Physiological and microbiome adaptation of coral Turbinaria peltata in response to marine heatwaves

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Abstract

Against the backdrop of global warming, marine heatwaves are projected to become increasingly intense and frequent in the future. This trend poses a potential threat to the survival of corals and has the capacity to lead to the destruction of entire coral reef ecosystems. Although many studies have confirmed the resilience of corals to heat stress, but their ability to withstand repeated heatwave events occurring in nature remains unclear. In this study, focusing on physiological and symbiotic microorganism responses, we examined the adaptation and resilience of Turbinaria peltata after repeated exposed to marine heatwaves. In the first heatwave, From physiological perspective, Turbinaria peltata showed the average values of Chl a and endosymbionts increased, while GST, Caspase-3, CAT, and SOD showed significant decreases ($p < 0.05$) upon repeated exposure to heatwaves compared to the initial exposure. In terms of bacteria, the abundance of Leptospira which functional prediction indicating potential pathogenicity and intracellular parasitism, increased significantly during the initial exposure to the heatwave. In contrast, probiotic bacteria such as Achromobacter arsenitoxydans and Halomonas desiderata, which might related to mobile elements, biofilm formation, stress-tolerant bacteria, Gram-positive bacteria, nitrogen uptake, and nitrate uptake, showed significant increases during the re-exposure to the heatwave. Overall, the results indicate that Turbinaria peltata adapts to marine heatwaves through physiological regulation and changes in the microbial community.

1. Introduction

Marine heatwaves (MHWs) are extreme climate events of anomalously high surface temperature, which might last for days to months from local to regional scales (Hughes et al., 2019). With the ongoing climate change, MHWs have become more frequent and severe, resulting in escalating damage to coral reefs (Hughes et al., 2018; Oliver et al., 2018; Dietzel et al., 2020). These MHWs lead to abnormally high water temperatures, causing corals to bleach by expelling the symbiotic algae within a short perioding of time, which would affect the survival and reproduction of corals, even destroying the entire coral ecosystem (Hoegh-Guldberg et al., 2007; Hughes et al., 2018).

It is worth noting that more and more studies have found that corals have some tolerance to high temperatures, and based on this tolerance, corals may survive after a short time of heat stress (Hughes et al., 2003). Studies have found that Corals might adjust their zooxanthellae density or ratio (Barker, 2018; Yu et al., 2020), modify the composition of their membrane lipids, particularly by increasing the presence of unsaturated fatty acids with double bonds, and enhance the production of antioxidant substances, enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST), as well as non-enzymatic antioxidants such as vitamins, carotenoids, and tocopherols to acclimate to the high temperature(Krueger et al., 2017; Kultz, 2020). These adaptive mechanisms bolster the coral's ability to withstand stress and protect the integrity of their cell membrane structures.

In addition to corals and zooxanthellae changes, there are also research findings that have highlighted the critical role of coral symbiotic bacteria in helping corals cope with heat stress (Claar et al., 2020; Sun et

al., 2023). Previous studies have found that the composition and function of coral's symbiotic bacteria might change to facilitate the coral's adaptation and ecological plasticity in adapting to rapid environmental changes (Roder et al., 2014; Frade et al., 2016; Lee et al., 2016; Neave et al., 2016).

Due to global warming, there is an increasing trend of MHWs (Frolicher et al., 2018; Oliver, 2019a; Oliver et al., 2019b). Although there have been numerous studies have reported on the mechanisms of coral response to MHWs, research on the impacts of repeated exposure to MHWs remains limited (Claar et al., 2020; Marzonie et al., 2023).

Turbinaria peltata is widely distributed in the Indo-Pacific region, and it is an essential ecological and dominant species in the South China Sea. Due to its resistance to environmental changes and stress, it can be used as an important model organism to study the response mechanism of coral holobiont to the environment. So in this study, we investigate the physiological and microbiological effects of repeated MHWs on reef-building coral *Turbinaria peltata* to assess its responses to extreme climatic conditions. These findings will provide valuable data and reference for understanding the response and adaptation mechanisms of corals to repeated MHWs.

