

Supplementary Table S1. Primer pairs (5'-3'), size of amplicons and protocols used in the amplification of the mitogenomes of *Gasterophilus pecorum* (Fabricius) and *Wohlfahrtia magnifica* (Schiner).

Primers	Sequence (5'-3')	Size (bp)	PCR conditions (annealing and extension)
0006_F/R	TGAATTGCCTGATAAAAAGG	~1500	44°C, 30s
	CTCCAATTAAGCTCCTGGATG		72°C, 1min, 50s
0007_F/R	GGTGGATTACCCCCATTTTTAGG	~1400	44°C, 30s
	TTAGCTAAAATTACTCCAGTTAATCCTCC		72°C, 1min, 50s
0008_F/R	GAGGAGATCCAATTCTCTATCAACA	~1400	56°C, 30s
	CAATGACAATTGGTATAAAGCTGTG		72°C, 1min, 50s
0009_F/R	CATTTGATTTGCATTCAAAAAGTATTG	~1200	37°C, 30s
	GCTGCTATAGCAGCTCCTACKCC		72°C, 1min, 50s
0010_F/R	CCATAAGTAGAAATATAAGGTATAAATCA	~1450	55°C, 30s
	TATTGATTTGTGGTATCAAAGATAAG		72°C, 1min, 50s
0011_F/R	CAGTAATTTTATTAACATGAATTGGAGC	~1350	64°C, 30s
	GCGACCTCGATGTTGGATTAAG		72°C, 1min, 50s
0012_F/R	GACATGTTTTTGTAAACAGGCG	~1450	45°C, 30s
	GTATAAAGTTTTATTTTGGCTTG		72°C, 1min, 50s
0013_F/R	TTCACTTCAAGATAGCTCATCTCCT	~1600	48°C, 30s
	GGTCATGGACTATAYTCTACTA		72°C, 2min
0014_F/R	GCAGTTCGATTAACAGCWAA	~1500	54°C, 30s
	AGTGATAAGCCTCTTTTTGGCTTC		72°C, 2min
0016_F/R	CAATTCTATTAATTAAGAAATTTCTCC	~1400	46°C, 30s
	CTTATTTTTGATTTACAAGACCAATG		72°C, 1min, 40s
0017_F/R	GAAAATAATCATTTCCATGAGTTCG	~1350	48°C, 30s
	AATCCTCCTCATACTCATTGTAC		72°C, 1min, 40s
0018_F/R	CCTTTACGAATYAAACACCC	~1150	44°C, 30s
	CTATCTTATGTTTTCAAACATATGC		72°C, 1min, 20s
0019_F/R	CTAAATTTATTGCACTAATCTGCC	~1150	56°C, 30s
	TTGTACCTTGTGTATCAGGG		72°C, 1min, 20s
0022_F/R	ACTAATCCCAATCCGTCTC	~1100	44°C, 30s
	TGGTTACCTAAGGCTCAT		72°C, 1min, 30s
0027_F/R	WTCYCCWTTYGAATGYGGAT	~800	50°C, 30s
	ATTTTGGRRGGTCAAMATTTA		72°C, 1min, 10s
0029_F/R	GCTGGCACAAATTTAATCAATACTC	~1350	54°C, 30s
	AATTGGGGTATGAACCCAGTAGCT		72°C, 2min

Primer pairs 0006_F/R–0019_F/R, 0029_F/R are from Weigl *et al.* [60]. Primer pair 0027_F/R is from Zhang *et al.* [67]. Primer pair 0022_F/R is designed using Primer Premier 5.0 [68].

Supplementary Table S2. Organization of the mitogenome of *Gasterophilus pecorum* (Fabricius) / *Wohlfahrtia magnifica* (Schiner).

