Technical Report HCSU-055

DISTRIBUTION AND PREVALENCE OF KNEMIDOKOPTIC MANGE IN HAWAIʻI ʻAMAKIHI ON THE ISLAND OF HAWAIʻI

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November 2014

This product was prepared under Cooperative Agreement CA11AC20269 for the Pacific Island Ecosystems Research Center of the U.S. Geological Survey.

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ABSTRACT

Knemidokoptic mange was first observed on two Hawai'i 'Amakihi (*Hemignathus virens*) mist netted in Manuka Natural Area Reserve (NAR) on the Island of Hawai'i in June 2007. Microscopic examination of skin scrapings from lesions of the infested individuals revealed the scaley-leg mite, *Knemidokoptes jamaicensis*. Continued surveillance at Manuka NAR (2007-2009) documented a 24% (15/63) prevalence of mange among Hawai'i 'Amakihi distributed from coastal habitat to 1,500 m above sea level (asl). From 2012-2014, we conducted an island-wide survey of wild passerine birds from several leeward sites (Manuka NAR, Kahuku Unit of Hawai'i Volcanoes National Park (HAVO), Pu'u Wa'awa'a Forest Bird Sanctuary, and Kipahoehoe NAR) and windward sites (Hakalau Forest National Wildlife Refuge, 'Āinahou Ranch of HAVO, Malama Ki Forest Reserve, and Keauohana Forest Reserve) to determine the current distribution and host range of knemidokoptic mange. We also determined the prevalence of malaria in Hawai'i 'Amakihi populations where mange was present and treated a subset of infested Hawai'i 'Amakihi mange with a single, topical dose of moxidectin. We mist netted and examined a total of 1,734 passerines, including 738 Hawai'i 'Amakihi. Mange was present in Hawai'i 'Amakihi at Manuka NAR (595 and 305 m asl), Kahuku Ranch Unit of HAVO (Glover site: 1,201 m asl and Kipuka Akala site: 1,532 m asl), Malama Ki Forest Reserve and Keauohana Forest Reserve (293 m asl). No other passerine birds (n = 995) were infected. Mange prevalence ranged from a high of 69% (40/58) in Keauohana Forest Reserve to a low of 2% (1/65) in the Kahuku Ranch Unit of HAVO (Kipuka Akala). At Manuka NAR prevalence had decreased from 26% in 2010 to 10% (7/81) in 2012–2014. We found no significant relationship between the prevalence of mange and the prevalence of avian malaria in mesic habitats at Manuka NAR ($P = 0.59$ (FET, n = 81)), but there was a significant association between the prevalence of mange and the prevalence of malaria in lowland wet forests in Puna Forest Reserves ($P < 0.01$ (FET, n = 72)). This apparent association may be a reflection of the high prevalence of malaria (>80%) in these areas. There was no difference in the frequency of recapture of birds that were infested versus un-infested at first capture at our long-term sites (Manuka NAR and Puna sites) ($\chi^2_{(1,\, n\, =\, 227)} = 1.51$, $P = 0.22$, but when all sites with mange present were pooled, there was a significant difference in the frequency of recaptures between infested and un-infested birds ($\chi^2_{(1,\, n\, =\, 424)} = 7.13$, $P = 0.01$). There was a significant association between parasitemia level (per 10,000 RBCs) and the ranked stage of mange present in infested individuals. We treated 24 Hawai'i 'Amakihi with moxidectin and upon recapture (n = 2), found a reduction in both the size and stage of mange lesions, such that a single dose, topical treatment of moxidectin appears to be an effective treatment for knemidokoptic mange in wild populations. Our results suggest that knemidokoptic mange is currently limited to Hawai'i 'Amakihi and prevalent in low elevation sites on both the windward and leeward sides of the island.

INTRODUCTION

The scaly-leg mite, *Knemidokoptes jamaicensis*, is responsible for knemidokoptic mange in domestic and wild passerine birds (Wade 2006, Pence 2009). This mite has been increasingly reported from wild passerines worldwide suggesting recent host shifts, increased movement of host species, or synergistic effects of environmental stressors (Pence *et al.* 1999, Latta 2003). This mite is currently known to affect over 37 avian host species in 13 passerine families with an extensive global geographical distribution including Oceania, North and Central America, Europe, Asia, and Africa (Dabert *et al.* 2013). Reported wild passerine hosts of *Knemidokoptes jamaicensis* include canaries, finches, and mynahs, but it has also been reported in crows, blackbirds, catbirds, grackles, towhees, warblers, and woodpeckers (Wade 2006).

Scaly leg mites cause dramatic deformity of the feet (Figure 1) that affects the general health of the bird. Kirmse's (1966) aviary study on Red-winged Blackbirds (*Agelaius phoeniceus*) found that the lesions impaired the ability to perch. In flocks of wild American Robins (*Turdus migratorius*), the prevalence of mange was as high as 80%, and individuals with advanced mange had difficulty walking, perching, appeared lethargic, and did not attempt to feed (Pence *et al*. 1999). Demographic studies in North and Central America indicate severe mite infestations may affect survivorship of the host (Latta 2003, Benkman *et al.* 2005). Latta (2003) studied Palm Warblers (*Dendroica palmarum*) in the Dominican Republic where the prevalence of mange was as high as 25%. He found that the overall body condition, as measured by mean pectoral muscle mass scores, decreased in infested birds and that birds with lower pectoral muscle mass scores were less likely to return from overwintering sites (Latta 2003). In a similar demographic study, estimated annual survival of Red Crossbills (*Loxia curvirosta*) was significantly lower for birds infested with mites (Benkman *et al.* 2005).

In June 2007, knemidokoptic mange was reported in Hawai'i 'Amakihi in Manuka Natural Area Reserve (NAR) on the Big Island of Hawai'i (Gaudioso *et al.* 2009b). This was the first report of *K. jamaicensis* infesting wild birds in the Hawaiian Islands. Between June 2007 and December 2008, we found Hawai'i 'Amakihi with knemidokoptic mange present from the coast to 1,585 m above sea level (asl). Prevalence was highest (15-22%) at lower elevations (< 600 m asl), and much lower (4-5%) at higher elevations (800-1,600 m asl). Knemidokoptic mange was not found in any other native or non-native species. Among the Hawai'i 'Amakihi affected in Manuka NAR, 53% exhibited early stage, 8% exhibited intermediate stage, and 39% exhibited the advanced stage mange. Knemidokoptic mange was also detected in Hawai'i 'Amakihi at Keauohana Forest Reserve over 100 km away. A Hawai'i 'Amakihi study by Gaudioso (2009) at 10 additional sites on the island of Hawai'i did not detect knemidokoptic mange, but subsequent mist netting at lower elevation sites in Manuka NAR (State Park and Lama sites) in 2009-2010 revealed a 18-27% prevalence among Hawai'i 'Amakihi (DAL, unpublished data).

The main objectives of this project were to determine the current geographical and altitudinal distribution, host species range, and prevalence of knemidokoptic mange in representative Hawaiian forest bird habitat on the island of Hawai'i. We also examined the association between knemidokoptic mange and *Plasmodium relictum*, temporal and geographical trends in mange prevalence, and the efficacy of a topical treatment with moxidectin in free-living Hawai'i 'Amakihi with knemidokoptic mange.

METHODS

Study Area

Hawai'i 'Amakihi were captured with mist nets from April 2012 to April 2014 at seven core sites on the leeward (Manuka Natural Area Reserve (NAR), Pu'u Wa'awa'a Forest Bird Sanctuary, Kipahoehoe NAR, Kahuku Unit of Hawai'i Volcanoes National Park (HAVO)-Nene Cabin site) and windward (Keauohana Forest Reserve, 'Āinahou Ranch of HAVO, and Nauhi Cabin at Hakalau Forest National Wildlife Refuge (NWR)) sides of the island of Hawai'i (Figure 2). Additional sites of this study were located in the Kahuku Unit of HAVO (Glover and Kipuka Akala) and the Malama Ki Forest Reserve in Puna (Figure 2). Sites ranged in elevation from sea level to 1,800 m asl with representative sites on both leeward and windward sides of the island at low (0-500 m asl), mid (956-1,335 m asl) and high (1,598-1,965 m asl) elevations. The core sites ranged from dry, ohia (*Metrosideros polymorpha*)-introduced co-dominant forest to very wet, ohia-dominated forest. Hakalau NWR, 'Āinahou Ranch in HAVO, the Kahuku Ranch Unit of HAVO, and Pu'u Wa'awa'a are previously ranched lands that have been recently managed as

Figure 1. The first adult male Hawai'i 'Amakihi captured at Manuka NAR in 2007, exhibiting advanced knemidokoptic mange lesions.

conservation land through fencing, ungulate control, and reforestation using outplantings and exclosures (Table 1).