2. Materials and methods

2.1 Sample collection

Turbinaria peltata specimens were collected from Xuwen Coral Reef National Nature Reserve (109°55'76E, 20deg16'N). The corals were temporarily housed in a 200 L tank with the following water conditions: temperature of 26 , pH of 8.0, and salinity of 33 for a duration of 7 days. Artificial seawater was prepared using coral salt from Aquarium Systems in France. The corals were illuminated using a full-spectrum LED lamp (Maxspect) with a light-dark ratio of 12 h:12 h and an effective radiation of 200 μmol ($m^{-2}s^{-1}$). After the acclimation, *Turbinaria peltata* colonies were fragmented into 48 pieces and placed in another 200 L tank for an additional week until the corals exhibited normal elongation.

2.2 Experimental design

To simulate the impact of heatwaves on corals, a heatwave treatment was conducted by manipulating the seawater temperature over a specific period (Fig. 1). Coral nubbins were randomly divided into 6 aquariums, each with a capacity of 20L. Two experimental groups were established: the control group ("C") and the heatwave group ("H"), based on historical high-temperature extremes recorded in Xuwen over the past 10 years (source: http://gd.cma.gov.cn).

From day 1 to day 5, a microcomputer digital thermostat (XH-W1308, Tyrell, Shenzhen, China) was used to gradually increase the seawater temperature from 26 °C to 32 °C, representing the first heatwave. Subsequently, from day 6 to day 10, the seawater temperature was reduced back to 26 °C, simulating the first recovery period. Starting from day 11 to day 15, the seawater temperature was increased again to 32 °C to model a second heatwave. Finally, from day 16 to day 20, the seawater temperature was lowered to 26 °C to simulate the second recovery period. In the control treatment, the seawater temperature was maintained constantly at 26 °C throughout the entire exposure period.

Specific dates were assigned as "day 5" (end of the first heatwave), "day 10" (end of the first recovery period), "day 15" (end of the second heatwave), and "day 20" (end of the second recovery period), and were denoted as "A", "B", "C", and "D", respectively. From each group, three corals were randomly selected for sampling.

Fig. 1. Design of the culture experiment.

2.3. Physiological indicators

The samples were rinsed with an equal volume of sterilized seawater, and divided into 6 portions, each containing 12 mL, and then subjected to centrifugation at 4 °C for 10 minutes at 4000 rpm. The precipitates were used to determine the density and Chl *a* content of the zooxanthellae, while, the supernatants were used

to measure the activity of various enzymes. After drying, the surface area of the skeletons was determined using the aluminum foil technique (Marsh, 1970; Fu et al., 2022).

For the analysis of endosymbiont density and Chl *a* content, three portions of the precipitate were washed and suspended in 5 mL of formaldehyde. The density of the endosymbiont was determined using a microscope with a blood counting plate. The remaining three samples were resuspended in 8 mL of methanol and extracted at 4 °C for 24 hours. After centrifugation at 4 °C for 10 minutes at 4000 rpm, Chl *a*content was determined using a UV-visible spectrophotometer (Ritchie, 2006).

Quantitative supernatants were collected for further analysis of GST, Caspase-3, SOD, and CAT activities using commercial dilution kits (AKPR013U, AKPR027-1, AKBL006C, AKEN001U, BOXBIO, Beijing, China). Finally, the protein concentrations were determined using a Bradford kit (AKPR015, BOXBIO, Beijing, China). The enzyme activity units were normalized to U $mgprot^{-1}$. Data conversion was performed using Excel 2016, while graphing was done using GraphPad Prism 8.0.2.

2.4 Microbiological analysis

Genomic DNA was extracted from a 0.5 g frozen coral sample using the TGuide S96 magnetic bead method and the DP812 DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China). The V3-V4 variable region of the bacterial 16S rRNA gene was PCR amplified with the forward primer 27 F (5'-AGRGTTTGATYNTGGCTCAG-3') and the reverse primer 1492 R (5'-TASGGHTACCTTGTTASGACTT-3') (Zhong et al., 2021). Library preparation was performed using the SMRTbell Template Prep Kit (PacBio, USA), and the PCR reaction was conducted in a Veriti 96-well thermal cycler (Applied Biosystems, USA). Sequencing was carried out on the Sequel II platform after purification.

