

Supplementary data for:

Towards a mitogenomic phylogeny of mud dragons (Kinorhyncha): two new mitogenomes from the Pycnophyidae family.

Authors: Lubośny M., Zalewska A., Herranz M., Sørensen MV., Grzelak K.

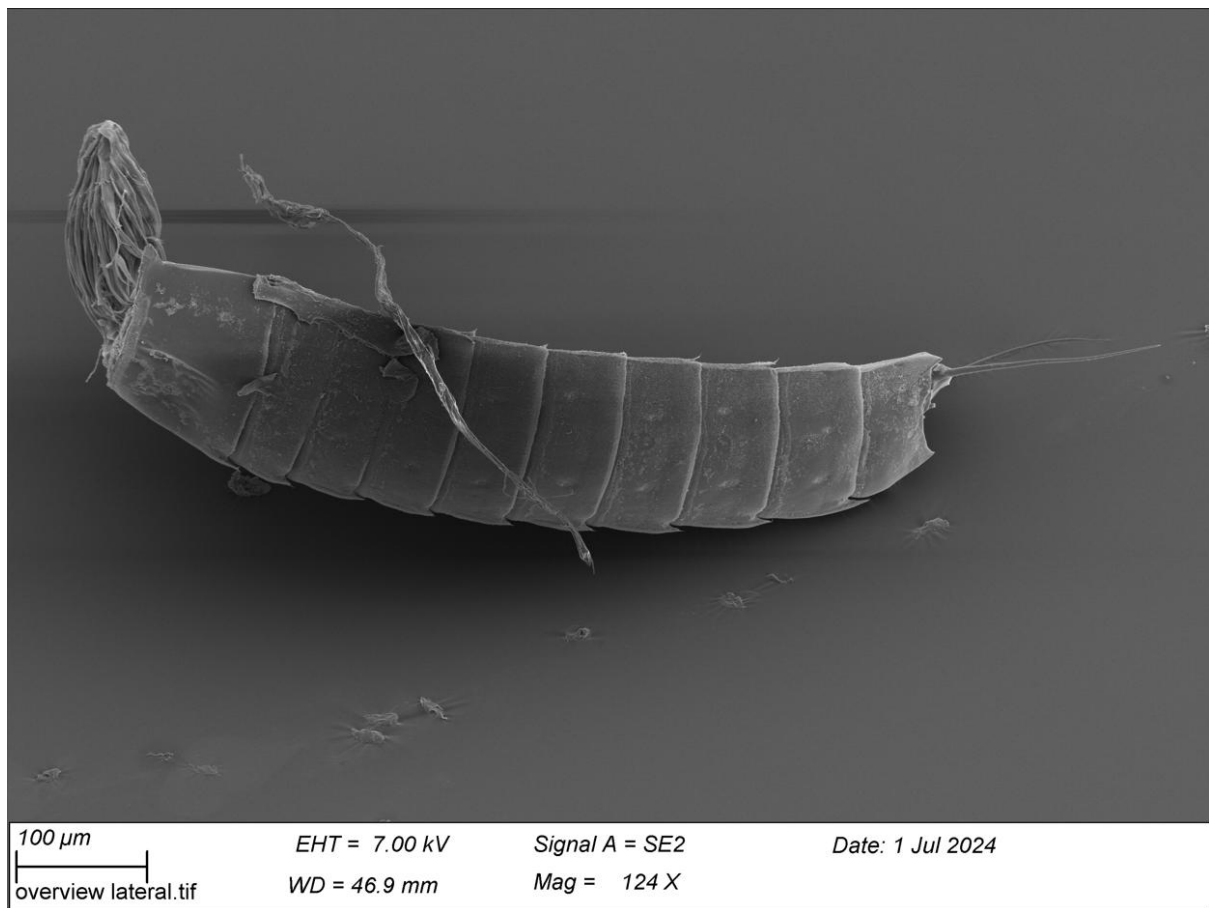


Fig S1. Scanning electron microscopy (SEM) image of *Cristaphyes cryopygus* individual used in this study.



