

Supplementary Information for:

Identification of 13 *Spirogyra* species (Zygnemataceae) by traits of sexual reproduction induced under laboratory culture conditions

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Supplementary Table S1. List of localities of samples from which 122 new strains of *Spirogyra* (S.) were collected.

Locality and habitat	Water temperature (°C)	pH	Strain*	Species and <i>rbcL</i> -type**	New <i>rbcL</i> genes used for present phylogenetic analysis	New <i>atpB</i> genes used for present phylogenetic analysis
A pond in Toneri park, Tokyo (35°47'52.1"N 139°46'23.0"E) (29 November 2013)	11	7.4	T8, T15	S. sp.*** JPS037		
			T9	S. sp. JPS050		
			<u>T11</u>	S. sp. JPS050	MK558113	MK558151
A pond in Ukima park, Toyko (35°47'33.5"N 139°41'31.7"E) (11 December 2013)	10	8.2	<u>Uki1 [=NIES-4302]</u>	<i>S. chungkingensis</i> Jao JPS001	MK558136	MK558167
			Uki2	S. sp. JPS001		
			Uki5, Uki9, Uki11	S. sp. JPS050		
A pond in Mitsugi park, Tokyo (35°46'21.3"N 139°41'37.6"E) (14 December 2013)	10	7.1	<u>mit0203</u>	S. sp. JPS030	MK558104	MK558176
			<u>mitA01</u>	S. sp. JPS031	MK558135	MK558186
			<u>mitA02</u>	S. sp. JPS031		
			mitA06, mitA07	S. sp. JPS030		
			mitA04, mitA05	S. sp. JPS001		
A pond in Arakaza Sizen park, Tokyo (35°44'24.5"N 139°47'06.8"E) (25 December 2013)	6	7.6	<u>A3</u>	S. sp. JPS016	MK558103	MK558175
			A6	S. sp. JPS016		
			A4	S. sp. JPS050		
			A5	S. sp. JPS050		
			<u>A2F [=NIES-4303]</u>	<i>S. corrugata</i> Transeau JPS002	MK558126	MK558170
			<u>Tpx8 [=NIES-4314]</u>	<i>S. punctata</i> Cleve JPS013	MK558127	MK558169
Paddy fields in Kamogawa city, Chiba1 (35°07'48.4"N 139°58'27.7"E) (16 May 2014)	22.6	9.0	sen0301, sen0402	S. sp. JPS002		
			senA1001, senA2004, sen01401, senB2002	S. sp. JPS015		
			<u>senA1602</u>	S. sp. JPS038	MK558108	MK558180
			senA2302, senA2305, senB2003, senB2005, senB2605	S. sp. JPS038		
			<u>senA2002</u>	S. sp. JPS039	MK558111	MK558150
			<u>senA2303</u>	S. sp. JPS040	MK558138	MK558172
			<u>senB2001</u>	S. sp. JPS041	MK558142	MK558185
			senB2004	S. sp. JPS041		
			<u>senB2603</u>	S. sp. JPS042	MK558120	MK558155
			<u>senB2604</u>	S. sp. JPS043	MK558122	MK558145
			<u>sen0102</u>	S. sp. JPS044	MK558110	MK558149
			sen0502	S. sp. JPS044		
			<u>sen0103</u>	S. sp. JPS045	MK558129	MK558181
			<u>sen01505</u>	S. sp. JPS046	MK558130	MK558191
			<u>sen0406</u>	S. sp. JPS047	MK558125	MK558156
A narrow stream	14.2	7.6	<u>kit0101</u>	S. sp. JPS027	MK558118	MK558154

in Kitayama park,
Toyko
(35°45'59.9"N
139°27'31.2"E)
(6 April 2015)

			<u>kit0201</u> [=NIES-4307] <u>kit0301, kit0401</u> <u>kit2</u> <u>kit42B</u>	<i>S. longata</i> (Vaucher) Kützing JPS006 <i>S. sp.</i> JPS006 <i>S. sp.</i> JPS028 <i>S. sp.</i> JPS029	MK558124 MK558094 MK558096	MK558143 MK558162 MK558159
Paddy fields in Yasu city, Shiga (35°05'31.9"N 136°04'21.9"E) (8 April 2015)	9.0	8.1	<u>shi1101</u> <u>shi1701</u> <u>shi0303</u> <u>shi0305</u> [=NIES-4311] <u>shi0308</u>	<i>S. sp.</i> JPS021 <i>S. sp.</i> JPS038 <i>S. sp.</i> JPS048 <i>S. mirabilis</i> (Hassall) Kützing JPS010 <i>S. sp.</i> JPS049	 MK558131 MK558133 MK558093	 MK558182 MK558174 MK558166
Lake Biwa, Shiga1 (35°04'17.7"N 135°56'18.3"E) (1 May 2015)	29.9	6	<u>biw0302</u> [=NIES-4305] <u>biw0601</u> <u>biw0602</u> <u>biw0604</u> <u>biw0703</u> biw0101, biw0201, biw0501, biw0802m, biw0802f	<i>S. hopeiensis</i> Jao JPS004 <i>S. sp.</i> JPS017 <i>S. sp.</i> JPS018 <i>S. sp.</i> JPS019 <i>S. sp.</i> JPS021 <i>S. sp.</i> JPS008	MK558112 MK558114 MK558107 MK558117 MK558119	MK558147 MK558146 MK558177 MK558148 MK558153
A pond in Takamatsu city, Kagawa (34°19'21.8"N 134°02'05.8"E) (27 May 2015)	25.3	9.4	<u>taka0101</u> <u>taka1201</u>	<i>S. sp.</i> JPS051 <i>S. sp.</i> JPS052	MK558095 MK558139	MK558163 MK558168
Lake Biwa, Shiga2 (35°03'10.5"N 135°52'32.0"E) (30 June 2015)	29.8	9.1	<u>biw06300601</u> <u>biw160601</u> <u>biw160602</u> <u>biw160604</u>	<i>S. sp.</i> JPS020 <i>S. sp.</i> JPS022 <i>S. sp.</i> JPS023 <i>S. sp.</i> JPS028	MK558128 MK558097 MK558101	MK558173 MK558194 MK558160
Paddy fields in Kamogawa city, Chiba2 (35°07'51.7"N 139°58'31.2"E) (15 April 2016)	25.4	7.8	<u>chi0101</u> <u>chi0102</u> [=NIES-4316] <u>chi0202</u> [=NIES-4308] <u>chi0305</u> [=NIES-4313] <u>chi0504</u> <u>chi0207</u> <u>chi0802</u>	<i>S. sp.</i> JPS024 <i>S. varians</i> (Hassall) Kützing JPS015 <i>S. majuscula</i> Kützing JPS007 <i>S. pseudomaxima</i> Kadłubowska JPS012 <i>S. sp.</i> JPS025 <i>S. sp.</i> JPS009 <i>S. sp.</i> JPS021	MK558091 MK558105 MK558140 MK558102 MK558132	MK558165 MK558178 MK558184 MK558161 MK558187
Paddy fields in Kamogawa city, Chiba3 (35°08'03.2"N 139°59'00.5"E) (28 February 2017)	13.8	6.2	<u>chiA101</u> [=NIES-4304 1 <u>chiA102</u> <u>chiA301</u> <u>chiA302</u> <u>chiA304</u> [=NIES-4315] <u>chiA305</u> [=NIES-4306] <u>chiA307</u> [=NIES-4312]	<i>S. dentireticulata</i> Jao JPS003 <i>S. sp.</i> JPS015 <i>S. sp.</i> JPS028 <i>S. sp.</i> JPS024 <i>S. semiornata</i> Jao JPS014 <i>S. longata</i> (Vaucher) Kützing JPS005 <i>S. pratensis</i> Transeau JPS011	MK558115 MK558116 MK558123 MK558134	MK558152 MK558144 MK558157 MK558193

