RRR2021 - Session 4.3 Sphagnum propagules

9th March 2021

Axenic *in-vitro* cultivation of 19 peat-moss (*Sphagnum*) species as a resource for basic biology, biotechnology and paludiculture

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Breeding and mass production of peat mosses for sustainable industrial production of horticultural substrates

Karlsruher Institut für Technologie

NIRA

Collection and genetic characterization of *Sphagnum* (peat moss)

Optimization of the process parameters and large-scale mass production

Non-axenic biomass production in the field

Natural *Sphagnum* as starting material is not realizable for large-scale implementation

- Lack of sufficient founder material for *Sphagnum* farming
- *Sphagnum* is a protected genus and growths predominantly in protected areas
- Contaminated with unwanted species

  → limits its use as raw material for horticultural growing media

→ Development of a production method for founder material

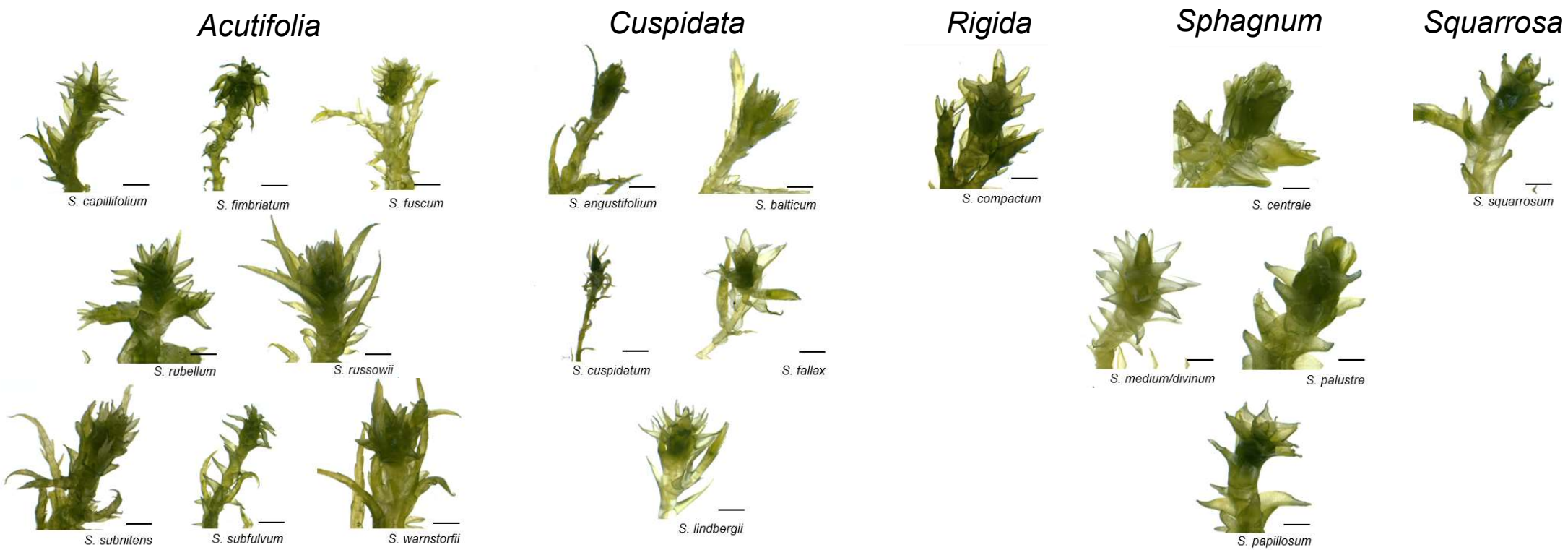
→ Selection of highly productive clones

→ Axenic, clonal cultures produce high quantities of biomass under laboratory conditions

