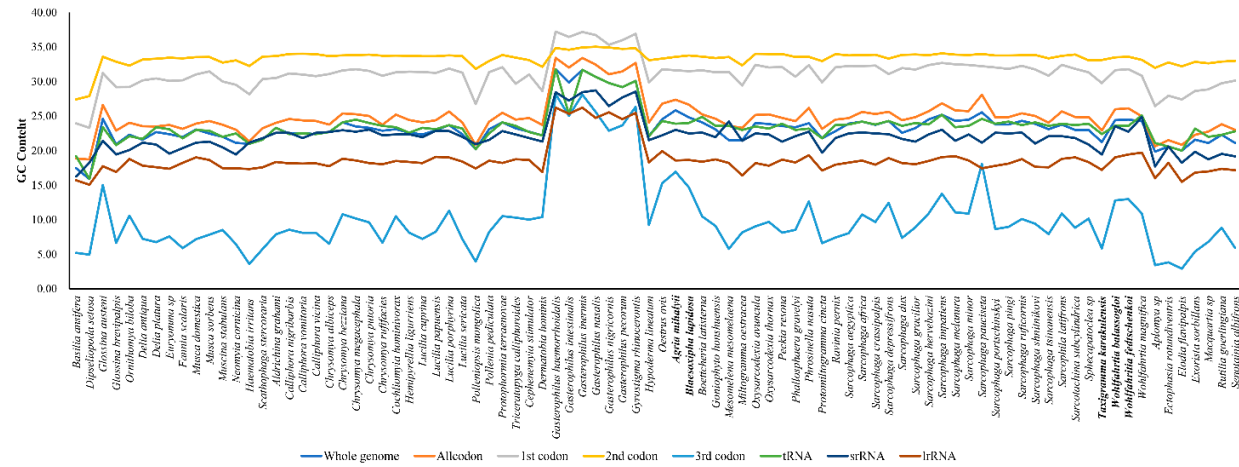# Supplementary Materials

Figure S1. GC content of 13 mitochondrial protein-coding genes in Calyptratae. (GC: Guanine and Cytosine). The sequences for species with names in bold were generated in this study.
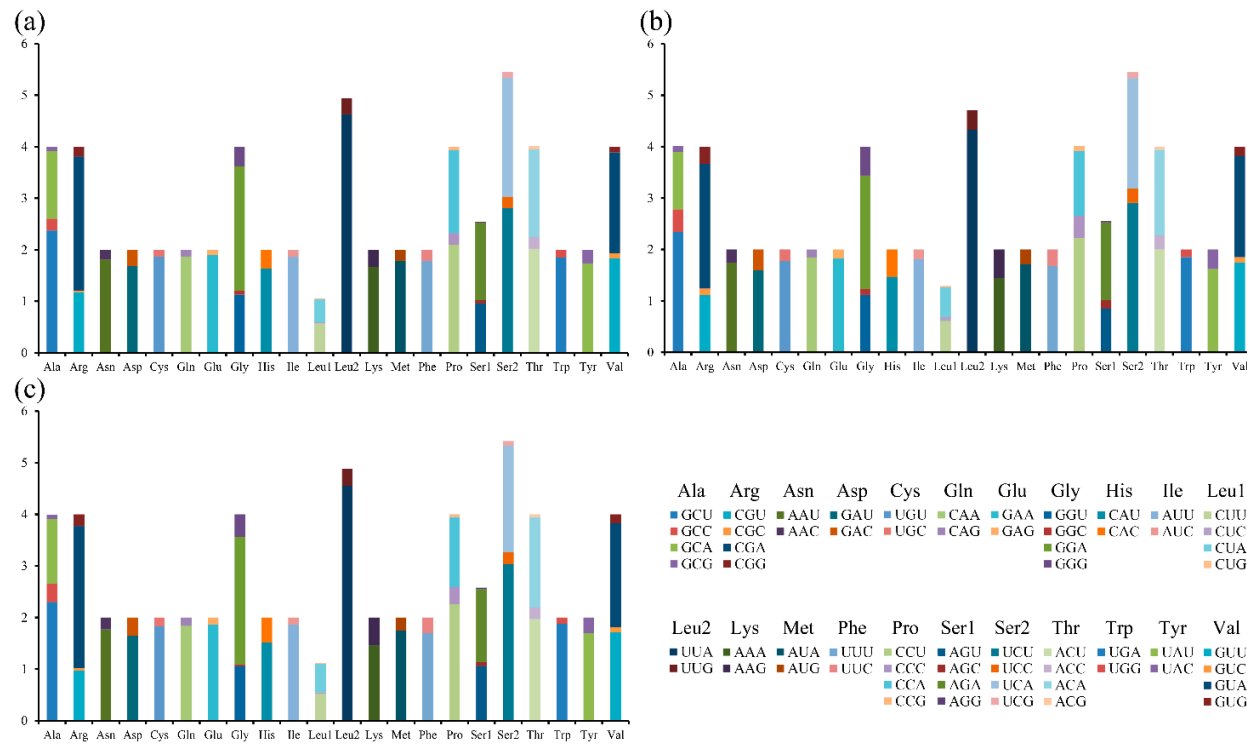
Figure S2. Codon usage analysis of the mitochondrial genomes from three subfamilies (a: Miltogrammatinae, b: Paramacronychiinae and c: Sarcophaginae).