Mist Netting and Sample Collection
From 2012-2014, we operated 10-15 mist nets (6 or 12 m long, 36 mm mesh) at each site supported by double-length (6 m) sections of electrical metal conduit. Nets were operated from 0700-1600 hours and checked every 30 minutes by a team of 3-5 banders. Nets were closed if drops of moisture beaded on the mesh, rain became heavy or persistent, or wind persisted above a score of three on the Beaufort wind scale. Birds were banded using U.S. Geological Survey aluminum bands, measured for morphometrics to obtain sex and age, and bled by brachial venipuncture using a sterile, 28-gauge needle and micro-hematocrit capillary tubes. A thin blood smear was made immediately after blood collection and fixed with methanol. The whole blood collected was spun in a battery-operated field centrifuge to separate plasma from packed blood cells. Microhematocrit tubes were broken with a file and plasma and packed cells were removed using a 200 ul pipet. Plasma was stored in 0.5 ml tubes and packed cells were dispensed in 50 ul of lysis buffer (0.1 M Tris, pH 8.0, 0.1 M EDTA, 2% SDS) in a 0.5 ml tube. All samples were transported to the Kīlauea Field Station (35-106 miles away) laboratory on wet ice and stored at -70°C.

Field Procedures
Hygienic Protocol
When birds with avian pox or knemidokoptic mange were encountered, all mist nets, bird bags, and banding tools were immediately treated with 1-Stroke Environ®, a broad spectrum germicidal detergent used in the animal industry to control for a number of bacterial, viral and fungal pathogens. A 1:256 dilution of 1-Stroke Environ was sprayed on the net section until thoroughly wetted and allowed to dry before reopening. The bird bags were flagged, turned inside out and sprayed wet as well. Bird bags were then stored with other used bags until

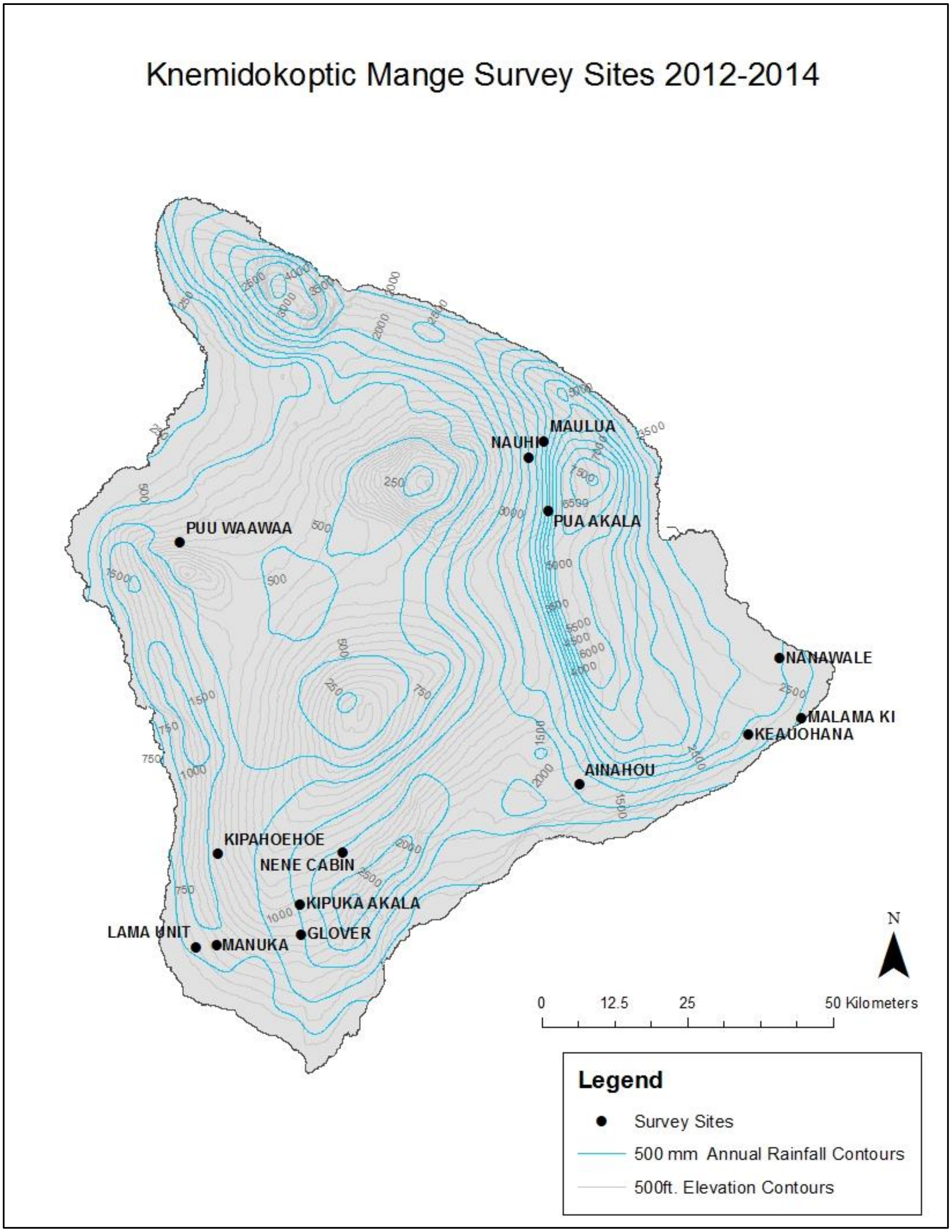

Figure 2. A map of knemidokoptic mange survey study sites on the island of Hawai‘i, 2012-2014 with 500 mm rainfall isohyets (from UH–Manoa Geography Dept., 2011).

machine washing with detergent and bleach. Banding tools and hard surfaces were disinfected with Environs spray for 10 minutes before rinsing with water and drying. Any bander that handled an infested bird disinfected their hands with a liberal application of a ≥ 60% alcohol-

Table 1. Geographical descriptions of knemidokoptic mange 2012–2014 survey sites.

Geographical Category	Site name Coordinates	Habitat description*** Moisture zone*; Canopy***	Elevation (meters)	Rainfall ** (millimeters)
Low-Leeward	Manuka NAR 19° 06′ 37.1″ N 155° 45′ 30.3″ W	Moderately dry/Seasonal mesic Ohia-introduced trees co-dominant Open canopy	450	875
Low-Windward	Keauohana Forest Reserve 19° 25′ 18.2″ N 154° 57′ 20.6″ W	Moderately wet Ohia-introduced trees co-dominant Scattered canopy	293	3,000
Mid-Leeward	Puʽu Waʽawaʽa Forest Bird Sanctuary 19° 44′ 06.7″ N 155° 52′ 14.3″ W	Moist mesic Ohia-koa co-dominant Open canopy	1,335	750
Mid-Leeward	Kipahoehoe NAR 19° 15′ 08.1″ N 155° 49′ 05.9″ W	Moist mesic Ohia-koa co-dominant Open canopy	1,298	1,000
Mid-Windward	ʽĀinahou Ranch (HAVO) 19° 15′ 03.3″ N 155° 36′ 50.1″ W	Moist mesic Ohia-mamane co-dominant Open canopy	1,000	2,250
High-Leeward	Kahuku Ranch (HAVO) Nene Cabin 19° 15′ 03.1″ N 155° 36′ 50.0″ W	Seasonal mesic Ohia-native tree co-dominant Open canopy	1,965	1,750
High-Windward	Hakalau Forest NWR Nauhi Cabin 19° 51′ 20.5″ N 155° 17′ 49.1″ W	Moderately wet Ohia dominant Open canopy	1,598	3,500

* Moisture zone designations were determined using Price *et al.* 2012.
** Mean annual rainfall is from the University of Hawaiʽi at Manoa, Department of Geography, 2011.
***Habitat description and canopy cover as per Judge *et al.* 2011.

based hand sanitizer before handling any other birds. All nets and/or banding equipment were disinfected with Environs solution before use at a new site.

Presumptive Diagnosis of Gross Lesions
Birds were examined for knemidokoptic mange and pox lesions on the feet and ectoparasites on the head, body, wings and tail. Knemidokoptic lesions were assessed by the same observer (JMG) each time, and assigned a stage of development (early, intermediate, or advanced) based on a set of established criteria (Figure 3). Observers were trained to determine pox versus mange lesions using documented gross lesion descriptions and photographs. For mange, observers referenced the following description:

"*The lesions of epizootic podoknemidokoptiasis caused by K. jamaicensis range from white powdery scaling to proliferative epidermal overgrowth with massive crusts and scab formation resulting from massive hyperkeratosis and intense dermal inflammation on the un-feathered part of the legs and feet. The skin may become markedly thickened and very rough, gray-white in color, desiccated, and fractured*" (Pence 2009).

For pox, observers referenced the following description:

"*Most avipox lesions in wild birds occur on one toe, with half that number on two toes and the leg. Lesions are few in number, appearing as innocuous warty growths on one or two toes, at the base of the bill or eyelid. In perching birds, lesions start as a swelling on the toe, leg or facial region. The swelling appears smooth, reddish, and dome shaped. Eventually the swelling cracks or bursts and the lesion will begin to form. The lesions heal following degeneration and sloughing of the abnormally proliferated epithelium. In some cases, toes or whole feet can be lost*" (van Riper and Forrester 2009).

Treatment of Knemidokoptic Mange
Moxidectin (Cydectin, Cyanamid) was topically applied to the skin of the inner thigh at a dose equivalent to 20-25 mg/kg (Toparlak *et al.* 1999). The average application was approximately 0.33 mg/bird. We treated approximately 40% of the infested birds captured and all recaptured, infested birds were re-examined and assigned an updated stage of mange. This treatment protocol had been previously tested on captive Hawai'i 'Amakihi and found safe and effective (LaPointe *et al.* 2012).

