First records of *Baetis vernus* Curtis (Ephemeroptera: Baetidae) in North America, with morphological notes

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ABSTRACT

The *Baetis vernus* group (Ephemeroptera: Baetidae) – which includes *B. brunneicolor* McDunnough, *B. bundyae* Lehmkuhl, *B. hudsonicus* Ide, *B. jaervii* Savolainen, *B. liebenauae* Keffermüller, *B. macani* Kimmins, *B. subalpinus* Bengtsson, *B. tracheatus* Keffermüller & Machel, and *B. vernus* Curtis – is both diverse and taxonomically tangled. Some members of the group – *B. brunneicolor*, *B. bundyae*, and *B. hudsonicus* – have been previously found in North America. The remainder of the group is known to be only of Palearctic distribution, including *B. vernus,* which has a wide trans-Palearctic distribution. We report the collection of specimens from the Northwest Territories and British Columbia that we have identified as *B. vernus* using DNA barcoding and morphological examination and provide characters to assist separation of the North American members of the group from *B. vernus*. A genetically cohesive Holarctic clade for *B. vernus* likely relates to a Beringian dispersal event. This substantial expansion of the known range of *B. vernus* adds new phylogeographic and ecological complexity, but it may also help to provide further clues to the evolutionary history of this group.

INTRODUCTION

Mayflies of the *Baetis vernus* group (Savolainen *et al.* 2007; Ståhls and Savolainen 2008; Drotz *et al.* 2012) are widespread across the Holarctic, but distributions of its members are challenging to determine because many are difficult to separate in the most commonly collected larval stage using morphological characters (Ståhls and Savolainen 2008; Drotz *et al.* 2012). This is due both to similarity of characters among group members and to high levels of variation relating to environmental conditions (Bauernfeind and Humpesch 2001; Ståhls and Savolainen 2008). Ståhls and Savolainen (2008) stressed the importance of combining molecular and morphological data to sort out species distributions in this group.

Until recently, only three species in this group were known in North America (McCafferty and Jacobus 2017). *Baetis brunneicolor* McDunnough is widespread in the Nearctic: it is reported from across Canada, including Arctic and Sub-Arctic zones (Harper and Harper 1981; Cordero *et al.* 2017; Giberson and Burian 2017) and is found in the northeastern, northwestern, and southeastern United States (USA; McCafferty and Jacobus 2017). *Baetis bundyae* Lehmkuhl has a generally northern distribution in Nearctic and Palearctic: in North America, it is widespread across the north but also extends into the northern USA (Giberson *et al.* 2007; Giberson and Burian 2017). *Baetis hudsonicus* Ide has so far been reported only in northern and far northern Canada (Cordero *et al.* 2017; Giberson and Burian 2017; McCafferty and Jacobus 2017).

Recent collecting efforts in northern British Columbia (Huber *et al.* 2019) and in the Northwest Territories (Cordero *et al.* 2017) revealed four specimens whose cytochrome

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oxidase I (COI) barcode matched Palearctic specimens of *Baetis vernus* Curtis, a species not previously reported in North America. *Baetis vernus* specimens showed many morphological similarities to *B. brunneicolor*, potentially causing confusion when determining the distribution of the two species in North America. Recently, Webb *et al.* (2018) recommended that *B. brunneicolor* and *B. vernus* be treated as a species complex (the *B. vernus* complex) and not identified further if identifying larvae using current morphologically-based keys. Here, we describe DNA barcode data of the Canadian *B. vernus* specimens, demonstrating their genetic similarity to Palearctic *B. vernus* and distance from other *B. vernus* group members, as well as the relevant morphology of those same specimens compared to North American *B. brunneicolor* characteristics.

METHODS AND MATERIALS

Mayfly larvae examined in this study resulted from recent aquatic insect sampling in river and lake habitats in northern British Columbia (BC), Yukon (YT) and Northwest Territories (NT), plus examination of archived specimens in the Canadian National Collection (CNCI) in Ottawa (Giberson and Burian 2017; Huber *et al.* 2019). Collection locality, voucher, and DNA sequence data for specimens that were collected and/or analyzed in this study are described in Table 1. The cytochrome oxidase I (COI) barcode region (Hebert *et al.* 2003; Ball *et al.* 2005) of the Crooked River, BC, specimen was sequenced at the Biodiversity Institute of Ontario, and other barcode sequence data were extracted from public databases. North American *B. vernus* group specimens were compared to other described members of the *B. vernus* for which sequence data were available (exceptions: *B. jaervii* Savolainen and *B. tracheatus* Keffermüller & Machel). A FASTA file of all sequences used in this study, including sequence ID and accession information, is available as supplemental data. All sequence data are publicly available as listed in Table 1 and as BOLD IDs (most sequences) or an NCBI accession number (Yellowknife specimen sequence) in Figure 1. Barcode sequences were aligned with ClustalW and visualized with FigTree v.1.4.3.

Specimens were observed for morphological characters, colouration, and colour patterns under Wild M5A stereoscopic and Bausch & Lomb phase contrast compound light microscopes (up to 1000x magnification). Mouth and body parts of the larvae were dissected in 80% alcohol and slide mounted in Hoyer's Mounting Media. Specimens were photographed using a Nikon D300s DSLR and the Nikon Camera Control Pro2® software. All measurements were made using a calibrated ocular micrometer (nearest 0.10 mm). Measurements were made from entire specimens and/or parts (not mounted on slides) that were held as flat as possible (without inducing distortion) using sections of broken glass microscope slides and coverslips.

Specimens were determined to species by comparing morphological characters to all pertinent descriptions and morphological studies of members of the *Baetis vernus* species group on a global basis, as well as Nearctic keys to species of *Baetis* (Ide 1937; Leonard 1950; Macan 1957; Keffermüller and Machel 1967; Müller-Liebenau 1969; Lehmkuhl 1973; Morihara and McCafferty 1979a, b; Jacob 2003; Wiersema *et al.* 2004; Savolainen 2009; Jacobus *et al.* 2014). In addition, reared specimens of *B. brunneicolor* from the USA and voucher specimens of larvae of *B. macani* and *B. jaeverii* (provided by E. Savolainen) from Finland were used to evaluate characters observed on *B. vernus* specimens from northern Canada.

Table 1

Collection data for *Baetis* specimens in this study. All specimens listed were verified to species by S.K. Burian, except BIOUG22893-F05 and BIOUG22893-F05 (verified by J.E. Sones) and the specimens listed in Harper and Harper (1981) (verified by P. Harper) and Cordero *et al.* 2017 (verified through barcoding). BC: British Columbia; NT: Northwest Territories; YT: Yukon Territory; MB: Manitoba; PQ: Province of Quebec; ON: Ontario.

Specimen code	Reg.	Site	Date	Species	Location Latitude/ Longitude	Sequence ID	Voucher location	Reference
E18-CR6	BC	Crooked River	18-Jun-14	Baetis vernus Curtis	54.328, –122.669	CREPH018-16	BIO (Univ of Guelph)	Huber et al. 2019
2011215 EYK00	NT	Near Yellowknife	10-Jun-11	Baetis vernus Curtis	62.53, –114.97	KJ675352	ROM (Toronto)	Cordero et al. 2017, Giberson and Burian 2017
BIOUG22893-F05	BC	Canyon Creek	08-Jul-14	Baetis vernus Curtis	54.768, –126.936	MG383307	BIO (Univ of Guelph)	Huber et al. 2019
BIOUG22893-F06	BC	Canyon Creek	08-Jul-14	Baetis vernus Curtis	54.768, –126.936	MG376386	BIO (Univ of Guelph)	Huber et al. 2019
1083318	NT	Harris River	30-Jun-72	Baetis brunneicolor McD.	61.867, –121.317		CNCI (Ottawa)	Giberson and Burian 2017
1083321	NT	Harris River	30-Jun-72	Baetis brunneicolor McD.	61.867, –121.317		CNCI (Ottawa)	Giberson and Burian 2017
1082864	YT	Caribou Bar Creek	14-Aug-73	Baetis brunneicolor McD.	67.467, –140.550		CNCI (Ottawa)	Giberson and Burian 2017
1082878	YT	Caribou Bar Creek	28-Aug-73	Baetis brunneicolor McD.	67.467, –140.550		CNCI (Ottawa)	Giberson and Burian 2017
1082880	YT	Caribou Bar Creek	04-Sep-73	Baetis brunneicolor McD.	67.467, –140.550		CNCI (Ottawa)	Giberson and Burian 2017
1082336	NT	Bosworth Creek	03-Jul-08	Baetis brunneicolor McD.	65.302, –126.675		CNCI (Ottawa)	Giberson and Burian 2017
1082337	NT	Moosehide Creek	03-Jul-08	Baetis brunneicolor McD.	65.266, –126.736		CNCI (Ottawa)	Giberson and Burian 2017
1082563	YT	Seaforth Creek	24-Jul-06	Baetis brunneicolor McD.	60.446, –133.582		CNCI (Ottawa)	Giberson and Burian 2017
-	MB	Churchill and area (loc.22)	8 July-19 Sept 1947–1957	Baetis brunneicolor McD.	58.768, –94.165		CNCI (Ottawa)	Harper and Harper 1981
-	PQ	SEBJ basin de la riviera du castor (loc.33)	8 July-19 Sept 1973–75	Baetis brunneicolor McD.	53.4194, –78.6134		SEBJ / Collection Entomo-logique de U de M	Harper and Harper 1981
2010568 EMO043	ON	Moos-enee	19 July – 26 July 2010	Baetis brunneicolor McD.	51.2792, –80.6615	KJ675138	ROM (Toronto)	Cordero et al. 2017
2010621 ESV011	NL	Labra-dor, near border with Scheffer-ville PC	10-Jul-10	Baetis brunneicolor McD.	54.6684, –66.7636	KJ675281	ROM (Toronto)	Cordero et al. 2017
1082761	YT	Caribou Bar Creek	28-Jul-72	Baetis n.sp. (vernus group)	67.650, –140.733		CNCI (Ottawa)	Giberson and Burian 2017
1082848	YT	Caribou Bar Creek	02-Aug-73	Baetis n.sp. (vernus group)	67.533, –140.583		CNCI (Ottawa)	Giberson and Burian 2017
1082348	NT	Kat Creek	06-Aug-10	Baetis n.sp. (vernus group)	64.940, –127.457		CNCI (Ottawa)	Giberson and Burian 2017
DG 18	NT	Heart Lk/ Butterfly Ck	07-Aug-10	Baetis n.sp. (vernus group)	64.961, –127.563		NEL (NE Ephem.Lab Southern Connecti-cut State U.)	Giberson and Burian 2017

