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Mermithid parasitism of Hawaiian *Tetragnatha* spiders in a fragmented landscape

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Abstract

Hawaiian *Tetragnatha* spiders inhabiting small forest fragments on the Big Island of Hawaii are parasitized by mermithid nematodes. This is the first report of mermithid nematodes infecting spiders in Hawaii, and an initial attempt to characterize this host–parasite interaction. Because immature mermithids were not morphologically identifiable, a molecular identification was performed. A phylogenetic analysis based on 18S small ribosomal subunit nuclear gene sequences suggested that Hawaiian spider mermithids are more closely related to a mainland presumptive *Aranimemis* species that infects spiders, than to an insect-infecting mermithid collected on Oahu, HI, or to *Mermis nigrescens*, also a parasite of insects. Measured infection prevalence was low (ranging from 0 to 4%) but differed significantly among forest fragments. Infection prevalence was associated significantly with fragment area, but not with spider density nor spider species richness. Results suggest that mermithid populations are sensitive to habitat fragmentation, but that changes in infection prevalence do not appear to affect spider community structure.
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1. Introduction

Habitat fragmentation has many impacts upon remnant populations and species. Species with very specific habitat requirements, low population sizes, and low dispersal abilities are most likely to decline or become extinct due to habitat fragmentation (Bierregaard and Stouffer, 1997; Brown and Hutchings, 1997; Gascon et al., 1999; Malcom, 1997). Predators and parasites are often the first to decline or disappear from isolated habitat fragments, perhaps due to relatively greater energy and area requirements and smaller population sizes (Gibb and Hochuli, 2002; Laurance et al., 2002). Disproportionate loss of higher trophic levels can lead to further alterations in species interactions (Terborgh et al., 2001). For example, loss of predators can lead to ecological release in prey populations (Kareiva, 1987). Additionally, habitat fragmentation changes the

behavior of some animals, further disrupting interactions among species. Taylor and Merriam (1996) found that the forest damselfly, *Calopteryx maculata*, altered its flight range in fragments, leading to lower risk of infection by a gregarine parasite. Ultimately, loss of top-down control in fragments may lead to "ecological distortions" that eventually result in a cascade of secondary extinctions, and loss of biodiversity beyond that predicted by loss of area alone (Terborgh, 1988; Terborgh et al., 1997).

We investigated the interaction between Hawaiian *Tetragnatha* spiders (Araneae, Tetragnathidae) and their mermithid parasites (Nematoda, Mermithidae) in naturally formed forest fragments on the Big Island of Hawaii. Mermithids that infect spiders had not previously been documented in the Hawaiian Islands. This study had two major goals. First, we used DNA sequence data to make a preliminary identification in a phylogenetic framework. Second, the prevalence of parasitism was compared among six forest fragments of different sizes where information on spider host density

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and species diversity is known (Vandergast, 2002; Vandergast and Gillespie, submitted). Here we investigated the relationships between prevalence of parasitism and fragment size, spider host density and *Tetragnatha* species richness and diversity, to determine whether habitat fragmentation has altered host–parasite interactions.

2. Materials and methods

This study was conducted within a system of forest fragments called *kipuka* on the slope of Mauna Loa Volcano, on the Big Island of Hawaii. Here, fragmentation due to volcanic activity has occurred throughout the history of the volcano, and these flows can be accurately dated based on radiocarbon dating (Carson and Clague, 1995; Lockwood et al., 1988). Forest re-growth on lava substrate is slow enough so that fragments separated by lava over one hundred years ago are still distinct. Our studies concentrated on a network of these forest *kipuka*, surrounded by a lava flow that originated in 1855. These forest fragments have been isolated from each other for 148 years.