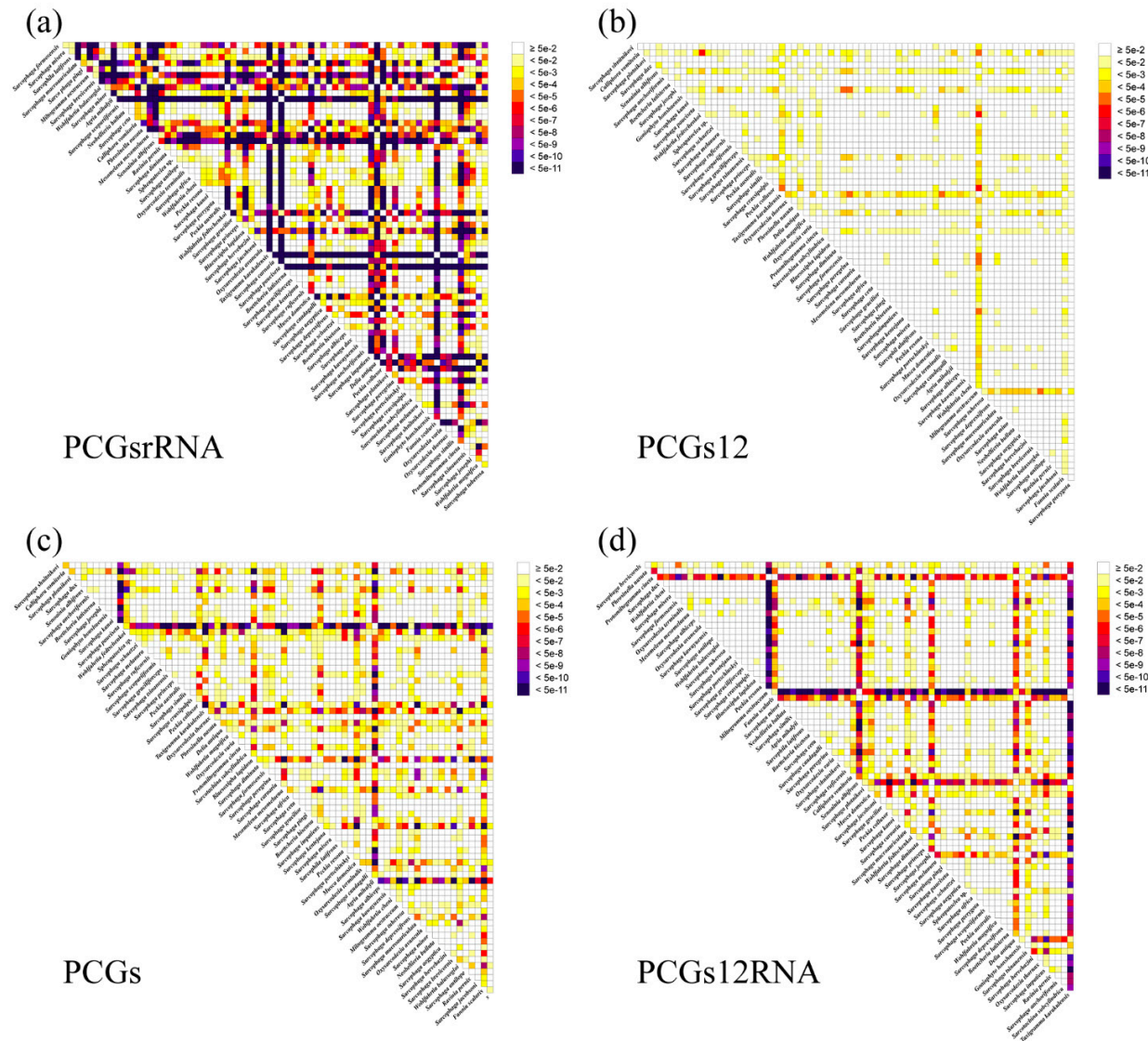
Figure S3. Heatmaps showing pairwise heterogeneous sequence divergence of matrices used for phylogenetic analyses. Abbreviations: PCGsrRNA (a), all 13 protein-coding genes and two rRNA; PCGs12 (b), 1st- and 2nd- codon positions of the 13 protein-coding genes; PCGs (c), all 13 protein-coding genes; PCGs12rRNA (d), 1st- and 2nd-codon positions of the 13 protein-coding genes and two rRNA.
