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BIOLOGY
CENTRE
CAS

Methodological challenges for studying fungus like organisms in water

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Funded by
the European Union

Who are the fungus like organisms?

Table 1: Lists of basal fungi according to two classification schemes

Tedersoo et al. 2018

Chytridiomycota 
Zoopagomycota
Olpidiomycota 
Blastocladiomycota 
Calcarisporiellomycota
Caulochytriomycota
Aphelidiomycota 
Basidiobolomycota
Glomeromycota
Rozellomycota (Class: Microsporidia) 
Neocallimastigomycota 
Entomophthoromycota
Mortierellomycota
Mucoromycota
Monoblepharomycota 
Kickxellomycota

Oomycetes

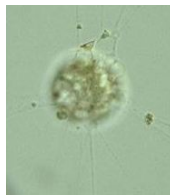
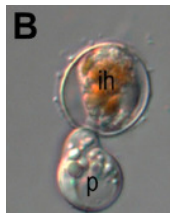
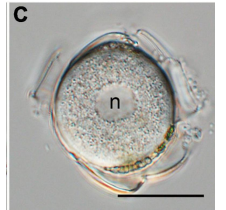
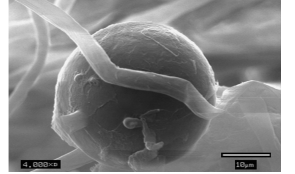
Labyrinthulomycetes

Perkinsozoa

Syndiniales

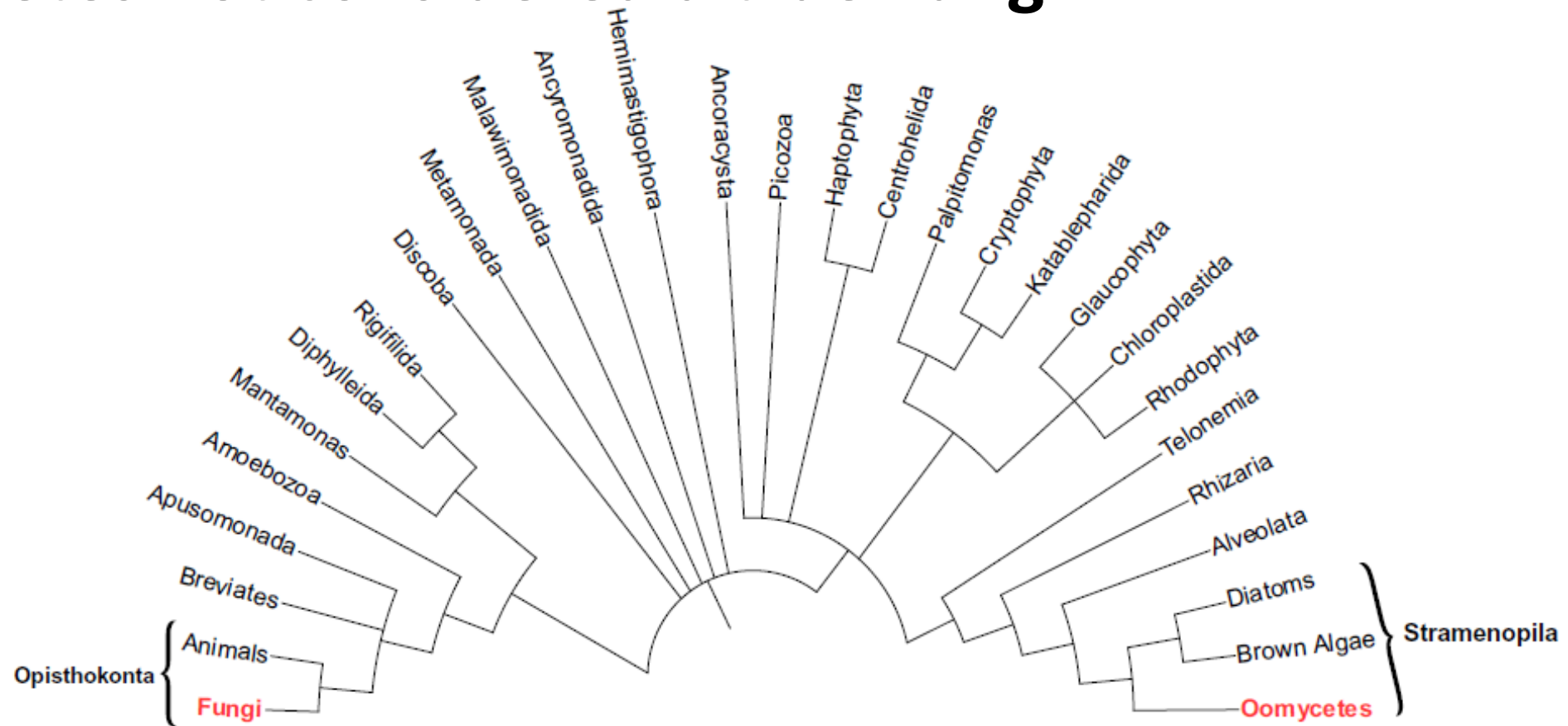
Cercozoa

Phytomyxea



Why are Oomycetes not considered a true Fungi?

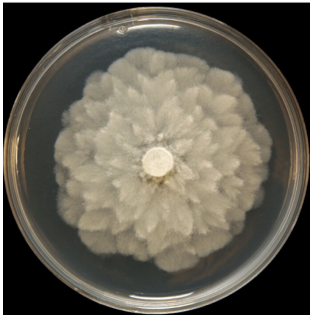
Belongs to the Kingdom Straminipila



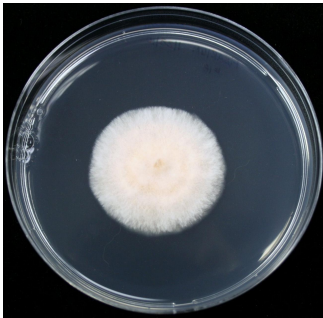
Adapted from Burki et al (2019)

Why are Oomycetes not considered a true Fungi?

Morphologically resemble fungi and often occupy similar niches



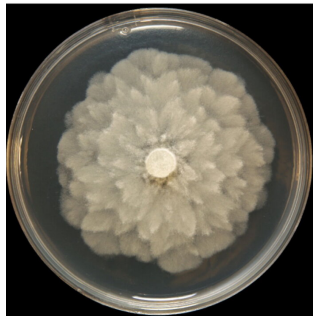
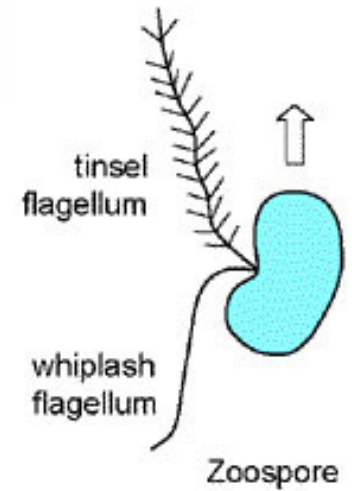
Phytophthora



Fusarium

Why are Oomycetes not considered a true Fungi?

