Chromosome-scale genome assembly for the duckweed *Spirodela intermedia*, integrating cytogenetic maps, PacBio and Oxford Nanopore libraries

Phuong TN Hoang<sup>1,2,+</sup>, Anne Fiebig<sup>1,+</sup>, Petr Novák<sup>3</sup>, Jiří Macas<sup>3</sup>, Hieu X Cao<sup>1,4</sup>, Anton Stepanenko<sup>5,6</sup>, Guimin Chen<sup>5,6</sup>, Nikolai Borisjuk<sup>5,6</sup>, Uwe Scholz<sup>1</sup>, Ingo Schubert<sup>1\*</sup>

<sup>1</sup> Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, D-06466 Stadt Seeland, Germany

<sup>2</sup> permanent address: Biology Faculty, Dalat University, District 8, Dalat City, Lamdong Province, Vietnam

<sup>3</sup> Biology Centre, Czech Academy of Sciences, Institute of Plant Molecular Biology, České Budějovice, CZ-37005 Czech Republic

<sup>4</sup> present address: Martin-Luther-University Halle-Wittenberg, Institute of Biology, D-06120, Germany

<sup>5</sup> Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake, School of Life Sciences, Huaiyin Normal University, Huai'an, 223300, China

<sup>6</sup> Jiangsu Collaborative Innovation Centre of Regional Modern Agriculture & Environmental Protection, Huaiyin Normal University, Huai'an, 223300, China

+These authors contributed equally to this work

\* Corresponding author: <u>schubert@ipk-gatersleben.de</u>, Tel: +49 394825239

IS: ORCID ID 0000-0002-6300-2068; AF: ORCID 0000-0003-3159-3593; US: ORCID 0000-0001-6113-3518; HC: ORCID ID 0000-0003-1230-4127; PTNH: ORCID ID 0000-0002-7418-9091; AS: ORCID ID 0000-0003-2326-6613; NB: ORCID ID 0000-0001-5250-9771; JM: ORCID ID 0000-0003-0829-1570; PN: ORCID ID 0000-0002-5068-9681;

## Table S1: Statistics of gene prediction and functional annotation

	S. intermedia clone 7747	S. intermedia clone 8410	S. polyrhiza clone 9509ª
Total number of gene models	22.245	21,594	20,659
3' truncated genes	38	8	149
5' truncated genes	2,981	327	3,350
Internal stop codons	1	0	277
Number of coding transcripts	22,116	21,546	16,877
Proteins with functional annotation	15,754	15,558	12,352
Protein coding genes with GO terms	11,166	10,234	8,398
Protein coding genes with eggNOG	16,162	16,493	11,327
Proteins with Pfam annotation	15,908	15,165	8,302

