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If you've seen one worm, have you seen them all? Spatial, community, and genetic variability of tubificid communities in Montana

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Abstract: Genetic studies are recognized increasingly as important for understanding naturally occurring disease dynamics and are used to predict host genetic diversity and coevolutionary processes and to identify species composition in ecological communities. *Tubifex tubifex*, the definitive host of the whirling disease parasite *Myxobolus cerebralis*, comprises 6 known lineages that vary widely in parasite susceptibility. We used 16S ribosomal DNA (16S rDNA) to identify relationships among genetic variability of 3 oligochaete genera (*T. tubifex*, *Rhyacodrilus* spp., and *Ilyodrilus* spp.; Oligochaeta:Tubificidae), oligochaete assemblage composition, and the presence of whirling disease in 9 locations across 4 watersheds in Montana, USA. We assessed genetic variability among 183 tubificid worms from locations classified as positive or negative for whirling disease based on 5 to 8 y of monitoring by the Montana Department of Fish, Wildlife, and Parks. Within genera, we found 2 groups of *T. tubifex* (lineages I and III), 2 groups of *Rhyacodrilus* spp., and 4 groups of *Ilyodrilus* spp., possibly suggesting cryptic species. The maximum genetic variability within taxa was relatively high (~10% sequence divergence) for all 3 genera, but haplotype diversity within groups with >5% sequence divergence was greater for *Ilyodrilus* spp. (0.719) than for *Tubifex* spp. (0.246) and *Rhyacodrilus* spp. (0.143). The variation was nonrandomly distributed over the landscape. Oligochaete genetic composition was more similar among locations in the same watershed than among locations with or without whirling disease. Thus, oligochaete assemblage composition did not appear to be related to the presence of the disease at this watershed spatial scale.

Key words: tubificid taxa, 16S, haplotype diversity, spatial variation, community assembly

Community assembly has intrigued biologists for decades (Elton 1946). Comparison of species diversity among communities sheds light on aspects of host–parasite ecology and evolution important for disease epidemiology, especially because epidemiological processes can be highly variable over space and time. Host spatial distribution may influence disease transmission, especially if the parasite is closely associated with a particular host species (Frantz et al. 2009). Interactions among hosts and parasites at the landscape level are particularly interesting because heterogeneous features and scale of habitat and environment affect many patterns of infectious disease (Archie et al. 2009).

Myxobolus cerebralis (Hofer 1903), the causative agent of whirling disease, was introduced to the USA from Europe in the 1950s and has spread to >25 states (Bartholomew and Reno 2002). In some areas of the Intermountain West ecoregion, it has caused catastrophic decline of salmonids (Vincent 1996). Despite continued research on the role of variability in the oligochaete host, salmonid, and environment (Hedrick et al. 1998, Gilbert and Granath 2003), much of the temporal and spatial variation in fish disease risk remains unexplained (Kerans and Zale 2002). For example, certain tributaries in Montana have tested consistently negative for the parasite despite close proximity to streams where Rainbow Trout populations have

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declined because of whirling disease. In some tributaries, whirling disease severity has remained low several years after introduction of the parasite. Comparisons of the biotic and abiotic factors that cause spatial variation in whirling disease risk exist among streams within the same watershed (de la Hoz and Budy 2004), but few comparisons have been made across multiple watersheds (but see McGinnis and Kerans 2013).

Tubifex tubifex (Müller, 1774), the definitive oligochaete host of *M. cerebralis*, is now viewed as central to the severity and eventual management of whirling disease for certain trout populations (Beauchamp et al. 2002, Elwell et al. 2006). Fish disease risk varies spatially, and a major biotic factor that contributes to this spatial variation is tubificid assemblage composition, a mixture of species composed of *T. tubifex*, *Limnodrilus hoffmeisteri*, *Ilyodrilus* spp., and *Rhyacodrilus* spp. (Kerans et al. 2004). Sympatric cryptic species, parthenogenetic clones, and polyploidy are common in these groups (Paoletti 1989). The existence of different genetic lineages in *T. tubifex* populations colonizing the same or different habitats (Lang and Langdobler 1979, Lafont 1984) also may contribute to spatial variation in fish disease risk (Krueger et al. 2006). The influence of variation in tubificid assemblage composition on disease severity is not well understood (Kerans et al. 2004), but quantification of tubificid assemblage composition across multiple watersheds would provide a starting point for better understanding the spatial variation of whirling disease risk.

