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ORIGINAL ARTICLE

Tracking the origins of lice, haemosporidian parasites and feather mites of the Galápagos flycatcher (*Myiarchus magnirostris*)

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ABSTRACT

Aim To discover the origins of the lice, haemosporidian parasites and feather mites found on or in Galápagos flycatchers (*Myiarchus magnirostris*), by testing whether they colonized the islands with the ancestors of *M. magnirostris* or if they were acquired by *M. magnirostris* after its arrival in the Galápagos Islands.

Location The Galápagos Islands (Ecuador) and north-western Costa Rica.

Methods We collected lice, feather mites and blood samples from *M. magnirostris* on seven of the Galápagos Islands (n = 254), and from its continental sister species, *M. tyrannulus*, in Costa Rica (n = 74), and identified them to species level using traditional taxonomy and DNA sequencing.

Results The blood parasites from the two bird species were different: *Plasmodium* was found only in *M. tyrannulus*, while a few individuals of *M. magnirostris* were infected by *Haemoproteus multipigmentatus* from Galápagos doves (*Zenaida galapagoensis*). *Myiarchus tyrannulus* was parasitized by three louse species, two of which (*Ricinus marginatus* and *Menacanthus distinctus*) were also found on *Myiarchus magnirostris*. We also collected one louse specimen from *M. magnirostris*, which was identified as *Brueelia interposita*, a species commonly found on finches and yellow warblers from the Galápagos, but never recorded on *M. tyrannulus*. The richness of mite species was lower for *M. magnirostris* than for *M. tyrannulus*; all mite species or genera from *M. magnirostris* were also sampled on *M. tyrannulus*, but *M. tyrannulus* had two additional mite species.

Main conclusions Our results revealed that two of the louse and three of the mite species we found on *M. magnirostris* are likely to have come to the archipelago with these birds' colonizing ancestors, but that one louse and one haemosporidian species were acquired from the Galápagos bird community after the arrival of the *M. magnirostris* lineage. We also confirmed that, for closely related hosts, island mite richness was lower than on the continent.

Keywords
Costa Rica, feather mites, Galápagos, Haemosporida, island biogeography, island colonization, *Myiarchus*, Phthiraptera.

INTRODUCTION

Studies of natural colonization of islands by living organisms have contributed greatly to the development of biogeography as a science (e.g. MacArthur & Wilson, 1967; Whittaker, 1998; Losos & Ricklefs, 2010). Discovering the geographical origins of colonists that successfully arrive and become established on isolated islands, often differentiating into new species, is an important part of these studies. Perhaps the best-described pattern generated from such studies is that, due to their isolation and limited size, islands present lower species diversity than larger continental areas (MacArthur & Wilson, 1967; Whittaker, 1998). Despite this extensive research, island biogeography of parasites has received minimal attention (Nieberding *et al.*, 2006), and the colonization histories of island parasites are mostly unknown.

The study of host–parasite relationships and distributions is important for an understanding of the biogeography of

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both groups (McDowall, 2000; Lafferty *et al.*, 2010). For instance, associations between different groups of fishes and their metazoan parasites have elucidated the colonization histories of the parasites (e.g. Plaisance *et al.*, 2008), the historical biogeography of the hosts (e.g. McDowall, 2000), and that of both the hosts and their parasites (Carney & Dick, 2000; Choudhury & Dick, 2001). These studies are also important because the pressure from parasites could influence the contraction and reduction of their host species (taxon cycling) in space and time (Ricklefs, 2011).

Here, we present a novel study about the origin of three taxonomic groups that are found in close association with the endemic Galápagos flycatcher (*Myiarchus magnirostris* (Gould, 1838); Passeriformes: Tyrannidae): lice, haemosporidian parasites and feather mites. We refer to feather mites as symbionts rather than parasites, because there is little evidence that they negatively affect their hosts' fitness (see Galván *et al.*, 2012).

The Galápagos Islands (Fig. 1) are separated by *c.* 1000 km of open water from the nearest mainland in Ecuador (Jackson, 1993; Geist, 1996). This archipelago has low species diversity in comparison with other islands or close continental landmasses (Linsley & Usinger, 1966; Jackson, 1993), representing a simpler community with fewer potential numbers of species interactions. Several lice, blood parasites and mites have been studied in Galápagos birds (see Table 1); these islands therefore present an interesting opportunity to understand the interactions between these symbionts and their bird hosts and also to understand their colonization histories. Studying the origins of parasites from an endemic island species is also important because island populations are considered to be vulnerable to new diseases (Parker *et al.*, 2006; Lindström *et al.*, 2009). Island species have small and isolated populations, and might have lost their resistance to pathogens because of the pronounced colonization bottleneck, through genetic drift, or due to the absence of selective pressure by parasites (Frankham, 1998). The introduction of non-native parasites and pathogens to the Galápagos is of great concern for the conservation of its unique and intact avifauna (Wikelski *et al.*, 2004; Parker *et al.*, 2006), so it is important to elucidate the colonization histories of the parasites themselves (Lindström *et al.*, 2009).

The distribution patterns of hosts and their parasites (and other symbionts) are influenced by both their intrinsic coevolutionary dynamics, such as cospeciation and host range expansion events, and also by ecological changes and the external environment (Thompson, 2005; Ricklefs, 2010). When host species colonize new locations, they can lose, transfer or gain parasites (Lafferty *et al.*, 2010) and other symbionts. We can therefore classify the bird symbionts (parasites and others) found on the Galápagos Islands into three groups according to their origin: (1) those that came to the islands with the ancestors of their host species; (2) those that were acquired following colonization from other host species in the native bird community; and (3) those that were introduced to the islands by humans. In order to define the origins of the symbionts found on an endemic species from the Galápagos, it is necessary to discover which of them are found on the continental closest relatives of their hosts and to compare them with the parasites and other symbionts found on the native bird community in the Galápagos. Here, we use this approach to find the origins of the chewing lice (Order Phthiraptera), feather mites (Hyporder Astigmata; Schatz *et al.*, 2011) and blood parasites (Order Haemosporida) of the Galápagos flycatchers.

