Population genetics and dispersal of the flatworm, *Polycelis coronata*: a test of the habitat stability hypothesis

Russell B. Rader, Peter J. Unmack & Jeffrey N. Moore

Taylor & Francis
Taylor & Francis Group

Population genetics and dispersal of the flatworm, *Polycelis coronata*: a test of the habitat stability hypothesis

Russell B. Rader[a], Peter J. Unmack[a,b] and Jeffrey N. Moore 🆔[a]

[a]Department of Biology, Brigham Young University, Provo, UT, USA; [b]Institute for Applied Ecology, University of Canberra, Australia

ABSTRACT

The *habitat stability* hypothesis states that species in spring-like habitats have little reason to disperse compared to species in temporary habitats. Planarians commonly inhabit springs around the world and they have long been considered poor dispersers. Recently, however, genetic analyses have shown contradictory results on the dispersal of planarians. Asexual planarians that can establish a new population by colonization of a single individual showed little genetic differentiation between sites separated by hundreds of kilometers, whereas species inhabiting springs showed deep differentiation between sites separated by hundreds of meters. The latter results are consistent with the *habitat stability* hypothesis. We used the cytochrome oxidase subunit I gene from 468 individuals of *Polycelis coronata*, an asexual species, collected from 50 sites, nested in 26 tributaries, in 4 catchments of the Wasatch Mountains of Utah, USA, to explore the dispersal capabilities of *P. coronata*. The longest distance between sites was 66 km. Despite this small spatial extent, we found that 77% of the 130 haplotypes were collected from a single site and 89% from a single catchment. F_{ST} values between local populations in the same tributary (0.221, 0.266, 0.389) were similar to the average F_{ST} values in different catchments for other headwater taxa. Also, variation among individuals accounted for the majority of genetic structuring with little differentiation beyond the scale of a single site. Dispersal is very slow in this species which is consistent with the *habitat stability* hypothesis. However, we suggest that other explanations also warrant consideration. We also identified two potential cryptic species suggesting a high degree of hidden variation at the level of species in this genus.

Introduction

Planariidae possess several traits that may limit their ability to disperse within stream ecosystems. They are obligate aquatic species without a terrestrial phase in their life cycle, which eliminates active, overland dispersal. Also, they may rarely encounter passive vectors (e.g. birds) because the adults are nocturnal and cryptic, and if they produce resting eggs, their cases are firmly attached to the undersides of rocks (Walter 1907; Kolasa et al. 2009). Plus, macro-turbellarians, like *Polycelis coronata*, do not swim (Kolasa et al. 2009) and they are rarely found in the drift (Minshall & Winger 1968). Consequently, biologists have long thought that planarians have a poor dispersal capability (e.g. Ball 1969, 1974). However, we still have much to learn about dispersal in flatworms because

CONTACT Russell B. Rader ✉ russell_rader@byu.edu

population genetic data are limited to <15 of the approximately 1300 species of freshwater Planariidae worldwide (Schockaert et al. 2008). Also, studies on the population genetics of stream planarians appear contradictory. That is, some species show deep differentiation between sites separated by hundreds of meters (Hebert & Payne 1985; Brändle et al. 2007), whereas others show little population differentiation between sites separated by hundreds of kilometers (Pongratz et al. 2003; Lázaro & Riutort 2013).

Recent research on Mediterranean Planariidae (e.g. *Dugesia* spp., *Schmidtea mediterranea*) and from headwater streams in Germany (*Crenobia alpina*) support the assertion that the degree of genetic connectivity in stream Planariidae depends on habitat stability and the prevalence of asexual reproduction (Brändle et al. 2007; Lázaro et al. 2009, 2011; Lázaro & Riutort 2013). Poorly connected species inhabited stable streams, whereas well-connected species were asexual and recently dispersed by human vectors (e.g. Lázaro et al. 2009; Lázaro & Riutort 2013). Stability in this context refers to springs or permanent spring-like streams that are not subject to extreme flow fluctuations. Compared to temporary environments, planarian species in stable habitats are thought to have little reason to disperse (e.g. Roff 1990). Consequently, we would expect some level of genetic structuring among sites separated by short distances (i.e. 1–10 km).

Some species of Planariidae are strictly sexual, some are strictly asexual and reproduce by fission and/or parthenogenesis, and some species combine sexual and asexual reproduction (e.g. D'Souza & Michiels 2010). Asexuality may have little effect on increasing rates of dispersal unless it is better at increasing population densities compared to sexual reproduction, and if large populations are more prone to disperse than small populations. However, reproduction by fission can certainly increase the probability of successful colonization because new populations can be established from a single individual, or even from a fragment of an individual (e.g. Calow et al. 1979). A high rate of colonization success combined with recent population expansion attributed to human-facilitated dispersal was thought to account for a lack of population differentiation among asexual planarians (e.g. Lázaro et al. 2009).

Polycelis coronata (Platyhelminthes: Turbellaria: Planariidae, Girard 1891) inhabits stable headwater streams across the western USA and Canada where summer temperatures rarely exceed ~12 °C (Beck 1954; Reynoldson 1961; Kenk 1973; Nixon 1978). According to the *stable habitat* hypothesis, *P. coronata* should show genetic structuring among populations separated by short distances. Indeed, Nixon and Taylor (1977) used protein electrophoresis to show a high level of genetic structuring between 22 populations of *P. coronata* in the Pacific Northwest (USA), where the straight-line distance between sites varied from about 20 to 600 km. However, *P. coronata* is also an asexual species that appears to primarily reproduce by fission (Kenk 1973; Nixon & Taylor 1977; Kolasa et al. 2009). Thus, *P. coronata* in other parts of its range may be a highly connected species because it can easily colonize new habitats.

We used a hierarchical sampling design to examine the genetic structure of *P. coronata* using a 763-bp fragment of the mitochrodrial cytochrome oxidase subunit I (COI) gene (mtDNA) from 541 individuals to draw inferences about its dispersal across 50 sites in 26 headwater segments in 4 adjacent catchments in the central portion of the Wasatch Mountains, UT, USA. The linear distance between the furthest sites was only 66 km. These sites are also within minutes of a heavily populated urban area and are connected by an extensive network of hiking trails. The trail-heads leading into the canyons of this study often occur inside urban neighborhoods. Thus, human-facilitated dispersal of this asexual species should be common. We hypothesized that dispersal would be frequent across these short distances (≤66 km) and that *P. coronata* would show no genetic differentiation among sites in the Central Wasatch Mountains. This result would suggest the importance of asexual reproduction in promoting colonization and connectivity in a species that otherwise has no traits that promote dispersal. We also collected *P. coronata* from distant locations (Logan River, UT; Salt River, WY; Colorado River, CO; Snake River, ID; Rio Grande River, NM) to test the hypothesis that some haplotypes found in the central Wasatch Mountains have dispersed over long distances. Long-range dispersal of *P. coronata* haplotypes would also suggest the importance of asexual reproduction in enhancing dispersal.

