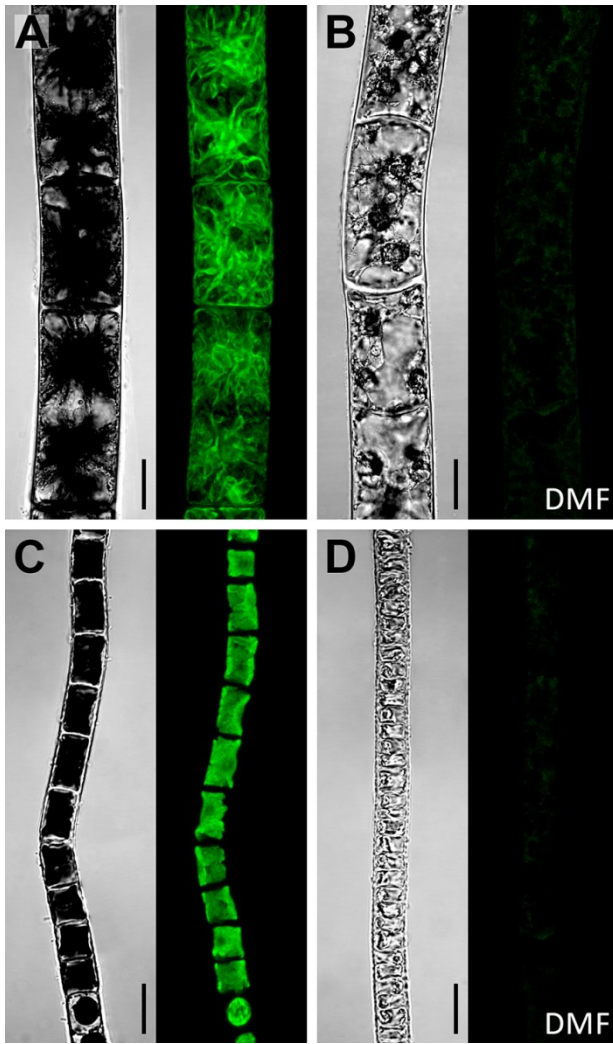


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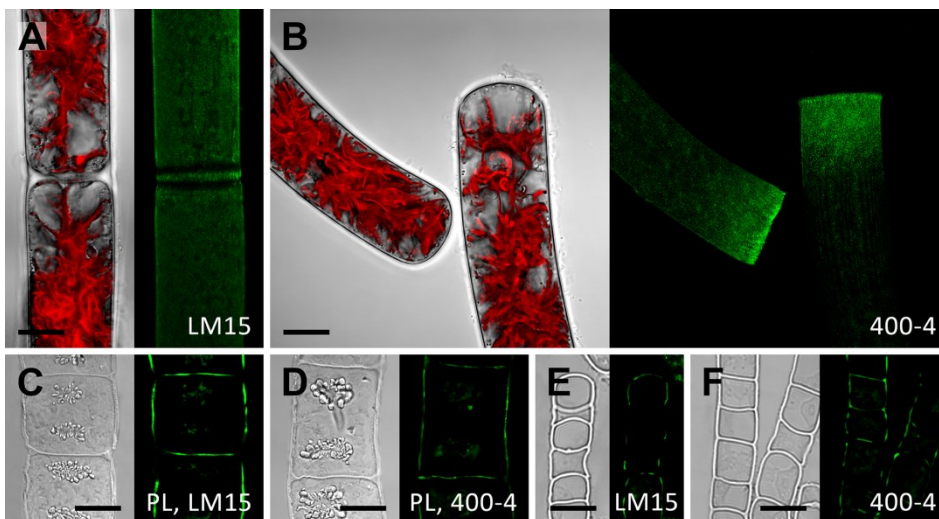


