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1 **DNA barcode-based survey of Trichoptera in the Crooked River reveals three new species**
2 **records for British Columbia**

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8

9 **Abstract**

10 Anthropogenic pressures on aquatic systems have placed a renewed focus on biodiversity of
11 aquatic macroinvertebrates. By combining classical taxonomy and DNA barcoding we identified
12 39 species of caddisflies from the Crooked River, a unique and sensitive system in the
13 southernmost arctic watershed in British Columbia. Our records include three species never
14 before recorded in British Columbia: *Lepidostoma togatum* (Lepidostomatidae), *Ceraclea*
15 *annulicornis* (Leptoceridae), and *Cheumatopsyche harwoodi* (Hydropsychidae). Three other
16 specimens may represent new occurrence records and a number of other records seem to be
17 substantial observed geographic range expansions within British Columbia.

18

19 **INTRODUCTION**

20 With accelerating anthropogenic climate change there is a renewed interest in assessing
21 biodiversity in freshwater ecosystems (Parmesan 2006). Freshwater ecosystems are especially
22 under cumulative threats as their summer temperatures rapidly warm, with increased demand for
23 fresh water, and by industrial in riparian zones (Meyer *et al.* 1999). Assessing insect biodiversity

24 is a challenging, but vital, activity in the face of these changes in order to understand aquatic
25 food webs, ecosystem services, and for use in aquatic environmental monitoring (Burgmer *et al.*
26 2007; Dobson and Frid 2009; Cairns and Pratt 1993).

27 Trichoptera taxonomy is primarily based male adult morphology, which often requires
28 experts for definitive identification. Taxonomy of the larvae is complicated and often
29 problematic as it is not always possible to distinguish between species of the same genus
30 (Burington 2011, Ruitter et al 2013). DNA barcoding and the use of sequence databases,
31 combined with classical taxonomy, can help to speed up this process by allowing rapid surveys
32 of novel regions (Ruitter et al 2013, DeSalle *et al.* 2005, Jinbo et al. 2011, Pauls et al. 2010, Zhou
33 et al 2007). The Barcode Of Life Database (BOLD) currently contains DNA barcodes for more
34 than 260, 000 species including ~4555 Trichoptera species, and facilitates the identification of
35 species based on subunit I of the cytochrome oxidase I (COI) DNA gene. In addition, recent
36 comprehensive work on barcode-assisted Trichoptera taxonomy (Zhou et al. 2009, 2010a,b,
37 2011, 2016) provides a solid foundation for biodiversity surveys of caddisflies in North America.
38 Trichoptera, Ephemeroptera (mayflies), Plecoptera (stoneflies), and often aquatic Diptera (true
39 flies) are used in well-developed protocols as indicators of aquatic ecosystem health (Lenat and
40 Barbour 1994). Due to their taxonomic richness, differential susceptibility to pollutants, and
41 abundance in almost all water bodies worldwide, shifts in their numbers, relative ratios, or
42 taxonomic diversity both temporally and/or geographically are used to observe stability and
43 disturbance of ecosystems (Houghton 2004; Pond 2012). Monitoring work is best accomplished
44 with good information on which species are present. Due to a lack of historical sampling in some
45 areas, managers often must rely on regional (often province- or state-level) checklists that may or
46 may not represent the taxonomic and functional diversity of smaller areas or specific sensitive

47 systems. The Crooked River (Figure 1) is the southernmost lotic system in British Columbia that
48 ultimately drains into the Arctic Ocean. It flows north from Summit Lake (which is just on the
49 north side of the continental divide) to McLeod Lake, connecting a series of lakes along the way.
50 From there its water flows via other systems to eventually end up in the Williston Reservoir – a
51 massive hydroelectric reservoir in the Rocky Mountain Trench that represents one of the largest
52 anthropogenic landscape modifications on earth.

53 The Crooked River is named for all the oxbows due to its slow meandering flow. This
54 river is also fed by underground springs, such as Livingston Springs in Crooked River
55 Provincial. This well-known spring supplies the river with water year round and moderates
56 annual temperature shifts. An extinct volcano (Teapot Mountain) is situated at its headwaters,
57 and likely provides mineral nutrient inputs. As a *bona fide* spring creek, the Crooked River has a
58 very flat gradient with swamp and marshland along much of its shoreline. During freshet the
59 river floods these marshes bringing more nutrients into the system. These factors result in high
60 fertility and a fairly stable year-round temperature which make the Crooked River unique
61 compared to neighbouring systems. Nearby river systems are more typical of British Columbia –
62 they are best described as oligotrophic freestone rivers that are highly susceptible to drastic
63 changes in discharge from spring freshets and that show considerable annual temperature
64 variation.

65 The Crooked River has been heavily used by European settlers for transport and trade for
66 much of their history in British Columbia (McKay 2000) – and it was doubtless used prior to that
67 by First Nations groups for sustenance and as a settlement location. Human-caused impacts
68 continue to this day and are, in fact, increasing. The river is in the direct path of planned
69 pipelines originating from northeastern British Columbia and Alberta that will run toward the

70 Pacific coast. In addition the watershed has been logged for years resulting in a network of
71 resource roads and bridges. A major highway and a rail line also run along much of its length,
72 and are at times only a few meters from the river's main channel. However, even with its unique
73 nature and high levels of anthropogenic impacts, our searches have revealed no recorded
74 biodiversity surveys on the Crooked River.

