Winter 2-9-2016

Phylogeography of Two Species of the Genus Apochthonius Chamberlin, 1929, in the Pacific Northwest (Arachnida, Pseudoscorpiones)

Brandi Lynn Welch
Portland State University

Phylogeography of Two Species of the Genus *Apochthonius* Chamberlin, 1929, in the Pacific Northwest (Arachnida, Pseudoscorpiones)

by

Brandi Lynn Welch

A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science
in
Biology

Thesis Committee:
Susan E. Masta, Chair
Luis A. Ruedas
Sarah M. Eppley

Portland State University
2016

Abstract

I used mitochondrial COI sequence data from forty one individuals to investigate phylogenetic relationships among populations of two morphologically similar species of the pseudoscorpion genus *Apochthonius*, *A. minimus* and *A. occidentalis,* in western Washington, Oregon, and northern California. My goal was to assess whether genetic structure in the two species was congruent with geography. Many plant and animal species in the Pacific Northwestern United States have shown patterns of genetic differentiation that follow both north-south and east-west trends, indicating that geologic and climatic events in the past separated populations to the extent that they became genetically differentiated . A distinct geographic pattern emerged within *A. occidentalis*, with at least one northern and two southern populations. A clade containing all *A. minimus* sequences was recovered. However, this clade falls within the larger clade of *A. occidentalis*, rendering *A. occidentalis* paraphyletic. Furthermore, the *A. minimus* sequences showed north-south geographic structuring within the clade. Population genetic analyses were performed based on geographic location within the Pacific Northwest. I found high genetic differentiation coupled with low gene flow between most populations, with the exception of the Portland and North Coast Range populations. These data suggest the presence of more than two species of *Apochthonius* in the Pacific Northwest.

Dedication

I dedicate this thesis to all of the amazing women I have had the divine privilege of witnessing throughout my life. To my mother, Lorie Welch, who has always demonstrated that I can do anything I need to do, usually with about half the resources people lead me to believe: I could never have accomplished so much without you and all you've taught me. To my wife, Jodie Lombardi, who supports me in all of my big dreaming and scheming with a level head, a voice of reason, and superb high-fives: I'm excited to return the favor for the rest of our lives. To my grandmothers, Margaret Childers and Nancy Bell, who believe in me (and are even proud of me) even though they don't believe in evolution: it is because of you I am able to love openly and hold space at my table for anyone who may need a seat. To my advisor, Dr. Susan Masta, who would not tolerate my attempts as self-sabotage: I admire your work, your precision, and your enthusiasm for all the wondrous things many people overlook. To the womyn of the Michigan Womyn's Music Festival, especially producer Lisa Vogel, who taught me from a young age that I am beautiful, capable, strong, and *worthy*: I hope I have done you proud, and that I continue to do so for all our years. And to Margot Jones, Maria Lamb, Nora Frala, Heather Pittenger, Tyler White, Cassia Gammill, Tavi Gupta, Rachel Camp, Amanda Martinez, Sutree Irving, and Barrie Brewer: I truly could not have done this without your friendship, your guidance, and your support. These represent only a small portion of the women who have held me up and kept me going to the end of this wild endeavor. Though it may put them all to sleep, this is for them.

Acknowledgements

I would like to thank my committee members, Susan Masta, Sarah Eppley, and Luis Ruedas, for the many hours of guidance through this research, for lending supplies and ears, and for keeping me on track through life's many surprises. I am deeply appreciative of the Forbes Lea Foundation, which provided funding for a portion of my research. I would also like to thank Mark Harvey for helping me learn how to look at pseudoscorpions, and for his countless contributions to the field of chelonethology, without which this project would have been nearly impossible. The late Ellen Benedict's work was also invaluable in structuring this research, as she provided a solid foundation for pseudoscorpion research in Oregon with her dissertation work in 1978. Many fellow students helped immensely in this work with hours of sorting samples and photographing and measuring specimens. I would especially like to thank them – James Carroll, Cherie James, and Jayson Durtschi. Finally, I am filled with gratitude for my wife, Jodie Lombardi, and our son Oliver Welch, who made it easy to do the work I needed to do to complete this thesis without guilt.

Table of Contents

List of Tables

List of Figures

Chapter 1: Phylogeography of two Pacific Northwest endemic pseudoscorpions of the genus *Apochthonius* Chamberlin, *A. occidentalis* and *A. minimus*

1. Introduction

The Pacific Northwestern United States has a complex geologic and climatic history, which has shaped and reshaped its biogeography since the land was young. Early land-building events included collisions with volcanic island arcs, mountain building, and fierce volcanic activity across the region for millions of years (Bishop, 2003). During the Pleistocene (~2.6my—11,700ya), glaciers covered large sections of the region in the north (Delcourt & Delcourt, 1993), while other areas, such as the Columbia Basin to the south of the Cordilleran ice sheet, underwent xerification (Brunsfeld *et al.*, 2001). Plant and animal species endemic to the mesic forests were unable to persist in all but a few areas. By occupying glacial refugia, the flora and fauna of the Pacific Northwest survived while uninhabitable areas separated populations. When glaciers receded to the north, recolonization became possible. Those populations that developed sufficient mating isolating mechanisms maintained their separate species identity following contact.

1.1 Phylogeography

Evidence of separation events and possible refugia is now detectable in the genomes of some extant Pacific Northwest species (Carstens *et al.*, 2005). Many plants and animals have exhibited genetic structuring which coincides with separation events caused by glaciation and xerification, such as the red alder *Alnus rubra,* three

Saxifragaceae, *Tolmiea menziesii*, *Tellima grandiflora* and *Tiarella trifoliata,* and the stink currant, *Ribes bracteosum* (reviewed in Soltis *et al.*, 1997); the lodgepole pine, *Pinus contorta* (Godbout *et al.*, 2008); the red tree vole, *Phenacomys longicaudus* (Miller *et al.*, 2006); the tailed frog, *Ascaphus truei* (Nielson *et al.*, 2001); and the arionid slug, *Prophysaon coeruleum* (Wilke & Duncan, 2004). These taxa exhibit a distinct north-south break in genetic architecture, with a clade containing all individuals from central Oregon to British Columbia and Alaska in the north, and a clade containing individuals from southern Oregon and northern California in the south.

Species with low dispersal rates would logically have slower recolonization rates than those that are more mobile. For this reason, low-dispersing invertebrates may constitute ideal systems to explore geographic structuring related to separation events. One little-studied order of Arachnida, Pseudoscorpiones, is abundant in the mesic forests of the Pacific Northwest. The most common species present are small (1-2mm), have low vagilities, and are fairly generalist in diet as well as habitat (Benedict, 1978). Species of the most common pseudoscorpion family in the Pacific Northwest, Chthoniidae, prefer habitats that are wet and shady, so xerification would likely have driven them out of regions such as the Willamette Valley during the Pleistocene.

1.2 Pseudoscorpiones

Pseudoscorpiones is an order of arachnids with over 3,500 extant species described (Harvey, 2011). Pseudoscorpions first appeared in the fossil record, in what is now North America, as early as the Middle Devonian (~380mya) (Schawaller *et al.*, 1991), when the supercontinents Gondwana and Laurasia were merging into Pangea (Veevers, 2004). Although these are among the earliest known terrestrial animals, there has been relatively little taxonomic work on the group, resulting in uncertainty as to their evolutionary relationships or even what species exist. Most systematics work has focused on higher-level taxonomic relationships (e.g. Murienne *et al.*, 2008), while genus- and species-level relationships have received little attention. There have been no phylogeographic studies of pseudoscorpions on the continent of North America other than those on the species *Cordylochernes scorpioides* from the Isthmus of Panama (Zeh *et al.*, 2003). A number of taxonomic and biogeographical studies of pseudoscorpions in North America north of Mexico were undertaken in the twentieth century (e.g. Chamberlin, 1929; Hoff, 1949, 1958; Hoff & Bolsterli, 1956; Muchmore, 1974; Nelson, 1975; Muchmore & Benedict, 1976; Buddle, 2005). All of these studies were carried out before DNA sequencing technology and phylogenetic theory had been developed. Benedict (1978) surveyed the taxa present in western Oregon in an effort to fill an information gap for North American pseudoscorpions. Benedict collected pseudoscorpions from all regions of Oregon, raising the regional count from thirteen species in three families to fifty species in nine families. For a number of genera, Benedict noted undescribed

species or discussed the need for redescription of species. No further taxonomic work of Pacific Northwest pseudoscorpions have been undertaken since then, nor have any molecular studies of pseudoscorpions specific to the region occurred.

1.3 Apochthonius

Chthoniidae (Hansen, 1893) is the most abundant family of pseudoscorpions in western Oregon's temperate rainforests, with members of the species *Apochthonius occidentalis* (Chamberlin, 1929) being most common (Benedict, 1978). *Apochthonius* is a species rich genus, with twenty-four described species. Most species of *Apochthonius* have a type locality of southeastern, western, or northwestern United States (Harvey, 2011). *A. occidentalis* inhabits the leaf litter and duff, moss, and rotting wood of the wet temperate forests of western Oregon. They are much less common in the xerophytic forests east of the summit of the Cascade Range (Benedict, 1978), but have been reported from California, New Mexico, Oregon, and Washington (Benedict, 1978).

The less common *Apochthonius minimus* (Schuster, 1966) is the only non-troglobitic congener to *A. occidentalis* known to occur in Oregon. *A. minimus* have been reported from Washington (Schuster, 1966), Oregon (Benedict, 1978), and British Columbia (Buddle, 2005). The two species are quite similar morphologically, distinguished only by slightly differing coxal spines (Fig 1) – a character that is not discussed in the description of *A. minimus* (Schuster, 1966). The coxal spines of *A. minimus* reportedly have an anterior projection, which varies greatly in size, while *A.*

occidentalis may have no projection, or a "very tiny" anterior projection (Benedict, 1978). Additionally, *A. minimus* tends to be about a third smaller than *A. occidentalis* where they co-occur (Benedict, 1978).

Habitat fragmentation events could impact lineages of *Apochthonius* by isolating populations from one another. These species are very small (1-2mm) and not known to practice phoresy – travel on other arthropods. They disperse solely by walking, and thus could require thousands of years to recolonize formerly occupied areas. That slow recolonization rate could lead to especially long periods of isolation and little or no gene flow between populations after vicariance events.

Work on other pseudoscorpion lineages has shown patterns of strong genetic differentiation with little if any detectable morphological variation (Wilcox *et al.*, 1997; Heerden *et al.*, 2013). Benedict (1978) stated that a redescription of *A. occidentalis* was in preparation, including notes on intraspecific variation, but there is no record of that manuscript in the literature. After extensive examination of hundreds of specimens of *Apochthonius*, Benedict further indicated that most non-cavernicolous species needed redescription, explicitly naming *A. minimus*. Neither species studied here has been redescribed, nor have their phylogenetic relationships been assessed. This study will investigate phylogenetic relationships among populations of both *A. occidentalis* and *A. minimus* in the Pacific Northwest, with an emphasis on populations in Oregon west of the Cascade Range.

2. Materials and methods

2.1 Taxon sampling and identification

We broadly sampled the habitats in which *Apochthonius* is known to occur in the Pacific Northwest. Sample sites were chosen based on the preference of *A. occidentalis* for shady, wet forests west of the Cascade Range (Benedict, 1978). One-gallon samples of leaf litter, moss, and rotting wood were collected in zip-close bags and local vegetation data and GPS coordinates were recorded. These samples were subsequently placed on Berlese funnels for a maximum of 48 hours, which extracted invertebrates, euthanizing them in 95% EtOH. Pseudoscorpions were separated from other invertebrates under a microscope, and identified following Chamberlin (1929), Benedict (1978), and Harvey (1992). Further examination of *A. occidentalis* and *A. minimus* in reference to the original species descriptions (Chamberlin, 1929; Schuster, 1966) was performed, and voucher photographs were taken. All pseudoscorpions were stored in 95% EtOH at -80°C prior to DNA extraction, and those not used in this study are stored in the invertebrate collection at Portland State University.

