Helminths of *Thomomys* Species (Rodentia: Geomyidae) of Oregon

Malorri Rene Hughes
*Portland State University*

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Helminths of *Thomomys* Species (Rodentia: Geomyidae) of Oregon

by

Malorri Rene Hughes

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

Doctor of Philosophy
in
Biology

Dissertation Committee:
Deborah A. Duffield, Chair
Virginia L. Butler
Annie R. Lindgren
Michael T. Murphy
Luis A. Ruedas

Portland State University
2022
Abstract

Although often viewed as inconsequential, parasites have significant ecological and evolutionary importance, and studying them can generate knowledge regarding their environments and their hosts. Helminths (intestinal parasites) infecting Western pocket gophers (*Thomomys* species) in Oregon had been documented based only on morphology prior to this study. Basing parasite identification solely on morphology is problematic because the characteristics used to identify species can be vague or similar, leading to misidentification or obscuring the true biodiversity present. Using molecular markers to verify species present not only is more reliable but also can be informative in terms of the host-parasite association. To more accurately quantify the diversity of helminths present in these hosts and to better understand their associations with their hosts and the environment, I performed the following three studies.

In the first study (Chapter 2), I documented the parasites found in *Thomomys* species in Oregon using a molecular approach. Partial nuclear (either the 18S rRNA gene or the ITS1 region) and mitochondrial (COI gene) sequences were used to construct phylogenetic trees for helminth specimens identified morphologically as *Trichuris fossor*, *Heligmosomoides thomomyos*, *Ransomus rodentorum*, and *Hymenolepis tualatinensis*. The results verified that each of these species represented a distinctive lineage, and that genetic variability was present for each group. Additionally, I documented the presence of what is likely an undescribed species of *Heligmosomoides*.

In the second study (Chapter 3), I used the COI mitochondrial gene to create phylogenetic trees for *Thomomys* species. The two subgenera, *Megascapheus* and
Thomomys formed well-supported monophyletic groups in both the maximum likelihood and Bayesian inference analyses. The COI gene was also used to test for coevolution between Thomomys hosts and the Heligmosomoides species parasitizing them. There was no statistical support for a coevolutionary relationship between Thomomys hosts and their Heligmosomoides species.

In the final study (Chapter 4), I investigated the role of intrinsic and extrinsic factors on helminth infections using a series of statistical analyses. A significant difference in prevalence of overall and H. thomomyos infections among host species was detected. The intensity of overall infections, T. fossor infections, and H. thomomyos infections did not vary among host species. The prevalence of T. fossor infections varied marginally among age classes. There was no significant difference in prevalence or intensities of overall infections, T. fossor infections, or H. thomomyos infections between host sexes. Prevalence and infection intensity did not vary by ecoregion for overall, T. fossor, or H. thomomyos infections. Overall infections, T. fossor infections, and H. thomomyos infections did vary significantly among townships (i.e., the closest town or city) and the intensity of overall infections varied among townships as well. Prevalence of overall infections varied between 2018 and 2019, although intensity of overall infections did not vary between the two collection years. The prevalence of overall infections varied marginally by season and the prevalence of T. fossor and H. thomomyos infections varied seasonally as well.

This dissertation provides further insight into the helminth biodiversity present within Thomomys species, factors that affect infections within these hosts, and the evolutionary relationship between Heligmosomoides species and their hosts.
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Chapter 1: Introduction

Coevolution is a critical process that generates and maintains the complex structure of biological communities and impacts the composition of biodiversity (Thompson, 1998; Guimarã et al., 2011). Relationships between mutualists, competing species, prey and predators, and parasites and hosts are all impacted through coevolution; in some instances, species become so specialized and mutually reliant that they cannot survive without one another (Thompson, 1998). Although coevolution has been well-documented, its ecological impact is only beginning to be understood (Thompson, 1998).

Reciprocal adaptations that are attributable to the presence of one another are an example of coevolution (Page, 1996; Light and Hafner, 2007). Parasites rely on their host(s) for essential resources. When hosts adapt in ways that better protect themselves, the parasite must counteract the increase in resistance and eventually an evolutionary arms race ensues (Hafner et al., 2003). Simultaneous study of host and parasite phylogenies in conjunction offers more insight into coevolution (Page, 1996) and can be enlightening in terms of the parasites’ virulence (degree of harmfulness) (Thompson, 1998; Best et al., 2009). Virulence can change considerably when parasites and hosts have differing mutation rates; even minor changes in the host or parasite can lead to upsurges in virulence (Best et al., 2009).

Pocket gophers (Rodentia: Geomyidae; Appendix A) are ideal coevolutionary study hosts (Hafner et al., 2003; Light and Hafner, 2007). They have low dispersal capacities, are restricted to habitable soils, and their fossorial, solitary lifestyles isolate populations, sequestering parasites on the host ancestry (Smith, 1998; Álvarez-Castañeda and Patton,
Parasites with direct life cycles (i.e., no intermediate host) and insignificant dispersal capabilities are ideal for exploring codivergence and cospeciation between a host and parasite (Light and Hafner, 2007; Callejón et al., 2010). When cospeciation is present, it is a reliable way to assess evolutionary rates among organismal groups (Spradling, 1997) and can eventually give rise to cophylogeny, or analogous host and “associate” phylogenies (Light and Hafner, 2007). Codivergence, cospeciation, and cophylogeny are well-known examples of coevolution from studies on pocket gophers and their ectoparasites (chewing lice; see Light and Hafner, 2007). Constrained distribution patterns produced comparable lice and pocket gopher phylogenies (Hafner et al., 1994). Although there are few studies on the topic, endoparasite-fossorial mammal assemblages seem ideal for coevolutionary studies given their infrequent interactions (Hafner et al., 2003). Endoparasite assemblages appear to have patterns of host specificity similar to those of lice (Gardner et al., 2020); a similar coevolutionary relationship could therefore exist between geomyids and their endoparasites. Given the diversity and potential high degree of host specificity, phylogenetic hypotheses should be formed for each host-endoparasite group (Gardner, 1985; 2001). The distinctive endoparasite assemblages among geomyids could be a result of deep evolutionary associations (Gardner, 1985; 2001).

Geomyids are endemic to the New World and are distributed from southern Canada to northern South America (Fernández et al., 2014). Fossils date to the late Miocene and are consistent with a wide-ranging distribution (Makarikov et al., 2012; Fernández et al., 2014). Extant species possess exterior, fur-lined cheek pouches, incisors projecting through the lips, small eyes and ears, short, sparsely-haired tails, fusiform bodies,
plantigrade and pentadactyl feet, and dense, dorsoventrally-flattened skulls (Verts and Carraway, 1998).

Despite these synapomorphies, Geomyidae is one of the most diverse mammal families (Verts and Carraway, 1998; Mathis et al., 2014). Of the five geomyid genera, Cratogeomys and Thomomys are the most speciose (Fernández et al., 2014). It is common for intraspecific phenotypic or genetic variation of geomyids to surpass those of distinct species or even genera of other mammals (Verts and Carraway, 1998; Mathis et al., 2014). Allozyme and karyotype data aided in separating some species of geomyids (Baker and Bradley, 2006). While mitochondrial divergence values are useful for detecting cryptic species in other mammal groups (Baker and Bradley, 2006), they are insufficient for geomyids due to abnormally high evolutionary divergence rates (Mathis et al., 2014). Multilocus approaches have tended to measure genetic isolation and divergence more accurately (Mathis et al., 2014).

Geomyids construct and live in complex belowground tunnel systems (Reichman and Seabloom, 2002). They are considered ‘ecosystem engineers’ or ‘bioengineers’ (Reichman and Seabloom, 2002; Kerley et al., 2004) because they physically alter their environments and, consequently, impact coexisting species and affect community dynamics (Decker et al., 2019). Bioengineers that alter soil characteristics are particularly valuable and have an array of ecosystem impacts that ultimately promote environmental heterogeneity (Kerley et al., 2004; Mallen-Cooper et al., 2019). For example, pocket gophers increase soil fertility (Verts and Carraway, 1998). Soil disturbances decrease erosion and run-off, and increase soil water availability, infiltration, permeability, nutrient abundance and dispersal, and enzymatic activity (Kerley et al., 2004; Decker et
Nutrient-rich soil ejecta are ‘hotspots’ for plants, which increases recruitment, productivity, abundance, and richness (Kerley et al., 2004; Decker et al., 2019; Mallen-Cooper et al., 2019). Eventually, “resource-rich”, secure areas are established and used by invertebrates and other vertebrates (Decker et al., 2019). Animals that exploit vacated burrows are more abundant when burrowing mammals are present (Stinson, 2020; Mallen-Cooper et al., 2019).

Loss of keystone species, such as bioengineers, can initiate decreases in ecosystem functions (Smith and Foggin, 2006; Decker et al., 2019). Although promoting rodent conservation can be challenging given that many people find them off-putting or regard them as pests (Fernández et al., 2014), pocket gophers are sensitive to habitat fragmentation and climatic changes (Hadly, 1997; IUCN, 2000) and are keystone species (Stinson, 2020).

*Thomomys* (western or smooth-toothed pocket gophers) occupy the western portion of the United States from southwest Canada to southern Mexico (Belfiore et al., 2008; Fig. 1). Dividing *Thomomys* species into two subgenera, *Megascapheus* and *Thomomys*, is supported by morphological and molecular evidence (Belfiore et al., 2008). However, uncertainty regarding species delineations within these groups remains due to high phenotypic and genetic variability among species (Belfiore et al., 2008).

have been used to separate species (see Belfiore et al., 2008), but these features vary widely among individuals and populations (Smith, 1998), so results from the literature can be ambiguous or contradictory. Furthermore, hybridization is also known to occur between some well-supported species (Thaeler, 1968, 1974; Patton and Dingman, 1968; Hoffmeister, 1969; Patton et al., 1979, 1984; Patton and Smith, 1989; Mathis et al., 2013a). When a species has recently derived from geographically isolated populations within the larger range of a parent species, the parent species can be consistently paraphyletic (Belfiore et al., 2008). Paraphyletic groups are ones that include the most recent ancestor for a set of organisms, but not all of the descendants. Conserved ancestral polymorphisms or divergent lineage reticulation can produce seemingly resolved trees that, in fact, depict erroneous phylogenetic relationships among species (Patton and Smith, 1994; Belfiore et al., 2008; Mathis et al., 2013a). For these reasons, resolving the taxonomy of *Thomomys* has proven to be difficult.

Five of the currently recognized species of *Thomomys* are found in Oregon: *Thomomys bottae, Thomomys townsendii, Thomomys bulbivorus, Thomomys talpoides,* and *Thomomys mazama* (Mammal Diversity Database, 2020; Figs. 1, 2). Geomyid distributions typically are allopatric or parapatric (Verts and Carraway, 1998). Oregon species are an exception in that each has a distribution that is (or recently was) sympatric with one or more other species (Verts and Carraway, 1998).

The subgenus *Thomomys* consists of *T. mazama, T. monticola,* and *T. talpoides,* while the subgenus *Megascapheus* contains *T. atrovarius, T. bottae, T. bulbivorus, T. townsendii, T. sheldoni,* and *T. umbrinus* (Appendix A). Within the subgenus *Megascapheus, T. bottae* and *T. townsendii* once were considered conspecific with *T.
and separating the three from one another is challenging (Hall, 1981; Jones and Baxter, 2004). Taxonomy of this complex remained in flux for over a century as a consequence of exceptionally high morphological and genetic diversity within the group (Jones and Baxter, 2004; Álvarez-Castañeda, 2010). *Thomomys bottae* alone has one of the highest levels of genetic diversity of any mammal species (Patton, 1972; Hall, 1981; Patton and Smith, 1990; Verts and Carraway, 1998; Smith, 1998; Patton, 2005; Belfiore et al., 2008), and allozyme and mitochondrial data have confirmed the existence of several distinct geographic units of *T. bottae* (Patton and Smith, 1990; Smith, 1998; Belfiore et al., 2008; Álvarez-Castañeda, 2010). Highly variable interpopulation genetics is typical for *Thomomys* species, with genetic drift and gene flow levels existing as products of stochastic events (Carraway and Kennedy, 1993). Genetic characterizations suggest speciation independent of genetic differentiation, that the degree of variation among *Thomomys* populations is unrelated to reproductive compatibility, and that the degree of heterozygosity does not appear to be related to size of species’ distributions (Carraway and Kennedy, 1993).

Interpopulation variability is low in *T. townsendii*, however (Carraway and Kennedy, 1993) and *T. townsendii* may have derived independently of neighboring *T. bottae* populations (Álvarez-Castañeda, 2010). In *T. townsendii*, disconnected populations align with historic Great Basin drainage boundaries in southeastern Oregon (Verts and Carraway, 2003).

*Thomomys bulbivorus*, the only monotypic *Thomomys* species occurring in Oregon, is endemic to the Willamette Valley and has a relatively small distribution (about 8,000 km$^2$) (Verts and Carraway, 1998; Figs. 1, 2). It is the largest member of the genus, with
adult males that can exceed 500 grams (Verts and Carraway, 1998; this study). Elliot (1903) erected the subgenus *Megascapheus* for *T. bulbivorus* based on body size disparity and unique skull features (Verts and Carraway, 1987). Genetically, *T. bulbivorus* is well separated from, and is the sister group to, the remaining species of *Megascapheus* (Belfiore et al., 2008). Carraway and Kennedy (1993) reported considerable diversity among sites and high levels of heterozygosity with limited inbreeding for *T. bulbivorus*.

*Thomomys talpoides* inhabits a broader range of habitats and is more widely distributed than some of the other Oregon species (Verts and Carraway, 1998, 1999). This species is found from southwestern Canada to the lower southwestern United States, and from the Midwest to the Pacific Northwest (Verts and Carraway, 1998, 1999). Interpopulation morphological, chromosomal, and genetic disparities indicate *T. talpoides* is likely a species complex (Verts and Carraway, 1999; Belfiore et al., 2008).

*Thomomys talpoides* and *T. mazama* are the only Oregon species belonging to subgenus *Thomomys* (Verts and Carraway, 1998; see Appendix A). They appear to be sister taxa based on nuclear and mitochondrial phylogenies, but lineage sorting may be incomplete (Belfiore et al., 2008). Based on diploid chromosome number variations and hypothesized biogeographic origins, *T. mazama* may be polyphyletic (Verts and Carraway, 2000). Some hypothesize that *T. mazama* speciated from isolated *T. talpoides* populations that were south-southwest of the Cascades during the Pleistocene and then dispersed to occupy the grasslands from southern Washington to northcentral California, where it is found today (see Verts and Carraway, 2000). Many historic populations of *T.*
mazama have been greatly reduced or have gone extinct, and several subspecies in Washington are endangered and under federal and state protection (see Stinson, 2020).

Defining taxonomic boundaries and monitoring genetic variability and gene flow are imperative for conservation management. Because this remains challenging for Thomomys due to the above-mentioned taxonomic issues, it is critical to evaluate genetic data from endoparasites of Thomomys to try to resolve host systematics and evaluate coevolutionary relationships with their parasites. In addition, such a study will fill gaps of knowledge regarding endoparasite biodiversity, evolutionary relationships, population dynamics, and host-associations.

Disparate host population structures or phylogenies can contribute to distinctive parasite assemblages (Krasnov et al., 2012). For instance, host-specific louse species do not cross T. bottae-T. townsendii hybridizing zones (Patton et al., 1984). Connected host populations will presumably have more similar parasite assemblages than populations that are isolated. An alternative hypothesis is that, unlike ectoparasites, endoparasites do not have life cycles that restrict them to a host population or host species. If host relationships correlate to helminth diversity, more closely related Thomomys species may have more similar helminth assemblages.

Although they occupy critical ecological niches and play prominent evolutionary roles, the diversity of parasites has been underestimated, and population dynamics, phylogenies, and host-associations remain unclear for many groups (Bordes and Morand, 2009; Brooks et al., 2014; Nichols and Gómez, 2011). Documenting parasite diversity has intrinsic value because parasites comprise most of the earth’s species diversity and are often at a higher risk of decline and extinction than free-living organisms (Nichols
Parasite conservation is considered unimportant by many, yet arguments established for preserving other groups of organisms also apply to parasites: medicinal uses of parasite species may be beneficial to humans (e.g., immunosuppression therapy), and the loss of a parasite can modify community or ecosystems interactions (Gompper and Williams, 1998; Hudson et al., 2006). Maintaining host-specific parasite populations is sometimes necessary for the survival of the host population because the loss of a species can modify competition among surviving parasites, possibly to the hosts’ disadvantage (e.g., opening a niche for other, potentially more harmful, parasites) (Gompper and Williams, 1998). To promote conservation, quantify and maintain biodiversity, and advance our knowledge of ecological relationships, comprehensive records of parasite species, including their host preferences, prevalence, and geographical origins, must be established (Brooks et al., 2014).

Parasite diversification arose through periodic climatic and environmental changes in concurrence with host switching, and today parasites are ubiquitous and found in abundance throughout earth’s ecosystems (Brooks et al., 2014). Documenting distribution patterns enhances our perception of historic fluctuations across temporal and spatial scales (Brooks et al., 2014). Environmental or ecological disruptions that cause successive surges of radiation with deviations from ancestors increasing exponentially over time can induce rapid host switching and disease expansion (Brooks et al., 2014). Additional research allows us to improve our ability to predict comparable shifts in other areas, behavior of close relatives under similar circumstances, and effects of climatic changes on infections (Brooks et al., 2014).
Parasites have long-standing relationships with their native vegetation, crop, livestock, wildlife, and/or human hosts (Brooks et al., 2014) and cause diseases that have socioeconomic effects on “hundreds of millions of” people worldwide each year (Cantacessi et al., 2012). Distribution data can be used to create models that predict habitable environments in understudied regions or how changes may alter distributions. Furthermore, distribution data can be used to create informed protocols to help detect, control, or prevent the spread or emergence of diseases (Brooks et al., 2014). Understanding parasite evolution and population genetics enhances the accuracy of these predictions (Blouin et al., 1995; Wu et al., 2009). They also can reveal information about other species’ evolutionary histories, coevolution, and enable the detection of on-going host-switching or colonization (Brooks et al., 2014).

Compared to other mammalian groups, geomyids have one of the most comprehensive parasitic nematode (‘roundworm’) datasets (Gardner, 2001). Six of the approximately eleven parasitic nematodes described are specific to Geomyidae (Gardner, 2001). *Trichuris fossor* (Trichuridae), *Heligmosoides thomomyos* (Heligmosomidae), *Ransomus rodentorum* (Strongylidae), and *Vexillata vexillata* (Ornithostrongylidae) have been documented in *T. bottae*, *T. bulbivorus*, and/or *T. talpoides* based on morphologies and host preferences (Hall, 1916; Chandler, 1945; Tryon, 1947; Lubinsky, 1957; Frandsen and Grundmann, 1961; Douglas, 1969; Todd et al., 1971; Jasmer, 1980; Gardner and Jasmer, 1983; Gardner, 1985).

The helminths, or intestinal ‘worms’, such as parasitic nematode or cestode species (Appendix B), of *T. bottae*, *T. bulbivorus*, and *T. talpoides* were described based on morphology (see Gardner, 1985, Gardner and Schmidt, 1988, and Makarikov et al., 2012.
for an overview). However, for these reports, only the *T. bulbivorus* hosts were from Oregon (Gardner and Jasmer, 1983; Gardner, 1985; Makarikov et al., 2012). Reports from *T. bottae* hosts are from California or Colorado (see Gardner, 1985, and Gardner and Schmidt, 1986) and reports from *T. talpoides* are from hosts from Alberta (Canada), California, Colorado, Montana, Utah, Washington, and Wyoming (see Gardner and Schmidt, 1986, 1988). To date, the remaining species in Oregon, *T. townsendii* and *T. mazama*, have not previously been surveyed for helminths, although Stinson (2020) speculated that *T. mazama* was likely to be infected with helminths based on *T. talpoides* and *T. bottae* accounts.

*Trichuris fossor* (a ‘whipworm’) has been reported only from hosts belonging to the genus *Thomomys* (Todd and Lepp, 1972; Gardner, 1985; but see Falcón-Ordaz, 1993), although *Trichuris* species (‘whipworms’) inhabit the ceca of a wide variety of mammalian hosts worldwide (Robles et al., 2018). Species descriptions and reports are based mostly on biometrics, morphology, host(s) infected, and/or geography (Robles et al., 2018; Eberhardt et al., 2019). These characteristics are not reliable in all instances because *Trichuris* species have similar attributes with overlapping ranges of measurements (Callejón et al., 2015) and host preference may be more variable than previously thought (Doležalová et al., 2015). Many of the 29 species reported from rodents exhibit comparable morphologies (Falcón-Ordaz et al., 2020; Ribas et al., 2020).

*Heligmosomoides thomomyos* has been documented only from *T. bottae* and *T. bulbivorus* (Gardner and Jasmer, 1983; Gardner, 1985), however, Gardner and Jasmer (1983) suspected *H. thomomyos* could occur in *T. mazama* and other Pacific Northwest geomyids because it is not host-specific to the species level.
Ransomus rodentorum has been reported from *T. bottae* (Jasmer, 1980), *T. bulbivorus* (Gardner, 1985), *T. talpoides* (Hall, 1916; Frandsen and Grundmann, 1961; Todd et al., 1971), *T. umbrinus* (Frandsen and Grundmann, 1961), and *Geomys bursarius* (Geomyidae; Bartel and Gardner, 2000). *Ransomus rodentorum* is found only at low intensities (below ten individuals/host; Grundmann et al., 1976; this study).

The cestodes (‘tapeworms’) *Arostrilepis horrida* (but see Makarikov et al., 2011), *Hymenolepis tualatinensis, Arostrilepis schilleri, Hymenolepis citelli,* and *Hymenolepis dimunita* (all belong to family Hymenolepididae) have been documented from *Thomomys* species that occur in Oregon (see Gardner et al., 2020). Additional rodent-infecting hymenolepidids (*Hymenolepis weldensis* and *H. diminuta*) have been successfully transmitted to *T. mazama* and *T. talpoides* in a laboratory setting (Gardner, 1985; Gardner and Schmidt, 1988; Gardner et al., 2020).

From the early 1900’s to the 1980’s and 1990’s, the assumption was that hymenolepidid cestodes infecting rodents had extensive, and frequently intercontinental, distributions, were noticeably different morphologically, and had no apparent host restrictions (Makarikov et al., 2015). Since then, however, it has become apparent that hymenolepidids are more specialized and much more diverse than previously thought—approximately 19 species of *Hymenolepis* (s. str.) that parasitize rodents alone now are recognized (Makarikov et al., 2015). Given this, there justifiably has been much uncertainty over hymenolepidid taxonomy, and in recent decades there have been several revisions (Makarikov et al., 2015). Given the amount of hidden or weakly separated hymenolepidid species revealed recently from small mammal groups, including rodents, substantial diversity, presumably, still is concealed (Makarikov et al., 2015).
*Hymenolepis tualatinensis* was described from *T. bulbivorus* by Gardner in 1985 and had not been documented again until the present study. *Arostrilepis schilleri* was described from *T. bulbivorus* by Makarikov et al. (2012) based on *T. bulbivorus* specimens collected in the 1980’s. New species of hymenolepidids from closely-related small Nearctic mammals, such as pikas (Order Lagomorpha) and other rodents (e.g., voles and deer mice, Family Cricetidae), including other geomyids (*Cratogeomys* and *Heterogeomys* species), have been described in recent years (e.g., Makarikov et al., 2011, 2015; Makarikov and Tkach, 2013; Gardner et al., 2020; see Makarikov et al., 2012). An extensive association with *Thomomys* species in Oregon could have led to differentiation among hymenolepidids either regionally (Makarikov et al., 2012) or by host groups, but this has not yet been evaluated.

