

Online Appendix 1. More detailed description of methods for the determination of the mitochondrial genomes and their properties like codon usage.

mtDNA Data Collection

Auchenoplax crinita and *Paralvinella sulfincola* – Mitochondrial genomes were amplified in four overlapping segments using species-specific primers. To generate these primers, conserved regions of *mLSU*, *cox1*, *cob* and *nad5* genes were amplified using taxonomically inclusive primers (Zhong et al. 2008), purified by QIAquick PCR purification kit (Qiagen), and products were sequenced in a CEQ8000 (Beckmann). From these data, four sets of species-specific primers for long PCRs were designed (Supplementary Table 1). HotStart long PCRs were performed on Eppendorf Mastercycler (Eppendorf) using Takara LA-Taq and the following protocol: 94 °C for 3min; then addition of polymerase followed by 35 cycles with 94°C for 30sec, 52°C for 30sec, and 70°C for 8min; final extension at 72°C for 10min and hold at 6°C. 50 µl long PCR reactions were set up as follows: 5µl 10×buffer, 8µl dNTP (2mM), 5µl MgCl₂ (25mM), 2µl of each long PCR specific primers (10µM each), 0.5µl Takara LA-Taq (5U/µl), 2µl DNA template and 25.5µl sterilized, distilled water. Both the *mLSU-cox1* and *cox1-cob* regions were about 4 kb in size. Both the *cob-nad5* fragment of *A. crinita* and the *nad5- mLSU* fragment of *P. sulfincola* were about 6-7 kb in size. The *nad5- mLSU* fragment of *A. crinita* and *cob-nad5* of *P. sulfincola* containing the UNK region were difficult to amplify consistent with previous reports for annelids (Boore and Brown 2000, Jennings and Halanych 2005, Zhong et al. 2008).

Pectinaria gouldi – Genome of *P. gouldi* was amplified in six segments. Conserved fragments of *mLSU*, *cox1*, *nad5* and *nad4* genes were amplified by taxonomically inclusive primers (Zhong et al. 2008) and sequenced. New primers were designed based on conserved regions in *cox3* (see Supplementary Table 1). Then specific long-PCR primers (Supplementary Table 1) were designed for amplification of *mLSU-cox1*, *cox1-cox3*, *nad5-nad4* and *nad4-mLSU*. The “universal” *cob* reverse primer “Cytb 876R” was used to pair with the *cox3* forward one to amplify *cox3-cob* fragment. Based on the sequence of this long fragment a specific *cob* forward long-PCR primer was designed to pair with the *nad5* reverse one to amplify the *cob-nad5* fragment. Long PCRs employed Takara LA-Taq as described above. The protocols of long PCR and purification were similar to above with some modifications of annealing temperatures based on different pairs of primers (Supplementary Table 1). All long fragments were sequenced directly by primers walking. We were unable to obtain a *cob-nad5* fragment, which presumably contains the UNK region. Information for all

sequencing primers for the three species can be found in the Supplementary Table 2.

Mitochondrial gene order

All three mitochondrial genomes (mtDNA) are uncompleted due to difficulty amplifying the UNK region. The partial mtDNA genome of *Auchenoplax crinita* (Ampharetidae) was 13,759 bp in length, of *Paralvinella sulfincola* (Alvinellidae) 13,640 bp, and of *Pectinaria gouldi* (Pectinariidae) 13,438 bp. From *A. crinita* we recovered 34 genes including 13 protein-coding genes, two rRNA genes and 19 tRNAs. There were 33 genes recovered in the *P. sulfincola* mtDNA including 12 protein-coding genes, two rRNA genes and 19 tRNAs, whereas the recovered *P. gouldi* genome contained 34 genes including 12 protein-coding genes, two rRNA genes and 20 tRNAs. Interestingly, the *P. gouldi* fragment possesses identical mitochondrial gene order as both the trichobranchid *Terebellides stroemi* and the terebellid *Pista cristata*. *P. gouldi* even possesses two adjacent copies of the methionine tRNA gene as found in the trichobranchid and terebellid (Zhong et al. 2008). Supplementary Figure 1 shows gene orders of all six available terebelliform mitochondrial genomes. All mitochondrial genes are transcribed from the same strand, similar to other annelids (Bleidorn et al. 2006a, Bleidorn et al. 2006b, Boore and Brown 2000, Boore and Staton 2002, Jennings and Halanych 2005, Zhong et al. 2008). Gene order of *A. crinita* is identical to the another published ampharetid *Eclysippe vanelli* except for the *trnF* between the *nad1* and *nad3* genes in *E. vanelli* (Zhong et al. 2008) that is presumably in the uncovered region of *A. crinita*.

Two adjacent *trnM* genes were discovered in the pectinariid *P. gouldi* as well as in terebellid and trichobranchid worms (Zhong et al. 2008), but not in an alvinellid and ampharetids. A unique duplication event was speculated based on their similar secondary structure, identical anticodons and the same sequences in stems. As Pectinariidae was highly supported as a basal clade of Terebelliformia, *trnM* duplication was noted to be a symplesiomorphy of terebelliforms instead of a synapomorphy for Trichobranchidae and Terebellidae (Zhong et al. 2008). Both Ampharetidae and Alvinellidae have secondarily lost one of the two *trnMs*. Besides, two adjacent *trnMs* were recently found in a complete mitochondrial genome of the sipunculid *Phascolosoma esculenta* (Shen et al. 2009). Very similar sequences in the D and anticodon arms between the two *trnMs* indicate they may be from a duplication event, like in our study. However, such a duplication event has not been found in the other two published mitochondrial genomes of sipunculids, either a complete one of *Sipunculus nudus* (Mwinyi et al. 2009) or a partial one of *Phascolopsis gouldi* (Boore and Staton 2002), which covers this region. Comparing the mtDNA gene order across the

three sipunculid lineages, an identical protein-coding and rRNA gene order is evident. Considering for both *Sipunculus* and *Phascolopsis*, that their gene orders are very similar with exception of one *trnE* translocation, it cannot be expected that an extra *trnM* exists in the unobserved region of the *Phascolopsis* mitochondrial genome. The phylogenetic references of sipunculids suggest that the basal lineage *Sipunculus* is sister to the other two (Maxmen et al. 2003, Schulze et al. 2005), indicating that the *trnM* duplication in the *Phascolosoma* lineage evolved potentially independently in Sipuncula from the one in Terebelliformia.