Axenic *in-vitro* cultivation methods of *Sphagnum*

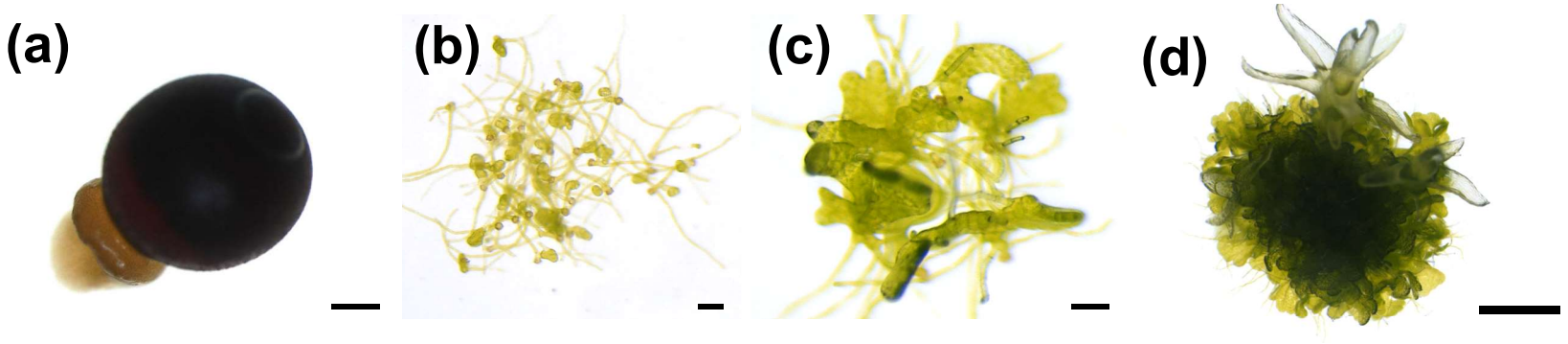
Sphagnum spec. in axenic cultures

19 different *Sphagnum* species from 5 sections in axenic cultures:



Heck et al. 2021, New Phytologist

From spore to clone



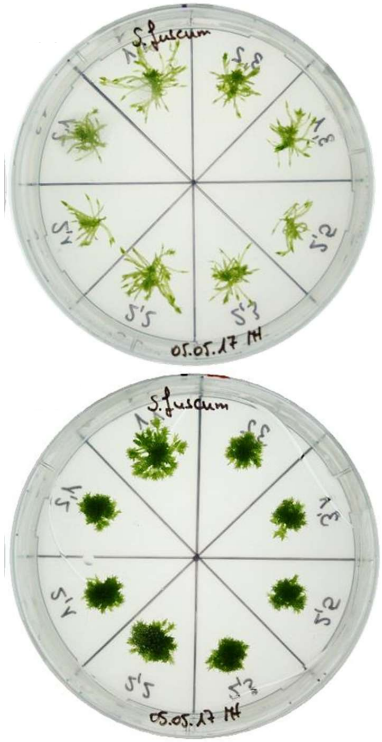
- Decontamination of capsule and spores with NaClO (0.6 - 2.4%)
- Spores germinate within a few weeks
- Separation of single plants

→ Cultivation of independent clones

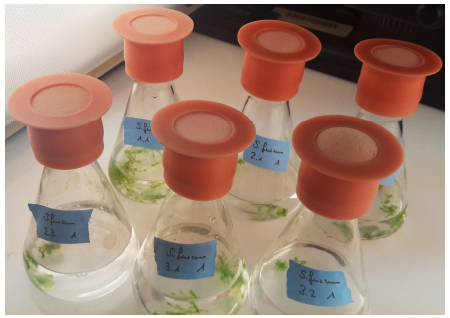
Beike et al. 2015, Plant Cell Tiss Organ Cult

