Long-distance dispersal, ice sheet dynamics, and mountaintop isolation underlie the genetic structure of glacier ice worms

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Running title: Population genomics of ice worms

Keywords: RAD sequencing, Pacific Northwest, phylogeography, *Mesenchytraeus solifugus*, global change biology, annelid

Abstract:

Disentangling the contemporary and historical factors underlying the spatial distributions of species is a central goal of biogeography. For species with broad distributions but little capacity to actively disperse, disconnected geographic distributions highlight the potential influence of passive, long-distance dispersal (LDD) on their evolutionary histories. However, dispersal alone cannot completely account for the biogeography of any species, and other factors–e.g., habitat suitability, life history–must also be considered. North American ice worms (*Mesenchytraeus solifugus*) are glacier-obligate annelids that inhabit coastal North American glaciers from Oregon

to Alaska. Previous studies identified a complex biogeographic history for ice worms, with evidence for genetic isolation, unexpectedly close relationships among geographically disjunct lineages, and evidence for contemporary migration across large (>1,500 km) areas of unsuitable habitat. In this study, we collected genome-scale sequence data for most of the known ice worm range. We found support for a deep divergence between populations along the Pacific Coast and the inland flanks of the Coast Mountains (mean $F_{ST} = 0.60$) as well as support for LDD from Alaska to Vancouver Island, perhaps mediated by migrating birds. Our results highlight the power of genomic data for disentangling complex biogeographic patterns, including the presence of LDD.

Introduction:

For more than a century, long-distance dispersal (LDD) among presumably isolated populations has intrigued biologists [1-4]. Historically considered rare and unpredictable, the idea that LDD can act as a general mechanism influencing the biogeography of presumably dispersal-limited, macroscopic organisms has gained traction in recent years, with examples accumulating for both plants [5] and invertebrates [6-9]. Many animal vectors play an integral role in plant and invertebrate LDD [e.g., 10, 11], however, in most cases, the resulting LDD is limited to < 10 km. For more extreme LDD events (e.g., > 100 km), the most common animal vector is likely migratory birds, as they seasonally move by the millions over broad spatial scales and geographic barriers, visiting similar habitats along the way [12, 13]. Through this mechanism, dispersal units (e.g., whole organisms, eggs, seeds, etc.) may be ingested and dispersed internally or by directly adhering to the bird's exterior [12]. Thus, as long as there is an opportunity for migratory birds and dispersal units to interact, the opportunity also exists for LDD. Physically quantifying LDD is difficult, however, because it requires real-time sampling and searching (internal and external) of migrating birds for hitchhiking dispersers. Moreover, because rare migratory events can affect species distributions [14] and influence genetic differentiation among populations [15], even thorough physical surveys of migratory birds that find no evidence for LDD cannot rule out its presence. Therefore, alternative approaches for detecting and characterizing LDD should be employed. Because population genomic tools are well-suited to detecting gene flow among populations [e.g., 16], these tools are also well-suited to the indirect detection of LDD, even in the absence of field observations.

Many mechanisms influence genetic relationships among taxa and a range of factors should be considered when attempting to reconstruct biogeographic patterns. For instance, pulses and contractions of glaciers and ice sheets have shaped the evolutionary histories of populations and species throughout Earth's history [17-20]. These ice sheet dynamics typically affect organisms as ice traverses the landscape, separating and reconnecting populations as it expands and contracts. However, some species are directly tied to ice sheets [e.g., the meltwater stonefly, 17] and are therefore much more susceptible to ice sheet influence on their evolutionary trajectories. Perhaps no species is more directly tied to ice sheets than the glacier ice worms, *Mesenchytraeus solifugus* in North America [21] and *Sinenchytraeus glacialis* in Tibet [22]. The geographic range of *M. solifugus* (hereafter "ice worm") follows a coastal arc from the Chugach Mountains in southeast Alaska to the Cascade Volcanoes of Washington and Oregon [24]. Ice worms can also not tolerate temperatures more than roughly ±7 °C from freezing and requires glacier ice for survival and reproduction [23]. With such unique ecology and physiology, and a dispersal-limited life history, the evolutionary history of ice worms since diverging from conspecifics [25, 26] should be relatively simple, with gene flow occurring during glacial periods and isolation (paired with genetic drift) driving divergence among mountaintop-isolated populations during interglacial periods. Natural systems, however, are often more complex than expected and indeed, the evolutionary history of ice worms challenges general expectations of gene flow and evolutionary dynamics in mountain systems.

