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Prevalence and diversity of avian malaria parasites in illegally traded white-winged parakeets in Peruvian Amazonas

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Abstract

Illegal or poorly regulated wildlife trade may enhance parasite spread worldwide, leading to pathogen outbreaks and the emergence of diseases affecting native wildlife, domestic animals and humans. The order Psittaciformes has the largest proportion of endangered species among all birds worldwide and is one of the most trafficked taxa in the pet trade. However, despite the large number of parrot species commercialized worldwide, the influence of illegally traded wild birds on the introduction of exotic pathogens is still poorly investigated. Here we molecularly examined the prevalence and genetic diversity of haemosporidian parasites in illegally traded white-winged parakeets (*Brotogeris versicolurus*), one of the most trafficked parrots in South America. We found that 18.5% of parakeets harboured *Plasmodium relictum* GRW04, a highly invasive malaria parasite provoking population decline and even extinctions in native avifauna when established outside its natural range. We also showed that malaria infected birds have lower body condition than uninfected parakeets, revealing the negative effects of malaria on their avian hosts. These outcomes highlight the risk of malaria spill over and disease outbreak in illegally traded wildlife. Our results also reveal epidemiological key concepts in disease transmission, such as the role of poorly studied parrot species as natural reservoir hosts of haemosporidians. These findings stress the importance of enforcing health control regulations and trade policies to fight wildlife trafficking effectively.

Introduction

Illegal and poorly regulated wildlife trade represents high-profile global health, conservation and animal-welfare challenges (Fukushima *et al*., 2021). This criminal activity has significantly increased in the last decades worldwide (UNODC, 2020). The annual earnings generated by the global illegal trade in wildlife have been estimated at between US$ 4–23 billion (’t Sas-Rolfes *et al*., 2019), with annual economic loses in governments up to US $12 billion in potential incomes (World Bank, 2019). Illegal wildlife trade negatively impacts local economies through the reduction or loss of ecosystem services, increasing vulnerability and poverty in indigenous and rural communities (Cardoso *et al*., 2021; Fukushima *et al*., 2021). Wildlife trafficking also has ecological consequences, such as population depletion of traded species. In this sense, a decline in about 62% of abundance is estimated in trafficked bird, mammal and reptile populations, which may drive to increased local extirpation and extinction risk in traded species (Morton *et al*., 2021). In addition, illegal animal trade may also provoke a flow of secondary effects impacting non-target species through disrupted interspecific interactions, community structures and ecosystem functioning (Cardoso *et al*., 2021). Moreover, translocated species by wildlife trade may foster parasite spread in the new area leading to pathogen

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outbreaks and the emergence of diseases affecting native wild fauna, domestic animals and humans (Bezerra-Santos *et al*., 2021; Rush, Dale, & Aguirre, 2021).

Birds are the most trafficked taxa in the pet trade (Rush, Dale, & Aguirre, 2021). Parrots (Order: Psittaciformes) have the largest proportion of endangered species among all birds worldwide, with 27% of the 379 parrot species in the world are threatened to some degree of extinction (IUCN, 2023). One of main factors provoking these population declines is the unsustainable poaching of parrots for legal and illegal trade, which can have serious implications for conservation, animal welfare and biosecurity, including the potential spread of infectious diseases (Fogell *et al*., 2018). One example of a wildlife species that is currently being unsustainably harvested in South America is the white-winged parakeet (*Brotogeris versicolurus*), Peru's most trafficked parrot species (Gastañaga *et al*., 2011; SERFOR, 2017). This is a small parrot native to the Amazon River basin and distributed from south-east Colombia and north-east Peru to the river's mouth in Brazil (del Hoyo & Collar, 2014). This species can be legally captured or poached in Peru for consumption, belief-based use, ornaments and as pets. However, the number of individuals for sale (more than 31 000 individuals per year) is much higher than numbers that can officially be legally sold (1250 individuals per year), suggesting that legally authorized Peruvian parrot trade may facilitate a much larger illegal parrot trade (Gastañaga *et al*., 2011). Although Amazon markets almost exclusively sell local species, these local markets are connected in a structured network that provides more opportunities for domestic and international trafficking (Ortiz-von Halle, 2018; Mendoza *et al*., 2022), favouring their introduction and the subsequent establishment of feral populations in many countries such as Ecuador (Freile *et al*., 2012), Puerto Rico (Falcón & Tremblay, 2018) and the USA (del Hoyo & Collar, 2014).

