Prevalence and Diversity of Avian Haemosporidians May Vary with Anthropogenic Disturbance in Tropical Habitats in Myanmar

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1. Introduction

Predicting and mapping the distribution, abundance, and diversity of species is fundamental for resource management and biodiversity conservation planning [1]. This is
particularly important in the current global change scenario, where processes such as climate change and land-use changes are globally impairing wildlife populations [2,3]. Biotic and abiotic factors are recognized as drivers in limiting species distributions. For example, interspecific interactions, as well as habitat availability and heterogeneity, have been proposed as the main determinants shaping the abundance and distribution of species [4,5]. However, most of the studies on interactions between organisms only include competition and predation as the major factors driving species diversity, whereas parasites have been comparatively poorly investigated [4]. This is particularly remarkable, given the abundance, ubiquity, and extraordinary diversity of parasites [6,7], and also because parasites might affect animal diversity in a similar way to predators [8]. Because host–parasite interactions represent a selective pressure maintaining genetic variability in host populations, characterizing the structure of parasite communities is crucial to understanding ecosystem diversity and functioning [9–11].

Avian malaria and related haemosporidians (genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) are widespread, abundant, and diverse apicomplexan parasites, with more than 4000 parasite lineages infecting most avian clades [12,13]. The life cycle of haemosporidian parasites is complex, including stages that occur within blood-sucking dipteran vectors and stages in tissues and circulating blood cells of vertebrate hosts [14]. These blood parasites exert pathogenic effects on their avian host by provoking tissue damage [15], diminishing survival [16–18], adversely affecting body condition [19,20], and reducing reproductive success [21,22]. These costs of haemosporidian infection could decimate host populations and be responsible for large declines or even extinctions of naïve populations after parasite introduction beyond their natural range [23,24].

Although avian haemosporidians are present in almost all geographical regions, these parasites have been irregularly studied across different biogeographical regions [13], and some host families have received less attention [25,26]. Whilst the number of molecular studies on avian haemosporidians has significantly increased in the last 20 years, some biogeographical areas, such as tropical regions, remain undersampled [27]. Because of its biodiversity-rich ecosystems and its high concentration of endemic species, Myanmar is recognized as a world biodiversity hotspot [28,29]. Moreover, Myanmar exhibits a great level of avian diversity, where new bird species are continuously being described [30,31]. Because of their high degree of endemism and/or their risk of extinction, many of these bird species are considered a priority in conservation policies [32,33]. However, notwithstanding the huge number of bird species present in this tropical country, very few studies have explored the genetic diversity of its bird haemosporidian parasites (see [34–36] for some exceptions), suggesting that a large part of the Myanmar haemosporidian diversity remains unexplored.

Anthropogenic activities such as deforestation, habitat fragmentation, and land use changes are major threats to tropical biodiversity [37,38]. These ecological changes may provoke alterations in temperature and microclimate conditions disturbing insect vector populations, thus, potentially affecting vector-borne disease epidemiology [39,40]. In this sense, several studies have explored the effects of habitat anthropogenic changes (i.e., habitat fragmentation and deforestation, urbanization, and agriculture intensification) in the abundance and diversity of avian haemosporidians, showing that these human-driven alterations have a profound impact on the overall composition of haemosporidian communities [41–46]. Myanmar exhibits one of the highest forest covers in mainland Southeast Asia [47]. However, these tropical forests are currently threatened by forest loss due to activities such as logging and cropland expansion [48–50], leading to biodiversity loss and changes in bird and vector species composition that could affect host–parasite interactions [51,52].

Here, we present a molecular-based study to explore infections by haemosporidian parasites in bird species from Myanmar. We screened more than 125 bird individuals belonging to 32 bird species (about 3.15% of the overall Myanmar bird diversity) from three Myanmar districts (Mawlamyine, Myeik, and Mandalay) to explore host–parasite assemblages and
to determine differences in prevalence and diversity of parasite lineages across areas. We also analyzed whether haemosporidian prevalence and diversity vary along a gradient of anthropogenic disturbance characterized by different land-use types (urban/suburban area, paddy field, and hill). We predicted that urban environments [53,54] and anthropized areas with greater availability of water sources (e.g., paddy fields) [37,55,56] could host birds with a high prevalence and diversity of parasites. Given the threats to tropical forests, the identification of avian haemosporidian diversity and its relationship with land-use change will provide essential knowledge for a better comprehension of host–parasite associations and biodiversity conservation in Myanmar.

2. Materials and Methods

2.1. Study Sites and Field Sampling of Birds

The study was carried out in three localities belonging to two different ecoregions [57,58]: Myeik and Mawlamyine (sampled in March 2019), within the Myanmar coastal rain forests (Eco ID: 250), and Mandalay (sampled in July 2019), located within the Ayeyarwady (also known as Irrawaddy) moist deciduous forests (Eco ID: 235). Both ecoregions belong to tropical and subtropical moist broadleaf forest biomes. Myanmar experiences a tropical monsoon climate with three seasons: cool, relatively dry northeast monsoon (late October to mid-February), the hot, dry inter-monsoonal season (mid-February to mid-May), and the rainy southwest monsoon (mid-May to late October) [59].

