



# Microorganismal diversity within lichens

Kristiina Mark



# Lichens

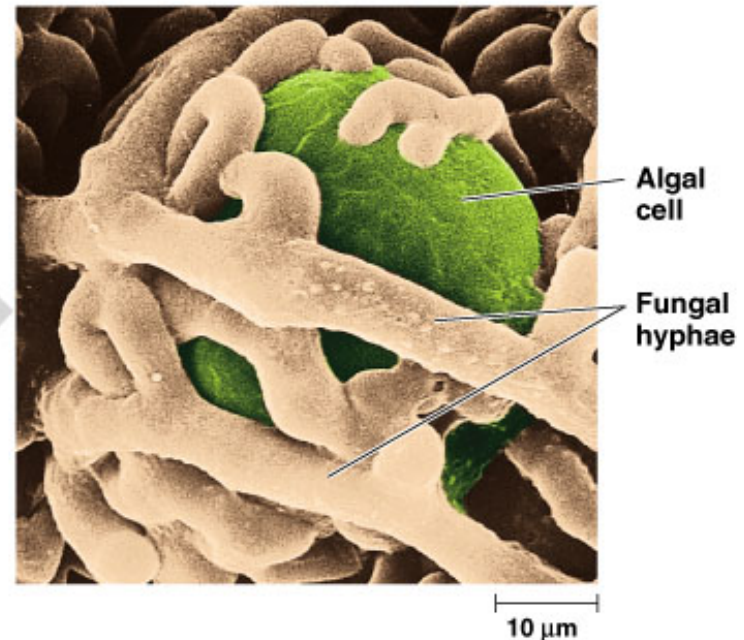
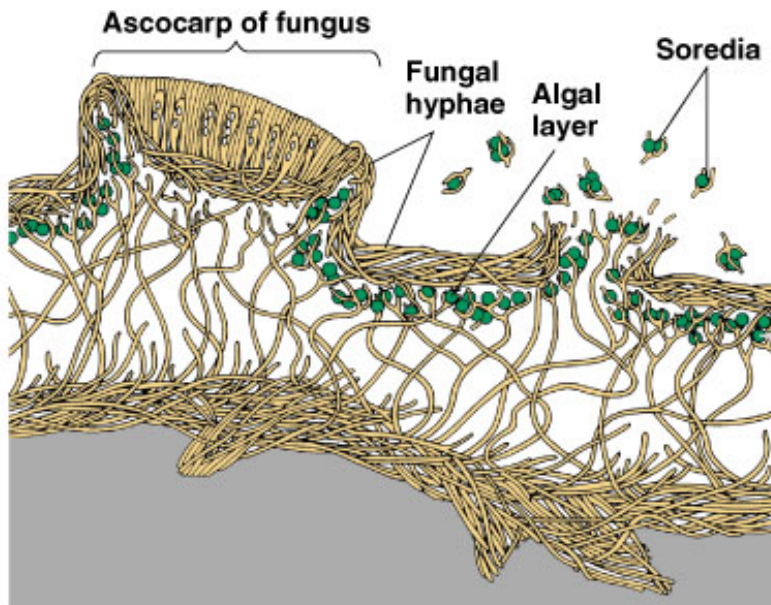
Simon Schwendener:  
lichens are a combination of fungi with algae or cyanobacteria (1867)





# Lichen symbiosis

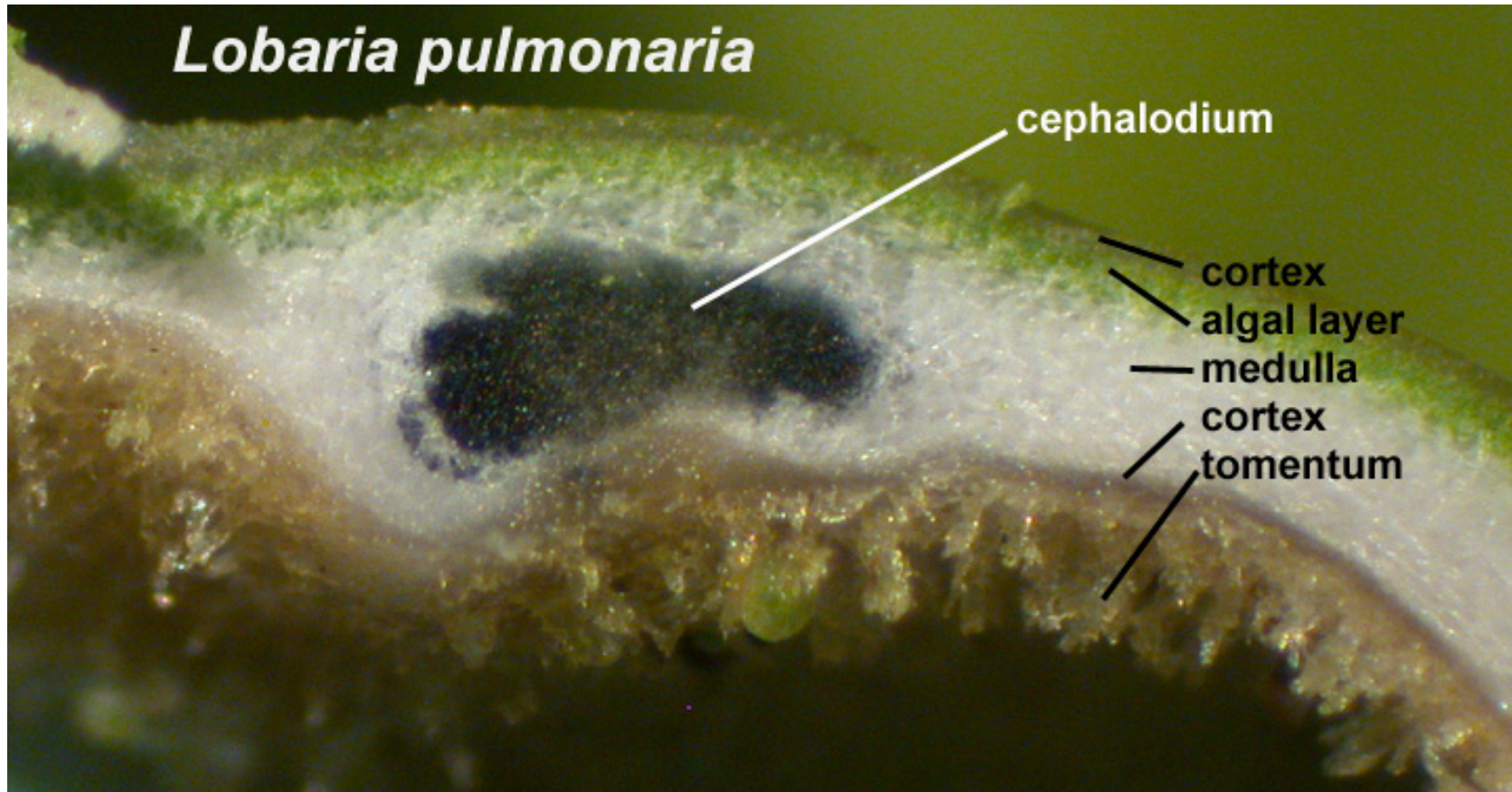
- Mutualism or controlled parasitism between the fungal partner (mycobiont) and photosynthetic partner/s (photobiont/s)





