Gut Fungal Communities of *Anser erythropus* Wintering at Shengjin Lake and Caizi Lake in China

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ABSTRACT

Diet is among the most relevant factors affecting gut microbial communities and affects the host gut microbiota composition and function. We investigated the gut fungal communities of *Anser erythropus* wintering at either Shengjin Lake or Caizi Lake, China, using high-throughput sequencing of the internal transcribed spacer gene region. We retained 1,302,562 valid tags corresponding to 2,102 operational taxonomic units (OTUs) from 20 fecal samples (ten per lake). The OTUs from the Shengjin Lake geese represented 7 phyla, 27 classes, 81 orders, 151 families, and 395 genera; those from the Caizi Lake geese comprised 7 phyla, 28 classes, 73 orders, 133 families, and 232 genera. Ascomycota, Basidiomycota, Zygomycota and Rozellomycota were the dominant gut fungal phyla in the geese. The alpha-diversity indices differed significantly between the Shengjin Lake and Caizi Lake geese. The Shengjin Lake geese ate mainly Poaceae species, whereas the Caizi Lake geese ate mainly *Carex* spp., suggesting that different diets might induce varied gut fungal communities between the geese. More fungal genera were significantly correlated with bacterial genera (*Sphingobacterium*, *Brevundimonas*, *Stenotrophomonas*, *Chryseobacterium*, *Acinetobacter*, and *Pseudomonas*) in pairwise populations, and *Ceratobasidium*, *Tomentella*, *Paurocotylis*, *Tuber*, *Podospora* and *Mortierella* were the core fungal genera among the geese from both lakes. Nine potentially pathogenic species were identified across all samples, and the relative abundances of potential pathogens were significantly higher in the Shengjin Lake samples than in the Caizi Lake samples. These findings suggest that the gut fungi were highly sensitive to the diets of the geese at both lakes. Potential pathogenic species of *A. erythropus* should be further studied.

INTRODUCTION

Vertebrate gut microbiotas are among the most densely populated and complex microbial assemblages and play important roles in maintaining host health (Fu *et al.*, 2020; Bodawatta *et al.*, 2021). Microbes help protect the gastrointestinal system and metabolism, eliminate toxic metabolites and drugs, protect and regulate immune system responses to pathogens, and aid in immune cell development and maturation (Turnbaugh *et al.*, 2006; Grond *et al.*, 2018; Fu *et al.*, 2020; Bodawatta *et al.*, 2021). Under different environmental conditions, gut microbiotas of the same species can differ in both their communities and diversity (Liu *et al.*, 2020). Environmental variations

cause rapid and significant changes in gut microbiomes (Chen *et al.*, 2017). Studies have shown that host genetic divergence strongly shapes the composition of the gut microbiome, while habitat, environment, human activities, sex, age, diet, host, season, and migration can significantly impact gut microbial compositions and functions (Zhang *et al.*, 2021). Fungi are important members of the animal gut microflora. Some fungal genera in the gut contribute to digestion and regulate the host's digestion efficiency, and gut fungi aid the host by providing crucial amino acids, facilitating lignocellulose breakdown, promoting absorption of nutrients from food resources, and triggering immune functions (Chen *et al.*, 2020; Xiang *et al.*, 2021). Much like bacterial populations, fungi are an important part of the environmental microflora but have also been associated with disease onset. Some potential pathogenic fungi reside in the host's gut, and some potential fungal pathogens are passed via host-environment interactions, which increases the risk of their cross-transmission because they share an environment with humans and other animals (Mahtab *et al.*, 2021). Birds provide interesting study groups for investigating the roles of the gut microbiota because they exhibit complex diversity and vary in life-history traits such as migratory behavior, flight capacity,

diet, mating systems, longevity and physiology, all of which may impact the gut microbiota (Grond *et al*., 2018; Xiang *et al*., 2021). Additionally, because birds fly and live in various habitats during migration, the composition and characteristics of their gut microbiotas differ from those of other animals. The gut microbial communities of wild birds have received attention and are interesting subjects for studying the gut microbiota (Xiang *et al*., 2021). Despite the great diversity and ecological significance of these microbes, research on avian gut microbiotas has lagged behind that of mammalian research. Studying microbial diversity and function contributes to better understanding avian physiology and adaptive capacity to variable environments (McWhorter *et al*., 2009). Migratory waterbirds experience more microbiota alterations than do other birds owing to their variable environments during migration. At different wintering-site habitats, migratory waterbirds forage in different habitats, especially for food resources that contain different nutrient contents; therefore, the birds' gut microbiotas may differ (Xiang *et al*., 2021). Diet and dietary sources are important factors affecting the gut microbiota, and an abundance of a preferred diet will affect the host's gut microbiota composition and function, which can lead to differences within the same species (Zhang *et al*., 2021). Changes in dietary patterns can directly affect the gut microbial community composition and function; for example, the gut bacterial communities of *Grus monacha* differed among those at different wintering lakes because their food resources differed (Dong *et al*., 2019; Zhang *et al*., 2021).