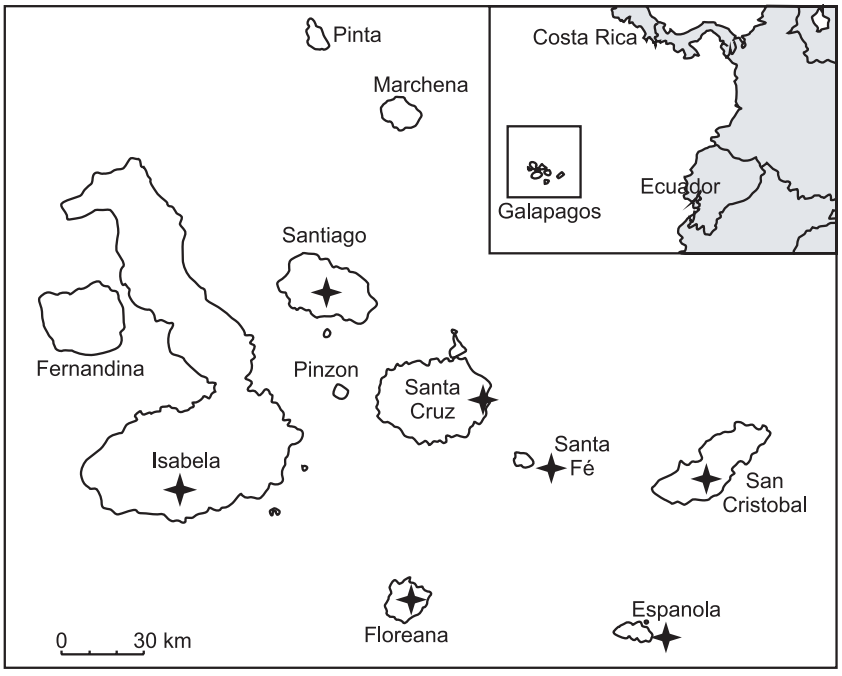

Figure 1 Map of the Galápagos archipelago showing the main islands where *Myiarchus magnirostris* is distributed. Sampled islands are indicated with stars. The insert shows the position of the Galápagos Islands relative to Costa Rica, where samples from *M. tyrannulus* were collected, and to continental Ecuador, its nearest mainland.

Table 1 Bird parasites and mites (symbionts) recorded from the Galápagos Islands that are relevant for this study. Within taxonomic groups, species are in alphabetical order.

Taxonomic group	Symbiont species	Hosts in the Galápagos	References
Blood parasites (Order Haemosporida)	*Haemoproteus (Haemoproteus) iwa*	Great frigatebird (*Fregata minor*); Magnificent frigatebird (*Fregata magnificens*).	Padilla *et al.* (2006); Levin *et al.* (2011).
	Haemoproteus (Haemoproteus) jenniae	Swallow-tailed gull (*Creagrus furcatus*)	Levin *et al.* (2012)
	Haemoproteus (Haemoproteus) multipigmentatus	Galápagos dove (*Zenaida galapagoensis*)	Santiago-Alarcon *et al.* (2010); Valkiūnas *et al.* (2010).
	Haemoproteus (Haemoproteus) sp.	Red-footed booby (*Sula sula*); Swallow-tailed gull (*Creagrus furcatus*); Nazca booby (*Sula granti*).	Padilla *et al.* (2006); Levin *et al.* (2011).
	Haemoproteus (Parahaemoproteus) sp.	Blue-footed booby (*Sula nebouxii*)	Levin *et al.* (2011)
	Plasmodium sp.	Galápagos penguin (*Spheniscus mendiculus*)	Levin *et al.* (2009)
Chewing lice (Order Phthiraptera)	*Brueelia chelydensis*	Large tree finch (*Camarhynchus psittacula*); Medium ground finch (*Geospiza fortis*); Small ground finch (*Geospiza fuliginosa*); Large cactus finch (*Geospiza conirostris*).	Linsley & Usinger (1966); Price *et al.* (2003).
	Brueelia galapagensis	Galápagos mockingbirds (*Mimus* spp.); Small ground finch (*Geospiza fuliginosa*).	Price *et al.* (2003); Štefka *et al.* (2011).
	Brueelia interposita	Yellow warbler (*Dendroica petechia aureola*).	Linsley & Usinger (1966); R.L. Palma, pers. comm.
	Colpocephalum turbinatum; *Craspedorrhynchus* sp.; *Degeeriella regalis.*	Galápagos hawk (*Buteo galapagoensis*).	Price *et al.* (2003); Whiteman *et al.* (2007, 2009).
	Columbicola macrourae; *Physconelloides galapagensis.*	Galápagos dove (*Zenaida galapagoensis*).	Johnson & Clayton (2002); Price *et al.* (2003).
	Columbicola macrourae; *Physconelloides galapagensis.*	Galápagos hawk (*Buteo galapagoensis*) – atypical host/straggler	Whiteman *et al.* (2004)
	Menacanthus distinctus	Galápagos flycatcher (*Myiarchus magnirostris*)	R.L. Palma, pers. comm.
	Myrsidea darwini	Large tree finch (*Camarhynchus psittacula*); Small ground finch (*Geospiza fuliginosa*); Large ground finch (*Geospiza magnirostris*).	Palma & Price (2010)
	Myrsidea nesomimi	Galápagos mockingbirds (*Mimus* spp.); and recorded straggling events to several Darwin finch species.	Palma & Price (2010); Štefka *et al.* (2011).
	Myrsidea ridulosa	Yellow warbler (*Dendroica petechia aureola*)	Palma & Price (2010)
	Philopterus insulicola	Galápagos vermillion flycatcher (*Pyrocephalus rubinus nanus*)	Linsley & Usinger (1966); Price *et al.* (2003).
	Ricinus marginatus	Galápagos flycatcher (*Myiarchus magnirostris*)	Price *et al.* (2003)
	Other louse species	Darwin finches	Price *et al.* (2003)
Feather mites (Hyporder Astigmata)	*Amerodectes atyeoi*; *Dermoglyphus* sp.; *Mesalgoides geospizae*; *Proctophyllodes darwini*; *Strelkoviacarus* sp.; *Trouessartia geospiza*; *Xolalges palmai.*	Darwin ground finches (*Geospiza* spp.)	Mironov & Pérez (2002); OConnor *et al.* (2005); Lindström *et al.* (2009).
	Analges spp. (four species)	Galápagos mockingbirds (*Mimus* spp.)	Štefka & Smith, pers. comm.; Štefka *et al.* (2011).
Parasitic flies (Order Diptera; Family Hippoboscidae)	*Microlynchia galapagoensis*	Galápagos dove (*Zenaida galapagoensis*); Galápagos mockingbird (*Mimus parvulus*).	Valkiūnas *et al.* (2010); Deem *et al.* (2011).
	Ornithoica vicina	Yellow warbler (*Dendroica petechia aureola*); Large tree finch (*Camarhynchus psittacula*); Darwin ground finches (*Geospiza* spp.); Galápagos mockingbirds (*Mimus* spp.); Short-eared owl (*Asio flammeus*).	Deem *et al.* (2011)