2.1. Hawaiian Tetragnatha

Spiders in the genus *Tetragnatha* constitute a nocturnally active, predatory component of Hawaiian forests. Of the few spider groups represented in Hawaii's native biota, the genus *Tetragnatha* has undergone the most impressive adaptive radiation, with approximately 50 species found throughout the islands (Gillespie, 1991, 1992, 1994, 2002). Usually, several species co-occur in any one area, each with marked differences in ecological association (Gillespie et al., 1997). Within the *kipuka* system, at least seven species inhabit remnant forest patches (Vandergast, 2002; Vandergast and Gillespie, submitted).

2.2. Mermithid life history and ecology

Although mermithid infections in spiders are commonly observed in mainland systems, to our knowledge, this is the first report of mermithid nematodes infecting spiders in the Hawaiian Islands. There are no records of any mermithid nematodes found in the *Fauna Hawaiiensis* (Sharp, 1913), or the Hawaii Biological Survey (Miller and Eldrede, 1996); and nematology and arthropod experts working in Hawaii have not previously encountered specimens from spiders (C. Womersely, Unversity of Hawaii, Manoa; F. Howarth, Bishop Museum, pers. commun.). However, mermithid nematodes have been observed in Hawaiian crickets (Orthoptera; K. Shaw, University of Maryland, pers. commun.), and other insects (this study) from the islands of Hawaii and Oahu. Preliminary morphological examination of mermithids found in Hawaiian spiders suggests that they most likely represent a new species or genus (C. Womersely; G. Poinar, Jr., Oregon State University, pers. commun.).

Systematic studies based on morphology suggest that spider mermithids are a distinct taxon specially adapted for infecting spider hosts (Poinar, 1987). Although mermithid parasites have been recorded from a variety of spiders worldwide, very little is known about the relationship between mermithids and their spider hosts. Poinar (1985) suggests that two possible life cycles may exist: (1) Mermithids infect spiders secondarily, after the spider preys upon an intermediate insect host. (2) Mermithids infect spider hosts directly. Both described species of spider mermithids, *Aranimermis aptispicula* (Poinar and Benton, 1986) and *A. gigantica* (Poinar and Early, 1990) are known to have indirect life cycles that involve a paratenic aquatic insect host. However, Poinar (1985) does not rule out the possibility that other spider mermithid species could have a direct life cycle.

2.3. Mermithid parasitism in a fragmented system

Mermithids generally have localized, discontinuous distributions (Petersen, 1985). This may be especially true when infection occurs in non-dispersive stages of the host. Spiders are generally thought of as good long-distance dispersers because many species can produce a long-strand of silk to "balloon" along air currents directly after hatching (Gillespie and Roderick, 2002). However ballooning occurs mostly in small, immature instars (Wise, 1993), before feeding; and therefore, most likely before contact with second stage juvenile mermithids or infected insect prey. Additionally, in the Hawaiian *kipuka* system, genetic evidence suggests that dispersal among fragments has become greatly reduced in some *Tetragatha* (Vandergast, 2002). For these reasons, it is unlikely that mermithids are able to disperse among fragments with their spider hosts in the *kipuka* system. If isolated, populations of mermithids may reach a new equilibrium size that is correlated with fragment area (sensu. MacArthur and Wilson, 1967). Further, if mermithid distributions prior to fragmentation were patchy, then by chance, lava fragmentation may have isolated some forest *kipuka* in which mermithids are entirely absent, creating refuges for spiders that reside there. Depending on the infection rate, this could in turn alter population densities in species that have escaped infection, and potentially alter the community composition of spiders.

2.4. Mermithid collections

In this study 994 spiders chiefly in the genus *Tetragnatha* were examined for mermithid infection. Spider specimens were collected from between 1997 and 2000 at

each of six isolated forest fragments (K1–6, Fig. 1), as well as several other locations across the island of Hawaii (Fig. 1). Spider abdomens were carefully dissected; nematodes were removed and preserved in 95% ethanol.

