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Diversity and prevalence of avian haemosporidians across Afrotropical urban and non-urban habitats

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ABSTRACT

Haemosporidian parasites are widespread among birds globally, with impacts ranging from severe disease to negligible effects, particularly in host species that have coevolved with their parasites. Despite their ecological importance, the diversity and prevalence of these parasites in the Afrotropical region remain poorly understood, especially in urban environments. Our study investigated the prevalence and diversity of haemosporidian parasites in Afrotropical avian hosts, focusing on differences between urban and non-urban habitats. We screened 95 birds from various species in Nigeria and investigated whether urbanization is associated with changes in the prevalence and richness of lineages of three haemosporidian parasites (*Haemoproteus*, *Plasmodium*, and *Leucocytozoon*). We found a haemosporidian prevalence of 36.8% with genus-specific differences between urban and non-urban habitats. The probability of *Haemoproteus* infection was higher in urban than non-urban habitats, but *Plasmodium* and *Leucocytozoon* did not differ between these habitats. Moreover, *Haemoproteus* lineages were exclusively found in urban habitats, while most *Plasmodium* lineages were restricted to non-urban habitats. Notably, we expanded the knowledge on diversity of haemosporidian lineages and avian hosts in the Afrotropics, with the first-ever record of hPYNJOC1 and pLUME2 lineages for the region, and the addition of new hosts for four *Haemoproteus* and two *Plasmodium* lineages. Our findings highlight the complexity of host-parasite relationships and the need for further research into the dynamics of haemosporidian parasites in Afrotropical avian hosts inhabiting diverse habitats. Overall, our study contributes to a better understanding of the prevalence, diversity, and distribution of haemosporidian parasites in the Afrotropics, emphasizing the importance of continued surveillance and monitoring to inform strategies for avian conservation and management.

1. Introduction

Urbanization, the transformation of natural areas into urban areas, has accelerated rapidly in recent decades (United Nations, 2016; McIntyre, 2021). This anthropogenic landscape modification is characterized by increased human population density, infrastructure development, and changes in land use, posing a significant challenge to natural habitats worldwide (Grimm et al., 2008; Gaston, 2010). Projections suggest that urban areas will continue to expand globally, with regions in the Global South, particularly the Afrotropics, experiencing a higher urbanization rate compared to those in the Global North (Angel et al., 2011; Seto et al., 2012; United Nations, 2024). Urbanization, by converting natural habitats into built environments, disrupts ecosystems by altering species interactions and threatens species survival (Faeth et al., 2011; Gil and Brumm, 2014; Piano et al., 2020), which has led to a profound global biodiversity loss (McKinney, 2006;

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Ibáñez-Álamo et al., 2017). However, although studies have typically focused on the impacts of urbanization on the diversity of some taxa, such as birds or plants (e.g., Chace and Walsh, 2006; McKinney, 2008; Ibáñez-Álamo et al., 2017), much less attention has been paid to other groups and processes, particularly haemosporidians and host-parasite interactions affecting non-human organisms (Delgado-V and French, 2012; Awoyemi and Ibáñez-Álamo, 2023).

Haemosporidians are a diverse group of parasitic protozoa of the phylum Apicomplexa, encompassing multiple genera, including avian malaria parasites (i.e., *Plasmodium*) and related malaria-like parasites (e.g., *Haemoproteus* and *Leucocytozoon*; Valkiūnas, 2005; Bensch et al., 2009). Various haematophagous dipterans transmit these parasites. Mosquitoes are the vectors of *Plasmodium*, biting midges (*Culicoides*) and louse flies transmit *Haemoproteus* parasites, while black flies transmit *Leucocytozoon* parasites (Valkiūnas, 2005). These parasites infect the blood cells of various vertebrates, including mammals, reptiles, and birds (Valkiūnas, 2005). Birds are host to an astonishingly diverse array of haemosporidian parasites, with over 5000 identified genetic lineages infecting most avian clades (data from MalAvi database; Bensch et al., 2009). Haemosporidian infections can have marked pathogenic effects on avian hosts, including tissue damage (Ilgunas et al., 2019), impaired body condition (Marzal et al., 2008), reduced reproductive success (Marzal et al., 2005; Knowles et al., 2010), and increased mortality, especially in immunologically naive birds (Yorinks and Atkinson, 2000; Atkinson and Samuel, 2010; Jia et al., 2018). The cumulative impact of these parasites can be devastating, contributing to significant declines or even local extinctions of native host populations (Samuel et al., 2011; Levin et al., 2013; Niebuhr et al., 2016).

The availability of modern molecular techniques has enhanced the understanding of complex interactions between haemosporidian parasites, their hosts, and the urban environment (Santiago-Alarcon et al., 2020; Jiménez-Peñuela et al., 2021; Caizergues et al., 2024; Figuerola et al., 2024). Urbanization can significantly alter the ecology of haemosporidian parasites and their hosts, leading to changes in parasite diversity, distribution, and prevalence (Evans et al., 2009; Caizergues et al., 2024; Figuerola et al., 2024), although such changes may depend on interannual variations (Jiménez-Peñuela et al., 2021). Changes in vector populations, such as mosquitoes and biting midges, can occur in urban environments. For example, urban areas may have less suitable breeding areas for haematophagous insects, which may reduce vector abundance and richness (Ferraguti et al., 2016), potentially altering the dynamics of parasite transmission and infection (Bradley and Altizer, 2007; Delgado-V and French, 2012). Additionally, urbanization can lead to shifts in host-parasite interactions. The global loss of biodiversity due to urbanization can change the distribution and abundance of susceptible avian host species (Bradley and Altizer, 2007; Ferraguti et al., 2018; Sumasgutner et al., 2023) or can increase parasite prevalence in some avian host populations, but not in others (Carbó-Ramírez et al., 2017; Santiago-Alarcon et al., 2019). However, the understanding of urban-induced variations in haemosporidians and avian hosts is restricted to Global North countries (Delgado-V and French, 2012; Ibáñez-Álamo et al., 2017), leaving significant knowledge gaps in areas of the Global South, such as the Afrotropics (Shackleton et al., 2021; Awoyemi and Ibáñez-Álamo, 2023). This mismatch could have far-reaching implications, given the overlap of vast biodiversity and high urban expansion in the Afrotropical region (Angel et al., 2011; Seto et al., 2012; Gatti et al., 2015). Despite this, there are some studies investigating the diversity of avian haemosporidian parasites in the area, all restricted to birds from natural rainforests (Valkiūnas et al., 2008; Beadell et al., 2009; Iezhova et al., 2011; Lutz et al., 2015; Chaisi et al., 2019; Musa et al., 2019; see Njabo et al., 2012 for a screening of vectors). Hence, there is a notable lack of research in Afrotropical urban areas (Delgado-V and French, 2012; Awoyemi and Ibáñez-Álamo, 2023).

