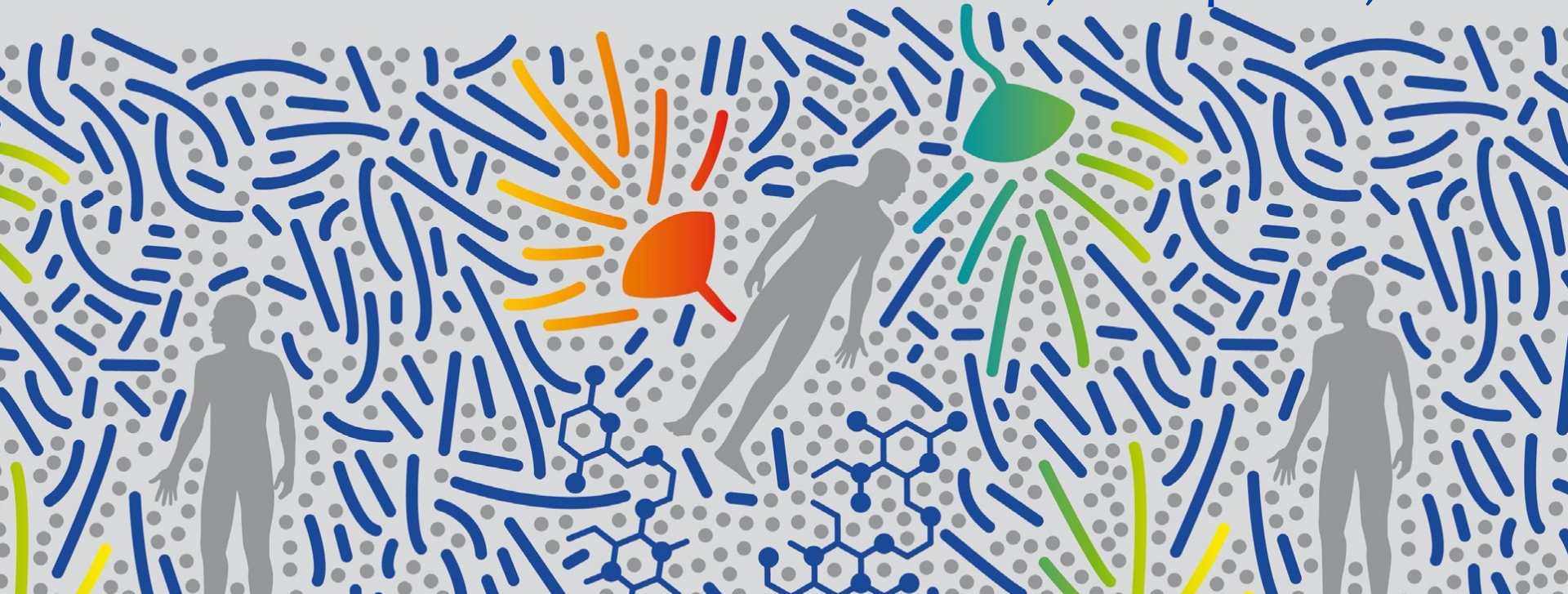


Research with strain collections: The advantage of strain multitudes using the example of diagnostics of fungal infections

Kerstin Voigt

ECCO XL, 28th September, 2022



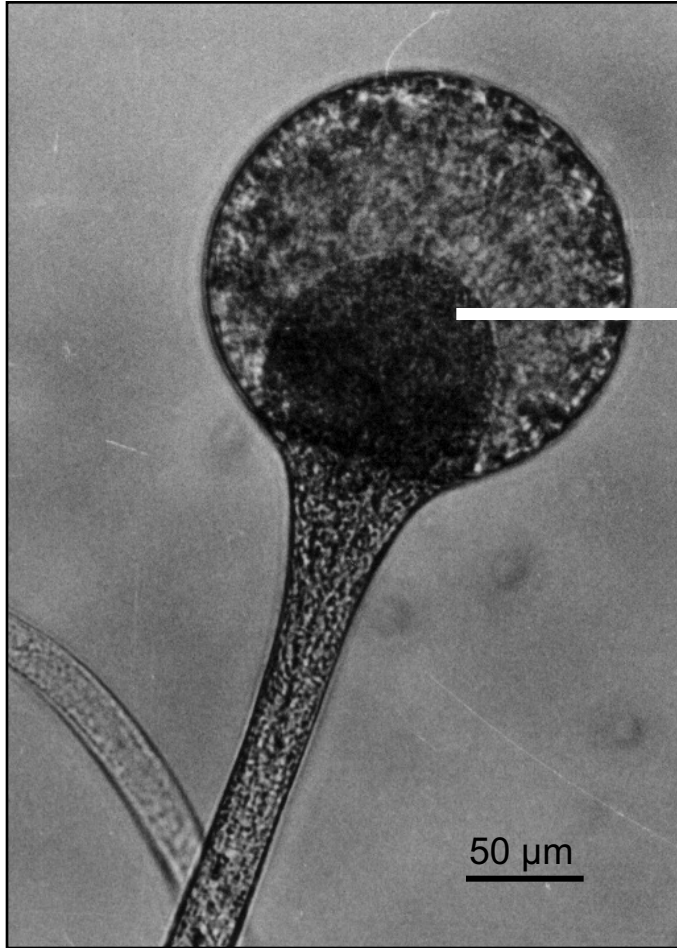
Mucorales are everywhere

- Basal filamentous fungi
- Ubiquitous saprobes (e.g. soil, hay; fruits - post-harvest pathogen)



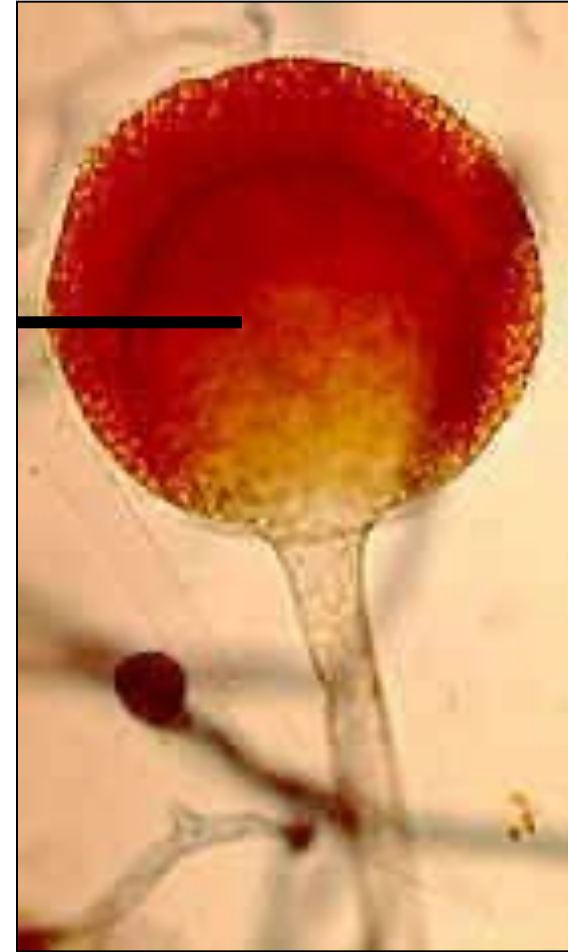
Photo courtesy: Grit Walther

The trade-mark of the Mucorales is a swollen extension of the sporangiophore called a columella, which protrudes like a balloon into the sporangium.