The cestode genus *Arostrilepis* was erected in 1997 from *Hymenolepis* (Mas-Coma and Tenora, 1997). Accounts of hymenolepidids that predate the genera being split (Mas-Coma and Tenora, 1997), and especially records of *A. horrida (= H. horrida)*, need to be reevaluated (Makarikov et al., 2012). Previously, *A. horrida* had been reported from several families of rodents worldwide although in actuality a complex of species was being referenced (Makarikov et al., 2011, 2015). The distribution of *Arostrilepis* within *Thomomys* hosts indicates either a broadly distributed species is present or that there is an extensive compilation of isolated species (Makarikov et al., 2012). Reports of *T. bulbivorus* and *T. bottae* as *A. horrida* hosts require substantiation because details on distinguishing morphological characteristics were limited or “insufficient” for definitive species identification and the museum specimens from original accounts cannot be reevaluated because they are missing or inaccessible (Makarikov et al., 2012).
Except for the *A. schilleri* description (Makarikov et al., 2012), which used museum specimens collected in the 1980’s, there have been no recent publications on helminths infecting geomyids in Oregon. There may still be undescribed species of hymenolepidids infecting geomyids (Makarikov et al., 2012), especially in host species that have not yet been studied. No studies of genetic diversity exist, but the genetic diversity of geomyid endoparasites will likely uncover new species (Gardner, 2001) and may reveal ancestral lineages.

To form connections and properly interpret, apply, and share data, it is important that the taxonomy and systematics of parasite groups are resolved and uniformly recognized. A sole reliance on morphological characteristics for species identification or phylogenetics can be imprecise due to morphological convergence or phenotypic variation based on host(s), geography, or environmental factors (Perkins et al., 2011). This can contribute to misestimations of biodiversity by obscuring cryptic species and does not always accurately depict finer-scale relationships (Perkins et al., 2011). Systematic and taxonomic studies of parasites incorporating molecular data result in more reliable results and can yield data that allow inferences about environments they inhabit, host-parasite evolutionary associations, and host histories to be made (Whiteman and Parker, 2005; Perkins et al., 2011).

Parasite genetics is an underutilized resource, especially when hosts have complicated population genetic structures or genealogies (Whitman and Parker, 2005). Evaluation of parasite DNA can potentially help resolve the host’s evolutionary history and relationships as well (Whiteman and Parker, 2005). Because parasites are inherited from previous generations in a similar manner to heritable genes and are subject to
comparable biological forces, their DNA can be thought of as an unexplored host gene (Demastes et al., 2003; Page, 2003). Parasite DNA tends to evolve more rapidly than that of the host, which can improve the resolution of convoluted host systematics (Whiteman and Parker, 2005). In addition, parasite evolution is exceedingly receptive to the hosts’ evolution (Best et al., 2009) and increased genetic variation for parasites relative to that of the host can be anticipated (Whiteman and Parker, 2005). Examining how genetic variation is distributed among host populations can expose the hosts’ phylogenies even before their own DNA has coalesced (Whiteman and Parker, 2005).

The conservation of a host species or group is made easier if a holistic view of their biological and natural histories, including their parasite fauna and variations or trends pertaining to parasitic infections, is obtained. Parasite infections can influence host population dynamics in a number of ways (Hudson et al., 2006). In some host-parasite systems, older hosts are more likely to be infected (Hamid et al., 2015; Hughes et al., 2018); in others, one host sex may be more commonly or more heavily parasitized (i.e., host sex bias; Córdoba-Aguilar and Munguia-Steyer, 2013). Different parasite groups can correlate with different geographic areas in hosts, particularly in host species with distributions spanning multiple ecosystems (Bafundo et al., 1980). Environmental variables, such as humidity or annual temperature, and presence of intermediate hosts, can affect infections as well (Jasmer, 1980; Córdoba-Aguilar and Munguía-Steyer, 2013; Hamid et al., 2015).

*Project Overview*
To assess taxonomy, biodiversity, systematics, and coevolution within *Thomomys* and their helminths, I collected 134 new specimens representing all of the *Thomomys* species occurring in Oregon between March 2018 and November 2019 (Fig. 2; Appendix C). Twenty-five *T. bulbivorus* were salvaged from professional trappers; the remaining 60 *T. bulbivorus*, 7 *T. bottae*, 5 *T. mazama*, 28 *T. talpoides*, and 9 *T. townsendii* were collected using lethal traps following American Society of Mammalogy Animal Care and Use Best Practices (Sikes et al., 2016; PSU IACUC #66, ODFW Permit 055-19). The intestinal tract of each pocket gopher was examined for helminths following procedures outlined by Gardner and Jasmer (1983).

During specimen preparations, standard body measurements (total body, tail, hind foot, and ear lengths), mass, sex, and reproductive condition (e.g., lactating, pregnant, testes conditions) were recorded, frozen tissue samples (typically heart, liver, and kidney) were taken, and ectoparasites and cheek pouch contents were stored in 95% EtOH. Skeletons were dehydrated, refrozen, and subsequently cleaned in a dermestid beetle colony. The infection status (un/infected), initial helminth species identifications (determined by previous host records, location within host, and morphology; see Gardner and Schmidt, 1988; Makarikov et al., 2012), and intensity (number of parasites/host) of infections per helminth species were recorded during intestinal examinations. Helminths were stored in 95% ethanol and frozen for sequencing. In many instances, a subset was also stored in 10% buffered formalin for subsequent mounting or deposition in museum collections.

**Chapter 2** used molecular data to document and verify helminth species present in *Thomomys* hosts in Oregon to test the hypothesis that the helminths *T. fossor, H.*
thomomyos, R. rodentorum, and H. tualatinensis represented distinctive lineages. This study represents the first sequence data for helminths taken from geomyid hosts (Eberhardt et al., 2019). Phylogenies were generated from nuclear (18S rRNA, ITS1 region) and mitochondrial (COI mtDNA) DNA sequences. Nuclear markers were used to confirm initial identifications and COI mtDNA was used to better test finer scale resolution of relationships.

Chapter 3 used COI mtDNA to reconstruct a host phylogeny for Thomomys species of Oregon and tests the hypothesis that coevolutionary associations among the Heligmosomoides-Thomomys assemblage exist by comparing host and parasite COI phylogenies. Corresponding branch lengths and patterns were used to test whether cospeciation or cophylogeny was occurring (Hafner et al., 1994; Light and Hafner, 2007). Congruence between Heligmosomoides and Thomomys phylogenies was tested by carrying out a goodness-of-fit test on superimposed phylogenies to detect significant associations between taxa using jackknife estimations (Balbuena et al., 2013). This is the first study to test coevolutionary relationships among geomyids and their helminths.

Chapter 4 combines data collected during field sampling efforts with specimen preparations to test the hypothesis that intrinsic and extrinsic variables impact the prevalence and intensity of helminth infections in Thomomys populations. Specifically, the effects of host species, age, sex, collection ecoregion, township, locality, year, and season are all examined to analyze their effect on the prevalence and intensity of Thomomys helminth infections.

Chapter 5 affords conclusions for Chapters 2, 3, and 4 and outlines future work that would continue to offer resolution on these areas.
Figure 1. Distributions for nine of the currently recognized *Thomomys* species. Spatial data were not available for *T. atrovarius, T. nayarensis, T. sheldoni*, which were more recently elevated to species but were previously considered to belong to the *T. umbrinus* group (Hafner et al., 2011; Mathis et al., 2013a, 2013b).
Figure 2. *Thomomys* species distributions in Oregon and collection sites for this study.
Chapter 2: Molecular Characterization and Phylogenies of Helminths Infecting *Thomomys* Species of Oregon

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**Introduction**

Genetic data add to knowledge of biodiversity and evolutionary relationships of living organisms. For certain groups, such as parasites, utilizing molecular data rather than morphological characters alone is a more reliable approach for species identification, taxonomic resolution, and inferring evolutionary histories (Blouin, 2002; Perkins et al., 2011; Callejón et al., 2015, 2016; Vejl et al., 2017). Such data not only enable the identification of cryptic species, but also help illuminate host-specificities. The aim of this chapter was to provide molecular characterizations to assess species taxonomy and phylogenetic relationships for three nematode species, *Trichuris fossor* (Trichuridae), *Heligmosomoides thomomyos* (Heligmosomatidae), and *Ransomus rodentorum* (Strongylidae), and two cestode species of the family Hymenolepididae, *Hymenolepis tualatinensis* and *Hymenolepis citelli*, from *Thomomys* hosts.

*Trichuris fossor*
Nematodes of the genus *Trichuris* (‘whipworms’) inhabit the ceca of a wide variety of mammalian host species worldwide (Callejón et al., 2015; Robles et al., 2018). Approximately 85 species are described based mostly on reports that have used biometrics, morphological features, host(s) infected, and/or biogeography (Guardone et al., 2013; Doležalová et al., 2015; Callejón et al., 2016; Eberhardt et al., 2019; Falcón-Ordaz et al., 2020; Ribas et al., 2020). These characters are not reliable in all instances because many species have similar attributes with overlapping ranges of measurements (Callejón et al., 2015, 2016; Vejl et al., 2017) and host preferences may be more variable than previously thought (Doležalová et al., 2015). Approximately 29 species have been reported from rodents, with many of the species exhibiting similar morphological attributes (Robles et al., 2014; Doležalová et al., 2015; Eberhardt et al., 2019; Falcón-Ordaz et al., 2020; Ribas et al., 2020). *Trichuris fossor* has been reported only from host species belonging to the genus *Thomomys* (Rodentia: Geomyidae; Todd and Lepp, 1972; Gardner, 1985; but see Falcón-Ordaz, 1993). Descriptions have been based on morphology and host preference and *Trichuris* from geomyid hosts had never been sequenced prior to this study (Eberhardt et al., 2019; Hughes et al., 2020).

Understanding the diversity and phylogeny of whipworms is important. False classification limits our acuity of biogeography and conceals the zoonotic potential of trichurids (Callejón et al., 2015; Doležalová et al., 2015). Certain species (e.g., *T. suis* and *T. trichiura*) are problematic in developing countries and have vast socioeconomic impacts via human or livestock infections; nearly 1 billion human trichuriasis infections are reported globally each year (Jex et al., 2014). Other species, such as *T. muris* have been gathering attention in biomedical research for potential use in immunosuppression.
therapy (Feliu et al., 2000). A more comprehensive understanding of relationships within this group would enable predictions about how close relatives interact with their host(s).

Relationships within Trichuridae have not been well-resolved using genetic approaches. Results differ depending on the gene(s) sequenced and the approach used for phylogenetic reconstructions (Callejón et al., 2015). Mitochondrial data, primarily cytochrome oxidase subunit I (COI), have been used commonly and has resulted in high resolution of closely related lineages; however, it may be less credible to use with Trichuris species due to the degree of hybridization and maternal mitochondrial heredity seen in this genus (Callejón et al., 2015; Doležalová et al., 2015). Nuclear data have provided higher support for relationships than mitochondrial data (Doležalová et al., 2015). The nuclear ITS1-ITS2 RNA genes offer markers that allow closely related species to be discriminated (Eberhardt et al., 2019) and ITS1-5.8S-ITS2 has been used to show relationships among ruminant- and rodent-infecting species (Doležalová et al., 2015). However, the number of variants of RNA genes (including the ITS2 region) makes their utility in disentangling the phylogeny of Trichuris less favorable, particularly given that the amount of trichurid ploidy is unknown (Doležalová et al., 2015). To date, both nuclear and mitochondrial data have suggested that Trichuris may be a polyphyletic genus; species or groups within the genus, e.g., T. trichiura and T. suis, also may be polyphyletic (Doležalová et al., 2015). However, the 18S rRNA gene has been used to infer the placement of trichurids within Nematoda as well as to elucidate relationships within Trichuridae and is less prone to result in unclear multiple alignments (Guardone et al., 2013; Callejón et al., 2013, 2015; Doležalová et al., 2015).
Heligmosomoides thomomyos

Jasmer (1980) reported the presence of an unidentified *Heligmosomoides* species (Heligmosomidae) in 23% of Botta’s pocket gophers, *Thomomys bottae*, from California. Gardner and Jasmer (1983) later described this as *Heligmosomoides thomomyos* based on morphological features and suspected that *H. thomomyos* could occur in other Pacific Northwest geomyid species. There has been one report of *H. thomomyos*, from *Thomomys bulbivorus* (Gardner, 1985), supporting the hypothesis that *H. thomomyos* is not host-specific to the species level.

The systematics and host specificities of species in the genus *Heligmosomoides* remain ambiguous (Cable et al., 2006; Behnke and Harris, 2010; Clough and Råberg, 2014), and North American forms are particularly understudied (Harris et al., 2015). Elucidating relationships within the genus are important because *Heligmosomoides* species are commonly used in immunological studies and as models for helminth infections in humans and livestock (Cable et al., 2006; Behnke and Harris, 2010; Maizels et al., 2012). Molecular studies can help quantify host specificities (Clough and Råberg, 2014) and resolve systematics-related issues by increasing the certainty of species delineations (Harris et al., 2015) as heligmosomatid species can be molecularly distinctive despite displaying morphological similarities (see Zaleśny et al., 2014). Specifically, the mitochondrial COI gene is sufficient for species-level identification of *Heligmosomoides* (Clough and Råberg, 2014).

Ransomus rodentorum
Ransomus rodentorum (Nematoda: Strongylidae) has been reported from Thomomys species and Geomys bursarius (Rodentia: Geomyidae) hosts based on morphology (Hall, 1916; Todd et al., 1971; Jasmer, 1980; Gardner, 1985; Bartel and Gardner, 2000). Given this, and the fact that few studies on this nematode exist, the classification of R. rodentorum is tentative. As the only member of this genus, clarifying its classification could be advantageous for resolving the systematics of strongylids.

Hymenolepis species

The family Hymenolepididae (Cestoda) also significantly affects human health issues on a global scale: Rodentolepis (= Hymenolepis) nana is the most prevalent human cestode worldwide and, although uncommon, Hymenolepis diminuta (the rat tapeworm) can also infect humans, especially children (Macnish et al., 2002a). Although as many as 75 million people are parasitized by R. nana worldwide (Macnish et al., 2002a), disagreement over the taxonomic status of this species, along with others within the family, still exists (Macnish et al., 2002b; Binkienė et al., 2019).

Hymenolepididae has recently undergone several revisions and the original genus, Hymenolepis, likely is comprised of numerous species complexes (Makarikov and Tkach, 2013). The genera Arostrilepis and Rodentolepis were erected recently from Hymenolepis (Macnish et al., 2002a; Makarikov and Tkach, 2013). Species still belonging to the genus Hymenolepis evidently form a strongly supported monophyletic group that infect a single hedgehog and several rodent species (Binkienė et al., 2019). Hymenolepidids are, with some exceptions, highly host-specific, with most showing specificity to at least to the level of host genus, but sometimes to the family or subfamily level (Makarikov and
Tkach, 2013). The genus *Hymenolepis* is significant because species infect hosts from two separate orders, Eulipotyphla (which includes the hedgehog) and Rodentia (Binkienė et al., 2019).

Because they have the potential to serve as reservoirs and intermediate hosts for some species of cestodes (Younis et al., 2021), it is worth studying the hymenolepidids that parasitize rodents. Understanding the phylogenetic history and host-associations of hymenolepidids is important given that host colonization and host switching events are apparent throughout the history of this family (Binkienė et al., 2011; Binkienė et al., 2019). An enhanced understanding of the systematics and host associations of hymenolepidids can help predict the probabilities of such events in the future (Brooks et al., 2014).

*Hymenolepis tualatinensis* was described by Gardner (1985) from *T. bulbivorus* but has not been documented since. *Hymenolepis citelli* and an unidentified *Hymenolepis* species were documented by Voge (1955) from *T. bottae* from California. Later, Jasmer (1980) reported *H. citelli* from *T. bottae* from Humboldt County, California. Although other hymenolepidids (i.e., *Arostrilepis* (= *Hymenolepis*) *horrida, Arostrilepis schilleri,* and *H. diminuta*) have been reported from *Thomomys* hosts in Oregon (Gardner, 1985; Gardner and Schmidt, 1988; Makarikov et al., 2012), many of these reports predate the recent taxonomic revisions for this family and require reevaluation (Makarikov et al., 2012). Thorough molecular studies are essential to delineate species boundaries, distributions, and host specificities among *Hymenolepis* species (Makarikov and Tkach, 2013).
Overview

Each of the helminth groups discussed has only been described based on morphology prior to this study (Hughes et al., 2020; Hughes et al., 2021). Examining molecular data is essential for understanding variations based on geography or hosts, to detect cryptic species, and to help resolve the phylogenies and systematics of both Nematoda and Cestoda. This study represents the first molecular characterization and phylogenetic analyses for the nematodes *T. fossor*, *H. thomomyos*, *R. rodentorum* and the cestode *H. tualatinensis* and contributes new, significant knowledge of biodiversity and evolutionary relationships for these taxa. Nuclear genes (18S rRNA or ITS1 region) were used to confirm initial identifications and a mitochondrial gene (COI mtDNA) was used for finer scale genetic resolution. Maximum likelihood (ML) and Bayesian inference (BI) phylogenies were generated using sequence data from each of these genes for all of the helminths discussed here.

Methods

Complete intestinal tracts of 134 *Thomomys* specimens (Fig. 2; Appendix C) were examined following procedures outlined by Gardner and Jasmer (1983). Collected parasites were stored in 95% ethanol and frozen for DNA extraction. Helminths were tentatively identified based on general morphology and previous records for the hosts (Hall, 1916; Chandler, 1945; Todd and Lepp, 1972; Jasmer, 1980; Gardner and Jasmer, 1983; Gardner, 1985; Gardner and Schmidt, 1988; Makarikov et al., 2012).

DNA extraction, amplification, and sequencing were undertaken on individual helminths. Before beginning isolation, specimens were transferred to 1.5 mL
microcentrifuge tubes and repeatedly rinsed with DI water to remove all traces of ethanol. They were then transferred to fresh PCR tubes and mechanically homogenized before DNA extraction using either the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer’s protocols or Sigma-Aldrich REDExtract-N-Amp™ Tissue PCR Kit. For extractions using the Sigma-Aldrich kit, 20 µL extraction buffer and 5 µL tissue preparation solution was added to each tube and the following protocols were run on a thermocycler: 10 minutes at 65°C, 10 minutes at 95°C, and 10 minutes at 10°C. Then, 30 µL neutralization solution was added to each tube.

PCR amplification of 35 DNA samples was carried out with PuReTaq Ready-To-Go PCR beads (Cytiva). Overlapping fragments of 18S rRNA or COI mtDNA were amplified using previously published primers (Table 1). PCR protocols for 18S rRNA for *T. fossor* followed Callejón et al. (2013), except that the denaturation temperature was increased to 95°C and the annealing temperature was decreased to 55°C. The PCR protocols that corresponded with the primer cocktails used to amplify the COI mtDNA of *T. fossor* and *R. rodentorum* (see Table 1) are outlined in Denham et al. (2021). The protocols followed for *H. thomomyos* for 18S rRNA PCR are outlined in Chilton et al. (2006) and those for COI rRNA PCR are described in Cable et al. (2006) with the exception of the annealing temperature, which was increased to 60°C. *Ransomus rodentorum* 18S PCR protocols were as follows: 95°C for 1 minute, 35 cycles of 95°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute, 30 seconds, and a final extension of 72°C for 7 minutes. *Hymenolepis* ITS1 PCR protocols are detailed in Macnish et al. (2002b) and COI mtDNA protocols are listed in Okamoto et al. (1997), except that the annealing temperature was increased to 53°C. After PCR products were visualized on a
1% agarose gel, they were SPRI-purified (Elkin et al., 2001) and prepared for direct end sequencing. Sanger sequencing reactions were processed by the Center for Genome Research and Biocomputing (CGRB; Oregon State University, Corvallis, OR).
Table 1. Primers targeted for PCR and sequencing of helminth 18S rRNA gene, ITS1 region, or COI mtDNA gene in this study. C_NemF1_t1 and C_NemR1_t1 represent cocktails consisting of three primers each (all of which are shown) which were used in a 1:1:1 ratio.

<table>
<thead>
<tr>
<th>Species</th>
<th>Gene</th>
<th>Primer</th>
<th>Primer sequence 5’ – 3’</th>
<th>Forward (F) or Reverse (R)</th>
<th>Citation</th>
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<td><em>Trichuris fossor</em></td>
<td>18S</td>
<td>G18S4</td>
<td>GCTTGCTCTCAAGAATAGGCC</td>
<td>F</td>
<td>Callejón et al., 2013</td>
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<tr>
<td></td>
<td>136</td>
<td>TCATCTTCTGCAGGTTCACCTAC</td>
<td></td>
<td>R</td>
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<tr>
<td></td>
<td>647</td>
<td>CATCTTGGCAAATGCTTTGCG</td>
<td></td>
<td>F</td>
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<tr>
<td></td>
<td>652</td>
<td></td>
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<td>R</td>
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</tbody>
</table>
|                              | COI      | C_NemF1_t1  | NemF1_t1: TGTAAAAACGAGCCGTGACGAGTTGTTG
NemF2_t1: TGTAAAAACGAGCCGTGACGAGTTG
NemF3_t1: TGTAAAAACGAGCCGTGACGAGTTG  | F                          | Prosser et al., 2013     |
|                              | (cocktail)| C_NemR1_t1  | NemR1_t1: CAGGAAACAGCTATGACTAAAACCTCCWGGRTGACCAAAAATCA
NemR2_t1: CAGGAAACAGCTATGACTAAAACCTCCWGGRTGACCAAAAATCA
NemR3_t1: CAGGAAACAGCTATGACTAAAACCTCCWGGRTGACCAAAAATCA  | R                          |                           |
<p>| <em>Heligmosomoides thomomyos</em> | 18S      | NC18SF1     | AAAGATTAAGCCCATGCA                                                                  | R                          | Chilton et al., 2006      |
|                              |          | NC5BR       | GCGAGTTCCCACTACGAT                                                                   | F                          |                           |
|                              | COI      | LCO1490     | GGTCACAAATCTATAAGATATTG                                                              | F                          | Folmer et al., 1994       |
|                              |          | HCO2198     | TAAACTTCAGGGTGACCAAAAATCA                                                            | R                          |                           |</p>
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<th><strong>Ransomus rodentorum</strong></th>
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<th>988F</th>
<th>CTCAAAGATTAAGCCATGC</th>
<th>F</th>
<th>Holterman et al., 2006</th>
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| COI | C_NemF1_t1 (cocktail) | NemF1_t1: TGTAAAACGACGGCCAGTCRACWGTWAATCAYAARAATA TTGG |
|     |                       | NemF2_t1: TGTAAAACGACGGCCAGTARAGATCTAATCATAAAGATAT YGG |
|     |                       | NemF3_t1: TGTAAAACGACGGCCAGTARAGTTCTAATCATAARGATAT TGG |

| COI | C_NemR1_t1 (cocktail) | NemR1_t1: CAGGAAACAGCTATGACTAAACTTCWGGRTGACCAAAAA ATCA |
|     |                       | NemR2_t1: CAGGAAACAGCTATGACTAWACYTCWGGRTGMCCAAAA AAYCA |
|     |                       | NemR3_t1: CAGGAAACAGCTATGACTAAACCTCWGGATGACCAAAAA ATCA |

<table>
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<th>ITS1</th>
<th>F3</th>
<th>GCGGAAGGATCATCATTACACGTTCC</th>
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<th>Macnish et al., 2002b</th>
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<tbody>
<tr>
<td></td>
<td>R3</td>
<td>GCTCGACTCTTCACTCAATCCACG</td>
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| COI | pr-a | TGGTTTTTTTTGTGATCTCTGAGGTTTA | F | Okamoto et al., 1997 |
|     | pr-b | AGAAAGAAGCTAATGAAAATGAGCAAC | R |                       |
Sequences were examined using MEGA v. 7.0.26 (Kumar et al., 2016). If adequate forward and reverse sequences were obtained for individual segments, they were combined after overlapping segments were identified by alignment using MUSCLE. Low-quality ends were trimmed and a BLAST search against the NCBI nr database was carried out. Previously published sequences identified from the BLAST matches were incorporated into the alignment for subsequent phylogenetic analysis. All sequences were aligned using MUSCLE with default parameters and ends were trimmed to attain sequences of equal lengths.

