DOI: 10.7589/2014-10-251

Journal of Wildlife Diseases, 51(3), 2015, pp. 651–663

KNEMIDOKOPTINID (EPIDERMOPTIDAE: KNEMIDOKOPTINAE) MITE INFESTATION IN WILD RED-CROWNED PARAKEETS (*CYANORAMPHUS NOVAEZELANDIAE*): CORRELATIONS BETWEEN MACROSCOPIC AND MICROSCOPIC FINDINGS

Bethany Jackson,[1,2,7] Allen Heath,[3] Cathy Harvey,[4] Carly Holyoake,[2] Richard Jakob-Hoff,[1] Arvind Varsani,[5,6] Ian Robertson,[2] and Kris Warren[2]

[1] Auckland Zoological Park, Motions Road, Western Springs, Auckland 1022, New Zealand
[2] School of Veterinary and Life Sciences, College of Veterinary Medicine, Murdoch University, Murdoch 6150, Western Australia, Australia
[3] Hopkirk Research Institute, Massey University, Private Bag 11008, Palmerston North 4442, New Zealand
[4] New Zealand Veterinary Pathology, Auckland Zoological Park, Motions Road, Western Springs, Auckland 1022, New Zealand
[5] School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
[6] Biomolecular Interaction Centre, University of Canterbury, Private Bag 4800, Christchurch, 8140, New Zealand
[7] Corresponding author (email: B.Jackson@murdoch.edu.au)

ABSTRACT: During a study on health and disease in Red-crowned Parakeets (*Cyanoramphus novaezelandiae*) on Tiritiri Matangi Island and Little Barrier Island (Hauturu-o-Toi) in New Zealand between 2011 and 2013, an outbreak of feather loss prompted the collection of skin biopsies ($n=135$) under anesthesia from the head of captured birds. A subset of samples ($n=7$) was frozen to obtain whole specimens for identification of ectoparasites. Mites (range 1–11) were observed in 79/135 (58.5%) skin biopsies, whereas feather loss was only found in 47/142 (33.1%) birds captured during the sampling period. Compact orthokeratotic hyperkeratosis and acanthosis were found in association with mites. *Procnemidocoptes janssensi* (Acari: Epidermoptidae, Knemidokoptinae) was identified from whole mites obtained from skin biopsies. We describe the presence, pathology, and stages of infestation for knemidokoptinid mange in a wild parrot population in New Zealand. Given the clinical and pathologic changes observed and poor knowledge of the parasite's New Zealand host and geographic distribution, further work is recommended for this and sympatric parrots, to understand relationships between the host, parasite, environment, and expression of disease. Results from this study reinforce the value of including biopsy samples for the investigation of skin disease in wild birds, particularly to link etiologic agents with pathologic changes.

Key words: Biopsy, *Cyanoramphus novaezelandiae*, disease outbreak, Knemidokoptinae, mange, mites, parrots, wildlife disease.

INTRODUCTION

Mites are the primary cause of parasitic skin and feather disease in birds (Harrison and Lightfoot 2006; Fletcher 2008), having evolved to adapt to different environments and hosts. The most prevalent mites in domestic birds are in the family Epidermoptidae, subfamily Knemidokoptinae (Harrison and Lightfoot 2006). These epidermoptid or skin mites cause mange in a broad avian assemblage, including Passeriformes, Galliformes, and Psittaciformes (Wade 2006). Their morphology and biology resemble the mammalian parasites, Sarcoptidae, with all life stages being present on the host and requiring close contact for transmission (Müllen and Durden 2009).

There are six genera and 17 species in the Knemidokoptinae (Fain and Elsen 1967; Fain 1974; Fain and Lukoschus 1979; Mironov et al. 2005). Historically, the literature has focused on those of significance to poultry and caged birds, including "scaly face" (*Knemidocoptes pilae*; Lavoipierre and Griffiths 1951) in budgerigars (*Melopsittacus undulates*; Yunker and Ishak 1957), depluming itch (*Neocnemidocoptes gallinae* and *Picicnemidocoptes laevis*; Pence 1972) in poultry and pigeons, respectively (Fain and Elsen 1967) and scaly leg (*Knemidocoptes mutans*; Robin and Lanquetin 1859) in

TABLE 1. Avian families commonly infested by the Knemidokoptinae, with species of mites found and area of the body affected during clinical disease. Translated and adapted to reflect current nomenclature (Fain and Elsen 1967).

Host family	Knemidokoptinae species reported	Areas affected
Galliformes	*Knemidokoptes mutans*	Legs
	Neocnemidocoptes gallinae[a]	Body
Columbiformes	*Picicnemidocoptes laevis*[b]	Body
Psittaciformes	*Knemidokoptes pilae*	Beak, legs, and rarely skin of body
	Procnemidocoptes janssensi	Body
Passeriformes	*Knemidokoptes fossor*	Base of beak
	Knemidokoptes jamaicensis	Legs
	Knemidokoptes intermedius	Legs

[a] Formerly reported as *Neocnemidocoptes laevis gallinae*.

[b] Formerly reported as *Neocnemidocoptes laevis laevis*.

poultry (Morishita et al. 2005). Knemidokoptinid mange has been considered uncommon in wild birds (Mainka et al. 1994); however, in the past 15 yr, there has been an increase in reports of these parasites in a range of wild birds (Mainka et al. 1994; Ladds 2009; Dabert et al. 2011; Dabert et al. 2013), particularly Passeriformes (Mason and Fain 1988; Jaensch et al. 2003; Holz et al. 2005; Low et al. 2007; Gaudioso et al. 2009).

