

Chromosomal integration of the *Spirodela polyrhiza* reference genome

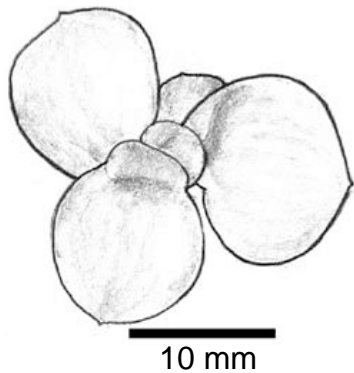
Hieu Cao

the 2nd Inter. Conference on Duckweed Research & Applications
New Brunswick, Aug 21-24, 2013

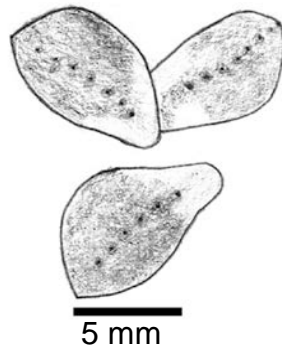


Lemnoideae (duckweeds)

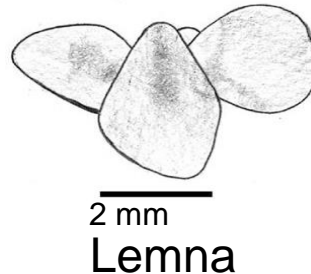
- 5 genera, 37 species; worldwide distribution, except arctic & antarctic zones
- Floating free on surface water; neotenous reduction
- Fast growing & rare sexual reproduction
- Genome size variation: 0.158 – 1.88 Gbp
- Chromosome numbers (2n): 20 - 126



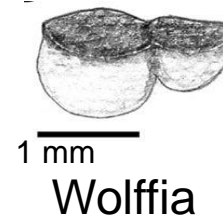
Spirodela



Landoltia



Lemna



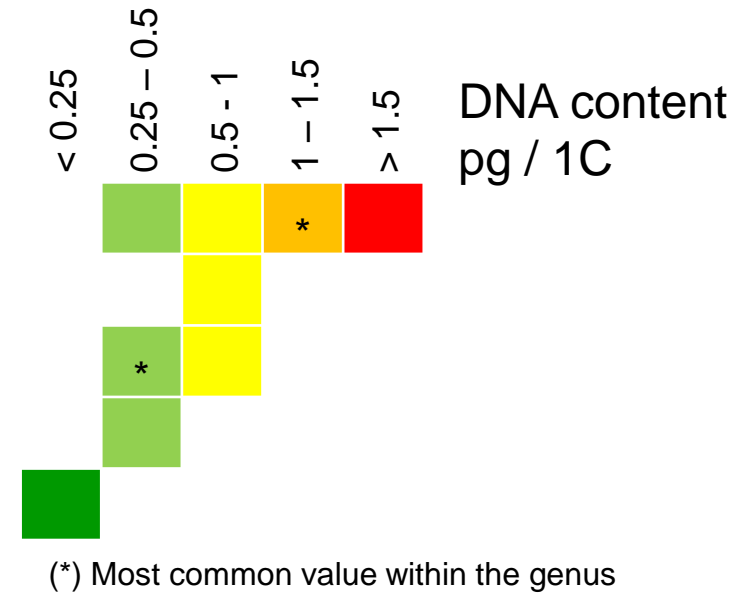
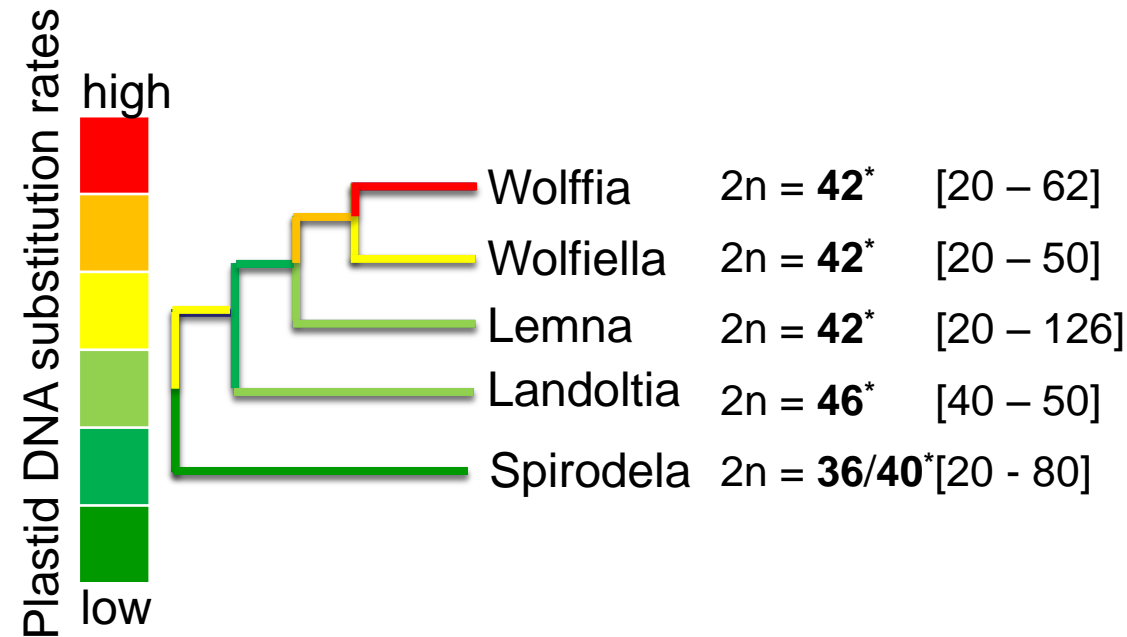
Wolffia



