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CRYPTIC HOST SPECIFICITY OF AN AVIAN SKIN MITE (EPIDERMOPTIDAE) VECTORED BY LOUSEFLIES (HIPPOBOSCIDAE) ASSOCIATED WITH TWO ENDEMIC GALÁPAGOS BIRD SPECIES

Noah Kerness Whiteman, Pablo Sánchez*, Jane Merkel†, Hans Klompen‡, and Patricia G. Parker†
Harvard University Museum of Comparative Zoology, 26 Oxford Street, Cambridge, Massachusetts 02138. *e-mail: nwhiteman@oeb.Harvard.edu*

ABSTRACT: Host specificity of vectors is an important but understudied force shaping parasite evolution and the relationship between hosts and parasites. Low vector specificity may allow a vectored parasite to invade new host species, whereas high specificity of vectors may reduce the host range of the parasite and favor specialization. The 'generalist' and widely distributed avian skin mite *Myialges caulotoon* Speiser (Acari: Epidermoptidae) is unusual because females require an insect vector to complete their life cycle. *Myialges caulotoon* was previously reported from 2 lousefly (Diptera: Hippoboscidae) species, *Olfersia sordida* and *Icosta nigra*, parasitizing flightless cormorants (*Phalacrocorax harrisi*) and Galápagos hawks (*Buteo galapagoensis*), respectively, within the Galápagos Islands. This is a surprising distribution, given that the 2 lousefly species involved are relatively host-specific. Mitochondrial DNA sequences revealed 2 reciprocally monophyletic *Myialges* clades that sorted out perfectly with respect to their vector species, regardless of whether they were in allopatry or sympatry. One clade was restricted to flies of hawks and the other to flies of cormorants. Females of the 2 *Myialges* groups were also separated consistently by the shape of the sternal surface sclerotization. Mites of hawk flies were more abundant than those of cormorant flies. Within the *Myialges* clade associated with hawks, genetic differentiation between 2 island populations mirrored its host's patterns of differentiation.

The role of host specificity of vectors in influencing the evolution of the parasites and pathogens they transmit is of broad interest, but remains enigmatic for most host–vector–parasite complexes, particularly in nonhuman systems (Fonseca et al., 2004). For example, although many studies examining prevalence and host specificity of vector-borne parasites and pathogens of birds explain their findings in light of the presence/absence, relative abundance, or host specificity of suitable vectors (e.g., Sehgal et al., 2001, 2005; Fallon et al., 2003; Ishtiaq et al., 2006), rarely are vector data collected during the study (for important exceptions, see Sol et al., 2000; Votýpka et al., 2002; Garvin and Greiner, 2003; Freed et al., 2005).

Parasites tend to be morphologically conservative relative to their hosts. Generally, this conservatism has resulted in use of host relationships to define parasite species limits (Eichler, 1948), and conversely has led to the artificial grouping of cryptic parasite species, including protozoans (Perkins, 2000), helminths (Hung et al., 1999; Criscione and Blouin, 2004), lycaenid butterflies (Als et al., 2004), chewing lice (Johnson, Williams et al., 2002; Whiteman et al., 2004), and ticks (McCoy et al., 2005). Fortunately, molecular genetic data have provided additional characters with which to evaluate relationships among parasites (e.g., McManus and Bowles, 1996; Johnson, Weckstein et al., 2002; Besansky et al., 2003).

In addition to its utility in differentiating among parasite species, molecular-genetic characterization of parasite lineages and populations also reveals information about the processes driving parasite diversification (Hebert et al., 2004; Criscione and Blouin, 2005; Whiteman and Parker, 2005). Many parasite species have complex life cycles involving intermediate hosts or free-living stages; these dynamics influence their population genetic structure and phylogenetic history (Nadler, 1995). Despite this, few studies have examined the molecular ecology of parasites (Criscione and Blouin, 2005).

Members of the mite subfamily Epidermoptinae within the Epidermoptidae (Acari: Astigmata) burrow into the upper skin layers of their avian hosts and cause dermatitis and mange, which may lead to host mortality, as reported in endangered seabird species (Evans et al., 1963; Fain, 1965; Greve, 1984; Gilardi et al., 2001). Individuals of most epidermoptid species are not vectored by other arthropods and most species are presumed to be highly host specific, based on morphological characters (Fain, 1965; Mironov et al., 2005). Female individuals of *Myialges* and *Microlichus* spp., however, must attach to ectoparasitic insects to successfully reproduce (Fain, 1965). Gravid females of *Myialges* and *Microlichus* spp. are exclusively found attached to louseflies or, less often, to lice, of birds (Fain, 1965). Inseminated adult female mites move from the bird onto the insect, to which they become permanently attached. Female mites oviposit while attached to the insect and eggs are anchored to the host insect's cuticle. The eclosed immature mites disperse from the insect vector onto an avian host to complete development where they feed on bird epidermal tissue and body fluids (Evans et al., 1963). Immature adult male and nongravid female mites live in the avian host's skin and have not been found on insects (Fain, 1965). Thus, the insects are cyclodevelopmental vectors for these 2 skin mite genera. The life cycle of *Myialges* sp. is depicted in Figure 1.

Species of *Myialges* and *Microlichus* are thought to lack host specificity, which ostensibly reflects the low specificity of their insect vectors. Another explanation for the apparent lack of specificity, however, is morphological conservatism. Currently named ''species'' of mites could in fact be species complexes in which genetically differentiated and host-specific lineages retain highly similar adult morphology. Certainly in other groups of parasites, previously defined ''generalist'' parasites have been shown to consist of host races or cryptic species (McManus and Bowles, 1996; McCoy et al., 2005).

Myialges caulotoon Speiser is a mite that has been reported from at least 9 hippoboscid species collected in association with at least 8 avian host species, comprising 3 avian orders and 5

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* Escuela de Biología, Pontifícia Universidad Católica del Ecuador, Quito, Ecuador.
† Saint Louis Zoo, One Government Drive, St. Louis, Missouri 63110.
‡ Department of Entomology, Ohio State University, 1315 Kinnear Road, Columbus, Ohio 43212.

