

Supplementary Material

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Supplementary Table S1. Strains of the *Euglena deses* complex used in this study and the GenBank accession numbers for their nuclear SSU, LSU and plastid SSU, LSU rDNA gene sequenced

Taxon	Strains	GenBank accession			
		Nuclear		Plastid	
		SSU	LSU	SSU	LSU
<i>E. adhaerens</i> Matvienko	ASW08138	EU750713	None	None	None
	Songdang042007A, Korea	KX889634	KX889653	KX889674	KX889698
<i>E. agilis</i> Carter	MI08, USA	FJ719607	KT304904	FJ719652	EF525322
	MI71, USA	KX889635	KX889654	KX889675	KX889699
<i>E. cantabrica</i> Pringsheim	SAG1224-25	AJ532412	KT304908	AY626047	EF525316
	SAG1224-40	AJ532413	KT304909	FJ719655	EF525315
	SAG26.93	AY626065	None	AY626048	EF525314
<i>E. catarae</i> (Pringsheim) Marin et Melkonian	SAG1224-22	KX889636	KT304910	KX889676	KT305131
<i>E. clara</i> Skuja	SAG25.98	AJ532423	DQ140114	FJ719654	EF525326
<i>E. deses</i> Ehrenberg (as <i>E. deses</i> v. <i>intermedia</i>)	ASW08044	AJ532408	None	KX889677	KX889700
	ASW08074	HM213844	None	KX889678	None
	Bihaksan031007A, Korea	KX889637	KX889655	KX889679	KX889701
	CCAC0090	KX889638	KX889656	KX889680	None
	Daeriji010910A, Korea	KX889639	KX889657	KX889681	KX889702
	Dongsan101709A, Korea	KX889640	KX889658	KX889682	KX889703
	Jeongsan092004A, Korea	KX889641	KX889659	KX889683	KX889704
	Jeongsan110104-5, Korea	None	KX889660	KX889684	KX889705
	Malinta012810E, Philippine	KX889642	KX889661	KX889685	KX889706
	Malmiji103109C, Korea	KX889643	KX889662	KX889686	KX889707
	MIJ04, MI, USA	KX889644	KX889663	KX889687	KX889708
	Philippine101309A, Philippine	KX889645	KX889664	KX889688	KX889709
	Psurononuma100609K, Japan	KX889646	KX889665	KX889689	KX889710
	Saeraewool102007R, Korea	KX889647	KX889666	KX889690	KX889711
	Saeraewool103109C, Korea	None	KX889667	KX889691	KX889712
(as <i>E. deses</i> var. <i>deses</i>)	SAG1224-19b	AJ532409	KT304911	AY626043	KT305132
(as <i>E. deses</i> var. <i>mesnii</i>)	SAG1224-20	AJ532410	KT304912	FJ719656	EF525328
	SAG1224-23	AJ532407	KT304913	EU750709	None
	Sinweoljunje120807A, Korea	KX889648	KX889668	KX889692	KX889713
	Yongsu042007D, Korea	None	KX889669	KX889693	KX889714
<i>E. geniculata</i> Dujardin	Daejeon101004, Korea	FJ719609	KT304914	FJ719657	EF525313
	SAG1224-4b	AY070249	KT304915	AF289241	KT305133
<i>E. gracilis</i> Klebs	SAG1224-5/25	AJ532426	None	NC001603	NC001603
<i>E. graculata</i> (Klebs) Schmitz	ACOI 921	AY626062	JQ398652	AF289242	KT305136
<i>E. laciniata</i> Pringsheim	SAG1224-8c	AJ532421	KT304919	EU221488	EF525295
	SAG1224-12b	DQ140149	KT304920	EU221491	EF525297
	SAG1224-31	AJ532420	None	EU370513	EF525396
<i>E. longa</i> (Pringsheim) Marin et Melkonian	UTEX512	AF112871	AY130223	NC002652	NC002652

