

Supplementary Information

Supplementary Table S1: Additional information on amount of reads, contigs after de novo assembly and length of the mitochondrial genome.

Species	Reads	Contigs	N50 [bp]	mt-Genome [bp]
<i>Chaetopterus variopedatus</i>	86.481.655	215.905	390	16.134
<i>Eurythoe complanata</i>	25.294.230	71.520	330	15.829
<i>Magelona johnstoni</i>	5.695.394	15.928	337	15.239
<i>Owenia fusiformis</i>	29.777.298	177.948	798	16.204
<i>Phyllochaetopterus</i> sp.	25.904.248	10.824	785	16.087

Supplementary Table S2: Partition schemes and best fitting substitution models for both mitochondrial datasets as inferred with IQ-TREE.

Data set	Partition #	Loci included	Substitution model
Data set 1 (40 taxa)	1	ATP6, ATP8, NAD1, NAD2, NAD3, NAD4, NAD4L, NAD5, NAD6	mtZOA+F+G
	2	COX1, COX2, COX3, CYTB	mtZOA+G
Data set 2 (48 taxa)	1	ATP6	mtZOA+F+G
	2	ATP8	mtZOA+F+G
	3	COX1	mtZOA+G
	4	COX2	mtZOA+G
	5	COX3	mtZOA+G
	6	CYTB	mtZOA+G
	7	NAD1	mtZOA+G
	8	NAD2	mtZOA+F+G
	9	NAD3	mtZOA+G
	10	NAD4	mtZOA+F+G
	11	NAD4L	mtZOA+G
	12	NAD5	mtZOA+F+G
	13	NAD6	mtART+G

Supplementary Table S3: Results of cross-validation analysis performed with PhyloBayes.

Data set	Replicate #	Log-likelihood for model		
		mtART	CAT-Poisson	CAT-GTR
Data set 1 (40 taxa)	1	17677.8	17390.3	17227.7
	2	18562.4	18287.3	18068.2
	3	17020.5	16874	16619.5
	4	18292.1	18031	17852.3
	5	17752.6	17521.3	17277.8
	6	18753.4	18401.1	18289.2
	7	18083.5	17801.7	17605.9
	8	19267.5	18955.1	18718.8
	9	18474.3	18141	17959.2
	10	17996.4	17816.2	17633.5
	mean	18188.1	17921.9	17725.2
Data set 2 (48 taxa)	1	19715.9	19483.2	19272
	2	20275.9	19941.1	19755.5
	3	21075.3	20748.3	20507.6
	4	20226.4	20011.9	19774.4
	5	20517.2	20135.6	19903.1
	6	21079.2	20844.2	20561
	7	21427.6	21118.6	20934.2
	8	19680.8	19479.2	19221.9
	9	20059.8	19735.4	19500.5
	10	19398.1	19025.1	18816.9
	mean	20345.6	20052.3	19824.7

Supplementary Table S4: Annotation of the complete mitochondrial genome of *Magelona mirabilis*

Name	Type	Start	End	Length [bp]	Strand	Anticodon
COX1	PCG	10	1521	1511	+	
COX2	PCG	1550	2224	674	+	
tRNA-D	tRNA	2238	2303	65	+	GTC
ATP8	PCG	2301	2459	158	+	
ATP8 (copy)	PCG	2483	2512	29	+	
ATP6	PCG	2622	3170	548	+	
tRNA-E	tRNA	3180	3240	60	+	TTC
COX3	PCG	3243	4019	776	+	
tRNA-K	tRNA	4035	4097	62	+	TTT
tRNA-A	tRNA	4101	4167	66	+	TGC
tRNA-R	tRNA	4167	4231	64	+	TCG
tRNA-N	tRNA	4235	4299	64	+	GTT
tRNA-G	tRNA	4300	4365	65	+	TCC
tRNA-Q	tRNA	4371	4439	68	+	TTG
Nad6	PCG	4440	4919	479	+	
CytB	PCG	4939	6060	1121	+	
tRNA-W	tRNA	6083	6149	66	+	TCA
tRNA-Y	tRNA	6167	6231	64	+	GTA
tRNA-C	tRNA	6232	6296	64	+	GCA
tRNA-M	tRNA	6298	6362	64	+	CAT
SrRNA	rRNA	6372	7237	865	+	
tRNA-V	tRNA	7231	7294	63	+	TAC
LrRNA	rRNA	7242	8610	1368	+	
tRNA-L1	tRNA	8577	8644	67	+	TAG
tRNA-L2	tRNA	8645	8707	62	+	TAA
NAD1	PCG	8693	9637	944	+	
tRNA-P	tRNA	9959	10021	62	-	TGG
tRNA-S2	tRNA	10025	10091	66	+	TGA
tRNA-T	tRNA	10109	10174	65	-	TGT
NAD4L	PCG	10218	10475	257	+	
NAD4	PCG	10511	11863	1352	+	
tRNA-H	tRNA	11868	11930	62	+	GTG
NAD5	PCG	11982	13640	1658	+	