We increased the spatial scale at which fish disease and tubificid assemblage composition have been studied from single (Krueger et al. 2006) to multiple watersheds to determine if variation in oligochaete assemblages would be related to the risk of Rainbow Trout whirling disease at the watershed scale. This hypothesis, the dilution effect, posits that high diversity in the tubificid community could dilute the effect of the most competent host (LoGiudice et al. 2003). To determine if fish disease and tubificid assemblage are related at the watershed scale, we used relative abundance and DNA-sequence data to test the relationship between tubificid assemblage composition and incidence of fish disease. We focused on tubificids with external morphology similar to *T. tubifex* (i.e., *Tubifex* spp., *Ilyodrilus* spp., and *Rhyacodrilus* spp.), which made up the greater part of tubificid assemblages in sampled watersheds.

We used the genetic species concept (species are genetically isolated) rather than the biological species concept (species are reproductively isolated) (Dobzhansky 1950, Baker and Bradley 2006) because the taxonomy of these genera is not well developed and cryptic species have been reported for *Tubifex* spp. (Sturmbauer et al. 1999) and *Rhyacodrilus* spp. (Martinsson et al. 2013). This species concept is useful for DNA-based studies.

However, identifying the threshold for separate species is problematic. Two lines of evidence suggest >5% sequence divergence in the 16S ribosomal DNA (rDNA) gene is meaningful for tubificids. First, Beauchamp et al. (2001) found that 16S rDNA sequence divergence for major lineages of *T. tubifex* ranged from 5.8 to 12.5%. Second, *Rhyacodrilus* spp. showed a similar 16S rDNA sequence divergence (Martin et al. 2010). *Rhyacodrilus aeternorum* and *Rhyacodrilus latinus* differ by 4.8%, and these sister species differ from *Rhyacodrilus abyssalis* by 18.5%.

We examined the distribution and abundance of *Tubifex* spp., *Ilyodrilus* spp., and *Rhyacodrilus* spp. in 4 western watersheds in Montana, USA, and simultaneously assessed their spatial genetic variability and its relationship to fish disease. We sampled multiple locations to estimate the large-scale taxon diversity and genetic variability of these 3 taxa. The Montana Department of Fish, Wildlife, and Parks had previously assessed whirling disease incidence in fish at these locations over a 5- to 8-y period. Our objectives were to: 1) assess the taxon diversity and genetic variability of *Tubifex* spp., *Ilyodrilus* spp., and *Rhyacodrilus* spp. in the study locations, and 2) relate the assemblage composition of these 3 genera to the watershed-scale incidence of whirling disease. In conducting this study, we also evaluate the possibility of cryptic species for *Ilyodrilus* spp. because ours is the first genetic study of this genus.

METHODS

Sampling locations

We examined tubificids from 9 locations in 4 watersheds (Fig. 1A–D): Belmont Creek (Bel), Chamberlain Creek (Cham), and Gold Creek (Gold) in the Blackfoot River watershed; Ross Fork (Ross), West Fork Rock Creek (WFR), and Willow Creek (Wil) in the Rock Creek watershed; Ledford (Led) Creek and Ruby River (Ruby) in the Ruby River watershed; and West Fork Madison River (WF) in the Madison River watershed (see Table S1 for a list with global positioning system coordinates). All watersheds had naturally reproducing populations of wild Rainbow Trout affected to varying degrees by whirling disease (Vincent 1996, T. E. McMahon, Montana State University, personal communication), but whirling disease was positive only at Bel, Cham, WF, Ross, Wil, and Led (Table 1).

We used estimated risk of whirling disease based on sentinel cage data available from the Montana Department of Fish, Wildlife and Parks over a 5- to 8-y period for all locations (Krueger et al. 2006). Briefly, these data were obtained as follows. Locations (i.e., the entire area draining to a sentinel cage) within single watersheds were selected such that they were statistically independent. Wire-mesh enclosures stocked with 50 Rainbow Trout fry were