Previous genetic studies based on one or two genetic markers identified three ice worm lineages: a "northern" clade in southern Alaska, a "central" clade in southeast Alaska and northern British Columbia (BC), and a "southern" clade ranging over much of BC to southern Oregon [23-25]. Surprisingly, phylogenetic evidence supported the northern and southern lineages as being most closely related to one another despite the central clade separating them geographically. The most curious aspect of ice worm biogeography, however, has been the repeated discovery of closely related ice worms on glaciers several hundred to thousands of kilometers south of their closest genetic relatives [23, 25; P. Wimberger, unpublished data]. These disjunct northern ice worms co-occurred with, but appeared genetically distinct from, their conspecifics (either central or southern clade ice worms) on the same glaciers. Dial *et al.* [23] laid out three possible explanations for this pattern: wind transport, passerine-mediated dispersal, or a more extensive previous range of the northern clade. While wind transport seems unlikely,

94 the potential for passerine-mediated dispersal is reasonable, particularly in light of other
95 examples of bird-mediated LDD [see 7, 27]. The third scenario, a more extensive distribution of
96 the northern clade with holdover lineages inhabiting the same glacier as more recent colonizers
97 could indeed result in more than one distinct lineage on the same glacier. However, if this recent
98 colonization occurred at the Last Glacial Maximum when ice sheet extent last peaked in the
99 region, recently colonizing lineages should not remain closely related to the source without at
100 least some degree of contemporary gene flow.

101 In this study, we leveraged a modern population genomic toolkit to add new perspective
102 to the age-old challenge of identifying LDD in wild populations. We also provide new insight
103 into how multiple factors can interact to shape the evolutionary history of species. We
104 hypothesized that the biogeographic history of ice worms stemmed from a confluence of three
105 main factors: extreme LDD, glacier dynamics, and mountaintop isolation. To test this hypothesis,
106 we generated a genome-wide single nucleotide polymorphism (SNP) data set to answer three
107 more-specific questions: (1) How do the clades previously diagnosed from a small number of
108 markers hold up to genome-wide scrutiny? (2) What, if any, genomic evidence exists for LDD in
109 ice worms? (3) How do the evolutionary relationships among ice worm populations and genetic
110 clusters align with glacial history in the region [e.g., 28]? Beyond a refined view of ice worm
111 evolution, our study confirms that LDD does occur in ice worms, providing an example of LDD
112 in an annelid and a rare population genomic exploration of the process. Moreover, while
113 considerable evidence details the existence of refugia in the Pacific Northwest (PNW) during the
114 Pleistocene [29], few studies have explored how ice sheet dynamics influenced the evolutionary
115 history of species directly tied to them [e.g., 17]. Our results reveal the profound impact that a
116 putative ice ridge that formed along the crest of the Coast Mountains [28] may have had on ice
117 worm evolution, possibly precipitating an ongoing speciation event. Broadly, our findings
118 highlight the power for population genomics to capture contemporary evidence of LDD while
119 also providing novel evidence for reconstructing the glacial history of a region.

Table 1. Sampling information and summary statistics for all ice worm populations included in this study. Abbreviations: *n* = sample size, π = nucleotide diversity, Het = heterozygosity, F_{IS} = inbreeding coefficient, AK = Alaska, BC = British Columbia, VI = Vancouver Island. π, Het, and F_{IS} were calculated for variable sites only.

Population	Latitude, longitude	State/Prov.	Elev. (m)	*n*	π	Het	F_{IS}
Learnard (LEA)	60.806, -148.721	AK	624	3	0.114	0.100	0.026
Davidson (DAV)	59.067, -135.551	AK	986	15	0.078	0.065	0.037
Treaty (TRE)	56.586, -130.151	BC	1376	8	0.026	0.033	-0.012
Bear (BEA)	56.096, -129.681	BC	648	6	0.039	0.046	-0.013
William Brown (WIB)	54.611, -129.129	BC	1260	6	0.056	0.057	0.000
Jacobson (JAC)	52.050, -126.072	BC	1249	5	0.108	0.106	0.005
White Mantle (WHM)	50.795, -125.153	BC	1764	8	0.131	0.135	-0.005
Comox (COM)	49.545, -125.355	BC (VI)	1881	4	0.091	0.072	0.034
Mariner (MAR)	49.460, -125.764	BC (VI)	1754	4	0.098	0.084	0.024