Wolffiella

Drawings: Dr. K. Sowjanya Sree, Amity University Delhi.

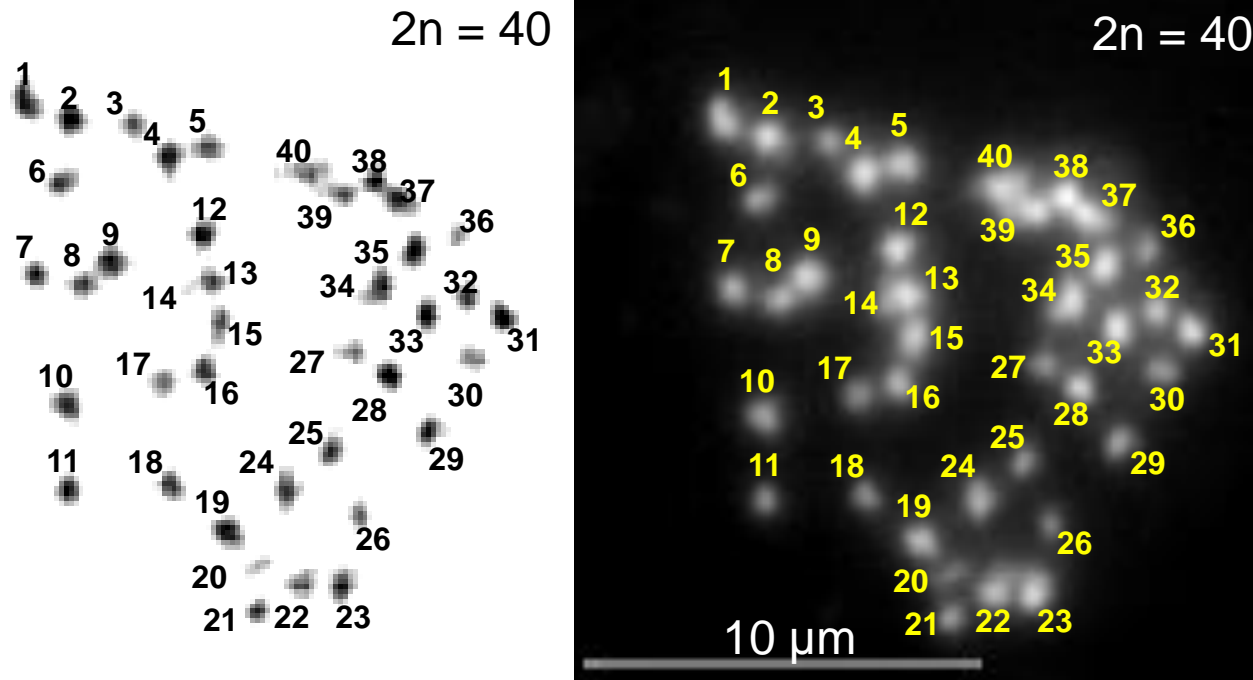
Lemnoideae are of interest for genome and karyotype evolution studies



Les et. al. Sys. Bot. 2002
 Nauheimer et. al. N. Phytol. 2012
 Appenroth et al. CIBJ 2013

