

Table S1. Primers used in the qRT-PCR analysis

Primers	Sequence (5'- 3')	Amplified gene
α Tub1	TGTCGTCCCCAAGGAT	α -tubulin
α Tub2	GTTCTCTGGTCTTGATGGT	
β act1	CTCTCTTCTACCTCCAAACT	β -actin
β act2	AGGACCAGATTCATCATATT	
GPx1A	CTTGGGCTGAGTCTGAAA	<i>TtGPx1</i>
GPx1B	GAAAAGAGGGAATTCTACGT	
GPx3A	TAGCAAATAATACTCCTGGAT	<i>TtGPx3</i>
GPx3B	GGTTTCCCTCAGCATT	
GPx9A	CCCGATGGTAAGGTCA	<i>TtGPx9</i>
GPx9B	CAGCTATCATATCGTTAGGAT	
GPx10A	GTGATGGTACAGTTCATAGTT	<i>TtGPx10</i>
GPx10B	AGGTTCAATTCTTTGGTT	
GPx11A	AAGGTAGACGTTAAATGGTCA	<i>TtGPx11</i>
GPx11B	AGAAGAATGTCTCTCAAGAA	
GPx12A	CTACTATACAAGATGATTCTG	<i>TtGPx12</i>
GPx12B	GTCTCTACTGAAAAGCTTAAT	

Table S2. Quantitative RT-PCR standard-curve parameters

Gene	Slope	Efficiency (%)	R ² ⁽¹⁾
α -tubulin	- 3.43	95.56	0.99
β -actin	- 3.35	98.98	0.99
<i>TtGPx1</i>	- 3.47	93.96	0.99
<i>TtGPx3</i>	- 3.58	90.16	0.99
<i>TtGPx9</i>	- 3.52	92.24	0.99
<i>TtGPx10</i>	- 3.50	92.92	0.99
<i>TtGPx11</i>	- 3.42	96.14	0.99
<i>TtGPx12</i>	- 3.33	99.77	0.99

⁽¹⁾ Correlation coefficient. Efficiency (E) is calculated from the slope value of the standard curve: E = $10^{(-1/\text{slope})-1}$

Table S3. Characteristics of ciliate GPx SECIS elements.

GPx	Location⁽¹⁾	Length (b)	DNA strand⁽²⁾	Type⁽³⁾	Score⁽⁴⁾	Grade⁽⁵⁾
TtGPx10	3'UTR (96 b -> Stop)	82	+	I	22.19 (C) 14.89 (I)	A
TbGPx6	3'UTR (63 b -> Stop)	77	+	I	27.17 (C)	A
TbGPx7	3'UTR (121 b -> Stop)	74	+	I	13.97 (I) 21.84 (C)	A
TmGPx9	3'UTR (48 b -> Stop)	74	+	I	13.54 (I) 23.89 (C)	A
TmGPx10	3'UTR (99 b -> Stop)	82	+	I	30.28 (C)	A
EocGPx1	3'UTR (29 b -> Stop)	71	+	I	14.49 (C)	A
TeGPx8	2 ^o intron	75	+	I	19.34 (I) 29.61 (C)	A
StyGPx5	ORF (337 b -> ATG)	59	-	I	13.89 (I) 1.35 (C)	B
StyGPx6	5'UTR (105 b -> Tel)	71	-	I	18.71 (I) 14.89 (C)	B

⁽¹⁾ Nine possible SECIS locations have been detected: in the 3'UTR region (the number of bases (b) from the stop codon to the SECIS element is indicated), within an intron (the intron number is indicated), in the ORF or coding region (the number of bases from the start codon to the SECIS element is indicated), or in the 5'UTR region (the number of bases from the telomere to the SECIS element is indicated). ⁽²⁾ Strand on which the SECIS element was found: (+): on the sequence as it was input, (-): on its reverse complement.