			chiA308 <u>chiA401</u>	S. sp. JPS011 S. sp. JPS026	MK558106	MK558192
A pond in Obuse Town, Nagano (36°41'27"N 138°19'49"E) (28 March 2017)	11.6	7	<u>nag101</u> [=NIES-4310]	<i>S. minuticrassoides</i> Yamagishi JPS009	MK558098	MK558164
A pond in Nagano city, Nagano1 (36°37'04.40"N 138°16'25"E) (30 March 2017)	11.8	7	<u>nag201</u>	S. sp. JPS032	MK558109	MK558179
A pond in Nagano city, Nagano2 (36°37'04.40"N 138°16'25"E) (30 March 2017)	10.0	7	<u>nag301</u> [=NIES-4309]	<i>S. majuscula</i> Kützing JPS008	MK558141	MK558183
A pond in Suzaka city, Nagano (36°37'47.4"N 138°17'22.7"E) (30 March 2017)	13.4	7	nag401	S. sp. JPS033		
Pond Ochikage-ike, Nagano (36°46'56.9"N 138°12'59.0"E) (18 May 2017)	-	-	nag501	S. sp. JPS008		
			nag503 <u>nag504</u>	S. sp. JPS028 S. sp. JPS033	MK558092	MK558190
Pond Tamizo-ike, Nagano (36°16'28.66"N 137°58'05.72"E) (6 June 2017)	24.9	-	<u>nag701</u>	S. sp. JPS034	MK558100	MK558188
			<u>nag702</u> nag703 nag704 nag706 <u>nag707</u>	S. sp. JPS035 S. sp. JPS028 S. sp. JPS007 S. sp. JPS036 S. sp. JPS037	MK558099 MK558121 MK558137	MK558189 MK558158 MK558171
Paddy fields in Matsumoto city, Nagano (36°12'40.91"N 137°59'25.53"E) (6 June 2017)	30.0	-	nag901	S. sp. JPS028		
Lake Nojiri, Nagano1 (36°49'59.97"N 138°14'32.71"E) (28 July 2017)	25.9	-	nagXII04	S. sp. JPS005		
			nagX03, nagXI02, nagXII03, nagXIII03	S. sp. JPS013		
Lake Nojiri, Nagano2 (36°49'59.97"N 138°14'32.71"E) (23 August 2017)	26.1	-	nagXV01	S. sp. JPS034		
			nagXIV03, nagXV04	S. sp. JPS013		
Pond Obasute-ooike, Nagano (36°29'17.03"N 138°04'59.82"E) (3 September 2017)	21.5	-	nagXVI01	S. sp. JPS013		
Lake Hijiri, Nagano (36°29'18.32"N 138°03'55.14"E) (3 September 2017)	23.1	-	nagXVIII01	S. sp. JPS040		
			nagXVIII02	S. sp. JPS001		
Pond Gongen-ike, Nagano	18.2	-	nagXIX04	S. sp. JPS005		

(36°29'36.24"N
138°01'20.05"E)
(3 September
2017)

			nagXIX06	S. sp. JPS006
Pond Daizahoushi-ike, Nagano (36°42'22.54"N 138°08'86.91"E) (11 September 2017)	22.8	-	nagXXI02	S. sp. JPS007
			nagXX01, nagXX03, nagXXI01, nagXX02	S. sp. JPS034 S. sp. JPS028
Lake Reisenji, Nagano (36°44'51.53"N 138°11'04.69"E) (11 September 2017)	22.7	-	nagXXIV01, nagXXIV04	S. sp. JPS031

*Fifty-two strains with different *rbcL*-types (underlined) were selected for conjugation experiments. Note that the 122 strains exhibit 52 different *rbcL* sequences or types.

**Scientific name with specific epithet represents a taxonomic species that was identified based on vegetative and reproductive characteristics observed under the present cultural conditions (Figs. 1-4). Taxonomically unidentified species (*S. sp.*) were distinguished and labeled based on their *rbcL*-types.

***Species that may belong to *Temnogyra* or *Sirogonium* because of lack of sexual reproduction characteristics.

Supplementary Table S2. List of primers used for sequencing *rbcL* genes.

Designation	Positions*	sequence (5'-3')
CHAR-RF-1**	1-23	ATGTCACCACAGACAGAACTAA
CHAR-RF-2**	560-581	GAGCTCTATATGAATGTCTTCG
CHAR-RR-3**	695-676****	GTTTCTGCTTGAGATTTATA
R42AE***	1403-1384*****	TC(AG)AA(CT)TT(AG)AT(CT)TC(CT)TTCCA

*Coordinate number from the *Chara connivens* Salzmänn ex A. Braun *rbcL* gene^{S1}.

**Primers from Sakayama *et al.*^{S2}.

***Degenerate primer from Shimada *et al.*^{S3}.

****Reverse primer.

Supplementary Table S3. List of primers used for sequencing *atpB* genes.

Designation	Positions*	sequence (5'-3')
<i>atpB</i> -175FZYG**	174-194	TRTWACYTGTGARGTACARCA
<i>atpB</i> -700F**	700-719	TATGGTCAAATGAATGAACC
<i>atpB</i> -866R**	884-866***	CCWACTGCAGAAGGCATAC
<i>atpB</i> -1404RZYG**	1423-1402***	CYARRTARAACGCGYTGTCTCTGG

*Coordinate number from the *Spirogyra maxima* (Hassall) Wittrock gene^{S4}.

**Primers from Stancheva *et al.*^{S5}.

***Reverse primer.

Supplementary Table S4. List of additional *rbcL* and *atpB* gene sequences to Table S1, included in the present phylogenetic analysis.