See also Deem *et al.* (2011) and Parker *et al.* (2006) for other compilations of Galápagos bird parasite studies.

The Galápagos flycatcher (*M. magnirostris*) is endemic to the Galápagos archipelago, where it inhabits a variety of habitats and a broad elevational range on most of the islands (Jackson, 1993). Recently, we proposed that the *Myiarchus* colonizers that gave origin to *M. magnirostris* arrived from Central America *c.* 850,000 years ago, and that its closest living relative is *Myiarchus tyrannulus* (Statius Müller, 1776) from Central and North America (Sari & Parker, 2012). In order to find the origin of the parasites and other symbionts from *M. magnirostris*, we have collected lice, feather mites and blood samples from this bird species and from *Myiarchus tyrannulus* in Costa Rica.

We hypothesize that (1) the parasites and feather mites that are present in both host species arrived in the Galápagos archipelago with the ancestors of *M. magnirostris*, and (2) those that are found on *M. magnirostris* but are not found on *M. tyrannulus* were acquired after *M. magnirostris* arrived on the Galápagos, either from another bird species native to the Galápagos or by human introduction. Furthermore, we hypothesize that the island bird host, *M. magnirostris*, will present lower parasite and mite species richness than the continental species *M. tyrannulus*. This is, to our knowledge, the first study that elucidates the origin of multiple taxonomic groups that live in close association with an island host.

MATERIALS AND METHODS

Collection of samples

We captured 254 Galápagos flycatchers (*M. magnirostris*) on seven of the Galápagos Islands (Fig. 1) and 74 brown-crested flycatchers (*M. tyrannulus*) in four localities in Costa Rica (Table 2). Birds were attracted to mist nets using recordings of each species' song and were released after samples were collection. Blood samples were collected from all birds using heparinized capillary tubes. A few drops of blood were used to make two or three blood smears and the rest was stored in lysis buffer until DNA extraction. Blood smears were fixed in methanol for 3 min at the end of each sampling day.

Lice and feather mites were sampled via dust ruffling of the birds using 1% pyrethroid insecticide (Zodiac Flea & Tick Powder; Wellmark International, Schaumburg, IL, USA). We worked approximately half to one teaspoon of the powder into birds' feathers and body (including the head), and let it sit while biometric measurements were taken, followed by ruffling of the feathers. During ruffling, birds were held over a clean plastic tray to collect dislodged lice and mites, which were collected from the tray using forceps and magnifying glasses and stored in 95% ethanol. Before dust-ruffling, all birds were visually examined, and lice and mites were collected opportunistically using entomological forceps. A total of 94 individuals of *M. magnirostris* and 63 of *M. tyrannulus* were dust-ruffled, but the visual examination and opportunistic collection of lice and mites were performed for 203 individuals of *M. magnirostris* and all 74 captured individuals of *M. tyrannulus*. Prevalence values were calculated for each species as the number of birds that carried that symbiont species divided by the total number of samples analysed (Table 3; Margolis *et al.*, 1982).

Table 2 Number of Galápagos flycatchers (*Myiarchus magnirostris*) and brown-crested flycatchers (*M. tyrannulus*) per island or locality sampled for haemosporidian parasites screening tests and for collection of lice and mites.

Locality	Haemosporidian screen	Visual exam. and/or dust-ruffle	Dust-ruffle
M. magnirostris			
Galápagos Islands	**254**	**203**	**94**
Española	26	26	11
Floreana	33	33	26
Isabela	39	35	20
San Cristóbal	55	47	6
Santa Cruz	70	35	17
Santa Fé	11	11	9
Santiago	20	16	5
M. tyrannulus			
Costa Rica	**74**	**74**	**63**
Palo Verde	19	19	17
Santa Rosa	37	37	33
Horizontes	2	2	2
El Hacha	16	16	11

Haemosporidian parasites screening

We used microscopy and polymerase chain reaction (PCR) techniques to detect the presence of haemosporidian parasites in the blood samples. The blood smears were stained using Giemsa stain as described by Valkiūnas (2005) and inspected for parasites by microscopy for 5 min at 200× magnification, followed by examination of 100 fields at 1000× magnification.

Table 3 Prevalence data (number of infected birds/total birds sampled) for blood parasites, lice and feather mites for the two bird species, Galápagos flycatchers (*Myiarchus magnirostris*) and brown-crested flycatchers (*M. tyrannulus*). Numbers for feather mites was calculated based on examination of all samples from the Galápagos (n = 203) and 34 samples from Costa Rica.