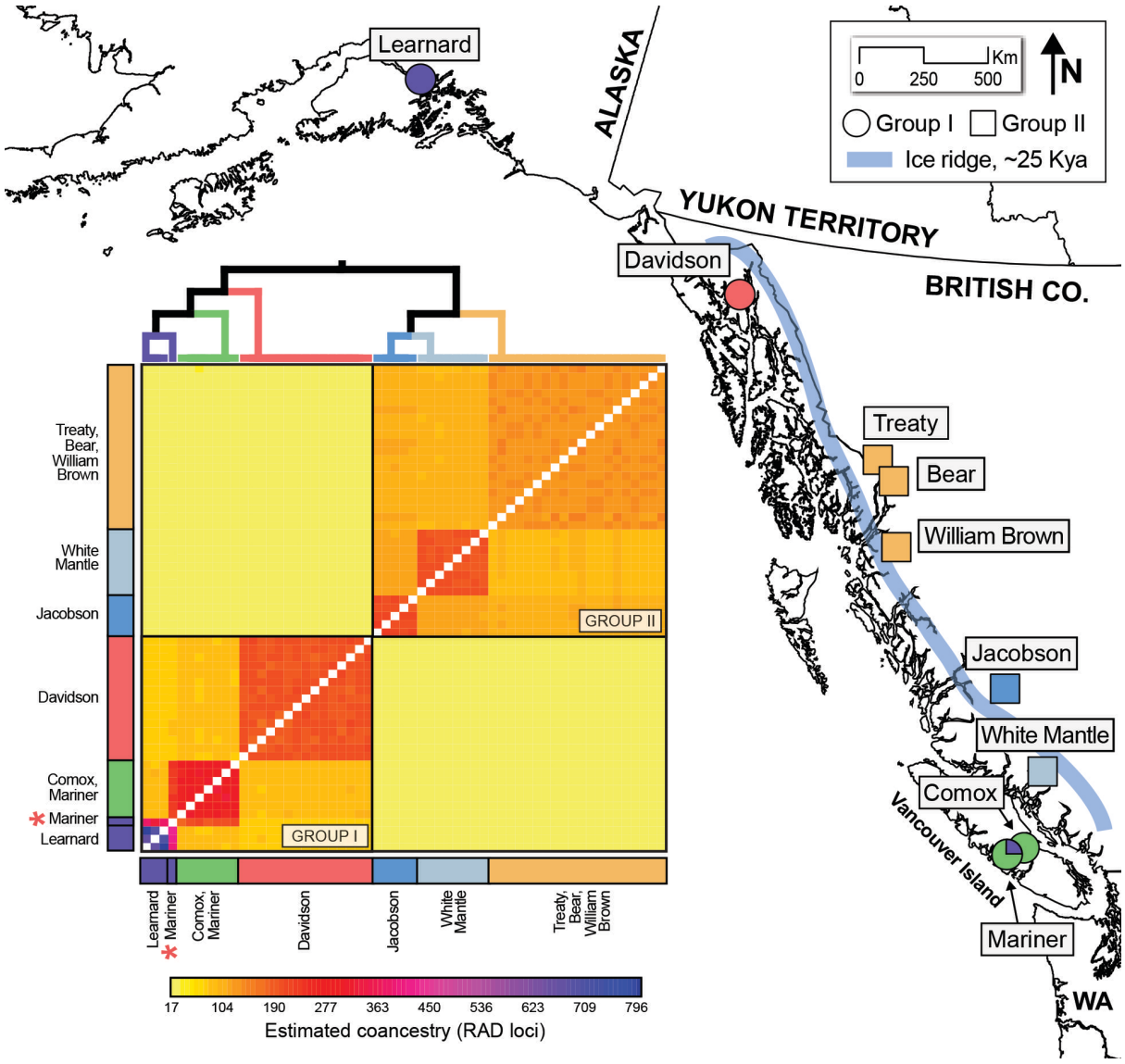
Methods:

Sample collection, library preparation, and SNP calling

During the summer of 2009, ice worms were collected from nine glaciers across most of their range (Figs. 1, S1; Table 1). Samples were stored in > 80% EtOH until DNA was extracted from 59 ice worms using a Qiagen DNEasy Blood and Tissue Kit. Double-digest restriction-site associated DNA sequencing libraries were prepared following Peterson *et al.* [30] with restriction enzymes EcoRI and NlaIII. During library preparation, samples were divided into two groups and each sample was assigned a unique, variable-length barcode [31] which was incorporated during adapter ligation. Size selection for a 350 bp ± 35 bp window was performed with a Pippen Prep (Sage Science), and both sample groups were subsequently amplified using PCR primers containing a group-specific barcode. The 59-sample library was sequenced on one lane of an Illumina HiSeq4000 at the University of Illinois High-Throughput Sequencing and Genotyping Unit with single-end, 100 bp chemistry.