Anser erythropus (family Anatidae, order Anseriformes) is a long-distance migratory waterbird and an important indicator species in wetlands. The species mainly breeds in northeastern Siberia to Fennoscandian Lapland and winters primarily in China, South Korea, and Japan. In China, empirical studies have indicated that these geese spend the long winter period (November to April) at Shengjin and Caizi Lakes (Bengtsson-Palme *et al*., 2013; Yang *et al*., 2016). During the winter, Shengjin and Caizi Lakes have begun to undergo degradation due to human interference, thus decreasing the available food for wintering birds. In winter, *A. erythropus* are omnivorous, but mainly feed on plants. Previous studies have shown large differences in the diets between the geese at the two lakes, with *A. erythropus* feeding almost exclusively on *Carex* spp. at Caizi Lake, while an extra Poaceae component was identified in the geese at Shengjin Lake (Zhao *et al*., 2012; Yang *et al*., 2016). Previous studies indicated that diet may be an important driver of gut bacterial community structure in the birds at Shengjin and Caizi Lakes (Liu *et al*., 2020). However, little is known about the gut fungal communities and functions within these

geese, especially regarding their wintering at Shengjin and Caizi Lakes, as well as the importance of geography in shaping gut fungal communities. Thus, understanding the gut fungal communities in *A. erythropus* may help clarify the ecological structure and functions of the fungi in these geese. The internal transcribed spacer (ITS) gene region is composed of two highly variable spacers (ITS1 and ITS2), which are usually species-specific, whereas the intercalary 5.8S gene is highly conserved and is the primary choice for molecular identification of fungi (Schoch *et al*., 2012; Bengtsson-Palme *et al*., 2013). Here, we performed high-throughput sequencing of the ITS region to analyze the gut fungal communities of wintering *A. erythropus* at Shengjin and Caizi Lakes. We assessed whether the gut fungal communities showed markedly different shift patterns, similar to those of gut bacteria, between *A. erythropus* wintering at Shengjin Lake and those wintering at Caizi Lake and further tested the impacts of diet on the gut fungi of the geese. We also examined potential pathogens in the guts of *A. erythropus* and hypothesized that these pathogens differ markedly between *A. erythropus* wintering at Shengjin Lake and those wintering at Caizi Lake.

MATERIALS AND METHODS

Ethical standards

No birds were harmed during this research. All experimental procedures complied with current laws regarding animal welfare and research in China and were specifically approved by the Animal Research Ethics Committee of Anhui Medical University.

Study areas and sample collection

We selected the wintering sites for *A. erythropus* at Shengjin and Caizi Lakes in China (30.25°–30.50°N, 116.92°–117.25°E and 30.75°–30.97°N, 117.00°–117.15°E, respectively). These lakes are shallow, river-connected lakes located in the middle and lower Yangtze River floodplain in Anhui Province, China. Both lakes are globally important stopover and wintering wetlands for migratory wading birds on the East Asian-Australasian Flyway (Mahtab *et al*., 2021; Chen *et al*., 2011). The lakes are surrounded by mud flats, grasslands, and paddy fields in the winter, and in addition to being stopovers for wading birds, they serve as stopovers and wintering sites for other migratory waterbirds such as cranes, ducks, and geese.

Twenty fecal samples from *A. erythropus* were collected from paddy fields at Shengjin and Caizi Lakes in December 2018 (ten samples per lake). Before collecting the fecal samples, we selected large groups of >150 geese after observing them with binoculars. The sample

collection distance was >5 m to avoid individual repetition. To avoid human disturbance, the fecal samples were collected immediately after the geese finished foraging, and all samples were obtained from the inside of the feces to avoid soil contaminants. All fecal samples were placed in sterile 50-ml centrifuge tubes, immediately transported to the laboratory, and stored at −80°C.

Fecal DNA extraction, species identification, and high-throughput sequencing

DNA was isolated from the fecal samples using a MagPure Soil DNA LQ Kit (Magen, Guangdong, China). Avian species were determined using the cytochrome c oxidase subunit I (*cox1*) gene with primers BIRDF1/BIRDR1. The DNA extraction, PCR reaction, and sequence identification were conducted as per Yang *et al.* (2016 and 2019). The cycling parameters were 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 55°C for 45 s, and 72°C for 90 s, with a final extension at 72°C for 10 min. The PCR products were sequenced, then blasted against the National Center for Biotechnology Information (NCBI) database to confirm that all fecal samples were *A. erythropus*. PCR amplification of the ITS region was performed in a 25-µl reaction using universal primer pairs, ITS1F-5′-CTT GGT CAT TTA GAG GAA GTA A-3′ and ITS2-5′-GCT GCG TTC ATC GAT GC-3′. The PCR products were purified with Agencourt AMPure XP beads (Beckman Coulter Co., USA). A Qubit dsDNA assay kit was used for quantification, and the concentrations were normalized for sequencing (Dong *et al.*, 2019). An Illumina NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) with two paired-end read cycles of 250 bases each was used as the sequence platform. Sequencing was conducted by OE Biotech Co., Ltd. (Shanghai, China). Data for the raw reads were submitted to the Sequence Read Archive of the NCBI (accession number SRR15328848).