Clinical signs of knemidokoptinid mange vary based on the host and parasite species (see Table 1). *Knemidokoptes pilae* infestation, most common in budgerigars, causes hyperkeratotic encrustations on the beak, cere, and legs (Koski 2002) that may affect perching and behavior (Beck 2000) or cause respiratory compromise in advanced cases (Ladds 2009). Severe hyperkeratosis leading to loss of whole digits and feet has been reported in American Robins (*Turdus migratorius*) with *Knemidokoptes jamaicensis* (Turk 1950), including reluctance to feed (Pence et al. 1999). Clinical signs are generally in featherless areas, such as the beak, legs, and feet, and characterized by hyperkeratosis of varying severity (Wade 2006). Proliferative papillary-like lesions (Kirmse 1966; Schulz et al. 1989) may be confused with other diseases, such as avianpox (Kirmse 1966). Exceptions to the hyperkeratotic presentation include depluming itch of fowl and pigeons, where lesions are found on the body and the main clinical symptom is feather loss, not hyperkeratosis (Fain and Elsen 1967).

Knemidokoptinid mange lesions are mostly confined to the stratum corneum, with mild to severe compact orthokeratotic hyperkeratosis and acanthosis (Fletcher 2008). Often tunnels are formed within the crusts and filled with mites (Blackmore 1963; Mason and Fain 1988), lending a honeycombed appearance that may be evident macroscopically. Dermal changes are less common and may include a perivascular mononuclear infiltrate (Mason and Fain 1988; Ladds 2009) or a heterophilic infiltrate (Fletcher 2008). Subcorneal pustules and mites burrowing beneath the stratum corneum have also been reported (Low et al. 2007).

Here, we present findings from an investigation of feather loss in two island populations of Red-crowned Parakeet (RCP; *Cyanoramphus novaezelandiae*), where pathologic changes were linked to a knemidokoptinid mite. We describe normal RCP skin, the pathology and stages of mange in this species, and key features that enabled identification and differentiation of the mite species from similar members of the Knemidokoptinae.

MATERIALS AND METHODS

Location and sampling design

Sampling took place in March and September 2012 on Tiritiri Matangi Island (36°36′

2″S, 174°53′24″E), and June 2013 on Little Barrier Island (LBI; Hauturu-o-Toi; 36°11′32″S, 175°4′29″E) in the Hauraki Gulf of New Zealand. We captured RCP using standard mist nets (9×2.6 m, 30-mm mesh, Avinet, Freeville, New York, USA), placed in single-use cotton bags (Prospectors Earth Sciences, Baulkham Hills, New South Wales, Australia), and taken to a field processing station. Birds were anesthetized with isoflurane at an initial flow rate of 5% in 1-L oxygen, reducing to 1–2% isoflurane in 1-L oxygen after approximately 30 s. A zero dead-space circuit was used to minimize anesthetic risk (Advanced Anesthesia Specialists, Ryde, New South Wales, Australia). Samples were collected for other studies (not reported here), including blood, feathers, and feces. A skin biopsy (3–5 mm) was taken from the caudal aspect of the base of the head, using fine sharp scissors and forceps, and placed in 10% neutral buffered formalin. The site was a common place for feather loss, could be easily standardized across birds, and was in a better location for healing than other areas of the head. In a subset of seven severely affected individuals from Tiritiri Matangi Island, a section of skin was stored frozen in 70% ethanol for extraction and identification of any skin parasites. Biopsy sites were closed with a single suture of 4-0 absorbable suture material (Visorb, SVS, Auckland, New Zealand), and antibacterial ointment applied. Birds were banded using metal and color bands (Department of Conservation, Wellington, New Zealand). No birds were resampled within a sampling session. A standard set of morphometric measurements were collected, and photos of the head (right and left lateral and front) and body (ventral and dorsal) were taken. Birds were allowed to recover in plastic bird boxes and released by the attending veterinarian or nurse. Birds were provided with heating pads during and after anesthesia when appropriate and given 0.5 mL of 0.9% normal saline (NaCl) subcutaneously.

Feather loss

Feather loss was categorized as follows: 0=no feather loss; 1=patchy or focal loss with no coalescing areas; 2=large areas of feather loss <50% of head; 3=>50% feather loss on head or feather loss on ventral neck and body.

Biopsies

Skin biopsies were processed routinely for histopathology (Labtest, Auckland, New Zealand), with three sections of 6 μm stained with H&E and examined under light microscopy. Images were taken using a camera mount for microscopes. Mites were only counted if sections of bodies (idiosoma) were visible. Mites were often visible across multiple sections and easily identified as the same individual based on size, location, and surrounding structures. These were not double counted.

Recovery and processing of mites

Frozen stored skin samples were examined under a dissecting microscope. Mites were recovered by placing skin samples on a glass slide, adding a drop of Hoyer's medium, and dissecting out whole specimens using microtools (Minitools, Australian Entomological Supplies, Coorabell, New South Wales, Australia). Each slide was examined by light microscopy after clearing, with images and measurements for later taxonomic purposes taken of each mite.

Serial section examination

To investigate mite distribution across the body of mange-affected individuals and relative mite abundance (i.e., number of mites per biopsy), a set of serial sections was obtained using a 3-mm punch biopsy from the frozen, stored body of a RCP with mange, found dead in October 2012 on Tiritiri Matangi Island. Sections of skin ($n=10$) were taken from the cheek, ventral and dorsal neck, back, ventral and dorsal aspect of the patagium, lateral body wall over the ribs, abdomen, cloaca, and the skin over the tibiotarsus. These were processed, as described previously for other frozen skin sections.