75 Besides that, to our knowledge no comprehensive recent assessment has been done on
76 Trichoptera in central or northern British Columbia. As the Crooked River is such a unique and
77 nutrient-rich system we questioned whether it may provide habitat to species not yet reported for
78 British Columbia. The aims of this study were to: (1) provide a comprehensive list of the
79 Trichoptera biodiversity in a unique and vulnerable river as a baseline for future work and
80 management; (2) explore the Trichoptera biodiversity of an historically unstudied region; and (3)
81 determine if the Crooked River contains species not yet recorded for British Columbia.

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84 **METHODS AND MATERIALS**

85 We collected specimens on a biweekly basis from eight locations (CR2 – 54.484°N, -
86 122.721°W, CR2B – 54.484°N, -122.721°W, CR3 – 54.643°N, -122.743°W, CR4 – 54.388°N, -
87 122.633°W, CR5 – 54.478°N, -122.719°W, CR6 – 54.328°N, -122.669°W, CR100BR –
88 54.446°N, -122.653°W, CR108 – 54.458°N, -122.722°W) along the edge of the Crooked River,
89 British Columbia between May and August 2014 using both hand and kick-net methods. This
90 study focused mainly on larvae to ensure that we collected caddisflies from the Crooked River
91 only and not from nearby water bodies. We completed collections under the British Columbia
92 Ministry of Environment Park Use Permit #107171 where required. We preserved specimens in
93 80% ethanol upon collection. We classified all 2204 caddisfly specimens that we collected to the

94 lowest possible taxonomic ranking (genus or family) based on published morphological keys
95 (Wiggins 1977; Clifford 1991; Schmid 1998). We selected morpho-species based on that visual
96 identification and 214 specimens were subsequently sent to the Biodiversity Institute of Ontario
97 (BIO) and its Barcode of Life Database (<http://www.boldsystems.org>) in Guelph, Ontario, to
98 have their barcode region (COI) sequenced for further classification. We received back 185
99 useable sequences (>400 bp., <5 miscalls, no contamination detected). We vouchered all
100 specimens set for sequencing at the Centre for Biodiversity Genomics at the University of
101 Guelph. Initial species identification was based on a 650 bp sequence in COI 5' region using the
102 BOLD platform with MUSCLE sequence alignments and a Kimura-2-parameter distance model.
103 The data for all collected specimens is available as dataset DS-CRTRI
104 [http://www.boldsystems.org/index.php/Public_SearchTerms?query=CRTRI].

105 Neighbor joining (NJ) analyses were performed on *Cheumatopsyche harwoodi*,
106 *Lepidostoma togatum* and *Ceraclea annulicornis* specimens from the Crooked River compared
107 to con- and heterospecific sequence data from the Barcode Of Life Database (BOLD).
108 Evolutionary distances were computed using the Kimura 2-parameter method bootstrapped
109 (5000 replications) after a MUSCLE alignment and were visualized in MEGA6.0 (Saitou and
110 Nei, 1987; Felsenstein, 1985; Kimura, 1980; Tamura et al., 2013). We cross-referenced the
111 Crooked River Trichoptera species list that we obtained from analysis of our BOLD data using
112 checklists, museums records and databases from the following: Canadian National Collection of
113 Insect, Arachnids and Nematodes (<http://www.canacoll.org/>); Strickland Museum at the
114 University of Alberta; Beaty Biodiversity Museum at the University of British Columbia;
115 Electronic Atlas of the Wildlife of British Columbia
116 (<http://ibis.geog.ubc.ca/biodiversity/efauna/>); Natureserve (<http://www.natureserve.org/>);

117 Canadensys (<http://www.canadensys.net/>), Global Biodiversity Information Facility
118 (<http://www.gbif.org/>); the Royal Ontario Museum, and the Royal British Columbia Museum
119 (<http://search-collections.royalbcmuseum.bc.ca/Entomology>).

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121 RESULTS & DISCUSSION

122 We used morphological keys to identify all 2204 collected specimens to family or genus,
123 after which we used successful barcodes and database searches to deduce the species identities of
124 185 individuals based on previous database annotations. In total we detected 41 caddisfly species
125 – found in 20 genera within 11 families – in the Crooked River system (Table 1). All barcode
126 data are publicly available at BOLD (DS-CRTRI,
127 http://www.boldsystems.org/index.php/Public_SearchTerms?query=CRTRI). Thirty five of the
128 41 species we identified were assigned to known species via database matches using a 2%
129 threshold for delineating species within Trichoptera, which is considered to be a reliable
130 approach (Zhou *et al.* 2009). COI sequences of specimens from the Crooked River with DNA
131 sequences matching 99.67% and 99.13% to *Lepidostoma cinereum* and *Neophylax rickeri*
132 respectively, were assigned to the aforementioned species.

