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Historical biogeography of the North American glacier ice worm, *Mesenchytraeus solifugus* (Annelida: Oligochaeta: Enchytraeidae)

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ABSTRACT

North American ice worms are the largest glacially-obligate metazoans, inhabiting coastal, temperate glaciers between southcentral Alaska and Oregon. We have collected ice worm specimens from 10 new populations, completing a broad survey throughout their geographic range. Phylogenetic analyses of 87 individuals using fragments of nuclear 18S rRNA, and mitochondrial 12S rRNA and cyctochrome c oxidase subunit 1 (CO1) identified 18 CO1 haplotypes with divergence values up to ~10%. Phylogeographic interpretations suggest a St. Elias Range, Alaskan ancestry from an aquatic mesenchytraeid oligochaete during the early-Pliocene. A gradual, northward expansion by active dispersal from the central St. Elias clade characterizes a northern clade that is confined to Alaska (with one exception on Vancouver Island, British Columbia), while a distinct southern clade representing worms from British Columbia, Washington and Oregon was likely founded by a passive dispersal event originating from a northern ancestor. The geographic boundary between central and southern clades coincides with an ice worm distribution gap located in southern Alaska, which appears to have restricted active gene flow throughout the species' evolutionary history.

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1. Introduction

Glacier ice worms are the largest metazoans known to complete their life cycle exclusively in glacier ice. Described species of glacier ice worms (Annelida: Enchytraeidae) currently include the North American *Mesenchytraeus solifugus* (Emery 1898) (with ssp. *M. solifugus ranierensis* Welch 1916) and the Asian *Sinenchytraeus glacialis* (Liang et al., 1979). Both species inhabit maritime glaciers, those with hydrated ice and year-round temperatures near 0 °C. Other enchytraeids tolerate colder temperatures in permafrost (<−20 °C), but do so through dormant phases involving dehydration or sugar accumulation (Holmstrup and Sjursen, 2001; Holmstrup et al., 2002). In contrast, glacier ice worms maintain static metabolic activity at 0 °C, and survive down to −6.8 °C (Edwards, 1986). The mechanisms by which ice worms have adapted to life on maritime glacial ice remain mostly unresolved, but recent evidence suggests that relatively high intracellular ATP levels may be a contributing factor (Napolitano et al., 2004; Morrison and Shain, 2008).

The known geographic range of the North American glacier ice worm, *M. solifugus*, extends from southcentral Alaska (AK) to central Oregon (OR), with relatively low elevation, coastal glaciers as their primary habitat (Tynen, 1970; Hartzell et al., 2005). Inland glaciers generally do not support ice worms, but this boundary has not been well defined in terms of landscape and climatic variables such as temperature and precipitation. Because ice worms are stenothermic, surviving within a narrow temperature range between ~5 °C and −6.8 °C (Edwards, 1986), glacier ice on inland terrain is likely too cold during winter months to sustain ice worm populations long term. Indeed, current ice worm distribution patterns may well be traced by the historic position and timing of relatively warm glaciation.

The evolutionary history of ice worms remains mostly unexplored. While certainly linked to extinction and colonization processes, the linkage between phylogeography and geologic history has not been made explicit. We have shown previously that ice worms comprise two major lineages, i.e., northern and southern, that are geographically and genetically distinct; moreover, phylogenetic analyses of northern- and southernmost populations suggest these regions represent leading edges of expansion from a more central stock (Hartzell et al., 2005). To define the geographic

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boundary between ice worm lineages, and to explore genetic relationships between and within ice worm populations, we collected ice worms throughout the central region of their geographic range (i.e., primarily British Columbia (BC) and southern AK). Our goal was to better elucidate the historical biogeography of the North American ice worm using three genes: fragments of nuclear 18S rRNA, and mitochondrial 12S rRNA and cyctochrome c oxidase subunit 1 (CO1). Our data indicate that the geographic boundary between central and southern ice worm lineages coincides roughly with the coastal AK/BC boundary, and that colonization of most BC populations occurred comparatively earlier than populations throughout northern and central lineages. Further, gene flow has not frequently crossed an ice worm distribution gap that separates central and southern lineages, but episodes of passive dispersal appear to have populated southern territory from northern/central ancestors.

2. Material and methods

2.1. Specimens

Glacier ice worms, *Mesenchytraeus solifugus* and ssp. *M. solifugus ranierensis*, were collected in the field during Summer, 2009, mostly in August. *Mesenchytraeus pedatus* specimens were generously provided by Steven Fend (US Geological Survey, Menlo Park, CA). Field specimens were fixed in 70–95% ethanol and stored in 1.8 ml eppendorf tubes. Live individuals from some populations were maintained in ice water as described previously (Hartzell et al., 2005). Access to remote glaciers was by helicopter, floatplane and/or by foot.

2.2. Molecular analyses

Worm DNA was extracted according to Barstead et al. (1991), with modifications. Briefly, 100 µl lysis buffer (50 mM KCl, 2.5 mM $MgCl_2$, 10 mM Tris pH 8.0, 0.45% Tween 20, 0.05% gelatin) was added to individual worms in respective eppendorf tubes. After incubation at −80 °C for at least 1 h, tubes were thawed and brought to a final concentration of 60 µg/ml proteinase K. Samples were incubated at 60 °C for 1 h with occasional mixing, and then 95 °C for 15 min. To remove debris, tubes were centrifuged at 14,000*g* for at least 2 min. Supernatants were removed and 1–2 µl was used directly for PCR amplifications. Mitochondrial cytochrome c oxidase subunit 1 (CO1) and 12S ribosomal RNA (rRNA), and nuclear 18S rRNA were amplified with primer sets GATTCTTTGGACATCCAGAAG/CTACGTTGGGCTGCGAATC (~600 bp; Hartzell et al., 2005), AAACTAGGATTAGATACCCTATTAT/AAGAGCGACGGGCGATGTGT (~400 bp), and CGGTAATTCCAGCTC/CAGACAAATCGCTCC (~800 bp), respectively, from individual worms. PCR conditions for CO1 and 12S rRNA fragments were: 94 °C for 2 min followed by 35 cycles of 94 °C (20 s), 52 °C (1 min), 72 °C (1 min), and final extension at 72 °C for 10 min. Conditions for 18S rRNA were identical, except annealing was at 55 °C. All reactions were in 25 µl using Titanium Taq polymerase (ClonTech) according to the manufacturer's specifications, and supplemented with 3 mM $MgCl_2$. Following PCR, samples were electrophoresed on a 0.8% agarose gel buffered with Tris/acetic acid. Amplified bands were excised and purified by GeneClean (MP Biomedicals, LLC). DNA sequencing was performed by GeneWiz (South Plainfield, NJ) using primers employed for PCR reactions.

