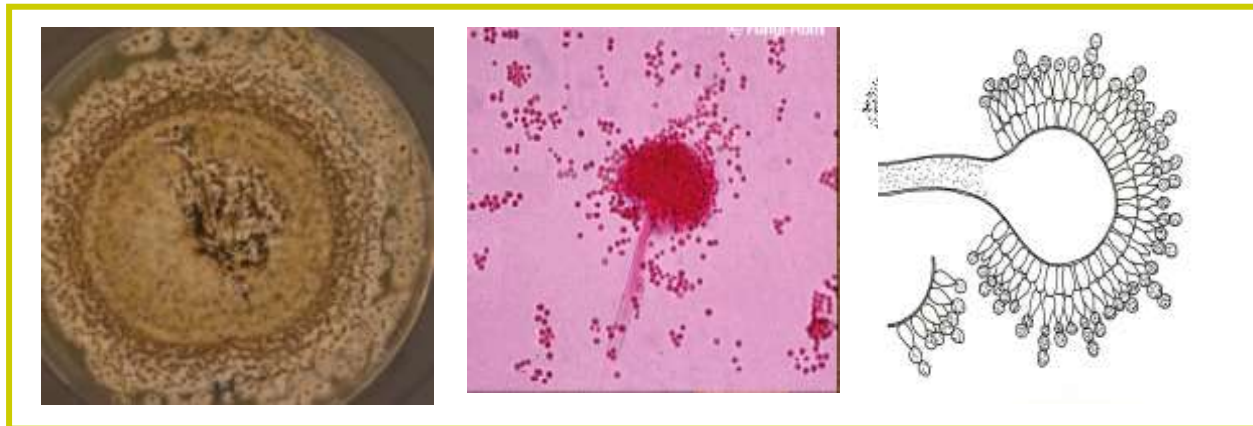
Aspergillus species distribution according to epidemiological surveys from Spain and the U.S.

Species	Section	Transnet		Spanish study	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1
<i>A. udagawae</i>	Fumigati	3	1.4	0	0.0
<i>A. pseudofischeri</i>	Fumigati	1	0.5	1	0.4
<i>A. viridinutans</i>	Fumigati	0	0.0	1	0.4
<i>A. fumigatiafinis</i>	Fumigati	0	0.0	1	0.4
<i>A. flavus</i>	Flavi	29	13.3	27	9.7
<i>A. alliaceus</i>	Flavi	0	0.0	3	1.1



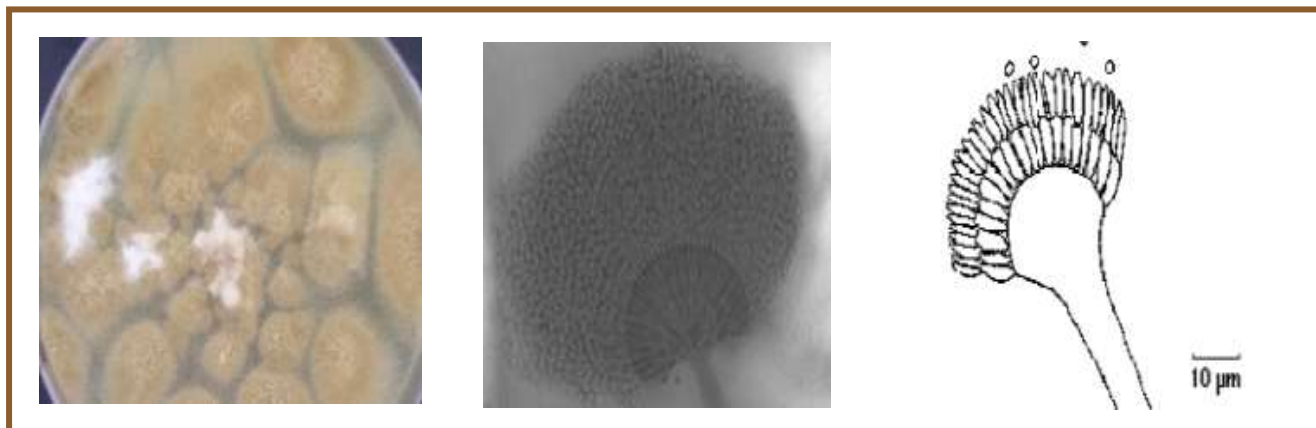
Aspergillus species distribution according to epidemiological surveys from Spain and the U.S.

