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Complete mitochondrial genome of *Orthetrum* dragonflies and molecular phylogeny of Odonata

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ABSTRACT

Dragonflies of the genus *Orthetrum* are members of the anisopteran family Libellulidae. To date, there are no reports on the phylogeny of *Orthetrum* dragonflies based on the complete mitochondrial genome (mitogenome). There is only a single entry of a nearly complete mitogenome for *O. melania*. We report here the complete mitogenome of *O. chrysis*, *O. glaucum*, *O. sabina* and *O. testaceum* and their phylogenetic relationships with other taxa of Libellulidae as well as Epiophlebiidae, Anisoptera and Zygoptera. The whole mitogenomes of these four species possessed 37 genes (13 protein-coding genes – PCGs, 2 rRNA and 22 tRNA genes) and a non-coding region. Molecular phylogeny based on 13 PCGs was concordant with 15 mitochondrial genes (13 PCGs and 2 rRNA genes). The Libellulidae (Anisoptera) was monophyletic with two lineages: (*Orthetrum*) + (*Brachythemis* + *Hydrobasileus*). It formed a sister group with Corduliidae. The Zygoptera was monophyletic with three lineages: (Calopterygidae) + (Euphaeidae + Pseudolestidae) + (Coenagrinidae + Platycnemididae). The enigmatic *Epiophlebia superstes* (Epiophlebiidae) forms a sister group with Zygoptera. The complete mitogenome is useful for determining the higher-level phylogenetic relationships of Odonata.

1. Introduction

Dragonflies of the genus *Orthetrum* (Newman, 1833) are members of the anisopteran family Libellulidae. Fifty-nine species are currently recorded in the World Odonata List (Schorr and Paulson, 2015). The number of species will increase with the documentation of additional cryptic species (Yong et al., 2014). Of the genus *Orthetrum*, two species complexes have been well resolved: (1) *O. japonicum* species complex comprising *O. japonicum* and *O. internum* (Futahashi, 2011; Karube et al., 2012); and (2) *O. triangulare* species complex comprising *O. triangulare* and *O. melania* (Sasamoto and Futahashi, 2013). Recently two

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taxa of *O. pruinosum* (*O. p. neglectum* and *O. p. schneideri*) have been reported to belong to distinct genetic lineages (Yong et al., 2014).

To date, studies on the molecular phylogeny of *Orthetrum* dragonflies are based on DNA nucleotide sequences of mitochondrial and nuclear genes (Futahashi, 2011; Karube et al., 2012; Sasamoto and Futahashi, 2013; Yong et al., 2014). As far as we are aware, there are no reports on the phylogeny of *Orthetrum* dragonflies based on the complete mitochondrial genome (mitogenome). There is only a single entry of a nearly complete mitogenome for *O. melania* (reported as *O. triangulare melania*) (Yamauchi et al., 2004) in the GenBank.

We report here the complete mitogenome of four species of the genus *Orthetrum* and their phylogenetic relationships with other taxa of Libellulidae as well as the Epiophlebiidae and suborders Anisoptera and Zygoptera.

2. Materials and methods

2.1. Ethics statement

The four species of *Orthetrum* dragonflies are common in gardens and roadside. They are not endangered or protected by law. No permits are required to study these dragonflies.

2.2. Specimen and mitochondrial DNA extraction

The dragonflies were collected by sweep net in the University of Malaya campus. Body parts (legs, thorax and abdomen) were preserved in absolute ethanol and stored in storage temperature until use. The mitochondrial DNA (mtDNA) was extracted using Mitochondrial DNA Isolation Kit (Abnova, Taipei, Taiwan) following the manufacturer's instructions.

2.3. Genome sequencing and analysis

Mitochondrial DNA extraction, sample and library preparation (using Nextera DNA Sample Preparation Kit), genome sequencing using the Illumina MiSeq Desktop Sequencer (2×150 bp paired-end reads) (Illumina, USA), and genome analysis were as described in Yong et al. (2015a,b; 2016).

2.4. Mitogenome identification, annotation and visualization

The mitogenome was annotated using MITOS (Bernt et al., 2013) followed by manual validation of the coding regions using the NCBI ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The sequin file generated from MITOS was edited and submitted to NCBI according to ORF Finder result (NCBI GenBank accession numbers: *O. chrysis* KU361233; *O. glaucum* KU361232; *O. sabina* KU361234; *O. testaceum* KU361235). The circular mitogenome was visualized with Blast Ring Image Generator (BRIG) (Alikhan et al., 2011).

