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MOLECULAR AND MORPHOLOGICAL DIVERGENCE IN A PAIR OF BIRD SPECIES AND THEIR ECTOPARASITES

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ABSTRACT: In an evolutionary context, parasites tend to be morphologically conservative relative to their hosts. However, the rate of neutral molecular evolution across many parasite lineages is faster than in their hosts. Although this relationship is apparent at the macroevolutionary scale, insight into the processes underpinning it may be gained through investigations at the microevolutionary scale. Birds and their ectoparasitic lice have served as important natural experiments in co-evolution. Here, we compared mitochondrial and morphological divergence in 2 recently diverged avian host lineages and their parasites. Gálapagos hawks (*Buteo galapagoensis*) are phenotypically divergent from their closest mainland relatives, the Swainson's hawk (*Buteo swainsoni*). Both species are host to a feather louse species of *Craspedorrhynchus* (Insecta: Phthiraptera: Ischnocera, Philopteridae). We sequenced the 5′ end of the mitochondrial gene cytochrome oxidase *c* subunit I (COI) from a set of hawks and lice. Although this fragment allowed unambiguous identification of host and parasite lineages on the islands and the mainland, only a single variable site was present in the 2 hosts, but 2 major *Craspedorrhynchus* clades divergent by ~10% were recovered that sorted perfectly with host species. We found significant population genetic structure within the Galápagos *Craspedorrhynchus* lineage. While the host species are highly differentiated phenotypically, the 2 *Craspedorrhynchus* louse lineages are phenotypically overlapping, although subtle but significant morphological differences exist.

Across species, morphological evolution tends to be more conservative in parasites than their hosts (Klassen, 1992). This tendency, coupled with the fact that some parasites are vertically transmitted across ecological and evolutionary time (Page, 2003; Nieberding and Olivieri, 2007), led parasitologists to use parasite distributions to infer host genealogical relationships (Klassen, 1992). In contrast to phenotypic change, the rate of neutral molecular evolution among lice tends to be faster than in their hosts (Hafner et al., 1994; Page et al., 1998). Parasites and their hosts could be compelling systems in which to test the prediction that rates of morphological evolution in co-evolved hosts and parasites are independent of genetic changes in those same lineages. Given that the rate of neutral evolution in the parasites is expected to exceed that in the host, and that parasites tend to be morphologically conservative with respect to their hosts, we expect less genetic divergence at homologous loci in 2 recently derived hosts than in their parasites, and more phenotypic divergence in the hosts than in the parasites.

The feather louse (Insecta: Phthiraptera) fauna of birds inhabiting the Galápagos Islands has a special and controversial role in the history of co-evolutionary biology (Kellogg and Kuwana, 1902; Klassen, 1992; Choudhury et al., 2002). Claims of rampant horizontal transfer of lice among Galápagos birds are noteworthy (reviewed in Palma, 1994). The endemic birds of Galápagos were central to the development of Darwin's theory of evolution by natural selection, and a key insight was that the forms he encountered in the islands were similar to, but distinct from, those on the mainland, i.e., "The archipelago is a little world within itself, or rather a satellite attached to America, whence it has derived a few stray colonists, and has received the general character of its indigenous productions" (Darwin, 1909). Kellogg and Kuwana (1902) reported that through their study of Phthiraptera collected during the Hopkins Stanford Galápagos Expedition, "It was hoped that the character of the parasites found on the strictly Galapagos Island bird hosts might throw some light on the relationships of these birds to continental genera and species. . ." However, Kellogg and Kuwana (1902) instead found that distantly related birds in the Galápagos, particularly land and seabirds, were host to the same louse species, which they attributed to increased avian population densities on islands resulting in increased contact among unrelated species leading to rampant straggling of lice (see Pilgrim and Palma [1982] for a definition of stragglers). Hopkins (1951) and Palma (1994) posited that claims of unusually high rates of straggling in Galápagos were dubious and suggested instead that the "abnormal phase of normal straggling" reported by Kellogg and Kuwana (1902) was the result of human error from storage of unrelated bird species in the same containers shortly after they were killed. Although the closest mainland relatives of many native Galápagos Island birds have been tentatively identified through molecular phylogenetics, additional insight into the colonization history of the islands may be gained by examining the distributions and evolutionary histories of the birds' parasites (Whiteman et al., 2006, 2007). Thus, Kellogg and Kuwana's (1902) question of whether parasites might reveal insight into the relationships between Galápagos birds and their mainland relatives remains an open one.

The Galápagos hawk (*Buteo galapagoensis*) is highly divergent, morphologically and behaviorally, from other *Buteo* species, which rendered identifying its closest mainland relative extremely difficult. Darwin (1909), perhaps misled by the fact that he seemed to believe that it fed primarily on carrion, observed that the Galápagos hawk was ". . .curiously intermediate in structure between a buzzard and the American group of carrion-feeding

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Polybori.'' Gould (1837), agreed: ''. . .it forms a beautiful intervening link between these genera (*Buteo* and *Polyborus*) as is evidenced by the scaling of the tarsi and the produced form of the beak, while its habits place it within the limits of the latter genus.'' Initially, Gould placed the Galápagos hawk in the genus *Polyborus*, and it was subsequently placed in the genera *Craxirex*, *Buteo*, *Astur*, and *Dromolestus* in the 19th century, before being consistently placed in *Buteo* (see de Vries, 1973). Given the taxonomic confusion resulting from its divergent phenotype, its closest living relative on the mainland remained unknown until recently. A molecular phylogeny placed the Galápagos hawk sister to the Swainson's hawk (*Buteo galapagoensis*) (Riesing et al., 2003) with high confidence; a subsequent phylogeographic study explored the Galápagos + Swainson's hawk node further and showed that the 2 species were so recently separated that mitochondrial DNA divergence between the 2 species was equivalent to divergence within each species (Bollmer et al., 2006). A more detailed study with broader sampling among Swainson's hawks indicated that the Galápagos hawk is a monophyletic lineage nested phylogenetically within Swainson's hawks, rendering Swainson's hawks paraphyletic with respect to Galápagos hawks (Hull, Savage et al., 2008).

The Swainson's hawk is host to all 3 species of Phthiraptera reported from the Galápagos hawk, including 1 in the suborder Amblycera, i.e., *Colpocephalum turbinatum* Denny, 1842, and 2 in the suborder Ischnocera, i.e., *Degeeriella regalis* (Giebel, 1866) (Price et al., 2003), and a species of *Craspedorrhynchus*. Two other louse species of the suborder Amblycera have been recorded from Swainson's hawks, i.e., *Laemobothrion maximum* (Scopoli, 1763) and *Kurodaia fulvofasciata* (Piaget, 1880) (Price et al., 2003), but they have not been found on the Galápagos hawks, despite our intensive sampling of over 250 birds from all extant island populations.

