Fulnečková et al. 2013. A broad phylogenetic survey unveils the diversity and evolution of telomeres in eukaryotes.

Supplementary material online

TRAP assay

Telomerases are known to protect broken non-telomeric DNA by addition of telomeric repeats *de novo*, and the ability of telomerases to utilize non-telomeric substrates for telomere repeat addition is used in the TRAP assay (fig. S2A). In this PCR-based method telomerase elongates a non-telomeric substrate oligonucleotide. Then the product is amplified in the second step by PCR using the same non-telomeric substrate primer and a telomeric oligonucleotide as a reverse primer. The telomerase-enriched fractions for TRAP assays were purified from the "crude extract" by precipitation with 10% PEG 8000. We compared telomerase activity in samples of crude protein extracts (without PEG precipitation), the telomerase-enriched fractions and the fractions of proteins not precipitated by PEG. The TRAP assay (fig. S2A) was performed in two phases (Sýkorová et al. 2003). In the extension step, 10 pmol of a substrate primer (47F; Fojtová et al. 2002) was elongated at 26ºC for 45 min in a reaction mix with the telomerase-enriched extract containing 0.1-1 μg of total protein. After the extension step, samples were heat-inactivated, then a mixture containing 10 pmol of a reverse primer (TELPR30-3A; Fulnečková et al. 2012) and 2 units of DyNazyme II Polymerase (Finnzymes) was added and PCR amplification of the TRAP product was performed.

Alternative template usage

The template region of any telomerase RNA subunit is a short sequence matching usually one and a half of the telomere repeat synthesized (fig. S2D, on right). Enzymatic properties of telomerases differ e.g. in the accuracy of telomere repeat synthesis (Sykorova et al. 2003; Weiss-Schneeweiss et al. 2004), limited usage of non-telomeric substrate oligonucleotides (Fitzgerald et al. 1996) or flexibility in annealing to the template region (Sykorova et al. 2006b). The RNA subunit of green algal telomerases has not been identified yet, but according to known properties of other telomerases (Cifuentes-Rojas et al. 2011; Chen et al. 2000) some prediction could be made. The TRAP assay (see (Fajkus 2006) for review) comprises two subsequent steps (i) elongation of the substrate primer by telomerase and (ii) amplification of elongated product by PCR. During the elongation step of the TRAP assay, the 3'-end of the substrate oligonucleotide anneals to the template region within the RNA subunit (fig. S2D, on right). The positioning of the substrate oligonucleotide onto the template region sequence is delimited by biochemical properties of the protein subunit. Then telomerase adds nucleotides up to the 3'-end of the template region using its reverse transcriptase activity. After that, the elongated substrate primer is moved into position at the

5' end of the template region and next round of annealing and synthesis is completed (see (Kelleher et al. 2002) for review). Synthesis of the first telomeric repeat sets a new and uniform 3'-end of the elongated substrate primer and this makes next synthesis regular. In the PCR step, telomerase-elongated products are amplified using a substrate primer and a telomere reverse primer. The TRAP products show a typical ladder of products with a periodicity matching the length of telomeric repeat synthesized, e.g. a 6-nucleotide periodicity for the human-type repeat TTAGGG and a 7-nucleotide periodicity for the *Arabidopsis*-type repeat TTTAGGG.

Fitzgerald et al. (Fitzgerald et al. 1996) reported the most important part of a non-telomeric substrate sequence at its 3'-end. The substrate primers 47F and pSSyF differ in their 3'-end sequence and thus use a different part of the putative template region. The pattern of TRAP products shown in fig. S2 suggests that the 47F primer anneals to a similar position within the template region of algal and *Arabidopsis* telomerases. However, using the substrate primer pSSyF, the TRAP assay displays the same result for *Arabidopsis* telomerase and *Jaagiella alpicola* (TEL84, Trebouxiophyceae) but different for *Arabidopsis* and four other algal strains representing Trebouxiophyceae (TEL88 *Heterochlorella luteoviridis*, TEL90 *Auxenochlorella protothecoides*), Chorophyceae (TEL98 *Chromochloris zofingiensis*), and Xanthophyceae (TEL202 *Pleurochloris meiringensis*)(fig. S2). A similar duality in template region usage was observed in plants (Sykorova et al. 2006b). The primer pSSyF could possibly anneal at two different sites within the template region (Fig. S2D, on right), resulting in addition of portions of the telomeric repeat differing by one nucleotide (fig. S2D, on left;). The telomere reverse primer TELP30-3A, which has the triplet AAA at the 3' end, would then anneal at different positions with respect to the end of the substrate primer, depending on the position of the triplet TTT most proximal to the substrate oligonucleotide. This would result in a different length of the shortest TRAP product (Fig. S2B, C, arrows). The preference of the substrate primer annealing site is driven by the telomerase protein subunit and could be very strong, showing a complete shift of the ladder (e.g. TEL98 *Chromochloris zofingiensis* and TEL202 *Pleurochloris meiringensis*), or weaker, showing a different amount of the two shortest TRAP products synthesized (e.g. TEL88 *Heterochlorella luteoviridis* and TEL90 *Auxenochlorella protothecoides*). Tight restriction to the substrate oligonucleotide sequence is apparent in Xanthophyceae (fig. S2C). The algal strain TEL204 *Heterococcus protonematoides* is not able to utilize the substrate oligonucleotide pSSyF in contrast to TEL202 *Pleurochloris meiringensis* that display a result similar to three green algal strains shown in fig. S2B.

Detection of telomere-like minisatellite repeats by Southern hybridization

The problem of multiple positive hybridization signals provided by different sequence variants of telomeric repeat probes was first addressed by Allshire et al. (Allshire et al. 1989) who compared

signals of $(TTGGGG)_n$, $(TTAGGG)_n$ and $(TTTAGGG)_n$ probes hybridized onto human telomeres. They found that the signal of $(TTGGGG)_n$ on human DNA corresponds to the presence of this sequence rather than to cross-hybridization. With more types of telomeric repeats identified in eukaryotic organisms, the question is repeatedly asked whether the candidate sequences might just be cross-hybridizing to a uniform tandem repeat or if there could be several types of repeat motifs which would contribute to a heterogeneous repetitive telomeric region. A specific hybridization with telomere minisatellite sequences representing seven telomere types have been demonstrated by Neplechová et al. (Neplechova et al. 2005) despite the sequence similarity among minisatellites. A high specificity of detection was achieved by various conditions of hybridization, washing, and probe preparation. The most problematic was evaluation of signals using the *Arabidopsis*-type probe (TTTAGGG) onto control samples containing the *Chlamydomonas*-type telomeric sequence (TTTTAGGG) and high stringency conditions for washing were recommended to avoid cross-hybridization. It could be hypothesized the cross-hybridization is caused by a high similarity between these sequence types.

Our collection comprised several algal strains with the *Chlamydomonas*-type of telomeric sequence (Klebsormidiophyceae), which was identified as the "true" telomeric repeat synthesized by telomerase (see Results and fig. 1G), and the strain TEL97 *Klebsormidium subtilissimum* with the TTTTAGG-type of telomeric repeat. Such a difference in the telomeric repeat type could be possible because the sequence synthesized by telomerase is purely defined in the template region of the telomerase RNA subunit and mutation within this region leads to synthesis of a variant type (Sykorova et al. 2003). A similar transition of the minisatellite sequence from the TTTAGGG-type to the TTTAGG-type has been reported in telomeres of the apicomplexan *Cryptosporidium parvum* (Liu et al. 1998). We investigated occurrence of the TTTTAGGG-type and TTTTAGG-type repeats in the genome of *Klebsormidium subtilissimum* (TEL97) using Southern hybridization on the BAL 31 digested samples (fig. S3) and various washing conditions (not shown). The pattern of both probes remained the same when using low stringency (2xSSC, 0.1% SDS; fig. S3) or two more stringent washing solutions (1xSSC, 0.1%SDS and 0.6xSSC, 0.1% SDS) and a gradual decrease of the overall signal strength corresponding to stringency of washing was observed. This result suggests most likely cross-hybridization of both probes and impossibility to distinguish between their signals using Southern hybridization. We presume that the telomerase of TEL97 could synthesize variant telomeric repeats as suggested by the TRAP assay (fig. 1G) and they may form a small portion at the chromosomal ends in comparison to a predominant *Chlamydomonas*-type in subtelomeres. Synthesis of a different telomere type and occurrence of ancestral telomeric minisatellites in subtelomeres and/or internal chromosomal regions was reported also in plants

