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The role of temperature in the distribution of the glacier ice worm, *Mesenchytraeus solifugus* (Annelida: Oligochaeta: Enchytraeidae)

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ABSTRACT

The North American glacier ice worm, *Mesenchytraeus solifugus* (Emery, 1898), is restricted to coastal glaciers in the American Pacific Northwest with a puzzling 400 km distribution gap along the Alaska–British Columbia border and several disjunct populations of northern clades in southern latitudes. We illustrate the role of minimum temperatures in ice worm behavior, abundance, and distribution. The study included 200 glaciers and 25 mitochondrial CO1 haplotypes from the species' 5×10^5 km^2 geographic range. Minimum winter temperatures on the previous summer surface appear to determine: (1) the elevations ice worms occupy, (2) the glaciers that can support them, and (3) the mountain ranges they inhabit. Ice worms do not inhabit glaciers with over-winter temperatures below −7 °C on the previous summer surface. An annelid molecular clock in a Bayesian phylogeny suggests ice worms diverged from an aquatic ancestor 2.23 Ma, emerging as three clades 1.6–1.7 Ma. Cold sensitivity, together with southeast Alaska's geography and past climate, likely created the distribution gap, a hypothesis supported by their phylogeography.

INTRODUCTION

The science of ecology generally tests hypotheses based on central tendency relative to variability, yet biological processes often respond critically to extremes (Gaines and Denny, 1993; Gutschick and BassiriRad, 2003). For example, where and how often a set of threshold climatic values are exceeded can determine geographic range (Araújo et al., 2008). This fact, conceptualized as the "fundamental niche" (Hutchinson, 1957), is applied as the climate envelope in niche modeling (Pearson and Dawson, 2003), but rarely is a geographic range set by the fundamental niche. Dispersal limitation and species interactions determine the realized niche in most complex ecosystems. In extreme environments, like ice fields and glaciers, however, a geographic range may be determined essentially by dispersal limitation to sites within the species' climate envelope.

Glaciers support a diversity of taxa morphologically and physiologically specialized for low temperatures (psychrophiles; Napolitano and Shain, 2004; Hodson et al., 2008). Glacier-dwelling psychrophilic algae (Chlorophyta and Cyanobacteria) in Alaska are most abundant near the equilibrium line altitude (Takeuchi, 2013), the elevation where glacier ice accumulation equals its ablation (ELA). Here we treat the ELA as the uppermost summer snow-line elevation. Many glacier psychrophiles, such as the various species of snow algae (Chlorophyta), overwinter on the previous summer surface. Glacier "ice worms" (Annelida: Oligochaeta: Enchytraeidae) require permanent, glacial ice for survival and reproduction. Their distribution is limited to maritime glaciers of northwest North America (*Mesenchytraeus solifugus* [Emery, 1898]; Dial et al., 2012) and monsoon glaciers of southeastern Tibet (*Sinenchytraeus glacialis* [Liang and Hsu, 1979]; Liang et al., 1979). *Mesenchytraeus solifugus* is known to graze on snow algae (Goodman, 1971) and generally surfaces on glaciers during summer nights (Shain et al., 2001). It is a darkly pigmented member (Moore, 1899) of an Holarctic genus, part of a radiation of species across Beringia generated by Pleistocene glacial cycling (Rota and Brinkhurst, 2000). The geographic range of *M. solifugus* extends from Alaska's Chugach Mountains 2400 km southeastward to Oregon's Three Sisters Mountains (Fig. 1; Hartzell et al., 2005). Previous biogeographic studies reported the species absent from the Rocky Mountains and Alaska's Wrangell, Talkeetna, Alaska, and Brooks Ranges (Hartzell, 2005; Hartzell et al., 2005; Dial et al., 2012), where Hartzell et al. (2005) hypothesized low temperatures limit the northern distribution of ice worms.

Cytrochrome c oxidase I (COI) is a mitochondrial gene used by all eukaryotes for aerobic respiration. Because it is generally conserved among conspecific animals and mutates relatively quickly, it has become a commonly used genetic marker for identifying species, such as in DNA barcoding. Based on molecular evidence and a presumed oligochaete mutation rate for the COI gene of 2.4% m.y.^{-1} (oligochaete "evolutionary rate" of 4.8% m.y.^{-1}; Chang et al., 2008), we previously proposed a biogeographic history and phylogeny (supported by mitochondrial genes COI and 12S) whereby ice worms diverged from an aquatic ancestor and then into three clades during the Pliocene (Dial et al., 2012). Each clade now occupies a mountain chain >4000 m above sea level (asl): the Northern clade in Alaska's Chugach and Kenai Mountains, the ancestral Central clade in the Saint Elias Range, and the Southern clade from central British Columbia (BC) to the Oregon Cascades (Fig. 1).

The more closely related Northern and Southern clades are geographically separated by 1000 km and the intervening Central clade. A 400 km distribution gap separates the Central and Southern clades, straddling apparently suitable, maritime habitat in southeast Alaska and northwest BC (Fig. 1, part B). Two disjunct populations of the Northern clade exist within the Southern clade range on Vancouver Island, BC, and the Olympic Peninsula, Washington (P. Wimberger, University of Puget Sound, personal communication), 1700 km distant from the main Northern range; they show no evidence of genetic mixing.

We pose three questions. First, why are ice worms found only on maritime glaciers? Second, what is the basis for the extensive distribution gap along the Alaska-BC border? And third, why are only Northern clade populations disjunct? While other enchytraeids survive to −15 °C (Slotsbo et al., 2008), ice worms tolerate a narrow ±7 °C range centered near freezing (–6.8 to 5 °C; Edwards, 1985). Here we show that low temperatures likely determine the distribution of ice worms, providing a case study in the relative roles of ecological limitation, regional extinctions, and dispersal across the geographic range of an obligate alpine species.