133 Among the 34 specimens identified to species with 100% database matches are
134 *Cheumatopsyche harwoodi*, *Lepidostoma togatum* and *Ceraclea annulicornis*, all three are new
135 species records for British Columbia.

136 There are currently six species within the genus *Cheumatopsyche*: *C. analis*, *C. campyla*,
137 *C. gracilis*, *C. oxa*, *C. pettiti* and *C. smithi* (<http://ibis.geog.ubc.ca/biodiversity/efauna>, Cannings
138 2007). We found a larva of *Cheumatopsyche harwoodi* (synonym *C. enigma*) at CR4 on May
139 16th 2014. Based on morphological keys we were only able to classify our specimen to genus

140 level. This is not surprising as morphology-based taxonomy of *C. harwoodi* larvae is
141 exceedingly difficult (Wiggins 1996). In some cases *C. harwoodi* larvae are indistinguishable
142 from other species within the genus (Burington 2011). Based on our phylogenetic tree-based
143 analysis the Crooked River *C. harwoodi* sequence groups with *C. harwoodi* sequences from
144 Ontario (JF434099, JF434097), New Brunswick (KR146677), and Manitoba (HM102631); and
145 not with any of the known species of *Cheumatopsyche* in British Columbia (Figure 2). The
146 Crooked River specimen also aligns 100% with a DNA sequence of *C. harwoodi* from Alberta
147 (HM102632), but also with a *C. gracilis* sequence from Wyoming (HQ560573) (Figure 2).

148 Identifying species based on DNA sequence is that it requires accurate morphological
149 identification to species level of physical specimen that is then sequenced – and ideally
150 replicated a number of times. Currently BOLD has 178 barcodes for specimens identified as
151 *C. harwoodi* and the Crooked River specimen aligns very closely to these with less than 0.6%
152 difference within the species as a whole, well below the 2% threshold suggested by Zhou and co-
153 workers in 2009. There are currently only two barcodes for *C. gracilis* and both these barcodes
154 group with the various *C. harwoodii* sequences. And these two *C. gracilis* specimens have a
155 1.3% difference based on our analysis. The preponderance of evidence, then, points to one of
156 three possibilities. First, the two *C. gracilis* specimens in BOLD are actually misidentified *C.*
157 *harwoodii* and our specimen is also *C. harwoodii*. Second, the specimens represent different
158 species but that difference is not reflected in the DNA barcode. And third, the taxonomic status
159 of both species should be reconsidered as potentially being one species. A more definitive
160 identification might be possible as BOLD is populated with more *C. gracilis* sequences that
161 helps delineate the two species.

162 On 14 July 2014 we found a larva for *Lepidostoma togatum* {synonyms *L. canadense*
163 (Banks, 1899) *L. pallidum* (Banks, 1897) *Mormomyia togatum* (Hagen, 1861), *Pristosilo*
164 *canadensis* (Banks, 1899), *Silo pallidus* (Banks, 1897)} at CR3. The DNA sequence of this
165 specimen aligns clearly with *L. togatum* sequences (Figure 3). Based on museum and database
166 records in Canada *L. togatum* is known to be present in the Northwest Territories, Alberta and
167 the Maritime Provinces of Canada. Our report is the first for this species west of the Rocky
168 Mountains.

169 On 13 August 2014 we found a specimen of *Ceraclea annulicornis* {(synonyms:
170 *Athripsodes annulicornis* (Stephens, 1836), *C. futilis* (Banks, 1914), *C. recurvata* (Banks, 1908),
171 *Leptocerus annulicornis* (Stephens, 1836), *L. futilis* (Banks, 1914)} at CR3 (Figure 1). The
172 phylogenetic tree-based analysis using sequences from Manitoba, Ontario, and New Brunswick
173 strongly suggest our specimen is *C. annulicornis* (Figure 4).

174 We found specimens belonging to three genera that had no significant matches at the
175 species level on either the Barcode of Life Database or at NCBI; therefore we only provide
176 genus-level identifications (Table 1). A specimen we putatively assign as *Micrasema* had only
177 one match in BOLD Genbank Accession# KR145307 (Zhou et al., 2016), but much further
178 south, on southern Vancouver Island. Images of this specimen are publicly available at BOLD
179 (BIOUG18683-F08).

180 A specimen putatively belonging to the genus *Hydroptila* had a number of 100% matches
181 to the Crooked River *Hydroptila* sp. in the BOLD database (Zhou et al., 2016), but none
182 identified to species level. Sequence alignments revealed 86% and 84. 74% similarity to *H. rono*
183 and *H. xera* respectively; both species are known to be present in British Columbia. The other
184 two known *Hydroptila* spp. in British Columbia, *H. arctia* and *H. consimilis*, are substantially

185 dissimilar from our specimen (81% and 82% match, respectively). Images of our specimen are
186 publicly available at BOLD (BIOUG18683-A06).

187 A third specimen putatively assigned to *Lepidostoma* resides in a BIN with only two
188 members (BOLD:ACL5324) –the Crooked River specimen and one other from British Columbia
189 (Genbank Accession # KX142483). Images of this specimen (adult) are publicly available at
190 BOLD (BIOUG18683-G10).