2.5. Mitogenomes from GenBank

The complete mitogenomes of Odonata available from GenBank – Corduliidae: *Cordulia aenea* JX963627 (Simon and Hadrys, 2013); Gomphidae: *Davidius lunatus* NC_012644 (Lee et al., 2009), and *Ictinogomphus* sp. KM244673; Libellulidae: *Brachythemis contaminata* NC_026305 (Yu et al., 2014), *Hydrobasileus croceus* NC_025758 (Tang et al., 2014), and *Orthetrum melania* AB126005 (Yamauchi et al., 2004); Calopterygidae: *Atrocalopteryx atrata* NC_027181 and *Vestalis melania* NC_023233 (Chen et al., 2014); Coenagrionidae: *Ischnura pumilio* NC_021617 (Lorenzo-Carballa et al., 2014); Euphaeidae: *Euphaea formosa* NC_014493 (Lin et al., 2010); Platycnemidae: *Platycnemis foliacea* NC_027180; Pseudolestidae: *Pseudolestes mirabilis* NC_020636; *Epiophlebia superstes* NC_023232 (Wang et al., 2015) were used for comparison, with species of Ephemeroptera (*Ephemera orientalis* NC_012645 and *Siphlonurus immanis* NC_013822) as outgroup taxa.

2.6. Phylogenetic analysis

Alignment of nucleotide sequences was carried out using MAFFT multiple sequence alignment software version 7 (Katoh and Standley, 2013). The optimal partitioning scheme and substitution models of each partition was selected using Partition Finder v1.1.1 (Lanfear et al., 2012) for the dataset (Table 1). The bayesian information criterion (BIC) with linked branch lengths and greedy algorithm were used to search for the best-fit partitioning model. Maximum likelihood (ML) and Bayesian Inference (BI) analyses were carried out based on the selected scheme recommended by Partition Finders. A maximum likelihood (ML) analysis was conducted using RAxML-HPC v.8.2.8 (Stamatakis, 2006) with GTRGAMMA model on the CIPRES Web Portal at http://www.phylo.org/portal2/(Miller et al., 2010). ML nodal support was calculated by conducting bootstrapping runs with 1000 replicates, using the rapid bootstrap feature (random seed value 12345). Bayesian analyses were carried out using MrBayes 3.2.6 on XSEDE utility on the CIPRES Web Portal http://www.phylo.org/portal2/(Miller et al., 2010), with 25% of trees discarded as burn-in and trees were sampled every 1000 generations.

Table 1
The best partitioning scheme selected by Partition Finder for 13PCGs, 2rRNAs and 13PCGs+2rRNAs.

Data set	Partition	Best partitioning scheme	Included nucleotides
13PCGs	1	TIM+I+G	position 1 of *atp6*, position 1 of *nad1*, position 1, 2, and 3 of *nad3*
	2	TVM+I+G	position 2 of *atp6*, position 2 of *cob*, position 3 of *cox3*, position 2 of *cox2*, position 1 of *cox1*
	3	TIM+I+G	position 3 of *atp6*, position 3 of *atp8*, position 3 of *cob*, position 1 of *cox3*, position 3 of *cox2*, position 2 of *cox1*
	4	K81uf+I+G	position 1 of *atp8*, position 2 of *atp8*, position 3 of *nad1*, position 1 of *nad4*, position 1 and 3 of *nad4l*, position 2 of *nad5*
	5	GTR+I+G	position 1 of *cob*, position 2 of *cox3*, position 1 of *cox2*, position 3 of *cox1*
	6	GTR+I+G	position 2 of *nad1*, position 3 of *nad4*, position 2 of *nad4l*, position 1 of *nad5*
	7	TrN+I+G	position 1 of *nad2*, position 2 of *nad6*
	8	TVM+I+G	position 2 of *nad2*, position 1 and 3 of *nad6*
	9	GTR+I+G	position 3 of *nad2*, position 2 of *nad4*, position 3 of *nad5*
2rRNAs	1	GTR+I+G	position 1,2 and 3 of *rrnL*, position 1,2 and 3 of *rrnS*
13PCGs+2rRNAs	1	TIM+I+G	position 1 of *atp6*, position 1 of *nad1*, position 1, 2, and 3 of *nad3*
	2	TVM+I+G	position 2 of *atp6*, position 2 of *cob*, position 3 of *cox3*, position 2 of *cox2*, position 1 of *cox1*
	3	TIM+I+G	position 3 of *atp6*, position 3 of *atp8*, position 3 of *cob*, position 1 of *cox3*, position 3 of *cox2*, position 2 of *cox1*
	4	K81uf+I+G	position 1 of *atp8*, position 2 of *atp8*, position 3 of *nad1*, position 1 of *nad4*, position 1 and 3 of *nad4l*, position 2 of *nad5*
	5	GTR+I+G	position 1 of *cob*, position 2 of *cox3*, position 1 of *cox2*, position 3 of *cox1*
	6	GTR+I+G	position 2 of *nad1*, position 3 of *nad4*, position 2 of *nad4l*, position 1 of *nad5*
	7	TrN+I+G	position 1 of *nad2*, position 2 of *nad6*
	8	TVM+I+G	position 2 of *nad2*, position 1 and 3 of *nad6*
	9	GTR+I+G	position 3 of *nad2*, position 2 of *nad4*, position 3 of *nad5*
	10	GTR+I+G	position 1, 2 and 3 of *rrnL*, position 1, 2 and 3 of *rrnS*

