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Forest Type and Body Size Drive Blood Parasite Infections in Afrotropical Ant-Following Birds

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Abstract

Forest fragmentation due to deforestation is a major threat to biodiversity in the Afrotropics, particularly affecting specialized avian guilds such as ant-following birds. While their population declines have been documented, the influence of habitat disturbance on their prevalence remains poorly understood. This study investigates the prevalence of five blood and tissue parasites (*Plasmodium*, *Haemoproteus*, *Leucocytozoon*, Trypanosome, and Microfilariae) in ant-following birds across a degraded tropical rainforest in South Western Cameroon, and explores the relationship between infection status, ant-following behavior and bird weight. Blood samples from 520 birds were analyzed using microscopy and PCR, which was run for only microscopy positive samples. *Plasmodium* was the most commonly encountered parasite genus with a prevalence of 76.5% in *Chamaetylas poliocephala*, followed by *Leucocytozoon* with 45.5% in *Neocossyphus poensis*, *Trypanosoma* with 40% in *Phyllastrephus xavieri*, *Haemoproteus* with 36.4% in *Neocossyphus poensis* and microfilariae with 27.3% in *Alethe castanea. Trypanosoma* prevalence was significantly higher in fragmented forests ($p = 0.0235$) in ant-followers, while the other parasites showed no significant difference across forest types. Ant-following birds consistently had higher parasite prevalence than none ant-followers. The ant-following nature of an avian host influences the prevalence of parasites it harbors. Logistic regression confirmed that higher bird weight significantly increased the odds of infection with several parasites, especially *Haemoproteus* ($p = 0.0009$) and *Leucocytozoon* ($p = 0.0022$) and Microfilariae ($p = 0.0036$). These findings underscore the subtle but important effects of habitat disturbance and host physiology on avian parasite ecology.

Keywords

Neocossyphus poensis, *Alethe castanea*, *Chamaetylas poliocephala*, *Phyllastrephus xavieri*, *Trypanosoma*, *Plasmodium*

1. Introduction

Tropical rainforests are rapidly disappearing, with serious consequences for birds, ecosystems, and the emergence of infectious diseases. Avian haemosporidians, blood parasites in birds, are often used as models to study human parasites due to their similarities in life cycles [1]-[3]. These parasites provide important insights into evolutionary and ecological processes. Protozoan blood parasites (or haemoparasites) have been reported in African rainforest birds [1] [4] [5] but there is limited information on how bird traits, such as ant-following behavior and morphology, affect parasite prevalence, particularly in forests undergoing deforestation.

Protozoan blood parasites belong primarily to the order *Haemosporida*. *Plasmodium* species are primarily transmitted by mosquitoes (family Culicidae) [6]. *Haemoproteus* species are vectored by biting insects including mosquitoes, biting midges (*Culicoides*), louse flies (*Hippoboscidae*), and horse flies (*Tabanidae*). *Leucocytozoon* species are transmitted by black flies (*Simuliidae*) [7]. Microfilariae (larval stages of filarial nematodes) are transmitted by blood-feeding arthropods—mainly Dipterans such as *Simulium* and *Culicoides*, but also other bloodsucking insects [8] [9]. At least 16 genera of filariae are known to infect birds [10]. Avian trypanosomes are also transmitted by a wide variety of arthropods (Simuliidae, Culicidae, Ceratopogonidae, Hippoboscidae, and Dermanyssidae) [11]-[14]. Compared to their mammalian counterparts, avian trypanosomes are poorly studied, especially in cases of co-infection with other avian haemoparasites [5] [15]. Despite numerous filarial species found in birds, little is known about their prevalence in ant-following birds.

Ant-following birds, common in tropical ecosystems, exhibit a unique foraging strategy: trailing army ant swarms to catch prey fleeing from the forest floor [16]. While this behavior offers feeding advantages, it may also increase exposure to parasites. These birds are among the most frequent and dominant swarm attendant [17]. Ant-following specialization is classified into three categories: occasional, regular, and professional [18]. Professional followers rely almost exclusively on a single ant species, *Eciton burchellii*, making them particularly vulnerable to environmental threats such as deforestation and climate change. In Western Kenya, Peters and Okalo [19], found that the abundance of army ants (*Dorylus wilverthi* and *D. molestus*) was positively correlated with the occurrence of specialized ant-followers (*Neocossyphus poensis*, *Alethe poliocephala*, and *Bleda syndactyla*). Craig [20], documented 52 regular ant-following bird species in Africa—38 of which belong to three overrepresented families: Muscicapidae (18 spp.), Pycnonotidae (13 spp.), and Turdidae (7 spp.). Because birds have shorter life spans than humans, they are ideal for studying disease dynamics across lifetimes and generations, helping us better understand host-vector-environment interactions.

