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ORIGINAL ARTICLE

Effect of introduced parasites on the survival and microbiota of nestling cactus finches (*Geospiza scandens*) in the Galápagos Islands

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Abstract

Introduced parasites and pathogens have colonized the Galápagos Islands of Ecuador. For example, a parasitic nest fly, *Philornis downsi,* was introduced to the Galápagos and has since caused significant nestling mortality for some endemic Darwin's finches. However, some larger bodied species of birds in the Galápagos, including vegetarian finches (*Platyspiza crassirostris*) and Galápagos mockingbirds (*Mimus parvulus*), can be less affected by the parasite. Our study explores the effects of *P. downsi* on a small population of common cactus finches (*Geospiza scandens*) across two breeding seasons on San Cristóbal Island, Galápagos. Specifically, we experimentally manipulated *P. downsi* abundance and characterized the gut and skin microbiota, and survival of nestling finches. Because cactus finches are medium-sized birds and small- and medium-sized birds are generally negatively affected by *P. downsi,* we predicted that the parasite would have a similar detrimental effect on fledging success. We also determined the effect of *P. downsi* on the microbiota because other studies have shown that the bacterial community can be beneficial for host health (e.g., conferring immunity and nutrient absorption). Although the overall skin and gut microbiota differed, we found that parasite treatment did not affect the microbiota in either tissue. However, nestlings from parasitized nests had significantly lower fledging success (25%, on average) compared to nestlings from non-parasitized nests (79%). We found that, similar to other medium-sized species, cactus finches are negatively affected by *P. downsi*, which could have implications for their risk of extirpation from the island. The results of this study should be considered when managing this population of concern.

Keywords Darwin's finch · Extirpation · Invasive species · Microbiome

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Zusammenfassung

Auswirkungen eingeschleppter Parasiten auf Überlebensraten und Mikrobiome von Nestlingen des Kaktusgrundfinks (*Geospiza scandens*) auf den Galapagosinseln

Die ekuadorianischen Galapagosinseln sind durch eingeschleppte Parasiten und Pathogene besiedelt. So wurde beispielsweise die parasitäre Fliege *Philornis downsi* auf Galápagos eingeführt und hat seither bei manchen endemischen Darwinfinkenarten Nestlingssterblichkeit von signifikantem Ausmaß ausgelöst. Jedoch können einige größere Vogelarten auf Galápagos, darunter Dickschnabel-Darwinfinken (*Platyspiza crassirostris*) und Galapagosspottdrosseln (*Mimus parvulus*), weniger durch den Parasiten betroffen sein. Unsere Studie befasst sich mit den Auswirkungen von *P. downsi* auf eine kleine Population des Kaktusgrundfinks (*Geospiza scandens*) während zweier Brutsaisons auf der Insel San Cristóbal, Galápagos. Konkret veränderten wir im Experiment die Häufigkeit von *P. downsi* und erfassten die Mikrobiome im Darm und auf der Haut sowie die Überlebensraten der Finkennestlinge. Da es sich bei Kaktusgrundfinken um mittelgroße Vögel handelt, und kleine und mittelgroße Vogelarten gemeinhin negativ durch *P. downsi* beeinflusst werden, erwarteten wir, dass der Parasit eine vergleichbar nachteilige Auswirkung auf ihren Ausfliegeerfolg haben würde. Außerdem bestimmten wir den Effekt von *P. downsi* auf die Mikrobiome, da in anderen Untersuchungen gezeigt wurde, dass diese Bakteriengemeinschaften der Wirtgesundheit förderlich sein können (beispielsweise durch die Verleihung von Immunität und durch Nährstoffabsorption). Obgleich sich die Mikrobiome von Haut und Darm insgesamt unterschieden, stellten wir fest, dass die Parasitenbehandlung die Mikrobiomzusammensetzung bei keinem der beiden Gewebstypen beeinflusste. Nestlinge aus parasitierten Nestern wiesen allerdings einen signifikant geringeren Ausfliegeerfolg (im Durchschnitt 25%) auf, verglichen mit Nestlingen aus parasitenfreien Nestern (79%). Wir stellten fest, dass Kaktusgrundfinken, ähnlich wie andere mittelgroße Vogelarten, durch *P. downsi* negativ beeinflusst werden, was Konsequenzen für das Risiko hat, dass sie von der Insel verschwinden. Daher sollten die Ergebnisse dieser Studie bei Managemententscheidungen bezüglich dieser gefährdeten Population berücksichtigt werden.