Morphologically resemble fungi and often occupy similar niches



Phytophthora



Fusarium

Main differences are:

Septa are rare, resulting in a multinucleate condition

Nuclei of vegetative cells are typically diploid

Cell wall is composed of β -1,3, and β -1,6 glucans

Biflagellated swimming spores

Ecology of Oomycetes – host range

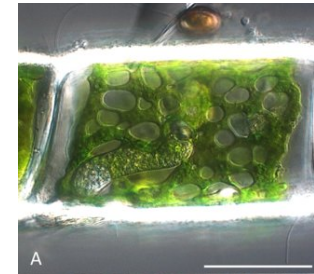
Pontisma lagenidioides – red algae



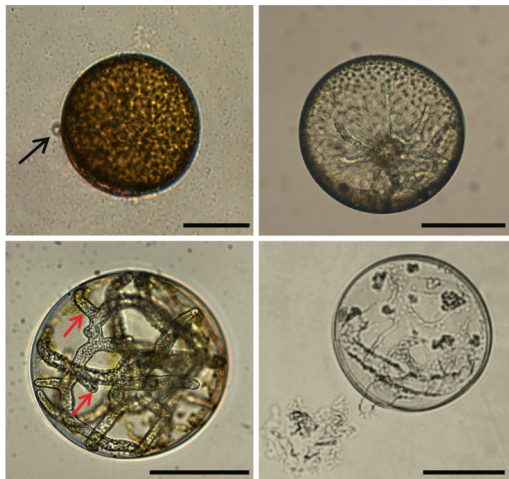
Anisolpidium sphacellarum – brown algae



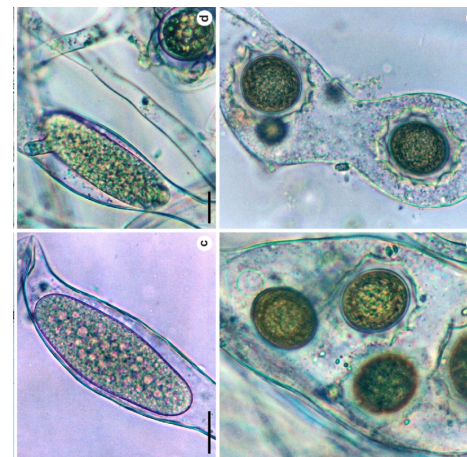
Sirolopidium bryopsidis – green algae



Lagenisma coscinodisci – diatoms



Olpidiopsis – oomycetes



Ecology of Oomycetes - distribution

They ..are likely all 'hard wired' for parasitism
(Beakes et al., 2012)

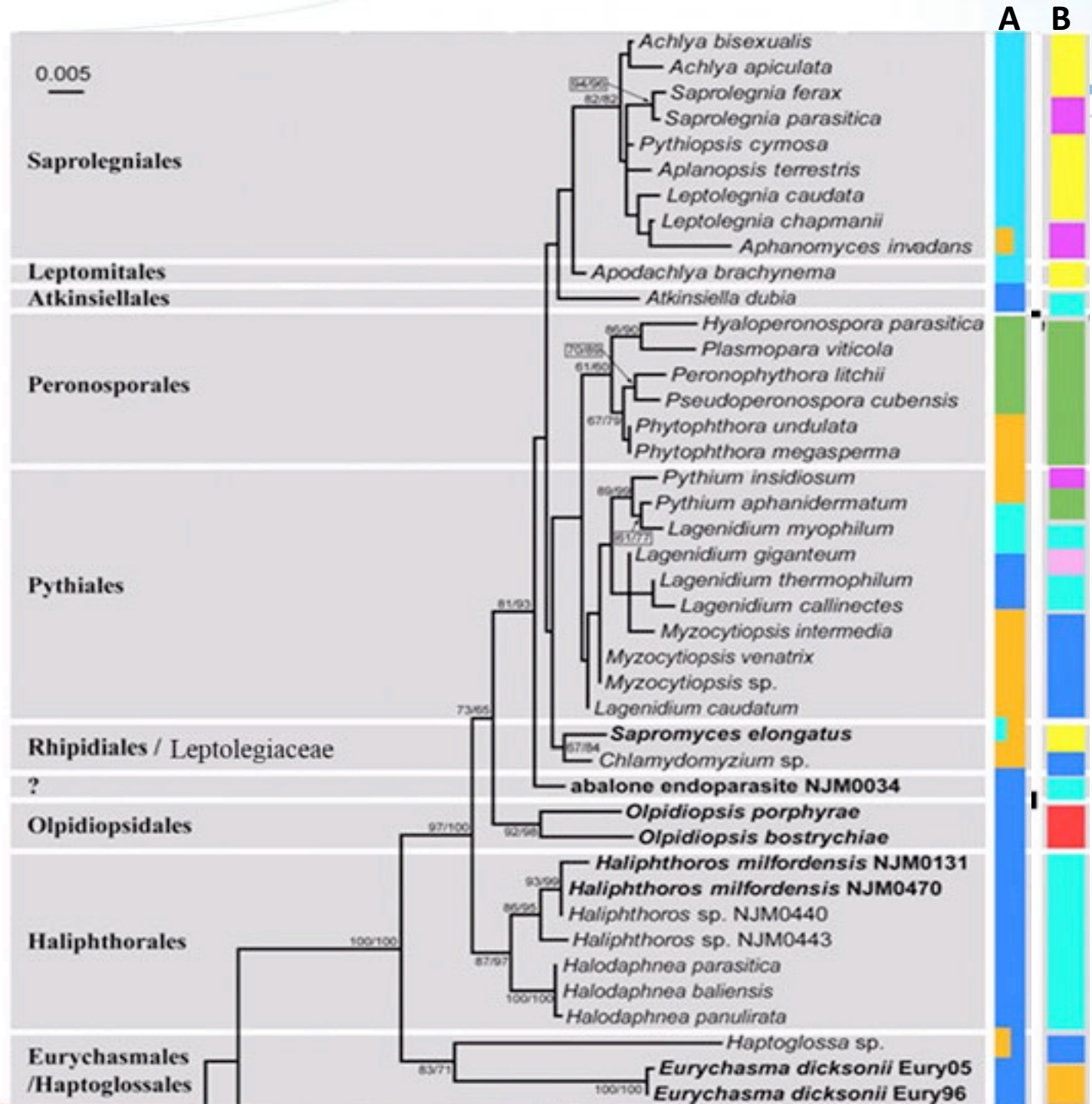
A Ecosystem

- Freshwater
- Marine
- Soil
- Terrestrial Leaves etc.