In the present study, we investigated how the prevalence and diversity of avian haemosporidians *Plasmodium*, *Haemoproteus* and *Leucocytozoon* vary across habitats (urban vs. non-urban) and seasons (dry vs. wet) in Nigeria—a rapidly urbanizing Afrotropical country with limited data on haemosporidians (Hellgreen et al., 2007, 2013; Chaisi et al., 2019). Previous research on urbanization in Nigeria has shown that this human-induced landscape change reduces bird taxonomic diversity by more than half relative to adjacent non-urban habitats, with greater declines during the dry season than the wet season (Awoyemi et al., 2024; Danmallam et al., 2024). Building on these findings and other regional evidence (see below), we hypothesize that avian haemosporidian prevalence and lineage diversity will be lower in urban than non-urban habitats due to reduced vector abundance and diversity. Because haemosporidian transmission depends on dipteran vectors, any reduction in vector availability or habitat suitability can directly limit parasite spread among avian hosts. Studies across the Afrotropics have reported this pattern, in which urbanization restricts suitable breeding habitats and decreases populations of insect vectors (Kigozi et al., 2015; Doumbe-Belisse et al., 2021; Bikangui et al., 2023). These trends are consistent with findings from North Global regions showing that urban landscapes often suppress vector activity and consequently lower parasite transmission (Bradley and Altizer, 2007; Ferraguti et al., 2016). Also, according to the more intense declines in bird diversity during the dry season (see above), we predict larger differences in prevalence and diversity between urban and non-urban areas during that season compared to the wet season. Because we sampled a multitude of avian host species and given that the Afrotropics has the most unique and threatened bird species globally (De Klerk et al., 2002; Matthews et al., 2022), we expect to detect previously unreported lineages and avian hosts (e.g., Musa et al., 2019). Hence, this research will contribute to the understanding of the diversity and prevalence of haemosporidian parasites, particularly in understudied regions with high urban expansion and biodiversity, such as the Afrotropics (Seto et al., 2012; Gatti et al., 2015; Awoyemi and Ibáñez-Álamo, 2023).

2. Materials and methods

2.1. Study area

This study was conducted in three paired urban and non-urban habitats (Ado, Ibadan, and Lagos) in Southwest Nigeria, Africa, a region characterized by a tropical climate with distinct seasonal variations (Ezealor, 2001). The area experiences almost equal months of dry (October–March) and wet (April–September) seasons, with mean temperatures ranging from 21 °C to 35 °C and annual rainfall varying between 150 mm and 3000 mm (Akinyemi et al., 2019). These climatic conditions allow lush vegetation growth, typical of tropical rainforests, featuring dense evergreen forests with towering trees and thick undergrowth (Richards, 1996; Hawthorne and Jongkind, 2006), which in turn enhance the associated avian diversity (Adegbola et al., 2024; Awoyemi et al., 2024).

We defined urban habitats as contiguous patches of built-up land exceeding 1 km^2, characterized by a high density of human-constructed features, including buildings (>10 buildings/ha), roads, and vehicles, as well as high human population density (>1600 inhabitants/km^2) (Niemelä, 1999; Marzluff, 2001; Schneider et al., 2010). In contrast, non-urban habitats were defined as regions with extensive wilderness or vegetation cover, interspersed with agricultural landscapes (Marzluff, 2001; MacGregor-Fors, 2011). To ensure the independence of avian communities, urban and non-urban study sites were located at least 20 km apart, following previously established guidelines (Liker et al., 2008; Awoyemi et al., 2024). Although this distance minimizes direct overlap in local bird communities, we acknowledge that seasonal or migratory movements could still facilitate occasional exchanges of individuals or parasites between habitats (Santiago-Alarcon et al., 2012; Lauron et al., 2015).

2.2. Field sampling

We conducted bird sampling in paired urban and non-urban habitats following the methodology used in previous studies (e.g., Figuerola et al., 2024). In detail, we capture urban and non-urban birds in Ado, Ibadan, and Lagos using mist-nets during the dry (January 8–12, 2024) and wet seasons (August 23 to September 16, 2023). The nets were set up at a single location within each urban and non-urban habitat, and birds were sampled from 7:00 to 11:00 local time. Upon capture, birds were immediately sampled: the underside of a wing was swabbed with ethanol-soaked cotton wool, and the brachial vein was punctured to collect blood (maximum 1% of bird body mass), which was then collected directly into an Eppendorf tube filled with absolute ethanol and stored at −20 °C until further procedure (Muriel et al., 2018; Garrido-Bautista et al., 2023). We used sterilized tools and consumables to prevent cross-contamination. Overall, we collected samples from 95 birds representing 31 species, distributed across three sites (Ado: 24, Ibadan: 37, and Lagos: 34), two habitats (urban: 49, non-urban: 46), and two seasons (dry: 72, wet: 23), as detailed in the supplementary material (Appendix Table S1). The A.P. Leventis Ornithological Research Institute (APLORI) in Jos, Nigeria, granted AGA (bird ringer ID: 16491; licensed by SAFRING, South Africa) the necessary ethical permit for fieldwork on September 26, 2022. Additionally, a Material Transfer Agreement was signed between APLORI and the University of Granada to facilitate the transportation of samples between Nigeria and Spain.

2.3. Molecular analysis

We used 10 μL of blood per bird for DNA extraction. Following the manufacturer's instructions, we isolated genomic DNA using the DNeasy Blood & Tissue kit (Qiagen, Germany), but the sample digestion time at 56 °C was increased from 10 min to 1 h. We measured the quantity and quality (260/280 nm ratio) of the isolated DNA using a NanoPhotometer P300 (Implen GmbH, Munich, Germany). The DNA samples were used to screen for avian haemosporidian parasites (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) using nested-PCR protocols targeting conserved regions of the haemosporidian cytochrome *b* gene (*CytB*) as described in Hellgren et al. (2004). In the first PCR, we used the primers HaemNFI (5′–CAT ATA TTA AGA GAA NTA TGG AG–3′) and HaemNR3 (5′–ATA GAA AGA TAA GAA ATA CCA TTC–3′) to target a 571 bp *CytB* fragment of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* parasites. In the nested PCRs, we used the primers HaemF (5′–ATG GTG CTT TCG ATA TAT GCA TG–3′) and HaemR2 (5′–GCA TTA TCT GGA TGT GAT AAT GGT–3′) to target a 478 bp *CytB* section of *Plasmodium* and *Haemoproteus*, and the primers HaemFL (5′–ATG GTG TTT TAG ATA CTT ACA TT–3′) and HaemR2L (5′–CAT TAT CTG GAT GAG ATA ATG GNG C–3′) to target a 476 bp *CytB* section of *Leucocytozoon*.