Codon usage

We also determined codon usage biases for the individual taxa using the average relative synonymous codon usage (RSCU). The RSCU of the protein-coding genes in all included annelids were calculated using the DNASTAR™ Lasergene program GENEQUEST (Burland 2000) (Supplementary Tables 3 & 4). We differentiated between degree of degeneration (4-fold or 2-fold), 3rd position nucleotides, and the location of corresponding tRNA (mitochondrial or nuclear genome). Thus, we had a total of ten classes: 4-fold degenerate NNA (mitochondrial), NNC, NNG, NNU (nuclear) and 2-fold degenerate NNA (mitochondrial), NNG (nuclear), NNA (nuclear), NNG (mitochondrial), NNC (mitochondrial), NNT (nuclear).

Ampharetidae used codons coding for 4-fold degenerated triplets less often than the other terebelliform taxa when codons possessed an adenine or cytosine at the 3rd position (Supplementary Fig. 2). The same was true for 2-fold degenerated codons ending on a cytosine. tRNAs of nuclear genomic origin with codons ending on guanine as well as 2-fold degenerated codons ending on uracil were used more often. Alvinellidae showed the opposite (Supplementary Fig. 2). Codons with an uracil at the 3rd position were used less often, while 4-fold degenerated codons ending on adenine and 2-fold ones ending on cytosine were used more often. Additionally, tRNAs of mitochondrial origin with 2-fold degenerated codons ending on guanine were used more often and, vice versa, 2-fold ones ending on adenine less often.

References

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SUPPLEMENTARY TABLE 1: Primers used in long-run PCR amplifications

Fragments	Primer name	Sequence	Annealing Temp.
<i>A. crinita</i>			
<i>mLSU-cox1</i>	16S-Ac-longF	5'—GCG GTA TCC TGA CTG TGC TAA GGT AGC GTG—3'	53
	CO1-Ac-longR	5'—AGA ATG AGC AAT ATT ACT AGA TAA GGG CGG—3'	53
<i>cox1-cob</i>	CO1-Ac-longF	5'—TTA ATT CGT GTT GAG CTT GGA CAG CCA GGC—3'	53
	Cytb-Ac-longR	5'—AGA GGG GTT ACT AGT GGA TTT GCT AAT AGC C—3'	53
<i>cob-nad5</i>	Cytb-Ac-longF	5'—ATT TTC TTG TGC CTT TTA TTA TGG TAG CAC—3'	54
	Nad5-Ac-789R	5'—ATC GCA CTC TAC CAA AGC TGA AAA CGC AGC—3'	54
<i>nad5-mLSU</i>	Nad5-Ac-645F	5'—CGC TAG TTC ATT CGT CTA CAC TTG TAA CAG C—3'	--
	16S-Ac-longR	5'—TGT CCC ACA CTT ACA TTC AGG TAT TTT CAC C—3'	--
<i>P. sulfincola</i>			
<i>mLSU-cox1</i>	16S-PS-longF	5'—GTT CTA ACT GCA TAT CAA GGC AAA ACC AGC C—3'	58
	CO1-PS-longR	5'—GCC TGC GTG TGC CAT GTT TCC TGC TAG TGG—3'	58
<i>cox1-cob</i>	CO1-PS-longF1	5'—AAT CTA CCC ACC ACT AGC AGG AAA CAT GGC—3'	55
	cob-PS-longR	5'—AAT TGA TCA GTT TTC TGG TTC TCC CAG GGC—3'	55
<i>cob-nad5</i>		5' —TGR GGN TAT CAR CCN GAR CG—3'	
	cob-PS-longF	5'—ACA ACC CTC TTG GTA TTA ACT CAG ACT CCG—3'	--
<i>nad5-mLSU</i>	Nad5-PS-longR	5'—AGT GTG GAT GAG TGG ACT AGT GCA GAG ACG—3'	--
	Nad5-PS-longF	5'—GAC TTC CAG CAG CAA TGG CAG CAC CTA CGC—3'	58
	16S-PS-longR	5'—ATC AGT TGT GCT TGT GTG GCT GGT TTT GCC—3'	58
<i>P. gouldi</i>			
<i>mLSU-cox1</i>	16S-PecG-longF	5'—AAA TCA TAG GAC AAG AAG ACC CCG TAG AGC—3'	58
	CO1-PecG-longR	5'—AAA AAT AGC AAG ATC CAC GGA AGG GCC TGC—3'	58
<i>cox1-cox3</i>	CO1-PecG-longF	5'—CTT AAT TCG TGT AGA ACT TGG TCA ACC AGG C—3'	52
	CO3-Ann-724R	5'—ACR TCS ACA AAR TGT CAR TAY CA—3'	52
<i>cox3-cob</i>	CO3-PG-long627F	5'—GAT TCC ACG GGC TTC ATG TTC TAA TTG GC—3'	52
	Cytb 876R	5'—GCR TAW GCR AAW ARR AAR TAY CAY—3'	52
<i>cob-nad5</i>	Cob-PG-midF	5'—TTG CTT CGA AAC CTT CAT GCT AAC GGA GC—3'	--
	Nad5-PG-675R	5'—AAT CGG ACT AAA AGA AAC ACT CCT GCC G—3'	--
<i>nad5-nad4</i>	Nad5-PG-453F	5'—TTA TAC TTC TTT TAT CTA TTG GAT GAG CCC—3'	53
	Nad4-PecG-longR	5'—ATT GGG AGG AGT TAA AAG AGT AAA GGA TTG C—3'	53
<i>nad4-mLSU</i>	Nad4-PecG-longF	5'—TTT AAC TCC TCC CAA TCC CTA TCT TTT AGC—3'	58
	16S-PecG-longR	5'—GTT TAG GTT AGG CGG GAT GCC TTA TTG CTC—3'	58