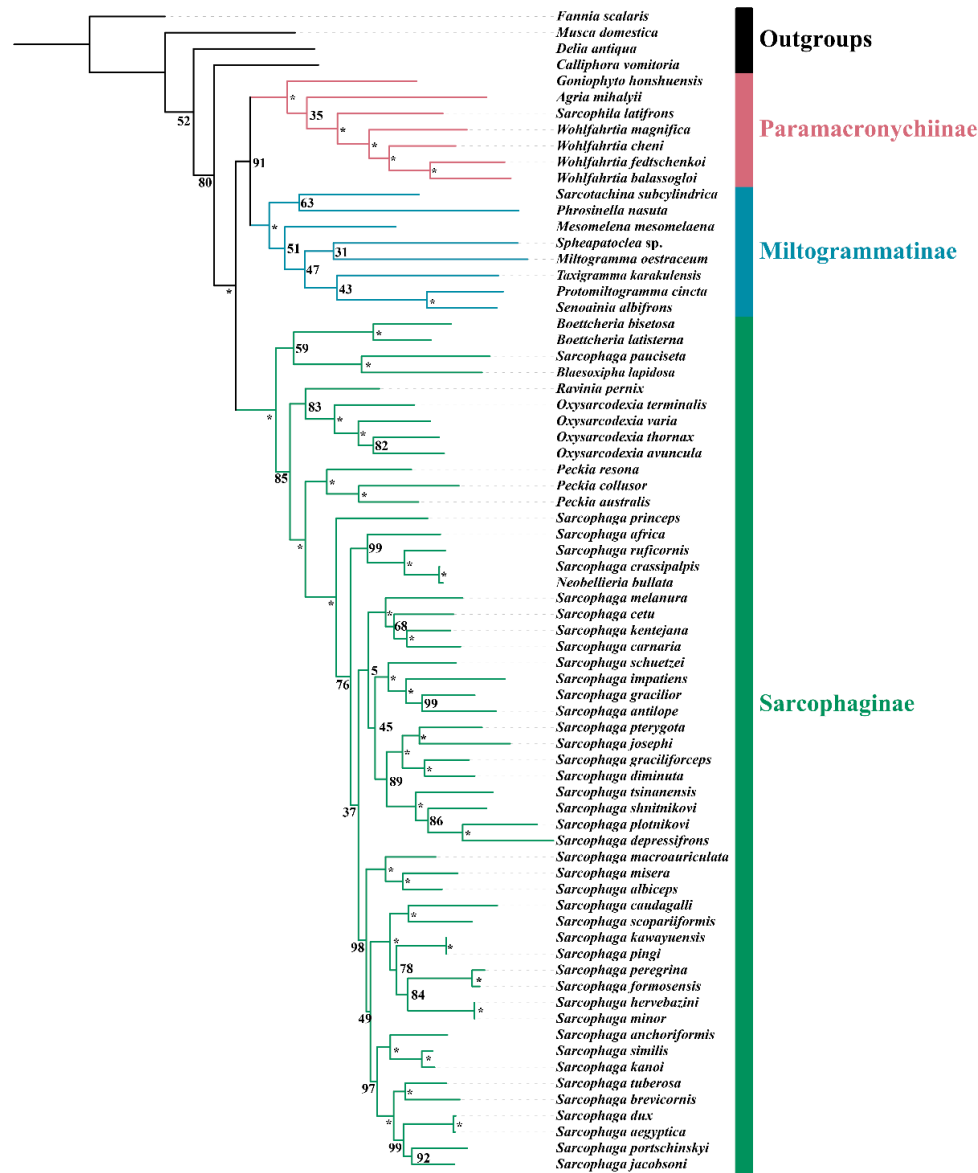
Figure S4. Maximum likelihood phylogenetic tree for Sarcophagidae based on concatenation of 13 mitochondrial protein-coding genes and two rRNA genes. Each node is provided with the support values of Maximum Likelihood bootstrap values (BS) for tree inference using matrix PCGsrRNA. Asterisks (*) represent full support.
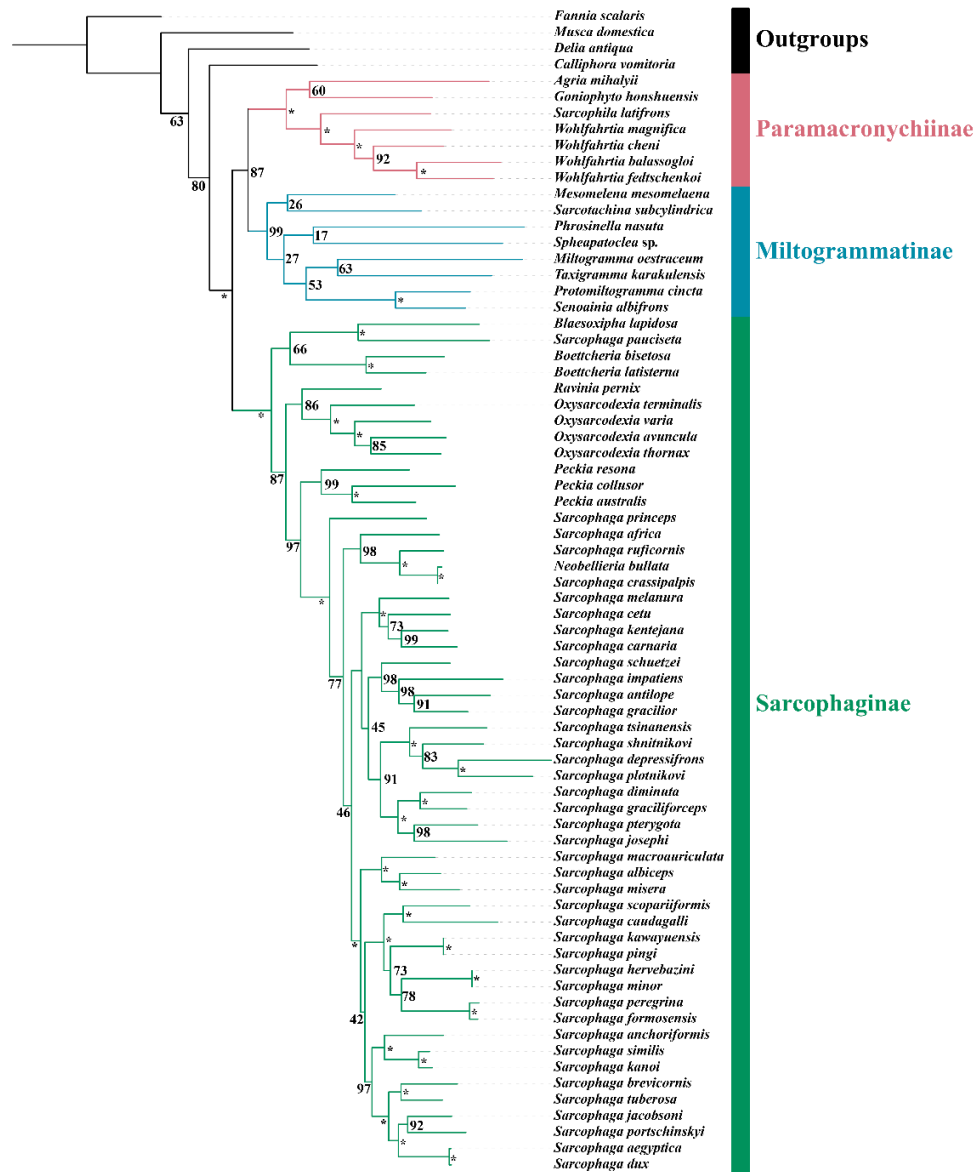
Figure S5. Maximum likelihood phylogenetic tree for Sarcophagidae based on concatenation of 13 mitochondrial protein-coding genes. Each node is