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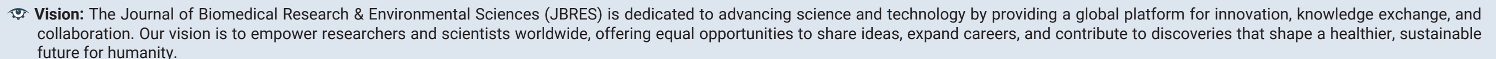

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RESEARCH ARTICLE

# *De Novo* Transcriptome Assembly and Annotation of the Temperate Asymbiotic Coral *Chromonephthea Hirotai*

**Lee K[1], Sung-Jin H[2], Lee T[1], Yum S[3] and Woo S[4]***

[1]Tropical & Subtropical Research Center, Korea Institute of Ocean Science and Technology, Jeju, Republic of Korea
[2]Department of Life Science, Woosuk University, Jincheon-gun, Republic of Korea
[3]Ecological Risk Research Department, Korea Institute of Ocean Science and Technology Geoje, Republic of Korea
[4]Marine Biotechnology & Bioresource Research Department, Korea Institute of Ocean Science and Technology, Busan, Republic of Korea

## Abstract

*Chromonephthea hirotai* (Anthozoa; Alcyonacea; Nephtheidae) is a little-known species of soft coral that inhabits the seas around South Korea, Japan, and the wider Indo-Pacific region. In Korea, the species occurs mainly in southern coastal areas. A marked decline in the coral's wild population has been witnessed in recent years due to climate change, and it has been identified as a species of important scientific value. However, there is little information on the genomic profile of this species, particularly regarding its stages of development, immune system, and ability to deal with environmental change. In this study, we performed a transcriptomic analysis of samples from a colony of *C. hirotai*. Three *C. hirotai* individuals were collected near Chujado, South Korea, and total RNA was extracted to construct the transcriptome assembly. The Illumina HiSeq 3000 platform was used for transcriptome sequencing, and the sequences were predicted by de novo assembly and analysis of the coding regions. Overall, 48,671 out of 129,086 unigenes were annotated in at least one public database, meaning that the overall annotation ratio of the *C. hirotai* assembly was 37.7%. The average length and N50 metric were 654.65 and 1118 base pairs, respectively. This study constitutes the primary documentation of the transcriptome assembly of *C. hirotai* in the colony phase, offering valuable insights into the genomics of this coral species. We anticipate that a framework will be developed to elucidate the mechanisms by which the azooxanthellate coral species *C. hirotai* responds to and develops in relation to various environmental stressors.

***Corresponding author(s)**

**Woo S,** Marine Biotechnology & Bioresource Research Department, Korea Institute of Ocean Science and Technology, Busan, Republic of Korea

**Email:** cwoo@kiost.ac.kr

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- Azooxanthellae

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## Introduction

Coral reef ecosystems are among the most ecologically and economically valuable marine habitats, supporting exceptionally high levels of biological diversity [1,2]. However, these ecosystems are increasingly threatened by ocean warming-A direct consequence of anthropogenic climate change-which has imposed significant thermal stress on coral communities. This stress has led to widespread coral bleaching and elevated mortality rates [3-6]. Recent studies on Scleractinia (Hard corals) have demonstrated that thermal stress can lead to a substantial reduction in genetic diversity and the erosion of adaptive genetic variation [7-9]. These findings underscore the critical importance of preserving genetic diversity as a foundation for enhancing the resilience and long-term persistence of coral reef ecosystems in the face of ongoing environmental change.

Beyond their structural complexity, coral ecosystems support a wide range of biotic interactions and ecological functions that extend beyond the coral species themselves [10]. These systems host a diverse array of associated fauna, including invertebrates and coral-associated fish assemblages that form obligate or dynamic relationships with the reef structure, relying on coral formations for habitat, nutrition, shelter, and reproduction [11,12]. The degradation of coral habitats, therefore, triggers cascading effects throughout the broader reef-associated community. These biotic associations are essential for maintaining biodiversity, ecological stability, and energy flow within reef ecosystems. Consequently, the loss of coral cover due to thermal stress and other anthropogenic pressures threatens not only the corals themselves but also the complex ecological networks they sustain.

