

Phylogeography of Termites (Isoptera) from Oregon and Washington

by

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ABSTRACT

A molecular genetics study involving DNA sequencing of a portion of the mitochondrial DNA 16S rRNA gene was undertaken to determine the extent of genetic variation and distribution of *Reticulitermes*, *Zootermopsis*, and *Incisitermes* termites from Oregon and Washington. Eighty seven samples were sequenced and evaluated. From 17 Oregon and Washington counties, a total of one *I. minor*, 15 *Z. angusticollis*, one *R. flavipes*, one *R. hageni*, 65 *R. hesperus*, two *R. okanaganensis*, and two undescribed *Reticulitermes* were identified. The corresponding numbers of haplotypes for represented taxa include one haplotype each for *R. hageni*, *R. flavipes*, an undescribed *Reticulitermes*, *I. minor*, and *Z. angusticollis*, two haplotypes of *R. okanaganensis*, and 4 haplotypes of *R. hesperus*. Glacial refugia theories about the distribution of termites occupying both Oregon and Washington provide possible clues into the possible mechanisms which have fostered genetic variability in *R. hesperus* haplotypes and restricted gene flow in *Z. angusticollis*. They also provide some understanding to the driving forces behind the identification of newly identified termite groups from the region.

Keywords: 16S rRNA, *Reticulitermes*, *Zootermopsis*, Pacific Northwest, DNA sequence.

INTRODUCTION

There have been recent research efforts to identify the population boundaries of different Nearctic *Reticulitermes* species throughout North America apply-

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ing various genetic markers. The application of genetic markers is preferred because of the clarity and number of unambiguous characters resulting in more resolute identification, particularly in instances where diagnostic castes are unavailable (Szalanski *et al.* 2003, Foster *et al.* 2004). Studies have focused on the south central, south western and central States (Austin *et al.* 2006b, Austin *et al.* 2004a,b,c, Copren *et al.* 2005, Szalanski *et al.* 2006, Tripodi *et al.* 2006), but no studies have focused on the phylogeography of termites from the Pacific Northwest.

Existing taxonomic studies provide information on only one *Reticulitermes* spp. in Oregon, *R. hesperus* (Banks), the western subterranean termite, which is the most common termite pest species found from southern British Columbia to central California (Snyder 1954, Weesner 1965). Other studies have implicated the existence of cryptic taxa in the west (Myles 2000, Jones 2004, Copren *et al.* 2005, Austin *et al.* 2005a, McKern *et al.* 2006, Tripodi *et al.* 2006), but there are no current identification or distribution studies of taxa from the Pacific Northwest. Distribution studies on Pacific Northwest species of *Reticulitermes* have been addressed by Castle (1928) and Light and Pickens (1934). Recently McKern *et al.* (2006) found *R. flavipes* and *R. hageni* in Oregon and Szalanski *et al.* (2006) found genetic evidence for a new species of *Reticulitermes*, *R. okanaganensis* n. sp., that is found in British Columbia, Washington, Nevada, and California.

The purpose of this research was to update known occurrences of termite species found in Oregon and Washington, corroborate identities applying molecular markers and measure their genetic variation compared to other studies of this type, and to compare their phyletic relationships.

MATERIALS AND METHODS

Termites were collected from various locations in Washington and Oregon and preserved in 100% ethanol (Table 1, Figs. 1-2). We solicited the assistance of Pest Management Professionals (PMPs) throughout these states for the purpose of interpreting the predominant species recovered from infested structures and from samples obtained during the 2002 National Termite Survey (Messenger 2002). Morphology of *Reticulitermes* collected was identified applying the keys of Krishna and Weesner (1969), Scheffrahn and Su (1994), and Hostettler *et al.* (1995). Voucher specimens preserved

Table 1. Collection data, haplotypes and number of samples from Oregon and Washington.

