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Genomic data reveal similar genetic differentiation in aquifer species with different dispersal capabilities and life histories

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Little is known about the life histories, genetic structure and population connectivity of shallow groundwater organisms. We used next-generation sequencing (RAD-seq) to analyse population genomic structure in two aquifer species: *Paraperla frontalis* (Banks, 1902), a stonefly with groundwater larvae and aerial (winged) adults; and *Stygobromus* sp., a groundwater-obligate amphipod. We found similar genetic differentiation in each species between floodplains separated by ~70 river km in the Flathead River basin of north-west Montana, USA. Given that *Stygobromus* lacks the above-ground life stage of *P. frontalis*, our findings suggest that connectivity and the magnitude of genetic structure cannot be definitively assumed from life history differences.

ADDITIONAL KEYWORDS: Amphipoda – aquifer ecosystem – groundwater fauna – Plecoptera – RAD-seq.

INTRODUCTION

Gravel-bed river floodplains have been described as the ‘ecological nexus’ of mountain regions because of their important above- and below-ground communities and processes (Hauer *et al.*, 2016). Stanford & Gaufin (1974) first reported stoneflies in the alluvial aquifers of Montana rivers. Researchers have since documented diverse communities of macroinvertebrates, meiofauna and microbes in shallow aquifers worldwide, and have established the enormous ecological importance of floodplain habitats (e.g. Tockner & Stanford, 2002; Stanford *et al.*, 2005; Boulton *et al.*, 2010; Hauer *et al.*, 2016). These communities include insects that remain underground as nymphs but emerge above ground for reproduction (amphibionts), as well as taxa (e.g. crustaceans, oligochaetes and mites) that are obligate residents of interstitial spaces in aquifers (stygobionts). These animals occur over 10 m beneath riverine floodplains and up to 5 km from the main river channels (Stanford & Ward, 1988).

The distribution of groundwater crustaceans in alluvial aquifers is driven by hydrogeological and geomorphic processes and has little relationship to distance from the river. This means that stygobionts are ubiquitous throughout the floodplain, whereas amphibionts are most common near the river (Stanford *et al.*, 1994; Ward *et al.*, 1994). Amphibiotic stoneflies (Insecta: Plecoptera) spend 1–3 years in the aquifer before leaving the groundwater to emerge as adults (Stanford *et al.*, 1994). In contrast, as stygobionts, *Stygobromus* (Crustacea: Amphipoda) individuals never leave the groundwater system. The life cycles of many stygobionts, including *Stygobromus*, are largely unknown, but they exhibit characteristics of all aquatic subterranean animals and obligate cave dwellers that render them maladapted to life above ground – being blind and lacking pigmentation (Culver *et al.*, 2010). *Stygobromus* is found in a variety of subterranean habitats including caves, phreatic lakes, seeps and epikarst (Culver *et al.*, 2010), as well as alluvial aquifers, and there is no evidence that they ever voluntarily leave the groundwater.

Shallow aquifers offer many challenges to resident organisms, including geologically bounded isolation, no light, variable water flow, and reduced availability of carbon, other nutrients and oxygen (Tockner *et al.*, 2010;

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DelVecchia *et al.*, 2016). Many factors, including bedrock geology, soil permeability, water chemistry and quality, groundwater levels, adjacent surface flows, riparian vegetation, and climate, can influence the spatial distribution and ecology of resident organisms (e.g. Johns *et al.*, 2015; Korbel & Hose, 2015). Previous genetic studies have identified widespread, longterm barriers to dispersal by groundwater species, even within drainages, despite potentially linking floods (Lefebure *et al.*, 2006; Cooper *et al.*, 2007, 2008; Finston *et al.*, 2007). For example, groundwater amphipods in Western Australia showed strong genetic differentiation between discrete pockets of groundwater, despite repeated Quaternary floods that covered the entire area (Cooper *et al.*, 2007).