Methods

Sampling design and study system

We sampled 26 headwater segments nested in 4 adjacent catchments (Provo, American Fork, Little Cottonwood, and Weber) to determine the distribution of haplotypes of *P. coronata* in the central portion of the Wasatch Mountains (Figure 1). All segments were permanent, and included 3 small third-order streams, 11 second-order tributaries, and 12 first-order tributaries. All segments were sampled at an upstream and downstream site typically separated by ∼500–1000 m except for two segments that contained a single site. Thus, we sampled a total of 50 sites in this study (Appendix 1). A site consisted of a stream reach ∼50 m in length with both riffles and pools. We defined the *local population* as the planarians collected at a site.

The Weber (6070 km²) and Provo (1761 km²) are two of the largest catchments in the central Wasatch Mountains, whereas Little Cottonwood (119 km²) and American Fork (107 km²) are more typical of smaller drainages (Shiozawa & Rader 2005). Each catchment eventually drains into The Great Salt Lake, and all catchments are separated by steep mountainous terrain. In addition to sampling tributaries, we conducted a thorough search for *P. coronata* in mainstem segments in each catchment, except for the Weber River where private property forced limited access. Also, *P. coronata* and several aquatic insect species were absent in the central portion of the Provo drainage because of frequent dewatering by agriculture (Figure 1).

During July and August of 2008, we collected 10 *P. coronata* by hand from the undersides of submerged rocks at each site. We also collected 10 individuals of *P. coronata* from five distant locations to determine how many haplotypes, if any, had a broad distribution within the region (Logan River, UT; Salt River, WY; Colorado River, CO; Snake River, ID; Rio Grande River, NM). We could show the ability of *P. coronata* to disperse over long distances, if some haplotypes are distributed between the central Wasatch Mountains and these distant locations.
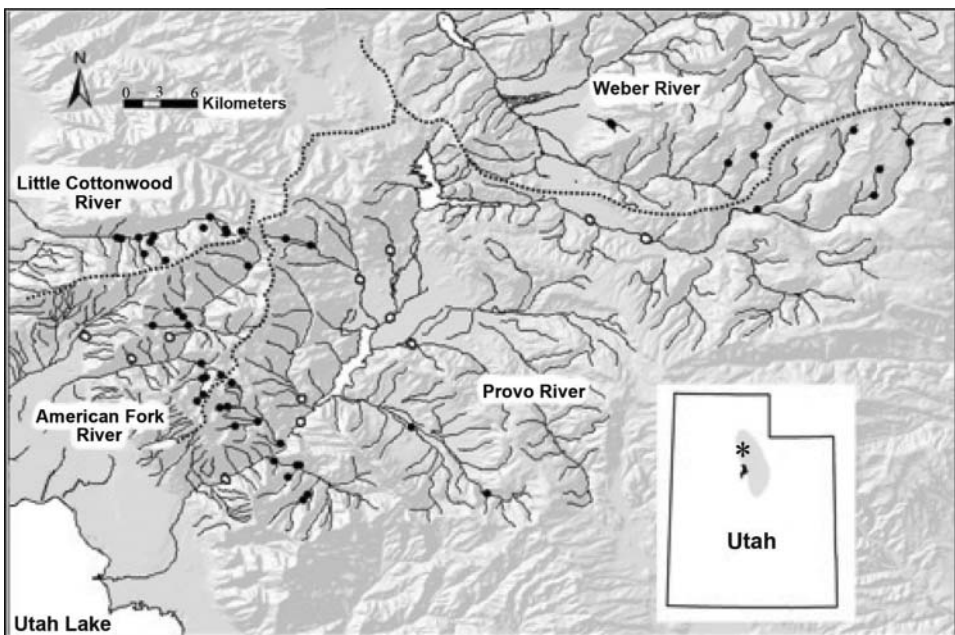
Figure 1. Map showing the sites within catchments of this study (Provo River, Weber River, American Fork River, and Little Cottonwood Canyon). Closed circles represent sites where *Polycelis coronata* occurred; open circles show sites where this species was absent. Dashed lines indicate the boundaries of each catchment. In the lower right corner is the state of Utah showing Salt Lake City (star), Utah Lake, and the extent of the Wasatch Mountains (shaded area).

We collected a timed (1 minute) benthic sample (D-frame sweep net with a 250-μm mesh) at each site to estimate the average relative abundance of *P. coronata* in the central Wasatch Mountains. We also made visual estimates of substrate stability (presence of bryophytes and embeddedness) and collected chemical data (pH, conductivity, and water temperature) as spot measurements during mid-day at each site. Stable sites would be spring-like and fed by a high proportion of groundwater. Thus, stable sites would show high conductivity, low pH, cool water temperatures, embedded substrate, and an abundance of bryophytes. Flow records were not available for any of these small tributaries. However, bryophytes are slow-growing plants indicative of rocks that have seldom been rolled by high flows and are thus a reliable indicator of stable substrate conditions (e.g. Bowden 1999; Suren & Duncan 1999; Milner et al. 2000).

Primer selection, mtDNA extraction and sequencing

All flatworms were initially stored in 95% ethanol in the field and transferred to 99% ethanol in the lab where they were stored at −80 °C. We extracted genomic DNA from tissue samples using the DNeasy Tissue Kit (Qiagen, Hilden, Germany). The mtDNA gene COI is the most commonly used marker in flatworm molecular studies (Pongratz et al. 2003; Vilas et al. 2005; Brändle et al. 2007; Álvarez-Presas et al. 2008). Mitochondrial DNA primers used in previous flatworm studies (e.g. Bessho et al. 1992) failed to amplify, thus we made several primers from two European *Polycelis* species (GenBank nos. AF178321.1 and DQ666049.1) and tried these in combination with different general invertebrate primers (Simon et al. 1994). The primer combination mtD-6 (5′-GGAGGATTTG-GAAAATGATTAGTTCC-3′) and *Polycelis* COI-R2 (5′-CATAAAAGAGCAACAGAATCATA-3′) produced some data which was then used as a template to design specific primers for *P. coronata*. Most individuals were amplified with the primer combination FW14F (5′-ACACCTGATATGATWT-TYCCTCG-3′) and FW.COI.2R (5′-GCTTTAGACAAAACTATTCCAG-3′). For those that failed to amplify, we used an alternative forward primer, FW20F (5′-GATATGATWTTYCCTCGTAC-3′) or mtD-6 in combination with FW.COI.2R. In a few instances where both forward and reverse primers were ineffective, we used primers FR500R (5′-TAAATTATTACATGAGATATAATACC-3′) and FW123F (5′-GTTGAACTGTTTATCCRCCTTTGAC-3′) in combination with the other forward and reverse primers to amplify two shorter overlapping fragments.

