

1 **Nuclear and mitochondrial genomic resources for the meltwater stonefly, *Lednia tumana***
2 **Ricker, 1952 (Plecoptera: Nemouridae)**

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12 **Abstract:**

13 With more than 3,700 described species, stoneflies (Order Plecoptera) are an important
14 component of global aquatic biodiversity. The meltwater stonefly *Lednia tumana* (Ricker, 1952;
15 Family Nemouridae) is endemic to alpine streams of Glacier National Park and has been
16 petitioned for listing under the U.S. Endangered Species Act (ESA) due to climate change-
17 induced loss of alpine glaciers and snowfields. Here, we present *de novo* assemblies of the
18 nuclear (~520 million base pairs [bp]) and mitochondrial (15,014-bp) genomes for *L. tumana*.
19 The *L. tumana* nuclear genome is the most complete stonefly genome reported to date, with
20 ~71% of genes present in complete form and more than 4,600 contigs longer than 10-kilobases
21 (kb). The *L. tumana* mitochondrial genome is the second for the family Nemouridae and the first
22 from North America. Together, both genomes represent important foundational resources, setting
23 the stage for future efforts to understand the evolution of *L. tumana*, stoneflies, and aquatic
24 insects worldwide.

25
26 **Keywords:** Plecoptera; Nemouridae; *Lednia*; genomics; nuclear genome; USA

27
28 **Introduction:**

29 Stoneflies are a diverse, globally distributed group of hemimetabolous insects that
30 diverged from their closest relatives (e.g., Orthoptera, Dermaptera, Zoraptera) at least 300
31 million years ago in the Carboniferous Period (Béthoux, Cui, Kondratieff, Stark, and Ren 2011).
32 With more than 3,700 described species, stoneflies account for a substantial portion of
33 freshwater biodiversity (DeWalt, Kondratieff, and Sandberg 2015). The meltwater stonefly,
34 *Lednia tumana* (Ricker, 1952; Plecoptera: Nemouridae), resides in alpine streams of Glacier
35 National Park (GNP), USA, where it is iconic of habitat loss due to climate change in the region
36 (Muhlfeld et al. 2011; Giersch, Hotaling, Kovach, Jones and Muhlfeld 2017). *Lednia tumana* is
37 one of four extant species in the genus *Lednia* which all exhibit alpine, cold-water distributions
38 in western North America (Baumann and Kondratieff 2010; Baumann and Call 2012). The
39 majority of *L. tumana*'s habitat is supported by seasonal melting of permanent ice and snow, a
40 habitat type that is under considerable threat of near-term loss as the global cryosphere recedes
41 (Hotaling, Finn, Giersch, Weisrock, and Jacobsen 2017; Hotaling et al. 2019). The recent
42 evolutionary history of *L. tumana* is closely tied to glacier dynamics with present-day genetic

43 clusters arising in parallel with ice sheet recession at the end of the Pleistocene (~20,000 years
44 ago, Hotaling et al. 2018). Genetic evidence has also highlighted a possible loss of mitochondrial
45 genetic diversity for the species on even more recent, decadal timescales (Jordan et al. 2016).
46 With such a narrow habitat niche in a small, mountainous region of the northern Rocky
47 Mountains, *L. tumana* has been recommended for listing under the U.S. Endangered Species Act
48 (US Fish & Wildlife Service 2016).

49 In this study, we present an assembly of the nuclear genome for *L. tumana*, the most
50 complete nuclear genome for the order Plecoptera reported to date. This resource also represents
51 one of only three high-coverage (> 50x) genomes for any EPT taxon (Ephemeroptera, *Ephemera*
52 *danica*, Polechau et al. 2014; Plecoptera, this study; Trichoptera, *Stenopsyche tienmushanensis*,
53 Luo, Tang, Frandsen, Stewart, and Zhou 2018), a globally important group of aquatic organisms
54 commonly used for biological monitoring (e.g., Tronstad, Hotaling, and Bish 2016). We also
55 present a nearly complete mitochondrial genome assembly (mitogenome) for *L. tumana*, the
56 fourth for the stonefly family Nemouridae after two previous studies (Chen and Du 2017; Cao,
57 Wang, Huang, and Li 2019).