All collections and field protocols were permitted under the terms of State of Hawai'i Protected Wildlife Permit WL13-07, Federal Bird Banding Permit 22613, a State of Hawai'i Natural Area Reserves Special Use Permit, and University of Hawai'i Animal Care and Use Protocol 09-893-4.

Laboratory Procedures
Diagnostics for Avian Malaria
We screened all Hawai'i 'Amakihi by real-time polymerase chain reaction (rtPCR) assays to establish infection status with *P. relictum* and measure intensity of infection. Purified DNA for PCR analysis was extracted from packed blood cells using DNeasy tissue extraction kits (Qiagen Inc., Valencia, CA) according to the manufacturer's protocols, but we increased the initial incubation times with Proteinase K to overnight to increase yield of DNA. DNA was recovered from extraction columns with Tris ethanolamine buffer, measured by spectrophotometry with a Nanodrop spectrophotometer to assess purity and determine DNA concentration, and stored frozen at -20°C until used in rtPCR reactions.

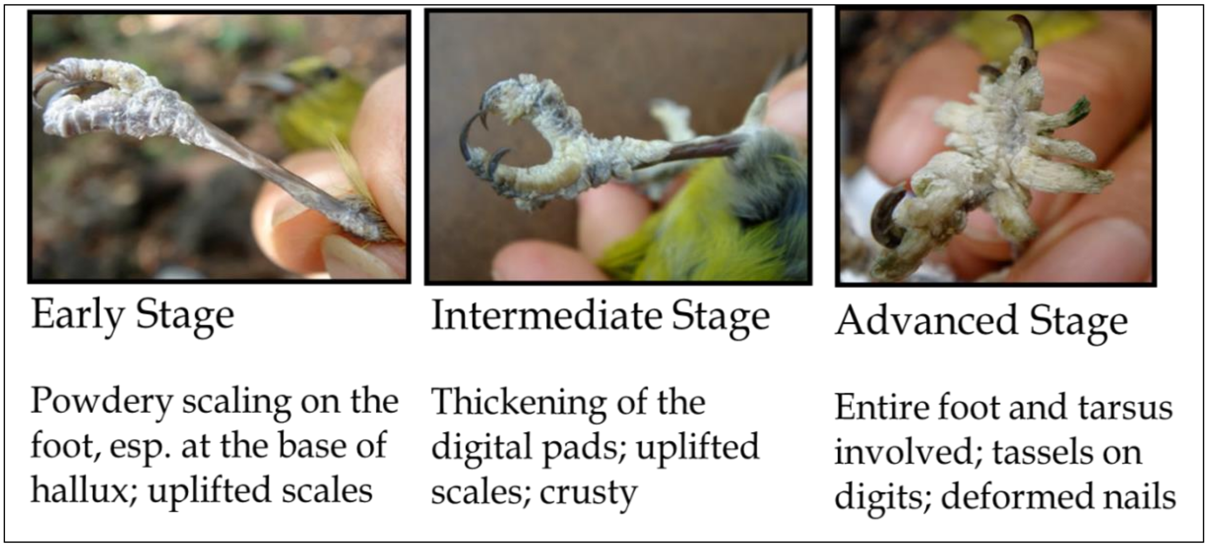

Figure 3. Hawai'i 'Amakihi exhibiting three different stages of knemidokoptic mange development (adapted from Gaudioso *et al.* 2009a).

We used a modification of the real-time assay described by Zehtindjiev *et al.* (2008) that uses SYBR-green based detection of a 105 bp region of the cytochrome b gene of *P. relictum* (lineage GRW4) during PCR amplification. We used primers GRW4/11F and GRW4/11R and cycling conditions described by Zehtindjiev *et al.* (2008) for the assay in a BioRad CFX96 thermocycler. Reactions were run in triplicate in 20 ul volumes for each sample using BioRad iTaq Universal Sybr Green Supermix (containing Mg+), a template concentration of 40-60 ng, and 0.4 uM of both forward and reverse primers per reaction. We generated a standard curve for estimating intensity of infection by making six serial 1:10 dilutions of extracted DNA from a blood sample from a naturally infected 'I'iwi (*Vestiaria coccinea*) with a parasitemia of 9.3 infected erythrocytes/10,000 erythrocytes. The serial dilutions were prepared with DNA from one or more blood samples from uninfected 'I'iwi (1 part infected DNA + 9 parts uninfected DNA) so that overall DNA concentration remained constant for each dilution. Parasitemia was calculated by counting number of infected and uninfected erythrocytes in fifty, 400X microscope fields using the image analysis program ImageJ to count erythrocyte nuclei (Gering and Atkinson 2004). Assays were considered valid if reaction efficiency was between 90 and 110% and the R^2 value of the standard curve was greater than 0.98. Samples were considered to be positive if all three replicates had detectable Cq values after 45 cycles. If only one or two of three replicates had detectable Cq values, the samples were re-tested and classified as positive only if half or more of the total replicates from both assays had detectable Cq values after 45 cycles.

Confirmation of Knemidokoptid Mites
Skin scrapings of presumptive knemidokoptic mange lesions were examined at 40x magnification under a zoom stereo microscope. Samples were scored positive for knemidokoptic mange if adult mites and/or if the characteristic honey-comb tunnels of mites were present (Figure 4).

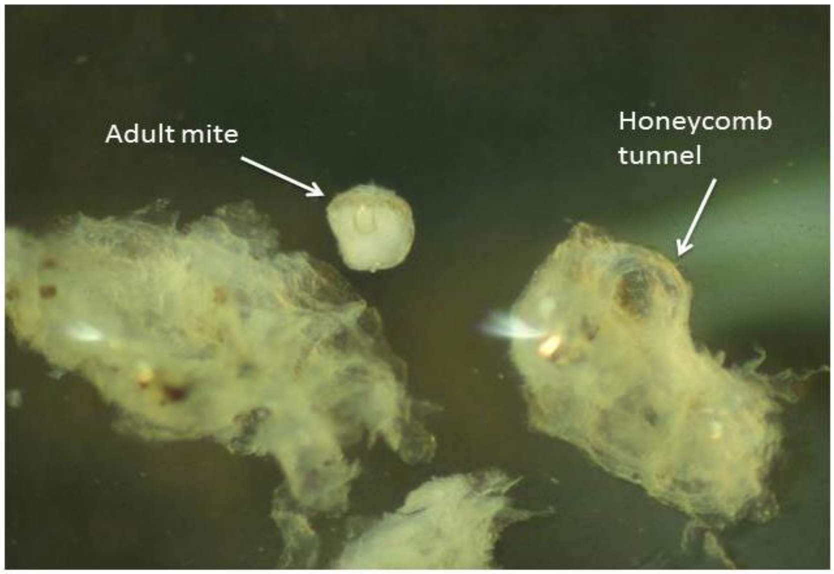

Figure 4. A photograph of an adult female knemidokoptid mite and lesion tissue with characteristic honeycomb-textured tunnel taken from an 'Amakihi exhibiting intermediate mange (magnification: 56X).

Statistical Methods

All Chi-square tests, Fisher's exact tests and t-tests were run in Systat 11 (SPSS Inc., Chicago 2004). We used Chi-square and Fisher's exact tests (FET) to determine the significance of the frequency of mange by the following categories: sex, the presence of ectoparasites, sampling time, recapture status, and treatment status. Fisher's exact tests were used in lieu of Chi-square tests when the presence of any one cell was less than five individuals. We also used FET to determine the significance of the frequency of mange by co-infection status of avian malaria and *Avipoxvirus*. We did not conduct Fisher's exact tests for sites which had mange present in fewer than five individuals. T-tests were conducted to determine if there were significant differences in fat score, weight, and the index of weight/tarsus length by mange status. To increase sample size by region, we combined the data from mange-positive sites into districts with similar rainfall, elevation, geography, and habitat which created the two groups of *Puna* and *Manuka*. Therefore, the Malama Ki and Keauohana sites were pooled to create the *Puna* sample. Likewise, we pooled the Lama Unit and Manuka State Park samples to create the *Manuka* sample.

Analysis of Variance (ANOVA) was conducted to examine the effect of age on mange prevalence. We used the following age classes: Hatch-Year (HY), After Hatch-Year (AHY), and After Second-Year (ASY). ANOVA was also used to examine the possible association between malaria parasetemia and the presence and severity (stage) of mange (0 = no mange, 1 = early, 2 = intermediate, 3 = advanced). ANOVA and post-hoc tests using Tukey's Honest Significant Differences (HSD) for multiple comparisons were conducted using the statistical package R 2.15.0 (R Core Team 2014). Results were considered significant when $P \leq 0.05$.

Graphs were created in Microsoft Excel 2010. Birds that lacked certain morphometric, aging, or sexing data were not included in analyses where those data were necessary for grouping or calculating means. All means are reported as the mean $\pm$ standard error. A *recapture* was defined as a bird that was caught at least one month (30 days) after its original capture date. Results were considered significant when $P \leq 0.05$.