B Host or lifestyle

- Plant pathogen
- Ochrophyte pathogen
- Rhodophyte pathogen
- Insect pathogen
- Vertebrate pathogen
- Crustacean pathogen
- Nematode pathogen
- Saprotroph
- Bacteriotrophic

OOMYCETES



Challenges to study fungus like:

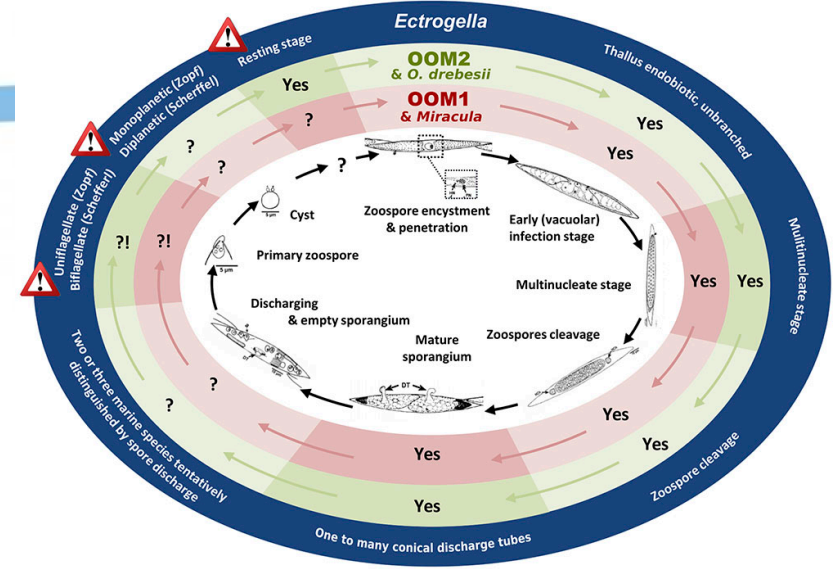
Complex life cycles

Morphological variability

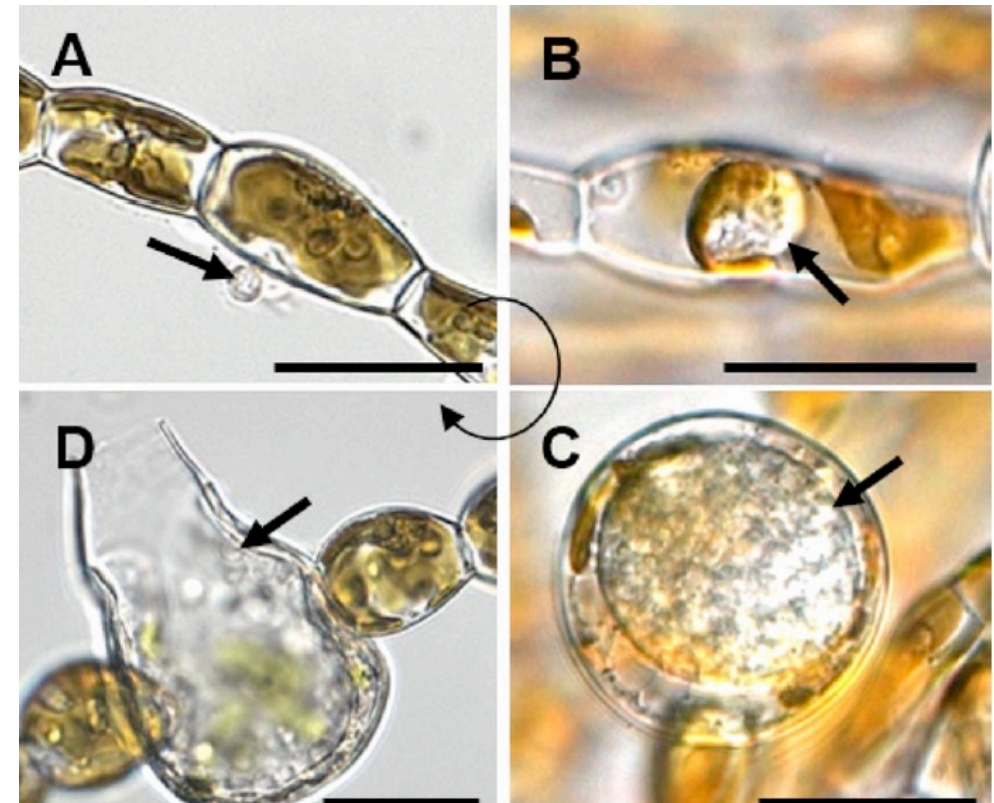
Better genetical markers

Gaps on databases

Lifestyles range: from biotrophy to necrotrophy, and obligate to facultative parasites.



Eurychasma dicksonii infecting brown algal (*Ectocarpus siliculosus*)