2.5. Morphological and molecular identification of mermithid specimens

Mermithid samples collected from Hawaiian spiders were sent to G. Poinar, Jr. (Oregon State University) for morphological identification. Because morphological identification of mermithids to genus and species relies on adult samples (2 molts after emergence; Poinar, 1985), specific identification was not possible. However,

based on characteristics present in the parasitic stage, Hawaiian samples most closely resembled specimens from the spider-infecting genus *Aranimermis* (Poinar, pers. commun.).

In addition to morphological identification, we used molecular sequence data to compare seven spider mermithids collected on Hawaii, one insect mermithid collected from an earwig (*Eurborellia annulipes*, Dermaptera: Labiduridae) on Oahu, one spider mermithid (collected in San Diego County, CA, from *Habronattus signatus*, Salticidae; likely *Aranimermis sp.*, G. Poinar, Jr., pers. commun.), and one published sequence from *Mermis nigrescens* of unknown geographic origin (Blaxter et al., 1998). *M. nigrescens* is known to para-
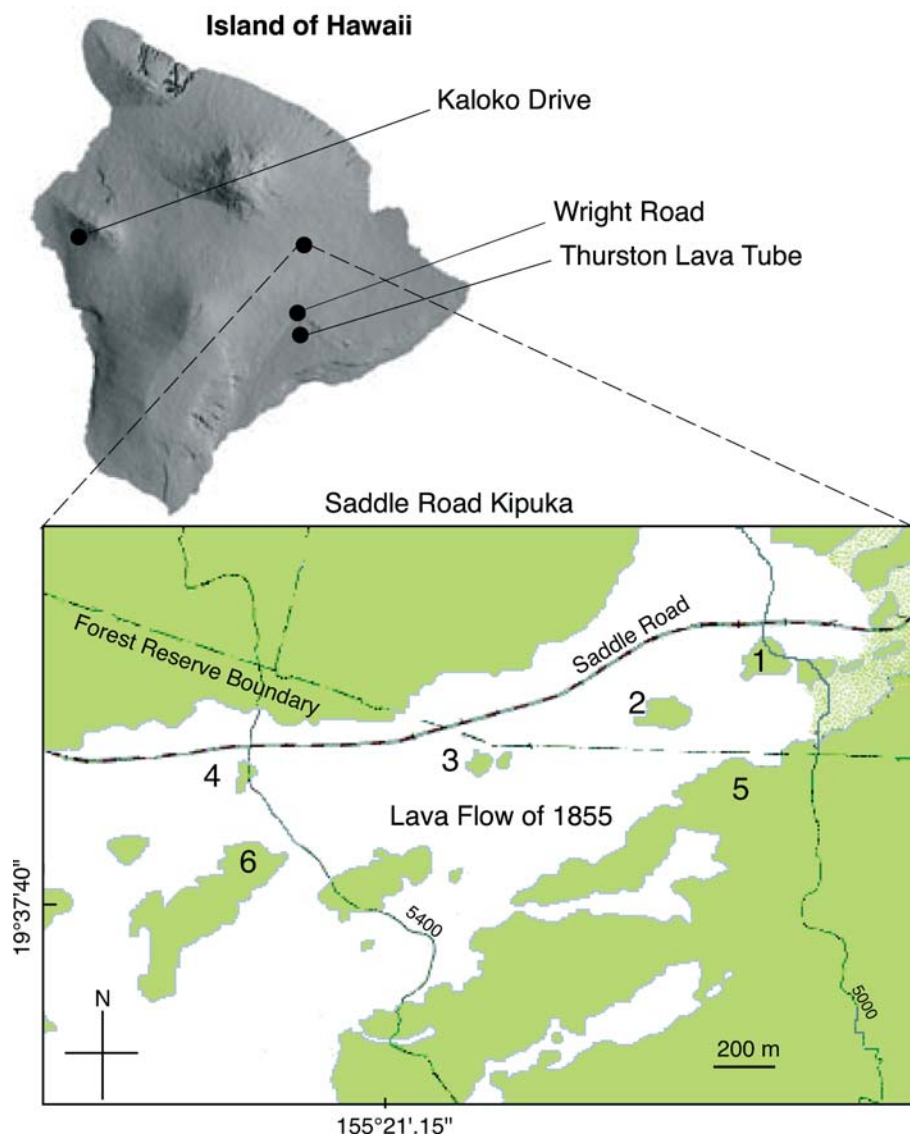