2.3. Phylogenetic analyses

DNA sequences were viewed and edited in Chromas (Queensland, Australia), and aligned in Clustal W (Larkin et al., 2007). Reliable sequence data on both strands were obtained as follows: 418 bp CO1, 313 bp 12S rRNA, 543 bp 18S rRNA. In total, 1274 bp were considered, containing 138 informative positions. Maximum-likelihood (ML) phylogenies, Monte Carlo Markov chain (Bayesian) modeling, and haploid network analyses were performed for DNA comparisons, using the pipeline sequence

Table 1
Description of field sites in Alaska (AK), Oregon (OR), Washington (WA) and British Columbia (BC).

Map key	Glacier	Location (decimal deg.)	Elevation (m)	Number (ind.)	Haplotypes
Worms	*Present*				
1	Eklutna (AK)	N 61.212 W 148.950	1400	6	1
2	Byron[a] (AK)	N 60.75 W 148.86			1
3	Bering[a] (AK)	N 60.394 W 142.972			1
4	Grand Pacific Junction[a] (AK)	N 59.07 W 137.88			2
5	Davidson (AK)	N 59.067 W 135.551	900	20	7
6	Treaty (BC)	N 56.586 W 130.151	1500	7	1
7	Bear (BC)	N 56.096 W 129.681	640	7	1
8	East Ridge, Mt. William Brown (BC)	N 54.580 W 129.114	1700	3	1
8	North Ridge, Mt. William Brown (BC)	N 54.611 W 129.129	1300	10	2
9	Jacobson (BC)	N 52.046 W126.066	1180	12	1
10	White Mantle (BC)	N 50.795 W 125.153	1800	7	1
11	Powder Mtn. (BC)	N 50.168 W 123.322	2200	8	1
12	Comox (BC)	N 49.545 W 125.355	1850	7	2
13	Overlord[a] (BC)	N 50.02 W 122.84			1
14	Easton[a] (WA)	N 48.75 W 121.83			1
15	Daniels[a] (WA)	N 47.57 W 121.18			1
16	Paradise[a] (WA)	N 46.81 W 121.72			1
17	Black Fin[a] (OR)	N 44.16 W 121.79			1
Worms	*Absent*				
A	Laughton[a] (WA)	N 59.53 W 135.1			
B	Mendenhall (AK)	N 58.496 W 134.540	700	–	–
C	Baird (AK)	N 57.168 W 132.622	500–2000	–	–
D	Eagle Ridge (BC)	N 56.782 W 129.878	1800	–	–
E	Glacier Gulch (BC)	N 54.8123 W 127.318	1900	–	–

[a] Haplotypes from Hartzell et al. (2005).

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MUSCLE (Edgar, 2004) for aligning corresponding sequences from multiple individuals or homologous DNA across species; Gblocks (Castresana, 2000) for alignment curation; FaBox for haplotype collapse; and ModelTest v3.06 in PAUP* for determining substitution models. Haplotype phylogenies were constructed as ML (100 bootstrapped ML trees each for CO1, 12S, and CO1 + 12S partitioned) in RAxML (Stamatakis et al., 2008; Guindon and Gascuel, 2003), as Bayesian in BEAST v1.6.2 (Drummond and Rambaut, 2007), and as haplotype network in ANeCA v1.2 (Panchal, 2007) and Network v4.6. Trees were drawn using FigTree V1.3.1. For CO1 Bayesian, we used BEAUTi v1.6.2 (part of the BEAST package) with an HKY + G (kappa = 2.2, alpha = 0.18) substitution model; an oligocheate molecular clock rate from Chang et al. (2008) as clock prior (relaxed clock, uncorrelated lognormal, mean divergence 2.4%/million years with sd = 0.05); and Yule speciation process as tree prior. For the Bayesian runs we used 10^7 steps with a 10% burn-in, retaining parameters at 1/200 frequency, yielding 45,000 trees for posterior probabilities and divergence time distributions. We viewed BEAST output in TRACER v1.5 to determine divergence time statistics (mean and 95% HPD intervals). Outgroups included several species of oligochaete worms, including *M. pedatus*, *M. pelicensis*, and *M. flavus*. Results are shown with *M. pelicensis* and *M. pedatus*, the species closest to the *M. solifugus*. Other species yielded similar topologies within the ice worm clade.

A haplotype network analysis applied the automated nested clade tools ANeCA v1.2 (Panchal, 2007), TCS v2.1 (Clement et al., 2000), and GeoDis v2.5 (Posada et al., 2000), based on the methods of Templeton et al. (1995). For the haplotype network we used all individuals in Table 1 from the current study plus six other haplotypes from a previous study (Hartzell et al., 2005) represented as singletons. In the geographic analysis we used five populations, three centered at Eklutna (radius, r = 500 km), Davidson (r = 500 km) and Comox (r = 100 km) Glaciers (Table 1), plus two additional, one centered among the BC samples (r = 250 km) and the other among the WA samples (r = 200 km). We used n = 10,000 permutations.