After obtaining the sequencing data, the raw reads underwent filtering to remove adapter sequences using Trimmomatic (version 0.33) and Cutadapt (version 1.9.1) software (Bolger et al., 2014; Martin, 2011) to obtain clean reads. Paired reads were merged using USEARCH (version 10) (Edgar, 2013), and chimeric sequences were removed using UCHIME (version 8.1). Subsequently, high-quality sequences were obtained for further analysis. The sequences were clustered at a 97 % similarity level using USEARCH (version 10.0) with a default OTU filtering threshold of 0.005 % of the total sequencing count. For ASV analysis, the DADA2 method in QIIME2 (version 2020.6) was employed to denoise the data, with the default filtering threshold of 0.005 % of the total sequencing count.

The obtained high-quality sequences were then annotated against databases such as Silva, Unite, Greengenes, NCBI, Fungene, and MaarjAM for taxonomic classification and functional prediction using BugBase and FAPROTAX. Alpha and beta diversity analyses, community composition analyses, and BugBase and FAPROTAX functional prediction analyses were conducted using BMKCloud (www.biocloud.net). Core OTU analysis was carried out using Venn diagrams to identify shared OTUs among different sample groups, and the composition and relative abundance differences of core OTUs between groups were analyzed using pie charts and heatmaps. Data visualization and figure generation were performed using GraphPad Prism 8.0.2 and RStudio. The data that support the findings of this study are openly available in figshare: https://doi.org/10.6084/m9.figshare.24152901.

3. Results

3.1Physiological response

The physiological response of *Turbinaria peltata* to MHWs stress was remarkable (Fig. 2). After the first MHWs, the density of zooxanthellae reduced about 79.44 % compared to the control group, and the concentration of Chl *a* decreased about 33.67 %. While, the levels of GST, Caspase-3, SOD, and CAT showed a significant increase. The effect of the second MHWs was less intense, as indicated by the slight decrease in density of zooxanthellae and concentration of Chl *a* , which showed about 69.71% and 17.63% decrease compared to the control group, respectively. Although there was an increase in levels of GST, Caspase-3, SOD, and CAT, it was not as high as those of the first heat stress. Compared to the first heat stress, the

levels of GST and Caspase-3 exhibited a significant decrease ($p < 0.05$), and the levels of CAT and SOD also decreased although it's not significant.

Fig. 2. Response of coral physiological indicators under different treatments. a: zooxanthellae, b: Chl a, c: GST, d: Caspase-3, e: CAT, f: SOD.

3.2 Diversity analysis of bacteria

After analyzing the high-throughput sequencing data, we got 318,431 optimized sequences without chimeras. The dilution curve of the Sobs index reached a plateau, indicating sufficient sequencing data and a wide range of detected microbial species, reflecting microbial diversity.

At the end of the first heatwaves, the Simpson index showed consistency compared to the control group. However, the Shannon index, Chao1 index, and Ace index were slightly higher than those of the control group (Fig. 3). In the second MHWs, each index showed a slight decrease compared to the control group.

Fig. 3. α-diversity analysis of coral-symbiotic bacteria under different treatments. a: Shannon. b: Simpson. c: Chao1. d: Ace.

The PCoA (Principal Coordinate Analysis) results showed that the first two axes accounted for about 69.39% of the variation in microbial community composition among all the samples. PC1 explained 52.74 % of the variation, while PC2 explained 16.65 % (Fig. 4). The three replicate samples within each group exhibited close clustering and high repeatability, and more variation was observed between groups than within groups.

Fig. 4. PCoA analysis of coral symbiotic microbial communities under different treatments.

3.3 Difference in Bacterial community composition

The analysis of co-occurring bacterial communities revealed the presence of 36 phyla, 88 orders, 242 classes, 413 families, 858 genera, and 1,422 species across all samples. As shown in Figure 5-a the top 10 bacterial phyla in terms of relative abundance were Proteobacteria, Bacteroidetes, Verrucomicrobia, Firmicutes, Actinobacteria, Campylobacterota, Dadabacteria, Vibrio vermicularis, Bdellovibrionota, and Desulfobacterota. The remaining 16 bacterial phyla were classified under the "other" category.

After the first MHWs, the composition of co-occurring bacterial communities was similar between the CA and HA groups. After the 5-day recovery, the relative abundance of Ascomycetes and Micrococcus warty decreased significantly, while the Mycobacterium anisopliae increased significantly in the HB group. After the second MHWs, the relative abundance of Ascomycetes and Warty Microbacteria phylum showed a significant decrease, while the Anabaena phylum exhibited a significant increase in the HC group.

