

Reproductive barriers in the *Seminavis robusta* species complex and their role in species diversification



Sam De Decker¹, Koen Van den Berge², Pieter Vanormelingen¹, Koen Sabbe¹, Wim Vyverman¹

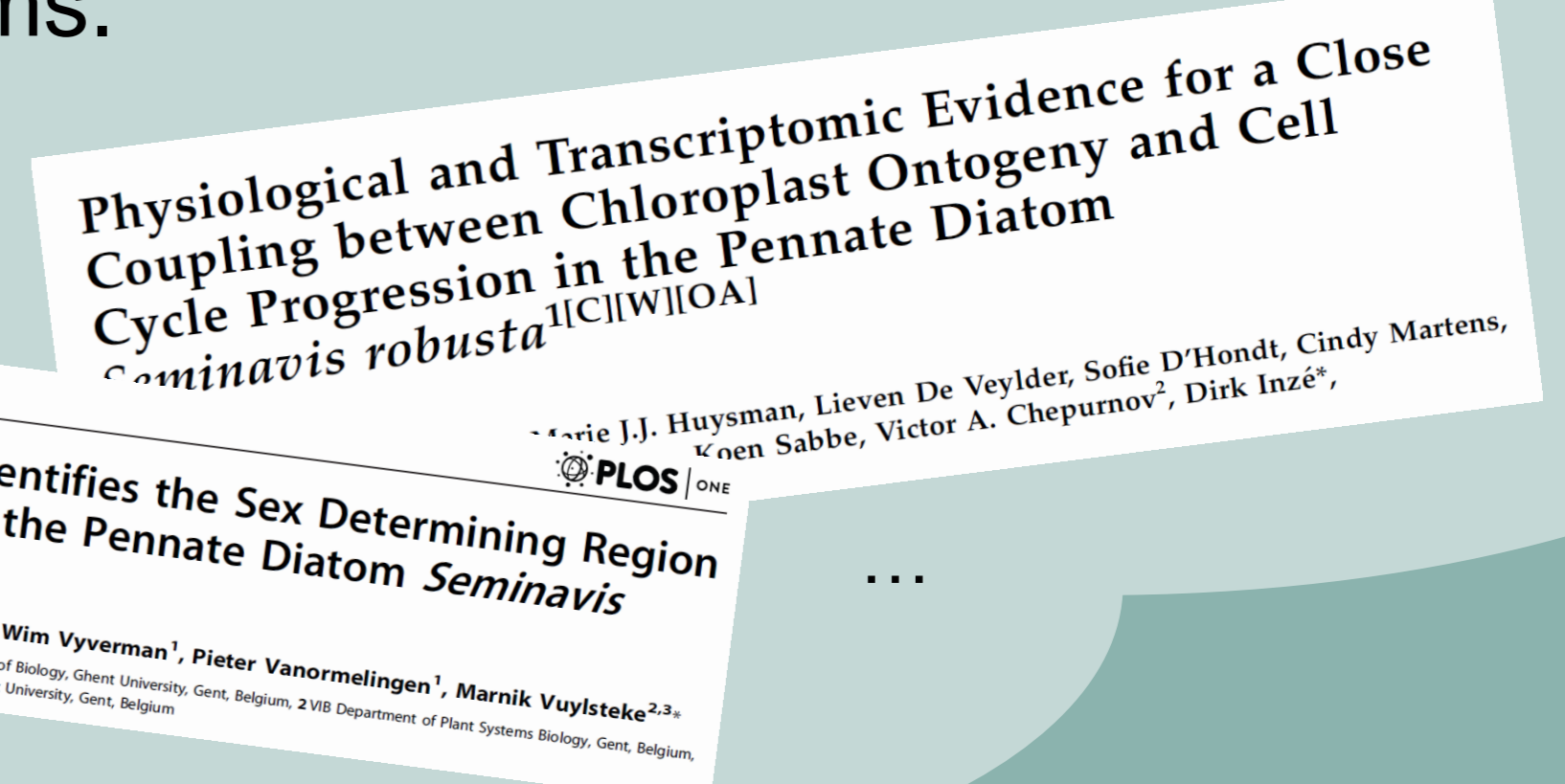
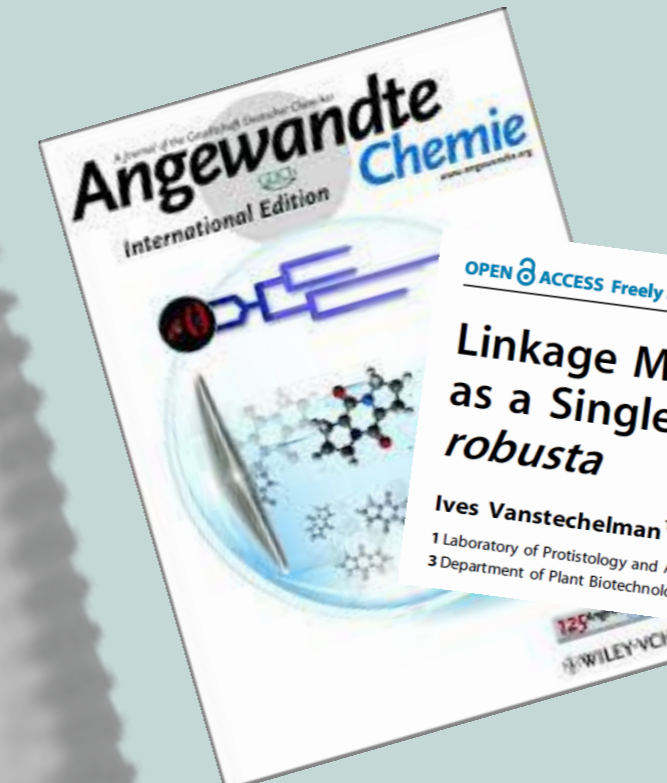
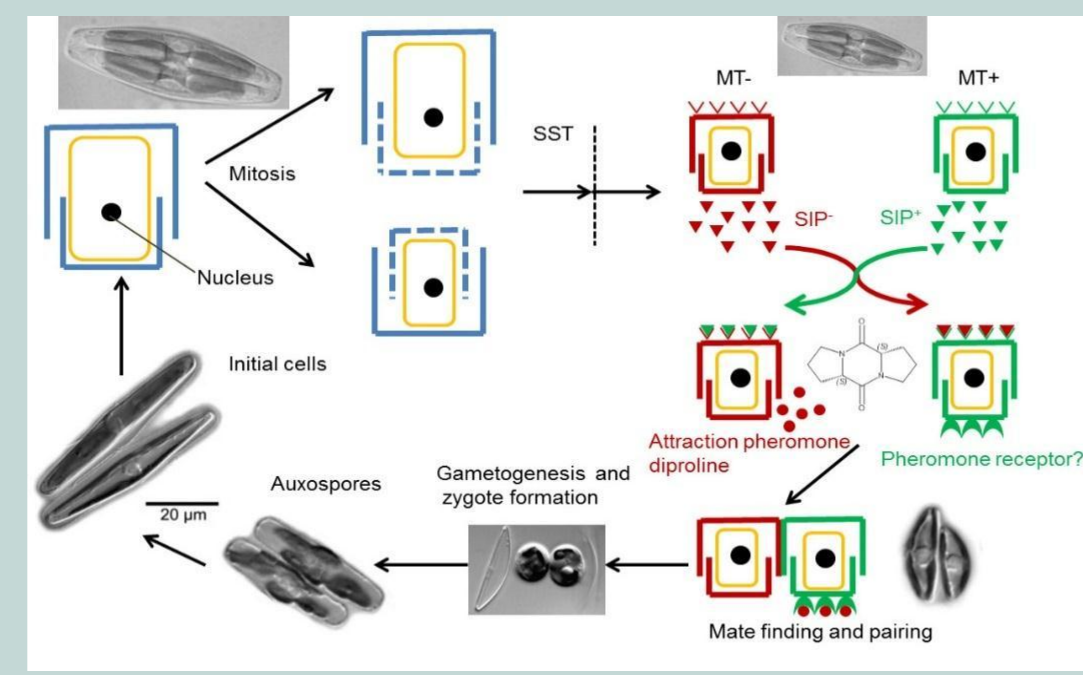
¹ Laboratory for Protistology & Aquatic Ecology, Ghent University, 9000 Ghent, Belgium