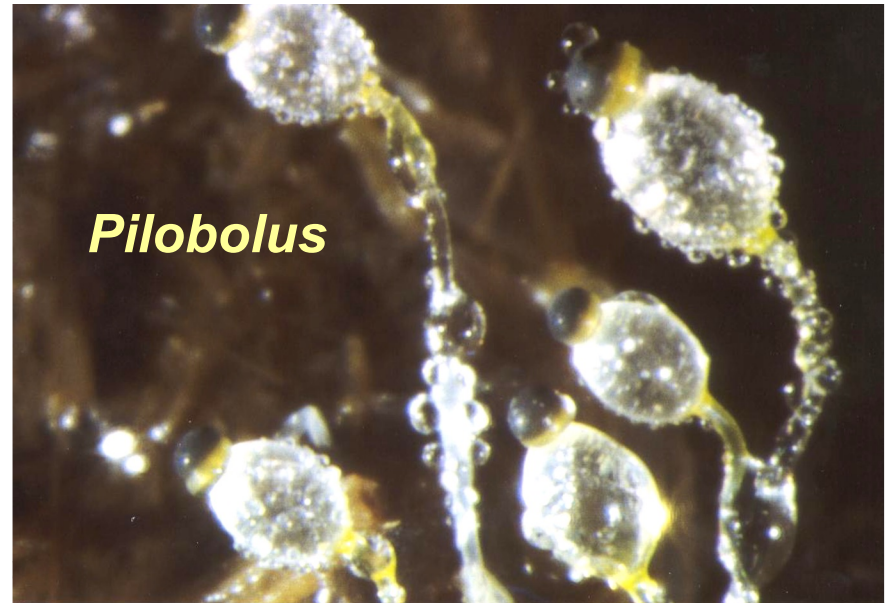
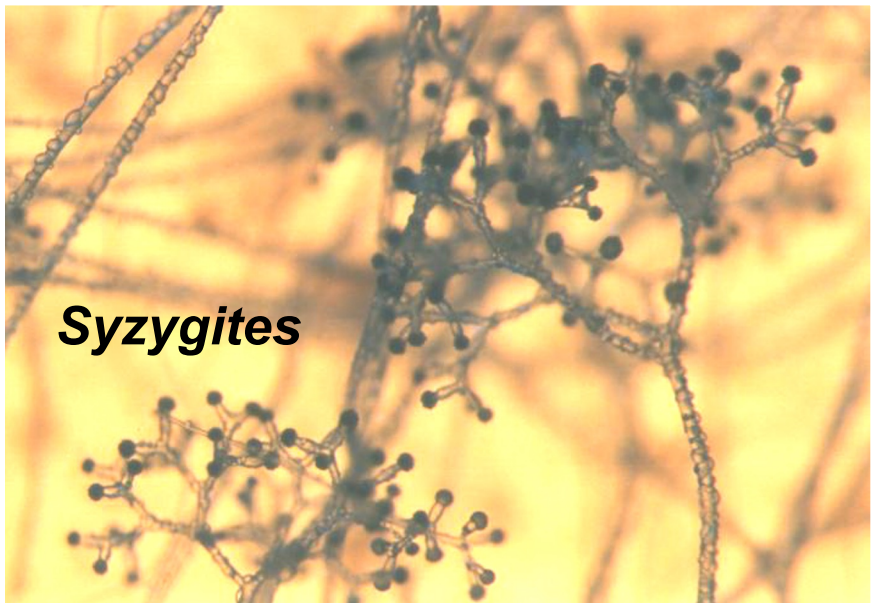
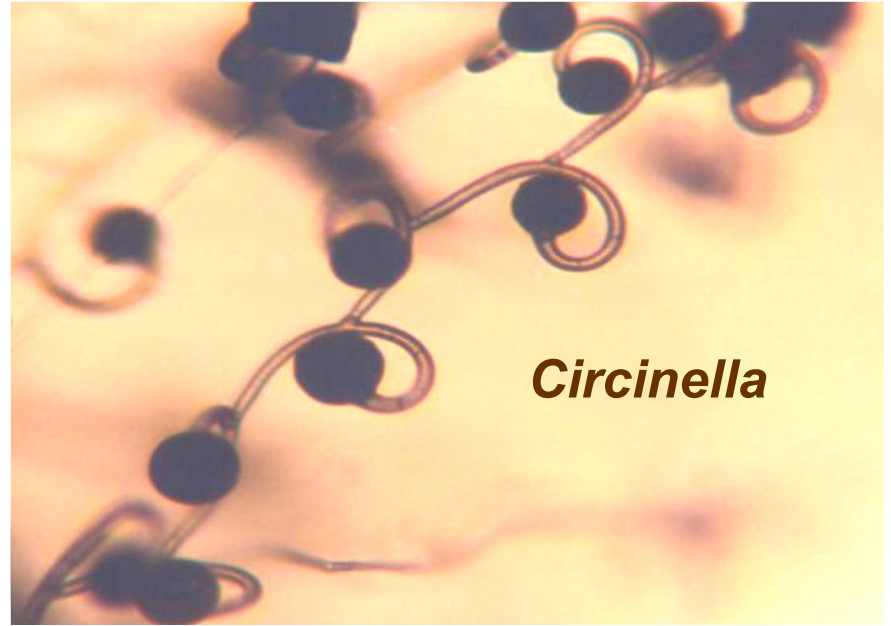
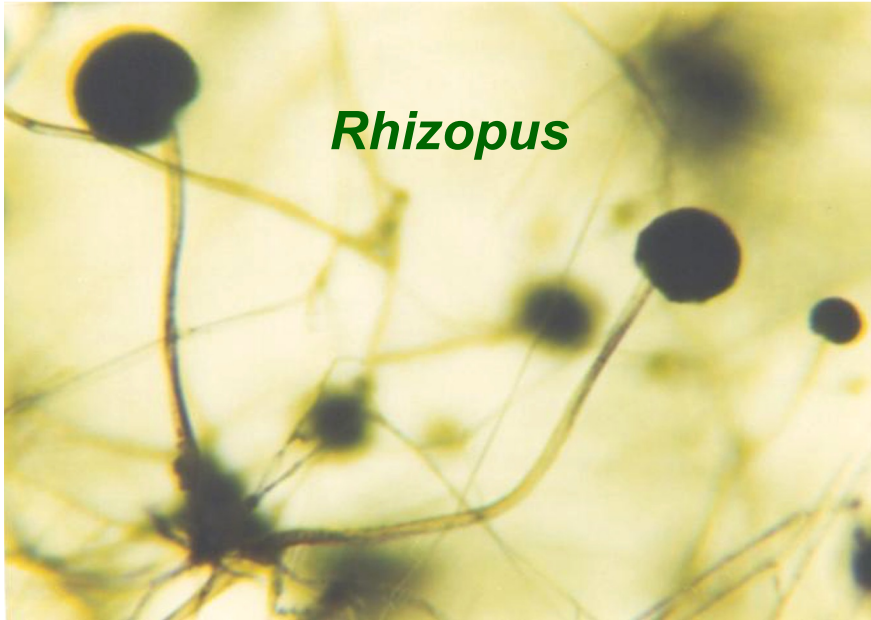


Columella

a bulbous vesicle at the sporangiophore apex

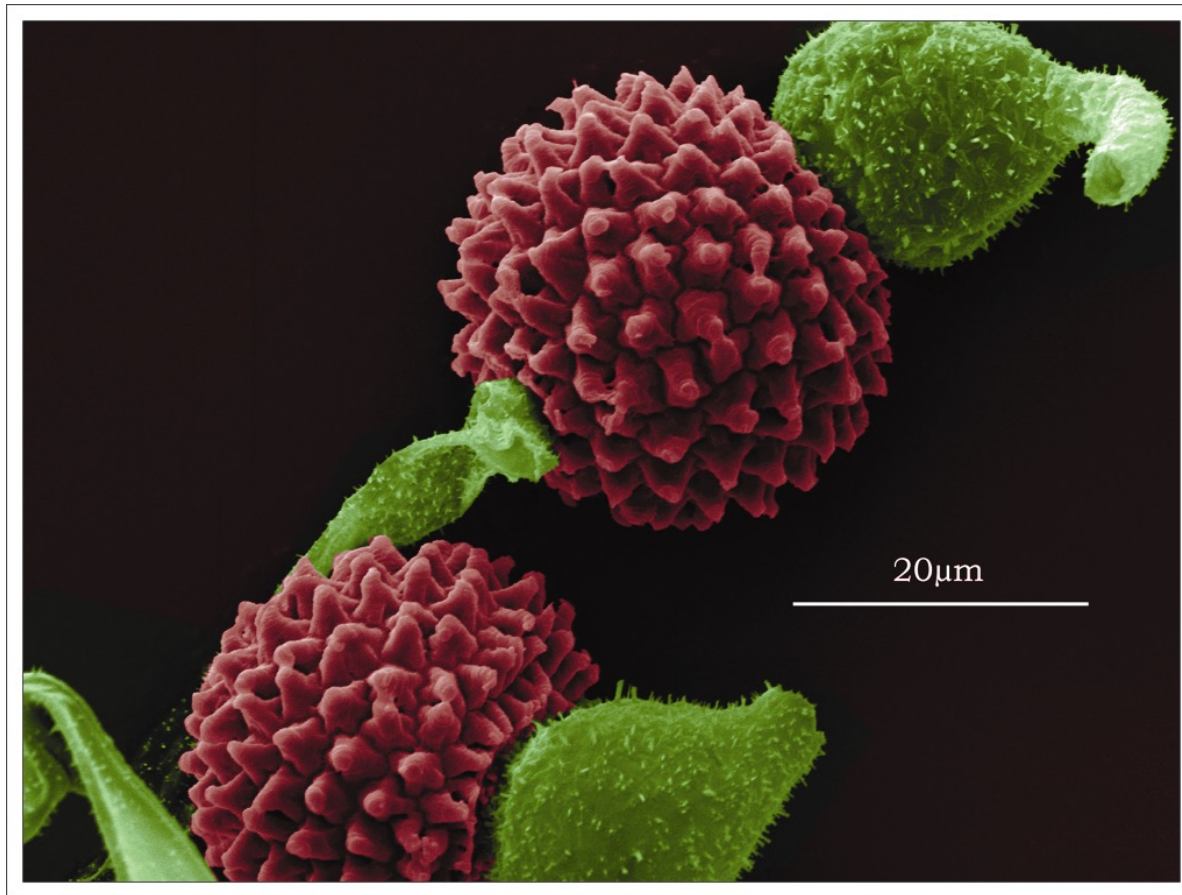


Mucorales propagate asexually *via* mitospores produced endogenously in sporangia