The 18S rRNA gene was sequenced from four specimens putatively identified as *T. fossor* collected from four *Thomomys* host species (*T. bottae*, *T. bulbivorus*, *T. mazama*, and *T. talpoides*; Table 2). The *Trichuris* 18S rRNA sequences were 1,644 base pairs long after trimming low-quality reads (prior to alignment) and excess ends (following alignment). Newly generated sequences were submitted to the GenBank database under accession numbers MT071351, MT071352, MT071353, and MT071354 (Table 2). Based on the BLAST search results, twenty-six additional species from GenBank were added to the 18S rRNA analysis. These included other members of Trichuridae (*Trichuris arvicolae*, *Trichuris muris*, *Trichuris vulpis*, *Trichuris suis*, *Trichuris trichiura*, *Trichuris discolor*, *Trichuris ovis*, *Trichuris leporis*, *Trichuris skrjabini*, and an unidentified *Trichuris* species), members of Capillaridae (an unidentified *Ecoleus* species, *Baruscapillaria obsignata*, *Pseudocapillaria tomentosa*, *Capillaria bursata*, *Aonchotheca paranalis*, *Aonchotheca musimon*, *Aonchotheca putorii*, an unidentified *Pearsonema* species, *Pearsonema plica*, *Calodium hepaticum*, *Aonchotheca riukiensis*, *Capillaria*...
suis), and members of Trichinellidae (Trichinella nativa, Trichinella spiralis, Trichinella murrelli, and Paralamyctes validus; Table 2).

The COI gene was sequenced from three T. fossor specimens from three separate Thomomys species (T. bulbivorus, T. mazama, T. talpoides) hosts (Table 2). Primers that are often used for Trichuris species COI mtDNA PCR (Callejón et al., 2013) frequently amplified the hosts’ DNA rather than T. fossor’s and the primer cocktail PCRs often were entirely ineffective. As a result, only three sequences were successfully obtained for the COI gene for T. fossor (Table 2). Eight additional species from GenBank were included in the COI mtDNA analysis: Trichuris mastomysi, T. muris, T. arvicolae, T. ovis, T. discolor, T. suis, T. trichiura, and Trichinella nativa (Table 2). The COI mtDNA sequences were 435 bp after low-quality and excess ends were trimmed. The Trichuris fossor COI sequences were deposited to GenBank under the accession numbers OM276505–OM276507 (Table 2). The final molecular datasets included 30 sequences from 23 known host species for the 18S analysis and 11 sequences from 11 host species for the COI analysis (Table 2).

Table 2. Species included in the 18S rRNA or COI mtDNA Trichuris fossor phylogenetic analyses.

<table>
<thead>
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<th>Species</th>
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<th>GenBank accession number</th>
</tr>
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33
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The 18S and COI genes were sequenced from two *H. thomomyos* from individual host specimens for *T. bottae* and *T. bulbivorus* (Table 3). For *T. talpoides*, four total *H. thomomyos* were sequenced from separate hosts (two from near Burns, Harney Co., and two from John Day, Grant Co.; Table 3; Appendix C). From the only infected *T. townsendii* (from Princeton, Harney Co.), one *H. thomomyos* was sequenced (*H. thomomyos* was not detected in any of the eight *T. townsendii* collected from Owyhee, Malheur Co.; Table 3; Appendix C). Sequences used for the 18S rRNA *Heligmosomoides* dataset were 1,523 bp long after low-quality or excess ends were trimmed. Nine
additional sequences from GenBank were added to the 18S rRNA analyses:

*Heligmosomoides polygyrus*, *Nicollina cameroni*, *Herpetostrongylus pythonis*, *Viannaia viannai*, *Carolinensis perezponcedeleoni*, *Chabaudstrongylus ninhae*, *Ostertagia leptospicularis*, *Haemonchus similis*, *Trichostrongylus colubriformis*, and *Tetrabothriostrongylus mackerrasae* (as the outgroup; Table 3). The COI mtDNA dataset was 530 bp after being trimmed. Two North American *Heligmosomoides* species from GenBank were included in the COI analyses: *Heligmosomoides americanus* and *Heligmosomoides vandegrifti; Trichostrongylus colubriformis* (Nematoda: Strongylida) was included as the outgroup (Table 3). Novel sequences were deposited to GenBank under the accession numbers MZ458407–MZ458413 and MZ458119–MZ 458120 for the 18S sequences and MZ441139–MZ441147 for the COI sequences (Table 3). The final molecular datasets included 19 sequences for the 18S analysis from 13 known host species and 12 sequences from 7 host species for the COI analysis (Table 3).

Table 3. Species included in the 18S rRNA or COI mtDNA *Heligmosomoides thomomyos* phylogenetic analyses.

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The 18S rRNA gene was sequenced from one *R. rodentorum* from a *T. bottae* host. I was unable to amplify this gene for any other specimens using the same primers (Table 1). Nineteen species from GenBank were incorporated into the 18S rRNA dataset (Table 4). These included *Oesophagostomum muntiacum*, *Cyclodontostomum purvisi*, *Chabertia ovina*, *Zonolaimus mawsonae*, *Hypodontus macropi*, *Labiostrongylus bipapillosus*, *Stephanurus dentatus*, *Strongylus equinus*, *Cylilocyclus insignis*, *Petrovinema poulatum*, *Necator americanus*, *Mammomonogamus laryngeus*, an unidentified *Mammomonogamus* species, *Mammomonogamus ierei*, *Mammomonogamus auris*, *Kalicephalus cristatus*, *Uncinaria stenocephala*, *Angiostrongylus costaricensis*, and *Ancylostoma caninum* (as the outgroup; Table 4). The sequences were 1,024 bp after trimming. Two COI mtDNA sequences were obtained for *R. rodentorum*: one was from the same *R. rodentorum* specimen from the *T. bottae* host, the other was from a *T. townsendii* host (Table 4). Ten additional species were added from GenBank to the COI mtDNA dataset (407 bp in length after trimming): an unidentified *Necator* species, *N. americanus*, an unidentified *Oesophagostomum* species, *Oesophagostomum colombianum*, *H. americanus*, *H. polygyrus*, *H. vandegrifti*, *Ancylostoma duodenale*, *Ancylostoma caninum*, and *Uncinaria sanguinis* as the outgroup; Table 4). The 18S sequence was deposited to GenBank under the accession number OM296295 and the COI sequences were deposited under the GenBank accession numbers OM302374–OM302375 (Table 4). In total, the final molecular datasets incorporated 20 sequences from 16 known host species for the 18S analysis and 12 sequences from 9 known host species for the COI analysis (Table 4).
Table 4. Species included in the 18S rRNA or COI mtDNA *Ransomus rodentorum* phylogenetic analyses.

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<td>Wallabia bicolor</td>
<td>18S</td>
<td>AJ920339</td>
</tr>
<tr>
<td><em>Labiostrongylus bipapillosus</em></td>
<td>Macropus giganteus</td>
<td>18S</td>
<td>AJ920337</td>
</tr>
<tr>
<td><em>Stephanurus dentatus</em></td>
<td>Sus scrofa</td>
<td>18S</td>
<td>AJ920345</td>
</tr>
<tr>
<td><em>Strongylus equinus</em></td>
<td>-</td>
<td>18S</td>
<td>DQ094176</td>
</tr>
<tr>
<td><em>Cylicocyclus insignis</em></td>
<td>Equus caballus</td>
<td>18S</td>
<td>AJ920342</td>
</tr>
<tr>
<td><em>Petrovinema poulcatum</em></td>
<td>Equus caballus</td>
<td>18S</td>
<td>AJ920343</td>
</tr>
<tr>
<td><em>Necator americanus</em></td>
<td>Homo sapiens</td>
<td>18S</td>
<td>AJ920348</td>
</tr>
<tr>
<td><em>Mammomonogamus laryngeus</em></td>
<td>Bubalus bubalis</td>
<td>18S</td>
<td>MF668006</td>
</tr>
<tr>
<td><em>Mammomonogamus sp.</em></td>
<td>Felis catus</td>
<td>18S</td>
<td>MF668045</td>
</tr>
<tr>
<td><em>Mammomonogamus ierei</em></td>
<td>Felis catus</td>
<td>18S</td>
<td>MF668043</td>
</tr>
<tr>
<td><em>Mammomonogamus auris</em></td>
<td>Felis catus</td>
<td>18S</td>
<td>MF668044</td>
</tr>
<tr>
<td><em>Kalicephalus cristatus</em></td>
<td>Austrelaps superbus</td>
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<td>AJ920349</td>
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<td><em>Angiostrongylus costaricensis</em></td>
<td>Sigmodon hispidus</td>
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<tr>
<td><em>Unicaria stenocephala</em></td>
<td>Canis latrans</td>
<td>18S</td>
<td>MN218457</td>
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<tr>
<td><em>Ancylostoma caninum</em></td>
<td>Canis lupus familiaris</td>
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<td>AJ920347</td>
</tr>
<tr>
<td><em>Ransomus rodentorum</em></td>
<td>Thomomys townsendii</td>
<td>COI</td>
<td>OM302374</td>
</tr>
<tr>
<td><em>Ransomus rodentorum</em></td>
<td>Thomomys bottae</td>
<td>COI</td>
<td>OM302375</td>
</tr>
<tr>
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<td>MH200977</td>
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<td><em>Necator americanus</em></td>
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<tr>
<td><em>Oesophagostomum sp.</em></td>
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<td>COI</td>
<td>MK282873</td>
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<td>COI</td>
<td>KF921077</td>
</tr>
<tr>
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<td>Apodemus sylvaticus</td>
<td>COI</td>
<td>KJ994545</td>
</tr>
<tr>
<td><em>Heligmosomoides vandegrifti</em></td>
<td>Peromyscus maniculatus</td>
<td>COI</td>
<td>MN927211</td>
</tr>
<tr>
<td><em>Ancylostoma duodenale</em></td>
<td>-</td>
<td>COI</td>
<td>AP017676</td>
</tr>
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<td><em>Ancylostoma caninum</em></td>
<td>Homo sapiens</td>
<td>COI</td>
<td>AB751618</td>
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<td>Uncinaria sanguinis</td>
<td>Neophoca cinerea</td>
<td>COI</td>
<td>KF693754</td>
</tr>
<tr>
<td>---------------------</td>
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</tr>
</tbody>
</table>

The first internal transcribed region (ITS1 region) was sequenced from four *Hymenolepis* specimens: three identified tentatively as *H. tualatinensis* from separate *T. bulbivorus* hosts and one unidentified *Hymenolepis* species from a *T. bottae* host (Table 5). Seven additional hymenolepidid species from GenBank were included in the ITS1 analyses: *H. diminuta, Pseudanoplocephala crawfordi, R. nana, Staphylocystis schilleri,* an unidentified *Staphylocystis* species, *Staphylocystis furcata,* and *Rodentolepis straminea* (as the outgroup; Table 5). This dataset was 488 bp after trimming. Three COI mtDNA sequences were obtained from *Hymenolepis* species from this study: two represented *H. tualatinensis* from individual *T. bulbivorus* hosts and the other was the unidentified *Hymenolepis* species from a *T. bottae* host (Table 5). Twelve additional species from GenBank were added to the COI mtDNA analyses; these included other hymenolepidids belonging to the genera *Hymenolepis, Arostrilepis, Passerilepis,* and *Citrilolepis,* and taeniids (Family Taeniidae) belonging to the genera *Echinococcus* and *Taenia*; Table 5). The COI mtDNA dataset was 385 bp after trimming. The new sequences were deposited to GenBank under the accession numbers OM304282–OM304285 for the ITS1 sequences and OM280143–OM280145 for the COI sequences (Table 5). The final molecular datasets consisted of 11 sequences from 8 known host species for the ITS1 analysis and 15 sequences from 12 known host species for the COI analysis (Table 5).
Table 5. Species included in the ITS1 region or COI mtDNA *Hymenolepis tualatinensis* phylogenetic analyses

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Gene/region</th>
<th>GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hymenolepis tualatinensis</em></td>
<td><em>Thomomys bulbivorus</em></td>
<td>ITS1</td>
<td>OM304282</td>
</tr>
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<td><em>Hymenolepis tualatinensis</em></td>
<td><em>Thomomys bulbivorus</em></td>
<td>ITS1</td>
<td>OM304283</td>
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<tr>
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<td>ITS1</td>
<td>OM304284</td>
</tr>
<tr>
<td><em>Hymenolepis tualatinensis</em></td>
<td><em>Thomomys bulbivorus</em></td>
<td>ITS1</td>
<td>OM304285</td>
</tr>
<tr>
<td><em>Hymenolepis diminuta</em></td>
<td>rodent (species not specified)</td>
<td>ITS1</td>
<td>JN258038</td>
</tr>
<tr>
<td><em>Pseudanoplocephala crawfordi</em></td>
<td>pig (species not specified)</td>
<td>ITS1</td>
<td>KJ150726</td>
</tr>
<tr>
<td><em>Rodentolepis nana</em></td>
<td><em>Rattus rattus</em></td>
<td>ITS1</td>
<td>MT454661</td>
</tr>
<tr>
<td><em>Staphylocystis schilleri</em></td>
<td><em>Sorex palustris</em></td>
<td>ITS1</td>
<td>KF257896</td>
</tr>
<tr>
<td><em>Staphylocystis sp.</em></td>
<td><em>Sorex vagrans</em></td>
<td>ITS1</td>
<td>KF257898</td>
</tr>
<tr>
<td><em>Staphylocystis furcata</em></td>
<td><em>Sorex araneus</em></td>
<td>ITS1</td>
<td>KF257897</td>
</tr>
<tr>
<td><em>Rodentolepis straminea</em></td>
<td><em>Apodemus sylvaticus</em></td>
<td>ITS1</td>
<td>JN258054</td>
</tr>
<tr>
<td><em>Hymenolepis tualatinensis</em></td>
<td><em>Thomomys bulbivorus</em></td>
<td>COI</td>
<td>OM280144</td>
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<td><em>Thomomys bulbivorus</em></td>
<td>COI</td>
<td>OM280145</td>
</tr>
<tr>
<td><em>Hymenolepis sp.</em></td>
<td><em>Thomomys bottae</em></td>
<td>COI</td>
<td>OM280143</td>
</tr>
<tr>
<td><em>Hymenolepis hibernia</em></td>
<td><em>Apodemus sylvaticus</em></td>
<td>COI</td>
<td>LC063180</td>
</tr>
<tr>
<td><em>Hymenolepis sp.</em></td>
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<td>COI</td>
<td>LC063183</td>
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<tr>
<td><em>Arostrilepis horrida</em></td>
<td><em>Clethrionomys glareolus</em></td>
<td>COI</td>
<td>DQ340978</td>
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<tr>
<td><em>Passerilepis zimbebel</em></td>
<td><em>Terpsiphone viridis</em></td>
<td>COI</td>
<td>MK463854</td>
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<tr>
<td><em>Echinococcus canadensis</em></td>
<td>-</td>
<td>COI</td>
<td>MK492626</td>
</tr>
<tr>
<td><em>Echinococcus ortlepti</em></td>
<td>-</td>
<td>COI</td>
<td>MN886287</td>
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<td><em>Citriolepis citrili</em></td>
<td><em>Crithagra citrinelloides</em></td>
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<td>MK463853</td>
</tr>
<tr>
<td><em>Taenia omissa</em></td>
<td><em>Puma concolor</em></td>
<td>COI</td>
<td>KR095314</td>
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<tr>
<td><em>Taenia regis</em></td>
<td><em>Panthera leo</em></td>
<td>COI</td>
<td>AB905198</td>
</tr>
<tr>
<td><em>Taenia hydatigena</em></td>
<td><em>Ovis aries</em></td>
<td>COI</td>
<td>MW316694</td>
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<tr>
<td><em>Taenia lynciscapreoli</em></td>
<td><em>Lynx lynx</em></td>
<td>COI</td>
<td>MK033479</td>
</tr>
<tr>
<td><em>Taenia ovis</em></td>
<td><em>Ovis aries</em></td>
<td>COI</td>
<td>MG594802</td>
</tr>
</tbody>
</table>

Phylogenetic analyses were carried out using MEGA and BEAST2 v. 2.6.1 (Bouckaert et al., 2019). I used MEGA to determine the best fit substitution model for the data (Tables 6, 7) based on Bayesian information criterion (BIC). An evolutionary history was then inferred based on the suggested model using the maximum likelihood (ML) method and a consensus tree was generated using 1,000 bootstrapping (BS) replicates in
MEGA. The Bayesian inference (BI) analyses were prepared using BEAUti v. 2.6.0 (Bouckaert et al., 2019) and undertaken using BEAST2 v. 2.6.0 (Bouckaert et al., 2019).

The nucleotide substitution models used for the 18S rRNA and COI mtDNA analyses are shown in Tables 6 and 7, respectively. In some instances, different models were used for the ML BS tree and the BI tree (see Tables 6 and 7) because the best-fit model was not available in BEAST2. The parameters for the BI tree model shown were the most comparable or had similar BIC scores to the ML BS model (Tables 6 and 7). The exception to this was the *Hymenolepis* COI BI tree; the model selected for this analysis yielded adequate effective sample size (ESS) values whereas the model suggested by MEGA did not. BI analyses ran for $1 \times 10^7$ generations.

Table 6. Nucleotide substitution models used for nuclear marker phylogenetic analyses. For all taxa except the *Hymenolepis* species, the models were for the 18S rRNA gene. For the *Hymenolepis* species, the models were for the ITS1 region.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>ML BS Model</th>
<th>BI Model</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichuris fossor</em></td>
<td>K2P+$I(0.004)+G(0.376)$</td>
<td>HKY with equal frequencies</td>
</tr>
<tr>
<td></td>
<td>(Kimura, 1980)</td>
<td>(Hasegawa et al., 1985)</td>
</tr>
<tr>
<td><em>Heligmosomoides thomomyos</em></td>
<td>K2P+$I(0.481)+G(0.05)$</td>
<td>HKY with equal frequencies</td>
</tr>
<tr>
<td></td>
<td>(Kimura, 1980)</td>
<td>(Hasegawa et al., 1985)</td>
</tr>
<tr>
<td><em>Ransomus rodentorum</em></td>
<td>T92+$I(0.818)+G(1.079)$</td>
<td>HKY with equal frequencies</td>
</tr>
<tr>
<td></td>
<td>(Tamura, 1992)</td>
<td>(Hasegawa et al., 1985)</td>
</tr>
<tr>
<td><em>Hymenolepis</em> species</td>
<td>K2 ($\text{Kimura, 1980}$)</td>
<td>HKY with equal frequencies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Hasegawa et al., 1985)</td>
</tr>
</tbody>
</table>

Table 7. Nucleotide substitution models used for COI mtDNA phylogenetic analyses.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>ML BS Model</th>
<th>BI Model</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichuris fossor</em></td>
<td>T92+$G(0.203)$</td>
<td>TN93 with equal frequencies</td>
</tr>
<tr>
<td></td>
<td>(Tamura, 1992)</td>
<td>(Tamura and Nei, 1993)</td>
</tr>
<tr>
<td><em>Heligmosomoides thomomyos</em></td>
<td>TN93+$I(0.646)+G(0.468)$</td>
<td>TN93+$I(0.646)+G(0.468)$</td>
</tr>
<tr>
<td></td>
<td>(Tamura and Nei, 1993)</td>
<td>(Tamura and Nei, 1993)</td>
</tr>
<tr>
<td><em>Ransomus rodentorum</em></td>
<td>TN93+$I(0.517)+G(0.237)$</td>
<td>TN93+$I(0.517)+G(0.237)$</td>
</tr>
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<td></td>
<td>(Tamura and Nei, 1993)</td>
<td>(Tamura and Nei, 1993)</td>
</tr>
<tr>
<td><em>Hymenolepis</em> species</td>
<td>TN93+$I(0.416)+G(0.360)$</td>
<td>TN93</td>
</tr>
<tr>
<td></td>
<td>(Tamura and Nei, 1993)</td>
<td>(Tamura and Nei, 1993)</td>
</tr>
</tbody>
</table>
Tracer v 1.7.1 (Rambaut et al., 2018) was used to evaluate convergence and ensure that ESS values for each parameter were met. ESS values were all >350. Tree files were combined in LogCombiner v. 2.6.0 (Bouckaert et al., 2019) and a maximum clade credibility (MCC) tree was constructed using TreeAnnotator v. 2.6.0 (Bouckaert et al., 2019) with posterior probabilities limited 50% and a burn-in percentage of 10%. FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize the MCC tree.

Results

*Trichuris fossor*

In 18S rRNA ML and BI analyses trees, *Trichuris, Trichinella* (Nematoda), and capillariid (Nematoda) species represented highly supported (100%) monophyletic groups (Figs. 3, 4). The capillariid and trichurid clades formed sister taxa with 100% support in both analyses. These findings are consistent with those of previous studies (Feldman and Ramirez, 2014; Borba et al., 2019). The new sequences fell within the *Trichuris* clade with 100% node support, forming an independent subclade (Subclade A) with 100% support in both analyses (Figs. 3, 4). Four subclades were present within *Trichuris*: Subclade A) the new sequences from *T. fossor*; Subclade B) *T. arvicolae, T. muris, T. vulpis;* Subclade C) *T. suis,* unidentified *Trichuris* sp. (= *T. colobae,* see Cutillas et al., 2014); and Subclade D) *T. trichiura, T. discolor, T. ovis, T. leporis,* and *T. skrabini* (Figs. 3, 4). The composition of previously studied species in Subclades B, C, and D is consistent with results from studies that used nuclear, mitochondrial, and/or concatenated data (Callejón et al., 2013, 2015; Doležalová et al., 2015; Feldman and Ramirez, 2014).
In the ML analysis, the relatedness of the *Trichuris* subclades to one another had low (≤70%) support or were unresolved (Fig. 3). The BI analysis offered better resolution among trichurids (Fig. 4). The *T. fossor* subclade (Subclade A) was most closely related to the *T. arvicola*, *T. muris*, and *T. vulpis* subclade (Subclade B); the remaining two subclades (Subclades C and D) were more closely related to one another than to the other two subclades (Figs. 3, 4). Species belonging to the families Capillaridae and Trichinellidae formed separate monophyletic groups in the ML analyses (Fig. 3). Members of the family Capillaridae formed a monophyletic clade in the BI analysis as well (Fig. 4). However, Trichinellidae did not represent a monophyletic group in the BI analysis; *Paralamyctes validus* (GenBank accession AF173243) was the outgroup to all remaining species included in the BI tree rather than grouping with the remaining trichinellid representatives (Fig. 4).