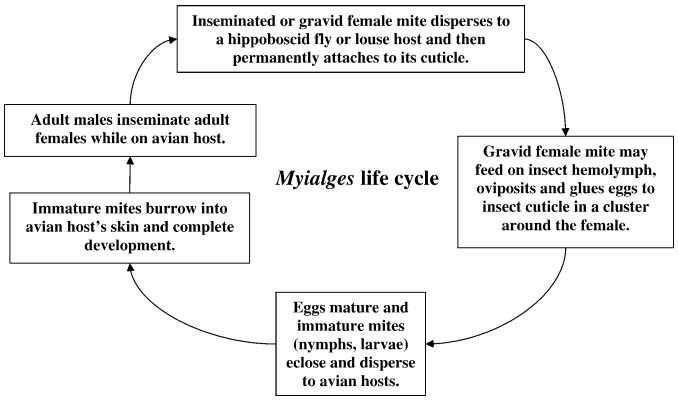

FIGURE 1. Life cycle of *Myialges* sp. mites (after Evans et al., 1963), which alternates between hippoboscid fly or louse hosts and avian hosts.

families (Table I). During a survey of parasitic mites from the Galápagos Islands (Harmon et al., 1990), hippoboscid flies were collected from Galápagos hawks (Falconiformes: Accipitridae: *Buteo galapagoensis*) on Isla Santiago, and from flightless cormorants (Pelecaniformes: Phalacrocoracidae: *Phalacrocorax harrisi*) and brown pelicans (Pelecaniformes: Pelecanidae: *Pelecanus occidentalis*) on Isla Fernandina. Mites found on flies from hawks (*Icosta nigra*) and the 2 pelicaniform birds (*Olfersia sordida*) were identified as *Myialges caulotoon* by A. Fain (Institut Royal des Sciences Naturelles de Belgique), the world authority on Epidermoptidae. However, Madden and Harmon (1998) noted that the Galápagos specimens differed morphologically from the type specimens (Fain, 1965) and that ''other less detectable morphological differences exist between this population and those previously described. The Galápagos population may thus be a clinal or ecotypic population of *M. caulotoon*.'' In fact, Phillips and Fain (1991) suggested that *M. caulotoon* may be a complex of species. Even so, Madden and Harmon (1998) suggested that ''*M. caulotoon* might not be a very good indicator of host phylogeny or ecology because it occurs on at least 2 hippoboscid species and 3 unrelated bird species with at least 2 very different niches in Galapagos.'' However, only 1 *M. caulotoon* individual was collected and examined from the *O. sordida* flies associated with flightless cormorants. The taxonomy of the species is confused (Speiser, 1907; Ferris, 1928; Thompson, 1936; Dubinin, 1953; Fain, 1965). For example, Fain (1965) listed 11 characters (10 of which are continuous morphological characters, e.g., ''Legs I longer'') by which *M. caulotoon* could be distinguished from other *Myialges* species. Only females of *M. caulotoon* are known; however, males are typically needed to confirm species limits in these mites (Phillips and Fain, 1991).

Despite the apparent broad geographic and host range of *M. caulotoon* (Fain, 1965; Madden and Harmon, 1998), several points suggest that cryptic host specificity may be present and detectable within the species. First, dispersal of *Myialges* sp. mites among avian hosts is likely to be largely restricted to

TABLE I. Published reports of lousefly species host to the avian skin mite (Epidermoptidae) *Myialges caulotoon* Speiser (sensu Fain, 1965) and the avian hosts from which the flies were collected.

Lousefly host (Hippoboscidae)	Avian host	Locality	Reference
Icosta ardeae (Macquart)	*Ardea purpurea* (Ciconiiformes, Ardeidae)	Tanganyika Territory (Kibonoto on Kilimandjaro and Tanga)	Speiser, 1907; Bequaert, 1953; Fain, 1965
	Botaurus lentiginosus (Ciconiiformes, Ardeidae)	St. Paul, Minnesota	Phillips and Fain, 1991
Icosta chalcolampra (Speiser)	Unknown	Solomon Islands	Phillips and Fain, 1991
Icosta duckei (Austen)	*Urotriorchis macrourus* (Falconiformes, Accipitridae)	Bantanga, Congo	Phillips and Fain, 1991
Icosta nigra (Perty)	Unknown	Isla Santa Cruz, Galápagos Islands, Ecuador	Hill et al., 1967; Phillips and Fain, 1991
	Buteo galapagoensis (Falconiformes, Accipitridae)	Isla Santiago (San Salvador), Galápagos Islands, Ecuador	Madden and Harmon, 1998
	Unknown	Tuscon, Arizona	Phillips and Fain, 1991
Olfersia fossulata Macquart	Unknown	Peru	Phillips and Fain, 1991
Olfersia fumipennis (Sahlberg)	*Pandion haliaetus* (Falconiformes. Accipitridae)	Belize	Phillips and Fain, 1991
Olfersia sordida Bigot	*Phalacrocorax harrisi* (Pelecaniformes, Phalacrocoracidae) *Pelecanus occidentalis* (Pelecaniformes, Pelecanidae)	Isla Fernandina, Galápagos Islands, Ecuador	Madden and Harmon, 1998
Olfersia spinifera (Leach)	*Fregata magnificens* (Pelecaniformes, Fregatidae)	Mathews, Jamaica	Phillips and Fain, 1991
Ornithomyia remota Walcher	Unknown	Chile	Bequaert, 1953

hippoboscid flies. Host specificity of *Myialges* sp. mites may mirror that of the lousefly vectors, as suggested by Phillips and Fain (1991). Host specificity of hippoboscids is highly variable across fly species, but most are restricted to 1 avian order or family, and many are highly host specific (Maa, 1963; Marshall, 1981). The lousefly *Icosta nigra* is typically restricted to members of the Falconiformes and *Olfersia sordida* to 2 families within the Pelecaniformes (Phalacrocoracidae and Pelecanidae; Maa, 1963). The Galápagos hawk, flightless cormorant, and brown pelican are the only resident birds from these avian lineages within the archipelago. *Icosta nigra* is likely restricted to Galápagos hawks (and possibly short-eared owls, *Asio flammeus galapagoensis*) and *O. sordida* to flightless cormorants and brown pelicans within the archipelago, a distribution that could prevent dispersal of *Myialges* sp. mites between avian host orders. Finally, if host specialization has occurred, genetic differentiation of parasites should be apparent between sympatric hosts (Jaenike, 1981; McCoy et al., 2001); morphological differentiation may also be possible. In this study, we tested these predictions, report data on geographic distribution of *Myialges* sp. within each fly host and relate these to data from Madden and Harmon (1998), and report attachment site data of mites on *I. nigra* louseflies.

