Molecular Characterization of Rotifers and Their Potential Use in the Biological Control of Biomphalaria

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Background: Schistosomiasis is one of the most important tropical parasitic diseases worldwide. Biomphalaria straminea, the intermediate host of Schistosoma mansoni, has invaded and spread to Southern China since 1974 and may pose enormous threats to public health. Controlling intermediate host snails is an effective strategy in schistosomiasis intervention. However, the only effective chemical molluscicide, niclosamide, currently recommended by WHO may cause environmental pollution, loss of biodiversity, and high costs. Thus, to counter intermediate hosts, a sustainable and environmentally friendly tool is urgently needed. Here, we conducted field investigations to collect and identify a potential snail competitor rotifer and evaluated its molluscicide effect.

Results: In this study, we collected two samples of rotifers from Shenzhen. We found both red and black phenotypic B. straminea snails at the sampling sites. We identified the rotifer population as a species of the genus Philodina according to the amplification and phylogenetic analysis results of cox1 gene. We found that rotifer exposure did not significantly affect the hatching rate of B. straminea eggs but promoted the killing of juvenile snails. Meanwhile, rotifer exposure did not significantly alter the fecundity of B. straminea quantified by the number of eggs per egg mass, the number of egg masses per snail, and the number of eggs per snail; but the snails exposed to rotifers showed lower fecundity performance than the control snails. Importantly, rotifer exposure could significantly affect the development of juvenile B. straminea, showing a smaller shell diameter of the exposed snails than that of the control snails. In addition, rotifer exposure affected the life span of B. straminea snails, showing a 16.61% decline in the average life span. After rotifer exposure, the S. mansoni-infected B. straminea snails died significantly faster than those without rotifer exposure. Similar findings were observed in S. mansoni-infected Biomphalaria glabrata snails. These results implied that rotifer exposure significantly promoted the mortality of S. mansoni-infected B. straminea and B. glabrata.
Conclusions: Our study demonstrated the potential molluscicide effect of rotifers on intermediate hosts under laboratory conditions. Our findings may provide new insights into the development of biocontrol strategies for snail-borne disease transmission.

Keywords: Biomphalaria straminea, Biomphalaria glabrata, Schistosoma mansoni, rotifer, Philodina, biocontrol strategy

INTRODUCTION

Schistosomiasis is one of the most important human parasitic diseases (Chitsulo et al., 2000), causing almost 240 million people infected worldwide, which may cause huge economic and social burdens globally (Colley et al., 2014). Among all human infected schistosomes, Schistosoma mansoni is the most widespread species. S. mansoni is distributed predominantly in South America, Africa, the Caribbean, and the Middle East (Crompton, 1999; Chitsulo et al., 2000; Colley et al., 2014). Biomphalaria snails, including Biomphalaria glabrata and Biomphalaria straminea, are the main intermediate hosts of S. mansoni (Colley et al., 2014). As an important intermediate host of S. mansoni (Coelho and Caldeira, 2016), the freshwater snail B. straminea has invaded Hong Kong, China, since 1974 and has spread widely in South China (Meier-Brook, 1974; Lin et al., 2020). In addition, this invasive snail can also transmit the zoonotic parasite Angiostrongylus cantonensis (Xu et al., 2019; Zhu et al., 2019). Considering the potential risk of transmission of S. mansoni and threats to human health in China (Colley et al., 2014; Lin et al., 2020), it is necessary to pay more attention to monitoring and controlling B. straminea with close surveillance and control strategies.

The strategy of controlling intermediate hosts has been proven to be an effective approach to interrupt the transmission of S. mansoni (Lardans and Dissous, 1998). The application of chemical molluscicides is a major strategy for snail control. Niclosamide is the only molluscicide recommended by the WHO (Yang et al., 2010). However, the environmental effects, high toxicity to non-target organisms, and high costs in most endemic countries have hampered the widespread use of chemical molluscicides (Ekabo et al., 1996; Oliveira-Filho and Paumgarten, 2000). In addition, the application of niclosamide may induce resistance (Dai et al., 2015). To achieve the UN Sustainable Development Goals (SDGs), alternative tools for intermediate hosts and schistosomiasis control are urgently needed. In recent years, biocontrol strategies have attracted significant research attention due to their low toxicity and environmentally friendly features (de Oliveira et al., 2004; Soberon et al., 2013; Wei et al., 2017). Therefore, low toxicity and environmentally friendly tools are urgently needed and suited for invasive snail control.

