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Supplementary Tables S1–S3; Figures S1–S6

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Research Article **OPEN ACCESS**

Huang Y., Feng J.-M., Liu W., Sun B.L., Shu X.-J., Li W.-X., Wang G.T., Hua C.-J. 2024: Comprehensive molecular characterisation of the complete mitogenome of *Ergasilus tumidus* and phylogenetic relationships of Copepoda inferred from mitogenomes. Folia Parasitol. 71: 002.

Table S1. Primers used to amplify and sequence the mitochondrial genome of *Ergasilus tumidus* Markevich, 1940.

Fragment No.	Gene or region	Primer name	Sequence (5'-3')	Length (bp)
F1	*COX2*	PDF1	TCCCCTGTGATAGAAGAGTT	575
		PDR1	CCGCAAATCTCGGAGCATTGGC	
F2	*COX2-16S*	PDF2	CACTAGTTAGAAGCTCCGATG	6,508
		PDR2	CCTTGAGGGGTCCAAGAGTAG	
F3	*16S*	PDF3	TTAGACGAGAAGACCCTA	177
		16SR	CTTAATTCAACATCGAGGTC	
F4	*16S-ND4*	PDF4	CTACTCTTGGACCCCTCAAGG	1,766
		PDR4	GAGTACTTCGGTTCTCTCCAC	
F5	*ND4*	PDF5	CGCTACCGAAGAGTACGCTA	234
		PDR5	CACTTATGGTTGCCTAAAGC	
F6	*ND4-CYTB*	PDF6	CTCTTCTAATGACCCCTCCTC	1,108
		PDR6	GGCTCTATCTGTAATAGTCC	
F7	*CYTB*	PDF7	TCTACAGGTCGGGCGCCGATTC	865
		PDR7	CCTGTGATGTTGAGTTGGCG	
F8	*CYTB-COX2*	PDF8	GTTACACCCACTATTCAAACCTC	4,345
		PDR8	GCTTGCCCTTCAAGTATTGAC	

Table S2. Composition and skewness of 34 species of mitogenomes in the present phylogenetice analysis.

Order	Species	A%	T%	G%	C%	A+T%	AT-Skew	GC-Skew
Calanoida	*Calanus sinicus*	32.1	29.6	19.3	19.1	61.7	0.041	0.006
	Calanus glacialis	31.7	28.8	19.9	19.6	60.5	0.048	0.008
	Calanus finmarchicus	28.8	29.7	18.8	22.7	58.5	-0.015	-0.095
	Calanus hyperboreus	31.1	35.1	19.5	14.2	66.2	-0.06	0.159
	Calanus simillimus	35.3	34.3	15.0	15.4	69.6	0.014	-0.012
	Lovenula raynerae	33.5	34.6	19.3	12.5	68.1	-0.016	0.214
	Phyllodiaptomus tunguidus	34.6	34.8	18.2	12.3	69.4	-0.003	0.194
	Labidocera rotunda	31.6	36.6	16.9	14.8	68.2	-0.073	0.068
	Eurytemora affinis	28.4	37.2	19.8	14.7	65.6	-0.135	0.150
Cyclopoida	*Paracyclopina nana*	37.5	33.3	13.9	15.2	70.8	0.060	-0.045
	Ergasilus tumidus	33.1	32.6	17.4	16.9	65.7	0.006	0.017
	Lamproglena orientalis	35.6	36.9	11.6	15.9	72.5	-0.018	-0.158
	Lamproglena chinensis	31.6	30.7	14.4	23.2	62.3	0.014	-0.235
	Lernaea cyprinacea	34.9	35.9	12.4	16.9	70.8	-0.015	-0.154
	Sinergasilus polycolpus	35.9	35.5	13.8	14.9	71.4	0.005	-0.040
	Sinergasilus undulatus	34.9	35.5	13.9	15.7	70.4	-0.007	-0.058
Harpacticoida	*Tigriopus japonicus*	25	35.3	25.1	14.5	60.3	-0.170	0.268
	Tigriopus kingsejongensis	26.7	27.0	19.4	26.8	53.7	-0.006	-0.160
	Tigriopus californicus	26.2	36.0	24.0	13.8	62.2	-0.158	0.270
	Schizopera knabeni	31.9	26.3	22.7	19.0	58.2	0.097	0.089
	Amphiascoides atopus	34.2	29.9	20.5	15.3	64.1	0.066	0.145
Siphonostomatoida	*Caligus rogercresseyi*	31.4	35.2	19.8	13.7	66.6	-0.057	0.181
	Caligus clemensi	36.3	39.2	14.6	9.8	75.5	-0.038	0.194
	Lepeophtheirus salmonis	32.8	33.1	20.6	13.5	65.9	-0.004	0.208
	Pennella sp.	30.9	37.4	19.6	12.1	68.3	-0.096	0.235
Outgroup	*Vulcanolepas fijiensis*	38.3	31.2	9.6	20.8	69.5	0.102	-0.366
	Metaplax longipes	37.6	33.8	10.6	17.9	71.4	0.053	-0.257
	Scyllarides squammosus	31.2	34.3	13.2	21.2	65.5	-0.047	-0.233
	Austinogebia edulis	37.1	36.5	10.1	16.3	73.6	0.008	-0.238
	Typhlatya galapagensis	37.5	31.6	10.6	20.3	69.1	0.085	-0.315
	Munidopsis verrilli	34.5	29.5	11.5	24.5	64.0	0.077	-0.363
	Longpotamon parvum	36.3	37.6	8.1	18.0	73.9	-0.018	-0.379
	Daphnia pulex	31.5	30.8	16.7	21.1	62.3	0.011	-0.116
	Artemia tibetiana	31.1	31.7	17.2	20.1	62.8	-0.010	-0.078

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Table S3. Relative synonymous codon usage (RSCU) in mitogenomes of 18 species of Copepoda.