191 These three specimens are thus most likely also new species records for British
192 Columbia. All known species in British Columbia belonging to *Micrasema* and *Hydroptila* have
193 DNA barcodes in BOLD, and ten of the 12 *Lepidostoma* species known to be in British
194 Columbia have DNA barcodes in BOLD. Only *L. quercina* and *L. stigma* do not, and it is
195 possible that our specimen belongs to one of these two species.

196 The presence of 41 species (20 genera, 11 families) of caddisflies in the Crooked River,
197 is comparable to to other rivers and regions. For instance sampling the Churchill, Manitoba area
198 – including the Churchill River, tundra ponds, lakes, and small streams – revealed 68 species
199 (Zhou *et al.* 2009). Sampling of the Ochre River, Manitoba revealed 33 species (8 families, 17
200 genera) (Cobb and Flannagan 1990). Broad-scale sampling across northern Canada from the
201 Ogilvie Mountains in the Yukon to Goose Bay in Newfoundland revealed 56 species (Cordero *et*
202 *al.* 2017). To our knowledge there is no study that provides comprehensive species checklist of
203 caddisflies for a specific tributary in British Columbia to which we could compare our data more
204 regionally.

205 In summary, our assessment of the Trichoptera inhabiting the Crooked River revealed
206 three new species records for British Columbia. Specifically, to our knowledge this is the first
207 report of *Cheumatopsyche harwoodi*, *Lepidostoma togatum* and *Ceraclea annulicornis*. Our

208 results also suggest at least two, and possibly three, new species records. This baseline
209 biodiversity data is vital for ongoing monitoring and management of this unique and highly
210 impacted located system and provides new data for managers and conservationists working in
211 this understudied system.