MATERIALS AND METHODS

Field methods

Because *Myialges* sp. mites occur under their avian host's skin, sampling from birds requires invasive procedures not feasible for threatened Galápagos hawks and flightless cormorants. Thus, we collected female *Myialges* sp. mites attached to the cuticle of louseflies (Fig. 2) associated with each avian host species from the entire breeding range of Galápagos hawks (2001–2003) and flightless cormorants (2004–2005). Sampling was associated with an ongoing avian disease survey involving the University of Missouri-St. Louis, the Saint Louis Zoo, Charles Darwin Research Station, and Galápagos National Park. In all cases, avian hosts were live-captured, sampled for subsequent disease testing, and released unharmed. Louseflies were collected from each host species on separate sampling trips. Eight Galápagos hawk populations on Española, Fernandina, Isabela, Marchena, Pinta, Pinzón, Santa Fe, and Santiago islands were sampled and *I. nigra* fly specimens were collected from birds by hand (Bollmer et al., 2005). *Olfersia sordida* flies were collected by hand from flightless cormorants from Islas Fernandina and Isabela. On Fernandina, sampling sites for cormorants included areas from which hawks were also sampled, e.g., coastal areas within hawk territories. All flies were placed in labeled vials of 95% ethanol, transported to the United States, and placed in a freezer at −20 C to maximize DNA preservation. Dr. B. J. Sinclair (Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany) identified representative specimens of flies from both hosts and retained voucher specimens, which were deposited at the Museum Alexander Koenig. *Olfersia sordida* specimens were not collected from brown pelicans, because this taxon had not yet been included in the collaborative avian disease survey. All field sampling procedures were approved by the University of Missouri-St. Louis Institutional Animal Care and Use Committee and the Galápagos National Park.

Specimen examination

Flies were thoroughly examined under a binocular dissecting microscope for the presence of *Myialges* sp. mites. If present, their attachment sites were recorded (see Figs. 2A, B, for examples of mite attachment sites). Basic metrics describing parasite population parameters, e.g., prevalence, abundance, intensity, and index *k*, were calculated for mites from each fly host species (from each population sampled) and compared between host species using the program Quantitative Parasitology 2.1 (QP; Reiczigel and Rózsa, 2001). For mites from *I. nigra* only (which had reasonably high *Myialges* sp. prevalences), comparisons of these measures among main body segments and among appendages of the thorax (legs I, II, III, wings, and main thorax) were carried out in QP. *Myialges* sp. prevalence data from Madden and Harmon's (1998) study were also included and analyzed for comparative purposes. Finally, 1 *I. nigra* specimen (with several *Myialges* sp. females attached) was dehydrated in an ethanol series, critical-point dried (SPI Jumbo critical point drier; Structure Probe, West Chester, Pennsylvania) and then gold–sputter-coated in a Polaron E5000 sputter coater (Quorum Technologies, Hailsham, U.K.). Images were taken on a Hitachi S450 scanning electron microscope (SEM; Tokyo, Japan) at 20 kV at the University of Missouri-St. Louis.

DNA extraction

The Cruickshank et al. (2001) voucher method was used to extract DNA from individual *Myialges* sp. mites collected from both *I. nigra* flies (from Galápagos hawks) and *O. sordida* flies (from flightless cormorants). Specifically, individual mites and their brood (if present) were removed from the alcohol-preserved hippoboscid fly hosts (e.g., Fig. 2) using sterile syringe needles. Because mitochondrial DNA (mtDNA) is typically maternally inherited in animals, mitochondrial sequences from mother mites and their brood should be identical; including the brood increased the quantity of DNA and, therefore, the quality of subsequent PCR amplicons. Mites were dried on the bench top in clean watch glasses for 5 min and the hysterosomas sliced into 2 approximately equal pieces with the beveled edge of a sterile needle tip. The mites were then individually transferred to 1.5-ml Eppendorf tubes and the animal tissue extraction instructions for the DNAeasy tissue extraction kit (Qiagen, Inc., Valencia, California) were followed with these modifications: (1) mites were left in incubation at 55 C for 2 nights and (2) the final elution step consisted of only a single 40-μl volume of warmed elution buffer (EB). Mite exoskeletons (that were 'cleared' by the extraction process) were retrieved from the Eppendorf tubes, further cleared, slide-mounted using standard protocols, and deposited in the Ohio State University Acarology Collection (accession numbers of mites from flies of hawks: OSAL 14034–36, 14038–41, 14043–45, 14048, 14051–60, 14065–68; mites from flies of cormorants: OSAL 14061–64). These specimens were compared to those in Madden and Harmon (1998) and line drawings of the female sternum were prepared to illustrate differences in the series of *Myialges* sp. from each host. Some mite exoskeletons or portions thereof were lost during the retrieval process owing to their small size. One female *Myialges* sp. mite from *O. sordida* collected in association with a flightless cormorant was slide-mounted intact, and thus its DNA was not extracted. However, DNA from that mite's brood was extracted following the protocol above. The *Neodermation* sp. mite specimens were extracted following the above procedure except that 3 batches of mites (4–5 individuals each) rather than individuals were extracted because of their extremely small size.

PCR, cleanup, and DNA sequencing

The invertebrate-specific primer pair LCO1490 (5′-GGTCAACAA ATCATAA-AGATATTGG-3′) and HCO2198 (5′-TAACTTCAGGGT GACCAAAAAATCA-3′) was used to PCR amplify a 658-bp fragment of the mitochondrial gene cytochrome oxidase *c* subunit I (COI; near the 5′ end; Folmer et al., 1994). This particular locus has been used successfully as a DNA barcode to identify arthropod species (Hebert et al., 2003). Each PCR tube contained 47 μl of a PCR master mix of the following components: 2.5 μl of PCR buffer (provided with DNA polymerase), 1.5 μl of BIOLASE Red DNA polymerase (Midwest Scientific, St. Louis, Missouri), 3.9 μl of 25 mM $MgCl_2$ (provided with DNA polymerase), 3 μl of each primer (diluted to 100 μM), 1.6 μl of 100 μM DNTPs, and 31.5 μl of sterile dH_2O. Three microliters of template mite DNA was added from each individually extracted mite, yielding a final PCR volume of 50 μl. Negative controls (tubes with no template DNA) were included in each set of reactions. The PCR amplifications for COI were performed using the following conditions: an initial denaturing step at 94 C for 4 min, followed by 35 cycles beginning with a denaturation step at 94 C for 1 min, an annealing step at 40 C for 1 min, an extension step at 70 C for 1 min, followed by a final extension step at 72 C for 7 min after the completion of the 35 cycles. Amplicon size was verified on 1–2% TBE agarose gels stained with ethidium bromide and visualized under UV light and then purified with QIAQuick PCR columns or agarose gel purified and then extracted using

FIGURE 2. Scanning electron micrographs of a hippoboscid fly specimen (*Icosta nigra*) with female *Myialges* sp. mites (with brood) attached to (**A**) the left wing (black arrow) and (**B**) the hind leg (white arrow indicating a developing mite in an egg laid by this female mite). The fly was collected from a Galápagos hawk (*Buteo galapagoensis*).