Supplementary Table S1. Continued

Taxon	Strains	GenBank accession			
		Nuclear		Plastid	
		SSU	LSU	SSU	LSU
<i>E. mutabilis</i> Schmitz	CCAC0005	KX889649	KX889670	KX889694	KX889715
	CCAC0098	KX889650	KX889671	KX889695	KX889716
	CCAC0131	KX889651	KX889672	KX889696	KX889717
	ELC1W	EU090196	None	None	None
	Mulryang091209A, Korea	KX889652	KX889673	KX889697	KX889718
	NJ, Sandy, USA	AY523038	KT304921	EU221489	EF525329
	RT8n7	AY082988	None	None	None
<i>E. pseudochadefaudii</i> Zakrys & Kosmala	SAG1224-9b	AJ532405	KT304922	AY626044	KT305137
	MI00, USA	AY523033	KT304923	FJ719663	EF525303
<i>E. pseudostellata</i> Zakrys & Kosmala	ACOI1254(2951)	AY626064	KT304924	AY626046	KT305138
<i>E. pseudoviridis</i> Chadefaud	ACOI1255(2956)	EU730490	KT304925	EU370507	KT305139
	SAG1224-17c	AY523037	None	FJ719665	KT305141
<i>E. satelles</i>	ASW08093	HM213851	None	None	None
<i>E. splendens</i> Dangeard	Cp110601, USA	FJ719613	None	FJ719662	EF525307
<i>E. stellata</i> Mainx	ACOI1158	EU370489	KT304928	EU370508	KT305143
	SAG1224-14	AJ532419	KT304930	AF289244	EF525304
	SAG1224-34b	AJ532418	None	EU370499	KT305144
<i>E. tristella</i> Chu	Yeonhwaji091407 B, Korea	FJ719614	None	FJ719664	FJ719718
<i>E. undulata</i> Kato	MI03, USA	DQ140148	KT304932	EU370494	EF525324
<i>E. viridis</i> Ehrenberg	NJ001 (ATCC PRA 110)	AY523036	KT304933	EU370502	EF525317
	SAG1224-17d	AJ532417	KT304934	AF289248	FJ719719
<i>Euglenaria anabaena</i> var. <i>minor</i> (Mainx) Karnkowska et Linton	NJ05, USA	DQ140155	KT304937	EU370512	EF525331
	SAG1224-2	AJ532429	KT304938	FJ719668	EF525332
<i>Er. caudata</i> var. <i>minor</i> (Deflandre) Karnkowska et Linton	SAG1224-26a	EU624027	None	KT305056	EF525333
	SAG1224-32	AJ532436	KT304939	KT305057	KT305150
<i>Er. clavata</i> (Skuja) Karnkowska et Linton	SAG1224-32	AJ532436	KT304939	KT305057	KT305150
<i>Colacium mucronatum</i> Bourrelly et chadefaud	Jjockji033107A, Korea	FJ719601	KT304893	FJ719645	FJ719710
<i>Cryptoglena pigra</i> Ehrenberg	SAG1211-1	AJ532441	KT304894	EU221482	EF525388
	ACOI1338	JQ356763	JQ356785	JQ356810	EF525631
<i>C. skujae</i> Kim, Triemer et Shin	CCAP1212-1	AJ532437	JQ356786	EU221483	EF525364
<i>Discoplastis adunca</i> (Schiller) R. E. Triemer	ASW08039	AJ532453	KJ158185	FJ719650	FJ719714
<i>D. spathirhyncha</i> (Skuja) R. E. Triemer	SAG1224-42	AJ532454	KJ158186	AY130236	EF525355
<i>Lepocinclis acus</i> var. <i>major</i> (Müller) Marin et Melkonian	SAG1224-1b	AJ532456	JN603872	EU221495	EF525351
<i>L. ovum</i> (Ehrenberg) Minkevich	SAG1244-8	AF110479	JN603873	AY221726	EF525352
<i>Monomorphina aenigmatica</i> (Drezepolski) Nudelman et Triemer	ACOI 1055	FJ719621	JN603874	EU221499	FJ719727
	ACOI2778 (= ACOI1299, ACOI2583)	FJ719623	JN603905	FJ719679	EF525359
<i>M. pyrum</i> (Ehrenberg) Mereschowski	ACOI1312	FJ719636	KF744148	FJ719694	FJ719736
<i>Phacus skujae</i> Skvortsov	SAG1261-1	AJ532477	KF744138	FJ719690	EF525336
<i>P. pleuronectes</i> (Müller) Nitzsch	SAG1280-1	AY015000	KT304968	EU221506	EF525384
<i>Strombomonas acuminata</i> (Schmarda) Deflandre	S10	DQ140131	KT304971	FJ719699	FJ719740
<i>S. borystheniensis</i> (Roll) Popowa	ACOI1323	EF999904	KT304981	EU221508	KT305176
<i>Trachelomonas armata</i> (Ehrenberg) Stein	T235, USA	DQ140141	KT304998	EU221518	KT305181
<i>T. scabra</i> Playfair					