(Hyacinthaceae, Asparagales) with the human-type repeat synthesized by telomerase and *Arabidopsis*-type minisatellites present in the genome (Adams et al. 2001; Sykorova et al. 2003). Comparison of the hybridization pattern revealed a different quality of genomic DNA (gDNA) samples and high-molecular-weigth DNA (HMW-DNA) samples prepared by proteinase K method in solution and in agarose blocks, respectively. We experienced very week signals of several gDNA samples used in a dot-blot hybridization experiment (fig. 3) and in a TRF analysis (not shown) when compared to HMW-DNA samples used in a BAL 31 digestion experiment (a representative sample shown in fig. S4A). The proteinase K method was previously suitable for preparation of genomic DNA from a collection of strains from the green algal phylum Chlorophyta (Fulneckova et al. 2012). The collection analysed here comprises a much wider spectrum of organisms and not surprisingly, we experienced problems with isolation of gDNA samples e.g. from Zygnematophyceae and rhodophytes. We presume that a poor quality of gDNA samples caused also the inconsistent Southern hybridization results mentioned above. In contrast to gDNA samples prepared in solution, the HMW-DNA samples are prepared using basically the same protocol but the material is embedded into agarose blocks. These blocks are incubated in extraction solution several times and washed during preparation so the impurities are effectively removed from HMW-DNA samples.

Supplementary tables

Table S1. Algal strains used for this study

Table S2. Oligonucleotide sequences

Table S3. Telomerase enrichment in protein fractions during PEG purification

Table S4. Negative TRAP assays

Table S5. Telomere sequences search in genome databases

Supplementary figures

Fig. S1. Analysis of inhibitory effect of algal proteins to *Arabidopsis thaliana* telomerase.

Fig. S2. Different primer usage.

Fig. S3. Analysis of telomeres in *Klebsormidium subtilissimum*.

Fig. S4. Analysis of telomeres in *Klebsormidium crenulatum* and *Vischeria punctata*.

Fig. S5. TRAP assay of rhodophytes.

Table S1. Algal strains used for this study

The assignment of the strains into phyla and classes has been confirmed by sequencing the 18S rDNA and/or ITS loci where appropriate (in cases where the morphology of the strain itself was inconclusive; data not shown). The strain CCAP 881/1 is assigned into *Heterotrichella gracilis* Reisigl by the CCAP collection, but it is not the authentic strain and its morphology does not fit the description of *H. gracilis*. The latter species presumably belongs to Xanthophyceae, whereas CCAP 881/1 is a green alga related to the genus *Raphidonema* in the class Trebouxiophyceae (data not shown). Therefore, we put the name "*Heterotrichella gracilis*" into quotation marks. The strains were obtained from the following culture collections: SAG – Sammlung von Algenkulturen, University of Goettingen (http://www.uni-goettingen.de/en/184983.html), CCAP – Culture Collection of Algae and Protozoa, SAMS, Oban (http://www.ccap.ac.uk/), UTEX – The Culture Collection of Algae at the University of Texas at Austin (http://web.biosci.utexas.edu/utex/), CAUP – Culture Collection of Algae of Charles University in Prague (http://botany.natur.cuni.cz/algo/caup.html), NCMA (formerly CCMP) – National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences (https://ncma.bigelow.org/).

Phylum	Class	TEL	***Species***	Strain number
Glaucophyta		**195**	**Glaucocystis nostochinearum**	SAG 45.88
Rhodophyta	**Porphyridiophyceae**	**131**	*Porphyridium purpureum*	CCAP 1380/3
	Rhodellophyceae	**213**	*Rhodella maculata*	SAG 45.85
	Stylonematophyceae	**214**	*Rhodosorus marinus*	SAG 116.79
Chlorophyta	**Chlorophyceae**	**104**	*Chlamydomonas hydra*	SAG 4.73
		87	*Scenedesmus vacuolatus*	SAG 211-8b
		89	*Muriella decolor*	SAG 249-2
		91	*Mychonastes homosphaera*	SAG 6.95
		98	*Chromochloris zofingiensis*	SAG 211-14
		108	*Neochloris conjuncta*	SAG 78.80
		123	*Chlorococcum hypnosporum*	SAG 213-6
		138	*Pseudomuriella aurantiaca*	SAG 249-1
		140	*Follicularia paradoxalis*	SAG 33.98
		188	*Bracteacoccus cohaerens*	UTEX 1272
	Trebouxiophyceae	**01**	*Chlorella vulgaris*	CCAP 211/11B
		84	*Jaagiella alpicola*	SAG 11.97
		85	*Asterochloris phycobiontica*	SAG 26.81
		88	*Heterochlorella luteoviridis*	SAG 211-4
		90	*Auxenochlorella protothecoides*	SAG 211-7A
		121	*Dictyochloropsis irregularis*	SAG 2036
		134	*„Heterotrichella gracilis"*	CCAP 881/1
	Chlorodendrophyceae	**211**	*Tetraselmis chui*	SAG 1.96
		212	*Tetraselmis striata*	SAG 41.85
	Ulvophyceae	**86**	*Planophila laetevirens*	SAG 2008
		94	*Pseudendocloniopsis botryoides*	SAG 465-1
		111	*Pseudendoclonium printzii*	SAG 467-1
		124	*Pseudendoclonium basiliense*	SAG 466-2
		137	*Desmochloris halophila*	UTEX 2073
		139	*Pirula salina*	SAG 1.95

Streptophyta	**Klebsormidiophyceae**	**187**	*Klebsormidium crenulatum*	SAG 37.86
		97	*Klebsormidium subtilissimum*	SAG 384-1
		100	*Klebsormidium dissectum*	SAG 2155
		101	*Klebsormidium flaccidum*	SAG 7.91
		103	*Klebsormidium nitens*	SAG 13.91
	Zygnematophyceae	**181**	*Zygnema circumcarinatum*	SAG 698-1a
		196	*Micrasterias crux-melitensis*	CAUP K-602
		198	*Mesotaenium endlicherianum*	SAG 12.97
Haptophyta	**Pavlovophyceae**	**210**	*Pavlova lutheri*	SAG 926-1
Alveolata		**233**	*Chromera velia*	CCMP 2878
Euglenozoa	**Euglenophyceae**	**185**	*Euglena anabaena*	SAG 1224-2
		206	*Euglena stellata*	SAG 1224-14
		207	*Euglena geniculata*	SAG 1224-4b
Ochrophyta	**Xanthophyceae**	**95**	*Xanthonema cf. hormidioides*	SAG 836-1
		202	*Pleurochloris meiringensis*	SAG 860-3
		203	*Xanthonema hormidioides*	SAG 836-1
		204	*Heterococcus protonematoides*	SAG 835-9
		205	*Botrydiopsis intercedens*	SAG 806-3
	Eustigmatophyceae	**133**	*Eustigmatos polyphem*	CCAP 860/8
		201	*Vischeria punctata*	SAG 887-1
	Bacillariophyceae	**231**	*Phaeodactylum tricornutum*	CCMP 2561

