

DONE

- **Active biomonitoring:** Observations on individual plant species (*Nicotiana tabacum*, *Trifolium pratense*) as bioindicators of a specific type of pollutants: photochemical smog (ozone);
- **Passive biomonitoring:** determination of a BIODIVERSITY Index of epiphytic lichen communities as indicators of air QUALITY;
- **Active and passive biomonitoring:** analysis of samples of generally mosses or lichens, and animals (bees, molluscs) as bioaccumulators of metals or persistent airborne organic pollutants

# What are lichens

Lichens are an extracellular, stable, multi-kingdom symbiosis between a fungus ("**mycobiont**") and one or more populations of algae and/or cyanobacteria ("**photobionts**") that live hosted within a stable morphological structure, the **lichen thallus**, which is formed by the hyphae of the mycobiont.

The thallus also hosts further accessory organisms, **from bacteria to other fungi** (which can live as saprophytes or parasites, both generalists or highly specialized).

Overall, the lichen thallus represents a **small ecosystem** in itself, made up of primary producers and consumers of different nature.



**<100 specie**



**~ 15.000 species**

~10% od lichens

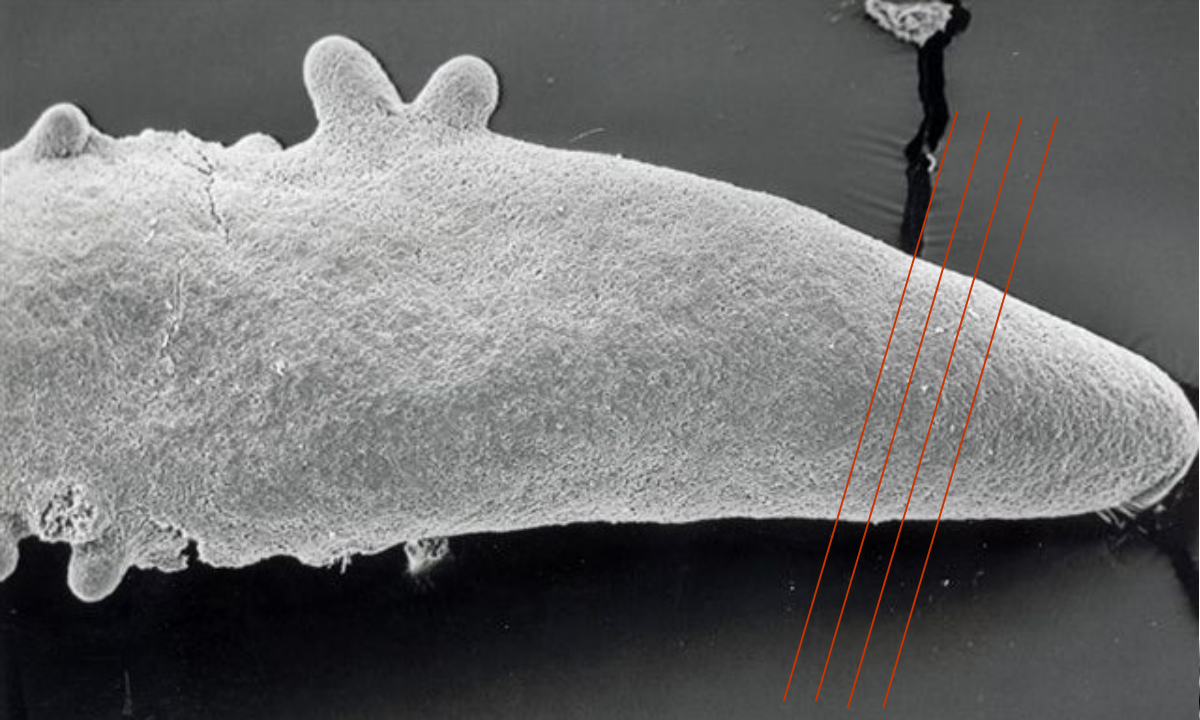
~90% of lichens



**<1.000 species**

The thallus of 45% of lichens is considered among the morphologically most complex structures formed by a fungus. In practice it is a small cultivation chamber of PHOTOBIONTS, which are placed in the best growth conditions, to the full advantage of the MYCOBIONT



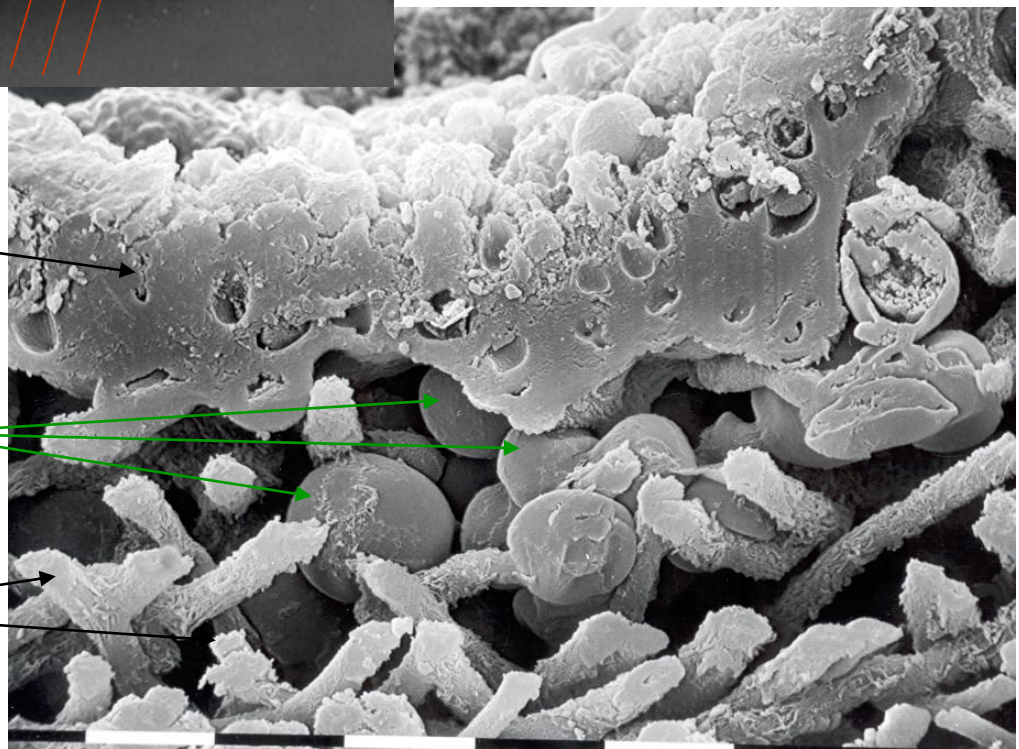


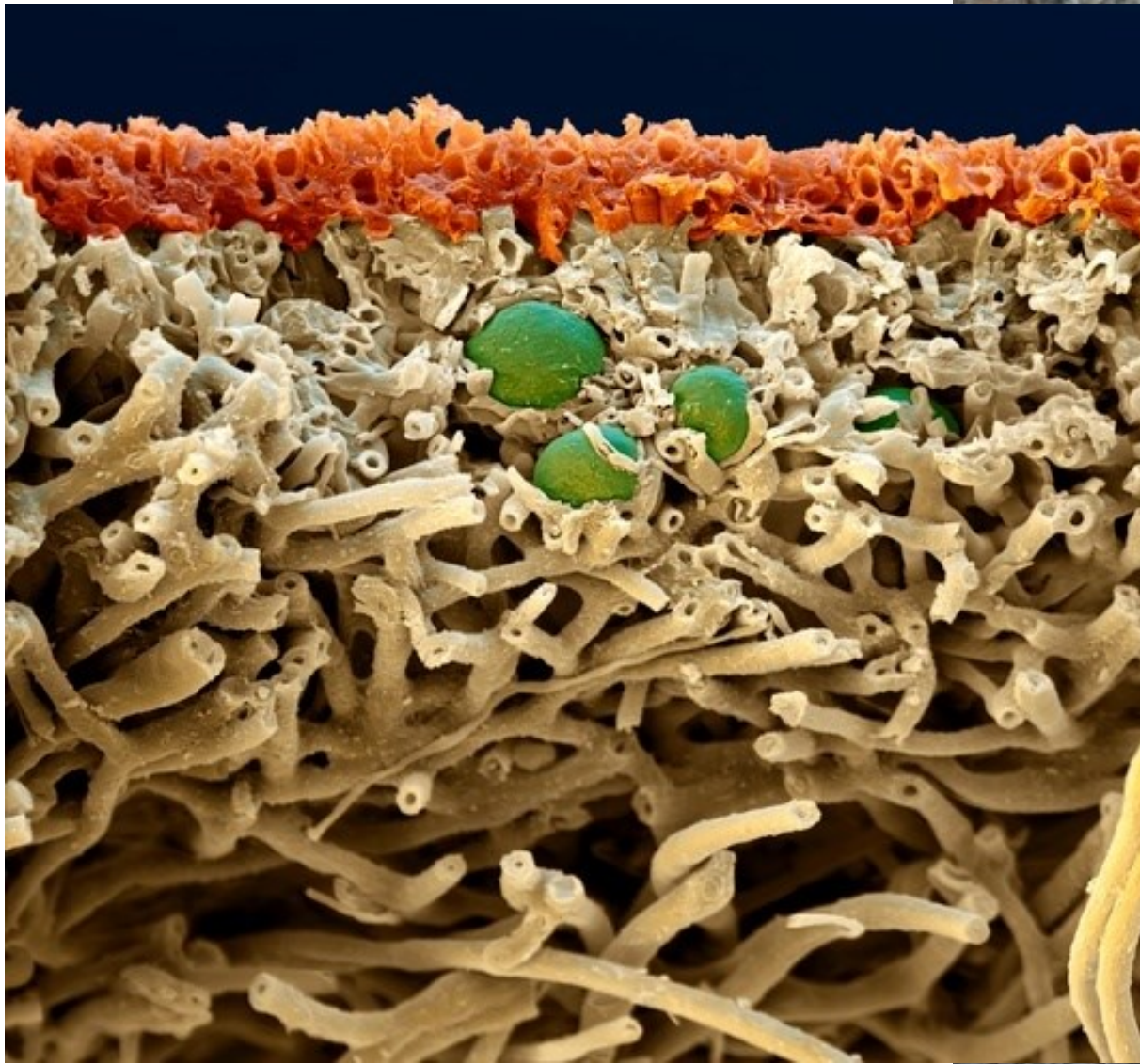
By transversally dissecting a lobe of a lichen, the hyphae of the fungus, and colonies of algae or cyanobacteria become evident

