The complete mitochondrial genome of the neotropical helicopter damselfly *Megaloprepus caerulatus* (Odonata: Zygoptera) assembled from next generation sequencing data

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ABSTRACT

Odonata (dragonflies and damselflies) is a small order at the base of flying insects (Pterygota). Resolving family-level phylogenetic relationships within this order receives great attention. Hereby, genetic data already resulted in various reorganizations of phylogenetic relationships (e.g. Dumont et al. 2010; Carle et al. 2015). However, today’s phylogenies still harbour open questions concerning family-level as well as deeper taxonomic positions. Mitochondrial genome projects in odonates may help to unravel such unresolved phylogenetic relationships by constructing more robust phylogenies based on complete gene and genome comparison (e.g. Simon & Hadrys 2013). One family that has received great attention in the past are the Pseudostigmatidae (giant damselflies) (e.g. Groeneveld et al. 2007; Ingleby et al. 2012). They were recently placed into the Coenagrionidae based on three sequence markers (Dijkstra et al. 2014). Here, more robust genomic data are needed not only to clarify their taxonomic position but also to facilitate genomic-based studies on Neotropical forest health indicators. In this work, we present a complete mitochondrial genome of *Megaloprepus caerulatus* as the first member of this group. *Megaloprepus caerulatus* was already included in many ecological (e.g. Fincke & Hedström 2008) and evolutionary studies in tropical habitats, but yet little research has been done on its genetics (e.g. Fincke & Hadrys 2001; Feindt et al. 2014).

Via the standard phenol–chloroform extraction, (Hadrys et al. 1992) total genomic DNA from the flight muscles of a single *M. caerulatus* individual collected at the Biological Research Station La Selva (OTS), Costa Rica (N 10°25’19.74”W 84°00’35.22”) was extracted. Library preparation and DNA sequencing (100 bp mate pairs with different insert sizes, Illumina HiSeq2500, Illumina Inc., San Diego, CA) was performed at the Weill Cornell Medical College in New York. The mitochondrial genome was assembled using Geneious vers. 8.1 (http://www.geneious.com); while mapping a fraction of the cleaned reads onto a seed sequence (here, *cox1*: KF895301.1 and *nad1*: KF895193.1) allowing strand extension using varying iters. Hereby, the settings included a minimum overlap of 60 bp, a minimal overlap identity of 90%, and a variable word size between 35 and 50. The mitochondrial genome was annotated using the MITOS WebServer (mitos.bioinf.uni-leipzig.de/index.py) and verified via BLAST (Altschul et al. 1990) against the NCBI database (http://www.ncbi.nih.gov) or additionally with published mitochondrial genomes of other odonate species. Transfer RNA genes were identified by a tRNA covariance model implied on the tRNAscan-SE vers. 1.21 Search Server (http://lowelab.ucsc.edu/tRNAscan-SE; Lowe & Eddy 1997) and ARWEN vers. 1.2 (http://mbio-serv2.mbioekol.lu.se/ARWEN, Laslett & Canbäck 2008).
Phylogenetic relationships were reconstructed using a selection of odonate mitochondrial genomes and a mayfly Parafronurus youi as outgroup (Figure 1). All 13 protein coding genes and rRNA genes were aligned independently, then concatenated and a maximum parsimony tree was calculated (1000 replicates) in PAUP (Swofford 2002).

The obtained complete mitochondrial genome of M. caerulatus (NCBI: KU958377) is the first of a tropical odonate species. It has a total length of 16,094 bp, and is the second largest aside of Vastalis melanica (16,685 bp, NC_023233). In all observed parameters, the presented mitochondrial genome shows a strong similarity to all other already published odonate mitochondrial genomes (e.g. Lorenzo-Carballa et al. 2014; Tang et al. 2014; Yu et al. 2014; Chen et al. 2015; Feindt et al. 2016; Herzog et al. 2016). It contains the common arrangement of 37 genes including 13 protein-coding genes, 2 rRNA (16S and 12S rRNA) genes, 22 tRNA genes, and an A + T rich (control) region of 1376 bp in length. Comparing with the previously released single genes, we observed 100% similarity to nad1 (DQ642992.1) (Groeneveld et al. 2007) and 16S RNA (DQ642987.1), and in nad1 (JQ966612.1), 16S (JQ966660.1) and 12S RNA (JQ966647.1) a similarity between 98% and 100% to genes illustrated in Ingley et al. (2012). The overall A + T content of the mitochondrial genome is 75.9% (A: 43.1%, C: 14.2%, G: 9.8%, T: 32.8%) and therefore it is similar to the base frequencies of the protein-coding genes (AT: 74.4%).

Hereby, atp8 encompasses the highest A + T content with 81.1% and cox1, as it was described in other odonates (Lorenzo-Carballa et al. 2014), with 68.9% the lowest. All protein coding genes start with characteristic invertebrate specific mitochondrial start codons: cox1, atp6, cox3, nad4, nad4L and cob use ATG; nad2, cox2 and nad6 start with ATA; nad3 and nad1 start with TTG; nad5 starts with ATT and atp8 starts with ATC. The standard stop codon TAA was used eight times (nad2, cox1, atp8, atp6, nad4L, nad6, cob, nad1), whereas TAG was used only once by nad3. An incomplete stop codon with a single T was found in four cases (cox2, cox3, nad5 and nad4). Length of the tRNA genes in M. caerulatus ranges from 64 bp to 74 bp except for trnL1 (Leu), trnS1 (Ser), trnG (Ser) all of which employ the typical clover-leaf secondary structures.

A difference in numbers of intergenic spacer regions is described in the two large odonate orders (Lorenzo-Carballa et al. 2014). They seem to provide a phylogenetic signal for the split of Anisoptera (dragonflies) from Zygoptera (damselflies). The lack of the intergenic spacer region s5 seems to be a damselfly specific character. In M. caerulatus, we detected four spacer regions at trnY/cox1, tmF/nad5, tmT/trnP and tmS2/nad1 with a total length of 106 bp. With this we proved that the unique spacer s4 between tmF and nad5, which is also present in I. pumilio (NC_021617), Pseudolestes mirabilis (NC_020636) and I. elegans (KU958378) might not only be a damselfly specific character for the Coenagrionidae. The phylogenetic tree (Figure 1) places M. caerulatus as a sister species to Ischnura spp. with low support. More mitochondrial genomic data is needed to resolve relationships within and between families. However, the mitochondrial genome presented in this study is a valuable resource for future population genomic studies and also owns potential for answering phylogenetic questions on higher taxonomic levels.

Disclosure statement

Collecting permit was processed from the Ministerio de Ambiente y Energía, Costa Rica (MINAE) and the permit for using the genetic material of the individual was approved by Comisión Nacional para la Gestión de
la Biodiversidad, Costa Rica (CONAGEBIO). Furthermore, the authors state no conflicts of interest.

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