There is a vast amount of zooplankton in rivers and oceans. As an important type of zooplankton, rotifers are widely distributed in freshwater bodies (Gilbert, 2017). Rotifers are an important food source of fishes in aquaculture (Stelzer, 2009; Dabrowski and Miller, 2018) and can also be indicators of environmental toxicity (Stelzer, 2009; Dabrowski and Miller, 2018; Colvin et al., 2021; Xu et al., 2021) and water quality (Jose et al., 2008; Picapedra et al., 2021). Nevertheless, rotifers can promote mortality by affecting the ingestion of shrimp (Yan et al., 2004; Yan et al., 2007) and cause tissue injury and fish death by attaching to the gills (Imai et al., 1991; Xu et al., 1999; Xu et al., 2000). Mass rotifers may rob food and nutrition from aquatic animals, inducing unhealthy status and mortality among aquatics (Meyabeme et al., 2010; Reyes-Prieto et al. 2014; Ranasinghe and Amarasinghe 2020). B. straminea and B. glabrata are important freshwater snails and invasive species globally. In addition, the identification of microbiota as food competitors, such as rotifers, could be a potential additional tool for mosquito control (Ranasinghe and Amarasinghe, 2020). However, whether rotifers can be competitors of freshwater snails is unclear. Few studies have focused on controlling intermediate hosts by rotifers. Therefore, we hypothesized that rotifers could affect the development and survival of Biomphalaria snails.

In the present study, we collected rotifer samples from field studies in South China from 2016 to 2017 and investigated the influence and survival of Biomphalaria snails affected by rotifer exposure. Our findings may promote the development of biocontrol strategies for intermediate hosts.

METHODS

Sample Collection
To collect the rotifer samples in Guangdong Province, we conducted systematic field surveys from 2016 to 2017. We collected about 100 snails from each sampling site. We found some rotifers were attaching to the surface of the B. straminea shell. Then, we collected samples and transferred alive rotifers to the laboratory. The name of the locality, Global Positioning System (GPS) coordinates, and date were recorded. We took pictures of the surroundings using a camera. The living specimens were maintained under laboratory conditions. We finally preserved several samples in 95% ethanol and stored them in −80°C for further processing.

DNA Extraction
We removed the shell from the snail before genomic DNA extraction. Total DNA was extracted separately from approximately 30 mg of head-foot or the entire rotifer. All samples were individually crushed using a bead mill in an enzyme-free Eppendorf tube.
with 1-mm-diameter inox beads (Qiagen, Germany). After removing the beads, we extracted total DNA using the hipure DNA mini kit (Magen, China) as previously described (Lin et al., 2020). Briefly, genomic DNA was extracted according to the protocol of the kit, and finally, total DNA was suspended in 30 μl of nuclease-free buffer and stored at −80°C until further processing. The DNA quality and quantity were examined using a NanoDrop instrument (Thermo Fisher Scientific, USA).

Amplification and Sequencing
The DNA samples were amplified for identification as described in the previous study (Lin et al., 2020). The universal cytochrome oxidase subunit (cox1) I primer set for rotifer identification was used: LCO1490 5′-GGTCAACAATCATAAGATATTGG-3′ and HCO2198 5′-TAAACTTCAAGGGTGAACAAAAATCA-3′. The PCR amplification system for the target gene comprises 1 μl of cDNA, 12.5 μl of a mixture, 1 μl of forward primer, 1 μl of reverse primer, and 9.5 μl of double deionized water. The PCR cycling conditions were carried out: initial denaturation step at 94°C for 5 min followed by 30 cycles of 94°C for 45 s, 48°C for 45 s, and 72°C for 45 s with a final extension step at 72°C for 10 min. In addition, The universal cox1 primer set was also used for the Biomphalaria species identification. The PCR conditions for the marker amplification were performed: denaturation at 94°C for 5 min, 30 cycles of 94°C for 50 s, 55°C for 50 s, 72°C for 50 s, and final extension at 72°C for 10 min. The PCR products were detected on 3% agarose gel electrophoresis and purified according to the protocol of the Qiagen gel extraction kit (Qiagen, Germany). The purified PCR products were sequenced on an ABI-3730 platform (Applied Biosystems) by the Majorbio company (Guangzhou, China).