2.2 DNA extraction, PCR, sequencing

We used the DNeasy blood and tissue kit (Qiagen, Valencia, CA) for all DNA extractions, following the manufacturer's protocols. Many specimens were crushed during extraction, others were lysed following Boyer et al. (2005), with the entire animal immersed in lysis buffer and left to dissolve overnight at a lower

temperature, enabling retention of the cuticle as a voucher after DNA extraction. At least one voucher cuticle was retained from each clade that was resolved in the phylogenetic analyses that follow. Remaining DNA is stored at -80°C, and cuticles are in 95% EtOH at -4°C at Portland State University.

A portion of the mitochondrial protein coding gene cytochrome c oxidase subunit I (COI) was amplified via polymerase chain reaction. Pseudoscorpion-specific primers PsdCOI-LR (TAAACTTCAGGATGACCAAAAAATCA) and PsdCOI-UF (CTACTAATCATAAAGATATTGGAAC) were designed based on preliminary data acquired with HCO-2198 and LCO-1490 from Vrijenhoek (1994). For amplification, 3µl of template DNA was used in each reaction with 1µl each primer at 10mM, 18.5µl DD H_2O, 2.5µl 10X PCR buffer (100mM Tris-HCl pH 8.3, 500mM KCl, 15mM $MgCl_2$, 0.01% w/v gelatin), 1µl 25mM $MgCl_2$, 1µl 10mM dNTPs, and 0.1µl Taq polymerase (5 U/µl). The reaction ran on a DYAD DNA Engine thermal cycler for 35 cycles, each consisting of a 30s denaturing phase at 94°C, a 45s annealing phase at 51°C, and a 60s extension phase at 72°C, with a final extension of 72°C for 7min. PCR cleanup was carried out using either QIAquick PCR purification kit (Qiagen, Valencia, CA) in accordance with the manufacturer's protocol, or enzyme digestion by mixing 2.5µl PCR product per 1µl ExoSAP-IT (Affymetrix, Santa Clara, CA).

Sanger sequencing reactions consisted of 30 cycles, each with a 10s denaturing phase at 96°C, 5s annealing phase at 50°C, and a 4min extension phase at 60°C. Each 10µl reaction contained 1.5µl 5x sequencing buffer (400 mM Tris pH 9.0, 10 mM MgCl2), .33µl 10µM primer PsdCOI-LR, 1µl Big Dye, 3-4µl PCR product, and H_2O to

volume. The sequences were cleaned via isopropanol precipitation, and dried pellets sent to Oregon Health & Science University for generation of chromatograms with quality readings. Chromatograms and sequences were edited by eye for call accuracy using the program Sequencher v. 5.3 (Gene Codes Corporation, Ann Arbor, MI).

2.3 Phylogenetic analysis

Forty-one *Apochthonius* specimens (Table 1) were included in the phylogenetic analysis. *Kleptochthonius sp.* was chosen as outgroup and the sequence obtained from GenBank (accession #: EU559518.1) because it is the hypothesized sister taxon to *Apochthonius* (Murienne *et al.*, 2008). A maximum likelihood (ML) tree was built with the program SeaView v.4.5.3 (Gouy *et al*., 2010). SeaView is an open source phylogeny program that uses the programs Clustal Ω to drive sequence alignment and PhyML v.3.1 to compute maximum likelihood trees. The Hasegawa-Kishino-Yano, 85 (HKY85) model of evolution was used initially in the study, as it is very similar to the model determined most appropriate for another pseudoscorpion taxon by Zeh *et al*. (2003). HKY85 is a substitution model that distinguishes between the rate of transitions and transversions and accounts for unequal base frequencies (Hasegawa *et al.*, 1985). We also used jModelTest to test the accuracy of the model. A model of evolution corresponding to GTR+I+G was suggested, and trees were constructed with both. Tree topology search of Nearest Neighbor Interchange (NNI) was selected with a starting tree of BioNJ. Ts/Tv ratio was fixed at 4.00. Nucleotide

equilibrium frequencies were set to "empirical." We performed 1000 bootstrap replicates to assess the level of support for the recovered clades.

2.4 Calculations of genetic diversity

Six sequence sets were chosen based on sampling locations and information gathered in a preliminary phylogenetic analysis. Genetic structure in early analyses informed the decision to separate the Northern Oregon clade into North Coast Range, Portland, and Cascades. Northern California and Central Coast constituted the remaining *A. occidentalis* populations, and *A. minimus* was considered one population.

I calculated measures of genetic diversity within and between populations. Pairwise distances between populations were calculated using MEGA v. 6.06 for MacOS (Tamura *et al*., 2013). To determine gene flow between populations, pairwise F_{ST} values were calculated using DnaSP v. 5.10.1 (Librado and Rozas, 2009), as well as genetic diversity measures within each population—sample size, distinct haplotypes, haplotype diversity, average number of differences, and genetic divergence with a Jukes Cantor correction.

2.5 Molecular clock dating

We used two molecular clocks to estimate a range of divergence times between populations; the widely accepted substitution rate of 2.3% per million years (Brower, 1994), and a rate of 3.54% per million years (Papadopoulou *et al.*, 2010), which was calibrated for insects across the Aegean Trench. These rates were chosen

in the absence of a reliable molecular clock for arachnid mtDNA, and are likely to provide dates of divergence that underestimate substitution rates. Dates of divergence were calculated by dividing the percent divergence between clades by each of the molecular clock rates to obtain an estimate in millions of years.

3. Results

3.1 Samples

We sequenced portions of the mitochondrial gene COI from forty-one specimens (Table 1) from western Oregon, northwestern California, and the Olympic Peninsula in Washington. Thirty-nine of the specimens were initially identified as *A. occidentalis,* but two of those individuals placed with the *A. minimus* clade and, upon further examination, were determined to be *A. minimus.* Total sequence length obtained was 688 nucleotides. Because some of the earlier sequences had shorter high quality regions, excluding alignment gaps left 179 sites, of which 76 were polymorphic and 52 were parsimony informative, defining 28 haplotypes.

3.2 Phylogenetic analyses

The two models of evolution yielded the same tree topology, so the simpler of the two, HKY85, was used. The Maximum Likelihood analysis produced a single most likely tree (–ln likelihood = -4365.3; Fig. 2). Four clades were discriminated in the tree, but largely with low support. Nevertheless, these will be considered clades due to consistent placement of taxa in repetitive trials of tree building with increasing sample size. The basal node separates the northern and southern portions of the sampling range with low bootstrap support. Many of the shallower nodes on the tree are well supported, while deeper nodes are consistently lacking in support. The Northern Oregon clade contains all of the sequences from North Coast Range, Portland, and Cascades, and is sister to the Central Oregon Coast, Northern

California, and *A. minimus* clades. The Northern California clade is sister to the Central Oregon Coast and *A. minimus* clades. *A. minimus* was resolved as one clade after reassignment of the two above-mentioned individuals, and this clade fell within the larger clade of *A. occidentalis,* rendering *A. occidentalis* paraphyletic.

A. minimus is widely dispersed across the sampling range, while the three *A. occidentalis* clades suggest geographic structuring (Fig. 2). There is a northwest-southeast break (Fig. 3) for all but *A. minimus*, resulting in one northern and two southern clades from the *A. occidentalis* sequences. *A. minimus* is embedded in the southern branch. There is geographic overlap (one individual) between the Northern California and Central Oregon Coast clades.

3.3 Calculations of genetic diversity

Measures of genetic diversity are reported in Table 2. Haplotype and nucleotide diversity were high overall (0.98 and 0.097, respectively). The lowest haplotype diversity was in the Portland population (0.80), which had a sample size of ten and five distinct haplotypes. This was also the population with the lowest diversity (.06) and the fewest average differences (10.38). Conversely, the North Coast Range and Cascades populations had the highest haplotype diversity (1.0). In these two populations, every individual had a distinct haplotype.

Pairwise F_{ST} values are quite high for most population relationships (Table 3). The North Coast Range and Portland populations appear to be panmictic (F_{ST} = 0.002), but exhibit high divergence, with a pairwise distance of 0.071. Northern California

and North Coast Range have the next lowest gene flow (F_{ST} = 0.033). The F_{ST} values for the remainder of relationships are greater that 0.15, with one, *A. minimus* vs. Portland, reaching 0.42. Pairwise distances are at or above 0.08 for all but North Coast Range vs. Portland and Cascade Range vs. Portland (0.071 and 0.074, respectively).

3.4 Molecular clock dating

Using the two insect-based rates of substitution , the divergence at the basal node of the *Apochthonius occidentalis* tree inferred to range from 3.6-5.6My in age. This separates the northern populations of *A. occidentalis* from all other sequences analyzed in this study. The remaining nodes range from 2.1-3.5My using the faster substitution rate to 3.2-5.3My with the slower substitution rate. The north-south split in the *A. minimus* clade is inferred to have occurred 2.2-3.3Mya. These divergence estimates predate Pleistocene disturbances.

4. Discussion

4.1 Phylogeographic patterns in Pacific Northwest Apochthonius *species and comparison with other flora and fauna of the region.*

Mitochondrial sequence data identified four poorly supported but consistent clades of *Apochthonius*. The deepest node divides northern and southern populations near 44° latitude, roughly the halfway point between Washington and California in Oregon. This position is consistent with the southern edge of the Willamette Valley and the Siuslaw River. Gene flow between populations was estimated using pairwise F_{ST}. Values ranged from 0.002 between North Coast Range and Portland to 0.42 between *A. minimus* and Portland. All but one of the values for other population pairs were over 0.15, indicating that there is very little gene flow between the populations.

Pairwise divergence values ranged from 7.4% between Cascade Range and Northern California to 12.8% between Cascade Range and Central Oregon Coast populations. These estimates are similar to the only other invertebrate with low dispersal capacity that has been similarly studied in the region, the slug *Prophysaon coeruleum*, which has a range of genetic divergence of *ca.* 7% to 26% (Wilke & Duncan, 2004). For comparison, the widely-dispersed spruce beetle, *Dendroctonus rufipennis,* has 3-4% divergence between populations (Maroja *et al.*, 2007), and the Rocky Mountain sky island grasshopper, *Melanoplus oregonensis*, sequence divergence ranged from 0.71-3% (Knowles, 2001). Other pseudoscorpions sampled

genetically yield results similar to *Apochthonius*. *Cordylochernes scorpioides* in Central and South America, for instance, has a range of divergence from *ca.* 4-27% (Zeh *et al.*, 2003). In South Africa, the genus *Horus* displays *ca.* 14-39% divergence between closely related lineages (Heerden *et al.*, 2013). Interestingly, neither divergence nor gene flow estimates in *Apochthonius* were consistently higher between congeners than conspecifics; *A. occidentalis* appears to be as divergent between populations as it is from *A. minimus.*

The genetic architecture found in this study is consistent with other fauna of the Pacific Northwest, which also largely exhibit a distinct north-south genetic architecture. For example, cytochrome b sequences analyzed from the red tree vole, *Phenacomys longicaudus* (Miller *et al.*, 2006), mitochondrial control region sequences from the spotted owl, *Strix occidentalis* (Barrowclough *et al.*, 1999), and cytochrome b and random amplified polymorphic DNA sequences from six Pacific Northwest salamanders (Wagner, 2000) all presented north-south breaks at varying latitudes near a boundary referred to as the Soltis line (Brunsfeld *et al.*, 2007). This boundary lies at roughly 44° latitude. Many taxa also exhibit an east-west split when populations from the Cascade Range and Rocky Mountains are analyzed (Carstens *et al.*, 2005), but our study range does not extend west to the Rockies. These shared patterns of genetic differentiation have been largely attributed to populations retracting during glacial maxima and related climatic changes. Estimated divergence times for lineages of *Apochthonius*, however, clearly predate Pleistocene glaciation.

