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SCIENTIFIC NOTE

Protoptila coloma (Trichoptera: Glossosomatidae): a new species record for Canada

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Abstract
Northern British Columbia, Canada, is an undersurveyed region for aquatic macroinvertebrates. We surveyed the Stellako River, a culturally and economically important river in the region, for adult caddisflies using Malaise traps, then identified species by using DNA barcoding, which revealed the presence of *Protoptila coloma* Ross (Trichoptera: Glossosomatidae). This is the first record of this species in Canada; it represents a 680-km northwards expansion of the species' currently known range.

The Trichoptera (caddisflies) represent one of the major orders of aquatic insects. Combined with Ephemeroptera (mayflies) and Plecoptera (stoneflies), caddisflies are routinely used in the assessment of ecosystem health (Lenat 1988; Sheffield *et al.* 2019). With the acceleration of anthropogenic climate change, assessing biodiversity is an increasing priority (Parmesan 2006).

Protoptila coloma Ross (Trichoptera: Glossosomatidae) was first described in the mid-twentieth century (Ross 1941). The adult male of this species is only 3 mm long. It belongs to the Protoptilinae (Ross 1963), which is the only subfamily of the Glossosomatidae found in the Neotropics (Robertson and Holzenthal 2013). The distribution of *Protoptila* spp. ranges from Canada to South America. Currently, only four species of *Protoptila* are recorded in Canada, of which only *P. tenebrosa* is previously known from British Columbia, Canada. However, the genus *Protoptila* has been recorded to have much greater diversity in the Neotropics (Wiggins 1996; Blahnik *et al.* 2023). *Protoptila coloma*, which is closely related to *P. tenebrosa* (Ross 1941), has been previously recorded in California, Colorado, Idaho, Montana, Nevada, Oregon, Utah, Washington, and Wyoming, United States of America (Ross 1941; Zack *et al.* 2006; Ruiter *et al.* 2014). More generally, Sheffield *et al.* (2019) estimate a further 8–15 species of Glossosomatidae remain unrecorded in Canada.

The use of DNA barcoding is a well-established approach for identifying insects, often to the species level, and allows for the rapid assessment of biodiversity (Hebert *et al.* 2003; Zhou *et al.* 2009, 2010). The Trichoptera have been extensively identified through DNA barcoding in combination with morphology-based taxonomy (Zhou *et al.* 2009, 2011; Ruiter *et al.* 2013), which makes this group particularly amenable to the barcoding methodology. Generally, a 2% difference in the cytochrome *c* oxidase subunit 1 mitochondrial gene (*CO*1) sequence among caddisflies

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supports the separation of species within the order. The Barcode of Life Database (BOLD) is routinely used for such assessments (Zhou *et al.* 2009; Ruiter *et al.* 2013; Ratnasingham and Hebert 2007, 2013).

The Stellako River, located west of Prince George, British Columbia is in the traditional territory of the Stellat'en First Nation, and it connects Francois Lake (Neda Bun) to Fraser Lake (Nee Tai Bun). The river is ecologically and culturally important and has been impacted by past economic activities (Rajala 2010). Every fall, sockeye salmon migrate into the Stellako to spawn, where they have been harvested by the Stellat'en First Nation for thousands of years (www.stellaten.ca). The Stellako River sockeye population is closely monitored by both the Stellat'en First Nation and Fisheries and Oceans Canada. In addition, the Stellako River and Stellako Lodge on its banks are world-renowned among fly anglers for the rainbow trout that inhabit the river year-round. With the Stellako being a culturally and economically important river, the rainbow trout population is closely monitored and studied by the BC Ministry of Water, Land and Resource Stewardship (Hagen *et al.* 2020).

Juvenile sockeye salmon and rainbow trout in the Stellako rely on aquatic insects as a food source. However, no work has been done on the biodiversity of the aquatic insects in the river. A thorough understanding of the river's macroinvertebrate community is important for informing the substantial and longstanding conservation management efforts along its course (Rajala 2010; Sanderson *et al.* 2015).