Final concentrations for the polymerase chain reaction (PCR) per 25 μL were 25 ng template DNA, 0.25 μM of each primer, 0.625 units of Taq DNA polymerase, 0.1 mM of each dNTP, 2.5 μL of 10X reaction buffer, and 2.5 mM MgCl$_2$. Amplification proceeded at 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 48 °C for 30 s, and 72 °C for 75 s. Following these cycles, final elongation was held at 72 °C for 7 min. We examined PCR products on a 1% agarose gel using SYBR safe DNA gel stain (Invitrogen, Eugene, OR, USA). We purified PCR products using a Montage PCR 96 plate (Millipore Billerica, MA, USA). Sequences were obtained via cycle sequencing with Big Dye 3.0 dye terminator ready reaction kits using 1/16th reaction size (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were run with an annealing temperature of 52 °C following the ABI manufacturer's protocol. We purified sequenced products using sephadex columns, and sequences were obtained using an Applied Biosystems 3730 XL automated sequencer at the Brigham Young University DNA Sequencing Center. All haplotypes were deposited in GenBank, accession numbers KY057039-KY057186.

Analysis of mtDNA sequence data

DNA sequences were edited using Chromas Lite 2.0 (Technelysium, Tewantin, Queensland, Australia) and imported into BioEdit 7.0.5.2 (Hall 1999), then aligned by eye. An alignment algorithm was not necessary because there were no gaps in the sequences. Sequences were checked for unexpected frame shift errors or stop codons in Mega 6.06 (Tamura et al. 2013). Individual sequences were then reduced to 148 haplotypes using DnaSP 5.10.01 (Librado & Rozas 2009). We used

RAxML 8.0.24 (Stamatakis 2014) for our maximum-likelihood (ML) analysis to examine the evolutionary relationships of our sequence data on the CIPRES cluster at the San Diego Supercomputer Center (Miller et al. 2010). We used the GTRGAMMA model for 1000 bootstrap replicates and for the final tree calculation. Due to a lack of more appropriate outgroups, trees were rooted with the European species, *P. felina* and *P. tenius* (GenBank accession numbers DQ666049 and AF178321, respectively). Mean *p*-distances for DNA were calculated using Mega based on the individual haplotypes.

All individuals from outside of our four catchments and those identified as potential cryptic species were eliminated from our analyses of *P. coronata* in the Wasatch Mountains. We used an analysis of molecular variance (AMOVA) implemented in the Vegan package of 'R' 2.8.1 (R Development Core Team 2010) with significance assessed using 10,000 random permutations to examine spatial patterns of genetic variation (Excoffier & Lischer 2010). We partitioned genetic variation into the following hierarchically nested groups: 'F_{IL}' was the variation among individuals (I) within a local population (L), while F_{ST} was the variation among local populations. F_{ST} was partitioned into F_{LS}, F_{SC}, and F_{CT}. 'F_{LS}' was the variation among local populations (L) nested within segments (S), 'F_{SC}' was the variation among segments (S) within catchments (C), and 'F_{CT}' was the variation among catchments (CT). We do not expect significant differentiation among local populations at any level of the spatial hierarchy if this species has been recently dispersed by humans.

We used a Mantel test in 'R' (R Development Core Team 2010) to test the hypothesis that pairwise genetic distances were either correlated with straight-line geographic distances between local populations, or distances following the stream network measured by ArcGIS 10 (Environmental Systems Research Institute, Redlands, CA). A positive correlation between F_{ST} and geographic distances would suggest the potential for passive overland dispersal by terrestrial vectors (e.g. humans) with a greater tendency to disperse between near sites than between far sites. However, a positive correlation between F_{ST} and stream distances would indicate the potential for dispersal through the stream network.

Results

Environmental conditions

Polycelis coronata occurred at 86% of the headwater sites within our study system. When present, it had an average relative abundance of 21% (±11%) of the total invertebrates collected at a site. Along with chironomid midges, Baetidae, Simuliidae, and *Neothremma alicia* (Trichoptera, Uenoidae), it was consistently one of the five most abundant taxa in this area. We did not find *P. coronata* from mainstem segments where the average water temperature, pH, and conductivity in July and August was 12.8 °C (SE ± 0.68 °C), 8.1 (SE ± 0.08), and 185 μS/cm (SE ± 11.4), respectively. This is compared to 10.4 °C (SE ± 0.68 °C), 7.6 (SE ± 0.06) and 311.6 μS/cm (SE ± 17.6) at our headwater sites. Embeddedness of cobbles and boulders was about 30% at headwater sites and about 10%, if at all, in mainstem segments, except for the Provo River. Embeddedness was greater in the Provo River than in other mainstem segments because of the effects of river regulation (armoring). Also, patches of bryophytes were common and extensive at headwater sites and nearly absent in mainstem segments. These data indicate that our headwater sites were cool, stable, spring-like habitats with a high influx of groundwater.

