DNA barcode-based survey of Trichoptera in the Crooked River reveals three new species records for British Columbia (#18714)

First revision

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

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

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Abstract

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

INTRODUCTION

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

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

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

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

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

METHODS AND MATERIALS

We collected specimens on a biweekly basis from eight locations (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) along the edge of the Crooked River, British Columbia between May and August 2014 using both hand and kick-net methods. This study focused mainly on larvae to ensure that we collected caddisflies from the Crooked River only and not from nearby water bodies. We completed collections under the British Columbia Ministry of Environment Park Use Permit #107171 where required. We preserved specimens in 80% ethanol upon collection. We ~~classified~~ all 2204 caddisfly specimens that we collected to the

lowest possible taxonomic ranking (genus or family) based on published morphological keys (Wiggins 1977; Clifford 1991; Schmid 1998). We selected morpho-species based on that visual identification and 214 specimens were subsequently sent to the Biodiversity Institute of Ontario (BIO) and its Barcode of Life Database (http://www.boldsystems.org) in Guelph, Ontario, to have their barcode region (COI) sequenced for further classification. We received back 185 useable sequences (>400 bp., <5 miscalls, no contamination detected). We vouchered all specimens set for sequencing at the Centre for Biodiversity Genomics at the University of Guelph. Initial species identification was based on a 650 bp sequence in CO1 5' region using the BOLD platform with MUSCLE sequence alignments and a Kimura-2-parameter distance model. The data for all collected specimens is available as dataset DS-CRTRI [http://www.boldsystems.org/index.php/Public_SearchTerms?query=CRTRI].

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

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

RESULTS & DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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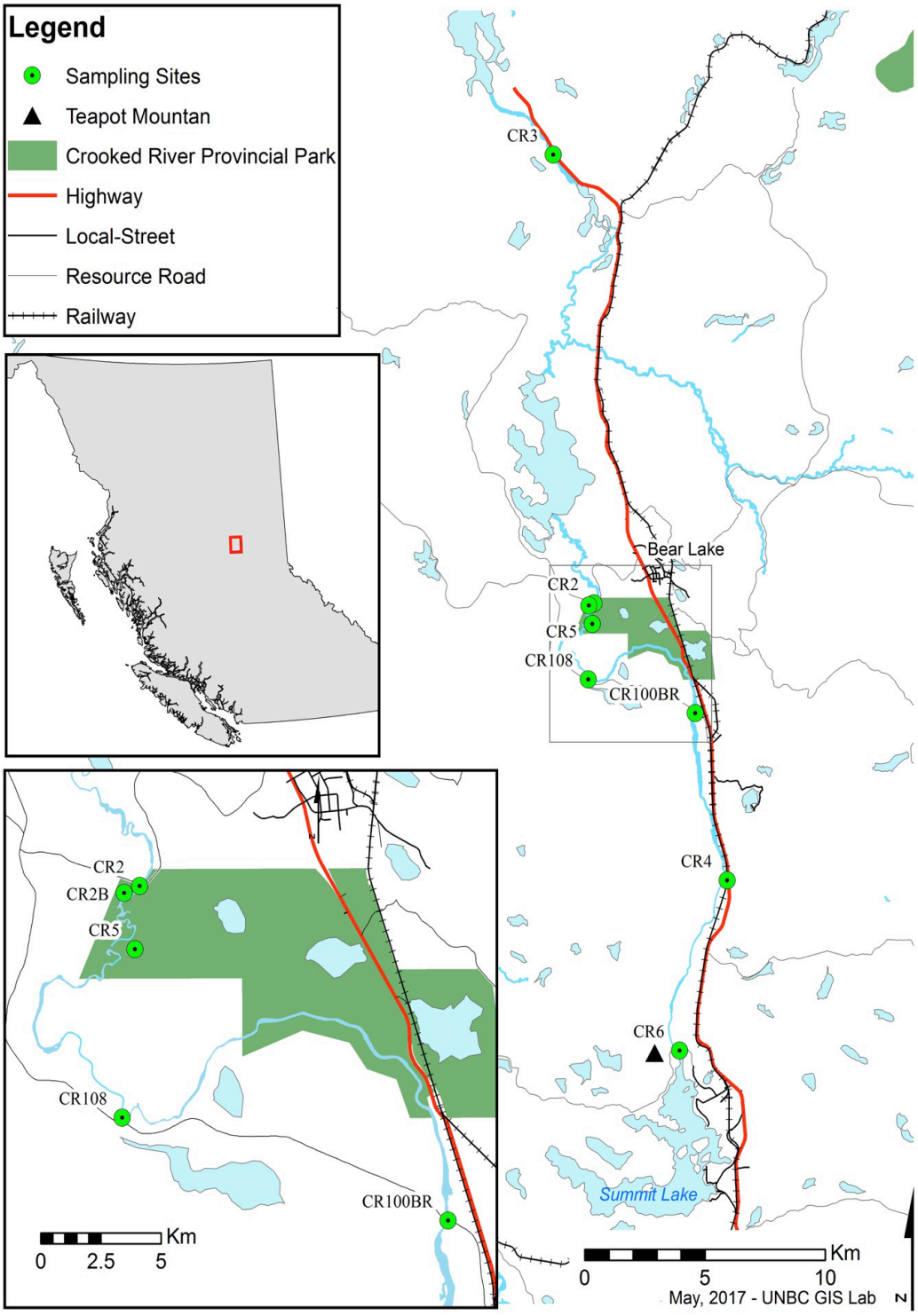
Figure 1: Map of sampling sites along the Crooked River, British Columbia. CR2: 54.485265ºN, -122.717974ºW; CR2B: 54.484474ºN, -122.721257ºW; CR3: 54.642963ºN, -122.743021ºW; CR4: 54.387709ºN, -122.633217ºW; CR5: 54.477975ºN, -122.719000ºW; CR6: 54.328038ºN, -122.669236ºW; CR100BR: 54.446455ºN, -122.653129ºW;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