a) data taken from Michael et al., (2017)<sup>25</sup>

 Table S2: Number of genes associated with eggNOG functional categories

eggNOG category	S. intermedia	S. intermedia	S. polyrhiza
	7747	8410	9509
CELLULAR PROCESSES AND SIGNALING			
[D] Cell cycle control, cell division,	268 (1.6%)	362 (2.0%)	199 (1.7%)
[M] Cell wall/membrane/envelope biogenesis	249 (1.5%)	224 (1.3%)	189 (1.6%)
[N] Cell motility	2 (0.0%)	2 (0.0%)	2 (0.0%)
[O] Post-translational modification, protein	1507 (9.0%)	1511 (8.6%)	1069 (9.1%)
[T] Signal transduction mechanisms	1523 (9.1%)	1523 (8.6%)	1065 (9.0%)
[U] Intracellular trafficking, secretion and	651 (3.9%)	600 (3.4%)	486 (4.1%)
[V] Defense mechanisms	133 (0.8%)	143 (0.8%)	82 (0.7%)
[W] Extracellular structures	56 (0.3%)	43 (0.2%)	41 (0.3%)
[Y] Nuclear structure	40 (0.2%)	37 (0.2%)	37 (0.3%)
[Z] Cytoskeleton	225 (1.3%)	235 (1.3%)	190 (1.6%)
INFORMATION STORAGE AND PROCESSING			
[A] RNA processing and modification	521 (3.1%)	693 (3.9%)	415 (3.5%)
[B] Chromatin structure and dynamics	156 (0.9%)	193 (1.1%)	116 (1.0%)
[J] Translation, ribosomal structure and	626 (3.7%)	723 (4.1%)	430 (3.6%)
[K] Transcription	1466 (8.7%)	1478 (8.4%)	1068 (9.1%)
[L] Replication, recombination and repair	535 (3.2%)	935 (5.3%)	275 (2.3%)
METABOLISM			
[C] Energy production and conversion	698 (4.2%)	540 (3.1%)	308 (2.6%)
[E] Amino acid transport and metabolism	435 (2.6%)	537 (3.0%)	306 (2.6%)
[F] Nucleotide transport and metabolism	134 (0.8%)	144 (0.8%)	114 (1.0%)
[G] Carbohydrate transport and metabolism	962 (5.7%)	993 (5.6%)	674 (5.7%)
[H] Coenzyme transport and metabolism	199 (1.2%)	202 (1.1%)	155 (1.3%)
[I] Lipid transport and metabolism	470 (2.8%)	511 (2.9%)	348 (2.9%)
[P] Inorganic ion transport and metabolism	431 (2.6%)	394 (2.2%)	338 (2.9%)
[Q] Secondary metabolites biosynthesis,	529 (3.1%)	556 (3.1%)	387 (3.3%)
POORLY CHARACTERIZED			
[S] Function unknown	4992 (29.7%)	5080 (28.8%)	3503 (29.7%)

Table S3: Average similarity, length and chromosomal assignment of whole BAC sequences from *S. polyrhiza* on *S. intermedia* assemblies from PacBio (clone 7747) and Hybrid ON/Illumina (clone 8410)

BAC	Sp9509 (ON)	Si	i7747 (PacBio)		Si8410 (Hyb	rid ON/Illun	nina)
DAC	ChrSp (=gi )	ChrSi_7747	Ave. simil	length	ChrSi_8410	Ave. simil	length
030D21	gi   1	Si01	87,46	60112	jcf159735_S01	87,61	59666
014L22	gi   1	Si01	87,12	81806	jcf159735_S01	87,15	88220
019A13	gi   1	Si01	87,1	59976	jcf159735_S01	87,17	60103
012014	gi   1	Si01	85,08	46416	jcf159735_S01	85,16	47916
032E03.	gi   1	Si01	86,56	45578	jcf159735_S01	86,17	48318
003D17	gi 1	Si01	85,91	50373	jcf159735_S01	85,75	51344
004D21	gi 2	Si02	86,27	53252	jcf159572_S02	86,3	58720
005N05	gi 2	Si02	86,53	74035	jcf159572_S02	97,39	67910
037B02	gi 2	Si02	87,35	68814	jcf159572_S02	87,64	69089
003H12	gi 2	Si02	84,25	30588	jcf159572_S02	84,72	28285
021D24	gi 3	Si04	85,37	52775	jcf159718_S04	85 <i>,</i> 57	52789
002E10.	gi 3	Si04	84,9	50015	jcf159718_S04	86,81	38546
002K06	gi 3	Si03	85,95	83901	jcf159755_S03	85,88	79771
011P20	gi 3	Si03	88,53	20882	jcf159755_S03	87,47	20336
010J02	gi 3	Si03	85,25	53304	jcf159755_S03	85	52948
006F09	gi 4	Si05	85,98	37528	jcf159694_S05	89,95	39275
013D18	gi 4	Si05	86,16	32980	jcf159694_S05	85,93	35398
035L13	gi 4	Si05	85,91	71580	jcf159694_S05	85,59	75905
017E03.	gi 4	Si05	85,49	49432	jcf159694_S05	85,82	46564
030K22	gi 5	Si06	83,8	35963	jcf159561_S06_3	84,25	35725
027D17	gi 5	Si06	85,39	27060	jcf159561_S06_3	85,17	28765
009A15	gi 5	Si06	85	53927	jcf159561_S06_3	85,67	45060
009020	gi 5	Si06	85,77	54215	jcf159561_S06_3	86,46	59510
023101	gi 6	Si06	86,17	45086	jcf159561_S06_3	86,13	45287
033A10	gi 6	Si06	83,85	116778	jcf159633_S07	83,35	5641
002104	gi 6	Si07	85,72	39814	jcf159633_S07	86,06	38432
026N01	gi 6	Si07	85	42591	jcf159633_S07	85,31	42343
035L19	gi 6	Si03	86,39	9519	jcf159755_S03	86,41	9518
014F03	gi 7	Si07	83,47	26249	jcf159633_S07	84,43	24575
035P21	gi 7	Si07	85,98	42029	jcf159633_S07	85,94	45303
004A04	gi 7	Si08	84,86	42563	jcf159638_S08	85,42	41477
030B11	gi 7	Si08	86,29	37369	jcf159638_S08	86,93	39912