Given the far-reaching ecological consequences of coral degradation, there is a growing need to develop effective strategies for reef restoration and conservation [13]. As coral loss disrupts complex biotic interactions and undermines ecosystem stability, understanding the biological mechanisms that enable coral survival under stress becomes increasingly vital [14]. In this context, transcriptomic analysis has emerged as a critical tool, enabling researchers to uncover the molecular pathways involved in coral resilience, adaptation, and stress response [14-16]. By examining the genetic basis of key physiological traits, this approach provides valuable insights into the adaptive capacity of corals, informing science-based interventions aimed at preserving both coral populations and the ecological communities they support [14].

Building on the importance of transcriptomic approaches in coral conservation, recent studies have demonstrated their utility in revealing critical molecular interactions and adaptive mechanisms that support coral resilience. For instance, interactions between specific bacterial communities and coral larvae have been found to be essential for larval settlement and metamorphosis which are Methods key developmental stages that underpin successful reef restoration efforts [17]. Comparative transcriptomic analyses across coral species have highlighted the conservation of stress-response genes and the emergence of lineage-specific adaptations, such as the upregulation of immune-related and protective molecular pathways under environmental stress [18]. Additionally, investigations into sex-specific gene expression, particularly those linked to male reproductive behaviour, offer insights into coral reproductive biology that could enhance restoration methods like transplantation and artificial fertilization [19]. Advances in long-read sequencing technologies have further improved genome assemblies, enabling high-resolution population genomics and multi-omics analyses that inform targeted conservation strategies [20]. Moreover, transcriptomic responses to sedimentation and thermal stress continue to

clarify the molecular mechanisms by which corals maintain resilience under increasingly adverse conditions [21,22]. These findings collectively strengthen the foundation for applying molecular data to practical reef management and restoration initiatives.

The soft coral species, *Chromonephthea hirotai* [23], a member of the family Nephtheidae, remains relatively understudied (Figure 1). Initially described from coastal waters of Japan, this temperate species is distributed along the coasts of both Korea and Japan [23-25]. Its colonies exhibit a distinctive red coloration, primarily due to the red calcareous sclerites within the polyps. In South Korea, *C. hirotai* mainly inhabits the subtidal zone along the southern coast, typically occurring on sloping to gently sloping rocky substrates at depths of up to 40 meters (Figure 1). The species is vulnerable to rising sea temperatures, which have contributed to population declines and local extinctions, leading to its recent designation as a protected marine organism in South Korea [26].

Considering the ecological importance and documented vulnerability, the present study aimed to generate high-quality transcriptome assemblies for this azooxanthellate soft coral. As a temperate species susceptible to thermal stress, *C. hirotai* provides a valuable model for investigating how non-reef-building corals respond at the molecular level to environmental challenges. The transcriptomic data generated herein serve as a foundational resource for future studies on gene expression related to stress tolerance, resilience, and potential adaptive mechanisms. These insights will ultimately support broader efforts to conserve temperate coral biodiversity and to develop informed strategies for mitigating the impacts of climate change on soft coral populations.