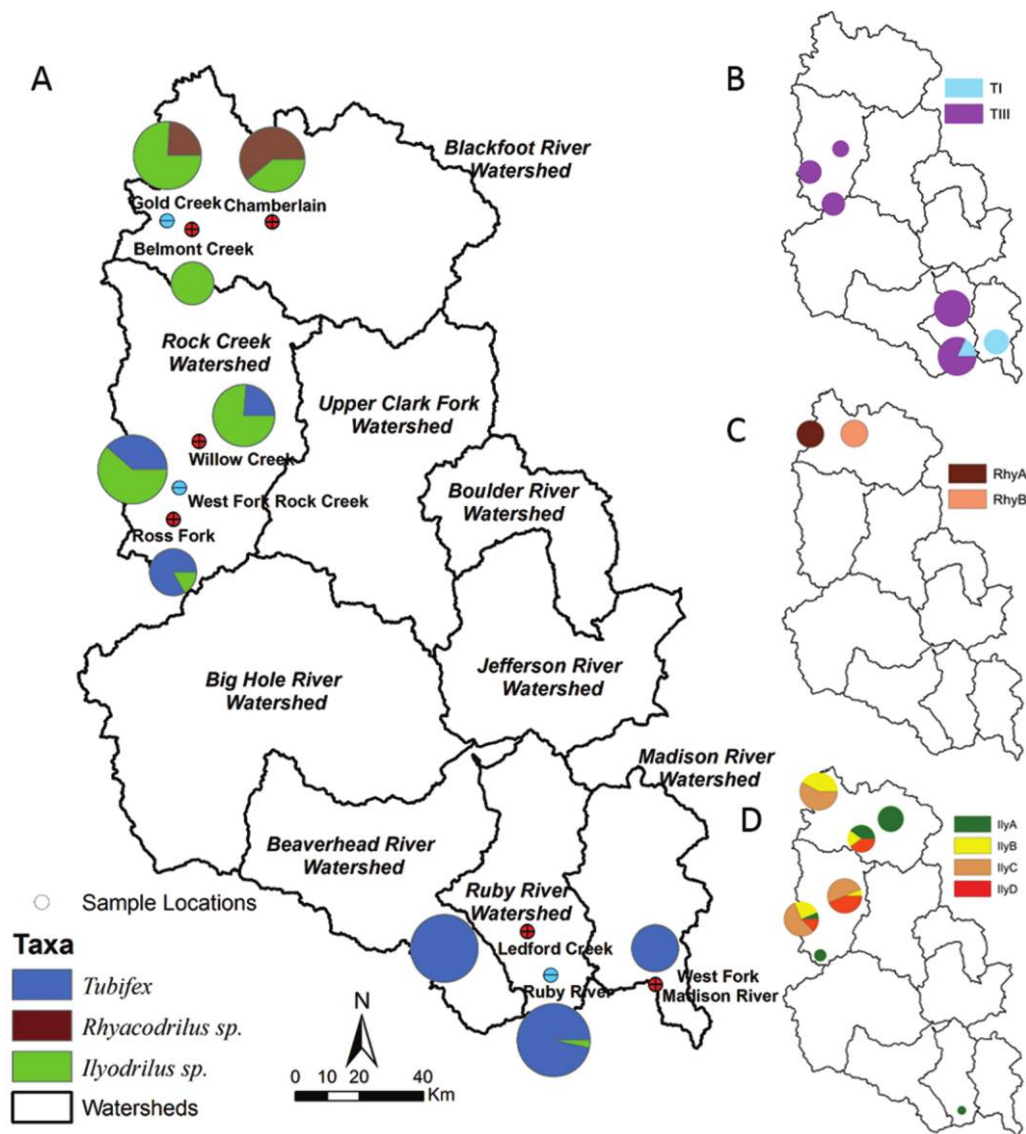


Figure 1. Oligochaete collection locations in 4 watersheds in western Montana showing assemblage composition of genera of tubificids with hair chaetae (*Tubifex* spp., *Rhyacodrilus* spp., and *Ilyodrilus* spp.) (A), and composition of groups with >5% genetic divergence within *Tubifex* (B), *Rhyacodrilus* (C), and *Ilyodrilus* (D). The size of the circles is proportional to sample size. + = presence and - = absence of whirling disease.

placed in streams for ~10 d. Fish were raised at state facilities for ~80 d to allow myxospore development, and then humanely killed and assayed for whirling disease severity (low, moderate, high). We designated locations having >50% of sentinel fish with moderate and high infection severities for whirling as positive. Two negative locations never had infected fish, and 1 negative location had 1 infected fish (2%) in 1 of the 8 y tested.

