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Comparative Transcriptomics Reveals Divergent Gene Expression Patterns Underlying Environmental Adaptation in Two Generations of *Neopyropia yezoensis*

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Abstract

Neopyropia yezoensis and other red algae in the order Bangiales possess a typical heteromorphic alternation of generations life cycle. However, the mechanisms underlying the alternation and evolution between the two distinct generations, the thallus and conchocelis, remain unclear. In this study, we analyzed transcriptomic data from the thallus and free-living conchocelis by RNA sequencing to identify key metabolic pathways and regulatory mechanisms underlying the alternation of generations, filamentous thallus growth, and maturation. A total of 12,255 genes were identified in *N. yezoensis*, among which 3,688 genes were screened as significantly differentially expressed genes (DEGs) with the criteria of fold change≥2 and adjusted p-value < 0.05, including 2,373 upregulated genes and 1,315 downregulated genes in the thallus relative to the conchocelis. The results showed that the differences in environmental stress factors and functional roles between the thallus and conchocelis of *N. yezoensis* determine their transcriptomic divergence. Specifically, genes related to the photosynthetic system (e.g., thylakoid membrane, photosynthetic membrane, photosystem I and II genes) were significantly upregulated in the thallus with fold change≥2; meanwhile, genes involved in protein phosphorylation and dephosphorylation (kinase activity, phosphotransferase activity genes) also exhibited higher expression levels in the thallus compared to the conchocelis, with the related DEGs enriched in the photosynthesis (ko00195) and photosynthesis-antenna proteins (ko00196) pathways of KEGG database. In contrast, the filamentous conchocelis displayed significant upregulation (fold change≥2) of genes regulating the membrane system (endoplasmic reticulum, endomembrane system, membrane and membrane part genes) and the actin cytoskeleton, with the core DEGs enriched in the regulation of actin cytoskeleton (ko04810), nitrogen metabolism (ko00910) and purine metabolism (ko00230) pathways, which facilitates its adaptation to the marine environment and maintenance of the slender morphological characteristics. Therefore, the differential expression of these regulatory pathway genes drives the alternation of generations between the thallus and conchocelis, resulting in significant differences in their life histories and environmental adaptation strategies. This study deepens our understanding of the molecular mechanisms underlying alternation of generation and environmental adaptation in *N. yezoensis* at the transcriptomic level.

Keywords

Neopyropia yezoensis, thallus, conchocelis, transcriptome, environmental adaptation

1. Introduction

Neopyropia yezoensis, a species of red algae, is classified under the phylum *Rhodophyta*, class *Bangiophyceae,* order Bangiales, family *Bangiaceae,* and genus *Neopyropia* [1-2]. It is a typical intertidal macroalga with a monostromatic cellular structure [3], widely distributed in the coastal intertidal zones north of the Yangtze River in China (e.g., Qingdao and Weihai) and in coastal areas of Jiangsu Province [4]. As a key representative of the order Bangiales, it serves as a classic model organism for investigating heteromorphic alternation of generations in red algae, with its life cycle comprising two developmentally distinct stages that differ drastically in morphology, physiology, and adaptive strategies to cope with the fluctuating intertidal marine environment [5]. Heteromorphic alternation of generations is a key evolutionary adaptation of marine algae to complex, fluctuating aquatic environments [6]. Deciphering its underlying molecular regulatory mechanisms is crucial for understanding the evolutionary origins and environmental adaptation strategies of red algae, which represent one of the earliest-diverging lineages of photosynthetic eukaryotes [7].

Transcriptomic studies on the molecular mechanisms and metabolic pathways of *N. yezoensis* have become a research focus in marine algal molecular biology, as they not only reveal the genetic basis of its developmental regulation and environmental stress response but also lay a robust molecular foundation for elucidating the evolutionary characteristics of alternation of generations in red algae [8-10]. Previous studies have identified a series of functional genes and metabolic pathways associated with environmental stress adaptation in this species, providing preliminary insights into its molecular response to abiotic stressors such as temperature, salinity, and light [11]. However, the core scientific question of how molecular regulatory networks drive morphological and physiological differentiation between the two generations and further mediate their adaptive divergence into distinct ecological niches remains to be addressed.

The complete life cycle of *N. yezoensis* comprises two distinct heteromorphic generations with typical alternation of generations: the gametophyte (GAM) and the sporophyte (SPO), which correspond to the thallus and the filamentous form, respectively [12]. The macroscopic thallus is the dominant stage in the intertidal rock habitat, while the microscopic filamentous conchocelis mainly inhabits the interior of mollusk shells or exists as free-living conchocelis in seawater [13]. These two generations face drastically different environmental stressors and ecological selection pressures in their respective habitats: the thallus is exposed to dynamic intertidal conditions while the conchocelis lives in a relatively stable microenvironment with low light and limited nutrient availability [14-15]. The morphological and physiological differentiation between the two generations results from long-term adaptive evolution, and the underlying molecular regulatory mechanisms are key to understanding the adaptation and evolution of this species, and of red algae as a whole [16].

During the growth and development of marine algae, they are exposed to a range of environmental and biological stressors, including temperature, freshwater, and light stress [17-19]. Extensive research worldwide has examined the molecular mechanisms and alterations in metabolic pathways associated with these environmental stressors. For example, genome-wide identification of NADPH oxidase (NOX) gene families in red algae has revealed the unique evolutionary characteristics and functional diversity of these genes in mediating reactive oxygen species (ROS) signaling and stress responses [20-21]. In addition, the daily light-dark cycle has been shown to regulate the expression of many genes in N. yezoensis, modulating its metabolic and physiological processes to adapt to the rhythmic changes in the intertidal environment [22-23]. Transcriptomic studies on Antarctic red algae have also identified key genes and pathways associated with adaptation to extreme light environments, providing a reference for exploring the environmental adaptation mechanisms of red algae in different habitats [24].