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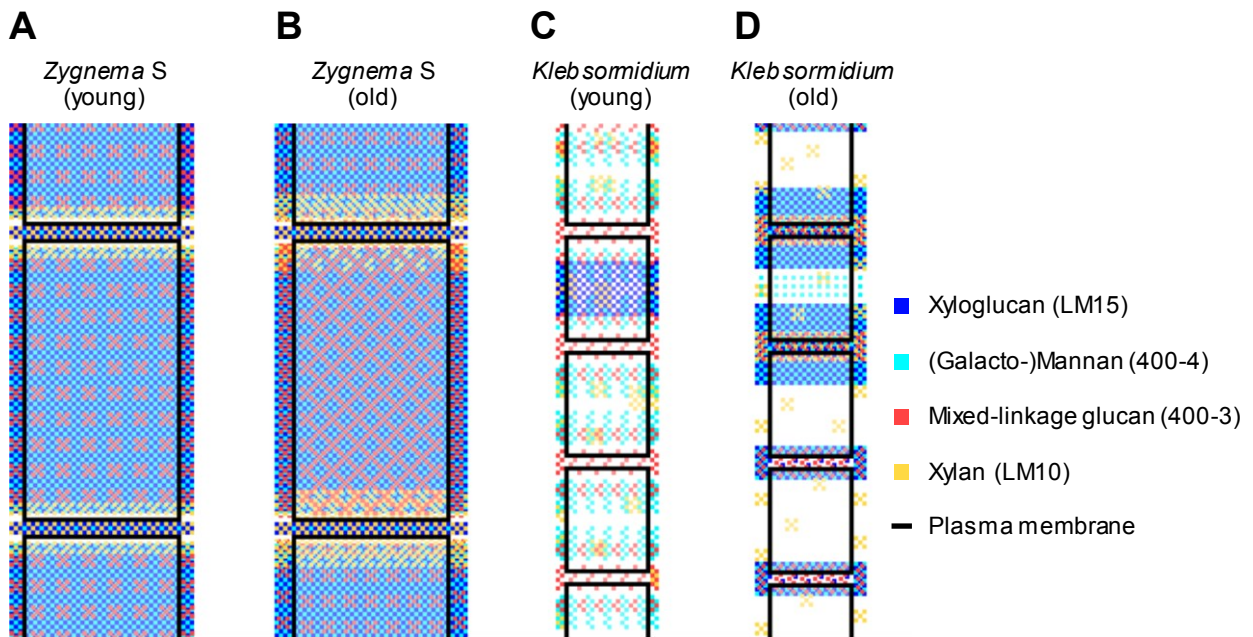
**University College Cork, Ireland**  
Coláiste na hOllscoile Corcaigh



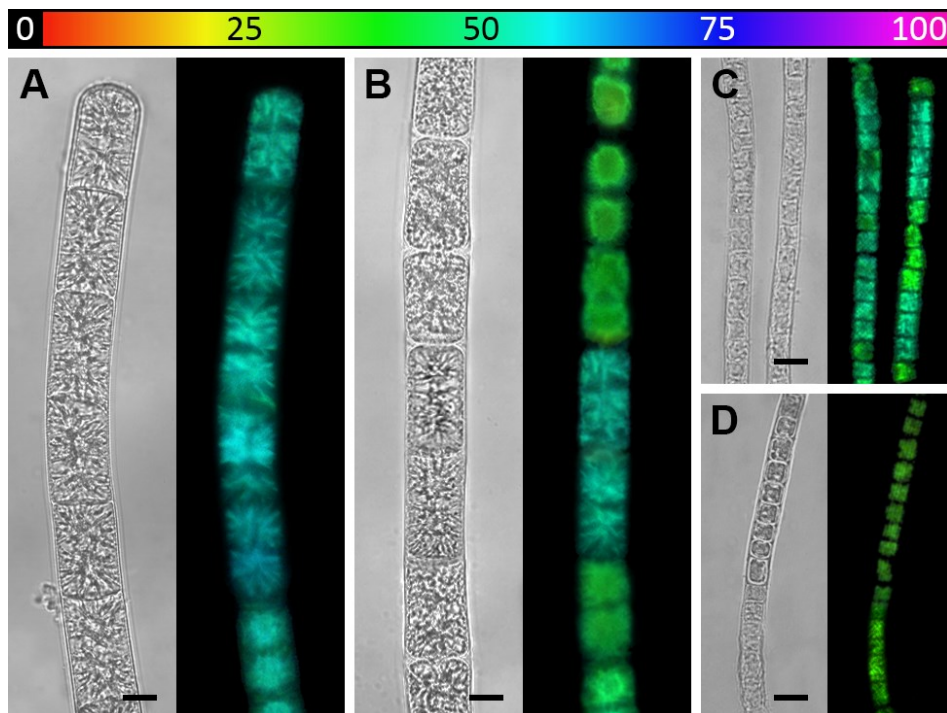
**Fig. S1. Effect of dimethylformamide (DMF) on chloroplast auto-fluorescence.** CLSM micrographs of *Zygnema S* (A, B) and *Klebsormidium crenulatum* (C, D) filaments before (A, C) and after (B, D) incubation in DMF. Bars = 10  $\mu$ m.