3. Results

3.1. Mitogenome analysis and features

The total length for the mitogenome ranged from 15,088 bp for *O. chrysis* to 15,184 bp for *O. glaucum* (Tables S1–S4). The total GC content was 26.8% for *O. chrysis*, 25.3% for *O. glaucum*, 26.5% for *O. sabina*, and 25.3% for *O. testaceum* (Table S5).

The mitogenomes of *O. chrysis*, *O. glaucum*, *O. sabina* and *O. testaceum* had similar gene order and contained 37 genes (13 protein-coding genes – PCGs, 2 rRNA genes, and 22 tRNA genes) and a non-coding region (A + T-rich control region) (Fig. 1, Tables S1–S4). The control region was flanked by *rrnS* and *trnI* genes, with 375 bp in *O. chrysis*, 456 bp in *O. testaceum*, 475 bp in *O. sabina*, and 487 bp in *O. glaucum*.

There were nine regions with spacing sequence ranging from 1 to 32 bp in *O. chrysis*, *O. testaceum* and *O. sabina*; *O. glaucum* had eight regions and lacked the intergenic sequence of 3 bp between *trnF* and *nad5* (Tables S1–S4). All four species had overlaps in 10 regions with overlapping base-pairs ranging from 1 to 8 bp.

Most of the PCGs in the four species had identical start codon, except ATC (instead of ATT) for *atp8* in *O. sabina*, ATC (instead of ATT) for *nad3* in *O. glaucum* and *O. testaceum*, and TTG (instead of ATA) for *nad1* in *O. sabina* (Table 2). The four species had identical stop codon for the respective PCGs, with incomplete T stop codon for *cox1-3*, *cob* and *nad5*.

The nucleotide compositions of the mitochondrial whole genome, protein-coding genes, rRNA genes and control region of *O. chrysis*, *O. glaucum*, *O. sabina* and *O. testaceum* are summarized in Table S5. All four species were A+T rich. The A + T content for PCGs was lowest in *cox1* (66.4% for *O. chrysis* and *O. sabina*) and highest in *atp8* (85.2% for *O. testaceum*, 84.6% for *O. glaucum*, 82.7% for *O. chrysis* and 80.9% for *O. sabina*). The A + T content of the non-coding control region ranged from 82.9% for *O. chrysis* to 89.3% for *O. testaceum*. For the two rRNAs, *rrnL* had a higher A + T content than *rrnS* (76.2%–76.7% vs 72.6%–73.9%). The GC skewness values for the whole genome, 10 PCGs (excepting *cox1* and *cox2*), and both rRNA genes in the four species were negative indicating bias toward the use of Cs over Gs. Although the AT skewness value was positive for the whole genome and rRNA genes, it was negative for the control region and variable for individual PCGs (Table S5).

