Parasitology

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A new pathogen spillover from domestic to wild animals: *Plasmodium juxtanucleare* infects free-living passerines in Brazil

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Research Article

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Abstract

Habitat modification may facilitate the emergence of novel pathogens, and the expansion of agricultural frontiers make domestic animals important sources of pathogen spillover to wild animals. We demonstrate for the first time that *Plasmodium juxtanucleare*, a widespread parasite from domestic chickens, naturally infects free-living passerines. We sampled 68 wild birds within and at the border of conservation units in central Brazil composed by Cerrado, a highly threatened biome. Seven out of 10 passerines captured in the limits of a protected area with a small farm were infected by *P. juxtanucleare* as was confirmed by sequencing a fragment of the parasite's cytochrome *b*. Blood smears from these positive passerines presented trophozoites, meronts and gametocytes compatible with *P. juxtanucleare*, meaning these birds are competent hosts for this parasite. After these intriguing results, we sampled 30 backyard chickens managed at the area where *P. juxtanucleare*-infected passerines were captured, revealing one chicken infected by the same parasite lineage. We sequenced the almost complete mitochondrial genome from all positive passerines, revealing that Brazilian and Asian parasites are closely related. *P. juxtanucleare* can be lethal to non-domestic hosts under captive and rehabilitation conditions, suggesting that this novel spillover may pose a real threat to wild birds.

Introduction

Emerging infectious diseases (EID) are those caused by the spread of parasites/pathogens within new hosts and geographical areas (Daszak *et al.*, 2000) and those which incidence has increased in recent times or that threatens to increase in the near future (Friend *et al.*, 2001). Current and projected habitat modification and overexploitation can increase the frequency of EID affecting humans, livestock and wild animals (Lafferty, 2003; Sehgal, 2010; Tompkins *et al.*, 2015), and these factors together are a major threat to the conservation of biodiversity (Smith *et al.*, 2009). There are several examples of population decimations following the spread of infectious diseases within wild animals (van Riper *et al.*, 1986; Robinson *et al.*, 2010; Preece *et al.*, 2017), and these negative effects can be favoured in human-modified landscapes (Mennerat *et al.*, 2010; Brearley *et al.*, 2013; Becker *et al.*, 2015; Tompkins *et al.*, 2015). However, detecting novel pathogens in natural populations before the onset of mortality episodes is challenging (Preece *et al.*, 2017).

Domestic animals are important sources of novel pathogens for naïve populations of wild animals, and disease spillover is facilitated due to the encroachment of livestock into natural areas (Daszak *et al.*, 2000; Deem *et al.*, 2012). Livestock activities usually maintain high densities of domestic animals (Lafferty and Gerber, 2002), and such areas can be important food and shelter sources for opportunistic free-living wild animals, where they gather at high densities (Gottdenker *et al.*, 2005; Carrete *et al.*, 2009; Tompkins *et al.*, 2015). Moreover, these areas can increase the abundance of important disease vectors by providing artificial water bodies for the development of bloodsucking dipterans (Norris, 2004; Reiter and LaPointe, 2007). Synanthropic and opportunistic wild animals can use areas with different levels of habitat integrity and they can act as a bridge, carrying pathogens from degraded areas where livestock is raised to preserved forests with a higher diversity of habitat-specialists animals (Karlsson *et al.*, 2015; Ferreira *et al.*, 2017). These epidemiological aspects make the interface between natural and anthropogenic-modified areas important hotspots for the emergence of novel host-pathogen interactions (Smith *et al.*, 2009; Karlsson *et al.*, 2015). The expansion of agricultural frontiers is reshaping these interactions (Sehgal, 2015), and one prediction, for instance, is that habitat modifications will change the distribution of avifauna populations,

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increasing the chances of disease emergence in wild birds (Friend *et al.*, 2001; Fuller *et al.*, 2012).

Haemosporidians are widely distributed protozoan parasites, and two main genera infecting birds are *Plasmodium*, transmitted by dipterans of the family Culicidae, and *Haemoproteus*, which vectors are members of the families Ceratopogonidae and Hippoboscidae (Valkiūnas, 2005). Haemosporidian infections on avian hosts have been linked to reductions on the reproductive success and longevity in chronic infections (Knowles *et al.*, 2010; Asghar *et al.*, 2015) and is also related to mortality outbreaks in populations with no evolutionary association with these parasites (Pacheco *et al.*, 2011*b*; LaPointe *et al.*, 2012; Vanstreels *et al.*, 2015). Avian haemosporidians can infect a wide number of bird species, and this host breadth can be labile across different geographical areas and temporal scales (Ellis *et al.*, 2015), with elevated chances of novel host–parasite interactions occurring at areas under anthropic alterations (Santiago-Alarcon *et al.*, 2012*a*).

Plasmodium gallinaceum and *P. juxtanucleare*, two species that naturally infect domestic chickens (*Gallus gallus domesticus*), have originated in the Asian continent, but only the second has global distribution (Valkiūnas, 2005). *Plasmodium juxtanucleare* was described for the first time in 1941 in Brazil (Versiani and Gomes, 1941) and since then it has been reported in Asia (Bennett and Warren, 1966; Chen *et al.*, 2015) and in many parts of the globe, including Africa (Earle *et al.*, 1991; Poulsen *et al.*, 2000) and other countries in the American continent, such as Mexico and Uruguay (Garnham, 1966). This parasite is widely distributed in Brazil, and prevalence varies from 4 to 100% in backyard chicken (Paraense, 1949; Krettli, 1972; Mota *et al.*, 2000; Prezoto *et al.*, 2001). The chronic phase of the infection is usually benign (Bennett and Warren, 1966; Silveira *et al.*, 2009), but anaemia, prostration and clinical signs such as incoordination and paralysis due to neurological lesions are common at the acute phase of infection, with some cases evolving to death under experimental conditions (Krettli, 1972; Silveira *et al.*, 2013).

Plasmodium juxtanucleare has been detected in several hosts of the family Phasianidae (order Galliformes), but experimentally infected Columbiformes (*Columba livia*), Anseriformes (*Anas platyrhynchos*) and Passeriformes (*Passer domesticus* and *Serinus canaria*) were shown to be refractive (Valkiūnas, 2005). This parasite was associated to an outbreak that killed five black-footed penguins (*Spheniscus demersus*, Sphenisciformes) under rehabilitation in South Africa (Grim *et al.*, 2003), and caused the mortality of a captive white eared-pheasant (*Crossoptilon crossoptilon*; Galliformes) in Japan (Murata *et al.*, 2008). Even though *P. juxtanucleare* infects non-domestic birds, there are no reports of this parasite infecting free-living bird communities in any part of the globe.

We captured wild birds at the border between a protected preserved area and modified landscapes to investigate the distribution of avian haemosporidians in different bird communities. Robust morphological and molecular analyses demonstrated that wild passerines are competent natural hosts of *P. juxtanucleare*. We detected the same parasite lineage in a backyard chicken sampled in the surroundings where infected passerines were captured, showing that avian malaria spillover to wild birds is possible. Determining whether this spillover could lead to an EID should be considered a priority for wildlife conservation in the Cerrado biome, one of the most threatened biodiversity hotspot in South America.

Material and methods

Study site and bird sampling

We conducted wild bird sampling within the limits of the Chapada dos Guimarães National Park (CGNP), and at its influence area in Chapada dos Guimarães municipality, which is covered by the Cerrado biome in the Brazilian central plateau in the State of Mato Grosso. Cerrado is a biodiversity hotspot (Myers *et al.*, 2000), but is also one of the most endangered eco-regions on Earth, with land conversion for agriculture constituting the main cause of habitat loss (Beuchle *et al.*, 2015).