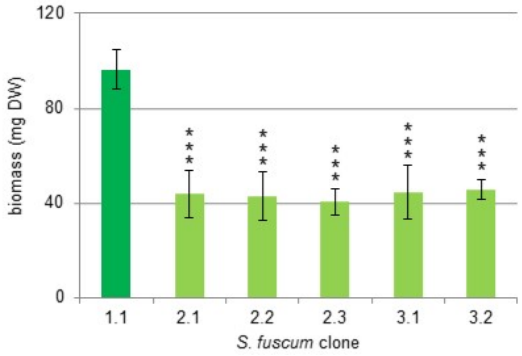
Identification of the best growing clone

clones on agar plates



clones in flasks with liquid growth medium*





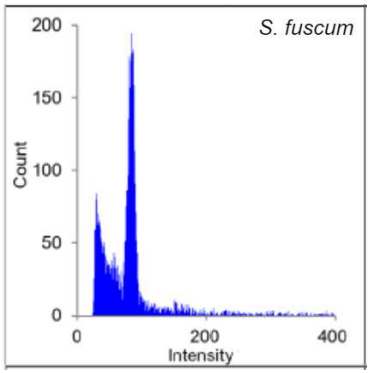
best clone in bioreactor

Heck et al. 2021, New Phytologist
* Beike et al. 2015, Plant Cell Tiss Organ Cult

plant-biotech.net

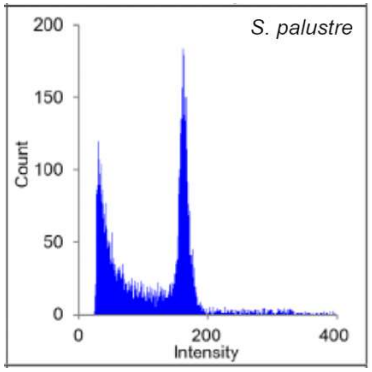
Ploidy measurement using flow cytometry

haploid



S. angustifolium
S. balticum
S. capillifolium
S. compactum
S. cuspidatum
S. fallax
S. fimbriatum
S. fuscum
S. lindbergii
S. medium/divinum
S. rubellum
S. squarrosum
S. subfulvum
S. subnitens
S. warnstorfii

diploid



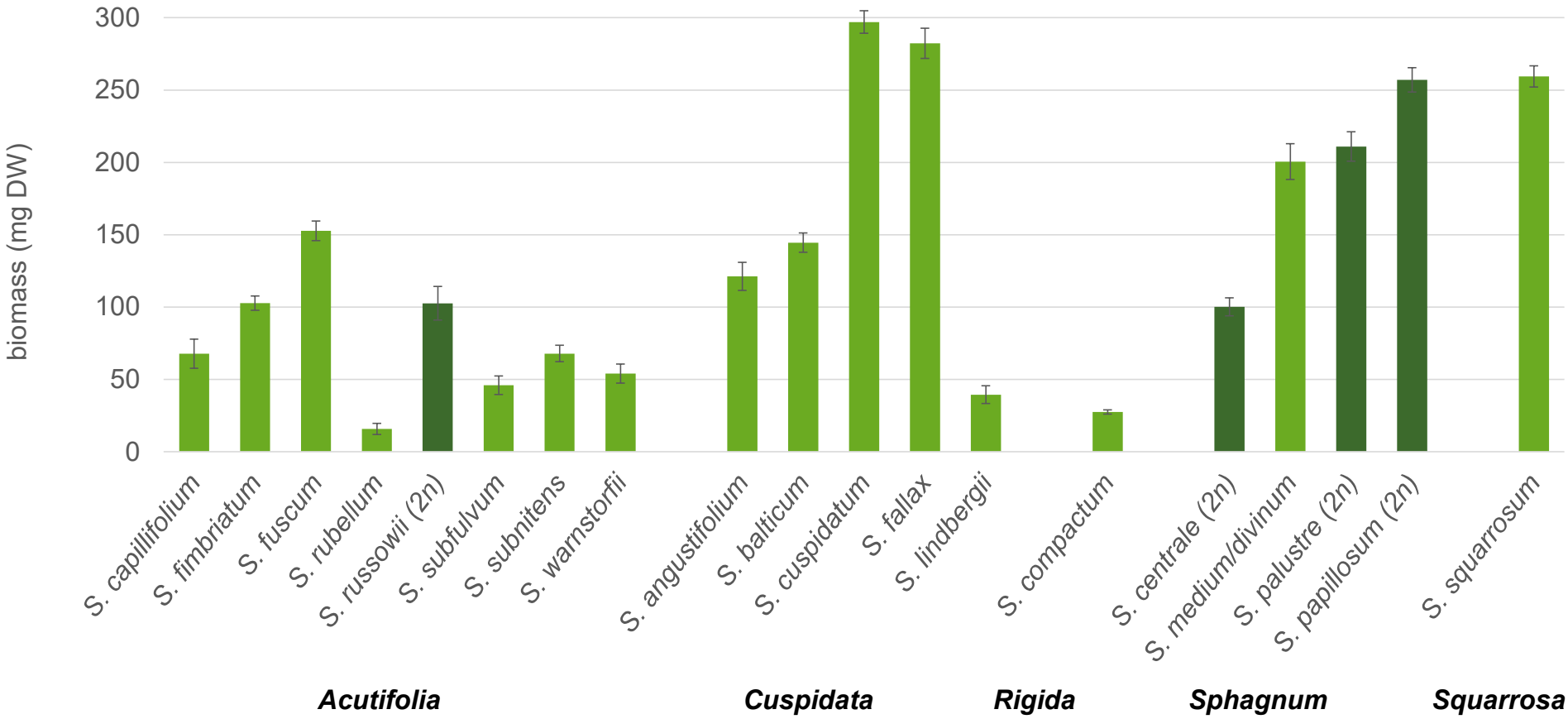
S. centrale
S. palustre
S. papillosum
S. russowii