As in other insects, the mitogenomes of *O. chrysis*, *O. glaucum*, *O. sabina* and *O. testaceum* had three main clusters of characteristic tRNAs (Fig. 1): (1) I-Q-M; (2) W-C-Y; and (3) A-R-N-S1-E-F. All four species lacked the DHU-stem in *trnS1* (Figs. S1–S4). TΨC-loop was absent in *trnN* and *trnH* of *O. chrysis* (Fig. S1); *trnN*, *trnI* and *trnF* of *O. testaceum* (Fig. S2); *trnN* and *trnF* of *O. glaucum* (Fig. S3); and *trnT* of *O. sabina* (Fig. S4).

3.2. Phylogenetic relationships and genetic divergence

The molecular phylogeny of *O. chrysis*, *O. glaucum*, *O. sabina* and *O. testaceum* in relation to other taxa of Libellulidae as well as the Epiophlebiidae and suborders Anisoptera and Zygoptera is depicted in Fig. 2. The phylogram based on concatenated 13

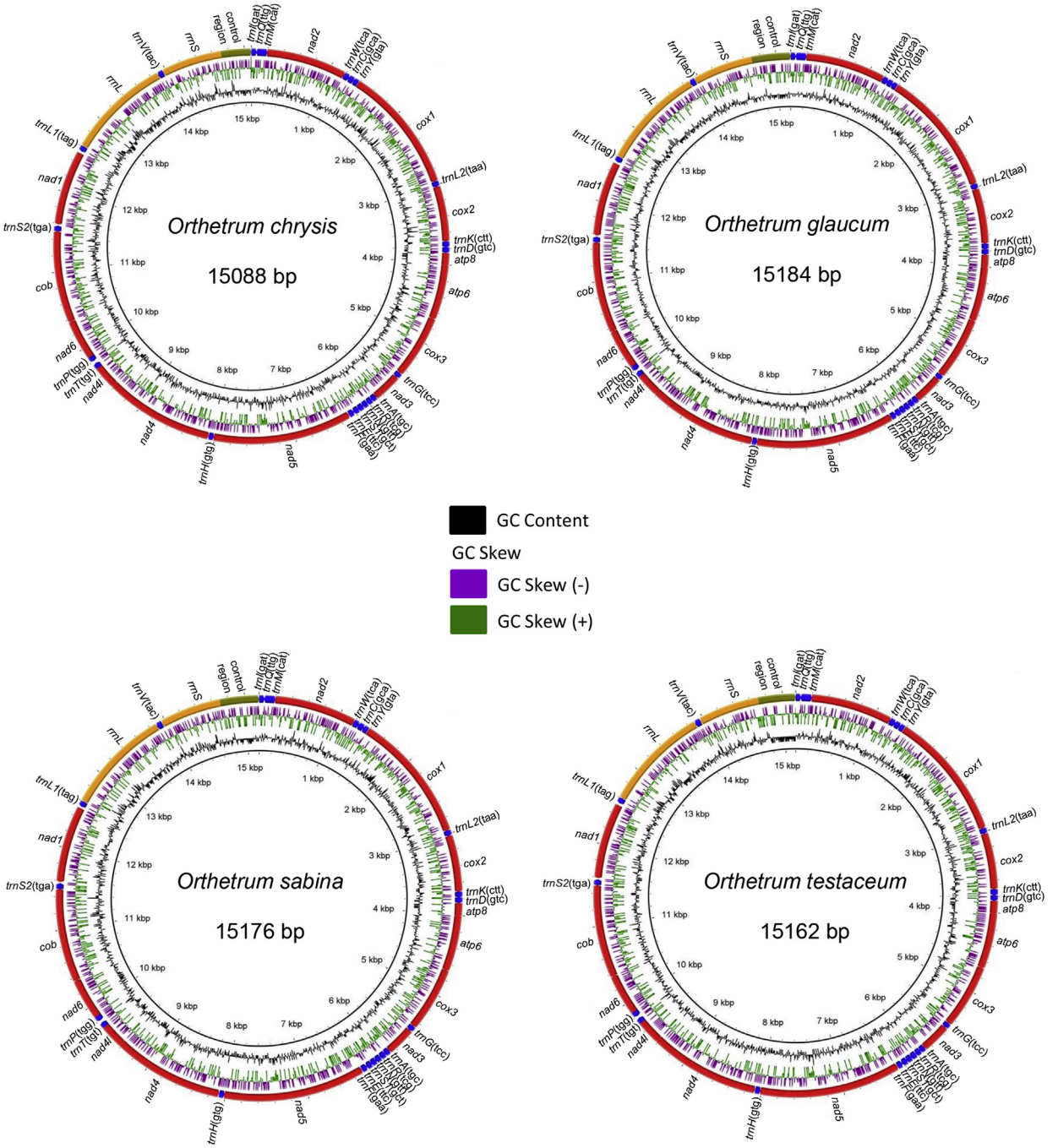

Fig. 1. Complete mitogenomes of *Orthetrum chrysis*, *O. testaceum*, *O. glaucum* and *O. sabina* with BRIG visualization showing the protein coding genes, rRNAs and tRNAs. GC skew is shown on the outer surface of the ring whereas GC content is shown on the inner surface.

PCGs was concordant with that based on 15 mt-genes. Nonetheless the genus *Orthetrum* was monophyletic, forming a clade with other taxa of the Libellulidae in which *Brachythemis contaminata* and *Hydrobasileus croceus* formed a sister group. Based on 15 mt-genes and 13 PCGs, *O. chrysis* and *O. testaceum* formed a sister group and *O. sabina* was basal to the other *Orthetrum* taxa (Fig. 2).

The genetic divergence between different pairs of *Orthetrum* taxa based on (a) 13 PCGs, (b) 2 rRNA genes, and (c) 13 PCGs + 2 rRNAs genes is summarized in Table 3.

Table 2
Start/stop codon of protein-coding genes (PCGs) of *Orthetrum* and other anisopteran taxa and *Epiophlebia superstes*. Bold codon indicates difference compared to *O. chrysis*. Oc, *O. chrysis*; Ot, *O. testaceum*; Os, *O. sabina*; Om, *O. melania*; Bc, *B. contaminata*; Hc, *H. croceus*; Ca, *C. aenea*; Dl, *D. lunatus*; Is, *Ictinogomphus* sp.; Es, *E. superstes*.

