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PROTOZOOLOGY - ORIGINAL PAPER

Avian haemosporidia in native and invasive sparrows at an Afrotropical region

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Abstract

Bio-invasions are a major threat to biodiversity and ecosystems globally and may contribute to the proliferation of emerging infectious diseases. We examined the prevalence and phylogenetic diversity of avian haemosporidian parasites infecting the non-native house sparrows (*Passer domesticus*) and the native southern grey-headed sparrows (*Passer diffusus*). Blood samples from 104 sparrows (74 house sparrows and 30 southern grey-headed sparrows) mist-netted inside and around the Kruger National Park were used. Genomic DNA was extracted from each blood sample and subjected to nested PCR analyses, Sanger sequencing and phylogenetic analyses. Overall, 35.57% (37/104) of the birds sampled were infected with at least one haemosporidian parasites. Southern grey-headed sparrows had a higher parasite prevalence (60%) than house sparrows (24.3%). A total of 16 parasite lineages were identified, of which eight were novel lineages. Whereas *Haemoproteus* spp. showed the highest lineage diversity, *Leucocytozoon* spp. were the most prevalent parasites, albeit with significant differences between sparrow species. A single *Plasmodium* sp. infection was recorded in a southern grey-headed sparrow. In support of the enemy release hypothesis, we found that prevalence on non-native house sparrows was lower than prevalence recorded in their region of origin and also that they were infected only by indigenous parasites lineages.

Keywords Avian haemosporidia · Enemy release hypothesis · Invasive alien species · Sparrows

Introduction

Invasive alien species (IAS) are regarded as a major threat to native biodiversity. IAS can alter ecosystem function, services and human health, causing population declines and disease spread and driving competitive exclusion that in extreme cases leads to the extinction of native taxa (Jeschke et al. 2014). IAS may also contribute towards the spread and maintenance of infectious diseases in the ecosystem, acting as vectors or reservoirs. On colonising a new area, IAS may not only introduce new pathogens, but they may lose their original pathogens prior, during or after introduction (MacLeod et al. 2010) and become infected with local parasites, thus giving them a competitive advantage (Lymbery et al. 2014). These mechanisms are explained by two hypotheses: the novel weapon hypothesis (NWH) and the enemy release hypothesis (ERH). The NWH suggests that non-natives may introduce pathogens against which native species have not evolved defences, potentially acting as "biological weapons" against native species (Lymbery et al. 2014). ERH, on the other hand, suggests that non-native species lose their original pathogens, obtaining thus an advantage against native competitors (Colautti et al. 2004; Marzal et al. 2011).

Avian malaria and other related haemosporidian parasites are suspected to be responsible for population declines and mortalities of numerous wild and captive bird

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species all over the world (Levin and Parker 2012). For instance, the avian malaria pathogen *Plasmodium relictum* is responsible for the population decline and extinction of endangered birds on the Island of Hawaii (Atkinson and Samuel 2010). This parasite is also currently suspected to be responsible for the population decline of numerous native bird species in England and New Zealand (Dadam et al. 2019; Niebuhr et al. 2016). To date, reports from negative effects of haemosporidian parasites in birds come mostly from insular bird species without prior exposure to these parasites and birds in captivity (Atkinson and Samuel 2010; Grilo et al. 2016; Jia et al. 2018; Niebuhr et al. 2016). The extent of the impact of these parasites on wild bird species in regular conditions remains poorly understood because of sampling bias and the under-reported cases of mortality.

Despite the increased interest in studies dealing with the impact of alien species in the environment, birds have not received as much attention as other taxa like plants, mammals and the aquatic taxa (Schirmel et al. 2016). Studies in birds have focused mainly on viruses and other zoonotic pathogens. Avian haemosporidia studies have focused on either a single species or a group of unrelated species (Clark et al. 2015; Marzal et al. 2011; Santiago-Alarcon et al. 2020; Sijbranda et al. 2016; Van Hemert et al. 2019). Few studies have compared the role species from the same family of native and alien origin, with more or less overlapping habitat, play in the spread of avian haemosporidia.

We examined the prevalence of avian haemosporidia in two sparrow species (family Passeridae, order Passeriformes), one native and one IAS, which partly overlap in habitat and habits within an Afrotropical Lowveld region in South Africa: the southern grey-headed sparrow (*Passer diffusus*) and the house sparrow (*Passer domesticus*). The southern grey-headed sparrow (herewith referred to as grey-headed sparrow) is a regional endemic species widely distributed in the southern Afrotropical region (Hockey et al. 2005). It is found mainly in savanna and woodlands but also gardens, farms, cultivation areas, villages and plantation edges. The distribution of this bird largely overlaps with its non-native counterpart, the house sparrow, which uses similar habitats, although it usually only occurs in or close to human settlements. The house sparrow is native to Europe but is recognised as the second most notorious urban invasive bird species after the common mynah (*Acridotheres tristis*) earning a classification as a category 3 invader species in South Africa, prohibiting and restricting its spread (Hanson et al. 2020; Magudu and Downs 2015). Although widely distributed in their introduced ranges, house sparrow populations have declined considerably in parts of their native ranges, especially in the UK, where avian malaria is suspected to be the causal factor (Angelier and Brischoux 2019; Sheldon and Griffith 2017).