Strain	ingroup/ outgroup	Species	<i>rbcL</i>	<i>atpB</i>
ACOI 901	ingroup	<i>Actinotaenium cucurbitinum</i> (Biss.) Teiling	EF371279	KC779063
JH0199	ingroup	<i>Bambusina borrieri</i> (Ralfs) Cleve	EF371283	KC779064
UTEX 1075	ingroup	<i>Closterium acerosum</i> Ehr. ex Ralfs	EF371285	KC779065
JH0013	ingroup	<i>Closterium ehrenbergii</i> var. <i>malinvernianum</i> (De Notaris) Rabenh.	EF371286	KC779066
JH0021	ingroup	<i>Closterium libellula</i> Focke	EF371287	KC779067
CFD300a1	outgroup	<i>Coleochaete divergens</i> Pringsh.	AY082330	AY082326
UTEX 1261	outgroup	<i>Coleochaete nitellarum</i> Jost	AY051140	AY082325
CFD57b6	outgroup	<i>Coleochaete pulvinata</i> A. Braun ex. Kütz.	AY082310	AY082307
SAG 3.90	outgroup	<i>Coleochaete scutata</i> Bréb.	AY082329	AY082324
CFD10d1	outgroup	<i>Coleochaete sieminskiana</i> Szymanska	AF408249	AF408791
UTEX 301	ingroup	<i>Cosmarium botrytis</i> Menegh.ex Ralfs	EF371288	KC779068
ACOI 95	ingroup	<i>Cosmocladium saxonicum</i> De Bary	EF371292	KC779069
UTEX 1259	ingroup	<i>Cylindrocystis brebissonii</i> (Ralfs) De Bary	EF371293	KC779070
JH0038	ingroup	<i>Cylindrocystis</i> sp.	EF371296	KC779071
UTEX 1925	ingroup	<i>Cylindrocystis</i> sp.	EF371295	KC779072
SVCK 108	ingroup	<i>Desmidium aptogonum</i> Bréb. ex Archer	EF463091	KC779073
SVCK 113	ingroup	<i>Desmidium grevillei</i> (Kütz.) De Bary	EF463090	KC779074
JH0018	ingroup	<i>Euastrum crassum</i> var. <i>michiganense</i> Prescottt	EF371300	KC779075
ACOI 350	ingroup	<i>Gonatozygon kinahani</i> (Archer) Rabenh.	AJ553945	KC779076
SVCK 158	ingroup	<i>Gonatozygon monotaenium</i> De Bary	KC779163	KC779077
SVCK 302	ingroup	<i>Haplotaenium minutum</i> (Ralfs) Bando	EF371326	KC779078
SVCK 428	ingroup	<i>Heimansia pusilla</i> (Hilse) Coesel	EF371291	KC779079
SAG 384.2	ingroup	<i>Hyalotheca dissiliens</i> (Smith) Bréb. ex Ralfs	KC779164	KC779080
UTEX 41	ingroup	<i>Mesotaenium caldariorum</i> (Lagerheim) Hansg.	EF371307	AF408798
UTEX 1024	ingroup	<i>Mesotaenium kramstai</i> Lemmerm.	EF371309	KC779082
JH0031	ingroup	<i>Mesotaenium</i> sp.	EF371310	KC779081
UTEX 1941	ingroup	<i>Micrasterias rotata</i> Ralfs	EF371312	KC779083
UTEX 758	ingroup	<i>Mougeotia</i> sp.	AF408252	AF408800
UTEX 561	ingroup	<i>Netrium digitus</i> (Bréb. ex Ralfs) Itzigsohn & Rothe	U38698	KC779084
ACOI 780	ingroup	<i>Penium cylindrus</i> Bréb. ex Ralfs	EF371320	KC779085
ACOI 330	ingroup	<i>Penium maragaritaceum</i> Bréb. ex Ralfs	EF371322	KC779086
SAG 47.89	ingroup	<i>Phymatodocis nordstedtianum</i> Wolle	KC779165	KC779087
ARL 700	ingroup	<i>Sirogonium melanosporum</i> (Randhawa) Transeau	L13484	KC779088
UTEX 1985	ingroup	<i>Sirogonium sticticum</i> (Sm.) Kütz.	DQ015924	KC779089
RSS007	ingroup	<i>Spirogyra borgeana</i> Transeau	KC779194	KC779128
RSS024	ingroup	<i>Spirogyra borgeana</i> Transeau	KC779209	KC779138
RSS025	ingroup	<i>Spirogyra borgeana</i> Transeau	KC779210	KC779139
RSS001	ingroup	<i>Spirogyra californica</i> Stancheva, J. D. Hall, McCourt et Sheath sp. nov.	KC779188	KC779123
UTEX 2462	ingroup	<i>Spirogyra communis</i> (Hassall) Kütz.	DQ015932	KC779090
UTEX 1744	ingroup	<i>Spirogyra condensata</i> (Vaucher) Kütz.	DQ015936	KC779091
JH0425	ingroup	<i>Spirogyra croasdaleae</i> Blum	KC779170	KC779107
RSS033	ingroup	<i>Spirogyra fluviatilis</i> Hilse	KC779218	KC779146
UTEX 1743	ingroup	<i>Spirogyra gracilis</i> (Hassall) Kütz.	DQ015937	KC779092
RSS011	ingroup	<i>Spirogyra grevilleana</i> (Hassall) Kütz.	KC779197	KC779130
RSS017	ingroup	<i>Spirogyra grevilleana</i> (Hassall) Kütz.	KC779202	KC779133
UTEX 477	ingroup	<i>Spirogyra grevilleana</i> (Hassall) Kütz.	DQ015938	KC779093
UTEX 1742	ingroup	<i>Spirogyra juergensii</i> Kütz.	DQ015939	KC779094

