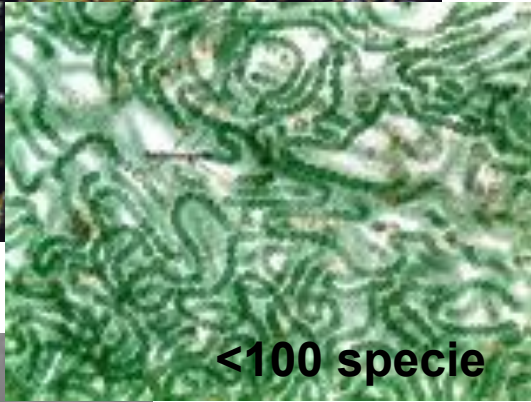
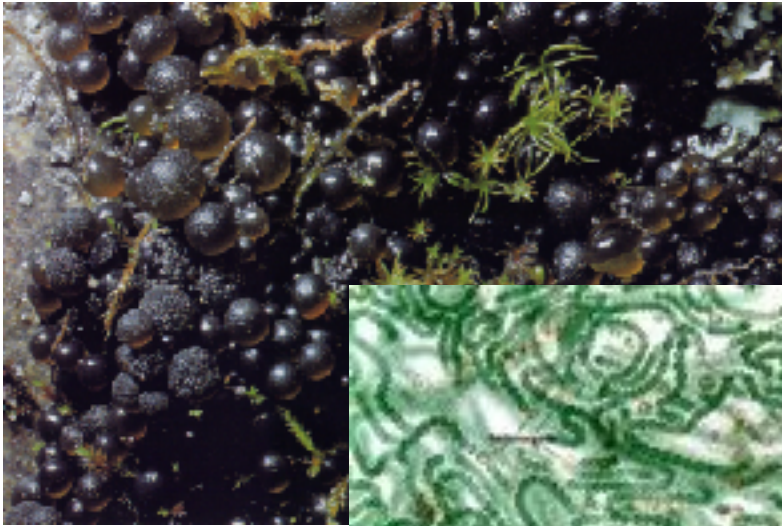


# LICHENI



**<100 specie**



**~ 15.000 specie**

~10% dei licheni

~90% dei licheni



**<1.000 specie**

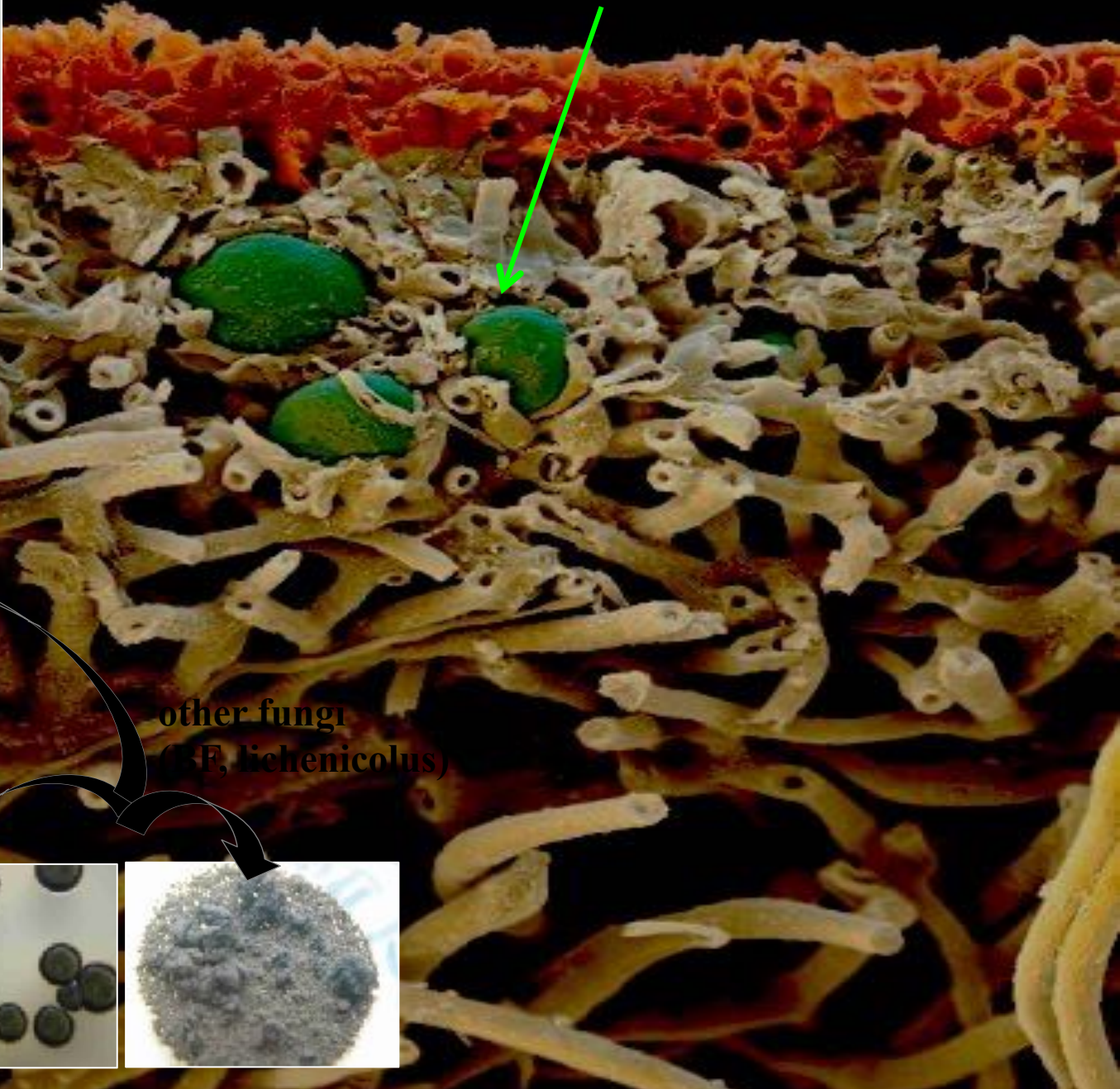
**Simbiosi  
tripartite:**  
micobionte +  
clorobionte +  
cianobionte  
(in cefalodi).

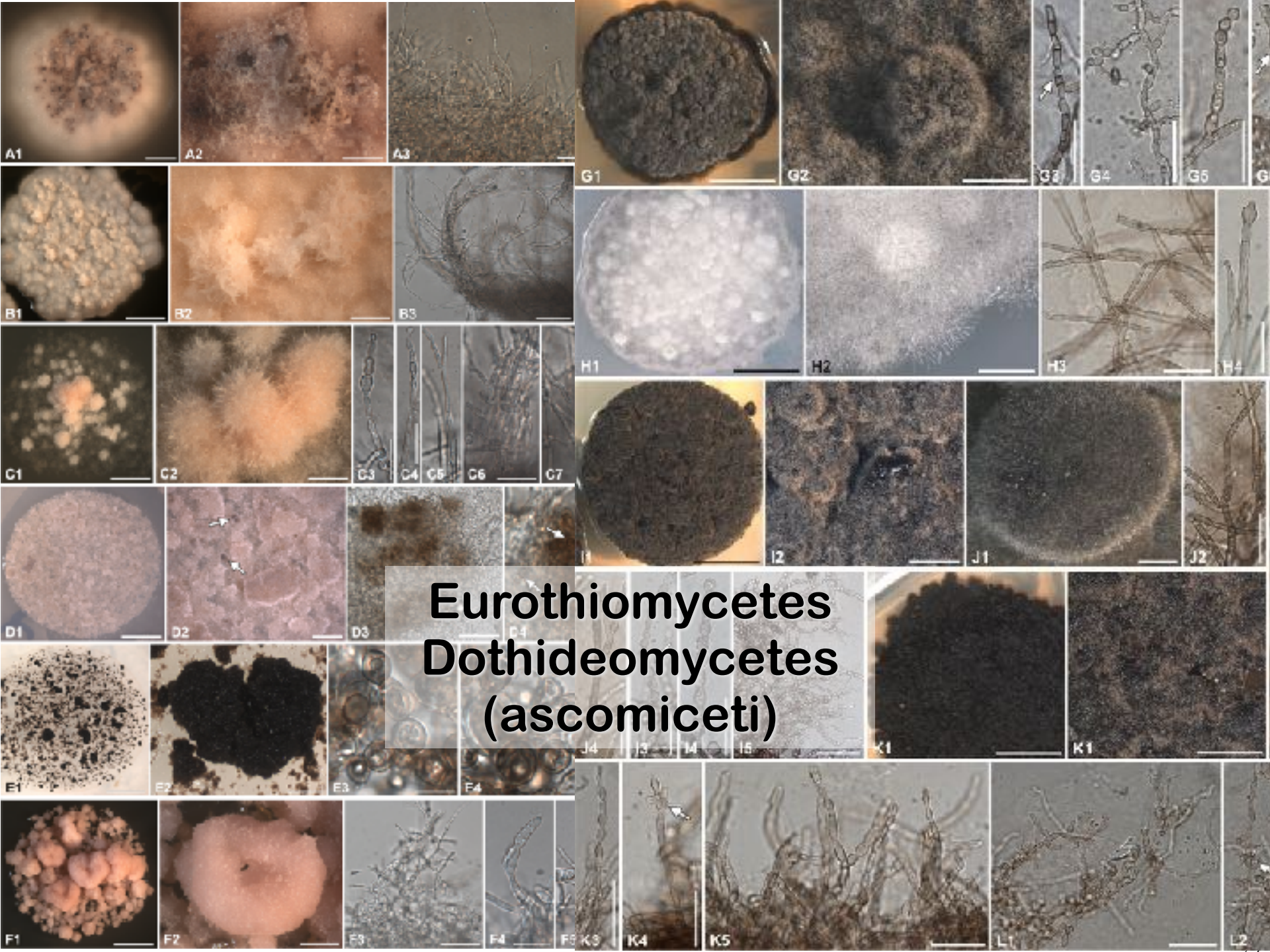


**FOTOBIONTE**

**MICOBIONTE**

**other fungi  
(SP, lichenicolus)**

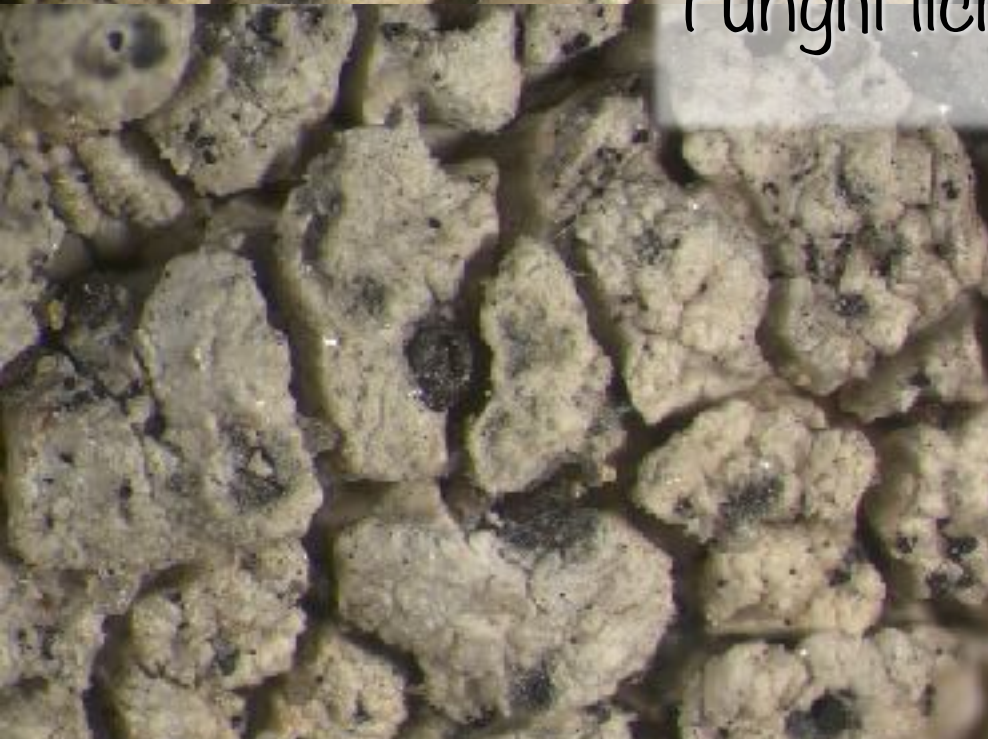




**Eurothiomycetes  
Dothideomycetes  
(ascomyceti)**

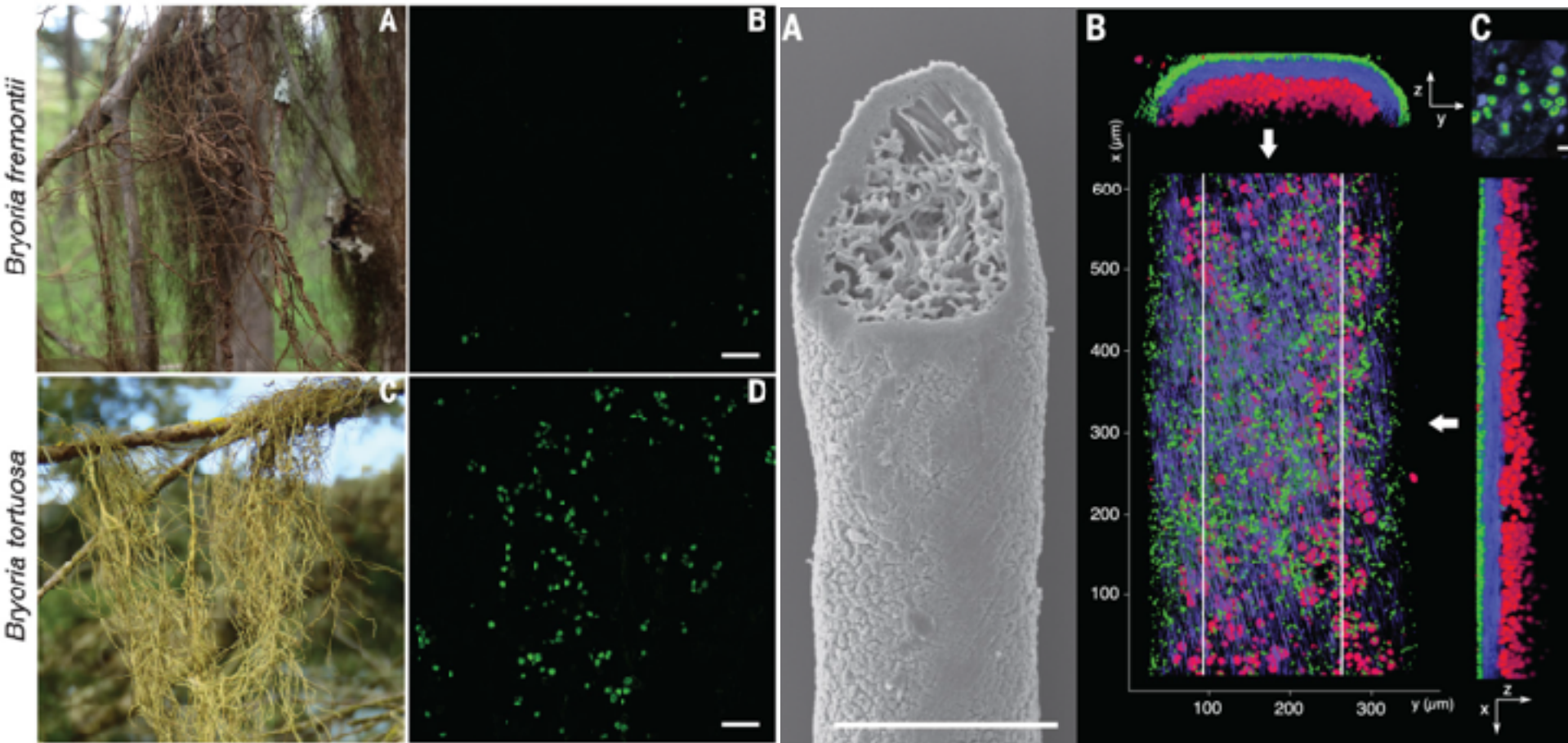


Funghi lichenicoli



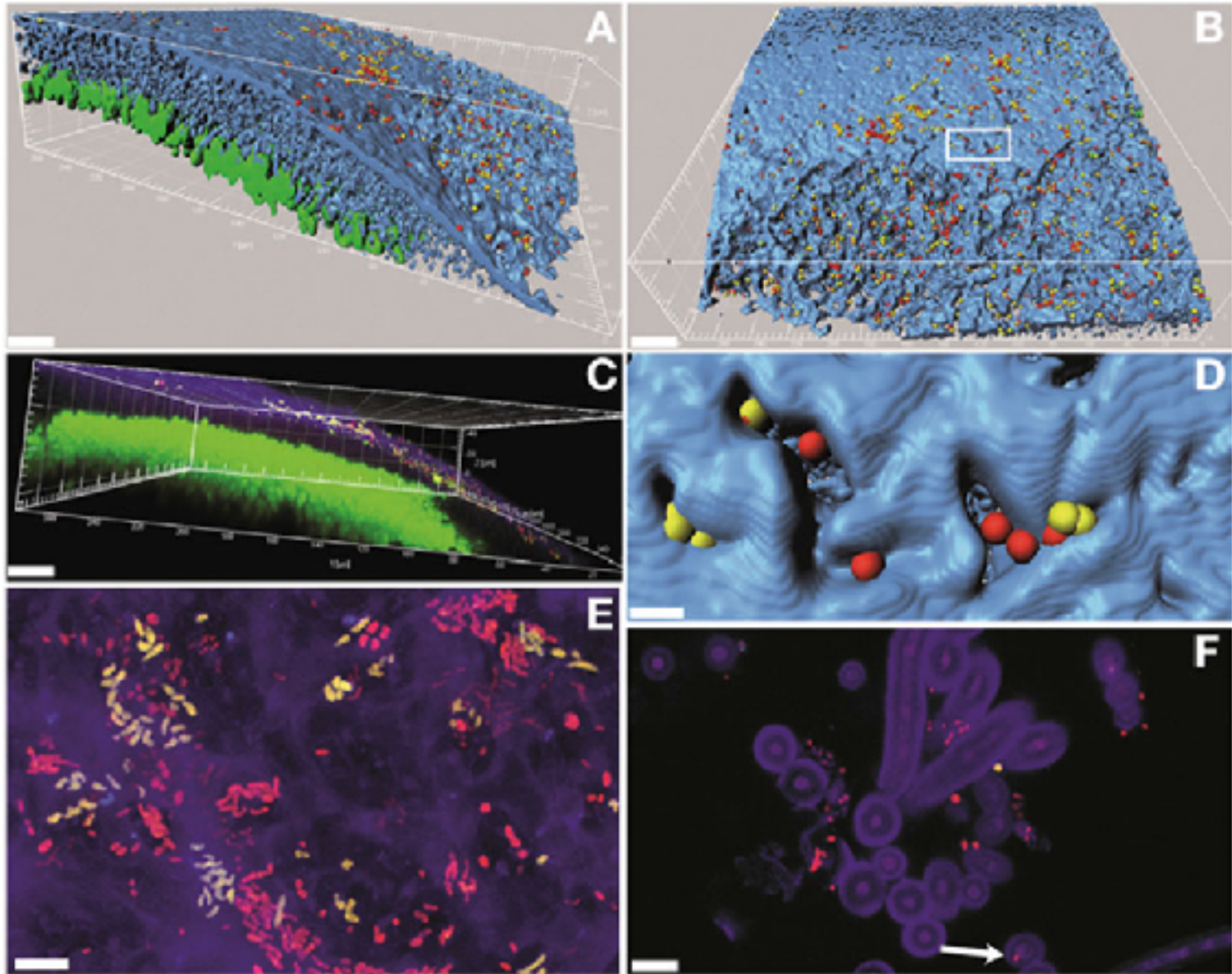
# Basidiomycete yeasts in the cortex of ascomycete macrolichens

Spribille *et al.* (2016) Science

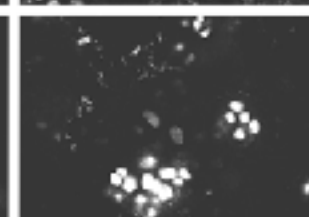
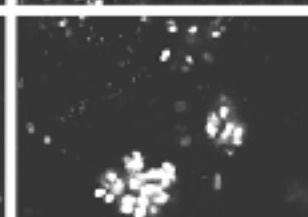
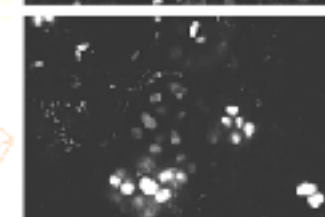
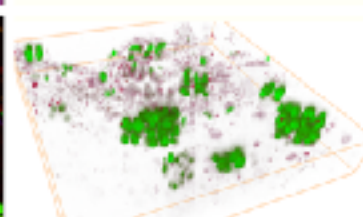
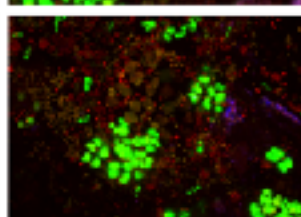
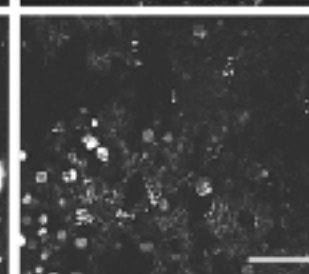
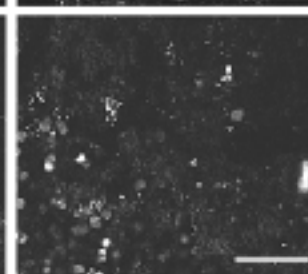
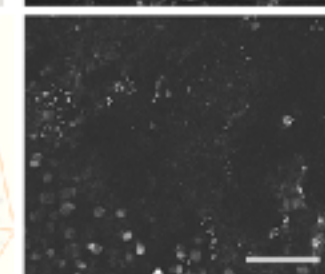
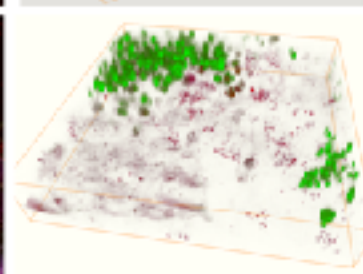
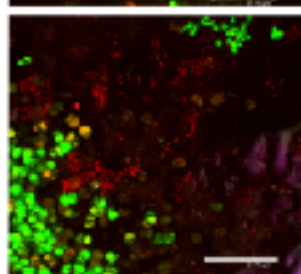
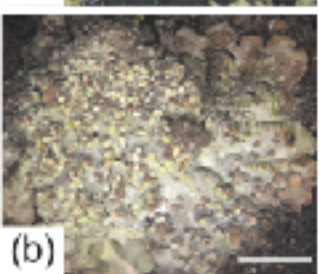
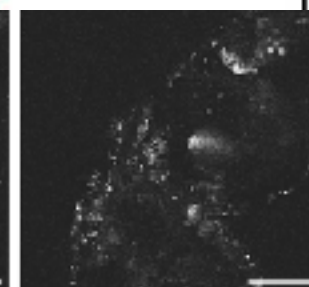
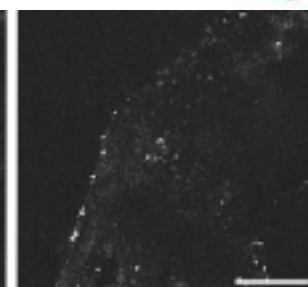
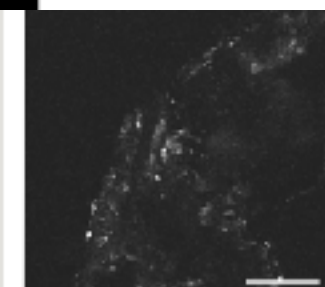
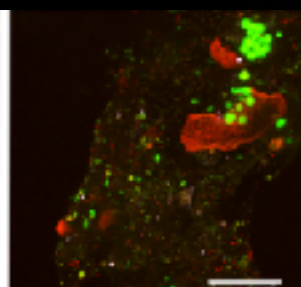
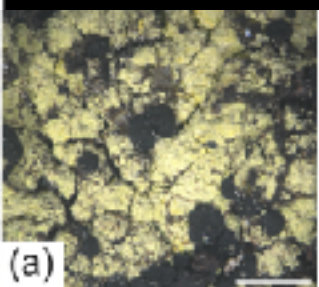
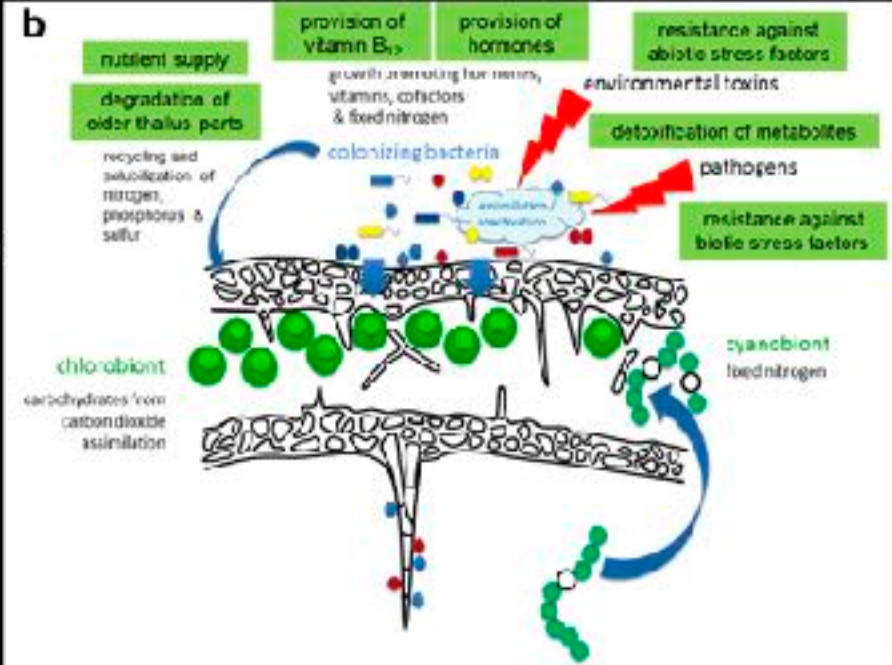
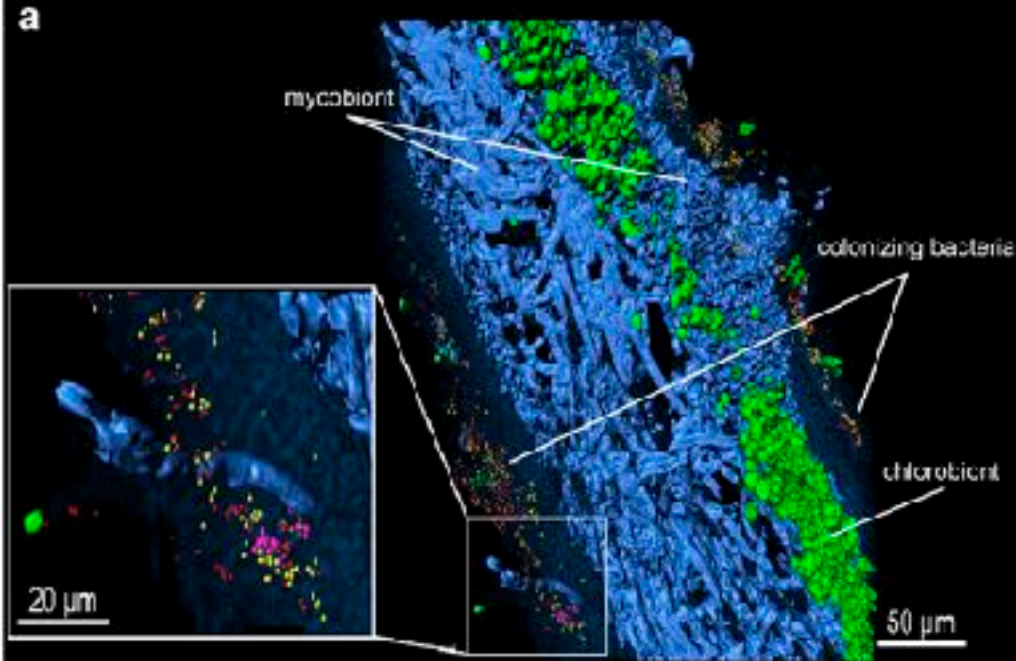


