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Congruent population genetic structure but differing depths of divergence for three alpine stoneflies with similar ecology and geographic distributions

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Abstract

1. Comparative population genetic studies provide a powerful means for assessing the degree to which evolutionary histories may be congruent among taxa while also highlighting the potential for cryptic diversity within existing species.
2. In the Rocky Mountains, three confamilial stoneflies (*Zapada glacier*, *Lednia tumana*, and *Lednia tetonica*; Plecoptera, Nemouridae) occupy cold alpine streams that are primarily fed by melting ice. *Lednia tumana* and *L. tetonica* are sister species diagnosed from systematic morphological differences, and they are endemic to areas surrounding Glacier National Park and Grand Teton National Park, respectively, in the U.S. Rocky Mountains. *Zapada glacier* is also present in alpine streams from Glacier National Park to the Teton Range, sometimes co-occurring with either *Lednia* species.
3. We used mitochondrial sequence data to clarify species boundaries, compare population genetic patterns, and test demographic models in a coalescent framework for the three stoneflies. We addressed four questions: (1) Is there genetic support for the morphology-based species boundaries in *Lednia*? (2) Is there genetic support for cryptic, or as-yet undescribed, diversity within *Z. glacier*? (3) Do similar geographic distributions and ecological requirements yield spatial congruence of genetic structure between high-elevation *Lednia* and *Z. glacier* populations? (4) Is there evidence for contemporary gene flow among isolated populations in either group?
4. Our results supported the existing taxonomy with *Z. glacier* and the two *Lednia* species differing in their depths of divergence among study regions (e.g. maximum sequence divergence within *Z. glacier* = 1.2% versus 5% between *L. tumana* and *L. tetonica*). However, spatial population genetic patterns were broadly congruent, indicating stonefly populations isolated on mountaintop islands. Coalescent modelling supported the possibility of rare, extremely limited contemporary gene flow among *Z. glacier* populations, with no support for gene flow between *L. tumana* and *L. tetonica*.

5. The focal stoneflies and associated assemblages occupy the highest elevation, coldest permanent alpine streams in the study region. This lotic habitat type faces an uncertain future under a diminishing alpine cryosphere. Given spatial congruence of genetic structure demonstrating unique biodiversity associated with individual alpine islands, we encourage conservation management strategies be developed and applied at corresponding spatial scales.

KEYWORDS
alpine stream, *Lednia*, Plecoptera, Rocky Mountains, *Zapada*

1 | INTRODUCTION

In freshwater biology, species boundaries between closely related taxa are traditionally inferred from systematic morphological differences. Species-level lineages can be obscured, however, by limited or undiagnosed morphological divergence (Bickford et al., 2007), and characterising this potential for cryptic biodiversity among macroinvertebrates is a pressing challenge in freshwater ecology and beyond (Jackson et al., 2014). Ideally, morphology-based species delimitations would be evaluated in the context of molecular data, preferably in a comparative framework with closely related species. Because every individual, population, and species has its own evolutionary history, comparative genetic frameworks can also provide key avenues for linking past processes to present-day variation (Hewitt, 2000; Whiteman, Kimball, & Parker, 2007). Indeed, a comparative approach can help discern if closely related or similar species (e.g. in terms of geographic distribution and/or ecological requirements) have responded similarly or differently at the genetic level to historical geological influence (e.g. glacial oscillation, Brunsfeld, Sullivan, Soltis, & Soltis, 2001), current landscape structure (Goldberg & Waits, 2010), and/or variance in dispersal ability (Lourie, Green, & Vincent, 2005). Often, co-distributed species with similar ecological requirements have congruent evolutionary trajectories (Lapointe & Rissler, 2005; Satler & Carstens, 2017; Whiteman et al., 2007), but ecological and/or life history variation can also substantially influence patterns of genetic differentiation (Hughes, Schmidt, & Finn, 2009; Miller, Blinn, & Keim, 2002; Phillipsen et al., 2014).

Three stoneflies (Plecoptera: Nemouridae), *Zapada glacier*, *Lednia tumana*, and *Lednia tetonica*, occupy the highest, coldest reaches of Rocky Mountain alpine streams in Wyoming and Montana, U.S.A. (Figure 1). All three stoneflies are phytophagous with short (probably <30 days) winged adult stages, and they inhabit (and sometimes co-occur within) streams fed by meltwater from permanent ice sources (e.g. glaciers or snowpack, Baumann, 1975; Muhlfeld et al., 2011; Baumann & Call, 2012; Giersch et al., 2015; Giersch, Hotaling, Kovach, Jones, & Muhlfeld, 2016). This type of mountaintop island distribution can be a driver of genetic divergence within aquatic (e.g. Finn, Encalada, & Hampel, 2016; Finn, Theobald, Black, & Poff, 2006) and terrestrial species (Floyd, Van Vuren, & May, 2005; Hime et al., 2016). Indeed, if the same pattern of differentiation is found across multiple species, there is a greater probability that the island nature of the species distributions is a major driver of diversification. The genus *Lednia* has been the focus of significant, recent taxonomic attention, with studies of adult morphology resulting in an expansion of the genus from one to four species, with each species inhabiting a different mountain range or sub-range (a subdivision of a more expansive mountain range) in North America (Baumann & Call, 2012; Baumann & Kondratieff, 2010). Morphological variation within *Z. glacier*, however, is poorly understood and no morphological comparison across its range has been performed, largely due to the difficulty of collecting adult specimens. *Zapada glacier* populations inhabit the same isolated Rocky Mountain sub-ranges as two of the described *Lednia* species, *L. tumana* (Glacier National Park [GNP] and vicinity) and *L. tetonica* (Teton Range of northwest Wyoming). Previous molecular analyses lent preliminary support to possible genetic divergence between GNP and Teton Range Z. glacier populations (Giersch et al., 2015, 2016). Therefore, these two groups (*L. tumana*/*L. tetonica* and co-distributed *Z. glacier* populations) and their mountaintop distributions provide a useful framework for applying comparative population genetic methods to clarify species boundaries, both existing and possibly undescribed, while also refining our understanding of the distributions, evolutionary history, and contemporary connectivity of high-elevation lotic taxa that are likely vulnerable to rapidly changing climate and hydrology.

Investigating the potential for cryptic speciation in rare and/or understudied taxonomic lineages has important conservation implications (Hime et al., 2016). Both *Z. glacier* and *L. tumana* have been petitioned for listing under the U.S. Endangered Species Act due to climate change-induced loss of alpine glaciers and permanent snowfields (U.S. Fish and Wildlife Service, 2016). However, the story of alpine cryosphere decline driving hydrological shifts in stream habitats and threats to resident biota is not limited to the Rocky Mountains, as it is playing out in high-altitude regions worldwide (Hall & Fagre, 2003; Hansen et al., 2005; Pederson, Graumlich, Fagre, Kipfer, & Muhlfeld, 2010; Roe, Baker, & Herla, 2016). Linked to these changes is the potential loss of entire communities of meltwater-dependent alpine organisms (Giersch et al., 2016; Hotaling, Finn, Giersch, Weisrock, & Jacobsen, 2017; Hotaling, Hood, & Hamilton, 2017; Hotaling, Tronstad, & Bish, 2017; Muhlfeld et al., 2011) and, in most cases, little to no

