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Influence of Bird Behavioural Traits and Habitat in Predicting Haemoparasite Infection

Grace Nyathi [1], Mduduzi Ndlovu [1,2,*] and Tshifhiwa C. Nangammbi [3]

[1] School of Biology and Environmental Sciences, University of Mpumalanga, Mbombela 1201, South Africa; 201915359@ump.ac.za
[2] Stellenbosch Institute for Advanced Study (STIAS), Wallenberg Research Centre at Stellenbosch University, Stellenbosch 7600, South Africa
[3] Department of Nature Conservation, Tshwane University of Technology, Pretoria 0001, South Africa; nangammbitc@tut.ac.za
* Correspondence: mduduzi.ndlovu@ump.ac.za

Abstract

Host-vector contact rates influence the spread of several vector-borne infections, including avian haemoparasites. To investigate the ecological mechanisms underlying avian disease dynamics, we examined haemoparasite prevalences in relation to bird life-history attributes. Using previously collected data of 1003 birds sampled from an Afrotropical region, we tested the hypothesis that a bird's behavioural traits and habitat do not influence the chances of infection. Overall, infection prevalence did not differ significantly between gregarious and solitary birds, nor across association categories (wild, mixed, anthropogenic). However, significant differences in infection were detected across haemoparasite genera. *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* showed distinct infection patterns in relation to host behavioural traits and habitats. Moreover, there were significant differences in infection prevalence based on movement patterns (resident, nomadic, migratory) and foraging strata (ground, mixed, aerial). These results enhance our avian parasitology theories, indicating that behavioural traits and habitat also have parasite-genus-dependent impacts on infection prevalence. Our research demonstrates that behavioural characteristics have an unequal impact on haemoparasite prevalence, indicating that no single factor can accurately predict the probability of infection at an Afrotropical setting.

Keywords: avian malaria; movement patterns; foraging strata; haemosporidian; host; parasites; prevalence

Academic Editor: Gary Voelker

Received: 14 August 2025
Revised: 16 October 2025
Accepted: 17 October 2025
Published: 18 October 2025

Citation: Nyathi, G.; Ndlovu, M.; Nangammbi, T.C. Influence of Bird Behavioural Traits and Habitat in Predicting Haemoparasite Infection. *Diversity* **2025**, *17*, 731. https://doi.org/10.3390/d17100731

1. Introduction

Several diseases that have a detrimental effect on the health of animals are caused by vector-borne infections [1]. Numerous hosts are susceptible to infection by these pathogens, and among prone host species, prevalence differs [2]. Notwithstanding the complexity of the mechanisms influencing host susceptibility variation, the spread of certain vector-borne infections is significantly influenced by host-vector contact rates [2]. A significant filter for the spread of vector-borne infections like avian malaria parasites may be provided by the life history characteristics and behaviours of the host, which have been linked to host-vector contact rates [3]. We can better understand the fundamental evolutionary and ecological mechanisms underpinning the virulence of pathogens, prevalence across populations, and the susceptibility of hosts across taxa by empirically determining the

role of host traits [4]. Determining which life history attributes have the strongest filtering impacts on transmission across systems of vector-borne diseases is especially intrusive [5].

In North America, avian models have yielded significant insights into the connections between host life history characteristics and vector-borne pathogen transmission, including the West Nile Virus (WNV) [6], St. Louis encephalitis [7], and the agent of bacterial Lyme disease, *Borrelia burgdorferi* [8]. The phylum Apicomplexa, which includes avian haemosporidian parasites, has provided valuable models for research on host–parasite relationships [9], parasite-mediated selection [10], as well as the epidemiology and genetics of human malaria [11]. Avian haemosporidia, which comprise the malarial parasite, *Plasmodium*, and the closely linked genera *Leucocytozoon* and *Haemoproteus*, have labile relationships with vertebral hosts that change throughout space and time [12]. Microscopy-based research [12] has significantly underestimated the diversity of haemosporidian parasites; however, an increasing number of molecular studies have now discovered more than 1500 distinct parasite lineages [13]. Haemoparasites are primarily spread by dipteran vectors, *Haemoproteus* through biting midges (Ceratopogonidae), *Leucocytozoon* by blackflies (Simuliidae), *Plasmodium* by mosquitoes (Culicidae), with dynamics of transmission impacted by several behavioural and ecological factors [14]. Understanding the behavioural and environmental drivers of parasite transmission is essential for the effective conservation and management of bird species.

Afrotropical bird communities, along with their haemosporidian parasites, can be used as an excellent model system to study the impact of avian life history characteristics on the probability of contracting vector-borne infections [15]. Afrotropical bird species inhabit diverse habitats, often situated near one another, and they display a wide range of flocking practices, nest placements, nest types, and habitat preferences [16]. As a result, this system is appropriate for evaluating the predictive power of peculiar host life history characteristics on parasitism rates in a wide range of hosts. The ability to comprehend the evolution and ecology of haemosporidia on a wider biogeographic scale will also be significantly impacted by better sampling of understudied Afrotropical avian haemosporidia, particularly those found in the southern hemisphere, which have received less attention than their temperate equivalents [13].

Foraging habitat and bird behavioural traits are two important factors affecting the occurrence of haemoparasites [17]. A bird's behavioural trait describes whether a species is solitary (independent) or gregarious (social), whereas foraging habitat comprises the kinds of environments wherein birds forage for food, whether it be on the ground, in mixed environments, or arboreal [18]. Both behavioural traits [19] and habitat [20] are believed to affect the risk of infection and vulnerability to haemoparasite vector carriers. Although little is known about the mechanisms linking avian ecology and haemoparasite infections, certain traits are likely to impact exposure risk [20]. For instance, while solitary species may have less exposure, gregarious behaviour may enhance vector contact rates [14]. The probability of encountering vector groups like blackflies, biting midges, or mosquitoes is further influenced by habitat utilisation and foraging strata (ground, mixed, aerial) [21]. Notwithstanding their ecological significance, very few studies have examined the direct effects of habitat associations and behavioural traits on infection prevalence in Afrotropical birds [2]. Our study intends to better understand wildlife disease ecology and improve conservation strategies in the context of environmental change by analysing these ecological characteristics to find trends in host–parasite relationships across various bird groups [22].