Table S2. Oligonucleotide sequences

Name	Sequence 5´-3´	Analysis	Ref.
TS21	GACAATCCGTCGAGCAGAGTT	TRAP substrate	(Fitzgerald et al. 1996)
CaMV	CGTCTTCAAAGCAAGTGGATT	TRAP substrate	(Fajkus et al. 1998)
47F	CGCGGTAGTGATGTGGTTGTGTT	TRAP substrate	(Fojtova et al. 2002)
pSSyF	CTTTTGAAAAATGGATGGGTTCCTTGCTTGGAATT	TRAP substrate	this work
GG(21)	CACTATCGACTACGCGATCAG	TRAP substrate	(Fitzgerald et al. 1996)
HUTC	AACCCTAACCCTAACCCTAAC	TRAP reverse	(Sykorova et al. 2006b)
TELPR	CCGAATTCAACCCTAAACCCTAAACCCTAAACCC	TRAP reverse	(Fajkus et al. 1998)
TELPR30-3A	CCGAATTCAACCTAAACCCTAAACCCTAAA	TRAP reverse	(Fulneckova et al. 2012)
T3AG2-C	AAACCTAAACCTAAACCTAAACCTA	TRAP reverse	(Fulneckova et al. 2012)
T4AG2-C	CCTAAAACCTAAAACCTAAAACCTA	TRAP reverse	this work
T4AG2-PR	CCGAATTCAACCTAAAACCTAAAACCTAAAACCTA	TRAP reverse	this work
TTATAG3-C	CTATAACCCTATAACCCTATAA	TRAP reverse	this work
CHTRTTRAPRev1	CCC TAA AAC CCT AAA ACC CTA AAA	TRAP reverse	this work
BOTPR-32	CCGAATTCATCCTAACCTAACCTAACCTAACC	TRAP reverse	this work
T2CG3-PR	CCGAATTCAACCGAACCCGAACCCGAACC	TRAP reverse	this work
T3G3-C	AACCCAAACCCAAACCCAAAC	TRAP reverse	(Fulneckova et al. 2012)
T4G3-C	AAACCCAAAACCCAAAACCCA	TRAP reverse	this work
TTAAG3-C	ACCCTTAACCCTTAACCCTTA	TRAP reverse	this work
TATAG3-C	ACCCTATACCCTATACCCTAT	TRAP reverse	this work
ATTTAG3-C	CTAAATCCCTAAATCCCTAAAT	TRAP reverse	this work
TATTAG3-C	CTAATACCCTAATACCCTAATA	TRAP reverse	this work
CATC	ACCCTAGACCCTAGACCCTAG	TRAP reverse	this work
RedALTPRV-1	CCGATATCCCATTCCCCCCATTCCC	TRAP reverse	this work
RedALTPRV-2	CCGATATCCCCATTCCCCCCATTCC	TRAP reverse	this work
RedALTPRV-3	CCGATATCCCCCATTCCCCCCATT	TRAP reverse	this work
RedALTPRV-4	CCGATATCCCCATTTCCCCCATTTCCC	TRAP reverse	this work
RedALTPRV-5	CCGATATCCCATTTTCCCCCATTTTCCC	TRAP reverse	this work
RedALTPRV-6	CCGATATCCCCTTTCCCCCCTTTCC	TRAP reverse	this work
GthRedPR	CCGAATTCTCCCCCCTTCTCTCTCTC	TRAP reverse	this work
ScRedPR	CCGAATTCTCTCCTCTCCCTCTCTC	TRAP reverse	this work
CHSB	GTTTTAGGGTTTTAGGGTTTTAGGGTTTTAG	Southern hybridization	(Sykorova et al. 2003)
HUSB	TTAGGGTTAGGGTTAGGGTTAGGGTTAG	Southern hybridization	(Sykorova et al. 2003)
ATSB	GGTTTAGGGTTTAGGGTTTAGGGTTTAG	Southern hybridization	(Sykorova et al. 2003)
TTTAGGC-SB	GCTTTAGGCTTTAGGCTTTAGGCTTTAG	Southern hybridization	(Fulneckova et al. 2012)
TTCAGGG-SB	TTCAGGGTTCAGGGTTCAGGGTTCAGG	Southern hybridization	(Fulneckova et al. 2012)
T4AG2-SB	TAGGTTTTAGGTTTTAGGTTTTAGGTTTTAGGTT	Southern hybridization	this work
Red alga-SB	CCCCCCATTCCCCCCATTCCCCCCATT	Southern hybridization	(Nozaki et al. 2007)
T2CG3-SB	GGGTTCGGGTTCGGGTTCGGGTT	Southern hybridization	this work
T3G3-SB	TTTGGGTTTGGGTTTGGGTTTGGGTTTG	Southern hybridization	this work

Table S3. Telomerase activity in protein fractions

Class	TEL number	crude	extract	supernatant
Glaucophyta	TEL 195	n.a.	+++	+++
Treboxiophyceae	TEL 84	+++	+++	+++
	TEL 88	+++	+++	+++
	TEL 90	n.a.	+++	+++
	TEL 121	+++	+++	+++
Chlorophyceae	TEL 98	+++	+++	+++
	TEL 108	+++	+++	+++
	TEL 140	+++	+++	+++
	TEL 188	n.a.	+++	+++
	TEL X-2	+++	+++	+++
Prasinophyceae	TEL 211	n.a.	+++	+++
	TEL 212	n.a.	++	++
Klebsormidiophyceae	TEL 97	n.a.	++	+/ -
	TEL 101	n.a.	++	-
	TEL 103	++	++	-
	TEL 187	+	++	-
Zygnemophyceae	TEL 181	n.a.	+	+/ -
	TEL 196	++	+++	+
	TEL 198	n.a.	+++	+
Xanthophyceae	TEL 95	n.a.	+	+/-
	TEL 110	++	++	+
	TEL 202	n.a.	+++	++
	TEL 203	+	++	+/-
	TEL 204	++	++	+
	TEL 205	++	++	++
Haptophyta	TEL 210	+/-	+++	+/-
Alveolata	Chromera	n.a.	++	-
Euglenophyta	TEL 185	n.a.	+++	+
	TEL 206	+++	+++	+
	TEL 207	n.a.	++	+

n.a. not analyzed, + low activity, +++ high activity, - not active

Table S4. Negative primer combinations

Class	primer combination	GG(21)	TS21	pSSyF
Rhodophyta	**RedALTPRV-1**	TEL131,213, 214		
	RedALTPRV-2	TEL131,213, 214		
	RedALTPRV-3	TEL131,213, 214		
	RedALTPRV-4	TEL131,213, 214		
	RedALTPRV-5	TEL131,213, 214		
	RedALTPRV-6	TEL131,213, 214		
	GthRedPR	TEL131,213, 214		
	ScRedPR	TEL131,213, 214		
	T2CG3-PR		TEL131,213, 214	
	HUTC	TEL131,213, 214		
Klebsormidiophyceae	**T3AG2-C**			TEL97,100, 103, 187
	T3G3-C			TEL97,100, 103, 187
	BOTPR-33			TEL97,100, 103, 187
	T4G3-C	TEL97,103,187		
	TTCTAG3-C	TEL97,103,187		
	TTAAG3-C	TEL97,103,187		
	TATAG3-C	TEL97,103,187		
	ATTTAG3-C	TEL97,103,187		
	TATTAG3-C	TEL97,103,187		
	TTATAG3-C	TEL97,103,187		
	T2CG3-PR		TEL187	
Ulvophyceae	**T3AG2-C**			TEL137
	T3G3-C			TEL137
	BOTPR-33			TEL92,93,94,124,137
	T4G3-C	TEL137		
	TTCTAG3-C	TEL137		
	TTAAG3-C	TEL137		
	TATAG3-C	TEL137		
	ATTTAG3-C	TEL137		
	TATTAG3-C	TEL137		
	TTATAG3-C	TEL137		
	T2CG3-PR		TEL94,124,137,139	
	T4AG2-C			TEL86, 94, 111, 124,137
Eustigmatophyceae	**T3AG2-C**			TEL133, 201
	T3G3-C			TEL133, 201
	BOTPR-33			TEL133, 201
	T4G3-C	TEL133, 201		
	TTCTAG3-C	TEL133, 201		
	TTAAG3-C	TEL133, 201		
	TATAG3-C	TEL133, 201		
	ATTTAG3-C	TEL133, 201		
	TATTAG3-C	TEL133, 201		
	TTATAG3-C	TEL133, 201		
	T2CG3-PR		TEL133, 201	
	T4AG2-C			TEL133, 201