Mite identification

Mites were identified to subfamily and species based on the criteria described by Fain and Elsen (1967) for the Knemidokoptinae (previously Knemidocoptidae but subsumed as a subfamily in the Epidermoptidae by Mironov et al. 2005). Mites of the Knemidokoptinae are small (<580 μm for females), with short legs and globose or oval bodies, and lacking spines (Fain and Elsen 1967). Unlike many mite species, adult females of the Knemidokoptinae are larviparous, so larval forms are frequently evident in gravid females. Only the female of *Procnemidocoptes janssensi* has been described. Key characteristics that differentiate female *P. janssensi* from the similar *K. pilae* are tarsal appendages on all legs and uninterrupted dorsal striations on the idiosoma. The male,

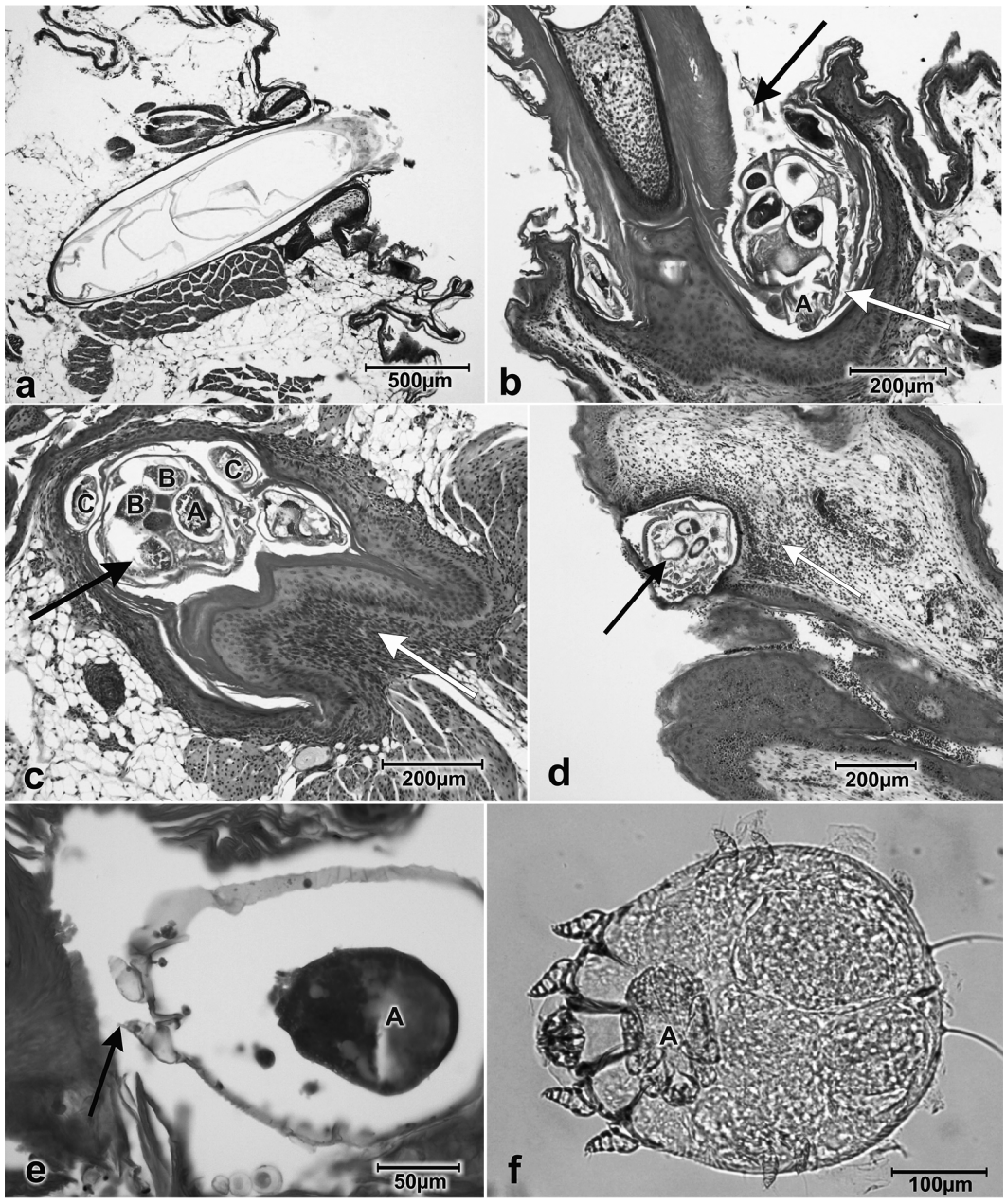

FIGURE 1. Stages of infestation with *Procnemidocoptes janssensi* in Red-crowned Parakeet (*Cyanoramphus novaezelandiae*) skin, including normal skin and a whole mite specimen. a) Normal skin demonstrating the thin epidermis, basket-weave keratin layer, and minimal cellularity to dermis. H&E stain. b) Widening of feather follicle due to presence of adult female mite at base (white arrow), with associated moderate acanthosis and hyperkeratosis. Feces evident (black arrow) behind the mite. Gnathosoma (A) visible with mouthparts. H&E. c) One large adult female mite (black arrow) with chitinous (A) and developing (B) larval forms inside. Body shape and short legs of adult female support identification as knemidokoptinid mite. Larvae are also evident adjacent to the female mite (C). Mites are overlying the dermal papilla (white arrow) of the feather follicle, with moderate surrounding acanthosis and hyperkeratosis. H&E. d) Marked mixed mononuclear and granulocytic diffuse dermal inflammation (white arrow) subjacent to mite penetrating the epidermis (black arrow). Marked acanthosis evident in adjacent epidermis. H&E. e) Pretarsal stalk and

larva, and nymph of *P. janssensi* are currently undescribed, although from our observations, they share many features in common with the other species within the Knemidokoptinae. Larvae have six legs, nymphs have eight legs and lack genitalia, and the adult male has characteristic genitalia, including an aedeagus.

Beak and feather disease virus screening

DNA was extracted from feather and blood samples using the iGenomic blood DNA extraction kit, with PCR performed for beak and feather disease virus (BFDV) using primers that target a ~605-base pair region of the replication associated gene, as previously described (Julian et al. 2012, 2013).

RESULTS

Sampling

In total, 142 birds were caught and sampled for the broader study between 2012 and 2013 on Tiritiri Matangi Island and LBI (Hauturu-o-Toi). Skin biopsies examined for mites were obtained from 135 birds.