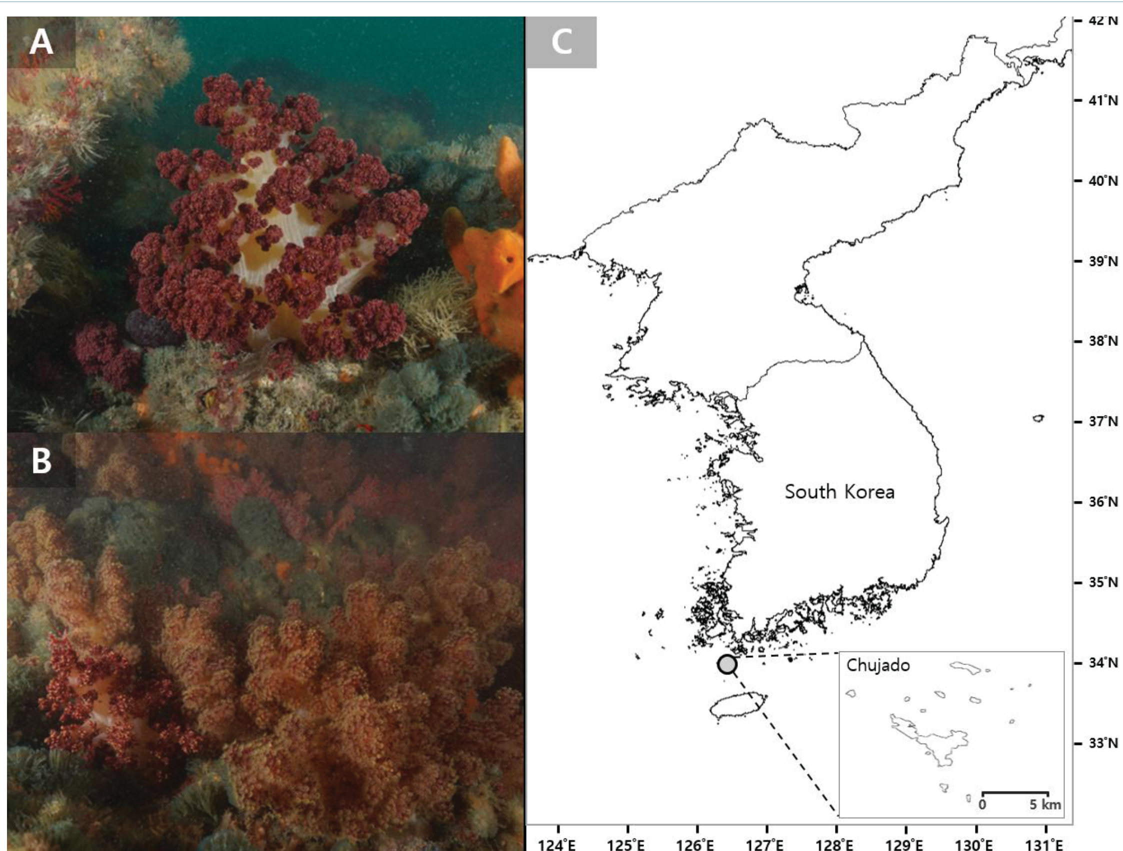


**Figure 1** *Chromonephthea hirotai* and collection site. A: Colony of *C.hirotai*; B: Coral population in a habitat; C: Collection site (Chujado) located on the southern coast of South Korea.

# Methods

## Sample collection and RNA preparation

Three individuals of *C. hirotai* were collected by scuba diving at water depths of 15-20 m around Chujado Island (33°59′19″N, 126°14′51″E) in the southern coastal area of South Korea. The sampling point seawater temperature was 20 °C. Scuba diving was performed to help conserve the *C. hirotai* colony. Segments of branches (Approximately 5-10 cm) were meticulously excised and collected from the *C. hirotai* colony, located at a depth of 10-20 m. The extent of sample collection was judiciously regulated to mitigate any adverse effects on the harvested colonies. The colonies were enclosed within polyethylene bags containing seawater and subsequently conveyed in an insulated icebox. An air compression system was used to infuse the interior of the polyethylene bag with pure oxygen, maintaining a concentration approximately 3-5 times greater than that of the adjacent seawater. To alleviate the stress associated with transport to the laboratory, the samples were left to settle for 24 h after arriving at the laboratory.