² Department of Applied Mathematics, Computer Science and Statistics, Ghent University, 9000 Ghent, Belgium

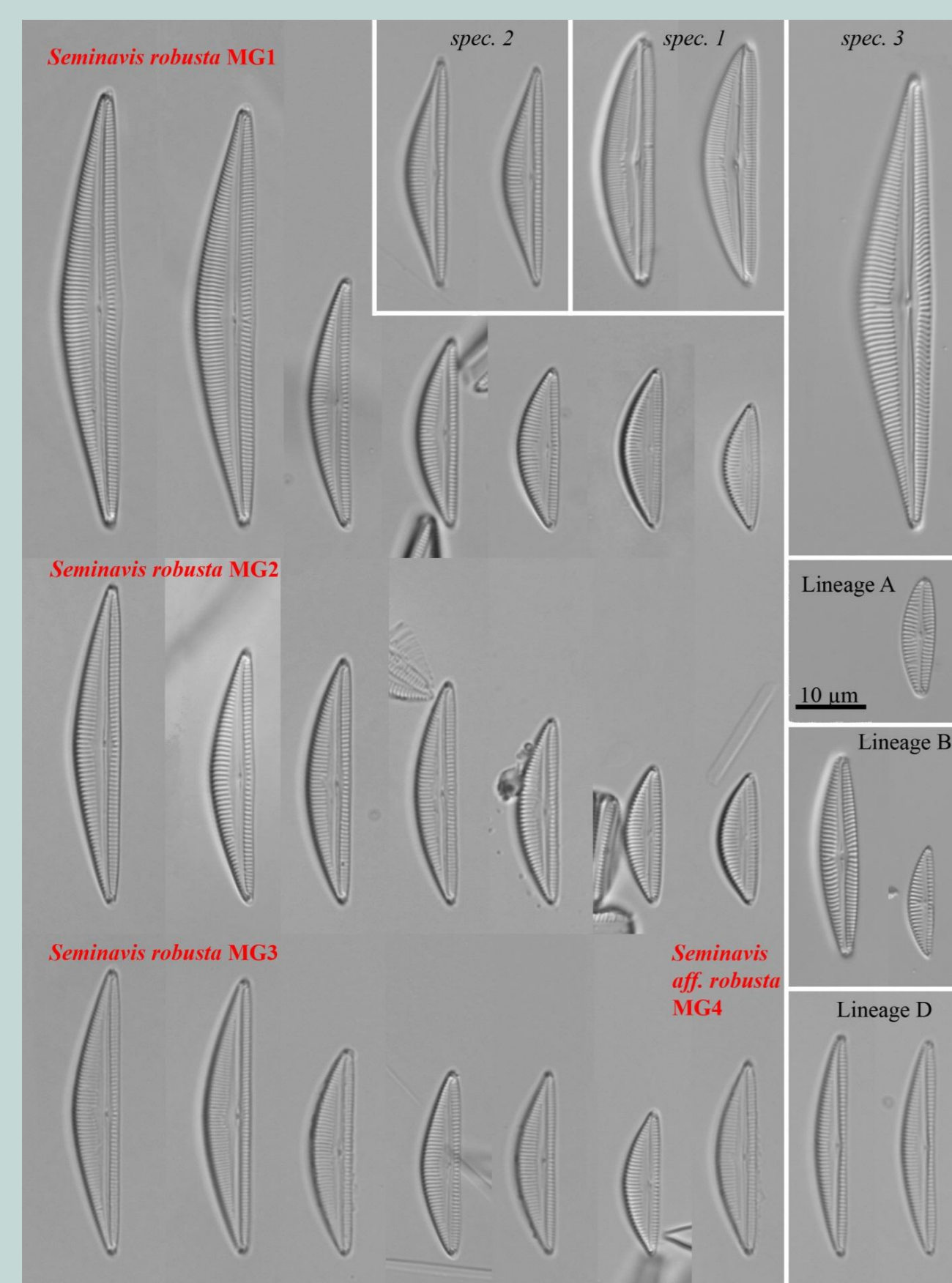
Why *Seminavis robusta*?