⁽³⁾ Eukaryotic SECIS Type-I or II. ⁽⁴⁾ The search method(s) predicted the SECIS element: by Covels (C) program and/or Infernal (I) program. ⁽⁵⁾ Marker for how good the SECIS prediction looks like. The SECIS grade can be A, B, C in decreasing order of goodness.

Table S4. Secondary structure characteristics of ciliate SECIS elements.

GPx	SECIS core ⁽¹⁾ (UGAN/KGAW)	Conserved A in Loop-II	Stem-I (bp)	Stem-II ⁽²⁾ (bp)	Loop-I (b)	Loop-II (b)	Mismatches bases (bp)	Non-canonical pairing (AG/GA)
TtGPx10	UGAA/UGAA	3	7	11	21	13	2	0
TbGPx6	UGAC/UGAA	3	6	11	11	16	1	0
TbGPx7	UGAC/UGAA	2	6	11	12	14	2	0
TmGPx9	UGAU/UGAA	3	11	11	6	15	2 + 1b	1
TmGPx10	UGAA/UGAA	3	7	11	19	15	2	0
EocGPx1	UGAU/UGAA	3	8	10	10	10	1 b	1
TeGPx8	UGAU/UGAA	3	11	12	6	15	1	1
StyGPx5	UGAU/UGAG	0	8	8	13	2	1	1
StyGPx6	UGAC/AGAU	3	9	11	6	13	2	1

⁽¹⁾ K: (U/G), W: (U/A), N: any nucleotide. ⁽²⁾ After removing the SECIS core.

Table S5. Relative-fold induction values \pm SD of selected *TtGPx* genes, under different stressful conditions.

Treatment	TtGPx gene					
	<i>TtGPx1</i>	<i>TtGPx3</i>	<i>TtGPx9</i>	<i>TtGPx10</i>	<i>TtGPx11</i>	<i>TtGPx12</i>
H ₂ O ₂ (1h)	74.76 \pm 1.45	9.09 \pm 0.15	6.26 \pm 0.89	5.77 \pm 0.09	1.94 \pm 0.06	4.9 \pm 0.88
MD (1h)	733.34 \pm 67.4	45.59 \pm 2.91	3802 \pm 296.17	40.62 \pm 5.23	6.91 \pm 0.55	3.24 \pm 1.78
PQ (1h)	224.9 \pm 6.93	7.42 \pm 0.22	664.95 \pm 16.9	52.14 \pm 2.43	8.21 \pm 0.28	26.21 \pm 0.95
PQ (24h)	218.58 \pm 80.31	33.19 \pm 3.46	168.46 \pm 58.79	58.34 \pm 21.01	29.9 \pm 3.72	113.92 \pm 11.86
CAM (1h)	0.91 \pm 0.09	9.22 \pm 1.38	6.31 \pm 0.63	9.17 \pm 2.3	4.14 \pm 0.58	13.71 \pm 2.1
CAM (24h)	19.62 \pm 0.72	561.87 \pm 24.65	1996.27 \pm 93.69	312.16 \pm 84.8	25.11 \pm 1.07	237.42 \pm 15.36
Cd (1h)	949.11 \pm 59.1	156.13 \pm 26.67	2175.83 \pm 656.27	216.16 \pm 63.98	26.97 \pm 8.34	81.01 \pm 41.45
Cd (24h)	15.22 \pm 1.05	17.87 \pm 0.98	42.22 \pm 2.57	29.64 \pm 6.28	9.78 \pm 0.75	318.12 \pm 17.12
Pb (1h)	141.44 \pm 3.94	73.04 \pm 2.2	138.27 \pm 5.65	41.79 \pm 1.08	18.74 \pm 0.56	45.25 \pm 6.32
Pb (24h)	119.55 \pm 7.25	52.06 \pm 1.85	134.88 \pm 7.07	126.84 \pm 6.39	24.2 \pm 1.2	158.88 \pm 8.87
Cu (1h)	2.98 \pm 0.61	47.67 \pm 8.76	12.39 \pm 2.22	36.98 \pm 7.24	10.4 \pm 1.97	71.2 \pm 7.22
Cu (24h)	2.67 \pm 0.42	33.85 \pm 4.01	25.83 \pm 3.39	64.79 \pm 6.87	9.97 \pm 2.14	35.9 \pm 3.03

SD: Standard deviation. MD: menadione. PQ: paraquat. CAM: camptothecin.

