

Supplementary information

Table S1 Universal primers for Sanger sequencing. List of primer pairs used for the PCR amplification and Sanger sequencing of *Baetis rutilocylindratus* (Baetidae) and *Habrophlebiodes zijinensis* (Leptophlebiidae).

Primer Name	Nucleotide sequence (5'-3')	Annealing temperature (°C)	No. of fragments
<i>Baetis rutilocylindratus</i>			
CO1F	ACAAATCATAAGGATATTGG	48	1
N-2329	ACTGTAAATATATGATGTGCTCA	48	1
C2-J-3696	GAAATTTGTGGAGCYAATCACAG	50	2
C3-N-5460	TGATACTGACATTTTGTGA	50	2
CB-J-11545	ACATGAATTGGAGCTCGACCAGT	53	3
LR-N-12945	GCGACCTCGATGTTGGATTAA	53	3
CRUST16SF	TAATTCAACATCGAGGTCGCAA	54	4
H13845-12Sb	GTGCCAGCAGCTGCGGTTA	54	4
CO1F	ACAAATCATAAGGATATTGG	49	5
RC2	GGGCAACTCCAGATTGACAC	49	5
FC2-3	CTTCTACAAGAGTGCTGGGA	51	6
RC2-3	CAACTAAGTGAAATGGGTGA	51	6
F-cob	ATTCCAAGGGCTTCACACT	49	7
RC3-cob	TAGAAAGTAAACCACCGTCA	49	7
F16n	TTAGAAAAGCCACCCCGAT	55	8
R16n	GGACGAGAAGACCCCATAGA	55	8
F16-C1	TAAATCCTTATGTACGGCACG	55	9
R16-C1	TAAATCCTTATGTACGGCACG	55	9
F12-C1	CCCCTTGCCCTCATCGTCTAA	59	10
R12-C1	CTCCGAATGCTTCCTTCTTGC	59	10
<i>Habrophlebiodes zijinensis</i>			
CO1F	ACAAATCATAAGGATATTGG	48	1
N-2329	ACTGTAAATATATGATGTGCTCA	48	1
N5-J-6579	TTCTCATCATCCTTGATC	51	2
N4-N-8484	GCTAATATGGCTGCTCCTCC	51	2
CB-J-11545	ACATGAATTGGAGCTCGACCAGT	53	3
LR-N-12945	GCGACCTCGATGTTGGATTAA	53	3
CRUST16SF	TAATTCAACATCGAGGTCGCAA	54	4
H13845-12Sb	GTGCCAGCAGCTGCGGTTA	54	4
TM-J-206	GCTAAGBTAAAGCTAATAGTTTCAT	47	5
ZR16-CI	TAGAAACACGAAACCGAGGGACCA	47	5
FCOI-CB1	TTTTCTCCCTCCATTTAGCC	49	6
N5-N-6562	GATCAAGGKTGAAGAGAA	49	6
FzN4-CB	ATAAACTCACGAACTCTCCAG	51	7
RzN4-CB	TTTTTGTGTGTGCCGTTGA	51	7
Fz1-5n	GCTGCCTGATACTGACATT	44	8
Rz1-5	ATTATGTTTTTAGGGTCAA	44	8

Table S2 Overview of ephemeropteran mitochondrial genomes. Total sequence length in base pairs (bp) and individual nucleotide compositions of mayfly mitochondrial genomes calculated based on the whole available sequences and the ones corrected for the incomplete mitochondrial genomes (see 2. Material and methods section)