Order	Species	Phe		Leu (TAA)		Leu (TAG)				Ile		Met		Val				Ser (TGA)		
		UUU	UUC	UUA	UUG	CUU	CUC	CUA	CUG	AUU	AUC	AUA	AUG	GUU	GUC	GUA	GUG	UCU	UCC	UCA
Calanoida	*Calanus simillimus*	1.52	0.48	2.58	0.42	1.06	0.27	1.29	0.38	1.56	0.44	1.54	0.46	1.10	0.39	1.87	0.64	1.72	0.70	1.25
	Phyllodiaptomus tunguidus	1.77	0.23	3.20	0.77	0.88	0.37	0.55	0.23	1.75	0.25	1.49	0.51	1.53	0.45	1.15	0.87	2.78	0.74	0.90
	Labidocera rotunda	1.34	0.66	3.24	0.50	0.87	0.2	0.93	0.25	1.57	0.43	1.64	0.36	1.42	0.33	1.65	0.60	1.40	0.38	1.76
	Calanus hyperboreus	1.48	0.52	2.24	0.71	0.61	0.51	1.28	0.64	1.38	0.62	1.37	0.63	1.52	0.44	1.12	0.92	1.61	0.88	1.69
	Eurytemora affinis	1.71	0.29	3.06	0.63	1.17	0.35	0.52	0.27	1.82	0.18	1.51	0.49	1.78	0.32	1.15	0.74	1.58	0.26	1.21
Cyclopoida	*Paracyclopina nana*	1.63	0.37	2.61	0.79	1.16	0.17	1.00	0.28	1.70	0.30	1.62	0.38	1.48	0.36	1.76	0.39	2.28	0.39	1.37
	Ergasilus tumidus	1.54	0.46	2.20	0.67	1.00	0.40	1.32	0.42	1.50	0.50	1.52	0.48	1.37	0.52	1.30	0.81	1.72	0.80	1.11
	Lamproglena orientalis	1.62	0.38	3.40	0.37	0.91	0.23	0.95	0.14	1.67	0.33	1.83	0.17	1.26	0.33	1.94	0.46	2.32	0.40	1.00
	Lamproglena chinensis	1.34	0.66	2.14	0.77	0.98	0.44	1.35	0.32	1.26	0.74	1.57	0.43	1.13	0.52	1.60	0.75	1.38	1.40	0.97
	Lernaea cyprinacea	1.69	0.31	3.51	0.56	0.78	0.26	0.77	0.13	1.69	0.31	1.81	0.19	1.68	0.19	1.50	0.62	1.86	0.62	1.12
	Sinergasilus polycolpus	1.66	0.34	3.70	0.47	0.67	0.14	0.86	0.15	1.62	0.38	1.72	0.28	1.08	0.29	2.00	0.64	2.36	0.49	1.06
	Sinergasilus undulatus	1.66	0.34	3.60	0.80	0.64	0.16	0.66	0.14	1.61	0.39	1.66	0.34	1.42	0.33	1.53	0.71	2.37	0.36	1.20
Harpacticoida	*Tigriopus japonicus*	1.53	0.47	1.76	1.72	0.97	0.31	0.52	0.72	1.66	0.34	1.03	0.97	1.50	0.40	0.93	1.17	1.38	0.39	0.43
	Tigriopus kingsejongensis	1.41	0.59	0.93	1.33	1.12	0.70	0.91	1.02	1.26	0.74	0.77	1.23	0.87	0.80	0.76	1.57	1.76	1.13	0.63
	Tigriopus californicus	1.60	0.40	2.28	1.33	1.10	0.21	0.69	0.40	1.62	0.38	1.49	0.51	1.42	0.35	1.00	1.23	1.33	0.68	0.55
	Amphiascoides atopus	1.66	0.34	2.74	0.60	0.93	0.57	0.84	0.32	1.52	0.48	1.27	0.73	1.24	0.70	0.96	1.10	2.59	0.79	0.91
Siphonostomatoida	*Lepeophtheirus salmonis*	1.51	0.49	2.87	0.73	0.97	0.28	0.94	0.21	1.57	0.43	1.59	0.41	1.17	0.35	1.72	0.76	1.66	0.79	0.68
	Pennella sp.	1.56	0.44	3.05	1.06	0.77	0.28	0.65	0.19	1.61	0.39	1.55	0.45	1.29	0.20	1.76	0.74	2.06	0.44	1.07