Adglutinated hyphae

Unicellular algae

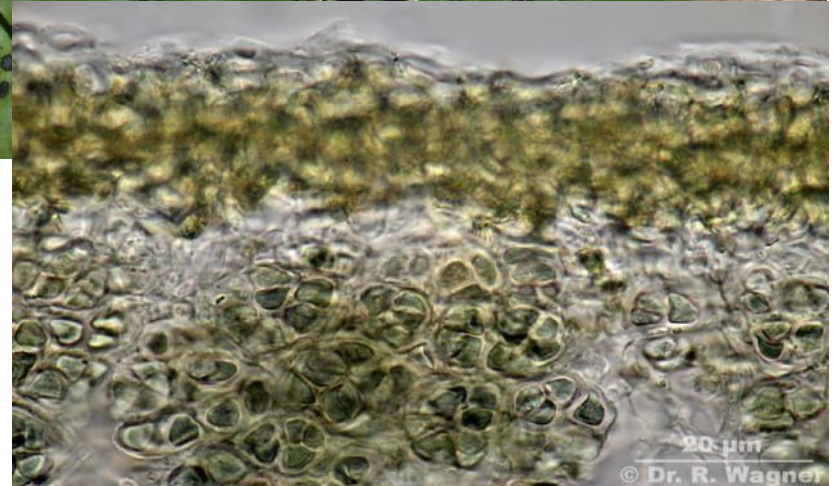
Web of hyphae



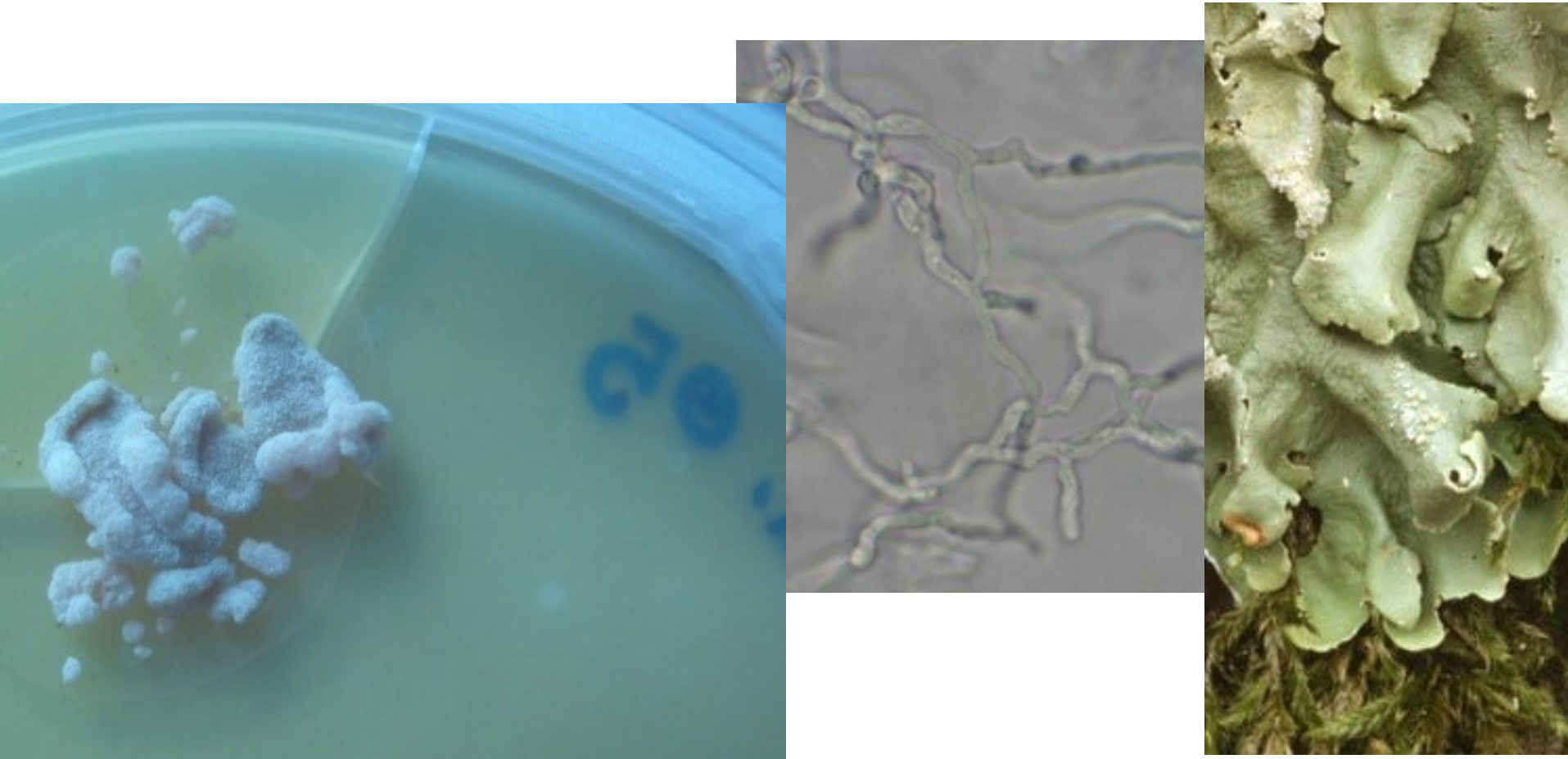


*Xanthoria elegans*, microfotografia SEM ai falsi colori.

In some lichens, cyanobacteria and green algae co-exist together, in different areas of the thallus, as in this *Peltigera*

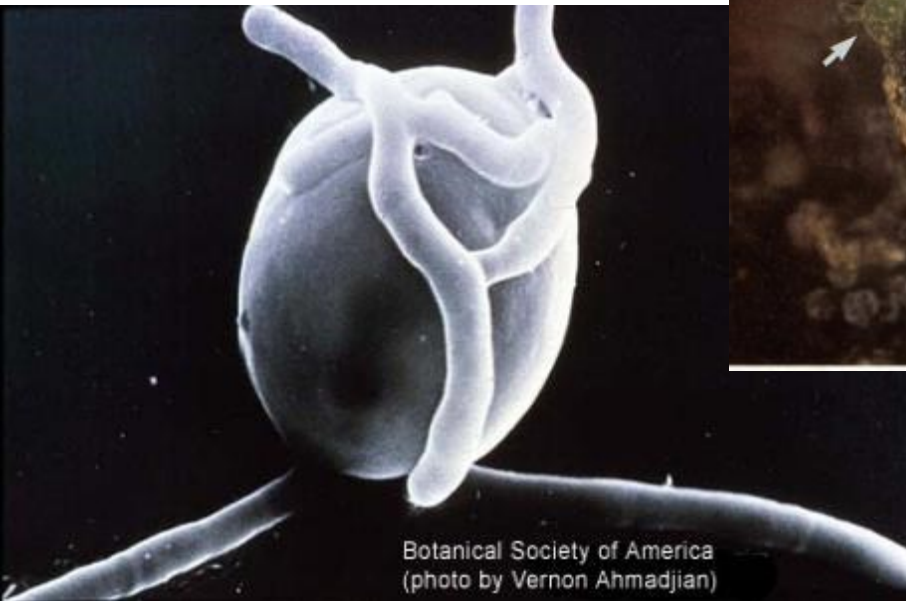
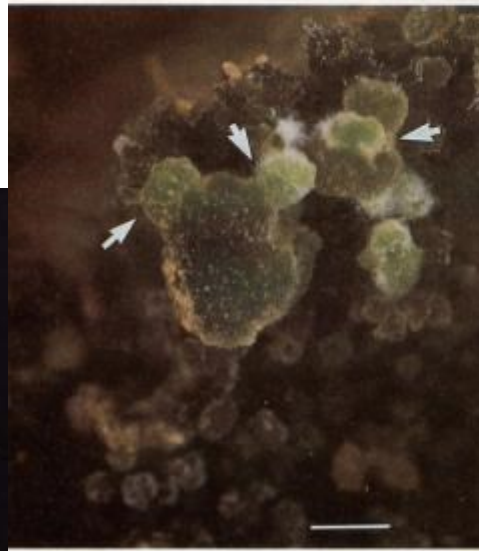
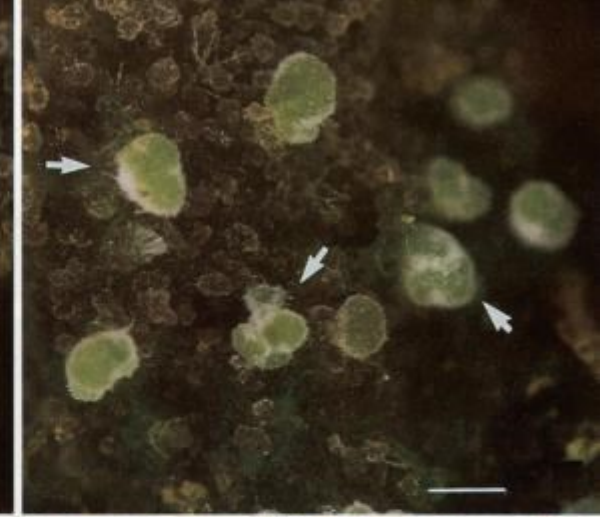
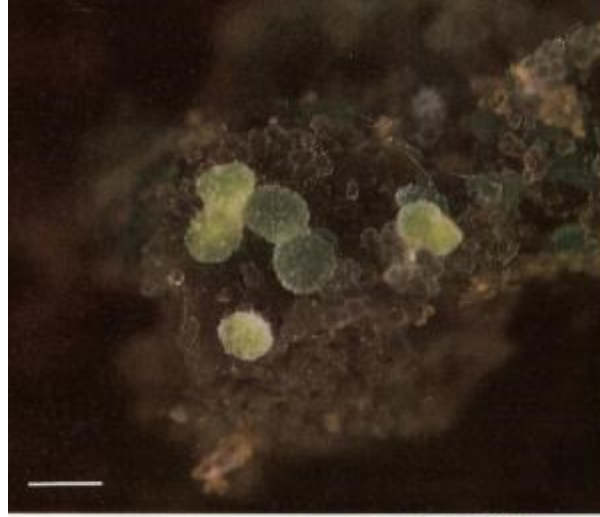


A striking fact is that this organization is only formed if the two partners grow together. When mycobionts are grown in artificial conditions alone (once it was thought that this was not even possible!), their appearance is very different: they form a classic fungal mycelium, poorly differentiated, with hyphae that move away to “explore” the substrate.





Morphogenetic processes start as the fungus is in contact with the right algae and/or cyanobacteria. As this happens, is still largely unknown.



