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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

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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). DNA barcoding combined with classical taxonomy can help to speed up this process (DeSalle *et al.* 2005). 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 a portion their cytochrome oxidase I (COI) DNA genes. In addition, recent comprehensive work on barcode-assisted Trichoptera taxonomy (Zhou et al. 2009, 2010a,b, 2011, 2016) provides a solid foundation for biodiversity assessments of caddisflies in North America.

Trichoptera, Ephemeroptera (mayflies), Plecoptera (stoneflies), and often aquatic Diptera (true flies) are used in well-developed indices as indicator 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 number or taxonomic diversity both temporally and/or geographically are often used as indicators of disturbance (Houghton 2004; Pond 2012). However 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 systems.

The Crooked River (Figure 1) is the southernmost Arctic watershed lotic system in British Columbia. It flows north from Summit Lake (which is just on the north side of the continental divide) to McLeod Lake, connecting a series of lakes along the way. From there its water flows via other systems to eventually end up in the Williston Reservoir – a massive hydroelectric

reservoir in the Rocky Mountain Trench that represents one of the largest anthropogenic landscape modifications on earth. The Crooked River is named for all the oxbows due to its slow meandering flow. This river is also fed by underground springs – Livingston Springs in Crooked River Provincial is a well-known spring that supplies the river with water year round and moderates annual temperature shifts. An extinct volcanic cone – currently named Teapot Mountain – is situated at its headwaters, likely providing mineral nutrient inputs. As a *bona fide* spring creek, the Crooked River has a very flat gradient with swamp and marshland along much of its shoreline. During freshet the river floods these marshes bringing more nutrients into the system.

The Crooked River has been heavily used by European settlers for transport and trade for much of their history in British Columbia (McKay 2000) – and it was doubtless used prior to that by First Nations groups for sustenance and as a settlement location. These human-caused impacts continue to this day in an increasing manner. The river is in direct path of planned pipelines originating from northeastern British Columbia and Alberta that will run toward the Pacific coast. In addition the area 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. Our searches have revealed no recorded biodiversity surveys on the Crooked River. In addition, to our knowledge no comprehensive recent assessment has been done on Trichoptera in central or northern British Columbia. It is therefore important to develop a baseline of aquatic species present in the Crooked River for ongoing and future monitoring work on this river and nearby systems.

METHODS AND MATERIALS

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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. 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. We identified specimens based on the 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 project CRTRI at http://v4.boldsystems.org/index.php/MAS_Management_DataConsole?codes=CRTRI.

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

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92 (http://ibis.geog.ubc.ca/biodiversity/efauna/); Natureserve (http://www.natureserve.org/);
93 Canadensys (http://www.canadensys.net/), Global Biodiversity Information Facility
94 (http://www.gbif.org/); the Royal Ontario Museum, and the Royal British Columbia Museum
95 (http://search-collections.royalbcmuseum.bc.ca/Entomology).
96
97 **RESULTS & DISCUSSION**
98 We used morphological keys to identify all 2204 collected specimens to family or genus,
99 after which we used successful barcodes and database searches to deduce the species identities of
100 185 individuals based on previous database annotations. In total we detected 41 caddisfly species
101 – found in 20 genera within 11 families – in the Crooked River system (Table 1). All barcode
102 data are available at the Barcode Of Life Database (BOLD). Thirty five of the 41 species we
103 identified were assigned to known species via database matches. Although originally arbitrary, a
104 2% threshold for delineating species within Trichoptera is considered to be a reliable approach
105 (Zhou *et al.* 2009). COI sequences from of specimens from the Crooked River with DNA
106 sequences matching 99.67% and 99.13% to *Lepidostoma cinereum* and *Neophylax rickeri*
107 respectively, were assigned to the aforementioned species.
108 Among the 34 specimens identified to species with 100% database matches are
109 *Cheumatopsyche harwoodi, Lepidostoma togatum* and *Ceraclea annulicornis,* all three are new
110 species records for British Columbia. We found a larva of *Cheumatopsyche harwoodi* (synonym
111 *C. enigma*) at CR4 on May 16th 2014 (Figure 2). On July 14th we found a larva for *Lepidostoma*
112 *togatum* {synonyms *L. canadense* (Banks, 1899) *L. pallidum* (Banks, 1897) *Mormomyia togatum*
113 (Hagen, 1861), *Pristosilo canadensis* (Banks, 1899), *Silo pallidus* (Banks, 1897)} at CR3 (Figure
114 3). On August 13th, 2014 we found a specimen of *Ceraclea annulicornis* {(synonyms:

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Athripsodes annulicornis (Stephens, 1836), *C. futilis* (Banks, 1914), *C. recurvata* (Banks, 1908), *Leptocerus annulicornis* (Stephens, 1836), *L. futilis* (Banks, 1914)} at CR3 (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 (Figure 5).

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. (Figure 6). 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).

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) (Figure 7).

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, compares similarly to other rivers and regions. For instance sampling the Churchill, Manitoba

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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) (Cobb and Flannagan 1990). To our knowledge there is no study that provides comprehensive species checklist of caddisflies for a specific tributary in British 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 two more, 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: 54.485265°N, -122.717974°W; CR2B: 54.484474°N, -122.721257°W; CR3: 54.642963°N, -122.743021°W; CR4: 54.387709°N, -122.633217°W; CR5: 54.477975°N, -122.719000°W; CR6: 54.328038°N, -122.669236°W; CR100BR: 54.446455°N, -122.653129°W; CR108: 54.458511°N, -122.721828°W.

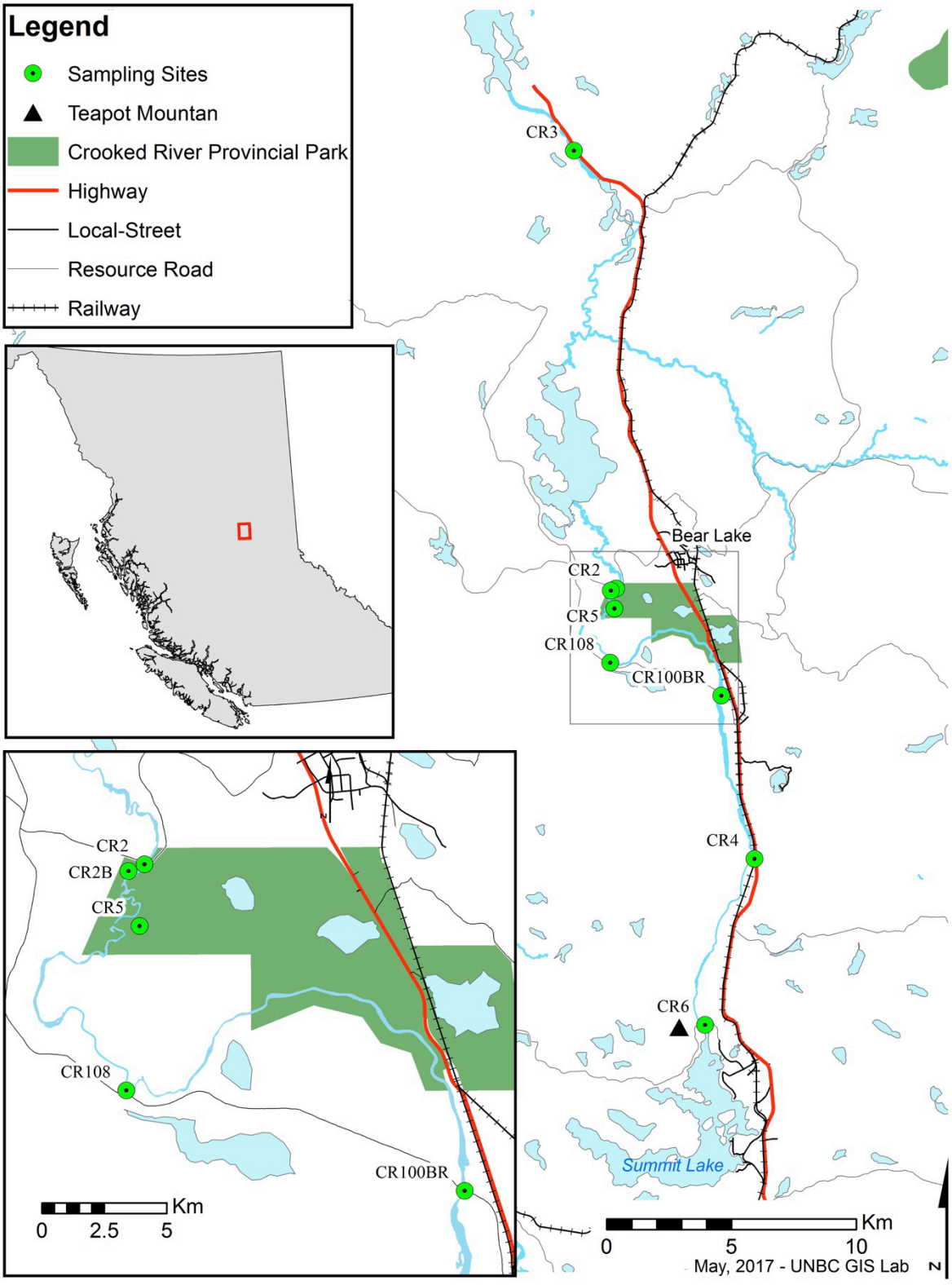

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Figure 2: Larva identified as *Cheumatopsyche harwoodi* collected at CR4 on 16 May 2014. Lateral (A), ventral (B), dorsal (C), mouthparts (D), dorsolateral head capsule (E), lateral head capsule (F). This specimen is vouchered at the Biodiversity Institute of Ontario (specimen ID: BIOUG18684-B09). All images: Creative Commons BY-NC-SA (2017), Centre for Biodiversity Genomics Photography Group.

