

# Mitochondrial DNA Part A

## DNA Mapping, Sequencing, and Analysis

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## MITOGENOME ANNOUNCEMENT

# The mitogenome of *Hydropsyche pellucidula* (Hydropsychidae): first gene arrangement in the insect order Trichoptera

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We describe the mitochondrial genome of *Hydropsyche pellucidula* Curtis 1834, which is first described for the suborder Annulipalpia and the first in the order Trichoptera to show a non-canonical gene order. The mitogenome was obtained by *de novo* assembly of shotgun sequenced total genomic DNA using Illumina Miseq technology, which produced an average coverage of 115× and a minimum coverage of 48×. The mitochondrial genome includes 13 protein-coding genes, 2 rRNAs and 22 tRNAs. The genome is characterized by a rearrangement in the relative position of protein-coding and ribosomal genes. This mitogenome sequence will be useful for studying the family Hydropsychidae, which is commonly used for freshwater pollution biomonitoring.

**Keywords**

Biomonitoring, Hydropsychidae,  
Mitochondrial rearrangement,  
mitochondrial metagenomics

**History**

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The insect order Trichoptera contains around 12 000 described species (Mackay & Wiggins, 1979). Both the aquatic larvae and terrestrial adult stages are essential components of aquatic and riparian communities and are used for biological assessment of water quality (de Moor & Ivanov, 2008; Mackay & Wiggins, 2003). We sequenced the mitogenome of *Hydropsyche pellucidula* BMNH-1425186 (Trichoptera: Hydropsychidae). The high bioindicator value of the genus *Hydropsyche* (Tszedel et al., 2015) makes this mitogenome a useful tool for biomonitoring approaches.

The voucher specimen was a larva manually collected from Adam's Pond, Richmond Park, London, UK. It was morphologically identified and deposited in the Natural History Museum of London (voucher code BMNH 1425186). The mitogenome was *de novo* assembled from a metagenomic library containing other aquatic macroinvertebrates following the mitochondrial metagenomics (MMG) approach (Crampton-Platt et al., 2015; Gillett et al., 2014; Tang et al., 2014). The mitochondrial contig of *Hydropsyche pellucidula* was identified through its *cox1* gene (barcode region) obtained by Sanger sequencing and by its 99.6% similarity to another barcode from Múrria et al. (2010) (HM134820). Gene annotations were predicted using MITOS (Bernt et al., 2013) and were manually refined.

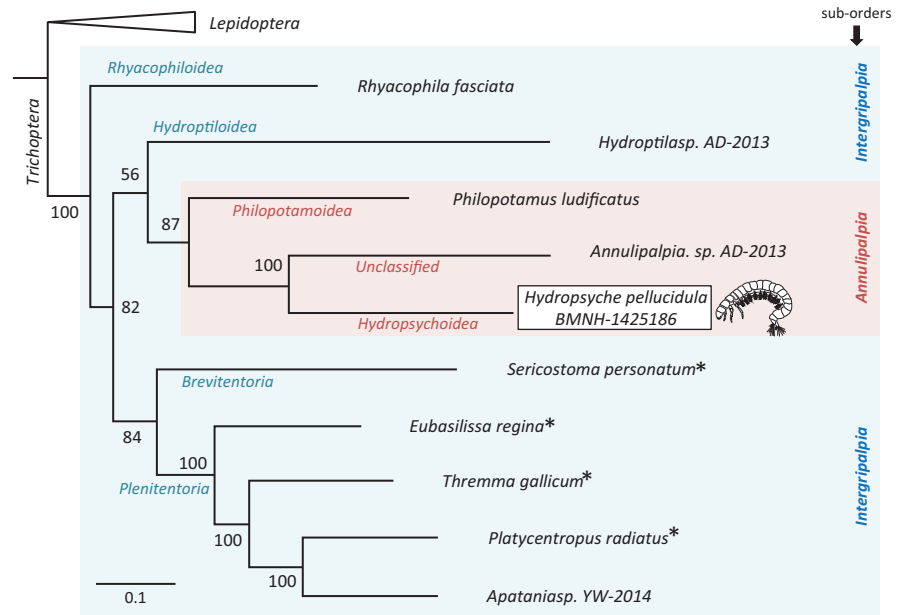
The resulting mitogenome is 25 004 bp long with an even read coverage of 115× on average (min: 48×; max: 280×). We

identified 13 protein-coding genes, 2 rRNAs and 22 tRNAs and a particularly long control region (~8 kb). A terminal AT-rich region did not allow us to circularize the genome with confidence. The gene order differs from canonical insect mitochondria. The *rrnS*, generally located between the *rrnL* and the control region, is displaced to a region between *cob* and *nad1* and is flanked by two regions of around 600 and 800 bp for which homology could not be established. Additionally, two tRNAs (*trnP*, *trnI*) are also found in non-canonical positions.

The sequence was validated by the construction of a phylogenetic tree for the order Trichoptera based on protein-coding and rRNA genes from all complete or nearly complete mitogenomes and transcriptomes retrieved from GeneBank (September 2015; Benson et al., 2014). The dataset included 9 different species sharing a set of mitochondrial loci (*atp6*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad3*, *nad4* and *rrnL*). Three Lepidoptera genomes were selected as outgroups. Multiple alignments were built for each locus with MAFFT (Katoh & Standley, 2013) and a maximum-likelihood phylogenetic analysis was conducted in RAxML-HPC2 (Stamatakis, 2006) on the CIPRES server (Miller et al., 2010). The best tree was selected over 100 alternative runs, using a GTR-cat model and node support was calculated with 10 000 bootstrap replicates.

As expected, *Hydropsyche pellucidula* is placed in the suborder Annulipalpia (Figure 1), which is recovered as monophyletic. In contrast, the suborder Integripalpia appears to be paraphyletic and contentious as previously reported (Ivanov, 2002; Kjer et al., 2002). The tree is also consistent with the recent phylogenomic analysis of Misof et al. (2014). The rearrangement cannot be assigned to a specific phylogenetic level, as other Annulipalpia data are based on transcriptomes and unknown gene order. The mitogenome was deposited in GeneBank:KT876876.

Figure 1. Phylogeny of the order Trichoptera based on 9 mitochondrial loci (*atp6*, *cob*, *cox1*, *cox2*, *cox3*, *nd1*, *nd3*, *nd4* and *rnl*). The new mitogenome is highlighted with a white box. Suborders are delimited by red (Annulipalpia) and blue (Integripalpia) backgrounds. Trichoptera species with a complete mitochondrial genome are marked by an asterisk. All other species are based on transcriptomic data, i.e. their relative gene order remains unknown.



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## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article. This work was founded by the NHM Biodiversity Initiative. PA was supported by a postdoctoral grant from The Royal Society (Newton International Program, UK).

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