The complete mitochondrial genome of the North American pale summer sedge caddisfly *Limnephilus hyalinus* (Insecta: Trichoptera: Limnephilidae)

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**ABSTRACT**

The pale summer sedge caddisfly, *Limnephilus hyalinus* Hagen, 1861 (Limnephilidae, the Northern Caddisflies), is widespread in North America. Genome skimming by Illumina sequencing allowed assembly of a complete 15,168 bp circular mitogenome from *L. hyalinus* consisting of 78.0% AT nucleotides, 22 tRNAs, 13 protein-coding genes, two rRNAs and a control region in the ancestral insect gene order. *Limnephilus hyalinus* COX1 features an atypical CGA start codon while ATP8, NAD1, NAD5, and NAD6 exhibit incomplete stop codons. The mtTERM binding site is conserved between the Trichoptera and the Lepidoptera. Phylogenetic reconstruction reveals a monophyletic Order Trichoptera, Family Limnephilidae, and genus *Limnephilus*.

The Living Prairie Mitogenomics Consortium is constructing a library of arthropod mitogenomes for improved DNA-based species identification and phylogenetics (Living Prairie Mitogenomics Consortium 2017, 2018; Marcus 2018) through an inquiry learning exercise in an undergraduate course (Marcus et al. 2010). Students who successfully analysed the class data (which were further curated by the instructor) belong to our consortium.

On 14–15 August 2015, we collected night-flying insects at the Living Prairie Museum (GPS 49.889607 N, –97.270487 W) using a USDA blacklight trap (Winter 2000). One adult specimen of the pale summer sedge caddisfly, *Limnephilus hyalinus* Hagen, 1861 (Insecta: Trichoptera: Limnephilidae, project specimen number 2015.08.14.076), was trapped, pinned, identified (Ruiter 1995; Schwiebert 2007), and deposited in the Wallis Roughley Museum of Entomology at the University of Manitoba (voucher JBWM0379996).

*Limnephilus* is a large genus with over 150 species that are frequently abundant in high latitude and high altitude lacustrine habitats (Ruiter 1995; Schwiebert 2007; McCullagh et al. 2015). *Limnephilus hyalinus* occurs from Alaska to Colorado, and from British Columbia to New England in lakes and rivers, with adults emerging July-September (Schwiebert 2007; Houghton 2012). Here, we describe the first complete mitogenome for a New World *Limnephilus* from *L. hyalinus*.

DNA was prepared (McCullagh and Marcus 2015) and sequenced by Illumina MiSeq (San Diego, CA) (Peters and Marcus 2017). We assembled the mitogenome of *L. hyalinus* (GenBank MK077681) with Geneious 10.1.2 from 7,020,834 paired 75 bp reads using an *Anabolia bimaculata* (Trichoptera: Limnephilidae) reference mitogenome (MF680449) (Peirson and Marcus 2017). Annotation was in reference to *A. bimaculata*, *L. decipiens* (AB971912), and *Eubasilliassa regina* (Trichoptera: Phryganeidae, NC023374) (Wang et al. 2014). The locations of mitochondrial tRNAs were determined using ARWEN v.1.2 (Laslett and Canback 2008). Also, the *L. hyalinus* nuclear rRNA repeat (GenBank MK077680) was assembled and annotated using *A. bimaculata* (MF680448) and *Triaenodes tardus* (Trichoptera: Leptoceridae, MG201853) (Lalonde and Marcus 2017) reference sequences.

The *L. hyalinus* circular 15,168 bp mitogenome assembly was composed of 10,042 paired reads with nucleotide composition: 39.5% A, 13.9% C, 8.1% G, and 38.4% T. Gene composition and order in *L. hyalinus* are identical to most other trichopteran mitogenomes (Marcus 2018). *Limnephilus hyalinus* COX1 begins with an aberrant start codon (CGA) like many other insects...
The mitogenome contains three protein-coding genes (ATP8, NAD1, NAD5) with single-nucleotide (T) stop codons and one gene (NAD6) with a two-nucleotide (TA) stop codon completed by post-transcriptional addition of 3' A residues. The structure and arrangement of tRNAs, rRNAs, and control region are typical for Trichoptera (Lalonde and Marcus 2017; Peirson and Marcus 2017). The mitogenome contains an mtTERM binding site (ATACTAATA) between tRNA-Ser and NAD1, similar to those described from sister Order Lepidoptera (McCullagh and Marcus 2015).

The mitogenomes from L. hyalinus, 16 other Trichoptera, and six Lepidoptera species were aligned in CLUSTAL Omega (Sievers et al. 2014) and analysed by maximum likelihood (ML) and parsimony in PAUP* 4.0b8/4.0d78 (Swofford 2002). Phylogenetic analysis reveals monophyletic Trichoptera and Limnephilidae and places L. hyalinus as sister to L. decipiens.

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Disclosure statement

The authors report no conflicts of interest and are solely responsible for this paper.

Figure 1. Maximum-likelihood phylogeny of superorder Amphiesmenoptera (GTR+I+G model, I = 0.1680, G = 0.8600, likelihood score 200095.94713) included complete mitochondrial genome sequences from Limnephilus hyalinus, 16 other Trichoptera species, and six representatives from sister clade Lepidoptera based on 1 million random addition heuristic search replicates (with tree bisection and reconnection). One million maximum parsimony heuristic search replicates also produced a single tree (43,649 steps) with a topology identical to the ML tree. Maximum-likelihood bootstrap values are above nodes and maximum parsimony bootstrap values are below nodes (each from 1 million random fast addition search replicates).
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