Family	Species	Length [bp]	A%	C%	G%	T%	GC%	AT%	Reference
Ameletidae	<i>Ameletus</i> sp1	15,141	33.2	13.7	20.6	32.5	34.2	65.7	(Tang et al., 2014)
		12,305	33.1	13.9	20.7	32.3	34.6	65.4	
Baetidae	<i>Alainites yixiani</i>	14,589 ^a	29.1	15.5	20.5	34.9	36.0	64.0	(Jia & Zhou unpublished)
		12,130	28.8	14.7	21.0	35.5	35.7	64.3	
Baetidae	<i>Baetis rutilocylindratus</i>	14,883 ^a	27.3	19.8	20.1	32.8	39.9	60.1	this study
		12,234	26.7	19.8	20.4	33.2	40.2	59.9	
Baetidae	<i>Cloeon dipterum</i>	14,355	30.6	14.7	16.3	38.4	30.9	69.0	this study
		12,197	29.9	14.5	16.9	38.7	31.4	68.6	
Caenidae	<i>Caenis pycnacantha</i>	15,351 ^a	32.4	20.8	14.2	32.5	35.1	64.9	(Jia & Zhou unpublished)
		12,301	31.8	21.4	15.3	31.6	36.7	63.4	
Ephemerellidae	<i>Ephemerella</i> sp.	14,896	30.4	21.9	16.3	31.4	38.2	61.8	(Tang et al., 2014)
		12,233	30.4	21.6	17.1	31.0	38.6	61.4	
Ephemerellidae	<i>Vietnamella dabieshanensis</i>	15,761 ^a	32.1	17.6	11.8	38.6	29.4	70.7	(Wang & Zhou unpublished)
		12,286	32.5	18.1	12.3	37.1	30.4	69.6	
Ephemerellidae	<i>Vietnamella</i> sp.	15,043	30.8	20.5	13.5	35.2	34.0	66.0	(Tang et al., 2014)
		12,788	30.8	21.0	13.9	34.3	34.9	65.1	
Ephemeridae	<i>Ephemera orientalis</i>	16,463 ^a	37.2	17.4	10.3	35.0	27.7	72.2	(Lee et al., 2009)
		12,366	36.3	18.2	11.4	34.1	29.6	70.4	
Heptageniidae	<i>Epeorus</i> sp.	15,456	31.7	22.1	13.8	32.3	35.9	64.0	(Tang et al., 2014)
		12,371	31.0	22.8	14.4	31.8	37.2	62.8	
Heptageniidae	<i>Paegniodes cupulatus</i>	15,715 ^a	32.5	20.7	13.7	33.1	34.4	65.6	(Wang & Zhou unpublished)
		12,366	32.2	20.8	13.6	33.4	34.3	65.6	
Heptageniidae	<i>Parafronurus youi</i>	15,481 ^a	32.7	20.5	13.1	33.7	33.6	66.4	(Zhang et al., 2008)
		12,361	32.9	20.2	12.9	34.0	33.0	66.9	
Isonychiidae	<i>Isonychia ignota</i>	15,105 ^a	30.5	23.4	14.9	31.2	38.3	61.7	(Qin et al. unpublished)
		12,318	30.6	23.6	15.0	30.8	38.6	61.4	
Leptophlebiidae	<i>Habrophlebiodes zijinensis</i>	15,407	33.8	20.2	11.0	35.1	31.2	68.9	this study
		12,417	33.6	20.6	11.5	34.3	32.1	67.9	
Potamanthidae	<i>Potamanthus</i> sp.	14,937	32.8	19.4	13.6	34.1	33.1	66.9	(Tang et al., 2014)
		12,289	32.5	19.5	14.2	33.9	33.6	66.4	
Siphonuridae	<i>Siphonurus immanis</i>	15,529 ^a	35.2	17.6	11.7	35.4	29.3	70.6	(Hua et al. unpublished)
		12,339	34.7	18.0	12.2	35.1	30.2	69.8	
Siphonuridae	<i>Siphonurus</i> sp.	14,745	31.5	20.9	14.7	32.9	35.6	64.4	(Tang et al., 2014)
		12,333	31.0	21.0	15.1	32.9	36.1	63.9	
Siphuriscidae	<i>Siphuriscus chinensis</i>	16,616	33.9	19.0	14.4	32.6	33.4	66.5	(Li et al., 2014)
		12,394	33.5	19.6	14.3	32.7	33.9	66.2	
Teloganodidae	sp.	15,252	30.1	21.9	14.4	33.6	36.3	63.7	(Tang et al., 2014)
		12,208	29.8	22.6	15.1	32.6	37.6	62.4	

^a, Indicates complete mitochondrial genomes; rest are incomplete mitochondrial genomes.