We georeferenced the 9 sampling locations (Table S1) with GPSmap (version 76CSx; Garmin, Olathe, Kansas). We downloaded a base map from nris.mt.gov/gis and identified upstream points with the editor's distance-distance

tool in ArcGIS (version 10; Environmental Systems Research Institute [ESRI], Redlands, California). We created the sampling-location map from the base map (GCS_North_American_1983_CSRS) and mapped the taxon distributions in ArcMap (version 9.3.1.3000; ESRI).

Oligochaete collection

Between November 2007 and January 2008, we took 2 oligochaete samples upstream of the sentinel cage at each location. We collected the 1st sample at the 1st road crossing upstream of the sentinel cage and the 2nd sam-

Table 1. The relative abundances of 3 oligochaete genera (*Tubifex* spp., *Rhyacodrilus* spp., and *Ilyodrilus* spp.) and presence or absence of whirling disease at 9 locations in 4 watersheds in Montana. Values for the highest abundance of each taxon are in bold.

Taxon	Blackfoot		Madison		Rock			Ruby		Total (All taxa)
	Belmont	Chamberlain	Gold	West Fork	Ross Fork	West Fork	Willow	Ledford	Ruby	
<i>Tubifex</i>	0	0	0	12 (100%)	10 (83%)	10 (62%)	5 (24%)	25 (100%)	28 (97%)	90 (49%)
<i>Rhyacodrilus</i>	0	14 (61%)	6 (24%)	0	0	0	0	0	0	20 (11%)
<i>Ilyodrilus</i>	10 (100%)	9 (39%)	19 (76%)	0	2 (17%)	16 (38%)	16 (76%)	0	1 (3%)	73 (40%)
Total	10	23	25	12	12	26	21	25	29	183
Whirling disease	Positive	Positive	Negative	Positive	Positive	Negative	Positive	Positive	Negative	

ple at the next upstream channel unit (40× the largest width measured along 3 random transects in the 1st sampling location). If an accessible upstream road crossing did not exist, the 1st sampling location occurred at the 1st point where the stream was ≤200 m from the road across public land. When an accessible road crossing did not exist ≤200 m from the stream, we sampled oligochaetes directly upstream of the sentinel cage.

Each sample consisted of four 2-min kick-net collections and was sorted by 1 to 4 people for a total of 30 person-min or until 200 oligochaetes were collected, whichever came first. We transported live oligochaetes to the laboratory in a cooler and kept them at 10°C in an incubator until processed further (usually within 24 h). In the laboratory, we selected a random subsample of 15 oligochaetes with hair chaetae (i.e., *Tubifex* spp., *Ilyodrilus* spp., and *Rhyacodrilus* spp.). When samples contained <15 individuals, we selected all oligochaetes with hair chaetae. We cut each oligochaete in half and slide-mounted the anterior section for morphological identification using published keys (Kathman and Brinkhurst 1998) and extracted DNA from the posterior section (as described in Kerans et al. 2004) for polymerase chain reaction (PCR) analysis of the 16S rDNA mitochondrial gene (Beauchamp et al. 2001). Thus, we used up to 30 oligochaetes with hair chaetae from each location (up to 15 from each of 2 samples from the 9 locations for a total of 183) to assess the spatial genetic pattern and measure the relative abundance of *Tubifex* spp., *Ilyodrilus* spp., and *Rhyacodrilus* spp. at each location. We did the analysis hierarchically based on: 1) genus (*Tubifex* spp., *Ilyodrilus* spp., and *Rhyacodrilus* spp.), 2) major groups within genera (>5% sequence divergence), and 3) haplotypes within groups.

DNA extraction and amplification

We extracted DNA from individuals by repeated freezing (−20°C) and thawing (37°C) of samples in lysis buffer with proteinase K, followed by incubation at 65°C for 90 min and 95°C for 15 min (Crottini et al. 2008). We quantified the extracted DNA with a NanoDrop (Thermo Scientific, Wilmington, Delaware) spectrophotometer, di-

luted it to 10 to 100 ng/μL with water, and stored it at −20°C.