SUPPLEMENTARY TABLE 2: Primers used for sequencing the mitochondrial genomes of *Auchenoplax crinita*, *Paravinella sulfincola* and *Pectinaria gouldi*.

Fragments	Primer name	Sequence
<i>A. crinita</i>		
<i>mLSU-cox1</i>	CO1-Auc1-startR	5'—GTC GCA TAT AAC CAA CGC—3'
	16S-Auc1-560F	5'—AAT CCT ACA TGA GCT GAG—3'
	16S-Auc1-midF	5'—AAT CCT ACA TGA GCT GAG—3'
	Nad2-Auc1-705R	5'—CTA CTT GTC TAG ATC TTC C—3'
	Nd2-Auc-189R	5'—CAA CTC AAA TAG CTA GCC—3'
	Nd2-Auc-508R	5'—TAA TAC TAC TTG TCT AGA TC—3'
	Nad1-Auc-37F	5'—TTT ATG CGC TAT ACT AGC—3'
	Nad3-Auc1-startR	5'—AGA AAT AAC CAT TAA CGC C—3'
	Nad1-Auc1-561F	5'—TGT TTC GGT TTT AGC TGA G—3'
	Nad1-Auc1-521R	5'—GAT AAT CAA ACT TGC CG—3'
	Nad2-Auc-599R	5'—AAT TAT TGA CCC TGC TAC—3'
	Nad2-Auc-588F	5'—AAT TTC TAG TGT TAG AGC—3'
	Nad1-Auc-startF	5'—TGG CAG ACT AGT GCG TTG G—3'
	Nad2-A1-5R	5'—AGG TTT TCC ATG TTA ATG C—3'
	Nd1-A1-500F	5'—TGT TTC GGT TTT AGC TGA G—3'
	<i>cox1-cob</i>	
	CO1-Auc1-591F	5'—TTC GTT ACC AGT ACT GGC—3'
	Cytb-Auc1-417R	5'—CCA TAA TAA ATT CCA CGC—3'
	Cob-Auc1-mid2R	5'—ACA TAC ACG CTG AGT AAC—3'
	CO3-Auc1-middR	5'—CTA CTG GCG GTC ATA CAC—3'
	Nad6-Auc1-156R	5'—TTC ATG ACG TTG ATT AGG—3'
	CO1-Auc1-1004F	5'—AAA ATA GTT TAT GAT CCT GC—3'
	Atp8-Auc1-startR1	5'—AAT AGG AGA CAA ATG AGG C—3'
	CO1-Auc-911R	5'—TTG ACA AAA TAA TCC CAG—3'
	Nad6-Auc-startR	5'—AAT CAA AAC CCT ATG TGC—3'
	Nad6-Auc-45F	5'—TAG TTT TAG TAT TAC TTG G—3'
	Atp8-A1-endR	5'—TAA TAG GAG ACA AAT GAG GC—3'
	CO3-A1-startR	5'—TAC TGT CAC ACC AGA AGC—3'
<i>cob-nad5</i>	Cob-Auc1-942F	5'—AGC AGA AGC GTA GAA GAC —3'
	Nd5-Auc1-436R	5'—AAC TAT GAT TAT CCC AGC—3'
	Atp6-Auc1-70F	5'—AGT TTC TGT AAT AGG AGT G—3'
	Cob-Auc1-ennddR	5'—AAA GTA CAC ATA AAG CTC C—3'
	Nad4-Auc-812R	5'—ACA AAC TTC CAT GTT GAG—3'
	Nad5-Auc-middR	5'—GAA GTT ATT ATC CCA GGC—3'
	Nad5-Auc-middF	5'—TTA ACT TAT ATG GCG TAT G—3'
	Nad4-Auc1-672R	5'—TAG ACC CAG CAA CAG GAG C—3'
	tRNAX-Auc-befNd5R	5'—AAC ATC GAA ACC ATG GGC—3'
	Atp6-Auc-220F	5'—TAT GTT TAC TCA GTC ACG—3'
	Atp6-Auc-235R	5'—ACG CTG ATA AAA CAC TTC—3'
	Nad4-A1-312R	5'—CTA AAA TTA TAG ACC CAG C—3'
	Atp6-A1-530F	5'—TAA GAG CAG GGC ATA TTG CG—3'
<i>P. sulfincola</i>		
<i>mLSU-cox1</i>	16S-PS-midF	5'—CTA CCT GCT ACA GTT CTC C —3'
	16S-PS-endF	5'—AAA ATA TGC CCT ACT AGG CTC—3'
	tRNAX-PS-R	5'—TTA TGA AGA CGA CTT TGA GG—3'
	Nad2-PS-235R	5'—GGA AAC CAT TGA TGA CAC GG—3'

