Effects of Small Hydropower Stations Along Rivers on the Distribution of Aquatic Biodiversity

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INTRODUCTION

Since 1950s, as an important clean and renewable energy, hydropower has played an important role in electricity supply, poverty reduction, rural economy and social development (Li et al., 2022), which is an indispensable guarantee for achieving the goals of “carbon peaking” and “carbon neutrality”. At the same time, small hydropower station projects are widely used in rural areas and remote mountainous areas because of their small investment, low risk, stable benefits and low operating costs (Fu et al., 2008). By 2021, more than 47,000 small hydropower stations have been built in China, with an annual power generation of 250 billion kilowatt-hours, equivalent to saving more than 70 million tons of standard coal and reducing carbon dioxide emissions by 180 million tons per year (Peng et al., 2022). It has also been pointed out that the construction of small hydropower stations has negative ecological impacts, such as affecting fish migration and reproduction (Cao, 2019); limiting the spread of animals and plants (Rivinoja et al., 2010); altering the hydraulic flow characteristics of the river (Guo et al., 2015); and changing the structure and genetic diversity of biological communities (Sundermann et al., 2011; Liu, 2020; Costea et al., 2021).
The impact of small hydropower stations on the aquatic environment can be monitored by aquatic biodiversity. As one of the main indicators of water environment assessment, aquatic biodiversity plays an important role in ecological restoration and health assessment of river basins (Sun et al., 2013; Gao et al., 2015), while the health of aquatic biota can directly or indirectly reflect the health of the water environment. Phytoplankton, zooplankton and large aquatic organisms are widely used in water environment monitoring and assessment. For example, Wei et al. (2012) analyzed the water quality of the Erhai Sea by investigating the species, number and distribution of phytoplankton; Zhao et al. (2008) evaluated and analyzed the status of the water ecological environment in the Huaihe River basin by investigating the composition and abundance of phytoplankton, zooplankton and benthic animal. Fish cover all trophic levels of consumer ecology and provide a powerful tool for assessing aquatic environments (Lin et al., 2021); plankton are an important link in the material and energy transfer of aquatic ecosystems, with phytoplankton playing a vital role in the food chain as the first trophic level (Zhang et al., 2010); zooplankton participate in the material cycle of aquatic ecosystems by feeding on phytoplankton, bacteria and other organisms (Bi et al., 2001; Li et al., 2004); benthic organisms are an essential part of the aquatic food web and provide the bait base for most fish in rivers, and are closely related to the ecological taxa and zonal composition of riverine fish (Šarauskienė et al., 2021); in addition, the species and abundance of microbial communities can reflect changes in aquatic ecosystems. Overall, the aquatic organisms mentioned above can indicate the health of the aquatic environment at a certain level (Xu, 1996; Araújo et al., 2000; Jakovčev-Todorović et al., 2005; Salmaso et al., 2006; Li, 2022).

At present, the assessment of aquatic ecological environment based on aquatic biodiversity is mainly focused on large and medium-sized reservoirs and hydropower stations (Otahelová and Valachovič, 2003; Bredenhand and Samways, 2009; Andrianova, 2020; Chen et al., 2020), and fewer studies have been reported on the disturbance of aquatic biodiversity by small hydropower stations. Therefore, it is meaningful to explore the impact of small hydropower stations on the characteristics, diversity and functional groups of aquatic communities to improve the ecological impact assessment index system of small hydropower stations. This study investigated the impacts of the construction of 15 small hydropower stations in Qionglai on the diversity of aquatic organisms (fish, plankton, benthic animal and microorganism). With a view to provide basic data and theoretical basis for the development of small hydropower stations and ecological environmental protection of river basins.

Since the sampling conditions were available during the low-water seasons, in December 2021, we collected samples from a total of 16 points at 15 small hydropower stations and the confluence of watersheds in the Baimo and Wenjing River basins in Qionglai City. The river is divided into four watersheds (Q1-Q4) according to its connectivity and location (Table 1). Among them, S0 is the confluence of rivers, and the remaining 15 sampling points are all diversion-type power stations. The geographical distribution of sampling points is shown in Figure 1. S1-S4 belong to basin Q1, S5 belongs to basin Q2, S6-S9 belong to basin Q3 and S10-S15 belong to basin Q4.