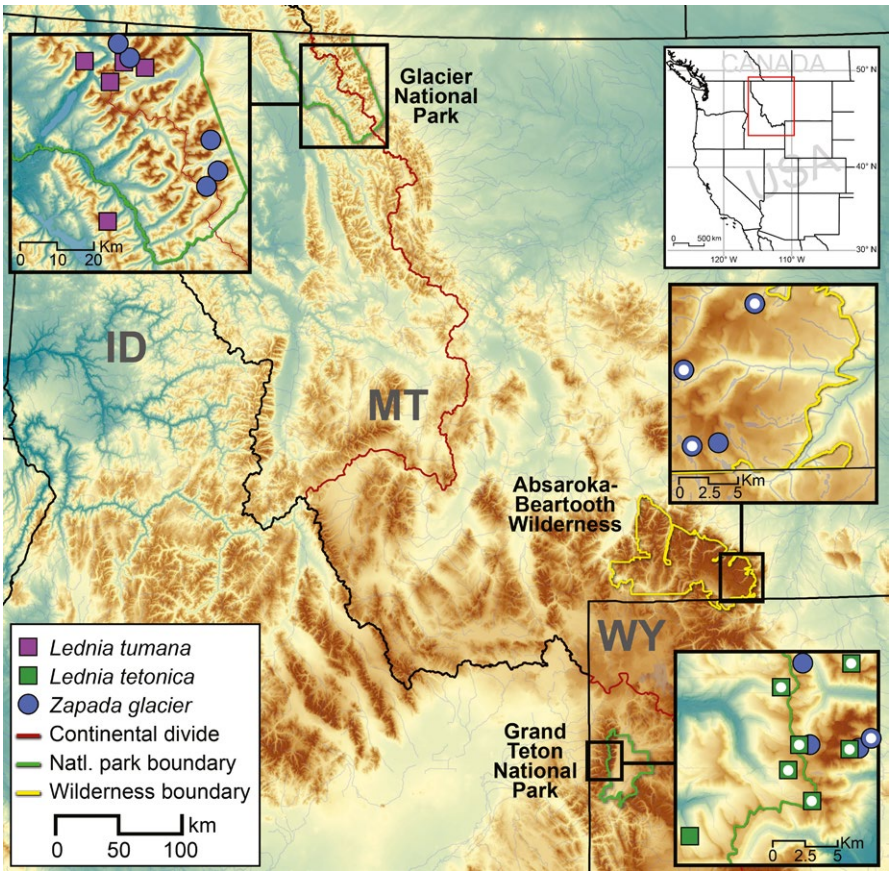

FIGURE 1 Collection localities for *Zapada glacier*, *Lednia tumana*, and *Lednia tetonica* specimens included in this study. The study area shown includes Glacier National Park, the Absaroka–Beartooth Wilderness, and Grand Teton National Park superimposed on an elevation gradient. Detailed locality information is included in Table 1. Circles with white fill indicate the 10 new populations (four of *Z. glacier*, six of *L. tetonica*) identified in this study. Although not explicitly shown, known ranges of all three species align with the sampling distributions shown here

systematic information regarding what could be lost, whether existing genetic diversity or species, exists (Bálint et al., 2011; Finn, Khamis, & Milner, 2013). As harbingers of climate change in North America, *Z. glacier* and *Lednia* are important indicator taxa of vulnerable, high-alpine ecosystems.

Here, we combined mitochondrial DNA (mtDNA) sequence data for three alpine stoneflies with our current understanding of their habitat and distributions to address four questions: (1) Is there genetic support for the existing morphology-based species boundaries in *Lednia*? (2) Is there evidence for corresponding, but undescribed, cryptic species diversity within *Z. glacier*? (3) Do similarities in geographic distribution (e.g. mountaintop isolation) and habitat requirements yield congruent spatial population genetic patterns for *Lednia* and *Z. glacier*? (4) Is there evidence for contemporary gene flow among isolated populations of either group? Our results highlight the utility of comparative population genetics for strengthening existing morphology-based species descriptions while also improving understanding of the evolutionary histories of ecologically similar, co-occurring aquatic species.

2 | METHODS

2.1 | Study species and field sampling

Zapada glacier (Supporting Information Figure S1a) is known to occur in three mountainous regions: GNP of northwest Montana, the Absaroka–Beartooth Wilderness (ABW) of southern Montana, and the Teton Range of northwest Wyoming (Figure 1; Giersch et al., 2016). Conversely, both focal *Lednia* species in this study are endemic to a single mountain sub-range: *L. tumana* (GNP and vicinity; Supporting Information Figure S1b) and *L. tetonica* (Teton Range; Figure 1, Supporting Information Figure S1c). All three focal species—*Z. glacier*, *L. tumana*, and *L. tetonica*—exhibit restricted, alpine distributions. In their respective ranges, both *Lednia* species co-occur with *Z. glacier*. Overall, the genus *Zapada* is widely distributed, with seven recognised species in the western United States (Baumann, 1975; Baumann, Gaufin, & Surdick, 1977), whereas *Lednia* includes just two other species that are also sub-range endemics: *L. borealis* of the Cascades in Washington and *L. sierra* of the Sierra Nevada in California (Baumann & Kondratieff, 2010). While no described *Lednia* species occur in sympatry, many *Zapada* species do. However, as nymphs, *Zapada* species are difficult to distinguish from one another morphologically (Baumann & Gaufin, 1971). We overcame this identification challenge through DNA barcoding of all *Zapada* nymphs collected for this study (see *DNA barcoding* below).

During the summers of 2015 and 2016, we sampled *Zapada* and *Lednia* specimens from alpine streams in GNP, ABW, and the Teton Range (Figure 1, Supporting Information Figure S1d). To provide broader phylogenetic and population genetic context for our focal species data set, we also obtained mtDNA sequences from *Zapada* specimens representing the full western taxonomy from mountain

Species	Stream	Sub-range	N	Latitude, longitude	Elev. (m)
Z. glacier	Piegan Pass	GNP	16	48.7294, −113.6972	1,911
Z. glacier	Upper Grinnell Lake	GNP	37	48.7574, −113.7248	1,951
Z. glacier	Appistoki Creek	GNP	87	48.4589, −113.3489	2,097
Z. glacier	Dry Fork Spring	GNP	55	48.5345, −113.3805	2,207
Z. glacier	Buttercup Park	GNP	3	48.4237, −113.3844	1,915
Z. glacier	*Jasper Lake	ABW	2	45.0233, −109.5785	3,216
Z. glacier	*Timberline Lake	ABW	5	45.1325, −109.5077	2,966
Z. glacier	Frosty Lake	ABW	6	45.0261, −109.5515	3,194
Z. glacier	*W. Fork Rock Creek	ABW	10	45.0962, −109.6040	3,001
Z. glacier	*Delta Lake	GRTE	1	43.7325, −110.7750	2,754
Z. glacier	Teton Meadows	GRTE	21	43.7259, −110.7904	2,824
Z. glacier	S. Cascade Creek	GRTE	6	43.7285, −110.8373	2,948
Z. glacier	Mica Lake Outlet	GRTE	7	43.7854, −110.8414	2,886
L. tumana	Lunch Creek	GNP	23	48.7052, −113.7046	2,156
L. tumana	Sexton Glacier	GNP	31	48.7003, −113.6281	1,992
L. tumana	Siyeh Bend	GNP	4	48.7115, −113.6751	1,943
L. tumana	Bearhat Mountain	GNP	10	48.6650, −113.7491	1,957
L. tumana	Heavens Peak	GNP	1	48.7102, −113.8427	2,042
L. tumana	Grant Glacier	GNP	1	48.3314, −113.7368	1,606
L. tetonica	*W. Buck Mtn	GRTE	6	43.6895, −110.8327	3,119
L. tetonica	*Sunset Lake	GRTE	6	43.7102, −110.8556	2,949
L. tetonica	*Schoolroom Glacier	GRTE	6	43.7286, −110.8440	3,039
L. tetonica	Wind Cave	GRTE	6	43.6657, −110.9561	2,676
L. tetonica	*Teton Meadows	GRTE	6	43.7258, −110.7931	2,845
L. tetonica	*N. Fork Teton Creek	GRTE	6	43.7681, −110.8615	2,780
L. tetonica	*Upper Paintbrush	GRTE	7	43.7852, −110.7941	2,805

TABLE 1 Sampling information for all *Zapada glacier*, *Lednia tumana*, and *Lednia tetonica* specimens included in this study. Sub-range refers to the primary geographic area where specimens were collected. *N* is the sample size for a given locality. Elevation is reported in meters. GNP: Glacier National Park; ABW: Absaroka–Beartooth Wilderness; GRTE: Grand Teton National Park/Teton Range. All lake locations are referring to inlet streams unless otherwise indicated. Complete sampling information for all taxa is included in Supporting Information Table S1. Asterisks next to stream names indicate populations newly identified in this study

streams in California, Washington, New Mexico, and Oregon as well as sequences from *L. sierra* collected in Cold Water Creek in central California and *L. borealis* from Snow Lake in Mount Rainier National Park, WA (Supporting Information Figure S2). Between our own and previous studies (Giersch et al., 2015, 2016), at least 300 streams have been surveyed for *Z. glacier* across our study area and only 13 populations (including this study) have been identified. For *Lednia*, despite considerable effort, the genus has not been observed in the ABW (J. J. Giersch and D. S. Finn, unpublished) nor in lower elevation streams (Tronstad, Hotaling, & Bish, 2016) or high-elevation lakes (Hotaling, Tronstad, et al., 2017) of the Teton Range. Sampling information for all localities and species included in this study is provided in Tables 1 and Supporting Information Tables S1–S2.