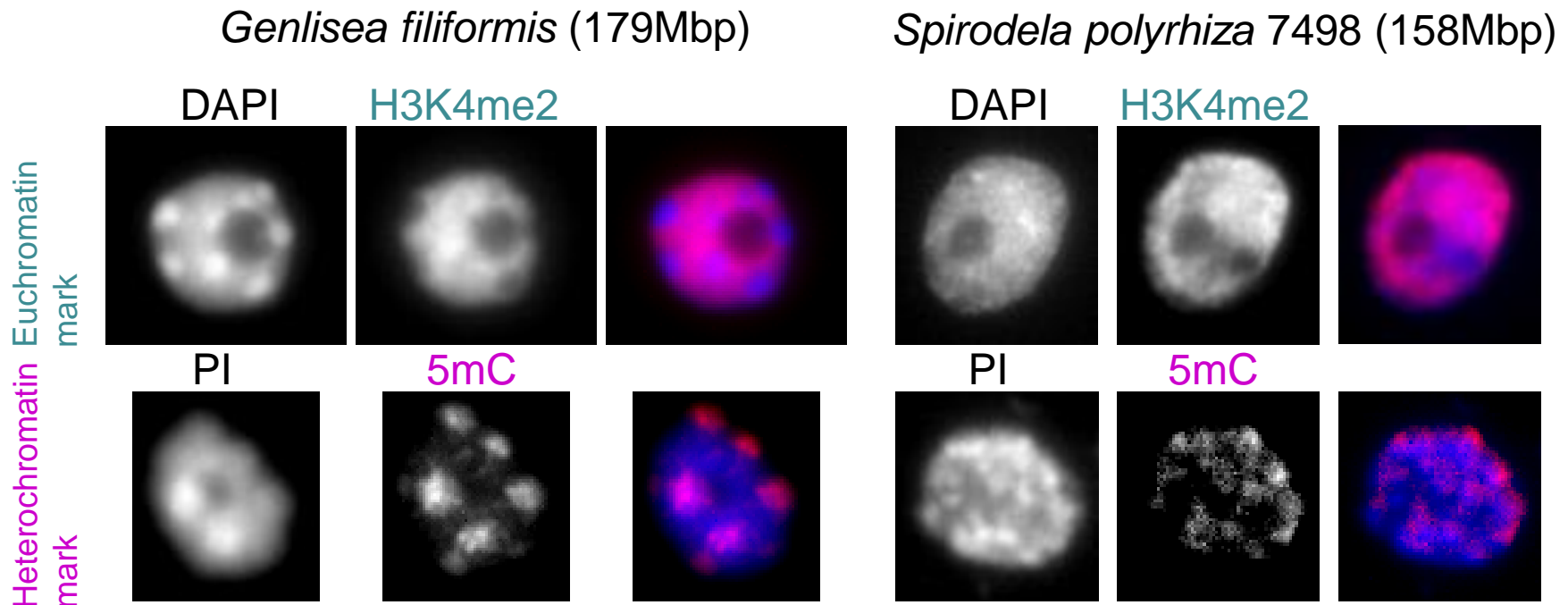
Wang et. al. J. Bot. 2011
 Landolt VGI-ETH 1986
 Geber Uni. Vienna 1989

Preliminary cytogenetic characterization of *Spirodela polyrhiza* clone 7498



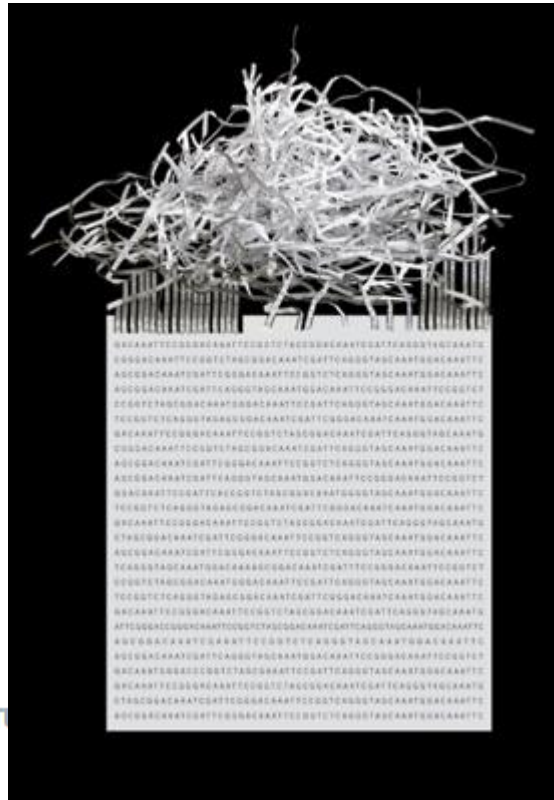
S. polyrhiza clone 7498 contains 20 chromosome pairs (somatic metaphase)

Preliminary cytogenetic characterization of *Spirodela polyrhiza* 7498



Typical heterochromatic chromocenters of small genomes are free of H3K4me2 but enriched in 5mC.

Constitutive heterochromatin which is visualized in *S. polyrhiza* interphase nuclei by propidium iodide (PI) staining is enriched in 5mC.