Fig S2. Light microscopy image of *Pycnophyes greenlandicus* individual used in this study.

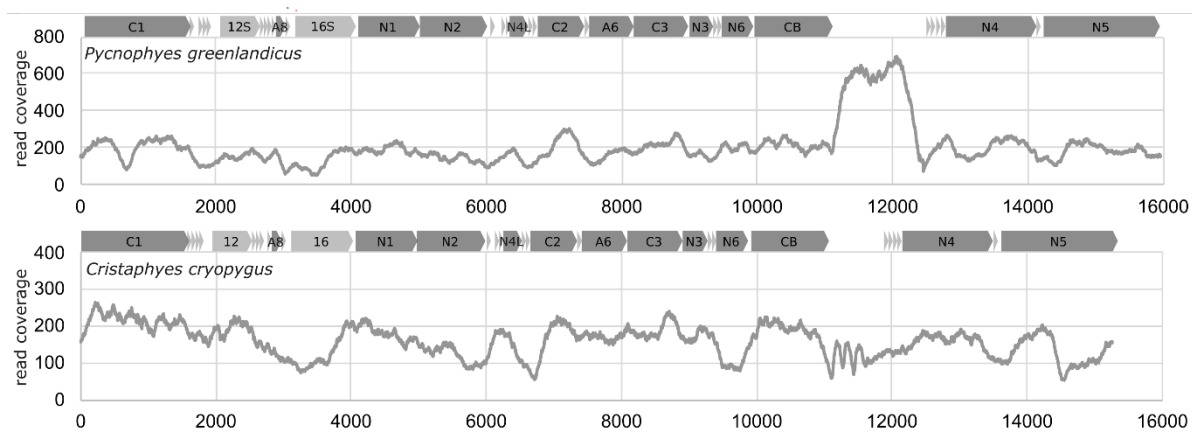


Fig S3. Sequencing coverage plot for *P. greenlandicus* and *C. cryopygus* mitogenomes.

Table S1. Gene coverage and polyadenylation signal coverage for *P. ilyocryptus* (SRR14509495) mitochondrial transcripts. Polyadenylation counted as the average number of adenine nucleotides in up to 20 bp window, excluding coverage spikes caused by adenines in non-polyadenylated reads in polycistronic transcripts.

<i>P. ilyocryptus</i>	Poly(A) coverage	Gene coverage	Poly(A)/Gene
<i>cox1</i> TAG (STOP)	50.38	89108.26	0.057%
<i>cox1</i> 4bp (downstream)	712.62	89108.26	0.800%
<i>cox2</i> TAG (STOP)	6.50	14665.62	0.044%
<i>cox2</i> 6bp (downstream)	1001.85	14665.62	6.831%
<i>cox3</i> TAG (STOP)	16.55	107787.63	0.015%
<i>cox3</i> 23bp (downstream)	2757.35	107787.63	2.558%
<i>atp6</i> TAA (STOP)	935.40	5891.97	15.876%
<i>cytb</i> TAG (STOP)	3.20	4170.09	0.077%
<i>nad1</i> TAA (STOP)	1171.71	4686.7	25.001%
<i>nad2</i> (3'end assembly problem)	n.d.	789.79	n.d.
<i>nad3</i> TAA (STOP)	1785.52	14588.03	12.240%
<i>nad4</i> TAA (STOP)	823.90	22387.22	3.680%
<i>nad5</i> TAA (STOP)	31.95	1031.31	3.098%
<i>nad6</i> TAG (STOP)	71.18	17862.57	0.398%
<i>nad6</i> 41bp (downstream)	1078.55	17862.57	6.038%

Table S2. Gene coverage and polyadenylation signal coverage for *P. giganteus* (SRR14509496) mitochondrial transcripts. Polyadenylation counted as the average number of adenine nucleotides in up to 20 bp window, excluding coverage spikes caused by adenines in non-polyadenylated reads in polycistronic transcripts.