Gene	Oc	Ot	Og	Os	Om	Bc	Hc	Ca	Dl	Is	Es
nad2	ATT/TAA	ATT/TAA	ATT/TAA	ATT/TAA	**CCG/T**	ATT/TAA	ATT/TAA	ATT/TAA	ATT/**T**	ATT/TAA	ATT/TAA
cox1	ATA/T	ATA/T	ATA/T	ATA/T	ATA/T	**ATC**/T	**TTG**/T	ATA/T	**TTG**/T	**ATG**/T	**ATG**/T
cox2	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T
atp8	ATT/TAA	ATT/TAA	ATT/TAA	**ATC**/TAA	**ATC**/TAA	**ATC**/TAA	ATT/TAA	**TTG**/TAA	**ATC**/TAA	**ATC**/TAA	**ATC**/TAA
atp6	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	**GTG/TA**	**ATA**/TAA	**ATA**/TAA	**ATC**/TAA	ATG/**TA**	**ATA**/TAA	ATG/TA
cox3	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/**TA**	ATG/T	ATG/**TAG**	ATG/T	ATG/T	ATG/T
nad3	ATT/TAG	**ATC**/TAG	**ATC**/TAG	ATT/TAG	**ATC/TA**	ATT/TAG	ATT/TAG	**ATA/TAA**	**ATC/T**	**ATC**/TAG	**ATA/TA**
nad5	ATT/T	ATT/T	ATT/T	ATT/T	ATT/T	ATT/T	ATT/T	ATT/**TA**	ATT/T	ATT/T	ATT/T
nad4	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/**TA**	ATG/**TAG**	ATG/**TAG**
nad4l	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA
nad6	ATC/TAA	ATC/TAA	ATC/TAA	ATC/TAA	ATC/TAA	**ATT**/TAA	**ATT**/TAA	ATC/TAA	ATC/TAA	**ATA**/TAA	ATC/TAA
cob	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/**TAA**	ATG/T	ATG/**TAA**	ATG/T	ATG/**TAA**	ATG/T
nad1	ATA/TAA	ATA/TAA	ATA/TAA	**TTG**/TAA	ATA/TAA	ATA/TAA	ATA/**TAG**	**TTG**/TAA	**ATG/TAG**	ATA/**TAG**	**TTG/TAG**

4. Discussion

Mitogenomes of insects have been extensively studied, particularly regarding phylogeny and evolution (Bernt et al., 2013; Cameron, 2013). The complete nucleotide sequences (PCG and PCG+RNA) of mitogenome could be used to resolve higher-level phylogeny of Paraneopteran insects (Li et al., 2015). To date, eleven complete and two incomplete mitogenomes are available for Odonata in the GenBank comprising eleven genera in eight families (Fig. 2). The present study has added the complete mitogenome of four species of the genus *Orthetrum* to the list.