TABLE II. Comparisons of *Myialges* sp. loads between 2 lousefly host species associated with 2 endemic Galápagos birds. Data are from present study and Madden and Harmon (1998). Numbers in parenthesis are 95% bootstrap confidence intervals. Prevalence differed significantly across hawk lousefly (*Icosta nigra*) populations ($\chi^2 = 26.32$, 6 df, $P < 0.001$), but not between the 2 populations of cormorant louseflies (*Olfersia sordida*; $\chi^2 = 0.14$, 1 df, $P > 0.05$).

Metric	Site	Prevalence of *Myialges* ex. *Icosta nigra* from Galápagos hawks	Prevalence of *Myialges* ex. *Olfersia sordida* from flightless cormorants	*P*	*t*
Mite prevalence	Fernandina	8/46 flies infected 17.4% (7.8–31.4%)	5/59 flies infected 8.5% (2.8–18.7%)	—	—
	Isabela	2/20 flies infected 10% (1.2–31.7%)	2/20 flies infected 10% (1.2–31.7%)	—	—
	Española	0/14 flies infected 0%	—	—	—
	Santa Fe	0/5 flies infected 0%	—	—	—
	Pinzón	2/18 flies infected 11.1% (1.4–34.7%)	—	—	
	Pinta	0/46 flies infected 0%	—	—	—
	Santiago	41/144 flies infected 28.5% (21.3–33.6%)	—	—	—
	Total present study	53/296 flies infected 17.9% (13.7–22.76%)	8/105 flies infected 7.6% (3.33–14.57%)	0.011*	—
	Madden and Harmon	7/13 flies infected 53.8% (25.13–80.78%)	1/15 flies infected 6.7% (0.16–31.95%)	0.011*	—
Mean mite abundance	Present study	0.43 (0.24–0.61%)	0.08 (0.02–0.11%)	0.025*	3.39
Mean mite intensity	Present study	2.396 (1.53–3.30%)	1	0.078	2.91
Median mite intensity	Present study	1 (1–2%)	1	0.018*	—

* Significant at the $P < 0.05$ level.

QIAQuick gel extraction kit following the manufacturer's instructions (Qiagen Inc., Valencia, California). Purified amplicons were then verified on an agarose gel following the above procedures. Direct sequencing was performed on both strands of each amplicon by Macrogen, Inc. (Seoul, Korea) using the primers above with ABI PRISM® BigDye Terminator PCR cycling conditions and sequenced on Applied Biosystems 3730xl DNA analyzers (Applied Biosystems Division, Foster City, California). We also amplified a 330–334-bp (the fragment size variation results from the presence of insertions or deletions) fragment of the 12S mitochondrial ribosomal RNA gene from the same samples (except from the *Neodermation* sp. samples) using the primer pair 12SAI (5′-CTAGGATTAGA-TACCCTATT-3′ and 12SBI (5′-AA GAGCGACGGGCGATG-3′) published previously (Simon et al., 1994). Volumes of reagents and template DNA in PCRs as well as purification methods (using QIAQuick PCR columns only) were identical to those described above. The PCR amplifications for 12S amplifications were performed using the following conditions: an initial denaturing at 94 C for 2 min, followed by 35 cycles beginning with a denaturation step at 94 C for 30 sec, annealing at 46 C for 30 sec, extension at 70 C for 30 sec, followed by a final extension step at 72 C for 7 min after the completion of the 35 cycles. Sequencing was carried out by Macrogen as described above (using 12SAI and 12SBI primers for sequencing).

DNA sequence analyses

Raw sequence chromatograms of forward and reverse strands were assembled for each amplicon in Seqman II (DNASTAR, Inc., Madison, Wisconsin). The entire length of each strand was evaluated by eye. Poor quality data and primer sequences were trimmed from both strands. Seqman II was used to assemble the consensus sequences, or contigs, resulting from the double-stranded sequences for each gene, which were aligned in Se-Al (Rambaut, 1996) or ClustalX (Thompson et al., 1997). We then returned to the original chromatograms to ensure that variable sites were unambiguously assigned. Sequences from the *Myialges* and *Neodermation* specimens have been deposited in GenBank (accession numbers for COI haplotypes from *Myialges* sp.: DQ503439–DQ503447; accession number for the COI haplotype from *Neodermation* sp.: DQ832189; accession numbers for 12S haplotypes from *Myialges* sp.: DQ503448–DQ503456).

Neighbor-joining analyses were performed on each dataset (COI, 12S, and combined) in PAUP*. To roughly test the monophyly of each *Myialges* clade identified, heuristic parsimony and neighbor-joining searches were performed in PAUP* on an alignment of the COI *Myialges* sp. sequences and 2 outgroup COI sequences: *Neodermation* sp. and *Varroa destructor*. 12S sequences, which are highly variable in length between invertebrate species, were not used for the analyses including distantly related outgroups. To examine cryptic genetic diversity within each putative *Myialges* lineage (those from hawks and cormorants), a statistical parsimony haplotype network was constructed using the TCS program (Clement et al., 2000) for each locus.

The pairwise F_{ST} value was calculated for *Myialges* sp. sequences obtained from flies of hawks (*I. nigra*) from Islas Fernandina and Santiago to test for population differentiation. To compare the level of genetic differentiation between the hawk mite and its avian host, we also calculated pairwise F_{ST} values between these islands for the Galápagos hawk using mtDNA sequences obtained previously (Bollmer et al., 2006). The preceding analyses were conducted in Arlequin (Schneider et al., 2000). Pairwise F_{ST} values from multilocus minisatellites were also obtained (Bollmer et al., 2005) from the same hawk populations for an estimate of differentiation of the host at nuclear loci.