**Fig. S2. Immunolabelling of whole cells and semithin sections (CLSM micrographs).** *Zygnema S* (A-D) and *Klebsormidium crenulatum* (E, F) filaments labelled with the monoclonal antibodies LM15 or 400-4. Whole cell labelling (A, B), labelling of semithin sections (C-F). Red autofluorescence is shown. (A) Weak fluorescence in outer and cross cell walls. (B) Recently fragmented filament with fluorescence close to terminal cross cell walls. (C-F) Labelling in outer and cross cell walls. Bars 10 =  $\mu$ m



**Fig. S3. Hemicellulose distribution in filamentous charophytes.** Schemes are based on immuno-localisations of hemicellulose epitopes (antibody names in brackets) in young (1 month) and old (12 months) filaments of (A, B) *Zygnema S* and (C, D) *Klebsormidium crenulatum*.



**Fig. S4. Photosynthetic performance of *Zygnema S* and *Klebsormidium crenulatum*.** NIR and corresponding Y(II) images (false colour) of young (A, C) and old (C, D) filaments of *Zygnema S* (A, B) and *Klebsormidium* (C, D). The colour bar at the top indicates the relative Y(II) as a percentage. (A) Vacuolated cells containing two stellate chloroplasts and high Y(II) values ( $\sim 0.64$ ). (B) Akinete cells dominate old filaments and exhibit a lower Y(II) (i.e.  $\sim 0.41$ ) than younger (i.e. vacuolated, thin-walled, growing) cells within the same filament ( $\sim 0.6$ ). (C) Cells of young filaments with a higher Y(II) ( $\sim 0.48$ - $0.59$ ) than cells of old filaments (D) ( $\sim 0.3$ ). Bars = 10  $\mu\text{m}$ .

**Tab. S1 Evidence for major cell wall polysaccharides in Charophytes.**

Cell wall component	Klebsormidiophyceae	Charophyceae	Zygnematophyceae
Cellulose	A <sup>1,5</sup> B <sup>7</sup> C <sup>6</sup>	A <sup>5</sup> B <sup>8,7</sup>	A <sup>5</sup> B <sup>3,5,7</sup>
Callose	A <sup>5</sup> B <sup>3,5</sup> C <sup>6</sup>	A <sup>5</sup> B <sup>5,8</sup>	A <sup>5</sup> B <sup>3</sup>
<b>Pectins</b>			
HG	A <sup>1,5*</sup> B <sup>5</sup> C <sup>6</sup>	A <sup>5,7</sup> B <sup>8</sup>	A <sup>5,7</sup> B <sup>4,9,10</sup>
RGI/RGII	-	A <sup>5,7†</sup>	A <sup>7†</sup>
<b>Hemicelluloses</b>			
Xyloglucan	B <sup>5</sup> C <sup>6</sup>	A <sup>5</sup> B <sup>5,8</sup>	B <sup>5,8</sup>
MLG	B <sup>5</sup>	B <sup>5,8</sup>	A <sup>4,5</sup> B <sup>4,5</sup>
Mannans	B <sup>5</sup>	B <sup>5,8</sup>	B <sup>5</sup>
Xylans	B <sup>5</sup>	B <sup>5,8</sup>	B <sup>5</sup>
<b>(Glyco)Proteins</b>			
Arabinogalactan proteins	-	B <sup>5</sup>	B <sup>5</sup>
Expansins	C <sup>6</sup>	C <sup>11</sup>	C <sup>11</sup>
Extensins	C <sup>12</sup>	-	B <sup>5</sup>

Key: A biochemical, B immunological/histochemical, C genetically (genomic/transcriptomic data), ‘-’ no evidence.

\*Biochemical evidence for HG in Klebsormidiophyceae is ambiguous. While Domozych et al. (1980) and Sorensen et al. (2011) detected galacturonic acid in *K. flaccidum*, O’Rourke et al. 2015 suggested its absence in *K. flaccidum* and *K. subtile*.

†Suggested by the co-occurrence of 1→5-Ara f, 1→4-Gal p and 2→4-Rha p.

<sup>1</sup>Domozych et al. 1980, <sup>2</sup>Popper and Fry 2003, <sup>3</sup>Herburger and Holzinger 2015, <sup>4</sup>Eder et al. 2008, <sup>5</sup>Sørensen et al. 2011, <sup>6</sup>Hori et al. 2011, <sup>7</sup>O’Rourke et al. 2015, <sup>8</sup>Domozych et al. 2009, <sup>9</sup>Domozych et al. 2006, <sup>10</sup>Eder and Lütz-Meindl 2010,

<sup>11</sup>Vannerum et al. 2011, <sup>12</sup>Liu et al. 2016

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