Bioinformatics, statistical analysis, bacteria-fungi association analysis and potentially pathogenic species identification

The fungal data from the raw sequencing were in FASTQ format and processed using Quantitative Insights into Microbial Ecology, version 2 (QIIME2) (Xiang *et al*., 2019). The paired-end reads were preprocessed using Trimmomatic to filter low-quality sequences. Reads with chimeras were filtered using VSEARCH (Hao *et al*., 2020). All representative reads were annotated and blasted against the Silva database (v.132) using the RDP classifier (confidence threshold: 70%) (Vishniac, 2002). Alpha-diversity indices (Chao1, Shannon, and Simpson) were evaluated using one-way analysis of variance for normally distributed data (Wrighton, 2019). For significant

differences ($p < 0.05$), we used Tukey's honestly significant difference test ($p < 0.05$) to perform post hoc pairwise multiple comparisons. The nonparametric Kruskal-Wallis test ($p < 0.05$) was used for non-normally distributed data, and post hoc pairwise multiple comparisons were performed using the Dunn-Bonferroni test. The UniFrac distance matrix determined by QIIME software was used for the unweighted UniFrac principal coordinates analysis (PCoA). Abundant fungi in the geese at the two lakes were identified using linear discriminant analysis effect size (LEfSe), using a nonparametric Kruskal-Wallis rank-sum test with the default settings (alpha: 0.05, effect size threshold: 2) to identify biomarkers (Londero *et al*., 2019). Venn analysis was performed to categorize the core fungal taxa using the Oebiotech tools at https://cloud.oebiotech.cn/task/. All identified fungal species were manually searched as keywords in the Web of Science database. Fungal species that had been reported as pathogens in humans or other animals were further analyzed, and differences in the pathogenic community compositions between the two lakes were estimated in SPSS 22.0. We analyzed the relationships between the fungal and bacterial communities using gut bacterial data from 20 fecal samples taken from Shengjin and Caizi Lakes in our previous study (Liu *et al*., 2020). Fungal and bacterial OTUs were selected to present a co-occurrence network analysis. Pairwise Spearman correlations, R scores, and *p*-values for the OTUs were calculated using the Hmisc package, version 4.1.1, in R. Only OTUs with a strong Spearman's correlation coefficient were considered.

RESULTS

General sequencing information

The ITS gene region was sequenced and analyzed from 20 fecal samples collected from *A. erythropus* wintering at Shengjin and Caizi Lakes. A total of 1,322,935 reads were retained, and after removing low-quality reads, 1,302,562 clean reads corresponding to 2,102 OTUs were retained. Each sample contained 65,128 ± 5,168 reads (range, 46,229–69,486 per sample) and an average of 147 OTUs (range, 57–774), and 96.5% (mean) were classified as fungal phyla. A total of 1761 OTUs were identified from the geese at Shengjin Lake, and 1228 OTUs were identified from the geese at Caizi Lake. Among all OTUs, 44.09% were shared in geese from both lakes (Fig. 1). Geese from Shengjin Lake had 41.58% unique OTUs; geese from Caizi Lake had 14.33% unique OTUs (Fig. 1).

Gut fungal community compositions

Gut fungi from the fecal samples collected from *A. erythropus* at Shengjin Lake comprised 7 phyla, 27 classes,

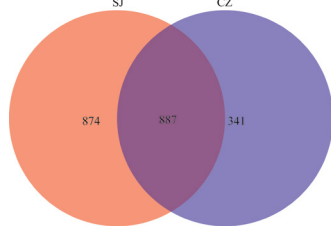81 orders, 151 families, and 395 genera; those from

Fig. 1. Venn diagram illustrating the number of OTUs from the gut fungi of *A. erythropus* from Shengjin (SJ) and Caizi (CZ) Lakes, China.
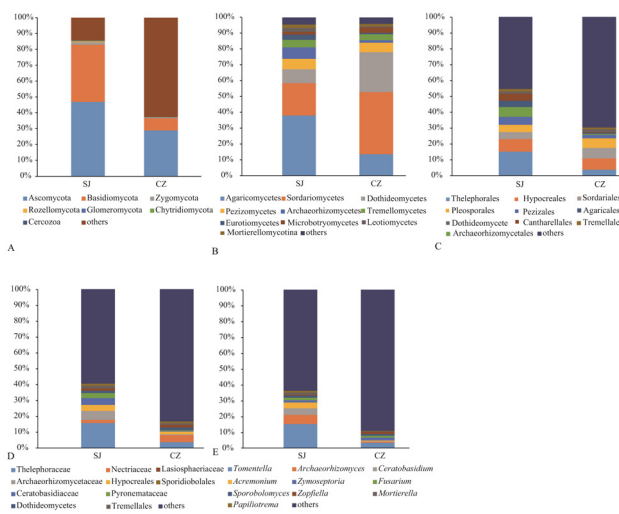

Fig. 2. Dominant gut fungal phyla (A), classes (B), orders (C), families (D), and genera (E) from *A. erythropus* at Shengjin (SJ) and Caizi (CZ) Lakes.