Recent studies of parasite prevalence in house sparrows found that birds from Europe harboured higher parasite prevalence than birds introduced in Brazil (Antonini et al. 2019), supporting the ERH. The objectives of this study were to (1) investigate the prevalence of avian haemosporidia in house sparrows and the grey-headed sparrows, (2) determine the genetic diversity of avian haemosporidia identified and finally (3) compare infections in both species. In line with the ERH, we predicted a high parasite prevalence and diversity in the native grey-headed sparrow compared to the invasive house sparrow found in the same vicinity. The assumption being that the invasive sparrows will "release" their infection burden to the native sparrows.

Materials and methods

Bird sampling

Birds were caught using birdcall lure enhanced mist nets (Ndlovu 2018) in the Kruger National Park and the surrounding settlement areas, as part of a larger study conducted in the Lowveld region of South Africa. The Kruger National Park is a 2,000,000 ha conservation area situated between the Mpumalanga and Limpopo provinces of South Africa. The surrounding area is dominated by private and provincial conservation areas as well as plantations, villages, semi-urban and urban densely populated towns. The area lies in the low-lying savanna of north-eastern South Africa, with elevations ranging from 250 to 800 m above sea level. The climate in this region is tropical to subtropical (Köppen climate classification subtype Bsh) with high mean summer temperatures (32 °C) and mean annual rainfall between 350 mm (in the north) and 750 mm (in the south) concentrated between October and April. The fauna in this area is very diverse with numerous mammals, reptiles, amphibians and bird species. The park and adjacent areas are classified as one of South Africa's Important Bird and Biodiversity Areas (IBA) with over 500 bird species recorded.

House sparrows and grey-headed sparrows were caught at two sites within the park (Satara [24.393° S, 31.779° E] and Shingwedzi [23.116° S, 31.433° E]) and two sites outside the park (Acornhoek [24.605° S, 31.087° E] and Hazyview [25.051° S, 31.136° E]; Fig. 1), between the months of March to April, June to July and October to November of the years 2015 to 2017. The captured birds were identified, sexed and aged when possible, ringed using a SAFRING aluminium ring, and morphological measurements were taken (head length, culmen length, wing length, tarsus length and tail length). Approximately 30 µl of blood was collected through brachial venipuncture using a capillary tube and placed in two tubes containing lysis buffer. For each bird caught, two blood smears were prepared, air-dried, fixed in