A single polyp was cut from each colony and placed in a 2 mL tube, and RNA was extracted by using the acid guanidinium thiocyanate phenol chloroform method [27-29]. Briefly, the homogenized sample was lysed and mixed with 1 M citrate buffer-saturated phenol at pH 4.3 and chloroform. Diethyl pyrocarbonate-treated water was added to dissolve the RNA pellet, which was then stored in a deep freezer below -70 °C. A spectrophotometer (NanoDrop ND-1000, Thermo Scientific, Waltham, MA, USA) was used to measure the yield and purity of RNA. The RNA integrity number (RIN) was determined with a bioanalyzer system (2100, Agilent Technology, Santa Clara, CA, USA). The cDNA library was generated using the TruSeq RNA Sample Prep Kit (Illumina, San Diego, CA, USA).

## Sequencing and *de novo* genome assembly

The total RNA from each sample was sequenced using a sequencing system (HiSeq 3000 System, Macrogen, Seoul, South Korea) to reconstruct the transcriptome sequence of *C. hirotai* in the absence of reference genome sequences. The Minimum Information about any (x) Sequences (MIxS) information, a standard description of sequence data, is given in table 1. The quality of raw reads was checked using FastQC v.0.11.7 [30]. Trimmomatic v0.38 [31] with the settings “PE -phred33 ILLUMINACLIP: adapters/TruSeq-PE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36” was used to remove sequencing

**Table 1**: MIxS specifications of the transcriptome of *C. hirotai*.

| Item | Description |
|---|---|
| Investigation type | Eukaryote |
| Project name | Transcriptome assembly of *Chromonephthea hirotai* |
| Organism | *Chromonephthea hirotai* |
| Classification | Animalia (Kingdom); Cnidaria (Phylum); Anthozoa (Class); Octocorallia (Subclass); Alcyonacea (Order); Alcyoniina (Suborder); Nephtheidae (Family); *Chromonephthea* (Genus) |
| Latitude and longitude | 33°59'19"N, 126°14'51"E |
| Geographic location name | South Korea, Jeju Island, Chujado |
| Collection_date | 2021-07-30 |
| Collector | Ki-hwan Lee, Seung-hwan Park |
| Environment (Biome) | Marine benthic biome (ENVO:0054) |
| Environment (Feature) | Sea shore (ENVO:0054) |
| Environment (Material) | Sea water (ENVO:0054) |
| Environmental package | Water |
| Sequencing method | Illumina |
| Transcriptome platform | Hiseq3000 System |
| Assembly method | Trinity v2.3.2 |
| Submitted to INSDC | Bioproject (PRJNA1062078) |
| | Biosample (SAMN39287690) |
| | SRA (SRR32016640) |

SRA: Sequence Read Archive.

errors and artificial adapters from the reads to maximize the quality of the de novo assembly. The assembly was constructed using Trinity v2.3.2 [32] with the default settings.

## Gene ontology annotation

For the functional annotation, we searched for the unigenes in the following databases: Gene Ontology (GO), UniProt, National Center for Biotechnology Information (NCBI) Non-Redundant Protein (NR), Protein Family (Pfam), Evolutionary Genealogy of Genes: Non-Supervised Orthologous Groups (EggNOG), NCBI Nucleotide (NT), and the Kyoto Encyclopedia of Genes and Genomes (KEGG). These searches were conducted using the NCBI BLASTN tool and the BLASTX tool in DIAMOND software, with the default E-value cutoff of 1.0E-5.