Name	Type	Start	End	Length	Strand	Anticodon
tRNA-F	tRNA	13651	13717	66	+	GAA
tRNA-I	tRNA	13752	13815	63	+	GAT
NAD3	PCG	13812	14165	353	+	
tRNA-S1	tRNA	14178	14246	68	+	GCT
NAD2	PCG	14292	15233	941	+	

Supplementary Table S5: Annotation of the complete mitochondrial genome of *Owenia fusiformis*

Name	Type	Start	End	Length	Strand	Anticodon
COX1	PCG	11	1555	1544	+	
COX2	PCG	1564	2232	668	+	
tRNA-D	tRNA	2262	2329	67	+	GTC
ATP8	PCG	2330	2503	173	+	
tRNA-Q	tRNA	2520	2586	66	+	TTG
tRNA-R	tRNA	2600	2668	68	+	TCG
NAD3	PCG	2690	3013	323	+	
NAD5	PCG	3186	4757	1571	+	
NAD5	PCG	3186	4757	1571	+	
tRNA-S1	tRNA	4892	4960	68	+	GCT
NAD2	PCG	5000	5908	908	+	
tRNA-F	tRNA	6069	6133	64	+	GAA
tRNA-H	tRNA	6149	6215	66	+	GTG
tRNA-C	tRNA	6242	6306	64	+	GCA
NAD1	PCG	6345	7238	893	+	
tRNA-P	tRNA	7266	7332	66	-	TGG
tRNA-S2	tRNA	7334	7401	67	+	TGA
NAD6	PCG	7403	7886	483	+	
CytB	PCG	7915	9006	1091	+	
tRNA-T	tRNA	9076	9140	64	-	TGT
NAD4L	PCG	9172	9435	263	+	
NAD4	PCG	9567	10808	1241	+	
tRNA-E	tRNA	10826	10893	67	+	TTC
tRNA-W	tRNA	10931	10997	66	+	TCA

Name	Type	Start	End	Length [bp]	Strand	Anticodon
tRNA-M	tRNA	10999	11065	66	+	CAT
SrRNA	rRNA	11079	11887	808	+	
LrRNA	rRNA	12096	13177	1081	+	
tRNA-L1	tRNA	13242	13308	66	+	TAG
tRNA-V	tRNA	13316	13381	65	+	TAC
tRNA-L2	tRNA	14154	14220	66	+	TAA
ATP6	PCG	14387	14920	533	+	
tRNA-N	tRNA	14935	15002	67	+	GTT
tRNA-Y	tRNA	15019	15083	64	+	GTA
tRNA-I	tRNA	15087	15156	69	+	GAT
tRNA-A	tRNA	15169	15235	66	+	TGC
tRNA-G	tRNA	15247	15313	66	+	TCC
COX3	PCG	15315	16091	776	+	
tRNA-K	tRNA	16119	16186	67	+	TTT

Supplementary Table S6: Annotation of the complete mitochondrial genome of *Eurythoe complanata*.