We determined the 16S rDNA sequence of each individual by DNA sequencing of PCR products. We used 2 primer sets. We amplified an ~550 base pair (bp) fragment using 16Sar (5'-CGCCTGTTTATCAAAAACAT-3') and 16Sbr (5'-CCGGTYTGAACCTCAGATCAYGT-3') (Palumbi et al. 1991) or an ~350-bp fragment using *T. tubifex*-specific Tub16SF (5'-AACGGCCGCGGTATCC-TG-3') and Tub16SR (5'-TAARCCAACATYAGAGTGCC-3') (Beauchamp et al. 2001). We ran the PCR amplification in 25 μL volume with 2× MangoMix (Bioline, Tauton, Massachusetts), 0.5 μL of 10 μM of each primer, 1 to 2 μL (20–100 ng/μL) of DNA and PCR-grade water (W1754; Sigma-Aldrich, St Louis, Missouri). The amplification profile was initial denaturation at 95°C for 5 min and 35 cycles at 95°C for 1 min, 65°C for 2 min 30 s, 72°C for 1 min 30 s, and a final extension at 72°C for 10 min. To confirm amplification and correct amplicon size, we visualized PCR products in 2% agarose gel stained with SYBR Green I nucleic acid gel stain (Invitrogen, Eugene, Oregon).

DNA sequencing and alignment

Sequencing was done by the DNA analysis facility at the University of Vermont or by Agencourt (Beckman Coulter Genomics, Danvers, Massachusetts). The cycling conditions for both facilities were denaturation for 3 min at 95°C and 25 cycles of amplification at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Automated sequencing was done in both directions with the same amplification primers used for PCR with BigDye Terminator (version 3.1; Applied Biosystems, Carlsbad, California). Sequences were determined with an ABI 377 DNA sequencer (Vermont) or ABI PRISM 3730xl (Agencourt) (both Applied Biosystems).

All sequences were trimmed to the ~320 bp between the primers (no insertions or deletions), aligned, and edited using Sequencer 4.10.1 (Gene Codes Corporation, Ann Arbor, Michigan). We deposited 1 sequence for each haplotype in GenBank (see Table S2 for a list of haplotypes and GenBank accession numbers). The taxonomic

identity of each individual was determined using the Basic Local Alignment Search Tool (BLAST) algorithm (National Center for Biotechnology Information [NCBI] BLAST) and compared to sequences of laboratory-reared tubificids of known taxa. Species-level identification was not possible for *Ilyodrilus* spp. and *Rhyacodrilus* spp. because they did not fit descriptions in taxonomic keys and similar sequences were not present in GenBank. We grouped these tubificids within genera based on genetic differences >5%.

Diversity assessment

We compared taxon diversity between locations with the Jaccard Index (Jaccard 1901), which estimates similarity on a scale of 0 (no taxa in common) to 1 (identical composition) between locations a and b :

$$J(a, b) = \frac{a \cup b}{a \cap b'}$$

where $a \cup b$ is the number of taxa shared between 2 locations and $a \cup b$ is the total number of taxa in the 2 locations. We examined taxon similarity among locations grouped by: 1) watershed and 2) presence (WD+) or absence (WD-) of whirling disease. We calculated Jaccard indices with EstimateS (version 9; University of Connecticut, Storrs, Connecticut) with an abundance-based probabilistic approach that depends primarily on relative abundance, assuming random mixing and equivalent detectability (Chao et al. 2005). We ran 1000 bootstrap replicates to calculate standard deviations.

Estimating relative abundance and nucleotide and haplotype diversity

We calculated the relative abundances of each genus and of groups with >5% sequence divergence within genera for each location and compared them with the Pearson χ^2 test (JMP®, version 9; SAS Institute, Cary, North Carolina). We estimated nucleotide diversity (π ; Nei 1987), haplotype diversity (h ; Nei and Tajima 1981), and the number of segregating sites (θ_w ; Watterson 1975) in DnaSP (version 5; Librado and Rozas 2009). We created a haplotype network for each genus in Network (version 4.516; available from: fluxus-engineering.com) (Fig. S1).

RESULTS

Spatial variation in community composition

Samples from our 9 locations included 2 lineages of *T. tubifex* (T_I and T_{III}), 2 groups of *Rhyacodrilus* spp. (*RhyA* and *RhyB*), and 4 groups of *Ilyodrilus* spp. (*IlyA*, *IlyB*, *IlyC*, *IlyD*) (Table S2, Fig. 1A–D). The distribution of the 3 genera was nonrandom across watersheds (likelihood ratio: $\chi^2 = 185$, $df = 6$, $p < 0.0001$) and among locations (likelihood ratio: $\chi^2 = 215$, $df = 16$, $p < 0.0001$).