<i>cox1-cob</i>	Nad1-PS-endF	5'—TGA ACG GAT AGC TCT GAT GC—3'
	Nad3-PS-211R	5'—TGT GGC TGA GTG CTT AGA CG—3'
	Nad1-PS-308R	5'—GTT GAT ATT GGA TAA TTG GC—3'
	CO1-PS-834F	5'—AGA CAC ACG AGC CTA CTT TAC CGC —3'
	Cob-PS-202R	5'—TGA GAA GGC TAG GTC TAC ATT TGG—3'
	CO1-PS-1500F	5'—CAC CAG CAT TCC ACT CAG GAG CCG—3'
	Nad6-PS-108R	5'—GTG TTA GAG TCG ATA GTC TGC—3'
	CO2-PS-497F	5'—AGC AGA CGT AAT TCA TTC ATG AGC C—3'
	CO3-PS-333R	5'—TTA AGA GTG GTA CTG CAA ATG GGT C—3'
	<i>nad5-mLSU</i> 12S-PS-endR	5'—ATG CAG TGA CAT GGT GGC TTG CTG CGG —3'
	Nad5-PS-1160F	5'—GTG CCG CAG CTG CCT ACT CAA CAC G —3'
	12S-PS-startR	5'—TTC AGT GTA AGT GAG TGG CAT CAC C—3'
	tRNAX-PS-R2	5'—TCT ATT TGG ACA TTT CGT TAA ACC G—3'
	Nad4-PS-200F	5'—ATT CAT CAT TGT AGT CTC TTT CGC—3'
<i>P. gouldi</i> <i>mLSU-cox1</i>	Nad4-PS-531F	5'—CGT TGC CGC ATC TTT ACC GC—3'
	Nad4-PS-1176R	5'—ATA AGA TTA AAG AGT ATG TAC C—3'
	16S-PG-720F	5'—GCC CAG CTA ATT GGC AGA CTA GTG C —3'
	CO1-PG-startR	5'—GAG ATA ACG TGG CAC AAA CCA AAG C—3'
	Nad1-PG-261F	5'—TCT GCG CTT TAA CCT TAG CCC TGC—3'
	Nad2-PG-495R	5'—CCC GTA TTT GGG TTT GAT TTA GC—3'
	Nad1-PG-656F	5'—ATT GAA TAT AGA AGT GGC AGC—3'
	Nad2-PG-2R	5'—TTT ATA GGG GCA GTC ATA GG—3'
	Nad2-PG-321F	5'—GTA CAA TTT CAA TCA ACC AG—3'
	<i>cox1-cox3</i> CO1-PG-900R	5'—CAT GGT TGC GGC AGT AAA ATA GGC —3'
	CO3-PG-204R	5'—CCT AAA AAA GTT CCC TCA CG —3'
	CO2-PG-591R	5'—GCA TTG ACC ATA GAA TAC TCC G—3'
	CO2-PG-startR	5'—TCT TGG AAG GAT AGT TGG GCT C—3'
	CO1-PG-1257F	5'—TTC TTC CCT CAA CAC TTC CTC GG—3'
<i>cox3-cob</i>	CO1-PG-1134R	5'—AGA GGA AAC CAG TGG TTA AAT GC—3'
	Cob-PG-midF	5'—TTG CTT CGA AAC CTT CAT GCT AAC GGA GC —3'
	Nad6-PG-273F	5'—CTA ATT GCA CTC CTT TTG TCA GGC—3'
	CO3-PG-L627F	5'—GAT TCC ACG GGC TTC ATG TTC TAA TTG GC —3'
<i>nad5-nad4</i>	Nad4-PG-186F	5'—TCT TAA CCT TAT GAA TTT CCA GCC—3'
	Nad4-PG-516R	5'—TTT ATG AGG ATC GAG TAA CTT GC—3'
	Nad4L-PG-75F	5'—ATG GCG ACC CTG ATT ATT CAA CG—3'
	Nd5-PG-1005R	5'—GTT TGC AAT TAA AAG GCA GGA C—3'
	Nd5-PG-1065F	5'—GCC GGG TTC TAC TCT AAA GAC C—3'
	Nd5-PG-1587F	5'—CAA GGT TGA AAC GAA ATA GCC GG—3'
	Nad4-PG-453R	5'—CTA TAA GGA GTG GTA GTG AAG C—3'
<i>nad4-mLSU</i>	Nad4-PG-846F	5'—CTT ATT GCT TAT TCC TCA GTT GGT C —3'
	Nad4-PG-1026F	5'—GGG TTA CTC ATT AAT ACT TTC CAG—3'
	12S-PG-startR	5'—AAT TTT TCA ATA GTT TAC TCA TGG AG —3'
	tRNAX-PG-F	5'—CAT GAC CCA AAA GTG GAA ACA ATC C—3'
	12S-PG-111F	5'—TGT CCT CTA ACT CGA TAA TCC ACG—3'
	12S-PG-midR	5'—ATT CCT GAT GCT ATG CTT TGT GGC—3'
	Nad4-PG-519R	5'—TGG CTG GAA AGT ATA TGA GTA ACC—3'

SUPPLEMENTARY TABLE 3: RSCU analyses for the three Terebelliformia taxa for which we obtained new mitochondrial data.