Name	Type	Start	End	Length [bp]	Strand	Anticodon
COX1	PCG	91	1602	1511	+	
tRNA-N	tRNA	1649	1713	64	+	GTT
COX2	PCG	1714	2388	674	+	
tRNA-D	tRNA	2399	2462	63	+	GTC
ATP8	PCG	2464	2619	155	+	
tRNA-Y	tRNA	2621	2684	63	+	GTA
tRNA-E	tRNA	2687	2750	63	+	TTC
tRNA-C	tRNA	2752	2813	61	+	GCA
tRNA-M1	tRNA	2815	2878	63	+	CAT
tRNA-M2	tRNA	2883	2948	65	+	CAT
SrRNA	rRNA	2949	3746	797	+	
tRNA-V	tRNA	3740	3806	66	+	TAC
LrRNA	rRNA	3868	5092	1224	+	
tRNA-L1	tRNA	5059	5122	63	+	TAG
tRNA-A	tRNA	5123	5187	64	+	TGC

Name	Type	Start	End	Length [bp]	Strand	Anticodon
tRNA-I	tRNA	5188	5254	66	+	GAT
tRNA-K	tRNA	5256	5320	64	+	TTT
NAD3	PCG	5322	5673	351	+	
tRNA-S1	tRNA	5676	5743	67	+	TCT
NAD2	PCG	5753	6745	992	+	
tRNA-G	tRNA	6753	6816	63	+	TCC
COX3	PCG	6814	7593	779	+	
tRNA-Q	tRNA	7604	7671	67	+	TTG
NAD6	PCG	7672	8136	464	+	
CytB	PCG	8150	9259	1109	+	
tRNA-S2	tRNA	9288	9354	66	+	TGA
tRNA-L2	tRNA	9355	9418	63	+	TAA
NAD1	PCG	9420	10325	905	+	
tRNA-W	tRNA	10357	10420	63	+	TCA
ATP6	PCG	10458	11114	656	+	
tRNA-R	tRNA	11122	11186	64	+	TCG
tRNA-H	tRNA	11189	11249	60	+	GTG
NAD5	PCG	11283	12962	1679	+	
tRNA-F	tRNA	12970	13034	64	+	GAA
tRNA-P	tRNA	13035	13098	63	+	TGG
tRNA-T	tRNA	13101	13167	66	+	TGT
NAD4L	PCG	13195	13455	260	+	
NAD4	PCG	13485	14798	1313	+	

Supplementary Table S7: Annotation of the complete mitochondrial genome of *Chaetopterus variopedatus*.

Name	Type	Start	End	Length [bp]	Strand	Anticodon
COX1	PCG	10	1837	1827	+	
tRNA-A	tRNA	1865	1928	63	+	TGC
tRNA-L2	tRNA	1931	1998	67	+	TAA
NAD1	PCG	1984	2901	917	+	
tRNA-R	tRNA	2934	3001	67	+	TCG

Name	Type	Start	End	Length	Strand	Anticodon
tRNA-T	tRNA	3008	3072	64	+	TGT
tRNA-I	tRNA	3101	3166	65	+	GAT
NAD6	PCG	3153	3641	488	+	
tRNA-G	tRNA	3678	3744	66	+	TCC
ATP6	PCG	3746	4444	698	+	
tRNA-Q	tRNA	4468	4534	66	+	TTG
tRNA-W	tRNA	4539	4599	60	+	TCA
tRNA-C	tRNA	4602	4668	66	+	GCA
tRNA-M1	tRNA	4670	4733	63	+	CAT
tRNA-Y	tRNA	4744	4807	63	+	GTA
tRNA-M2	tRNA	4808	4871	63	+	CAT
SrRNA	rRNA	4870	5734	864	+	
tRNA-V	tRNA	5727	5791	64	+	TAC
LrRNA	rRNA	5758	7130	1372	+	
tRNA-L1	tRNA	7145	7208	63	+	TAG
NAD3	PCG	7237	7584	347	+	
tRNA-S1	tRNA	7588	7656	68	+	GCT
NAD2	PCG	7693	8619	926	+	
tRNA-P	tRNA	8655	8726	71	+	TGG
NAD3 (copy)	PCG	9144	9254	110	+	
CytB	PCG	9365	10480	1115	+	
tRNA-S2	tRNA	10505	10571	66	+	TGA
NAD4L	PCG	10581	10850	269	+	
NAD4	PCG	10934	12214	1280	+	
tRNA-H	tRNA	12235	12297	62	+	GTG
NAD5	PCG	12388	13998	1610	+	
tRNA-E	tRNA	14089	14151	62	+	TTC
tRNA-F	tRNA	14162	14227	65	+	GAA
COX3	PCG	14227	15006	779	+	
tRNA-K	tRNA	15008	15074	66	+	TTT
COX2	PCG	15076	15753	677	+	
tRNA-D	tRNA	15769	15834	65	+	GTC
ATP8	PCG	15837	15989	152	+	
tRNA-N	tRNA	16010	16076	66	+	GTT