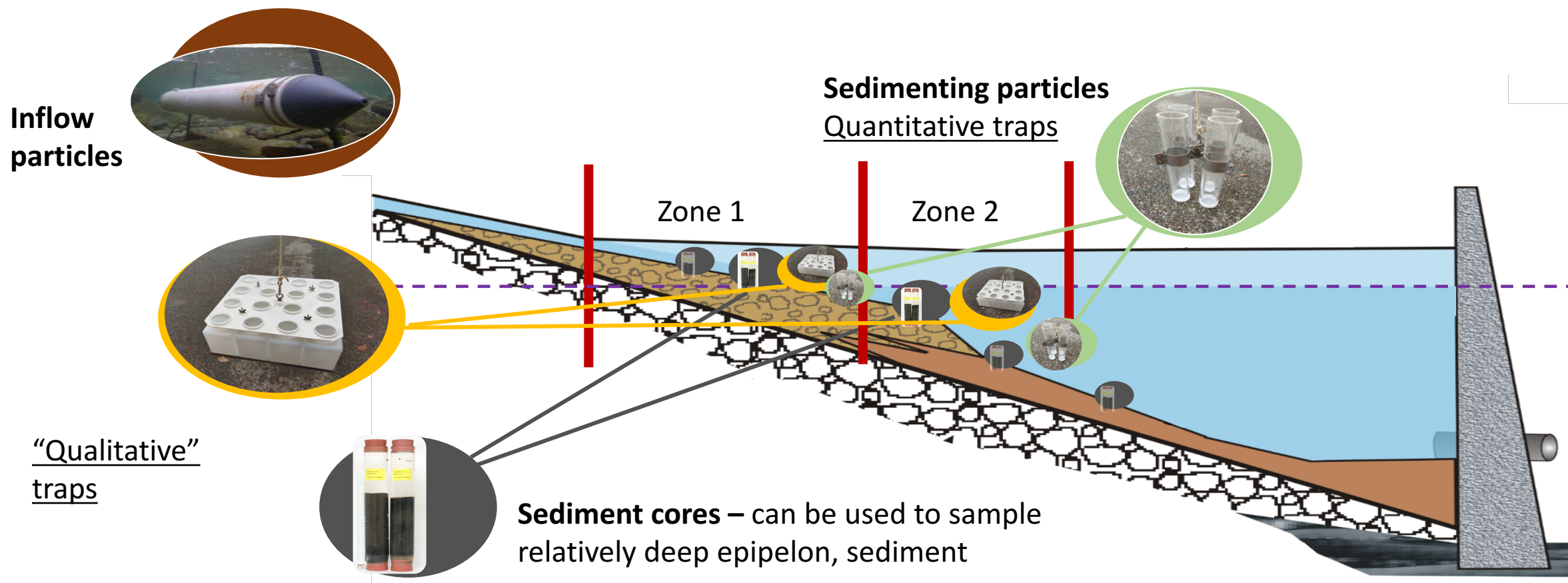


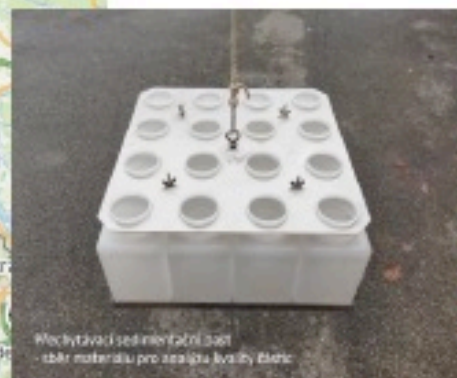
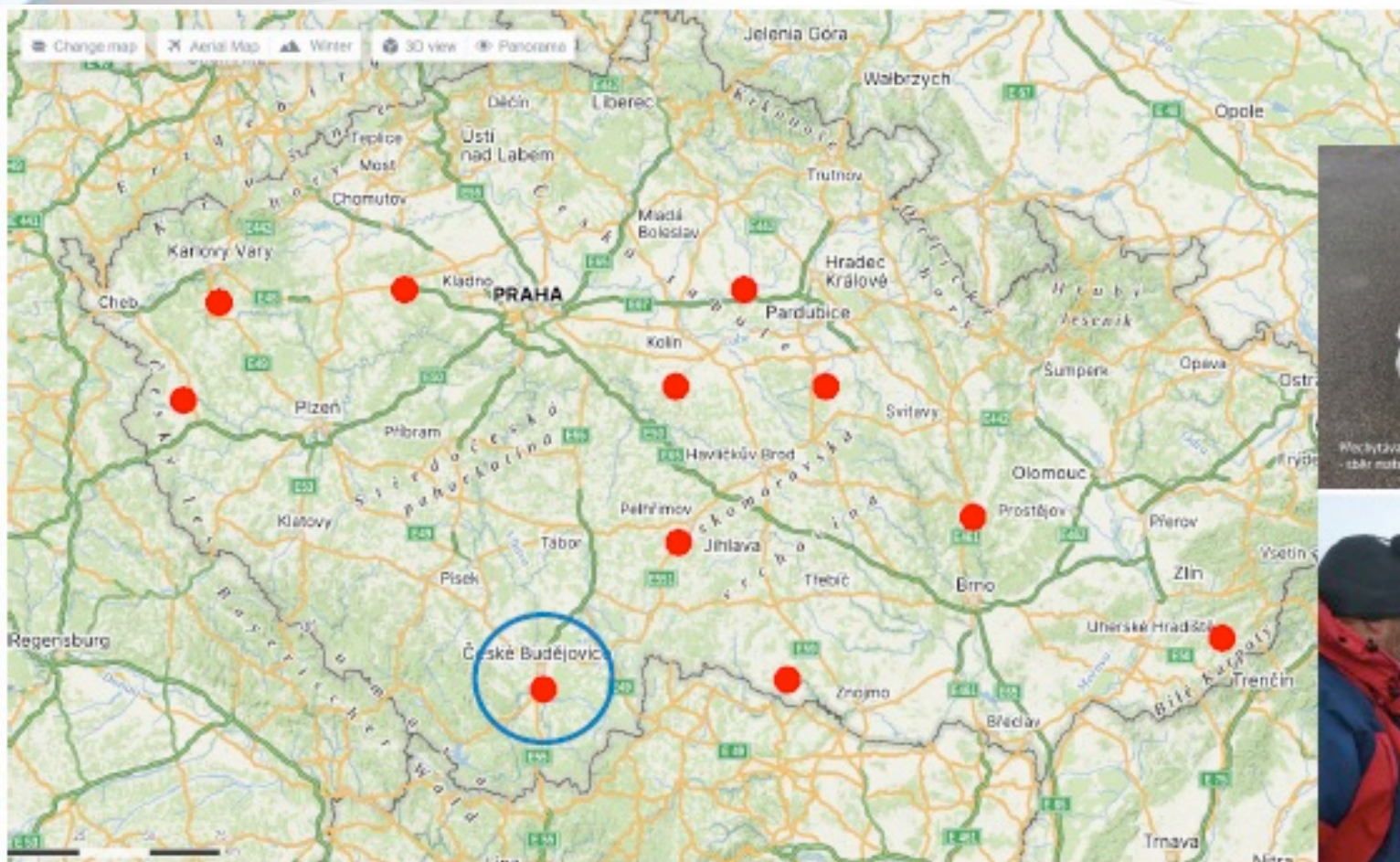
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Inter COST project „INFERA“





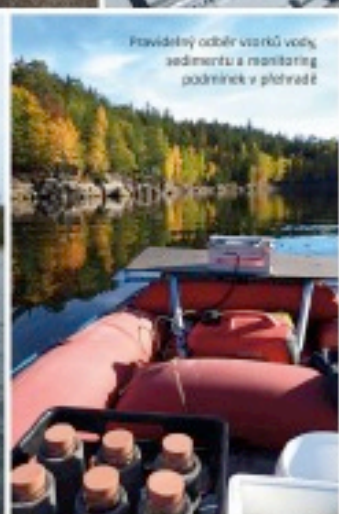
Wektýřová sedimentační past
- sběr materiálu pro analýzu kvalitativní



Sběr dat z instalovaných reálných stanic



Quantitativní sedimentační past
- stanovení množství sedimentujícího materiálu



Pravidelný odběr vzorků vody
sedimentu a monitoring
podmínek v hloubce



Úkleda odebraných sedimentů v
plastových trubkách z jednotlivých
zón raketové stanice

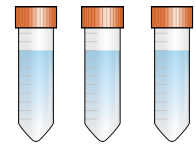


Sběrná příkalková blána domácnosti
- instalovaná na příkalku

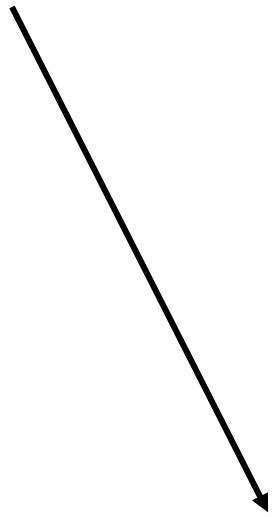


Monitorovací čidlo při lovení
celého sedimentu nad sedimentem

FURTHER STEPS:



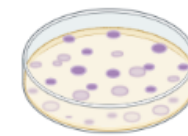
SAMPLES



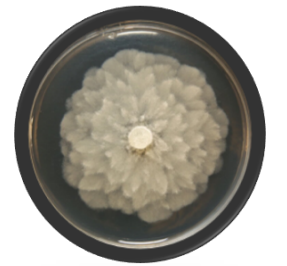
SERIAL DILUTION



PLATING



ISOLATION



STRAIN



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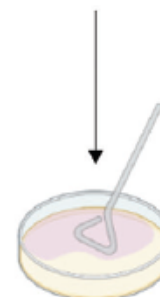
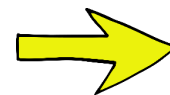