The first PCRs, including HaemNFI–HaemNR3, were run for 20 cycles with 1 μL DNA as template, and the nested PCRs (HaemF–HaemR2 and HaemFL–HaemR2L) were run for 35 cycles with 1 μL PCR product from the first PCRs. The PCRs started with an initial denaturation for 2 min at 94 °C, followed by 20 cycles (first PCR)/35 cycles (nested-PCR) with 30 s at 94 °C, 30 s at 50 °C, 1 min at 72 °C, and a final extension for 10 min at 72 °C. All PCRs were performed in 25 μL volumes, and negative controls (nuclease-free water) and positive controls for *Haemoproteus* and *Leucocytozoon* (single-infected adult Blue Tits *Cyanistes caeruleus* from southeastern Spain; unpubl. data) were included in all PCR experiments.

Positive amplifications were then purified and sequenced in both directions using the primers of the nested-PCRs. The obtained sequences were edited by visual inspection of the electropherograms and aligned using BioEdit v 7.7.1 (Hall, 1999). Mixed infections were identified based on double base calling in the electropherograms (Pérez-Tris and Bensch, 2005), and the corresponding ambiguous characters were inserted in the sequences in BioEdit. The aligned and cleaned (without primers) sequences were blasted against the MalAvi database (Bensch et al., 2009) to identify the haemosporidian parasite lineages. The obtained sequences were deposited in NCBI GenBank under the accession numbers: PX716799 to PX716829. *Leucocytozoon* lineages could not be identified given the low quality of electropherograms.

2.4. Haemosporidian phylogeographic analysis

To examine the geographic and host distribution of the haemosporidian lineages found, we collected all available information on *CytB* lineages from GenBank and the MalAvi database (accessed: April 7, 2025) (Bensch et al., 2009). We created pie charts to show the distribution per country and host species for each lineage found, except for *Plasmodium elongatum* GRW06 and pGRW09, because these lineages are globally distributed across a multitude of hosts (Bensch et al., 2009). Pie charts were edited using Inkscape 1.4 Software.

2.5. Statistical analysis

We examined whether the probability of infection of haemosporidian groups and lineage richness varied with habitat type and season using Generalized Linear Mixed-Effect Models (GLMM) using the package 'lme4' (Bates et al., 2022). The individual prevalence (presence/absence) of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* was the dependent variable in the corresponding model. At the same time, the habitat type (urban and non-urban) and season (dry and wet) were the fixed factors. We included the city (Ado, Ibadan, and Lagos) as a random factor in each model to account for site-specific variation. In these three GLMMs, we applied a binomial distribution with a logit link because the response variable was binary (presence/absence of infection). We run another GLMM using the richness of haemosporidian lineages per individual host as the dependent variable and the habitat type and season as fixed factors. The city was also included as a random factor. This GLMM was run with a Poisson distribution and a log function because the count data were not overdispersed (i.e., variance < mean). Richness was defined as the sum of distinct haemosporidian lineages per infected bird. Thus, the three birds infected with *Plasmodium* sp. (see Table 1) were excluded while calculating richness because we did not know whether these birds were single- or co-infected. The *Leucocytozoon*-infected birds were assumed to harbour a *Leucocytozoon* lineage as electropherograms failed.

Model assumptions were systematically evaluated by inspecting residuals and checking for overdispersion, which was not detected. The distributional choices of GLMMs follow recommended practices for modelling binary and count responses in ecological datasets (Bolker et al., 2009; Zuur et al., 2009). We created plots using the package 'ggplot2' (Wickman, 2016). All analyses were performed in the R Statistical Software 4.4.2 (R Development Core Team, 2024).

3. Results

3.1. Prevalence and diversity of haemosporidians

We screened 95 birds representing 31 species and 14 families (Appendix Table S1). In total, 35 of the 95 sampled birds (mean = 36.8%; 95% CI = 27–47%) were infected with haemosporidian parasites, including 26 *Leucocytozoon*, 16 *Haemoproteus*, and 16 *Plasmodium*. Meanwhile, a Black-necked Weaver (*Ploceus nigricollis*) showed amplification with the HaemF–HaemR2 primers. However, the electropherograms failed, preventing us from knowing whether this bird was single- or co-infected, and consequently, no lineages were found. Meanwhile, most *Leucocytozoon* produced co-infections (14/26 with *Haemoproteus* and 10/26 with *Plasmodium*) compared to single infections occasioned by 2/26 *Leucocytozoon*. The minority of the *Haemoproteus* and *Plasmodium* infections occurred singly (2/16 *Haemoproteus* and 6/16 *Plasmodium*). The haemosporidian lineages (*Haemoproteus* and *Plasmodium*) and their prevalence, confirmed by

Table 1
Haemosporidian lineages detected in bird communities sampled from Southwest Nigeria (West Africa).

Lineage	Avian host species	Number of infected individuals (present study)	Prevalence (%)	Previous African records	Previous non-African records	Accession number
Haemoproteus						
H. micronuclearis VILWE1	Village Weaver (*Ploceus cucullatus*)	7	20.00	2	0	DQ847181
H. sanguinis BUL1	Common Bulbul (*Pycnonotus barbatus*)	1	2.86	3	2	DQ847194
H. sanguinis BUL2	Common Bulbul (*Pycnonotus barbatus*)	1	2.86	9	1	DQ847195
hPAGRI01	Northern Grey-headed Sparrow (*Passer griseus*)	1	2.86	2	0	JX196863
hPYNJOC1	Common Bulbul (*Pycnonotus barbatus*)	1	2.86	0	1	MK493387
hRBQ07	Village Weaver (*Ploceus cucullatus*)	1	2.86	2	0	EF117230
hTURABY01	Blue-spotted Wood Dove (*Turtur afer*)	1	2.86	1	0	MG018657
hVILWE3	Village Weaver (*Ploceus cucullatus*)	1	2.86	7	0	DQ847201
hVIMWE1	Village Weaver (*Ploceus cucullatus*)	1	2.86	25	0	NA
New lineages	Village Weaver (*Ploceus cucullatus*)	3	8.57	0	0	–
Plasmodium						
pGRW09	Little Greenbul (*Eurillas virens*)	1	11.43	129	48	DQ060773
	Grey-headed Bristlebill (*Bleda canicapillus*)	1				
	Snowy-crowned Robin-chat (*Cossypha niveicapilla*)	2				
pLUME2	Common Bulbul (*Pycnonotus barbatus*)	1	8.57	0	1	DQ368384
	African Thrush (*Turdus pelios*)	2				
pRECOB4	Collared Sunbird (*Hedydipna collaris*)	2	5.71	33	2	DQ847260
pAEMO01	Green Hylia (*Hylia prasina*)	1	2.86	17	14	FJ355919
pBT8	Black-necked Weaver (*Ploceus nigricollis*)	1	2.86	10	9	AY393792
pCAMBRA02	Green-backed Camaroptera (*Camaroptera brachyura*)	1	2.86	1	0	MG018677
P. elongatum GRW06	Green-backed Camaroptera (*Camaroptera brachyura*)	1	2.86	16	149	DQ368381
Plasmodium sp.	Olive Sunbird (*Cyanomitra olivacea*)	1	8.57	–	–	–
	African Thrush (*Turdus pelios*)	2				

This list includes the host bird species, number of infected individuals, prevalence, number of previous records in African and non-African countries, and accession number of the lineage in MalAvi database (Bensch et al., 2009).

sequencing, are listed in Table 1. All except three *Plasmodium*-infected birds (an Olive Sunbird *Cyanomitra olivacea*, and two African Thrushes *Turdus pelios*; Table 1) were successfully sequenced and identified.

