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Article *in* The Canadian Entomologist · August 2015

DOI: 10.4039/tce.2014.46

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Extended family: a caddisfly new to Saskatchewan, Canada with notes on the life history of *Neophylax splendens* (Trichoptera: Thremmatidae)

Brittney M. Hoemsen,[1] Iain D. Phillips, Dale W. Parker, Aaron J. Bell, Jordyn A. Bergsveinson, James S. Armstrong, Douglas P. Chivers

Abstract—Streams draining the Cypress Hills support unique and understudied macroinvertebrate communities in Saskatchewan, Canada. Here, we report the discovery of a species of caddisfly new to the Cypress Hills and Saskatchewan, *Neophylax splendens* Denning (Trichoptera: Thremmatidae). Larvae were collected early in May 2012, and are found to enter pre-pupal diapause in mid-June until mid-September. Larvae were identified as *N. splendens* by morphological characters and verified with genetic analysis. Its occurrence strengthens the biogeographical link between the montane regions in British Columbia, Canada and Utah, United States of America with the southwest corner of Saskatchewan. This study highlights the importance of seasonal sampling, resolute species level identifications in biological surveys and the use of genetic analyses to obtain this level of identification.

Introduction

Species-level investigations are invaluable to aquatic ecology studies of community structure, including food web research, biodiversity surveys, and ecosystem health assessments (Rosenberg *et al*. 1986; Lenat and Resh 2001). Although the need for accurate species-level identifications is increasing in the field of aquatic entomology, the number of expert taxonomists is declining (New 1996; Stribling *et al.* 2003). Thus, accurate identifications using morphological characters alone often prove difficult to obtain due to lack of taxonomic expertise, significant variation in regional phenotypes and damaged specimens (Packer *et al.* 2009). Furthermore, aquatic studies often prove challenging because the commonly collected immature stages are difficult to identify to species as many are cryptic and taxonomic species level keys often do not exist and/or are designed for only the male or adult stage.

Cryptic species are indistinguishable morphologically, however, the use of morphological features in conjunction with DNA barcoding can lead to definitive identifications. (Pauls *et al.* 2010; Pramual *et al.* 2011). DNA barcoding (Hebert *et al*. 2003a) is the analysis of a standardised molecular identification system that provides

Received 12 January 2013. Accepted 14 March 2014.

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Subject Editor: Justin Schmidt
doi:10.4039/tce.2014.46

species-level identifications when morphological identification is difficult. Mitochondrial molecular markers are ideal for species level identification because mitochondrial DNA (mtDNA) has a faster mutation rate than nuclear DNA, often revealing genetic differences between species where many nuclear molecular markers would remain identical. The most commonly used molecular marker for DNA barcoding is a short (~650 base pairs) segment of the mitochondrial cytochrome *C* oxidase subunit 1 (COI) gene (Hebert *et al*. 2003b). The COI gene has been used to successfully identify aquatic invertebrates such as springtails (Collembola) (Hogg and Hebert 2004), mosquitoes (Kumar *et al*. 2007), Chironomidae (Diptera) (Pfenninger *et al*. 2007; Sinclair and Gresens 2008), black flies (Diptera: Simuliidae) (Rivera and Currie 2009), mayflies (Ephemeroptera) (Ball *et al*. 2005; Webb *et al*. 2012), and caddisflies (Trichoptera) (Parker 2000; Zhou *et al*. 2009, 2011).

Many areas in Saskatchewan, Canada are inhabited by rare organisms that provide great insight into the biodiversity and ecosystem health of the region (Phillips *et al.* 2008). The Cypress Hills in southwestern Saskatchewan, in particular, supports rich and unique biodiversity that remains understudied. The area is a flat-topped plateau acting as a montane refuge in the prairies for unique species (Phillips *et al.* 2013). Many of these species are deemed pre-glacial relicts (Hilton 1985; Campbell and Peck 1990), anomalies for the prairies (Shorthouse 1991), representative of Rocky Mountain fauna (Russell 1951; Bird 1962; Newsome and Dix 1968), or "Sonoran" in origin (Lehmkuhl 1980), originating from the Colorado system in Utah, United States of America. Focussed studies on such unique ecological systems and taxa, especially those including life history of aquatic insects, are highly valuable to our knowledge of the ecology of streams in the Northern Great Plains (Benke *et al*. 1984).

During a focussed ecological study of Pine Cree Creek in the Cypress Hills we discovered a new family (Thremmatidae) and species of Trichoptera for Saskatchewan, *Neophylax splendens* Denning. Here we describe our methods of combining morphological identification and genetic barcoding to identify *N. splendens,* and provide detailed observations about its life history.