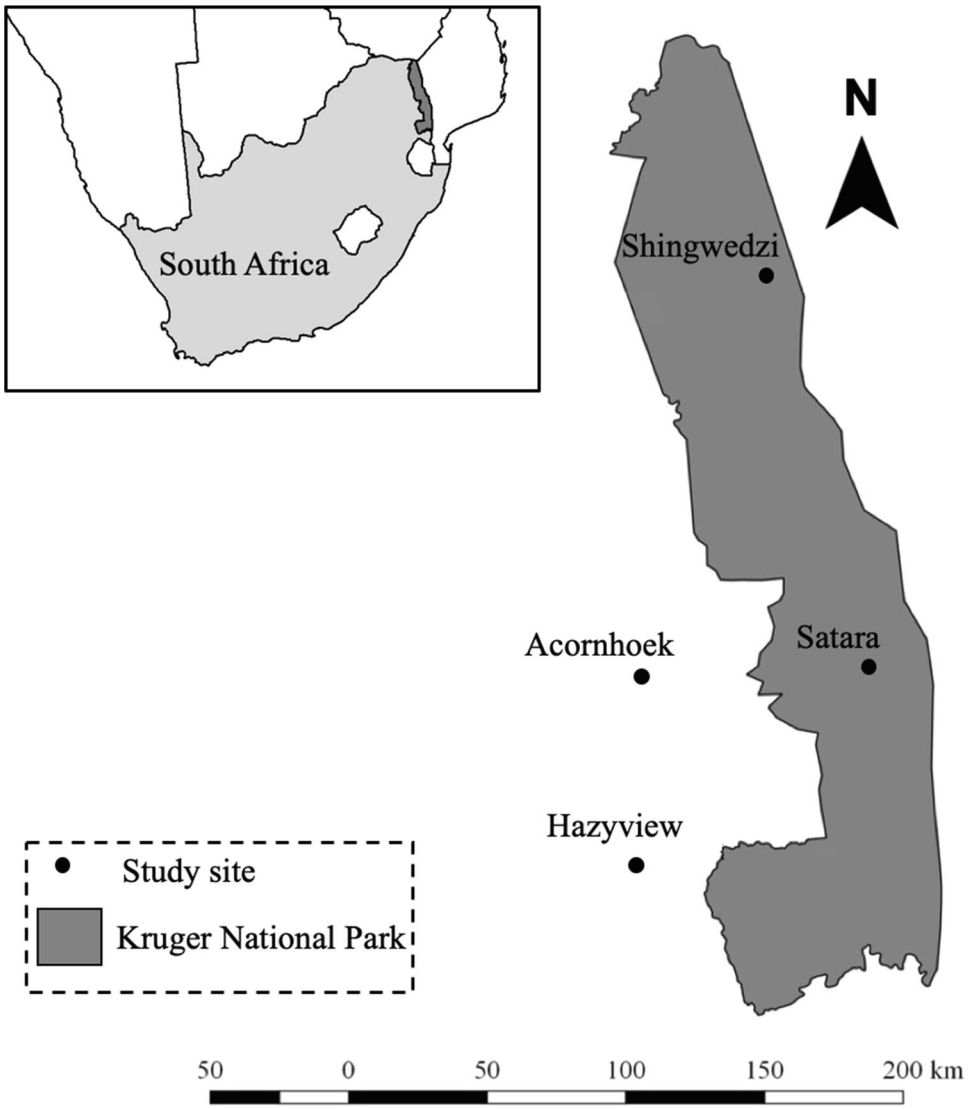

Fig. 1 Map of the study area showing sampling sites situated inside the Kruger National Park (Shingwedzi and Satara) and outside the park (Acornhoek and Hazyview)

absolute methanol and stained in a 10% working solution of Giemsa for microscopic observation. Blood smears were observed under an Olympus CX41 compound microscope (Olympus Optical, Japan) mounted with a digital camera at × 400 and × 1000 magnification, for avian haemosporidia. Observed parasites were identified at the genus level according to Valkiūnas (2004).

Molecular detection of blood parasites

Genomic DNA was extracted from the 104 sparrow blood samples using the Invisorb Spin Blood Mini Kit (Stratec Molecular, Berlin, Germany). The DNA elution stage was conducted twice: first with 200 µl DNA elution buffer and then with 50 µl. Niebuhr and Blasco-Costa (2016) observed that second eluates may contain a higher parasite to host DNA ratio. In this study, the second eluate of 50 µl was used for further analyses. The DNA extraction process did not include a control. After quantification of the DNA using the NanoDrop 2000 Spectrophotometer (ThermoFisher), the extracted DNA samples were diluted with TE buffer to a working concentration of approximately 25 ng/µl. The DNA working solutions were subjected to a sexing PCR protocol (Griffiths et al. 1998) to identify sexes and test DNA sample quality. A nested PCR protocol described by Hellgren et al. (2004) to screen for positive parasite infections was later conducted. The sexing PCR protocol amplifying introns of the CHD1Z and CHD1W genes was performed using the primers 0057F and 002R. This PCR reaction was performed with the GoTaq® G2 Flexi DNA Polymerase Kit (Promega Ltd., USA). Each reaction included 2 µl of 5X PCR buffer, 1.2 µl of MgCl2 (25 mM), 1 µl dNTP (1.25 mM), 0.4 µl of each primer (10 µM), 0.08 µl Taq polymerase, 3.42 µl double distilled water (ddH_2O) and 1.5 µl DNA template (25 ng/µl concentration), for a total reaction volume of 10 µl. The following cycling conditions were used: an initial denaturing

step at 94 °C for 1 min followed by a "touch-down" scheme for 10 cycles where the annealing temperature was lowered by 1 °C per cycle, starting from 60 to 50 °C, then 25 additional cycles at a constant annealing temperature of 50 °C. During each cycle, denaturation was at 94 °C for 30 s, annealing for 30 s and extension at 72 °C for 45 s. A final elongation was run at 72 °C for 10 min after the last cycle. A positive control (DNA template from a known sexed bird) and a negative control (double distilled water) were included for every 24 samples run.

The nested PCR protocols amplify a 479 bp fragment of the parasites' cytochrome *b* gene. The first amplification was carried using primers HaemNFI and HaemNR3. The total volume of the reaction was 25 µl and made use of the GoTaq® G2 Flexi DNA Polymerase Kit (Promega Ltd., USA). Each reaction well included 5 µl of 5X PCR buffer, 1.1 µl of MgCl2 (25 mM), 2.5 µl dNTP (1.25 mM), 1 µl of each primer (10 µM), 0.1 µl Taq polymerase, 12.3 µl double distilled water (ddH_2O) and 2 µl DNA template (25 ng/µl concentration). The following cycling conditions were used: 3 min of initialisation at 94 °C, followed by 20 cycles of denaturation at 94 °C for 30 s, 50 °C annealing for 30 s and 72 °C extension for 45 s and end with an elongation step at 72 °C for 10 min. The second nested PCR was carried out with 2.0 µl of the first PCR's product using the same reaction mix as above, with the identical thermal profile, except performed over 35 cycles instead of 20 cycles. The second PCR used the primer set HaemF and HaemR2 to amplify *Plasmodium* sp. and *Haemoproteus* sp. and primer set HaemFL and HaemR2L to amplify *Leucocytozoon* sp. A positive control (DNA template from a known infected bird) and a negative control (distilled water) were included for every 24 samples in a 96-well plate. The amplification products (1.5 µl) were run in 2% agarose gel stained with GelRed (Biotium, USA). The PCR amplification was repeated if the samples showed unclear bands. All positive PCR products were sent to Macrogen Inc. (Macrogen, Amsterdam, the Netherlands) for forward sequencing using primers HaemF for *Plasmodium* sp. and *Haemoproteus* sp. and primers HaemFL for *Leucocytozoon*. Due to shortage of funds, a selected number of PCR products were reverse sequenced using primer HaemR2 for *Plasmodium* sp. and *Haemoproteus* sp. and primer HaemR2L for *Leucocytozoon.*