# Tripartite lichens

Green algae + cyanobacteria as photobionts



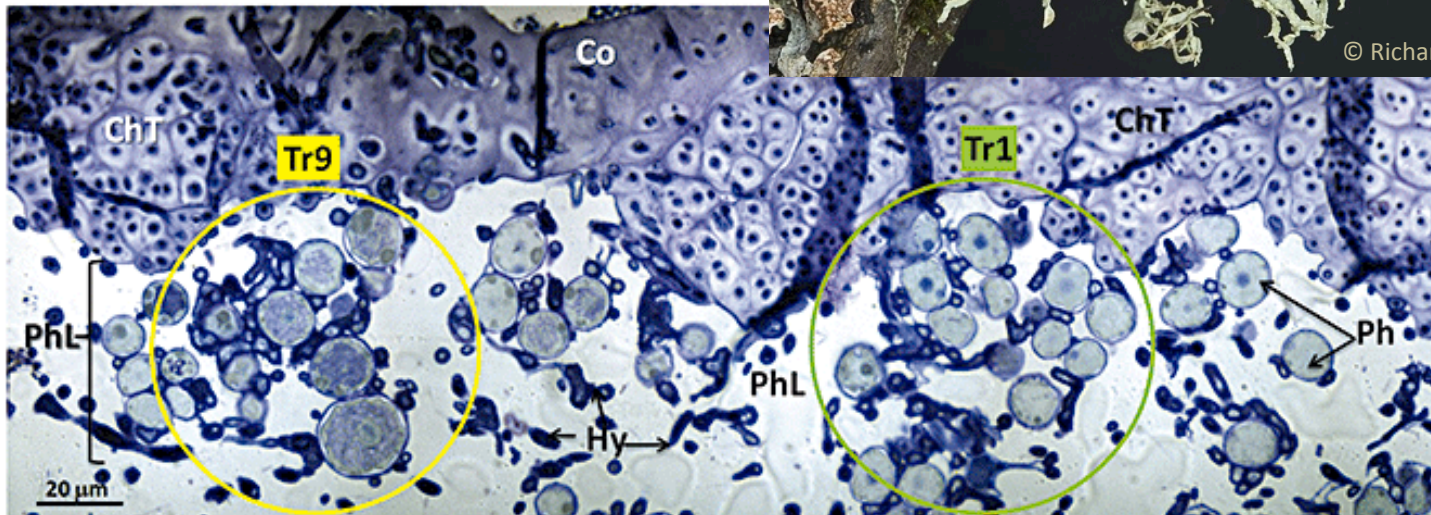


# Tripartite lichens

Two *Trebouxia* species in *Ramalina farinacea*



© Richard Droker



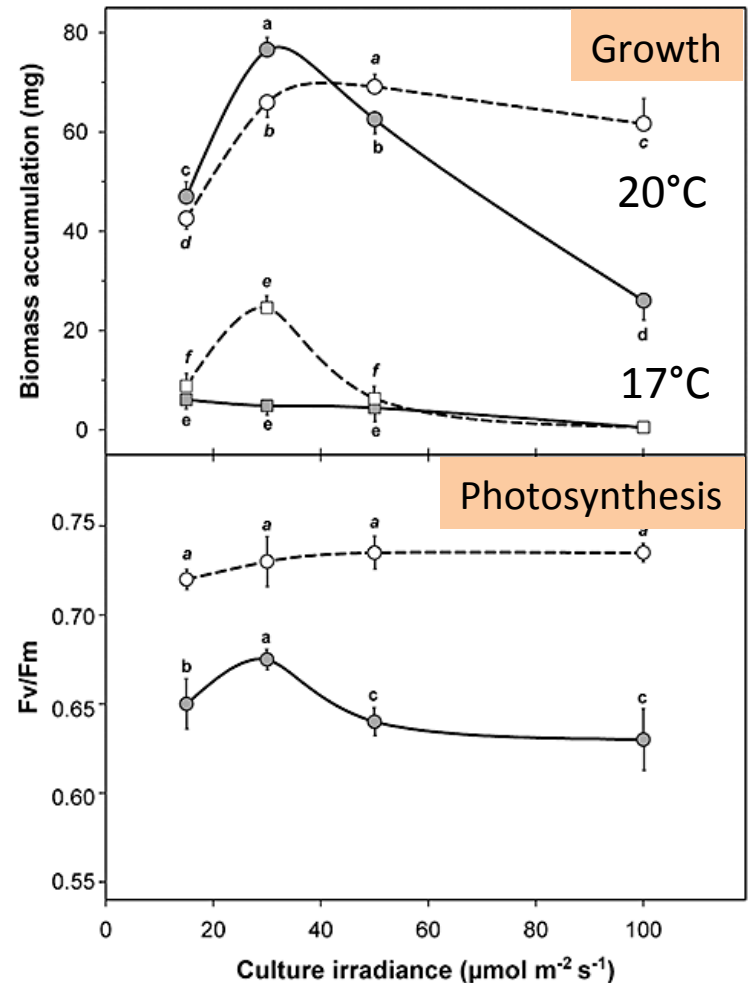
Casano et al. (2011) Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus Competition?. *Environmental Biology* 13(3): 806-818.

# Tripartite lichens

Two *Trebouxia* species in *Ramalina farinacea*

– Different physiological performances in response to environment

– TR9 performed better in higher temperature and radiance





# Additional fungal symbiont?

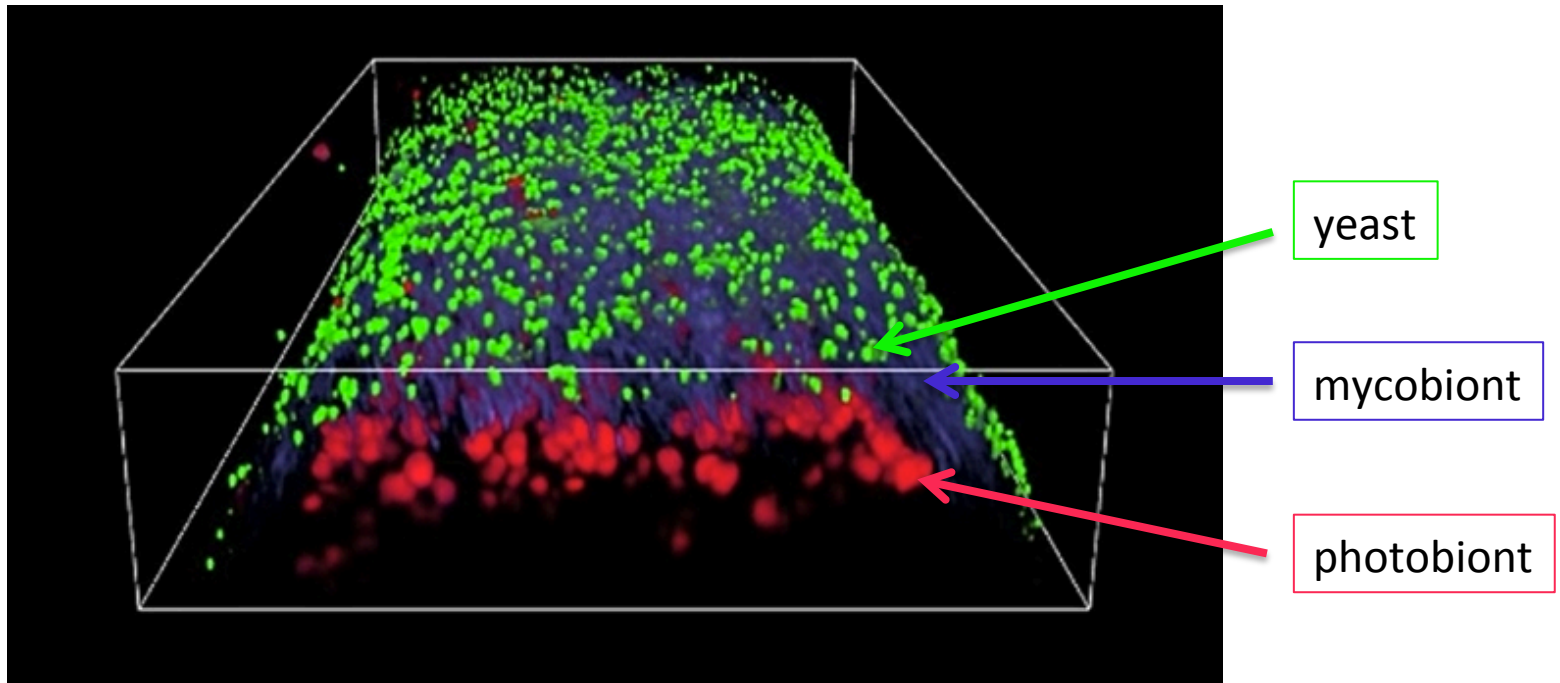
Science

REPORTS

Cite as: Spribille *et al.*, *Science*  
10.1126/science.aaf8287 (2016).

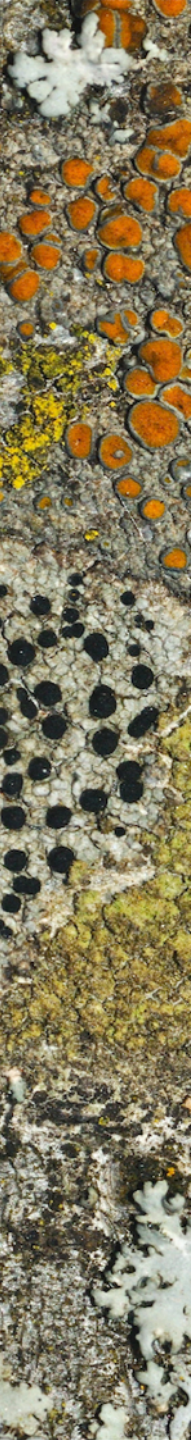
## Basidiomycete yeasts in the cortex of ascomycete macrolichens

Toby Spribille,<sup>1,3\*</sup> Veera Tuovinen,<sup>3,4</sup> Philipp Resl,<sup>1</sup> Dan Vanderpool,<sup>3</sup> Heimo Wolinski,<sup>5</sup> M. Catherine Aime,<sup>6</sup>  
Kevin Schneider,<sup>1,†</sup> Edith Stabentheiner,<sup>1</sup> Merje Toome-Heller,<sup>6,‡</sup> Göran Thor,<sup>4</sup> Helmut Mayrhofer,<sup>1</sup> Hanna  
Johannesson,<sup>2</sup> John P. McCutcheon<sup>3,7</sup>



# Additional fungal symbiont?

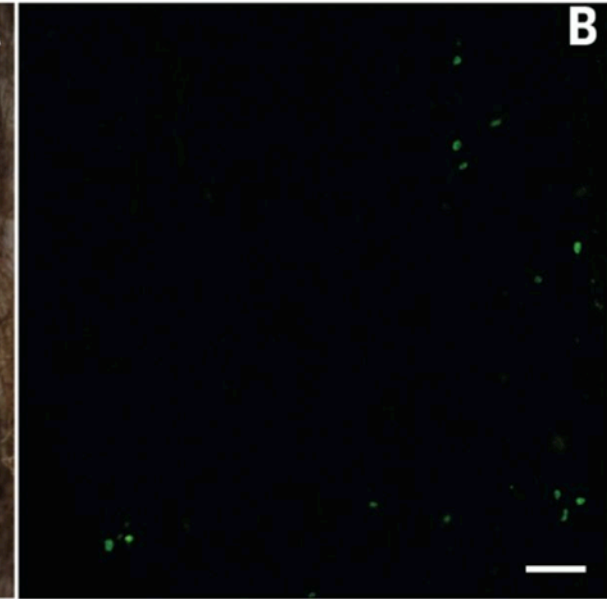
The abundance of yeast correlated with previously unexplained variation in lichen phenotype