<i>P. giganteus</i>	Poly(A) coverage	Gene coverage	Poly(A)/Gene
<i>cox1</i> TAA (STOP) in tRNA-E	12.57	12168.17	0.103%
<i>cox1</i> 15bp (upstream) GAA	95.17	12168.17	0.782%
<i>cox1</i> 20bp (upstream) ATA	12.29	12168.17	0.101%
<i>cox1</i> 30 (upstream) TACT	8.2	12168.17	0.067%
<i>cox2</i> TAA (STOP)	98.76	14135.74	0.699%
<i>cox2</i> 14bp (downstream)	1076.62	14135.74	7.616%
<i>cox3</i> TAG (STOP)	11.67	19546.15	0.060%
<i>cox3</i> 6bp (downstream)	1437.00	19546.15	7.352%
<i>atp6</i> TAA (STOP)	402.40	1704.21	23.612%
<i>cytb</i> TAG (STOP)	11.00	636.43	1.728%
<i>nad1</i> TAA (STOP)	103.32	398.48	25.928%
<i>nad2</i> TAA (STOP)	1.50	962.33	0.156%
<i>nad2</i> 21bp (downstream)	142.72	962.33	14.831%
<i>nad3</i> TAA (STOP)	141.43	969.18	14.593%
<i>nad4</i> TAG (STOP)	2.90	3984.65	0.073%
<i>nad4</i> 37bp (downstream)	288.57	3984.65	7.242%
<i>nad5</i> TAA (STOP)	244.33	1719.22	14.212%
<i>nad6</i> TAG (STOP)	0.14	1510.35	0.009%

