Additional information for

Culicidae evolutionary history focusing on the Culicinae subfamily based on mitochondrial phylogenomics

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Supplementary table 1. Annotation of draft mitogenomes using MITOS.

Species	*nad2*	*COI*	*COII*	*atp8*	*atp6*	*COIII*	*nad3*	*nad5*	*nad4*	*nad4l*	*nad6*	*cytb*	*nad1*	*rrnL*	*rrnS*	*tRNA*	*PCG*	*rRNA*	Total of genes
Ad. squamipennis	1	1	1	-	-	1	1	1	**1**	-	-	1	1	**1**	1	16	9	2	27
Ae. alboannulatus	1	1	1	-	-	**1**	1	1	1	**1**	1	**1**	**1**	1	1	13	11	2	26
Ae. camptorhynchus	1	1	1	1	-	**1**	1	**1**	1	**1**	1	**1**	**1**	1	1	16	12	2	30
Ae. detritus	1	1	1	1	1	**1**	1	**1**	**1**	**1**	1	**1**	**1**	1	1	16	13	2	31
Ae. fluviatilis	1	1	1	1	1	1	-	**1**	1	**1**	1	**1**	1	1	-	4	12	1	17
Ae. polynesiensis	1	1	1	**1**	-	**1**	1	**1**	**1**	1	1	**1**	**1**	1	1	20	12	2	34
Ae. riversi	-	1	1	1	1	1	1	**1**	1	-	-	**1**	1	1	-	6	10	1	17
Ae. scapularis	1	**1**	1	1	**1**	1	-	**1**	1	-	1	**1**	1	-	1	13	11	1	25
Ae. taeniorhynchus	1	1	1	1	1	1	1	1	1	**1**	1	1	1	1	1	22	13	2	37
An. albimanus	1	1	1	**1**	-	**1**	1	**1**	**1**	**1**	1	**1**	**1**	1	1	20	12	2	34
An. aquasalis	1	1	1	1	-	**1**	-	1	1	-	-	**1**	1	1	**1**	3	9	2	14
An. freeborni	1	1	1	**1**	-	**1**	1	**1**	1	**1**	1	**1**	**1**	1	1	19	12	2	33
An. nuneztovari	1	1	1	1	-	**1**	1	**1**	1	1	1	**1**	1	1	1	9	12	2	23
An. quadriannulatus	1	1	1	**1**	-	**1**	1	**1**	**1**	**1**	1	**1**	**1**	1	1	20	12	2	34
Cq. albicosta	1	1	1	**1**	1	1	1	1	**1**	**1**	1	1	1	1	1	21	13	2	36
Cq. chrysonotum	**1**	1	1	-	1	1	1	1	1	1	1	**1**	1	1	1	20	12	2	34
Cq. hermanoi	**1**	1	1	1	1	1	1	**1**	1	-	-	1	-	1	1	14	10	2	26
Cq. juxtamansonia	1	1	1	-	1	1	-	1	1	-	-	1	-	1	1	8	8	2	18
Cq. venezuelensis	1	1	1	1	1	1	1	**1**	**1**	**1**	1	1	1	1	1	19	13	2	34
Cx. amazonensis	1	1	1	**1**	1	1	1	1	1	1	1	1	1	1	1	21	13	2	36
Cx. australicus	1	1	1	1	1	1	1	**1**	1	**1**	1	**1**	1	1	1	17	13	2	32
Cx. corniger	1	1	**1**	1	-	1	-	1	1	1	-	1	-	1	**1**	9	9	2	20
Cx. globocoxitus	1	1	1	1	-	**1**	1	**1**	1	**1**	1	**1**	**1**	1	1	17	12	2	31
Cx. hortensis	1	1	1	-	-	**1**	1	**1**	1	1	1	**1**	**1**	1	1	12	11	2	25
Cx. pipiens molestus	**1**	1	1	1	1	1	1	**1**	1	**1**	1	**1**	1	1	1	6	13	2	21
Cx. nigripalpus	1	1	1	1	1	1	1	1	**1**	**1**	1	1	1	1	1	22	13	2	37
Cx. tarsalis	1	1	1	1	-	**1**	1	**1**	**1**	**1**	1	**1**	**1**	1	1	19	12	2	33
Cx. torrentium	1	1	1	1	1	1	1	**1**	1	**1**	1	**1**	1	1	1	11	13	2	26
Ma. wilsoni	1	1	1	1	1	1	1	1	**1**	1	-	1	1	1	1	14	12	2	28
Ma.humeralis	-	1	**1**	-	1	-	1	**1**	-	-	-	1	1	1	1	5	7	2	14
Ma.titillans	**1**	1	**1**	-	1	1	1	1	**1**	-	1	1	1	1	1	14	11	2	27
Ps. cingulata	**1**	1	1	1	1	1	1	1	**1**	1	**1**	**1**	**1**	1	1	21	13	2	36
Ps.albipes	1	1	1	1	-	**1**	1	**1**	**1**	**1**	1	1	**1**	1	1	19	12	2	33
Tp. aranoides	1	1	-	-	-	**1**	-	1	-	1	-	**1**	1	1	1	-	7	2	9
Tr. digitatum	**1**	1	1	-	1	1	1	1	**1**	**1**	-	**1**	-	**1**	1	16	10	2	28
Tx. amboinensis	1	1	1	1	1	**1**	1	**1**	**1**	**1**	1	**1**	1	**1**	1	19	13	2	34
Ur. pulcherrima	1	**1**	1	-	1	1	1	1	1	1	1	**1**	1	1	1	18	12	2	32

Legend: Bold names represent sequenced mitogenomes. Disrupted or duplicated gene are represented by bold numbers. Unidentified gene are represented by -.

Supplementary table 2. Predicted TMRCA values from Bayesian inference analysis for studied species clades.

Node*	HPD 95%[1]	Median dating[1]	PP of analysis [1]	PP of analysis [2]	UFboot of analysis [2]	PP of analysis [3]	PP of analysis [4]	PP of analysis [5]	UFboot of analysis [5]	PP of analysis [6]	UFboot of analysis[7]
A	243.79-332.41	273.74	1	1	-	1	1	1	-	1	-
B	145.88-232.95	182.75	1	1	-	1	1	1	-	1	-
F	128.09-204.91	160.86	1	-	-	-	-	-	-	-	-
I	124.77-200.69	157.02	0.49	0.81	-	0.49	-	0.96	69	0.99	-
H	117.92-195.06	151.36	0.5	-	-	-	-	-	-	-	-
C	114.58-187.29	145.83	1	1	100	1	1	1	100	1	100
N	101.87-168.21	130.49	1	1	93	1	1	0.99	92	1	79
J	88.78-146.1	113.73	1	1	100	1	1	-	-	0.81	83
D	87.14-141.71	110.73	1	1	100	1	1	1	100	1	100
U	79.07-135.87	103.9	1	1	100	1	1	1	100	1	100
O	81.02-132.17	102.87	1	1	100	1	1	1	100	1	100
Q	74.02-121.02	94.04	1	1	100	1	1	1	100	1	-
K	66.37-115.22	88.32	1	1	100	1	1	1	100	1	100
L	66.31-109.73	85.29	1	1	100	1	1	1	100	1	100
S	53.53-88.54	68.94	1	-	100	1	1	-	-	-	-
P	45.84-84.94	63.25	1	1	100	1	1	1	100	1	100
T	43.26-73.19	56.37	0.92	-	65	0.95	-	-	-	-	-
R	24.82-45.09	33.9	1	1	100	1	1	1	100	1	100
V	16.73-28.91	22.09	1	1	100	1	-	-	100	0.85	89
Z	15.05-26.45	20.08	1	1	100	1	0.85	0.86	97	-	98
Y	9.82-17.37	13.15	1	1	-	1	1	-	-	0.96	99
X	7.78-16.85	11.85	1	1	100	1	1	1	100	1	99
M	3.13-8.92	5.71	1	1	100	1	1	1	100	1	100
E	2.35-4.42	3.27	1	1	100	1	1	1	100	1	100
G	1.43-2.81	2.04	1	1	100	1	1	1	-	1	-

The HPD95% values represent the confidence intervals for each node. PP represents the probability posterior. UFboot represents ultrafast bootstrapping. * Nodes present in Fig. 3 and Fig. 4. [1] Analysis based on partitioned PCG with codon partition (1st + 2nd and 3rd separately). [2] Analysis based on complete mitogenome sequences. [3] Analysis based on partitioned PCG without codon partition. [4] Analysis based on Partitioned PCG without 3rd codon positions. [5] Analysis based on concatenated predicted amino acid sequences. [6] Analysis based on partitioned predicted amino acid sequences. [7] Analysis based on concatenated PCG without 3rd codon position.

Supplementary table 3. Information of sequenced samples.

