Doctoral programme in Environmental Life Sciences, XXXVII ciclo (a.a. 2021/2022)

# Interazioni micobionte-fotobionte nella simbiosi lichenica

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# INTRODUZIONE

## La simbiosi lichenica

**Micobionte**

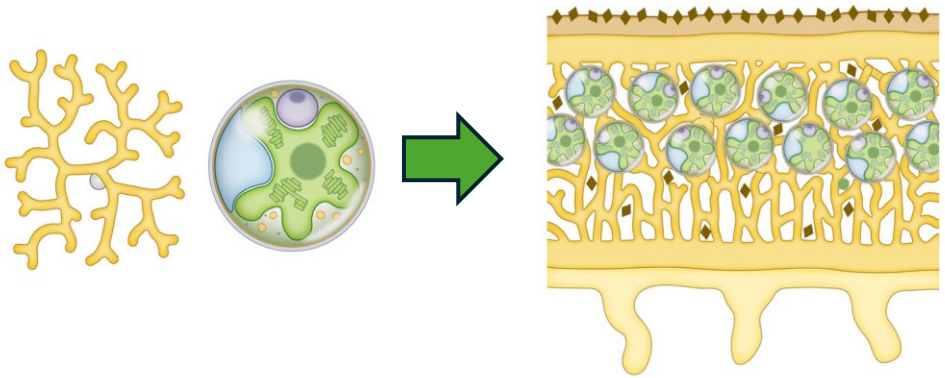
**+**

**Fotobionte**

**+**

**Microrganismi**

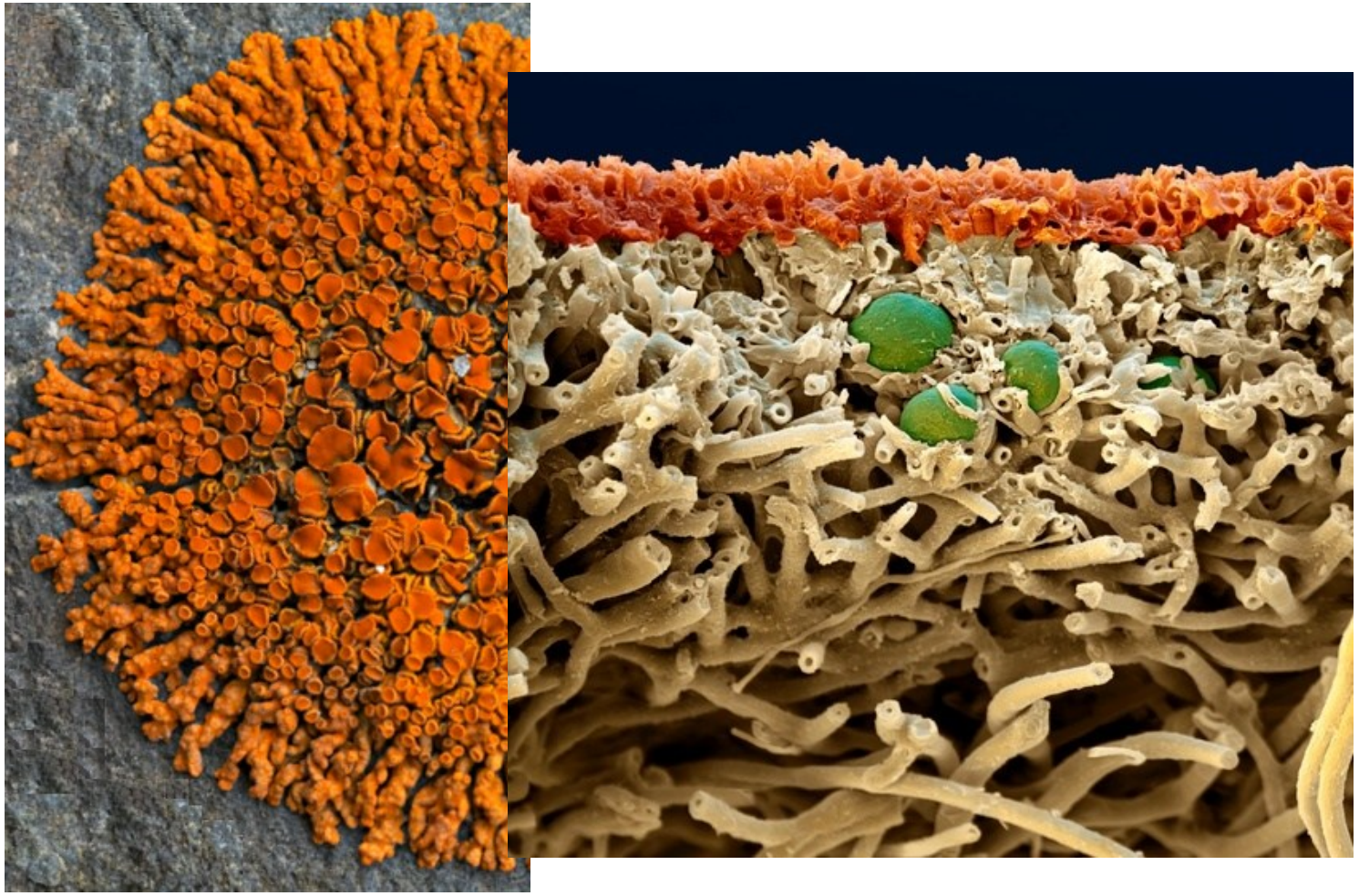
(Hawksworth & Grube, 2020)



Da Pichler et al. (2023) *New Phytologist 238: 1362–1378*

# INTRODUZIONE

I talli delle specie più evolute offrono un esempio di una delle strutture più complesse del mondo dei funghi



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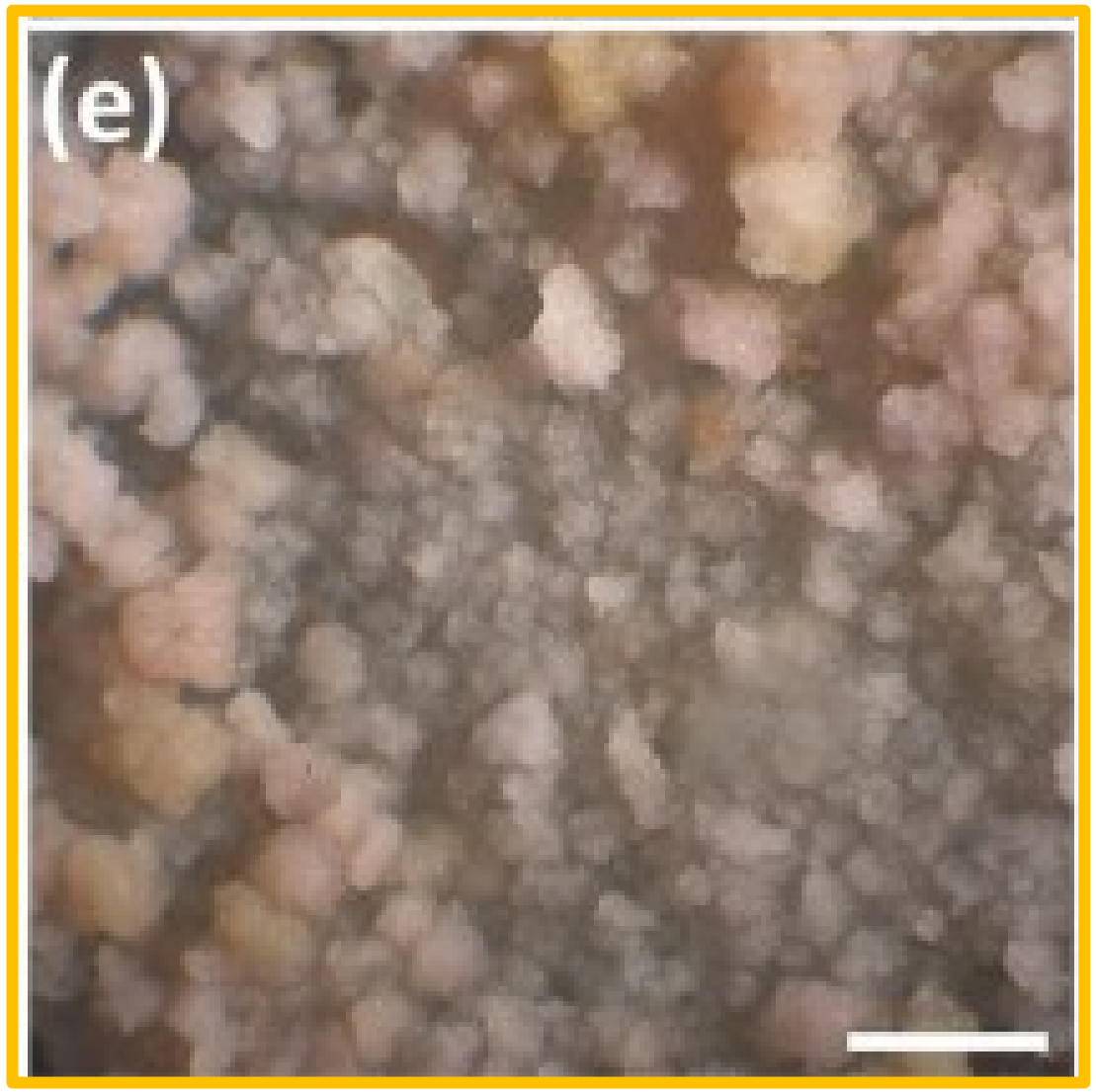
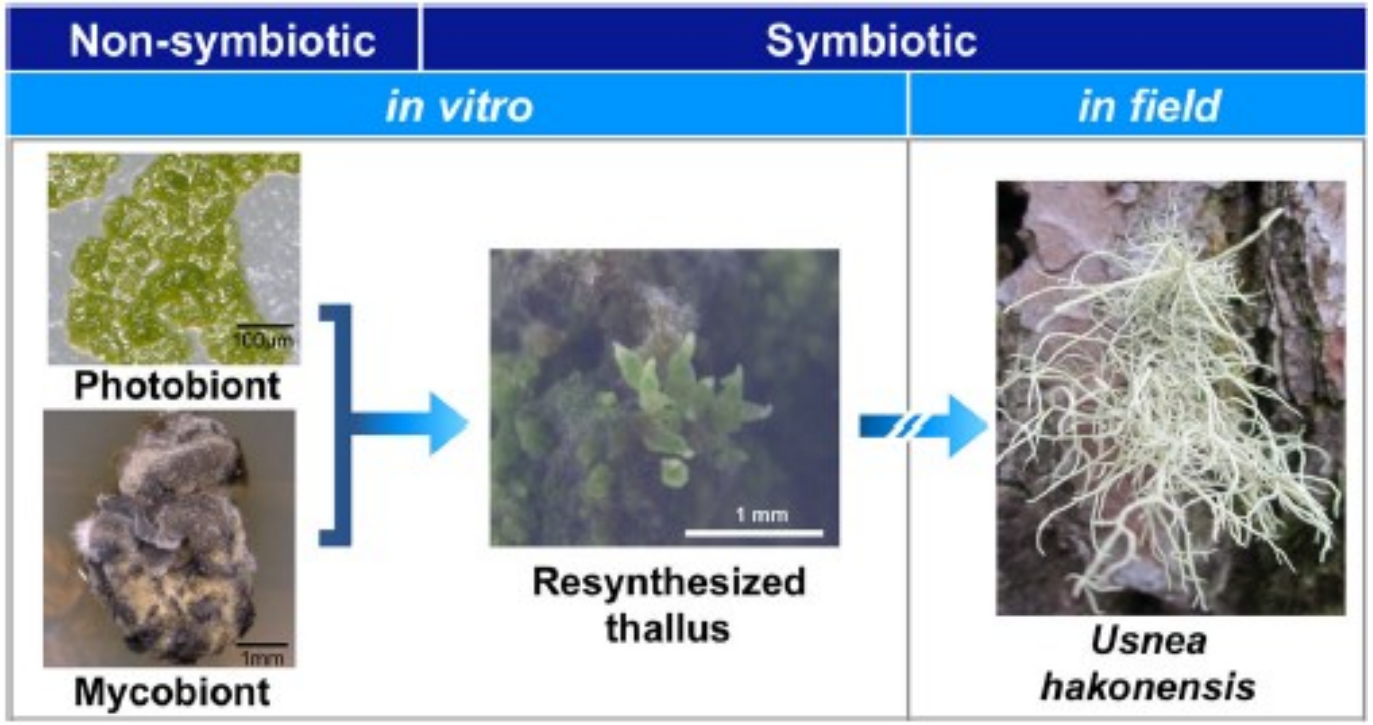


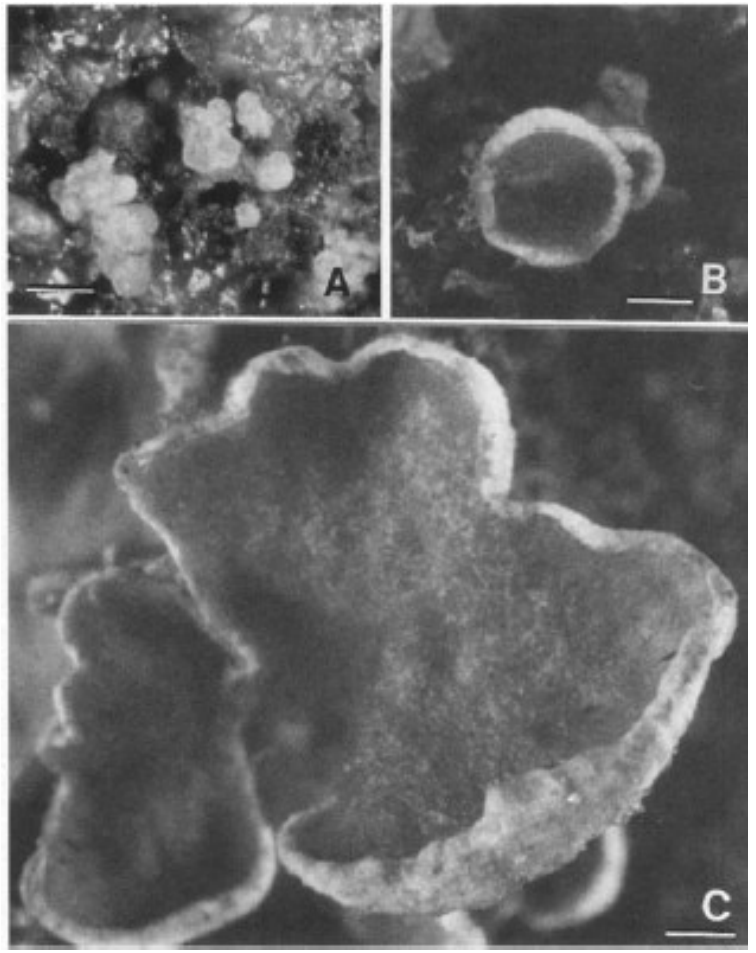

Modificato da Pichler G. et al., 2020. *Symbiosis* **82**, pp. 95-108

La complessa struttura del tallo deriva necessariamente dalle **interazioni** fra i due simbionti

# Morfogenesi del tallo




Da Kono et al. (2020) *BMC Genomics 21:* 671–687




Da Stocker-Wörgötter (2001) *The Bryologist 104:* 576–581

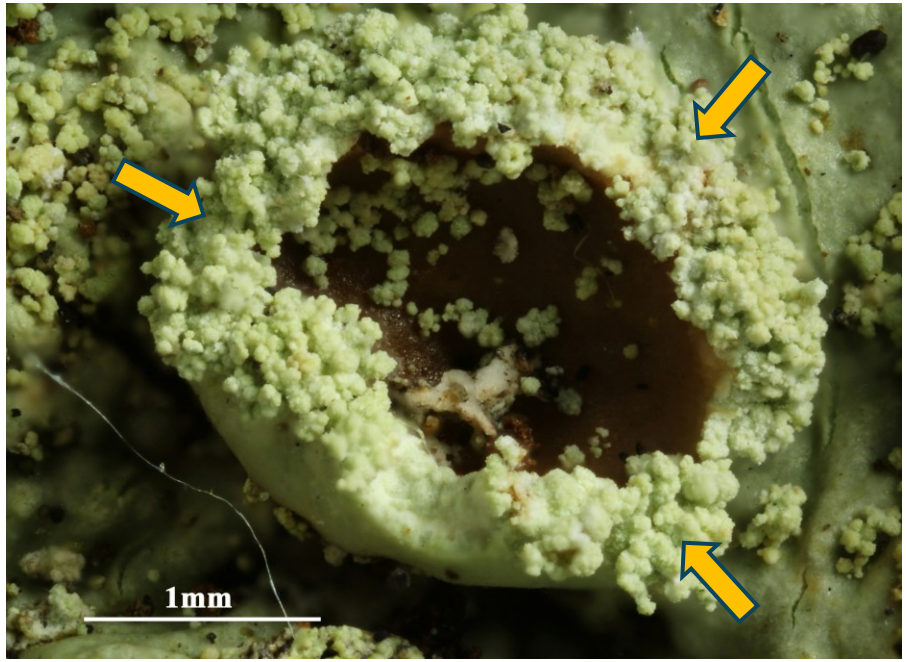
# INTRODUZIONE

## Riproduzione asessuale del micobionte

Frammentazione del tallo

Strutture riproduttive (e.g. soredi e isidi)

**Propaguli**



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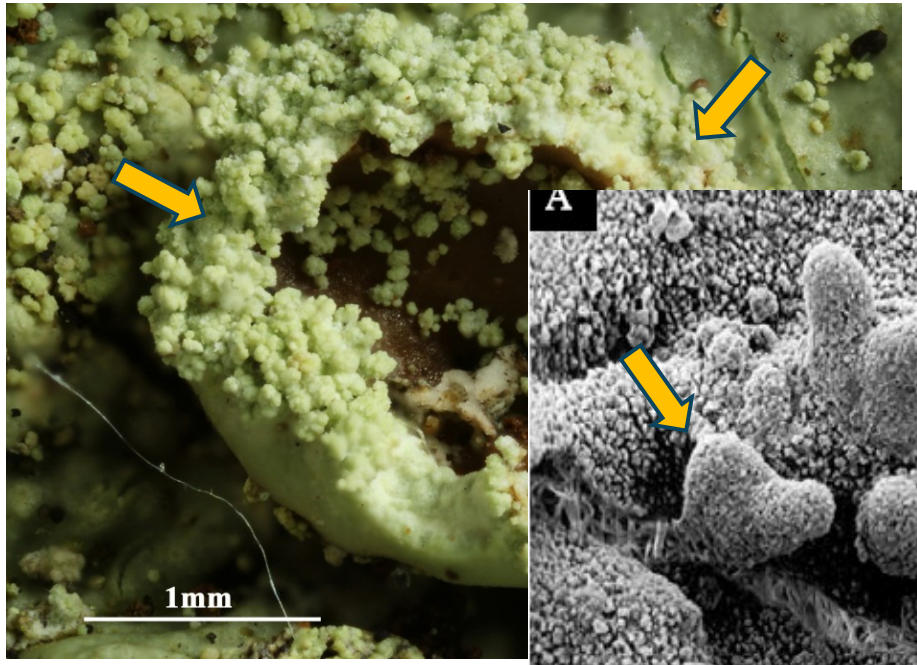
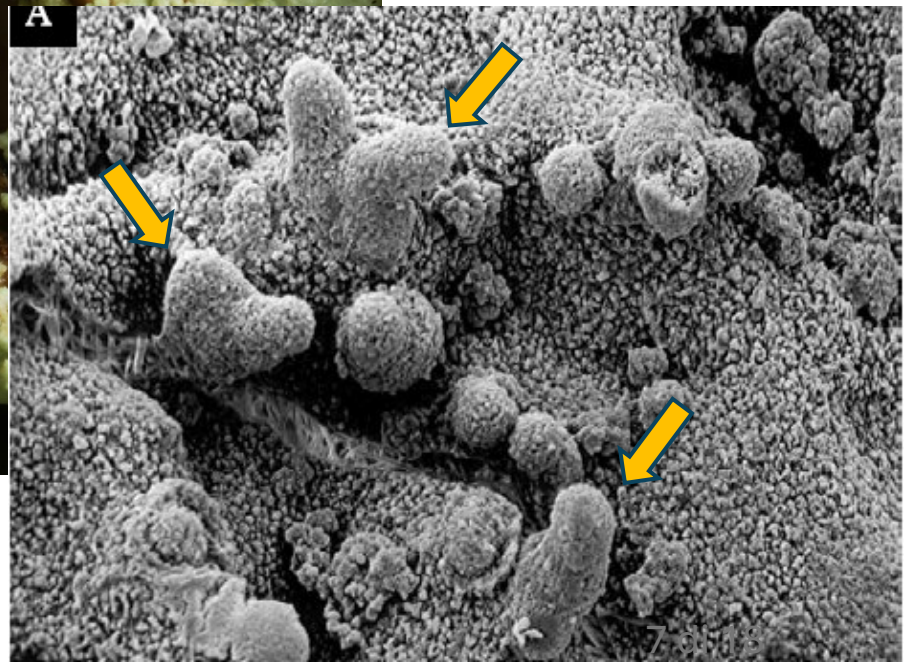


# INTRODUZIONE

## Riproduzione asessuale del micobionte

La maggior parte dei funghi lichenizzati si riproduce **sessualmente** e deve quindi ristabilire la simbiosi ad ogni ciclo riproduttivo (Honegger, 2008)

