



Stoneflies in the genus *Lednia* (Plecoptera: Nemouridae): sentinels of climate change impacts on mountain stream biodiversity

Matthew D. Green¹ · Lusha M. Tronstad² · J. Joseph Giersch³ · Alisha A. Shah⁴ · Candace E. Fallon⁵ · Emilie Blevins⁵ · Taylor R. Kai⁶ · Clint C. Muhlfeld^{3,7} · Debra S. Finn⁸ · Scott Hotaling⁹

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Abstract

Rapid recession of glaciers and snowfields is threatening the habitats of cold-water biodiversity worldwide. In many ice-sourced headwaters of western North America, stoneflies in the genus *Lednia* (Plecoptera: Nemouridae) are a prominent member of the invertebrate community. With a broad distribution in mountain streams and close ties to declining glacier cover, *Lednia* has emerged as a sentinel of climate change threats to high-elevation aquatic biodiversity. *Lednia tumana*, which is endemic to Glacier National Park, USA and the surrounding mountains, is the most well-studied species in the genus and in 2019 became federally protected under the U.S. Endangered Species Act (ESA) due to climate-induced loss of meltwater habitats. Three other *Lednia* species have also been described, and like *L. tumana*, each is endemic to a mountain region of western North America: *Lednia sierra* in the Sierra Nevada, *Lednia borealis* in the Cascade Range, and *Lednia tetonica* in the Teton Range. In this review, we provide a comprehensive overview of *Lednia* ecology, genetics, and physiology, with an emphasis on the conservation outlook for the group and species with similar headwater distributions. We highlight substantial progress made in the last decade to better understand the ecology and evolution of *Lednia*, including the identification of 140 *Lednia*-containing streams (an increase from 12 streams in 2010), and a more complete understanding of the degree to which warming streams may imperil species in the genus. In light of the ESA listing of *L. tumana*, we show that similar conservation threats likely face all extant *Lednia* species. However, substantial gaps in our knowledge remain, primarily centering around their distributions (and the potential for as yet undescribed species), life history, ecophysiology, and trophic ecology. We conclude by describing pressing questions for *Lednia* that when addressed will expand knowledge of the genus and its conservation as well as broader understanding of climate risks to mountain stream biodiversity worldwide.

Keywords Alpine stream · Glacier recession · Endemic · Meltwater stonefly · Global change · Stream ecology

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Extended author information available on the last page of the article

Introduction

Climate change is having a multi-faceted impact on alpine habitats worldwide. For alpine freshwaters, receding glaciers and snowfields (Rauscher et al. 2008; Pederson et al. 2010) along with changing precipitation regimes (Herbst and Cooper 2010) are altering hydrological regimes, species distributions, the availability of basal resources, and threatening the existence of habitats and species (Hotaling et al. 2017). Alpine streams harbor substantial biodiversity due to their unique spatial position in the uppermost branches within river networks and high environmental heterogeneity (Finn et al. 2011). However, despite the importance of alpine streams as water resources and the vulnerability of these ecosystems to climate change, biodiversity in the highest, coldest headwaters is poorly known. In North America, stoneflies in the genus *Lednia* are sentinels of climate impacts on headwater ecosystems due to their affinity for high-elevation coldwater streams. In this review, we provide an overview of *Lednia* research with an emphasis on the progress that has been made in the last ~ 10 years. Throughout, we highlight how *Lednia* can act as a conservation surrogate for issues facing mountain stream species worldwide.

The genus *Lednia*: past and present

Since its first description from a glacial stream in Glacier National Park (GNP), USA, *Lednia tumana* (Ricker, 1952) and congeneric species have remained a largely overlooked component of alpine aquatic fauna. The last decade, however, has been marked by a surge of *Lednia* research with the description of three new species, *Lednia borealis*, *Lednia tetonica*, and *Lednia sierra* (Baumann and Kondratieff, 2010; Baumann and Call, 2012). Since their discovery and description, subsequent research has established links between glacier cover and *Lednia* distributions (Muhlfeld et al. 2011; Giersch et al. 2017), examined physiological tolerances (Treanor et al. 2013; Hotaling et al. 2020a, 2021a), and resulted in the first genetic perspectives for the genus (Hotaling et al. 2019b, c). Alongside this progress, and no doubt one of its drivers, has been the increased attention paid to *L. tumana* as a harbinger of climate-induced habitat loss (Rumsey et al. 2003; Stagliano et al. 2007; Muhlfeld et al. 2011; Giersch et al. 2017). In November 2019, *L. tumana* was federally listed as threatened under the U.S. Endangered Species Act due to habitat loss that is primarily being driven by declining stream flows and rising water temperatures (US Fish and Wildlife Service 2019).

Though *Lednia* research has greatly expanded in recent years, three points should be acknowledged when discussing this progress. First, aside from morphology-based systematics and species distributions, nearly all *Lednia* research has stemmed from studies of *L. tumana* (but see Hotaling et al. 2019b, 2020b; Fallon et al. in press). While much has been learned from these efforts, better understanding of the ecological similarities between *L. tumana* and other *Lednia* species will shed important light on how knowledge gained from *L. tumana* can be applied to other species in the genus. Second, the existing *Lednia* taxonomy is based on morphology and phylogenetic insight from a single marker (*cytochrome oxidase I*, Hotaling et al. 2019b). A more robust phylogenomic perspective on interspecies relationships within *Lednia* and the family Nemouridae is needed to give context to the deeper relationships revealed by a recent phylogenomic study of Plecoptera in North America (South et al. 2021). This is particularly relevant given the potential for cryptic

species diversity to be present even when morphological variation is lacking (e.g., Leache and Fujita 2010; Hotaling et al. 2016; Hime et al. 2016). Third, the number of discovered *Lednia* populations has increased dramatically during the past decade, and this expansion has been driven primarily by increases in sampling effort. Continued research and monitoring efforts will undoubtedly reveal additional *Lednia* localities and perhaps new species.

Systematics

In 1952, Ricker described a new subgenus of *Nemoura*, and the first species within the subgenus, *Nemoura tumana* (Ricker 1952), which is now recognized as *Lednia tumana*. The circumstances of the collection of the type material—a misty day near a glacier-fed stream in GNP—inspired the species' etymology (*Lednia* from the Russian “led” meaning “ice,” and *tumana* from the Russian “túmán” meaning “mist,” Ricker 1992). Later revision of Plecoptera elevated *Lednia* to a genus (Illies 1966). A second *L. tumana* locality from GNP was described by Gaufin et al. (1972) and in Baumann's (1975) revision of the Nemouridae, a cladistic analysis of morphological characters placed *Lednia* as the sister taxon to *Visoka*. Soon after, *L. tumana* was documented in Waterton Lakes National Park (WNP), Alberta, representing the first record from Canada (Donald and Anderson 1977). Decades later, Kondratieff and Lechleitner (2002) reported a significant range extension of *L. tumana* to Mount Rainier National Park, Washington, USA. However, these specimens were later confirmed to be a new species—*L. borealis*—as were specimens from the Sierra Nevada, California, USA—*L. sierra* (Baumann and Kondratieff 2010). During the same year, a putative fossil *Lednia* species—*Lednia zilli*—was described from an inclusion in Eocene Baltic amber collected in present-day Poland (Caruso and Wichard 2010). Two years later, the fourth extant species—*L. tetonica*—was described from the Teton Range, Wyoming, USA (Baumann and Call 2012).