Fig. 1. Location of collecting sites on the island of Hawaii with detailed map of the *kipuka* study site, located on the Saddle Road. Forested areas are in green, lava flows are in white. *Kipuka* under study are labeled 1–6. *Kipuka* 1–4 are small forest patches ranging in diameter from about 100–250 m. *Kipuka* 5 is a large stretch of continuous forest. All *kipuka* are surrounded by an 1855 lava flow originating from Mauna Loa Volcano. Elevation ranges from 5000 to 5400 feet. Forests are classified as mesic to wet *ohia* forest with other native trees and a tree fern and native shrub understory (Jacobi, 1990; Map modified from USGS 7.5 minute series topographic, Upper Piihonua Quadrangle, Island of Hawaii, 1981).

sitize insects in the orders Orthoptera, Dermaptera, Coleoptera, and Lepidoptera.

Genomic DNA was isolated from mermithid samples using the Qiagen DNEASY Tissue Kit (Qiagen, Valencia, CA), following the protocol for extraction of animal tissues. Eight hundred base pairs of the 18S small ribosomal subunit (SSU) nuclear gene were sequenced from samples obtained, using primers 18S-5F (5′GCGAAAGCATTT GCCAAGAA) and 18S-9R (5′GATCCTTCCGCAGGT TCACCT). Twenty-five microliters of PCR amplifications contained 0.5 U AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, CA), 1.8 mM MgCl₂, 0.2 mM each dNTP, and 0.4 mM each primer. A "touchdown" PCR protocol was employed. The first 20 cycles were performed with 95 °C/30 s denaturing, a 30 s annealing with temperatures starting at 55 °C and incrementally decreasing each cycle to 45 °C, and 72 °C/40 s extension. An additional 20 cycles were performed with an annealing temperature of 45 °C. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). PCR products were cycle-sequenced in both primer directions using Big Dye Terminator (Applied Biosystems) and sequenced on an ABI 377 automated sequencer. Electrochromatograms were compared using Sequence Navigator (Applied Biosystems). All seven Hawaiian spider mermithids had identical SSU sequences. GenBank Accession Nos. are as follows: A4374415–A4374417.

Thirty-six published SSU sequences of nematode taxa (including *M. nigrescens*) and one outgroup (*Chordodes morgani*, Nematomorpha) were obtained from GenBank for comparison with this study (Accession Nos. U81506, U81574, U81579, U81581, U81582, AFO36586–AFO36612, and AFO36612–AFO36644). These sequences were originally gathered as part of a phylogenetic review of the Nematoda (Blaxter et al., 1998). Sequences were aligned using a multiple alignment algorithm (Gap cost 15, Extension Cost 6.66, Transition weight 0.50) in the program ClustalX (Thompson et al., 1997).

Relationships among taxa were examined by generating a phylogenetic tree using both parsimony and maximum likelihood algorithms in the program PAUP* v 4.0b10 (Swofford, 1993) Trees were rooted with the outgroup *C. morgani* used in Blaxter et al.'s (1998) analysis. Statistical confidence of branches within the tree was assessed by performing bootstrap resampling (200 replicates; Felsenstein, 1988; Swofford, 1993). Resulting phylogenetic trees were compared to each other and with the published maximum parsimony phylogeny of the Nematoda (Blaxter et al., 1998) to determine any discrepancies in taxon placement.