4.2 Molecular clock dating of Apochthonius *divergences*

Lacking a reliable molecular clock for pseudoscorpion mtDNA, we cannot confidently rule out any dates of divergence. The pseudoscorpion mt genome exhibits high levels of gene rearrangement in the two taxa whose mt genome has been sequenced (Ovchinnikov & Masta, 2012). Some unusual patterns emerged in the aforementioned study, including rearrangement of protein coding genes and some genes moving to the opposite strand from the ancestral arrangement. This may contribute to a faster rate of evolution in pseudoscorpion mitochondria. Nonetheless, for the divergences calculated in our study to align with Pleistocene glaciation, and subsequent xerification and repetitive flooding of the Willamette Valley, *Apochthonius* would have to have a rate of evolution of 85.7% per million years. As this is highly unlikely, we must accept the results from the standard molecular clocks for the time being, and look for correlations with geologic and climatic disturbances of the times estimated.

Our estimates place all divergences between 2.1 and 5.6Mya, encompassing the Pliocene and the very beginning of the Pleistocene. The Pliocene was a time of great shifts in climate, with drastically increased atmospheric carbon dioxide first causing a warming of the planet, then the closing of the Isthmus of Panama (*ca.* 3Mya) leading to a drastic cooling as the warmer waters of the Atlantic could no longer mix with the colder Pacific Ocean (Bishop, 2003). This sparked the beginning of the last ice age. Around the same time, in Oregon, many small volcanic eruptions took place across the Portland Basin and the Cascade foothills to the north and west of Mount

Hood. The resulting lava flows and habitat fragmentation are more likely the drivers of the sequence evolution we see in *Apochthonius.* As pseudoscorpions have low vagility, it seems the populations of *Apochthonius* that separated during the Pliocene and early Pleistocene did not recolonize quickly enough to reflect those later Pleistocene disturbances in their genomes. More comprehensive sampling could, however, lead to evidence of more recent divergence within populations.

4.3 Multiple species?

Several concepts have been proposed for determining species boundaries. Most concepts focus on a single most important requirement to define a species. The unified species concept (de Queiroz, 2007), however, identifies species simply as separately evolving lineages. Some of the sample populations of *A. occidentalis* appear to fulfill this requirement with high divergence and low gene flow. With no opportunity to interbreed, these lineages are becoming separated. The level of geographic structuring seen within the three *A. occidentalis* clades suggests the presence of three species or more rather than one. A divergence range of 7% - 12.8% is high for populations considered to belong to one species. Further, the presence of higher divergence between two populations of *A. occidentalis* than between certain populations of *A. occidentalis* and *A. minimus*, and higher gene flow between *A. minimus* and some *A. occidentalis* populations indicates that either *A. occidentalis* is actually multiple species, or *A. minimus* should be revised and considered *A. occidentalis.*

There are no obvious morphological characters that separate these clades (See appendix). We measured length and width of three palp segments and three other regions of the body and compared these across populations using simple boxplots. All populations showed overlap in every trait measured, including ratios of length to width in the palpal femur – a character often used in pseudoscorpion description and identification. Discreet morphological characters such as chelal and cheliceral dentition, epistome shape, or chaetotaxy (positioning of setae) may prove more useful in delimiting these species.

Cryptic speciation could take place in taxa that are in morphological stasis – under no selection pressure for morphological change. It is possible that if characters are under selection, they are undetectable by our methods. Pseudoscorpions are highly chemosensory organisms, with many setae and complex mouthparts. Fine differences in these features could drive speciation, as they are traits related to finding mates and food. Unlike most other arachnids, genitalia are not considered in the description or identification of the *Apochthonius* species discussed here, though they do possess complex genitalia and mate using spermatophores. Size and structure of spermatophore could be a diagnostic feature, but determining this would require keeping live specimens, which can be quite difficult and costly.

Conclusions

Geologic events may have shaped the dispersal of *Apochthonius* such that populations, specifically northern and southern, have been isolated long enough to undergo lineage separation. More rigorous sampling to the edges of the range of both species could determine whether these patterns hold further north and south. Alleviating gaps in sampling could lend new evidence about gene flow between these populations. The Northern Oregon clade is comprised of all sampled populations from the Cascades to the North Coast Range. This was the region with the most continuous sampling scheme. Had the southern region been more continuously sampled, the tree topology in southern clades may have been quite different. The placement of *A. minimus*, with its broad dispersal, as sister to the Central Oregon Coast clade opens up a new set of inquiries for further investigation. A larger sample size for *A. minimus* could unveil geographic structuring that remains undetected in our study.

As we have analyzed only a single mitochondrial gene, some speculation is in order. Additional markers and morphological features could provide other sources of data to further support lineage separation within *Apochthonius*. Bootstrap support is exceedingly low for much of the observed topology, specifically at deeper nodes. Low bootstrap support is not entirely unexpected, as we are studying closely related species and conspecifics. However, tree topology was consistent each time a new tree was built. Adding more sequences increased bootstrap support at many nodes, thus increasing sample size may lead to a better-supported tree.

While these data provide a clue that multiple species are described under *A. occidentalis,* further sampling and investigation are required. There is a clear north-south break in the populations sampled, which, though older, coincides with similar structuring in other organisms in the region. This work could provide a solid foundation on which to expand, ultimately leading to description of new *Apochthonius* species.

Future Directions

This research represents the beginning steps in creating a full picture of *Apochthonius* evolution in the Pacific Northwest. A great many questions remain unanswered, but we have set the foundation for future work. The next logical step will be to examine the morphology of specimens from each of these genetic clades and geographic populations. Any of a number of morphological characters could separate these lineages; chelal and cheliceral dentition, chaetotaxy, mouthpart morphology, epistome structure, and genitalia should all be studied in a systematic fashion. Should these return no patterns of variation, the species described from this work will be cryptic.

There is also a need to fill sampling gaps and to determine the boundaries of distribution for these species of *Apochthonius* in the Pacific Northwest. Specifically, sampling the southern Cascade Range and Coast Range, as well as the northern Cascades and the Olympic Peninsula, will provide the data needed to understand movement of *Apochthonius* during and after historical disturbances. Sampling the Rocky Mountains would add important information to Pacific Northwest phylogeography research on the whole, as low-dispersing organisms will provide a clearer picture of both long past and more recent separation events. There is at least one report of *A. occidentalis* in New Mexico (Benedict, 1978), and this should be investigated. Given the genetic differentiation across the range of our samples, it is unlikely that a New Mexico *Apochthonius* is the same species as a Pacific Northwest *Apochthonius.*

Tables and Figures

Table 1: Specimens included in our analyses and their locations. Specimens for which a cuticle was retained as a voucher are indicated.

Coll. Number	Species	County	State	Latitude	Longitude	Population	Cuticle
SEM2418	*A. occidentalis*	Clackamas	OR	45.40740	-121.78560	Cascades	
SEM2469	*A. occidentalis*	Multnomah	OR	45.50270	-122.69050	Portland	
SEM2478	*A. occidentalis*	Lane	OR	44.04867	-122.21557	Cascades	
SEM2495	*A. occidentalis*	Multnomah	OR	45.47500	-122.71799	Portland	
SEM2516	*A. occidentalis*	Tillamook	OR	45.76546	-123.96934	N. Coast	
SEM2537	*A. occidentalis*	Douglas	OR	43.66189	-123.48974	Cen. Coast	
SEM2553	*A. occidentalis*	Del Norte	CA	41.80951	-124.04618	N. CA	x
SEM2555	*A. occidentalis*	Del Norte	CA	41.80951	-124.04618	N. CA	
SEM2632	*A. occidentalis*	Del Norte	CA	41.92832	-124.08091	N. CA	
SEM2634	*A. occidentalis*	Del Norte	CA	41.92832	-124.08091	N. CA	
SEM2660	*A. minimus*	Jackson	OR	42.16817	-122.67905	*A. min.*	
SEM2661	*A. minimus*	Jackson	OR	42.16817	-122.67905	*A. min.*	x
SEM2663	*A. minimus*	Jackson	OR	42.16817	-122.67905	*A. min.*	
SEM2682	*A. occidentalis*	Clatsop	OR	45.88626	-123.61641	N. Coast	
SEM2701	*A. occidentalis*	Del Norte	CA	41.81479	-124.08615	N. CA	
SEM2702	*A. occidentalis*	Del Norte	CA	41.81479	-124.08615	N. CA	
SEM2703	*A. occidentalis*	Del Norte	CA	41.81479	-124.08615	N. CA	x
SEM2710	*A. occidentalis*	Del Norte	CA	41.81008	-124.04643	N. CA	x
SEM2719	*A. minimus*	Jefferson	WA	47.59916	-123.16220	*A. min.*	
SEM2721	*A. minimus*	Jefferson	WA	47.59916	-123.16220	*A. min.*	x
SEM2741	*A. occidentalis*	Del Norte	CA	41.80909	-124.04662	N. CA	
SEM2841	*A. occidentalis*	Lincoln	OR	44.93367	-123.85184	Cen. Coast	
SEM2882	*A. minimus*	Clatsop	OR	45.50560	-123.45580	*A. min.*	
SEM2882	*A. occidentalis*	Clatsop	OR	45.50560	-123.45580	N. Coast	
SEM2904	*A. occidentalis*	Tillamook	OR	45.77023	-123.97069	N. Coast	
SEM2921	*A. occidentalis*	Multnomah	OR	45.50220	-122.69320	Portland	
SEM2922	*A. occidentalis*	Multnomah	OR	45.50220	-122.69320	Portland	
SEM2923	*A. occidentalis*	Multnomah	OR	45.50220	-122.69320	Portland	
SEM3053	*A. occidentalis*	Multnomah	OR	45.53639	-122.21861	Portland	
SEM3072	*A. occidentalis*	Hood River	OR	45.60278	-121.88000	Cascades	
SEM3094	*A. occidentalis*	Hood River	OR	45.60194	-121.87944	Cascades	
SEM3106	*A. occidentalis*	Linn	OR	44.40972	-122.05833	Cascades	
SEM3136	*A. occidentalis*	Multnomah	OR	45.53275	-122.71498	Portland	
SEM3137	*A. occidentalis*	Multnomah	OR	45.53275	-122.71498	Portland	
SEM3138	*A. occidentalis*	Multnomah	OR	45.53275	-122.71498	Portland	x
SEM3180	*A. occidentalis*	Multnomah	OR	45.60367	-122.81976	Portland	
SEM3202	*A. occidentalis*	Clatsop	OR	45.82780	-123.77857	N. Coast	x
SEM3219	*A. occidentalis*	Linn	OR	44.34352	-122.98692	Cascades	x
SEM3235	*A. occidentalis*	Douglas	OR	43.61893	-124.18076	Cen. Coast	
SEM3243	*A. occidentalis*	Coos	OR	43.37080	-124.22994	Cen. Coast	
SEM3269	*A. occidentalis*	Coos	OR	43.61893	-124.18076	Cen. Coast	x
SEM3402	*A. occidentalis*	Lane	OR	43.92079	-124.11164	Cen. Coast	

Table 2: Gene flow and genetic differentiation within each population as represented by sample size (N), distinct haplotypes (H), haplotype diversity (H_d), average number of differences (K), and nucleotide divergence with a Jukes Cantor correction (PiJC).

Population	**N**	**H**	**H_d**	**K**	**PiJC**
Northern California	9	5	0.8889	12.72	0.07599
Central Coast	6	4	0.8667	18.27	0.11165
North Coast	4	4	1	14.83	0.08825
Portland	10	5	0.8	10.38	0.06178
A. minimus	6	4	0.8667	15.2	0.09217
Cascades	6	6	1	12	0.07045

Table 3: Genetic differentiation between populations of *Apochthonius.* Pairwise F_{ST} is shown above diagonal and pairwise-distance below.