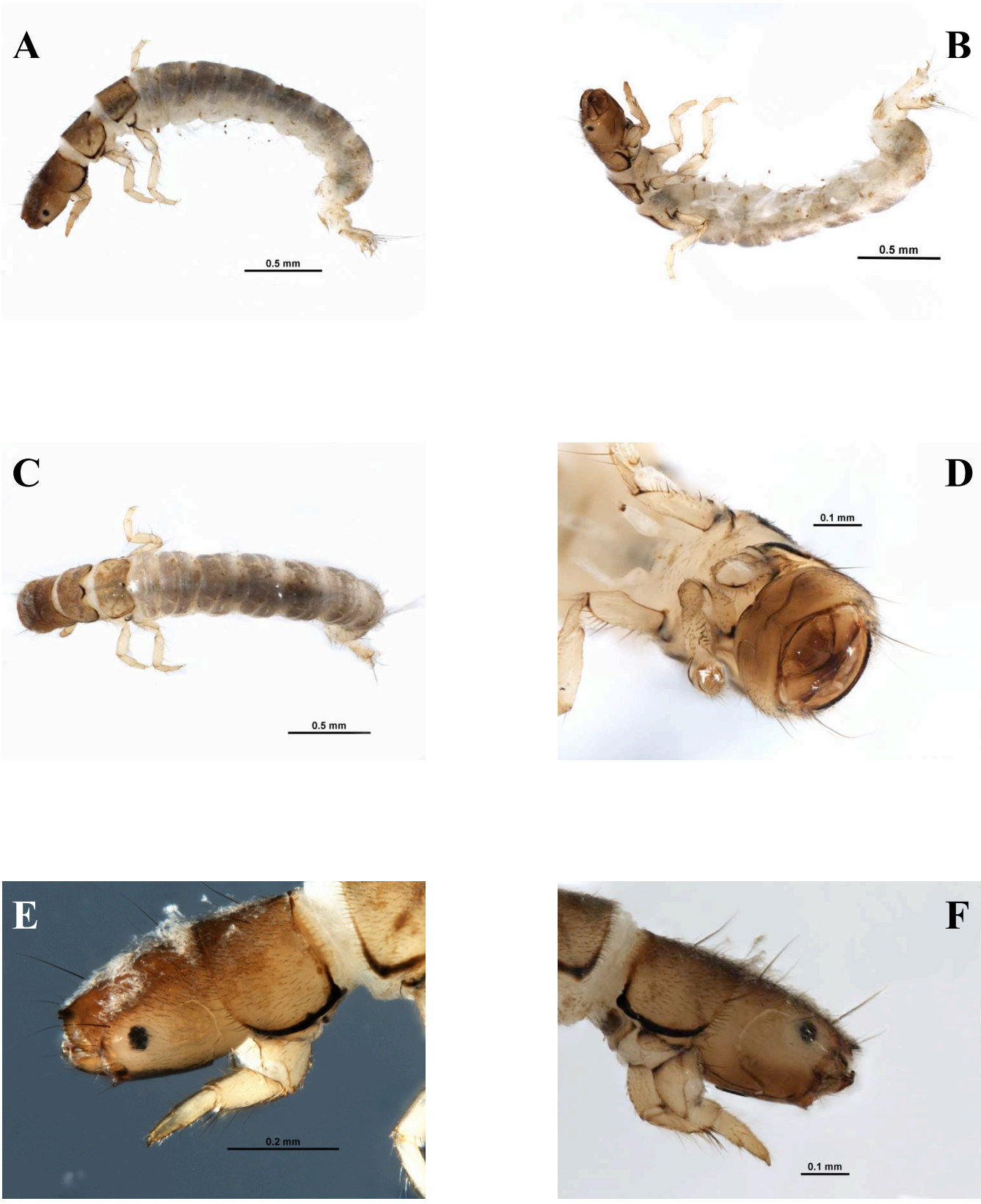

Figure 3: Larva identified as *Lepidostoma togatum* collected at CR3 on 14 July 2014. Lateral (A), ventral (B), dorsal (C), mouthparts (D), dorsolateral head capsule (E), dorsal head capsule (F). This specimen is vouchered at the Biodiversity Institute of Ontario (specimen ID: BIOUG18684-D02). All images: Creative Commons BY-NC-SA (2017), Centre for Biodiversity Genomics Photography Group.