In each locality (Myeik, Mawlamyine, and Mandalay), we performed bird captures on four selected areas with different anthropogenic disturbance (downtown, university campus, paddy fields, and hill) (Table S1, Figure 1). The degree of anthropogenic disturbance was characterized based on differences in housing density (see classification in Supplementary Materials [60]). Downtown (>70% impervious surface) was the most anthropized area, followed by the university campus (moderately developed: 30–40%), the hill area (sparsely developed: <30%), and the paddy fields (rural agricultural areas), which was the area with the least human disturbance in the four-class system. Due to the difficulty of capturing birds in the downtown (for example, in Myeik and Mawlamyine only one individual was captured in this area), we decided to pool the samples obtained in the downtown and the university campus in a new category (urban area) since they were very close to each other (less than 6 km of distance) and their level of urban development was greater than 30–40%. Thus, the statistical analyses related to area comparisons were made based on three land-use types: urban area, paddy fields, and hill area. We performed one capture session per area on each locality. On every capture session, four 9 m long and three 6 m long mist nets (16 mm gauge, 2.6 m height) were set up before dawn. Nets were opened from 06:00 a.m to 11:00 a.m. Mist nets were checked at least every 40 min to avoid birds injuring themselves and to protect them from high temperatures and predators. This work abided by the guidelines on ethical standards of the Ministry of Higher Education in Myanmar and the ministry endorsed the study and granted permissions. All samples were taken in accordance with national Myanmar law and the animal protection laws of the EU (directive 2010/63/EU of the European Parliament). Methods were approved by the Research Ethics and Animal Welfare Committee on Animal Experimentation of the University of Extremadura (reference 101/2020).
Figure 1. Map of Myanmar depicting the sampling localities (Mandalay, Mawlamyine, and Myeik) and the four areas under different anthropogenic disturbance where the mist-nets were set up for capturing birds (blue marks: downtown, pink: university campus, yellow: paddy fields, and green: hill).

2.2. Blood Sampling

A small blood volume (ca. 30–40 µL) was collected from the bird’s jugular vein using a 0.5 mL insulin syringe with a 31-gauge ultrafine needle (Insumed 31G Insulin Syringe 31G × 8 mm; Picsolution, Artsana, Grandate, Italy). Blood samples were immediately added to 500 µL of SET buffer (0.015 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH 8.0) in Eppendorf tubes, and stored at 4 °C until DNA extraction in the lab. An additional drop of blood was smeared on one individually marked microscope slide. Blood samples from smears were air-dried, fixed by 3 min immersion in 100% methanol, and stained using commercial Giemsa diluted with PBS pH 7 (1:2). Slides were examined under the microscope (Motic BA310, Barcelona, Spain) for 10–15 min at low magnification (×400), and then at least 100 fields were studied at high magnification using the oil immersion objective (×1000). Blood smears from birds captured in Myeik were spoiled due to the use of an inappropriate reagent for fixation. Only those fixed with 100% methanol in Mawlamyine and Mandalay had good quality to be observed under the light microscope.

2.3. Molecular Parasite Screening

Genomic DNA was extracted from all blood samples collected in this study using GeneJET™ Genomic DNA Purification Kit (Thermo Scientific Inc., reference #K0722) according to the manufacturer’s instructions. Haemosporidian infections were detected from blood samples using molecular methods [61]. The obtained sequences of 478 bp of the cyt b were edited, aligned and compared in a sequence identity matrix by BLAST (Basic Local Alignment Search Tool) implemented in MalAvi database (version 2.4.7, 6 October 2020, [12] to identify parasite lineage. New lineages (sequences not previously published in GenBank) were also sequenced from the reverse end using the primer HaemR2. Parasites with sequences differing by one nucleotide substitution were considered to represent evolutionary independent lineages [12,62]. New lineages were coded following the nomenclature of the MalAvi database [12] and deposited in GenBank under the accession numbers MW351708—MW351710 (Table 1).
2.4. Phylogenetic Analyses

The genetic relationship between the parasites was investigated by analyzing the avian haemosporidian cytochrome b sequence divergence using a maximum likelihood method with 1000 bootstrap replicates in the program Geneious version 6.1.6 [63]. The phylogenetic tree was rooted with a sequence from Leucocytozoon schoutedeni as the outgroup (GenBank # KM056646) [64]. We used a relative substitution rate model in order to define the rate at which each of the transitions and transversions occur in an evolving sequence. The phylogenetic tree was edited by using R language v. 3.5.3 [65] and libraries ggplot [66] and ggtree [67].