*Bryoria fremontii*



A

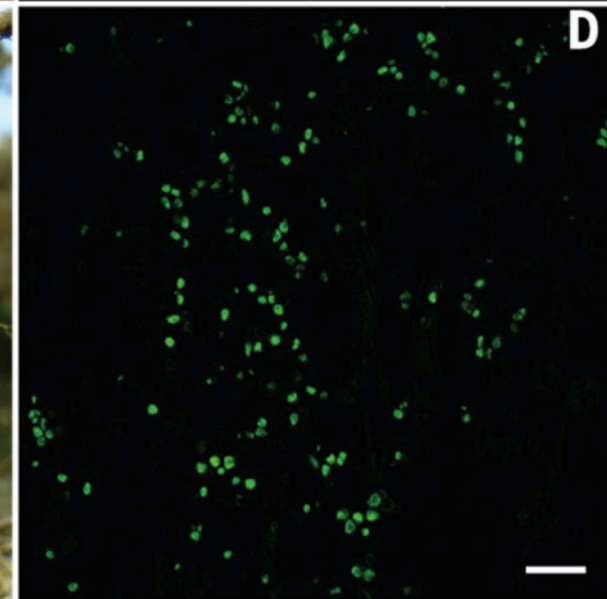


B

*Bryoria tortuosa*



C

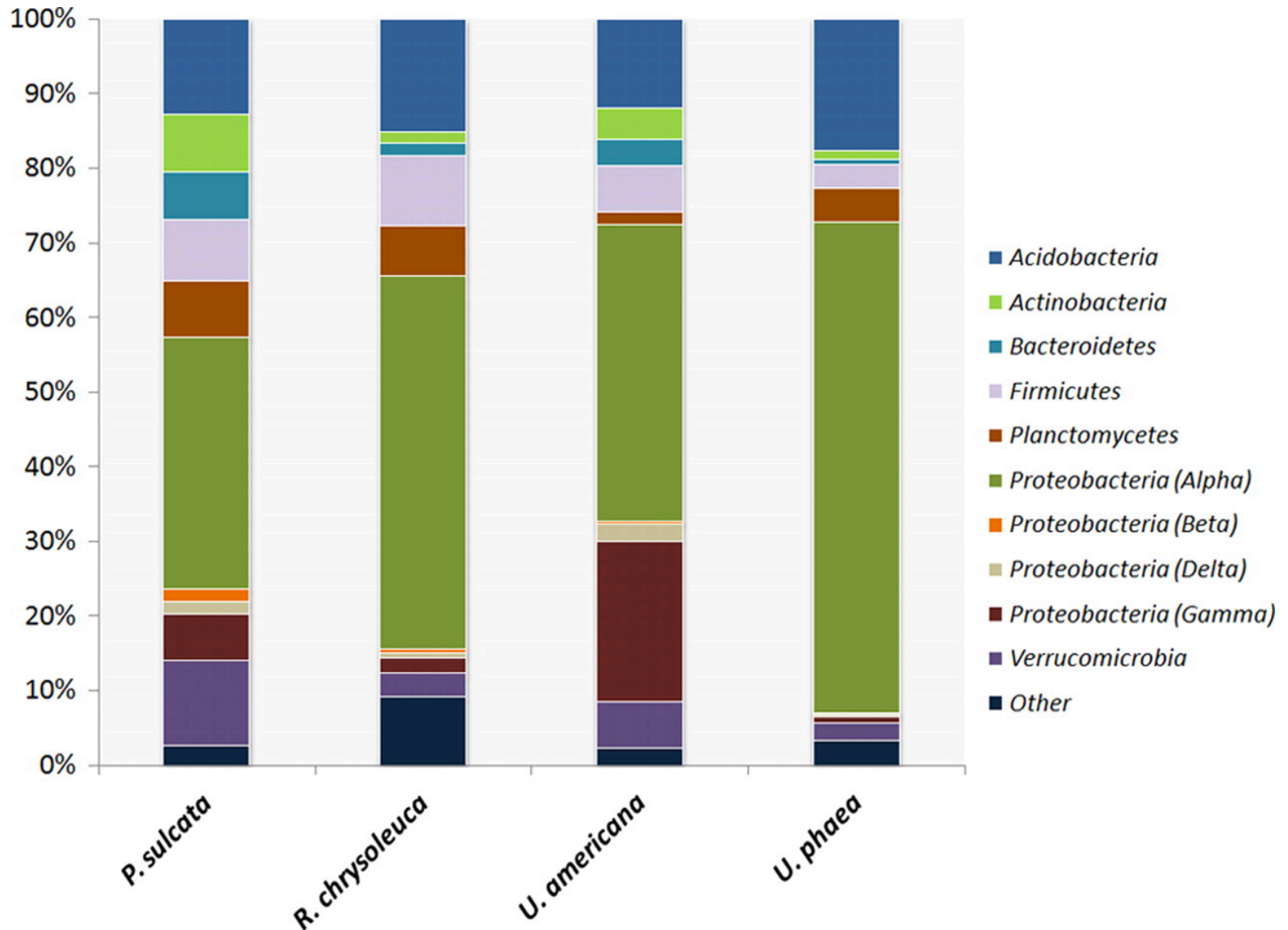


D

Spribile et al. (2016) Bacidiomycete yeasts in the .cortex of ascomycete macrolichens Sciencel Reports.

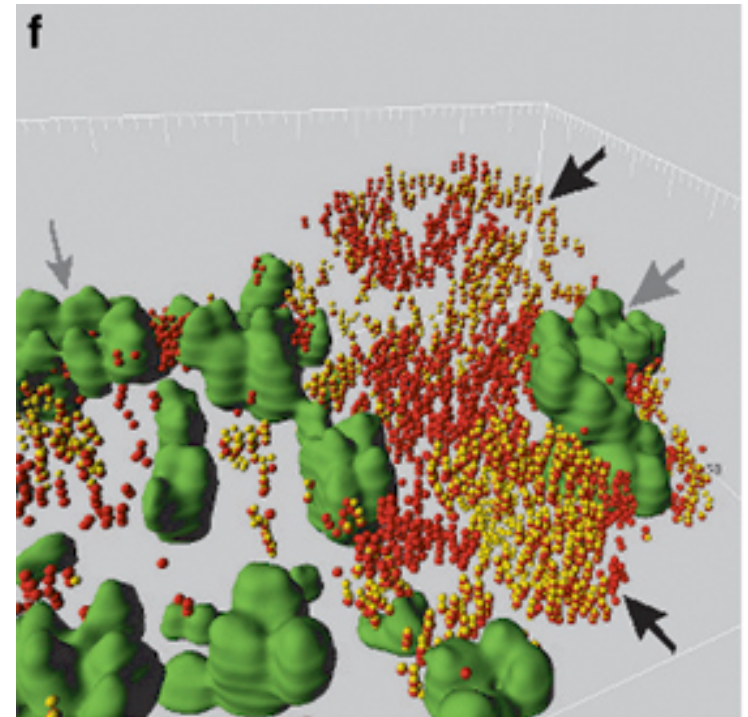
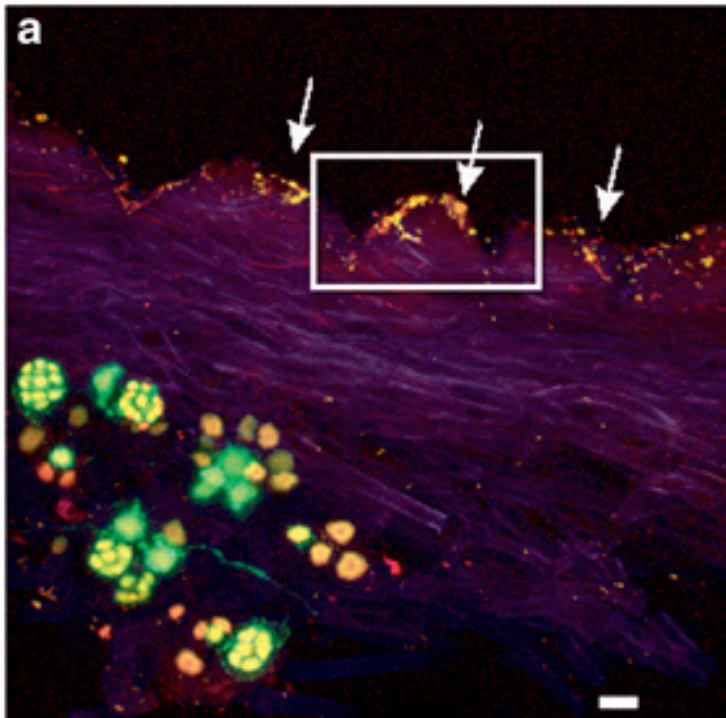