Table S5. Telomere sequences search in genome databases

Major taxonomic group	subgroup	*species*	telomere	database search	accession or database	reference
Metazoa	Vertebrata	*Homo sapiens* and other vertebrata	TTAGGG			(Podlevsky et al. 2008) and references herein
	Tunicata	*Oikopleura dioica*	TTAGGG	???	CABV01000000, CABW01000000	(Podlevsky et al. 2008) and references herein
	Cephalochordata	*Branchiostoma floridae*	TTAGGG	TTAGGG	JGI	(Costa Castro and Holland 2002)
	Echinodermata	*Strongylocentrotus purpuratus*	TTAGGG	TTAGGG	AAGJ04000000	(Podlevsky et al. 2008) and references herein
		Holothuria tubulosa	TTAGGG			(Plohl et al. 2002)
	Arthropoda	*Drosophila melanogaster*	Het-A, TART			(Podlevsky et al. 2008) and references herein
		Anopheles gambiae	recombination			(Podlevsky et al. 2008) and references herein
		Apis melifera	TTAGG	TTAGG	AADG06000000	(Podlevsky et al. 2008) and references herein
		Tribolium castaneum	TCAGG	TCAGG	AAJJ01000000	(Mravinac et al. 2011)
		Tenebrio molitor	TCAGG			(Mravinac et al. 2011)
		Penaeus japonius	TTAGG			(Lang et al. 2004)
		Bombyx mori	TTAGG			(Podlevsky et al. 2008) and references herein
		Acyrthosiphon pisum	TTAGG	TTAGG	ABLF02000000	(Monti et al. 2011) (2010)
		Megoura viciae	TTAGG			(Monti et al. 2011)
		Myzus persicae	TTAGG			(Monti et al. 2011)
		Rhopalosiphum padi	TTAGG			(Monti et al. 2011)
		Ephestia kuehniella	TTAGG			(Traut et al. 2007)
		Chironomus tenants	satellites			(Podlevsky et al. 2008) and references herein
	Nematoda	*Ascaris lumbricoides*	TTAGGC			(Podlevsky et al. 2008) and references herein
		Caenorhabditis elegans	TTAGGC			(Podlevsky et al. 2008) and references herein
		Caenorhabditis remanei		TTAGGC	AAGD02000000	
		Parascaris univalens	TTGCA			(Podlevsky et al. 2008) and references herein
	Mollusca	*Donax trunculus*	TTAGGG			(Plohl et al. 2002)
		Mytilus galloprovincialis	TTAGGG			(Plohl et al. 2002)
		Haliotis rufescens	TTAGGG			(Gallardo-Escarate et al. 2005)
		Argopecten irradians	TTAGGG			(Estabrooks 1999)
Metazoa	Annelida	*Platynereis dumerilii*	TTAGGG			(Jha et al. 1995)

		Pomatoceros lamarckii	TTAGGG			(Jha et al. 1995)
		Octodrilus complanatus	TTAGGG			(Vitturi et al. 2002a)
		Haemopis sanguisuga	TTAGGG			(Vitturi et al. 2002b)
	Platyhelminthes	*Schistosoma mansoni*	TTAGGG			(Hirai and LoVerde 1996)
		Schmidtea mediterannea	TTAGGG			(Tan et al. 2012)
	Ctenophora	*Pleurobrachia pileus*	TTAGGG			(Traut et al. 2007)
	Cnidaria	*Madracis auretenra*	TTAGGG			(Zielke and Bodnar 2010)
		Madracis decactis	TTAGGG			(Zielke and Bodnar 2010)
		Aurelia aurita	TTAGGG			(Traut et al. 2007)
		Chrysaora hysoscella	TTAGGG			(Traut et al. 2007)
		Cyanea lamarcki	TTAGGG			(Traut et al. 2007)
		Sanderia malayensis	TTAGGG			(Traut et al. 2007)
		Tripedalia cystophora	TTAGGG			(Traut et al. 2007)
		Acropora surculosa	TTAGGG			(Sinclair et al. 2007)
		Nematostella vectensis	TTAGGG			(Traut et al. 2007)
		Hydra vulgaris	TTAGGG			(Traut et al. 2007)
		Hydra magnipapillata	TTAGGG			(Anokhin et al. 2010)
	Placozoa	*Trichoplax adhaerens*	TTAGGG			(Traut et al. 2007)
	Porifera	*Leucosolenia sp.*	TTAGGG			(Traut et al. 2007)
		Sycon sp.	TTAGGG			(Traut et al. 2007)
		Eunapius fragilis	TTAGGG			(Traut et al. 2007)
		Suberites domuncula	TTAGGG			(Koziol et al. 1998)
		Geodia cydonium	TTAGGG			(Koziol et al. 1998)
		Leucetta chagosensis	TTAGGG			(Sakai et al. 2007)
		Halichondria japonica	TTAGGG			(Sakai et al. 2007)
		Halichondria panicea	TTAGGG			(Sakai et al. 2007)
Choanoflagellata		*Monosiga brevicollis*	TTAGGG	TTAGGG	JGI	(Robertson 2009)
		Codosiga gracilis	TTAGGG			(Traut et al. 2007)
Fungi	Basidiomycota	*Coprinopsis cinerea*	TTAGGG	TTAGGG	AACS02000000	(Stajich et al. 2010)
		Phanerochaete chrysosporium		TTTAGGG	AADS01000000	(Ramirez et al. 2011)
		Ceriporiopsis subvermispora	TTAGGG			(Fernandez-Fueyo et al. 2012)
		Pleurotus ostreatus	TTAGGG			(Perez et al. 2009)
		Dacryopinax sp.		TTAGGG	AEUS01000000	
		Cryptococcus neoformans var. neoformans	TTAGGGGG	TTAGGGGG	AAEY01000000	(Edman 1992)
		Ustilago maydis	TTAGGG	TTAGGG	AACP01000000	(Sanchez-Alonso et al. 1996)
		Puccinia triticina		TTAGGG	ADAS01000000	
		Helicobasidium mompa	TTAGGG			(Aimi et al. 2003)
		Rhodotorula glutinis		TTAGGG	AEVR01000000	
Fungi	Basidiomycota	*Wallemia sebi*		TTAGG	AFQX01000000	

	Taphrinomycotina	*Schizosaccharomyces pombe*	G2–8TTAC(A)			(Podlevsky et al. 2008) and references herein
	Saccharomycotina	*Yarrowia lipolytica*	GGACGATTG			(Podlevsky et al. 2008) and references herein
		Debaryomyces hanseni	ATGTTGAGGTGTAGGG			(Podlevsky et al. 2008) and references herein
		Candida albicans	ACGGATGTCTAACTTC TTGGTGT			(Podlevsky et al. 2008) and references herein
		Pichia stipitis	GGATCTTTTCACGTCT TGCGGTA			(Podlevsky et al. 2008) and references herein
		Saccharomyces cerevisiae	T(G)2-3(TG)1-6	T(G)2-3(TG)1-6	AAEG01000000	(Podlevsky et al. 2008) and references herein
		Kluyveromyces lactis	ACGGATTTGATTAGGTA TGTGGTGT			(Podlevsky et al. 2008) and references herein
		Eremothecium gossypii	GTGTGGTGTATGGGTC TCTCAGCG			(Podlevsky et al. 2008) and references herein
	Pezizomycotina	*Botryotinia fuckeliana (Botrytis cinerea)*	TTAGGG	TTAGGG	AAID01000000	(Levis et al. 1997)
		Sclerotinia sclerotiorum	TTAGGG	???	AAGT01000000	(Levis et al. 1997)
		Hypocrea jecorina (Trichoderma reesei)	TTAGGG	TTAGGG	AAIL02000000	(Martinez et al. 2008)
		Nectria haematococca		TTAGGG	JGI	
		Gibberella zeae	TTAGGG	TTAGGG?	AACM02000000	(Cuomo et al. 2007)
		Acremonium alcalophilum		TTAGGG	JGI	
		Neurospora crassa	TTAGGG			(Podlevsky et al. 2008) and references herein
		Neurospora tetrasperma		TTAGGG	JGI	
		Podospora anserina	TTAGGG			(Podlevsky et al. 2008) and references herein
		Chaetomium thermophilum		TTAGGG	BORK	
		Thielavia terrestris		TTAGGG	JGI	(Berka et al. 2011)
		Thielavia heterothallica		TTAGGG	JGI	(Berka et al. 2011)
		Magnaporthe oryzae (=Magnaporthe grisea)	TTAGGG	TTAGGG	AACU03000000	(Levis et al. 1997)
		Aspergillus fumigatus	TTAGGG	TTAGGG	AAHF01000000	(Podlevsky et al. 2008) and references herein
		Aspergillus oryzae	TTAGGGTCAACA	???		(Kusumoto et al. 2003)
		Emericella nidulans		TTAGGG	AACD01000000	
		Uncinocarpus reesii		TTAGGG	AAIW01000000	
		Coccidioides immitis		TTAGGG	AAEC01000000	
		Coniosporium apollinis		TTAGGG	AJKL01000000	
		Exophiala dermatitidis		TTTAGGG	AFPA01000000	
		Xanthoria parietina		TTAGGG	JGI	
		Beauveria bassiana	TTAGGG			(Viaud et al. 1996)
Fungi	Pezizomycotina	*Rosellinia necatrix*	TTAGGG			(Aimi et al. 2002)
		Paecilomyces strain	TTAGGG			(Inglis et al. 2005)