Cryptic species

Editing resulted in a 763-bp fragment of COI from 541 individuals, which collapsed into 148 haplotypes. ML recovered one tree with a −ln score of −5046.65 (Figure 2). We identified two potential cryptic species shown in the upper left of Figure 2. The first branching in-group lineage, herein called *Polycelis* sp. A, and the sister lineage to *P. coronata*, herein referred to as *Polycelis* sp. B
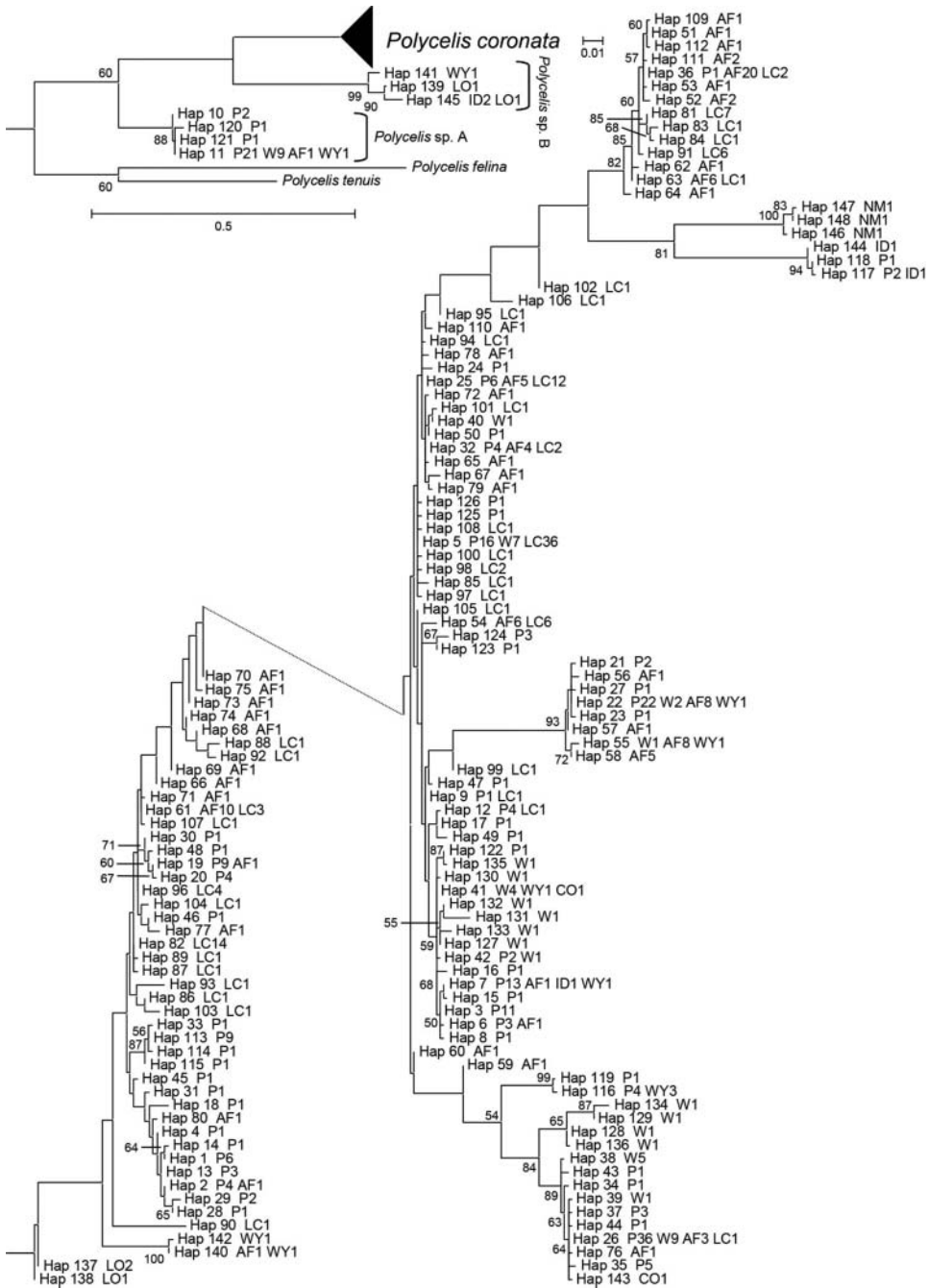
Figure 2. Maximum likelihood phylogram of all 148 COI haplotypes generated in this study. In the upper left corner are two European species used to root the tree and two potential cryptic species. The remaining tree is a blow-up showing relatedness of 130 *P. coronata* haplotypes. The scales for the branching lengths differ between the cryptic species (0.5) and the blow-up for *P. coronata* (0.01). This tree is split for ease of display as indicated by the dotted line. Bootstrap values were obtained from 1000 replicates, only values above 50 are shown. Each haplotype is followed by the number of individuals from the catchment(s) it occurred in: Provo (P), Weber (W), American Fork (AF), and Little Cottonwood (LC) or, from the state it was collected from for haplotypes of distant populations: Wyoming (WY), Colorado (CO), Idaho (ID), New Mexico (NW), and Logan (LO), Utah.

Table 1. Mean between-lineage cytochrome oxidase I nucleotide p-distances (as percentages) for *Polycelis*. Mean within-lineage *p*-distance is on the diagonal.

	P. coronata	Polycelis sp. B	Polycelis sp. A	P. felina	P. tenius
P. coronata	3.8				
Polycelis sp. B	13.8	3.3			
Polycelis sp. A	14.9	14.3	0.7		
P. felina	17.7	17.8	16.3	n/a	
P. tenius	17.7	19.6	17.2	17.7	n/a

Note: n/a is not applicable.

(Figure 2), were both strongly divergent from *P. coronata*. That is, the average *p*-distance between them and *P. coronata* was 14.4% as compared to average within species divergences for *Polycelis* sp. A, *Polycelis* sp. B, and *P. coronata* equal to 2.6% (Table 1). The difference between *P. coronata*, *Polycelis* sp. A, and *Polycelis* sp. B was nearly as great as the difference between *P. coronata* and the two European species of *Polycelis* used to root the tree (Table 1). Thirty-five of the 36 individuals of sp. A were collected from within our study system (Figure 2). Of the 22 haplotypes collected from outside our study system, 5 were sp. B, whereas the remaining 17 haplotypes were *P. coronata*.

Haplotype distribution and relatedness in P. coronata

Of the 763 base pairs, 622 characters were constant, 121 characters were parsimony informative, and 20 were parsimony uninformative. Eight of the 15 haplotypes of *P. coronata* from distant sites were not collected from any of the four catchments (Haps 137, 138, 142, 143, 144, 146, 147, 148) showing that there is considerable genetic diversity within this species outside our study system. We deleted haplotypes from distant sites and from *Polycelis* sp. A and sp. B, and used the remaining 130 haplotypes from 468 individuals of *P. coronata* to test our first hypothesis of no genetic differentiation across our study system.

Relationships within *P. coronata* were poorly resolved with little bootstrap support (Figure 2). Most haplotypes were quite closely related being separated from others by only one or two base pairs. Although most haplotypes were confined to a single catchment, several were closely related to other haplotypes in different basins, especially between the Provo and Weber catchments (lower right corner) and the American Fork and Little Cottonwood catchments (upper right corner).

Given the short distances between sites in this study (≤66 km), a well-connected species would show little differentiation even between catchments but especially between sites within segments. Contrary to the first hypothesis, the AMOVA showed the opposite result. The difference between local populations within segments (F_{LS}) was significant suggesting that there was little gene flow between populations in the same stream segment (Table 2). Also, differences among individuals within a local population explained the greatest amount of the total genetic variation (F_{IL} in Table 2). Of the 10 individuals collected at a site, the number of haplotypes ranged from 1 to 9 with an average of 5. That is, 50% of the individuals at a site had different haplotypes.

Table 2. Hierarchical analysis of molecular variance of *P. coronata*. 'F_{IL}' was the variation among individuals (I) within a local population (L) and F_{ST}, the variation among local populations was partitioned into F_{LS}, F_{SC}, and F_{CT}: 'F_{LS}' was the variation among local populations (L) nested within segments (S); 'F_{SC}' was the variation among segments (S) within catchments (C); 'F_{CT}' was the variation among catchments (CT).