2.5. Statistical Procedure

Statistical analyses were performed in the R language v. 3.5.3 [65], and the significance level was set at $\alpha = 0.05$ for all tests; marginally significant results ($0.05 < p < 0.1$) were also indicated. All calculations about parasite lineage and avian diversity indices (richness, abundance, rarefaction, Shannon–Wiener index, evenness and effective diversity) were performed using the vegan package [68]. We calculated richness and abundance for both parasite lineages and avian species by locality and sampling areas with different anthropogenic disturbance. Parasite lineage and bird species richness refers to the total number of lineages or species in each locality and sampling area considered, whereas abundance refers to the number of individuals (see [69]). Since lineage and species richness are highly correlated with sample size, we used rarefaction [70] to estimate the number of species expected from a sample in a sub-sample of a given number of individuals. Thus, the effect of differential sampling intensity on diversity estimates is reduced. The “rarefy” function was used to compute rarefied species diversity and the standard error for each sampling area. We used counts for each parasite lineage as individual-based abundance data regardless of host species identity, which should result in a conservative estimate of species richness. The Shannon–Wiener index was calculated to estimate the “equitability” or “evenness” of lineage and species abundances for each of our sampling zones. We used Pielou’s evenness to compare the actual diversity value (such as the Shannon–Wiener Index) to the maximum possible diversity value. In this way, we assessed the homogeneity or evenness of a community based on the abundances of its species, which may vary among communities in ways that correlate with biotic or abiotic differences (see [69]). Finally, since not all host-taxa had equal abundance of individuals, we calculated the effective (true) diversity, which refers to the number of equally abundant types needed for the average proportional abundance of the types to equal that observed in our dataset [71]. Unfortunately, we were unable to sequence the amplified parasite for the Ayeyarwady bulbul (Pycnonotus blanfordi), due to technical problems. Therefore, this sample was not considered in the diversity analysis of parasite lineages, but it was included in those related to prevalence. Additionally, we used generalized linear models (GLMs) (Poisson distribution) to analyze if the effective diversity of parasite lineage was affected by the avian species effective diversity found in each locality and different anthropogenic disturbance areas. In these models, we used avian species diversity as an “offset” to control for its effects on parasite lineage diversity.

The map of Myanmar, showing the sampling localities and areas (Figure 1), was generated from the exact geographical coordinates (see Supplementary Table S1) using ggspatial, maturalearth, and ggplot packages [66]. Then, specific maps for each location were produced with ggmap [72] and leaflet [73] packages. The cross-sectional analysis to compare the infection status between different localities were modelled using binomial generalized linear models (GLMs) with a logit link function using the package MASS [74]. Tukey’s multiple comparisons were carried out using the function “glht” in multcomp package. Analyses to compare the infection status between different land-use types were modelled using binomial generalized linear mixed models (GLMMs) with a logit link function using the package MASS [74], where locality was defined as a random effect.
| L       | A     | Bird Species | N (Total/Infected) | Genus | Lineage | GenBank Acc. N | Alt. Host | Alt. Locat. | Reference |
|---------|-------|--------------|--------------------|-------|---------|----------------|----------|-------------|-----------|
| MANDALAY |
| H       |       | Acridotheres burmannicus * | 2/1 | H | AFR084 $ | KM056470 | Gracupica contra | India [75] |
|         |       | Argya gularis | 2/2 | H | TURSTR02 $ | MF565817 | Argya striata | India [75] |
|         |       | Aegithina tiphia | 2/0 | - | - | - | - | - |
|         |       | Botaurus stellaris | 1/1 | P | IXOMIN02 | KU752579 | Japan [76] |
|         |       | Cisticola juncidis | 1/0 | - | - | - | - | - |
| PF      |       | Merops orientalis | 2/1 | H | MERORI02 $ | MW351709 |            |           |
|         |       | Ploceus hypoxanthus * | 1/1 | H | PLOHYP01 $ | MW351710 |            |           |
|         |       | Pycnonotus blanfordi | 2/0 | - | - | - | - | - |
|         |       | Pycnonotus cafer | 2/0 | - | - | - | - | - |
|         |       | Cinnaris asiaticus | 1/0 | - | - | - | - | - |
|         |       | Passer domesticus | 17/0 | - | - | - | - | - |
| U       |       | Pycnonotus blanfordi | 1/0 | - | - | - | - | - |
|         |       | Pycnonotus cafer | 1/0 | - | - | - | - | - |
|         |       | Argya gularis * | 3/3 | H | TURSTR02 $ | MF565817 | Argya striata | India [75] |
| MAWLAMYINE |
| H       |       | Copysychus malabaricus | 1/0 | - | - | - | - | - |
|         |       | Phylloscopus fuscatus | 1/0 | - | - | - | - | - |
|         |       | Pycnonotus jocosus | 1/0 | - | - | - | - | - |
|         |       | Acrocephalus aedon | 1/1 | H | ACAED03 $ | MW351708 |            | Myanmar [34,35] |
|         |       | Aegithina tiphia | 1/1 | P | AEGTIP01 | DQ659581 | Dicrurus leucophaeus |            |
| PF      |       | Orthotomus sutorius | 1/0 | - | - | - | - | - |
|         |       | Phylloscopus fuscatus | 1/0 | - | - | - | - | - |
|         |       | Pycnonotus blanfordi * | 2/1 | - | - | - | - | - |
|         |       | Copysychus saularis | 1/0 | - | - | - | - | - |
| U       |       | Dicrurus macrocercus | 1/0 | - | - | - | - | - |
|         |       | Lanius cristatus | 2/0 | - | - | - | - | - |
|         |       | Passer montanus | 12/0 | - | - | - | - | - |
| L | A  | Bird Species                  | N (Total/Infected) | Genus | Lineage | GenBank Acc. N | Alt. Host | Alt. Locat. | Reference |
|---|-----|-------------------------------|-------------------|-------|---------|----------------|-----------|-------------|-----------|
| MYEIK | H   | Alcedo atthis                 | 1/0               | -     | -       | -              | -         | -           | -         |
|     |     | Hirundo tahitica              | 1/0               | -     | -       | -              | -         | -           | -         |
|     |     | Lanius cristatus              | 1/0               | -     | -       | -              | -         | -           | -         |
|     |     | Lonchura punctulata           | 8/0               | -     | -       | -              | -         | -           | -         |
|     |     | Lonchura striata              | 14/0              | -     | -       | -              | -         | -           | -         |
|     |     | Passer montanus               | 10/0              | -     | -       | -              | -         | -           | -         |
|     |     | Acrocephalus sp.              | 2/0               | -     | -       | -              | -         | -           | -         |
|     |     | Cisticola juncidis            | 1/0               | -     | -       | -              | -         | -           | -         |
|     |     | Ficedula albicilla *          | 1/1               | H     | FIPAR02 $\dagger$ | EF380197 | Ficedula parva | Myanmar  | [35]       |
|     |     | Passer montanus               | 2/0               | -     | -       | -              | -         | -           | -         |
|     |     | Acridotheres javanicus        | 1/0               | -     | -       | -              | -         | -           | -         |
|     |     | Acridotheres tristis          | 12/1              | P     | FANTAIL01 | AY714196 | -              | Singapore, | [77,78]    |
|     | U   | Columba livia                  | 1/1               | H     | HAECOL1  | AF495554       | -         | India       | [79]      |
|     |     | Dicrurus macrocercus          | 6/0               | -     | -       | -              | -         | -           | -         |
|     |     | Halcyon smyrnensis            | 1/0               | -     | -       | -              | -         | -           | -         |
|     |     | Pycnonotus finlaysoni *       | 1/1               | P     | ORW1 $\dagger$ | AF254963 | Ficedula parva, Jynx torquilla, Tephrodornis pondicerianus | Myanmar  | [35]       |