RESULTS

Fly collections

In total, 296 *I. nigra* specimens were collected from 8 island populations of *B. galapagoensis* hosts, which constitute the entire breeding range of these endemic birds (Table II). We did not observe or collect any *I. nigra* specimens from any of the 26 hawk individuals sampled on Isla Marchena. In total, 105 *O. sordida* specimens were collected from Islas Isabela and

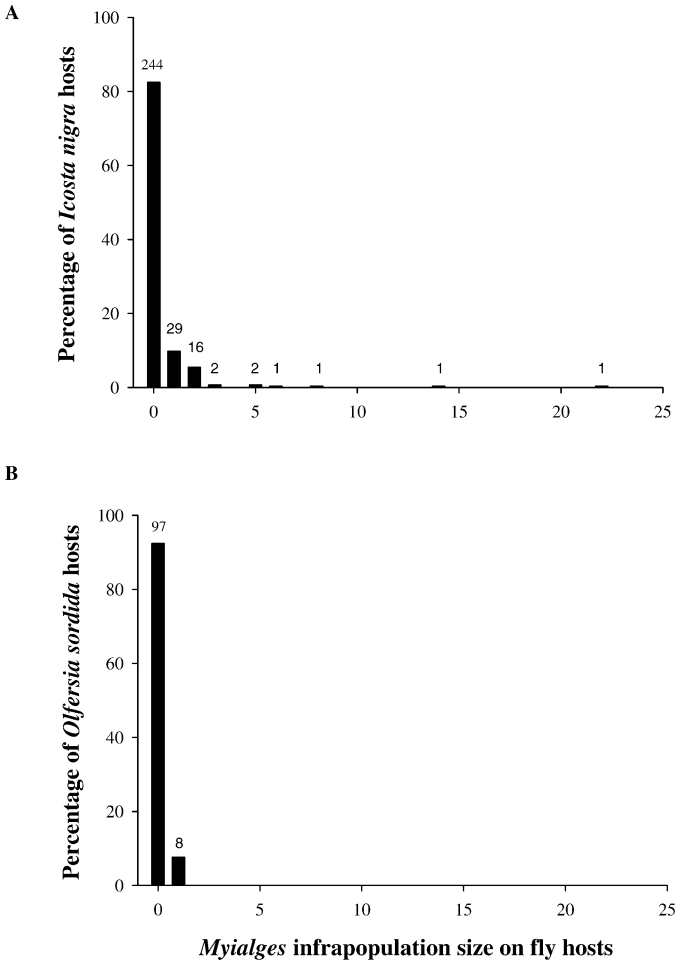

FIGURE 3. Frequency histogram of *Myialges* mite distribution among (**A**) 296 *Icosta nigra* lousefly individuals from 7 island populations of Galápagos hawk hosts and (**B**) 105 *Olfersia sordida* lousefly individuals from 2 island populations of flightless cormorant hosts. Mites were significantly more prevalent on flies of hawks than on flies of cormorants.

Fernandina (and several small islets associated with Isabela), which constitute the entire species range of *P. harrisi* hosts (Table II).

Mite collections, distributions, and morphological differentiation

A total of 127 *Myialges* sp. individuals were found attached to 53 of the 296 *I. nigra* specimens from 4 of the 8 islands on which hawk hosts were sampled (Islas Fernandina, Isabela, Santiago, and Pinzon). Seven *Myialges* sp. individuals were found attached to 7 of the 105 *O. sordida* specimens (from both Isabela and Fernandina) and 1 *Myialges* sp. individual was found floating free in the collection vial associated with 3 of the 105 *O. sordida* specimens and is assumed to have been present on 1 of those fly individuals prior to immersion in alcohol. Prevalence of mites on *I. nigra* (hawk louseflies) differed significantly among islands (Table II), whereas prevalence of mites on *O. sordida* (cormorant louseflies) did not differ significantly among islands (Table II). However, this could be an artifact of the low sample size of mites on flies of cormorants.

TABLE III. Attachment site data of 127 female *Myialges* sp. mite individuals attached to 53 *Icosta nigra* louseflies associated with Galápagos hawks (*Buteo galapagoensis*).

Mite attachment site	Prevalence	Mean abundance	Mean intensity	Median intensity
Head	9.4%	0.09	1	1
Thorax	79.2%	1.08	1.36	1
Abdomen	47.2%	1.23	2.6	1
P	< 0.001			
Within thorax only				
Main thorax	21.4%	0.24	1.11	1
Wing	28.3%	0.28	1	1
Legs I	7.5%	0.08	1	1
Legs II	13.2%	0.17	1.29	1
Legs III	32.1%	0.36	1.12	1
P	< 0.01			

In both Madden and Harmon's (1998) study and the present one (using a combined data set among islands), mites were significantly more prevalent and abundant on *I. nigra* (from hawks) than on *O. sordida* (from cormorant) fly hosts (Table II). Distributions of mites among flies approximated a negative binomial distribution (Fig. 3). Within *I. nigra* flies infected with mites, female *Myialges* sp. were most prevalent on the thorax, followed by the abdomen and head (Table III). Considering only the thorax for flies infected with *Myialges* sp., mites were most prevalent on the hind legs, followed by attachment to the wings, main thorax, middle legs, and fore legs (Table III).

Reexamination of *Myialges* sp. females from flies of hawks and flies of cormorants revealed that the 2 groups can be separated consistently by the shape of the surface sclerotization around the sternum (Fig. 4). The drawing by Madden and Harmon (1998) of material recovered from flies of hawks (*I. nigra*) fits very well with our material from the same host. Moreover, the sternal morphology of both Galápagos forms differs from that figured by Fain (1965) for the lectotype of *M. caulotoon*, associated with *Ardea purpurea* from Tanzania.

DNA sequence, population genetic, and phylogenetic analyses

We obtained double-stranded sequences of 658 bp of COI and the 330–334 bp of 12S mtDNA from 28 *Myialges* sp. individuals (sample size by island: n = 2, Isabela; n = 1, Pinzon; and n = 25, Santiago) associated with 28 *I. nigra* louseflies from 24 different Galápagos hawks and 8 *Myialges* sp. individuals (n = 3, Isabela; n = 5, Fernandina) associated with 8 *O. sordida* louseflies from 8 different flightless cormorants. In the case of the *I. nigra* samples, 1 mite each was sampled from 2 different louseflies collected from the same hawk in 4 cases (hawk bands G44, G16, and N40 from Isla Santiago and hawk band R5E from Isla Fernandina). Alignments of both loci were unambiguous within *Myialges* sp. sampled from Galápagos hosts. The inferred amino acid residues translated (using the arthropod mtDNA code) from 658 bp of the protein-coding COI sequences resulted in an open reading frame comprising 219 codons (in the second frame). The non–protein-coding 12S fragments amplified from *Myialges* sp. collected from flies of

