

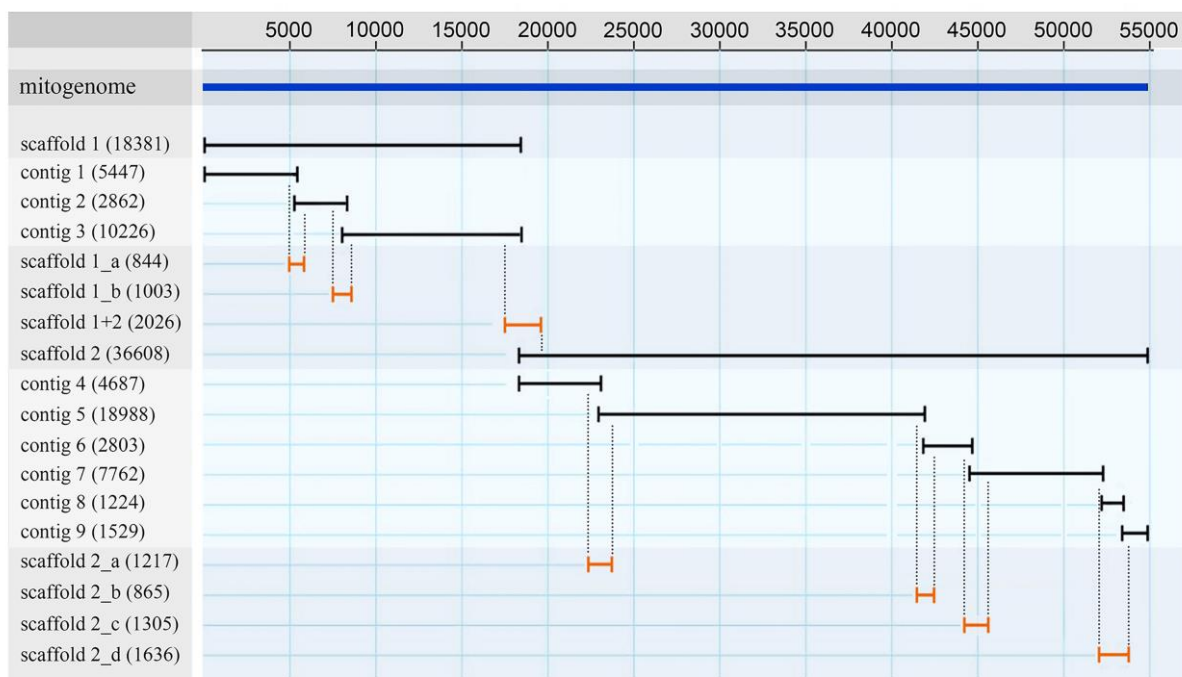


Figure S1. Schematic maps demonstrating how whole mitogenome was confirmed in *Strombidium cf. sulcatum*. Black lines indicate the mitochondrial contigs recovered from genomic assembly of *Strombidium cf. sulcatum*. Red lines represent PCR results used to link mitochondrial contigs. The vertical dashed lines indicate the binding positions of PCR products. Numbers in parentheses suggest the lengths (bp) of the fragments.