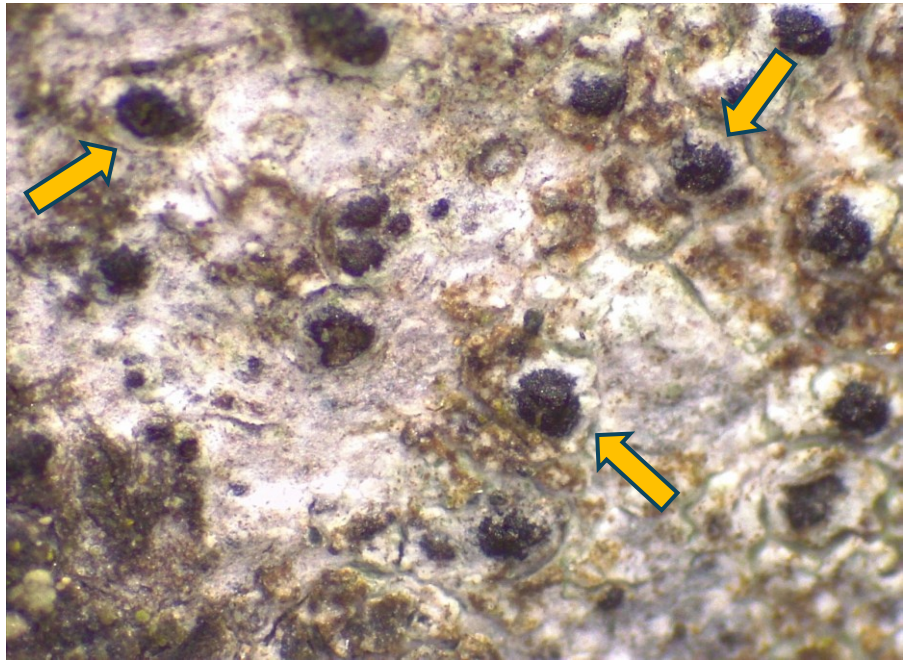
# INTRODUZIONE

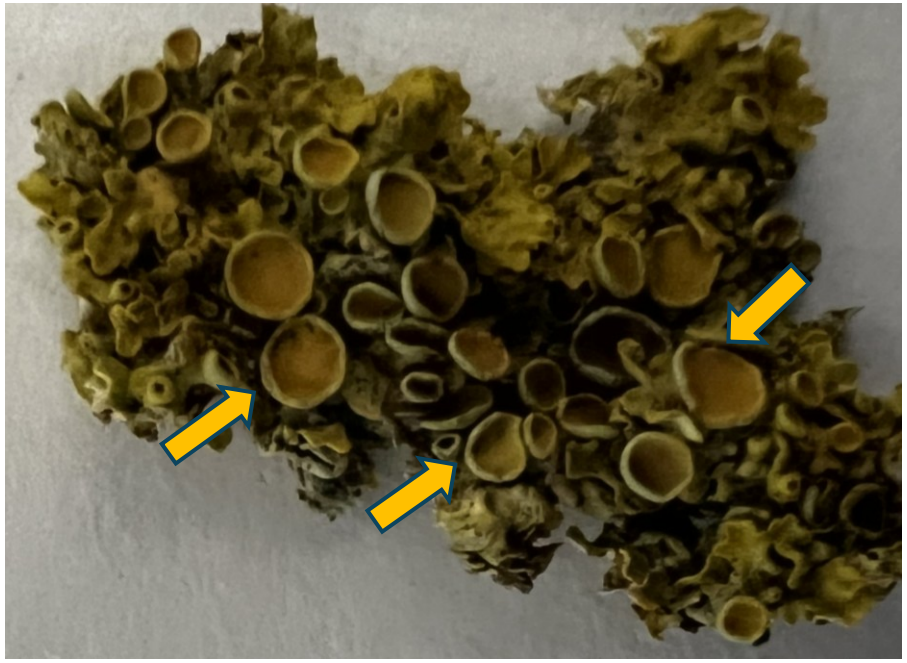
## Riproduzione sessuale del micobionte

Riproduzione sessuale (e.g. periteci e apoteci)

⇩

**Ascospore**





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Riproduzione sessuale (e.g. periteci e apotec

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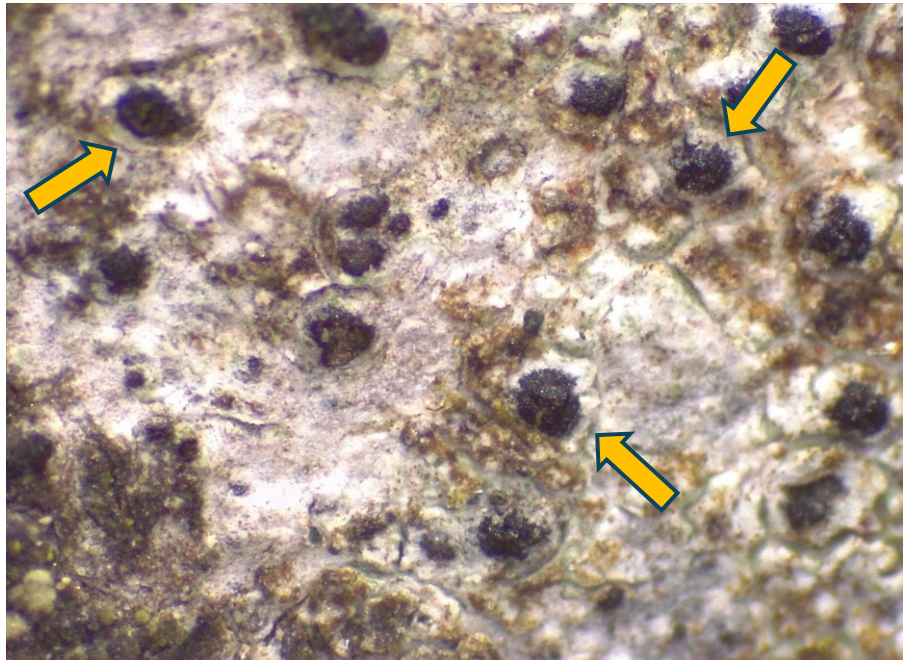

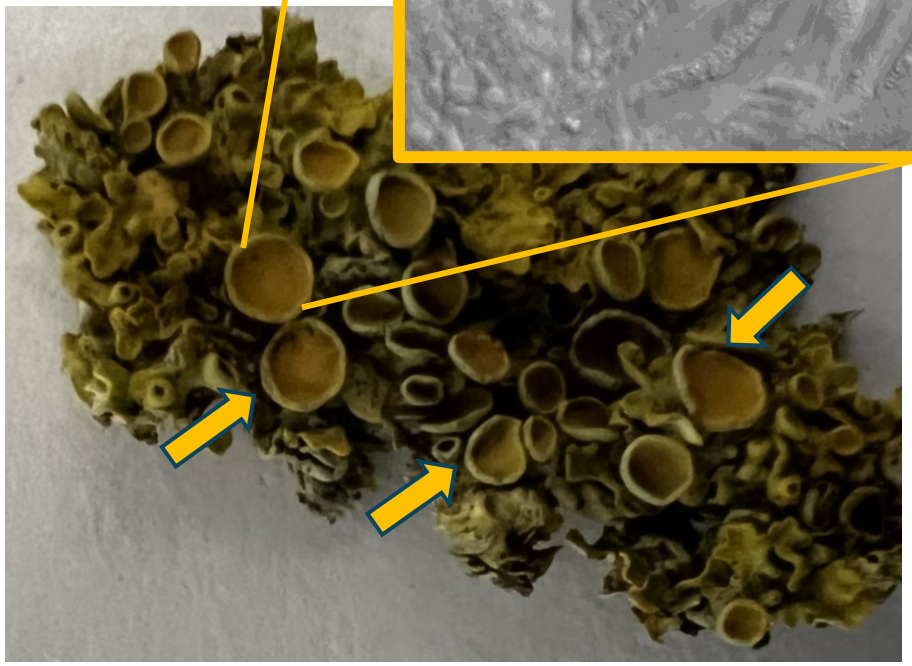

# INTRODUZIONE

**Su posti insoliti…**

Vero non solo per i licheni che si riproducono asessualmente (isidi, soredi), ma anche per quelli che si **riproducono sessualmente**

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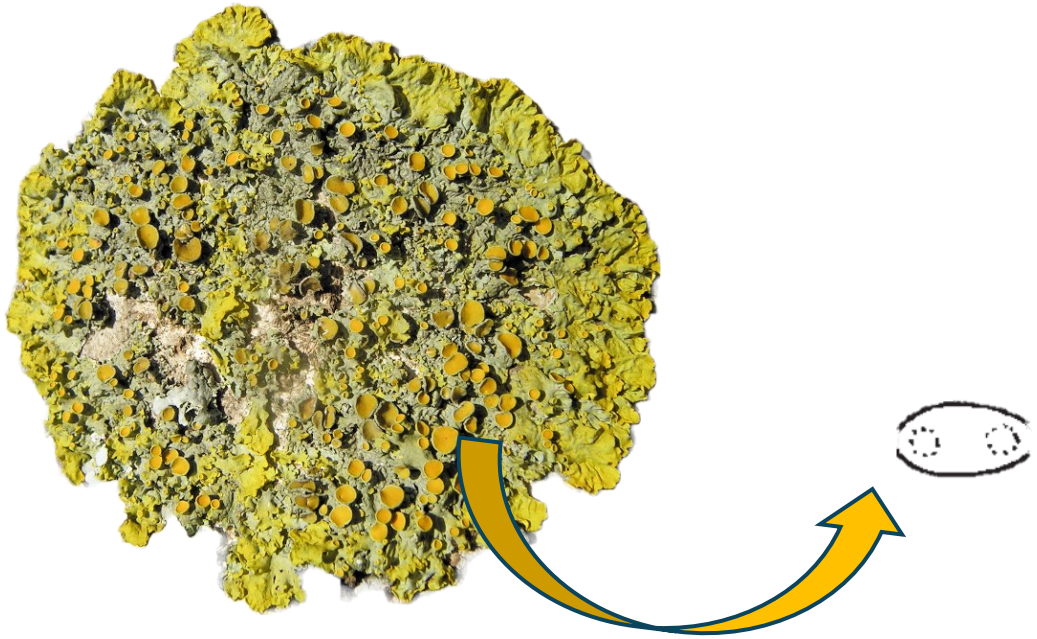

*Xanthoria parietina* (L.) Th. Fr.

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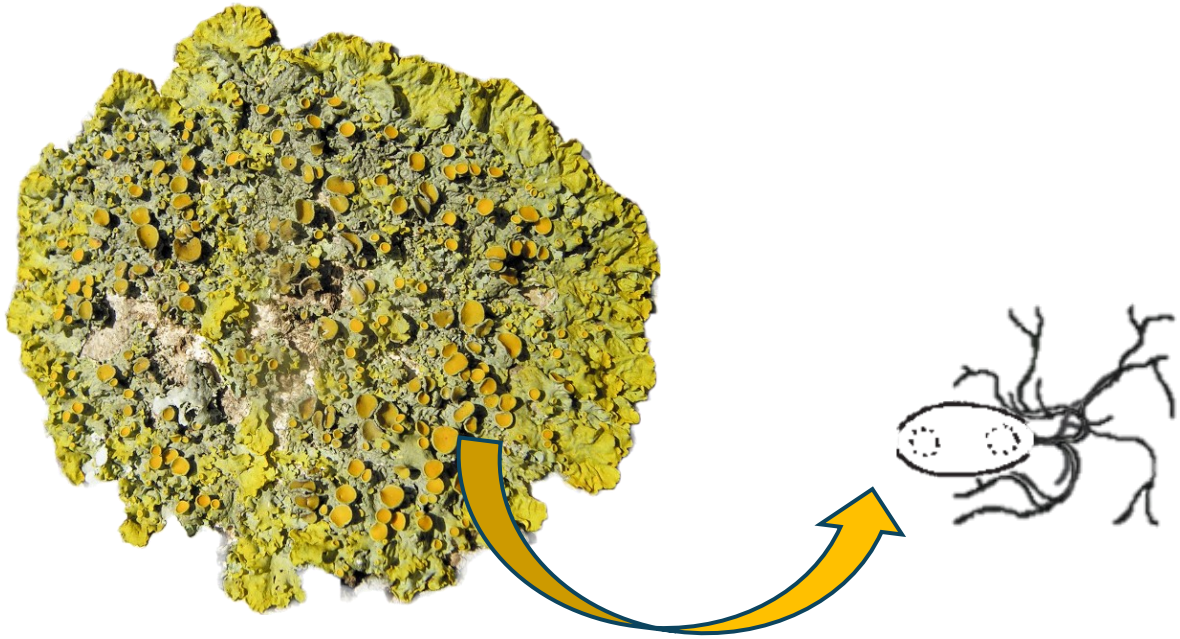

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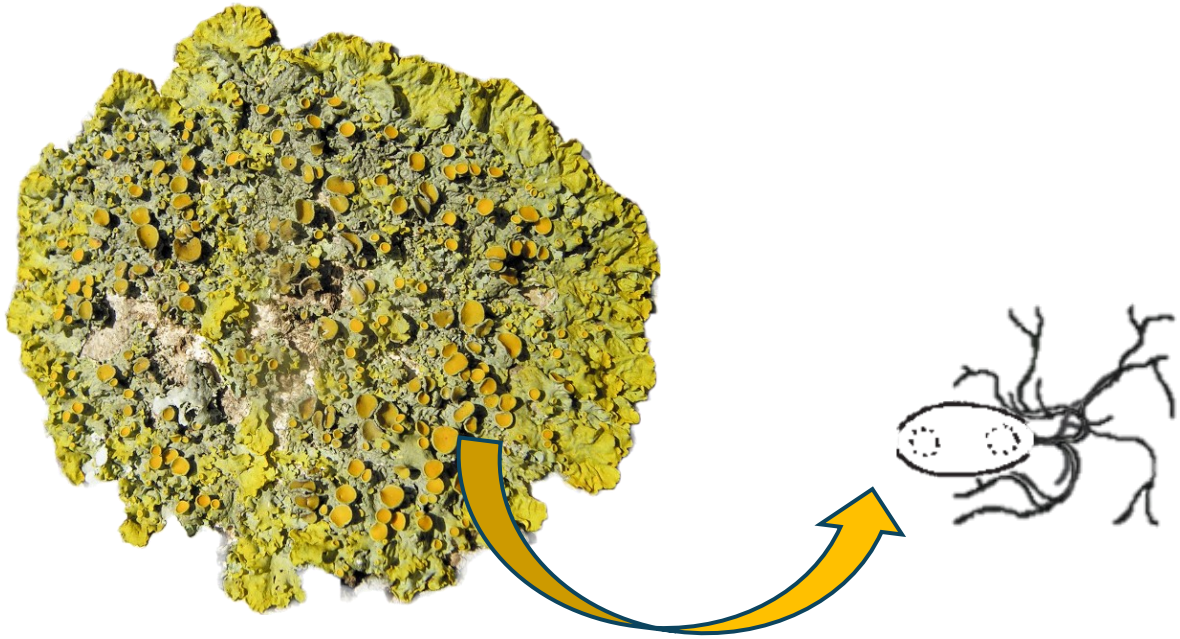
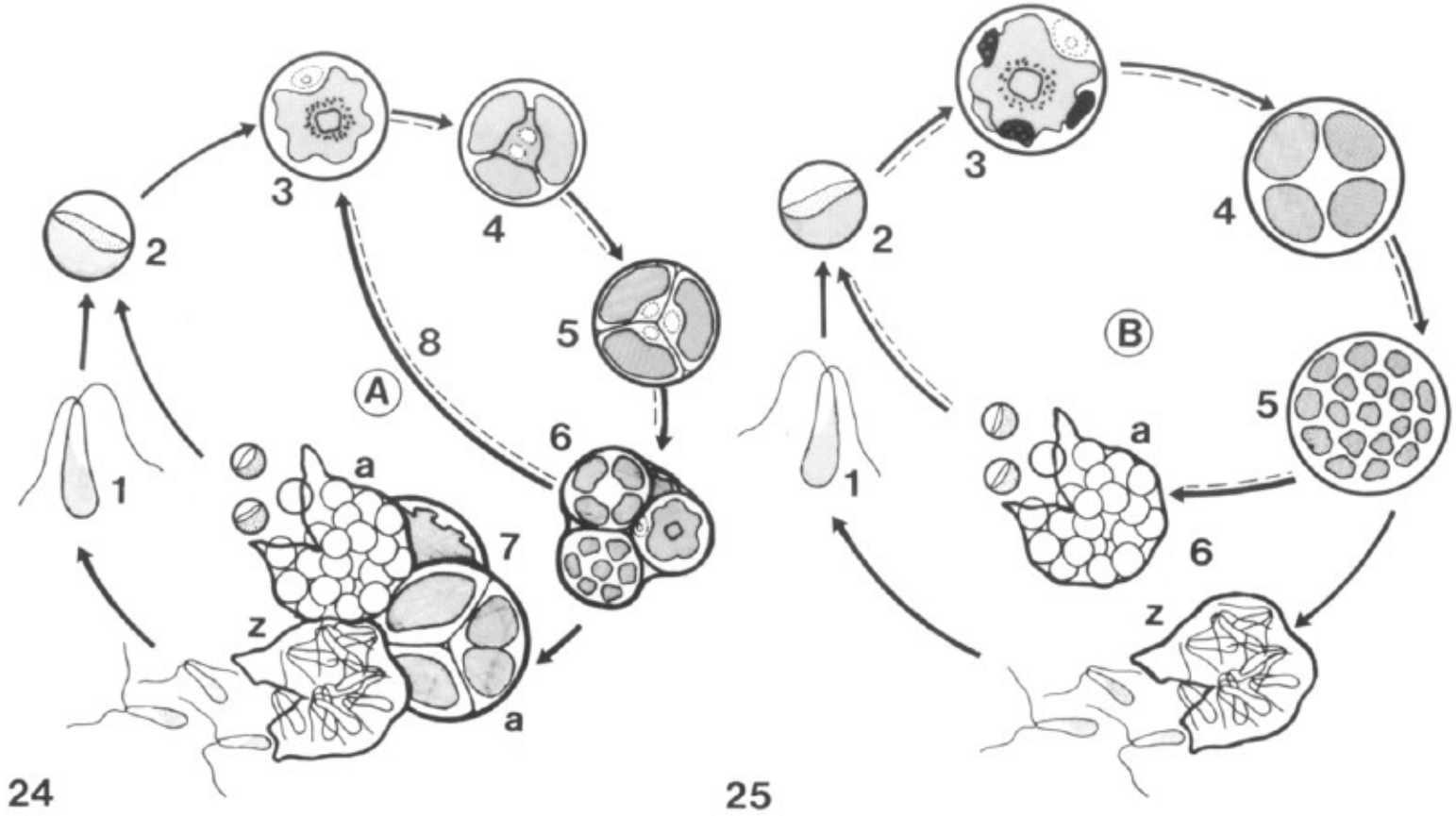


*Xanthoria parietina* (L.) Th. Fr.

*Trebouxia decolorans* Ahmadjian

# INTRODUZIONE

## Riproduzione asessuale del fotobionte




Modificato da Friedl T., 1993. *Arch. Protistenkd.* **143**, pp. 153-161

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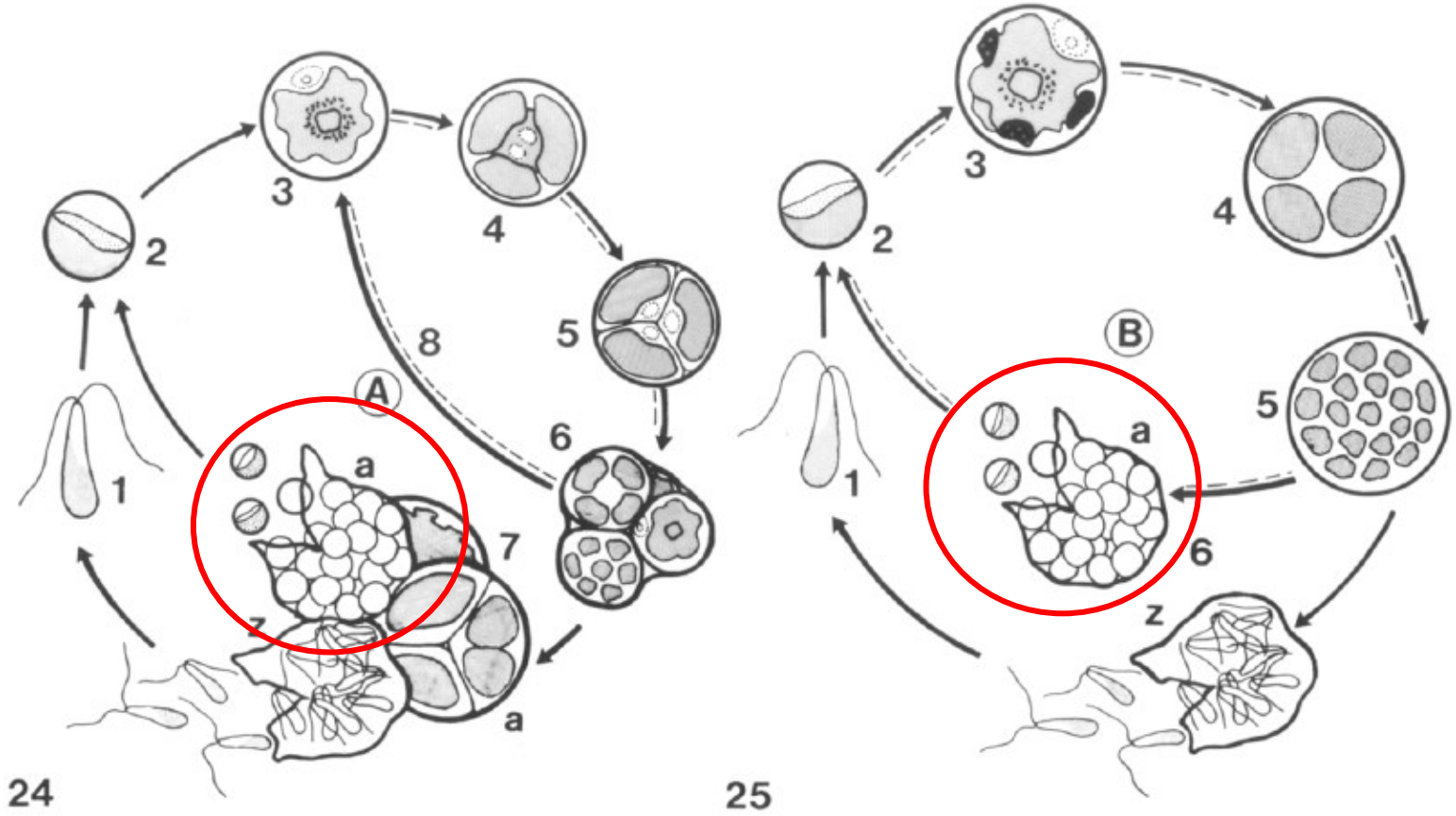


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aplanospore vegetative (immobili)