Caizi Lake included 7 phyla, 28 classes, 73 orders, 133 families, and 232 genera. The dominant fungal phyla from both groups were Ascomycota, Basidiomycota, Zygomycota, Rozellomycota, Glomeromycota, and Chytridiomycota. Ascomycota, Basidiomycota, Zygomycota and Rozellomycota accounted for 61.60%, 35.60%, 1.84%, and 0.30% of the OTUs, respectively (Fig. 2A). Glomeromycota and Chytridiomycota accounted for less than 0.001% of all OTUs. Fecal samples collected from Shengjin Lake had significantly higher relative abundances of Basidiomycota, Zygomycota, Rozellomycota, and Glomeromycota than did those collected from Caizi Lake. The relative abundances of Ascomycota and Chytridiomycota did not significantly differ between the samples. The dominant fungal classes from both groups were Agaricomycetes, Sordariomycetes, Dothideomycetes, Pezizomycetes and Archaeorhizomycetes, accounting for 30.77%, 26.77%, 14.33%, 6.58% and 5.67% of the OTUs, respectively (Fig.

2B). Fecal samples collected from Shengjin Lake geese had significantly higher relative abundances of Agaricomycetes, Sordariomycetes and Archaeorhizomycetes than did those collected from Caizi Lake, and the relative abundances of Dothideomycetes and Pezizomycetes did not significantly differ between the samples. The top 10 fungal orders from both groups were Thelephorales, Hypocreales, Sordariales, Pleosporales, Pezizales, Archaeorhizomycetales, Agaricales, Cantharellales and Dothideomycetes, accounting for 20.22%, 15.50%, 11.19%, 10.94%, 7.38%, 6.67%, 4.98%, 4.96%, 3.50% and 2.96% of the OTUs, respectively (Fig. 2C). The top 10 fungal families were Thelephoraceae, Nectriaceae, Archaeorhizomycetaceae, Hypocreales, Ceratobasidiaceae, Pyronemataceae, Dothideomycetes, Lasiosphaeriaceae, Tremellales and Sporidiobolales, accounting for 28.98%, 9.58%, 9.56%, 8.20%, 7.11%, 5.40%, 5.02%, 4.83%, 4.08% and 3.80% of the OTUs, respectively (Fig. 2D). The top 10 fungal genera from both groups were *Tomentella*, *Archaeorhizomyces*, *Ceratobasidium*, *Acremonium*, *Zymoseptoria*, *Fusarium*, *Sporobolomyces*, *Zopfiella* and *Mortierella*, accounting for 26.71%, 8.98%, 6.49%, 5.93%, 4.71%, 3.89%, 3.43%, 2.79%, 2.37% and 2.07% of the OTUs, respectively (Fig. 2E). To explore the variations in the fungal community compositions between the two lake populations, we performed LEfSe tests to detect differences in the relative abundances of the fungal taxa across samples. Basidiomycota, Agaricomycetes, and Thelephoraceae were significantly enriched in the Shengjin Lake geese, while the Nectriaceae and Sordariales were significantly enriched in the Caizi Lake geese (Fig. 3). Spearman's rank correlation was used to identify the co-occurrence relationships among the top 30 genera of gut fungi from *A. erythropus* from both Shengjin Lake and Caizi Lake (Fig. 4). *Zopfiella* and *Staphylotrichum* had a very strong co-exclusionary relationship (R = 0.982, $p < 0.001$), whereas *Tomentella* and *Staphylotrichum* had a very weak co-exclusionary relationship (R = −0.270, $p = 0.253$; Fig. 4).

Alpha and beta diversity
     Alpha diversity of the gut fungi was estimated via the observed Chao1, Simpson and Shannon indices, which differed significantly between the Shengjin Lake-associated and Caizi Lake-associated geese (Fig. 5). One-way analysis of variance results showed that alpha diversity in the gut was significantly higher in the Shengjin Lake geese than in the Caizi Lake geese ($X^2 = 5.17$, $p < 0.05$). Fungal beta diversity was estimated via the observed PCoA analysis, which indicated that the gut fungal communities of most samples from Shengjin and Caizi Lakes were well matched with their sampling lakes. Gut fungi from the Shengjin Lake geese were strongly clustered together, and those from the Caizi Lake geese