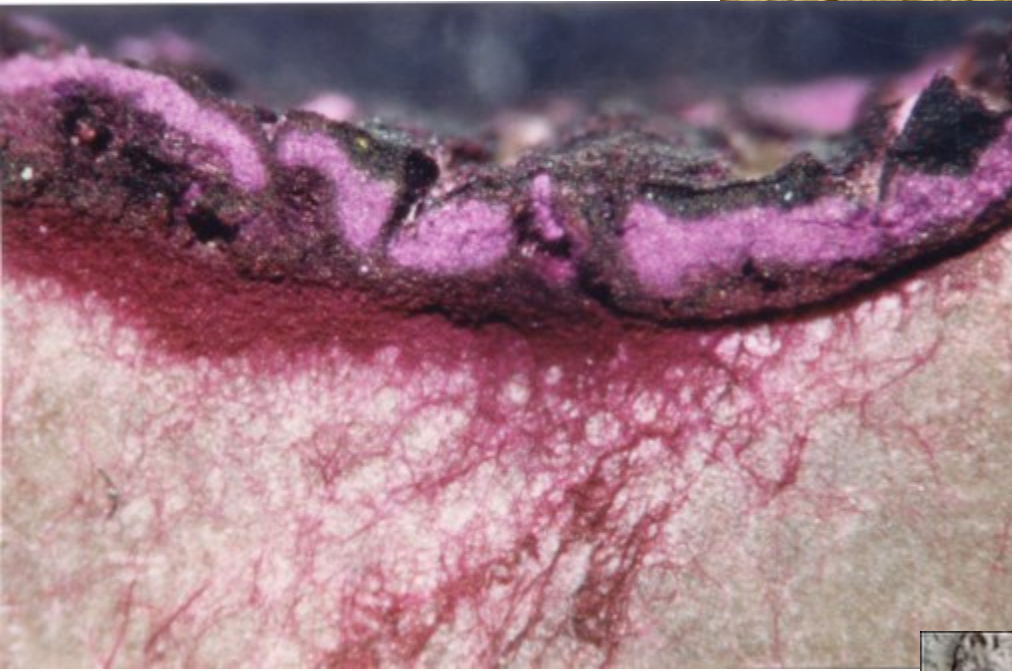
# FRUTICOSE



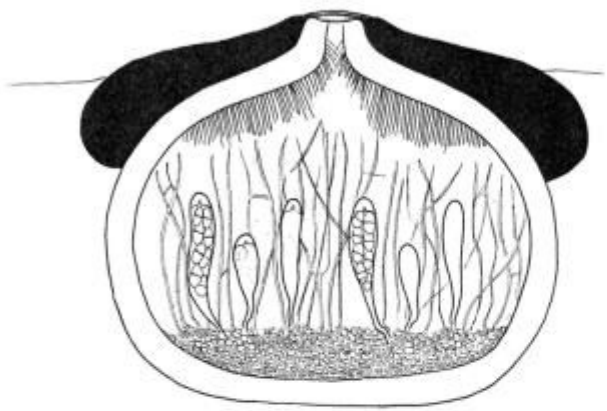
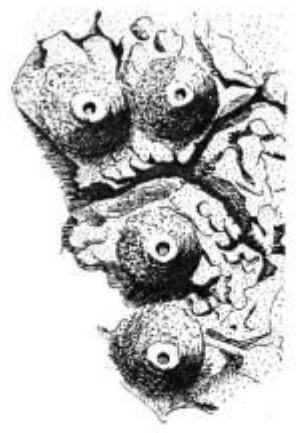
# FOLIOSE



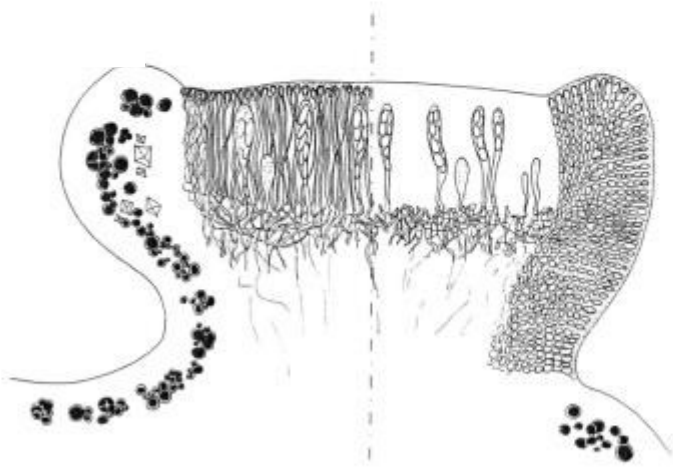
# CRUSTOSE



Sexual reproduction is the prerogative of the mushroom alone. Algae (and cyanobacteria) can only reproduce vegetatively, by division.