RESULTS AND DISCUSSION

DNA barcodes for *B. vernus* specimens collected by us (Cordero *et al.* 2017; Huber *et al.* 2019) and others from British Columbia and the Northwest Territories were virtually identical to each other and to sequences of *B. vernus* collected in Finland. The sequences were substantially different [much greater than 2% (Zhou et al. 2009; Webb et al. 2012; Cordero et al. 2017)] from other *B. vernus* group members, including group members found in North America (Fig. 1). Morphological examination of the Northwest Territories and Crooked River, BC, specimens revealed traits similar to *B. brunneicolor,* such that the specimens keyed to *B. brunneicolor* in the most recent key to *Baetis* spp. in North America (Wiersema *et al.* 2004, updated with recent couplet patches found in Jacobus *et al.* 2014).

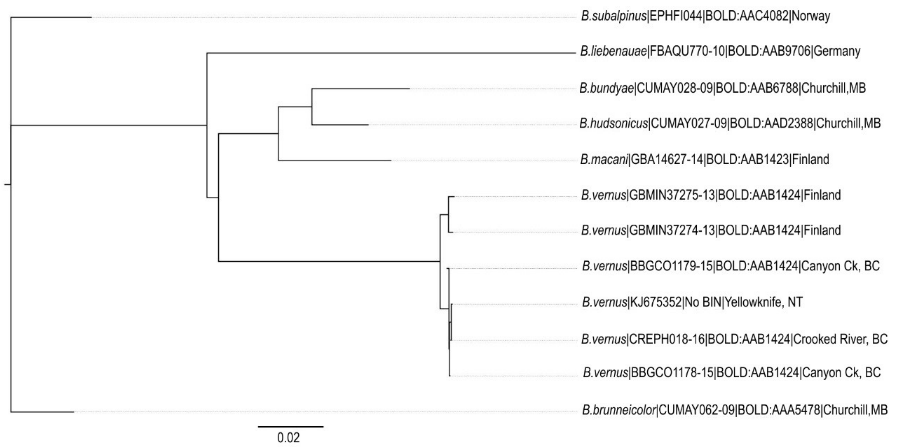

Figure 1: A DNA barcode comparison of Palearctic and Nearctic specimens of *B. vernus* and other closely related baetid species derived from our own collections (Yellowknife and Crooked River *B. vernus* specimens) and from the BOLD database (Ratnasingham and Hebert 2017). The sequences were aligned with Clustal W and visualized with FigTree 1.4.3. Approximate collection locations in Canada and Europe are listed next to each specimen along with that specimen's BIN (Ratnasingham and Hebert 2013). Sequence data are publicly available using the BOLD IDs (most specimens) or NCBI accession number (Yellowknife specimen) associated with each specimen.

These combined results prompted us to look at other *B. vernus* group specimens collected in northwest Canada. These consisted of specimens labeled "*B. brunneicolor*", "*B. bundyae*", and "*Baetis* n. sp. (*vernus* group)" collected from northern Yukon (Porcupine River drainage), southern Yukon (streams along the Alaska Highway), and streams in the Mackenzie Mountains west of the Mackenzie River Valley in the Northwest Territories (Table 1). *Baetis bundyae* and *B. hudsonicus* specimens were usually easy to distinguish from *B. brunneicolor* and *B. vernus* due to the presence of narrow gills on the abdomen. A third group of specimens collected from northern Yukon and from the Mackenzie Mountains west of the Mackenzie River (Table 1) appeared to show characteristics of both groups, with narrow gills like *B. bundyae* but other characters more consistent with *B. vernus.* Due to the age and/or storage conditions of these latter specimens, DNA barcoding was not possible, so the primary concern was distinguishing the larvae of the widespread Nearctic *B. brunneicolor* and the newly discovered *B. vernus*. Morphological features are described for each species below, including a summary of characters that can be used to distinguish the two species in northwestern Canada.

***Baetis brunneicolor* – General Morphological Description of Larva** (Figs. 2–13):

Head: Frons – General shape subtriangular with blunt apex, lateral edges straight or slightly concave (Fig. 2). **Antennae** – Scape and pedicel with many small hair-like setae, no robust setae present. Small hair-like setae seem to be restricted to distal half of scape (Figs. 3a, 3b). No apparent pattern of small setae on pedicel.

Figure 2. *Baetis brunneicolor*: dorsal view of frons.

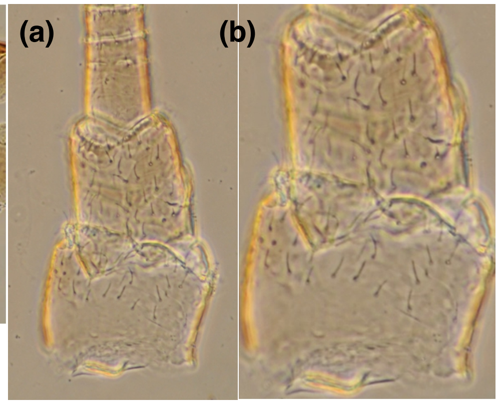

Figure 3. *Baetis brunneicolor*: antennal scape and pedicel; (b) is enlargement of lower section of (a).

Mouthparts: Labrum – Lateral edges tend to be rounded, never appearing straight (Fig. 4a). Dorsal setal pattern 1 long median pair, a gap then row of 4–5 smaller setae (Fig. 4b) extending to edge of anterior margin (i.e., 1 + 4–5). Dorsal surface with many setae, most concentrated near posterior corners. Middle of dorsal surface with somewhat rounded raised area surrounded with small surface setae, no obvious dark marking associated with raised medial area. **Right Mandible** – First tooth of outer incisor larger than second tooth and with squared-off outer edge; second tooth larger than third tooth, and with blunt outer edge; and third tooth smallest of three with rounded outer edge (Fig. 5a, left). This is the "new" condition after moulting, worn teeth are much more similar in size and shape (Fig. 5a, right). Prostheca with pectinate tip, most apical setae about same size and equally spaced (Fig. 5b). **Left Mandible** – One or two small auxiliary teeth present between molar teeth and large apical projection on anterior margin (Fig. 6a). Outer incisor with first tooth slightly larger than second tooth and with squared-off edge; second tooth distinctly larger than third tooth and both teeth with irregularly pointed apices (Fig. 6b, left). This is the "new" condition after moulting, worn teeth are much more similar in size and shape (Fig. 6b, right). **Maxillae –** Four maxillary canines present that lack serrations (Fig. 7a, 7b). Dense brush of long setae along anterior margin of galea-lacinia below canines; margin below setae slightly concave (Fig. 7b). Maxillary palpi two segmented and both segments with many small hair-like setae (Fig. 7a, 7c). Segment 1 of maxillary palpi as long as segment 2. Tips of maxillary palpi extend about one-third of their total length above the tips of canines. **Labium** – Paraglossae broad with mostly straight margins approaching the apices (Fig. 8a, 8b). Apices of paraglossae with 12–15 long setae in two rows. Glossae with broadly pointed apices, ventral surface with single row of about nine long setae located along medial edge (Fig. 8b). Segment 2 of labial palpi with either well developed inner apical lobe and distinctly concaved margin below lobe (Fig. 8a) or moderately developed inner apical lobe and only slightly concave margin below lobe (Fig. 8c).

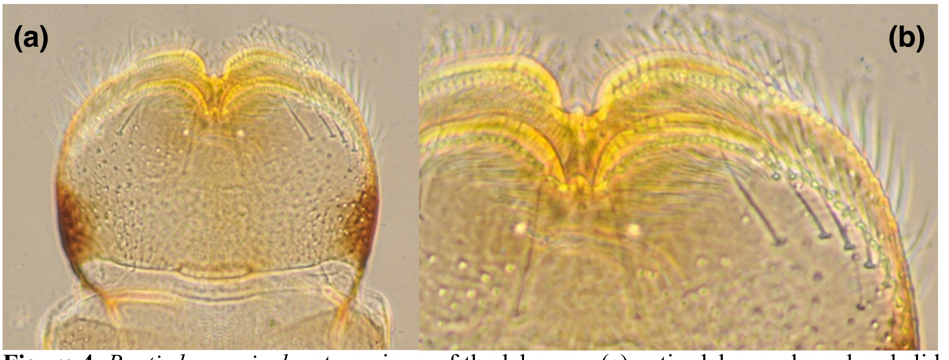

Figure 4. *Baetis brunneicolor*: two views of the labrum – (a) entire labrum cleared and slide-mounted; (b) anterior area enlarged to show setal pattern.

