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Nuclear and mitochondrial genomic resources for the meltwater stonefly, *Lednia tumana* (Plecoptera: Nemouridae)

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Abstract:

With more than 3,700 described species, stoneflies (Order Plecoptera) are an important component of global aquatic biodiversity. The meltwater stonefly *Lednia tumana* (Family Nemouridae) is endemic to alpine streams of Glacier National Park and has been petitioned for listing under the U.S. Endangered Species Act (ESA) due to climate change-induced loss of alpine glaciers and snowfields. Here, we present *de novo* assemblies of the nuclear (~520 million base pairs [bp]) and mitochondrial (13,752 bp) genomes for *L. tumana*. The *L. tumana* nuclear genome is the most complete stonefly genome reported to date, with ~71% of genes present in complete form and > 4,600 contigs longer than 10 kilobases (kb). The *L. tumana* mitochondrial genome is the second for the family Nemouridae and the first from North America. Together, both genomes represent important foundational resources, setting the stage for future efforts to understand the evolution of *L. tumana*, stoneflies, and aquatic insects worldwide.

Keywords: stonefly genome, nuclear genome, mitochondrial genome, genome assembly, global change biology, alpine stream

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Introduction:

Stoneflies are a diverse, globally distributed group of hemimetabolous insects that diverged from their closest relatives (e.g., Orthoptera, Dermaptera, Zoraptera) at least 300 million years ago in the Carboniferous Period (Béthoux, Cui, Kondratieff, Stark and Ren, 2011). With more than 3,700 described species, stoneflies account for a substantial portion of freshwater biodiversity (DeWalt, Kondratieff and Sandberg, 2015). The meltwater stonefly, *Lednia tumana* (Plecoptera: Nemouridae), resides in alpine streams of Glacier National Park (GNP), USA, where it is iconic of habitat loss due to climate change in the region (Giersch, Hotaling, Kovach, Jones and Muhlfeld, 2017). *Lednia tumana* is one of four extant species in the genus *Lednia* which all have alpine, cold-water distributions in western North America (Baumann and Kondratieff, 2010; Baumann and Call, 2012). The majority of *L. tumana*'s habitat is supported by seasonal melting of permanent ice and snow, a habitat type that is under considerable threat of near-term loss as the global cryosphere recedes (Hotaling, Finn, Giersch, Weisrock and Jacobsen, 2017; Hotaling et al., in press). The recent evolutionary history of *L. tumana* is closely tied to glacier dynamics with present-day genetic clusters arising in parallel with ice sheet recession at the end of the Pleistocene (~20,000 years ago, Hotaling et al., 2018). Genetic evidence has also highlighted a possible loss of mitochondrial genetic diversity for the species on even more recent, decadal timescales (Jordan et al., 2016). With such a narrow habitat niche in a small, mountainous region of the northern Rocky Mountains, *L. tumana* has been recommended for listing under the U.S. Endangered Species Act (US Fish & Wildlife Service, 2016).

In this study, we present an assembly of the nuclear genome for *L. tumana*, the most complete nuclear genome for the order Plecoptera reported to date. This resource also represents one of only three high-coverage genomes for any EPT taxon (Ephemeroptera, Plecoptera, and Trichoptera), a globally important group of aquatic organisms commonly used for biological monitoring (e.g., Tronstad, Hotaling and Bish, 2016). We also present a nearly complete

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mitochondrial genome assembly (mitogenome) for *L. tumana*, the second for the stonefly family Nemouridae after *Nemoura nankinensis* (Chen and Du, 2017).

Materials and Methods:

Genomic DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit from a single *L. tumana* nymph collected in 2013 from Lunch Creek in GNP. Prior to extraction, both the head and as much of the digestive tract as possible were removed. A whole-genome shotgun sequencing library targeting a 250 bp fragment size was constructed and sequenced by the Florida State University Center for Genomics. The library was sequenced twice on 50% of an Illumina HiSeq2500 flow cell each time with paired-end, 150 bp chemistry, resulting in 242,208,840 total reads. The size of the *L. tumana* nuclear genome was estimated using a kmer-based approach in sga preQC (Simpson, 2014). Read quality was assessed with fastQC (Andrews, 2010) and low-quality reads were either trimmed or removed entirely using TrimGalore (Krueger, 2015) with the flags: --stringency 3 --quality 20 --length 40. We assembled the nuclear genome using SPAdes v3.11.1 with default settings (Bankevich et al., 2012) and generated summary statistics with the Assemblathon2 perl script (assemblathon_stats.pl, Bradnam et al., 2013). The completeness of our nuclear assembly was assessed by calculating the number of conserved single copy orthologs (BUSCOs) in the assemblies using BUSCO v3 and the 1,658 “insecta_ob9” set of reference genes (Simão, Waterhouse, Ioannidis, Kriventseva and Zdobnov, 2015). These BUSCO analyses provided a proxy for how complete genic regions are in both our own and the two existing stonefly genomes. To compare the completeness of the *L. tumana* genome in the context of other stoneflies, we downloaded the two other publicly available stonefly genomes for *Isoperla grammatica* (Family Perlodidae) and *Amphinemura sulcicollis* (Family Nemouridae) which are deposited under GenBank BioProject PRJNA315680 (Macdonald et al., 2016; Macdonald et al., 2017) and ran the same Assemblathon2 and BUSCO analyses.