- All clones from one species have the same ploidy level

  → no correlation between ploidy and productivity

Heck et al. 2021, New Phytologist

Comparsion of *Sphagnum* biomass increase



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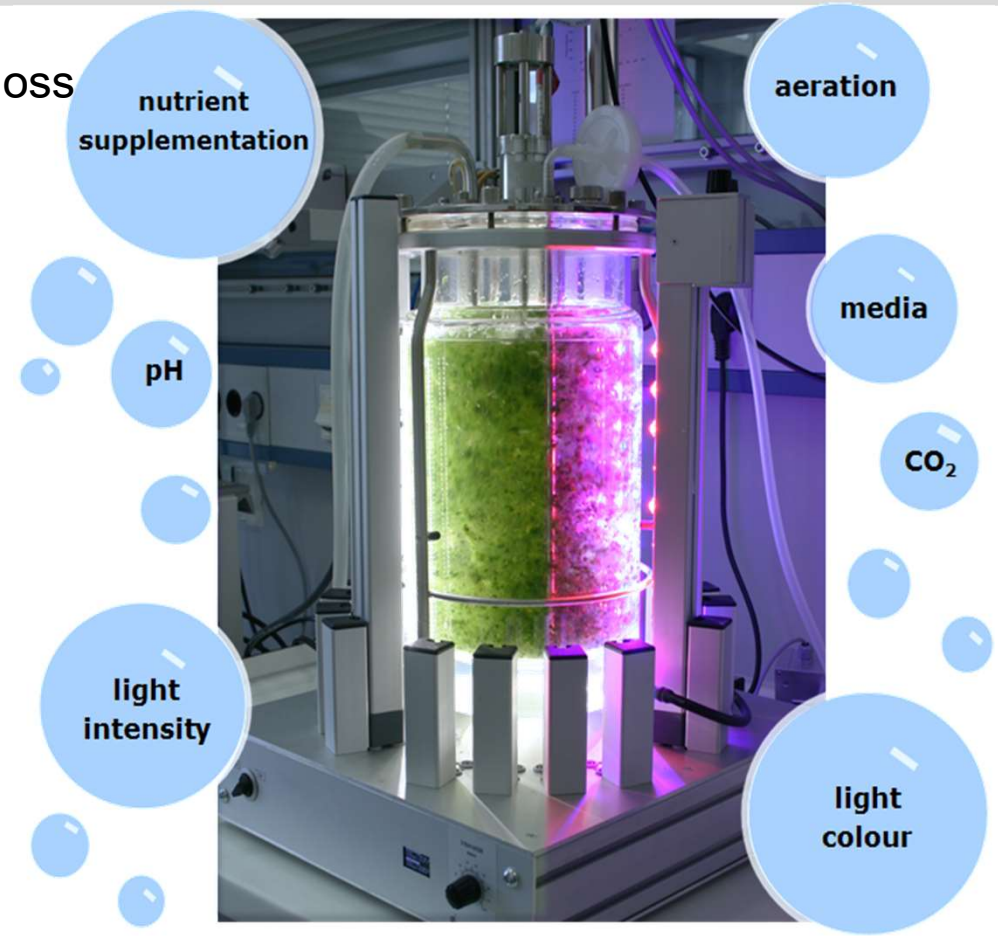
Heck et al. 2021, New Phytologist