Haemoproteus parasites featured nine known lineages and three tentatively new lineages infecting three Village Weavers (*Ploceus cucullatus*; Table 1). *Haemoproteus micronuclearis* VILWE1 was the most frequently found lineage, in all cases infecting Village Weavers. One of the seven Village Weavers infected with *H. micronuclearis* VILWE1 showed a co-infection with hRBQ07. The remaining *Haemoproteus* lineages were found infecting different individuals (Table 1); in this case, a Common Bulbul (*Pycnonotus barbatus*) was simultaneously co-infected by hPYNJOC1 and *H. sanguinis* BUL1. Regarding the three tentatively new *Haemoproteus* lineages found: (1) differed at two base pairs (bp) from hVIMWE1 (DQ847181) in positions 20/478 and 102/478; (2) differed at one bp from hVILWE3 (DQ847201) in position 434/478; and (3) differed at five bp from both hVIMWE1 and hPLONEL01 (MF442606) in several positions.

The *Plasmodium* parasites featured seven lineages (Table 1). Of this total, pGRW09 infected four birds of three species, including a Little Greenbul (*Eurillas virens*), a Grey-headed Bristlebill (*Bleda canicapillus*) and two Snowy-crowned Robin-chats (*Cossypha niveicapilla*). pLUME2 infected three birds of two species (a *P. barbatus* and two *T. pelios*), pRECOB4 infected two Collared Sunbirds (*Hedydipna collaris*), while the remaining *Plasmodium* lineages infected single individuals (Table 1). No *Plasmodium* lineages co-occurred with *Haemoproteus* parasites.

3.2. Geographic and host distribution of haemosporidians

The pie charts show the distribution of each *Haemoproteus* (Fig. 1) and *Plasmodium* (Fig. 2) lineage across countries and host species. The records presented in the pie charts incorporate the data from the birds sampled in this study. For the lineages found in the present study, Table 1 shows the number of previous records from African and non-African countries.

Haemoproteus micronuclearis VILWE1 was previously found infecting a Village Weaver and a Black-headed Weaver (*Ploceus melanocephalus*) from Nigeria and Uganda, respectively. Our study added seven new records for this lineage in the Village Weaver. Both *H. sanguinis* BUL1 and BUL2 were recorded in several *Pycnonotus* species, mainly from the Afrotropics (Benin, Malawi, Nigeria) and Southern African islands of Madagascar and Comoros, and indeed, we found these two lineages in two Common Bulbuls. The hPAGRI01 lineage was reported exclusively in Northern Grey-headed Sparrow (*Passer griseus*) from the Afrotropics (Benin, Nigeria). Notably, we also detected this lineage in a Northern Grey-headed Sparrow. Regarding hPYNJOC1, we reported this lineage for the first time in the Afrotropics, and in a new host species (*P. barbatus*; Table 1). This lineage was only previously reported once in a Red-whiskered Bulbul (*Pycnonotus jocosus*) sampled in India. We also added new host species, Village Weaver and Blue-spotted Wood Dove (*Turtur afer*), in Afrotropics for the lineages hRBQ07 and hTURABY01, respectively (Table 1). We added a new record for hVILWE3, since this lineage was found infecting only the genus *Ploceus* from Afrotropical countries. Lastly, although hVIMWE1 has been recorded in 25 birds across Afrotropical countries, we added a new host species, the Village Weaver, for this lineage (Table 1).

Plasmodium pLUME2 has been previously reported only once in a single European Robin (*Luscinia megarhynchos*) from Germany (Hellgreen et al., 2007). We report this lineage in the Afrotropics for the first time and added two additional host species, African Thrush and Common Bulbul (Table 1). The pRECOB4 lineage has been reported in 35 bird species, mostly from African countries. While our study did not identify new avian hosts, it confirmed the presence of pRECOB4 in two Collared Sunbirds (*Hedydipna collaris*). We added a new host species, the Green Hylia (*Hylia prasina*), for pAEMO01 since this lineage was reported in other migratory and resident bird species from the Afrotropics and Europe (Table 1). We report pBT8 in Nigeria for the first time, but in