Supplementary Table S8: Annotation of the complete mitochondrial genome of *Phyllochaetopterus* sp.

Name	Type	Start	End	Length [bp]	Strand	Anticodon
COX1	PCG	20	1498	1478	+	
tRNA-L2	tRNA	1563	1626	63	+	TAA
NAD1	PCG	1621	2541	920	+	
tRNA-T	tRNA	2566	2631	65	+	TGT
tRNA-A	tRNA	2658	2723	65	+	TGC
tRNA-P	tRNA	2753	2818	65	+	TGG
tRNA-R	tRNA	2878	2944	66	+	TCG
tRNA-D	tRNA	2945	3007	62	+	GTC
CytB	PCG	3739	4839	1100	+	
tRNA-S2	tRNA	4850	4917	67	+	TGA
NAD4L	PCG	4933	5199	266	+	
NAD4	PCG	5205	6557	1352	+	
tRNA-H	tRNA	6569	6628	59	+	GTG
NAD5	PCG	6656	8326	1670	+	
tRNA-I	tRNA	8350	8415	65	+	GAT
NAD6	PCG	8416	8895	479	+	
tRNA-G	tRNA	8909	8973	64	+	TCC
ATP6	PCG	9181	9669	488	+	
tRNA-Q	tRNA	9680	9746	66	+	TTG
tRNA-W	tRNA	9749	9809	60	+	TCA
tRNA-Y	tRNA	9815	9882	67	+	GTA
tRNA-C	tRNA	9884	9939	55	+	GCA
tRNA-M	tRNA	9944	10008	64	+	CAT
SrRNA	rRNA	10008	10893	885	+	
tRNA-V	tRNA	10894	10961	67	+	TAC
LrRNA	rRNA	10922	12303	1381	+	
tRNA-L1	tRNA	12262	12326	64	+	TAG
NAD3	PCG	12324	12671	347	+	
tRNA-S1	tRNA	12681	12745	64	+	TCT
NAD2	PCG	12794	13759	965	+	
tRNA-E	tRNA	13797	13857	60	+	TTC
tRNA-F	tRNA	13859	13922	63	+	GAA
COX3 (copy)	PCG	13925	13999	74	+	

