Neglected parasite reservoirs in wetlands: Prevalence and diversity of avian haemosporidians in waterbird communities in Northeast China

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ABSTRACT

The diversity of waterbirds is threatened, and haemosporidian parasite infection is considered one of the most important causative factors. However, to date, only a few studies focusing on specific parasite species have been carried out, which cannot reflect the general patterns at the community level. To test whether the reported haemosporidian diversity in waterbirds is underestimated, we estimated the prevalence and lineage diversity of avian haemosporidian parasites in 353 waterbirds from 26 species in the Tumuji National Nature Reserve, Northeast China, as well as the host-parasite associations. According to the molecular analysis of cytochrome *b* (*cyt b*) barcode sequences, 28.3% of the birds were infected by 49 distinct parasite lineages, including 11 *Plasmodium*, 12 *Haemoproteus*, and 26 *Leucocytozoon* lineages, of which 39 were novel. The highest prevalence was contributed by *Leucocytozoon* (13.31%), followed by *Plasmodium* (13.03%) and *Haemoproteus* (4.25%), which suggested that waterbirds were infected to a lesser extent by *Haemoproteus* than by the other two genera. Among the most sampled birds, species belonging to Anatidae appeared to be susceptible to *Leucocytozoon* but resistant to *Plasmodium*, while Rallidae presented the opposite pattern. On the phylogenetic tree, most of the *Leucocytozoon* lineages detected in Anatidae clustered together and formed two well-supported clades, while lineages restricted to Gruidae were distantly related to other parasites in all three genera. SW5 was the most abundant lineage and therefore might be a major threat to waterbirds; among the hosts, the common coot harboured the highest diversity of parasite lineages and thus could act as a reservoir for potential transmission. This is the first study of avian haemosporidian infections in a wild waterbird community in Asia. Our findings have doubled the number of lineages recorded in waterbirds, broadened our understanding of host-parasite associations, and addressed the importance of studying haemosporidian infections in wild waterbird conservation.

1. Introduction

The diversity of waterbirds is under threat, especially in Asia (Syroechkovskiy, 2006; De Boer et al., 2010). For many years, extensive researches have been carried out on the conservation of waterbirds, mainly focusing on habitat loss, degradation of stopover sites and climate change that can severely affect waterbird health (Syroechkovskiy, 2006; De Boer et al., 2010; Szabo and Mundkur, 2017; Si et al., 2018; Gaget et al., 2020; Xia et al., 2020), but few studies have evaluated the pathogen diversity inducing common infectious diseases of wild waterbirds, such as avian malaria and related syndromes. Birds possess a high diversity of malaria and related haemosporidian parasites, which may cause infectious diseases and have been implicated in mass mortality events (Valkiūnas, 2004; Eastwood et al., 2019).

Avian haemosporidian parasites, including three main genera, *Haemoproteus*, *Leucocytozoon*, and *Plasmodium*, are protozoan parasites transmitted by blood-sucking dipteran vectors. They are detected in nearly 2000 bird species on all continents except Antarctica (Bensch

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et al., 2009) and are considered to be important threats to wild birds by reducing the host body condition (Merino et al., 2000; Marzal et al., 2008), reproductive success and lifespan, subsequently affecting their fitness (Lachish et al., 2011; Asghar et al., 2015). Moreover, those detrimental effects can be exacerbated when the parasite is transmitted from one host species to another in a cascade (Waldenström et al., 2002; Garcia-Longoria et al., 2020). Therefore, knowledge of the haemosporidian infection patterns in waterbirds may be helpful to comprehensively assess their health condition and develop appropriate conservation plans. Given that parasites often present density-dependent transmission among clustered birds (Hochachka and Dhondt, 2000; Mccallum, 2001), it is important to focus on the potential risk of cross-species dissemination of haemosporidian parasites, particularly at the community level. In this sense, for effective and valued conservation of waterbird communities, one priority is to understand the patterns of haemosporidian infections, including the variations in prevalence and diversity (Smith and Ramey, 2015), as well as the susceptibility of hosts and the specificity of parasites (Valkiūnas, 2004; Chaisi et al., 2018).