Phylogenetic Analysis
The sequences obtained from sequencing and the National Center for Biotechnology Information (NCBI) databases (https://www.ncbi.nlm.nih.gov/) were aligned and concatenated by the neighbor-joining method using the molecular evolutionary genetics analysis (MEGA) 7 (Kumar et al., 2016). We performed the parsimony analysis by generating 1,500 bootstrap replicates.

Maintenance of the Snails in the Laboratory
The Biomphalaria snails were raised under laboratory conditions as described in the previous study (Lin et al., 2020). Each snail was exposed to 10 S. mansoni miracidia. The procedures for infecting snails with miracidia were described in the previous study (Keiser et al., 2014). The S. mansoni-exposed snails were maintained with shading treatment. The infection rate was measured as described in the previous study (Fernandez and Thiengo, 2002). The release of cercaria from Biomphalaria was previously described (Lin et al., 2020).

Exposure Experiment Design
To investigate the effect of rotifers, we used eggs, juvenile, and mature snails to perform further experiments. i) We randomly divided eggs or snails on the same developmental stage into two groups: normal snails being exposed with or without rotifers. ii) S. mansoni miracidia-infected snails were also randomly divided into two groups: snails being exposed with or without rotifers. We selected the 2-week-old Biomphalaria snails for S. mansoni-infected experiments. After S. mansoni miracidia exposure experiments, both the exposed and unexposed snails were maintained under the same conditions. The survival rate was measured. The snail releasing S. mansoni cercaria was considered an infected snail.

Influence of Parameters Measured
The fecundity (the number of eggs per egg mass, the number of egg masses per snail, and the number of eggs per snail) and fertility (rate of eggs hatched per mass) were measured as previously described (Costa et al., 2004). The number of hatching embryos was examined in 2 weeks, and subsequently, the egg hatchability was calculated. The survival and growth rates (shell diameter) were measured. The snails being measured for the shell diameter were randomly picked out from the alive juvenile snails.

Statistical Analysis
We calculated the results using GraphPad Prism version 6.0 (GraphPad Software, USA). Data are expressed as the mean ± standard error of the mean (SEM). The differences between groups were analyzed by Student’s t-test using SPSS 19.0 software (SPSS Inc., USA). The survival rates between groups were analyzed using the chi-square test. *p < 0.05 was considered statistically significant.

RESULTS

Sampling Site Study
We found that some rotifers were attached to the surface of the shell of Biomphalaria snails in field studies. Then, we collected rotifer samples from the sites of the Guanlan River (22°40′18″ N and 114°2′25″ E) and Donghu Park (22°33′26″ N and 114°8′38″ E) in Shenzhen in South China (Figure 1A). Pictures of the surroundings of these sampling sites are shown (Figures 1B, C). In addition, maximum-likelihood trees showed that both the red and black phenotypic Biomphalaria snails collected from Shenzhen were similar to the South American B. straminea strain (Figures 1D, E).

Species Identification of Rotifer
We found that the rotifers were mainly attached to the navel of Biomphalaria snails (Figure 2A). To determine the species identification of rotifers from sampling sites, cox1 gene was amplified and sequenced for phylogenetic reconstruction. The PCR fragments of cox1 gene of rotifer were amplified and resolved in an agarose gel (Figure 2B). The five referenced sequences of mitochondrial genes obtained from the NCBI database included EF650549.1 (uncultured bdelloid rotifer), DQ078567.1 (Philodina sp.), HM032977.1 (Philodina sp. Pha3), DQ078584.1 (Philodina sp.), and MT895717.1 (Philodina sp.).
We found that our samples clustered on the same branch, similar to the branches of uncultured bdelloid rotifers and Philodina sp. (Figure 2C). The sequence similarity between these two clusters was greater than 97% according to the BLAST results (https://blast.ncbi.nlm.nih.gov/Blast.cgi). These results showed that our rotifer samples belonged to the genus Philodina. Therefore, we named our rotifer samples collected from Shenzhen Philodina sp. sz1 and Philodina sp. sz2.

Rotifer Exposure Did Not Significantly Influence the Hatching Rate of B. straminea Egg Masses

To investigate the effect of rotifers on the hatching rate of B. straminea, we randomly divided egg masses of B. straminea into two groups. We observed gelatinous intima and extima on the egg masses (Figure 3A). Rotifers were only attached on the edge of the Biomphalaria egg mass and segregated into eggs by the extima and intima (Figure 3A). We found no significant difference in the hatching rates between groups infected with (0.7839 ± 0.03658) or without (0.8267 ± 0.02927) rotifers, but on average, the hatching rate declined in the rotifer-infected masses (Figure 3B).