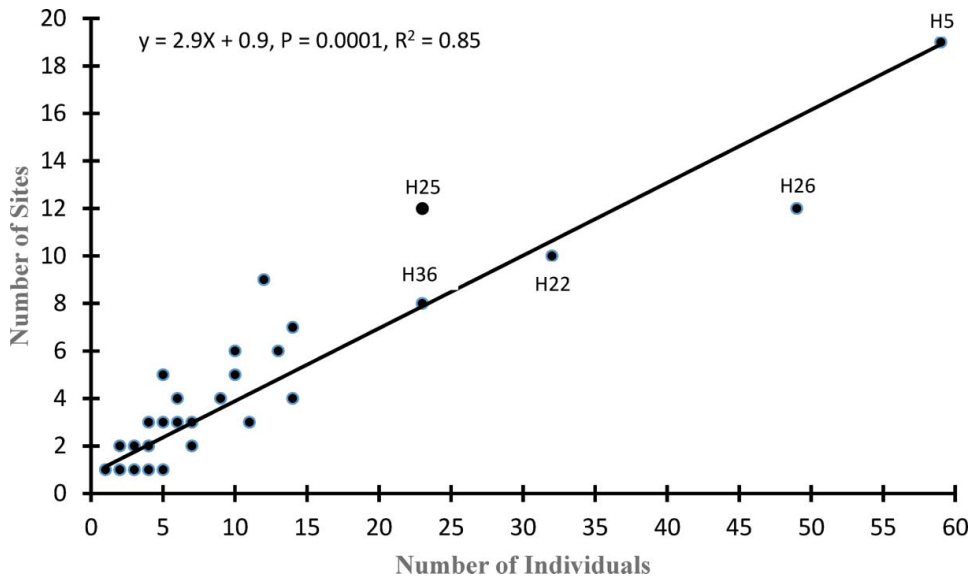| Source of variation	df	Percentage variation	Fixation indices	p
Among catchments	3	6.9	F_{CT}: 0.070	<0.3650
Among segments within catchments	21	9.8	F_{SC}: 0.120	<0.0900
Among local populations within segments	38	27.0	F_{LS}: 0.325	<0.0001
Within local populations	442	56.3	F_{IL}: 0.438	<0.0001

Figure 3. Scatter plot showing a positive relationship between the abundance and the distribution of 130 COI haplotypes of *Polycelis coronata* in four catchments located in the central portion of the Wasatch Mountains. Many of the haplotypes are overlain on top of each other. The number assigned to the five most abundant haplotypes corresponds to haplotype numbers in Figure 2.

There was a positive relationship between haplotype abundance and haplotype distribution. The majority of *P. coronata* haplotypes were rare in our study system. Seventy-one percent (92 haplotypes) were represented by a single individual (Figure 3), whereas 12 haplotypes were considered abundant ($\geq$10 individuals). The abundant haplotypes were distributed across the greatest number of sites (Figure 3). However, most of the abundant haplotypes were confined to one or rarely two catchments, and only one was collected from all four drainages (Hap 26). Thus, the majority of haplotypes were very rare and had a very restricted distribution. That is, 77% of the haplotypes of *P. coronata* in our study system were collected from a single site and 89% occurred within a single catchment (Figure 2), which is also inconsistent with our first hypothesis that all haplotypes could disperse to all sites.

Of the 15 *P. coronata* haplotypes from distant sites (Figure 2), 7 from Wyoming, Idaho, and Colorado were also collected from our study system (Haps 7, 22, 41, 55, 116, 117, 140). This confirms our second hypothesis and shows the potential for long-range dispersal in this species. However, we found no relationship between genetic distances (F_{ST} values) and distances following the stream channel ($R^2 = 0.16$; $p = 0.24$) suggesting that *P. coronata* typically does not disperse through the stream network. Instead, we found a weak positive correlation between genetic distances and the straight-line distance between sites ($R^2 = 0.43$; $p = 0.001$) indicating a slight degree of spatial autocorrelation. That is, dispersal appears slightly more common among near sites than distant sites.

Discussion

Polycelis coronata in the central Wasatch Mountains is a poorly connected species. That is, 77% of the haplotypes were confined to a single site and 89% were restricted to a single catchment. Also, average F_{ST} values for *P. coronata* between local populations in the same tributary (0.221, 0.266, 0.389) were similar to the average F_{ST} values in different catchments (0.17, 0.318, 0.38, 0.39, 0.57) for other headwater taxa (Blephariceridae, Simuliidae, shrimp) that also poorly disperse (Hughes et al. 1995, 1996; Wishart & Hughes 2001; Finn & Adler 2006, Finn & Adler 2006). These data show that dispersal is rare in *P. coronata* even between sites in the same tributary.

Rare dispersal is consistent with the *habitat stability* hypothesis, which states that species in permanent habitats are not likely to disperse compared to species from temporary habitats (e.g. Roff 1990; Lázaro et al. 2009). All of the sites in this study were permanent but stability can also be defined by flow characteristics which create sheer stress and dislodged substrate (e.g. Mérigoux & Dolédec 2004, Biggs et al. 2005). Our sites undoubtedly showed a gradient from very stable seasonal flows to quasi-stable conditions where smaller substrate particles may be dislodged during spring run-off. However, these sites were very different from mainstem segments and the presence of moss is indicative that most particles were rarely disturbed. Plus, most studies examining the habitat stability hypothesis show a similar gradient in flow conditions within stable sites as in this study (e.g. Baggiano et al. 2011).

Although aquatic species in temporary habitats may frequently disperse to avoid desiccation (e.g. Bilton et al. 2001), it is risky to assume that species rarely disperse from stable habitats. A variety of mechanisms might promote dispersal in stable habitats (e.g. Gandon & Michalakis 1999). For example, high population densities may result in competition causing individuals to disperse to find more abundant resources (e.g. Baguette et al. 2013). As an alternative explanation to the *stable habitat* hypothesis, low rates of dispersal in *P. coronata* may be related to habitat specialization. Species should have a low tendency to disperse if there is a low probability of finding a particular type of habitat (e.g. Wishart & Hughes 2001). Narrow thermal tolerance, as in *P. coronata*, may select against dispersal if there is a high probability of landing in inhospitable habitats (Dobzhansky 1950; Vannote & Sweeney 1980) such as, warm downstream reaches. Species that are habitat specialists and transported by vectors may frequently land in unsuitable habitats, which may drive their evolution away from a dispersive phenotype (e.g. Jacob et al. 2015).

Vectors may be the only means of dispersal in *P. coronata* because it is an obligate aquatic species, it does not swim, and it is rarely found in the drift. Plus, it has a narrow temperature tolerance which probably prevents it from dispersing though warmer downstream reaches. Although it may have dispersed through the stream network during glacial periods, we found no correlation between genetic distances and the distance between sites following the stream channel. Instead, we found a weak correlation between genetic distance and the straight-line, overland distance between sites. Dispersal by terrestrial vectors (e.g. birds and mammals) is most consistent with this pattern. If vectors are the primary means of dispersal, then we should not be surprised that there was a weak relationship between genetic distances and geographic distances because individuals of *P. coronata* would be transported various distances to a variety of habitats by different vectors thus, introducing a lot of variation.