Species	Section	Transnet		Spanish study	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1
<i>A. udagawae</i>	Fumigati	3	1.4	0	0.0
<i>A. pseudofischeri</i>	Fumigati	1	0.5	1	0.4
<i>A. viridinutans</i>	Fumigati	0	0.0	1	0.4
<i>A. fumigatiafinis</i>	Fumigati	0	0.0	1	0.4
<i>A. flavus</i>	Flavi	29	13.3	27	9.7
<i>A. alliaceus</i>	Flavi	0	0.0	3	1.1

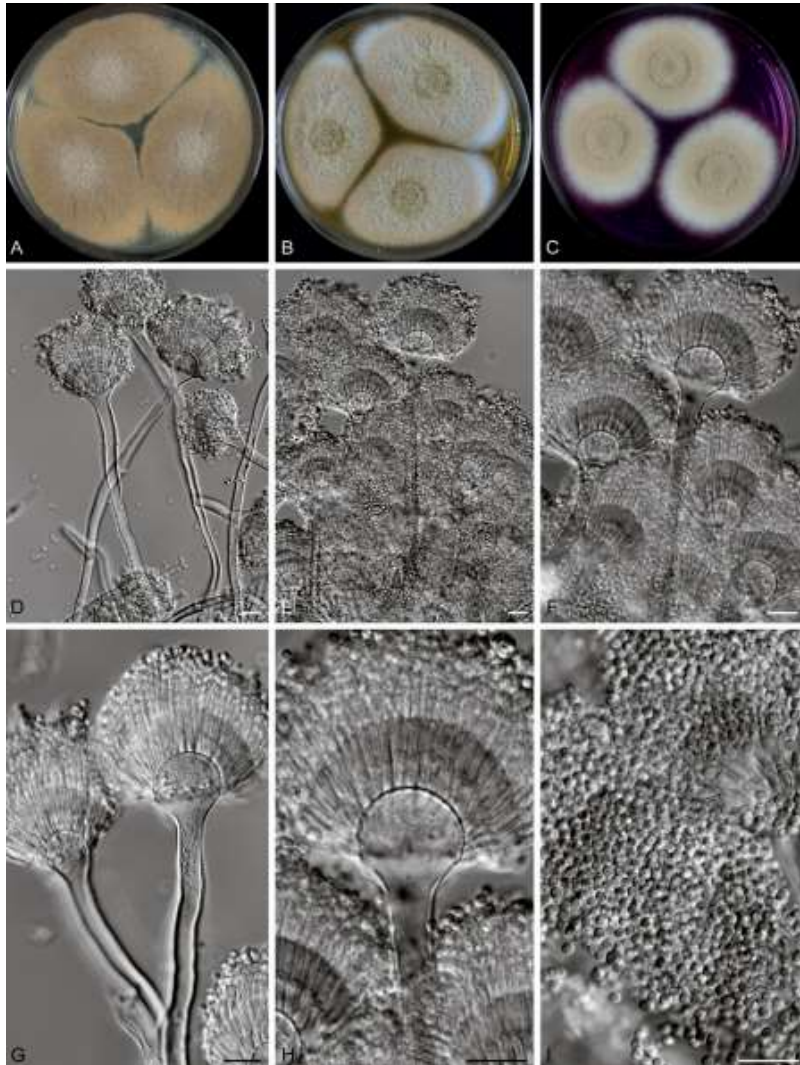


Aspergillus species distribution according to epidemiological surveys from Spain and the U.S.

