Mitochondrial DNA

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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of the stonefly *Dinocras cephalotes* (Plecoptera, Perlidae)

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Abstract

The complete mitochondrial genome of the perlid stonefly *Dinocras cephalotes* (Curtis, 1827) was sequenced using a combined 454 and Sanger sequencing approach using the known sequence of *Pteronarcys princeps* Banks, 1907 (Pteronarcyidae), to identify homologous 454 reads. The genome is 15,666 bp in length and includes 13 protein-coding genes, 2 ribosomal RNA genes, 22 transfer RNA genes and a control region. Gene order resembles that of basal arthropods. The base composition of the genome is A (33.5%), T (29.0%), C (24.4%) and G (13.1%). This is the second published mitogenome for the order Plecoptera and will be useful in future phylogenetic analysis.

Keywords

Dinocras cephalotes, insect, mitogenome, plecoptera, stoneflies

History

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Dinocras cephalotes (Curtis, 1827) is a large predatory stonefly of the family Perlidae. It's habitat are unpolluted headwater streams of European mountain ranges. Stoneflies are sensitive towards environmental pollutants and thus play an important role as indicator organisms in water quality assessments. The *D. cephalotes* specimen used in this study was collected in May 2011 in the Henne stream of the Sauerland region, a low mountain range in western Germany (51°10′25.90″N, 8°32′28.08″E). DNA was sequenced on a 454 GS Junior sequencer (Roche) as part of a population genetics project (see Elbrecht et al., in press). The mitogenome of the stonefly *Pteronarcys princeps* (Genbank AY687866, Stewart & Beckenbach, 2006) was used to identify sequences of mitochondrial origin in the quality trimmed 454 data using the discontinuous megablast algorithm within BLASTn (BLAST suite 2.2.26+, e-values $<e^{-20}$, Camacho et al., 2009). Relevant reads were extracted from the data set using a custom R script (available on request) and the genome was assembled *de novo* with Geneious Pro 6.0.6 (Kearse et al., 2012). The first draft genome was subsequently used as a search query to identify additional reads in the 454 read database. Furthermore, Sanger sequencing was used to close gaps and to validate accuracy of weakly supported regions. Primers were developed using the Primer3 (Rozen, 2000) software implemented in Geneious and PCRs were conducted in 25 µl reactions with the following conditions: 1 × PCR buffer (HotMaster, 5-Prime), 0.2 mM dNTPs, 0.5 µM of each primer, 0.02 U/µl HotMasterTaq (5-Prime) and 1 µl DNA, filled up to a total volume of 25 µl with HPLC H_2O. PCR program: 94 °C/120 s, 36 cycles of (94 °C/20 s, annealing temperature depending on T_m of primer pair /30 s, 65 °C/60 s), 65 °C/7 min. 10 µl PCR product were ExoI/FastAP purified (Werle et al., 1994) and Sanger sequenced on an ABI 3730 sequencer (Applied Biosystems) by GATC Biotec (Konstanz, Germany) and the sequencing core facility (Department of Receptor Biochemistry) of the Ruhr University Bochum, Germany.

The mitogenome was annotated for CDS, 18S, 16S and control region by comparison with the *P. princeps* genome and using the ORF finder function in Geneious. For tRNA prediction the programs tRNAscan-SE 1.21 and ARWEN v1.2.3 were used (Laslett & Canback, 2008; Lowe & Eddy, 1997).

By using a combination of 454 and Sanger sequences we were able to assemble the complete mitogenome of *D. cephalotes* with a length of 15,666 bp and good overall coverage, except for parts of the control region (Figure 1). The final assembly contains 184 of the 454 reads and 40 Sanger sequences. Average sequence coverage is 6.0 (SD = 2.1). The mitochondrial genome includes 13 protein-coding genes, 2 ribosomal RNA genes, 22 transfer RNA genes and a control region. The base composition of the genome is A (33.5%), T (29.0%), C (24.4%) and G (13.1%) with a GC content of 37.5%. The annotated mitogenome is available online in NCBI (GenBank accession number KF484757).

The completed mitochondrial genome of *D. cephalotes* is slightly shorter than the genome of *P. princeps* (16,004 bp) mainly due to deletions in the control region. Predicted coding features of both stoneflies are identical in number and gene order following the basal arthropod mitochondrial gene structure. Pairwise sequence identity of coding regions is 73.0% (SD = 5.1%). Initial phylogenetic analyses of the protein-coding genes support the monophyly of both plecopterans in the mitochondrial insect tree (Poettker, unpublished data). In this article, we provided the second mitochondrial genome of a stonefly. This new mitochondrial genome will be a useful resource for future phylogenetic analyses of arthropods.

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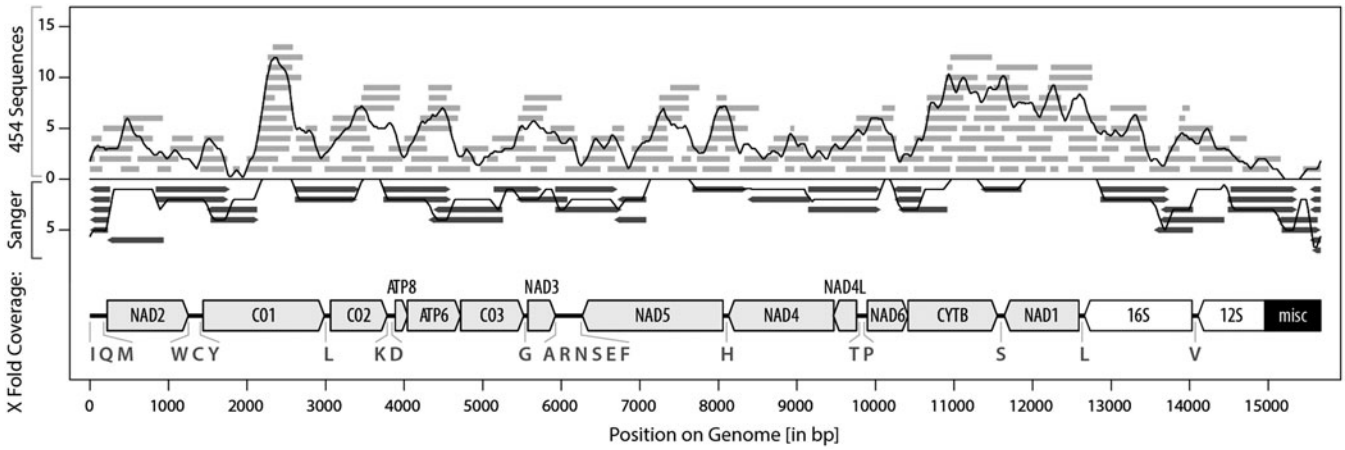

Figure 1. Gene annotations for the mitochondrial genome of *D. cephalotes* (bottom) and sequencing depth for 454 and Sanger reads (top). Genes are annotated with boxes, highlighting the direction of the CDS. tRNAs are annotated with letters in light gray. Sequence coverage is shown in light gray for 454 reads and dark gray for Sanger sequences including the sequencing direction indicated by arrows. The average coverage is shown with black lines using a 100 bp sliding window.

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Declaration of interest

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