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Genetic variation within a broadly distributed chewing louse genus (*Thomomydoecus*)

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GENETIC VARIATION WITHIN A BROADLY DISTRIBUTED CHEWING LOUSE GENUS (*THOMOMYDOECUS*)

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Abstract

No broad study has been conducted to examine the genetics of *Thomomydoecus* species and their patterns of geographic variation. Chewing lice and their parasite-host relationships with pocket gophers have been studied as a key example of cophylogeny (Demastes et al., 2012). Despite this, genetic data on interspecific and intraspecific variation in *Thomomydoecus* is unexplored, and prior studies consisted within the narrow frame of one complex or species and its relative host gopher. This project collected, and analyzed genetic data, then generated phylogenetic trees. Many of the existing relationships between *Thomomydoecus* species was confirmed, and there were a number of unexpected findings, and the dispersal of *Thomomydoecus* louse species is one that diversifies based on an isolating geographic landscape, rather than gopher host species. This will have future use in studies comparing phylogeography and genetic variation of *Thomomydoecus* to that seen in species of *Thomomys* pocket gophers.

Keywords: Phylogeography, Thomomydoecus, mitochondrial DNA

Introduction

Pocket gophers (Rodentia: Geomyidae) are fossorial mammals with asocial behavior, and as such spend much of their life cycle in their own tunnel systems and hoarding food. The only exceptions to this asocial way of life are mating and the rare territorial behaviors. Consequently, these gophers often remain isolated from other populations across a geographic range with reduced gene flow depending on distance, and this allows an increased number of speciation events within the family (Page, 2003). Isolated with them are populations of parasites that wholly depend on the gophers for biological requirements. Fleas, chewing lice, mites, and other ectoparasitic species have developed adaptations or lost specific traits such as jumping ability or eyesight as a result of this extended ecological association with pocket-gophers in their enclosed tunnels. Out of them all, the chewing louse has been known to develop specific characteristics suited to its host species, leading to a relationship that continues to develop over time (Nadler et al., 1990). This makes them optimal specimens for studies that explore the genetic relationship between two different species after extended interaction over millennia.

This is in part because of the feeding behaviors of lice, as their appendages must be suited to clinging and traveling over hairs, exact anatomical shape is of importance when grasping hairs that vary in texture, size, and length depending their location on an animal. They also have a competition mating system when in such close proximity with other male competitors on a gopher, which are only 6 to 8 inches in body length (Macdonald, 2006). This leads to diversity in reproductive organs, especially that of male genitalia. Combining all the different types of specialization louse species can undergo, it leads to an astounding amount of

diversity even when restrained to just the family Geomyidae. To add even more complexity, due to the asocial behavior of pocket gophers there is limited opportunity for these louse populations to undergo host-switching, which leads to restricted gene flow and constitutes a rare case of parapatric speciation and codivergence over evolutionary time. This is when a small population is isolated from the majority, and this subpopulation differentiates over time to the point that it becomes a new species. These populations are not separated by a geographical area, but a shift in habitat, and in this case that would be pocket gopher hosts and the localities at which they reside. It is important to note that geographical barriers can still play a role with gopher (host) dispersal and speciation.

Most louse transfer from host to host is believed to be vertical, from mother to offspring or during mating. It has specified to the point that many pocket gophers only have one species of louse on them, although there are cases where 2 or 3 species reside (Hellenthal and Price, 1991). As expected from this unique relationship, even within North America there are countless species and subspecies of lice and pocket gophers. This dynamic where the host changes over evolutionary time and the parasite adapts to suit the host, leading to an extended host-parasite interaction, is a prime example of a cophylogenic relationship (Light & Hafner, 2007a, Page, 2003). This is exemplified by multiple genus of chewing louse worldwide, but this study focuses on those in North American.

There are two genera of chewing lice that have colonized North American pocket gophers: *Geomydoecus* and *Thomomydoecus*. There are an astounding total of 122 species (although this number will potentially shift in the future) and subspecies of chewing lice in the genera *Geomydoecus* and *Thomomydoecus* hosted by pocket gophers (Page et al., 1995). This

study's focus was on *Thomomydoecus*. What is interesting about this genus comparative to *Geomydoecus* is that while the genus *Geomydoecus* has successfully colonized the family Geomyidae throughout North America, *Thomomydoecus* presumably emerged more recently and only on the gopher genus *Thomomys*.