State (number of samples)	Species	City or County (Haplotype-number from that location)
Oregon	(1) <i>I. minor</i>	Multnomah 1(IM)
	(1) <i>R. flavipes</i>	Marion 1(FF)
	(1) <i>R. hageni</i>	Marion 1(H1)
	(57) <i>R. hesperus</i>	Clackamas 4(HE3), 11(HE4), 1(HE23); Jackson 1(HE4); Linn1(HE4); Marion 2(HE4); Multnomah 2(HE3), 17(HE4), 1(HE23); Umatilla 1(HE4); Wasco 1(HE4); Washington 1(HE3), 10(HE4); Yamhill 1(HE3), 2(HE4)
	(2) <i>R. okanaganensis</i>	Jackson 1(RO8); Klamath 1(RO3)
	(15) <i>Z. angusticollis</i>	Clackamas 5(ZA); Marion 1(ZA); Multnomah 7(ZA); Washington 2(ZA)
Washington	(8) <i>R. hesperus</i>	King 5(HE4), 1(HE11); Pierce 3(HE4)
	(2) <i>R. n. sp. 2</i>	Jackson 1(Rnsp2); Yakima 1(Rnsp2)

in 100% ethanol are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

Alcohol-preserved specimens were allowed to dry on filter paper, and DNA was extracted according to Liu and Beckenbach (1992) on individual worker termites with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 µl of Tris:EDTA and stored at -20°C. Polymerase chain reaction (PCR) of a 428bp fragment of the 16S rRNA gene was conducted using the primers LR-J-13007 forward: 5'-TTA CGC TGT TAT CCC TAA-3' (Kambhampati & Smith 1995) and LR-N-13398 reverse: 5'-CGC CTG TTT ATC AAA AAC AT-3' (Simon *et al.* 1994).

The PCR reactions were conducted with 1 µl of the extracted DNA (Szalanski *et al.* 2000), having a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 60 s. Amplified DNA from individual termites was purified and concentrated with minicolumns (Wizard PCRpreps, Promega) according to the manufacturer's instructions. Samples were sent to the DNA core sequencing facility at The University of Arkansas Medical, Little