These studies have conducted in-depth analyses of the metabolic pathways and regulatory mechanisms of algae, especially red algae, in response to environmental stress from multiple perspectives, including transcriptomics and proteomics. However, for *N. yezoensis*, a red algal species with a typical heteromorphic alternation of generations [25], the differences in metabolic regulatory networks between its thallus and conchocelis generations, as well as the molecular mechanisms driving the transformation and evolutionary adaptation between these two heteromorphic stages, remain poorly understood. Additionally, the specific molecular pathways underlying the shell-boring growth and maturation of the conchocelis filament, which are critical for completing its life cycle, have not been elucidated.

In this study, *N. yezoensis* samples of different generations were obtained by fragmenting pure-line filamentous thalli and inoculating them onto clam shells for artificial cultivation. We optimized the RNA extraction protocol for shell-associated conchocelis thalli. We performed transcriptome sequencing and analysis across three biological forms: the leafy thallus, the shell-boring filamentous thallus, and the free-living filamentous thallus. The primary objectives of this study were to identify the key metabolic pathways and regulatory genes underlying alternation of generations, shell-boring growth, and filament maturation in N. yezoensis, and to reveal the molecular mechanisms underlying adaptive divergence between its two generations. This study aims to deepen the understanding of the molecular basis of alternation of generations and environmental adaptation in red algae, and provide new insights into the evolutionary adaptation strategies of marine intertidal macroalgae.

2. Materials and Methods

2.1 Cultivation of Neopyropia Samples

Filamentous thalli of *N. yezoensis* were fragmented and inoculated onto *Meretrix meretrix* shells in beakers, with three biological replicates set for each cultivation group in the experiment. The cultures were maintained at 20 °C under a light intensity of 40 μmol photons·m^{-2}·s^{-1} and a photoperiod of 12 h light:12 h dark (12L:12D). Upon confirmation of successful shell colonization, the samples were transferred to a 24 °C incubator, where temperature difference stimulation was applied to induce the swelling of algal conchocelis.

A small piece of the shell was sampled and immersed in Perenyi's decalcification solution. The sample was then observed under a microscope to verify filamentous thallus swelling. Upon confirmation of filament swelling, the samples were transferred to a 20 °C incubator for aerated culture.

Daily maintenance involved shell wiping and water replacement, with 200 μL of culture medium sampled each day to monitor conchospore release density. Upon massive conchospore release, sterilized seed ropes were placed into beakers and removed after 24 hours to verify conchospore attachment. Subsequently, the seed ropes were transferred to 1-L aerated flasks for continued cultivation at 20°C.

Upon the thallus seedlings reaching a naked-eye-visible size, they were transferred to a 10 °C incubator and cultured under 50 μmol photons·m^{-2}·s^{-1} light intensity and a 12L:12D photoperiod. When the seedlings grew to approximately 5 mm in length, they were detached from the seed ropes and further cultured at 10 °C to obtain *N. yezoensis* samples.

2.2 RNA Extraction

Fifty milligrams of *N. yezoensis* samples from each biological replicate were ground thoroughly into a fine powder in liquid nitrogen, then mixed with 500 μL of NucleoZOL and gently pipetted to ensure homogeneity. Two hundred microliters of RNase-free water was added to the lysate, which was then vortexed vigorously for 15 seconds and incubated at room temperature for 5 minutes. Subsequently, the mixture was centrifuged at 12,000 × g for 15 minutes.

500 μL of the supernatant was transferred to a new centrifuge tube, and 500 μL of 100% isopropanol was added. The mixture was incubated at room temperature for 10 minutes, then centrifuged at 12,000 × g for 10 minutes. The supernatant was discarded, and the crude RNA sample was collected. Subsequently, 500 μL of 75% ethanol was added, and the mixture was centrifuged at 8,000 × g for 3 minutes. The supernatant was discarded, and this washing step was repeated once.

An appropriate volume of RNase-free water was added to dissolve the RNA, adjusting the total RNA concentration to approximately 1μg/μL. The mixture was then vortexed at room temperature for 3 minutes to fully solubilize the precipitate.

2.3 Bioinformatics Analysis

The raw sequencing data were processed to filter out low-quality reads, adapters, and reads with ambiguous base calls, yielding clean data. Subsequently, the clean data were aligned to the genomes of *N.*

yezoensis and *N. haitanensis* using Hisat2 (v2.0.5)-108. The number of read pairs mapped to each gene was quantified with featureCounts v1.5.0-p3.

Gene expression levels were quantified using the FPKM (Fragments Per Kilobase of transcript sequence per Million base pairs sequenced) metric, where a gene was defined as expressed if its FPKM value exceeded 5.

Differential expression analysis was performed using the DESeq2 R package (v1.16.1). The screening criteria for differentially expressed genes (DEGs) were set as follows: fold change ≥ 2 (corresponding to $\log_2$ (fold change) ≥ 1 or ≤ -1) and adjusted p-value (FDR-corrected) < 0.05. The false discovery rate (FDR) is a statistical method primarily used in DEG analysis to control the proportion of false positive results in the final dataset.

Differentially expressed genes (DEGs) were subjected to functional annotation and enrichment analysis using the ClusterProfiler R package, by mapping them against the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. For each group, the top 10–20 most significantly enriched terms were selected for visualization.