provided with the support values of Maximum Likelihood bootstrap values (BS) for tree inference using matrix PCGs. Asterisks (*) represent full support.
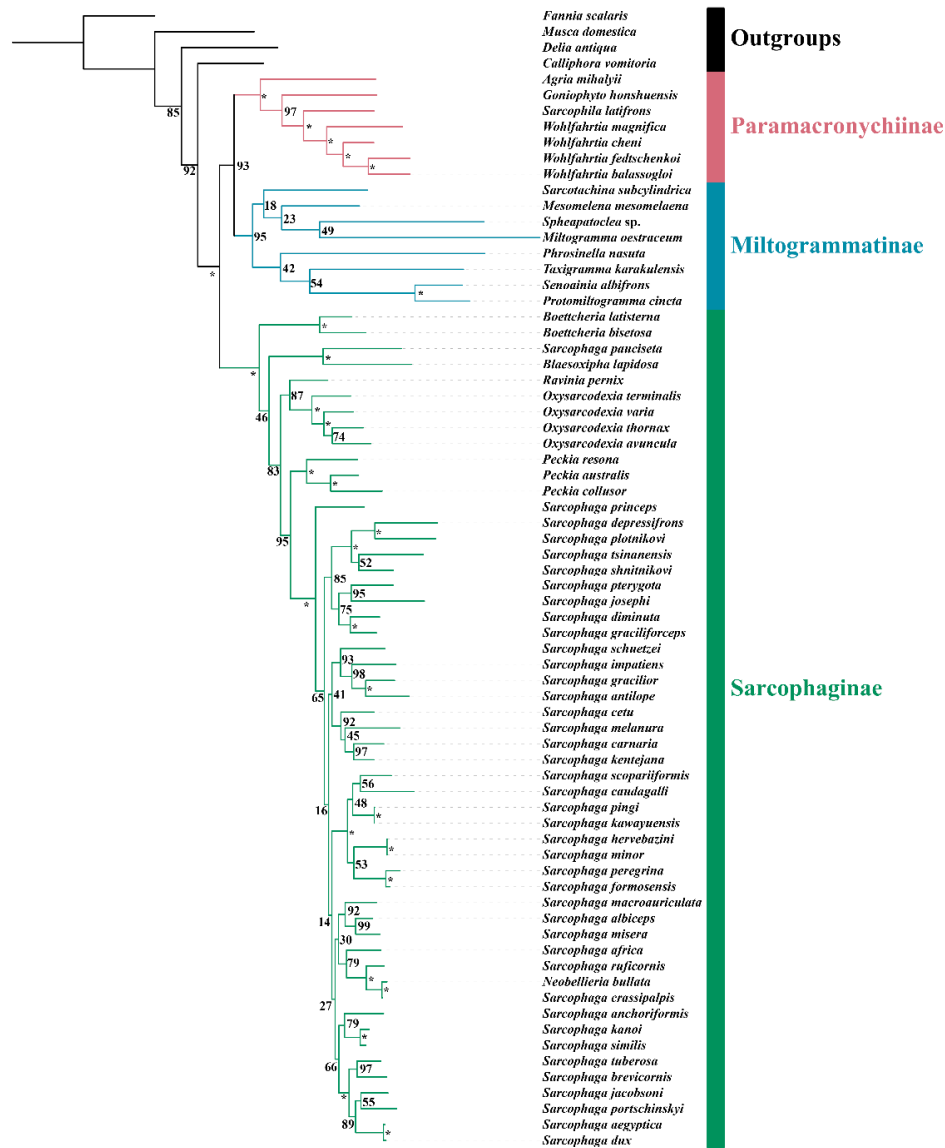
Figure S6. Maximum likelihood phylogenetic tree for Sarcophagidae based on concatenation of 13 mitochondrial protein-coding genes excluding 3rd-codon positions and two rRNA genes. Each node is provided with the support values of Maximum Likelihood bootstrap values (BS) for tree inference using matrix PCGs12rRNA. Asterisks (*) represent full support.