		Leptophaeria maculans	TTAGGG			(Leclair et al. 1996)
		Cladosporidium fulvum	TTAGGG			(Coleman et al. 1993)
	Glomeromycota	*Glomus intraradices*	TTAGGG			(Hijri et al. 2007)
	Mucoromycotina	*Rhizopus oryzae*	TTGTGG			(Ma et al. 2009)
		Mucor circinelloides		TTAGGG	JGI	
	Mortierellomycotina	*Mortierella alpina*		TTTTTTAGGG	ADAG01000000	
	Blastocladiomycota	*Allomyces macrogynus*		T(2-6)AGG	ACDU01000000	
		Catenaria anguillulae		TTTAGG	JGI	
	Chytridiomycota	*Batrachochytrium dendrobatidis*		TTAGGG	ADAR01000000	
		Spizellomyces punctatus		TTAGGG	ACOE01000000	
		Gonapodya prolifera		TTAGGG	JGI	
Microsporidia		*Encephalitozoon cuniculi*	G(A/G)GCCT(C/T)CT, GAGCCTTGTTT, GAGACGCAGTGTTGC CAGGATG	???	AEWD01000000	(Podlevsky et al. 2008) and references herein
Amoebozoa	Dictyostelida	*Dictyostelium discoideum*	palindrome arm	???	AAFI02000000	(Heidel et al. 2011)
		Polysphodylium pallidum	TAAGGG			(Heidel et al. 2011)
		Dictyostelium fasciculatum	TTAGGG	TTAGGG	ADHC01000000	(Heidel et al. 2011)
	Myxogastria	*Physarum polycephalum*	TTAGGG			(Podlevsky et al. 2008) and references herein
	Lobosea	*Acanthamoeba castellanii*		TTAGGG	AEYA01000000	
Excavata	Fornicata	*Giardia lamblia*	TAGGG	TAGGG	ACVC01000000	(Le Blancq et al. 1991)
	Euglenozoa	*Trypanosoma brucei*	TTAGGG			(Van der Ploeg et al. 1984)
		Trypanosoma cruzi	TTAGGG			(Van der Ploeg et al. 1984)
		Leishmania major	TTAGGG			(Fu and Barker 1998)
		Bodo saltans		TTAGGG?	WTSI (http://www.sanger.ac.uk/resources/downloads/protozoa/bodo-saltans.html)	
	Heterolobosea	*Naegleria gruberi*		TTTGGG	JGI	
	Jakobida	*Andalucia godoyi*		TTAGGG	Eliáš et al., unpublished genome sequence assembly	
	Malawimonadida	*Malawimonas californiana*		TTAGGG	Eliáš et al., unpublished genome sequence assembly	
Chloroplastida	Embryophyta	*Arabidopsis thaliana*	TTTAGGG			(Podlevsky et al. 2008) and references herein
Chloroplastida	Embryophyta	*Populus trichocarpa*	TTTAGGG			(Podlevsky et al. 2008)

		Vitis vinifera	TTTAGGG			and references herein (Podlevsky et al. 2008)
		Oryza sativa	TTTAGGG			and references herein (Podlevsky et al. 2008)
		Aloe sp.	TTAGGG			and references herein (Podlevsky et al. 2008)
		Iris tectorum	TTAGGG			and references herein (Sykorova et al. 2003)
		Ipheion uniflorum	TTAGGG			(Sykorova et al. 2006a)
		Selaginella martensii	TTTAGGG			(Fuchs and Schubert 1996)
		Psilotum nudum	TTTAGGG			(Suzuki 2004)
		Marchantia paleacea var. diptera	TTTAGGG			(Suzuki 2004)
		Pellia epiphylla	TTTAGGG			(Fuchs et al. 1995)
		Zamia furfuracea	TTTAGGG			(Fuchs et al. 1995)
		Barbula unguiculata	TTTAGGG			(Suzuki 2004)
		Physcomitrella patens	TTTAGGG			(Rensing et al. 2008; Shakirov et al. 2010)
	Mamiellophyceae	*Ostreococcus lucimarinus*	TTTAGGG			(Derelle et al. 2006)
		Ostreococcus tauri	TTTAGGG	TTTAGGG	JGI	
		Micromonas pusilla		TTTAGGG	ACCP01000000	
	Trebouxiophyceae	*Chlorella variabilis*		TTTAGGG	ADIC01000000	
		Coccomyxa subellipsoidea C-169	TTTAGGG	TTTAGGG	AGBL01000000	(Higashiyama et al. 1995)
		Asterochloris sp.		TTTAGGG	JGI	
	Chlorophyceae	*Chlamydomonas reinhardtii*	TTTTAGGG			(Petracek et al. 1990)
		Volvox carteri		TTTTAGGG	JGI	
Rhodophyta	Cyanidiophyceae	*Cyanidioschyzon merolae*	AATG6	AATG6	TOKYO	(Nozaki et al. 2007)
		Galdieria sulphuraria		TTTATT(T)AGGG	Galdieria genome database (http://genomics.msu.edu/galdieria/)	
	Bangiophyceae	*Porphyra umbilicalis*		???TTAGGG	NCBI-SRA Ilumina	
Glaucophyta		*Cyanophora paradoxa*		TTAGGG	Rutgers	
Alveolata	Ciliata	*Tetrahymena thermophila*	TTGGGG	TTGGGG	AAGF03000000	(Podlevsky et al. 2008) and references herein
		Paramecium tetraurelia	TT(T/G)GGG	TT(T/G)GGG	CAAL01000000	(Podlevsky et al. 2008) and references herein
		Ichthyophthirius multifiliis		TTGGGG	AEDN01000000	
		Nyctotherus ovalis	TTTTGGGG			(Ricard et al. 2008)
		Sterkiella nova (Oxytricha)	TTTTGGGG			(Podlevsky et al. 2008) and references herein
Alveolata	Ciliata	*Euplotes aediculatus*	TTTTGGGG			(Podlevsky et al. 2008) and references herein
		Metopus es	TTTTGGGG			(McGrath et al. 2007)

		Chilodonella uncinata	TTTGGG			(McGrath et al. 2007)
		Uroleptus sp.	TTTTGGGG			(Chang et al. 2006)
	Apicomplexa	*Cryptosporidium parvum* Iowa II	TTTAGG	TTTAGG	AAEE01000000	(Podlevsky et al. 2008) and references herein
		Toxoplasma gondii		TTTAGGG	AAYL01000000,	
		Hammondia hammondi strain H.H.34		TTTAGGG	AHJH01000000	
		Plasmodium falciparum Dd2	TT(T/C)AGGG	TT(T/C)AGGG	AASM01000000	(Podlevsky et al. 2008) and references herein
		Theileria parva	TTTTAGGG/TTTAGGG	TTTTAGGG/TTTAGGG	AAGK01000000	
		Theileria annulata	TTTTAGGG			(Podlevsky et al. 2008) and references herein
		Babesia bovis T2Bo		TTTAGGG	AAXT01000000	
		Eimeria tenella	TTTAGGG			(Ling et al. 2007)
	Perkinsea	*Perkinsus marinus* ATCC 50983		TTTCGGG	AAXJ01000000	
	Dinoflagellata	*Cryptecodinium cohnii*	TTTAGGG			(Fojtova et al. 2010)
		Karenia papilionacea	TTTAGGG			(Fojtova et al. 2010)
		Prorocentrum micans	TTTAGGG			(Alverca et al. 2007)
		Amphidinium carterae	TTTAGGG			(Alverca et al. 2007)
		Symbiodinium sp.	TTTAGGG			(Zielke and Bodnar 2010)
Stramenopiles	Labyrinthulida	*Aurantiochytrium limacinum* ATCC MYA-1381		TTAGG	JGI	
	Opalinata	*Blastocystis hominis*		TTAGGG	CABX01000000	
	Oomycetes	*Phytophthora sojae*		TTTAGGG	JGI	
		Phytophthora capsici		TTTAGGG	JGI	
		Phytophthora infestans	TTTAGGG	TTTAGGG	JGI	(Pipe and Shaw 1997)
		Hyaloperonospora arabidopsidis Emoy2		TTTAGGG	ABWE02000000	
		Pseudoperonospora cubensis		TTTAGGG	AHJF01000000	
		Pythium ultimum		TTTAGGG	ADOS01000000	
	Ochrophyta: Bacillariophyceae	*Thalassiosira pseudonana*	TTAGGG	TTAGGG	JGI	(Armbrust et al. 2004)
		Phaeodactylum tricornutum		TTAGGG	JGI	
		Fragilariopsis cylindrus		TTAGGG	JGI	
	Ochrophyta: Eustigmatophyceae	*Nannochloropsis gaditana* CCMP526		TTAGGG??	AGNI01000000	
		Nannochloropsis oceanica		TTAGGG??	AEUM01000000	
	Ochrophyta: Pelagophyceae	*Aureococcus anophagefferens*		TTAGGG	JGI	
Stramenopiles	Ochrophyta: Phaeophyceae	*Ectocarpus siliculosus*	TTAGGG		CABU01000000	(Cock et al. 2010)
Rhizaria		*Bigelowiella natans*	TTAGGG (TCTAGGG)	TTAGGG	JGI	(Gilson and McFadden

						1995)
Haptophyta		*Emiliania huxleyi* *Phaeocystis antarctica*		TTAGGG TTAGGG???	JGI TraceArchive	
Cryptomonadea	Cryptophyceae	*Guillardia theta*	TTTAGGG (red alga like)	TTTAGGG	JGI	(Zauner et al. 2000)
	Goniomonadida	*Goniomonas avonlea*		TTTAGGG	Kim et al., unpublished genome sequence assembly	

Fig. S1. Analysis of inhibitory effect of algal proteins to *Arabidopsis thaliana* telomerase.