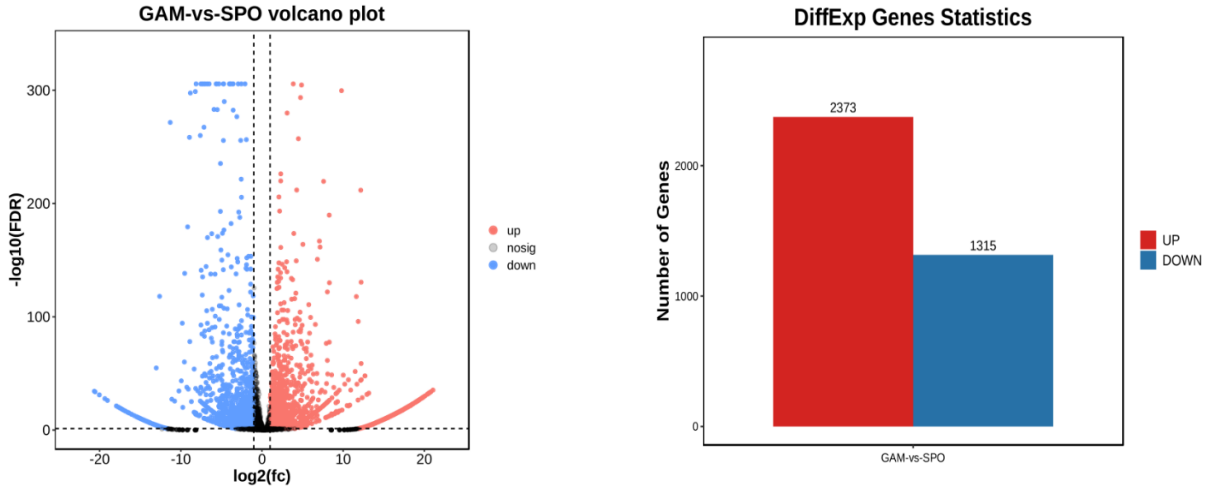
A heatmap was generated from the log2(fold change) values of differentially expressed genes between the thallus and the filament of N. yezoensis. The analysis was performed with R software (v4.2.1) using the pheatmap package (v1.0.12) as the core tool, supplemented by ggplot2 (v3.3.6) for visualization adjustment, dplyr (v1.0.9) for data preprocessing, and RColorBrewer (v1.1-3) for color palette design. The visualization was also validated using the Biomarker Online Cloud Platform (www.biomarker.com.cn), and no normalization was performed during graphing.

3. Results

3.1 Identification of Differentially Expressed Genes

A total of 12,255 genes were identified from *Neopyropia yezoensis* via data analysis, among which 3,688 genes exhibited significant differential expression (FDR < 0.05) (Figure 1). Volcano plot analysis revealed that 2,373 genes were significantly upregulated in the thallus relative to the filament ($\log_2$ (fold change) > 1, FDR < 0.05), whereas 1,315 genes were significantly downregulated in the thallus relative to the filament ($\log_2$ (fold change) < -1, FDR < 0.05). The bar chart clearly demonstrated that the number of upregulated genes was nearly twice that of downregulated genes.

Figure 1: Differential gene expression between GAM and SPO. (a) Volcano plot: red dots represent upregulated genes, and blue dots represent downregulated genes in thallus relative to conchocelis; (b) Bar chart: the number of upregulated (red) and downregulated (blue) genes is visually depicted.



(a) (b)