Even though interest in the epidemiology and ecology of avian haemoparasites is increasing, there is still an array of important research gaps that significantly constrain our knowledge [23]. Most of the current research is geographically skewed, concentrating mostly on temperate areas and on a few species of birds, thus ignoring the parasite load and

rich biodiversity present in subtropical and tropical regions [24]. Furthermore, the ecology of dipteran vectors (such as blackflies, mosquitoes, and biting midges), the dynamics of co-infection, host immunological responses, and the evolutionary background of host–parasite interactions remain poorly understood [14].

This study sought to ascertain the prevalence of avian haemoparasites in and around the Kruger National Park (KNP) as well as to evaluate how host life history traits, including sociality, foraging stratum, movement patterns, and habitat associations, influence infection risk patterns.

2. Materials and Methods

2.1. Study Area

The research utilised data samples previously collected for a 2024 study that focused on avian haemosporidian diversity and distinct parasite lineages [25]. The data contained information from birds that were sampled from nine selected locations within (Shingwedzi, Shangoni, Phalaborwa, Satara, and Skukuza) and around (Acornhoek, Hazyview, Mkhuhlu, and Malelane) the KNP (Figure 1). Selected sites were evenly located along the breadth of the park to capture habitat, climate, and latitudinal differences. The park is a protected area of approximately 2,000,000 hectares spanning the provinces of Limpopo and Mpumalanga in South Africa's north-eastern region. It lies in low-lying savannas (Lowveld), with elevations ranging from 250–800 m above sea level [26]. To the east, the park borders Mozambique, and to the west, it adjoins South African private game reserves, communal residential areas, and small-scale agricultural land.

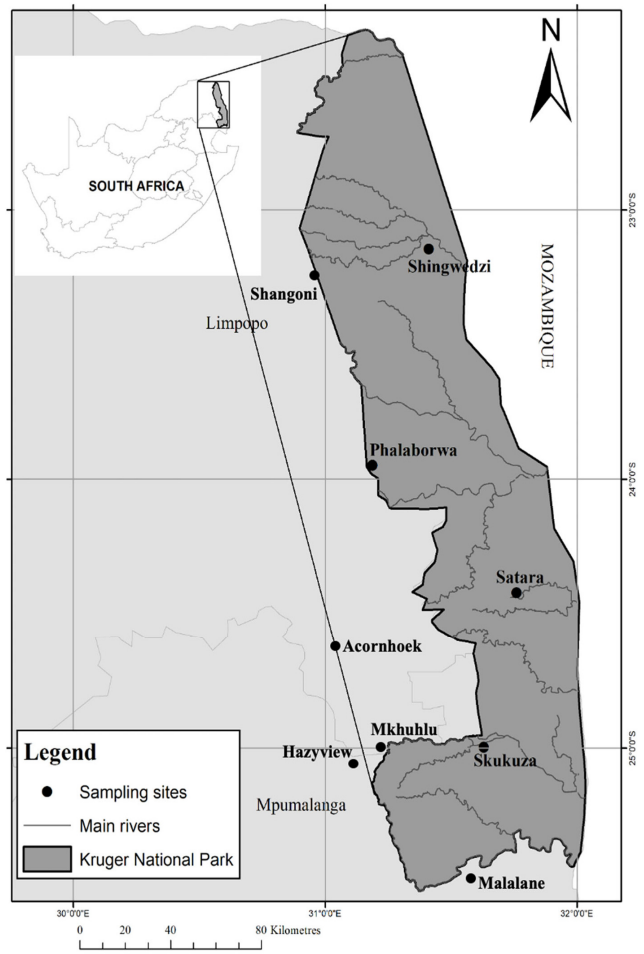

Figure 1. Geographical map of the study sites, both inside and outside the Kruger National Park [25].

The Lowveld region has a subtropical climate [26], with summer temperatures frequently exceeding 38 °C during hot, humid days. Winters are moderate and typically without frost. The wet summer season lasts from September to May, and rainfall in the KNP decreases gradually from the south (750 mm annually) to the northern part (350 mm annually) [27]. Despite this precipitation gradient, there remains a persistent drought in this protected area [28]. There are vast savanna vegetation types in the southern region of the park, while the north is dominated by Mopani woodlands [26]. The park sustains an extensive diversity of over 520 bird species due to the variety of microhabitats [29]. There are approximately 20 bird orders in which these species are found, including Strigiformes (owls), Bucerotiformes (hornbills), Accipitriformes (hawks and eagles), Coraciiformes (rollers and kingfishers), and Passeriformes (perching birds) [29]. Half of the birds that occur in the area are resident, 94 are cyclical, 109 are obscure, 107 have a restricted distribution range, and 18 are exclusively nocturnal [29].

2.2. Bird Sampling

Birds were caught using mist nets and birdcall lures as described by [30] between April 2015 and November 2017. The 32-month sampling period included both the dry (April–October) and the wet (November–March) seasons. Captured birds were identified and sexed. They were also ringed with an aluminium SAFRING ring, and their morphological measurements (culmen, wing, tarsus, head, and tail lengths) were taken. A capillary tube was used to collect approximately 30 µL of blood using branchial venipuncture, and the blood was deposited into a cryotube with lysis buffer solution (1 M Tris-HCI, pH 8.0, 0.5 M EDTA, 5 M NaCl, 10% SDS, and distilled water). Samples were stored at room temperature until extraction.