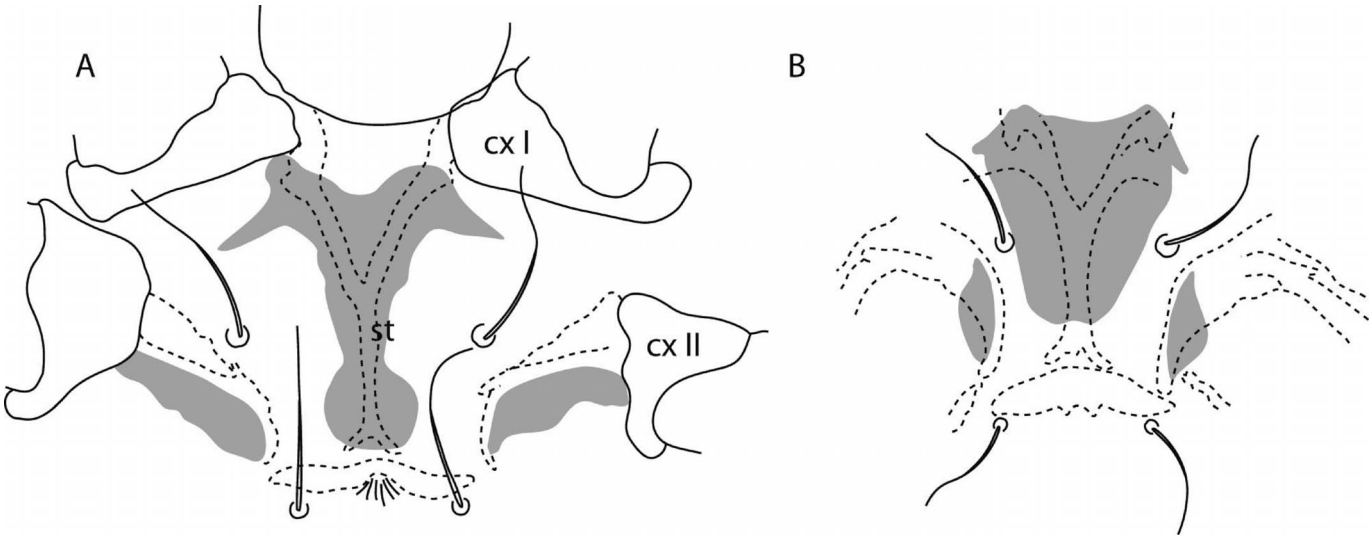

FIGURE 4. Ventral view of anterior part of the body of *Myialges* females. Surface sclerotization indicated by gray shading. (**A**) Female from *Icosta nigra*. (**B**) Female from *Olfersia sordida*. Abbreviations: cx I, cx II: coxa I and II; st: sternum (fused section of coxal apodemes I).

cormorants were 4 bp shorter (330 bp) than the fragment amplified from *Myialges* sp. collected from flies of hawks (334 bp). The concatenated alignment (COI + 12S) was 988 bp in length (gaps were inserted in the cormorant *Myialges* sp. sequences). A neighbor-joining analysis using the combined 988-bp alignment and an analysis of the 12S data set only from the 35 *Myialges* sp. individuals revealed trees essentially identical to the NJ tree resulting from an analysis of only COI data shown in Figure 5. We chose to display the COI tree (Fig. 5) because we were able to align out-group sequences unambiguously with the in-group sequences. In all of the trees (COI, 12S, and combined), 1 main lineage comprised only *Myialges* sp. collected from flies of cormorants (*O. sordida*) and these haplotypes were separated from the other main lineage, comprising only *Myialges* sp. collected from flies of hawks (*I. nigra*), by 16.05–16.55% uncorrected *p*-distance. For the COI phylogeny, the monophyly of the 2 host-specific *Myialges* spp. was highly supported in both NJ and parsimony bootstrap analyses (100% in each case; Fig. 5). The cormorant *Myialges* sp. lineage was further composed of 2 clades, separated by ~1% uncorrected *p*-distance at COI (7 variable sites) and ~0.3% uncorrected *p*-distance at 12S (1 variable site). Mite sequences collected from flies of cormorants on both Fernandina and Isabela were recovered in each clade (no island-specific lineages). Sequences of *Myialges* sp. mites from flies of hawks sampled from the same hawk host individuals (but from 2 different hippoboscid individuals within each bird) were identical at both loci. A Fernandina-specific hawk *Myialges* clade comprised 5 *Myialges* sp. individuals (see haplotype network below) was also recovered. Eight sites varied across the 988-bp alignment within the hawk *Myialges* sp. lineage.

Haplotype networks for each lineage (cormorant *Myialges* and hawk *Myialges* sp.) showed very different patterns (Fig. 6). Whereas the cormorant *Myialges* clade only harbored 2 haplotypes separated by 8 mutational steps, the hawk *Myialges* clade was starlike and individual haplotypes were all closely related, with 1 central common haplotype that may be ancestral. All 6 COI mutations within the hawk *Myialges* clade were synonymous. Within the cormorant *Myialges* clade, 6 COI mutations were synonymous and 1 was nonsynonymous.

Myialges sp. populations collected from hawks on Islas Fernandina and Santiago were differentiated from each other (F_{ST} = 0.33, $P < 0.00001$) based on the combined (COI + 12S) mtDNA dataset. Hawk populations from these 2 islands were completely differentiated (though invariant at the homologous 5′ end of COI used here) from each other based on nearly 3 kb of mtDNA (F_{ST} = 1, $P < 0.00001$) obtained for hawk individuals sampled previously (n = 23, Santiago; n = 22, Fernandina) (Bollmer et al., 2006). Bollmer et al. (2005) also showed that hawks from Santiago (n = 37) and Fernandina (n = 20) were significantly differentiated at multilocus minisatellite (nuclear) loci (F_{ST} = 0.123).

DISCUSSION

Ecologically simplified systems, such as those of oceanic archipelagoes, are excellent sites for studying host-parasite interactions (Whiteman et al., 2004, 2006). We genetically characterized and quantified the distributions of epidermoptid mite populations associated with endemic flightless cormorants and Galápagos hawks in the Galápagos Islands. To determine if these mites exhibited cryptic host specificity or instead were being transmitted between host species, we sampled mites from locations where the 2 avian hosts were sympatric and shared habitat (Isla Fernandina) and from locations where only the Galápagos hawk was present, including Isla Santiago. Islas Fernandina (the sympatric setting) and Santiago were also the island populations from which *M. caulotoon* mites were collected from louseflies of flightless cormorant and Galápagos hawks, respectively, by Madden and Harmon (1998). Significant genetic divergence (~17% uncorrected *p*-distance) and reciprocal monophyly was revealed between mite populations collected from each host species.