Figure S1. Multiple-sequence alignment of 101 GPx from ciliates and other organisms. Only regions containing residues of the catalytic tetrad (U/C, Q, W, N) are shown, indicated by ▼. Highly conserved residues in the regions adjacent to the tetrad are indicated by grey shading and +. See Table 1 for species names.



	1030	1040	1030	1040	
	++ ++ +++ ▼++ +			++ ++ +++ ▼++ +			
TtGPx1	SQGLEILGFP	CNQFGAQEPW	AESEILSYTQ	StyGPx7	EKGFEILIAFP	CNQFMKQEPK	TNQEIKTFLQ
TtGPx2	SQGLEVLAFF	CNQFGEQEPW	AESEILSYTQ	StyGPx8	SKGLEILIAFP	CNQFHKQEPPL	NDNEIKVNAQ
TtGPx3	DKGLEILIAFP	CNQFFNOEPF	DEPAIKEFVK	StyGPx9	DQGFEILIAFP	CNQFMSQEPG	SNQEIKQFVR
TtGPx4	SQGLEILIAFP	CNQFGQQEPW	AESEILSYTQ	OxyGPx1	-----	-----ESGNPL	RNRDLAILLK
TtGPx5	SQGLEVLAFF	CNQFGEQEPW	AESEILSYTQ	OxyGPx2	DQGFEILIAFP	CNEFFSTEPG	DSKQIQLSLR
TtGPx6	SQGLEVLAFF	CNQFGEQEPW	AESEILSYTQ	OxyGPx3	QHGLEIVGFP	CNQFQSQEGK	TNDEFQFVC
TtGPx7	SRGFEILIAFP	TNDFMEQEPW	DNNKIKKEYVQ	OxyGPx4	AQGFEILIAFP	CNQFNKEEPW	NDHEIKQHV1
TtGPx8	SRGFEILIAFP	TNDFMEQEPW	DNNKIKKEYVQ	OxyGPx5	DQGFEIVAFP	CNQFGSQESK	PNETIYEFVC
TtGPx9	SQGFEILIAFP	ANQFMGQEPW	DNAKIKEYVV	OxyGPx6	SQGFDVLAFF	CNQFLQEPG	SAEEIQKFAC
TtGPx10	HLTTTLSQWK	CIRNTNREVV	KFLHSLPINL	OxyGPx7	DKGFEILIAFP	CNQFMRQEPR	SNQEIKEYVI
TtGPx11	KDKLEILIAFP	CNQFYN-EPS	NFKTIKDYS	OxyGPx8	EQGFEVLAFF	CNQFGSQEPD	SNASILDVFV
TtGPx12	DSGLEILGFP	CNQFMSQEPW	AEPKIKDFIT	OxyGPx9	DQGFEILIAFP	CNQFMNQEPG	TPEEIKKF1K
TbGPx1	AQGLEILGFP	CNQFGAQEPW	DEKEILSYTK	EvGPx1	HKGQFILAFP	CNQFLGQESC	SNDDINEFVR
TbGPx2	DQGLEILGFP	CNQFLSQEPW	DEPKIQEFIG	EvGPx2	DKGQFQIAFP	CNQFGAQEPG	TNEEIRAFQAQ
TbGPx3	TQGFEILAFP	ANQFMGQEPW	DNAKIKEYIV	SteGPx1	DKEFEILGFP	CNQFFHQEPAG	TSSEIKDYIR
TbGPx4	NRGFEILAFP	TNDFMQQEPW	DSKKIKKEYVQ	SteGPx2	SEGLCILIAFP	CNQFNNREPQ	TDEQIVAFAK
TbGPx5	DQGLEILIAFP	CNQFMNQEPF	DEPQIKEFVK	SteGPx3	SRGLEILSFP	CNQFWREYS	EQETIKSYLI
TbGPx6	---LEILGFP	CNQFLSQEPW	AEPKIKDFIT	SteGPx4	SKGLEILIAFP	CNQFLHQEPG	SDEEIEKFAR
TbGPx7	SRGFEILAFF	CNQFMGQEPW	DPPQIKEFVV	SteGPx5	KEGLIIILAFP	CNQFNNQEPG	TNAEIQEFR
TbGPx8	KDQLEVIAFP	CNQFYN-EPS	NFKGIQENYA	SteGPx6	SRGLEILIAFP	CNQFWREYS	THNEIKDYL5
TeGPx1	QQGLEILGFP	CNQFGAQEPW	SESEILSYTQ	SteGPx7	SRGLCILIAFP	CNQFQFYQERG	TSDQIKDFIT
TeGPx2	SQGLEILGFP	CNQFGSQEPW	AESEILSYTQ	PtGPx1	-LPYQVILFP	KCDH----TF	TY----KQ