2.2 | DNA barcoding

We sequenced the *DNA barcoding* portion of the mtDNA genome, a 658-bp region of the cytochrome *c* oxidase I (COI) subunit, for 79 newly collected specimens of *Zapada* spp. ($n = 34$), *L. tetonica* ($n = 43$), *L. sierra* ($n = 1$), and *L. borealis* ($n = 1$). COI is commonly used in DNA barcoding as it is variable both within and among species, yet retains conserved primer binding sites (Hebert, Cywinska, & Ball, 2003). Barcoding was performed by the Canadian Center for DNA Barcoding (CCDB) following established protocols for extraction (Ivanova, Dewaard, & Hebert, 2006), polymerase chain reaction (PCR), and sequencing (Dewaard, Ivanova, Hajibabaei, & Hebert, 2008; Hajibabaei et al., 2005). For PCR, the primer sets

LCO1490/HCO2198 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) were used to amplify the target fragment of COI. Successful PCR amplicons were checked on a 2% agarose gel, and products were cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA, U.S.A.). Purified amplicons were cycle-sequenced using a Big Dye v3.1 dye termination kit, purified using Sephadex, and sequenced bidirectionally on an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, U.S.A.). Additional information on the methods and pipelines used for barcoding by CCDB are available at http://ccdb.ca/resources/. Sample information, photographs, and sequences of newly barcoded specimens are available through the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007; project name = *LDZP*). After barcoding, COI sequences were visually inspected, corrected, and aligned using MUSCLE (Edgar, 2004) as implemented in Geneious version 6.1.8 (Kearse et al., 2012).

To confirm the identity of *Z. glacier* specimens and generate a complete genetic data set for our focal stoneflies, we combined the 79 new COI sequences with data from three published studies: two focused on *Zapada* spp. (Giersch et al., 2015, 2016) and one on *L. tumana* (Jordan et al., 2016). GenBank and BOLD accession information for all new and previously published sequence data can be found in Supporting Information Table S2. To limit any influence of temporal genetic change (e.g. loss of haplotypes, Jordan et al., 2016), only specimens collected after 2010 were included in this study with the exception of six *Z. glacier* samples from ABW that were collected in 2000. For *Zapada*, the final data set contained 460 specimens: 256 sequences for *Z. glacier* and 204 sequences representing all other species in the western *Zapada* taxonomy. For *Lednia*, the final data set contained 115 specimens: 70 *L. tumana* sequences, 43 *L. tetonica* sequences, and one sequence each for *L. borealis* and *L. sierra*.

2.3 | Gene tree estimation, haplotype network construction, and population genetic analyses

For phylogenetic analyses, we analysed the *Zapada* and *Lednia* data sets separately with *Visoka cataractae* (Plecoptera: Nemouridae) serving as the outgroup for all *Zapada* specimens and *Z. glacier* as the outgroup for *Lednia*. To construct trees, we first used an Akaike information criterion (AIC) test implemented in MrModeltest (Nylander, 2004) to select the best-fit model of DNA substitution (GTR + I + G). Next, we used MrBayes version 3.2.4 (Ronquist et al., 2012) to generate mtDNA gene trees for each data set with five chains analysed for 10-million generations preceded by a 1-million generation burn-in. Samples were taken every 10,000 generations for two replicates. Convergence was determined by inspecting values of effective sample size (ESS > 200) in Tracer v1.6.0 (Rambaut & Drummond, 2007). Retained posterior distributions for each replicate were combined to generate a majority-rule consensus tree. Our 34 newly barcoded *Zapada* specimens were identified based upon which clade they belonged to in the consensus *Zapada* tree.

We constructed haplotype networks by compressing sequences into common haplotypes using the ALTER web server (Glez-Peña, Gomez-Blanco, Reboiro-Jato, Fdez-Riverola, & Posada, 2010) and generating networks in POPART (Leigh & Bryant, 2015) with the TCS implementation (Clement, Posada, & Crandall, 2000). We performed a nested analysis of molecular variance (AMOVA) in Arlequin 3.5 (Excoffier & Lischer, 2010) to assess how genetic variation was partitioned across multiple sampling levels (among sub-ranges, among populations within sub-ranges, and within populations). AMOVAs were performed separately on the *Z. glacier* and *L. tumana* + *L. tetonica* data sets. We assessed significance and 95% confidence intervals using 5,000 bootstrap replicates. We also calculated nucleotide diversity (π) for four spatially defined groups: *Z. glacier* across its full range, *Z. glacier* by mountain sub-range, *L. tumana*, and *L. tetonica*.

2.4 | Demographic model selection and gene flow estimation

For both the *Z. glacier* and *L. tumana* + *L. tetonica* data sets, we tested a range of demographic models and characterised gene flow parameters (when applicable) in a coalescent framework with Migrate-n v3.6 (Beerli & Felsenstein, 2001). For *Z. glacier*, we tested eight 3-lineage models, which ranged from isolation to panmixia (Figure 2a). For *L. tumana* and *L. tetonica*, we tested five similar, two-lineage models (Figure 2b). For all Migrate-n analyses, initial parameter values were calculated using F_{ST} and model averaging was used to estimate migration rate (m) and population size (θ). For the two models without migration (*Z. glacier*: Models 7 and 8, *L. tumana* + *L. tetonica*: Models 4 and 5; Figure 2), we followed Beerli and Palczewski (2010) in specifying a very small (m = 0.001), uniform custom migration rate among groups. We estimated the transition/transversion ratio (ti/tv) from sequence alignments for each group via maximum likelihood model selection in jmodeltest2.1.10 (Darriba, Taboada, Doallo, & Posada, 2012). These ratios were 4.70 and 15.63 for *Z. glacier* and *L. tumana* + *L. tetonica*, respectively. For all runs, a static heating strategy with four short chains (temperatures of 1.0, 1.5, 3.0, and 1.0×10^6) and one long chain was used. We recorded 25,000 steps every 100 generations with 10,000 steps discarded as burn-in. To ensure Markov chain stationarity, we examined ESS values for each parameter with a minimum threshold of 200. To select among models, we used the Bezier approximation score to calculate log Bayes factors (LBFs) and probabilities for each model following Beerli and Palczewski (2010). We calculated number of migrants per generation using the equation, $Nm = M \times \theta$.

3 | RESULTS

3.1 | *Zapada* barcoding, phylogenetics, and population genetics

Our final COI alignment for *Zapada* was 658-bp long with 2.49% missing data across all specimens and 1.95% missing data for *Z. glacier* only. Phylogenetic analyses supported the seven recognised western North American *Zapada* species as monophyletic with posterior probabilities (PPs) of 1.0 (Figure 3a). Of our 34 newly barcoded *Zapada* specimens, 18 were identified as *Z. glacier*. These new specimens

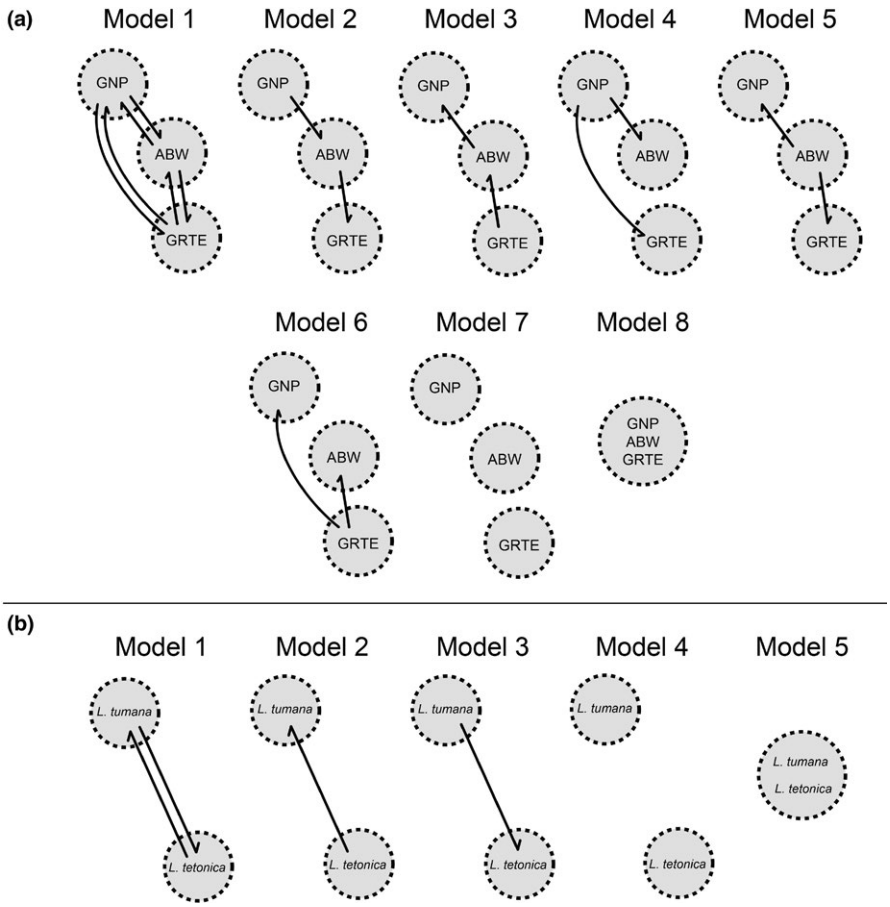

FIGURE 2 Phylogeographic models tested in Migrate-n for (a) *Zapada glacier* and (b) *Lednia tumana* and *Lednia tetonica*. GNP: Glacier National Park; ABW: Absaroka–Beartooth Wilderness; GRTE: the Teton Range. Black arrows indicate the direction of gene flow