The first record of a louse in the Galápagos Islands that we now recognize as belonging to *Craspedorrhynchus* was published by Kellogg (1906) as *Docophorus taurocephalus* Kellogg, 1896, and the petrel *Puffinus subalaris* Ridgway, 1897 was reported as its host; this is most likely another case of cross-contamination of lice between genetically and ecologically unrelated hosts; *Craspedorrynchus* species are specific to Falconiformes birds (see Palma, 1994). In their list of insects from the Galápagos Islands, Linsley and Usinger (1966) correctly transferred this louse species to the *Craspedorrhynchus*, but they neglected the fact that *C. taurocephalus* had already been demoted to a junior synonym of *C. dilatatus* (Rudow, 1869) by Hopkins and Clay (1952). Linsley and Usinger (1966) mentioned 'Baltra' as the island where Kellogg's (1906) record had originated, but they did not include any host name for *C. taurocephalus*, nor for the other 57 louse species they listed. Parker et al. (2006) were the first to associate *Craspedorrhynchus* sp. lice with the Galápagos hawk. However, this is the first report of *Craspedorrhynchus* lice from the Swainson's hawk.

Craspedorrhynchus lice are highly host specific (Mey, 2001) and typically occur on a single host species. This makes them potentially useful as evolutionary tags. In this study, we asked whether the degree of phenotypic differentiation and neutral molecular evolution were different in *Craspedorrhynchus* spp. lice found on Galápagos and Swainson's hawks. We also asked whether these ectoparasites tracked the hosts' population structure across the Galápagos archipelago.

MATERIALS AND METHODS

Field methods

Using a dust-ruffling technique (Walther and Clayton, 1997) modified for Galápagos hawks (Whiteman and Parker, 2004a, 2004b), we collected *Craspedorrhynchus* spp. lice from the head and nape regions of 35 Galápagos hawk individuals across 6 islands within the Parque Nacional Galápagos, Ecuador (the islands of Española, Fernandina, Pinta, Pinzón, Santa Fe, and Santiago) in 1992 and 2003–2004. We also sampled lice (using the same methods) from the heads of breeding and fledgling Swainson's hawks in New Mexico (2002) and Manitoba, Canada (2002), and from an over-wintering population in Córdoba, Argentina (2003). A blood sample for host DNA analysis was taken from each bird captured and preserved in Longmire's solution (see Bollmer et al., 2005). In all cases, avian subjects were live-captured, sampled, and released unharmed as in Whiteman et al. (2007).

Molecular genetics

Hosts: DNA was extracted from whole blood using a modified phenol-chloroform method following Bollmer et al. (2005). We amplified 496 bp near the 5′ end of the COI fragment (the homologous region was sequenced in the lice) from 22 Galápagos hawks (n = 2 from each island: Española, Fernandina, Marchena, Pinta, Pinzón, Santa Fe, Santiago; n = 4 from each island: Isabela, Santa Cruz) and from 5 Swainson's hawks (all from Argentina), as described elsewhere (Bollmer et al., 2006) using the primers L6615 and H7539; sequencing was performed using L6615 or H7181 (primer sequences are listed in Bollmer et al., 2006). Single-stranded sequences from hawks were obtained using ABI BigDye Terminator version 3.1 and an ABI PRISM 3100-Avant genetic analyzer (PE Applied Biosystems, Foster City, California; University of Florida, Gainesville, Florida). A subset of individuals was sequenced in both directions.

Lice: Prevalence and intensity of *Craspedorrhynchus* spp. infestations are generally low; these lice are infrequently encountered when sampling from birds. Thus, we were able to extract DNA from a total of 35 *Craspedorrhynchus* spp. lice from Galápagos hawks, including lice from Fernandina (n = 10), Santiago (n = 10), Pinta (n = 9), Española (n = 2), Santa Cruz (n = 1), Santa Fe (n = 1), and Pinzón (n = 1). We extracted DNA from a total of 9 *Craspedorrhynchus* spp. lice from Swainson's hawks, including lice from Argentina (n = 5), New Mexico (n = 3), and Manitoba (n = 1). The voucher method of Cruickshank et al. (2001) was used to extract DNA from individual lice at the University of Missouri–St. Louis following Whiteman et al. (2006, 2007). For each extraction, an individual louse was removed from a preservation vial containing 95% ethanol (stored at −20 C). It was then dried on the bench top in a clean watch glass for 5 min and sliced laterally through the thorax with the beveled edge of a sterile needle tip. Both pieces of each louse were then individually transferred to 1.5-ml Eppendorf tubes, and the animal tissue extraction instructions for the DNeasy Tissue Extraction Kit (Qiagen, Inc., Valencia, California) were followed with these modifications: (1) lice were left in incubation at 55 C for 2 nights, and (2) the final elution step consisted of only a single 50-μl volume of warmed elution buffer (EB). Louse exoskeletons (which were 'cleared' by the extraction process) were retrieved from the Eppendorf tubes and stored in 70% ethanol. Each exoskeleton was then slide-mounted at the Museum of New Zealand Te Papa Tongarewa and deposited in its insect collection.

The primer pair LCO1490 and HCO2198 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 to aid in the identification of avian louse species (Whiteman et al., 2004) and in population genetics studies of lice (Whiteman et al., 2007; Toon and Hughes, 2008). PCR reaction conditions, amplicon clean-up, and double-stranded sequencing followed Whiteman et al. (2007).

DNA sequence analyses

For host and parasite sequences, 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 contigs

FIGURE 1. Rooted maximum likelihood tree (score = 1459.201) of *Craspedorrhynchus* sp. COI sequences from a heuristic search in Paup* (HKY+G model). Numbers at nodes are parsimony bootstrap values (100 replications). Alpha-numeric codes represent hawk bands. Lowercase letters represent multiple louse individuals sampled from a single host.

(consensus sequences) 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 in each case; if any ambiguity existed within a sequence, that sequence was discarded from the alignment. The entire alignment was then trimmed to the shortest sequence, resulting in an alignment of 494 bp for the louse and 497 bp for the hawks. Sequences were deposited in GenBank under the accession numbers GQ922118-GQ922132 for the louse and AY870866-AY870867 for the hawk.