In the MalAvi database, which presents compiled information on all reported avian haemosporidian lineages (Bensch et al., 2009), only approximately 2% of cases had been based on wild waterbird data, which is out of proportion to the higher species diversity (approximately 8% of all birds) and conservation significance of waterbirds. Several case studies on the prevalence and diversity of haemosporidian parasites among single avian species were conducted in Europe (Fourcade et al., 2014) and North America (Ramey et al., 2016). In Asia, only a few studies have been carried out focusing on specific avian species, such as migratory waterbirds belonging to Anatidae and Phalacrocoracidae in Mongolia (Seimon et al., 2016) and rescued waterbirds in Japan (Inumaru et al., 2017); however, long-term studies at the community level are still lacking. To test whether the diversity of haemosporidian parasites in waterbirds has been underestimated and to uncover the potential specific host-parasite specificity, it is necessary to conduct systematic surveys in species-rich communities from wetlands. Our study was thereby conducted in Tumuji National Nature Reserve, China, which is a large wetland harbouring nearly 60 species of waterbirds (Zhang et al., 2016; Wang et al., 2019). With a typical temperate continental monsoon climate, this location is one of the most important stopover sites on the East Asian-Australasian flyway (EAAF) and an important breeding place for many endangered waterbird species. A variety of studies have been carried out on population size, habitat selection, behavioural ecology, and other aspects of waterbirds in this region (Zhang et al., 2016), offering a very good system to study the relationship between waterbird communities and haemosporidian parasites.

The majority of waterbirds flock during migration and breeding seasons (Budka and Osiejuk, 2013), and this behaviour benefits density-dependent pathogen transmission (Møller and Erritzø; Waldenström et al., 2002; Smith and Ramey, 2015). Therefore, the whole avian community can be considered a natural reservoir for parasites (Xu et al., 2016), providing an ideal model to determine the relationship between haemosporidian parasites and waterbird communities.

In this study, we assessed the prevalence and diversity of haemosporidian parasites with molecular methods in the Tumuji National Nature Reserve, aiming at answering the following questions: (i) How does the prevalence and diversity of haemosporidian parasites vary among wild waterbird species and families? (ii) Were the parasite lineages equally distributed in the community, or do they follow any certain aggregation pattern? And if the latter, (iii) was the host-parasite association pattern related to host or parasite phylogeny?

2. Materials and methods

2.1. Sampling and data collection

Wild birds were captured mainly between the spring and autumn migration seasons in 2012, 2014 and 2015 (Supplementary Table 1) using specialized traps in Tumuji National Nature Reserve, Inner Mongolia, China (46°04′12″ to 46°25′47″N and 122°44′13″ to 123°10′24″E). Blood samples (50 μl–100 μl) were collected from the brachial vein immediately after capture and stored in anhydrous ethanol until DNA extraction. DNA was extracted using a TIANamp Genomic DNA kit (Tiangen Biotech Ltd., Beijing) following the manufacturer's protocol and dissolved in 100 μl of TE buffer.

The detection of haemosporidian parasites was conducted following a general nested PCR protocol (Hellgren et al., 2004) amplifying a partial *cyt b* gene from the parasite's mitochondrial genome. PCR of each sample was repeated twice to eliminate false negatives and reduce the effect of amplification randomness in mixed infections. At least one negative control (adding ddH$_2$O as the template instead of DNA samples) was included in each reaction to avoid false positives (McClintock et al., 2010). Positive amplifications were distinguished by 1% agarose gel scanning, and products were sequenced bidirectionally using a 3730XL automatic sequencer (Applied Biosystems, USA). The obtained sequences were edited using the software CodonCode Aligner v.5.1.5 (CodonCode Corporation, USA), and the sequences containing one or more ambiguous nucleotides were considered coinfections; i.e., the tested individual was infected by two or more parasites at the same time and separated using Geneious primer v.11.0.9 (Kearse et al., 2012). To avoid overestimation of lineage diversity, coinfections with two undefined lineages were discarded. Parasite taxa were identified using the BLAST module in the MalAvi database.

2.2. Phylogenetic analysis

Phylogenetic relationships among the identified parasite lineages were obtained by constructing a Bayesian analysis tree. Haplotypes were generated using DnaSP v5.10.01 (Librado and Rozas, 2009) and aligned in MEGA v5.1 (Tamura et al., 2013) together with a partial *cyt b* sequence of *Hepatocystis* sp. (GenBank No. KC262867.1) as an outgroup. All morpho-species of *Plasmodium* and *Haemoproteus* recorded in waterbirds and two common morpho-species of *Leucocytozoon* were also included to determine the phylogenetic position of new lineages. A haplotype with at least 1 bp difference from lineages compiled in the MalAvi database was defined as a new lineage. All obtained sequences were uploaded to GenBank (Accession No. MW882263-MW882311).