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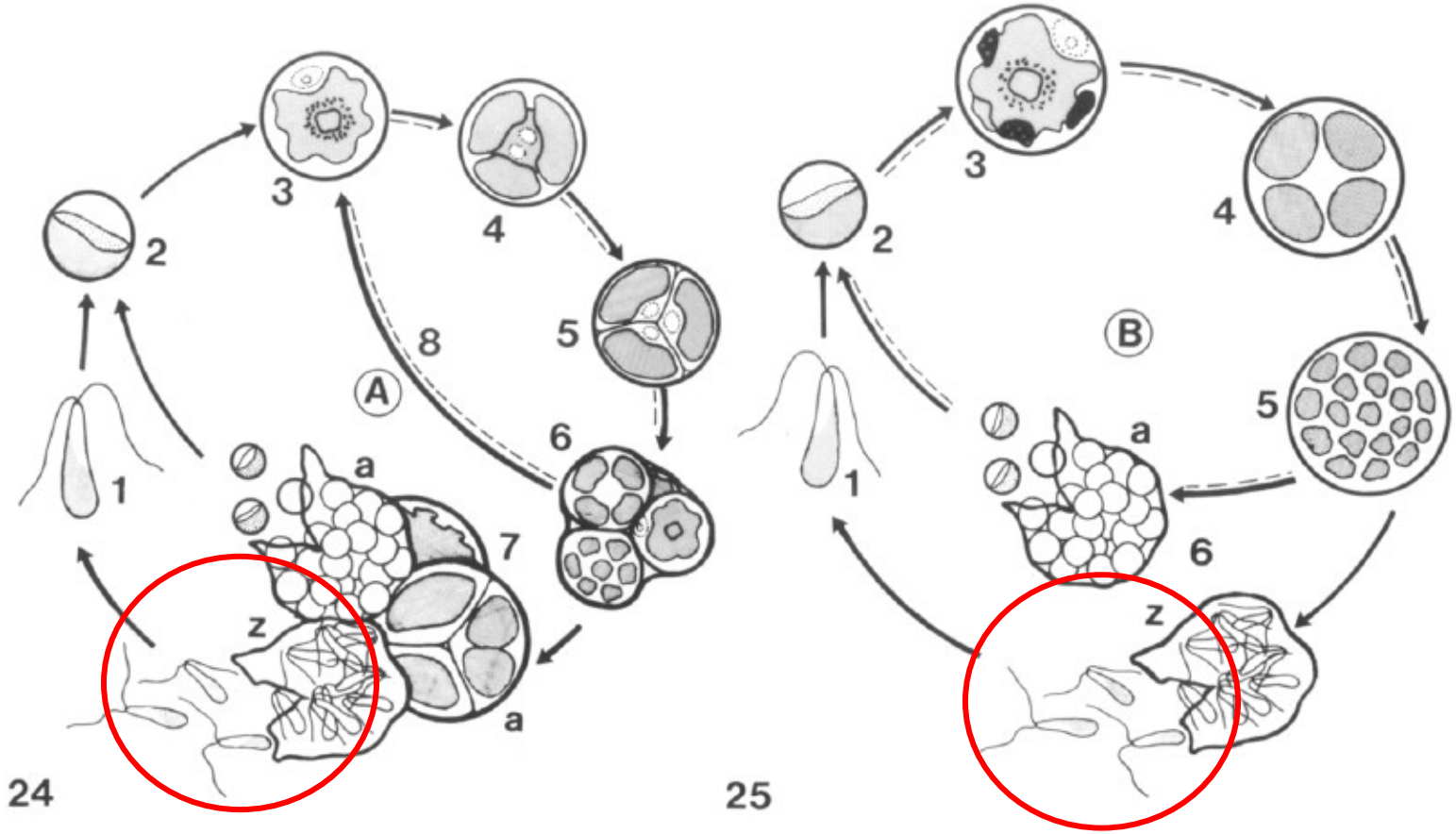


Modificato da Friedl T., 1993. *Arch. Protistenkd.* **143**, pp. 153-161

aplanospore vegetative (immobili)

**zoospore** (motili con flagelli)

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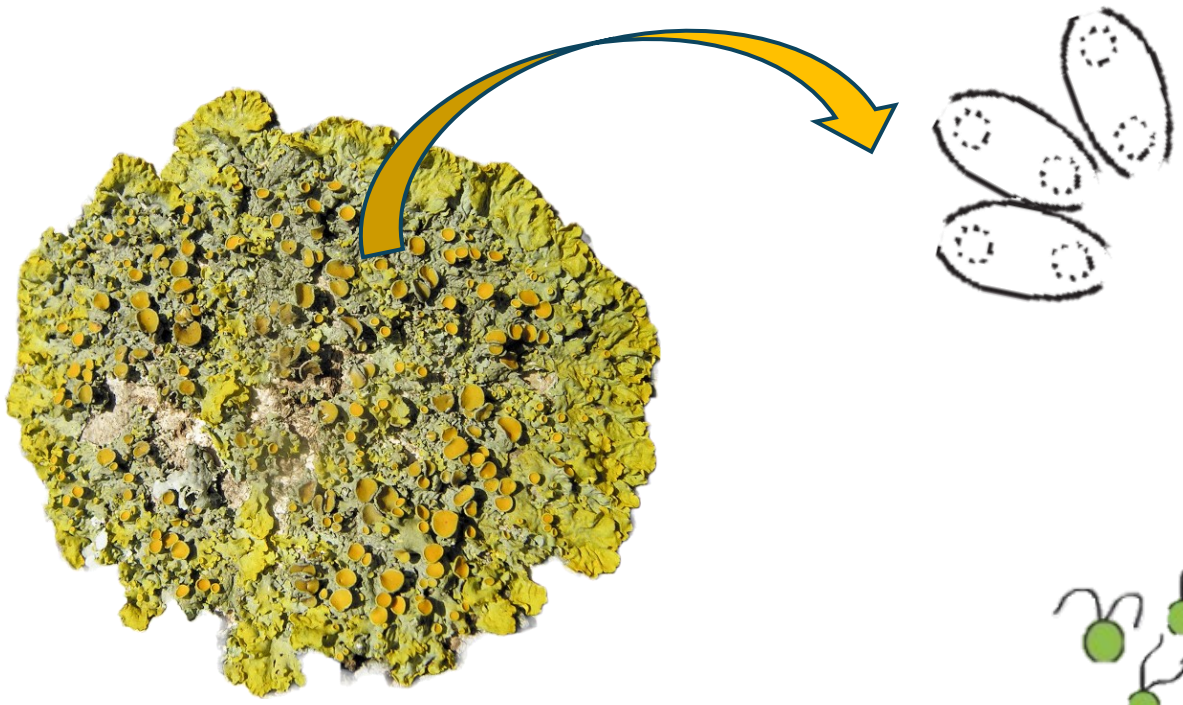

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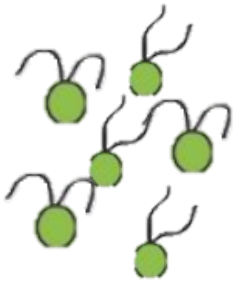

… the transient presence of liquid water could provide the means by which algal zoospores migrate and settle near fungal spores and mycelia. **The exudates** of these microorganisms **might be** a sufficient **attractant** or **stimulus for a zoospore to settle**. (Sanders, 2005).

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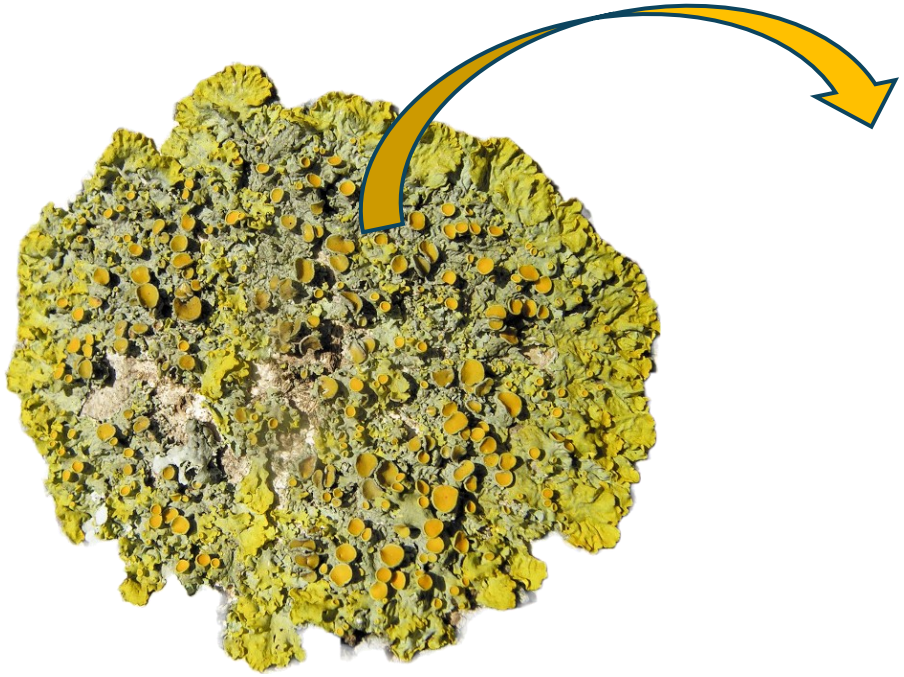

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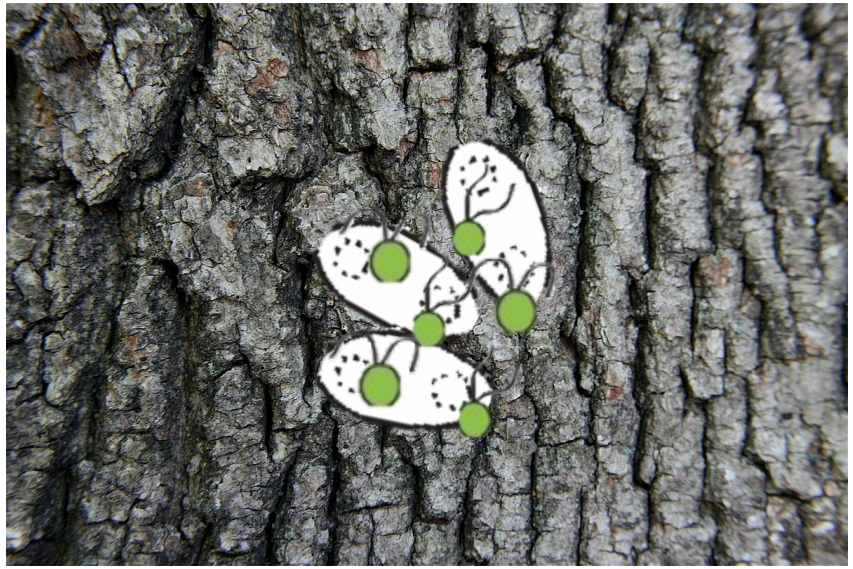


# OBIETTIVI

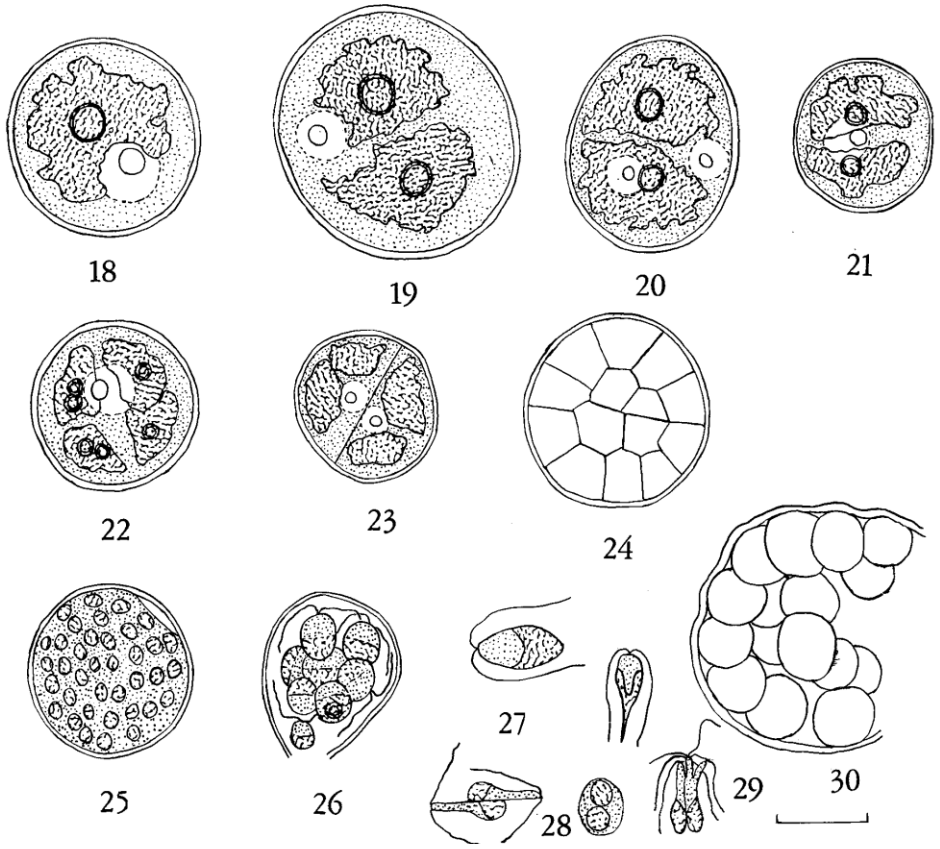
1. Studiare il ciclo vitale di *Trebouxia* al variare di diversi stimoli ambientali per identificare le condizioni di rilascio delle zoospore.

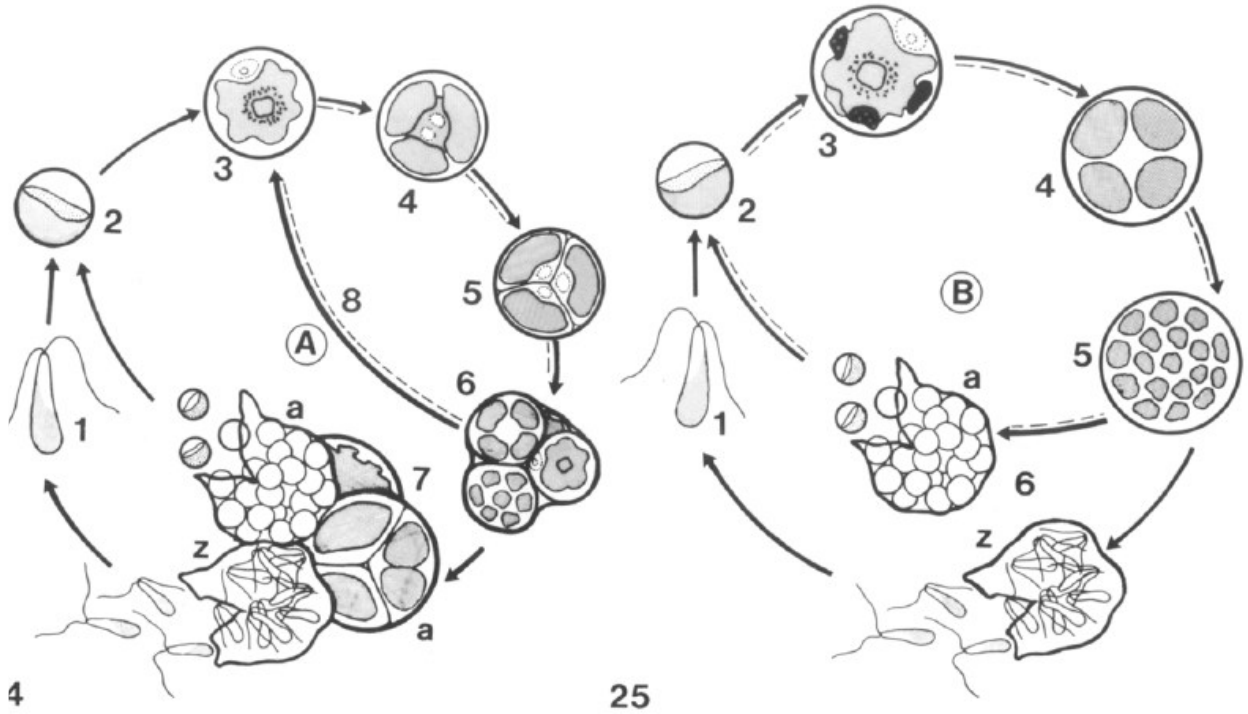
2. Sviluppare protocolli per l'isolamento delle ascospore dai talli lichenici.

3. Esperimenti di co-coltura tra ascospore fungine e zoospore algali per verificare se queste si muovono selettivamente verso le ascospore del fungo simbionte.

## Capitolo 1 - Background

# Ciclo cellulare di *Trebouxia* – Cosa si sa…



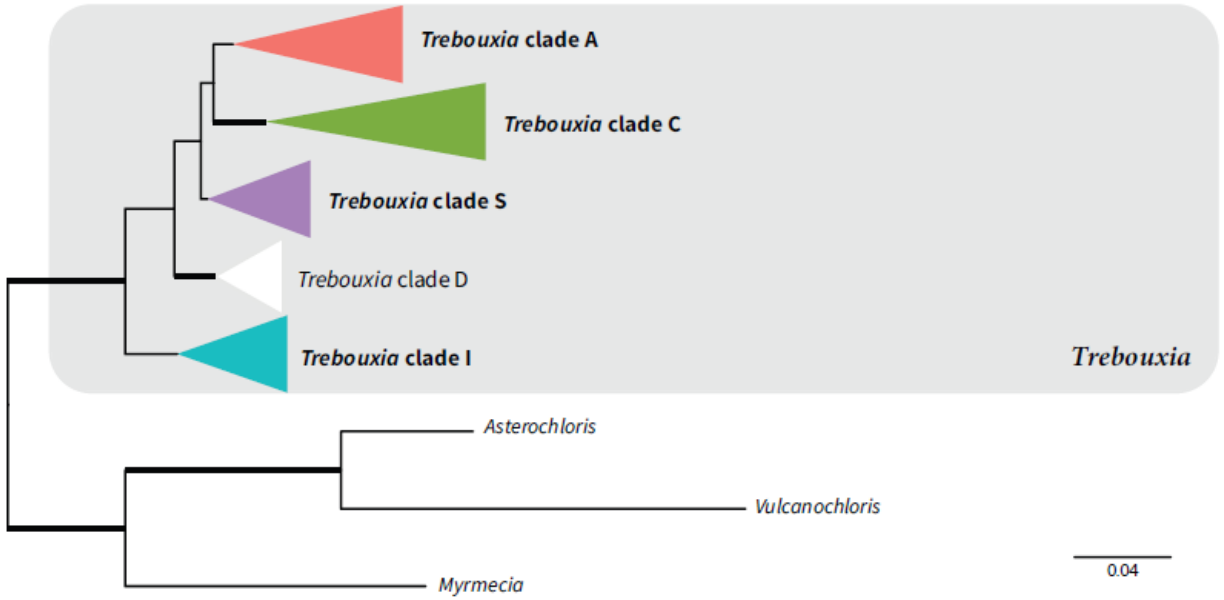

Ahmadjian (1960) *American Journal of Botany* 47: 677–683

Friedl (1993) *Arch. Protistenkd.* 143: 153–161

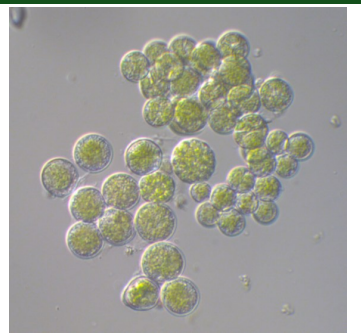
# Zoospore diversity and sexual reproduction in *Trebouxia*: from neglected evidence to new facts

## Capitolo 1 – Materiali e metodi

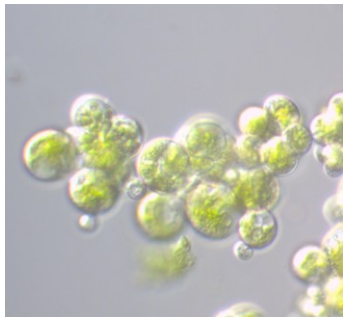
### Specie di *Trebouxia*:




Medeiros et al. (2021) *Front. Microbiol.* 12, 774839



*T. decolorans*



*T. vagua*

*T. angustilobata*

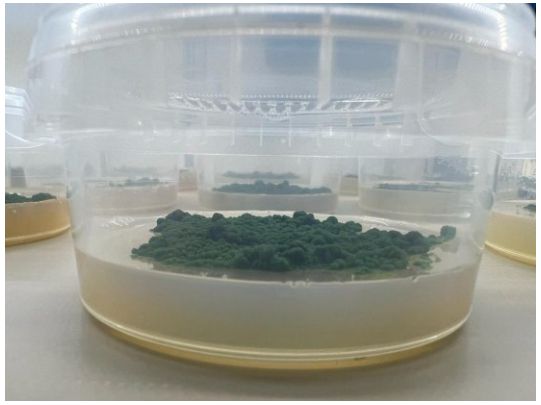
*T. gelatinosa*

# Zoospore diversity and sexual reproduction in *Trebouxia*: from neglected evidence to new facts

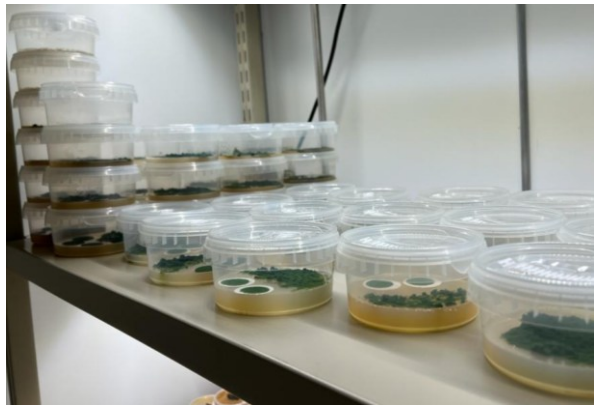
## Capitolo 1 – Materiali e metodi

Condizioni ambientali **standard** per la crescita di *Trebouxia* spp.