# Lichen–Bacterial Interactions

Grube *et al.* (2009-2016) Environmental and Microbial Relationships, 3rd Edition, The Mycota IV. I.S. Druzhinina and C.P. Kubicek (Eds.) Springer International Publishing Switzerland 2016





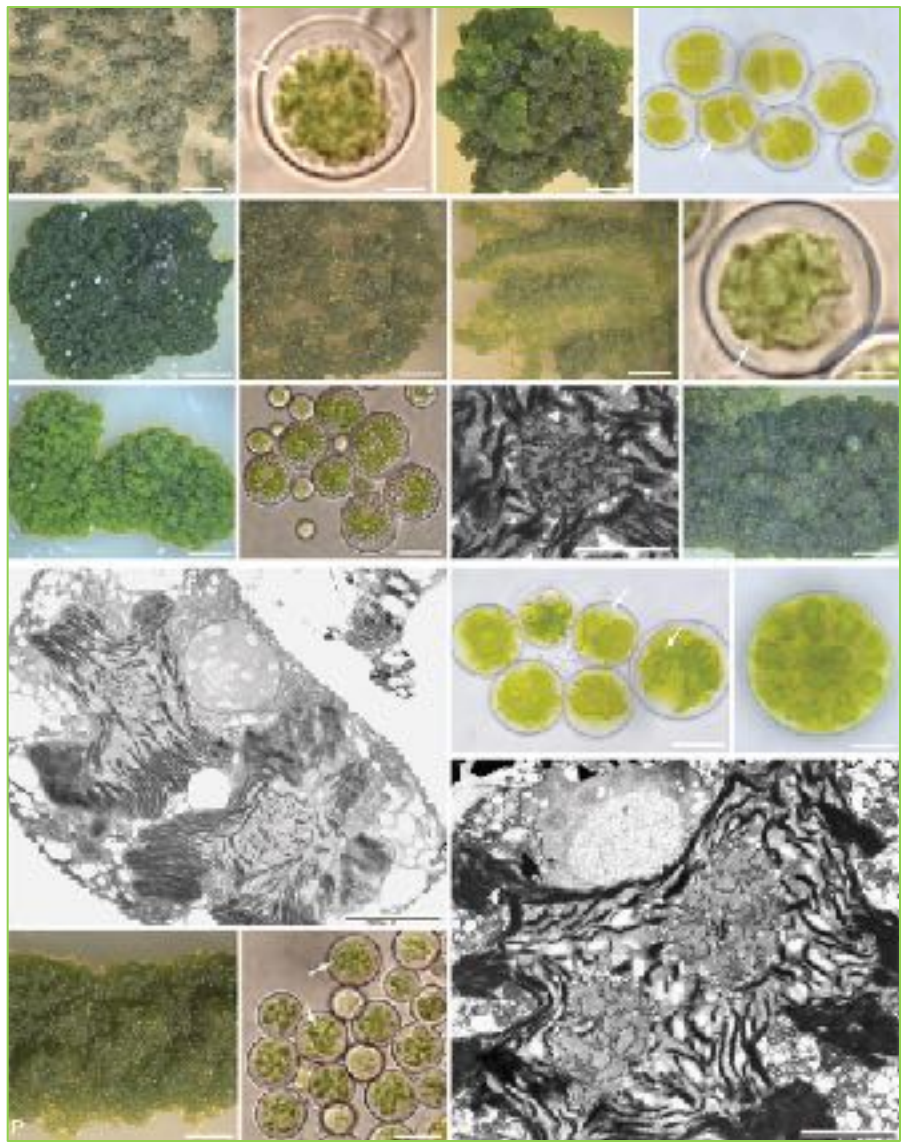
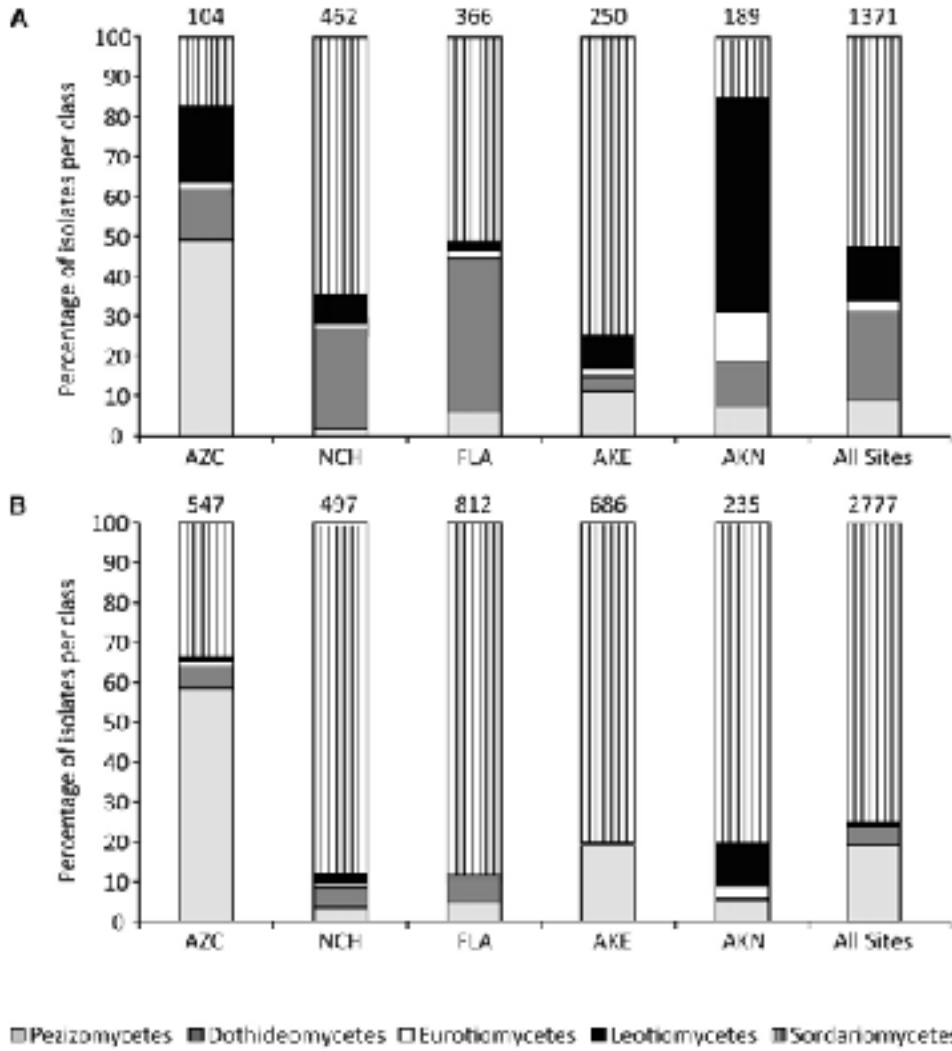


# A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification?

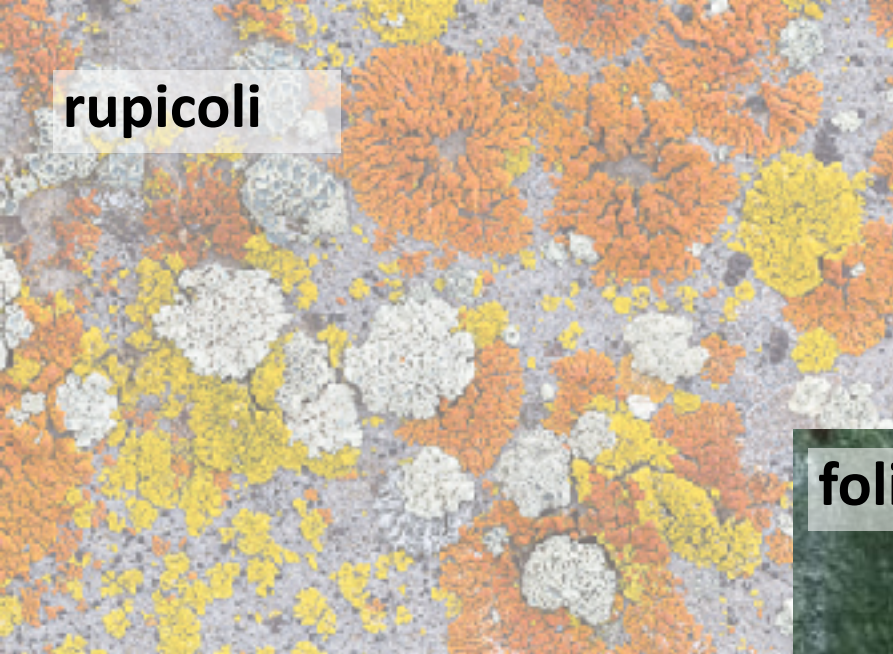
Arnold et al. (2009) Systematic Biology 58: 283–297.

# Multiplicity of photobionts within individual lichen thalli

Muggia et al. (2008-2014)  
Barreno et al. (2009-2016)



**rupicoli**



**terricoli**



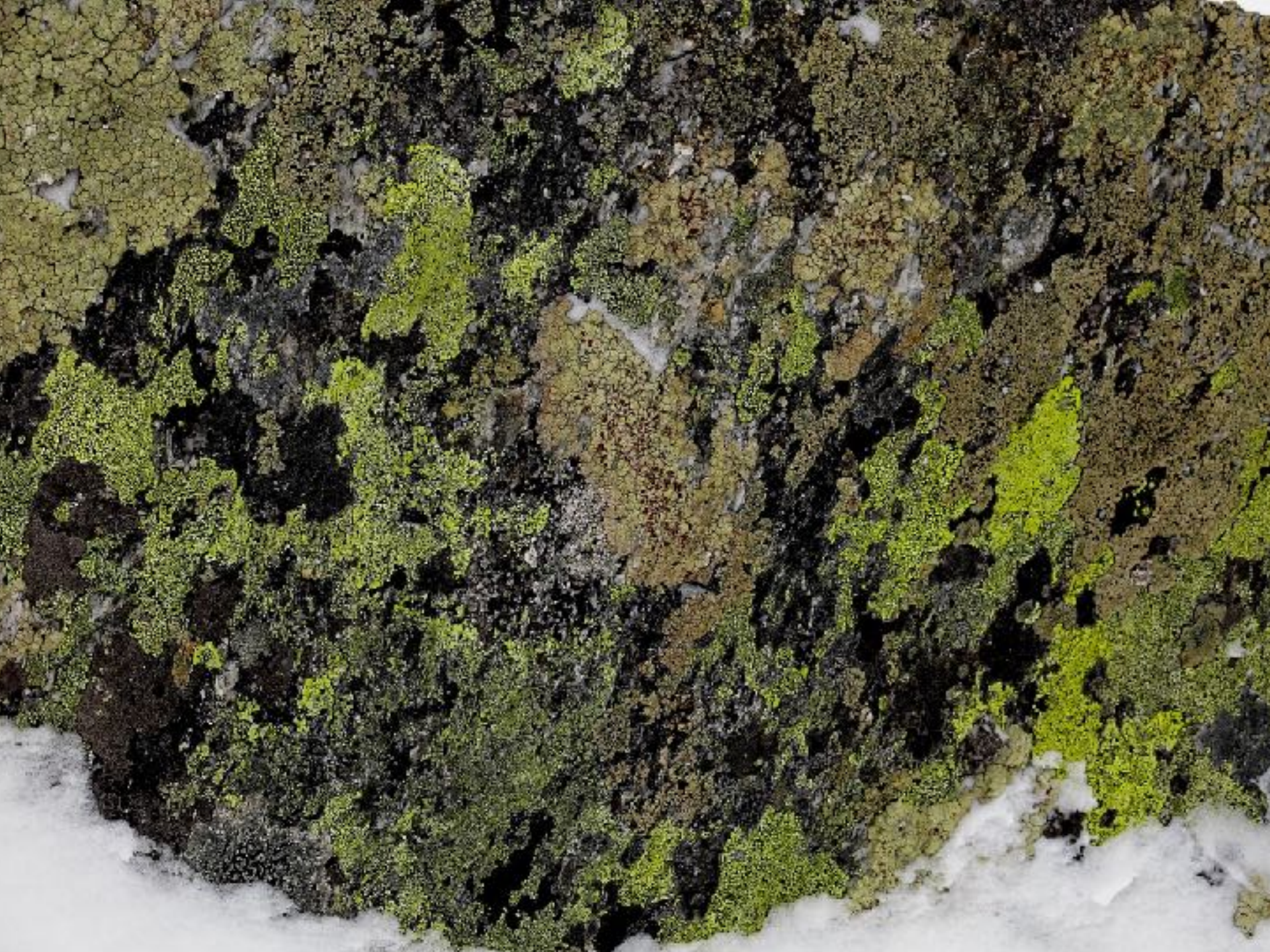
**foliicoli**



**lignicoli**

**muscicoli**



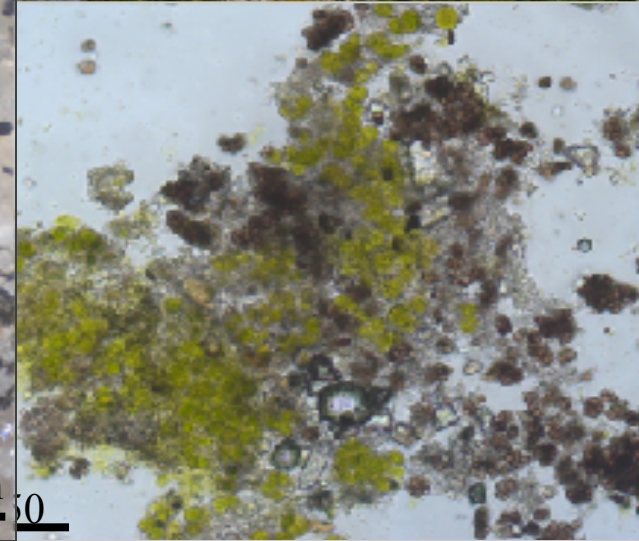
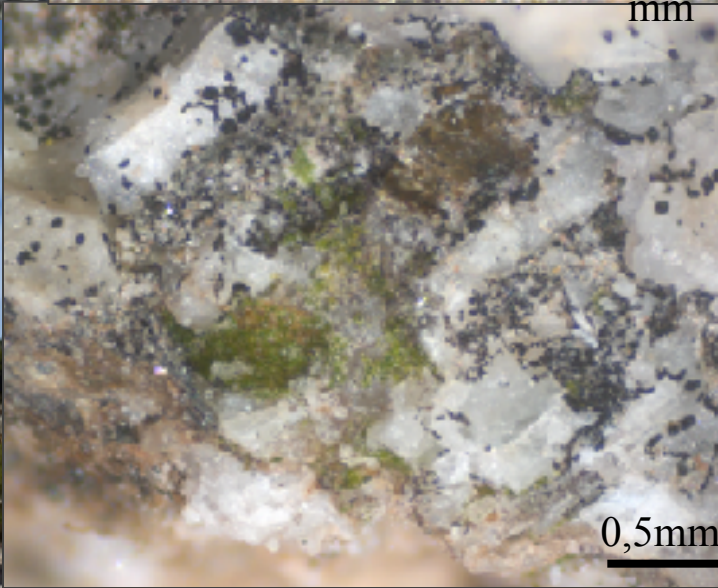
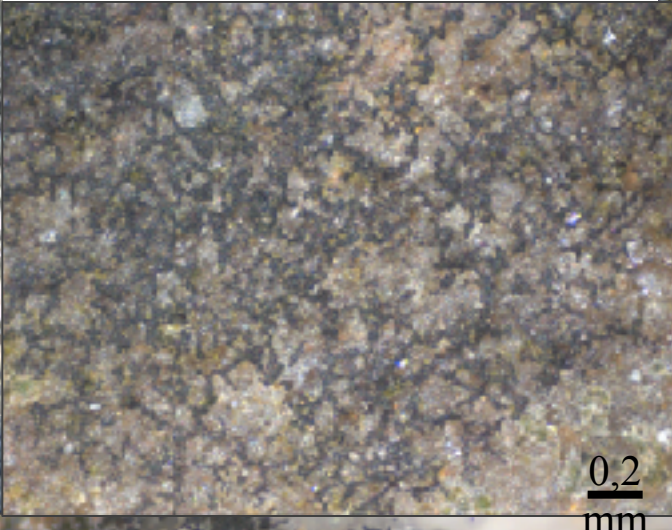


In alcuni deserti la copertura lichenica può essere imponente  
↔ apporto di umidità (nebbie),  
e.g. regione costiera del Namib  
(Africa), deserto di Atacama (tra  
Cile e Bolivia), deserto del Sonora  
(Messico nord-occidentale).





Specie licheniche più primitive colonizzano ambienti “estremi” (rocce nude, alta radiazione UV, alte temperature)







Nelle regioni artica e boreale la biomassa lichenica rappresenta una frazione elevata ed importante → cicli biogeochimici di molti elementi, sostanze e  $H_2O$ .



Il numero di specie non è necessariamente elevato.... poche specie, con tantissimi individui!!



.... i licheni sono in grado di colonizzare addirittura il vetro, la plastica o i metalli.



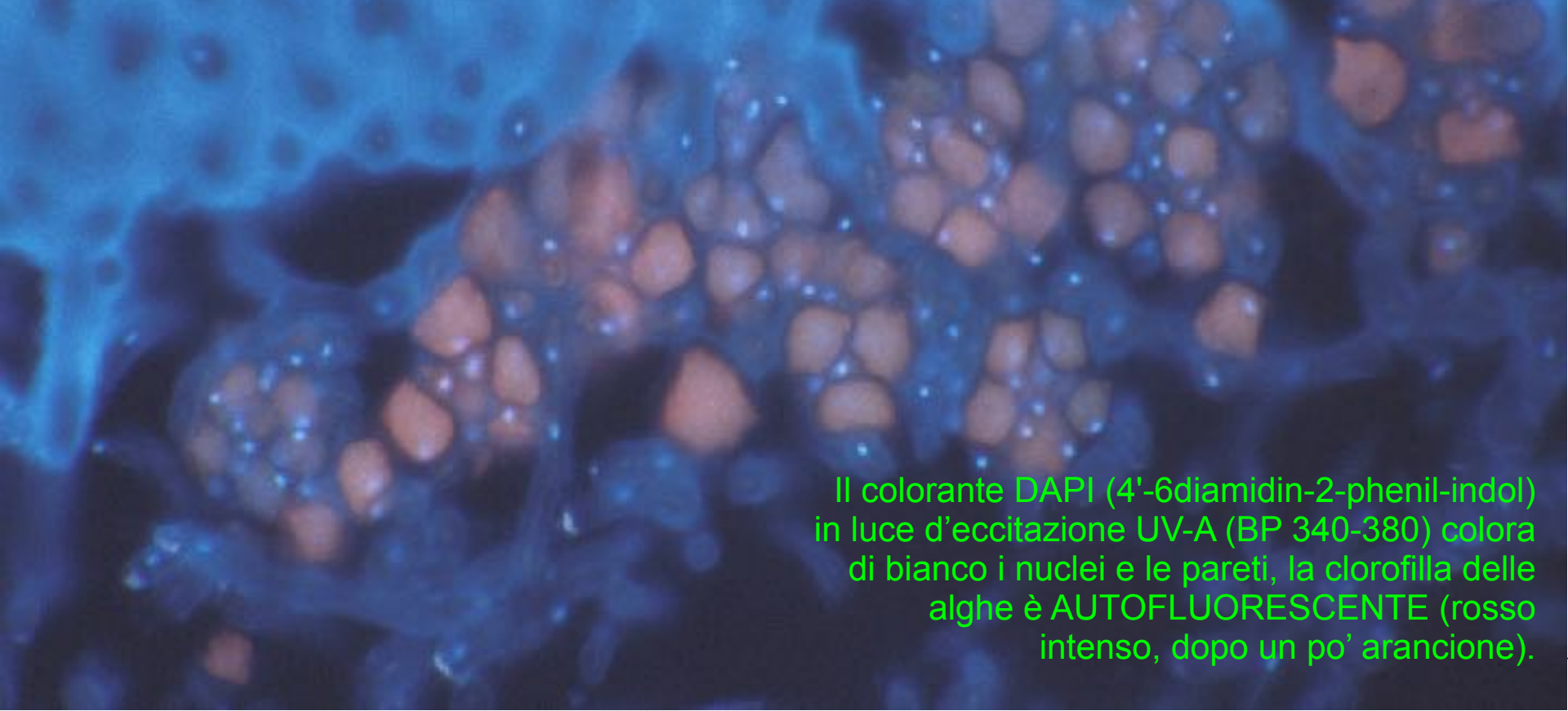
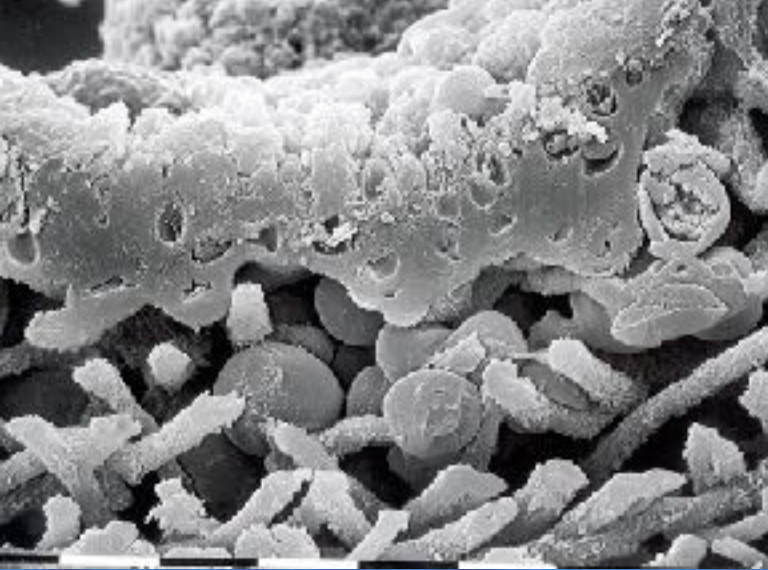


.....siamo circondati da licheni, sugli alberi, sui muri e ... sui monumenti !



**“deserti lichenici”:**  
inquinamento ambientale troppo elevato di anidride solforosa ( $\text{SO}_2$ ) ossidi di azoto ( $\text{NO}$ ,  $\text{NO}_2$ ,  $\text{NO}_x$ ), acido fluoridrico, ecc.,

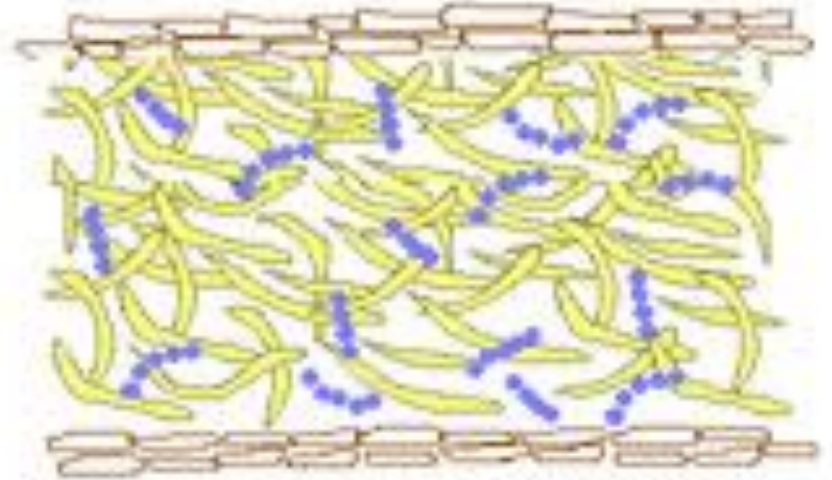
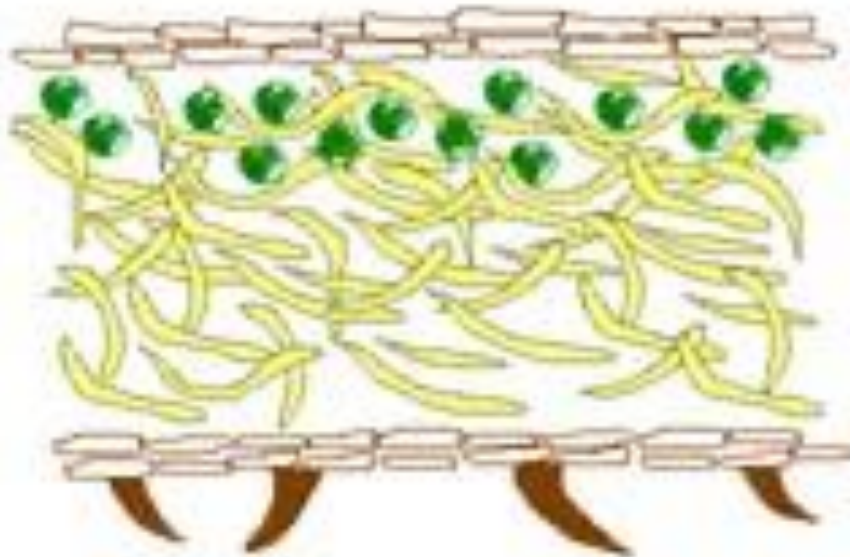
## Sezione trasversale del tallo



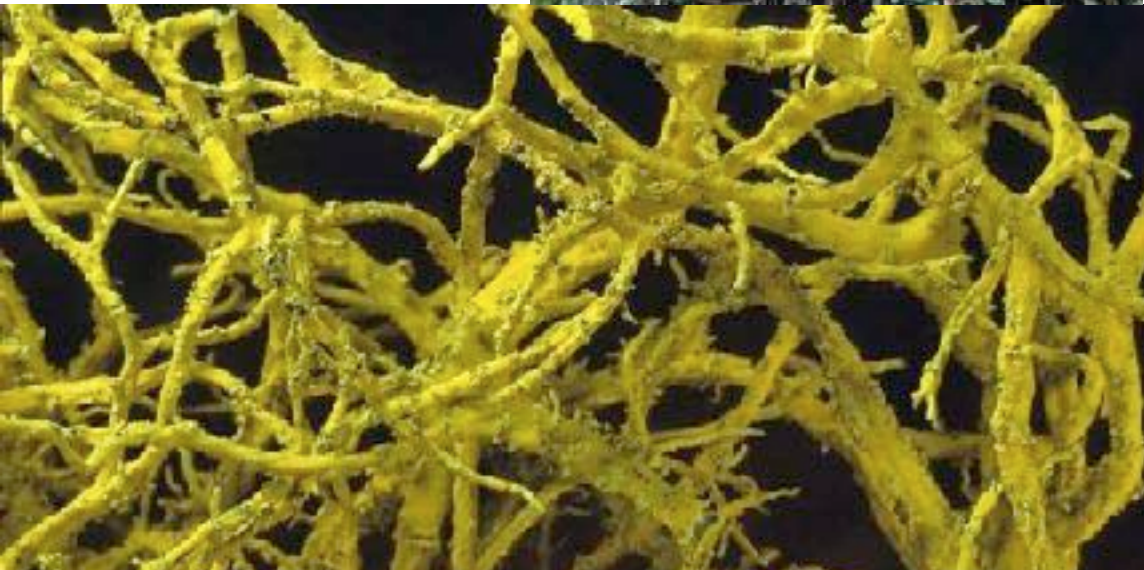
Il colorante DAPI (4'-6diamidin-2-phenil-indol) in luce d'eccitazione UV-A (BP 340-380) colora di bianco i nuclei e le pareti, la clorofilla delle alghe è AUTOFLUORESCENTE (rosso intenso, dopo un po' arancione).