Avian malaria and related haemosporidians (genera *Haemoproteus*, *Plasmodium* and *Leucocytozoon*) are vector borne parasites infecting birds from many different taxa (Valkiūnas, 2005; Santiago-Alarcón & Marzal, 2020). The life cycle of haemosporidian parasites is complex, including sexual stages that occur within invertebrate vectors and asexual stages in tissues and circulating blood cells of vertebrate hosts. To date, only species of blood-sucking dipteran insects (Diptera) have been described as vectors for haemosporidian parasites (Ferreira, Santiago-Alarcón, & Braga, 2020). Culicidae mosquitoes from five genera (*Anopheles*, *Culex*, *Aedes*, *Culiseta* and *Coquillettidia*) are the main vectors transmitting avian *Plasmodium* parasites, whereas *Leucocytozoon* species are vectored by black flies (Simuliidae) (Ferreira, Santiago-Alarcón, & Braga, 2020). Biting midges (mostly of the genus *Culicoides*) and louse flies (Hippoboscidae) transmit *Haemoproteus* parasites (Ferreira, Santiago-Alarcón, & Braga, 2020).

Haemosporidian parasites are known to exert negative effects on the life traits of their avian hosts. For example, several studies have reported a decrease in lifespan and survival of haemosporidian infected birds (Martínez-de la Puente *et al*., 2010; Asghar *et al*., 2015; Ilgūnas *et al*., 2016; Marzal *et al*., 2016). Also, it has been documented that malaria parasites may decrease the body condition of birds (Marzal *et al*., 2008; Coon *et al*., 2016). Moreover, some studies have revealed a lower mating success of birds infected with these blood parasites (Bosholn *et al*., 2016; Höglund *et al*., 2017). In addition, experimental studies have demonstrated that haemosporidian parasites may impair the reproductive success of their avian hosts, by reducing their clutch size, hatching success and fledging success (Merino *et al*., 2000; Marzal *et al*., 2005). Although more than 4800 malaria parasite lineages have been described across all continents except Antarctica (MALAVI database version 2.5.6, March 2023, Bensch, Hellgren, & Pérez-Tris, 2009), avian haemosporidian diversity and distribution vary across latitudinal gradient (Fecchio *et al*., 2019) and biogeographical regions (Clark, 2018; Garcia-Longoria *et al*., 2021). However, with human assistance, some haemosporidian parasites that spread beyond their natural range may switch into native bird species and infect them in new habitats (Marzal & García-Longoria, 2020). Due to the lack of evolved protected immunity in native birds (Schmid-Hempel, 2021), these co-transported parasites are expected to be highly virulent in naïve host populations in new environments (Lymbery *et al*., 2014).

Despite the large number of wildlife species commercialized worldwide, the influence of illegally traded wild birds on the introduction of exotic pathogens is still poorly investigated (Bezerra-Santos *et al*., 2021; Rush, Dale, & Aguirre, 2021). It has been shown that white-winged parakeet can be a pathway for introduction and spread of viral infectious diseases (Daut *et al*., 2016), but the potential risk of illegally trafficked white-winged parakeets on the introduction of other pathogens has not been explored yet. Moreover, parrot species have been poorly examined yet in search for malaria parasites. For example, more than 4800 haemosporidian lineages infecting more than 2180 species representing most bird clades have been described, but only 20 haemosporidian lineages have been found infecting Psittaciform species. Furthermore, only 8% of parrot species in the world have been described infected with haemosporidians (MALAVI database version 2.5.6, March 2023, Bensch, Hellgren, & Pérez-Tris, 2009). Here we molecularly examined the prevalence and genetic diversity of haemosporidian parasites to assess the presence of potentially invasive pathogens in illegally traded white-winged parakeets in San Martín region (Peru), a Tropical Andes Biodiversity Hotspot region with the highest conservation priority worldwide (Myers *et al*., 2000).