**Microbox**

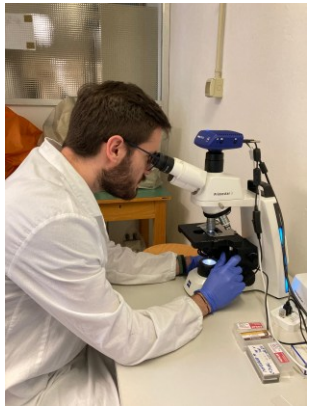


**Camera di crescita**

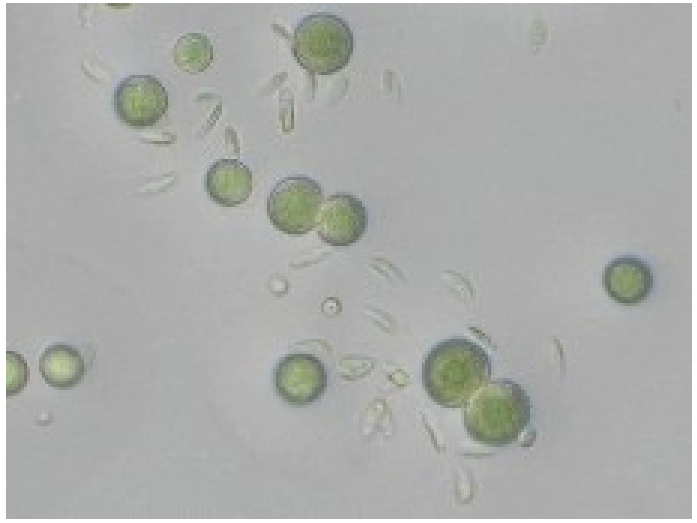


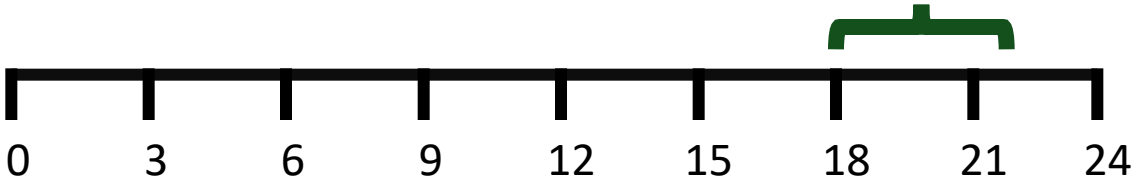
T = 16 ± 1 °C
Fotoperiodo = 12/12 h light/dark
Intensità luminosa = 22.5 ± 0.8 µmol $s^{-1}$ $m^{-2}$
Tipo di luce = Luce bianca
Terreno di crescita = BBM vs **TM**

**Microscopio ottico** per foto e video



**Microscopio Elettronico a Scansione (SEM)**





# Zoospore diversity and sexual reproduction in *Trebouxia*: from neglected evidence to new facts

## Capitolo 1 – Risultati

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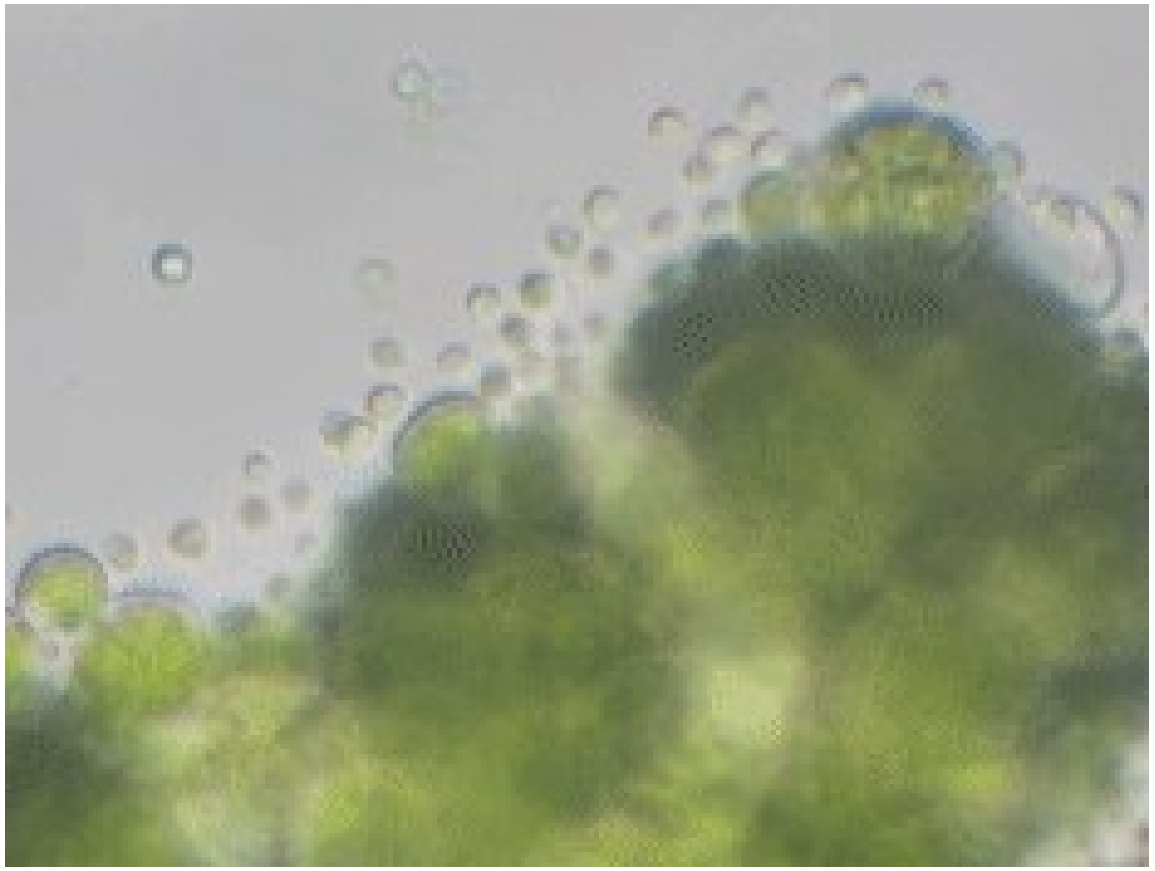

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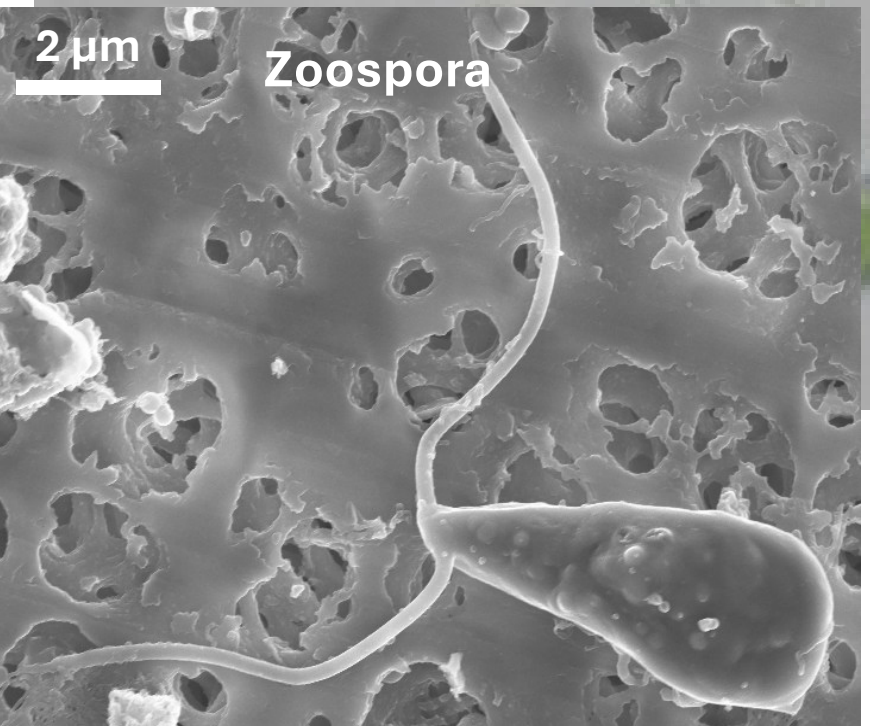


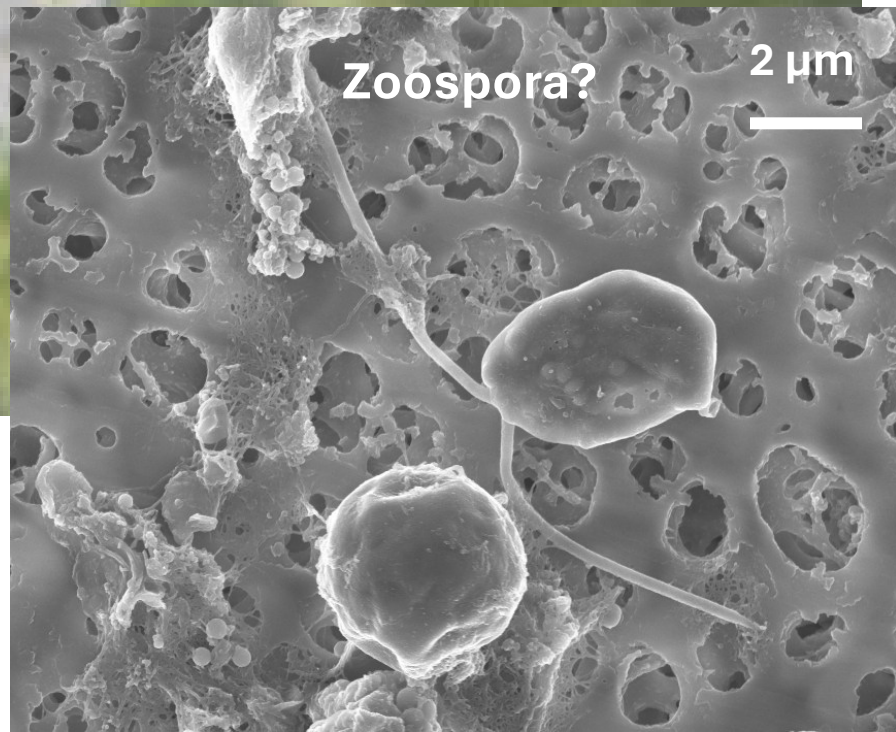


Differenti morfotipi in:
***T. decolorans***
***T. vagua***
***T. angustilobata***
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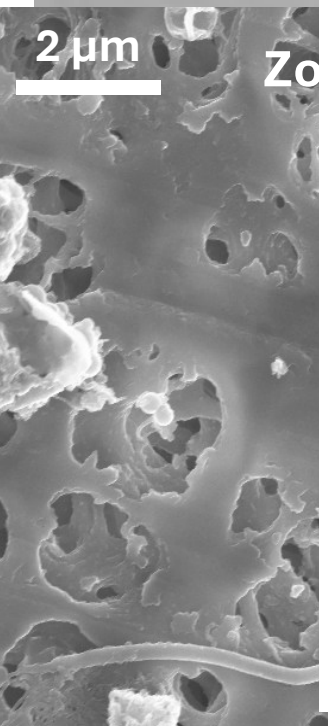
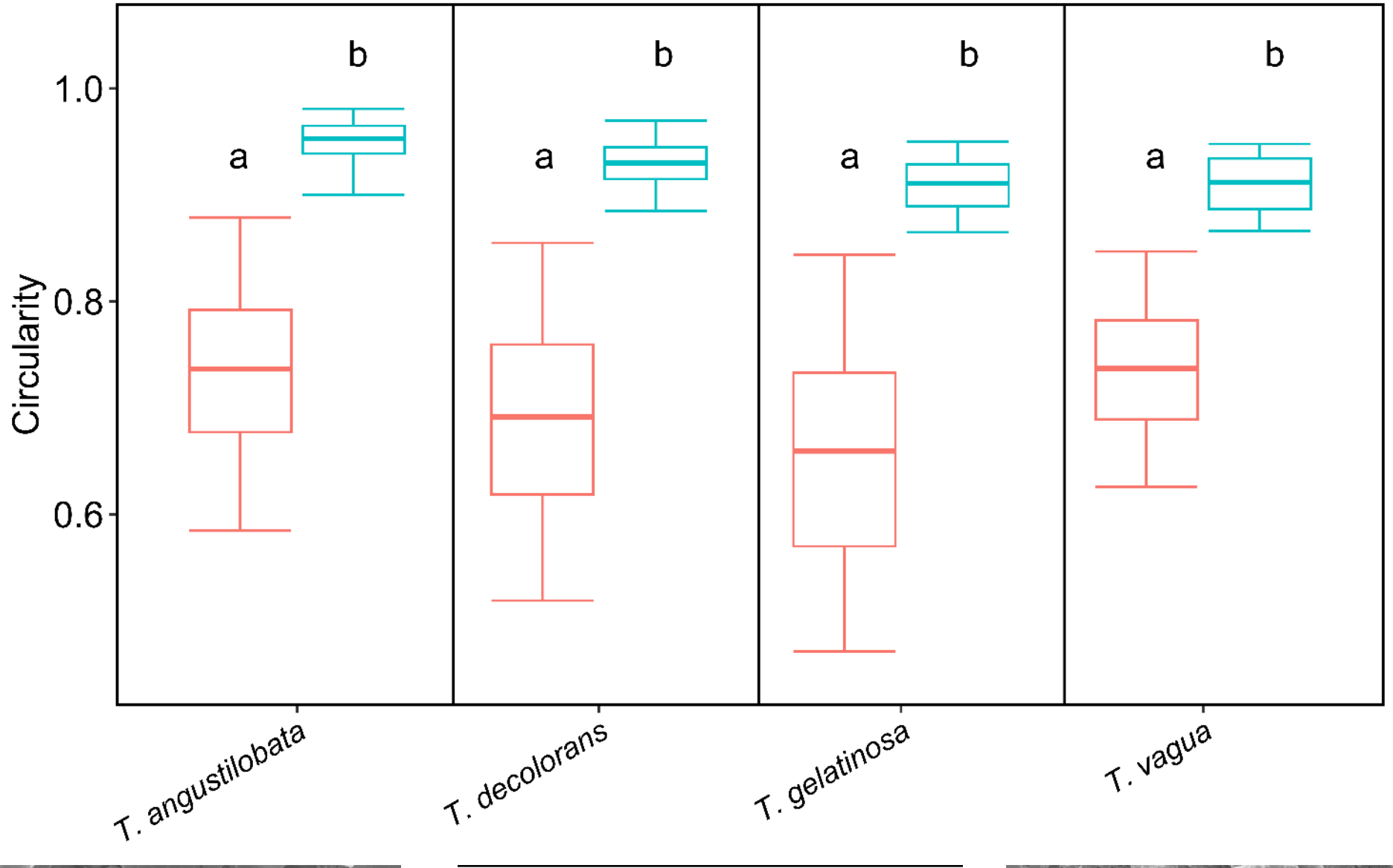
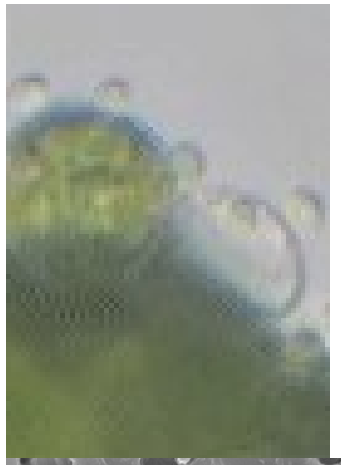
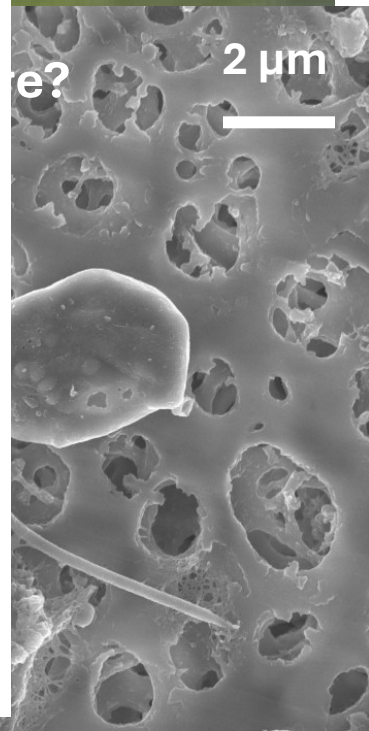


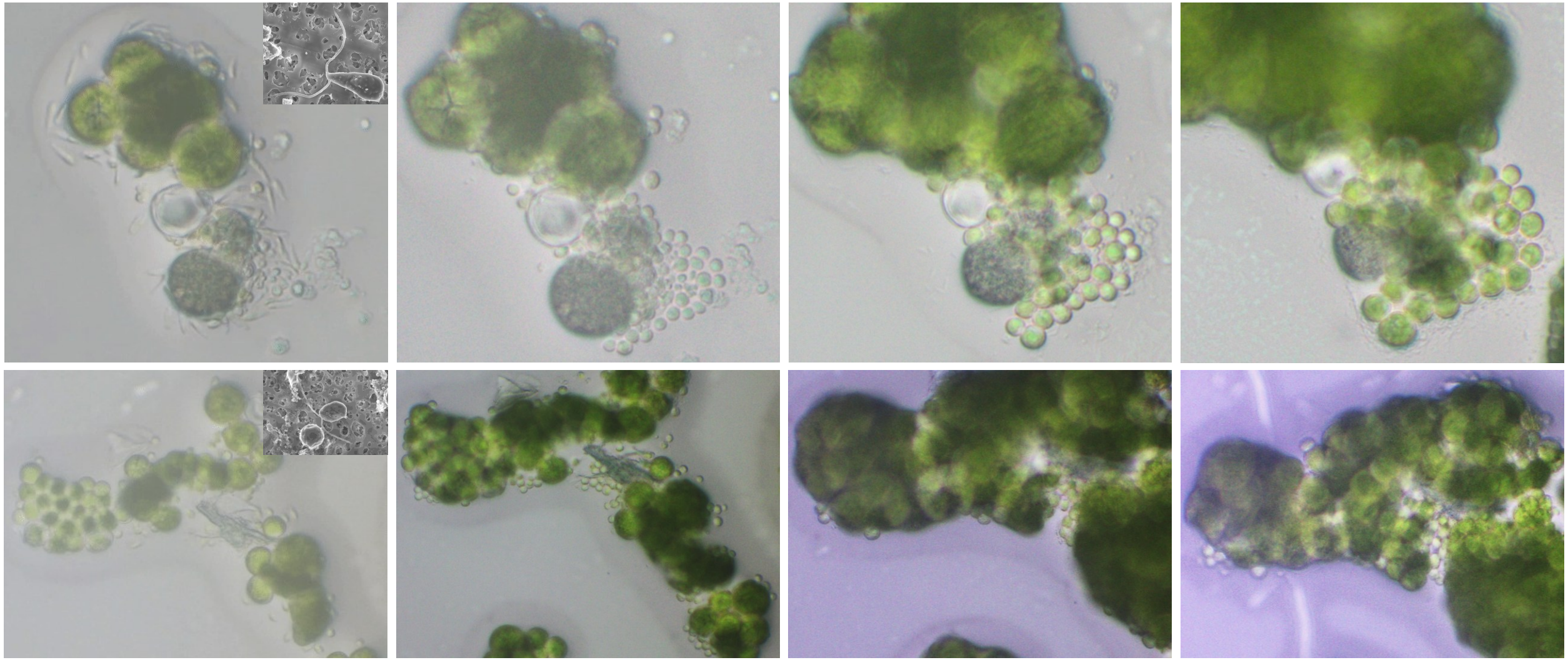





# Zoospore diversity and sexual reproduction in *Trebouxia*: from neglected evidence to new facts

## Capitolo 1 – Risultati

**Giorno 0** | **Giorno 1** | **Giorno 4** | **Giorno 9**



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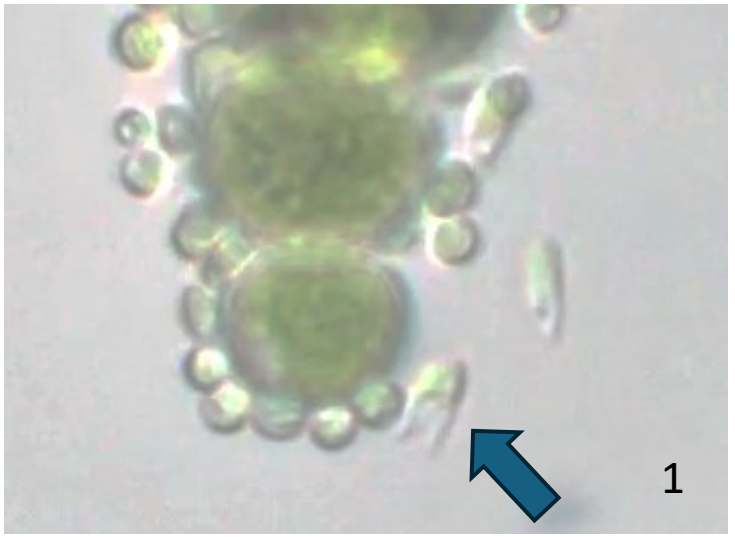