were from four streams where *Z. glacier* had not previously been recorded: three in ABW and one in the Teton Range (Figure 1; Table 1), bringing the total number of streams known to contain *Z. glacier* to 13. A haplotype network connecting all *Z. glacier* specimens (n = 256) included 20 haplotypes from three sub-ranges: GNP (n = 198 specimens; 14 haplotypes), ABW (n = 23 specimens; two haplotypes), and the Teton Range (n = 35 specimens; five haplotypes). Each sub-range was generally characterised by a distinct haplotype group; however, haplotypes were relatively shallowly diverged within sub-ranges (maximum = 0.6% divergence within GNP) and only slightly more diverged among them (maximum = 1.2% divergence between any two *Z. glacier* haplotypes, Figure 4a). Interestingly, one unique haplotype was found at both the Grinnell Glacier site in GNP (n = 1) and all four sites in ABW (n = 22). When the full western *Zapada* taxonomy was connected in a haplotype network (Supporting Information Figure S3), relationships reflected those in the mtDNA gene tree (Figure 3a). Described and potentially cryptic species-level *Zapada* lineages differed from closely related taxa by 4.26% (e.g. *Z. glacier* to *Z. haysi*) to 8.35% (*Z. cinctipes* to *Z. columbiana*; Supporting Information Figure S3).

Differentiation among sub-ranges explained 58.7% of the total observed variation and within-population variation explained 41.1%, with little variation explained by populations within sub-ranges (0.2%). Overall, Φ_{ST} (0.59) and Φ_{CT} (0.59) were significant (Table 2), revealing that the majority of population structure in *Z. glacier* was explained by isolation among sub-ranges, rather than isolation among populations occupying the same sub-range. Nucleotide diversity (π) for *Z. glacier* was 0.0696 and for each sub-range: GNP (π = 0.0203), ABW (π = 0.0003), and the Teton Range (π = 0.0066; Table 2).

3.2 | *Lednia* barcoding, phylogenetics, and population genetics

Our final COI alignment for *Lednia* (n = 115) was 658-bp long with 1.27% missing data. We confirmed the presence of *L. tetonica* at its only previously known location, the outlet stream from Wind Cave (Baumann & Call, 2012), and new field surveys expanded this distribution to seven streams, all still within the Teton Range (Figure 1, Table 1). For other *Lednia* species, we did not identify any new localities beyond those previously described (Baumann & Call, 2012; Baumann & Kondratieff, 2010; Giersch et al., 2016; Jordan et al., 2016; Muhlfeld et al., 2011). Phylogenetic analyses strongly supported the existing, morphology-based *Lednia* taxonomy with PPs of 1.0 for all nodes and described species resolved as monophyletic (Figure 3b). *Lednia tetonica* and *L. tumana* were resolved as sister species, with *L. borealis* as the sister species to the *L. tetonica* + *L. tumana* clade, and *L. sierra* as the outgroup to the other three (Figure 3b).

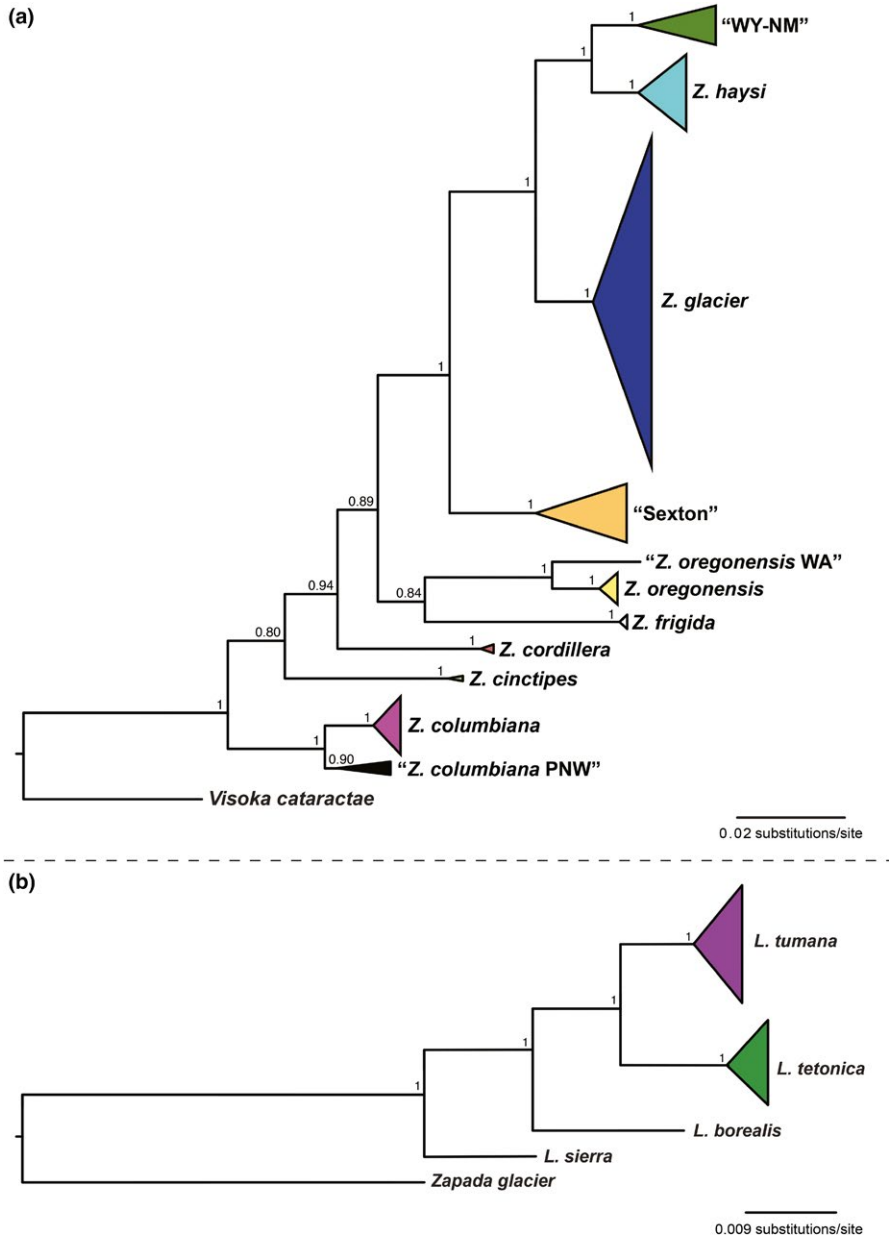

FIGURE 3 Cytochrome oxidase c subunit I (COI) gene trees of (a) western North American *Zapada*, and (b) the genus *Lednia* including 70 specimens from Jordan et al. (2016) and 45 newly barcoded specimens. Terminal nodes were compressed into triangles and scaled according to number of specimens. Numbers above nodes indicate posterior probabilities

Across all *Lednia* specimens, we identified five *L. tumana* haplotypes, seven *L. tetonica* haplotypes, and one haplotype each for the single specimens of *L. borealis* and *L. sierra*. The *Lednia* haplotype network revealed substantial divergence among described species (and, by proxy, among sub-ranges; Figure 4b). These divergences ranged from a minimum of 4.6% between *L. tumana* and *L. tetonica* to a maximum of 11.9% between *L. tumana* and *L. sierra* (Figure 4b). For *L. tumana* and *L. tetonica*, among species differentiation explained 95.3% of the total variation observed and within-population variation explained 4.4%, with little variation explained by populations within species (0.3%). Both Φ_{ST} (0.95) and Φ_{CT} (0.96) were significant. Like *Z. glacier*, the majority of genetic structure in *L. tumana* + *L. tetonica* was explained by isolation among sub-ranges (i.e. described species), rather than isolation among populations within sub-ranges (Table 2). Nucleotide diversity (π) for *L. tumana* and *L. tetonica* was 0.0035 and 0.0013, respectively (Table 2).