Parasites and mites	*M. magnirostris*	*M. tyrannulus*
Haemoproteus multipigmentatus	2.0% (5/254)	0
Plasmodium sp.	0	52.7% (39/74)
Brueelia interposita	0.5% (1/203)	0
Menacanthus distinctus	0*	14.9% (11/74)
Tyranniphilopterus rufus	0	60.8% (45/74)
Ricinus marginatus	0.5% (1/203)	17.6% (13/74)
Amerodectes sp.	0	2.9% (1/34)
Analgidae	0	8.8% (3/34)
Nycteridocaulus spp.	0.5% (1/203)	26.5% (9/34)
Trouessartia sp.	7.4% (15/203)	76.5% (26/34)
Tyrannidectes berlai	2.5% (5/203)	73.5% (25/34)

*Species recorded for *Myiarchus magnirostris* by R.L. Palma (Museum of New Zealand, Wellington, New Zealand).

Genomic DNA was extracted from blood samples as described in Sari & Parker (2012). We used the method of Waldenström *et al.* (2004) to detect haemosporidian parasites from the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, by amplifying the first region (580 bp) of the mitochondrial gene cytochrome *b* (*cytb*). All samples were screened twice, using slightly different PCR conditions (see below). In each PCR reaction, both a positive control (*Plasmodium*-infected sample) and one or several negative controls (blanks) were used. All samples that amplified parasite DNA only once were retested for confirmation.

For the first DNA amplification, 1 μL of total genomic DNA was used in a 25-μL reaction with 0.625 units of *Ex Taq* DNA polymerase (0.125 μL), 1× *Ex Taq* buffer without $MgCl_2$, 0.2 mM each dNTP, 1.75 mM $MgCl_2$ (*Taq* and reagents from Takara Bio, Shiga, Japan), and 0.4 μM each external primer (HaemNF and HaemNR2; all primers from Sigma-Aldrich, St Louis, MO, USA). The amplification programme comprised 20 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 45 s, followed by a final extension step at 72 °C for 10 min. 1 μL of the amplicon from this reaction was used for a nested reaction with the same reagent concentrations, but using the internal primers HaemF and HaemR2. The amplification programme was the same, but repeated for 35 cycles. For the second PCR screening, the PCR programmes also included a 3-min denaturation step at 94 °C (as in Waldenström *et al.*, 2004) and a lower annealing temperature (48 °C), in order to enhance the detection of parasites.

Amplified internal DNA fragments (524 bp) were detected on a 2.0% agarose gel in TBE (Tris–borate–EDTA) buffer stained with GelStar (Lonza, Walkersville, MD, USA). PCR products were purified with exonuclease 1 and Antarctic phosphatase (New England BioLabs, Ipswich, MA, USA) and sequenced using ABI BigDye Terminator Kit (Applied BioSystems/Life Technologies, Carlsbad, CA, USA) with 30 cycles at 95 °C for 20 s, 50 °C for 10 s and 60 °C for 4 min, and run in an ABI 3100 automatic sequencer (Applied BioSystems/Life Technologies). DNA fragments from all samples were sequenced in both directions using HaemF and HaemR2 (or HemoR; Perkins & Schall, 2002).

We used SeqManII 4 (1989–1999, DNASTAR, Madison, WI, USA) to analyse sequence traces and create contigs. Sequences were aligned using Clustal W with default parameters, as implemented in mega 4.0 (Tamura *et al.*, 2007). Haemosporidian parasite lineages were identified by searching GenBank for sequences that were similar to those we obtained, using the megablast search algorithm.

Louse and mite identification and molecular analyses of lice and their hosts

Mites and lice were initially sorted to morphospecies using a dissecting microscope. For species identification, representative specimens of each morphospecies were slide-mounted and examined by specialists on each taxonomic group (lice, Ricardo L. Palma, Museum of New Zealand, Wellington, New Zealand; mites, H. Klompen; see voucher numbers in Appendix S1 in Supporting Information) using a compound microscope. Afterwards, we used a dissecting microscope to sort and identify to species a total of 204 feather mites and 2 lice from *M. magnirostris*, and 892 feather mites and 496 lice from *M. tyrannulus*.

We used a molecular approach to compare the one *Brueelia* louse specimen we collected from one individual of *M. magnirostris* to sequences of *Brueelia galapagensis* from Galápagos mockingbirds (*Mimus* spp.; Štefka *et al.*, 2011) available on GenBank, and of *Brueelia interposita* from one Galápagos yellow warbler (*Dendroica petechia aureola*) that we sampled opportunistically. DNA was extracted using the voucher method (Cruickshank *et al.*, 2001). We amplified and sequenced a fragment of approximately 650 bp from the mitochondrial gene cytochrome oxidase *c* subunit I (*COI*) using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). PCR conditions were similar to those used for Haemosporida (see above), but including 0.08 mg mL^{-1} bovine serum albumin (BSA). The amplification programme started with denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 60 s, 40 °C for 60 s and 72 °C for 60 s, followed by a final extension step at 72 °C for 7 min.

We also used this molecular approach to obtain *COI* sequences from *Ricinus marginatus* lice collected from the two host species: *M. tyrannulus* and *M. magnirostris*. We wanted to estimate the genetic distance between lice found on both host species and to compare their distance with the genetic divergence between the bird species. We used DNA from four individuals of *Ricinus*: three collected from different *M. tyrannulus* individuals and one from *M. magnirostris*.

We also obtained sequences from *COI* (1550 bp) from *M. magnirostris* ($n = 5$) and *M. tyrannulus* ($n = 4$) following Chaves *et al.* (2008) and using the same PCR conditions as those we used for the louse *COI*, but with an annealing temperature of 61 °C. Sequence characteristics and genetic distances for lice and birds, between samples collected on the Galápagos and on the continent, were calculated in mega 4, using the Tamura–Nei (TN) substitution model. GenBank accession numbers are given in Appendix S2.