NATURE METHODS | TECHNOLOGY FEAT

De novo genome assembly: what every biologist should know

Monya Baker

“As more genomes are assembled from scratch, scientists are struggling to assess and improve their quality”

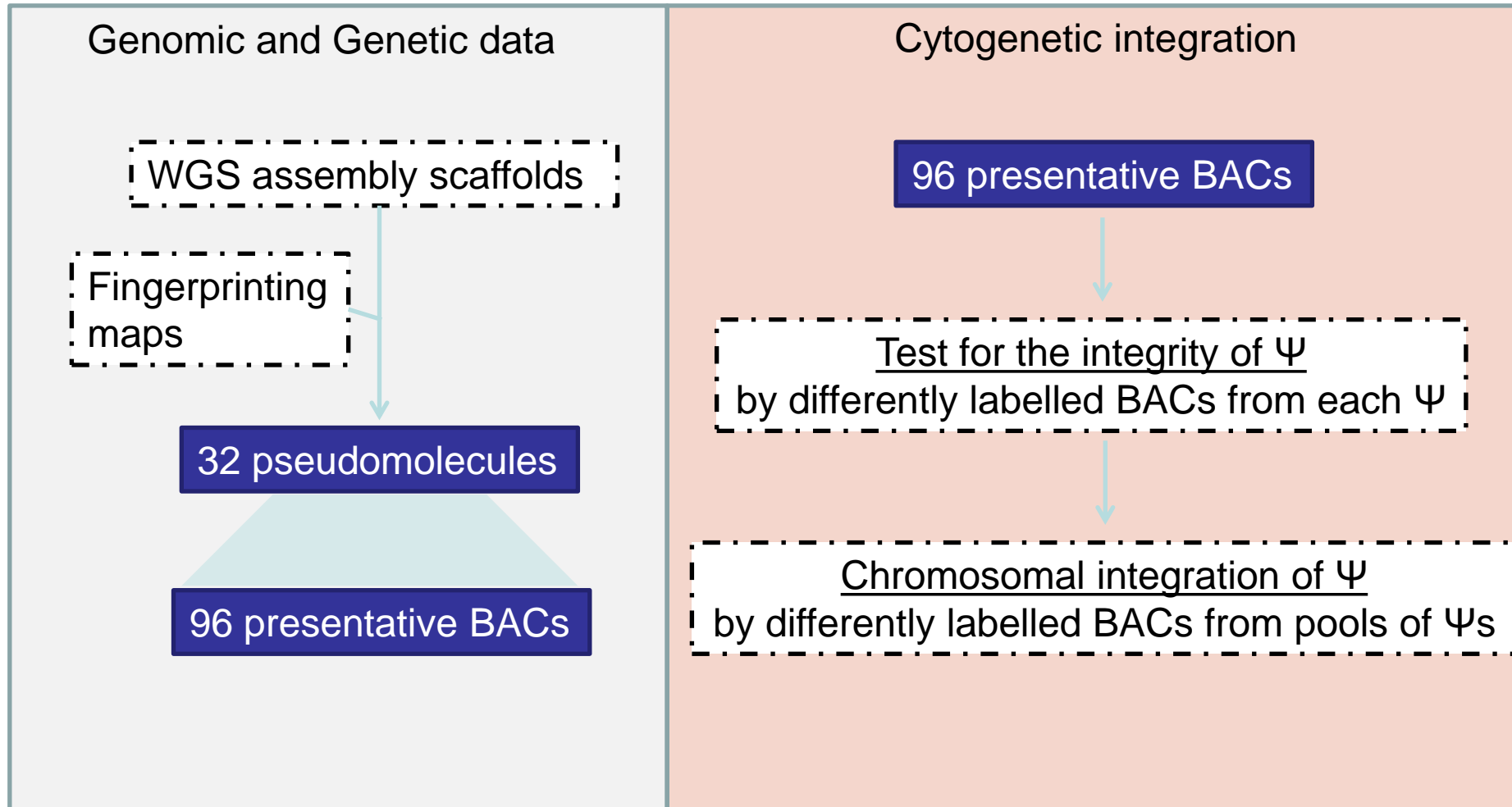
Nature Methods **9**, 333–337 (2012) | doi:10.1038/nmeth.1935