58

59 **Materials and Methods:**

60 Genomic DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit from a single
61 *L. tumana* nymph collected in 2013 from Lunch Creek in GNP. Prior to extraction, both the head
62 and as much of the digestive tract as possible were removed. A whole-genome shotgun
63 sequencing library targeting a 250-bp fragment size was constructed and sequenced by the
64 Florida State University Center for Genomics. The library was sequenced twice on 50% of an
65 Illumina HiSeq2500 flow cell each time with paired-end, 150-bp chemistry, resulting in
66 242,208,840 total reads. The size of the *L. tumana* nuclear genome was estimated using a kmer-
67 based approach in sga preQC (Simpson 2014). Read quality was assessed with fastQC (Andrews
68 2010) and low-quality reads were either trimmed or removed entirely using TrimGalore (Krueger
69 2015) with the flags: --stringency 3 --quality 20 --length 40. We assembled the nuclear genome
70 using SPAdes v3.11.1 with default settings (Bankevich et al. 2012) and generated summary
71 statistics with the Assemblathon2 perl script (assemblathon_stats.pl, Bradnam et al. 2013). The
72 completeness of our nuclear assembly was assessed by calculating the number of conserved
73 single copy orthologous genes [Benchmarking Universal Single-Copy Orthologs (BUSCOs)] in

74 the assembly using BUSCO v3 and the 1,658 “insecta_ob9” set of reference genes (Simão,
75 Waterhouse, Ioannidis, Kriventseva, and Zdobnov 2015). To compare the completeness of the *L.*
76 *tumana* genome in the context of other stoneflies, we downloaded the two other publicly
77 available stonefly genomes for *Isoperla grammatica* (Poda, 1761; Perlodidae) and *Amphinemura*
78 *sulcicollis* (Stephens, 1836; Nemouridae) which are deposited under GenBank BioProject
79 PRJNA315680 (Macdonald, Cunha, and Bruford 2016; Macdonald, Ormerod, and Bruford
80 2017). We performed the same BUSCO and Assemblathon2 analyses on the *I. grammatica* and
81 *A. sulcicollis* genomes as we did for *L. tumana* above.

82 We assembled the *L. tumana* mitogenome with NOVOPlasty v2.6.7 (Dierckxsens,
83 Mardulyn, and Smits 2016) using an 872-bp segment of the *L. tumana cytb* gene (GenBank
84 KX212756.1) as the “seed” sequence. After assembly, the mitogenome was annotated through a
85 combination of the MITOS web server with default settings (Bernt et al. 2013) and comparison
86 to the *Nemoura nanikensis* mitogenome (Plecoptera: Nemouridae; Chen and Du 2017).
87 However, in this initial assembly, the 16S gene was fragmented and the 12S gene was missing
88 entirely. To mitigate this, we extracted sequences for both genes from the *N. nanikensis*
89 mitogenome (Plecoptera: Nemouridae; Chen and Du 2017) and mapped our raw reads to these
90 reference sequences for each gene with BWA-MEM v0.7.12-r1039 (Li 2013) using the default
91 settings. Next, we used bcftools v1.9 (Li, Orti, Zhang, and Lu 2009) to collect summary
92 information on the read mapping and genotype likelihoods (‘mpileup’ with default settings),
93 called consensus nucleotides (‘call’ with -m flag), and output the consensus sequence
94 (‘consensus’ with default settings). Finally, we used samtools v1.7 (Li et al. 2009) to calculate
95 coverage depth per nucleotide for each sequence and masked consensus bases with no coverage
96 (i.e., bases with no information from the *L. tumana* read mapping). We manually integrated our
97 16S and 12S sequences into the *L. tumana* mitogenome through comparison with the *N.*
98 *nanikensis* mitogenome (Chen and Du 2017) and re-annotated the assembly with the MITOS
99 web server (Bernt et al. 2013) as described above.