$\dagger$: Indicates additional information or notes related to the reference.
3. Results

3.1. Prevalence of Haemosporidian Infection

We screened a total of 127 individuals belonging to 32 bird species from Myanmar in the search for haemosporidian parasites. Sixteen out of the 127 individuals were infected with haemosporidians (overall prevalence = 12.59%). All PCR-positive samples for haemosporidian infections showed evidence of gametocytes in their blood smears (with the exception of the four birds from Myeik, which did not have suitable blood smears). Results from cross-sectional analyses predicting infection status based on the distribution of birds by sampling localities showed significant differences (chi-square test: $\chi^2 = 6.31$, df = 2, $p = 0.043$). Post hoc analysis showed that birds captured in Mandalay had higher haemosporidian prevalence (23.7%) than birds from Myeik (6.3%; $p = 0.043$), but none of these localities had significant differences with birds from Mawlamyine (12%) (see Figure 2 and Table S2 in the Supplementary Material). Similarly, results from cross-sectional analyses predicting infection status based on the distribution of birds by sampling areas showed that there was a marginally significant difference ($\chi^2 = 5.54$, df = 2, $p = 0.062$). Post hoc analysis revealed that communities of birds inhabiting paddy fields had marginally significant higher haemosporidian prevalence (29.2%) than birds from urban areas (9.8%; $p = 0.081$) and those from the hills (7.1%; $p = 0.064$) (Figure 2 and Table S2 in the Supplementary Material). However, no comparison showed a significant variation.

![Figure 2. Bar chart showing the avian haemosporidian prevalence between localities and land-use types. Tukey post-hoc tests were utilized to determine significance among localities or land-use types (ns = not significant, ms = marginally significant ($0.05 < p < 0.1$), * = $p < 0.05$).](image)

Of the 16 birds that tested positive, 75% were infected with *Haemoproteus* (subgenus *Parahaemoproteus*) and 25% were infected with *Plasmodium*. No individual was infected with *Leucocytozoon* (Table 1, Figure 3). In Mandalay, four individuals were infected with *Haemoproteus* and one individual was infected with *Plasmodium*. In Mawlamyine, one individual was infected with *Plasmodium* and one individual was infected with *Haemoproteus* spp. In Myeik, two individuals were infected with *Plasmodium* spp. and two individuals were infected with *Haemoproteus* (Table 1, Figure 3). *Haemoproteus* was the only genera found among infected birds in hills (three infected birds). In paddy fields and urban areas, we found similar results in the number of infected individuals in relation to haemosporidian genera (four birds infected with *Haemoproteus* and two birds harbouring a *Plasmodium* infection) (Table 1, Figure 3).
3.2. Estimates of Parasite Lineage and Bird Diversity