METHODS

Field Sites

We compiled ice worm presence/absence on 200 glaciers through literature and fieldwork. Fieldwork consisted of walking or skiing over glacier surfaces between the terminus and the accumulation zone searching for worms on the surface and in meltwater pools during warm, summertime nights. Consistent with anecdotes from mountaineers and glaciologists, we found no worms in the Alaska Range (n = 25 glaciers), the Brooks Range ("Brooks"; n = 6), or Talkeetna Mountains ("Talkeetnas"; n = 5). Of 31 glaciers visited in south-central Alaska's (Fig. 1, part A) western Chugach ("Chugach"; n = 14) and Kenai Mountains ("Kenai"; n = 17), 21 had ice worms and 10 did not. Ice worms in this region were generally present on larger glaciers and glaciers close to others with ice worms present, and absent on small and isolated glaciers (Fig. 2). Molecular data suggest that ice worms rarely disperse between noncontiguous glaciers (Hartzell et al., 2005) in ecological time, but were likely subject to long-distance dispersal over evolutionary time (Dial et al., 2012).

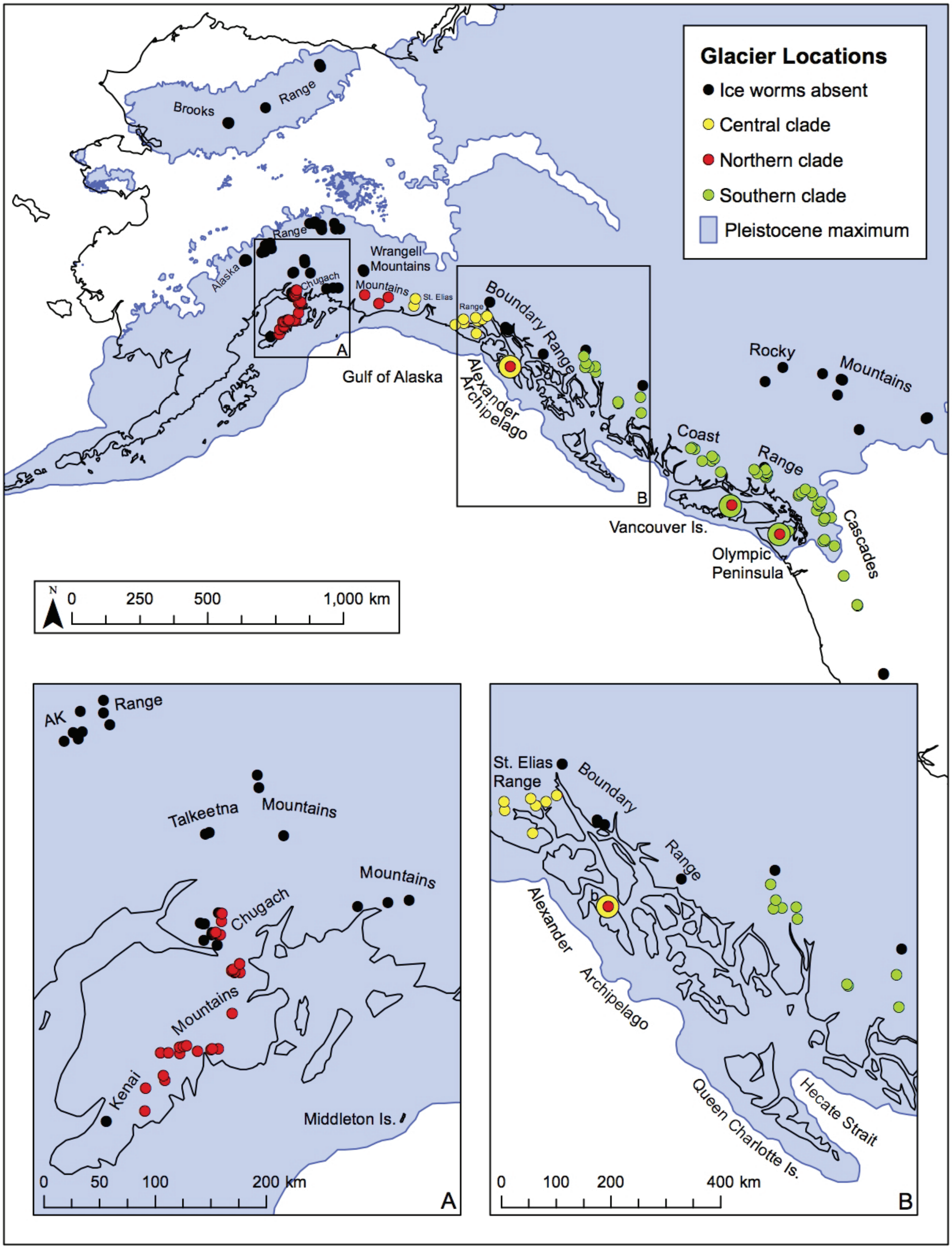

FIGURE 1. North American glacier ice worm (*Mesenchytraeus solifugus*) distribution overlaid on Pleistocene maximum glaciation (light blue) with modern mountain ranges and other geographic references (black text). Black bullets represent glaciers without ice worms; colored bullets indicate glaciers with ice worms (red–Northern clade; yellow–Central clade; green–Southern clade). Large bullet with smaller enclosed bullet indicates location with two clades. (A) Inset map showing south-central Alaska. (B) Inset map showing southeast Alaska and adjacent Canada. The small "b" adjacent to large yellow bullet with red center indicates Baranof Island population. Albers projection.