Because Galápagos and Swainson's hawks were invariant within each species at the 5′ end of COI, and only different between species at 1 site, we did not explore these data further. For the lice, we determined the most likely model of nucleotide substitution across the COI alignment using the Modeltest program (v. 3.71; Posada and Crandall, 1998). The HKY+G model was chosen as the most likely using the likelihood ratio test. We then performed a maximum likelihood heuristic search using stepwise addition in Paup* (v. 4.0b10; Swofford, 2002) to evaluate the phylogenetic relationships among the *Craspedorrhynchus* spp. sequences. A likelihood bootstrap search was performed in Paup* under the same parameters using 100 replications, and it produced a single tree (Fig. 1).

The alignments were analyzed in DNAsp (Rozas et al., 2003) to calculate standard population genetic parameters (Tables I, II) and deduce the amino acid sequences from the COI sequences. DNAsp was also used for calculation of overall (combined across islands) F_{ST} values for the 3 Galápagos populations with sufficient sample sizes (islands Fernandina, Pinta, and Santiago). Two statistical parsimony haplotype networks (Figs. 2, 3) were constructed using the TCS 1.8 program (Clement et al., 2000) for the COI to determine the extent of geographic structuring within each major clade of *Craspedorrhynchus*.

Morphological analysis

Hosts: Sampling localities and methods used in capturing and measuring host morphological characters are described elsewhere (Bollmer

TABLE I. Population genetic parameters of *Craspedorrhynchus* lice from Galápagos and Swainson's hawks.

Population genetic parameters	*Craspedorrhynchus* sp. from *Buteo galapagoensis* (n = 35)	*Craspedorrhynchus* sp. from *Buteo swainsoni* (n = 9)
Aligned length	494 bp	494 bp
No. polymorphic sites	8	14
Nucleotide diversity	0.0028	0.0086
No. of haplotypes	8	7
Haplotype diversity	0.733	0.917
Theta per sequence from S (Watterson's estimator)	1.943	5.151
No. of synonymous/ nonsynonymous substitutions	6/2	13/1

TABLE II. Population genetic structure within Galápagos *Craspedorrhynchus* spp.

Island comparisons	Geographic distance (km)	F_{st}
Fernandina–Santiago	57	0.13
Fernandina–Pinta	120	0.86
Santiago–Pinta	76	0.87

et al. [2003] for Galápagos hawks; Hull, Anderson et al. [2008] for Swainson's hawks). In total, 118 male and 85 female Galápagos hawks and 42 male and 26 female Swainson's hawks were measured for 4 characters: wing chord, culmen chord, hallux chord, and body mass.

Lice: We used a compound microscope to record the following morphological measurements on slide-mounted specimens of *Craspedorrhynchus* spp. (to nearest 0.01 mm): head capsule length at the midline, head capsule width (at the temples), prothorax width, and pterothorax width. In total, 8 males and 8 females collected from *B. swainsoni* and 12 males and 12 females from *B. galapagoensis* were measured, including some of the specimens used in the genetic analyses.

Principal components analyses

For each data set (lice and hosts), we subjected the morphological measurements to a principal components analysis (PCA) in SPSS v. 10 (Chicago, Illinois) to determine the degree of overlap between Galápagos and mainland forms in morphospace. Separate analyses were conducted for individuals of each louse sex and for each host sex. We retained 2 components in each analysis (Table III for lice; Table IV for hosts), and eigenvalues were rotated using Varimax. We then compared PC scores (within each sex for each species) to test for significant differences between lice collected from the 2 host species using Mann–Whitney *U*-tests in SPSS.

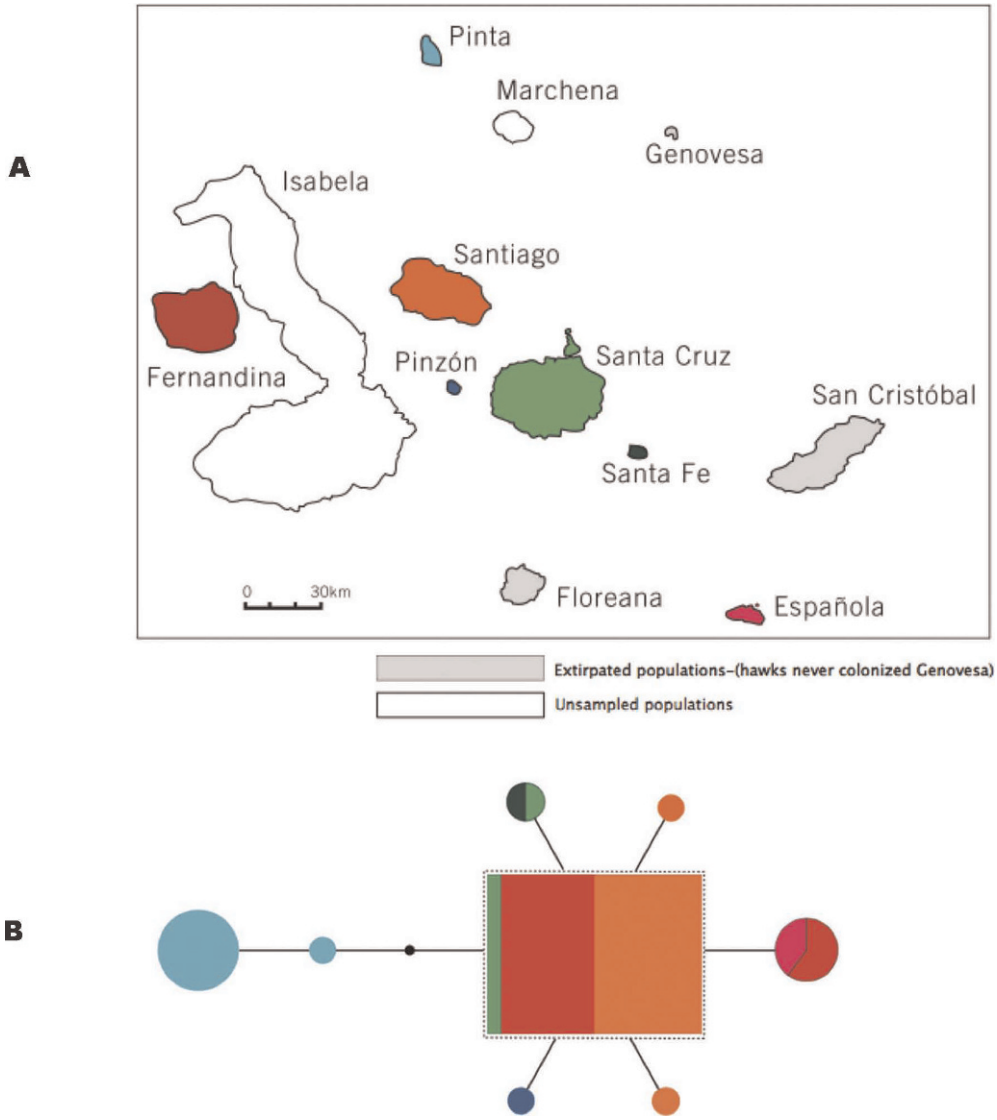

FIGURE 2. (**A**) Map of Galápagos Islands, Ecuador, where each island population sampled is given a different color/grayscale shade. (**B**) A 95% statistical parsimony haplotype network of mitochondrial cytochrome oxidase *c* subunit I sequences from *Craspedorrhynchus* sp. sampled from Galápagos hawks in the Galápagos Islands. Geographical locations are color-coded in the accompanying map. Each connection (dash) between haplotypes represents one mutational step, and small black circles are inferred (unsampled or extinct) haplotypes. Sampled haplotypes are represented by circles or rectangles; squares represent the putative oldest haplotype. If >1 island population harbored a haplotype, its frequency in each is indicated by the pie diagrams or the proportionally divided rectangles.