were strongly clustered together. Gut fungi were distant between the two lakes (Fig. 6).
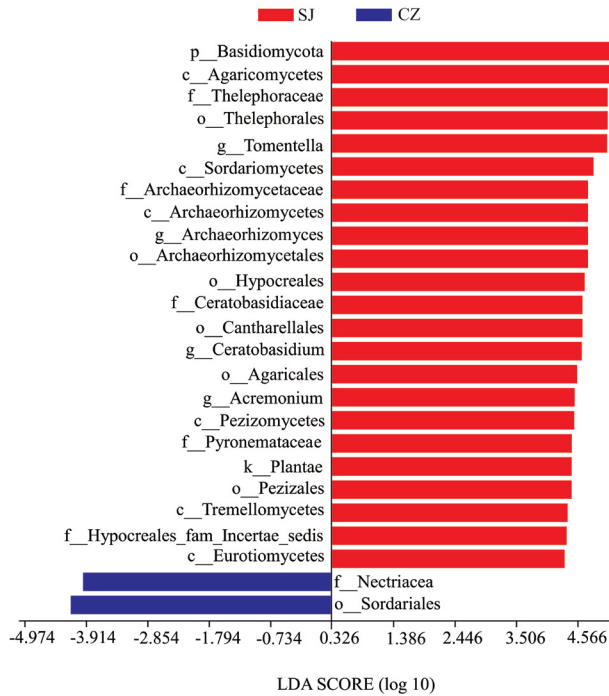

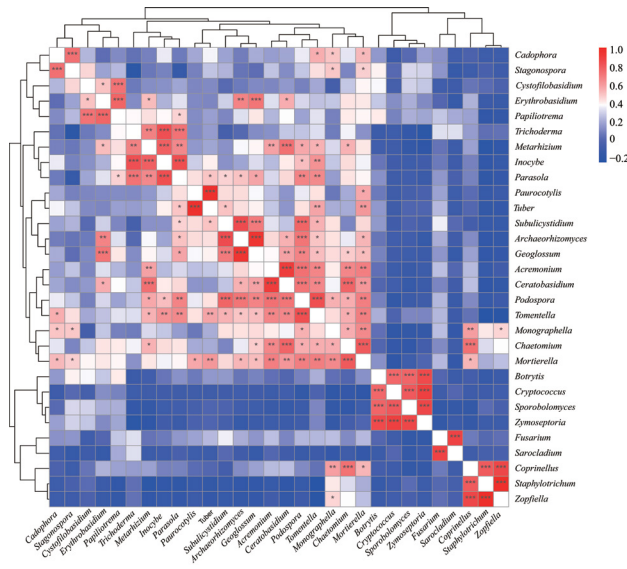Fig. 3. LEfSe analysis of the gut fungi from *A. erythropus* at Shengjin (SJ) and Caizi (CZ) Lakes.

Fig. 4. Correlations among the top 30 core genera across the 20 samples from *A. erythropus* at Shengjin (SJ) and Caizi (CZ) Lakes, determined by Spearman's rank correlation analysis. Note: *$p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Red: positive correlation; blue: negative correlation.
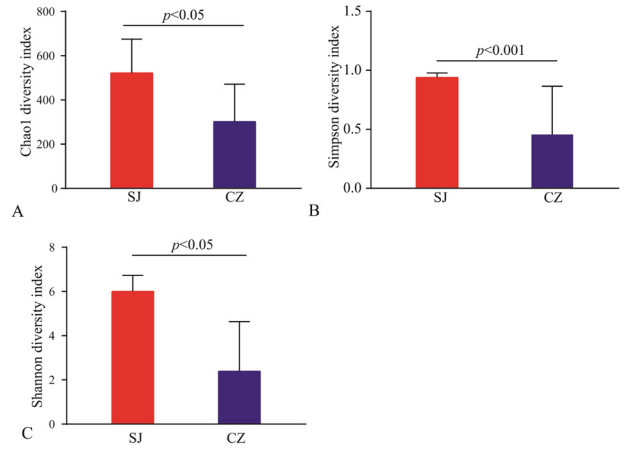

Fig. 5. Chao1 (A), Simpson (B), and Shannon (C) indices for the gut fungi from *A. erythropus* Shengjin (SJ) and Caizi (CZ) Lakes.
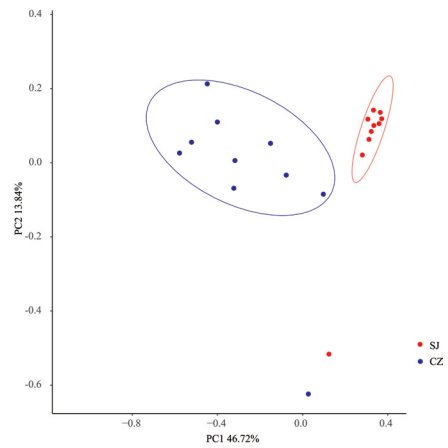

Fig. 6. PCoA results from the feces of *A. erythropus* sampled from Shengjin (SJ) and Caizi (CZ) Lakes. Red: fecal samples from geese from Shengjin (SJ) Lake; blue: fecal samples from geese from Caizi (CZ) Lake.