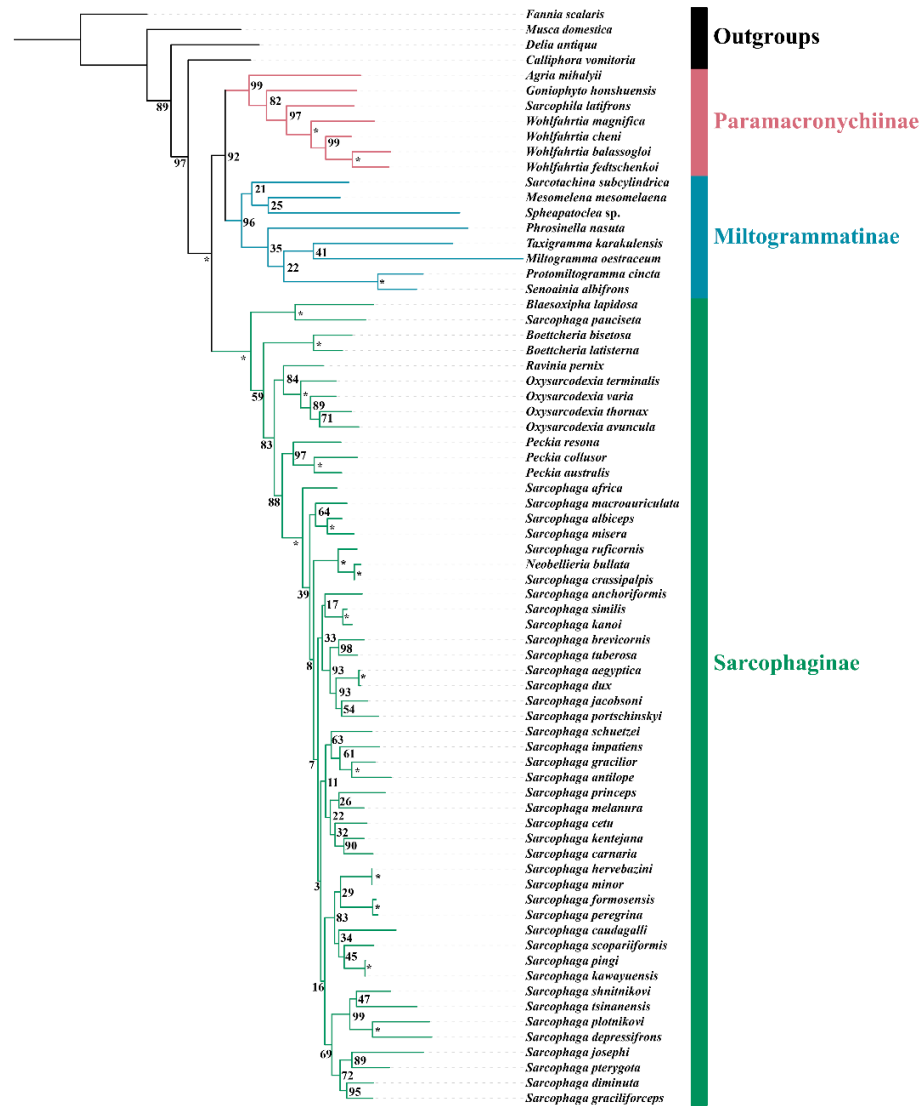
Figure S7. Maximum likelihood phylogenetic tree for Sarcophagidae based on concatenation of 13 mitochondrial protein-coding genes excluding 3rd-codon positions. Each node is provided with the support values of Maximum Likelihood bootstrap values (BS) for tree inference using matrix PCGs12. Asterisks (*) represent full support.