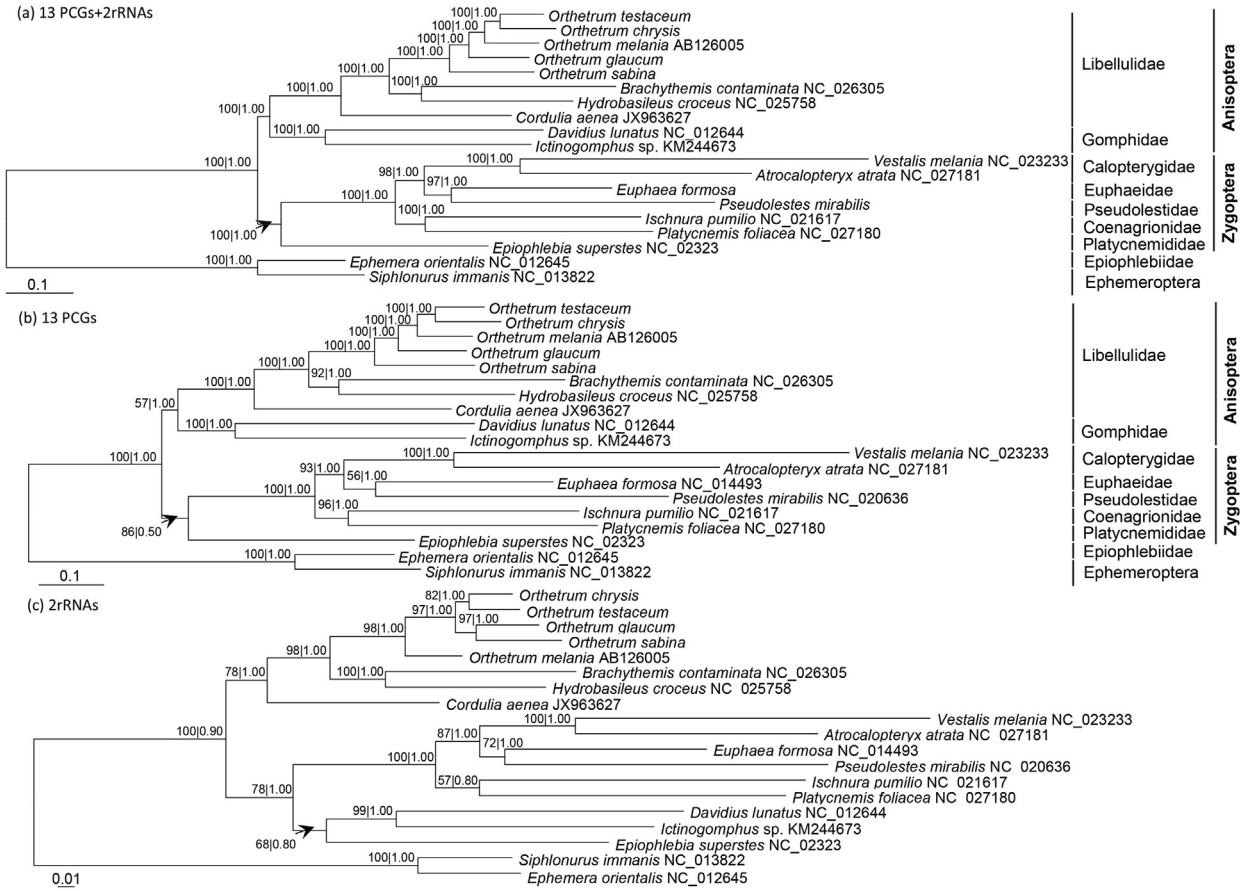

Fig. 2. Bayesian inference and maximum likelihood tree based on (a) 13 PCGs and 2 rRNA genes, (b) 13 protein-coding genes and (c) 2 rRNA genes of the whole mitogenomes of *Orthetrum* and other odonate taxa with Ephemeroptera taxa as outgroup. Numeric values at the nodes are ML bootstrap/Bayesian posterior probabilities.