### Sampling Methods

#### Fish Collection and Identification

The collection of fish resources included field sampling and visiting surveys, on-site identification of fish specimens as far as possible, determination of biological basic data, looking through common fish atlases to determine fish species, and taking scales and other materials for age identification. For the identification of fish, reference the *Chinese Fish Atlas* (Li, 2015) to determine the species of fish.

#### Phytoplankton Collection and Identification

1 liter of water sample was collected with glass water sampler into polyethylene bottles, stored in 1.5% Lugol’s solution and brought back to the experiment for analysis. Firstly, it needed to settle for 48 h, and then using a siphon device, the water sample was concentrated to 30 mL, 0.1 mL of the shaken concentrated water sample was taken on a plankton counting plate, and the morphological analysis of the algal species was observed by microscopic counting method under a light microscope. For the identification of algae, reference the *The system, taxonomy and ecology of Chinese freshwater algae* (Hu and Wei, 2006).

### MATERIALS AND METHODS

#### Sample Collection

Qionglai City is located in Sichuan Province of western China, at the upper reaches of the Yangtze River, and has been “the first state in southern Sichuan” since ancient times. There are numerous small hydropower stations in the Baimo and Wenjing River basins in Qionglai.

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### TABLE 1 | Sampling sites.

| Watershed | Sample | Name |
|-----------|--------|------|
| Q1        | S1     | Tang Shuimo Hydropower Station |
|           | S2     | Niu Xin Hydropower Station    |
|           | S3     | Long Menkou Hydropower Station |
|           | S4     | Fu Xiang Hydropower Station   |
| Q2        | S5     | Yan Zi Hydropower Station     |
|           | S6     | Zen Xishan Hydropower Station |
|           | S7     | Ma Ping Hydropower Station    |
|           | S8     | He Ping Hydropower Station    |
|           | S9     | Xiang Shuhan Hydropower Station |
| Q4        | S10    | Xiao Jiawan Hydropower Station |
|           | S11    | Zheng Fa Hydropower Station   |
|           | S12    | Tian Tai Hydropower Station   |
|           | S13    | Tian Chepo five-stage Hydropower Station |
|           | S14    | Tian Chepo four-stage Hydropower Station |
|           | S15    | Hong Yan Hydropower Station   |

| Watershed confluence | S0 |
|----------------------|----|
| Ma Qihukou            |    |
Zooplankton Collection and Identification
Fifty liters of water sample were collected at the same place, and the mixed water samples were filtered through a No. 25 plankton net (pore size of 64 µm), and finally the concentrated solution was transferred to a polyethylene bottle, and the samples were stored in 5% formaldehyde solution, protected from light and low temperature. The shaken concentrated water samples were taken 0.1 mL on a plankton counting plate, covered with a coverslip, and the samples were identified and counted for species under a light microscope. Referring to the Atlas of Freshwater Microorganisms (Zhou, 2005) for classification and identification of species.

Benthic Animal Collection and Identification
Benthic animals were collected by a mud collector for 3-5 times, with the sampling area of 0.09 m². And the samples in the mud collector were passed through a 40 mesh sieve, washed and picked out as suspected benthic animals, and loaded into specimen bottles containing 30% alcohol. The samples were brought back to the laboratory and the benthic animals were then picked
out on dissecting trays and stored in 75% alcohol at 4°C. The alcohol was replaced once a week pending identification. For quantitative benthic analysis, sample species identification and enumeration were carried out under a light microscope, mainly with reference to the Atlas of Chinese Animals (Tong, 1982), and identified to species.

Microorganism Collection and Analysis
The collected microbial water samples were filtered with a water body extractor (0.45 μm filter membrane) for microbial enrichment, and if the samples are turbid, centrifugation is used for enrichment. The enriched microbial water samples were sent to Shanghai Personalbio Technology Co., Ltd., for testing, and the microbial community-related data were obtained by high-throughput sequencing (HTS) and polymerase chain reaction (PCR) amplification of 16sRNA gene-specific fragments.

Data Analysis
Physical and chemical properties of water quality and spatial distribution of phytoplankton density and biomass use Personalbio for analysis. Distribution of each sampling point at different division levels uses Origin 2022 for analysis.