The pennate diatom *Seminavis robusta*, is a marine, benthic species with a well-documented life cycle. In a recent study, the **importance of chemical signaling** during the early phases of the heterothallic sexual reproduction process was shown.^[1] Earlier studies provided **valuable resources** (genome sequence, metabolomics and pheromone characterisation ^[1], transcriptome data ^[2]), resulting in the strong emergence of *S. robusta* as a **model species** for molecular cell and life cycle research in diatoms.

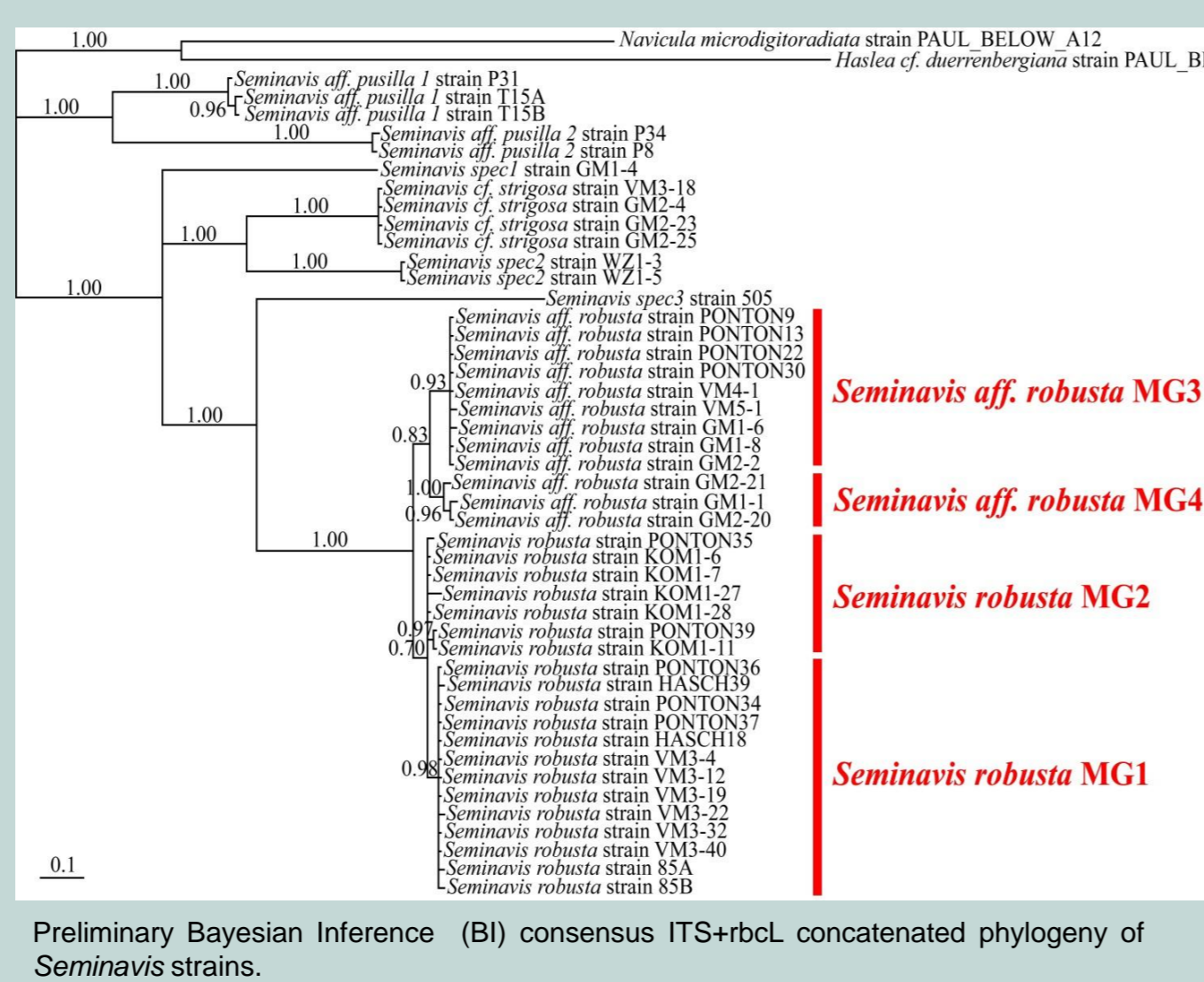
The life cycle of *Seminavis robusta*, modified after (3) with microscopic images from (4) and (5). During the vegetative phase, average cell size decreases. Once below the SST, both MT⁻ and MT⁺ cells start the production of pheromones, SIP⁺ and SIP⁻ respectively. Transcriptomic analysis revealed that SIP⁺ triggers the switch from mitosis-to-meiosis in the opposing mating type, coupled with the transcriptional induction of proline-biosynthesis genes, correlating with the accumulation of the proline-derived attraction pheromone (2). MT⁺ probably expresses a diproline receptor. Diproline signaling then leads to mate finding and pair formation, after which gametes and zygotes are formed. The specialized zygotes (auxospores) elongate to initial cell size. The induction of a cell cycle arrest by a chemically distinct pheromone than the one used to attract the opposite mating type highlights the existence of a sophisticated mechanism to increase chances of mate finding while keeping the metabolic losses associated with the release of an attraction pheromone to a minimum.