The best nucleotide substitution model was selected in jModelTest v2.1.1 software (Darriba et al., 2012) according to the Akaike information criterion (AIC) and Bayesian information criterion (BIC) comparison. Bayesian phylogenetic inference was set up in Beauti v2.0 software (Drummond and Rambaut, 2007) with the best-fit model, with a strict molecular clock and Yule process for tree prior. Markov chain Monte Carlo (MCMC) was set to ten initialization attempts, with the length of the chain set as 2×10^7 and log parameters as every 1×10^3 generations. Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/) was used to test the convergence of MCMC chains until the ESS of all parameters was higher than 200. The first 2,000 trees were abandoned as burn-ins, and the maximum credibility tree was selected by TreeAnotator v1.7.5 and visualized in Figtree v1.3.1 (http://tree.bio.ed.ac.uk/softw are/figtree/).

2.3. Statistical analysis

To assess the difference in avian haemosporidian prevalence among host species, chi-square tests were implemented in R v. 3.5.3 (R Core Team, 2019. https://www.R-project.org) using a significance cut-off of $P < 0.05$ (Chagas et al., 2017). To visualize the infection pattern, a

heatmap was generated using the R package pheatmap (Kolde and Kolde, 2015), and a Sankey diagram was processed in Sankeymatic (http://sankeymatic.com/build/).

To further investigate the aggregation pattern of each parasite lineage among different waterbird hosts, we calculated the host and parasite diversity indexes separately. A host species diversity index (S_H) was calculated using the Shannon–Wiener index algorithm for lineages that infected no less than five individuals.

$$S_H = \sum_i^H (H_i)(\text{In } H_i)$$

Where H is the number of infected host species and for the host species i ($0 < i < H$), H_i refers to the proportion of infected individuals, i.e., supposing the number of infected individuals of the certain lineage is N, and that number in the bird species i is n_i, then $Hi = n_i/N$.

The calculation of the parasite diversity index (S_P) is similar to that of S_H for hosts with more than four sampled individuals.

$$S_P = \sum_i^P (P_i)(\text{In } P_i)$$

Where P is the number of infected lineages and P_i refers to the proportion of infected lineages.

3. Results

3.1. Prevalence of parasites

We sampled 353 waterbirds belonging to 26 species of the orders Anseriformes, Podicipediformes, Gruiformes, Charadriiformes, Suliformes and Pelecaniformes (Supplementary Table 1). One hundred of the birds tested positive for haemosporidian parasites, including 22 identified mixed infections. The overall prevalence was 28.3% (Table 1). Among the three genera, the prevalence of *Leucocytozoon* (13.31%) and *Plasmodium* (13.03%) were similar, while that of *Haemoproteus* (4.25%) was significantly lower comparing to *Leucocytozoon* ($\chi^2 = 16.99$, df = 1, $P < 0.001$) and *Plasmodium* ($\chi^2 = 16.15$, df = 1, $P < 0.001$).

The majority of the sampled waterbirds were from the families Anatidae (43.34%) and Rallidae (40.22%), and different infection patterns were observed in these two families (Fig. 1). The prevalence of *Leucocytozoon* in Anatidae was significantly higher than that in Rallidae ($\chi^2 = 32.91$, df = 1, $P < 0.001$), while the prevalence of *Plasmodium* in Rallidae was significantly higher than that in Anatidae ($\chi^2 = 0.01$, df = 1, $P < 0.05$). No significant difference was found in the prevalence of *Haemoproteus* among all sampled waterbird families in our study ($\chi^2 = 5.42$, df = 5, $P = 0.38$).

Table 1
Haemosporidian parasite diversity and prevalence in birds from the Tumuji National Nature Reserve, lineage number of each parasite genera and the prevalence in percentage are given for the host species and orders.