Raw reads were demultiplexed, quality-filtered, and RAD loci were assembled *de novo* using the *process_radtags* and *denovo_map* functions of the Stacks v1.46 pipeline [32]. We allowed a maximum distance between stacks of 2 and a minimum read depth of 10. Next, we applied a stringent filtering scheme to identify high-confidence SNPs that were shared among many individuals. We only included SNPs if they were present in ≥ 5 populations, genotyped in ≥ 50% of individuals per population, and were in Hardy-Weinberg equilibrium with a minor allele frequency of ≥ 0.025 overall. We further restricted analyses to one random SNP per locus for all analyses except fineRADstructure (see below). All post-Stacks filtering steps were

146 performed in PLINK v1.07 [33] and the commands used in this study are provided on GitHub
147 (https://github.com/scotthotaling/ice_worm_ddRAD).



148

149 **Figure 1.** Ice worm populations sampled for this study. Color-coding reflects the results of a
150 fineRADstructure coancestry analysis. Group I (circles) and II (square) populations were generally
151 defined by their presence to the east or west of a putative ice ridge that formed during the Pleistocene
152 ~25,000 years ago [28] as well as their distance to the Pacific Ocean. The deep divergence between
153 groups I and II is clearly evident along with more recent differentiation within each group. One individual
154 from the Mariner Glacier (asterisk) is admixed between the Mariner/Comox (Vancouver Island) and
155 Learnard (southern Alaska) clusters, indicating recent LDD.
156

157 *Population genetic and phylogenetic analyses*

158 For each population, we calculated nucleotide diversity (π), heterozygosity (Het), and the
159 inbreeding coefficient (F_{IS}). We also calculated a pair-wise AMOVA F_{ST} for all population

combinations [34] – using the *populations* module in Stacks. To test for a signature of isolation-by-distance [IBD, 35], we estimated Euclidean distances among sites with Google Earth and tested the correlation between geographic distance and F_{ST} with four Mantel tests performed in GenoDive v2.0b27 [36]. The first Mantel test included all nine populations and the second excluded both Vancouver Island (VI) populations from the Mariner and Comox Glaciers to assess whether unique histories for those populations were significantly altering results. The third and fourth Mantel tests focused on signatures of IBD within groups "I" and "II" (see Results). We also measured Euclidean distances for each population to the Pacific Ocean in Google Earth Pro as an additional spatial comparison of groups I and II. We determined if mean distances to the Pacific Ocean differed between the two groups with a one-way ANOVA.

Population structure was inferred in two ways: a maximum likelihood-based method using ADMIXTURE 1.3.0 [37] and a discriminant analysis of principal components (DAPC) with the R package *adegenet* [38]. ADMIXTURE analyses were performed with default settings, a range of clusters (K) from 1-12, and 25 replicates per K with the current time as the random seed. The cross-validation (CV) error for each K was plotted to identify the best-fit K (minimized CV across the mean of all replicates for each K). After identifying the best-fit K, we considered the replicate that minimized CV across all 25 replicates for all K's to be the best-fit solution overall. However, because all runs did not converge on the same result, we also inspected best-fit solutions for other replicates of $K = 7$ (the best-fit K overall) to clarify the distribution of best-fit solutions. For DAPC, we first used the *find.clusters* function to identify the optimal K [i.e., the K with the lowest Bayesian Information Criterion (BIC)]. Next, to avoid over-fitting of the model, we retained the appropriate number of principal components (PCs) according to the α-score [PCs retained = 6, Fig. S2; 38]. We performed a final DAPC analysis using the best-fit K and optimal number of PCs identified in the previous two steps.

We extended our population structure analyses to infer both shared ancestry and phylogenetic relationships in two ways: a nearest neighbor haplotype approach to infer coancestry with fineRADstructure [39] and phylogenetic relationships inferred from singular value decomposition estimates for quartets of tips using SVDQuartets [40] as implemented in PAUP* v4.0a159 [41]. For fineRADstructure, we used 100,000 burn-in iterations followed by 100,000 iterations sampled every 1,000 steps for the Markov chain Monte Carlo clustering algorithm. Next, we used 10,000 iterations of the tree-building algorithm to assess genetic

191 relationships among clusters. Since fineRADstructure is a haplotype-based approach, analyses
192 were performed using all SNPs for a given RAD locus (i.e., a haplotype) rather than randomly
193 selected single SNPs per locus. For SVDQuartets, we performed exhaustive sampling of all
194 possible quartets (every combination of four tips) and branch support was estimated with 100
195 nonparametric bootstrap replicates.