Axenic biomass production in the bioreactor

- Promising system for biomass production of peat moss as founder material
- Cultivation under fully controlled conditions:

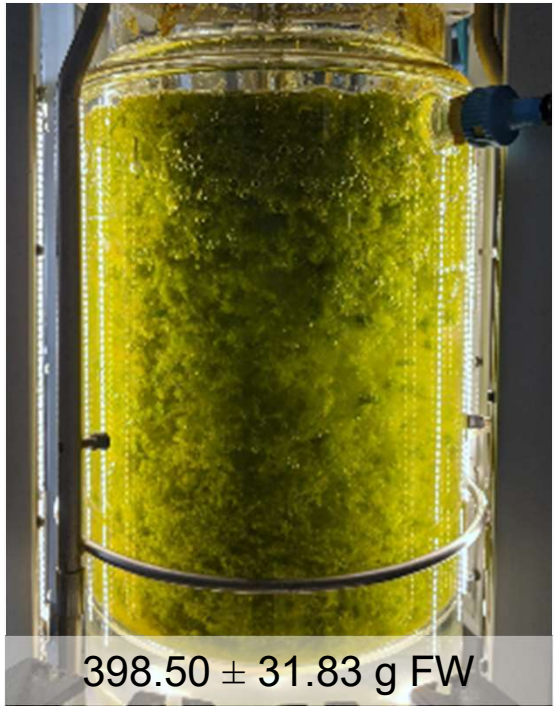
Light cycle	20/4 h
Light intensity	150 - 500 µE
	(stepwise increase until day 7)
Temperature	22ºC
Aeration	0,3 vvm + 2% CO_2
Media	optimized media with sucrose
pH	not adjusted, but tracked