RESULTS

Knemidokoptic Mange Prevalence and Distribution

Capture Summary

Between April 2012 and April 2014, we captured, banded, bled and examined 1,734 wild passerines including 738 Hawai'i 'Amakihi during mist netting (8,138 total net hours) at the following sites: Manuka NAR (236 birds), Keauohana Forest Reserve (147 birds), Kahuku Ranch Unit of HAVO (Glover site: 138 birds, Kipuka Akala: 165 birds), Nene cabin (175 birds), Malama Ki Forest Reserve (85 birds), Kipahoehoe NAR (96 birds), 'Āinahou Ranch of HAVO (241 birds), Hakalau NWR (Nauhi cabin site: 306 birds) and Pu'u Wa'awa'a Forest Bird Sanctuary (145 birds; Table 2).

Spatial and Temporal Patterns of Knemidokoptic Mange Prevalence

Knemidokoptic mange was present on both the windward and leeward sides of Hawai'i Island and was most prevalent in the windward, lowland sites in Puna (Malama Ki: 33%; Keauohana: 69%; Table 3, Figures 5 and 6). Mange was also present in Ka'u on the leeward side from 305 m asl at the Lama Unit of Manuka NAR to 1,532 m asl at Kahuku-Kipuka Akala. We found no knemidokoptic mange in Hawai'i 'Amakihi from most of our mid elevation sites (HAVO-'Āinahou Ranch or Pu'u Wa'awa'a) or our high elevation sites (Hakalau-Nauhi, Kipahoehoe, or Kahuku-Nene Cabin), although we did have some detections at Glover and Kipuka Akala in the Kahuku Ranch Unit of HAVO. Additional detections of mange (4/6 Hawai'i 'Amakihi) were made by a colleague mist netting at Nanawale Forest Reserve (K. McClure, personal communication). Annual prevalence of mange in Manuka State Park was stable from 2007-2010 but decreased by nearly 60% in 2012-2014 (Table 3). The mean prevalence for both sites at Manuka NAR was 10% (8/81), which was significantly lower ($\chi^2_{(1,\ n=134)} = 5.24$, $P = 0.02$) than 2010 when it was 26% (14/53).

Knemidokoptic Mange Prevalence by Sex and Age Classes

There was a significant difference in the prevalence of mange dependent on age class when all sites were pooled. The prevalence of mange was significantly higher for AHY birds as compared to HY birds ($P = 0.04$), and for ASY birds as compared to HY birds ($P < 0.01$), but ASY and AHY ($P = 0.06$) were not significantly different (Table 4). There was no significant difference between the prevalence of mange of males and females ($\chi^2_{(1,\ n=231)} = 1.86$, $P = 0.17$).

Knemidokoptes Identification by Lesion Scrapings

We collected scrapings of 32 early lesions, 22 intermediate lesions and 11 advanced lesions in the field. We confirmed 75% (49/65) of cases that were sampled in the field by microscopic examination of lesion scrapings. Lesion material was collected from Hawai'i 'Amakihi (n = 56), 'Apapane (*Himatione sanguinea*; n = 1), Hawai'i 'Elepaio (*Chasiempis sandwichensis*; n = 1), Northern Cardinal (*Cardinalis cardinalis*; n = 1), Japanese White-eye (*Zosterops japonicas*; n = 5), and Red-billed Leothrix (*Leothrix lutea*; n = 1). No intermediate or advanced lesions were observed in any other species besides Hawai'i 'Amakihi, and no suspect early stage lesions were confirmed in any species other than Hawai'i 'Amakihi. Adult mites and/or the characteristic honeycomb textured tissue were found in all of the intermediate and advanced lesion scrapings, but were found in only 53% (17/32) of the early stage mange scrapings (Figure 4).

Table 2. Number of captures by species and by site for the 2012–2014 surveys.

	Windward Hawai'i Sites				Leeward Hawai'i Sites					
Species	Hakalau-Nauhi	'Āinahou	Keauohana	Malama Ki	Kahuku - Nene	Kipahoehoe	Pu`u Wa`awa`a	Manuka NAR	Kahuku-Kipuka Akala	Kahuku - Glover
'Apapane	30	11	2	0	93	25	4	1	71	8
'Akepa	1	0	0	0	0	0	0	0	0	0
'Elepaio	20	0	0	0	0	4	0	4	0	0
Hawai'i 'Amakihi	82	143	58	18	65	35	102	81	65	89
Hawai'i Creeper	4	0	0	0	0	0	0	0	0	0
House Finch	0	3	0	7	0	0	1	37	12	0
'I'iwi	89	2	0	0	0	10	1	0	4	0
Japanese white-eye	24	76	76	50	15	16	17	79	10	37
Melodious Laughing Thrush	0	0	1	1	0	0	0	0	0	0
Northern Cardinal	2	3	10	5	0	1	2	6	2	0
Nutmeg Manikin	0	3	0	2	0	0	5	0	0	3
'Ōma'o	24	0	0	0	0	0	0	0	1	0
Red-billed Leothrix	29	0	0	0	0	3	5	20	0	0
Warbling Silverbill	0	0	0	0	0	0	5	0	0	0
Yellow-billed Cardinal	0	0	0	2	0	0	0	0	0	0
Yellow-fronted Canary	1	0	0	0	2	1	2	5	0	1
Zebra Dove	0	0	0	0	0	0	0	3	0	0
TOTAL	306	241	147	85	175	96	145	236	165	138

Table 3. Knemidokoptic mange prevalence by site in Hawai'i 'Amakihi from 2007–2014 surveys.

	Year(s) of survey			
Study sites	2007-2008[1]	2008-2009[2]	2010[3]	2012-2014[4]
Malama Ki FR	0% (0/32)	-	-	33% (6/18)
Keauohana FR	6% (2/36)	25% (2/8)	-	69% (40/58)
Manuka NAR: Lama	-	15% (2/13)	25% (1/4)	10% (5/50)
Manuka NAR: State Park	26% (7/27)	22% (8/36)	27% (13/49)	10% (3/31)
'Āinahou Ranch (HAVO)	0% (0/49)	-	-	0% (0/143)
Kahuku Ranch (HAVO) Glover	-	-	-	4% (4/89)
Kipahoehoe NAR	-	-	-	0% (0/35)
Pu'u Wa'awa'a Forest Bird Sanctuary	0% (0/74)	-	-	0% (0/102)
Kahuku Ranch (HAVO) Kipuka Akala	-	-	-	2% (1/65)
Hakalau Forest NWR Nauhi	-	-	-	0% (0/82)
Kahuku Ranch (HAVO) Nene Cabin	-	-	-	0% (0/65)

[1]Gaudioso 2009; [2]Gaudioso *et al.* 2009; [3]LaPointe *et al.* 2012; [4]Current study

Disease Diagnostics and Co-infection Frequencies

Avian Malaria and Avipoxvirus

The prevalence of malaria was 26% (25/95) at Kahuku Ranch Unit of HAVO-Glover site, 14% at Manuka NAR (11/81), 93% (51/55) at Keauohana Forest Reserve, and 56% (10/18) at Malama Ki Forest Reserve. We did not find a significant association between the prevalence of knemidokoptic mange and avian malaria in the Hawai'i 'Amakihi sampled from Manuka NAR (FET; $P = 0.59$; n = 81). In fact, there were no cases in which an individual was infected with malaria and knemidokoptic mange at Manuka NAR. At Keauohana Forest Reserve, we found that 66% (37/55) of the birds with mange also tested positive for malarial infection, but there was no significant association of co-infection (FET; $P = 0.58$; n = 55). At Malama Ki Forest Reserve, we found that 88% (7/8) of the 'Amakihi with mange also tested positive for malarial infection and there was a significant association of co-infection (FET; $P = 0.04$, n = 17). When both sites in Puna were combined, we found a significant association of co-infection of mange and malaria (FET; $P < 0.01$; n = 72), but this may be a reflection of the high prevalence of malaria in Puna. At the Kahuku Ranch Unit of HAVO-Glover site, there was no significant association of co-infection (FET; $P = 1.0$, n = 89). We found the prevalence of active *Avipoxvirus* of all species over all sites surveyed from 2012-2014 ranged from 0-3% (Table 5). The highest prevalence of *Avipoxvirus* was seen at Keauohana Forest Reserve (3%) and Manuka NAR (3%). There was a significant association of the frequency of *Avipoxvirus* and knemidokoptic mange in Hawai'i 'Amakihi from sites sampled from 2012-2014 where mange was present (FET; $P = 0.05$; n = 311).