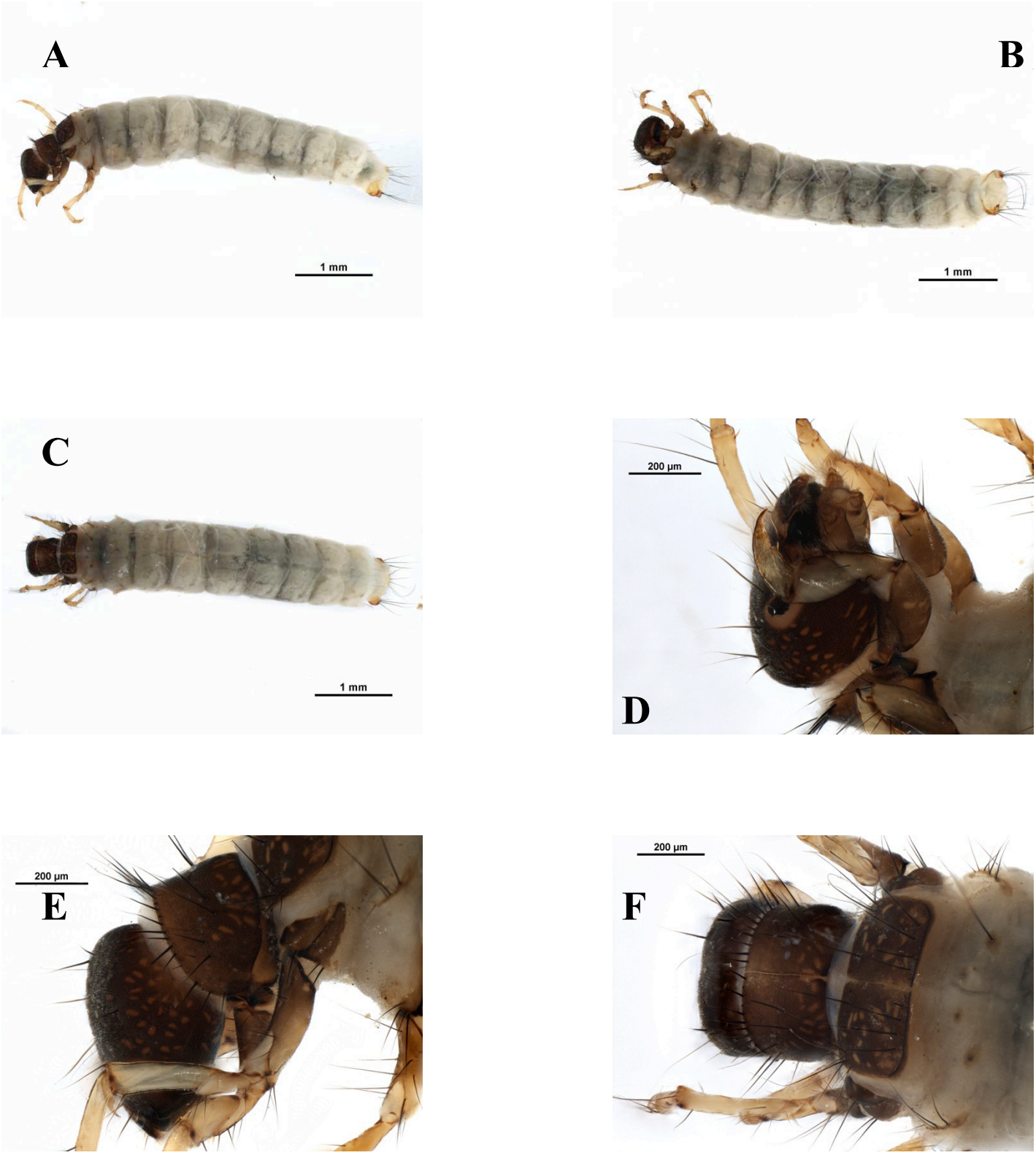

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Figure 4: Larva identified as *Ceraclea annulicornis* collected at CR3 on 13 August 2014. Dorsal (A), dorsal head capsule (B), ventral (C), mouthparts (D). This specimen is vouchered at the Biodiversity Institute of Ontario (specimen ID: BIOUG18683-B02). All images: Creative Commons BY-NC-SA (2017), Centre for Biodiversity Genomics Photography Group.