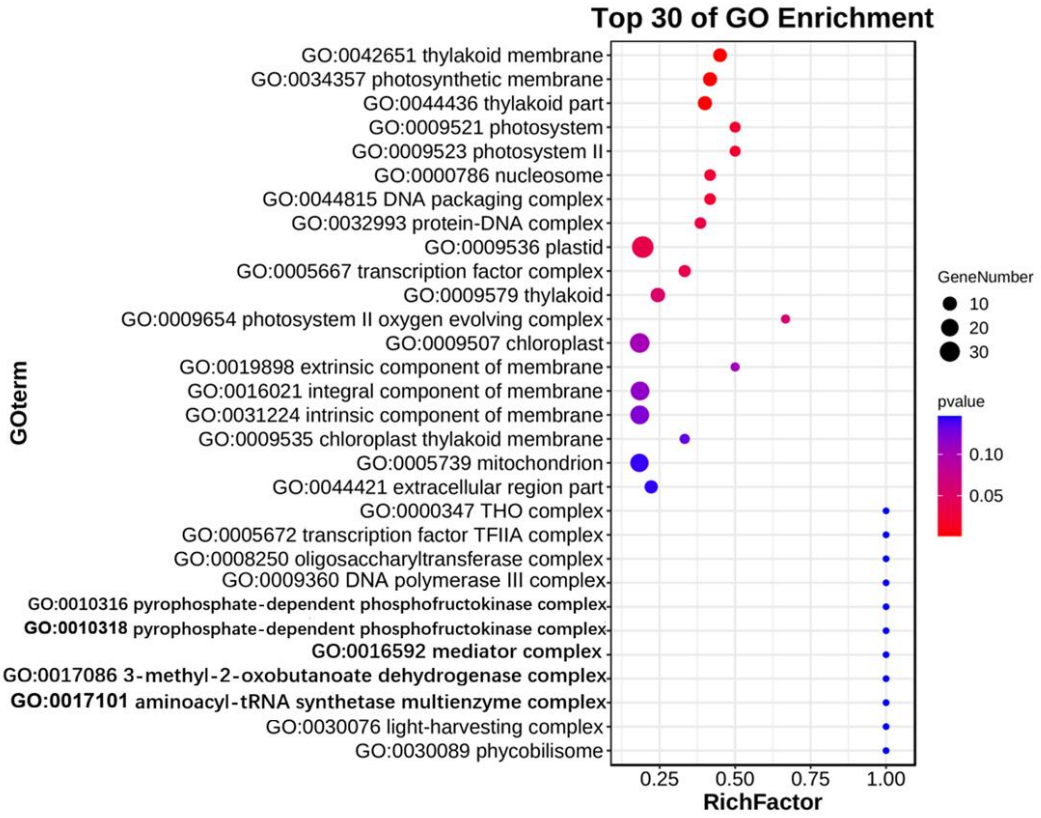
3.2 Functional Enrichment Analysis

3.2.1 Frond-preferential high expression

(1) GO Enrichment

At the cellular component category of the GO database, 247 genes with significant upregulation in thallus relative to conchocelis were enriched ($p < 0.05$) (Figure 2). Among these upregulated genes, 9 were annotated to the thylakoid membrane (GO:0042651), 10 to the photosynthetic membrane (GO:0034357), and 8 to photosystem (GO:0009521) and photosystem II (GO:0009523). Notably, these genes are all functionally associated with photosynthesis in the gametophytic thallus.

Figure 2: Functional annotation of cellular component terms for genes upregulated in thallus relative to conchocelis, based on the GO database. Only the top 30 most significant terms are displayed. In the plot, larger dots indicate a greater number of enriched genes (Gene Number), while redder colors indicate higher significance (lower p-values)



At the molecular function category of the GO database, a total of 388 genes were enriched for upregulation in thallus relative to conchocelis, among which 263 showed significant upregulation ($p < 0.05$) (Figure 3). Specifically, these genes were significantly upregulated: 9 annotated to chlorophyll binding (GO:0016168), 46 to kinase activity (GO:0016301), 32 to phosphotransferase activity (alcohol group as acceptor; GO:0016773), 6 to secondary active transmembrane transport (GO:00115291), and 2 to phosphoglycerate kinase activity (GO:0004618).

At the biological process category of the GO database, a total of 343 genes were enriched for upregulation in thallus relative to conchocelis (Q-value < 0.05) (Figure 4). Specifically, among these significantly upregulated genes, 4 were annotated to the pantothenate metabolic process (GO:0015939) and another 4 to the pantothenate biosynthetic process (GO:0015939). A key characteristic of these genes is their functional association with the pantothenate metabolic pathway in *Neopyropia yezoensis*. Pantothenate metabolism-related genes are involved in the tricarboxylic acid (TCA) cycle and fatty acid synthesis; in intertidal-dwelling *N. yezoensis*, their upregulation enhances tolerance to high-temperature and desiccation stress by promoting antioxidant enzyme activity and osmotic regulation.

Figure 3: Functional annotation of molecular function terms for genes upregulated in thallus relative to conchocelis, based on the GO database

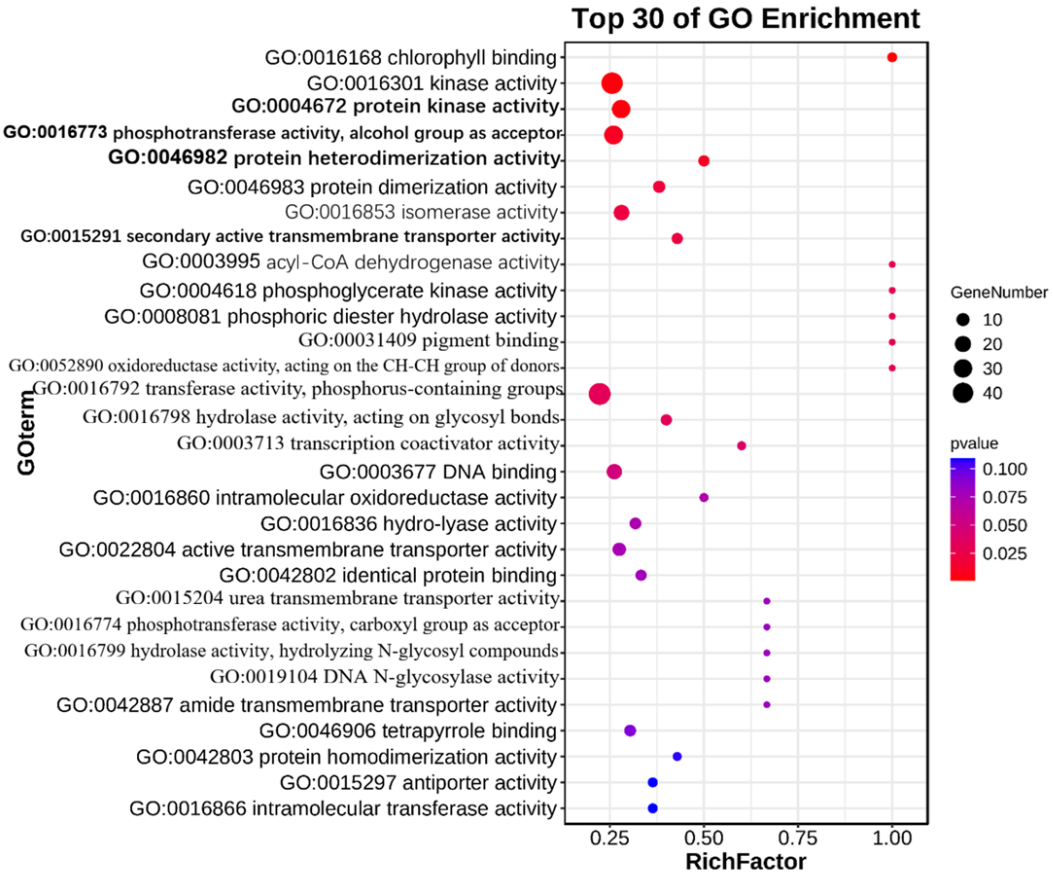
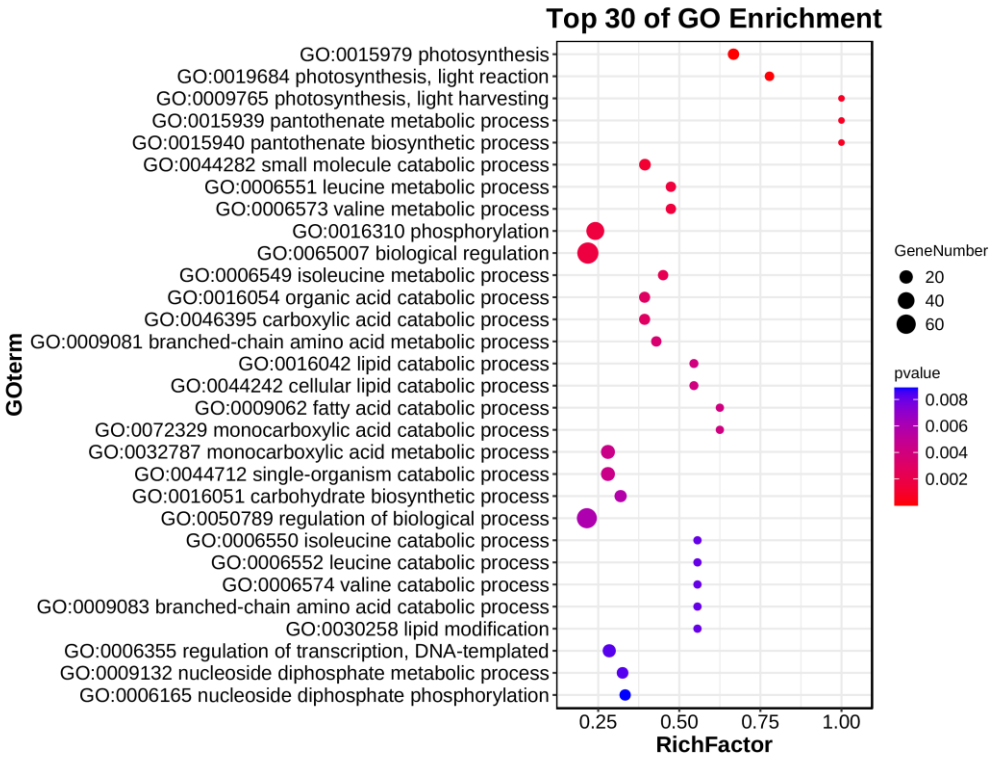