3.3 | Demographic model selection and gene flow estimation

For *Z. glacier*, the most supported demographic model was a *north-to-south* model, which included gene flow parameters for migration from GNP into ABW and ABW into the Teton Range (model 2, model probability ~1; Figure 2a, Table 3). All other models were strongly rejected (LBFs ≥ 12, model probabilities ≤ 2.4×10^{-3}). Interestingly, a no-migration model was among the least supported models (model 7; LBF = 47.3, model probability = 5.5×10^{-11}). For the best-fit model, the mean number of migrants per generation from GNP into ABW (Nm = 1.02, 95% confidence interval = 0–5.27) was estimated at twice that of ABW into the Teton Range (Nm = 0.5, 95% confidence interval = 0–2.75; Supporting Information Table S3). These estimates should be interpreted with caution, however, as both 95% confidence intervals included Nm = 0.

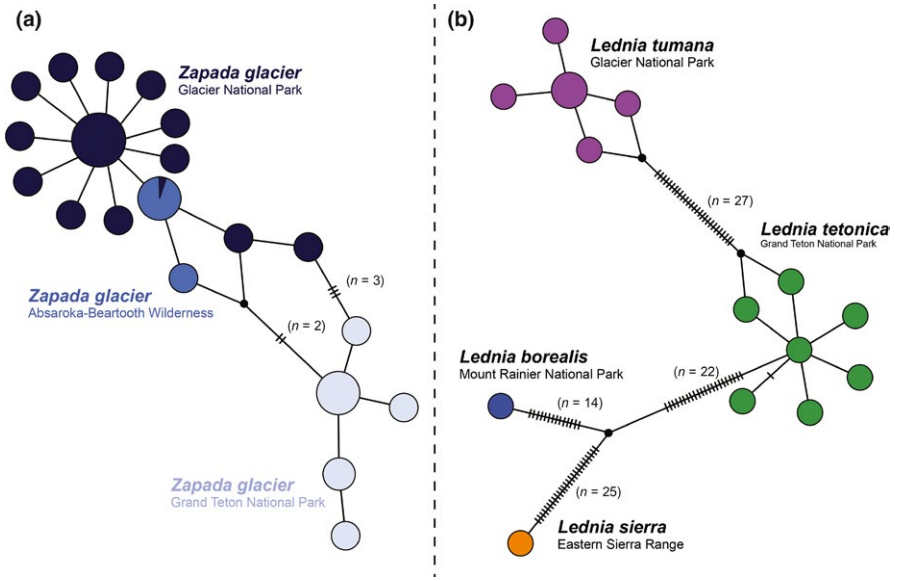

FIGURE 4 A Cytochrome oxidase c subunit I haplotype network of (a) all *Zapada glacier* specimens and (b) all representatives of the current *Lednia* taxonomy. Colored circles represent haplotypes (with circle size scaled by frequency). Hash marks between haplotypes represent one substitution step (i.e. one nucleotide difference)

TABLE 2 Population genetic diversity metrics and results of a nested AMOVA for specimens grouped by populations within sub-ranges. Symbols include: Φ_{CT} = among sub-range structure, Φ_{SC} = within sub-range structure, Φ_{ST} = population-level structure across the full study extent irrespective of group structure, and π = nucleotide diversity averaged over the entire COI locus. For "*Zapada glacier*, by range" the given π is for all *Z. glacier* specimens. Bold values are significant at $p \leq .05$

Group	π	Φ_{CT}	Φ_{SC}	Φ_{ST}
Z. glacier, by range	0.0696	**0.59**	0.01	**0.59**
Z. glacier, GNP	0.0203			
Z. glacier, ABW	0.0003			
Z. glacier, Teton Range	0.0066			
Lednia sp., by species		**0.96**	0.08	**0.95**
L. tumana	0.0035			
L. tetonica	0.0013			

For *L. tumana* and *L. tetonica*, the most supported demographic model included no migration between species (model 4, model probability ~1; Figure 2b, Table 3). All models including a gene flow parameter were rejected (LBFs ≥ 142.9, model probabilities ≤ 9.3×10^{-32}) as was the panmixia model (model 5, LBF = 529.5, model probability = 1.1×10^{-115}). Because the best-fit model did not include a gene flow parameter, we did not estimate migration rates between *L. tumana* and *L. tetonica*.

4 | DISCUSSION

Understanding the degree to which similar habitat requirements and geographic distributions extend to shared evolutionary histories is an important question in evolutionary biology. Previous studies have shown that shared distributions (Barber, Erdmann, Palumbi, & Ayre, 2006; Lapointe & Rissler, 2005) and ecological traits (Satler & Carstens, 2017; Whiteman et al., 2007) can both influence genetic differentiation, and either can drive spatial congruence of genetic structure. When framed in the context of multi-species comparisons across a study area, comparative population genetic studies can also provide a useful mechanism for uncovering the potential for cryptic species diversity within a group of interest. In this study, we first asked whether existing morphology-based species boundaries were supported by genetic data for the previously described *L. tumana* and *L. tetonica*. Next, we explored the reverse question of whether isolated populations of *Z. glacier* occurring in sympatry with the focal *Lednia* species may contain cryptic species-level diversity. We then considered whether similar geographic distributions, including mountaintop isolation, and ecological requirements for these three stoneflies have translated to spatial congruence of genetic structure and demographic history.

We found support for the existing delimitation of *L. tumana* and *L. tetonica* as separate species, with monophyly, deep evolutionary divergence, and no evidence for contemporary gene flow between them. Our results also supported the existing description of *Z. glacier* as a single species, with the species comprised of isolated populations associated with mountain sub-ranges. Our results, however, did support the potential for cryptic diversity in other lineages of *Zapada*, and future studies with additional genetic and taxonomic sampling across the genus are needed to explore this possibility (Figure 3a). The demographic history of *Z. glacier* was best described by a *north-to-south* migration model, with minimal (and perhaps non-existent) contemporary gene flow among sub-ranges (Supporting Information Table S3). Our support for a north-to-south migration model lends another line of evidence to a broader biogeographic hypothesis in North America, specifically that an immigration corridor existed along the spine of the Rocky Mountains from a Beringian refugium deep into the western U.S.A. (DeChaine & Martin, 2005;

TABLE 3 Phylogeographic model descriptions and selection results for (a) *Lednia tumana* and *Lednia tetonica*, and (b) *Zapada glacier* tested in Migrate-n. BAS: Bezier approximation score (log marginal likelihood). LBF: log Bayes factor; GNP: Glacier National Park; ABW: Absaroka–Beartooth Wilderness. LBFs and model probabilities calculated following Beerli and Palczewski (2010). Arrows (>) indicate the direction of migration for a given model. The best-fit model is highlighted in bold

Model	Description	BAS	LBF	Probability	Choice
(a) Lednia tumana and *Lednia tetonica*					
1	Full migration	−1,341.2	255.6	3.0×10^{-56}	4
2	Unidirectional: *L. tetonica* > *L. tumana*	−1,294.4	161.4	8.9×10^{-36}	3
3	Unidirectional: *L. tumana* > *L. tetonica*	−1,284.9	142.9	9.3×10^{-32}	2
4	**No migration**	**−1,213.4**	**–**	**~1**	**1**
5	Panmixia	−1,478.1	525.5	1.1×10^{-111}	5
(b) Zapada glacier					
1	Full migration	−1,317.5	64.5	1.0×10^{-14}	7
2	**North to south: GNP > ABW > Teton Range**	**−1,285.3**	**–**	**~1**	**1**
3	South to north: Teton Range > ABW > GNP	−1,291.3	12.1	2.4×10^{-3}	2
4	Out of GNP: GNP > ABW, GNP > Teton Range	−1,292.3	14.0	9.0×10^{-4}	3
5	Out of ABW: ABW > GNP, ABW > Teton Range	−1,301.0	31.5	1.4×10^{-7}	4
6	Out of the Teton Range: Teton Range > GNP, Teton Range > ABW	−1,315.3	60.0	9.2×10^{-14}	6
7	No migration	−1,308.9	47.3	5.5×10^{-11}	5
8	Panmixia	−1,393.0	215.4	1.7×10^{-47}	8

Finn & Adler, 2006). Our results should be interpreted with caution, however, as we only evaluated mtDNA, which reflects female-mediated gene flow and is a single genetic marker, independent of the nuclear genome. Discordance in population genetic inference between mitochondrial and nuclear genomes is relatively common (Gompert, Forister, Fordyce, & Nice, 2008; Toews & Brelsford, 2012; Weisrock, Shaffer, Storz, Storz, & Voss, 2006). As such, multi-locus nuclear data paired with coalescent-based species delimitation methods are needed before robust molecular conclusions can be drawn regarding both species boundaries and population genetic patterns (Grummer, Bryson, & Reeder, 2014; Hotaling et al., 2016; Yang & Rannala, 2010). A more concerted effort to collect and compare adults is also needed to assess the degree to which systematic morphological differences among *Z. glacier* populations and other major lineages of the genus may exist.