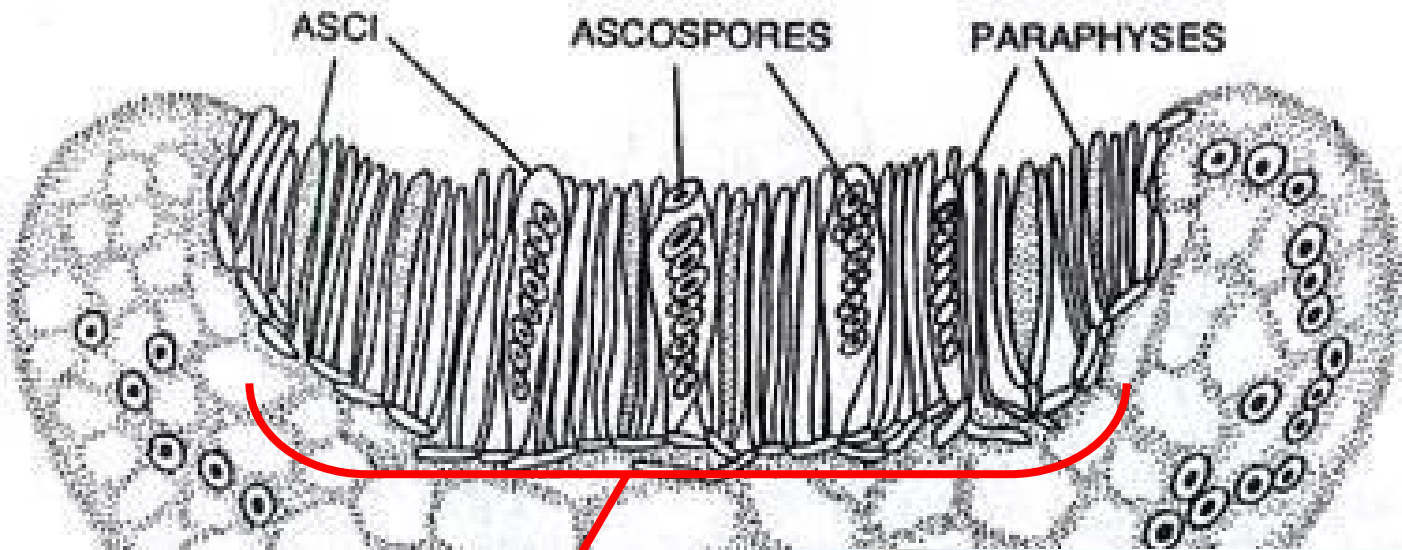


**PERITECIA**, a sort of flask opening through a pore

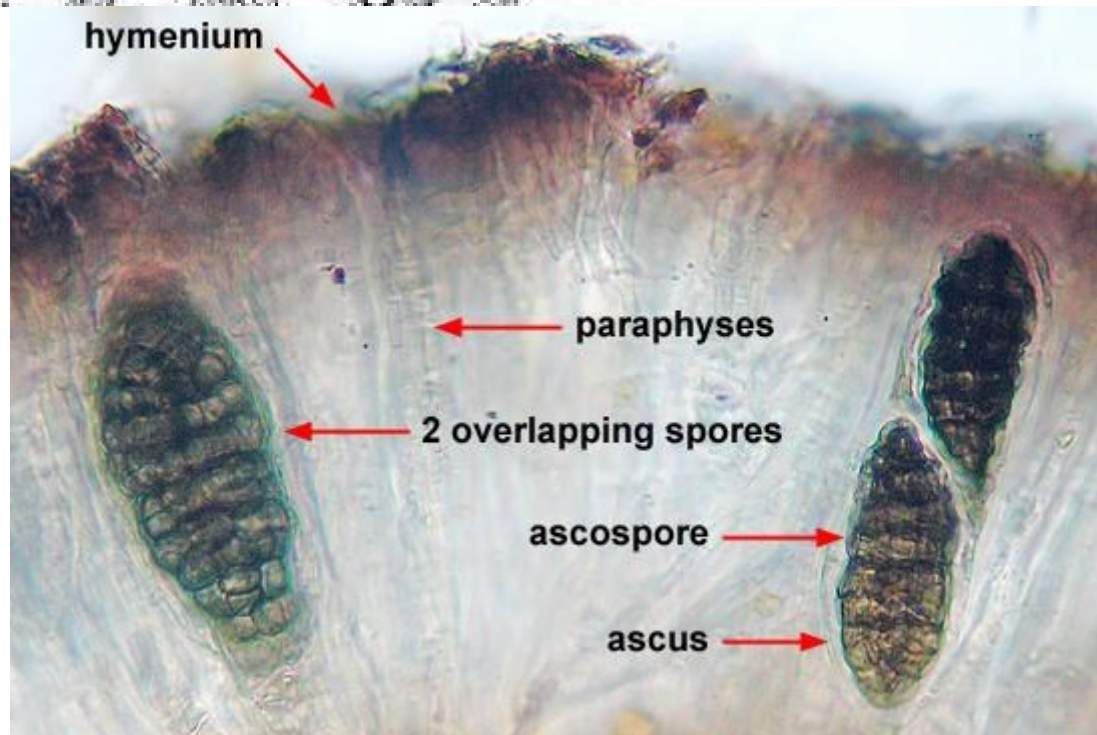


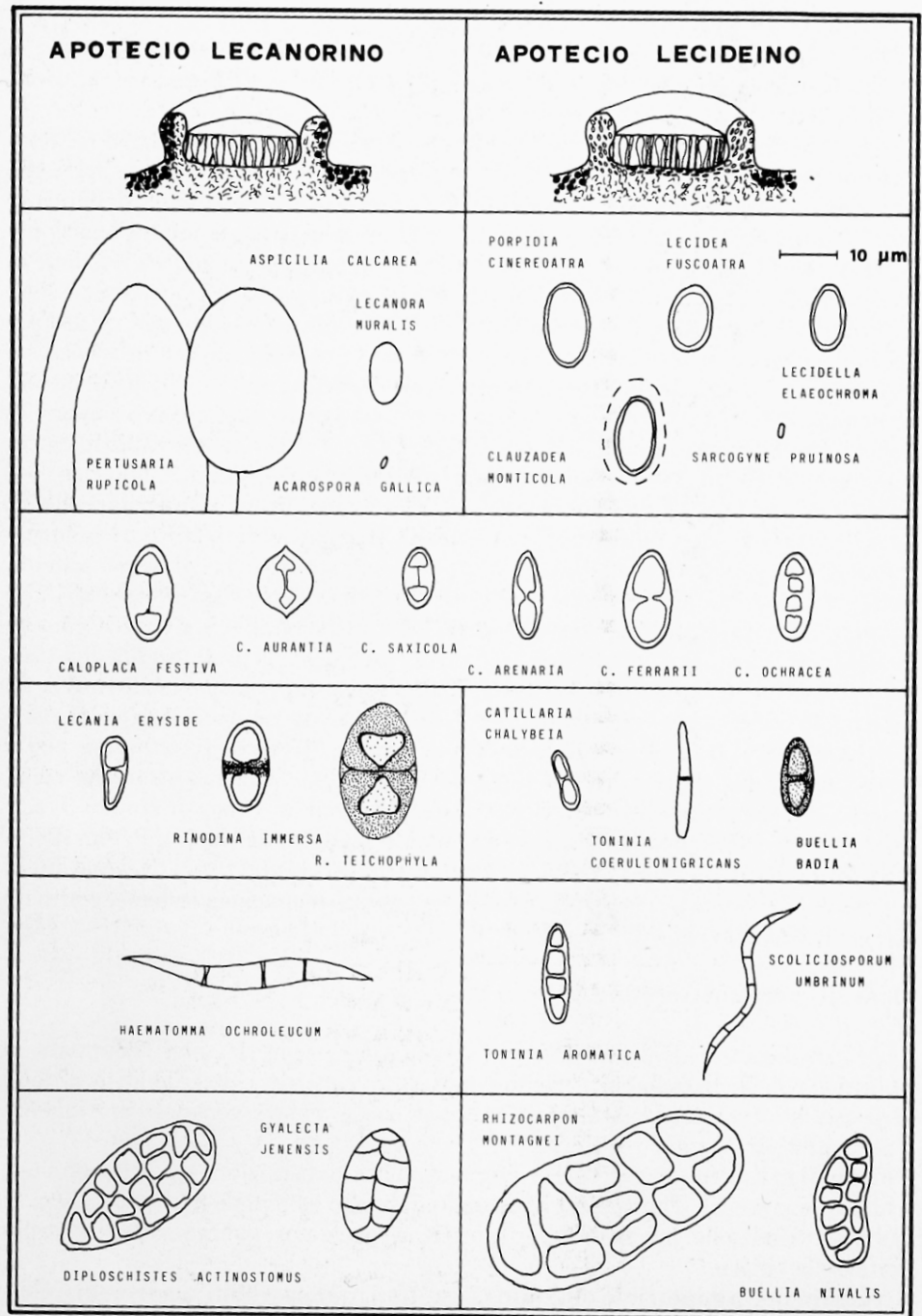
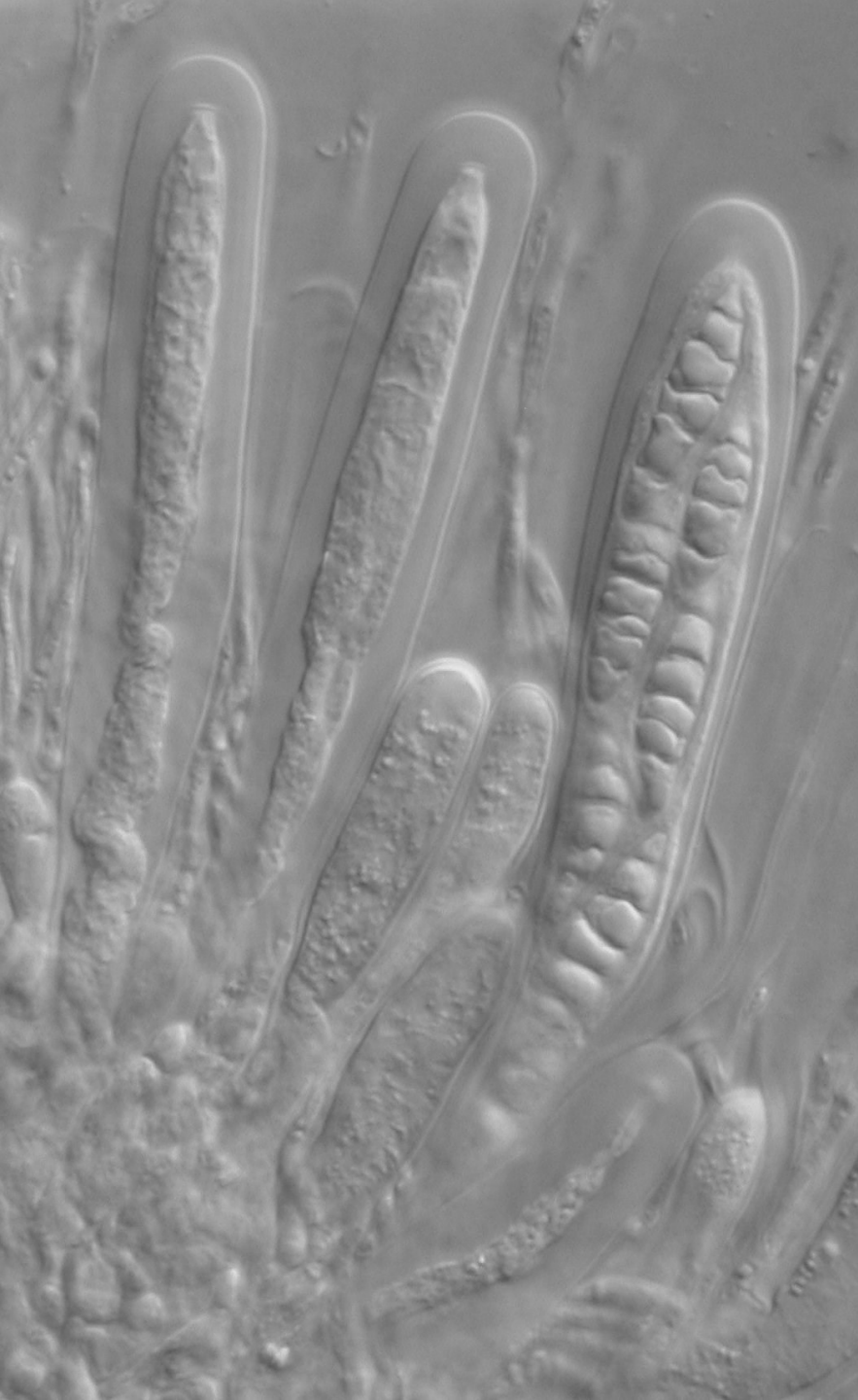
**APOTECIA**, flat disks usually with a margin

**In 99% of species, these structures are long-living**



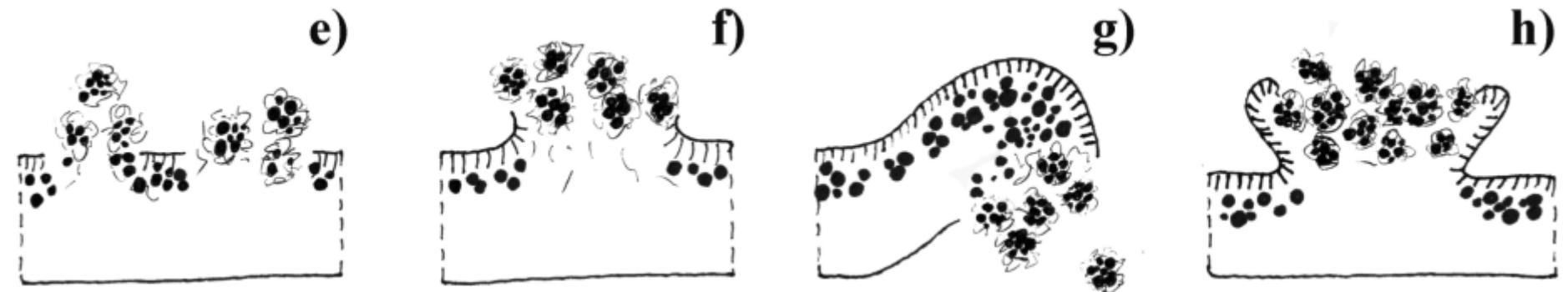
hymenium



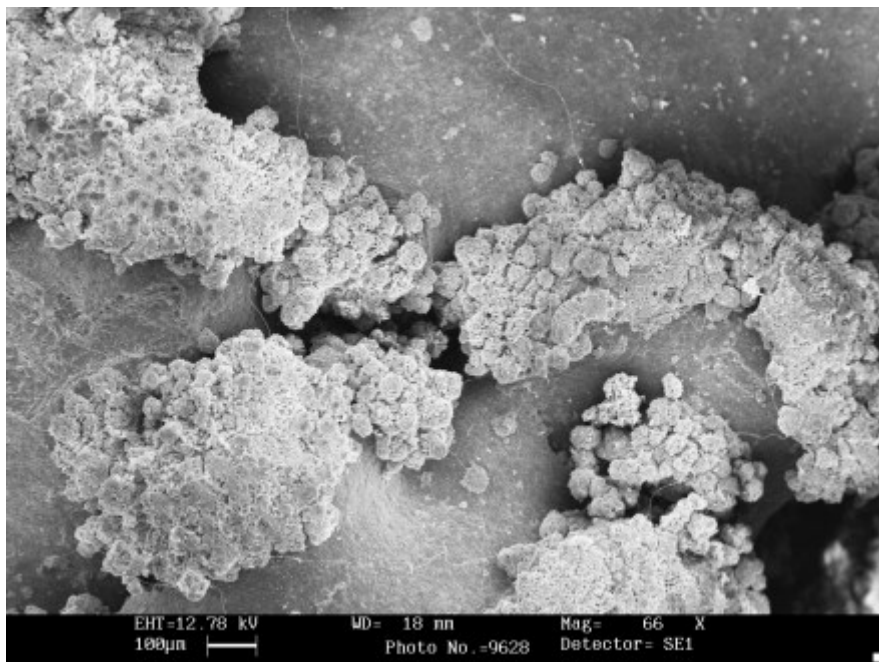
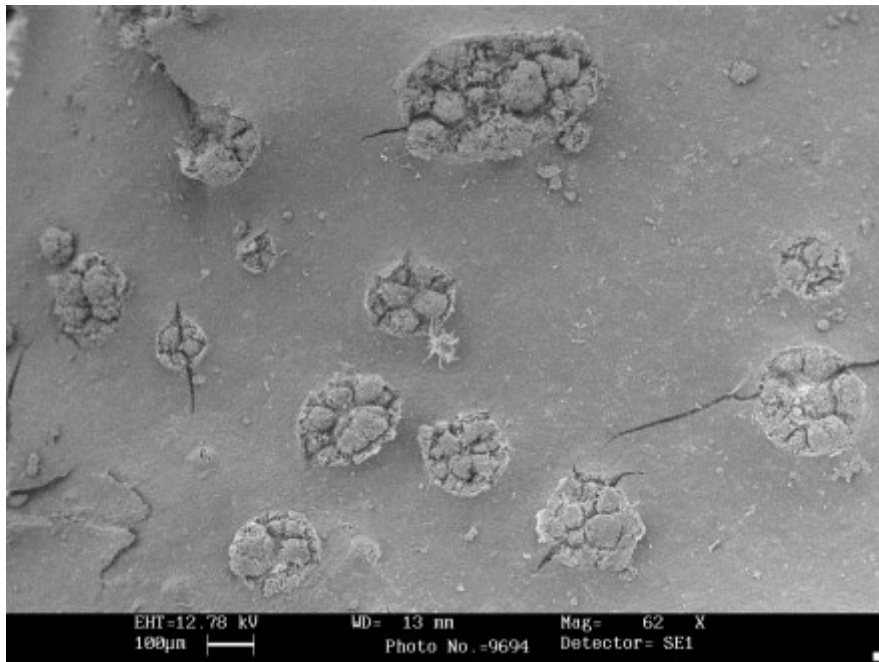


Tav. E Diversi tipi di spore di specie ad apotecio lecanorino (sinistra) e lecideino (destra).