2.6. Prevalence of parasitism in Hawaiian kipuka

The number and percent of spiders carrying mermithid parasites were calculated over all spiders collected from each of the six *kipuka* under study. Because no species-specific infection patterns were evident (i.e., there was no sequence diversity among the Hawaiian spider mermithids sampled) and previous studies had found wide host ranges in other spider-infecting mermithids (Poinar and Benton, 1986; Poinar and Early, 1990), spider species were lumped to calculate the prevalence of infection in spiders. An exact χ^2 test was used to compare the number parasitized in each *kipuka* with the program StatXact v. 5.0 (Cytel Software Corporation). The prevalence of spiders parasitized in each *kipuka* was plotted against measurements of habitat area, spider species density, spider species richness, and spider diversity to determine possible correlating and causal factors affecting the rate of parasitism. The area of each *kipuka* was calculated by measuring the diameter of each forest fragment on a digitalized USGS topographic map using ArcView 3.0 (Environmental Systems Research Institute, 1997). Spider species richness and diversity (Shannon diversity index; Magurran, 1988) were calculated from standardized transect collections performed in each *kipuka* (see Vandergast, 2002 for methods). Spider density was calculated by pooling all spiders collected across transects in a *kipuka* and dividing by transect area.

3. Results

3.1. Molecular identification

Phylogenetic trees from parsimony and maximum likelihood analyses were identical with high bootstrap support in the placement of the clade containing the Longidoridae, Mermithida, and Monochida (Hawaiian spider mermithid, *Aranimermis* sp. San Diego, Hawaiian insect mermithid, *M. nigrescens*, *Mylonchulus arenicolus*, *Xiphinema rivesi*, and *Longidorus elongates*). Therefore, only the maximum parsimony tree is presented (Fig. 2). The trees obtained in this study closely resemble the previously published Nematoda tree, with no differences in the clade comprising the Mermithidae. Based on these sequences, the Hawaiian spider mermithid appears to be more closely related to the presumed *Aranimermis* sp. than to the Hawaiian insect-infecting nematode or *M. nigrescens*, supporting the morphological identification as suggested by Poinar.

3.2. Prevalence of infection

Although spiders were examined from several locations across the island of Hawaii, mermithid parasitism was only detected in the *kipuka* populations, and occurred in multiple spider species (Table 1). The intensity of infection was the same in all cases, consisting of one mermithid per spider. The prevalence of infection in
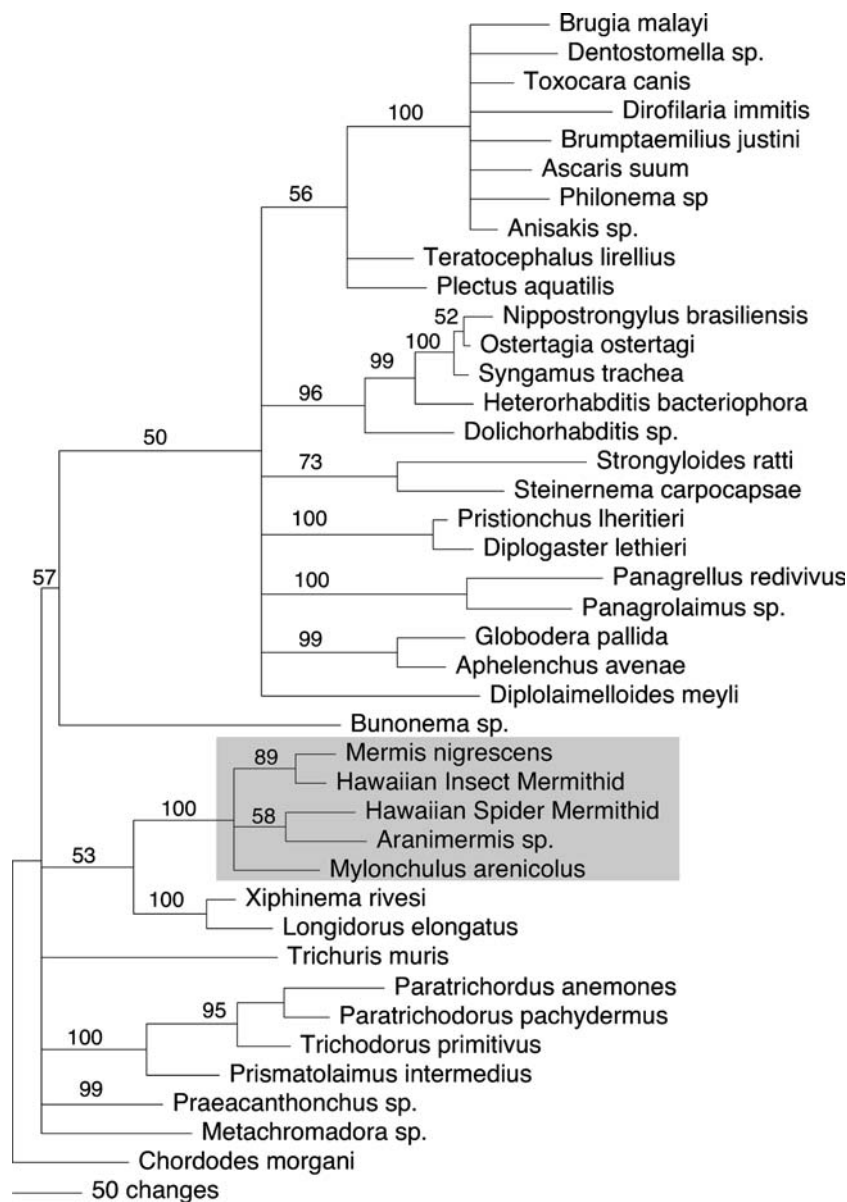
Fig. 2. Maximum parismony analysis of SSU (18 S ribosomal DNA) sequences from 39 nematode taxa and one outgroup, *C. morgani*. As many as 1231 characters (bases plus gaps) were aligned and processed, with 737 parsimony informative characters in MP analysis. One shortest tree (score of 3225) was found. Bootstrap values are presented from 200 bootstrap replicates. The clade of interest (shaded in grey) showed identical tree topology whether including or excluding gaps, and in maximum likelihood analysis, with similar levels of bootstrap support.