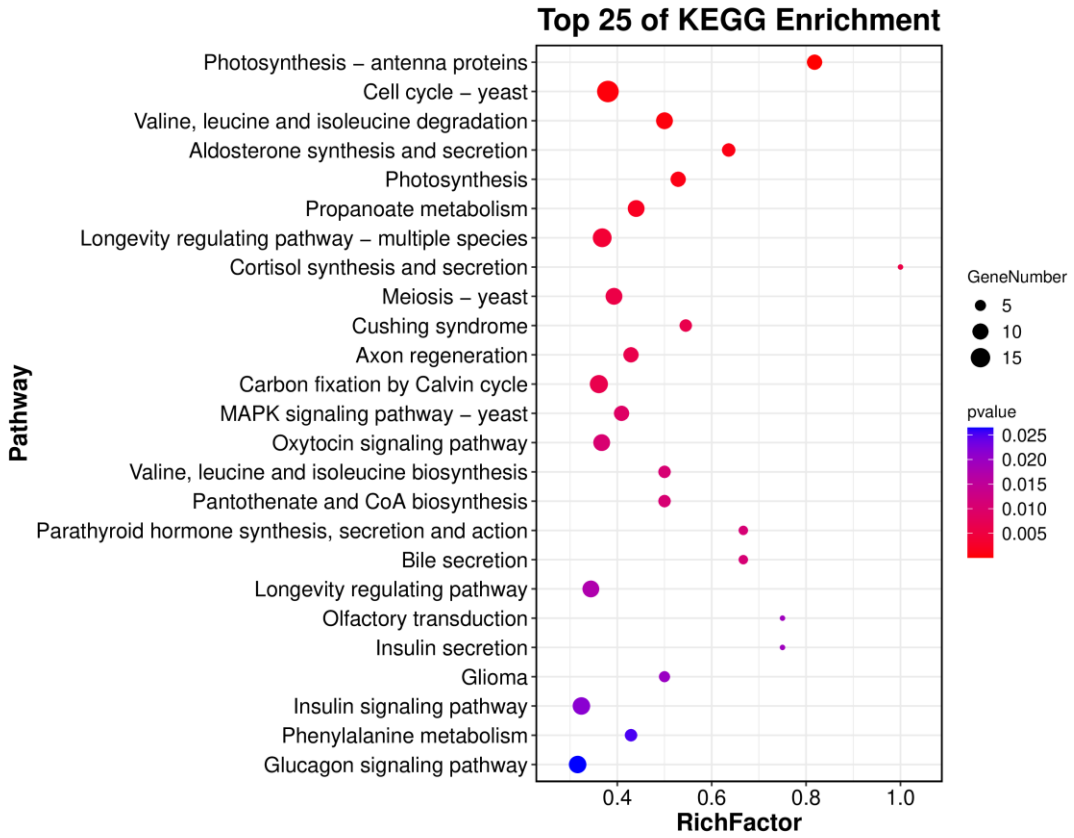
Figure 4: Functional annotation of biological process terms for genes upregulated in thallus relative to conchocelis, based on the GO database



(2) KEGG Enrichment

Following comparative analysis against relevant datasets within the KEGG database, a total of 343 genes exhibiting significantly higher expression in thallus relative to conchocelis were identified (FDR < 0.05) (Figure 5). Among these pathways with significantly elevated expression, the photosynthesis-antenna proteins pathway (Pathway ID: ko00196) encompassed 9 genes. In comparison, the photosynthesis pathway (Pathway ID: ko00195) included 4 genes, all of which are involved in photosynthetic processes. Additionally, the aldosterone synthesis and secretion pathway (Pathway ID: ko04925) contained 7 genes.