Parasite sequences obtained from Macrogen Inc. were edited and aligned manually using BioEdit version 7.0.5.2 (Hall 1999). The sequence chromatograms were also visually inspected for "double peaks", and where these were observed, the nucleotide letter of the longest peak was assigned to the sequence. Final sequences were subjected to a Basic Local Alignment Search Tool (BLAST) search on MalAvi (Bensch et al. 2009) and GenBank databases to identify parasite lineages. The parasite sequences were assigned the MalAvi lineage name if identified as a 100% match to an existing lineage. All sequences that differed by one or more bases (< 100% identity) from known parasite lineages were identified as novel lineages (Hellgren et al. 2004). Novel lineages were given a unique lineage name according to the MalAvi nomenclature described by Bensch et al. (2009). New parasite lineages recovered in this study were deposited in GenBank (accession number, MW546958-65) and MalAvi databases.

Phylogenetic analyses

We conducted a phylogenetic analysis of unique parasite sequences (representing 16 lineages) identified in this study with the closest identified lineage obtained from MalAvi and GenBank databases. We also included previous lineages identified in house sparrows and grey-headed sparrows from the African continent as recorded in the MalAvi database. Thirty-four parasite lineages (13 *Haemoproteus* sp., 12 *Plasmodium* sp. and 9 *Leucocytozoon* sp.) were included in the analyses, and *Plasmodium falciparum* lineage from China (GenBank accession number AF069609.1) was used as outgroup to root the tree. The phylogenetic reconstruction for the three parasite genera was conducted using the maximum likelihood (ML) method. The best-fit DNA substitution model for ML was selected as general time reversible model with gamma distribution and evolutionary invariable sites (GTR + G + I) as determined by the model with the lowest Bayesian information criterion (BIC). The ML analysis made use of 1000 bootstrap replications, and all analyses were conducted using MEGA X (Kumar et al. 2018). The cut-off value of 50% was used for the final condensed tree.

Statistical analyses

Parasite infection prevalence was calculated as the proportion of infected birds in the population of interest (number infected divided by the total individuals sampled in the population) as detected by each screening method and also merged (a bird recorded as infected if it was positive by at least one of the methods). Parasite prevalence between bird species and parasite genera were compared using the chi-square test (a significance level of $p < 0.05$ was used). *Plasmodium* sp. was excluded from the analyses as it was recorded only once. Parasite richness was determined as the number of unique parasite lineages detected in each bird species, whilst parasite lineage diversity per host species was calculated by using Simpson's diversity index (expressed as D_L) in the *Vegan* package (Oksanen et al. 2019). All statistical analyses were conducted in R software (R Core Team 2015).

Results

Prevalence

A total of 74 house sparrows and 30 grey-headed sparrows were caught. All house sparrows were caught outside the Park, whilst grey-headed sparrows were caught inside the Park. Of the 104 birds, 37 were infected (35.57%) with at least one parasite genus. Prevalence measured through PCR screening differed significantly between bird species ($\chi^2 = 26.113$, $df = 1$ and $p < 0.0001$) but not between *Haemoproteus* and *Leucocytozoon* infections ($\chi^2 = 1.851$, $df = 1$ and $p = 0.174$). Eighteen house sparrows were infected (24.32% prevalence) against 18 grey-headed sparrows (60% prevalence; Table 1). *Leucocytozoon* was the most common infection (23.07%), whilst *Haemoproteus* recorded a 13.46% prevalence. Co-infections of *Plasmodium* and *Haemoproteus* could not be confirmed because both parasite genera were amplified using the same primer pair. There was only one record of *Plasmodium* in a grey-headed sparrow. Parasites screening through microscopy detected infections in sixteen house sparrows (21.6% prevalence) and twenty-one grey-headed sparrows (70% prevalence).