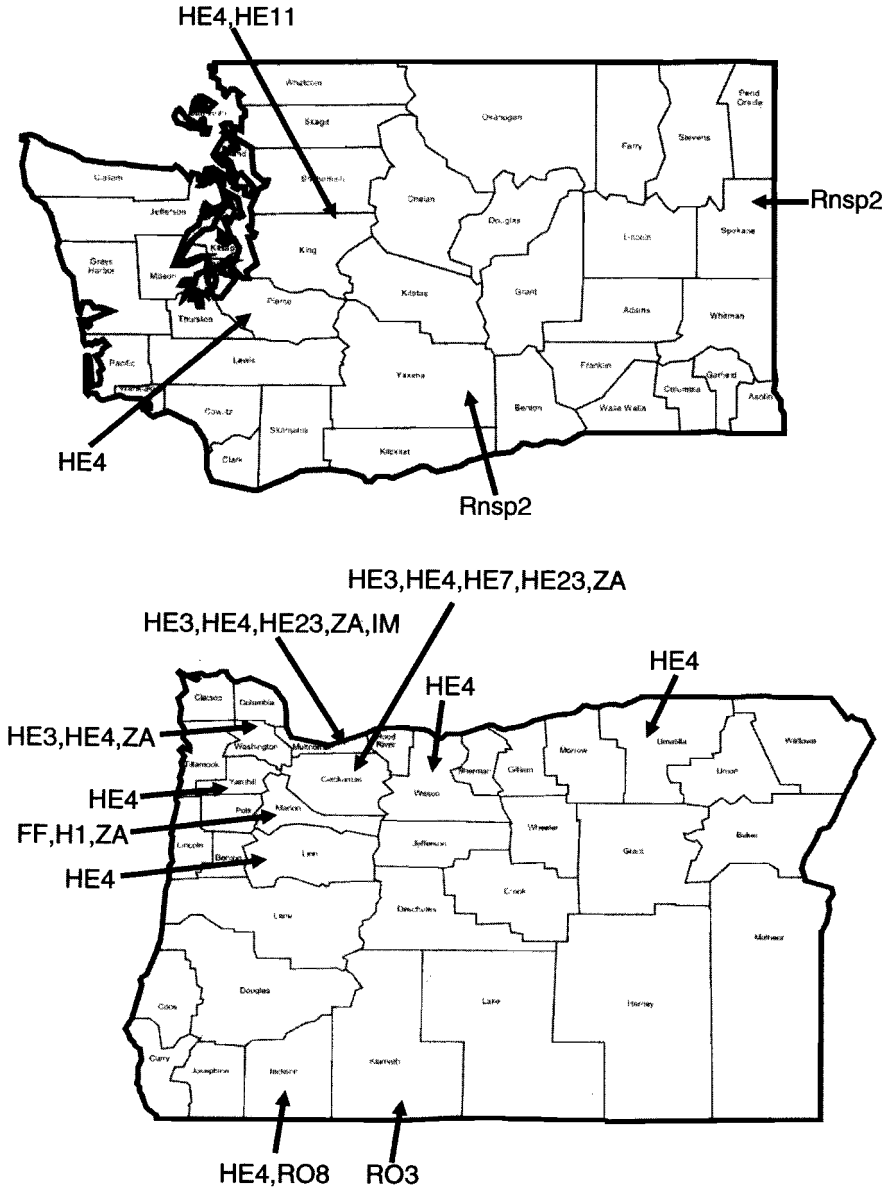


Fig. 1. Distribution of termite haplotypes found in Washington (upper) and Oregon (lower). Key to haplotypes is provided in Table 1.

Rock for direct sequencing in both directions. GenBank accession numbers were EF543142 to EF543146 for new termite haplotypes found in this study. DNA sequences were aligned using BioEdit v5.89 (Hall 1999) and adjusted manually. Mitochondrial DNA haplotypes were aligned using MacClade v4 (Sinauer Associates, Sunderland, MA).

The distance matrix option of PAUP* 4.0b10bv (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model of sequence evolution (Kimura 1980). Additional western *Reticulitermes* taxa that were used by Tripodi *et al.* (2006) and McKern *et al.* (2006) along with two outgroup taxa (Fig. 3) were included to corroborate relationships within the genus for our phylogenetic analysis. Maximum parsimony analysis on the alignments were conducted with PAUP* 4.0b10 (Swofford 2001). Gaps were treated as a fifth character state. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings and used the Branch and Bound algorithm of PAUP*.

RESULTS

Eighty seven samples were sequenced and evaluated. From 17 Oregon and Washington counties, a total of one *I. minor*, 15 *Z. angusticollis*, one *R. flavipes*, one *R. hageni*, 65 *R. hesperus*, two *R. okanaganensis*, and two undescribed *Reticulitermes* were identified (Table 1, Figs. 1-2). The corresponding numbers of haplotypes for represented taxa include one haplotype each for *R. hageni*, *R. flavipes*, an undescribed *Reticulitermes*, *I. minor*, and *Z. angusticollis*, two haplotypes of *R. okanaganensis*, and 4 haplotypes of *R. hesperus*.

The aligned *Reticulitermes* DNA data matrix, including the outgroup taxa resulted in a total of 437 characters. Of these characters, 105 (24%) were variable and 75 (17%) were phylogenetically informative. This dataset had only one most parsimonious tree (Fig. 3), (length = 248, CI = 0.609), as documented using the Branch and Bound search algorithm of PAUP*. Bootstrap analysis of the aligned *Reticulitermes* species and the outgroup taxa resulted in a consensus tree with all branches demonstrating the monophyletic nature of species within the genus. These relationships have been observed repeatedly in other genetic studies using both Cytochrome oxidase II gene sequences (Austin *et al.* 2002, Jenkins *et al.* 1998, 1999) and 16S rRNA (Austin *et al.* 2004a, b, c). The undescribed *Reticulitermes* species from Spokane county,

Washington formed a sister group with *R. okanaganensis*. There was no apparent genetic structure associated with geography based on such a small sample size for the observed haplotypes in the present study.