196 **Table 2.** Above the diagonal: Pair-wise AMOVA F_{ST} values for all localities populations included in this
197 study. Mean F_{ST} (bottom row) refers to the average pair-wise differentiation for columnar populations
198 versus all others. Below the diagonal (in gray): mean pair-wise shared loci for the fineRADstructure
199 coancestry analysis (see Fig. 1).

	LEA	DAV	TRE	BEA	WIB	JAC	WHM	COM	MAR
LEA	--	0.333	0.634	0.626	0.608	0.586	0.557	0.347	0.344
DAV	74.9	--	0.567	0.557	0.541	0.527	0.515	0.290	0.295
TRE	19.8	20.0	--	0.153	0.207	0.275	0.250	0.656	0.652
BEA	19.0	19.9	127.2	--	0.165	0.250	0.222	0.650	0.645
WIB	20.1	20.5	119.8	123.0	--	0.216	0.201	0.630	0.628
JAC	21.0	20.4	99.7	99.5	104.7	--	0.172	0.604	0.600
WHM	21.6	19.1	90.7	90.9	93.0	107.8	--	0.576	0.572
COM	84.8	86.1	21.8	21.3	23.0	22.9	22.1	--	0.160
MAR	164.5	82.9	21.2	21.3	22.3	21.8	21.4	262.3	--
Mean F_{ST}	0.504	0.453	0.424	0.408	0.399	0.404	0.383	0.489	0.487

200

201 **Results:**

202 We generated 343,875,880 reads with an average of 5,828,404 sequences per individual
203 (min. = 446,872 and max. = 40,982,490). Our total RAD data set included 360,534 unique loci.
204 After filtering, we retained 6,019 loci and 10,392 SNPs (mean = 1.73 SNPs per locus). This final
205 data set had genotype calls for ~65% of all SNPs. Nucleotide diversity (π) was highest in the
206 White Mantle and Learnard populations (0.131 and 0.114, respectively) and lowest in the Treaty,
207 Bear, and William Brown populations (0.026-0.056; Table 1). Heterozygosity followed the same
208 pattern as π (Table 1). The inbreeding coefficient (F_{IS}) was highest in the Davidson and Comox
209 populations (0.037 and 0.034, respectively) and lowest in Bear (-0.013) and Treaty (-0.012;
210 Table 1). Mean differentiation (F_{ST}) for all pairwise comparisons was 0.439. The Learnard
211 population from southern Alaska was, on average, the most differentiated from all others (mean
212 F_{ST} = 0.504) and White Mantle was the least differentiated (mean F_{ST} = 0.383; Table 2). We
213 detected no association between genetic and geographic distances in either study area-wide
214 Mantel tests (Mantel's *r*, all populations = -0.04, *P* = 0.42; Mantel's *r*, no VI populations = 0.15,

215 $P = 0.36$). There was, however, a signature of IBD within group II (Mantel's r, group I = 0.839,
216 $P = 0.025$) but not within group I (Mantel's r, group II = 0.839, $P = 0.082$).
217 Our DAPC analyses supported $K = 6$ as the optimal number of genetic clusters (Figs. 2A,C). For
218 ADMIXTURE, our results supported $K = 7$ as the best-fit to the data (Figs. 2B,C) and the
219 SVDQuartets phylogeny largely mirrored both lines of population structure evidence (Fig. 2D).
220 All analyses supported multiple independent genetic clusters of ice worms, many more than three
221 clades identified in previous studies [e.g., 25]. Our DAPC and ADMIXTURE results differed in
222 two ways: (1) DAPC analyses grouped the Treaty, Bear, and William Brown populations into
223 one cluster (as did SVDQuartets) whereas ADMIXTURE split William Brown into its own
224 cluster, and this accounted for the $K = 6$ versus $K = 7$ discrepancy between the two approaches.
225 (2) ADMIXTURE also split Learnard (southern Alaska) and the two Vancouver Island
226 populations – Comox and Mariner – into two clusters with one sample, MS5 (red asterisks in
227 Fig. 2), almost evenly split in terms of ancestry between the two. Conversely, DAPC split
228 Learnard and Comox+Mariner into two clusters with MS5 completely assigned to the Learnard
229 cluster, despite MS5 being sampled from the Mariner glacier far to the south (Figs. 1,2). Aside
230 from the best-fit solutions, we also observed a second common ADMIXTURE solution for $K = 7$
231 which differed from the best-fit solution in one way; rather than splitting Treaty+Bear and
232 William Brown into two clusters (Fig. 2B), Bear specimens were almost evenly admixed
233 between Treaty and William Brown (Fig. S3), its two closest neighbors to the north and south,
234 respectively. Finally, because our SNP filtering focused on overarching patterns in the data set,
235 and likely overlooked some degree of population-specific detail, our results are likely
236 conservative estimates of genetic structure in the group.