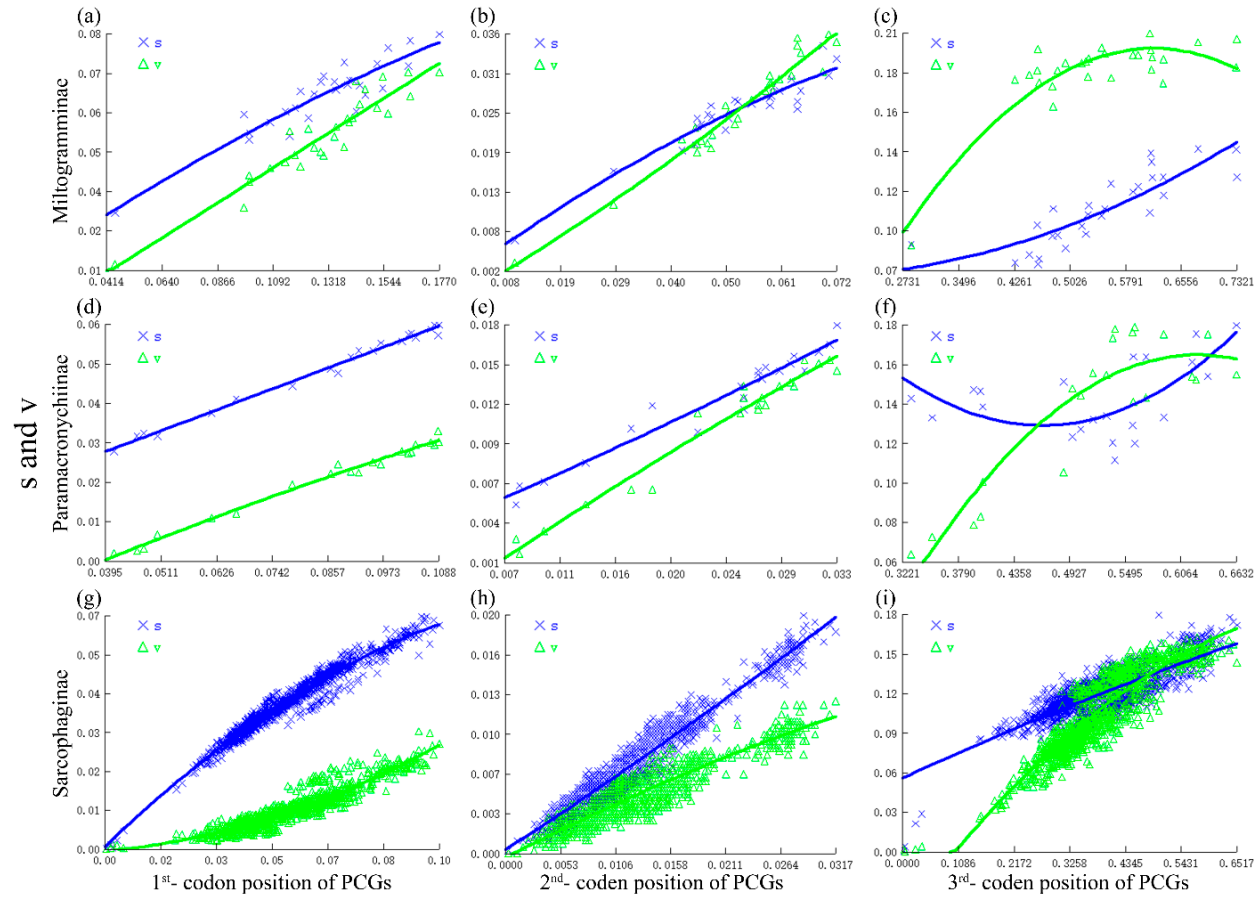
Figure S8. Nucleotide substitution saturation plots of all the 13 PCGs of three subfamilies based on 1st-, 2nd-, and 3rd- codon positions (a, b, c: Miltogrammatinae; d, e, f: Paramacronychiinae; g, h, i: Sarcophaginae). Transition and transversion are represented by blue and green plots, respectively.

Table S1. Collection information of specimens of flesh flies used for DNA extraction in this study.

Species	Collection Location	Collection Time
Agria mihalyii	Songshan, Beijing, China	5-Jun-16
Blaesoxipha lapidosa	Kalamaili Nature Reserve, Xinjiang, China	1-Jul-15
Taxigramma karakulensis	Kalamaili Nature Reserve, Xinjiang, China	29-Apr-16
Wohlfahrtia balassogloi	Kalamaili Nature Reserve, Xinjiang, China	23-Apr-15
Wohlfahrtia fedtschenkoi	Kalamaili Nature Reserve, Xinjiang, China	16-May-15

Table S2. The sequence characteristics of mitochondrial genome of flesh flies.

Gene		Agria_mihalyii				Blaesoxipha_campestris				Taxigramma_karakulensis				Wohlfahrtia_balassogloi				Wohlfahrtia_fedtschenkoi		
	Strand	Location^a	Length(bp)	Codon Start/Anti Stop	Strand	Location^a	Length(bp)	Codon Start/Anti Stop	Strand	Location^a	Length(bp)	Codon Start/Anti Stop	Strand	Location^a	Length(bp)	Codon Start/Anti Stop	Strand	Location^a	Length(bp)	Codon Start/Anti Stop
tRNA^{Ile}	+	1-66	66	AAT	+	1-66	66	AGT	+	1-65	66	AAT	+	1-66	66	AAT		1-66	66	AAT
tRNA Gln	-	64-132	69	TAT	-	64-132	69	TAT	-	63-131	69	TAT	-	64-132	69	TAT	-	64-132	69	TAT
tRNA Met	+	132-200	69	AAA	+	135-203	69	AAA	+	131-199	69	AAA	+	135-203	69	AAA	+	140-208	69	AAA
ND2	+	201-1217	1017	ATT/TAA	+	204-1220	1017	ATT/TAA	+	200-1216	1017	ATT/TAA	+	204-1220	1017	ATT/TAA	+	209-1225	1017	ATT/TAA
tRNA Trp	+	1216-1283	68	AAG	+	1219-1286	68	AAG	+	1215-1281	67	AAG	+	1219-1286	68	AAG	+	1224-1291	68	AAG
tRNA Cys	-	1276-1338	63	GGC	-	1279-1342	64	AGC	-	1274-1335	62	GGC	-	1279-1341	63	GGC	-	1284-1348	65	GGC
tRNA^{Tyr}	-	1352-1417	66	GAT	-	1351-1416	66	GAT	-	1349-1413	65	GAT	-	1350-1415	66	GAT	-	1357-1422	66	GAT
COI	+	1416-2949	1534	TCG/T	+	1415-2948	1534	TCG/T	+	1412-2945	1534	TCG/T	+	1414-2947	1534	TCG/T	+	1421-2954	1534	TCG/T
tRNA Leu	+	2950-3015	66	TCT	+	2949-3014	66	TCT	+	2946-3011	66	TCT	+	2948-3013	66	TCT	+	2955-3020	66	TCT
COII	+	3023-3710	688	ATG/CTT	+	3019-3703	685	ATG/T	+	3015-3699	685	ATG/T	+	3019-3706	688	ATG/T	+	3026-3713	688	ATG/T
tRNA Lys	+	3711-3780	70	CAT	+	3707-3777	71	CAT	+	3703-3773	71	CAT	+	3707-3776	70	CAT	+	3714-3784	71	CAT
tRNA Asp	+	3781-3847	67	AAA	+	3777-3843	67	AAA	+	3783-3847	65	AAA	+	3777-3844	68	AAA	+	3785-3851	67	AAA
ATP8	+	3848-4012	165	ATT/TAA	+	3844-4008	165	ATT/TAA	+	3848-4012	165	ATT/TAA	+	3845-4006	162	ATC/TAA	+	3851-4012	162	ATC/TAA
ATP6	+	4006-4683	678	ATG/TAA	+	4002-4679	678	ATG/TAA	+	4006-4677	672	ATG/TAA	+	4000-4677	678	ATG/TAA	+	4006-4683	678	ATG/TAA