Wild birds were sampled in five field campaigns of five days each from April 2013 until May 2014. We operated 10 mist-nets (10 m long × 3 m high, with 20 mm mesh size) for 5 h starting at sunrise, and nets were checked every 30 min. In April 2013, we captured 16 passerines inside the limits of CGNP (15°19′5.08″ S, 55°53′2.18″ W), a protected conservation site of 32 600 ha created in 1989 which is currently inside a strict nature reserve of 251 848 ha. In September 2013, and in February 2014, we captured 42 passerines in a gallery forest not protected by Brazilian laws (15°29′55.80″ S, 56°10′30.30″ W). However, this area is partially preserved and has integrity levels and forest structure compared with the CGNP. In August 2013 and in May 2014, we captured 10 passerines in a secondary forest contiguous with a pasture area belonging to a small farm (15°21′30.34″ S, 55° 27′23.99″ W). This sampling point is situated between the strict nature reserve and an area of sustainable use of natural resources encompassing 39 500 ha (Fig. 1). In December 2014, we sampled 30 backyard chickens raised in a small farm, located 50 m distant from the area where we mist-netted the birds. Flock size in this farm commonly fluctuates between 40 and 80 chickens, where they breed locally for meat and egg production. Individual chickens are kept until egg production ceases, and roosters are managed for more than 1 year. Sampled birds did not present physical alterations and estimated age ranged between three to 6 months. We could not sample younger chicks and older females.

Captured passerines and chickens were physically restrained and we obtained blood samples through brachial venipuncture. We prepared two blood smears per passerine and the material was air dried and fixed with absolute methanol for 1 min within 12 h of preparation. We did not obtain blood smears from sampled chickens. We stored the remaining volume of blood samples in absolute ethanol at room temperature for a maximum of five days and the material was ultimately kept at −20 °C until DNA was extracted. Before release, passerines were tagged with individual aluminium leg-rings.

Blood smear analysis

Blood smears were stained with 10% Giemsa solution for 40 min within two weeks of preparation. An Olympus CX31 light microscope equipped with an Olympus Q-Color5 imaging system (Olympus, Tokyo, Japan) together with Q-Capture Pro7 imaging software (QImaging, Surrey, Canada) were used to examine passerine blood smears and to capture images. We analysed blood films only from birds positive at the screening PCR. At least 200 microscopic fields under 1000 × magnification were examined for the detection of parasites which were identified following Valkiūnas (2005). Parasitaemia was estimated based on the actual counting of infected erythrocytes in a total of 10 000 observed erythrocytes.

Molecular analyses

Approximately 10 μL of blood was transferred to 1.5 mL microtubes and samples were dried at 37 °C for subsequent DNA extraction, for which we used a conventional phenol–chloroform method with isopropanol precipitation (Sambrook and Russell, 2001). The genomic DNA pellet was resuspended in 50 μL of ultrapure water and quantified using a NanoDrop 2000 (Thermo Scientific, Waltham, United States). Between 50 and

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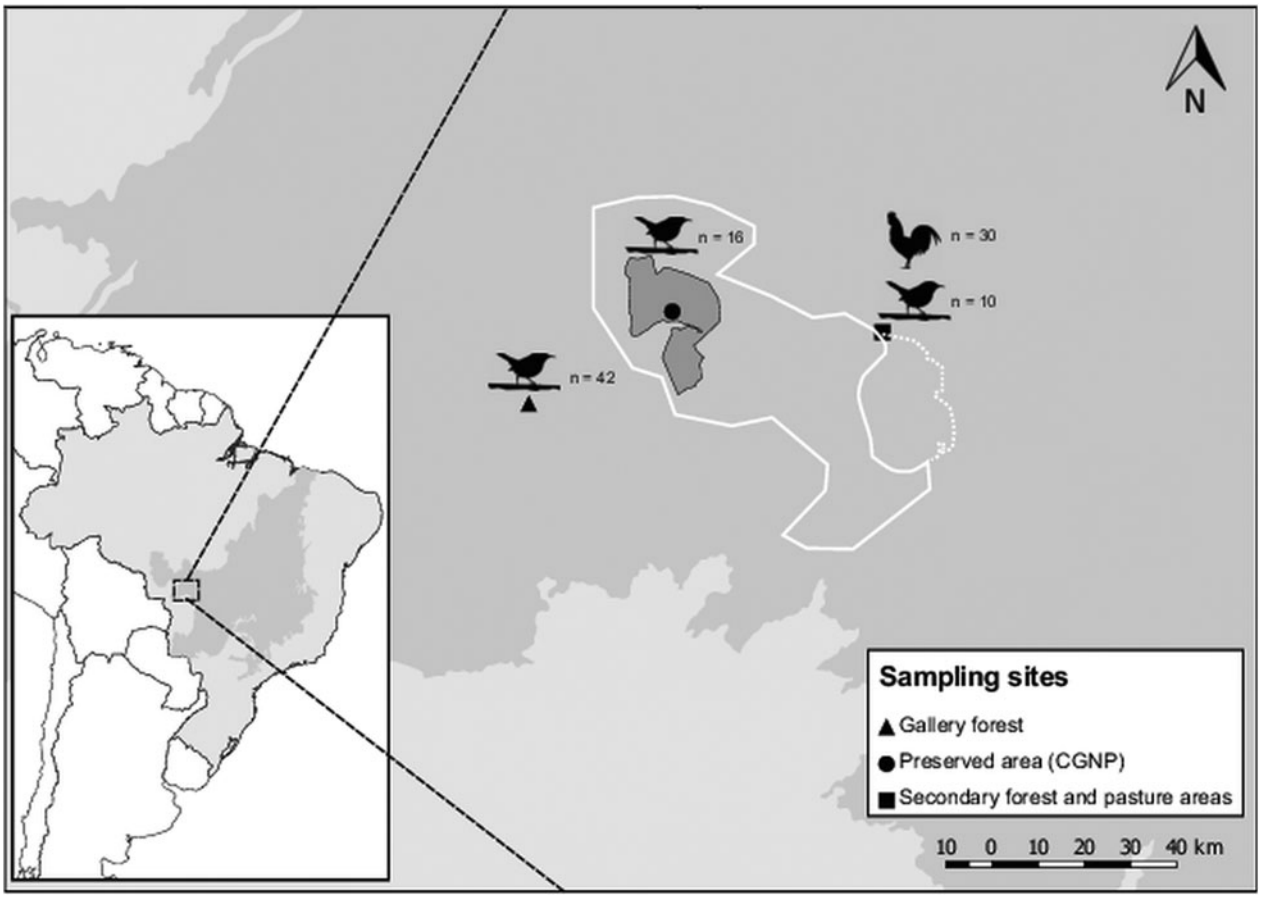

Fig. 1. Map of Chapada dos Guimarães National Park (dark grey area) in Mato Grosso, Brazil, showing sampling areas. A medium grey area indicates the distribution of the Cerrado biome. The white solid line represents the strict nature reserve and the white dashed line represents the area of sustainable use of natural resources.

100 ng of the extracted DNA was used for a screening PCR that amplifies a 154-nucleotide segment (excluding primers) of ribosomal RNA coding sequence within the mitochondrial DNA (mtDNA) of *Plasmodium* and *Haemoproteus* in a single reaction. We used the primers 343F (5′-GCTCACGCATCGCTTCT-3′) and 496R (5′-GACCGGTCATTTTCTTTG-3′) designed by Fallon *et al.* (2003) under PCR conditions and amplification analysis described by Roos *et al.* (2015).