Phylogenetics, morphology, and the fossil record

The common ancestors of stoneflies diverged from other insect groups (e.g., the clade containing Orthoptera, Dictyoptera, and allies) ~275–350 million years before present (ybp) near the boundary of the Permian and Pennsylvanian periods (Béthoux et al. 2011; Misof et al. 2014; Tong et al. 2015). Within Plecoptera, molecular analyses have placed Nemouridae as sister to the small winter stoneflies (Capniidae; Terry 2004; South et al. 2021). Within Nemouridae, *Lednia* is closely related to *Podmosta* and *Prostoia* (South et al. 2021). Nymphs and adults of extant *Lednia* are small, darkly pigmented, and roughly 4.5–7.5 mm long (Fig. 1a–c). Species are differentiated in the adult stage by morphological differences in the male epiproct (Ricker 1952; Baumann and Call 2012). External gills are absent in *Lednia* nymphs (Baumann and Kondratieff 2010).

Differentiating *Lednia* from most other nemourids as adults can be done by assessing the 9th abdominal sternite as only two nemourid genera, *Lednia* and *Paranemoura*, lack the vesicle of the 9th abdominal sternite. Adults of these two genera can then be differentiated by wing venation. For *Lednia*, the terminal costal crossvein Sc2 joins radius R1, whereas in *Paranemoura* Sc2 joins Sc1 (Caruso and Wichard 2010). However, since *Lednia* and *Paranemoura* species have yet to be included in the same molecular phylogeny, it remains unclear if this diagnostic character is indicative of shared ancestry or has occurred independently in both groups.

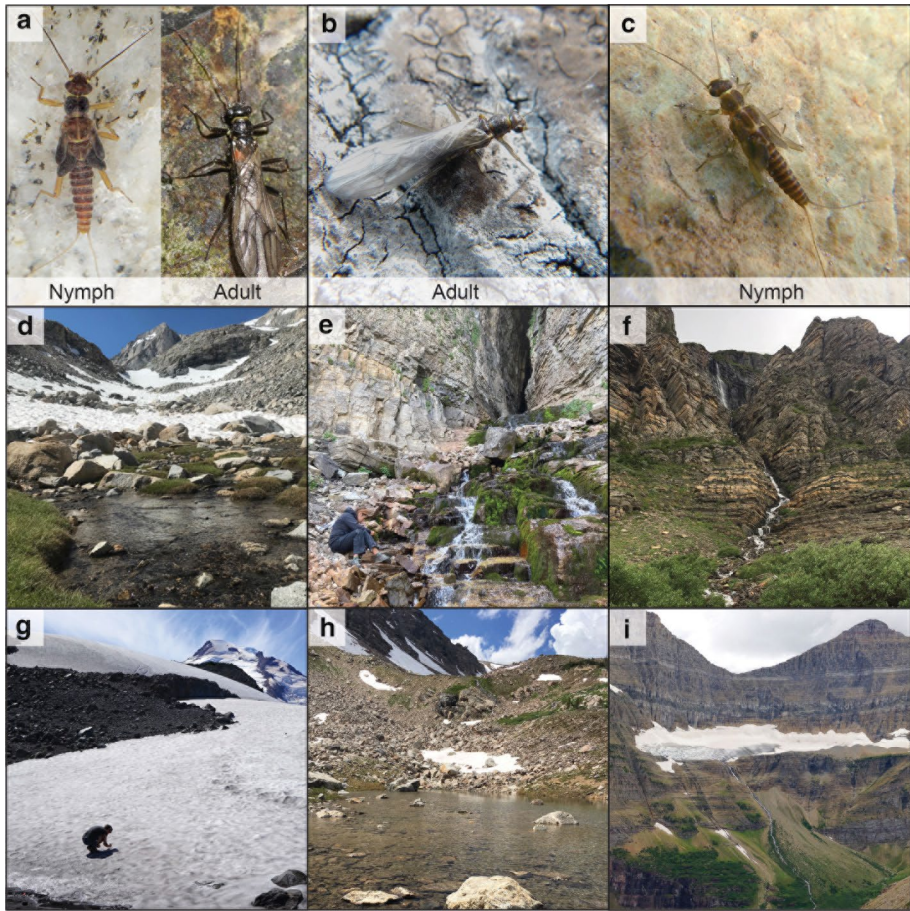


Fig. 1 Examples of *Lednia* and the high-elevation habitats they reside in. **a–c** Nymphs and adults across species (**a** *Lednia tetonica*, **b–c** *Lednia tumana*). **d** A snowmelt-fed stream, Rock Creek, in the high Sierra Nevada, California, USA, hosts a population of *Lednia sierra*. **e** A stream fed by subterranean ice emanating from Wind Cave in the Teton Range, Wyoming, USA, and the type locality for *L. tetonica*. **f** A snowmelt-fed stream in Glacier National Park, Montana, USA containing *L. tumana*. **g** A researcher collecting *Lednia borealis* adults emerging from a meltwater seep on the margins of the Sholes Glacier, Washington, USA. **h** A meltwater pond unofficially referred to as “Tetonica Pond” where a population of *L. tetonica* resides in Grand Teton National Park, Wyoming, USA. **i** A meltwater stream emerging from the Sexton Glacier (center of the photo) in Glacier National Park, Montana, USA. Despite harsh conditions, *L. tumana* are dominant members of the non-Dipteran aquatic invertebrate community in this stream (Giersch et al. 2017). Photos by: **a–c** Joe Giersch, **d** Matthew Green, **e, f, h, i** Scott Hotaling, and **g** Emilie Blevins

The putative fossil *L. zilli* specimen identified by Caruso and Wichard (2010) bore the characteristic 9th abdominal sternite (lack of a vesicle) and wing venation characteristic (the terminal costal crossvein Sc2 joins radius R1) of *Lednia* but differed significantly in features of the male epiproct from *L. tumana*, the only *Lednia* species at the time of description (Caruso and Wichard 2010). Given that all extant *Lednia* are known from western North America, the description of a European species from ~34 to 56 million ybp in the Eocene is striking and suggests the genus may have historically been much more widely

distributed. Alternatively, however, the Caruso and Wichard (2010) specimen, despite similar morphology, may not represent a species of *Lednia*. It is plausible, and perhaps likely, that rather than *Lednia* once spanning a Nearctic and Palearctic distribution, the *L. zilli* specimen may have belonged to a common ancestor of *Lednia* and similar Palearctic species. However, without more fossil evidence, identification of contemporary *Lednia* outside of North America, or further study using advanced technologies (e.g., X-ray microtomography, Pepinelli and Currie 2017), the placement of *L. zilli* in *Lednia* should remain tentative.

Geographic distributions

Lednia species appear to be restricted to cold streams in mountainous areas of western North America. All known populations are in the contiguous United States (USA) with the exception of one *L. borealis* population in central British Columbia, Canada, and a *L. tumana* population in southern Alberta, Canada, just north of the core range for the species in GNP (Fig. 2, Table S1; Donald and Anderson 1977). Across all locations, the number of streams containing each species are: *L. tumana* ($N=111$), *L. tetonica* ($N=16$), *L. borealis* ($N=17$), and *L. sierra* ($N=8$; Fig. 2; Tables S1). Prior to 2010, only 12 streams were known to harbor *Lednia* and three species would eventually be described from these localities (*L. borealis*, *L. sierra*, *L. tumana*). In the years since, 140 new *Lednia* populations and one new species (*L. tetonica*) have been documented (Fig. 3). Beyond increases in population number, expanded search efforts have broadened the geographic range of each species, most notably for *L. borealis* and *L. tumana*. For *L. borealis*, new records from Washington, USA, in the Alpine Lakes and Glacier Peak Wildernesses filled in distributional gaps between Mount Rainier and North Cascades National Parks (Fallon et al. in press; Fig. 2). The identification of a new *L. borealis* population from central British Columbia in this study (see “Molecular ecology and genomics” below) represents an ~600 km northern expansion for the species and the first record outside of the United States. Recently discovered populations have reconfirmed *L. tumana*’s presence in Waterton Lakes National Park in Canada, as well as expanded the known distribution in GNP while also extending its range by ~45 km to the south (Fig. 2; Giersch et al. 2017).