2.3. Molecular Screening and Parasite Detection

Genomic DNA was extracted from blood samples using two commercial kits, namely the DNeasy (Qiagen, Valencia, CA, USA) and Invisorb Spin Blood Mini Kit (Stratec molecular, Berlin, Germany), following the manufacturers' protocols. DNA quality and concentration were assessed using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE 19810 USA running the NanoDrop 2000 operating software), and molecular sexing PCR was conducted as a quality control step. Only samples yielding positive amplifications were included in parasite screening. Haemosporidian detection was performed using a validated SYBR Green-based real-time PCR protocol targeting a 182 bp fragment common to *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* (see Bell et al. [31]). Each reaction included positive and no template controls, and samples were run in duplicate. Positive samples were defined by amplification before the 36th cycle in both replicates. A synthetic *Plasmodium relictum* DNA fragment was used as an inter-run calibrator. All positive PCR products were sent for Sanger sequencing and lineage identification via a Basic Local Alignment Search Tool (BLAST, version 2.7.1) searches against the MalAvi and GenBank databases.

Extracted DNA samples were screened using a validated SYBR Green-based real-time PCR protocol targeting a 182 bp mitochondrial fragment common to *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* [31]. The primers R330F and R480RL were previously optimized at 500 Nm concentrations. Reactions were run using PowerUp SYBR Green Master Mix on an AB StepOnePlus system. Each plate included positive and no-template controls. A synthetic *Plasmodium relictum* gBlock (GenBank NC012426) served as an Inter-Run Calibrator (IRC), producing a melt peak at 79.6 °C, and generated 10-fold dilution curves to ensure consistency. Only samples with Ct < 36 in both replicates were considered positive.

All positive samples were sent to Macrogen Inc. (Seoul, Republic of Korea) for sequencing. Obtained sequences were altered and manually aligned with BioEdit version 7.0.5.2 [32]. BLAST analysis on MalAvi was performed [33] to determine parasite lineages present in the final sequences of the GenBank databases. When the parasite's genomes were found to be a perfect match for an already existing lineage, it was assigned MalAvi's lineage name [33]. Sequences that differed from existing parasite lineages either by a single base or more were all classified as novel lineages [34]. The novel lineages were assigned a distinctive lineage name following the MalAvi nomenclature outlined by Bensch et al. The new lineages of parasites that were discovered in this research were entered in the MalAvi databases and GenBank (see [25]).

2.4. Data Analysis

The current study analysed an existing dataset to investigate behavioural and ecological drivers of infection (gregariousness, foraging strata, movement patterns, and habitat association). Only bird species with five or more individuals sampled were considered, and prevalence was analysed at the haemoparasite genus level, i.e., *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* spp. Parasite prevalence was calculated from the number of infected birds divided by the total number of sampled individuals in the population. Following the Shapiro–Wilk test, neither the overall prevalence nor the prevalence of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* was normally distributed. Based on these findings, non-parametric tests were applied. We evaluated whether infection incidence (positive/negative) differed according to avian family using a Chi-square test of independence. Only bird families with $\geq$20 individuals were analysed. A Mann–Whitney U test was performed to compare overall infection prevalence as well as the prevalence for each infection genus, *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* in gregarious and solitary birds. To compare overall infection prevalence, *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* prevalences between ground, mixed, and aerial foraging birds, among resident, sedentary, and migratory birds, and between three groups of wild, mixed, and anthropogenically associated birds, Kruskal–Wallis tests were used, respectively. All statistical analyses were performed with the Jamovi statistical software (Jamovi 1.6, R 4.0) and tested at the 5% level of significance.

3. Results

A total of 1035 birds were sampled, but after screening the data for the required sample sizes of the target bird species, we retained 1003 individuals, representing 36 species, 28 genera, 22 families, and 8 orders. At least one haemosporidian genus was detected in 283 birds (Table 1). Three genera were identified using a conventional PCR protocol, namely: *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*. Overall infection prevalence was 28.2%, comprising 16.7% *Haemoproteus*, 4.5% *Plasmodium*, and 9.4% *Leucocytozoon.*

Haemoproteus (18.31%) was most prevalent in the dry season, followed by *Leucocytozoon* (7.28%). Both parasite genera were frequently detected even in the wet season, with prevalences of 14.73% and 10.17% for *Haemoproteus* and *Leucocytozoon* spp., respectively. *Plasmodium* prevalence was low in both the dry (3.94%) and wet (4.77%) seasons. Six of the study sites had high overall haemosporidian prevalence, two sites displayed moderately lower prevalence, and Hazyview had the lowest (Figure 2).

The Southern Grey-headed Sparrow (*Passer diffusus*, order Passeriformes) had the highest infection prevalence (59.23%) among birds with a sample size of $\geq$20, while the Red-billed Quelea (*Quelea quelea*, Passeriformes) had the least infection prevalence (1.54%). The avian family with the highest overall haemoparasite prevalence (14/25 = 56%) was Fringillidae (Finches, Passeriformes), and the least infection (3/70 = 4.29%) was detected

in Bucerotidae (Hornbills, Bucerotiformes). The most common parasite was *Haemoproteus* spp, whereas *Plasmodium* spp. were the least common. *Haemoproteus* was found to be most prevalent (63/244 = 27.87%) in Starlings (Sturnidae, Passeriformes). Red-billed Oxpeckers (Buphagidae, Passeriformes) had the highest (and only) *Leucocytozoon* infection prevalence (13/30 = 43.33%). *Plasmodium* and *Leucocytozoon* infections were not detected in Swallows (Hirundinidae, Passeriformes). There was significant evidence of association between avian family and infection presence (X^2 = 51.26, d.f. = 9, $p < 0.001$). Examination of standardized Pearson residuals suggested that deviations from independence were not uniformly distributed across families, consistent with heterogeneity in infection prevalence across avian families. (Table 2).

Table 1. Summary of sampled birds with species names, behaviour (association, foraging strata, movement, sociality), and corresponding infection prevalence (%) by haemosporidian genera (*Plasmodium*, *Haemoproteus*, *Leucocytozoon*). Title headings: *n* = sample size; Infected = number of birds where at least one type of infection was detected; *H* = *Haemoproteus*; *P* = *Plasmodium*; *L* = *Leucocytozoon*. Prevalence (%) calculations per avian species are a percentage proportion of infected birds out of the total birds sampled. PS: Totals of haemosporidian genus infections do not add up to the total infected numbers, as some individual birds had co-infections of more than one parasite genus.