	1	2	3	4	5	6
1 Northern California	-	0.28731	0.03314	0.19356	0.32203	0.25168
2 Central Coast	0.121	-	0.22118	0.30362	0.26176	0.34203
3 North Coast	0.08	0.119	-	0.00154	0.29883	0.16796
4 Portland	0.08	0.115	0.071	-	0.41603	0.15978
5 *A. minimus*	0.115	0.127	0.12	0.122	-	0.32469
6 Cascades	0.092	0.128	0.09	0.074	0.113	-

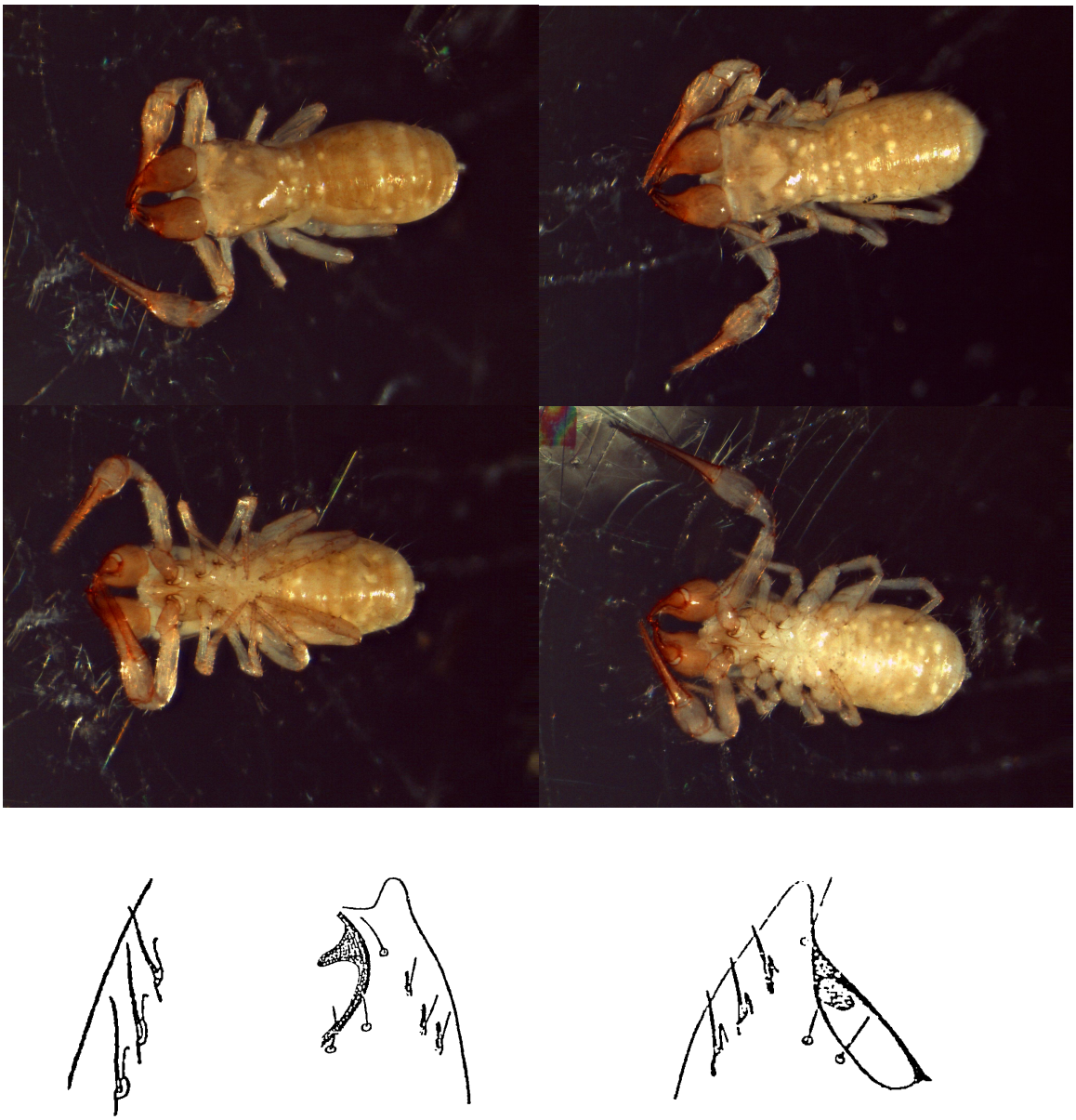

Figure 1: *A. occidentalis* (left) and *A. minimus*. Dorsal view of these two species is shown at top, ventral in center row, and comparison of coxal spines in the key to pseudoscorpions of Oregon (Benedict, 1978) at bottom. Note the very slight differences in coxal spine morphology as depicted in the key, and the extreme similarity of these two congeners in dorsal and ventral views.

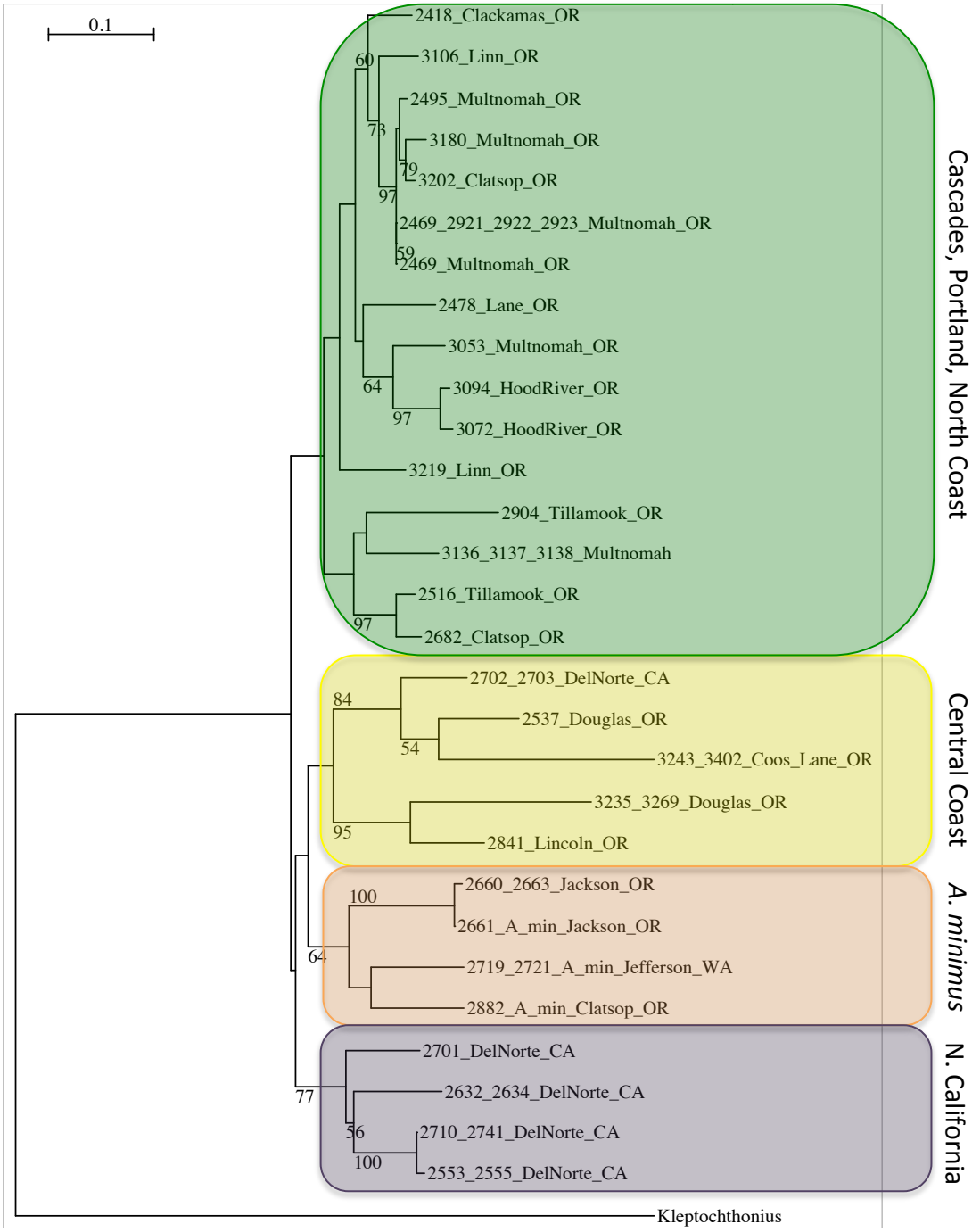

Figure 2: Maximum likelihood tree constructed with 1000 replicates (-ln(L) = -4365.3). Note four distinct clades, and the embedding of what is described as *A. minimus* (orange) within what is described as *A. occidentalis* (all others). Clades are vertically labeled with their names. Bootstrap values greater than 50 are next to nodes. See Fig. 3 for geographic distribution of these clades.

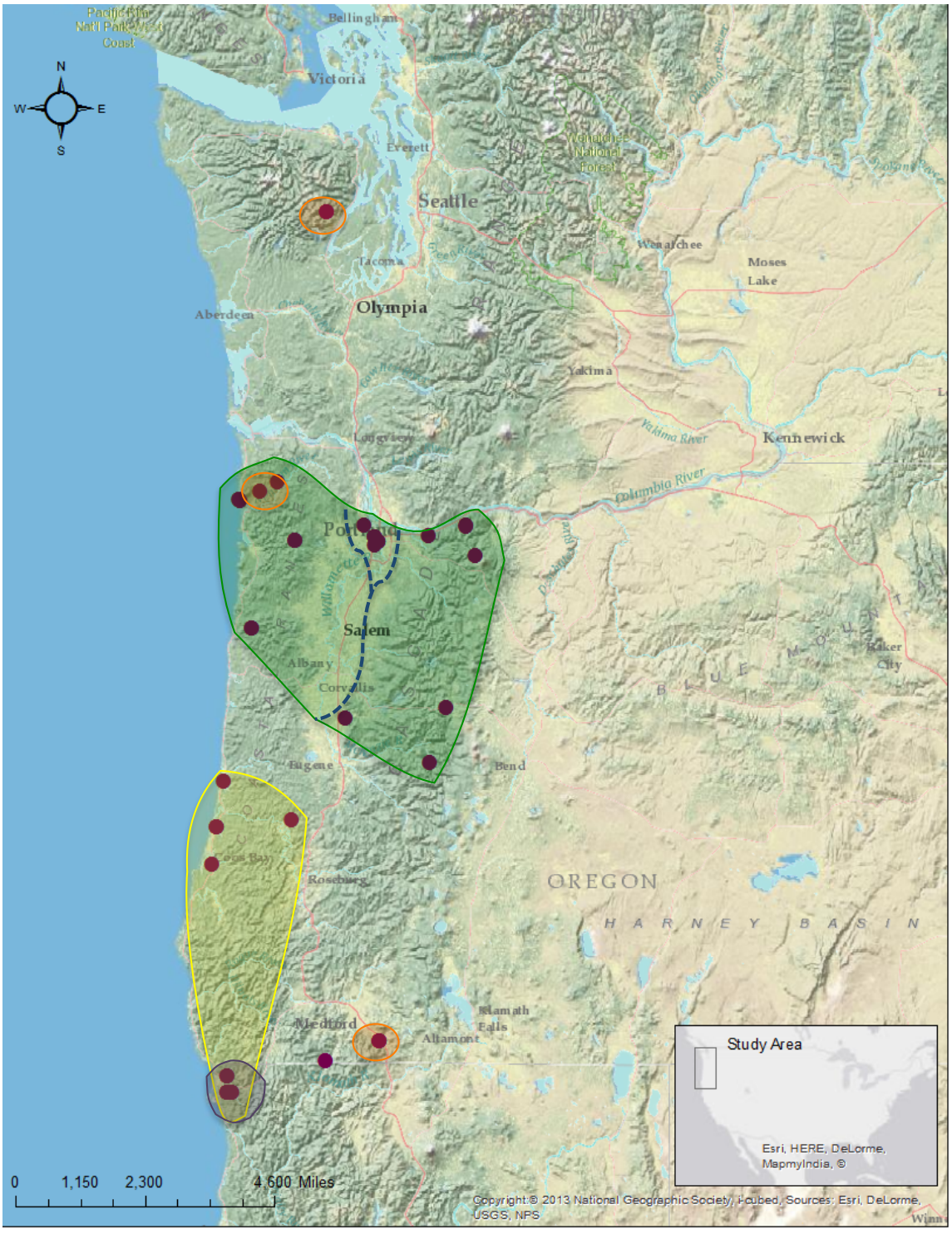

Figure 3: Collection map of *Apochthonius* specimens used in phylogenetic analysis, with clades (Fig. 1) highlighted in their corresponding colors. Geographic overlap between the Del Norte and Central Coast clades occurs in one individual collected at a site where both clades are represented – in the more southern of the Del Norte clade's sampling sites. Boundaries for separating populations in the Northern Oregon clade are indicated by the dashed line

Literature cited

Barrowclough, G., Gutierrez & Groth, J. 1999. Phylogeography of spotted owl (*Strix occidentalis*) populations based on mitochondrial DNA sequences: gene flow, genetic structure, and a novel biogeographic pattern. *Evolution, 919-931.*

Benedict, EM. 1978. A biogeographical study of currently identified Oregon pseudoscorpions with an emphasis on western Oregon forms.