Gene	Strand ^a	Location	Length (bp)	IGN ^b	Codon		AT%
					Start / Anti	Stop	
tRNA ^{Ile}	+ / N ^c	1-67 / N	67 / N	-3 / N	GAT / N		74.6 / N
tRNA ^{Gln}	- / -	65-133 / 17-85	69 / 70	13 / 3	TTG / TTG		82.6 / 81.2
tRNA ^{Met}	+ / +	147-215 / 89-158	69 / 70	0 / 0	CAT / CAT		71.0 / 71.4
ND2	+ / +	216-1229 / 159-1175	1014 / 1017	-2 / -2	ATT / ATT	TAA / TAA	71.8 / 78.7
tRNA ^{Trp}	+ / +	1228-1295 / 1174-1241	68 / 68	-8 / -8	TCA / TCA		76.5 / 75.0
tRNA ^{Cys}	- / -	1288-1350 / 1234-1297	63 / 64	-11 / 16	GCA / GCA		69.8 / 70.3
tRNA ^{Tyr}	- / -	1360-1426 / 1314-1379	67 / 66	-2 / -2	GTA / GTA		76.1 / 71.2
COI	+ / +	1425-2963 / 1378-2911	1539 / 1534	-5 / 0	TCG / TCG	TAA / T	63.5 / 68.1
tRNA ^{Leu(UUA)}	+ / +	2959-3023 / 2912-2977	65 / 66	3 / 5	TAA / TAA		69.2 / 71.2
COII	+ / +	3027-3714 / 2983-3670	698 / 688	0 / 0	ATG / ATG	T / T	67.6 / 74.6
tRNA ^{Lys}	+ / +	3715-3785 / 3671-3741	71 / 71	-1 / -1	CTT / CTT		66.2 / 69.0
tRNA ^{Asp}	+ / +	3785-3850 / 3741-3805	66 / 65	0 / 0	GTC / GTC		86.4 / 87.7
ATP8	+ / +	3851-4012 / 3806-3967	162 / 162	-7 / -7	ATT / ATC	TAA / TAA	70.4 / 81.5
ATP6	+ / +	4006-4683 / 3961-4638	678 / 678	-1 / -1	ATG / ATG	TAA / TAA	66.2 / 72.4
COIII	+ / +	4683-5471 / 4638-5426	789 / 789	6 / 2	ATG / ATG	TAA / TAA	63.1 / 69.5
tRNA ^{Gly}	+ / +	5478-5542 / 5429-5493	65 / 65	-3 / -3	TCC / TCC		78.5 / 80.0
ND3	+ / +	5540-5896 / 5491-5847	357 / 357	-2 / 2	ATA / ATA	TAG / TAA	70.3 / 79.3
tRNA ^{Ala}	+ / +	5895-5959 / 5850-5914	65 / 65	-2 / -1	TGC / TGC		75.4 / 73.8
tRNA ^{Arg}	+ / +	5960-6022 / 5914-5976	63 / 63	2 / 0	TCG / TCG		69.8 / 71.4
tRNA ^{Asn}	+ / +	6025-6089 / 5977-6042	65 / 66	0 / 0	GTT / GTT		70.8 / 72.7

tRNA^{Ser(AGC)}	+/+	6090-6157 / 6043-6110	68 / 68	0 / 0	GCT / GCT		70.6 / 73.5
tRNA^{Glu}	+/+	6158-6223 / 6111-6176	66 / 66	20 / 18	TTC / TTC		90.9 / 92.4
tRNA^{Phe}	-/-	6244-6310 / 6195-6259	67 / 65	0 / 0	GAA / GAA		65.7 / 72.3
ND5	-/-	6311-8030 / 6260-7979	1720 / 1720	15 / 15	ATT / ATT	T / T	70.3 / 76.3
tRNA^{His}	-/-	8046-8109 / 7995-8060	64 / 66	-1 / 0	GTG / GTG		78.1 / 81.8
ND4	-/-	8109-9449 / 8061-9399	1341 / 1339	-1 / -7	ATG / ATG	TAA / T	72.2 / 77.4
ND4L	-/-	9449-9733 / 9393-9689	285 / 297	-4 / 2	AAG / ATG	TAA / TAA	75.3 / 81.5
tRNA^{Thr}	+/+	9736-9800 / 9692-9756	65 / 65	0 / 0	TGT / TGT		76.9 / 83.1
tRNA^{Pro}	-/-	9801-9865 / 9757-9822	65 / 66	2 / -4	TGG / TGG		75.4 / 77.3
ND6	+/+	9868-10395 / 9825-10349	528 / 525	-1 / -1	ATT / ATT	TAA / TAA	73.9 / 81.5
CYTB	+/+	10395-11531 / 10349-11485	1137 / 1137	-2 / -2	ATG / ATG	TAG / TAG	64.7 / 70.1
tRNA^{Ser(UCA)}	+/+	11530-11596 / 11484-11551	67 / 68	18 / 17	TGA / TGA		80.6 / 79.4
ND1	-/-	11615-12553 / 11568-12506	939 / 939	10 / 10	ATA / ATA	TAG / TAA	70.1 / 76.1
tRNA^{Leu(CUA)}	-/-	12564-12627 / 12517-12581	64 / 65	-1 / -1	TAG / TAG		81.3 / 80.0
lrRNA	-/-	12627-13941 / 12581-13899	1315 / 1319	4 / 2			75.4 / 80.3
tRNA^{Val}	-/-	13946-14017 / 13902-13973	72 / 72	-1 / -1	TAC / TAC		76.4 / 80.6
srRNA	-/-	14017-14749 / 13973-14702	732 / 730	0 / N			72.3 / 75.2
Control region	- / N	14750-15750 /N	1001 / N				80.8 / N