Furthermore, recent studies delineating *Thomomydoecus* and host relationships have typically dealt with a specific complex or species, and no broad-scale study has been conducted that compares *Thomomydoecus* genetics across its entire geographic range. This study's aim was to make a comprehensive phylogeny with genetic (mitochondrial) data of *Thomomydoecus* chewing louse across North American and compare these with the range of *Thomomys* host species, then discuss the mechanisms by which this louse diversifies across a geographic range. Interspecific and intraspecific variation of the species was also compared. In order to have a better understanding of what this research entailed, some background information into the groups within the genus *Thomomydoecus* and their host gophers is necessary.

Literature Review

The Western pocket gopher is widespread throughout the Western part of North America and is called *Thomomys bottae* (*T. bottae*). This gopher species has an unusual genetic pattern that does not appear to follow the usual mechanism of reproductive isolation leading to changes in alleles frequency usually supported in other species. It does not appear to be constructed by immediate distance towards other populations of gophers nor geographic constraints (Patton & Yang, 1977). *Thomomys umbrinus* (*T. umbrinus*) is also known as the Southern pocket gopher and distributed from southeastern Arizona and southwestern New

Mexico southward into the Trans-Mexico Volcanic Belt (TMVB) and can be divided into a number of subspecies (Verity et al., 2014). When *T. bottae* and *T. umbrinus* are combined, they host two major complexes of *Thomomydoecus* species, the *neocopei* complex and *minor* complex (Price & Hellenthal, 1980). Pocket gopher species *Thomomys atrovarius (T. atrovarius)*, *Thomomys sheldoni (T. sheldoni)*, and *Thomomys nayarensis* (*T. nayarensis*) have a neotropical distribution reaching southward to the Trans Mexican Volcanic Belt (TMVB) in Central Mexico. *Thomomys talpoides* and associated subspecies (also known at times as the *talpoides* complex due to unsettled taxonomy) is also relevant as it is the Northernmost pocket gopher, capable of living in mountainous or tundra environments (Long, 2003). They host the *wardi* complex of *Thomomydoecus*, which includes the species *Thomomydoecus barbarae (T. barbarae), Thomomydoecus arleneae (T. arleneae)*, and *Thomomydoecus wardi (T. wardi)* (Hellenthal & Price, 1989). These species can also be found on two populations of *T. bottae*. This may seem confounding, but in regions where the two species of gopher overlap, there is the possibility of two different species interacting to mate, which would allow one species of louse to transfer to a different gopher species in a host-switching event. In the north, *T. barbarae* is the only *Thomomydoecus* species that ranges far into a cold climate, near the border of Canada, although the sampling in this study only extends through North Dakota. These make up the major relevant groups within this study.

The formal classification of the chewing lice of pocket gophers (Family Trichodectidae) has undergone several revisions. Originally, the *wardi, minor*, and *neocopei* complexes were regarded as part of the genus *Geomydoecus*, but sufficient evidence was able to clarify that it was entirely a separate group (Hellenthal & Price, 1984). In general, the morphology of

Thomomydoecus varies to some degree from *Geomydoecus*, even by eye. *Thomomydoecus* males are generally slenderer and more elongated in the abdominal region, and also come to a sharper point at the tail end of the abdomen, compared to *Geomydoecus* with a rounded body. Most microscope identification of either louse genus is done by inspecting traits such as male genitalia and setae (Price & Hellenthal, 1980). Altogether, it makes for a large amount of complexity within just one genus of chewing louse and provides an insightful study for evolutionary questions and cophylogenic relationships (Hafner and Page, 1995).

The fact that *Thomomydoecus* lice possibly colonized *Thomomys* pocket gophers after *Geomydoecus* also adds interest to this study. It is rare for one genus to occupy the same niche as another when intense competition usually results in the genus with more fitness becoming predominant over evolutionary time. This research aimed to better understand how *Thomomydoecus* was able to occupy this niche and examined the validity of the current morphology-based classification of *Thomomydoecus* by creating a phylogeny for the genus. This will be useful for understanding not only pocket-gophers and parasite cophylogeny, but the biogeography of North America as a whole. Finally, the genetic work done in this broad-scale study will be a foundation for future tests of *Thomomydoecus*-*Thomomys* group cophylogeny in the future.

Methods

Pocket gophers have been sampled in a range extending from South Dakota and Montana, the far coast of California, and extending through the base of Mexico and Trans-Mexican Volcanic Belt (TMVB). Each gopher is prepared as a study specimen and undergoes a 'brushing' to collect the louse bodies into a numbered sample vial . I then checked for

Thomomydoecus lice as some gophers have no lice or only *Geomydoecus*. When feasible, 3 *Thomomydoecus* lice were sampled from each gopher in the study. This sampling included 48 gophers, and of these 70 individual louse samples had DNA successfully amplified and were used in creating the final phylogenetic trees and figures. Gopher sample locality and subsequent louse identifications and info can be viewed in the *Appendix*.