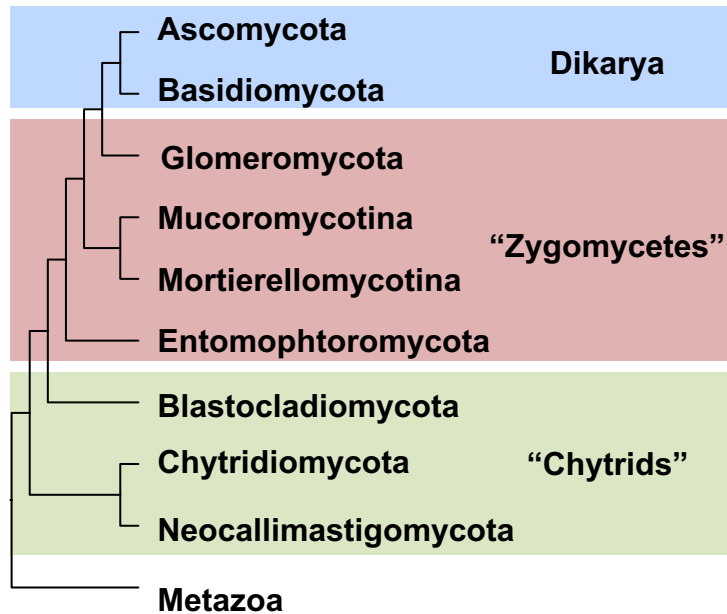
Sexual Interaction – Gametangiogamy

Conjugation of non-differentiated, coenocytic gametangia leads to the formation of zygotes (zygospores), which are associated with a pair of two yoke-shaped suspensors

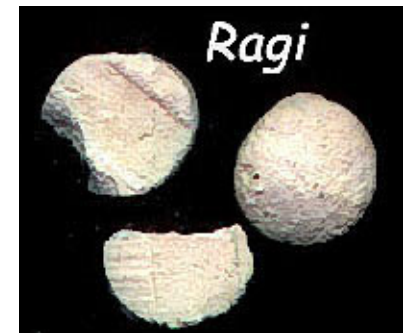
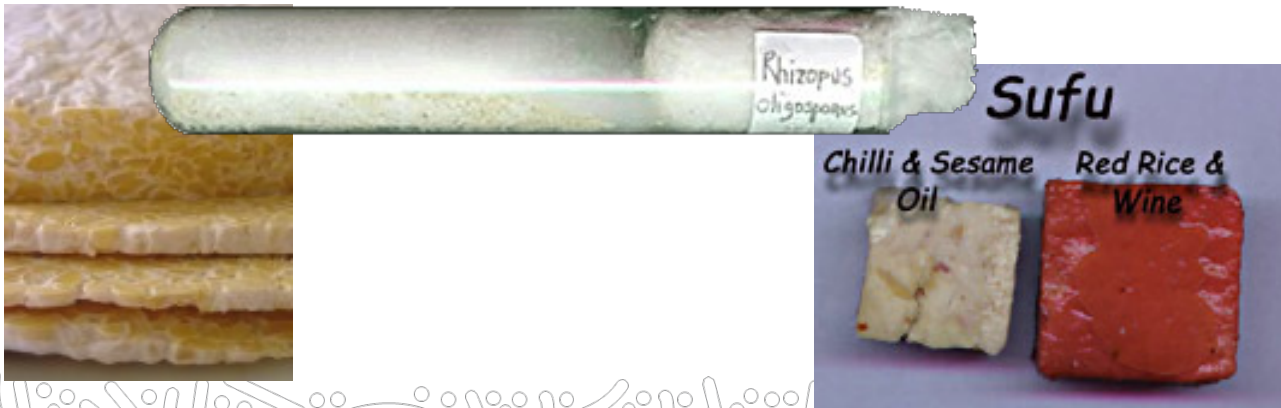


Mucor moellerii (ex: *Zygorhynchus moellerii*)

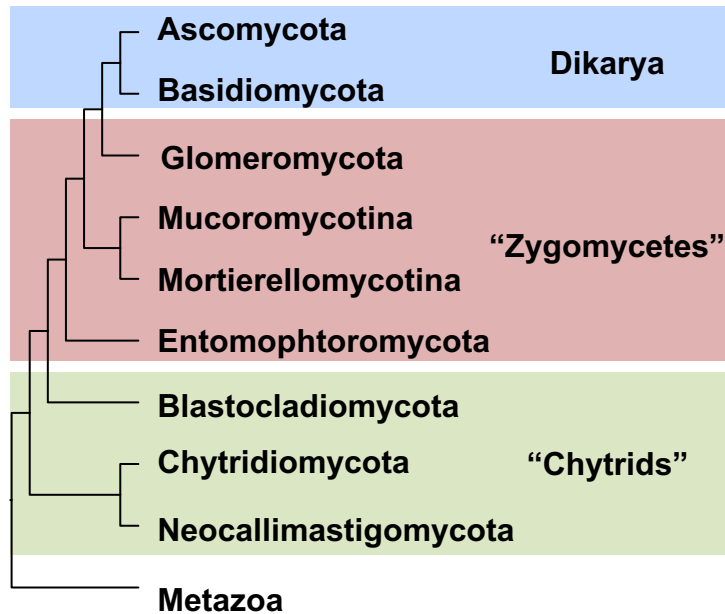
Mucorales



- Food production (Asian cuisine)
- Tempeh, soy sauce etc.



Mucorales



- Ancient terrestrial filamentous fungi
- Mainly ubiquitous saprotrophs
- Food production (Asian cuisine)

Invasive infections	Cases per year
Aspergillosis	200,000
Candidiasis	400,000
Mucormycosis	10,000