Our findings are generally congruent with other alpine stream population genetic studies. Observed patterns of differentiation in both *Z. glacier* and *Lednia* corresponded with a signature of mountaintop isolation (Finn & Adler, 2006; Finn et al., 2006, 2016; Jordan et al., 2016). Our results also support the possibility of underlying differences in timing, rate of divergence, and/or degree of contemporary gene flow between two highly similar species groups. Comparative population genetic studies are rare in alpine streams (Hotaling, Finn, et al., 2017; Hotaling, Hood, et al., 2017; Hotaling, Tronstad, et al., 2017), and of the few that have been conducted, the majority have emphasised comparisons of ecologically distinct but co-occurring species, with hypothesised links between patterns of genetic differentiation and dispersal ability or other biological traits that influence gene flow (Dussex, Chuah, & Waters, 2016; Monaghan, Spaak, Robinson, & Ward, 2002).

Variation in life-history (e.g. timing of emergence, voltinism) or other species traits (e.g. dispersal capacity) may underlie the differing depths of divergence between *Z. glacier* and *Lednia* observed in this study. The seasonal window for growth in the alpine is short, and *Z. glacier* and the two *Lednia* species emerge at different times. As with most nemourids, *Z. glacier* adults emerge early in summer (e.g. June), immediately after stream channels become exposed by snowmelt. In contrast, very few *Lednia* adults have been collected earlier than mid-August (Baumann & Call, 2012; Baumann & Kondratieff, 2010; Giersch et al., 2015, 2016), and *L. tumana* adults have been collected into October (Baumann & Kondratieff, 2010). The earlier emergence of *Z. glacier* may be more conducive to dispersal as there is a longer window of mild, summer weather versus the autumnal snows that probably end the reproductive season for many *Lednia* adults (e.g. Finn & Poff, 2008). Differences in voltinism (e.g. a faster generation time in Lednia), could also accelerate the accumulation of genetic drift leading to a signature of deeper divergence in the same or a shorter amount of time. In terms of dispersal capacity, *Lednia* adults may be weaker fliers than their *Zapada* counterparts. This possibility is supported by dispersal studies in the Colorado Rocky Mountains where *Zapada cinctipes* was the only stonefly caught actively crossing high ridgelines (D. S. Finn, personal observation). Variation in life-history traits and/or dispersal among co-occurring, closely related species (i.e. *Lednia* and *Zapada*) is not unprecedented,

having been observed for both congeneric caddisflies (Jackson & Resh, 1991) and other aquatic taxa (Finn & Poff, 2008; Monaghan et al., 2002).

Finally, differing depths of divergence may reflect genus-specific evolutionary trajectories. It is possible, and perhaps even likely, that *L. tumana* and *L. tetonica* have a longer history as cold-water specialists isolated in glacier associated refugia than *Z. glacier* (e.g. before the last glacial maximum in the Rocky Mountains ~20,000 years ago, Carrara, 1987). This timeline is supported by estimates of divergence timing among *L. tumana* genetic clusters in GNP, which placed intraspecies splits as occurring in the last ~18,000 years (Hotaling et al., 2018). Conversely, *Z. glacier* may have more recently invaded headwaters, possibly due to range contraction into higher elevations to follow the retreat of glaciers (e.g. Giersch et al., 2015) and/or to avoid competition with lower elevation species (Khamis, Brown, Hannah, & Milner, 2014). Moreover, all four species of *Lednia* are cold-water specialists (Baumann & Call, 2012; Baumann & Kondratieff, 2010), suggesting cold stenothermy as an ancestral trait to the clade, whereas *Z. glacier* is the only meltwater-dependent specialist within the widely distributed and more speciose *Zapada* genus. Future studies are required to clarify the relative influences of life-history variation as well as historical biogeography and time since divergence on contemporary patterns of genetic differentiation in *Lednia* and *Z. glacier*.

In light of the recent U.S. Fish and Wildlife Service recommendation to list *Z. glacier* and *L. tumana* under the Endangered Species Act due to climate change threats (U.S. Fish and Wildlife Service, 2016), the results of our field surveys in the Teton Range and ABW provide important refinement of the geographic distributions of *Z. glacier* as well as its understudied sister species, *L. tetonica*. We expanded the known distribution of *L. tetonica* from its type locality (Wind Cave, WY) to seven headwater streams in the Teton Range (Table 1), all of which are fed by permanent ice (either subterranean ice sources or surface glaciers). We also identified four populations of *Z. glacier* that were previously unknown: three in ABW and one in the Teton Range. *Zapada glacier* has now been documented in 13 alpine streams across the three sub-ranges (Table 1). Support for a north-to-south migration model for *Z. glacier* from GNP through ABW and into the Teton Range also highlights the potential importance of ABW as a dispersal stepping stone, recent and/or historical, between GNP and the Teton Range. The single *Z. glacier* haplotype shared between GNP and ABW and shallow sequence divergence between GNP and ABW populations (relative to the more deeply diverged Teton Range populations) also suggests that additional stepping-stone populations of *Z. glacier* might exist in other small, high-elevation sub-ranges between GNP and the ABW.

Beyond the focal stoneflies included in this study, entire assemblages of organisms tightly associated with meltwater-fed alpine streams are likely vulnerable to regional-scale extinction as climate change proceeds (e.g. Hotaling, Finn, et al., 2017; Hotaling, Hood, et al., 2017; Hotaling, Tronstad, et al., 2017; Jacobsen & Dangles, 2017; Wilhelm, Singer, Fasching, Battin, & Besemer, 2013). Weakly dispersing taxa are particularly at risk, as they are more susceptible to becoming caught in summit traps as they track colder conditions to higher elevations (Hotaling, Finn, et al., 2017; Hotaling, Hood, et al., 2017; Hotaling, Tronstad, et al., 2017; Sauer, Domisch, Nowak, & Haase, 2011; Sheldon, 2012). Given limited resources, a major question in conservation biology concerns the effectiveness of managing for one or a few indicator or umbrella species; that is, individual taxa whose conservation will, in turn, theoretically protect a multitude of co-occurring taxa (e.g. Roberge & Angelstam, 2004). Assessing the spatial congruence of population genetic patterns for more than one taxon provides an evolutionary approach to answering this question. All three species included in this study exhibited genetic isolation at the mountain sub-range scale, indicating that sub-ranges contain unique biodiversity components and should be managed as such. However, only a conservation plan developed for *Z. glacier* across its range would also protect both *Lednia* species. The reciprocal, a conservation plan developed for either *L. tumana* or *L. tetonica* would only protect *Z. glacier* across part of its range and provide no benefit to the other Lednia species. With global cryosphere decline proceeding with no signs of slowing down, an additional management emphasis should include the identification of alpine streams most likely to maintain at least small patches of permanent meltwater habitat in the near future as these streams may represent vital refugia for cold-adapted taxa (cf. Hotaling, Finn, et al., 2017; Hotaling, Hood, et al., 2017; Hotaling, Tronstad, et al., 2017; Morelli et al., 2016).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Bálint, M., Domisch, S., Engelhardt, C. H. M., Haase, P., Lehrian, S., Sauer, J., … Nowak, C. (2011). Cryptic biodiversity loss linked to global climate change. *Nature Climate Change*, *1*, 313–318. https://doi.org/10.1038/nclimate1191

Barber, P. H., Erdmann, M. V., Palumbi, S. R., & Ayre, D. (2006). Comparative phylogeography of three codistributed stomatopods: Origins and timing of regional lineage diversification in the coral triangle. *Evolution*, *60*, 1825–1839. https://doi.org/10.1111/j.0014-3820.2006.tb00526.x

Baumann, R. W. (1975). Revision of the stonefly family Nemouridae (Plecoptera): A study of the world fauna at the generic level. *Smithsonian Contributions to Zoology*, *211*, 1–74. https://doi.org/10.5479/si.00810282.211

Baumann, R. W., & Call, R. G. (2012). *Lednia tetonica*, a new species of stonefly from Wyoming (Plecoptera: Nemouridae). *Illiesia*, *8*, 104–110.