Generally speaking, the discovery of new *Lednia* species and populations has followed a consistent rule: the greater the search effort, the more populations have been discovered (Fig. 3). In light of climate-induced alteration of headwater streams, there is a pressing need for dedicated surveys of potential habitat. New efforts should focus on regions known to contain small numbers of *Lednia* populations (e.g., Sierra Nevada, California, USA; Alpine Lakes and Glacier Peak Wildernesses, Washington, USA) as well as mountain ranges within the broader distribution of *Lednia* that have not been surveyed. Likely candidates include: the Wallowa Mountains (northeastern Oregon, USA), the Olympic Range (western Washington, USA), the Sawtooth Range (central Idaho, USA), the Cabinet Mountains (western Montana, USA), and the Wind River Range (central Wyoming, USA). However, with the discovery of *L. borealis* in central British Columbia, perhaps the most pressing areas to survey are the Canadian Rockies and Coast Mountains in western Canada.

When possible, future surveys should leverage emerging ecological tools. For instance, species distribution models that incorporate environmental conditions known to affect *Lednia* populations (e.g., distance from source and stream temperature, Giersch et al. 2017) with remote sensing data could be used to inform survey efforts on a range-by-range basis. The publicly accessible Google Earth platform is a powerful and

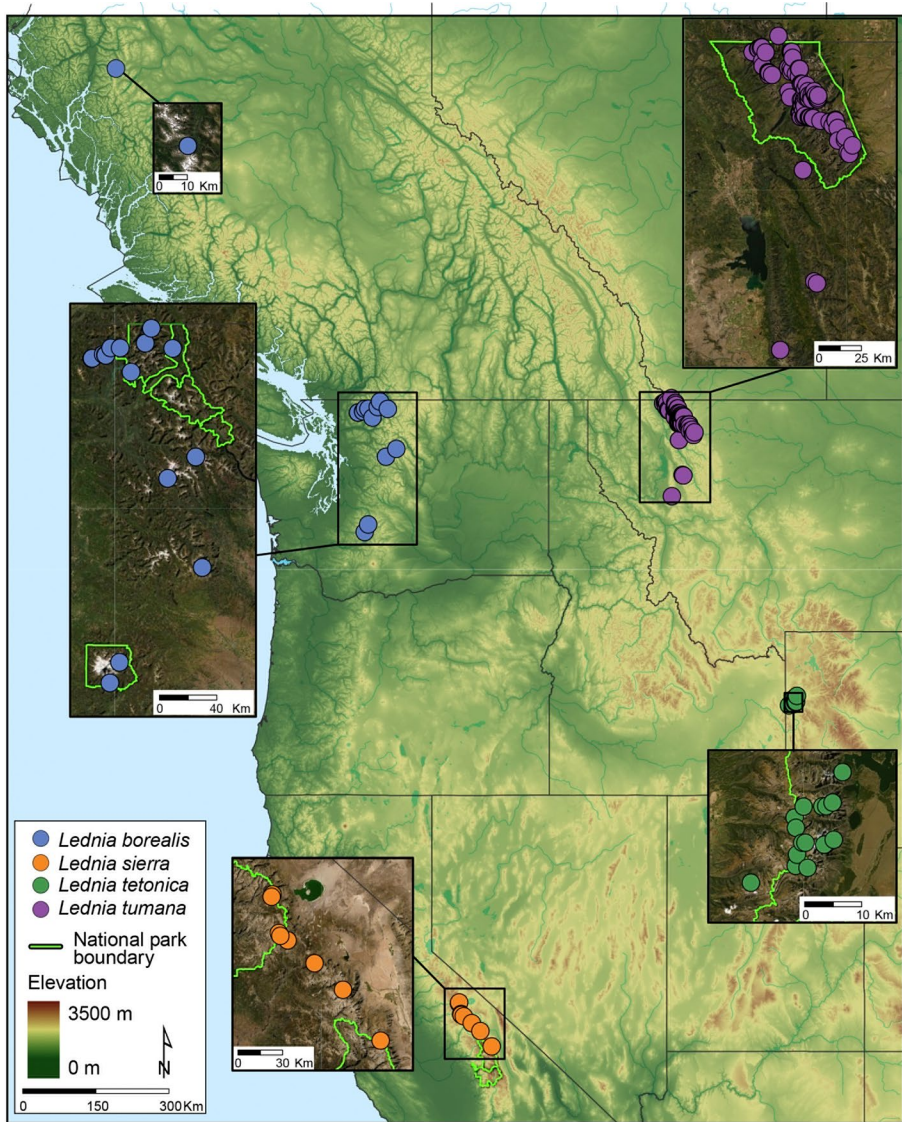


Fig. 2 All known extant populations of *Lednia*, color-coded by species. Green outlines in inset views of terrain represent national park boundaries. Locality information is included in Table S1 and stems from an array of published (Ricker 1952; Gaufin et al. 1972; Donald and Anderson 1977; Kondratieff and Lechleitner 2002; Baumann and Kondratieff 2010; Muhlfeld et al. 2011; Kubo et al. 2013; Giersch et al. 2017; Hotaling et al. 2018; Hotaling et al. 2018; Tronstad et al. 2020; Fallon et al. in press) and unpublished data

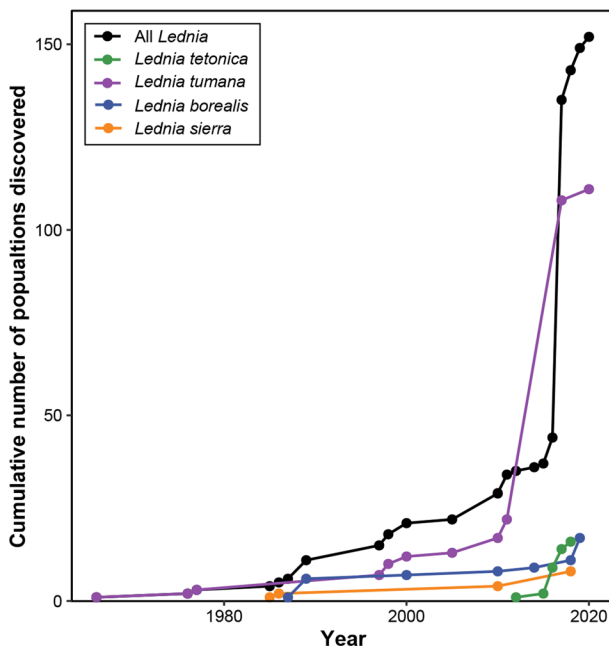


Fig. 3 The cumulative number of populations discovered through time for all *Lednia* and each species, respectively. The year of discovery corresponds to the publication year for the study detailing the findings (if published) and is included for each population in Table S1

accessible tool for reconnaissance of rare species habitats. Passive sampling techniques, particularly the use of environmental DNA (eDNA; Thomsen and Willerslev 2015), which has been applied to rare stoneflies elsewhere (Mauvisseau et al. 2019), also hold considerable potential to refine our understanding of distributional ranges.

Ecological links to glaciers and snowfields

Lednia populations are associated with high-elevation streams, often emanating from glaciers, perennial snowfields, cold springs, or subterranean ice (e.g., rock glaciers; Giersch et al. 2017; Tronstad et al. 2020; Brighenti et al. 2021; Fallon et al. in press; Fig. 1d–i). Populations living in the outflows of subterranean ice—termed “icy seeps” (Hotaling et al. 2019c)—are of particular recent interest as these streams are predicted to respond more slowly to climate change than those fed by surface ice and snow, and thus, may act as key climate refugia for *Lednia* and other coldwater taxa (Brighenti et al. 2021).