Diversity and phylogenetic relationship

Sixteen parasite lineages were identified, of which eight were novel lineages recorded for the first time. Among the identified lineages, nine belonged to the *Haemoproteus* genus, six belonged to *Leucocytozoon* and only one to *Plasmodium*. House sparrows were infected by seven parasite lineages, whilst grey-headed sparrows were infected by twelve lineages (Table 2). Three parasites lineages were recorded in both bird species (PAMEL01, RS4 and PASDIF03). Grey-headed sparrows recorded a higher parasite lineage diversity ($D_L = 0.88$) than house sparrows ($D_L = 0.51$). The most common parasite lineage recorded in both bird species was from *Leucocytozoon* sp. lineage RS4, recorded in 18 individual birds (13 house sparrows and five grey-headed sparrows). There were two novel parasite lineages in house sparrows (PADOM39 and PASDIF03) and seven in grey-headed sparrows (PASDIF01, PASDIF02, PASDIF03, PASDIF04, PASDIF05, PASDIF06 and PASDIF07). Although *Leucocytozoon* sp. was common, there was a higher lineage diversity among *Haemoproteus* sp. lineages.

The phylogenetic tree of parasite lineages identified and the ones from MalAvi included in the ML analyses based on the GTR + G + I model of evolution produced a clustering by parasite genera (Fig. 2) with strong support indicated by bootstrap values (86 to 93%). The *Leucocytozoon* clade (I) grouped all recorded parasite lineages into one large sub-clade (91% bootstrap) linked to *Leucocytozoon gentili*. The two newly described *Leucocytozoon* lineages (PASDIF02 and PASDIF03) differed from each other by three nucleotides and showed a similarity percentage of 99.79% and 99.78%, respectively, to *L. gentili*. It is however worthy to note the presence of two sub-clusters within this large *Leucocytozoon* sub-clade where lineage PASDIF03 aligned with the existing lineage AFR170 (69% bootstrap value) and the existing lineages RECOB3 and RS4 clustered together with 52% bootstrap value. The *Plasmodium* clade (II) revealed two sub-clades where the only *Plasmodium* parasite lineage recorded and newly described in this study (PASDIF04) aligned with lineages SYBOR10 and ERU-379P with 99%

Table 1 Prevalence of avian haemosporidian parasite infections in house sparrows and southern grey-headed sparrows

Species	*n*	Prevalence (%)	Parasite genera		
			H	*P*	*L*
Passer diffusus	30	60.00 (19/30)	13	1	9
Passer domesticus	74	24.32 (18/74)	3	0	16
Total	104	35.57 (37/104)	16	1	25

There was a significant difference in infection prevalence between bird species (χ^2 (1, $n = 104$) = 26.113, $p < 0.0001$)

Column headings: n = number of birds sampled; Prevalence = proportion of infected birds; *H* = *Haemoproteus*; *P* = *Plasmodium*; *L* = *Leucocytozoon*

Table 2 Parasite lineages detected in sparrow species. The number of birds infected by each lineage is shown

Parasite lineage	New/existing	*Passer diffusus*	*Passer domesticus*	Total
Haemoproteus				
AFR042	Existing	1	0	1
PAMEL01	Existing	4	1	5
PAMEL02	Existing	2	0	2
PASDIF01	New	3	0	3
PASDIF06	New	1	0	1
PASDIF05	New	1	0	1
PASDIF07	New	1	0	1
PADOM39	New	0	1	1
VILWE2	Existing	0	1	1
Leucocytozoon				
AFR164	Existing	0	1	1
AFR173	Existing	1	0	1
PASDIF02	New	2	0	2
PASDIF03	New	1	1	2
REB7	Existing	0	1	1
RS4	Existing	5	13	18
Plasmodium				
PASDIF04	New	1	0	1