DNAs from positive individuals were submitted to a nested-PCR targeting the amplification of a 478 bp region of the cytochrome *b* (*cyt b*) gene. For the first reaction, we used primers HaemNFI (5′-AGACATGAAATATTATGGITAAG-3′) and HaemNR3 (5′-GAAATAAGATAAGAAATACCATTC-3′) (Hellgren *et al.*, 2004) with 50–100 ng of genomic DNA. A 1-μL aliquot of this PCR product was then used as a template for the second reaction with the primers HaemF (5′-CTTATGGTGTCGATATATGCATG-3′) and HaemR2 (5′-CGCTTATCTGGAGATTGTAATGGTT-3′) (Bensch *et al.*, 2000). Both reactions contained 1 × buffer, 4 mM of $MgCl_2$, 0.3 mM of each dNTP, 1 unit of Taq (Phoneutria, Belo Horizonte, Brazil), 0.4 mM of each primer, and nuclease-free water in 25 μL reaction volumes. DNA extracted from blood samples of chickens experimentally infected with *P. gallinaceum* and ultrapure water was used as positive and negative controls, respectively. These nested-PCRs followed the protocol by Hellgren *et al.* (2004).

Products from all positive nested-PCRs were purified with Polyethylene Glycol 8000 (Sambrook and Russell, 2001) and bi-directionally sequenced with dye-terminator fluorescent labelling in an ABI Prism 3100 sequencer (Applied Biosystems, Foster City, USA). DNA sequences were aligned, checked for the presence of mixed infections (the presence of double peaks in the eletrochromatograms), edited using ChromasPro 2.0.6 (Technelysium Pty Ltd, Helensvale, Australia), and compared with data available in the public databases GenBank and MalAvi (Bensch *et al.*, 2009). Detected *cyt b* sequences were deposited in GenBank under accession numbers MG598389–MG598398 and MG598406.

In addition to the *cyt b* gene, seven almost complete mitochondrial genomes (mtDNA) of *P. juxtanucleare* were amplified, cloned and sequenced from the following positive bird samples: pearly-vented tody-tyrant (*Hemitriccus margaritaceiventer*, *n* = 2), a red-crested finch (*Coryphospingus cucullatus*), eastern slaty thrush (*Turdus subalaris*, *n* = 2), a short-crested flycatcher (*Myiarchus ferox*) and a plain-crested elaenia (*Elaenia cristata*). In order to avoid potential mixed infections, PCR products were amplified and cloned as followed: the primers forward 5′-GAGGATTCTCTCCACACTTCAATTCGTACTTC and reverse 5′CAGGAAAATWATAGACCGAACCTTGGACTC were used to amplify 5904 base pairs of mtDNA genome with TaKaRa LA TaqTM Polymerase (TaKaRa Mirus Bio Inc., Shiga, Japan) as described by Pacheco *et al.* (2011*a*). Wherever the parasitaemia was low, a nested PCR was performed using the internal oligos forward 5′-TTTCATCCTTAAATCTCGTAAC-3′/reverse 5′-GACCG AACCTTGGACTCTT-3′. PCR amplifications for both PCR (outer and inner) were carried out in a 50 μL volume using 20 ng of total genomic DNA. The PCR conditions were: a partial denaturation at 94 °C for 1 min and 30 cycles of 30 s at 94 °C and 7 min at 68 °C, followed by a final extension of 10 min at 72 °C. Following manufactory directions, two independent PCR products (bands of approximately 6 kb) were excised from the gel, purified using QIAquick® Gel extraction kit (Qiagen, GmbH, Hilden, Germany) and cloned in the pGEM®-T Easy Vector systems (Promega, Madison, USA). For at least three clones, both strands

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were sequenced using an Applied Biosystems 3730 capillary sequencer. There were no inconsistencies among the clones. The mtDNA genome sequences were submitted to GenBank under accession numbers MG598399–MG598405.

Phylogenetic analyses

Nucleotide alignment was produced by using ClustalX v2.0.12 and Muscle as implemented in SeaView v4.3.5 (Gouy *et al.*, 2010) with manual editing. The alignment was constructed with a total of 17 mtDNA genome sequences (5356 bp excluding gaps) belonging to three genera (*Leucocytozoon*, *Haemoproteus* and *Plasmodium*). Then, the alignment was divided into six partitions corresponding to the three non-coding regions between the ORFs (fragmented SSU rRNA and LSU rRNA) and the three coding genes, keeping their order in the mtDNA genome (non-coding, *cox3*, non-coding, *cox1*, non-coding, *cytb*, non-coding). Then, the phylogenetic relationship was inferred based on the alignment using the Bayesian methods implemented in MrBayes v3.2.6 with the default priors (Ronquist and Huelsenbeck, 2003). A general time reversible model with gamma-distributed substitution rates and a proportion of invariant sites (GTR + Γ + I) was used for each partition; which was the model with the lowest Bayesian Information Criterion (BIC) scores for both alignments and each partition as estimated by MEGA v7.0.14 (Kumar *et al.*, 2016). Bayesian support for the nodes was inferred in MrBayes by sampling every 500 generations from two independent chains lasting 10^4 Markov Chain Monte Carlo (MCMC) steps. As a 'burn-in', 50% of the sample was then discarded once convergence was reached. The chains were assumed to have converged once the value of the potential scale reduction factor (PSRF) was between 1.00 and 1.02 and the average standard deviation of the posterior probability was below 0.01 (Ronquist and Huelsenbeck, 2003). In addition, the effective sample sizes (ESSs) for all the parameters were checked by using Tracer v1.6 (Rambaut *et al.*, 2015). ESSs are recommended to be >200. In our case, all ESS values were higher than 750 (ranging from 769 to 5245), confirming a good mixing and convergence of the chains.

Results

Haemosporidian screening

We analysed blood samples from 68 wild birds, comprising 31 species from 13 families (Supplementary Table 1). Although our sample sizes did not allow estimating parasite prevalence in particular host species, the screening PCR targeting the rRNA from *Plasmodium*/*Haemoproteus* showed that 19.1% of the samples were positive. One out of 16 birds captured in the protected area of CGNP was positive (6.3%), five out of 42 birds sampled in the gallery forest were positives (12%), and seven out of 10 birds sampled in the transition between a secondary forests and pasture areas were positives (70%).

Molecular detection of P. juxtanucleare *and other haemosporidian parasites*

We sequenced a 478 bp fragment of the parasite's *cyt b* from 10 positive samples, as we could not obtain high-quality sequences from one bird sampled in the protected area and from two birds sampled in the gallery forest, even though these samples were positive in the nested-PCR. All seven samples from the secondary forest near pasture areas were successfully sequenced and they had 100% identity with a *P. juxtanucleare* strain isolated from chickens in southeastern Brazil (GenBank accession number KC142195) (Silveira *et al.*, 2013). Infected birds species were pearly-vented tody-tyrant (*H. margaritaceiventer*, $n = 2$), a red-crested finch (*C. cucullatus*), eastern slaty thrush (*T. subalaris*, $n = 2$), a short-crested flycatcher (*M. ferox*) and a plain-crested elaenia (*E. cristata*). This parasite lineage had also been detected in Malaysia (unpublished work, KT290910) and in Thailand (Tattiyapong *et al.*, 2016), and has a single nucleotide polymorphism in relation to the *P. juxtanucleare* associated to the death of one captive white eared-pheasant (*C. crossoptilon*) in Japan (AB302893) (Murata *et al.*, 2008).