Habitat disturbance and conversion to plantations reduce the diversity of birds, ants, and haemosporidian parasites. Forest fragmentation often leads to declines in army ant populations, which in turn impacts the bird species that depend on them [21]. These cascading effects influence parasite prevalence. Ant diversity generally declines with disturbance [22] [23], likely due to changes in vegetation and microclimate. Forests converted into mixed-crop fields also show lower ant diversity [24]. Southern Cameroonian forests had significantly higher ant diversity compared to fallow or crop fields. Long-term Amazonian studies have shown that ant-following bird communities remain stable in undisturbed forests [25]. In Cameroon, forest degradation has led to sharp declines in insectivorous birds—especially ant-followers—and delayed recolonization of regenerating forests [16] [19] [26]-[29]. Ocampo-Ariza *et al.* [28] reported significantly lower ant-following bird richness and encounter rates in oil palm plantations than in protected or agroforestry landscapes. However, no study has examined how parasite prevalence in these birds correlates with body mass.

Research on bird haemosporidians in Africa has focused largely on *Eurillas latirostris* and *Cyanomitra olivacea* [30]-[32], while much less is known about ant-following birds in disturbed habitats. Bonneaud *et al.* [30] reported higher *Plasmodium* prevalence in pristine forests than in disturbed ones. Similarly, Chasar *et al.* [31] found higher *Haemoproteus* and *Leucocytozoon* prevalence in undisturbed areas. Tchoumbou *et al.* [4] noted that parasite prevalence decreased with deforestation, but *Haemoproteus* slightly rebounded following the establishment of palm oil plantations, while *Plasmodium* continued to decline. There remains a clear knowledge gap regarding protozoan parasite prevalence in ant-following birds in forests undergoing active deforestation.

In Cameroon, mature forests are shrinking due to logging and slash-and-burn agriculture, while secondary forest cover is expanding [33]. The Talangaye rainforest in the South West region is undergoing rapid deforestation from palm oil development, making it emblematic of the country's broader deforestation crisis. This loss of forest not only threatens bird biodiversity but may also alter parasite dynamics. While ant-following birds have been well studied in the Neotropics [34]-[38], little is known about their parasite dynamics in African tropical forests undergoing active deforestation.

This study focuses on five groups of avian blood parasites, *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, trypanosomes, and microfilariae, in ant-following birds. These parasites have important effects on host behavior, fitness, and survival. We hypothesize that:

1) Parasite prevalence will differ between pristine and fragmented forests in ant-following birds.

2) Ant-following behavior will influence the prevalence of parasites.
3) Host body mass will influence the likelihood of parasite infection.

2. Methodology

2.1. Sample Site and Data Collection

These samples were collected during large-scale deforestation for the development of a palm oil plantation in the Talangaye rainforest, Koupé-Manengouba sub-division (5˚08'N to 5˚20'N and 9˚22'E to 9˚24'E), in the years 2016 and 2017. Data from Camp 2 (5.17530N, 9.34882E) in January 2016 (pristine forest) and January in 2017 (fragmented forest) were used in this study. All research authorization/ permits were obtained from the competent local administration and authorities of the oil palm plantation

We captured birds using traditional mist netting technique [27], weighed, identified, measured, ringed, bled, and released them alive. The banded birds were identified and classified into host families using Borrow and Demey [39]. Ant-following birds were identified based on previous studies [19] [20] [40], into three host families Muscicapidae, Pycnonotidae and Turdidae. Approximately 50 μL of blood from the brachial vein of each bird was also collected into cryo tubes containing lysis buffer (10 mMTris-HCl pH 8.0, 100 mM ethylene-diaminetetraacetic acid, 2% sodium dodecyl sulphate) and stored at −20˚C for subsequent molecular analysis [41] in the Clinical Diagnostic Laboratory of the University Buea, Cameroon. In addition, we prepared two thin blood films for each bird, air-dried with the aid of a battery-operated hand fan [6], fixed in 100% methanol for 1 minute, packed into slide boxes and stained with Giemsa in the laboratory.

2.2. Microscopic and Molecular Analysis

Fixed thin blood smears were stained with Giemsa as described by [6]. They were then studied using an Olympus BX40 light microscope equipped with a canon microphotographic camera at the highest magnification (1000× for 30 - 45 min) [2] and the intensity of infections determined as recommended by Godfrey *et al.* [42].