100

101 **Results and Discussion:**

102 The size of the *L. tumana* nuclear genome was estimated to be 536.7-megabases (Mb)
103 from raw sequence data. Our final *L. tumana* genome assembly was 520.2-Mb with 50% of the
104 assembly in contigs \geq 4.69-kilobases (kb; Figure 1a, Table 1). The assembled genome size is in

105 line with the only other publicly available stonefly genomes, *I. grammatica* (509.5 Mb) and *A.*
106 *sulcicollis* (271.9 Mb). The *L. tumana* genome assembly also includes ~3,800 more contigs > 10
107 kb than the *A. sulcicollis* genome and ~4,600 more than the *I. grammatica* assembly (Figure 1a,
108 Table 1). All associated data for the resources detailed in this study, including both raw reads and
109 assemblies, are available as part of GenBank BioProject PRJNA472568 (mitogenome:
110 MH374046, nuclear genome: SAMN09295077, raw reads: SRP148706).

111 The meltwater stonefly's nuclear genome is similarly A/T-rich (58.4%) to the other
112 stoneflies (58–60%; Table 1), ants (55–67%; Gadau et al. 2012), *Drosophila melanogaster*
113 (58%), *Anopheles gambiae* (56%), and the honeybee, *Apis mellifera* (67%, The Honeybee
114 Genome Sequencing Consortium 2006). However, the *L. tumana* genome is far more complete in
115 terms of genic regions than both existing stonefly assemblies with 92.8% of BUSCO reference
116 genes either complete (70.6%) or fragmented (22.2%) versus 80.8% for *A. sulcicollis* (50.5%
117 complete, 31.3% fragmented) and just 50.1% for *I. grammatica* (13.3% complete, 36.8%
118 fragmented; Figure 1b, Table 1).

119 The *L. tumana* mitogenome is nearly complete, covering 15,014-bp, including all 13
120 protein-coding genes, 21 tRNA genes, both rRNA genes, and is only missing the control region
121 (Figure 2). The organization of the *L. tumana* mitogenome is similar to that of *N. nankinensis*,
122 the only other mitogenome available for the family Nemouridae. In *N. nankinensis*, the control
123 region is ~1-kb which indicates that the complete *L. tumana* mitogenome is likely around 16-kb.
124 This predicted size would fall in line with mitogenome sizes reported for other stoneflies (Chen
125 and Du 2017). Our inability to resolve the control region is also unsurprising. In *N. nankinensis*,
126 the control region contains a large ~1-kb repeat region which is inherently difficult to resolve
127 without targeted long-range PCR re-sequencing or longer read high-throughput sequencing.

128

129 **Conclusion:**

130 The increasing availability of genome assemblies for a wide array of organisms is rapidly
131 expanding the scope and comparative power of modern genome biology (Hotaling and Kelley
132 2019). With more than 4,600 contigs longer than 10-kb and ~70% of genes assembled in their
133 complete form, the *L. tumana* nuclear genome provides new opportunity for exploring genome
134 evolution within Plecoptera, a highly diverse, globally distributed insect order, or at higher levels
135 of taxonomic organization (e.g., across all insects). Specifically, the *L. tumana* genome could be

136 mined for genes for phylogenomic studies (e.g., Li et al. 2007; Borowiec, Lee, Chiu, and
137 Plachetzki 2015) or more targeted, comparative assessments of specific genes or gene families
138 across many taxa to clarify evolutionary shifts and/or copy number variation (e.g., Baalsrud et al.
139 2017).

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

151 Future efforts to refine both assemblies, including the incorporation of longer reads (e.g.,
152 generated using Pacific Biosciences sequencing technology, Utturkar et al. 2014) will yield
153 greater insight into the genome biology of *L. tumana*, stoneflies, and aquatic insects broadly.
154 Still, the resources provided here, and particularly the most complete stonefly nuclear genome
155 published thus far, represent an important step towards empowering modern stonefly research, a
156 globally relevant group of aquatic insects that has been largely overlooked in the genomic age.
157

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163 State University Center for Institutional Research Computing.