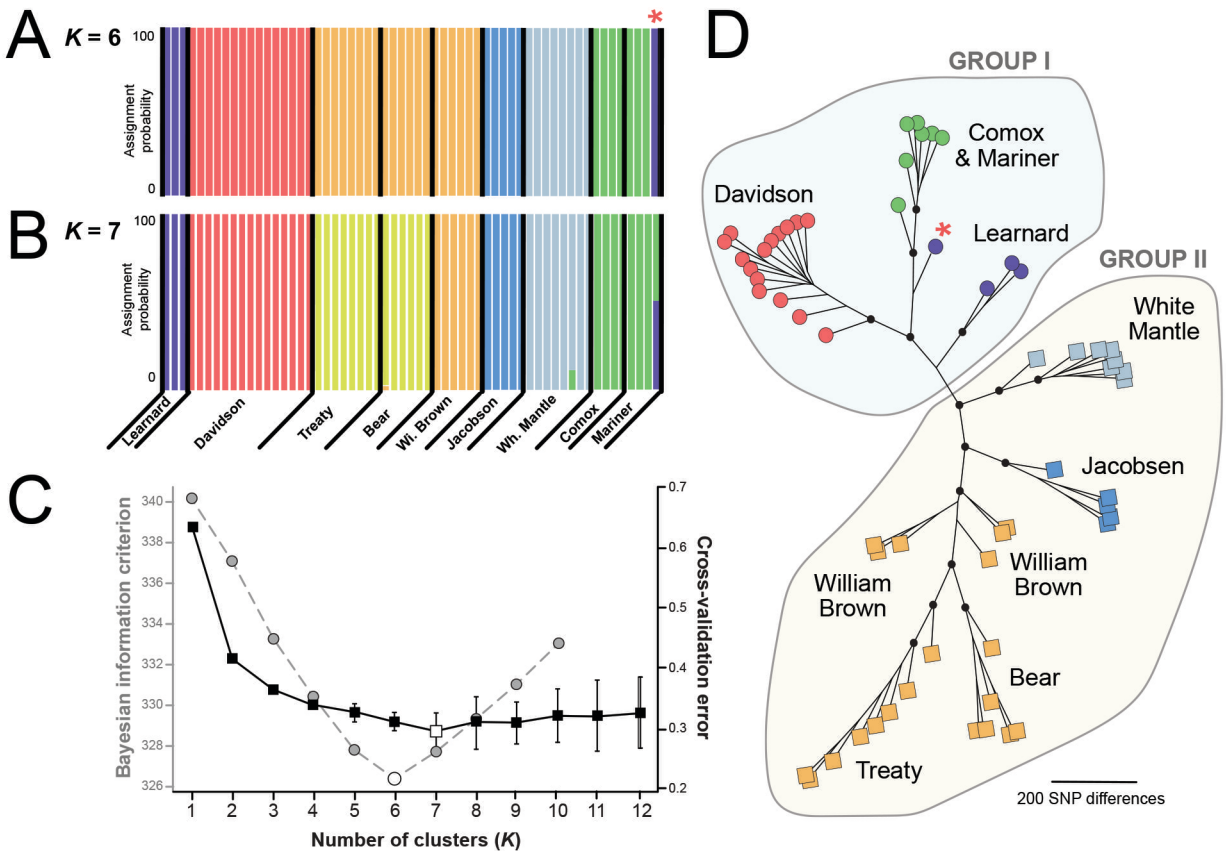


Figure 2. Population genetic structure of ice worms based upon (A) a discriminant analysis of principal components (DAPC) for $K = 6$ and (B) ADMIXTURE results for $K = 7$. (C) Comparisons of support for different values of K for DAPC (Bayesian information criterion, BIC; gray dashed line, left y-axis) and Admixture (cross-validation error, CV; dark line, right y-axis). The best-fit K (white square, ADMIXTURE; white circle, DAPC) corresponds to the K at which CV (ADMIXTURE) or BIC (DAPC) was minimized, respectively. For ADMIXTURE, vertical white bars represent the standard deviation of values for each K across 25 replicates. (D) An unrooted phylogeny of ice worms generated with SVDQuartets. Tip colorations reflect assignments in (A). Dark circles indicate nodes with > 95% bootstrap support. Specimens in group I (circles) and II (squares) are denoted with different symbols. Asterisks highlight a single specimen, MS5, which showed evidence of shared ancestry across geographically disjunct populations, indicating LDD.