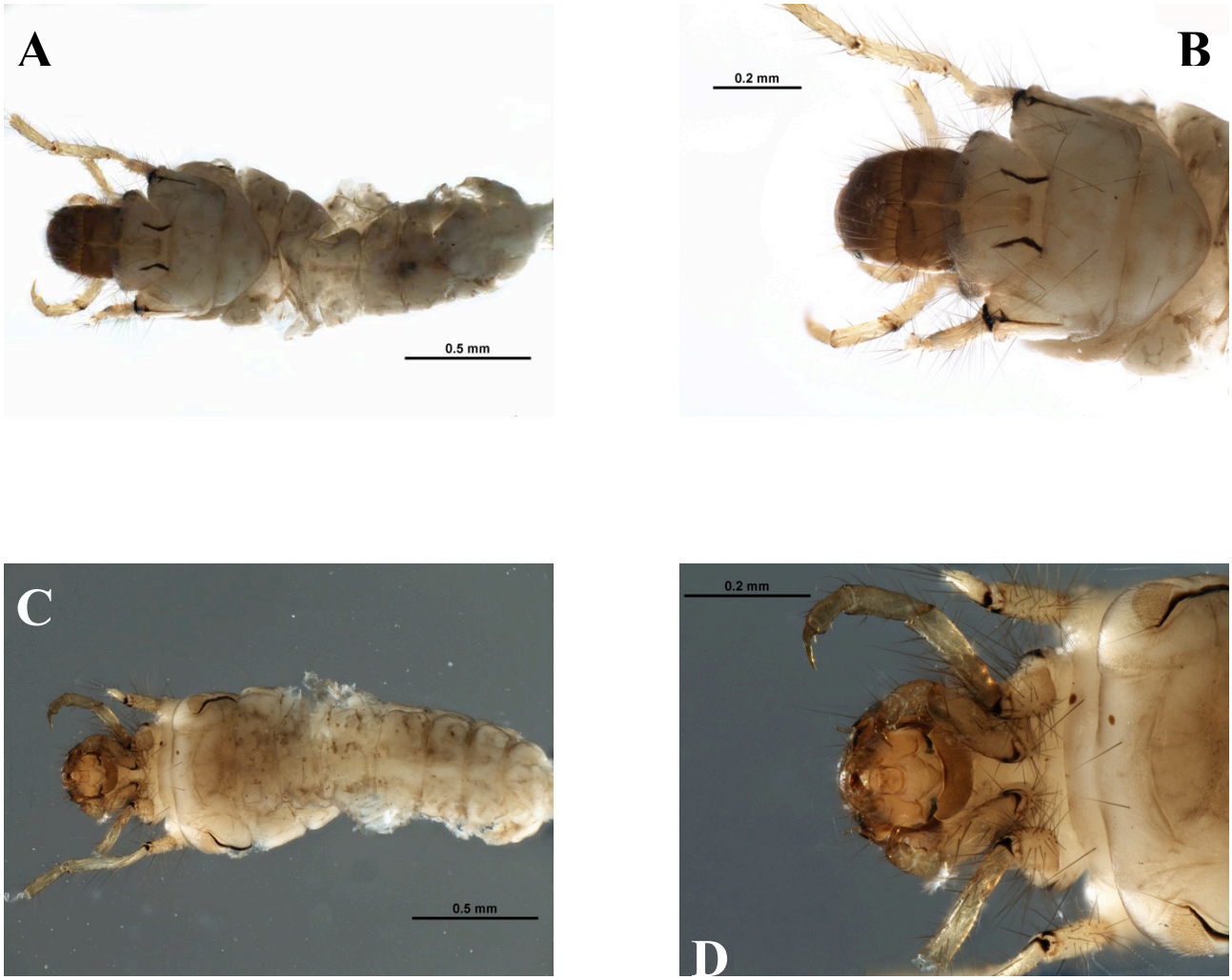

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Figure 5: Larva assigned to *Micrasema* sp. collected at CR2 on 18 June 2014. Lateral (A), dorsal (B), mouthparts (C), head (D). This specimen is vouchered at the Biodiversity Institute of Ontario (specimen ID: BIOUG18683-F08). All images: Creative Commons BY-NC-SA (2017), Centre for Biodiversity Genomics Photography Group.