Species	*n*	Behaviour				Infected	Prevalence (%)	*H*	*P*	*L*
		Association	Foraging	Movement	Sociality					
Actophilornis africanus	5	Wild	Ground	Migratory	Solitary	0	0	0	0	0
Anaplectes rubriceps	10	Wild	Aerial	Resident	Solitary	6	60	4	0	2
Buphagus erythrorhynchus	30	Mixed	Aerial	Resident	Gregarious	14	43	0	0	13
Colius striatus	6	Wild	Aerial	Resident	Gregarious	0	0	0	0	0
Creatophora cinerea	38	Wild	Ground	Resident	Gregarious	16	42	13	3	0
Crithagra mozambica	25	Anthropogenic	Ground	Resident	Gregarious	17	56	9	1	7
Dicrurus adsimilis	14	Mixed	Mixed	Resident	Solitary	2	29	2	0	0
Euplectes orix	9	Wild	Ground	Resident	Gregarious	0	0	0	0	0
Halcyon albiventris	5	Wild	Aerial	Migratory	Solitary	1	20	1	0	0
Hirundo rustica	28	Anthropogenic	Aerial	Migratory	Gregarious	1	7	1	0	0
Hirundo smithii	22	Anthropogenic	Aerial	Migratory	Gregarious	4	18	4	0	0
Lamprotornis chalybaeus	191	Mixed	Ground	Resident	Gregarious	64	34	45	18	2
Lamprotornis nitens	15	Mixed	Ground	Resident	Gregarious	5	33	5	0	0
Merops bullockoides	6	Mixed	Aerial	Nomadic	Gregarious	0	0	0	0	0
Motacilla aguimp	7	Mixed	Ground	Resident	Gregarious	0	0	0	0	0
Passer diffusus	31	Wild	Ground	Resident	Solitary	20	65	14	1	10
Passer domesticus	84	Anthropogenic	Ground	Resident	Gregarious	18	21	3	0	16
Ortygornis sephaena	10	Wild	Ground	Nomadic	Solitary	5	50	0	3	3
Ploceus capensis	7	Mixed	Ground	Resident	Gregarious	4	57	3	1	0
Ploceus cucullatus	69	Wild	Mixed	Resident	Gregarious	38	55	18	15	8
Ploceus intermedius	23	Wild	Mixed	Resident	Gregarious	4	17	4	0	0
Ploceus velatus	8	Mixed	Mixed	Resident	Gregarious	3	38	2	1	0
Prinia subflava	7	Wild	Ground	Resident	Solitary	1	14	1	0	0
Prionops plumatus	5	Wild	Ground	Resident	Gregarious	0	0	0	0	0
Pycnonotus tricolor	17	Anthropogenic	Ground	Nomadic	Gregarious	8	47	4	1	7
Quelea quelea	65	Mixed	Ground	Migratory	Gregarious	1	2	1	0	0
Spermestes cucullata	5	Mixed	Ground	Resident	Gregarious	0	0	0	0	0
Spilopelia senegalensis	102	Anthropogenic	Ground	Nomadic	Solitary	35	34	22	0	20
Streptopelia decipiens	54	Wild	Ground	Nomadic	Solitary	2	4	2	0	0
Streptopelia semitorquata	8	Wild	Ground	Resident	Solitary	3	38	3	0	1
Terpsiphone viridis	9	Wild	Mixed	Migratory	Solitary	1	11	1	0	1
Tockus erythrorhynchus	40	Wild	Ground	Resident	Gregarious	3	5	1	1	0
Tockus leucomelas	30	Wild	Ground	Nomadic	Gregarious	1	3	1	0	0
Trachyphonus vaillantii	5	Mixed	Ground	Resident	Solitary	2	40	2	0	1
Turdoides jardineii	6	Wild	Ground	Resident	Gregarious	3	50	0	0	3
Uraeginthus angolensis	7	Mixed	Mixed	Resident	Gregarious	1	14	1	0	0
Total	1003					283		167	45	94