RESULTS AND DISCUSSION

Water Quality Conditions in the Study Basin
Water samples from S0, which is at the confluence of Baimo and Wenjing River, and 3 representative power stations (S4, S9, and S15) are selected to measure physical and chemical indexes, and the comparison maps of pH, water temperature (WT), conductivity (Cond), dissolved oxygen (DO) and transparency of each sampling site are drawn (Figure 2). As can be seen from Figure 2, the pH of water in the study basin varies between 8.5 and 8.8, belonging to weakly alkaline water quality; WT varies between 11.6°C and 14.3°C; Cond varies from 30.3 ms/m to 43.0 ms/m; DO varies between 12.99 ppm and 13.5 ppm; transparency varies between 0.4 m and 1.3 m. There are no significant differences in pH, WT, and DO among the sampling sites.

Fish Composition and Distribution Characteristics
The results of the fish survey are detailed in Supplementary Table 1. There are 30 species of fish in total. Among them, 6 species are obtained by on-site sampling, namely Misgurnus anguillicaudatus, Monopterus albus, Pelteobagrus fulvidraco, Cyprinus carpio, Pseudorasbora parva and Carassius auratus. A total of 10 species are found after checking the data, mainly including Rhodeus sinensis, Rhodeus ocellatus, Abbottina rivularis and Hemibarbus maculatus, etc. Through the interview survey, it is found that there are 14 kinds of fish, including Silurus asotus, Hypophthalmichthys molitrix, Silurus meridionalis and Aristichthys nobilis, etc.

A total of 6 species of fish are collected on site, among which Misgurnus anguillicaudatus, Cyprinus carpio and Carassius auratus are highly resistant to pollution and are medium pollution zone indicator fish, indicating that there may be a certain degree of pollution in the watershed. Zhu et al. (2021) collected 71 species of fish in the nearshore Jingjiang section of the Yangtze River near the mouth; Wang et al. (2020) collected 64 species of fish in the Xinzhou waters of Anqing of the Yangtze River; Zheng et al. (2019) collected 56 species of fish in the Wuhan section of the Yangtze River, the fish resources in the survey basin are relatively small compared to the aquatic resources in the upper reaches of the Yangtze River, it is possible that the construction of small hydropower stations has led to changes in river volume and flow rate, which has affected the survival and reproduction of fish (Sun and Liu, 2020), reducing the space in the water column and affecting the species composition of fish (Benejam et al., 2016). The construction of small hydropower stations has a greater impact on fish diversity than other aquatic organisms.

Phytoplankton Composition and Distribution Characteristics
The results of the phytoplankton survey in the study basin are shown in Table 2. The phytoplankton shows Q3 > Q4 > Q1 > Q2 in terms of species abundance and diversity. The proportion of phytoplankton in the three divisions of Bacillariophyta, Cyanophyta and Chlorophyta in the four watersheds is shown in Figure 3. The Bacillariophyta is mainly found in the basin Q1 and Q4, especially in the 4 sampling sites S1, S2, S10, and S11. And the Cyanobacteria and Chlorophyta are mainly distributed in the Q3 basin.

A total of 26 species of common phytoplankton are found in Q1, among which the Bacillariophyta is the most abundant with 22 species, mainly including Diploneis elliptica and Navicula sp., etc., followed by Chlorophyta, Cyanobacteria with 2 species each, namely Scenedesmus bijugatus, Ulothrix sp. and Pseudaonabaena sp., Aphanocapsa sp. A total of 10 species of common phytoplankton are found in the basin Q2, among which Bacillariophyta are the most abundant with 9 species, mainly including Cymbella affinis and C. cymbiformis, etc., followed by Chlorophyta with only 1 species of C. fracta. A total of 32 species of common phytoplankton are found in Q3, among which 14 species of Chlorophyta, mainly including Scenedesmus quadricauda, etc., followed by the Bacillariophyta with 11 species, mainly including Fragilaria capucina, etc., there are 7 species of Cyanobacteria, mainly including Phormidium faveolarum, etc.

A total of 28 species of common phytoplankton are found in Q4, among which Bacillariophyta is the most abundant with 22 species, mainly including Diploneis elliptica and Navicula sp., etc., followed by 3 species each of Cyanobacteria and Chlorophyta. In general, The largest relative abundance is in the Bacillariophyta, with 55.9%, followed by the Chlorophyta, with 28.8%, and the Cyanophyta, with 15.3% (Supplementary Table 2).