013104	gi 8	Si09	86,95	54696	jcf159258_S09	86,3	61589
006P24	gi 8	Si09	84,6	22078	jcf159258_S09	84,57	21724
032L08	gi 8	Si09	86,25	67943	jcf159258_S09	86,17	73293
034K03	gi 8	Si09	86	76355	jcf159258_S09	86,22	77363
004E01.	gi 8	Si09	84,2	30708	jcf159258_S09	84,63	30343
006L17	gi 8	Si09	84,74	59271	jcf159258_S09	85,1	59976
037H06	gi 9	Si10	85,94	84892	jcf159563_S10	85,96	81218
025016	gi 9	Si10	85,68	59148	jcf159563_S10	85,85	58340
019G12	gi 9	Si10	86,05	30432	jcf159563_S10	85,77	44605
024C02	gi 9	Si10	85,63	65769	jcf159563_S10	85,7	64611
036C07	gi 9	Si10	86,17	69591	jcf159563_S10	86,33	71742
001D13	gi 9	Si10	84,51	31843	jcf159563_S10	84,35	38379
035P14	gi 10	Si11	84,88	33727	jcf159580_S11	85,03	37048
002B12	gi 10	Si11	85,33	51252	jcf159580_S11	85,39	64004
037K21	gi 10	Si11	86,68	44661	jcf159580_S11	86,4	53612
008G11	gi 10	Si11	83,42	40388	jcf159580_S11	84,1	40591
020G09	gi 10	Si12	85,73	50729	jcf159635_S12	85,77	43981
028B20	gi 10	Si12	84,89	45472	jcf159635_S12	84,95	47152
026G16	gi 10	Si12	86,92	55062	jcf159635_S12	87,03	52791
019103	gi 11	Si13	86,83	56535	jcf159540_\$13	86,8	56717
010P10	gi 11	Si13	85,3	34147	jcf159540_\$13	85,82	34902
004G03	gi 11	Si13	84,37	33178	jcf159540_S13	83,87	36537
011L15	gi 11	Si13	87,11	39402	jcf159540_S13	83,87	36537
001C15	gi 11	Si13	86,66	67380	jcf159540_S13	86,89	65754
028L12	gi 12	Si14	86,43	27269	jcf159533_S14	86,5525	27264
035F17	gi 12	Si14	86,53	74050	jcf159533_S14	86,36	82780
030D24	gi 12	Si14	84,89	26996	jcf159533_S14	88,6	29763
010L16	gi 13	Si15	86,95	57898	jcf159593_S15	86,07	69048
008B06	gi 13	Si15	83,88	19711	jcf159593_S15	84,19	20862
031B15	gi 13	Si15	84,76	35280	jcf159593_S15	85,19	36491
020L20	gi 13	Si15	86,1	60590	jcf159593_S15	86,1	63574
003B08	gi 14	Si07	84,94	68038	jcf159633_S07	86,1	69838
036F14	gi 14	Si07	86,23	57142	jcf159633_S07	86,51	53981
040G15	gi 14	Si07	85	71317	jcf159633_S07	85,2	72711
037118	gi 14	Si03	85,5	22859	jcf159755_S03	85,34	25230
006D12	gi 14	Si03	84,21	42787	jcf159755_S03	84,54	29381
011C23	gi 15	Si16	80	19792	jcf159680_S16	79	25137