Materials and methods

Study sites and sample collection

We sampled 135 white-winged parakeets seized from illegal wildlife trade by Autoridad Regional Ambiental (ARA) and Servicio Nacional Forestal y de Fauna Silvestre (SERFOR). Recovered individuals were housed in aviaries with water and food *ad libitum* in three wildlife recovery centres from SERFOR and ARA located in the vicinities of Tarapoto (San Martin, Peru): Chullachaqui (N = 46; 6°27′56″S, 76°19′03″W), Fundo Copa (N = 55; 6°28′43″S, 76°19′22″W) and Yacumaman (N = 36; 6°28′53″S, 76°21′21″W). Samples from Chullachaqui were collected in May 2016 and June 2017, whereas samples from Fundo

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Copa and Yacumaman were collected in June 2016 and June 2018 respectively.

From each individual we measured the tarsus length with a digital calliper to the nearest 0.01 mm and their body mass with a digital balance to the nearest 0.1 g. We used body mass and tarsus length to calculate scaled body mass index (Peig & Green, 2009), which is a reliable estimate of animal physical condition that standardizes body mass at a fixed value of a linear body measurement based on the scaling relationship between mass and length (Peig & Green, 2010). We also took a blood sample (c. 40–60 μL, according to its body size) in heparinized microcapillaries by puncturing the brachial vein and stored in 1.5 mL Eppendorf tubes containing 500 μL SET-buffer (0.015 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH 8.0) until molecular analysis. All samples and measurements were collected no later than 1 week since individuals were seized from illegal wildlife trade by ARA and SERFOR.

All samples were taken in accordance with national Peruvian law (200-2016-SERFOR/DGGSPFFS) and the animal protection laws of the EU (directive 2010/63/EU of the European Parliament). Methods were approved by the Research Ethics and Animal Welfare Committee on Animal Experimentation of the University of Extremadura (reference 101/2016).

Haemosporidian molecular detection

Haemosporidian parasites (*Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp.) were detected from blood samples using molecular methods targeting conserved regions of the haemosporidian cytochrome b gene (*cyt b*) (Hellgren, Waldenström, & Bensch, 2004). DNA from the avian blood samples was extracted in the laboratory using the GeneJETTM Genomic DNA Purification Kit (Thermo Scientific Inc., reference #K0722). The quality of extracted DNA (260/280 nm ratio) was assessed using a NanoDrop One UV–Vis Spectrophotometer (Thermo Fisher Scientific). Diluted genomic DNA (25 ng/μL) was used as a template in a polymerase chain reaction (PCR) assay for detection of the parasites using nested-PCR protocols described by Hellgren, Waldenström, & Bensch (2004). The procedure started with an initial PCR (20 cycles) using primers located outside the target fragment (HaemNFI and HaemNR3). We used 2 μL of the first PCR reaction as the template for two specific PCRs (35 cycles) to selectively amplify species of *Plasmodium*/*Haemoproteus* and *Leucocytozoon*. These two final amplifications used the primers HaemFL and HaemR2L to amplify *Leucocytozoon* spp., and HaemF and HaemR2 for *Haemoproteus* spp./*Plasmodium* spp. The amplification was evaluated by running 2.5 μL of the final PCR on a 2% agarose gel. All PCR experiments contained one negative control for every eight samples and two positive controls (lineage *Leucocytozoon* spp. RS4 from Eurasian blackcap *Sylvia atricapilla* and lineage *Plasmodium relictum* SGS1 from house sparrows *Passer domesticus*) for every 24 samples. Parasites detected by a positive amplification were sequenced using the procedures described by Hellgren, Waldenström, & Bensch (2004). Amplified fragments were sequenced from 5′-end with forward primers HaemF and HaemFL. The obtained sequences of 478 bp of the *cyt b* were edited, aligned and compared in a sequence identity matrix using the programs BioEdit (Hall, 1999) and Geneious (Kearse *et al*., 2012). Finally, the aligned sequences were blasted against GeneBank and the MalAvi databases (version 2.5.4, June 2022, Bensch, Hellgren, & Pérez-Tris, 2009) in order to identify parasite lineages. Parasites with sequences differing by one nucleotide substitution were considered to represent evolutionary independent lineages (Ricklefs *et al*., 2005).