The distribution of phytoplankton population density and biomass at each sampling point is shown in Figure 4. Quantitative analysis statistics of phytoplankton from 15 sampling sites shows that the density of phytoplankton ranges from 8.0 pcs/L to 7869.1 pcs/L, with an average of 2967.1 pcs/L.
The highest sampling point is S6 with 7869.1 pcs/L, the second highest is S9 with 7396.8 pcs/L, and the lowest is S5 with 8 pcs/L. The biomass of phytoplankton ranges from 0.0071 mg/L to 0.9908 mg/L, with an average of 0.3468 mg/L. The highest sampling point is S6 with 0.9908 mg/L, the second highest is S8 with 0.9227 mg/L, and the lowest is S5 with 0.0071 mg/L. The difference between sampling zones is significant ($p < 0.05$). Phytoplankton density in Q1 ranges from 11 pcs/L to 15 pcs/L, with an average of 12.8 pcs/L and biomass ranges from 0.0194 mg/L to 0.0234 mg/L, with an average of 0.0215 mg/L; phytoplankton density and biomass of Q2 are 8 pcs/L and 0.0071 mg/L, respectively; phytoplankton density in Q3 ranges from 7228.5 pcs/L to 7869.1 pcs/L, with an average of 7450.8 pcs/L and biomass ranges from 0.1759 mg/L to 0.9908 mg/L, with an average of 0.6838 mg/L; phytoplankton density in Q4 ranges from 2004.3 pcs/L to 2979.9 pcs/L, with an average of 2445.5 pcs/L and biomass ranges from 0.1759 mg/L to 0.2796 mg/L, with an average of 0.2507 mg/L.

A total of 59 species of 3 phytoplankton divisions are identified in the study basin. Tan et al. identified 95 phytoplankton species from 6 phyla in five sections from the upper reaches of the Yangtze River to Jiangjin (Tan et al., 2017); Min et al. found 79 phytoplankton species from 6 phyla in the Yangtze River source area (Min et al., 2020); Cui et al. recorded 175 phytoplankton species from 8 phyla in the main stream and its tributaries in the Yangtze River source area (Cui et al., 2020). In comparison, fewer phytoplankton species were found in this survey. The phytoplankton in the Q1, Q2, and Q4 basins are dominated by Bacillariophyta, occupying 84.6, 90.0, and 78.6%, respectively,
consistent with the theory that Bacillariophyta dominate the phytoplankton of many lowland rivers (Devercelli and O’Farrell, 2013). The Bacillariophyta has the largest biomass in winter (56.1%), followed by the Chlorophyta (29.8%), which is similar to the results of Tian et al. (2017) and Wu et al. (2014). Generally speaking, diatoms is the dominant species in clean water, green
algae is the representative of medium-sized eutrophic water, and blue algae is the dominant species in eutrophic water (Zhen and Zhang, 2017). Green algae and blue algae accounted for 15.4% in Q1, 10.0% in Q2, 65.6% in Q3 and 21.4% in Q4 basin, which indicated that there is some pollution in this area.

**Zooplankton Composition and Distribution Characteristics**

Through identification, a total of 16 species of zooplankton in 4 categories are detected. And they are Protozoa, Cladocera, Copepoda and Rotifera, respectively. The largest relative abundance is Protozoa (43.8%), followed by Rotifera (31.3%), Copepoda (18.8%), and Cladocera (6.3%). The distribution of zooplankton species in the study basin in each phylum is shown in Figure 5. A total of 7 common zooplankton species in 4 categories are detected in Q1. The Protozoa includes *Arcella discoides* and *Epistyli* sp.; the Rotifera includes *Lepadella patella* and *Keratella cochlearis*; the Copepoda includes *Cyclopoid larva* and *Nauplius*; the Cladocera includes *Moina* sp. 6 common zooplankton species in 4 categories are detected in Q2. The Protozoa includes *Arcella vulgaris*, *Centropyxis aerophile* and *Paramecium caudatum*; the Rotifera includes *Keratella cochlearis*, *Notholca squamula* and *Brachionus calyciflorus*. 4 common zooplankton species in 2 categories are detected in Q3, among which the Protozoa is the dominant phylum with 3 species, including *Difflugia* sp., *Centropyxis aculeata* and *Arcella discoides*; 1 species of Copepoda is *Nitosra* sp. 8 common zooplankton species in 4 categories are detected in Q4, among which Rotifera is the dominant phylum with 3 species, including *Lepadella patella*, *Filinia longiseta* and *Keratella cochlearis*; the Protozoa