Methods

Description of Pine Cree Creek

Pine Cree (49°36'32.1"N, 108°45'43.0"W) is a first order, spring-fed stream that flows through the Pine Cree Regional Park of the Cypress Hills (altitude = 1070 m; Fig. 1). The riparian zone includes mixed wood forest of white spruce (*Picea glauca* (Moench) Voss (Pinaceae)), lodgepole pine (*Pinus contorta* Douglas ex Loudon (Pinaceae)), trembling aspen (*Populus tremuloides* Michaux (Salicaceae)), and balsam poplar (*Populus balsamifera* Linnaeus (Salicaceae)). The creek has a mean annual specific conductivity of 638 µS (±38 µS), mean pH of 7.02 (±0.80), mean summer temperature of 4.5 °C (May–September) with maximum temperature of 16.4 °C. Riparian health averages 89% and is deemed healthy following Prairie Conservation Action Plan (2008) riparian health assessments.

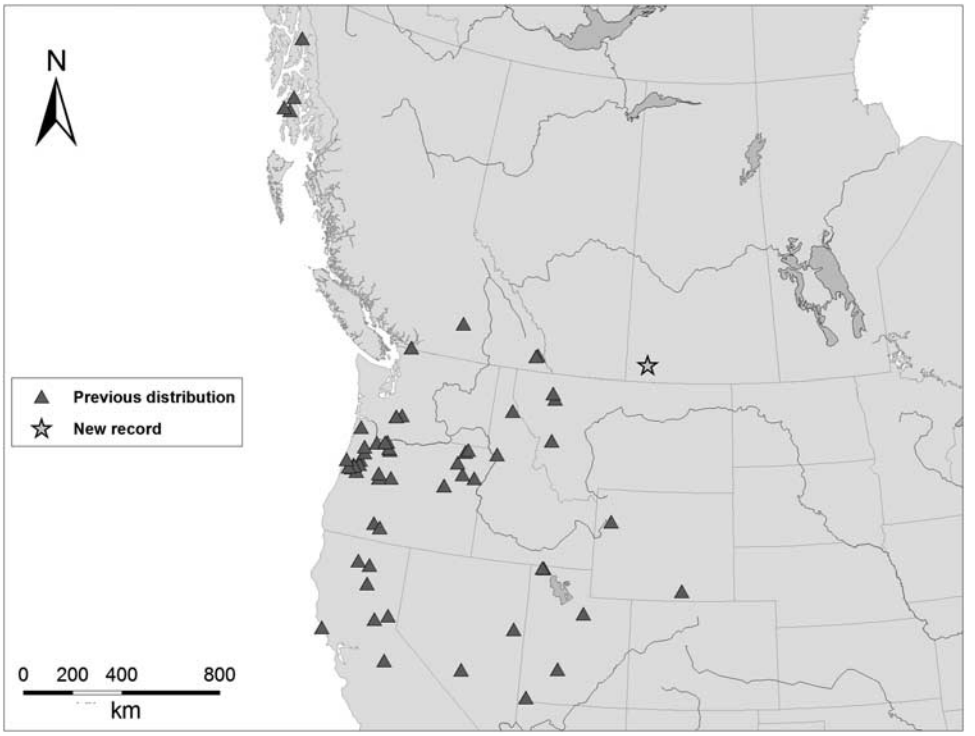
Sampling methods

We have conducted on-going annual fall sampling of Pine Cree Creek and other streams in the Cypress Hills since 2006. A 500 µm mesh size D-frame net was used to sample riffle, run, and pool habitats. The site was visited monthly from May–October 2012, and three samples were collected in each riffle, run, and pool habitat using a 500 µm mesh size Hess sampler. In addition, an emergence trap was suspended over a productive pool habitat upstream of the Pine Cree Regional Park and emptied monthly. Samples were placed in 70% ethanol and sorted under 7× magnification. Specimens were identified using the keys of Clifford (1991), Vineyard (2005), and Merritt *et al*. (2008) and unique taxa were separated for further investigation. Diet was established through dissection of the gut and observation under 100× magnification.

Identification

Third instars (based on case length; Vineyard 2005) were collected in 5 May 2012. Specimens were sent to Michael Floyd at the United States Fish and Wildlife Service for verification and species identification. However, as the specimens were early instars with key characters including the setae on the submarginal row indiscernible, species-level identification was inconclusive (M. Floyd, United States Fish and Wildlife Service, personal communication). We therefore conducted genetic analysis

Fig. 1. Known distribution of *Neophylax splendens* (triangle markers) showing new record of the species from Pine Cree Creek, Saskatchewan, Canada (star marker). Records obtained through Barcode of Life Database (BOLD) (http://www.boldsystems.org) and Vineyard (2005).



to complement morphological characters for species determination (Flint and Kjer 2011). Specimens were selected for genetic analysis based on the following family and generic characters: long basal spurs on the tarsal claws, reduced metathorax Sa1 and sclerotised mesothoracic plate (Wiggins *et al.* 1985; Vineyard and Wiggins 1988; Vshivkova *et al.* 2007).