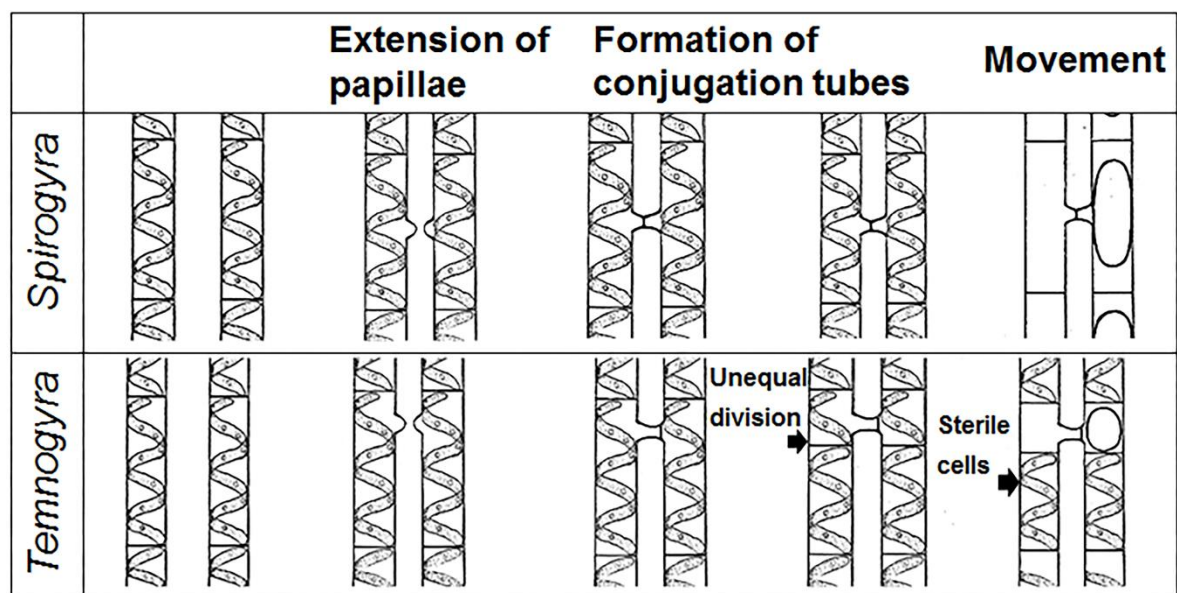
RSS015	ingroup	<i>Spirogyra juliana</i> Stancheva, J. D. Hall, McCourt et Sheath <i>sp. nov.</i>	KC779200	KC779131
UTEX 1745	ingroup	<i>Spirogyra liana</i> Transeau	DQ015940	KC779095
RSS031	ingroup	<i>Spirogyra longata</i> (Vaucher) Kütz.	KC779216	KC779144
RSS003	ingroup	<i>Spirogyra lutetiana</i> P. Petit	KC779190	KC779125
RSS018	ingroup	<i>Spirogyra lutetiana</i> P. Petit	KC779203	KC779134
RSS006	ingroup	<i>Spirogyra majuscula</i> Kütz.	KC779193	KC779127
RSS026	ingroup	<i>Spirogyra maxima</i> (Hassall) Kütz.	KC779211	KC779140
RSS032	ingroup	<i>Spirogyra maxima</i> (Hassall) Kütz.	KC779217	KC779145
UTEX 2495	ingroup	<i>Spirogyra maxima</i> (Hassall) Kütz.	DQ015941	AF408797
RSS008	ingroup	<i>Spirogyra notabilis</i> Taft	KC779195	KC779129
JH0002	ingroup	<i>Spirogyra parvula</i> (Transeau) Czurda	KC779166	KC779101
UTEX 1746	ingroup	<i>Spirogyra pratensis</i> Transeau	DQ015949	KC779098
UTEX 928	ingroup	<i>Spirogyra pratensis</i> Transeau	DQ015948	KC779097
JH0058	ingroup	<i>Spirogyra</i> sp.	DQ015954	KC779102
JH0130	ingroup	<i>Spirogyra</i> sp.	DQ015955	KC779104
JH0263	ingroup	<i>Spirogyra</i> sp.	KC779168	KC779105
JH0278	ingroup	<i>Spirogyra</i> sp.	KC779169	KC779106
JH0429	ingroup	<i>Spirogyra</i> sp.	KC779171	KC779108
JH0643	ingroup	<i>Spirogyra</i> sp.	KC779173	KC779110
JH0728	ingroup	<i>Spirogyra</i> sp.	KC779174	KC779111
JH0744	ingroup	<i>Spirogyra</i> sp.	KC779175	KC779112
JH0763	ingroup	<i>Spirogyra</i> sp.	KC779176	KC779113
JH0941	ingroup	<i>Spirogyra</i> sp.	KC779179	KC779115
JH0977	ingroup	<i>Spirogyra</i> sp.	KC779182	KC779118
JH0979	ingroup	<i>Spirogyra</i> sp.	KC779183	KC779119
JH0987	ingroup	<i>Spirogyra</i> sp.	KC779185	KC779121
RSS020	ingroup	<i>Spirogyra</i> sp.	KC779205	KC779135
RSS036	ingroup	<i>Spirogyra</i> sp.	KC779220	KC779148
RSS021	ingroup	<i>Spirogyra submaxima</i> Transeau	KC779206	KC779136
ACOI 1925	ingroup	<i>Spirogyra tenuissima</i> (Hassall) Kütz.	KC779187	KC779099
JH1015	ingroup	<i>Spirogyra tenuissima</i> (Hassall) Kütz.	KC779186	KC779122
RSS027	ingroup	<i>Spirogyra teodoresci</i> Transeau	KC779212	KC779141
UTEX 479	ingroup	<i>Spirogyra varians</i> (Hassall) Kütz.	DQ015951	KC779100
RSS004	ingroup	<i>Spirogyra weberi</i> Kütz.	KC779191	KC779126
SVCK 365	ingroup	<i>Spondylosium pulchellum</i> (Archer) Archer	AM911261	KC779153
JH0014	ingroup	<i>Staurostrum arctiscon</i> (Ehr.) Lundell	EF371343	KC779154
SAG 679.1	ingroup	<i>Staurostrum punctulatum</i> Bréb.	FN432117	NC_008116
UTEX 2508	ingroup	<i>Staurodesmus convergens</i> (Ehrenb.) Lillieroth	EF371281	KC779155
SAG 25.88	ingroup	<i>Teilingia granulata</i> (Roy & Bissett) Bourr.	EF371350	KC779156
JH0054	ingroup	<i>Xanthidium antilopaeum</i> var. <i>polymazum</i> Nordst.	EF378638	KC779157
SAG 698-2	ingroup	<i>Zygnema cylindricum</i> Transeau	EF371357	KC779158
JH0007	ingroup	<i>Zygnema</i> sp.	EF371359	KC779159
JH0044	ingroup	<i>Zygnema</i> sp.	JF965517	KC779160
ACOI 60	ingroup	<i>Zygnemopsis minuta</i> Randhawa	EF371363	KC779161
UTCC 136	ingroup	<i>Zygogonium tunetanum</i> Gauth.-Lièvre	JQ780057	KC779162

Supplementary Table S5. Information of vegetative cells about 52 strains in this study.