3. Results

Independent searches for glacier ice worms were conducted at 14 geographically distinct glacial field sites ranging from southcentral AK to southern BC (Fig. 1; Table 1). Ice worms were observed and collected from 10 glaciers (i.e., Bear, Comox, Davidson, Eklutna, Jacobson, E. Ridge Mt. William Brown, N. Ridge Mt. William Brown, Powder Mtn., Treaty, White Mantle), and were not found on four (i.e., Baird, Eagle Ridge, Glacier Gulch and Mendenhall). Excepting an ice worm distribution gap comprising Alaskan glaciers from approximately Skagway, AK to Petersburg, AK (see Fig. 1), coastal, maritime glaciers predictably supported ice worms while inland, continental glaciers did not.

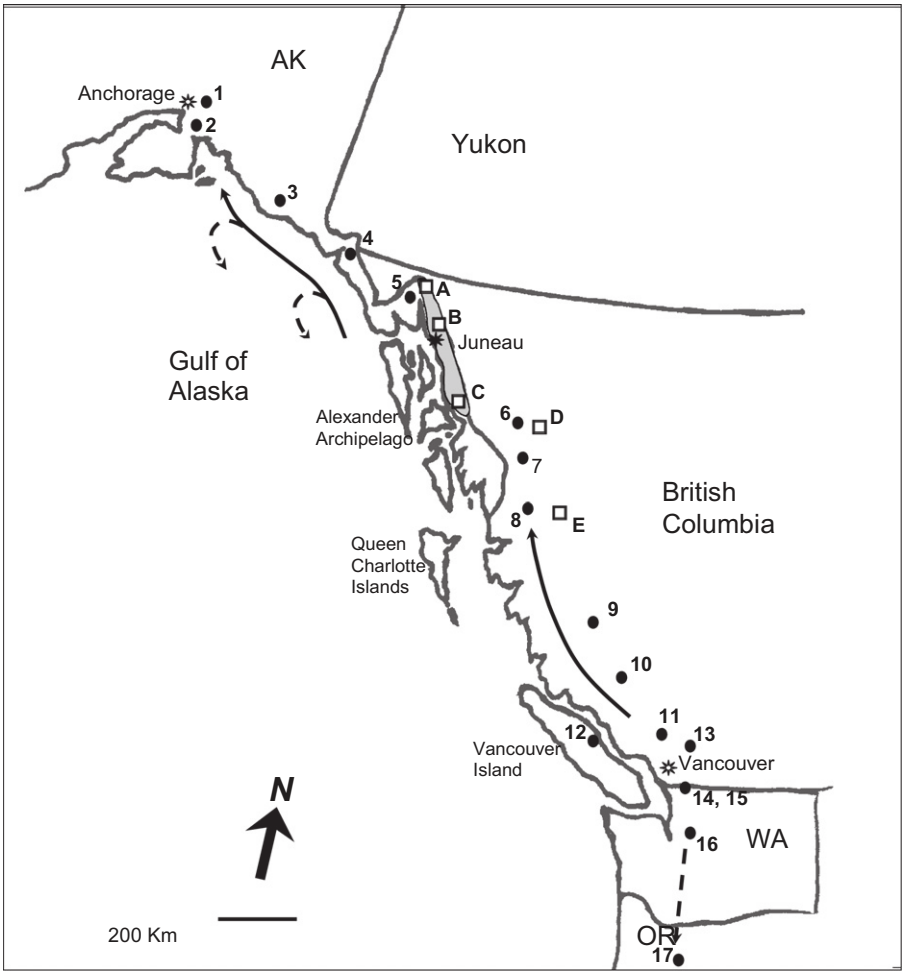

Fig. 1. Geographic range of North American glacier ice worms. Solid dots identify field sites where ice worms were found; open squares where ice worms were not found. The shaded area is a distribution gap. Putative active (solid arrows) and passive (dashed arrows) dispersal routes are shown. The lower, dashed arrow in WA represents a putative ancestral passive dispersal event that founded the OR population (Hartzell et al., 2005); more recently, the upper, dashed arrow gave rise to the Comox Glacier population on Vancouver Island. Glacier field sites match numbers and letters in Table 1.

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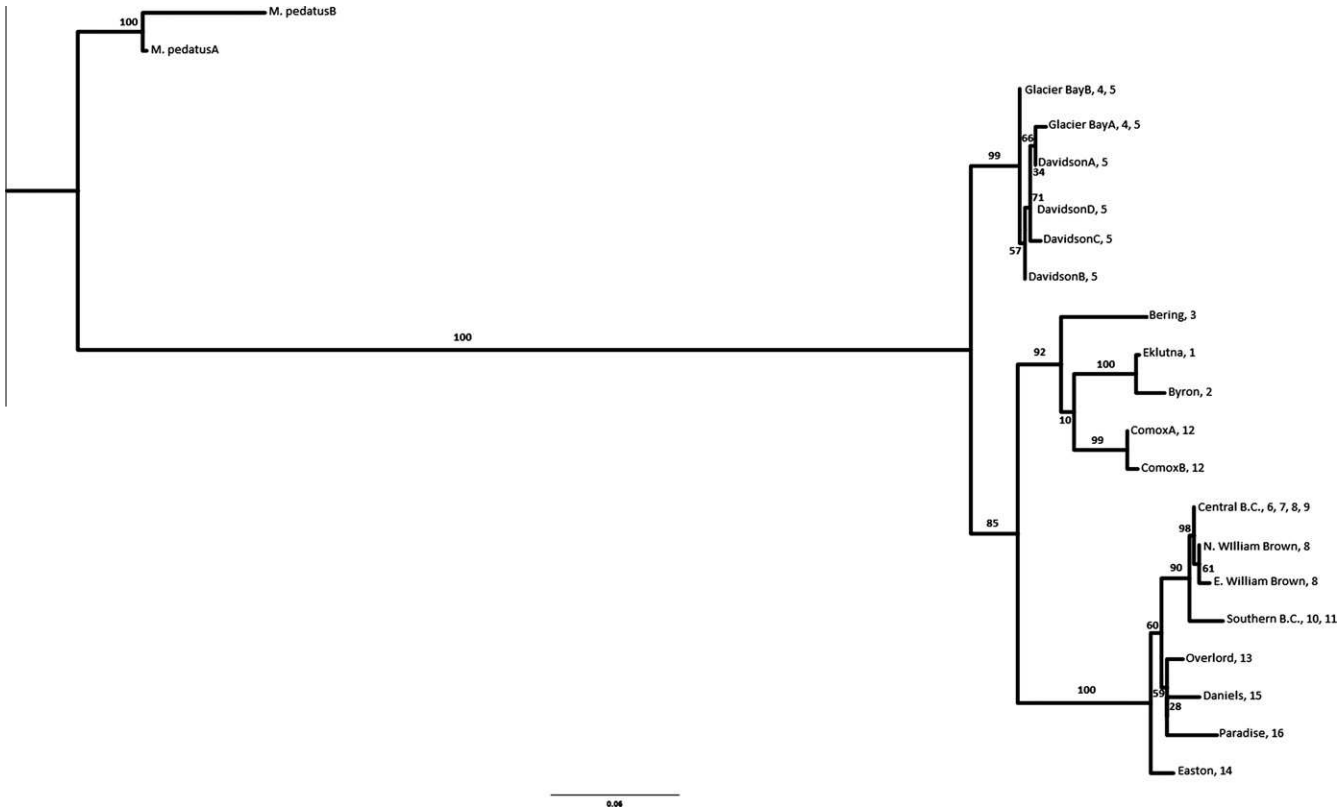