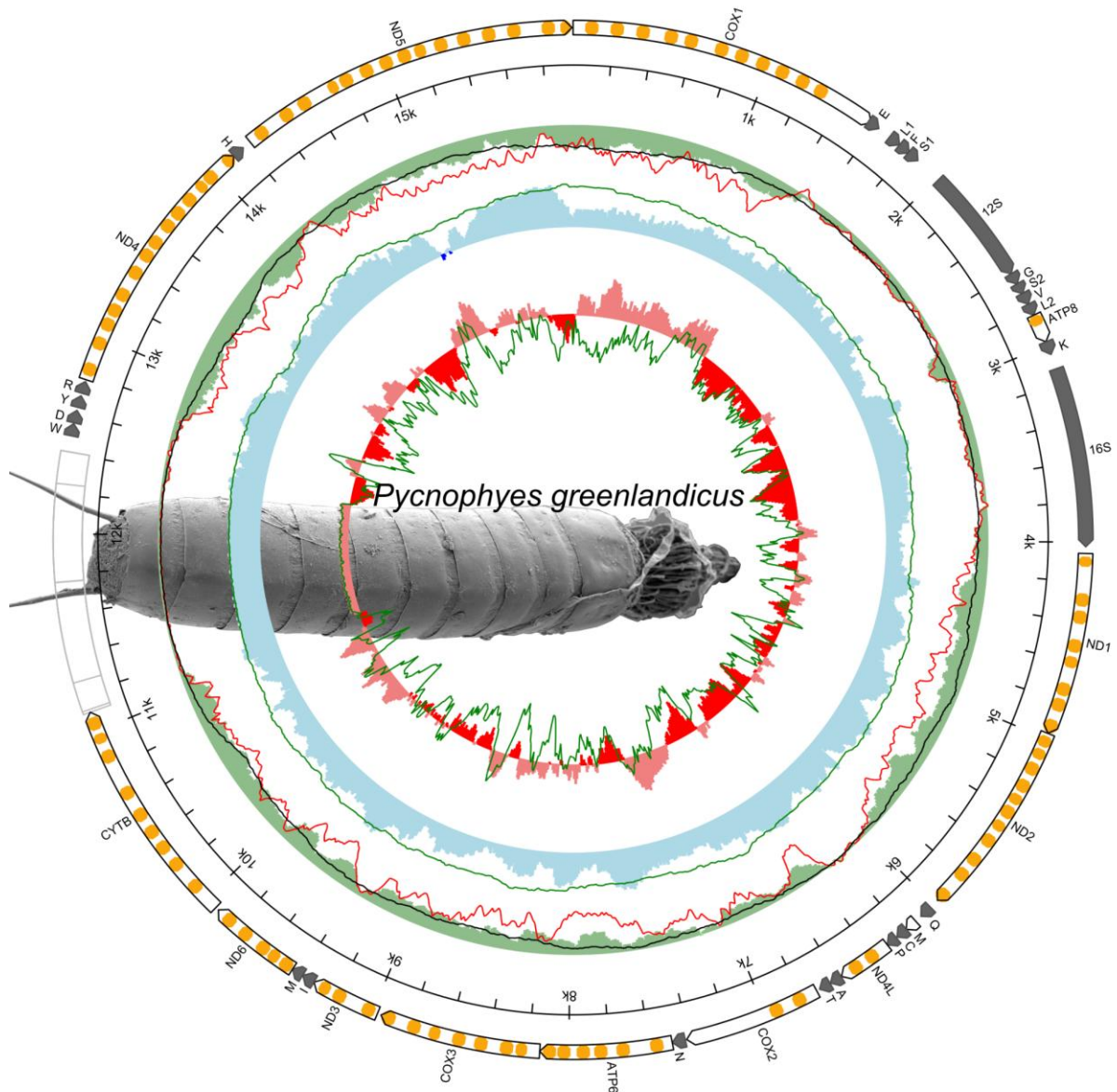


Fig S4. Genetic map of the *Pycnophyes greenlandicus* mitogenome. The presented features follow the convention of MITOCONSTRICATOR. Orange ovals on arrows represent identified transmembrane domains. Grey arrows represent rRNA and tRNA. Transparent white short arrow represent duplicated tRNA-Met. Transparent light-gray boxes represent repetitive sequences in non-coding regions. Sequence local compositional bias is represented by inner circles, calculated in a 200 bp long sliding window with 25 bp steps. The first inner green circle represents local AT-skew; blue circle represents local GC-skew; red circle represents local GC content relative to average of the whole mitogenome. The red line represents filtered AT-skew at non-coding regions, and second codon position. Black and two green lines represent filtered AT-skew, GC-skew and GC content respectively, at neutral sites, calculated in a 1.000 bp long sliding window.