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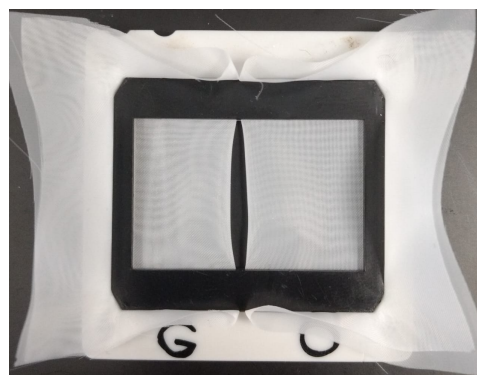
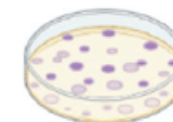


Serial dilution



Spreading

Incubation



Cellophane



Onion skin



Pollen



Exuviae
(insects, crustacean or arachnids)



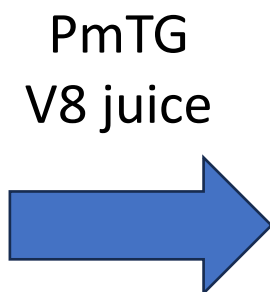
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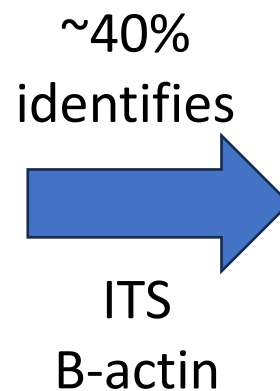
BIOLOGY
CENTRE
CAS

Institute of Hydrobiology

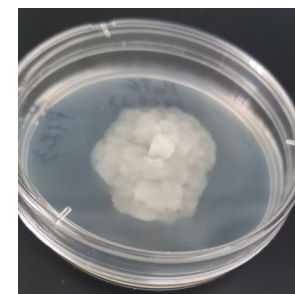
Sediment litter
Sediment trap
Artificial system
Shallow lakes
River
Macrophyte