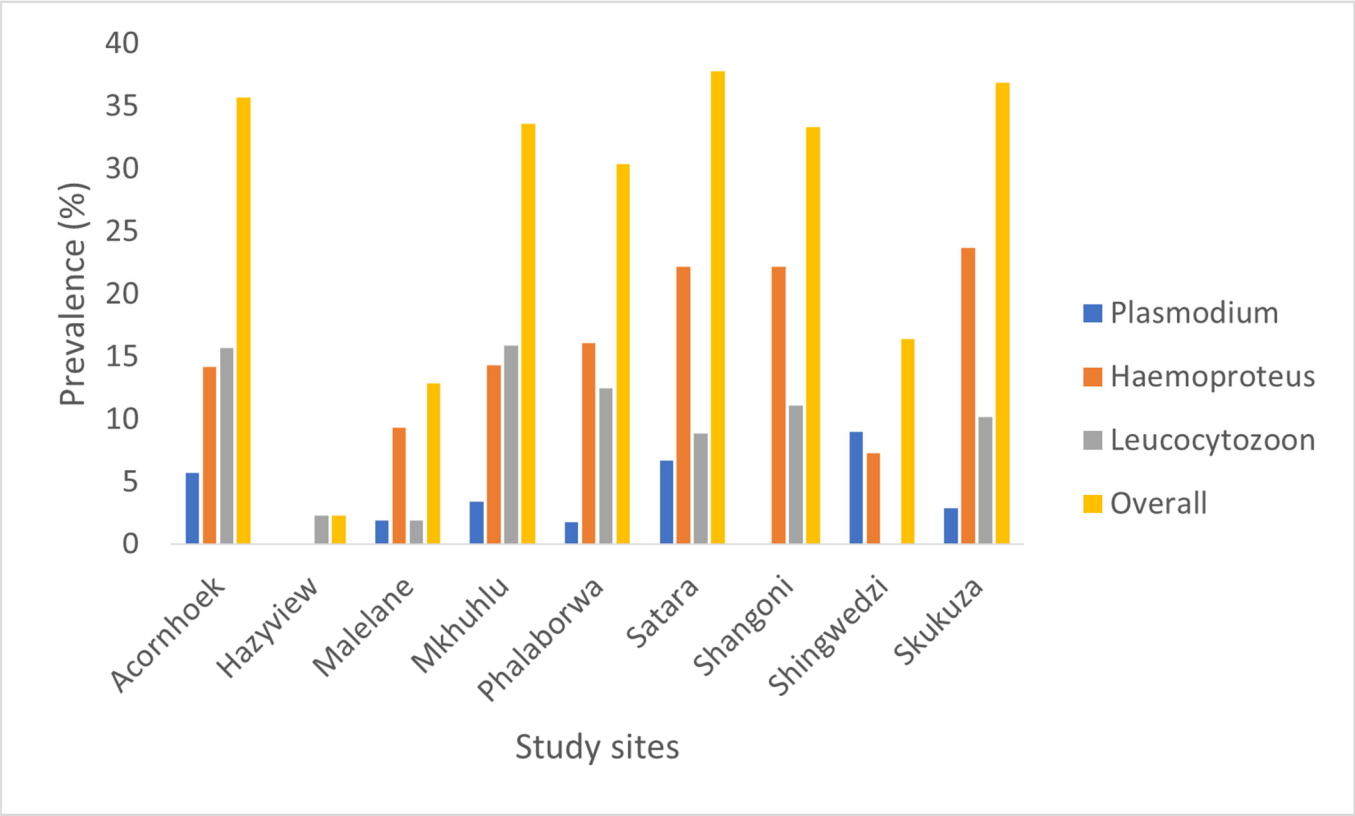

Figure 2. Diversity and haemoparasite prevalence at each sampling site.

Table 2. Avian Family and corresponding parasite infection prevalence. Only bird families with ≥20 sampled individuals were analysed and are presented. Column headings *n* = sample size.

Avian Family	*n*	Infected	Infection Prevalence %			
			Overall	*Haemoproteus*	*Plasmodium*	*Leucocytozoon*
Fringillidae	25	14	56.00	36.00	4.00	28.00
Buphagidae	30	13	43.33	-	-	43.33
Sturnidae	244	86	35.25	25.82	8.61	0.82
Passeridae	115	38	33.04	14.78	0.87	22.61
Ploceidae	191	56	29.32	16.75	8.90	5.24
Columbidae	164	40	24.39	16.46	-	12.80
Hirundinidae	50	5	12.00	10.00	-	-
Bucerotidae	70	3	4.29	2.86	1.43	-

Parasite genus diversity was highest among Dark-capped Bulbul (*Pycnonotus tricolor*), Greater Blue-eared Starlings (*Lamprotornis chalybaeus*), Village Weavers (*Ploceus cucullatus*), and Yellow-fronted Canary (*Crithagra mozambica*), harbouring all three parasites (Table 3). A total of 16 birds (5.8%) had double infections (birds with infections from two distinct genera of haemoparasites). Among the combinations were *Plasmodium* + *Leucocytozoon* (*n* = 4) and *Haemoproteus* + *Leucocytozoon* (*n* = 12). Most double infections were recorded in Laughing Doves, *Spilopelia senegalensis* (*n* = 6) and Dark-capped Bulbul (*n* = 3).

Sampled birds had a disproportionately larger number of gregarious (*n* = 743), than solitary (*n* = 260) birds. *Plasmodium* prevalence was highest in gregarious birds. *Haemoproteus* and *Leucocytozoon* prevalences were highest in solitary birds (Table 4). There was no significant difference (U = 87,722, p = 0.141) in overall parasite prevalence between gregarious (*n* = 743, Mdn = 29.8%) and solitary (*n* = 260, Mdn = 30.0%) infected birds. In contrast, genus-specific infections had significant differences in *Plasmodium* (U = 88,380,

$p = 0.005$), *Haemoproteus* ($U = 87{,}049$, $p = 0.039$), and *Leucocytozoon* ($U = 87{,}865$, $p = 0.025$) prevalence between gregarious and solitary birds (Table 4).

Table 3. Avian species with the highest diversity of infections. Column headings *n* = sample size. Labels for haemoparasite presence: *P* = *Plasmodium*, *H* = *Haemoproteus*, and *L* = *Leucocytozoon.* The number of birds infected with the corresponding parasite is indicated by values in parentheses.

Common Name	Species	*n*	Detected Haemoparasites
Dark-capped Bulbul	*Ploceus capensis*	7	*P* (1), *H* (3), *L* (7)
Greater Blue-eared Starling	*Lamprotornis chalybaeus*	191	*P* (18), *H* (45), *L* (2)
Village Weaver	*Ploceus cucullatus*	69	*P* (15), *H* (18), *L* (7)
Yellow-fronted Canary	*Crithagra mozambica*	25	*P* (1), *H* (8), *L* (7)
Total		302	

Table 4. Comparison of infection prevalence between Gregarious and Solitary birds according to the Haemosporidian genus. Row heading "Overall" indicates where any genus infection was detected.

Infection Genus	Prevalence (%)		Mann–Whitney *U*	*p* Value
	Gregarious	Solitary		
Plasmodium	5.52 (41/743)	1.54 (4/260)	88,380	0.005
Haemoproteus	15.48 (115/743)	20.00 (52/260)	87,049	0.039
Leucocytozoon	7.54 (56/743)	14.61 (38/260)	87,865	0.025
Overall	29.82 (205/743)	30.00 (78/260)	87,722	0.141

Overall infection prevalence comparison between ground, mixed, and aerial foraging birds revealed a significant difference ($H = 16.06$, $p < 0.001$) among the three groups. Similarly, there were genus-specific differences in prevalence for *Plasmodium* ($H = 23.50$, $p < 0.001$) and *Haemoproteus* ($H = 8.26$, $p = 0.016$), but no significant differences for *Leucocytozoon* ($H = 5.02$, $p = 0.081$) among ground, mixed, and aerial foraging birds. Post-hoc pairwise comparisons for overall prevalence revealed significant differences between Ground and Mixed birds ($W = 5.543$, $p < 0.001$) and between Aerial and Mixed birds ($W = 4.094$, $p = 0.011$), while no significant difference was detected between Ground and Aerial birds ($W = -0.106$, $p = 0.997$). Plasmodium prevalence in the Mixed group differed significantly from both the Ground ($W = 5.71$, $p < 0.001$) and Aerial groups ($W = 5.18$, $p < 0.001$), no significant difference was detected between the Ground and Aerial groups ($W = -2.86$, $p = 0.107$). *Haemoproteus* prevalence in the Mixed group was significantly different from both the Ground group ($W = 3.46$, $p = 0.038$) and the Aerial group ($W = 3.40$, $p = 0.043$), while no significant difference was detected between the Ground and Aerial groups ($W = -1.78$, $p = 0.420$).