# TALLO ETEROMERO

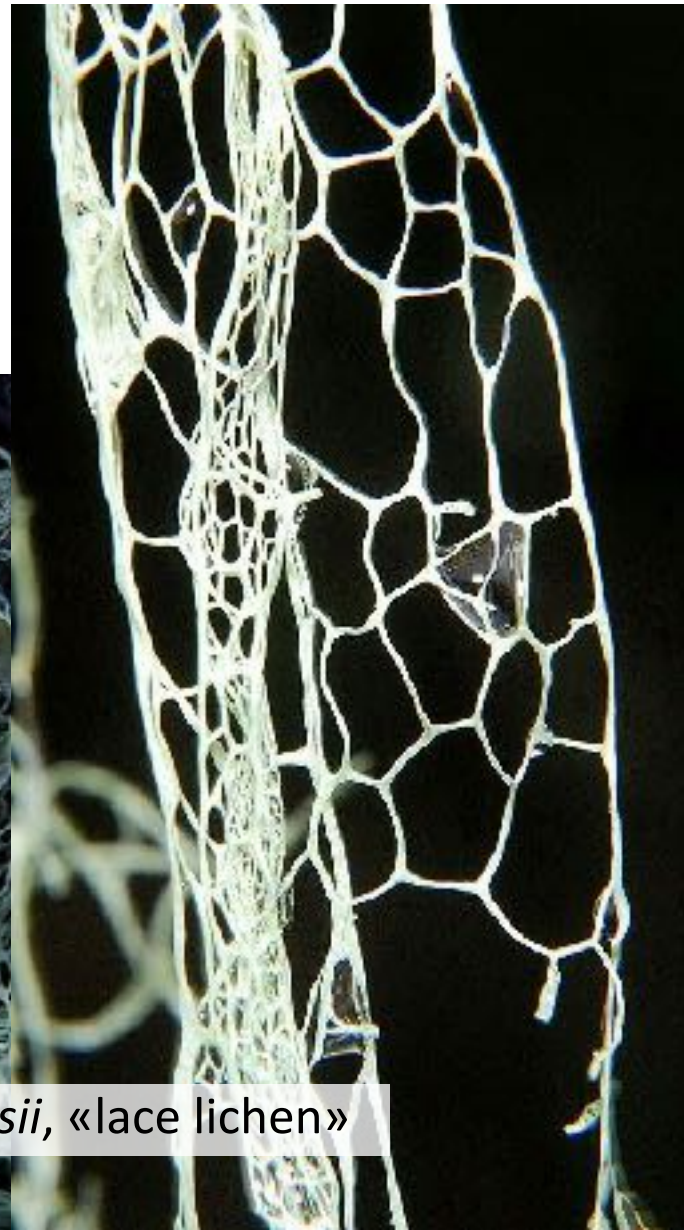
# TALLO OMEOMERO



# FRUTICOSO



*Cladia retipora*



*Ramalina menziensii*, «lace lichen»

# FOGLIOSO





# CROSTOSO

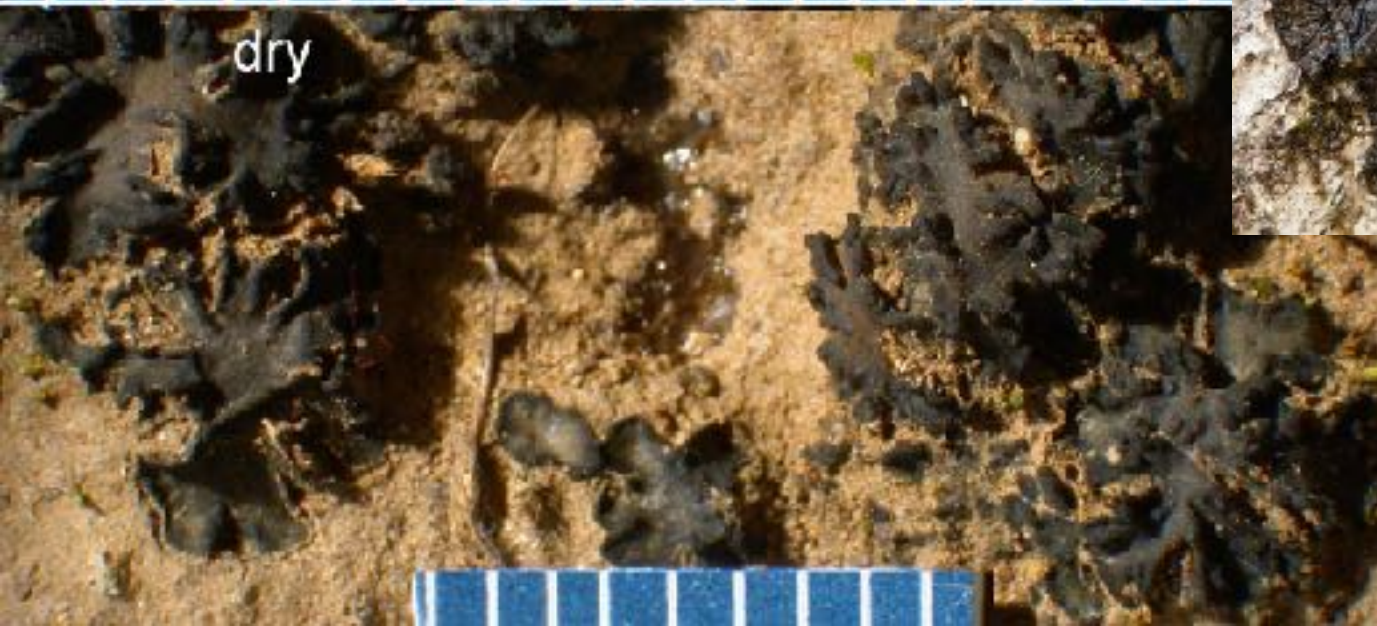
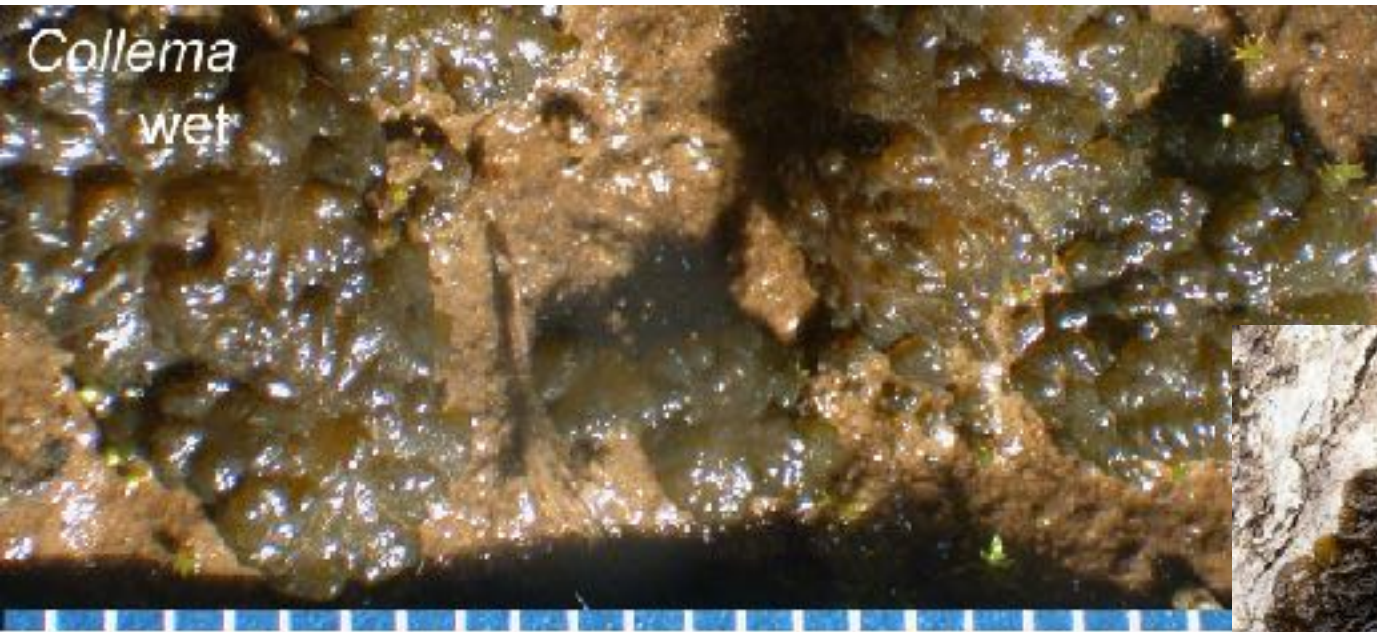


**FOLIICOLOUS lichens**



5 mm

# GELATINOSO o non gelatinoso (tallo omeomero)



L' alga (*Trentepohlia* sp.)  
determina la forma del  
lichene.

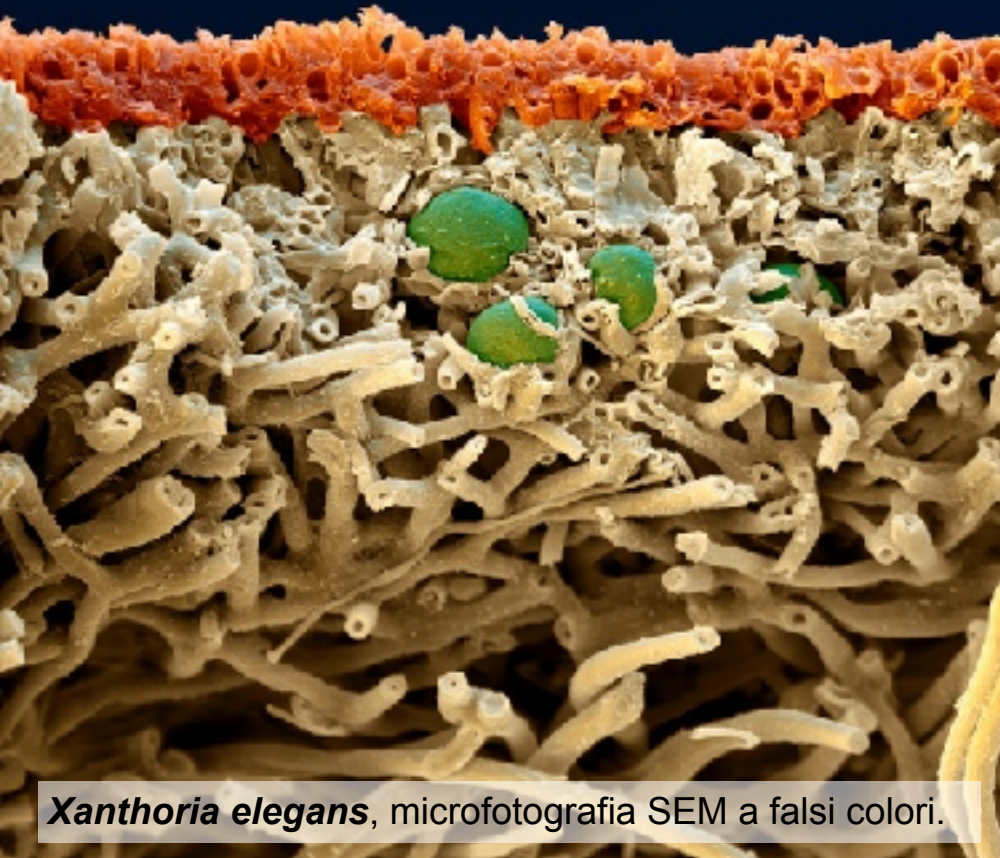


*Cystocoleus  
ebeneus*



*Racodium  
rupestre*

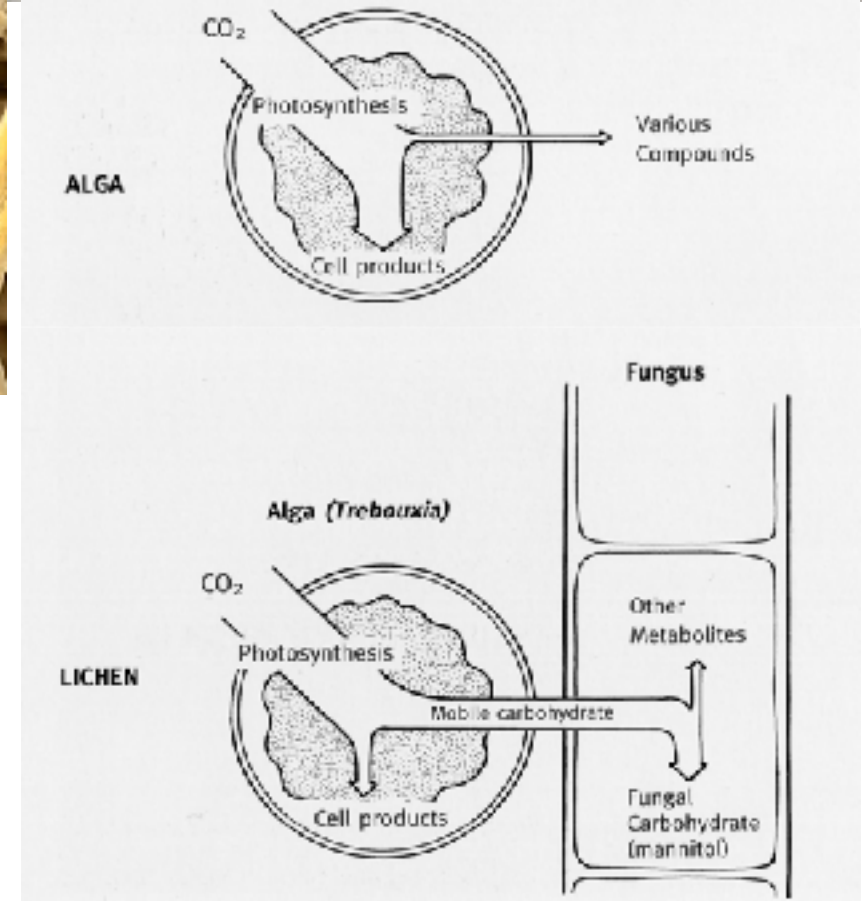


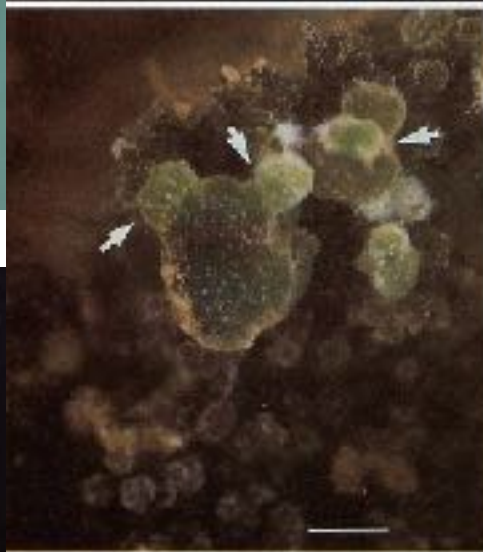
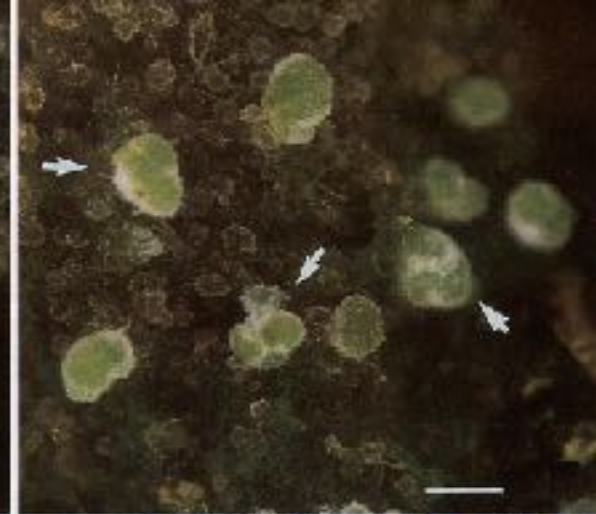
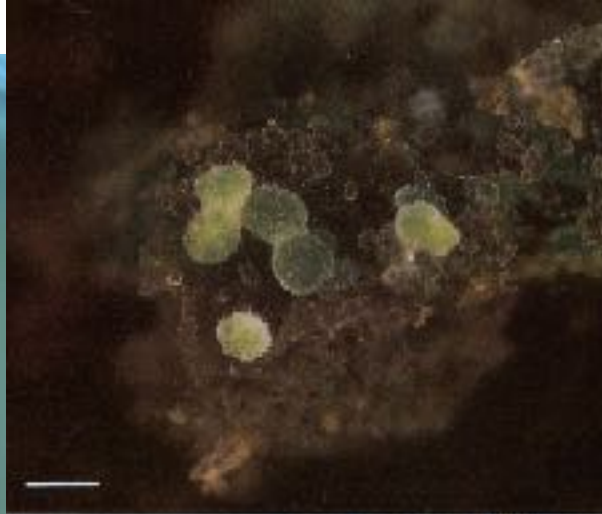


*Xanthoria elegans*, microfotografia SEM a falsi colori.



- **Green algae:** sugar alcohols (polyols) – vapor water
- **Cyanobacteria:** glucose – liquid water

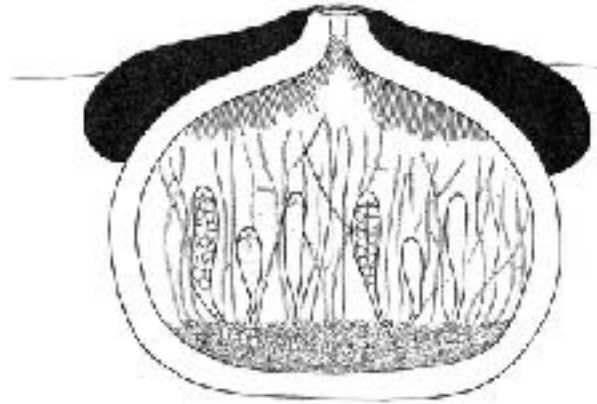




Botanical Society of America  
(photo by Vernon Ahmadjian)

I processi morfogenetici iniziano quando un'ifa incontra il partner giusto.

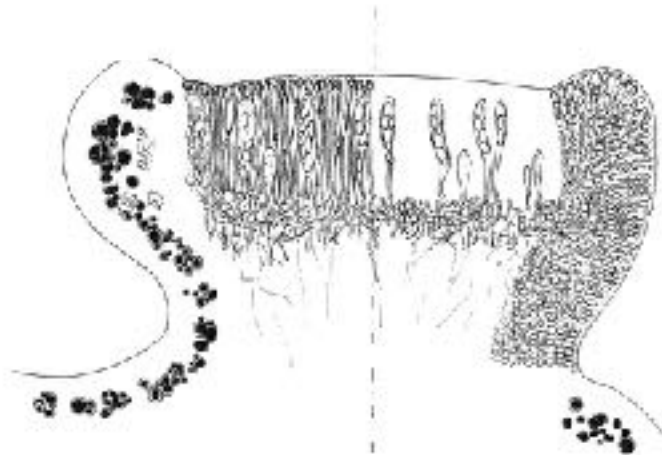
Solo il fungo si riproduce sessualmente. Le alghe (e i cianobatteri) si riproducono vegetativamente.



**PERITECI,**

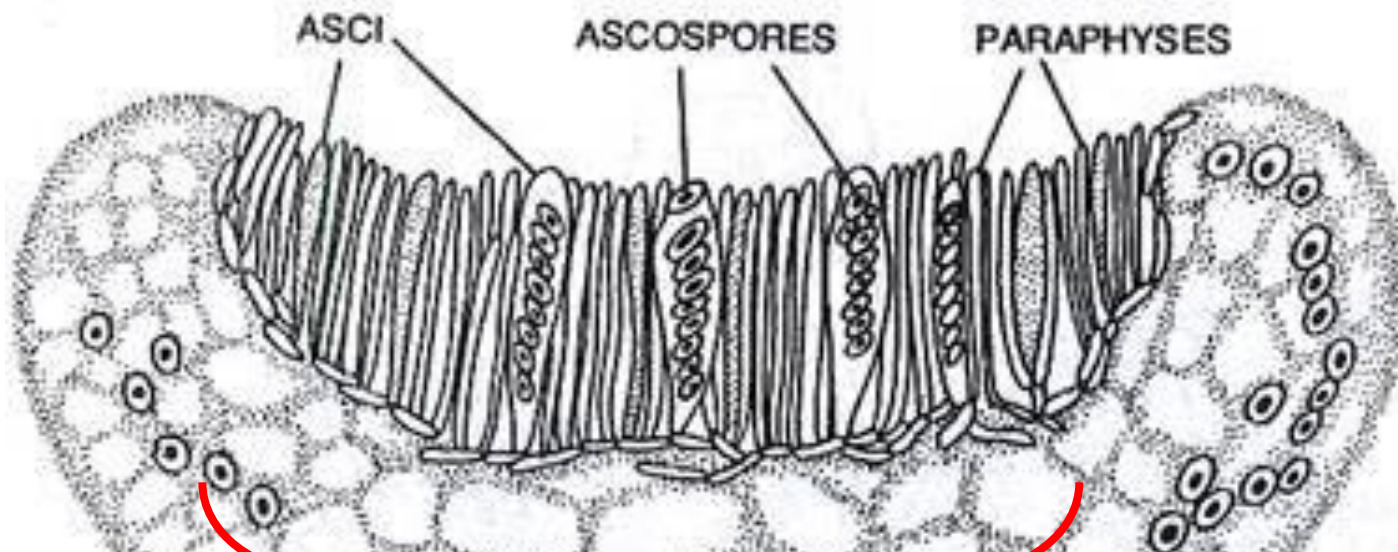


Fig. 10.10.10. Perithecia of the lichen *Peziza apiculata*. The lichen is a cup-shaped fungus that grows on a rock. The cup-shaped structures are the fruiting bodies. The fungus, *Peziza apiculata*, is a member of the Ascomycota.