021P03	gi 15	Si16	87	28053	jcf159680_S16	86,22	33699
020E12.	gi 15	Si16	84,13	67518	jcf159680_S16	84,68	71876
035L20	gi 15	Si16	85	26169	jcf159680_S16	84,68	32086
009J15	gi 16	Si11	85,4	57130	jcf159580_S11	85,8	57445
002L10	gi 16	Si11	84,89	14497	jcf159580_S11	84,46	36142
009007	gi 17	Si04	86,52	30662	jcf159718_S04	87,82	47243
004N06	gi 17	Si04	82,02	177344	jcf159718_S04	84,63	116454
009L02	gi 17	Si04	85,86	59364	jcf159718_S04	85,92	52240
037B13	gi 18	Si09	86,57	39229	jcf159258_S09	87,1	36333
029K19	gi 18	Si09	86,27	37344	jcf159258_S09	86,1	41667
015L03	gi 19	Si17	80,5	23281	jcf159710_S17	80,3	43506
015J20	gi 19	Si17	83,1	74308	jcf159710_S17	83,2	46935
037H19	gi 19	Si17	85,4	47128	jcf159710_S17	85,29	55129
008A12	gi 19	Si17	88,35	309	jcf159710_S17	88,39	310
006C19	gi 20	Si18	86,5	67472	jcf159617_S18	86,1	67065
007A03	gi 20	Si18	87,22	94416	jcf159617_S18	86,1	95006
013004	gi 20	Si16	80,4	166821	jcf159680_S16	80,3	76462

Table S4: BLAST data of whole BAC sequences (029A10, 028I16 and 037J09) against assemblies for *S. polyrhiza* clones 7498 (Cao et al., 2016) and 9509 (Michael et al., 2017)

BAC			S	p7498 asse	mbly					g	p9509 asso	embly		
DAC	sseqid	pident	length	qstart	qend	sstart	send	sseqid	pident	length	qstart	qend	sstart	send
		99.53	22983	68796	91756	5822175	5799227		99.62	22173	118016	140148	5754022	5731861
		99.73	19450	91701	111136	5798870	5779431		99.67	7259	110726	117974	5761320	5754063
		99.41	16845	1	16811	5891712	5874872		99.81	8457	102185	110637	5769858	5761406
		99.44	15999	47861	63838	5843071	5827104		99.71	25957	76217	102154	5795842	5769889
		99.72	13817	111090	124885	5779242	5765428		99.66	8635	67463	76079	5804617	5795983
		99.44	13126	23910	37014	5867460	5854348		98.44	2812	64625	67430	5807531	5804728
		99.49	6476	41412	47887	5849805	5843358		99.48	7465	57275	64735	5814834	5807380
029A10	pseudo1	99.57	5640	128685	134314	5760759	5755130	CP019095.1	99.74	5039	52166	57202	5819952	5814919
		99.69	4789	16742	21528	5874847	5870060		99.76	1691	50392	52081	5821718	5820028
		99.16	4414	36977	41378	5854226	5849828		99.50	8912	41410	50312	5830711	5821829
		98.61	3964	64729	68670	5826171	5822215		99.82	2811	38560	41370	5833540	5830732
		99.63	2731	137420	140148	5751910	5749185		98.21	1728	35258	36971	5836919	5835201
		99.63	2424	21531	23951	5869905	5867484		99.74	12742	22467	35207	5849697	5836970
		99.61	2075	126653	128723	5763620	5761548		99.85	21478	1	21476	5872187	5850713
		99.10	1991	135482	137470	5754028	5752050							
		99.59	24197	19758	43939	5446478	5422300		99.61	46314	73255	119503	5284597	5238337
		99.35	12352	44831	57169	5420842	5408501		99.86	2933	84400	87331	5368531	5365600
		98.75	11240	72780	83999	5395318	5384128		98.85	6181	77798	83969	5375172	5369018
028116	pseudo1	99.44	6081	13707	19784	5452735	5446667	CP019095.1	99.73	3745	73028	76766	5379992	5376248
		99.77	5730	3005	8729	5463838	5458112		99.42	5312	66925	72213	5386154	5380843
		99.64	5582	66903	72480	5400918	5395339		99.79	1881	64852	66732	5388291	5386412