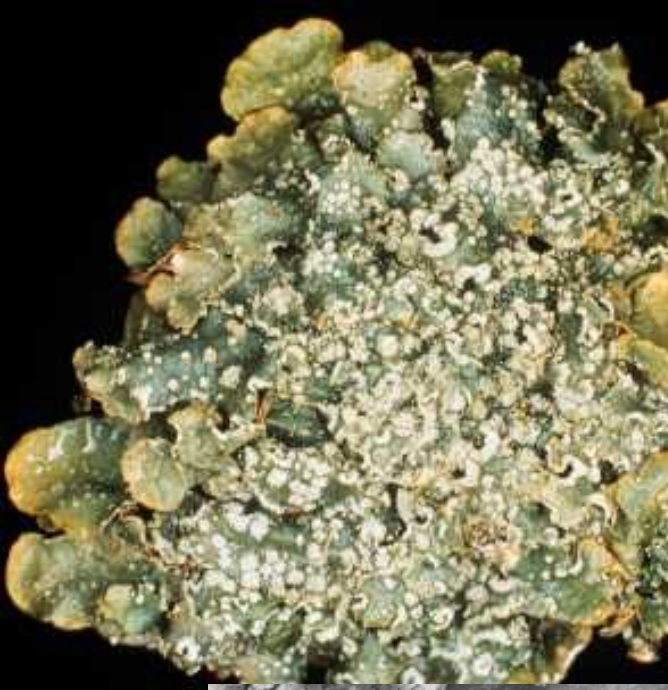
**SOREDIA:** they are NON-cortical structures, often formed in well-defined areas of the thallus (“**soralia**”). They originate from interruptions in the cortex, through which the hyphae of the medulla proliferate, “capturing” some algal cells.



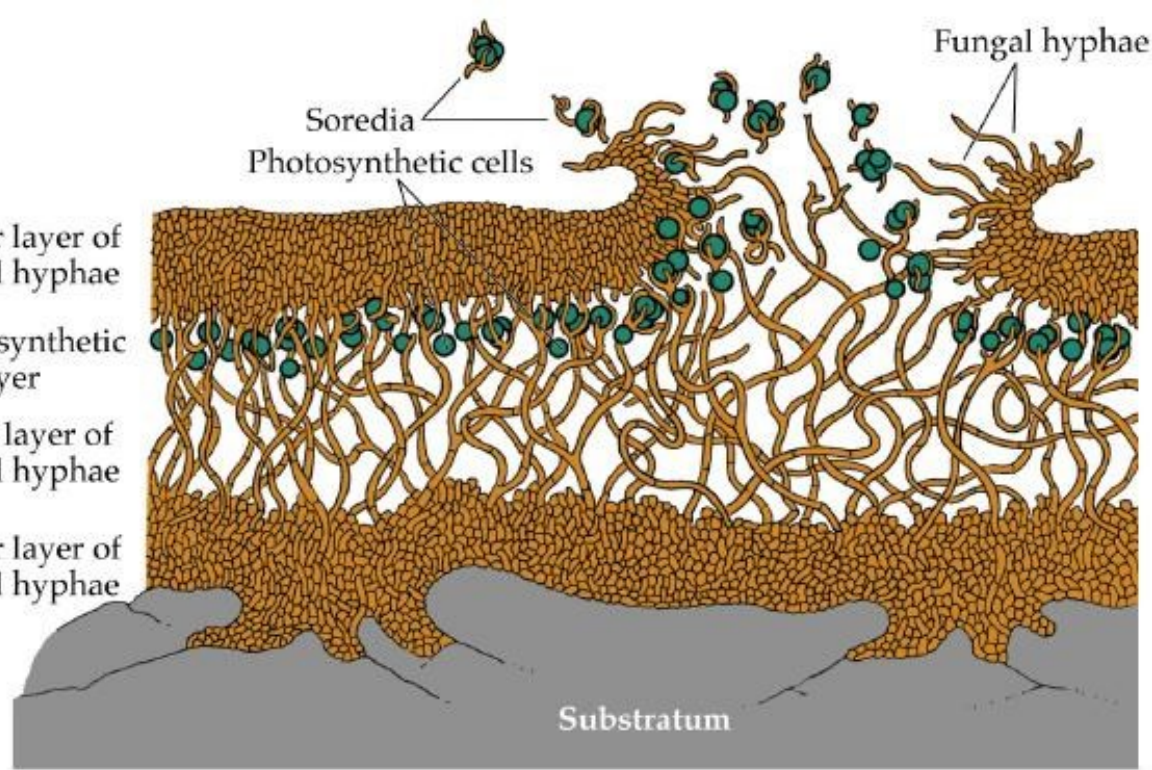




The **soralia** often appear as small islands, lines or whitish and powdery areas, which contrast with the variously colored surface of the thallus.

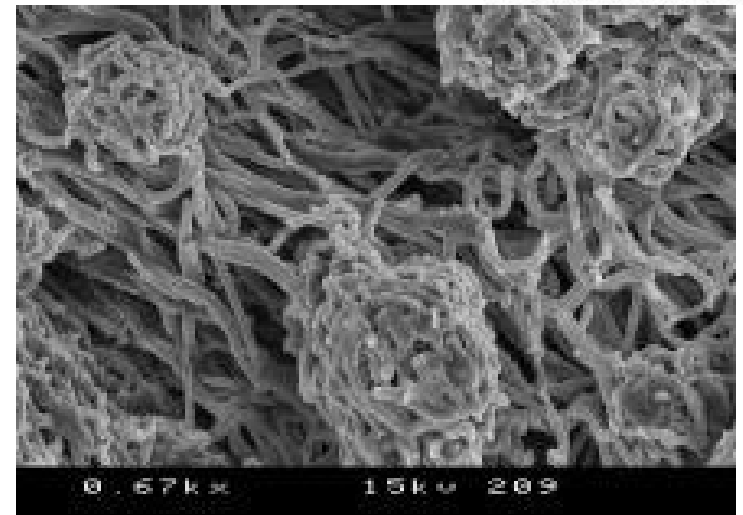
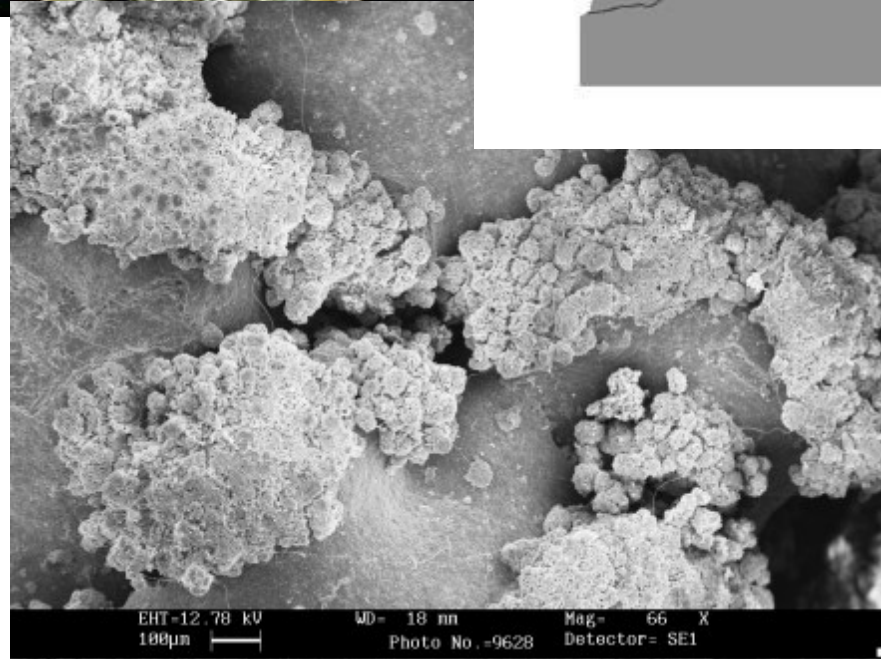


Upper layer of fungal hyphae  
 Photosynthetic cell layer  
 Loose layer of fungal hyphae  
 Lower layer of fungal hyphae



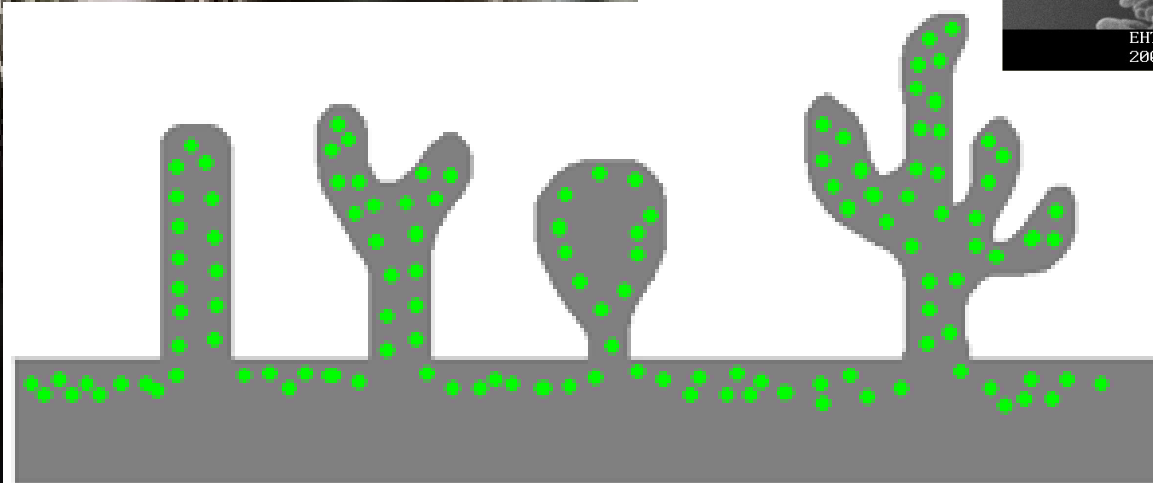
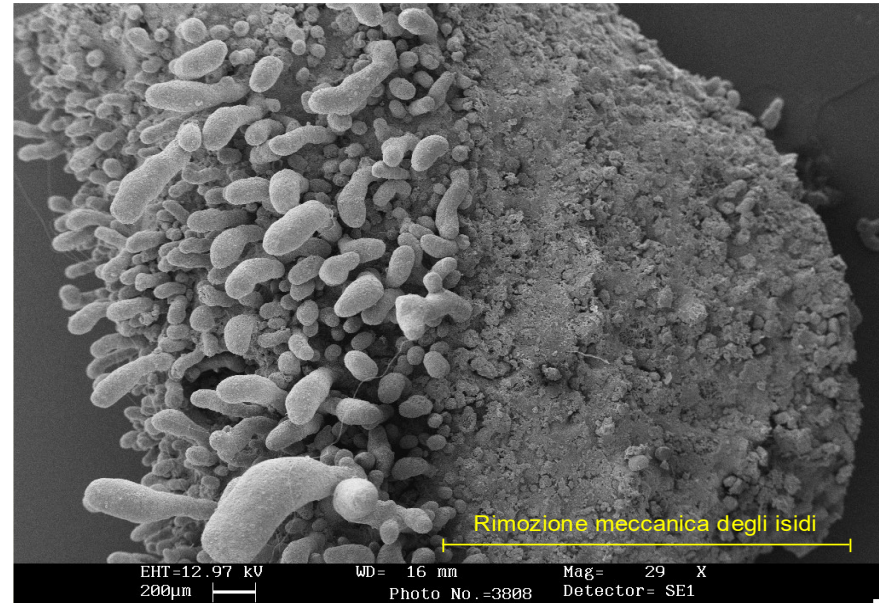
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# SOREDIA