Several studies have shown that organisms move non-randomly through the environment to settle in habitats that may enhance their individual performance (e.g. Edelaar et al. 2008, Dreiss et al. 2012). This is called the *habitat matching* hypothesis (Edelaar et al. 2008). Passive dispersal on vectors should be more stochastic than habitat matching because it depends on the behavior and habitat selection of other species. Planarians transported by vectors cannot control where they land (e.g. Hebert & Payne 1985). Thus, contrary to the *habitat matching* hypothesis, the distribution of haplotypes and patterns of gene flow should be more random where passive dispersal on vectors is the primary means of dispersal.

Why do individuals account for the majority of genetic structuring with little differentiation at larger scales? Stochastic dispersal by vectors combined with little connectivity between sites would reduce genetic structuring at larger scales. That is, the initial stochastic distribution of *P. coronata*, possibly attributed to dispersal by vectors would be maintained if there was little dispersal among segments and catchments to homogenize the distribution of haplotypes at larger scales. For example, if haplotypes could disperse within a catchment but not between catchments, then we would expect significant genetic structuring at the scale of catchments. But, if haplotypes rarely disperse beyond a site, then we would expect individuals within sites to account for the majority of genetic structuring, which is what we found in this study. Thus, perhaps we should not expect to find genetic structuring at larger scales when species rarely disperse by vectors.

We found a positive relationship between the distribution and abundance of *P. coronata* haplotypes, plus some haplotypes had a very broad distribution occurring in our study system and at least as far away as Idaho, Wyoming, and Colorado. Why are a few haplotypes widely distributed when the vast majority have a restricted distribution? There are at least two related possibilities. First, the most widely distributed haplotypes may be the most abundant haplotypes. That is, abundant haplotypes should have the greatest chance of being transported by vectors. Any vector must come in contact with individuals of *P. coronata*, and that possibility increases for the most abundant haplotypes. Second, the most widely distributed haplotypes of *P. coronata* may have expanded their distribution during early post-glacial climates. Cooler temperatures throughout the stream network may have facilitated the dispersal of *P. coronata* before warming restricted its distribution to upper reaches in tributary segments. Catchments in this study were free of extensive ice sheets starting about 12,000 years ago (e.g. Laabs et al. 2006) whereas, warming and desertification became evident at about 9000 years ago (Rhode 2000). This leaves approximately 3000 years of cooler temperatures for some haplotypes of *P. coronata* (possibly the most abundant) to expand their distribution.

Species that rarely disperse should show low haplotype diversity at local scales because novel haplotypes would not be able to readily disperse across our study system (Connor & Hartl 2004). Free dispersal would increase the number of haplotypes at any given site as all newly derived haplotypes within the region could colonize all sites. We found, on average, 5 different haplotypes out of 10 individuals per site with a few sites showing as many as 9 out of 10. Since dispersal appears to be rare in this species, how did several haplotypes of *P. coronata* accumulate at local scales? Over time, isolated populations can accumulate haplotypes by mutation and slow lineage sorting. Lineage sorting can be a slow process in large populations and *P. coronata* was one of the five most abundant species in our study. As population size increases, the rate at which rare alleles are lost via drift decreases (Wright 1931), whereas the rate at which novel haplotypes are generated via mutation increases, which would result in increased levels of mtDNA polymorphisms (Templeton 2006). A slow rate of lineage sorting also explained a large number of haplotypes at local scales in Nematodes (Blouin et al. 1992) and Blackflies (Finn & Adler 2006), which are also species that attain high population densities and show a low degree of connectivity.

Also, asexual reproduction can increase the effective population size for mtDNA. Only females pass their mtDNA to the next generation in species with obligate sexual reproduction. In populations that reproduce by fission, all of the individuals pass their mtDNA to the next generation, thus preserving a greater proportion of variant mtDNA fragments (Bessho et al. 1992). Both factors (large populations and asexual reproduction) can cause incomplete lineage sorting and prolong the persistence of haplotypes at local scales, which may account for the high number of haplotypes at local scales in *P. coronata*.

The genus *Polycelis* has a very uniform morphology potentially hiding several new species (e.g. Kenk 1973). Identifying new species in asexual flatworms can be especially difficult because the morphology of the penis, which can be obscured in asexual species, is a critical diagnostic character (e.g. Kenk 1973). We discovered what appears to be two cryptic species based on mtDNA. *Polycelis* sp. A was widespread across the Provo Basin, being found at seven sites, although usually in low abundance. However, it was common at one site in the Provo catchment (Marjorie Creek) and at one site in the Weber catchment (Shingle Creek). Otherwise, we collected a single individual of *Polycelis* sp. A from the American Fork catchment and one individual from Wyoming (Fort Bridger). Only five individuals of *Polycelis* sp. B were found from the Logan, (UT) Snake (ID), and Upper Colorado (WY) basins all outside our study system.

Two other subspecies of *P. coronata* are currently recognized, *P. c. brevipenis* (from western Colorado) and *P. c. monticola* (from California, near Lake Tahoe); while a third form, *P. borealis* (described from Alaska), has been synonymized with *P. coronata* (Kenk 1989). We were not able to include samples from their respective type localities to determine if any of these existing names apply to the cryptic species in our study. However, our data suggest the validity of previous observations (Braithwaite 1962), that continued sampling across the entire range of this species might reveal

a large complex of unidentified species currently grouped with *P. coronata*. Future research should expand the spatial extent of this research to include the entire range of this species. Ignoring cryptic diversity distorts our perception of general diversity patterns and can obscure our understanding of a species niche requirements and functional roles (e.g. Smith et al. 2006).

Polycelis coronata is an obligate aquatic species that inhabits cool, headwater tributaries at the tips of the stream network in catchments of the central Wasatch Mountains. Our intensive, hierarchical sampling design over a very small spatial extent leaves little doubt that this is a poorly connected species. Thus, we found no evidence to suggest that asexual reproduction and recent transport by humans have played a role in increasing the dispersal of this species in the central Wasatch Mountains. However, low connectivity may not be attributed to habitat stability, and multiple explanations should be explored. For example, habitat specialization could also select against a dispersive phenotype. Finally, the effect of dispersal by vectors on the distribution of haplotypes warrants further investigation. Do vectors produce a strong stochastic component to dispersal and how does that effect the distribution of haplotypes? Do individuals at a local site become the greatest source of genetic variation with little structuring at larger scales if vectors are the primary means of dispersal?

Notes on contributors

Russell B. Rader is an aquatic ecologist interested in streams, wetlands, and the marine intertidal zone. His research spans genetic, population, community, and ecosystem dynamics.

Peter J. Unmack is a molecular geneticist with a focus on phylogeography and conservation of desert fishes.

Jeffrey N. Moore was a graduate student at the time of this research.