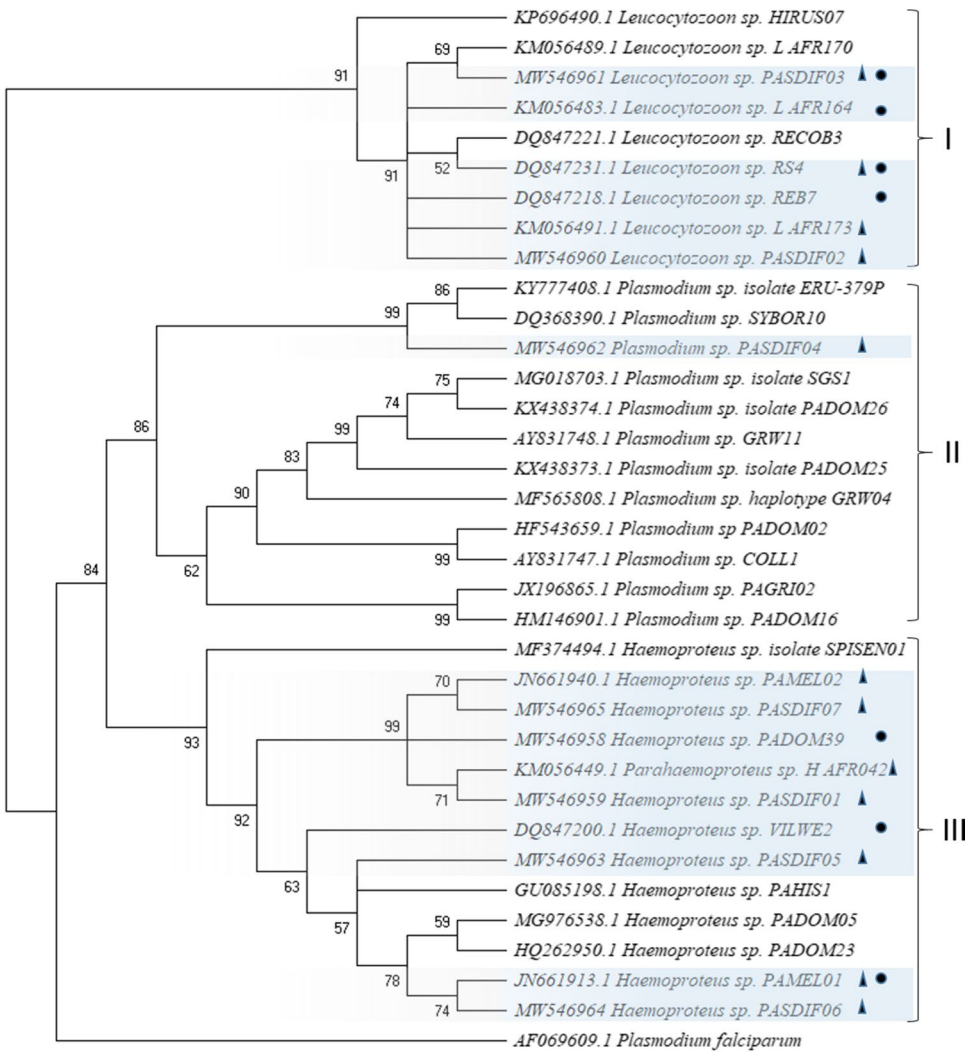

Fig. 2 Maximum likelihood phylogenetic tree of lineages found in this study (indicated by markers and shaded in light blue) and reference lineages (indicated by GenBank accession numbers), with bootstrap values (> 50%) displayed. Black triangle indicates lineages found in southern grey-headed sparrows, and black circle indicates lineages found in house sparrows from this study. I, II and III represent clusters

bootstrap value owing to only three nucleotides difference in their sequences. The remaining *Plasmodium* sp. lineages recorded in other studies are grouped in a large cluster with 62% bootstrap value. The third clade (III) revealed the presence of two sub-clades with 99% and 63% bootstrap values. The sub-clade with 99% bootstrap values grouped five parasite lineages recorded in this study among which three are novel (PADOM39, PASDIF05 and PASDIF07). This sub-clade revealed up to 98.95% similarity to *Haemoproteus homobelopolskyi*. In the second sub-clade, the remaining four lineages are recorded in this study and included two novel lineages (PASDIF05 and PASDIF06), with close similarity to *Parahaemoproteus passeris*. It is worthy to note here the presence of a sub-cluster that included PADOM05, PADOM23, PAMEL01 and PASDIF06 that grouped together owing to a 78% bootstrap value. The differences in relative base pair numbers observed between parasite lineages in the *Haemoproteus* clade were higher than those observed in the *Plasmodium* and *Leucocytozoon* clades indicative of a high lineage diversity.

Discussion

On the African continent, this is the first study reporting on the prevalence of avian haemosporidian infections in the native grey-headed sparrow and the invasive house sparrow. This study made use of both microscopy and molecular screening methods, to decipher the diversity of the infections in both bird hosts and make inferences on the role of the invasive in the spread of avian haemosporidian infections. This study took place in the Lowveld region of South Africa, and the host species were sampled inside the Kruger National Park and in residential areas outside the Park. Our findings revealed that the native grey-headed sparrows had a higher prevalence of infection by haemosporidians than the invasive house sparrows. Grey-headed sparrows also recorded the highest diversity of avian haemosporidians, and *Leucocytozoon* sp. infections were the most frequent among both bird species, whilst *Haemoproteus* sp. recorded a higher diversity among parasite lineages.