Introduction

Invasive parasites and pathogens can have major effects on host fitness worldwide, causing population decline, or, in some cases, extinction (Daszak et al. 2000). For example, the introduced avian malaria parasite and the amphibian chytrid fungus are responsible for the decline and extinction of multiple host species (van Riper et al. 1986; Stuart et al. 2004; Warner 2007; Atkinson and LaPointe 2009). Furthermore, even hosts that survive the infection can suffer sublethal effects from the parasites, such as reduced growth and altered behavior (Davidson et al. 2007). One explanation for these interactions is that naïve hosts cannot effectively defend themselves against novel parasites; evidence suggests that over time, host species can develop effective defenses to survive and thrive despite the infection (Daszak et al. 2004; Keesing et al. 2010).

Parasites can also affect the commensal organisms within the host, such as the microbiota, which can shape their development, physiology, and behavior (e.g., Round and Mazmanian 2009; Morgan et al. 2012; Sampson and Mazmanian 2015; Wilkinson et al. 2017; Grond et al. 2018). Parasites can indirectly and directly influence the gut and skin microbiota of the host. For example, gut and skin parasites can consume or compete with the microbiota (Cogen et al. 2008; Zaiss and Harris 2016; Leung et al. 2018). Alternatively, parasites can affect circulating, non-specific immune molecules, such as granulocytes or macrophages, which can then contact and disrupt the gut and skin microbiota (Agaisse and Perrimon 2004; Sorci and Faivre 2009). Due to the microbiota's influence on many processes in the host, identifying the effect of novel parasites on the gut and skin microbiota could have important conservation implications (Oliver et al. 2003).

Several introduced diseases and parasites have already been established in the Galápagos in wild birds. For example, the introduced parasitic nest fly *Philornis downsi* can cause up to 100% mortality in nestling Darwin's finches and has the potential to drive endemic bird species to extinction (reviewed in Kleindorfer and Dudaniec 2016; Fessl et al. 2018; McNew and Clayton 2018). Adult flies are non-parasitic and lay their eggs in the nests of landbirds (Fessl and Couri 2001; Fessl and Tebbich 2002). Once the fly eggs hatch, the larvae feed on the blood of the nestlings and brooding female birds (Koop et al. 2013b; Cimadom et al. 2014). Identifying the effects of *P. downsi* on populations of Darwin's finches that are threatened with extirpation is important to inform potential management measures of the species (Knutie et al. 2014).

The common cactus finch (*Geospiza scandens*) is found throughout the Galápagos Islands, but only a small (~30 individuals), geographically restricted population is present on San Cristóbal Island (Dvorak et al. 2019). *Philornis downsi* can significantly affect other species of Darwin's finches, such as Small Ground Finches (*G. fuliginosa*), Medium Ground Finches (*G. fortis*), Warbler Finches (*Certhidea olivacea*), Small Tree Finches (*Camarhynchus parvulus*), Medium Tree Finches (*C. pauper*), and Mangrove Finches (*C. heliobates*) (Fessl and Tebbich 2002; Dudaniec et al. 2006; Fessl et al. 2010; O'Connor et al. 2010; Koop et al. 2013a, b, 2016). Larger bodied Galápagos land birds, such as Vegetarian Finches (*Platyspiza crassirostris*) (~ 34 g) and Mockingbirds (*Mimus parvulus*) (~ 54 g), seem to be tolerant to the parasite (i.e., can compensate for parasite damage without killing the parasite) when compared to medium or smaller sized birds, such as Medium Tree Finches (~ 20 g), Small Ground Finches (~ 14 g), Medium Ground Finches (~ 22 g) and Small Tree Finches (~ 13 g) (Grant 1999; Dudaniec et al. 2007; O'Connor et al. 2010; Cimadom et al. 2014; Knutie et al. 2016; Heimpel et al. 2017). The body mass of common cactus finches falls in the medium-bodied range, with an average mass around 21 g (Grant 1999); therefore, cactus finches could be affected similar to other medium- or small-bodied birds. Determining the effect of *P. downsi* on the small population of cactus finches on San Cristóbal is important to potentially prevent extirpation. Furthermore, as gut and skin microbiota can play a role in the physiology and behavior of hosts (Round and Mazmanian 2009; Morgan et al. 2012; Sampson and Mazmanian 2015), it is important to understand the potential effects of *P. downsi* on the microbiota.