Host species	No. individuals	Prevalence (%)	Plasmodium		Haemoproteus		Leucocytozoon	
			No. lineages	Prevalence (%)	No. lineages	Prevalence (%)	No. lineages	Prevalence (%)
Anseriformes								
Anser cygnoid	2							
Anser anser	1	100					1	100
Aix galericulata	8	12.5					2	12.5
Mareca strepera	7	14.29	1	14.29			1	14.29
Mareca falcata	18	88.89	1	5.56			8	88.89
Anas zonorhyncha	49	14.29	1	4.08	3	6.1	2	4.08
Anas platyrhynchos	34	52.94	1	23.53	2	2.94	10	35.29
Anas crecca	6	33.33					2	33.33
Spatula clypeata	6	16.67					2	16.67
Spatula querquedula	3							
Sibirionetta formosa	4	75					2	75
Aythya ferina	15	20	2	20				
Total	153	34.64	3	9.8	4	2.61	21	25.49
Podicipediformes								
Tachybaptusruficollis	11	9.09	1	9.09				
Podiceps cristatus	20	15.00			1	5.00	2	10.00
Total	31	12.90	1	3.23	1	3.23	2	6.45

| Host species	No. individuals	Prevalence (%)	Plasmodium		Haemoproteus		Leucocytozoon	
			No. lineages	Prevalence (%)	No. lineages	Prevalence (%)	No. lineages	Prevalence (%)
Gruiformes								
Gallinula chloropus	27	48.15	5	48.15				
Fulica atra	115	17.39	6	12.17	6	4.35	3	1.74
Grus leucogeranus	1	100			1	100		
Grus grus	4	75	1	25	1	50	1	50
Total	147	25.17	9	19.05	7	5.44	4	2.72
Charadriiformes								
Vanellus vanellus	1							
Pluvialis fulva	1							
Total	2							
Suliformes								
Phalacrocorax carbo	6	33.33	1	16.67			1	16.67
Total	6	33.33	1	16.67			1	16.67
Pelecaniformes								
Botaurus stellaris	4							
Nycticorax nycticorax	2	100			2	100		
Ardea cinerea	2							
Ardea purpurea	5	40	1	20			1	20
Ardea alba	1							
Total	14	28.57	1	7.14	2	14.29	1	7.14
Total	353	28.33	11	13.03	12	4.25	26	13.31
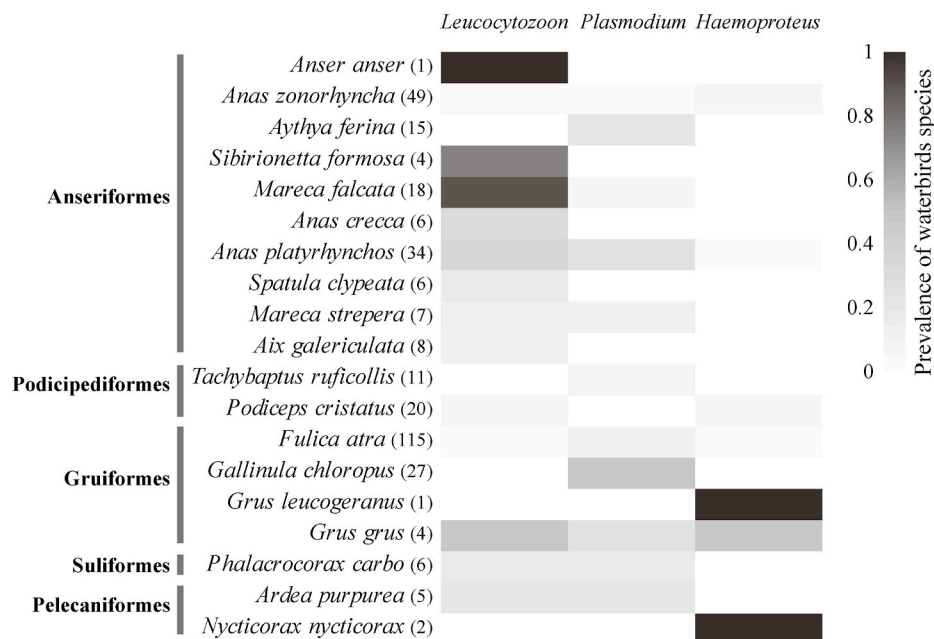
Fig. 1. Heatmap of the apparent prevalence of waterbird species in the Tumuji National Nature Reserve. Presenting infected waterbird species (left, sorted by order) with prevalence (indicated by colour gradient, scale from 0 to 1). The sample size is shown in parentheses.