Figure 5: KEGG pathway enrichment analysis of genes upregulated in gametophytic thallus. Only the top 25 most significant pathways are visualized. In the plot, larger points indicate a greater number of enriched genes (Gene Number), while redder colors indicate higher significance (lower p-values)



3.2.2 Filament-preferential high expression

(1) GO Enrichment

At the cellular component level of the GO database, a total of 14 genes with significantly higher expression in thallus relative to conchocelis were enriched ($p < 0.05$) (Figure 6). Among these significantly upregulated genes, 3 were annotated to the endoplasmic reticulum (GO: 0005783), 4 to the endomembrane system (GO: 0012505), and a combined 15 genes to the membrane (GO: 0016020) and membrane part (GO: 0044425) terms.

At the biological process level of the GO database, a total of 19 genes with significantly higher expression in conchocelis relative to thalli were enriched ($p < 0.05$) (Figure 7). Notably, among these significantly upregulated genes, one gene was annotated to the phosphoenolpyruvate transport term (GO: 0015714).

Figure 6: Cellular component classification of genes with significantly higher expression in conchocelis relative to thallus, based on the GO database

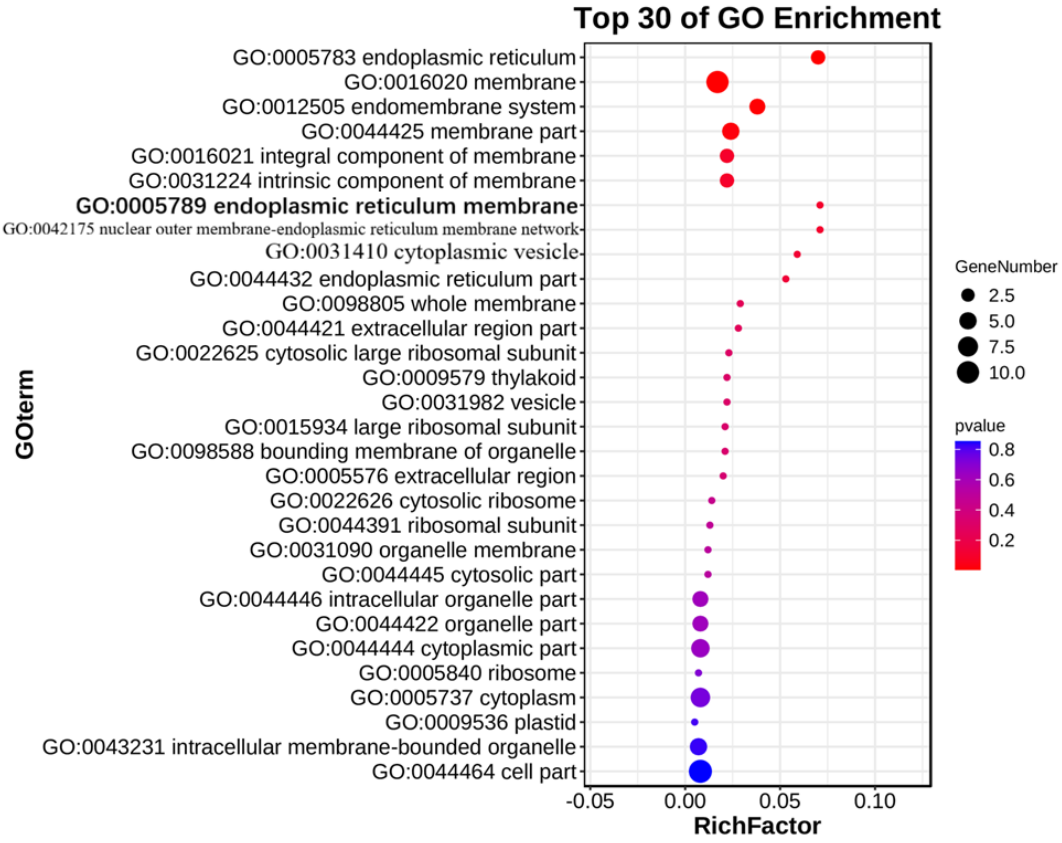
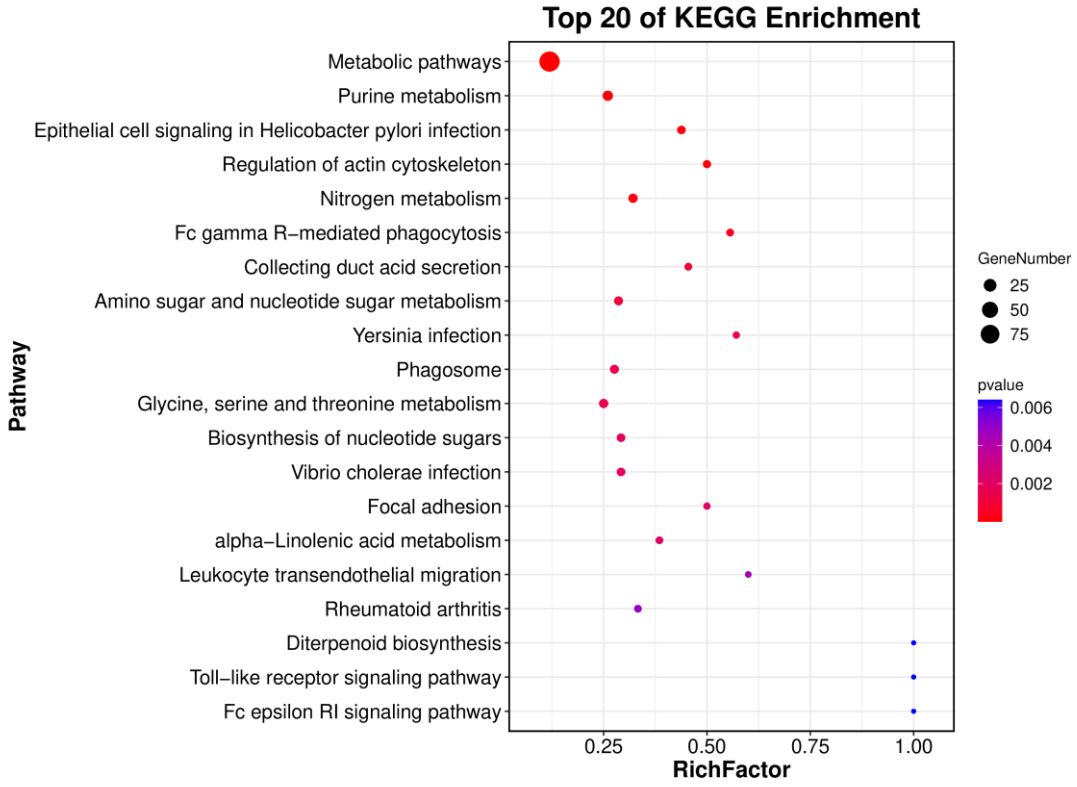