The topologies for the COI mtDNA ML and BI trees were identical, although the BI tree had higher support values for a greater number of nodes (Fig. 5) than the ML tree (not shown). There were four distinctive *Trichuris* subclades in the COI mtDNA trees (Fig. 5). The *T. fossor* sequences formed a highly supported clade (Subclade A) in the COI mtDNA ML tree (96%) and BI tree (100%; Fig. 5). *Trichuris fossor* from the *T. mazama* host was the sister group to *T. fossor* from *T. talpoides* and, as in the 18S analyses, *T. fossor* from *T. bulbivorus* was successive sister taxon. The sister group to the *T. fossor* clade, Subclade B, consisted of *T. mastomysi*, *T. muris*, and *T. arvicola*, all of which are Old World species. The sister taxa comprising the remaining two subclades (C and D) as well as their relationships to one another for both COI mtDNA trees were consistent with previous studies (Callejón et al., 2013; Petružela et al., 2021).
*Heligmosomoides thomomyos*

All *Heligmosomoides* species (from this study and the GenBank reference sequence) formed a monophyletic group with high BS support (89%) in the 18S ML tree (Fig. 6) and high posterior probability support (100%) in the 18S BI tree (Fig. 7). Within this group, two distinct *Heligmosomoides* clades, A and B, were supported, also with high posterior probabilities (100% and 83%; Fig 7). In the ML BS tree, Clade B had high bootstrap support (76%) while Clade A did not (Fig. 6). The majority of our *Heligmosomoides* sequences were most similar to the *H. polygyrus* sequence from GenBank (accession AJ920355), as evidenced by its placement within Clade A (Figs. 6, 7). However, bootstrap support values and posterior probabilities within Clade A were too low to infer fine-scale relationships using the 18S gene. The topologies of Clade A were different between the two analyses (Figs. 6, 7). In the ML tree, *H. thomomyos* from the same location were not monophyletic (Fig. 6) while in the BI tree, *H. thomomyos* from the same location were monophyletic with the exception of *H. thomomyos* from *T. talpoides* from John Day (Fig. 7). Two *Heligmosomoides* sequences from *T. talpoides* hosts collected near Burns formed a separate clade (Clade B) in both analyses (Figs. 6, 7).

Similar to the 18S trees, all *Heligmosomoides* formed a monophyletic group in the COI ML and BI trees (Figs. 8, 9). However, the COI trees yielded a more detailed perspective on intrageneric relationships. Four distinct clades were supported with high bootstrap support (87–100%) or posterior probabilities (99–100%); Clade D in the BI tree was associated with a low (<70%) posterior probability (Fig. 8, 9). In every instance, *Heligmosomoides* sequences from the same location were monophyletic (Figs. 8, 9). The
*Heligmosomoides* sequence from *T. townsendii* was sister to those of *T. talpoides* from John Day hosts, and together these three sequences formed Clade A with 99% bootstrap and 100% posterior probability support (Figs. 8, 9). Clades B and C each contained sequences only from a single host species, *T. bulbivorus* and *T. bottae*, respectively (Figs. 8, 9). The *Heligmosomoides* sequences from this study were sister to the *H. vandegrifti* sequence from GenBank (accession MN927211) in the ML tree (Fig. 8). In the BI tree, *H. americanus* (GenBank accession KF921077) and *H. vandegrifti* formed Clade D with the *H. thomomyos* from *T. talpoides* from Burns, although this clade had a low posterior probability support (Fig. 9).

*Ransomus rodentorum*

In both 18S trees, *R. rodentorum* formed a clade with species belonging to the superfamily Strongyloidea, although the clade was not well-supported (Figs. 10, 11). Chabertiidae species formed a well-supported (79% bootstrap and 99.9% posterior probability) monophyletic clade in both analyses (Figs. 10, 11). *Stephanurus dentatus* (GenBank accession AJ920345), which belongs to the family Stephanuridae, was the sister taxa to the Chabertiidae clade, with high posterior probability support in the BI tree (Figs. 10, 11). Together, *R. rodentorum*, the chabertiids, and *S. dentatus* represented Clade A in the ML tree (Fig. 10). *Ransomus rodentorum* was equally related to *S. dentatus* and the chabertiids in the ML tree, although this relationship did not have high bootstrap support (Fig. 10). In the BI tree, Clade A also consisted of the chabertiids and *S. dentatus*, although it did not include *R. rodentorum* (Fig. 11). In fact, in the BI tree, *R. rodentorum* was not included in any of the three clades (Fig. 11). The sister group to
Clade A in the ML and BI trees, Clade B, contained members of the family Strongylidae (Figs. 10, 11). Clade B had high bootstrap support (83%) in the ML tree (Fig. 10) and high posterior probability support (99.9%) in the BI tree (Fig. 11). The superfamily Strongyloidea was not monophyletic in either tree due to the presence of *Necator americanus* (GenBank accession AJ920348) within Clade C (Figs. 10, 11). The superfamily Ancylostomatoidea was polyphyletic in both analyses (Figs. 10, 11).

The *R. rodentorum* specimens were not monophyletic in the COI ML tree (Fig. 12). The *R. rodentorum* sequence from the *T. townsendii* host formed a subclade with a *Necator* species from GenBank (accession MH200977), and the *R. rodentorum* from *T. bottae* was sister to this subclade (Subclade A, Fig. 12). However, neither of these nodes had high bootstrap support (Fig. 12). In the BI tree, the *R. rodentorum* sequences formed a monophyletic subclade (Subclade A) with high posterior probability support (95.7%; Fig. 13). In the ML tree, the *R. rodentorum* and *Necator* species subclade, Subclade A, was equally related to Subclade B and to Subclade C. Subclade B contained *Necator americanus* (GenBank accession MT074036) and two *Oesophagostomum* species (GenBank accessions MK282872 and MK 282873) while Subclade C contained three *Heligmosomoides* species (GenBank accessions KF921077, KJ994545, MN927211). However, these nodes also all had low bootstrap support (Fig. 12). In the BI tree, the *R. rodentorum* subclade, Subclade A, was equally related to Subclades B, C, and D, which contained *Oesophagostomum* species, *Heligmosomoides* species, and *Necator* species, respectively (Fig. 13). This node had high posterior probability support (99.8%; Fig. 13).

*Hymenolepis* species
The ITS1 ML tree (not shown) and BI tree (Fig. 14) had identical topologies. The new sequences formed a subclade (Subclade A) with high bootstrap (100%) and posterior probability (100%) support (Fig. 14). *Hymenolepis tualatinensis* was paraphyletic in both analyses due to the placement of the *Hymenolepis* sequence from the *T. bottae* host (Fig. 14). In both trees, the *Hymenolepis* species from GenBank (accession JN258054) and *Pseudanoplocephala crawfordi* (GenBank accession KJ150726) formed a subclade (Subclade B) with high support values (100% in both analyses) and the *Staphylocystis* species formed a highly supported (≥94%) monophyletic subclade (Subclade C, Fig. 14). In both trees, *Rodentolepis* was polyphyletic (Fig. 14). *Rodentolepis nana* formed a highly supported (≥95%) clade with the *Hymenolepis* species and *P. crawfordi* while *R. straminea* was the outgroup for these analyses (Fig. 14).

The new *Hymenolepis* sequences formed a well-supported (100% in both analyses) subclade (Subclade A) in both the COI mtDNA ML and BI trees (Figs. 15, 16). The composition of species in the sister group to Subclade A, Subclade B, varied between the analyses (Figs. 15, 16). In both trees, the *Hymenolepis* sequences from GenBank (accessions LC063180 and LC063183) were included in Subclade B (Figs. 15, 16). In the BI analysis, these were the only sequences that fell within Subclade B (Fig. 15). In the ML tree, this clade also contained *Arostrilepis horrida* (GenBank accession DQ340978), although the clade had low BS support (Fig. 15). In the BI tree, Subclade B had high posterior probability support (99.7%; Fig. 16). Hymenolepididae was not monophyletic in either tree due to the placement of *Citrilolepis citrili* (GenBank accession MK463853), which was more closely related to members of the family Taeniidae than to members of the family Hymenolepididae (Figs. 15, 16).
Discussion

*Trichuris fossor*

My results verify that, based on molecular data, *T. fossor* is a distinct species because it represented a monophyletic group in each of the analyses. This is the first report of *T. fossor* from a *T. mazama* host (Hughes et al., 2020). In the 18S rRNA BI analysis, *T. fossor* from *T. bottae* and *T. talpoides* were monophyletic and sister to the specimen from *T. mazama*. In both COI analyses, *T. fossor* from *T. bottae* was not included, and instead *T. fossor* from *T. mazama* and *T. fossor* from *T. talpoides* formed monophyletic sister clades. In each analysis, *T. fossor* from the *T. bulbivorus* host was an outgroup to all remaining *T. fossor* sequences. Although the 18S rRNA gene is not reliable for determining whether genetic distances among *Trichuris* fall within the range of intraspecific variation, the COI mtDNA gene shows substantial interspecific divergence (Guardone et al., 2013). The COI analyses identified similar relationships among the *T. fossor* sequences, demonstrating that variability likely exists among *T. fossor* from different host species. Based on the degree of divergence observed, it is likely that *T. fossor* from *T. bulbivorus* is a distinctive subspecies, or possibly even a separate species, from the *T. fossor* from the remaining *Thomomys* hosts based on the results of these analyses. A morphological study examining the differences between *T. fossor* from various *Thomomys* hosts would help to determine definitively if, in fact, these are the same species of *Trichuris*.

My work thus represents a preliminary step in investigating the phylogeny of *T. fossor*. Examining more molecular data, including different genes, likely will result in
increased resolution of the phylogenetic relationships of *T. fossor*. Comparing other markers of nuclear and organellar DNA (Doležalová et al., 2015), and incorporating *T. fossor* from different host species and from different geographic areas also would be valuable to investigating lineages within the *T. fossor* subclade (Callejón et al., 2010). A thorough morphological analyses of *T. fossor* from various hosts could be beneficial as well (Falcón-Ordaz et al., 2020) and would help to definitively discern whether this group represents a single species or multiple species and the significance of the variation within these lineages. This study not only showed that *T. fossor* represents a distinctive monophyletic lineage, but also revealed that there is variation present within the clades *T. fossor* formed in each analysis.

*Heligmosomoides thomomyos*

I hypothesized that *H. thomomyos* sequences would form a monophyletic clade in both the 18S and COI analyses, and that sequences from the same host species would be sister taxa in the COI analysis. However, the 18S BI tree deviated from this hypothesis, because the placement of the *H. polygyrus* sequence from GenBank created a paraphyletic relationship among *H. thomomyos* samples. Furthermore, low support values (posterior probabilities <50%) within Clade A of the 18S tree did not allow for finer-scale resolution of my *H. thomomyos* sequences. Given the slow mutation rate of the 18S gene in comparison to the COI gene, I had not anticipated that this analysis would yield this unexpected result because I had morphologically identified all nematodes from each of the *Thomomys* hosts as a single species, *H. thomomyos*. Thus, the position of *H.
*thomomyos* from Burns in the phylogenetic tree was surprising. This suggests that there likely are cryptic *Heligmosomoides* species present within *Thomomys* host species.

The COI BI tree revealed a paraphyletic relationship for my *H. thomomyos* samples. However, the sister taxon relationships of *H. thomomyos* from the same host species in the COI tree aligned more with the relationships I anticipated. Based on my analyses, *H. thomomyos* could be paraphyletic or, more likely, multiple cryptic *Heligmosomoides* species could be present in *Thomomys* host species. I suspect that the *Heligmosomoides* from the *T. talpoides* from Burns, at least, represents an undescribed species. Other genes, in particular the ITS1 and ITS2 regions, the 5.8S rRNA gene, and the 28S rRNA gene, as well as a thorough morphological analysis, should be evaluated to definitively ascertain this. Further studies that survey a broader distribution also would help establish host specificities and systematics of the *Heligmosomoides* complex within rodent hosts (Clough and Råberg, 2014).

This study verifies that, based on molecular data, *H. thomomyos* represents a distinct lineage. All of the sequences except those from *T. talpoides* hosts from Burns formed a monophyletic group comprised of *H. thomomyos*. This investigation also revealed that a cryptic species of *Heligmosomoides* likely is present within *T. talpoides* hosts from Burns. Thus, it is likely that my study has identified the presence of two *Heligmosomoides* species within these *Thomomys* host species.

*Ransomus rodentorum*

*Ransomus rodentorum* represented a distinctive lineage in these analyses. The superfamilies were not monophyletic, which is consistent with previous research that
used the 18S gene (Chilton et al., 2006). Chilton et al. (2006) also found strong support for strongylids and ancylostomatids forming a clade, as was the case in this study. However, the family to which *R. rodentorum* belongs is unclear based on my analyses. *Ransomus rodentorum* was initially placed in family Strongylidae by Hall (1916). Frandsen and Grundmann (1961) and Jasmer (1980) also considered *R. rodentorum* a member of Strongylidae. My 18S results suggest that *R. rodentorum* belongs to the superfamily Strongyloidea, but it did not nestle within the strongylid clade in either of the 18S trees resulting from this study. Family-level identification therefore could not be confirmed. *Ransomus rodentorum* was either equally related to the chabertiid clade and the stephanurid clade (ML tree) or to the chabertiid clade and the strongylid clade (BI tree) in the 18S analyses.

The COI trees included members of Chabertiidae, Heligmosomatidae, and Ancylostomatidae, but not Stephanuridae or Strongylidae. In both COI trees, Ancylostomatidae was polyphyletic. This is consistent with studies based on the 18S rRNA gene (Chilton et al., 2006), but inconsistent with ancylostomatid studies that used a combination of nuclear and mitochondrial genes (Xie et al., 2017) or a combination of mitochondrial genes (Xie et al., 2019). In the COI trees, *R. rodentorum* fell within one of the ancylostomatid subclades (with *Necator* sp. in the ML tree), or it formed a subclade of its own that was equally related to the chabertiids, heligmosomatids, and one of the ancylostomatid subclades (BI tree). The COI ML tree had low support for all nodes, the BI tree therefore most likely is a truer depiction of the relationship of *R. rodentorum* to these taxa.
Further study on the systematics of *R. rodentorum* is warranted given the sample size was small and only two partial genes were examined in this study. Other genetic markers could prove helpful in uncovering the phylogeny of this nematode. After additional genetic markers have been assessed and a broader study has been undertaken, reevaluating the systematics of *R. rodentorum* may be necessary. However, this is the first study to document the presence of *R. rodentorum* using molecular markers and it represents a preliminary step in uncovering the true taxonomy and systematics of *R. rodentorum*.

*Hymenolepis* species

Consistent with the ITS1 analyses in this study, *Rodentolepis* did not represent a monophyletic group in other studies that used 28S rRNA (Greiman and Tkach, 2012; Neov et al., 2019), 18S rRNA (Neov et al., 2021), and COI mtDNA (Neov et al., 2021). In a study based on the ITS2 region, *Rodentolepis nana* formed a clade with *Staphylocystis* species (Sharma et al., 2016), which was not the case in my analyses. *Hymenolepis diminuta* formed a clade with *P. crawfordi* in other studies (Zhao et al., 2015; Sharma et al., 2016) as well as in this one. *Hymenolepis* and *Staphylocystis* also formed highly supported clades in a study by Neov et al. (2019), using 28S rRNA data.

The results from my analyses demonstrate that there is variation among *Hymenolepis* sequences from these *Thomomys* hosts. The *H. tualatinensis* sequence that was the sister to the other sequences from this study in the ITS1 trees was included in the COI trees, where it was monophyletic with the other *H. tualatinensis* sequence. Based on the ITS1 and COI trees, all the specimens sequenced in this study potentially could
represent the same species given they did form a monophyletic group. This would mean that this is the first account of *H. tualatinensis* from a *T. bottae* host. However, it also is possible that these represented multiple distinctive species. This would be unsurprising given that certain hymenolepidids that parasitize rodents (e.g., *A. horrida, R. nana*) have recently been shown to represent species complexes (Macnish et al., 2002b; Makarikov et al., 2015). If this were the case, then it is possible the *H. tualatinensis* that was the sister to the other *Hymenolepis* sequences from this study represents an undescribed species.

Rausch and Tiner (1948) suggested that *H. citelli* was synonymous with *H. diminuta* because they are difficult to differentiate morphologically. Although later studies demonstrated that they most likely are separate species (Voge, 1956), the validity of *H. citelli* as a distinctive species is, to date, still uncertain (Gardner et al., 2020). Due to this, I was hesitant to assign a species identification to the *Hymenolepis* from the *T. bottae* host based on morphology. I suspected it most likely was *H. citelli* based on host records (Voge, 1955; Jasmer, 1980), however an unidentified *Hymenolepis* species also was reported previously by Voge (1955). Because there are currently no *H. citelli* sequences on GenBank, I was unable to include this species (when identified with more certainty) in these analyses. It is worth repeating these analyses with sequences from specimens identified as *H. citelli*, including those from other rodent hosts (cricetids or sciurids), to confirm the identity of the new *Hymenolepis* sp. sequence or to confirm that it is a species distinct from *H. tualatinensis*. The 28S gene also would be useful to sequence given there are more hymenolepidid 28S sequences on GenBank.

This study is the first to document *Hymenolepis* from geomyid hosts using molecular data. It represents an initial step in uncovering the diversity present within
Thomomys hosts and verifies that multiple species are present in Thomomys species of Oregon.

The aim of this chapter was to use molecular data to document the helminths parasitizing Thomomys species of Oregon. I verified that T. fossor, H. thomomyos, R. rodentorum, and H. tualatinensis represent distinct lineages based on molecular data. These analyses also revealed that additional undescribed helminth species may be present in these hosts based on the amount of variation observed and resulting topological patterns in the phylogenies. For each species or group documented here, future studies should examine additional genetic markers and morphology to determine the extent of the helminth biodiversity present in Thomomys hosts.
Figure 3. 18S rRNA bootstrap consensus tree (1,000 replicates) generated using the maximum likelihood method based on the K2P+I+G model. Bootstrap support values of 70% or greater are indicated next to nodes. Hosts are included next to the *Trichuris fessor* sequences. Subclades A through D are indicated by brackets.
Figure 4. 18S rRNA maximum clade credibility consensus tree generated using the Bayesian inference method under the HKY model. Posterior probabilities for nodes >70% are displayed. Hosts are included next to the *Trichuris fossor* sequences. Subclades A through D are indicated by brackets.
Figure 5. This maximum clade credibility consensus tree based on COI mtDNA sequences was generated using Bayesian inference method and the TN93 model. For the *Trichuris fossor* sequences, the *Thomomys* host is listed. GenBank accessions are listed next to all other taxa. Posterior probabilities $>70\%$ are shown next to nodes and maximum likelihood bootstrap values $>70\%$ are shown in bold. Subclades A through D are indicated by brackets.
Figure 6. Partial 18S rRNA sequences (1,523 bp) were used to construct this maximum likelihood bootstrap consensus tree based on the aT3P+I+G model. Support values >70% are shown near nodes. For sequences from this study, the host is listed, and, for *Thomomys talpoides* hosts, the nearest township is included. Clades A and B are indicated by brackets.
Figure 7. Partial nuclear 18S rRNA sequences (1,523 bp) were used to construct this hypothesis. This Bayesian inference maximum clade credibility tree was generated using the HKY model. Support values \( \geq 70\% \) are shown near nodes. For new sequences, the host species are listed, and, for *Thomomys talpoides*, the nearest township is specified. Clades A and B are indicated by brackets.
Figure 8. Partial COI mtDNA sequences (576 bp) were used to construct this hypothesis. This maximum likelihood bootstrap consensus tree was generated using a GTR+I+G model. Support values ≥70% are shown near nodes. For new sequences, the host (and nearest town for Thomomys talpoides) is shown. For sequences from GenBank, the accession number is given. Clades A through D are indicated by brackets.
Figure 9. Partial COI mtDNA sequences (576 bp) were used to construct this Bayesian inference maximum clade credibility tree. This hypothesis used a TN93+I+G model. Posterior probabilities ≥70% are shown near nodes. Sequences from this study list the host name, and, for H. thomomyos, the nearest township in Oregon. Clades A through D are indicated by brackets.
Figure 10. This maximum likelihood bootstrap tree used 18S rRNA sequences and was generated using T92+I+G model. Bootstrap values ≥70% are shown near nodes. The *Ransomus rodentorum* specimen was from a *Thomomys bottae* host. GenBank accession numbers are shown next to the remaining taxa. Clades A through D are indicated by brackets.
Figure 11. This 18S rRNA maximum clade credibility tree was generated using the Bayesian inference method and the HKY+I+G model. Posterior probabilities \( \leq 70\% \) are not shown. The \textit{Ransomus rodentorum} specimen was from a \textit{Thomomys bottae} host. The remaining sequences were downloaded from GenBank; accession numbers are shown next to taxa. Clades A through D are indicated by brackets.
Figure 12. COI mtDNA tree constructed using the maximum likelihood method. This bootstrap consensus tree (1,000 replicates) was based on the TN93+I+G model. For Ransomus rodentorum sequences, the host is indicated. For sequences from GenBank, the accession number is shown. Support values \( <70\% \) are not shown. Subclades A through C are indicated by brackets.
Figure 13. COI mtDNA maximum clade credibility tree based on the Bayesian inference method. This phylogeny used the TN93+I+G model. Posterior probabilities $>70\%$ are shown next to nodes. For the new sequences, the hosts are indicated. For sequences from GenBank, the accession number is shown. Subclades A through E are indicated by brackets.
Figure 14. This Bayesian inference method maximum clade credibility tree was based on ITS1 sequences and used the HKY model with equal frequencies. Posterior probabilities <70%, and bootstrap support values <70% (in bold) are not shown. For new sequences, the *Thomomys* host is listed. For sequences from GenBank, the accession number is shown. Subclades A and B are indicated by brackets.
Figure 15. Maximum likelihood method bootstrap (1,000 replicates) consensus tree based on COI mtDNA sequences. This reconstruction used the TN93+I+G model. Bootstrap values ≥70% are shown next to nodes. For new sequences, the *Thomomys* host is indicated. For sequences from GenBank, the accession number is listed. Subclades A and B are indicated by brackets.
Figure 16. Bayesian inference maximum clade credibility consensus tree based on COI mtDNA sequences. This tree used the TN93 model. Posterior probabilities ≥70% are shown next to nodes. For sequences newly generated for this study, the *Thomomys* host is shown. For sequences from GenBank, the accession is shown next to the taxa. Subclades A and B are indicated by brackets.
Chapter 3: Phylogenetics of *Thomomys* Species of Oregon and a Coevolutionary Analyses of *Heligmosomoides* and Their *Thomomys* Hosts

**Introduction**

Coevolution, or pairwise evolution in interacting species, is an important phenomenon that impacts the composition of biodiversity worldwide and contributes to the maintenance of intricate biological communities (Thompson, 1998; Guimarã et al., 2011). Relationships between interacting species are influenced by coevolution (Thompson, 1998). Coevolution has been well-documented, although we are only beginning to understand the ecological effects of this process (Thompson, 1998).

In extreme instances, coevolution can lead to cospeciation of lineages. Phylogenies can test for cospeciation (parallel speciation) between parasites and hosts by comparing branch lengths and patterns and cladistic relationships (Page, 1996). Corresponding branch lengths and branch patterns indicate that cospeciation or cophylogeny is occurring (Hafner et al., 1994; Light and Hafner, 2007). The quintessential example of cophylogeny is pocket gophers (Rodentia: Geomyidae) and their chewing lice (Hafner et al., 1994; Hafner et al., 2003; Light and Hafner, 2007; Hafner et al., 2019; Popinga et al., 2019). Parallel phylogenies have resulted from the constrained distributional pattern of pocket gophers and, subsequently, their chewing lice (Hafner et al., 1994). Pocket gophers are solitary and exist in isolated populations (Hall, 1981; Daly and Patton, 1990; Verts and Carraway, 1998), making them an ideal host system for studies of coevolution (Hafner et al., 2003; Light and Hafner, 2007). Due to their antisocial dispositions, small home
ranges, and low dispersal capabilities, parasites are effectively trapped on the host lineage (Light and Hafner, 2007).