Order	Species		Pro				Thr				Ala				Tyr		His		Gln	
		UCG	CCU	CCC	CCA	CCG	ACU	ACC	ACA	ACG	GCU	GCC	GCA	GCG	UAU	UAC	CAU	CAC	CAA	CAG
Calanoida	*Calanus simillimus*	0.49	2.22	0.70	0.83	0.25	1.96	0.78	0.97	0.29	1.63	0.96	1.07	0.34	1.35	0.65	1.07	0.93	1.32	0.68
	Phyllodiaptomus tunguidus	0.31	2.28	0.64	0.64	0.44	1.88	0.72	1.07	0.33	1.59	0.72	1.08	0.62	1.44	0.56	1.24	0.76	1.51	0.49
	Labidocera rotunda	0.23	2.17	0.54	1.02	0.27	1.83	0.51	1.29	0.38	1.93	0.58	1.10	0.39	1.35	0.65	1.18	0.82	1.31	0.69
	Calanus hyperboreus	0.44	0.96	0.96	1.54	0.54	1.58	0.84	1.18	0.39	1.25	1.28	1.08	0.39	1.32	0.68	1.09	0.91	1.00	1.00
	Eurytemora affinis	0.26	2.16	0.67	0.79	0.38	1.96	0.66	1.24	0.14	1.91	0.64	0.92	0.53	1.39	0.61	1.28	0.72	1.29	0.71
Cyclopoida	*Paracyclopina nana*	0.14	1.56	0.85	1.07	0.52	1.72	0.47	1.51	0.31	1.66	0.62	1.39	0.33	1.41	0.59	1.28	0.72	1.31	0.69
	Ergasilus tumidus	0.37	2.26	0.51	0.84	0.39	1.83	1.04	0.95	0.18	1.75	0.73	0.79	0.73	1.10	0.90	1.26	0.74	1.33	0.67
	Lamproglena orientalis	0.06	2.22	0.37	1.26	0.15	1.78	0.62	1.38	0.22	1.84	0.92	1.09	0.15	1.34	0.66	1.36	0.64	1.61	0.39
	Lamproglena chinensis	0.39	1.55	1.10	0.75	0.60	1.42	1.12	1.26	0.20	1.05	1.73	0.66	0.56	1.03	0.97	0.86	1.14	1.48	0.52
	Lernaea cyprinacea	0.16	1.90	0.70	1.17	0.22	1.57	0.84	1.40	0.19	1.88	0.80	0.94	0.38	1.47	0.53	1.24	0.76	1.84	0.16
	Sinergasilus polycolpus	0.11	2.13	0.51	1.08	0.29	2.21	0.39	1.27	0.13	2.22	0.59	0.84	0.35	1.32	0.68	1.59	0.41	1.20	0.80
	Sinergasilus undulatus	0.08	2.35	0.44	0.95	0.25	2.21	0.46	1.15	0.18	1.86	0.80	0.84	0.50	1.19	0.81	1.37	0.63	1.78	0.22
Harpacticoida	*Tigriopus japonicus*	0.47	1.65	0.77	0.81	0.77	1.59	0.85	1.04	0.52	1.99	0.49	0.87	0.65	1.30	0.70	1.38	0.62	0.92	1.08
	Tigriopus kingsejongensis	0.52	1.13	1.55	0.59	0.73	1.29	1.11	0.95	0.65	1.08	1.29	0.77	0.85	0.80	1.20	0.89	1.11	0.68	1.32
	Tigriopus californicus	0.36	1.90	0.73	0.89	0.48	1.75	0.50	1.07	0.68	1.53	0.62	1.15	0.69	1.33	0.67	1.29	0.71	1.16	0.84
	Amphiascoides atopus	0.38	1.78	1.19	0.68	0.35	1.55	0.96	0.89	0.59	1.85	0.97	0.82	0.36	1.18	0.82	1.21	0.79	1.79	0.21
Siphonostomatoida	*Lepeophtheirus salmonis*	0.23	2.18	1.11	0.46	0.25	2.11	0.69	0.96	0.24	1.89	0.70	0.87	0.54	1.28	0.72	1.40	0.60	1.10	0.90
	Pennella sp.	0.23	2.20	0.53	0.83	0.43	2.19	0.68	0.95	0.19	1.93	0.60	1.05	0.43	1.30	0.70	1.36	0.64	1.15	0.85

Table S3. continuation...