212

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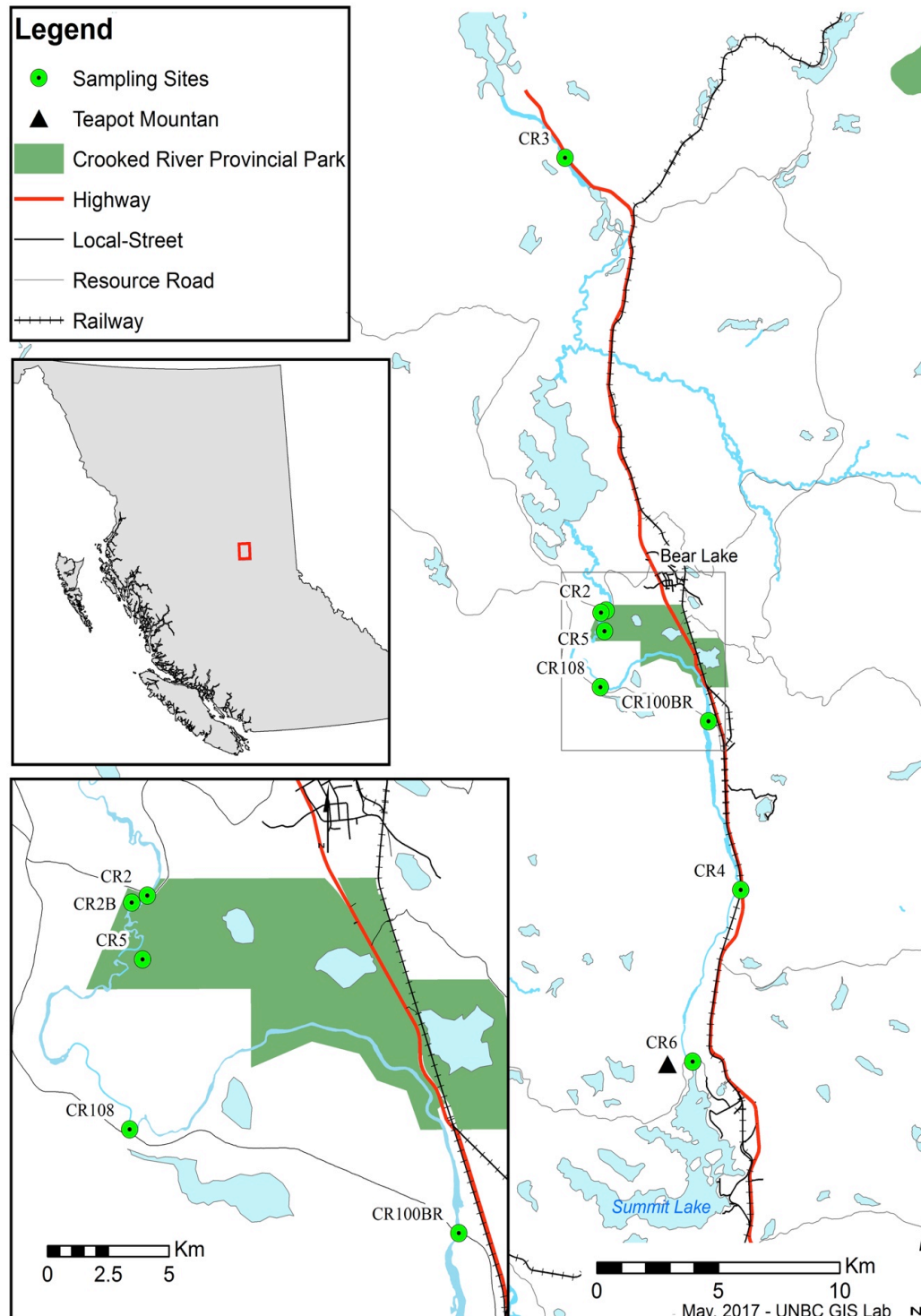
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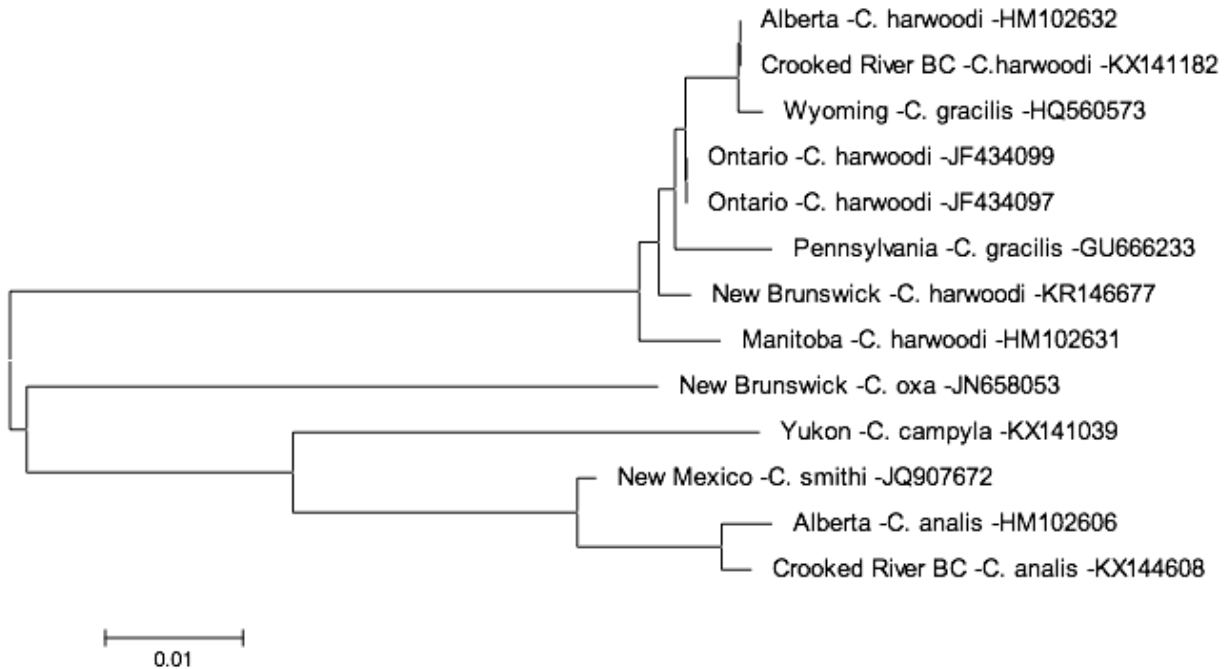
344 **Figure 1:** Map of sampling sites along the Crooked River, British Columbia. CR2: 54.485265°N, -
 345 122.717974°W; CR2B: 54.484474°N, -122.721257°W; CR3: 54.642963°N, -122.743021°W; CR4:
 346 54.387709°N, -122.633217°W; CR5: 54.477975°N, -122.719000°W; CR6: 54.328038°N, -122.669236°W;
 347 CR100BR: 54.446455°N, -122.653129°W; CR108: 54.458511°N, -122.721828°W.