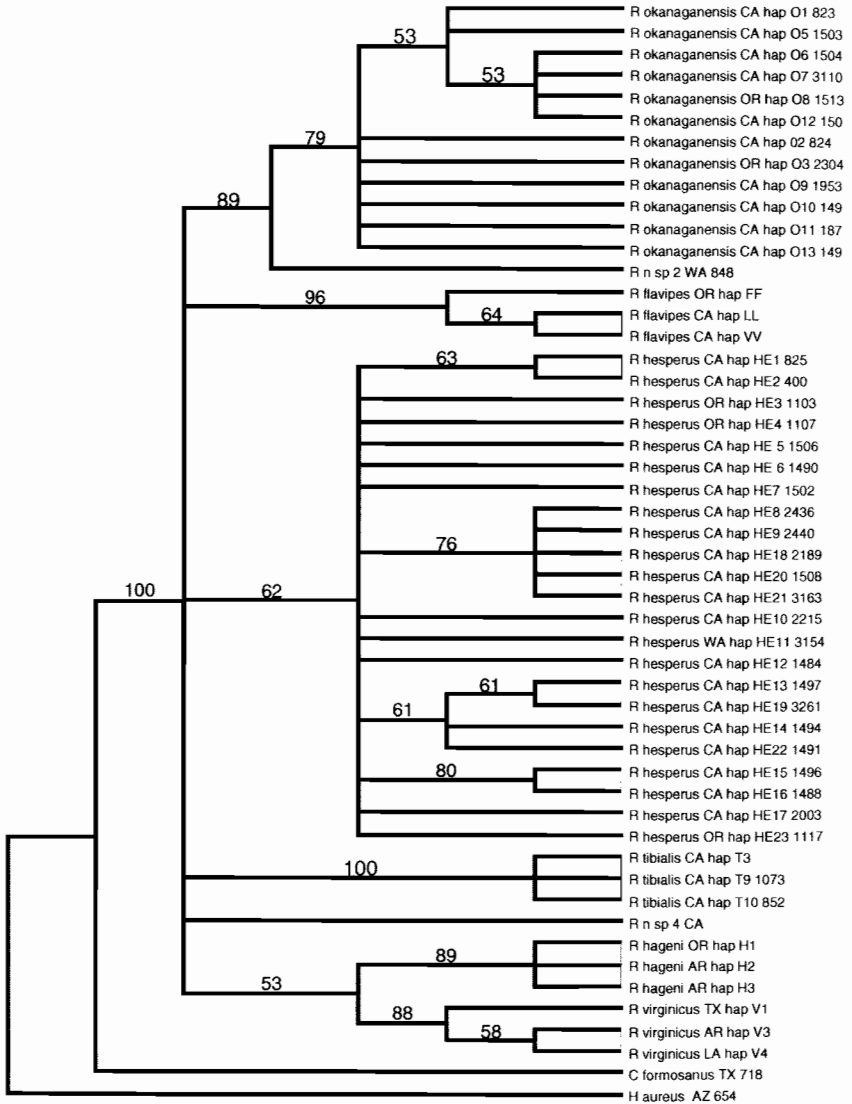


Fig. 3. 16S rRNA single most parsimonious tree for *Reticulitermes* during a branch and bound search using PAUP*. Bootstrap values for 1,000 replicates are listed above the branches supported at $\geq 50\%$.

DISCUSSION

There are five rather distinct faunal areas in the Nearctic with respect to termite species and *Reticulitermes* are present in all five (Krishna & Weesner 1970). The Pacific coastal area extends from the upper portion of Baja California through the area of Vancouver Island and adjacent mainland coastal areas of Canada. This area includes the Cascade-Sierra Nevada Mountains, and, in the north, extends as far inland as Montana. The offshore Pacific islands of the Baja California area are included here (Krishna & Weesner 1970). The Pacific coastal area has been described as having a limited fauna, including two species of hodotermitids (*Z. angusticollis* and *Z. nevadensis*), one rhinotermitid (*R. hesperus*), and two kalotermitids (*Incisitermes minor* and *I. fruticavus* Rust, (in Rust, Reiersen & Scheffrahn 1979) (Rees & Gaufin 1939, Krishna & Weesner 1970). The 2002 Termite Survey (Messenger 2002) lists samples of *Z. nevadensis* procured from PMPs from Garden City, ID, Battleground, WA, and Oregon City, OR; *Z. angusticollis* from Milwaukie, OR, Federal Way, Roy, and Tacoma, WA; *Reticulitermes* from Oregon include samples from Ashland, Beaverton, Keizer, Klamath Falls, Milwaukie, Myrtle Point, Portland and Sweet Home; *Reticulitermes* from Washington include samples from Auburn, Des Moines, Federal Way, Kent, Sunnyside, Taxoma, Yakima, and Zillah.

