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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of the giant casemaker caddisfly *Phryganea cinerea* (Insecta: Trichoptera: Phryganeidae)

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ABSTRACT

The rush sedge caddisfly *Phryganea cinerea* Walker, 1852 (Phryganeidae, the giant casemakers), is a widespread and adaptable North American caddisfly. Genome skimming by Illumina sequencing permitted the assembly of a complete 15,043 bp circular mitogenome from *P. cinerea* consisting of 78.2% AT nucleotides, 22 tRNAs, 13 protein-coding genes, 2 rRNAs and a control region in the ancestral insect gene order. *Phryganea cinerea COX1* features an atypical CGA start codon and *COX1, NAD1, NAD4,* and *NAD5* exhibit incomplete stop codons completed by the addition of 3′ A residues to the mRNA. Phylogenetic reconstruction reveals a monophyletic Order Trichoptera and Family Phyrganeidae.

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Illumina sequencing; mitogenomics; inquiry-based learning; Trichoptera; Phryganeidae

The Living Prairie Mitogenomics Consortium is a project to construct a library of arthropod mitogenomes for improved DNA-based species identification and phylogenetics (Living Prairie Mitogenomics Consortium 2017; Marcus 2018). Mitogenome sequences were annotated by undergraduates in a course inquiry-based learning exercise (Marcus et al. 2010). Students who analyzed the data successfully (which were further curated by the instructor) belonged to our consortium.

On 30–31 July 2015, a USDA blacklight trap (Winter 2000) was deployed to collect night-flying insects at the Living Prairie Museum (LPM, GPS 49.889607 N, -97.270487 W). Nearby aquatic habitats include Sturgeon Creek (0.57 km) and the Assiniboine River (1.92 km) (Marcus 2018). One adult specimen of the rush sedge caddisfly *Phryganea cinerea* Walker, 1852 (Insecta: Trichoptera: Phryganeidae, the giant casemakers, project specimen number 2015.07.30.014) was trapped, pinned and deposited in the Wallis Roughley Museum of Entomology at the University of Manitoba (voucher JBWM0360830).

Phryganea cinerea larvae are common in North American marshes and littoral zones of lakes (Williams and Penak 1980), but are also found at depths of up to 100 m (Selgeby 1974) and in sluggish streams (Hilsenhoff 1970). Larvae preferentially make cases from shredded vegetation, but will use many other materials (Neave 1933; Williams and Penak 1980). To lay eggs, adult female *P. cinerea* will fly up to 10 m in the air and dive-bomb the water surface, sending up small splashes, mimicking rain (LaFontaine 1981). Presented here is the first complete New World mitogenome for family Phryganeidae from *P. cinerea.*

DNA was prepared (McCullagh and Marcus 2015) and sequenced by Illumina MiSeq (San Diego, CA) (Peters and Marcus 2017). The mitogenome of *P. cinerea* (Genbank MG980616) was assembled by Geneious 10.1.2 from 6,093,858 paired 75 bp reads using a *Eubasilissa regina* (Trichoptera: Phryganeidae) reference mitogenome (NC023374) (Wang et al. 2014). Annotation was in reference to *E. regina* and *Anabolia bimaculata* (Trichoptera: Limnephilidae, MF680449) mitogenomes (Peirson and Marcus 2017). The *T. tardus* nuclear rRNA repeat (Genbank MG986214) was also assembled and annotated using *A. bimaculata* (MF680448) and *Triaenodes tardus* (Trichoptera: Leptoceridae, MG201853) (Lalonde and Marcus 2017) reference sequences.

The *P. cinerea* circular 15,043 bp mitogenome assembly was composed of 19,655 paired reads with nucleotide composition: 39.2% A, 14.0% C, 7.7% G, and 39.0% T. Gene composition and order in *P. cinerea* is identical to most other trichopteran mitogenomes (Marcus 2018). *Phryganea cinerea COX1* begins with an aberrant start codon (CGA) that is typical of insects (Liao et al. 2010). The mitogenome contains four protein-coding genes (*COX1, NAD1, NAD4, NAD5*) with single-nucleotide (T) stop codons completed by post-transcriptional addition of 3′ A residues. The tRNAs, rRNAs, and control region are typical for Trichoptera (Lalonde and Marcus 2017; Peirson and Marcus 2017).