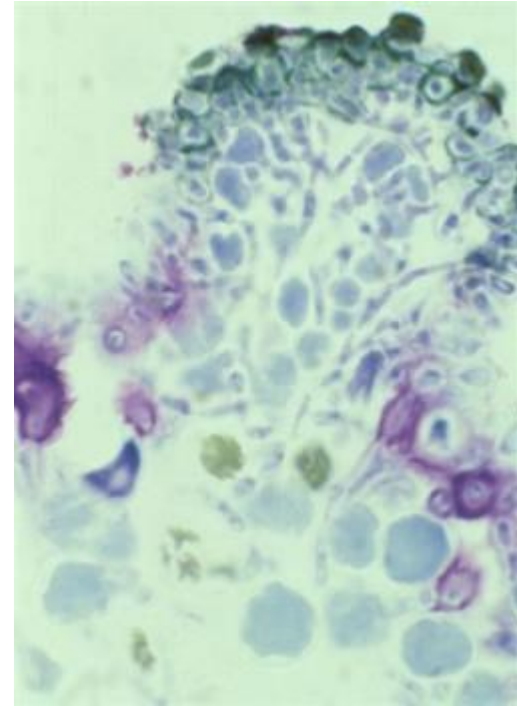
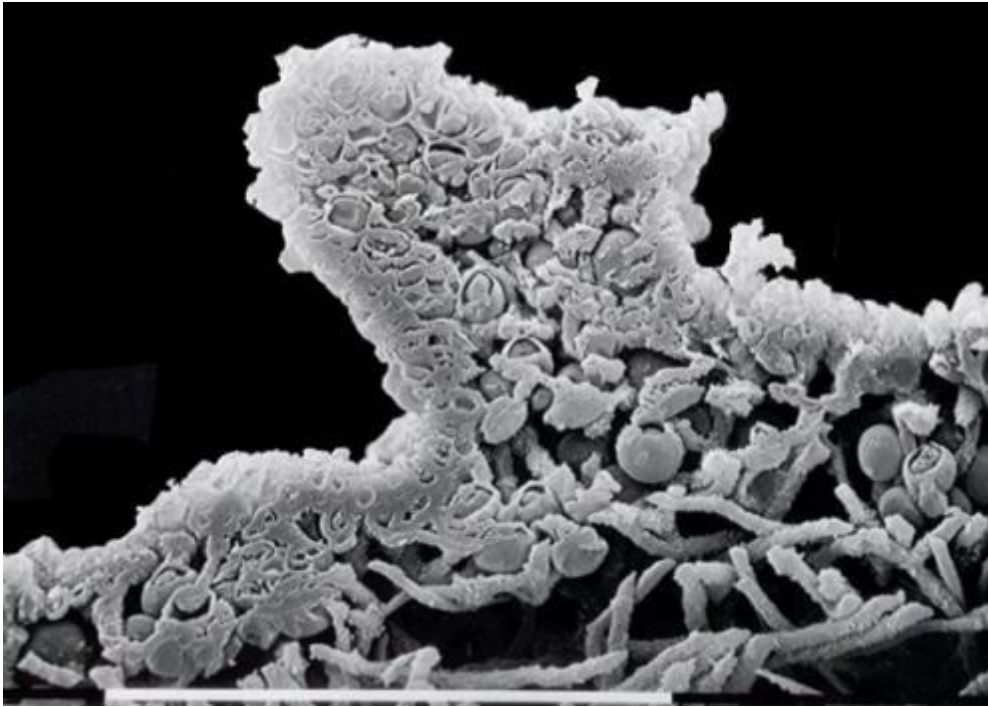




**ISIDIA:** are corticated propagules, the same color as the external surface, or darker, especially at the apex.



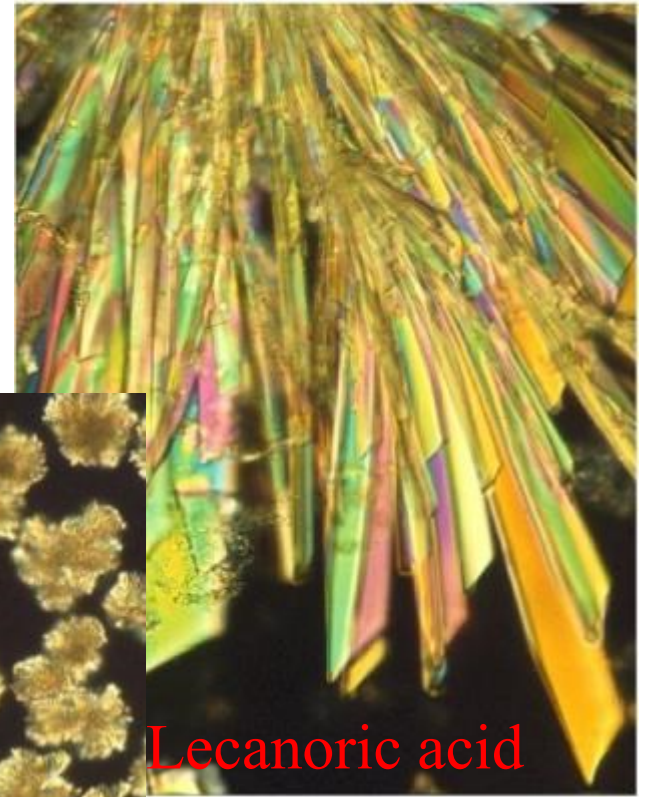
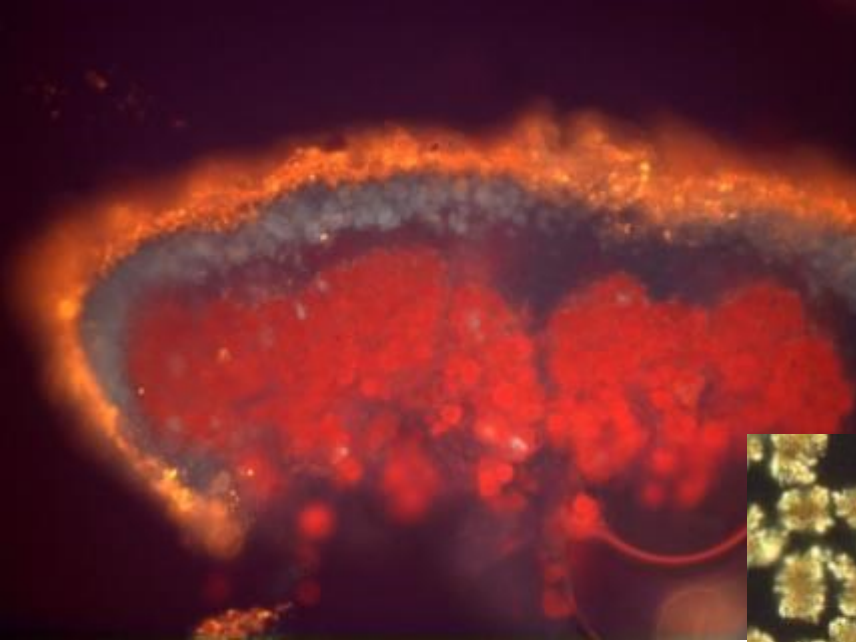
At the lens, they look like small lumps, fingers, pills...



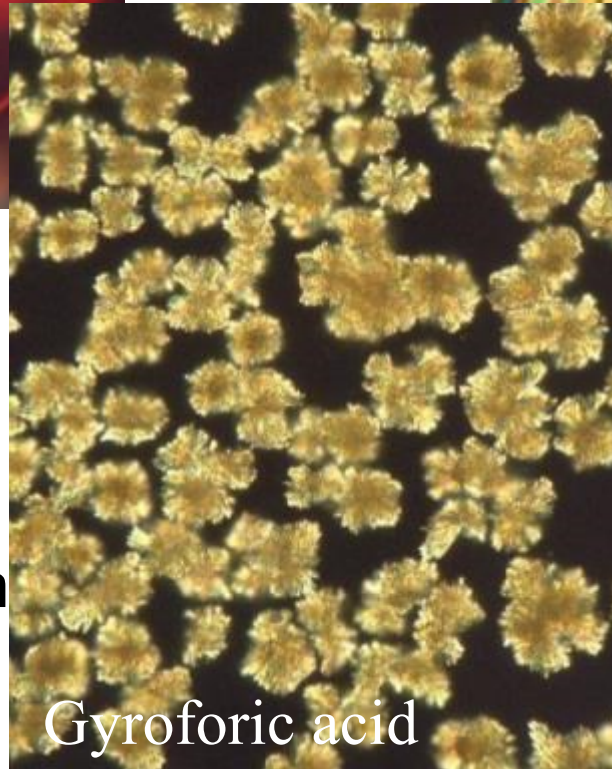
**ISIDIA** always contain cells of the photobiont.

Contrary to what was stated until recently, they also originate from a proliferation of medullary hyphae, which however is accompanied by a growth of the hyphae of the cortex, so they are **always corticated**.