The highest infection prevalence by any haemosporidian genus, was detected in mixed-foraging birds (prevalence = 39.23%), namely Blue Waxbill (*Uraeginthus angolensis*), African Paradise Flycatcher (*Terpsiphone viridis*) and Fork-tailed Drongo (*Dicrurus adsimilis*), followed by birds that forage on the ground (prevalence = 25.72%), namely African Mourning Dove (*Streptopelia decipiens*), Arrow-marked Babbler (*Turdoides jardineii*) and Cape Glossy Starling (*Lamprotornis nitens*), and lastly aerial foragers (prevalence = 24.30) had the least haemosporidian-infected birds, e.g., Red-headed Weaver (*Anaplectes rubriceps*), Wire-tailed Swallow (*Hirundo smithii*), and Red-billed Oxpecker (*Buphagus erythrorhynchus*). All three haemosporidian genera were detected in ground and mixed foragers. Mixed foragers had the highest prevalence of *Haemoproteus*, while aerial foraging birds had the highest prevalence of *Leucocytozoon* (Table 5).

Table 5. Prevalence of haemoparasites in relation to foraging strata.

Parasite	Prevalence (%)			Kruskal–Wallis (x^2, df, p)
	Aerial	Ground	Mixed	
Plasmodium	0 (0/107)	3.66 (28/766)	11.54 (15/130)	23.50, 2, <0.001
Haemoproteus	10.28 (11/107)	16.01 (123/766)	23.08 (30/130)	8.26, 2, 0.016
Leucocytozoon	14.02 (15/107)	8.09 (62/766)	6.92 (9/130)	5.02, 2, 0.081
Overall	24.30 (26/107)	25.72 (197/766)	39.23 (51/130)	16.06, 2, <0.001

Overall infection prevalence differed significantly (H = 47.0, $p < 0.001$) between migratory, nomadic, and resident birds. There were also significant differences in prevalence for *Plasmodium* (H = 13.7, p = 0.001), *Haemoproteus* (H = 16.0, $p < 0.001$), and *Leucocytozoon* (H = 14.7, $p < 0.001$) amongst the three movement patterns. Post-hoc pairwise comparisons showed that overall prevalence differed significantly across all movement categories, Migratory vs. Resident (W = 9.31, $p < 0.001$), Migratory vs. Nomadic (W = 6.00, $p < 0.001$), and Resident vs. Nomadic (W = –4.05, p = 0.012). Significant differences were found for *Plasmodium* between Migratory and Resident (W = 4.12, p = 0.010) and Resident and Nomadic birds (W = –3.43, p = 0.041), but not between Migratory and Nomadic birds (W = 2.24, p = 0.253). A comparable trend was displayed for *Haemoproteus*, with significant contrasts between Migratory and Resident (W = 4.80, p = 0.002) and Resident and Nomadic birds (W = –3.56, p = 0.032), but no variation across Migratory and Nomadic birds (W = 2.16, p = 0.278). For *Leucocytozoon*, there was a significant difference between migratory and resident birds (W = 4.55, p = 0.004) and migratory and nomadic birds (W = 5.65, $p < 0.001$), but not between Resident and Nomadic birds (W = 2.16, p = 0.278). Highest infections were recorded in resident (prevalence = 33.23%) followed by nomadic (prevalence = 22.37%) and lastly migratory (prevalence = 6.27%) birds. All bird movement type groups were infected by all three parasite genera. Resident birds had the highest prevalence of *Plasmodium* and *Haemoproteus*, *Leucocytozoon* prevalence was highest in nomadic birds (Table 6).

Table 6. The relationship between movement patterns (resident, nomadic, and migratory) and haemoparasite prevalence.

Parasite	Prevalence (%)			Kruskal–Wallis (x^2, df, p)
	Resident	Nomadic	Migratory	
Plasmodium	6.00 (39/650)	1.83 (4/219)	0 (0/134)	13.7, 2, 0.001
Haemoproteus	19.69 (128/650)	12.33 (27/219)	6.72 (9/134)	16.0, 2, <0.001
Leucocytozoon	8.64 (57/660)	12.79 (28/219)	0.75 (1/134)	14.7,2, <0.001
Overall	33.23 (216/650)	22.37 (49/219)	6.72 (9/134)	47.0, 2, <0.001

Lastly, there was no significant difference in overall parasite prevalence (H = 2.718, p = 0.257) or *Haemoproteus* prevalence (H = 0.409, p = 0.815) between wild, mixed, and anthropogenic-associated birds. In contrast, significant differences were observed for *Plasmodium* (H = 11.815, p = 0.003) and *Leucocytozoon* (H = 32.831, $p < 0.001$). Post-hoc pairwise comparisons for *Plasmodium* prevalence showed significant differences in infection prevalence between birds from wild and anthropogenic habitats (W = −4.796, p = 0.002) and between mixed and anthropogenic habitats (W = −4.624, p = 0.003), while no significant difference was detected between birds associated with wild and mixed habitats (W = −0.466, p = 0.942). *Leucocytozoon* prevalence showed significant differences in infection between birds associated with wild and anthropogenic habitats (W = 5.68, $p < 0.001$) and between mixed and anthropogenic habitats (W = 7.32, $p < 0.001$), while no significant difference was detected between birds from wild and mixed habitats (W = –1.66, p = 0.467). For overall prevalence and *Haemoproteus*, none of the pairwise comparisons were significant. All three

parasites were present in all bird groupings (anthropogenically associated, mixed, and wild), with sample sizes of 278, 360, and 357, respectively. Overall prevalence was highest in wild birds. *Plasmodium* and *Haemoproteus* prevalence was highest in birds found in both anthropogenic and wild settings, *Leucocytozoon* was more common in wild birds associated with wild environments (Table 7).