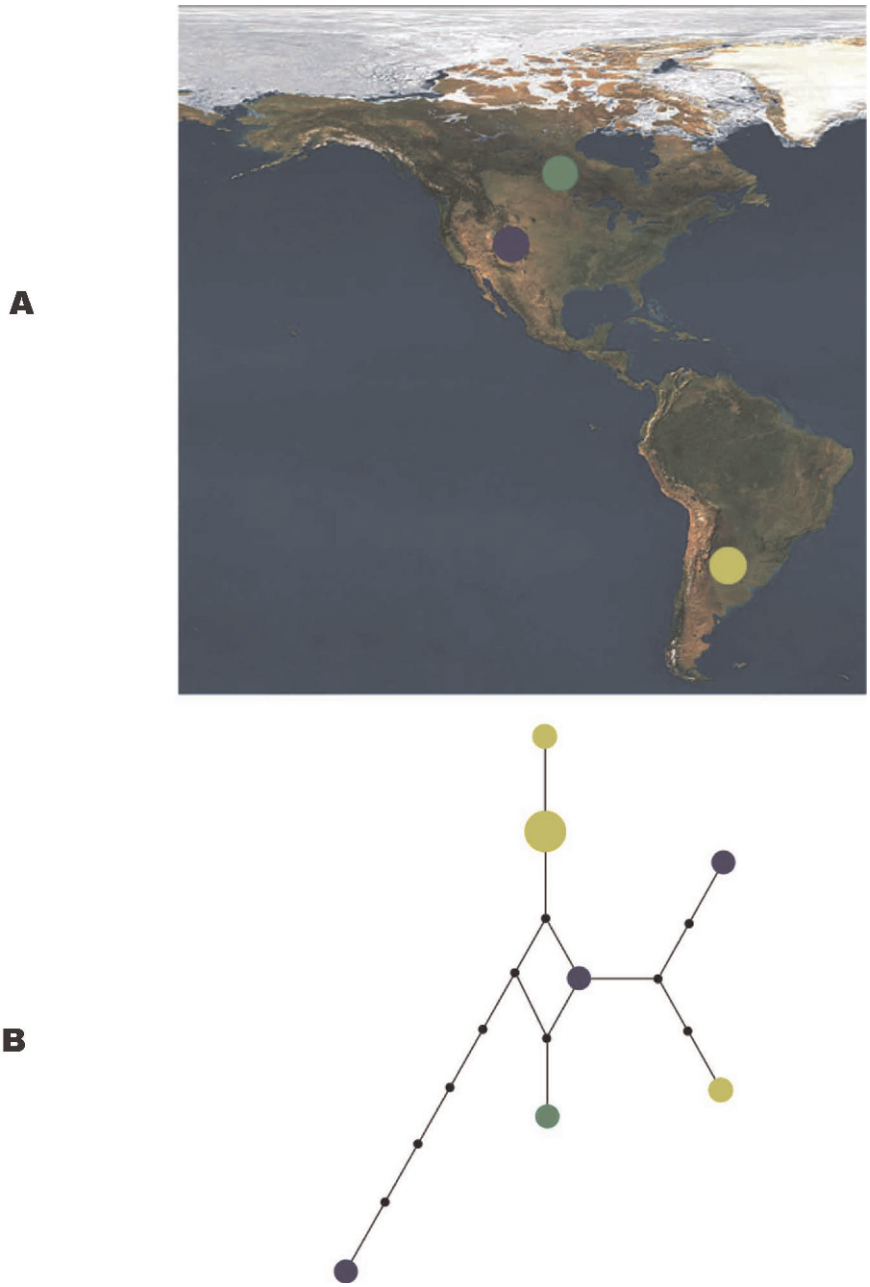

FIGURE 3. (**A**) Map of North and South America showing approximate sampling localities. (**B**) A 95% statistical parsimony haplotype network of mitochondrial cytochrome oxidase *c* subunit I sequences from *Craspedorrhynchus* sp. sampled from Swainson's hawks breeding on the North American mainland (New Mexico in blue and Manitoba in green) and over-wintering on the South American mainland (Argentina in yellow) (Colors are online/grayscale shades in print). Geographical locations are color-coded in the accompanying map. Each connection (dash) between haplotypes represents one mutational step, and small black circles are inferred (unsampled or extinct) haplotypes. Sampled haplotypes are represented by circles; small sample size did not permit estimation of the oldest haplotype. If >1 island populations harbored a haplotype, its frequency in each is indicated by the pie diagrams.

RESULTS

Phylogenetics

Uncorrected COI p-distances among the 44 sequences of lice ranged from 0 to 10.93%. Within lice from Galápagos hawks, genetic distance ranged from 0 to 0.81%, and genetic distance within lice from Swainson's hawks ranged from 0 to 1.62%. The genetic distance between lice from the 2 hosts ranged from 9.99 to 10.93%. The maximum likelihood search in Paup* yielded a single most likely tree (Fig. 1). Two main *Craspedorrhynchus* lineages sorted out perfectly with respect to host species (Fig. 1); each host-specific clade is separated by ~10% uncorrected p-distance. A bootstrap analysis showed strong support for the monophyly of 2 *Craspedorrhynchus* clades, 1 from Swainson's hawks and the other from Galápagos hawks. Moderate support was also obtained for sub-clades within each of the 2 main clades, including a clade restricted to those lice from the island of Pinta.

TABLE III. Results of principal components analysis on male and female *Craspedorrhynchus* lice.