Species	Section	Transnet		Spanish study	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1
<i>A. udagawae</i>	Fumigati	3	1.4	0	0.0
<i>A. pseudofischeri</i>	Fumigati	1	0.5	1	0.4
<i>A. viridinutans</i>	Fumigati	0	0.0	1	0.4
<i>A. fumigatiafinis</i>	Fumigati	0	0.0	1	0.4
<i>A. flavus</i>	Flavi	29	13.3	27	9.7
<i>A. alliaceus</i>	Flavi	0	0.0	3	1.1
<i>A. terreus</i>	Terrei	11	5.0	26	9.4
<i>A. carneus</i>	Terrei	0	0.0	1	0.4

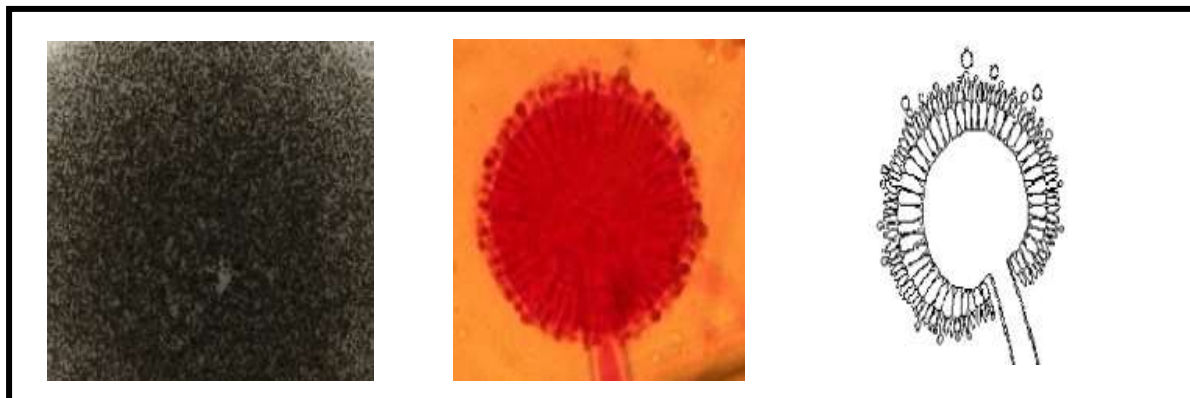


New species in *Aspergillus* section *Terrei*



Aspergillus species distribution according to epidemiological surveys from Spain and the U.S.

Species	Section	Transnet		Spanish study	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1
<i>A. udagawae</i>	Fumigati	3	1.4	0	0.0
<i>A. pseudofischeri</i>	Fumigati	1	0.5	1	0.4
<i>A. viridinutans</i>	Fumigati	0	0.0	1	0.4
<i>A. fumigatiafinis</i>	Fumigati	0	0.0	1	0.4
<i>A. flavus</i>	Flavi	29	13.3	27	9.7
<i>A. alliaceus</i>	Flavi	0	0.0	3	1.1
<i>A. terreus</i>	Terrei	11	5.0	26	9.4
<i>A. carneus</i>	Terrei	0	0.0	1	0.4
<i>A. tubingensis</i>	Nigri	6	2.8	22	7.9
<i>A. niger</i>	Nigri	13	6.0	21	7.6