Distinct lineages?



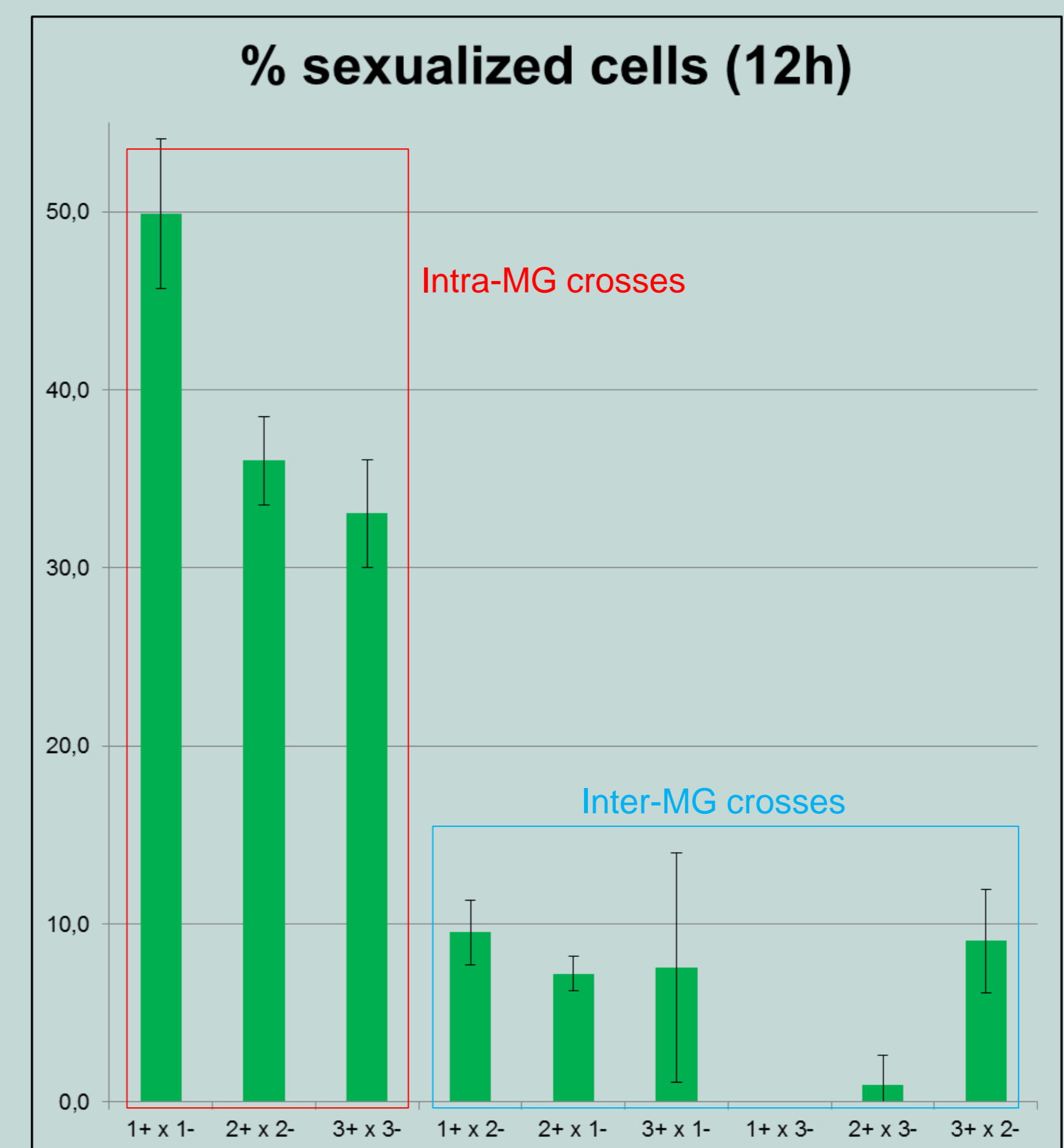
Phylogenetic and morphological analysis of a set of new strains showed that *S. robusta* forms at least **4 very closely related but distinct cryptic lineages**.