The ability of groundwater organisms to actively disperse within and between adjacent habitats remains unclear. Life history probably plays a role in population connectivity. For example, the retention of ephemeral, winged life stages by some amphibionts offers dispersal advantages over taxa that never leave the groundwater. Current genomic tools can facilitate the study of local adaptation to atypical environments (Luikart *et al.*, 2003) and resolve finescale differentiation in aquatic insects (Hotaling *et al.*, 2018). A better understanding of dispersal in these systems would greatly benefit biological understanding of connectivity along the river corridor, a major theme in river ecology (e.g. Stanford *et al.*, 2005).

Here we address these issues by using RAD-seq (restriction site associated DNA sequencing) datasets for two co-occurring groundwater species with vastly different life histories from two floodplains of the Flathead River in north-west Montana. We hypothesized that the groundwater-obligate *Stygobromus* would have fewer opportunities for gene flow and thus vastly higher genetic differentiation between floodplains than the amphibiont *Paraperla frontalis* (Banks, 1902).

METHODS

Study SiteS

We sampled two Flathead River sites ~70 km apart: the Nyack and Kalispell Floodplains (Fig. 1). These floodplains have been the focus of long-term research (e.g. Helton *et al.*, 2014), and their aquifers are probably not connected in the subsurface because the floodplains are bounded by bedrock knickpoints (Hauer *et al.*, 2007). Each aquifer is known to contain a diverse array of meiofauna and macroinvertebrates (Stanford *et al.*, 1994; Gibert *et al.*, 1994; DelVecchia *et al.*, 2016).

taxa and Sampling We sampled two taxa that exemplify major, different life-history strategies: *P. frontalis*, an aquifer stonefly with a winged adult stage, and *Stygobromus* sp., a blind, pigmentless, groundwater-obligate crustacean. We used mechanical pumping of seven permanent wells to collect these species in June 2011 and 2012 (Fig. 1). We extracted DNA from 96 individuals of each species and confirmed species identifications based on cytochrome *c* oxidase subunit I (*COI*) gene barcoding before proceeding with RAD-seq.

Snp calling and filtering

We prepared RAD-seq libraries following standard protocols using the restriction enzyme SbfI, an 8-base cutter, and unique 6-bp barcodes (Miller *et al.*, 2012). We sequenced 192 individuals on two lanes of an Illumina HiSeq 2500 sequencer with 100-bp, single-end chemistry. Raw sequences were filtered with 90% of the bases required to have a quality score ≥20 using the FASTQ Quality Filter (http://hannonlab.cshl.edu/fastx_toolkit/). Reads for *Stygobromus* were trimmed to 80 bp to maximize quality. We used the process_radtags script in Stacks v.1.19 (Catchen *et al.*, 2013) to demultiplex reads by barcode, removing any with uncalled bases, and to call single nucleotide polymorphisms (SNPs) with a read depth (-m) of 5 and a maximum of two mismatches (-n) per locus and between catalogue loci (-N). Additional SNP calling details are provided in the Supporting Information.

To investigate the influence of data scale (SNP number) and missing data on our results, we constructed four datasets for downstream analyses. All filtering steps applied to the two species and all datasets received the baseline filtering described above. Dataset 1: we removed all individuals with >50% missing data, and all loci genotyped in <60% of individuals. We also removed all loci that were not present in at least 50% of individuals in each population. For datasets 2–4, we removed any individuals with more than one standard deviation of missing data above the mean in the raw SNP dataset. Dataset 2: no additional filters. Dataset 3: we only included loci genotyped in >25% of individuals. Dataset 4: we only included loci genotyped in >75% of individuals.

pairwiSe differentiation and population Structure

We calculated pairwise F_{ST} values using GENEPOP (Rousset, 2008) for all sample–well pairs using dataset 1. We pooled samples with pairwise $F_{ST} = 0$ for subsequent analyses of population structure