kipuka was quite low, ranging from 0 to 4.12%. Among *kipuka*, the number of parasitized spiders varied significantly ($\chi^2 = 11.64$, 5 d.f., exact $p = 0.017$; from 10,000 Monte Carlo simulations). The percent of spiders parasitized was significantly associated with fragment area (regression: $R^2 = 54.8\%$, 4 d.f., $p = 0.09$; Spearman rank correlation: $r = 0.829$, 4 d.f., $p \leqslant 0.05$, two-tailed; Fig. 3), but was not associated with spider species richness ($R^2 = 0.0\%$, 3 d.f., $p = 0.98$; Spearman $r = 0.359$, $p > 0.5$), spider species diversity ($R^2 = 25.5\%$, 3 d.f., $p = 0.39$; Spearman $r = 0.700$, $p \leqslant 0.10$), or spider density ($R^2 = 1.7\%$, 3 d.f., $p = 0.84$; Spearman $r = 0.100$, $p > 0.5$).

4. Discussion

4.1. Molecular identification

Because nematodes are difficult to identify with certainty using morphological techniques at certain developmental stages, molecular identification offers a powerful alternative (Szalanski et al., 2001). Such techniques have been successfully developed with a high degree of accuracy for use in identification of economically and medically important nematode taxa (McKeand, 1998; Zhu et al., 2001). For example, Floyd et al. (2001) used SSU sequences to create molecular "bar

Table 1
Specimens examined, including number of spiders dissected, number of mermithids found and rates of parasitism for *kipuka*s and other areas on Hawaii Island