COIII	+	4683-5471	789	ATG/TAA	+	4679-5467	789	ATG/TAA	+	4677-5465	789	ATG/TAA	+	4677-5465	789	ATG/TAA	+	4683-5471	789	ATG/TAA
tRNA Gly	+	5478-5542	65	ATC	+	5474-5538	65	ATC	+	5468-5532	65	ATT	+	5468-5532	65	ATC	+	5478-5542	65	ATC
ND3	+	5543-5896	354	ATT/TAA	+	5536-5892	357	ATA/TAA	+	5533-5886	354	ATT/TAA	+	5533-5886	354	ATT/TAA	+	5543-5896	354	ATT/TAA
tRNA Ala	+	5921-5988	65	AGG	+	5895-5959	65	AGG	+	5887-5949	63	AGG	+	5889-5952	64	AGG	+	5899-5963	65	AGG
tRNA Arg	+	5989-6051	63	GAA	+	5959-6022	64	GAA	+	5950-6013	64	GAA	+	5953-6015	63	GAA	+	5964-6026	63	AAT
tRNA Asn	+	6052-6116	65	TTA	+	6024-6088	65	TTA	+	6015-6078	64	TTA	+	6017-6081	65	TTA	+	6026-6090	65	TTA

Gene	Strand	Position	Size	Codon	Strand	Position	Size	Codon	Strand	Position	Size	Codon	Strand	Position	Size	Codon	Strand	Position	Size	Codon
tRNA-Ser	+	6117-6184	68	GAA	+	6088-6157	70	AGA	+	6079-6146	68	GAA	+	6082-6149	68	GAA	+	6091-6158	68	GAA
tRNA-Glu	+	6185-6251	67	ATT	+	6157-6222	66	ATT	+	6154-6218	65	ATT	+	6151-6217	67	GTT	+	6159-6226	68	ATT
tRNA-Phe	-	6270-6335	66	ATT	-	6241-6306	66	ATT	-	6237-6303	67	ATT	-	6236-6300	65	ATT	-	6245-6309	65	ATT
ND5	-	6336-8055	1720	ATT/T	-	6307-8023	1717	ATT/T	-	6304-8023	1720	ATT/T	-	6301-8020	1720	ATC/T	-	6310-8029	1720	ATC/T
tRNA-His	-	8071-8134	64	ATT	-	8039-8101	63	TTA	-	8039-8102	64	ATT	-	8036-8101	66	ATT	-	8040-8105	66	AAT
ND4	-	8133-9473	1341	ATG/TAT	-	8104-9442	1339	ATG/T	-	8101-9441	1341	ATG/TAT	-	8102-9440	1339	ATG/T	-	8111-9449	1339	ATG/T
ND4L	-	9467-9763	297	ATG/TAA	-	9436-9732	297	ATG/TAA	-	9435-9731	297	ATG/TAA	-	9434-9730	297	ATG/TAA	-	9443-9739	297	ATG/TAA
tRNA-Thr	+	9766-9830	65	GTT	+	9735-9799	65	GTT	+	9734-9798	65	GTT	+	9733-9797	65	GTT	+	9742-9806	65	GTT
tRNA-Pro	-	9831-9896	66	CAA	-	9800-9864	65	CAA	-	9799-9863	65	CAA	-	9798-9863	66	CAA	-	9807-9872	66	CAA
ND6	+	9899-10423	525	ATT/TAA	+	9867-10391	525	ATT/TAA	+	9866-10390	525	ATT/TAA	+	9866-10390	525	ATT/TAA	+	9875-10399	525	ATT/TAA
CYTB	+	10423-11559	1137	ATG/TAG	+	10391-11527	1137	ATG/TAG	+	10390-11526	1137	ATG/TAG	+	10390-11526	1137	ATG/TAG	+	10399-11535	1137	ATG/TAG
tRNA-Ser	+	11558-11625	68	AGT	+	11526-11593	68	AGT	+	11525-11589	65	AGT	+	11525-11592	68	AGT	+	11534-11601	68	AGT
ND1	-	11642-12580	939	ATA/TAA	-	11610-12548	939	ATA/TAA	-	11611-12531	921	ATA/TAA	-	11609-12547	939	ATA/TAA	-	11618-12556	939	ATA/TAA
tRNA-Leu	-	12591-12654	64	ACT	-	12559-12623	65	GCT	-	12545-12609	65	ACT	-	12558-12623	66	ACT	-	12567-12632	66	ACT
lrRNA	-	12651-13979	1329		-	12620-13947	1328		-	12606-13934	1329		-	12621-13944	1324		-	12632-13953	1322	
tRNA-Val	-	13976-14047	72	CAA	-	13948-14019	72	CAA	-	13935-14006	72	CAA	-	13945-14016	72	CAA	-	13954-14025	72	CAA
srRNA	-	14048-14830	783		-	14020-14804	785		-	14007-14790	784		-	14017-14800	784		-	14026-14815	790	

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

Table S3. Nucleotide composition of the mitogenomes of flesh flies.