Fig. 2. Maximum likelihood phylogeny for glacier ice worms based on CO1 nucleotide data (mid-point rooted). Bootstrap values for 100 replicates. All haplotypes from the current study are shown (GenBank accession numbers JF788608–JF788625; *M. pedatus* AY873846 and AY73847); remaining sequences are from Hartzell et al. (2005). Scale gives nucleotide substitutions per site. Substitution model used: HKY + G. Label numbers as in Fig. 1.

Cytochrome c oxidase subunit 1 (CO1) fragments were successfully amplified from 87 individuals representing all examined populations (Table 1). A 418 bp CO1 fragment containing 106 informative positions was compared between individuals. In total, 19 unique CO1 fragments were identified, with Davidson Glacier population displaying the largest haplotype diversity (seven haplotypes in 20 examined specimens) and a central BC haplotype most widespread (found on four glaciers). Phylogenetic analysis using ML (Fig. 2), Bayesian (Fig. 3), and haplotype network (Fig. 4) based on CO1 haplotypes, supplemented with representative CO1 haplotypes throughout the geographic range of North American glacier ice worms (Hartzell et al., 2005), resolved three clades roughly corresponding to three geographic regions. A purely central clade in the St. Elias Range comprising all Davidson Glacier haplotypes and geographically proximal populations from Grand Pacific Glacier formed a basal node of the tree. A purely southern clade, containing populations throughout the Cascades and south and central BC Coast Ranges, contributed the distal-most branches of the tree. A third, mostly northern clade contained northernmost AK populations (e.g., Byron, Eklutna), as well as a population from Vancouver Island, BC (i.e., Comox Glacier). ML and Bayesian phylogenies located northern and southern clades on a branch sister to the central clade. Divergence values of CO1 haplotypes were consistently ~10% between central and southern clade individuals, and ~12% between ice worms and their aquatic relative, *Mesenchytraeus pedatus*. Excepting the Comox Glacier population, ice worms in BC diverged by less than ~2%, and by less than ~6% throughout the southern clade. In contrast, northern and central clade individuals were up to ~9% divergent, displaying strong correspondence between genetic and geographic distances (e.g., Eklutna and Davidson Glacier individuals diverged by ~9%, but were genetically and geographically equidistant from the Bering Glacier population between them; see Figs. 1 and 2).

Divergence times using Bayesian trees with a 2.4%/my oligochaete clock (Chang et al., 2008) prior and two outgroups (*M. pedatus* plus *M. pelicensis*) estimated the ice worm mean origin at 4.9 million years ago (mya) (lower 95% HPD to upper 95% HPD = 0.9–10.2 mya) from an ancestor shared with *M. pedatus* (Fig. 3). The mean time of central clade divergence from the sister northern + central clade was 3.8 mya (0.6–7.6 mya) and the northern and southern clades divergence was 3.2 mya (0.3–4.8 mya). Model runs with separate single species outgroups was similar. Divergence times using a slower 1%/my clock placed the mean origin date at ~9 mya (1.4–20.4 mya) with mean clade divergences at 6.7 mya (1.3–14.5 mya) and 5.4 mya (1.1–11.6 mya).

Using the automated inference keys of ANeCA v1.2 (TCS v2.1 + GeoDis v2.5) produced the result that the ice worm clade as a whole showed restricted gene flow with long distance dispersal over intermediate, unoccupied areas. Haplotype network analysis resolved the same three highest order clades (Fig. 4) as ML and Bayesian, excepting that Eklutna was included in the central clade, the result of allopatric fragmentation. Furthermore the southern clade showed evidence of contiguous range expansion.