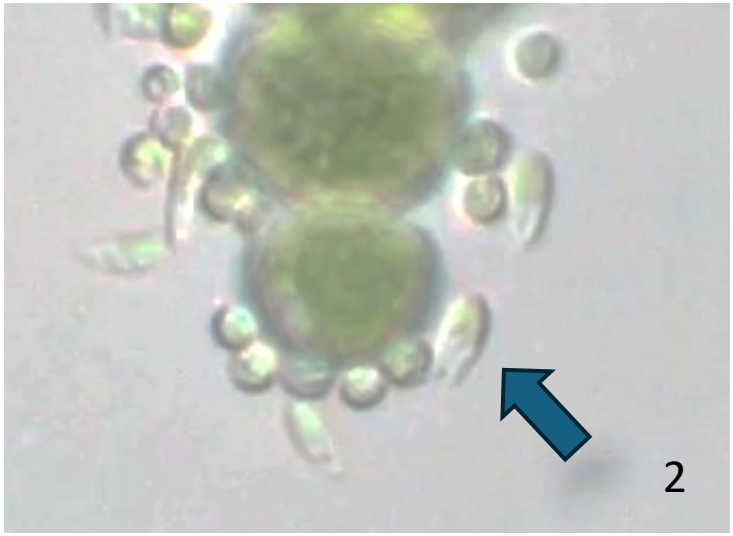


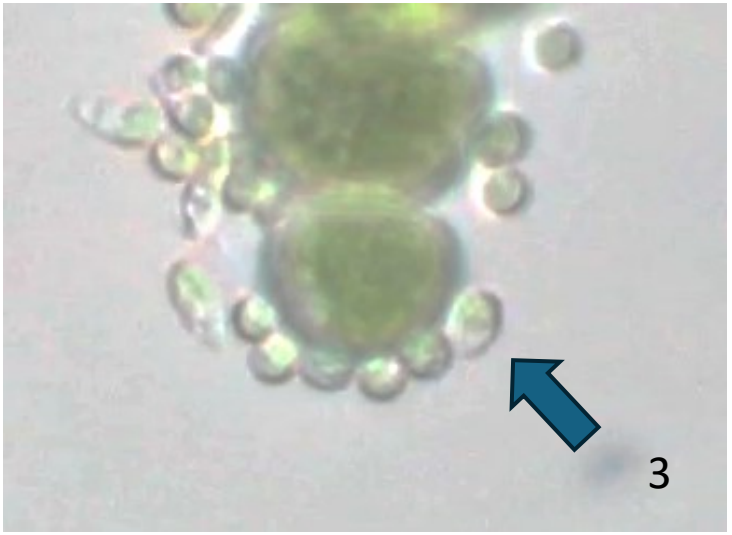


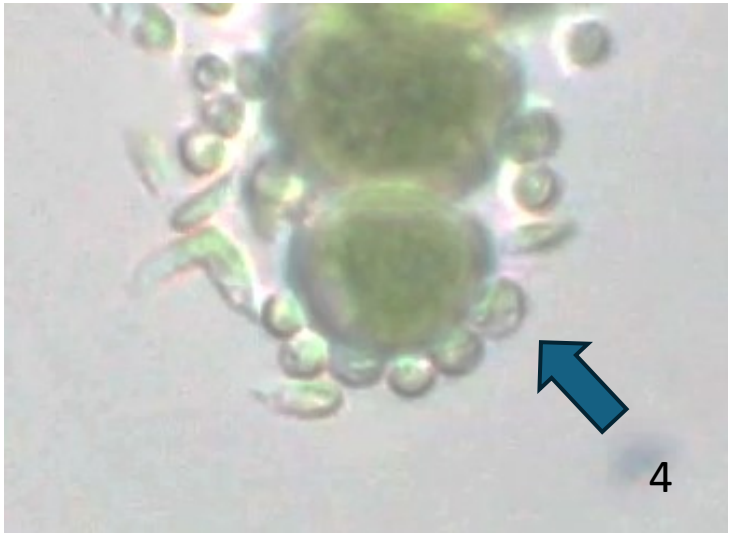

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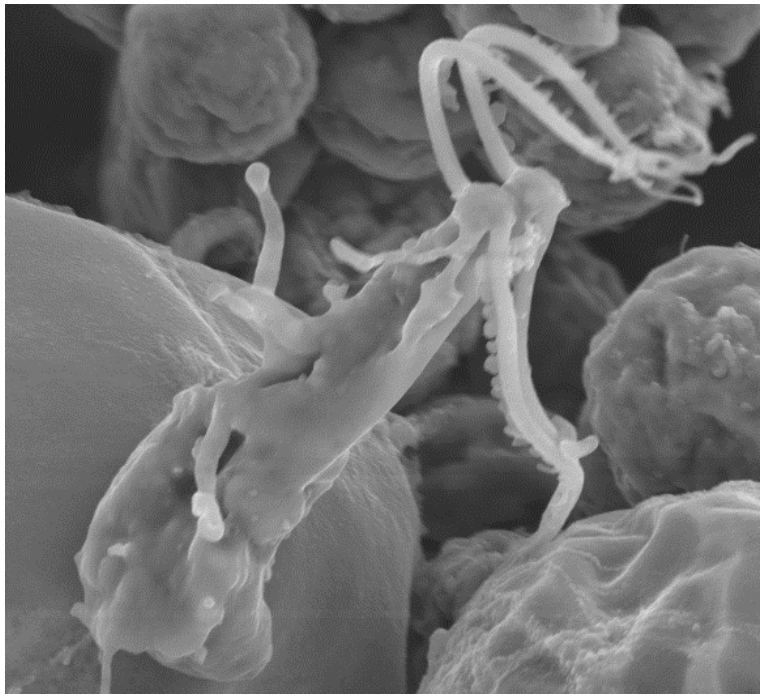
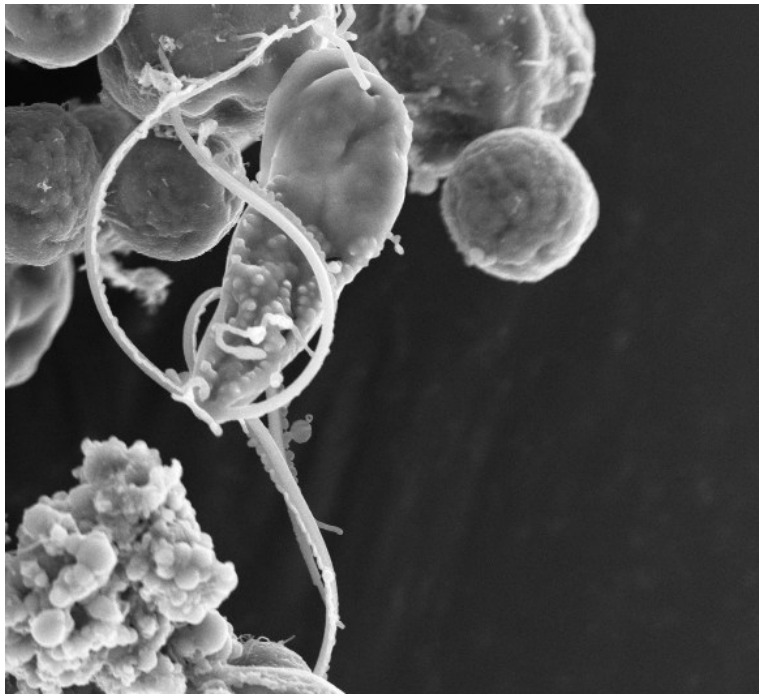
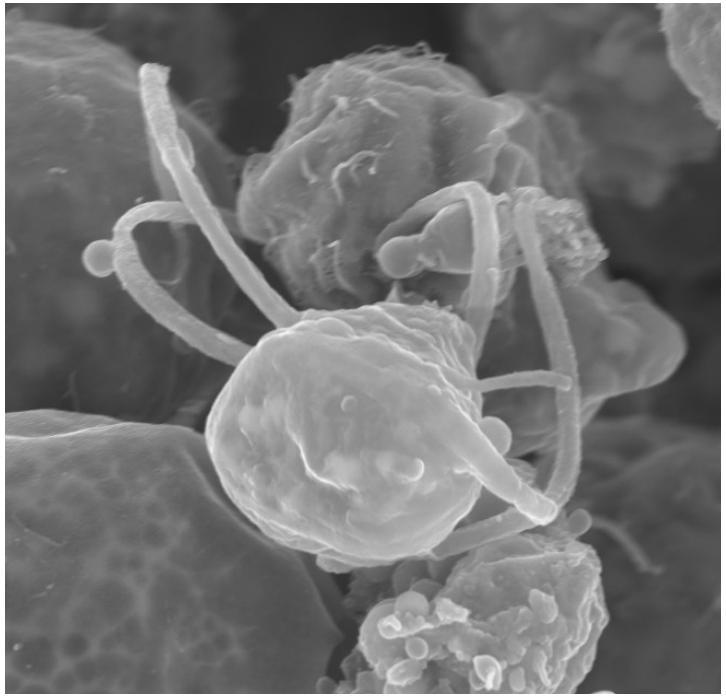


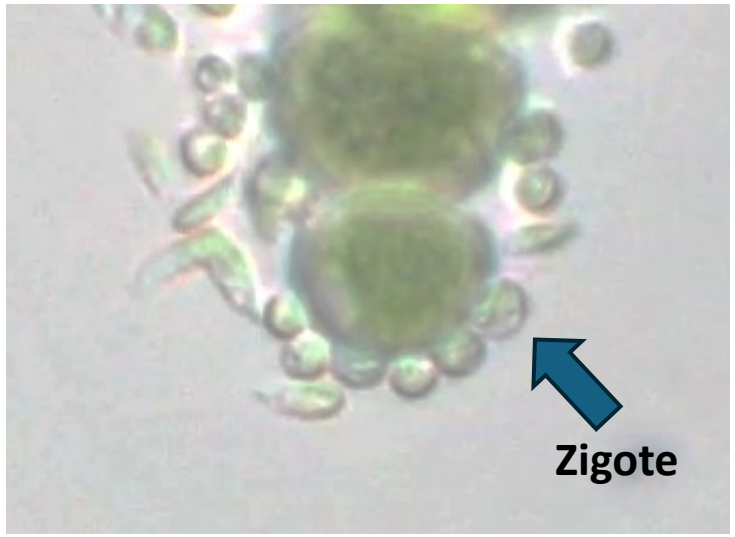




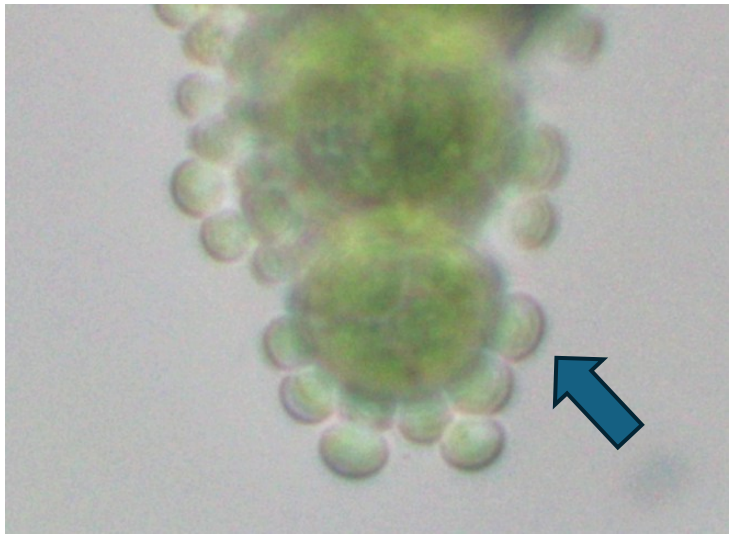
**Diverse fasi del processo plasmogamico (progressivamente più fuse)**