# Results and Discussion

## Transcriptome of *C. hirotai*

The short read dataset from the sequencing result of *C. hirotai* was deposited in the Sequence Read Archive under the accession number SRR32016640 and the assembly statistics are summarized in table 2. Overall, 48,671 out of a total of 129,086 unigenes were annotated in at least one public database (Supplementary Data 1), meaning the overall annotation ratio of the *C. hirotai* assembly was 37.7%. We summarized the annotation results using public databases in

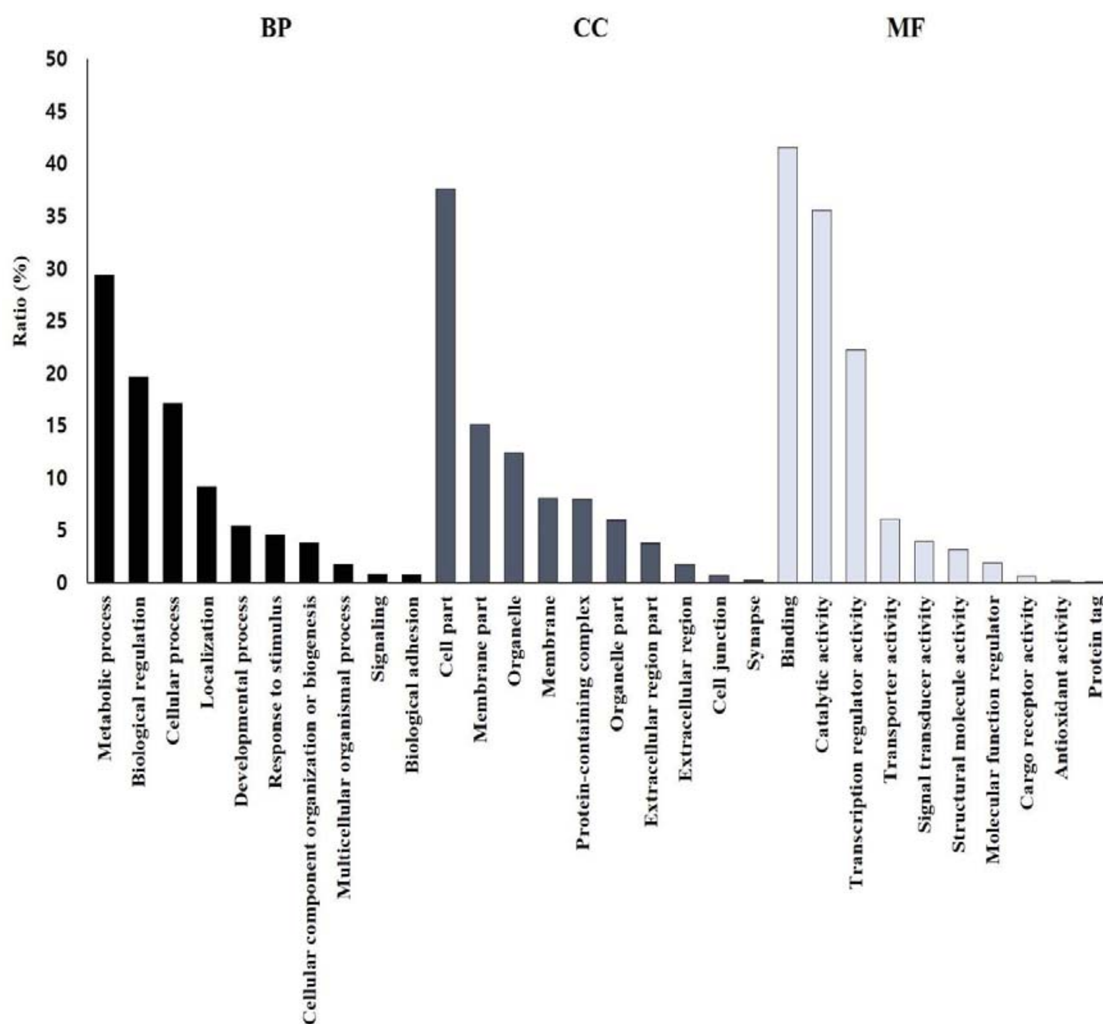


**Figure 2** Gene Ontology classification of *C. hirotai*. The histogram shows the results of unigene classification for three major GO categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF).