Taxon	**Genus**	**Subgenus**	**Tribe**	**Nº of specimens**	**Location**	**Nº of reads (Mi)**
Ad. squamipennis	Aedeomyia	Aedeomyia	Aedeomyiini	3	Aldeia-PE+REC[2]	3.9
Ae. scapularis	Aedes	Ochlerotatus	Aedini	7	Juazeiro-BA	7
Ae. taeniorhynchus	Aedes	Ochlerotatus	Aedini	10	São Luís -MA	11.3
Cq. hermanoi	Coquillettidia	Rhynchotaenia	Mansoniini	1	Aldeia-PE	1.8
Cq. chrysonotum	Coquillettidia	Rhynchotaenia	Mansoniini	14	PDI[1]	6.2
Cq. albicosta	Coquillettidia	Rhynchotaenia	Mansoniini	3	PDI[1]	2.4
Cq. juxtamansonia	Coquillettidia	Rhynchotaenia	Mansoniini	2	REC[2]	5.5
Cq. venezuelensis	Coquillettidia	Rhynchotaenia	Mansoniini	4	PDI[1]+REC[2]	4.3
Cx. amazonensis	Culex	Aedinus	Culicini	1	REC[2]	2.5
Cx. corniger	Culex	Phenacomyia	Culicini	5	Mato Grosso	3.9
Cx. nigripalpus	Culex	Culex	Culicini	6	PDI[1]	4.8
Ma. titillans	Mansonia	Mansonia	Mansoniini	3	REC[2]	6.6
Ma. wilsoni	Mansonia	Mansonia	Mansoniini	2	REC[2]	8.2
Ma. humeralis	Mansonia	Mansonia	Mansoniini	1	Aldeia-PE	2.2
Ps. cingulata	Psorophora	Grabhamia	Aedini	3	Mato Grosso	4.9
Tr. digitatum	Trichoprosopon	Trichoprosopon	Sabethini	1	Mato Grosso	7.6
Ur. pulcherrima	Uranotaenia	Uranotaenia	Uranotaeniini	1	REC[2]	1.1

PE refers to state of Pernambuco, Brazil. MA refers to state of Maranhão, Brazil. BA refers to state of Bahia, Brazil. Mi refers to million of reads. [1] Parque Estadual Dois Irmãos-PE . [2] Reserva Ecológica de Carnijó-PE

Supplementary table 4. NCBI accession number of mitochondrial genomes retrieved from NCBI.

Species	Genus	Subgenus	Tribe	Access	Length (bp)	References *
Aedes aegypti	Aedes	Stegomyia	Aedini	NC_010241.1	16,655	1
Aedes albopictus	Aedes	Stegomyia	Aedini	NC_006817.1	16,665	-
Aedes notoscriptus	Aedes	Finlaya	Aedini	KM676218.1	15,846	2
Aedes vigilax	Aedes	Ochlerotatus	Aedini	KP995260.1	15,877	3
Anopheles albitarsis	Anopheles	Nyssorhynchus	-	HQ335344.1	15,413	4
Anopheles bellator	Anopheles	Kerteszia	-	KU551287.1	15,668	5
Anopheles christyi	Anopheles	Cellia	-	NC_028214.1	14,967	6
Anopheles cruzii	Anopheles	Kerteszia	-	KJ701506.1	15,449	-
Anopheles culicifacies	Anopheles	Cellia	-	NC_027502.1	15,330	7
Anopheles darlingi	Anopheles	Nyssorhynchus	-	NC_014275.1	15,386	8
Anopheles deaneorum	Anopheles	Nyssorhynchus	-	HQ335347.1	15,424	4
Anopheles dirus	Anopheles	Cellia	-	JX219731.1	15,404	9
Anopheles epiroticus	Anopheles	Cellia	-	NC_028217.1	15,379	6
Anopheles farauti	Anopheles	Cellia	-	NC_020770.1	15,412	9
Anopheles funestus	Anopheles	Cellia	-	NC_008070.1	15,354	10
Anopheles gambiae	Anopheles	Cellia	-	NC_002084.1	15,363	11
Anopheles homunculus	Anopheles	Kerteszia	-	KU551283.1	15,739	5
Anopheles janconnae	Anopheles	Nyssorhynchus	-	HQ335348.1	15,425	4
Anopheles koliensis	Anopheles	Cellia	-	JX219743.1	15,412	9
Anopheles melas	Anopheles	Cellia	-	NC_028219.1	15,366	6
Anopheles merus	Anopheles	Cellia	-	NC_028220.1	15,365	6
Anopheles punctulatus	Anopheles	Cellia	-	NC_028222.1	15,322	6
Anopheles quadrimaculatus	Anopheles	Anopheles	-	NC_000875.1	15,455	12
Anopheles sinensis	Anopheles	Anopheles	-	NC_028016.1	15,076	-
Anopheles stephensi	Anopheles	Cellia	-	NC_028223.1	15,387	6
Bironella hollandi	Bironella	Brugella	-	NC_037796.1	15,772	13
Chagasia sp.	Chagasia	-	-	MF381717.1	15,717	13
Culex bidens	Culex	Culex	Culicini	NC_037809.1	15,583	13
Culex gelidus	Culex	Culex	Culicini	KX753344.1	15,600	14
Culex bilineatus	Culex	Culex	Culicini	NC_037819.1	15,599	13
Culex brami	Culex	Culex	Culicini	NC_037828.1	15,586	13
Culex camposi	Culex	Culex	Culicini	NC_036008.1	15,570	15

Culex coronator	Culex	Culex	Culicini	NC_036006.1	15,576	15
Culex declarator	Culex	Culex	Culicini	NC_037822.1	15,575	13
Culex dolosus	Culex	Culex	Culicini	MF381620.1	15,851	13
Culex chidesteri	Culex	Culex	Culicini	NC_037826.1	16,052	13
Culex lygrus	Culex	Culex	Culicini	MF381718.1	15,572	13
Culex mollis	Culex	Culex	Culicini	MF381705.1	15,576	13
Culex pipiens pallens	Culex	Culex	Culicini	KT851543.1	15,617	16
Culex pipiens pipiens	Culex	Culex	Culicini	NC_015079.1	14,856	-
Culex quinquefasciatus	Culex	Culex	Culicini	NC_014574.1	15,587	1
Culex surinamensis	Culex	Culex	Culicini	MF381615.1	15,568	13
Culex tritaeniorhynchus	Culex	Culex	Culicini	KT851544.1	14,844	16
Culex usquatissimus	Culex	Culex	Culicini	NC_036007.1	15,574	15
Culex usquatus	Culex	Culex	Culicini	NC_036005.1	15,573	15
Drosophila melanogaster	Drosophila	Drosophilinae	Drosophilini	U37541.1	19,517	17
Haemagogus janthinomys	Haemagogus	Haemagogus	Aedini	NC_028025.1	15,698	18
Sabethes belisarioi	Sabethes	Sabethes	Sabethini	MF957171.1	15,911	19
Sabethes chloropterus	Sabethes	Sabethoides	Sabethini	NC_037499.1	15,609	19
Sabethes glaucodaemon	Sabethes	Sabethoides	Sabethini	NC_037500.1	15,620	19

* References listed at the end of the document.

Supplementary table 5. Information about retrieved raw sequencing datasets from NCBI SRA database.

Species	Genus	Subgenus	Tribe	SRA accession number	Original study
Ae. alboannulatus	Aedes	Finlaya	Aedini	SRR5665565	RNA-Seq (Species virome)
Ae. camptorhynchus	Aedes	Ochlerotatus	Aedini	SRR5665568	RNA-Seq (Species virome)
Ae. detritus	Aedes	Ochlerotatus	Aedini	SRR6489869	RNA-Seq (Differential gene expression)
Ae. fluviatilis	Aedes	Ochlerotatus	Aedini	SRR3574348	RNA-Seq (Differential gene expression)
Ae. polynesiensis	Aedes	Stegomyia	Aedini	ERR1706659	RNA-Seq (Comparative transcriptomes)
Ae. riversi	Aedes	Stegomyia	Aedini	ERR1706661	RNA-Seq (Comparative transcriptomes)
An. albimanus	Anopheles	Nyssorhynchus	-	SRR314655	Genome sequencing
An. aquasalis	Anopheles	Nyssorhynchus	-	SRR927456	RNA-Seq (Transcriptome)
An. freeborni	Anopheles	Anopheles	-	SRR908289	RNA-Seq (Saliva transcriptome)
An. nuneztovari	Anopheles	Nyssorhynchus	-	SRR6471062	RNA-Seq (Saliva transcriptome)
An. quadriannulatus	Anopheles	Cellia	-	SRR529986	Genome sequencing
Cx. australicus	Culex	Culex	Culicini	SRR5665566	RNA-Seq (Species virome)
Cx. globocoxitus	Culex	Culex	Culicini	SRR5665567	RNA-Seq (Species virome)
Cx. hortensis	Culex	Maillotia	Culicini	SRR1324883	RNA-Seq (Comparative transcriptomes)
Cx. pipiens molestus	Culex	Culex	Culicini	SRR1462325	RNA-Seq (Comparative transcriptomes)
Cx. tarsalis	Culex	Culex	Culicini	SRR5149179	RNA-Seq (Transcriptome)
Cx. torrentium	Culex	Culex	Culicini	SRR1324895	RNA-Seq

					(Comparative transcriptomes)
Ps.albipes	Psorophora	Janthinossoma	Aedini	SRR908278	RNA-Seq (Saliva transcriptome)
Tp. aranoides	Tripteroides	Rachionotomyia	Sabethini	SRR6155938	RNA-Seq (Transcriptome)
Tx. amboinensis	Toxorhynchites	Toxorhynchites	Toxorhynchitini	SRR2061845	RNA-Seq (Transcriptome)

Supplementary table 6. Calibration points used in BEAST 1.8.4

Clade	**Represented taxa**	**Minimum bound**	**Maximum bound**	**References**
Diptera	*Culicidae* and *D. melanogaster*	240	241	KRZEMIŃSKI, KRZEMIŃSKA & PAPIER, 1994[20]
Culicidae	*Culicinae* e *Anophelinae*	90	99	BORKENT & GRIMALDI, 2004[21]
Culicinae	*Culicinae*	76.5	79	POINAR, ZAVORTINK., PIKE & JOHNSTON, 2000[22]
Anophelinae	*Anophelinae*	15	34	ZAVORTINK & POINAR, 2000[23]

Supplementary table 7. Evolutionary models used in BEAST analysis.