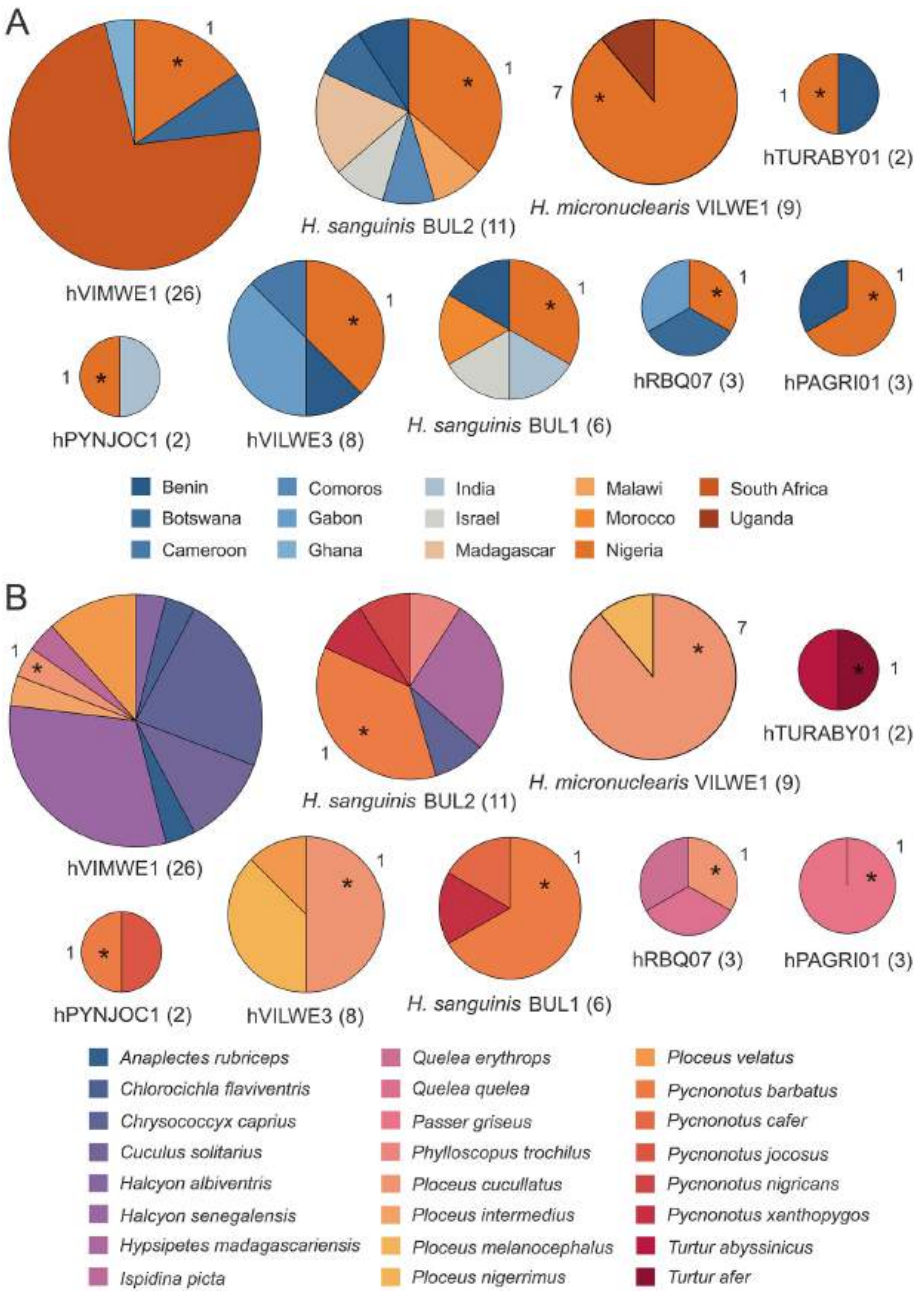

Fig. 1. Pie charts showing the geographic (**A**) and avian host (**B**) distribution of *Haemoproteus* lineages found in birds sampled in the present study. The asterisks show the birds examined in the present study. The number of infected birds from the present study is displayed near each pie chart.

a host species already reported (Table 1). pCAMBRA02 was only found once in a Green-backed Camaroptera (*Camaroptera brachyura*) individual from Benin, and we also detected this lineage in this same host species. Lastly, we detected *P. elongatum* GRW06 in a Green-backed Camaroptera individual, and pGRW09 in three host species (one Little Greenbul, one Grey-headed Bristlebill, two Snowy-crowned Robin-chat). These two latter *Plasmodium* lineages were previously recorded in a multitude of host species distributed worldwide (*P. elongatum* GRW06: 166 birds from 101 species, mainly in non-African countries; pGRW09: 177 birds from 89 species, mainly in African countries; Table 1).

3.3. Haemosporidians, habitat type, and season

The probability of infection by *Haemoproteus* was significantly higher in the urban habitat (mean = 32.0%; 95% CI = 19–45%) than in the non-urban habitat (mean = 2.2%; 95% CI = 0–7%; Fig. 3; Table 2). This contrasted with the results on probabilities of infection by *Plasmodium* and *Leucocytozoon*, which were similar between urban and non-urban habitats (Fig. 3; Table 2). Meanwhile, we did not find any significant effect of season on the probability of infection by any of the haemosporidian genera (Table 2).

The mean richness of haemosporidians was 0.58 ± 0.09 and 1.71 ± 0.10 (mean ± SE) considering all individuals and infected-individuals, respectively. Richness did not significantly vary with habitat or season (Table 2). Although the mean number of distinct haemosporidian lineages (i.e., richness) was similar between urban and non-urban habitats, the haemosporidian community differed between urban and non-urban habitats. All *Haemoproteus* lineages except hTURABY01, including the three tentatively new species, were found in urban habitats (Fig. 4). In contrast, only pLUME2 and pBT8 were the only two of seven *Plasmodium* lineages restricted to non-urban habitats (Fig. 4). There were no

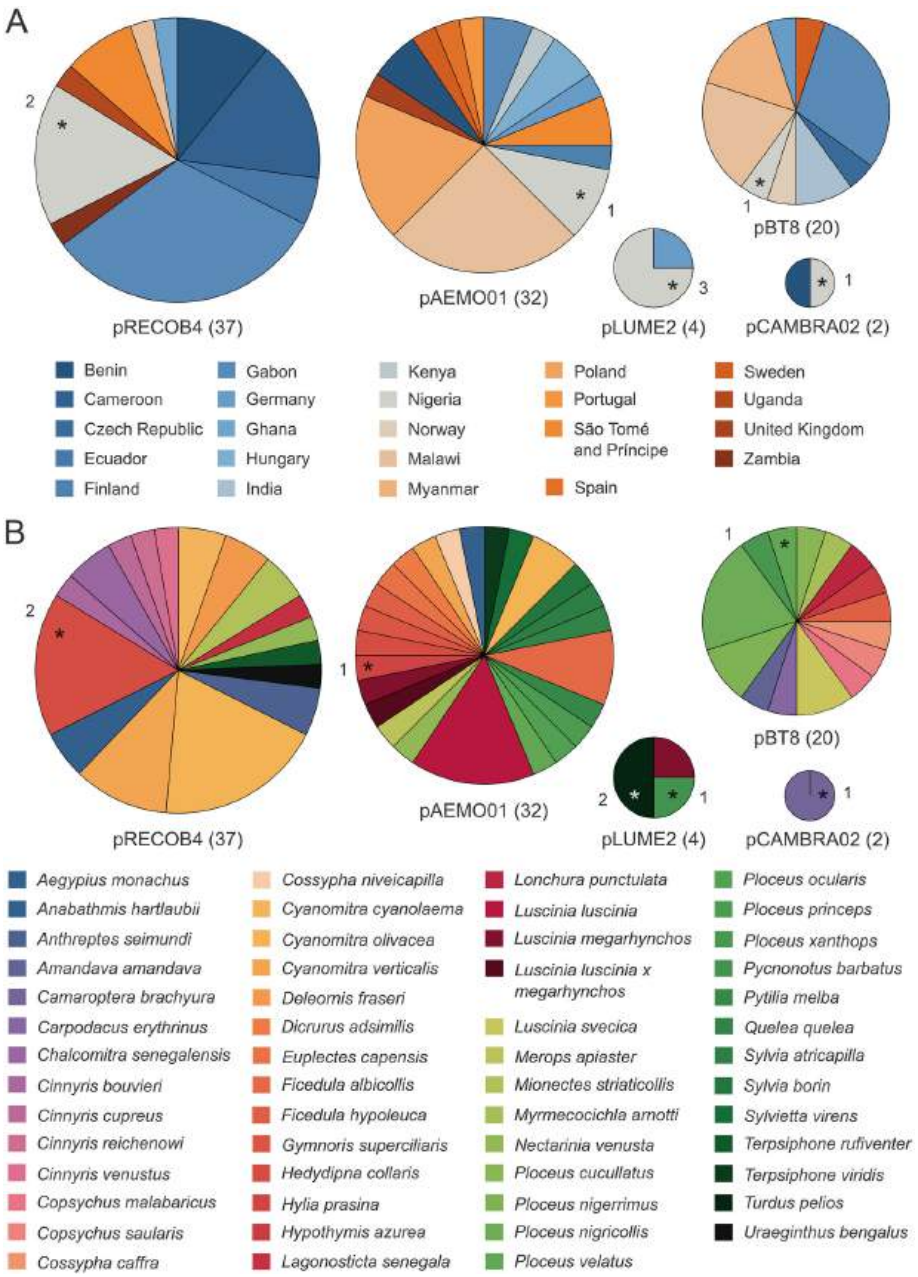

Fig. 2. Pie charts showing the geographic (**A**) and avian host (**B**) distribution of *Plasmodium* lineages found in birds sampled in the present study. The asterisks show the birds examined in the present study. The number of infected birds from the present study is shown near each pie chart.

haemosporidian lineages found simultaneously in both habitat types.