A similar relationship might exist between pocket gophers and their endoparasites, particularly considering certain helminth species have high degrees of host-specificity (Gardner, 1985). Fossorial mammals, such as pocket gophers, and their endoparasites are ideal candidate systems for coevolutionary study due to their remarkable degree of interdependence (Page, 2003). Whether a coevolutionary relationship is present between Thomomys hosts and their helminths has yet to be investigated. One way to test for coevolution involves constructing host and helminth phylogenies and comparing the topologies.

Phylogenetic studies on Thomomys species have been conducted by Smith (1998), Belfiore et al. (2008), and Mathis et al. (2013a, 2013b), among others. The cytochrome b gene is frequently used and has proven helpful for uncovering relationships at the genera and subgenera level (Smith, 1998; Mathis et al., 2013a; Mathis et al., 2013b). The COI gene has been used by others for phylogenetic (Mathis et al., 2013a; Mathis et al., 2013b) and coevolutionary studies (Hafner et al., 1994). Within the genus Thomomys, morphological and molecular analyses consistently recover two well-supported subgenera: Megascapheus and Thomomys (see Appendix A; Patton and Smith, 1981; Smith, 1998; Verts and Carraway, 1999; Belfiore et al., 2008). Morphological, karyotypic, and genetic evidence have been used to identify Thomomys species, and both nuclear and mitochondrial markers have been used in phylogenetic studies (Smith, 1998; Belfiore et al., 2008; Mathis et al., 2013a; Mathis et al., 2013b). Delineating species boundaries within the genus, however, is challenging because of rapid diversification.
events, on-going hybridization, incomplete lineage sorting, and the high degree of genetic diversity exhibited by *Thomomys* species (Belfiore et al., 2008).

Five *Thomomys* species are currently recognized in Oregon. Only one previous study, which used nuclear loci, included all the species of Oregon in a phylogenetic study (Belfiore et al., 2008). Here, I use the COI gene to examine the phylogenetic relationships of *Thomomys* species, with a special focus on those occurring in Oregon. This adds to the existing database of *Thomomys* sequences to be used in molecular analyses. Additionally, I analyze the potential for coevolution between *Heligmosomoides* species and their *Thomomys* hosts. The first aim of this study was to generate a phylogeny of *Thomomys* of Oregon based on COI mtDNA to use in a coevolutionary analysis. The second aim was to test the hypothesis that a coevolutionary relationship existed between *Heligmosomoides* and *Thomomys* using a distance-based analysis and assessing their phylogenies for congruence. This contributes to our understanding of host-parasite associations and the evolutionary histories of these taxa.

**Methods**

*Thomomys Phylogenetic Analyses* — Collection methods for *Thomomys* specimen collection were outlined in Chapter 1. The COI gene was sequenced for 19 *Thomomys* specimens collected from 15 sites (Fig. 17). The final dataset included the following specimens: 2 *T. bottae*, 10 *T. bulbivorus*, 2 *T. mazama*, 4 *T. talpoides*, and 1 *T. townsendii* (Fig. 17). DNA was extracted with the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer’s protocols or Sigma-Aldrich REDExtract-N-Amp™ Tissue PCR Kit. For extractions using the Sigma-Aldrich kit, 100 µL extraction
buffer and 25 µL tissue preparation solution were added to a fresh microcentrifuge tube and 1 mg of tissue (either heart, liver, or kidney) was submerged in the mixture. The following protocols were then run on a thermocycler: 10 minutes at 65°C, 10 minutes at 95°C, and 10 minutes at 10°C. Next, 125 µL neutralization buffer was added to the tubes. Standard PCR was carried out with PuReTaq Ready-To-Go PCR beads (Cytiva) using the primers CO1-5285F (5’- CCYCTGTNYTTAGATTACAGTCT -3’) and CO1-6929R (5’- ACAARGTTATGTAATDDTTTTACTA -3’; Spradling et al., 2004). The protocols specified in Spradling et al. (2004) were followed, with the exception that the annealing temperature was decreased to 49°C and the number of cycles varied from 30–40 (depending on the sample). PCR products were visualized on 1% agarose gels. Purification and sequencing methods for Thomomys PCR products followed those outlined in Chapter 2 for helminths.

Sequences were examined using MEGA v. 7.0.26 (Kumar et al., 2016). Forward and reverse sequences were combined in MEGA using MUSCLE. The concatenated sequences were deposited to GenBank under the accession numbers OK501245–OK501263 (Table 8, Appendix C). After performing a BLAST search using the newly acquired sequences, 9 previously published Thomomys sequences from GenBank were added to the dataset (Table 8). These sequences represented the following species: T. atrovarius (KC525221), T. bottae (AY331088), T. bulbivorus (AY331090), T. mazama (AY331092), T. monticola (AY506565), T. sheldoni (KC589035), T. talpoides (AY331091 and JX520545), and T. umbrinus (AY331089; Table 8). Only partial sequences of T. townsendii COI mtDNA were available on the GenBank database and they were too short to include in this analysis. Orthogeomys heterodus (KC680020) was
included as the outgroup. Sequence alignment methods are the same as those outlined in Chapter 2 for helminths. The *Thomomys* COI mtDNA dataset was 1,428 bp after trimming low quality and excess ends. MEGA determined that the GTR+I_(0.58)+G_(2.09) model was the best fit substitution model based on Bayesian information criterion (BIC). A maximum likelihood (ML) tree was generated in MEGA, and a Bayesian inference (BI) tree was generated in BEAST2 v. 2.6.1 using this model. The BI analyses ran for $1 \times 10^7$ generations. Tracer v 1.7.1 was used to assess convergence and ensure that effective sample size (ESS) values were met for all parameters. ESS values all were > 350. Tree files were combined in LogCombiner v. 2.6.0 and a maximum clade credibility (MCC) tree was created using TreeAnnotator v. 2.6.0 with posterior probabilities limited 50% and a burn-in percentage of 10%. FigTree v. 1.4.4 was used to visualize the MCC tree.

Table 8. Species included in the *Thomomys* phylogenetic analyses. Asterisks indicate sequences acquired during this study.

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<td>Thomomys sheldoni</td>
<td>KC589035</td>
<td>Durango, Mexico</td>
</tr>
<tr>
<td>Thomomys talpoides</td>
<td>AY331091</td>
<td>New Mexico: Sandoval Co.</td>
</tr>
<tr>
<td>Thomomys talpoides</td>
<td>JX520545</td>
<td>New Mexico: Cibola Co.</td>
</tr>
<tr>
<td>Thomomys talpoides*</td>
<td>OK501259</td>
<td>Oregon: Harney Co.</td>
</tr>
<tr>
<td>Thomomys talpoides*</td>
<td>OK501260</td>
<td>Oregon: Harney Co.</td>
</tr>
<tr>
<td>Thomomys talpoides*</td>
<td>OK501261</td>
<td>Oregon: Grant Co.</td>
</tr>
<tr>
<td>Thomomys talpoides*</td>
<td>OK501262</td>
<td>Oregon: Grant Co.</td>
</tr>
<tr>
<td>Thomomys townsendii*</td>
<td>OK501263</td>
<td>Oregon: Malheur Co.</td>
</tr>
<tr>
<td>Thomomys umbrinus</td>
<td>AY331089</td>
<td>Durango, Mexico</td>
</tr>
<tr>
<td>Orthogeomys heterodus</td>
<td>KC680020</td>
<td>Cartago, Costa Rica</td>
</tr>
</tbody>
</table>

Coevolutionary Analysis – Intestinal examination procedures followed those outlined by Gardner and Jasmer (1983). DNA extraction, PCR, purification, and sequencing methods for COI mtDNA were outlined in Chapter 2 for Heligmosomoides and above for Thomomys. In Chapter 2, I hypothesized that H. thomomyos and a new, undescribed species were present in these Thomomys hosts. In this chapter I refer to them as Heligmosomoides or Heligmosomoides species given that there are likely multiple species present within Thomomys hosts.

For these analyses, only Heligmosomoides sequences that had a corresponding Thomomys host COI gene sequenced were included. This led to a smaller dataset compared to that in Chapter 2 because they did not have corresponding host COI sequences, and vice versa. The final dataset with paired host and helminth sequences included a sample size of six Heligmosomoides and their six Thomomys hosts. One Heligmosomoides was sequenced from a T. bulbivorus host, 2 from separate T. bottae hosts, and 3 from separate T. talpoides hosts.

Host and parasite datasets were restricted to 530 bp of the COI gene, which was identified by aligning Heligmosomoides and Thomomys sequences using MUSCLE.
Using MEGA, the HKY model with a gamma distribution was determined to be the best fit substitution model based on BIC for both datasets (HKY+G(0.05) for both datasets; Hasegawa et al., 1985). Maximum likelihood (ML) trees then were generated for *Heligmosomoides* and *Thomomys*, respectively, following the methods outlined in Chapter 2. To construct a tanglegram, these trees then were uploaded in Inkscape (Inkscape Project, 2020) where dashed lines were added to connect the respective hosts to their helminths.

The hypothesis that coevolutionary relationships exist between these helminths and their *Thomomys* hosts was tested first using a distance-based analysis, ParaFit (Legendre et al., 2002) (999 permutations), in the R package ape (Paradis and Schliep, 2019). Next, the *Thomomys* and *Heligmosomoides* COI phylogenies were assessed for congruence using the PACo package (Balbuena et al., 2013) in R. The methods followed were outlined in Balbuena et al. (2013). In short, PACo generates Procrustean-fit residual sum of squares ($m^2_{xy}$) that evaluates similarities of host-parasite phylogenies and performs a goodness-of-fit test (100,000 permutations) to assess significance (Balbuena et al., 2013). PACo also performs a jackknife estimation, which allows significant associations among taxa to be distinguished (Balbuena et al., 2013).

**Results**

**Thomomys Phylogenetic Analyses** – Subgenus *Megascapheus* was monophyletic, with high support (98% BS and 100% posterior probability), in both trees (Figs. 18, 19). Within the *Megascapheus* clade, *T. bulbivorus* from this study and from GenBank formed a monophyletic group with 100% bootstrap and posterior probability support (Figs. 18,
As evidenced by the formation of subclades within the *T. bulbivorus* clade, there was some degree of variation observed for this species alone (Figs. 18, 19). The *T. bulbivorus* clade was sister to a clade that contained the remaining *Megascapheus* species (*T. atrovarius*, *T. bottae*, *T. sheldoni*, *T. townsendii*, and *T. umbrinus*; Figs. 18, 19). The node supporting the relationship of these sister clades had high bootstrap (98%) and posterior probability (100%) support (Figs. 18, 19). *Thomomys bottae* from this study did not form a monophyletic group with the *T. bottae* from GenBank in either analysis (Figs. 18, 19). Instead, the *T. bottae* sequences from this study were sister to a clade that contained the *T. atrovarius* and *T. bottae* sequences from GenBank and the *T. townsendii* sequence from this study (Figs. 18, 19). *Thomomys umbrinus* and *T. sheldoni* were sister taxa with 100% support in both analyses (Figs. 18, 19). The clade formed by these two species was sister to the *T. atrovarius*, *T. bottae*, and *T. townsendii* clade (Figs. 18, 19).

Species belonging to subgenus *Thomomys* formed a highly supported (99% BS and 100% posterior probability) monophyletic group in both analyses (Figs. 18, 19). In the ML tree, *T. talpoides* was paraphyletic; novel *T. talpoides* sequences from Oregon were more closely related to *T. mazama* than they were to the *T. talpoides* sequences from GenBank (from New Mexico) although the clade *T. mazama* and *T. talpoides* formed had low BS support (Fig. 18). In the BI tree, however, *T. talpoides* formed a highly supported (98.3%) monophyletic group (Fig. 19). The 3 *T. mazama* sequences were monophyletic in both trees with high (100% BS and posterior probability) support (Figs. 18, 19). *Thomomys monticola* was the sister to the remaining members of subgenus *Thomomys* with high (99%) bootstrap support in the ML tree (Fig. 18). In the BI tree, *T. monticola*
instead formed a clade with *T. mazama*, also with high (97.5%) posterior probability support (Fig. 19).

**Coevolutionary Analyses** – The *Thomomys* hosts COI ML tree topology was identical to that of the *Heligmosomoides* COI ML tree (Fig. 20). The John Day *T. talpoides* sequences were sister taxa and formed a clade with the *T. bulbivorus* sequence (Fig. 20). The *T. talpoides-T. bulbivorus* clade was sister to a second clade, which contained the *T. bottae* sequences. The *T. talpoides* sequence from Burns was sister to both clades. These relationships were mirrored in the *Heligmosomoides* tree (Fig. 20). Corresponding branching patterns, like the pattern observed here, are consistent with the view that a coevolutionary relationship exists between host and parasite (Hafner et al., 1994; Page, 1996; Light and Hafner, 2007).

The ParaFit results were not significant for *Heligmosomoides* and their *Thomomys* hosts (*p*-value = 0.115). The goodness-of-fit test (calculated with PACo) was also not significant (*m^2 = 0.041, p*-value = 0.252). Based on the jackknife analyses results, the links related to all samples except for the *T. talpoides* from Burns contributed moderately to the residual sum of squares, particularly in the case of the *T. bottae* host-parasite relationship (Fig. 21). This is indicative of a coevolutionary pattern for all *Thomomys-Heligmosomoides* associations except for the *T. talpoides-Heligmosomoides* from Burns. However, because the confidence intervals for the *T. talpoides* from John Day and the *T. bulbivorus* host-parasite links were broad, interpretation of their influence on the overall cophylogenetic pattern is less certain (Balbuena et al., 2013; Fig. 21).

**Discussion**
Thomomys Phylogenetic Analyses – This is only the second study to include all of the *Thomomys* species occurring in Oregon in a broader phylogenetic analysis of the genus (Belfiore et al., 2008) and the first containing all these species to use the COI gene. Given that many genetic studies have focused on *T. bottae* and *T. umbrinus* (Verts and Carraway, 1993), this study contributes new molecular evidence for additional species in Oregon that can be used in future genetic research and conservation projects.

Subgenera *Megascapheus* and *Thomomys* formed well-supported monophyletic groups in both phylogenetic analyses. This is unsurprising given that these groups were resolved as well-supported in studies based on morphology (Thaeler, 1980), molecules (Thaeler, 1980; Patton and Smith, 1981), and previous phylogenetic studies (Smith, 1998; Spradling et al., 2004; Belfiore et al., 2008; Mathis et al., 2014).

*Thomomys bulbivorus* forming the sister lineage to the remaining *Megascapheus* species also is consistent with prior research (Spradling et al., 2004; Belfiore et al., 2008). Although there is clearly some genetic diversity present among *T. bulbivorus* (Verts and Carraway, 1993; this study), these results still support the monotypic designation for this species because of the monophyletic groups recovered in each analysis and the short branch lengths within the monophyletic *T. bulbivorus* clade in the BI analyses. In Spradling et al. (2004), *T. bottae, T. bulbivorus,* and *T. umbrinus* also formed a well-supported clade.

The paraphyly exhibited by *T. bottae* may be attributable to rapid radiation within this species complex (Smith, 1998). The *T. bottae* sequence from GenBank was obtained from a specimen from Colorado and represented a different subspecies than the *T. bottae* sequenced in this study; this may have also contributed to the paraphyletic relationship
observed in these analyses. These results further suggest that *T. bottae* may represent a species complex, as shown by Smith (1998). In studies that used the cytochrome *b* gene, *T. bottae* also was paraphyletic (Patton and Smith, 1994; Smith, 1998; Belfiore et al., 2008).

*Thomomys atrovarius, T. sheldoni, and T. umbrinus* formed a clade with *T. bottae* as the sister taxon in a study based on 3 mitochondrial and 5 nuclear genes (Mathis et al., 2014). The relationships of these species to one another within this clade, however, were not resolved (Mathis et al., 2014). In the present study, *T. atrovarius* and the *T. bottae* from GenBank were sister taxa with *T. townsendii* as the successive sister group, and *T. sheldoni* and *T. umbrinus* represented sister taxa. Although this study was only based on one gene, it provides further insight into the relationships of these species.

Relationships among the species within the subgenus *Thomomys* were not identical between the two analyses in this study. *Thomomys monticola* and *T. talpoides* were more closely related to one another and *T. mazama* was the sister group in Álvarez-Castañeda and Patton (2004). This conflicts with both the BI and ML results in this study. *Thomomys mazama* and *T. monticola* forming a clade in the BI tree is congruent with the results recovered by Smith (1998). Spradling et al. (2004), however, found that *T. mazama* and *T. talpoides* consistently formed a clade and were sister taxa in the majority (4 of the 6) of their analyses (they formed polytomies with *T. monticola* in the remaining analyses), which is consistent with the results shown in the ML tree (Fig. 18). The paraphyly of *T. talpoides* in the ML tree aligns with the findings of Belfiore et al. (2008). Belfiore et al. (2008) suggested that *T. talpoides* likely was polytypic; my results provide further support for this assertion. The *T. talpoides* sequences from GenBank were from
specimens collected in New Mexico and all of my sequences were obtained from Oregon. This may have contributed to the paraphyletic relationship exhibited by *T. talpoides* in the ML tree as well.

Due to the short amount of time since the divergence of some species, reticulation may still be possible for some members of the genus *Thomomys* (Belfiore et al., 2008). Because of this and other factors influencing diversification (e.g., inadequate isolation mechanisms pressure), phylogenetic reconstructions remain problematic for taxa undergoing rapid radiation such as *Thomomys* species (Belfiore et al., 2008). High genetic differentiation is typical for *Thomomys* (Verts and Carraway, 1993) and mitochondrial genetic variation in particular is known to be extremely high within the genus (Mathis et al., 2014). The COI gene is not typically used independently for *Thomomys* at the intrageneric level, i.e., when used, it is usually concatenated with other genes for phylogenetic reconstructions (Spradling et al., 2004; Mathis et al., 2014). A multilocus approach has been shown to be effective at estimating *Thomomys* phylogenies (Mathis et al., 2014), thus, it would be advantageous to examine other markers in addition to the COI gene for all of the species included in these analyses. Additionally, increasing sampling efforts throughout the state of Oregon would be beneficial for uncovering the true relationships among these taxa.

**Coevolutionary Analyses** – Based on the tanglegram, it initially appeared that my hypothesis that a coevolutionary relationship existed between these *Heligmosomoides* species and their *Thomomys* hosts was supported. The jack-knife squared residuals plot also indicated there might be a pattern of coevolution between some of these host-parasite groups, although these results were more ambiguous. The results for the
remaining statistical analyses, however, did not provide strong support for such a relationship between these taxa. The ParaFit results suggested that the null hypothesis, that these hosts and helminths evolved independently (Hommola et al., 2009) and the individual host-parasite links are random, cannot be rejected. The null hypothesis for the goodness-of-fit test, that the host clades and the parasite clades are not associated with one another (Balbuena et al., 2013), was accepted in this instance as well. Both of these results indicate that there is not a consistent pattern of coevolution between Heligmosomoides and Thomomys species. Gardner (2001) stated that “evidence of phylogenetic coevolution of nematode parasites and their pocket gopher hosts” was scarce to completely absent. This study provides further support for this assertion. However, these results should be interpreted with caution due to small sample size.

Other factors may have obscured the true nature of the Heligmosomoides-Thomomys association. It is likely that multiple Heligmosomoides species were represented in this analysis (see Chapter 2). Thus, in addition to a small sample size, ‘the problem of multiple lineages’, or the presence of multiple parasite species (Page, 1993), could have led to a misrepresentation of these host-parasite associations (Light and Hafner, 2007). Replicating this analysis with a larger sample size potentially could yield different conclusions. Heligmosomoides thomomyos is putatively specific to Thomomys species (Gardner and Jasmer, 1983), but the specificity of the unidentified Heligmosomoides sp. is unknown. Eliminating the unknown species from the analysis along with more robust sampling efforts for H. thomomyos may also be useful. Hybridization, varying population sizes, and “degree and temporal length of isolation” may further complicate a coevolutionary analysis of Thomomys and their parasites.
(Patton and Smith, 1981). Lastly, a better understanding of the distribution and host specificities of these *Heligmosomoides* species is crucial to fully understand their associations with their hosts. If these *Heligmosomoides* species are cospeciating with their host species, it would be expected that their distributions would be congruent with that of their hosts (Hafner et al., 2019). However, there are currently insufficient data to determine the extent of the distributions of these *Heligmosomoides* species.

This study generated phylogenies for *Thomomys* species of Oregon based on the COI gene and, using the same gene, performed a coevolutionary analysis for *Thomomys* species and the *Heligmosomoides* that parasitize them. The results of the phylogenetic analyses were consistent with previous studies and there was no statistical support recovered for a coevolutionary relationship between *Thomomys* and *Heligmosomoides* species.
Figure 17. Distributions of *Thomomys* spp. of Oregon and collection sites for *Thomomys* spp. sequenced for this study.
Figure 18. *Thomomys* phylogeny based on the maximum likelihood method. This tree was generated using the GTR+I+G model. The accession numbers are shown next to taxa for sequences from GenBank. Bootstrap values $\geq 70\%$ are shown next to nodes.
Figure 19. *Thomomys* phylogenetic tree constructed using the Bayesian inference method. This maximum clade credibility tree was created using the GTR+I+G substitution model. Accession numbers are shown next to taxa downloaded from GenBank. Posterior probabilities ≥70% are shown next to nodes.
Figure 20. Tanglegram of Thomomys (left) and Heligmosomoides (right) COI sequences. Coexisting hosts and their helminths are linked by dashed lines. Tbo = T. bottae; Tbu = T. bulbivorus; Tt B = T. talpoides from Burns; Tt JD = T. talpoides from John Day.
Figure 21. Jack-knife squared residual with upper 95% confidence intervals (error bars) for *Heligmosomoides* and their *Thomomys* hosts. PACo was applied to HKY85 genetic distances. The median squared residual value is represented with the dashed line.
Chapter 4: The Influence of Host Intrinsic and Extrinsic Factors on Helminth Infection Prevalence and Intensity in Western Pocket Gopher (*Thomomys* spp.) Hosts

**Introduction**

The helminths *Ransomus rodentorum* and *Trichuris* spp., *Heligmosomoides* spp., and *Hymenolepis* spp. have life cycles with stages that occur outside of any host(s) (Jasmer, 1980; Hernandez and Sukhdeo, 1995; Schantz, 1996; Leroux et al., 2018; Appendix B). Given this phenomenon, extrinsic factors (i.e., those not directly pertaining to the host), such as environmental conditions, can be detrimental or fatal to eggs or larvae (Larsen and Roepstorff, 1999; Bogitsh et al., 2013; Gillingham, 2015). These factors can affect the prevalence (percentage of infected individuals) or intensity (number of parasites per individual host) of infections.

For example, Jasmer (1980) found that temperature and rainfall both influenced *R. rodentorum* larval prevalence in *Thomomys bottae* (Appendix A) in California. He hypothesized that the subsurface soil temperature likely had a considerable influence on the infectivity of *R. rodentorum* larvae given that infection intensities were higher in the spring. Subsequently, Gardner (1985) reported that *R. rodentorum* prevalence was significantly different between two *Thomomys bulbivorus* study sites within the Willamette Valley, Oregon, which he suspected might be attributable to microhabitat differences.