205 isolates



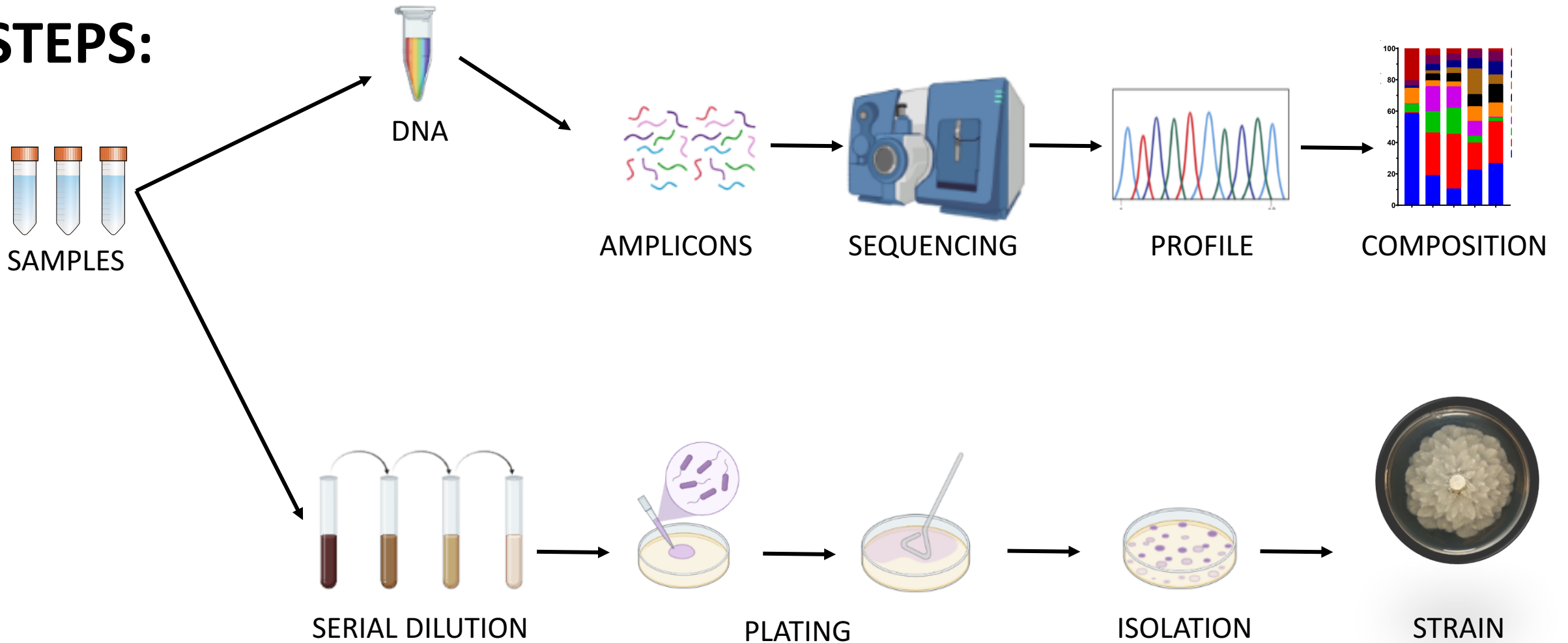
37% of
Oomycetes

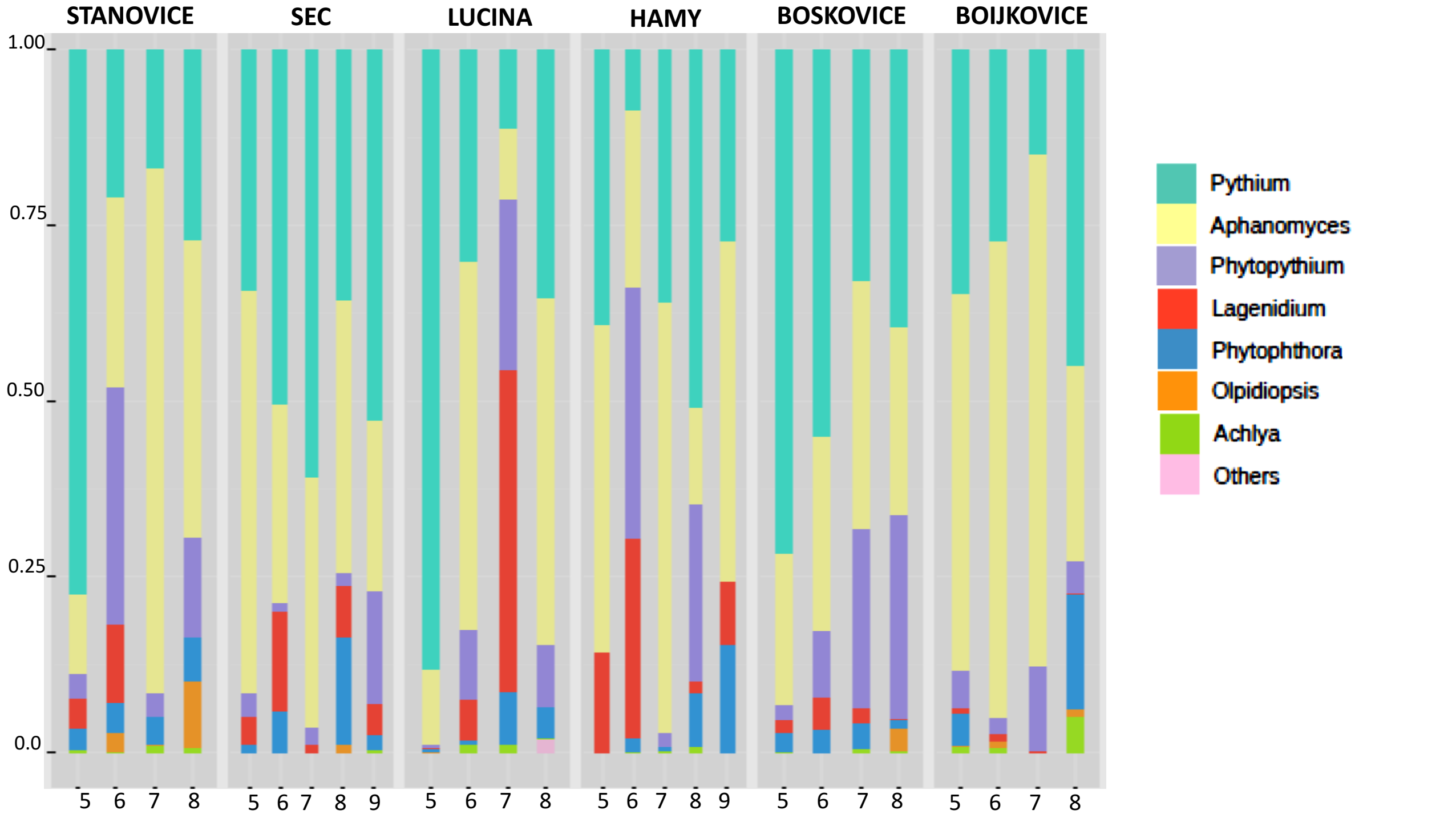


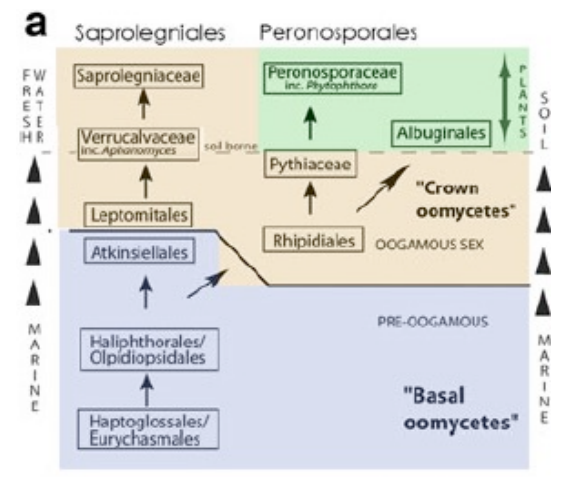
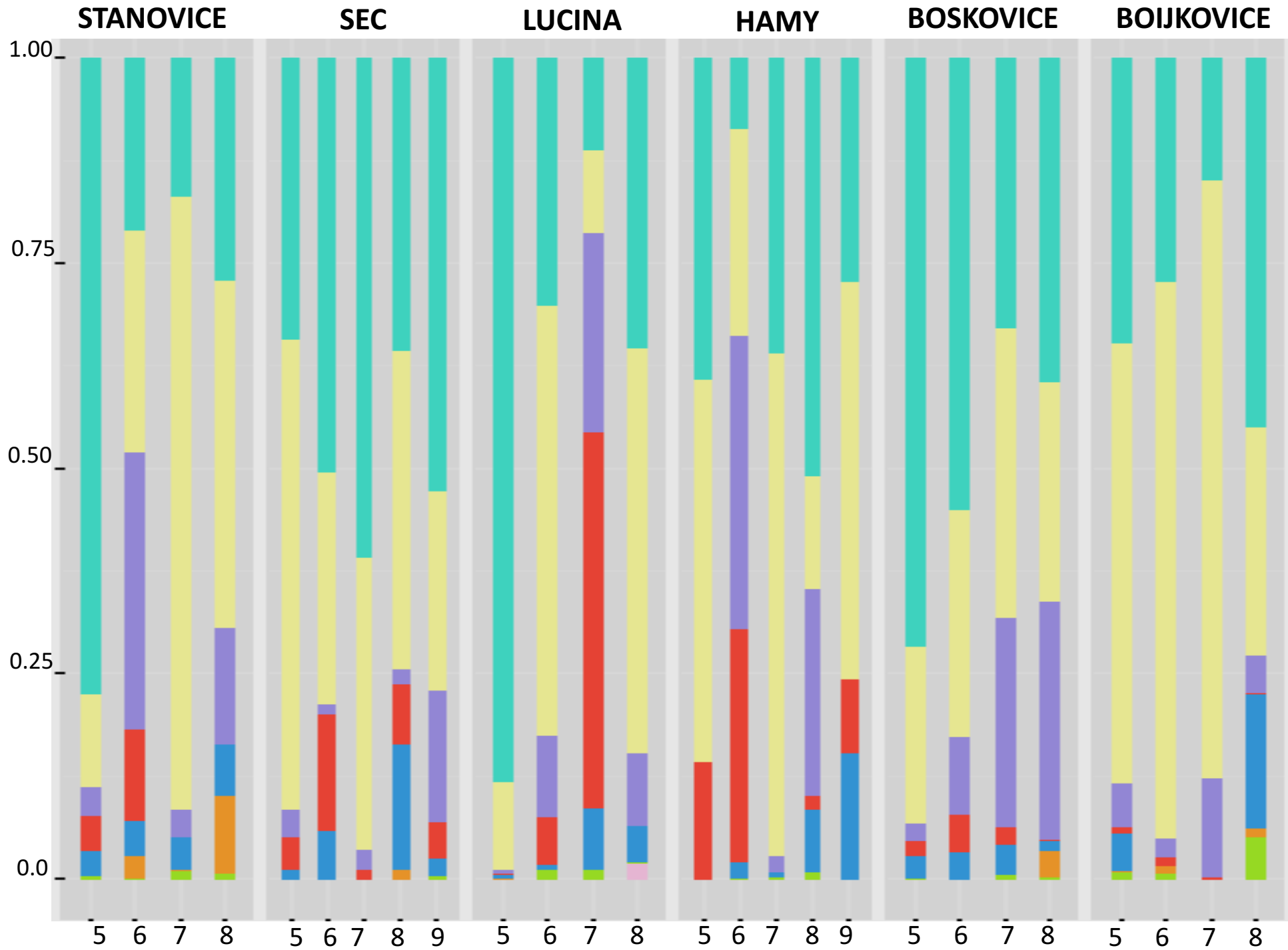
Phytophthora sp.