Microscopy was first done for all samples and then PCR-based methods were employed on all microcopy positive samples to determine the true haemosporidian species composition in each naturally infected individual host. We extracted DNA from blood samples using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Each sample was simultaneously screened for *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* blood parasites using a nested-PCR method described by Hellgren *et al.* [43] which amplifies a 479 bp fragment of the parasite's mitochondrial DNA (mtDNA) cytochrome b (cytb) gene. Nested1 Primers set was HaemNF/HaemNR2 which amplified gene segments that are similar in both parasite species; while Nest2 Primers set was HaemF /HaemR2 which amplified specific gene segments to *Haemoproteus*/*Plasmodium* spp. [43] [44]. PCR cycling was performed as primary denaturation at

95˚C (5 min), annealing at 50˚C (30 s), extension at 72˚C (45 s), and finally followed by a final extension at 72˚C (10 min) which was run for 20 cycles in 1st nested PCRs and 35 cycles in 2nd nested PCRs. Positive amplicons of the previous study and ultra-pure ddH2O were utilized as positive and negative controls for each PCR reaction set. PCR products were visualized on 2% agarose gel. Purification and sequencing were carried out using 15 μl of PCR products (479 bp) by BIONEER, South Korea.

3. Statistical Analyses

Prevalence was calculated for each parasite across forest types. Chi-square tests assessed differences in infection prevalence between habitats. T-tests compared bird weights of infected vs. non-infected individuals. Logistic regression models assessed the influence of forest type, ant-following behavior and body weight, on infection probability. All analyses were conducted using R, with significance set at $p < 0.05$.

4. Results

4.1. Prevalence of Protozoan Parasites across Forest Types

A total of 520 birds representing 42 species were captured in this study with an overall parasite prevalence of 47%. In the pristine forest 316 birds were sampled and 204 from the fragmented forest with 47% and 49% prevalence of parasites respectively. The protozoan parasites detected in this study were as follows: *Plasmodium* (P), *Haemoproteus* (H), *Leucocytozoon* (L), Microfilariae (M) and *Trypanosoma* (T). Only *Trypanosoma* prevalence varied significantly between pristine and fragmented forest ($\chi^2 = 6.37$, $p = 0.0116$). Pristine forest had higher prevalence of infection for *Plasmodium* and *Leucocytozoon* parasites while the fragmented forest had higher prevalence for *Haemoproteus*, *Trypanosoma* and microfilariae (**Figure 1**). *Plasmodium* infections were slightly higher in pristine forests (32.8%) than fragmented ones (29.85%). *Trypanosoma* infections were more than twice as high in fragmented forests (15.67%) compared to pristine (7.41%). Microfilariae show slightly higher prevalence in fragmented forests (13.43%) than pristine forest (11.11%). *Plasmodium* was the most commonly encountered parasite genus with a prevalence of 76.5% in *Chamaetylas poliocephala*, followed by *Leucocytozoon* with 45.5% in *Neocossyphus poensis*, *Trypanosoma* with 40% in *Phyllastresphus xavieri*, *Haemoproteus* with 36.4% in *Neocossyphus poensis* and microfilariae with 27.3% in *Alethe castanea* (**Figure 2**).

4.2. Prevalence of Parasites by Ant-Follower Behavior

Sixteen species of birds were identified as ant-followers (**Table 1**). Ant-following birds made up 67.5% (351/520) of all the birds sampled. Among these, 47% of ant-following birds tested positive for at least one parasite, compared to 29% prevalence of infection in non ant-following birds. Parasite prevalence varied significantly with ant-following behavior ($p < 0.01$). The prevalence of protozoan blood