Partition	Best nucleotide model [1]	Best protein model [2]
Nucleotide mitogenome alignment	GTR+G+I	-
Concatenated protein alignment	-	mtREV+G+I
Atp6	GTR+G+I	mtREV+G+I
Atp8	GTR+G	JTT+G+I
COIII	GTR+G+I	mtREV+G+I
COII	GTR+G+I	mtREV+G
COI	GTR+G+I	JTT+G+I
CytB	GTR +G+I	mtREV+G+I
Nad1	GTR +G+I	JTT+G+I
Nad2	GTR +G+I	JTT+G
Nad3	GTR +G+I	mtREV+G+I
Nad4L	GTR +G+I	mtREV+G
Nad4	GTR +G+I	JTT+G+I
Nad5	GTR +G+I	JTT+G
Nad6	GTR +G+I	mtREV+G
rrnL	GTR +G+I	-
rrnS	GTR +G+I	-

[1] Best model as suggested by SMS. [2] More likely model suggested by Prottest and present in BEAST 1.8.4.

Supplementary file 1. Test of substitution saturation of mitochondrial PCG.

```
 Analysis performed on all sites.
Testing whether the observed Iss is significantly lower than Iss.c.
  IssSym is Iss.c assuming a symmetrical topology.
  IssAsym is Iss.c assuming an asymmetrical topology.
Note: two-tailed tests are used.
```

COI 1st+2nd codon positions:

```
NumOTU     Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
    4    0,119     0,821  72,061  1023  0,0000    0,789  68,777  1023  0,0000
    8    0,120     0,789  64,934  1023  0,0000    0,684  54,715  1023  0,0000
   16    0,124     0,773  64,019  1023  0,0000    0,576  44,550  1023  0,0000
   32    0,147     0,750  59,214  1023  0,0000    0,445  29,334  1023  0,0000
```

COI 3rd codon positions:

```
NumOTU     Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
    4    0,537     0,796  11,883   510  0,0000    0,762  10,330   510  0,0000
    8    0,580     0,753   7,928   510  0,0000    0,641   2,812   510  0,0051
   16    0,600     0,722   5,837   510  0,0000    0,513   4,163   510  0,0000
   32    0,608     0,703   4,751   510  0,0000    0,378  11,490   510  0,0000
```

COII 1st+2nd codon positions:

```
NumOTU     Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
=============================================================================
    4    0,258     0,793  31,684   456  0,0000    0,759  29,701   456  0,0000
    8    0,224     0,747  30,309   456  0,0000    0,636  23,846   456  0,0000
   16    0,274     0,713  25,787   456  0,0000    0,504  13,505   456  0,0000
   32    0,285     0,697  25,475   456  0,0000    0,371   5,276   456  0,0000
```

COII 3rd codon positions:

```
NumOTU     Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
    4    0,630     0,777   4,490   227  0,0000    0,762   4,033   227  0,0001
    8    0,673     0,732   1,878   227  0,0617    0,631   1,319   227  0,1885
   16    0,718     0,654   2,087   227  0,0380    0,459   8,498   227  0,0000
   32    0,750     0,685   2,250   227  0,0254    0,363  13,387   227  0,0000
```

COIII 1st+2nd codon positions:

```
NumOTU     Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
    4    0,230     0,797  34,999   525  0,0000    0,764  32,946   525  0,0000
    8    0,236     0,754  32,263   525  0,0000    0,643  25,328   525  0,0000
   16    0,261     0,725  29,985   525  0,0000    0,515  16,431   525  0,0000
   32    0,281     0,705  29,101   525  0,0000    0,378   6,659   525  0,0000
```

COIII 3rd codon positions:

```
NumOTU     Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
    4    0,651     0,778   4,004   262  0,0001    0,758   3,356   262  0,0009
```

```
   8    0,716     0,732  0,487   262   0,6265     0,627   2,761   262   0,0062
  16     0,748     0,666   2,525   262   0,0121     0,465   8,773   262   0,0000
  32     0,796     0,682   3,501   262   0,0005     0,356  13,527   262   0,0000
```

ND1 1st+2nd codon positions:

```
NumOTU       Iss  Iss.cSym       T    DF        P Iss.cAsym       T    DF        P
==============================================================================
   4    0,232     0,803  41,973   627   0,0000     0,772  39,681   627   0,0000
   8    0,270     0,763  31,688   627   0,0000     0,653  24,621   627   0,0000
  16     0,257     0,741  33,136   627   0,0000     0,530  18,739   627   0,0000
  32     0,307     0,715  27,312   627   0,0000     0,388   5,436   627   0,0000
```

ND1 3rd codon positions:

```
NumOTU       Iss  Iss.cSym       T    DF        P Iss.cAsym       T    DF        P
==============================================================================
   4    0,616     0,782   5,860   313   0,0000     0,756   4,938   313   0,0000
   8    0,773     0,735   1,302   313   0,1939     0,626   5,017   313   0,0000
  16     0,787     0,682   3,693   313   0,0003     0,475  10,927   313   0,0000
  32     0,776     0,683   3,322   313   0,0010     0,354  15,165   313   0,0000
```

ND2 1st+2nd codon positions:

```
NumOTU       Iss  Iss.cSym       T    DF        P Iss.cAsym       T    DF        P
==============================================================================
   4    0,462     0,806  19,700   683   0,0000     0,775  17,927   683   0,0000
   8    0,505     0,768  14,386   683   0,0000     0,658   8,401   683   0,0000
  16     0,505     0,747  13,360   683   0,0000     0,538   1,812   683   0,0704
  32     0,598     0,721   6,691   683   0,0000     0,396  10,982   683   0,0000
```

ND2 3rd codon positions:

```
NumOTU       Iss  Iss.cSym       T    DF        P Iss.cAsym       T    DF        P
==============================================================================
   4    0,778     0,784   0,226   341   0,8212     0,756   0,810   341   0,4185
   8    0,828     0,737   3,275   341   0,0012     0,627   7,222   341   0,0000
  16     1,031     0,689  12,631   341   0,0000     0,481  20,299   341   0,0000
  32     1,037     0,685  13,325   341   0,0000     0,356  25,823   341   0,0000
```

ND3 1st+2nd codon positions:

```
NumOTU       Iss  Iss.cSym       T    DF        P Iss.cAsym       T    DF        P
==============================================================================
   4    0,367     0,777  16,360   233   0,0000     0,761  15,719   233   0,0000
   8    0,332     0,732  15,880   233   0,0000     0,630  11,819   233   0,0000
  16     0,379     0,656  10,371   233   0,0000     0,460   3,005   233   0,0029
  32     0,415     0,684  10,433   233   0,0000     0,362   2,066   233   0,0400
```

ND3 3rd codon positions:

```
NumOTU       Iss  Iss.cSym       T    DF        P Iss.cAsym       T    DF        P
==============================================================================
   4    0,669     0,793   2,773   116   0,0065     0,821   3,396   116   0,0009
   8    0,770     0,773   0,069   116   0,9452     0,707   1,383   116   0,1694
  16     0,826     0,599   4,990   116   0,0000     0,469   7,845   116   0,0000
  32     0,950     0,763   4,278   116   0,0000     0,512  10,022   116   0,0000
```

ND4L 1st+2nd codon positions:

```
NumOTU      Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
   4    0,330     0,780  17,564   157   0,0000     0,784  17,747   157   0,0000
   8    0,334     0,745  14,305   157   0,0000     0,658  11,299   157   0,0000
  16    0,349     0,623  10,092   157   0,0000     0,454   3,879   157   0,0002
  32    0,432     0,712  10,365   157   0,0000     0,417   0,545   157   0,5863
```

ND4L 3rd codon positions:

```
NumOTU      Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
   4    0,659     0,833   3,248    78   0,0017     0,903   4,560    78   0,0000
   8    0,707     0,848   2,665    78   0,0093     0,823   2,189    78   0,0316
  16    0,793     0,573   4,465    78   0,0000     0,524   5,454    78   0,0000
  32    0,839     0,891   1,139    78   0,2580     0,739   2,217    78   0,0296
```

ND4 1st+2nd codon positions:

```
NumOTU      Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
   4    0,344     0,816  31,298   895   0,0000     0,785  29,198   895   0,0000
   8    0,357     0,782  24,785   895   0,0000     0,675  18,555   895   0,0000
  16    0,402     0,765  18,447   895   0,0000     0,563   8,178   895   0,0000
  32    0,407     0,740  15,626   895   0,0000     0,428   0,971   895   0,3318
```

ND4 3rd codon positions:

```
NumOTU      Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
   4    0,682     0,792   4,404   447   0,0000     0,759   3,068   447   0,0023
   8    0,750     0,747   0,111   447   0,9118     0,635   4,355   447   0,0000
  16    0,811     0,711   3,659   447   0,0003     0,502  11,350   447   0,0000
  32    0,834     0,696   4,726   447   0,0000     0,369  15,958   447   0,0000
```

ND5 1st+2nd codon positions:

```
NumOTU      Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
   4    0,390     0,826  28,458  1161   0,0000     0,794  26,372  1161   0,0000
   8    0,375     0,796  23,303  1161   0,0000     0,692  17,559  1161   0,0000
  16    0,413     0,780  17,244  1161   0,0000     0,587   8,194  1161   0,0000
  32    0,473     0,758  11,503  1161   0,0000     0,461   0,452  1161   0,6514
```

ND5 3rd codon positions:

```
NumOTU      Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
==============================================================================
   4    0,723     0,801   3,337   580   0,0009     0,769   1,964   580   0,0501
   8    0,823     0,759   2,356   580   0,0188     0,648   6,451   580   0,0000
  16    0,885     0,734   4,978   580   0,0000     0,524  11,951   580   0,0000
  32    0,973     0,710   7,593   580   0,0000     0,382  17,075   580   0,0000
```