Sequenced parasites not related to *P. juxtanucleare* obtained from birds captured at the gallery forest were an already described *Plasmodium* sp. lineage (BAFLA04; acc. no. JX029861) in an eastern slaty thrush (*T. subalaris*) and newly described lineages related to *Haemoproteus* sp. (acc. no. MG598390) and to *Plasmodium* sp. (acc. no. MG598391) detected in *Monasa nigrifrons*, order Galbuliformes (Supplementary Table 1).

After detecting *P. juxtanucleare* in passerines, we sampled and tested 30 backyard chickens maintained at 50 m from the area where these passerines were captured. The screening PCR revealed one positive sample (3.3%), which was confirmed as *P. juxtanucleare* by sequencing 478 base pairs of the *cyt b*, with 100% similarity with the parasite detected in the passerines.

Then, we sequenced the almost complete mitochondrial genome (17 sequences and 5356 bp excluding gaps) from parasites detected in all seven passerines infected by *P. juxtanucleare*. Results were identical between samples and these parasites had polymorphisms at four sites when compared with a *P. juxtanucleare* isolated from chickens in Japan (Omori *et al.*, 2007), the single isolate with the complete sequence at the mitochondrial level. Only one synonymous substitution occurred in *cyt b* gene, two substitutions occurred in two large subunit ribosomal RNAs and one substitution occurred in the RNA11 region (Fig. 2).

Morphological confirmation of complete development of P. juxtanucleare *in passerines*

Parasite identity was morphologically confirmed as *P. juxtanucleare* for all seven positive samples infected with this parasite in the sequencing results (Fig. 3). Parasitaemia ranged from 0.12–0.23%, and trophozoites, meronts, macro and microgametocytes were visualized in all blood smears (Table 1), except in a *T. subalaris*, in which sample we did not find gametocytes despite detecting a 0.19% parasitaemia. Features used to identify this parasite included trophozoites of generally small size, with scanty cytoplasm and usually adhering to the erythrocyte nucleus; meronts with scanty cytoplasm adhering to the subpolar portion of the nucleus, with three or four merozoites; macro and microgametocytes usually roundish, but that sometimes display oval to irregular shape, with mature forms never exceeding the size of the erythrocyte nucleus. Mature macro and microgametocytes possess a maximum of three roundish pigment granules clumped at the edge of the parasites. The overall proportion of infected wild birds with *P. juxtanucleare* was 10.3%.

Discussion

We demonstrated, using molecular and microscopy analyses, that *P. juxtanucleare*, a parasite typically found in domestic fowl, naturally infects free-living passerines in Brazil. We confirmed the presence of mature gametocytes in the blood of five bird species from four different families, revealing these passerines as competent hosts of *P. juxtanucleare*. This same parasite lineage was also detected in a backyard chicken managed close to the area where infected passerines were captured, revealing that wild and domestic birds share the same haemosporidian at the limits of a strict

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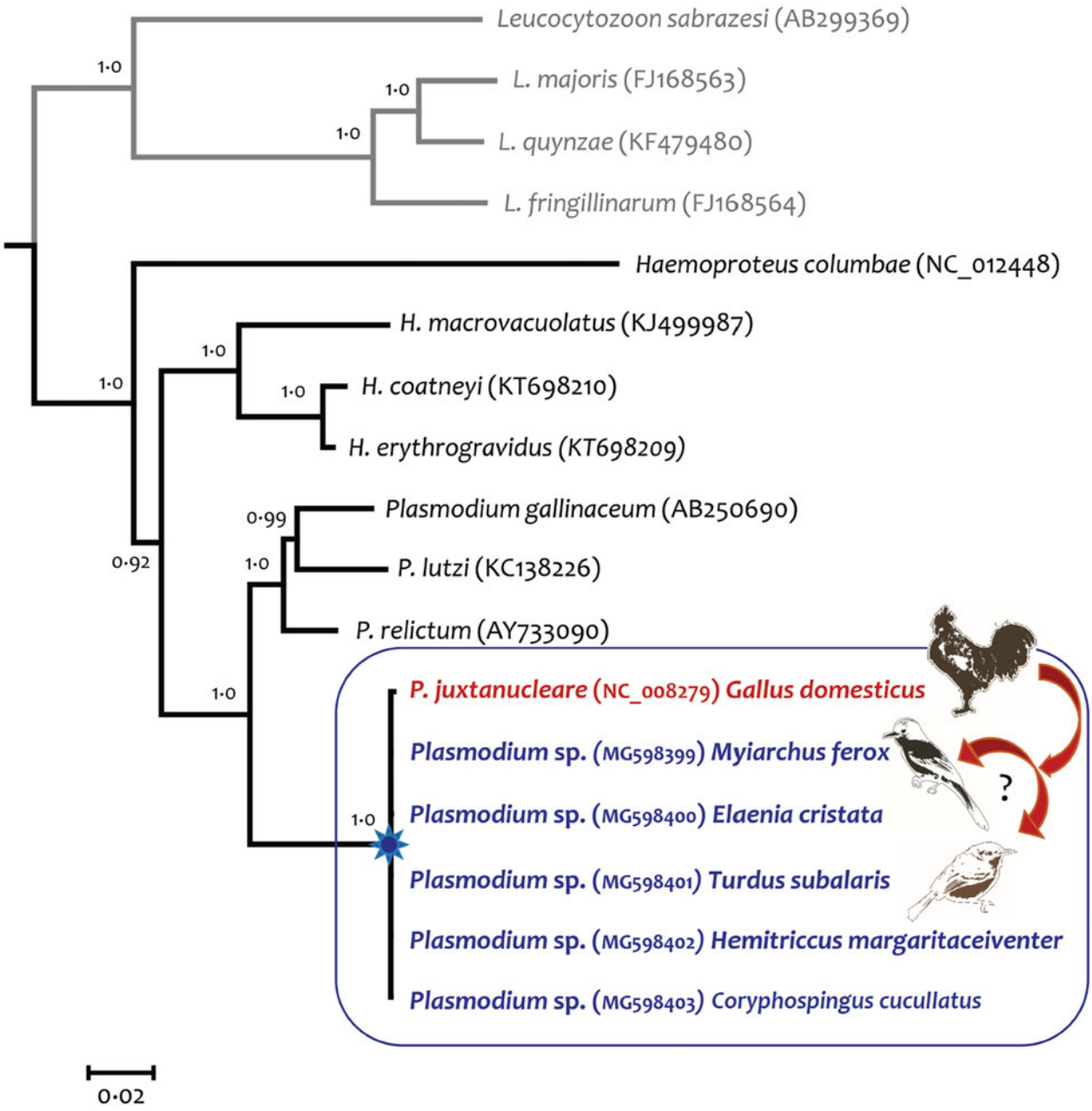

Fig. 2. Bayesian phylogenetic tree showing the relationships between *Plasmodium juxtanucleare* detected in passerines and in domestic chicken from Japan. Bayesian inference was conducted based on the almost complete mitochondrial genome (17 sequences and 5356 bp excluding gaps) of avian haemosporidians. Values above branches are posterior probabilities and the *Leucocytozoon* genus was used as an outgroup. All sequences obtained from passerine birds were identical but differed at four sites from the lineage isolated from *Gallus gallus domesticus*.

nature reserve. Our study shows a new parasite spillover from domestic livestock to wild birds in a highly threatened ecosystem from a megadiverse country. *Plasmodium juxtanucleare* can be highly pathogenic for non-domestic birds under captive conditions (Grim *et al.*, 2003; Murata *et al.*, 2008), thus these novel host–parasite associations may pose a real threat to the avifauna in Brazil.