Statistical procedures

The probability of being infected with haemosporidians was modelled by using a logistic regression analysis. Scaled body mass index, year of sampling and location of sampling were included as predictors in the logistic regression in order to understand which factors were influencing the probability that a white-winged parakeet was infected. We performed Bonferroni post-hoc tests to determine if the prevalence of haemosporidian parasites varied among localities of sampling.

We used a general linear model (GLM) to investigate the factors explaining variation in the scaled body mass index for individual white-winged parakeets. Haemosporidian infection (uninfected or infected), year of sampling and location of sampling were included in the analysis as predictor variables. The interactions between haemosporidian infection and year of sampling, and between haemosporidian infection and location of sampling were also included as predictors in the model. We also used Bonferroni post-hoc test to explore for differences in scaled body mass index among parakeets housed in different locations. All analyses were performed using PASW Statistics 22 statistical package for Windows.

Results

Prevalence and genetic diversity of malaria infection

We screened a total of 135 white-winged parakeets in search for haemosporidian parasites. Twenty-six of the 135 individuals were infected with haemosporidians (overall prevalence = 19.26%). We detected two different *Plasmodium* lineages infecting white-winged parakeets. *Plasmodium relictum* GRW4 (GenBank accession number: AF254875) was the most prevalent malaria lineage found infecting parakeets (96% of all malaria infections). Additionally, one individual was infected with *Plasmodium nucleophilum* DENPET 03 (GenBank accession number: AY640137). No individual was infected with *Haemoproteus* or *Leucocytozoon*.

Factors determining malaria infection in white-winged parakeets

Scaled body mass index explained variation in haemosporidian infection in white-winged parakeets (Table 1). Specifically, uninfected individuals showed higher scaled body mass indexes (mean ± SE = 61.80 ± 7.89) than malaria-infected parakeets (mean ± SE = 57.16 ± 5.49) (Fig. 1).

Locality of sampling also significantly explained variation in haemosporidian infection. In short, haemosporidian

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Table 1 Results of a logistic regression analysis of factors determining haemosporidian infection of white-winged parakeets

Variable	*B*	SE	Wald	d.f.	*P*	Exp (*B*)
Location of sampling	−0.963	0.416	5.365	2	**0.021**	0.382
Year of sampling	−0.133	0.411	0.104	2	0.747	0.876
Scaled body mass index	−0.102	0.039	5.454	1	**0.020**	0.912
Intercept	274.807	828.191	0.110	1	0.998	2.225E+119

Year of sampling, location of sampling and scaled body mass index were included as predictor variables. Sample size was 135 individuals. Significant factors are highlighted in bold.

prevalence varied among localities in which white-winged parakeets were housed (Table 1). Post-hoc analyses showed that birds housed in Chullachaqui and Fundo Copa had similar haemosporidian prevalence (25.0 and 27.45% respectively; $P = 0.782$); however, malaria prevalence in parakeets from Chullachaqui and Fundo Copa were significantly higher than haemosporidian prevalence in individuals from Yacumaman (0%; $P < 0.001$ in both post-hoc analyses).

Factors determining scaled body mass index in white-winged parakeets

Haemosporidian infection and location of sampling significantly explained variation in scaled body mass index in white-winged parakeets (Table 2). As mentioned above, uninfected individuals showed higher scaled body mass indexes than malaria-infected parakeets (Fig. 1). In addition, post-hoc analyses revealed that parakeets housed in Fundo Copa showed lower values of scaled body mass index ($N = 51$; mean ± SE = 56.55 ± 6.15) than individuals kept in Chullachaqui ($N = 48$; mean ± SE = 63.10 ± 5.95) and Yacumaman ($N = 36$; mean ± SE = 64.54 ± 8.52) (all $P < 0.001$), but there were no differences in scaled body mass index between parakeets housed in Chulachaqui and Yacumaman ($P = 0.602$) (Fig. 2).

Finally, neither the interaction between haemosporidian infection and year of sampling nor the interaction between haemosporidian infection and location of sampling significantly influenced the scaled body mass index of white-winged parakeets (Table 2).