To collect genetic data on mitochondrial DNA, the methods of DNA extraction, amplification, and sequencing follow Light and Hafner (2007). DNA isolation is done using Qiagen DNeasy Tissue Kit (Qiagen, Valencia, California), then a T-Gradient Biometra thermocycler is used for polymerase chain reaction (PCR). The main primers used to amplify the mitochondrial DNA were LCO 1490 and HCO 2198 (Folmer et al., 1994). To yield more results, primers *Thomomydoecus* 1490 and *Thomomydoecus* 2198 were also used, which were based on the general primers noted previously, but modified to fit the *Thomomydoecus* species. The primer that was most successful at amplifying DNA varied depending on the louse sample. The program used for PCR begins with 95° C for 2 minutes to denature the doubled-stranded DNA, then consist of 40 cycles of 94° C for 45 seconds, 45° C for 45 seconds, and 72° C for 45 seconds. Finally, it transitions to 72° C for 10 minutes to finish DNA annealing and into 15° C until the samples are collected. This process amplifies the originally miniscule DNA sample from the louse so that it can be at a number viable for use in gel electrophoresis and the final sequencing. Finally, the resulting PCR products were prepared for sequencing using Exosap-it (USB, Cleveland, Ohio), put on a plate, then sent to the Iowa State University DNA Sequencing Facility (Ames, Iowa) for sequencing.

Once we received the sequence data, it was analyzed using the program Geneious which aligns the DNA sequences. Modeltest version 3.7 (Posada et al., 1998) was used to select the appropriate nucleotide substitution model (GTR+G). This data was run in the MrBayes 3.1.1 (Ronquist et al., 2003) that generates Bayesian probability trees, and was also tested using Randomized Axelerated Maximum Likelihood (RaxML) analysis (Stamatakis, 2014) via the CIPRES Gateway (Miller et al. 2010). These probabilities were then combined to make a phylogenetic tree that delineates relationships between the *Thomomydoecus* complex (Figure 1). Probabilities under .75 for Bayes were excluded from the final figure, and those under .70 for maximum likelihood.

Locality information was gathered from recorded data at the collection time and place. Louse sample localities used in the final phylogram were mapped geographically using R programming (R core team, 2020) and ggplot (Wickham, 2009). Comparison with previous studies on *Thomomydoecus* host species were used to color and block the resulting tree in groups based on host species and their localities (Patton and Yang, 1977, Verity et al., 2014). Finally, the phylogeny was re-formatted for ease of viewing using the program FigTree, version 1.4.2, to create the final product (Rambaut, 2010). A few of the DNA samples were excluded from the final figure because they were confirmed to be in the genus *Geomydoecus*.