ORCID

Jeffrey N. Moore http://orcid.org/0000-0002-9391-309X

References

Álvarez-Presas M, Baguñà J, Riutort M. 2008. Molecular phylogeny of land and freshwater planarians (Tricladida, Platyhelminthes): from freshwater to land and back. Mol Phylogenet Evol. 47:555–568.

Baggiano O, Schmidt DJ, Sheldon F, Hughes JM. 2011. The role of altitude and associated habitat stability in determining patterns of population genetic structure in two species of *Atalophlebia* (Ephemeroptera: Leptophlebia). Freshwater Biol. 56:230–249.

Baguette M, Blanchet S, Legrand D, Stevens VM, Turlure C. 2013. Individual dispersal, landscape connectivity and ecological networks. Biol Rev. 88:310–326.

Ball IR. 1974. A contribution to the phylogeny and biogeography of the freshwater triclads (Platyhelminthes, Turbellaria). In: Morse NW, editor. Biology of the Turbellaria. New York: McGraw-Hill; p. 339–401.

Ball IR, Fernado CH. 1969. Freshwater Triclads (Platyhelminthes, Turbellaria) and continental drift. Nature. 221:1143–1144.

Beck DE. 1954. Distributional and natural history notes on *Polycelis coronata* (Girard) in Utah. Proc Utah Acad Sci. 31:79–82.

Bessho Y, Ohama T, Osawa S. 1992. Planarian mitochondria.1. Heterogeneity of cytochrome-c-oxidase subunit-I gene sequences in the fresh-water planarian, *Dugesia japonica*. J Mol Evol. 34:324–330.

Biggs BJF, Nikora VI, Sneider TH. 2005. Linking scales of flow variability to lotic ecosystem structure and function. River Res Appl. 21:283–298.

Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. Annu Rev Ecol Syst. 32:159–181.

Blouin MS, Dame JB, Tarrant CA, Courtney CH. 1992. Unusual population genetics of a parasitic nematode: mtDNA variation within and among populations. Evolution. 46:470–476.

Bowden WB. 1999. Role of bryophytes in stream ecosystems. J North Am Benthol Soc. 18:151–184.

Braithwaite LF. 1962. The taxonomic problem of *Polycelis* in the United States [M.S. thesis]. Provo (UT): Brigham Young University.

Brändle M, Heuser R, Marten A, Brandl R. 2007. Population structure of the freshwater flatworm *Crenobia alpina* (Dana): old lineages and low gene flow. J Biogeogr. 34:1183–1192.

Calow P, Beveridge M, Sibly R. 1979. Heads and tails - adaptational aspects of asexual reproduction in freshwater triclads. Am Zool. 19:715–727.

Conner JK, Hartl DL. 2004. A primer of ecological genetics. Sunderland (MA): Sinauer Associates Publishers.

Dobzhansky T. 1950. Evolution in the tropics. Am Sci. 38:209–221.

Dreiss AN, Antoniazza S, Burri R, Fumagalli L, Sonnay C, Frey C, Goudet J, Roulin A. 2012. Local adaptation and matching habitat choice in female barn owls with respect to melanic coloration. J Evol Biol. 25:103–114.

D'Souza TG, Michiels NK. 2010. The costs and benefits of occasional sex: theoretical predictions and a case study. J Hered. 101:S34–S41.

Edelaar P, Siepielski AM, Clobert J. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. Evolution. 62:2462–2472.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 10:564–567.

Finn DS, Adler PH. 2006. Population genetic structure of a rare high-elevation black fly, *Metacnephia coloradensis*, occupying Colorado lake outlet streams. Freshw Biol. 51:2240–2251.

Gandon S, Michalakis Y. 1999. Evolutionarily stable dispersal rate in a metapopulation with extinctions and kin competition. J Theor Biol. 199:275–290.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 41:95–98.

Hebert PDN, Payne WJ. 1985. Genetic variation in populations of the hermaphroditic flatworm *Mesostoma lingua* (Turbellaria, rhabdocoela). Biol Bull. 169:143–151

Hughes JM, Bunn SE, Hurwood DA, Choy S, Pearson RG. 1996. Genetic differentiation among populations of Caridinazebra (Decapoda: Atyidae) in tropical rainforest streams, northern Australia. Freshw Biol. 36:289–296.

Hughes JM, Bunn SE, Kingston DM, Hurwood DA. 1995. Genetic differentiation and dispersal among populations of *Paratya australiensis* (Atyidae) in rainforest streams in southeast Queensland, Australia. J North Am Benthol Soc. 14:158–173.

Jacob S, Bestion E, Legrand D, Clobert J, and Cote J. 2015. Habitat matching and spatial heterogeneity of phenotypes: implications for metapopulation and metacommunity functioning. Evol Ecol. 29:851–871.

Kenk R. 1973. Freshwater triclads (Turbellaria) of North America. V: the genus Polycelis. Washington (DC): Smithsonian Institution Press.

Kenk R. 1989. Revised list of the North American freshwater planarians (Platyhelminthes: Tricladida: Paludicola). Smithson Contrib Zool. 476:1–10.

Kolasa J, Tyler S, James HT, Alan PC. 2009. Flatworms: Turbellarians and Nemertea. In: Thorp JH, Covich AP, editors. Ecology and classification of North American freshwater invertebrates. 3rd ed.. San Diego (CA): Academic Press. p. 143–161.

Laabs BJC, Plummer MA, Mickelson DM. 2006. Climate during the last glacial maximum in the Wasatch and southern Uinta Mountains inferred from glacier modeling. Geomorphology. 75:300–317.

Lázaro EM, Harrath AH, Stocchino GA, Pala M, Baguná J, Riutort M. 2011. Schmidtea mediterranea phylogeography: an old species surviving on a few Mediterranean islands? BMC Evol Biol. 11:274–288.

Lázaro EM, Riutort M. 2013. Dugesia sicula (Platyhelminthes, Tricladida): the colonizing success of an asexual Planarian. BMC Evol Biol. 13:268–281.

Lázaro EM, Sluys R, Pala M, Stochino GA, Baguñá J, Riutort M. 2009. Molecular barcoding and phylogeography of sexual and asexual freshwater planarians of the genus Dugesia in the Western Mediterranean (Platyhelminthes, Tricladia, Dugesia). Mol Phylogenet Evol. 52:835–845.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25:1451–1452.

Mérigoux S, Doledec S. 2004. Hydraulic requirements of stream communities: a case study on invertebrates. Freshw Biol. 49:600–613.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE); 2010 Nov 14; New Orleans (LA): IEEE. p.1–8. Available from: www.phylo.org.

Milner MM, Knudsen EE, Solseth C, Robertson AL, Schell D, Phillips IT, Magnusson K. 2000. Colonization and development of stream communities across a 200-year gradient in Glacier Bay National Park, Alaska, U.S.A. Can J Fish Aquatic Sci. 57:2319–2335.