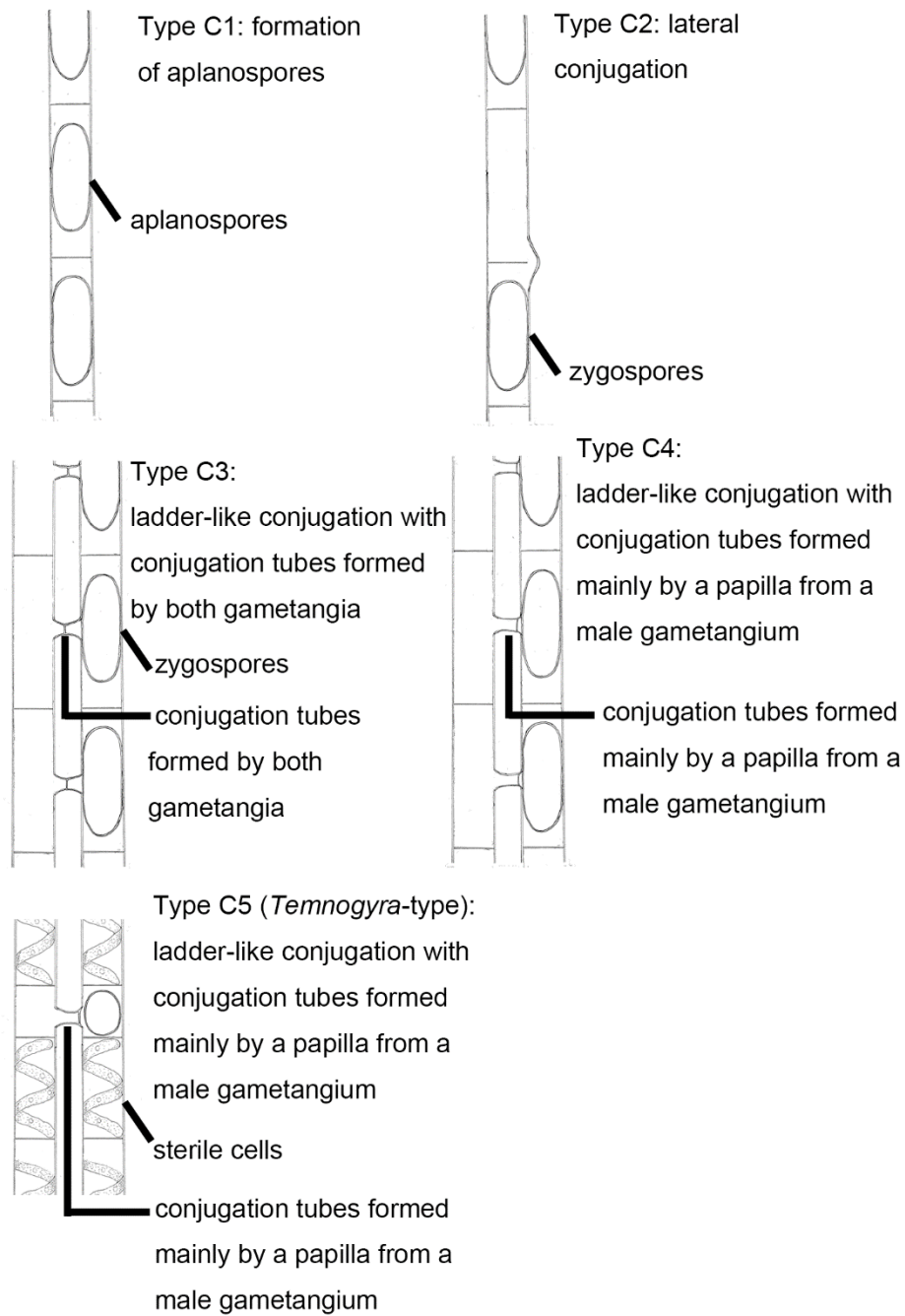
Strain (<i>rbcL</i> -type)	Species	Width (μm)	Length (μm)	End wall	Chloroplasts	
					per cell	Turns per cell
Uki1 [=NIES-4302] (JPS001)	<i>S. chungkingensis</i> Jao	24-27	150-300	plane	3	4-5
A2F [=NIES-4303] (JPS002)	<i>S. corrugata</i> Transeau	28-34	200-600	plane	2-3	3-5
chiA101 [=NIES-4304] (JPS003)	<i>S. dentireticulata</i> Jao	20-24	200-340	replicate	1	4-5
biw0302 [=NIES-4305] (JPS004)	<i>S. hopeiensis</i> Jao	24-28	110-260	replicate	1	5-6
chiA305 [=NIES-4306] (JPS005)	<i>S. longata</i> (Vaucher) Kützinger	26-30	110-260	plane	1	4-5
kit0201 [=NIES-4307] (JPS006)	<i>S. longata</i> (Vaucher) Kützinger	27-32	110-250	plane	1	3-6
chi0202 [=NIES-4308] (JPS007)	<i>S. majuscula</i> Kützinger	70-80	170-300	plane	6	1.5-2
nag301 [=NIES-4309] (JPS008)	<i>S. majuscula</i> Kützinger	70-80	110-270	plane	6	1-2
nag101 [=NIES-4310] (JPS009)	<i>S. minuticrassoides</i> Yamagishi	95-112	295-515	plane	6	2.5-3
shi0305 [=NIES-4311] (JPS010)	<i>S. mirabilis</i> (Hassall) Kützinger	20-26	105-270	plane	1	6-8
chiA307 [=NIES-4312] (JPS011)	<i>S. chenii</i> Jao	17-22	160-270	plane	1	3-5
chi0305 [=NIES-4313] (JPS012)	<i>S. pseudomaxima</i> Kadłubowska	135-140	190-430	plane	7-8	1-1.5
Tpx8 [=NIES-4314] (JPS013)	<i>S. punctata</i> Cleve	26-30	150-300	plane	1	5-7
chiA304 [=NIES-4315] (JPS014)	<i>S. semiornata</i> Jao	26-32	250-380	replicate	1	5-6
chi0102 [=NIES-4316] (JPS015)	<i>S. varians</i> (Hassall) Kützinger	28-33	125-200	plane	1	4-8
A3 (JPS016)	<i>S. sp.*</i>	10-12	90-180	plane	1	4-5
biw0601 (JPS017)	<i>S. sp.</i>	15-20	210-350	replicate	1	5-7
biw0602 (JPS018)	<i>S. sp.</i>	24-30	120-190	plane	1	3-5
biw0604 (JPS019)	<i>S. sp.</i>	8-12	120-160	replicate	1	4-7
biw06300601 (JPS020)	<i>S. sp.</i>	21-25	170-300	plane	1	5-7
biw0703 (JPS021)	<i>S. sp.</i>	42-52	32-590	plane	3-4	2-4
biw160601 (JPS022)	<i>S. sp.</i>	-	-	-	-	-
biw160602 (JPS023)	<i>S. sp.</i>	135-148	400-610	plane	5	2-3
chi0101 (JPS024)	<i>S. sp.</i>	22-28	110-270	plane	2	3-5
chi0504 (JPS025)	<i>S. sp.</i>	20-25	80-130	plane	1	3-4
chiA401 (JPS026)	<i>S. sp.</i>	22-30	105-240	plane	1	4-5
kit0101 (JPS027)	<i>S. sp.</i>	13-16	125-185	replicate	1	4-5
kit2 (JPS028)	<i>S. sp.</i>	66-88	140-240	plane	3	1.5-2
kit42B (JPS029)	<i>S. sp.</i>	32-45	110-180	plane	3	2-3
mit0203 (JPS030)	<i>S. sp.</i>	10-17	30-115	plane	1	3-4
mitA01 (JPS031)	<i>S. sp.</i>	-	-	-	-	-
nag201 (JPS032)	<i>S. sp.</i>	22-28	120-240	plane	1	3-5

nag504 (JPS033)	S. sp.	-	-	-	-	-
nag701 (JPS034)	S. sp.	132-142	285-440	plane	6-7	3-4
nag702 (JPS035)	S. sp.	-	-	-	-	-
nag706 (JPS036)	S. sp.	30-42	115-340	plane	1	3-6
nag707 (JPS037)	S. sp.	51-57	130-280	plane	3-4	2-3
senA1602 (JPS038)	S. sp.	20-23	75-150	plane	1	1-3
senA2002 (JPS039)	S. sp.	14-19	185-300	replicate	1	5-7
senA2303 (JPS040)	S. sp.	45-55	215-320	plane	4	2-5
senB2001 (JPS041)	S. sp.	64-74	190-360	plane	4	1-3
senB2603 (JPS042)	S. sp.	19-25	250-400	replicate	1	5-7
senB2604 (JPS043)	S. sp.	18-24	250-540	replicate	1	4-6
sen0102 (JPS044)	S. sp.	12-15	200-380	replicate	1	4-7
sen0103 (JPS045)	S. sp.	14-18	94-340	plane	1	4-7
sen01505 (JPS046)	S. sp.	20-26	150-210	plane	1	5-6
sen0406 (JPS047)	S. sp.	22-28	280-410	plane	1	4-8
shi0303 (JPS048)	S. sp.	13-20	110-230	plane	1	4-6
shi0308 (JPS049)	S. sp.	39-44	160-250	plane	3	2-4
T11 (JPS050)	S. sp.	11-14	110-195	replicate	1	3-5
tak0101 (JPS051)	S. sp.	80-86	100-140	plane	4-5	1-2
tak1201 (JPS052)	S. sp.	36-47	200-510	plane	1	6-7

* Species that may belong to *Temnogyra* or *Sirogonium* because of lack of sexual reproduction characteristics.