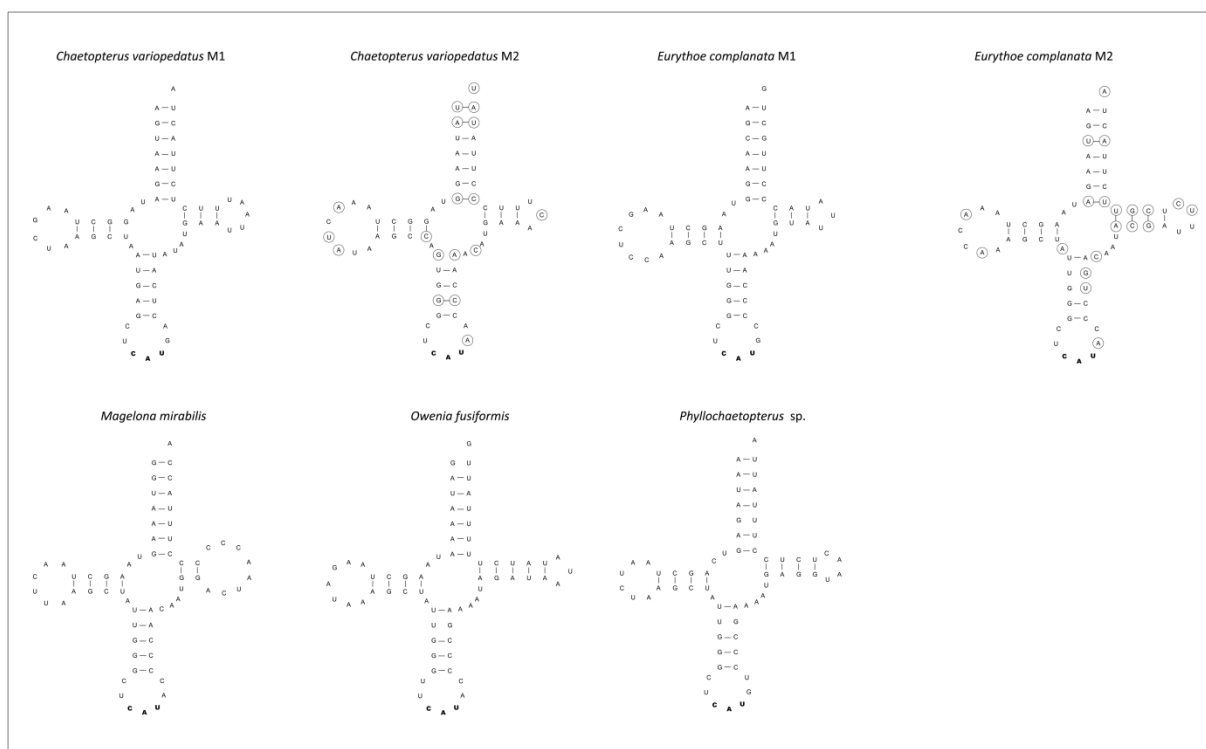
Name	Type	Start	End	Length [bp]	Strand	Anticodon
COX3	PCG	14272	14970	698	+	
COX2	PCG	15003	15659	656	+	
ATP8	PCG	15709	15828	119	+	
tRNA-N	tRNA	15868	15931	63	+	GTT
tRNA-K	tRNA	15935	16000	65	+	TTT

Supplementary Table S9: Comparison of mitochondrial gene order of basal branching annelids with patterns of each sister group, Pleistoannelida and Lophotrochozoa determined with CREx with tRNAs excluded. Rearrangement scenarios to transform one gene order in the other are abbreviated as followed: Tp – Transposition, rTp – Reversal transposition, R – Reversal, Tdrl – Tandem duplication random loss. *Italic letters represent genes transcribed on the “-” strand.*