Only birds captured in a secondary forest at the boundaries of a farm managing chickens were positive for *P. juxtanucleare*. Previous studies demonstrated that wild birds captured closer to poultry farms are more likely to be infected by general haemosporidians even though *P. juxtanucleare* was not detected (Gonzalez-Quevedo *et al.*, 2014; Padilla *et al.*, 2017). Here, we show that the infection by *P. juxtanucleare* also seems to be related to the proximity to poultry farms, and this is expected because chickens are a major reservoir for this parasite. Farms managing backyard chickens can act as ecological traps for wild birds (Carrete *et al.*, 2009; Becker *et al.*, 2015), where they gather in high densities (Gottdenker *et al.*, 2005) and may present an increased likelihood of being infected by a parasite that we demonstrate to be more generalist than previously thought. Seven out of eight passerines captured in this area in May 2014 were positive for *P. juxtanucleare*, suggesting that such environmental conditions created by livestock production might favour parasite transmission.

Determining whether this parasite will become a wildlife health problem requires additional investigations, as new host–parasite interactions do not necessarily lead to EIDs (Ebert and Herre, 1996; Pacheco *et al.*, 2013; Hillman *et al.*, 2015; Tompkins *et al.*, 2015). Passerines found infected in our study had parasitaemias similar to levels at which chicks experimentally infected with *P. juxtanucleare* presented physiological alterations

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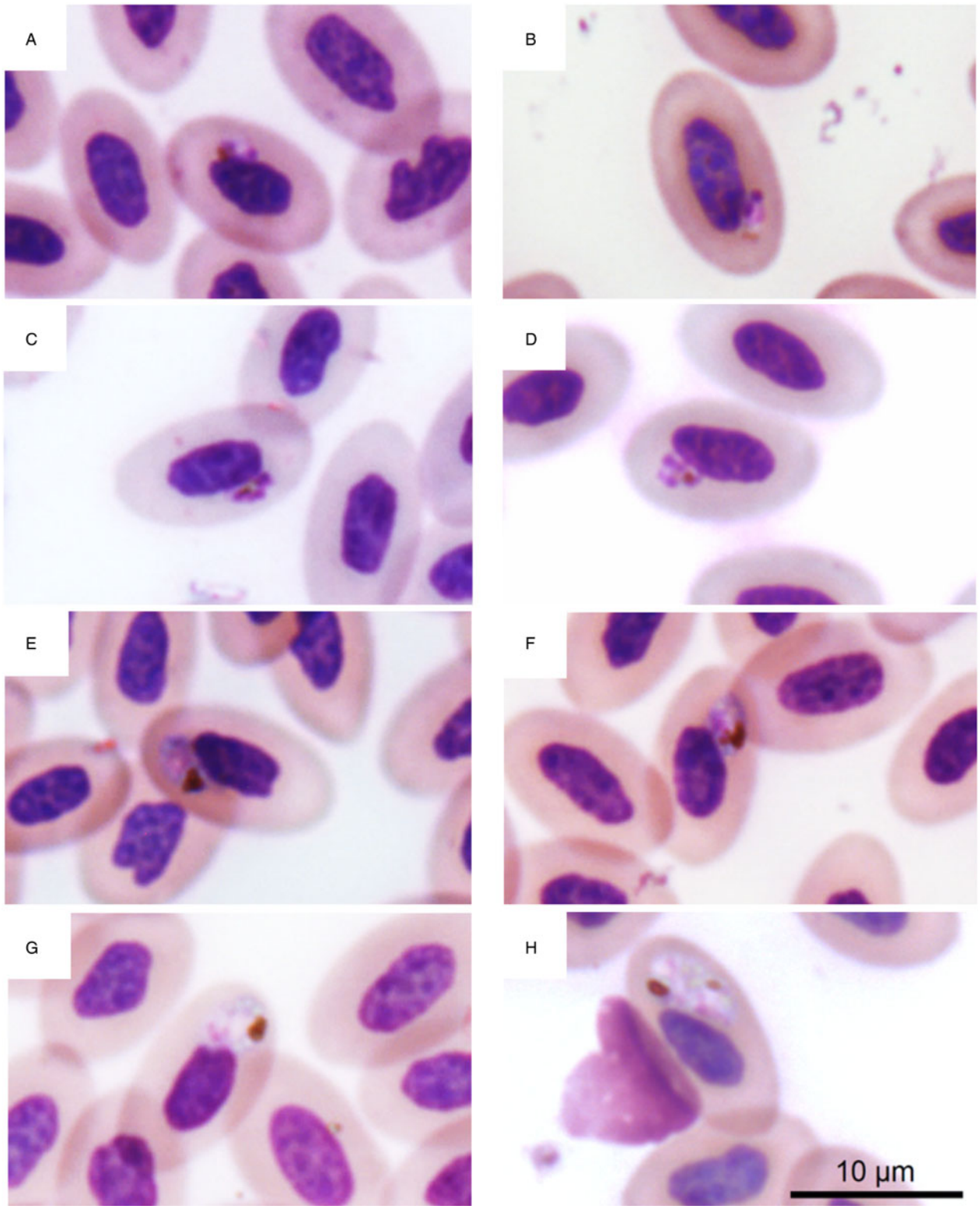

Fig. 3. Blood stages of *Plasmodium juxtanucleare* from passerines captured in the interface between preserved and modified landscapes. (A, B) trophozoites; (C, D), meronts with three and four merozoites, respectively; (E, F), macrogametocytes; (G, H), microgametocytes.

such as anaemia (Silveira *et al.*, 2013). This indicates that wild birds can survive the infection by a pathogenic parasite and regain locomotor activity at natural conditions, what had been demonstrated in experimental conditions (Mukhin *et al.*, 2016). Consequently, passerines can disperse *P. juxtanucleare* across different biomes and countries in South America due to their wide geographic range (Sick, 1997). The eastern slaty thrush (*T. subalaris*), for example, is a migratory species wintering at central Brazil (our area of study) that use the southernmost region of the country as their breeding sites (Vogel, 2014). Long and short-distance migrants disperse avian haemosporidians within the American continent, sharing a high proportion of parasites with resident species in both wintering and breeding grounds (Roos *et al.*, 2015; Ricklefs *et al.*, 2017), and the eastern slaty thrush may be important at dispersing this parasite in a broad geographical range. Additionally, *P. juxtanucleare*-infected bird species use habitat at different integrity levels, such as advanced secondary forests and preserved Cerrado, as well as they are found in

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Table 1. Parasite forms and parasitaemia of *Plasmodium juxtanucleare* detected in wild birds sampled in the Brazilian Cerrado

Bird species	Family	Parasite forms detected	Parasitaemia (%)	GenBank acc. No.
Hemitriccus margaritaceiventer	Rhynchocyclidae	Troph., Meron., Macrog., Microg.	0.21	MG598402
Hemitriccus margaritaceiventer	Rhynchocyclidae	Troph., Meron., Macrog., Microg.	0.15	MG598404
Coryphospingus cucullatus	Thraupidae	Troph., Meron., Macrog., Microg.	0.23	MG598403
Turdus subalaris	Turdidae	Troph., Meron., Macrog., Microg.	0.23	MG598401
Turdus subalaris	Turdidae	Troph., Meron.	0.19	MG598405
Myiarchus ferox	Tyrannidae	Troph., Meron., Macrog., Microg.	0.12	MG598399
Elaenia cristata	Tyrannidae	Troph., Meron., Macrog., Microg.	0.13	MG598400

Troph., trophozoites; Meron., meronts, Macrog. macrogametocytes; Microg., microgametocytes.
Parasitemia estimated in 20 000 erythrocytes counted at 1000 × magnification under optical microscope.

peri-urban areas around the protected area of the park (Lopes *et al.*, 2009), what may facilitate the dissemination of *P. juxtanucleare* within bird communities that do not get into close contact with domestic chickens.