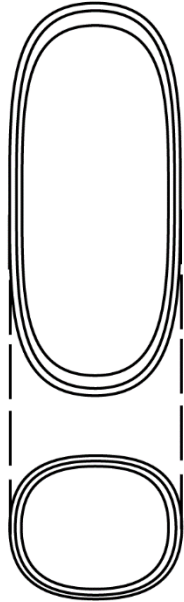
Supplementary Figure S1. Comparison of conjugation processes in *Spirogyra* and *Temnogyra*. Based on Yamagishi^{S6}.



Supplementary Figure S2. Diagrams of five types of formation of zygospores or aplanospore observed in this study.

Type Z1: ovoid

zygospore/aplanospore



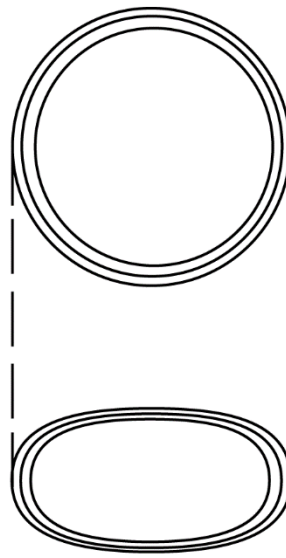
Type Z2: ellipsoid

zygospore



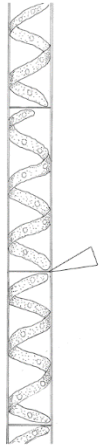
Type Z3: lenticular

zygospore

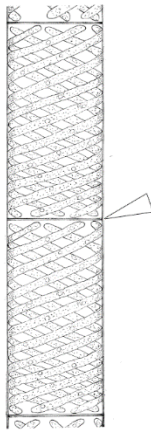


Supplementary Figure S3. Diagrams of three types of zygospores or aplanospores observed in this study. Terminology is based on Transeau^{S7}.

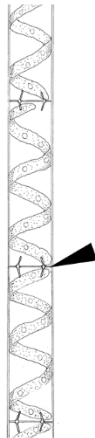
Type V1: plane
transverse wall and
single chloroplast



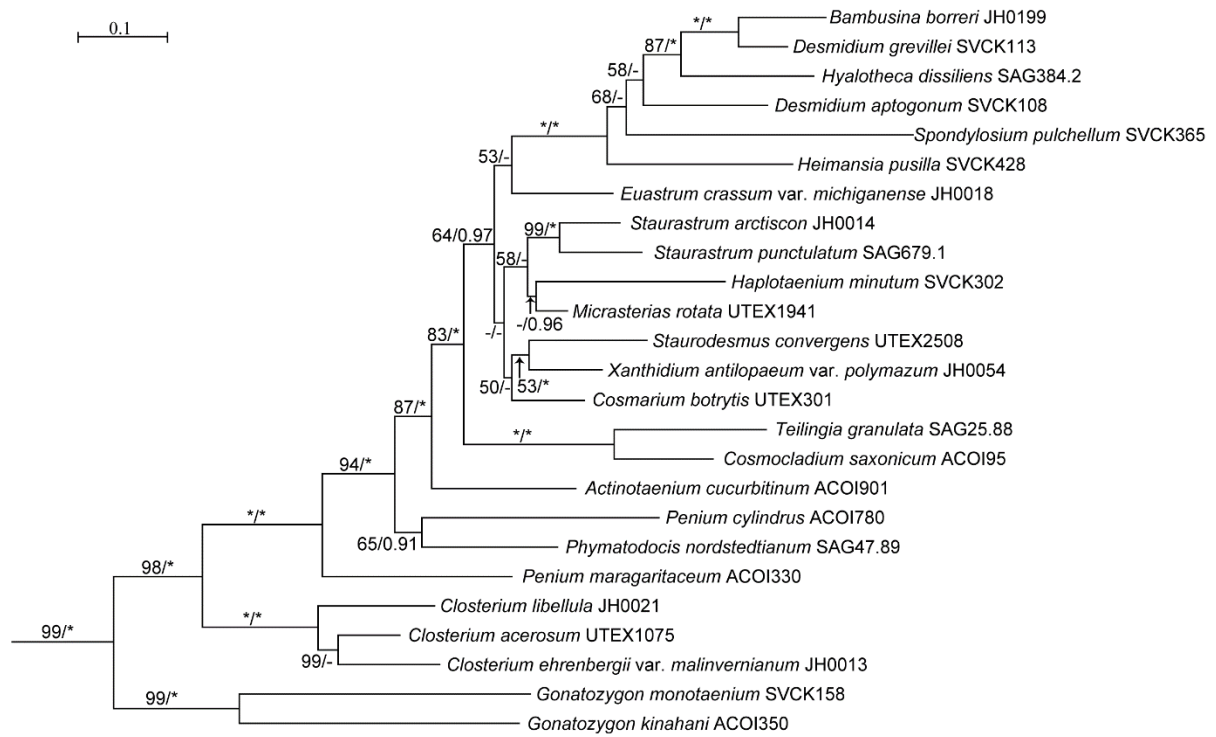
Type V2: plane
transverse wall
and multiple
chloroplasts



Type V3: replicate
transverse wall and
single chloroplast



Supplementary Figure S4. Diagrams of three types of vegetative cells observed in this study. The open arrowheads show plane transverse wall and the closed arrowhead shows replicate transverse wall.



Supplementary Figure S5. Details of “Desmidiales” in ML tree (Fig. 5a). For details of the explanation of the tree, see Figure 5.

Supplementary Notes

Supplementary Note S1. Taxonomic accounts

Spirogyra chungkingensis Jao 1935: 600^{S8}. (Figs. 2a-d)

Species description: Vegetative cells 24-27 µm wide, 150-300 µm long; transverse walls plane; chloroplasts 3 per cell (Fig. 2a). Conjugation scalariform (like ladder); conjugation tubes formed by both gametangia; fertile female gametangia slightly inflated (Fig. 2b). Zygospores ellipsoid, 33-35 × 55-83 µm; mesospore two-layered, outer brownish, wrinkled; inner brown, irregularly reticulate (Figs. 2c, d).

Distribution: China and Japan^{S6,S9} (Table S1).

Strain examined: Uki1 (JPS001).

Remarks: *Spirogyra chungkingensis* was originally described by Jao^{S8} based on the field-collected material from China. The present Japanese material agrees with the species concept of *S. chungkingensis* by Jao^{S8} in having plane transverse walls, 3 chloroplasts and two-layered mesospores, of which the outer is thin and wrinkled, the inner irregularly reticulate (Figs. 2c, d).

Spirogyra corrugata Transeau 1934: 229^{S10}. (Figs. 4a-e)

Synonym: *Temnogyra corrugata* (Transeau) Yamagishi 1963: 207^{S11}.