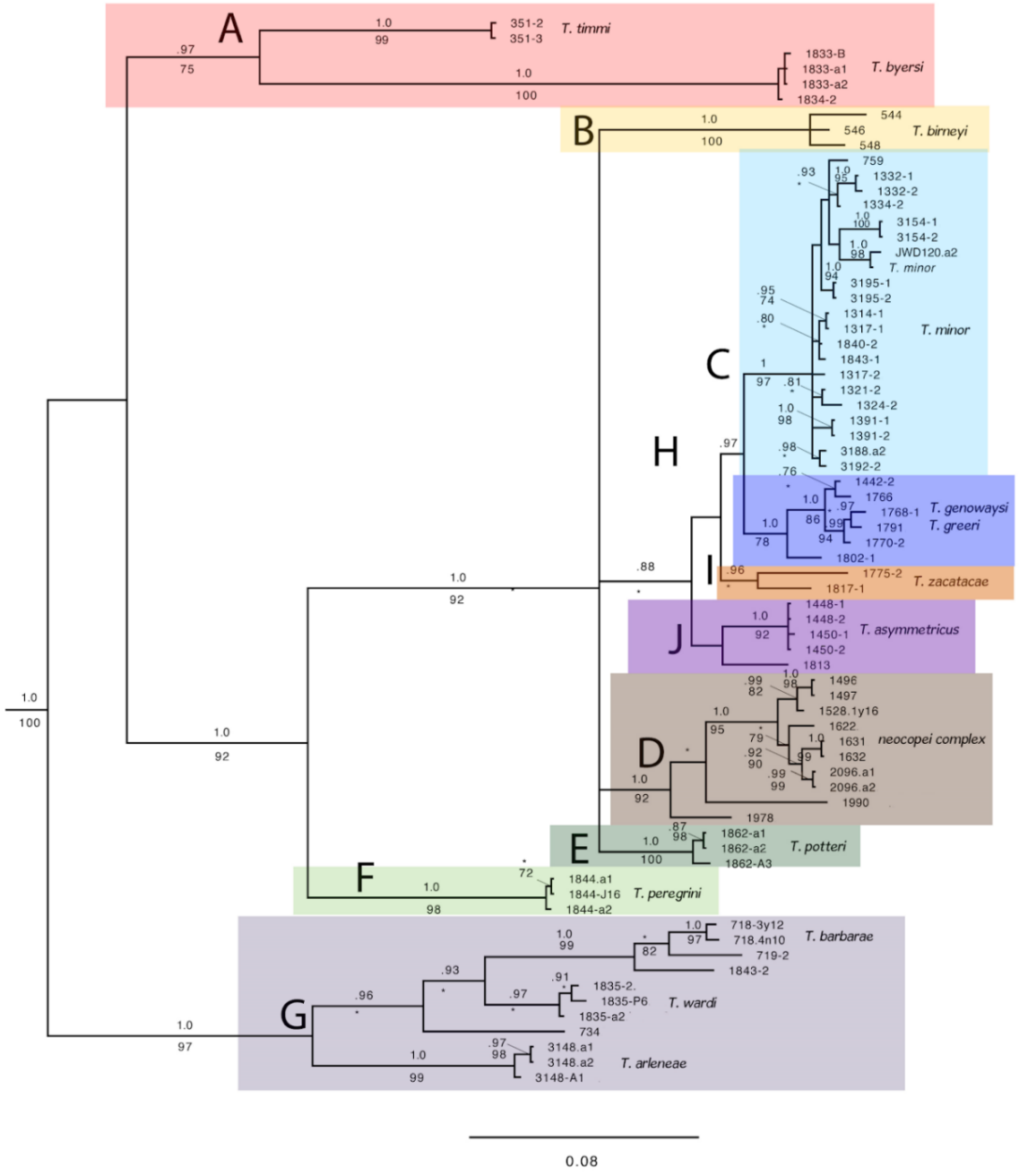
Results

Major complexes and species relationships were supported (Fig. 1). Values generated by Bayesian analysis or Maximum Likelihood are marked at each node, and those that were beneath the acceptable cut off are marked with an asterisk. The splits that were not supported by either analysis were left blank and do not affect the placement of clades. To verify the

authenticity of the clades and louse species, microscope identification was done by hand on the majority of the samples, using anatomical descriptions and references available in previous studies (Price & Hellenthal, 1980; 1989).

Figure 1

Phylogram of Thomomydoecus clades based on Bayesian and maximum likelihood support



Note. Bayesian phylogram for *Thomomydoecus* based on 70 samples of mitochondrial DNA. Clades between significant branching events are denoted with the letters (A-J), and major complexes and species are delineated with color and name (right). Nodes with Bayesian and Maximum Likelihood probabilities are denoted, starred values were below the cut off (.75 and 70). The four numbers before the dash or period are the gopher of which the louse sample was obtained, then numbers afterward indicate the specific louse sample.

The *Wardi* complex has a split between *T. arleneae, T. barbarae,* and *T. wardi* with decent support (clade G). Sample 734 has lacking support in its split from the *wardi* group and is likely also *T. wardi. T. byersi* may be located in a different group (see discussion).

The *neocopei* complex was partially grouped (clade H), but also distinctly includes the groups of *T. genowaysi, T. asymmetricus ,T. greeri, T. peregrini*, and *T. potteri* (clades J; D; E; F). Of them, *T. peregrini* is distinctly separated from the rest of the group with strong support (clade F).