To determine the effect of *P. downsi* on a restricted population of the common cactus finch on San Cristóbal Island, we experimentally manipulated *P. downsi* abundance in the nests of the birds and then quantified nestling survival (fledging success) and characterized the skin and gut microbiota (bacterial diversity, community structure and membership, and relative abundance of phyla and genera). Because cactus finches are a medium-sized species of Darwin's finch, we predicted that the parasitized nestlings would have lower fledgling success rates to non-parasitized nestlings (Knutie et al. 2016; Heimpel et al. 2017; McNew and Clayton 2018). Second, we predicted that the skin and fecal microbial communities would differ because others have found differences in the microbiota between tissue types (Costello et al. 2009; Godoy-Vitorino et al. 2012; Huttenhower et al. 2012; Grond et al. 2018). Because parasites can affect the microbiota of avian hosts, we predicted that the gut and skin microbiota would differ between parasitized and non-parasitized nestlings (Knutie 2018, 2020). Overall, our study explores the effects of *P. downsi* on a small, restricted population of finches to determine whether these birds are well defended against the parasite or could potentially face extirpation on San Cristóbal.

Methods

Study system

The study was conducted during February–April 2018 and 2019 at the Jardín de Opuntias (0° 56′ 18.92" S, 89° 32′ 54.93" W) on the island of San Cristóbal in the Galápagos Islands. The site is a 1.4 by 0.12 km area in the arid coastal zone, which is characterized by Darwin's cotton (*Gossypium darwinii*), Galápagos croton (*Croton scouleri*), Galápagos acacia (*Acacia rorudiana*), and prickly pear cactus (*Opunita megasperma*). The common cactus finch, a specialist species, feeds primarily on the pulp, fruit, and flowers of prickly pear cactus (Grant and Grant 1980, 1981; Millington and Grant 1983). Prickly pear cacti are rare on San Cristóbal and one of the few locations on the island where they can be found is within the Jardín de Opuntias. Cactus finches typically build dome-shaped nests, which are made of coarse and fine grasses, in prickly pear cacti. Consequently, this area is likely the primary location on the island where cactus finches breed (Dvorak et al. 2019). Nest building typically begins after the first rain of the season (~ January) and pairs can lay up to two broods in a breeding season. Clutch size ranges from one to four eggs, which are incubated for about 12 days. The nestlings develop in the nests for about 2 weeks, and are fed nectar, pollen, and insects by both the male and female parents who feed them via regurgitation (Boag and Grant 1984). Adult birds were opportunistically mist-netted and weighed in 2018 ($n = 13$) and 2019 ($n = 2$) at the field site; the mean mass (± SE) of an adult cactus finch was 18.63 ± 0.81 g ($n = 15$).

Experimental manipulation of parasites

The field site was searched daily for evidence of nest building by cactus finches. Once eggs were laid, nests were checked every other day until the nestlings hatched. Within two days of nestling hatching, *P. downsi* abundance was experimentally manipulated. The experimental nests were treated with a 1% permethrin solution (Permacap) and will now be referred to as "non-parasitized" (2018: $n = 5$ nests, 2019: $n = 6$ nests). The control nests were sham-fumigated with water and will now be referred to as "parasitized" (2018: $n = 5$ nests, 2019: $n = 7$ nests). Because the sample size was low for each year, we combined years for all analyses. When the nestlings had just hatched, the contents of the nests were removed (including the nestlings, unhatched eggs, and the nest liner), and the nest was sprayed and/or injected with either the permethrin solution or water. After treatment, the dry nest liner was returned, and nestlings were placed back into the nest. Nestlings did not come into contact with the insecticide, and adults returned to the nests with no cases of abandonment due to the treatment.