Discussion

The number of studies examining the prevalence and genetic diversity of haemosporidian parasites in Psittaciformes is scarce when compared to other bird orders (MALAVI database version 2.5.6, March 2023, Bensch, Hellgren, & Pérez-Tris, 2009). Moreover, the few studies exploring avian malaria in these birds usually analyse a limited number of individuals (e.g. Durrant *et al.*, 2006; Chagas *et al.*, 2017; Fecchio *et al.*, 2018, 2019; Olias *et al.*, 2011; Ishtiaq *et al.*, 2007; Anjos *et al.*, 2021; but see Ortiz-Catedral *et al.*, 2019 for some exception), which could lower the accuracy of prevalence estimates (Jovani & Tella, 2006). We found an overall haemosporidian prevalence of about 20% in white-winged parakeets. Chagas *et al.* (2017) analysed the haemosporidian infection on several species of captive Psittaciformes (the golden parakeets *Guaruba guarouba*, the

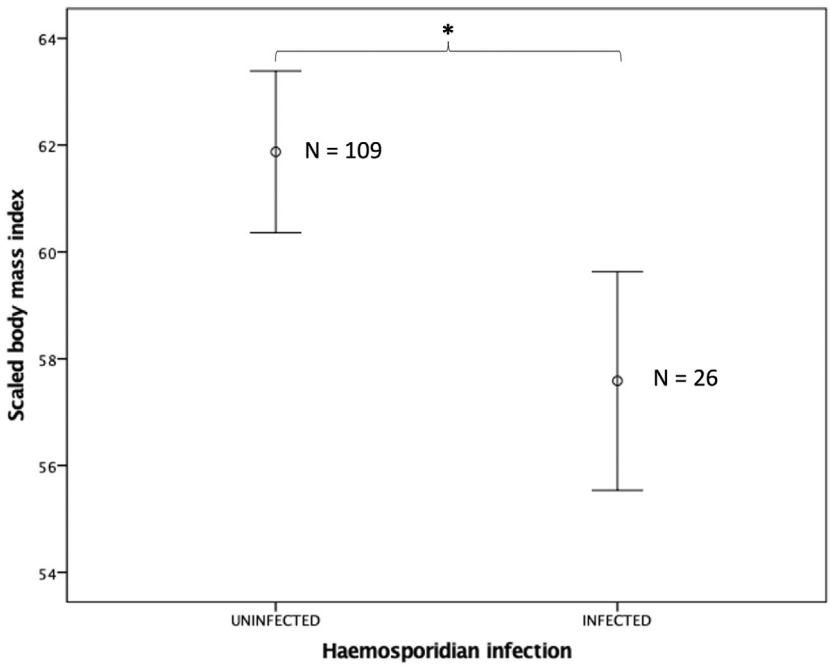

Figure 1 Barplots with error bars showing scaled body mass index values for uninfected and infected white-winged parakeets. One asterisk (*) indicates *P* value smaller than 0.05 ($P < 0.05$). Sample sizes are shown in the figures.

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Table 2 Factors explaining variation in the scaled body mass index for individual white-winged parakeets

Independent variable	Square-sum III	d.f.	*F*	*P*
Haemosporidian infection	250.424	1	5.640	**0.019**
Year of sampling	4.102	2	0.092	0.762
Location of sampling	237.043	3	5.339	**0.022**
Infection × year	52.340	1	1.179	0.280
Infection × location	19.641	1	0.442	0.507

Haemosporidian infection, year of sampling, location of sampling and the interactions between haemosporidian infection and year of sampling, and haemosporidian infection and location of sampling were included in the analysis as predictor variables. Sample size was 135 individuals. Significant factors are highlighted in bold.

turquoise-fronted Amazon *Amazona aestiva*, the hyacinth macaw *Anodorhynchus hyacinthinus* and the Spix's Macaw *Cyanopsitta spixii*), showing prevalences of 14.2, 8.3, 9 and 25% respectively. Also, Fecchio *et al.* (2018) analysed six wild individuals from three species of Psittaciformes from Brazilian Amazonia (the jandaya parakeet *Aratinga jandaya*, the brown-throated parakeet *Aratinga pertinax* and the pearly parakeet *Pyrrhura lepida*), showing an overall prevalence of haemosporidian infection of 42.9%. However, we should be cautious with our interpretations because the observed prevalence in our study could underestimate true prevalence. Following this idea, it is estimated that about 75% of parrots illegally poached from the wild may die from stress, disease or malnourishing during capture and transport before reaching the consumer (Cantú Guzmán *et al.*, 2007; Baker *et al.*, 2013). This mortality could be enhanced by malaria infections. In this sense, it has been shown that capture and transport may provoke stress in birds. For example, Dickens, Earle, & Romero (2009) empirically demonstrated that the capture and transport provoked changes in the physiological stress response in chukar partridge (*Alectoris chukar*). Moreover, Loiseau *et al.* (2008) documented that nestling house sparrows (*Passer domesticus*) injected with corticosterone, the hormone released during the stress response, showed a weaker immune response than control birds. Because this