# Zoospore diversity and sexual reproduction in *Trebouxia*: from neglected evidence to new facts

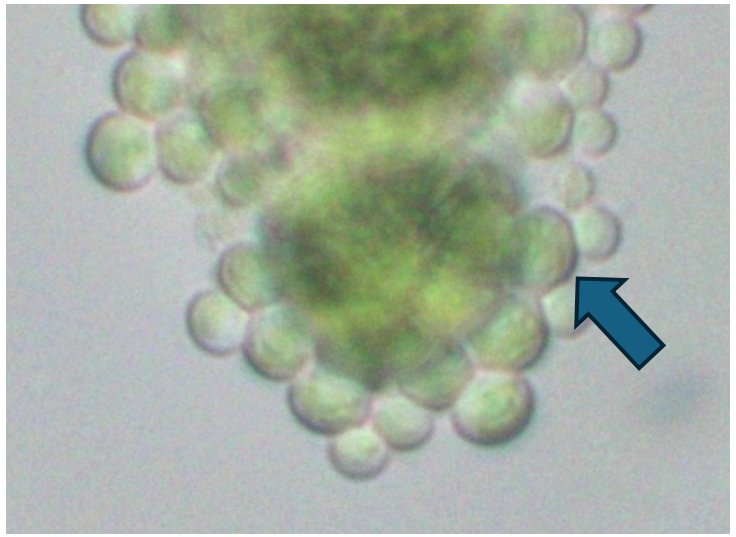
## Capitolo 1 – Risultati



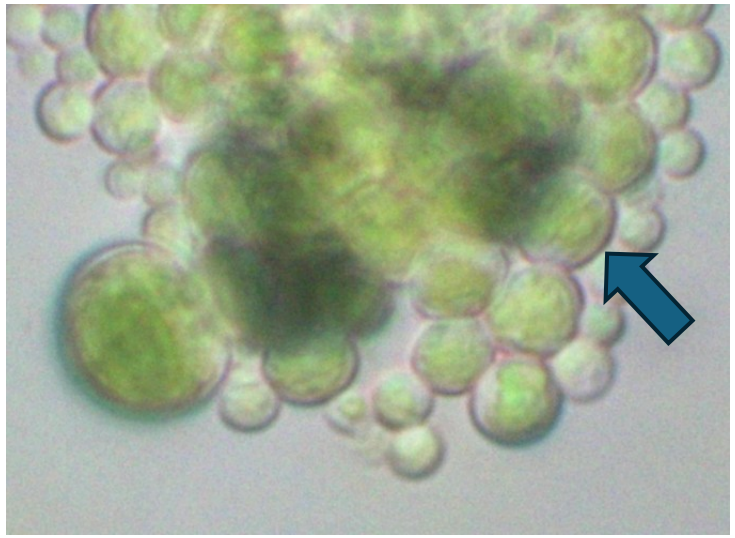

Giorno 0



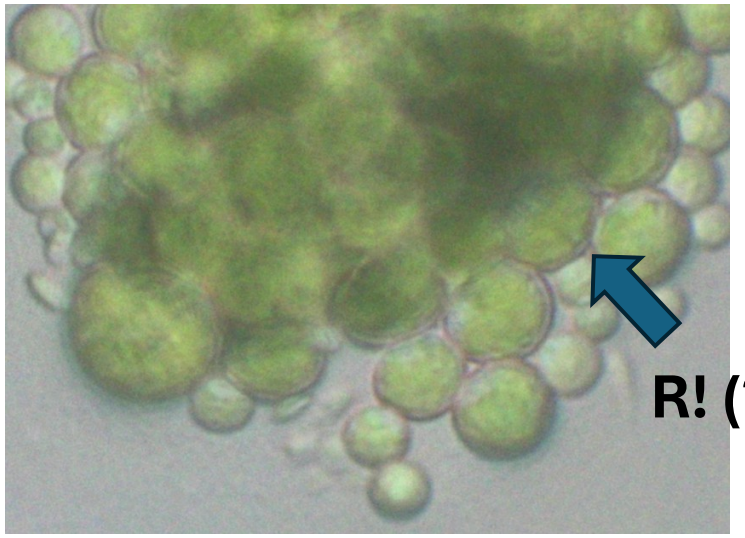
Giorno 1



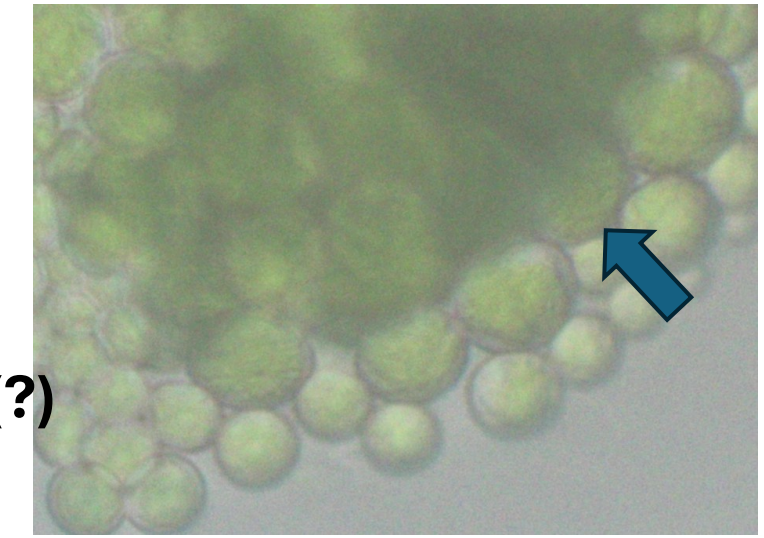
Giorno 3



Giorno 6



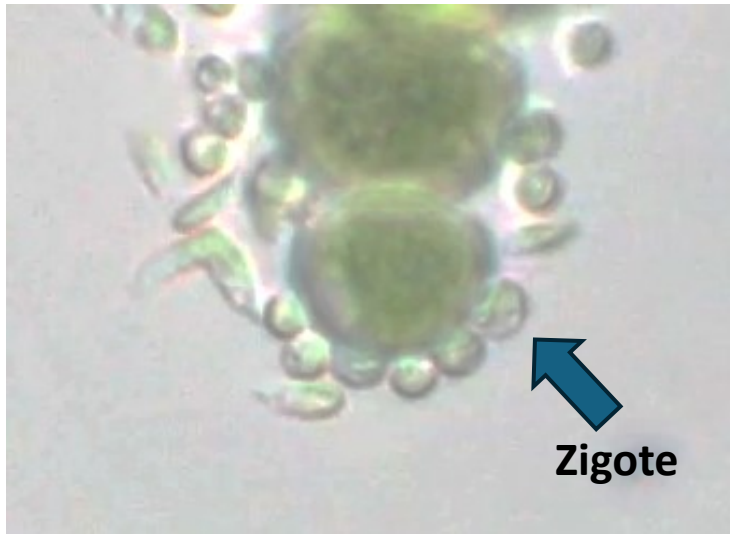

Giorno 10



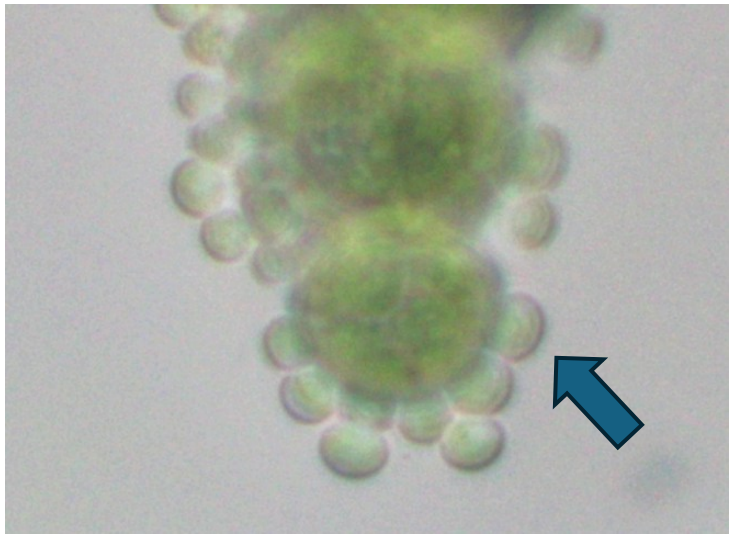
Giorno 14

# Zoospore diversity and sexual reproduction in *Trebouxia*: from neglected evidence to new facts

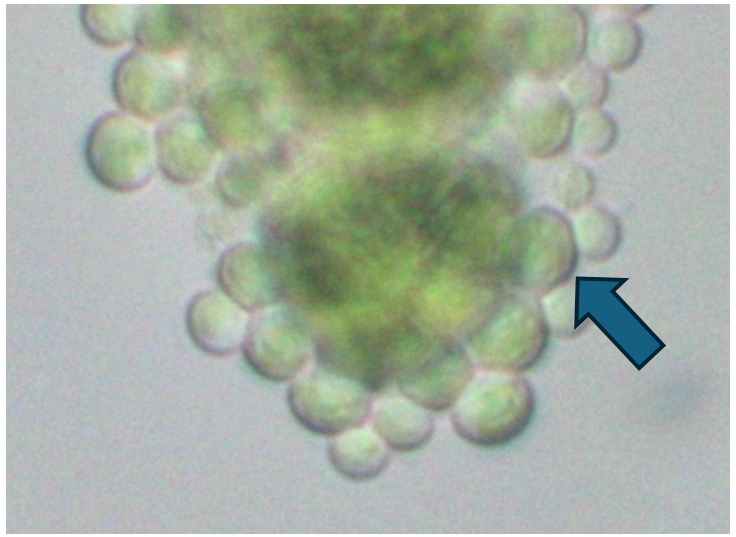
## Capitolo 1 – Risultati



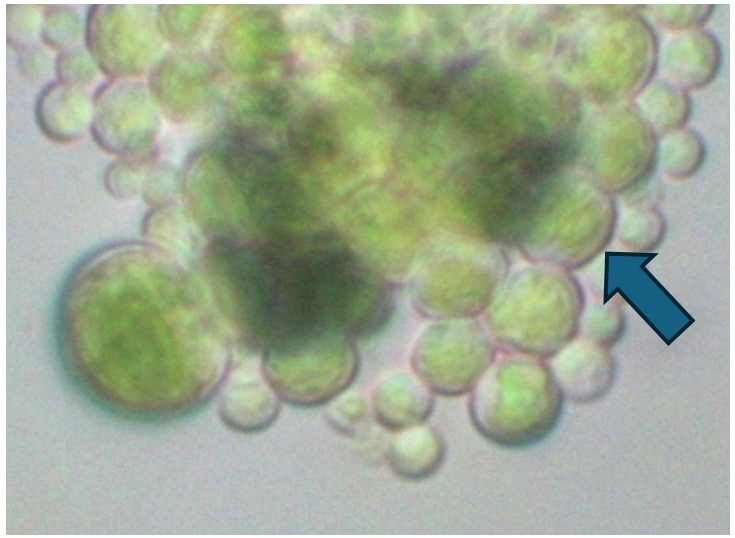

Giorno 0



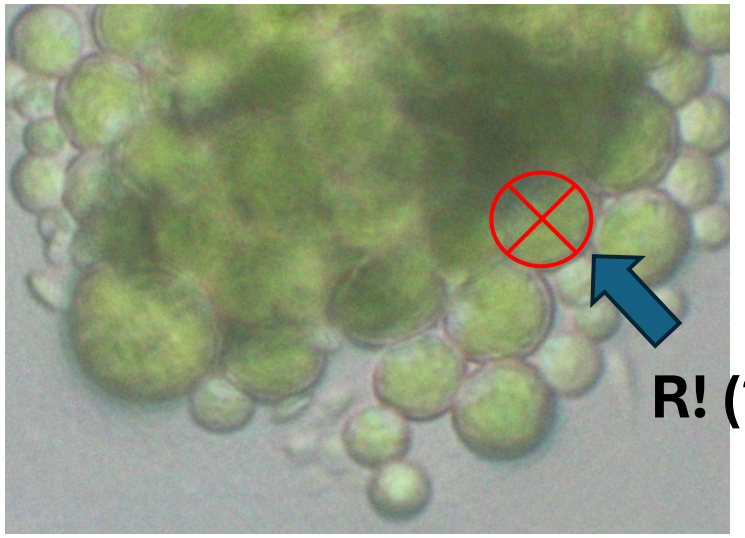
Giorno 1



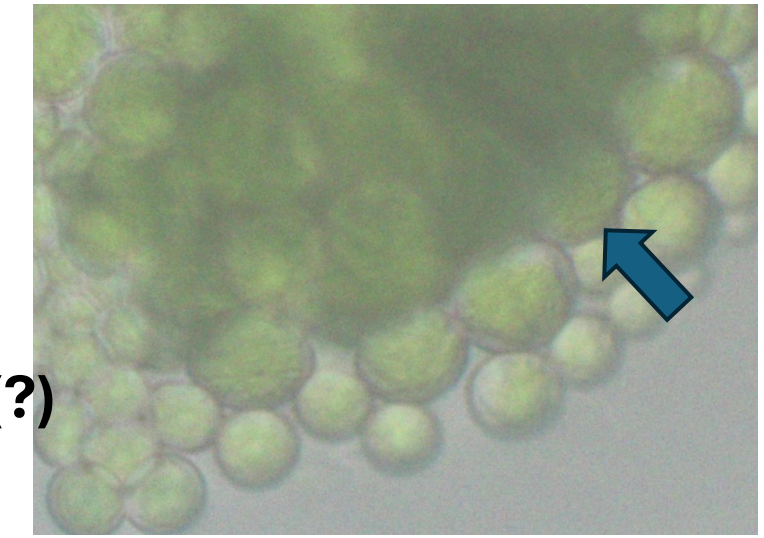
Giorno 3



Giorno 6




Giorno 10



Giorno 14

## Capitolo 1 – Risultati

# Indagine genetica… ricerca dei geni essenziali per la **meiosi**

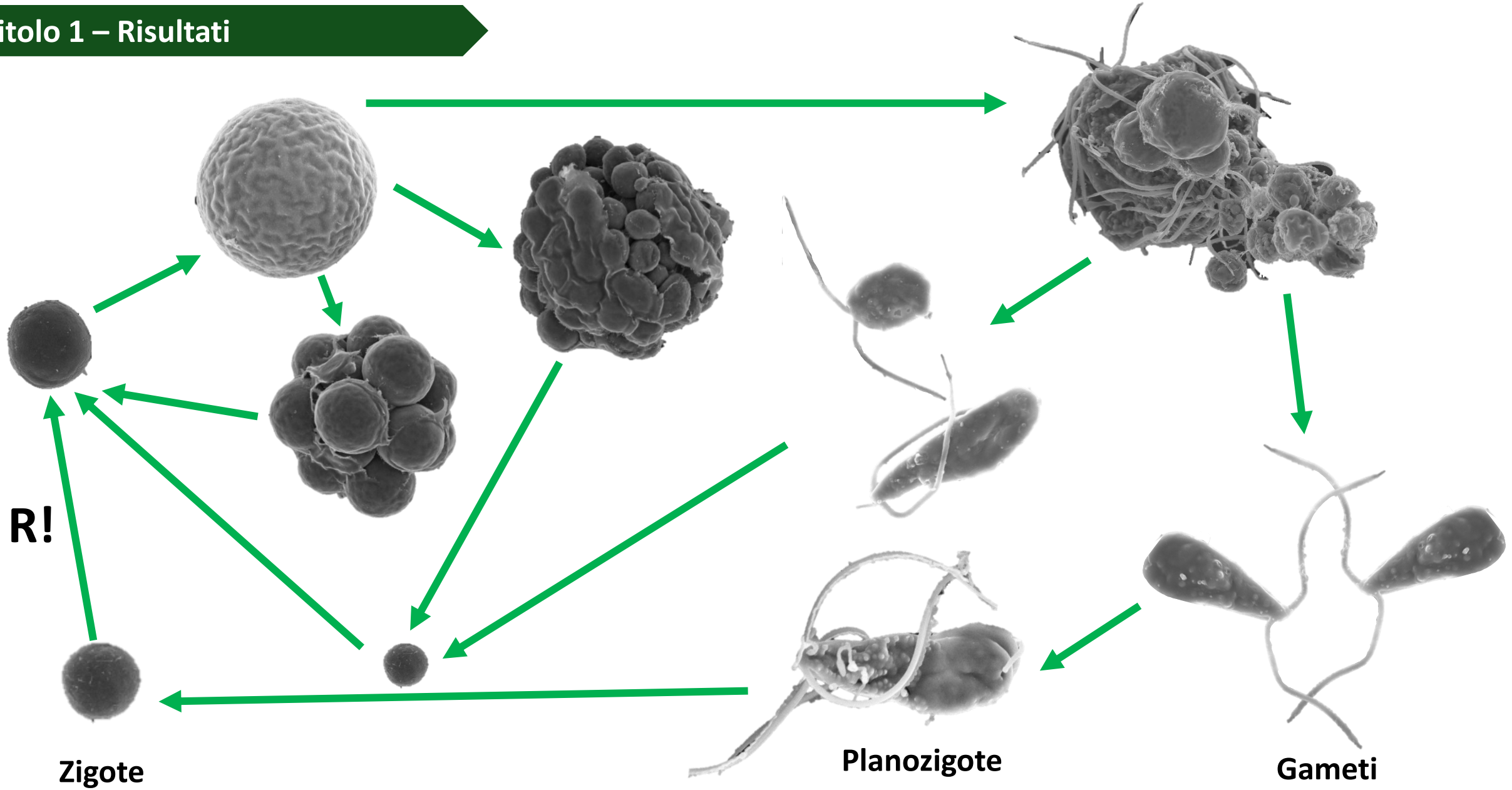
| Genes | Trebouxiophyceae genomes | *T. gelatinosa* transcriptome |
|---|---|---|
| **DMC1** | + | - |
| **RAD51** | + | + |
| **HOP1/2/3** | + | + |
| **MER3** | + | - |
| **MND1** | + | + |
| **MSH4** | + | + |
| **MSH5** | + | + |
| **REC8/RAD21** | + | + |
| **SPO11** | + | + |

Fučíková et al. (2015) *J. Phycol.* 51

Candotto Carniel et al. (2016) *Plant Mol. Biol.* 91

# Zoospore diversity and sexual reproduction in *Trebouxia*: from neglected evidence to new facts

## Capitolo 1 – Risultati



# Zoospore diversity and sexual reproduction in *Trebouxia*: from neglected evidence to new facts

## Capitolo 1 – Conclusioni

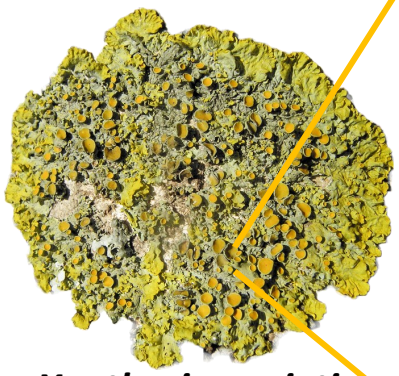
- Entrambi i morfotipi osservati (allungato e subsferico) possono svilupparsi in una cellula vegetativa, risultando in ciò che si definisce ‘zoospora’;

- Le cellule mobili allungate possono subire un'isogamia, formando uno zigote che poi si divide (potenzialmente con un evento meiotico). Se così fosse, il ciclo biologico avrebbe tutte le caratteristiche di un ciclo aplonte (comune nelle alghe verdi);

- Le cellule allungate sono sia zoospore che gameti oppure sono gameti che possono avere uno sviluppo vegetativo?

# Do zoospores play a role in lichen symbiosis? Zoospores-ascospores interaction in the lichen *Xanthoria parietina*

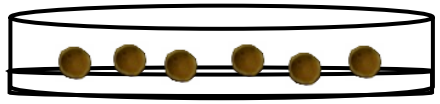
## Capitolo 2 – Materiali e metodi

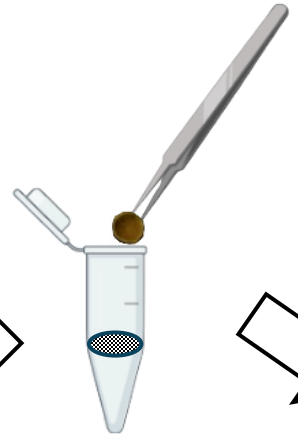


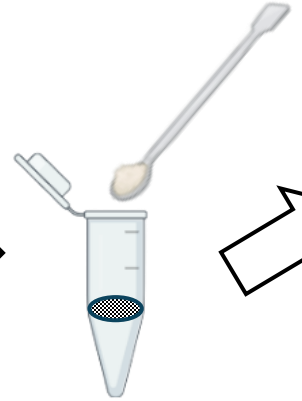
***Xanthoria parietina* (compatibile)**

***Morchella esculenta* (non compatibile)**

Reidratazione apoteci (12 h – 1 d)







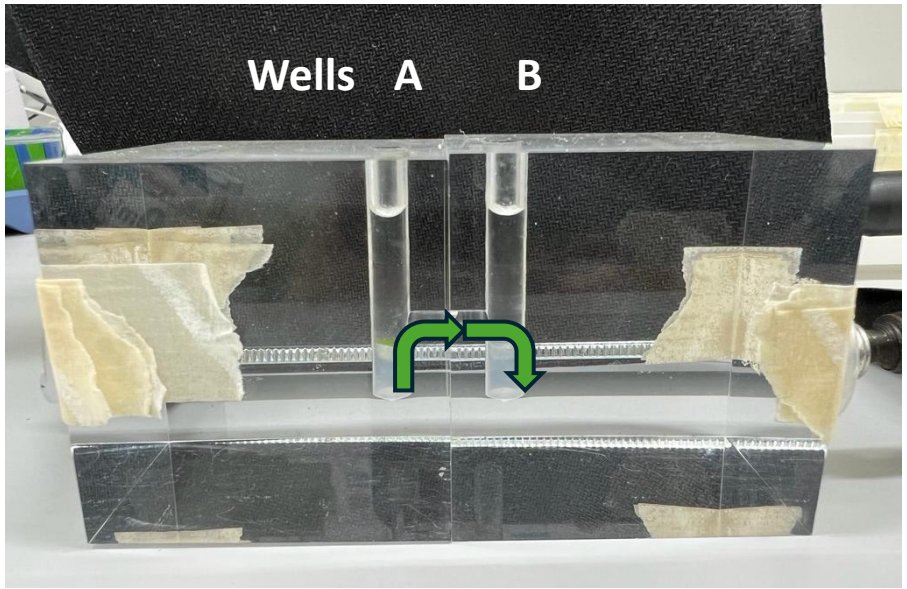
3000 rpm
6 min


**Le spore sono state messe a germinare in terreno liquido (BBM)**

# Do zoospores play a role in lichen symbiosis? Zoospores-ascospores interaction in the lichen *Xanthoria parietina*

## Capitolo 2 – Materiali e metodi

Entrambi i pozzetti sono stati riempiti con terreno minerale (BBM)

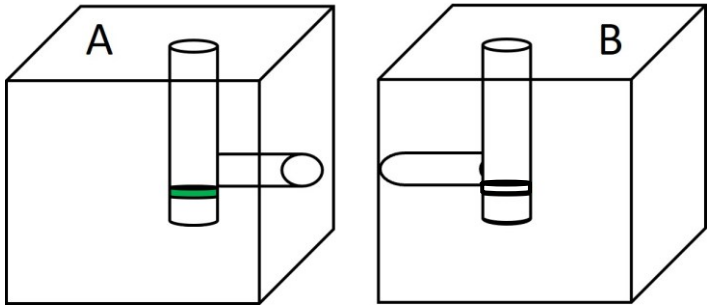


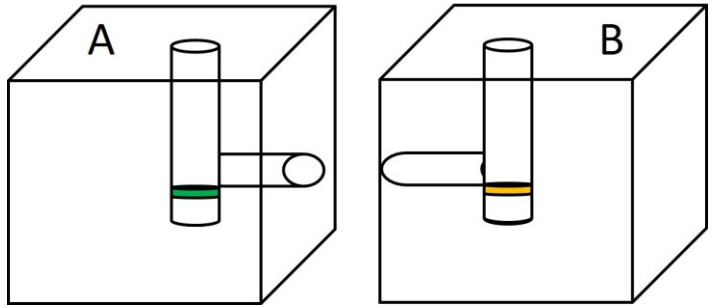

**A**

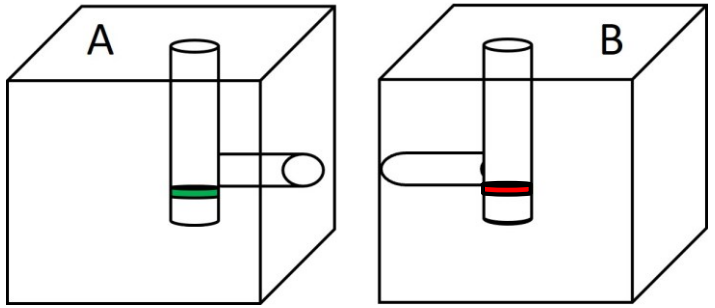
*T. decolorans* con zoospore

**B**

- Vuoto (CTRL)
- Ascospore di *X. parietina*
- Ascospore di *M. esculenta*
- TM
- TM - G
- TM - P



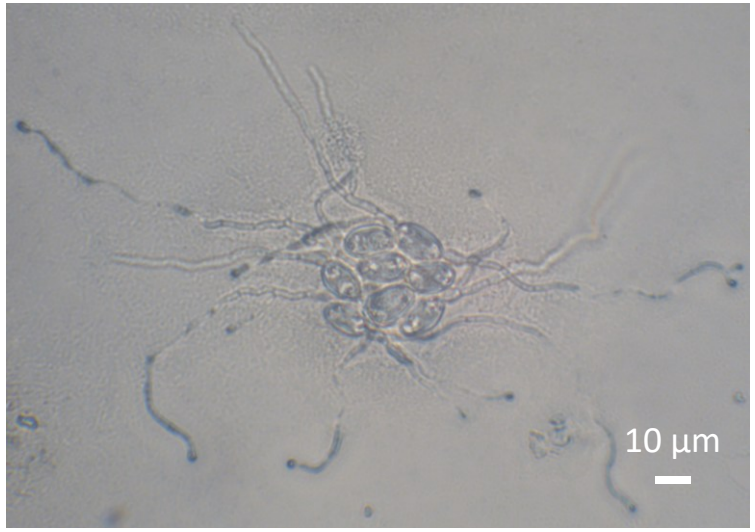
# Do zoospores play a role in lichen symbiosis? Zoospores-ascospores interaction in the lichen *Xanthoria parietina*

## Capitolo 2 – Risultati

Le ascospore sono germinate dopo 1 giorno su BBM a 20 °C.


**Giorno 1**


**Giorno 3**



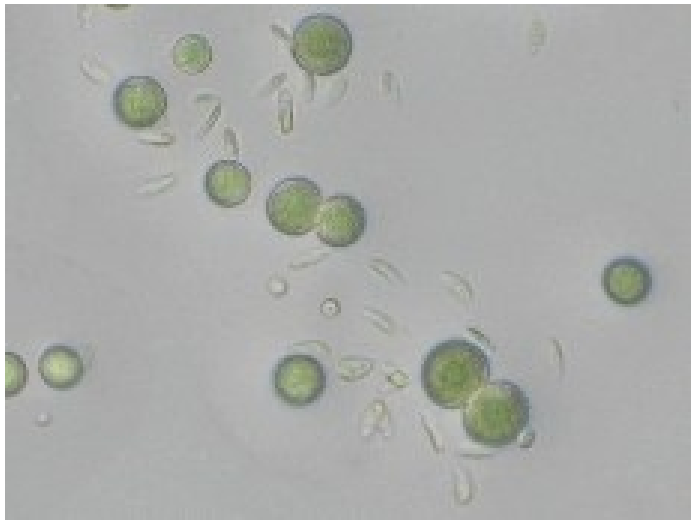

**Giorno 6**

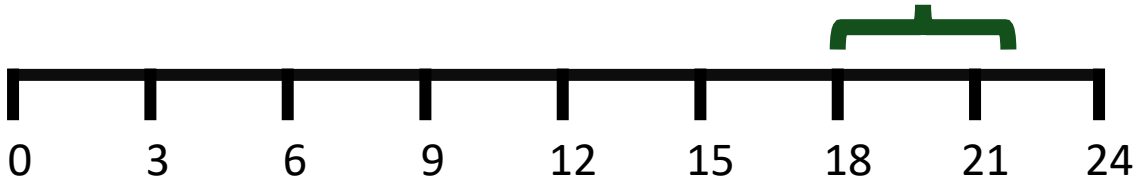
# Do zoospores play a role in lichen symbiosis? Zoospores-ascospores interaction in the lichen *Xanthoria parietina*

## Capitolo 2 – Risultati

Le colture di *T. decolorans* sono state raccolte al rilascio delle zoospore

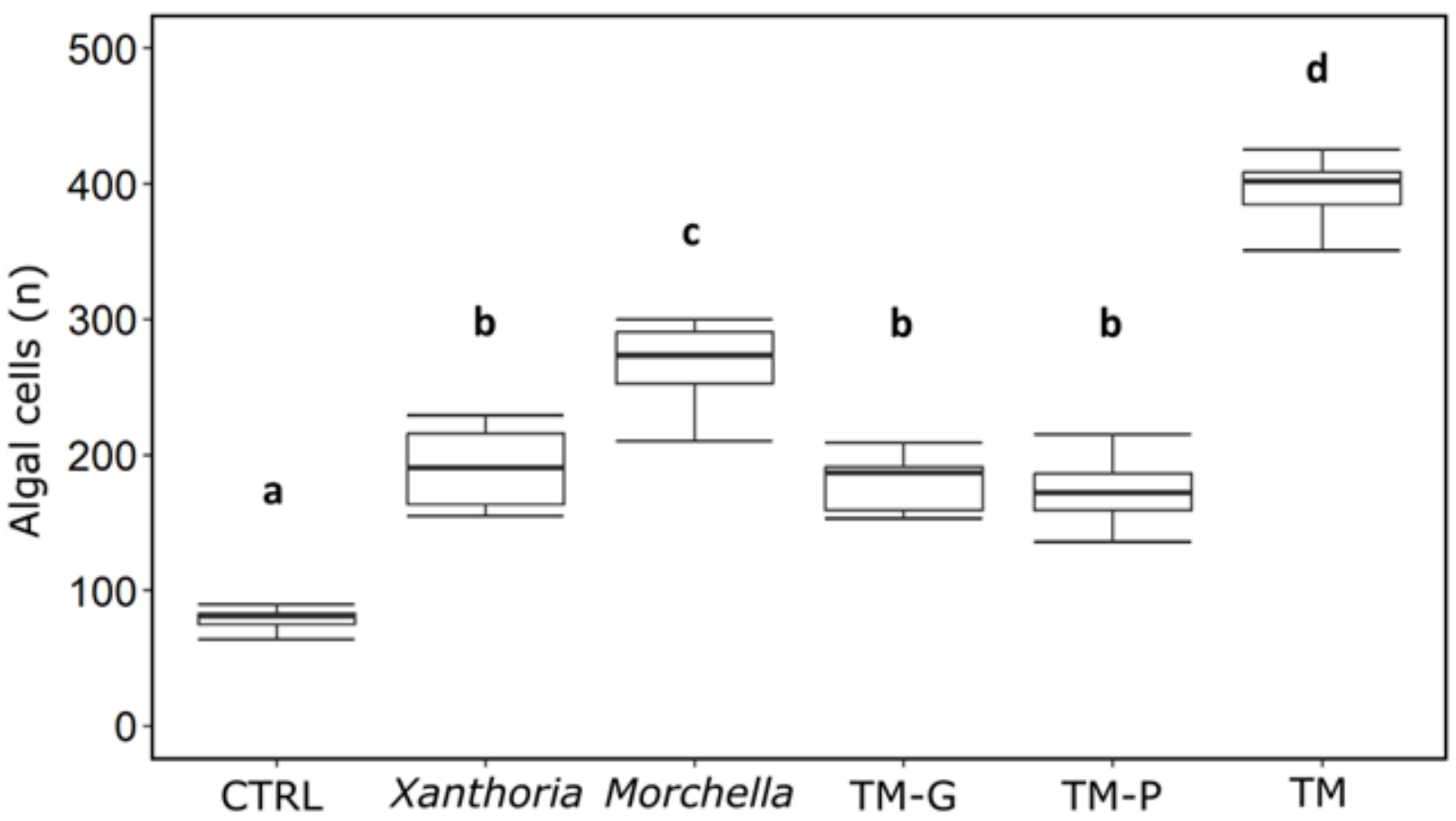
*T. decolorans*

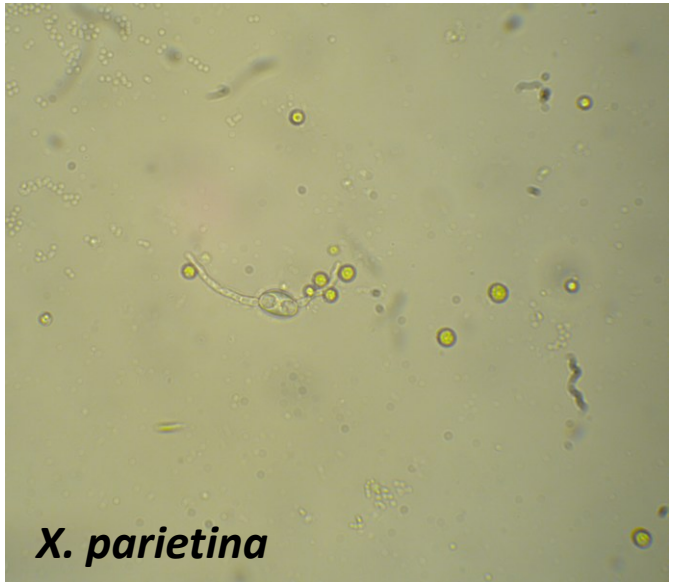


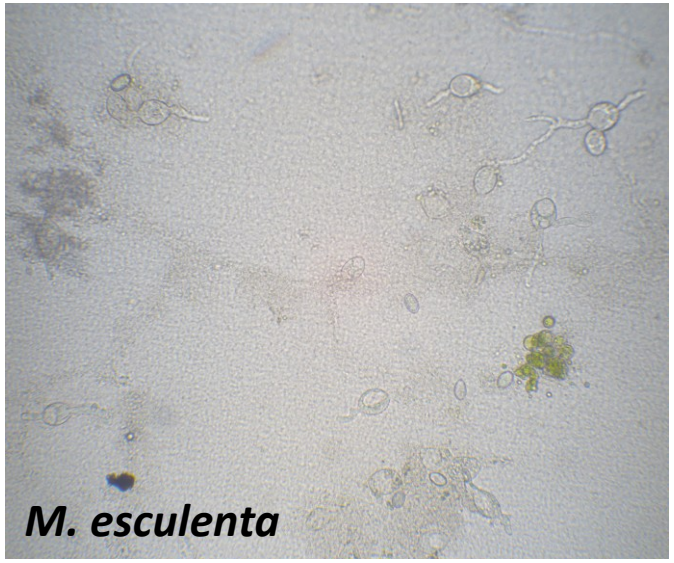


# Do zoospores play a role in lichen symbiosis? Zoospores-ascospores interaction in the lichen *Xanthoria parietina*

## Capitolo 2 – Risultati



# Do zoospores play a role in lichen symbiosis? Zoospores-ascospores interaction in the lichen *Xanthoria parietina*

## Capitolo 2 – Conclusioni

- Le zoospore hanno mostrato una preferenza per i terreni ricchi di nutrienti organici (ad es. TM) rispetto a quelli minerali (CTRL), suggerendo di seguire un gradiente di composti organici: glucosio e/o peptone.

- Le zoospore sono state attratte dalle ascospore, indipendentemente dalla specie fungina. Potrebbero essere attratte dai composti organici rilasciati dalle ascospore in germinazione (glucosio da trealosio?).

- Quando nell'ambiente naturale è presente un velo d'acqua, le zoospore possono essere attratte dalle ascospore del partner compatibile, facilitando l'instaurarsi di una nuova simbiosi.