Table 7. The frequency of haemoparasites by association.

Parasite	Prevalence (%)			Kruskal–Wallis (x^2, df, *p*)
	Anthropogenic	Mixed	Wild	
Plasmodium	0.72 (2/278)	5.56 (20/360)	5.75 (21/365)	11.815, 2, 0.003
Haemoproteus	15.10 (42/278)	17.50 (63/360)	16.16 (59/365)	0.409, 2, 0.815
Leucocytozoon	16.91 (47/278)	4.44 (16/360)	6.30 (23/365)	32.831, 2, <0.001
Overall	28.84 (79/278)	26.94 (97/360)	26.84 (98/365)	2.718, 2, 0.257

4. Discussion

Overall prevalence of haemoparasite infection in Afrotropical birds, from in and around the KNP of South Africa, was less than a third of the sample (28.2%). This was comparably lower than a study in Malawi, which reported a prevalence of 78.1%. Infections were detected at all sites, with prevalence varying across habitats, consistent with previous findings that haemosporidian infections often exhibit spatial variation driven by ecological and environmental factors [35–39]. Environmental and ecological drivers, including host communities, vector availability, and habitat, are usually cited as the causes of these variations. Geographic disparities in infection rates can also be attributed to climatic factors like rainfall and temperature patterns, which are known to affect parasite development and vector dynamics [39]. These factors drive the spatial distribution of avian haemoparasite prevalence.

Although double infections were few in our study, they offer valuable information about the intricacy of infections by avian haemoparasites. *Leucocytozoon* spp. were involved in most of these co-infections. Considering their subclinical presentations and wide host range, *Leucocytozoon* spp. are possibly able to coexist and persist alongside other haemoparasites without being noticed [19]. Co-infections can exacerbate the dynamics of diseases, potentially altering transmission patterns, increasing parasite virulence, or leading to increased morbidity [19].

Comparison of parasite prevalence between solitary and gregarious birds revealed notable trends. Although overall haemosporidian prevalence did not differ significantly, gregarious birds exhibited a higher overall prevalence than solitary birds. This non-significant difference suggests that social living could increase the likelihood of parasite exposure, but these risks might be offset by other physiological and ecological factors, resulting in similar total infection rates across groups. These findings support the growing understanding that host–parasite interactions are context-dependent and influenced by a variety of interconnected factors, such as vector ecology, host immunity, and habitat structure [5,40].

At the genus level, gregarious birds had considerably higher haemoparasite prevalences than solitary species. These results are consistent with [41], who reported higher parasitaemia and *Haemoproteus* prevalence in gregarious birds. This supports the theory that sociality increases vulnerability to vectors, leading to genus-specific differences despite similar overall prevalence. This pattern supports the notion that social interaction can enhance encounters with vector-borne parasites. Aggregations such as roosts, communal breeding assemblies, or flocks may draw higher concentrations of vectors, thereby increasing genus-specific transmission within sociable birds [42,43]. Solitary species had consistently lower infection prevalence, likely because of fewer opportunities for transmis-

sion associated with reduced host density. Similar patterns have been reported by [41], who identified a lower prevalence of *Haemoproteus* in solitary birds compared to gregarious birds. Conversely, ref. [44] found 100% infection prevalence in Eurasian Jays (*Garrulus glandarius*), highlighting that non-gregarious living does not necessarily reduce the risk of infection. Overall, these results suggest that independent living provides a limited level of protection, while social behaviour elevates exposure risk.

Although genus-specific rates of infection were higher in sociable birds, overall prevalence did not differ, prompting intriguing ecological questions. This could reflect uneven infection among parasite genera. *Plasmodium* often exhibits generalist traits, infecting several host species and proliferating in high-density populations [25,45]. On the contrary, habitat specificity and vector distribution restrict *Leucocytozoon* and *Haemoproteus* [25,46]. The disproportionate growth of parasite genera in sociable birds could result from an amalgamation of these processes, balancing the overall prevalence.

The prevalence of haemosporidians varied markedly within foraging guilds, with mixed foragers demonstrating the greatest overall infection rates, followed by ground and aerial foragers. This finding contradicts previous studies indicating that ecological generalists such as mixed foragers would encounter lower infection rates owing to their extensive habitat utilisation and adaptable behaviours that mitigate exposure to certain vectors [2]. Rather, our data indicate that generalist foraging tendencies might increase the risk of infection by increasing exposure to a greater variety of parasite vectors found across various vertical strata.

At the genus level, *Haemoproteus* and *Plasmodium* exhibited much higher prevalence in mixed foragers than in ground and aerial specialists. The findings may indicate cumulative exposure to several vector species, including biting midges and *Culex* spp. mosquitoes, which could be more uniformly dispersed within various strata in savanna–lowveld ecosystems [47]. Earlier research in Neotropical forests found that single-stratum specialists exhibited a higher prevalence of *Plasmodium* [48]. However, our results emphasize the significance of habitat structure and regional vector ecology in determining infection patterns.

The prevalence of *Leucocytozoon* did not significantly differ among foraging strata; however, compared to other groups, aerial foraging birds showed slightly higher infection rates. Our results align with earlier studies from Amazonian regions that revealed relatively uniform *Leucocytozoon* occurrence across bird guilds [21], possibly due to the widespread presence of blackfly vectors. The absence of a clear ecological pattern may result from methodological limitations in detecting *Leucocytozoon*, especially in cases of low parasitaemia or co-infection, which could obscure small differences among host groups.

Overall, these findings challenge the contention that ecological generalism provides ongoing defence against haemosporidian infection. Instead, our findings imply that generalist species might experience increased cumulative vector exposure in certain settings, which could raise their risk of infection. This emphasizes how host behaviour, habitat structure, and local vector ecology must all be considered when assessing host–parasite dynamics.