	99.45	4361	9352	13707	5457181	5452830		99.09	4052	60074	64100	5396082	5392035
	99.77	3031	1	3031	5467227	5464200		99.38	9140	50541	59676	5405666	5396538
	99.28	2934	84399	87331	5383451	5380533		99.68	5554	44935	50483	5411269	5405722
	99.36	2661	57169	59822	5408303	5405649		99.69	5829	38709	44529	5417618	5411795
	96.94	687	8716	9402	5458170	5457498		99.75	13444	25037	38476	5431348	5417914
								99.79	9377	15604	24979	5440783	5431409
								99.04	1143	14399	15539	5441999	5440864
								99.44	5008	8716	13711	5447729	5442727
								99.68	8734	1	8729	5456401	5447671
	99.68	22753	107896	130634	5260327	5237617		99.82	5636	132201	137835	5222680	5217046
	99.61	18693	28622	47292	5346162	5327484		99.74	10705	120518	131220	5234356	5223666
	99.57	11934	8337	20263	5364343	5352425		97.71	2268	69628	71877	5288109	5285850
	99.32	10980	89724	100672	5278776	5267803		99.83	10044	59797	69834	5297991	5287948
	99.66	7641	62154	69788	5312112	5304473		98.29	2401	55331	57725	5302503	5300122
	99.46	7222	130621	137835	5237591	5230376	CD010005 1	99.38	7555	47340	54894	5310532	5303021
	99.63	6147	20223	26359	5352325	5346183	CP019095.1	99.36	4694	42535	47225	5315344	5310653
	99.69	6052	79488	85532	5288615	5282568		99.19	3929	38822	42743	5319251	5315342
037J09 pseudo1	99.41	5428	1	5414	5373174	5367752		99.61	9682	28647	38324	5329400	5319727
	99.86	4323	103574	107896	5264870	5260548		99.69	7414	21179	28590	5336957	5329556
	99.37	3956	48408	52361	5326259	5322325		99.66	13713	7456	21145	5350686	5336985
	99.26	3537	85703	89235	5282315	5278780		99.36	7445	1	7431	5358179	5350747
	99.57	3058	74494	77544	5293937	5290881							
	99.89	2620	100651	103270	5267561	5264943							
	98.96	2681	5705	8369	5367163	5364484							
	99.64	2523	52310	54831	5321873	5319352		sseqid	Subject S	eq-id (ID of	the databa	ise hit)	
	98.84	2246	55326	57559	5318933	5316696		pident	Percentag	ge of identi	cal matche	S	

	99.53	2112	60048	62159	5314325	5312214	length	Alignment length					
	97.68	2203	69676	71853	5304636	5302439	qstart	Start of al	ignment in	query			
	98.07	1662	58418	60068	5315977	5314333	qend	End of alignment in query					
	99.11	447	47966	48410	5326679	5326233	sstart	Start of alignment in subject (database hit)					
	96.77	155	74343	74497	5294056	5293904	send	End of ali	gnment in	subject (da	tabase hit)		
	98.18	55	89444	89498	5968585	5968531							
	100.00	50	86637	86686	5281330	5281281							
	100.00	50	86686	86735	5281379	5281330							

Fig. S1: DAPI-stained metaphase chromosome complements of *S. intermedia* clones 7747 and 8410 both showing 36 chromosomes. Scale bars: 5µm





Fig. S2: Percental distribution of genes associated with eggNOG functional categories within three *Spirodela* genomes.