	% Variation explained by 1st and 2nd component	Variable rotated component matrix	Rotated component matrix	
			Component 1	Component 2
Males	49.72, 31	Head length	0.843	0.250
		Head width	−0.018	0.919
		Prothorax width	0.240	0.834
		Pterothorax width	0.926	−0.022
Females	58.87, 20.75	Head length	0.913	0.101
		Head width	0.086	0.933
		Prothorax width	0.854	0.282
		Pterothorax width	0.493	0.642

Population genetics

The 494 bp fragment of COI yielded 64 segregating sites and 66 mutations over all 44 louse individuals from both host species. Among 15 total haplotypes, 8 were unique to those lice collected from Galápagos hawks, and 7 were unique to those lice collected from Swainson's hawks (Table I). Among the 66 total mutations, 61 were synonymous, while 5 were replacement changes. Lice from Galápagos hawks exhibited less nucleotide and haplotype diversity than the population from Swainson's hawks (Table I). Whereas 25% of the substitutions in the Galápagos population were not synonymous, only 7.14% of those in the Swainson's hawk population were not synonymous. There was population genetic structure among islands within the Galápagos *Craspedorrhynchus* spp. lineage.

The haplotype network for *Craspedorrhynchus* spp. within the Galápagos Archipelago indicates that the oldest haplotype is the most common haplotype found in lice from the large, centrally situated islands of Fernandina, Santa Cruz, and Santiago. A private haplotype from lice sampled on Pinta Island was fixed in almost all individuals (Fig. 2). The network for *Craspedorrhynchus* spp. within Swainson's hawks showed that haplotypes were more distantly related than in the Galápagos lineage. Haplotypes from breeding and fledgling Swainson's hawks in New Mexico were scattered throughout the haplotype network (Fig. 3). Two closely related haplotypes were found from lice collected from over-wintering Swainson's hawks in Argentina.

Morphology

Hosts: The first 2 principal components explained ~90% of the morphological variation within male and ~88% of the variation within female hosts. When these 2 factor loadings were plotted, individuals from 1 host species strongly clustered in morphospace, and there was virtually no overlap (Fig. 4). We found significant differences between Galápagos and Swainson's hawks in the first and second principal components, for both males and females ($P < 0.001$). This likely reflects differences in overall body size in the first component.

The 2 host species are distinct morphologically (Fig. 5). Although dark-phase Swainson's hawks are similar in plumage color to that in Galápagos hawks, regardless of plumage color, each is readily diagnosable based on body size alone, because Galápagos hawks are significantly larger than Swainson's hawks. Specifically, body mass, hallux length, and culmen chord do not overlap between the 2 hawk species, in either sex (Fig. 4).

Lice: The first 2 principal components explained ~80% of the morphological variation within male and female *Craspedorrhynchus* spp. When these 2 factor loadings were plotted, individuals from 1 host species loosely clustered in morphospace, but there was some degree of overlap (Fig. 6). We found significant differences between lice collected from Galápagos and Swainson's hawks in the first principal component of female lice ($P < 0.001$) and in the second principal component in the male lice ($P < 0.01$), which likely reflect differences in overall body size.

Craspedorrhynchus spp. lice are very conserved morphologically. In addition to the samples from the 2 species of *Buteo* mentioned already, we compared specimens of 3 species (*Craspedorrhynchus pachypus* [Giebel, 1874], *C. melittoscopus* Nitzsch [in Giebel, 1874], and *C. reichelti* Mey, 2001) from hosts belonging to 3 other genera related to *Buteo* (*Haliastur*, *Pernis*, and *Aquila*). While morphological differences are certainly present among these species, they are not outstanding. We cannot

TABLE IV. Results of principal components analysis on male and female Galápagos and Swainson's hawks.

	% Variation explained by 1st and 2nd component	Variable rotated component matrix	Rotated component matrix	
			Component 1	Component 2
Males	64.1, 25.6	Wing chord	0.121	0.993
		Culmen length	0.922	0.117
		Hallux chord	0.931	0.112
		Mass	0.912	0.107
Females	59.2, 28.6	Wing chord	0.280	0.958
		Culmen length	0.894	0.190
		Hallux chord	0.883	0.291
		Mass	0.842	0.327