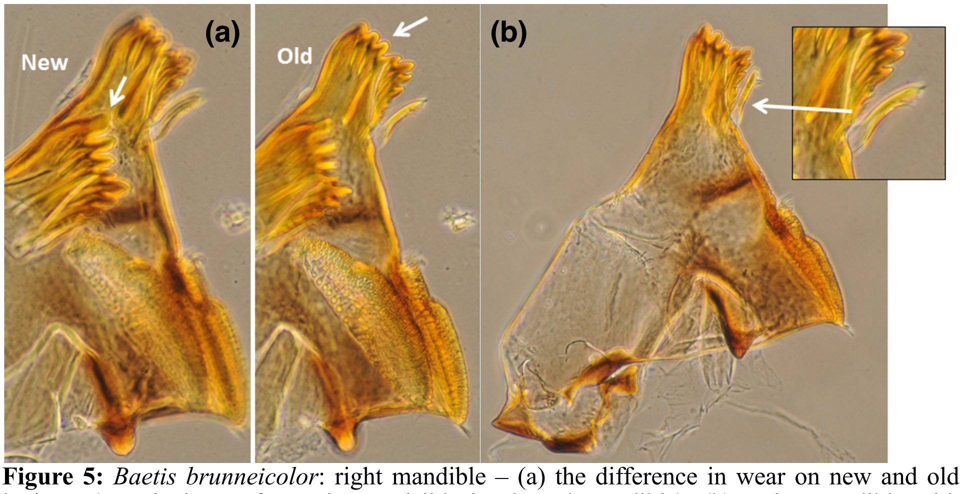

Figure 5: *Baetis brunneicolor*: right mandible – (a) the difference in wear on new and old incisors (new incisors of next instar visible in cleared mandible); (b) entire mandible with prostheca enlarged in inset.

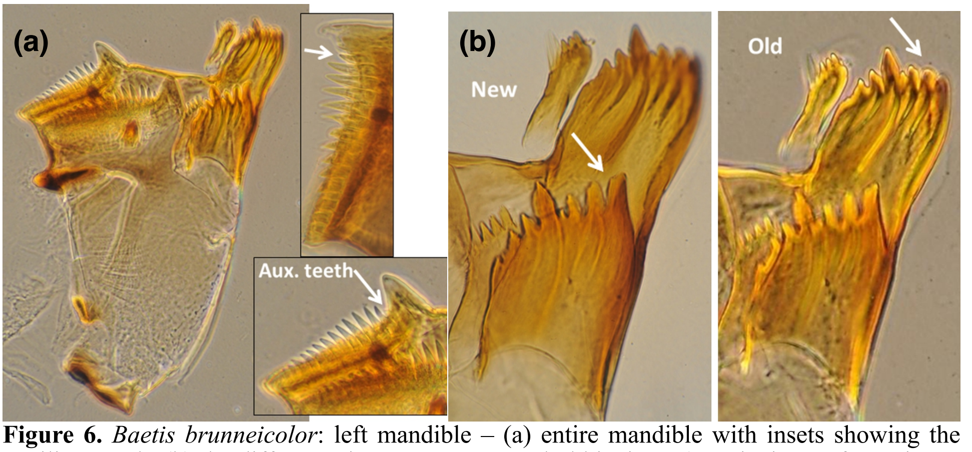

Figure 6. *Baetis brunneicolor*: left mandible – (a) entire mandible with insets showing the auxiliary teeth; (b) the difference in wear on new and old incisors (new incisors of next instar visible in cleared mandible).

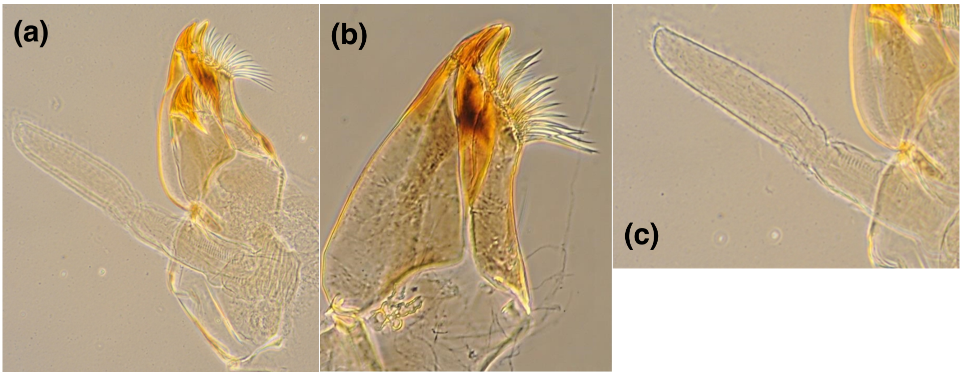

Figure 7. *Baetis brunneicolor*: maxilla – (a) entire maxilla; (b) canines; (c) maxillary palp.

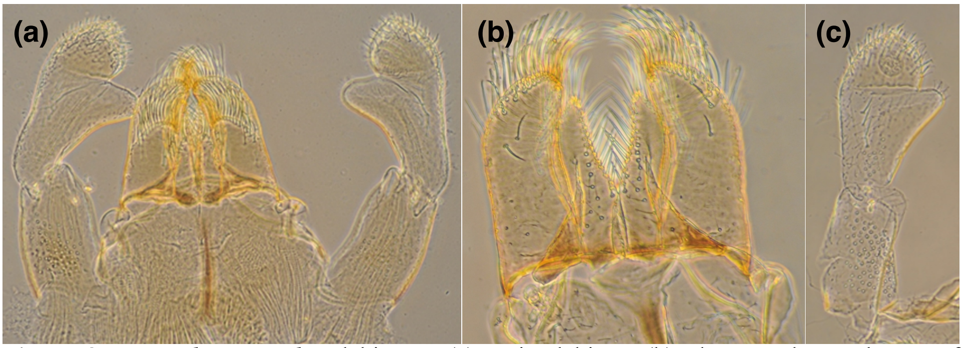

Figure 8. *Baetis brunneicolor*: labium – (a) entire labium; (b) glossa and paraglossae of labium; (c) labial palp.

Forelegs: Femora broadest near midpoint of segment (Fig. 9a). Outer edge with two staggered rows of long, blunt setae that have uniform width from base to tip, setae become more widely spaced and fewer in number near apex of segment (Fig. 9a). Tibia and tarsus generally seem to be much stouter (i.e., wider and shorter) compared to those of *B. vernus* (compare Fig. 9b to Fig. 21b). Foreleg claw with about nine denticles that progressively become larger from base toward apex, apex of claw appears slightly attenuated (i.e., narrowed) (Fig. 9b).

Abdomen: Abdominal Tergite V – Shape typical for abdomen with outer edge of tergite slightly tapering posteriorly (Fig. 10, top). Posterior lateral corners with minimal dark brown colour in fresh specimens at gill insertion. Numerous scale setae present and few scattered hair-like setae between scale setae. Surface with faint cuticular ridges (i.e., weakly grainy) (Fig. 10, bottom). Posterior margins with spinules, but not darkly pigmented compared to rest of tergite (Fig. 10, top). **Abdominal Gills 4 and 5** – Gill 4 larger than gill 5, but both have same basic oval shape with smoothly curved dorsal edge and outer margin (Fig. 11). Both gills have marginal teeth; at 20X magnification gill 4 has about 8 teeth/0.05 mm of edge and gill 5 has 8–9 teeth/0.05 mm of edge. Both gills have distinct central trachea with one or two smaller side branches visible (Figs. 11). Gill 4 length slightly less than twice the width (i.e., width x 1.8=length). Gill 5 has same length/width relationship as gill 4. **Paraprocts –** Inner apical edges with regular, large spines (Fig. 12). Surface with scattered long, hair-like setae that are more or less uniformly distributed over surface (Fig. 12). No other distinctive surface features or textures.

Colour Pattern of Body: Overall body colour uniform brown with less distinct contrasting lighter areas (Fig. 13a). Pronotum lacking bi-lobed brown spots, but large somewhat "c-shaped" diffuse blotches are sometimes present (Fig. 13b). Meso- and metanotum of thorax mostly brown with some lighter streaks and spots, especially on the mesonotum. Abdominal terga with submedian paired brown spots; these are faint on some specimens (Fig. 13a). A medial pale spot with paired lateral pale spots separated by brown background colour seems a common pattern on terga. Posterolateral edges of terga pale compared to brown medial part of terga.