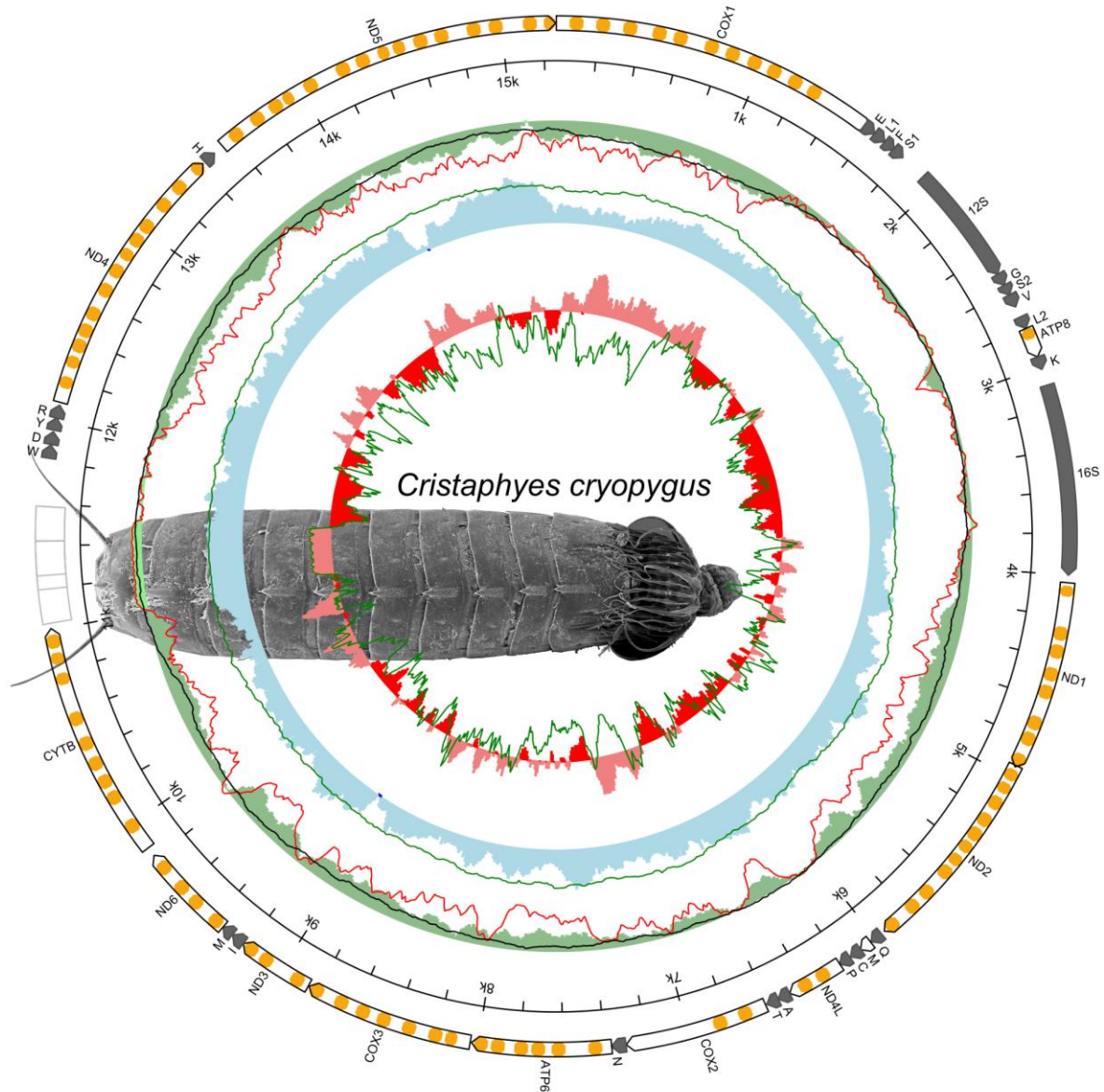


Fig S5. Genetic map of the *Cristaphyes cryopygus* mitogenome. The presented features follow the convention of MITOCONSTRUCTOR. Orange ovals on arrows represent identified transmembrane domains. Grey arrows represent rRNA and tRNA. Transparent white short arrow represent duplicated tRNA-Met. Transparent light-gray boxes represent repetitive sequences in non-coding regions. Sequence local compositional bias is represented by inner circles, calculated in a 200 bp long sliding window with 25 bp steps. The first inner green circle represents local AT-skew; blue circle represents local GC-skew; red circle represents local GC content relative to average of the whole mitogenome. The red line represents filtered AT-skew at non-coding regions, and second codon position. Black and two green lines represent filtered AT-skew, GC-skew and GC content respectively, at neutral sites, calculated in a 1,000 bp long sliding window.

Table S3. The codon usage for *Cristaphyes cryopygus* mtDNA genes. Fields marked with color indicate codons that are not used in a particular gene. Codons marked with red indicate codons found at the 3' end of *cox1* open reading frame.

	AAA (K)	AAC (N)	AAG (K)	AAT (N)	ACA (T)	ACC (T)	ACG (T)	ACT (T)	AGA (S)	AGC (S)	AGG (S)	AGT (S)	ATA (M)	ATC (I)	ATG (M)	ATT (I)
<i>cox1</i>	2	1	4	15	1	0	1	28	7	0	4	9	22	1	5	35
<i>cox2</i>	3	1	2	9	0	0	1	5	6	0	3	2	15	0	3	22
<i>cox3</i>	2	0	1	8	2	0	0	11	7	2	5	2	10	0	1	18
<i>cytb</i>	10	1	2	11	2	0	0	9	10	0	5	5	18	3	6	20
<i>atp6</i>	2	0	2	4	2	0	0	4	8	0	4	5	19	0	2	11
<i>atp8</i>	1	1	0	1	0	0	0	0	4	0	1	3	3	0	1	3
<i>nd1</i>	5	0	3	5	0	1	0	3	4	0	1	1	13	1	5	27
<i>nd2</i>	4	1	3	6	3	0	0	2	20	1	10	7	19	6	7	20
<i>nd3</i>	3	0	0	1	0	0	0	1	1	0	2	1	7	0	1	12
<i>nd4</i>	11	1	1	6	1	0	0	4	12	1	8	12	28	2	10	22
<i>nd4l</i>	0	0	1	1	0	0	0	1	2	0	2	1	6	1	2	10
<i>nd5</i>	9	2	6	14	5	1	0	8	17	1	10	8	39	1	10	34
<i>nd6</i>	1	1	3	0	0	0	0	1	2	0	1	3	10	1	3	8