3.2. Lineage diversity and host-parasite associations

A total of 49 unique parasite lineages were identified, of which 11 belonged to *Plasmodium*, 12 to *Haemoproteus*, and 26 to *Leucocytozoon*. Thirty-nine lineages were novel, of which 18 were separated from coinfections with one recorded lineage. Novel lineages were mostly from the genus of *Leucocytozoon* (n = 21), followed by *Plasmodium* (n = 8) and *Haemoproteus* (n = 10) (Fig. 2a). Seven species were first reported with haemosporidian infections, and sixty-nine new host-parasite associations were detected when compared to the MalAvi database (Supplementary Table 1). In all three parasite genera, we detected similar lineage aggregation patterns: one or two dominant lineages were responsible for the majority of the infections, while most of the remaining lineages were recorded only once in the parasite assemblage (Fig. 2b).

On the phylogenetic tree, parasite lineages clustered in three robust clades corresponding to the three genera. The lineages belonging to *Leucocytozoon* formed five major clades. Clade L1 and clade L4 specialized in Anatidae (Figs. 2a and 3), and L2 was only detected in cranes. Clades L3 and L5 were detected in multiple host families in our study. No clear host-related clade was detected in *Plasmodium*, and the most frequently detected lineages were SW5 (*Plasmodium circumflexum*) and GALCHL02, the latter was closely related to SW2 (*Plasmodium homonucleophilum*) (Fig. 3). There were three major clades in *Haemoproteus*. The majority of the lineages clustered in clade H2, which infected multiple host species and thus can be considered the main culprit of infections in the waterbirds from the Tumuji National Nature Reserve. Clade H1, which included only the lineage GRUAME01 (*Haemoproteus antigonis*), was found in Gruidae and was clearly separated from other lineages with high support. Clade H3 was formed by two novel lineages, both only detected once in Anatidae (Fig. 3).

For the common waterbird species in our dataset (n ≥ 5), the common coot (*Fulica atra*) had the highest parasite lineage diversity index ($S_P = 2.49$), but considerably low prevalence of all the lineages, while the common moorhen (*Gallinula chloropus*) had the lowest parasite lineage diversity index ($S_P = 1.27$) (Supplementary Table S2). For the haemosporidian parasites, the host diversity index was highest in *Leucocytozoon* ($S_H = 2.08$), followed by *Plasmodium* ($S_H = 1.84$) and *Haemoproteus* ($S_H = 1.77$). Among the common lineages (infecting at least

five individuals), SW5 had a higher host diversity index ($S_H = 1.64$) than all the others (Supplementary Table S3), while ANSPLA01 ($S_H = 1.64$) and ANACRE02 ($S_H = 1.04$) had the highest host diversity index in *Haemoproteus* and *Leucocytozoon*, respectively.

4. Discussion

In this study, we conducted the first survey of the prevalence and lineage diversity of haemosporidian parasites in a wild waterbird community in China involving 353 host individuals from 26 species. A total of 49 parasite lineages were detected, of which nearly 80% were newly reported, supporting the assumption that the recorded haemosporidian diversity in waterbirds was largely underestimated. Several host-specific clades infecting Anatidae and Rallidae were identified, especially in the genera *Leucocytozoon* and *Haemoproteus*. Our investigations have revealed a number of new host-parasite associations in waterbirds (Supplementary Table 1) and provide insightful guidance for wild waterbird conservation.

All sampled waterbirds in this study showed high resistance to *Haemoproteus* in terms of both prevalence and host diversity index. This is consistent with the limited previous studies in single avian species in North America (Villar et al., 2013; Ramey et al., 2016), Europe (Fourcade et al., 2014) and Asia (Seimon et al., 2016). Therefore, on the basis of the single species to the community level, it is likely that waterbirds may not be susceptible to *Haemoproteus*. Considering the phylogenetic dispersion of different waterbird taxa, it is tempting to speculate that ecological specificity (Lootvoet et al., 2013), rather than host phylogenetic similarity, leads to the relatively lower compatibility between *Haemoproteus* and waterbirds. On the other hand, vector composition and preferences may change depending on local environmental conditions and thus shape the heterogeneous prevalence among parasites (Santiago-Alarcon et al., 2012). *Culicoides*, the main vector that transmits *Haemoproteus* parasites, might prefer forestry wetlands over grassland environments where most waterbirds are distributed. This may induce a lower observed prevalence of *Haemoproteus* than the other two genera in waterbirds (Wood et al., 2007).