Table 3. The unigenes were classified according to GO terms using an in-house script. The main GO terms were Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). GO terms could be assigned to 20.12% of the unigenes in the annotated transcriptome; 6.96% of the unigenes were assigned to the BP category, 6.15% to the CC category, and 7.01% to the MF category. Although the GO annotation information remains in flux, the functional roles of many genes have not been completely elucidated or recorded. This situation is frequently observed for organisms that have not been extensively researched, and explains the high no-hit proportion of 79.88%, meaning many genes in this *C. hirotai* assembly could not be identified or annotated.

Gene Ontology (GO) analysis was performed to systematically categorize the annotated genes into three principal domains: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) (Figure 2). Within the BP category, the most prominent terms encompassed metabolic process, biological regulation, and cellular process, collectively representing more than 65% of the annotated functionalities. Additional enriched terms comprised localization, developmental process, and response to stimulus. In the CC category, the preponderance of annotated genes was linked to cell part, membrane part, and organelle, with supplementary representation from membrane and protein-containing complexes. In the MF category, binding and catalytic activity emerged as the most significant terms, accounting for over 75% of the annotations. Furthermore, other functional terms such as transporter activity, signal transducer activity, and structural molecule activity were also identified. A comprehensive enumeration of the top 10 enriched GO terms within each category, accompanied by their respective proportions, is depicted in figure 2.

**Table 2**: Statistics of the transcriptome dataset and assembly.

| Description | Statistics |
|---|---|
| Species (Sample name) | *Chromonephthea hirotai* (Ch_A) |
| Number of raw reads | 121,938,074 |
| Number of raw read bases | 12,315,745,474 |
| Number of processed reads | 119,788,528 |
| Number of processed read bases | 12,027,970,617 |
| Assembly statistics | |
| Guanine-cytosine content (%) | 38.55 |
| Minimum length (Base pairs) | 201 |
| Maximum length (Base pairs) | 30,756 |
| Average length (Base pairs) | 654.65 |
| N20 (Base pairs) | 3171 |
| N50 (Base pairs) | 1118 |
| N70 (Base pairs) | 475 |
| Total number of genes | 129,086 |

**Table 3:** Annotation results of various databases.

| Assembly | *Chromonephthea hirotai* |
|---|---|
| Total number of unigenes | 129,086 |
| Number of annotated unigenes per database | |
| GO | 25,969 (20.12%) |
| UniProt | 22,933 (17.77%) |
| NCBI NR | 45,208 (35.02%) |
| Pfam | 25,681 (19.89%) |
| EggNOG | 32,269 (25.0%) |
| NCBI NT | 26,639 (20.64%) |
| KEGG | 25,683 (19.9%) |
| Total number of annotated unigenes | 48,671 (37.7%) |

EggNOG: Evolutionary Genealogy of Genes: Non-Supervised Orthologous Groups; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; NCBI: National Center for Biotechnology Information; NR: Non-Redundant Protein; NT: Nucleotide; Pfam: protein family.

## Functional genes of C. hirotai

For the predicted genes identified in this study, homology searches were performed as a first step in the functional annotation. Proteins homologous to each *C. hirotai* protein were identified using the NR proteins of NCBI as the targets; 45,208 of the 48,671 *C. hirotai* protein sequences (92.87%) had at least one homologous hit in nr (e-value cutoff: 10-4). The three taxa containing the greatest numbers of homologous proteins were Dendronephthya gigantea (26,163

proteins, 57.87%), Paramuricea clavata (9,432 proteins, 20.97%), and Acropora millepora (1,738 proteins, 3.84%). Top 2 taxa, *D. gigantea* and *P. clavata* are soft corals in class Octocoralia like as *C. hirotai* and A. millepora showing approximate 4 % of homologous protein sequence belongs to class Hexacoralia. The result presented much higher sequence homology found between similar octocoral species and relatively lower protein sequence homology were found between octocoral and hexacoral species.