Lednia species vary in their elevational distributions likely due to differences in latitude and annual snowfall (and thus the elevation of permanent snowpack in a given region). *Lednia sierra* inhabit the highest elevations (mean=3273 m), followed by *L. tetonica* (mean=2872 m), *L. tumana* (mean=2023 m), and *L. borealis* (mean=1589 m; Fig. 4a, Tables S2–S3). However, despite significant differences in the average elevation of populations, no difference exists among species in their average distance to a stream source (Fig. 4b; Tables S2–S3). Across all populations and species, *Lednia* are on average just 383.5 m (SD=659.1) from their hydrological source.

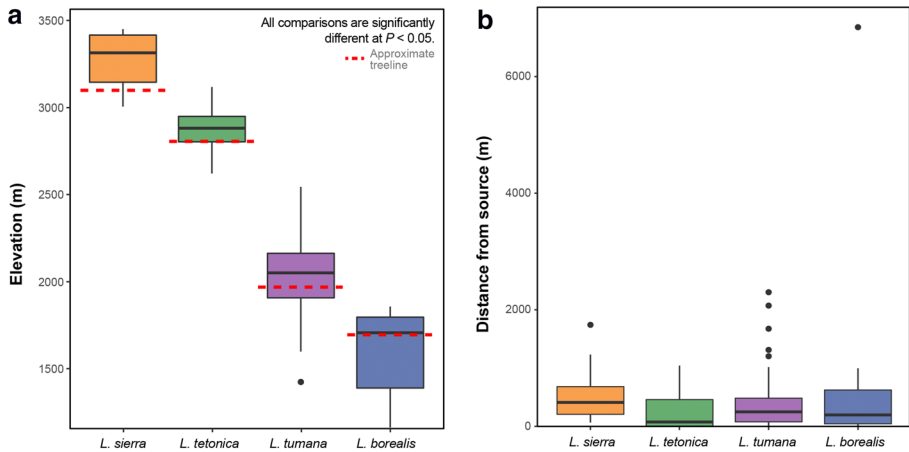


Fig. 4 Variation in **a** elevation and **b** distance to source across *Lednia* species. Complete methods and results for these figures are provided in Supporting Information and Tables S2–S3, respectively. Approximate treeline in (a) was visually estimated using Google Earth imagery

While *Lednia* may exhibit species-specific variation in habitat preferences, too few populations of species other than *L. tumana* have been identified to draw strong conclusions. However, some general rules appear to exist for the genus: *Lednia* inhabit higher elevation, perennial flowing waters and shallow lentic habitats (less than ~4000 m² in area). For the best-studied species, *L. tumana*, populations inhabit the full array of alpine hydrology from heavily glacier-influenced streams to groundwater springs, subterranean icy seeps, and the margins of small meltwater ponds. Densities of *L. tumana* decline with increasing distance from a stream source and increasing water temperatures; however, the modeled effect of water temperature explains just half the variability compared to that of distance from source (Giersch et al. 2017). *Lednia borealis* generally occurs in forested stream ecosystems up to the melting edges of snowfield, glaciers, and permanent ice (Fallon et al. in press). *Lednia borealis* is also the only species regularly found below treeline (Fig. 4a), likely due to the lower elevation of meltwater sources in the Pacific Northwest, USA, stemming from substantial annual snowfall.

For *L. borealis*, *L. sierra*, and *L. tetonica*, a link between population occurrence and the presence of a meltwater source (e.g., a glacier, snowfield, or rock glacier) has been hypothesized (e.g., Tronstad et al. 2020) but not formally tested. However, for *L. tumana*, Muhlfeld et al. (2011) established a clear link between *L. tumana* distributions and permanent, but declining, glacier masses in the northern Rocky Mountains. Later, Giersch et al. (2017) used expanded sampling to confirm and extend the conclusions of Muhlfeld et al. (2011), showing that the occurrence and abundance of *L. tumana* declines precipitously with increased stream temperatures and distance from source. More recently, Muhlfeld et al. (2020) explicitly tested how the scale and timeline of glacier cover has influenced the entire community of alpine macroinvertebrates in GNP and the surrounding mountains. A key pattern emerged: a coldwater community, of which *L. tumana* is a major component, has persisted in catchments that have not been glaciated for at least ~170 years (Muhlfeld et al. 2020). Thus, for at least *L. tumana*, runoff from surface glaciers appears important but not required for persistence.

Life history

The life histories of *Lednia* species, beyond anecdotal field observations of emergence timing, are poorly known. Given the importance of life history in shaping species distributions, as well as informing management decisions and evolutionary models, this is a significant research gap. Current knowledge suggests *L. tumana* has a semivoltine, two-year life cycle comprised of 15–18 instars (Treanor et al. 2013; J.G., personal observation). This is supported by co-occurring mature, late-instar nymphs and smaller, early-instar nymphs late in the summer season (e.g., September; J.G., personal observation). Limited evidence, however, suggests life histories may vary among *Lednia* species. For instance, *L. tetonica* populations tend to exhibit a single nymph size class during summer collection periods and thus appears univoltine (L.M.T., personal observation).

All *Lednia* appear to emerge as adults in mid- to late-summer (late July to early September; Baumann and Call 2012) with variation among streams, likely depending on thermal regime and snow cover. *Lednia sierra* adults have only been collected in August and September; however, as the least studied species in the genus, this may reflect limited observations rather than a truly narrow emergence window. Conversely, *L. tumana*, the most-studied species in the genus, has been observed emerging as early as mid-July and as late as mid-October (Baumann and Kondratieff 2010). Alpine aquatic insects can have temperature-dependent emergence patterns based upon sex (Nebeker 1971; Finn and Poff 2008). For example, *Zapada haysi* males emerge first then swarm later-emerging females (Cather and Gaufin 1976; Finn and Poff 2008). *Lednia tumana* likely also display temperature-dependent emergence patterns based upon sex; of the 96 nymphs collected by Treanor et al. (2013) in late August, only five were male.

Variation in emergence timing, whether due to sex, snow cover variation, or other factors, may also affect the connectivity of *Lednia* populations and the potential for gene flow across stream types which could, in turn, influence patterns of local adaptation. While dispersal capacity for *Lednia* has not been quantified, nemourid stoneflies are generally considered weak fliers with particularly low rates of female dispersal (Finn and Poff 2008; but see DeWalt and South 2015). However, genomic data do support some degree of connectivity within and across basins; for instance, *L. tumana* populations within 1–2 km of each other in the GNP alpine exhibit very low levels of differentiation (Hotaling et al. 2018).

Biotic interactions

The diet of *Lednia* has not been studied, but current knowledge suggests nemourid stoneflies primarily consume decaying plant material, biofilms, and other organic material (Ledger and Hildrew 2000; Feeley and Kelly-Quinn 2015; Merritt et al. 2019). *Lednia* occurs in many locations that lack conspicuous vegetation and with limited biofilm growth on stream substrate. An important food resource for macroinvertebrates in other alpine environments comes from glaciers, ice, snow and their associated algal communities, which may also be used by *Lednia* (Skidmore et al. 2000; Milner et al. 2001; Hågvar et al. 2016; Ono et al. 2021). Gut content analyses of *Lednia* nymphs, stable isotopes of insect body tissues to identify food web linkages (e.g., Coat et al. 2009), and molecular identification of gut contents through metabarcoding (e.g., Jo et al. 2020) would all offer crucial trophic insights for *Lednia* and similar species.