Fragments of mitochondrial 12S rRNA and nuclear 18S rRNA were obtained from a subset of individuals representing major CO1 haplotypes from examined ice worm populations. 18S sequences 543 bp in length were identical across ice worm populations. 12S sequences were 313 bp in length with 32 informative

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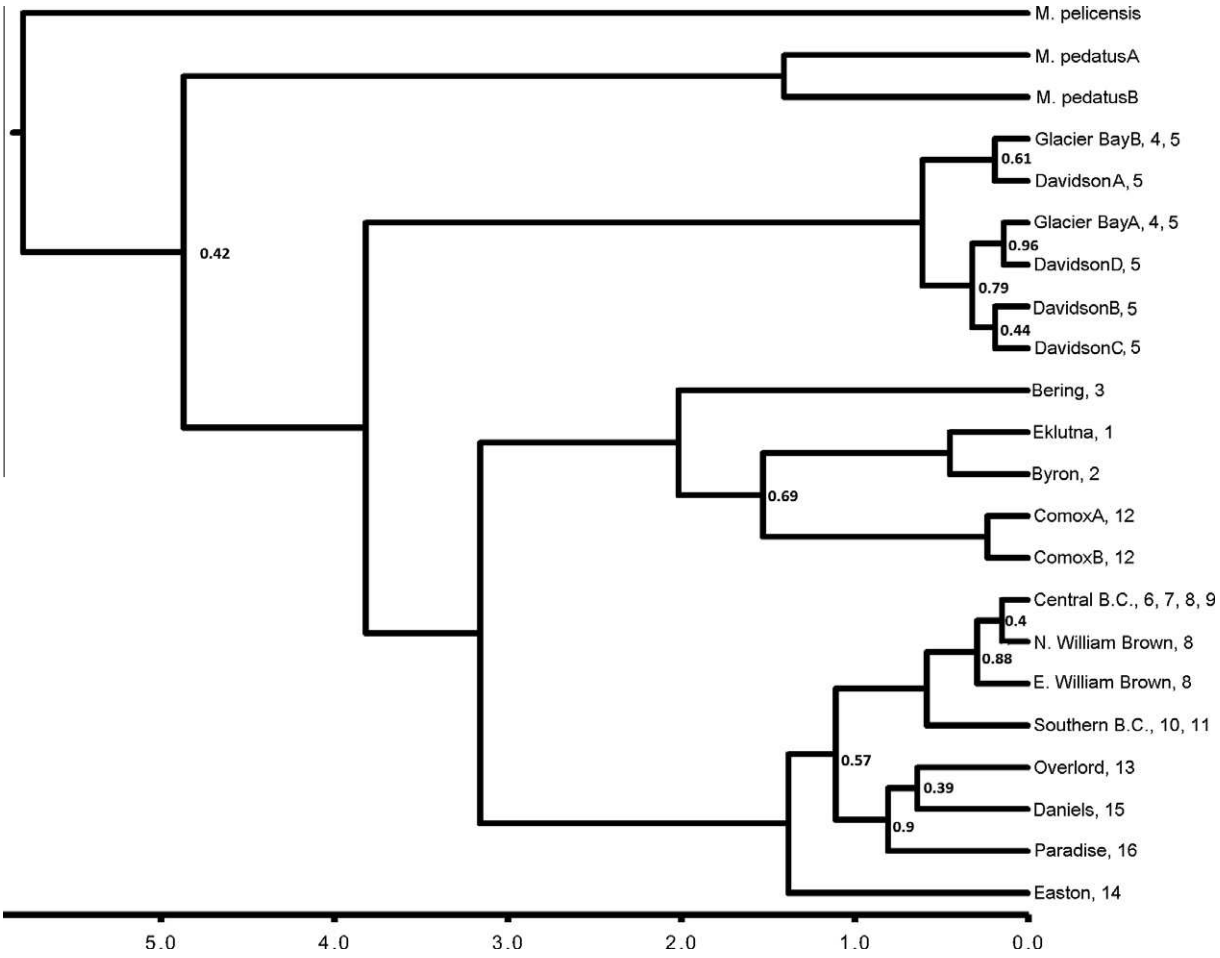

Fig. 3. Bayesian phylogeny based for glacier ice worms based on CO1 nucleotide data. Only posterior probabilities < 1 are shown. All haplotypes from the current study are shown (GenBank accession numbers JF788608–JF788625, *M. pedatus* AY873846 and AY73847, *M. pelicensis* GU453375); remaining sequences from Hartzell et al. (2005). Scale gives divergence age in millions of years. Substitution model used: HKY + G. Label numbers as in Fig. 1.

positions, and combined CO1/12S sequences were 431 bp in length with 138 informative positions. ML trees constructed with these data sets generated topologies concordant with that of CO1 phylogeny (Fig. 5A and B; *cf.* Fig. 2).

4. Discussion

Results presented here could be interpreted as three phylogenetic species of North American ice worm, each corresponding to a major mountain region over 4300 m above sea level (the St. Elias, Chugach, and Cascade) that may offer allopatric refugia during interglacial periods. The high CO1 divergence rates among the three clades (~9%) support a species level classification (Bely and Weisblat, 2006), as ice worms and their nearest known relative, the aquatic *Mesenchytraeus pedatus*, diverge by only ~12%. While differences occur at the 12S locus, the lack of 18S variation in the ice worm does not allow more definitive statements concerning speciation, and as yet, no morphological evidence, other than body size (Hartzell et al., 2005), or ITS data support reclassification.

Despite geography as a reliable indicator for ice worm presence in the Pacific NW (Goodman, 1971; Tynen, 1970; Hartzell et al., 2005), a distribution gap occurs at the boundary between the central and southern clades (Fig. 1). Neither this nor previous studies (Hartzell et al., 2005) have observed ice worms on suitable glaciers between Skagway and Petersburg, AK. Genetic data provides strong evidence that this distribution gap has restricted active gene flow between the central and southern clades for at least ~4 million years. The underlying cause of the ice worm distribution gap in geological time is unclear, but may result from past climate. During the Wisconsinan Glaciation (14–16 kya), for example, the Cordilleran Ice Sheet reached sea level (Dyke, 2005). At that time, the 500 km long Alexander Archipelago rose as a 60 km wide coastal range (> 1700 m asl). This historic coastal range blocked maritime air from regions now covered with maritime ice (e.g., Juneau Icefield). The Wisconsinan age ice fields along the Alaska-Canadian border were also subject to cold, dry easterlies (Dyke, 2005), climate unsuitable for ice worms. We speculate that these historic climatic conditions resulted in the current ice worm distribution gap in southern AK. Other genetic studies indicate an unglaciated refugium in the border region of AK and BC (Conroy et al., 1999; Cook and MacDonald, 2001; Shafer et al., 2010).