Gardner (1985) also reported seasonal variation in prevalence of *Trichuris fossor* infections in *T. bulbivorus* and hypothesized that these seasonal fluctuations were due to biotic and abiotic factors affecting the larvae’s infectivity or even the host’s
predisposition to infections. Studies on other trichurids (i.e., *T. vulpis*, *T. trichiura*, and *T. arvicolae*) have shown that individual hosts inhabiting drier, less humid environments had lower infection prevalence (Onorato, 1932; Torres et al., 2003; Ok et al., 2009).

Conversely, *Heligmosomoides* transmission may be more advantageous in arid climates (Milazzo et al., 2010). Prevalence of *H. polygyrus* infections in wood mice (*Apodemus sylvaticus*) in England varied significantly among collection years (Behnke et al., 1999) and intensity of infections varied seasonally, with higher intensities in the spring (Gregory, 1992).

The prevalence of some infections of *Hymenolepis* species (e.g., *H. diminuta*) also have been shown to vary seasonally as well (Ahmad et al., 2014), although *H. tualatinensis* prevalence did not vary by season in a study by Gardner (1985).

*Hymenolepis tualatinensis* is specific to *T. bulbivorus* (Gardner, 1985), which is endemic to the Willamette Valley. Gardner (1985) did, however, note variation in prevalence of *H. tualatinensis* between two *T. bulbivorus* collection sites within the Willamette Valley.

Whether the prevalence of *R. rodentorum*, *T. fossor*, and *H. thomomyos* varies in different climates has yet to be investigated. Evaluating the potential for a disparity among hosts collected from various regions can increase our knowledge of distributions and climatic conditions that are more favorable for the developing and infective ova.

Numerous intrinsic host factors, such as the age or sex of the host, can influence helminth infections (Behnke et al., 1999). In certain host-parasite systems, older individuals are more likely to be infected than younger ones (Roepstorff and Jorsal, 1989; Hamid et al., 2015; Villarreal et al., 2016; Hughes et al., 2018), although in other systems, there is no difference in prevalence of infections among age cohorts (Behnke et
al., 1999). Furthermore, inherent differences between host sexes such as morphology, physiology, or behavior can increase the likelihood of one sex being more frequently parasitized than the other (Poulin, 1996).

Understanding these extrinsic and intrinsic dynamics is critical to anticipate how climatic and environmental changes will affect rates of helminth infections in their hosts. The aim of this chapter is to investigate the effects of three intrinsic factors (host species, age, and sex) and four extrinsic factors (ecoregion, township, year, and season) on helminth infections in Western pocket gophers (Thomomys species) in Oregon. I hypothesize that: 1) there would be significant variation in the prevalence of infections among host species; 2) the prevalence of infections would vary among age classes; 3) the prevalence of infections would vary between male and female hosts; 4) variation among ecoregions may exist; 5) detecting variation in the prevalence and intensity of infections between years would be unlikely given the short time period over which this study took place; and 6) there would be seasonal fluctuations in the prevalence and/or intensity of infections. This study adds to existing knowledge regarding the impact of intrinsic and extrinsic factors on infection status of Thomomys species and further contributes to general understanding of helminth infections.

Methods

Thomomys specimen were collected using methods outlined in Chapter 1. Locality and date were recorded for each field sampling event and for most of the salvaged T. bulbivorus specimens (Fig. 2). Six of the T. bulbivorus specimens salvaged from professional trappers in the spring of 2018 had unknown localities and the remaining 80
*T. bulbivorus* were salvaged or collected from 21 locations throughout the Willamette Valley between March 2018 and November 2019 (Figs. 22, 23; Appendix C). The remaining species were collected from fewer localities (Fig. 22). Seven *T. bottae* were collected in August 2019 from Brookings, Curry County, and five *T. mazama* were collected in August 2019 from Prospect, Jackson County (Fig. 22). *Thomomys talpoides* was collected from three sites in Eastern Oregon: Burns and Frenchglen in Harney County, and John Day in Grant County (Fig. 22). One *T. talpoides* was captured in Frenchglen in August 2018, four specimens were collected from John Day in August 2019, and the remaining 23 *T. talpoides* specimens were from the Burns site. The Burns site was sampled in August of both 2018 and 2019 (Fig. 22). One of the nine *T. townsendii* was collected in August 2018 from Princeton, Harney County, the remaining specimens were from Owyhee, Malheur County, and were captured in August 2019 (Fig. 22).

*Thomomys* collected for this study were prepared as museum specimens and are currently housed at the Portland State University Museum of Vertebrate Biology. During specimen preparation, data recorded included standard body measurements (total body, tail, hind foot, and ear lengths), mass, sex, and reproductive condition (e.g., lactating, pregnant, presence of implantation scars, testes measurements, etc.). Ectoparasites and cheek pouch contents were stored in 95% EtOH and frozen tissue samples (typically heart, liver, and kidney samples) were collected during specimen preparation for future molecular studies (except for a few instances where salvaged specimens were too decomposed). Skeletons were subsequently cleaned in a dermestid beetle colony for storage in the Portland State University Museum of Vertebrate Biology.
Complete intestinal tracts of 134 *Thomomys* specimens were examined for helminths following methods outlined by Gardner and Jasmer (1983). During intestinal examinations, infection status (un- or infected), initial helminth species identifications (determined by locality within host, previous host records, and morphology), and intensity of infections (number of parasites/individual host) per helminth species were recorded. Helminths were stored in 95% ethanol and frozen for sequencing and, for some, a subset of helminths also were stored in 10% buffered formalin for subsequent mounting or for deposition in a museum collection.

Upon completion of dermestid cleaning, 50 skulls (32 *T. bulbivorus*, 4 *T. bottae*, 4 *T. mazama*, 8 *T. talpoides*, and 2 *T. townsendii*) were examined and assigned a relative age class based on suture ossification (Thaeler, 1968). The infection status of these specimens was unknown during the suture scoring process, which used a modification of the five cranial suture technique outlined by Thaeler (1968). The palatine-pterygoid, maxilla-palatine, maxilla-alisphenoid, alisphenoid-squamosal, and parietal-squamosal sutures were examined, but instead of recording each suture as either open or fused, they were assigned a score of 1–3 for open, fusing, or fused, respectively. The sum of the scores for each of the five sutures was used as the relative age class: the lowest score that could be assigned was a 5, while the highest score possible was a 15. Individuals with 2 or more unfused sutures received a score of 13 or lower and were considered young of the year; those that scored higher than a 13 were considered adults. This allowed for finer-scale partitioning of age classes.

The effects of host species, age, and sex as well as collection ecoregion, locality (township), year, and season on the prevalence and intensity of infections were evaluated
using R software (R Development Core Team, 2020). Prevalence was evaluated for ‘overall infections’, i.e., all helminth species combined, and for each helminth species separately whenever sample sizes were large enough \((n = 5\) or greater) to compare statistically with accuracy. Host and environmental variables were examined for all host and all helminth species combined, for separate helminth species with all hosts combined, independently for each host species with all helminth species combined, and/or, lastly, for individual helminth species for each host species. Intensity of infections was analyzed in the same manner. Intensity was recorded for 65 of the *Thomomys* specimens; those with low confidence in intensity counts were excluded. ‘Overall intensity’ is the sum of the intensities for all helminth species found within a single host. For statistical analyses that assume normality, outliers (based on interquartile range criterion) were removed from the overall intensity and from the *H. thomomyos* intensity datasets because numerous transformations failed to normalize the distribution of these variables. Without transformation or removal of outliers the results would not have been reliable because they would have violated the assumption of normality for the statistical analyses that were undertaken.

*Host species* – Variation in prevalence of infections among host species was assessed for all helminth species combined and for *T. fossor* and *H. thomomyos* separately using either a Pearson’s Chi-squared or a Fisher’s Exact test, depending on group sample sizes. *Ransomus rodentorum* and *Hymenolepis* species could not be compared due to small sample sizes or because they are highly host specific. To test for a significant difference in overall intensity among all hosts, a one-way ANOVA was carried out. A separate one-way ANOVA tested for variation in intensity of *T. fossor*
infections among all hosts. Lastly, a one-way ANOVA also was used to test for variation in intensity of *H. thomomyos* infections among *T. bottae, T. bulbivorus*, and *T. talpoides* (sample sizes were too small to compare for other hosts).

*Age* – Fisher’s Exact test was used to assess if a difference in prevalence of overall infections was present among age classes for all hosts, and in *T. bulbivorus* and *T. talpoides* independently. Subgroup sample sizes were too small for all other hosts to be analyzed independently. Separate Fisher’s Exact tests also were completed to determine whether the prevalence of *T. fossor* or *H. thomomyos* infections varied among age classes for all hosts and whether the prevalence of *H. tualatinensis* varied among *T. bulbivorus* age classes. Intensity was recorded for 24 of the *Thomomys* that were aged. The effect of host age on intensity of overall infections for all *Thomomys* hosts was assessed using a generalized linear model fitted using the equation \( \hat{y}_i = -71.907 + (6.747 \times \text{age}) \).

*Sex* – Sex was recorded for 128 individuals. Depending on sample size, a Pearson’s Chi-squared test (in some instances with simulated p-values due to small subgroup sizes) or a Fisher’s Exact test were used to analyze the influence of sex on prevalence for various groups. Separate Welch’s *t*-tests were used to test for significant differences between males and females in overall, *T. fossor*, and *H. thomomyos* infection intensities.

*Ecoregions* – Level III ecoregions sampled included the Willamette Valley (*T. bulbivorus*), Coast Range (*T. bottae*), Cascades (*T. mazama*), Northern Basin and Range (*T. talpoides* and *T. townsendii*), Blue Mountains (*T. talpoides*), and Snake River Plains (Fig. 22). The Blue Mountains ecoregion was excluded due to small sample size. A generalized linear model was used to determine whether a difference in infection prevalence existed among ecoregions. The model was fitted using the equation \( \hat{y}_i = \).
A separate generalized linear model was used to determine if infection intensity varied by ecoregions and was fitted to the data using the equation $\hat{y}_i = 23.750 + (-16.750_{(\text{Cascades})}) + (-19.150_{(\text{Coast Range})}) + (-16.630_{(\text{Northern Basin and Range})}) + (-1.404_{(\text{Willamette Valley})})$.

*Townships* – Localities also were grouped into townships (defined by nearest town or city). There were 18 total townships for all *Thomomys* species, of which 10 had sample sizes large enough to compare statistically for variance in overall infection prevalence: Brookings (Curry Co.), Burns (Harney Co.), Forest Grove (Washington Co.), John Day (Grant Co.), Newberg (Yamhill Co.), Owyhee (Malheur Co.), Prospect (Jackson Co.), Portland (Multnomah Co.), Sheridan (Yamhill Co.), and Sherwood (Washington Co.; Figs. 22, 23). Five of these townships (those from Yamhill and Washington Counties) represented collection sites for *T. bulbivorus* (Fig. 23). A Pearson’s Chi-squared test followed by pairwise comparisons using either a Fisher’s Exact test or a Pearson’s Chi-squared test (depending on subgroup sample sizes) with Bonferroni adjustments for multiple comparisons assessed whether a difference existed among townships in the prevalence of overall infections, infections by *T. fossor*, or those by *H. thomomyos*, for all host species. A Pearson’s Chi-squared test also was used to test whether a difference in prevalence of *H. tualatinensis* infections among townships occurred (for *T. bulbivorus* hosts only). Whether variation in intensity of overall infections among townships occurred was tested using a generalized linear model that was fitted to the data using the equation $\hat{y}_i = 7.611 + (-3.011_{(\text{Brookings})}) + (-5.111_{(\text{Forest Grove})}) + (16.139_{(\text{John Day})}) + (40.639_{(\text{Newberg})}) + (-1.754_{(\text{Owyhee})}) + (-0.611_{(\text{Prospect})}) + (20.389_{(\text{Sheridan})}) + (-5.111_{(\text{Sherwood})})$. 

95
Years – *Thomomys bulbivorus* and *T. talpoides* were sampled during multiple years with sufficiently large sample sizes to be statistically compared by year. Both species first were combined to analyze if there was a difference in overall infection prevalence between 2018 and 2019 using a Pearson’s Chi-squared test. Annual prevalence for overall infections then was evaluated independently for each host species: a Pearson’s Chi-squared was used for the *T. bulbivorus* interannual analysis and a Fisher’s Exact test was used for the *T. talpoides* annual analysis. A Welch’s t-test was used to test for variation in intensity of overall helminth infections for *T. bulbivorus* and *T. talpoides* (combined) between 2018 and 2019. To determine whether overall intensity of infections varied between 2018 and 2019 for each of these host species, a Mann-Whitney U test was carried out for *T. bulbivorus*, and a Welch’s t-test for *T. talpoides*. The different analyses used were due to differences in sample sizes for each of the species.

*Seasons* – Seasons were defined as three-month periods: fall = September 1–November 31; winter = December 1–February 28; spring = March 1–May 31; and summer = June 1–August 31. Only *T. bulbivorus* was evaluated in the seasonal analyses because it was the only species sampled throughout the year, the remaining species were collected in the summer (Table 9). A Pearson’s Chi-squared test was used to evaluate whether prevalence of overall helminth infections varied among seasons. Pearson’s Chi-squared test also was used to test whether prevalence of *T. fossor* infections varied by season. This analysis was followed either by Fisher’s Exact test or Pearson’s Chi-squared tests (depending on subgroup sample sizes) for pairwise comparisons between seasons with a Bonferroni adjustment for multiple comparisons. A Pearson’s Chi-squared test was used to determine if *H. thomomyos* or *H. tualatinensis* infection prevalence varied by
season. Seasonal variation could not be analyzed for *R. rodentorum* due to small subgroup sample sizes. Variation in intensity of overall helminth infections, and of *T. fossor*, *H. thomomyos*, and *H. tualatinensis* infections in *T. bulbivorus* each were assessed using one-way ANOVAs. Sample sizes for other helminths infecting *T. bulbivorus* were too small to statistically analyze for seasonal intensity variance.

Table 9. Sample size per season and year for all *Thomomys* specimens collected for this study. *Thomomys bulbivorus* was sampled year-round and was the only species considered for the seasonal analyses on infections.

<table>
<thead>
<tr>
<th></th>
<th><em>Thomomys bulbivorus</em></th>
<th><em>Thomomys bottae</em></th>
<th><em>Thomomys mazama</em></th>
<th><em>Thomomys talpoides</em></th>
<th><em>Thomomys townsendii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>27</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Summer</td>
<td>13</td>
<td>15</td>
<td>7</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Fall</td>
<td>14</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Winter</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>40</td>
<td>7</td>
<td>5</td>
<td>18</td>
</tr>
</tbody>
</table>

**Results**

Helminths were detected in 75 (55.97%) of the 134 *Thomomys* specimens: 6 (85.71%) *T. bottae*, 35 (40.70%) *T. bulbivorus*, 5 (100%) *T. mazama*, 22 (78.57%) *T. talpoides*, and 7 (77.78%) *T. townsendii* were infected with one or more helminth species (Fig. 24). Forty-four *Thomomys* were infected with *T. fossor* (32.8%), 32 were infected with *H. thomomyos* (23.9%), 11 were infected with *R. rodentorum* (8.2%) and 6 were infected with a *Hymenolepis* species (4.5%). Four (57.1%) of the *T. bottae* were infected with *T. fossor*, 5 (71.4%) were infected with *H. thomomyos*, and 1 (25%) was infected with *R. rodentorum* (Fig. 25). One *T. bottae* specimen (25%) also was infected with what I suspected was *Hymenolepis citelli* (Fig. 25). In the *T. bulbivorus* specimens, *T. fossor* was detected in 27 (31.4%), *H. thomomyos* in 5 (5.8%), *R. rodentorum* in 1 (1.2%), and
Heligmosomoides thomomyos was detected in 19 (67.9%) T. talpoides, while 10 (35.7%) had T. fossor infections (Fig. 25). No other helminths were detected in T. talpoides. No T. fossor were detected in any of the 9 T. townsendii examined, but R. rodentorum was detected in 7 (77.8%) and H. thomomyos was detected in 1 (11.1%; Fig. 25). No cestodes were detected in any of the examined T. mazama, T. talpoides, or T. townsendii (Fig. 25).

Host species – There was a significant difference in prevalence for overall infections among some Thomomys species (Table 10). Post-hoc pairwise comparison tests with Bonferroni adjustments for multiple comparisons revealed that overall infections differed significantly between T. bulbivorus and T. talpoides (Table 10). There was no significant difference in prevalence among host species for T. fossor infections (Table 10). There was, however, a significant difference in prevalence of H. thomomyos infections among host species: T. bulbivorus was significantly different from T. bottae and from T. talpoides; T. talpoides and T. townsendii also differed significantly from one another (Table 10).

Table 10. Host and helminth species groups, sample sizes, test performed, and results for infection prevalence variation among host species analyses. P-values were adjusted for multiple comparisons using a Bonferroni correction. Significant values are indicated in bold and with an asterisk.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Helminth species</th>
<th>n</th>
<th>Test</th>
<th>Analyses results</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>All (overall</td>
<td>134</td>
<td>Pearson's Chi-</td>
<td>$X^2(1) = 21.536, p-value = 0.0005^*$</td>
</tr>
<tr>
<td></td>
<td>infections)</td>
<td></td>
<td>squared</td>
<td></td>
</tr>
<tr>
<td>T. bulbivorus vs. T. talpoides</td>
<td>All</td>
<td>113</td>
<td></td>
<td>$X^2(1) = 10.334, p-value = 0.001$ (adjusted = 0.013$)</td>
</tr>
<tr>
<td>T. bulbivorus vs. T. bottae</td>
<td></td>
<td>92</td>
<td></td>
<td>$X^2 = 5.193, p-value = 0.041$ (adjusted = 0.409)</td>
</tr>
</tbody>
</table>
Overall intensities of infections ranged from 1 to 209 ($\bar{x} = 14.03$, SD = 29.376; Fig. 26). For *T. bulbivorus*, overall intensity was recorded for 26 individuals and ranged from 1 to 209 ($\bar{x} = 22.346$, SD = 42.823; Fig. 26). All other host species had a narrower range of infection intensities (Fig. 21). Intensity was recorded for 5 *T. bottae* ($\bar{x} = 4.6$, SD = 2.51, range = 1–7), 5 *T. mazama* ($\bar{x} = 7.0$, SD = 4.062, range = 1–12), 22 *T. talpoides* ($\bar{x} = 10.545$, SD = 16.916, range = 1–74), and 9 *T. townsendii* ($\bar{x} = 5.857$, SD = 3.132. range =

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>N</th>
<th>Test</th>
<th>$X^2$</th>
<th>p-value</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. bulbivorus</em> vs. <em>T. mazama</em></td>
<td>90</td>
<td>$X^2 = 6.618$</td>
<td>0.016</td>
<td>(adjusted = 0.16)</td>
<td></td>
</tr>
<tr>
<td><em>T. bulbivorus</em> vs. <em>T. townsendii</em></td>
<td>94</td>
<td>$X^2 = 4.411$</td>
<td>0.083</td>
<td>(adjusted = 0.825)</td>
<td></td>
</tr>
<tr>
<td><em>T. bottae</em> vs. <em>T. talpoides</em></td>
<td>35</td>
<td>Fisher's Exact test</td>
<td>p-value = 1 (adjusted = 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. mazama</em> vs. <em>T. talpoides</em></td>
<td>12</td>
<td>p-value = 0.556 (adjusted = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. bottae</em> vs. <em>T. mazama</em></td>
<td>16</td>
<td>p-value = 1 (adjusted = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. bottae</em> vs. <em>T. townsendii</em></td>
<td>14</td>
<td>p-value = 0.506 (adjusted = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. mazama</em> vs. <em>T. townsendii</em></td>
<td>37</td>
<td>p-value = 1 (adjusted = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All <em>T. fossor</em></td>
<td>134</td>
<td>Pearson's Chi-squared</td>
<td>$X^2 = 7.930$, p-value = 0.081</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>132</td>
<td>Pearson's Chi-squared</td>
<td>$X^2 = 63.882$, p-value = 0.0005*</td>
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<tr>
<td><em>T. bulbivorus</em> vs. <em>T. talpoides</em></td>
<td>113</td>
<td>$X^2 = 56.053$, p-value = 0.0005 (adjusted = 0.004*)</td>
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<tr>
<td><em>T. bulbivorus</em> vs. <em>T. bottae</em></td>
<td>92</td>
<td>$X^2 = 32.624$, p-value = 0.0005 (adjusted = 0.004*)</td>
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<tr>
<td><em>T. bulbivorus</em> vs. <em>T. mazama</em></td>
<td>88</td>
<td>$X^2 = 3.692$, p-value = 0.186 (adjusted = 1)</td>
<td></td>
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<td></td>
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<tr>
<td><em>T. bulbivorus</em> vs. <em>T. townsendii</em></td>
<td>94</td>
<td>$X^2 = 0.663$, p-value = 0.428 (adjusted = 1)</td>
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<td></td>
</tr>
<tr>
<td><em>T. bottae</em> vs. <em>T. talpoides</em></td>
<td>35</td>
<td>Fisher's Exact test</td>
<td>p-value = 1 (adjusted = 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. bottae</em> vs. <em>T. mazama</em></td>
<td>10</td>
<td>p-value = 1 (adjusted = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. bottae</em> vs. <em>T. townsendii</em></td>
<td>16</td>
<td>p-value = 0.035 (adjusted = 0.315)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. mazama</em> vs. <em>T. talpoides</em></td>
<td>31</td>
<td>p-value = 1 (adjusted = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. mazama</em> vs. <em>T. townsendii</em></td>
<td>12</td>
<td>p-value = 0.506 (adjusted = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. talpoides</em> vs. <em>T. townsendii</em></td>
<td>37</td>
<td>p-value = 0.002 (adjusted = 0.020*)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2–10; Fig. 26). A one-way ANOVA determined that total intensity did not vary significantly among host species \((F_{[4,53]} = 0.428, p\text{-value} = 0.787)\).

Intensity of *Trichuris fossor* infections was recorded for each of the 4 infected *T. bottae* \((\bar{x} = 6.25, SD = 3.594\) range 3–11), 21 *T. bulbivorus* \((\bar{x} = 20.81, SD = 45.063,\) range 1–208), each of the 3 infected *T. mazama* \((\bar{x} = 8.333, SD = 3.055,\) range 5–11), and 10 *T. talpoides* \((\bar{x} = 5.7, SD = 9.764,\) range 1–33; Fig. 27). Intensity of *T. fossor* infections did not vary significantly among host species \((F_{[3, 34]} = 1.667, p\text{-value} = 0.192)\).

Intensity of *H. thomomyos* infections were recorded for 32 Thomomys specimens \((\bar{x} = 7.25, SD = 10.411,\) range 1–43). Five *T. bottae* \((\bar{x} = 7, SD = 10.700,\) range 1–26), 4 *T. bulbivorus* \((\bar{x} = 1.75, SD = 1.5,\) range 1–4), 2 *T. mazama* \((\bar{x} = 3.5, SD = 3.536,\) range 1–6), 20 *T. talpoides* \((\bar{x} = 8.9, SD = 11.863,\) range 1–43), and 1 *T. townsendii* \((\bar{x} = 5, SD = 0,\) range 5) were infected (Fig. 28). Despite the disparities in the ranges among host species, the intensity of *H. thomomyos* infections did not differ significantly among *T. bottae, T. bulbivorus, or T. talpoides* \((F_{[2,26]} = -0.714, p\text{-value} = 0.499)\).

