

Supplementary Tables

Table S1. List of volvoclean strains and *MAT3* sequences used in this study.

Taxon (sexual reproduction)	Strain designation	Gender (mating type)	Accession number of <i>MAT3</i>	Characteristics of full length open reading frames of <i>MAT3</i>		
				Number of nucleotides	Number of deduced amino	<i>MAT3</i> locus [reference]
<i>Gonium pectorale</i> (isogamy)	K41 ^a	<i>plus</i>	AB771928	3,084	1,028	within <i>MT</i>
	NIES-1711 (Kaneko4)	<i>plus</i>	AB771929			[Hamaji T et al. unpublished]
	K34 ^a	<i>minus</i>	AB771930	3,084	1,028	
	NIES-1710 (Kaneko3)	<i>minus</i>	AB771931			
<i>Yamagishiella unicocca</i> (isogamy)	NIES-1861 (Hasu-4)	<i>plus</i>	AB771932	3,726	1,242	ND
	NIES-1859 (Hasu-1)	<i>minus</i>	AB771933	3,726	1,242	
<i>Eudorina</i> sp. (anisogamy)	2010-623-F1-E2 ^b	male	AB771934	3,522	1,174	within or near <i>MT</i>
			AB771935			[the present study]
	2010-623-F1-E8 ^b	female	AB771936 AB771937	3,522	1,174	
<i>Pleodorina starii</i> (anisogamy)	2010-0513-F2-P10 ^c	female	AB771938	3,102	1,034	ND
	2010-0513-F2-P11 ^c	male	AB771939	3,102	1,034	
<i>Volvox africanus</i> (oogamy)	UTEX 1890 (Darra 6 ^d)	male	AB771940	3,375	1,125	ND
	UTEX 1891 (Darra 4 ^d)	female	AB771941	3,375	1,125	
<i>Volvox obversus</i> (oogamy)	UTEX 1865	male	AB771942			

<i>Volvox carteri</i> f.	Adam ^e	male	GU784916	3,339	1,113	within <i>MT</i> [S1]
<i>nagariensis</i>	Eve ^e	female	XM_002954427	3,321	1,107	
(oogamy)						
<i>Volvox carteri</i> f.	NIES-722	male	AB771943			
<i>kawasakiensis</i>	NIES-733	female	AB771944			
(oogamy)						
<i>Volvox carteri</i> f.	NIES-866	male	AB771945			
<i>weismannia</i>	(=UTEX 1875, Wt 4					
(oogamy)	male ^f)					
	UTEX 1876	female	AB771946			
	(Wt 3 female ^f)					
<i>Chlamydomonas</i>	8511A ^g	<i>plus</i>	AB771947	3,627	1,209	autosome near <i>MT</i>
<i>reinhardtii</i>	8511B ^g	<i>minus</i>	AB771948	3,627	1,209	[S2]
(isogamy)						
<i>Chlamydomonas</i>	CC-1870 ^h		AY781411	3,639	1,213	
<i>globosa</i>						
(isogamy)						

^aF1 strains of Kaneko3x4 [S3].

^bF1 strains of male and female strains (2006-703-E14xE15; [S4]).

^cF2 strains of male and female strains [S5].

^dIsolated from the same mud sample [S6].

^eNot examined in this study.

^fOriginating from Waterford, Australia [S7]

^gSaito et al. [S8].

^hPreviously misidentified as *C. incerta* [S9].

Table S2. Gender-based divergence of *MAT3* genes from *Chlamydomonas reinhardtii* and six species of the colonial Volvocales.

Taxon	S	N	dN	SE	dS	SE	dN/dS
<i>Chlamydomonas reinhardtii</i>	662.1	2964.9	0.0020	0.0008	0.0262	0.0064	0.0773
<i>Gonium pectorale</i>	837	2247	0.0004	0.0004	0.0012	0.0012	0.3723
<i>Yamagishiella unicocca</i>	1000.5	2722.5	0	0	0	0	NA
<i>Eudorina</i> sp.	1043.3	2478.7	0.0004	0.0004	0.001	0.001	0.4208
<i>Pleodorina starrii</i>	885	2214	0	0	0	0	NA
<i>Volvox africanus</i>	916.9	2455.1	0.0086	0.0019	0.0166	0.0043	0.519
<i>Volvox carteri</i> f. <i>nagariensis</i>	859.7	2320.3	0.3621	0.0152	1.6465	0.1444	0.2199

S, number of synonymous positions; N, number of nonsynonymous positions; dN, number of substitutions per nonsynonymous site; dS, number of substitutions per synonymous site. For details, see supplementary table S1 and supplementary information (Supplementary Material online).

Supplementary References

- S1. Ferris P, Olson BJSC, De Hoff PL, et al. (15 co-authors). 2010. Evolution of an expanded sex-determining locus in *Volvox*. *Science* 328: 351–354.
- S2. Umen JG, Goodenough, UW. 2001. Control of cell division by a retinoblastoma protein homolog in *Chlamydomonas*. *Genes Dev.* 15: 1652–1661.
- S3. Mogi Y, Hamaji T, Suzuki M, Ferris P, Mori T, Kabeya Y, Miyagishima S, Nozaki H. 2012. Evidence for tubular mating structures induced in each mating type of heterothallic *Gonium pectorale* (Volvocales, Chlorophyta). *J Phycol.* 48: 670–674.
- S4. Yamada TK, Tsuchikane Y, Wu J-T, Sekimoto H, Miyaji K, Nozaki H. 2008. Morphology and molecular phylogeny of *Eudorina* sp. (Volvocaceae, Chlorophyceae) from Taiwan. *Hikobia* 15: 135–143.
- S5. Nozaki H. 2008. Zygote germination in *Pleodorina starrii* (Volvocaceae, Chlorophyta). *Biologia* 63/6: 774–776.
- S6. Starr RC. 1971. Sexual reproduction in *Volvox africanus*. In Parker BC, Brown RM, editors. *Contributions to phycology*. Lawrence (KS): Allen Press. pp. 59–66.
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- S9. Nakada T, Shinkawa H, Ito T, Tomita M. 2010. Recharacterization of *Chlamydomonas reinhardtii* and its relatives with new isolates from Japan. *J Plant Res.* 123: 67–78.