Figure 7: Biological process annotation of genes with significantly higher expression in conchocelis relative to thallus, based on the GO database

(2) KEGG Enrichment

Through comparative pathway analysis of genes in conchocelis relative to thalli using the KEGG database, a total of 150 genes with significantly higher expression in conchocelis were enriched (Q-value < 0.05) (Figure 8). Notably, among these significantly upregulated genes: 6 were annotated to the "regulation of actin cytoskeleton" pathway (pathway ID: ko04810); 9 to "nitrogen metabolism" (pathway ID: ko00910); and 13 to "purine metabolism" (pathway ID: ko00230).

Figure 8: Pathway enrichment analysis of genes with significantly higher expression in conchocelis via the KEGG database. Only the top 20 most significant pathways are displayed. In the plot, larger points indicate a greater number of enriched genes (Gene Number), while redder colors indicate higher significance (lower p-values)



4. Discussion

4.1 Molecular Mechanism Underlying the Environmental Adaptability of Thallus

The thallus of *Neopyropia* utilizes chloroplasts as the site for photosynthesis. Protein complexes, which harbor photosynthetic pigments and electron transport chain components essential for the light-dependent reactions, are localized on the thylakoid membranes of chloroplasts. During photosynthesis, photochemical reactions occur on the thylakoid membranes. Thus, the thylakoid membrane serves as the primary site, providing the spatial context for the conversion of light energy to chemical energy [26].

GO enrichment analysis revealed the following findings: 9 genes were annotated to the thylakoid membrane (GO: 0042651), 10 genes to the photosynthetic membrane (GO: 0034357), and a total of 8 genes to photosystem (GO: 0009521) and photosystem II (GO: 0009523). Notably, all these genes are associated with photosynthesis in the thallus. These expression differences were further corroborated by KEGG enrichment analysis: 9 genes were enriched in the photosynthesis-antenna proteins pathway (ID: ko00196), and 4 genes in the photosynthesis pathway (ID: ko00195). The enriched pathways are responsible for the photosynthesis of the frondose body, and their numbers are relatively higher than those of the filamentous body.

Morphologically, the filamentous and frondose stages of *Neopyropia* differ: the thallus stage is characterized by large, flattened blades that inhabit intertidal rocks. Due to its inherently larger surface area compared to the filamentous stage, the thallus can capture more light energy, making it better adapted for photosynthesis and thus enabling the accumulation of materials and energy to support the entire life cycle of *N. yezoensis* [27-28].

Furthermore, GO enrichment analysis at the molecular function level revealed that 46 genes were annotated to kinase activity (GO: 0016301) and 32 genes to phosphotransferase activity (GO: 0016773). Excluding chlorophyll-binding genes associated with photosynthesis and genes encoding phosphoglycerate kinase that supply partial substrates for photosynthesis, the remaining genes are collectively involved in coordinating cellular life activities and enhancing stress tolerance under adverse environmental conditions.

Protein phosphorylation is among the most extensively studied areas of protein post-translational modification and serves as a key regulator of signal transduction [29]. By precisely orchestrating the interaction network between kinases and phosphatases, phosphorylation enables fine-tuned regulation of cell growth, proliferation, and stress responses to intracellular biological stimuli [30]. Protein kinase genes primarily function to elevate protein phosphorylation levels in thallus cells. In contrast, phosphotransferases encoded by phosphotransferase activity genes are responsible for transferring phosphate groups from one molecule to another protein. Collectively, these two classes of genes synergistically enhance cellular signal transduction.

As an intertidal macroalga, *Neopyropia* is inevitably exposed to environmental stressors, such as elevated temperatures and desiccation during emersion at low tide. When *Neopyropia* thalli are subjected to adverse environmental conditions in their natural habitat, protein phosphorylation plays a pivotal role in stress responses, modulating the activity of antioxidant enzymes, the expression of heat shock proteins, and the biosynthesis of diverse metabolites [31]. Under stress, cells require rapid perception and adaptation to environmental fluctuations. As a core mechanism of cellular signal transduction, protein phosphorylation enables rapid transmission of stress signals and the activation of downstream stress-responsive genes to counter abrupt environmental changes [32]. Additionally, high phosphotransferase activity facilitates the regulation of photosynthetic enzyme activity and enhances photoprotective capacity, thereby mitigating potential photodamage induced by reactive oxygen species (ROS) accumulation during photosynthesis [33]. The upregulated expression of genes governing phosphorylation and dephosphorylation—relative to the filamentous stage—enhances the stress tolerance of thallus on intertidal rocks, enabling their high adaptability to the dynamic intertidal environment.