^a Strand indicates the strand by which the genes are encoded; plus (+) and minus (-) symbols represent major and minor strands, respectively.

^b IGN: Intergenic nucleotide, minus indicates overlapping between genes. tRNA^X: where X is the abbreviation of the corresponding amino acid.

^c N = Not available.

Supplementary Table S3. Comparison of mitochondrial nucleotide composition in Calyptratae.

Species	Whole sequence ^a			PCG		rRNA	
	Length(bp)	A%	AT%	Codons	AT%	Codons	AT%
<i>Aldrichina grahami</i> ^b	14903	39.1	76.8	11182	76.0	2107	80.1
<i>Calliphora vicina</i>	16112	39.6	77.6	11176	75.7	2111	80.2
<i>Chrysomya albiceps</i>	15491	39.7	77.3	11179	76.2	2113	80.4
<i>Chrysomya bezziana</i>	15236	39.2	75.9	11179	74.6	2116	79.6
<i>Chrysomya megacephala</i>	15271	38.6	76.0	11178	74.8	2114	79.7
<i>Chrysomya pinguis</i>	15838	38.7	76.1	11178	74.2	2114	79.8
<i>Chrysomya putoria</i>	15837	39.1	76.7	11185	74.9	2114	80.0
<i>Chrysomya rufifacies</i>	15412	39.6	77.3	11179	76.2	2114	80.2
<i>Chrysomya saffrana</i>	15835	38.9	76.4	11178	74.6	2114	79.8
<i>Cochliomyia hominivorax</i> ^b	16022	39.8	76.9	11190	74.8	2110	79.8
<i>Delia platura</i> ^b	14858	38.9	77.3	11191	76.5	2107	80.9
<i>Dermatobia hominis</i>	16360	40.6	77.8	11190	75.3	2112	81.4
<i>Elodia flavipalpis</i>	14932	40.1	79.9	11193	79.1	2120	83.5
<i>Euryomma</i> sp. ^b	15315	39.2	77.7	11187	76.3	2121	81.6
<i>Gasterophilus pecorum</i>	15750	38.4	70.7	11177	68.5	2048	74.3
<i>Haematobia irritans</i>	16078	39.7	79.1	11183	77.7	2105	81.2
<i>Hemipyrellia ligurriens</i>	15938	39.5	77.3	11184	75.6	2115	80.1
<i>Hypoderma lineatum</i>	16354	40.6	77.8	11181	75.9	2101	80.5
<i>Lucilia cuprina</i>	15951	39.5	77.6	11179	75.9	2113	80.2
<i>Lucilia porphyria</i>	15877	39.1	76.3	11184	74.3	2115	79.6
<i>Lucilia sericata</i> ^b	15939	39.4	77.6	11183	76.0	2112	80.0
<i>Musca domestica</i>	16108	39.4	76.9	11178	76.1	2110	80.0
<i>Muscina stabulans</i>	15933	40.1	77.9	11181	76.2	2148	81.4
<i>Phormia regina</i>	15506	38.8	76.2	11183	74.9	2105	80.0
<i>Pollenia rudis</i> ^b	14340	38.9	76.5	11178	75.9	N ^c	N
<i>Protophormia terraenovae</i>	15170	38.6	75.8	11179	74.5	2112	80.1
<i>Ravinia pernix</i>	15778	39.6	77.2	11185	75.5	2114	80.4
<i>Rutilia goerlingiana</i>	15329	40.4	77.7	11156	76.2	2101	81.8
<i>Sarcophaga africa</i>	15144	39.4	75.7	11184	74.4	2111	79.9
<i>Sarcophaga crassipalpis</i>	15415	39.8	76.2	11186	74.7	2109	80.0
<i>Sarcophaga impatiens</i>	15169	39.4	74.8	11185	73.1	2113	79.5
<i>Sarcophaga melanura</i>	15190	39.4	75.6	11165	74.1	2108	80.1
<i>Sarcophaga peregrina</i> ^b	14922	38.9	75.0	11170	73.7	2108	79.8
<i>Sarcophaga portschinskyi</i>	14929	38.9	76.2	11168	75.2	2109	80.4
<i>Sarcophaga similis</i>	15158	39.5	76.4	11168	75.3	2107	80.3
<i>Scathophaga stercoraria</i>	16223	39.6	78.4	11185	75.7	2114	80.8
<i>Stomoxys calcitrans</i> ^b	16790	39.4	78.9	11179	76.9	2107	81.4
<i>Wohlfahrtia magnifica</i> ^b	14701	39.2	76.5	11178	74.6	2049	78.5