In addition, *Plasmodium* and *Leucocytozoon* presented different infection patterns in different waterbird families. Anatidae had a higher prevalence of *Leucocytozoon* and a lower prevalence of *Plasmodium* than
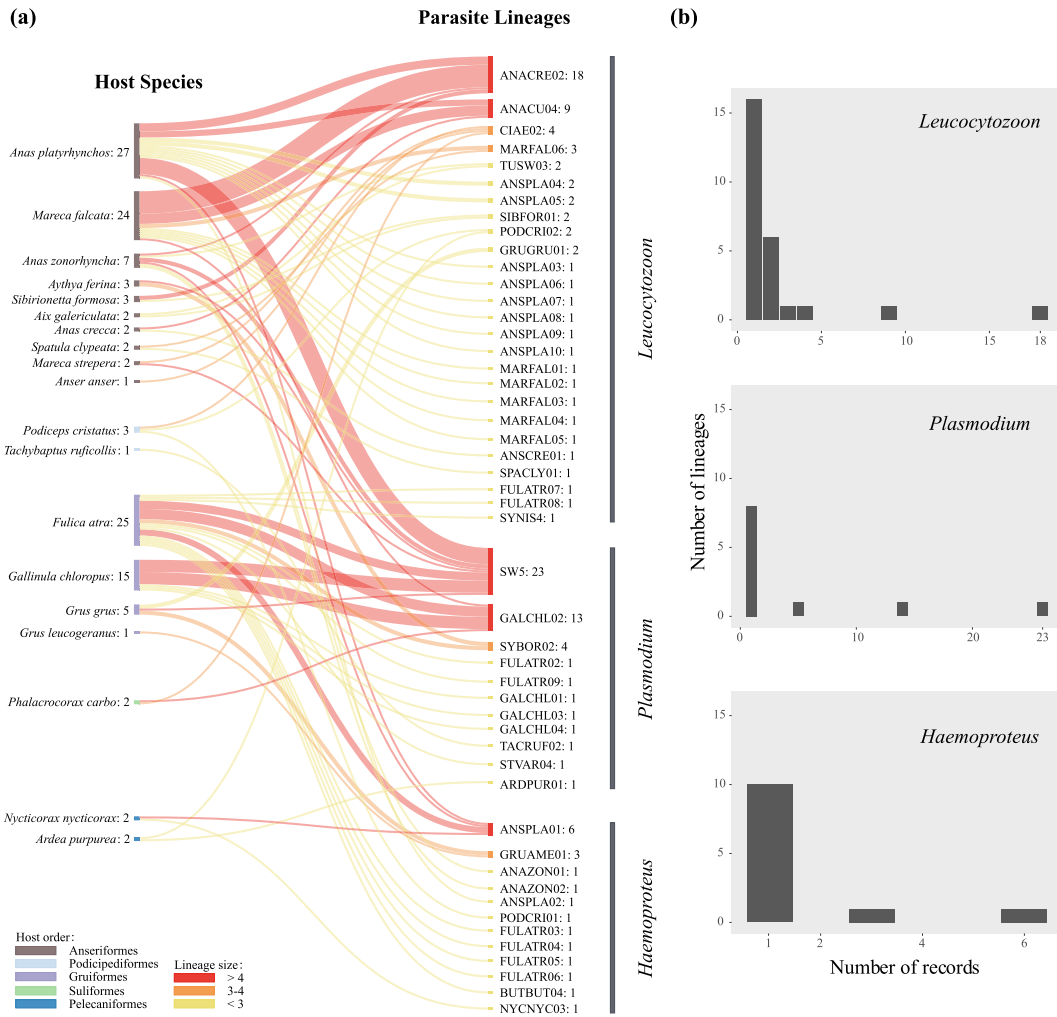
Fig. 2. Diversity (a) and frequency (b) of haemosporidian parasite lineages obtained from waterbirds in Tumuji, China. Sankey diagrams of the correlation between waterbirds (left, sorted by order) and identified haemosporidian lineages (right). The width of the lines indicates proportion to the infection recordings in waterbirds, and the colour of the lines indicates the range of the lineage size. The numbers represent infection cases. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
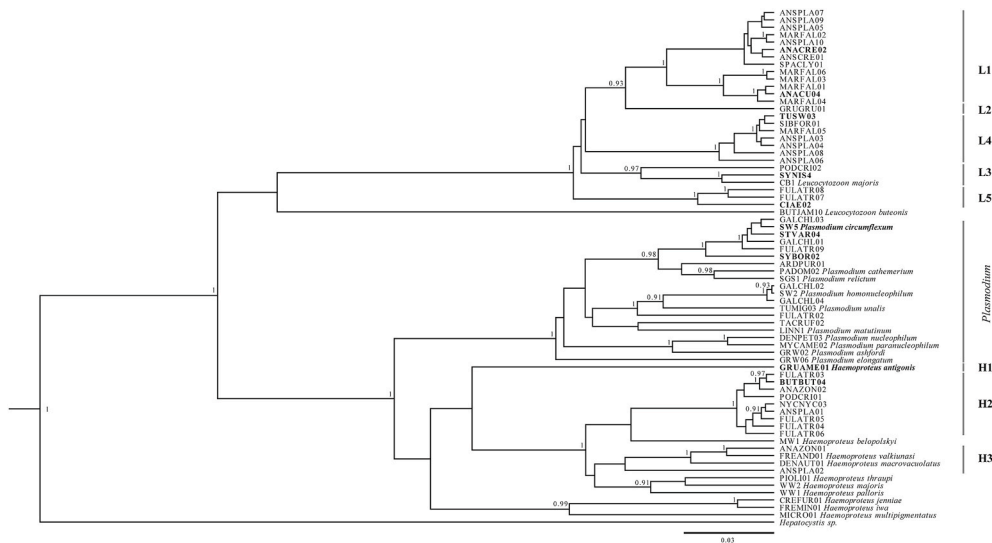