Normal RCP skin

There were 21 skin samples without mites or other changes. Normal RCP skin had a thin epidermis, 1–2 cell layers thick, with a basket-weave keratin layer of 3–5 layers, and minimal cellularity to the dermis (Fig. 1a).

Feather loss

Feather loss was observed in 47/97 (49%; 95% confidence interval [CI]: 38.2–58.8%) of RCP on Tiritiri Matangi Island, and 0/45 (0%; 95% CI: 0–7.9%) of RCP on LBI (Hauturu-o-Toi). Overall prevalence of feather loss for both islands was 47/142 (33%, 95% CI: 25.4–41.5%). Feather loss ranged in severity from mild periocular changes to loss of most feathers on the head, ventral neck, and sternum (Fig. 2). Encrustations of the legs, beak, or cere were not observed in the RCP examined. In some birds, featherless areas were mildly scaly, pigmented, or lichenified. There were eight individuals with feather loss in which mites were not found; however, there was hyperkeratosis and acanthosis suggestive of mange.

Histopathologic findings—general

Prevalence and range data for mites found in skin biopsies are presented in Table 2. Mites were significantly more likely to be located intrafollicularly in these birds ($P<0.0001$, z-test). Mite numbers in skin biopsies were strongly positively skewed in the density histogram (Fig. 3). Mite numbers in biopsies correlated to severity of feather loss, with the odds of having grade 3 feather loss significantly associated with >3 mites present in a skin biopsy (odds ratio [OR]=6.2; 95% CI=1.74–22.05; $P=0.004$, Fisher's exact test).

Hyperkeratosis and acanthosis were present in the majority of cases where mites or feather loss were noted, and were significantly associated with these explanatory variables (Table 3). Inflammatory changes in the dermis ranged from perivascular to regionally extensive infiltrates involving predominantly mixed mononuclear cell lines with occasional granulocytic dominance. There was, however, no significant relationship between these changes and the presence of mites (OR=0.97; 95% CI=0.46–2.07; $P=0.9$).

Histopathologic findings—mite stages and features

Small mites of a size consistent with larvae were found at the epidermal junction of the feather follicle in most cases (except for two mites), with varying responses of mild underlying acanthosis and hyperkeratosis, epidermal atrophy, or spongiosis. Individual mites appeared to

←
pulvillus (arrow) evident on second leg of adult female mite, definitive for *P. janssensi* compared with other knemidokoptinids. Female mite has developing embryo (A). H&E. f) Cleared whole adult female mite with identifying features of *P. janssensi*, including chitinous larval form inside (A). Hoyer's medium.

FIGURE 2. Red-crowned Parakeets (*Cyanoramphus novaezelandiae*) with feather loss on Tiritiri Matangi Island, New Zealand. Female with grade 3 feather loss affecting >50% of head and mild scale formation (a), and male with grade 3 feather loss, ventral neck and keel severely affected (b).

be in the process of moving down the feather shaft toward the base of the follicle (Fig. 1b). Mites of all sizes, including gravid females, were observed in compact hyperkeratotic plugs at the base of feathers, overlying the dermal papilla and often widening the feather follicle itself, with acanthosis of the feather follicle epithelium (Fig. 1b).

Mites of all sizes were observed extrafollicularly, including gravid females, with compact orthokeratotic hyperkeratosis and acanthosis in the adjacent epidermis. Some mites were found free floating next to skin sections. Extrafollicular mites were also seen in small groups forming a compact hyperkeratotic plug within the epidermis (Fig. 1c). A marked diffuse inflammatory

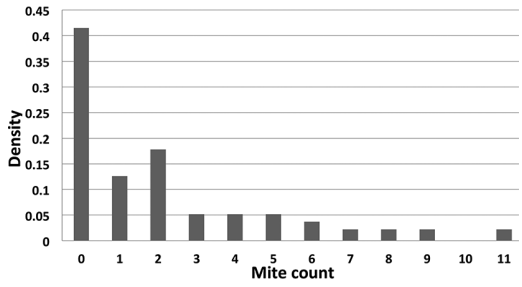

FIGURE 3. Density histogram for mite counts from skin biopsies of Red-crowned Parakeets (*Cyanoramphus novaezelandiae*) on Tiritiri Matangi Island and Little Barrier Island (Hauturu-o-Toi), New Zealand, 2012–13.

infiltrate of both granulocytic and mononuclear cells was seen in the dermis on the rare occasion that mites were found penetrating the epidermis (see Fig. 1d).

Sections of mites in skin were often longitudinal or transverse, enabling reasonable size and shape inferences; thus, in most cases identification of life stage and mite family could be determined. Key characteristics typical of the Knemidokoptinae were observed, such as larval forms within adult females and cross sections, demonstrating short legs relative to round bodies (Fig. 1b, c). Rarely, the pretarsal stalk and pulvillus characteristic of *P. janssensi* females was observed, which in combination with other features mentioned, enabled specific identification (Fig. 1e). Feces were regularly seen in sections and appear as round, pink clumps of keratin surrounded by a sheath (Fig. 1b).

Whole mite results

Results from examination of whole mites retrieved from frozen stored biopsies of seven birds (Table 4) demonstrate the dominance of *P. janssensi*. During dissection, mites were observed along the feather shaft, within hyperkeratotic plugs in the epithelium, or at the base of feathers, consistent with the histopathologic findings.

Adult females had defining features of the Knemidokoptinae described in the Materials and Methods section (Fig. 1f). Larval, nymph, and nongravid female mites were seen in all sections, with features consistent with the Knemidokoptinae, generally, and the adult female *P. janssensi*, specifically. Features were consistent across the mites found, supporting identification by association, despite lacking a formal description in the literature for the subadult stages of *P. janssensi*.