Species description: Vegetative cells 28-34 µm wide, 200-600 µm long; transverse walls plane; chloroplasts 2-3 per cell (Fig. 4a). Conjugation scalariform; conjugation tubes formed by male gametangia; fertile female gametangia inflated (Fig. 4b). Gametangia adjoining sterile cells in both male and female filaments (Fig. 4b). Zygospores ovoid, 50-54 × 97-111 µm; mesospore two-layered, the outer thin, corrugate and the inner thick, reticulate (Figs. 4d, e).

Distribution: United States, China, Africa, and Japan^{S6,S7,S9,S12-14} (Table S1).

Strain examined: A2F (JPS002).

Remarks: This species was originally described by Transeau^{S10} as *Spirogyra corrugata*, and was transferred to *Temnogyra*, as *Temnogyra corrugata* by Yamagishi^{S11} based on the presence of sterile cells adjoining gametangia. However, no subsequent authors recognize the genus *Temnogyra*. Transeau^{S7} described morphological details of this species such as having plane transverse walls, 1-3 chloroplasts, and two-layered mesospores. The present Japanese material agrees with the species concept of *T. corrugate* by Transeau^{S7}. The present phylogenetic analysis demonstrated that this species is not separated from species of

Spirogyra and *Sirogonium* sensu Yamagishi^{S11}. Thus, this species should be classified to the genus *Spirogyra*.

Spirogyra dentireticulata Jao 1935: 611^{S8}. (Figs. 1a-d)

Species description: Vegetative cells 20-24 µm wide, 200-340 µm long; transverse walls replicate; chloroplasts single per cell (Fig. 1a). Conjugation scalariform; conjugation tubes formed by both gametangia; fertile female gametangia slightly enlarged (Fig. 1b). Zygospores ovoid, 30-34 × 70-76 µm; mesospore yellow-brown, reticulate with coarse ridges (Figs. 1c, d).

Distribution: China, USA, Kazakhstan and Japan^{S7,S9,S13} (Table S1).

Strain examined: chiA101 (JPS003).

Remarks: *Spirogyra dentireticulata* was originally described by Jao^{S8} based on the field-collected material from China. The present Japanese material agrees with the species concept of *S. dentireticulata* by Jao^{S8} and Transeau^{S7} in having replicate transverse walls, single chloroplasts and reticulate mesospore with spinulose-dentate reticulations at the intersections. Although Jao^{S8} and Transeau^{S7} reported both lateral and scalariform conjugation in this species, we observed only scalariform conjugation of Japanese material under the present culture conditions. This species has not previously been recorded from Japan^{S6}.

Spirogyra hopeiensis Jao 1935: 608^{S8}. (Figs. 1e-h)

Species description: Vegetative cells 24-28 µm wide, 110-260 µm long; transverse walls replicate; chloroplasts single per cell (Fig. 1e). Conjugation scalariform; conjugation tubes formed by male gametangia; fertile female gametangia inflated mostly on the conjugation sides (Fig. 1f). Zygospores ellipsoid, 30-34 × 59-62 µm; mesospore yellow-brown, smooth (Figs. 1g, h).

Distribution: Kazakhstan, China and Japan^{S6,S9,S13} (Table S1).

Strain examined: biw0302 (JPS004).

Remarks: *Spirogyra hopeiensis* was originally described by Jao^{S8} based on the field-collected material from China. The present cultured material agrees with the description of *S. hopeiensis* by Jao^{S8} and Yamagishi^{S6} in having replicate transverse walls, single chloroplasts, conjugation tubes formed by male gametangia, female gametangia inflated on the inner side and smooth single-layered mesospore.

Spirogyra longata (Vaucher) Kützing 1843: 279^{S15}. (Figs. 1i-l)

Basionym: *Conjugata longata* Vaucher 1803: 71^{S16}.

Species description: Vegetative cells 26-32 μm wide, 110-260 μm long; transverse walls plane; chloroplasts single per cell (Fig. 1i). Conjugation scalariform; conjugation tubes formed by both gametangia; fertile female gametangia cylindrical, sometimes slightly enlarged (Fig. 1j). Zygospores ellipsoid to ovoid, 28-32 $\times$ 48-74 μm ; mesospore yellow-brown, smooth (Figs. 1k, l).

Distribution: Widely distributed in the United States, Europe, China and Japan^{S6,S7,S9,S12-14} (Table S1).

Strain examined: chiA305 (JPS005) and kit0201 (JPS006).

Remarks: *Spirogyra longata* was originally described by Vaucher^{S16} as *Conjugata longata*, and was transferred to *Spirogyra*, as *Spirogyra longata* by Kützing^{S15}. Transeau^{S7} described morphological details of this species such as having plane transverse walls, single chloroplasts, cylindrical female gametangia and smooth mesospores. The present Japanese material agrees with the species concept of *S. longata* by Transeau^{S7}. Although Transeau^{S7} reported both lateral and scalariform conjugation in this species, we observed only scalariform conjugation of Japanese material under the present culture conditions.

Spirogyra majuscula Kützing 1849: 441^{S17}. (Figs. 3a-d)

Species description: Vegetative cells 70-80 μm wide, 110-300 μm long; transverse walls plane; chloroplasts 6 per cell (Fig. 3a). Conjugation scalariform; conjugation tubes formed by both gametangia; fertile female gametangia slightly inflated on the outer side (Fig. 3b). Zygospores lenticular, 58-78 $\times$ 58-78 $\times$ 43-55 μm ; mesospore brown, smooth (Figs. 3c, d).

Distribution: Widely distributed in Europe, America, Asia, Africa, and Australia^{S6,S7,S9,S12-14} (Table S1).

Strain examined: chi0202 (JPS007) and nag301 (JPS008).

Remarks: *Spirogyra majuscula* was originally described by Kützing^{S17}. Transeau^{S7} characterized this species by detailed vegetative and reproductive morphology based on the field-collected material from United States. The present Japanese material agrees with the species concept of *S. majuscula* by Transeau^{S7} in having plane transverse walls, 5-8 chloroplasts and smooth mesospore. Although Transeau^{S7} reported both lateral and scalariform conjugation in this species, we observed only scalariform conjugation of Japanese material under the present culture conditions.

Spirogyra minuticrassoidea Yamagishi 1963: 216^{S18}. (Figs. 3e-h)

Species description: Vegetative cells 95-112 µm wide, 295-515 µm long; transverse walls plane; chloroplasts 6 per cell (Fig. 3e). Conjugation scalariform; conjugation tubes formed by both gametangia; fertile female gametangia cylindrical (Fig. 3f). Zygospores compressed ellipsoid, 100-107 × 125-144 × 68-90 µm; mesospore yellow-brown, smooth (Figs. 3g, h).

Distribution: Japan^{S6} (Table 1).

Strain examined: nag101 (JPS009).

Remarks: Since Yamagishi^{S18} described *Spirogyra minuticrassoidea* based on the field-collected material from Aichi prefecture, Japan, no further records of this species have previously been reported. The present Japanese material agrees with the species concept of *S. minuticrassoidea* by Yamagishi^{S18} in having plane transverse walls, 6-7 chloroplasts, compressed ellipsoid zygospores and smooth mesospore. This species is morphologically similar to *S. crassoidea* Transeau and *S. ellipsospora* Transeau according to Transeau^{S7}. However, this species differs from both species in having a smaller compressed ellipsoid zygospore and narrower vegetative cells.