Table 3
Percentage of uncorrected 'p' genetic distance between different pairs of *Orthetrum* taxa based on 13 protein-coding genes (PCGs), 2 rRNA genes and 13 PCGs + 2 rRNA genes.

Species pair	13 PCGs	2 rRNAs	13 PCGs + 2 rRNAs
O. chrysis vs *O. testaceum*	10.1	5.1	9.3
O. chrysis vs *O. glaucum*	12.5	7.1	11.6
O. chrysis vs *O. sabina*	14.4	7.5	13.3
O. chrysis vs *O. melania* AB126005	10.6	5.2	9.8
O. testaceum vs *O. glaucum*	11.3	7.1	10.7
O. testaceum vs *O. sabina*	13.9	8.5	13.1
O. testaceum vs *O. melania* AB126005	9.7	5.3	9.0
O. glaucum vs *O. sabina*	13.5	7.8	12.6
O. glaucum vs *O. melania* AB126005	10.7	6.3	10.0
O. sabina vs *O. melania* AB126005	13.2	6.6	12.2

The mitogenome size of *O. glaucum* (15,184 bp), *O. sabina* (15,176 bp) and *O. testaceum* (15,162 bp) is larger than that of *O. chrysis* (15,088 bp) and those of two other Libellulid taxa (*B. contaminata* with 15,056 bp and *H. croceus* with 15,088 bp). Among the other Anisoptera taxa available in GenBank, *D. lunatus* (Gomphidae) has the largest mitogenome size of 15,913 bp. Only one odonate species has a mitogenome size of over 16,000 bp, viz. *V. melania* (Zygoptera) with 16,685 bp.

Of the 13 PCGs, only *cox2* and *nad4l* have invariant start and stop codons for the 10 taxa of Anisoptera and *E. superstes* (Table 2). Several PCGs have incomplete stop codons (Table 2): T for (1) *cox1* and *cox2* in all 10 anisopteran taxa and *E. superstes*, (2) *cox3* in *E. superstes* and 8 anisopteran taxa except *B. contaminata* with TA and *C. aenea* with TAG, (3) *cob* in *E. superstes* and 7 anisopteran taxa except TAA in *B. contaminata*, *C. aenea* and *Ictinogomphus* sp., (4) *nad3* only in *D. lunatus* and TA in *O. melania* and *E. superstes*, and (5) *nad5* in *E. superstes* and 9 anisopteran taxa except TA in *C. aenea*; and TA for *nad4* in *D. lunatus* and *atp6* in *O. melania*, *D. lunatus* and *E. superstes*. The incomplete stop codons (T and TA) can be converted to TAA by post-translational polyadenylation (Ojala et al., 1981).

Among the tRNAs, *trnS1* has aberrant cloverleaf structure in all taxa (Table 4) – lacking (1) DHU-stem in *O. chrysis*, *O. glaucum*, *O. sabina*, *O. testaceum*, *C. aenea*, *D. lunatus* and *E. superstes*; (2) DHU-loop in *O. melania*; and (3) DHU-arm in *B. contaminata*, *H. croceus* and *Ictinogomphus* sp. Omitting *C. aenea* with incomplete mitogenome, *trnS1* is the only tRNA with aberrant cloverleaf structure in Gomphidae and Epiophlebiidae. In Libellulidae, six other tRNAs (*trnN*, *trnH*, *trnI*, *trnF*, *trnP*, *trnT*) lack the TΨC-loop, with one or more aberrant tRNAs in each taxon (Table 4).

As in most anisopteran mitogenomes, the intergenic spacer between *nad1* and *trnL1* genes of *Orthetrum* taxa is large – 28 bp in *O. chrysis* and *O. testaceum*, and 25 bp in *O. glaucum*, *O. melania* and *O. sabina* (Tables S1–S4, Table S6). An exception for the Anisoptera is the *D. lunatus* mitogenome with 1 bp separating the *nad1* and *trnL1* genes (Table S6). This condition is also present in the *E. superstes* mitogenome (1 bp), and all the known zygopteran mitogenomes have 1–2 bp separating these genes (Table S6).