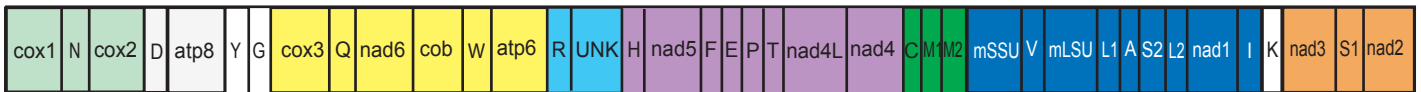
AA	Codon	<i>A. crinita</i>		<i>P. gouldi</i>		<i>P. sulfincola</i>		AA	Codon	<i>A. crinita</i>		<i>P. gouldi</i>		<i>P. sulfincola</i>	
Ala	GCA	63	1.223	63	0.966	141	2.029	Lys	AAA	45	1.286	61	1.584	62	1.797
	GCC	2	0.039	94	1.441	82	1.180		AAG	25	0.714	16	0.416	7	0.203
	GCG	41	0.796	15	0.230	6	0.086	Met	AUA	165	1.480	147	1.531	105	1.160
	GCU	100	1.942	89	1.364	49	0.705		AUG	58	0.520	45	0.469	76	0.840
Arg	CGA	21	1.200	36	2.361	29	2.035	Phe	UUC	13	0.089	89	0.695	126	1.135
	CGC	1	0.057	5	0.328	12	0.842		UUU	280	1.911	167	1.305	96	0.865
	CGG	15	0.857	6	0.393	2	0.140	Pro	CCA	44	1.313	49	1.153	104	2.337
	CGU	33	1.886	14	0.918	14	0.982		CCC	2	0.060	55	1.294	19	0.427
Asn	AAC	11	0.227	60	1.091	83	1.495		CCG	8	0.239	15	0.353	20	0.449
	AAU	86	1.773	50	0.909	28	0.505		CCU	80	2.388	51	1.200	35	0.787
Asp	GAC	14	0.424	31	1.016	31	1.000	Ser	AGA	88	2.071	38	0.897	39	1.091
	GAU	52	1.576	30	0.984	31	1.000		AGC	5	0.118	13	0.307	17	0.476
Cys	UGC	11	0.415	15	1.000	21	1.135	Trp	AGG	38	0.894	7	0.165	8	0.224
	UGU	42	1.585	15	1.000	16	0.865		AGU	38	0.894	18	0.425	12	0.336
Gln	CAA	33	1.294	56	1.723	61	1.694		UCA	62	1.459	68	1.605	90	2.517
	CAG	18	0.706	9	0.277	11	0.306		UCC	6	0.141	68	1.605	41	1.147
Glu	GAA	39	1.000	50	1.493	45	1.429	Ter(.)	UCG	16	0.376	10	0.236	14	0.392
	GAG	39	1.000	17	0.507	18	0.571		UCU	87	2.047	117	2.761	65	1.818
Gly	GGA	52	0.908	54	1.207	78	1.880		UAA/U	12	2.000	9	1.800	9	1.636
	GGC	12	0.210	41	0.916	38	0.916		UAG	0	0.000	1	0.200	2	0.364
	GGG	121	2.114	43	0.961	19	0.458	Thr	ACA	63	1.839	59	1.198	150	2.317
	GGU	44	0.769	41	0.916	31	0.747		ACC	4	0.117	65	1.320	58	0.896
His	CAC	7	0.203	51	1.133	67	1.489		ACG	8	0.234	4	0.081	12	0.185
	CAU	62	1.797	39	0.867	23	0.511		ACU	62	1.810	69	1.401	39	0.602
Ile	AUC	21	0.166	73	0.638	105	0.847	Trp	UGA	53	1.082	66	1.451	68	1.563
	AUU	232	1.834	156	1.362	143	1.153		UGG	45	0.918	25	0.549	19	0.437
Leu	CUA	32	0.396	111	1.271	194	2.456	Tyr	UAC	15	0.219	45	0.891	59	1.311
	CUC	2	0.025	71	0.813	58	0.734		UAU	122	1.781	56	1.109	31	0.689
	CUG	10	0.124	24	0.275	54	0.684	Val	GUA	117	1.505	63	1.527	91	1.906
	CUU	57	0.705	153	1.752	88	1.114		GUC	7	0.090	36	0.873	28	0.586
	UUA	321	3.971	137	1.569	67	0.848		GUG	48	0.617	17	0.412	15	0.314
	UUG	63	0.779	28	0.321	13	0.165		GUU	139	1.788	49	1.188	57	1.194
2fold	NNA	57.8	1.357	64.8	1.597	58.3	1.547	4fold	NNA	60.0	1.332	54.0	1.402	98.8	2.084
	NNG	30.8	0.643	18.8	0.403	22.2	0.453		NNC	4.7	0.095	49.3	1.029	39.5	0.808
	NNC	13.1	0.249	52.0	0.924	70.3	1.202		NNG	40.2	0.809	16.7	0.405	12.3	0.272
	NNU	125.1	1.751	73.3	1.076	52.6	0.798		NNU	76.3	1.764	52.2	1.165	37.5	0.836

SUPPLEMENTARY TABLE 4: RSCU analyses for the other taxa in our analyses.