By analyzing genetic diversity of haemosporidian parasites, we identified four unique lineages of *Plasmodium* and seven of *Haemoproteus*. Three out of seven *Haemoproteus* lineages had not been previously recorded (ACAED03, MERORI02 and PLOHYP01) (Table 1, Figure 3). Moreover, we found that five out of 12 infected bird species analyzed in this study had not been previously documented as positive for haemosporidian parasites in molecular studies. Two of these bird species documented for the first time as infected with haemosporidian parasites are endemic species, such as the white-throated babbler (*Argya gularis*, Figure 4A) and the Ayeyarwady bulbul (*Pycnonotus blanfordi*). Multiple infections, identified by double peaks in sequence chromatograms, were not present in this study.

Figure 3. Consensus phylogenetic tree from haemosporidian lineages previously detected in Myanmar [34,35] (no colored dots), and those detected in this study for Mandalay (green dots), Mawlamyine (orange dots) and Myeik (purple dots). Node tips are labelled with abbreviation for parasite genus (*H_* = *Haemoproteus*, and *P_* = *Plasmodium*), followed by the lineage name. *Leucocytozoon schoutedeni* (*L_GALLUS06*, GenBank KM056646) was used as an out-group. New lineages (sequences not previously published in GenBank) are indicated in bold.

Figure 4. (A) White-throated babbler (*A. gularis*) photographed in Mandalay hill, and (B) two macrogametocytes of lineage *H_TURSTR02* (arrows), observed in a white-throated babbler’s Giemsa-stained blood smear using oil immersion objective lens (scale bar 10 µm). Picture credits: Jaime Muriel (A) and Sergio Magallanes (B).
We analyzed several diversity indexes among different localities (Table 2, Figure 5). We found a similar pattern in Shannon–Wiener and effective diversity indexes for both parasite lineages and bird species, with Myeik representing the locality showing the highest diversity values, whereas Mandalay and Mawlamyine showed similar values (Table 2, Figure 5). Regarding the homogeneity of parasite lineages per localities, Mawlamyine showed the highest evenness values, indicating that this locality was more homogeneous than Mandalay or Myeik (Table 2, Figure 5). Concerning the homogeneity of bird communities, Mandalay showed the highest evenness values, representing the locality with more even communities, whereas birds from Mawlamyine or Myeik were less equally distributed (Table 2, Figure 5). We also checked if the effective diversity of parasite lineages varied among localities when controlling for the effect of avian species diversity. The results showed that haemosporidian lineage diversity was not affected by bird diversity among localities ($\chi^2 = 0.001, \text{df} = 1, p = 0.980$).

**Table 2.** Estimates of (A) parasite lineage diversity and (B) avian diversity based on the number of birds handled in three localities and three land-use types.

| Locality  | Abundance | Shannon–Wiener Index | Evenness | Effective Diversity |
|-----------|-----------|----------------------|----------|--------------------|
| Mandalay  | 5         | 1.303                | 0.398    | 3.680              |
| Mawlamyine| 2         | 0.693                | 0.721    | 2.000              |
| Myeik     | 4         | 1.386                | 0.541    | 4.000              |
| Urban     | 2         | 0.637                | 0.641    | 1.889              |
| Paddy fields | 6 | 1.792                | 0.465    | 6.000              |
| Hill      | 4         | 1.242                | 0.481    | 3.464              |
| Mandalay  | 11        | 1.675                | 0.316    | 6.523              |
| Mawlamyine| 11        | 1.859                | 0.308    | 6.422              |
| Myeik     | 16        | 2.020                | 0.308    | 9.026              |
| Urban     | 11        | 1.847                | 0.328    | 6.344              |
| Paddy fields | 14 | 2.520                | 0.344    | 12.43              |
| Hill      | 14        | 2.043                | 0.313    | 7.718              |

![Figure 5. Bar chart showing the effective (true) diversity indexes for both parasite lineages (black bars) and avian species (white bars) in three localities and land-use types.](#)

We explored whether estimates for parasite lineage diversity and avian diversity varied among areas with different anthropic disturbance. Paddy fields showed the highest Shannon–Wiener and effective diversity indexes, whereas hill areas showed medium values, and urban environments exhibited the lowest values for these estimates (Table 2, Figure 5). By analyzing the homogeneity of parasite lineages per areas with different land-use types, urban areas showed the highest evenness values, indicating that these areas were more homogeneous than paddy fields or hills (Table 2, Figure 5). With respect to the homogeneity
of bird community, paddy fields showed the highest evenness value, thus representing the areas with more homogeneous communities, while urban and hill areas were less equally distributed (Table 2, Figure 5). In addition, we tested whether the effective diversity of parasite lineages varied among areas with different anthropic disturbance when controlling for the effect of avian species diversity, showing that haemosporidian lineage diversity was not affected by bird diversity among areas ($\chi^2 = 0.462, df = 1, p = 0.498$).