Tubifex spp. were widely distributed (i.e., in 3 of the 4 watersheds). *Tubifex* spp. were not found in Blackfoot River watershed, but this was the only watershed in which *Rhyacodrilus* spp. were found. *Ilyodrilus* spp. were found in 3 of the 4 watersheds, and were absent from the Madison River watershed (Table S2, Fig. 1A).

We never found all 3 genera together. Three locations (Bel, Led, and WF) had 1 genus, and the other 6 locations had 2 (Fig. 1A). Assemblage composition at the 6 locations with 2 genera ranged from dominance by 1 genus to a fairly even distribution of abundance (Fig. 1A). When considering groups with >5% sequence divergence, the Rock Creek (6 groups) and Blackfoot River (5 groups) watersheds had the highest richness compared to 3 groups for Ruby River watershed and only 1 for the Madison River watershed. Highest group richness occurred at Rock Creek (6 groups), and 2 locations (Led and WF) had only 1 group (Fig. 1B–D).

Tubifex tubifex was present at only 6 locations. Four of these locations (WF, Ross, Wil, Led) were WD+, and 2 (Rock, Ruby) were WD-. Two locations that lacked *T. tubifex* were WD+ (Bel, Cham) (Table 1, Fig. 1A).

Biotic and abiotic associations

Assemblage similarity at the genus level (*Tubifex* spp., *Rhyacodrilus* spp., and *Ilyodrilus* spp.) was greater on average (0.58–1.00) for locations grouped by watershed (geography) than for locations grouped by presence or absence of whirling disease (WD+: 0.12–0.59, WD-: 0.27–0.76; Fig. 2A). For example, on average, Led (WD+) was more similar to other locations in the same watershed ($J[\text{Led, Ruby}] = 0.97$) than to other WD+ locations (e.g., $J[\text{Led, Gold}] = 0$, $J[\text{Led, Cham}] = 0$, $J[\text{Led, Ross}] = 0.83$, and $J[\text{Led, Will}] = 0.24$; average $J[\text{Led, WD+}] = 0.27$). The same pattern was seen when considering groups with >5% sequence divergence, but J -values were lower (see Table S4 for values, Fig. 2B).

Spatial variation in taxa and cryptic species

The 16S rDNA gene was useful for identifying taxonomic groups, but not for examining variation within groups with >5% sequence divergence, as shown by the overall low genetic diversity (Table 2). Genetic diversity measures generally were 1.5× higher for *Ilyodrilus* spp. than for the other genera. The 32 to 50 mutations within each genus resulted in similar θ_w , h , and π values for *Tubifex* spp., *Rhyacodrilus* spp., and *Ilyodrilus* spp. (Table 2). All diversity indices were lower for T_{III} than *IlyD* (Table 2). *IlyD* was more variable than the other *Ilyodrilus* groups, *Tubifex* spp., and *Rhyacodrilus* spp. Six *Ilyodrilus* spp. haplotypes were found from 21 sequences resulting in a haplotype diversity of 0.719. The number of segregating sites per sequence within *IlyD* was ~4× that within T_{III} (Table 2).