Mites from the 2 hosts were also distinguishable by differences in the shape of the surface sclerotization around the sternum of the adult females. These observations are consistent

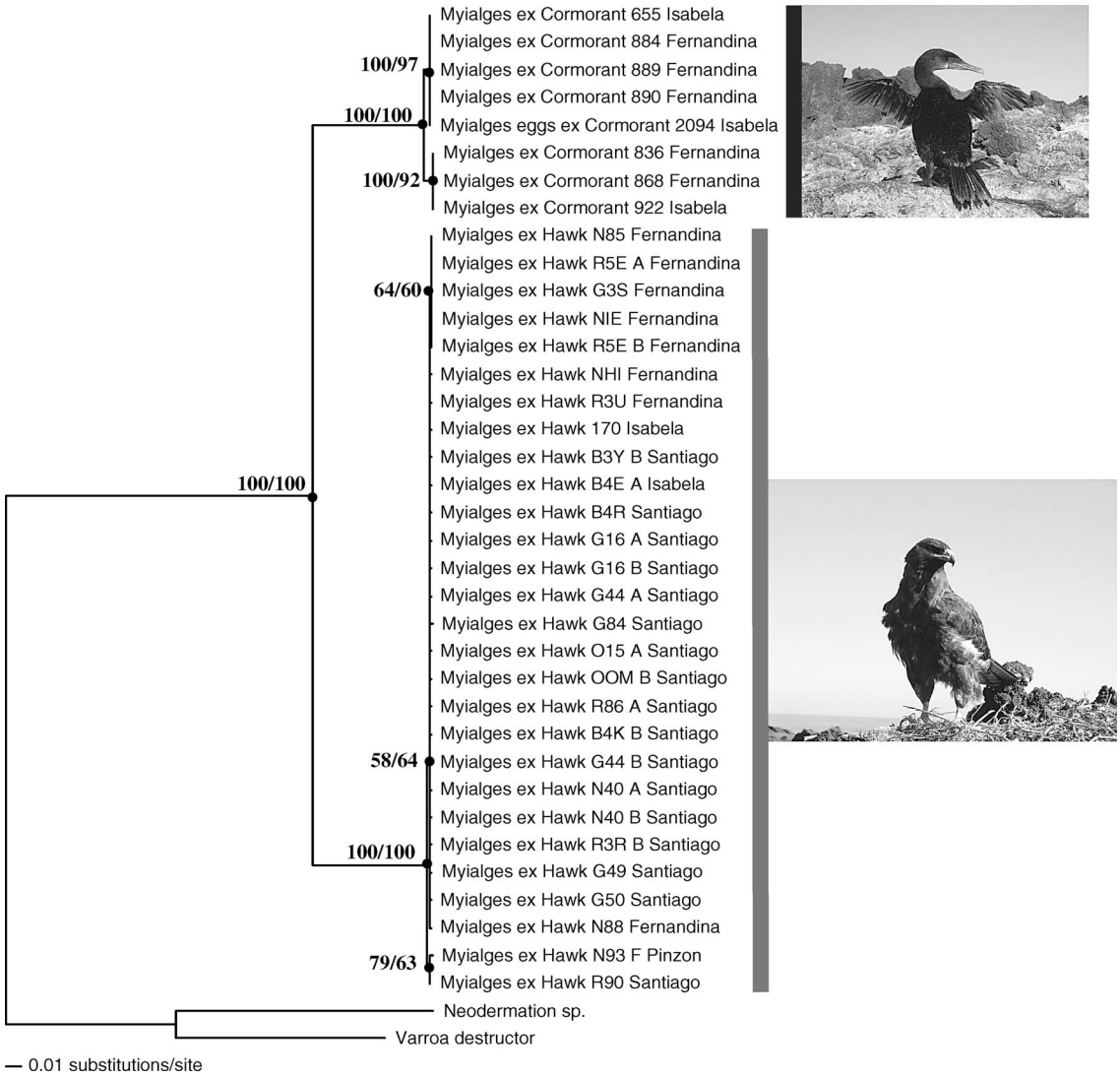

FIGURE 5. Tree from neighbor-joining (NJ) analysis of 658 bp of mitochondrial cytochrome oxidase *c* subunit I (COI) sequences from 7 female and 1 brood cluster of *Myialges* sp. removed from *Olfersia sordida* louseflies associated with flightless cormorants (*Phalacrocorax harrisi*) and 28 female *Myialges* sp. individuals removed from *Icosta nigra* louseflies associated with Galápagos hawks (*Buteo galapagoensis*). Homologous COI sequences from *Varroa destructor* (GenBank accession number AJ493124), a mesostigmatid mite, and a *Neodermation* sp. (Neodermationidae) individual, which is a close relative of the Epidermoptidae, were included as outgroups in the analyses. A heuristic parsimony bootstrap analysis yielded a tree with a topology nearly identical to the NJ tree (all nodes common to both trees are indicated by black dots). Numbers on the tree represent bootstrap support values for nodes, estimated using 10,000 replications for the NJ tree and 1,000 replications for the parsimony tree implemented in PAUP* v.4.0b (values left to right: NJ values/parsimony values).

with the results of the molecular analyses, suggesting that ''*Myialges caulotoon*'' may be a species complex. Formal descriptions of these forms will be postponed until appropriate material from other instars can be obtained. We found further genetic variation within each major host-specific *Myialges* clade, including an island-specific clade of *Myialges* sp. from hawks on Isla Fernandina and 2 relatively divergent non–island-specific clades among *Myialges* sp. sequences from cormorants.

The haplotype network indicates that the most widespread haplotype among the hawk-associated *Myialges* sp. is most likely the oldest DNA sequence (Castelloe and Templeton, 1994). Interestingly, this haplotype is most abundant on Isla Santiago, and rare on Isla Fernandina. Together with the F_{ST} data, this suggests that these island populations of *Myialges* sp. are genetically isolated from one another just as their host populations are differentiated between these 2 islands.

Mites were significantly more prevalent among flies of hawks than flies of cormorants, and exhibited a pattern of distribution typical for that of most parasites (most hosts have no parasite individuals and few have many parasites). Within flies of hawks, prevalence of female mites varied across island populations, mirroring the isolation of most hawk populations. Among infected flies of hawks (all island populations combined), prevalence also varied across the major fly body segments and within the main thoracic structures. Nearly 80% of infected flies of hawks harbored mites on the thorax, where the most common attachment site (based on prevalence) was the rear legs. Several possible explanations for the nonrandom *Myi-*