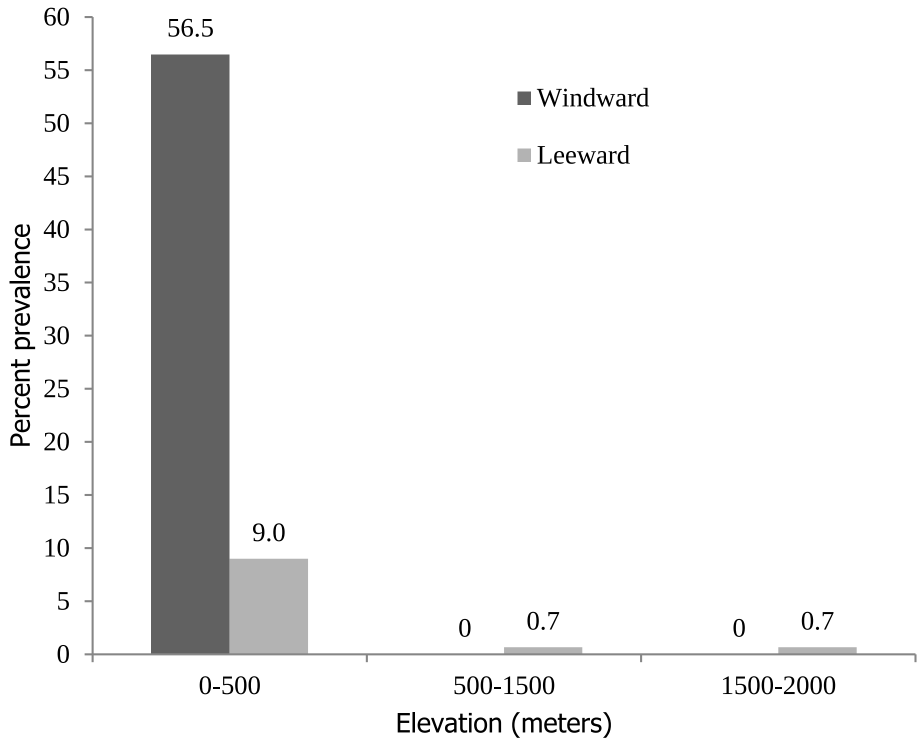

Figure 5. Mange prevalence by elevation and windward (dark gray) versus leeward (light gray) sites sampled from 2012-2014.

The parasitemia (number of erythrocytes infected with *Plasmodium relictum* per 10,000 erythrocytes) varied significantly with ranked stage of mange in Puna Forest Reserves ($F_{(3, 69)}$ = 5.42, $P < 0.001$). The *post-hoc* Tukey's HSD test showed that there is a significant difference in the parasitemia level of the advanced stage birds versus the un-infested, early, and intermediate stage birds in Puna. This relationship was not significant at Manuka NAR. When the two outliers of parasitemia levels >18 were dropped from the data set, there was no significant difference in the parasitemia level by the stage of mange ($F_{(3, 67)}$ = 1.14, P = 0.34). A two-sample t-test that compared the parasitemia level between infested and un-infested Hawai'i 'Amakihi in the Puna Forest Reserves showed infested birds had a significantly higher parasitemia level (1.67 $\pm$ 0.61) than un-infested birds (0.42 $\pm$ 0.19; t_{71} = -1.59, P = 0.05). The mean parasitemia level of infected, un-infested birds was 0.415 $\pm$ 0.98, and the mean parasitemia level of infected, infested birds was 1.67 $\pm$ 4.08.

Feather Ectoparasites Prevalence
Of the 265 Hawai'i 'Amakihi from all sites we examined for feather mites and lice, 85 of them had feather mites and/or lice present on their wings, tail, or body. There was a significant difference in the frequency of the presence of these ectoparasites on birds with mange versus birds without mange ($\chi^2_{(1, n = 265)}$ = 35.7, $P < 0.01$), with infested birds being more likely to have feather ectoparasites present.

Recapture Rates, Body Condition and Moxidectin Treatment
Recapture Rates and Mange
The average number of days between recaptures for Hawai'i 'Amakihi was 385 days, with a median of 190 days. Our overall recapture rate for Hawai'i 'Amakihi from sites where mange was present from 2007-2014 was 13% (60/455). From sites where birds were banded from

2007-2014, there were a total of 52/301 (17%) Hawai'i 'Amakihi that were recaptured (15 at Keauohana, 8 at Malama Ki, and 29 at Manuka). For the two sites where birds were banded only from 2012-2014, there were a total of 8/154 (5%) recaptured Hawai'i 'Amakihi (four at Kahuku Ranch Unit Glover and four at Kahuku Ranch Unit Kipuka Akala). Of these 52 recaptured Hawai'i 'Amakihi, 12 of them went from being un-infested to infested during their capture history. The time that lapsed for birds that were un-infested at first capture, and then became infested ranged from 8-1,308 days. No recaptured birds went from being infested to being totally clear of signs of mange, or un-infested without treatment.

There was no difference in the frequency of recapture of birds that were infested versus un-infested at first capture at our long-term sites (Manuka NAR and Puna sites; $\chi^2_{(1,\, n = 227)} = 1.51$, $P = 0.22$), but when all sites with mange present were pooled, there was a significant difference in the frequency of recaptures between infested and un-infested birds ($\chi^2_{(1,\, n = 424)} = 7.13$, $P = 0.01$). The frequency of recapture did not differ between treated and untreated infested birds at the three sites where we administered moxidectin treatments (Keauohana, Manuka NAR, and Kahuku Unit of HAVO-Glover; $\chi^2_{(1,\, n = 75)} = 0.04$, $P = 0.85$).

Morphometrics and Mange

We found that there was a significant difference in the fat score rank (1-5, lowest to highest fat content) between infested and un-infested Hawai'i 'Amakihi captured at all sites where mange was present in at least five individuals ($F_{(3,145)} = 4.52$, $P < 0.01$). According to the post-hoc Tukey's HSD test, there was a significant difference in the fat scores between un-infested birds and those with advanced mange ($P = 0.04$). Un-infested birds had a mean fat score of 2.54 $\pm$ 0.09, while the infested birds had a mean fat score of 1.70 $\pm$ 0.15 ($t = -4.78$, $P < 0.001$). Birds with advanced mange (stage = 3) had the lowest mean fat score (0.75 $\pm$ 0.96; Figure 7). There was no significant difference in the weight between infested and non-infested birds ($t = 0.07$, $P = 0.94$) at all sites combined. When we looked at each individual site, there was a significant difference in fat scores between infested and un-infested birds at Manuka NAR. Infested birds had a mean fat score of 1.2 $\pm$ 0.191, while un-infested birds had a mean fat score of 2.3 $\pm$ 0.116 ($t = 4.63$, $P < 0.01$). However, there was no significant difference in mean fat scores between infested and un-infested birds in the Puna Reserves. There was no significant difference in body weight between infested and un-infested birds at the Puna Forest Reserves ($t = -0.49$, $P = 0.63$) or Manuka NAR ($t = 0.59$, $P = 0.56$). When a body mass index of weight/tarsus length was used, there was no significant difference in the index between infested and un-infested birds at the Puna Forest Reserves ($t = -0.17$, $P = 0.86$), or at Manuka NAR ($t = 1.68$, $P = 0.09$).

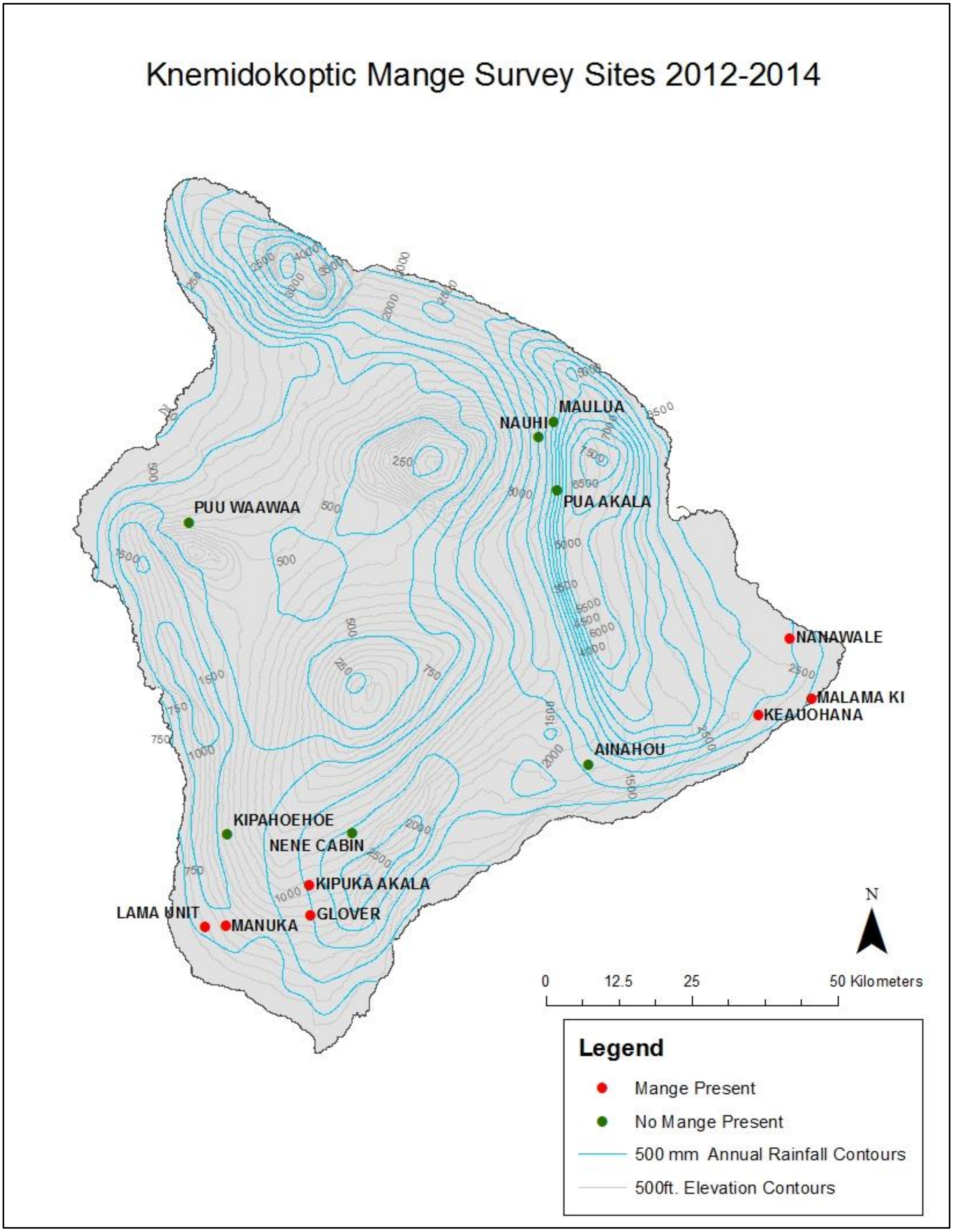

Figure 6. A map showing where mange is present (red) and not present (green) on Hawai'i Island according to the 2012-2014 surveys.