# Lichen-specific bacterial communities



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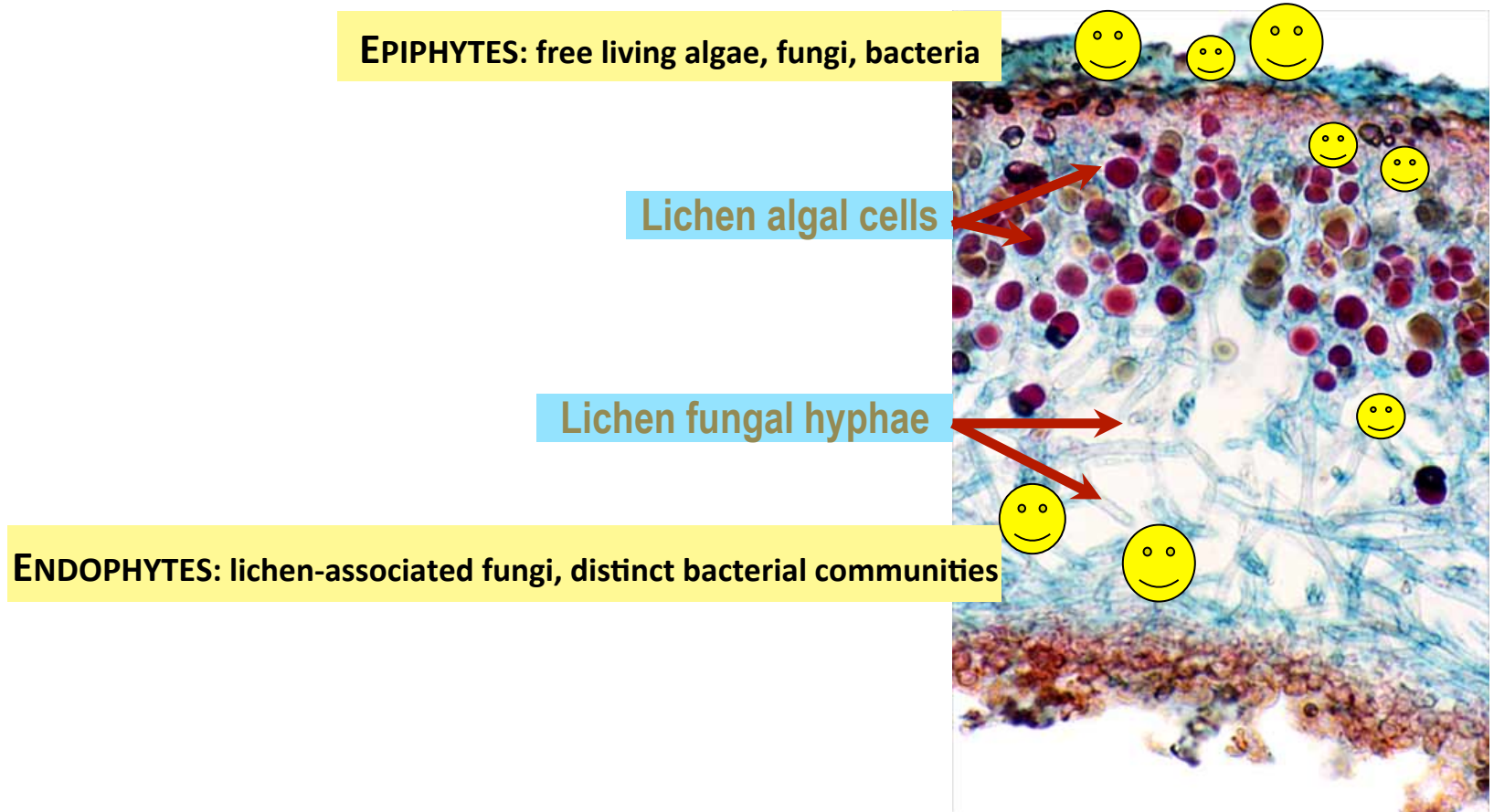
- Highly structured, biofilm-like on fungal surfaces
- Mostly *Alphaproteobacteria*
- Distinct microbial patterns for each lichen species
- Involvement in nutrient cycling, lytic activities, hormone production, phosphate mobilization, and antagonistic activity toward other microorganisms





# Lichen as a microhabitat

- Protection from high radiance & drying
- Unique antibacterial and antifungal chemistry



# Lichen-associated fungi

– Symptomatic (incl. lichenicolous fungi)

Necrotrophic to biotrophic interactions

*Biatoropsis usnearum* on *Usnea subfloridana*



*Vouauxiella verrucosa* on *Lecanora campestris*



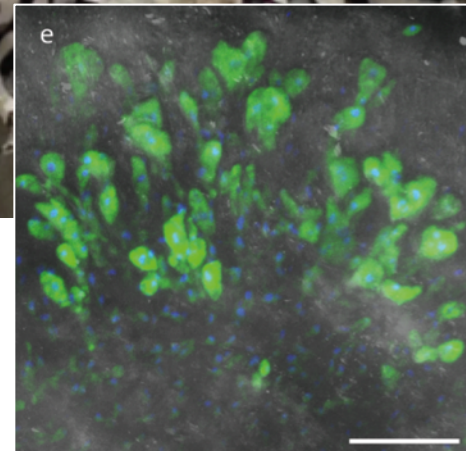
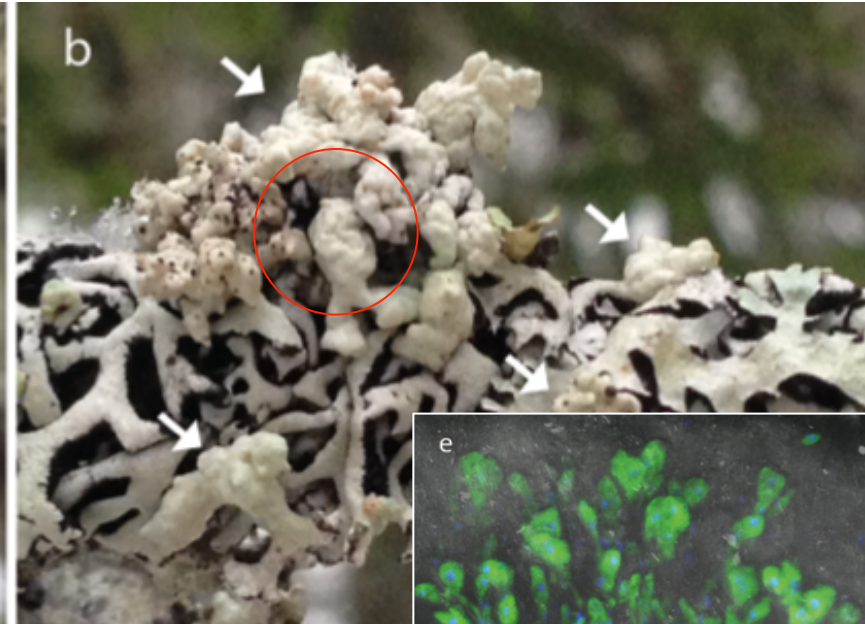


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Necrotrophic to biotrophic interactions

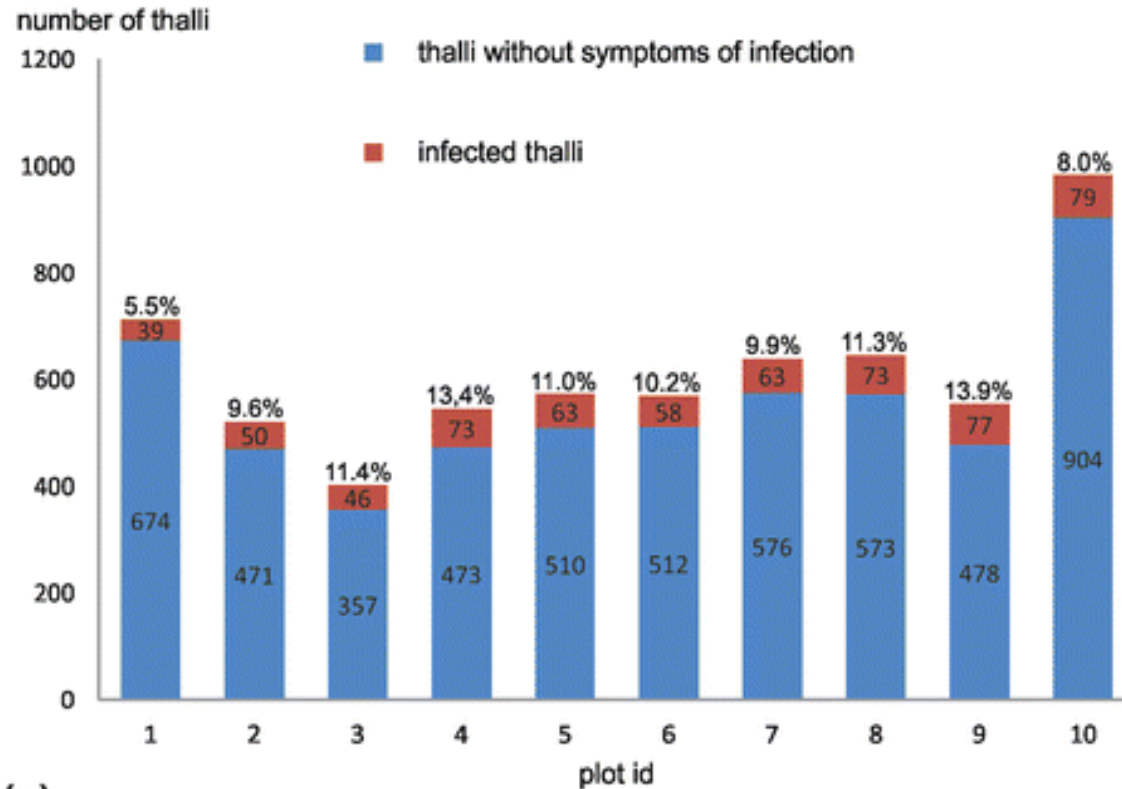
*Cyphobasidium hypogymniicola* in the regular thallus (a) and galls (b) of *Hypogymnia physodes*