Order	Species	Asn		Lys		Asp		Glu			Cys		Trp		Arg				Ser (TCT)	
		AAU	AAC	AAA	AAG	GAU	GAC	GAA	GAG	UGU	UGC	UGA	UGG	CGU	CGC	CGA	CGG	AGA	AGG	AGU
	Calanus simillimus	1.43	0.57	1.44	0.56	1.28	0.72	1.11	0.89	1.48	0.52	1.33	0.67	1.00	0.62	1.77	0.62	1.53	0.74	1.13
	Phyllodiaptomus tunguidus	1.54	0.46	1.75	0.25	1.31	0.69	1.15	0.85	1.21	0.79	1.43	0.57	0.88	0.64	1.52	0.96	1.50	0.86	0.49
Calanoida	*Labidocera rotunda*	1.35	0.65	1.52	0.48	1.37	0.63	1.28	0.72	1.53	0.47	1.65	0.35	1.33	0.07	2.18	0.42	2.79	0.67	0.59
	Calanus hyperboreus	1.43	0.57	1.03	0.97	1.29	0.71	0.72	1.28	0.97	1.03	1.11	0.89	0.94	0.47	1.10	1.49	1.38	1.02	0.69
	Eurytemora affinis	1.44	0.56	1.49	0.51	1.53	0.47	1.13	0.87	1.50	0.50	1.16	0.84	0.98	0.14	2.18	0.70	1.69	1.34	1.21
	Paracyclopina nana	1.54	0.46	1.55	0.45	1.21	0.79	1.40	0.60	1.37	0.63	1.62	0.38	0.85	0.26	2.21	0.68	2.15	0.59	0.76
	Ergasilus tumidus	1.19	0.81	1.25	0.75	1.16	0.84	0.96	1.04	1.00	1.00	1.66	0.34	1.06	0.73	1.47	0.73	1.66	0.80	0.94
	Lamproglena orientalis	1.50	0.50	1.82	0.18	1.60	0.40	1.46	0.54	1.60	0.40	1.66	0.34	0.82	0.41	2.53	0.24	2.43	0.40	0.91
Cyclopoida	*Lamproglena chinensis*	1.18	0.82	1.45	0.55	1.26	0.74	1.10	0.90	1.09	0.91	1.24	0.76	0.87	0.61	1.39	1.13	1.23	0.99	0.73
	Lernaea cyprinacea	1.58	0.42	1.77	0.23	1.43	0.57	1.48	0.52	1.73	0.27	1.44	0.56	1.55	0.73	1.27	0.45	2.50	0.62	0.87
	Sinergasilus polycolpus	1.34	0.66	1.68	0.32	1.47	0.53	1.39	0.61	1.81	0.19	1.53	0.47	1.28	0.34	1.87	0.51	2.22	0.57	0.72
	Sinergasilus undulatus	1.27	0.73	1.76	0.24	1.20	0.8	1.20	0.80	1.57	0.43	1.46	0.54	0.72	0.24	2.16	0.88	1.76	0.88	0.98
	Tigriopus japonicus	1.56	0.44	1.07	0.93	1.36	0.64	0.76	1.24	1.28	0.72	0.98	1.02	1.36	0.71	1.07	0.86	1.84	1.99	0.86
Harpacticoida	*Tigriopus kingsejongensis*	1.01	0.99	1.14	0.86	1.01	0.99	0.83	1.17	0.54	1.46	1.12	0.88	0.52	0.85	1.25	1.38	1.36	1.45	0.59
	Tigriopus californicus	1.38	0.62	1.02	0.98	1.42	0.58	0.88	1.12	1.40	0.60	1.07	0.93	1.40	0.07	1.05	1.47	1.97	1.76	1.03
	Amphiascoides atopus	1.28	0.72	1.65	0.35	1.00	1.00	1.27	0.72	1.12	0.88	1.43	0.57	1.09	0.51	1.67	0.73	1.33	0.95	0.38
Siphonostomatoida	*Lepeophtheirus salmonis*	1.38	0.62	1.51	0.49	1.26	0.74	1.24	0.76	1.41	0.59	1.39	0.61	0.76	0.19	1.81	1.24	2.14	1.45	0.79
	Pennella sp.	1.38	0.62	1.51	0.49	1.59	0.41	0.99	1.01	1.43	0.57	1.19	0.81	1.25	0.17	1.33	1.25	1.76	1.41	0.74

Order	Species		Gly			
		AGC	GGU	GGC	GGA	GGG
	Calanus simillimus	0.45	1.40	0.53	1.33	0.75
	Phyllodiaptomus tunguidus	0.41	0.76	0.41	1.36	1.47
Calanoida	*Labidocera rotunda*	0.19	1.17	0.51	1.62	0.71
	Calanus hyperboreus	0.29	0.82	0.73	1.10	1.34
	Eurytemora affinis	0.46	1.32	0.49	0.72	1.48
	Paracyclopina nana	0.31	0.95	0.39	1.41	1.24
	Ergasilus tumidus	0.60	0.55	0.61	1.17	1.67
	Lamproglena orientalis	0.47	0.76	0.48	1.65	1.11
Cyclopoida	*Lamproglena chinensis*	0.92	0.62	0.86	0.81	1.72
	Lernaea cyprinacea	0.25	1.31	0.42	1.40	0.88
	Sinergasilus polycolpus	0.49	0.91	0.43	1.28	1.38
	Sinergasilus undulatus	0.36	0.85	0.37	1.07	1.71
	Tigriopus japonicus	0.64	0.80	0.42	0.95	1.83
Harpacticoida	*Tigriopus kingsejongensis*	0.55	0.25	0.83	0.83	2.10
	Tigriopus californicus	0.32	0.65	0.29	1.09	1.96
	Amphiascoides atopus	0.67	0.53	0.74	1.55	1.18
Siphonostomatoida	*Lepeophtheirus salmonis*	0.26	0.60	0.52	1.38	1.50
	Pennella sp.	0.29	0.81	0.13	1.12	1.93