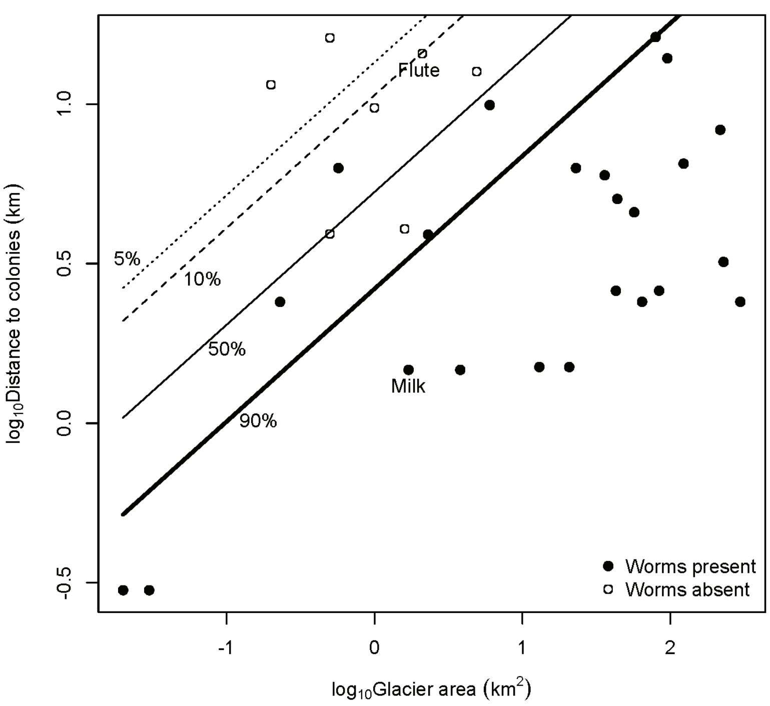

FIGURE 2. Presence (solid bullets) and absence (open circles) of ice worms from glaciers in the western Chugach and Kenai Mountains as plotted by log area and log distance to nearest glacier with ice worms. Lines give probabilities of occupancy based on an additive logistic regression model of log(area) and log(distance). In this treatment Milk Glacier supports an ice worm population (Shain et al., 2001).

Extensive glaciation resulting from warm, wet, cyclonic systems from the Gulf of Alaska characterize coastal south-central Alaska (Bailey, 1995). Large, flat glacial landscapes <1200 m a.s.l., including the 1800 km^2 Harding Icefield ("Harding") and its outlet glaciers (Adalgeirsdottir et al., 1998), cover much of the eastern Kenai. At the Kenai Peninsula isthmus, 90 km northeast of Harding, a small, avalanche-cone glacier ("Byron"; Shain et al., 2001) near Byron Glacier was the site of autumn observations. Wolverine Glacier ("Wolverine"), 50 km north of Harding, is a United States Geological Survey (USGS) benchmark glacier (Fountain et al., 1997). Northeast of Kenai, maritime-climate glaciers drain the Chugach Mountains (<4000 m a.s.l.) southward and continental-climate glaciers drain northward. Glaciers in the Chugach vary from small, disconnected cirque glaciers to long (>30 km) connected glacier systems. Eklutna Glacier ("Eklutna") provided several study plots. A weather station (Campbell Science, Sensor CS215 model) installed at the equilibrium line altitude (ELA) recorded hourly temperature and radiation 2 m above the snow surface. The Talkeetnas, less than 20 km directly north of Chugach, Alaska Range ≈50 km north of the Talkeetnas, and Brooks 500 km further north, each experience greater temperature extremes and less precipitation than coastal ranges (Bailey, 1995). Large, low-elevation icefields are absent north of the Chugach, but several long glaciers exist in the Alaska Range. The Talkeetnas and Brooks Range glaciers are small valley (<20 km) or cirque glaciers. Gulkana Glacier ("Gulkana") in the eastern Alaska Range is a USGS benchmark glacier (Fountain et al., 1997; Takeuchi, 2013). Ablation (ABL) and accumulation (ACC) zones are below and above the ELA, respectively, functionally defined as bare ice (ABL) and snow over firn (ACC) at the end of the melt season. Glaciologists (M. Loso, Alaska Pacific University, and S. O'Neel, USGS, personal communications) calculated the true ELA for Eklutna and USGS benchmark glaciers.

Seasonal Activity

Early Spring (May)

To investigate where ice worms overwinter, we dug snow pits 22–26 May 2010 (before their first seasonal surface appearance) at three elevations on Eklutna: ABL (1133 m a.s.l.), ELA (1395 m a.s.l.), and ACC (1532 m a.s.l.). The ACC pit was shoveled to 2 m depth and a 5-cm-diameter snow auger advanced to the summer surface 4 m below the pit floor (depth = 6.73 m). In the ELA (depth = 4.16 m) and ABL pits (depth = 1.93 m) the previous summer surface was visible as 2 m^2.

Late Spring (June)

Ice worms become seasonally active on glacier surfaces in late spring. To examine weather factors that may initiate surface activity on Eklutna, we used interval photography to photo-capture their first and subsequent appearances for 33 days, 9 June–11 July 2010 at the ELA weather station (1395 m a.s.l.). A waterproof, 10 megapixel digital camera (Pentax, Optio W80 model) mounted to the weather station ≈0.5 m from the snow surface captured a 50 × 70 cm field of view every 1.5 h.

Summer (August)

We recorded snow surface temperature and video of ice worm surface activity simultaneously. Recordings were made on Chugach's Raven Glacier near the ELA at 1279 m a.s.l. A 10 megapixel digital camera (Pentax, model Optio W80) mounted on a tripod 25 cm above the snow surface video-recorded ice worm activity. We used data loggers (Onset, HOBO Pendant Temperature/Light Data MicroDAQ model) on the snow surface to record temperature concurrent with video recordings of ice worm activity for 41 minutes (20:22–21:03, 26 August 2011). Temperatures at the glacier surface decreased from 7 °C to nearly 0 °C under light winds and patchy cloud cover. Ice worm activity over 30 s intervals was characterized by three classes of behavior: "burrowing," "surface-movement," and "surface-no-movement."

Autumn and Early Winter (October–December)

We visited glacial avalanche cones (105 m a.s.l.) below the terminus of Byron Glacier on 15 dates between October and December over three years ($n = 8$ visits in 2009, $n = 2$ in 2010, $n = 5$ in 2011). Daily minimum temperatures from the Portage Glacier Visitor Center (2.4 km north at 48 m a.s.l.; http://www.ncdc.noaa.gov/cdo-web/datatools/findstation) were similar among years (2009: –28.9 to 9.4 °C; 2010: –27.8 to 6.7 °C to; 2011: –29 to 4 °C). Visits to near the melting edge of the avalanche cones on surface snow, stranded icebergs, and the inside of ice tunnels were generally 2–4 hours long in the late afternoon to early evening.