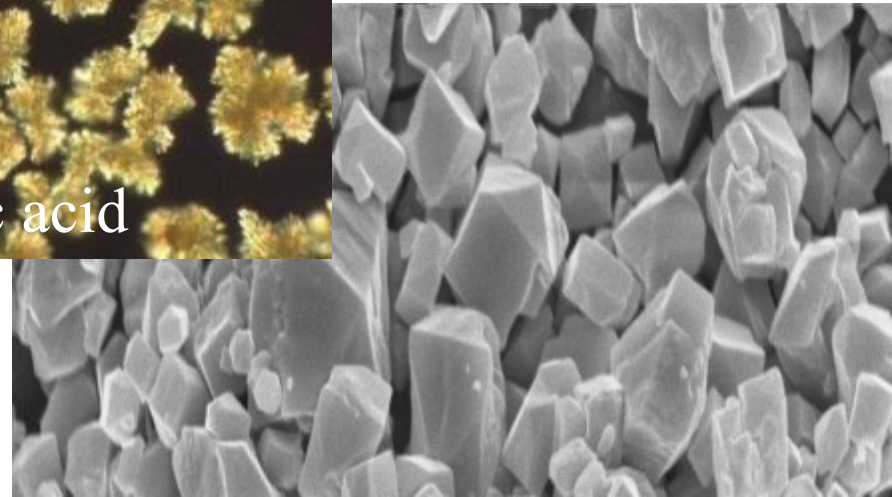




Lecanoric acid



Gyroforic acid



Lichens produce a wide range of secondary metabolites, collectively called "lichen acids" or "lichen substances", with very different functions, which are accumulated in the apoplastic space, on the hyphae.



Lichens are capable of colonizing environments in which the two partners alone would be incapable of surviving. A fungus normally penetrates into the substrate, living in the darkness, to emerge with its sexual structures.

In the case of a lichen, on the contrary, the fungus is forced to live fully exposed to the light, which is fundamental for the photosynthetic process of its partner.

Lichens are able to colonize even glass, plastic or metals.



Lichens are «desiccation tolerant», i.e., they normally survive to a deep dehydration of their cytoplams (less than 5% of the water at cell turgor), recovering normal metabolic activity within minutes as soon as water become available.

### “Homoiochlorophyllous poikylhydric”

This feature is shared by many orther organisms, from mosses (this photo), to many pollens and seeds, to tardigrads and nematods.



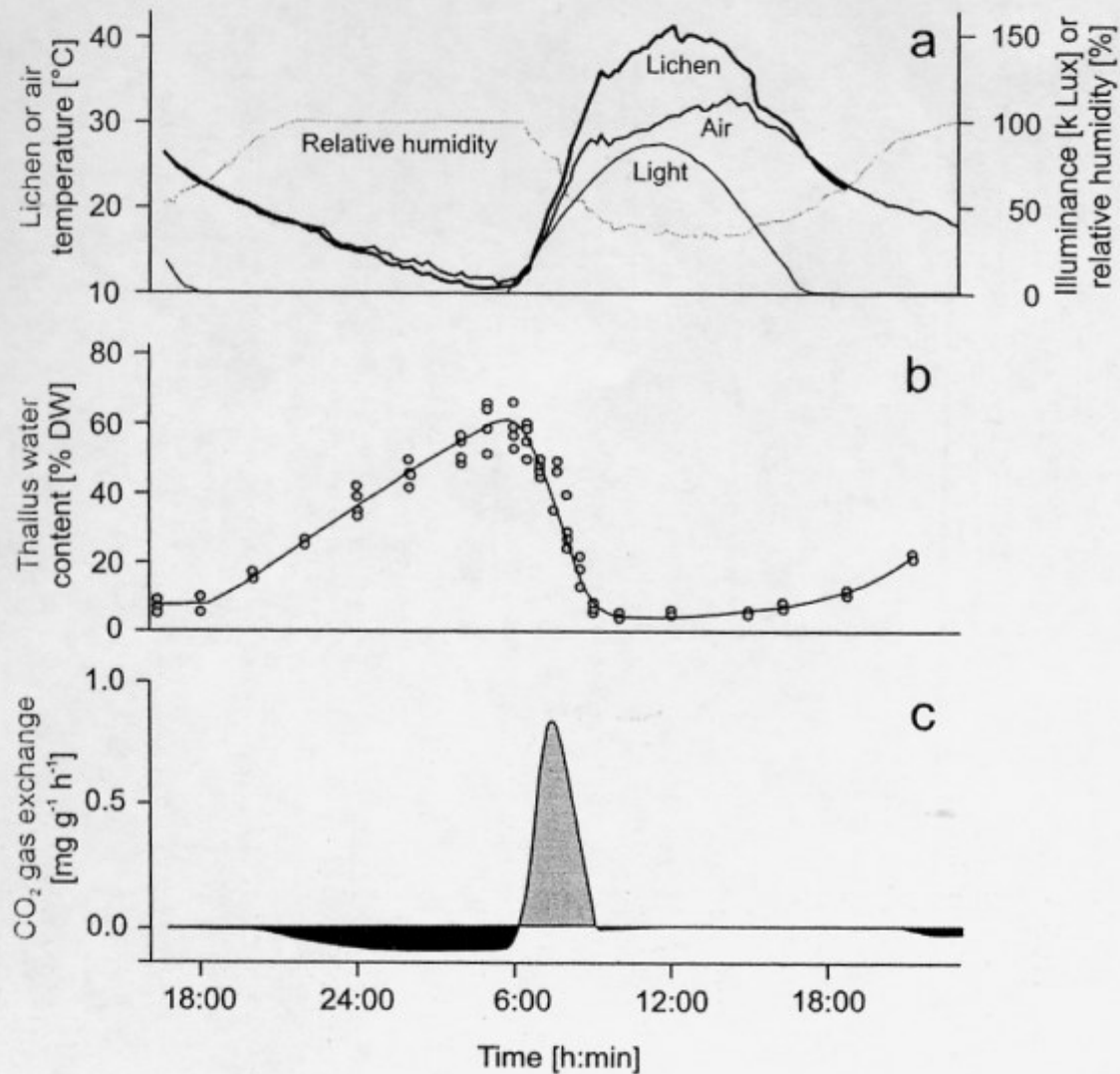


bagnato



secco

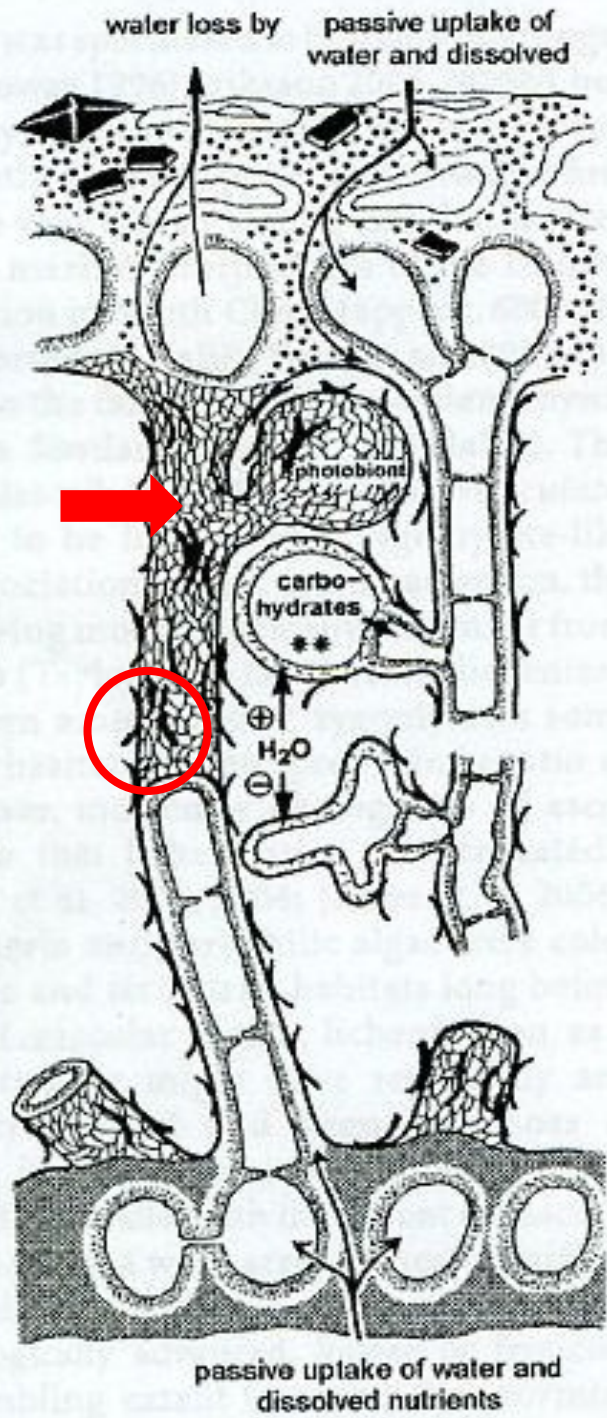




**Figure 1.** CO<sub>2</sub> gas exchange of *Ramalina maciformis* in relation to environmental parameters during and after dewfall in the Negev Desert. Figure redrawn and modified after Lange (1970). (a) Lichen and air temperature, illuminance and

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.





**Hydrophilic**

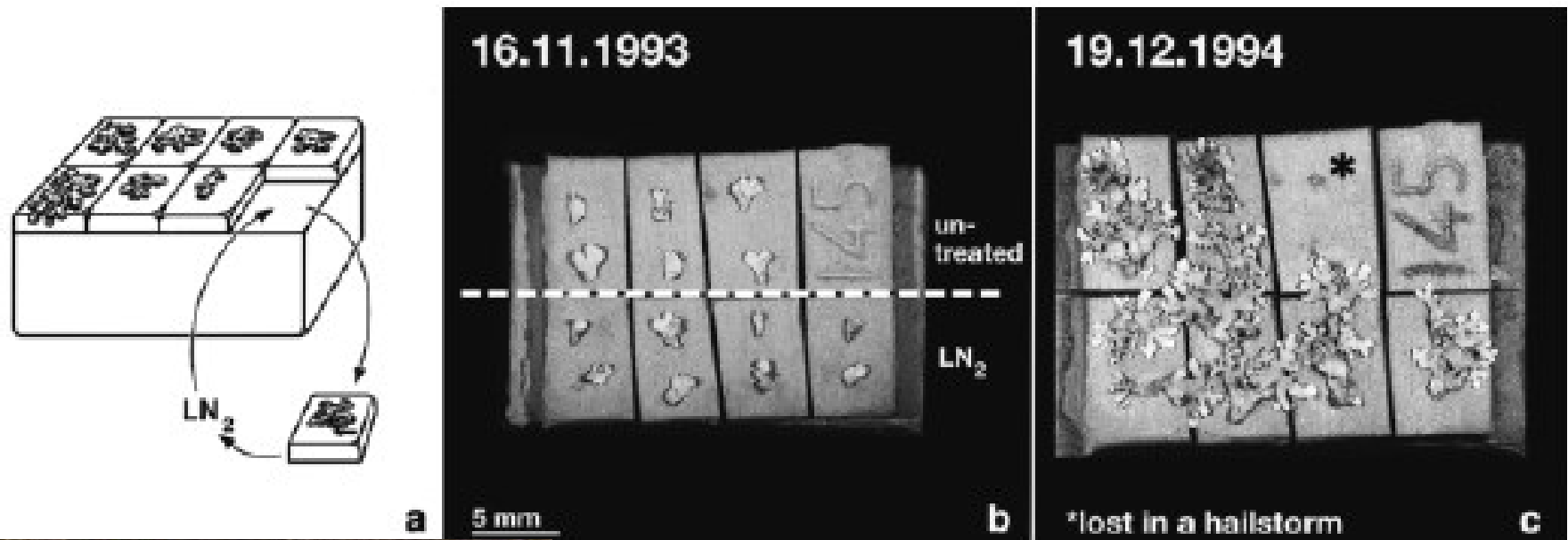
Except for the few species sensitive to desiccation, repeated “dry-wet” cycles are fundamental for the survival of the symbiosis, as they allow the exchange of substances between the two (or more) partners through the water mass flow.