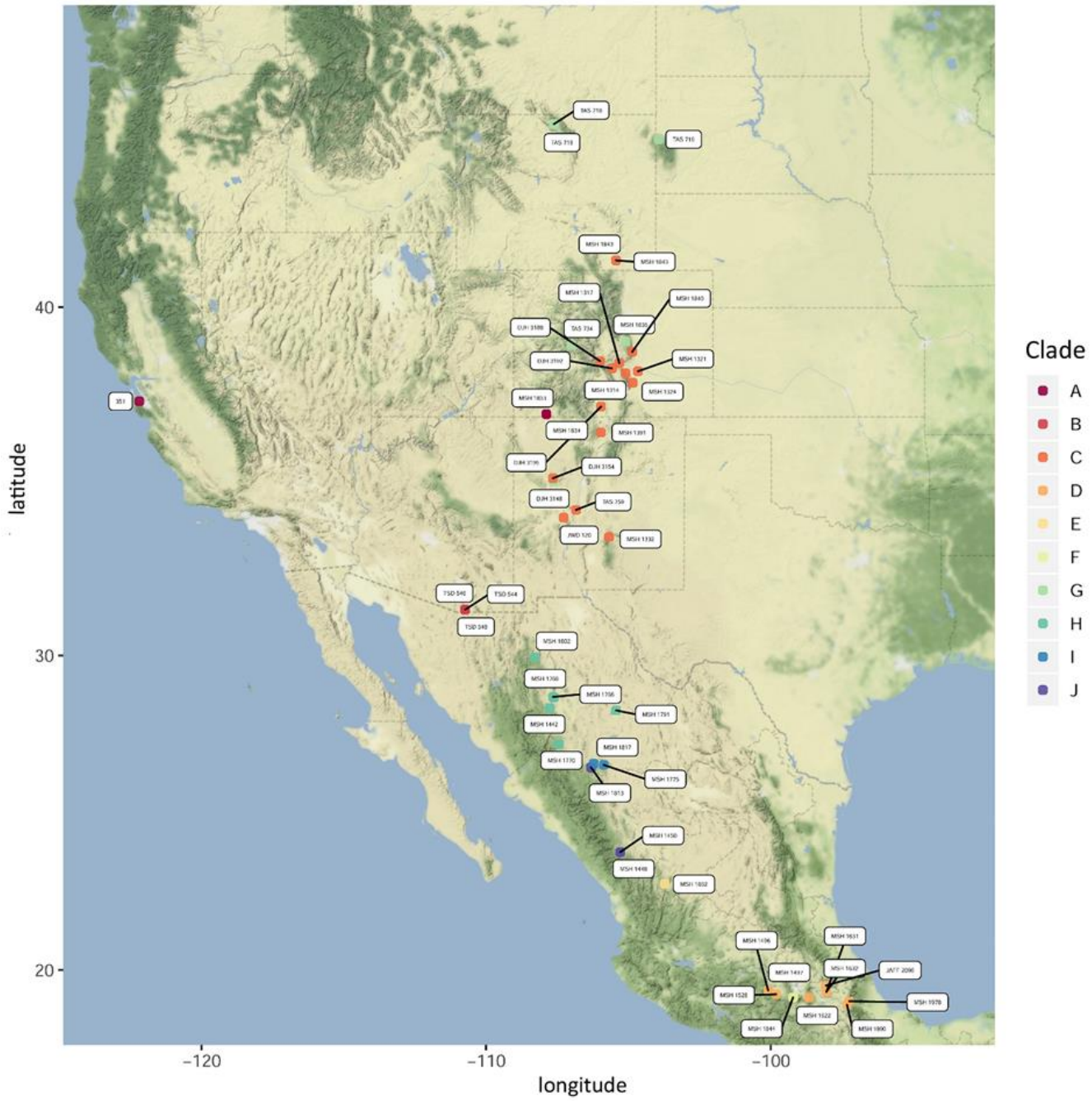
The *minor* complex is the largest, although there is little support for many of the lesser branching present, including *T. birneyi* and *T. zacatacae* as separate nodes, and the stand-out from this group is *T. timmi*, which by far has the most interspecific variation from the rest of the *minor* group (clades A; B; C).

Overall, intraspecific variation between species is large enough that it almost makes identification (if there was no microscope confirmation) confounding. A number of louse samples, even from a different host in the same locality, can create a strong group apart from

the same species. Interspecific variation is more diverse than expected, with some species completely outside the genetic group of their original complex.

Figure 2

Thomomydoecus species genetic diversity across their geographic range



Note. The clades in Fig. 1 (A-J) are further visualized by placing them in localities. Clades diversify towards the equator. Genetically distinct *T. peregrini* is located within the scattered population of clade D, the *neocopei* complex at the base of Mexico. Clade A, which includes *T. byersi* and *T. timmi*, the range of *T. timmi* extends from the west coast of California to Colorado, but only one gopher sample is included in this study. The complex of *wardi* is farthest north on *T. talpoides*, but also clearly occurs within the majority of the *minor* complex on select populations of *T. bottae* in the midst of clade C.

A map made with R programming was used to place these samples and clades with their respective localities and is helpful in gaining insight on how these *Thomomydoecus* species are distributed (Fig. 2). *Thomomydoecus wardi* is the northernmost, *T. minor* is concentrated in the central plains to the west coast of California, and *T. neocopei* is dispersed throughout the central plains down the TMVB. The most genetic similarity is found in the *minor* complex (C), and clades diversify as one progresses down towards the equator, an evolutionary mechanism not supported by only climate but the terrain throughout the TMVB which frequently has splits and breaks that isolates populations of pocket gophers more than a relatively flat terrain.