Bacteria-fungi association analysis

Some fungal genera were significantly correlated with bacterial genera from the 20 samples (Fig. 7). The abundances of the bacterial genera *Sphingobacterium*, *Brevundimonas*, *Stenotrophomonas*, *Chryseobacterium*, *Acinetobacter*, and *Pseudomonas* were positively correlated with the abundances of the fungal genera *Tuber*, *Paurocotylis*, *Mortierella*, *Archaeorhizomyces*, *Acremonium*, *Subulicystidium*, *Podospora*, *Ceratobasidium*, *Tomentella*, *Erythrobasidium*, and *Cystofilobasidium*. Additionally, *Sporosarcina* and *Lactobacillus* were negatively correlated with *Paurocotylis*, *Mortierella*, *Archaeorhizomyces*, *Acremonium*, *Subulicystidium*,

Podospora, *Ceratobasidium*, and *Tomentella* (Fig. 7). Bivariate comparisons between Shengjin and Caizi Lakes revealed significant Pearson correlation coefficients for the fungal and bacterial communities (R = −0.52, *p* < 0.05). We built genus-level correlation networks for both the bacteria and fungi and found that *Sphingobacterium*, *Stenotrophomonas*, *Pseudomonas*, *Brevundimonas* and *Chryseobacterium* were the hub bacterial genera, and *Ceratobasidium*, *Tomentella*, *Paurocotylis*, *Tuber*, *Podospora* and *Mortierella* were the more abundant fungal genera (Fig. 8).
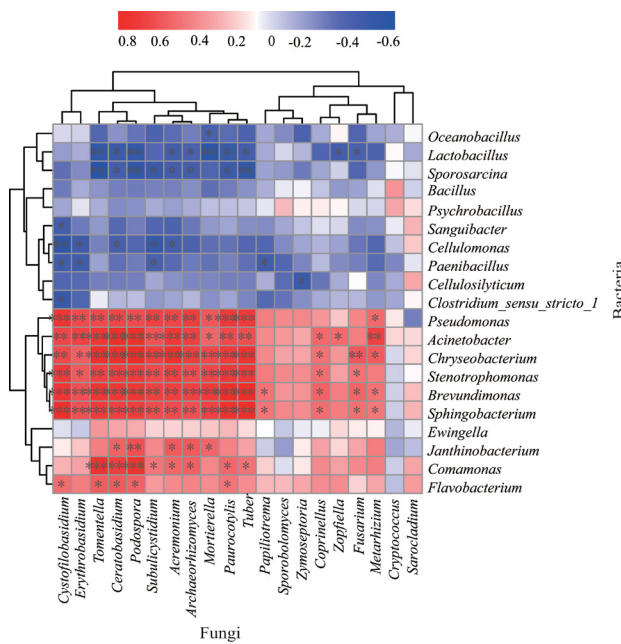

Fig. 7. Specific bacterial-fungal correlation analysis results for the 20 samples from *A. erythropus* from Shengjin (SJ) and Caizi (CZ) Lakes. Note: *p* < 0.05, ** *p* < 0.01 and *** *p* < 0.001. Red: positive correlation; blue: negative correlation.

Potential pathogens

We identified nine potential pathogenic species across all *A. erythropus* gut samples (Fig. 9A). The relative abundances of potential fungal pathogens were significantly higher in the Shengjin Lake samples than in the Caizi Lake samples (Fig. 9B). Basidiomycota (*Malassezia restricta*, *Cryptococcus heimaeyensis*, *Papiliotrema laurentii*, and *Apiotrichum veenhuisii*) and Ascomycota (*Graphium basitruncatum*, *Purpureocillium avendulum*, *Aspergillus flavus*, *Monocillium indicum*, and *Hirsutella vermicola*) were the dominant potentially pathogenic fungal phyla and species in the guts from the geese at both lakes. The potential fungal pathogen species with the highest relative abundance was *Graphium basitruncatum* (53.65%) and
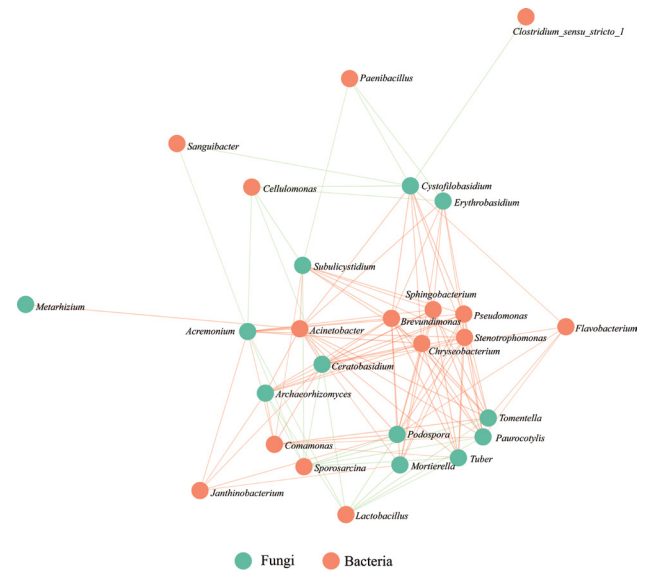