Significant differences in haemosporidian prevalence were found among movement patterns, but not in the expected direction. Migrants are frequently expected to maintain the highest rates of infection due to exposure across wide geographic scales [34]. In contrast, our findings showed that residents had the highest overall prevalence, followed by nomads, while migrants had the lowest rates. This reversal implies that methodology and local ecology mediate infection trends, cautioning against broad extrapolations.

The residents' consistent exposure to local vector communities likely sustains transmission and chronic infections [47]. Migrants may show reduced prevalence if migration mortality disproportionately excludes highly infected birds [49] or if severe infections are eliminated before departure. Interpretation is further limited by the small sample of

migrants, which reduced statistical power. Nomads' irregular exposure to vectors could account for their moderate prevalence.

Haemoproteus and *Plasmodium* were most prevalent among residents, consistent with stable populations of mosquitoes and biting midges. On the contrary, nomads showed the highest *Leucocytozoon* prevalence, likely reflecting patchy blackfly breeding sites. They frequently exploit ephemeral wetland or riverine areas with locally high vector abundance, but sporadic opportunities for infection. Alternatively, biases in detection might be involved. *Leucocytozoon* often shows low parasitaemia or manifests in co-infections that obscure its detection in molecular assays [50]. If such circumstances were more prevalent among migrant birds, subtle distinctions between groups might be obscured or exaggerated.

Significant habitat-associated differences were found for *Leucocytozoon* and *Plasmodium*, but not for overall prevalence or *Haemoproteus*. *Plasmodium* prevalence was considerably lower in anthropogenic than in natural habitats, contrasting with Afrotropical and Neotropical studies reporting increased prevalence in altered landscapes [51]. This reverse trend could reflect the absence of competent *Culex* spp. larval habitats, the dominance of disturbance-tolerant bird species with lower susceptibility [48], or management techniques including drainage and vegetation modification, reducing vector availability.

The ecological makeup of blackfly vectors (Simuliidae) aligns with higher *Leucocytozoon* prevalence in natural and mixed habitats. These flies need fast-flowing, clean, and shaded streams for reproducing [12], conditions typical of natural rather than urban habitats. Prior research also showed that intact riparian or forest systems promote *Leucocytozoon* transmission, while disruption reduces vector abundance [9,48]. Therefore, the decreased prevalence in anthropogenic settings in our study conforms to anticipated patterns.

Neither the overall prevalence of infection nor that of *Haemoproteus* varied significantly across habitats. This may reflect the extensive ecological adaptability of *Haemoproteus* vectors, which are more impacted by microhabitat features or host-specific factors than by major habitat differences [5]. Also, aggregating parasite groups into overall prevalence can conceal biologically significant trends by pooling across parasites with different vector dynamics and ecological requirements. The inconsistent results could potentially be explained by methodological factors. Without direct measurement of vector distributions, it is challenging to assess whether vector-related interactions drive reported prevalence patterns in the absence of corresponding entomological data. Additionally, PCR-based haemosporidian identification may misrepresent true disease prevalence in mixed infections or low parasitaemia cases, thereby masking subtle habitat-linked changes [52]. Establishing a truly infected bird is possible by detecting gametocytes in blood smears under microscopy. This study did not use this validation technique, but a previous study at a similar setting revealed that the efficiency of microscopy vs. nested PCR was very similar [53]. We therefore assumed that nested PCR was a very good proxy for true infection, and hence these findings do carry some merit.

5. Conclusions

In conclusion, these results refine established theories of avian parasitology that predict distinct differences in haemoparasite prevalence based on behavioural traits and habitat. In Afrotropical bird populations sampled in and around the KNP, infection possibility could not be predicted by social organization alone. Nonetheless, genus-specific analyses revealed higher infection in gregarious birds, suggesting a subtler role of social behaviour. Habitat effects also varied among genera, with *Haemoproteus* and *Plasmodium* showing the strongest association. Thus, behavioural traits and habitat had a parasite-genus dependent effect on infection patterns. Our findings add precedence to the hypothesis that ecological and behavioural factors alone cannot fully predict infection risk, despite their interactions

with vector dynamics and parasite life histories. For a more thorough understanding of the mechanisms influencing haemoparasite distributions in Afrotropical birds, subsequent studies should integrate phylogenetically informed methodologies with immunological, behavioural, and ecological data.

Author Contributions: All authors contributed to the design of the study, data collection, analysis and writing of this manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for this research came from the National Research Foundation (Grant number: IFR190125410940), the Foundational Biodiversity Information Programme (Grant number: FBIP190314423449), and the University of the Free State.

Institutional Review Board Statement: The study was approved by the South African National Park (Research Permit No: NDLM1262) and the University of the Witwatersrand Animal Ethics Screening Committee (Clearance Certificate No: 2015/02/B). Other ethical clearance was obtained for the overarching project registered by MN at the University of the Free State (UFS-AED2017/0004). Permission to conduct this research in terms of the South African Section 20 of the Animal Disease Act 1984 (Act No 35 of 1984) was obtained from the Department of Agriculture, Forestry, and Fisheries of South Africa (Ref. number 12/11/1/4). All bird capture and ringing were conducted in accordance with the SAFRING (South African Ringing Scheme) bird ringing manual under the supervision of MN.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data for this study is freely available from the South African Foundational Biodiversity Information Programme (FBIP) repository and is deposited at the following link: https://figshare.com/articles/dataset/Data_01_12_2020_xlsx/13317293 (accessed on 17 October 2025). Accession numbers of new parasite lineages detected are available in GenBank and MalAvi databases.

Acknowledgments: We thank several former students who contributed to field data collection, namely Tinotendashe Pori and Maliki Wardjomto. Antón Perez-Rodríguez assisted with the lab work. The South African National Parks (SANParks) and the Mpumalanga local Municipalities kindly granted permission for the birds to be captured in Kruger National Park and the sites adjacent to the park, respectively.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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