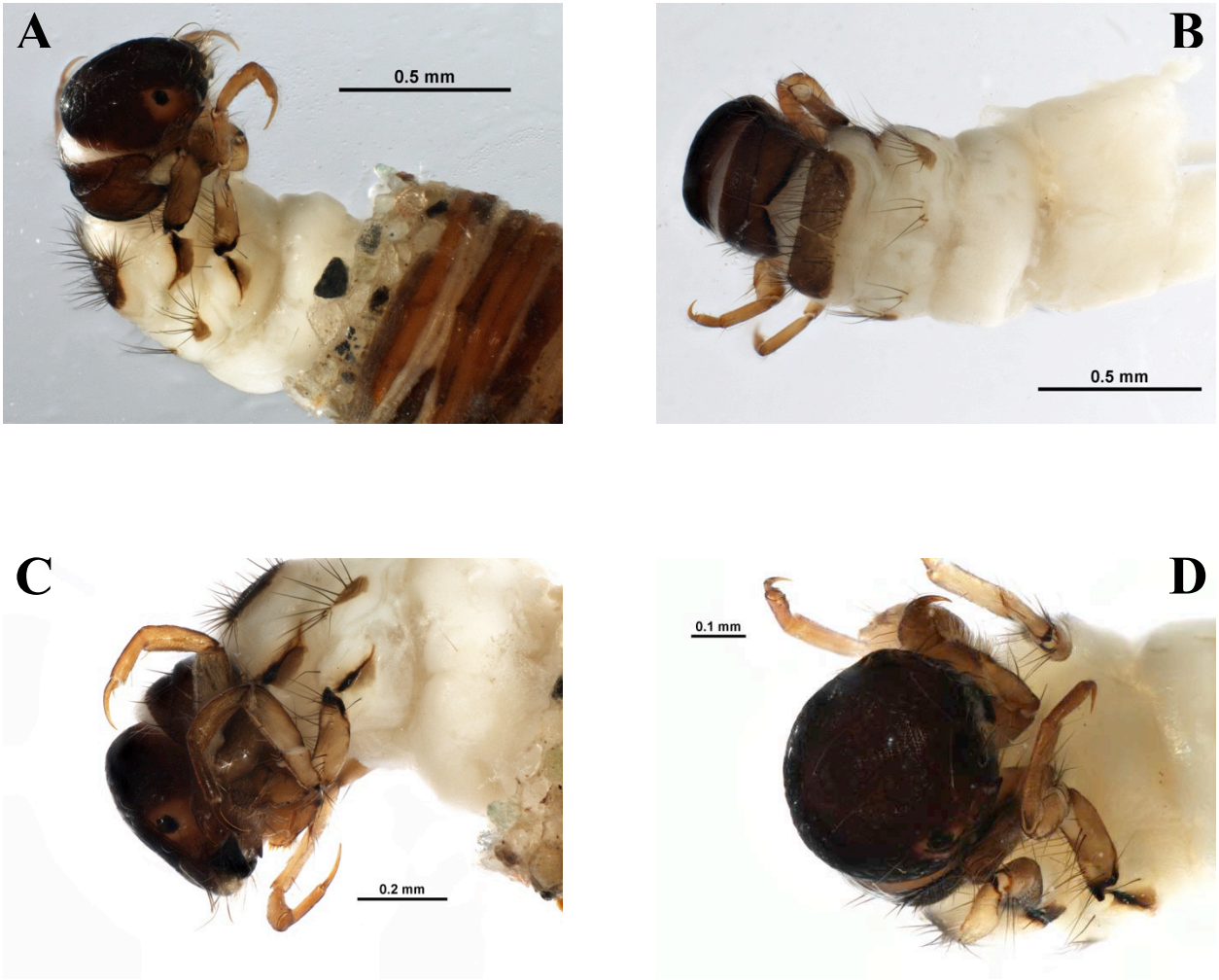

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Figure 6: Larva assigned to *Hydroptila* sp. collected at CR2 on 18 June 2014. Lateral (A), dorsal (B), mouthparts (C), head (D). This specimen is vouchered at the Biodiversity Institute of Ontario (specimen ID: BIOUG18683-A06). All images: Creative Commons BY-NC-SA (2017), Centre for Biodiversity Genomics Photography Group.

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Figure 7: Adult assigned to *Lepidostoma* sp. collected at CR2 on 4 August 2014. Lateral (A), dorsal (B), mouthparts (C), head (D). This specimen is vouchered at the Biodiversity Institute of Ontario (specimen ID: BIOUG18683-G10). All images: Creative Commons BY-NC-SA (2017), Centre for Biodiversity Genomics Photography Group.

A

B

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557
558 **Table 1:** Trichoptera collected along the Crooked River, British Columbia and associated COI
559 DNA barcode-assigned identifications along with date ranges of collection. Locations of
560 collection sites are given in the footnotes. All sequence data are available in public repositories
561 as listed, and all specimens are vouchered at the University of Guelph – Centre for Biodiversity
562 Genomics.

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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	

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		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	

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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 CRTRI project DOI:

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.

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