Fecal collection

Nestlings were banded with a unique color band combination and a numbered metal band when they were 6–8 days old. Feces and skin swabs were also collected from nestlings opportunistically at this time (parasitized, feces: $n = 10$

nestlings from 7 nests; skin: $n=3$ nestlings from 3 nests; non-parasitized, feces: $n=12$ nestlings from 7 nests; skin: $n=4$ nestlings from 4 nests). To collect feces, nestlings were removed from the nest and held over a sterile weigh boat until they defecated. The fecal sample was then moved from the tray to a sterile tube, placed on ice in the field for up to 6 h, and then stored in a – 20 ˚C freezer until the bacterial DNA was extracted. The samples were then transported to the University of Connecticut and stored in a – 80 ˚C freezer for downstream 16S sequencing. Although studies show that the bacterial community in avian feces does not always represent the entire digesta of the host (e.g., in the cecum; Wilkinson et al. 2017), fecal samples are generally representative of the bacterial community in the large intestines (Wilkinson et al. 2017; Videvall et al. 2018) and are used when hosts cannot be euthanized (Kohl 2017).

A skin swab was also collected from the nestlings using a sterile swab applicator. First, the sterile swab was lightly moistened in nuclease-free water. The nestling's belly, which had not been touched by the observer, was then inspected for wounds and swabbed back and forward ten times. The swab was then immediately placed in a sterile tube, placed on ice, and processed similarly to the fecal samples.

Quantifying fledging success and parasite abundance

When nestlings were approximately 12 days old, nests were checked for fledging every other day until the nest was empty. After the nest was empty, it was collected and placed in a sealed plastic bag. Nests were carefully dissected within 8 h of collection. Parasite abundance was quantified as the number of second and third instar *P. downsi* larvae, pupae, and eclosed pupal cases (Koop et al. 2013b). Larvae and pupae were reared to the adult stage to confirm that they were *P. downsi* (Dodge and Aitken 1968).

Bacterial DNA extraction and sequencing

Total DNA was extracted from nestling feces and skin swabs using a Qiagen PowerFecal DNA Isolation Kit. DNA extractions were then sent to the University of Connecticut Microbial Analysis, Resources and Services for sequencing with an Illumina MiSeq platform and v2 2 × 250 base pair kit (Illumina, Inc.). We also sequenced a laboratory blank to control for kit contamination and found no detectable sequences. Bacterial inventories were conducted by amplifying the V4 region of the16S rRNA gene using primers 515F and 806R and with Illumina adapters and dual indices (Kozich et al. 2013). Raw sequences were demultiplexed with onboard bcl2fastq and then processed in Mothur v1.40.5 (Schloss et al. 2009) according to the standard MiSeq protocol (Kozich et al. 2013). Briefly, forward and reverse sequences were merged. All sequences with any ambiguities, that did not align to the correct region, or that did not meet length expectations, were removed. Sequences were then aligned to the Silva nr_v128 alignment, and grouped into operational taxonomic units (OTUs) based on a 97% similarity level (Quast et al. 2013). OTUs were identified using the Ribosomal Database Project Bayesian classifier (Wang et al. 2007). Non-bacterial sequences that classified as chloroplasts, mitochondria, or unknown (i.e., did not classify to the level of kingdom) were removed. Chimeric reads were also removed using UCHIME (Edgar et al. 2011). Alpha and beta diversity statistics were calculated by averaging 1,000 random subsampling of 9,000 reads per sample. We calculated the sobs index, which indicates the total number of species observed in a sample. We calculated the Shannon and the Simpson diversity indices, which are both measures of diversity that take into account the number of species present and their relative abundances, with the Simpson index giving more weight to common taxa. We also calculated the Shannon evenness index, which shows how evenly spread the species are in a sample. The resulting data sets included a total of 1,186,836 sequences and an average of 40,925 ± 4,327 reads per sample (min: 9,314, max: 96,082).

Statistical analyses

Negative binomial and binomial generalized linear models (GLMs) were used to determine the effect of treatment on parasite abundance and fledging success, respectively. Generalized linear mixed models (GLMMs) were used to compare the bacterial diversity between tissue types (skin and feces) and parasite treatment (parasitized and non-parasitized) for feces; nest was used as a random effect to control for the non-independence of nestlings within a nest. A GLM with Gaussian errors was used to determine the effect of parasite treatment on skin microbiota since there was only one sample per nest. Analyses were conducted in R (2017, version 3.6.3). Analyses were conducted using the glm (GLM) and glmer (GLMM) functions within the lme4 package (Bates et al. 2015). Probability values were calculated using log-likelihood ratio tests using the ANOVA function in the car package (Fox and Weisberg 2019).