Rotifer Exposure Affected the Development of Juvenile B. straminea Snails

As our results showed before, there was no significantly different effect on the hatchability. However, the required times for juvenile snails to hatch from the egg mass were not similar, ranging from 5 to 14 days. The juvenile snails hatching from egg masses were exposed to rotifers (Figure 4A), and the juvenile snails immediately become infected with rotifers after hatching.
We found that the survival rate of juveniles in the infected group declined significantly compared with that of the control snails (Figure 4B). In addition, rotifers affected the development of juveniles, which showed a significantly smaller shell diameter than the control snails (Figure 4C).

Rotifer Exposure Affects the Fecundity and Sexual Maturation Time of *B. straminea*

We found no significant differences in the number of egg masses per snail per day (Figure 5A), the number of eggs per snail (Figure 5B), or the number of eggs per mass (Figure 5C). However, rotifer
exposure significantly affected the oviposition time of juvenile *B. straminea*, indicating that rotifers may delay the sexual development of *B. straminea* (Figure 5D and Table 1). Our study showed that there was no significant difference in the fecundity of snails infected with or without rotifers.

**Rotifer Exposure Affected the Life Span of *B. straminea* Snails**

To test whether rotifer exposure could impact aging and affect life span, we treated juvenile *B. straminea* with rotifers. We measured the life spans of both exposed snails and control snails (without rotifer exposure). Since our previous results showed that rotifer exposure significantly affected the survival rate of juvenile *B. straminea*, we selected 5-week-old snails for further studies. We found that exposed *B. straminea* experienced dramatic life span shortening as compared with the control group (Figure 6, Table 2), showing a 16.61% decline in median life span after rotifer exposure.

**Rotifer Exposure Affected the Survival of *S. mansoni*-Infected *B. straminea* and *B. glabrata* Snails**

As an intermediate host of *S. mansoni*, *B. straminea* plays an important role in the transmission of *S. mansoni*. Therefore, reducing the transmission risk of *S. mansoni* can be helpful for disease control. However, we found that rotifer exposure did not significantly alter the infection rate of *S. mansoni*-exposed *B. straminea* snails (Figure 7A). To conduct further experiments, we used positive snails that can release the cercaria of *S. mansoni*. After rotifer exposure, the *S. mansoni*-infected *B. straminea* died faster than the unexposed snails (Figure 7B and Table 3), showing a decline in the release time of cercaria from intermediate hosts.

*B. glabrata* is an important model organism for researching the interaction mechanism between *S. mansoni* and mollusks. We also detected the effect of rotifer exposure on the survival of *S. mansoni*-infected *B. glabrata*. We found that rotifer exposure did not significantly alter the infection rate of *S. mansoni*-exposed *B. glabrata* snails (Figure 8A). Since the infection rate was not 100% after *S. mansoni* miracidia exposure, we used positive snails that could release the cercaria of *S. mansoni* for further studies. We found that the *S. mansoni*-infected *B. glabrata* died significantly more than unexposed snails after 15 weeks of *S. mansoni* infection (Figure 8B). Rotifer exposure may accelerate the death of Biomphalaria snails infected with *S. mansoni*. Our results showed that rotifer exposure did not significantly alter the infection rate but significantly promoted...
the mortality of S. mansoni-exposed Biomphalaria snails, indicating the potential use of rotifer exposure on snail-borne disease transmission.