and

390 Evolutionary history is based on the Neighbour-Joining Method bootstrapped (5000 replicates)
391 and the Kimura-2 method to calculate distances. Each species is identified by the geographic
392 region of collection, species, and Genbank accession number for the COI-5P DNA sequence.
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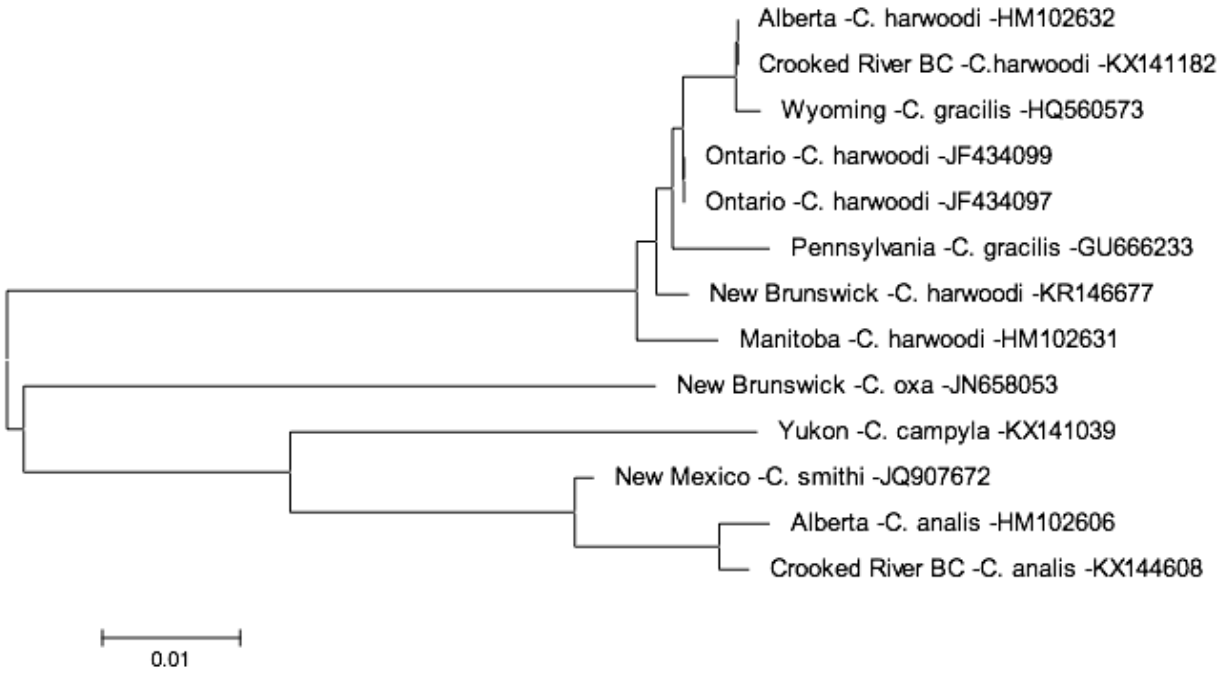