^a Whole length reported on NCBI.^b Incomplete genomes with absent / incomplete control region, or incomplete rRNA.^c Not available (unknown or incomplete data).

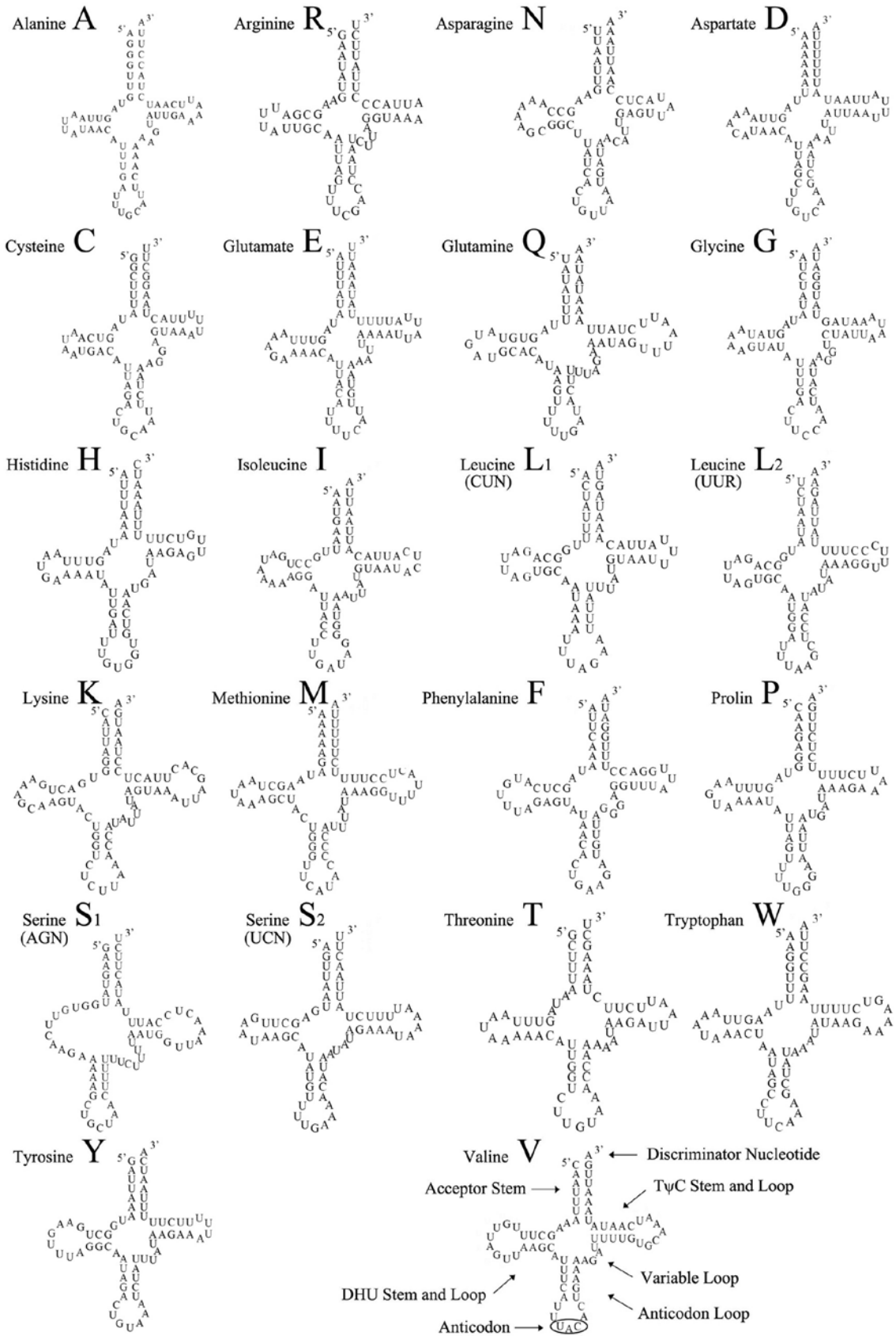
Supplementary Table S4. The average *p*-distances of mitochondrial protein-coding genes and rRNA genes at different taxonomic levels within Calyptratae.