4. Discussion

Our study provides valuable insights into the prevalence and diversity of haemosporidian parasites infecting Afrotropical avian hosts, adding novel information on the variation between urban and non-urban habitats. Contrary to our prediction, we found that the diversity of haemosporidians, estimated as richness of lineages, was similar between urban and non-urban habitats, but the haemosporidian community differed between these areas. The *Haemoproteus* lineages were exclusively found in urban habitats, while most *Plasmodium* lineages were restricted to non-urban habitats. This habitat-specific distribution suggests that urbanization plays a crucial role in shaping the haemosporidian community, allowing a certain level of habitat specialization for these parasites. This trend was observed in previous studies, most of them restricted to the Global North (Jiménez-Peñuela et al., 2021; Figuerola et al., 2024; but see Caizergues et al., 2024). Notably, the probability of *Haemoproteus* infection was significantly higher in urban habitats (32%) compared to non-urban habitats (2%), contradicting our initial prediction. In contrast, *Plasmodium* and *Leucocytozoon* infection probabilities did not vary significantly between urban and non-urban habitats. Several studies found lower prevalence of haemosporidians in urban areas (Geue and Partecke, 2008; Evans et al., 2009; Santiago-Alarcon et al., 2020; Figuerola et al., 2024), but others found the opposite pattern or even no differences (Jiménez-Peñuela et al., 2019, 2021; Caizergues et al., 2024), depending on the parasite genera. The higher *Haemoproteus* infection rate in urban habitats could be attributed to several factors. One possibility is that Afrotropical urban areas support a greater diversity and/or abundance of main vectors of *Haemoproteus* (i.e., biting midges) than adjacent non-urban habitats because of a higher availability of artificial breeding areas (e.g., Ferraguti et al.,

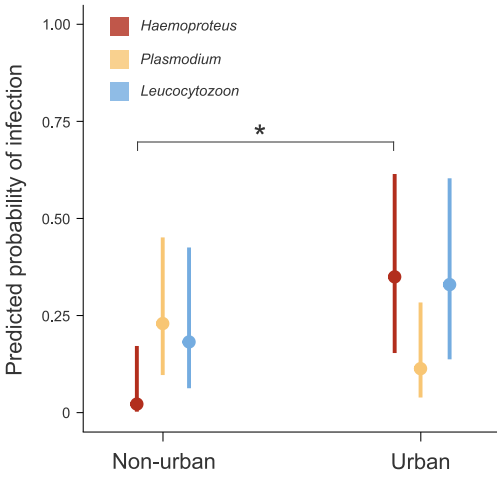

Fig. 3. Predicted probability of infection of birds by haemosporidians (red: *Haemoproteus*, yellow: *Plasmodium*, blue: *Leucocytozoon*) between urban and non-urban habitats in Nigeria, obtained from three different models detailed in Table 2. The bars represent confidence intervals (50% CI inner interval, 89% CI outer interval). The asterisk shows significant differences in the predicted probability of *Haemoproteus* infection between urban and non-urban habitats. Both *Plasmodium* and *Leucocytozoon* infections did not significantly differ between the urban and non-urban habitats.

Table 2
Results of separate Generalized Linear Mixed-Effect Models analyzing the difference in the probability of infection by *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* and haemosporidian richness between habitats and season.

Explanatory variables	Estimate	Standard error	*z*-value	*p*-value
Model for *Haemoproteus* prevalence				
Intercept	−3.793	1.108	−3.424	0.001
Habitat type (urban)	**3.173**	**1.081**	**2.936**	**0.003**
Season (wet)	−1.315	0.883	−1.490	0.136
Random factor (city)	Variance = 0.497, standard deviation = 0.705			
Model for *Plasmodium* prevalence				
Intercept	−1.204	0.533	−2.261	0.024
Habitat type (urban)	−0.841	0.613	−1.371	0.171
Season (wet)	−0.309	0.735	−0.421	0.674
Random factor (city)	Variance = 0.338, standard deviation = 0.581			
Model for *Leucocytozoon* prevalence				
Intercept	−1.503	0.617	−2.435	0.015
Habitat type (urban)	0.793	0.532	1.491	0.136
Season (wet)	−0.778	0.658	−1.183	0.237
Random factor (city)	Variance = 0.582, standard deviation = 0.763			
Model for richness				
Intercept	−0.883	0.368	−2.401	0.016
Habitat type (urban)	0.505	0.303	1.665	0.096
Season (wet)	−0.665	0.406	−1.640	0.101
Random factor (city)	Variance = 0.212, standard deviation = 0.461			

Reference categories for habitat and season are non-urban and dry, respectively. Significant parameters are marked in bold.

2016), despite non-urban areas having a more diverse avian host community in Nigeria (Awoyemi et al., 2024). In fact, mosquitoes are less generalist than biting midges in their breeding habitats, hence urban habitats may support reduced populations of mosquitoes compared to biting midges, as previously found in other African regions (Kigozi et al., 2015; Doumbe-Belisse et al., 2021; Bikangui et al., 2023). Additionally, *Plasmodium* infections could have been underestimated in this study because of PCR assay sensitivity (see below). Alternatively, birds in urban environments may be more susceptible to haemoproteosis due to compromised body condition and health, as suggested by previous studies (Jiménez-Peñuela et al., 2019). Further research is needed to explore these hypotheses and understand the complex interactions between haemosporidian parasites, their vectors, hosts, and the urban environment.