Discussion

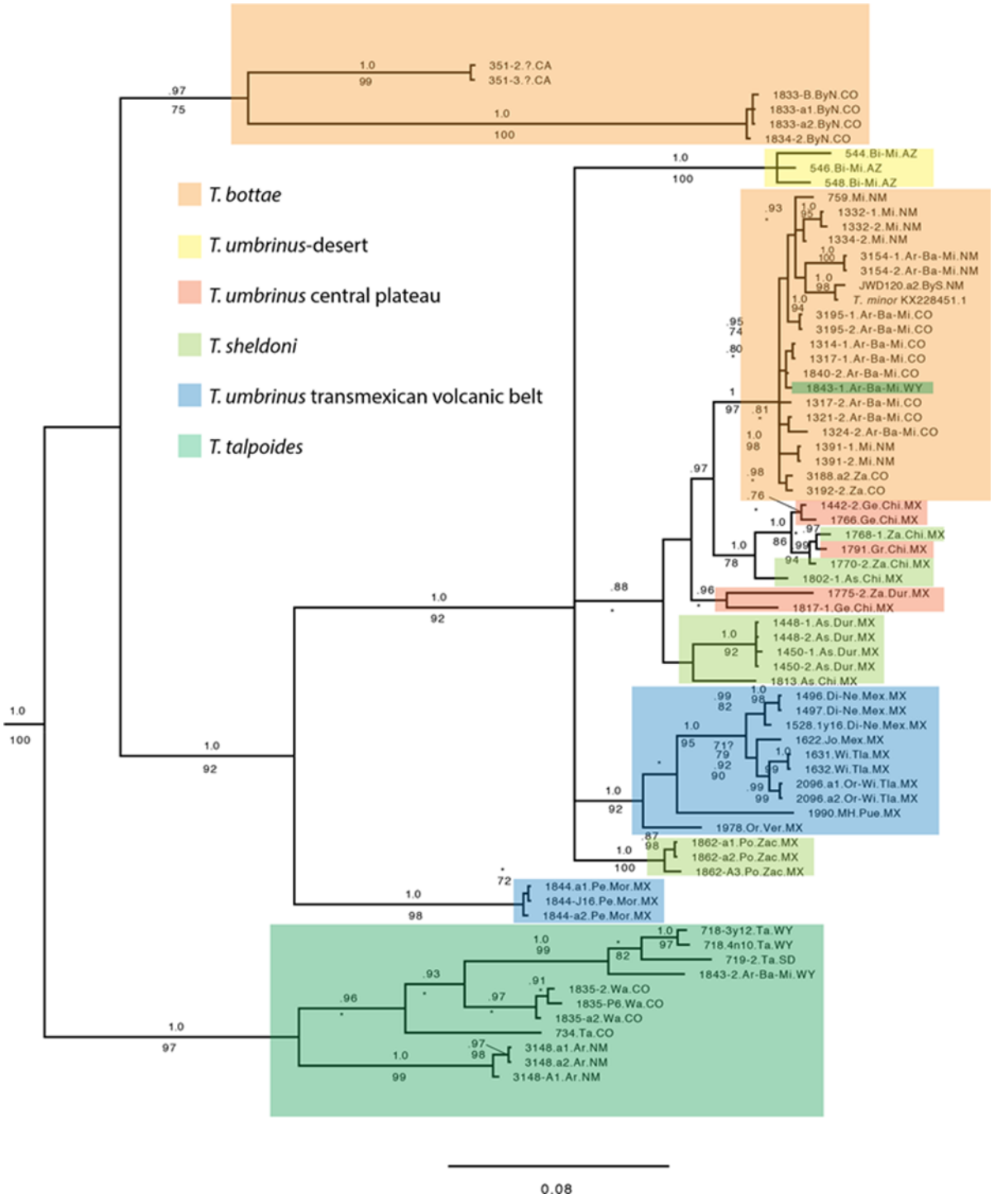
The main goal of this study was to see how *Thomomydoecus* diversifies across its geographic range, interspecific and intraspecific variation, and a comparison to the genus *Geomydoecus*. Although there are certainly signs of codivergence and host specificity in *Thomomydoecus*, this study finds that compared to *Geomydoecus*, *Thomomydoecus* depends more heavily on geographic boundaries. This is to say that in *Geomydoecus* chewing lice, host-

switching to a different gopher species or subspecies can merit a change in *Geomydoecus* species, but *Thomomydoecus* does not follow this trend as closely (Demastes et al., 2012). This is most supported by comparison with *Thomomys* hosts (Fig. 3). The louse clade does not always match with the one species of gopher, some complexes rely on multiple hosts across a population. The best evidence for this is the *neocopei* complex (clade H) which includes the species *T. greeri* and *T. genowaysi* with the host species of *T. sheldoni* and two different subspecies of *T. umbrinus*. The *neocopei* complex is especially complex because of its dispersal over the TMVB. The geography of the region, a volcanic belt that peaks into mountains of high elevation under which it gains the name Sierra Nevada, creates strict borders between populations of pocket gophers, and these subpopulations then diversify over an extended period of time (Verity et al., 2014). Rather than *T. neocopei* being present on two scattered subpopulations of *T. umbrinus* that are separated by mountains, *T. neocopei* would likely populate one side of the mountainous terrain and appear on another host species in the same area such as *T. sheldoni*. A *Geomydoecus* species, on the other hand, based on the mechanisms of codivergence would usually be found on either side of the mountain range and on the same host species (Page, 2003).