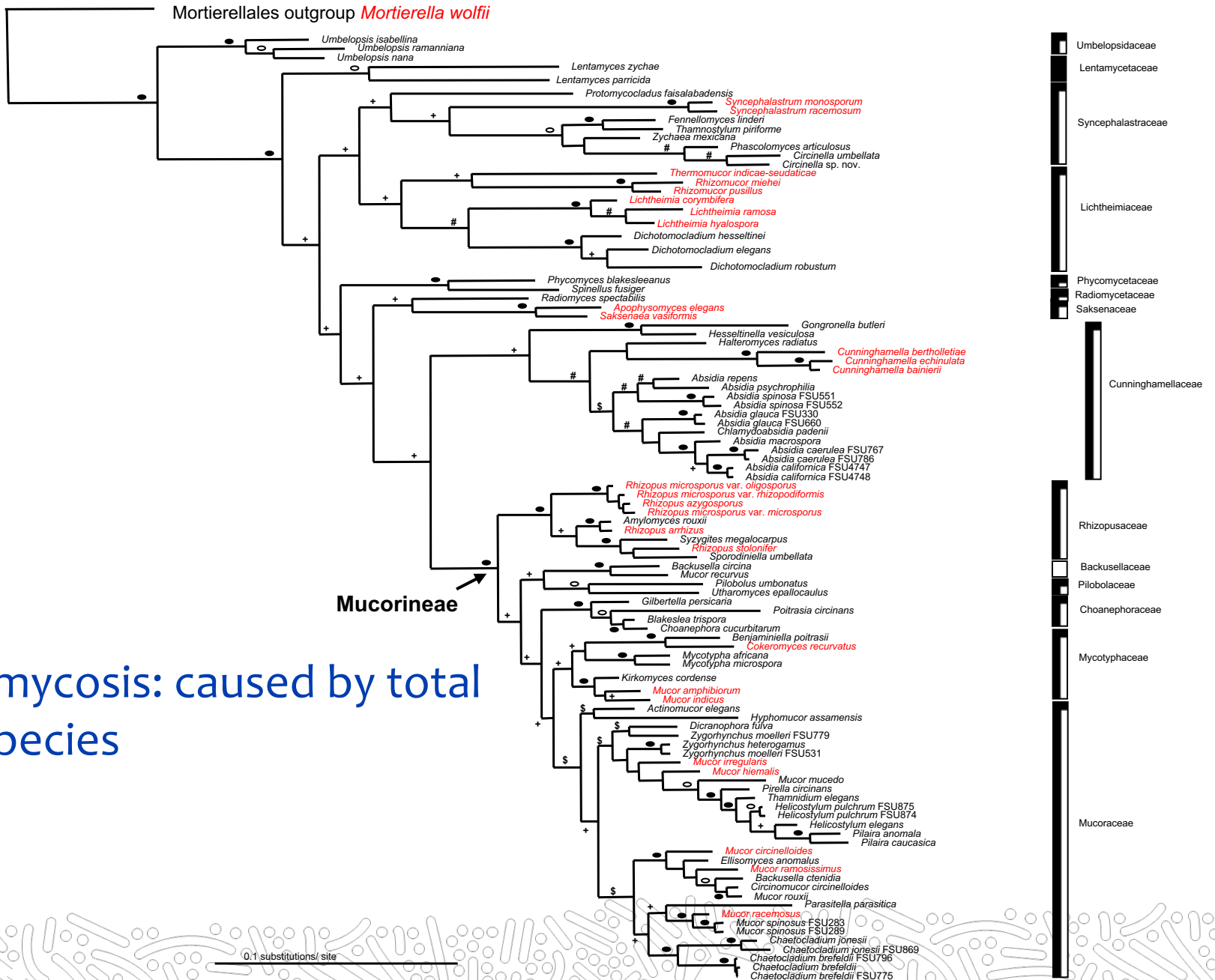


Lidor & Nunley, 1997, N Engl J Med



Petrikkos et al. 2012, CID

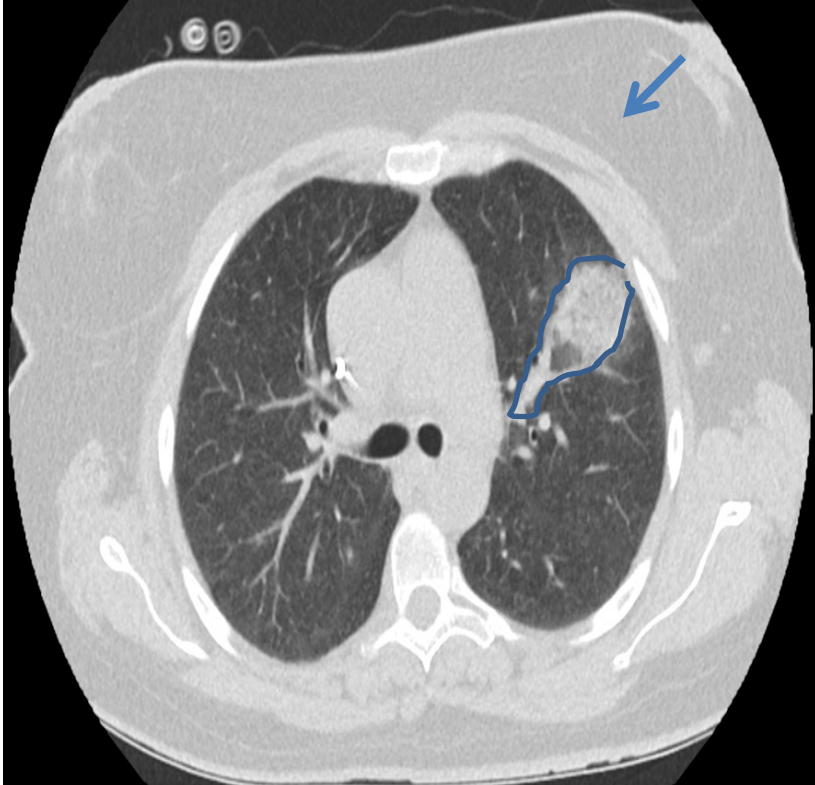
- Emerging fungal infections
- COVID-19 associated
- Fast progress, high mortality rates
- Reliable diagnostics is mandatory for therapy



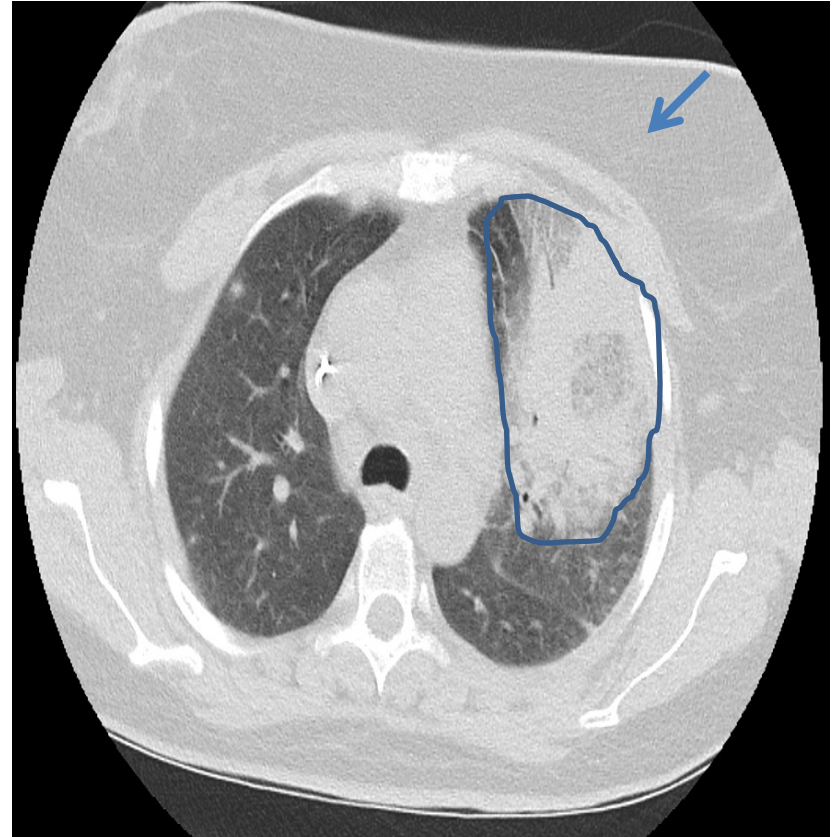
Mucormycosis: caused by total of 26 species

Mucormycosis: pulmonary infection

Female patient: 38 yrs., admitted to UKJ, KIM II, Int. Oncology, Sept. 2017



t = 0

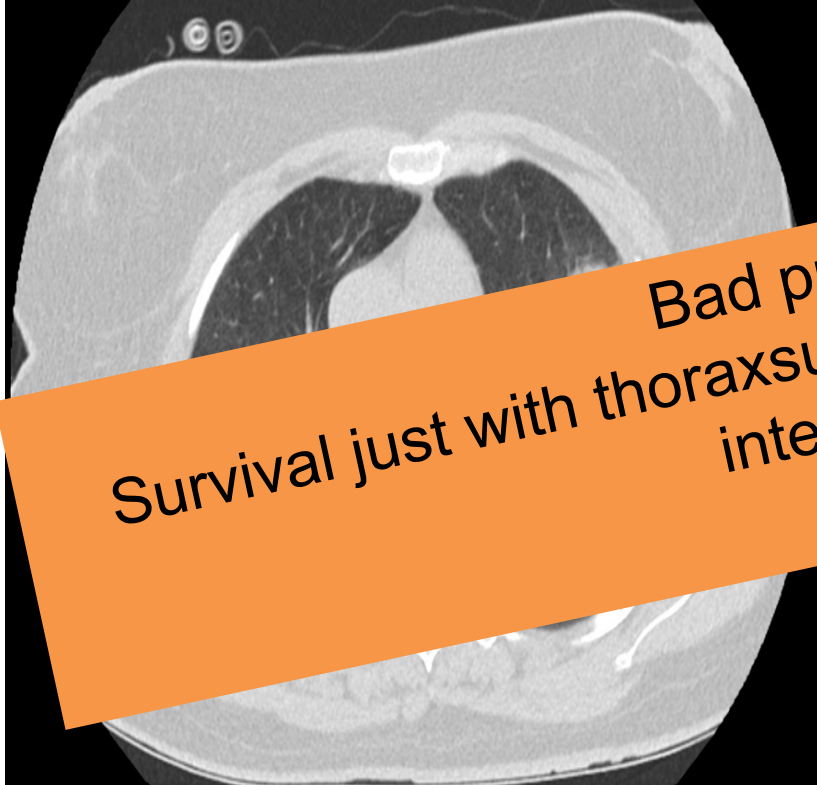


t = 1 week later - > 5x augmentation of lesion



Mucormycosis: pulmonary infection

Female patient: 38 yrs., admitted to UKJ, Sept. 2017



t = 0



t = 1 week later

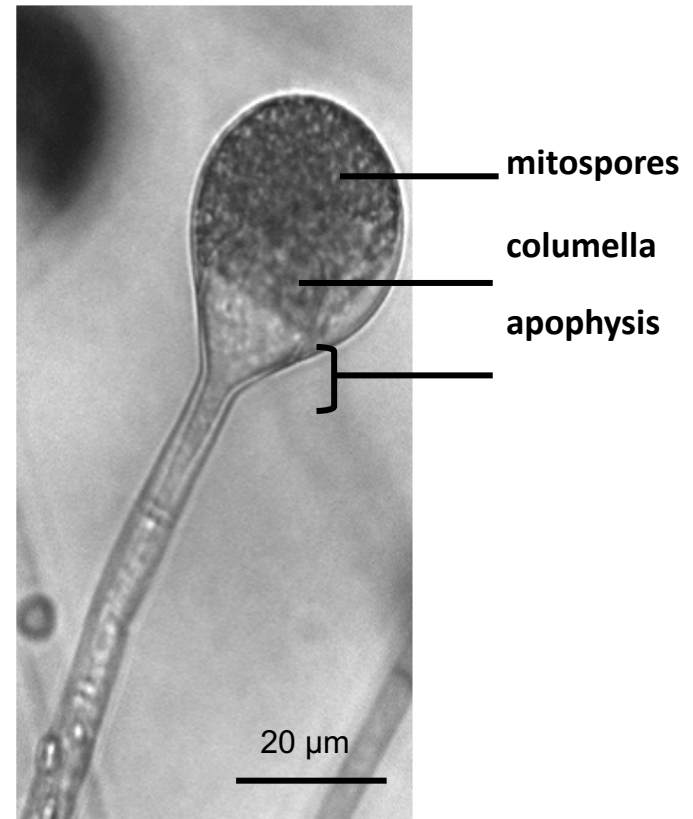
Bad prognosis
Survival just with thoraxsurgical and chemotherapeutic
intervention