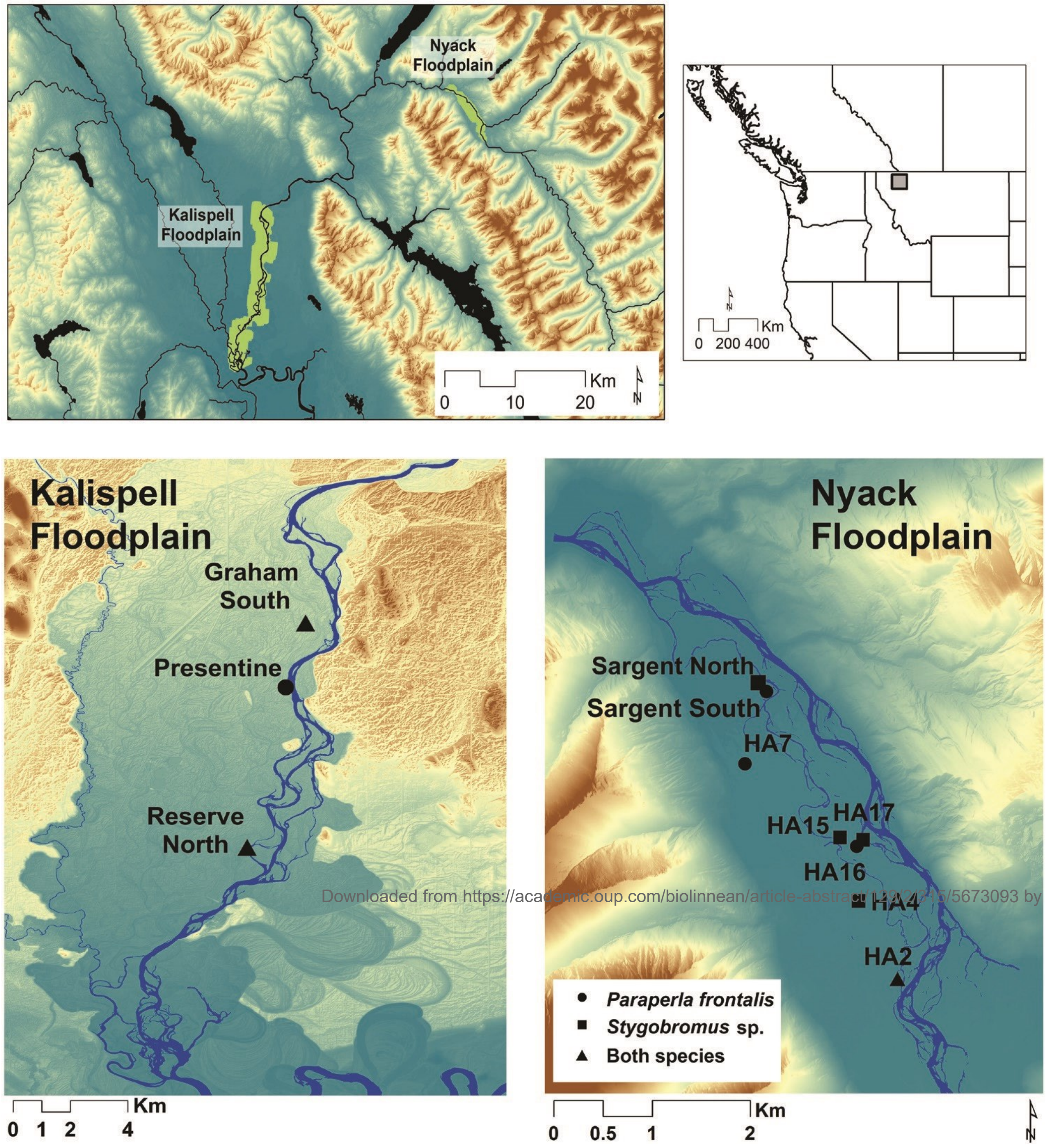

Figure 1. Sampling locations for two species of groundwater invertebrates, with sampled floodplains shown in light green, and individual wells coded according to the taxa obtained from each.

using datasets 2–4. For these analyses, we performed a discriminant analysis of principal components (DAPC) using the R package *adegenet* (Jombart,

RESULTS

Sequencing and genotyping

2008). We tested *K* = 1–7 for *P. frontalis* and *K* = 1–6 Using *COI* gene sequences, we identified one for *Stygobromus*.