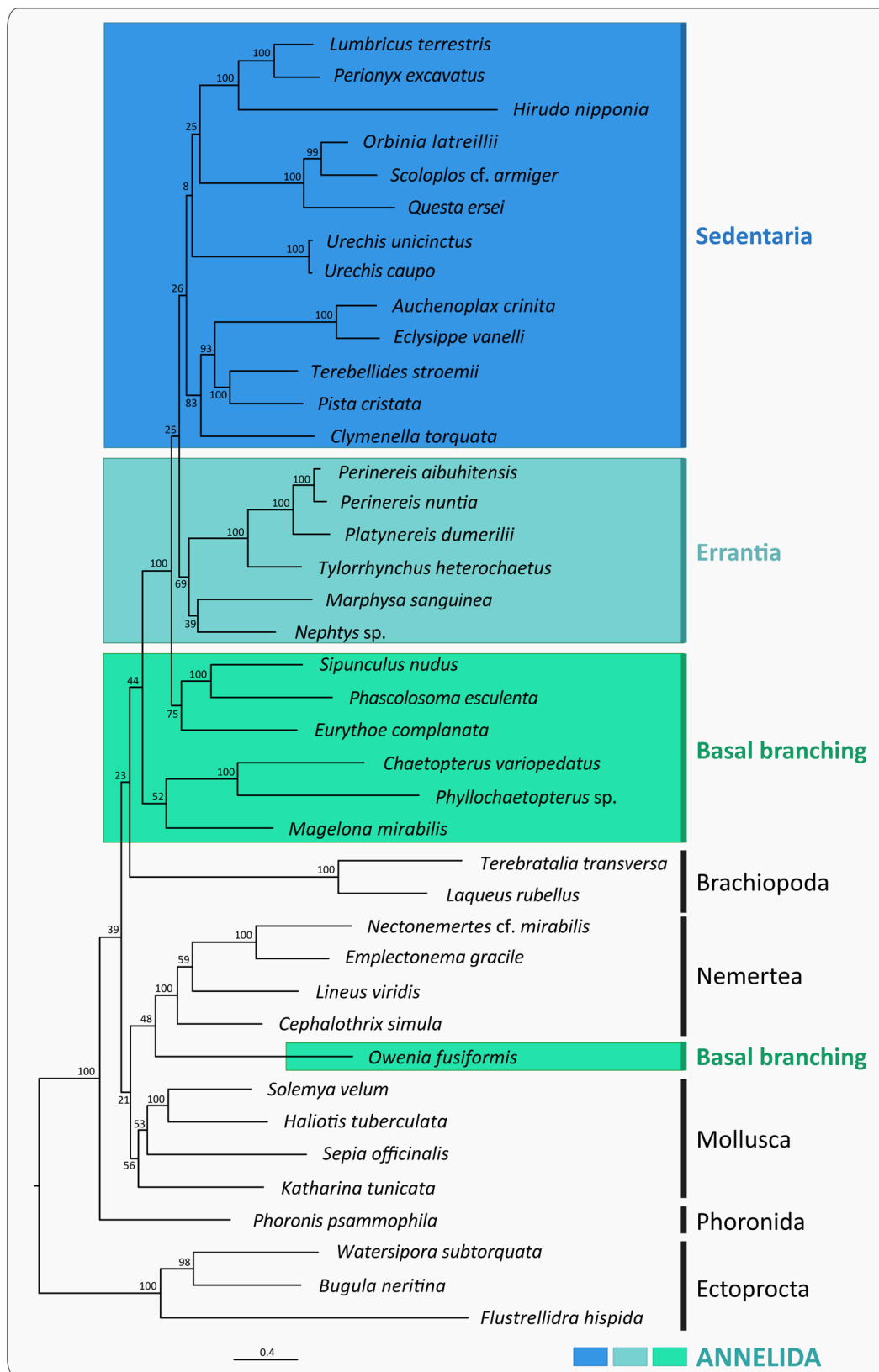
Comparing taxa	Scenario	Gene region
<i>Eurythoe complanta</i>		
Pleistoannelida	Tdrl Tp	COX3-NAD6-CytB, NAD1 ATP6-NAD5-NAD4L-NAD4
Lophotrochozoa	Tp Tp Tp Tp Tdrl	NAD1 NAD5 COX3 NAD6-CytB-NAD1 ATP6, NAD5-NAD4L-NAD4
Sipuncula	Tp Tdrl	NAD4L-NAD4 SrRNA-LrRNA-NAD3, NAD2
<i>Chaetopterus variopedatus</i>		
Pleistoannelida	Tp rTp R	NAD5 NAD6-CytB-ATP6-NAD4L-NAD4-NAD5-SrRNA- LrRNA-NAD1-NAD3-NAD2 <i>NAD2-NAD3-NAD1-LrRNA-SrRNA-NAD5-NAD4- NAD4L-ATP6-CytB-NAD6</i>
Lophotrochozoa	Tdrl Tp	CytB, NAD4L-NAD4-NAD5, NAD1 CytB, NAD4L-NAD4-NAD5
<i>Phyllochaetopterus</i> sp.	Tp	CytB-NAD4L-NAD4-NAD5