Beyond *Lednia*'s trophic role, a standing question in alpine stream biology centers around the degree to which species require cold conditions for survival or if they are instead tolerating harsh habitats as a means of escaping biotic stressors (Hotaling et al. 2020a). *Lednia* may be poor competitors that are outcompeted for space and resources at lower elevations by taxa that are cold intolerant (e.g., Flory and Milner 1999). In addition to competition, many insects are predators in mountain stream habitats (Niedrist and Füreder 2017) and may exhibit high plasticity in prey species affinity depending upon environmental conditions (Zah et al. 2001; Füreder et al. 2003). An experimental test of predation on alpine stream community structure highlighted the potential for a large predatory stonefly transplanted to a new stream to alter densities of native prey (Khamis et al. 2015). Thus, biotic interactions in lower elevation streams by cold intolerant taxa could constrain *Lednia* to higher elevation habitats (see “[Thermal physiology](#)” below).

Molecular ecology and genomics

With the growing ease of generating molecular data for non-model species (e.g., Hotaling et al. 2021b), substantial advances have been made in the genetics of alpine biodiversity (e.g., Dussex et al. 2016; Hotaling et al. 2018). For *Lednia*, we have learned a great deal about relationships among species (Hotaling et al. 2019b), the underlying drivers and temporal changes in differentiation within species (Jordan et al. 2016; Hotaling et al. 2018), and how patterns of genetic structure in *Lednia* compare to related species (e.g., *Zapada glacier*, Hotaling et al. 2019b).

In the first population genetic study of *L. tumana*, Jordan et al. (2016) compared mitochondrial DNA (mtDNA) haplotypes of two genes, *cytochrome oxidase I* (COI) and *cytochrome b*, for *L. tumana* specimens collected in 2010 versus historical samples (collected 10 or more years earlier). The 2010 samples exhibited reduced haplotype diversity (a proxy for genetic diversity and/or population size) versus historical samples, indicating that contemporary recession of glaciers and snowfields may be driving a loss of genetic diversity (Jordan et al. 2016). Next, Hotaling et al. (2018) used genome-scale data collected with restriction-site associated DNA sequencing (RADseq) to provide fine-scale resolution of genetic structure within *L. tumana* and the origins of population-level differentiation within the species. Analyses revealed three genetic clusters that generally aligned with geography and diverged despite gene flow ~13,000–18,000 ybp, likely as continental ice sheets receded into the high mountains at the end of the Wisconsin glaciation (~20,000 ybp, Carrara 1987).

Later, Hotaling et al. (2019b) added the first comparative genetic study of *Lednia* species. Using COI, Hotaling et al. (2019b) showed that *L. tumana* and *L. tetonica* are sibling species, with *L. sierra* as the outgroup to the rest of the genus (Fig. 5). When compared to a confamilial stonefly with a similar distribution in the high Rocky Mountains—the ESA-listed *Zapada glacier*—genetic divergence between *L. tumana* and *L. tetonica* was nearly an order of magnitude greater than divergence among *Z. glacier* populations across the same area. Given the overlapping distributions of the two groups (*L. tumana* and *L. tetonica* vs. *Z. glacier*)—both geographically and environmentally—this result highlights the importance of not assuming underlying genetic patterns from distributional or phylogenetic evidence alone.

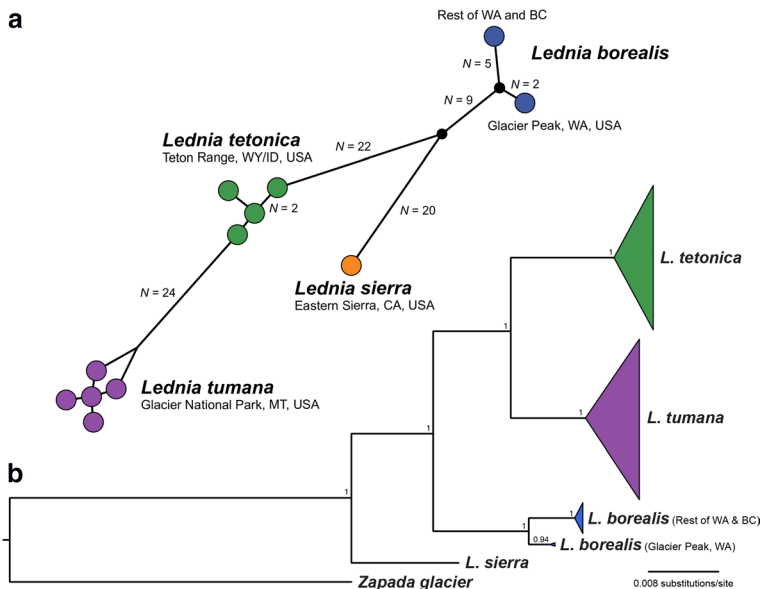


Fig. 5 A re-analysis of sequence data included in Hotaling et al. (2019b) with new samples of *Lednia borealis* added (see Supporting Information). **a** Cytochrome oxidase c subunit I (COI) haplotype network of all available *Lednia* sequences. Colored circles represent haplotypes and *N* indicates the number of substitution steps (i.e., one nucleotide difference) between haplotypes. **b** COI gene trees for all *Lednia* sequences. Terminal nodes for everything except *L. borealis* (the new sequences included in this study) were compressed into triangles and scaled according to the number of sequences. Numbers above nodes are posterior probabilities

Here, we re-analyzed the data of Hotaling et al. (2019b), adding 11 new sequences available on the Barcode of Life Database (BOLD; Ratnasingham and Hebert 2007) that were either morphologically identified as *L. borealis* (*N*=9) or a close relative *Prostoia besametsa* (*N*=2). In addition to *L. borealis*, we were particularly interested in the *P. besametsa* sequences because they clustered with *L. borealis* despite being collected from central British Columbia, the furthest north of any known *Lednia* range. Phylogenetic and haplotype network analysis of the expanded data set and communications with the collectors of the putative *P. besametsa* samples revealed two key findings: (1) new *L. borealis* samples from the Glacier Peak Wilderness represented a distinct genetic cluster that is more diverged from conspecifics than any other *Lednia* populations (Fig. 5); (2) the *P. besametsa* specimens were originally misidentified and instead represent *L. borealis* samples from central British Columbia. This analysis provided the data to infer an expanded known distribution of *L. borealis* by ~800 km to the northwest.

In addition to new genetic insight, we have also recently gained a first look into the genome of *L. tumana* (Hotaling et al. 2019c), the first for any stonefly (Hotaling et al. 2020b). This first nuclear genome assembly for *L. tumana* comprised ~520.2 million basepairs and its mtDNA genome was ~16.7 thousand basepairs (Hotaling et al. 2019c), the latter being similar to that found in other stonefly mtDNA genomes (Chen and Du 2017). Both assemblies, and particularly the nuclear genome, will greatly empower genomic

research in the system, including future efforts to understand the genetic basis of *L. tumana*'s extreme life history.