More recently, an apparently indigenous species of *Microcerotermes* has been described from San Diego County (Setter & Myles 2005). One species, *Zootermopsis angusticollis* (Hagen), occurs in the forested areas along the Pacific coast, west of the continental divide and is quite abundant. This species has been described as occurring on Vancouver and the Gulf Islands, British Columbia, and on the Canadian mainland as far north as the Powell River (Spencer 1945); southward it extends into Baja California (Castel 1934). Although the size of founding colonies of *Z. angusticollis* are reportedly small and their general infestations are found in decaying logs, they occasionally attack structural timbers, especially in the northern ranges of their distribution (e.g., personal collections from Paradise, CA by D. Kistner (Krishna & Weesner 1970). In these occurrences, pine, fir, and occasionally redwood have been attacked (Castel 1934) and transport of *Z. angusticollis* in lumber shipments from the northwest Pacific have been reported.

Another termite, *Incisitermes minor* (Hagen) has a wide geographical and ecological range, but is most frequently found occupying the coastal areas of California, Oregon, and Washington (Light 1933). This drywood-dwelling form is often associated with dead portions of living trees, but may also be found in structural wood in some areas, causing significant economic loss. Because this species can endure a wide range of temperature fluctuations and can persist in dry wood, its transport to numerous areas throughout the United States is essentially unabated and accidental human-aided dispersal is a common dilemma.

Among the species identified in this present study, none are more pervasive and difficult to control than those belonging to *Reticulitermes*. In the Pacific west, *R. hesperus* occurs along the Pacific coast from Canada into northern Baja California (Krishna & Weesner 1970). The northern limit of its range occurs in the upper Frazer valley from Lytton to Kamploope, British Columbia (Spencer 1937, 1945). Although thought to be the exclusive species to the western United States, recent termite surveys have revealed that this species has frequently been misidentified with other congeners from the west. Identification of eastern Nearctic incursions of *Reticulitermes* to western states have been demonstrated for *R. flavipes* in California (Austin *et al.* 2005a, 2006c), *R. flavipes* and *R. hageni* to Oregon (McKern *et al.* 2006) and the identification of new groups which likely constitute species in need of proper descriptions (Szalanski *et al.* 2006), following distinct biogeographic patterns (Copren *et al.* 2005, Tripodi *et al.* 2006).

Because the present study has relied heavily on material submitted by PMPs, a clear bias associated with the frequency of occurrences from various *Reticulitermes* species that attack structures is reflected. This study represents the first attempt to collectively update the current geographic distribution and genetically categorize termites from the Pacific Northwest. Most of the inferred relationships had strong quantitative support as indicated by bootstrap analysis. The relationships among taxa inferred from maximum parsimony analysis was congruent with presently accepted groupings. Samples which could be identified morphologically did not contradict our molecular findings, but it should be noted that there is significant morphological variation which frequently precludes the application of many existing dichotomous keys for the identification of *Reticulitermes* species from the region.

Identifications of cryptic *Reticulitermes* species from the western Palearctic have provided the impetus to renew collecting efforts there and intensify our understanding of the natural distributions occupying this geographic location. One of the most plausible explanations concerning the distribution of species in the northwestern Nearctic is that it may be due to postglacial colonization routes (of termites) that follow specialized ecological patterns. Comes and Kadereit (1998) demonstrated that climatic oscillations in the Quaternary have played a major role in changing the geographical distribution of plant species there. In their work, Comes and Kadereit (1998) demonstrated a North-south partitioning of two major cpDNA lineages in a herbaceous perennial (*Tolmiea menziesii*, Saxifragaceae) from the Pacific Northwest of North America, and a possible postglacial scenario involving colonization from isolated northern and southern refugia appears to be the principle means of distribution there.