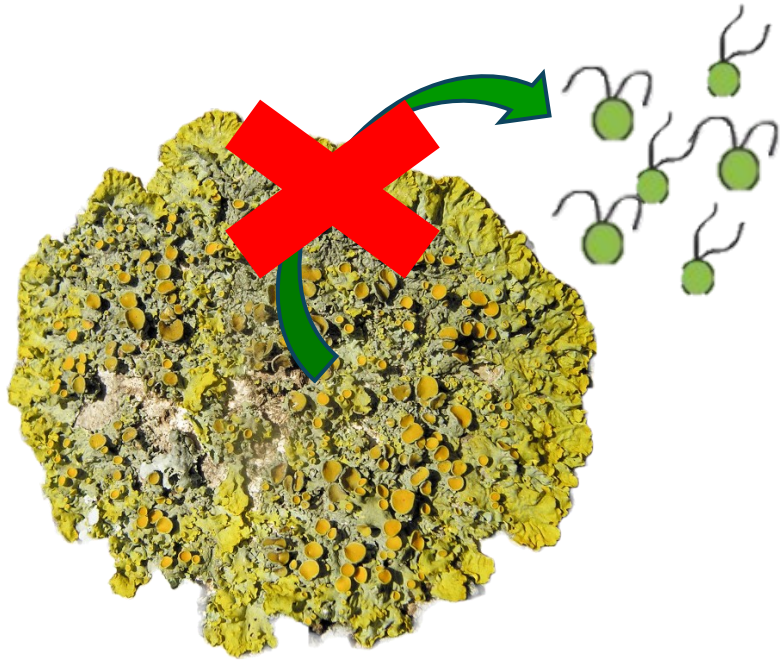
# Zoosporogenesis in *Trebouxia*: the role of culture conditions

## Capitolo 3 – Background

La formazione di zoospore è solitamente soppressa nel tallo (Slocum et al. 1980).

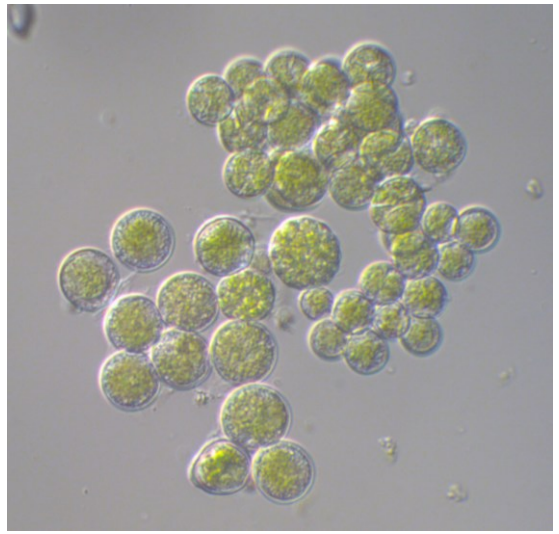
Perché e come i licheni influenzano il ciclo vitale di *Trebouxia*?

Quali stimoli ambientali innescano la zoosporogenesi?



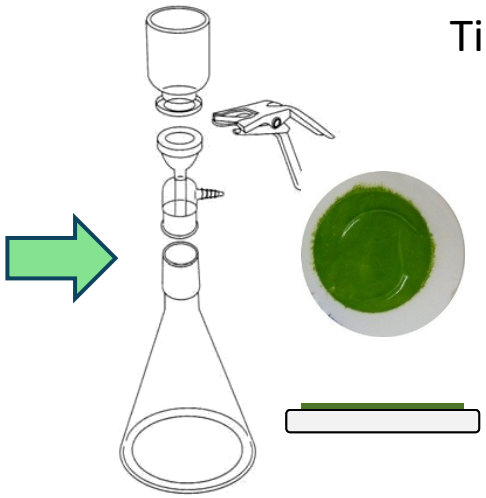
# Zoosporogenesis in *Trebouxia*: the role of culture conditions

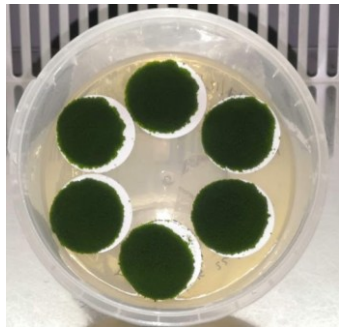
## Capitolo 3 – Materiali e metodi



*T. decolorans*

Fotoperiodo = 12/12 h luce/buio
Intensità luminosa= 22.5 ± 0.8 µmol $s^{-1}$ $m^{-2}$
Tipo di luce= Luce bianca calda (600 nm)





- Disponibilità di nutrienti
- Potenziale osmotico ($\Psi_s$)
- Temperature

CTRL: 16 °C; TM

BBM: 16 °C; BBM

2TM: 16 °C; 2TM

PEG75: 16 °C; BBM + PEG [75 g/kg]

PEG137.5: 16 °C; BBM + PEG [137.5 g/kg]

T12: 12 °C; TM

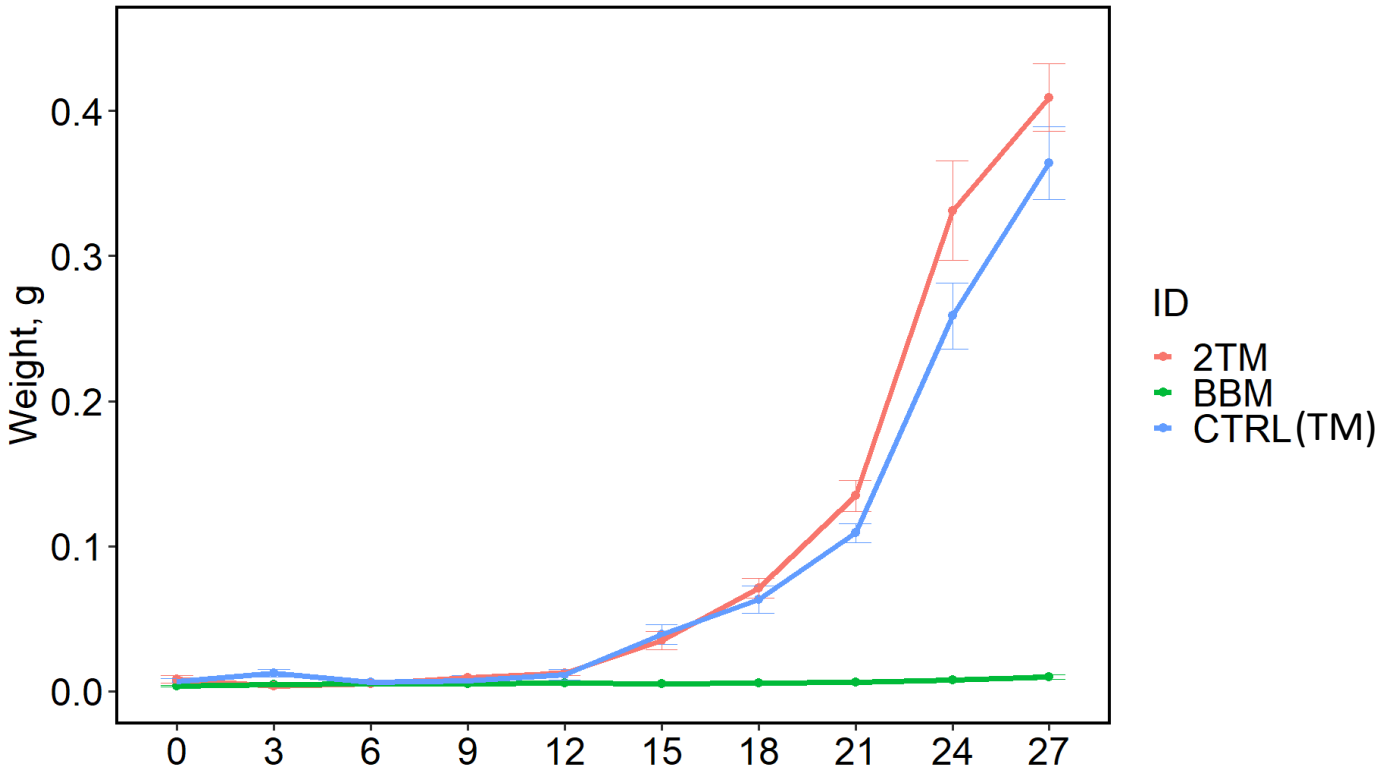
CTRL: 16 °C; TM

T20: 20 °C; TM

# Zoosporogenesis in *Trebouxia*: the role of culture conditions

## Capitolo 3 – Risultati

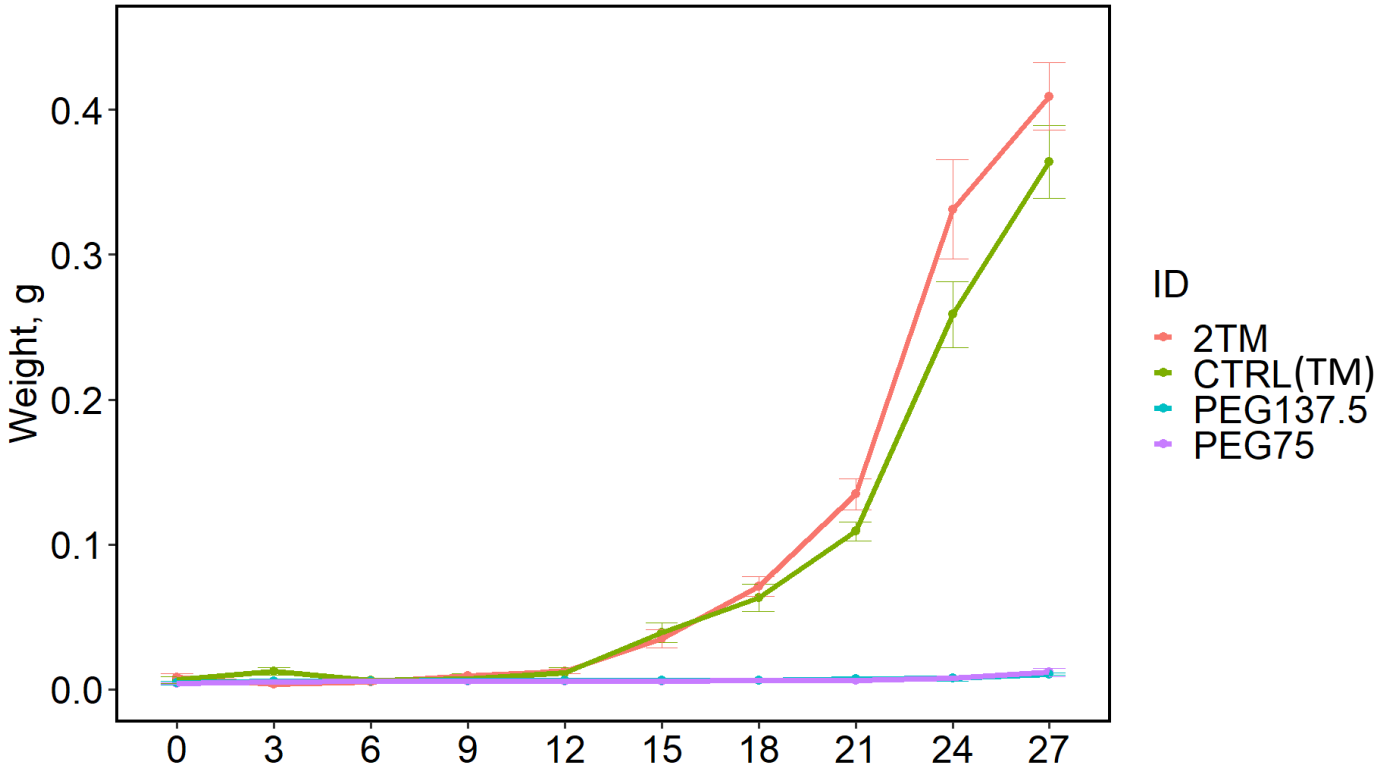
*T. decolorans*



# Zoosporogenesis in *Trebouxia*: the role of culture conditions

## Capitolo 3 – Risultati

### *T. decolorans*



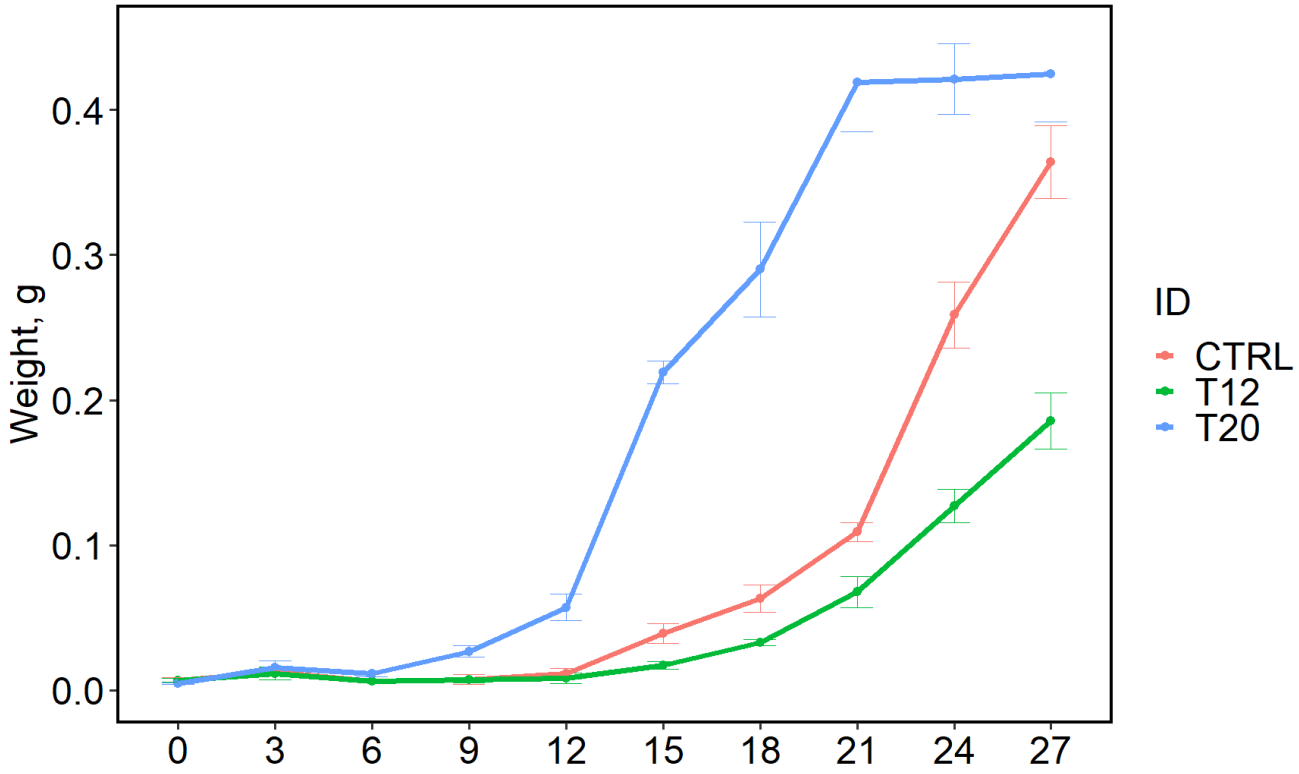

$\Psi_s$ CTRL: -0.59 MPa
$\Psi_s$ PEG75: -0.56 MPa

$\Psi_s$ 2TM: -1.04 MPa
$\Psi_s$ PEG137.5: -0.98 MPa

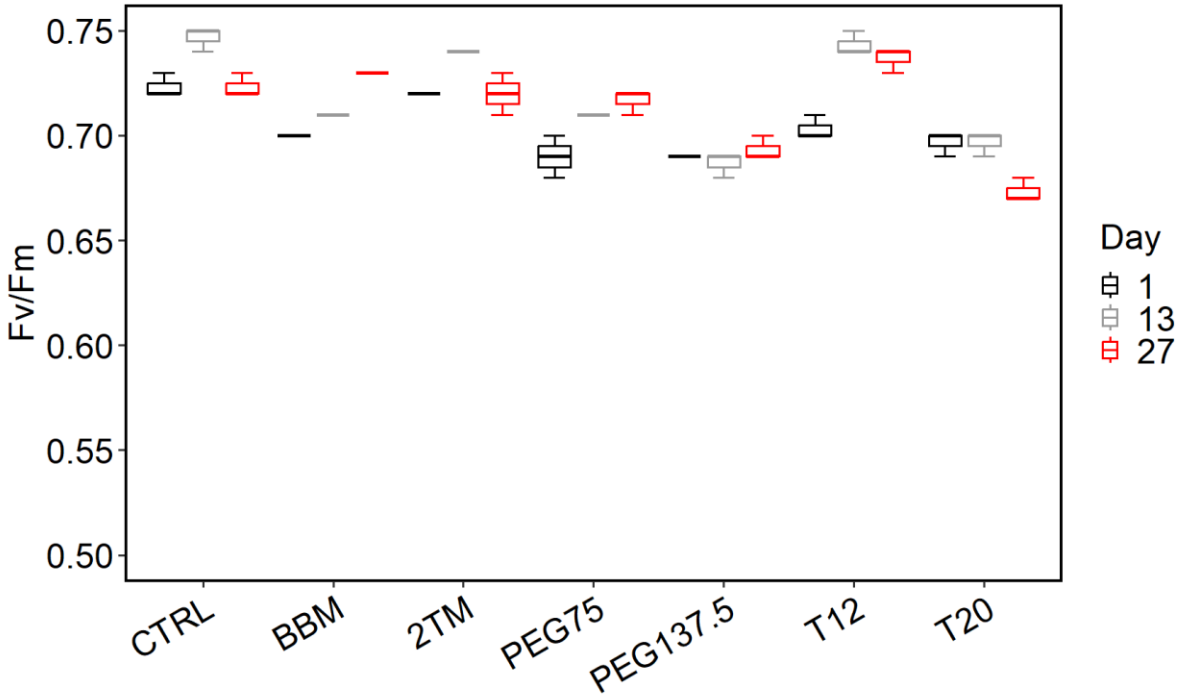
# Zoosporogenesis in *Trebouxia*: the role of culture conditions

## Capitolo 3 – Risultati

### *T. decolorans*

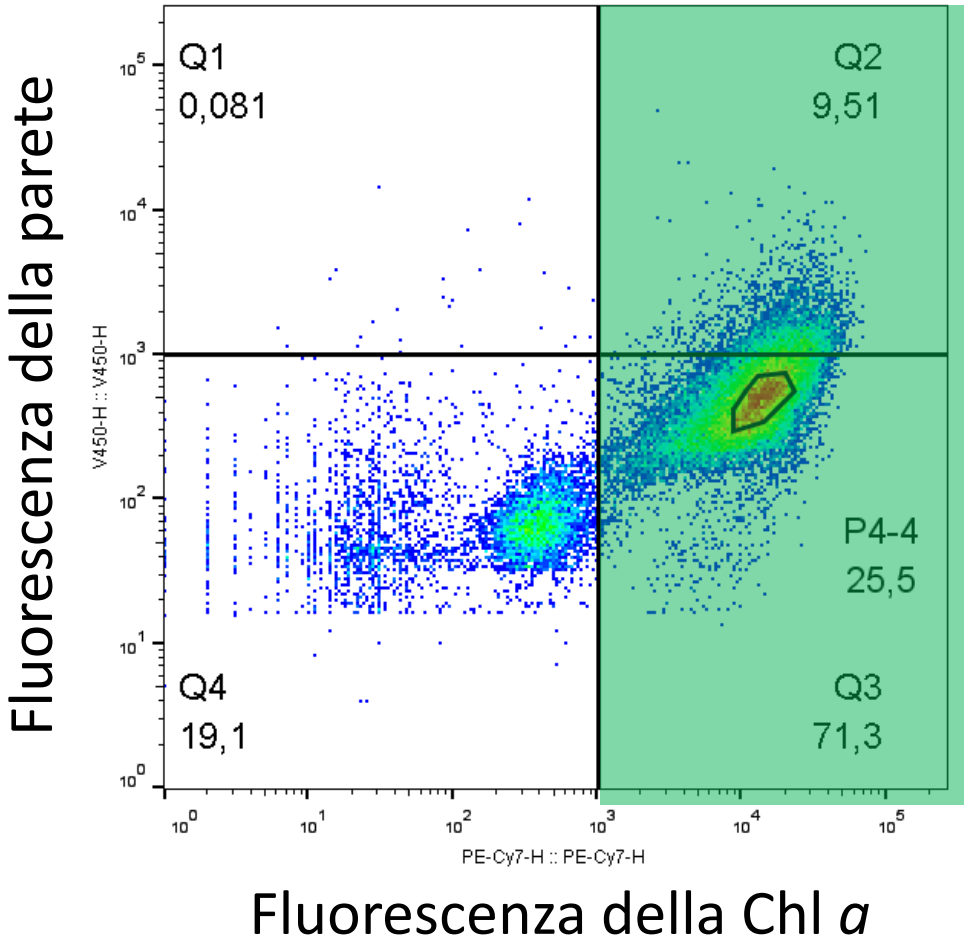


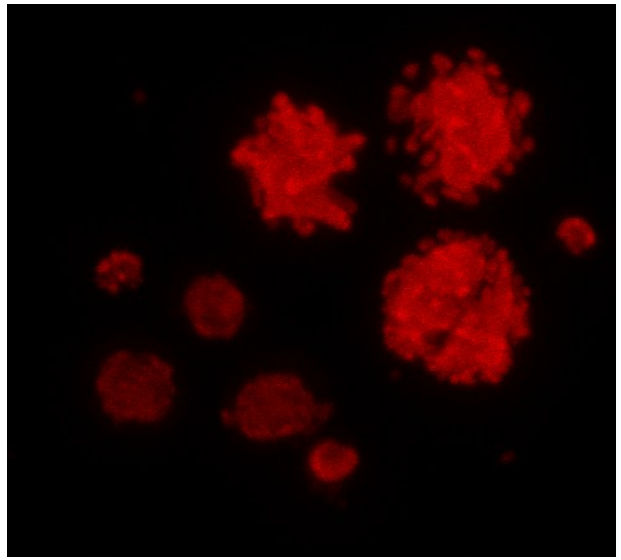

T12 : 12 °C
CTRL : 16 °C
T20: 20 °C



# Quantificazione delle zoospore

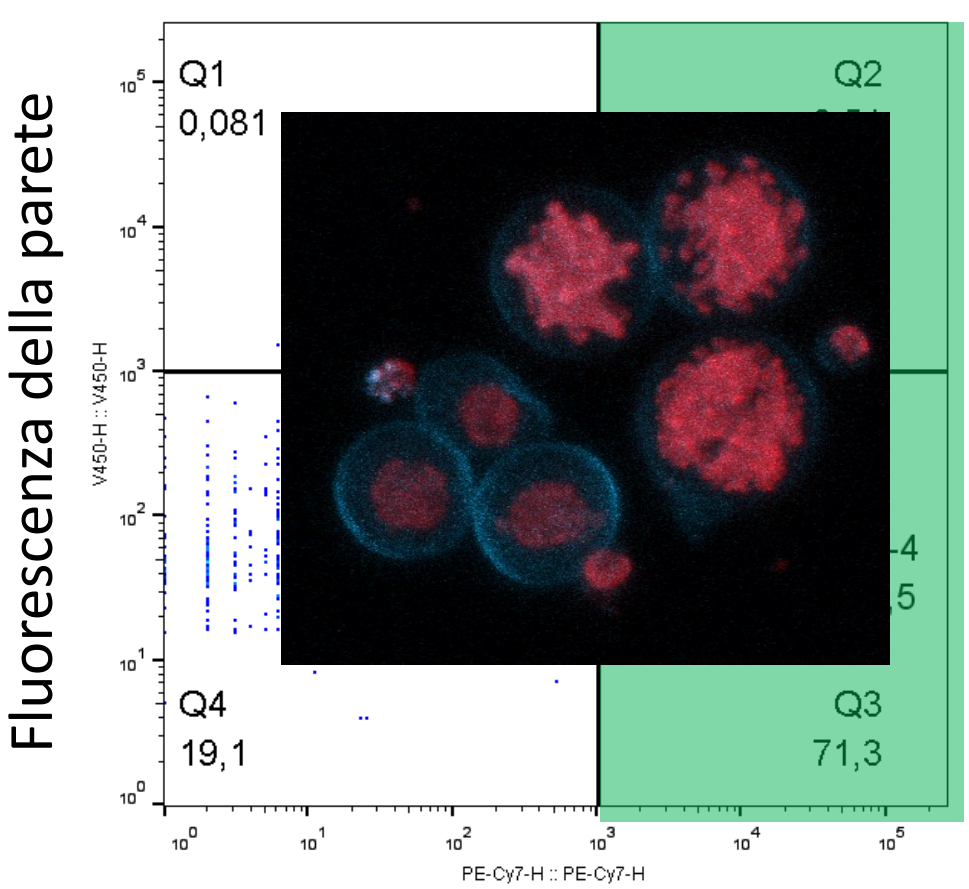
Cellule non marcate (CFW)