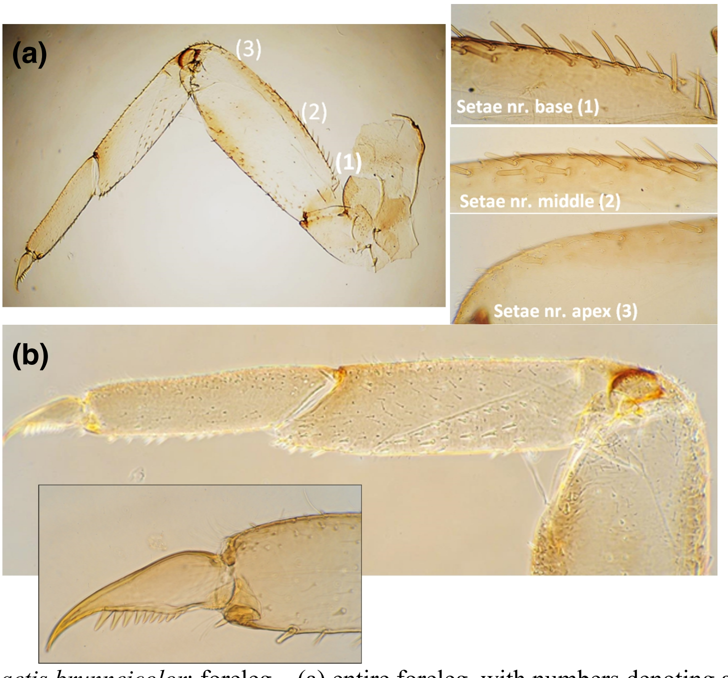

Figure 9. *Baetis brunneicolor*: foreleg – (a) entire foreleg, with numbers denoting areas of the femur with setal patterns shown at right; (b) tibia and tarsus, with inset showing denticles on claw.

General Shape of Abdomen: The overall shape of the abdomen, viewed dorsally, is one of a gradually tapering cylinder that is widest at segment I and narrowest at segment X (Fig. 13a). The shape results from a change in the width/length ration from anterior segments to posterior segments. Segment I is about three times as wide as long and segment X is almost as wide as long.

***Baetis vernus* – General Morphological Description of Larva** (Figs. 14–26):

Head: Frons – General shape subtriangular with blunt apex, lateral edges either straight or slightly concave (without slide mounting intact specimens can even appear slightly convex) (Fig. 14). **Antennae** – Scape and pedicel with many small hair-like setae, no robust setae present. Small hair-like setae seem to be restricted to distal part of scape (Fig. 15). No apparent pattern of small setae on pedicel.

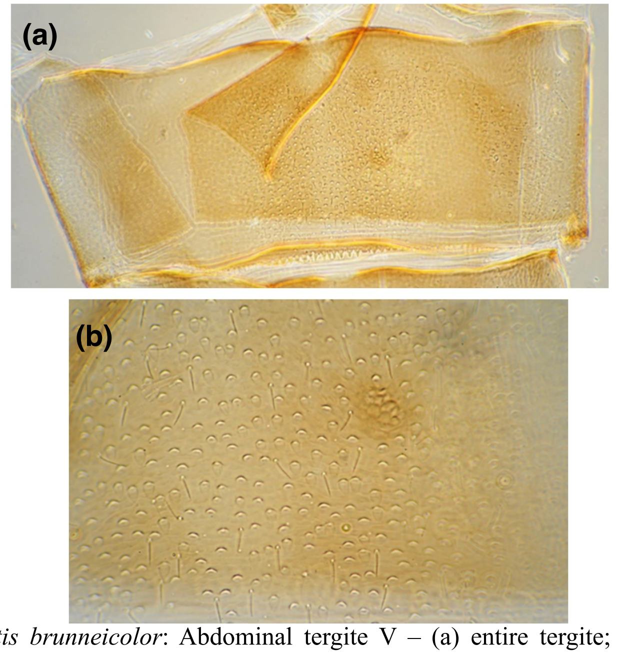

Figure 10. *Baetis brunneicolor*: Abdominal tergite V – (a) entire tergite; (b) enlargement showing cuticular patterns.

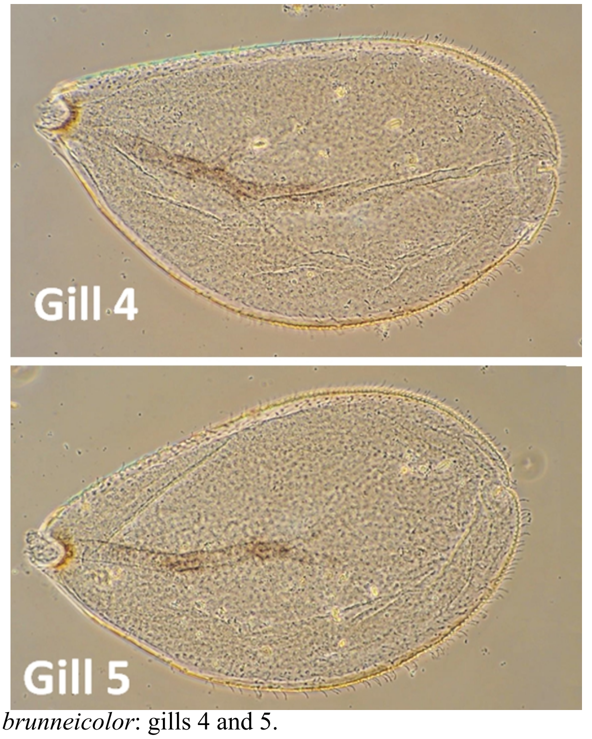

Figure 11. *Baetis brunneicolor*: gills 4 and 5.

Figure 12. *Baetis brunneicolor*: paraprocts, with inset at right showing detail of the lower paraproct at left.

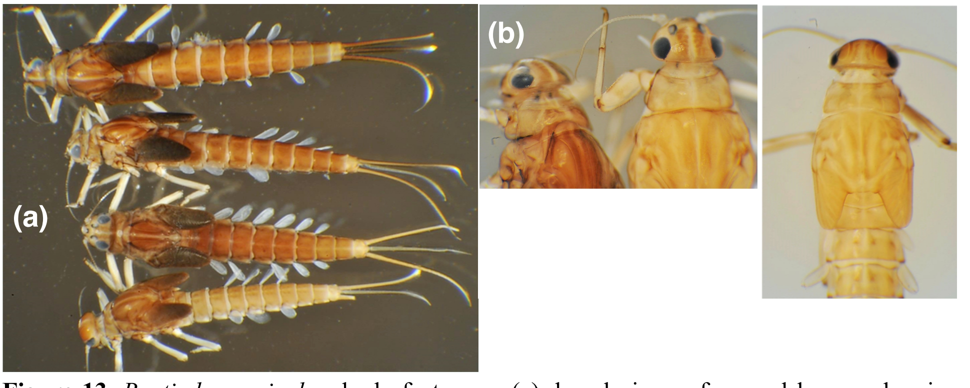

Figure 13. *Baetis brunneicolor*: body features – (a) dorsal views of several larvae, showing colour patterns and body shape; (b) detail of anterior sections of larvae showing colour patterns on thorax.

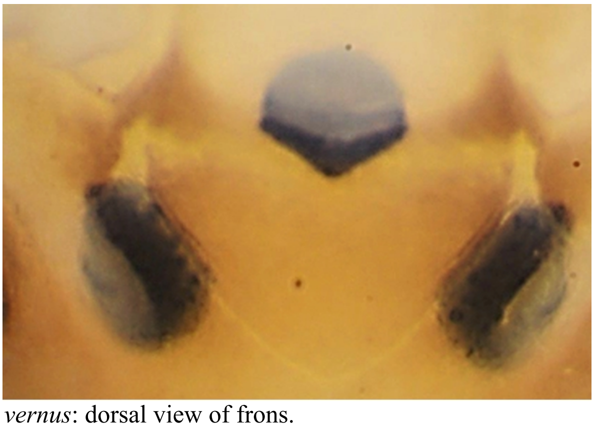

Figure 14. *Baetis vernus*: dorsal view of frons.

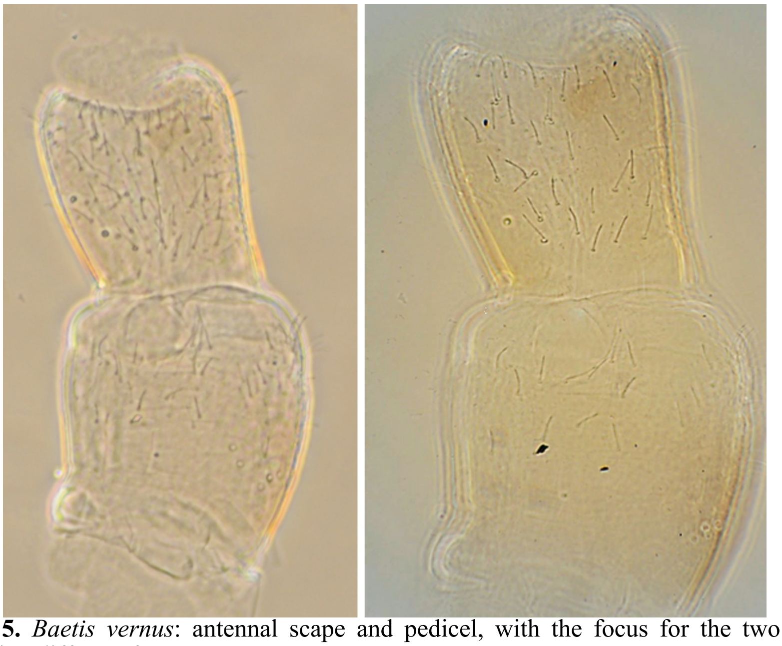

Figure 15. *Baetis vernus*: antennal scape and pedicel, with the focus for the two images highlighting different features.