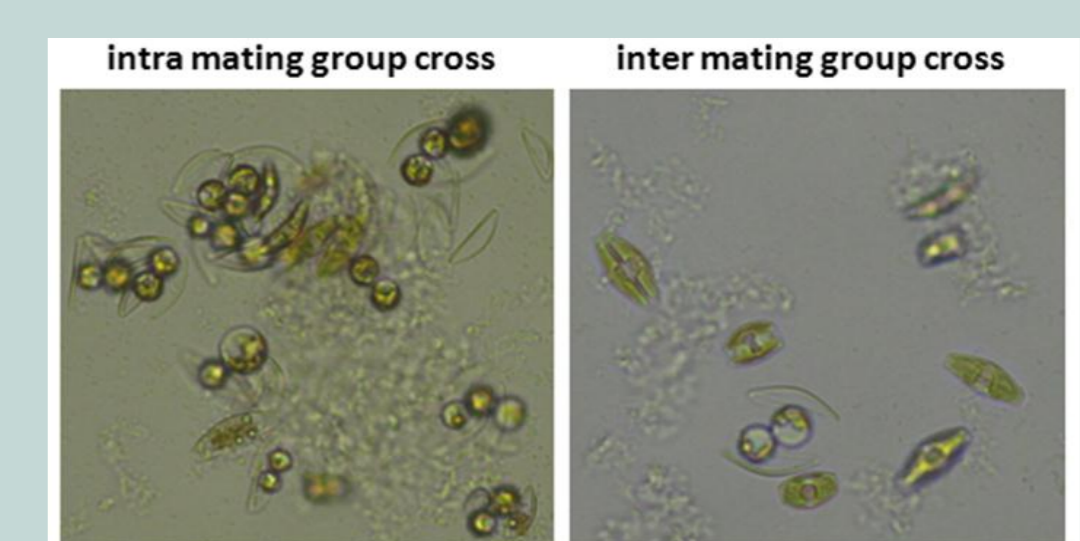
LM pictures of *Seminavis* strains focusing on the 4 closely related cf. *Seminavis robusta* lineages MG1, MG2, MG3, MG4

Reproductive barriers?