At the same time, we analyzed the distribution of symbiotic microbial communities at the genus level, which is depicted in Figure 5-b. The top 15 bacterial genera in terms of relative abundance were u. Flavobacteriaceae, *Marivita* , u. Legionellaceae, *Ruegeria* ,*Achromobacter* , *Methylotenera* , *Limnobacter* ,*Mycoplasma* , *Halomonas* , *Donghicola* ,*Rubritalea* , u. Helicobacteraceae, u. Bacteria, *Coxiella* , and u. Rhizobiaceae.

Considering the relative abundance of coral symbiotic bacteria, after the first MHWs, the HA group exhibited an increase of u. Legionellaceae,*Ruegeria* , and *Achromobacter* , while a decrease of u. Flavobacteriaceae, *Marivita* , *Methylotenera* ,*Limnobacter* , *Donghicola* , and *Coxiella* . Following the 5-day recovery, the HB group showed an increase in the relative abundance of u. Flavobacteriaceae, u. Legionellaceae, and*Achromobacter* , along with a decrease of *Marivita* ,*Ruegeria* , *Methylotenera* , and *Rubritalea* . After the second MHWs, the HC group displayed an increase in the relative abundance of u. Flavobacteriaceae, u. *Legionellaceae* ,*Achromobacter* , and *Halomonas* , while *Marivita* ,*Ruegeria* , *Methylotenera* , and *Rubritalea* showed decreased. Finally, after the secondary 5-day recovery, the HD group exhibited an increase in the relative abundance of *Rubritalea* , whereas the relative abundance of u. Flavobacteriaceae, *Marivita* , and *Ruegeria* .

Fig. 5. Coral symbiotic bacterial community structure, a: histogram of community analysis at the phylum level, b: histogram of community analysis at the genus level. Unclassified species are indicated by u.

(unclassified) plus the family name.

3.4 Changes in bacterial phenotypes and functional groups

3.4.1 BugBase Forecast

BugBase analysis is capable of predicting the phenotypes of prokaryotic bacteria in environmental samples. In Figure 6-a, the predominant phenotypes observed in all samples at the end of the experiment were identified as Gram Negative, Stress Tolerant, Oxygen demand (Aerobic, Anaerobic, Facultatively Anaerobic), Gram-Positive, Potentially Pathogenic, Contains Mobile Elements, and Forms Biofilms. The study showed that the abundance of different phenotypes of microorganisms changed at different treatment periods. Following the initial MHWs, an increase in the relative abundance of Potentially Pathogenic and Gram-Positive bacteria was observed in the HA group. After the 5-day recovery, the HB group displayed an increase in the relative abundance of Stress Tolerant and Gram-Positive bacteria. Subsequently, after the second round of MHWs, the HC group showed an increase in the relative abundance of Mobile Elements, Forms Biofilms, Stress Tolerant, and Gram-Positive bacteria. Similar patterns were also observed in the HD group, while there was an elevation in the relative abundance of Anaerobic bacteria at the same time.

3.4.2 FAPROTAX Forecast

In this study, we analyzed the ecological functions of prokaryotic bacteria with FAPROTAX. Based on Figure 6-b, the experimental group of symbiotic bacteria displayed a wide range of ecological functions. These functions included intracellular parasites, nitrogen respiration, nitrate respiration, nitrate reduction, and other unidentified functions. These findings provide insights into the functional versatility and contributions of symbiotic bacteria within the ecosystem. The symbiotic microbial functions exhibited distinct changes throughout the experiment. At the end of the initial MHWs, the HA group showed a marked increase in the relative abundance of intracellular parasite bacteria., On the contrary, after the 5-day recovery, the HB group maintained a significantly high abundance of intracellular parasite bacteria. Following the subsequent MHWs, the HC group demonstrated a notable increase in the relative abundance of nitrogen respiration, nitrate respiration, and nitrate reduction bacteria. Additionally, after a 5-day recovery period, the HD group exhibited an elevated relative abundance of nitrate reduction and Other bacteria.

Fig. 6. Functional analyses of coral symbiotic microbial communities, a: BugBase bacterial phenotypic structure analyses, b: eco-functional composition analyses of FAPROTAX community.