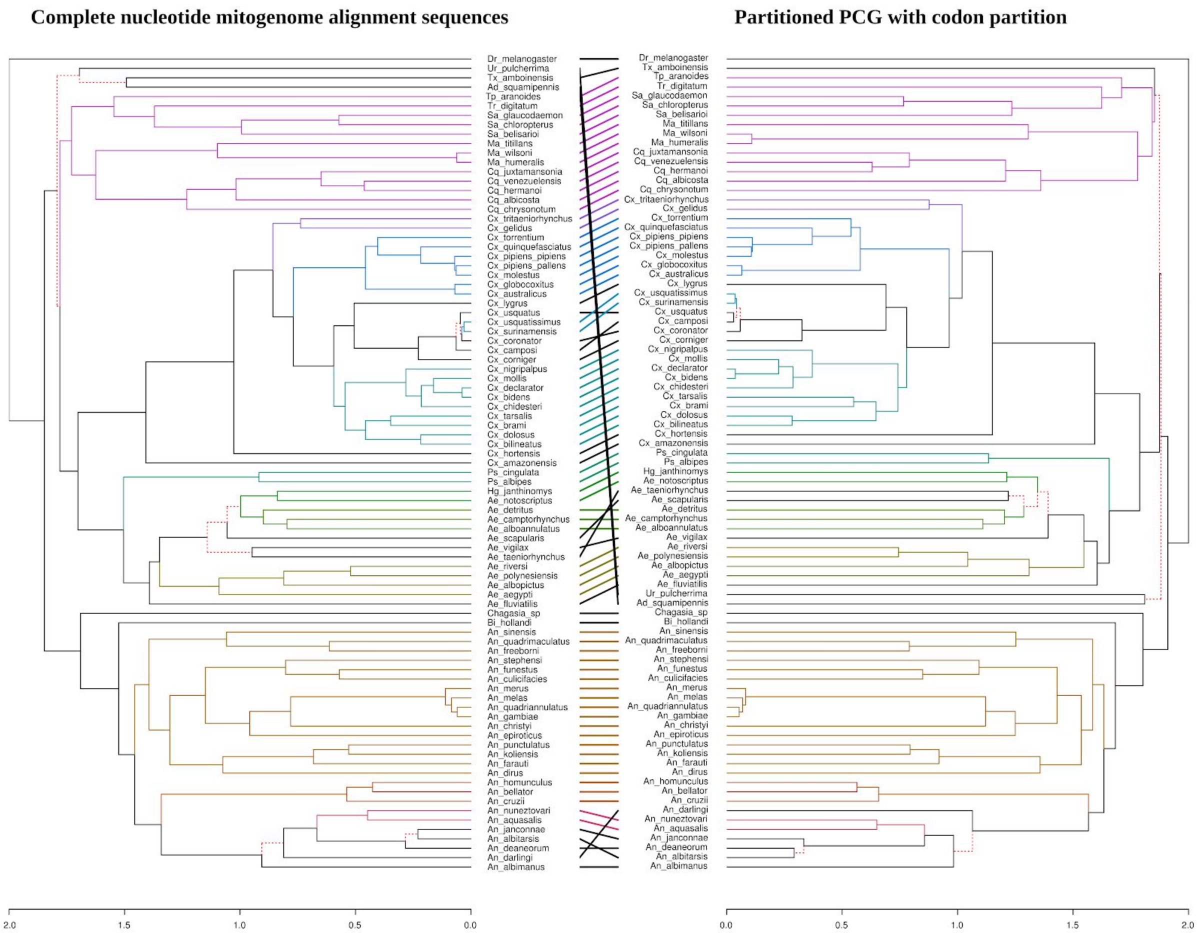
ND6 1st+2nd codon positions:

```
NumOTU      Iss  Iss.cSym      T    DF       P Iss.cAsym       T    DF       P
```

```
=====================================================================================
   4    0,463     0,781  12,219   307   0,0000     0,756  11,237   307   0,0000
   8    0,528     0,734   7,426   307 111,3362     0,626   3,521   307   0,0005
  16     0,564     0,680   4,021   307728760384     0,474   3,118   307   0,0020
  32     0,632     0,683   1,713   307   0,0877     0,354   9,373   307   0,0002
```

ND6 3rd codon positions:

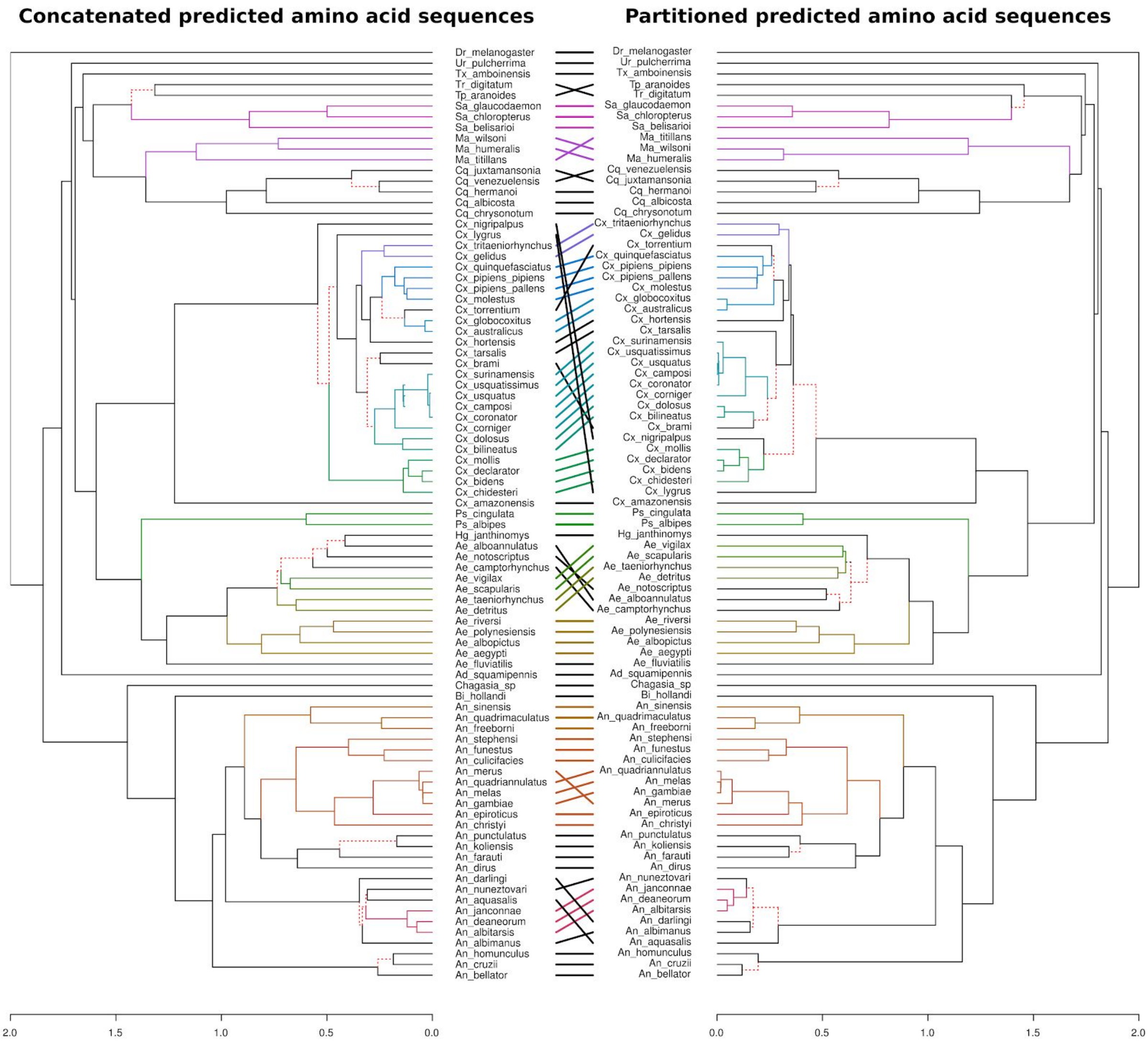
```
NumOTU       Iss  Iss.cSym       T    DF        P Iss.cAsym       T    DF        P
====================================================================================
   4    0,847     0,781   1,620   152   0,1073     0,788   1,454   152   0,1479
   8    0,986     0,747   5,532   15213484792,     0,662   7,495   152 510,3412
  16     1,049     0,620  10,045   152   0,0002     0,455  13,912   152   0,0000
  32     1,118     0,716   9,303   152   0,0014     0,425  16,021   152   0,0000

Interpretation of results:
              Significant Difference
              ----------------------
              Yes               No
-------------------------------------------------------
Iss < Iss.c    Little            Substantial
              saturation         saturation
-------------------------------------------------------
```