In other words, *Geomydoecus* is more closely tied to host genetics whereas *Thomomydoecus* seems to correspond more to geography. This idea can be more thoroughly tested when the present parasite data can be compared to a comparable host dataset when it becomes available.

Figure 3.

Thomomydoecus phylogram compared to general pocket gopher host



Note. Indicates major host species groups (left) for comparison with species and clades of *Thomomydoecus* (Fig. 1). Bayesian and ML probabilities remain the same. The listed host names are extremely, and each group in desert, central plateau, TMVB, central plains *T. bottae*, and northern *T. talpoides*, have subspecies or other species that are not included within this figure, but can be grouped based on geographic ranges.

Thomomydoecus is a newer genus than *Geomydoecus*, so the current trends could change over a span of evolutionary time, but this relationship also holds when one examines the upper ranges in North Dakota through Utah. In comparison to the multitude of species in the TMVB with small ranges, the biological ranges of different *Thomomydoecus species* in the North trends on wider dispersal (Fig. 2) in a flatter, less interrupted terrain. *Thomomydoecus talpoides* hosts the *wardi* complex, but so can populations of *T. bottae* that are close to the end of *T. talpoides* range. Generally speaking, the genus *Thomomydoecus* is not as host specific and has the closest correlation to geographic location and terrain for species dispersal.

From the phylogeny alone (Fig. 1), there is an intricate amount of diversity within the genus of *Thomomydoecus,* with varying amounts of interspecific and intraspecific variation in complexes. Species that are still within the same complex can be less genetically related than one would predict, for example in the traditionally classified *neocopei* complex, the groups *T. greeri* and *T. genowaysi* stand out because of their closer relationship to the *minor* complex (C) rather than the *neocopei* complex (D). Most of all, *T. peregrini* appears to have diverged from the other species earlier. In particular, this species is more closely related to the rest of the

neocopei complex, but it could have undergone genetic drift or another pressure that has led it to diverge slightly from the genetics of the rest of its complex.

Of note are louse samples 1843-1 and 1843-2 from a *T. talpoides* gopher brushing. Louse 1843-1 appears in the *minor* complex, while 1843-2 appears in the *wardi* complex as *T. barbarae*. It is possible for one gopher to host multiple species of *Thomomydoecus* (as discussed in introduction) but the *T. minor* would be out of the range denoted in previous locality maps, which could imply that its range is spreading northward, or that a rare host-switching event occurred where *T. minor* colonized a *T. talpoides*.

There is one incongruence seen in clade A, *T. timmi* and *T. byersi.* Although *T. byersi* should belong to the *wardi* complex, it was most similar to *T. timmi* in the minor complex. It could be that this sample was a mistaken *T. timmi,* despite being from the same locality as a *T. byersi* hosting gopher, or it could indicate that its mitochondrial DNA is more closely aligned with *T. timmi.* Indeed, on its own, *T. timmi* already has a distinct evolutionary distance from the rest of the minor complex that is strongly supported. Unfortunately, the voucher specimen was not of high enough quality to allow microscopic identification.

There is also the possibly that the anomalies discussed above were due to a collection error. In cases where the genetic data received was not of high quality (below 70% consensus), they were excluded from the final results, however, other samples still have possibility to falsely align with a sequence they were not related to. Checking this study's results with further samples from the same gopher host would help reinforce some of the results. This is especially so considering that some of the species represented on the final figures were from a single host

or individual louse sample. This can be done with the use of an additional gene or examine nuclear genes to ensure a species tree rather than a tree based off a single gene.

Studies that extend this research could also investigate the relationship of *T. timmi* and *T. byersi*, as well as look into the genetic difference between *T. peregrini* and the rest of the *neocopei* complex by collecting a larger number of samples. To further the genetic data already gathered in this study, tissue samples of host gophers and associated subspecies should be taken and also used to produce a phylogram, to see to what extent it mirrors that of the *Thomomydoecus* complexes, then estimates of cophylogeny in these host-parasite relationships can be better understood and affirmed. Although, previous studies have typically noted that several species of *Thomomydoecus* already have multiple hosts (Page, 2003), a wide-scale study has the potential to see new discoveries than a study over a single species and host.