Baumann, R. W., & Gaufin, A. R. (1971). New species of *Nemoura* from western North America (Plecoptera: Nemouridae). *Pan-Pacific Entomologist*, *47*, 270–278.

Baumann, R. W., Gaufin, A. R., & Surdick, R. F. (1977). The stoneflies (Plecoptera) of the Rocky Mountains. *Memoirs of the American Entomological Society*, *31*, 1–208.

Baumann, R. W., & Kondratieff, B. C. (2010). The stonefly genus *Lednia* in North America (Plecoptera: Nemouridae). *Illiesia*, *6*, 315–327.

Beerli, P., & Felsenstein, J. (2001). Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 4563–4568. https://doi.org/10.1073/pnas.081068098

Beerli, P., & Palczewski, M. (2010). Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics*, *185*, 313–326. https://doi.org/10.1534/genetics.109.112532

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., … Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*, 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Brunsfeld, S., Sullivan, J., Soltis, D., & Soltis, P. (2001). Comparative phylogeography of northwestern North America: A synthesis. *Special Publication-British Ecological Society*, *14*, 319–340.

Carrara, P. E. (1987). Holocene and latest Pleistocene glacial chronology, Glacier National Park, Montana. *Canadian Journal of Earth Sciences*, *24*, 387–395. https://doi.org/10.1139/e87-041

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, *9*, 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*, 772. https://doi.org/10.1038/nmeth.2109

DeChaine, E. G., & Martin, A. P. (2005). Historical biogeography of two alpine butterflies in the Rocky Mountains: Broad-scale concordance and local-scale discordance. *Journal of Biogeography*, *32*, 1943–1956. https://doi.org/10.1111/j.1365-2699.2005.01356.x

Dewaard, J. R., Ivanova, N. V., Hajibabaei, M., & Hebert, P. D. (2008). Assembling DNA barcodes. In C. C. Martin (Ed.), *Methods in molecular biology: Environmental genomics* (pp. 275–293). Totowa, NJ: Humana Press.

Dussex, N., Chuah, A., & Waters, J. M. (2016). Genome-wide SNPs reveal fine-scale differentiation among wingless alpine stonefly populations and introgression between winged and wingless forms. *Evolution*, *70*, 38–47. https://doi.org/10.1111/evo.12826

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797. https://doi.org/10.1093/nar/gkh340

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Finn, D. S., & Adler, P. H. (2006). Population genetic structure of a rare high-elevation black fly, *Metacnephia coloradensis*, occupying Colorado lake outlet streams. *Freshwater Biology*, *51*, 2240–2251. https://doi.org/10.1111/j.1365-2427.2006.01647.x

Finn, D. S., Encalada, A. C., & Hampel, H. (2016). Genetic isolation among mountains but not between stream types in a tropical high-altitude mayfly. *Freshwater Biology*, *61*, 702–714. https://doi.org/10.1111/fwb.12740

Finn, D. S., Khamis, K., & Milner, A. M. (2013). Loss of small glaciers will diminish beta diversity in Pyrenean streams at two levels of biological organization. *Global Ecology and Biogeography*, *22*, 40–51. https://doi.org/10.1111/j.1466-8238.2012.00766.x

Finn, D. S., & Poff, N. L. (2008). Emergence and flight activity of alpine stream insects in two years with contrasting winter snowpack. *Arctic, Antarctic, and Alpine Research*, *40*, 638–646. https://doi.org/10.1657/1523-0430(07-072)[finn]2.0.co;2

Finn, D. S., Theobald, D. M., Black, W. C., & Poff, N. L. (2006). Spatial population genetic structure and limited dispersal in a Rocky Mountain alpine stream insect. *Molecular Ecology*, *15*, 3553–3566. https://doi.org/10.1111/j.1365-294x.2006.03034.x

Floyd, C. H., Van Vuren, D. H., & May, B. (2005). Marmots on Great Basin mountaintops: Using genetics to test a biogeographic paradigm. *Ecology*, *86*, 2145–2153. https://doi.org/10.1890/04-1227

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*, 294–299.

Giersch, J. J., Hotaling, S., Kovach, R. P., Jones, L. A., & Muhlfeld, C. C. (2016). Climate-induced glacier and snow loss imperils alpine stream insects. *Global Change Biology*, *23*, 2577–2589.

Giersch, J. J., Jordan, S., Luikart, G., Jones, L. A., Hauer, F. R., & Muhlfeld, C. C. (2015). Climate-induced range contraction of a rare alpine aquatic invertebrate. *Freshwater Science*, *34*, 53–65. https://doi.org/10.1086/679490

Glez-Peña, D., Gomez-Blanco, D., Reboiro-Jato, M., Fdez-Riverola, F., & Posada, D. (2010). ALTER: Program-oriented conversion of DNA and protein alignments. *Nucleic Acids Research*, *38*, W14–W18. https://doi.org/10.1093/nar/gkq321

Goldberg, C. S., & Waits, L. P. (2010). Comparative landscape genetics of two pond-breeding amphibian species in a highly modified agricultural landscape. *Molecular Ecology*, *19*, 3650–3663. https://doi.org/10.1111/j.1365-294x.2010.04673.x

Gompert, Z., Forister, M. L., Fordyce, J. A., & Nice, C. C. (2008). Widespread mito-nuclear discordance with evidence for introgressive hybridization and selective sweeps in Lycaeides. *Molecular Ecology*, *17*, 5231–5244. https://doi.org/10.1111/j.1365-294x.2008.03988.x

Grummer, J. A., Bryson, R. W., & Reeder, T. W. (2014). Species delimitation using Bayes factors: Simulations and application to the *Sceloporus scalaris* species group (Squamata: Phrynosomatidae). *Systematic Biology*, *63*, 119–133. https://doi.org/10.1093/sysbio/syt069

Hajibabaei, M., Dewaard, J. R., Ivanova, N. V., Ratnasingham, S., Dooh, R. T., Kirk, S. L., … Hebert, P. D. N. (2005). Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society B-Biological Sciences*, *360*, 1959–1967. https://doi.org/10.1098/rstb.2005.1727

Hall, M. H. P., & Fagre, D. B. (2003). Modeled climate-induced glacier change in Glacier National Park, 1850-2100. *BioScience*, *53*, 131–140. https://doi.org/10.1641/0006-3568(2003)053[0131:mcigci]2.0.co;2

Hansen, J., Nazarenko, L., Ruedy, R., Sato, M., Willis, J., Del Genio, A., … Tausnev, N. (2005). Earth's energy imbalance: Confirmation and implications. *Science*, *308*, 1431–1435. https://doi.org/10.1126/science.1110252

Hebert, P. D. N., Cywinska, A., & Ball, S. L. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*, 313–321. https://doi.org/10.1098/rspb.2002.2218

Hewitt, G. (2000). The genetic legacy of the quaternary ice ages. *Nature*, *405*, 907–913. https://doi.org/10.1038/35016000

Hime, P. M., Hotaling, S., Grewelle, R. E., O'Neill, E. M., Voss, S. R., Shaffer, H. B., & Weisrock, D. W. (2016). The influence of locus number and information content on species delimitation: An empirical test case in an endangered Mexican salamander. *Molecular Ecology*, *25*, 5959–5974. https://doi.org/10.1111/mec.13883

Hotaling, S., Finn, D. S., Giersch, J. J., Weisrock, D. W., & Jacobsen, D. (2017). Climate change and alpine stream biology: Progress, challenges, and opportunities for the future. *Biological Reviews*, *92*, 2024–2045. https://doi.org/10.1111/brv.12319

Hotaling, S., Foley, M. E., Lawrence, N. M., Bocanegra, J., Blanco, M. B., Rasoloarison, R., … Weisrock, D. W. (2016). Species discovery and validation in a cryptic radiation of endangered primates: Coalescent-based species delimitation in Madagascar's mouse lemurs. *Molecular Ecology*, *25*, 2029–2045. https://doi.org/10.1111/mec.13604