	CAA (Q)	CAC (H)	CAG (Q)	CAT (H)	CCA (P)	CCC (P)	CCG (P)	CCT (P)	CGA (R)	CGC (R)	CGG (R)	CGT (R)	CTA (L)	CTC (L)	CTG (L)	CTT (L)
<i>cox1</i>	2	0	2	18	3	1	1	14	7	0	4	2	1	0	1	12
<i>cox2</i>	5	0	0	4	1	0	0	9	2	0	4	2	1	0	0	3
<i>cox3</i>	5	0	1	10	1	0	1	4	1	0	4	1	5	0	2	1
<i>cytb</i>	2	2	5	6	0	0	2	12	4	0	1	3	5	1	1	2
<i>atp6</i>	0	0	2	2	2	0	0	3	2	0	1	0	2	0	0	1
<i>atp8</i>	0	0	1	0	0	0	0	2	0	0	0	0	1	0	0	0
<i>nd1</i>	2	0	1	1	2	1	1	4	4	0	2	2	3	0	0	4
<i>nd2</i>	1	0	0	1	0	1	0	3	2	0	1	1	4	0	1	3
<i>nd3</i>	1	0	1	1	0	0	0	3	0	0	0	0	2	0	0	0
<i>nd4</i>	1	0	2	5	0	0	1	7	1	0	4	5	7	0	0	9
<i>nd4l</i>	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0
<i>nd5</i>	3	0	5	3	0	0	0	8	3	0	3	2	2	0	2	5
<i>nd6</i>	0	0	0	0	0	1	0	1	1	0	0	0	3	1	0	3

	GAA (E)	GAC (D)	GAG (E)	GAT (D)	GCA (A)	GCC (A)	GCG (A)	GCT (A)	GGA (G)	GGC (G)	GGG (G)	GGT (G)	GTA (V)	GTC (V)	GTG (V)	GTT (V)
<i>cox1</i>	6	2	6	14	8	0	0	14	28	2	15	21	15	1	9	22
<i>cox2</i>	5	1	2	8	1	0	0	2	6	1	9	2	8	1	5	10
<i>cox3</i>	5	1	1	3	2	0	1	6	8	1	4	7	11	2	7	8
<i>cytb</i>	5	1	1	8	4	1	3	10	8	1	9	10	14	1	5	19
<i>atp6</i>	3	1	1	4	0	0	1	4	1	2	5	4	9	1	2	17
<i>atp8</i>	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	3
<i>nd1</i>	7	0	5	5	6	0	0	5	8	1	8	8	11	0	6	11
<i>nd2</i>	2	0	6	4	0	0	0	1	3	2	11	7	11	0	9	12
<i>nd3</i>	4	0	2	3	1	0	1	0	1	1	4	1	5	0	2	2
<i>nd4</i>	9	0	4	4	1	0	0	9	18	1	8	12	14	1	7	11
<i>nd4l</i>	3	1	1	1	1	0	1	1	3	0	2	1	5	0	3	3
<i>nd5</i>	5	1	6	17	6	1	1	10	19	0	17	15	11	0	5	23
<i>nd6</i>	1	0	1	2	1	0	0	2	5	0	3	7	3	0	1	10