TeGPx3	SQGFEVLAFF	ANQFMGQEPW	DNAKIKEYTV	PtGPx2	AQGLEILGFP	CNQFMGQESK	PEPEIKEFVL
TeGPx4	SRGFEILAFP	TNDFMEQEPW	DNQKIKKEYVQ	PtGPx3	AQGLEILGFP	CNQFMNQESK	PEPEIKEFVI
TeGPx5	SKGFEILAFP	TNDFMQQEPW	DNKKIKKEYVQ	PtGPx4	DQGLEILGFP	CNQFRNQESK	PEPEIKNYVT
TeGPx6	SKGFEILAFP	TNDFMEQEPW	DNKKIKKEYVQ	PtGPx5	AQGLEILGFP	CNQFMGQESQ	PEPEIKEFVI
TeGPx7	DKGLEILAFF	CNQFLNQEPW	DEPQIKEFVK	McGPx1	DKGFEIFIAFP	CNQFLSQEPG	SNEDIKKFAR
TeGPx8	-HGLEVLGFP	CNQFMSQEPW	AEPKIKEFIT	McGPx2	DQGFEIFIAFP	CNQFMSQEPG	THEQIKKFAQ
TeGPx9	SRGLEILAFP	CNQFMEQEPW	DPPQIKEFVV	McGPx3	DHGFEIFIAFP	CNQFMSQEPG	THEQIKKFAQ
TeGPx10	NDKLEILAFP	CNQFYN-EPS	NFKTIKEQYS	PcpGPx1	-----	-----	EIKQFLN
TmGPx1	SQGLEILGFP	CNQFGAQEPW	SESEILSYTQ	PcpGPx2	KREVKVVAIS	CNDVKTHKDW	IKD-IEHY--
TmGPx2	SQGLEILAFP	CNQFGSQEPW	AESEILSYTQ	PcpGPx3	-----	-----	EIQHVV
TmGPx3	TQGLEILAFP	CNQFGQQEPW	DESEILSYTQ	PcpGPx4	DQGFEILIAFP	CNQFGKQEPL	PNDQILEAAR
TmGPx4	SQGFEILAFF	ANQFMGQEPW	DNAKIKEYVV	PcpGPx5	KKGVKVIAS	CNDADTHKEW	IKD-VDHY--
TmGPx5	SRGFEILAFP	TNDFMEQEPW	DNKKIKKEYVQ	PcpGPx6	QKGLEIFIAFP	CNQFGAQEPN	PNNEILDAAR
TmGPx6	SRGFEILAFP	TNDFMEQEPW	DNKKIKKEYVQ	PcpGPx7	NNGFEILCFP	CNQFYN-EPG	TFNSNLKQIYL
TmGPx7	SRGFEILAFP	TNDFMEQEPW	DNKKIKKEYVQ	EocGPx1	DKGFEIFIAFP	CNQFLSQESC	SNEHIKNFVQ
TmGPx8	DKGLEILAFF	CNQFLNQEPF	DEPAIKEFVK	EocGPx2	DKGFQIFIAFP	CNQFMKQEPG	SNEDIKKFAQ
TmGPx9	DSGLEILGFP	CNQFMSQEPW	AEPKIKDFII	EocGPx3	-----	-----	-----
TmGPx10	SRGLEILAFP	CNQFMGQEPW	DPPQIKEFVV	EocGPx4	DKGFQIFIAFP	CNQFMGQESK	CNLDIKKYAQ
TmGPx11	KDKLEILAFF	CNQFYN-EPL	NFKNIKDYS	EocGPx5	DKGLRVFGLP	TTDVLLIGSTK	LTYD-
ImGPx1	---FTILAFF	ANQFMSQEPW	DPPQIKDFVI	CrGPx5	DRGLVILGFP	CNQFGGQEPG	DASAIGEFCQ
ImGPx2	-----ANQ-----	-----	-----	CrGPx1	ATDLTIVAFP	CNQFGGQEPG	TNAEIKAFAS
StyGPx1	EQGFEILAFF	VNQFFSQEPG	TNQQIQLSLR	ScGPx1	DEGFTIIGFP	CNQFGHQEPG	SDEEIAQFCQ
StyGPx2	DQGFEVLAFF	CNQFGAQEPG	SNSQIFEFVC	TcGPx1	PRGFTIILAFP	CAQFANQEPK	SNEEIAVWAQ
StyGPx3	DQGLEILAFP	CNQFMNQEPG	SNLQILEYAR	PfGPx1	ARGLEILAFP	TSQLNQEFD	NTKDICTFNE
StyGPx4	PQGFEVMAFP	CNQFGSQEPG	TNKQILDVFVC	AtGPx6	GHGFEILAFF	CNQFGNQEPG	TNEEIVQFAC
StyGPx5	ERGFCVY GAL	PNDVAGGNPL	RNKEIANTLK	DmGPx1	ERGLVILNFP	CNQFGSQMPF	ADGEAMVCHL
StyGPx6	DQGFEILTFF	CNQFGGQEPG	TNQEVKQFIR	HsGPx1	PRGLVVLGFP	CNQFGHQENA	KNEEILNSLK
				HsGPx5	PYGLVVLGFP	CNQFGKQEPG	DNKEILPLGLK
				HsGPx4	EFAAGYNVFK	DMFSKICVNG	DDAHPLWKWM