Activity of *A. thaliana* (A.th.) telomerase was not affected by addition of protein extracts from algal strains (Alga) representing groups without detected telomerase activity – Eustigmatophyceae (TEL133, TEL201), Bacillariophyceae (TEL231), Ulvophyceae (TEL94, TEL124), and Rhodophyta (TEL213, TEL214). Final protein amount (200 ng) was achieved by combination of protein extracts from *A. thaliana*, algal strains and bovine serum albumin (BSA); protein amounts in ng, including negative (-) and positive (+) controls.

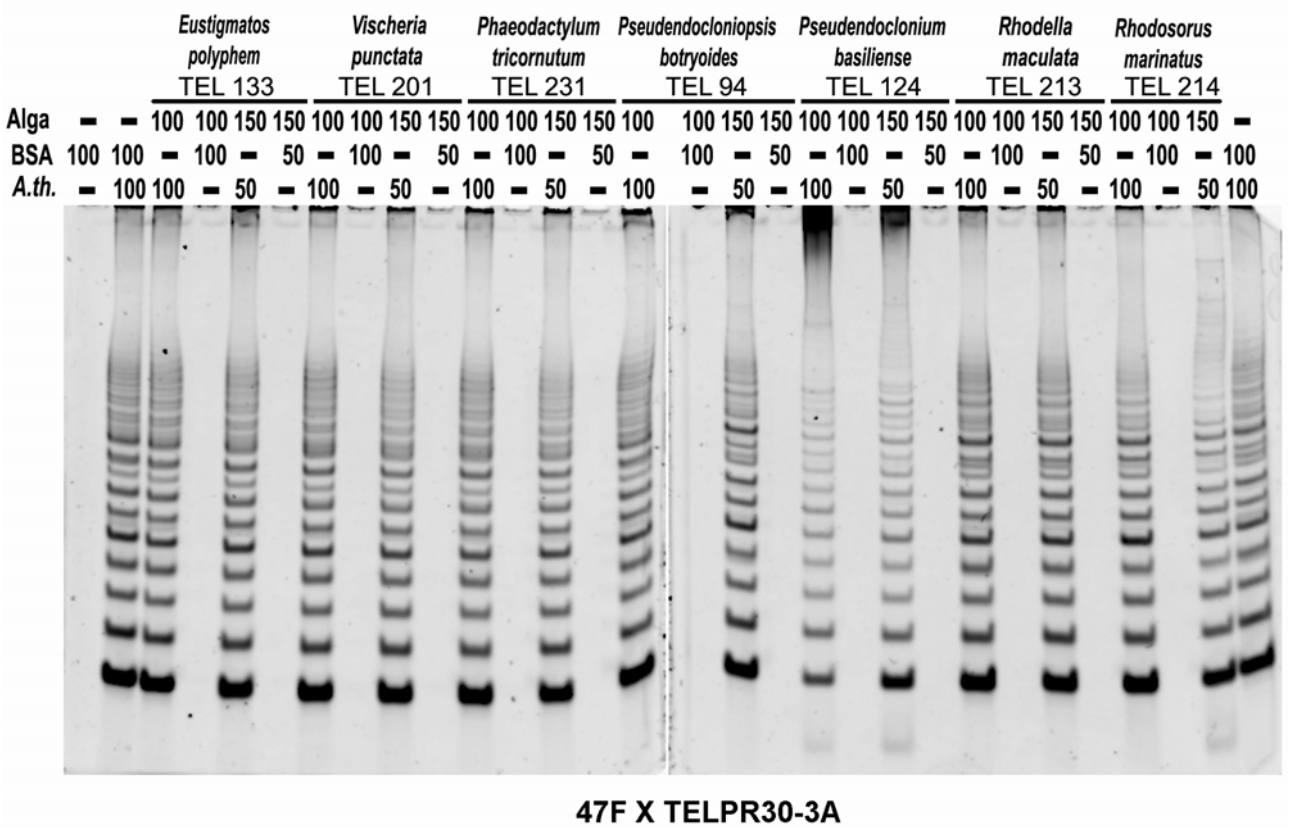


Fig. S2. TRAP assay (A) and different primer usage in Trebouxiophyceae, Chlorophyceae (B) and Xanthophyceae (C) in telomerase template region (D). The TRAP assay is performed in two steps (A). In the extension step, telomerase adds telomeric repeats to the 3' end of a substrate primer producing a mixture of single-stranded molecules differing by the number of added telomeric repeats. Products are then amplified in the second (PCR) step using a substrate primer in combination with a telomeric reverse primer. The periodicity of TRAP products resolved on PAA gel depends on minisatellite added by telomerase and its repeat length (shown for the *Arabidopsis*-type telomeric sequence with the 7-nt periodicity). Investigation of telomerase activity revealed different usage of the substrate primer pSSyF by representative algal telomerases and *Arabidopsis thaliana* telomerase. A significant difference in the ladder of TRAP products was observed (arrows) when the substrate primer pSSyF (B, C) was used in contrast to the substrate primer 47F (B). We presume that the difference resulted from different preferences of primer usage of algal telomerases (D) in comparison to the *Arabidopsis* telomerase. In contrast to *Jaagiella alpicola* (TEL84) that showed a similar TRAP pattern as the control *A. thaliana* (TTTAGGG), other Trebouxiophyceae displayed a shifted ladder of TRAP products (B). The Xanthophyceae strains (C) revealed a shifted ladder (arrows) in *Pleurochloris meiringensis* (TEL202) and no TRAP products in *Heterococcus protonematoides* (TEL204) when the substrate primer pSSyF was used. Examples of telomerase action are shown in (D) comprising a representative sequence of cloned TRAP products (on the left) and annealing onto putative RNA template region (on the right) for both types of TRAP ladders. The shortest TRAP product results from elongated substrate primer amplified with the reverse primer on the first possible site. The substrate oligonucleotide 47F (C) showed uniform TRAP products suggesting a similar annealing to the template region of *A. thaliana* and algal telomerase RNA subunits. The efficiency of telomerase purification (summarized in supplementary table S3) during preparation in protein extract (100 ng and 1 μg, triangles; or 1 μg of total protein) was monitored without PEG precipitation (crude, cr.), and in fractions non-precipitated (supernatant, sup.) and precipitated by PEG (telomerase extract, ex.). A telomerase-enriched extract (50 ng of

total protein) from *Chlamydomonas hydra* (+TTTTAGGG) was used as a positive control; negative control (-), no extract.