Elevational Gradients

We used ice worm counts collected on Harding (2001, 2005, 2009, and 2011) and Eklutna (2009) to estimate maximum ice worm surface density (greatest density observed of all samples) as a function of elevation class. We sampled density across elevational gradients using sample frames of 0.05–1.25 m^2 placed during ski surveys across Eklutna, Harding, and eight glaciers draining Harding (Exit, East Skilak, Skilak, Bear, Holgate, Tustumena, Chernof, Dinglestadt).

Winter (September–May) Temperatures on Previous Summer Surface

Regional Distribution

We compared temperatures at buried summer surfaces of glaciers, air temperatures adjacent to glaciers, and lowland air temperatures in a 65 km^2 area of the Chugach. Ice worms were observed in summer on "worms-present" Raven Glacier (ELA 1279 m). Shain et al. (2001) reported worms on "worms-lost" Milk Glacier (ELA 1273 m), but none were observed during this study (6 September 2010 and 15 August 2011). Similarly, no worms were observed on "worms-absent" Flute (ELA 1585 m) or Calliope (ELA 1427 m) Glaciers following multiple summer surveys from 2009 to 2011. We installed data loggers near the ELA on the summer surfaces of these four glaciers and at the ELA on moraine rocks adjacent to Crow Glacier ("moraine," ELA 1425 m a.s.l.). The data logger on Calliope was lost. Air temperatures from a nearby (≈10 km SSE), low elevation (469 m a.s.l.) SNOTEL site (Mount Alyeska; 60.967°N, 149.083°W) were downloaded (http://www.wcc.nrcs.usda.gov/snotel/Alaska/alaska.html).

Geographic Distribution

No ice worms have been documented in the Talkeetna Mountains, Brooks Range, or Alaska Range, despite repeated searches (Fig. 1; Hartzell et al., 2005; N. Takeuchi, Chiba University, personal communication; this study). To investigate temperature differences between mountain ranges, we compared USGS temperature measurements from previous summer surfaces on two USGS benchmark glaciers (Fountain et al., 1997): Gulkana in the Alaska Range, where ice worms were absent, and Wolverine in the Kenai Mountains, where they were present (S. O'Neel, USGS, personal communication). Data loggers installed on summer surfaces (R. S. Marsh, USGS, unpublished data) at two elevations each on Gulkana (1350 m, ABL; 1675 m, ELA) and Wolverine (550 m, ABL; 1055 m, ELA) provided 10–11 years of temperature data (hydrologic years 1997–2010). Data loggers had a low temperature cutoff (–5 °C) to increase data capacity.

Phylogeny and Divergence Estimation

In summer 2013 we obtained 10 ice worms collected from a single glacier (6 km^2) on Cross Mountain, Baranof Island, southeast Alaska, the tallest and most glaciated island in the Alexander Archipelago. These are the first worms documented south of Glacier Bay and north of BC. We sequenced mitochondrial DNA in the COI gene following methods outlined in Dial et al. (2012) and found 6 haplotypes (CrossMt_1, CrossMtn_2_6, CrossMt_3_4_8_9, Cross_Mt_5, CrossMt_7, CrossMt_10) in the population; 22 additional haplotypes were retrieved from GenBank including 3 haplotypes representing two outgroup taxa (*M. solifugus* JF788608–JF788625; *M. pedatus* AY873846 and AY73847; *M. pelicensis* GU453375). All sequences (417 bp) were manually aligned, then translated to amino acids and examined for internal stop codons to uncover pseudogenes. A hierarchical likelihood ratio test was run to determine the best model of DNA substitution using MrModeltest v.2.3 (Nylander, 2004). Parameters were set in BEAUti, part of the BEAST (v.1.6.2) software package (Drummond and Rambaut, 2007). Substitution model was set to HKY+G using empirical base frequencies and partitions into codon positions. We tested both strict and relaxed clock models and several tree processes using default priors and operators and analyzed Bayes Factors (if ESS values for tests were >300) in Tracer v1.4 (Rambaut and Drummond, 2007). A relaxed clock with a lognormal distribution and uncorrelated rates consistently provided minus log-likelihood scores nearest zero. The Yule speciation process provided a Bayes factor >4.9 compared to other tree priors available in BEAST v.1.6.2. Divergence times were estimated using a 4.8% $m.y.^{-1}$ substitution rate to match Chang et al.'s (2008) ambiguous "evolutionary rate" for an oligochaete annelid. Dial et al. (2012) assumed a 4.8% divergence rate (= 2.4% substitution rate), giving a Pliocene origin, likely too early for a glacier-obligate psychrophile. We ran two independent analyses with an MCMC chain of length 100 million, logging trees every 10,000. The resulting topology was visualized in FigTree v.1.3.1 (Rambaut, 2009). As a previous CO1 phylogeny was supported by mitochondrial 12S and we were unable to resolve differences among populations with nuclear 18S (Dial et al., 2012), we applied only COI.

RESULTS

Seasonal Activity and Temperature

Early Spring (May)

On Eklutna we shoveled snow pits to the previous summer surface at the ABL, ELA, and ACC in May 2010. No ice worms were found in the ABL or ACC pits. However, on 22 May three live, adult ice worms, approximately 2 cm long, were found 4.16 m deep on the previous summer surface in the ELA pit.

Late Spring (June)

Springtime surface activity of ice worms depended on sufficiently warm nighttime temperatures. No worms were observed when illumination was >50.0 W m^{-2} or when air temperature at 2 m above snow surface (^{air}T) was below freezing. The first appearance of ice worms on the surface (captured with interval photography at 23:00 21 June 2010) occurred once the daytime hourly minimum ^{air}T exceeded 3.9 °C. Prior to first appearance, ^{air}T was between –3.4 °C and 2.1 °C. Of the 31 nights photo-sampled, 15 nights were considered "long, warm nights" (^{air}T > 0.47 °C and illumination < 50.0 W m^{-2} for seven consecutive hours or more). We photo-captured worm activity during 12 of the 15 long, warm nights and during one of 16 non-long-warm nights. Overall, a model of long, warm nights correctly predicted worm presence and absence on 87% of the 31 nights.