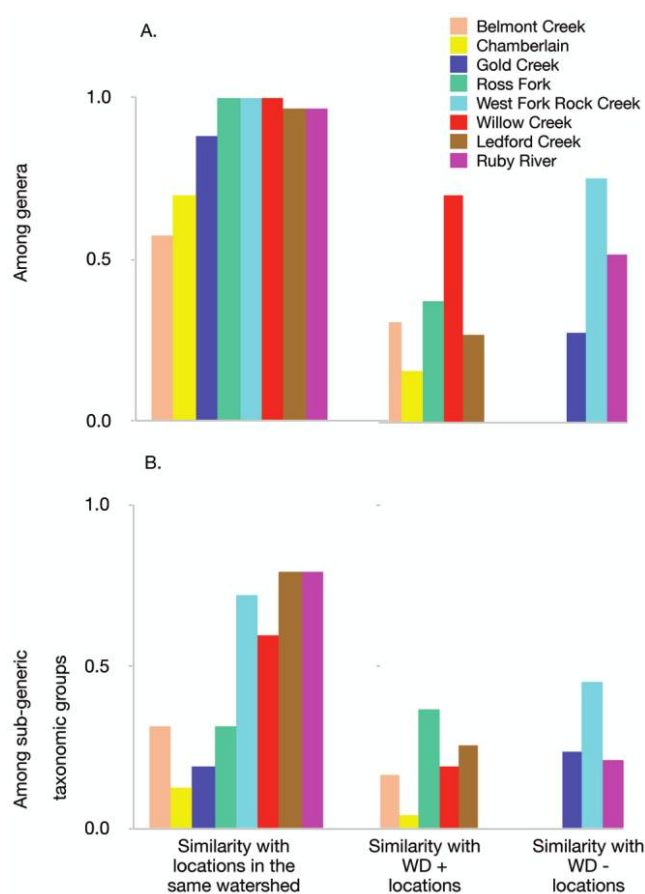


Figure 2. Mean values of Jaccard's Index of Similarity among assemblages of oligochaete genera (A) and groups with >5% genetic divergence (B) at sampling locations grouped by watershed and by presence of whirling disease (WD+ or WD-). Only one stream (West Fork Madison) was sampled in the Madison River watershed, so this site was excluded from these analyses.

Tubifex spp. ($n = 90$) consisted of 2 groups, T_I and T_{III} , which diverged by $\sim 10\%$ (36–38 mutations). *Tubifex* spp. had 4 haplotypes (see Fig. S1A for the haplotype network). T_I had only 1 haplotype and was found in the 2 southern watersheds. The 3 T_{III} haplotypes were only 2 mutations apart. T_{III} was found in the Rock Creek watershed and the 2 southern watersheds (Fig. 1B).

Rhyacodrilus spp. ($n = 20$) consisted of 2 major groups, *RhyA* and *RhyB*, which differed by $\sim 10\%$ (31–32 mutations) (Table 2). *Rhyacodrilus* had 3 haplotypes (Table S2). *RhyA* consisted of 1 haplotype found in 6 individuals, all from Gold Creek. *RhyB* was found only at Chamberlain, where 13 individuals shared a haplotype, and 1 individual differed (Table S2, Fig. 1C).

Ilyodrilus spp. ($n = 76$) consisted of 4 major groups (*IlyA*–*IlyD*) separated by 5 to 10% (15–31 mutations) (Table 2, Fig. S1B). This result suggests the existence of multiple *Ilyodrilus* spp. lineages and perhaps cryptic species. *IlyD* had 6 haplotypes, whereas *IlyA*, *IlyB*, and *IlyC* had only 1 or 2 (Table 2, Fig. S1B). The single *IlyA* haplotype was widely distributed, but rare in the southern part of the study area. *IlyB*, *IlyC*, and *IlyD* were restricted to the Blackfoot and Rock watersheds (Fig. 1D).

DISCUSSION

Relationship between tubificid assemblage composition and risk of whirling disease

Spatial variation in infection prevalence can be caused by variation in the genetic makeup of the host and by abiotic and biotic factors. We investigated the hypothesis that variation in oligochaete assemblages, particularly the distribution and abundance of *Tubifex* spp., *Rhyacodrilus* spp., and *Ilyodrilus* spp., would be related to the risk of Rainbow Trout whirling disease because high abundance provides ample host habitat for *M. cerebralis* (Krueger

Table 2. Genetic variability of an ~ 350 base pair segment of 16S ribosomal DNA from tubificid assemblages sampled from 9 locations in 4 watersheds in Montana. Variability is shown for 3 genera (*Tubifex* spp., *Rhyacodrilus* spp., and *Ilyodrilus* spp.), *T. tubifex* lineage III (T_{III}), *Rhyacodrilus* sp. group B (*RhyB*), and *Ilyodrilus* sp. groups C (*IlyC*) and D (*IlyD*). N = number of specimens, π = nucleotide diversity, θ_w = segregating sites per sequence and h = haplotype diversity.