Wild birds infected with *P. juxtanucleare* presented circulating gametocytes, meaning that they can be the source of infection for vectors, which could subsequently infect other wild birds without the need to feed on domestic chickens. We cannot determine if parasite transmission within wild birds is dependent on the presence of domestic chickens or if the transmission is sustained within passerines. However, nine individuals from four species found infected with *P. juxtanucleare* near to the farm were not infected by this parasite when captured in the Gallery forest, indicating that the conditions created at backyard chicken production are important for parasite persistence among passerines.

The detection of gametocytes in passerines indicates that *P. juxtanucleare* can be transmitted from wild to domestic birds as well. This parasite seems to be established within these populations and transmission is likely to occur in both directions. Local asynchronous breeding in the farm analysed here provide susceptible hosts year round, favouring a consistent transmission cycle that may persist over time. Consequences of this infection are usually mild for domestic chickens (Krettli, 1972; Silveira *et al.*, 2009), although high mortality rates (Versiani and Gomes, 1943; Garnham, 1966) and reduction in egg production may occur (Massard, 1982), showing that *P. juxtanucleare* epizootic cycle might affect food production and income in small farms.

Mosquitoes from the genus *Culex* are major vectors of *P. juxtanucleare* in Asia (Bennett *et al.*, 1966; Chen *et al.*, 2015), and *C. saltanensis* is the only confirmed vector for this parasite in Brazil (Lourenço-de-Oliveira and de Castro, 1991). This mosquito species is highly ornithophilic, and inhabit secondary forests and areas under anthropic modifications (Lourenço-de-Oliveira *et al.*, 1986; Consoli and de Oliveira, 1994). This habitat flexibility can facilitate parasite transmission between habitat generalists and forest specialist birds, favouring host switching even in the absence of infected domestic chickens. Haemosporidian vectors can have broad host preferences in forests under the anthropic influence, favouring parasite shifts between different groups of hosts (Santiago-Alarcon *et al.*, 2012*b*). *Culex saltanensis* is also found in Argentina, Panama, and Venezuela (Laurito *et al.*, 2008), suggesting that transmission of *P. juxtanucleare* can take place in different areas in South America. However, additional investigations are required to confirm whether *C. saltanensis* is the vector of this parasite in our study area or if other mosquito species can transmit this parasite as well.

Whole mitochondrial sequencing revealed that *P. juxtanucleare* infecting passerines in Brazil are very similar to the ones from Asia (Omori *et al.*, 2007), the region where this parasite is likely to have been originated. Previous observations suggested that American strains of *P. juxtanucleare* are more pathogenic (Valkiūnas, 2005), but we lack genetic information to address whether this high virulence is a phenotype linked to particular genetic characteristics of those parasite populations (e.g. unknown virulent factors), circumstances surrounding host exposure, or to other host-related factors.

The novel interaction between birds and parasites reported here have unpredictable conservational consequences. Understanding the epidemiology and transmission cycle of *P. juxtanucleare* within and between passerines and domestic chickens can provide valuable information to assess disease risk in wild bird populations due to the spillover of this pathogen. Reducing cross-species transmission of pathogens by limiting contact with domesticated animals may significantly reduce the risk of pathogen transmission to wildlife (Pedersen *et al.*, 2007). However, this can be unachievable in the avian malaria system, as free-living backyard chicken production is widespread in rural areas worldwide. This control is also difficult given that this is a mosquito-borne disease, and vectors can move freely between areas at different levels of habitat integrity (Consoli and de Oliveira, 1994; Ferreira *et al.*, 2016), having access to birds that do not get into close contact with domestic fowl.

Common pathogens in poultry production such as Avian paramyxovirus 1 (Garcia *et al.*, 2013) and *Mycoplasma gallisepticum* (Luttrell *et al.*, 2001) had been demonstrated to spill over to wild birds, causing clinical disease and mortality episodes. Here, we describe a new pathogen to be considered in future studies assessing diseases transmission risk between wild and domestic birds, such as in programmes for the reintroduction of endangered species (Deem *et al.*, 2012) or in wild animals translocations (Ewen *et al.*, 2012; Sainsbury and Vaughan-Higgins, 2012). Screening for *P. juxtanucleare* would aim to avoid the introduction of this parasite into new geographical areas or would avoid the introduction of birds in locations where *P. juxtanucleare* is present in wild birds or in backyard chickens.

Although previous investigations in Brazil did not detect *P. juxtanucleare* in wild birds captured in urban parks and in the interface between urbanized and preserved areas in the Cerrado biome (Belo *et al.*, 2011; Fecchio *et al.*, 2013), future studies should monitor whether our findings indicate a common or rather a transient and geographically isolated avian malaria spillover. On the other hand, *P. juxtanucleare* was detected in 2013 by employing partial *cyt b* sequencing in samples from wild passerines in the Brazilian Pantanal, a wetland-type biome, showing that this spillover may not be restricted to the Brazilian Cerrado (Richard C. Pacheco, unpublished observations). Continuous surveillance with capture-mark-recapture of birds together with haemosporidian screening can detect a possible fluctuation in population densities due to parasite infection (Podmokła *et al.*,

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2017) and may detect the impact of *P. juxtanucleare* at host community levels.

Experimental studies should be conducted to assess at which levels *P. juxtanucleare* is pathogenic for native avifauna throughout the globe, to predict current and future impacts of a pathogen spillover that may occur in a broad range. Furthermore, future studies should elucidate which factors may have driven this spillover to be detected only 70 years after the first description of *P. juxtanucleare*, to understand whether this is due to recent or local modifications in host-vector-parasite relationships or if it was simply due to failures to detect this parasite in the wild.

In conclusion, *P. juxtanucleare* spillover can be considered another detrimental impact derived from land conversion into agriculture areas in the Brazilian Cerrado, where an epizootic cycle seems to be established among and between domestic and wild birds. With this in mind, the distribution of *P. juxtanucleare* among free-living birds should be evaluated at large scale given the global distribution of this parasite in domestic livestock. Our study emphasizes that it is important to combine molecular and morphological analyses in blood hematozoa studies since we could only confirm host competence for a novel pathogen relationship after detecting mature gametocytes in blood smears from free-living passerines. Conducting epidemiological surveillance in transition areas between protected areas and livestock production can demonstrate the emergence of pathogens that can threaten wildlife conservation.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S003118201800077X

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Conflicts of interest. None.

Ethical standards. This study was approved by the Ethics Committee on Animal Research of the Federal University of Mato Grosso (Protocol #23108.033602/12-0) and by Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO 57491).

References

Asghar M *et al.* (2015) Hidden costs of infection: chronic malaria accelerates telomere degradation and senescence in wild birds. *Science* **347**, 436–438.

Becker DJ, Streicker DG and Altizer S (2015) Linking anthropogenic resources to wildlife-pathogen dynamics: a review and meta-analysis. *Ecology Letters* **18**, 483–495.

Belo NO *et al.* (2011) Prevalence and lineage diversity of avian Haemosporidians from three distinct Cerrado habitats in Brazil. *PLoS ONE* **6**, e17654.

Bennett GF and Warren M (1966) Biology of the Malaysian strain of *Plasmodium juxtanucleare* Versiani and Gomes, 1941. I. Description of the stages in the vertebrate host. *Journal of Parasitology* **52**, 565–569.

Bennett GF, Warren M and Cheong WH (1966) Biology of the Malaysian strain of *Plasmodium juxtanucleare* Versiani and Gomes, 1941. II. The sporogonic stages in *Culex* (*Culex*) *sitiens* Wiedmann. *The Journal of Parasitology* **52**, 647–652.