Fig. 3. Bayesian phylogenetic reconstruction of 479 bp haemosporidian *cyt b* lineages from waterbirds in Tumuji, China, with *Hepatocystis* sp. as an outgroup, and several morpho-species were included for a higher resolution of phylogenetic patterns. Posterior probabilities higher than 0.90 are shown by the node. Lineages that were previously recorded and detected in this study are marked in bold. Major monophyletic clades with high support are labelled behind the line (*Leucocytozoon*: L1-L5; *Haemoproteus*: H1–H3).

other taxa in our research. Similar patterns were observed in previous studies of wild tundra swan (*Cygnus columbianus*) in North America (Ramey et al., 2012) and rescued Anatidae in Japan (Inumaru et al., 2017). Thus, we can infer that Anatidae is possibly susceptible to *Leucocytozoon* but more resistant to *Plasmodium*. In contrast, *Plasmodium* was responsible for the majority of infections in Rallidae, while *Leucocytozoon* was relatively rare. Such a pattern was reported previously in corn crake (*Crex crex*) (Fourcade et al., 2014), common coot, and water rail (*Rallus aquaticus*) (Inumaru et al., 2017) in different regions, indicating that Rallidae may be more susceptible to *Plasmodium* than to *Leucocytozoon*. Taken together, these findings suggest that different waterbirds in similar niches can have different susceptibilities to parasites, which may be due to innate genetic heterogeneity in immunity. Therefore, in addition to ecological similarity, host-parasite associations can also be shaped by phylogenetic divergence in long-term evolution, especially immune-related gene diversity, such as the differentiation of MHC genes (Spurgin and Richardson, 2010). Anatidae might exhibit a low diversity of MHC genes related to *Leucocytozoon* while Rallidae might have a similar low diversity of MHC genes related to *Plasmodium*, and vice versa. Alternatively, this pattern may result from differences in tolerance to infection. Low tolerance may lead to severe disease or even rapid mortality post infection (Mukhin et al., 2016); as it is almost impossible to sample heavily infected individuals in the field, and we would observe a lower prevalence of parasites than truly occur (Zehtindjiev et al., 2008). In addition, differences in the behaviour and habitat niche of hosts frequently influence host-parasite associations (Clark and Clegg, 2017). In our case, Anatidae prefers to live in interior wetlands, while Rallidae more commonly appear on the shore or in shallow water. Whether these niches possess different vector compositions and further determine the parasite transmission rate requires future investigation.