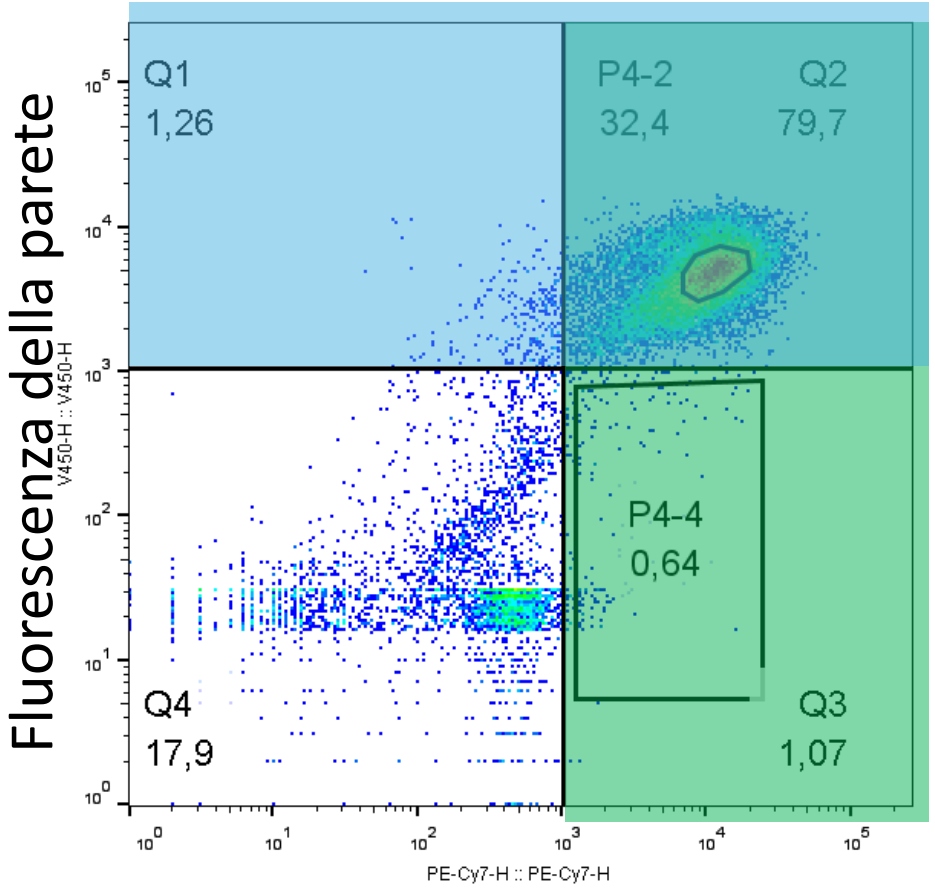



# Quantificazione delle zoospore

Cellule non marcate (CFW)

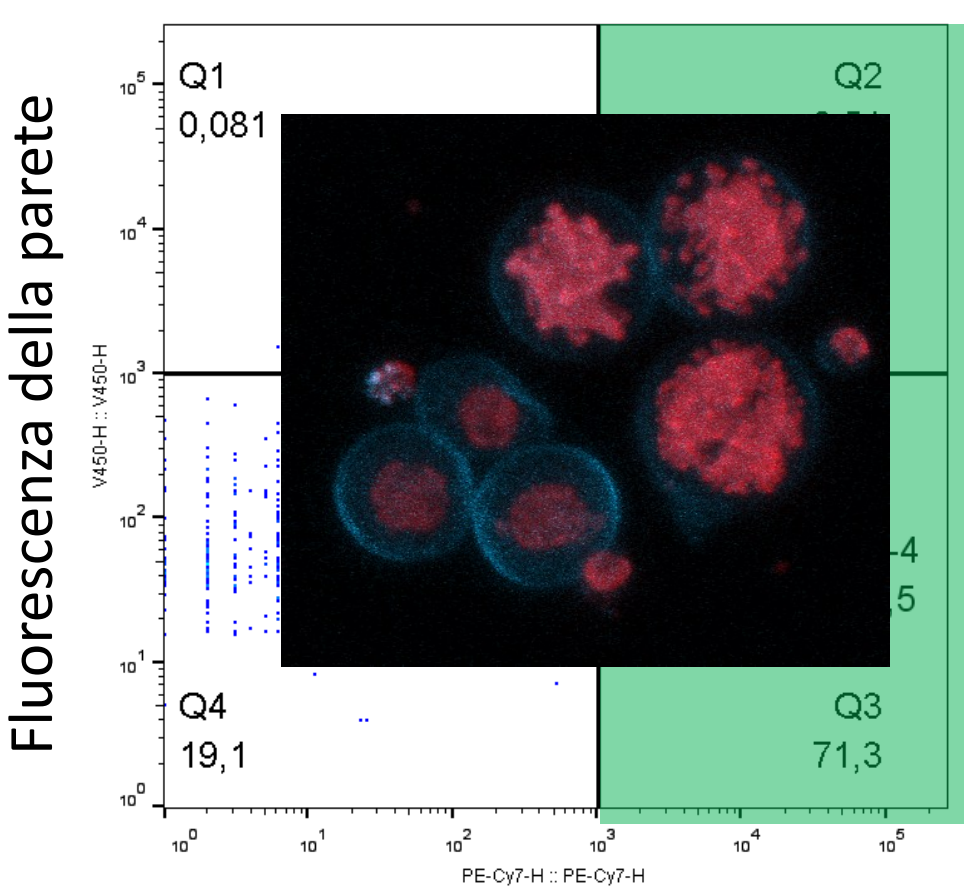



Cellule marcate (CFW)



# Quantificazione delle zoospore

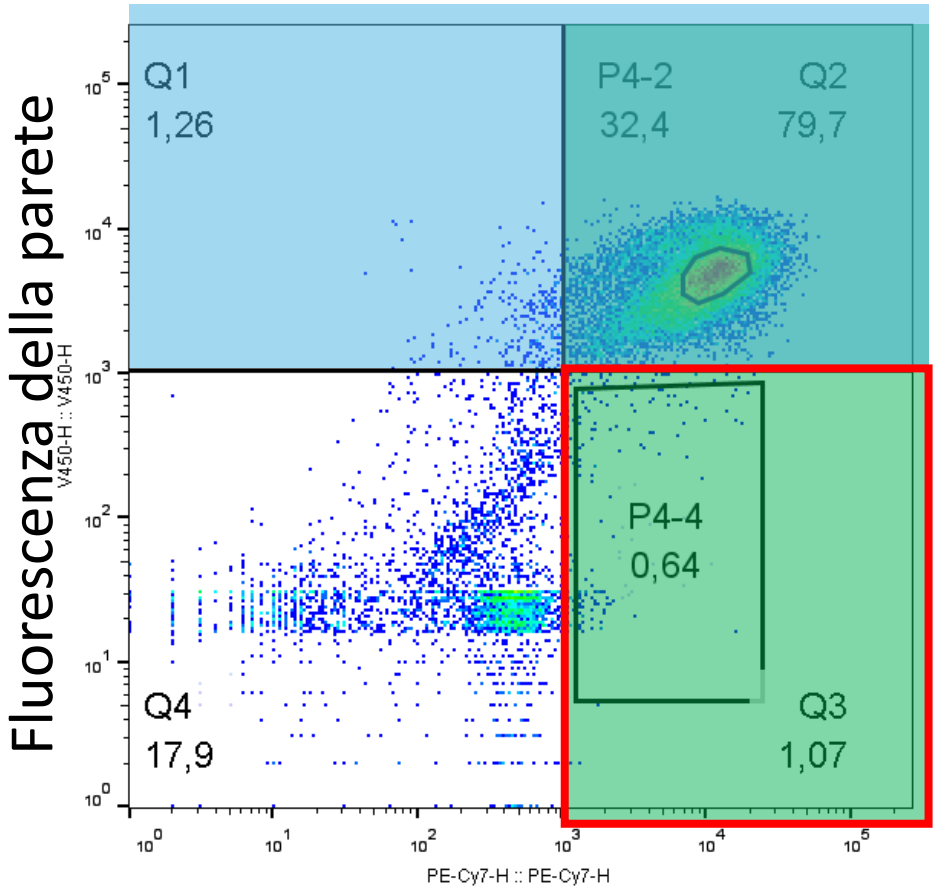
Cellule non marcate (CFW)




Fluorescenza della parete

Fluorescenza della Chl *a*

Cellule marcate (CFW)




Fluorescenza della parete

Fluorescenza della Chl *a*

# CONCLUSIONI GENERALI

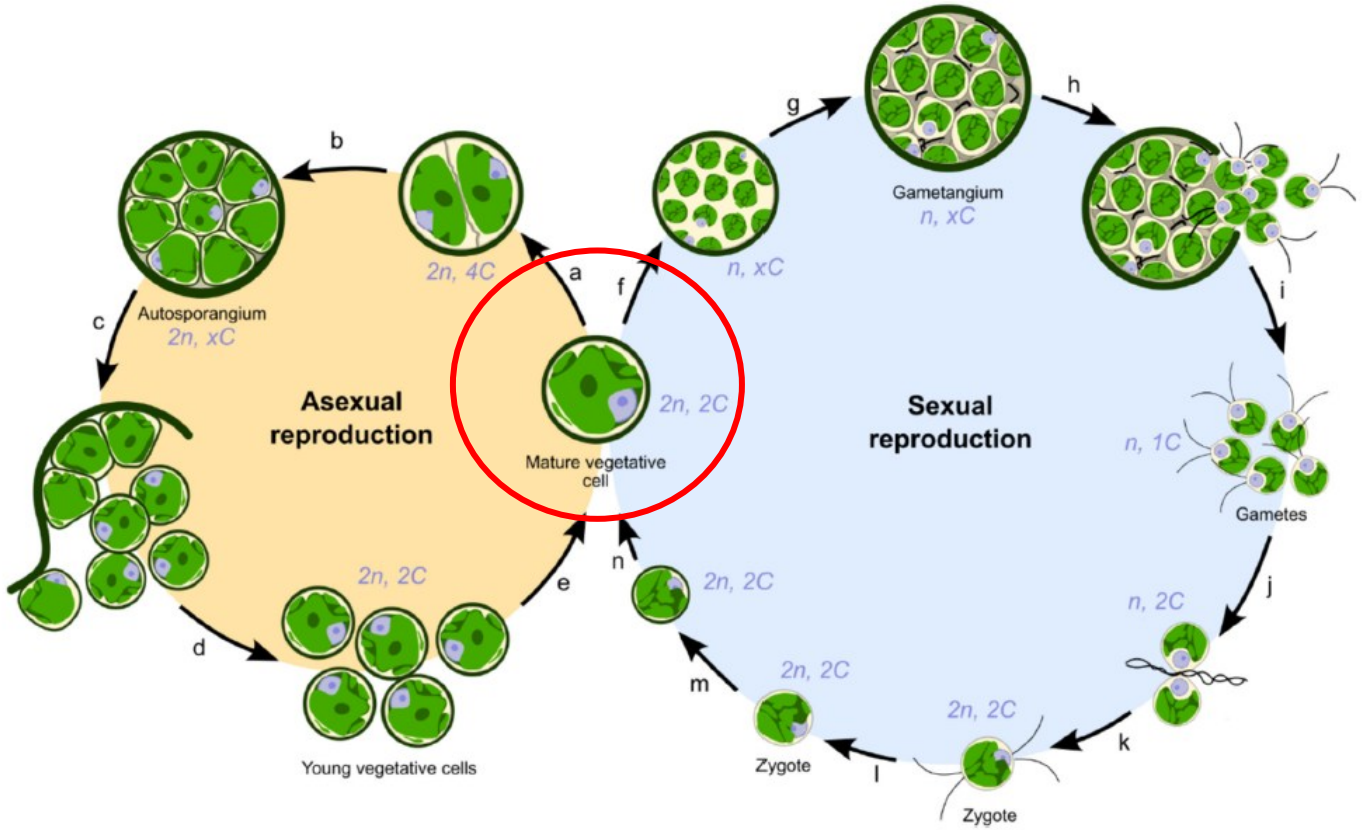
- Abbiamo fornito prove dirette della riproduzione sessuale in *Trebouxia* e proponiamo il suo ciclo vitale come un ciclo aplonte (comune nelle alghe verdi).

- Le zoospore seguono probabilmente gradienti di concentrazione dei composti organici rilasciati dalle ascospore in germinazione.

- La temperatura e la disponibilità di nutrienti hanno influenzato in modo significativo il tasso di crescita di *Trebouxia* e, di conseguenza, potrebbero influenzare anche i tempi e l'intensità della zoosporogenesi.

# E ORA?...

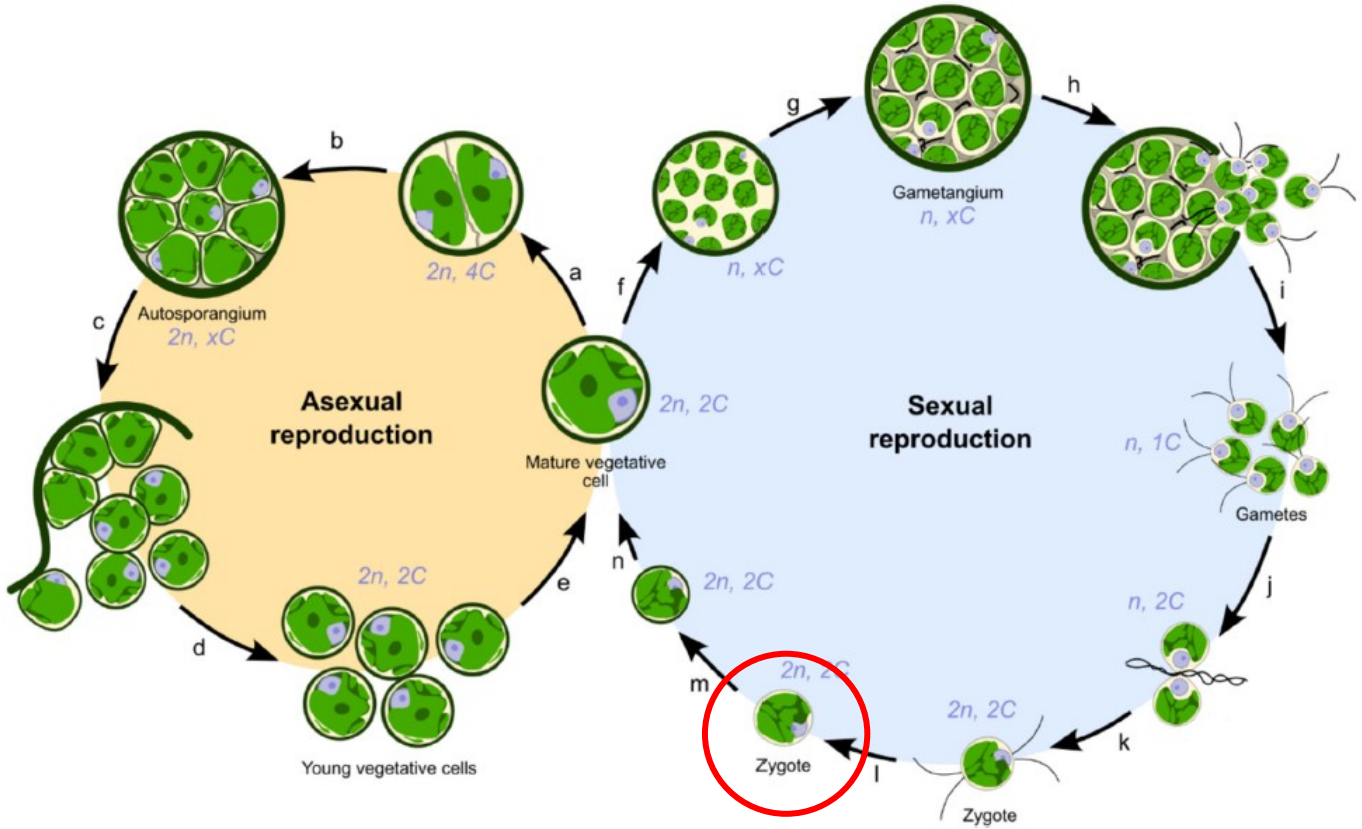
- qPCR per quantificare l'espressione dei geni della **meiosi**… Avviene prima o dopo la produzione delle cellule flagellate?




Gazquez et al. (2024) *Algal Research* 84

# E ORA?...

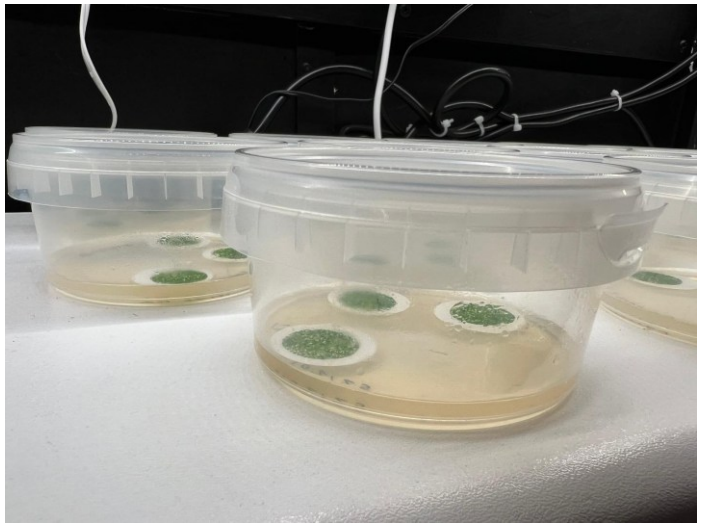
- qPCR per quantificare l'espressione dei geni della **meiosi**… Avviene prima o dopo la produzione delle cellule flagellate?

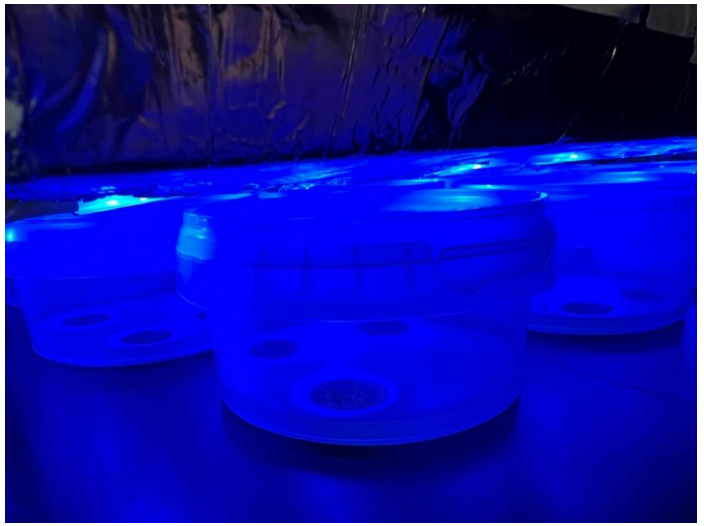


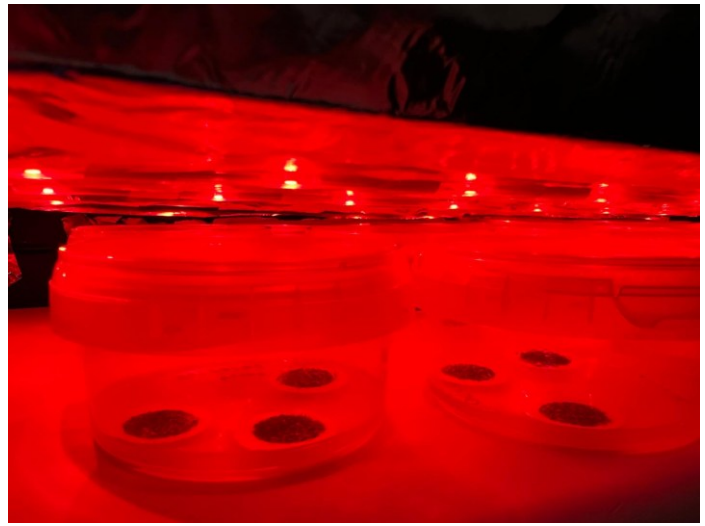

Gazquez et al. (2024) *Algal Research* 84

# E ORA?...

- Quali altri fattori possono influire sulla **produzione di cellule flagellate**?







Differenti λ della luce di crescita

# Grazie!

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