Summer (August)

Observations of ice worm activity with surface temperatures (^{sur}T) between 0 °C and 7 °C showed a general increase in activity with higher temperatures and corresponding decrease with lower temperatures. Most worms (6 of 11 individuals) burrowed when $^{sur}T < 0.7$ °C. All worms showed surface movement for at least one 30 s interval when $^{sur}T > 0.7$ °C, but only two worms were observed moving at $^{sur}T < 0.7$ °C.

Autumn and Early Winter (October–December)

Autumn observations of ice worm behavior showed that activity was temperature dependent. In autumn, worms were only seen during daylight hours; nighttime temperatures were below freezing. Worm activity on surface snow decreased with decreasing air temperatures. Among 15 visits between October and December over three years, we found that daily minimum ^{air}T on days with worms moving on snow surfaces ($n = 11$ of 15 visits) ranged from +3.9 to –3.3 °C. When ice worms were not observed as active on the snow surface ($n = 4$ visits), the daily minimum ^{air}T ranged from –6.7 to –15 °C. On some days when no ice worms were visible on the glacial surface, including a 6 December 2009 visit, they could be found in or adjacent to thin films of water flowing across near-vertical ice surfaces inside subglacial tunnels.

Elevational Gradients in Abundance

The size distribution and abundance of ice worms depended on elevation, with maximum density (≈10^2–10^3 individuals m^{-2}) and body size range (≈ 2–20 mm in length) consistently found near the ELA (Harding ≈1100 m a.s.l.; Eklutna ≈1400 m a.s.l.). Generally ice worms were absent, or rare and large sized (>10 mm) in the accumulation zone 200 m or more above the ELA and absent in the lower ablation zone, but often very abundant (≈10^2–10^3 individuals m^{-2}) in supraglacial streams and pools in the upper ablation zone. Glaciers without ice worms were higher on average (mean±sd ELA = 1270 ± 282 m a.s.l., $n = 10$) than glaciers with ice worms present (1069 ± 405 m a.s.l., $n = 21$).

Winter (September–May) Temperatures on Previous Summer Surface

Regional Distribution

We did not find worms on Chugach glaciers where the overwinter minimum temperature at the previous summer surface (^{pss}T) was colder than the lower lethal threshold (Fig. 3). In general, ^{pss}T on the worms-present glacier was warmer throughout the winter than on glaciers where we did not find ice worms. On all glaciers the seasonal minimum ^{pss}T occurred in late September, not midwinter. Overwinter minimum ^{pss}T on the worms-present glacier (–4.8 °C, Raven Glacier) was over 3 °C warmer than on the worms-lost glacier (–7.9 °C, Milk Glacier) and almost 4 °C warmer than on the worms-absent glacier (–9.5 °C, Flute Glacier). The mean of the daily minimum ^{pss}T averaged over winter on the worms-present glacier (–1.9 °C) was higher than on the worms-lost (–2.4 °C) and worms-absent (–3.0 °C) glaciers. Following the early autumn, region-wide drop in temperature, ^{pss}T rose to nearly 0 °C on all glaciers by 2 October 2010, at the first heavy snowfall, then steadily decreased to about –4 °C until 15 April 2011. The worms-absent glacier had 33 cumulative "cold days" when minimum daily $^{pss}T < -4$ °C. The worms-lost glacier had five cold days and the worms-present glacier had four cold days, all of which occurred in September. After 2 October the temperatures at the previous summer surface on all three glaciers were as much as 15 °C warmer and much less variable than surface air temperatures (Fig. 3) recorded at a nearby moraine site (mean daily min $^{sur}T = -16.6$ °C), apparently buried in snow after 1 January, and air temperature at a nearby lowland Snowtel site (mean daily min $^{air}T = -4.1$ °C).

Geographic Distribution

We compared 10 years of minimum daily ^{pss}T on two glaciers, a Kenai and an Alaska Range benchmark glacier (Fig. 4). There were nearly three times the number of "cold days" (minimum daily $^{pss}T < -4$ °C) on the previous summer surfaces of the Alaska Range glacier (ELA mean = 2.2 days) as the Kenai glacier (ELA mean = 0.8 days). The mean monthly min ^{pss}T on the Alaska Range glacier fell below –4 °C during every winter month at both elevations, while the glacier in the Kenai Mountains did so only from September to January. In contrast to an environmental lapse expectation and consistent with winter inversions, there were more cold days on average from September–May at the lower elevation ABL than at the higher ELA for both glaciers (Fig. 4).