Bensch S, Hellgren O and Pérez-Tris J (2009) Malavi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome *b* lineages. *Molecular Ecology Resources* **9**, 1353–1358.

Bensch S *et al.* (2000) Host specificity in avian blood parasites: a study of *Plasmodium* and Haemoproteus mitochondrial DNA amplified from birds. *Proceedings of the Royal Society B: Biological Sciences* **267**, 1583–1589.

Beuchle R *et al.* (2015) Land cover changes in the Brazilian Cerrado and Caatinga biomes from 1990 to 2010 based on a systematic remote sensing sampling approach. *Applied Geography* **58**, 116–127.

Brearley G *et al.* (2013) Wildlife disease prevalence in human-modified landscapes: wildlife disease in human-modified landscapes. *Biological Reviews* **88**, 427–442.

Carrete M *et al.* (2009) Goats, birds, and emergent diseases: apparent and hidden effects of exotic species in an island environment. *Ecological Applications* **19**, 840–853.

Chen T-H *et al.* (2015) Avian Plasmodium infection in field-collected mosquitoes during 2012–2013 in Tarlac, Philippines. *Journal of Vector Ecology* **40**, 386–392.

Consoli RAGB and de Oliveira RL (1994) *Principais Mosquitos de Importância Sanitária no Brasil*. Rio de Janeiro, RJ: FIOCRUZ.

Daszak P, Cunningham AA and Hyatt AD (2000) Emerging infectious diseases of wildlife--threats to biodiversity and human health. *Science (New York, N.Y.)* **287**, 443–449.

Deem SL *et al.* (2012) Diseases of poultry and endemic birds in Galapagos: implications for the reintroduction of native species. *Animal Conservation* **15**, 73–82.

Earle RA *et al.* (1991) Occurrence of *Plasmodium juxtanucleare* in greywing francolin: short communication. *South African Journal of Wildlife Research* **21**, 30–32.

Ebert D and Herre EA (1996) The evolution of parasitic diseases. *Parasitology Today* **12**, 96–101.

Ellis VA *et al.* (2015) Local host specialization, host-switching, and dispersal shape the regional distributions of avian haemosporidian parasites. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 11294–11299.

Ewen JG *et al.* (2012) Parasite management in translocations: lessons from a threatened New Zealand bird. *Oryx* **46**, 446–456.

Fallon SM *et al.* (2003) Detecting avian malaria: an improved polymerase chain reaction diagnostic. *Journal of Parasitology* **89**, 1044–1047.

Fecchio A *et al.* (2013) Structure and organization of an avian haemosporidian assemblage in a Neotropical savanna in Brazil. *Parasitology* **140**, 181–192.

Ferreira FCJ *et al.* (2016) Searching for putative avian malaria vectors in a seasonally Dry tropical forest in Brazil. *Parasites & Vectors* **9**, 587.

Ferreira Jr FC *et al.* (2017) Habitat modification and seasonality influence avian haemosporidian parasite distributions in southeastern Brazil. *PLoS ONE* **12**, e0178791.

Friend M, McLean RG and Joshua Dein F (2001) Disease emergence in birds: challenges for the twenty-first century. *The Auk* **118**, 290–303.

Fuller T *et al.* (2012) The ecology of emerging infectious diseases in migratory birds: an assessment of the role of climate change and priorities for future research. *EcoHealth* **9**, 80–88.

Garcia SC *et al.* (2013) Molecular epidemiology of newcastle disease in Mexico and the potential spillover of viruses from poultry into wild bird species. *Applied and Environmental Microbiology* **79**, 4985–4992.

Garnham PCC (1966) *Malaria Parasites and Other Haemosporidia*. Oxford, England: Blackwell Scientific.

Gonzalez-Quevedo C, Davies RG and Richardson DS (2014) Predictors of malaria infection in a wild bird population: landscape-level analyses reveal climatic and anthropogenic factors. *Journal of Animal Ecology* **83**, 1091–1102.

Gottdenker NL *et al.* (2005) Assessing the risks of introduced chickens and their pathogens to native birds in the Galápagos archipelago. *Biological Conservation* **126**, 429–439.

Gouy M, Guindon S and Gascuel O (2010) Seaview version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27**, 221–224.

Grim KC *et al.* (2003) *Plasmodium juxtanucleare* associated with mortality in black-footed penguins (*Spheniscus demersus*) admitted to a rehabilitation center. *Journal of Zoo and Wildlife Medicine* **34**, 250–255.

https://doi.org/10.1017/S003118201800077X Published online by Cambridge University Press

Hellgren O, Waldenström J and Bensch S (2004) A New PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. *Journal of Parasitology* **90**, 797–802.

Hillman AE, Lymbery AJ and Thompson RCA (2015) Is *Toxoplasma gondii* a threat to the conservation of free-ranging Australian marsupial populations? *International Journal for Parasitology: Parasites and Wildlife* **5**, 17–27.

Karlsson EA *et al.* (2015) Non-Human primates harbor diverse mammalian and avian Astroviruses including those associated with human infections. *PLoS Pathogens* **11**, e1005225.

Knowles SCL, Palinauskas V and Sheldon BC (2010) Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *Journal of Evolutionary Biology* **23**, 557–569.

Krettli AU (1972) *Plasmodium juxtanucleare* in the state of Minas Gerais, Brazil. Studies on its prevalence and some aspects of its biology. *Revista do Instituto de Medicina Tropical de Sao Paulo* **14**, 235–245.

Kumar S, Stecher G and Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

Lafferty KD (2003) Is disease increasing or decreasing, and does it impact or maintain biodiversity? *Journal of Parasitology* **89**, s101–s105.

Lafferty KD and Gerber LR (2002) Good medicine for conservation biology: the intersection of epidemiology and conservation theory. *Conservation Biology* **16**, 593–604.

LaPointe DA, Atkinson CT and Samuel MD (2012) Ecology and conservation biology of avian malaria. *Annals of the New York Academy of Sciences* **1249**, 211–226.

Laurito M, Visintin AM and Almirón WR (2008) *Culex saltanensis* morphological redescription of the immature and adult stages. *Journal of the American Mosquito Control Association* **24**, 203–210.

Lopes LE *et al.* (2009) Aves da Chapada dos Guimarães, Mato grosso, Brasil: uma síntese histórica do conhecimento. *Papéis Avulsos de Zoologia* **49**, 9–47.

Lourenço-de-Oliveira R and de Castro FA (1991). *Culex saltanensis* Dyar, 1928: natural vector of *Plasmodium juxtanucleare* in Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz* **86**, 87–94.

Lourenço-de-Oliveira R *et al.* (1986) Alguns aspectos da ecologia dos mosquitos (Diptera, Culicidae) de uma área de planície (granjas Calábria), em Jacarepaguá, Rio de Janeiro: V. Criadouros. *Memórias do Instituto Oswaldo Cruz* **81**, 265–271.

Luttrell MP *et al.* (2001) *Mycoplasma gallisepticum* in house finches (*Carpodacus mexicanus*) and other wild birds associated with poultry production facilities. *Avian Diseases* **45**, 321–329.

Massard CL (1982) Caracterizacao do parasitismo por *Plasmodium juxtanucleare* (Haemosporidea:Plasmodiidae) em criacao de Gallus gallus da raca Leghorn Branca. *Arquivos da Universidade Federal Rural do Rio de Janeiro* **5**, 141–146.

Mennerat A *et al.* (2010) Intensive farming: evolutionary implications for parasites and pathogens. *Evolutionary Biology* **37**, 59–67.