Our fineRADstructure results mirrored those from DAPC, identifying the same six genetic clusters with MS5 assigned to the Learnard cluster rather than the Comox/Mariner cluster. Still, MS5 again exhibited substantial evidence for shared ancestry between the Learnard and Comox/Mariner genetic clusters (Fig. 1). On average, MS5 shared 411 loci with Learnard ice worms and 220 loci with ice worms from the Comox and Mariner ice worms (Fig. 1), a roughly 60/40 split between the two clusters. Notably, MS5 also exhibited the highest heterozygosity of any individual in the study (and this result did not stem from outsized

coverage, Fig. S4). Our fineRADstructure analysis also revealed the potential for an overarching, deep divergence between two groups of ice worm populations (groups I and II; Fig. 1). This split was corroborated by both SVDQuartets (Fig. 2D) and F_{ST} comparisons. Mean pairwise F_{ST} among populations within groups I and II were 0.21 and 0.30, respectively. Across groups, however, mean pairwise F_{ST} was 0.60. Mean distance to the Pacific Ocean also differed between groups by 100.5 km (group I = 73.3 km, group II = 173.8 km; ANOVA, $P < 0.001$).

To be clear, the geographic groupings (I and II) described in this study are with respect to the Coast Mountains, and specifically a putative ice ridge that formed along the crest of the range during the Pleistocene [28]. All populations, regardless of grouping, followed the known pattern of ice worms inhabiting relatively coastal glaciers (see Fig. S1). Interestingly, while the geographic location of 8 of 9 populations fell on either side of the putative ice ridge, one population did not: White Mantle. Our resolution of a deep, two-group split within ice worms adds new clarification to the three-clade result identified by earlier studies [e.g., 23, 25], showing that the previously recognized northern and central clades should likely be lumped together (now group I).

Discussion:

Historical and contemporary factors, both biotic and abiotic, interact to shape the present-day genetic structure of populations and species. Disentangling their varied contributions can be difficult, however, particularly when evolutionary histories are muddled by unexpected events (e.g., LDD of an organism with limited potential for active dispersal). The modern population genomic toolkit provides historically unprecedented power to resolve biogeographical complexity by allowing more quantitative perspectives of relatedness and greatly improved resolution of genetic independence or similarity [42]. In this study, we used a population genomic data set to refine understanding of the evolution history of the extremophile, glacier-obligate ice worm, *M. solifugus*. Our results provide a clear genomic perspective of LDD, showing unequivocally that migration has occurred between southern Alaska and the glaciers of Vancouver Island ~1,900 km to the south and across the Pacific Ocean. We also provide an independent line of biological evidence in support of the geological hypothesis that an ice ridge formed along the crest of the Coast Mountains during the Pleistocene [28].

The recent biogeographic history of ice worms appears to have been shaped by three main factors: i) ice sheet dynamics (likely during the Pleistocene), ii) mountaintop isolation from conspecifics following the retreat of Pleistocene ice into higher elevations, and iii) LDD.

i. Evidence for the first, overarching factor that has defined the recent evolution of ice worms lies in our overwhelming support for divergence between two overarching groups of ice worms (I and II). During the Pleistocene, ice was generated in the high peaks of the Coast Mountains and subsequently flowed west to the Pacific and east to the BC interior [28, 43, 44]. This potential western-eastern divergence in ice worms aligns with this divide, suggesting that each group diverged in allopatry from their conspecifics. At its maximum, the Cordilleran ice sheet was a ~2,000-3,000 m high convex dish with gentle interior slopes that steepened at its periphery [28, 45]. Given the sensitivity of ice worms to extreme cold [23], populations likely only persisted on the margins of the ice sheet, as their present-day occurrences on the lower flanks of higher elevation, low-latitude glaciers suggest. It is possible that, as suggested previously [23, 25], the Boundary Ranges (the most northern subrange of the Coast Mountains), are actually the biogeographic barrier that drove the observed deep divergence in ice worms described in this study. However, without more fine-scale population genomic sampling on both sides of the Coast Mountains (including the Boundary Ranges), this nuance of ice worm biogeography will remain unclear. In the same vein, the strong genetic similarity of White Mantle to populations east of the proposed ice ridge (Fig. 1), despite falling on its western side indicates that either the ice ridge actually formed more to the west than previously thought [28], the White Mantle population has migrated west since the end of the Pleistocene, or the ice ridge itself was not a barrier driving differentiation (as discussed above).