4. Discussion

Myanmar exhibits one of the richest and most diverse bird communities in mainland Southeast Asia, with 1017 out of 1225 avian species described in the Indochinese Peninsula [80]. However, there is a lack of information about the diversity of avian haemosporidian parasites in this tropical region of Southeast Asia. Among the 1017 bird species described in Myanmar, haemosporidian infection has only been documented by molecular methodologies in two domestic [36,81] and 44 wild bird species [34,35]. Here, we analyzed 32 different wild bird species in search of haemosporidian parasites in areas with different degrees of anthropic disturbance based on differences in housing density (urban, hill, and paddy fields) in three Myanmar localities. We showed that (1) 27% of the haemosporidian lineages infecting birds had not been previously described; (2) five of the 12 infected bird species had either not been previously screened for haemosporidians, or had not been found to harbor haemosporidian parasites in preceding studies; (3) 64% of the observed bird–parasite assemblages represent new host records for these haemosporidian parasites; (4) haemosporidian prevalence was higher in birds from Mandalay than in birds from other localities, and (5) birds from paddy fields showed slightly higher haemosporidian prevalence and diversity than birds from urban and hill areas.

4.1. Genetic Diversity of Bird Haemosporidians in Myanmar

Tropical biodiversity is currently threatened by multiple human activities (i.e., deforestation, habitat fragmentation, land-use change), potentially affecting the prevalence, diversity and pathogenicity of avian haemosporidian parasites [46]. Moreover, the high rates of habitat alteration in Southeast Asia have led to host-shifts and the emergence of new pathogens provoking infectious diseases affecting humans and wildlife [82]. Therefore, the identification of parasites in areas that have undergone transformations of natural habitats is an urgent need. To date, over 4000 unique avian malaria and related haemosporidian lineages have been characterized by molecular barcoding methods in more than 1900 bird species worldwide (MalAvi database Version 2.4.7, 6 October 2020, [12]). However, these parasites have been unevenly studied across different biogeographical regions [13]. For example, in spite of the remarkable bird diversity in Asia (more than 20% of world bird species) [83], only 2.39% of known avian haemosporidian lineages have been recorded in this region (MalAvi database version 2.4.7, 6 October 2020, [12]). These differences could be even more pronounced, as currently most of the bird species that have been studied inhabit temperate regions. In contrast, bird communities from tropical areas, which are considered to be the ecosystems harboring the world’s greatest diversity of avian species [84], have received comparatively less attention [27]. Because of the greater abundance and diversity of parasites in the tropics [85,86], it is expected that an important number of avian haemosporidian lineages are still undiscovered in Asia, and thus, deserve more attention. Our findings agree with these predictions. By analyzing genetic diversity of avian haemosporidians in Myanmar, we discovered that three (ACAED03, MERORI02, and PLOHYP01) out of 11 haemosporidian lineages we found had not been identified in previous studies. Moreover, 45% of the infected bird species in this study (P. finlaysoni, P. blanfordi, F. albicilla, P. hypoxanthus, and A. burmannicus) had not been reported as infected by haemosporidians in previous studies. Furthermore, most of the bird–haemosporidian associations (64%) found in this investigation had not been described in previous studies (MalAvi database version 2.4.7, 6 October 2020, [12]). Hence, we have identified new host records for these haemosporidian parasites. These new bird–haemosporidian associations
are made up of the three newly described haemosporidian lineages, plus four parasite lineages (AFR084, TURSTR02, FIPAR02, and ORW1) previously identified as infecting alternative hosts. The new diversity records on host–parasite interactions provided in this study will be valuable for detecting host range and transmission areas of haemosporidian parasites, and will improve our knowledge on the mechanisms of adaptation of avian haemosporidians to new hosts.

There are some noticeable host–parasite associations that are worthy of being highlighted. First, the prevalence and lineage diversity of the genus *Haemoproteus* (subgenus *Parahaemoproteus*) was higher than that of *Plasmodium*, perhaps because abiotic factors may have favored vector availability and abundance of *Parahaemoproteus* vectors (ceratopogonid midges) compared to those that transmit *Plasmodium* (culicid mosquitoes) [75]. This outcome agrees with results from studies on avian haemosporidians across tropical regions during the last 100 years (see review [27]). Second, lineage TURSTR02 is a *Haemoproteus* lineage detected in all the sampled white-throated babblers (*A. gularis*, formerly described as *Turdoides gularis* [87]) from both urban and hill habitats in Mandalay. This haemosporidarian lineage has previously been found in the Himalayan foothills with a high incidence in another species of the same genus (*Argya striata*, formerly described as *Turdoides striata* [75]). Finally, we detected *Plasmodium* haplotype FANTAIL01 in the broad-ranging avian invader, the common myna (*Acridotheres tristis*) [88]. This has been previously described in this bird species in Singapore [77] and Australia [78]. This is a generalist parasite infecting up to 12 avian hosts [12], and it could be highly virulent in new avian hosts [89]. Because pathogenic haemosporidarian parasites carried by invasive bird species to new environments can decimate native avifauna [24,90], the observed presence of this highly virulent *Plasmodium* parasite in our study could compromise the conservation of local birds in Myanmar.