Mota RA *et al.* (2000) *Plasmodium juxtanucleare* (Versiani e Gomes, 1941) em galinhas (*Gallus gallus* L., 1857) de criações rústicas no Estado de Pernambuco. *Revista Brasileira de Ciência Veterinária* **7**, 188–190.

Mukhin A *et al.* (2016) The strategy to survive primary malaria infection: an experimental study on behavioural changes in parasitized birds. *PLoS ONE* **11**, e0159216.

Murata K *et al.* (2008) *Plasmodium* (*Bennettinia*) *juxtanucleare* infection in a captive white eared-pheasant (*Crossoptilon crossoptilon*) at a Japanese zoo. *The Journal of Veterinary Medical Science* **70**, 203–205.

Myers N *et al.* (2000) Biodiversity hotspots for conservation priorities. *Nature* **403**, 853–858.

Norris DE (2004) Mosquito-borne diseases as a consequence of land Use change. *EcoHealth* **1**, 19–24.

Omori S *et al.* (2007) Complete nucleotide sequences of the mitochondrial genomes of two avian malaria protozoa, *Plasmodium gallinaceum* and *Plasmodium juxtanucleare*. *Parasitology Research* **100**, 661–664.

Pacheco MA *et al.* (2011*a*) Timing the origin of human malarias: the lemur puzzle. *BMC Evolutionary Biology* **11**, 299.

Pacheco MA *et al.* (2011*b*) Haemosporidian infection in captive masked bobwhite quail (*Colinus virginianus ridgwayi*), an endangered subspecies of the northern bobwhite quail. *Veterinary Parasitology* **182**, 113–120.

Pacheco MA *et al.* (2013) Malarial parasite diversity in chimpanzees: the value of comparative approaches to ascertain the evolution of *Plasmodium falciparum* antigens. *Malaria Journal* **12**, 328.

Padilla DP *et al.* (2017) Factors affecting the distribution of haemosporidian parasites within an oceanic island. *International Journal for Parasitology* **47**, 225–235.

Paraense WL (1949) A survey on the occurrence of "*Plasmodium juxtanucleare*" in Bambuí (State of minas Gerais). *Memórias do Instituto Oswaldo Cruz* **47**, 355–359.

Pedersen AB *et al.* (2007) Infectious diseases and extinction risk in wild mammals. *Conservation Biology: The Journal of the Society for Conservation Biology* **21**, 1269–1279.

Podmokła E *et al.* (2017) Effect of haemosporidian infections on host survival and recapture rate in the blue tit. *Journal of Avian Biology* **48**, 796–803.

Poulsen J *et al.* (2000) Prevalence and distribution of gastro-intestinal helminths and haemoparasites in young scavenging chickens in upper eastern region of Ghana, West Africa. *Preventive Veterinary Medicine* **45**, 237–245.

Preece ND *et al.* (2017) A guide for ecologists: detecting the role of disease in faunal declines and managing population recovery. *Biological Conservation* **214**, 136–146.

Prezoto HHS *et al.* (2001) Aspectos do parasitismo de *Plasmodium* (*Novyella*) *juxtanucleare* Versiani & Gomes, 1941 em *Gallus gallus* L., 1758 em criação rústica no município de Santa Bárbara do Tugúrio – MG. *Revista Brasileira de Ciência Veterinária* **8**, 65–67.

Rambaut A *et al.* (2015) *Tracer v1*. 6. Retrieved from Molecular evolution, phylogenetics and epidemiology website: http://tree.bio.ed.ac.uk/software/tracer/ (accessed 12 February 2018).

Reiter ME and LaPointe DA (2007) Landscape factors influencing the spatial distribution and abundance of mosquito vector *Culex quinquefasciatus* (Diptera: Culicidae) in a mixed residential-agricultural community in Hawai'i. *Journal of Medical Entomology* **44**, 861–868.

Ricklefs RE *et al.* (2017) Avian migration and the distribution of malaria parasites in New World passerine birds. *Journal of Biogeography* **44**, 1113–1123.

Robinson RA *et al.* (2010) Emerging infectious disease leads to rapid population declines of common British birds. *PLoS ONE* **5**, e12215.

Ronquist F and Huelsenbeck JP (2003) Mrbayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (oxford, England)* **19**, 1572–1574.

Roos FL *et al.* (2015) Prevalence and diversity of avian malaria parasites in migratory black skimmers (*rynchops Niger*, Laridae, charadriiformes) from the Brazilian Amazon basin. *Parasitology Research* **114**, 3903–3911.

Sainsbury AW and Vaughan-Higgins RJ (2012) Analyzing disease risks associated with translocations. *Conservation Biology* **26**, 442–452.

Sambrook J and Russell DW (2001) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Santiago-Alarcon D, Palinauskas V and Schaefer HM (2012*a*) Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. *Biological Reviews* **87**, 928–964.

Santiago-Alarcon D *et al.* (2012*b*) Bloodmeal analysis reveals avian *Plasmodium* infections and broad host preferences of *Culicoides* (Diptera: Ceratopogonidae) vectors. *PLoS ONE* **7**, e31098.

Sehgal RNM (2010) Deforestation and avian infectious diseases. *Journal of Experimental Biology* **213**, 955–960.

Sehgal RNM (2015) Manifold habitat effects on the prevalence and diversity of avian blood parasites. *International Journal for Parasitology: Parasites and Wildlife* **4**, 421–430.

Sick H (1997) *Ornitologia Brasileira*. Nova Fronteira, Rio de Janeiro, RJ.

Silveira P, DaMatta RA and Dagosto M (2009) Hematological changes of chickens experimentally infected with *Plasmodium* (*Bennettinia*) *juxtanucleare*. *Veterinary Parasitology* **162**, 257–262.

Silveira P *et al.* (2013) Interactions of *Plasmodium juxtanucleare* and chicken anaemia virus: establishing a model. *Parasitology* **140**, 1777–1788.

Smith KF, Acevedo-Whitehouse K and Pedersen AB (2009) The role of infectious diseases in biological conservation. *Animal Conservation* **12**, 1–12.

Tattiyapong M *et al.* (2016) Molecular characterization of *Plasmodium juxtanucleare* in Burmese red junglefowls (*Gallus gallus spadiceus*) in Thailand. *The Journal of Protozoology Research* **26**, 1–10.

Tompkins DM *et al.* (2015) Emerging infectious diseases of wildlife: a critical perspective. *Trends in Parasitology* **31**, 149–159.

Valkiūnas G (2005) *Avian Malaria Parasites and Other Haemosporidia, 1st Edition*. Boca Raton, Florida: CRC Press.

van Riper CI *et al.* (1986) The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecological Monographs* **56**, 327–344.

https://doi.org/10.1017/S003118201800077X Published online by Cambridge University Press

Vanstreels RET *et al.* (2015) Epidemiology and pathology of avian malaria in penguins undergoing rehabilitation in Brazil. *Veterinary Research* **46**, 30.

Versiani V and Gomes BF (1941) Sobre um novo hematozoario da galinha, *Plasmodium juxtanucleare* n. sp. (Nota previa). *Revista Brasileira de Biologia* **1**, 231–233.

Versiani V and Gomes BF (1943) *Plasmodium juxtanucleare*, parasita da galinha doméstica (Nota adicionais). *Revista Brasileira de Biologia* **3**, 113–117.

Vogel HF (2014) Occurrence of the eastern slaty thrush (Turdidae) in southern Brazil during the non-breeding season. *Brazilian Journal of Ornithology* **22**, 260–264.

https://doi.org/10.1017/S003118201800077X Published online by Cambridge University Press