Looking ahead, although research has moved beyond morphology for understanding *Lednia* taxonomy and population relatedness, considerable potential for molecular insight remains. First, a calibrated, multi-locus *Lednia* phylogeny is needed to confirm the existing topology (e.g., Hotaling et al. 2019b) and understand the group's evolutionary timeline. Additional lines of systematic evidence are also necessary because cryptic, undescribed species are common among mountain stream insects (e.g., Bálint et al. 2011; Hotaling et al. 2019b). For instance, a calibrated phylogeny could reveal whether *Lednia* species evolved in response to mountain-building in their respective ranges, similar to the evolutionary history of the glacier ice worm *Mesenchytraeus solifigus* (e.g., Lang et al. 2017) or whether they iteratively invaded mountain headwaters more recently. Such an approach could also shed light on whether the extinct *Lednia* species (*L. zilli*) described from Europe should be grouped with extant *Lednia* by providing an age for the most recent common ancestor for the genus. If this age is more recent than the fossil species, then it likely lived before the emergence of the genus and thus, should not be classified within it. Second, because *Lednia* habitats vary among and within species due to difference in their primary hydrological source (see Hotaling et al. 2017; Brighenti et al. 2021), *Lednia* populations may be locally adapted (Whitlock 2015). If evidence for local adaptation is found, linking these results to improved genomic resources to understand the genetic variation underlying local adaptation would provide a powerful means for identifying which populations may be most at risk under climate change. Finally, given the potential for a multi-year life history in *Lednia* species, the possibility of molecular differences existing between cohorts should be considered (e.g., Leys et al. 2017).

Thermal physiology

The link between *Lednia* (particularly *L. tumana*) and cold, high-elevation stream habitats (e.g., Muhlfeld et al. 2011; Giersch et al. 2017; Fallon et al. in press) suggests that *Lednia* nymphs are cold stenotherms, capable of surviving only at very low temperatures, and perhaps intolerant of warmer streams (e.g., $> 10^{\circ}\text{C}$). This widely accepted hypothesis has led to predictions of extreme vulnerability for *Lednia* and similar high-elevation stream taxa to warming (Birrell et al. 2020). For reference, the mean August temperatures of *L. tumana* localities across 43 sites rarely exceed 5.5°C (Giersch et al. 2017). However, an extant, cold-water distribution does not necessarily imply adaptation to cold climates or stenothermy but simply highlights that *Lednia* are tolerant of cold, harsh streams. As previously discussed, an alternative hypothesis to cold adaptation is that *Lednia* are relegated to high-elevations because of biotic interactions in lower elevation, warmer stream reaches that are more distant from the source (Richter et al. 1997).

At a minimum, disentangling these alternative hypotheses requires experimental measurements of *Lednia* thermal limits. Treanor et al. (2013) measured two physiological metrics, the critical thermal maximum (CT_{MAX}) and upper lethal limit (ULT), in late-instar nymphs collected from a snowmelt-fed stream (Lunch Creek, GNP). CT_{MAX} is a non-lethal metric of tolerance of acute heat stress and represents the temperature at which an organism's locomotor activity becomes disorganized and involuntary, akin to seizures. The

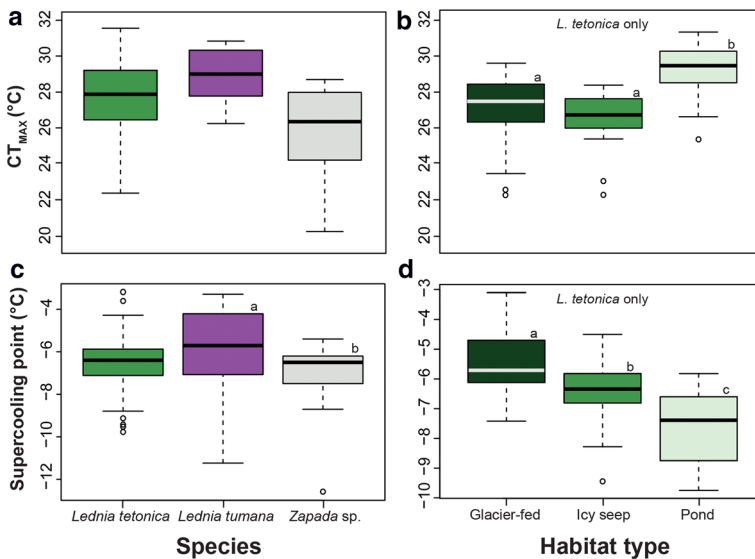


Fig. 6 Critical thermal maximum (CT_{MAX}) for **a** *L. tetonica*, *L. tumana*, and *Zapada* sp. And **b** *L. tetonica* only (grouped by stream type). Supercooling points of **c** *Lednia tetonica*, *Lednia tumana*, and another alpine stonefly, *Zapada* sp. And **d** *L. tetonica* only (grouped by stream type). Lower-case letters within plots indicate significant differences between groups at $P < 0.05$. Data re-plotted from Hotaling et al. (2020b) and Hotaling et al. (2021a, b)

second metric, ULT, is the temperature at which death occurs. *Lednia tumana* nymphs from Lunch Creek had surprisingly high tolerance to heat stress (CT_{MAX} = 20.6–21.85 °C, ULT = 31.1–32.3 °C) and could withstand temperatures nearly three times those which naturally occur in their habitat (Treanor et al. 2013).

The high thermal limits of *Lednia* observed by Treanor et al. (2013) could be the result of adaptation to thermal regimes typical of snowmelt rather than glacier-fed streams, which tend to have larger temperature fluctuations and higher summer maximums (Hotaling et al. 2017). Depending on the source, other streams in the high Rocky Mountains remain extremely cold even in summer. More recently, Hotaling et al. (2020b) assessed population-level variation in CT_{MAX} in three nemourid species, *L. tumana*, *L. tetonica*, and *Zapada* sp., a confamilial taxon that also occurs in high-elevation streams (Fig. 6a, b). CT_{MAX} of *L. tetonica*—the only taxon with multiple populations included in the study—correlated with maximum stream temperatures such that populations from colder streams had lower heat tolerance than those from warmer streams (Hotaling et al. 2020a). Thus, population level thermal physiology in *Lednia* appears to vary within species, even across small geographic scales, and may be linked to local conditions. Overall, *Lednia* exhibited relatively high CT_{MAX} values (~28°C; Fig. 6a, b), which were comparable to other high-elevation aquatic insects (Shah et al. 2017a) as well as the previous Treanor et al. (2013) study on *L. tumana*.

Unlike other studies of heat stress in aquatic insects, Hotaling et al. (2020b) linked thermal physiology to underlying cellular processes via RNA sequencing and gene expression comparison. Heat shock proteins (HSPs) are common in animals and high levels of HSP

expression are typical in organisms experiencing heat or other stressful conditions (King and MacRae 2015). Surprisingly, comparisons of *L. tetonica* exposed to normal ($\sim 3^{\circ}\text{C}$) versus hot ($\sim 28^{\circ}\text{C}$) water temperatures revealed no differences in expression of HSPs. Thus, it appears cold temperatures may be just as stressful for *Lednia* as hot temperatures. This important result, along with measures of high heat tolerance limits, suggests that *Lednia* are not cold stenotherms, but instead are unusually tolerant of cold conditions. Thus, identifying the mechanism(s) underlying the distribution of *Lednia* in cold, high-elevation streams warrants further investigation. As speculated above, it is possible that biotic interactions at lower elevations could explain *Lednia*'s high-elevation distribution—an important consideration as climate change shifts species distributions in mountain ecosystems worldwide (Shah et al. 2020a)—but this hypothesis has not been tested.