# Lichen-associated fungi

## – Asymptomatic

High, mostly undescribed diversity; various interactions

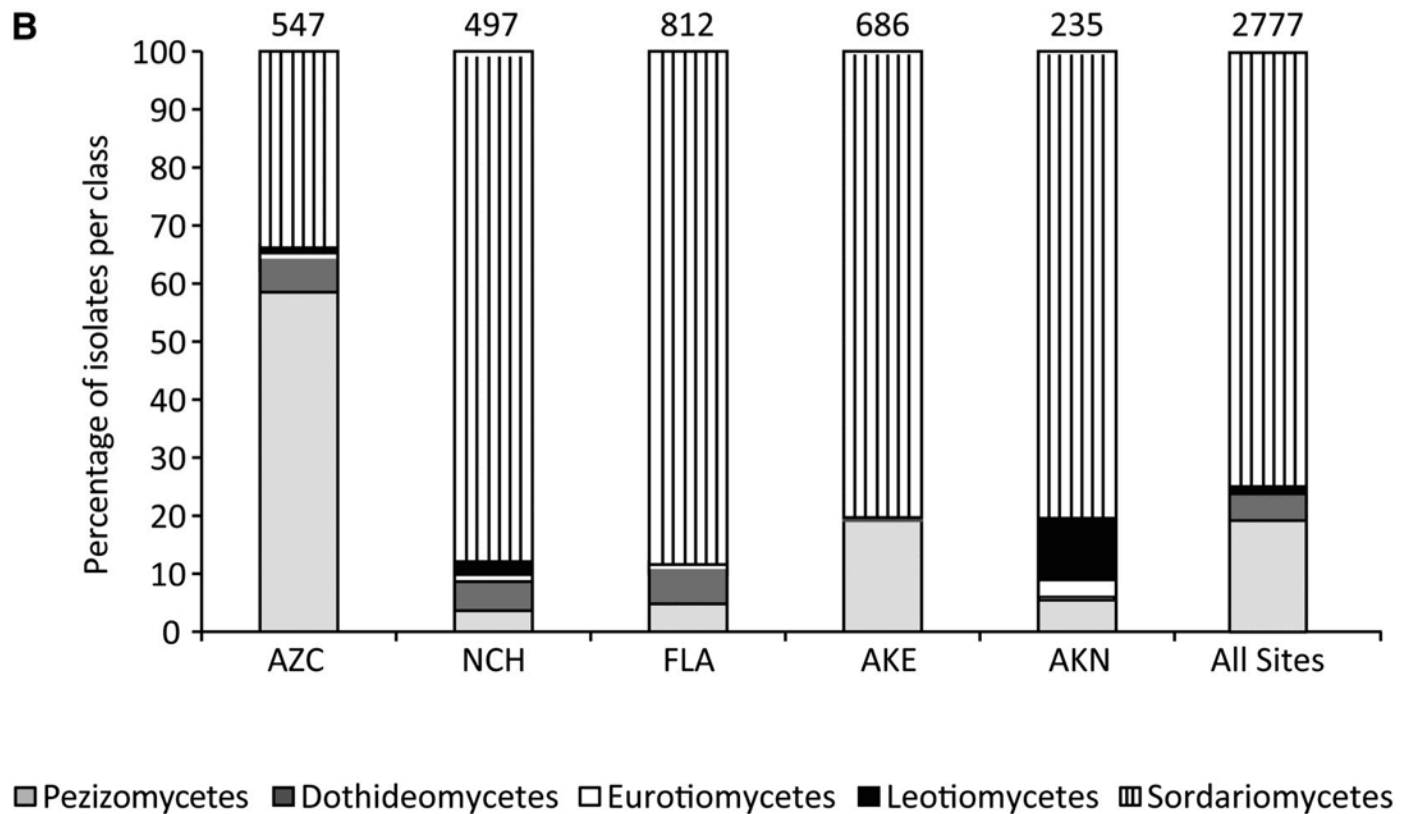




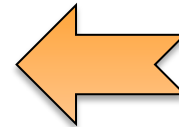
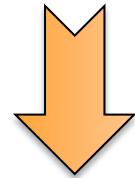
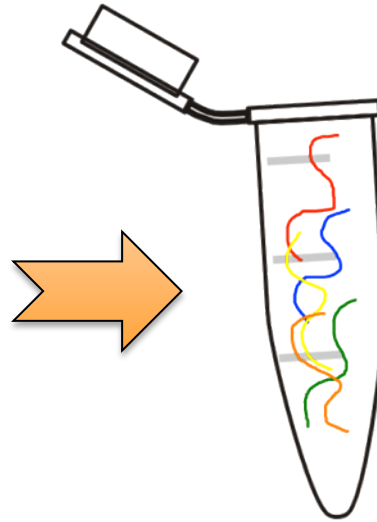
# Lichen-associated fungi

## – Asymptomatic

species richness and composition highly variable in sites and species



# Metabarcoding



1. Species A
2. Species B
3. Species C



# Molecular methods

100 lichen specimens

[1] DNA extraction

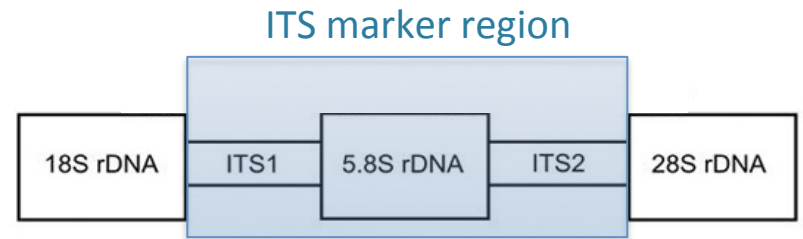
[2] PCR amplification

Hi-Fidelity polymerase

Standard fungal specific ITS primers: *ITS1F* & *ITS4*

[3] Amplicon sequencing in Roche/454 GS FLX+ system

[4] Data processing and OTU clustering (97%)





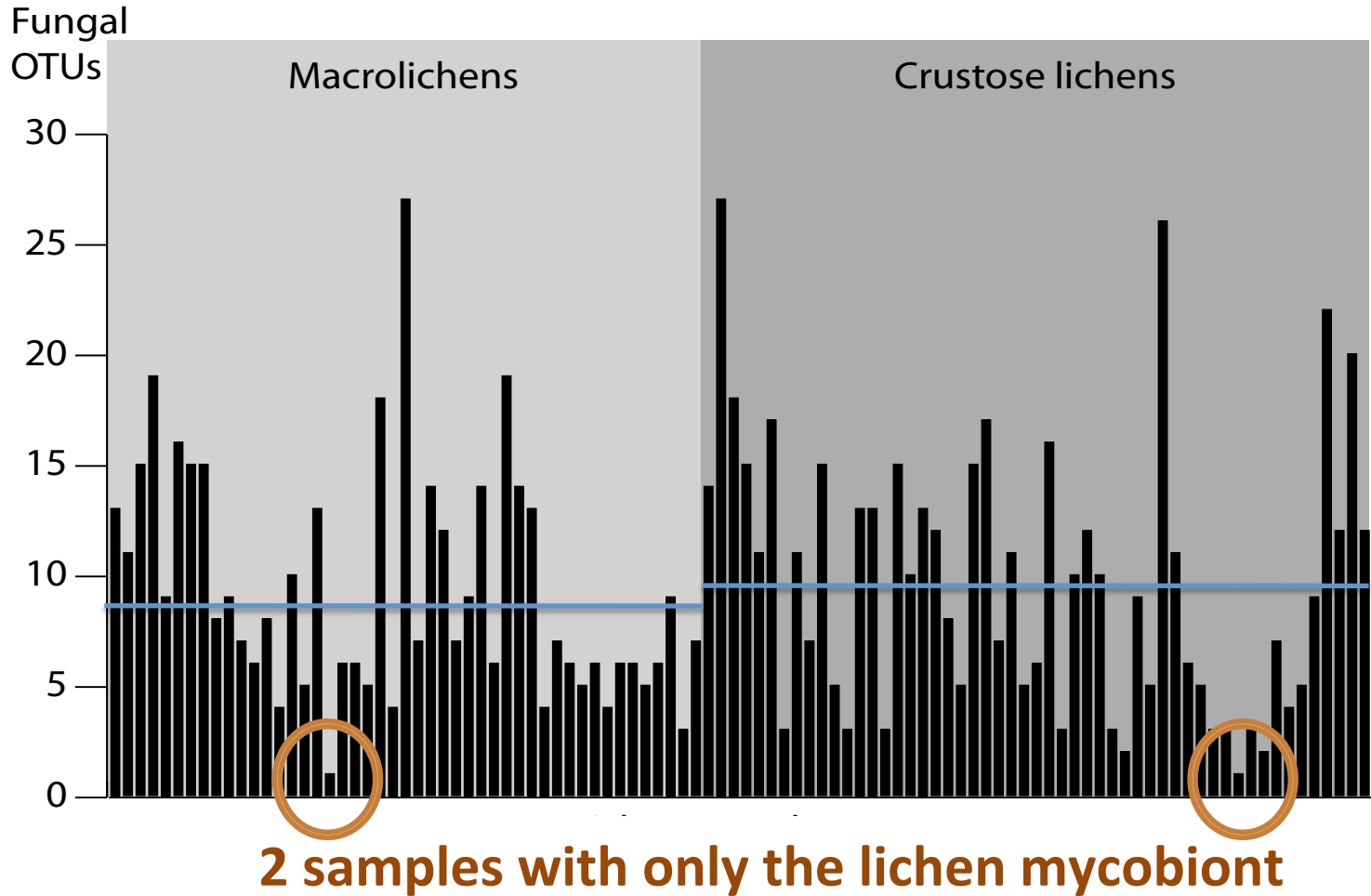
# Methods: Lichen-associated fungi

PCR products	100
Sequences total	128 449
Mean no of reads per sample	1285
Average length (bp)	615
Average quality score	32.8
Average no of clusters per sample at 95%	88
Barcodes	1171
Fungal OTUs	567