Mouthparts: Labrum – Lateral edges tend to be straight, or at most slightly rounded (Fig. 16a), making the labrum appear somewhat rectangular. Dorsal setal pattern 1 long medial pair, a gap then row of 3–4 smaller setae (Fig. 16b) extending to edge of anterior margin (i.e., 1 + 3–4). Dorsal surface with relatively few scattered setae, most tend to be concentrated near edges of somewhat triangular raised area that is flanked by dark bands (bands faint on some specimens) (Fig. 16a). **Right Mandible** – First tooth of outer incisor larger than second tooth and with squared-off outer edge; second tooth only slightly larger than third tooth and both with irregularly pointed tips (Fig.17a, left). This is the "new" condition after moulting, worn teeth are much more similar in size and shape (Fig. 17a, right). Prostheca pectinate with a single row of setae along inner edge near apex (Fig. 17b). Setae of variable lengths and some form a cluster near apex of prostheca. **Left Mandible** – One or two small auxiliary teeth present between molar teeth and large apical projection on anterior margin (Fig. 18a). Outer incisor with first tooth slightly larger than second tooth and with squared-off edge; second tooth only slightly larger than third tooth and both teeth with irregularly pointed apices (Fig. 18b, left). This is the "new" condition after moulting, worn teeth are much more similar in size and shape (Fig. 18b, right). **Maxillae –** Four maxillary canines present that lack serrations (Figs. 19a, 19b). A dense brush of long setae along anterior margin of galea-lacinia below canines; margin below setae straight or only slightly concave (Figs. 19a, 19b). Maxillary palpi two segmented and both segments with many small hair-like setae (Fig. 19a). Segment 1 of maxillary palpi as long as segment 2. Tips of maxillary palpi extend about one-third of their total length above the tips of canines (Fig. 19a). **Labium** – Paraglossae with broad curved apices (Figs. 20a, 20b). Apices of paraglossae with 11–12 long setae in two rows (Fig. 20b). Glossae with narrowly pointed apices, ventral surface with single row of about six long setae located along medial edge (Fig. 20b). Segment 2 of labial palpi with moderately developed inner apical lobe and slightly concaved margin below lobe (Fig. 20a).

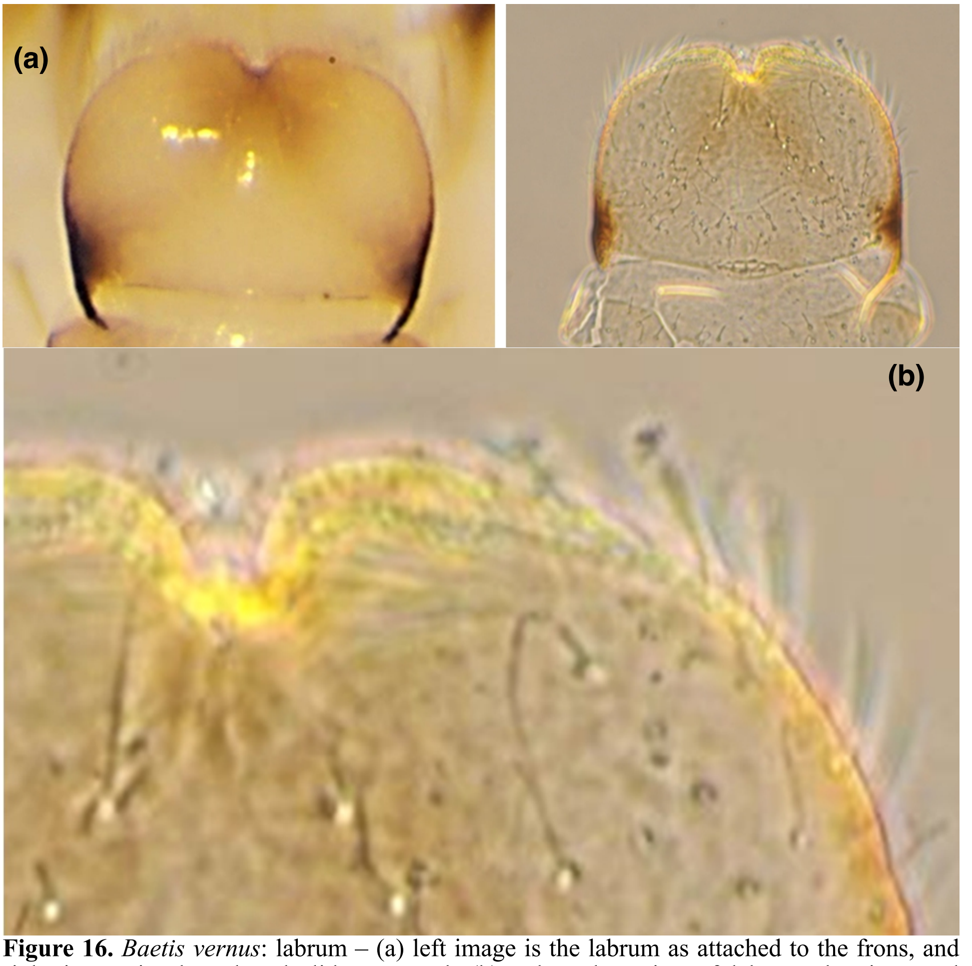

Figure 16. *Baetis vernus*: labrum – (a) left image is the labrum as attached to the frons, and right image is cleared and slide mounted; (b) enlarged portion of labrum showing setal pattern.

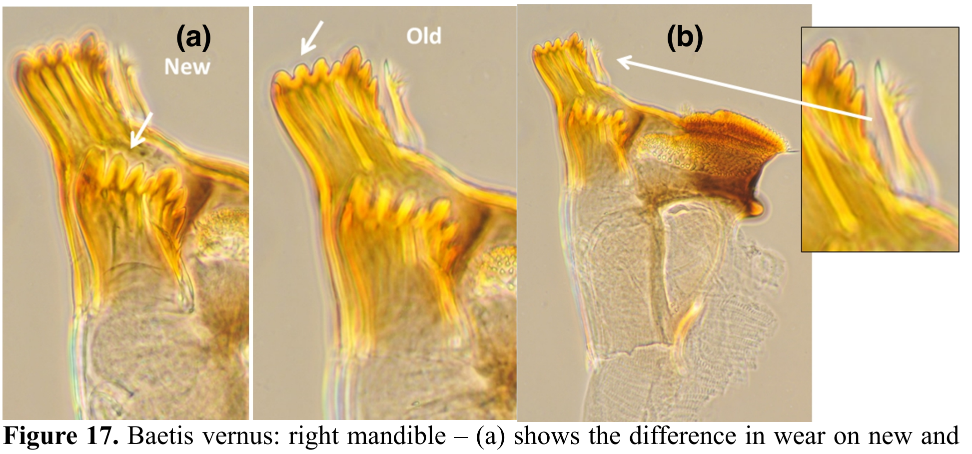

Figure 17. Baetis vernus: right mandible – (a) shows the difference in wear on new and old incisors (new incisors of next instar visible in cleared mandible); (b) entire mandible with inset showing prostheca.

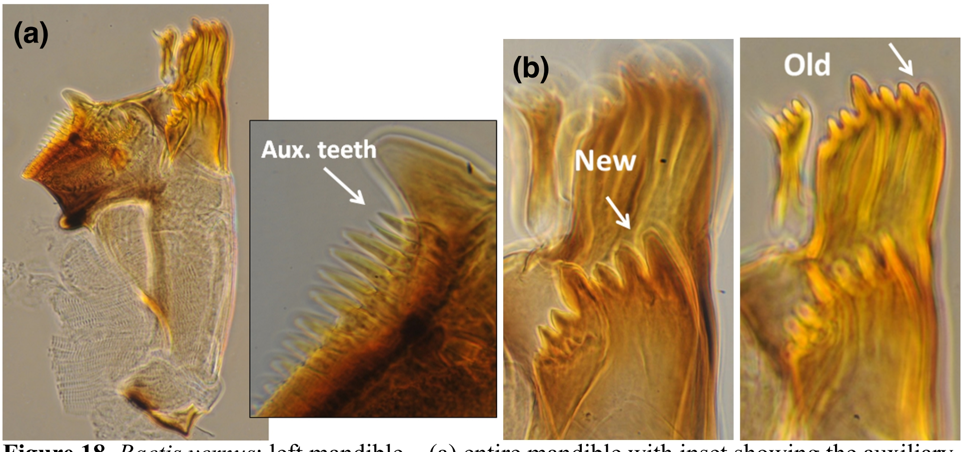

Figure 18. *Baetis vernus*: left mandible – (a) entire mandible with inset showing the auxiliary teeth; (b) the difference in wear on new and old incisors (new incisors of next instar visible in cleared mandible).