Spirogyra mirabilis (Hassall) Kützing 1849: 438^{S17}. (Figs. 2e-h)

Basionym: *Zygnema mirabile* Hassall 1843: 23^{S19}.

Species description: Vegetative cells 20-26 µm wide, 105-270 µm long; transverse walls plane; chloroplasts single per cell (Fig. 2e). Reproduction by aplanospores; sporangia inflated (Fig. 2f). Aplanospores ovoid, 23-32 × 41-53 µm; mesospore yellow-brown, smooth (Figs. 2g, h).

Distribution: Widely distributed in the United States, Europe, China and Japan^{S6,S7,S9,S12-14} (Table S1).

Strain examined: shi0305 (JPS010).

Remarks: Transeau^{S7} described morphological details of *Spirogyra mirabilis* such as its plane transverse walls, single chloroplasts, ovoid aplanospores and smooth mesospores. Transeau^{S7} reported very rare scalariform conjugation in this species. We have not observed sexual reproduction in the present Japanese material under the present culture conditions.

Spirogyra chenii Jao 1935: 587^{S8}. (Figs. 2i-m)

Species description: Vegetative cells 17-22 µm wide, 160-270 µm long; transverse walls plane; chloroplasts single per cell (Fig. 2i). Conjugation lateral and scalariform; conjugation

tubes formed by male gametangia; fertile female gametangia inflated on the both sides (Fig. 2k). Zygospor es ellipsoid, $24\text{--}27 \times 42\text{--}52 \mu\text{m}$; mesospore yellow, smooth (Figs. 2l, m).

Distribution: China and Japan^{S6,S9} (Table S1).

Strain examined: chiA307 (JPS011).

Remarks: The present cultured material agrees with the original description of *S. chenii* by Jao^{S8} and Yamagishi^{S6} in having plane transverse walls, single chloroplasts, conjugation tubes formed by male gametangia, female gametangia inflated on the both side and smooth single-layered mesospore wall.

Spirogyra pseudomaxima Kadłubowska 1979: 57^{S20}. (Figs. 3i-n)

Species description: Vegetative cells $135\text{--}140 \mu\text{m}$ wide, $190\text{--}430 \mu\text{m}$ long; transverse walls plane; chloroplasts 7-8 per cell (Fig. 3i). Conjugation scalariform; conjugation tubes formed by both gametangia; fertile female gametangia cylindrical (Fig. 3j). Zygospor es lenticular, $112\text{--}124 \times 112\text{--}124 \times 90\text{--}95 \mu\text{m}$; mesospore two-layered, outer thin, with branched ribs; inner brown, reticulate (Figs. 3k-n).

Distribution: Denmark and Japan^{S12} (Table S1).

Strain examined: chi0305 (JPS012).

Remarks. *Spirogyra pseudomaxima* was originally described by Kadłubowska^{S20} based on the field-collected material from Denmark. The present Japanese material agrees with the species concept of *S. pseudomaxima* by Kadłubowska^{S20} in having plane transverse walls, 5-7 chloroplasts, compressed ellipsoid zygospor es and two-layered mesospore. This species has not previously been recorded from Japan^{S6}. This species is similar to *S. maxima* in having reticulate mesospore, but this species has a two-layered mesospore (Fig. 3n) whereas the mesospore of *S. maxima* is single-layered according to Transeau^{S5} and Kadłubowska^{S12}.

Spirogyra punctata Cleve 1868: 23^{S21}. (Figs. 4f-j)

Synonym: *Temnogyra punctata* (Cleve) Yamagishi 1963: 207^{S11}.

Species description: Vegetative cells $26\text{--}32 \mu\text{m}$ wide, $150\text{--}300 \mu\text{m}$ long; transverse walls plane; chloroplasts single per cell (Fig. 4f). Conjugation scalariform; conjugation tubes formed by both gametangia; fertile female gametangia cylindrical, sometimes slightly inflated (Fig. 4h). Gametangia adjoining sterile cells in both male and female filaments (Fig. 4g). Zygospor es ellipsoid, $31\text{--}34 \times 54\text{--}65 \mu\text{m}$; mesospore yellow-brown, punctate (Figs. 4i, j).

Distribution: Widely distributed in Europe, America, Asia, Africa, and Australia^{S6,S7,S9,S12-14} (Table S1).

Strain examined: Tpx8 (JPS013).

Remarks: Transeau^{S7} described morphological details of this species such as having plane transverse walls, single chloroplasts, and punctate mesospores. The present Japanese material agrees with the species concept of *T. punctata* by Transeau^{S7}. As *Spirogyra punctata*, this species was classified to the genus *Temnogyra* by Yamagishi^{S11} because it has sterile cells adjoining gametangia. The present phylogenetic analysis either did not support the separation of this species from the genus *Spirogyra*.

Spirogyra semiornata Jao 1935: 604^{S8}. (Figs. 1m-p)

Species description: Vegetative cells 26-32 µm wide, 250-380 µm long; transverse walls replicate; chloroplasts single per cell (Fig. 1m). Conjugation scalariform; conjugation tubes formed by both gametangia; fertile female gametangia cylindrical, sometimes slightly enlarged (Fig. 1n). Zygospores ellipsoid with obtuse ends, 35-42 × 78-107 µm; mesospore yellow-brown, smooth (Figs. 1o, p).

Distribution: Europe, Africa, China and Japan^{S6,S7,S9,S12-14} (Table S1).

Strain examined: chiA304 (JPS014).

Remarks: *Spirogyra semiornata* was originally described by Jao^{S8} based on the field-collected material from China. The present Japanese material agrees with the original description of *S. semiornata* by Jao^{S8} in having replicate transverse walls, single chloroplasts and smooth mesospore. Although Jao^{S8} reported both lateral and scalariform conjugation in this species, we observed only scalariform conjugation in Japanese material under the present culture conditions.

Spirogyra varians (Hassall) Kützing 1849: 439^{S17}. (Figs. 2n-q)

Basionym: *Zygnema varians* Hassall 1843: 431^{S19}.

Species description: Vegetative cells 28-33 µm wide, 125-200 µm long; transverse walls plane; chloroplasts single per cell (Fig. 2n). Conjugation scalariform; conjugation tubes formed by male gametangia; fertile female gametangia inflated on the conjugation side (Fig. 2o). Zygospores ellipsoid, 30-34 × 50-53 µm; mesospore yellow-brown, smooth (Figs. 2p, q).

Distribution: Widely distributed in Europe, America, Asia, Africa, and Australia^{S6,S7,S9,S12-14} (Table S1).

Strain examined: chi0102 (JPS015).

Remarks: Transeau^{S7} described morphological details of this species such as having plane transverse walls, single chloroplasts, inflated female gametangia and smooth mesospores.

The present Japanese material agrees with the species concept of *S. varians* by Transeau^{S7}. Transeau^{S7} reported both lateral and scalariform conjugation in this species, we observed only scalariform conjugation of the Japanese material under the present culture conditions.

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