4.2. Differences in Prevalence of Infection among Localities

We found a total haemosporidian prevalence of 12.6%, which is lower than the 37.3% reported by Ishtiaq et al. [35] in a previous study of Myanmar birds. Many biotic and abiotic factors have been proposed to influence haemosporidian infections. For example, these differences in overall prevalence between studies could be explained by variations in the composition of analyzed bird communities, since infection status could be determined by the interaction between host species and parasite lineages, where tolerance and/or susceptibility to parasites may play an essential role [91,92]. In fact, only 6% (eight out of 133) of the species analyzed by Ishtiaq et al. [35] were also screened for haemosporidian parasites in our study. Beyond the variation in prevalence associated with host identity, landscape features (e.g., altitude, slope, urbanization, water reservoirs, etc.) could play a key role in explaining the difference in haemosporidian distribution between studies [93]. In this sense, most of the birds from Ishtiaq et al. [35] were sampled from high altitudes, whereas we sampled birds in lowland areas.

We also reported differences in avian haemosporidian prevalence between Mandalay and Myeik. Birds from Mandalay showed a higher prevalence of infection than individuals sampled in Myeik. Similarly, Mandalay birds had more infected individuals than those from Mawlamyine, although these differences were not statistically significant. These divergences in prevalence among localities can be explained by differences in the date in which the birds were sampled [43,94,95]. Following this idea, observed seasonally changes in haemosporidian prevalence have been attributed to specific patterns in the life cycle of these parasites, whose infective forms in the bloodstream are usually present during the reproductive period of birds, but gradually disappear in other periods of the year because they are sequestered in internal organs [13]. In addition, insect vector life cycles are heavily dependent on seasonal variation in climate, particularly temperature and rainfall [96–98], hence influencing vector availability and haemosporidian transmission [99,100]. Our study was carried out in Myeik and Mawlamyine during the hot–dry season, while we sampled birds in Mandalay during the rainy season, coinciding with the highest abundance of
mosquitoes and haemosporidian transmission [101]. Alternatively, the highest prevalence found in Mandalay bird community could be attributed to the decrease in prevalence of haemosporidian parasites associated with coastal environments [102,103]. Birds living in freshwater inland habitats show significantly higher prevalence of haemosporidian than birds in marine coastal habitats [103], probably due to the scarcity of suitable vectors in marine and saline environments [37,104]. Hence, sampling points from Mawlamyine and Myeik (encompassed in a coastal ecoregion) could show lower haemosporidian vector abundance than those from Mandalay.

4.3. Anthropic Disturbance and Avian Haemosporidian Infection

Land use changes, such as deforestation, urbanization, or agricultural encroachment, may influence the dynamics of wildlife diseases by affecting the distribution of vectors [40,51] and hosts [54]. In agreement with this prediction, we found that birds from paddy fields showed slightly higher haemosporidian prevalence than birds from urban and hills. The likelihood of acquiring an infection in the bird–haemosporidian–vector scenario may be driven by both the effects of habitat alteration on vector abundance, and/or the effects of land-use changes on the health status and condition of the host, potentially influencing infection risk [40,42,105]. We propose two non-exclusive hypotheses to explain these outcomes. First, vector abundance could be higher in paddy fields, therefore increasing the haemosporidian prevalence in birds from these areas. In this sense, a paddy field is a flooded field of arable land extensively practiced in Myanmar for growing semiaquatic crops [106]. This irrigation-based agricultural practice provides optimal environmental conditions for vector proliferation and increases the risk of the spread of vector-borne diseases, as it has been shown in Thailand [107,108]. Additionally, Rosà et al. [109] reported that proximity to rice fields predicted higher total abundance of *Culex pipiens*, one of the main vectors for haemosporidian parasites [13]. Second, birds living in paddy fields may show a compromised immune system, thus, favoring the acquisition of haemosporidian infection. For example, the alterations of habitats have been identified as stressing factors for birds, which may negatively impact the host immune response [110] and increase the likelihood of becoming infected by haemosporidian parasites [111]. In support of this hypothesis, Chávez-Zichinelli et al. [112] analyzed the corticosterone levels in two species of land birds, the Canyon Towhee (*Meliozone fusca*) and Inca Dove (*Columbina inca*), occupying three habitats of a subtropical mountain landscape with different degrees of human disturbance (forest edges, croplands, and urban sites). Their outcomes showed that stress levels (corticosterone concentrations) were higher in individuals living in cropland areas for both bird species. Additionally, pollutants, such as those accumulated by some intensive agricultural activities like rice cultivation [113], may impair immune function and prone individuals to acquire new haemosporidian infections [114,115].