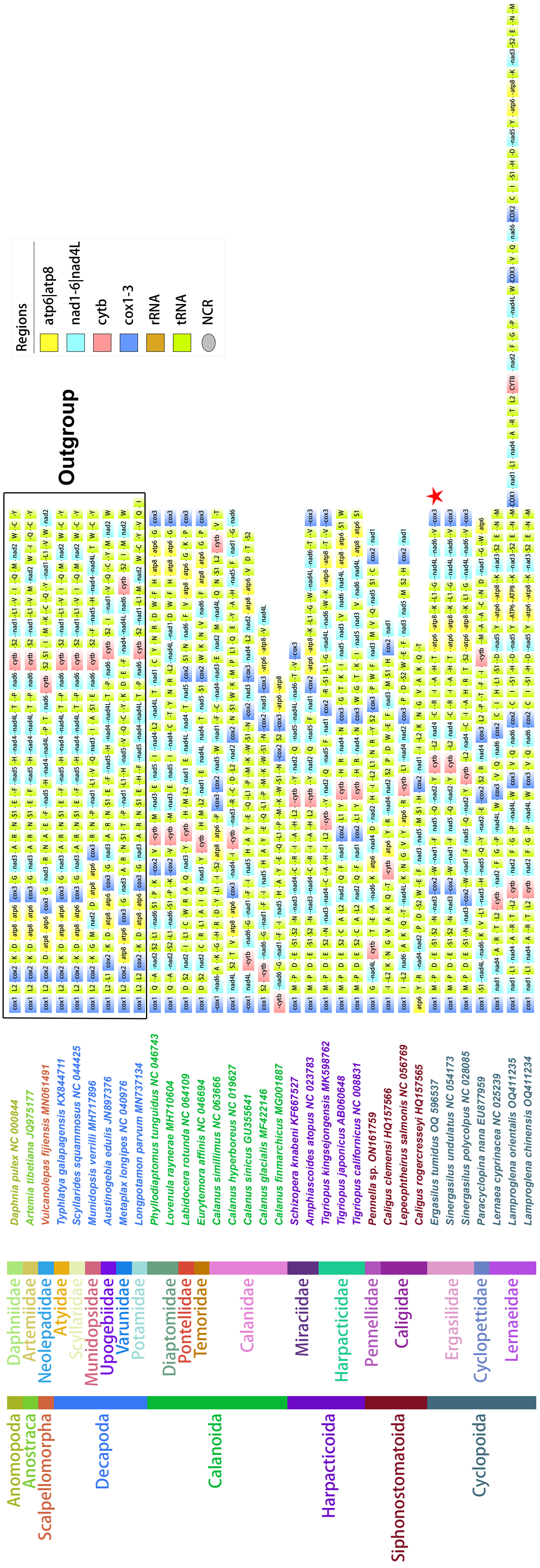

Fig. S1. Comparison of mitochondrial gene arrangements of 34 copepod mito genomes. Asterisk indicates the mitogenome of *Ergasilus tumidus* Markevich, 1940.

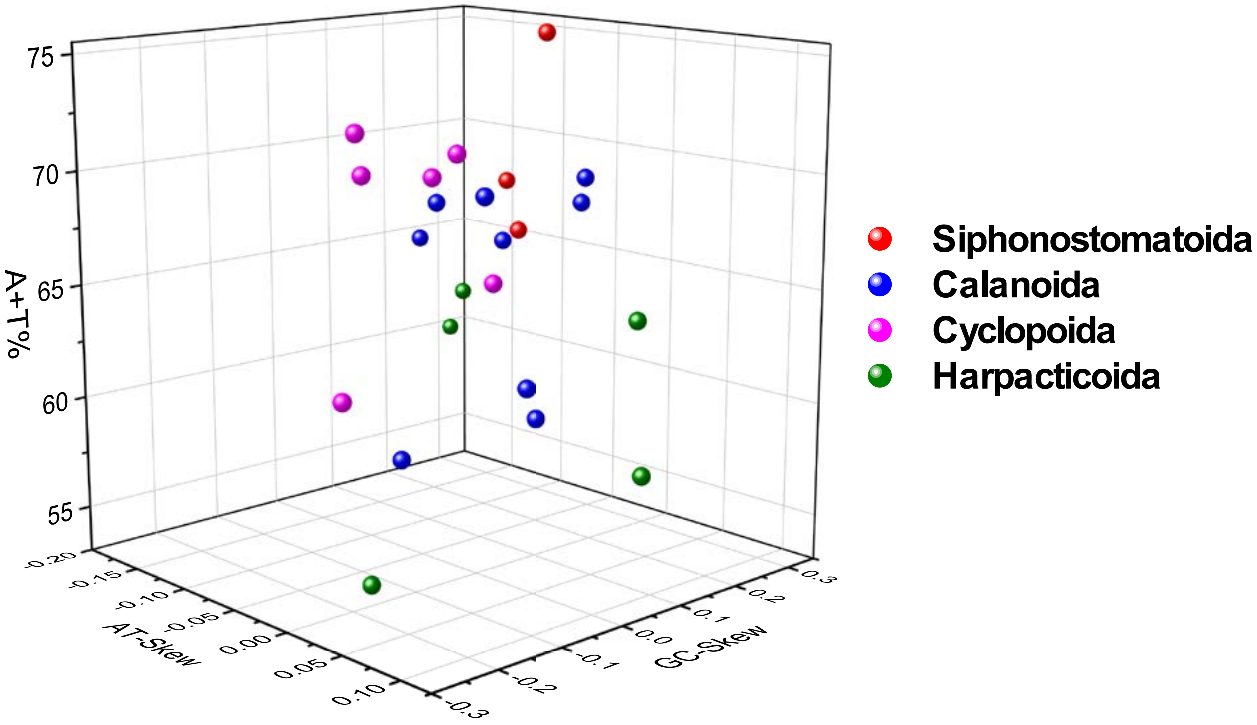

Fig. S2. Three-dimensional scatter plot of the AT-skew, GC-skew, and AT% of 25 copepod mitogenome sequences.