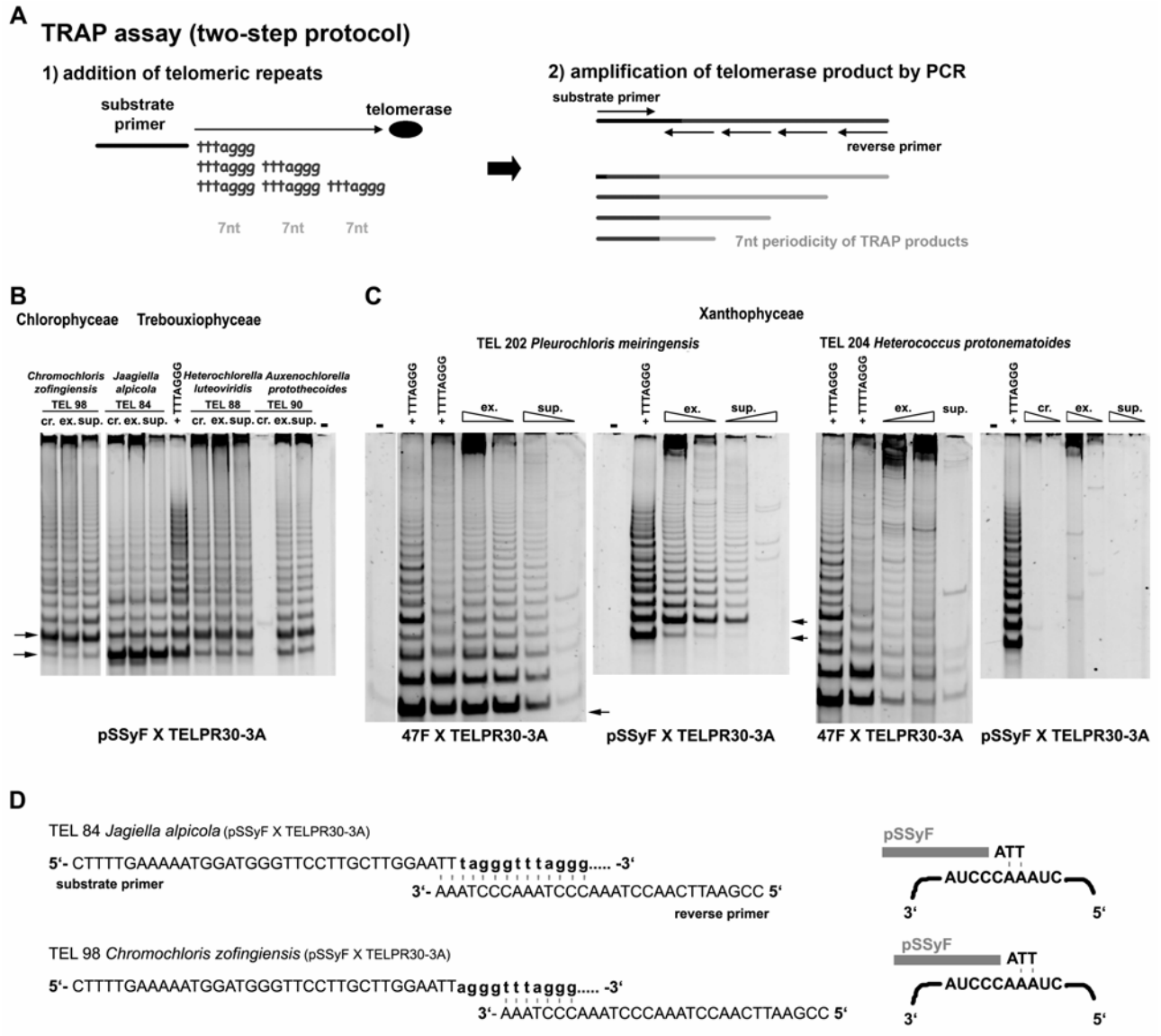
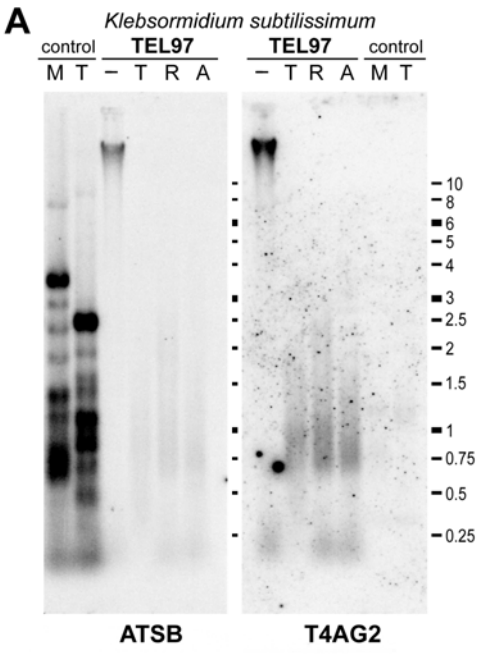


Fig. S3. Analysis of telomeres in *Klebsormidium subtilissimum*. An *Arabidopsis*-type probe (A, the left panel) produces only a negligible background signal, while the probe T4AG2 (A, right panel) provides a stronger signal ranging from 0.7 to 1.5 kb with restriction fragments produced by frequently cutting enzymes *Taq*I, *Rsa*I, and *Alu*I (lanes T, R and A, respectively); control samples of *Chlorella vulgaris* were digested with *Mbo*I and *Taq*I (lanes M, T). In subsequent analyses, high molecular weight samples were treated with BAL31 nuclease for 0, 15 or 60 min, and then digested by *Sma*I to produce longer TRFs (B). Shortening of high molecular weight fragments with duration of BAL31 digestion demonstrates terminal position of the fragments hybridizing with T4AG2*- and *Chlamydomonas*-type (CHSB*) probes. Hybridization patterns of both probes are identical both in higher molecular weight fraction (B, the upper panel) and the lower molecular weight fraction of TRFs (B, the lower panel).


A
Klebsormidium subtilissimum
control
TEL97
TEL97
control
M T – T R A – T R A M T
10
8
6
5
4
3
2.5
2
1.5
1
0.75
0.5
0.25
ATSB
T4AG2

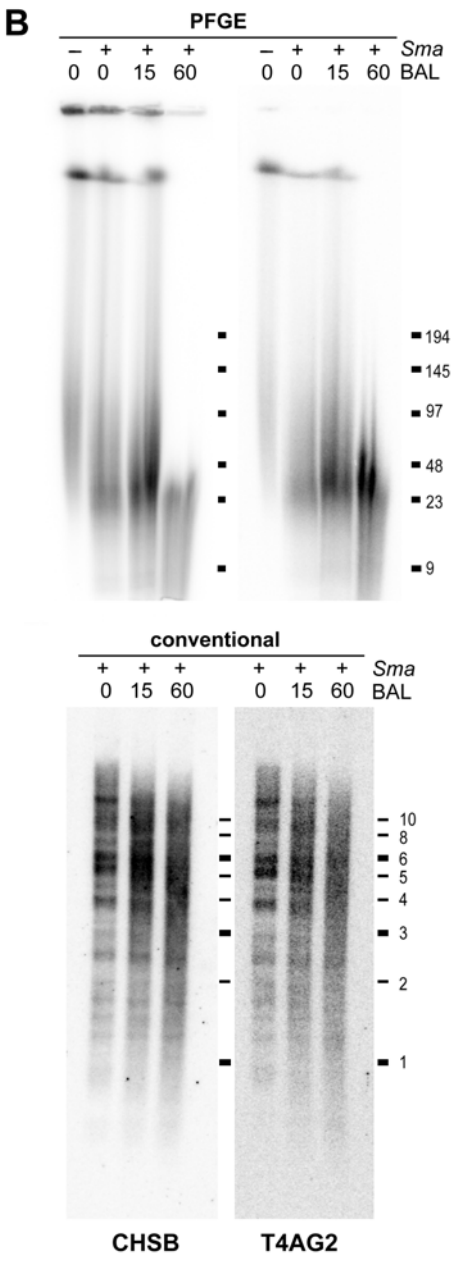
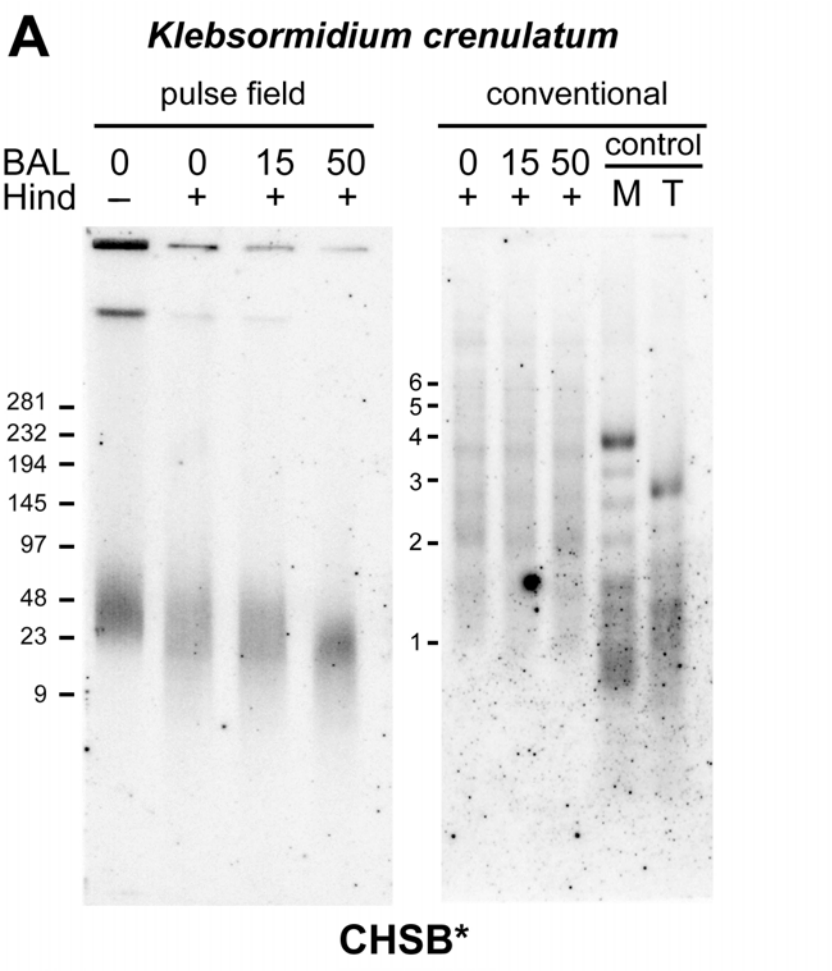