Fig. 8. Correlation network between fungi and bacteria from *A. erythropus* at Shengjin (SJ) and Caizi (CZ) Lakes. Orange circles: fungi; green circles: bacteria.
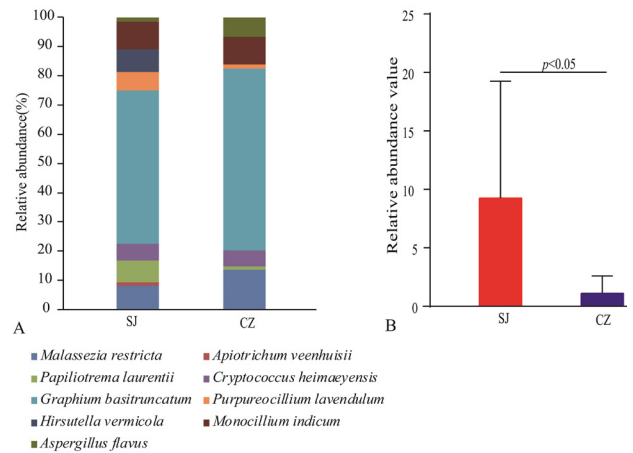
Fig. 9. Compositions of potential pathogenic species (A) and pathogenic diversity (B) of the gut fungi from *A. erythropus* at Shengjin (SJ) and Caizi (CZ) Lakes. Statistically significant differences by the Mann-Whitney-Wilcoxon test.

with the lowest relative abundance was *Apiotrichum veenhuisii* (1.04%) in both groups. *Apiotrichum veenhuisii* and *Hirsutella vermicola* were identified only in the Shengjin Lake geese. Of the aforementioned potential pathogens, *Malassezia restricta* can cause Crohn's disease, *Cryptococcus heimaeyensis* can cause leukemia, and *Papiliotrema laurentii* can cause immune system diseases in humans (Vishniac, 2002; Wrighton, 2019; Londero *et*

al., 2019; Hao *et al*., 2020). *Apiotrichum veenhuisii* can potentially cause skin diseases (Lara *et al*., 2019), and *Graphium basitruncatum*, *Purpureocillium avendulum*, *Aspergillus flavus*, *Monocillium indicum* and *Hirsutella vermicola* can propagate widely via the gut and increase the risk of disease in humans and other animals.

DISCUSSION

Previous studies have indicated that animal gut microbiotas exhibit strong plasticity. Additionally, environmental factors, diet, age, habitat and behavior can influence the microbial composition and structure (Hammons *et al*., 2010; Hird *et al*., 2015; Barbosa *et al*., 2016; Wang *et al*., 2016, 2017; Liu *et al*., 2020). Research has shown that the structure and function of the gut microbiota differ significantly among different geographical populations of the same species owing to food resource heterogeneity (Liu *et al*., 2020). A recent study found that *A. erythropus* guts contained many bacteria obtained over vast distances and that dramatic shifts occurred between geese at Shengjin and Caizi Lakes owing to different food resources (Liu *et al*., 2020). Here, we confirmed these two hypotheses and found significant differences in the gut fungal communities between *A. erythropus* wintering at Caizi and Shengjin Lakes. These differences were similar to those of bacterial communities, suggesting that food resources are an important factor influencing changes in the gut microbial community (Liu *et al*., 2020). The results also showed that the potential pathogens in the guts of *A. erythropus* differed markedly between *A. erythropus* wintering at Shengjin Lake and those wintering at Caizi Lake, similar to the differences in the bacterial and fungal communities (Liu *et al*., 2020).

Diet is the main factor influencing microbial composition, and food components may shape the fungal communities in waterbird guts (Bolnick *et al*., 2014; Xiang *et al*., 2021). At different wintering sites, wild birds forage for food resources with different nutrient contents. The alpha diversity of the gut fungal communities differed significantly between the *A. erythropus* wintering at Shengjin Lake and those wintering at Caizi Lake; this result was similar to that for the gut bacterial communities, thus supporting that the gut fungal communities were strongly associated with gut environmental filtering, especially owing to the different food resources (Grond *et al*., 2018; Liu *et al*., 2020). Shengjin and Caizi Lakes provide abundant and diverse food sources for wintering *A. erythropus*; however, the geese diets differed largely between the two lakes. Geese at Shengjin Lake ate mainly Poaceae; geese at Caizi Lake ate mainly *Carex* spp. (Hammons *et al*., 2010; Yang *et al*., 2016; Lara *et al*.,

2019). Therefore, the gut fungal community compositions of *A. erythropus* differed significantly between the two lakes, likely owing to variable food resources (Liu *et al*., 2020).