Fig. S3: Conserved location of 5S and 45S rDNA on S. intermedia clones 8410 and 7747. (a) 5S rDNA is located on ChrSi 15 (corresponding to ChrSp 13) (b) 45S rDNA is located on ChrSi 01 (corresponding to ChrSp 01); Scale bars =  $5\mu m$ 



Fig. S4: Sequence alignment of two 5S rDNA units of *S. intermedia* clones 7747 and 8410. The 5S rRNA gene (in grey) is followed by the of non-transcribed spacer.

7747-1 gggtgcgatcataccagcactaatgcaccggatcccatcagaactccgaagttaagcgtg 8410-1 gggtgcgatcataccagcactaatgcaccggatcccatcagaactccgaagttaagcgtg 7747-2 gggtgegatcataccagcactaatgcaccggatcccatcagaactccgaagttaagegtg 8410-2 gggtgegateataccagcactaatgeaccggateccateagaacteegaagttaagegtg 7747-1 cttqqqcqaqaqtaqtactaqqatqqqtqacctcctqqqaaqtcctcqtqttqcaccccc8410-1 cttqqqcqaqaqtaqtactaqqatqqqtqacctcctqqqaaqtcctcqtqttqcaccccc7747-2 cttqqqcqaqaqtaqtactaqqatqqqtqacctcctqqqaaqtcctcqtqttqcaccccc8410-2 ettqqqeqaqaqtaqtaetaqqatqqqtqaceteetqqqaaqteeteqtqttqeaceeec 7747-1 ttccccctttttgcttgttgc---ttgttgctgccgcggaagtcgccgacagaccaaccc 8410-1 tteeccetttttgettgttge---ttgttgetgeegeggaagtegeegacagaceaacee 7747-2 8410-2 \*\*\*\*\*.\*.\*\*\*\*\*\*\* \*\*\*\*\*\*\*\*\*\* \*\*\*\*\*\*\*\*\*\* 7747-1 ccctatcctactactactacccctqtgagcgaggcgacttcctcqtccgctcggctct 8410-1 ccctatcctactactactacccctgtgagcgagcgacttcttcgtccgctcggctct 7747-2 ----ccccctaccttattacccttgtgagcgaggcgacttcttcgtgcggtcggctct 8410-2 -----ccccctaccttattacccttgtgagcgaggcgacttcttcgtgcggtcggctct 7747-1 etacagegegttgtegetacgaagetaaggacaagegeaagetaactaactttegggagt 8410-1 7747-2 ctacagegegttgtegetaegaagecaaggacaagtgcaagetaactaactttegggagt 8410-2 ctacagegegttgtegetacgaagecaaggacaagtgeaagetaactaactttegggagt 7747-1 gtttcgggcacgtttgggcgcttaggaagggactcggaagacgggggccgcgcgttatgg 8410-1 gtttcgggcacgtttgggcgcttaggaagggactcggaagacgggggccgcgcgttatgg 7747-2 gtttcgggcacgtttgggcgcttaggaaggg-ctcggaagacgggggccgcgcgttatgg 8410-2 gtttcgggcacgtttgggcgcttaggaaggg-ctcggaagacgggggccgcgcgttatgg \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* 7747-1 gtgcggtgcgacctcccccagcggcgagagccatctcgcatctgcgctgctcggaggggc 8410-1 gtgeggtgegaceteececageggegagagecatetegeatetgegetgeteggagggge 7747-2 gtgcggtgcgacctcccccagcggcgagagccatctcgcatctgcgctgcttgga-gggc 8410-2 gtgcggtgcgacctcccccagcggcgagagccatctcgcatctgcgctgctcggaggggc 7747-1 gtcagcctccctcctcgtgggtcgatcgtctcacgggcccggcccggcccggcttggcga 8410-1 7747-2 8410-2 gtcagcctccctcctcgtgggtcgatcgtctcacgggcccggcccggcccggcttggcga 7747-1 tggaggtggaggggagagagggggggggagagaatgagaccggatgcggcggatgtgacatc 8410-1 tqqaqqtqqaqqqqqqqqqqqqqqqaqaatqaqaccqqatqcqqcqqatqtqacatc 7747-2 8410-2 tggaggtggagagagagaggggggggggagagaatgagaccggatgcggcggatgtgacatc