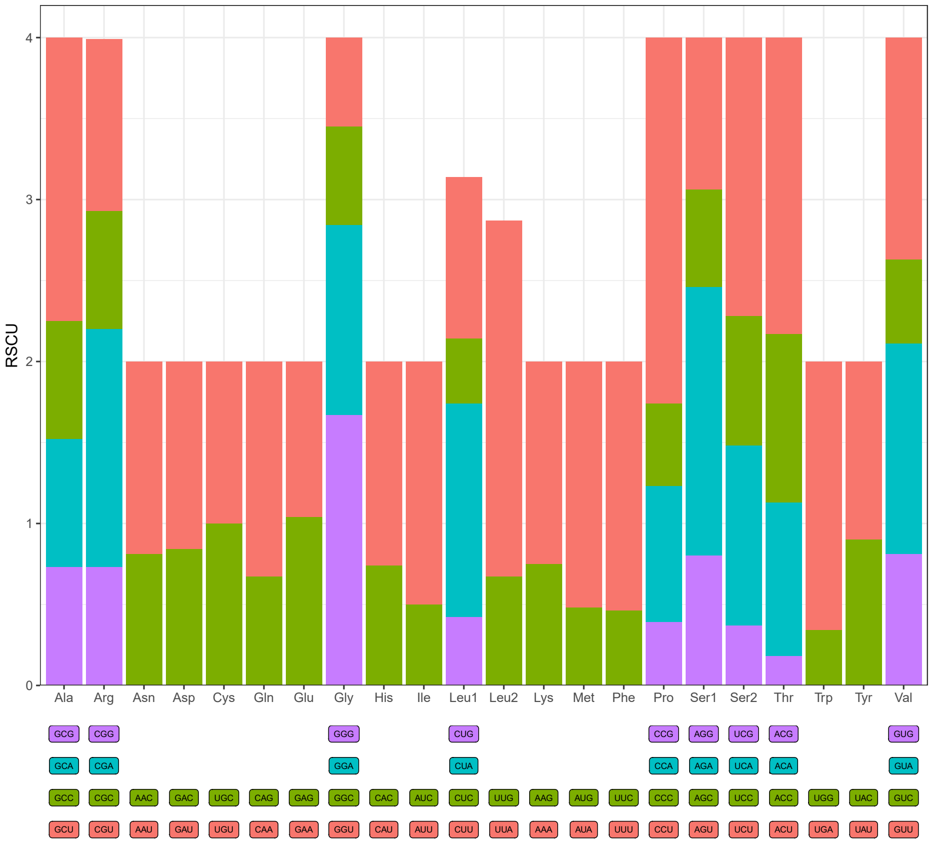

Fig. S3. Relative synonymous codon usage of mitogenomes of copepods. Codon families are labelled on the x-axis.

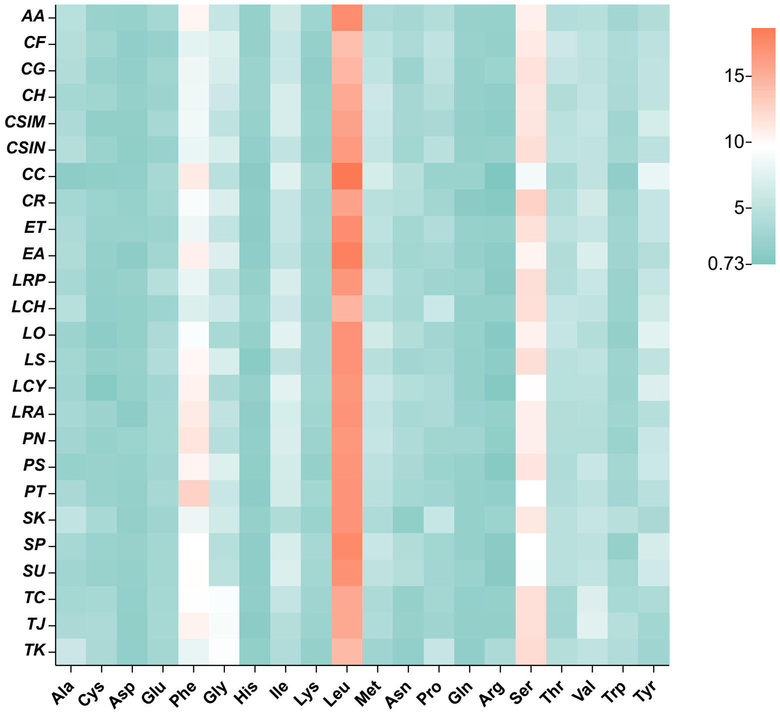

Fig. S4. Frequency percentage of each of 20 amino acids in 25 mitochondrial genome sequences of copepods. AA: *Amphiascoides atopus*, CF: *Calanus finmarchicus*, CG: *Calanus glacialis*, CH: *Calanus hyperboreus*, CSIM: *Calanus simillimus*, CSIN: *Calanus sinicus*, CC: *Caligus clemensi*, CR: *Caligus rogercresseyi*, ET: *Ergasilus tumidus*, EA: *Eurytemora affinis*, LRP: *Labidocera rotunda*, LCH: *Lamproglena chinensis*, LO: *Lamproglena orientalis*, LS: *Lepeophtheirus salmonis*, LCY: *Lernaea cyprinacea*, LRA: *Lovenula raynerae*, PN: *Paracyclopina nana*, PS: *Pennella* sp., PT: *Phyllodiaptomus tunguidus*, SK: *Schizopera knabeni*, SP: *Sinergasilus polycolpus*, SU: *Sinergasilus undulatus*, TC: *Tigriopus californicus*, TJ: *Tigriopus japonicus*, TK: *Tigriopus kingsejongensis*.