Comparing taxa	Scenario	Gene region
<i>Phyllochaetopterus</i> sp. Pleistoannelida Lophotrochozoa <i>Chaetopterus variopedatus</i>	Tp Tp R R R R Tdrl Tp Tp Tp Tp Tp	NAD5 NAD1 COX2-ATP8 COX3 NAD1-NAD6-CytB-ATP6-NAD4L-NAD4-NAD5-SrRNA-LrRNA-NAD3-NAD2 ATP8-COX2-COX3-NAD2-NAD3-LrRNA-SrRNA-NAD5-NAD4-NAD4L-ATP6-CytB-NAD6-NAD1 NAD6, ATP6 NAD6 ATP6-SrRNA-LrRNA COX3 COX2-ATP8 CytB-NAD4L-NAD4-NAD5
<i>Magelona mirabilis</i> Pleistoannelida Lophotrochozoa <i>Owenia fusiformis</i>	Tp R R R R Tp Tp Tp R R R R Tp Tp	ATP6 NAD5 NAD4L-NAd4 SrRNA-LrRNA-NAD1 NAD5-NAD4-NAD4L-NAD1-LrRNA-SrRNA NAD6-CytB COX3 NAD5 ATP6-COX3 NAD3-NAD5-NAD2 COX3-ATP6-NAD6-CytB-SrRNA-LrRNA-NAD1-NAD4L-NAD4-NAD2-NAD5-NAD3 NAD4-NAD4L-NAD1-LrRNA-SrRNA-CytB-NAD6 NAD6-CytB SrRNA-LrRNA
<i>Owenia fusiformis</i> Pleistoannelida	rTp R Tdrl Tdrl Tp	NAD6-CytB-ATP6-NAD5-NAD4L-NAD4-SrRNA-LrRNA-NAD1-NAD3-NAD2 NAD2-NAD3-NAD1-LrRNA-SrRNA-NAD4-NAD4L-NAD5-ATP6-CytB-NAD6 NAD6-CytB, NAD5-NAD4L-NAD4-SrRNA-LrRNA, NAD2 NAD1, NAD6-CytB, NAD4L-NAD4-SrRNA-LrRNA ATP6