AA	Codon	<i>Neptlys</i> sp.	<i>G. brachiosum</i>	<i>H. robusta</i>	<i>C. torquata</i>	<i>S. cf. armiger</i>	<i>O. latreillii</i>	<i>U. caupo</i>	<i>L. terrestris</i>	<i>R. pachypilla</i>	<i>Phascolopsis gouldi</i>	<i>P. dumerilii</i>											
Ala	GCA	69	0.914	36	1.674	58	1.771	100	1.581	90	1.545	78	1.139	121	1.698	81	1.178	68	1.439	48	1.371	90	1.343
	GCC	101	1.338	7	0.326	31	0.947	58	0.917	71	1.219	112	1.635	75	1.053	80	1.164	51	1.079	32	0.914	68	1.015
	GCG	17	0.225	0	0.000	5	0.153	6	0.095	3	0.052	6	0.088	14	0.196	15	0.218	1	0.021	5	0.143	30	0.448
	GCU	115	1.523	43	2.000	37	1.130	89	1.407	69	1.185	78	1.139	75	1.053	99	1.440	69	1.460	55	1.571	80	1.194
Arg	CGA	22	1.397	14	1.436	29	2.900	41	2.645	40	2.712	33	2.063	46	2.788	32	2.032	38	2.868	22	2.095	25	1.724
	CGC	17	1.079	3	0.308	5	0.500	4	0.258	8	0.542	8	0.500	5	0.303	7	0.444	5	0.377	5	0.476	13	0.897
	CGG	6	0.381	1	0.103	0	0.000	4	0.258	2	0.136	4	0.250	9	0.545	9	0.571	0	0.000	0	0.000	10	0.690
	CGU	18	1.143	21	2.154	6	0.600	13	0.839	9	0.610	19	1.188	6	0.364	15	0.952	10	0.755	15	1.429	10	0.690
Asn	AAC	65	1.008	12	0.238	33	0.667	70	0.909	61	0.961	59	0.937	65	1.040	66	0.971	35	0.654	27	0.720	66	0.957
	AAU	64	0.992	89	1.762	66	1.333	84	1.091	66	1.039	67	1.063	60	0.960	70	1.029	72	1.346	48	1.280	72	1.043
Asp	GAC	43	1.246	5	0.263	28	1.167	32	0.985	35	1.094	38	1.152	37	1.254	34	0.919	25	0.943	18	0.857	37	1.121
	GAU	26	0.754	33	1.737	20	0.833	33	1.015	29	0.906	28	0.848	22	0.746	40	1.081	28	1.057	24	1.143	29	0.879
Cys	UGC	9	0.600	4	0.364	6	0.600	16	1.000	22	1.128	24	1.067	11	0.759	21	1.077	13	0.839	7	0.778	13	0.619
	UGU	21	1.400	18	1.636	14	1.400	16	1.000	17	0.872	21	0.933	18	1.241	18	0.923	18	1.161	11	1.222	29	1.381
Gln	CAA	54	1.543	37	2.000	42	1.953	74	1.873	74	1.873	75	1.829	84	1.750	84	1.719	55	1.897	40	1.905	58	1.589
	CAG	16	0.457	0	0.000	1	0.047	5	0.127	5	0.127	7	0.171	12	0.250	9	0.281	3	0.103	2	0.095	15	0.411
Glu	GAA	55	1.507	48	2.000	39	1.592	60	1.600	57	1.932	64	1.882	73	1.759	41	1.155	58	1.871	31	1.771	60	1.481
	GAG	18	0.493	0	0.000	10	0.408	15	0.400	2	0.068	4	0.118	10	0.241	30	0.845	4	0.129	4	0.229	21	0.519
Gly	GGA	74	1.495	69	2.464	47	1.679	69	1.401	96	2.313	82	1.763	126	2.483	51	1.103	101	2.525	47	1.694	77	1.548
	GGC	66	1.333	3	0.107	12	0.429	39	0.792	25	0.602	62	1.333	27	0.532	34	0.735	19	0.475	24	0.865	48	0.965
	GGG	36	0.727	1	0.036	23	0.821	55	1.117	28	0.675	12	0.258	36	0.709	62	1.341	10	0.250	15	0.541	48	0.965
	GGU	22	0.444	39	1.393	30	1.071	34	0.690	17	0.410	30	0.645	14	0.276	38	0.822	30	0.750	25	0.901	26	0.523
His	CAC	48	0.950	5	0.175	24	0.842	30	0.714	40	0.930	60	1.111	55	1.250	59	1.242	22	0.620	32	0.914	50	1.111
	CAU	53	1.050	52	1.825	33	1.158	54	1.286	46	1.070	48	0.889	33	0.750	36	0.758	49	1.380	38	1.086	40	0.889
Ile	AUC	58	0.404	33	0.254	64	0.601	91	0.543	85	0.526	123	0.769	100	0.667	105	0.702	59	0.463	44	0.611	83	0.502
	AUU	229	1.596	227	1.746	149	1.399	244	1.457	238	1.474	197	1.231	200	1.333	194	1.298	196	1.537	100	1.389	248	1.498
Leu	CUA	81	0.822	39	0.711	99	1.880	151	1.601	178	1.884	223	2.172	223	2.295	177	1.907	127	1.558	56	1.249	136	1.603
	CUC	90	0.914	6	0.109	22	0.418	54	0.572	88	0.931	117	1.140	62	0.638	74	0.797	45	0.552	40	0.892	31	0.365
	CUG	17	0.173	1	0.018	7	0.133	14	0.148	8	0.085	15	0.146	38	0.391	32	0.345	5	0.061	2	0.045	19	0.224
	CUU	201	2.041	77	1.404	48	0.911	109	1.155	146	1.545	161	1.568	105	1.081	95	1.023	116	1.423	80	1.784	100	1.179
UUA	UUA	188	1.909	203	3.702	128	2.430	226	2.396	141	1.492	98	0.955	145	1.492	150	1.616	191	2.344	91	2.030	191	2.251
	UUG	14	0.142	3	0.055	12	0.228	12	0.127	6	0.063	2	0.019	10	0.103	29	0.312	5	0.061	0	0.000	32	0.377
2fold	NNA	73.167	1.587	58.000	1.917	54.000	1.740	90.333	1.768	83.167	1.832	84.500	1.821	81.500	1.614	75.000	1.571	67.667	1.822	38.833	1.890	84.333	1.649
	NNG	18.833	0.413	2.500	0.083	9.500	0.260	12.167	0.232	5.667	0.168	9.333	0.179	18.833	0.386	24.833	0.429	6.500	0.178	2.500	0.110	21.667	0.351
	NNC	49.714	0.800	14.286	0.249	35.000	0.760	51.000	0.766	56.143	0.920	72.143	1.045	63.857	1.003	106.714	1.053	38.571	0.690	28.286	0.750	53.714	0.826
	NNU	95.429	1.200	102.286	1.751	64.857	1.240	104.571	1.234	83.143	1.080	74.143	0.955	76.429	0.997	82.286	0.947	81.571	1.310	51.714	1.250	92.286	1.174