![Figure 5](image-url)
includes *Arcella discoides* and *Epistyliis* sp.; the Copepoda includes *Cyclopoid larva* and *Nauplius*; the Cladocera includes *Moina* sp. (Supplementary Table 3).

The results show that Protozoa and Rotifera dominate the study basin, with *Arcella discoides* and *Keratella cochlearis* being the most widely distributed. Q3 has the lowest number of zooplankton species (4 species) and Q4 has the highest number of zooplankton species (8 species). Among other Yangtze River basins, Sun et al. (2021) collected 74 species of zooplankton in the middle reaches of the Yangtze River; Dai et al. collected 74 species of zooplankton in 4 categories in the Yangtze River Dolphin Protection Zone in Zhenjiang, Jiangsu (Dai et al., 2011), Dai et al. (2019) collected 46 species of zooplankton in 4 categories in the Xinzhou waters of Anqing, Yangtze River, in contrast, the zooplankton in the surveyed watersheds are less diverse and less abundant than those in the other basins of the Yangtze River, and species diversity is low, probably because the water temperature in the Qionglai area is low (11.2°C-14.3°C), which is not conducive to the growth and reproduction of zooplankton (Sun et al., 2021), while the steeper slope and faster current in the Qionglai area make it difficult for phytoplankton to survive, and the number of zooplankton feeding on phytoplankton is thus reduced (Liu et al., 2017).

**Benthic Animal Composition and Distribution Characteristics**

The species distribution of each phylum of benthic animals is shown in Figure 6. Among them, Arthropoda is widely distributed in all watersheds, with the most in watershed Q3, while only 1 species of Annelida is detected in Q3, and **1**
species of Mollusca. 10 species of 2 common phyla are detected in the Q1 watershed, among which the Arthropoda is the dominant phylum with 9 species, mainly including the *Baetis* sp. and *Tipula* sp., etc.; followed by the Mollusca, with 1 species, which is *Linnoperna fortunei*. Q2 watershed detected common benthic animals 1 phylum 4 species, for the Arthropoda, including stonefly, *Rhyacophila* sp., etc. Q3 watershed detected common benthic animals 2 phylum 16 species, among which the Arthropoda is the dominant phylum with 15 species, including *Ephemera* sp. and *Baetis* sp., etc., followed by the Annelida with 1 species, which is *Limnodrilus hoffmeisteri*. 9 species of 1 common phylum were detected in the Q4 watershed, the Arthropoda, including *Corixidae* sp. and *Baetis* sp., etc. (Supplementary Table 4).

The survey results show that the benthic animals are mainly composed of Arthropoda, with the most widespread distribution of *Baetis* sp., *Tipula* sp., *Polypedilum* sp., *orthocladius* sp., and *Corixidae* sp. Studies have shown that *Ephemeroptera* are sensitive groups, mostly distributed in clean water, while *Chironomidae* and *Limnodrilius* are pollution-resistant groups, generally distributed in polluted areas (Wu et al., 2011). These three kinds of benthic animals are found in Qionglai watershed, of which *Ephemeroptera* account for 10.0% and *Chironomidae* account for 40.0% in Q1; *Ephemeroptera* account for 50.0% in Q2; *Ephemeroptera* account for 18.8%, *Chironomidae* and *Limnodrilius* account for 31.3% in Q3; *Ephemeroptera* account for 11.1% and *Chironomidae* accounted for 55.6% in Q4, therefore, there is a certain degree of pollution in the basin under investigation.