	1140	1150		1140	1150
	+ ▼ ▲ +	+++		+ ▼ ▲ +	+++
TtGPx1	AATKIPW	NF-AKFLVDG	OxyGPx1	RAYRIKE	SY-AKFLCNR
TtGPx2	SATKIPW	NF-AKFLIDG	OxyGPx2	KVKQIPW	NF-SKFLVDS
TtGPx3	FQGYIQW	NF-AKFLVNA	OxyGPx3	NFNKIGW	NF-GKFLVNQ
TtGPx4	SATKIPW	NF-AKFLIDG	OxyGPx4	NIKEIPG	NF-AKFLVDR
TtGPx5	YATKIPW	NF-AKFLIDG	OxyGPx5	KVGKIPW	NF-AKFLGDQ
TtGPx6	SATKIPW	NF-AKFLIDG	OxyGPx6	GITRIEW	NF-GKFLVDS
TtGPx7	KTRQIPW	NF-AKFLIDP	OxyGPx7	GVKEVPW	NF-SKFLVNK
TtGPx8	KTRQIPW	NF-AKFLINP	OxyGPx8	RIAKITW	NF-GKFLVNK
TtGPx9	KTRQIPW	NF-AKFLIGP	OxyGPx9	EVKEIPW	NF-AKFLVDQ
TtGPx10	NLAQILF	SL-VKQMLTE	EvGPx1	KLKSIPF	NF-SKFLINS
TtGPx11	NGAKITE	DF-SKFLINT	EvGPx2	TTGDIWP	NF-AKFLINS
TtGPx12	KAKQIPW	NF-SKFVVDR	SteGPx1	DGKRICW	NF-GKFLVDR
TbGPx1	AAQKIPW	NF-AKFLIDG	SteGPx2	KGSGIDW	NF-GKFLVDK
TbGPx2	KSKQIPW	NF-GKFIINK	SteGPx3	NGRKIGM	NF-CKFLVDR
TbGPx3	KTRQIPW	NF-AKFLIGP	SteGPx4	DGGKIGW	NF-GKFIVSR
TbGPx4	KTRQIPW	NF-AKFLIDP	SteGPx5	KGREIGW	NF-TKFLVNR
TbGPx5	FQGYIQW	NF-AKFLINS	SteGPx6	NSTQIGL	NF-GKFIVDK
TbGPx6	KAKQIPW	NF-SKFLVNQ	SteGPx7	NRRKICW	NF-GKFLIDR
TbGPx7	KAKQIPW	NF-AKFLIQP	PtGPx1	NGRQIKQ	DF-CKFLISE
TbGPx8	NGEKITE	DF-TKFLVNT	PtGPx2	EAKEVPW	NF-GKFLLNS
TeGPx1	AATKIPW	NF-AKFLVDG	PtGPx3	SARQVPW	NF-GKFLLDS
TeGPx2	AATKIPW	NF-AKFLIDG	PtGPx4	EVKYVPW	NF-AKFLLDA
TeGPx3	KTRQIPW	NF-AKFLIGP	PtGPx5	EAKEVPW	NF-GKFLLNS
TeGPx4	KTRQIPW	NF-AKFLINP	McGPx1	TIQNIPW	NF-AKFLIDE
TeGPx5	KTRQIPW	NF-AKFLINP	McGPx2	VVQNIPW	NF-AKFLIDN