Aspergillus *Nigri*

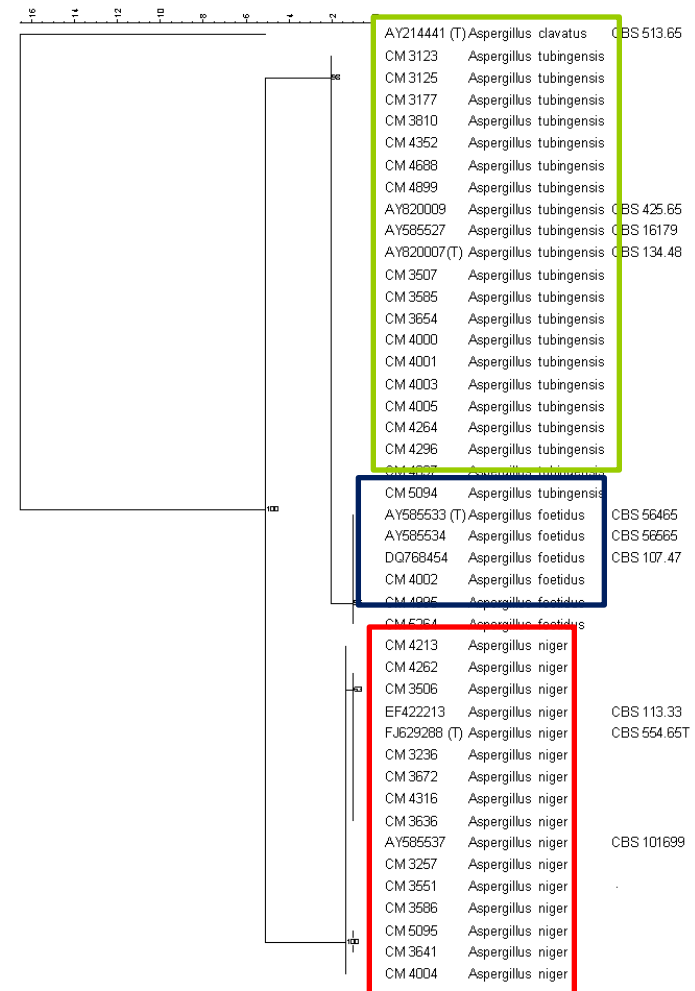


A. niger

A. tubingensis

A. awamori

A. foetidus



New and revisited species of *Aspergillus* section *Nigri*



Fig. 8. *Aspergillus acidalis* A–C. Colonies incubated at 25 °C for 7 d, A. CYA, B. MEA, C. CREA, D–I. Conidiophores and conidia. Scale bars = 10 µm.

Aspergillus species distribution according to epidemiological surveys from Spain and the U.S.

Species	Section	Transnet		Spanish study	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1

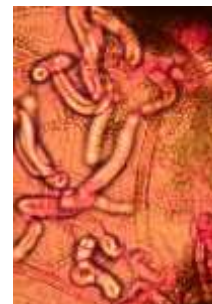
EUKARYOTIC CELL, Apr. 2008, p. 630–638
 1535-9778/08/\$08.00+0 doi:10.1128/EC.00425-07
 Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Vol. 7, No. 4

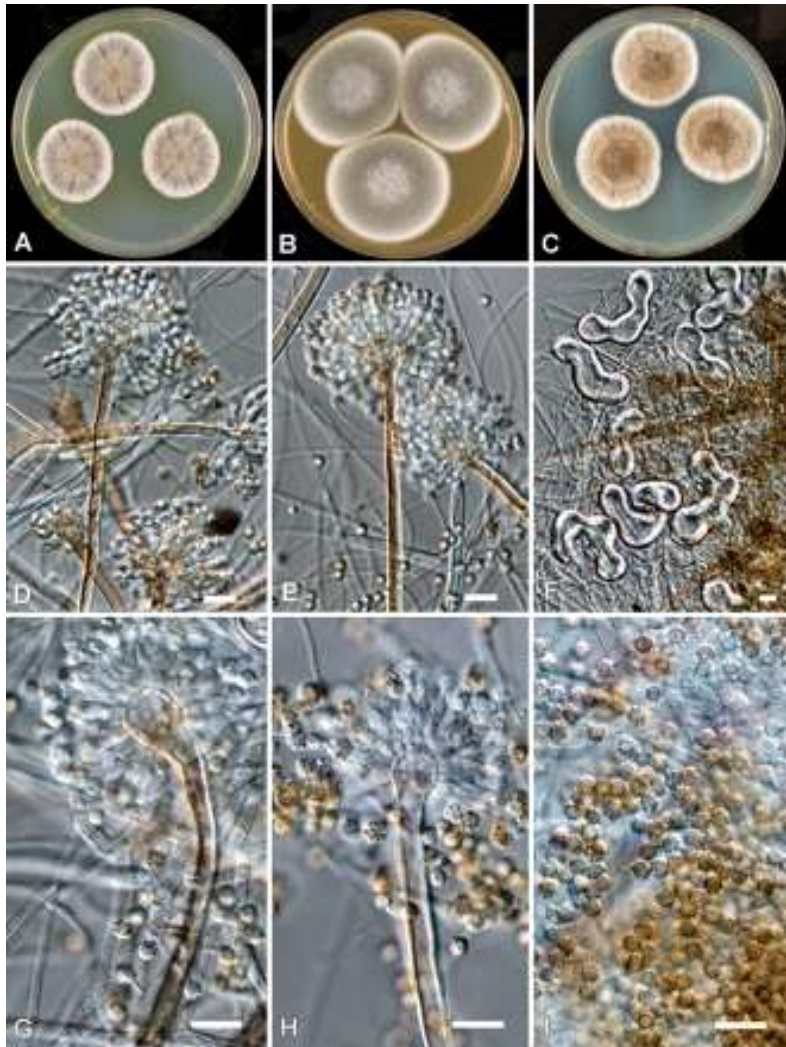
Aspergillus calidoustus sp. nov., Causative Agent of Human Infections Previously Assigned to *Aspergillus ustus*[▽]