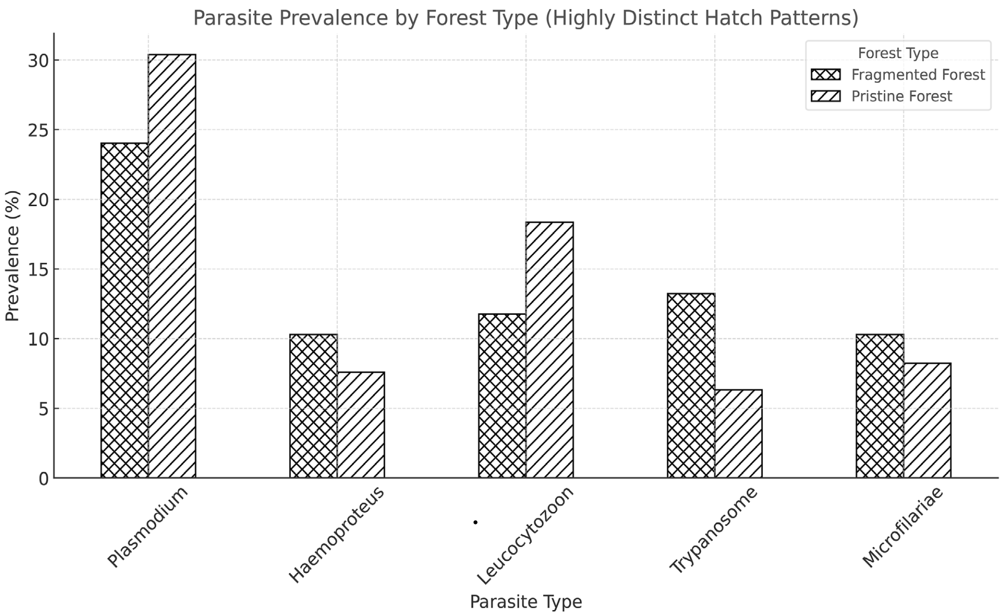
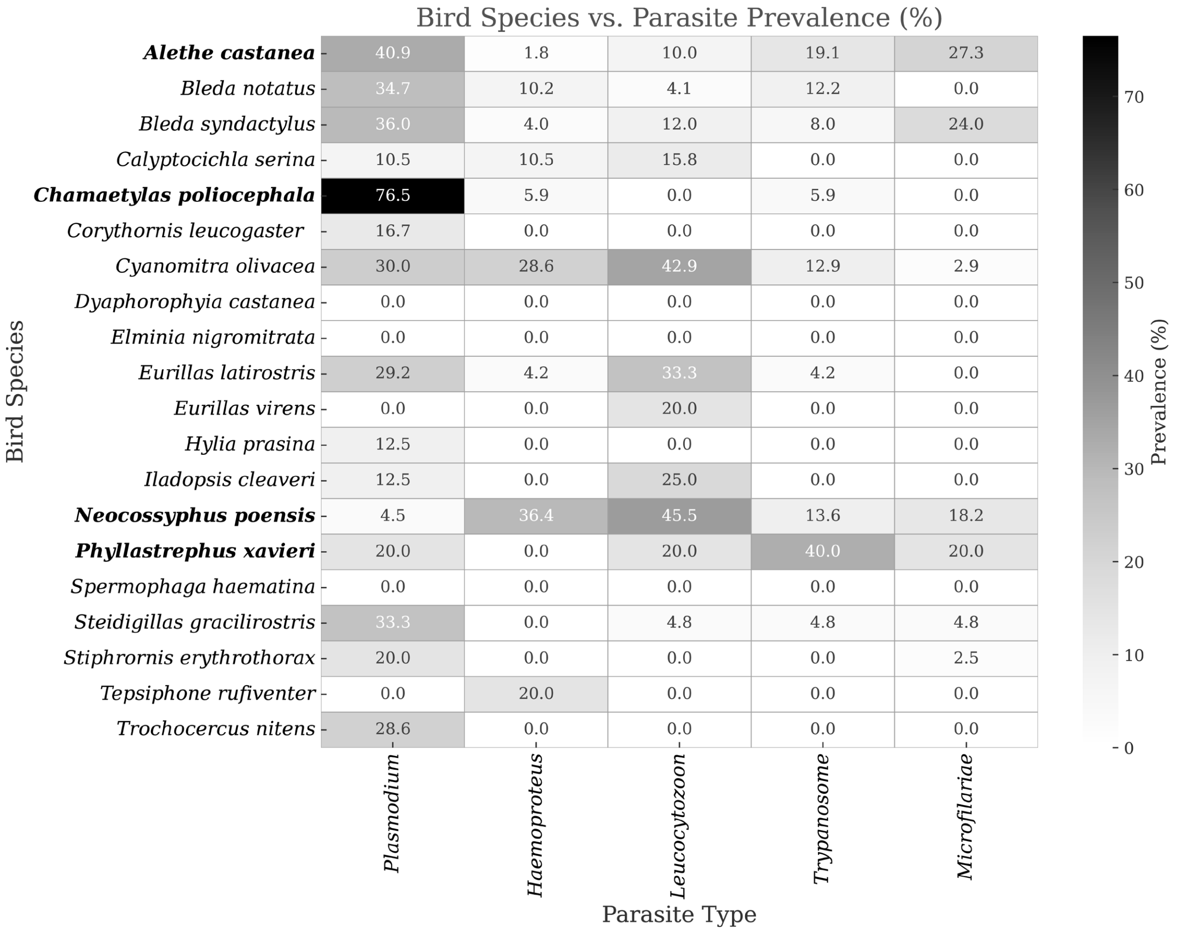


Figure 1. Prevalence of parasites across forest types. Only Trypanosome was significantly different across forest types.