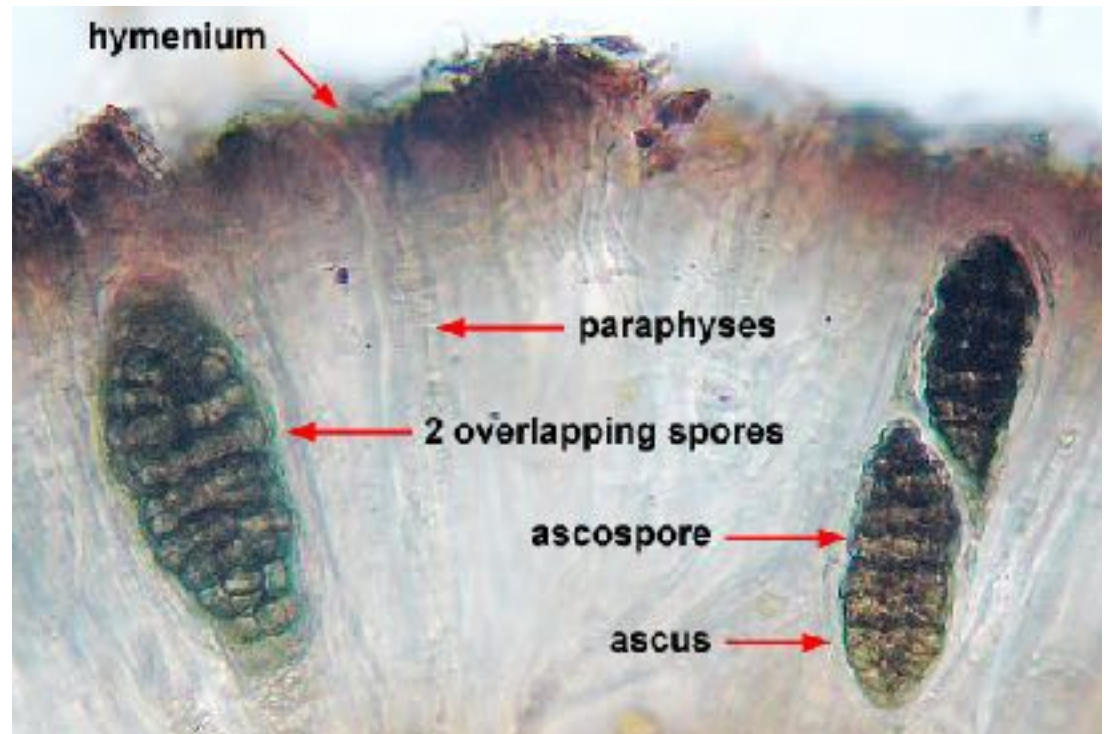


**APOTECI**

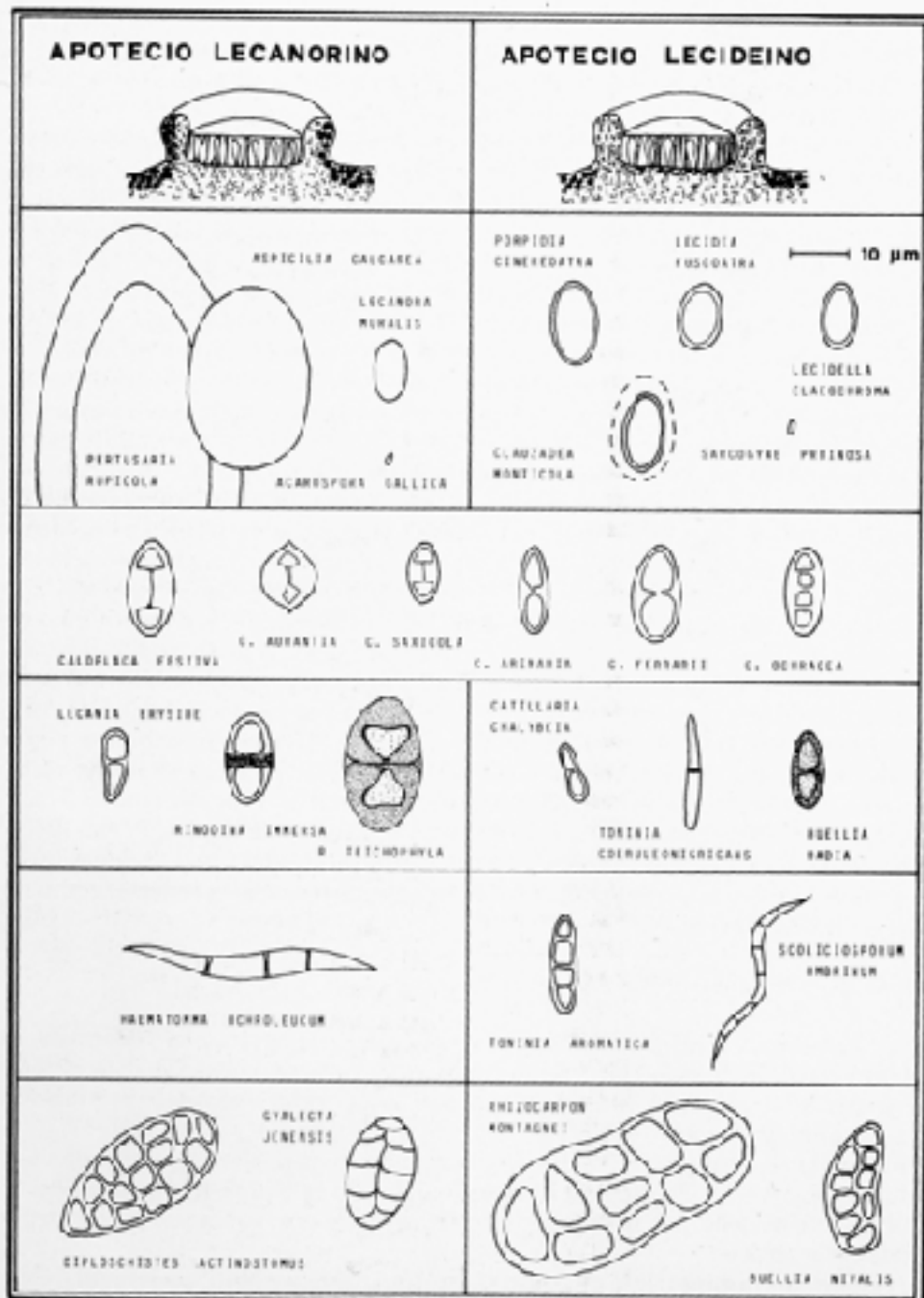
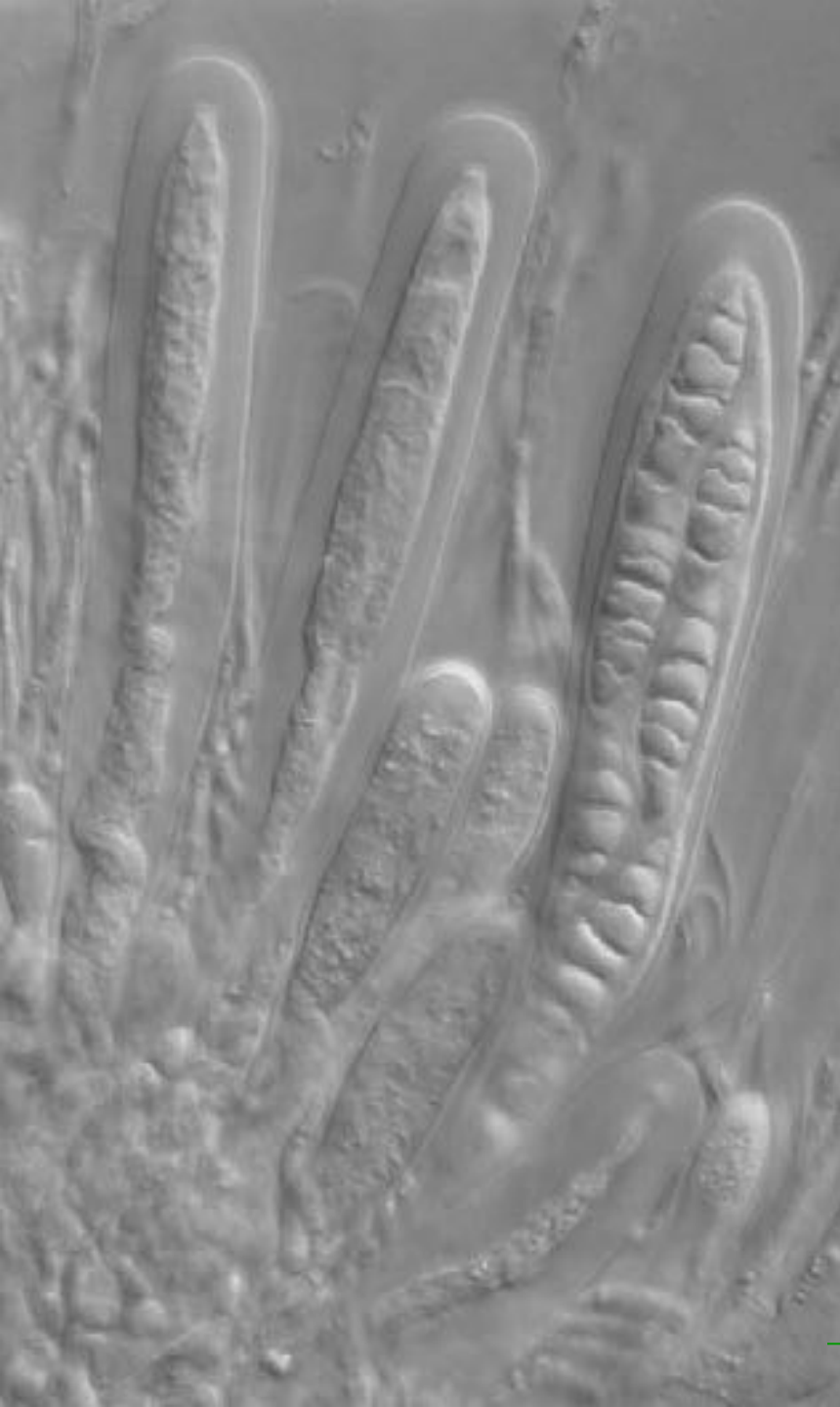
**Nel 99% dei casi sono strutture perennanti (a differenza dei funghi non lichenizzati!!!).**



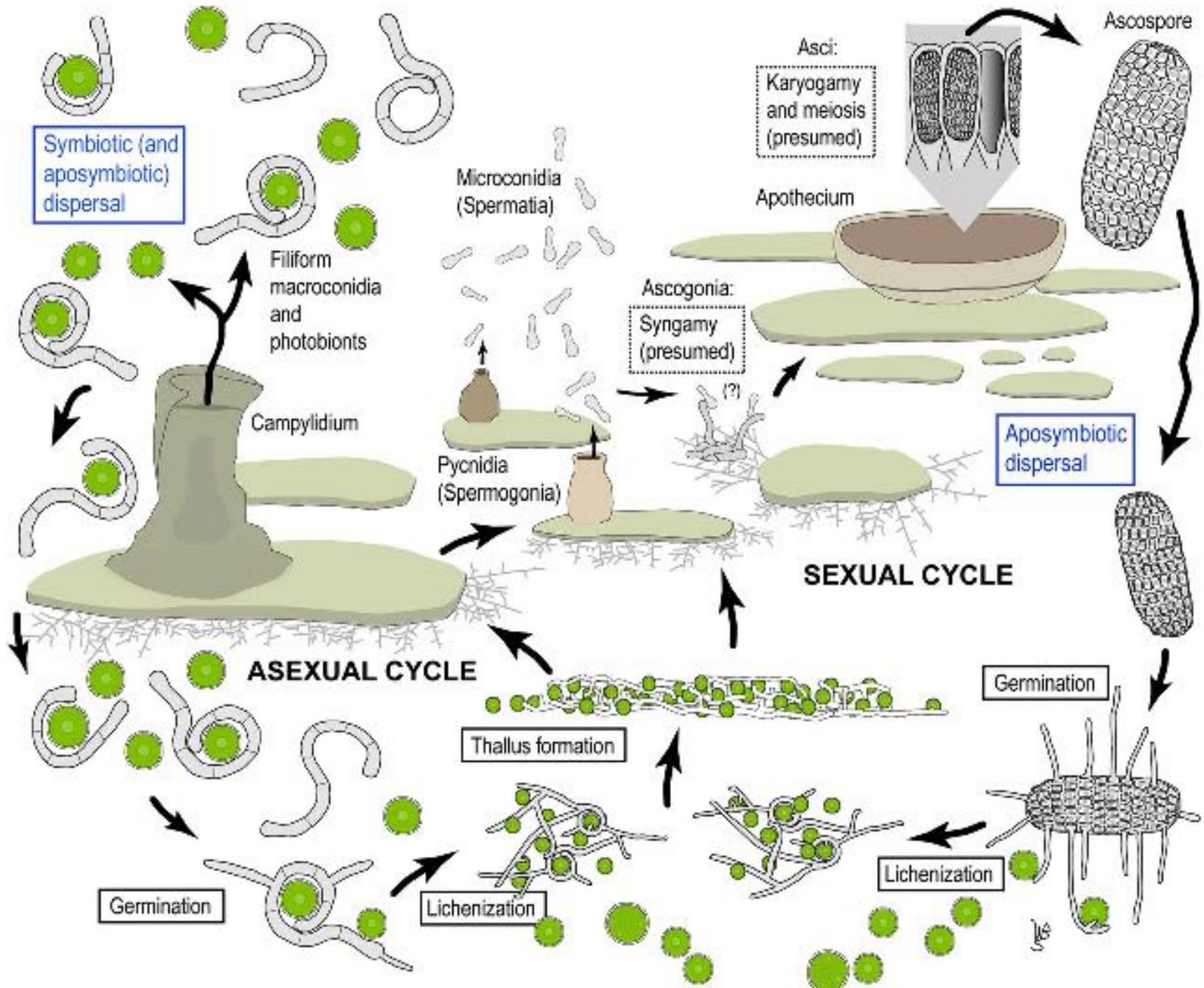
hymenium





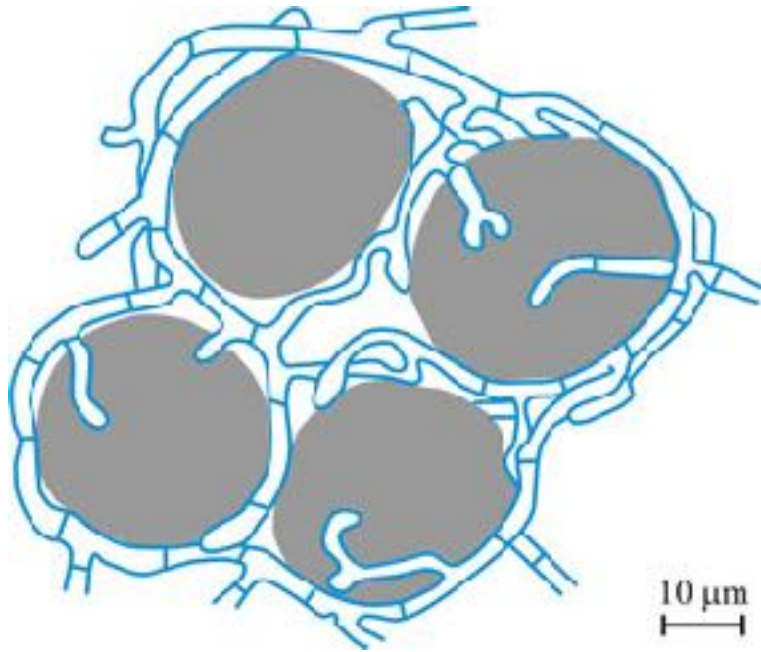


Tav. 2 Diversi tipi di spore di specie ad apotecio lecanorino (sinistra) e lecideino (destra).



5/04

**SOREDI: diaspore**, sono strutture NON corticate, spesso formate in zone delimitate del tallo (**sorali**).



Prendono origine da interruzioni del cortex, attraverso cui proliferano verso l'esterno le ife della medulla che intrappolano le cellule algali.

e)



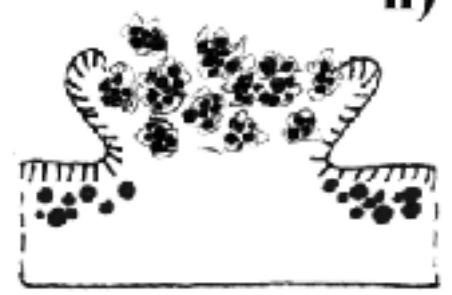
f)

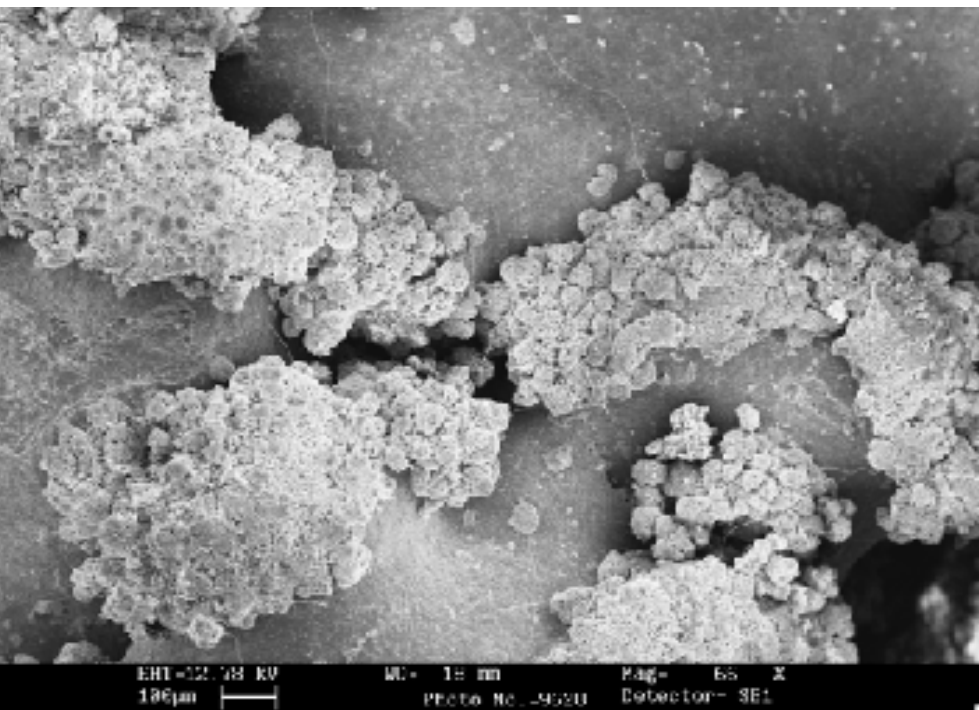
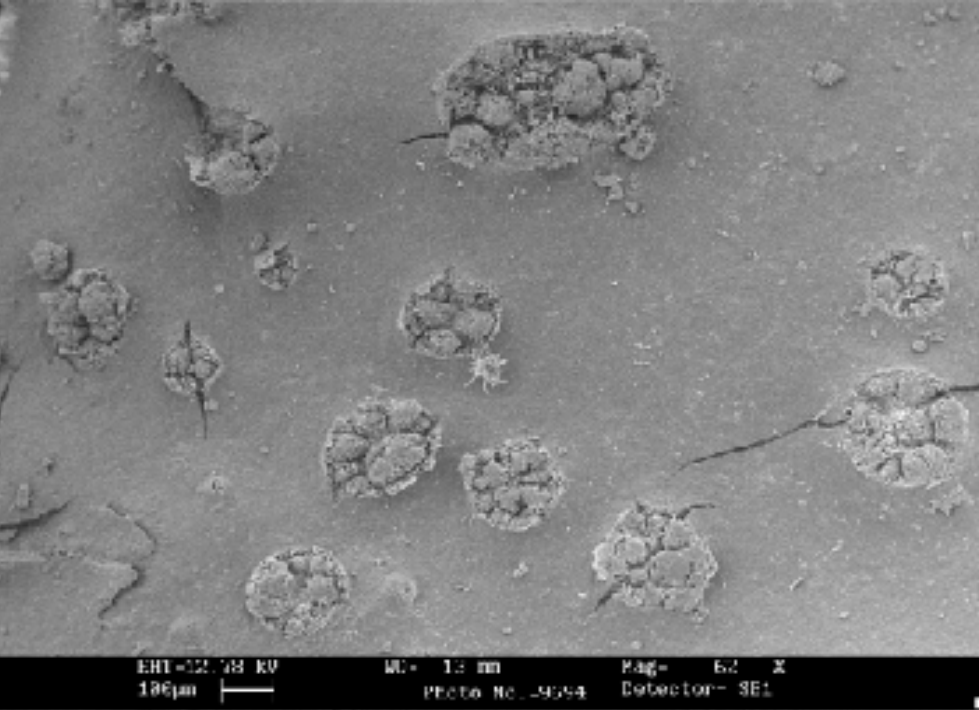


g)

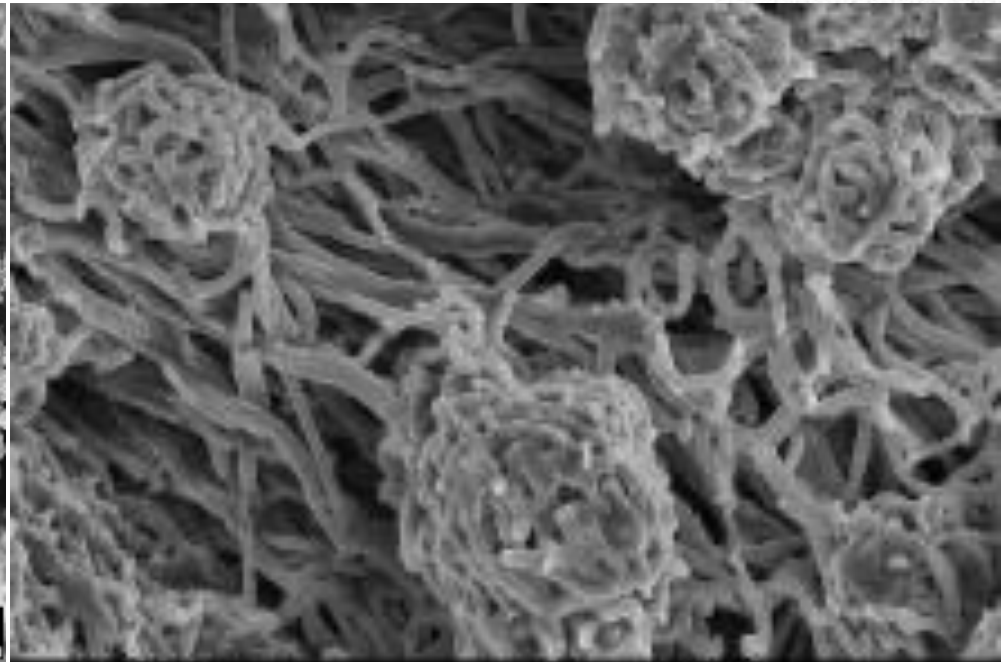
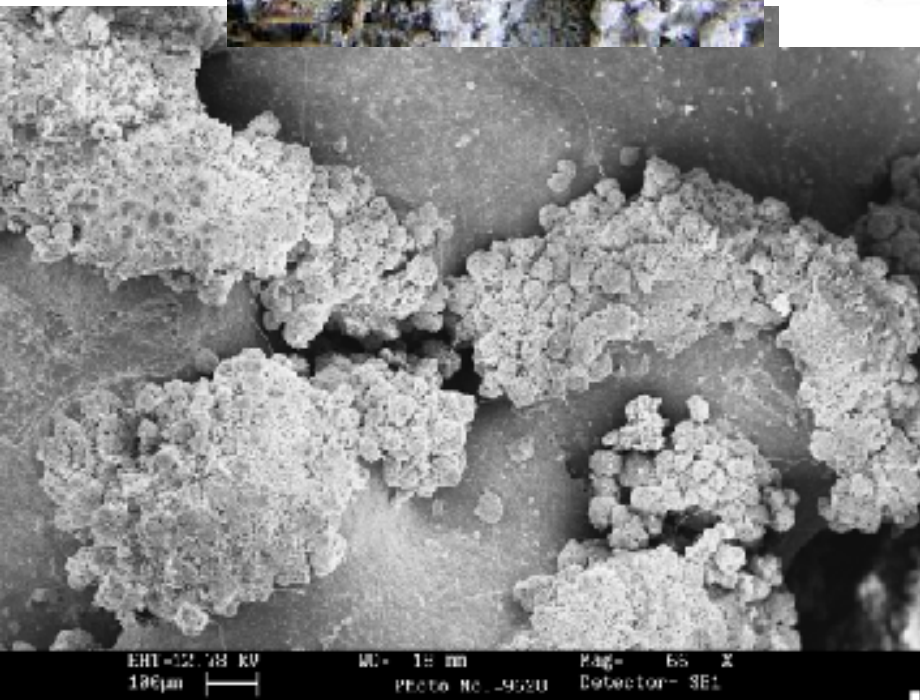
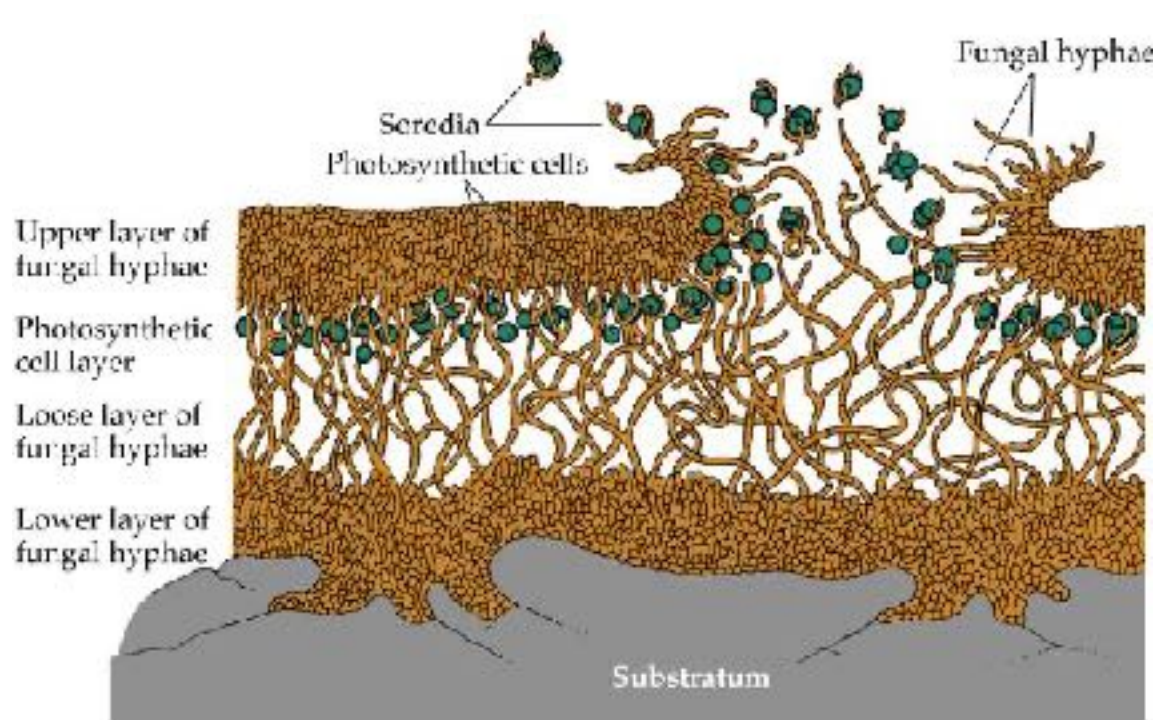


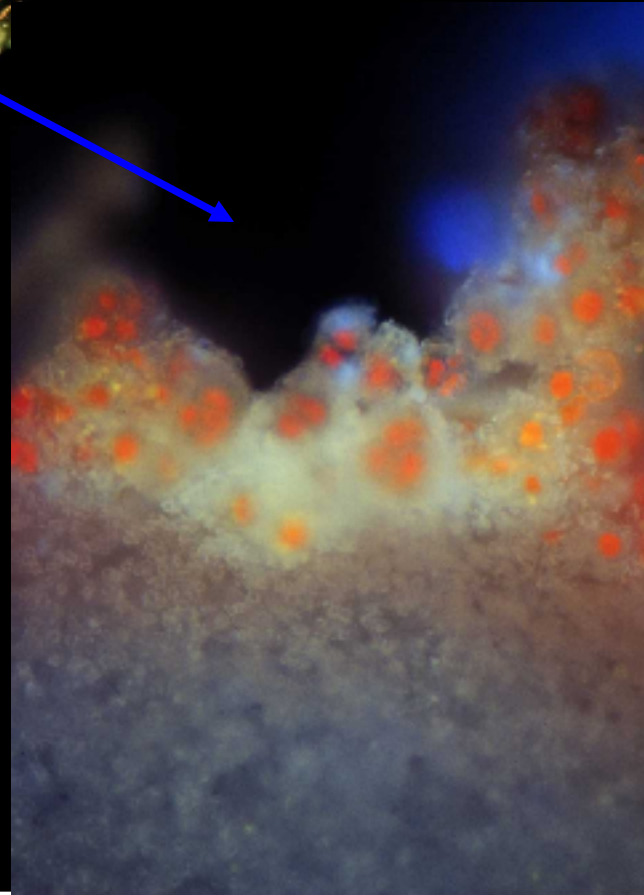
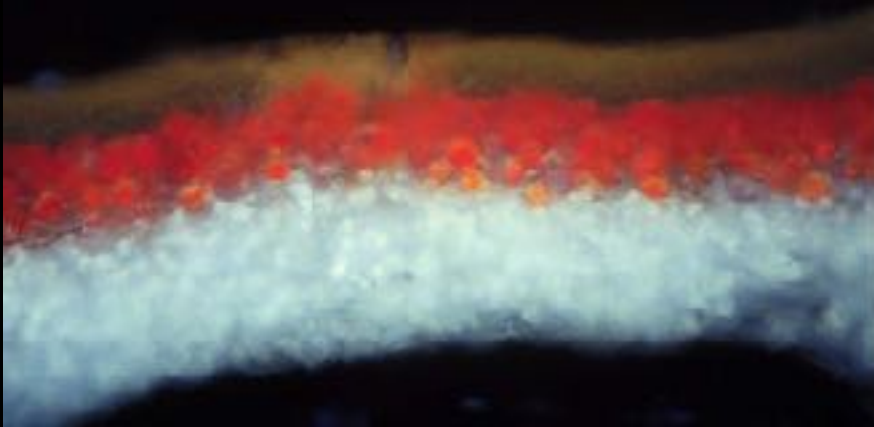
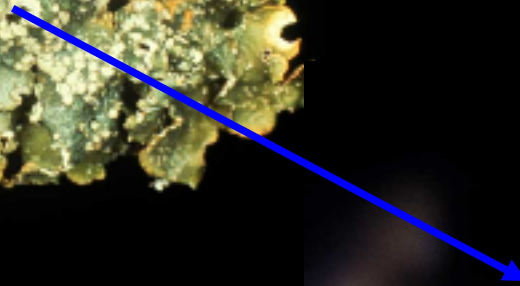
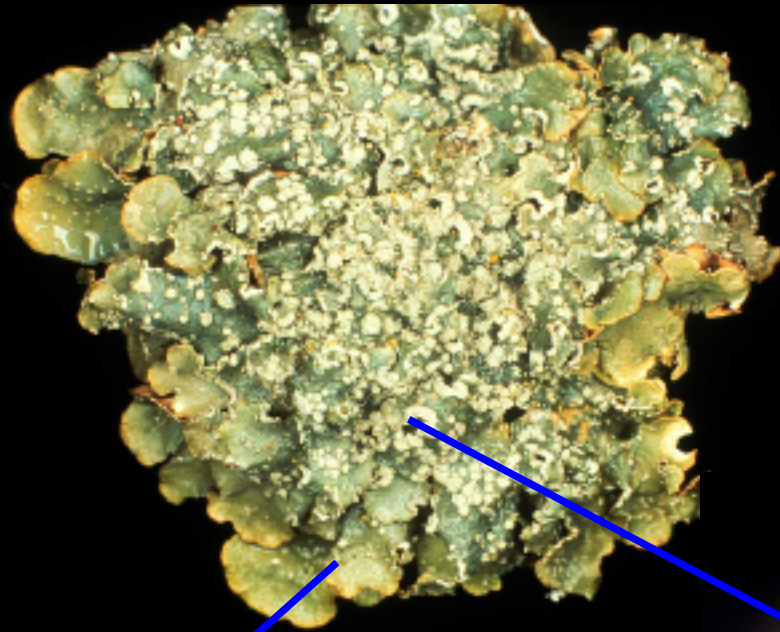
h)