Owing to their lower abundances and lack of well-characterized reference genome data, fungi are largely unexplored in birds. Consequently, knowledge of the gut fungal contributions in birds is limited (Palamidi and Mountzouris, 2018; Li *et al*., 2020). However, gut fungi play important roles in the metabolism and intestinal health of their host birds, including roles in energy metabolism, intestinal barrier function and immunity (Li *et al*., 2017, 2020). Thus, understanding the gut fungi of wild birds is important for clarifying the functions of these fungi in their hosts. *A. erythropus* requires high energy for normal physiological functioning; therefore, these birds may rely on their gut microbiotas during the winter. Bird guts harbor fungal taxa along with bacteria. In the present study, the gut fungal communities of *A. erythropus* were dominated by Ascomycota (61.60%), Basidiomycota (35.60%), Zygomycota (1.84%) and Rozellomycota (0.30%); these communities are similar to those of *Grus monacha* wintering at Shengjin Lake (Xiang *et al*., 2021; Mahtab *et al*., 2021). These fungi produce hydrolytic enzymes to degrade plant material, thus increasing the host's digestion efficiency (Ljungdahl, 2008). Ascomycota is the largest phylum in the fungal kingdom; it is essential in the physiology, growth, and metabolic mechanisms of its host species and produces several crucial enzymes that aid in digesting complex carbohydrates (Wu *et al*., 2018; Siriyappagouder *et al*., 2018). Ascomycota and Basidiomycota dominated the *A. erythropus* guts; these fungi help birds digest and absorb higher abundances of roots and leaves during the wintering period at both lakes. *Archaeorhizomyces*, *Tomentella* and *Ceratobasidium* dominated the *A. erythropus* guts at both lakes; these genera differed from those of *Grus monacha* at Shengjin Lake, with *A. erythropus* at Shengjin Lake feeding almost exclusively on Poaceae, whereas *Grus monacha* ate mainly wild *Vallisneria natans* and *Potamogeton malaianus* (Liu *et al*. 2020; Xiang *et al*., 2021). *Tomentella* may be associated with acetate production in bird guts, suggesting that the gut fungi might be involved in metabolizing dietary polysaccharides (Xiang *et al*., 2021). *Archaeorhizomyces* and *Ceratobasidium* might help increase host digestion and immunity. Our results suggested that *A. erythropus* might depend more on their gut fungi to acquire nutrients and enhance immunity (Xiang *et al*., 2021).

Some potential fungal pathogens are passed via host-environment interactions. Birds are an important disease source and vector in the environment and can spread many diseases through water, soil or air contamination to poultry

and humans (Zhao *et al*., 2017; Bodawatta *et al*., 2021). As migratory birds, *A. erythropus* fly long distances and live in various habitats throughout their life; thus, they can carry and spread many pathogens. In this study, we found nine pathogenic fungal OTUs in the *A. erythropus* fecal samples from the two lakes; these pathogenic fungi may cause serious diseases in humans and/or other animals. *A. erythropus* from Shengjin Lake carried higher abundances of potential pathogenic gut fungi than did those from Caizi Lake, suggesting that the species might suffer more pathogenic stress under harsh living conditions wintering at Shengjin Lake. Shengjin and Caizi Lakes are important wintering habitats for waterbirds, and a niche overlap exists between migratory birds and domestic birds, which can lead to cross-transmission of fungal pathogens. Shengjin and Caizi Lakes are crucial habitats for poultry and livestock, and fecal pathogens from *A. erythropus* can easily contaminate the water, air and soil. Additionally, poultry often forage together with wintering waterbirds in paddy fields at both lakes; thus, they can pass the gut pathogens to each other and increase the risk of disease in other sympatric animals (Yang *et al*., 2016). The risk of cross-transmission of potential fungal pathogens between migratory birds, domestic poultry, and other wild birds might be higher during the extended wintering period. Some potentially pathogenic species detected in this study also cause infections in humans. Local residents have a lot of contact with wintering waterbirds and domestic poultry; thus, the gut fungal pathogens of *A. erythropus* may propagate to humans either directly or indirectly during the winter (Dong *et al*., 2019; Xiang *et al*., 2021). Therefore, humans should avoid mixed groups of wintering waterbirds and poultry to prevent spreading these pathogens to other humans (Kevin, 2012; Fu *et al*., 2020).

In conclusion, the gut fungal community compositions and diversity of *A. erythropus* differed significantly between those wintering at Shengjin Lake and those wintering at Caizi Lake in China. The environment, homogeneity and different food resources might have caused the divergence in the gut fungal communities of *A. erythropus* wintering at Shengjin and Caizi Lakes. Furthermore, *A. erythropus* from Shengjin Lake migrated from a different breeding area than did those from Caizi Lake (Liu *et al*., 2020). *A. erythropus* might be at high risk for cross-transmission of fungal pathogens at the wintering sites. Additional research should focus on gut pathogens in wild birds and domestic poultry because these pathogens can cause diseases in humans and other animals.

This study had some limitations. We collected only 20 samples and did not consider spatiotemporal factors. These factors may influence gut fungal community compositions. These limitations should be addressed in future studies.

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