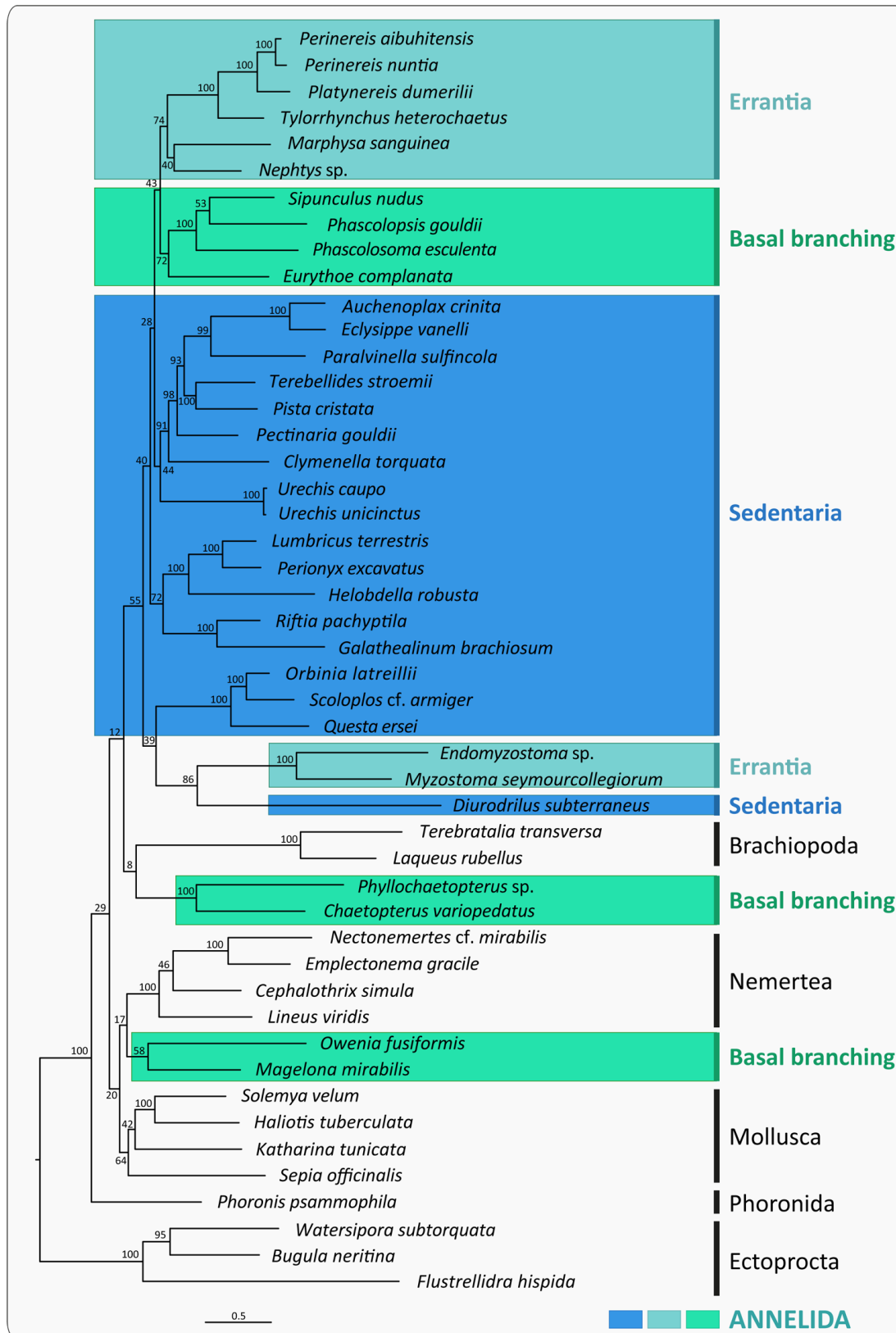
Comparing taxa	Scenario	Gene region
<i>Owenia fusiformis</i>		
Lophotrochozoa	R	NAD1-NAD6-CytB-NAD4L-NAD4
	R	SrRNA-LrRNA
	R	ATP6
	R	<i>NAD4-NAD4L-CytB-NAD6-NAD1-LrRNA-SrRNA-ATP6</i>
	Tp	NAD5
	Tp	NAD3-NAD2
<i>Magelona mirabilis</i>	Tp	NAD5
	R	ATP6-COX3
	R	NAD3-NAD5-NAD2
	R	<i>COX3-ATP6-NAD6-CytB-SrRNA-LrRNA-NAD1-NAD4L-NAD4-NAD2-NAD5-NAD3</i>
	R	<i>NAD4-NAD4L-NAD1-LrRNA-SrRNA-CytB-NAD6</i>
	Tp	NAD6-CytB
	Tp	SrRNA-LrRNA



Supplementary Figure S4: Putative secondary structures for methionine encoding tRNAs of basal branching annelids. *Chaetopterus variopedatus* and *Eurythoe complanata* each possess two tRNA-M. Watson-Crick pairs are indicated by lines and differences in base composition of copy 2 in comparison to copy 1 of each taxon are circled.



Supplementary Figure S5: Phylogenetic relationships of Annelida based on mitochondrial genome data. Best tree of the Maximum Likelihood analysis based on the partitioned data set 1 with the best fitting amino acid models (Supplementary Table S2) and comprising only complete mitochondrial genomes (40 taxa, 3654 amino acid positions). Bootstrap support values (BS) are shown for each node.



Supplementary Figure S6: Phylogenetic relationships of Annelida based on mitochondrial genome data. Best tree of the Maximum Likelihood analysis based on the partitioned data set 2 with the best fitting amino acid models (Supplementary Table S2) and comprising complete and partial annelid mitochondrial genomes (48 taxa, 3630 amino acid positions). The tree was built from the posterior samples of the two runs with the highest mean log likelihood. Bootstrap support values (BS) are shown for each node.