Sphagnum growth rate in the bioreactor

S. squarrosum

~ 25x in 24 days



398.50 ± 31.83 g FW

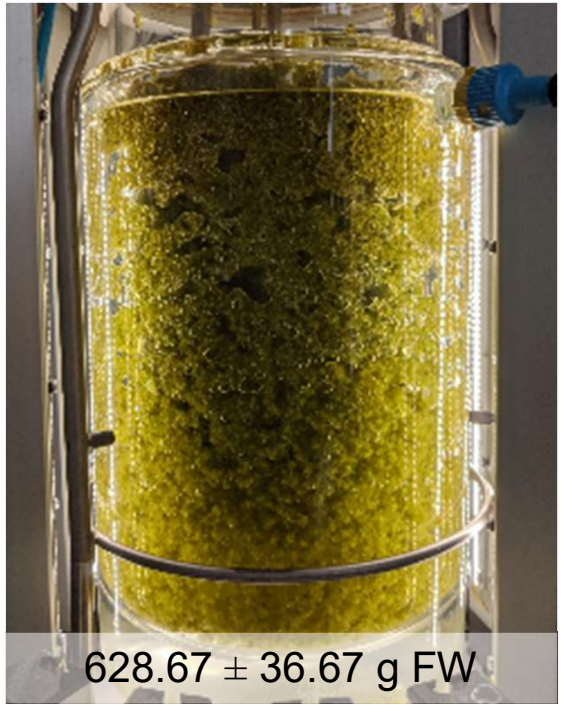
S. palustre

~ 40x in 24 days

576.33 ± 6.24 g FW

S. fuscum

~ 50x in 24 days



628.67 ± 36.67 g FW

Heck et al. 2021, in preparation

Bioreactor moss as starting material for *Sphagnum* farming

6 months later

Fotos: Anja Prager, University of Greifswald

Acknowledgement

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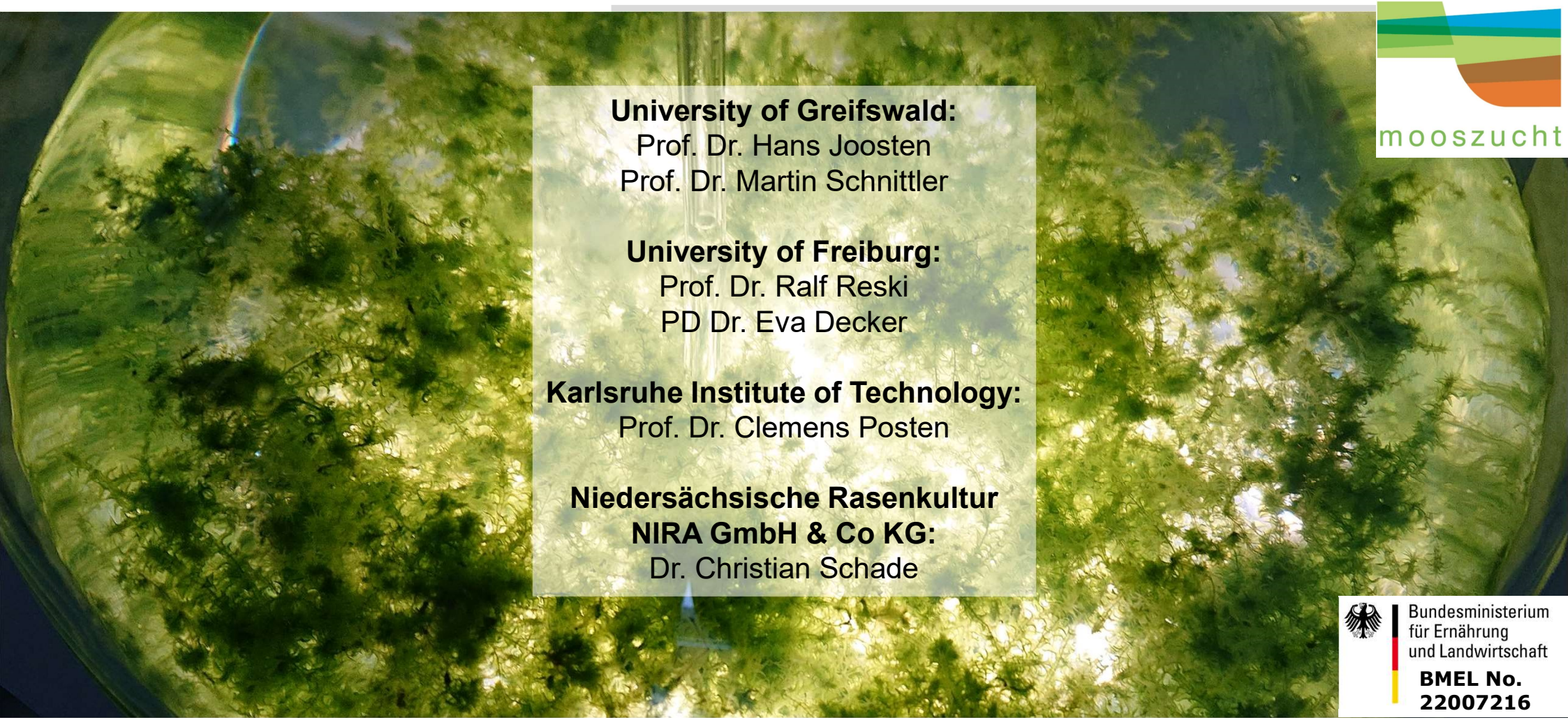

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