We found that the effective diversity of parasite lineages was higher for birds captured in paddy fields than individuals from urban and hill areas. This is comparable to the observed pattern in haemosporidian prevalence across sites. Previous studies have reported similar results, showing that anthropogenic landscape change increased wildlife parasite diversity [53,54,116]. Similarly, other studies found a lower haemosporidian diversity in undisturbed environments [45,117]. Patterns in the diversity of parasites may be explained by both host and vector availability [118]. On the one hand, the higher diversity of parasite lineages detected in birds captured in the paddy fields could be related to the higher diversity of bird species found in these habitats [60,119–121]. However, we did not find a significant association between bird species and parasite lineages diversity. On the other hand, haemosporidian diversity is shown to be positively related to the wetness of the host bird habitat, likely mediated by vectors [9], because the greater availability of water sources favors the development of blood-sucking insects [37,55,56].

Finally, we found that different lineages were more evenly distributed in urban areas, while those of hill areas and paddy fields had a more heterogeneous parasite community, which could be related to the parasite strategies (specialists–generalists) in heterogeneous
host communities [122]. Evenness is, therefore, an important factor to consider when analyzing communities since it may give a general sense about how parasite lineages or host species are distributed among different areas [123].

To conclude, we investigated the infection and host association of haemosporidian parasites in birds from areas in Myanmar with different degrees of anthropic disturbance, revealing the unexplored diversity of avian haemosporidian parasites in the tropical environments of Southeast Asia. We also found slightly higher haemosporidian prevalence and diversity levels in birds from paddy fields than in individuals from urban and hill areas, implying that human alterations of these natural environments in Myanmar may affect the dynamics of vector-borne diseases. Discovering new susceptible hosts and new areas of distribution, as well as having a greater understanding of the diversity of lineages, is essential to understanding host–parasite evolution, community dynamics, and disease transmission risk. These results establish a better comprehension of host–parasite associations in Myanmar, and ultimately could provide valuable insights for biodiversity management in threatened tropical ecosystems.

Supplementary Materials: The following are available online at https://www.mdpi.com/1424-2818/13/3/111/s1, Table S1: Geographical coordinates (decimal) of the different sampling points in each land use area grouped by locality, Table S2: Tukey post-hoc tests showing differences in haemosporidian prevalence between localities and land-use types.

Author Contributions: Conceptualization, J.M., A.M. and S.C.R.; methodology, all authors; software, J.M., L.G.-L., S.M. and A.M.; validation, J.M., L.G.-L., S.M. and A.M.; formal analysis, J.M., L.G.-L., S.M. and A.M.; investigation, all authors; resources, A.M. and S.C.R.; data curation, J.M., L.G.-L., S.M., S.C.R. and A.M.; writing—original draft preparation, J.M. and A.M.; writing—review and editing, all authors; data analysis, J.M.; map of Myanmar depicting the sampling locations and consensus phylogenetic tree, L.G.-L.; field sampling, all authors; supervision, A.M. and S.C.R.; project administration, A.M., M.S.-R., P.J.B., and S.C.R.; funding acquisition, A.M. and S.C.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Spanish Ministry of Economy and Competitiveness (ref. CGL2015-64650P) and Junta de Extremadura (ref. IB16121). J.M. was supported by a postdoctoral contract from the University of Extremadura (Junta de Extremadura—IB16121) (from May 2018 to September 2019) and a postdoctoral grant from the Juan de la Cierva Subprogram (FJCI-2017-34109), with the financial sponsorship of the MICINN (September 2019—ongoing). S.M.A. was supported by Junta de Extremadura (GR18047 research group BBB028 and IB16121) and the project “Aves y Enfermedades Infecciosas Emergentes: impacto de las especies exóticas y migratorias en la transmisión de malaria aviar y el virus del Nilo Occidental” from the Ayudas Fundación BBVA a Equipos de Investigación Científica 2019. L.G.-L. was supported by Junta de Extremadura (PO17024, Post-Doc grant). The 1st zoological section of The Natural History Museum Vienna supported the study by paying the article-processing charges.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the University of Extremadura (reference 101/2020).

Informed Consent Statement: Not applicable.

Data Availability Statement: http://dx.doi.org/10.20350/digitalCSIC/13781.

Acknowledgments: We thank Thida Win, former Rector of the University of Mandalay; Aung Myat Kyaw Sein and Mie Mie Sein, Rector and Pro Rector (respectively) of Mawlamyine University; and Ni Ni Oo, Rector of Myeik University for their encouragement and support. We are grateful to the many university students and researchers (Yee Yee Htay, Sandar Phyo, Zin May Moe Moe, Khaing Yweh Zaw, Ei Kyewi Soe, Phoe Ei Kyaw, Cho Sin Win, Khin Yuzana, Cherry Mon, La Ring, Su Myat Aung, Si Si Myint, Khwar Nyoo Zin, Naing Naing Aung, Aung Min Thu, Zin Mar Win, May Zane Thein, Nan Ei Thegi, Sabai, Jar San, Daw Phyu Pyar Tin, Ma Ye Mon) for collaboration in collecting samples in Myanmar. We also thank the three anonymous journal reviewers and Associate Editor for their invaluable comments on earlier drafts of the article. Technical and human support provided
by Facility of Bioscience Applied Techniques of SAIUEx (financed by UEX, Junta de Extremadura, MICINN, FEDER and FSE).

Conflicts of Interest: The authors declare no conflict of interest.

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