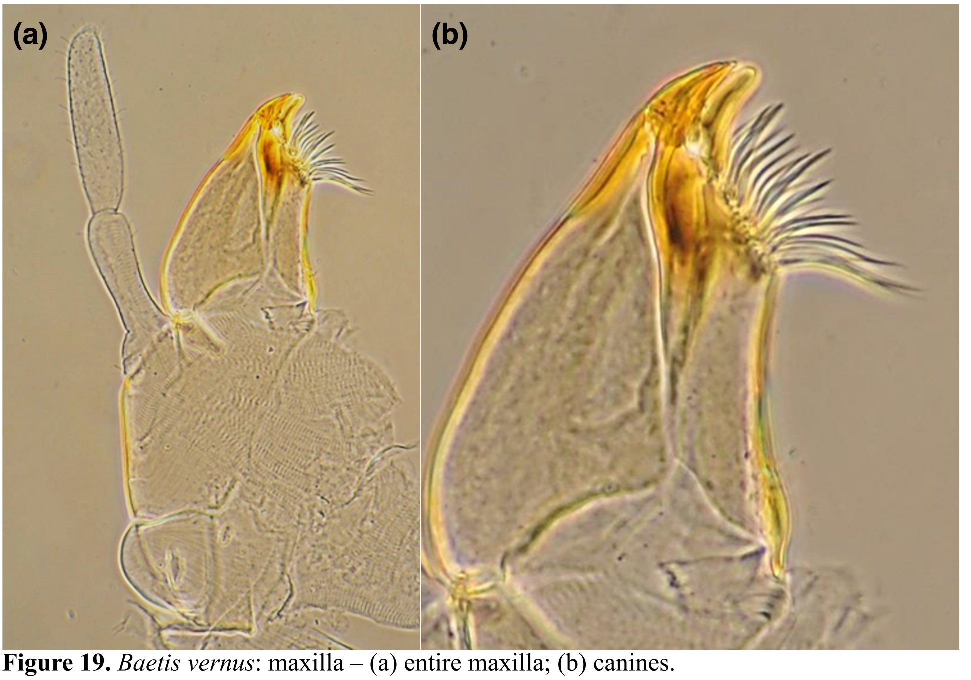

Figure 19. *Baetis vernus*: maxilla – (a) entire maxilla; (b) canines.

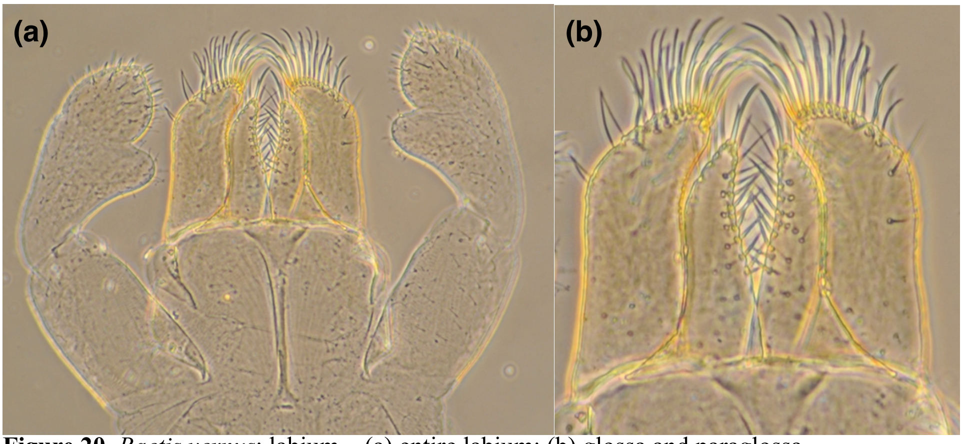

Figure 20. *Baetis vernus*: labium – (a) entire labium; (b) glossa and paraglossa.

Forelegs: Femora about same width from base to apex (Fig. 21a). Outer edge with two staggered rows of long, blunt setae, many of which have narrow bases and broad ends, but some setae have uniform width from base to tip (Fig. 21a). Setae on outer edge of femora are numerous near the base of the segment and gradually become fewer in number approaching joint with tibia, stopping entirely just before the joint with the tibia (Fig. 21a, right hand panels). Tibia and tarsus are thinner and more delicate compared to those of *B. brunneicolor* (compare Fig. 21b to Fig. 9b). Foreclaw with 8–10 denticles, small near base of claw and only gradually become larger toward apex making the row appear more uniform over its length (Fig. 21b). Apex of claw thicker and not noticeably attenuated as in *B. brunneicolor* (compare Figs. 9b and 21b)

Abdomen: Abdominal Tergite V – Shape typical for abdomen with outer edges of tergite nearly parallel (Fig. 22). Posterior lateral corners with distinct dark brown colour at gill insertions (Fig. 22). Numerous scale setae present, but widely spaced over surface of cuticle and with few scattered hair-like setae between scale setae (Fig. 22). Surface with distinct cuticular ridges (i.e., moderately grainy). Posterior margins with spinules, pigmented darker brown compared to lighter brown colour of rest of tergite (Fig. 22). **Abdominal Gills 4 and 5** – Gill 4 is larger than gill 5 and shaped distinctly different shape compared to gill 5 (Fig. 23). Gill 4 more subtriangular with a bulged dorsal margin. Gill 5 closer to sub-oval shape typical of *B. brunneicolor* gills (Fig. 23). Both gills have marginal teeth; at 20X magnification, gill 4 has about 8 teeth/0.05 mm of edge and gill 5 has 8–9 teeth/0.05 mm of edge. Gill 4 has only faint traces of the central trachea and gill 5 has no visible trachea (Fig. 23). Gill 4 almost exactly twice as long as wide. Gill 5 length is slightly less than twice the width. **Paraprocts –** Inner apical edges with irregular row of large spines, which become smaller around the apical corner (Fig. 24). Surface with few scattered hair-like setae and dense cluster of small cuticular scales near outer apical edge (Fig. 24).

Colour Pattern of Body: Overall body colour of Northwest Territories (NT) specimen is much more contrasting compared to the BC specimen (Figs. 25, 26). Generally, body somewhat brown with large pale areas. Pronotum with distinct paired medial brown spots or blotches, lateral edges dark brown, but rest of surface pale (Figs. 25, 26). Thorax with several large pale areas and smaller distinct brown spots or blotches (BC specimen seems closer in thoracic colour patterning to *B. brunneicolor* than NT specimen) (compare Figs. 25, 26 to Fig. 13). Abdominal terga I–IV of NT specimen mostly brown with large paired pale spots and a smaller medial spot (Fig. 25). Tergite V mostly white with limited brown marks at anterior margin and laterally. Terga VI–IX similar in colour to preceding terga. Tergite X white. The BC specimen had a much less-contrasting overall colour pattern but almost the same pattern of marks and spots (Fig.

26). However, tergite VI on the BC specimen was not pale, but patterned similar to other terga. Also, tergite X was uniformly light brown, not pale as in the NT specimen.

General Shape of Abdomen: The overall shape of the abdomen, viewed dorsally, seemed to change more gradually over its length, not appearing distinctly tapered as in *B. brunneicolor* (compare Figs. 25, 26 to Fig. 13). The change in width/length relationship was less per segment, which resulted in the appearance of a more uniformly shaped abdomen. Edges of individual tergites seemed less tapered compared to *B. brunneicolor*. On the BC specimen (Fig. 26), where gills were lost, it was clear that the abdomen did taper from anterior to posterior, segment I was approximately 2.3 times as wide as long. Segment X was slightly wider than long.

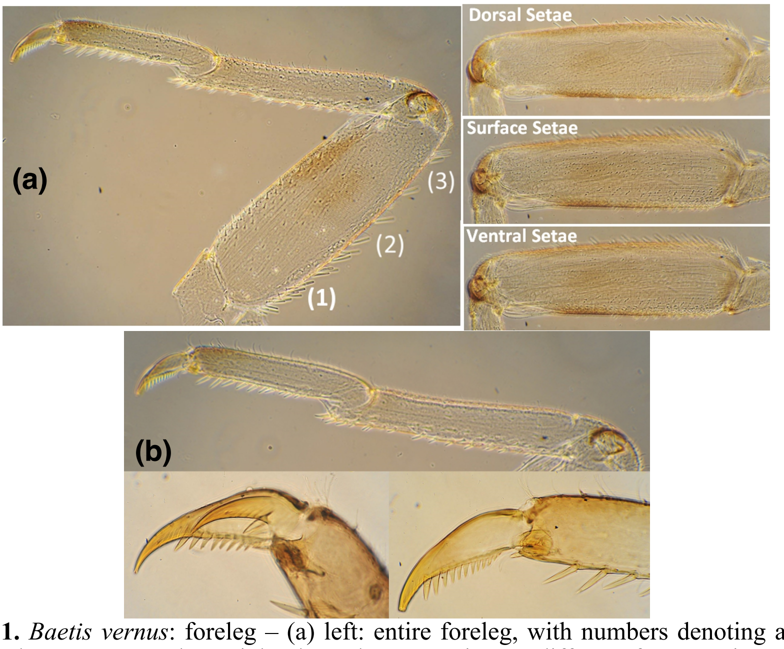

Figure 21. *Baetis vernus*: foreleg – (a) left: entire foreleg, with numbers denoting areas for femur setal patterns; panels at right show the same view at different focus settings to show setal patterns; (b) tibia and tarsus, with insets showing denticles on claw.