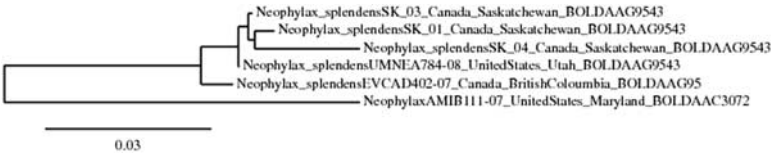
A single leg was removed from three selected specimens for DNA extraction and purification using DNeasy® Blood & Tissue Kit (Qiagen, Toronto, Ontario, Canada). Using the extracted DNA as a template, a 658 base pair region of the mitochondrial cytochrome oxidase I (COI) gene was targeted and amplified using two primers, IDT LCO1490 (5′-GGT CAA CAA ATC ATA AAG AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA – 3′). The polymerase chain reaction products were purified using ExoSAP-IT® procedure, and sequenced at the National Research Council Canada, Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada. The DNA sequences were entered into the Barcode of Life Database (BOLD) (Ratnasingham and Hebert 2007) for taxonomic identification. All 191 *Neophylax* sequences in the BOLD database as of 15 January 2014 were downloaded as FASTA files and included in the analysis. Sequences were aligned using MUSCLE alignment (Edgar 2004) and TreeDyn (Dereeper *et al.* 2008, 2010) was used to generate a neighbour-joining tree.

Larval voucher specimens are deposited in collections at the Royal Saskatchewan Museum, Regina, Saskatchewan, Canada, and the Water Security Agency of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Results and discussion

Larvae of *Neophylax splendens* were collected at Pine Cree Regional Park in the Cypress Hills, Saskatchewan, Canada 6 May 2012 ($n = 3$) and 6 June 2012 ($n = 17$) by I.D.P., B.M.H., and A.J.B. Thus, representing the first record of the family

Fig. 2. Neighbour-joining tree depicting relationships between *Neophylax* specimens publicly available on Barcode of Life Database (BOLD) (BIN: BOLD:AAG9543) (http://www.boldsystems.org). Specimens recorded from Saskatchewan, Canada are new to the database. Species names are followed by their BOLD process ID number, the country and state in which they are recorded, and the BOLD BIN number. The scale bar indicates the length of a branch estimated to have experienced 0.03 substitutions per site.



Thremmatidae in Saskatchewan and extending the known distribution of this family over 500 km east of the Rocky Mountains (Fig. 1). Prepupal aggregations were observed 3 October 2012. No adult specimens were found in emergence traps nor through active searching in early October.

Morphological features identified the specimens as *Neophylax* species and COI sequences confirmed *N. splendens* (over 99.4% similarity) using the BOLD (http://www.boldsystem.org) with BIN: BOLD:AAG9543. Specimen and locality information are also available on GenBank (http://www.ncbi.nlm.nih.gov/genbank/) under the accession numbers KF367633–KF367635. Publicly available sequences within the same cluster as our specimens were used to generate a neighbour-joining tree (Fig. 2).

Life cycle

Neophylax splendens is typically found in small, first order streams at high elevations (Vineyard 2005), similar to Pine Cree Creek, Saskatchewan. All species of *Neophylax* are univoltine, hatching from eggs in early spring and emerging as adults in late fall (Vineyard 2005). In Pine Cree Creek, this caddisfly appears to have peak larval abundance in early June then enters pre-pupal diapause from late August until early October. A long diapause can allow larvae to avoid high summer temperatures, synchronise emergence (Corbet 1964; Masaki 1980), take advantage of increased food availability in fall (Beam and Wiggins 1987), or defer oviposition until the height of the drought period is over (Ross 1944). Pre-pupal aggregations were found on the undersides of rocks or debris in Pine Cree Creek in late September. These aggregations allow for avoidance of predation and parasitism (Otto and Svensson 1981) and can facilitate colonisation of other invertebrates (McCabe and Gotelli 2003). Pupation typically extends from two to six weeks with shorter emergence in cooler waters (Beam and Wiggins 1987). Adults generally emerge synchronously in early fall (Vineyard 2005). At Pine Cree Creek, no adults were collected in emergence traps, suggesting emergence was later than 3 October.