Published online 27 March 2012

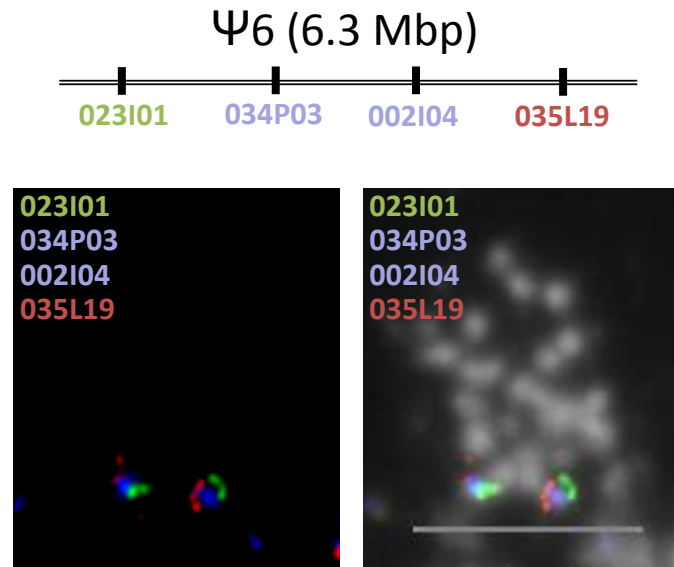
FISH as a finishing genome tool

- **Challenges:** Genome size, complexity (heterozygosity and/or ploidy), repetitive sequence content and composition
- Integration of multiple sequencing technologies, mapping and transcriptomics datasets
- Finishing genome: gap closure, assembly validation and refinement
- Multicolor Fluorescence in situ Hybridization (mcFISH):
 - to map genomic fragments/ library clones to specific chromosomal regions
 - to validate the integrity of the WGS scaffolds
 - to validate and support the integration of WGS and physical maps
 - to detect chromosome rearrangements, chromosome homeology and karyotype evolution in related non-sequenced species
- *Spirodela polyrhiza* / duckweeds contains:
 - high number of small chromosomes
 - no/very difficult to obtain genetic maps