House sparrows have been the subject of numerous studies related to the prevalence and phylogenetic diversity of haemosporidian parasites around the world mainly because of their wide distribution and role as invaders in multiple regions of the world (Antonini et al. 2019; Dadam et al. 2019; Marzal et al. 2018; Santiago-Alarcon et al. 2020). With the exception of Beadell et al. (2009), this is the first study to examine the prevalence of avian haemosporidian parasites in grey-headed sparrows at this scale (although our sample size is limited) in southern Africa, as these birds are mainly restricted to this region. This study is even more important as the two bird species studied here have similar habitat requirements and preferences and with overlapping distribution within the Afrotropical region. Furthermore, the house sparrow is suspected to displace native bird species around urban areas by their increasing numbers and competition for resources (Sheldon and Griffith 2017; Shochat et al. 2010). In this study, house sparrows were mainly sampled in sites outside the park, whilst grey-headed sparrows were mainly found in sites within the Park. The observed species distribution pattern, however, is not necessarily due to exclusion of one species by the other but could be driven by habitat preference which may drive bird host abundance at different sites. Grey-headed sparrows, although frequently found near settlements and plantations like the house sparrows, have a preference for woodlands, where house sparrows are less common (Hockey et al. 2005). House sparrows, on the other hand, are urban exploiters that are highly dependent on urban environments for food, shelter, reproduction and survival, although their numbers have been observed to decline in polluted cities throughout the world (Dadam et al. 2019; Herrera-Dueñas et al. 2017). This difference in niche exploitation and preference may have been the cause of the difference observed in the distribution of the two species. House sparrows, by their abundance and anthropo-dependent habits, may be more easily encountered during trapping sessions outside natural areas, whilst grey-headed sparrows have a preference for natural and semi-natural environments.

The enemy release hypothesis (ERH) posits that non-indigenous species in their introduced range should exhibit lower parasite prevalence than in their native range (Antonini et al. 2019). This hypothesis further predicts that introduced species exhibit higher parasite prevalence in their habitat of origin than in their introduced range. In essence, it suggests that invasive species may lose their parasites of origin during or after introduction into a new habitat and get infected with parasites found in their new habitat. As a consequence, these species may gain an added advantage over endemic species which may explain their ability to successfully colonise new habitats. As predicted in this study, the observed avian haemosporidia prevalence of 24.32% in house sparrows in the Lowveld region of South Africa is lower than that observed in their native European range by Marzal et al. (2011) and Antonini et al. (2019). House sparrows in their native range consistently exhibit higher prevalence than the one observed in this study ($\chi^2 = 55.423$, $df = 3$ and $p < 0{,}0001$). Antonini et al. (2019) recorded a total haemosporidia prevalence of 47% in Portugal whilst Marzal et al. (2011) recorded a total prevalence of 49% in European regions (SW Europe, SE Europe and N Europe). Marzal et al. (2018) also recorded a prevalence of 36.23% in house sparrows from Spain, Dadam et al. (2019) recorded a 74% prevalence in *Plasmodium relictum* in London (UK), and between 35 and 40% prevalence was recorded in southern France by Bichet et al. 2020. A similar tendency of lower prevalence was observed in other introduced bird species in Australia and New Zealand (Clark et al. 2015; Sijbranda et al. 2016). These results are in line with our prediction and those of the ERH. It is therefore evident that introduced passerine birds generally exhibit a lower avian haemosporidian parasites prevalence, thus acquiring a fitness advantage in their new habitat. In further support of the ERH, none of the parasite lineages infecting house sparrows in Europe was recovered in this study.

It is noteworthy to mention that numerous studies also found that climatic conditions, seasonality and locational disparities can affect the prevalence of avian haemosporidia (Bichet et al. 2020; Cadena-Ortiz et al. 2019; Hernandez-Lara et al. 2017). In this study, however, given the limited sample size and geographical proximity of the study sites, possible disparities associated with location and seasonality were not explored further. It is, however, noted that the local bird community would be more diverse inside the park than outside, potentially providing more opportunities for the grey-headed sparrows (recorded only inside Park) to be infected by a more diverse parasite community than the house sparrows (recorded solely outside the park). Large-scale sampling may provide clarity in the role of localities (inside versus outside the park), but climate is not expected to affect prevalence in this region as van Wilgen et al. (2016) found no significant difference in long-term climatic pattern in the region.

In this study, we found as much novel parasite lineages as previously described lineages from the MalAvi database. It was, however, noted that seven out of the eight novel parasite lineages were recorded on the grey-headed sparrow, despite the limited sample size (n = 30). Among the parasite lineages recorded on the grey-headed sparrow, only four were previously described in the MalAvi database. This result suggests that a larger number of novel lineages should be expected with a larger sample size. Outlaw et al. (2016) suggest that more intensive and systematic sampling in tropical sub-Saharan Africa will yield not only higher parasite diversity but high prevalence and high levels of lineage endemism. The grey-headed sparrow remains poorly sampled in relation to avian haemosporidian parasites. Broader sampling into

different habitats and broader climatic regions within the Southern African region is expected to yield greater parasite diversity among and within taxa.