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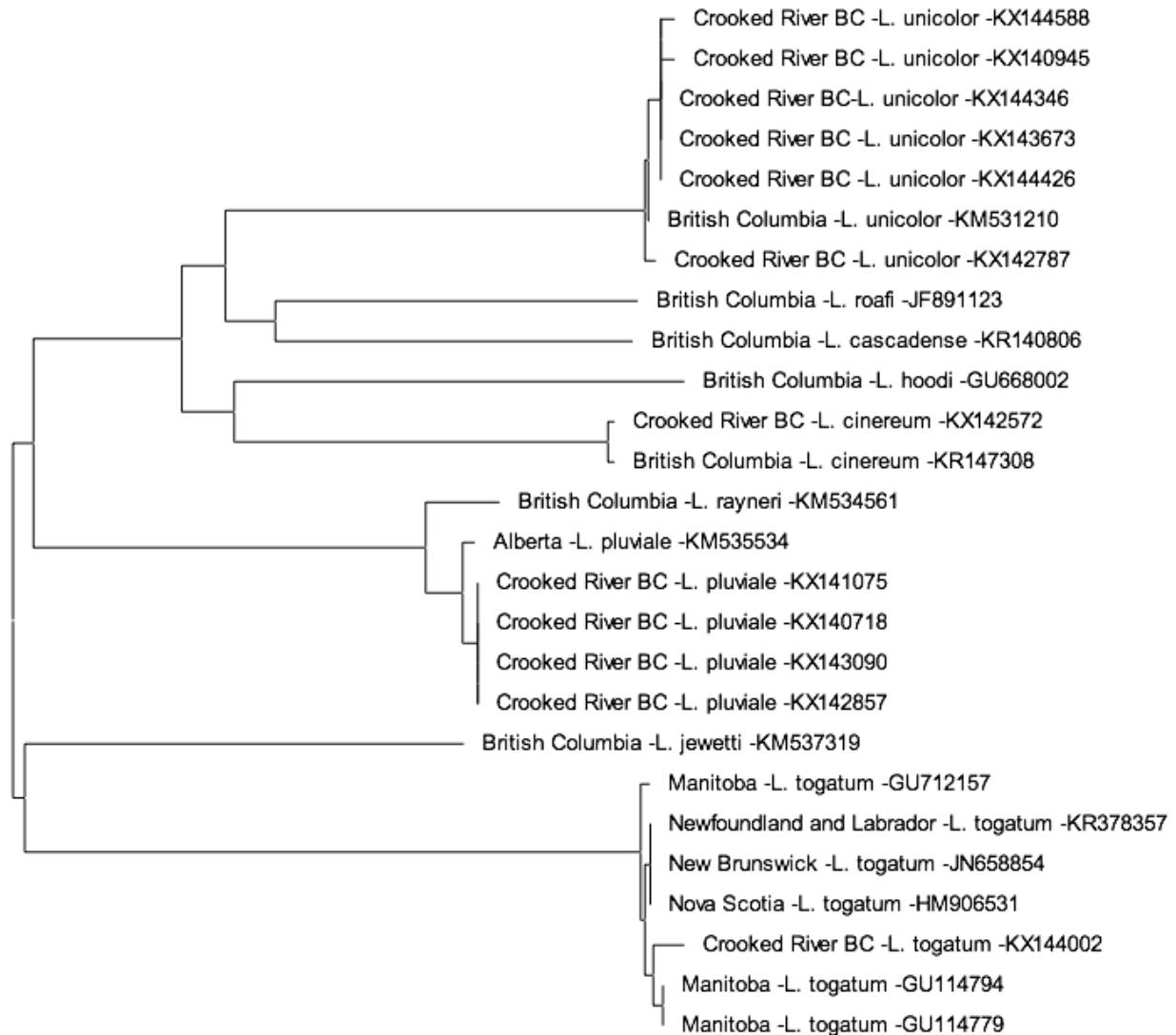
and

391 Evolutionary history is based on the Neighbour-Joining Method bootstrapped (5000 replicates)
392 and the Kimura-2 method to calculate distances. Each species is identified by the geographic
393 region of collection, species, and Genbank accession number for the COI-5P DNA sequence.
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399 **Figure 3:** Phylogenetic tree of *Lepidostoma* spp. collected from the Crooked River and
 400 congeneric COI-5P DNA sequences of *Lepidostoma* species with DNA barcodes. Evolutionary
 401 history is based on the Neighbour-Joining Method bootstrapped (5000 replicates) and the
 402 Kimura-2 method to calculate distances. Each species is identified by the geographic region of
 403 collection, species, and Genbank accession number for the COI-5P DNA sequence.
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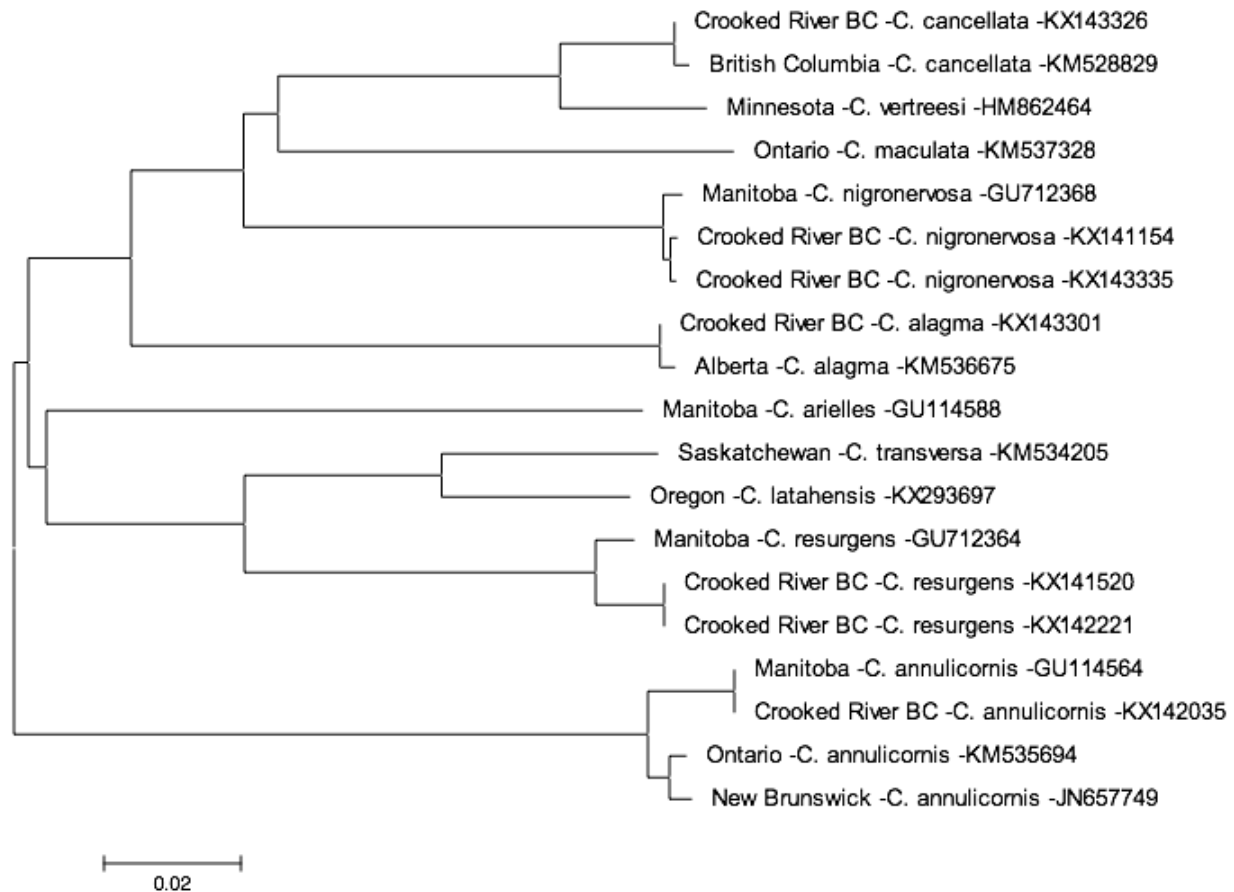