Although warming temperatures pose obvious risks to mountain biodiversity, an equally important but often overlooked trait for species like *Lednia* is cold temperature tolerance. To investigate tolerance to cold stress and freezing in *Lednia*, Hotaling et al. (2021a) measured the supercooling points (SCPs) of *L. tumana*, *L. tetonica*, and *Zapada* sp. inhabiting multiple stream types in the Rocky Mountains (Fig. 6c,d). Stream types were defined by landscape geomorphology and primary hydrological source (e.g., glaciers or subterranean ice). Briefly, the SCP is the temperature at which an organism's body transitions to a frozen state (Renault et al. 2002). During this transition, the formation of internal ice results in a rapid release of heat, which can be detected by thermocouples placed in contact with the organism's body. *Lednia tumana* from Lunch Creek, GNP, exhibited a slightly higher mean SCP ($-5.9 \pm 2^{\circ}\text{C}$) than the overall mean SCP for five *L. tetonica* populations representing a variety of stream types ($-6.6 \pm 2^{\circ}\text{C}$; Fig. 6c). These values fall within the upper range of supercooling points measured for terrestrial insects (Moore and Lee 1991; Sinclair 1999) but are higher than many aquatic insects, such as *Diamesa mendotae* (SCP: -21.6°C) and other stonefly species like *Allocapnia granulata* (SCP: $-13.15 \pm 0.44^{\circ}\text{C}$) and *Allocapnia pygmaea* (SCP: $-11.95 \pm 0.47^{\circ}\text{C}$; Danks 2008; Bouchard et al. 2009). A population of *L. tetonica* from a small, high-elevation alpine pond exhibited the lowest mean SCP of any population in the study ($-7.5 \pm 2^{\circ}\text{C}$, Fig. 6d; Hotaling et al. 2021a). This finding was particularly compelling as the geomorphology and hydrology of high-elevation ponds may induce lower SCPs. Generally, high-elevation ponds are shallow ($< 1\text{ m}$) with flat bottoms and extremely slow-moving water. Unlike steeper, fast-flowing streams, high-elevation pond populations are more likely to experience freezing stress (Hotaling et al. 2021a). Thus, much like the upper thermal limits, there is variation in SCP across species, populations, and habitat types in *Lednia* and closely related species that can be linked, at least in part, to local stream conditions. Although SCPs can be indicative of biochemical adaptations to internal ice-formation, most aquatic insects likely do not survive exposure to subzero temperatures below their supercooling points (Irons et al. 1993, but see Walters et al. 2009). Hotaling et al. (2021a, b) assessed tolerance to ice enclosure for late-instar *L. tumana* nymphs from Lunch Creek, GNP. No *L. tumana* nymphs survived the experiment, indicating that contact with ice in the environment is lethal. As a result, understanding how (and where) *Lednia* nymphs overwinter to either avoid or cope with freezing temperatures remains an open question.

Although efforts to understand the thermal ecology of *Lednia* represent important first steps, the results should be interpreted with caution. Metrics like CT_{MAX} and ULT only

measure response to short-term thermal stress and are often sensitive to methodological parameters (e.g., rates of temperature change, Rezende et al. 2011). Whether *Lednia* can withstand higher temperatures throughout development or whether there are trade-offs that become apparent at specific life stages are just some of the gaps that remain to be filled. Future studies should emphasize understanding the link between warming in alpine streams and organismal fitness, particularly when multiple factors are considered simultaneously. Warming will likely affect shifts in growth trajectories, developmental timelines, egg production, and adult flight performance (McCauley et al. 2015). Links between *Lednia*, stream temperature, and metabolic rates will also provide a comprehensive framework for predicting how subtle temperature change will affect energy budgets and organismal performance (Shah et al. 2020b). Warmer temperatures and lower stream flows will reduce the oxygen content of stream water (Birrell et al. 2020) and can result in decreased tolerance to heat stress (Pörtner and Knust 2007). Evidence for this reduced tolerance to heat stress has been observed for a lower elevation stonefly (Frakes et al. 2021), and the joint effects of warming and decreased flow may be especially problematic at high elevation, where low atmospheric partial pressure exacerbates oxygen limitations (Jacobsen 2020). However, like many other organisms, *Lednia* may escape or mitigate unfavorable stream conditions through intrinsically broad thermal tolerance, thermal plasticity (e.g., Shah et al. 2017b), or behavioral thermoregulation (McNamara et al. 2020). Whether *Lednia* acclimates to warmer conditions or physically moves within the stream to avoid higher temperatures is unknown. Similarly, during winter when *Lednia* habitats are snow-covered with temperatures near freezing, *Lednia* may seek refuge in the interstitial spaces of the streambed to avoid contact with ice (Danks 2008; Wissinger et al. 2016). If *Lednia* move around the stream to behaviorally thermoregulate in winter, they may also do so during the hottest times of the summer.

The future of *Lednia* and alpine stream ecosystems

Anthropogenic climate change is driving massive recession of glaciers and perennial snowfields in mountain ecosystems (Riedel et al. 2015; Zemp et al. 2015). For example, of the 150 glaciers documented in GNP and the surrounding area in 1850, only 25 remain, and these may vanish within the twenty-first century (Hall and Fagre 2003). As glaciers retreat, cold water habitats will be greatly reduced (Muhlfeld et al. 2011), although, the degree to which *L. tumana*, *L. tetonica*, and other *Lednia* species require cold meltwater for their persistence has been complicated by conflicting ecological, physiological, and molecular evidence (Hotaling et al. 2020a; Muhlfeld et al. 2020). Still, loss of coldwater habitat is likely to decrease gene flow and genetic diversity, hampering the potential for adaptive evolution, and increasing the risk of local extirpation and extinction for *Lednia*.

Thus far, predictions of climate change threats to *Lednia* and other alpine stream biota rests on the assumption that perennial streams will continue to flow on a deglaciated landscape. This may not be true (Haldorsen and Heim 1999), especially in the Teton Range and the Sierra Nevada where springs fed by groundwater aquifers, which are less likely to be impacted by cryosphere decline, are uncommon (Tronstad et al. 2020; M.G., personal

observation). However, though groundwater-fed springs are rare in landscapes like the high Teton Range, many of these places have a rich distribution of geological features that commonly harbor internal ice (e.g., rock glaciers); these “cold rocky landforms” essentially act as aquifers (Brighenti et al. 2021). At any rate, with restricted distributions and the potential for multi-year life histories within *Lednia*, streams transitioning from high-flow to intermittency or no flow will have dire consequences for the group.

Altered flow and habitat conditions are likely already influencing *Lednia* distributions. Range contractions have been demonstrated for another alpine nemourid stonefly, *Zapada glacier*, which can be sympatric with *Lednia* species. (Giersch et al. 2015; Hotaling et al. 2019b). *Zapada glacier* populations appear to be retreating upstream to track colder conditions driven by ongoing loss of glacial coverage in GNP (Giersch et al. 2015). However, the specific drivers of these range shifts remain unclear. Species may move uphill to track cooler conditions, move downhill to balance the effects of rising temperatures and oxygen availability (Jacobsen 2020), or move in unpredictable ways due to the pressures of biotic interactions (e.g., predation or competition; Khamis et al. 2015). The challenge of understanding and predicting these range shifts is intensified by the fact that organismal responses to changing alpine stream conditions will likely be species, and perhaps even population, specific (Shah et al. 2020b).

Conservation of *Lednia* and headwater biodiversity

The listing of *L. tumana* as threatened under the U.S. Endangered Species Act highlights the risks facing *Lednia* and other high-elevation species under climate change (e.g., *Z. glacier*; US Fish and Wildlife Service 2019). While this policy decision highlights the need for *L. tumana* to receive considerable research attention going forward, the high degree of ecological similarity among *Lednia* species paired with fewer known locations for non-*L. tumana* species highlights that the entire genus is likely facing similar threats. According to the NatureServe Explorer database of conservation statuses for rare and endangered species in the Americas, *L. borealis*, *L. tetonica*, and *L. tumana* are both classified as G1: critically imperiled (NatureServe 2021). *Lednia sierra* has not been ranked.