	Within subfamily		Within family		Within superfamily	
	average <i>p</i> -distance	STDEVA*	average <i>p</i> -distance	STDEVA	average <i>p</i> -distance	STDEVA
All PCGs	0.0799	0.0200	0.0957	0.0286	0.1274	0.0380
ND2	0.0878	0.0248	0.1079	0.0372	0.1497	0.0488
COI	0.0740	0.0216	0.0912	0.0262	0.1159	0.0308
COII	0.0658	0.0215	0.0849	0.0307	0.1174	0.0367
ATP8	0.0592	0.0294	0.0862	0.0525	0.1419	0.0776
ATP6	0.0872	0.0252	0.1001	0.0288	0.1326	0.0382
COIII	0.0734	0.0233	0.0906	0.0297	0.1228	0.0371
ND3	0.0927	0.0283	0.1044	0.0388	0.1437	0.0453
ND5	0.0808	0.0181	0.0993	0.0303	0.1293	0.0419
ND4	0.0758	0.0208	0.0855	0.0275	0.1153	0.0363
ND4L	0.0498	0.0216	0.0686	0.0374	0.1110	0.0523
ND6	0.1085	0.0329	0.1308	0.0410	0.1766	0.0510
CYTB	0.0938	0.0222	0.1054	0.0268	0.1346	0.0364
ND1	0.0713	0.0164	0.0820	0.0230	0.1043	0.0364
rRNA	0.0338	0.0163	0.0484	0.0270	0.0760	0.0364
lrRNA	0.0338	0.0163	0.0484	0.0270	0.0757	0.0363
srRNA	0.0327	0.0133	0.0491	0.0279	0.0746	0.0370
tRNA	0.0172	0.0071	0.0248	0.0148	0.0396	0.0196