Bishop, EM. 2003. In search of ancient Oregon: a geological and natural history.

Boyer, SL, Karaman, I & Giribet, G. 2005. The genus Cyphophthalmus (Arachnida, Opiliones, Cyphophthalmi) in Europe: a phylogenetic approach to Balkan Peninsula biogeography. *Molecular Phylogenetics and Evolution, 36(3), 554-567*.

Brunsfeld, SJ, Miller, TR & Carstens, BC. 2007. Insights into the biogeography of the Pacific Northwest of North America: evidence from the phylogeography of *Salix melanopsis*. *Systematic Botany*.

Brunsfeld, SJ, Sullivan, J & Soltis, DE. 2001. Comparative phylogeography of northwestern North America: a synthesis. *Special Publication-British Ecological Society, 14, 319-340.*

Buddle, C. 2005. A primer on pseudoscorpions and taxonomic status in Canada. *Newsl. Biol. Surv. Canada*.

Cartens, B. C., Brunsfeld, S. J., Demboski, J. R., Good, J. M., & Sullivan, J. 2005. Investigating the evolutionary history of the pacific northwest mesic forest ecosystem: hpotehesis testing within a comparative phylogeographic framework. *Evolution*, *59*(8), 1639-1652.

Chamberlin, JC. 1929. V.—A synoptic classification of the false scorpions or chela-spinners, with a report on a cosmopolitan collection of the same.—Part I. The Heterosphyronida (Chthoniidæ)(Archnida-Chelonethida). *Journal of Natural History*, *4*(19), 50-80.

Chamberlin, J.C. (1931). The arachnid order Chelonethida. Stanford University Publication of Biological Science 7: 1-284.

de Queiroz, K. 2007. Species concepts and species delimitation. *Systematic Biology, 56(6), 879-886.*

Godbout, J, Fazekas, A, Newton, CH & Yeh, FC. 2008. Glacial vicariance in the Pacific Northwest: evidence from a lodgepole pine mitochondrial DNA minisatellite for multiple genetically distinct and widely separated refugia. *Molecular Ecology*, *17*(10), 2463-2475.

Harvey, MS. 2011. Pseudoscorpions of the World, version 2.0. Western Australian Museum, Perth.http://www.museum.wa.gov.au/catalogues/pseudoscorpions

Hasegawa, M., Kishino, H. & Yano, T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of molecular evolution, 22(2), 160-174.*

Heerden, J., Taylor, P. & Heerden, C. 2013. Genetic Differentiation in Horus Chamberlin (Arachnida: Pseudoscorpiones: Olpiidae) as Indicated by Mitochondrial DNA Analysis. *African Zoology, 48(2), 351-358.*

Hoff, CC. 1949. The pseudoscorpions of Illinois. *Illinois Natural History Survey Bulletin; v. 024, no. 04.*

Hoff, CC & Bolsterli, JE. 1956. Pseudoscorpions of the Mississippi River drainage basin area. *Transactions of the American Microscopical Society, 155-179.*

Hoff, C.C. 1958. List of the pseudoscorpions of North America north of Mexico. American Museum novitates; no. 1875. New York, NY: American Museum of Natural History.

Knowles, L. L. 2001. Did the Pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshopprers. *Molecular Ecology*,*10*(3), 691-701.

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

Maroja, L. S., Bogdanowicz, S. M., Wallin, K. F., Raffa, K. F., & Harrison, R. G. 2007. Phylogeography of spruce beetles (Dendroctonus rufipennis kirby)(Curculionidae: Scolytinae) in North America. *Molecular Ecology*, *16*(12), 2560-2573.

Mayden, R. L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In M. F. Claridge, H. A. Dawah & M. R. Wilson (eds.), Species: The units of diversity. Chapman and Hall 381–423.

Mayr, E. 1942. *Systematics and the origin of species, from the viewpoint of a zoologist*. Harvard University Press.

Miller, M. P., Bellinger, M. R., Forsman, E. D., & Haig, S. M. 2006. Effects of historical climate change, habitat connectivity, and vicariance on genetic structure and diversity across the range of the red tree vole (Phenacomys longicaudus) in the Pacific Northwestern United States. *Molecular Ecology*,*15*(1), 145-159.

Muchmore, WB. 1974. Pseudoscorpions from Florida. 3. Epactiochernes, a new genus based upon Chelanops tumidus Banks (Chernetidae). *Florida Entomologist*.

Muchmore, W. B. 1990. Pseudoscorpionida. *Soil Biology Guide. (DL Dindal ed.). John Wiley and Sons, New York*, 503-527.

Muchmore, WB & Benedict, EM. 1976. Redescription of Apochthonius moestus (Banks), type of the genus Apochthonius Chamberlin (Pseudoscorpionida, Chthoniidae). *Journal of the New York Entomological Society, 67-74.*

Murienne, J., Harvey, M.S. & Giribet, G. 2008. First molecular phylogeny of the major clades of Pseudoscorpiones (Arthropoda: Chelicerata). *Molecular phylogenetics and evolution* 49: 170–184. Elsevier.

Nelson, S. Jr. 1975. A systematic study of Michigan Pseudoscorpionida (Arachnida). *American Midland Naturalist, 257-301.*

Nielson, M., Lohman, K. & Sullivan, J. 2001. Phylogeography of the tailed frog (Ascaphus trui): Implications for the biogeography of the Pacific Northwest. *Evolution, 55(1), 147-160.*

Ovchinnikov, S., & Masta, S. E. (2012). Pseudoscorpion mitochondria show rearranged genes and genome-wide reductions of RNA gene sizes and inferred structures, yet typical nucleotide composition bias. *BMC evolutionary biology*,*12*(1), 31.

Papadopoulou, A., Anastasiou, I., & Vogler, A. P. (2010). Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution*, *27*(7), 1659-1672.

Schawaller, W, Shear, WA & Bonamo, PM. 1991. The first Paleozoic pseudoscorpions (Arachnida, Pseudoscorpionida). American Museum novitates; no. 3009.

Schuster, R.O. 1966. New Species of Apochthonius from Western North America. *The Pan-Pacific Entomologist* 42: 178–183.

Sequencher® version 5.3 sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA http://www.genecodes.com

Soltis, DE, Gitzendanner, MA & Strenge, DD. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution, 206(1-4), 353-373.*

Soltis, DE, Soltis, PS, Kuzoff, RK, & Tucker, TL. (1992). Geographic structuring of chloroplast DNA genotypes in Tiarella trifoliata (Saxifragaceae). *Plant Systematics and Evolution*, *181*(3-4), 203-216.

Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725-2729.

Veevers, JJ. 2004. Gondwanaland from 650–500 Ma assembly through 320 Ma merger in Pangea to 185–100 Ma breakup: supercontinental tectonics via stratigraphy and radiometric dating. *Earth-Science Reviews*, *68*(1), 1-132.

Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology, 3(5), 294-299.*

Wagner, RS. 2000. Phylogeography, evolution, and conservation in forest-associated Pacific Northwest salamanders. ir.library.oregonstate.edu

Wilcox, TP, Hugg, L, Zeh, JA & Zeh, DW. 1997. Mitochondrial DNA sequencing reveals extreme genetic differentiation in a cryptic species complex of neotropical pseudoscorpions. *Molecular Phylogenetics and Evolution, 7(2), 208-216.*

Wilke, T & Duncan, N. 2004. Phylogeographical patterns in the American Pacific Northwest: lessons from the arionid slug *Prophysaon coeruleum*. *Molecular Ecology, 13(8), 2303-2315.*

Zeh, J. A., Zeh, D. W., & Bonilla, M. M. (2003). Phylogeography of the harlequin beetle-riding pseudoscorpion and the rise of the Isthmus of Panamá. *Molecular Ecology*, *12*(10), 2759-2769.

Appendix

Morphological measurements of *Apochthonius occidentalis* and *A. minimus.*

We performed preliminary morphological analyses on Pacific Northwest specimens of *Apochthonius*, using some features that are commonly used in description and identification of the Chthoniidae. Groups were chosen based on preliminary phylogenetic analyses in conjunction with sampling location, following the same pattern as in Chapter 1. Length and width of the chelicerae, carapace, and abdomen were measured, as well as length and width of the palpal femur, patella, and chela, or palp (Fig. 4). These characters were chosen based on historical use of body and leg segment ratios in pseudoscorpion descriptions and dichotomous keys. Preliminary morphological analyses (performed with the statistical analysis program R through RStudio v. 0.98.1091) included simply constructing boxplots of measurements to compare data distribution across populations. These revealed no strong trends across populations of *A. occidentalis* and *A. minimus* (Figs. 5-10). While these measurements may not prove useful in differentiating populations, I am including them here for future use (Table 4). The full dataset is stored on the server for the Masta lab, Portland State University.

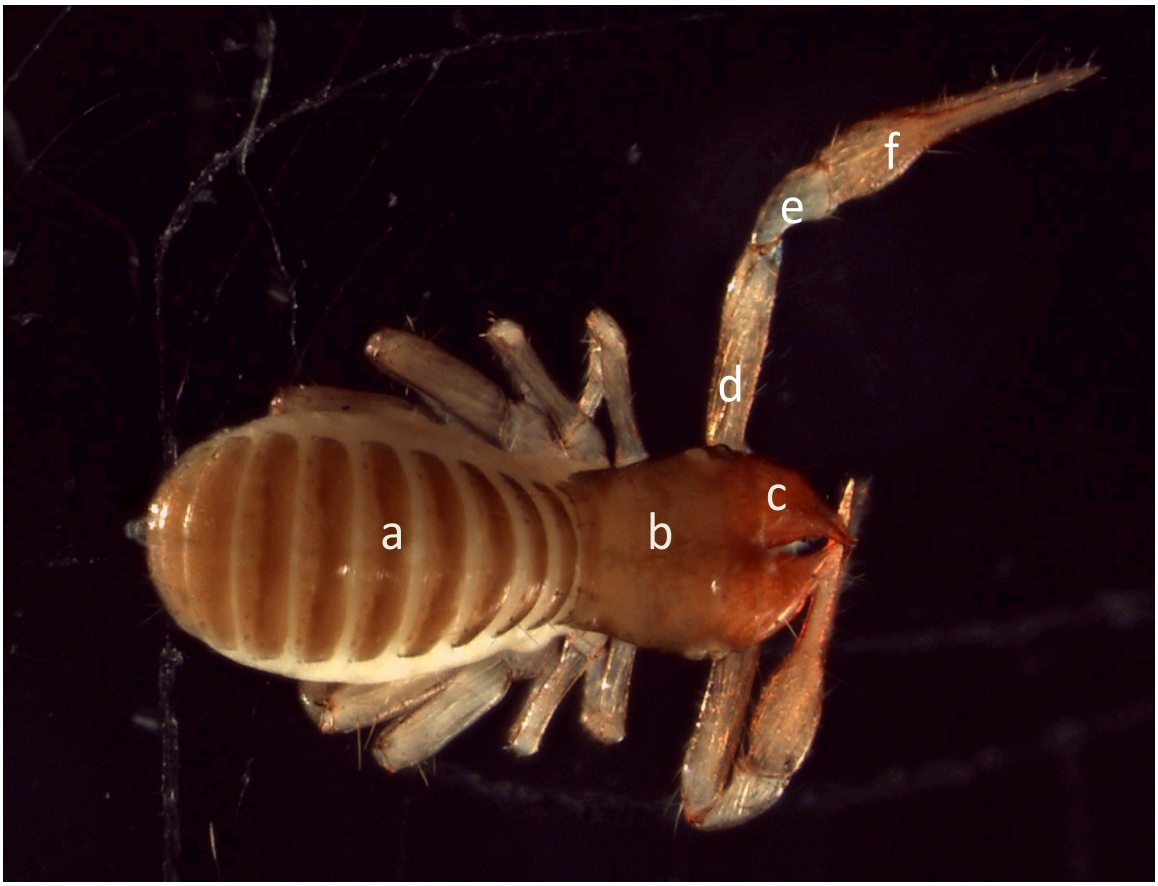

Figure 4: *A. occidentalis* at 40x with measured segments indicated – abdomen (a), carapace (b), chelicera (c), palpal femur (d), patella (e), palp (f).