Diet

Larvae were collected in aggregations under woody debris and rocks in riffle habitat. Gut content analysis of *N. splendens* larvae from Pine Cree Creek revealed diatoms, periphyton, and fine organic particles of aufwuchs. Mandibles of *Neophylax* are simple scrapers with a brush of 10–15 long, slender setae along the mesal edge that serve to brush small particles into the mouth (Slack 1936). This supports their classification as obligate grazers (Sedell 1971; Cummins 1973).

Stream insect community

Many other Trichoptera were collected from Pine Cree Creek at the same time as *N. splendens*, including representatives of *Glossosoma* Curtis (Glossossomatidae), *Rhyacophila* Pictet (Rhyacophila), *Hydropsyche* Pictet (Hydropsychidae), *Lepidostoma* Rambur (Lepidostomatidae), *Onocosmoecus* Banks (Limnephilidae), *Hesperophylax* Banks (Limnephilidae), and *Cheumatopsyche* Wallengren (Hydropsychidae). These genera also have limited distributions in the northern and southern boreal region of Saskatchewan (Smith 1984). *Neophylax rickeri* and *N. splendens* are sister species (Vineyard 2005) and are typically found in sympatry but distributed along a longitudinal gradient, with *N. splendens* occurring upstream of *N. rickeri* (Vineyard 2005; Mendez and Resh 2008). However, we did not detect *N. rickeri* in our samples.

Distribution

Neophylax splendens is widely distributed in western North America, from southeastern Alaska to southern California and extending east in the Rocky Mountains into western Colorado and southcentral Wyoming (Fig. 1). It is the most common species of *Neophylax* in western North America but has not previously been recorded in the central part of the continent (Houghton *et al.* 2001). Much of the work on Trichoptera in Saskatchewan has focussed on the boreal streams and the North and South Saskatchewan Rivers (Smith 1975, 1984). No previous records of this species currently exist for Saskatchewan (Smith 1975); Royal Saskatchewan Museum collection; Water Security Agency of Saskatchewan voucher collection; AquaTax Consulting (Dale Parker, personal communication).

Neophylax splendens is typically found in cool, fast flowing streams (Vineyard 2005). The restricted locality of *N. splendens* in Saskatchewan could be due to the exceptional water quality of Pine Cree Creek and unique characteristics of the Cypress Hills, including high elevation. Based on tolerance values for the northwest, *N. splendens* has a tolerance value of 3 (Barbour *et al.* 1999) indicating their sensitivity to human disturbance. Elevation could be the driver of the localised distribution of caddisflies (Williams 1991) in Pine Cree Creek, as it is at comparable elevations to the Utah, United States of America and British Columbia, Canada records (2040 and 1122 m, respectively). The occurrence of this species in the Cypress Hills strengthens the biogeographical link with montane regions such as Utah and British Columbia population. This supports Lehmkuhl's (1980) hypothesis of the insect fauna in the Cypress Hills being similar to montane regions such as Utah, forming the "Sonoran Connection".

Pine Cree Creek has been sampled each fall since 2006 as part of Saskatchewan's biomonitoring programme (Davies and Hanley 2010). Because this coincides with pre-pupal diapause or emergence time, this may explain why *N. splendens* has not been recorded to date. In the interests of expanding our knowledge of biodiversity in Saskatchewan, monitoring efforts should incorporate smaller order lotic systems. Currently, springs are understudied in Saskatchewan as sampling efforts focus largely on studying higher order systems due to their economical importance. However, it is imperative to study the springs from which these rivers originate to better understand freshwater systems as a whole (Danks and Williams 1991). These smaller order streams can support rare and sensitive species, as we have shown with *N. splendens*. This species is adapted to cold springs and information relating to its life history and distribution is especially valuable in studies of biomonitoring and ecosystem health.

Conclusion

This study highlights the value of species-level identification in biological surveys and the use of genetic analysis in obtaining this taxonomic resolution. It also illustrates the inadequacy of annual, single season/date, sampling regimes to assess stream biodiversity.

Acknowledgements

The authors are grateful for the support provided by Joan Hodgins at Pine Cree Regional Park and the expertise provided by Michael Floyd, Christy Geraci, and Xin Zhou. The Saskatchewan Wildlife Federation, Saskatchewan Summer Student Works, Natural Science and Engineering Research Council of Canada (NSERC) scholarships to B.M.H. and I.D.P., RBC Blue Water Group, EcoFriendly Sask EcoAction Grant, and the Entomological Society of Saskatchewan provided funding that supported this research. Furthermore, they would like to thank two anonymous reviewers for their comments on earlier drafts of this manuscript.

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