The effects of tissue and parasite treatment on bacterial community dynamics in parasitized nestlings were examined using the Bray–Curtis dissimilarity and Jaccard similarity distance matrices. The matrices were created using the vegdist function in the vegan package in R (Oksanen et al. 2019). We then used the adonis2 function in the vegan package to perform PERMANOVAs to assess the differences in bacterial community structure and membership between tissue types and treatment groups. Both Bray–Curtis and Jaccard are dissimilarity matrices, with Bray–Curtis taking into account the relative abundances of shared taxa while

Jaccard only considers the presence or absence of such taxa. Principal Coordinates Analyses (PCoA), where the distances among the samples are converted onto a graph, were done to compare and visualize differences between groups. Relative abundances (arcsine square root transformed; (Shchipkova et al. 2010; Kumar et al. 2012)) of bacterial phyla and genera were compared between skin and fecal groups, between fecal treatment groups, and between skin treatment groups. Data were manipulated using packages tidyr, reshape2, and plyr in R (Wickham 2007, 2011; Wickham and Henry 2020), and ANOVAs were run in the car package in R (Fox and Weisberg 2019); false discovery rate (FDR) tests were used to control for multiple analyses. All figures were created in Prism (2017, version 7).

Results

Effect of treatment on parasite abundance and fledging success

Permethrin-treated nests had zero parasites, which was significantly fewer than the control nests (Fig. 1a) (non-parasitized: mean ± SE: 0.00 ± 0.00; parasitized: 23.42 ± 7.10; GLM, $\chi^2 = 37.82$, $df = 1$, $P < 0.0001$). Parasitized nests had lower fledging success than non-parasitized nests (Fig. 1b) (non-parasitized: 78.91 ± 9.28%, parasitized: 25.00 ± 13.06%; GLM, $\chi^2 = 14.10$, $df = 1$, $P < 0.001$).

Microbiota of the skin and feces

Bacterial diversity, as measured by Sobs and Shannon index, did not differ significantly between the skin and feces of nestlings (Table S1). The Simpson index and Shannon evenness differed between tissue types, but the difference was non-significant (Table S1). Bacterial community structure (Fig. 2a) ($F_{1,28} = 3.49$, $P = 0.001$) and membership (Fig. 2b)

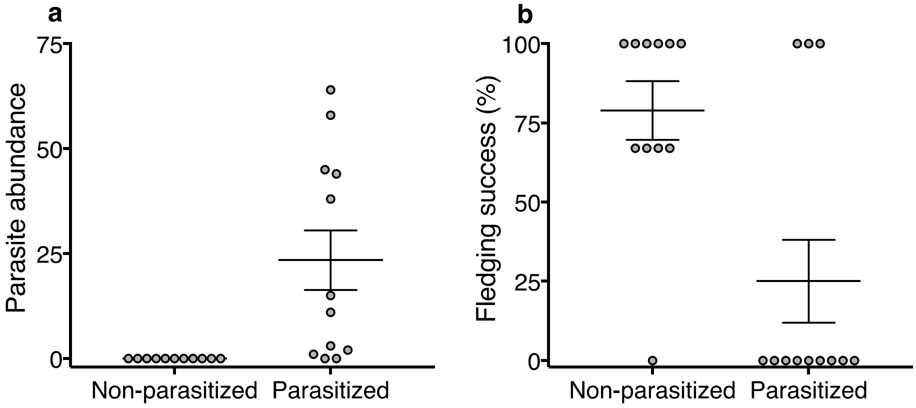

Fig. 1 Mean ± SE for **a** parasite abundance and **b** fledging success from non-parasitized and parasitized nests. Each point represents a nest within the treatment