RESULTS

Haemosporidian parasites

We obtained a 480-bp alignment of *cytb* sequences from haemosporidian parasites. We detected a high prevalence of *Plasmodium* sp. in *M. tyrannulus* samples from Costa Rica, but the prevalence detected by microscopy (13.5%; 10/74) was lower than the prevalence detected by PCR (52.7%; 39/74). *Plasmodium* was not found in any *M. magnirostris* sample, but we detected *Haemoproteus multipigmentatus* (Valkiūnas *et al.*, 2010) in 5 out of 254 *M. magnirostris* screened by PCR (2% prevalence) (Table 3). No parasites were seen in blood smears from *M. magnirostris*. All DNA sequences from *Plasmodium* obtained from *M. tyrannulus* ($n = 39$) were

identical, and the haplotype found was also described from a variety of bird species around the world (see Beadell *et al.*, 2006).

Lice

The overall prevalence of lice on *M. tyrannulus* was 66% (49/74) when considering all captured birds, and 73% (46/63) when considering only the birds that were dust-ruffled, much higher than the prevalence of lice observed for *M. magnirostris*: 1% (2/203). The lice collected from *M. tyrannulus* belonged to three species: *Ricinus marginatus* (Children, 1836) (Amblycera: Ricinidae), *Menacanthus distinctus* (Kellogg & Chapman, 1899) (Amblycera: Menoponidae), and *Tyranniphilopterus rufus* (Kellogg, 1899) (Ischnocera: Philopteridae), the last presenting the highest prevalence: 60.8% (45/74) (Table 3). These three species have been previously recorded from *Myiarchus tyrannulus* and other species from the genus *Myiarchus* and the family Tyrannidae (Oniki, 1999; Price *et al.*, 2003).

We collected only two individual lice from *M. magnirostris*, including one *Ricinus marginatus* on Santa Cruz, and one *Brueelia interposita* (Kellogg, 1899) (Ischnocera: Philopteridae) on San Cristóbal. While *Ricinus marginatus* has previously been found on *M. magnirostris*, *Brueelia* has never been recorded for *Myiarchus* flycatchers (Price *et al.*, 2003). In addition to *Ricinus marginatus*, specimens of *Menacanthus distinctus* have also been collected from *M. magnirostris* (R. L. Palma, pers. comm. 2011), but we did not find this species in our collections.

Lice and host genetic divergence

Our *COI* sequences of *Brueelia interposita* (677 bp) from *M. magnirostris* and from the Galápagos yellow warbler were identical, but there were 104 segregating sites between these and sequences of *Brueelia galapagensis* from Galápagos mockingbirds (18.1% TN genetic distance), confirming that our specimens are not *B. galapagensis*.

We found 36 polymorphic sites when comparing *Ricinus* from the Galápagos ($n = 1$) and from Costa Rica ($n = 3$) using 608 bp of *COI*. The net genetic distance between lice from these two regions was estimated as $6.27 \pm 0.99\%$. For the hosts (*M. magnirostris* and *M. tyrannulus*), using 611 bp from the same *COI* region that we sequenced for the lice, we found seven polymorphic sites. The net genetic distance between *M. magnirostris* ($n = 5$) and its sister species *M. tyrannulus* ($n = 4$) was estimated as $0.66 \pm 0.31\%$, almost ten times smaller than the genetic distance between the lice.

Mites

Feather mites were found with a total prevalence of 85.1% (63/74) for *M. tyrannulus* and 11.3% (23/203) for *M. magnirostris*. Five species of feather mites were collected from *M. tyrannulus*: *Trouessartia* sp. (Trouessartiidae), *Amerodectes* sp., *Nycteridocaulus* sp. nr. *lamellus* Atyeo, 1966 and *Tyrannidectes berlai* Mironov, 2008 (all Proctophyllodidae), and one species of Analgidae that was not further identified (Table 3). Of these, *Trouessartia* sp., *Tyrannidectes berlai* and *Nycteridocaulus lamellus* were commonly found, but the other two species were only rarely collected.

Three species of mites were collected from *M. magnirostris*: *Trouessartia* sp., *Tyrannidectes berlai* and *Nycteridocaulus* sp. (Table 3); the first two appear to be the same as the species found on *M. tyrannulus*. Identification of *Trouessartia* to species level, however, is hampered by the fact that this group has not been revised recently. In the most comprehensive species-level keys for the genus (Santana, 1976), the *Trouessartia* from *Myiarchus* keys close to *Trouessartia corolligera* (Gaud, 1968), from South Pacific starlings (*Aplonis* spp.), but this revision lacks any records of *Trouessartia* species from Tyrannidae. Valim *et al.* (2011) listed *Trouessartia* as associated with Tyrannidae, but we could not find records of specific identification for these *Trouessartia* species.

The third species collected from *M. magnirostris*, *Nycteridocaulus* sp., does not appear to be identical to the corresponding species in Costa Rica, which is probably *Nycteridocaulus lamellus*. Identification in this group is largely based on males, and although we obtained a few males from Costa Rica, we have none from the Galápagos. However, based on consistent differences in the dorsal ornamentation in females (Fig. 2) we conclude that the specimens from the Galápagos are probably not conspecific with those from *M. tyrannulus* from Costa Rica.