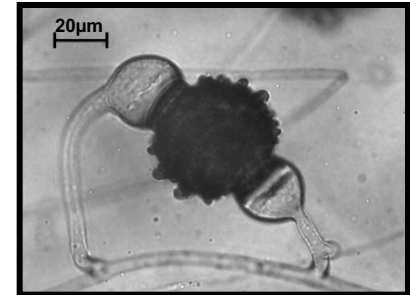
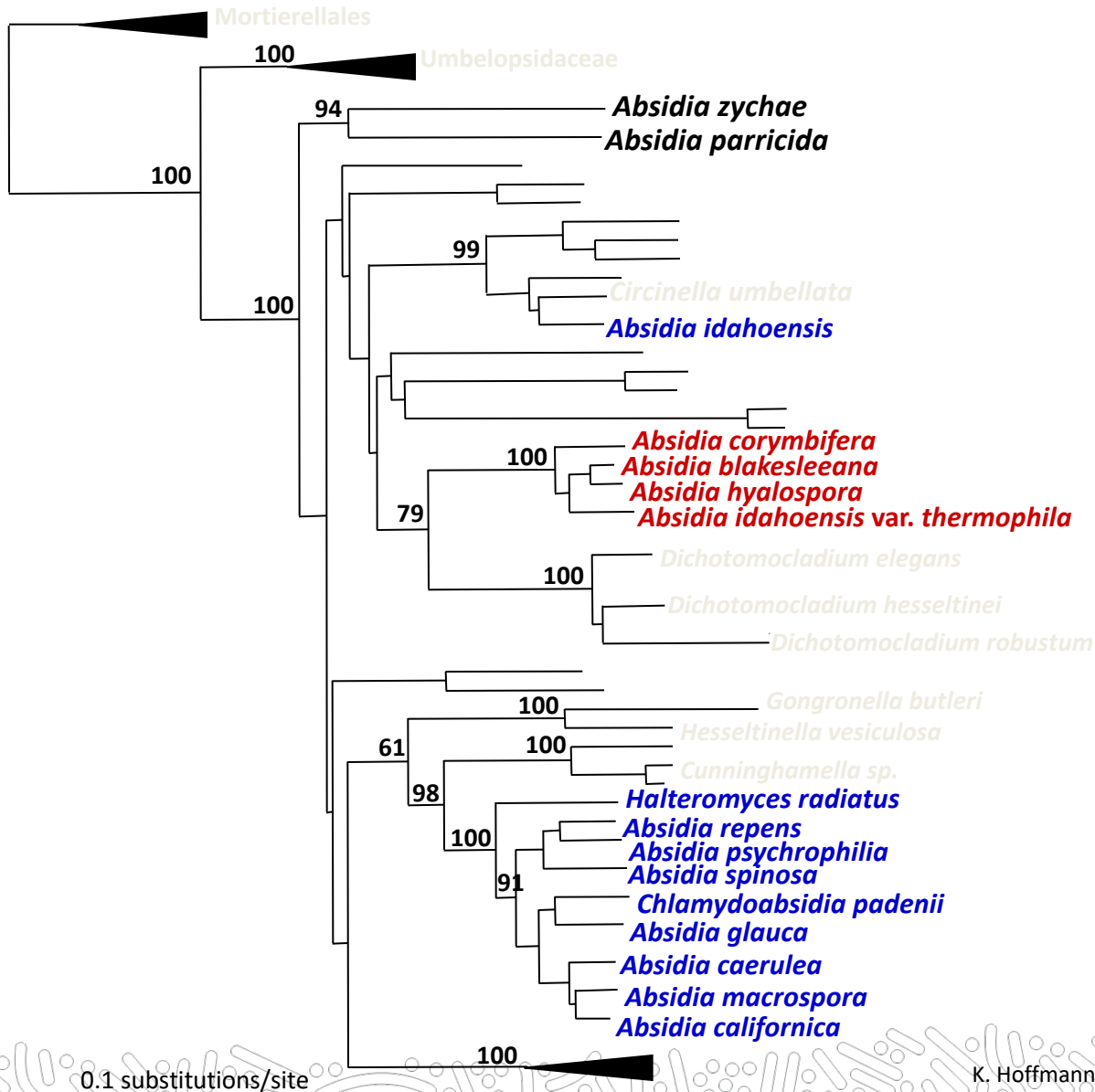
Absidia corymbifera: 1st species reported as causative agent of mucormycosis

- *Absidia corymbifera* (Cohn)
Sacc. & Trotter, in Saccardo,
Syll. fung. (Abellini) 21: 825
(1912)
- Basionym: *Mucor corymbifer*
Cohn in Lichtheim, *Z. klin.
Med.* 7: 149 (1884)

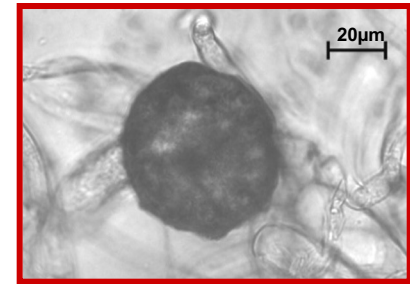
-pyriform, apophysate sporangia



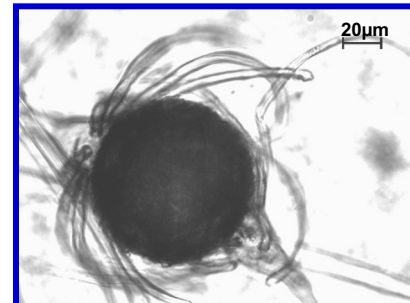
Absidia: polyphyletic origin



Zygospores: *Mucor*-like, papillate

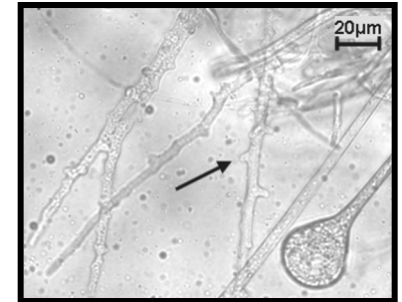
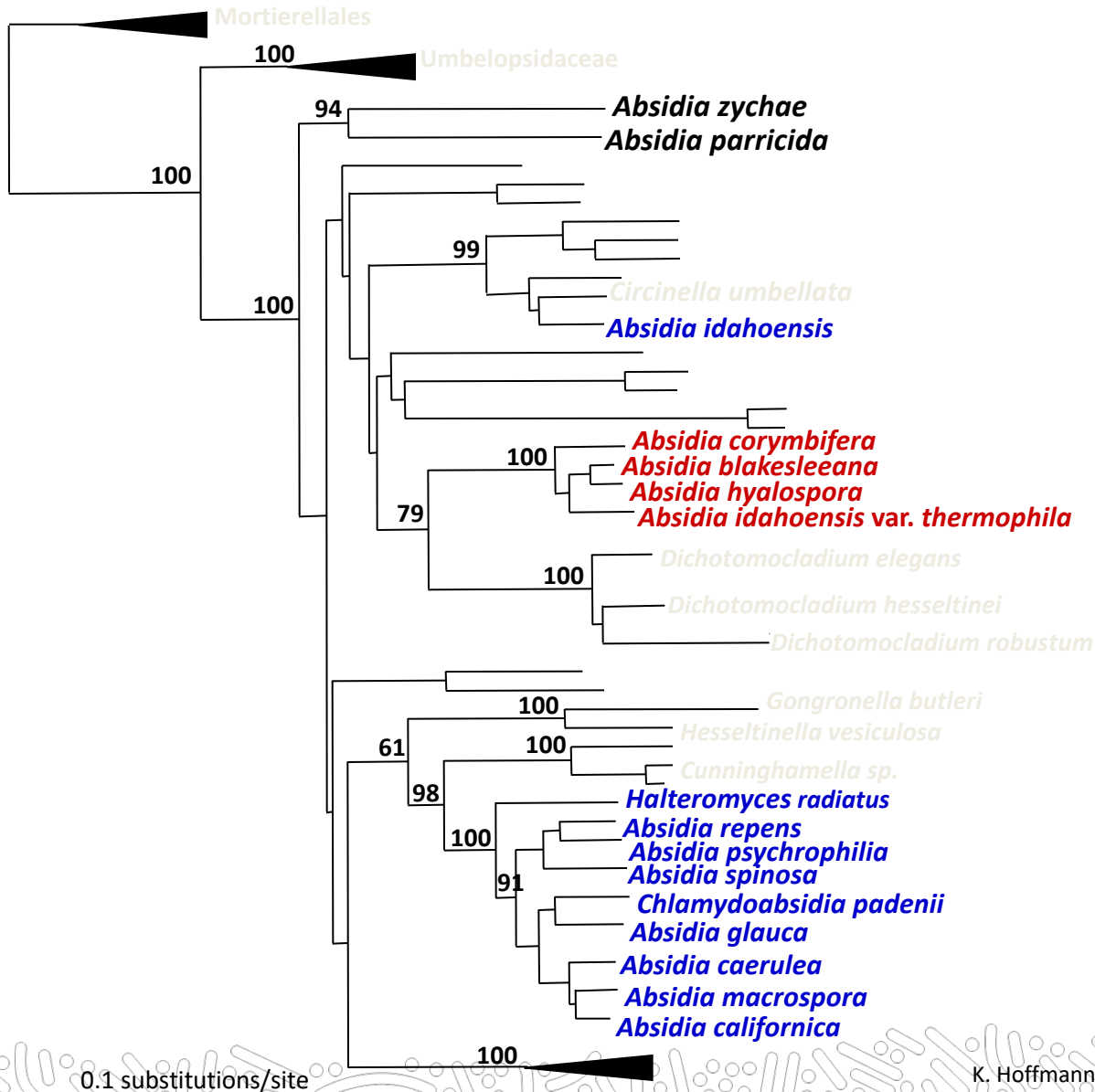