AA	Codon	<i>Nephys</i> sp.	<i>G. brachiosum</i>	<i>H. robusta</i>	<i>C. torquata</i>	<i>S. cf. armiger</i>	<i>O. latreillii</i>	<i>U. caupo</i>	<i>L. terrestris</i>	<i>R. pachyptila</i>	<i>Phascolopsis gouldi</i>	<i>P. dumerilii</i>											
Lys	AAA	64	1.662	56	1.898	48	1.714	82	1.907	90	1.957	84	1.931	77	1.692	70	1.522	62	1.908	32	1.882	66	1.692
	AAG	13	0.338	3	0.102	8	0.286	4	0.093	2	0.043	3	0.069	14	0.308	22	0.478	3	0.092	2	0.118	12	0.308
Met	AUA	156	1.529	137	1.851	129	1.613	233	1.792	178	1.816	174	1.649	170	1.532	194	1.504	138	1.683	66	1.970	233	1.596
	AUG	48	0.471	11	0.149	31	0.388	27	0.208	18	0.184	37	0.351	52	0.468	64	0.496	26	0.317	1	0.030	59	0.404
Phe	UUC	78	0.538	33	0.262	50	0.595	74	0.503	103	0.880	134	1.085	115	0.833	400	1.429	93	0.791	46	0.630	82	0.678
	UUU	212	1.462	219	1.738	118	1.405	220	1.497	131	1.120	113	0.915	161	1.167	160	0.571	142	1.209	100	1.370	160	1.322
Pro	CCA	20	0.455	37	1.437	75	2.970	80	1.730	66	1.412	74	1.430	80	1.584	53	1.184	46	1.150	40	1.553	64	1.497
	CCC	57	1.295	5	0.194	4	0.158	18	0.389	43	0.920	51	0.986	44	0.871	55	1.229	34	0.850	20	0.777	52	1.216
	CCG	3	0.068	1	0.039	2	0.079	13	0.281	6	0.128	5	0.097	6	0.119	16	0.358	1	0.025	2	0.078	8	0.187
	CCU	96	2.182	60	2.330	20	0.792	74	1.600	72	1.540	77	1.488	72	1.426	55	1.229	79	1.975	41	1.592	47	1.099
Ser	AGA	39	0.886	39	1.357	25	1.047	65	1.600	46	1.067	44	1.020	62	1.455	63	1.381	53	1.477	23	1.278	57	1.378
	AGC	24	0.545	1	0.035	5	0.209	12	0.295	3	0.070	14	0.325	15	0.352	14	0.307	6	0.167	5	0.278	26	0.628
	AGG	8	0.182	3	0.104	5	0.209	13	0.320	4	0.093	2	0.046	7	0.164	13	0.285	3	0.084	0	0.000	19	0.459
	AGU	15	0.341	6	0.209	11	0.461	16	0.394	9	0.209	17	0.394	7	0.164	20	0.438	6	0.167	4	0.222	25	0.604
	UCA	59	1.341	57	1.983	83	3.476	107	2.634	130	3.014	91	2.110	74	1.736	94	2.060	83	2.314	23	1.278	78	1.885
	UCC	67	1.523	8	0.278	19	0.796	38	0.935	68	1.577	74	1.716	65	1.525	74	1.622	51	1.422	30	1.667	42	1.015
	UCG	9	0.205	1	0.035	5	0.209	3	0.074	5	0.116	8	0.186	3	0.070	8	0.175	3	0.084	0	0.000	18	0.435
	UCU	131	2.977	115	4.000	38	1.592	71	1.748	80	1.855	95	2.203	108	2.534	79	1.732	82	2.286	59	3.278	66	1.595
Ter(.)	UAA/U	10	1.538	7	1.750	7	1.750	12	1.846	9	1.500	11	1.692	9	1.385	13	2.000	8	1.600	7	2.000	13	2.000
	UAG	3	0.462	1	0.250	1	0.250	1	0.154	3	0.500	2	0.308	4	0.615	0	0.000	2	0.400	0	0.000	0	0.000
Thr	ACA	79	1.179	34	1.432	77	2.216	139	2.180	102	1.729	87	1.338	93	1.404	90	1.434	64	1.399	42	1.631	98	1.468
	ACC	94	1.403	6	0.253	25	0.719	44	0.690	67	1.136	90	1.385	91	1.374	70	1.116	42	0.918	23	0.893	75	1.124
	ACG	7	0.104	0	0.000	0	0.000	5	0.078	4	0.068	5	0.077	5	0.075	11	0.175	2	0.044	1	0.039	23	0.345
	ACU	88	1.313	55	2.316	37	1.065	67	1.051	63	1.068	78	1.200	76	1.147	80	1.275	75	1.639	37	1.437	71	1.064
Trp	UGA	100	1.739	63	2.000	59	1.815	81	1.588	91	1.916	99	1.941	76	1.567	77	1.525	85	1.977	57	1.810	76	1.535
	UGG	15	0.261	0	0.000	6	0.185	21	0.412	4	0.084	3	0.059	21	0.433	24	0.475	1	0.023	6	0.190	23	0.465
Tyr	UAC	47	0.855	8	0.186	40	0.851	44	0.704	47	0.922	67	1.196	64	1.219	62	1.033	23	0.517	24	0.738	45	0.796
	UAU	63	1.145	78	1.814	54	1.149	81	1.296	55	1.078	45	0.804	41	0.781	58	0.967	66	1.483	41	1.262	68	1.204
Val	GUA	52	1.112	39	2.229	81	2.551	86	2.060	68	2.211	80	2.013	99	2.041	27	0.688	76	2.321	42	1.732	99	1.895
	GUC	28	0.599	3	0.171	11	0.346	23	0.551	22	0.715	31	0.780	28	0.577	44	1.121	19	0.580	20	0.825	18	0.344
	GUG	10	0.214	1	0.057	6	0.189	10	0.240	4	0.130	5	0.126	18	0.371	38	0.968	2	0.061	4	0.165	23	0.440
	GUU	97	2.075	27	1.543	29	0.913	48	1.150	29	0.943	43	1.082	49	1.010	48	1.223	34	1.038	31	1.278	69	1.321
4fold	NNA	52.667	1.092	38.167	1.779	61.167	2.348	85.833	1.933	77.000	1.987	72.333	1.624	94.167	2.000	55.667	1.270	65.500	1.950	40.167	1.679	75.500	1.579
	NNC	60.500	1.175	4.500	0.226	14.667	0.517	31.000	0.600	39.333	0.856	59.000	1.103	45.000	0.785	48.333	0.968	28.333	0.713	20.667	0.792	45.667	0.927
	NNG	13.167	0.287	0.667	0.039	6.000	0.207	15.500	0.345	7.833	0.198	6.167	0.149	14.667	0.336	25.167	0.605	2.667	0.067	4.500	0.161	23.667	0.512
	NNU	72.667	1.447	40.833	1.956	26.500	0.929	54.167	1.123	43.167	0.959	54.167	1.124	48.667	0.879	55.833	1.157	49.500	1.270	34.000	1.368	50.500	0.982