	Plasmodium	*Haemoproteus*	*Leucocytozoon*	*Trypanosome*	*Microfilariae*
Alethe castanea	40.9	1.8	10.0	19.1	27.3
Bleda notatus	34.7	10.2	4.1	12.2	0.0
Bleda syndactylus	36.0	4.0	12.0	8.0	24.0
Calyptocichla serina	10.5	10.5	15.8	0.0	0.0
Chamaetylas poliocephala	76.5	5.9	0.0	5.9	0.0
Corythornis leucogaster	16.7	0.0	0.0	0.0	0.0
Cyanomitra olivacea	30.0	28.6	42.9	12.9	2.9
Dyaphorophyia castanea	0.0	0.0	0.0	0.0	0.0
Elminia nigromitrata	0.0	0.0	0.0	0.0	0.0
Eurillas latirostris	29.2	4.2	33.3	4.2	0.0
Eurillas virens	0.0	0.0	20.0	0.0	0.0
Hylia prasina	12.5	0.0	0.0	0.0	0.0
Iladopsis cleaveri	12.5	0.0	25.0	0.0	0.0
Neocossyphus poensis	4.5	36.4	45.5	13.6	18.2
Phyllastrephus xavieri	20.0	0.0	20.0	40.0	20.0
Spermophaga haematina	0.0	0.0	0.0	0.0	0.0
Steidigillas gracilirostris	33.3	0.0	4.8	4.8	4.8
Stiphrornis erythrothorax	20.0	0.0	0.0	0.0	2.5
Tepsiphone rufiventer	0.0	20.0	0.0	0.0	0.0
Trochocercus nitens	28.6	0.0	0.0	0.0	0.0

Figure 2. Parasite prevalence heat map by bird species. The highest parasite prevalence are written in bold.

parasites in ant-following bird families were as follows; Ant-following birds (Y) showed higher prevalence of *Plasmodium*, Trypanosome, and Microfilariae and non ant-followers (N) had higher *Haemoproteus* and *Leucocytozoo*n infections (**Figure 3**). We found the highest prevalence in Turdidae, Muscicapidae, and then Pycnonotidae ant-following bird families (**Table 2**). Turdidae had the highest prevalence (63.6%) though from a smaller sample size. Significantly higher infection prevalence in ant-followers for *Plasmodium* ($\chi^2 = 8.09$, p = 0.0044), *Haemoproteus* ($\chi^2 = 8.73$, p = 0.0031), *Leucocytozoon* ($\chi^2 = 9.27$, p = 0.0023) and Microfilariae ($\chi^2 = 12.38$, p = 0.0004) were recorded. There was no significant difference in Trypanosome ($\chi^2 = 2.43$, p = 0.1189) prevalence between both groups. Box plot revealed that ant-following birds had higher loads of parasites than non ant-followers (**Figure 4**).

Table 1. Summary of 16 ant-following bird species screened for Protozoan blood parasites.

Species (Scientific Names)	Examined	Infected	Prevalence (%)
Alethe castanea	110	64	36.58
Bleda notatus	49	25	14.29
Stiphrornis erythrothorax	40	9	5.14
Bleda syndactylus	25	12	6.86
Eurillas latirostris	24	12	6.86
Neocossyphus poensis	22	14	8
Steidigillas gracilirostris	21	9	5.14
Calyptocichla serina	19	7	4
Chamaetylas poliocephala	17	13	7.43
Eurillas virens	10	2	1.14
Phyllastrephus xavieri	5	4	2.29
Criniger chloronotus	3	2	1.14
Stizorhina fraseri	3	1	0.57
Cossypha cyanocampter	1	0	0
Eurillas curvirostris	1	0	0
Muscicapa sethsmithi	1	1	0.57
	351	175	

Table 2. Prevalence of Protozoan blood parasites within infected ant-following bird families.