In the present study based on concatenated 13 PCGs and 15 mt-genes, the Libellulidae is monophyletic with two lineages: (*Orthetrum*) – (*Brachythemis* + *Hydrobasileus*) (Fig. 2). It forms a sister group with Corduliidae. This finding concurs with those based on morphology and DNA sequences (Blanke et al., 2013; Bybee et al., 2008; Carle et al., 2015; Fleck et al., 2008; Kim et al., 2014). The Anisoptera is also monophyletic (Fig. 2).

Based on 13 PCGs and 15 mt-genes, the enigmatic *E. superstes* (Epiophlebiidae) in this study with Ephemeroptera as outgroup taxa formed a sister group with Zygoptera (Fig. 2). However, based on 2 rRNA genes the anisopteran Gomphidae formed a lineage with (Zygoptera + *Epiophlebia*) instead of with Libellulidae and Corduliidae (Fig. 2). Earlier studies which designated Epiophlebiidae as a sister lineage of Anisoptera in the suborder Epiprocta (Lohmann, 1996) have been supported by morphological and molecular data (Rehn, 2003; Hasegawa and Kasuya, 2006; Bybee et al., 2008; Fleck et al., 2008; Davis

Table 4
Absence of TΨC-loop, DHU-stem and DHU-arm in the transfer RNAs of *Orthetrum* and other anisopteran taxa and *Epiophlebia superstes*. ● indicates absence; *incomplete mitogenome: *O. melania* without *trnI* and *trnM*; *C. aenea* without *trnQ, trnI, trnM* and *trnP*.

Taxon	*trnN* TΨC-loop	*trnH* TΨC-loop	*trnI* TΨC-loop	*trnF* TΨC-loop	*trnP* TΨC-loop	*trnT* TΨC-loop	*trnS1* DHU-stem	*trnS1* DHU-loop	*trnS1* DHU-arm
O. chrysis	●	●					●		
O. testaceum	●		●	●			●		
O. glaucum	●			●			●		
O. sabina						●	●		
*O. melania**	●			●				●	
B. contaminata		●			●				●
H. croceus				●					●
*C. aenea**							●		
D. lunatus							●		
Ictinogomphus sp.									●
E. superstes							●		

et al., 2011; Kim et al., 2014). The extensive literature has been well reviewed (Kim et al., 2014). Other studies have placed Epiophlebiidae as a sister group of Zygoptera (Büsse et al., 2012) or as suborder Anisozygoptera (Dijkstra et al., 2013). The present study based on 13 PCGs and 15 mt-genes supports the finding of *Epiophlebia* forming a siter group with Zygoptera (Fig. 2). This concurs with the finding that the mitogenomic organization of *E. superstes* is more similar to that of Zygoptera than to that of Anisoptera (Wang et al., 2014).

The monophyly of Zygoptera in our study agrees with findings supporting this status (Rehn, 2003; Dumont et al., 2010; Davis et al., 2011; Dijkstra et al., 2013; Kim et al., 2014). Our limited sample reveals three lineages: (Calopterygidae) + (Eupaeidae + Pseudolestidae) + (Coenagrionidae + Platycnemididae). These lineages agree with the grouping of Calopterygidae, Euphaeidae and Pseudolestidae as members of the superfamily Calopterygoidea, and Coenagrionidae and Platycnemididae as members of the superfamily Coenagrionoidea (Dijkstra et al., 2013).

In summary, we have successfully sequenced the whole mitochondrial genomes of *O. chrysis*, *O. testaceum*, *O. glaucum* and *O. sabina* by next generation sequencing. The genome features are similar to other dragonflies. The phylogenetic tree based on 13 PCGs is concordant with that based on 15 mt-genes. Based on concatenated 13 PCGs and 15 mt-genes of the mitogenome, the Libellulidae (Anisoptera) is monophyletic with two lineages: (*Orthetrum*) − (*Brachythemis* + *Hydrobasileus*). It forms a sister group with Corduliidae. The Zygoptera is monophyletic with three lineages: (Calopterygidae) + (Eupaeidae + Pseudolestidae) + (Coenagrionidae + Platycnemididae). The enigmatic *E. superstes* (Epiophlebiidae) forms a sister group with Zygoptera. The complete mitogenome is useful for determining the higher-level phylogenetic relationships of Odonata.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2016.09.002.

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