### DISCUSSION

The freshwater snail B. straminea, which plays an important role in the transmission of S. mansoni, is one of the most widely distributed species in the genus Biomphalaria and originated from the southeastern part of South America (Colley et al., 2014; Yang et al., 2018). During the last decades, B. straminea has been reported in tropical countries, including Brazil, Paraguay, Argentina, Uruguay, Colombia, and Costa Rica (Lin et al., 2020). B. straminea was first reported to be introduced into Hong Kong in 1974 and has now spread to Shenzhen, Dongguan, Huizhou, and Puning in South China (Colley et al., 2014; Yang et al., 2018). Historically, China has been a non-endemic area for blood flukes of S. mansoni. However, with the increasing imported schistosomiasis cases in China (Dai et al., 2020; Wang et al., 2020) and the spread of the intermediate host (Meier-Brook, 1974; Lin et al., 2020), the potential risk of transmission of S. mansoni is increasing. Considering the potential threats to human health, we should pay more attention...
and make efforts to manage these snails. Controlling intermediate hosts is considered an effective approach to interrupt the transmission of *S. mansoni* and control snail-borne disease schistosomiasis (Lu et al., 2018). Using chemical molluscicides to control snails was the major strategy. However, chemical molluscicides such as niclosamide are highly toxic to other aquatic animals. Therefore, environmentally friendly tools are urgently needed for intermediate host control. In the present study, we detected potential biocontrol strategies for the intermediate host of *S. mansoni*.

We reported the isolation of populations of rotifers collected from Shenzhen for biocontrol tools to the intermediate host of *S. mansoni*. According to *coxI* gene sequence analysis, we positioned these rotifers within the genus *Philodina*. Since these identity levels are greater than the genomic definition of a species based on *coxI* gene, we proposed to name our rotifer samples collected from Shenzhen rotifer *Philodina* sp. sz1 and rotifer *Philodina* sp. sz2. As important zooplankton, rotifers are distributed in all kinds of water bodies, though mainly in freshwater bodies (Lu et al., 2018). Rotifers naturally coexist with aquatic organisms and are an important food source of fish and shrimp (Stelzer, 2009; Dabrowski and Miller, 2018). However, previous studies have shown that rotifers can also affect the development and survival of fish and shrimp (Imai et al., 1991; Xu et al., 1999; Xu et al., 2000; Yan et al., 2004; Yan et al., 2007). Yet whether rotifers affect the development and survival of Gastropoda, such as *B. straminea* snails, is unknown.

*B. straminea* has already spread to Hong Kong and Guangdong provinces in South China (Lin et al., 2020). Our findings revealed that both the red and black phenotypic *Biomphalaria* snails collected from Shenzhen were similar to the South American *B. straminea* strain, implying that these two sites exhibited two kinds of invasive freshwater snail phenotypes, *B. straminea*. The population level of the intermediate host *B. straminea* may be associated with the number of snails exposed

![FIGURE 7](image-url) The effect of rotifer exposure on the survival of Schistosoma mansoni-infected Biomphalaria straminea snails. (A) Rotifer exposure did not significantly affect the infection rate of *B. straminea* to *S. mansoni*. (B) Rotifer exposure decreased the survival rate of *S. mansoni*-infected *B. straminea*. Unexposed; the *S. mansoni*-infected snail was not exposed to rotifers. Exposed; the *S. mansoni*-infected snail was exposed to rotifers. ns, Not statistically significant.

**TABLE 3** | The average difference in the life span of Schistosoma mansoni-infected Biomphalaria straminea after rotifer exposure.

| Item             | Unexposed (n = 5) | Exposed (n = 4) | Significance |
|------------------|-------------------|----------------|--------------|
| Life span (days) | 66.80 ± 2.417     | 76.50 ± 2.723  | *p* < 0.05   |

![FIGURE 8](image-url) The effect of rotifer exposure on the survival of Schistosoma mansoni-infected Biomphalaria glabrata snails. (A) Rotifer exposure did not significantly affect the infection rate of *B. glabrata* to *S. mansoni*. (B) Rotifer exposure decreased the survival rate of *S. mansoni*-infected *B. glabrata* in 15 weeks. ns, Not statistically significant.
to *S. mansoni* in the field: the more snails that exist, the more snails that are infected (de Souza et al., 1981; Fernandez and Pieri, 2001; Gandasegui et al., 2018). We found that rotifer exposure did not significantly affect the hatching rate of *Biomphalaria* eggs, and we hypothesized that a gelatinous membrane may cover the eggs, protecting them from pathogens. The hatching rate of *B. straminea* in our study was similar to that in previous studies (Scherrer et al., 1976; Costa et al., 2004) but was lower than that of *Biomphalaria pfeifferi* (Kengne-Fokam et al., 2016). In addition, rotifer exposure did not significantly affect the fecundity of *B. straminea*, showing no difference, but declines in the number of eggs per egg mass, the number of egg masses per snail, and the number of eggs per snail. We hypothesized that the reason for these declines was that there was enough food to supply snails and protect the fecundity of snails. The fecundity performance of *B. straminea* in our work was lower than that of *B. glabrata* (Rozenberg et al., 1992; Costa et al., 2004). These results suggested that the fertility of the genus *Biomphalaria* snails may be associated with genotype.