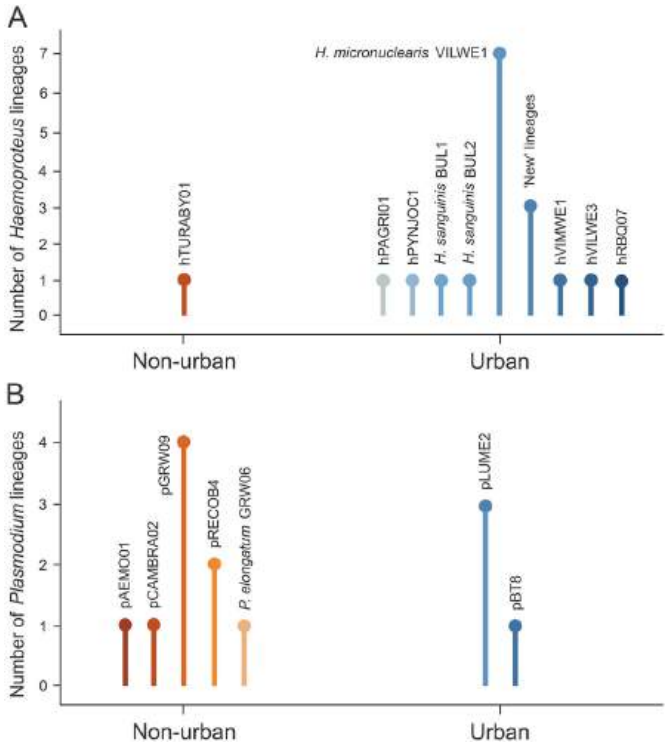

Fig. 4. Number of haemosporidian lineages of *Haemoproteus* (**A**) and *Plasmodium* (**B**) between urban and non-urban habitats in Nigeria.

Besides the role of urbanization in shaping the avian haemosporidian community, a crucial finding was the high diversity of haemosporidian lineages in the Afrotropics. The birds sampled in our study harbored a rate of haemosporidian infections of 36.8%, which is in line with those reported by previous studies on avian haemosporidians in the Afrotropics, including Nigeria (Table 3). The prevalence of avian haemosporidians typically varies with avian host life history, geographical location, seasonality, and even methodology (Levin et al., 2013; Lutz et al., 2015; Smith and Ramey, 2015; Schmid et al., 2017; Chaisi et al., 2019), potentially explaining observed differences between studies. For instance, mist nets generally under-sample acutely infected birds (because of reduced mobility) but capture non-infected individuals or individuals in the chronic stage of infection (Valkiūnas, 2005), which may influence capture rate vis-à-vis the prevalence rate obtained in this study. Previous studies finding extraordinarily high prevalence of avian haemosporidians in Afrotropical birds (79–83%) used alternative trapping methods, including shotguns (Lutz et al., 2015) and spring traps baited with worms (Chaisi et al., 2019). In contrast, the high diversity of haemosporidian lineages found here (12 *Haemoproteus* and 7 *Plasmodium* lineages in 35 birds of 13 species; *Leucocytozoon* samples could not be assigned to a lineage) is consistent with previous multi-species studies from the Afrotropics (Valkiūnas et al., 2008; Beadell et al., 2009; Lutz et al., 2015; Chaisi et al., 2019; Musa et al., 2019). Lutz et al. (2015) suggested that the number of distinct avian malaria lineages within a geographical area should be approximately the number of avian host species, whenever the sampling is sufficient. Our study, as well as previous studies conducted in the Afrotropics, corroborate this idea. The results suggest that the Afrotropics is an area of extremely high haemosporidian diversity and, possibly, endemicity (e.g., Lutz et al., 2015).

We expanded the known diversity of haemosporidian lineages and avian hosts in the Afrotropics, with the discovery of three novel *Haemoproteus* lineages infecting urban Village Weavers. This finding is not surprising given that the Afrotropics, despite being a hotspot of bird diversity, is a poorly studied geographic region in terms of avian haemosporidian infections. Most previous studies discovered new,

Table 3
Avian haemosporidians prevalence in the Afrotropics, including the present study.

Genus	Country	Prevalence (%)	Number of infected and total individuals	Reference
Haemoproteus	Benin	22.6	45/199	Harvey and Voelker (2017) [a]
	Cameroon	13.2	112/851	Chasar et al. (2009)
	Cameroon and Gabon	22.6	119/527	Beadell et al. (2009)
	Ghana	15.5	33/213	Loiseau et al. (2010)
	Ghana and Nigeria	82.3	14/17	Chaisi et al. (2019) [b]
	Madagascar	23.6	17/72	Ivanova et al. (2018)
		24.5	262/1067	Musa et al. (2019)
		9.4	39/414	Musa et al. (2022) [c]
	Malawi	25.0	134/535	Lutz et al. (2015)
	Nigeria	16.8	16/95	This study
		17.6	24/136	Hellgreen et al. (2013)
	Uganda	43.7	7/16	Iezhova et al. (2011)
		15.3	47/309	Valkiūnas et al. (2005)
Plasmodium	Benin	34.2	68/199	Harvey and Voelker (2017) [a]
	Cameroon	31.4	267/851	Chasar et al. (2009)
		30.8	177/575	Loiseau et al. (2002)
	Cameroon and Gabon	45.2	238/527	Beadell et al. (2009)
	Cameroon and Ghana	46.6	305/655	Valkiūnas et al. (2009)
	Ghana	30.0	64/213	Loiseau et al. (2010)
	Ghana and Nigeria	82.3	14/17	Chaisi et al. (2019) [b]
	Madagascar	18.0	13/72	Ivanova et al. (2018)
		30.7	328/1067	Musa et al. (2019)
		12.6	52/414	Musa et al. (2022) [c]
		11.8	81/686	Schmid et al. (2017)
	Malawi	38.9	208/535	Lutz et al. (2015)
	Nigeria	16.8	16/95	This study
		13.2	18/136	Hellgreen et al. (2013)
	Tanzania	12.6	51/406	Loiseau et al. (2002)
Leucocytozoon	Benin	6.5	13/199	Harvey and Voelker (2017) [a]
	Cameroon	41.9	357/851	Chasar et al. (2009)
	Cameroon and Gabon	6.6	35/527	Beadell et al. (2009)
	Ghana and Nigeria	64.7	11/17	Chaisi et al. (2019)
	Madagascar	59.7	43/72	Ivanova et al. (2018)
		5.5	59/1067	Musa et al. (2019)
		6.3	26/414	Musa et al. (2022) [c]
	Malawi	32.5	174/535	Lutz et al. (2015)
	Nigeria	27.4	26/95	This study
		17.6	24/136	Hellgreen et al. (2013)

This list includes the country, prevalence, number of infected and total sampled individuals, and reference for the three haemosporidian genus used in this investigation. In all studies, haemosporidian detection was conducted in blood by molecular assays, except otherwise indicated. The table reports the studies from which we could recover prevalence data.

[a] In Harvey and Voelker (2017), haemosporidian detection was made in both blood and muscle.

[b] In Chaisi et al. (2019), the combined prevalence of *Plasmodium* and *Haemoproteus* was reported.