Table 4: Morphological measurements of *Apochthonius* taken with a Leica EC3 camera at 40x magnification. Palpal segments were measured from a ventral aspect with palp extended, body regions and were measured from a dorsal aspect, with each specimen's anterior to the left. Palp measurements taken were the femur (Fem), patella (Pat), and chela (Che); body regions measured were the carapace (Car), abdomen (Abd), and chelicera (Cheli). Length (l) and width (w) were measured to obtain a ratio or l:w for analysis. Total length was calculated by adding abdomen and carapace length. Specimens used in the phylogenetic analysis (Chapter 1) are indicated, as these shaped the population assignment of all individuals. A dataset complete with GPS data for each specimen, elevation, and calculated ratios is stored on the server in the Masta lab at Portland State University. Table continued on pages 34 and 35.

SEM #	Species	County	Sex	Fem l	Fem w	Che l	Che w	Pat l	Pat w	Car l	Car w	Abd l	Abd w	Cheli l	Cheli w	Population	Phylo
2017	*occidentalis*	Clackamas	female	0.48	0.113	0.69	0.166	0.133	0.131	0.525	0.453	0.711	1.15	0.417	0.236	Cascades	
2388	*occidentalis*	Tillamook	male	0.395	0.111	0.65	0.122	0.124	0.089	0.389	0.389	0.918	0.484	0.284	0.178	North Coast	
2392	*occidentalis*	Tillamook	male	0.412	0.0967	0.68	0.136	0.178	0.11	0.477	0.391	0.96	0.521	0.351	0.183	North Coast	
2393	*occidentalis*	Tillamook	male	0.376	0.107	0.608	0.158	0.107	0.0912	0.399	0.412	0.941	0.499	0.284	0.168	North Coast	
2394	*occidentalis*	Tillamook	male	0.375	0.107	0.655	0.148	0.094	0.0972	0.447	0.413	0.829	0.516	0.332	0.203	North Coast	
2395	*occidentalis*	Tillamook	male	0.361	0.0986	0.658	0.125	0.138	0.0895	0.434	0.403	0.967	0.469	0.244	0.175	North Coast	
2396	*occidentalis*	Tillamook	male	0.385	0.0967	0.65	0.138	0.143	0.0967	0.44	0.405	1.03	0.516	0.315	0.184	North Coast	
2397	*occidentalis*	Tillamook	male	0.324	0.106	0.656	0.142	0.141	0.102	0.429	0.45	0.849	0.505	0.331	0.209	North Coast	
2398	*occidentalis*	Tillamook	male	0.42	0.0874	0.635	0.152	0.124	0.186	0.466	0.435	0.802	0.502	0.326	0.218	North Coast	
2399	*occidentalis*	Tillamook	male	0.43	0.108	0.659	0.16	0.109	0.115	0.428	0.421	0.788	0.515	0.338	0.203	North Coast	
2400	*occidentalis*	Tillamook	male	0.439	0.101	0.614	0.145	0.17	0.127	0.407	0.418	0.823	0.455	0.3	0.202	North Coast	
2401	*occidentalis*	Lane	male	0.491	0.0992	0.716	0.142	0.187	0.106	0.426	0.46	1.1	0.586	0.397	0.195	Cascades	
2418	*occidentalis*	Clackamas	male	0.395	0.0977	0.584	0.132	0.156	0.0952	0.331	0.409	0.678	0.5	0.318	0.191	Cascades	x
2482	*occidentalis*	Lane	female	0.378	0.0996	0.533	0.14	0.138	0.104	0.402	0.364	0.866	0.513	0.293	0.173	North Coast	
2484	*occidentalis*	Tillamook	female	0.342	0.113	0.586	0.16	0.139	0.0943	0.495	0.444	0.734	0.434	0.335	0.208	North Coast	
2485	*occidentalis*	Tillamook	female	0.427	0.12	0.681	0.18	0.155	0.108	0.455	0.476	0.846	0.488	0.377	0.219	North Coast	
2498	*occidentalis*	Tillamook	male	0.378	0.0895	0.587	0.143	0.209	0.0966	0.368	0.396	0.938	0.476	0.27	0.192	North Coast	
2499	*occidentalis*	Tillamook	male	0.483	0.123	0.814	0.17	0.207	0.135	0.362	0.412	0.8	0.45	0.334	0.198	North Coast	
2500	*occidentalis*	Tillamook	male	0.343	0.12	0.579	0.155	0.0857	0.133	0.379	0.413	0.841	0.498	0.341	0.209	North Coast	
2501	*occidentalis*	Tillamook	female	0.58	0.156	0.923	0.232	0.213	0.196	0.412	0.478	1.2	0.749	0.235	0.218	North Coast	
2502	*occidentalis*	Tillamook	male	0.49	0.142	0.794	0.173	0.17	0.131	0.342	0.4	0.775	0.501	0.301	0.187	North Coast	
2503	*occidentalis*	Tillamook	male	0.439	0.0965	0.63	0.131	0.165	0.125	0.381	0.406	0.866	0.463	0.295	0.207	North Coast	
2504	*occidentalis*	Tillamook	male	0.497	0.134	0.794	0.166	0.162	0.115	0.39	0.414	0.859	0.544	0.31	0.214	North Coast	
2505	*occidentalis*	Tillamook	male	0.438	0.113	0.591	0.128	0.16	0.125	0.416	0.415	0.963	0.476	0.287	0.198	North Coast	
2506	*occidentalis*	Tillamook	female	0.485	0.12	0.675	0.164	0.183	0.12	0.424	0.436	0.955	0.547	0.365	0.217	North Coast	
2507	*occidentalis*	Tillamook	male	0.481	0.142	0.76	0.176	0.158	0.145	0.368	0.392	0.775	0.465	0.31	0.191	North Coast	
2508	*occidentalis*	Tillamook	juv	0.353	0.176	0.64	0.176	0.153	0.11	0.403	0.481	0.871	0.471	0.372	0.228	North Coast	
2509	*occidentalis*	Tillamook	male	0.423	0.108	0.641	0.125	0.15	0.106	0.284	0.396	0.704	0.488	0.318	0.203	North Coast	
2510	*occidentalis*	Tillamook	male	0.452	0.11	0.674	0.138	0.165	0.108	0.329	0.333	0.822	0.524	0.351	0.206	North Coast	
2511	*occidentalis*	Tillamook	male	0.432	0.1	0.659	0.14	0.122	0.0966	0.318	0.399	0.776	0.476	0.316	0.191	North Coast	
2512	*occidentalis*	Tillamook	male	0.14	0.127	0.617	0.14	0.14	0.111	0.398	0.421	0.834	0.453	0.312	0.215	North Coast	
2513	*occidentalis*	Tillamook	male	0.4	0.104	0.634	0.129	0.118	0.0964	0.366	0.42	0.823	0.482	0.341	0.195	North Coast	
2514	*occidentalis*	Tillamook	male	0.43	0.106	0.632	0.138	0.147	0.097	0.295	0.385	0.825	0.535	0.3	0.199	North Coast	
2515	*occidentalis*	Tillamook	male	0.419	0.104	0.601	0.121	0.145	0.127	0.374	0.399	0.864	0.471	0.31	0.198	North Coast	
2553	*occidentalis*	Del Norte	female	0.564	0.118	0.796	0.196	0.225	0.13	0.402	0.529	0.8	0.616	0.412	0.244	Northern CA	x
2554	*occidentalis*	Del Norte	male	0.463	0.11	0.752	0.133	0.185	0.1	0.415	0.45	0.836	0.597	0.336	0.213	Northern CA	
2631	*occidentalis*	Del Norte	female	0.53	0.115	0.786	0.167	0.128	0.115	0.446	0.511	0.605	0.595	0.388	0.242	Northern CA	
2632	*occidentalis*	Del Norte	female	0.552	0.117	0.809	0.195	0.223	0.156	0.484	0.545	1.16	0.665	0.298	0.289	Northern CA	x
2634	*occidentalis*	Del Norte	female	0.479	0.125	0.707	0.127	0.171	0.118	0.44	0.444	0.93	0.618	0.333	0.216	Northern CA	x

Table 4 continued.