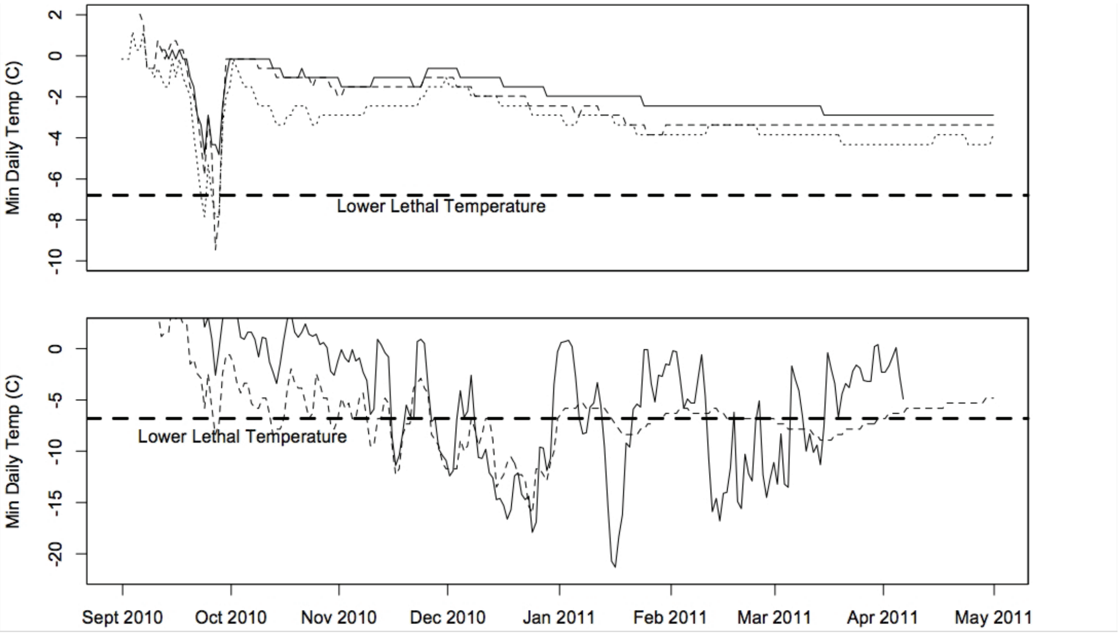

FIGURE 3. (Top panel) Minimum daily temperature time series on the previous-summer-surface for three glaciers in the western Chugach during September 2010 through May 2011. Solid line: Raven Glacier (ice worms present). Dashed line: Milk Glacier (ice worms present in 2000 but not been seen 2010–2011). Dotted line: Flute Glacier (ice worms absent). (Lower panel) Air temperatures at lowland, Alyeska Snowtel site (solid line) and a moraine boulder adjacent to the equilibrium line altitude on the Crow Glacier (dashed line). In both panels the heavy horizontal dashed line gives the lower lethal temperature for ice worms.

Phylogeny

New molecular samples from a glacier on Baranof Island in the Alexander Archipelago of southeast Alaska, together with the 22 haplotype sequences from Dial et al. (2012), provided three well-supported clades: Northern, Central, and Southern (Fig. 5). Ice worm coalescence time to most recent common ancestor (TMRCA) with *M. pedatus* (Eisen, 1904) took place 2.23 Ma. The TMRCA for all ice worms was estimated at 1.7 Ma. The sister relationship between Northern and Central clades was weakly supported (posterior probability = 0.25). Coalescence of the Northern clade was oldest at ≈1.1 Ma followed by the Central clade (0.83 Ma) and Southern clade (0.73 Ma). The worms from a single glacier on Baranof Island reflect the only known locality represented by both Northern and Central clades and one of three known localities of sympatry (Fig. 1).

Discussion

We set out to determine the relationship of glacier ice worms (*Mesenchytraeus solifugus*) to cold. In doing so, we constructed the first full, annual activity cycle based on temperature. Our results support a hypothesis that low autumn and winter temperatures at the previous summer surface limit the elevational, regional, and geographic distribution of ice worms. This hypothesis of ecological cold limitation serves as a basis to understand certain biogeographic patterns (Fig. 1).

A reapplication of an oligochaete molecular clock (Chang et al., 2008; Dial et al., 2012) with additional samples placed the divergence of the ice worm lineage at 2.23 Ma (Fig. 5), 0.5 m.y. later than the onset of Pleistocene glaciation (2.7 Ma; Bintanja and van de Wal, 2008). Considering the obligate glacier habitat of ice worms, the timing of initial divergence with the start of the current ice age is reasonable. Among ice worm lineages there is only weak support for basal relationships and we cannot reject the possibility that all three major clades resulted from fragmentation of a single continuous distribution early in the species' evolutionary history (Tynen, 1970) around 1.70 Ma.

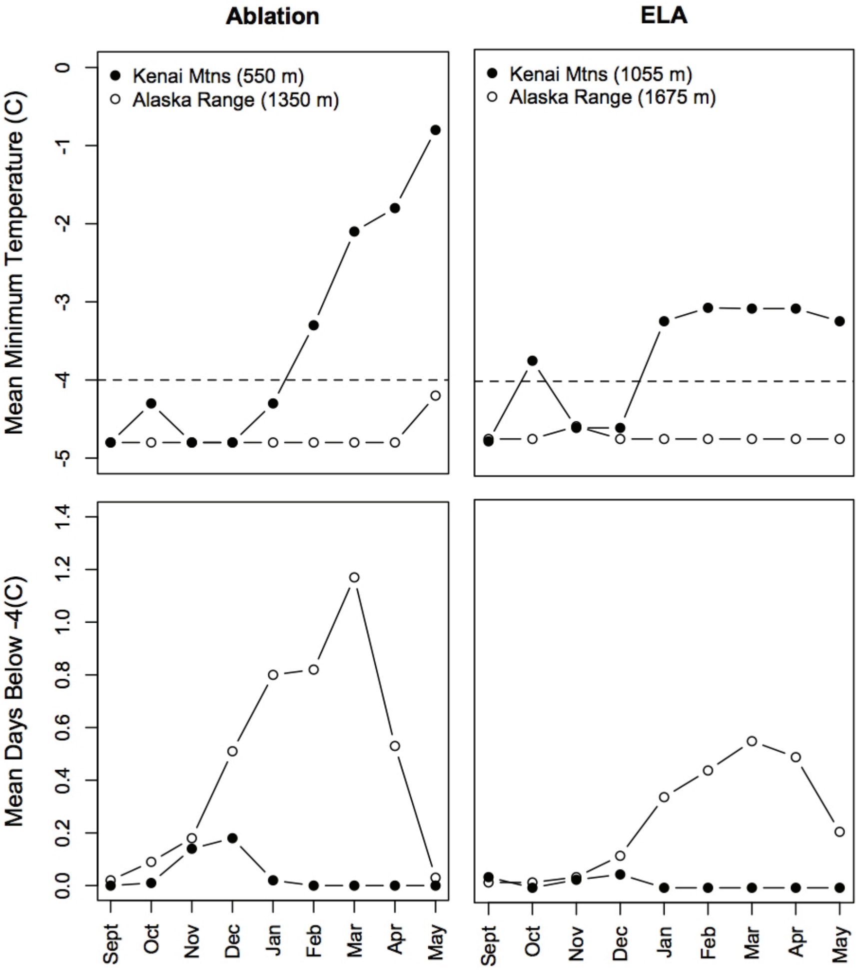

FIGURE 4. **Summary statistics (Upper panel: mean minimum temperature; Lower panel: mean number of days per month with minimum temperature < –4 °C) on previous-summer-surface temperatures (1997–2010) from Gulkana Glacier in the Alaska Range without ice worms (open circles) and Wolverine Glacier in the Kenai Mountains with ice worms (bullets) from the ablation zone (left column) and the equilibrium line altitude (ELA; right column). Numbers in parentheses give elevation in m a.s.l. Data loggers had a low temperature cutoff (–5 °C) to increase data capacity.**