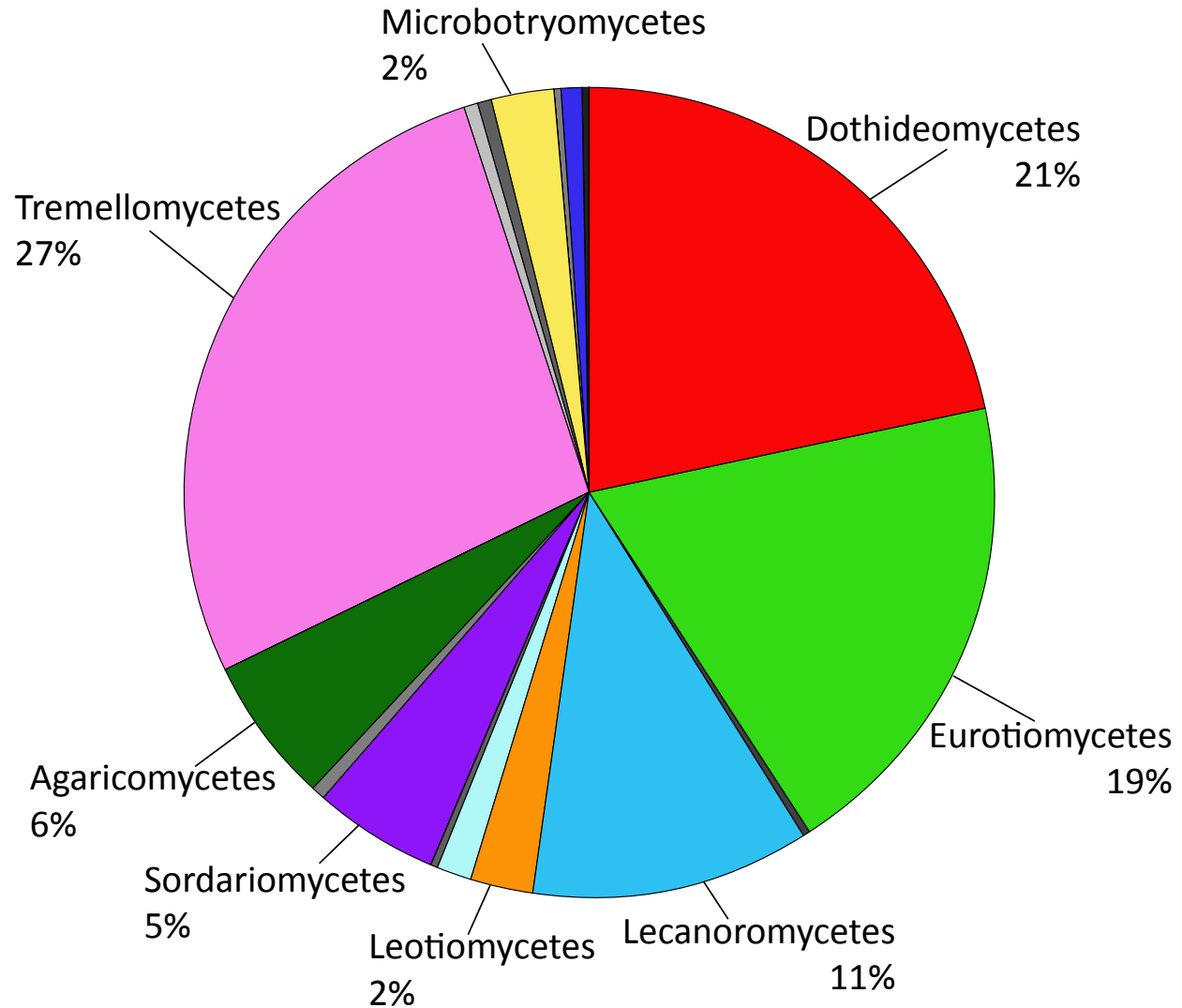
# RESULTS: Lichen-associated fungi

On average about 10 OTUs per lichen sample



# RESULTS: Lichen-associated fungi

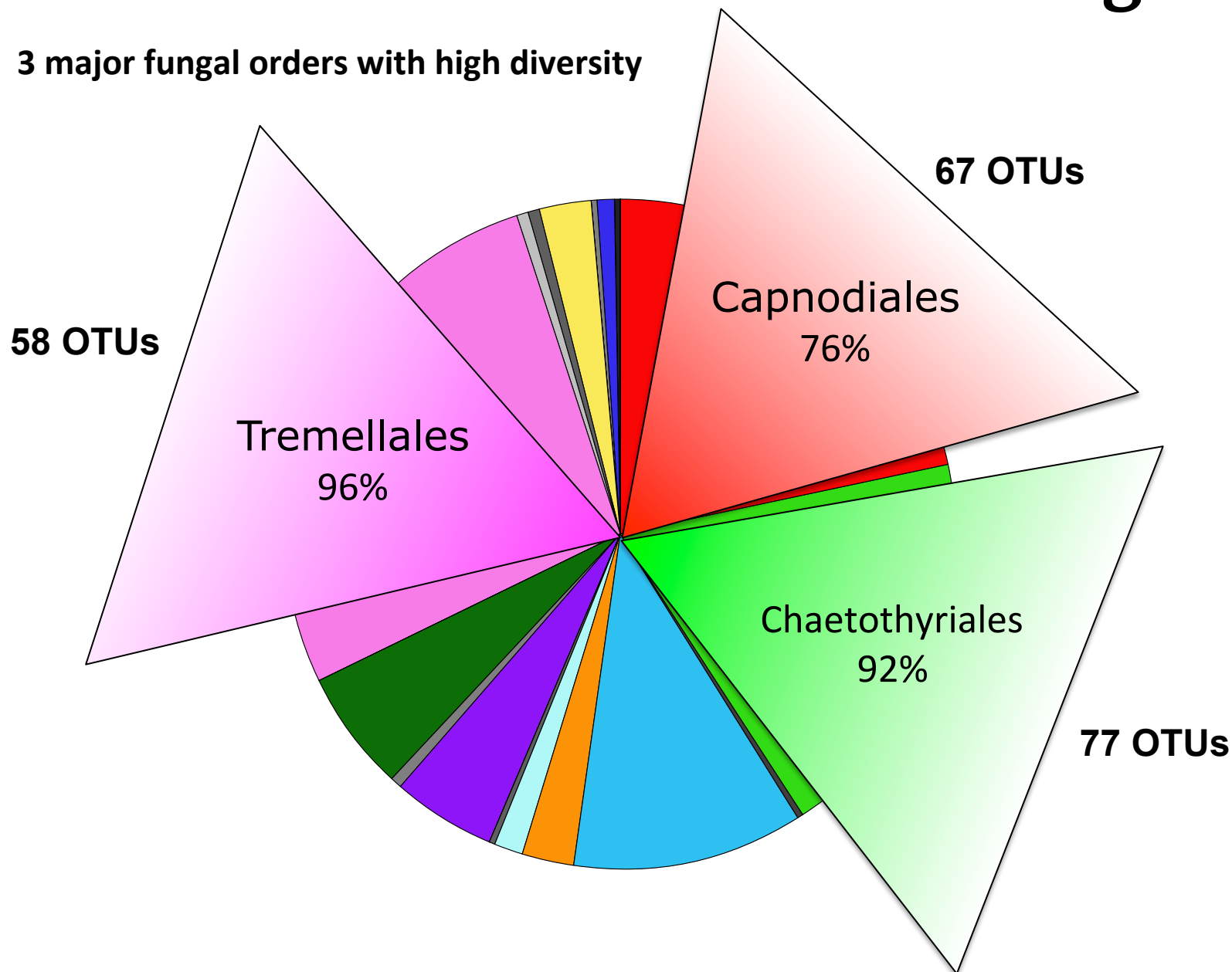
22 different fungal classes





# RESULTS: Lichen-associated fungi

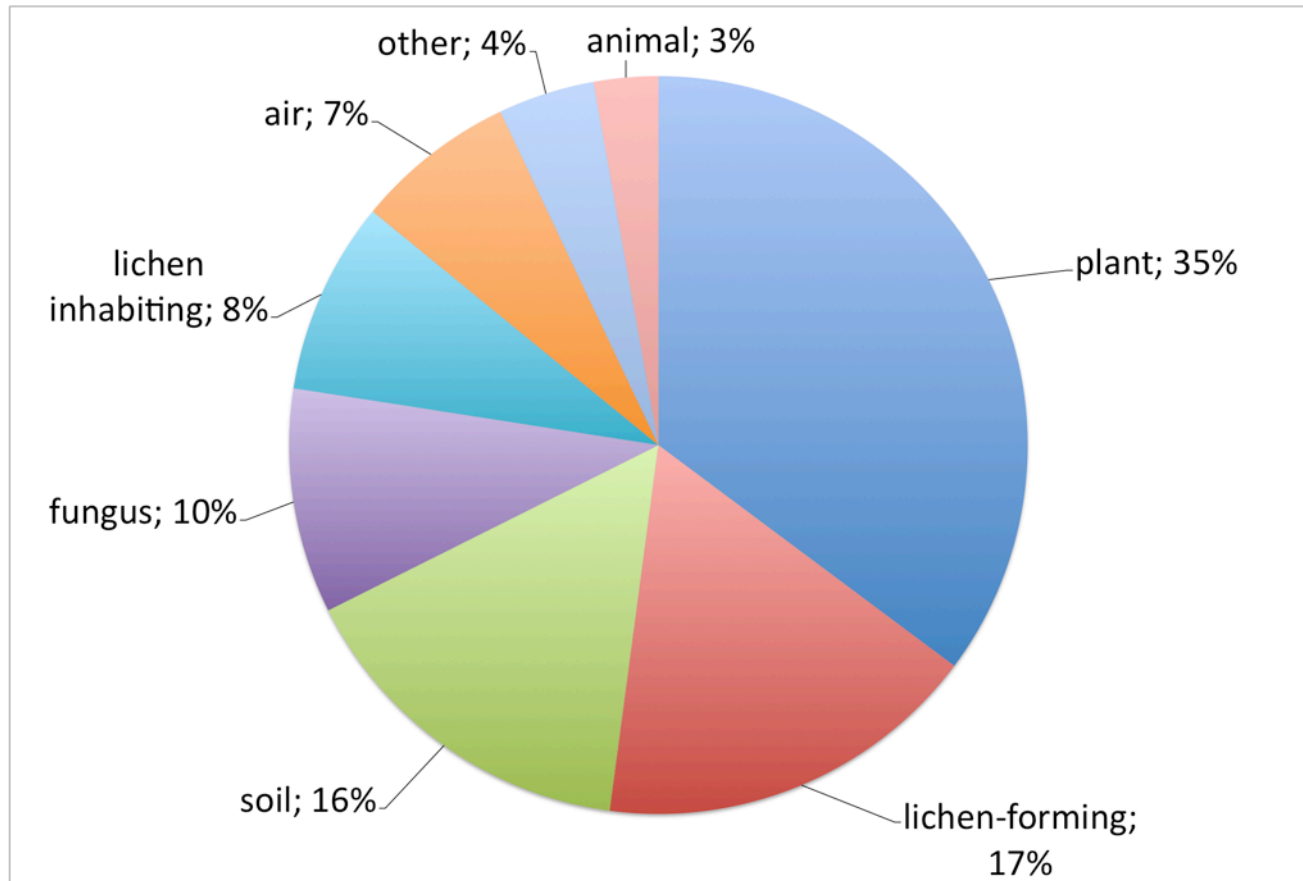
3 major fungal orders with high diversity



# RESULTS: Lichen-associated fungi

- 71 OTUs identified with  $\geq 97\%$  similarity to the species level

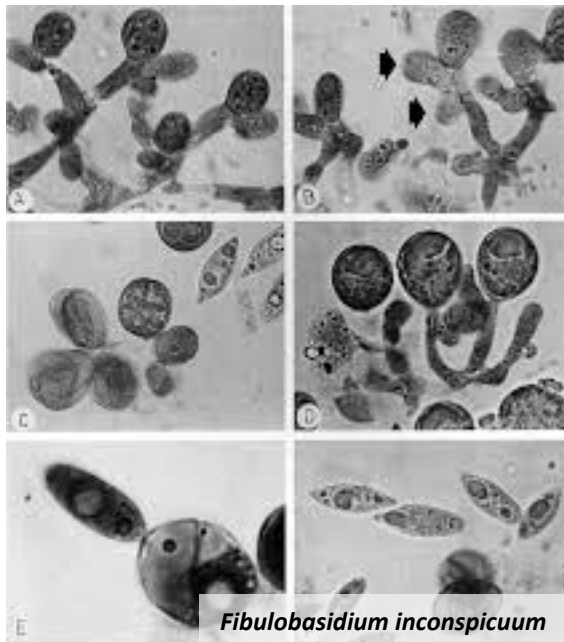
Isolation source of GenBank best match :



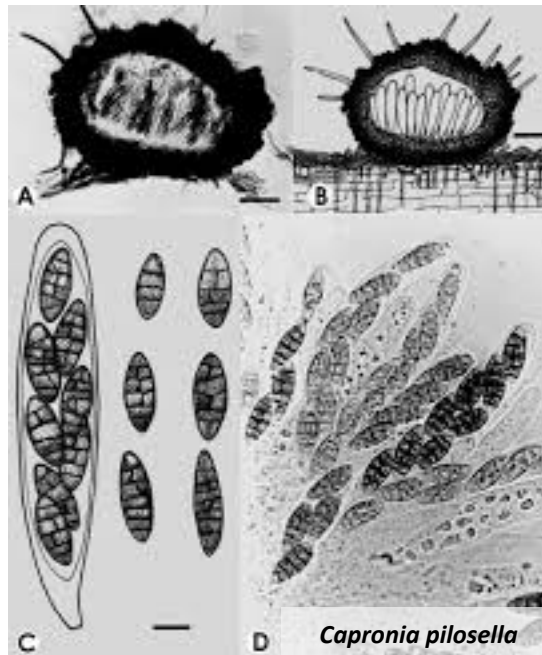


# RESULTS: Most frequent lichen-inhabiting fungi

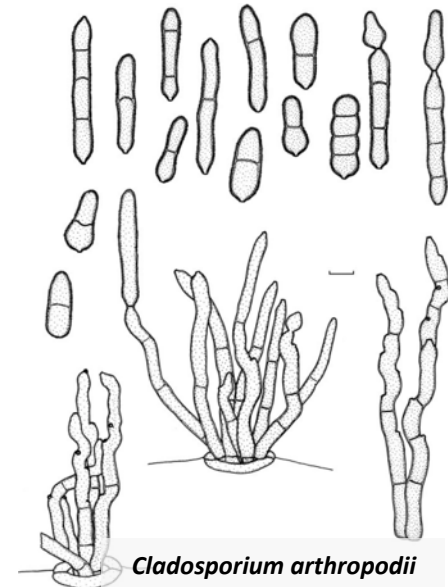
ID	Reads	Lichen samples	Identity (%)	GenBank#	Match	Isolation source
OTU_037	1219	18	99	KF823589	uncultured <i>Fibulobasidium</i>	dead wood of <i>Fagus sylvatica</i>
OTU_230	189	14	98	EU139148	<i>Capronia</i> sp. 94006a	<i>Umbilicaria mammulata</i>
OTU_043	1151	13	91	KF296787	uncultured <i>Chaetothyriales</i>	soil
OTU_119	349	11	99	AM999722	uncultured <i>Tremellales</i>	bryophyte
OTU_091	384	9	93	GU122904	uncultured <i>Capnodiales</i>	wood chips
OTU_133	184	9	99	EF521252	uncultured <i>Chaetothyriales</i>	soil in spruce forest
OTU_225	117	7	99	KJ867418	<i>Cladosporium arthropodii</i>	<i>Umbellularia californica</i> leaves
OTU_239	114	7	98	KC965446	uncultured <i>Hypocreales</i>	soil
OTU_273	107	7	86	KF225852	uncultured <i>Cryptococcus</i>	forest soil
OTU_072	405	6	99	GU187504	<i>Athelia arachnoidea</i>	<i>Populus</i> sp. leaves