Collection site	Number of spiders	Number of mermithids	Percent parasitized	Species parasitized	Area (Ha)	Spider species richness	Spider species diversity	Spider density per m²
Kipuka 1	108	2	1.85	*T. brevignatha* *T. quasimodo* *T. anuenue*	2.4	8	1.83	0.175
Kipuka 2	121	5	4.13	*T. brevignatha*	3.14	9	1.88	*0.200*
Kipuka 3	161	1	0.62	*T.* n.sp. "golden dome"	1.23	11	1.7	0.148
Kipuka 4	184	0	0		0.6	7	1.47	0.194
Kipuka 5	218	8	3.67	*T. brevignatha* *T. quasimodo* *T. anuenue*	43.6	8	1.58	0.125
Kipuka 6	47	1	2.13	*T. quasimodo*	9.42	*Unk.*	*Unk.*	*Unk.*
Volcanoes National Park	26	0	0					
Wright Road	103	0	0					
Kaloko Drive	26	0	0					

Mermithids were detected only in the *kipuka* study site area and were found in multiple species. The habitat area is listed for each *kipuka*. Spider species richness and density were determined from previous transect studies of spider abundance (Vandergast, 2002), but did not include *kipuka* 6.
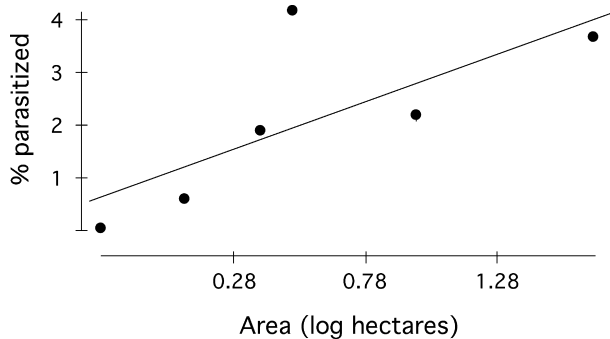

Fig. 3. Relationship between percent spiders parasitized and fragment area for *kipuka* sampled in this study.

codes" for soil nematode identification. Groups of identical or very similar sequences were designated as molecular operational taxonomic units to eventually be compared to a database of sequences from morphologically defined taxa. Genetic data from this study suggest that the Hawaiian spider mermithids are most closely related to other spider-infecting mermithids in the genus *Aranimermis*. However, without the inclusion of a wider array of mermithid taxa, it is difficult to assert this relationship with certainty. Although mermithids have been observed as parasites in over 30 spider genera worldwide (Poinar, 1987), these have only recently been studied in any taxonomic detail (Poinar and Benton, 1986; Poinar and Early, 1990), and a full systematic review has yet to be performed. When these data are available it may be possible to more accurately place the Hawaiian taxon.

If the Hawaiian taxon proves to be a close relative of *Aranimermis*, then the *Aranimermis* clade may have a wider ecological range than previously recorded. In their work on *Aranimermis*, Poinar and Colleagues (1986, 1990) found that individuals in this genus required an initial aquatic insect host, and after emergence from the secondary spider host, returned to water to complete their life cycle. In the Hawaiian *kipuka* where mermithids were found, no streams or ponds (permanent or temporary) were observed in four years of field work. (Forest soils of *kipuka* are composed of a thin layer of organic material on top of porous volcanic rock.) However, the area generally experiences a high level of rainfall, and the forest understory is consistently moist (Vandergast, 2002; Vandergast and Gillespie, submitted). Some Hawaiian insects with aquatic stages have evolved unique adaptations to cope with this type of environment. For example, larvae of the Hawaiian damselfly *Megalagrion koelense* develop in leaf axels where small amounts of water accumulate and another, *Megalagrion nestiotes*, has larvae that develop in damp leaf litter (Polhemus, 1997). It is possible that mermithids may develop through aquatic stages in a similar manner. Poinar (1985) suggests that some spider mermithids may infect spiders directly rather than through an intermediate or paratenic host. This is also a possibility for Hawaiian taxa, as an insect host has yet to be identified, and the mermithid collected thus far from an insect in Hawaii was unrelated to those found in spiders.