Temperature and Distribution

Ice worm respiration rate drops with temperature (Goodman, 1971; Napolitano and Shain, 2004). We found that ice worms overwinter on the previous summer surface in a state of dormancy as seen in Washington's Cascade Mountains (Doughton, 2006). Worms resurface in May or June when surface temperatures warm sufficiently and meltwater reaches the previous summer surface. Summer surface activity is related to a combination of darkness and sufficient near-atmospheric warmth. Like Goodman (1971), we did not find worms above surface crusts during subfreezing nights, behavior consistent with positive thermotaxis (Shain et al., 2001). As daytime temperatures seasonally drop toward freezing, ice worm activity shifts from nocturnal to diurnal, centering on liquid water, often concentrating worms locally. Once free-water freezes, worms cease activity. The concentration of ice worms in aquatic habitats at the ELA and below, such as supraglacial streams and meltwater pools in the ablation zone, also reflects the security of above-lethal temperatures in snow-free habitat. In bare ice habitats, subfreezing temperatures threaten ice worms at night, and without snow-cover ice worms may seek refuge from UV and other harmful radiation through submergence in liquid water. An alternative hypothesis explaining their behavior would be aquatic ancestry.

Autumn low temperatures likely limit ice worm abundance and distribution, locally on a given glacier and regionally within a mountain range. The previous summer surface usually experiences the lowest temperatures of the year during autumn when snow cover is at an annual minimum and extreme cold temperature events occur (Fig. 3). Shallow snow cover and very low winter air temperatures (<–30 °C) of interior mountain ranges explain the lack of ice worms found there.

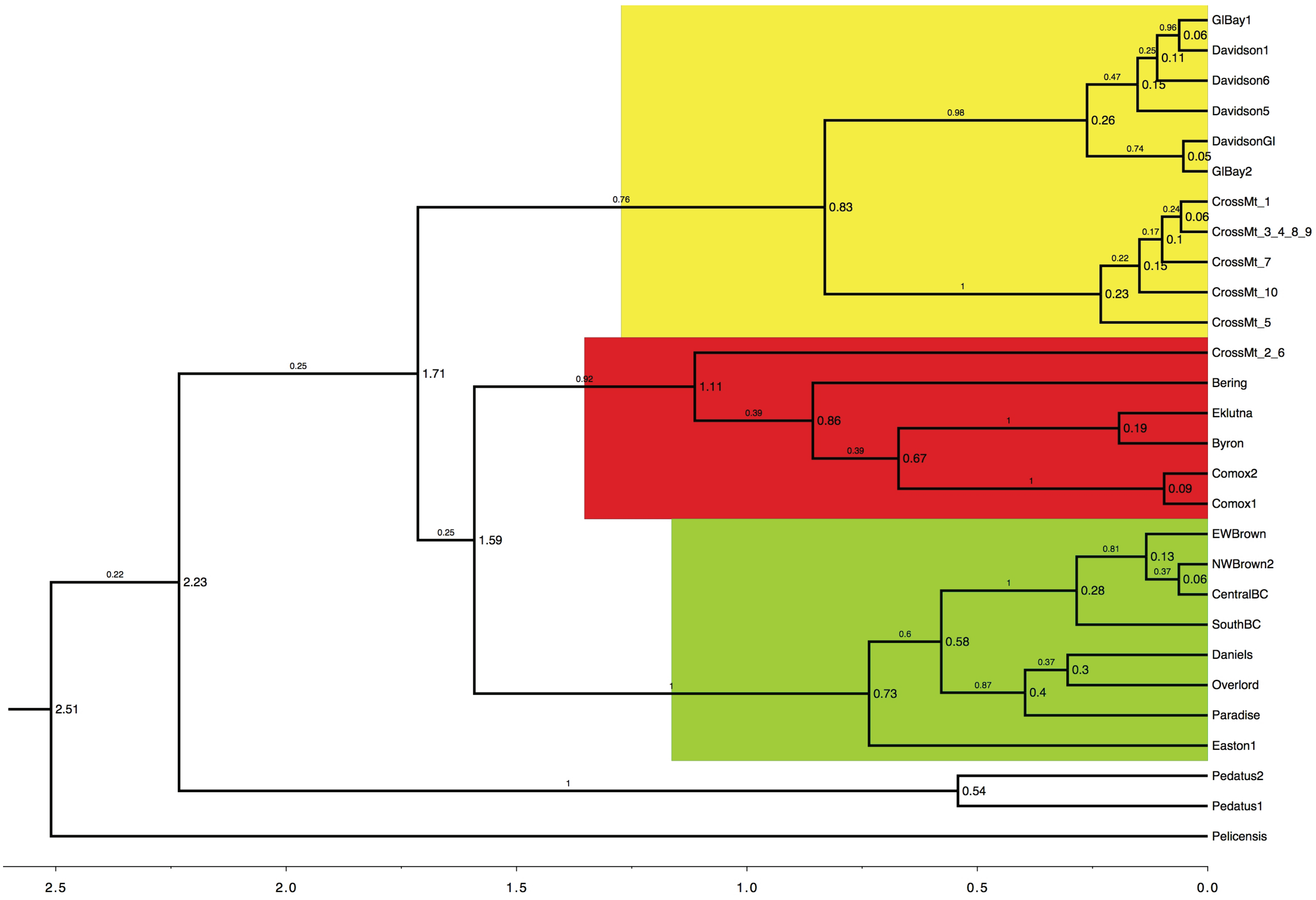

FIGURE 5. Bayesian phylogeny for 25 *Mesenchytraeus solifugus* haplotypes and three outgroup haplotypes (*M. pedatus* and *M. pelicensis* Issel, 1905). Large font numbers on nodes give divergence dates in Ma; small font numbers on branches give posterior probabilities. Yellow indicates Central clade, green Southern clade, and red Northern clade as shown in Figure 1. Locations for haplotypes as in Dial et al. (2012) with exception of haplotypes prefixed as "Cross_Mtn", collected at Cross Mountain (57.08930°N, 134.97468°W), Baranof Island, Alaska. Horizontal axis gives years before present as Ma.