Zygospores: naked, equatorial ring

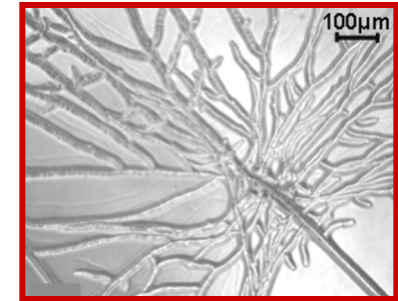


Zygospores: finger-like appendages

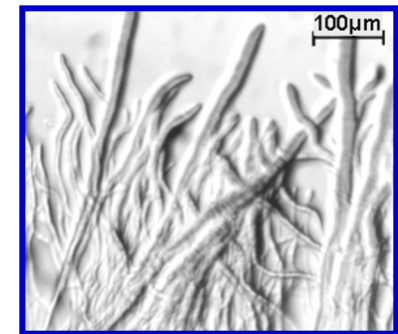
Absidia: polyphyletic origin



Substrate mycelium: sucker-like

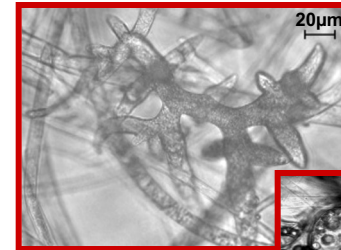
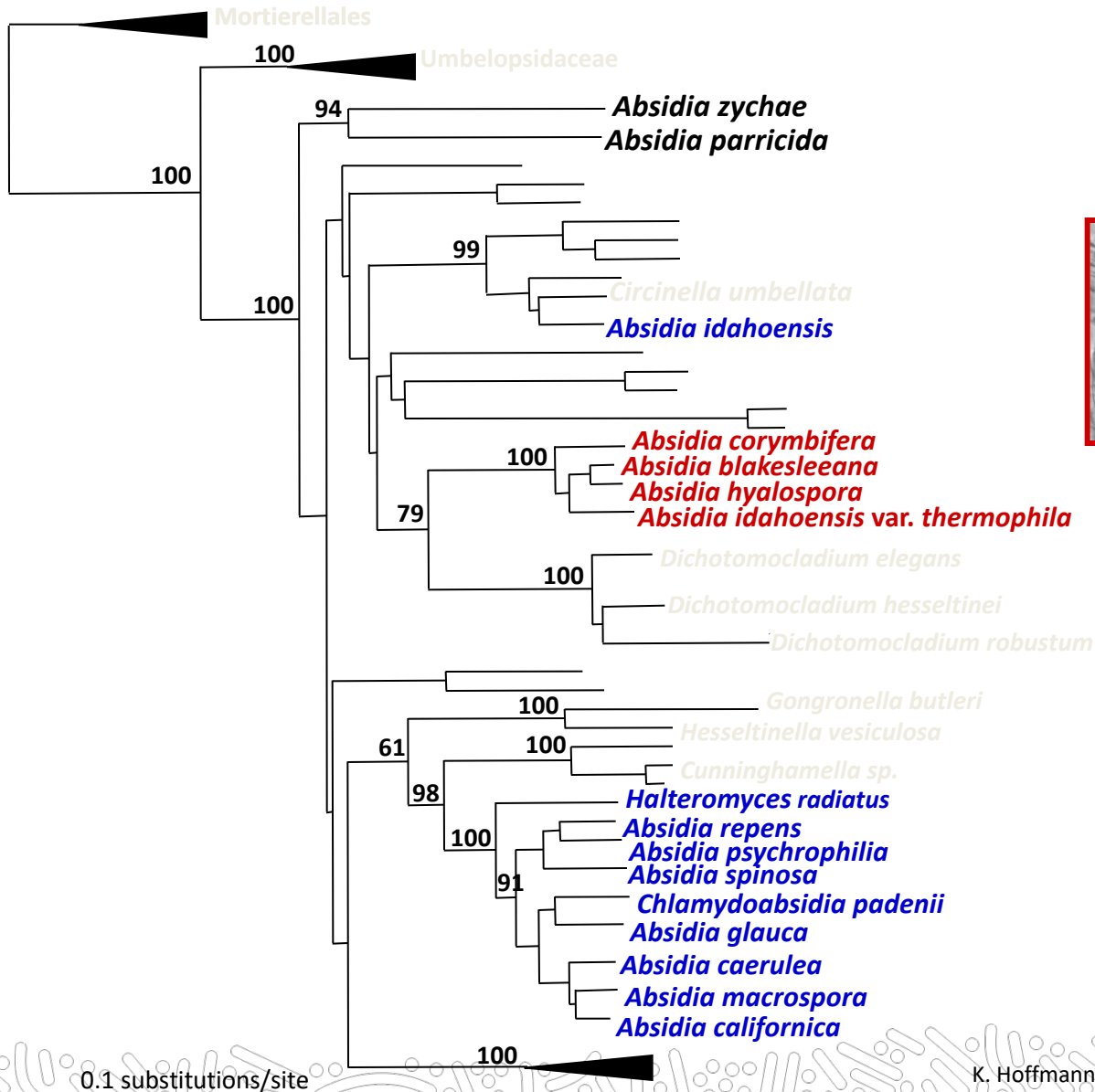