Table 4. Infested Hawai'i 'Amakihi captured in 2012–2014 by sex and age classes, as determined in the field.

Site name	Sex			Age[1]		
	Males	Females	Unknown	HY	AHY/SY	ASY
Manuka NAR	11% (4/44)	13% (3/23)	7% (1/14)	0% (0/2)	7% (4/57)	18% (4/22)
Keauohana FR	71% (27/38)	72% (8/11)	66% (6/9)	0% (0/1)	75% (30/40)	65% (11/17)
Kahuku Ranch Glover	10% (4/42)	0% (0/15)	0% (0/32)	0% (0/20)	4% (2/55)	14% (2/14)
Kahuku Ranch Kipuka Akala	0% (0/30)	0% (0/20)	7% (1/15)	10% (1/10)	0% (0/47)	0% (0/8)
Malama Ki FR	45% (5/11)	0% (0/3)	50% (2/4)	0% (0/2)	36% (5/14)	100% (2/2)
TOTAL	24% (40/165)	15% (11/72)	14% (10/74)	3% (1/35)	19% (41/213)	30% (19/63)

[1]HY = Hatch year, AHY = After hatch year, SY = Second year

Table 5. *Avipoxvirus* prevalence of all species captured at sites surveyed from 2012–2014.

Site name	*Avipoxvirus* prevalence
Manuka NAR: Manuka State Park	3% (3/108)
Manuka NAR: Lama Unit	0% (0/128)
Keauohana FR	3% (5/147)
Kahuku Ranch (HAVO): Glover site	1% (2/138)
Malama Ki FR	0% (0/85)
Kipahoehoe NAR	0% (0/96)
'Āinahou Ranch (HAVO)	0.4% (1/241)
Pu'u Wa'awa'a Forest Bird Sanctuary	1% (1/144)
Kahuku Ranch (HAVO): Nene Cabin	1% (1/175)
Kahuku Ranch (HAVO): Kipuka Akala	1% (2/165)
Hakalau NWR: Nauhi Cabin	0.7% (2/306)

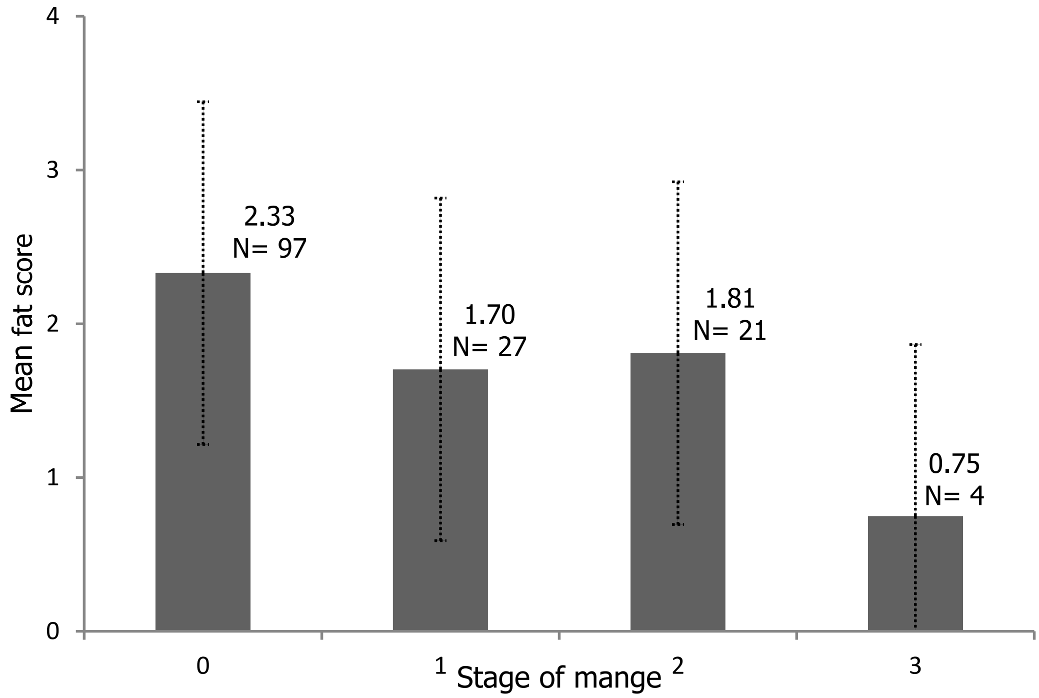

Figure 7. Mean fat scores + SE by stage of knemidokoptic mange for all sites sampled from 2012-2014 where mange was present (n = 149).

Status of Treated Birds

We treated a total of 24 infested Hawai'i 'Amakihi with moxidectin at three sites: Manuka NAR (1 with intermediate stage and 2 with advanced stage; n = 3), Keauohana Forest Reserve (7 with early stage, 11 with intermediate stage, and 1 with advanced stage; n = 19), and Kahuku Ranch Unit of HAVO (Glover site; 1 with early stage and 1 with advanced stage; n = 2). Of the 24, 4 of them exhibited advanced mange, 12 of them exhibited intermediate mange, and 8 of them exhibited early mange when first treated. One treated individual with intermediate mange was recaptured twice. It was treated at first capture when it exhibited intermediate mange. At recapture, one week later, the lesions had diminished in size, extensive growth on the intertarsal (tibiotarsal) joint and most digits had resolved. The bird was treated again and by the second recapture, two weeks after the first capture, lesions were only evident on the halluces and resembled early stages of infection. Another treated individual with intermediate mange was recaptured after three weeks post treatment, but it was not treated again. The intermediate stage lesions had regressed to only uplifted scales on the halluces present and no crusting of digits, indicative of early stage mange (Figure 8).

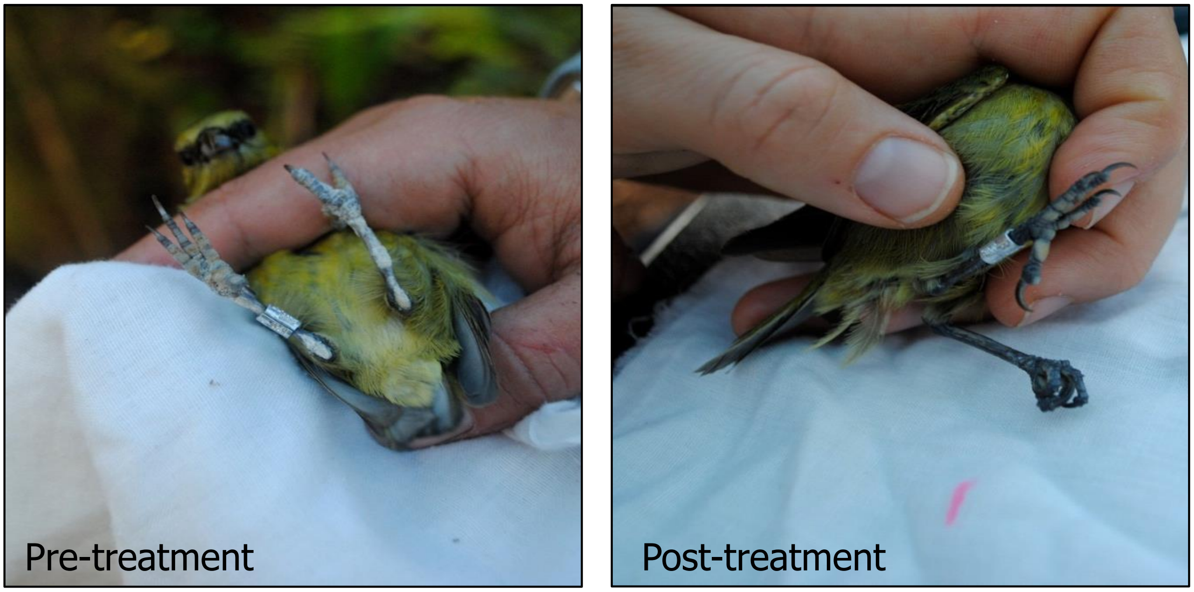

Figure 8. A treated Hawai'i 'Amakihi, first captured on 30 January 2013 with intermediate mange (left) and treated with moxidectin on both inner thighs, and recaptured on 20 March 2013, with regressed early stage mange (right). Photo: C. Gesmundo.