The annotation result of *C. hirotai* revealed 268 GO terms linked to BP, 94 linked to CC, and 152 linked to MF. A Fold-Enrichment (FE) of > 1.0 and $p < 0.05$ were used to define statistical significance. To increase the accuracy of the GO term identification, the terms were organized into a -log10 reference graph, and Table 4 shows the remaining gene counts and their respective FE values. In pursuit of more precise functional annotations, we focused on genes in the top 10 -log10 values, despite additional GO terms

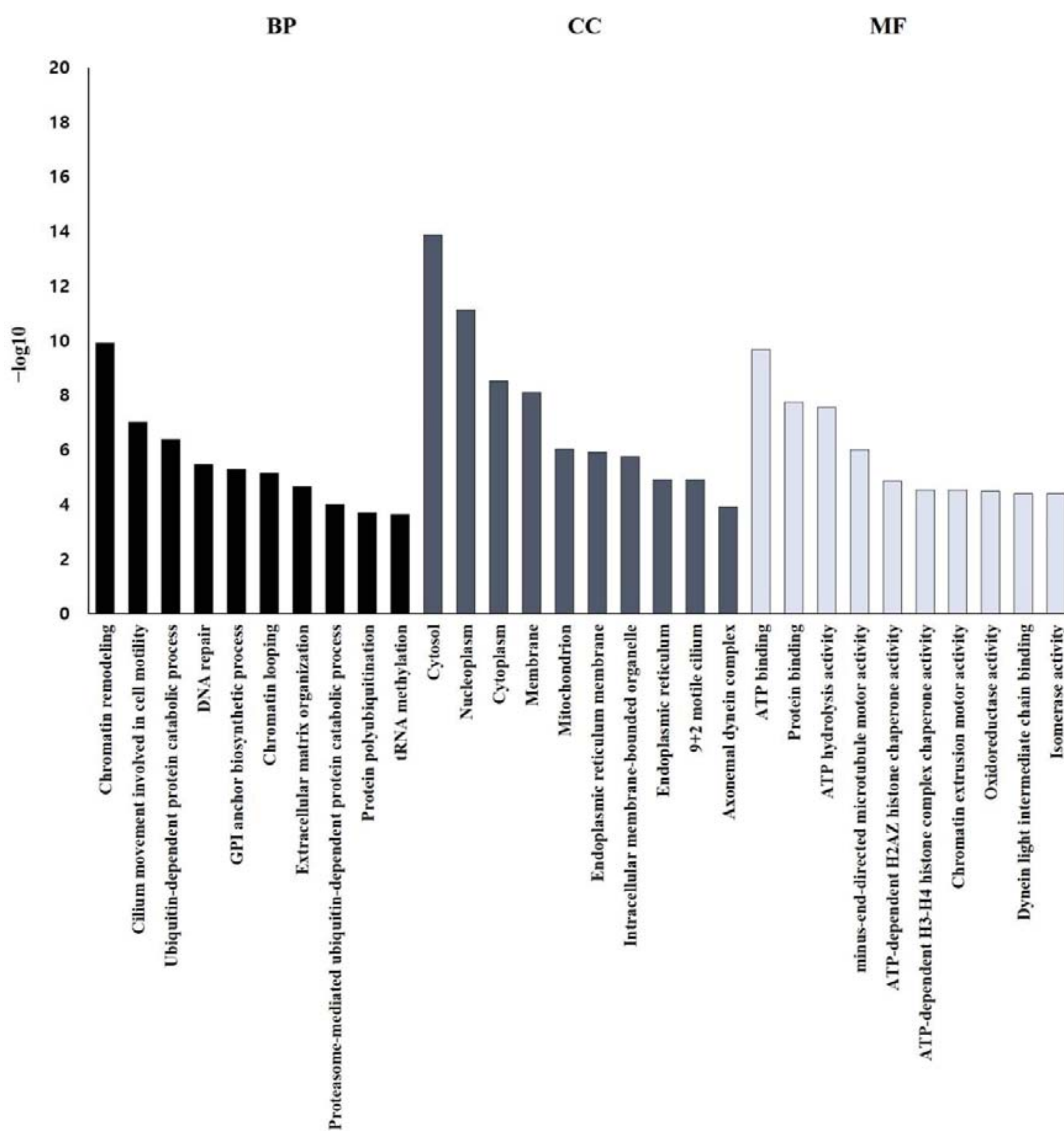


**Figure 3** Top 10 GO terms derived from the gene enrichment analysis identified based on the - log10 value and *p*-value. The top 10 terms were determined for each of the three main categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF).

exhibiting high FE values, for each of the three major categories (Figure 3). In the BP category, the FE was highest for the term "Cilium movement concentration in cell motility," with a value of 5.8. The ability of cilia to move is essential for cell movement and is particularly important for coral larvae. The larvae of many coral species use cilia to swim and locate optimal settlement sites [33]. This observation suggests that the larvae of *C. hirotai* may have been integrated into the breeding colony and the sample was collected during the reproductive cycle. Although there are no prior reports of *C. hirotai*, scanning electron microscopy has been used to observe the embryos and larvae of Acropora tenuis and Acropora millepora. These imaging studies demonstrated that cilia are crucial for removing sediment and for locomotion of these species [34]. Furthermore, in mature corals, cilia assume a significant function in the process of nutrient acquisition. These are diminutive hair-like structures located on the coralline surface that facilitate the generation of subtle yet persistent oceanic currents within the adjacent aquatic environment, thereby enhancing the likelihood that nutrient-laden waters will interact with the coral surface [35]. The next highest FE was for "Glycosylphosphatidylinositol (GPI) anchor biosynthetic processes," with a value of 4.4. GPI anchors are intricate glycolipids that tether proteins critical for the standard functioning of eukaryotic cells to their membranes [36,37]. Like other organisms, *C. hirotai* cells use GPI-anchored proteins for many cellular functions, which may