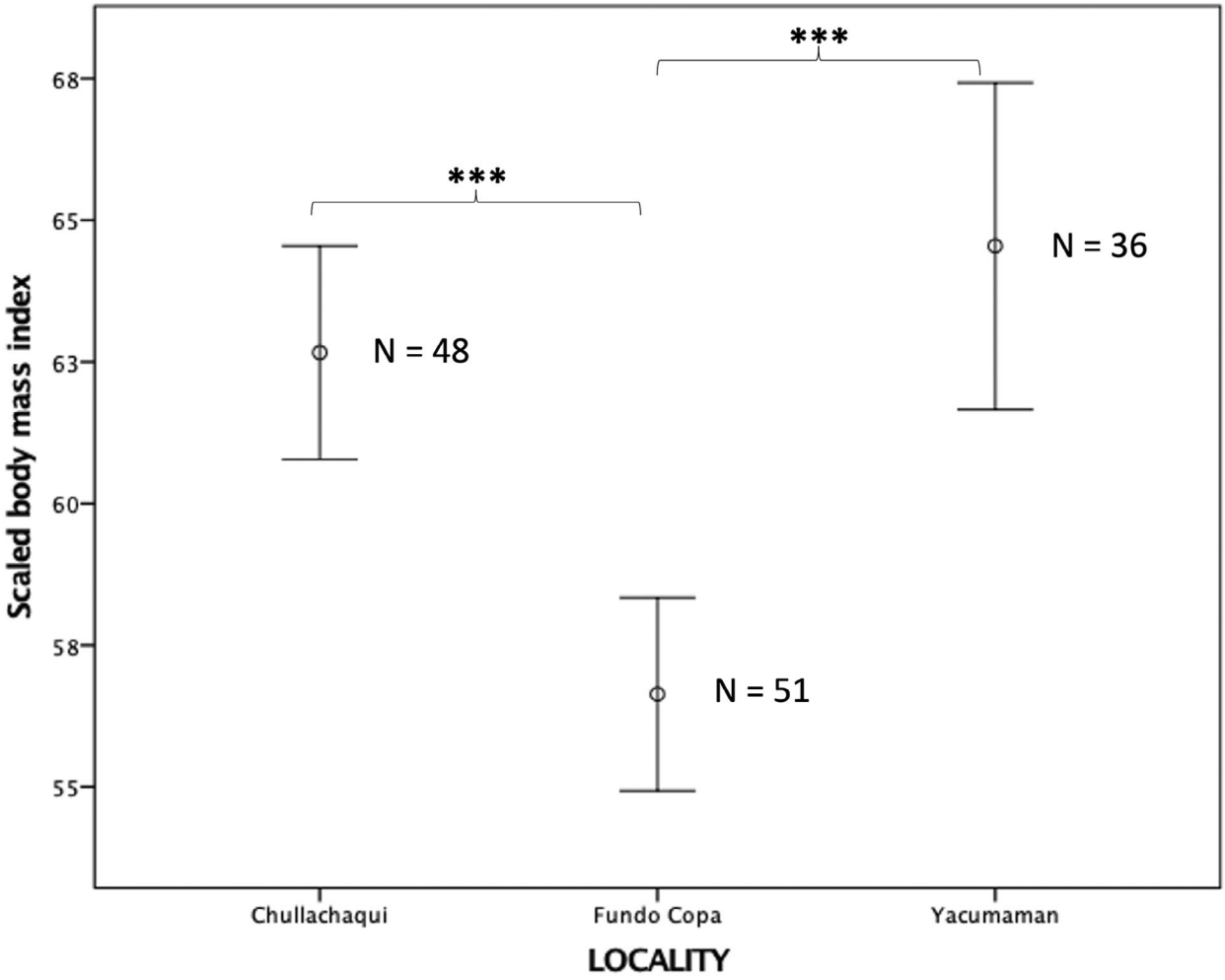

Figure 2 Barplots with error bars showing scaled body mass index values for white-winged parakeets sampled in Fundo Copa, Chullachaqui and Yacumaman. Three asterisks (***) indicate *P* value smaller than 0.001 ($P < 0.001$). Sample sizes are shown in the figures.

