Zootaxa 3866 (1): 131–137
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3866.1.7
http://zoobank.org/urn:lsid:zoobank.org:pub:99869EF6-062A-46AA-A417-BD25F729741F

Discovery of new populations and DNA barcoding of the Arapahoe snowfly A*rsapnia arapahoe* (Plecoptera: Capniidae)

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Abstract

The Arapahoe Snowfly, *Arsapnia arapahoe* (Nelson & Kondratieff) was recently discovered in six different first-order streams outside of the Cache la Poudre River Basin where it was previously considered endemic. Specimens of *A. arapahoe* were always collected in much lower relative abundance, 1.09% (±2.3SD), than other sympatric adult capniids. The first mitochondrial deoxyribonucleic acid (DNA) barcodes for *A. arapahoe* and *A. coyote* (Nelson & Baumann) are presented and compared with those of *A. decepta.* DNA barcoding was not able to differentiate between *A. arapahoe* and *A. decepta* Banks but it was able to indicate that *A. coyote* is specifically distinct.

Key words: stoneflies, aquatic insects, *Arsapnia*, *Capnia arapahoe*, COI, DNA barcoding, integrative taxonomy

Introduction

Aquatic ecosystems in the Rocky Mountains have experienced human alterations since the middle 1800's (Kenney 2010, Wohl 2006). Climate change constitutes additional threats that are predicted to modify temperatures and precipitation patterns resulting in altered stream thermal and hydrologic regimes that may impact aquatic species distributions, abundances, diversity and community function (Hauer et al. 1997, Meyer et al. 1999, Malmqvist & Rundle 2002, Kenney 2010). Consequently, it is imperative to document and monitor present-day distributions of aquatic macroinvertebrate assemblages and ecologically important aquatic species for future management of these systems.

The snowflies (Plecoptera: Capniidae) (Stark et al. 2012) are an ecologically important group of aquatic insects. Adults can serve as a food source for terrestrial predators during winter and early spring in temperate regions when few other groups of arthropods are present in riparian areas. Some snowfly species are widespread and occur in various stream conditions while other closely related species are highly localized and apparently have very specific habitat requirements. Their biology is poorly understood since much of their life cycle is spent as larvae in the hyporheic zone (Stanford & Ward 1993). Moreover, many snowfly larvae are presently indistinguishable morphologically for even skilled taxonomists (Stewart & Stark 2002).

The Arapahoe Snowfly, *Arsapnia arapahoe* (Nelson & Kondratieff 1988) was described from two adult males collected in 1986 and 1987 and placed in the *Capnia decepta* species group sensu Nelson & Baumann (1989). Those specimens were taken from Elkhorn Creek and Young Gulch, two first-order tributaries of the Cache la Poudre River along the Front Range of northern Colorado (Nelson & Kondratieff 1988). Despite repeated sampling at the type locality, Elkhorn Creek, and several other streams in and around the Cache la Poudre drainage, this species was not reported again until March 2009 when the female was collected for the first time in addition to a series of males (Heinold & Kondratieff 2010). Heinold & Kondratieff (2010) described the female allotype from those specimens.

The Coyote Snowfly, *Arsapnia coyote* (Nelson & Baumann), is known only from Los Angeles and San

Accepted by B. Kondratieff: 18 Aug. 2014; published: 22 Sept. 2014

conducting a search to document the distribution of *A. arapahoe* in other Front Range streams of northern Colorado.

Arsapnia coyote is known only from the San Gabriel Mountains of Los Angeles and San Bernardino Counties in southern California. Although not occurring in the same streams, *A. decepta* is known to the south and east of these localities (Nelson & Baumann 1987).

Barcode sequences were not able to differentiate between male specimens of *A. arapahoe* and *A. decepta* available on BOLD (Ratnasingham & Hebert 2007) despite distinct morphological differences in male genitalia traditionally used to separate Plecoptera species. This leads us to consider a variety of alternative hypotheses including: 1) ongoing hybridization between *A. arapahoe* and *A. decepta*, 2) recent divergence of these two lineages accompanied by incomplete lineage sorting, 3) *Wolbachia* infection, or 4) variation among these specimens is simply polymorphism and these populations collectively represent one biological species. We propose that logically we can rule out hypotheses 1, 3, and 4 because no intermediate morphs of these species are found (ruling out hybridization), the BOLD database detects *Wolbachia* and our records have not been flagged, and the stark contrast in morphology of the genitalia among these taxa exceeds that deemed acceptable by morphological taxonomists as the same species of Plecoptera.

The DNA barcode for the single *A. arapahoe* female matched that of *C. wanica* but was morphologically different. Both known females of *A. arapahoe* lack the distinctive Y-shaped sclerite that is present on sternum seven of the *C. wanica* female and curiously, the female *A. arapahoe* specimen used for DNA sequencing appeared to have nine abdominal segments instead of 10 like other capniids. Wing venation and the subgenital plate indistinctly resembled *C. wanica*. Consequently, we still consider the *A. arapahoe* female to be morphologically distinct from other sympatric capniids and the original description of the female of *A. arapahoe* by Heinold & Kondratieff (2010) to be valid.

The failure of DNA barcoding to distinguish between two related species, *A. decepta* and *A. arapahoe*, that have morphologically dissimilar male genitalia, prompted the question if it could distinguish between species with morphologically similar male genitalia, *A. decepta* and *A. coyote*. Barcoding was able to distinguish between these taxa. Alternatively, the haplotypes were almost the same for some of our specimens of *A. arapahoe* and *A. decepta.*

Understanding the discrepancies between morphology and DNA barcode sequences among these species require further investigation. We suggest a rigorous phylogenetic study incorporating both mitochondrial and nuclear markers to resolve the aforementioned complexities. Acquiring live individuals to observe mate selection may help strengthen our understanding of the morphology, distribution, and genetic relationships among these taxa.

Acknowledgements

GDPE provided money from a small grant for sequencing. Molecular work was supported by NSF DEB "Dimensions of Biodiversity" Award 1046408 to Poff et al., Colorado State University's Graduate Degree Program in Ecology and an NSF Graduate Research Fellowship to B.A. Gill. We thank Dr. Boris Kondratieff for reviews of early manuscripts, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins.

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