Examination of whole mites from serial sections of an opportunistically obtained dead bird with mange demonstrated large numbers of *P. janssensi* across the entire body (Table 5), despite clinical signs of feather loss being restricted to the head and ventral neck in this individual. Only one male was found from 245 *P. janssensi* examined (0.4%; 95% CI=0–2.3%). This, together with other material obtained in this study, provides the first specimens of the larva, nymph, and male of *P. janssensi*.

Of all mites recovered and examined from skin sections, including frozen skin (n=92) and the serial sections (n=153), 245/250 (98%; 95% CI=95.4–99.3%) were definitively identified as *P. janssensi*. The others were feather mites (Analgoidea; n=4) and a single unidentified epidermoptid, possibly *Myialges (Promyialges) macdonaldi* (Evans, Fain, and Bafort 1963),

TABLE 2. Prevalence (95% confidence interval [CI]) and range data for *Procnemidocoptes janssensi* mites in skin biopsies of Red-crowned Parakeets (*Cyanoramphus novaezelandiae*) in New Zealand, including locations of mites found. "Overall mites" indicates proportion of skin biopsies (n=135) with mites observed, whereas intrafollicular and extrafollicular mites are expressed as a proportion of mite positive biopsies (n=79).

	x/*n* (biopsies)	Prevalence (%)	95% CI (%)	Range
Overall mites	79/135	58.5	49.7–66.6	1–11
Intrafollicular mites	68/79	86	76.5–92.8	1–9
Extrafollicular mites	37/79	46.8	35.5–58.4	1–6

TABLE 3. Odds ratios (OR), 95% confidence interval (CI), and Fisher's exact *P*-value for relationships between outcome of combined hyperkeratosis/acanthosis and feather loss, mites, or feather loss and mites combined for Red-crowned Parakeets (*Cyanoramphus novaezelandiae*) in New Zealand, 2012–13.

Outcome of hyperkeratosis/acanthosis	OR	95% CI	*P* value
Mites in biopsy	8.93	4–19.91	<0.001
Feather loss	5.48	2.51–11.95	<0.001
Feather loss and mites in biopsy	31	9.23–104.14	<0.001

which has been found on Hippoboscidae associated with RCP (Heath 2010).

BFDV results

Two birds were positive for BFDV between 2012 and 2013; however, neither had feather loss. Mite counts were zero and five for these two birds.

DISCUSSION

Our results demonstrate a clear association between the presence of mites in skin biopsies and histopathologic changes of hyperkeratosis and acanthosis. These findings are consistent with a form of avian mange, although gross manifestations of feather loss varied, suggesting a host-parasite-environment relationship that dictates the likelihood of developing clinical disease. Carrier states and a spectrum of host effects are common findings in populations affected by mange, both avian (Yunker 1955; Blackmore 1963) and mammalian (O'Brien 1999; Kolodziej-Sobocińska et al. 2014). Relative abundance measures of mite burden revealed an aggregated, overdispersed pattern typical of macroparasite frequency distributions (Shaw et al. 1998), where the majority of birds had 0–3 mites, and few birds harbored 4–11 mites per skin biopsy. Given almost all whole mites examined from a subset of birds with clinical mange were *P. janssensi*, as well as histopathologic evidence of knemidokoptinid mites at varying stages of infestation of both feather follicles and the skin, the evidence strongly supports the correlation between this mite and the clinical mange observed. There is no information on the breeding biology and ecology of *P. janssensi*, and it can only be assumed that like other knemidokoptinids, it spends its entire life cycle on the host and requires close contact for transmission. Likewise, it is unknown whether RCP are natural hosts for this species or are infected through spillover events from sympatric species, such as the Eastern Rosella (*Platycercus eximius*) or even other avian families.

Procnemidocoptes janssensi has not been reported since the original description of a single nongravid female mite

TABLE 4. Species, stage, and total numbers of mites found in skin biopsies from the back of the head of seven Red-crowned Parakeets (*Cyanoramphus novaezelandiae*) during cross-sectional study on Tiritiri Matangi Island, New Zealand, 2012–13. NA = not applicable.

	Procnemidocoptes janssensi					
Bird No.	Gravid females	Nongravid females and nymphs	Larvae	Total	*n* (histopath)	Other mites (*n*)
1	6	11	12	29	5	—
2	3	4	6	13	2	—
3	1	4	10	15	8	Feather mite (2)
4	0	1	0	1	1	—
5	0	0	4	4	2	—
6	1	5	16	22	4	Epidermoptidae (1)
7	1	1	6	8	NA	—

TABLE 5. Adult, nymph, and larval forms of *Procnemidocoptes janssensi* recovered from serial 3-mm biopsy sections of skin from the body of a deceased New Zealand Red-crowned Parakeet (*Cyanoramphus novaezelandiae*) with mange.

Section	*Procnemidocoptes janssensi*				Other mites (*n*)
	Gravid females	Nongravid females and nymphs	Larvae	Total	
Cheek	2	8	4	14	—
Dorsal neck	7	11	10	28	—
Ventral neck	1	0	1	2	—
Abdomen	1	2	10	13	—
Back	3	4	6	13	Feather mite (1)
Lateral body	0	0	0	0	—
Dorsal patagium	6	2	26	34	Feather mite (1)
Ventral patagium	0	0	0	0	—
Cloaca	3	2	32	37	—
Tibiotarsus	3	7	2	12	—

from a Black-cheeked Lovebird (*Agapornis nigrigenis*) that died several days after being imported to Belgium from Zambia in 1966 (Fain 1966). Without knowing the provenance of the bird (wild or captive) or how the bird was housed on arrival in Belgium, it is not possible to confirm the mite is from Zambia, nor from the wild. No confirmed reports of knemidokoptinid mites in wild parrots were found during a literature search. Most knemidokoptinids reported in captive parrots are budgerigars infested with *K. pilae* (Lavoipierre and Griffiths 1951; Oldham and Beresford-Jones 1954; Newton and O'Sullivan 1956; Blackmore 1963; Rao et al. 1967). Reports of knemidokoptinids in captive parrots other than budgerigars are uncommon (Oldham and Beresford-Jones 1954; Garrett and Haramoto 1967; Schultz 1978). A knemidokoptinid morphologically different from *K. pilae* (species undescribed) was found in association with feather loss of the head, neck, wings, and thighs in 71% (95% CI=52.5–84.9%) of a group of captive RCP imported from Europe to Israel (Shoshana 1993). The clinical similarities between the infested RCP in this study and the report by Shoshana (1993) suggest these mites could have been *P. janssensi*, although original specimens would be needed to confirm this.