While ages of divergence are as unreliable as the molecular clocks used to determine them (Knowlton et al., 1993; Soto-Adames, 2002), applying an oligochaete CO1 clock (Chang et al., 2008) with a Bayesian model places the ice worm derivation from a non-ice worm ancestor at the start of the Pliocene with subsequent divergence among central, northern and southern clades in the mid-Pliocene. If a life cycle near 0 °C reduces mutation and reproduction, then a substantially slower clock rate applies, placing the origin event as early as mid-Miocene, during an early mountain building period and possible glaciation in AK (Marincovich, 1990).

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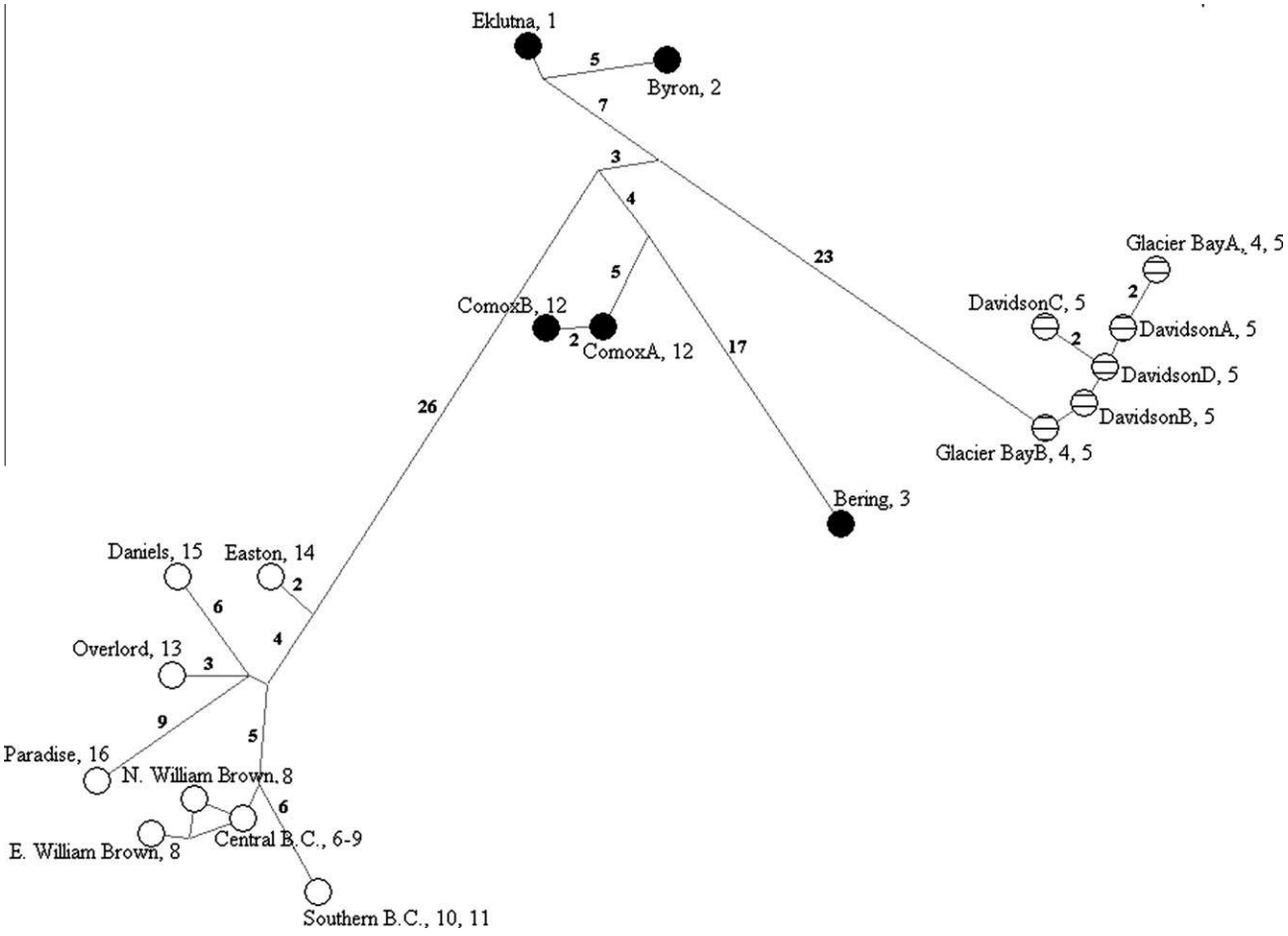

Fig. 4. Haplotype network for 19 unique haplotypes from 92 individual glacier ice worms. Open circles represent the southern clade; solid the northern and hatched the central. Branch values represent distances between node in substitutions. Only sampled haplotype nodes are shown and frequency data are omitted here but used in the analysis. Label numbers as in Fig. 1.

All three phylogenies (i.e., maximum likelihood, Bayesian, and haplotype network) show (1) the central clade as ancestral; (2) the southern and northern clades as sister to one another; and (3) of the latter two, the northern clade is older than the southern. Network analysis also suggests that the central clade gave rise to the northern through allopatric fragmenation, the northern clade gave rise to the southern, possibly through long distance dispersal (too few clades to resolve this with certainty), and the southern clade shows signs of contiguous range expansion. The central and northern geographic ranges are now, and have been, nearly linked by continuous low elevation, coastal ice (only one river separates the two) suggesting active dispersal from the oldest, central clade toward the north. The presence of the Bering haplotype, genetically and geographically intermediate between northern and central clades, supports this interpretation. The question is posed, then, where did the ancestor of the southern clade arise – or more precisely how did northern ancestors colonize southern glaciers?

The southern and central clades have long been separated by a distribution gap (see above). If northern worms actively dispersed toward the south, as central clade worms appear to have toward the north, then the current species distribution should show extant populations that are intermediate between the two in both geography and age; i.e., the central part of the range, where only the oldest and unrelated central clade persists. Future discoveries of intermediate haplotypes would support this hypothesis.