Minshall GW, Winger PV. 1968. The effect of reduction in stream flow on invertebrate drift. Ecology. 49:580–582.

Nixon SE. 1978. Freshwater Triclads (Turbellaria) of Washington. Northwest Sci. 52:51–56.

Nixon SE, Taylor RJ. 1977. Large Genetic distances associated with little morphological variation in *Polycelis coronata* and *Dugesia tigrina* (Planaria). Syst Biol. 26:152–164.

Pongratz N, Storhas M, Carranza S, Michiels N. 2003. Phylogeography of competing sexual and parthenogenetic forms of a freshwater flatworm: patterns and explanations. BMC Evol Biol. 3:23.

R Development Core Team. 2010. "R" a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available from: http://www.R-project.org.

Reynoldson TB. 1961. Environment and reproduction in freshwater triclads. Nature. 189:329–330.

Rhode D. 2000. Middle and late Wisconsin vegetation history in the Bonneville Basin. In: Madsen DB, editor. Vol. 130, Late quaternary paleoecology in the Bonneville Basin. Salt Lake City (UT): Utah Geological Survey Bulletin; p. 137–148.

Roff DA. 1990. The evolution of flightlessness in insects. Ecol Monogr. 60:389–421.

Schockaert ER, Hooge M, Sluys R, Schilling S, Tyler S, Artois T. 2008. Global diversity of free living flatworms (Platyhelminthes, "Turbellaria") in freshwater. Hydrobiologia. 595:41–48.

Shiozawa DK, Rader RB. 2005. Great basin rivers. In: Benke AC, Cushing CB, editors. Rivers of North America. Burlington (MA): Elsevier Academic Press; p. 655–685.

Simon C, Frati F, Beckenbach A, Crespi B, Hong L., Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitchondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomological Soc of America. 87:651–701.

Smith MA, Woodley NE, Janzen DH, Hallwachs W, Hebert PDN. 2006. DNA barcodes reveal cryptic host specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). Proc Nat Acad Sci (USA). 103:3657–3662.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30:1312–1313.

Suren AM, Duncan MJ. 1999. Rolling stones and mosses: effect of substrate stability on bryophyte communities in streams. J North Am Benthol Soc. 18:457–467.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 30:2725–2729.

Templeton AR. 2006. Genetic drift in large populations and coalescence. Somerset (NJ): Wiley.

Vannote RL, Sweeney BW. 1980. Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. Am Nat. 115:667–695.

Vilas R, Criscione CD, Blouin MS. 2005. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. Parasitology. 131:839–846.

Walter HE. 1907. The reactions of planarians to light. J Exp Zool. 5:35–162.

Wishart MJ, Hughes JM. 2001. Exploring patterns of population subdivision in the net-winged midge, *Elporia barnardi* (Diptera: Blephariceridae), in mountain streams of the southwestern cape, South Africa. Freshw Biol. 46:479–490.

Wright S. 1931. Evolution in Mendelian populations. Genetics. 16:97.

Appendix 1. Site locations and elevations.

Site	Catchment	Segment	GPS Coordinates	Elevation (m)
1	Provo	1	N 40°34.135' W 111°33.414'	2386
2	Provo	1	N 40°34.099' W 111°33.311'	2377
3	Provo	2	N 40°24.240' W 111°37.245'	2315
4	Provo	2	N 40°24.268' W 111°36.780'	2181
5	Provo	3	N 40°23.167' W 111°36.370'	2241
6	Provo	3	N 40°23.424' W 111°35.055'	1907
7	Provo	4	N 40°25.669' W 111°36.581'	2419
8	Provo	4	N 40°22.135' W 111°33.721'	1624
9	Provo	5	N 40°19.295' W 111°24.995'	2089
10	Provo	5	N 40°20.192' W 111°33.284'	2158
11	Provo	6	N 40°20.858' W 111°32.728'	1831
12	Provo	6	N 40°18.820' W 111°32.368'	1683
13	Provo	7	N 40°19.183' W 111°32.103	1921
14	Provo	7	N 40°20.842' W 111°32.582'	1914
15	Provo	8	N 40°21.111' W 111°34.057'	1656
16	Provo	8	N 40°40.861' W 111°54.835'	1596
17	Provo	9	N 40°39.783' W 111°56.792'	2793
18	Provo	9	N 40°40.562' W 111°59.485'	2904
19	Provo	10	N 40°35.832' W 111°05.743'	3077
20	Provo	10	N 40°40.900' W 111°04.439'	2303
21	Provo	11	N 40°40.046' W 111°05.988'	2862
22	Provo	11	N 40°38.151' W 110°58.540'	2793
23	Weber	1	N 40°38.151' W 110°58.540'	3054
24	Weber	1	N 40°36.676' W 110°58.900'	2901
25	Weber	2	N 40°40.749' W 111°14.226'	2224
26	Weber	2	N 40°40.917' W 111°14.369'	2202
27	Weber	3	N 40°40.749' W 111°14.226'	2618
28	American Fork	1	N 40°32.563' W 111°35.597'	2353
29	American Fork	2	N 40°29.841' W 111°39.737'	2182
30	American Fork	2	N 40°29.524' W 111°39.398'	2085
31	American Fork	3	N 40°29.063' W 111°41.155'	2680
32	American Fork	3	N 40°29.078' W 111°39.117'	2051
33	American Fork	4	N 40°24.636' W 111°38.589'	2635
34	American Fork	4	N 40°26.025' W 111°38.083'	2389
35	American Fork	5	N 40°25.000' W 111°38.130'	2389
36	American Fork	5	N 40°26.835' W 111°38.335'	2023
37	American Fork	6	N 40°43048' W 111°63886'	2636
38	American Fork	6	N 40°26.025' W 111°38.083'	2230
39	Little Cottonwood	1	N 40°35.220' W 111°37.490'	2615
40	Little Cottonwood	1	N 40°34.272' W 111°41.098'	2615
41	Little Cottonwood	2	N 40°34.590' W 111°36.009''	2853
42	Little Cottonwood	2	N 40°34.739' W 111°38.195'	2805
43	Little Cottonwood	3	N 40°34.451'' W 111°36.882'	2842
44	Little Cottonwood	3	N 40°34.716' W 111°36.923'	2821
45	Little Cottonwood	4	N 40°32.839' W 111°40.394'	2892
46	Little Cottonwood	4	N 40°33.959' W 111°41.330'	2484
47	Little Cottonwood	5	N 40°33.211' W 111°41.709'	2663
48	Little Cottonwood	5	N 40°34.202' W 111°42.019'	2184
49	Little Cottonwood	6	N 40°34.121' W 111°43.029'	2014
50	Little Cottonwood	6	N 40°34.172' W 111°43.282'	1997