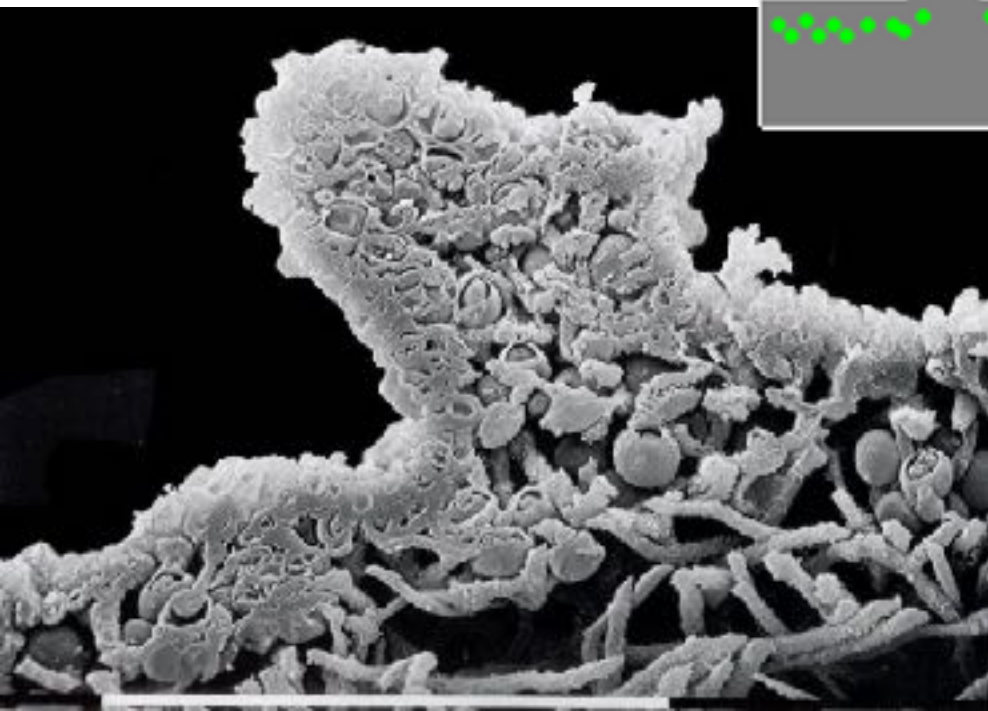
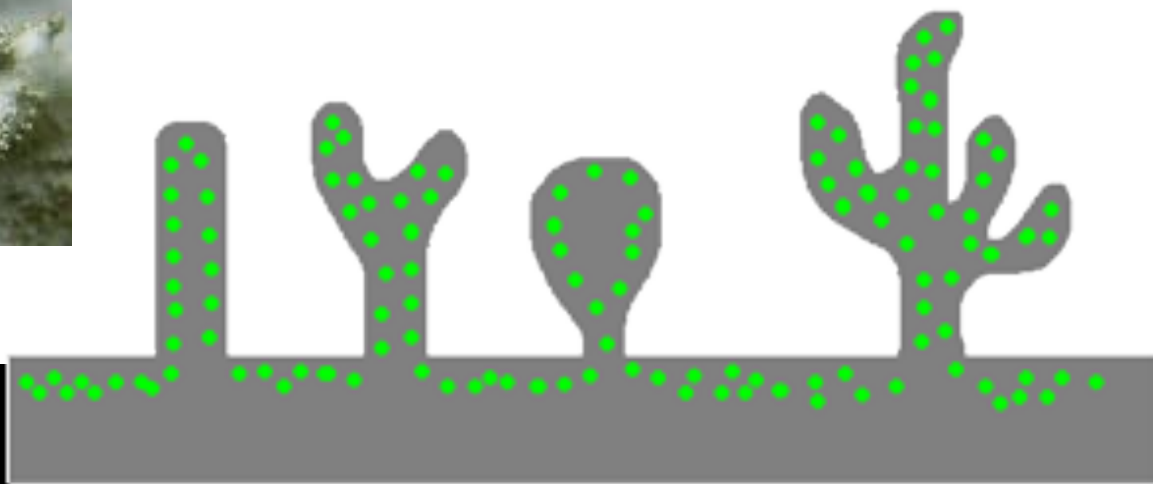
Hanno l'aspetto di granuli soffici e spesso idrofobi. I **sorali** spesso appaiono come delle punteggiature, linee o aree biancastre e pulverulente, che contrastano con la superficie del tallo.



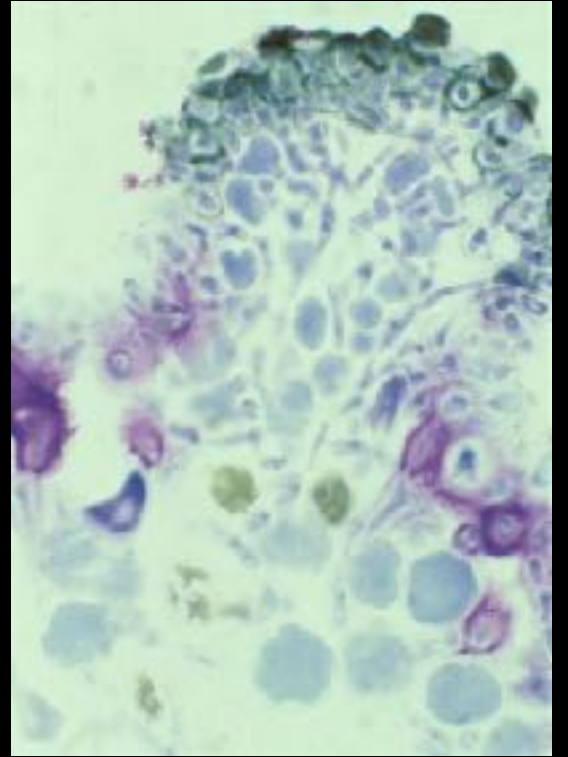
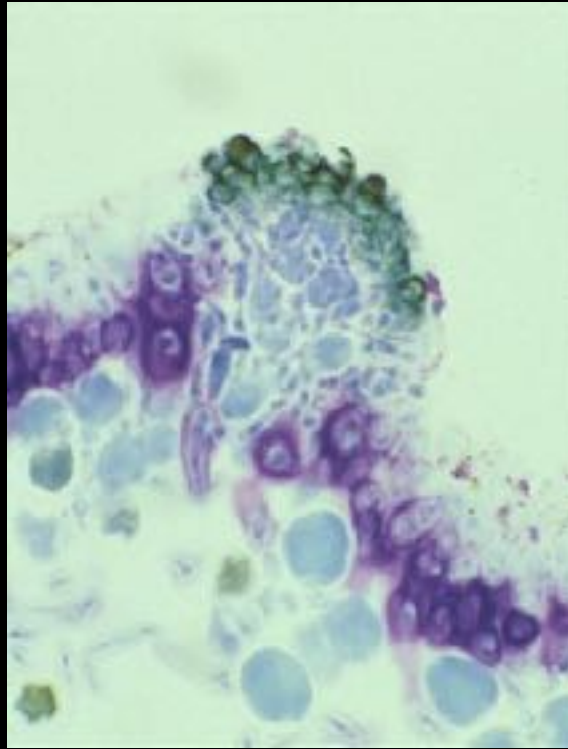
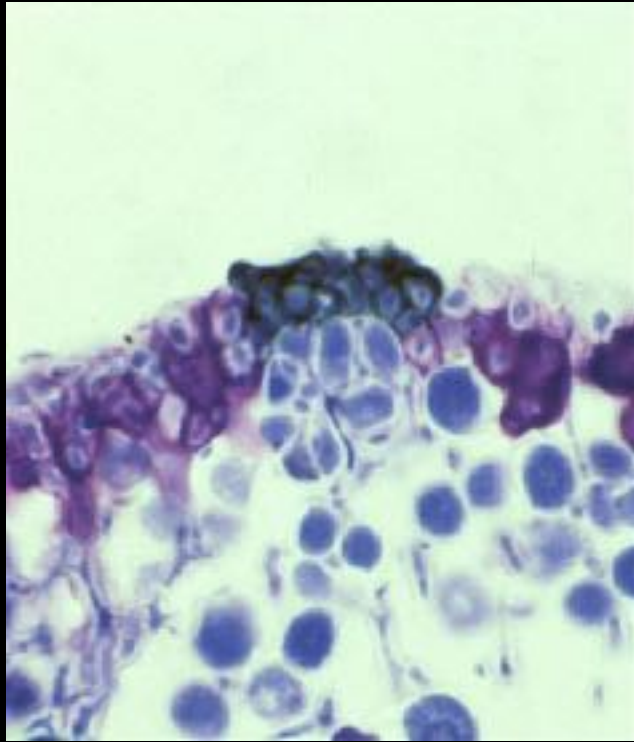




**ISIDI: diaspore**, sono strutture corticate, dello stesso colore della superficie esterna, o più scuri, soprattutto all'apice.

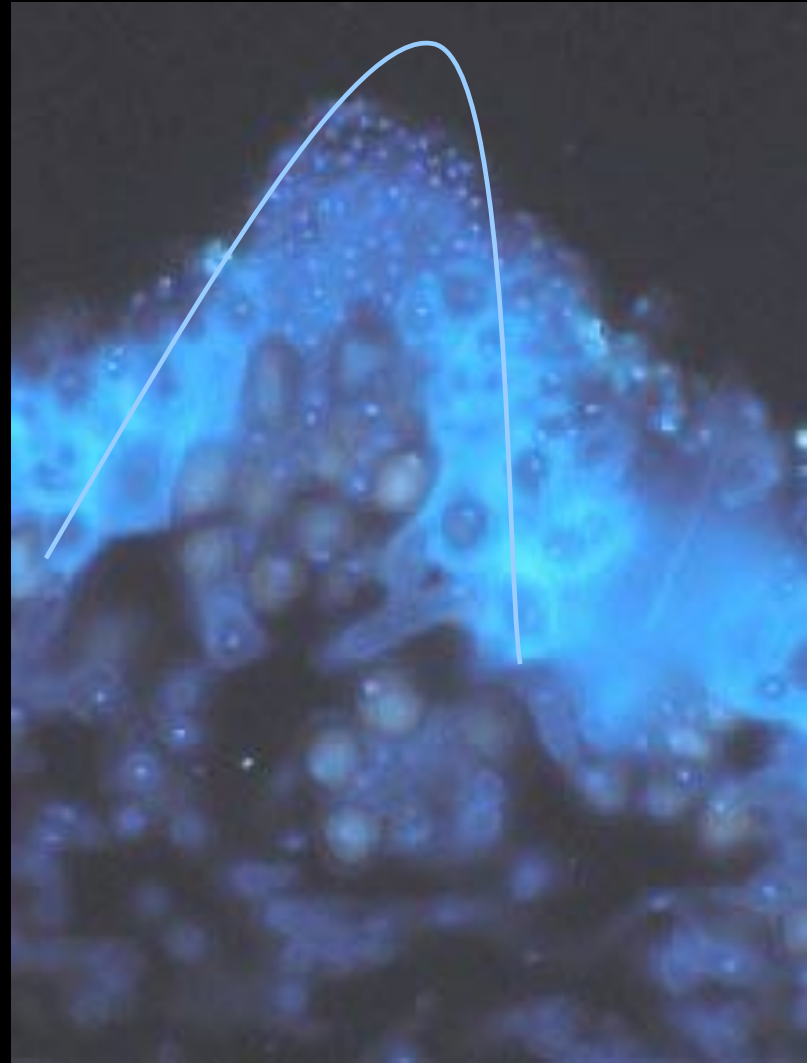
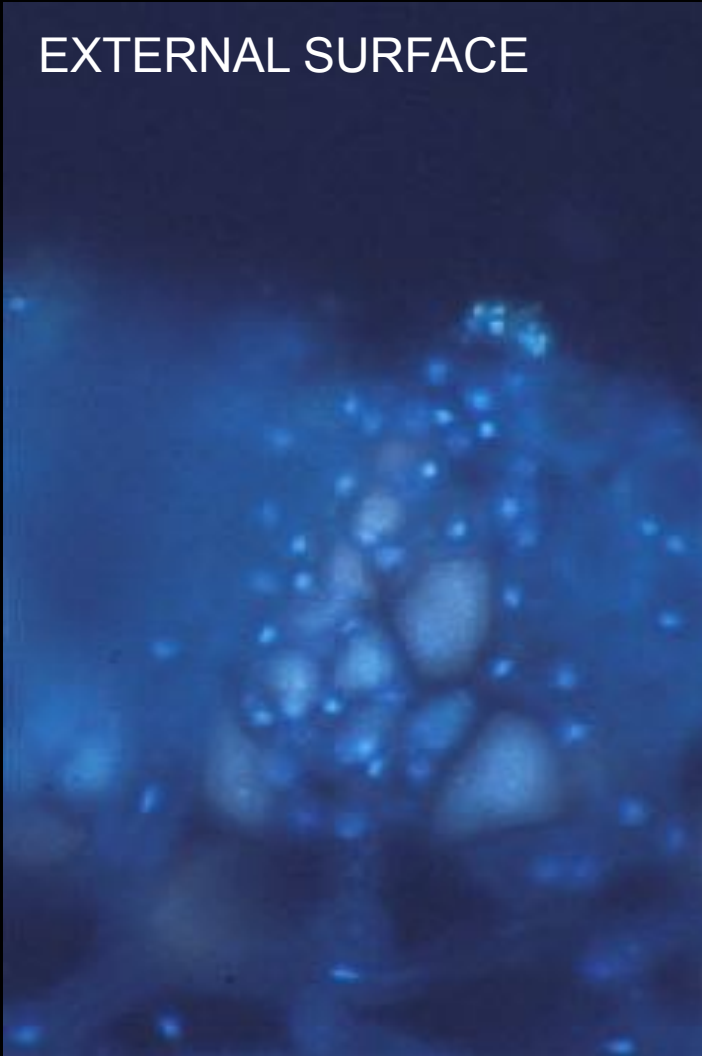


Originano per una proliferazione di ife medullari, che si accompagna ad una crescita delle ife del cortex, per cui essi sono sempre corticati.

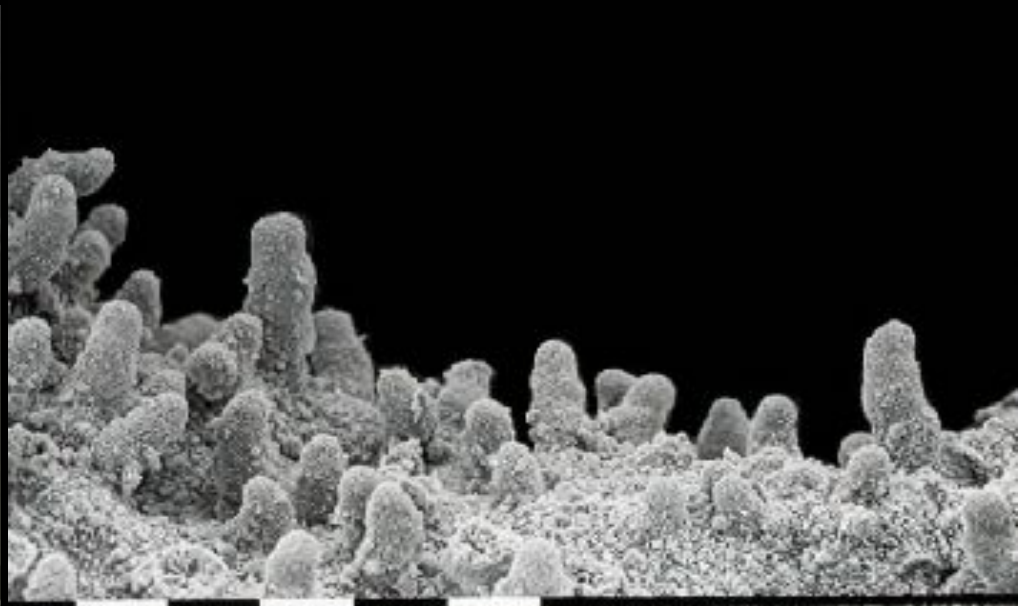
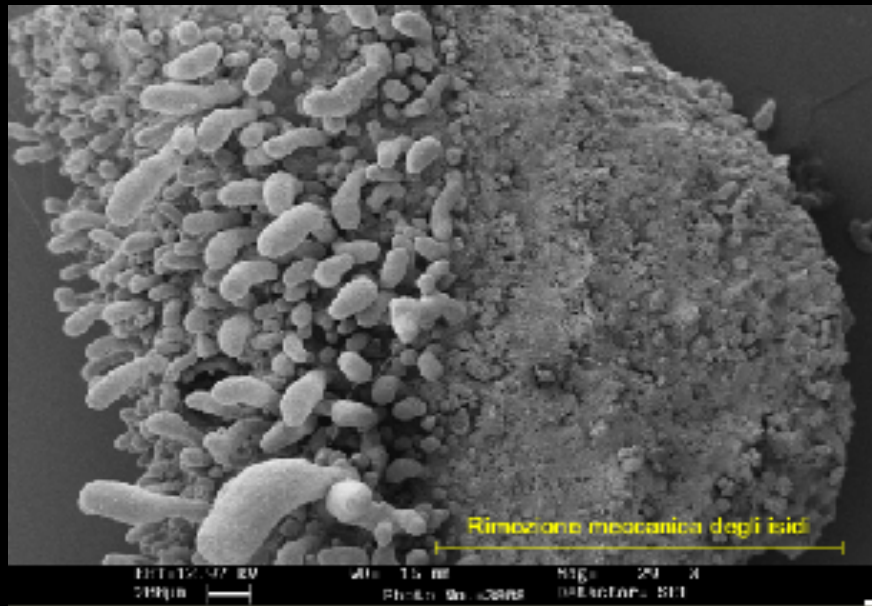


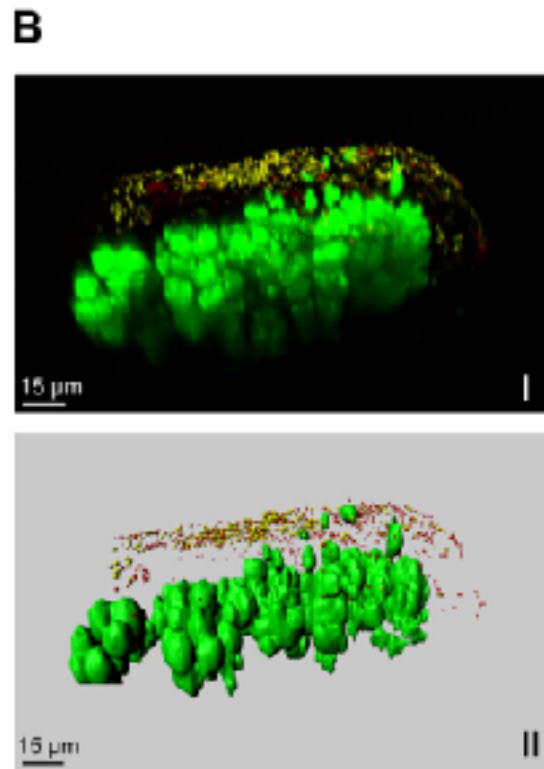
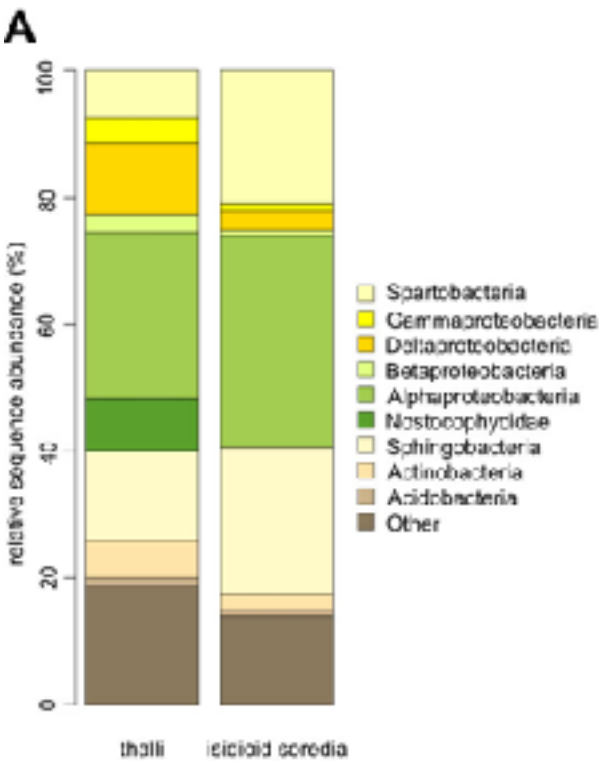


## EXTERNAL SURFACE



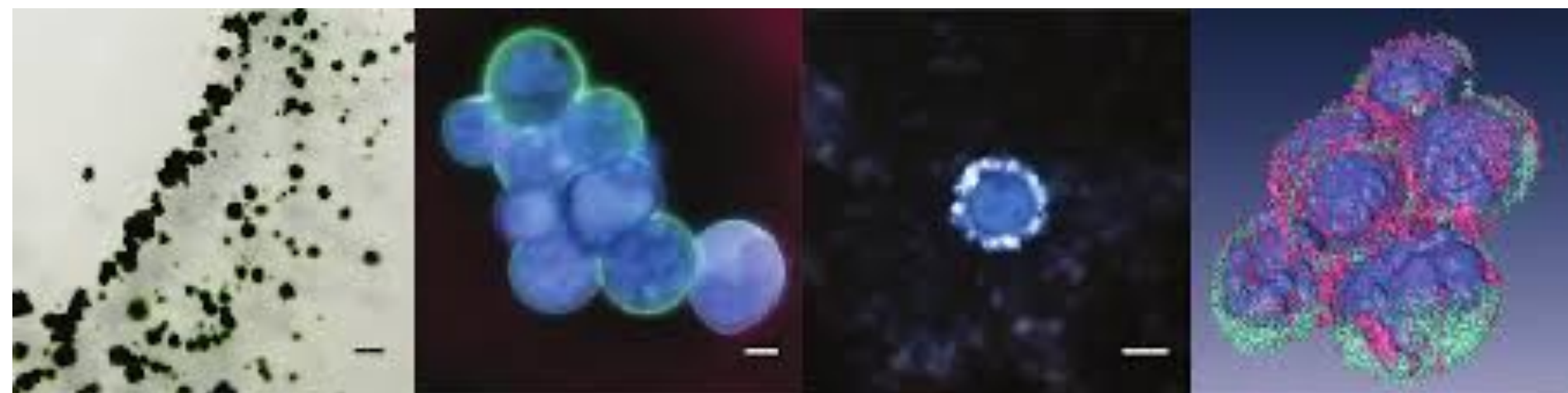
DAPI (4'-6diamidin-2-phenil-indol) in UV-A excitation light (BP 340-380)  
Technovit-encasted sections - DAPI was used to stain nuclei, being specific for double-stranded DNA; also cell walls may appear whitish.





- Isidia come “microbial cargo” nella trasmissione verticale di batteri co-simbionti (Aschenbrenner et al. 2016).

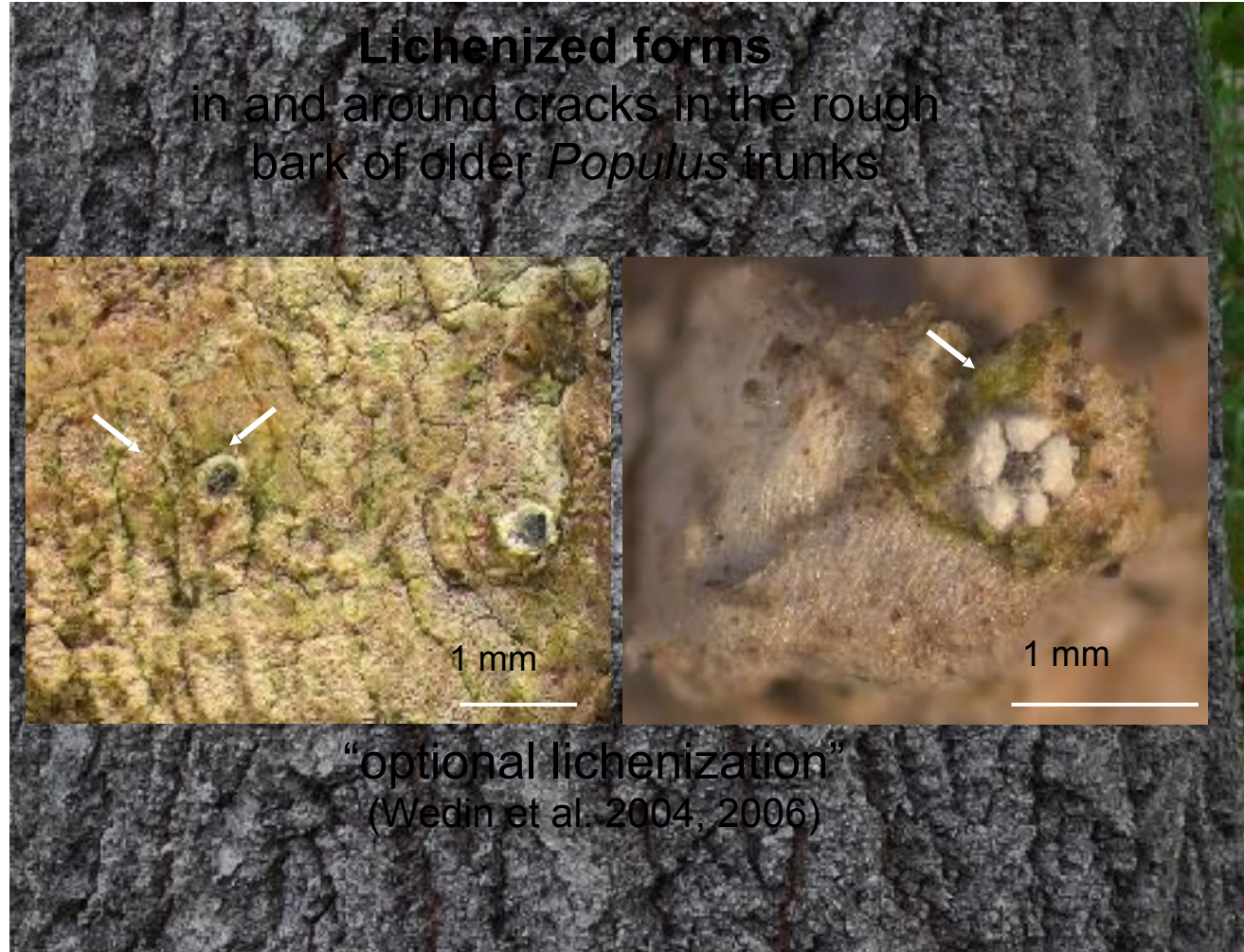
- *Trebouxia* alga and an Alphaproteobacterium species from *Usnea hakonensis*.
- The bacterium was able to use ribitol, glucose and mannitol. (Kono et al. 2017)



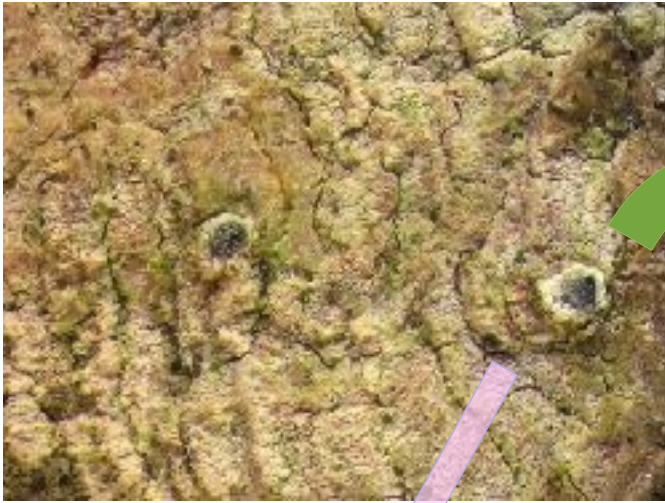
# *Schizoxylon albescens*

Stictidaceae (Ostropales, Lecanoromycetes): saprotrophic, parasitic and lichenized species; several taxa were found to live either as saprotrophs or as lichens.