Table 1. Sample sizes of the amphipod *Stygobromus* sp. and the stonefly *Paraperla frontalis*, including total numbers of individuals with DNA initially extracted and those that met the quality requirements for the final RAD-seq datasets (1 and 2–4)

Floodplain	Population	*Stygobromus* sp.			*P. frontalis*		
		Extracted	Dataset 1	Datasets 2–4	Extracted	Dataset 1	Datasets 2–4
Nyack	Sargent South				26	25	22
	Sargent North	15	3	3			
	Ha2	14	15	15	14	12	12
	Ha4	13	3	2			
	Ha7				14	14	14
	Ha15	18	11	9			
	Ha16				14	13	10
	Ha17	8	8	8			
Kalispell	Gra[illegible]	16	1	1	14	12	12
	Reserve North	12	9	8	8	8	8
	Presentine Bar				6	6	6
	Total	96	50	46	96	90	84

Complete details of DAPC analyses numerically dominant, but probably undescribed, are provided in the Supporting Information. *Stygobromus* species, with all individuals sharing a single mitochondrial DNA (mtDNA) haplotype. We observed lower coverage depth and fewer SNPs despite a much larger SNP catalogue (e.g. two to three times more RAD loci) for *Stygobromus* vs. *P. frontalis*. This is probably because the genome of *Stygobromus* is much larger than that of *P. frontalis*. While genome sizes of our study taxa have not been measured, estimates of genome size in the freshwater amphipod infraorder Gammarida, which includes *Stygobromus*, range from 2.1 to 16.2 gb (Gregory *et al.*, 2007; Jeffery *et al.*, 2017) while genomes for *Plecoptera* range from 0.35 to 2.1 gb (Gregory, 2005). For dataset 1, we identified 806 SNPs for 90 *P. frontalis* individuals and 314 SNPs for 50 *Stygobromus* individuals. For datasets 2–4, we included 84 and 46 individuals of *P. frontalis* and *Stygobromus*, respectively (Table 1). The total number of SNPs in datasets 2–4 ranged from 167 to 3187 (Fig. 2). Data and scripts have been archived in a Dryad dataset (https://doi.org/10.5061/dryad.98sf7m0dr). pairwiSe differentiation and population Structure

We calculated pairwise F_{ST} values within and between floodplains for both species using dataset 1 (Table 2). However, because of small sample sizes from the two Kalispell wells, we did not calculate pairwise F_{ST} values for *Stygobromus* within that floodplain. We found genetic differentiation between floodplains to be low for both species ($F_{ST} = 0.004$ for *Paraperla* and $F_{ST} = 0.000$ for *Stygobromus*). Population pairwise F_{ST} values within floodplains were of a similar magnitude to those between floodplains (Table 2).

DAPC analyses revealed similar levels of population structure for both species. For *P. frontalis*, the optimal K ranged from $K = 2$ (datasets 2 and 3) to $K = 3$ (dataset 4; Fig. 2A). For *Stygobromus*, the best-fit DAPC K was either $K = 5$ (dataset 2), $K = 4$ (dataset 3) or $K = 3$ (dataset 4; Fig. 2B), with all K values highlighting no obvious patterns of geographical structuring.