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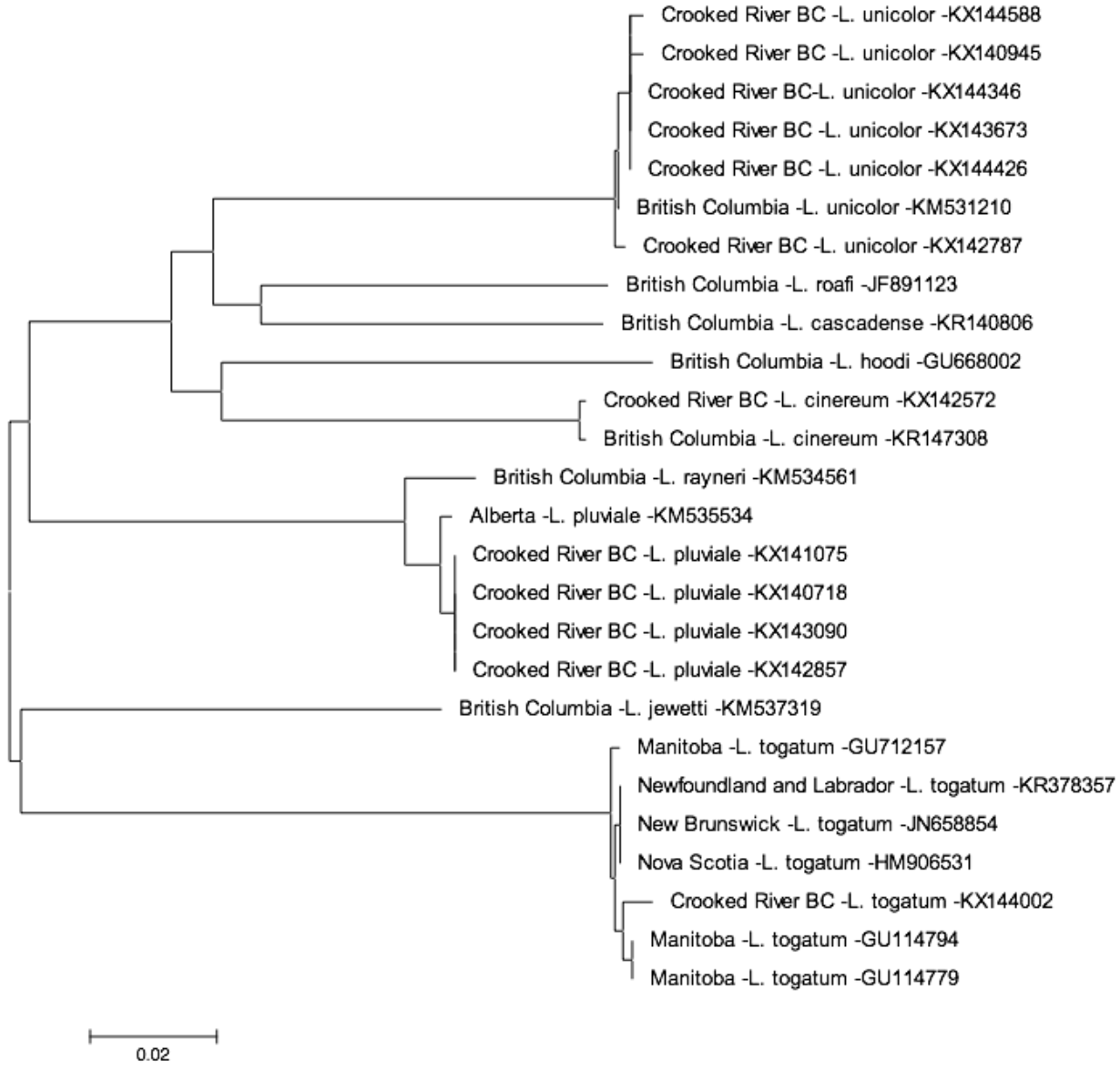
Figure 3: Phylogenetic tree of *Lepidostoma* spp. collected from the Crooked River and congeneric COI-5P DNA sequences of *Lepidostoma* species with DNA barcodes. Evolutionary history is based on the Neighbour-Joining Method bootstrapped (5000 replicates) and the Kimura-2 method to calculate distances. 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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409 **Figure 4:** . Phylogenetic tree of *Ceraclea* spp. collected from the Crooked River and congeneric
410 COI-5P DNA sequences of *Ceraclea* species with DNA barcodes. Evolutionary history is based
411 on the Neighbour-Joining Method bootstrapped (5000 replicates) and the Kimura-2 method to
412 calculate distances. Each species is identified by the geographic region of collection, species, and