Bandoni 1979



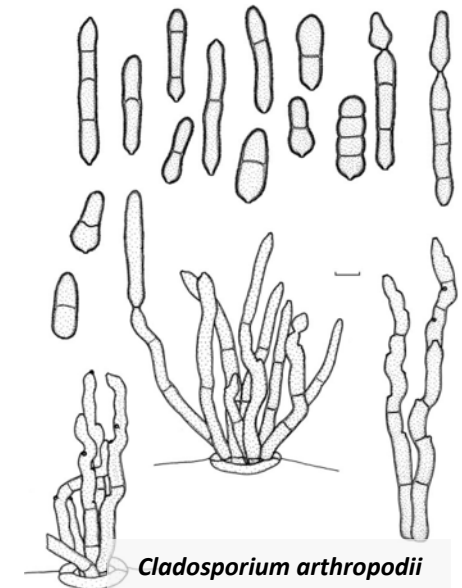
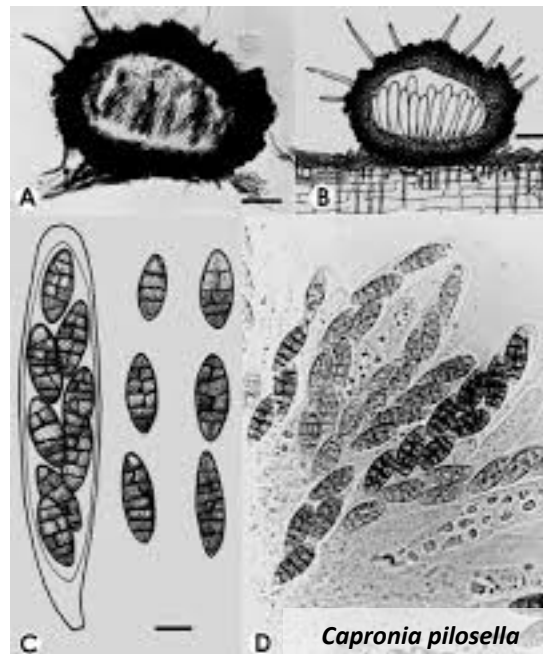
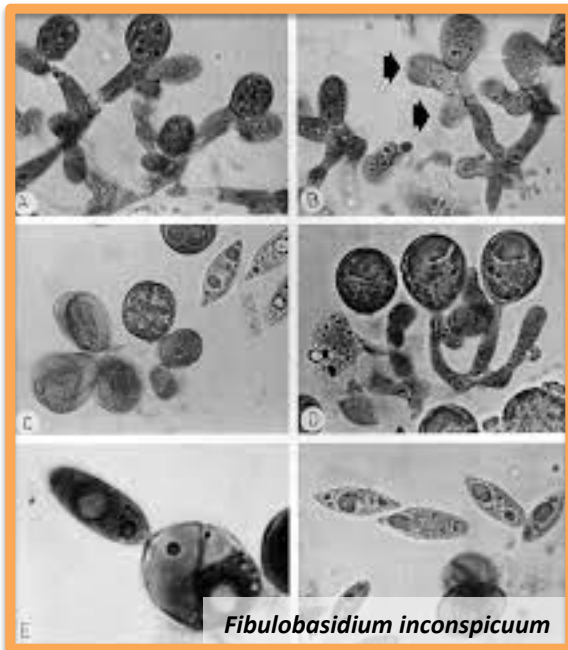
W.H. Hsieh



Bensch et al. 2012

# RESULTS: Most frequent lichen-inhabiting fungi

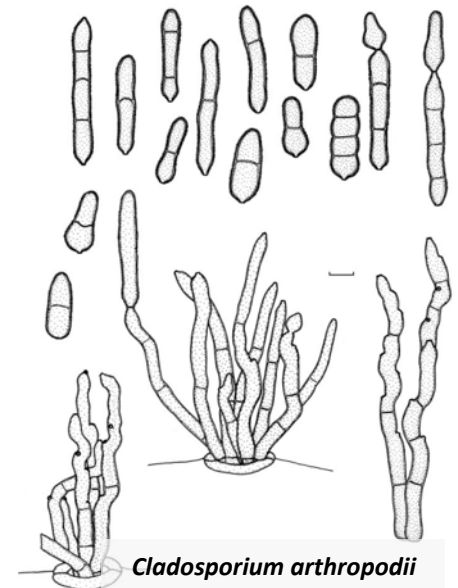
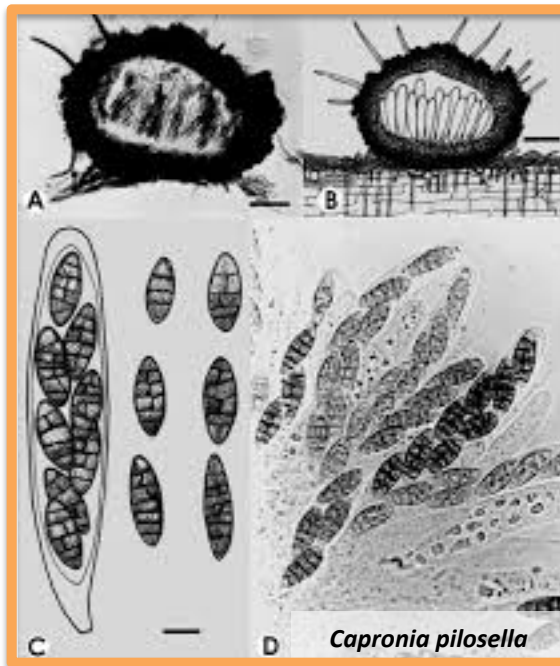
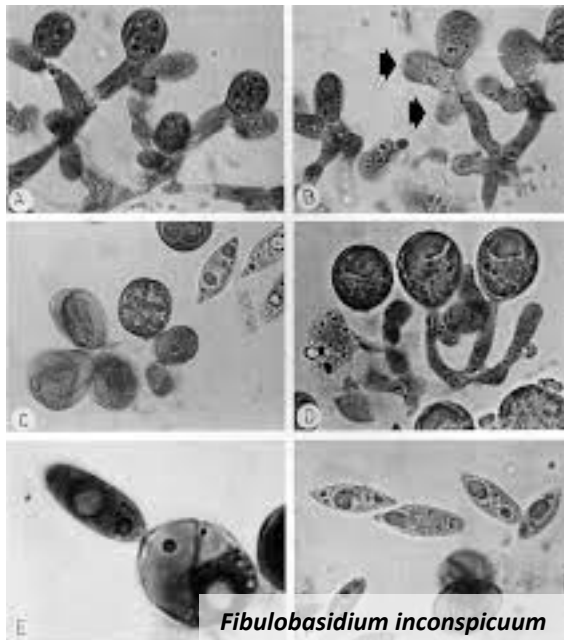
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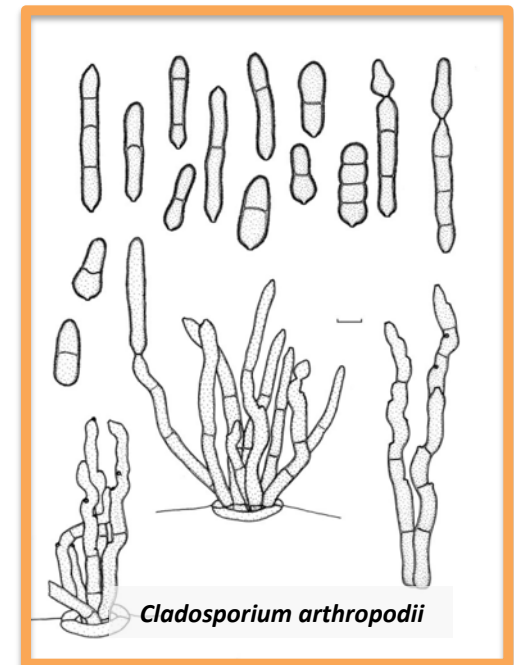
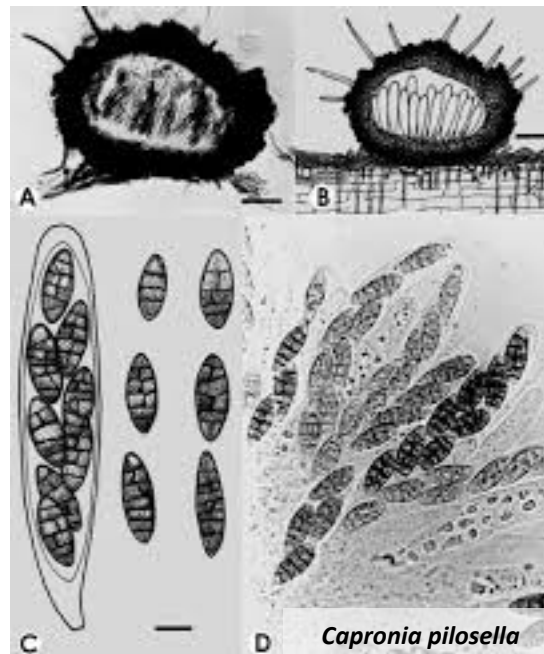
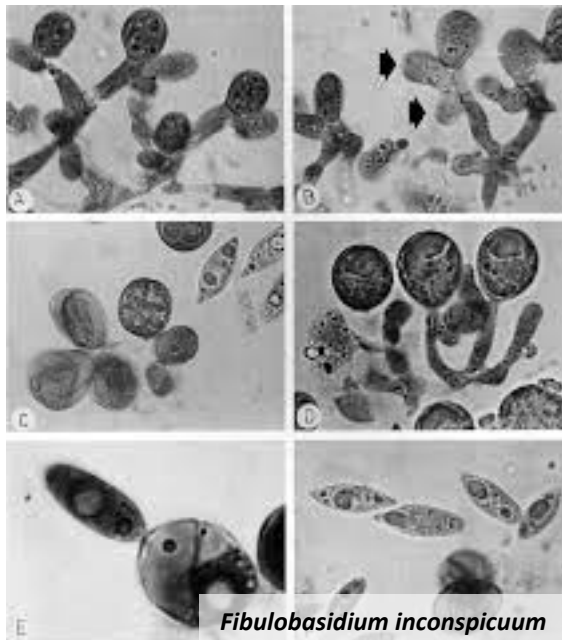
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