János Varga,^{1,2} Jos Houbraken,¹ Henrich A. L. Van Der Lee,³
 Paul E. Verweij,³ and Robert A. Samson^{1*}

<i>A. tubingensis</i>	Nigri	6	2.8	22	7.9
<i>A. niger</i>	Nigri	13	6.0	21	7.6
<i>A. calidoustus</i>	Usti	6	2.8	4	1.4
<i>A. insuetus</i>	Usti	0	0.0	1	0.4
<i>A. keveii</i>	Usti	0	0.0	1	0.4



Polyphasic taxonomy of *Aspergillus* section *Usti*

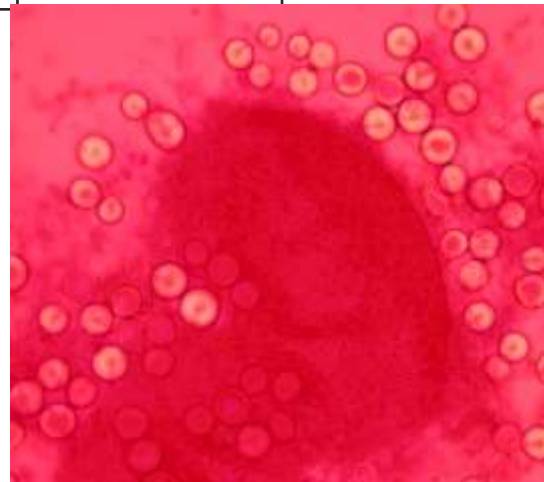
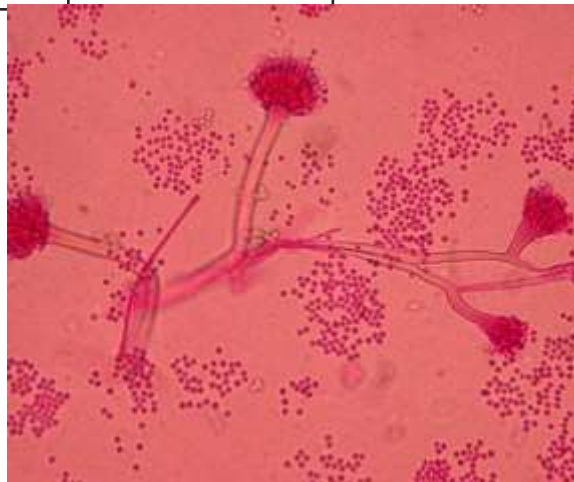
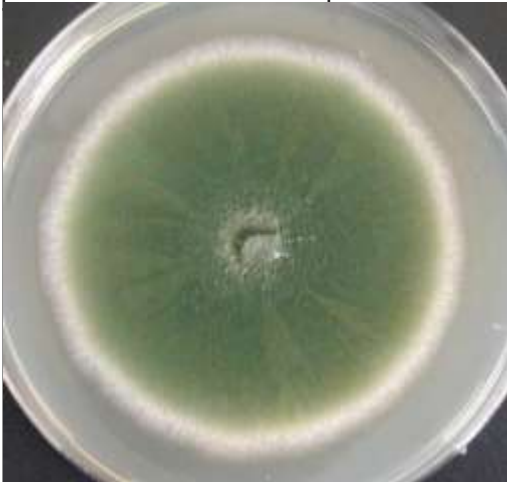


Aspergillus species distribution according to epidemiological surveys from Spain and the U.S.