The mitogenomes from *P. cinerea,* 14 other Trichoptera, and 6 species from sister order Lepidoptera were aligned

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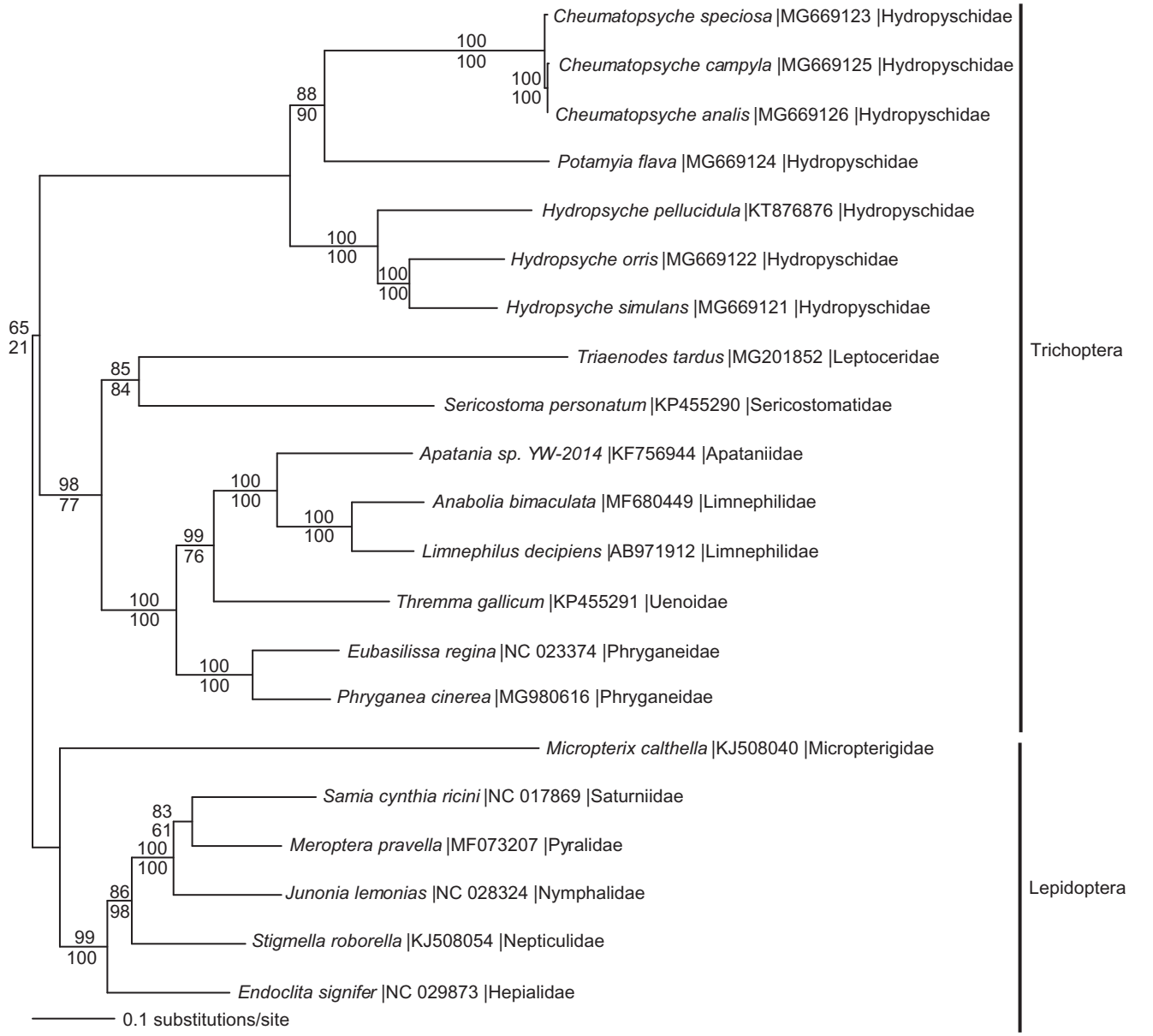

Figure 1. Maximum likelihood phylogeny of superorder Amphiesmenoptera (GTR + I + G model, I = 0.1730, G = 0.9090, likelihood score 186552.81293) included complete mitochondrial genome sequences from *Phryganea cinerea*, 14 other Trichoptera species, and 6 representatives from sister clade Lepidoptera 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 (40,342 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).

in CLUSTAL Omega (Sievers et al. 2011) and analyzed by maximum likelihood (ML) and parsimony in PAUP* 4.0b8/4.0d78 (Swofford 2002) (Figure 1). Phylogenetic analysis reveals a monophyletic Trichoptera and places *P. cinerea* as sister to *E. regina* in monophyletic family Phryganeidae.

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Disclosure statement

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

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