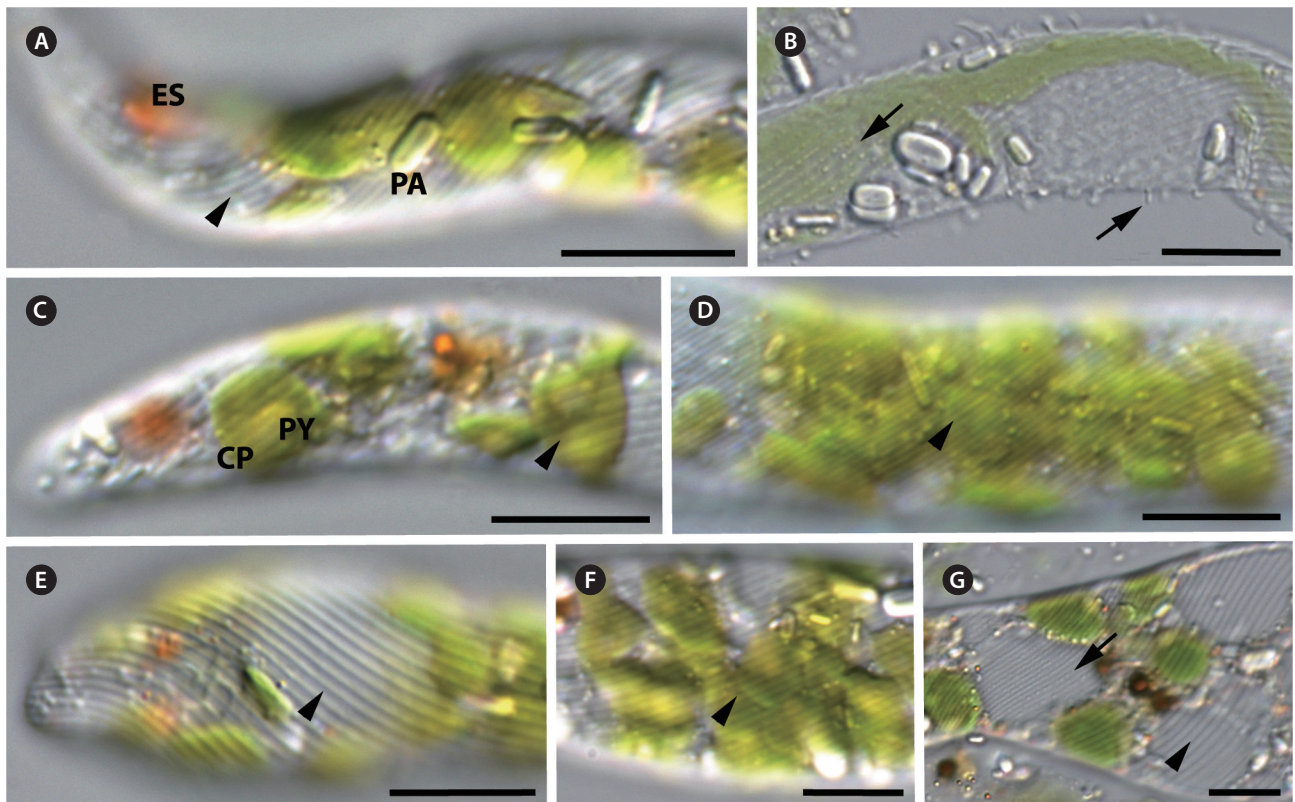
New sequences are indicated in bold type.

SSU, small subunit; LSU, large subunit; ACOI, Coimbra Collection of Algae, Coimbra, Portugal; ASW, Algenkulture-Sammlung an der Universität Wien, Vienna, Austria; ATCC, America Type Culture Collection, Manassas, VA, USA; CCAC, Culture Collection of Algae at the University of Cologne, Köln, Germany; CCAP, Culture Collection of Algae et Protozoa, Scotland; Korea, Korea isolate Woongghi lab, Korea; MI, Michigan isolate Triemer lab, USA; NJ, New Jersey isolate Triemer lab, USA; SAG, Sammlung von Algenkulturen Pflanzenphysiologisches Institut der Universität Göttingen, Germany; UTEX, Culture Center of Algae, Austin, TX, USA.

Supplementary Table S2. Evolutionary models, log likelihood values (-lnL), and model parameters resulted from each phylogenetic analyses for the combined data sets

Model parameter / data set	Bayesian	RxML
	GTR + I + G	GTR + G
-lnL	67,889.01	68,018.4
I	0.3449	-
G	0.6298	0.3197
Base frequencies (estimation)		
A	0.2691	0.2667
C	0.2067	0.2170
G	0.2690	0.2831
T	0.2552	0.2332
Substitution rate		
A-C	0.0858	0.9959
A-G	0.2736	3.2235
A-T	0.1004	1.3180
C-G	0.0497	0.5527
C-T	0.4121	5.0823
G-T	0.0785	1.0000

-lnL, negative log likelihood; A, C, G and T, frequency of nucleotides; I, fraction of unchangeable nucleotides; G, shape parameter of gamma (G) distribution rates; A-C, A-G, A-T, C-G, C-T, and G-T, rates of reversible nucleotide substitutions.



Supplementary Fig. S1. Light micrographs of *Euglena deses* strains showing eyespot (ES), chloroplast (CP), paramylon (PA), pyrenoid (PY), pellicular strip (arrowheads), and papillae (arrows). All representative strains have well defined pellicular strips without periplast ornamentations. (A & B) Light micrographs of Saeraewool103109C strain. (A) Ellipse-shaped paramylon grains and pellicular strips without ornamentations. (B) Pellicular strips covered with numerous rows (arrows) of inner cellular materials (described as papillae by Karnkowska-Ishikawa et al. 2011) when a cover slide pressed the cell by evaporating water. (C) Pellicular strips of the SAG1224-19b. (D) Pellicular strips of ASW08074. (E) Strong pellicular strips at anterior part of the Jeongsan092004A cell. (F & G) Light micrographs of ASW08044. (F) Smooth pellicular strips and chloroplast (arrowhead). (G) Papillae-like dots (left side, arrow) and smooth pellicular strips (right side, arrowhead). Scale bars represent: A-G, 10 μ m.