3.5 Core microbiological analysis

In order to examine the changes in symbiotic bacteria within corals, a total of 24 samples from the two MHWs treatments were analyzed. The distribution of core operational taxonomic units (OTUs) was assessed, and a total of 28 core OTUs were identified (Fig. 7a). At the genus level, the distribution of these core OTUs is depicted in Figure 7-b: u. Flavobacteriaceae (23.58 %), *Marivita* (9.43 %), *Ruegeria*(5.92 %), u. Legionellaceae (5.90 %), *Achromobacter* (3.95 %), u. Methylotenera (2.12 %), *Limnobacter* (1.60 %),*Rubritalea* (1.43 %), *Mycoplasma* (1.38 %),*Donghicola* (1.30 %), u. Bacteria (1.21 %), u. Helicobacteraceae (1.18 %), u. Dadabacteriales (1.03 %), and *Coxiella* (1.00 %), indicating their dominant presence within the core OTUs.

As shown in Figure 7-c, the core OTUs species-level microbial relative abundance expression heat map was constructed based on the top 30 species with average abundance. we can find that, *Leisingera caerulea* exhibited an increase in the HA group. while the HB group displayed an increase in u. Leptospira, *Halomonas desiderata* , and u. Flavobacteriaceae. After the second MWHs, the HC group exhibited elevated levels of u. Flavobacteriaceae, u. Coxiella, *Halomonas desiderata* , and *Achromobacter arsenitoxydans* . After 5 days of recovery, the HD group demonstrated a significant increase in the relative abundance of *Rubritalea tangerina* , u. Dadabacteriales, u. Woeseia, uncultured *delta proteobacterium* , u. Actinomarinales, and u. DEV007 bacteria.

These results suggest that the MHWs would lead to significant differences in coral-symbiotic microbial

communities, and the core microbial communities would be different between the two heatwaves and the recovery period.

Fig.7. Core bacterial community structure, a: Venn diagram of differences in community structure at the OTU level. b: Pie chart of community analysis at the genus level of core bacteria, with bacteria less than 1% of the genus level classified as others. c: Heat map of community analysis at the genus level of core bacteria. Those not identified to genus level are indicated by u. (unclassified) plus family name.

4. Discusstion

With the ongoing of global warming trend, the frequency of MHWs is expected to increase. So, the ability of corals to adapt to or recover from extreme heat stress will be crucial for their survival in the future. We found that *Turbinaria peltata* was adaptable to high temperatures, which was demonstrated by a reduced coral response to the second heatwave strike, indicated by the decreased loss of the density of zooxanthellae and the content of antioxidant-related enzymes. Decreased abundance of pathogenic bacteria and increased abundance of beneficial bacteria in symbiotic bacteria.

4.1 Physiological Adaptation of *Turbinaria peltata* to marine heatwaves

Recent studies have provided increasing evidence for the adaptive capacity of corals to heat stress (Yu et al., 2020; Hackerott et al., 2021; Schoepf et al., 2022). This adaptability primarily relies on the thermal tolerance of their symbiotic algae, with susceptible populations being expelled while those remaining show enhanced metabolic activity and photosynthetic efficiency to cope with high temperatures (Perniceet al., 2012; Arandia-Gorostidi et al., 2017; Stanley and van de Schootbrugge, 2018). In our study, the initial exposure to MHWs resulted in a significant decrease in the density of diatoms ($p < 0.05$). However, the second exposure to MHWs provided relief from this impact. Furthermore, corals regulate the synthesis and activity of antioxidant enzymes, such as superoxide SOD, CAT, and GST, as well as apoptotic proteases like Caspase-3, to counteract acute heat stress (Bhagooli and Hidaka, 2004; Lesser, 2006; Thummasan et al., 2021). The initial exposure to MHWs led to a significant increase ($p < 0.05$) in the levels of GST, Caspase-3, SOD, and CAT. However, the effects were alleviated upon subsequent exposure to MHWs. This regulatory process helps in clearing excessive reactive oxygen species (ROS), thereby maintaining normal cellular function and physiological state. Consequently, repetitive exposure to heatwaves imposes less physiological stress on corals compared to their initial exposure (Xu et al., 2021), indicating potential physiological adaptations of*Turbinaria peltata* to withstand MHWs.