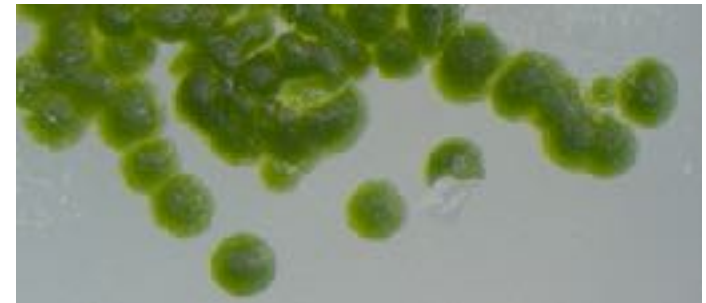
**Saprotrophic morphs**  
on dead, decorticated  
*Populus*- and *Salix*-  
branches



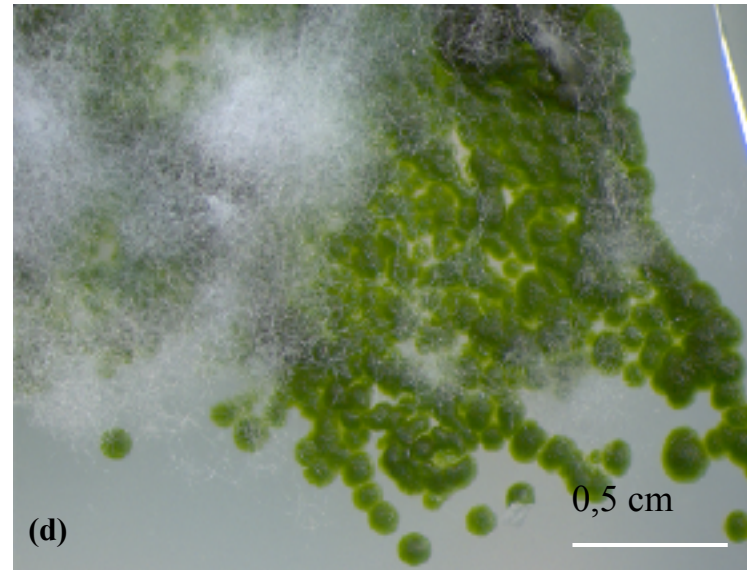
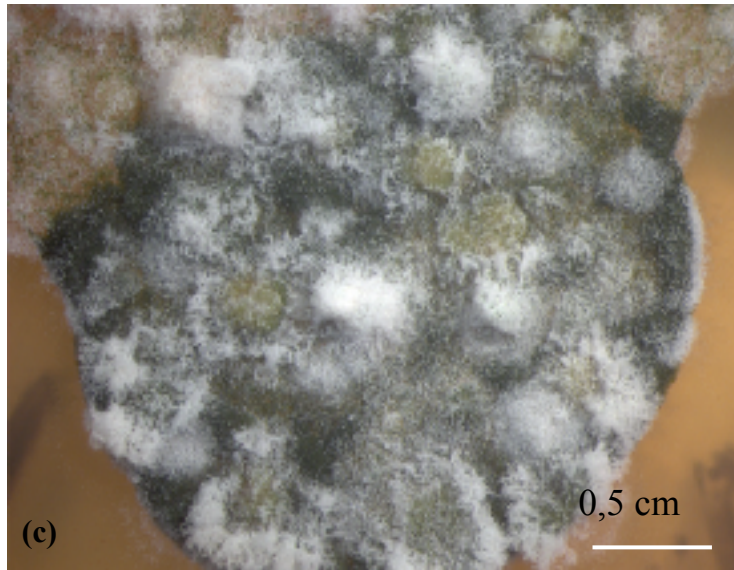
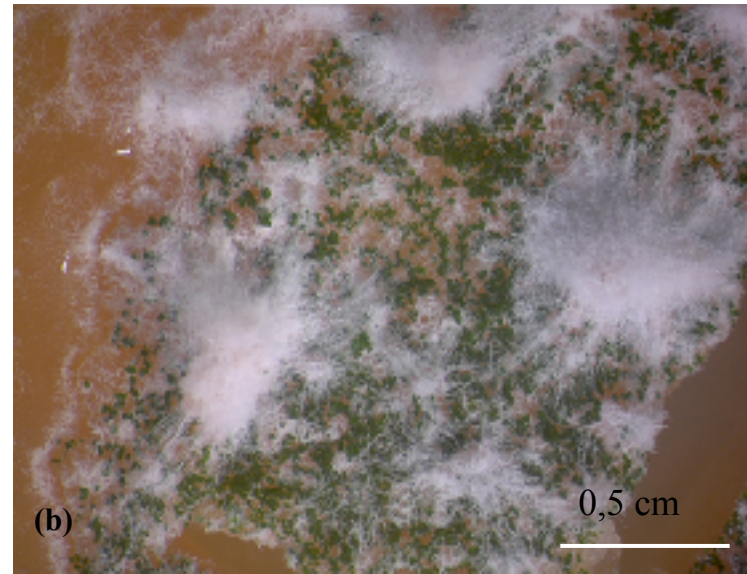
# Isolation of the fungus and the algae in axenic culture



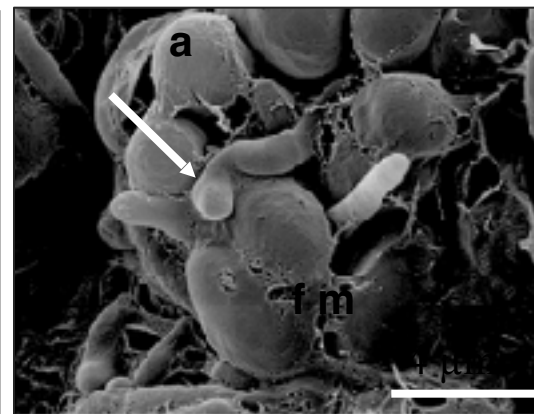
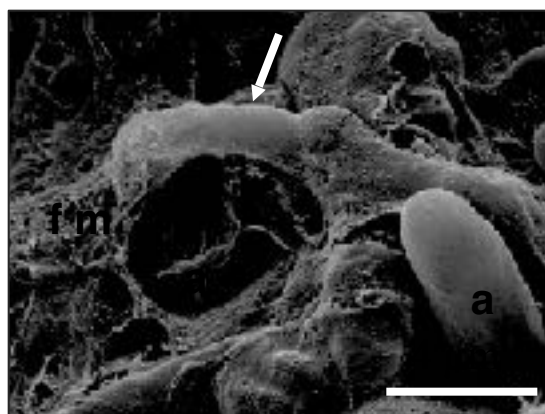
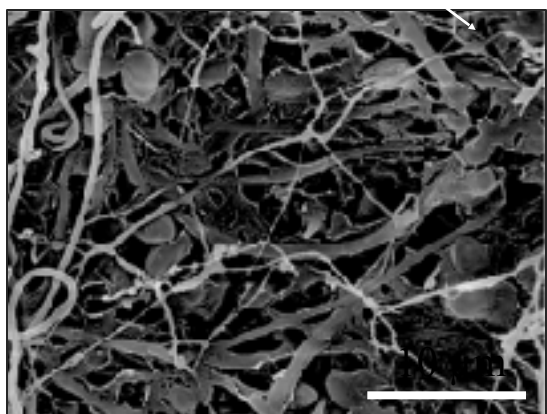
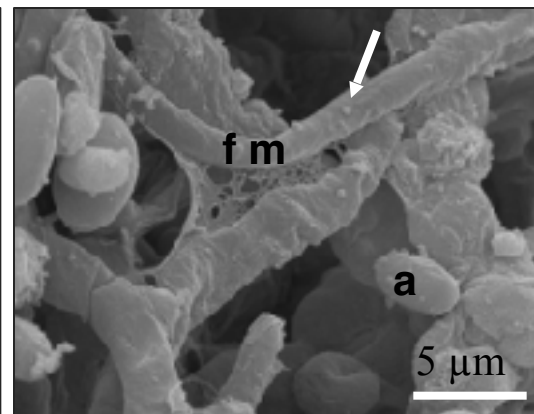
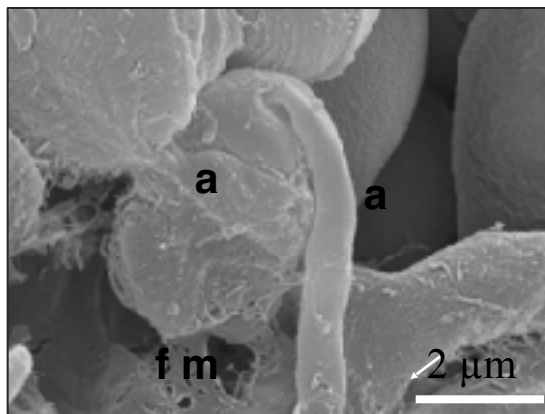
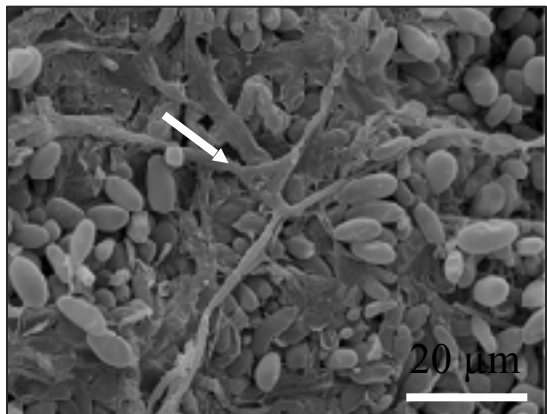
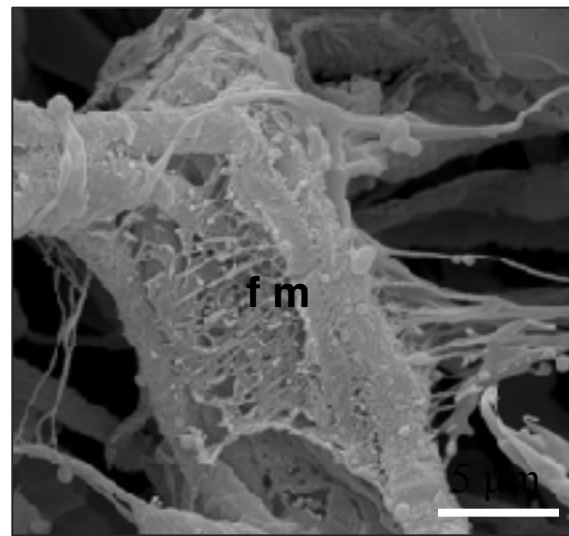
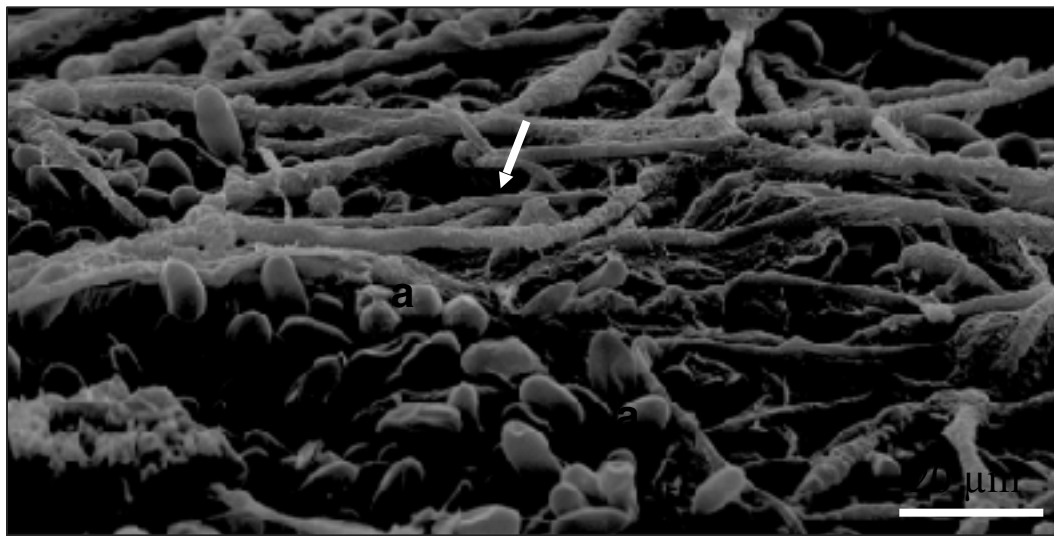
- i) one fruiting body was taken with a sterile razor blade, isolation according Yamamoto *et al.* (2002);
- ii) algal clumps were removed with a sterile needle and transferred directly on the agar medium;
- iii) algae clumps were diluted in sterile water and plated by pipetting.

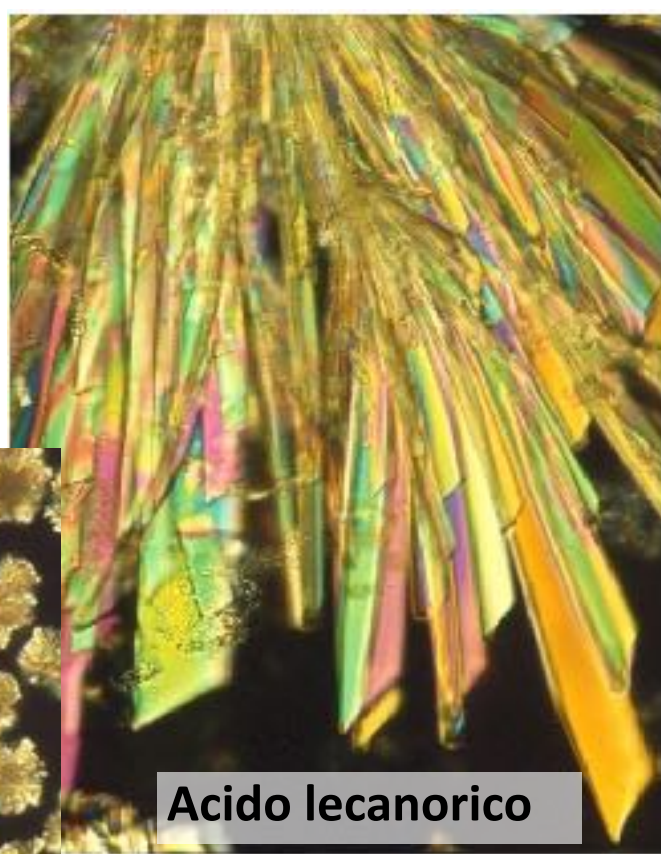


# Interaction fungus-algae in culture

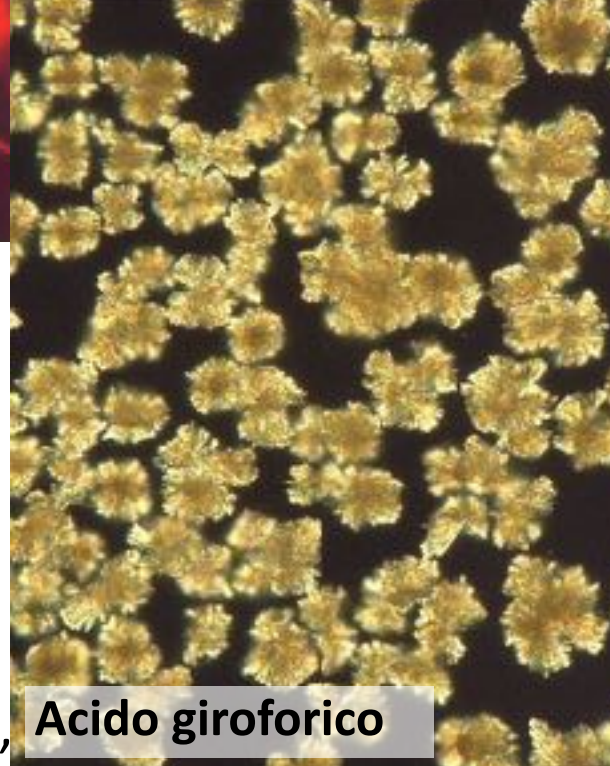


LB, MY, TM media, also on bark pieces

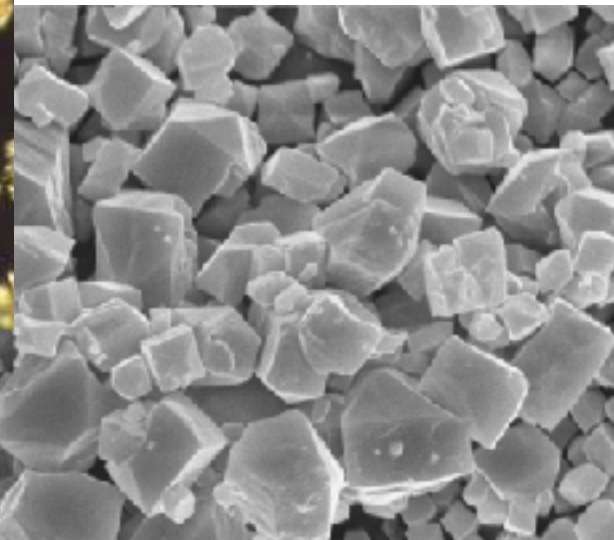




**Acido lecanorico**

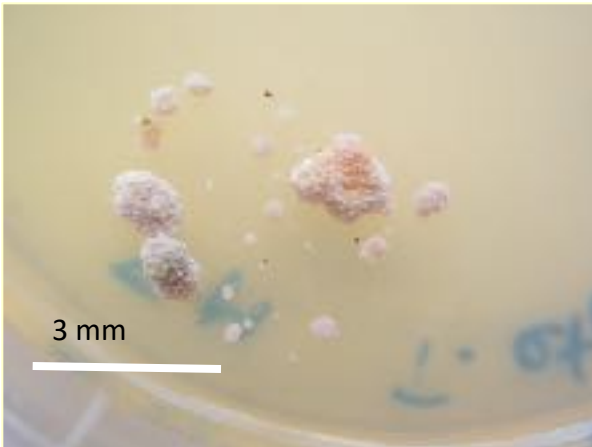


**Acido giroforico**

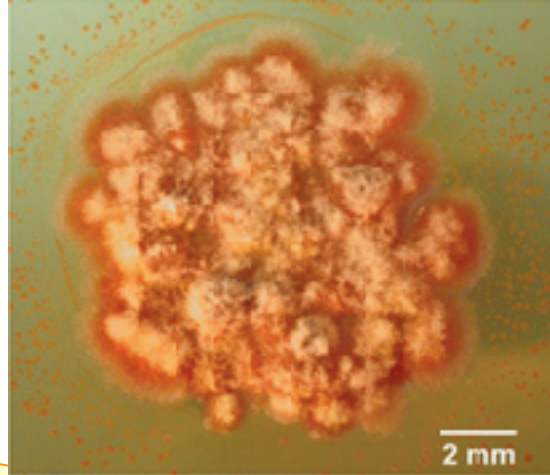


I licheni producono una ampia gamma di **metaboliti secondari**, chiamati «**acidi lichenici**», con funzioni molto diverse, accumulati nello spazio apoplastico.

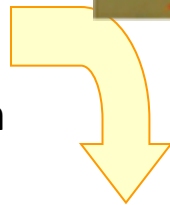




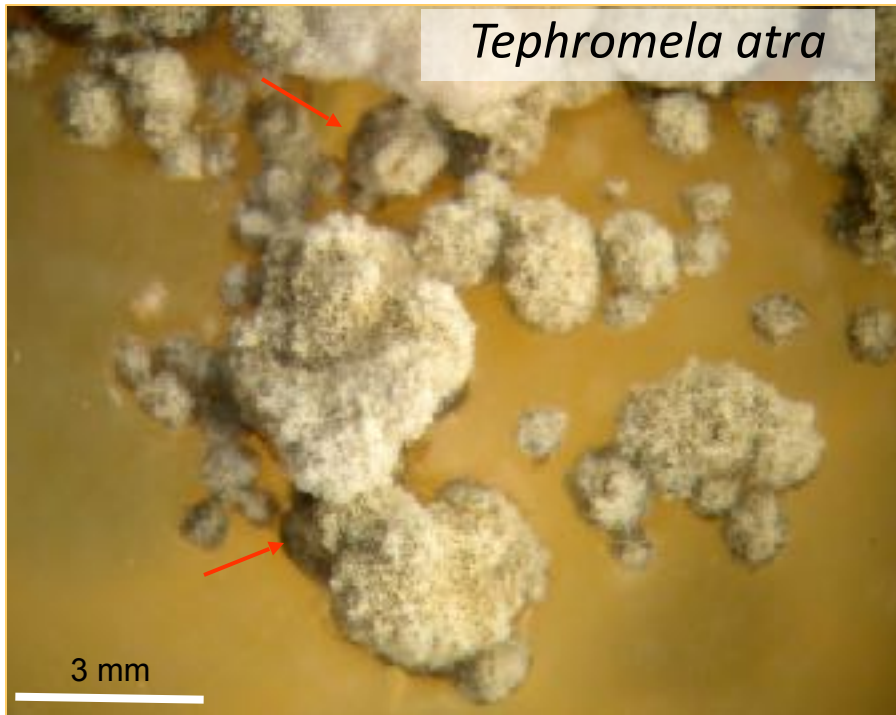
...after almost 1 year of growth in culture (MY medium)



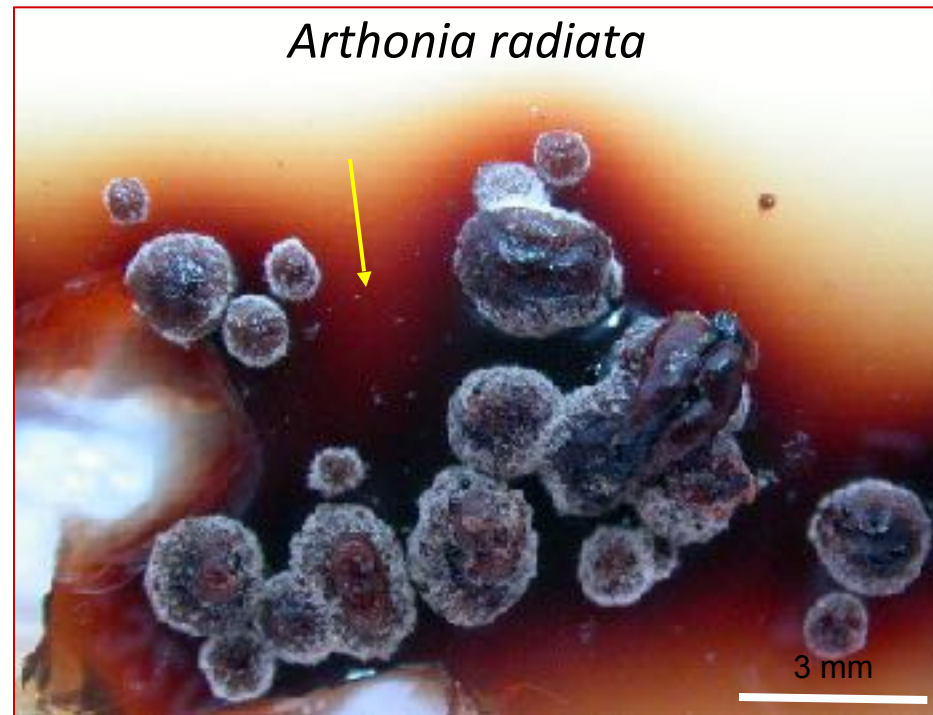
!!! The metabolic pattern of the cultured mycobiont can differ from the one in the symbiosis.



*Tephromela atra*



*Arthonia radiata*



I LICHENI SONO TOLLERANTI AL DISSECCAMENTO:

licheni sensibili al disseccamento (pochissimi), licheni mediamente tolleranti (tanti), licheni incredibilmente tolleranti (un discreto numero); occupano nicchie ecologiche diverse.