Reports of knemidokoptinid mites in parrots of New Zealand include *K. pilae* from captive budgerigars (O'Grady 1960), and a captive Yellow-crowned Parakeet (*Cyanoramphus auriceps*) with beak and cere lesions. This latter record was initially ascribed to an RCP host (Bishop and Heath 1998), but a later investigation confirmed the error. Knemidokoptinid mange was found in 19 wild-caught but captive-held Eastern Rosellas with scaly lesions of the head and legs (Gartrell et al. 2003). It is unknown if the birds acquired the infestation in captivity or the wild or which knemidokoptinid species caused the lesions. Morphologic similarities between *P. janssensi* and *K. pilae*, coupled with a general lack of available expertise for morphologic identification of parasites, could possibly have led to an underreporting or misidentification of *P. janssensi* to date in New Zealand and globally.

Epidermoptidae and Knemidokoptinae appear to have adapted to specific microhabitats on hosts (Dabert et al. 2011), as well as to certain host families (Fain and Elsen 1967), and this governs the histopathologic and clinical response that will be observed. *Knemidokoptes pilae* in Psittaciformes is found on unfeathered skin, often causing severe hyperkeratotic beak, cere, and leg lesions (Wade 2006), although a histopathologic study of the stages of infestation noted presence of mites in feather follicles of the face (Yunker and Ishak 1957). *Knemidokoptes*

mutans causes similar encrustations restricted to the legs and feet in Galliformes, as do *K. jamaicensis* and *Knemidocoptes intermedius* in Passeriformes (Dabert et al. 2011). *Picicnemidocoptes laevis* and *N. gallinae* are associated with feather loss on the body of Columbiformes and Galliformes, respectively (Fain and Elsen 1967; Rajabzadeh et al. 2008). The distribution and nature of the lesions in RCP from this study suggests *P. janssensi* behaves differently than *K. pilae* and, in fact, produces a clinical syndrome more akin to the depluming mites *N. gallinae* and *P. laevis*. Recent research examining morphologic variation in combination with molecular barcoding of *K. jamaicensis* has suggested this species may be a multispecies complex exploring a wide host range (Dabert et al. 2013). The potential for multispecies complexes and cryptic host and geographic specificity needs further investigation in *P. janssensi* and other knemidokoptinids by using morphologic and molecular techniques.

Our major histopathologic findings in association with mites were hyperkeratosis and acanthosis, which are nonspecific changes that may be associated with a range of etiologic agents, including parasites, viruses (Raidal 1995), and bacterial or fungal disease (Fletcher 2008). In this study, no other etiologic agents were identified in skin sections, and the observed changes were underlying or adjacent to parasites. Screening of blood and feathers from all birds ruled out BFDV as a causative agent, supported by the lack of histopathologic changes expected with this infection (Pass and Perry 1984; Fletcher 2008). A larger sample of BFDV-positive birds would be necessary to infer any relationship between infection with this virus and mite numbers.

Obtaining biopsies of all birds was critical to detect the range of pathology related to the mite, the stages of mite infestation, and to provide baseline descriptions of normal skin. Mites found at the base of feather follicles likely break the keratin bridges that play a significant role in attachment and strength of the feather (Fletcher 2008) as they move down the feather to this position. Feathers were often absent when mites were located at the base of a feather, and the observed widening of the base may interfere with new feather growth. It appears mites feed on keratin, given the appearance of eosinophilic feces in section, and in line with other Knemidokoptinae (Fain and Elsen 1967). We observed a range of dermal inflammatory changes; however, they were not significantly correlated with the presence of mites, although occasionally mites invading the epidermis led to an influx of both granulocytic and mononuclear cell lines. The absence of an association between dermal inflammatory changes and mites correlates with other studies of knemidokoptinid mange in avian species (Mainka et al. 1994) and demonstrates the value of a large cross-sectional study that includes noncases for comparative purposes.

As *P. janssensi* was predominantly found in feather follicles, skin scrapings used to detect knemidokoptinid mites in other species and studies (Dabert et al. 2011) are unlikely to be as sensitive, although this was not evaluated in this study. Issues of detection sensitivity for Knemidokoptinae using skin scrapes have been reported (Low et al. 2007), highlighting the importance of obtaining the right specimen based on the biology of the mite species involved. Shoshana (1993) successfully used 25 plucked feathers to look for mites in RCP with feather loss. Although this may have worked in the current study, it would not have allowed a description of the histopathologic changes associated with the presence of the mites, nor provided as many whole mites for identification as the biopsy method. However, there are recognized limitations to skin biopsies as they give information on a single point in a temporally dynamic disease process. It is possible to either miss the key lesion, or capture a developing or regressing lesion at

a point in which the inciting cause is no longer present (Sleiman et al. 2013). A larger biopsy or multiple biopsies may have provided more information on mite abundance and associated pathology and improved detection sensitivity. Standardization of the biopsy site meant it was not always possible to sample the recommended leading edge of a lesion (Seltzer 2007). A further limitation of biopsies is the need for appropriate chemical restraint, which, while necessary from a welfare perspective, adds additional logistic and personnel requirements to field studies. In this study however, the use of general anesthesia enabled a wide range of sampling and examinations with minimal stress to the animal, a level of investigation currently uncommon in wild bird studies of mange.