164

165 **Declaration of Interest:**

166 The authors declare no financial interest or benefit stemming from this research.

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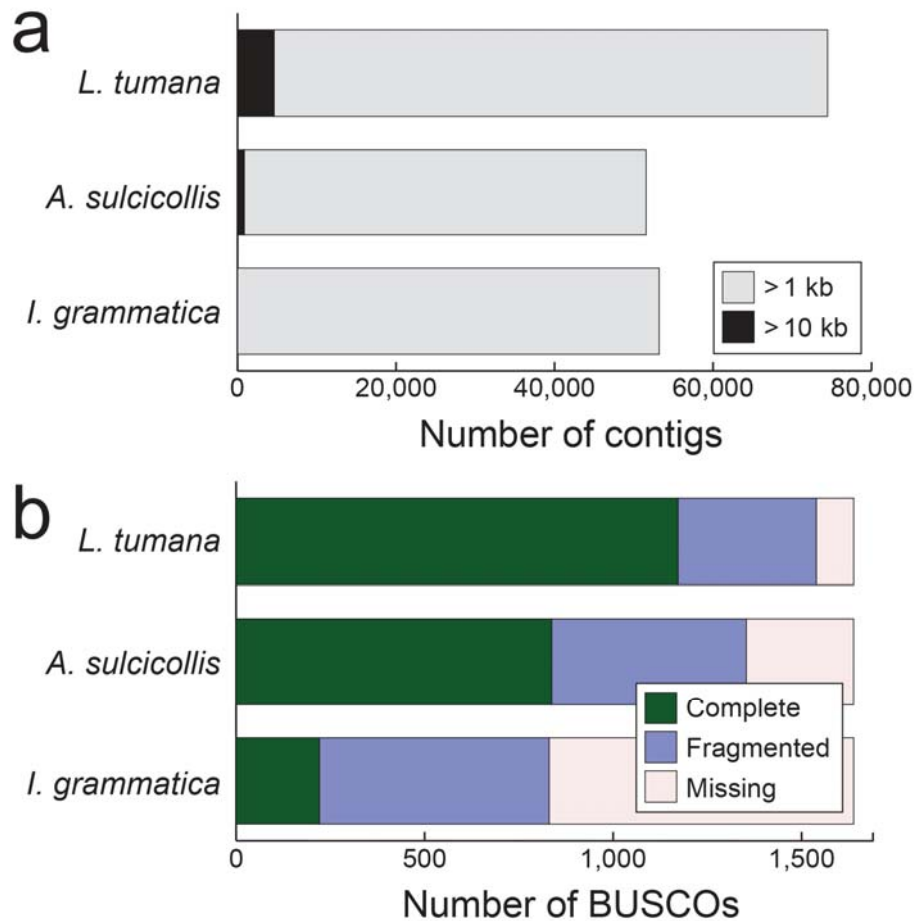
270 **Tables:**

271 Table 1. Assembly statistics for the nuclear genome of *Lednia tumana* (Ricker, 1952; Plecoptera:
272 Nemouridae) and two other stonefly species, *Amphinemura sulcicollis* (Stephens, 1836) and
273 *Isoperla grammatica* (Poda, 1761; Macdonald et al. 2016; Macdonald et al. 2017). BUSCOs:
274 Single-copy, orthologous genes known to be highly conserved among insects. A total of 1,658
275 BUSCOs were searched.