The MalAvi database (accessed on 26th February 2020) (Bensch et al. 2009) has the record of only six grey-headed sparrows sampled from Botswana infected with the same parasite lineage PAMEL01 (Beadell et al. 2009; Ishtiaq et al. 2012). This lineage was recorded in both house sparrows and grey-headed sparrows in this study. It is the first record of the PAMEL01 lineage infection in house sparrows. The most common parasite lineage recorded in this study was RS4 (*Leucocytozoon* sp.; GenBank accession number: DQ847231.1). This lineage was previously recorded in five migratory bird species in Europe (Spain, Sweden and Serbia) wintering in Africa and two resident bird species on the island of Madagascar (Hellgren et al. 2007; Ivanova et al. 2018; Rojo et al. 2014). It's been suggested that migratory birds moving between breeding and wintering sites may encounter and spread a great variety of parasites during migration (Ciloglu et al. 2020). This is the first record of RS4 lineage in both sparrow bird species from this study. This lineage was first recorded in the common redstart (*Phoenicurus phoenicurus*) and the Eurasian blackcap (*Sylvia atricapilla*) in Spain and Sweden, respectively, both birds wintering in Africa. This finding may suggest the possibility of pathogen spill-over of the RS4 lineage, a European strain to African birds. This does not necessarily support the novel weapon hypothesis (NWH), which proposes that introduced species possess pathogenic parasites against which native species have not evolved defences (Prenter et al. 2004). In essence, *Leucocytozoon* lineages AFR164, AFR173 and REB7 which were detected for the first time in house sparrows and grey-headed sparrows in this study had previously been recorded in resident bird species on the African continent only (Hellgren et al. 2007; Loiseau et al. 2010; Lutz et al. 2015). Although *Leucocytozoon* is not considered as the most pathogenic genus of the protozoan blood parasites of birds, the pathogenicity of the lineage RS4 (identified to be similar to *L. gentili*) has not been tested here. Such studies are difficult to assess in the wild since sick or dying birds fall victim to predators and carcasses are scarcely detected or recovered for post-mortem analyses.

The second most common infection lineage, PAMEL01 (*Haemoproteus* sp.; GenBank accession number, JN661913.1), was previously recorded in three bird species (grey-headed sparrow *Passer diffusus*, Cape sparrow *Passer melanurus* and lesser striped swallow *Cecropis abyssinica*) on the African continent, in Botswana (Ishtiaq et al. 2012) and South Africa (Okanga et al. 2014). Another lineage that was recorded in this study and was previously recorded on the African continent is AFR042 (*Parahaemoproteus* sp.; GenBank accession number, KM056449.1). These lineages seem to have a narrow range of hosts and were recorded in the yellow-throated bush sparrow (*Gymnoris superciliaris*) in Malawi (Lutz et al. 2015) and are suspected to be resident lineages on the African continent.

The single *Plasmodium* sp. lineage (PASDIF04) recorded in this study was a new parasite lineage recorded in the grey-headed sparrow in a co-infection with a novel *Leucocytozoon* sp. (PASDIF03). PASDIF04 aligned with SYBOR10 which was previously recorded in seven bird species across Africa (two species), Asia (two species) and Europe (three species) none of which were sparrow species. Lineage AFR170 (which aligned with PASDIF03) was recorded in only one bird species, the baglafecht weaver (*Ploceus baglafecht*), in Malawi. Host specificity studies report *Plasmodium* to be mainly generalist (Bensch et al. 2009) although several studies have shown certain *Plasmodium* lineages to be host-specific (Garcia-Longoria et al. 2019; Musa et al. 2019). We suspect that both PASDIF03 and PASDIF04 to be host-specific.

It is possible that the nested PCR method might impede the detection of multiple distinct co-amplified genetic variants or mixed infections, meaning that haemosporidian co-infections may have been missed, particularly when one haemosporidian specie was present at a lower abundance than the other. We recommend a multiplex PCR protocol (e.g. Ciloglu et al. 2019) or a whole genome amplification (Videvall 2019) to elucidate and confirm co-infections by multiple lineages of the same parasite genera.

In conclusion, we found that the prevalence and diversity of avian haemosporidian parasites were markedly higher in grey-headed sparrows than in house sparrows. The most prevalent infection in both bird species was from *Leucocytozoon* sp. with lineage RS4 infecting more bird species, whilst *Haemoproteus* sp. recorded the highest diversity. House sparrows were mainly infected with local parasite lineages, whilst the large majority of novel parasite lineages were detected on the grey-headed sparrows. Only one *Plasmodium* sp. infection was observed. It was evident in this study that house sparrows and grey-headed sparrows did not use the same habitat despite having similar habitat requirements. The results from this study showed partial support for the enemy release hypothesis as native grey-headed sparrows recorded higher parasite prevalence than the invasive house sparrows. These findings contribute to improving our understanding of the relationship between the parasite and the host in relation to native and alien invasive bird species in the Afro-tropics and provide baseline data to build upon.

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Data availability Data for this study is freely available from the South African Foundational Biodiversity Information Programme (FBIP) repository.

Declarations

Ethics approval 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 clearances were delivered by the University of the Free State to M Ndlovu (UFS-AED2017/0004) and MB Wardjomto (UFS-AED2018/0012). Permission to conduct research in terms of Sect. 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). The Bushbuckridge Municipality in Mpumalanga province granted access to sampling sites outside the Park.

Conflict of interest The authors declare no competing interests.

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