Complete nucleotide mitogenome alignment sequences

Partitioned PCG with codon partition

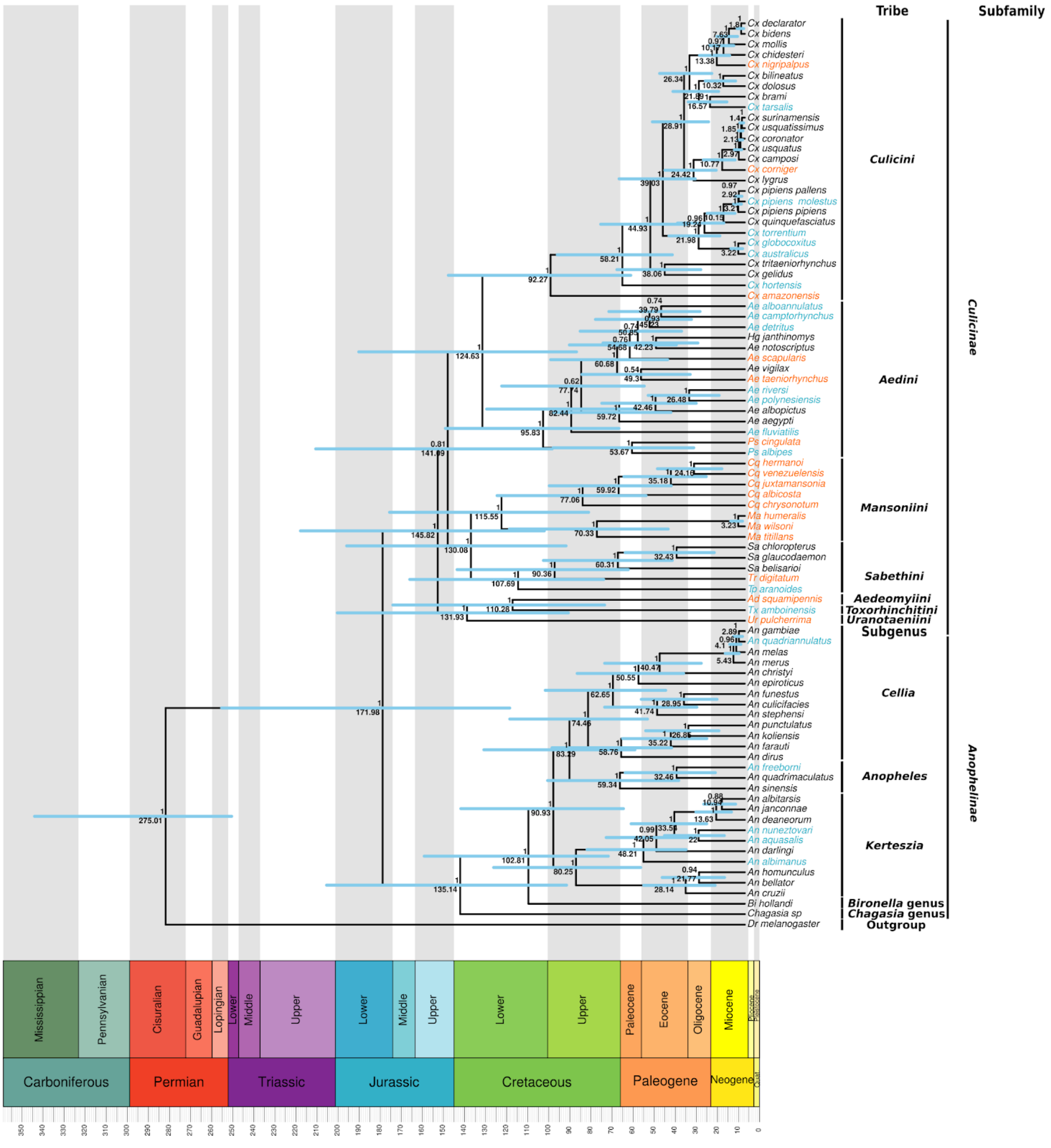
Supplementary figure 1. Comparative dendrogram topologies of trees obtained from different approaches using complete nucleotide mitogenomes and PCG with codon partition in the BEAST analysis. Dashed red lines show the branches that were different between the approaches used.



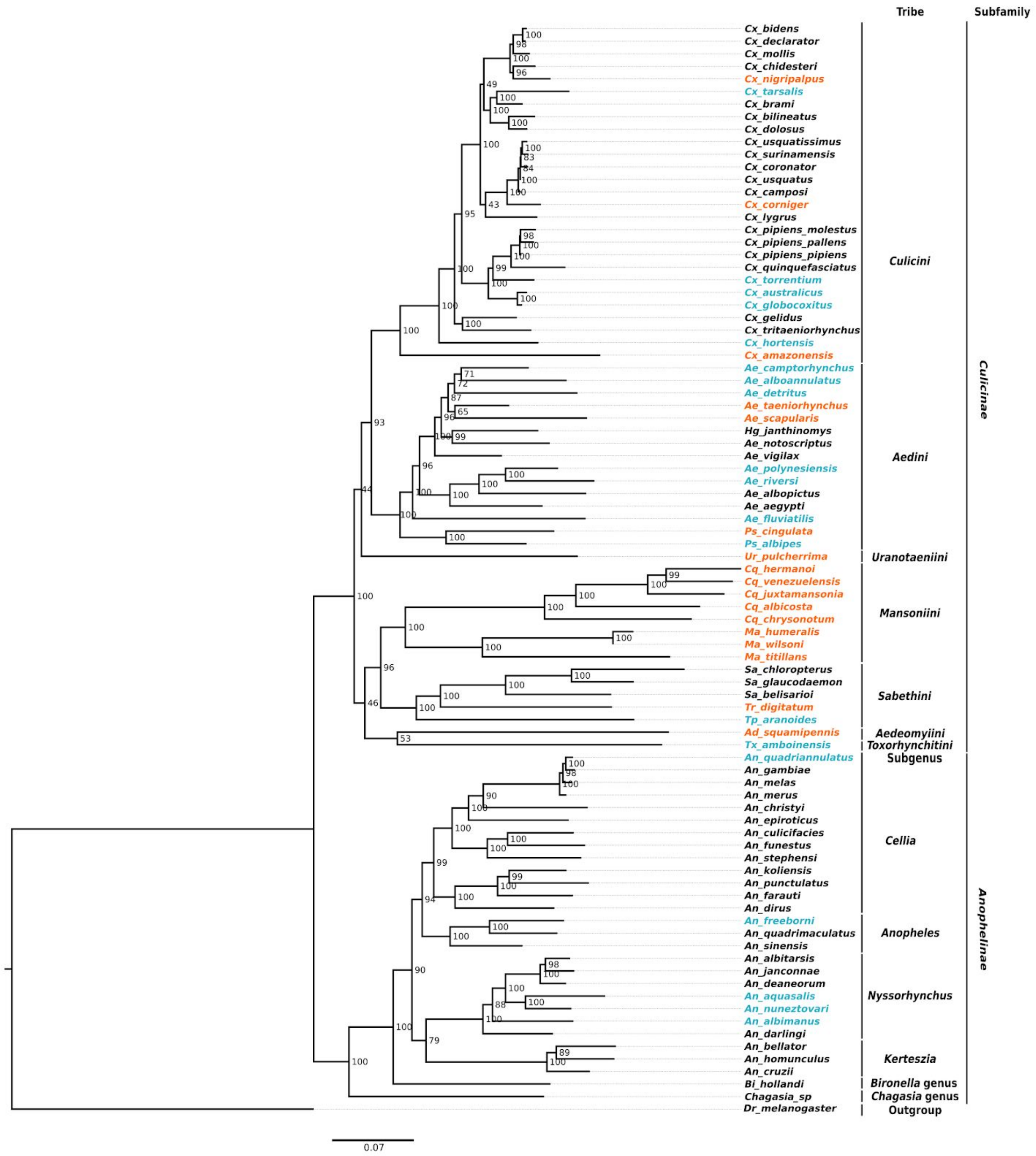
Concatenated predicted amino acid sequences

Partitioned predicted amino acid sequences

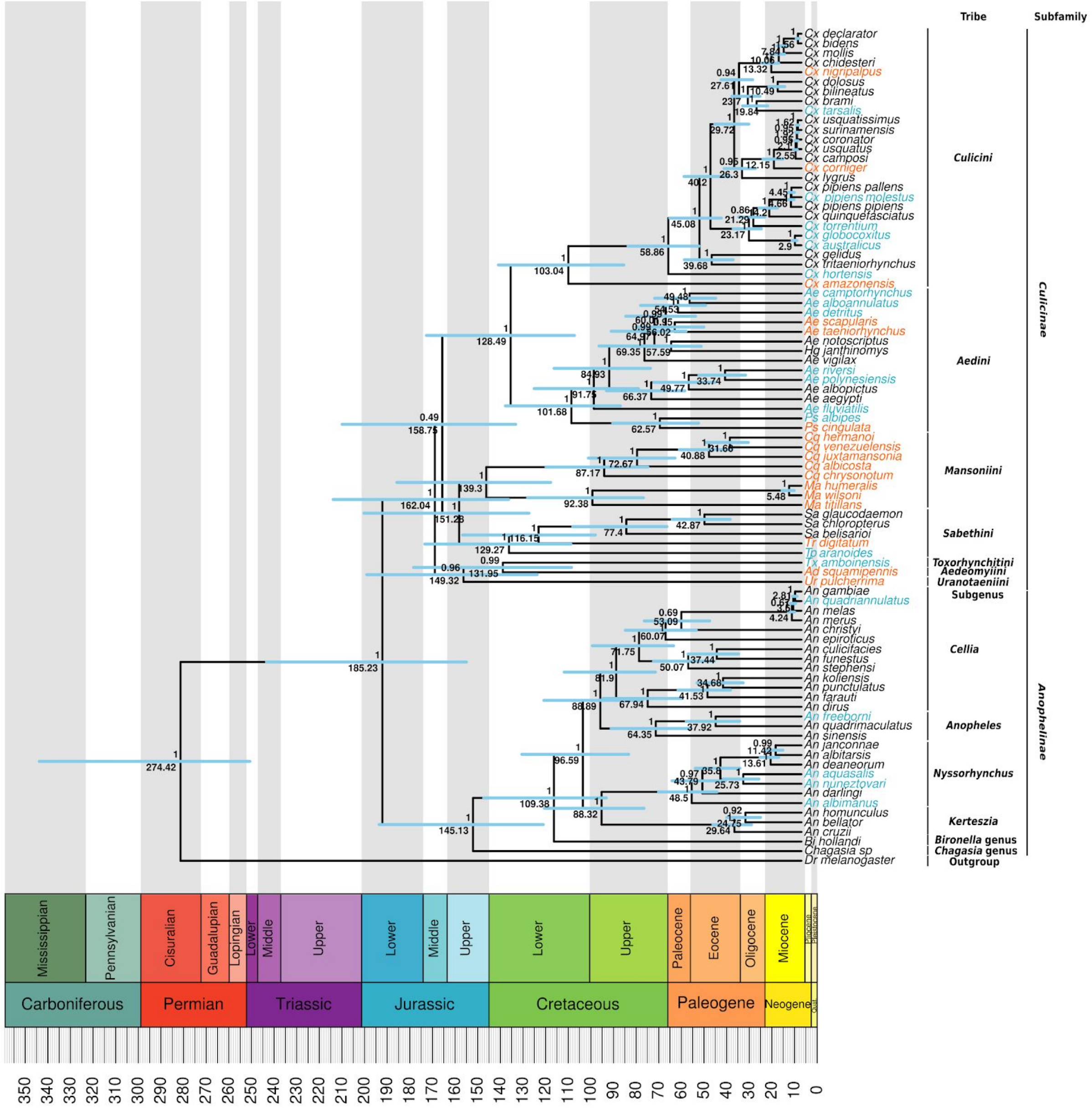
Supplementary figure 2. Comparative dendrogram topologies of trees obtained from different approaches using concatenated predicted amino acid sequences and partitioned predicted amino acid sequences in the BEAST analysis. Dashed red lines show the branches that were different between the approaches used.