4.2 Effects of the initial marine heatwave on bacteria

Symbiotic bacteria play a crucial role in coral reef ecosystems by maintaining a balanced bacterial community (Ziegler et al., 2017). However, environmental changes, especially temperature fluctuations, can alter the composition of these bacterial communities, particularly in response to temperature fluctuations (Ziegler et al., 2017, Lima et al., 2020; Zhu et al., 2022). Our findings indicate that after the initial marine heatwave, there was a significant increase in the abundance of a bacterial family called u. Legionellaceae, with functional predictions suggesting their potential as "Potentially Pathogenic" and "intracellular parasites". Legionellaceae are commonly found in coral reef ecosystems and have associations with coral pathogens (Vega et al., 2009), indicating that assumed beneficial bacterial groups within the coral symbiont community may transition into potentially pathogenic and intracellular parasites under high-temperature conditions (Sweet et al., 2013; McDevitt-Irwin et al., 2017).

At the end of the first recovery phase, *Leptospira* ,*Halomonas desiderata* , and u. Flavobacteriaceae maintained a relatively high abundance. *Leptospira* is an intracellular parasite commonly found in the environment (Kavela et al., 2023), and is speculated based on FAPROTAX predictions, to have detrimental effects on corals. Marine heatwaves can lead to increased nitrogen and nitrate levels in coral reef areas (Howells et al., 2012; WIEDEMANN et al., 2013; Grottoli et al., 2018). *Halomonas desiderata* are opportunistic microbes that form a symbiotic relationship with corals, residing on the coral surface and utilizing organic matter present in the coral reef area, which helps corals resist invasion by pathogenic microorganisms (Wang

and Shao, 2021). The symbiotic relationship between Flavobacteriaceae and corals is complex and sensitive (Meyer et al., 2016; Vega et al., 2008; Sweet et al., 2011; Certner and Vollmer, 2018). However, based on functional predictions, the primary function of u. Flavobacteriaceae in this study was an organic matter decomposition. These results indicate that during the recovery period, although potentially pathogenic bacterial groups are present within the core community, beneficial bacterial groups rapidly increase to aid in coral recovery (Sun et al., 2023).

4.3 Bacteria Adaptation during marine heatwaves

The Microbiome Flexibility Hypothesis of Metaorganism Adaptation suggests that changes in microbial communities contribute to the response and adaptation of organisms to environmental fluctuations (Voolstra and Ziegler, 2020). Our results demonstrate that following the second marine heatwave, there was a significant increase in the abundance of certain bacteria.

Specifically, there was an increase in the abundance of u. Coxiella, u. Flavobacteriaceae, *Achromobacter arsenitoxydans* , and*Halomonas desiderata* . Coxiella has been shown to cause diseases in invertebrates and is closely associated with coral health and disease (Antonio et al., 2000; Casas et al., 2004). u. Flavobacteriaceae, as a core community from the first to the second recovery phase, indicates its importance for coral health. Additionally, previous studies have demonstrated that *Achromobacter arsenitoxydans* and*Halomonas desiderata* enhance coral's resistance to environmental stress as stress-tolerant bacteria (Abrego et al., 2008; Rosado et al., 2019).

These findings highlight the complex dynamics of the microbial community during the recovery phases. Although repeated heatwaves can lead to the emergence of pathogenic bacteria, the abundance of beneficial bacterial communities also increased. At the end of the second recovery phase, there was a significant increase in the abundance of u. Dadabacteriales, u. Woeseia, *Rubritalea tangerine* , *uncultured delta proteobacterium* , and other beneficial microorganisms. These bacteria have important roles in coral health and growth status (Roder et al., 2014; Radecker et al., 2015; Röthig et al., 2016; Jiang et al., 2019).

These strategic changes in the microbial community might represent corals adaptive responses to the stress, where the microbial community undergoes adjustments to thrive within the marine heatwave environment (van Oppen and Blackall, 2019). This adaptability of the microbial community is crucial for supporting coral health and resilience in the face of stressful events like marine heatwaves.

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Credit authorship contribution statement

Xin Zhai: Data curation, Writing – original draft, Investigation, Methodology, Formal analysis. YanPing Zhang: Writing – review & editing, Funding acquisition. Jie Zhou: Writing – review & editing. Hao Li: Writing – review & editing. Ao Wang: Writing – review & editing. Liu Li: Writing – review & editing, Conceptualization, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data that support the findings of this study are openly available in figshare: https://doi.org/10.6084/m9.figshare.24152901.

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