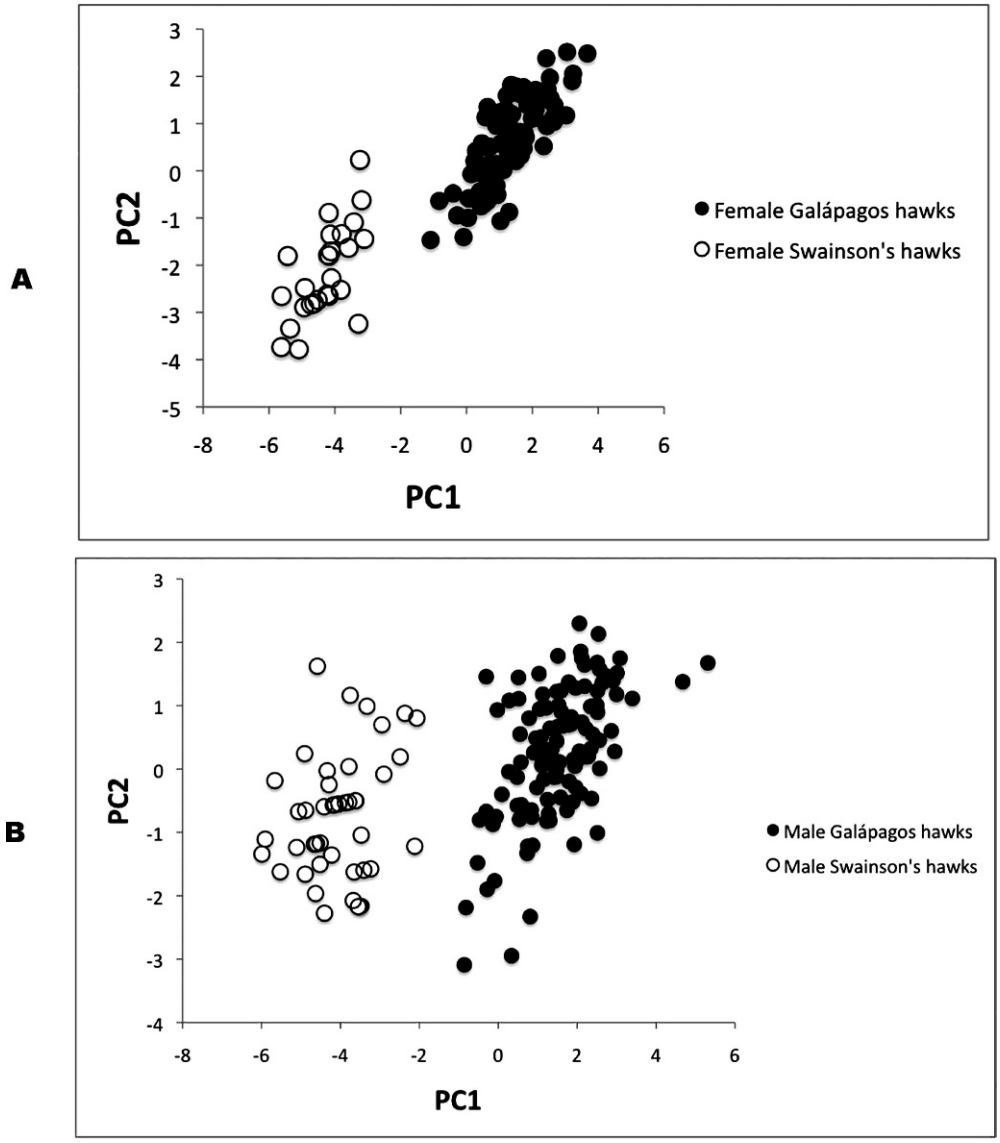

FIGURE 4. Principal components analysis of morphological variation in female (**A**) and male (**B**) Gálapagos and Swainson's hawks.

ascertain, at present, to which species the *Craspedorrhynchus* lice collected for this study belong. Both sexes of the 2 *Craspedorrhynchus* populations parasitic on *Buteo galapagoensis* and *B. swainsoni* can be distinguished morphologically by subtle differences in the preantennal area of the head (males are shown in Fig. 7, upper), especially the shape of pigmented areas of the premarginal carina and the shape of the clypeal signature (dorsal anterior plate). The mesosomal sclerites of male genitalia (Fig. 7, lower) and the sclerites of female terminalia also exhibit subtle, but consistent, differences between specimens from those 2 louse populations.

DISCUSSION

At the macroevolutionary scale, neutral molecular evolution in Phthiraptera and many other parasites proceeds more rapidly than in their hosts, while parasites tend to be morphologically conservative relative to their hosts. In this study, we provide evidence that this pattern is also found at the microevolutionary scale in 2 species of feather lice and their hawk hosts. On the one hand, the 2 louse lineages are very similar morphologically, yet they are separated by ~10% mitochondrial genetic distance. Moreover, within each louse clade, there is significant genetic variation at the same locus, which is structured geographically within the Galápagos Islands, consistent with genetic data from the host (Bollmer et al., 2005, 2006) and 4 other ectoparasite species (Whiteman et al., 2006, 2007), suggesting that water is an effective barrier to host and parasite dispersal. Parasites from each host species clustered loosely in morphospace according to host, but there was still some degree of overlap such that the 2 species are not easily diagnosable morphologically. On the other hand, the hosts are so distinct morphologically that their close evolutionary relationship was only recently confirmed (Riesing et al., 2003; Hull, Savage et al., 2008), yet they differed by only 1 substitution across the 5′ end of COI, a marker used widely to identify animal species (the DNA barcode; Hebert et al., 2003),

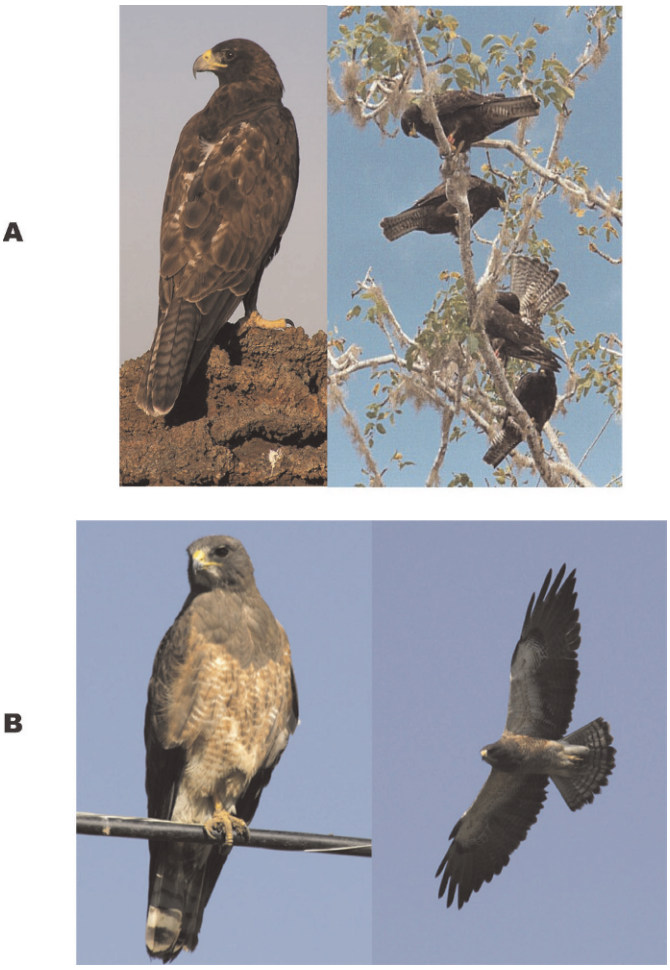

FIGURE 5. Photographs of Galápagos hawks (**A**) and Swainson's hawks (**B**).

including both lice (Whiteman et al., 2004) and birds (Hebert et al., 2004; Kerr et al., 2007). Within each host lineage, no genetic variation was observed; the single substitution is fixed within each lineage, in stark contrast to the variability within each louse clade. Nonetheless, this also shows that this single, fixed substitution in the 5′ region of COI successfully diagnosed each *Buteo* species.

The substitution rate difference between host and parasite is perhaps not surprising given that macroevolutionary studies involving birds and lice have produced similar patterns. However, because the rate difference is far greater in the parasites relative to the hosts, alternative explanations must be evaluated. Direct comparisons between divergence estimates of this island and mainland host–parasite pair assume that the *Craspedorrhynchus* lineage on Galápagos hawks was brought to the islands with the original hawk founders. This is a reasonable assumption because all members of the *Craspedorrhynchus* lineage are only reported from falconiform birds, with the exception of the clear case of straggling between birds in game bags discussed previously herein. The Galápagos hawk is the only resident falconiform known to have inhabited the Galápagos Islands based on historical records and subfossils (Steadman and DeLeon, 1999). Nonetheless, it is impossible to confirm whether this louse lineage was indeed brought to the archipelago with the Galápagos hawk founders. However, all island populations of hawks appear to have this species. Moreover, the lineage is monophyletic within Galápagos, individuals from Galápagos and Swainson's hawks are almost indistinguishable morphologically, and all other members of this lineage are highly host specific. It is possible that Galápagos hawks acquired individuals of the *Craspedorrhynchus* lineage currently parasitizing its island populations from other bird species living in the Galápagos Islands at the time of colonization, or thereafter, which would give rise to the pattern observed in the molecular analysis. Because COI substitutions saturate rapidly over evolutionary time, other loci, including nuclear loci from a broader sampling of Neotropical *Craspedorrhynchus*, would help test this alternative hypothesis. Although the early reports of straggling of lice between distantly related bird species within the Galápagos have been shown to be highly dubious in several analyses, it is still possible that there is increased straggling in island populations of birds relative to those on the mainland (Whiteman et al., 2004), and it is possible that hosts other than *B. galapagoensis* also harbor *Craspedorrhynchus* species in the Galápagos, despite intensive sampling from nearly all endemic and introduced birds (N. Whiteman and R. Palma, pers. obs.). Thus, although the present evidence favors the scenario in which species of *Craspedorrhynchus* lice co-colonized the Galápagos Islands with their hosts, it remains a hypothesis to be tested.