The vast majority of *Lednia* populations occur on federally managed lands (e.g., National Park Service; Fig. 2). While this distribution affords the group with some degree of protection, the ultimate fate of *Lednia* may be inextricably linked to anthropogenic climate change. Thus, a practical challenge remains: what concrete steps can be taken to conserve aquatic insects living in high-elevation streams whose sources are imperiled by climate change? To date, no *Lednia* species has been successfully reared in captivity so supporting populations through breeding programs is not currently feasible. Another option is identifying suitable habitat outside of existing ranges of *Lednia* species for managed relocation (also referred to as “assisted migration”, Schwartz et al. 2012). However, managed relocation raises a host of ecological and ethical concerns as it is essentially an intentional invasive species introduction (Ricciardi and Simberloff 2009). For a group as poorly known as *Lednia*, proper mitigation of these concerns while ensuring a high likelihood of post-relocation success likely presents a challenge too great to overcome—at least until the ecology of the species is better understood.

Perhaps the best practical solution for *Lednia* conservation will be identification, management, and monitoring of existing in situ climate refugia within the range of each species (Morelli et al. 2020). Climate refugia are areas of habitat within a species' range that are expected to be maintained in their current condition while nearby habitats degrade and become unsuitable (e.g., due to climate change, Ashcroft 2010). Rock glaciers and related "cold rocky landforms" (CRLs) that support subterranean ice and cold outflow streams (termed "icy seeps", Hotelling et al. 2019c) in mountain ecosystems may provide climate refugia for *Lednia* and other alpine biota (Brighenti et al. 2021). For example, research suggests that rock and debris-covered glaciers will likely persist on the landscape longer than surface glacier and snowfields due to thermal buffering afforded by debris cover (Clark et al. 1994; Anderson et al. 2018; Knight et al. 2019), and cold rocky landforms are common in the western United States and outnumber surface glaciers and perennial snowfields by a margin of ~2:1 (Fountain et al. 2017; Johnson 2018). Additionally, the biotic communities supported by rock glaciers and related features commonly overlap with nearby glacier-fed streams (Brighenti et al. 2021). Thus, an effective approach for *Lednia* conservation going forward could center around conducting surveys to identify currently unmapped *Lednia* populations, identifying the degree to which they are linked to CRLs, targeting management towards CRL-linked populations and similar *Lednia* climate refugia, and followed by regular monitoring of population sizes and environmental conditions to monitor stability through time (see Brighenti et al. 2021). This approach could be empowered at multiple levels by molecular (e.g., metabarcoding, eDNA) and modeling (e.g., SDM) approaches to improve search efficacy and accuracy of detection as well as the use of demographic stability metrics (or related approaches) to identify populations that are most likely to persist through future habitat change (e.g., Finn et al. 2009).

Future directions

Research in the last decade has greatly expanded our knowledge of *Lednia*; three new species were described, known populations of the genus expanded from 12 to 154, and a major extension of the *L. borealis* range was discovered in the mountains of British Columbia, Canada. We have also learned a great deal about the ecological and evolutionary characteristics of individual species and the genus overall. Along the way, this research transformed our capacity to predict climate change impacts on alpine stream biodiversity and transitioned *Lednia* from an enigma of the Rocky Mountain alpine streams to one of the best-known mountain stream residents in western North America and a sentinel of climate change impacts on headwaters. Still, our knowledge of *Lednia* and ecologically similar species remains incomplete. In Table 1, we offer a range of pressing areas for future research across the disciplines described above.

Table 1 Key areas for future *Lednia* research

Research area	Need	Benefit(s)
Distributions	More field surveys in likely <i>Lednia</i> habitat throughout western North America	More accurate understanding of <i>Lednia</i> species distributions; habitat conservation applications
Distributions	Develop passive detection approaches (e.g., eDNA) for <i>Lednia</i>	Increased capacity for detecting new <i>Lednia</i> populations; Nonlethal sampling of rare or listed species
Phylogenetics	Time-calibrated multi-locus phylogeny for <i>Lednia</i> and related species	Clarify evolutionary timeline and relationships; framework for detecting cryptic species
Ecology & physiology	Clarifying genus- and species-specific links to receding glaciers and snowfields	Better understanding of the factors driving <i>Lednia</i> distributions; Clarified climate change risks to species persistence
Life history	Estimation of <i>Lednia</i> life history timing and reproductive capacity	Improved basic understanding of <i>Lednia</i> ecology; conservation applications
Trophic ecology & physiology	Role of temperature and/or biotic interactions determining <i>Lednia</i> distributions	Understanding the relative importance of biotic interactions and/or temperature preferences in determining <i>Lednia</i> distributions
Trophic ecology	Characterize <i>Lednia</i> diet and place species in a broader food web context	Improved basic understanding of <i>Lednia</i> ecology and food web dynamics in mountain streams; Refined predictions of how biotic changes may affect <i>Lednia</i> persistence
Molecular ecology	Test for genomic signatures of local adaptation across <i>Lednia</i> populations across a range of hydrological regimes	Determine if <i>Lednia</i> populations are locally adapted to specific habitat conditions, and thus, the degree which climate change may affect populations differentially
Genomics	Generate high-quality, annotated genome assemblies for each <i>Lednia</i> species	Key resource for future evolutionary understanding and comparison of <i>Lednia</i>
Physiology	Disentangle the effects of single and multiple factors (e.g., temperature and oxygen availability) on <i>Lednia</i> fitness	Refined predictions of how environmental change will affect <i>Lednia</i> populations
Physiology	Test for physiological vulnerability to climate change across life stages	Identification of life stages (egg, nymph, or adult) that may be particularly sensitive to climate change
Conservation	Identify and monitor <i>Lednia</i> populations living in putative in situ climate refugia	Empirical understanding of how habitat change influences <i>Lednia</i> persistence

Conclusion

As mountain streams rapidly change around the world, we are faced with an increasingly pressing challenge to predict how these changes will affect *Lednia* and other alpine stream taxa. While recent studies (e.g., Hotaling et al. 2020a; Muhlfeld et al. 2020; Brighenti et al. 2021) suggest that stream biodiversity may be more tolerant of mountain cryosphere decline than previously anticipated, major research gaps remain. Ultimately, we need better predictive knowledge of the abiotic and biotic factors influencing the persistence of mountain stream communities. Going forward, we expect coordinated efforts, empowered by a growing interest in long-term ecological monitoring of mountain streams, to greatly improve understanding of high-elevation aquatic ecosystems and the unique biota they contain.

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Authors and Affiliations

Matthew D. Green¹  · **Lusha M. Tronstad**² · **J. Joseph Giersch**³  · **Alisha A. Shah**⁴  · **Candace E. Fallon**⁵  · **Emilie Blevins**⁵  · **Taylor R. Kai**⁶ · **Clint C. Muhlfeld**^{3,7}  · **Debra S. Finn**⁸  · **Scott Hotaling**⁹ 

✉ Scott Hotaling
scott.hotaling@wsu.edu

¹ Department of Evolution, Ecology, and Organismal Biology, University of California-Riverside, Riverside, CA, USA

² Wyoming Natural Diversity Database, University of Wyoming, Laramie, WY, USA

³ U.S. Geological Survey, Northern Rocky Mountain Science Center, West Glacier, MT, USA

⁴ Division of Biological Sciences, University of Montana, Missoula, MT, USA

⁵ The Xerces Society for Invertebrate Conservation, Portland, OR, USA

⁶ College of Medicine, University of Kentucky, Lexington, KY, USA

⁷ Flathead Lake Biological Station, Division of Biological Sciences, The University of Montana, Polson, MT, USA

⁸ Department of Biology, Missouri State University, Springfield, MO, USA

⁹ School of Biological Sciences, Washington State University, Pullman, WA, USA