A total of 23 species from three phyla of benthic animals are identified in the study basin. Zhang et al. (2022a) collected 38 species of macrobenthic animals from 3 phyla in the Qiaobian River, a first-class tributary of the Yichang section of the Yangtze River; Zhang et al. (2022b) collected 31 species of benthic animals from 3 phyla in the Huangbai River basin, a first-class tributary on the left bank of the middle reaches of the Yangtze River.
Li et al. (2020) collected 28 species of benthic animals from 3 phyla in the Qiaobian River, a tributary of the Yangtze River (Li et al., 2020). In contrast, the species abundance of benthic animals in the surveyed watershed is low and the overall resources are poor. It may be due to the construction of small hydropower stations that the water pollution is aggravated, the water depth and water flow velocity are changed (Premstaller et al., 2017), at the same times, the responses of different benthic animals to environmental changes are different (Ren et al., 2015), so the biomass and abundance of macrobenthos are decreased.

Microorganism

Microbial Community Diversity

The samples are clustered by euclidean distance of species composition data for UPGMA (default clustering algorithm), and the top 20 species are selected to draw species composition heat maps at the phylum level (Figure 7A) and genus level (Figure 7B). At the phylum level, Planctomycetes, WPS-2 and Armatimonadetes are highly expressed in S0; Dependenciae and Elusimicrobia are highly expressed in S4; Proteobacteria and Bacteroidetes are highly expressed in S9. Verrucomicrobia, Margulisbacteria, Chlamydiae, Nitrospirae, Chloroflexi and Hydrogenedentes are highly expressed in S15. At the genus level, Polynucleobacter, TRA3-20, Candidatus_Planktophila and Candidatus_Methylophilus are highly expressed in S0; Limnohabitans is highly expressed in S4; Nevskia, NS11-12_marine_group, Sediminibacterium, NS9_marine_group, Alkanibacter, Panacagrimonas and Sphingorhabdus are highly expressed in S9; Tychonema_CCAP_1459-11B, Rhodoferax and Pedobacter are highly expressed in S15. Microbial diversity and species abundance are high at all sampling sites, and the construction of small hydropower stations had a low impact on microorganisms due to their small size and wide distribution, and because they are less affected by environmental factors than benthic invertebrates (Vilmi et al., 2020).

Alpha and Beta Diversity Analysis

Alpha diversity analysis of microorganisms in water samples from 4 sampling points at S0 which at the confluence of Wenjing and Baimo River and three representative power stations (S4, S9, and S15) are performed, and the results are shown in Figure 8. From the Chao1 index, the number of species is high at all sampling sites, of which S9 has the highest number of sampling sites. The Shannon index, Simpson index and Observed species index show that the species richness and diversity of each sampling site are high, and the species are evenly distributed, among which the species richness of S15 is higher than the other 3 sampling sites.

Beta diversity analysis shows (Figure 9) that S4 is more similar to the top 10 species in terms of the abundance to S0. The relative abundance of hgcl_clade (27.30%) and Sporichthyaceae (8.17%) are higher in S0; the relative abundance of hgcl_clade (21.55%) and Chloroplast (20.70%) are higher in S4; the relative abundance of NS11.12_marine_group (32.70%) is the highest in S9; the relative abundance of Chloroplast (18.53%) and hgcl_clade (6.59%) are higher in S15.

CONCLUSION

This study of the impact of small hydropower stations on aquatic organisms in the Qionglai watershed, which is located in the
upper reaches of the Yangtze River, shows that the biodiversity of the Baimo and Wenjing River in Qionglai is at a low level, and the results of the fish diversity survey shows relatively few fish resources and poor species diversity, with only 6 species of fish found in the field sampling, mainly including Cyprinidae; 23 species of benthic animals are detected, and Arthropoda (91.3%) is dominant; the number of zooplankton species is less and the diversity is low, and only 16 species are detected in the study watershed; the biomass of Bacillariophyta accounts for the largest proportion of phytoplankton (56.1%), followed by Chlorophyta (29.8%) and Cyanobacteria (4%); both the diversity and species abundance of microorganisms are high, and the impact of small hydropower stations is the lowest. In summary, small hydropower stations have an impact on the biodiversity of the study watershed, showing an order of fish > benthic > plankton > microorganism.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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AUTHOR CONTRIBUTIONS

PG, TW, and YX conceived to the contribution and design of the study. YY and YZ organized the database. FL performed the statistical analysis. HM and JL wrote the first draft of the manuscript. ZZ, KY, and QL wrote sections of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.940606/full#supplementary-material
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