Whole mites from frozen skin sections were critical to accurately identify *P. janssensi*, assess relative abundance, and identify and enumerate other species or groups of mites present in RCP skin. Although features of the Knemidokoptinae were observed in histopathologic sections, it was extremely rare to find a feature such as a tarsal appendage on a female mite to distinguish them from *K. pilae*, the other common species in parrots. We found only one male mite. This may reflect a species that is parthenogenetic, a technique bias, or a genuine low relative abundance, with male mites of the Knemidokoptinae being difficult to detect (Oldham and Beresford-Jones 1954; Newton and O'Sullivan 1956).

We describe a knemidokoptinid mange outbreak in wild parrots and provide histopathologic evidence of the stages of infestation and guidance on methods for identifying mites in sections. Wildlife disease research is increasingly common, however, particularly where parasites are involved, such studies may be hampered by a lack of available expertise in morphologic identification, pending the more widespread availability of molecular tools. Further studies should focus on the epidemiology of disease expression and the biology of the mites. We also recommend targeted sampling of all wild parrot species in New Zealand to determine host species and geographic distribution of *P. janssensi*, including remote islands that may provide insight into whether the mite is a native parasite of parrots in this region or has been introduced to the wild through release of captive birds. It is unknown what impact this parasite may have on the conservation status and long-term viability of RCP and the broader parrot community in New Zealand. Given the predicted expression of disease in relation to adverse environmental and resource conditions, mange prevalence may act as a barometer for overall health in wild populations at a given time.

ACKNOWLEDGMENTS

We thank the following organizations for providing funds or in-kind support for this work: Auckland Zoo Conservation Fund, Murdoch University Research and Development Fund, Supporters of Tiritiri Matangi (SoTM), Forest and Bird New Zealand (JS Watson Fund), the Department of Conservation, and 360 Degrees Ferries. Steve Lawrence, aviculturalist, Outram, New Zealand, is thanked for clarifying the origin of Yellow-crowned Parakeets (*Cyanoramphus auriceps*) from which *Knemidocoptes pilae* were collected and originally erroneously ascribed to a Red-crowned Parakeet (*Cyanoramphus novaezelandiae*) host. Field support was generously provided through volunteers from the following organizations: Auckland Zoo, Massey University, SoTM, Department of Conservation and the Auckland Regional Council. All work was carried out in accordance with institutional guidelines and permission from the Murdoch University Animal Ethics Committee (permit: W2390/11) and the Auckland Zoo Animal Ethics Committee.

LITERATURE CITED

Beck W. 2000. Schnabelräude durch *Knemidocoptes pilae* (Acaridida: Knemidocoptidae) beim Wellensittich - Erregerbiologie, Pathogenese, Klinik, Diagnose und Therapie. *Kleintierpraxis* 45:453–456.

Bishop D, Heath A. 1998. Special issue: Parasites of birds in New Zealand—Checklist of ectoparasites. *Surveillance* 25:13–31.

Blackmore D. 1963. Some observations on *Cnemidocoptes pilae*, together with its effect on the budgerigar (*Melopsittacus undulatus*). *Vet Rec* 75:592–594.

Dabert J, Dabert M, Gal AF, Miclâus V, Mihalca AD, Sándor AD. 2013. Multidisciplinary analysis of *Knemidocoptes jamaicensis* parasitising the Common Chaffinch, *Fringilla coelebs*: proofs for a multispecies complex? *Parasitol Res* 112:2373–2380.

Dabert J, Mihalca AD, Sándor AD. 2011. The first report of *Knemidocoptes intermedius* Fain et Macfarlane, 1967 (Acari: Astigmata) in naturally infected European birds. *Parasitol Res* 109:237–240.

Fain A. 1966. *Procnemidocoptes janssensi* g.n., sp. n. et *Neocnemidocoptes* g.n. Remaniement des Knemidokoptidae, parasites cuticoles des Oiseaux (Acarina, Sarcoptiformes). *Rev Zool Bot Afr* 73:390–396.

Fain A. 1974. Notes sur les Knemidokoptidae avec description de taxa nouveaux. *Acarologia* 16:182–188.

Fain A, Elsen P. 1967. Les acariens de la famille Knemidocoptidae producteurs de gale ches les oiseaux. *Acta Zool Pathol Antverp* 47:1–145.

Fain A, Lukoschus F. 1979. Two new parasitic mites (Acari, Astigmata) from the skin of Australian vertebrates. *Mitt Hambg Zool Mus Inst* 76:387–393.

Fletcher OJ. 2008. *Avian histopathology*. 3rd Ed. American Association of Avian Pathologists, Raleigh, North Carolina, 438 pp.

Garrett L, Haramoto F. 1967. A catalog of Hawaiian Acarina. *Proc Hawaiian Entomol Soc* 19:381–414.

Gartrell B, Stone M, King C, Wang J. 2003. An outbreak of disease caused by psittacinepoxvirus in rosellas. *Surveillance* 30:11–13.

Gaudioso JM, Lapointe DA, Hart PJ. 2009. Knemidokoptic mange in Hawaiian Amakihi (*Hemignathus virens*) on the island of Hawaii. *J Wildl Dis* 45:497–501.

Harrison G, Lightfoot T. 2006. *Clinical avian medicine*. Spix Publishing, Palm Beach, Florida, 1008 pp.

Heath A. 2010. Checklist of ectoparasites of birds in New Zealand: Additions and corrections. *Surveillance* 37:12–17.

Holz P, Beveridge I, Ross T. 2005. *Knemidocoptes intermedius* in wild superb lyrebirds (*Menura novaehallandiae*). *Aust Vet J* 83:374–375.

Jaensch SM, Raidal SR, Hobbs R. 2003. *Knemidocoptes intermedius* in a wild currawong (*Strepera graculina*). *Aust Vet J* 81:411.