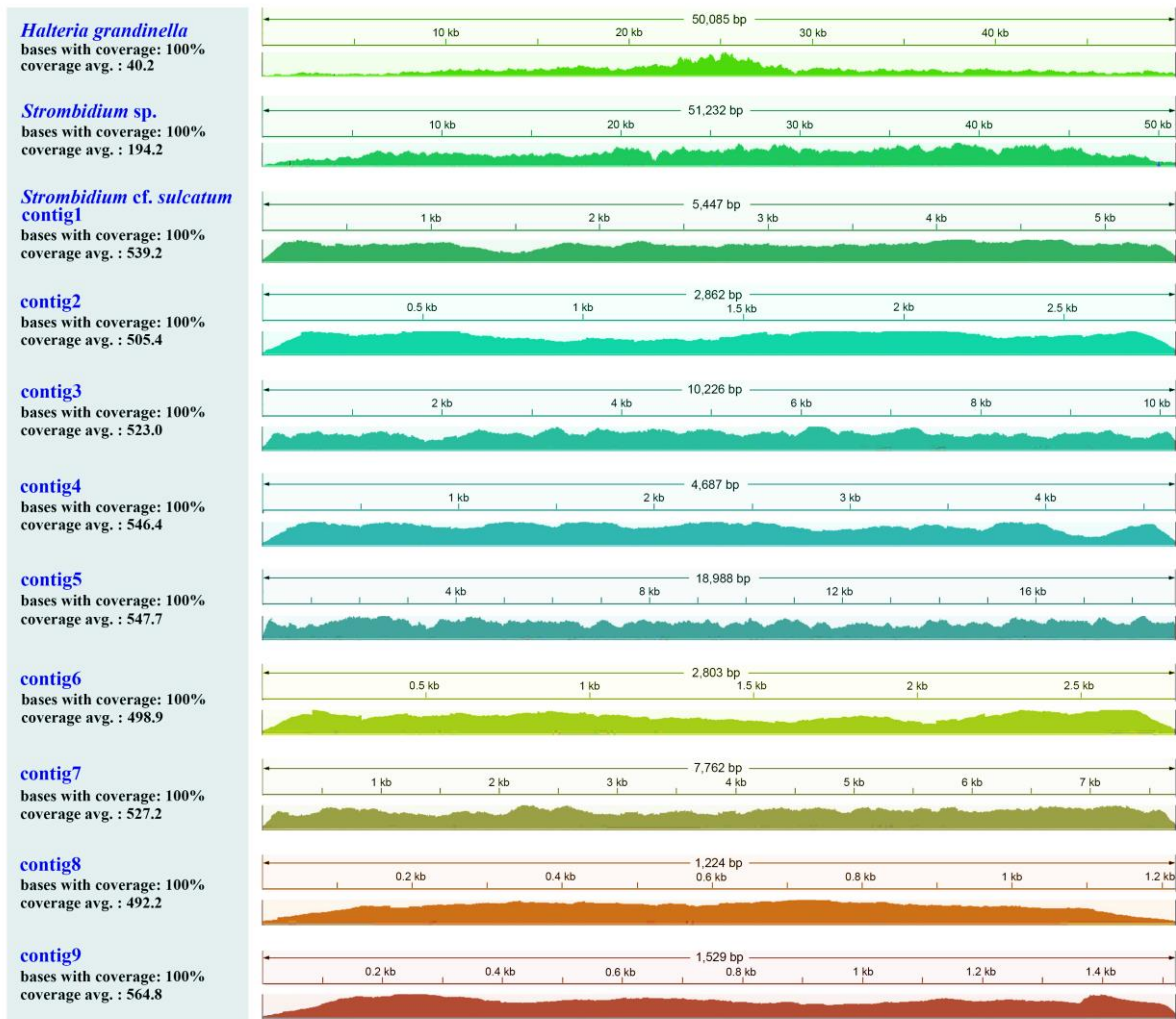


Figure S2. Coverage plots for mitochondrial contigs of *Halteria grandinella*, *Strombidium* sp. and *Strombidium* cf. *sulcatum*. The horizontal and vertical axes indicate contig length and coverage, respectively.

Table 1. Primers for PCR amplification to confirm mitogenome of *Strombidium* cf. *sulcatum*.

PCR fragments	Primers	Sequences (5'–3')
Scaffold 1_a	413_F1	CAGTAGCCAAGAATATAGCG
	1340_R1	ACTTCTAACTGAGTAGCATC
Scaffold 1_b	423_F1	CAAGACCTATCCATATCTCCCC
	1514_R2	GCCTTTTGTAGCAAGCCCATCA
Scaffold 2_a	195_F1	ATTTACAGTCTGCCGCTTTCG
	1518_R1	TGACTTTGGTAAGGCGTAGG
Scaffold 2_b	295_F1	GACTTTGAGCAGGTTGATACC
	1250_R1	TACTTCTTCGTAGTCGGGAG
Scaffold 2_c	503_F1	TAAGTATTCGATTATCCGCAGG
	1926_R1	ACCCGATTACAGAGGTTACG
Scaffold 2_d	1033_F3	CTGCCACATTCAAGTAGAC
	2668_R2	ATGGAATCCCTTATCCTGAA
Scaffold 1+2	51_F1	ACGATAGGGAGTGAATACGG
	1488_R1	ACTCGCCTGTATCAAGTCGG

Table S2. GenBank accession numbers of mitochondrial protein sequences used for the phylogenetic trees. Newly characterized sequences are in bold.