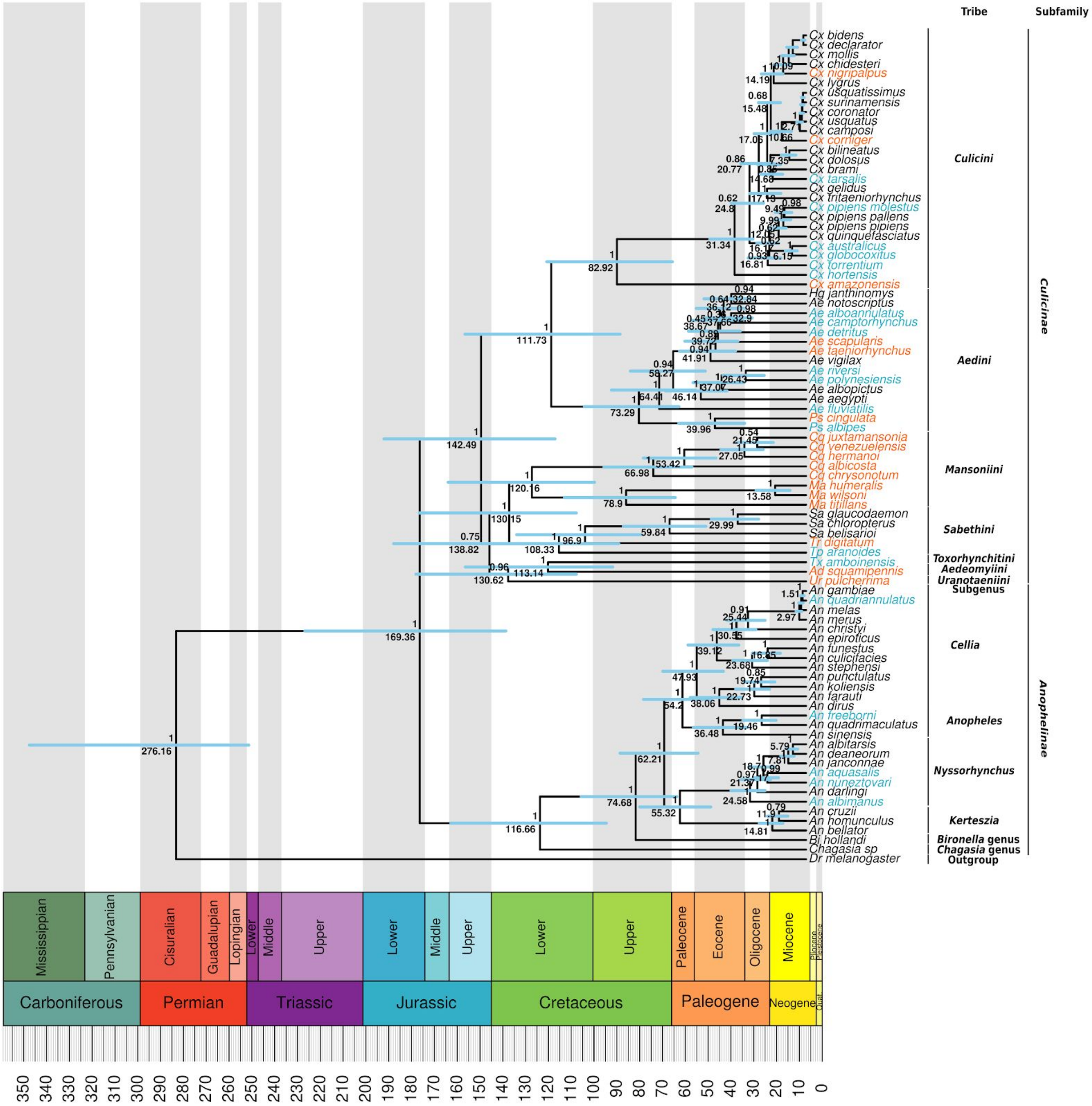
Supplementary figure 3. Evolutionary timescale of the Culicidae family based on BEAST analysis using complete mitogenome sequences. Blue bars represent the HPD95%. The numbers above and below the bars show the posterior probability and the predicted median dating respectively for each node. Light blue tip names represent mitogenomes characterized from SRA data. Orange tip names represent sequenced mitogenomes from this study.



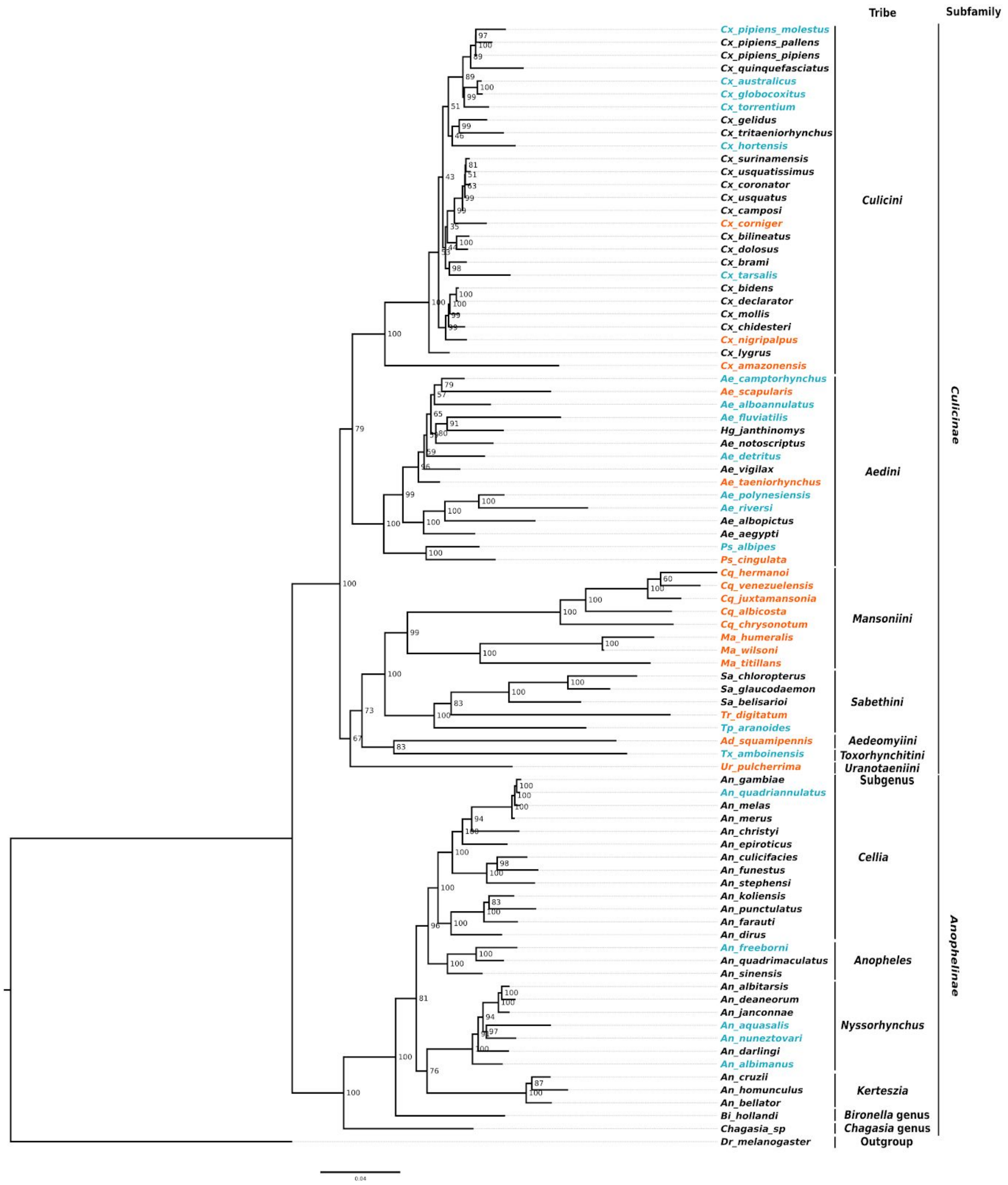
Supplementary figure 4. Maximum likelihood phylogenetic tree reconstructed with IQ-TREE version 1.6.12 based on complete mitogenome sequences using the GTR+F+I+G4 evolutionary model and the ultrafast bootstrapping with 1000 replicates. Light blue tip names represent mitogenomes characterized from SRA data. Orange tip names represent sequenced mitogenomes from this study.