DISCUSSION

Shallow alluvial aquifers are refugia for diverse, functionally unique species that are inherently difficult to study (Stanford & Ward, 1988). Thus, we are only just beginning to understand such fundamental attributes as their dispersal ability, population structure, mating habits and adaptability (Gibert *et al.*, 1994; Boulton *et al.*, 2010). Our study compared winged stoneflies

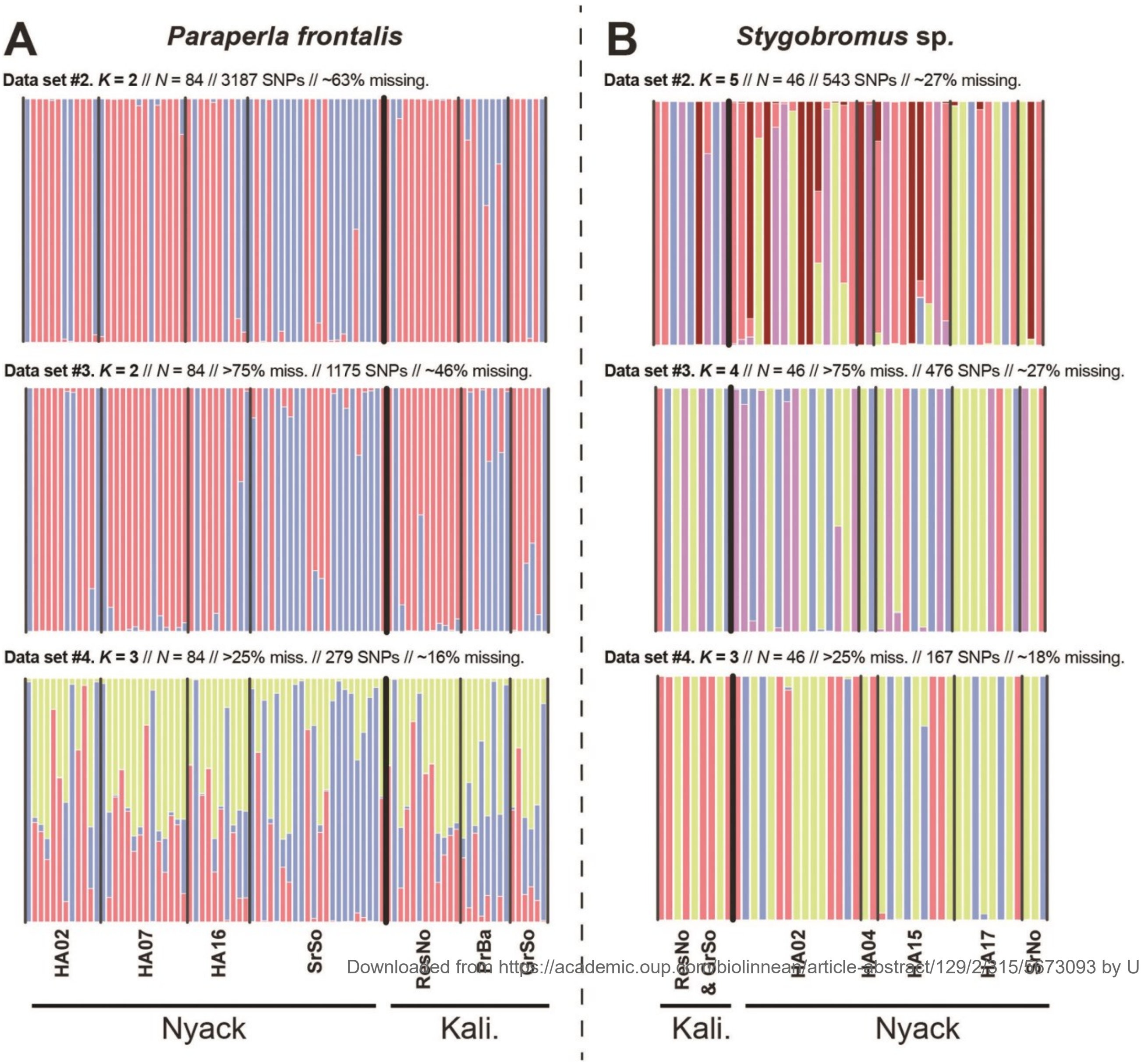

Figure 2. Comparisons of population structure for discriminant analysis of principal components (DAPC) of each of datasets 2–4, which include varying numbers of SNPs for (A) *Paraperla frontalis* and (B) *Stygobromus* sp. For each plot, the number of clusters supported (*K*), filters employed, number of SNPs and percentage of missing data are included. Filter abbreviations: >75% miss. and >25% miss., removal of any loci with greater than 75% or 25% missing data, respectively.