Dataset	Species	A%	T%	AT%	AT Skew	G%	C%	GC%	GC Skew
Whole genome	*Agria mihalyii*	38.51	35.92	74.43	0.03	9.91	15.66	25.57	-0.23
	Blaesoxipha lapidosa	39.06	36.11	75.17	0.04	9.52	15.31	24.83	-0.23
	Taxigramma karakulensis	40.08	39.07	79.15	0.01	8.68	12.17	20.85	-0.17
	Wohlfahrtia balassogloi	38.66	37.36	76.02	0.02	9.16	14.82	23.98	-0.24
	Wohlfahrtia fedtschenkoi	38.74	37.19	75.93	0.02	9.25	14.82	24.07	-0.23
All codon	*Agria mihalyii*	29.84	41.97	71.81	-0.17	13.61	14.58	28.19	-0.03
	Blaesoxipha lapidosa	31.26	42.02	73.28	-0.15	13.22	13.50	26.72	-0.01
	Taxigramma karakulensis	32.62	44.39	77.01	-0.15	11.62	11.36	22.99	0.01
	Wohlfahrtia balassogloi	30.91	42.43	73.34	-0.16	13.02	13.64	26.66	-0.02
	Wohlfahrtia fedtschenkoi	30.80	42.35	73.15	-0.16	13.06	13.79	26.85	-0.03
tRNA	*Agria mihalyii*	38.98	37.13	76.11	0.02	11.47	12.42	23.89	-0.04
	Blaesoxipha lapidosa	38.16	37.88	76.04	0.00	12.08	11.88	23.96	0.01
	Taxigramma karakulensis	38.80	38.80	77.60	0.00	11.03	11.37	22.40	-0.02
	Wohlfahrtia balassogloi	38.98	37.41	76.38	0.02	11.54	12.08	23.62	-0.02
	Wohlfahrtia fedtschenkoi	38.80	37.58	76.38	0.02	11.64	11.98	23.62	-0.01
lrRNA	*Agria mihalyii*	39.95	41.46	81.41	-0.02	12.26	6.32	18.59	0.32
	Blaesoxipha lapidosa	39.53	41.72	81.25	-0.03	12.58	6.17	18.75	0.34
	Taxigramma karakulensis	39.80	42.96	82.77	-0.04	11.21	6.02	17.23	0.30
	Wohlfahrtia balassogloi	39.80	41.16	80.97	-0.02	12.61	6.42	19.03	0.33
	Wohlfahrtia fedtschenkoi	39.49	41.07	80.56	-0.02	12.78	6.66	19.44	0.32
srRNA	*Agria mihalyii*	36.91	39.59	76.50	-0.04	15.58	7.92	23.50	0.33
	Blaesoxipha lapidosa	37.45	39.62	77.07	-0.03	15.54	7.39	22.93	0.36
	Taxigramma karakulensis	41.07	38.90	79.97	0.03	12.63	7.40	20.03	0.26
	Wohlfahrtia balassogloi	37.24	38.65	75.89	-0.02	15.43	8.67	24.11	0.28
	Wohlfahrtia fedtschenkoi	37.59	38.86	76.46	-0.02	15.19	8.35	23.54	0.29
Control region	*Agria mihalyii*	42.33	41.04	83.37	0.02	7.51	9.13	16.63	-0.10
	Blaesoxipha lapidosa	49.07	39.75	88.82	0.10	3.42	7.76	11.18	-0.39
	Taxigramma karakulensis	47.40	42.44	89.85	0.06	3.65	6.50	10.15	-0.28
	Wohlfahrtia balassogloi	44.61	43.60	88.21	0.01	3.86	7.94	11.79	-0.35
	Wohlfahrtia fedtschenkoi	46.12	42.01	88.13	0.05	3.99	7.88	11.87	-0.33