TeGPx6	KTRQIPW	NF-AKFLINP	McGPx3	VVQNIPW	NF-AKFLIDE
TeGPx7	FQGFIQW	NF-AKFLVDG	PcpGPx1	NTNNIPW	NF-AKFVVYD
TeGPx8	KAKQIPW	NF-SKFIINR	PcpGPx2	DPQGLPM	TVRSVYVI GP
TeGPx9	NSKQIPW	NF-AKFLIQN	PcpGPx3	ENPNIAY	----VTIKSN
TeGPx10	GGIKITE	DF-SKFLINN	PcpGPx4	KTKNILW	NF-GKFLIDK
TmGPx1	AATKIPW	NF-AKFLIDG	PcpGPx5	DAKGLPM	TVRSVYII GP
TmGPx2	SATKIPW	NF-AKFLIDG	PcpGPx6	KCEDIKW	NF-GKFLVDG
TmGPx3	SATKIPW	NF-AKFLIDG	PcpGPx7	SAEPIVE	DF-SKFLIDQ
TmGPx4	KTRQIPW	NF-AKFLIGP	EocGPx1	TMQSIPW	NF-TKFLIDE
TmGPx5	KTRQIPW	NF-AKFLIDP	EocGPx2	TLQNIPW	NF-AKFLIDE
TmGPx6	KTRQIPW	NF-AKFLINP	EocGPx3	-----	-----
TmGPx7	KTRQIPW	NF-AKFLIDP	EocGPx4	EIQSIPW	NF-TKFLIND
TmGPx8	FQGYIQW	NF-AKFLVNA	EocGPx5	KSRRINT	NF-NKFLCDR
TmGPx9	KAKQIPW	NF-SKFVVDR	CrGPx5	MMEMIKW	NF-EKFLVDK
TmGPx10	NSKQIPW	NF-AKFLIQS	CrGPx1	--SDIGW	NF-GKFLVRP
TmGPx11	NGAKITE	DF-SKFLINT	ScGPx1	GLRGIKW	NF-EKFLVDK
ImGPx1	KAKNIPW	NF-GKFLINK	TcGPx1	--GPIRW	NY-TKFICDR
ImGPx2	NGIKINE	DF-CKFLVDQ	PfGPx1	TLKSIGW	NF-GKFLVDK
StyGPx1	QVKRIPW	NF-SKFLVNK	AtGPx6	-GDGIKW	NF-AKFLVDK
StyGPx2	GLDQVTW	NF-GKFLIDQ	DmGPx1	-GSGIKW	NF-TKFLVNK
StyGPx3	KVKALPW	NF-CKFILDQ	HsGPx1	CRNDVAW	NF-EKFLVGP
StyGPx4	RLARITW	NF-GKFLVNQ	HsGPx5	KVHDIRW	NF-EKFLVGP
StyGPx5	KAIRVKE	NY-SKLLCNR	HsGPx4	LGNAIKW	NF-TKFLIDK
StyGPx6	EVKEIPW	NF-AKFLVNE			
StyGPx7	DIRMIPW	NF-SKFLVDS			
StyGPx8	KIKDIPG	NF-TKFLVDR			
StyGPx9	EVKEIPW	NF-AKFLVNE			