The 26 *Leucocytozoon* lineages detected in this study clustered in five major clades, two of which (L1 and L4) were found mainly in Anatidae. Among the previously reported lineages within these two clades, TUSW03 and ANACRE02 were also detected only in Anatidae (Ramey et al., 2013, 2016). ANACU04 was recorded once in a common kestrel (*Falco tinnunculus*) in addition to various Anatidae species. However, the infected samples from the kestrel were negative according to blood smear scanning and showed extremely low infection intensity (Huang et al., 2020), likely indicating an abortive infection (Valkiūnas and Iezhova, 2017). Therefore, we can infer that clades L1 and L4 are possibly specific to Anatidae. The majority of the novel lineages in *Leucocytozoon* were detected in Anatidae and clustered together, suggesting that they might be newly diverged specialist parasites. Combined with the high *Leucocytozoon* prevalence in Anatidae, it is feasible to predict that high susceptibility leads to local adaptation and further accelerates the evolution towards high host specificity and within-host speciation. In the Tumuji waterbird community, Anatidae may act as the main reservoir for persistence, divergence, and further transmission of *Leucocytozoon* lineages. Among the *Haemoproteus* parasites, all lineages in clade H2 were mostly found in waterbirds, except for BUTBUT04, which was recorded in another common kestrel in the same study recording ANACU04, also with low infection intensity indicative of an abortive infection. It is notable that the clade H1 contained only one lineage, GRUAME01 (*H. antigonis*), which was at a great phylogenetic distance from the other lineages. In previous studies, this lineage was detected in whooping crane (*Grus americana*) in North America (Bertram et al., 2017), captive white-naped crane (*Grus vipio*) and Siberian crane (*Grus leucogeranus*) in China (Jia et al., 2018). Here, we contribute the first report of this lineage in wild common cranes and the Siberian crane, implying active transmission of this lineage in our sampling region. Together with this information, we can infer that *H. antigonis* specializes in Gruidae and might be a common pathogen to these species with high conservation priority. Usually populations with depleted genetic diversity may be particularly susceptible to pathogens (Spielman et al., 2004). In the sense of conservation, our findings call for further assessment of the deleterious effects of *H. antigonis* in wild crane populations, including morphological characteristics and other life stage data.

For *Plasmodium*, the most frequent lineage, SW5, has been previously reported in a diverse set of hosts worldwide (Ventim et al., 2012; Ramey et al., 2016; Inumaru et al., 2017). As a presumed generalist parasite lineage, it is not surprising that SW5 presented the highest host diversity among all detected lineages herein and is a potential threat to most of the waterbirds in this community, including endangered species. The other frequent lineage, GALCHL02, was much more specialized, mostly detected in Rallidae (*Fulica atra* and *Gallinula chloropus*), with single records in two other species. As GALCHL02 has not been reported previously, it is uncertain whether the observed pattern was affected by sampling bias or spill-over effects. This lineage clustered together with *P. homonucleophilum*, but due to the lack of blood smears, we cannot confidently define this lineage as the same morpho-species. Our work in wild waterbirds expands further comprehension of these lineages, but further studies are still needed to uncover the transmission pattern in the community and the potential risk of cross-species infection to non-competent hosts.

Notably, the largest reservoir for different parasite lineages in the Tumuji waterbird community was the common coot, in which we identified 15 parasite lineages; however, a higher number of lineages did not present a higher prevalence than in mallard ducks and falcated ducks, which harboured fewer parasite lineages. Similar results have been observed in blue-black grassquits (*Volatinia jacarina*) in Brazil (Fecchio et al., 2021), which may be due to host genetic characteristics. Whether high diversity and low prevalence are a result of trade-offs in the host immune system or are purely the result of sampling bias remains unknown; thus, future studies are needed to address this issue.

5. Conclusion

The present study first reported infection patterns of avian haemosporidians in wild waterbirds at the community level in Asia. In all, 49 parasite lineages were detected, and nearly 80% were novel. All sampled waterbird species presented a low prevalence of *Haemoproteus*. The prevalence and diversity of *Leucocytozoon* and *Plasmodium* were similar in general but differed among host families. Despite these differences, the lineage aggregation pattern was similar in all three parasite genera, with one or two dominant lineages infecting the majority of hosts, while the other lineages were rarely detected. Further investigations on those dominant lineages are important for waterbird conservation. Among all lineages, SW5 belonging to the *Plasmodium* genus was detected in the most host species, suggesting that it might be a potential threat to wild waterbirds in this community. Common coots seem to be the largest reservoir of haemosporidian parasites in this community, making them ideal models to study host-parasite associations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2021.04.013.

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