Conclusion

This study aimed to test the current morphology-based classification of *Thomomydoecus* and examine genetic variation within the genus. No broad scale study of *Thomomydoecus* chewing louses and their host *Thomomys* had been undertaken before, with all previous studies taking place on a much smaller scale, within a single complex or between a single louse species or clade. Some of the established taxonomy between *Thomomydoecus* species was confirmed, but in multiple cases, a species was more genetically distinct from its designated group than expected. These results form the foundation for future tests of cophylogeny in the *Thomomys*-*Thomomydoecus* assemblage, which can be accomplished by comparing it to a phylogeny of the host gophers when it is available in the future. Research can then work towards establishing a complete cophylogenic record of the genus *Thomomydoecus*

and associated host-genus *Thomomys*. This valuable to understanding the biogeography of North America and how cophylogenic relationships form and are maintained over a period of time. This is especially true because of the possibly that *Thomomydoecus* colonized the genus *Thomomys* after *Geomydoecus*, and research that clarifies this relationship can eventually lead to insight into how this came to be.

Appendix

Field No.	Species of *Thomomydoecus*	LAT	LON	Clade
MSH 1834	*byersi*	37.062	-107.881	A
MSH 1833	*byersi*	37.062	-107.881	A
TAS 351	*timmi*	37.422	-122.186	A
TSD 544	*birneyi*	31.385	-110.741	B
TSD 546	*birneyi*	31.385	-110.741	B
TSD 548	*birneyi*	31.385	-110.741	B
JWD 120	*minor* complex	34.105	-107.275	C
TAS 759	*minor* complex	34.33	-106.84	C
MSH 1332	*minor* complex	33.54	-105.68	C
DJH 3154	*minor* complex	35.244	-107.65	C
DJH 3195	*minor* complex	37.275	-105.96	C
MSH 1314	*minor* complex	38.202	-105.103	C
MSH 1317	*minor* complex	38.482	-105.321	C
MSH 1840	*minor* complex	38.79	-104.865	C
MSH 1321	*minor* complex	38.255	-104.664	C
MSH 1324	*minor* complex	37.935	-104.848	C
MSH 1391	*minor* complex	36.545	-105.965	C
MSH 1843-1	*minor* complex	41.251	-105.436	C
DJH 3192	*minor* complex	38.333	-105.579	C
DJH 3188	*minor* complex	38.535	-105.998	C
MSH 1528	*dickermani*	19.198	-99.81	D
MSH 1496	*dickermani*	19.326	-100.09	D
MSH 1497	*dickermani*	19.326	-100.09	D
MSH 1622	*johnhafneri*	19.085	-98.646	D
MSH 1990	*markhafneri*	18.847	-97.318	D
MSH 1978	*orizabae*	18.966	-97.241	D
JAFF 2096	*orizabae*	19.49	-98.058	D
MSH 1632	*willamsi*	19.281	-98.043	D
MSH 1631	*williamsi*	19.281	-98.043	D
MSH 1862	*potteri*	22.827	-103.72	E
MSH 1844	*peregrini*	19.094	-99.214	F
DJH 3148	*arleneae*	35.143	-107.64	G
TAS 718	*barbarae*	44.751	-107.618	G
TAS 719	*barbarae*	44.368	-103.936	G
MSH 1843	*barbarae*	41.251	-105.436	G
MSH 1843-2	*barbarae*	41.251	-105.436	G
TAS 734	*wardi*	38.981	-107.005	G
MSH 1835	*wardi*	39.077	-105.093	G
MSH 1802	*genowaysi* or *greeri*	29.945	-108.289	H
MSH 1766	*genowaysi* or *greeri*	28.731	-107.648	H
MSH 1442	*genowaysi* or *greeri*	28.388	-107.764	H
MSH 1768	*genowaysi* or *greeri*	27.714	-107.608	H
MSH 1770	*genowaysi* or *greeri*	27.269	-107.446	H
MSH 1791	*greeri*	28.316	-105.431	H
MSH 1817	*zacatacae*	26.655	-106.22	I
MSH 1775	*zacatae*	26.615	-105.864	I
MSH 1813	*asymmetricus*	26.538	-106.315	J
MSH 1448	*asymmetricus*	23.844	-105.288	J
MSH 1450	*asymmetricus*	23.844	-105.288	J

Note. All field numbers denote gopher sample use in study. “Or” indicates that the species is not confirmed, but estimates were made based on locality and genetic similarity. *Minor* complex has been simplified and does not list individual species.

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