SEM #	Species	County	Sex	Fem l	Fem w	Che l	Che w	Pat l	Pat w	Car l	Car w	Abd l	Abd w	Cheli l	Cheli w	Population	Phylo
2657	*minimus*	Jackson	female	0.405	0.1367	0.286	0.151	0.12	0.104	0.335	0.313	0.55	0.375	0.286	0.151	A. minimus	
2658	*minimus*	Jackson	female	0.356	0.116	0.632	0.163	0.14	0.0999	0.288	0.373	0.749	0.511	0.32	0.173	A. minimus	
2659	*minimus*	Jackson	male	0.346	0.0992	0.589	0.123	0.115	0.103	0.367	0.378	0.658	0.42	0.306	0.187	A. minimus	
2660	*minimus*	Jackson	male	0.426	0.089	0.638	0.14	0.144	0.107	0.274	0.32	0.554	0.389	0.28	0.145	A. minimus	x
2661	*minimus*	Jackson	male	0.381	0.106	0.572	0.137	0.127	0.0734	0.287	0.331	0.595	0.376	0.241	0.136	A. minimus	x
2682	*occidentalis*	Clatsop	female	0.401	0.107	0.684	0.161	0.192	0.132	0.327	0.379	0.686	0.352	0.281	0.187	North Coast	x
2683	*occidentalis*	Clatsop	female	0.326	0.0844	0.478	0.129	0.119	0.0743	0.388	0.379	0.749	0.415	0.218	0.154	North Coast	
2693	*occidentalis*	Clatsop	male	0.476	0.0952	0.705	0.151	0.132	0.102	0.398	0.439	0.837	0.637	0.378	0.198	North Coast	
2694	*occidentalis*	Clatsop	male	0.437	0.0992	0.642	0.125	0.156	0.097	0.309	0.367	0.669	0.638	0.292	0.178	North Coast	
2695	*occidentalis*	Clatsop	male	0.457	0.11	0.751	0.122	0.194	0.094	0.401	0.452	0.903	0.638	0.346	0.234	North Coast	
2696	*occidentalis*	Clatsop	male	0.402	0.0889	0.588	0.117	0.147	0.0992	0.281	0.369	0.663	0.548	0.315	0.172	North Coast	
2701	*occidentalis*	Del Norte	female	0.437	0.123	0.736	0.194	0.206	0.138	0.465	0.537	0.967	0.612	0.494	0.24	Northern CA	
2702	*occidentalis*	Del Norte	male	0.496	0.094	0.592	0.117	0.134	0.0802	0.351	0.368	0.813	0.401	0.309	0.178	Northern CA	x
2703	*occidentalis*	Del Norte	male	0.282	0.0935	0.63	0.119	0.134	0.0854	0.32	0.378	0.698	0.417	0.301	0.17	Northern CA	x
2710	*occidentalis*	Del Norte	female	0.571	0.142	0.913	0.183	0.179	0.145	0.49	0.603	0.486	0.613	0.506	0.279	Northern CA	x
2719	*minimus*	Jefferson	female	0.409	0.112	0.72	0.182	0.235	0.182	0.3	0.384	0.894	0.458	0.287	0.186	A. minimus	x
2720	*minimus*	Jefferson	female	0.412	0.1	0.612	0.157	0.145	0.124	0.402	0.419	0.839	0.445	0.34	0.188	A. minimus	
2721	*minimus*	Jefferson	male	0.311	0.0992	0.613	0.112	0.168	0.0844	0.36	0.383	0.834	0.367	0.311	0.157	A. minimus	x
2722	*minimus*	Jefferson	female	0.338	0.104	0.615	0.133	0.163	0.109	0.326	0.441	0.834	0.619	0.354	0.2	A. minimus	
2723	*minimus*	Jefferson	male	0.371	0.0973	0.466	0.102	0.129	0.0863	0.295	0.384	0.943	0.435	0.302	0.152	A. minimus	
2724	*minimus*	Jefferson	female	0.341	0.102	0.607	0.142	0.0924	0.0988	0.393	0.445	0.917	0.508	0.345	0.203	A. minimus	
2725	*minimus*	Jefferson	male	0.381	0.104	0.66	0.112	0.122	0.0794	0.347	0.385	0.894	0.42	0.293	0.17	A. minimus	
2726	*minimus*	Jefferson	male	0.341	0.0889	0.571	0.109	0.153	0.107	0.345	0.375	0.718	0.438	0.325	0.158	A. minimus	
2727	*minimus*	Jefferson	female	0.376	0.117	0.618	0.137	0.196	0.104	0.367	0.427	0.952	0.633	0.316	0.193	A. minimus	
2728	*minimus*	Jefferson	male	0.376	0.104	0.603	0.107	0.12	0.0941	0.352	0.399	0.919	0.478	0.313	0.174	A. minimus	
2729	*minimus*	Jefferson	male	0.347	0.104	0.636	0.127	0.201	0.111	0.341	0.393	0.814	0.445	0.33	0.169	A. minimus	
2730	*minimus*	Jefferson	female	0.354	0.0923	0.626	0.125	0.174	0.128	0.387	0.402	0.916	0.543	0.342	0.185	A. minimus	
2741	*occidentalis*	Del Norte	female	0.384	0.122	0.789	0.201	0.198	0.113	0.47	0.562	0.913	0.862	0.343	0.249	Northern CA	x
2762	*occidentalis*	Josephine	female	0.471	0.112	0.767	0.178	0.22	0.122	0.439	0.488	0.62	0.524	0.415	0.231	NA	
2770	*occidentalis*	Wasco	female	0.414	0.103	0.621	0.153	0.2	0.13	0.308	0.354	0.861	0.695	0.28	0.192	Cascades	
2771	*occidentalis*	Wasco	female	0.323	0.0712	0.526	0.132	0.158	0.0902	0.305	0.367	0.911	0.631	0.272	0.168	Cascades	
2772	*occidentalis*	Wasco	male	0.37	0.0747	0.512	0.119	0.115	0.0977	0.247	0.339	0.613	0.481	0.253	0.158	Cascades	
2773	*occidentalis*	Wasco	male	0.319	0.094	0.541	0.11	0.115	0.0826	0.259	0.324	0.814	0.385	0.175	0.126	Cascades	
2790	*occidentalis*	Columbia	male	0.371	0.0892	0.697	0.13	0.185	0.124	0.43	0.419	0.879	0.508	0.335	0.183	North Coast	
2792	*occidentalis*	Columbia	male	0.411	0.094	0.526	0.129	0.142	0.104	0.398	0.428	0.665	0.445	0.334	0.181	North Coast	
2841	*occidentalis*	Lincoln	female	0.397	0.0902	0.628	0.124	0.157	0.0996	0.309	0.417	0.766	0.664	0.297	0.189	Central Coast	x
2842	*occidentalis*	Lincoln	male	0.337	0.102	0.604	0.135	0.146	0.101	0.332	0.351	0.813	0.43	0.284	0.168	Central Coast	
2842	*occidentalis*	Lincoln	male	0.337	0.102	0.604	0.135	0.146	0.101	0.332	0.351	0.813	0.43	0.284	0.168	Central Coast	
2843	*occidentalis*	Lincoln	male	0.331	0.0789	0.598	0.118	0.118	0.0992	0.388	0.398	0.816	0.539	0.294	0.176	Central Coast	
2843	*occidentalis*	Lincoln	male	0.331	0.0789	0.598	0.118	0.118	0.0992	0.388	0.398	0.816	0.539	0.294	0.176	Central Coast	
2871	*occidentalis*	Columbia	female	0.466	0.125	0.719	0.173	0.164	0.131	0.396	0.49	0.971	0.843	0.378	0.226	North Coast	
2872	*occidentalis*	Columbia	female	0.41	0.122	0.701	0.166	0.172	0.133	0.399	0.432	0.851	0.588	0.373	0.189	North Coast	
2882	*minimus*	Clatsop	female	0.404	0.125	0.71	0.147	0.168	0.113	0.402	0.464	0.79	0.467	0.345	0.178	A. minimus	x

Table 4 continued

SEM #	Species	County	Sex	Fem l	Fem w	Che l	Che w	Pat l	Pat w	Car l	Car w	Abd l	Abd w	Cheli l	Cheli w	Population	Phylo
2883	*minimus*	Clatsop	male	0.42	0.102	0.679	0.131	0.146	0.0953	0.325	0.405	0.785	0.537	0.329	0.215	A. minimus	
2884	*minimus*	Clatsop	male	0.411	0.0988	0.613	0.131	0.166	0.0889	0.368	0.196	0.671	0.452	0.311	0.196	A. minimus	
2891	*occidentalis*	Clatsop	female	0.52	0.124	0.755	0.175	0.168	0.105	0.449	0.505	1.02	0.809	0.389	0.238	North Coast	
2892	*occidentalis*	Clatsop	female	0.447	0.11	0.669	0.152	0.149	0.0931	0.402	0.463	0.79	0.638	0.321	0.208	North Coast	
2893	*occidentalis*	Clatsop	male	0.362	0.113	0.661	0.132	0.117	0.108	0.301	0.399	0.724	0.61	0.326	0.183	North Coast	
2904	*occidentalis*	Tillamook	female	0.445	0.123	0.699	0.185	0.187	0.118	0.406	0.509	1.08	0.789	0.355	0.225	North Coast	x
2920	*occidentalis*	Multnomah	female	0.507	0.129	0.813	0.208	0.217	0.133	0.369	0.451	0.961	0.543	0.382	0.221	Portland	
2921	*occidentalis*	Multnomah	male	0.434	0.104	0.634	0.13	0.193	0.123	0.353	0.42	0.904	0.564	0.334	0.193	Portland	x
2922	*occidentalis*	Multnomah	female	0.413	0.112	0.648	0.148	0.137	0.103	0.386	0.425	0.997	0.683	0.3	0.193	Portland	x
2923	*occidentalis*	Multnomah	male	0.354	0.1	0.584	0.126	0.15	0.0864	0.38	0.374	0.918	0.489	0.292	0.173	Portland	x
2924	*occidentalis*	Multnomah	female	0.522	0.131	0.713	0.152	0.0902	0.119	0.452	0.448	0.907	0.725	0.387	0.236	Portland	
2925	*occidentalis*	Multnomah	female	0.493	0.109	0.7	0.149	0.158	0.097	0.398	0.446	0.991	0.525	0.38	0.226	Portland	
3072	*occidentalis*	Hood River	female	0.519	0.132	0.762	0.172	0.176	0.11	0.434	0.493	0.978	0.874	0.422	0.229	Cascades	x
3094	*occidentalis*	Hood River	male	0.472	0.097	0.656	0.134	0.112	0.101	0.409	0.421	0.865	0.498	0.307	0.174	Cascades	x
3106	*occidentalis*	Linn	male	0.39	0.121	0.639	0.126	0.135	0.0973	0.412	0.388	0.761	0.516	0.256	0.162	Cascades	x
3135	*occidentalis*	Multnomah	female	0.769	0.211	1.12	0.23	0.321	0.203	0.773	0.754	1.16	0.843	0.583	0.368	Portland	
3136	*occidentalis*	Multnomah	female	0.713	0.157	1.08	0.254	0.201	0.166	0.755	0.772	1.47	0.793	0.499	0.338	Portland	x
3137	*occidentalis*	Multnomah	female	0.65	0.188	1.08	0.268	0.233	0.199	0.754	0.709	1.49	0.931	0.558	0.33	Portland	x
3138	*occidentalis*	Multnomah	female	0.79	0.194	1.05	0.271	0.349	0.209	0.832	0.769	1.29	0.707	0.548	0.37	Portland	x
3180	*occidentalis*	Multnomah	male	0.584	0.137	0.722	0.202	0.193	0.091	0.358	0.419	0.691	0.549	0.311	0.217	Portland	x
3219	*occidentalis*	Linn	male	0.491	0.106	0.717	0.127	0.125	0.0901	0.445	0.407	0.994	0.583	0.346	0.183	Cascades	x
3222	*occidentalis*	Linn	male	0.475	0.112	0.718	0.108	0.152	0.098	0.401	0.41	0.976	0.598	0.343	0.196	Cascades	
3236	*occidentalis*	Coos	female	0.423	0.0852	0.643	0.14	0.156	0.12	0.33	0.439	0.867	0.631	0.333	0.202	Central Coast	
3243	*occidentalis*	Coos	male	0.314	0.0961	0.584	0.107	0.147	0.0752	0.335	0.35	0.717	0.419	0.246	0.163	Central Coast	x
3252	*occidentalis*	Linn	female	0.458	0.117	0.709	0.167	0.139	0.125	0.509	0.534	0.916	0.525	0.4	0.224	Cascades	
3269	*occidentalis*	Douglas	female	0.448	0.132	0.853	0.191	0.174	0.158	0.434	0.536	0.936	0.765	0.401	0.248	Central Coast	x
3270	*occidentalis*	Douglas	female	0.495	0.133	0.77	0.16	0.144	0.099	0.375	0.487	1.05	0.73	0.342	0.237	Central Coast	
3346	*occidentalis*	Lincoln	female	0.58	0.112	0.849	0.213	0.199	0.131	0.53	0.569	1.14	0.667	0.395	0.272	Central Coast	x
3402	*occidentalis*	Lane	male	0.4	0.101	0.609	0.137	0.139	0.117	0.324	0.399	0.754	0.488	0.297	0.196	Central Coast	x
3403	*occidentalis*	Lane	male	0.437	0.0945	0.632	0.142	0.169	0.125	0.34	0.387	0.77	0.479	0.323	0.182	Central Coast	

Preliminary Data Exploration

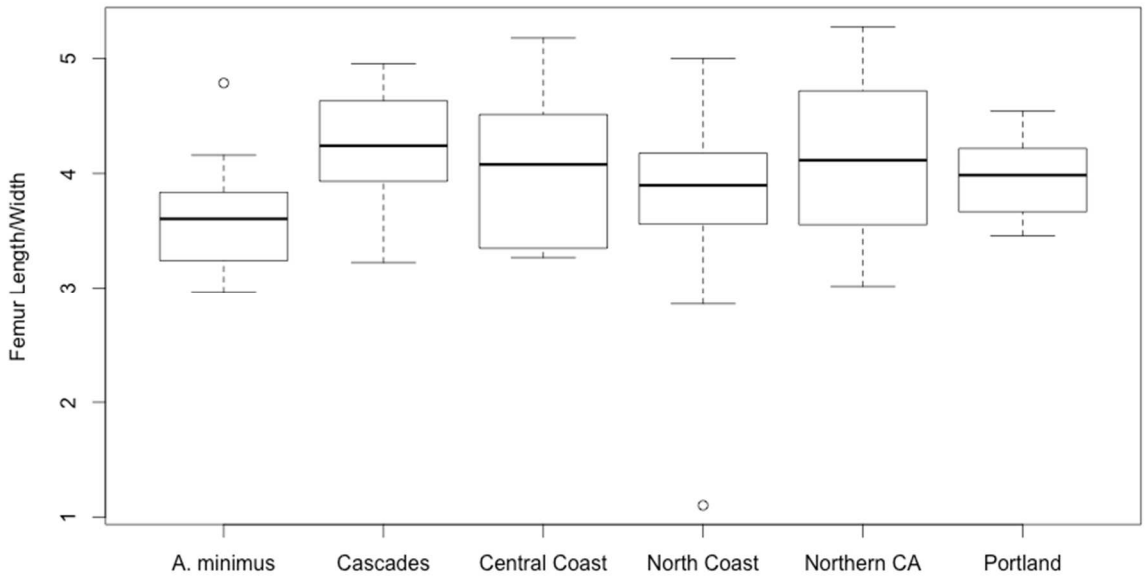

Figure 5: Across-population comparison of palpal femur length to width ratio. The populations listed on the X axis are those described in Chapter 1.