## “Homoiochlorophyllous poikylolydric”

- HDTs retain their chlorophyll on desiccation,
- PDTs (poikilochlorophyllous), desiccation results in the loss of chlorophyll, which must be reformed following remoistening



bagnato



secco



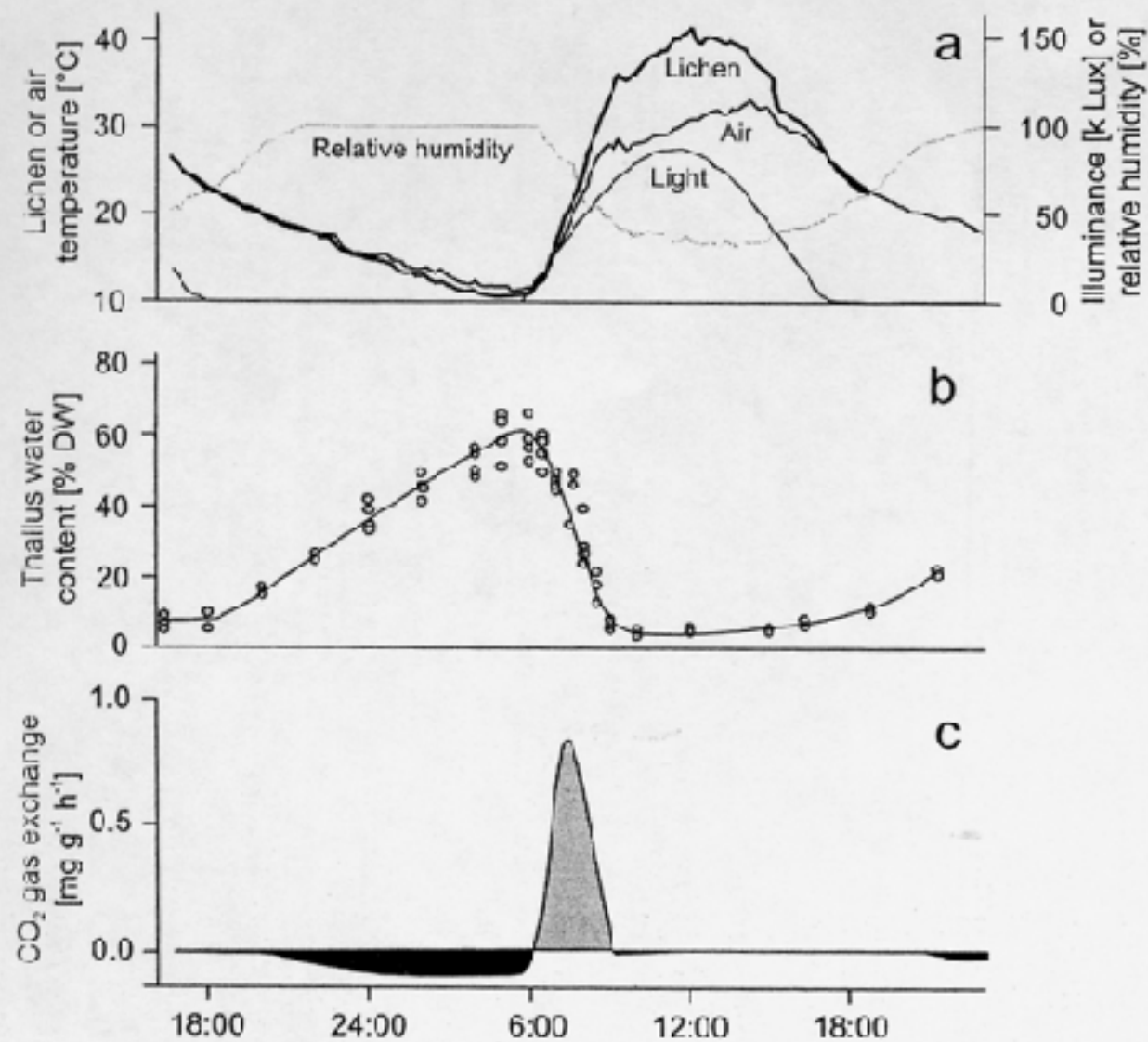
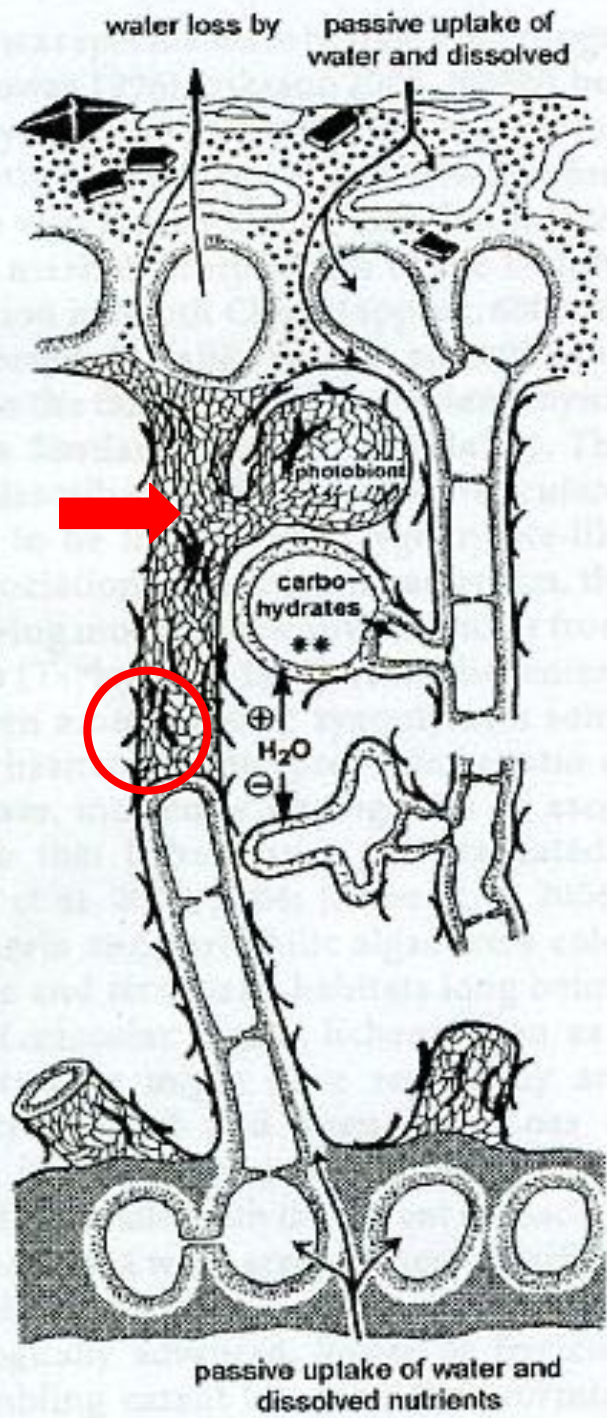


Figure 1. CO<sub>2</sub> gas exchange of *Ramalina maciformis* in relation to environmental parameters during and after dewfall in the Negev Desert. Figure redrawn and modified after Lange (1970). (a) Lichen and air temperature, illuminance and

Time

relative humidity (thick to thin lines) at the experimental site. (b) Thallus water content given as a percentage of dry weight; (c) CO<sub>2</sub> balance curves; values below zero (black) indicate net respiration and above zero (gray) they denote net photosynthesis.





**IDROFILO**

Tranne che per quelle poche specie intolleranti al disseccamento, i ripetuti cicli “secco-umido” sono fondamentali per la sopravvivenza della simbiosi, in quanto permettono lo scambio di sostanze tra i due (o più) partner.

**IDROFOBICO  
(micobionte)**

- Parete con sottile strato idrofobico di natura proteica
- Metaboliti secondari in cristalli sulla superficie della parete

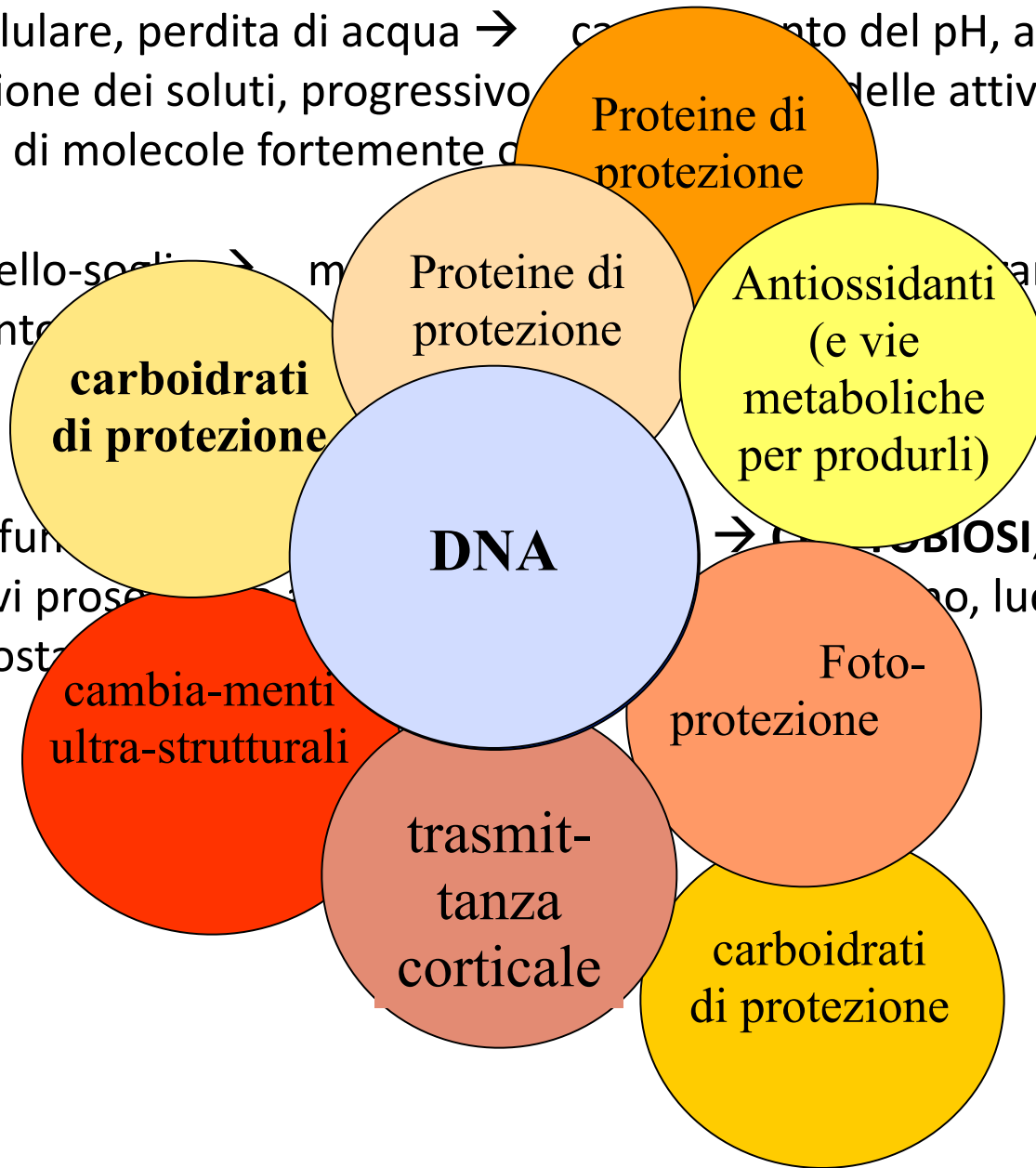
**IDROFILO**

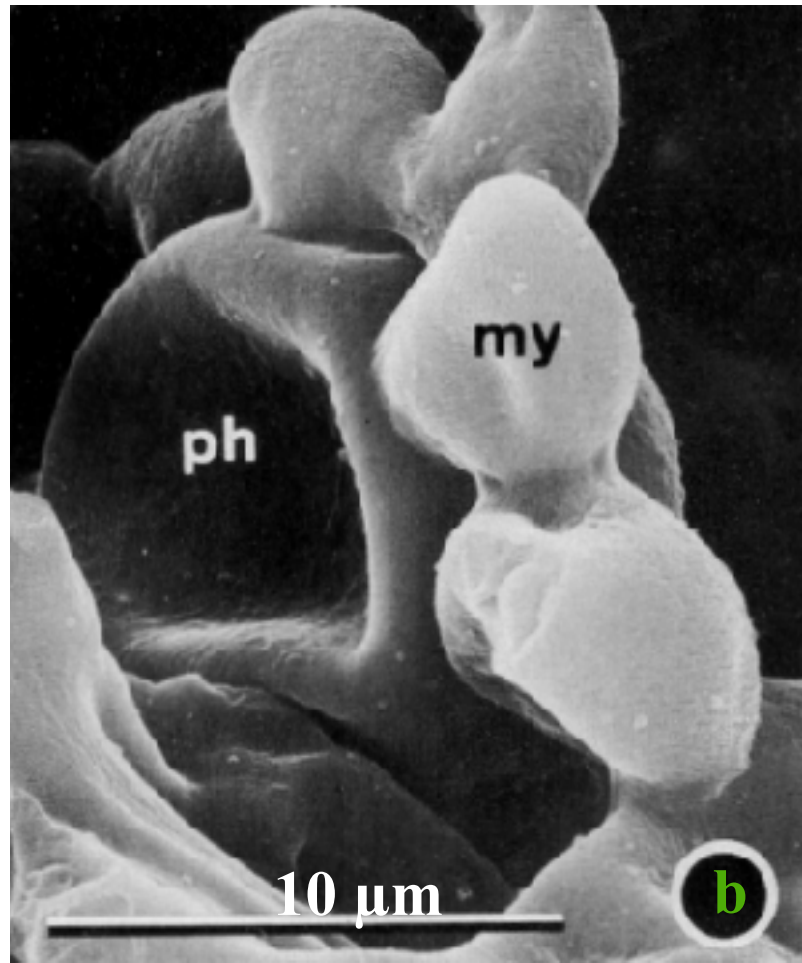
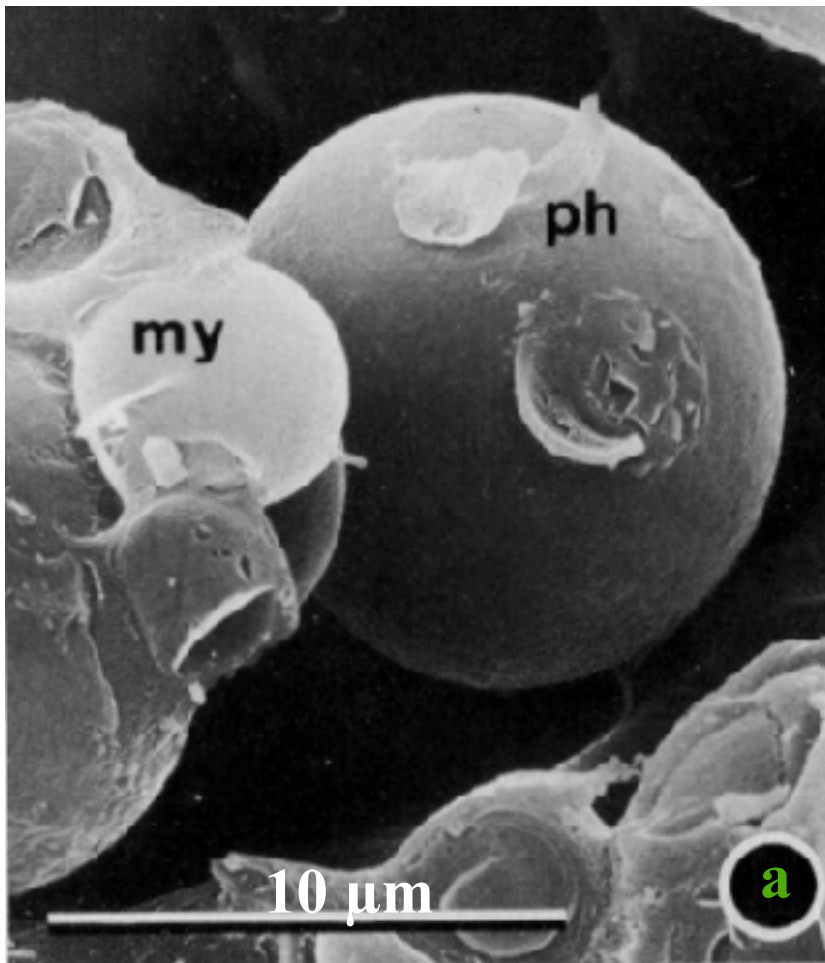
# EFFETTI DEL DISSECCAMENTO SUL METABOLISMO DEL LICHENE

A livello cellulare, perdita di acqua → aumento del pH, aumento della concentrazione dei soluti, progressivo arresto delle attività enzimatiche, formazione di molecole fortemente c

Oltre un livello-soglia → morte cellulare (per collassamento delle membrane e proteine) →

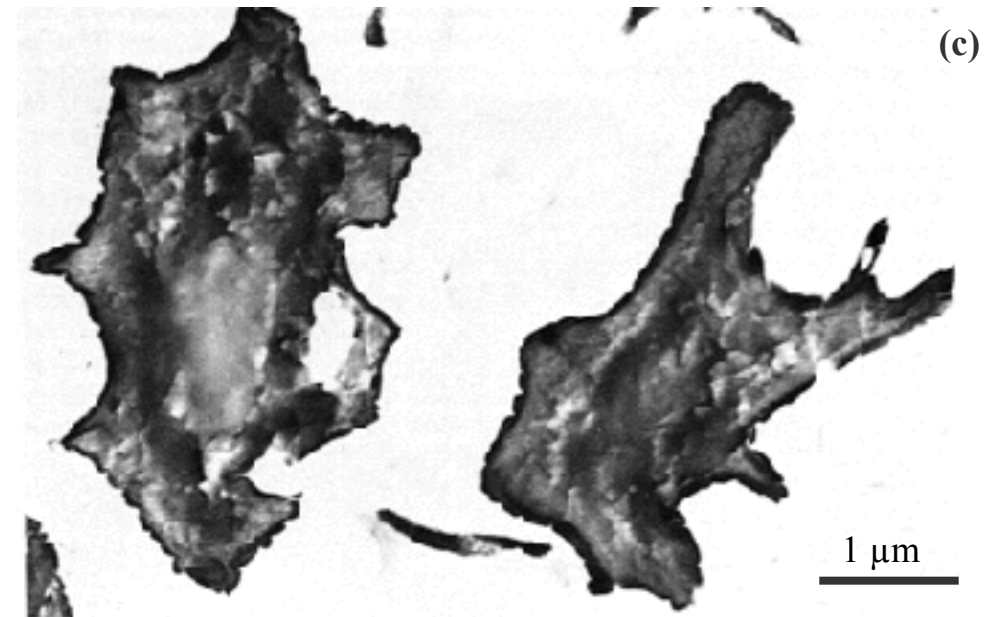
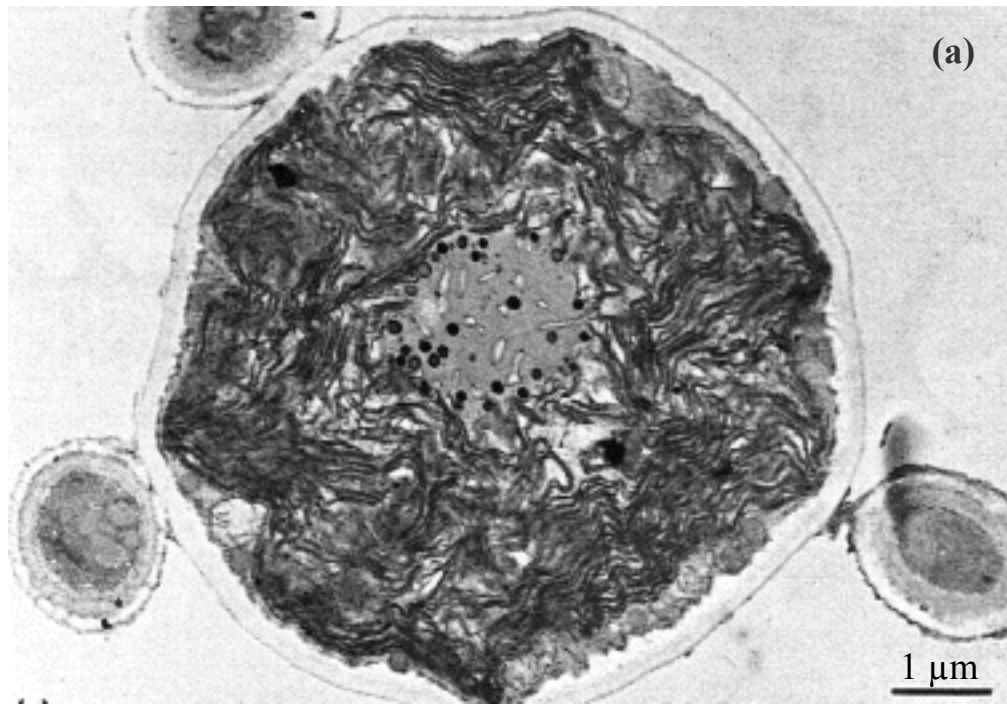
Cessano le funzioni metaboliche, si verificano processi degenerativi progressivi, si accumulano eventuali sostanze tossiche → **PROBIOSI**, i processi di morte, luce, ed





Immagini al LTSEM del lichene foglioso *Xanthoria parietina* perfettamente idratato (c. 150% di contenuto idrico relativo) (a) e secco (c. 30% di contenuto idrico relativo) (b). Da Honegger, *New Phytol.* 125: 659-677, modif.

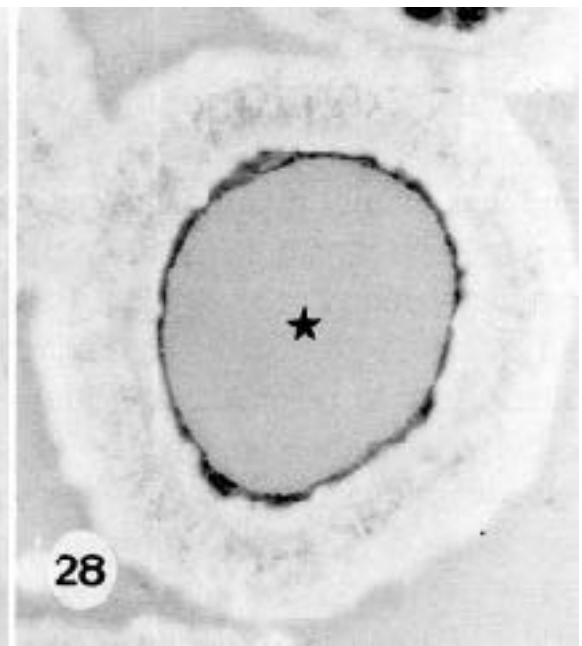
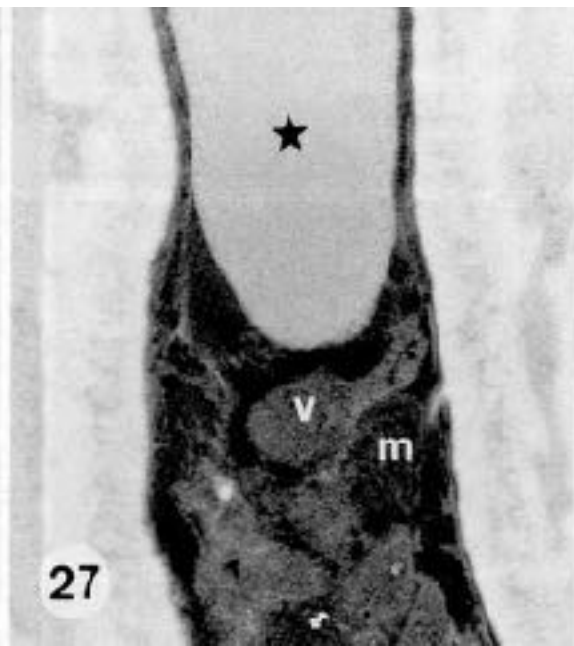
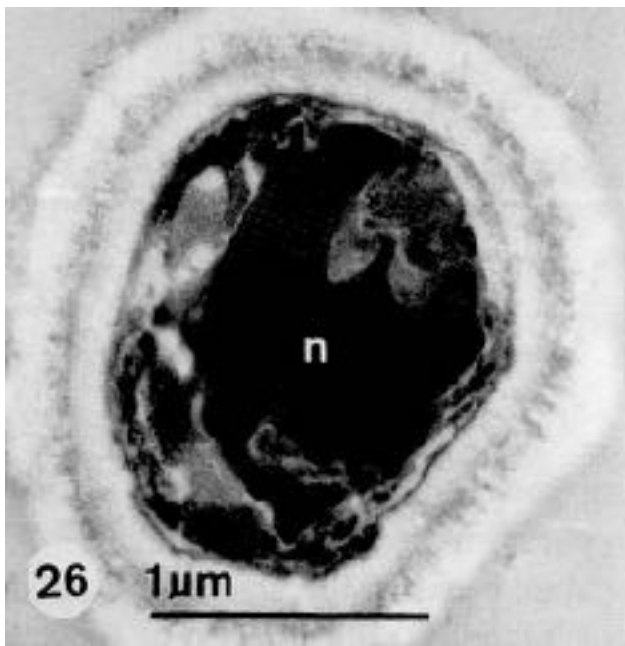




Fotobionti del genere *Trebouxia* fissati in glutaraldeide in tampone fosfato al 100%RH (a) e al 0%RH (b), e con vapori di tetrossido di osmio allo 0%RH (c).

Brown DH, Rapsch S, Beckett A, Ascaso C (1987) The effect of desiccation on cell shape in the lichen *Parmelia sulcata* Taylor. *New Phytologist* **105**: 295-299.



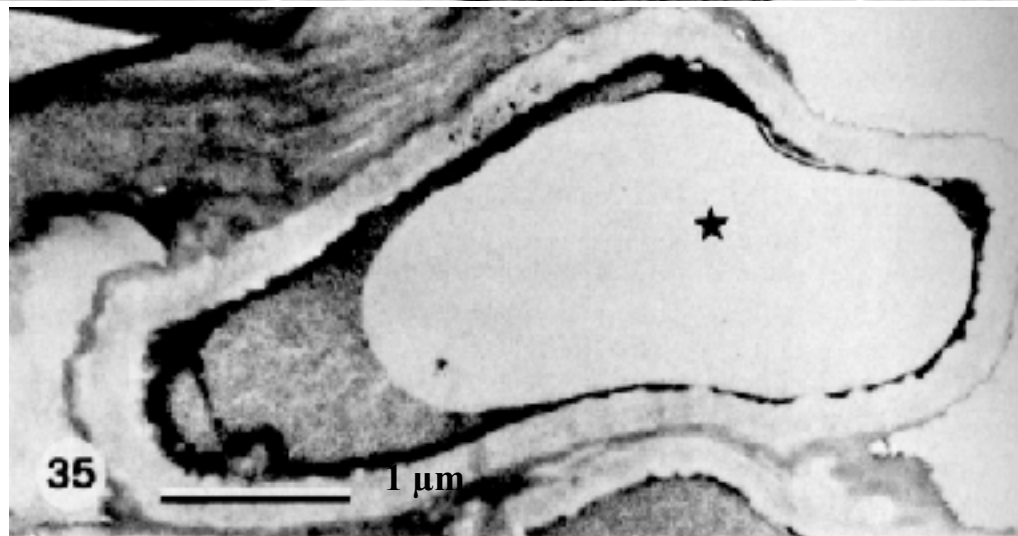


n: nucleo

v: vacuolo

m: mitocondrio

\*: bolla gassosa



Il pericolo forse maggiore del disseccamento, insieme alla denaturazione delle proteine enzimatiche, è legato alla **formazione di molecole fortemente ossidanti**, in particolare di **ROS, “Reactive Oxygen Species”**.

- I radicali liberi sono atomi o molecole con elettroni spaiati e sono particolarmente reattivi;
- prodotti da reazioni metaboliche (respirazione aerobia, fotosintesi), fenomeni di stress e molecole ossidanti (es.  $O_3$ ;  $H_2O_2$ )
- i radicali dell'ossigeno includono:

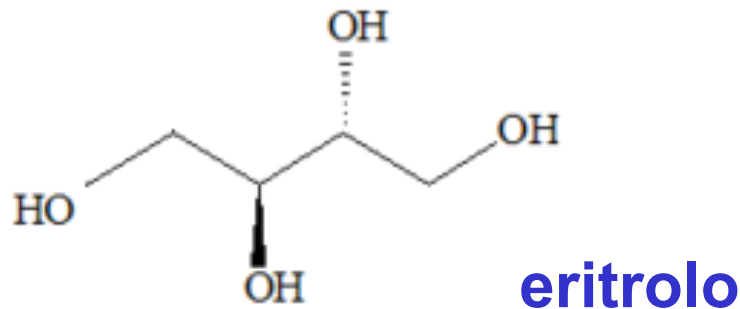
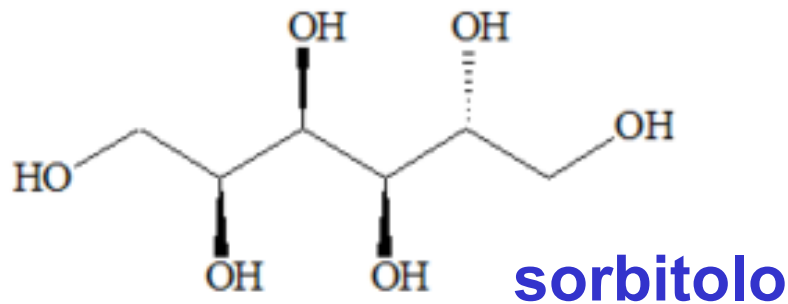
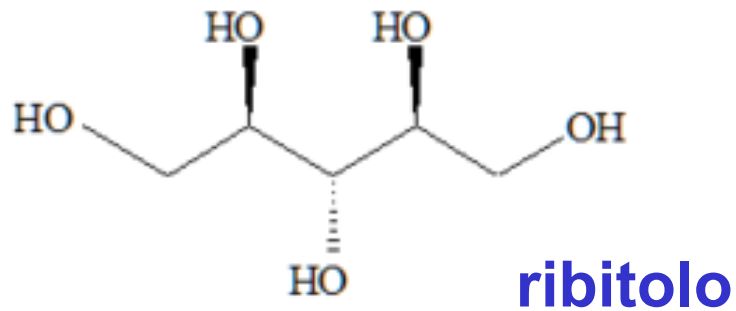


**Eliminare i ROS** – è compito di complessi enzimatici e molecole organiche a basso peso molecolare, quali il tripeptide GLUTATIONE(GSH), l'ASCORBATO, i TOCOFEROLI.