4.2. Prevalence of infection

This study represents one of the first attempts to quantify the prevalence of mermithid infection in spiders. Infection prevalence tends to vary widely across species and areas studied (Becnel and Johnson, 1998; Blackmore, 1994; Choo and Kaya, 1994; Dowd et al., 1995; McInnes and Tschinkel, 1996). For example, in a survey of five habitats containing populations of the mermithid *Romanomermis culicivorax*, the prevalence of infection in mosquitoes ranged from 8 to 42% when averaged over habitats, with prevalence in individual populations as high as 80% (Petersen and Willis, 1971). Similarly, Phelps and De Foliart (1964) studied host–parasite interactions in blackflies and mermithids in four Wisconsin streams. Prevalence of infection was reported from 10 to 90% and the authors suggested that localized parasite populations may attain levels of infection sufficient to virtually eliminate some host populations. Little work has been done to document the incidence of mermithid infections in spider populations. Poinar (1987) reported anecdotally that infection prevalence may range from 1 to 9%. In the *kipuka* system, the prevalence of infection was low ($\leqslant 4\%$). Consequently, mermithid infection most likely does not influence spider abundance as much as other trophic interactions, or habitat structure and dispersal ability (Vandergast, 2002; Vandergast and Gillespie, submitted). The prevalence of infection differed among *kipuka* and these differences may be attributable to the effects of fragmentation.

4.3. Effects of fragmentation

The percentage of spiders infected was related to fragment area, suggesting that mermithid parasites are more sensitive to fragmentation than their spider hosts. In previous studies of species richness and area in this system, spider species richness was not correlated with habitat area (Vandergast, 2002). Stronger fragmentation effects for higher trophic levels have been widely reported in other studies (Didham et al., 1998; Gibb and Hochuli, 2002; Kruess and Tscharntke, 2000; Laurance et al., 2002). In the *kipuka* system, this difference may reflect smaller initial population sizes in parasites than in hosts, or a greater sensitivity to changes in microhabitat associated with the process of fragmentation. Studies of forest fragmentation have demonstrated that smaller fragments can have microclimates more similar to the surrounding habitat than do larger areas (Kapos, 1989). In the *kipuka* system, surrounding lava flows are dry and sparsely vegetated in comparison to forest fragments, and edge effects penetrate further into small forest *kipuka* than in larger ones (Vandergast and Gillespie, submitted). Therefore, it is possible that smaller forest *kipuka* have drier soil conditions throughout their inte-

riors than larger ones. If Hawaiian mermithids develop in wet soils or in plant phytotelmata as hypothesized, they may be limited to larger fragments with more optimal environmental conditions. Alternatively, if Hawaiian mermithids rely on an intermediate or paratenic insect host, they may be limited in distribution by the insect host's range and dispersal capabilities. For example, distribution of the roundworm parasite, *Baylisascaris procyonis*, was found to be significantly related to forest area (Page et al., 2001). These changes in infection prevalence were explained by changes in movement and density of the definitive host (raccoons) in response to habitat fragmentation, resulting in altered encounter rates for secondary host (mice). In response, mice showed higher rates of parasitism, regardless of their population density. In the *kipuka* system, infection prevalence may be correlated with insect host movement and density, rather than spider host density. We found that the proportion of spiders parasitized by mermithids was not correlated with spider density.

Likely due to the low rates of parasitism, there was no detectable correlation between the percent of spiders parasitized and measurements of spider species richness. There is no evidence that spiders experience changes in species composition as a result of a release from parasitism.

5. Conclusions

This study is the first documentation of spider mermithids in the Hawaiian Islands. Results suggest that the prevalence of parasitism in this system has been altered due to fragmentation, with prevalence decreasing as fragment size decreases. Further work remains, including completing a definitive morphological species identification, determining microhabitat associations for free-living adult and juvenile stages, and identifying possible intermediate hosts. Once the species identification and life history is known, it will be possible to explore the extent of mermithid spider parasitism on other islands as well as establish whether these mermithids represent a unique Hawaiian lineage, or a more recent colonization.

was permitted by the Hawaii Department of Land and Natural Resources.

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