Recent molecular work has provided new insights into the location of glacial refugia and routes of postglacial expansion in Europe (Luchetti *et al.* 2005, Austin *et al.* 2006a). This change of geographical distribution, as observed in genetic surveys of the genus (Austin *et al.* 2006c), may have provided opportunities for speciation through isolation, and hybridization after secondary contact. This may partially explain the segregation of distinct geographic (and genetic) boundaries between *R. hesperus* (occupying a more coastal distribution in the western Nearctic) versus the cryptic group we currently have named *R. okanaganensis* (Szalanski *et al.* 2006). This isolation may have contributed to more rare groups of *Reticulitermes* which can only be found in specific locations. A recent investigation of Florida *Reticulitermes* has revealed tremendous genetic variation with a significant number of distinct 16S haplotypes that have only been found in that state alone (A.L.S. & J.W.A, unpublished), when broader comparisons for specific species have been conducted (Austin *et al.* 2005a). Several *R. hesperus* haplotypes, (e.g., HE4 and HE23) do not occur outside of Oregon or Washington. The geographic isolation and limited dispersal opportunity (given climatic conditions) are contributing factors. Likewise, this third undescribed group, identified herein as *R. n. sp.2* from south central and western Washington may have acquired its discrete genetic nature from other isolation events. An alternative scenario invokes glacial survival and subsequent northwards colonization from only a

southern refugium (or several southern refugia), as other investigators have found evidence for multiple new species occupying several different ecological niches in western states (Copren *et al.* 2005, Tripodi *et al.* 2006, Szalanski *et al.* 2006). These types of isolating mechanisms were recently used to describe transitions in the abundance and distribution of *Reticulitermes* species in the central Nearctic (Austin *et al.* 2006c). Intensive sampling from Oregon and Washington may provide the additional demographic information needed to identify the distribution and potential mechanisms underlying the occurrence of *R.n.sp.2* from this region. Among the relatively limited number of plant phylogeographic studies, the majority have relied on tree species in order to reveal glacial refugia, postglacial migration routes and loss of genetic diversity (Comes & Kadereit 1998). Similarly, termites would likely follow similar migration and establishment routes with a preferred food source such as trees. As genetic diversity of some termite food sources (e.g., trees) became more restricted, so too would their likely assemblages. This may partially explain the lack of genetic variation observed in *Z. angusticollis*.

Inbreeding and reduced heterozygosity are generally detrimental to fitness (Keller & Waller 2002). However, secondary reproductives (which contribute to inbreeding) are common in *Zootermopsis* colonies that have lost one or both primaries (Castle 1934; Light & Illg 1945), probably reducing allelic variation in their offspring (Husseneder *et al.* 1999; Thorne *et al.* 1999; Vargo 2003; DeHeer & Vargo 2004). *Zootermopsis* species constitute one-piece type species (Abe 1987), whereby the wood serves both as nest and food source throughout the colony's inception and maturation, and thus generally do not forage outside of their nests. Rosengaus and Traniello (2002) suggest that the ability of (*Zootermopsis*) termites to locally adapt to disease could influence the genetic identity of primary reproductives and the extent of inbreeding in termite populations. Could microbial and fungal development in rotten or moist wood, the preferred wood sources of *Z. angusticollis*, trigger a humoral response to infection which potentially influences pathogens direct activity on this species, thus skewing the reproductive composition of nests (e.g., *Wolbachia* infections which induce cytoplasmic incompatibility resulting in a gender-biased population composition) resulting with the reduced heterogeneity that other researchers observe in the species? Perhaps, but this would be a gross oversimplification of this species' unusual nesting habits.

Nevertheless, it may provide some explanation for the reduced heterozygosity in the populations of *Zootermopsis* evaluated in this study. Termite nesting ecology (Hamilton 1972), predation on alates (Lepage 1991; Matsuura & Nishida 2002) and disease risk (Rosengaus & Traniello 1993; Rosengaus *et al.* 2000; Calleri *et al.* 2005, 2006) may jointly limit dispersal, which could also increase inbreeding (Roisin 1999). A single 16S rRNA haplotype, ZA, was identified from 15 disjunct locations in Northwest Oregon (Table 1). Additional collection sites or a slightly larger genetic marker may reveal greater genetic variation within this species, as presumably greater differences would be seen with geographic distance (Mayr & Ashlock 1990). However, as seen in a recent study by Austin *et al.* (2005a, 2006b), even some indigenous populations of termites may exhibit no or little genetic variation over large geographic spatial scales. The limited dispersal of *Z. angusticollis* in the Northwest region of Oregon may likely demonstrate glacial refugia hypotheses stated earlier, hence further limiting the dispersal of this group and fostering greater overall homozygosity among their colonies. Further sampling should be encouraged to determine the nature of this reduced heterozygosity in *Z. angusticollis* from this region.

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