We assembled the *L. tumana* mitogenome with NOVOPlasty v2.6.7 (Dierckxsens, Mardulyn and Smits, 2016) using an 872 bp segment of the *L. tumana cytb* gene (GenBank KX212756.1) as the “seed” sequence. After assembly, the mitogenome was annotated through a

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87 combination of the MITOS web server with default settings (Bernt et al., 2013) and comparison
88 to the *Nemoura nankinensis* mitogenome (Plecoptera: Nemouridae; Chen and Du, 2017).
89
90 **Results and Discussion:**
91 The size of the *L. tumana* nuclear genome was estimated to be 536.7 megabases (Mb)
92 from raw sequence data. Our final *L. tumana* genome assembly was 520.2 Mb with 50% of the
93 assembly in contigs ≥ 4.69 kilobases (kb; Figure 1a, Table 1). The assembled genome size is in
94 line with the only other publicly available stonefly genomes, *I. grammatica* (509.5 Mb) and *A.*
95 *sulcicollis* (271.9 Mb). The *L. tumana* genome assembly also includes ~3,800 more contigs > 10
96 kb than the *A. sulcicollis* genome and ~4,600 more than the *I. grammatica* assembly (Figure 1a,
97 Table 1). All associated data for the resources detailed in this study, including both raw reads and
98 assemblies, are available as part of GenBank BioProject PRJNA472568 (mitogenome:
99 MH374046, nuclear genome: SAMN09295077, raw reads: SRP148706).
100 The meltwater stonefly's nuclear genome is similarly A/T-rich (58.4%) to the other
101 stoneflies (58.2-59.9%; Table 1), ants (55-67%; Gadau et al., 2012), *Drosophila melanogaster*
102 (58%), *Anopheles gambiae* (56%), and the honeybee, *Apis mellifera* (67%, The Honeybee
103 Genome Sequencing Consortium, 2006). However, the *L. tumana* genome is far more complete
104 in terms of genic regions than both existing stonefly assemblies with 92.8% of BUSCO reference
105 genes either complete (70.6%) or fragmented (22.2%) versus 80.8% for *A. sulcicollis* (50.5%
106 complete, 31.3% fragmented) and just 50.1% for *I. grammatica* (13.3% complete, 36.8%
107 fragmented; Figure 1b, Table 1).
108 The *L. tumana* mitogenome is nearly complete, covering 13,752 bp, including all 13
109 protein-coding genes, 21 tRNA genes, the 12S rRNA gene, and is only missing the 16S rRNA
110 gene and control region (Figure 2). The organization of the *L. tumana* mitogenome is similar to
111 that of *N. nankinensis*, the only other mitogenome available for the family Nemouridae. In *N.*
112 *nankinensis*, the regions missing from the *L. tumana* mitogenome assembly are ~3 kb, indicating
113 that the complete *L. tumana* mitogenome is likely around 16.7 kb, which is similar to
114 mitogenome sizes reported for other stoneflies (Chen and Du, 2017). Our inability to resolve the
115 control region is unsurprising as the *N. nankinensis* control region contains a large, ~1 kb repeat

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region which is difficult to resolve without targeted long-range PCR re-sequencing or longer read high-throughput sequencing.

Conclusion:

The increasing availability of genome assemblies for a wide array of organisms is rapidly expanding the scope and comparative power of modern genome biology (Hotaling and Kelley, 2018). With more than 4,600 contigs longer than 10 kb and ~70% of genes assembled in their complete form, the *L. tumana* nuclear genome provides new opportunity for exploring genome evolution within Plecoptera, a highly diverse, globally distributed insect order, or at higher levels of taxonomic organization (e.g., across all insects). Specifically, the *L. tumana* genome could be mined for genes for phylogenomic studies (e.g., Li et al., 2007, Boroweic et al., 2015) or more targeted, comparative assessments of specific genes or gene families across many taxa to clarify evolutionary shifts and/or copy number variation (e.g. Baalsrud et al., 2017).