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Figure 4: . Phylogenetic tree of *Ceraclea* spp. collected from the Crooked River and congeneric COI-5P DNA sequences of *Ceraclea* species with DNA barcodes. Evolutionary history is based on the Neighbour-Joining Method bootstrapped (5000 replicates) and the Kimura-2 method to calculate distances. Each species is identified by the geographic region of collection, species, and Genbank accession number for the COI-5P DNA sequence.



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421 **Table 1:** Trichoptera collected along the Crooked River, British Columbia and associated COI
422 DNA barcode-assigned identifications along with date ranges of collection. Locations of
423 collection sites are given in the footnotes. All sequence data are available in public repositories
424 as listed, and all specimens are vouchered at the University of Guelph – Centre for Biodiversity
425 Genomics.

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	Genus ¹	Species ¹	Sample IDs ²	BIN	NCBI accession ³	Collection site(s) ⁴	Collection date range ⁵	Notes
Brachycentridae	<i>Brachycentrus</i>	<i>americanus</i>	BIOUG18684-B11 and 22 others	BOLD:ABX6535	KX144627	CR2, CR2B, CR4, CR108	11-JUN to 13-AUG	
		<i>occidentalis</i>	BIOUG18683-H05 and 5 others	BOLD:AAE0281	KX144012	CR3, CR100BR	04-JUN to 13-AUG	
	<i>Micrasema</i>	<i>bactro</i>	BIOUG18683-F09.1	BOLD:AAC4650	KX143689	CR4	11-JUN	
		sp.	BIOUG18683-F08	BOLD:ACC4912	KX142261	CR2	18-JUN	Potential new B record
Hydropsychidae	<i>Arctopsyche</i>	<i>grandis</i>	BIOUG18683-A11.1 and 6 others	BOLD:AAB3049	KX143192	CR2, CR108	09-JUL to 13-AUG	
	<i>Cheumatopsyche</i>	<i>analis</i>	BIOUG18684-B10	BOLD:AAA5695	KX144608	CR100BR	28-JUL	
		<i>harwoodi</i>	BIOUG18684-B09	BOLD:AAA2316	KX141182	CR4	16-MAY	New BC record
		sp.	BIOUG18684-E05	BOLD:ACE5262	KX142965	CR108	09-JUL	
		sp.	BIOUG18684-E08 and 4 others	BOLD:AAA3891	KX142829	CR3	29-JUL to 13-AUG	
	<i>Hydropsyche</i>	<i>alhedra</i>	BIOUG18683-H03 and 2 others	BOLD:AAC1650	KX143172	CR4, CR108	04-JUN to 11-JUN	
		<i>alternans</i>	BIOUG18683-C12 and 14 others	BOLD:AAA3236	KX140968	CR3, CR100BR	10-JUN to 13-AUG	