**“Halliwell/Foyer/Asada cycle”** - Nel 1976 è stato proposto un ciclo, che vede l'azione integrata di superossido dismutasi, glutatione, ascorbato, glutatione reduttasi, ascorbato perossidasi, mono- e di-deidroascorbato reduttasi.

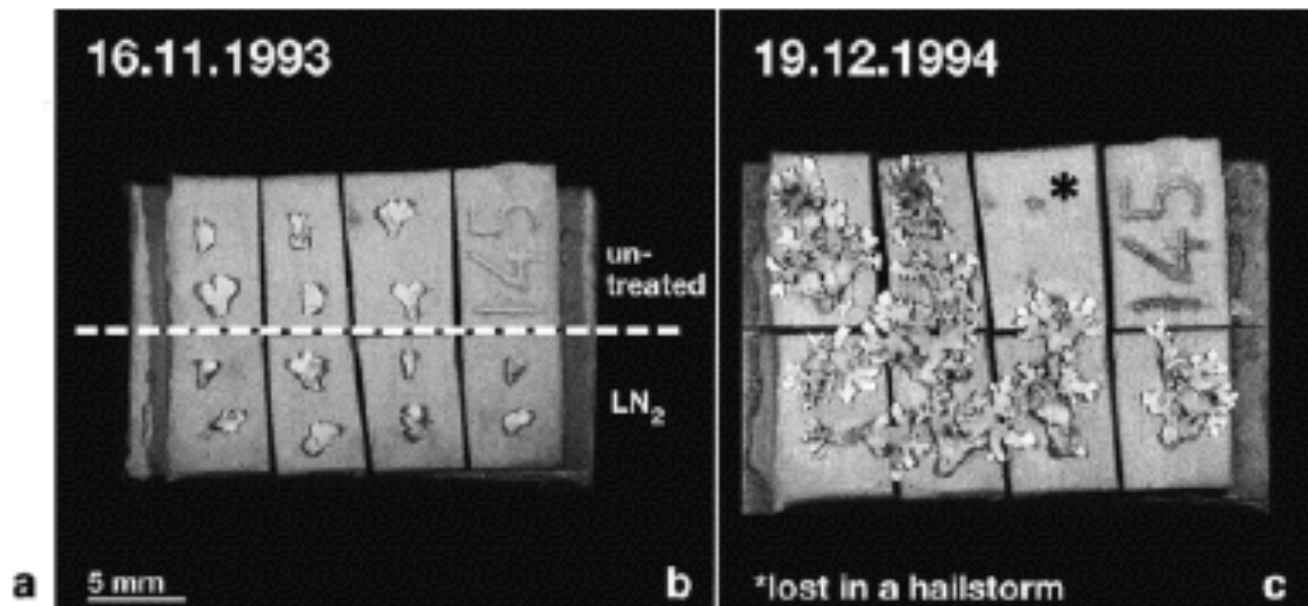
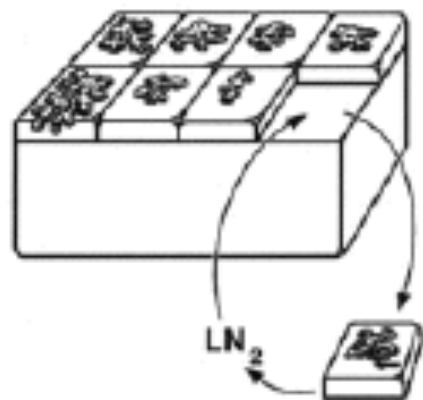
**Nei licheni** - La capacità di far fronte ai ROS dipende da:

- tempo richiesto dagli enzimi per diventare funzionanti con la reidratazione del tallo;
- capacità di mobilizzare potere riducente (NADPH);
- quantità di molecole anti-ROS costitutive, in particolare la coppia GSH - GSSG.



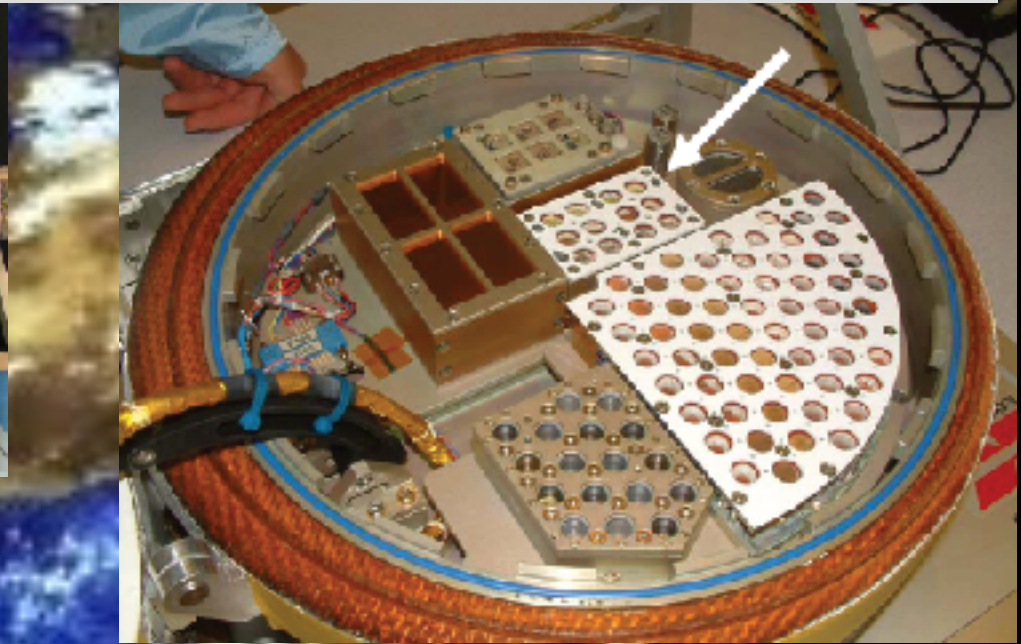
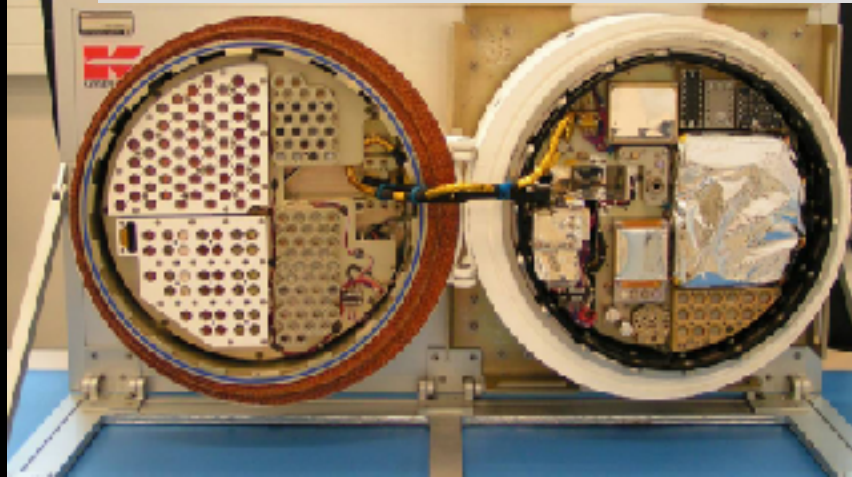
GSH is a major tissue antioxidant that provides reducing equivalents for the glutathione peroxidase (GPx) catalyzed reduction of lipid hydroperoxides to their corresponding alcohols and hydrogen peroxide to water.

In the GPx catalyzed reaction, the formation of a disulfide bond between two GSH molecules gives rise to oxidized glutathione (GSSG). The enzyme glutathione reductase (GR) recycles GSSG to GSH with the simultaneous oxidation of  $\beta$ -nicotinamide adenine dinucleotide phosphate ( $\beta$ -NADPH<sub>2</sub>).



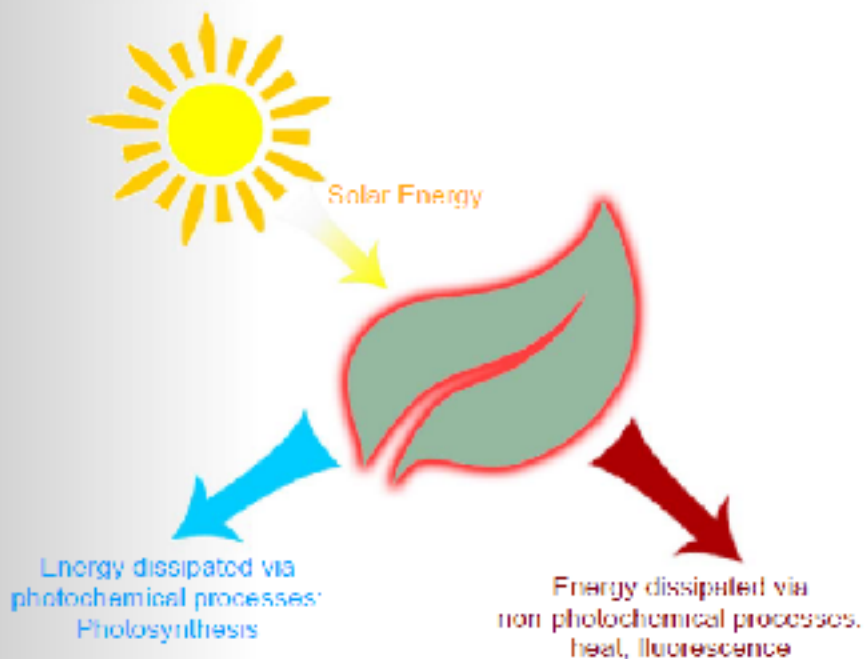
**Fig. 4** Reviviscence and growth of lobules of *X. parietina* after one week of  $LN_2$  storage (lower half in **b,c**) in comparison with untreated specimens (upper half in **b,c**). Lobules were fixed to detachable ceramic slabs with cyanoacrylate glue (**a**).

Biological test systems of the **Lithopanspermia/Biopan experiment**; all samples were exposed to space vacuum, cosmic radiation and selected wavelength ranges of solar extraterrestrial electromagnetic radiation (UV/VIS).

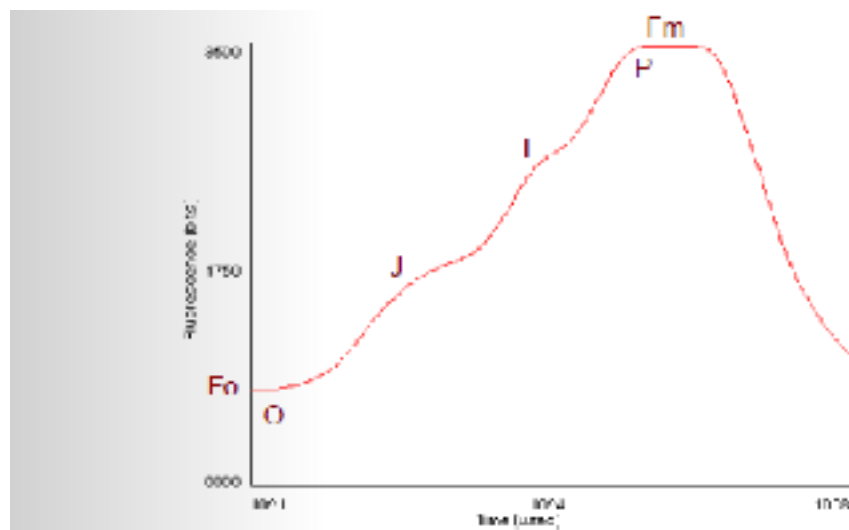


***“The data from the Lithopanspermia/Biopan experiment clearly demonstrates the extraordinary survival capacity of lichens in outer space.”***

## What is Chlorophyll Fluorescence?



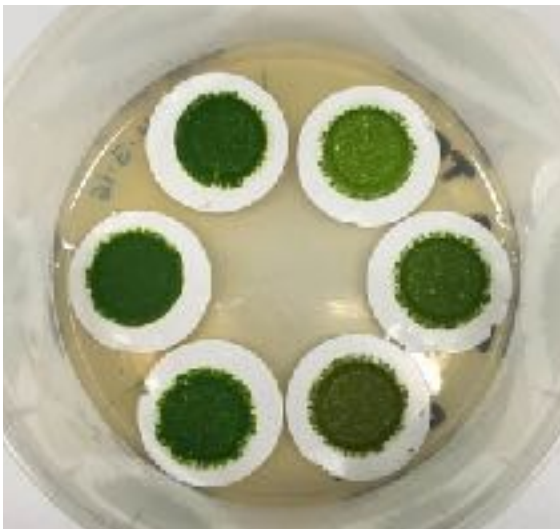
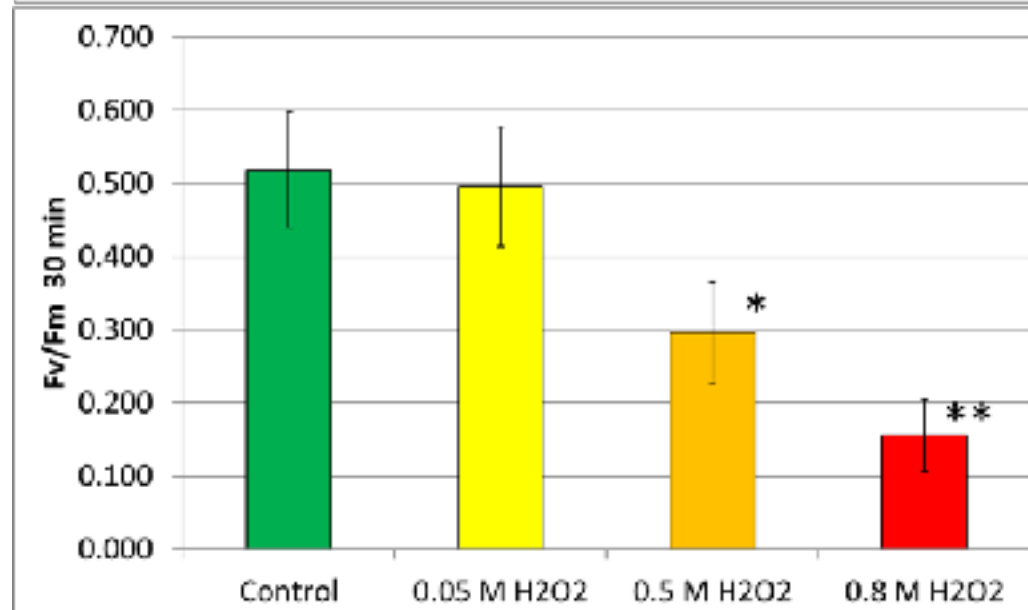
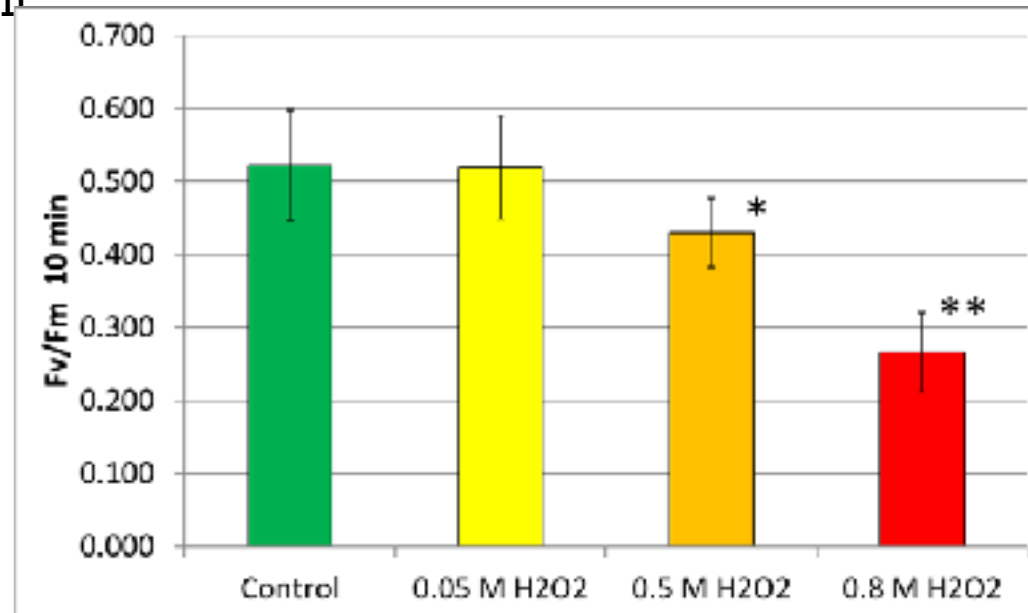
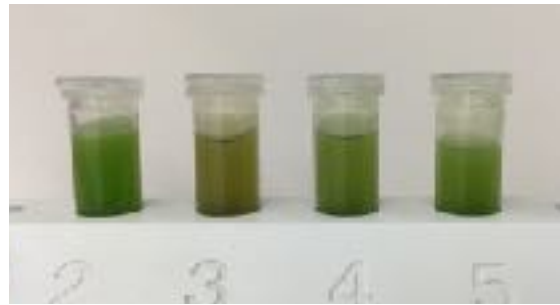
## The Kautsky Fluorescence Induction.



# Case study: oxidative stress in a lichen photobiont

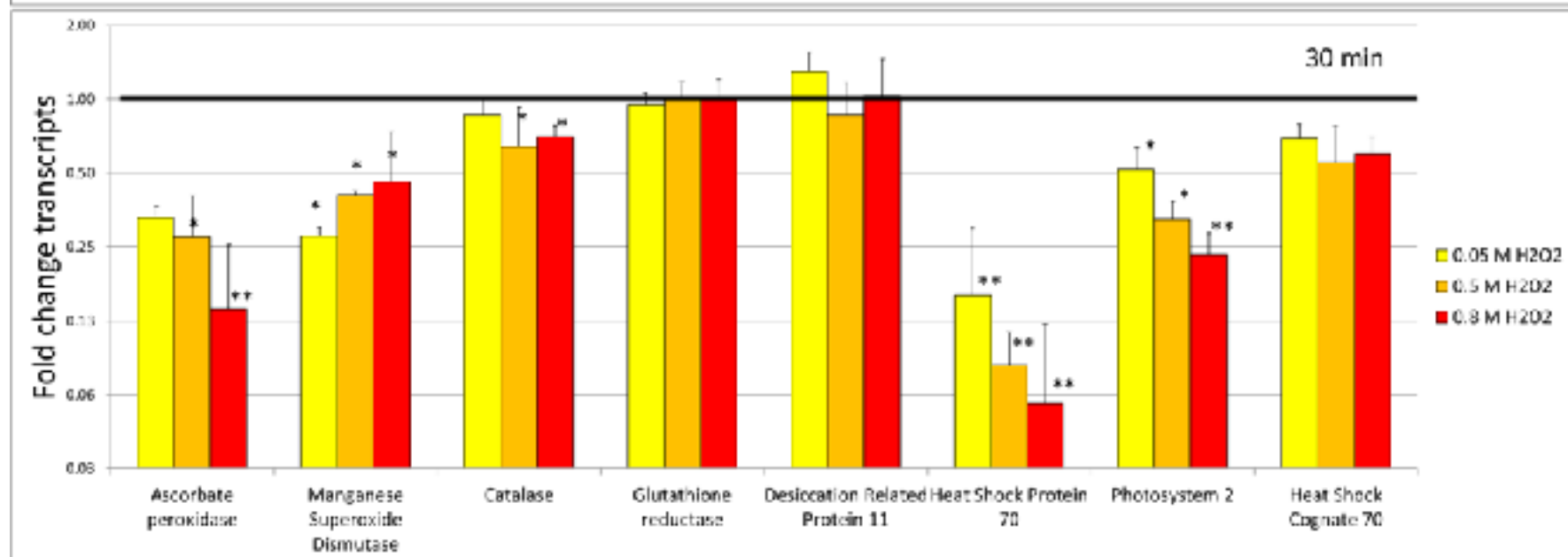
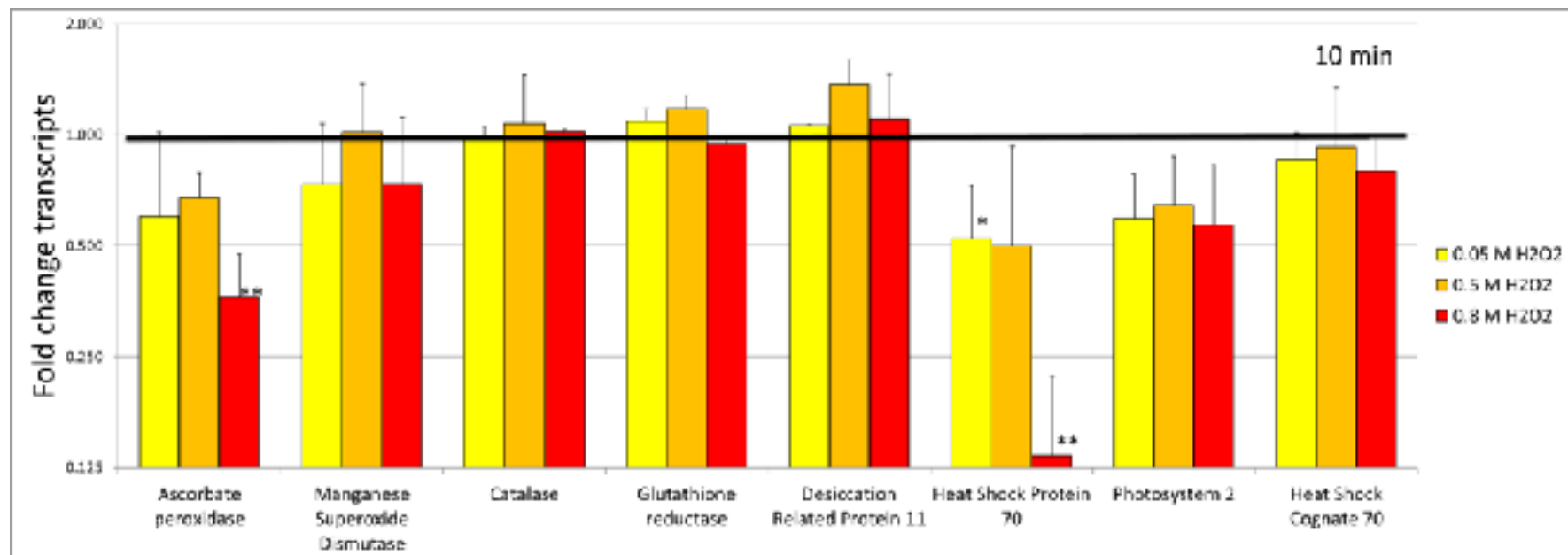
Handy PEA (Hansatech)

- *Trebouxia gelatinosa* algal suspension
- 3 H<sub>2</sub>O<sub>2</sub> concentrations
- 2 exposure times (10' and 30')

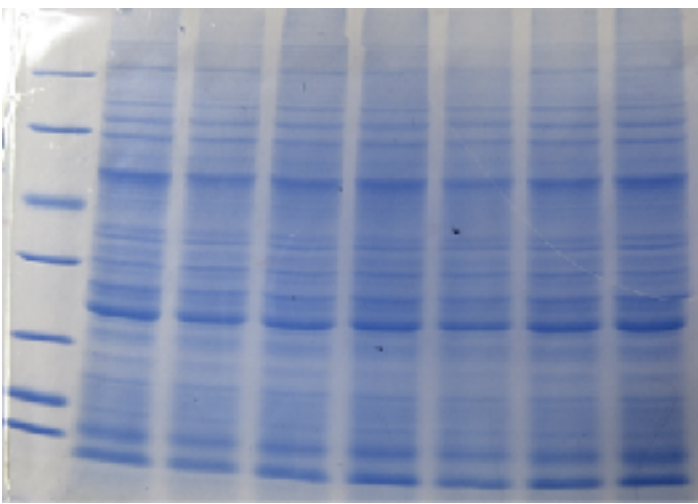




# Gene expression at transcript level – HSP70

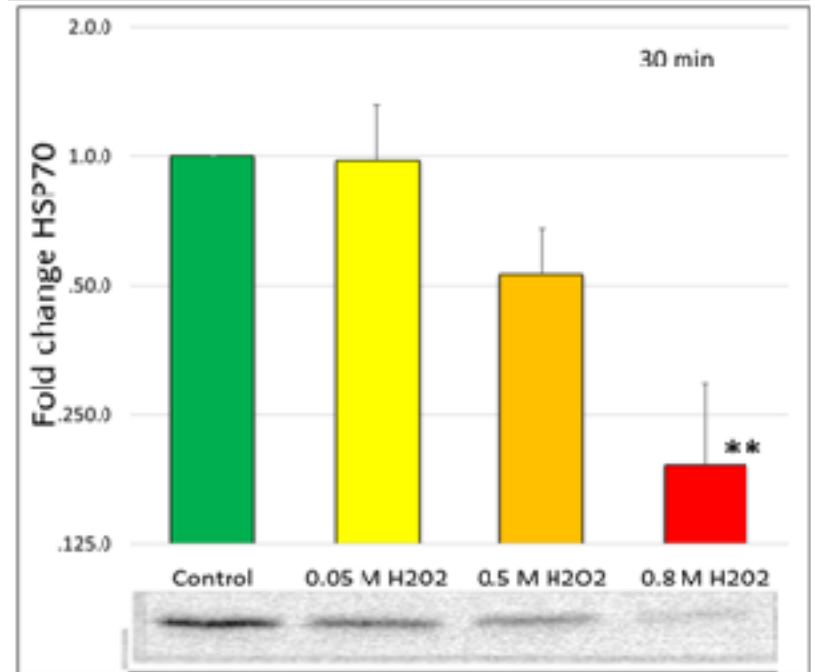
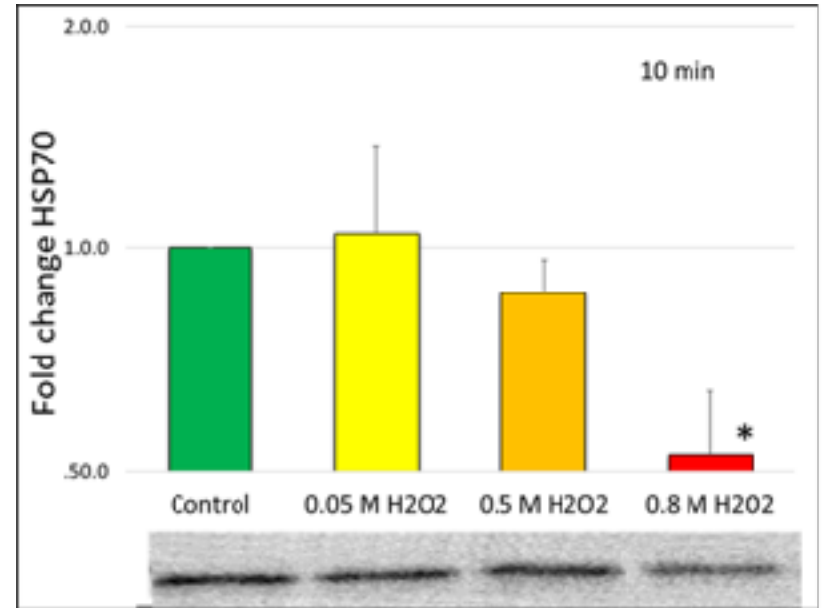


# Gene expression at protein level – HSP70



Coomassie staining

Western blot





- La **Società Lichenologica Italiana** è stata fondata proprio a Trieste, nel 1987.
- Il DSV ha il più grande erbario lichenologico d'Italia, ed il più numeroso gruppo di ricercatori che studiano licheni in Italia.
- Siamo un centro di eccellenza per questo soggetto di ricerca, soprattutto per lo studio della biodiversità, della biologia e degli aspetti applicativi (es.: biomonitoraggio ambientale).