Finally, the data revealed that 4 genes were annotated to the pantothenate metabolic process (GO: 0015939) and 4 genes to the pantothenate biosynthetic process (GO: 0015939). These genes regulate pantothenate metabolism-related genes to participate in the TCA cycle and fatty acid biosynthesis. For *N. yezoensis* growing in the intertidal zone, the upregulated expression of pantothenate metabolic genes can enhance antioxidant enzyme activity and osmotic adjustment, thereby improving its tolerance to high-temperature and desiccation stress.

4.2 Molecular Mechanism Underlying the Environmental Adaptation of Conchocelis

The data derived from GO analysis revealed that: the endoplasmic reticulum (GO: 0005783) is associated with 3 genes; the endomembrane system (GO: 0012505) encompasses 4 genes; and the membrane (GO: 0016020) and membrane part (GO: 0044425) together involve 15 genes. These findings suggest that the identified genes are functionally linked to the membrane system of filamentous cells. During the rapid growth phase of filamentous structures, extensive cell division and biosynthetic activities are requisite. Relative to thallus, the high expression of membrane system-related genes facilitates the rapid turnover of cell membranes and the proper functioning of organelles, thereby promoting cell division and growth [34]. Filamentous structures require efficient nutrient uptake capacity to support their early growth and development.

The elevated expression of membrane system-related genes contributes to enhanced cell membrane permeability and nutrient transport capacity, thereby improving nutrient uptake efficiency [35]. Meanwhile, free-living filamentous structures in seawater require adequate motility and plasticity to maintain their morphological traits and physiological homeostasis. The increased cell membrane fluidity resulting from the

high expression of membrane system genes provides a favorable structural basis for fulfilling these functional requirements.

In addition, analysis of KEGG enrichment data revealed the following: the actin cytoskeleton regulation pathway (ID: ko04810) contains 6 genes; nitrogen metabolism (ID: ko00910) involves 9 genes; and the purine metabolism pathway (ID: ko00230) includes 13 genes. These findings indicate that the filamentous structures of *N. yezoensis* exhibit greater complexity and slenderness, necessitating the maintenance of cellular toughness. Consequently, the expression levels of genes associated with the actin cytoskeleton regulatory pathway are relatively higher in filamentous forms compared to thallus. This heightened expression is critical for the effective survival of *N. yezoensis* as spores under environmental stress.

Concurrently, pathways significantly enriched by genes with relatively high expression in filamentous forms include nitrogen metabolism. For instance, nitrogen and purine metabolism may enhance nitrogen uptake, utilization, and storage by filamentous forms under nutrient-limited conditions.

5. Conclusion

The transcriptomic divergence between the two heteromorphic generations (thallus and conchocelis) of *Neopyropia yezoensis* is fundamentally driven by the distinct environmental stressors and functional ecological niches they occupy, with the differential expression of specific functional genes and metabolic pathways constituting the molecular basis for their unique environmental adaptation strategies and the implementation of heteromorphic alternation of generations.

The thallus, which colonizes the dynamic intertidal rocky habitat characterized by high light intensity and frequent abiotic stress, exhibits significant upregulation of genes associated with the photosynthetic apparatus, including those annotated to the thylakoid membrane (GO:0042651), photosynthetic membrane (GO:0034357), and photosystem I/II (GO:0009521/GO:0009523). These genes are significantly enriched in the photosynthesis (ko00195) and photosynthesis-antenna proteins (ko00196) pathways, which maximize the efficiency of light energy capture and conversion to sustain the material and energy metabolism required throughout the species' life cycle. Concurrently, the upregulated expression of genes related to protein phosphorylation and dephosphorylation, including kinase activity (GO:0016301) and phosphotransferase activity (GO:0016773) genes, as well as genes in the pantothenate metabolic pathway (GO:0015939), endows the thallus with efficient intracellular signal transduction capacity and enhanced tolerance to intertidal stressors such as high temperature and desiccation.

In contrast, the conchocelis, which inhabits a relatively stable microenvironment with low light and limited nutrient availability, achieves adaptive survival and maintenance of its slender filamentous morphology through the high expression of membrane system-related genes (endoplasmic reticulum, GO:0005783; endomembrane system, GO:0012505) and the significant enrichment of genes in the regulation of actin cytoskeleton (ko04810), nitrogen metabolism (ko00910), and purine metabolism (ko00230) pathways. These molecular features collectively promote rapid cell division and growth, improve nutrient uptake and utilization efficiency, and maintain cellular structural toughness in the conchocelis.

This study identifies the core functional genes and specific metabolic pathways regulating environmental adaptation and alternation of generations in *N. yezoensis* at the transcriptomic level. It clarifies the molecular correlation between differential gene expression and ecological niche adaptation of its two generations.

The findings provide a direct molecular foundation for elucidating the mechanism of heteromorphic alternation in red algae and their evolutionary adaptation strategies to the marine environment, and lay a solid groundwork for subsequent investigations into the regulatory networks of key genes involved in generation alternation and environmental adaptation. Follow-up research can combine gene editing and other functional verification technologies to characterize further the specific molecular functions of these core genes and their regulatory cascades.

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Conflicts of Interest

The authors declare no conflict of interest.

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