Our study revealed that rotifer exposure can significantly affect the development of *B. straminea* snails according to the shell diameter results, implying a potential influence on the reproduction and maturity of *Biomphalaria* snails. Previous studies revealed that there was an increase in growth inhibition in *S. mansoni*-infected snails (Looker and Etges, 1979; Meier and Meier-Brook, 1981; Cardoso and Coelho, 1990). Therefore, the growth of rotifer-exposed snails was similar to that of *S. mansoni*-infected snails. However, the association between growth alterations and parasite infection is unclear. Further studies on the mechanism of growth inhibition by rotifers are needed.

Rotifer exposure affected the survival rate of *B. straminea* snails, mainly juvenile snails. Importantly, rotifer exposure caused a significant decline in the average life span of *B. straminea* snails. Although previous studies have attempted to explore control strategies for intermediate hosts in China, they have mainly focused on chemical molluscicides, including salicylanilidate (He et al., 2017) and pyridylphenylurea derivatives (Wang et al., 2018). We focused on environmentally friendly tools to control intermediate hosts. Our findings suggested that rotifers may become a potential biocontrol tool for the intermediate host of *S. mansoni*. As one of the biocontrol strategies, pathogenic bacteria, including *Candidatus Paenibacillus glabratella* (Duval et al., 2015), *Bacillus thuringiensis* (Soberon et al., 2013), and *Beauveria bassiana* (Wei et al., 2017), have been further studied and have become potential alternative tools in disease intervention. Although the application of chemical molluscicides such as niclosamide is the most widely used method for snail control (Lardans and Dissous, 1998), we believe that environmentally friendly tools for intermediate hosts will be obtained with increasing research on biocontrol strategies.

Our work revealed that rotifers did not significantly affect the survival rate of adult *Biomphalaria* snails or the infection rates of *S. mansoni*-exposed snails. The susceptibility of the *Biomphalaria* snails mainly depends on their immune system, not foreign organisms (Hanington et al., 2010; Pila et al., 2016). Our findings demonstrated that rotifers promoted the killing of *S. mansoni*-infected *Biomphalaria* snails, including *B. straminea* and *B. glabrata*, implying that rotifer exposure may decrease the releasing cercaria of *S. mansoni* over time and contribute to disease control. Trematode parasites and their molluscan hosts produce antioxidants and oxidants to maintain the cellular redox balance, which may explain their survival in the late stage of parasite infection (Bayne et al., 2001; Bayne, 2009; Mourao et al., 2009). Rotifers not only grab food from other species but also receive foreign DNA from the animal kingdom, fungi, plants, and bacteria (Gladyshiev et al., 2008; Boscetti et al., 2012; Szydlowski et al., 2015). Therefore, rotifer exposure may increase the burden on the survival of *Biomphalaria* snails and ultimately induce an imbalance. These results implied that rotifer exposure may interrupt the immune balance between *S. mansoni* and host snails, leading to snail mortality. However, the mechanisms of these findings are unclear, and further studies are needed.

**CONCLUSION**

In our study, we identified a species of the genus *Philodina* rotifer collected from Shenzhen, South China. Rotifer exposure can alter the fecundity and significantly affect the fertility and life span of *B. straminea*, promote the death of juvenile snails, and significantly promote the mortality of *S. mansoni*-infected *B. straminea* and *B. glabrata*. Overall, our study demonstrated that rotifers may contribute to snail control and disease intervention by affecting the development and population quantity of *Biomphalaria* snails, in addition to *S. mansoni*-infected snails. Our results implied that rotifers may be a potential use and supplement in controlling snail-borne schistosomiasis transmission.

**DATA AVAILABILITY STATEMENT**

The data presented in the study are deposited in the GenBank repository, accession number (OK156495-OK156499). Please contact the author for additional data requests.

**AUTHOR CONTRIBUTIONS**

ZW, XS, and DL conceived and designed the study. DL carried out the experiments, prepared the manuscript, and handled the statistical analysis and interpretation of the data. DL, SX, BS, and YL critically revised the draft version of the paper. All authors contributed to the article and approved the submitted version.

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