	TAC (Y)	TAT (Y)	TCA (S)	TCC (S)	TCG (S)	TCT (S)	TGA (W)	TGC (C)	TGG (W)	TGT (C)	TTA (L)	TTC (F)	TTG (L)	TTT (F)
<i>cox1</i>	1	15	3	2	1	17	11	0	8	3	40	1	12	48
<i>cox2</i>	2	11	1	0	1	5	4	1	6	6	9	0	9	10
<i>cox3</i>	0	8	3	0	3	7	7	1	5	1	25	1	3	33
<i>cytb</i>	3	22	1	0	2	2	8	0	4	5	33	2	12	39
<i>atp6</i>	1	5	2	0	0	4	3	1	3	5	26	2	7	29
<i>atp8</i>	0	3	0	0	0	2	2	0	0	0	3	0	1	8
<i>nd1</i>	2	10	3	1	1	10	4	0	2	2	28	4	11	48
<i>nd2</i>	3	13	1	2	0	5	3	0	2	2	39	3	7	57
<i>nd3</i>	1	3	3	0	0	7	2	0	1	1	16	2	2	20
<i>nd4</i>	3	17	4	0	0	13	6	0	5	14	46	2	14	59
<i>nd4l</i>	0	2	0	1	0	1	4	0	1	2	7	0	4	12
<i>nd5</i>	1	22	2	1	2	20	10	0	6	5	55	1	27	81
<i>nd6</i>	1	7	2	1	2	3	1	0	2	2	19	0	5	31

Table S4. The codon usage for *Pycnophyes greenlandicus* mtDNA genes. Fields marked with color indicate codons that are not used in a particular gene. Codons marked with red indicate codons found at the 3' end of *cox1* open reading frame.

	AAA (K)	AAC (N)	AAG (K)	AAT (N)	ACA (T)	ACC (T)	ACG (T)	ACT (T)	AGA (S)	AGC (S)	AGG (S)	AGT (S)	ATA (M)	ATC (I)	ATG (M)	ATT (I)
<i>cox1</i>	2	1	5	14	4	0	2	24	10	0	4	1	20	0	7	34
<i>cox2</i>	3	1	1	7	0	0	1	4	8	0	2	1	8	1	7	21
<i>cox3</i>	4	1	0	6	1	1	0	8	12	0	1	4	8	1	2	14
<i>cytb</i>	6	1	6	10	0	0	1	8	10	0	8	7	18	3	4	19
<i>atp6</i>	3	0	1	3	0	0	0	5	7	1	8	3	18	1	3	13
<i>atp8</i>	0	0	1	0	0	0	0	0	2	0	3	2	1	0	0	1
<i>nd1</i>	8	0	1	5	0	0	1	3	4	0	3	0	13	3	5	30
<i>nd2</i>	4	1	7	4	0	0	1	2	12	0	7	7	20	0	7	28
<i>nd3</i>	2	0	0	1	1	0	0	1	3	1	0	2	9	0	1	9
<i>nd4</i>	5	0	3	11	1	0	0	6	10	0	11	7	25	2	7	28
<i>nd4l</i>	0	1	0	0	0	0	0	0	4	0	0	1	6	0	1	12
<i>nd5</i>	11	1	6	13	3	1	0	9	14	1	9	7	28	1	6	39
<i>nd6</i>	3	0	1	2	1	0	0	3	3	1	2	2	7	1	4	8