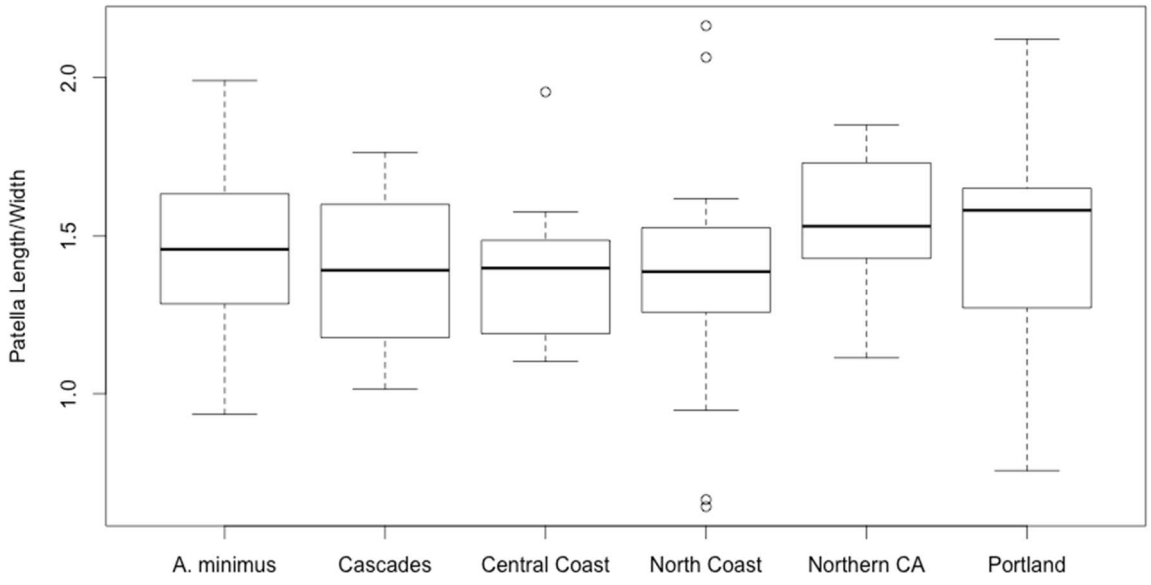

Figure 6: Across-population comparison of palpal patella length to width ratio.

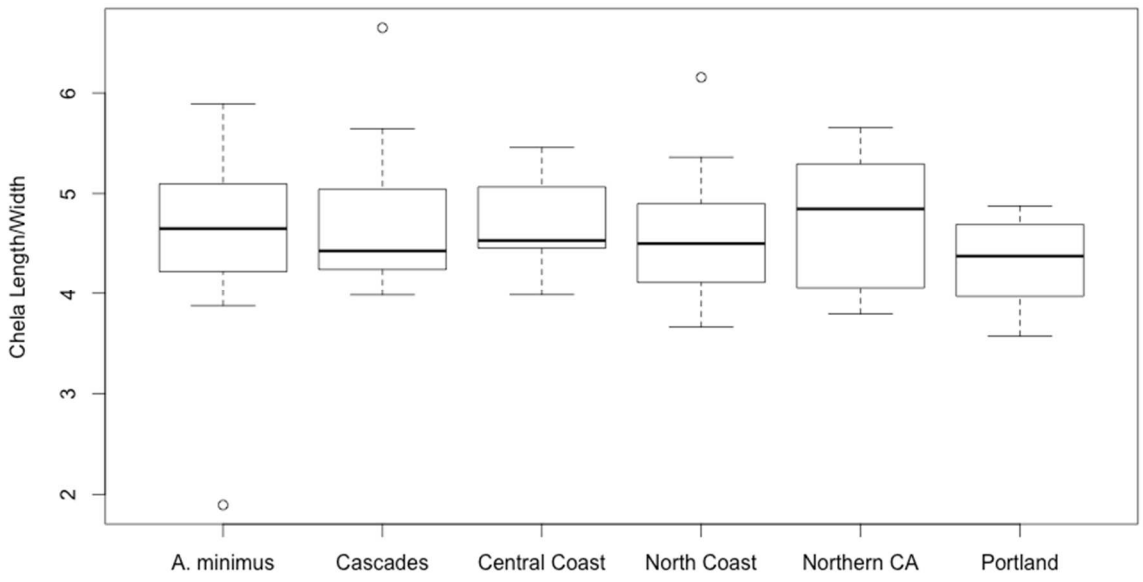

Figure 7: Across-population comparison of chela, or palp, length to width ratio.

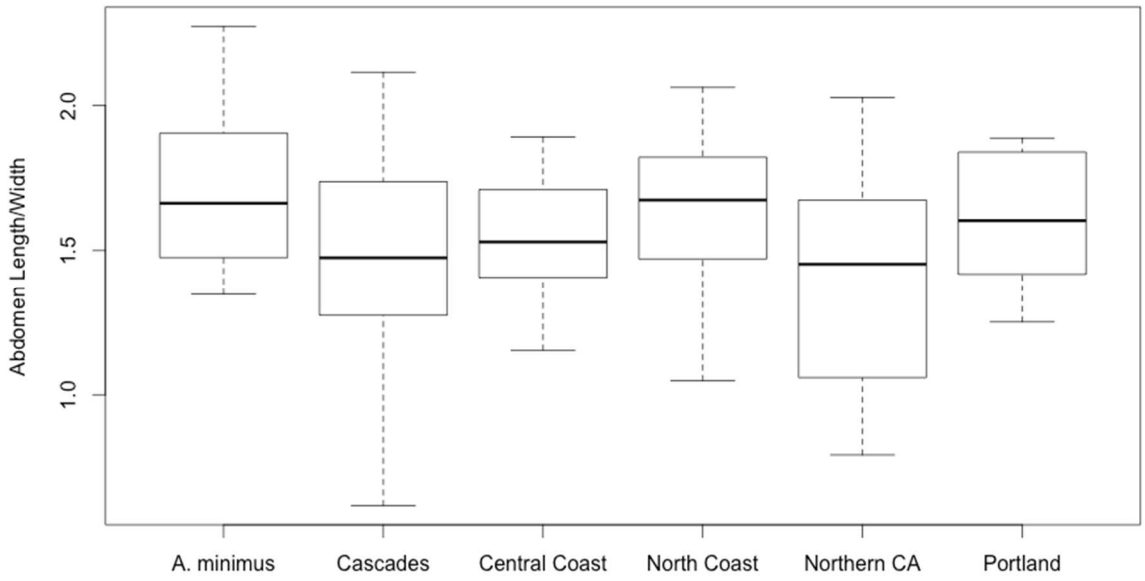

Figure 8: Across-population comparison of abdomen length to width ratio. This was determined an unfit parameter, as it will change drastically if an individual has recently eaten or is gravid.

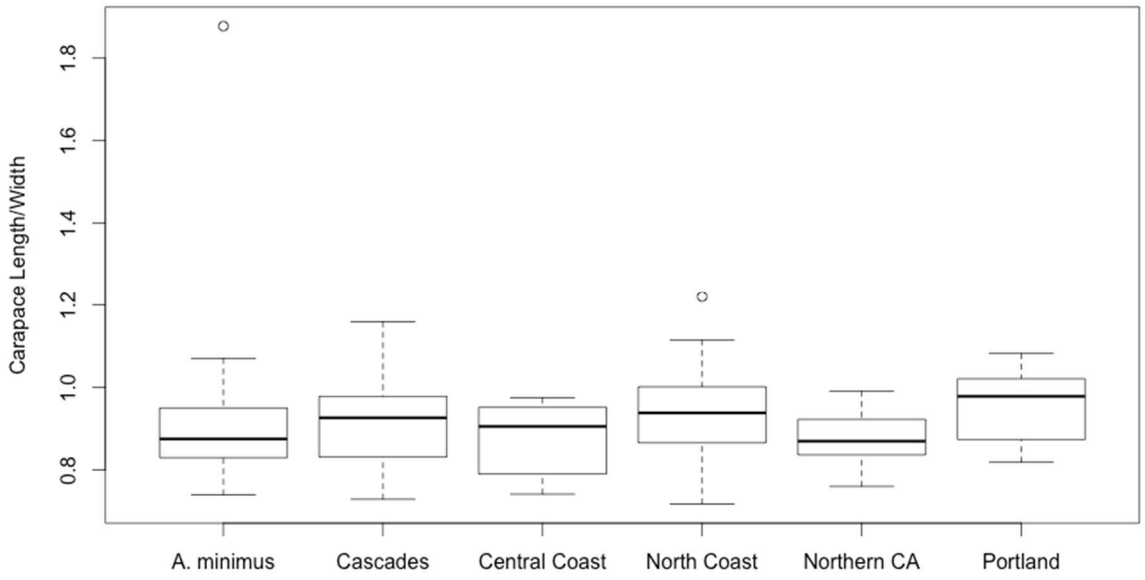

Figure 9: Across-population comparison of carapace length to width ratio.

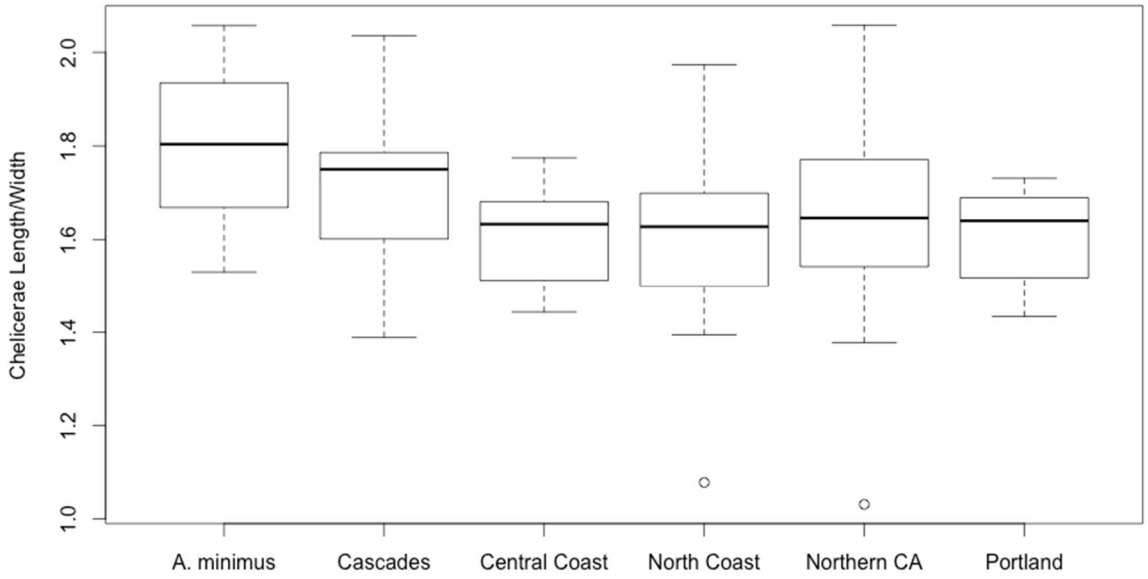

Figure 10: Across-population comparison of chelicerae length to width ratio.