Our favored alternative is motivated by two notable disjunct populations in the southern part of the range. One population (at ~1.4 my, the oldest of the of southern clade haplotypes) occupies the central OR mountains (Mt. Jefferson and the Sisters Range), over 200 km south of the nearest population, a gap that has never been glaciated (Hartzell et al., 2005). The second disjunct population (at 250 ky, the youngest of the northern clade) occupies Comox Glacier on Vancouver Island, BC, over 1700 km south of the nearest northern clade population. The consistent inclusion of Comox Glacier ice worms with worms nearly 2000 km NW in the northern clade is corroborated with other, independent studies (P. Wimberger, unpublished). Both of these disjunct populations appear to be the result of long distance, passive dispersal by an unknown vector, most probably avian (e.g., Edwards and Bohlen, 1996; Milbrink, 2003; Miura et al., 2011). We hypothesize that the southern clade, too, was founded by a long distance event between 1.5 and 3 mya, most likely during a mid- to late-Pliocene interglacial event, whereas the northern clade was founded by active dispersal of the central clade during early Pliocene glaciations (White et al., 2007).

In conclusion, the glacier ice worm's sensitive dependence upon near 0 °C temperatures has played a central role in the species' historical biogeography during both glacial and interglacial periods. Although several evolutionary scenarios may be compatible with these phylogenetic data, the most parsimonious sequence of events involves an ancestral population within the St. Elias Range (the oldest and highest mountains within the ice worm's geographic range). The genetic diversity and distribution patterns of glacier ice worms reflect the flux of glacier ice from the early

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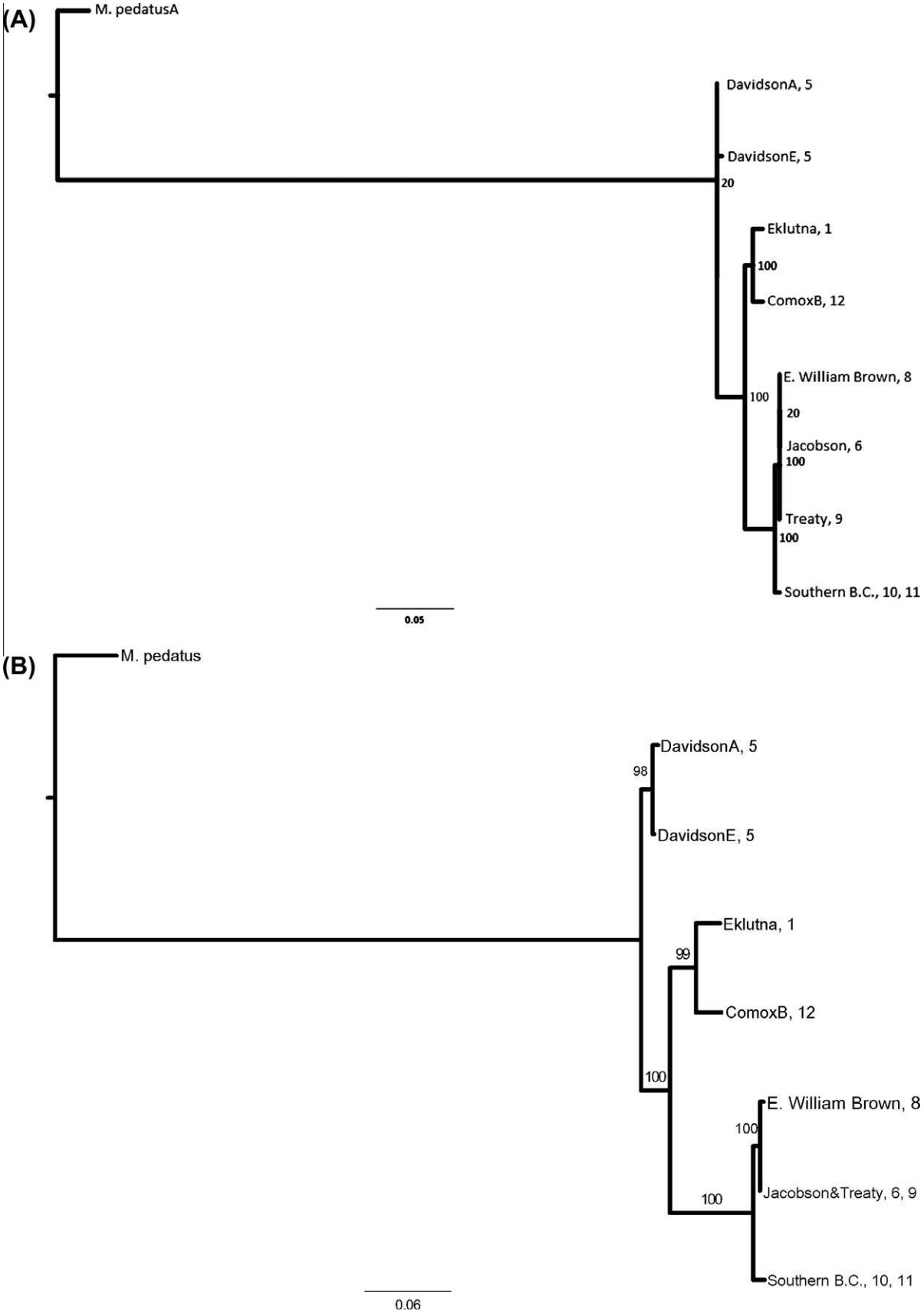

Fig. 5. Maximum likelihood phylogeny (mid-point rooted) for glacier ice worms based on (A) mitochondrial 12S and (B) partitioned 12S and CO1 sequences. Bootstrap values for 100 replicates. (GenBank accession numbers JF788590–JF788598; JF788599–JF788607, respectively). Representative haplotypes are shown. Label numbers as in Fig. 1.