		<i>cockerelli</i>	BIOUG18683-A03	BOLD:AAC3057	KX143078	CR4	16-MAY	
		<i>morosa</i>	BIOUG18684-E01 and 5 others	BOLD:AAA3679	KX143491	CR3	28-JUL	
		<i>slossonae</i>	BIOUG18684-E06 and 12 others	BOLD:AAA2527	KX143429	CR2, CR4, CR100BR, CR108	11-JUN to 13-AUG	
Hydroptilidae	<i>Hydroptila</i>	<i>arctia</i>	BIOUG18683-F10.1	BOLD:AAE5200	KX141605	CR108	25-JUN	
		sp.	BIOUG18683-A06	BOLD:AAK3416	KX142062	CR2	18-JUN	Potential new B record
Lepidostomatidae	<i>Lepidostoma</i>	<i>pluviale</i>	BIOUG18684-D07.1 and 3 others	BOLD:ACF2295	KX142857	CR100BR	18-JUN to 13-AUG	
		sp.	BIOUG18683-G10	BOLD:ACL5324	KX144650	CR2	4-AUG	Potential new B record
		<i>togatum</i>	BIOUG18684-D02	BOLD:AAA2325	KX144002	CR3	14-JUL	New BC record
		<i>cinereum</i>	BIOUG18683-C07.1 and 3 others	BOLD:AAK7943	KX142572	CR2, CR2B, CR4	25-JUN to 4-AUG	
		<i>unicolor</i>	BIOUG18684-H04 and 8 others	BOLD:AAC5923	KX142875	CR4, CR108	11-JUN to 4-AUG	
Leptoceridae	<i>Ceraclea</i>	<i>alagma</i>	BIOUG18683-F06 and two others	BOLD:AAA5876	KX143301	CR6, CR100BR, CR108	16-MAY to 14-JUL	
		<i>annulicornis</i>	BIOUG18683-B02	BOLD:AAA5429	KX142035	CR3	13-AUG	New BC record
		<i>cancellata</i>	BIOUG18684-A01	BOLD:ABZ0710	KX143326	CR4	4-AUG	

		<i>nigronervosa</i>	BIOUG18683-H09 and 1 other	BOLD:AAC3781	KX141154	CR100BR	10-JUN
		<i>resurgens</i>	BIOUG18683-F07.1 and 2 others	BOLD:ACG9704	KX142221	CR3	14-JUL to 28-JUL
Limnephilidae	<i>Amphicosmoecus</i>	<i>canax</i>	BIOUG18683-D09 and 5 others	BOLD:AAE2491	KX143314	CR2B, CR4, CR100BR	11-JUN to 9-JUL
	<i>Clistoronia</i>	<i>magnifica</i>	BIOUG18683-F05 and 1 other	BOLD:AAC1848	KX141495	CR3, CR4	28-JUL to 13-AUG
	<i>Dicosmoecus</i>	<i>atripes</i>	BIOUG18683-G05 and 2 others	BOLD:AAC5045	KX140940	CR4	11-JUN
		<i>gilvipes</i>	BIOUG18684-H07 and six others	BOLD:AAI9526	KX142636	CR2B, CR4, CR100BR	16-MAY to 9-JUL
	<i>Limnephilus</i>	<i>externus</i>	BIOUG18683-F12 and 1 other	BOLD:AAA2803	KX141731	CR2B, CR6	11-JUN to 18-JUN
	<i>Onocosmoecus</i>	<i>unicolor</i>	BIOUG18684-H04 and 8 others	BOLD:AAC5923	KX142875	CR4, CR108	11-JUN to 4-AUG
	<i>Psychoglypha</i>	<i>alascensis</i>	BIOUG18683-G07 and 7 others	BOLD:ACH0278	KX141905	CR4, CR5	9-MAY to 4-AUG
		<i>subborealis</i>	BIOUG18683-D11.1 and 2 others	BOLD:AAE0945	KX144814	CR4	9-JUL to 4-AUG
Philopotamidae	<i>Wormaldia</i>	<i>gabriella</i>	BIOUG18684-C03 and 4 others	BOLD:AAC1539	KX143731	CR2, CR108	21-JUL to 13-AUG
Phryganeidae	<i>Agrypnia</i>	<i>improba</i>	BIOUG18683-C01	BOLD:ACK0044	KX143489	CR2	13-AUG
Polycentropodidae	<i>Neureclipsis</i>	<i>bimaculata</i>	BIOUG18683-A08 and 3 others	BOLD:AAE2683	KX141945	CR3	14-JUL to 28-JUL
	<i>Plectrocnemia</i>	<i>cinerea</i>	BIOUG18684-A08	BOLD:AAA3441	KX141515	CR6	14-JUL

Rhyacophilidae	<i>Rhyacophila</i>	<i>brunnea</i>	BIOUG18683-B12 and 11 others	BOLD:AAB3088	KX141430	CR4, CR100BR, CR108	18-JUN to 2-AUG
		sp.	BIOUG18684-A07 and 3 others	BOLD:ACL4744	KX140935	CR2, CR100BR	13-AUG
Uenoidae	<i>Neophylax</i>	<i>rickeri</i>	BIOUG18683-G08	BOLD:AAG9543	KX144032	CR4	4-JUN

1- determined from morphological keys and BOLD database match.

2- if more than one specimen, longest sequence from BOLD with an NCBI accession number; other sample data are available at BOLD dataset CRTRI.

3- for the sample specified in the fourth column.

4- CR2 – 54.484°N, -122.721°W; CR2B – 54.484°N, -122.721°W; CR3 – 54.643°N, -122.743°W; CR4 – 54.388°N, -122.633°W; CR5 – 54.478°N, -122.719°W; CR6 – 54.328°N, -122.669°W;
CR100BR – 54.446°N, -122.653°W; CR108 – 54.458°N, -122.722°W

5- first collection date and (if applicable) last collection date in 2014.