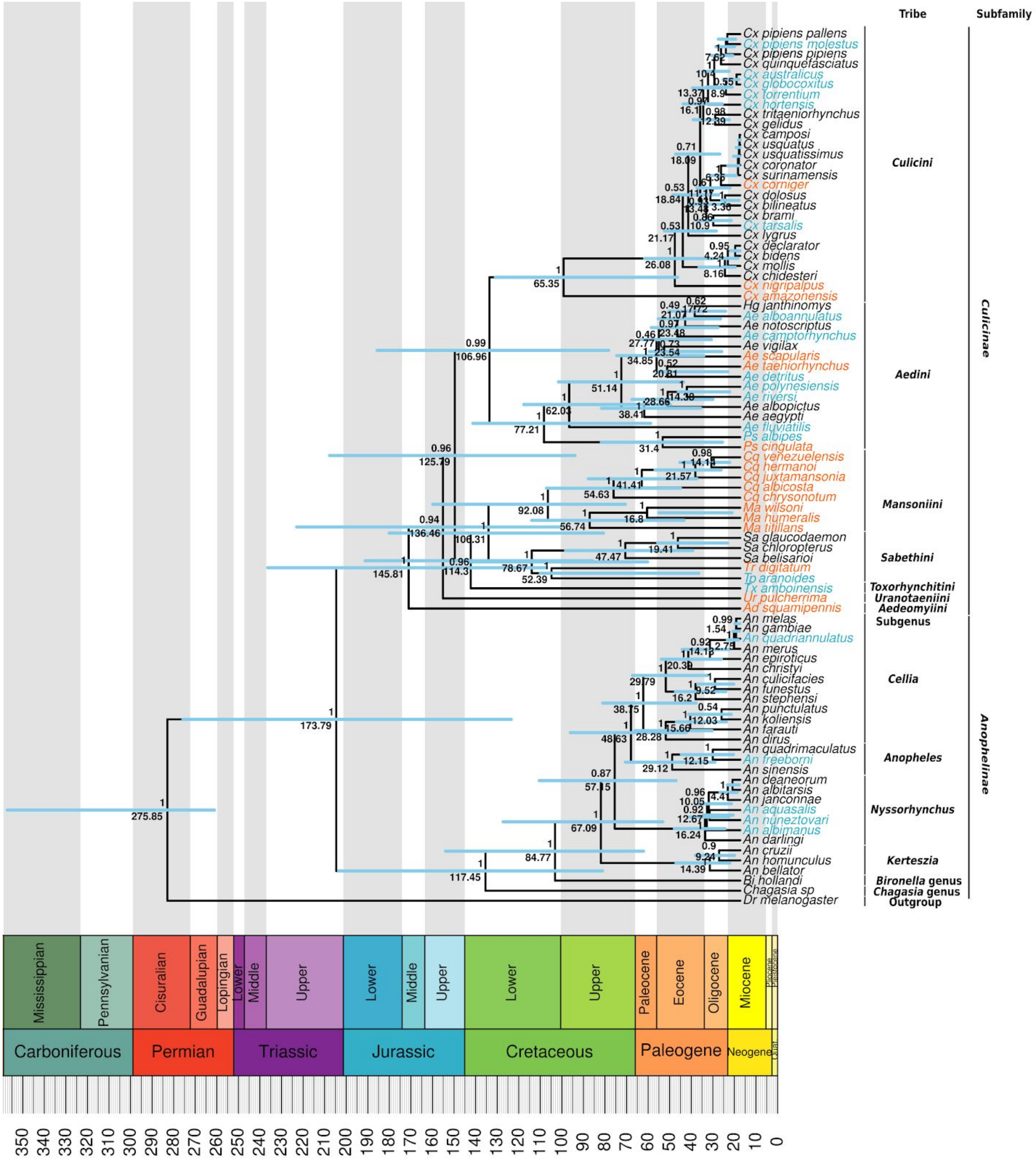
Supplementary figure 5. Evolutionary timescale of Culicidae family based on BEAST analysis using a partitioned scheme of PCG without codon partition. Blue bars represent the HPD95%. The numbers above and below the bars show the posterior probability and the predicted median dating respectively for each node. Light blue tip names represent mitogenomes characterized from SRA data. Orange tip names represent sequenced mitogenomes from this study.



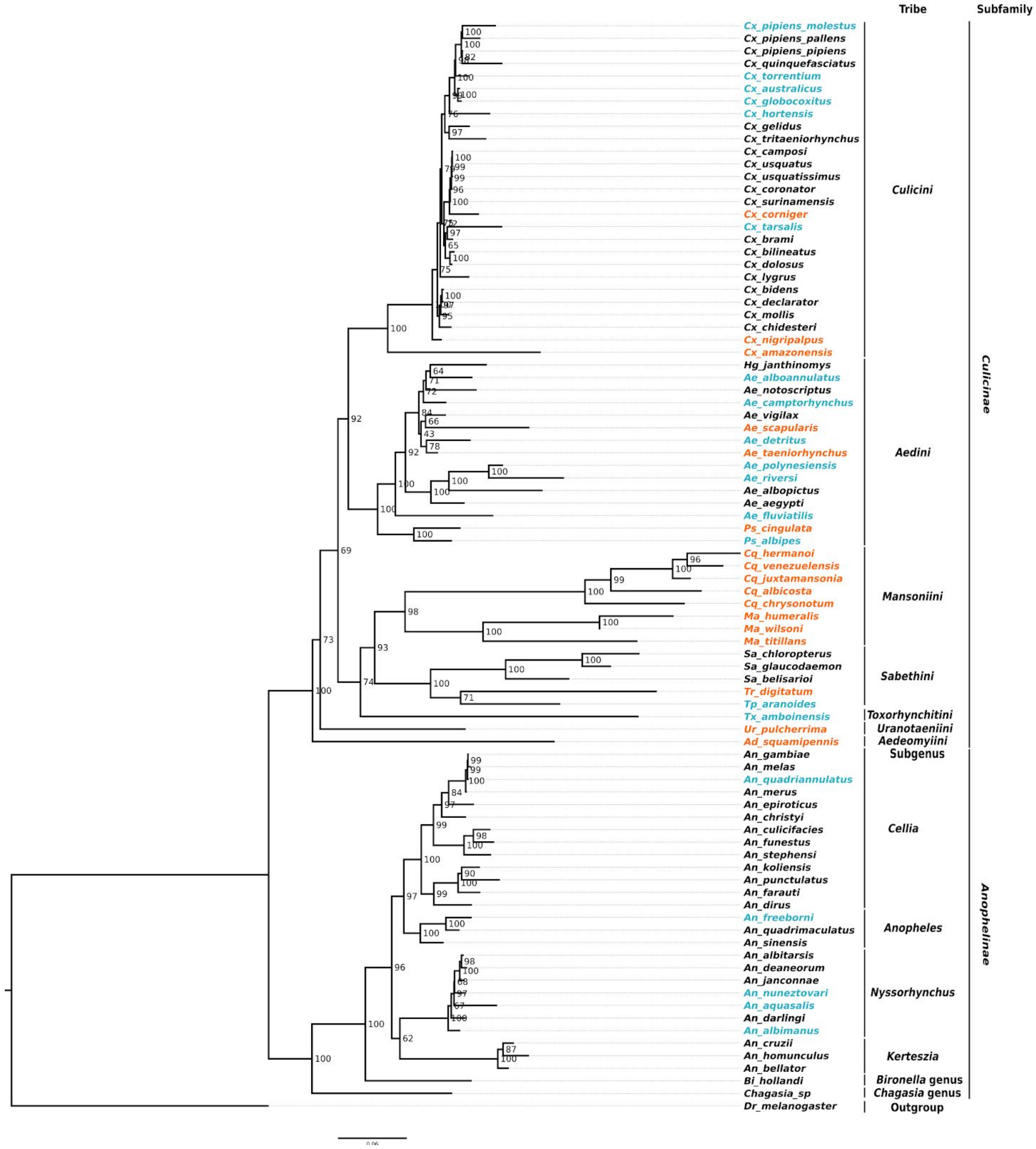
Supplementary figure 6. Evolutionary timescale of Culicidae family based on BEAST analysis using a partitioned scheme of PCG without 3rd codon positions. Blue bars represent the HPD95%. The numbers above and below the bars show the posterior probability and the predicted median dating respectively for each node. Light blue tip names represent mitogenomes characterized from SRA data. Orange tip names represent sequenced mitogenomes from this study.