Terebellides stroemi (Trichobranchidae)



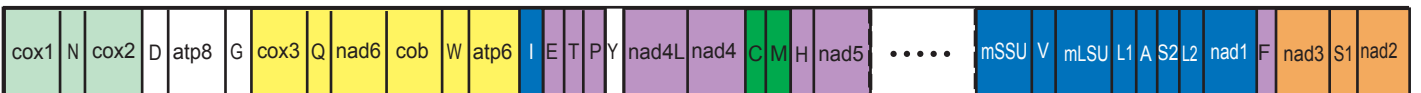
Pista cristata (Terebellidae)



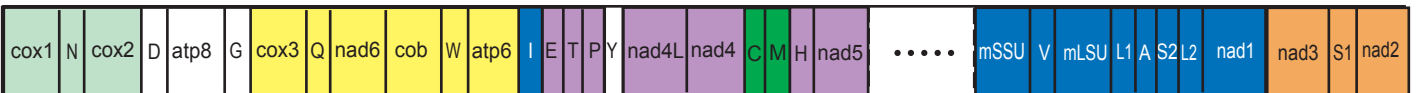
Pectinaria gouldi (Pectinariidae)



Eclysiptpe vanelli (Ampharetidae)



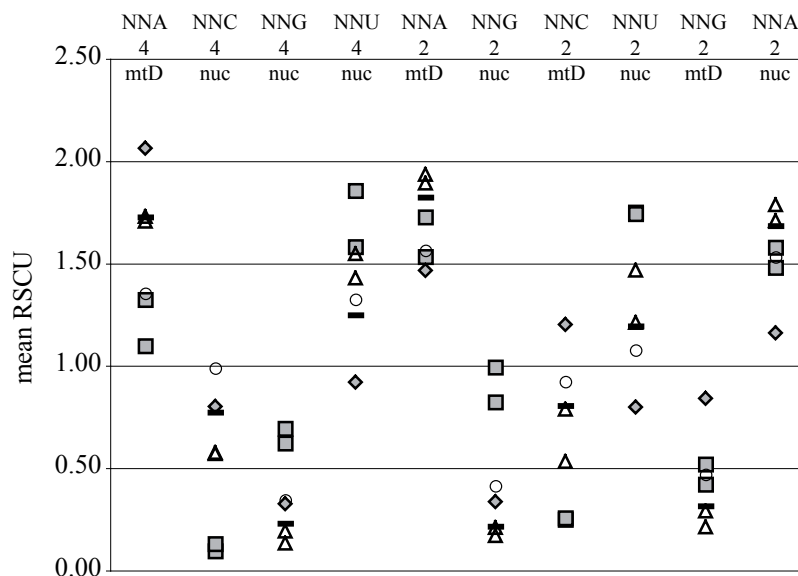
Auchenoplax crinita (Ampharetidae)



Paralvinella sulfincola (Alvinellidae)



SUPPLEMENTARY FIGURE 1. Mitochondrial gene order of six Terebelliformia worms. Different colors shows conserved gene clusters. Dots indicate missing regions.



SUPPLEMENTARY FIGURE 2. Analyses of the relative synonymous codon usage in the mitochondrial protein-coding genes for Ampharetidae (grey squares), Alvinellidae (grey diamonds), Pectinariidae (open circles), Trichobranchidae and Terebellidae (both open triangles) as well as the mean values of outgroup taxa (black bar). NNA, NNC, NNG, and NNU indicate the 3rd position of the codon, 2 and 4 if the codon was 2-fold or 4-fold degenerated and mtD and nuc if the corresponding tRNA is located in the mitochondrial or nuclear genome, respectively.