**Hydrofobic (micobiont)**

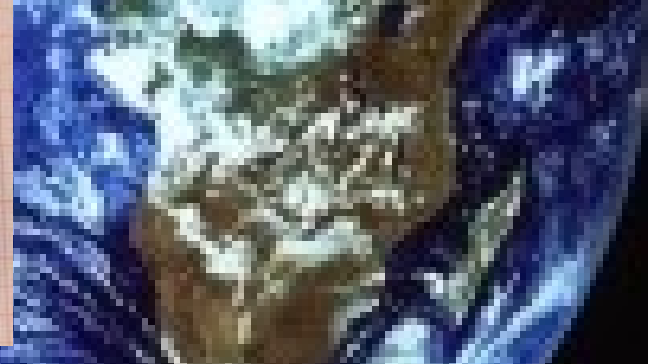
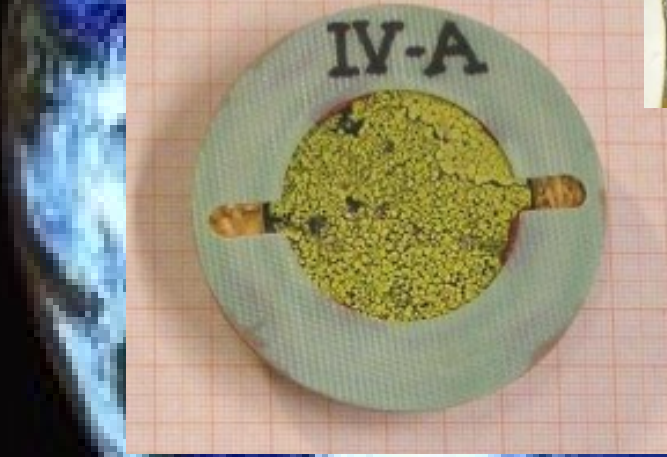
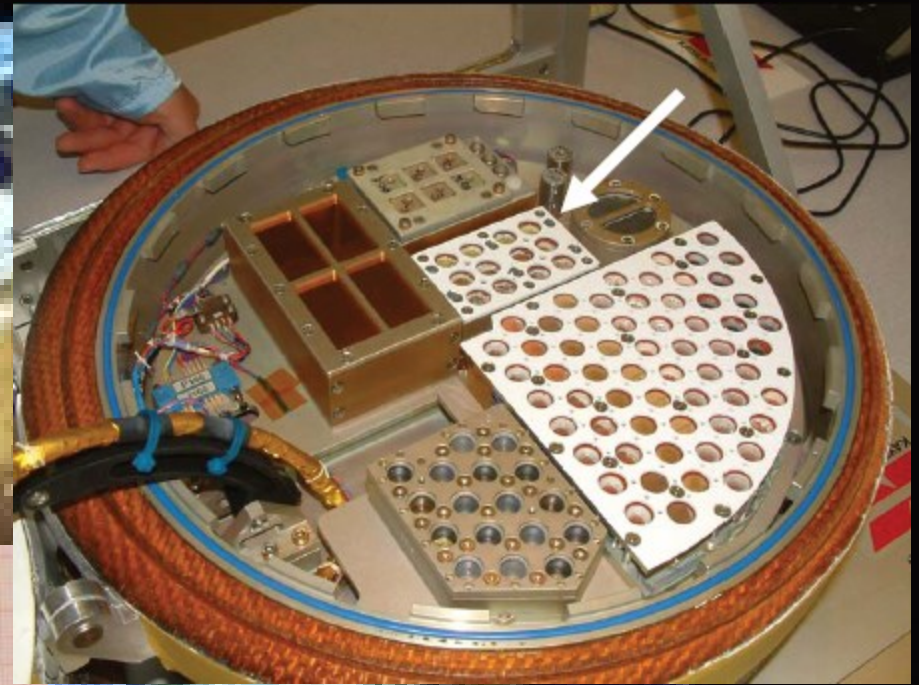
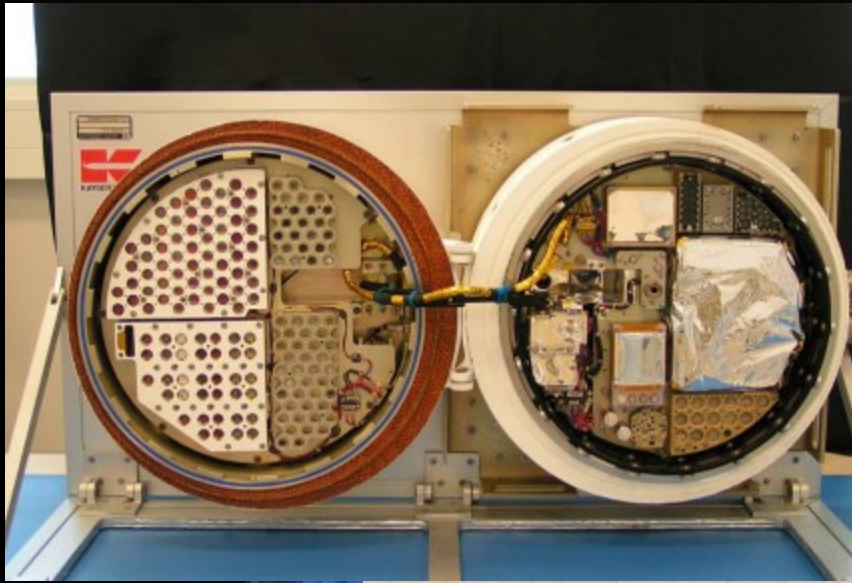
- The internal face of the cell wall is covered by a thin layer of hydrofobic proteins («hydrophobins»)
- Hydrophobic lichen substances frequently cover the outer surface of the cell wall (photobionts included)

**Hydrophilic**

When deeply dry, they are extremely resistant to extreme environmental conditions, e.g. to low temperatures.



**Fig. 4** Reviviscence and growth of lobules of *X. parietina* after one week of LN<sub>2</sub> 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).

On the contrary, if they are kept fully hydrated, they suffer irreversible damage if exposed to moderate heat shock treatments (e.g. 55 °C for one hour): they are thermo-sensitive if wet.

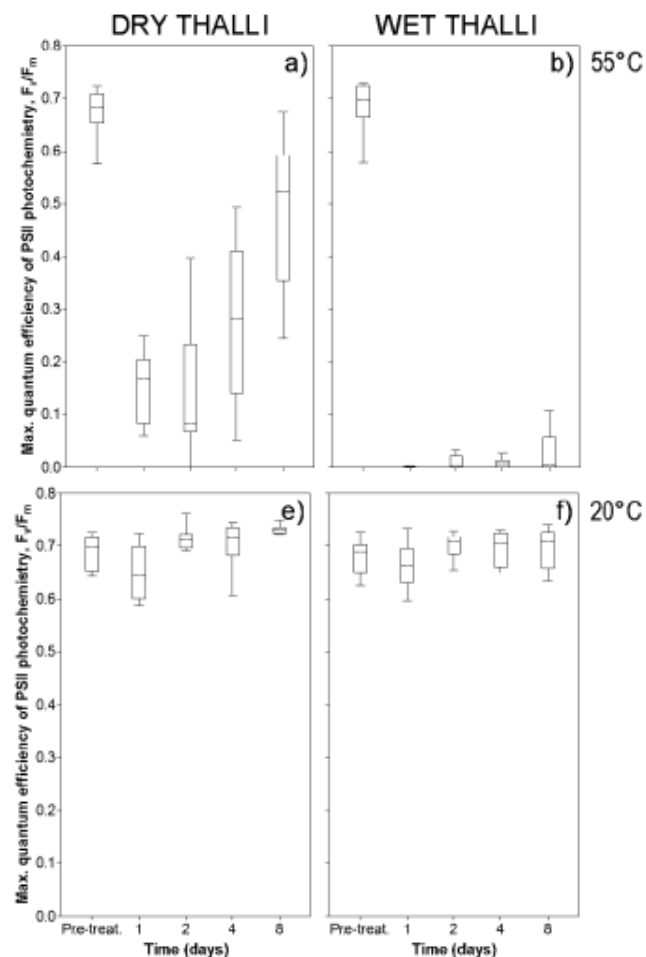
## Heat Shock Treatments: A New Safe Approach against Lichen Growth on Outdoor Stone Surfaces

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Supporting Information

**ABSTRACT:** The control of lichen growth, particularly important in the field of stone conservation of outdoor monuments, largely depends on the use of biocides, that may be dangerous for the users, the environment and the substratum. A new, alternative approach is proposed, which makes the most of a poorly known peculiarity of poikilohydrous organisms: they are thermo-tolerant (up to 65–70 °C) when dry, but thermo-sensitive when wet. The efficacy of thermal treatments (range: 20–55 °C), in parallel to the application of three biocides, was verified in the laboratory with six epi- and endolithic lichens. Chlorophyll *a* fluorescence emission was checked in treated and nontreated samples of all the species, whereas histochemical observations with a dead cell stain were carried out on one of them. The feasibility of the thermal treatments in the field was verified with a seventh species. The results confirm that a 6 h treatment at 55 °C is sufficient to kill the lichens if they are kept fully hydrated. At 40 °C the organisms are damaged: in this case biocides at concentrations 10× lower than in normal applications can profitably be used. The new protocol is simple, the field equipment cheap, and the negative effects associated with standard biocide treatments are absent.



**Figure 1.** Effects of a 24 h thermal treatment at 20 °C (e,f), 40 °C (c,d) and 55 °C (a,b) applied to dry (left column: a,c,e) and wet (right column: b,d,f) thalli of the epilithic lichen *Verrucaria nigrescens* on  $F_v/F_m$ , the maximum quantum efficiency of Photosystem II (PSII) photochemistry. Median, 25–75 percentiles, and nonoutlier minimum and maximum are reported; pretreat.: pretreatment value;  $n = 12$ .

We are surrounded by lichens, on the trees, on the walls and... on the monuments.

The only areas without lichens suffer from excessive environmental pollution: sulfur dioxide ( $\text{SO}_2$ ) is their main enemy, together with nitrogen oxides ( $\text{NO}$ ,  $\text{NO}_2$ ,  $\text{NO}_x$ ), hydrofluoric acid, etc.

This happens, for example, in the largest urban agglomerations, and in large areas of the Po Valley.