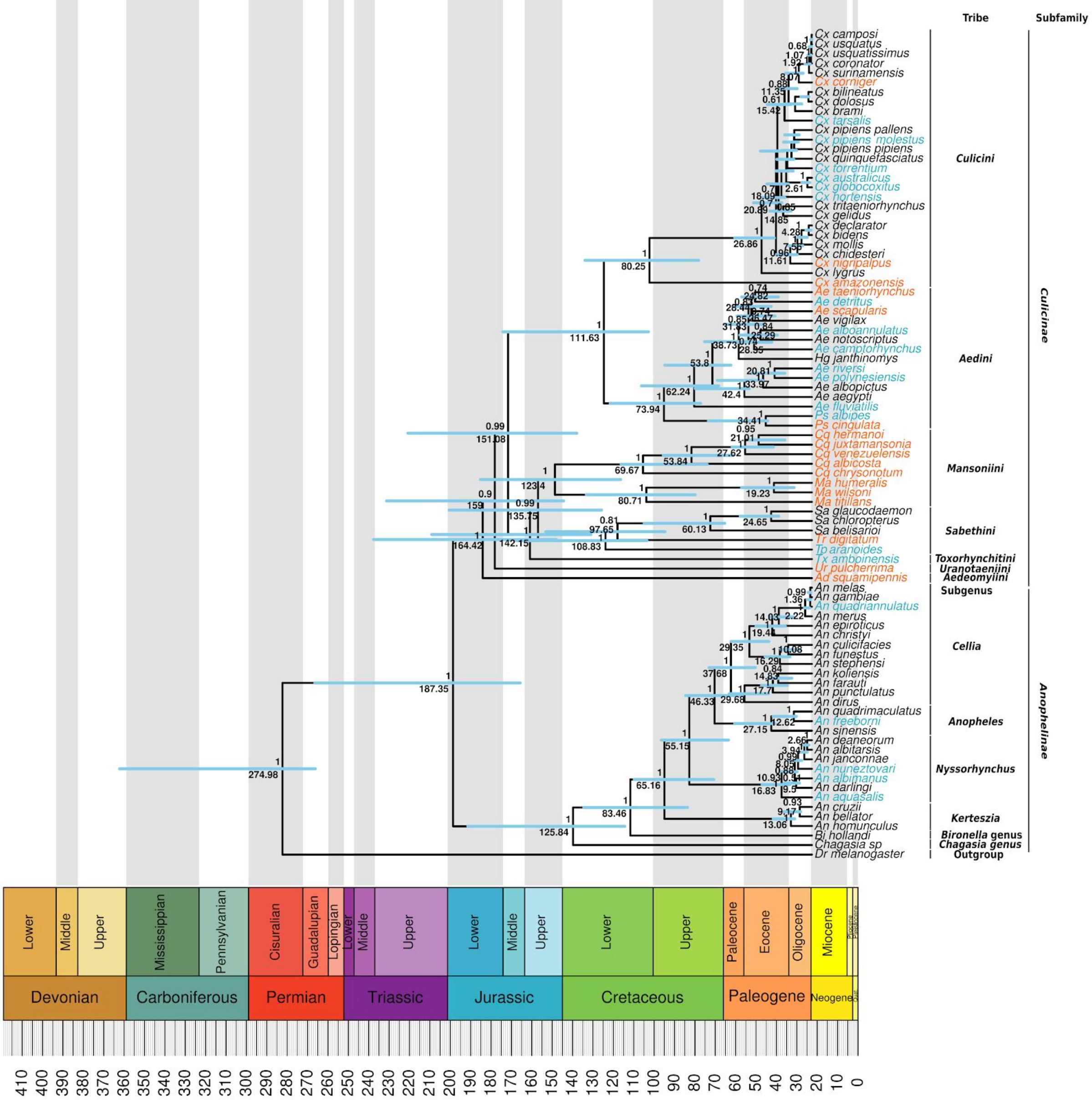
Supplementary figure 7. Maximum likelihood phylogenetic tree reconstructed with IQ-TREE version 1.6.12 based on concatenated PCG without 3rd codon positions using the GTR+F+I+G4 evolutionary model and the ultrafast bootstrapping with 1000 replicates. Light blue tip names represent mitogenomes characterized from SRA data. Orange tip names represent sequenced mitogenomes from this study.



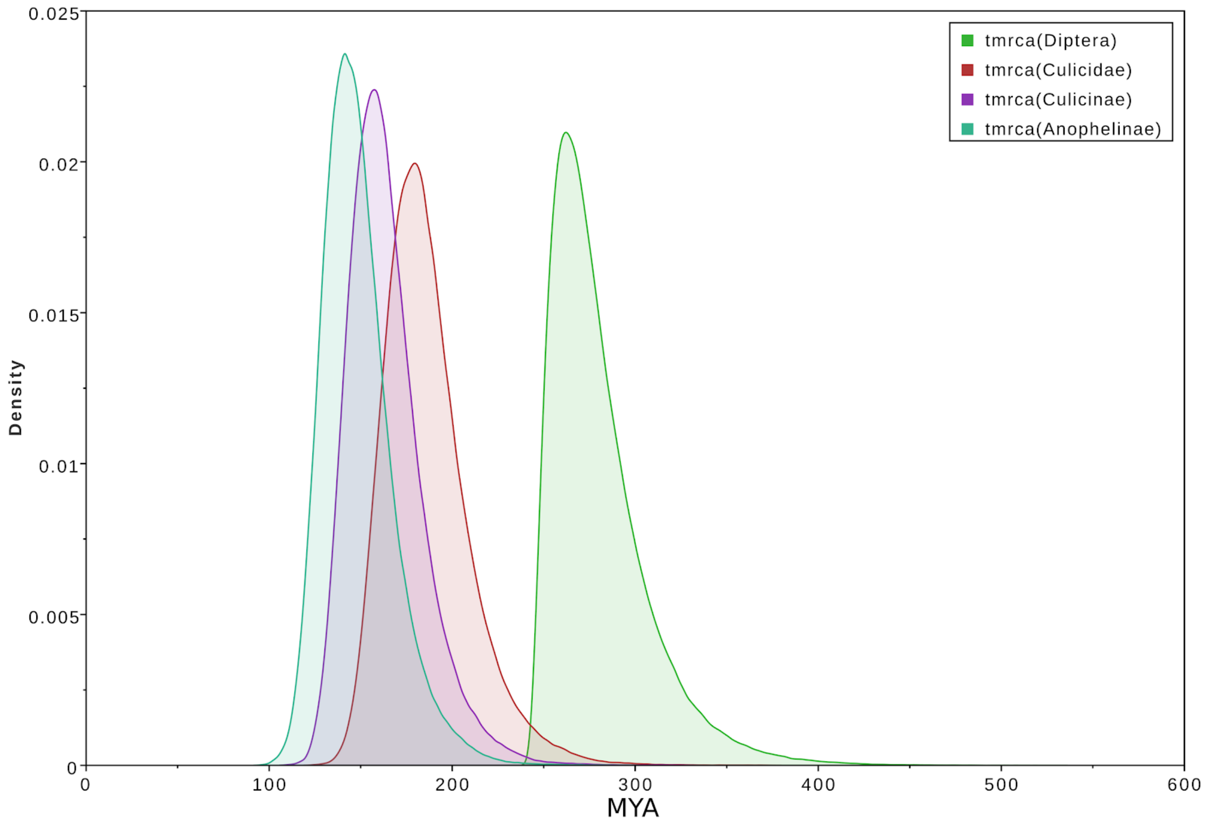
Supplementary figure 8. Evolutionary timescale of Culicidae family based on BEAST analysis using a concatenated predicted amino acid sequence. Blue bars represent the HPD95%. The numbers above and below the bars show the posterior probability and the predicted median dating respectively for each node. Light blue tip names represent mitogenomes characterized from SRA data. Orange tip names represent sequenced mitogenomes from this study.



Supplementary figure 9. Phylogenetic tree of Maximum likelihood reconstructed with IQ-TREE version 1.6.12 based on concatenated predicted amino acid sequences using the mtInv+I+G4 evolutionary model and the ultrafast bootstrapping with 1000 replicates. Light blue tip names represent mitogenomes characterized from SRA data. Orange tip names represent sequenced mitogenomes from this study.

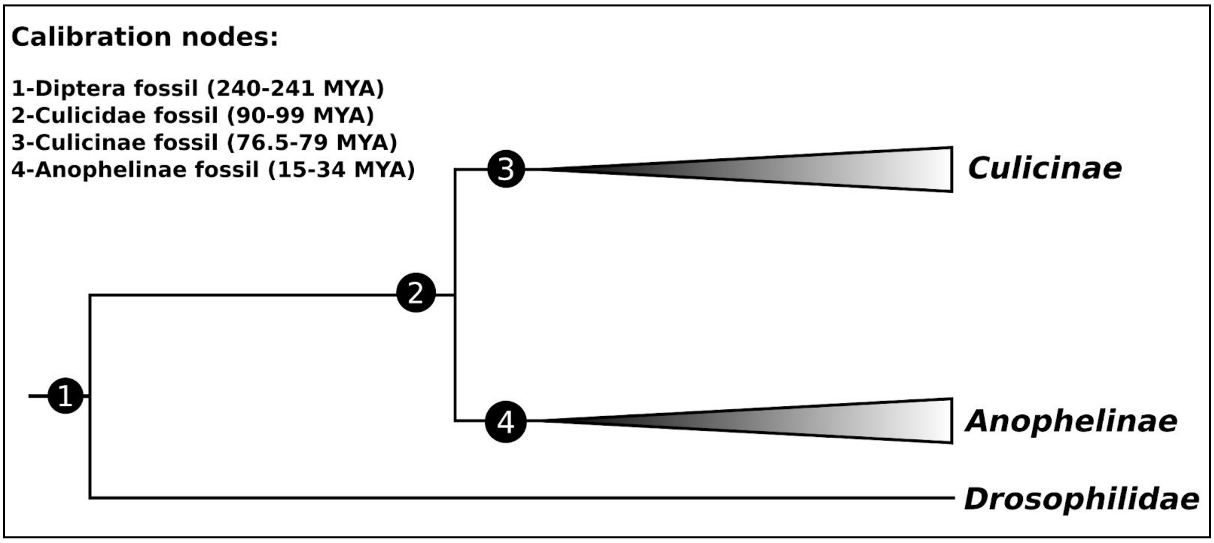


Supplementary figure 10. Evolutionary timescale of Culicidae family based on BEAST analysis using a partitioned scheme of predicted amino acid sequences. Blue bars represent the HPD95%. The numbers above and below the bars show the posterior probability and the predicted median dating respectively for each node. Light blue tip names represent mitogenomes characterized from SRA data. Orange tip names represent sequenced mitogenomes from this study.



Supplementary figure 11. Density plot showing the 95% posterior distribution of ancestors time estimate based on the alignment of partitioned mitochondrial PCG with codon partition.

Supplementary figure 12. Quality of raw reads generated from mosquito samples. Figure generated from FasqQC results and summarized by MultiQC tool. The yellow line represents results from *Cq. albicosta* reads and green lines represent the remaining species.



Supplementary figure 13. Schematic tree representing fossil dating used as calibration points priors in the molecular clock bayesian analyses. Circles with numbers inside represent each calibration point used based on the literature shown in Supplementary table 6.

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