Species	nad4	nad4L	nad5	nad7	nad9	nad10	cob	cox1	cox2	rps3	rps12	rpl2	rpl14	rpl16
<i>Strombidium</i> sp.	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Strombidium</i> cf. <i>sulcatum</i>	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Halteria grandinella</i>	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Oxytricha trifallax</i>	AEV66679	AEV66697	AEV66639	AEV66667	AEV66686	AEV66677	AEV66649	AEV66698	AEV66701	AEV66694	AEV66678	AEV66675	AEV66600	AEV66681
<i>Stylonychia lemnae</i>	ASY95723	ASY95740	KX524144	ASY95733	ASY95748	ASY95752	ASY95741	ASY9574	ASY9574	KX524144	ASY95728	ASY95727	ASY95731	ASY95746
<i>Laurentiella strenua</i>	APW82405	APW82422	APW82424	APW82402	APW82406	APW82435	APW82441	APW8248241	APW8248242	APW8242425	APW824431	APW82423	APW82412	APW82420
<i>Paraurostyla</i> sp.	ASY95711	ASY95717	ASY95710	ASY95698	ASY95690	ASY95719	ASY95713	KX524143	ASY95718	KX524143	ASY95704	ASY95706	ASY95700	ASY95694
<i>Urostyla grandis</i>	APW82389	APW82397	APW82373	APW82388	APW82396	APW82372	APW82384	APW8238238	APW8238239	APW8232394	APW823386	APW82380	APW82382	APW82385
<i>Pseudourostyla cristata</i>	QCU82614	QCU82604	QCU82636	QCU82622	QCU82609	QCU82616	QCU82631	QCU8263	QCU8261	QCU8262607	QCU82615	QCU82618	QCU82602	QCU82613
<i>Euplotes minuta</i>	ACX30958	ACX30950	ACX30940	ACX30966	ACX30954	ACX30960	ACX30945	ACX3093094	ACX3093094	ACX3090952	ACX30959	ACX30962	ACX30948	ACX30957
<i>Euplotes crassus</i>	ACX30996	ACX30985	ACX30976	ACX31004	ACX30990	ACX30998	ACX30980	ACX3093098	ACX3093098	ACX3090988	ACX30997	ACX31000	ACX30983	ACX30995
<i>Tetrahymena pyriformis</i>	AAD41955	AAD41940	AAD41944	AAD41924	AAD41941	AAD41921	AAD41943	AAD4194195	AAD4194195	AAD4191917	AAD41922	AAD41919	AAD41953	AAD41935
<i>Tetrahymena thermophila</i>	AAK77603	AAK77588	AAK77593	AAK77572	AAK77589	AAK77569	AAK77592	AAK7757759	AAK7757759	AAK7757565	AAK77570	AAK77567	AAK77602	AAK77583
<i>Tetrahymena malaccensis</i>	ABI51668	ABI51652	ABI51657	ABI51636	ABI51653	ABI51633	ABI51656	ABI51651663	ABI51651658	ABI5165629	ABI516534	ABI51631	ABI51666	ABI51647
<i>Tetrahymena paravorax</i>	ABI51712	ABI51697	ABI51701	ABI51681	ABI51698	ABI51678	ABI51700	ABI51701707	ABI51701702	ABI5170674	ABI517079	ABI51676	ABI51710	ABI51692
<i>Tetrahymena pigmentosa</i>	ABI51756	ABI51741	ABI51745	ABI51725	ABI51742	ABI51721	ABI51744	ABI51741751	ABI51741746	ABI5174718	ABI517222	ABI51720	ABI51754	ABI51736
<i>Tetrahymena rostrata</i>	QGS65290	QGS65275	QGS65279	QGS65259	QGS65276	QGS65256	QGS65278	QGS6526528	QGS6526528	QGS6525252	QGS65257	QGS65254	QGS65288	QGS65270
<i>Ichthyophthirius multifiliis</i>	AEL89289	AEL89274	AEL89279	AEL89261	AEL89275	AEL89258	AEL89278	AEL8929284	AEL8929280	AEL8929254	AEL89259	AEL89256	AEL89287	AEL89270
<i>Uronema marinum</i>	AXJ93331	AXJ93357	AXJ93360	AXJ93343	AXJ93358	AXJ93340	AXJ93359	AXJ93333326	AXJ93333361	AXJ9333336	AXJ933341	AXJ93338	AXJ93339	AXJ933353
<i>Paramecium caudatum</i>	CAZ66798	CAZ66836	CAZ66808	CAZ66819	CAZ66837	CAZ66816	CAZ66809	CAZ6686680	CAZ6686680	CAZ6686812	CAZ66817	CAZ66814	CAZ66800	CAZ66832
<i>Paramecium aurelia</i>	NP_039496	NP_039468	NP_039486	NP_039475	NP_039467	NP_039478	NP_039485	NP_03943949	NP_03943948	NP_03949481	NP_0394477	NP_039480	NP_039495	**
<i>Paramecium tetraurelia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paramecium sexaurelia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paramecium multimicronucleatum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paramecium biaurelia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-