Strategy for chromosomal integration of pseudomolecules (Ψ s) by FISH

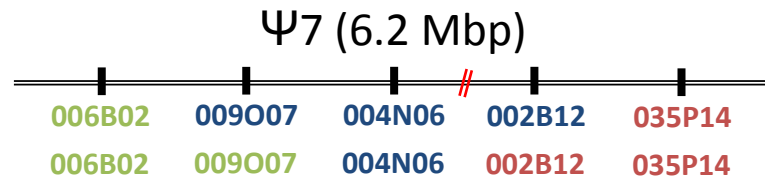


Validation of the integrity of the pseudomolecules



$\Psi 6$ is present on one chromosome pair

Validation of the integrity of the pseudomolecules



1st FISH

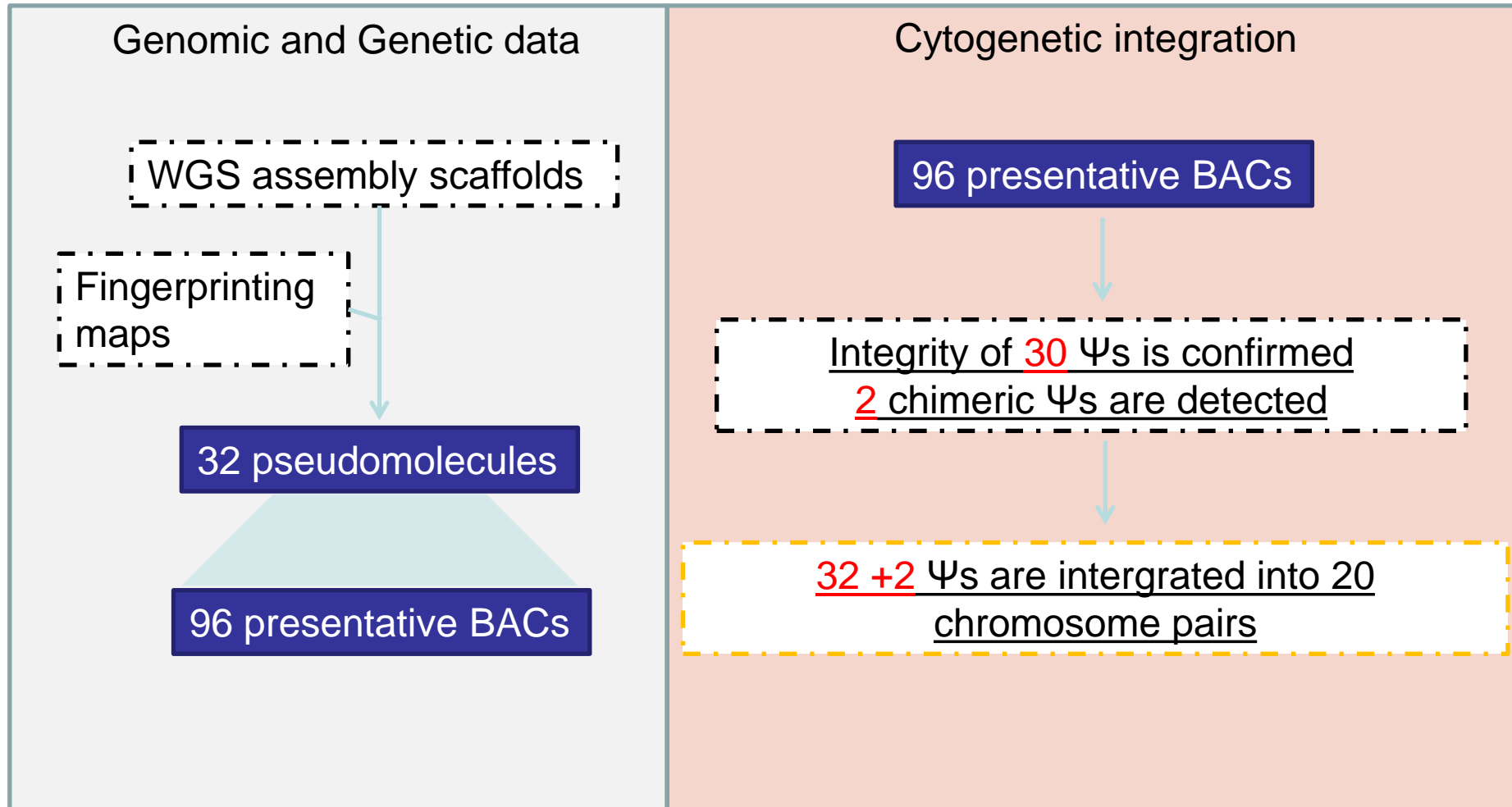


2nd FISH



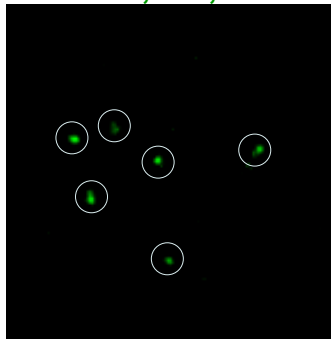
$\Psi 7$ is split into two chromosome pairs

Results of chromosomal integration of pseudomolecules (Ψ) by FISH

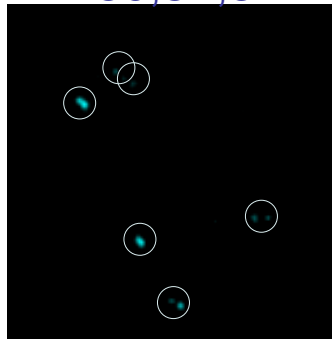


Integration of 32 + 2 Ψ s into 20 chromosomes

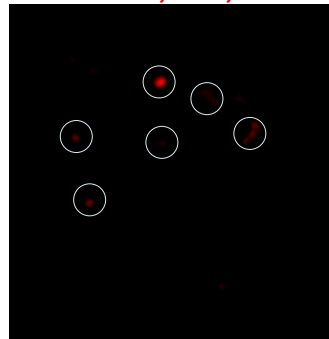
Ψ 24,25,26



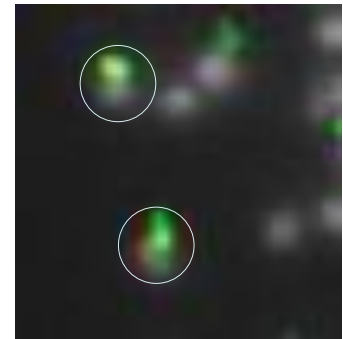
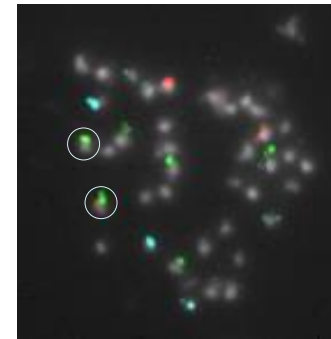
Ψ 30,31,32



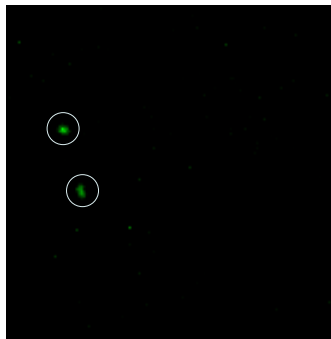
Ψ 27,28,29



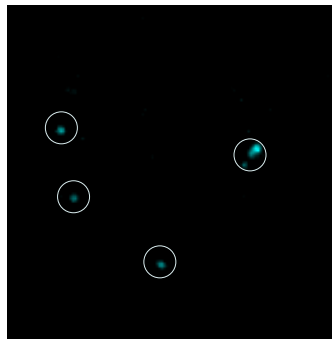
1st FISH



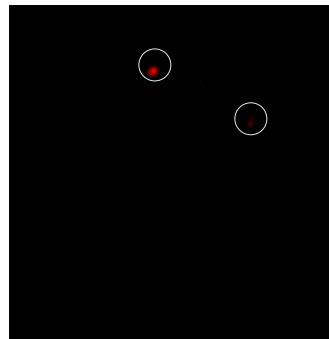
Ψ 25



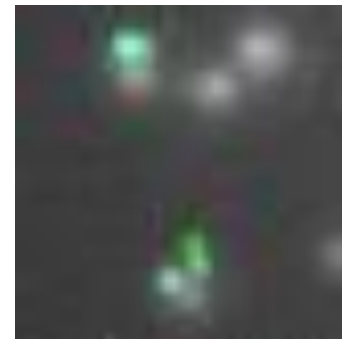
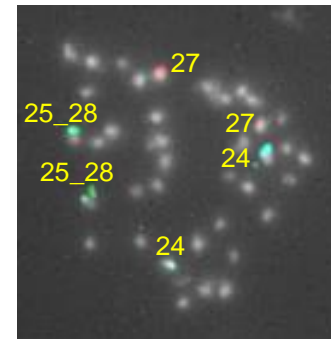
Ψ 24 & 28