Species	Section	Transnet		Spanish study	
		N isolates	%	N isolates	%
<i>A. fumigatus</i>	Fumigati	139	63.8	156	56.1
<i>A. lentulus</i>	Fumigati	4	1.8	3	1.1
<i>A. udagawae</i>		3			0.0
<i>A. pseudoflavus</i>		1			0.4
<i>A. viridinutans</i>		0			0.4
<i>A. fumigatus</i>		0			0.4
<i>A. flavus</i>		29			9.7
<i>A. alliaceus</i>		0			1.1
<i>A. terreus</i>		11			9.4
<i>A. carneus</i>		0			0.4
<i>A. tubingae</i>		6			7.9
<i>A. niger</i>	Nigri	13	6.0	21	7.6
<i>A. calidoustus</i>	Usti	6	2.8	4	1.4
<i>A. insuetus</i>	Usti	0	0.0	1	0.4
<i>A. keveii</i>	Usti	0	0.0	1	0.4
<i>A. sydowii</i>	Versicolores	2	0.9	1	0.4
<i>A. versicolor</i>	Versicolores	3	1.4	0	0.0
<i>E. quadrilineata</i>	Nidulantes	1	0.5	0	0.0
<i>A. nidulans</i>	Nidulantes	0	0.0	8	2.9
<i>A. westerdijkiae</i>	Circumdati	0	0.0	1	0.4
Total		218	100	278	100

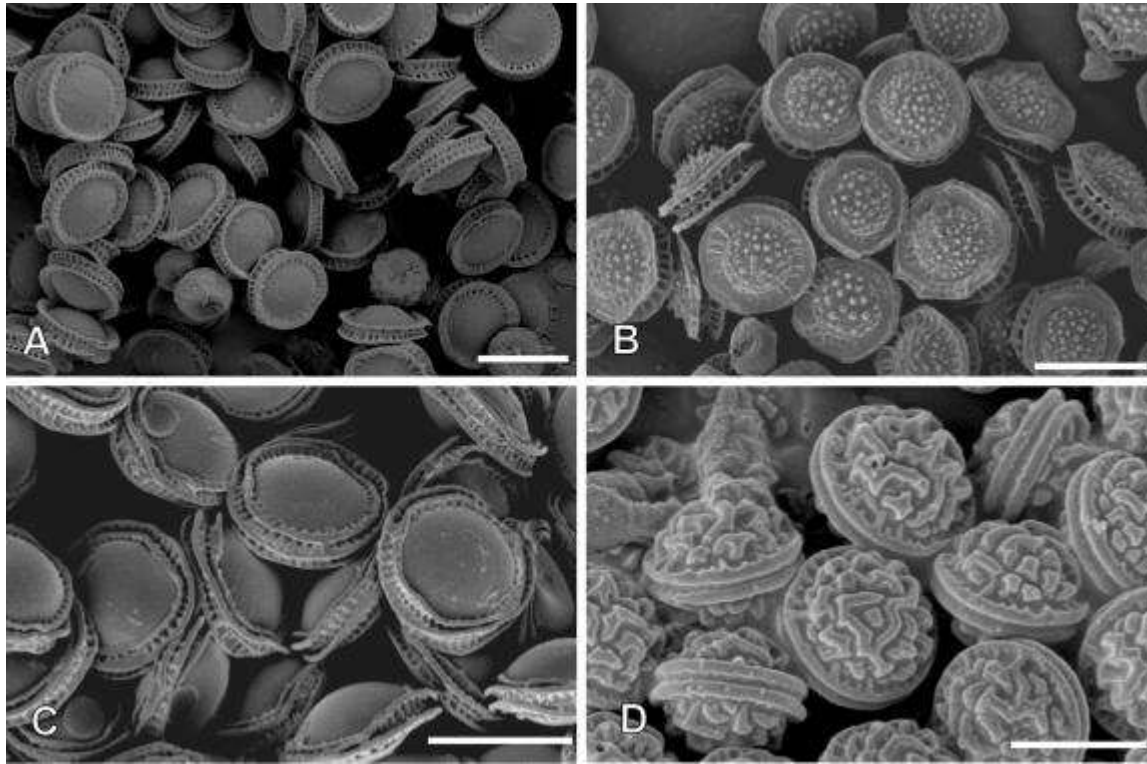


Aspergillus species distribution according to epidemiological surveys from Spain and the U.S.

