The complete mitochondrial genome of the emperor dragonfly *Anax imperator* LEACH, 1815 (Odonata: Aeshnidae) via NGS sequencing

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**ABSTRACT**

Here we report the complete mitochondrial genome of the emperor dragonfly, *Anax imperator* (Odonata: Aeshnidae) as the first of its genus. Data were generated via next generation sequencing (NGS) and assembled using an iterative approach. The typical metazoan set of 37 genes (13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes) was detected in the same gene order as in other odonate mitogenomes. However, only three intergenic spacer regions are present in *A. imperator* lacking the distinct s5 spacer, which was regarded as informative feature of the odonate suborder Anisoptera (dragonflies) but absent in Zygoptera (damselflies). With 16,087 bp, it is the longest anisopteran mitogenome to date, mainly due to the long A+T-rich control region of 1291 bp.

The emperor dragonfly, *Anax imperator*, is a widespread and common species in the old world inhabiting all types of standing- and slow-running freshwater ecosystems. It was one of the first odonate species for which a recent range shift northwards (e.g. Parr 2010) and towards higher altitudes (Westermann 2003; Hunger et al. 2006) was noticed due to global climate change. The first records of this species in Sweden were 2002 (Ott 2010). In only 11 years *A. imperator* crossed a distance of 970 km northwards through Scandinavia (Nielsen 1998; Lejfelt-Sahlen 2007). The larvae of this large dragonfly species are known to be very aggressive (e.g. Beutler 1985) and will invade and significantly influence the native species composition of freshwater ecosystems. Genetic and comparative genomic studies on range shift, expansion, and adaptive potential of this species are of great interest to further elucidate the impact of global change on flying insects. To date for *A. imperator*, a panel of 10 nuclear microsatellite loci and partial mitochondrial genes (*cox1*, *nad1*, and both *rRNAs*) were established so far to serve in various phylogenetic studies (Misof et al. 2001; Hadrys et al. 2007; Fleck et al. 2008; Rach et al. 2008; Bergmann et al. 2013). To consequently proceed towards a comparative genomic approach one first step is the unravelling and comparison of mitogenomes, e.g. their gene content, arrangements, and genealogical relationships.

As for the *A. imperator* mitogenome, a standard phenol–chloroform protocol by Hadrys et al. (1992) was used to extract total genomic DNA from flight muscles of a single individual collected in Southern France (43°36’17.7”N 4°48’34.4”E). DNA was submitted for library preparation and whole genome sequencing on an Illumina HiSeq2000 (75 bp paired-end reads) to the Yale Center for Genome Analyses (YCGA, http://www.ycga.yale.edu). Different mitochondrial gene sequences containing partial *nad1*, *cox1*, 12S rRNA, and 16S rRNA genes (accession numbers: KC912228.1, KF584974.1, EU477652.1 and EU183256.1) were used as reference seeds for a subsequent assembly employing Genious v.8.1.5 (http://www.geneious.com/). For mitochondrial genome annotation, the MITOS WebServer (mitos.bioinf.uni-leipzig.de/index.py) was applied and results were checked manually using BLAST (Altschul et al. 1990) and available odonate mitochondrial genomes (e.g. Yu et al. 2014; Chen et al. 2015). Transfer RNA genes were predicted using both, the tRNAscan-SE v.1.21 Search Server (Lowe & Eddy 1997) and ARWEN v.1.2 (Laslett & Canbäck 2008).

The complete circular mitochondrial genome sequence of *A. imperator* (GenBank accession number #KX161814) with the length of 16,087 bp is the largest known mitogenome among Anisoptera. It exhibits the standard metazoan gene content of 37 genes, comprising 13 protein-coding genes, 22 tRNA genes, and two rRNA genes which are identically arranged as in the few other odonate mitochondrial genomes (e.g. Simon & Hadrys 2013; Lorenzo-Carballa et al. 2014; Chen et al. 2015; Yu et al. 2014; Feindt et al. 2016). Overall base frequency is 76.0%...
AT-biased, for the 1291 bp long control (A + T rich) region even 93.5%. All standard mitochondrial invertebrate start codons are found, in detail ATT (nad5), ATA (nad2, nad3), TTG (cox1, nad1), ATC (atp8, nad6), and ATG (cox2, atp6, cox3, nad4, nad4l, cob). Two proteins (cox2, nad5) possess a single T as an incomplete stop codon, requiring post-transcriptional polyadenylation whereas all others protein-coding genes use TAA as stop codon (Table 1). The gene length of tRNA genes ranges from 65 bp to 73 bp and all tRNAs can be folded in the typical cloverleaf structure, except the D-replacement tRNA trnS1.

Further, two pseudo-tRNA genes were detected by the tRNA prediction software ARWEN v.1.2 (Laslett & Canbäck, 2008) which were both D-Loop tRNAs and located inside the cox2 sequence and in trnA/trnR, respectively. Therefore, their functionality remains questionable.