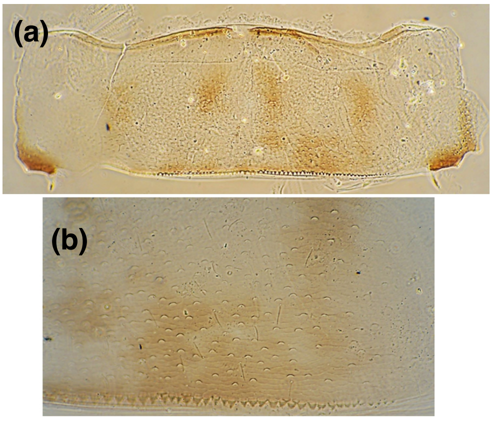

Figure 22. *Baetis vernus*: Abdominal tergite V – (a) entire tergite; (b) enlargement showing cuticular patterns.

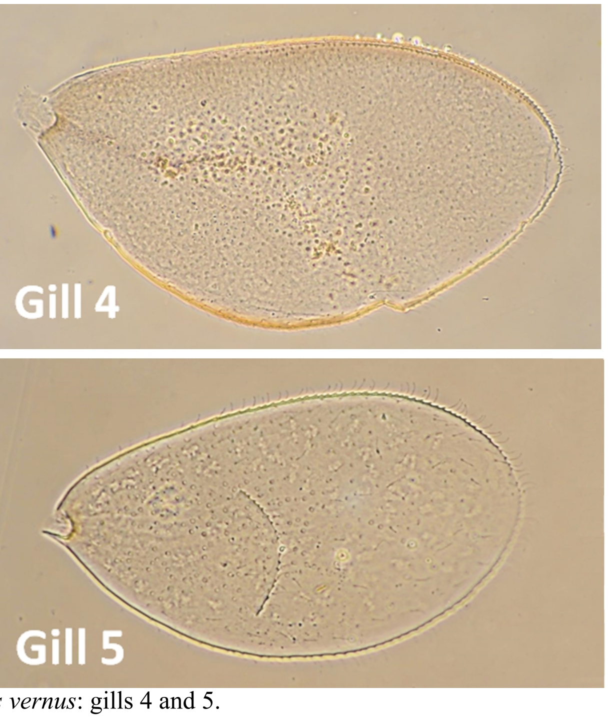

Figure 23. *Baetis vernus*: gills 4 and 5.

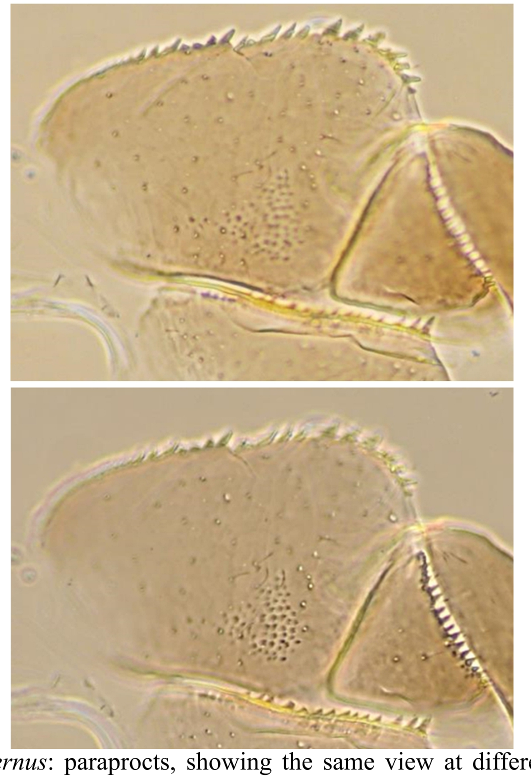

Figure 24. *Baetis vernus*: paraprocts, showing the same view at different focus settings to show setal patterns.

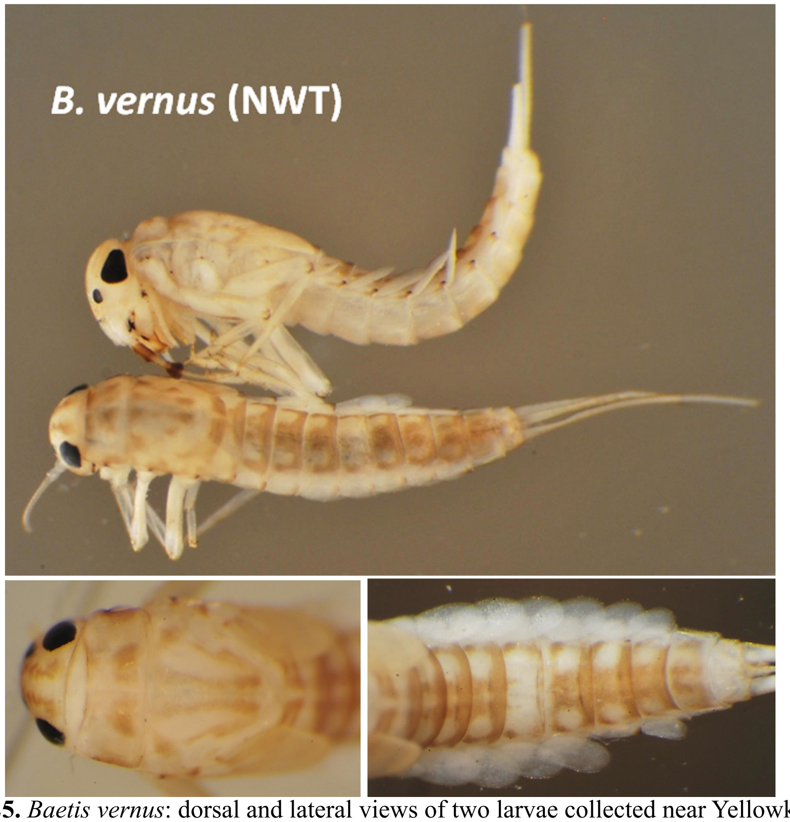

Figure 25. *Baetis vernus*: dorsal and lateral views of two larvae collected near Yellowknife in Northwest Territories, showing colour patterns and body shape.

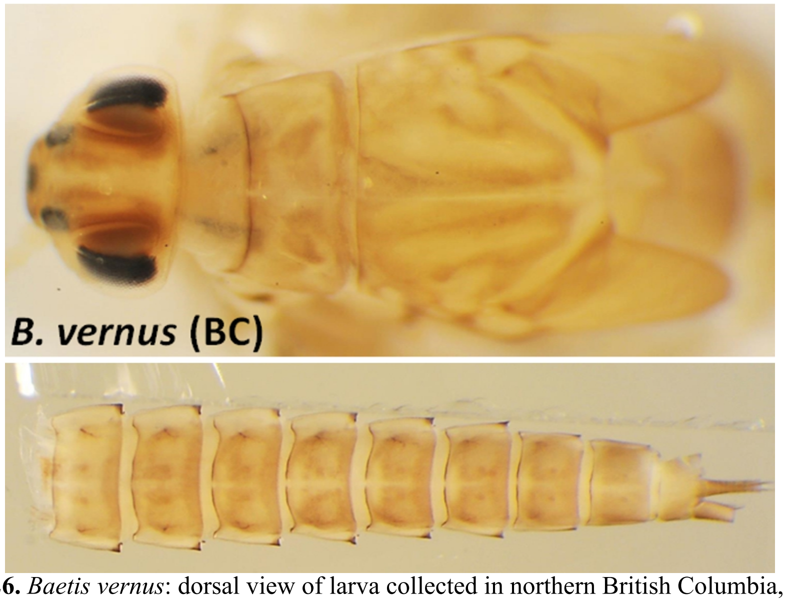

Figure 26. *Baetis vernus*: dorsal view of larva collected in northern British Columbia, showing colour patterns and body shape.

Diagnosis of larvae of *Baetis vernus* group species in North America:

In North America, the *Baetis vernus* group includes: *B. brunneicolor*, *B. bundyae*, *B. hudsonicus*, and *B. vernus*. Larvae of *B. bundyae* and *B. hudsonicus* can be separated from those of *B. brunneicolor* and *B. vernus* by the elongate abdominal gills, which are distinctly longer than twice their width. *Baetis bundyae* can be separated from *B. hudsonicus* by the presence of a short terminal filament, usually much shorter than lengths of adjacent cerci, whereas *B. hudsonicus* has a long terminal filament that is about equal to the length of adjacent cerci (secondarily, populations of *B. hudsonicus* seem to be completely parthenogenetic, no males have been detected).

Larvae of *Baetis vernus* can be separated from those of *B. brunneicolor* by the presence of the following combination of characters:
(1) Dorsal surface of body with distinctive contrasting colour pattern of brown with large pale spots and marks (Fig. 27), especially on abdominal terga, terga V and X mostly pale but other terga brown with large paired pale submedian spots,
(2) General shape of abdomen from dorsal perspective somewhat cylindrical appearing to very gradually taper from segments I to X,
(3) Dorsal surface of labrum with subtriangular raised area flanked by two brown bands that converge medially near base of notch in anterior margin, dorsal setal formula 1+3–4,
(4) Prostheca of right mandible with cluster of setae along inner edge near apex,
(5) Paraglossae of labium with apices distinctly curved inward,
(6) Femora with large blunt setae along outer edge with narrow bases and broad ends,
(7) Foretibia and -tarsus slender,
(8) Foreclaw with 8–10 denticles that gradually enlarge from base of claw toward tip,
(9) Tip of claw not attenuated (i.e., narrowed) beyond denticles,
(10) Abdominal terga with distinctive dark brown shading around gill insertions and spinules along posterior margins dark brown,
(11) Cuticle of abdominal terga moderately grainy with many distinct cuticular ridges among bases of scale setae, and
(12) Abdominal gills 2–4 with only faint traces of the medial trachea and trachea not visible on other gills.