Host family	Number screened	Number Positive	Prevalence
Muscicapidae	172	88	51.2%
Pycnonotidae	157	73	46.5%
Turdidae	22	14	63.6%

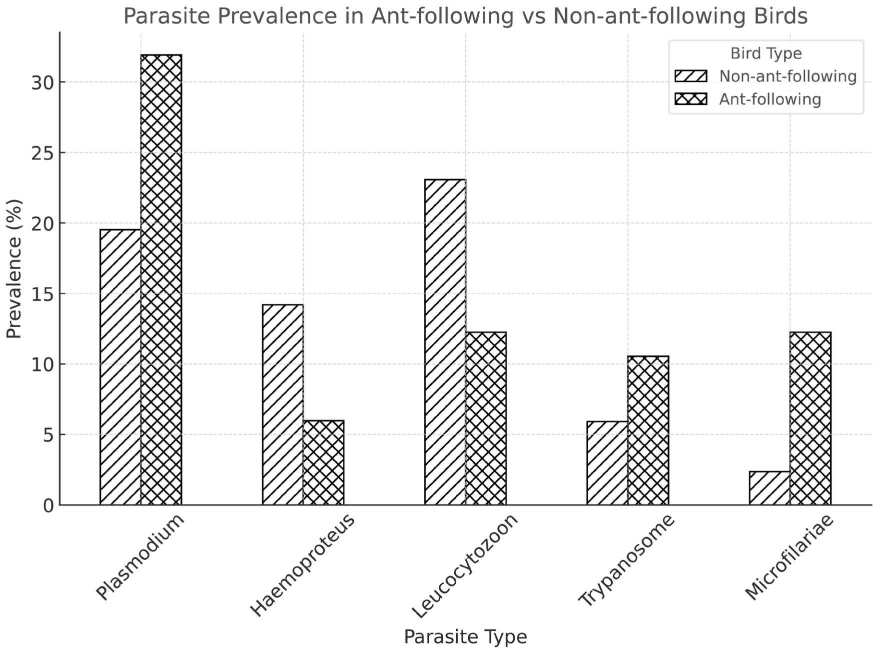


Figure 3. Prevalence of parasites in ant-following versus non ant-following birds.

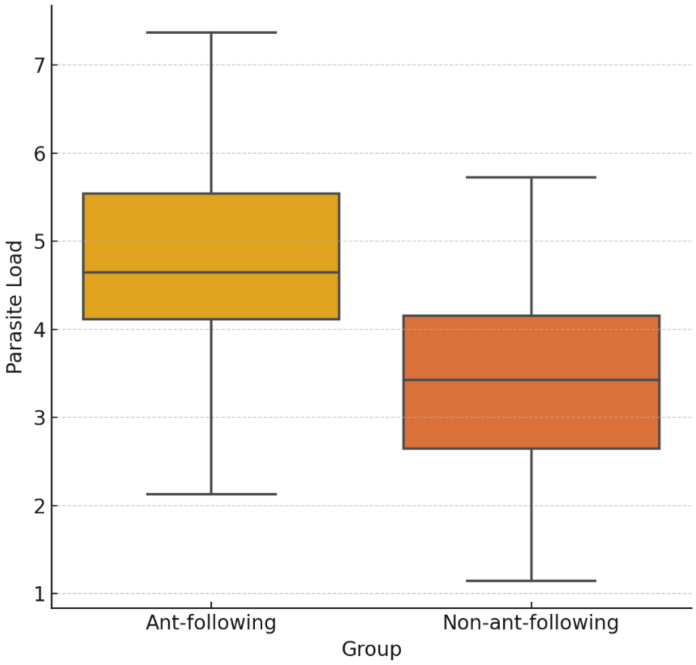


Figure 4. Parasite Prevalence by ant-following behavior.

4.3. Morphometric Traits

Body mass was measured for all bird species captured and the largest ant-following bird was *Neocosyphus poensis* (with a mean weight of 52.61 g) and the smallest ant-following bird was *Stiphrornis erythrothorax* (with a mean weight of 17 g). We analyzed whether birds infected with specific parasites had significantly different weights compared to uninfected birds. For *Plasmodium* and microfilariae, infected birds were significantly ($P < 0.05$) heavier than uninfected birds (**Table 3**). Box plots showing the distribution of bird weights for infected versus uninfected birds for each parasite observed are represented in **Figure 5**. Logistic regression confirmed that higher bird weight significantly increased the odds of infection with several parasites, especially *Haemoproteus* ($p = 0.0009$) and *Leucocytozoon* ($p = 0.0022$).

Table 3. p-values and interpretation of parasites in infected birds.