<i>Paramecium octaurelia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paramecium novaurelia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paramecium decaurelia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paramecium dodecaurelia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paramecium quadecaurelia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paramecium jenningsi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Nyctotherus ovalis</i>	ADN858	ADN858	ADN858	ADN858	ADN858	ADN858	*	*	*	*	ADN858	ADN858	ADN858	**
	77	81	87	92	69	79					97	90	84	
<i>Stentor coeruleus</i>	**	**	**	**	**	**	**	**	**	*	**	**	**	*
<i>Gruberia lanceolata</i>	QIB7198	QIB7197	QIB7196	QIB7198	QIB7197	QIB7197	QIB7198	QIB7	QIB7	QIB71	QIB719	QIB7198	QIB7199	QIB7199
	3	0	6	8	7	6	2	1965	1967	978	72	0	6	3

Note: - indicates the mitogenome data of ten *Paramecium* species are not available on NCBI database but can be accessed on Zenodo (<https://doi.org/10.5281/zenodo.2539699>); * indicates absent; ** indicates mitochondrial proteins of *Stentor coeruleus* (acc. no. MPUH01000652) and rpl16 of *Paramecium aurelia* (acc. no. NC001324) and *Nyctotherus ovalis* (acc. no. GU057832) are not available on NCBI database but annotated in the present work based on mitogenome data.

Table S3. Overlapped mitochondrial genes of three newly sequenced species in the present work.

Species	Overlapped Genes	Overlapped Length (bp)
<i>Strombidium</i> sp.	<i>nad1_a</i> + <i>orf_s2</i>	14
	<i>rps8</i> + <i>tRNA_Gln</i>	15
	<i>rpl2</i> + <i>orf_s4</i>	10
	<i>orf_s4</i> + <i>nad10</i>	25
	<i>nad4</i> + <i>rpl16</i>	19
	<i>rps10</i> + <i>tRNA_Glu</i>	42
	<i>rps3_b</i> + <i>tRNA_Trp</i>	46
	<i>rps2</i> + <i>nad5</i>	25
	<i>rps8</i> + <i>tRNA_Gln</i>	43
	<i>orf_s3</i> + <i>rps14</i>	7
<i>Strombidium</i> cf. <i>sulcatum</i>	<i>rps4</i> + <i>rps13</i>	62
	<i>nad9</i> + <i>tRNA_Phe</i>	82
	<i>orf578</i> + <i>tRNA_Tyr</i>	81
	<i>rps3_b</i> + <i>tRNA_Trp</i>	25
	<i>orf192</i> + <i>ccmf_ii</i>	43
	<i>orf535</i> + <i>tRNA_His</i>	73
	<i>orf535</i> + <i>cob</i>	11
	<i>cob</i> + <i>tRNA_His</i>	4
	<i>rps8</i> + <i>tRNA_Gln</i>	43
	<i>rps14</i> + <i>nad7</i>	4
<i>Halteria grandinella</i>	<i>rps4</i> + <i>rps13</i>	10
	<i>rps13</i> + <i>rps19</i>	25
	<i>rps19</i> + <i>rpl2</i>	4
	<i>rpl2</i> + <i>orf561</i>	32
	<i>orf561</i> + <i>nad10</i>	190
	<i>rps10</i> + <i>tRNA_Glu</i>	58
	<i>nad9</i> + <i>tRNA_Phe</i>	8
	<i>rps3_b</i> + <i>tRNA_Trp</i>	60
	<i>nad4L</i> + <i>cox1</i>	13