ii. The Cordilleran ice sheet was seeded by alpine glaciers and throughout western North America, the ice sheet's signature persists in mountainous regions. While the specific dynamics of deglaciation on valley and drainage scales are unknown, a safe assumption is that glaciers retreated from valleys into higher elevations, likely with ice worm populations in tow. Increasing mountaintop isolation and subsequent genetic drift likely precipitated more recent differentiation within groups I and II

since their initial split. Evidence for IBD within group II supports this hypothesis. The lack of support for IBD in group I may simply reflect a smaller sample size or LDD maintaining connections over larger spatial scales than a purely IBD model would predict.

iii. Despite limited sampling, we were able to identify one instance of recent LDD among geographically disparate ice worm populations. Indeed, with a roughly 60/40 ancestry split between the Mariner/Comox and Learnard clusters, one specimen (MS5), is likely the progeny of recent hybridization between the two. This indicates that LDD is both ongoing in ice worms and perhaps not particularly rare. The most plausible mechanism for ice worm LDD is passive dispersal of mucous-coated ice worm cocoons on southward migrating birds [e.g., 12]. Several passerines (e.g., Gray-crowned rosy finches, *Leucosticte tephrocotis*) have been observed feeding on ice worms [46, 47; S.H., personal observation] and the presence of an ice worm clitellum [48] indicates that ice worms, like other *Mesenchytraeus* species, reproduce by egg-laden cocoons [23]. The seemingly exclusive north-to-south pattern of ice worm LDD also has temporal support from bird migratory behavior. Indeed, late autumn ice worm reproduction [at the end of the productive season on mountain glaciers, 49] likely occurs in concert with southward-migrating birds stopping to feed on glaciers free of seasonal snow. In contrast, returning spring migrants pass over the same glaciers when seasonal snowfall still covers overwintering ice worms [23], limiting the potential for LDD in the reverse direction.

One question remains, however, if birds are precipitating LDD in ice worms, why has it only been observed for populations west of the Coast Mountains? This curiosity ties in to an important question in North American biogeography: to what extent have ice sheets driven present-day patterns of speciation and genetic differentiation among fauna of the northwest? For ice worms, we hypothesize that ice worm populations comprising groups I and II have accumulated some degree of reproductive isolation and are either already species-level lineages or on their way to this conclusion. Thus, we predict a zygotic barrier may be limiting inter-group migrants from leaving a genomic signature of their presence. It is also possible, and perhaps likely, that LDD in ice worms is merely the result of vector migration patterns. For instance, *L.*

tephrocotis, like other songbirds [50], may preferentially follow coastlines during migration. However, until more is known about the specific interactions of ice worms with various bird species, and thus their potential to act as LDD vectors, relating bird migrations to ice worm distributions will remain difficult. Beyond ice worms, ice sheets have been implicated as a key driver of speciation in boreal birds [51] and phylogeographic structure of many taxa, from nematodes to gray wolves [17, 29, 52-54], and our results clearly support these broader implications for biodiversity accumulation and maintenance in North America.

Conclusions

In this study, we leveraged population genomic data to unravel the complex evolutionary history of the North American ice worm, *M. solifugus.* Our results add new clarity to previous perspectives on ice worm biogeography while also lending genomic support to the existence of contemporary, passerine-mediated LDD in the group. While the phylogenetic data used in this study (i.e., the lack of an outgroup) preclude us from diagnosing groups I and II as monophyletic, given the results of previous studies [23-25], we predict that future efforts will diagnose them as such, perhaps even representing two distinct species. Finally, our genomic data lend support to the glaciological record in the region, adding a biological line of evidence to a postulated north-south ice ridge that formed along the crest of the Coast Mountains during the Pleistocene [28]. This potential for genomics to inform the geological record is intriguing and ice worms, as a rare glacier-obligate macroinvertebrate, may be an ideal taxon for similar studies in the future.

Acknowledgements: We thank Joe Giersch for map making assistance and the University of Kentucky Center for Computational Sciences for high-performance computing resources. This research was partially funded by NSF award #IOS-082050 to D.H.S.

Competing interests: We have no competing interests.

Author contributions: S.H., D.H.S., L.M.T, and D.W.W. conceived of and funded the study. S.H., D.H.S., S.A.L, R.K.B., and D.W.W. collected the data. S.H. and J.L.K. analyzed the data and wrote the manuscript with input from D.H.S., R.K.B., and D.W.W. All authors approved the final version.

Data accessibility: Raw sequence data for this study has been submitted to GenBank under BioProject #PRJNA479335 and code to reproduce the analyses is deposited on GitHub (https://github.com/scotthotaling/ice_worm_ddRAD).

Funding: Field sampling for this study was supported by NSF award #IOS-0820505 to D.H.S.

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