Figure S2. Phylogram of all *T. thermophila* GPx amino acid sequences. Catalytic tetrads are indicated. Numbers indicate bootstrap values from 2000 replicates. Branch lengths are drawn to scale as indicated by the scale bar.

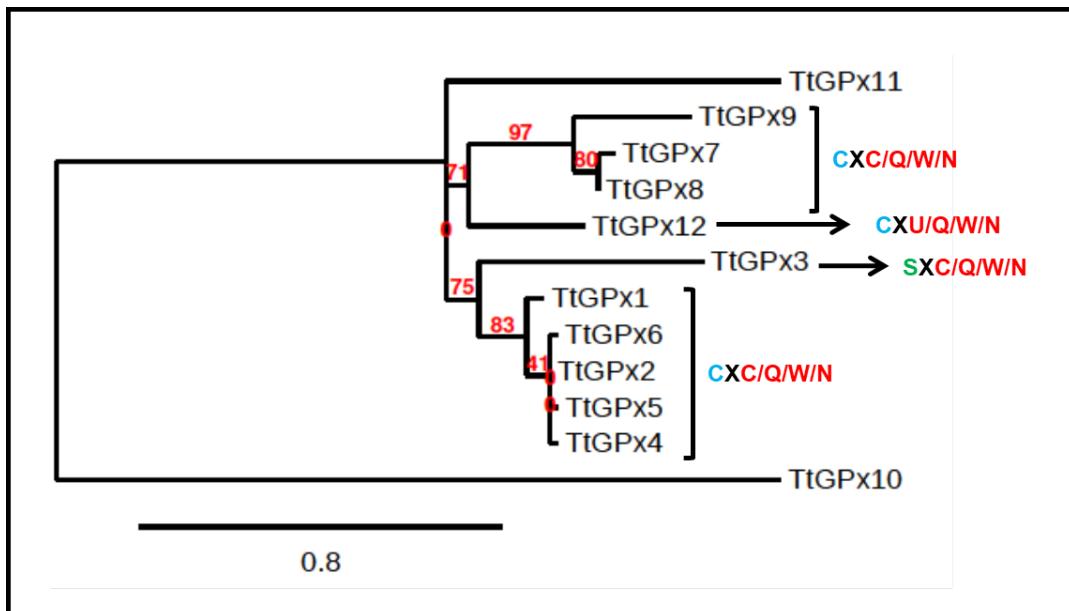


Figure S3. Phylogram of all ciliate putative GPx isoforms. Catalytic tetrads are indicated. No residue of the GPx catalytic tetrad are highlighted within a red square. Species with SECIS elements are indicated by a red star. Numbers indicate bootstrap values from 2000 replicates. Branch lengths are drawn to scale as indicated by the scale bar. See Table 1 for ciliate species identification.