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negative impact of stress on the immune response in birds, chronic malaria infections could be more likely to relapse when birds are exposed to stressful situations (Applegate, 1970), thus leading to mortality of infected birds (Asghar *et al*., 2015). In consequence, our estimates of prevalence could be lower because chronically infected birds could die due to the acute phase of parasitaemia in relapses provoked by stressful situations such as poaching and transport. We should also accept the fact that the number of infected birds arriving to the new region should be even lower if introduced white-winged parakeets may lose their native malaria parasites during the colonization process (MacLeod *et al*., 2010), as it has been shown in previous studies exploring the prevalence and genetic characterization of haemosporidian parasites of established populations of exotic birds outside their natural range (Antonini *et al*., 2019; Lopes *et al*., 2018; Muriel *et al*., 2023). Therefore, the negative impact of the introduced parakeets on the native avian communities could be more limited.

More than 4840 haemosporidian lineages have been described in more than 2200 bird host species belonging to most of avian orders. Despite this huge diversity, the number of haemosporidian lineages found in Psittaciformes is still low, where only 0.4% of the lineages that have been found in all avian taxa have been described in this group of birds (MALAVI database version 2.5.6, March 2023, Bensch, Hellgren, & Pérez-Tris, 2009). Here we found two haemosporidian lineages infecting white-winged parakeets. As far as we know, our study shows the first records of haemosporidian lineages infecting white-winged parakeets. These blood parasites have been previously found infecting other parrot species. For example, Baron *et al*. (2014) reported adult captive budgerigars (*Melopsittacus undulatus*) infected with *P. relictum* GRW04 in New Zealand. In addition, Durrant *et al*. (2006) found wild red-shouldered Macaw (*Diopsitta nobilis*) infected with *P. nucleophilum* DENPET 03 in Guyana. Also, Chagas *et al*. (2017) showed one golden parakeet infected with *P. nucleophilum* DENPET 03 out of the 12 parakeets analysed in captive birds from the São Paulo Zoo (Brazil).

Exotic species introduced beyond their natural geographical range may harbour and spread pathogens provoking emerging diseases with tremendous costs in terms of global health (García-Longoria *et al*., 2015; Marzal & García-Longoria, 2020). *P. relictum* lineages are highly invasive parasites, as they have been reported infecting birds beyond their native ranges (Clark *et al*., 2015; Marzal *et al*., 2015; Theodosopoulos *et al*., 2021). For these reasons, the International Union for Conservation of Nature (IUCN) considers avian malaria *P. relictum* within the 100 world's worst invasive species (Lowe, Browne, & Boudjelas, 2000). *P. relictum* GRW04 was the most prevalent malaria lineage found infecting white-winged parakeets, accounting for 96% of all malaria infections in our study. This is a wide generalist parasite that is mainly transmitted outside Europe (Hellgren *et al*., 2015; Ferraguti *et al*., 2023), and is thought to be responsible for devasting epizooties reported worldwide (Atkinson & Samuel, 2010). For example, after the successful establishment of *P. relictum* and its vector in Hawaii around the 1920s, a large number of endemic bird species have been driven to extinction (Warner, 1968; Van Riper III *et al*., 1986; Lapointe, Atkinson, & Samuel, 2012). This higher mortality in native birds provoked by exotic invasive parasites could be explained by the expected low virulence of parasites in co-evolved hosts, whereas naïve bird hosts lack evolved protective immunity (Schmid-Hempel, 2021). In this sense, some studies have reported that malaria has negligible negative effects on the survival of non-native birds that have a long evolutionary association with the parasite, which also have a limited period of effective disease transmission (Samuel *et al*., 2011; Samuel *et al*., 2015). In contrast, many endemic Hawaiian species, particularly Hawaiian honeycreepers, are highly susceptible to avian malaria, chronically infected and can act as life-long reservoirs and effectively transmit the diseases (Atkinson *et al*., 2000; Atkinson & LaPointe, 2009; Atkinson & Samuel, 2010). In support of this argument, Lymbery *et al*. (2014) examined the virulence of 16 co-introduced parasites that have switched to native hosts, revealing that 85% of these co-introduced parasites showed higher relative virulence in native hosts than in the co-introduced alien host. Therefore, the presence of the virulent malaria parasite *P. relictum* GRW04 in illegally traded white-winged parakeets may have catastrophic effects on native avifauna in areas where parakeets would be commercialized. Accidental or intentional releases by pet owners and traders have led to the introduction of several species of Psittaciformes beyond their natural range (Blackburn, Lockwood, & Cassey, 2009). For example, established self-sustaining feral populations of white-winged parakeets have been documented in metropolitan areas of cities from both North and South America such as Lima (Schulenberg *et al*., 2007), Los Angeles, San Francisco and Miami (Garrett, 1997; Brightsmith, 1999) and Guayaquil (Freile *et al*., 2012). Moreover, white-winged parakeets have greatly expanded their range in recent years in Puerto Rico (Falcón & Tremblay, 2018). Further studies analysing the haemosporidian infection in white-winged parakeets in these introduced population and nearby native birds would be desirable to assess potential spill-over risk of invasive avian malaria.