Phytophthora gonapoidydes
Phytophthora lacustris
Globisporangium sp
Phytophthium litorale
Phytophthium montanum
Phytophthora sp
Pythium sp

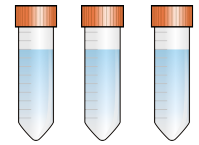
FURTHER STEPS:







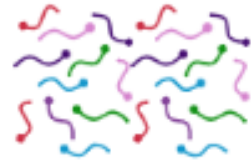
FURTHER STEPS:



SAMPLES



DNA



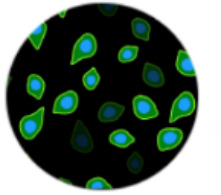
MARKERS



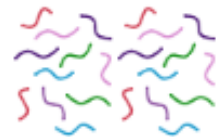
LABELLING



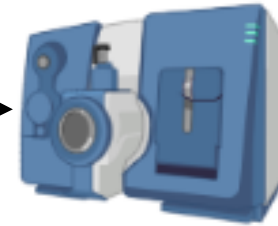
MICROSCOPY



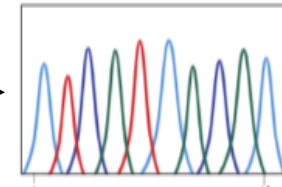
IMAGES



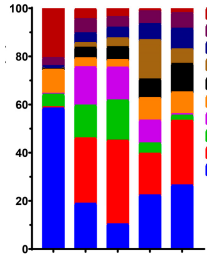
AMPLICONS



SEQUENCING



PROFILE



COMPOSITION



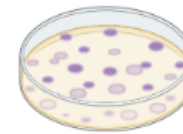
SERIAL DILUTION



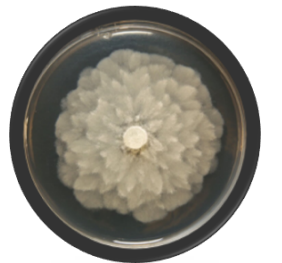
PLATING



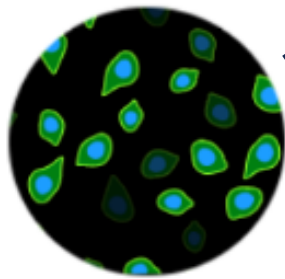
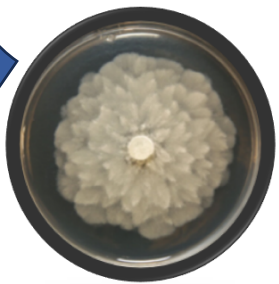
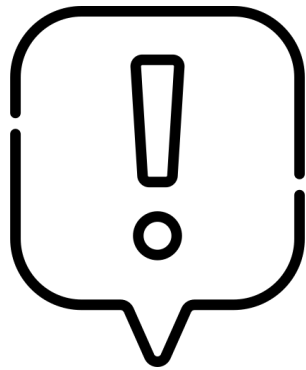
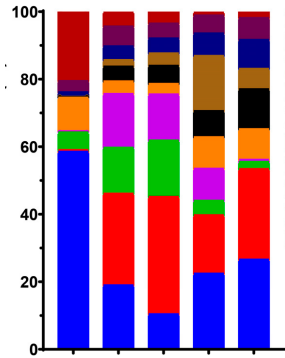
ISOLATION



STRAIN



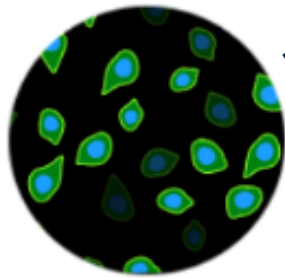
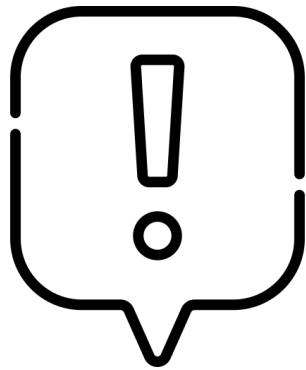
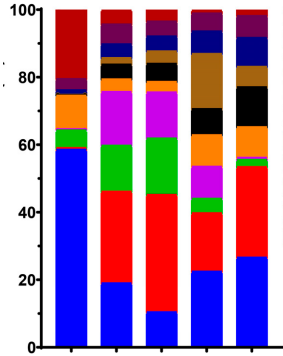
FURTHER STEPS:



ISOLATION

- Suitable methods
- Culture media
- Co-culture

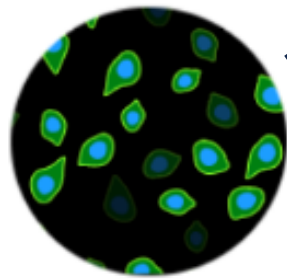
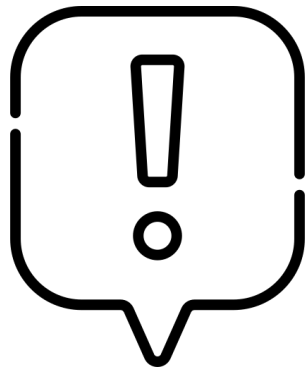
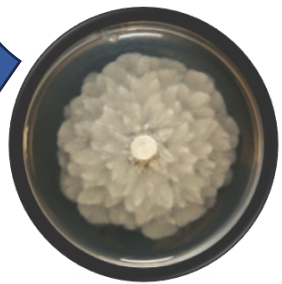
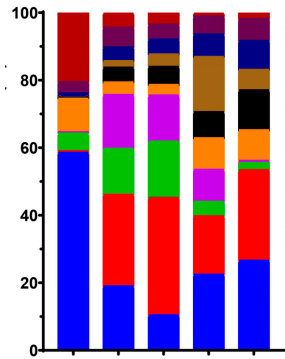
FURTHER STEPS:



SEQUENCING

- Sample source
- Time sampling
- Good primers (ITS, cox I and II, β -tubulin...)

**FURTHER
STEPS:**



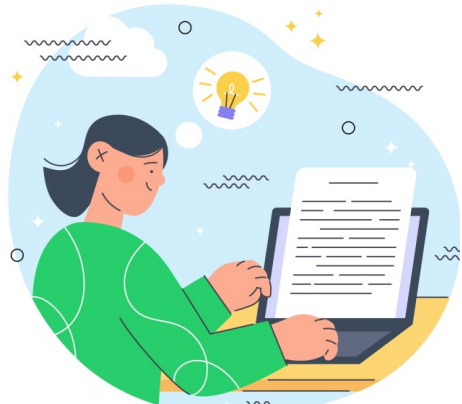
MICROSCOPY

- Sample fixation
- Staining
- Probes

WHAT NOW?