Taxon name	N	No. of variable sites	No. of haplotypes	% polymorphic sites	π	θ_w /sequence	h
<i>Tubifex</i>	90	38	4	0.112	0.037	7.693	0.471
<i>Rhyacodrilus</i>	20	32	3	0.096	0.001	9.020	0.511
<i>Ilyodrilus</i>	76	50	10	0.148	0.041	10.420	0.809
T_{III}	73	2	3	0.006	0.065	0.411	0.246
<i>RhyB</i>	14	1	2		0.006		0.143
<i>IlyC</i>	23		2				0.087
<i>IlyD</i>	21	6	6	0.018		1.668	0.719

et al. 2006). This hypothesis, the dilution effect, posits that high diversity in the tubificid community could dilute the effect of the most competent host (LoGiudice et al. 2003). *T_{III}* is highly susceptible and *T_I* is moderately susceptible to *M. cerebralis* (Beauchamp et al. 2002, DuBey et al. 2005, Lodh et al. 2011). *Rhyacodrilus* spp. (R. Lamb, Montana State University, unpublished results) and *Ilyodrilus* spp. (Kerans et al. 2004) are not suitable hosts, but might contribute in some way to variability in infection prevalence (e.g., by interacting with *T. tubifex* to influence its distribution). Therefore, we measured the genetic variability for these 3 taxa and evaluated what this variation could tell us about the disease dynamics. We found that group composition of the tubificid assemblage was not related to presence of whirling disease. Similarity in tubificid assemblages between streams was much more strongly related to geographic proximity than to presence or absence of fish disease, i.e., streams within the same watershed had similar tubificid assemblages, regardless of presence or absence of disease. In fact, we did not collect either *T_{III}* or *T_I* at 2 locations (Belmont and Chamberlain, both in the Blackfoot River watershed) where whirling disease had been previously found; we speculate *Tubifex* spp. may be rare in this watershed and the worm samples collected for this study were further away from the cage locations than in Krueger et al. (2006).

Spontaneous phylogeographic structure can arise in a parasite system (Real and Biek 2007) because the parasite relies on its host for dispersal (Criscione and Blouin 2007). Spatial variation and environmental factors (Kaeser et al. 2006, Anlauf and Moffitt 2008, Alexander et al. 2011) and the presence of susceptible and resistant lineages of *T. tubifex* play an important role in the spatial distribution of fish disease (Beauchamp et al. 2005). However, the variation among watersheds in the relative abundance and genetic variability of *Tubifex* spp., *Rhyacodrilus* spp., and *Ilyodrilus* spp. leads us to conclude that although previous investigators found that fish disease was related to the tubificid assemblage at the local scale (Krueger et al. 2006, Lodh et al. 2011), the tubificid community may not be a good predictor of fish disease at the watershed scale. Moreover, we found little relationship between the presence of *T. tubifex* and risk of whirling disease at the watershed scale.

Cryptic diversity in the tubificid assemblage

Cryptic species have been reported for both *Tubifex* spp. (Sturmbauer et al. 1999) and *Rhyacodrilus* spp. (Martinsson et al. 2013), but to our knowledge, not for *Ilyodrilus* spp. *Ilyodrilus* spp. were more variable than *Tubifex* spp. and *Rhyacodrilus* spp. in terms of genetic variation within and between groups with <5% sequence divergence and the number of locations at which the lineages and taxa were found. Moreover, haplotype diver-

sity was higher in *Ilyodrilus* spp. than in *Tubifex* spp. or *Rhyacodrilus* spp. (Table S2). All of the *Ilyodrilus* spp. individuals examined in our study were morphologically similar to *Ilyodrilus templetoni*, but the large amount of genetic variation leads us to conclude that cryptic species exist in this genus.

The high amount of genetic variation within *Ilyodrilus* spp. is surprising, especially given the relatively small spatial scale of our sampling. Verdonshot (2006) sampled across Europe and found only *Ilyodrilus templetoni*. In contrast, we found *Ilyodrilus* spp. to be more genetically variable than *T. tubifex* and *Rhyacodrilus* spp. at the >5% and <5% levels of genetic variation. Possible explanations for this difference include that *Ilyodrilus* spp. are more variable in the closely adjacent watersheds in Montana than across Europe for some unknown reason. Additional research on *Rhyacodrilus* spp. and *Ilyodrilus* spp. may tell us about the physiological traits that determine their distribution or accompany their genetic variability.

Our results show a spatial component to the distribution and abundance of these taxa, and highlight the importance of spatial context and spatial scale when studying host–parasite interactions. They also leave us with questions about the relationship between oligochaete assemblage composition and risk of whirling disease. Future research, with more intensive sampling within watersheds, could examine intermediate spatial scales for the relationship between oligochaete assemblage composition and risk of whirling disease.

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