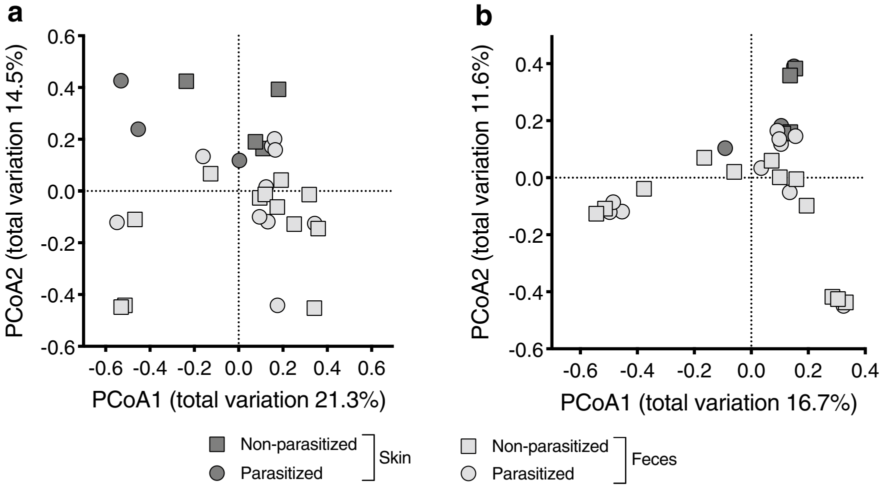

Fig. 2 Bacterial **a** community structure, and **b** membership based on the Bray–Curtis dissimilarity matrix and Jaccard similarity matrix, respectively, from feces and skin tissue of nestling finches. Different colors indicate different samples, while different shapes indicate different treatments

($F_{1,28} = 2.61$, $P = 0.002$) differed between skin and fecal samples. Relative abundances of several phyla were higher in skin as compared to feces, including phyla Gemmatimonadetes, Bacteroidetes, and Acidobacteria (Table S2). Relative abundances of several genera were higher in skin than feces, including genera *Bradyrhizobium*, *Pseudomonas*, *Leucobacter*, *Proteus*, *Acinetobacter*, *Roseomonas*, and *Methylobacterium* (Table S2).

Effect of parasite treatment on fecal microbiota

Bacterial diversity, as measured by Sobs, Shannon index, Shannon evenness, and Simpson index, from the fecal samples did not differ significantly for the nestlings in the parasitized and non-parasitized treatments (Table S3). Parasite treatment also did not affect the bacterial community structure (Fig. 2a) ($F_{1,21} = 1.03$, $P = 0.39$) and membership (Fig. 2b) ($F_{1,21} = 0.98$, $P = 0.44$) or the relative abundances of bacterial phyla and genera ($P > 0.05$ for all analyses).

Effect of parasite treatment on skin microbiota

Bacterial diversity, as measured by Sobs, Shannon index, Shannon evenness, and Simpson index, from the skin samples did not differ significantly for the nestlings in the parasitized and non-parasitized treatments (Table S4). Parasite treatment also did not affect the bacterial community structure (Fig. 2a) ($F_{1,6} = 0.69$, $P = 0.71$) and membership (Fig. 2b) ($F_{1,6} = 0.76$, $P = 0.80$) or the relative abundances of bacterial phyla and genera ($P > 0.05$ for all analyses).

Discussion

We examined the effects of *P. downsi* on common cactus finches, which have approximately 30 individuals in their breeding population on San Cristóbal Island. We found that *P. downsi* reduced fledgling success of cactus finches by over 50% compared to non-parasitized finches. In addition, although the microbiota differed between the skin and feces in nestling finches, parasitism did not affect the microbiota of either tissue. Our results show that *P. downsi* is problematic for cactus finches on San Cristóbal Island, which could have consequences for the future of this small population of Darwin's finches.

Nestling survival was predicted to be negatively affected by *P. downsi* because cactus finches are a medium-bodied host species, which are often affected by the parasite. Indeed, most parasitized cactus finch nests in our study failed, similar to other medium and smaller sized finches (Dudaniec et al. 2006; Fessl et al. 2006; O'Connor et al. 2014). Other studies have found that larger-sized birds, such as vegetarian finches and Galápagos mockingbirds, do not suffer a negative effect of *P. downsi* on fledgling success (Knutie et al. 2016; Heimpel et al. 2017). Together, these studies suggest that effective defenses could be related, in part, to the body mass of the host species. However, three parasitized cactus finch nests with 15–64 parasites per nest did not fail, suggesting that some individuals within the population might be well defended against the parasite. Future conservation efforts for *P. downsi* should consider the body mass of the bird species or population, and the effect of *P. downsi* on the host at an individual level.