What might explain the morphological results? Island adaptation of Galápagos hawks likely resulted from strong natural selection on morphology (Hull et al., 2008), but it is less likely that parasites inhabiting the head feathers of this host experienced strong natural selection on morphology. Although there was less genetic variation in the Galápagos *Craspedorrhynchus* clade than in those sampled from Swainson's hawks, there appeared to be more population structure in the Galápagos clade, consistent with data from the host and 3 other ectoparasite species, which indicate significant population structure within the Galápagos. However, definitive conclusions regarding the degree of population genetic structure within each major clade are premature until more hosts and populations are sampled. Sample sizes for several of the island populations were extremely small, including 1 or 2 individuals (Española, Pinzon, Santa Cruz, and Santa Fe), and the island populations of Marchena and Isabela were not sampled. Nonetheless, even from the relatively small sample sizes on the mainland, there is considerably more genetic diversity among the *Craspedorrhynchus* spp. from Swainson's hawks than among those from Galápagos hawks.

The 2 main *Craspedorrhynchus* lineages (Fig. 1) are clearly genetically isolated from each other and are likely distinct subspecies or species. Johnson et al. (2002) showed that COI variation between species within dove louse genera varied from 8.9 to 25.6%. The discrete morphological differences between the *Craspedorrhynchus* spp. populations from *Buteo galapagoensis* and *B. swainsoni*, although not obvious without detailed examination, are consistent from a taxonomic viewpoint, and, although there is considerable overlap of quantitative traits in morphospace, each population forms a cluster. Interestingly, within the Galápagos *Craspedorrhynchus* clade, the proportion of nonsynonymous substitutions (25% of the total) relative to synonymous substitutions was much larger than in the mainland clade (7% of the total), suggesting a potentially strong role of genetic drift in these small island populations. Genetic drift, a potent evolutionary force in small populations, can cause slightly deleterious mutations to become fixed at a faster rate in small

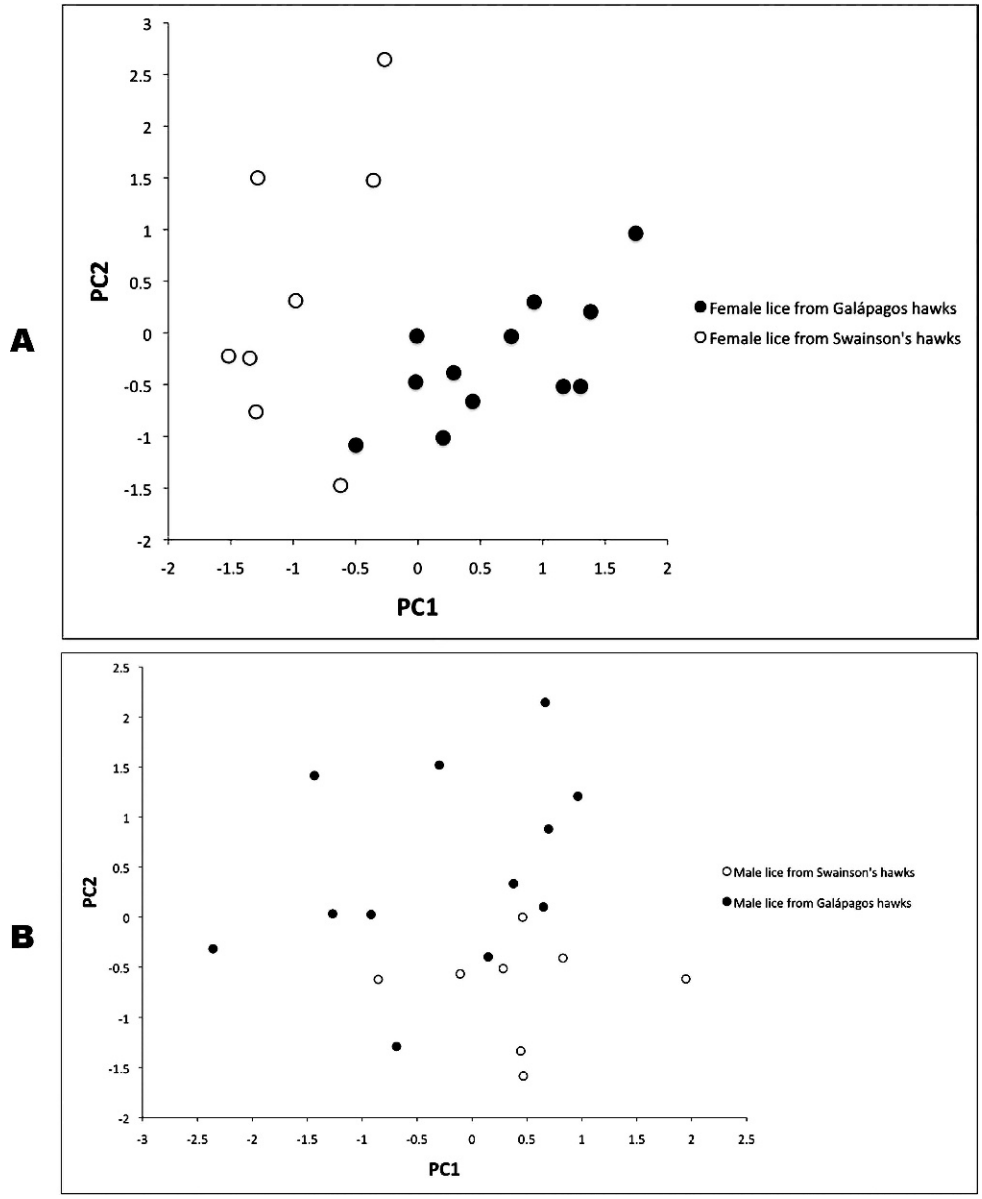

FIGURE 6. Principal components analysis of morphological variation in female (**A**) and male (**B**) *Craspedorrhynchus* sp. lice from Gálapagos and Swainson's hawks.

populations than in larger ones (Johnson and Seger, 2001), which may contribute to the observed pattern. This pattern was also observed at the 3′ end of COI within Galápagos hawks (Bollmer et al., 2006), in which 4 of 5 substitutions were not synonymous, while the single substitutions observed within the Swainson's hawk population were synonymous. The pattern for the 5′ end of COI from Galápagos *Craspedorrhynchus* sp. is consistent with that found in 2 other louse species of the Galápagos hawk, *C. turbinatum*, in which 25% of substitutions were not synonymous, and *D. regalis*, in which 31% were not synonymous (Whiteman et al., 2007) at the same locus. This could suggest similar demographic dynamics in the recent evolutionary history of this host–parasite community.