[c] In Musa et al. (2022), the prevalence is reported using combined results of multiplex and nested PCR.

previously undocumented lineages of avian haemosporidians across the Afrotropics (e.g., Valkiūnas et al., 2008; Beadell et al., 2009; Iezhova et al., 2011; Chaisi et al., 2019), including the exceptional case from Malawi, where the greatest diversity of avian haemosporidian lineages was reported in Afrotropical birds (Lutz et al., 2015). We defined at least one new *Haemoproteus* lineage based on single-nucleotide differences based on the MalAvi database (Bensch et al., 2009). However, there is no consensus on the naming of new lineages based on *CytB* sequences (Schmid et al., 2017), and some authors argue that a divergence of more than two base pairs is necessary to define a new lineage (Levin et al., 2013). Moreover, we lack morphological data of blood stages of such *Haemoproteus* lineages, preventing us from linking taxonomic and genetic information (Valkiūnas et al., 2008; Iezhova et al., 2011). Thus, we termed the three new *Haemoproteus* lineages as 'tentatively' new lineages.

Our findings showed high haemosporidian diversity, suggesting complex and potential host-specific relationships, similar to other identified parasite-host associations in the Afrotropics (Beadell et al., 2009; Lutz et al., 2015). In this respect, it is worth mentioning the specific pattern in *Haemoproteus* infection: high lineage diversity restricted to a few avian host species, with all lineages of *Haemoproteus* detected from a single host species. Although the limited sample size for most host species precluded us from determining host specificity of the obtained lineages, the Village Weaver harboured the greatest diversity of haemosporidian lineages—four known *Haemoproteus* lineages and three tentatively new ones—all detected in urban individuals. Moreover, the most common lineage (*H. micronuclearis* VILWE1) occurred exclusively in this species. The Village Weaver is an abundant, highly adaptable bird that thrives in anthropogenic habitats, nesting in urban gardens, farmlands, and suburban areas across sub-Saharan Africa (Fry and Keith, 2004; Yisau et al., 2014; Mgelwa et al., 2018). Its synanthropic behaviour, colonial nesting, and tolerance of human presence likely increase its exposure to diverse vector communities and facilitate parasite maintenance in urban ecosystems. Similar patterns have been observed in other urban-tolerant species such as the Eurasian Blackbird (*Turdus merula*), which often sustain a higher haemosporidian diversity and prevalence than urban-sensitive birds (Carbó-Ramírez et al., 2017; Figuerola et al., 2024; Santiago-Alarcon et al., 2019). Thus, the Village Weaver may play an analogous ecological role in African cities—acting as a key reservoir species that links urbanization processes with haemosporidian transmission dynamics. In contrast, *Plasmodium* lineages appeared to be more generalists, infecting several host species across multiple families; some *Plasmodium* lineages, for instance, pGRW09,

were recovered from three separate host species. The ubiquitous nature of mosquitoes, the primary vectors of *Plasmodium*, suggests that both urban and non-urban habitats in the study area may provide suitable environmental conditions for vector proliferation and parasite transmission. However, our lack of significant habitat or seasonal effects on *Plasmodium* prevalence should not be interpreted as evidence of uniform vector abundance. Previous studies have shown that mosquito density and species composition can vary substantially with habitat characteristics such as vegetation cover, water availability, and pollution (Kigozi et al., 2015; Doumbe-Belisse et al., 2021). These findings highlight the need for further research integrating entomological and parasitological data to clarify how urban environmental conditions shape haemosporidian transmission dynamics.

Overall, these findings underscore the need for further research into the dynamics of haemosporidian parasites in Afrotropical avian hosts and emphasize the importance of continued surveillance and monitoring of haemosporidian parasites. Furthermore, we reported the first records of hPYNJOC1 and pLUME2 lineages in the Afrotropics, which infected urban Common Bulbuls and urban African Thrushes. Although these lineages had been previously documented in single individuals of other species in India (hPYNJOC1) and Germany (pLUME2) (Hellgreen et al., 2007; Gupta et al., 2019), our findings suggest that these lineages are rare, or at least not sufficiently explored in the Afrotropics. This rarity supports the notion that the Afrotropical region remains understudied (Seto et al., 2012; Gatti et al., 2015; Awoyemi and Ibáñez-Álamo, 2023), emphasizing the importance of continued research into the diversity and distribution of haemosporidian parasites in this region. Moreover, we added new hosts for several *Haemoproteus* and *Plasmodium* lineages, including Common Bulbul (hPYNJOC1 and pLUME2), Village Weaver (hRBQ07 and hVIMWE1), Blue-spotted Wood Dove (hTURABY01), African Thrush (pLUME2), and Green Hylia (pAEMO01). Our discovery highlights the vast, unexplored diversity of haemosporidians in the Afrotropics.

While this study provides novel insights into the diversity and prevalence of avian haemosporidian parasites in a largely unexplored Afrotropical region, we acknowledge certain limitations. The relatively small sample size (95 birds) may have constrained our ability to detect less prevalent lineages and fully assess host–parasite associations across habitats and seasons. Additionally, although the nested-PCR protocol developed by Hellgren et al. (2004) is widely recognized for its efficiency in detecting *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, it also has some biases. For example, it can ineffectively detect co-infections between parasites of the genera *Haemoproteus* and *Plasmodium* due to differences in intensity of infection (Valkiūnas et al., 2006; Martínez et al., 2009; Bernotienė et al., 2016). Furthermore, the protocol introduced some primer non-specificity, potentially affecting *Leucocytozoon* amplification and lineage recovery. Future studies incorporating larger sample sizes, *Leucocytozoon*-specific primers (e.g., Ciloglu et al., 2019; Musa et al., 2024), and broader temporal sampling would improve parasite detection and strengthen inferences on host–parasite dynamics in Afrotropical bird communities.

In conclusion, our study contributes to a better understanding of the prevalence, diversity, and distribution of haemosporidian parasites in Afrotropical avian hosts. The divergence of haemosporidian lineages along urban and non-urban habitats—with *Haemoproteus* lineages found primarily in urban areas and most *Plasmodium* lineages in non-urban areas—underscores the need for further research into the dynamics of these parasites in the region. Continued study of haemosporidian parasites in the Afrotropics will provide valuable insights into the ecology and evolution of host–parasite relationships, ultimately informing strategies for conserving and managing avian populations in this highly biodiverse part of the world.

CRediT authorship contribution statement

Adewale G. Awoyemi: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Data curation. **Jorge Garrido-Bautista:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Yahkat Barshep:** Writing – review & editing, Investigation. **Juan Diego Ibáñez-Álamo:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Ethics statement

The A.P. Leventis Ornithological Research Institute (APLORI) in Jos, Nigeria, granted AGA (bird ringer ID: 16491; licensed by SAFRING, South Africa) the necessary ethical permit for fieldwork on September 26, 2022. Additionally, a Material Transfer Agreement was signed between APLORI and the University of Granada to facilitate the transportation of samples between Nigeria and Spain.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.avrs.2025.100330.

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