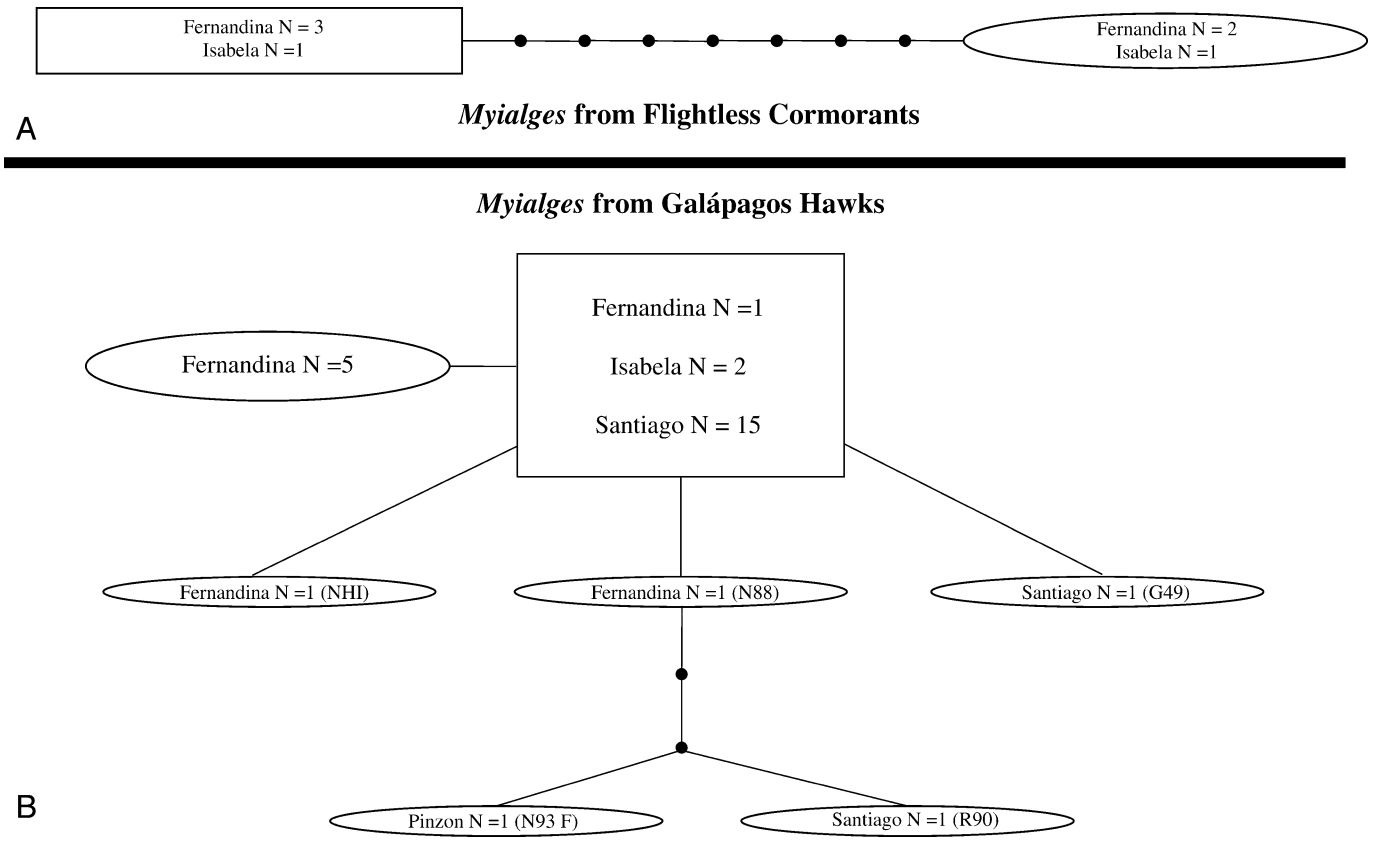

FIGURE 6. Ninety-five percent statistical parsimony haplotype networks estimated in TCS for 988 bp of combined cytochrome oxidase *c* subunit I and 12S data from *Myialges* sp. mites collected from (**A**) *Olfersia sordida* louseflies associated with flightless cormorants and (**B**) *Myialges* sp. mites collected from *Icosta nigra* louseflies associated with Galápagos hawks. Haplotype frequencies are listed by island population within each haplotype. Haplotypes drawn as squares were estimated as the most ancestral (most likely root) haplotype in TCS.

alges sp. distributions exist, including competition among mites for attachment sites (Hayashi and Ichiyanagi, 2005) and differences in the host's integument among sites (McAloon and Durden, 2000). However, we are unable to determine the cause of the patterns found here.

The genetic and distributional data of *Myialges* sp. within and between host species suggest that hippoboscid flies, which in this case are typically limited to Falconiformes (*I. nigra*) and Pelecaniformes (*O. sordida*) hosts, also limit maternal dispersal of *Myialges* sp. mites. Moreover, interisland dispersal of hawk-associated *Myialges* sp. is limited, and concordant with that of their hawk hosts. Vercammen-Grandjean (1966) first suggested that studies of mite host specificity may be particularly helpful in informing our understanding of host ecological and evolutionary history within taxa inhabiting the Galápagos Islands. Without Madden and Harmon's (1998) study, we would not have extensively collected flies from both of these hosts, which enabled the genetic characterization of the mite populations from each host species. Their survey of parasitic mites in Galápagos hosts served as a springboard for further research, and together, these studies show how little is known about parasite biodiversity (Whiteman and Parker, 2005).

Notably, a new species of epidermoptid, *Myialges pelecani*, was recently described from male specimens taken from Galápagos brown pelicans (Fain and Bochkov, 2003). Given that the lousefly *O. sordida* is also found on this bird, this name might apply to the *Myialges* sp. from *O. sordida* analyzed in this study (OConnor et al. 2006). This question could be resolved by collecting male *Myialges* sp. mites from flightless cormorants or through genetic characterization of *M. pelecani* from the pelicans.

Although the endemic avifauna of the Galápagos Islands remains relatively intact, the human population is rapidly expanding, causing concern that exotic wildlife diseases will be introduced into the archipelago, precipitating a Hawaii-like extinction among the native vertebrates (Warner, 1968; Wikelski et al., 2004). Therefore, understanding parasite transmission dynamics within these island populations is essential to reduce the threat posed by invasive disease agents, but little information is presently available on dynamics of either invasive or native disease agents (Whiteman et al., 2004, 2006; Wikelski et al., 2004; Gottdenker et al., 2005; Thiel et al., 2005), despite the important role transmission mode plays in the evolution of virulence (Clayton and Tompkins, 1994). Understanding the transmission dynamics of *Myialges* sp. mites is of wildlife health importance in this case, because epidermoptid mange may cause significant mortality in island birds (Gilardi et al., 2001). We have shown that hawk and cormorants are epidemiologically isolated with respect to these skin mites, even though both birds often occur together. Clearly, basic information on parasite identity, host specificity, and transmission is essential for informed conservation and public policy management deci-

sions, in addition to informing our understanding of parasite diversification.

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