Hotaling, S., Hood, E., & Hamilton, T. L. (2017). Microbial ecology of mountain glacier ecosystems: Biodiversity, ecological connections, and implications of a warming climate. *Environmental Microbiology*, *19*, 2935–2948. https://doi.org/10.1111/1462-2920.13766

Hotaling, S., Muhlfeld, C. C., Giersch, J. J., Ali, O. A., Jordan, S., Miller, M. R., … Weisrock, D. W. (2018). Demographic modeling reveals a history of divergence with gene flow for a glacially-tied stonefly in a changing post-Pleistocene landscape. *Journal of Biogeography*, *45*, 304–317. https://doi.org/10.1111/jbi.13125

Hotaling, S., Tronstad, L. M., & Bish, J. (2017). Macroinvertebrate diversity is lower in high-elevation lakes versus nearby streams: Evidence from Grand Teton National Park, Wyoming. *Journal of Natural History*, *51*, 1657–1669. https://doi.org/10.1080/00222933.2017.1353149

Hughes, J. M., Schmidt, D. J., & Finn, D. S. (2009). Genes in streams: Using DNA to understand the movement of freshwater fauna and their riverine habitat. *BioScience*, *59*, 573–583. https://doi.org/10.1525/bio.2009.59.7.8

Ivanova, N. V., Dewaard, J. R., & Hebert, P. D. N. (2006). An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, *6*, 998–1002. https://doi.org/10.1111/j.1471-8286.2006.01428.x

Jackson, J. K., Battle, J. M., White, B. P., Pilgrim, E. M., Stein, E. D., Miller, P. E., & Sweeney, B. W. (2014). Cryptic biodiversity in streams: A comparison of macroinvertebrate communities based on morphological and DNA barcode identifications. *Freshwater Science*, *33*, 312–324. https://doi.org/10.1086/675225

Jackson, J. K., & Resh, V. H. (1991). Periodicity in mate attraction and flight activity of three species of caddisflies (Trichoptera). *Journal of the North American Benthological Society*, *10*, 198–209. https://doi.org/10.2307/1467578

Jacobsen, D., & Dangles, O. (2017). *Ecology of high altitude waters*. Oxford, UK: Oxford University Press. https://doi.org/10.1093/oso/9780198736868.001.0001

Jordan, S., Giersch, J. J., Muhlfeld, C. C., Hotaling, S., Fanning, L., Tappenbeck, T. H., & Luikart, G. (2016). Loss of genetic diversity and increased subdivision in an endemic alpine stonefly threatened by climate change. *PLoS ONE*, *11*, e0157386. https://doi.org/10.1371/journal.pone.0157386

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Drummond, A. (2012). Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Khamis, K., Brown, L. E., Hannah, D. M., & Milner, A. M. (2014). Experimental evidence that predator range expansion modifies alpine stream community structure. *Freshwater Science*, *34*, 66–80.

Lapointe, F. J., & Rissler, L. J. (2005). Congruence, consensus, and the comparative phylogeography of codistributed species in California. *The American Naturalist*, *166*, 290–299. https://doi.org/10.1086/431283

Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*, 1110–1116. https://doi.org/10.1111/2041-210x.12410

Lourie, S., Green, D., & Vincent, A. (2005). Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: Hippocampus). *Molecular Ecology*, *14*, 1073–1094. https://doi.org/10.1111/j.1365-294x.2005.02464.x

Miller, M. P., Blinn, D. W., & Keim, P. (2002). Correlations between observed dispersal capabilities and patterns of genetic differentiation in populations of four aquatic insect species from the Arizona White Mountains, USA. *Freshwater Biology*, *47*, 1660–1673. https://doi.org/10.1046/j.1365-2427.2002.00911.x

Monaghan, M. T., Spaak, P., Robinson, C. T., & Ward, J. V. (2002). Population genetic structure of 3 alpine stream insects: Influences of gene flow, demographics, and habitat fragmentation. *Journal of the North American Benthological Society*, *21*, 114–131. https://doi.org/10.2307/1468304

Morelli, T. L., Daly, C., Dobrowski, S. Z., Dulen, D. M., Ebersole, J. L., Jackson, S. T., … Nydick, K. R. (2016). Managing climate refugia for climate adaptation. *PLoS ONE*, *11*, e0159909. https://doi.org/10.1371/journal.pone.0159909

Muhlfeld, C. C., Giersch, J. J., Hauer, F. R., Pederson, G. T., Luikart, G., Peterson, D. P., … Fagre, D. B. (2011). Climate change links fate of glaciers and an endemic alpine invertebrate. *Climatic Change*, *106*, 337–345. https://doi.org/10.1007/s10584-011-0057-1

Nylander, J. A. A. 2004) MrModeltest v2. Evolutionary Biology Centre, Uppsala University, Sweden.

Pederson, G. T., Graumlich, L. J., Fagre, D. B., Kipfer, T., & Muhlfeld, C. C. (2010). A century of climate and ecosystem change in western Montana: What do temperature trends portend? *Climatic Change*, *98*, 133–154. https://doi.org/10.1007/s10584-009-9642-y

Phillipsen, I. C., Kirk, E. H., Bogan, M. T., Mims, M. C., Olden, J. D., & Lytle, D. A. (2014). Dispersal ability and habitat requirements determine landscape-level genetic patterns in desert aquatic insect. *Molecular Ecology*, *24*, 54–69.

Rambaut, A., & Drummond, A. (2007). *Tracer v 1.4*. Program distributed by the authors. Retrieved from beast.bio.ed.ac.uk/Tracer. The University of Edinburgh.

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The barcode of life data system (http://www.barcodinglife.org). *Molecular Ecology Notes*, *7*, 355–364.

Roberge, J., & Angelstam, P. (2004). Usefulness of the umbrella species concept as a conservation tool. *Conservation Biology*, *18*, 76–85. https://doi.org/10.1111/j.1523-1739.2004.00450.x

Roe, G. H., Baker, M. B., & Herla, F. (2016). Centennial glacier retreat as categorical evidence of regional climate change. *Nature Geoscience*, *10*, 95.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Hohna, S., … Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542. https://doi.org/10.1093/sysbio/sys029

Satler, J. D., & Carstens, B. C. (2017). Do ecological communities disperse across biogeographic barriers as a unit? *Molecular Ecology*, *26*, 3533–3545. https://doi.org/10.1111/mec.14137

Sauer, J., Domisch, S., Nowak, C., & Haase, P. (2011). Low mountain ranges: Summit traps for montane freshwater species under climate change. *Biodiversity and Conservation*, *13*, 3133–3146. https://doi.org/10.1007/s10531-011-0140-y

Sheldon, A. L. (2012). Possible climate-induced shift of stoneflies in a southern Appalachian catchment. *Freshwater Science*, *31*, 765–774. https://doi.org/10.1899/11-135.1

Toews, D. P., & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, *21*, 3907–3930. https://doi.org/10.1111/j.1365-294x.2012.05664.x

Tronstad, L. M., Hotaling, S., & Bish, J. C. (2016). Longitudinal changes in stream invertebrate assemblages of Grand Teton National Park, Wyoming. *Insect Conservation and Diversity*, *9*, 320–331. https://doi.org/10.1111/icad.12169

U.S. Fish and Wildlife Service (2016). Endangered and threatened wildlife and plants; 12-month finding on a petition to list the western glacier

stonefly as an endangered or threatened species; proposed threatened species status for Meltwater Lednian Stonefly and Western Glacier Stonefly. *Federal Register*, *81*, 68379–68397.

Weisrock, D. W., Shaffer, H. B., Storz, B. L., Storz, S. R., & Voss, S. R. (2006). Multiple nuclear gene sequences identify phylogenetic species boundaries in the rapidly radiating clade of Mexican ambystomatid salamanders. *Molecular Ecology*, *15*, 2489–2503. https://doi.org/10.1111/j.1365-294x.2006.02961.x

Whiteman, N. K., Kimball, R. T., & Parker, P. G. (2007). Co-phylogeography and comparative population genetics of the threatened Galápagos hawk and three ectoparasite species: Ecology shapes population histories within parasite communities. *Molecular Ecology*, *16*, 4759–4773. https://doi.org/10.1111/j.1365-294x.2007.03512.x

Wilhelm, L., Singer, G. A., Fasching, C., Battin, T. J., & Besemer, K. (2013). Microbial biodiversity in glacier-fed streams. *The ISME Journal*, *8*, 1651. https://doi.org/10.1038/ismej.2013.44

Yang, Z., & Rannala, B. (2010). Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 9264–9269. https://doi.org/10.1073/pnas.0913022107

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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