DISCUSSION

Host Range, Temporal and Geographical Patterns in Knemidokoptic Mange

Despite the examination of over 1,715 native and non-native passerine birds we did not find any species other than Hawai'i 'Amakihi with confirmed clinical signs of knemidokoptic mange. This is similar to previous reports of single-species epizootics in American Robin (*Turdus migratorius*; Pence *et al*. 1999), Evening Grosbeak (*Coccothraustes vespertinus*; Carothers *et al.* 1974), and Red Crossbill (*Loxia curvirostra*; Benkman *et al.* 2005). Other studies have documented knemidokoptic mange affecting a number of species in an avian community. Typically in these multiple species epizootics, one species will have a higher prevalence than the rest; for example, Red-wing Blackbird (*Agelaius phoeniceus*) in Ontario (Kirmse 1966), Eurasian Tree Sparrow (*Passer montanus*) in Hong Kong (Mainka *et al.* 1994) and Palm Warbler (*Dendroica palmarum*) in the Dominican Republic (Latta and O'Connor 2001). Among passerine birds worldwide, members of the finch family (Fringillidae) are the most commonly reported hosts of knemidokoptid mites (Dabert *et al.* 2013). The Hawaiian honeycreepers are considered a subfamily (Drepanidinae) within the Fringillidae so the occurrence of knemidokoptic mange in Hawai'i 'Amakihi is consistent with the apparent susceptibility of this family of passerine birds. Why we did not observe knemidokoptic mange in other Hawaiian honeycreeper species remains uncertain but may be related to the more restricted altitudinal distribution of 'Apapane, 'I'iwi, and the endangered species. Although Hawai'i 'Amakihi have been extensively studied since the 1970s, knemidokoptic mange was only first reported in Hawai'i 'Amakihi in 2007. Van Riper (1991) examined 1,768 Hawai'i 'Amakihi mist netted from wet forests on Mauna Loa and xeric forests on Mauna Kea from 1972-1980 but did not detect knemidokoptic mange by gross or histological examination of foot lesions.

The recent epizootic of knemidokoptic mange in Hawai'i 'Amakihi may represent a host jump from a number of non-native finches co-occurring with Hawai'i 'Amakihi. The House Finch (*Carpodacus mexicanus*) and Yellow-fronted Canary (*Serinus mozambicus*) are potential sources. These two finch species are common at all sites where mange was present and were captured at 3/5 sites where mange was present. Both species are taxonomically similar to species reported to be susceptible to knemidokoptic mange (Dabert *et al.* 2013). The origin of *Knemidokoptes jamaicensis* in wild bird communities in the Hawaiian Islands is unknown but may be related to these two naturalized species and the domesticated common canary (*Serinus canaria*). *Knemidokoptes jamaicensis* is known to parasitize common canaries (Kaschula 1950) and knemidokoptic mange in domestic canaries is routinely seen by veterinary practices in Hawai'i.

Our survey found knemidokoptic mange present in additional sites in the Ka'u (Kahuku Ranch Unit of HAVO; Glover and Kipuka Akala) and the Puna (Nanawale Forest Reserve) districts, not previously surveyed during 2007-2010. These new detections of mange were confirmed by microscopic examination of scrapings. At both Malama Ki Forest Reserve and Keauohana Forest Reserve, there were large increases in mange prevalence since the earlier surveys in 2007-2009, with current prevalence of mange at an epizootic level, as high as 69%. The new detections show the distribution of mange is broader than originally documented, but still appears to be limited to lower elevations of the Ka'u and Puna districts. Mange was not detected in Nanawale and Malama Ki prior to the 2012-2013 survey (JMG, personal observation), suggesting that the spread to these sites occurred sometime between 2008 and 2011. The Kahuku Ranch Unit of HAVO had not been previously surveyed, so we cannot determine when mange first appeared in this population.

The observed annual prevalence rates in this study were comparable to other single species studies where prevalence ranged from 25% in Evening Grosbeaks (*Coccothraustes vespertinus*; Carothers *et al.* 1974) to 42% in Red Crossbills (Benkman *et al.* 2005). Higher prevalence rates as seen in Hawai'i 'Amakihi at Keauohana (69%) have only been reported among migrating American Robins in Oklahoma (Pence *et al.* 1999). However, few studies have examined changes in prevalence over time. Mange prevalence in Manuka NAR significantly decreased 58% since the earlier surveys conducted in 2007-2010. This may be, in part, due to the removal of 13 infested Hawai'i 'Amakihi in 2010 for a captive aviary study (LaPointe *et al.* 2012). A similar explanation was given for a decline in the prevalence of knemidokoptic mange in a wild population of Evening Grosbeaks where infested birds were culled from a mist netted population (Carothers *et al.* 1974). Alternatively, the decrease in the mange prevalence among Hawai'i 'Amakihi at Manuka NAR may represent a natural epizootic progression in a naive population where infected individuals die off or become immune. Such a cyclic reduction in prevalence was seen in resident Red Crossbills over time in a seven-year study (Benkman *et al.* 2005). Over a greater period of time we might expect to see enhanced immuno-competence of the host through natural selection (Råberg *et al.* 2009, Atkinson *et al.* 2013). The reduction in mange prevalence may also be explained by a shift toward environmental conditions that limit transmission of new infestations (Latta & O'Connor 2001, Latta 2003).

For example, Latta and Connor (2001) found a higher prevalence of knemidokoptic mange among birds in dry desert scrub than at higher elevation or in wetter habitats. We also found a lower prevalence of knemidokoptic mange at higher elevation, however unlike Latta and O'Connor (2001) we found a higher prevalence of mange among birds on the windward or wetter side of the island. While the exact mechanism of transmission in knemidokoptic mites is unknown, it is assumed that prolonged close or direct contact is required (Wade 2006). Cooler temperatures found at higher elevations might increase the degree of contact among roosting or brooding birds but the expected higher prevalence is inconsistent with field observations. Although there is no evidence that insects aid in the dispersal of knemidokoptic mites, hippoboscid flies can serve as phoretic vectors of epidermoptid skin mites (Jovani *et al.* 2001). Environmental influence on the phoretic dispersal of *K. jamaicensis* might explain geographical and temporal patterns in prevalence but the nearly three-fold difference in prevalence observed across the island of Hawai'i is more likely due to the stage in the epizootic cycle, the presence of alternative hosts or the general health of the local host population as influenced by concomitant disease and habitat quality.

Individual Factors and Concomitant Infections Influencing Knemidokoptic Mange

Age and Sex

Similar to our findings in 2007-2010, we found no significant sex bias for the prevalence of mange in Hawai'i 'Amakihi. Our finding is contrary to Benkman *et al.* (2005) who found a male-biased incidence of knemidokoptid mites in Red Crossbills, which supports the theory that parasitism becomes increasingly male-biased as sexual size dimorphism increases (Moore and Wilson 2002). We did, however, find a significant difference in the prevalence of mange by age. Mange was more common in older birds (AHY and ASY) than hatch-year birds. This result was similar to our finding during the earlier surveys in 2007-2010 and suggests that either hatch-year Hawai'i 'Amakihi daily behaviors do not allow for exposure to knemidokoptid mite infestation early in life, and/or that infestations require time to develop before clinical signs of lesions are evident. Although very little is known about host to host transmission, since the mite spends its entire life cycle on the host it is likely that close contact promotes transmission (Wichmann and Vincent 1958). While prolonged, direct contact does occur during brooding on the nest, the incidence of infestation in hatch-year birds is low, therefore if they are exposed to mites on the nest, it must take some time before they show clinical signs. If a compromised immune system is essential to the development of mange lesions, then young birds might not display clinical signs of infestation until well after fledging when their immune system may become compromised by environmental stressors. Other researchers observed that unlike other parasitic infections in birds, clinical infestation of knemidokoptic mange occurs in older birds (Latta 2003, Wade 2006). Wade (2006) suggested that not all nestlings will become clinically infected and that genetic susceptibility, stressors or a compromised immune system play a larger role in the clinical manifestation of knemidokoptic mange.

Co-infection of Avipoxvirus, Avian Malaria, and Mange

Avian malaria and *Avipoxvirus* are common in lowland Hawai'i 'Amakihi (Woodworth *et al.* 2005) and both infections may overtax or suppress their host's immune system. Like demodectic and sarcoptic mange in mammals, knemidokoptic mange likely occurs in immune-compromised hosts or inbred populations (Pence and Ueckermann 2002). Past research has documented case reports of co-infection between papillomavirus and knemidokoptic mange (Literak *et al.* 2005), however, co-infections with *Knemidokoptes jamaicensis* are infrequently reported. We observed cases of avian pox-*Knemidokoptes* co-infection in Hawai'i 'Amakihi and avian malaria-*Knemidokoptes* co-infections in this study. Although the frequency of co-infection between mange and avian malaria was not significant in Manuka NAR, the frequency of co-infection of mange and malaria in the Puna sites—where mange appeared more recently—was significant. Unfortunately, we did not encounter enough co-infected individuals to evaluate the possible association between *Avipoxvirus* and mange. While it is possible that the effects of one infection may predispose the individual to the secondary infection, given the chronic nature of avian malaria in Hawaiian honeycreepers, it is difficult to conclude which is the primary infection in our system. Confounding the potential role of co-infections with knemidokoptic mange is the finding that lowland Hawai'i 'Amakihi populations are more immuno-competent than populations at higher elevations (Atkinson and Paxton 2013, Atkinson *et al.* 2013). While we did find the association between avian malaria and mange to be significant at the Puna sites, this significance may be merely a reflection of the very high prevalence of chronic malarial infection at Malama Ki (56%) and Keauohana Forest Reserve (93%).