We captured insects using SLAM Malaise traps (BugDorm BT1004; MegaView Science Co. Ltd., Taichung, Taiwan) hung from trees along the edge of the Stellako River from May 2023 until September 2023. Malaise traps were set up at the level of the high-water mark. Trap bottles contained 95% (v/v) ethanol, and traps were set at three locations: Glenannan Bridge, 54° 0′ 33.86″ N, 125° 0′ 15.83″ W; Cabin Pool, 54° 0′ 40.27″ N, 124° 59′ 47.40″ W; and Millionaires Pool, 54° 1′ 5.99″ N, 124° 58′ 7.44″ W. Bottles were collected every two weeks. The specimens were sorted based on morphology, and several individuals of each morphotype were submitted for sequencing by the Canadian Centre for DNA Barcoding at the University of Guelph (Guelph, Ontario, Canada). The DNA barcode sequences can be accessed in a BOLD data set (dx.doi.org/10.5883/DS-STELLAKO), and specimens were vouchered at the Royal BC Museum, Victoria, British Columbia.

To determine distribution of *P. coloma,* the following databases were consulted: www.trichoptera.org, https://trichopt.app.clemson.edu/welcome.php, BOLD (http://www.boldsystems.org); 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/); and the Royal BC Museum collections (http://search-collections.royalbcmuseum.bc.ca/Entomology). In addition, the following primary sources were also consulted: Ross 1963; Nimmo 1974, 1977; Schmid 1982; Wymer and Morse 2000; Robertson and Holzenthal 2013; and Genco and Morse 2017.

Initial species identification was based on the 650-bp sequence in the *CO*1 5′ region using the bioinformatic tools within BOLD, followed by phylogenetic analysis using Molecular Evolutionary Genetics Analysis (MEGA), version 11.0, software to set up neighbour-joining trees based on the Kimura-2 model (Tamura *et al.* 2021; Supplementary material, data 1–4). To place our specimens' DNA sequences in context, publicly available DNA sequences were obtained from BOLD for *P. coloma* and *P. tenebrosa* of specimens with barcodes from the nearest geographical location to our specimens.

We captured 25 *Protoptila* spp. adults, of which four were sequenced. The DNA sequence analyses revealed that these four specimens (STRBC123-24, STRBC121-24, STRBC109-24, and STRBC120-24) were *P. coloma* (Fig. 1). Based on the *CO*1 tree analysis of the DNA barcodes, our specimens cluster closely with *P. coloma* from Washington State and Montana but separately from *P. tenebrosa*. *Protoptila tenebrosa* has been identified previously in British Columbia, Alberta, and Manitoba, Canada; however, DNA barcodes for *P. tenebrosa* in Canada are available only from Manitoba. Our analyses showed at least 4% barcode divergence between our specimens and those

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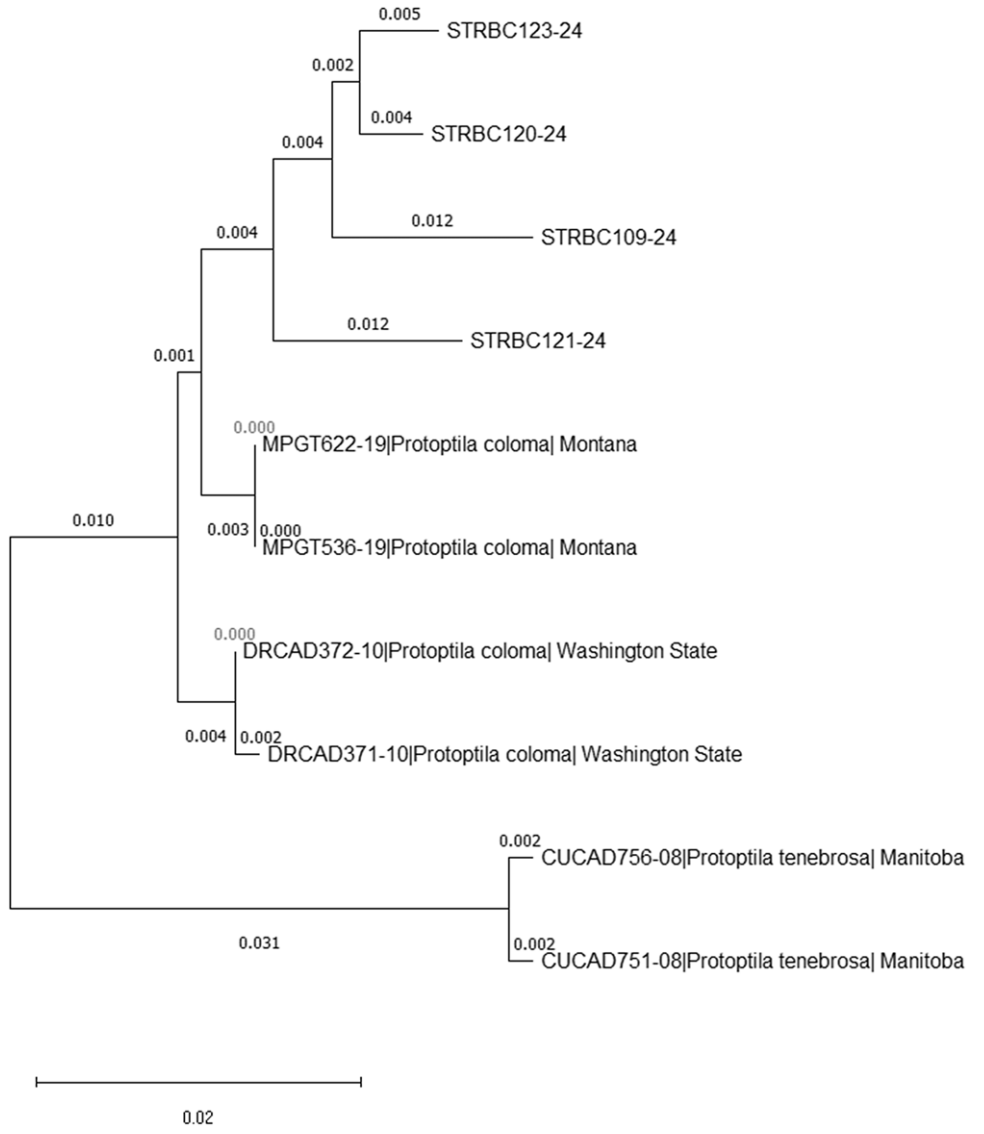