Table 4. ORFs with unknown function encoded in mitogenomes of the class Spirotrichea.

Group	ORFs	<i>Strombidium</i> <i>m. sp.</i>	<i>Strombidium</i> <i>cf. sulcatum</i>	<i>Halteria</i> <i>grandinella</i>	<i>Oxytricha</i> <i>trifallax</i>	<i>Stylonyc</i> <i>hia</i> <i>lemnæ</i>	<i>Laurentiella</i> <i>strenua</i>	<i>Paraurostyl</i> <i>a sp.</i>	<i>Urostyla</i> <i>grandis</i>	<i>Pseudourostyl</i> <i>a cristata</i>	<i>Euplotes</i> <i>minuta</i>	<i>Euplotes</i> <i>crassus</i>
Homologous ORFs	<i>orf535</i>	+	+	+	+	*	+	*	*	*	*	*
	<i>orf546</i>	+	+	+	+	+	+	*	+	<i>orf393</i>	<i>orf380</i>	*
	<i>orf578</i>	+	+	+	+	+	+	+	*	<i>orf439</i>	*	*
	<i>orf583</i>	+	+	+	+	+	+	+	+	<i>orf373</i>	<i>orf268</i>	<i>orf311</i>
	<i>orf592</i>	+	+	+	+	+	+	+	*	<i>orf262</i>	<i>orf78</i>	<i>orf129</i>
	<i>orf192</i>	*	*	+	*	*	*	*	*	+	*	*
	<i>orf549</i>	*	*	+	+	+	*	*	*	*	*	*
	<i>orf561</i>	*	*	+	+	+	+	+	*	<i>orf256</i>	<i>orf267</i>	<i>orf297</i>
	<i>orf584</i>	*	*	+	+	+	+	+	*	<i>orf178</i>	*	*
	<i>orf547</i>	*	*	*	+	*	*	+	+	*	*	*
	<i>orf_s1</i>	+	+	*	*	*	*	*	*	*	*	*
	<i>orf_s3</i>	+	+	*	*	*	*	*	*	*	*	*
	<i>orf_s4</i>	+	+	*	*	*	*	*	*	*	*	*
	<i>orf_s5</i>	+	+	*	*	*	*	*	*	*	*	*
	<i>orf_s6</i>	+	+	*	*	*	*	*	*	*	*	*
	<i>orf_s7</i>	+	+	*	*	*	*	*	*	*	*	*
	<i>orf259</i>	*	*	*	*	*	*	*	*	*	+	<i>orf449</i>
	<i>orf163</i>	*	*	*	*	*	*	*	*	*	+	<i>orf197</i>
	<i>orf187</i>	*	*	*	*	*	*	*	*	*	+	<i>orf134</i>
	<i>orf96</i>	*	*	*	*	*	*	*	*	*	+	<i>orf101</i>
	<i>orf111</i>	*	*	*	*	*	*	*	*	*	+	<i>orf175</i>
	<i>orf155</i>	*	*	*	*	*	*	*	*	*	+	<i>orf156</i>
Non-homologous ORFs		1	0	0	50	0	0	0	0	14	6	3

Note: + denotes present; * denotes absent; numbers indicate the number of non-homologous ORFs. Homologous ORFs identified according to blastp searches and gene position on mitogenome are shown in the same row.