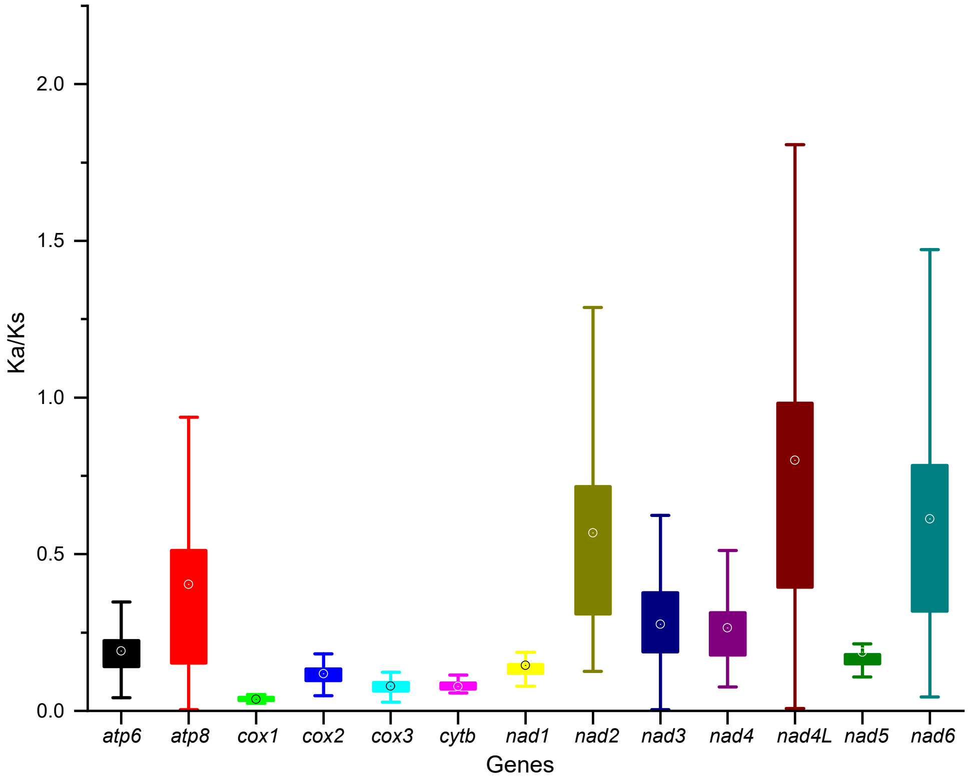

Fig. S5. Evolutionary rates of 13 protein-coding genes (PCGs) of 18 mitogenomes of copepods. Ka: non-synonymous mutation rate; Ks: synonymous mutation rate; Ka/Ks: the ratio of non-synonymous mutation rate to synonymous mutation rate. Neutral evolution (Ka/Ks = 1), purifying selection (Ka/Ks < 1), positive selection (Ka/Ks > 1). The plot summarises the median (central bar), the position of the upper and lower quartiles (central box), minimum (lower bar), and maximum value (upper bar).