Malaria-infected parakeets showed lower scaled body mass index than non-infected birds. Although some studies have failed to report detrimental effects of haemosporidian infections in the body condition of birds (Palinauskas *et al*., 2008; Granthon & Williams, 2017; Muriel *et al*., 2023), our results agree with other investigations revealing negative effects of malaria on the life history of their avian hosts. For example, Valkiūnas *et al*. (2006) experimentally infected nestling blackcaps (*Sylvia atricapilla*) with sporozoites of *Haemoproteus belopolskyi* developed in their vectors, revealing a significant weight loss in inoculated birds compared to control individuals. Also, Carlson *et al*. (2016) reported a poor body condition in canaries (*Serinus canaria*) intravenously inoculated with *Plasmodium* spp. In addition, Marzal *et al*. (2008) showed a reduced body condition in house martins (*Delichon urbicum*) infected with haemosporidian parasites.

Finally, haemosporidian infection and scaled body mass index varied among location of sampling. Although the

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prepatent period (the time from the inoculation of sporozoites into birds until the maturation of the first generation of metacryptozoites) of haemosporidian parasites varies among bird species and even within individuals of the same species (Valkiūnas, 2005; Valkiūnas *et al*., 2018), this period normally exceeds 1 week after the infection with sporozoites for *P. relictum* GRW04 and *P. nucleophilum* DENPET03 (Valkiūnas, 2005; Zehtindjiev *et al*., 2008). Because all individuals were sampled within 7 days since they were seized from illegal wildlife trade by local authorities, we could exclude the possibility of becoming infected in location of sampling. Possible road and river routes for wildlife trafficking have been identified in Peru, including the trafficking nodes Pucallpa–Tingo María–Lima, and Iquitos-Yurimaguas-Chiclayo (Mendoza *et al*., 2022). Unfortunately, we do not know the local areas where parakeets were illegally extracted, so we cannot infer if the observed haemosporidian infections could reflect local prevalence in wild parakeets.

Summarizing, we revealed the infection by *P. relictum* GRW04 in illegally traded white-winged parakeets. The presence of this highly pathogenic malaria parasite in these birds implies a serious disease outbreak risk in areas where parakeets are commercialized, since this invasive haemosporidian parasite is known to spill over to native avifauna with fatal consequences. Our outcomes stress the importance of the implementation of effective legislation, policies and interdisciplinary collaborations among scientists through a One Health approach aiming to reduce health risks from wildlife trade.

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Author contributions

All authors contributed to the study conception and design. AM obtained the funding and resources for the study. Material preparation, data collection and molecular analysis were performed by all authors. The first draft of the manuscript was written by **A. Marzal**, **A. Díez-Fernández** and **L. García-Longoria**. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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