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Pliocene through the Pleistocene, with glacial refugia in the St. Elias, Chugach, and Cascade ranges consistently maintaining ice worm populations during interglacial periods, and active colony expansion occurring during glacial maxima. Long distance, passive dispersal (e.g., avian) likely contributed to current ice worm distribution, and was probably the mechanism by which the southern clade was founded by a northern ancestor.

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References

Barstead, R.J., Kleiman, L., Waterston, R.H., 1991. Cloning, sequencing, and mapping of an alpha-actinin gene from the nematode *Caenorhabditis elegans*. Cell Motil. Cytoskeleton 20, 69–78.

Bely, A.E., Weisblat, D.A., 2006. Lessons from leeches: a call for DNA barcoding in the lab. Evol. Dev. 8, 491–501.

Castresana, J., 2000. Selection of conserved blocks for multiple alignments for their use in phylogenetic alignments. Mol. Biol. Evol. 17, 540–552.

Chang, C.H., Lin, S.M., Chen, J.H., 2008. Molecular systematics and phylogeography of the gigantic earthworms of the *Metaphire formosae* species group (Clitellata, Megascolecidae). Mol. Phylog. Evol. 49, 958–968.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1660.

Conroy, C.J., Demboski, J.R., Cook, J.A., 1999. Mammalian biogeography of the Alexander Archipelago of Alaska: a north temperate nested fauna. J. Biogeograhy 26, 343–352.

Cook, J.A., MacDonald, S.O., 2001. Should endemism be a focus of conservation effort along the North Pacific Coast of North America? Biol. Conserv. 97, 208–213.

Drummond, A., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Dyke, A.S., 2005. Late Quaternary vegetation history of Northern North America based on pollen, macrofossil, and faunal remains. Geogr. Phys. Quart. 59, 211–262.

Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 32, 1792–1797.

Edwards, J.S., 1986. How small ectotherms thrive in the cold without really trying. Cryo. Lett. 6, 388–390.

Edwards, C.A., Bohlen, P.J., 1996. The Biology and Ecology of Earthworms. Chapman and Hall, London.

Goodman, D., 1971. Ecological Investigations of Ice Worms on Casement Glacier, Southeastern Alaska. Institute of Polar Studies Report No. 39. Ohio State University Research Foundation, Columbus.

Guindon, S., Gascuel, O., 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Hartzell, P.L., Nghiem, J.V., Richio, K.J., Shain, D.H., 2005. Distribution and phylogeny of glacier ice worms (*Mesenchytraeus solifugus* and *Mesenchytraeus solifugus rainierensis*). Can. J. Zool. 83, 1206–1213.

Holmstrup, M., Sjursen, H., 2001. Freeze inducted glucose accumulation in the enchytraeid, Fredericia ratzeli, from Greenland. Cryo. Lett., 272–276.

Holmstrup, M., Bayley, M., Ramlow, H., 2002. Supercool or dehydrate? An experimental analysis of overwintering strategies in small permeable arctic invertebrates. Proc. Natl. Acad. Sci. USA 99, 5716–5720.

Knowlton, N., Weigt, L.A., Solóranzo, L.A., Mills, D.K., Bermingham, E., 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. Science 260, 1629–1632.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Liang, Y., Hsu, C., Chang, T., 1979. A new genus and species of Enchytraeidae from Tibet. Acta Zootaxinom. Sinica 4, 312–317.

Marincovich, L.J., 1990. Molluscan evidence for early middle Miocene marine glaciation in southern Alaska. GSA Bulletin 102, 1591–1599.

Milbrink, G., 2003. Distributional patterns of freshwater tubificid oligochaetes and the capacities for long-way dispersal of tubificis and other freshwater invertebrates with particular reference to—alien□ species and birds as vectors of dispersal. In: Verdonschot, P.F.M. (Ed.), Proceedings of the 9th International Symposium on Aquatic Oligochaeta, Wageningen, the Netherlands.

Miura, O., Torchin, M.E., Bermingham, E., Jacobs, D.K., Hechinger, F., 2011. Flying shells: historical dispersal of marine snails across Central America. Proc. Roy. Soc. B. doi:10.1098/rspb.2011.1599.

Morrison, B.A., Shain, D.H., 2008. An AMP nucleosidase gene knockout in *Escherichia coli* elevates intracellular ATP levels and increases cold tolerance. Biol. Lett. 4, 53–56.

Napolitano, M.J., Nagele, R.O., Shain, D.H., 2004. The ice worm, *Mesenchytraeus solifugus,* elevates adenylate levels at low physiological temperature. Comp. Biochem. Physiol. Part A 137, 227–235.

Panchal, M., 2007. The automation of nested clade phylogeographic analysis. Bioinformatics 23, 509–510.

Posada, D., Crandall, K.A., Templeton, A.R., 2000. GeoDis: a program for the cladistic nested analysis of the geographic distribution of genetic haplotyes. Mol. Ecol. 9, 487–488.

Shafer, A.B., Cullingham, C.I., Cote, S.D., Coltman, D.W., 2010. Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. Mol. Ecol. 19, 4589–4621.

Soto-Adames, F.N., 2002. Molecular phylogeny of the Puerto Rican *Lepidocyrtus* and *Pseudosinella* (Hexapoda: Collembola), a validation of Yoshii's color pattern species. Mol. Phylog. Evol. 25, 27–42.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web-servers. Syst. Biol. 75, 758–771.

Templeton, A.R., Routman, E., Philips, C.A., 1995. Separating population structure from population history: a cladistic analysis of the geographic distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140, 767–782.

Tynen, M.J., 1970. The geographical distribution of ice worms (Clitellata: Enchytraeidae). Can J. Zool. 48, 1363–1367.

White, J.M., Ager, T.A., Adam, D.P., Leopold, E.B., Liu, G., Jette, H., Schweger, C.E., 2007. An 18 million year record of vegetation and climate change in northwestern Canada and 30 Alaska: Tectonic and global climatic correlates. Palaeogeogr. Palaeoclim. Palaeoecol. 130, 293–306.