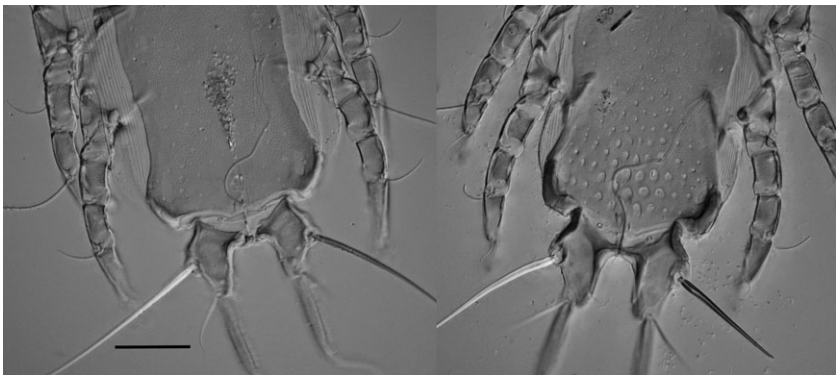

Figure 2 Female specimens of the mite *Nycteridocaulus* (Proctophyllodidae) collected in Costa Rica from *Myiarchus tyrannulus* (left) and in the Galápagos Islands from *M. magnirostris* (right). Between the two host species, morphological differentiation of dorsal ornamentation can be observed for this lineage of mites. The scale bar represents 50 μm.

DISCUSSION

We were interested in understanding the origins of the lice, blood parasites and mites found on or in the Galápagos flycatchers (*M. magnirostris*). We found that different parasite species have different origins. We infer that the haemosporidian parasites detected in *M. magnirostris* were acquired, after its arrival on the islands, from the endemic Galápagos doves (*Zenaida galapagoensis*), that two of the louse species on *M. magnirostris* came to the islands with the ancestors of this host, and that one louse species was acquired from the native bird community. Finally, we found that three species of mites from *M. magnirostris* probably came with the ancestors of these birds to the Galápagos, but morphological differentiation (and perhaps speciation) was observed in one of these mite species.

We also detected a much lower prevalence of parasites and mites for *M. magnirostris* than for *M. tyrannulus* (Table 3). In addition, the total number of mite species found on *M. magnirostris* ($n = 3$) was lower than the number of species found on *M. tyrannulus* ($n = 6$), which supports the expected pattern of lower species diversity on islands compared to continental areas (MacArthur & Wilson, 1967; e.g. Smith & Carpenter, 2006). These findings might reveal an island syndrome, but could also be related to differences in environmental conditions between the Galápagos and Costa Rica; it has been proposed that birds that live in drier environments have fewer lice than birds in more humid locations (Moyer *et al.*, 2002).

Haemosporidian parasites

Haemoproteus multipigmentatus, which we detected in *M. magnirostris*, belongs to the subgenus *Haemoproteus* and is found to parasitize Galápagos doves with very high prevalence and intensity, and also parasitizes dove species from the New World (Santiago-Alarcon *et al.*, 2010; Valkiūnas *et al.*, 2010). The competent host for this parasite in the Galápagos is the fly *Microlynchia galapagoensis* (Hippoboscidae) – a species associated only with the Galápagos doves in the Galápagos (Valkiūnas *et al.*, 2010) – and there are no reports of this fly on a flycatcher.

The *Haemoproteus* parasites that are commonly found parasitizing passerine birds elsewhere in the world belong to the subgenus *Parahaemoproteus* (Beadell *et al.*, 2006; Martinsen *et al.*, 2008). No blood parasites have been reported before for Galápagos passerines (e.g. Lindström *et al.*, 2009), but parasites from the subgenus *Parahaemoproteus* were detected in five blue-footed boobies (*Sula nebouxii*) in the Galápagos (Levin *et al.*, 2011). Parasites from the subgenus *Haemoproteus*, such as *H. multipigmentatus*, were thought to be specific to Columbiformes (doves and pigeons; Santiago-Alarcon *et al.*, 2010), but recently Levin *et al.* (2011) reported the association of this subgenus with frigatebirds and gulls. These parasites, however, have rarely (if ever) been reported for passerines.

Our detection of *H. multipigmentatus* in *Myiarchus magnirostris* was only by PCR, never by microscopy. The absence of meronts or gametocytes (the reproductive stage of a haemosporidian parasite) in blood smears from *M. magnirostris* indicates that these parasites may not be completing their life cycle (Valkiūnas, 2005), and that this bird species might not be a competent host. The occurrence of *H. multipigmentatus* in *M. magnirostris* could be the result from a 'spill-over', where, in rare cases, a hippoboscid fly that has bitten an infected dove could leave its typical host and inject *Haemoproteus* sporozoites into another bird species. These sporozoites could then be detected by PCR, but not in blood smears (Valkiūnas *et al.*, 2009).

The great majority of our *M. magnirostris* samples were collected during the months of July and August, during the Galápagos dry season, but our five samples of *Haemoproteus* were found among the few birds ($n = 27$) captured during the wet season (February–April). Transmission of blood parasites is expected to be more frequent during the wet season because of the increased number of vectors; the greater number of flies available could hence be associated with the spill-over of *Haemoproteus multipigmentatus* into *M. magnirostris*. Also, the five birds with *Haemoproteus* were captured on the island of Santa Cruz, in the city of Puerto Ayora, one of the most urbanized areas of the Galápagos archipelago. We believe that this spill-over could also be caused by environmental disturbance, indicating that human activities could be actively changing the species interactions of the Galápagos natural community. Unfortunately, our data do not allow us to test this hypothesis. Furthermore, Santa Cruz was the island where we collected the greatest number of samples ($n = 70$; Table 1) and our detection of *H. multipigmentatus* in *M. magnirostris* only on that island could therefore be biased.