*Ransomus rodentorum* intensity was recorded for 2 *T. bulbivorus* \((\bar{x} = 1, SD = 0,\) range 1), 1 *T. mazama* \((\bar{x} = 2, SD = 0,\) range 2), and 7 *T. townsendii* \((\bar{x} = 5.143, SD = 2.854,\) range 2–10). Sample sizes were too small to compare statistically to assess whether significant differences in intensities existed among hosts.

*Hymenolepis tualatinensis* intensity was recorded (by best estimation given cestodes are segmented and specimens broke apart easily) for 4 *T. bulbivorus* and ranged from 2 to 61 \((\bar{x} = 33, SD = 31.36)\). For the *H. tualatinensis* intensity of 61, however, the count was based on scolexes and therefore is more reliable.
**Age** – The lowest age class score assigned, a 9, was an uninfected individual (Fig. 24). For both age classes 10 and 11, two of the six hosts (33.33%) were infected with helminths (Fig. 24). For age class 12, all four aged individuals (100%) were infected (Fig. 29). Age classes 13 and 14 had more infected than uninfected hosts; age class 13 had an infection prevalence of 71.43% and age class 14 had an infection prevalence of 75% (Fig. 29). The last age class, 15, had a prevalence of 55.56% (Fig. 29). Based on the Fisher’s Exact test, I determined that there was no significant difference in prevalence of overall infections among age classes when all aged hosts were analyzed together (p-value = 0.197) or when *T. bulbivorus* or *T. talpoides* were analyzed independently (p-values = 0.361 and 0.556, respectively). The prevalence of *T. fossor* was marginally significantly different among age classes while *H. thomomyos* prevalence did not vary among age classes when all host species were analyzed together (p-values = 0.054 and 0.664, respectively). *Trichuris fossor* was more common in older individuals than in younger ones. Lastly, prevalence of *H. tualatinensis* did not vary among age classes for *T. bulbivorus* hosts (p-value = 0.516).

The host that was infected with 209 helminths was assigned to the oldest age class (suture score = 15; Fig. 30). All outliers for overall intensity of infections belonged to individuals assigned to age class 13 or higher (Fig. 30). Although intensity of infections appeared to vary among age classes, the results of the statistical analyses were not significant (p-value = 0.255).

**Sex** – There was no significant difference in prevalence of infections between males and females (Fig. 31) in any of the groups analyzed (Table 11). Intensity of overall helminth infections (Fig. 32), *T. fossor* infections, and *H. thomomyos* infections for all
host species did not vary significantly between male or female hosts ($t_{[52.641]} = -0.012$, $p$-value = 0.991; $t_{[26.541]} = -1.63$, $p$-value = 0.184; $t_{[24.735]} = -0.460$, $p$-value = 0.650, respectively).

Table 11. Sample size, test used, and results for each of the groups analyzed for influence of sex on prevalence of infections. * = *Ransomus rodentorum* was not detected any the *T. talpoides*, all other *Thomomys* species were combined.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Helminth species</th>
<th>n</th>
<th>Test</th>
<th>Analyses results</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>All</td>
<td>128</td>
<td>Pearson's Chi-squared</td>
<td>$X^2(1) = 0.982$, $p$-value = 0.322</td>
</tr>
<tr>
<td></td>
<td>T. fossor</td>
<td></td>
<td></td>
<td>$X^2(1) = 0.386$, $p$-value = 0.535</td>
</tr>
<tr>
<td></td>
<td>H. thomomyos</td>
<td>126</td>
<td></td>
<td>$X^2(1) = 3.335$, $p$-value = 0.068</td>
</tr>
<tr>
<td></td>
<td>R. rodentorum*</td>
<td>66</td>
<td>Fisher's Exact</td>
<td>$p$-value = 0.166</td>
</tr>
<tr>
<td>T. bulbivorus</td>
<td>All</td>
<td>82</td>
<td>Pearson's Chi-squared</td>
<td>$X^2(1) = 0.562$, $p$-value = 0.453</td>
</tr>
<tr>
<td></td>
<td>T. fossor</td>
<td></td>
<td></td>
<td>$X^2(1) = 1.214$, $p$-value = 0.271</td>
</tr>
<tr>
<td></td>
<td>H. thomomyos</td>
<td></td>
<td></td>
<td>$X^2(1) = 0.858$, $p$-value = 0.618</td>
</tr>
<tr>
<td></td>
<td>H. tualatinensis</td>
<td></td>
<td></td>
<td>$X^2 = 0.122$, $p$-value = 1</td>
</tr>
<tr>
<td></td>
<td>R. rodentorum</td>
<td>37</td>
<td>Fisher's Exact</td>
<td>$p$-value = 0.460</td>
</tr>
<tr>
<td>T. bottae</td>
<td>All</td>
<td>7</td>
<td>Fisher's Exact</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>T. fossor</td>
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<td>0.429</td>
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<tr>
<td></td>
<td>H. thomomyos</td>
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<tr>
<td></td>
<td>R. rodentorum</td>
<td></td>
<td></td>
<td>0.25</td>
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<tr>
<td>T. mazama</td>
<td>All</td>
<td>5</td>
<td>Fisher's Exact</td>
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<td>T. fossor</td>
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<td>R. rodentorum</td>
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<tr>
<td>T. talpoides</td>
<td>All</td>
<td>25</td>
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<td>0.653</td>
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<tr>
<td></td>
<td>T. fossor</td>
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<td>0.087</td>
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<tr>
<td></td>
<td>H. thomomyos</td>
<td></td>
<td></td>
<td>0.667</td>
</tr>
<tr>
<td>T. townsendii</td>
<td>All</td>
<td>9</td>
<td></td>
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<td></td>
<td>H. thomomyos</td>
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<td></td>
<td>0.222</td>
</tr>
<tr>
<td></td>
<td>R. rodentorum</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

_Ecoregions_ – Neither prevalence nor intensity of overall infections varied significantly among ecoregions (all host species combined; $p$-values all > 0.05; Figs. 33, 34).
Townships – A variation in overall infection prevalence for all host species was detected among townships \( (X^2 = 53.05, p\text{-value} = 0.0005) \). Post-hoc tests determined the difference in variance were between Burns and Sherwood (adjusted \( p\text{-value} = 0.002 \)), Burns and Forest Grove (adjusted \( p\text{-value} = 0.006 \)), and Forest Grove and Newberg (adjusted \( p\text{-value} = 0.009 \)). For \( T. \ bulbivorus \) collection sites only, there was variation in prevalence of overall infections among townships as well \( (p\text{-value} = 0.0005; \text{Fig. 35}) \). Pairwise comparisons with Bonferroni adjustments revealed Newberg and Sherwood (adjusted \( p\text{-value} = 0.010 \)), Forest Grove and Newberg (adjusted \( p\text{-value} = 0.002 \)), and Forest Grove and Portland (adjusted \( p\text{-value} = 0.040 \)) differed significantly in prevalence of overall infections. The prevalence of \( T. \ fossor \) infections for all host species varied significantly among townships \( (X^2 = 60.654, p\text{-value} = 0.0005) \), and pairwise comparisons with Bonferroni adjustments showed that the significance was between Forest Grove and Newberg (adjusted \( p\text{-value} = 0.002 \)). \( Heligmosomoides \ thomomyos \) prevalence varied significantly among townships as well \( (X^2 = 56.351, p\text{-value} = 0.0005) \); Brookings and Forest Grove (adjusted \( p\text{-value} = 0.014 \)), Burns and Forest Grove (adjusted \( p\text{-value} = 0.00002 \)), Burns and Owyhee (adjusted \( p\text{-value} = 0.017 \)), and Burns and Sherwood (adjusted \( p\text{-value} = 0.022 \)) varied significantly from one another. There was no variation in prevalence of \( H. \ tualatinensis \) infections among \( T. \ bulbivorus \) sites \( (X^2 = 5.343, p\text{-value} = 0.229) \). Intensity of overall infections for all hosts varied by township: Newberg was significantly different than other townships \( (p\text{-value} = 0.003, \text{all other } p\text{-values } > 0.05; \text{Fig. 36}) \).

Years – There was a significant difference in prevalence of overall infections between 2018 and 2019 when \( T. \ bulbivorus \) and \( T. \ talpoides \) where combined \( (X^2 = 5.343, p\text{-value} = 0.229) \).
3.953, df = 1, $p$-value = 0.047; Fig. 37) and when *T. bulbivorus* was analyzed independently ($X^2 = 6.500$, $p$-value = 0.011). Infections were more prevalent in 2018 for both species combined (Fig. 37) and for *T. bulbivorus* only. There was no significant difference in prevalence of infections between 2018 and 2019 for *T. talpoides* ($p$-value = 0.375). Intensity of overall infections for *T. bulbivorus* and *T. talpoides* (combined) did not vary between 2018 and 2019 ($t [45.648] = 1.2449$, $p$-value = 0.2195; Fig. 38).

Intensity of overall infections did not vary between years for *T. bulbivorus* or for *T. talpoides* when each was analyzed independently of one another ($p$-values > 0.05).

*Seasons* – The prevalence of overall helminth infections for *T. bulbivorus* varied marginally among seasons ($p$-value = 0.051; Fig. 39). After applying Bonferroni adjustments for multiple comparisons, however, I was unable to detect which seasons significantly varied from one another. *Trichuris fossor* infection prevalence varied significantly among seasons ($p$-value = 0.030), with spring and summer differing significantly from one another ($X^2 = 9.294$, adjusted $p$-value = 0.035). Infections were significantly more prevalent in the spring (51.35%) than in the summer (22.22%).

*Heligmosomoides thomomyos* infection prevalence also varied by season ($X^2 = 19.53$, $p$-value = 0.007) though, after correcting for multiple seasonal comparisons, there was no significant difference among any of these seasons. The prevalence of *Hymenolepis tualatinensis* infection prevalence did not vary by season ($X^2 = 2.887$, $p$-value = 0.453). Intensity of overall helminth infections did not vary among seasons ($F_{[3, 22]} = 0.653$, $p$-value = 0.590; Fig. 40), nor did intensity of *T. fossor* ($F_{[3, 17]} = 0.409$, $p$-value = 0.749), *H. thomomyos* ($F_{[2, 1]} = 0.25$, $p$-value = 0.816), or *H. tualatinensis* infections ($F_{[2, 1]} = 0.634$, $p$-value = 0.664).
Discussion

Prior to this study, no reports of helminth parasitism existed for *T. mazama* or *T. townsendii* (Hughes et al., 2020; Hughes et al., 2021). This work represents new host records for *T. fossor* (*T. mazama* only), *H. thomomyos* (both host species), and *R. rodentorum* (both host species). Because sample sizes were small for both host species, with only one to two sites sampled for each, there could be additional helminth species parasitizing these hosts, particularly those inhabiting other areas throughout their distributions.

This also is the first study to report overall prevalence and intensity of infections for *T. bottae* and *T. bulbivorus*. Jasmer (1980) reported prevalence by site and helminth species individually for *T. bottae* that he examined from Humboldt County, California. Likewise, Gardner (1985) reported prevalence and intensities of individual helminth species only for *T. bulbivorus* from two sites in the Willamette Valley. Todd et al. (1971) reported that 45 of the 46 (97.83%) *T. talpoides* they examined from Wyoming were infected with helminths. The overall prevalence observed by Todd et al. (1971) is higher than that observed for *T. talpoides* in the present study (78.57%). Todd et al. (1971) also reported a more diverse assemblage of helminths than detected in this study: they recovered seven total helminth species whereas I only detected two for *T. talpoides*.

Only one report previously documented the presence of *T. fossor* in *T. bottae* (from California; Chandler, 1945), and that author did not report the prevalence of infections. Jasmer (1980) did not detect *T. fossor* in any of the 89 *T. bottae* examined from California. The prevalence reported here for *T. fossor* in *T. bottae* should be viewed
cautiously given the small sample size and the fact that only one site was sampled. The prevalence of *Trichurus fossor* in *T. bulbivorus* in this study (31.40%) was comparable to that reported by Gardner in 1985 (26.03%). but the prevalence of *T. fossor* in *T. talpoides* in this study (35.71%) is lower than that of *T. fossor* in *T. talpoides* from Wyoming (65.22%; Todd et al., 1971). In addition to different sampling areas, the Todd et al. (1971) study had a larger sample size (46 versus 28 for this study). Given this, the prevalence Todd et al. (1971) reported might be more accurate for *T. fossor* in *T. talpoides* overall.

Chandler (1945) did not report the range or average intensity of *T. fossor* infections in *T. bottae* from California, thus there are no prior reports to which this study can be compared. The range of *T. fossor* infections in *T. bulbivorus* was much broader in this study (1–208) than in Gardner’s (1985; range = 1–36). Higher intensities may be typical for certain areas within the Willamette Valley, such as Newberg, which was not sampled by Gardner (1985), or, more likely, this was an irregularity as the intensity of 208 for *T. fossor* was a major outlier. The range of *T. fossor* infections in *T. talpoides* from Wyoming was 1–24 (Todd et al., 1971), which was comparable to that observed in this study (1–33).

*Heligmosomoides thomomyos* was present in 16.85% of *T. bottae* in Jasmer’s (1980) study in California, although it only was detected at one of the three sites. The prevalence reported here is much higher (71.4%), although my sample size was much smaller than his, and the *T. bottae* examined in this study were collected during a single event rather than over the span of several months (Jasmer, 1980). The difference between these studies can be mostly attributed to the fact that my specimens were collected during
the summer, when the prevalence of infections likely is higher (Jasmer, 1980). These differences could also be regional given that Jasmer (1980) only detected *H. thomomyos* at one collection site. It is possible that only certain areas or microhabitats within *T. bottae*’s range, such as my study site, are suitable for the life cycle and/or transmission of *H. thomomyos*. Gardner (1985) reported a prevalence of 9.6% for *H. thomomyos* in *T. bulbivorus* from both of the sites he sampled, which is higher than the 5.8% I observed. The present report also represents the first report of *H. thomomyos* from *T. talpoides* (Hughes et al., 2021), thus there are no previous studies to compare these findings with.

Jasmer (1980) did not report the intensity for *H. thomomyos* infections in *T. bottae* and neither did Todd et al. (1971) for *T. talpoides*. The present is the first report to document *H. thomomyos* intensities for these host species. The range and average intensity of *H. thomomyos* infections in *T. bulbivorus* from this study was comparable to that of Gardner’s (1985) survey (1–5, \( \bar{x} = 3 \)).

Jasmer (1980) found *R. rodentorum* in 75.3% of *T. bottae* from California while *R. rodentorum* was detected in 8.2% of the *T. bottae* examined in this study. Gardner (1985) reported a prevalence of 11.0% for *R. rodentorum* for *T. bulbivorus*, which is much higher than the 1.2% reported here. Todd et al. (1971) reported a prevalence of 81% for *R. rodentorum* in Wyoming *T. talpoides*, although I did not detect this helminth in any of the *T. talpoides* examined. *Ransomus rodentorum* was more prevalent in *T. townsendii* from Owyhee than in any other species.

*Hymenolepis citelli* was present in 11.2% of *T. bottae* collected in the spring in Jasmer’s (1980) study. It was only present in one (25%) of the *T. bottae* examined in this study. The smaller sample size in this study may account for the difference in prevalence
between these two studies. In addition, Jasmer (1980) found *H. citelli* at two of his three sites while only one location was sampled here.

Gardner (1985) observed *H. tualatinensis* in 10% of the *T. bulbivorus* he examined. Prevalence of *H. tualatinensis* in this study in contrast was only 5.8%. Although our sample sizes were similar (his was 73, mine was 86), I sampled more areas throughout the Willamette Valley than Gardner (1985), who only surveyed two sites. Additional areas throughout the Willamette Valley should be surveyed to understand whether there are temporal or microhabitat differences for *H. tualatinensis*. Another hypothesis that also warrants further investigation is that *H. tualatinensis* is in decline (S. L. Gardner, pers. comm., 26 April 2018).

Discrepancies between this and previous studies in prevalence of overall or individual helminth infections likely are due to regional and/or temporal fluctuations. Broader sampling efforts, particularly for taxa with extensive distributions such as *T. bottae* and *T. talpoides*, could explain why differences in assemblages and prevalence of helminth infections were observed. In addition, the use of toxic chemicals, such as pesticides, also might play a role in transmissibility, prevalence, or intensity of helminth infections (Dhakal et al., 2020; Edo-Taiwo and Aisien, 2020). Future studies should also attempt to document whether chemicals are used where *Thomomys* specimens are collected.

It was surprising that the overall prevalence of infections varied significantly only between *T. bulbivorus* and *T. talpoides* (p-value = 0.013) given the higher overall prevalence in other species as well. For instance, *T. bottae’s* overall prevalence was 85.7% and *T. mazama’s* overall prevalence was 100%. The difference in overall infection
prevalence likely is attributable to small sample sizes for the remaining species. *Thomomys bulbivorus* and *T. talpoides* had the largest sample sizes, the remaining host groups compared may have been too small to detect any true differences in prevalence of overall or individual helminth species infections. The prevalence and intensity of *Trichuris fossor* did not vary among host species, which provides further evidence that *T. fossor* is not host-specific to the species level. However, small sample sizes could be obscuring any real trends among *T. fossor* and *Thomomys* hosts. *Heligmosomoides thomomyos* prevalence did vary between certain species, although intensities of infections did not. The variation among species in prevalence of *H. thomomyos* infections could be attributable to on-going host specialization or differences in host immunity, although on-going studies are necessary to test both of these hypotheses.

Most of the individuals whose age was assessed belonged to older age classes, which initially appeared to be more frequently parasitized than younger groups (based on Fig. 24). However, there was no statistically significant difference in prevalence of overall infections among age classes. *Trichuris* species tend to be more prevalent in younger individuals in other hosts (Deter et al., 2007; Gul and Tak, 2016), which was the case in the present study. *Heligmosomoides thomomyos* did not vary significantly among *Thomomys* age classes, which is consistent with other studies on *Heligmosomoides* spp. (e.g., *H. polygyrus* in wood mice; Behnke et al., 1999). Other hymenolepidids (e.g., *Rodentolepis nana*) are more prevalent among older age groups (Hamid et al., 2015). On the basis of this widespread observation, I initially hypothesized there may be a difference in age of hosts of *H. tualatinensis*, although no difference in prevalence among age classes was observed. A larger sample size that included more individuals belonging
to younger age classes might have shown a different relationship between infections and age. The sample size for the intensity on age was also small, thus the results from the analysis on the effect of age on the intensity of infections should be interpreted with caution.

A higher prevalence in one host sex has been demonstrated for some close relatives of *T. fossor*, *H. thomomyos*, and *H. tualatinensis* (Behnke et al., 1999; Tasawar et al., 2004; Sanchez et al, 2011; Hamid et al., 2015). I therefore hypothesized significant variation in prevalence of infections between male and female hosts would exist. Based on the findings here (Fig. 26), it appeared initially that, when all host species were combined, males were more frequently parasitized than females. However, there was no statistically significant difference between sexes. The same was true for a study on *H. polygyrus* in wood mice in England: the prevalence of infections was slightly higher in females than in males, although infections were not significantly different between sexes (Behnke et al., 1999). Intensity of *H. polygyrus* infections did not vary between sexes in that study, or in a study on yellow-necked mice (*Apodemus flavicollis*) from the Italian Alps (Luong et al., 2010), which is consistent with my results.

Given the differences in climate among Oregon’s ecoregions and previous work on other trichurids and heligmosomatids (Onorato, 1932; Torres et al., 2003; Ok et al., 2009; Milazzo et al., 2010), I hypothesized that variation should exist among ecoregions and that there would be significant variation in prevalence of infections among these host species inhabiting different ecoregions. However, this study did not support the hypothesis that ecoregions influence infections for these helminth species. For example, Todd et al. (1971) noted that the prevalence of *T. fossor* and *R. rodentorum* in *T.*
talpoides in Wyoming decreased as elevation increased. Here, there was no significant difference in the prevalence of T. fossor between the Willamette Valley and ecoregions occurring at higher elevations.

It was not surprising that the prevalence of infections for the Forest Grove township differed from Burns and Newberg for all host species’ collection sites or from Newberg and Portland for sites where T. bulbivorus were captured because most (13 of the 16 or 81.25%) of the T. bulbivorus examined from Forest Grove were not infected with any helminths. The difference in prevalence between Sherwood and Burns also is unsurprising given that most of the T. bulbivorus from Sherwood (25 of the 33 or 75.76%) were uninfected, while the majority of the T. talpoides from Burns (18 of the 23 or 78.26%) were infected. Variation in the prevalence of T. fossor infections between sites also was observed in the study by Gardner (1985). Interestingly, in Gardner’s (1985) study, a site near Gaston, Oregon had a higher prevalence than the other site sampled near Corvallis, Oregon. Although Gaston is close to Forest Grove, the opposite was true for my study (Forest Grove had a lower prevalence than Newberg). Variation in H. thomomyos prevalence among townships is not surprising given the prevalence of H. thomomyos infections also varied between species inhabiting those townships. Unlike the Gardner (1985) study, prevalence of H. tualatinensis did not vary among T. bulbivorus collection sites in this study. Hymenolepis tualatinensis could be in decline and might exist only in scattered pockets throughout the Willamette Valley, which would explain this discrepancy.

In communications with the landowner, I learned that insecticides were used at the Forest Grove site, which may have contributed to the lower prevalence observed. I
suspect that for other sites with lower prevalence or intensity of infections pesticide use also may have played a role, and that the lack of pesticide use may have also contributed to the significantly higher intensity of overall infections at the Newberg site. I was not able to collect sufficient data from landowners on whether chemicals were used on their properties was therefore unable to evaluate whether this additional factor truly impacted infections.

I hypothesized that detecting annual variation in prevalence or intensity of infections between collection years would be unlikely given that this study took place over a two-year time period. However, prevalence of overall infections was significantly higher in 2018 than in 2019 when *T. bulbivorus* and *T. talpoides* were analyzed together and when *T. bulbivorus* was analyzed independently. In both 2018 and 2019, Oregon experienced much higher than average annual temperatures (NOAA, 2019; NOAA, 2020). In 2018, however, Oregon experienced below average annual precipitation (NOAA, 2019) while in 2019, statewide precipitation was near average (NOAA, 2020). It is possible that these differences contributed to the variance in infections observed between these two years.

It appeared that overall infection prevalence for *T. bulbivorus* was higher in the spring than in other seasons, but I was unable to detect which seasons were significantly different from one another after Bonferroni adjustments were applied. This is likely due to small sample sizes for some of the other seasons (for instance, winter had a sample size of four). *Trichuris fossor* infections of *T. bulbivorus* did vary by season: spring had a significantly higher prevalence than summer (46.0% versus 11.1%, respectively). Significant variation between spring and summer also was observed by Gardner (1985),
although prevalence for *T. fossor* actually was higher in the summer than in the spring. *Heligmosomoides thomomyos* and *H. tualatinensis* infections did not significantly vary by season in Gardner’s (1985) study.

In summary, for prevalence of infections, overall and *H. thomomyos* infections varied significantly among host species; overall, *T. fossor*, and *H. thomomyos* infections varied significantly among townships, overall infections varied significantly between 2018 and 2019, and *T. fossor* and *H. thomomyos* infections varied seasonally. Intensity varied significantly among townships. The results for all other analyses undertaken were not statistically significant.