Species	Section	Transnet		Spanish study	
		N isolates	%	N isolates	%
					
<i>A. carneus</i>	Terrei	0	0.0	1	0.4
<i>A. tubingenis</i>	RESEARCH				7.9
<i>A. niger</i>					7.6
<i>A. calidoustus</i>					1.4
<i>A. insuetus</i>					0.4
<i>A. keveii</i>					0.4
<i>A. sydowii</i>					0.4
<i>A. versicolor</i>	Versicolores	3	1.4	0	0.0
<i>E. quadrilineata</i>	Nidulantes	1	0.5	0	0.0
<i>A. nidulans</i>	Nidulantes	0	0.0	8	2.9
<i>A. westerdijkiae</i>	Circumdati	0	0.0	1	0.4
Total		218	100	278	100

***Emericella quadrilineata* as Cause of Invasive Aspergillosis**

Paul E. Verweij,* János Varga,†‡ Jos Houbraken,† Antonius J.M.M. Rijs,* Frans M. VerduynLunel,*
Nicole M.A. Blijlevens,* Yvonne R. Shea,§ Steven M. Holland,§ Adilia Warris,* Willem J. G. Melchers,*
and Robert A. Samson†



Scanning electronmicroscopy of ascospores of *Emericella* species.
A. *E. nidulans*; B. *E. echinulata*; C. *E. quadrilineata*; D. *E. rugulosa*
Scale bars = 5 μm

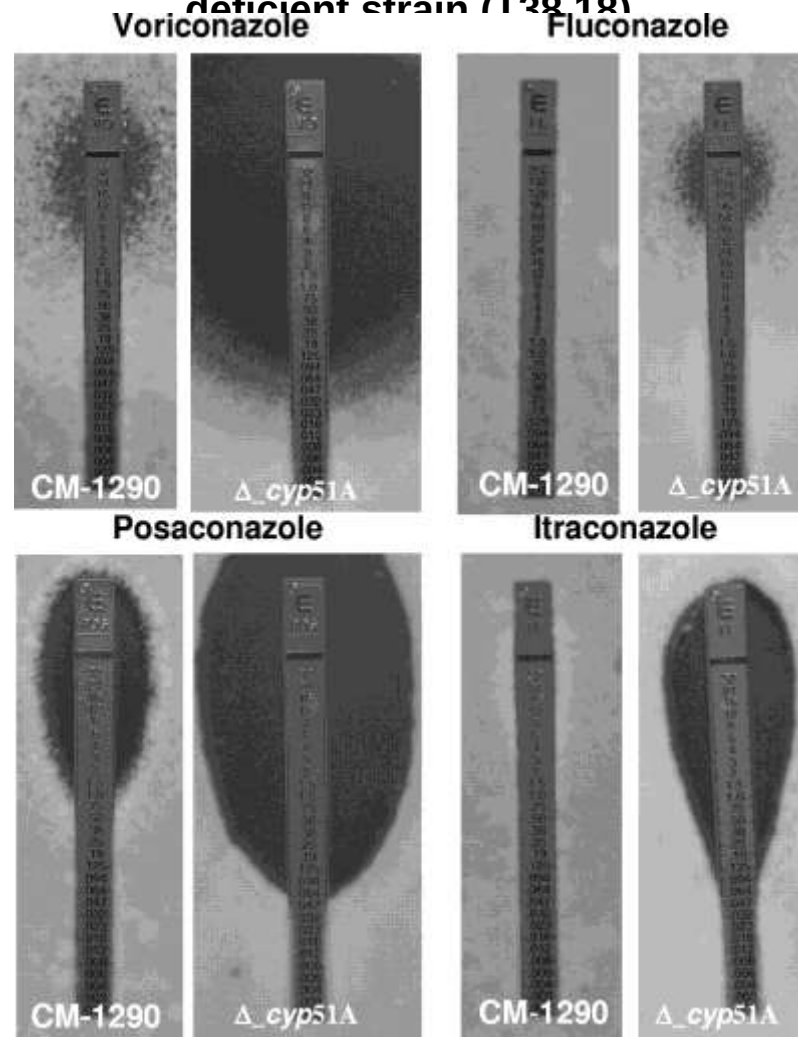
Infections caused by these fungi have been associated with a lower response rate and poorer outcomes.

The reduced susceptibility of *Flavi* can be attributed to *A. alliaceus*, which has been described as resistant to this drug.