Substrate mycelium: tree-like



Substrate mycelium: straight

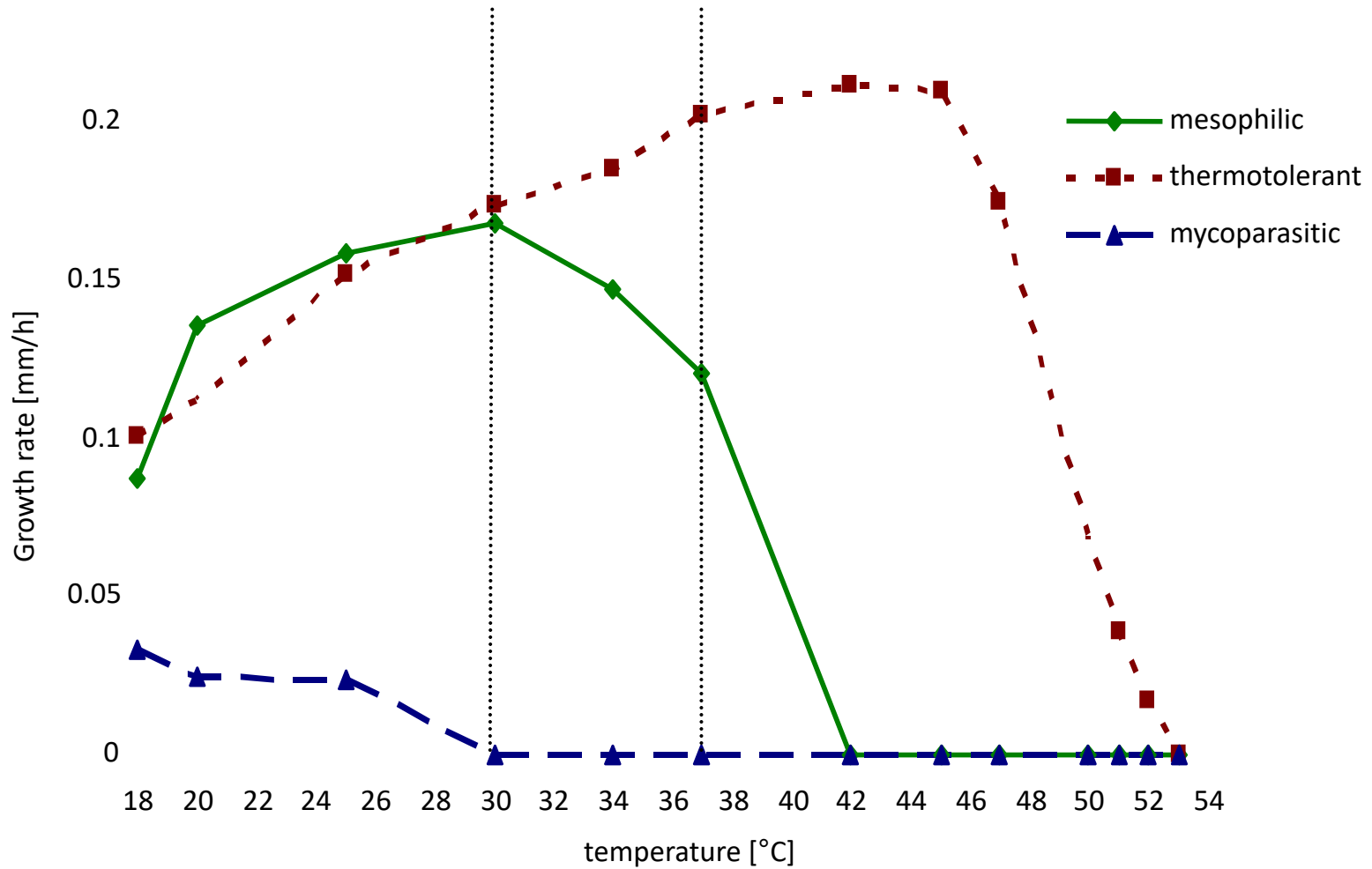
Absidia: polyphyletic origin



Giant cells: species dependent antler-like or bulbous; abundant

Absidia: growth physiology

Mortierellales



Signature sequences – Motifs of the nuc ITS 1

3`-end 18S rDNA 5`-end of ITS1 region

<i>Absidia coerulea</i> FSU 767(+)	GGATCATT	CAAATGGCGAGGTGGACGATTTATCCGACCCTTCAC
<i>Absidia coerulea</i> FSU 786(-)	GGATCATT	CAAATGGCGAGGTGGACGATTTATCCGACCCTTCAC
<i>Absidia cylindrospora</i> FSU 309	GGATCATT	CAAATGGCGAGGTGGACGATTTATCCGACCCTTCAC
<i>Absidia cylindrospora</i> FSU 906	GGATCATT	CAAATGCGGGGGTGGCTCTCTTTTCGGGGAGCTTTCT
<i>Absidia glauca</i> FSU 329(+)	GGTACATT	CAATGGCGGGGTAAGGCTTGCCTTCTCCCCAAATC
<i>Absidia glauca</i> FSU 330(-)	GGATCATT	CAATGGCGGGGTAAGGTTTCGCCCTTCTCCCCAAATC
<i>Absidia glauca</i> FSU 659(+)	GGTACATT	CAATGGCGGAATAAGGTTTCGCCCTTTTCCCCAAAT
<i>Absidia glauca</i> FSU 660(-)	GGTACATT	CAATGGCGGGGTGAAGACTTGCTCTTCTCCCCAAAT
<i>Absidia glauca</i> FSU 661(+)	GGTACATT	CAAATGGCGGGTAGAAGGTTTCGCCCTTTTCCCCAAA
<i>Absidia glauca</i> FSU 662(-)	GGTACATT	CAAATGGCGGGGTGAAGACTTGCACTTCTCCCCAAA
<i>Absidia orchidis</i> FSU 1608(-)	GGTACATT	CAAATGGCGAGGTGGAAGATTTATACGACCCTTCAC
<i>Absidia orchidis</i> FSU 323(-)	GGATCATT	CAAATGTAAGAGTGGATTAAGTATACGAGATATGCT
<i>Absidia repens</i> FSU 939	GGTACATT	CAAATGCGGCTCTGGTTCTCTTTTCGGGAGGAGGGCA
<i>Absidia spinosa</i> FSU 550	GGTACATT	CAGATGTGGGGTGGCCATAGGTCCTTACTTTTTTAA
<i>Absidia spinosa</i> FSU 551	GGTACATT	CAAATGGATTGGGTTTGCTATACGGTAATCCGATTT CARWKGBVD
<i>Absidia parricida</i> FSU 547	GGATCATT	CATGTTTATGACTGAGCAATTTCTCTGAAAAGAGGA
<i>Absidia parricida</i> FSU 917	GGTACATT	CATGTTTATGACTGAGCAATTTCTCTGAAAAGAGGG
<i>Absidia blakesleeana</i> FSU 2684(+)	GGTACATT	CTGAGAGGT CTCTTCTGTTCTCCAGTATGTGCAAT
<i>Absidia corymbifera</i> [AB054042]*	GGATCATT	CTGAGAGGT CTAAAAAACCACTAGTTGGGGGTCTCT
<i>Absidia corymbifera</i> FSU 787(+)	GGTACATT	CTGAGAGGT TAAAAACCGCATAGCGGGATATTCCT
<i>Absidia corymbifera</i> FSU 788(-)	GGTACATT	CTGAGAGGT AAAACCACTAGCGGGAATCTTTCCCT
<i>Absidia corymbifera</i> FSU 938(+)	GGTACATT	CTGAGAGGT TATTAAGCCTTGGCGGGTTGGCCTAAC

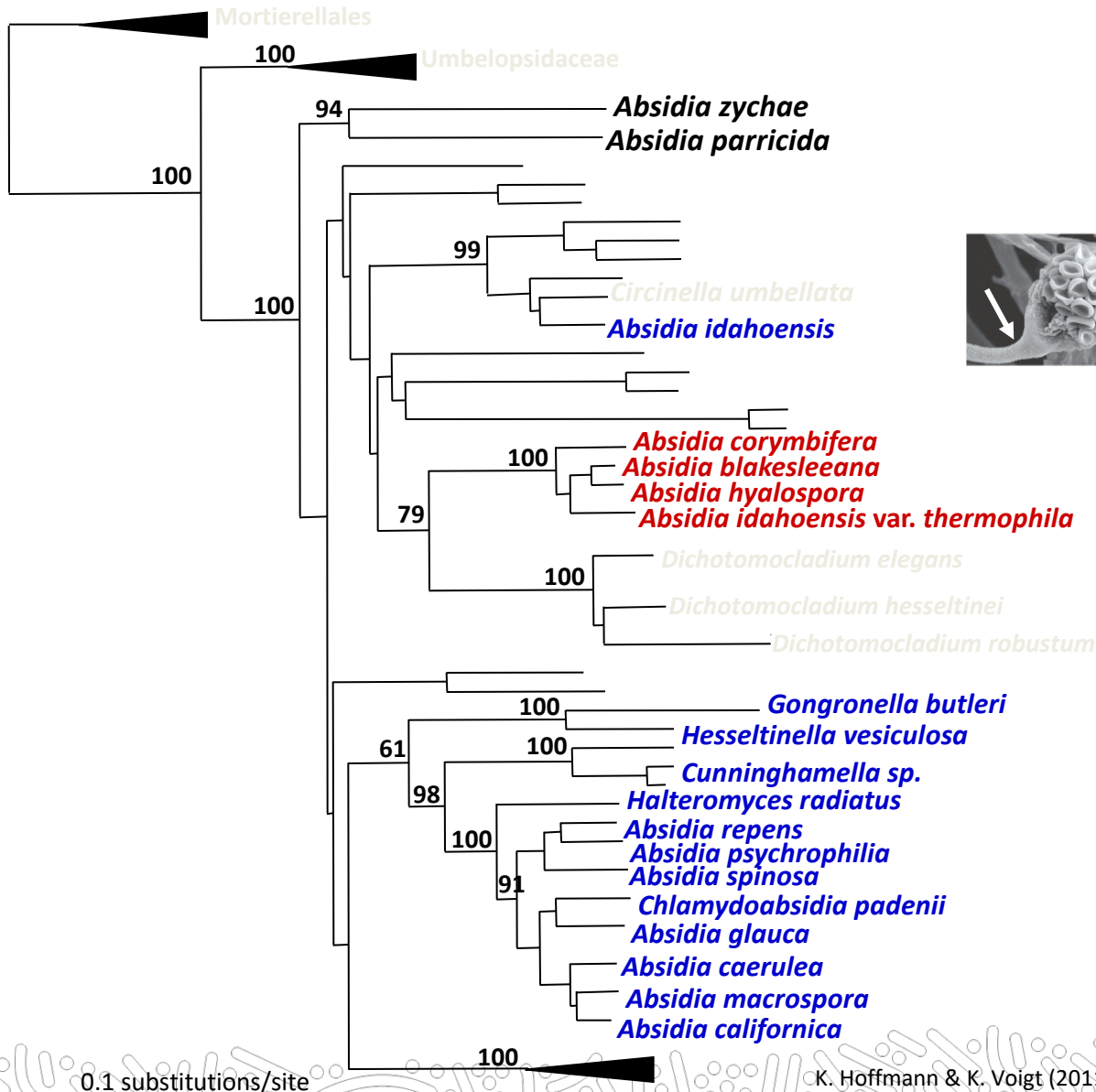
mesophilic

mycoparasitic

thermotolerant

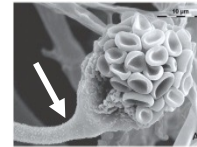
*... obtained from The National Centre for Biotechnology Information at www.ncbi.nlm.nih.gov

Lichtheimia: the introduction of Lichtheimiaceae fam. nov.