Sexual reproduction can be induced with high efficiency in intra-MG crosses within MG1, MG2 and MG3, while inter-MG mating success drops significantly. Interestingly, **phylogenetic distance correlates with interbreeding capacity** between the mating groups. These observations show the presence of (incomplete) **reproductive barriers**.

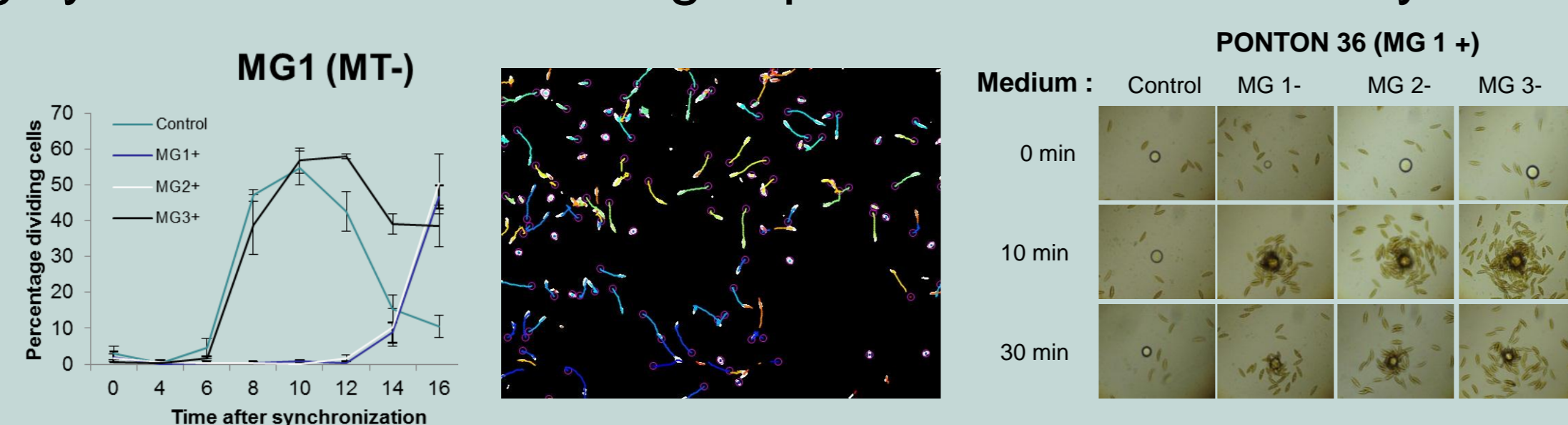


Comparison of the percentage of cells engaging in sexual reproduction upon crossing. For each mating type (+, -) of three mating groups (1, 2, 3), 8 strains were pooled in equal cell densities. These pools can be considered representative for the different incipient species. The pooled cultures were dark-synchronized for 24h before mixing equal cell densities for each cross. After 12h of light, for each cross at least 250 cells were counted and the percentage of cells engaging in the initial stages of the sexual process were determined. The mean percentages for 3 biological replicates are shown ± SD.



Ongoing work & future prospects?

- We are currently determining the **nature of this reduced mating** through a combination of highly standardized crossing experiments and bioassays.



Example results of three bioassays testing the initial phases of the sexual process as potential (pre-zygotic) reproductive barriers. Left: testing the potential of SIP⁺ of MG1, MG2 and MG3 to induce a G1-phase cell cycle arrest in dark-synchronized MG1⁻ cultures. Middle: testing the increase in motility of SIP⁺-conditioned MT⁺ cultures upon addition of 100nM diproline in the medium. Right: Testing the attraction of SIP⁺-conditioned MT⁺ cultures to diproline-coated silica beads.

- To assess the presence of possible **post-zygotic reproductive barriers**, viability and fertility of within-MG cross progeny will be compared to between-MG cross progeny.
- Genotyping-by-sequencing (GBS)** of a hybrid progeny mapping population in combination with **genome-wide association studies (GWAS)** of a large set of strains of the 3 mating groups will allow us to link the reproductive barriers to their underlying genomic regions.

References

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