Alustruey- Izquierdo A et al. 2012, Ann N Y Acad Sci. 1273:18-24
Balajee SA et al. 2007, J Clin Microbiol. 45: 2701-2703
Ozhak-Baysan B et al. 2010, Med Mycol. 48: 995-999

Some of these new species are more resistant to current antifungal drugs, making the identification to species level of the etiologic agent of an infection decisive for the correct management of the patients.

Etest susceptibility testing of itraconazole (ITC), voriconazole (VCZ), fluconazole (FCZ), and posaconazole (POS) for the *A. lentulus* wild-type strain (CM-1290) and an *A. lentulus* cyp51A-deficient strain (T2818)



Mellado E et al. Antimicrob. Agents Chemother.
2011;55:5459-5468

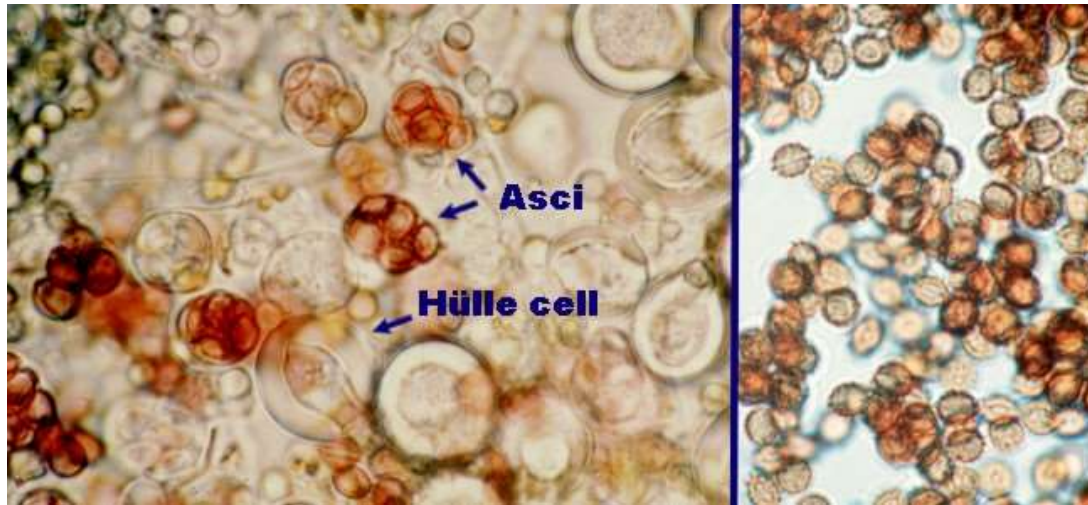
Antimicrobial Agents and Chemotherapy

This pattern of resistance has been also described in species from other complexes, such as *A. tubingensis* (*A. niger* complex) and *A. calidoustus* (*A. ustus* complex) that have an azole multiresistant susceptibility profile.

Pleomorphism

Anamorph + Teleomorph = Holomorph

asexual	sexual	whole fungus
imperfect	perfect	



However, it is difficult to have users switch to different names, and there is no consensus on which (teleomorph or anamorph) name to use.

Teleomorphs of *Aspergillus*

The genus *Aspergillus* can produce several teleomorph. The teleomorph of the majority of species is not known and current taxonomic knowledge has revealed that many homothallic exist although recently several heterothallic species have been discovered.

Chaetosartorya

Dichotomomyces

Emericella

Eurotium

Fennellia

Hemicarpenteles

Neocarpenteles

Neopetromyces

Neosartorya

Penicilliopsis

Petromyces

Raperia

Sclerocleista

Warcupiella

Chaetosartorya (*A. wentii*)
Emericella (*A. nidulans*)
Eurotium (*A. glaucus*)
Fennellia (*A. terreus*)
Hemicarpenteles (*A. paradoxus*)
Neocarpenteles (*A. clavatus*)
Neosartorya (*A. fumigatus*)
Petromyces (*A. flavus* and possibly *A. niger*)
Sclerocleista (*A. ornatus*)
Warcupiella (*A. spinnulosus*)

Fungal Nomenclature

- 2011
- 2012
- 2013
- 2014



CBS Symposium



CBS Symposium



CBS Symposium





Thank you for our attention!