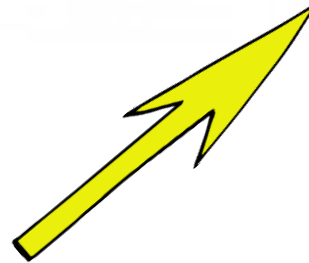
Find interested persons



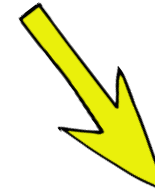
Output: paper, communication, guide



Compile main used methods



Share experiences and challenges

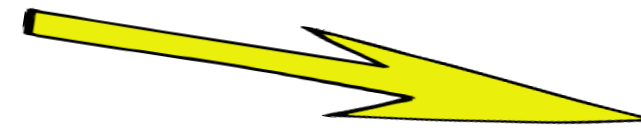


Brainstorm: Main points to go further

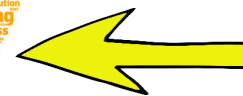


NETWORKS LEARNING

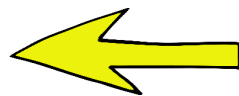
Network group



Design new plans and perspectives



New ideas and data



Good solutions from plant parasites:

Stages in water monitoring	Water monitoring techniques									
	Membrane filters	Baiting	Colony Forming Units	Zoospore-Trapping Immunoassay	Dipsticks	Lateral Flow Devices	Enzyme-Linked Immuno-Sorbent Assay	PCR	qPCR	Meta-barcoding
Concentration /capture	●	●	○	●	●	○	○	○	○	○
Detection/ identification	○	●	●	●	●	●	●	●	●	●
Viability	○	●	●	●	●	○	○	○	○	○
Quantification	○	●	●	●	●	●	●	○	●	●


KEY

○	Not effective
●	Moderately effective to good
●	Good
●	Very good to excellent

Good solutions from plant parasites:

ITP Identification Technology Program IDtools Search IDaids Bugwood ITP Node IDpic FSM PPQ IDaids imageID

IDphy: molecular and morphological identification of *Phytophthora* based on the types

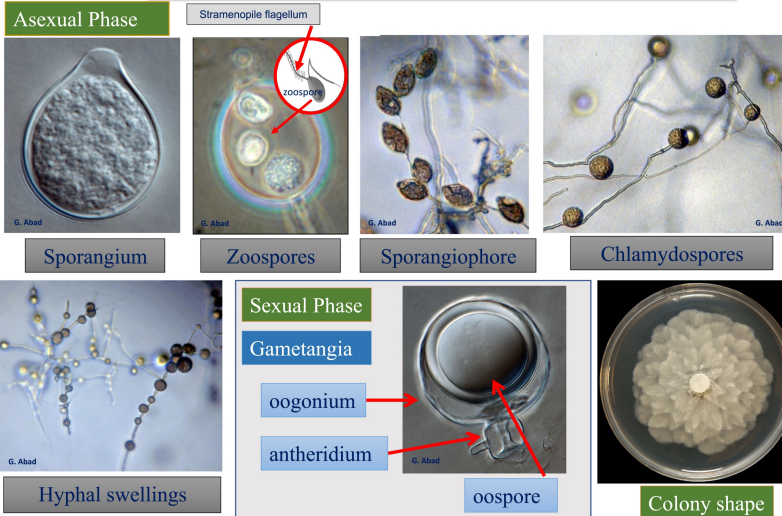


Molecular and morphological identification of *Phytophthora* species based on the types and other well-authenticated specimens

IDphy was developed to facilitate accurate and efficient identification of *Phytophthora* to species, using type specimens from the original descriptions for reference wherever possible. *IDphy* emphasizes species of high economic impact and species of

IDphy: molecular and morphological identification of <i>Phytophthora</i> based on the types		
	MOLECULAR IDENTIFICATION	
IDENTIFYING PHYTOPHTHORA UNKNOWNNS		
STEP 1) Go to NCBI BLAST blastn suite and perform a nucleotide BLAST analysis of the <i>Phytophthora</i> unknown's FASTA sequence for ITS rDNA or COI.	STEP 2) Under "Description", look for the species that have the closest alignment(s) with your query/unknown sequence. This example appears to match <i>P. boehmeriae</i> . The next steps will help confirm whether the matching sequence is from a well-authenticated specimen.	STEP 3) First, use the clade table to find the clade where the possible species positions. For this example, <i>P. boehmeriae</i> positions in clade 10b.
STEP 4) Once you have found the clade, check the appropriate accession number table (scroll down or check in the appendix of SOP-PID-05 for the tables) to find the accession number for the ex-type of the possible species.	STEP 5) Go to NCBI BLAST blastn suite and select " Align two or more sequences ". Paste the FASTA sequence of the <i>Phytophthora</i> unknown in the UPPER box and the accession number of the possible species match in the LOWER box (example <i>P. boehmeriae</i> in clade 10, MG783382). Perform the BLAST and	STEP 6) ... check the alignment. Does it align 99-100%? If so, we should confirm it is a good reference specimen.
STEP 7) It is very important to verify that the reference sequence is from a type or other well-authenticated specimen. Check the fact sheet for the accession number you used as the reference for your unknown (click the accession number link in the alignment results, as shown above).		
CONFIRM IDENTITY USING A FULL CLADE ANALYSIS		
STEP 8) Go to NCBI BLAST blastn suite " Align two or more sequences " and paste the FASTA sequence of the <i>Phytophthora</i> unknown in UPPER box. Copy and paste all accession numbers in the clade (from the appropriate accession number table) in the LOWER box (for this example, all species in clade 10).	STEP 9) Check the alignment of the unknown/query with all species in the clade. Do you observe 99-100% alignment with the same species as before? Check the alignment for each of the closest species. The best match appears to be <i>P. gondwanensis</i> , but before we select the best match, we should evaluate using a draft distance tree.	STEP 10) Prepare a distance tree through NCBI. In the results for the full clade multi-sequence alignment, click "Distance tree of results" under "Other reports." For "Tree method," select "Neighbor joining." For "Sequence label," select "Taxonomic name." Unknown will be marked in yellow. This example shows the best match is <i>P. gondwanensis</i> , as confirmed by the distance tree.

Morphological Characters of *Phytophthora*



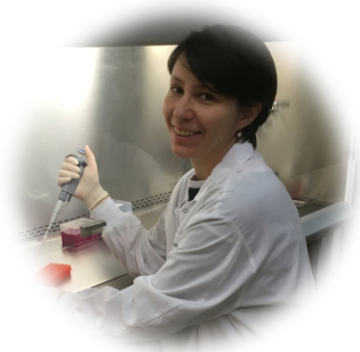
Asexual Phase

- Sporangium
- Zoospores
- Sporangiophore
- Chlamydospores

Sexual Phase

- Gametangia
- Hyphal swellings
- oogonium
- antheridium
- oospore
- Colony shape

Thank You



Dagmara Sirová



Caio César Pires de Paula



Jiří Bárta



Mariia Dimova



BIOLOGICKÉ
CENTRUM
AV ČR, v. v. i.



University of South Bohemia
in České Budějovice
Faculty
of Science



INSTITUTE OF HYDROBIOLOGY
Biology Centre CAS



GAČR
GRANTOVÁ AGENTURA ČESKÉ REPUBLIKY

Contact: caio.piresdepaula@hbu.cas.cz