There are currently 41 valid species included in *Craspedorrhynchus* (see Price et al., 2003; Valim, 2006), but a complete and detailed revision of all species in the genus is not available. In addition, most published descriptions are not detailed enough to show unambiguous diagnostic differences among species, especially in their illustrations of male genitalia. Therefore, we believe it would be unwise to apply a species name to the *Craspedorrhynchus* populations from *B. galapagoensis* and *B. swainsoni* before a complete revision of the genus, including the examination of type material or material from type hosts for all species, becomes available. Although a definitive conclusion must wait until more *Craspedorrhynchus* species are included in a phylogenetic analysis, the 2 louse populations from Galápagos and Swainson's hawks are considered the same species using morphological characters and are well within the genetic distances typically observed for chewing lice. Nonetheless, we agree with Johnson et al. (2007), that morphological and molecular data from these parasites can be used to reciprocally illuminate and identify taxonomic boundaries in feather lice.

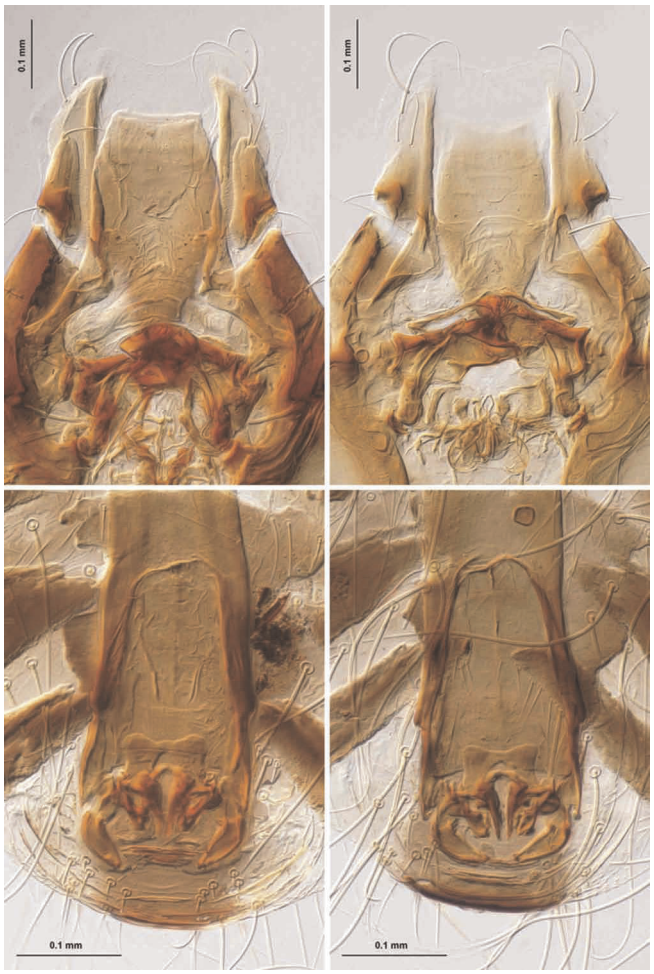

FIGURE 7. Micrographs of slide-mounted *Craspedorrhynchus* sp. male lice from Swainson's hawks (**left**) and Galápagos hawks (**right**). **Upper**: preantennal area of head. **Lower**: genitalia.

Although Kellogg and Kuwana (1902) wrote with disappointment that the results of their survey of lice on Galápagos birds were not able to illuminate ". . . the relationships of these birds to continental genera and species. . .," Clay (1958), in her revision of *Degeeriella* spp. lice, pointed out that *D. regalis* was found in the New World only on *Buteo galapagoensis* and *B. swainsoni*, providing the first piece of parasitological evidence that these 2 hosts were closely related. Here, in addition to the recent molecular evidence from species of *Buteo* (Riesing et al., 2003; Bollmer et al., 2006; Hull, Savage et al., 2008), we add another piece of evidence, i.e., a species in the highly host-specific lice *Craspedorrhynchus* spp., previously unreported from Swainson's hawks, also appears to reflect a close evolutionary relationship between these 2 *Buteo* species. However, given that ~0% sequence divergence exists between these 2 louse populations, and only 0.20% divergence exists between the hosts at the homologous locus, the 2 louse lineages, whether nominally conspecifics, are diverging at this locus more rapidly than their hosts.

Our results also suggest that *Craspedorrhynchus* spp. from islands closer to one another geographically (Fernandina and Santiago) were less genetically differentiated than those individuals from islands that were farther apart geographically (Table II). Host individuals from Pinta, which are the most genetically isolated of the 3 islands compared (Bollmer et al., 2005), also had the most genetically isolated *Craspedorrhynchus* sp. population (Table II), consistent with other lice on these birds (Whiteman et al., 2007). This is consistent with water serving as an effective barrier to *Craspedorrhynchus* sp. dispersal at relatively small distances, a pattern found to varying degrees in 3 other co-occurring parasites specific to the Galápagos hawk (Whiteman et al., 2007) and in the host (Bollmer et al., 2005, 2006). At a broader geographic scale, the few samples of *Craspedorrhynchus* sp. we were able to analyze suggest that there is little population genetic structure, but high genetic diversity, in lice collected from Swainson's hawks, which is consistent with patterns in the host, at least with respect to breeding populations in North America (Hull et al., 2008), which exhibited high diversity and weak population genetic structure. Nonetheless, it would be interesting to determine if, with larger sampling effort, there is population genetic structure in *Craspedorrhynchus* spp. lice across the breeding range of Swainson's hawks. Future studies should focus on testing the monophyly of *Craspedorrhynchus* from Galápagos and Swainson's hawks, which, if confirmed, would suggest that this louse lineage co-colonized the archipelago with the Galápagos hawk's founders, which will require a taxonomic revision and robust phylogenetic hypothesis including all extant species.

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