Parasite treatment did not affect the gut microbiota of nestling finches. One potential mechanism by which ectoparasites could interact with the gut microbiota is through the immune system (Thaiss et al. 2016; Knutie 2020). For example, reactive oxygen species and reactive nitrogen oxide species are both produced in response to a parasite and can cause an inflammatory response, which could then alter the microbiota (Sorci and Faivre 2009). One explanation for why we did not find an effect of *P. downsi* on the gut microbiota of nestlings is that their immune system is not developed enough to mediate this interaction. Studies have found that the IgY antibody response of nestling Darwin's finches is not detectable prior to fledging, which could explain our results (Koop et al. 2013b; Knutie et al. 2016). However, other immune parameters could be developing sooner than fledging, such as a transfer of maternal antibodies to the nestlings (Grindstaff et al. 2003). Another reason we did not find an effect of *P. downsi* on the gut microbiome could be because the parasite and the gut microbiota do not interact, or that body size influences the role of parasitism on the gut microbiota. In one study, *P. downsi* did not affect the gut microbiota of medium ground finches (medium-sized species), but did have an effect on the gut microbiota of mockingbirds (Knutie 2018). Similarly, the microbiota of the cactus finch, which is a similar size to the medium ground finch, was not affected by the parasite. Therefore, perhaps the effect of *P. downsi* on the microbiota of the host is also based on body size, with the microbiota of larger hosts being more affected by the parasite than smaller hosts. Since different Galápagos species harbor different microbiota communities (Knutie 2018; Michel et al. 2018; Loo et al. 2019), the host–microbiota–parasite relationship should be explored in other host species.

Host-associated microbiota differed between the skin and the gut microbiota of nestlings. Other studies have shown that microbiota can differ across tissue types, which could be due to the difference in microbial function (digestion in the gut vs. barrier to pathogens on the skin) and environment (anaerobic vs. aerobic) in each tissue (Costello et al. 2009; Godoy-Vitorino et al. 2012; Huttenhower et al. 2012; Grond et al. 2018). The skin can produce antimicrobial proteins that can eliminate or prevent the growth of harmful pathogens, while the gut hosts microbes that can aid in the digestion of food the host might not otherwise be able to break down (Zhu et al. 2011; Gallo and Hooper 2012). However, similar to the gut microbiota, parasite treatment did not affect the skin microbiota of nestling finches. We predicted that ectoparasitism by *P. downsi* would change the bacterial community on the skin surface because some studies do show that the skin microbiota is different between infected and non-infected hosts (Federici et al. 2015; Tomás et al. 2018). Additionally, the skin microbiota might alter the immune response, as certain immune cells, such as Langerhans cells and dendritic epidermal T cells, can accumulate near the surface of the skin. These cells then provide an inflammatory response that could alter the survival outcomes of the parasite (Owen et al. 2009; Pasparakis et al. 2014; Bukhari et al. 2019). Since the sample size for skin microbiota was relatively low, future studies could further explore the direct consequences of an ectoparasite on the skin microbe community with increased samplings.

Without the ability to effectively defend themselves against *P. downsi,* or without human intervention, finches may face extirpation. Small isolated populations are at risk because of a loss of genetic diversity due to genetic drift or bottlenecks, making the persistence of populations uncertain. Our study provides more evidence of the detrimental effects of *P. downsi* on endemic birds in the Galápagos. Because the population size of the San Cristóbal cactus finch is so small, the rapid evolution of effective defenses against the parasite to maintain the population is unlikely. Therefore, human intervention is needed to prevent the extirpation of this finch population.

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Author contributions SAK conceived the study; AMA and SAK conducted the data analyses, and wrote the manuscript; JAH, GJV, LA, TA, KC, SS, TBV collected data; JC provided logistical support. All authors revised and approved the manuscript.

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Data availability All raw data are available on FigShare (DOI: https://doi.org/10.6084/m9.figshare.12585497) and sequences have been uploaded to GenBank (BioProject accession number: PRJNA640644).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Our work in 2018–2019 was done under GNP permits PC 03-18 and PC 28-19 and Genetic Access permit MAE-DNB-CM-2016-0041.

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