References

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Fig. S1 Phylogenetic relationships of insect major orders using Bayesian inference reconstruction based on mitochondrial genome data using the concatenated amino acid sequences of the all_taxa matrix (see Table 1). Filled circles indicate well-supported nodes; whereby black circles represent Bayesian posterior probability (BPP) = 1, and grey circles BPP ≥ 0.95 . Scale bar indicates substitutions per site. Coloured tip labels refer to the orders, whereby light blue = Archaeognatha (AR), dark blue = Blattodea (BL), light green = Ephemeroptera (EP), dark green = Grylloblattodea (GR), red = Isoptera (IS), light orange = Mantodea (MT), dark red = Mantophasmatodea (MP), dark orange = Odonata (OD), light purple = Orthoptera (OR), dark purple = Phasmatodea, yellow = Plecoptera (PL), and brown = Zygentoma (ZY). Five taxa being excluded from the optimized_taxa matrix are coloured in dark grey.

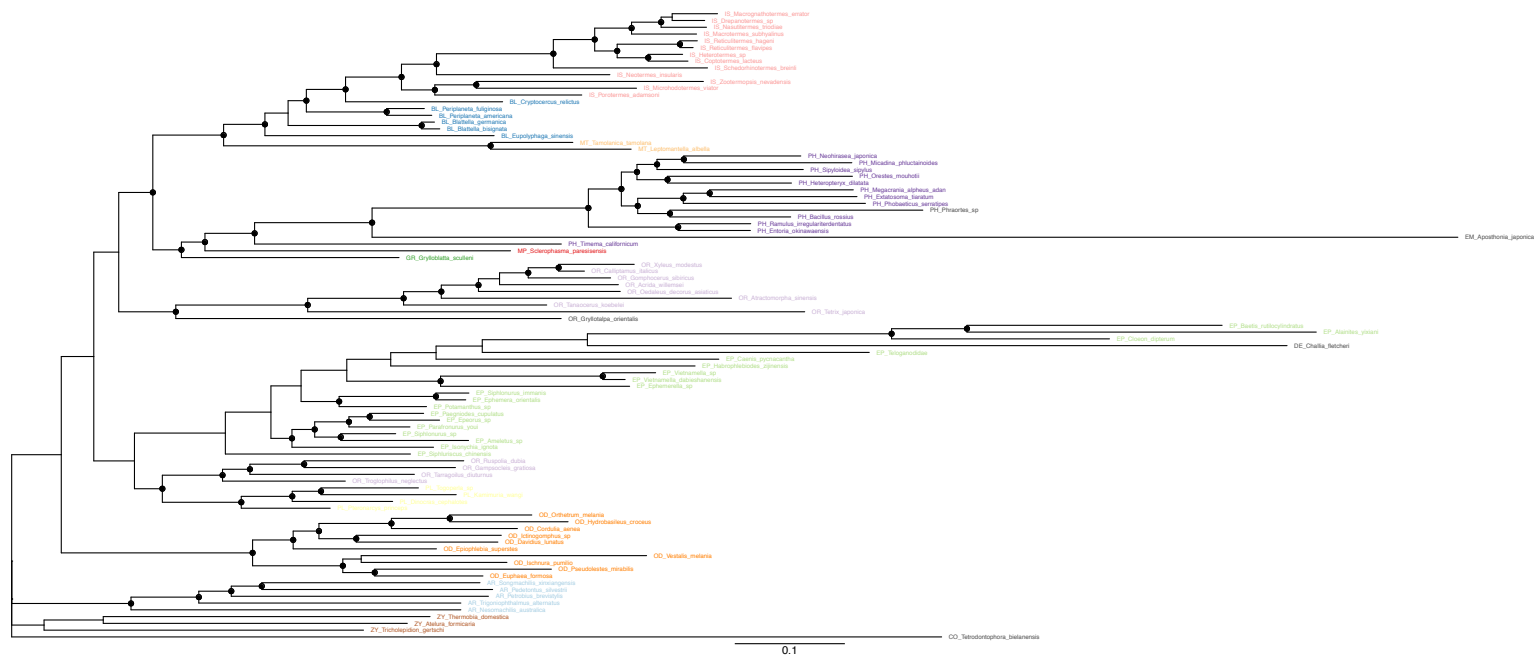


Fig. S2 Phylogenetic relationships of insect major orders using maximum likelihood reconstruction based on mitochondrial genome data using the concatenated amino acid sequences of the optimized_taxa matrix (see Table 1). Filled circles indicate well-supported nodes; whereby black circles represent Bootstrap Support (BS) ≥ 90 , and grey circles BS ≥ 80 . Scale bar indicates substitutions per site. Coloured tip labels refer to the orders (for details see Fig. S1).