Figure 1. Phylogenetic tree of *Protoptila coloma* from the Stellako River, British Columbia (STRBC123-24, STRBC121-24, STRBC109-24, and STRBC120-24), Montana (MPGT622-19 and MPGT536-16), and Washington State (DRCAD372-10 and DRCAD371-10), and *P. tenebrosa* (CUCAD756-08 and CUCAD751-08) from Manitoba.

of *P. tenebrosa*, supporting the hypothesis that our specimens belong to *P. coloma* and that the specimens collected during the present study represent the first record of *P. coloma* in Canada. This was also confirmed by comparing the male genitalia of our specimens to published keys for *P. coloma* (Ross 1941; Fig. 2).

Protoptila coloma is known to exist in many western states of the United States of the America: California, Colorado, Idaho, Montana, Nevada, Oregon, Utah, Washington, and Wyoming. Currently, only four *Protoptila* species are known in Canada: *P. tenebrosa, P. erotica* (Ross), *P. lega* (Ross) and *P. maculata* (Hagen), of which only *P. tenebrosa* had previously been reported for British Columbia. Our finding adds a fifth *Protoptila* species to Canada and a second to British Columbia.

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Figure 2. Male genitalia (lateral aspect) of *Protoptila coloma* that were collected from the Stellako River, British Columbia.

The low number of Canadian *Protoptila* species is in stark contrast with the neotropics, where much smaller geographic regions such as Panama and Costa Rica contain 15 and 19 *Protoptila* spp., respectively (Blahnik *et al.* 2023). The lack of diversity of *Protoptila* spp. in Canada may be due to Canada's northern geography. The genus prefers warmer streams (Wiggins 1996). However, bioassessment surveys most often use only the larval stage and morphology-based taxonomy, and caddisfly species identification is almost entirely based on the adult stage (Wiggins 2004). It is possible that surveys to sample macroinvertebrates often inadvertently select for the larger caddisfly species because they are easier to collect and identify than extremely small *Protoptila* spp. The larval cases of *Protoptila* spp. are only 4 mm (Wiggins 1996). Such small species can be missed in surveys because they are physically harder to collect and identify, leading to less focus on *Protoptila* spp. and other small trichopteran taxa in Canada. Focused collection efforts in undersurveyed regions, in combination with the use of the substantial reference resources available for the Trichoptera, including highly comprehensive DNA barcode databases such as IBOL and BOLD (Zhou *et al.* 2016), could significantly increase our understanding of Canadian aquatic invertebrate fauna.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.4039/tce.2024.31.

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