Parasite	Infected birds	p-value	Interpretation
Plasmodium	Slightly heavier	**0.049**	Significant
Haemoproteus	Slightly lighter	0.097	Not significant
Leucocytozoon	Lighter	0.055	Not significant
Trypanosoma	Heavier	0.090	Not significant
Microfilariae	Much heavier	**0.000006**	Highly significant

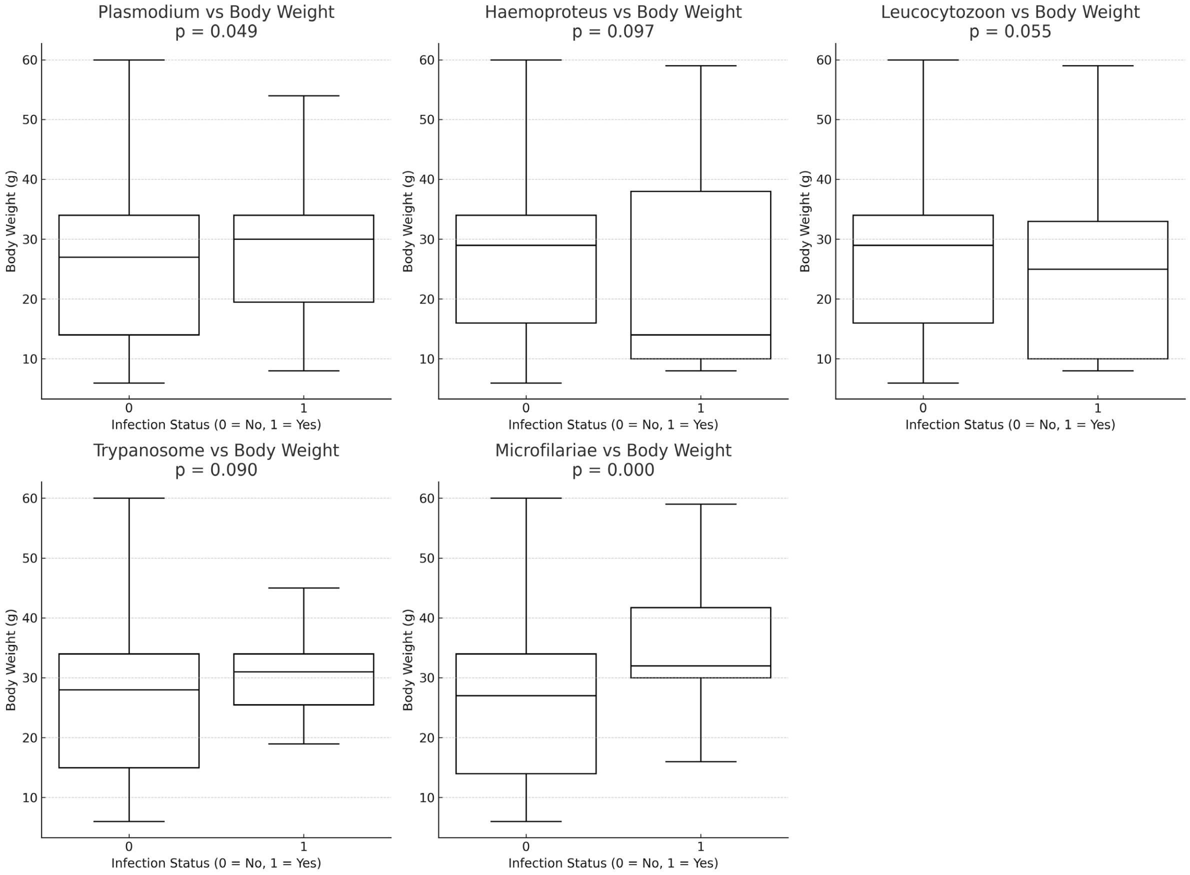


Figure 5. Distribution of bird weights for infected versus uninfected birds.

5. Discussion

In this study, we examined the prevalence of protozoan blood parasites in the three most common ant-following bird families in Afrotropical forests. Overall, birds from pristine forests exhibited a higher prevalence of protozoan parasites, particularly *Plasmodium* and *Leucocytozoon*, except in the case of *Haemoproteus*, *Trypanosoma*, and microfilariae. The high prevalence of *Plasmodium* and *Leucocytozoon* in undisturbed forests is consistent with previous findings from Cameroon [30] [31]. Interestingly, *Trypanosoma* prevalence was significantly higher in fragmented forests ($p = 0.0235$) among ant-following birds, whereas the other parasites did not show significant differences across forest types. Bonneaud *et al.* [30] similarly reported that forest type was significantly associated with variation in *Plasmodium* prevalence in three non ant-following bird species (*Andropadus latirostris*, *Andropadus virens*, and *Cyanomitra obscura*). Our study identified 16 species of regular ant-following birds: 4 in the family Muscicapidae, 7 in Pycnonotidae, and 2 in Turdidae. This represents 42% of the 38 regular ant-followers identified by Craig [20], who documented 18 Muscicapidae, 13 Pycnonotidae, and 7 Turdidae species. Notably, members of the Turdidae family have shown haemosporidian prevalence as high as 94% in temperate regions such as Germany [45].