Ψ 27

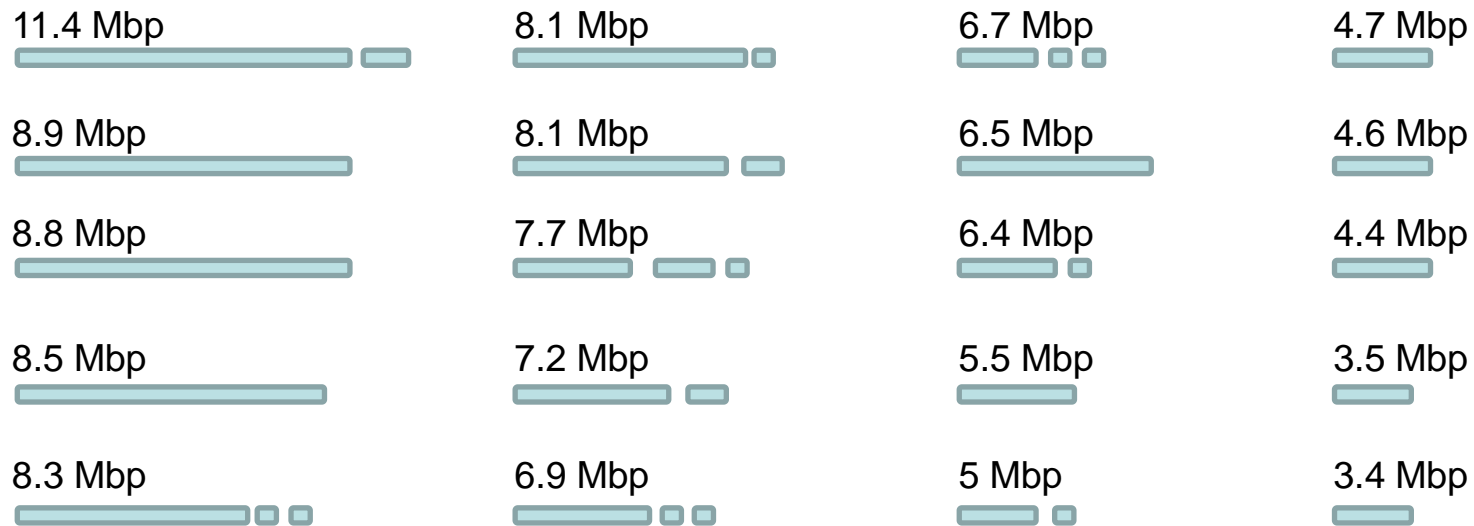


2nd FISH



Ψ 25 & 28 belong one chromosome pair

Integration of 32 + 2 Ψs into 20 chromosomes



Smallest size: 3.4 Mbp

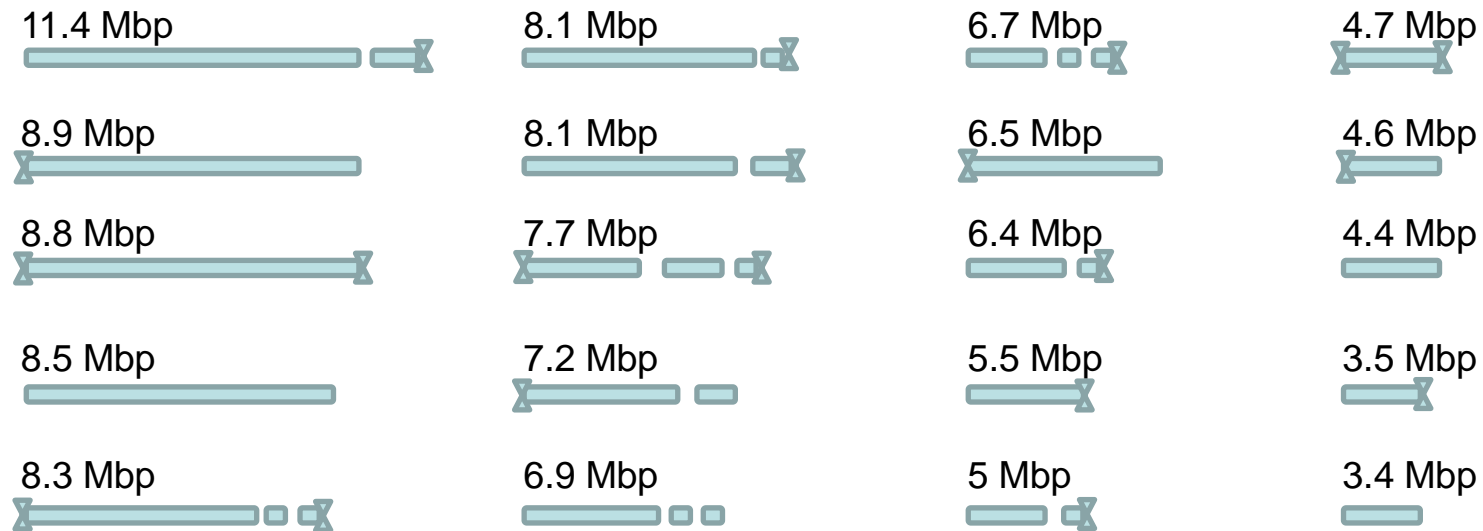
Largest size: 11.4 Mbp

Average size: 6.73 Mbp

Total size: 134.6 Mbp

 34 Ψs

Integration of 32 + 2 Ψs into 20 chromosomes



 34 Ψs

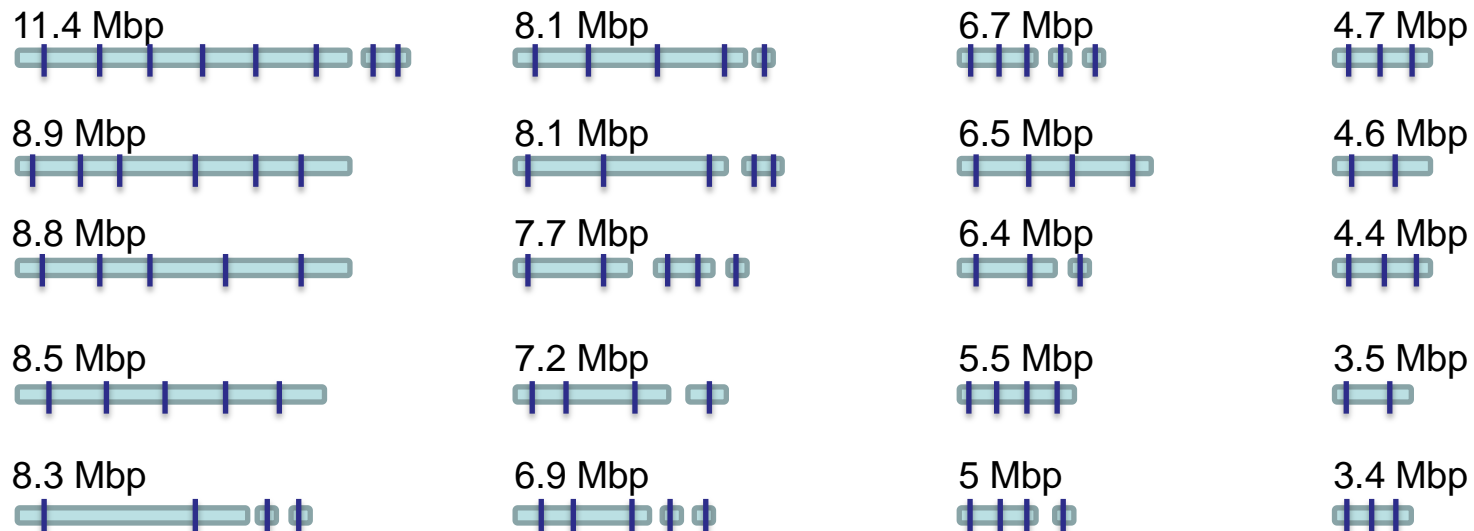
 Telomere repeats

20 predicted telomere repeats

4 chromosomal linkages flanked by 2 telomere repeat termini

12 chromosomal linkages flanked by 1 telomere repeat terminus

Spirodela cytogenetic map



Smallest number of markers per chromosome: 2

— 34 Ψ

Largest number of markers per chromosome: 8

| 85 BAC markers

Average number of markers per chromosome: 4

Total number of markers: 85

Future tasks

- Cytogenetic characterization of further Lemnoideae species
- Introducing more BAC markers in order to (1) define the break points of 2 chimeric Ψ s; (2) determine order & orientation of the Ψ s
- Studying chromosome homeology, genome and karyotype evolution within species of the same and other genera of Lemnoideae by means of comparative chromosome painting
- Establishing an optimized system/pipeline enabling a quick detection of chromosomal rearrangements in duckweed species/clones

Acknowledgement

- ❖ IPK, Gatersleben, Germany
 - Ingo Schubert
 - Joerg Fuchs
 - Martina Kuehne
 - Joachim Bruder
 - Rocío Rubio
- ❖ University of Jena, Germany
 - Klaus Appenroth
- ❖ Waksman Institute of Microbiology, Rutgers University, USA
 - Wenqin Wang
 - Joachim Messing

Spirodela polyrrhiza (L.) Schleid.
[as *Lemna polyrrhiza* L.]
Sowerby, J.E., English Botany, or
Coloured Figures of British Plants,
3th ed., vol. 9: t. 1397 (1869)