Moreover, single-copy orthologous genes compared among species can provide a means for quantifying differences in evolutionary rates across diverse taxa (e.g. Honeybee Genome Sequencing Consortium, 2006) and/or to identify rapidly evolving genes that underlie evolutionary transitions of interest. In the case of stoneflies and aquatic biodiversity generally, little is known of the evolutionary changes underlying the shift to an aquatic larval stage that is common among many orders (e.g., Plecoptera, Ephemeroptera, Trichoptera). With the addition of the *L. tumana* nuclear genome reported here to the recently published caddisfly (*Stenopsyche tienmushanensis*, Order Trichoptera, Luo et al., 2018) and mayfly (*Ephemera danica*, Order Ephemeroptera, Polechau et al., 2014) genomes, the stage is now set for broad, genome-scale investigations of how a major life history transition occurred across three globally distributed insect orders.

Future efforts to refine both assemblies, including the incorporation of longer reads (e.g., generated using Pacific Biosciences sequencing technology, Utturkar et al., 2014) will yield greater insight into the genome biology of *L. tumana*, stoneflies, and aquatic insects broadly. Still, the resources provided here, and particularly the most complete stonefly nuclear genome

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144 published thus far, represent an important step towards empowering modern stonefly research, a
145 globally relevant group of aquatic insects that has been largely overlooked in the genomic age.
146

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153

154 **Declaration of Interest:**
155 The authors declare no financial interest or benefit stemming from this research.

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247 **Tables:**

248 Table 1. Assembly statistics for the nuclear genome of *Lednia tumana* (Plecoptera: Nemouridae)
249 and two other stonefly species, *Amphinemura sulcicollis* and *Isoperla grammatica* (Macdonald et
250 al., 2016; Macdonald et al., 2017). BUSCOs: Single-copy, orthologous genes known to be highly
251 conserved among insects. A total of 1,658 BUSCOs were searched. For *L. tumana*, the assembly
252 size was 304,502,267 bp when only contigs larger than 500 bp were included.

	L. tumana	*A. sulcicollis*	*I. grammatica*
Estimated genome size	536,700,000	n/a	n/a
Assembly size	520,200,814	271,924,966	509,522,935
Coverage	~60x	~1.4x	~0.7x
Contigs > 1 kb	74,445	51,555	53,204
Contigs > 10 kb	4,608	849	4
Contig N50	4.69 kb	0.85 kb	0.46 kb
% A/T	58.4	58.2	59.9
% G/C	41.5	42.8	40.1
% N	0.1	0	0
Complete BUSCOs	1172 (70.6%)	837 (50.5%)	221 (13.3%)
Fragmented BUSCOs	368 (22.2%)	519 (31.3%)	610 (36.8%)
Missing BUSCOs	118 (7.2%)	302 (18.2%)	827 (49.9%)

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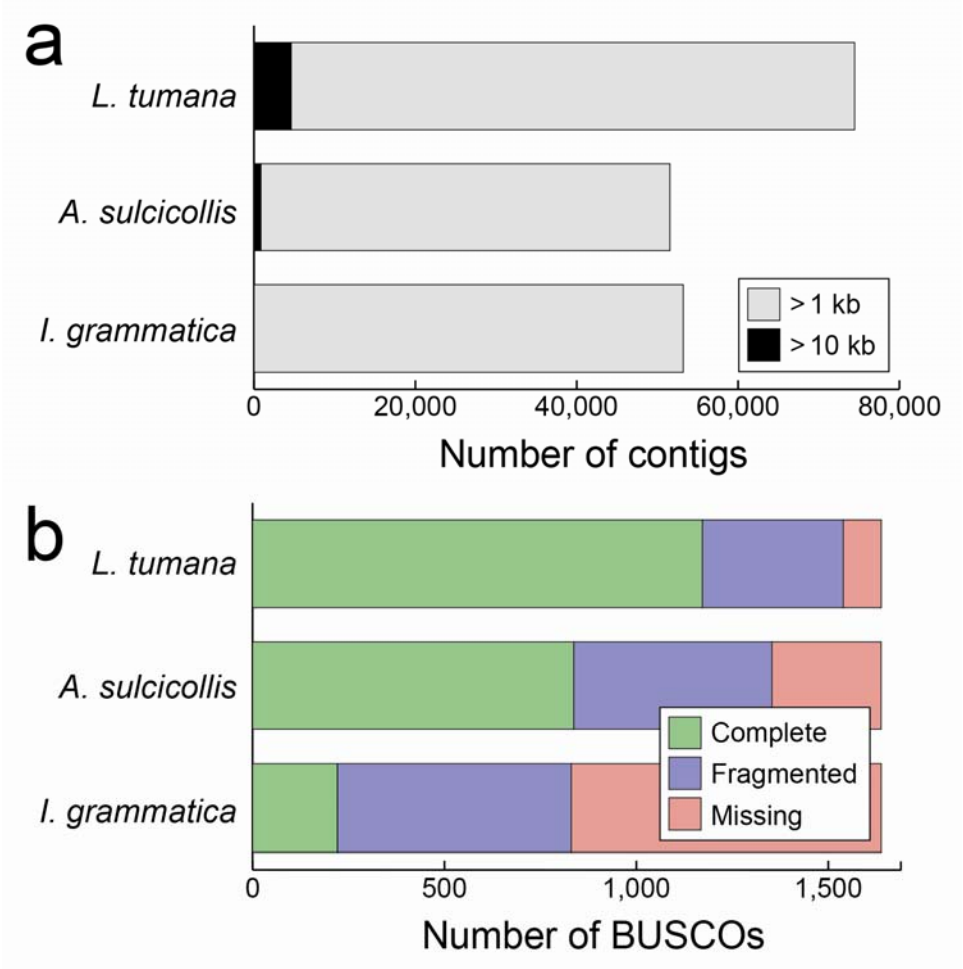
254 **Figures:**