	CAA (Q)	CAC (H)	CAG (Q)	CAT (H)	CCA (P)	CCC (P)	CCG (P)	CCT (P)	CGA (R)	CGC (R)	CGG (R)	CGT (R)	CTA (L)	CTC (L)	CTG (L)	CTT (L)
<i>cox1</i>	3	0	1	18	1	2	0	16	4	0	3	6	2	0	2	9
<i>cox2</i>	3	0	2	4	1	0	0	9	3	0	2	3	2	0	0	3
<i>cox3</i>	3	0	2	11	0	0	0	6	1	1	3	1	1	0	0	5
<i>cytb</i>	4	1	2	7	2	1	2	9	2	0	2	4	0	1	0	4
<i>atp6</i>	0	1	3	1	1	0	0	4	2	0	1	0	3	0	1	1
<i>atp8</i>	1	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0
<i>nd1</i>	2	0	1	1	1	1	0	6	3	0	4	1	1	0	0	5
<i>nd2</i>	1	0	0	0	0	1	0	3	3	0	1	0	3	0	2	8
<i>nd3</i>	2	0	0	1	0	0	0	3	0	0	0	0	0	0	0	2
<i>nd4</i>	3	0	0	5	1	1	0	6	1	0	3	4	4	0	0	11
<i>nd4l</i>	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0
<i>nd5</i>	6	1	3	2	1	1	1	4	1	0	2	5	3	0	1	13
<i>nd6</i>	0	0	0	0	0	0	0	2	0	0	0	1	2	0	1	1

	GAA (E)	GAC (D)	GAG (E)	GAT (D)	GCA (A)	GCC (A)	GCG (A)	GCT (A)	GGA (G)	GGC (G)	GGG (G)	GGT (G)	GTA (V)	GTC (V)	GTG (V)	GTT (V)
<i>cox1</i>	9	2	4	14	6	1	2	14	19	4	22	20	16	1	10	26
<i>cox2</i>	4	0	5	8	0	0	2	1	8	0	6	7	14	0	2	10
<i>cox3</i>	1	0	4	6	4	0	0	5	9	1	6	4	12	0	5	16
<i>cytb</i>	2	1	3	10	1	2	0	12	9	0	12	11	11	2	10	18
<i>atp6</i>	1	1	2	5	0	0	0	4	10	0	3	1	7	1	6	15
<i>atp8</i>	1	0	1	1	0	0	0	0	2	0	1	0	1	0	1	2
<i>nd1</i>	7	1	5	4	3	0	1	5	10	1	7	6	7	1	7	11
<i>nd2</i>	6	0	5	5	1	0	0	2	7	0	11	8	13	3	4	13
<i>nd3</i>	4	0	2	3	1	0	0	0	1	0	3	3	4	1	0	6
<i>nd4</i>	5	0	6	7	1	1	1	7	14	4	9	18	6	3	6	26
<i>nd4l</i>	3	0	1	1	0	0	0	2	3	0	5	1	4	0	2	6
<i>nd5</i>	4	4	10	13	6	1	1	10	22	0	17	15	14	3	9	24
<i>nd6</i>	0	0	3	1	2	0	0	2	8	0	3	2	4	0	6	9

	TAC (Y)	TAT (Y)	TCA (S)	TCC (S)	TCG (S)	TCT (S)	TGA (W)	TGC (C)	TGG (W)	TGT (C)	TTA (L)	TTC (F)	TTG (L)	TTT (F)
<i>cox1</i>	0	16	3	2	2	15	10	1	8	2	38	1	16	45
<i>cox2</i>	2	9	2	2	0	4	7	1	3	6	12	0	5	12
<i>cox3</i>	3	4	2	1	2	8	7	1	7	2	25	3	3	31
<i>cytb</i>	3	20	0	0	0	7	5	0	6	4	19	5	25	46
<i>atp6</i>	0	7	2	0	0	3	3	0	1	5	25	2	5	26
<i>atp8</i>	0	3	0	0	0	1	0	0	0	1	5	0	3	11
<i>nd1</i>	1	11	3	1	0	9	4	0	2	5	20	3	17	50
<i>nd2</i>	2	13	3	0	0	9	1	0	5	1	27	3	12	56
<i>nd3</i>	0	5	0	1	1	4	2	0	1	2	12	2	6	14
<i>nd4</i>	1	20	0	0	0	15	8	1	3	11	34	2	18	65
<i>nd4l</i>	0	3	0	1	0	1	4	0	1	2	8	1	6	7
<i>nd5</i>	2	18	2	0	1	20	9	1	5	7	61	2	19	81
<i>nd6</i>	0	7	3	0	0	2	4	0	2	4	15	3	6	25