Ant-following birds are insectivorous species that rely on swarms of army ants, particularly driver ants of the subfamily Dorylinae in tropical Africa, to flush arthropods and small vertebrates from the forest floor [16] [46]. Among African passerines, these species are considered especially vulnerable to forest loss and fragmentation [16] [26]. Because many of the flushed arthropods are vectors of protozoan parasites, this foraging strategy likely increases parasite exposure [6]-[9].

Five groups of protozoan blood parasites were detected in this study, with an overall infection prevalence of 47%. All five parasite types have previously been recorded in Afrotropical birds [1] [4] [5] [46]. Specifically, 47% of ant-following birds tested positive for at least one parasite, compared to only 29% in non-ant-following birds. This difference was statistically significant ($p < 0.01$), supporting earlier suggestions that ant-following behavior increases parasite risk [40].

Previous studies have reported even higher parasite prevalence in Afrotropical birds (68.8% - 82.8%) [47]. However, Chaisi *et al.* [47] relied on qPCR and nested PCR rather than microscopy, which can detect gametocytes and distinguish active from latent infections. In contrast, our study used nested PCR only on samples that tested positive via microscopy. Among the 16 bird species sampled, 14 tested positive for protozoan parasites. Only two species, *Cossypha cyanocampter* and *Eurillas curvirostris*, showed no infections, but as only one individual was sampled per species, no conclusions can be drawn regarding their parasite status. Larger sample sizes will be necessary to determine whether these species are truly free of protozoan infections.

Among ant-following species with a sample size ≥ 15, the most parasite-rich species were *Chamaetylas poliocephala*, *Neocossyphus poensis*, *Phyllastrephus xavieri*, and *Alethe castanea*. This threshold was used to minimize bias due to differing sample sizes across host species [48].

The high prevalence of microfilariae in *Alethe castanea* is consistent with previous studies [1] [40]. This ground-dwelling Muscicapid occurs in all forest types. Sehgal *et al.* [1] reported a microfilariae prevalence of 61.5% in *A. castanea*, with none detected in its close relative, *Chamaetylas poliocephala* (brown-chested alethe). Waltert *et al.* [49] also noted that *A. castanea* was the most frequent and consistent ant-follower at their study site. Given the high prevalence of both filariae and trypanosomes in this species, we recommend it as a candidate model for drug testing and further research on filarial and trypanosomal infections. Additional studies are needed to identify the specific filarial species involved and to better understand the origin and transmission of nematode infections in *A. castanea*.

High prevalence of *Leucocytozoon* and *Haemoproteus* was also found in *Neocossyphus poensis*, possibly due to its status as the most specialized African ant-follower in Kenya's Kakamega Forest [19]. It is important to note that ant-following specialization varies: occasional ant-followers feed opportunistically when swarms cross their path, while obligate followers rely heavily on this strategy for survival [18] [50].

We also observed generally low parasitemia levels. This may be due to the use of mist nets, which are more likely to capture birds in the chronic phase of infection, while acutely infected individuals may be less active and less likely to be sampled [6]. However, parasitemia is not necessarily indicative of disease severity, as even low-level infections can disrupt blood cell production and impair fitness [51]. The observed correlation between body mass and parasite load may reflect greater blood volume or immunosuppression in larger birds, or simply longer exposure due to age.

In summary, our findings provide strong evidence that ant-following behavior significantly increases the risk of protozoan infection in Afrotropical birds. These birds range widely across forest landscapes and forage near the ground, increasing their exposure to blood-feeding vectors such as mosquitoes (*Culicidae*) and biting midges (*Ceratopogonidae*) [46] [50]. Further research on these understudied species is crucial to better understand host-vector-parasite interactions in tropical forests undergoing rapid environmental change.

6. Conclusion

Forest fragmentation is associated with increased Trypanosome infection in ant-following birds, while heavier birds are more susceptible to several parasite types. Forest type was a significant predictor only for Trypanosomes (p = 0.0125). There were statistically significant differences in the prevalence of four parasites (Microfilariae, *Leucocytozoon, Haemoprotues* and *Plasmodium*) between ant-following and non ant-following birds. Higher bird weight significantly increased the odds of infection with several parasites, especially *Haemoproteus* (p = 0.0009) and *Leucocytozoon* (p = 0.0022) and Microfilariae (p = 0.0036). Ant-following birds have higher parasitemia than non-ant followers. These results highlight the need to integrate parasitological surveillance into avian conservation strategies.

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Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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