My study provides further support that microhabitat and temporal variations may exist for some of these helminth species, although continued monitoring is necessary to definitively determine if this is the case. It also provides further evidence that, as in closely related species, *T. fossor* infections vary seasonally. This represents another step in the continued monitoring of these helminth species to better understand intricate interactions they have with their hosts and environments and how both intrinsic and extrinsic factors influence the prevalence and intensity of infections.
Figure 22. Map of Oregon displaying *Thomomys* species collection sites for this study overlain on level III (US Environmental Protection Agency) ecoregions.
Figure 23. *Thomomys bulbivorus* distribution and collection sites. Townships that had sample sizes large enough to statistically compare are labeled.
Figure 24. Prevalence of overall helminth infections (all helminth species combined) by host species.

Figure 25. Prevalence of individual helminth infections by host species.
Figure 26. Intensity (number of helminths per host) of overall infections by host species. Major outliers were excluded from this figure.
Figure 27. Intensity (number of helminths per host) of *Trichuris fossor* infections by host species. Major outliers were excluded from this figure.
Figure 28. Intensity (number of helminths per host) of *Heligmosomoides thomomyos* infections by host species. Major outliers were excluded from this figure.
Figure 29. Prevalence of overall helminth infections by host age classes (estimated using the sum of five suture scores) for all Thomomys species combined ($n = 50$).
Figure 30. Intensity (number of helminths per host) of overall helminth infections by age classes (estimated using the sum of five sutures scores) for Thomomys specimens. Major outliers were excluded from this figure.

Figure 31. Prevalence of overall helminth infections by host sex.
Figure 32. Intensity (number of helminths per host) of overall helminth infections by host sex for all *Thomomys* species. F = females, M = males, NA = individuals that were not sexed. Major outliers were excluded from this figure.

Figure 33. Prevalence of overall helminth infections by ecoregion.
Figure 34. Total intensity (number of helminths per host) of infections by ecoregion for all *Thomomys* specimens. Major outliers were excluded from this figure.

Figure 35. Prevalence of overall *Thomomys bulbivorus* helminth infections by location.
Figure 36. Intensity (number of helminths per host) of overall infections by township. Major outliers were excluded from this figure.

Figure 37. Prevalence of overall infections for *Thomomys bulbivorus* and *Thomomys talpoides* (combined) by collection year.
Figure 38. Intensity (number of helminths per host) of overall helminth infections for *Thomomys bulbivorus* and *Thomomys talpoides* (combined) by collection year. Major outliers were excluded from this figure.

Figure 39. Prevalence of overall infections for *Thomomys bulbivorus* by collection season.
Figure 40. Intensity (number of helminths per host) of overall infections for *Thomomys bulbivorax* by season. Major outliers were excluded from this figure.
Chapter 5: Conclusions

My work provides further understanding of the helminth assemblages parasitizing *Thomomys* species of Oregon. Prior to this study, these helminths had not been sequenced and identification had been based solely on morphology (see Jasmer, 1980, Gardner, 1985, Gardner and Schmidt, 1988, and Makarikov et al., 2012). I have provided genetic data for each of the helminth species documented and for their *Thomomys* hosts. Although previous work (e.g., Jasmer, 1980 and Gardner, 1985) evaluated some extrinsic factors, my dissertation provides new data on the effects of several intrinsic and extrinsic factors on *Thomomys* helminth infections. My dissertation explored the influence of several factors on the prevalence and intensity of helminth infections in *Thomomys* species. For the first time, the COI gene was used to construct phylogenies that incorporated all of the *Thomomys* species in Oregon. Finally, the host-parasite associations had never before been explored for *Thomomys* and their helminths. My dissertation represents the first step in understanding the evolutionary relationship between *Thomomys* and *Heligmosomoides* species.

In Chapter 2, I used molecular markers to test the hypothesis that the helminths *T. fossor*, *H. thomomyos*, *R. rodentorum*, and *H. tualatinensis* represented distinctive lineages. As part of this work, I also uncovered the presence of an unidentified *Heligmosomoides* species as well as a potentially unidentified *Hymenolepis* species in these *Thomomys* hosts. Furthermore, I documented new host records for *T. fossor*, *H. thomomyos*, and *R. rodentorum*. Lastly, I provided evidence that there are potentially cryptic species present within each of the helminth groups assessed (except for *R. rodentorum*) based on the divergence estimates in my analyses. This study showed that
the diversity of helminth species present in *Thomomys* is likely greater than what was previously thought. The findings reported in Chapter 2 suggest that other genetic markers should be analyzed, and a thorough morphological analysis should take place for each of these species or groups in order to uncover the true biodiversity present in these hosts.

In **Chapter 3**, I tested the hypothesis that coevolution is occurring between host and parasite lineages. I also tested existing hypotheses of *Thomomys* evolution by incorporating new taxa and sequence data. First, I generated new phylogenies for *Thomomys* species of Oregon based on the COI mitochondrial gene. These phylogenetic analyses divided the taxa into two subgenera, which was consistent with previous literature (see Belfiore et al., 2008). The two methods (Maximum Likelihood and Bayesian Inference) used to generate the COI mtDNA trees yielded different results in terms of the relationships within the *Thomomys* subgenus. In the ML tree, *T. talpoides* was paraphyletic while in the BI tree it was monophyletic. The relationship of *T. monticola* to the remaining members of subgenus *Thomomys* also varied. Other researchers have emphasized that mitochondrial data alone are a poor indication of species boundaries for geomyids given the unusually high evolutionary rate (Mathis et al., 2014). Using a multilocus approach and repeating this analysis with each of the taxa represented here would likely provide deeper insight into relationships among the *Thomomys* species of Oregon.

Second, I conducted a coevolutionary analyses between *Thomomys* hosts and the *Heligmosomoides* species that parasitize them based on phylogenies generated using the COI gene. I did not definitively provide evidence for a coevolutionary relationship between these taxa, although some of my analyses indicated one may exist. The
tanglegram suggested that cospeciation and cophylogeny might be occurring, and the jackknife estimation results were ambiguous. However, the remaining statistical analyses were not significant, indicating there is not a coevolutionary relationship present, given the available data. Additional studies are necessary to truly discern the relationship between *Heligmosomoides* species and their *Thomomys* hosts. Future work should include a larger sample size and a clearer comprehension of all the *Heligmosomoides* species represented in the investigation.

In **Chapter 4**, I tested the hypothesis that some intrinsic and extrinsic factors influence the prevalence and intensity of helminth infections in *Thomomys* hosts of Oregon. The prevalence of overall helminth infections varied among *Thomomys* species, townships, and by year, and the intensity of overall infections also varied by townships. Specifically, I found that *Trichuris fossor* prevalence varied by townships and by season while intensity of *T. fossor* infections was not significantly different for any of the analyses. The seasonal variation in prevalence for *T. fossor* infections I observed is congruent with results reported by Gardner (1985). The prevalence of *H. thomomyos* infections varied among host species and by townships while intensity of infections did not vary significantly in any analyses. Given that this study had small subgroup sample sizes and only took place over a two-year period, there could be fluctuations in prevalence and intensities of infections that were not detected in my analyses. Future studies should sample more broadly and throughout the year for each of the *Thomomys* species represented in this analysis rather than just *T. bulbivorus*. Studies should also take place over a longer period of time to reflect the true relationships of these helminths with
each of the variables I examined and to enable the identification of seasonal or temporal patterns.

In conclusion, this work is significant in terms of quantifying helminth biodiversity in the state of Oregon, understanding variables that impact helminth infection prevalence and intensity, and comprehending the *Thomomys-Heligmosomoides* association. The first study I conducted indicated there may be undescribed helminth species present within Oregon *Thomomys* species, the second study suggested there is not a coevolutionary relationship among *Thomomys* and the *Heligmosomoides* species parasitizing them, and the final study revealed intrinsic and extrinsic factors are important in terms of infection status.
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Table 12. General taxonomy and classification for rodents (Class Mammalia: Order Rodentia), pikas (Class Mammalia: Order Lagomorpha), and the hedgehog (Class Mammalia: Order Eulipotyphla). U.S. states are abbreviated. Asterisks (*) denote species of focus for this study. Species is abbreviated to sp. for singular, unknown, or unidentified species or to spp. for multiple species.

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<td>T. bulbivorus, camas pocket gopher</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thomomys townsendii*</td>
<td>2</td>
<td>northern Great Basin region</td>
<td>T. townsendii</td>
</tr>
<tr>
<td></td>
<td>Thomomys sheldoni</td>
<td></td>
<td></td>
<td>2</td>
<td>Sierra Madre Occidental, Mexico</td>
<td>T. sheldoni</td>
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<tr>
<td></td>
<td>Thomomys umbrinus</td>
<td></td>
<td></td>
<td>approx. 21</td>
<td>southern AZ, Animas Mountains, NM, Mexico</td>
<td>T. umbrinus</td>
</tr>
<tr>
<td><strong>Thomomys</strong></td>
<td><strong>Thomomys mazama</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td>approx. 15</td>
<td>Pacific Northwest, WA to northern CA</td>
<td><strong>T. mazama</strong></td>
<td></td>
<td></td>
</tr>
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<td>---</td>
<td>---</td>
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<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Thomomys monticola</strong></td>
<td>none (monotypic)</td>
<td>CA, NV</td>
<td><strong>T. monticola</strong></td>
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<td></td>
</tr>
<tr>
<td></td>
<td><strong>Thomomys talpoides</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td>approx. 56</td>
<td>plains throughout western North America</td>
<td><strong>T. talpoides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Geomys</strong></td>
<td></td>
<td></td>
<td>southeastern US, northern Mexico plains throughout central North America</td>
<td><strong>Geomys sp./spp.</strong></td>
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<tr>
<td></td>
<td><strong>Geomys bursarius</strong></td>
<td>approx. 8</td>
<td>plains throughout central North America</td>
<td><strong>G. bursarius</strong></td>
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<td></td>
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<tr>
<td><strong>Heterogromys</strong></td>
<td></td>
<td>Nearctic</td>
<td><strong>Heterogromys sp./spp.</strong></td>
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<td></td>
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<tr>
<td><strong>Orthogromys</strong></td>
<td></td>
<td>Mexico, Central America, Colombia</td>
<td><strong>Orthogromys sp./spp.</strong></td>
<td></td>
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<tr>
<td></td>
<td><strong>Orthogromys heterodus</strong></td>
<td></td>
<td>Costa Rica</td>
<td><strong>O. heterodus</strong></td>
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<tr>
<td><strong>Ochotonidae</strong></td>
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<tr>
<td><strong>Ochotona</strong></td>
<td></td>
<td>Asia, North America</td>
<td>Pikas</td>
<td></td>
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<tr>
<td><strong>Muridae</strong></td>
<td></td>
<td>Worldwide</td>
<td>murids, murid sp./spp.</td>
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<tr>
<td><strong>Apodemus</strong></td>
<td></td>
<td>Old World</td>
<td><strong>Apodemus sp./spp.</strong></td>
<td></td>
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<tr>
<td></td>
<td><strong>Apodemus flavicollis</strong></td>
<td></td>
<td>Europe, Asia</td>
<td><strong>A. flavicollis</strong>, yellow-necked mice</td>
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</table>
Apodemus sylvaticus

Europe, Africa

A. sylvaticus, wood mouse
Table 13. General classification and abbreviations or alternate terms used for helminths mentioned in this study. ‘Helminth’ is a broad term used to refer to parasites, in these instances belonging to Phylum Nematoda or Cestoda, that inhabit the intestines (intestinal ‘worms’) of other organisms. Asterisks (*) denote species of focus for this study. Species is abbreviated to sp. for singular, unknown, or unidentified species, or to spp. for multiple species.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Associated Terms/Abbreviations</th>
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<tr>
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<td></td>
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<td>nematode sp./spp., nematodes, 'roundworms'</td>
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<td>Ancylostoma</td>
<td>Ancylostoma sp./spp.</td>
<td>ancylostomatids</td>
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<tr>
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<td></td>
<td>Ancylostoma caninum</td>
<td>A. caninum</td>
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</tr>
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<td></td>
<td></td>
<td>Ancylostoma duodenale</td>
<td>A. duodenale</td>
<td></td>
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<tr>
<td>Necator</td>
<td></td>
<td>Necator americanus</td>
<td>N. americanus</td>
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<td></td>
<td></td>
<td>Uncinaria</td>
<td>Uncinaria sp./spp.</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>Uncinaria sanguinis</td>
<td>U. sanguinis</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Uncinaria stenocephala</td>
<td>U. stenocephala</td>
<td></td>
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<td>Angiostrongylidae</td>
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<td>Angiostrongylus sp./spp.</td>
<td>angiostrongylids</td>
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<td></td>
<td>Angiostrongylus costaricensis</td>
<td>A. costaricensis</td>
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<tr>
<td>Capillariidae</td>
<td></td>
<td></td>
<td></td>
<td>capillariids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aonchotheca</td>
<td>Aonchotheca sp./spp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aonchotheca musimon</td>
<td>A. musimon</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Aonchotheca paranalis</td>
<td>A. paranalis</td>
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</tr>
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<td></td>
<td></td>
<td>Aonchotheca putorii</td>
<td>A. putorii</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aonchotheca riukiuensis</td>
<td>A. riukiuensis</td>
<td></td>
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<tr>
<td>Baruscapillaria</td>
<td></td>
<td>Baruscapillaria</td>
<td>Baruscapillaria sp./spp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calodium</td>
<td>Calodium hepaticum</td>
<td>C. hepaticum</td>
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</table>

Appendix B. General Classification for Helminths in This Study
<table>
<thead>
<tr>
<th>Order</th>
<th>Genus</th>
<th>Species</th>
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</thead>
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<td></td>
<td>Capillaria</td>
<td>Capillaria sp./spp.</td>
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<tr>
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<td>Capillaria</td>
<td>C. bursata</td>
</tr>
<tr>
<td></td>
<td>Capillaria</td>
<td>C. suis</td>
</tr>
<tr>
<td></td>
<td>Eucoleus</td>
<td>Eucoleus sp./spp.</td>
</tr>
<tr>
<td></td>
<td>Pearsonema</td>
<td>Pearsonema sp./spp.</td>
</tr>
<tr>
<td></td>
<td>Pseudocapillaria</td>
<td>Pseudocapillaria sp./spp.</td>
</tr>
<tr>
<td>Chabertiidae</td>
<td>Chabertia</td>
<td>Chabertia sp./spp.</td>
</tr>
<tr>
<td></td>
<td>Chabertia</td>
<td>C. ovina</td>
</tr>
<tr>
<td></td>
<td>Cyclodontostomum</td>
<td>Cyclodontostomum sp./spp.</td>
</tr>
<tr>
<td></td>
<td>Hypodontus</td>
<td>Hypodontus sp./spp.</td>
</tr>
<tr>
<td></td>
<td>Labiostrongylus</td>
<td>Labiostrongylus sp./spp.</td>
</tr>
<tr>
<td></td>
<td>Stephanurus</td>
<td>Stephanurus sp./spp.</td>
</tr>
<tr>
<td></td>
<td>Oesophagostomum</td>
<td>Oesophagostomum sp./spp.</td>
</tr>
<tr>
<td></td>
<td>Oesophagostomum</td>
<td>O. columbianum</td>
</tr>
<tr>
<td></td>
<td>Oesophagostomum</td>
<td>O. muntiacum</td>
</tr>
<tr>
<td></td>
<td>Zoniolaimus</td>
<td>Zoniolaimus sp./spp.</td>
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<tr>
<td>Diaphanocephalidae</td>
<td>Zoniolaimus mawsonae</td>
<td>Z. mawsonae</td>
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<tr>
<td></td>
<td>Kalicephalus</td>
<td>Kalicephalus sp./spp.</td>
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<tr>
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<td>Kalicephalus</td>
<td>K. cristatus</td>
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<td>Heligmosomatidae</td>
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<td>heligmosomatids</td>
</tr>
<tr>
<td><strong>Heligmosomoides</strong></td>
<td><strong>Heligmosomoides sp./spp.</strong></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Heligmosomoides americanus</strong></td>
<td><strong>H. americanus</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Heligmosomoides polygyrus</strong></td>
<td><strong>H. polygyrus</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Heligmosomoides thomomyos</strong>*</td>
<td><strong>H. thomomyos</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Heligmosomoides vandegrifti</strong></td>
<td><strong>H. vandegrifti</strong></td>
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</tbody>
</table>

**Strongylidae**

<table>
<thead>
<tr>
<th><strong>Cylicocyclus</strong></th>
<th><strong>Cylicocyclus</strong> sp./spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cylicocyclus insigne</strong></td>
<td><strong>C. insigne</strong></td>
</tr>
</tbody>
</table>

**Petrovinema**

| **Petrovinema pocusatum** | **P. pocusatum** |

**Ransomus**

| **Ransomus rodentorum*** | **R. rodentorum** |

**Strongylus**

| **Strongylus equinus** | **S. equinus** |

**Syngamidae**

<table>
<thead>
<tr>
<th><strong>Mammomonogamus</strong></th>
<th><strong>Mammomonogamus sp./spp.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mammomonogamus auris</strong></td>
<td><strong>M. auris</strong></td>
</tr>
<tr>
<td><strong>Mammomonogamus ierei</strong></td>
<td><strong>M. ierei</strong></td>
</tr>
<tr>
<td><strong>Mammomonogamus laryngeus</strong></td>
<td><strong>M. laryngeus</strong></td>
</tr>
</tbody>
</table>

**Trichinella**

<table>
<thead>
<tr>
<th><strong>Trichinella</strong></th>
<th><strong>Trichinella</strong> sp./spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trichinella murrelli</strong></td>
<td><strong>T. murrelli</strong></td>
</tr>
<tr>
<td><strong>Trichinella nativa</strong></td>
<td><strong>T. nativa</strong></td>
</tr>
<tr>
<td><strong>Trichinella spiralis</strong></td>
<td><strong>T. spiralis</strong></td>
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</tbody>
</table>

**Trichuridae**

<table>
<thead>
<tr>
<th><strong>Trichuris</strong></th>
<th><strong>see Trichuridae, Trichuris sp./spp.</strong></th>
</tr>
</thead>
</table>

* species

**Strongylids**

<table>
<thead>
<tr>
<th><strong>Cylicocyclus</strong></th>
<th><strong>C. insigne</strong></th>
</tr>
</thead>
</table>

**Petrovinema**

| **Petrovinema sp./spp.** | **P. pocusatum** |

**Ransomus**

| **Ransomus rodentorum*** | **R. rodentorum** |

**Strongylus**

| **Strongylus sp./spp.** | **S. equinus** |

**Syngamids**

<table>
<thead>
<tr>
<th><strong>Mammomonogamus</strong></th>
<th><strong>Mammomonogamus sp./spp.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mammomonogamus auris</strong></td>
<td><strong>M. auris</strong></td>
</tr>
<tr>
<td><strong>Mammomonogamus ierei</strong></td>
<td><strong>M. ierei</strong></td>
</tr>
<tr>
<td><strong>Mammomonogamus laryngeus</strong></td>
<td><strong>M. laryngeus</strong></td>
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</tbody>
</table>

**Trichinellids**

<table>
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<th><strong>Trichinella</strong></th>
<th><strong>Trichinella</strong> sp./spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trichinella murrelli</strong></td>
<td><strong>T. murrelli</strong></td>
</tr>
<tr>
<td><strong>Trichinella nativa</strong></td>
<td><strong>T. nativa</strong></td>
</tr>
<tr>
<td><strong>Trichinella spiralis</strong></td>
<td><strong>T. spiralis</strong></td>
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</tbody>
</table>

**Trichurids, trichurid sp./spp., 'whipworms'**

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<th><strong>see Trichuridae, Trichuris sp./spp.</strong></th>
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</thead>
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<td>Genus</td>
<td>Scientific Name</td>
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<tr>
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<tr>
<td><em>Trichuris</em></td>
<td>arvicolae</td>
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<tr>
<td><em>T.</em></td>
<td>arvicolae</td>
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<td><em>Trichuris</em></td>
<td>discolor</td>
</tr>
<tr>
<td><em>T.</em></td>
<td>discolor</td>
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<tr>
<td><em>Trichuris</em> fossor*</td>
<td>T. fossor</td>
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<tr>
<td><em>Trichuris</em> leporis</td>
<td>T. leporis</td>
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<tr>
<td><em>Trichuris</em> mastomysi</td>
<td>T. mastomysi</td>
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<tr>
<td><em>Trichuris</em> ovis</td>
<td>T. ovis</td>
</tr>
<tr>
<td><em>Trichuris</em> skrjabini</td>
<td>T. skrjabini</td>
</tr>
<tr>
<td><em>Trichuris</em> suis</td>
<td>T. suis</td>
</tr>
<tr>
<td><em>Trichuris</em> trichiura</td>
<td>T. trichiura</td>
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<tr>
<td><em>Trichuris</em> vulpis</td>
<td>T. vulpis</td>
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<tr>
<td>Ornithostrongylidae</td>
<td>Vexillata</td>
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<tr>
<td><em>Vexillata</em></td>
<td>vexillata</td>
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<tr>
<td><em>Vexillata</em> vexillata</td>
<td>V. vexillata</td>
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<td>Cestodes, cestode sp./spp., 'tapeworms'</td>
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<td>Hymenolepidids, hymenolepidid sp./spp., hymenolepiasis (disease)</td>
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<td>A. schilleri</td>
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<td>Citrilolepis sp./spp.</td>
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<td>C. citrili</td>
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<td>H. hibernia</td>
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<td>H. tualatinensis</td>
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<td>H. weldensis</td>
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<td>P. zimbebel</td>
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<td>Echinococcus ortleppi</td>
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Table 14. Specimens examined in this study. PN refers to the personal preparation number. For specimens from which the COI gene was sequenced, the GenBank Accession is listed.

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<th>Species</th>
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<th>Longitude</th>
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<td>-124.193049</td>
<td>22 August, 2019</td>
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<td>42.003436</td>
<td>-124.193049</td>
<td>22 August, 2019</td>
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<td>Thomomys bulbivorus</td>
<td>AAD 1</td>
<td>Yamhill Co.; Newberg</td>
<td>45.296887, -122.99727</td>
<td>20 October, 2018</td>
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<td></td>
<td>JMY 1</td>
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<td>Thomomys bulbivorus</td>
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<td>Thomomys mazama</td>
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<p>| Thomomys talpoides | WAC 1 | Harney Co.; Frenchglen | 42.827362 | -118.915018 | 21 August, 2018 |
| Thomomys talpoides | MRH 266 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 21 August, 2018 |
| Thomomys talpoides | DGMD 3 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 21 August, 2018 |
| Thomomys talpoides | MRH 267 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 22 August, 2018 |
| Thomomys talpoides | JWS 4 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 22 August, 2018 |
| Thomomys talpoides | AJC 3 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 22 August, 2018 |
| Thomomys talpoides | MRH 259 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 22 August, 2018 |
| Thomomys talpoides | JGD | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 22 August, 2018 |
| Thomomys talpoides | MRH 260 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 22 August, 2018 |
| Thomomys talpoides | MRH 261 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 22 August, 2018 |
| Thomomys talpoides | AJC 4 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 22 August, 2018 |
| Thomomys talpoides | AJC 6 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 22 August, 2018 |
| Thomomys talpoides | ECWD 6 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 22 August, 2018 |
| Thomomys talpoides | GBM 10 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 23 August, 2018 |
| Thomomys talpoides | MRH 263 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 23 August, 2018 |
| Thomomys talpoides | MRH 262 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 23 August, 2018 |
| Thomomys talpoides | AJC 5 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 23 August, 2018 |
| Thomomys talpoides | ARD 3 | Harney Co.; 35 mi E Burns | 43.616111 | -118.608899 | 23 August, 2018 |</p>
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<th>Longitude</th>
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