Julian L, Lorenzo A, Chenuet J-P, Bonzon M. 2012. Evidence of multiple introductions of beak and feather disease virus into the Pacific islands of Nouvelle-Caledonie (New Caledonia). *J Gen Virol* 93:2466–2472.

Julian L, Piasecki T, Chrzastek K, Walters M. 2013. Extensive recombination detected among beak and feather disease virus isolates from breeding facilities in Poland. *J Gen Virol* 94:1086–1095.

Kirmse P. 1966. Cnemidocoptic mite infestations in wild birds. *Bull Wildl Dis Assoc* 2:86–99.

Kolodziej-Sobocińska M, Zalewski A, Kowalczyk R. 2014. Sarcoptic mange vulnerability in carnivores of the Białowieża Primeval Forest, Poland: Underlying determinant factors. *Ecol Res* 29:237–244.

Koski MA. 2002. Dermatologic diseases in psittacine birds: An investigational approach. *Semin Avian Exot Pet* 11:105–124.

Ladds P. 2009. *The pathology of Australian native wildlife*. Commonwealth Scientific and Industrial Research Organisation Publishing, Collingwood, Australia, 640 pp.

Lavoipierre M, Griffiths R. 1951. A preliminary note on a new species of *Cnemidocoptes* (Acarina) causing scaly leg in a budgerigar (*Melopsittacus undulatus*) in Great Britain. *Ann Trop Med Parasit* 45:253–254.

Low M, Alley MR, Scott I. 2007. Pruritic facial dermatitis in a population of free-living stitchbirds. *J Wildl Dis* 43:262–268.

Mainka SA, Melville DS, Galsworthy A, Black SR. 1994. *Knemidocoptes* sp. on wild passerines at the Mai Po Nature Reserve, Hong Kong. *J Wildl Dis* 30:254–256.

Mason R, Fain A. 1988. *Knemidocoptes intermedius* identified in forest ravens (*Corvus tasmanicus*). *Aust Vet J* 65:260.

Mironov SV, Bochkov AV, Fain A. 2005. Phylogeny and evolution of parasitism in feather mites of the families Epidermoptidae and Dermationidae (Acari: Analgoidea). *Zool Anz–J Comp Zool* 243:155–179.

Morishita T, Johnson G, Johnson G, Thilsted J, Promsopone B, Newcomer C. 2005. Scaly-leg mite infestation associated with digit necrosis in bantam chickens (*Gallus domesticus*). *J Avian Med Surg* 19:230–233.

Müllen G, Durden L. 2009. *Medical and veterinary entomology*. Elsevier, Amsterdam, The Netherlands, 637 pp.

Newton LG, O'Sullivan PJ. 1956. A note on the occurrence of the mite *Cnemidocoptes pilae* on budgerigars in Queensland. *Aust Vet J* 32:89–90.

O'Brien DJ. 1999. Treatment of psoroptic mange with reference to epidemiology and history. *Vet Parasitol* 83:177–185.

O'Grady B. 1960. Knemidokoptic mange of the budgerigar (*Melopsittacus undulatus*) in New Zealand. *N Z Vet J* 8:118.

Oldham J, Beresford-Jones W. 1954. Observations on the occurrence of *Cnemidocoptes pilae* in Budgerigars and a parakeet. *Br Vet J* 110: 29–30.

Pass DA, Perry RA. 1984. The pathology of psittacine beak and feather disease. *Aust Vet J* 61:69–74.

Pence DB, Cole RA, Brugger KE, Fischer JR. 1999. Epizootic podoknemidokoptiasis in American robins. *J Wildl Dis* 35:1–7.

Raidal SR. 1995. Viral skin diseases of birds. *Semin Avian Exot Pet* 4:72–82.

Rajabzadeh M, Razavi SM, Homayounimehr AR. 2008. Depluming itch in native fowls in the Iran, a potential risk for commercial chickens. *Trop Anim Health Prod* 40:1–3.

Rao S, Ghafoor M, Narsapur V. 1967. *Cnemidocoptes pilae* (Lavoipierre and Griffiths 1951) a causative agent of scaly leg in Budgerigar (*Melopsittacus undulatus*) in India together with the description of the larval forms. *Indian Vet J* 44: 206–208.

Schultz D. 1978. Diseases of parrots. *Univ Syd Proc Post Grad Comm Vet Sci* 36:223–245.

Schulz T, Stewart J, Fowler M. 1989. *Knemidokoptes mutans* (Acari: Knemidocoptidae) in a great-horned owl (*Bubo virginianus*). *J Wildl Dis* 25:430–432.

Seltzer J. 2007. Skin biopsies in mammals. *Lab Anim* 36:23.

Shaw D, Grenfell B, Dobson A. 1998. Patterns of macroparasite aggregation in wildlife host populations. *Parasitology* 117:597–610.

Shoshana R. 1993. Knemidokoptes mites on feathers of the red fronted parakeet. In: *Proceedings of the European cconference on avian medicine and surgery*, Utrecht, The Netherlands, 3–6 March, pp. 329–331.

Sleiman R, Kurban M, Abbas O. 2013. Maximizing diagnostic outcomes of skin biopsy specimens. *Int J Dermatol* 52:72–78.

Wade L. 2006. Knemidocoptiasis in birds. *Vet Med* 101:782–790.

Yunker CE. 1955. Apparent extrinsic variation in *Knemidokoptes pilae* Lavoipierre and Griffiths, 1951 (Acarina:Sarcoptiformes). *J Parasitol* 41:642–643.

Yunker CE, Ishak KG. 1957. Histopathological observations on the sequence of infection in Knemidokoptic mange of budgerigars (*Melopsittacus undulatus*). *J Parasitol* 43:664–672.

Submitted for publication 20 October 2014.
Accepted 2 February 2015.