The significantly elevated parasitemia in infested birds suggests that they may have been immuno-compromised by mange, but these increases in parasitemia were relatively small in scale relative to the dynamics of acute malarial infections, where parasitemia can be as high as 200 per 10,000 RBC (Atkinson *et al.* 2013). When we removed the apparent outliers from the ANOVA, the effect was no longer significant. It is unclear whether the elevated parasitemia represent immune-suppressed individuals. Additional research under controlled experimental conditions is needed to understand the significance of moderately elevated parasitemia; and whether or not these parasitemias are influenced by knemidokoptic mange.

Impact of Knemidokoptic Mange on Hawaiʽi ʽAmakihi

Recaptures and Mange

There was no significant difference in the frequency of recapture between infested and un-infested birds at each of our long-term sites (Manuka NAR and Puna), however, when all sites with mange present were pooled, there was a significant difference in the frequency of recaptures between infested and un-infested birds. For those sites where knemidokoptic mange was detected at low prevalence little recapture effort was made and the large number of never-recaptured (n = 371) birds likely confounded the analysis. Unfortunately, we have no clear indication that mange has an effect on survivorship based upon our mark-recapture data. Other longer-term studies have shown some impact to survivorship in individuals with knemidokoptic mange (Latta *et al.* 2003, Benkman *et al.* 2005) and potential population level impacts (Carothers 1974, Bonter and Harvey 2008). Latta (2003) found that the presence of mites had a profound negative impact on the annual return rates of palm and prairie warblers. In fact no birds infested with mites ever returned the following year. Ectoparasites like *K. jamaicensis* may overburden migrating birds (Latta 2003). *K. jamaicensis* infestation also reduced annual adult survivorship among a resident population of red crossbill in Idaho so the impact of knemidokoptic mange may not solely rely on the stress of migration (Benkman *et al.* 2005).

Morphometrics and Mange

There was a significant difference in fat score between infested and un-infested birds over all sites and the fat score differed between infested and un-infested birds at Manuka NAR. The fat score of infested birds was significantly lower than that of un-infested birds in both cases, similar to emaciated American Robins collected from an epizootic event during the mid-1990s (Pence *et al.* 1999). Fat score can be considered a measure of overall body condition in birds (Brown 1996), but because fat can differ dramatically by season or even within the day (Gosler 1996), fat scores are not a reliable estimate of condition or survivorship. Knemidokoptid mite infestations can cause a significant decrease in mean pectoral mass scores, indicating a negative effect on overall body condition and physiological stress (Latta 2003). We did not collect data on pectoral mass in this study, so we cannot clearly access the effect of mange on the physiological condition of our infested birds. We also looked at the association between mite infestation and body weight and infestation and the index of weight/tarsus for infested versus un-infested birds but our data did not show a strong association between mite infestation and morphometrics.

Co-infestation of Feather Mites and Mange

We found a significant difference in the frequency of the presence of feather mites and lice on infested versus un-infested birds. Given our high frequency (161/265 birds, 60%) of un-infested birds without feather ectoparasites, it is difficult to conclude if there is a clear co-infestation of knemidokoptid mites and feather ectoparasites on Hawai'i 'Amakihi, due to the overall low prevalence of both types of ectoparasites over all sites combined. In addition, there may be some site-specific environmental conditions at play that would drive ectoparasites' colonization of the host (Marshall 1981, Poulin 1991, Merino and Potti 1996). Ectoparasite increases have been documented on birds with experimentally impaired preening (DeVaney 1979). Also, during a co-infestation of multiple ectoparasites, one of the parasites may predispose the host to other parasites (Literak *et al.* 2005), such as affecting the ability of the host to preen, and therefore the intensity and prevalence of the parasite load will increase (Clayton *et al.* 2010).

Treatment of Mange in Wild Populations

Knemidokoptic mange in wild birds is thought to be a chronic, progressive infection; not undergoing remission without treatment (Pence *et al.* 1999). Of the 24 birds we treated with moxidectin, only two were recaptured but both individuals showed a reduction in the severity and size of the lesions. Unfortunately with this limited number of recaptures we were unable to detect any difference in the frequency of recapture between treated and untreated birds. Toparlak *et al.* (1998) found that a single dose of moxidectin (25 ug/bird) was effective in reducing the severity and size of lesions in captive Budgerigars (*Melopsittacus undulatus*) and in our earlier captive study, we found few clinical signs of mange remaining three weeks post-treatment with a single dose of moxidectin (20 ug/bird; LaPointe *et al.* 2012). While the topical treatment of wild Hawai'i 'Amakihi with knemidokoptic mange has been shown to reduce lesion severity and size, we do not know how many birds would need to be treated to limit further spread.

CONCLUSIONS

Our survey of knemidokoptic mange has documented the current host species, distribution and spread of *K. jamaicensis* on the island of Hawai`i. Although there may be additional hosts of *K. jamaicensis* on island, such as domesticated birds, we only found knemidokoptic mange to be present in the wild in Hawai'i 'Amakihi. More attention should be given to surveying both domesticated and wild non-native birds for the presence of mange on island. We found knemidokoptic mange to be present from the coast to the montane forest at 1,585 m asl at Manuka NAR and at 1,532 m asl at the Kahuku Ranch Unit of HAVO. We also found knemidokoptic mange in lowland forests on both the leeward and windward flanks of Mauna Loa of Hawai'i Island. This broad geographical distribution suggests that *K. jamaicensis* is not limited by elevation or moisture. Prevalence rates varied across our sites but were highest in Hawai'i 'Amakihi populations inhabiting lowland forests in Puna. These differences in observed prevalence most likely reflect the epizootic cycle. Recently invaded populations will have a rapidly increasing prevalence as mites infest immunologically naive birds. As individuals die or recover from infestations, the prevalence of knemidokoptic mange will slowly decrease. The distribution, prevalence and timeline of knemidokoptic mange on the island of Hawai'i suggests an initial outbreak at the Manuka State Wayside section of Manuka NAR that has spread along the entire altitudinal extent of the Manuka NAR and eastward to the Puna lowlands. The effect of environmental factors on transmission rates may also be contributing to island-wide variation in prevalence and our data suggest that prevalence is associated with fragmented habitats at low-elevation, with warmer temperatures, and mean annual rainfall above 850 mm. We also detected an association of co-infection between avian malaria and mange in Puna, but the potential role of malaria in driving mange epizootics is unclear. While our study was unable to document any clear impacts on survivorship, we did document differences in fat scores and recapture rates between un-infested and infested birds.

Further research will improve our understanding of knemidokoptic mange impacts to endemic Hawaii forest birds. To estimate survivorship and access potential population impacts we recommend a long-term (four-year) intensive demographic study of a Hawaiʻi ʻAmakihi population where epizootic knemidokoptic mange is present. The invasion of a new pathogen in the native Hawaiian forest bird community is a reminder of the value of continuous monitoring of forest bird populations. Annual monitoring of Hawaiʻi ʻAmakihi populations at key conservation areas with (Keauohana Forest Reserve and Manuka NAR) and without (Puʻu Waʻawaʻa Forest Bird Sanctuary and HAVO-ʻĀinahou Ranch) knemidokoptic mange would provide data on the spread of this emerging infectious disease and also serve as an early warning for potential new diseases such as mycoplasmal conjunctivitis which affects a number of finch species across North America (Fischer *et al.* 1997, Farmer *et al.* 2005). The in-field treatment of knemidokoptic mange with moxidectin by researchers that routinely handle native Hawaiian birds may reduce the local transmission and reduce further spread of knemidokoptic mange. The adoption of an approved standard operating procedure for the disinfection of nets, banding equipment, bird bags and banders' hands would prevent further spread of this ectoparasite. A long-term (four-year) intensive demographic study of an infested Hawaiʻi ʻAmakihi population would provide a more complete picture of the potential population impacts of knemidokoptic mange on Hawaiʻi ʻAmakihi. There does appear to be an association of co-infection between avian malaria and mange in Puna. The association between higher parasitemia and later stages of mange suggest that birds are immunocompromised by one or both infections. Additionally, the co-infection mechanism should be investigated using experimental trials to determine the relationship between these diseases and directionality of susceptibility and infection in the wild.

ACKNOWLEDGEMENTS

We thank the following people for their assistance with specimen collection and data management: Maia Lipschutz, Callie Gesmundo, Matt Fairall, Rudy Levita, and Alexandria Vickery. We thank Kevin Brinck for statistical assistance. We also thank Ariel Gaffney and Ariel Egan for assisting with malarial diagnostics. We acknowledge the State of Hawaii Department of Land and Natural Resources—Division of Forestry and Wildlife, as well as the U.S. Department of Agriculture Forest Service as land managers and administrators for the Hawaii Experimental Tropical for their collaboration and access to field sites. This project was funded by U.S. Fish and Wildlife Service, Pacific Islands Office, Ecological Services and the U.S. Geological Survey, Ecosystems Mission Area.

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