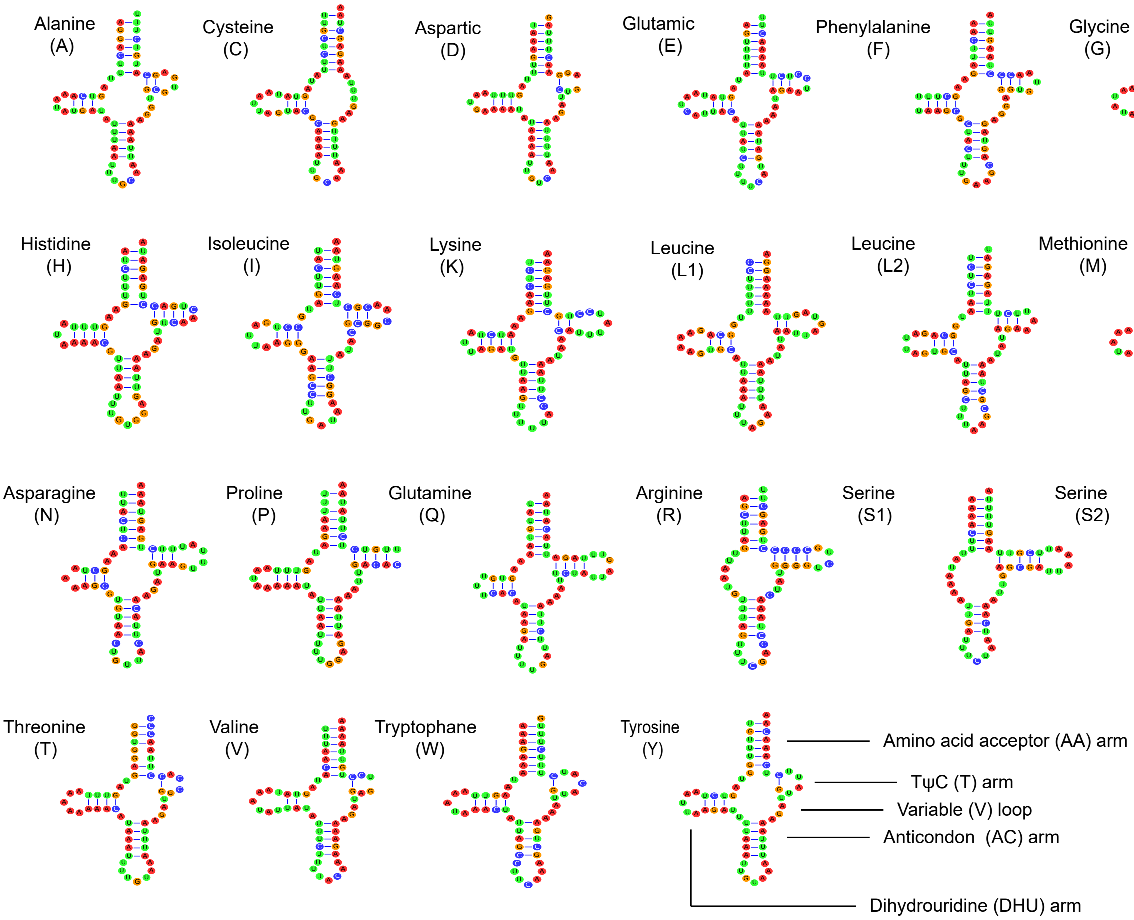

Fig. S6. The predicted secondary structure of 22 tRNAs of *Ergasilus tumidus* Markevich, 1940

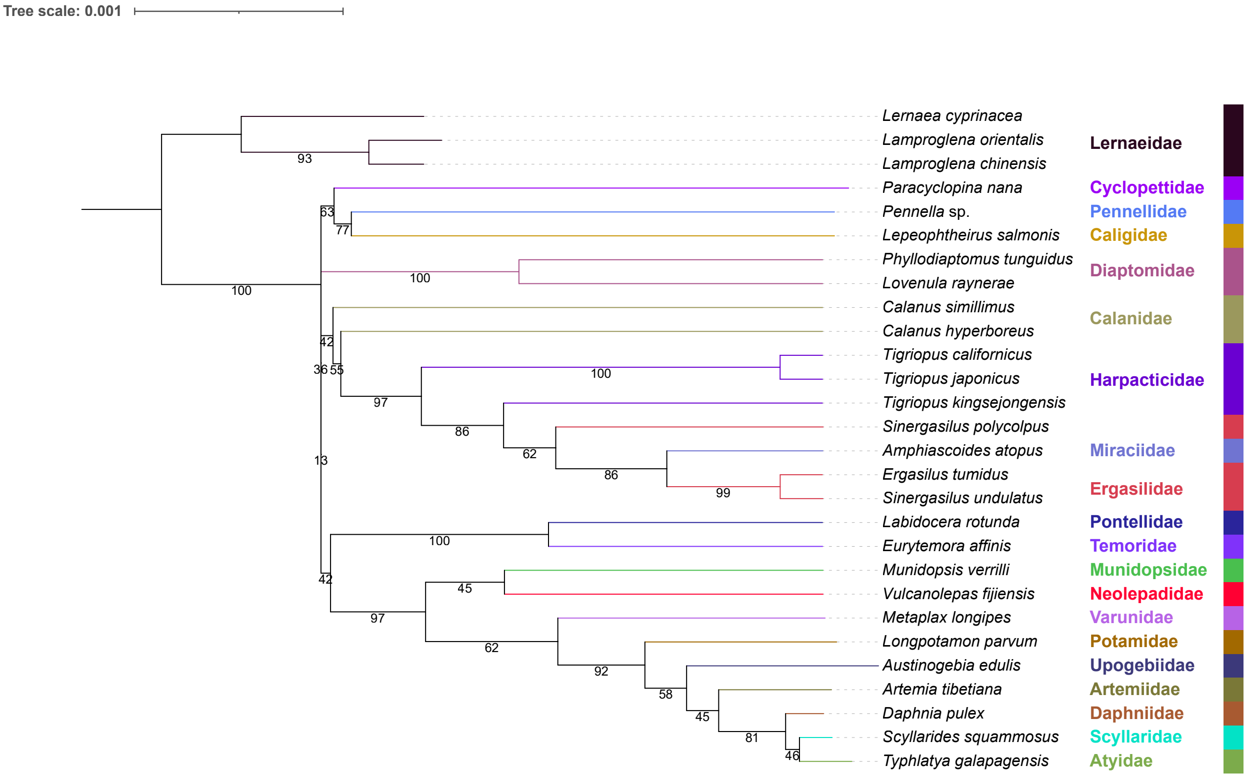

Fig. S7. Phylogenetic tree on the basis of the gene order dataset. Taxonomic identity (families) is shown on the right.