B
PFGE
– + + + – + + + Sma
0 0 15 60 0 0 15 60 BAL
194
145
97
48
23
9
conventional
+ + + + + + Sma
0 15 60 0 15 60 BAL
10
8
6
5
4
3
2
1
CHSB
T4AG2

Fig. S4. Analysis of telomeres in *Klebsormidium crenulatum* (A) and *Vischeria punctata* (B). The high molecular weight fraction of restriction fragments hybridizing with *Chlamydomonas*-type probe (CHSB*) is sensitive to BAL31 treatment (A, the left panel), while the low molecular weight fragments are BAL31-resistant, which reflects their intrachromosomal (non-telomeric) positions (A, the right panel). In *Vischeria punctata* (B), an *Arabidopsis*-type probe (ATSB*) does not provide any specific hybridization signal with either high-molecular-weight genomic DNA or its fragments produced by BAL31 and *Hin*dIII enzymes.


A
Klebsormidium crenulatum
pulse field
conventional
BAL 0 0 15 50 0 15 50 control
Hind – + + + + + + M T
281
232
194
145
97
48
23
9
6
5
4
3
2
1
CHSB*

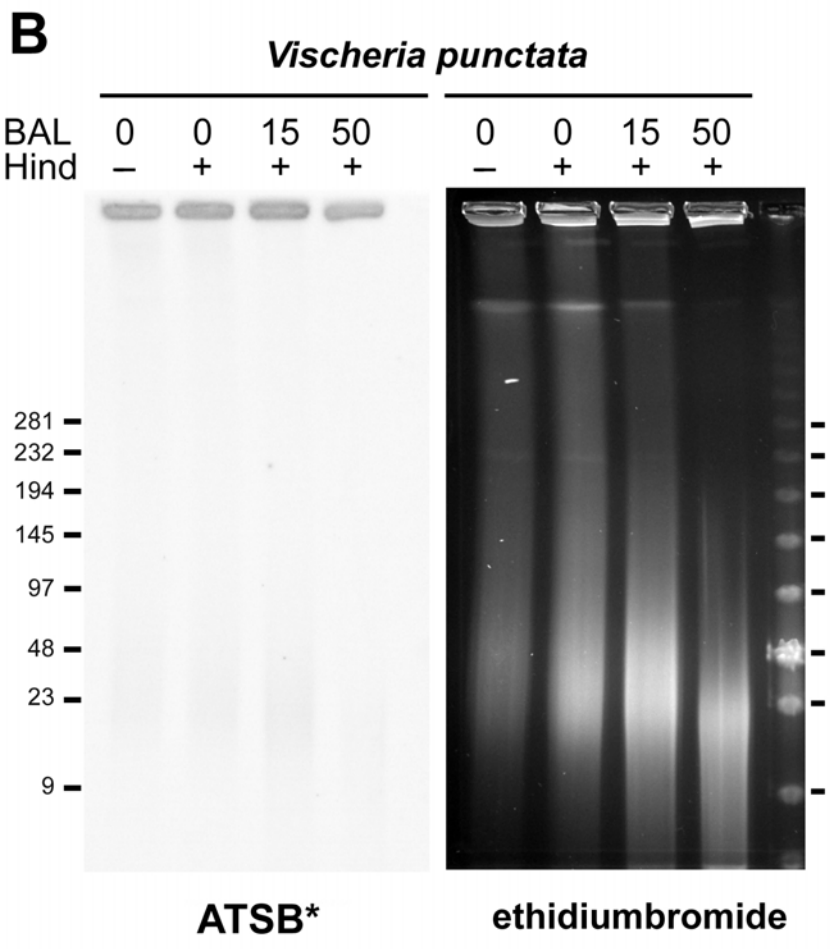
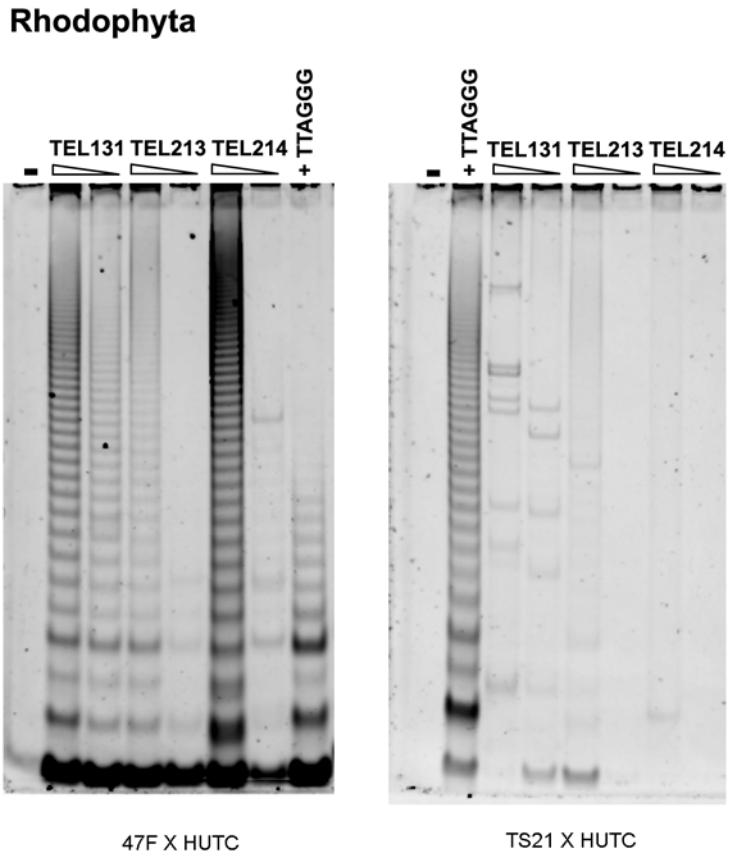

B
Vischeria punctata
BAL 0 0 15 50 0 0 15 50
Hind – + + + – + + +
281
232
194
145
97
48
23
9
ATSB*
ethidiumbromide

Fig. S5. TRAP assay of rhodophytes. Different results of a TRAP assay for *Porphyridium purpureum* (TEL131), *Rhodella maculata* (TEL213), and *Rhodosorus marinus* (TEL214) are shown, using sets of substrate primers 47F or TS21 and the human-type specific reverse primer HUTC. Red agal telomerase-enriched extracts containing 0.1 and 0.5 µg of total protein (indicated with triangle) were analyzed. Amplification of the TRAP products in all three red algal species was achieved only using the substrate primer 47F. A verification experiment with 47F substrate primer and another reverse primer (also able to amplify the human telomeric sequence) excluded a substrate primer preference of telomerase and revealed a false-positive result. Telomerase-enriched extract (0.1µg of total protein) from *Euglena anabaena* (TEL185) was used as a pattern control of six-nucleotide periodicity ladder (TTAGGG); negative control (-), no extract.



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