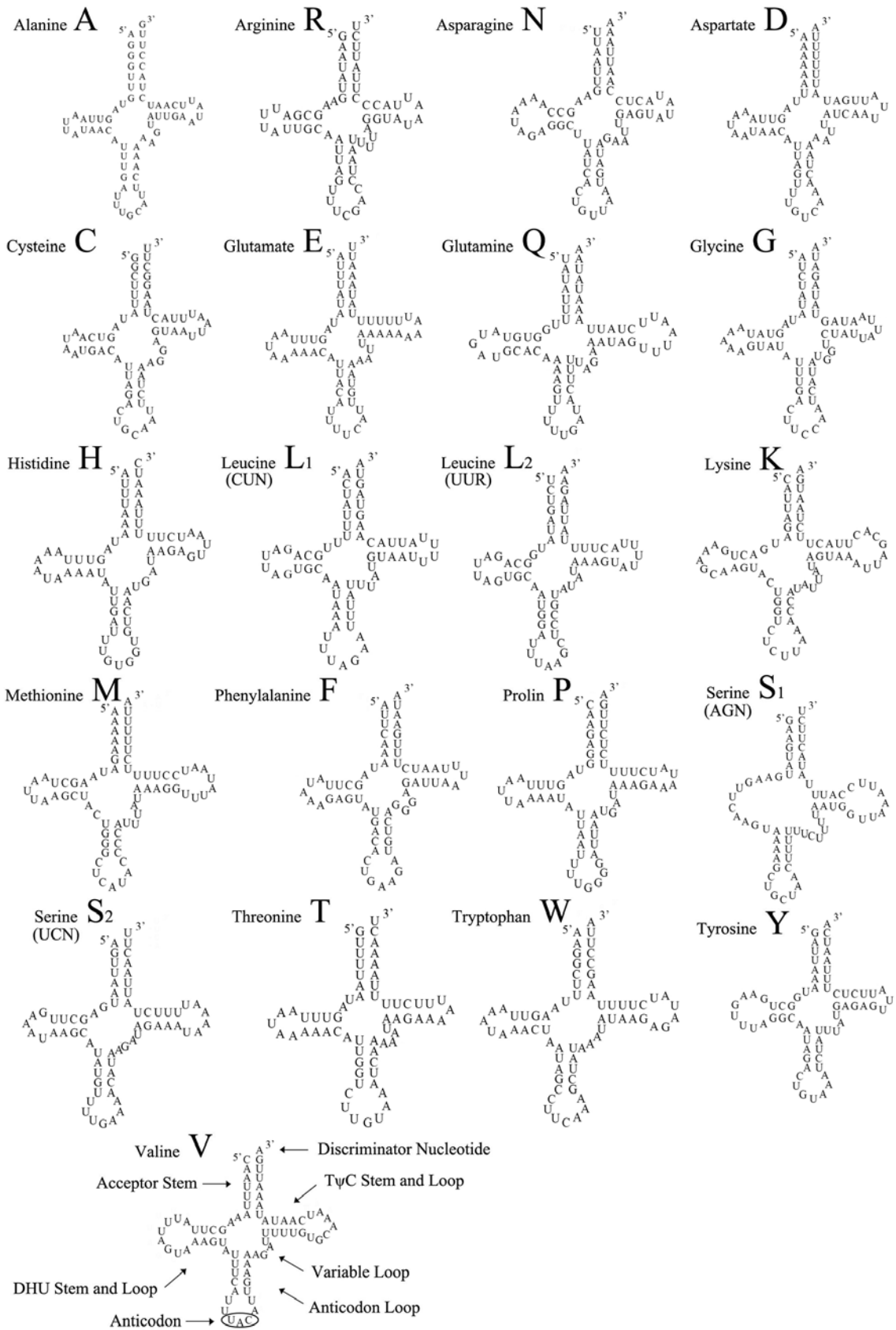
* STDEVA = standard deviation value.

Supplementary Table S5. Positions experiencing gaps, and invariable sites in the alignments of the 13 protein-coding genes.

Genes	Length	Gaps		Invariable sites	
		number	percentage	number	percentage
COI	1020	20	1.96	418	40.98
ND1	1539	5	0.32	924	60.04
COIII	690	14	2.03	365	52.90
ATP6	165	12	7.27	77	46.67
CYTB	678	20	2.95	372	54.87
COII	792	3	0.38	435	54.92
ND4	360	8	2.22	175	48.61
ND5	1728	8	0.46	824	47.69
ND4L	1341	2	0.15	696	51.90
ND3	297	12	4.04	154	51.85
ATP8	528	6	1.14	211	39.96
ND2	1137	2	0.18	603	53.03
ND6	951	24	2.52	533	56.05



Supplementary Figure S1a. Putative secondary structures of tRNAs found in the mitochondrial genome of *Gasterophilus pecorum* (Fabricius). All tRNAs can be folded into the usual clover-leaf secondary structure.



Supplementary Figure S1b. Putative secondary structures of tRNAs found in the mitochondrial genome of *Wohlfahrtia magnifica* (Schiner). The tRNA^{Ile} is absent because the control region could not be amplified. All tRNAs can be folded into the usual clover-leaf secondary structure.