Mature larvae of *B. brunneicolor* can usually be separated from those of *B. vernus* by the presence of the following combination of characters:
(1) Dorsal surface of body relatively uniformly brown, lacking large distinct contrasting pale spots or marks, especially on abdominal terga where some small paired dark marks are present (Fig. 27), Tergite X is usually a uniform light brown on mid-instar larvae but can be pale on black wing pad larvae,
(2) General shape of abdomen from dorsal perspective conical appearing to taper more distinctly from segments I to X,
(3) Dorsal surface of labrum with rounded raised area with no associated dark bands, dorsal setal formula 1+4–5,
(4) Prostheca of right mandible with single row of uniformly spaced setae along inner edge near apex,
(5) Paraglossae of labium with apices straight or only slightly curving inward,
(6) Femora with large blunt setae that usually have uniform width from base to tip,
(7) Foretibia and -tarsus stout,
(8) Foreleg claw with only about nine denticles that appear to change more abruptly in size from base of claw toward tip,
(9) Tip of claw attenuated (i.e., narrowed) beyond denticles,
(10) Abdominal terga with only small areas of brown shading around gill insertions and spinules along posterior margins not darker that rest of surface,
(11) Cuticle of abdominal terga weakly grainy with few widely spaced cuticular ridges among bases of scale setae, and
(12) Abdominal gills 2–6 with distinct medial trachea, lateral trachea also visible on larger gills.

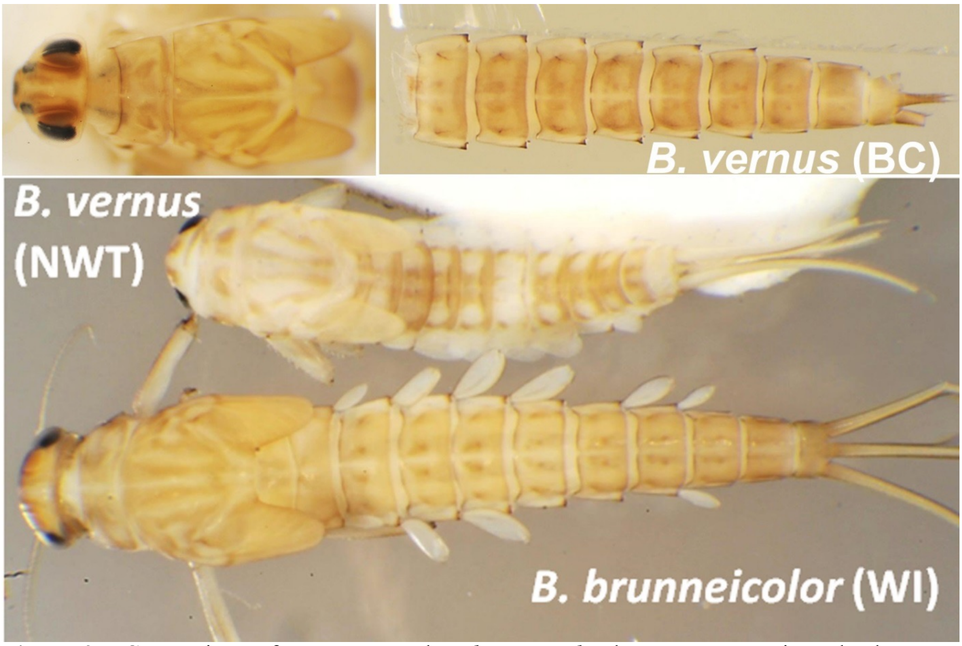

Figure 27. Comparison of *B. vernus* and *B. brunneicolor* larvae at approximately the same stage of development. The *B. vernus* specimens were collected from near Yellowknife in Northwest Territories and from the Crooked River in northern British Columbia, and the *B. brunneicolor* larva is from Wisconsin.

Distribution of *B. vernus* in Canada

The distribution of *B*. vernus in Canada is still unclear, as there are currently only four verified specimens of *B. vernus* from North America. Their distribution ranges from central British Columbia to the south–central Northwest Territories (Fig. 28, Table 1). However, *B. vernus* overlaps in distribution with *B. brunneicolor* (Fig. 28), so some specimens previously identified as *B. brunneicolor* from this region may be *B. vernus*, which could extend the distribution considerably. The specimens mapped in Fig. 4 (and listed in Table 1) were confirmed through comparison with confirmed specimens of *B. brunneicolor* and descriptions of *B. vernus* from the Palearctic. Another potential source of confusion stems from a group of specimens from northern Yukon and the Mackenzie Mountains that show characters of both species and may represent hybrid forms or a new species in the *B. vernus* group (Table 1). We show that a combination of DNA barcoding and morphological examination can resolve the two species, but targeted collecting should occur through northern Canada to obtain specimens for molecular examination to assess distribution patterns for species within the entire *Baetis vernus* group.

Phylogeography

Morphological analyses and DNA barcoding now confirm the Nearctic presence of *B. vernus*, and the locations of the currently known specimens of *B. vernus* indicate a widespread distribution in North America. Such a Holarctic distribution is not surprising as it is analogous to the distribution of *B. bundyae* (Giberson *et al.* 2007; Savolainen *et al.* 2014) and other Ephemeroptera (Kjærstad *et al.* 2012) and because *Baetis* spp. may be particularly pre-adapted for rapid dispersal due to their wing-loading characteristics (Corkum 1987). These results may also imply a widespread northern Asian distribution for *B. vernus* – or a common ancestor of it and other members of the *B. vernus* group – leading to a Beringian dispersal event. Members of the *B. vernus* group seem to be variably tolerant of a range of lentic (standing water) and lotic (running water) habitats

(Bauernfeind and Humpesch 2001; Giberson *et al.* 2007; Savolainen *et al.* 2007; Drotz *et al.* 2012), and differential use of such habitats may be a driver of structured populations or speciation (Drotz *et al.* 2012; Ståhls and Savolainen 2008). From our direct knowledge of collections (Cordero *et al.* 2017; Huber *et al.* 2019) or extrapolation from GPS coordinates (BOLD specimens in this study), the North American *B. vernus* specimens were collected in a range of situations, including a marshy area (Cordero *et al.* 2017), a slow-moving outflow of a lake (Huber *et al.* 2019), and seemingly typical lotic environments (BOLD specimens). *Baetis vernus* in Europe is mostly – but not exclusively – known from lotic systems (Savolainen *et al.* 2007). This seeming ability to reproduce and survive in a variety of habitats may have also aided *B. vernus*' dispersal ability.

DNA barcoding was vital for the initial detection of this species in North America and remains a valuable tool for distinguishing between *B. brunneicolor* and *B. vernus* (as well as potential new species in the group) in northern Canada. Morphological work on these specimens has revealed new questions regarding *B. vernus* group taxonomy and phylogeograhy, and these results highlight the need for substantial further collection of the *B. vernus* group in northern Canada. The growing use of eDNA surveys of likely habitat will be important for extending our knowledge of this and other mayfly species.

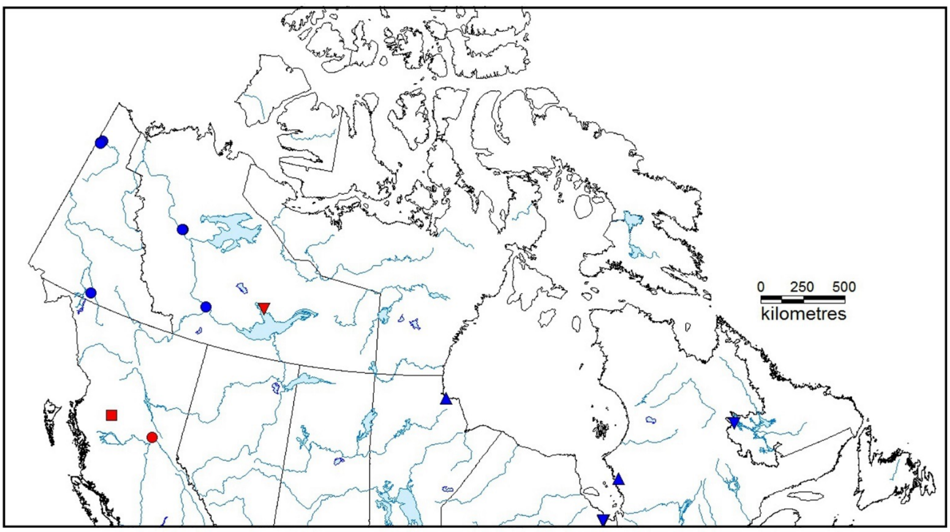

Figure 28: Confirmed record locations for *B. vernus* and *B. brunneicolor* across northern Canada. Red symbols = *B. vernus*, with different symbol shapes denoting different collections [red inverted triangle: Cordero *et al.* (2017); red circle: Huber *et al.* 2019; red square: BOLD-mined *B. vernus* data (two specimens)]. Blue symbols = *B. brunneicolor*, with different symbol shapes denoting different collections [blue circles: Giberson and Burian (2017), confirmed through comparison with eastern *B. brunneicolor* specimens; blue inverted triangles: Cordero *et al.* (2017); blue triangles: adult specimens reported in Harper and Harper 1981].

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