	<i>L. tumana</i>	<i>A. sulcicollis</i>	<i>I. grammatica</i>
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	1,172 (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%)

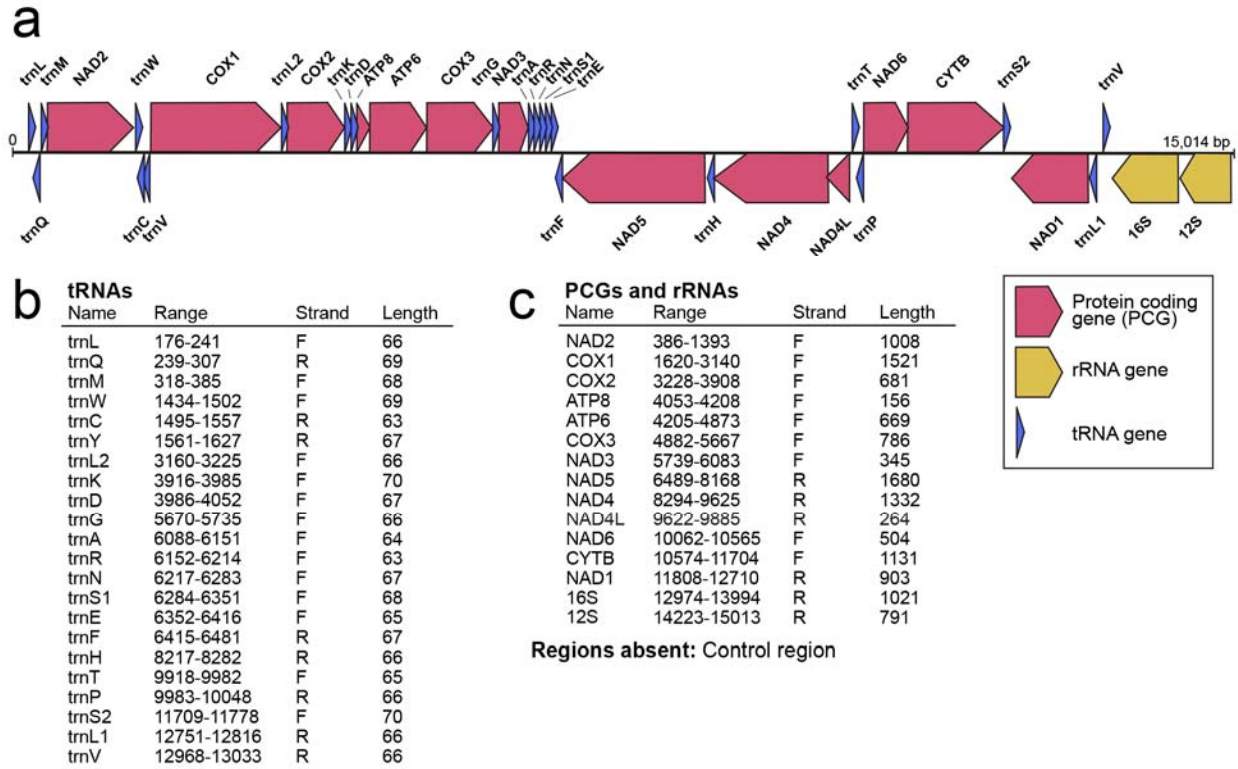
276

277 **Figures:**



278

279 Figure 1. Comparisons of the gene content and contiguity of the *Lednia tumana* (Ricker, 1952;
280 Plecoptera: Nemouridae) nuclear genome to the two other previously published stonefly
281 genomes for *Amphinemura sulcicollis* (Stephens, 1836) and *Isoperla grammatica* (Poda, 1761;
282 Macdonald et al. 2016; Macdonald et al. 2017). (a) The number of contigs > 1-kb and > 10-kb
283 across the three stonefly genomes. For *I. grammatica*, the assembly contains just four contigs
284 greater than 10-kb. (b) Presence of highly conserved, single-copy orthologous genes (BUSCOs)
285 across the three stonefly genomes.



286

287 Figure 2. (a) The mitogenome of *Lednia tumana* (Ricker, 1952; Plecoptera: Nemouridae). (b)

288 Locations of tRNAs. (c) Locations of protein coding (PCGs) and rRNA genes.