413 Genbank accession number for the COI-5P DNA sequence.
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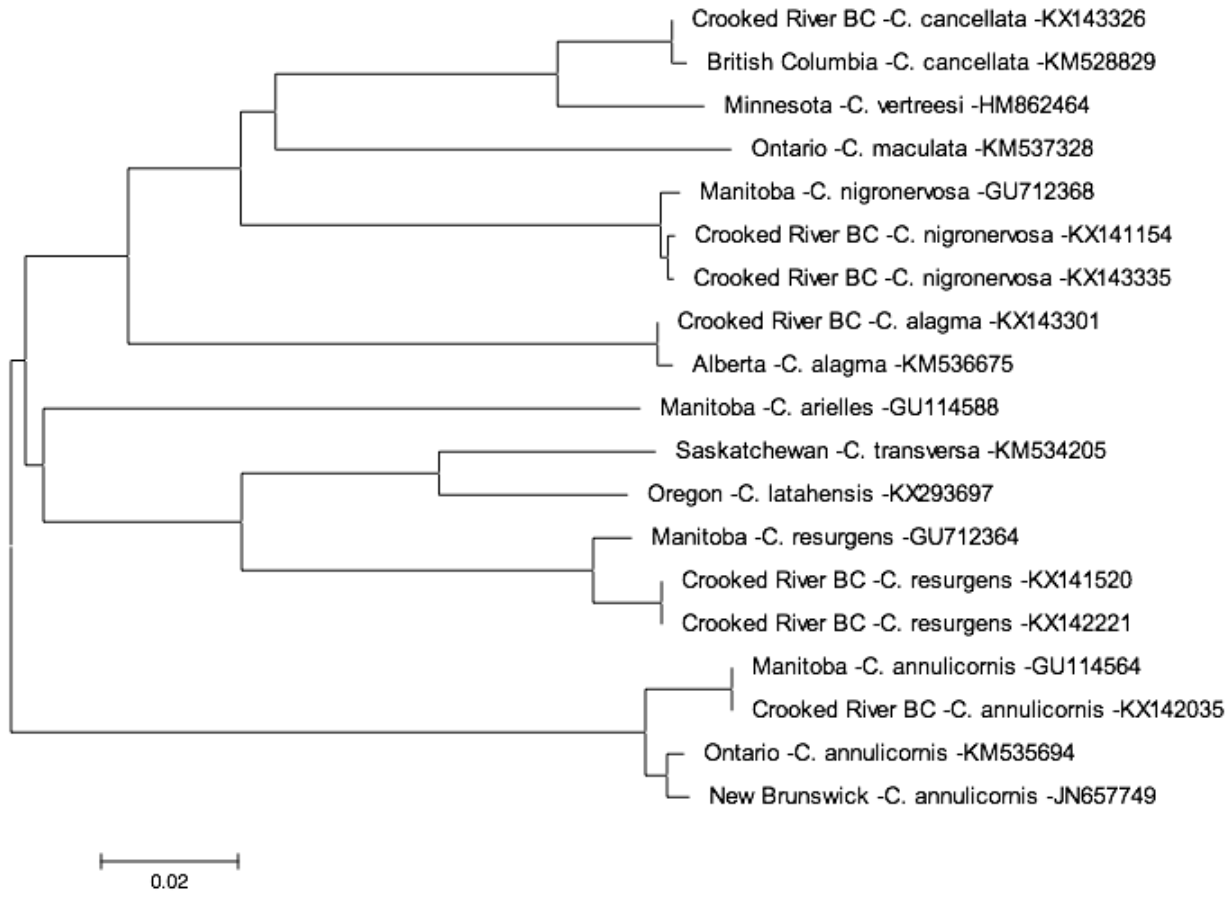

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Table 1: Trichoptera collected along the Crooked River, British Columbia and associated COI DNA barcode-assigned identifications along with date ranges of collection. Locations of collection sites are given in the footnotes. All sequence data are available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

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

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

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

Rhyacophilidae	*Rhyacophila*	*brunnea*	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	*Neophylax*	*rickeri*	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.