However, in contrast to the known other anisopteran mitogenomes, only three intergenic spacer regions were discovered (see Table 1). These are located between trnY/cox1, trnT/trnP, and trnS2/nad1. They are also present in other odonates (Anisoptera and Zygoptera), e.g. Ischnura elegans (Feindt et al. 2016), Ischnura pumilio (Lorenzo-Carballa et al. 2014), Megaloprepus caerulatus (Feindt et al. 2016), or Brachythemis contaminata (Yu et al. 2014). The latter, an anisopteran species additionally shows a fourth spacer region between nad1/trnL2 that is asserted to be typical for Anisopterans and lacking in Zygopterans (Lin et al. 2010). This spacer, commonly called s5 (though counting and numbering spacer regions is not consistent between most mitogenome publications) is not present in Anax. Consequently, the absence of this spacer refutes the theory of being a putative distinctive feature between Anisoptera and Zygoptera and stresses the necessity to analyze more mitogenomes within Odonata to allow stronger, reliable assumptions about phylogenetically informative mtDNA characteristics. The phylogenetic position of A. imperator in the context of all available anisopteran mitogenomes to date (3 May 2016) is displayed in Figure 1 and so far consistent with other gene tree phylogenies.

Table 1. Mitochondrial genome organization and gene content of A. imperator with detailed description of gene boundaries, strand, gene length (in bp) as well as start and stop codons for protein-coding genes and anticodons for tRNA genes, respectively.

| Gene/region   | Strand | Start position | Stop position | Length (bp) | Anti/start codon | Stop codon |
|---------------|--------|----------------|---------------|-------------|------------------|------------|
| trnI          | +      | 214            | 281           | 68          | GAT              | /          |
| trnQ          | -      | 278            | 347           | 70          | TTG              | /          |
| trnM          | +      | 352            | 420           | 69          | CAT              | /          |
| nad2          | +      | 424            | 1419          | 996         | ATA              | TAA        |
| trnW          | +      | 1417           | 1487          | 71          | TCA              | /          |
| trnC          | -      | 1479           | 1543          | 65          | GCA              | /          |
| trnY          | -      | 1545           | 1613          | 69          | CTA              | /          |
| s1            | NA     | 1614           | 1653          | 40          | /                | /          |
| cox1          | +      | 1634           | 3192          | 1539        | TTG              | TAA        |
| trnL2         | +      | 3187           | 3256          | 70          | TAA              | /          |
| cox2          | +      | 3256           | 3943          | 688         | ATG              | T(aa)      |
| trnK          | +      | 3944           | 4016          | 73          | CTT              | /          |
| trnD          | +      | 4016           | 4084          | 69          | GTC              | /          |
| atp8          | +      | 4084           | 4245          | 162         | ATC              | TAA        |
| atp6          | +      | 4239           | 4916          | 678         | ATG              | TAA        |
| cox3          | +      | 4916           | 5704          | 789         | ATG              | TAA        |
| trnG          | +      | 5704           | 5768          | 65          | TCC              | /          |
| nad3          | +      | 5,766          | 6,122         | 357         | ATA              | TAA        |
| trnA          | +      | 6122           | 6190          | 69          | TGC              | /          |
| trnR          | +      | 6189           | 6,258         | 70          | TCG              | /          |
| trnI          | +      | 6258           | 6324          | 67          | GTC              | /          |
| trnS1         | +      | 6325           | 6392          | 68          | GCT              | /          |
| trnE          | +      | 6392           | 6460          | 69          | TTC              | /          |
| trnF          | -      | 6459           | 6526          | 68          | GAA              | /          |
| nad5          | -      | 6525           | 8254          | 1730        | ATT              | T(aa)      |
| trnH          | -      | 8255           | 8322          | 68          | GTG              | /          |
| nad4          | -      | 8322           | 9665          | 1344        | ATG              | TAA        |
| nad4l         | -      | 9659           | 9952          | 294         | ATG              | TAA        |
| trnT          | +      | 9954           | 10,022        | 69          | TGT              | /          |
| s2            | NA     | 10,023         | 10,045        | 23          | /                | /          |
| trnP          | -      | 10,046         | 10,111        | 66          | TGG              | /          |
| nad6          | +      | 10,113         | 10,634        | 522         | ATC              | TAA        |
| cox2          | +      | 10,634         | 11,767        | 1134        | ATG              | TAA        |
| trnS2         | +      | 11,766         | 11,832        | 67          | TGA              | /          |
| s3            | NA     | 11,833         | 11,849        | 17          | /                | /          |
| nad1          | -      | 11,850         | 12,800        | 951         | TTG              | TAA        |
| trnL1         | -      | 12,801         | 12,868        | 68          | TAG              | /          |
| i-rRNA        | -      | 12,810         | 14,180        | 1371        | /                | /          |
| trnV          | -      | 14,167         | 14,236        | 70          | TAC              | /          |
| s-rRNA        | -      | 14,239         | 15,008        | 770         | /                | /          |
| A+T-rich (control) region | NA | 15,009 | 212 | 1291 | / |

Transfer RNAs are given in the one-letter amino acid code with the corresponding anticodons. Intergenic spacer regions are numbered (s1–s3).

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Figure 1. Neighbour-Joining Tree of A. imperator within all available anisopteran odonate species (03 May 2016): Orthetrum triangulare (AB126005.1), Hydrobasileus croceus (NC_025758.1), B. contaminata (NC_026305.1), Ictinogomphus sp. (KM244673) and Davidius lunatus (NC_012644.1). The phylogeny was reconstructed based on 13 mitochondrial protein-coding genes via Paup with 1000 bootstrap replicates and Euphea formosa (NC_014493.1) as an outgroup.
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