The effect of subfreezing temperatures on cells is principally dehydrating (Halfpenny and Ozanne, 1989; Marchand, 2014), making the dermal permeability of oligochaetes (Barnes, 1987) problematic in icy environments. Whereas terrestrial *Enchytraeus* have been shown experimentally to survive to –15 °C (Slotsbo et al., 2008), other Enchytraeids are drought-sensitive (Nielsen, 1955; Maraldo and Holmstrup, 2009). *Mesenchytraeus* shows the most aquatic adaptations in the family (Christensen and Glenner, 2010). The evolutionary history of *M. solifugus* (Dial et al., 2012) implies an ancestor similar to the aquatic *M. pedatus* (Healy and Fend, 2002). The drought-sensitive morphology, physiology, and water balance of ice worms contrasts with another glacially obligate macro-invertebrate, the Himalayan wingless midge (Diptera: Chironomidae: *Diamesa* sp.), active to –16 °C (Kohshima, 1984), whose larvae are aquatic, dwelling in supraglacial streams and cryoconite holes.

Ice Worms in a Warming Climate

Ice worm survivorship is greatest near the glacier's snowline (ELA). Below this altitude insufficient snow depth and temperature inversions expose ice worms to lethal temperatures in autumn and winter (Fig. 4). Higher in the accumulation zone, the snowpack is frozen and too dry. Taken together, these temperature considerations explain why ice worm density is greatest at intermediate elevations (Moore, 1899), a pattern similar to snow algae in Alaska (Takeuchi, 2013). A warming climate may increase mortality by exacerbating a thinning glacier snow cover that retreats during long summer days faster than ice worms can travel during short summer nights (≈3 m h^{-1}; Shain et al., 2001). Ice worms on the Harding Icefield and surrounding glaciers were often seen concentrated on the down-glacier edge of crevasses, consistent with up-glacier movement. Populations stranded without cover can be locally extirpated, particularly on flat icefields where a small rise in ELA exposes a large area. Low-elevation, stranded populations often appear denser than nearby colonies on snow, an artifact of their unveiling. In addition, ice worms seeking shelter from nighttime freezing and daytime radiation in supraglacial aquatic habitats may be doomed, if cold autumn temperatures precede snowfall. Very small glaciers, often completely bare ice at the end of the ablation season, also expose ice worms to lethal autumn temperatures, explaining the absence of ice worms there (Fig. 2); larger glaciers with ample snow cover generally support ice worms.

Distribution Gap in Boundary Range of Alaska–British Columbia

The 400 km distribution gap between the Central and Southern clades along the mainland Alaska-BC border (Fig. 1) includes the heavily glaciated, maritime Boundary Range (≈3000 m a.s.l.), covered by extensive icefields and drained by low-elevation, tidewater glaciers. The Boundary Range parallels the lower (≈1700 m a.s.l.), 500-km-long Alexander Archipelago. The archipelago supports few, small alpine glaciers on only two of its 2000 islands (Carrara et al., 2007). The absence of ice worms on the well-studied Juneau Icefield (Miller and Pelto, 1999) at the northern end of the Boundary Range has long been puzzling, considering that available habitat is within the ice worm's temperature envelope. We propose the following historical explanation. During prior glacial maxima, the Boundary Range was not coastal (Carrara et al., 2007), but separated from tidewater by 100 km of mountains consisting of the Alexander Archipelago embedded in the Cordilleran Ice Sheet (Fig. 1). Modern analogs include the Talkeetna and Rocky Mountains that lack ice worms separated from the coast by the ice worm inhabiting Chugach and Coast Ranges. The Alexander Archipelago likely harbored a more extensive distribution of ice worms during the glacial maxima. This hypothesis is supported by the recent discovery of ice worms in the Alexander Archipelago that are not members of the Southern clade. Why worms did not disperse inland during the end of the ice age could be due to the rapid violence of retreating tidewater glaciers (Meier and Post, 1987) within the archipelago's fjordlands, leaving ice worms on isolated alpine glaciers. We speculate that the high, cold, and dry Cordilleran Ice Sheet, together with glacial refugia formed by the Hecate Straight forebulge (Hetherington et al., 2003), presented a barrier to northward, active dispersal of the Southern clade. Individuals of the

Central clade could have actively dispersed 150 km southward along a coastal route.

Disjunct Populations and Dispersal

We conclude with a speculation of the asymmetry in disjunct populations. Northern clade worms could have arrived to the Alexander Archipelago and further south with passerines on trans-Gulf migrations (Swarth, 1920) during autumn cyclonic storms (L. DeCicco, USGS, personal communication), although we do not dismiss the possibility of a previously more extensive range of the Northern clade along this coastline or wind transport; other taxa also exhibit multiple lineages in Southeast Alaska (e.g., Cook et al., 2001). *Mesenchytraeus* generally deposit mucous adhesives on cocoons (Christensen, 1956) holding one large egg (Christensen and Glenner, 2010). The presence of a clitellum on ice worms (Shain et al., 2000) suggests they, too, reproduce by cocoons. If ice worms do reproduce with mucous-coated cocoons in autumn, then cocoons could adhere to southward-migrating birds that visit bare ice surfaces. In contrast to autumn migrations, most passerines migrate northward before ice worms have surfaced in spring, hence the apparent southward-only direction of transport.

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