Lice

The two louse species from both *M. magnirostris* and *M. tyrannulus* – *Ricinus marginatus* and *Menacanthus distinctus* – most probably came to the Galápagos with the ancestors of *Myiarchus magnirostris*. Many species of chewing louse are found only on a single bird host species (Johnson & Clayton, 2003), but here we have an example of host speciation without the speciation of two species of body louse (Amblycera). The same pattern is observed for the endemic Galápagos hawk (*Buteo galapagoensis*), which shares all louse species with its continental sister species, Swainson's hawk (*Buteo swainsoni*; Price *et al.*, 2003; Whiteman *et al.*, 2007, 2009). The Galápagos hawk diverged from its sister species only about 180,000 years ago (Bollmer *et al.*, 2006), while *M. magnirostris* and *M. tyrannulus* diverged about 850,000 years ago, suggesting that the process of speciation for lice can take much longer than it takes for their hosts, as mentioned by McDowall (2000). Another corroborating example of this pattern is seen for the Galápagos dove, which diverged from its continental sister species *c.* 2 Ma (Johnson & Clayton, 2000), and yet the two species share a species of louse (Johnson & Clayton, 2002; Price *et al.*, 2003).

We revealed that the genetic divergence between *Ricinus* (6.27%) collected from the two *Myiarchus* species is

approximately 10 times larger than the divergence between their host species (0.66%). It is possible that, if *Ricinus* has high genetic variation within each *Myiarchus* species, our estimate of the genetic divergence between *Ricinus* from Galápagos and Costa Rica may be somewhat overestimated. We may not have used a large enough sample size to capture this variation, especially as we only collected one *Ricinus* specimen from the Galápagos. Our *Ricinus* samples from Costa Rica collected at three different locations, however, presented no genetic variation. Despite this small sample size, the genetic distance between *Ricinus* from the two *Myiarchus* species would still be much larger than that of their hosts. When Whiteman *et al.* (2009) compared the genetic distance between the head louse *Craspedorrhynchus* found on the Galápagos hawk and on its sister species with the distance between the two host species, they found a difference of the same magnitude. This trend can be explained by the faster generation time for the lice in comparison to their hosts: each generation of a flycatcher (1 year) corresponds to about six generations of a louse (40–60 days; Johnson & Clayton, 2003). In addition, it is thought that louse mitochondrial DNA has a much faster evolutionary rate than the homologous molecules in birds (Page *et al.*, 1998).

This higher genetic divergence obtained between the lice in comparison to their hosts, paired with the invariable morphology for the lice, might be a result of the differences between the environments that hosts and their parasites experience. The speciation of *M. magnirostris* can be explained by drift and also by natural selection, due to the colonization of a new area with a different environment. While drift was also involved in the genetic differentiation of the lice on these two bird sister species, the environments for the lice are the feathers and body of their hosts, which have had little to no structural change and therefore do not represent a selective pressure that would invoke morphological changes in the lice. However, we have not explored the morphology of the louse samples we collected, and it is possible that the taxonomy of *Ricinus* needs to be revisited. Based on our findings, we suggest that general taxonomy of island parasites deserves a closer look.

Finally, we collected a *Brueelia interposita* louse from one individual of *M. magnirostris* but not from *M. tyrannulus*. Three *Brueelia* species are known to occur in the Galápagos: *Brueelia galapagensis* on Galápagos mockingbirds, *Brueelia chelydensis* on four Darwin's finches, and *Brueelia interposita* on Galápagos yellow warblers and three Darwin's finches (Price *et al.*, 2003; e.g. Štefka *et al.*, 2011; R. L. Palma, pers. comm. 2011). Because the *Brueelia* specimens from *M. magnirostris* and from the Galápagos yellow warbler are morphologically and genetically identical, we believe that this represents a classic example of a parasite that was acquired by *M. magnirostris* after its arrival on the islands and interaction with the local community.

Brueelia presents high dispersal ability through phoresis (transport), in which it moves to different hosts by attaching to parasitic flies (Diptera: Hippoboscidae) (Harbison & Clayton, 2011; Štefka *et al.*, 2011). Wing louse species like *Brueelia* frequently present evolutionary histories less associated with their hosts, with fewer cospeciation events (Johnson *et al.*, 2002; Harbison & Clayton, 2011). Štefka *et al.* (2011) studied the phylogeography of Galápagos mockingbirds and three of their ectoparasite species and noted that *Brueelia* had the least population structure, implying that its phoresis on hippoboscid flies in the Galápagos is substantial. Deem *et al.* (2011) reported the presence of the hippoboscid fly *Ornithoica vicina* on several Galápagos terrestrial birds, including the yellow warbler (Table 1) but not *M. magnirostris*. The *Brueelia* we collected could have been transported by this hippoboscid fly from a warbler to *M. magnirostris*. This non-specific dispersal of hippoboscid flies is consistent with our finding that a fly-transmitted blood parasite specific to Galápagos doves (*H. multipigmentatus*) was detected in Galápagos flycatchers.

Mites

Two of the five mite species we identified were identical on *M. tyrannulus* and *M. magnirostris*: *Trouessartia* sp. and *Tyrannidectes berlai*. *Tyrannidectes berlai* was described for *M. tyrannulus* from Brazil and it seems to be specific to hosts in the genus *Myiarchus* (Mironov *et al.*, 2008; Valim & Hernandes, 2010; Valim *et al.*, 2011); its presence in Costa Rica and Galápagos does, however, represent a significant range extension. Similarly, *Trouessartia* can be quite host-specific and, even though we could not get to species identification, the specimens from *M. tyrannulus* and *M. magnirostris* are different from the other *Trouessartia* species reported for the Galápagos, *Trouessartia geospiza* from the small ground finch *Geospiza fuliginosa* (OConnor *et al.*, 2005). In addition, the *Trouessartia* specimens ($n = 25$) we collected opportunistically from the other tyrannid from the Galápagos, *Pyrocephalus rubinus* ($n = 1$), are very similar to each other but differ from those collected from *Myiarchus*.

It is interesting that, among the three mite genera shared between *M. tyrannulus* and *M. magnirostris*, *Nycteridocaulus* is the only one in which morphological differentiation, and perhaps speciation, has occurred after colonization. Genetic studies comparing these three lineages of mites would be insightful to understand their rate of diversification in relation to each other and to their hosts.

Why did some parasites and mites from *M. tyrannulus* not colonize the Galápagos?