Each vertical bar represents one individual.

with groundwater-obligate amphipods, species we expected to exhibit vast differences in dispersal capacity. Using hundreds to thousands of SNPs, we have shown a similar magnitude of weak or no local and regional structure in both our study species, a surprising result given their different life histories, predicted dispersal differences and the barriers (geological and geographical) separating populations.

There are several possible explanations for this parallel

ReserveNorth

talis

−0.0114

P.

GrahamSouth

SargentNorth

Kalispell

06SNPsfor90

−0.0010198

0.0347

SargentSouth

0.0080040 −0.0016 HA17

−0.0183 0.0023

−0.003900030212 HA15

0.0056113 −0.0058

omus

Ha16

nd

−0.0059 0.0090030103 −0.0045 HA04

−0.003507007400

ntalis

ividuals

Ha07

obromus

−0.004610000290.0050 0.00160.0045 HA02

−0.003001900072 0.0016 0.0090

Nyack

Ha02

lesof

SNPsfor50

F_{ST}

wise

GrahamSouth(KAL)

ReserveNorth(KAL)

sp.

KAL,KalispellFloodplain.

Presentine(KAL)

SargentSouth

Stygobromus

SargentNorth

frontalis

le2.

Kalispell

Ha07Ha16

HA04HA15HA17

P.

lack of genetic structure. First, groundwater ecosystems are highly interconnected through alluvial and phreatic (deep bedrock) pathways (Gibert *et al.*, 1994), which could possibly facilitate dispersal. However, the environment of phreatic channels would be challenging to shallow aquifer taxa, with longer residence times and potentially limiting temperatures, carbon availability and oxygen levels (Larned, 2012). Second, it is possible that *Stygobromus* individuals occasionally enter the river current through upwelling and then passively disperse beyond bedrock knickpoints to become established and reproductive in downstream floodplains. Large flood events may contribute to their movement as well, if individuals are brought out of the shallow aquifer, although this stands in contrast to other amphipod studies (Cooper *et al.*, 2007). Third, the effective population size (N_e) of each species might play a role. *Stygobromus* sometimes occurs in large numbers, with hundreds of individuals being pumped from wells over 1–2 min. If N_e for *Stygobromus* is high, these populations will experience virtually no local genetic drift, and relatively little gene flow could maintain the surprisingly low F_{ST} values we observed (Allendorf & Luikart, 2007).

In summary, our results suggest that differences in genetic structure cannot be reliably inferred from life history differences, even as extreme as those between stygobionts and amphibionts. This is in contrast to previous studies of a variety of freshwater invertebrates (including Plecoptera and Amphipoda) that generally found a correlation between dispersal ability and degree of genetic structure (e.g. Miller *et al.*, 2002; Alp *et al.*, 2012). McCulloch *et al.* (2009) found that two stonefly species with vastly different dispersal abilities also showed highly divergent genetic structure, although one species, with wing morphologies ranging from wingless to fully winged, showed no variability between morphotypes in phylogeographical structure. In the future, we expect to better resolve the historical and ecological drivers of groundwater genomic and phylogenetic diversity using more robust taxonomic, genomic and geographical sampling.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website. All data and scripts for this study are available from the Dryad data repository: https://doi.org/10.5061/ dryad.98sf7m0dr

SHARED DATA

All data and scripts for this study are available from the Dryad data repository: https://doi.org/10.5061/ dryad.98sf7m0dr (Jordan *et al*., 2019).