255

256 Figure 1. Comparisons of the gene content and contiguity of the *Lednia tumana* nuclear genome
257 to the two other previously published stonefly genomes for *Amphinemura sulcicollis* and
258 *Isoperla grammatica* (Macdonald et al., 2016; Macdonald et al., 2017). (a) The number of
259 contigs > 1 kb and > 10 kb across the three stonefly genomes. For *I. grammatica*, the assembly
260 contains just four contigs > 10 kb. (b) Presence of highly conserved, single-copy orthologous
261 genes (BUSCOs) across the three stonefly genomes.

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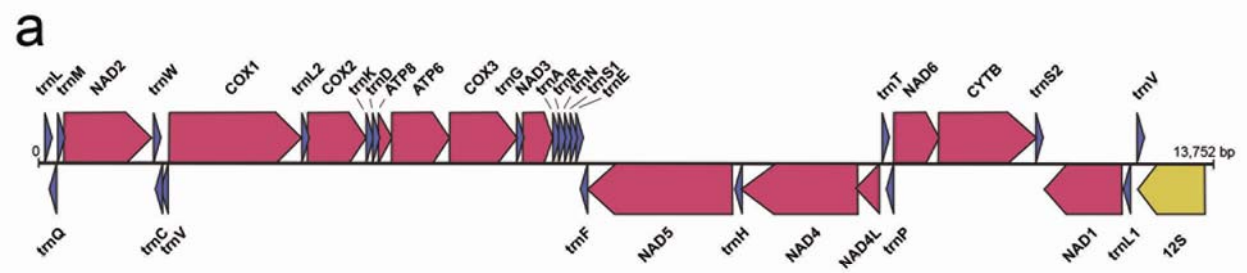

b **tRNAs**

Name	Range	Strand	Length
trnL	176-241	F	66
trnQ	239-307	R	69
trnM	318-385	F	68
trnW	1434-1502	F	69
trnC	1495-1557	R	63
trnY	1561-1627	R	67
trnL2	3160-3225	F	66
trnK	3916-3985	F	70
trnD	3986-4052	F	67
trnG	5670-5735	F	66
trnA	6088-6151	F	64
trnR	6152-6214	F	63
trnN	6217-6283	F	67
trnS1	6284-6351	F	68
trnE	6352-6416	F	65
trnF	6415-6481	R	67
trnH	8217-8282	R	66
trnT	9918-9982	F	65
trnP	9983-10048	R	66
trnS2	11709-11778	F	70
trnL1	12751-12816	R	66
trnV	12891-12956	R	66

c **PCGs and rRNAs**

Name	Range	Strand	Length
NAD2	386-1393	F	1008
COX1	1620-3140	F	1521
COX2	3228-3908	F	681
ATP8	4053-4208	F	156
ATP6	4205-4873	F	669
COX3	4882-5667	F	786
NAD3	5739-6083	F	345
NAD5	6489-8168	R	1680
NAD4	8294-9625	R	1332
NAD4L	9622-9885	R	264
NAD6	10062-10565	F	504
CYTB	10574-11704	F	1131
NAD1	11808-12710	R	903
12S	12897-13680	R	784

Regions absent: 16S, control region

Figure 2. (a) The mitogenome of *Lednia tumana* (Plecoptera: Nemouridae). (b) Locations of tRNAs. (c) Locations of protein coding (PCGs) and rRNA genes.