Lower parasite diversity on islands can result from the founder effect inherent in the colonization process, in which colonizing hosts may reach islands carrying only a subset of their native parasite community (Nieberding *et al.*, 2006; Lafferty *et al.*, 2010). Our results support this idea, in that we recorded some parasites and mites on *M. tyrannulus* in Costa Rica that we could not find in the Galápagos. We detected *Plasmodium* sp. in *M. tyrannulus* from Costa Rica with high

prevalence (53%), but we did not detect this parasite in any samples of *M. magnirostris*. We can think of three explanations for the absence of *Plasmodium* in *M. magnirostris*. First, the common ancestors of *M. tyrannulus* and *M. magnirostris* were not infected by *Plasmodium* because this parasite only started interacting with the *M. tyrannulus* lineage after its split from the *M. magnirostris* lineage *c.* 850,000 years ago. Another possibility is that *Plasmodium* was present in the common ancestors of these *Myiarchus* species, but the birds that arrived on the Galápagos either were not infected or were infected but were not able to successfully colonize the islands. *Plasmodium* can be pathogenic and negatively impacts host fitness and survival. The colonization of a new environment is a very stressful event, and birds with higher fitness had a better chance of successfully establishing on the Galápagos. Finally, *Plasmodium* could have arrived in the Galápagos with the ancestors of *M. magnirostris* but have gone extinct because of the absence of a competent vector in which it could complete its life cycle and be transmitted to other hosts. Although *Plasmodium* has been detected in Galápagos penguins (*Spheniscus mendiculus*; Levin *et al.*, 2009), the responsible vector has not yet been identified. There are three species of mosquitoes in the Galápagos that could potentially be vectors for this parasite but none of them was present before 200,000 years ago (Whiteman *et al.*, 2005; Bataille *et al.*, 2009), long after the estimated arrival date for *Myiarchus* flycatchers to the Galápagos (Sari & Parker, 2012).

Tyranniphilopterus rufus was the louse we found with the highest prevalence (60.8%) on *M. tyrannulus*, but it has never been found on *M. magnirostris* either by us or by other researchers. Among the three louse species we collected from *M. tyrannulus*, *T. rufus* is probably the one with the most specialization to stay attached to the host's feathers; it belongs to the suborder Ischnocera while the other two louse species belong to the suborder Amblycera, which generally comprises more mobile lice that can leave their host in search of a new one (Johnson & Clayton, 2003). Similarly to our discussion for *Plasmodium*, it is possible that the ectoparasite community of *M. tyrannulus* has changed through time, and *T. rufus* might not have been present on the common ancestors of *M. tyrannulus* and *M. magnirostris* when these two lineages split approximately 850,000 years ago. On the other hand, because lice can have a patchy distribution on their hosts, *T. rufus* could have been absent just from the *Myiarchus* individuals that colonized the Galápagos by chance only (i.e. they 'missed the boat'; see Paterson *et al.*, 1999). Another explanation could be associated with the relative damage that *T. rufus* could cause to host feathers. It is thought that ischnoceran lice can cause enough damage to the birds' feathers to result in thermoregulatory costs for the birds and, consequently, reduce the fitness of parasitized individuals (Clayton *et al.*, 1999). In this case, the birds that were parasitized by *T. rufus* may not have successfully arrived and established on the Galápagos.

For *M. tyrannulus*, we have detected two species of feather mites that we did not find on *M. magnirostris*. These were detected on one or very few specimens of *M. tyrannulus*, while the three mite species that were found on both bird species had a much higher prevalence on *M. tyrannulus*. There is little evidence that feather mites can affect their hosts' fitness (Galván *et al.*, 2012), so probably the two mite species that did not colonize the Galápagos were not present on the ancestors of *M. magnirostris*.

CONCLUSIONS

Our study suggests that most of the parasite and other symbiont species carried by the Galápagos flycatchers (*M. magnirostris*) came with the ancestors of these birds to the Galápagos, while others have spilled over to flycatchers from other native hosts. We also confirmed that the colonization of a new area by a host and the interactions of this host with the local community can change host–parasite interactions and the specificity of parasites. We did not note any parasites or feather mites in or on *M. magnirostris* that could be characterized as introduced by humans, but the knowledge about which parasites are native to a host is equally important for the conservation of this host species and also the community with which it interacts. The characterization of the origins of these symbionts is essential for our understanding about the evolutionary history of species interactions in the Galápagos community.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Voucher numbers for mite and louse species collected from *Myiarchus* flycatchers. Mites are deposited in the Ohio State University Acarology Collection (OSAL) and lice are deposited in the Museum of New Zealand Te Papa Tongarewa (AI).

Appendix S2 GenBank accession numbers for the sequences obtained in this study. Codes after host species are band numbers (for *Myiarchus magnirostris* and *Dendroica petechia aureola*) or personal identification numbers (for *M. tyrannulus*).

BIOSKETCHES

Eloisa H. R. Sari is interested in the population genetics, disease ecology and diversification of Neotropical birds and their parasites. This work was completed when she was a PhD student at the University of Missouri-St. Louis (UMSL), where she studied the colonization history and the pathogen pressure of the Galápagos flycatcher and its sister species, the brown-crested flycatcher, in Costa Rica.

Patricia G. Parker studies the evolutionary, disease and behavioural ecology of birds. She has focused on Galápagos birds and their pathogens and parasites since joining the faculty of UMSL as the Des Lee Professor of Zoological Studies and as Senior Scientist at the Saint Louis Zoo.

Hans Klompen studies systematics in mites, concentrating on the evolution of associations and developmental patterns. He is director of the Ohio State University Acarology Collection.

Author contributions: E.H.R.S. and P.G.P. conceived the ideas and collected the data; H.K. identified the mites and analysed the mite data; E.H.R.S. analysed the lice and haemosporidian data and led the writing.

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