} **Lentamyces gen. nov.**

K. Hoffmann & K. Voigt (2009) Plant Biology 11: 537-554.



Mis-application
Circinella idahoensis

Lichtheimia

} **Lichtheimiaceae
fam. nov.**

K. Hoffmann, G. Walther
& K. Voigt (2009) Mycol.
Res. 113: 277-278.

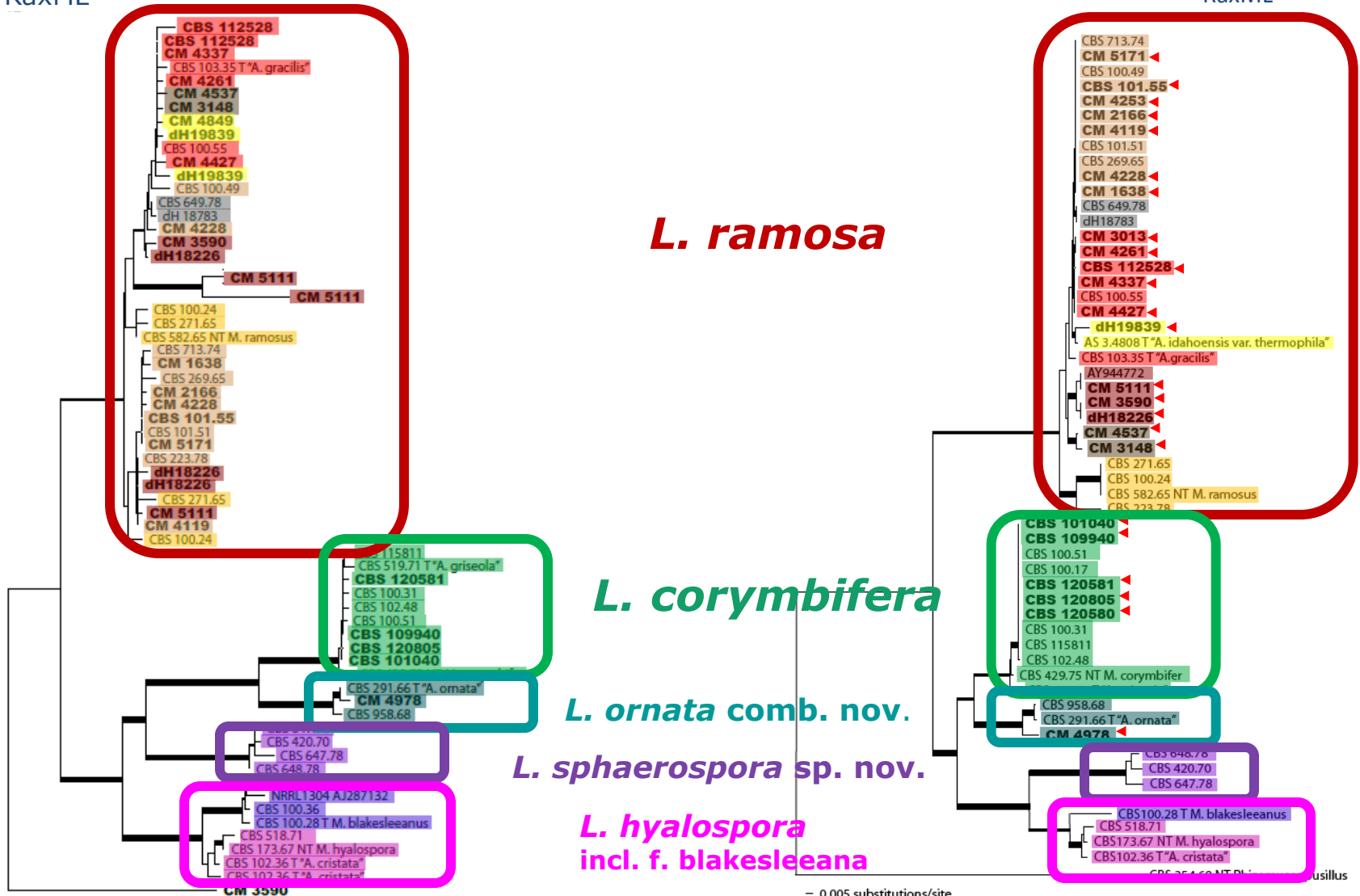
} **Cunninghamellaceae**

Naumov ex R.K. Benj., *Aliso* 4: 415 (1959)

Phylogenetic species recognition

Actin, Nj
Bt: Nj;
RaxML

ITS, Nj
Bt: Nj;
RaxML



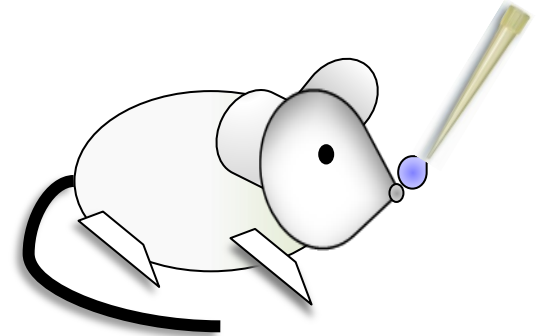
Establishment of a mouse model

Isolate	Designation in strain collection	Designation in this study	Source
<i>L. corymbifera</i>	JMRC:FSU:09682	Reference strain	Environment
<i>L. corymbifera</i>	JMRC:FSU:10164	LCJ5	Environment
<i>L. corymbifera</i>	JMRC:FSU:10061	LCJ3	Human
<i>L. corymbifera</i>	JMRC:FSU:10240	LCJ9	Human
<i>L. ramosa</i>	JMRC:FSU:11	LCJ22	Human

788; Luo *et al.*

Schulze *et al.* (2017) Ketoacidosis alone does not predispose to mucormycosis by *Lichtheimia* in a murine pulmonary infection model. *Virulence*. doi: 10.1080/21505594.2017.1360460

Intranasal infection



L. corymbifera type strain

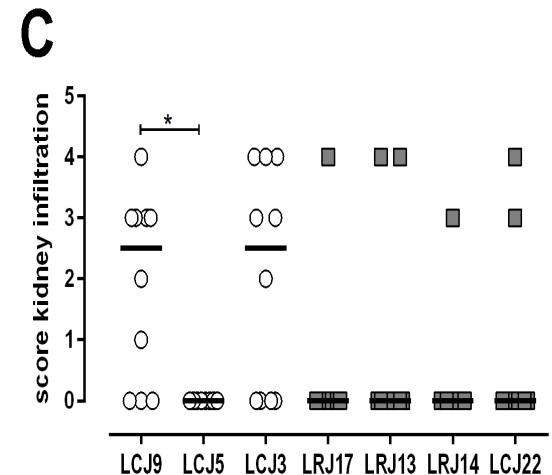
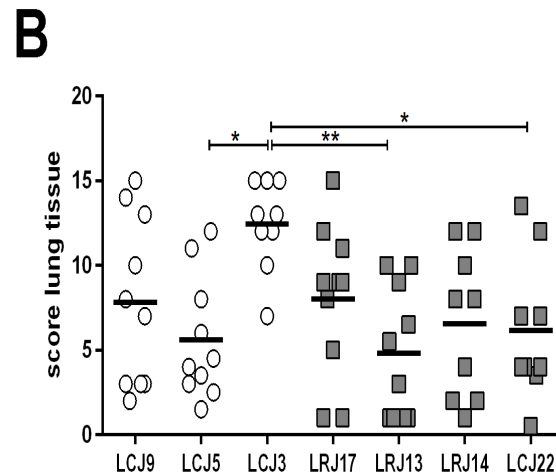
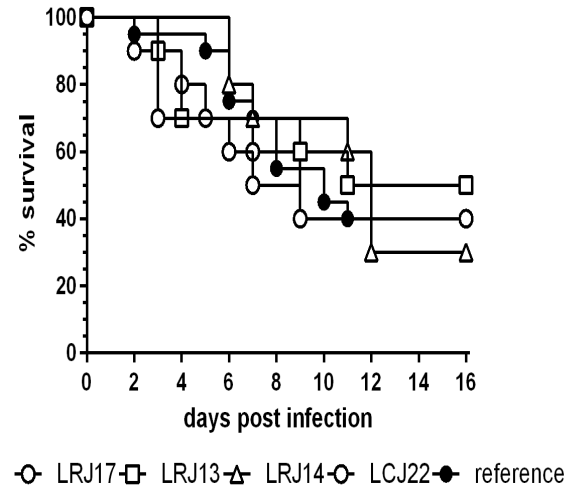
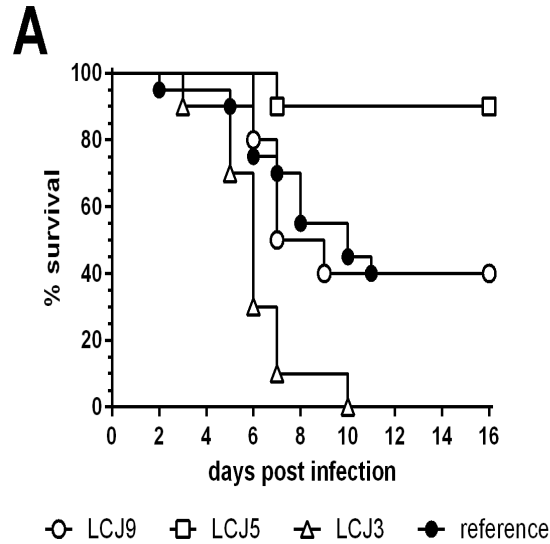
- average and reproducible virulence potential (clinical species)
- average *in vitro* phenotype (growth, stress resistance)
- Mice: Immunocompetent vs. streptozotocin vs. cortison
- n=5-10 mice/group/experiment

Strain specific virulence of different *Lichtheimia* isolates in a mouse model of pulmonary infection

(A) Survival of immunocompromised female CD-1 mice intranasally infected with 5×10^6 spores of *L. corymbifera* and *L. ramosa* strains. Combined data from two independent experiments (n=5 mice/group/experiment). ** $p < 0.01$, determined by Log-rank (Mantel-Cox) test.

(B) Analysis of histological scores from lung and kidneys

(C) (n=8-10 mice/group). Statistical significance was calculated using one-way ANOVA with a Tukey's multiple comparison test, * $p < 0.05$ and ** $p < 0.01$.



Summary

- Proper diagnostics requires many isolates
- Growth kinetics is indicative for the systematics of *Lichtheimia*
- Isolates of *Lichtheimia* differ in virulence in mouse
- Haploid genomes are common, diploid genomes are exceptional in *Lichtheimia* due to decreased stress tolerance and virulence



Thank you



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und Infektionsbiologie
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Olaf Kniemeyer
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Infection Biology

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Applied Systems Biology

Marc Thilo Figge
Zoltán Cseresnyés

Microbial Immunology

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Thank you

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