influence the coral's response to environmental stressors, particularly those induced by elevated water temperatures. Furthermore, the FE for the term "tRNA methylation" was 3.9, underscoring its role in maintaining the stability and proper functionality of tRNA molecules, which in turn affect protein synthesis and ultimately cellular metabolism [38,39]. Although there has been little research into the role of tRNA methylation in coral species, it is important for adaptation to stress and maintaining cellular stability, implying that tRNA methylation might be vital for increasing coral resilience to environmental changes. In the CC category, the term "9+2 motile cilium" showed the highest FE of 4.5. The 9+2 structure refers to the specific arrangement of microtubules within cilia, which is crucial for their motility. Coral larvae, such as A. millepora, use 9+2 motile cilia for locomotion [33]. Assuming that the sampled population does not contain larvae, the motile cilia in *C. hirotai* colonies may facilitate material transport across the coral surface by generating vortex flows [40]. The term with the highest FE in the MF category was "minus-end-directed microtubule motor activity," with a value of 6.5. Studies into the functions of negative terminal motor proteins, including dynein and kinesin-14, have elucidated their potential roles in cellular processes in corals. Integral to microtubule organization, these motor proteins are critical in numerous core cellular functions, including cell division and intracellular transport [41]. Another highly ranked term was "isomerase activity," with a FE of 3.3. Isomerases, particularly those involved in glucose metabolism and protein folding, are essential for sustaining cellular function and structural integrity in marine organisms [42,43]. Studies focusing on isomerase activities in a greater variety of marine creatures are expected to provide insights into coral function, especially its likely impact on stress management and adaptation to shifts in the ecosystem.

In this study, we generated the transcriptomic data of temperate asymbiotic octocoral species. This transcriptomic data can allow us to investigate the physiological responses of this soft coral species and also we expect that our findings will help researchers identify disparities in the gene expression profile of *C. hirotai* through the analysis of transcriptomic data, particularly in studies comparing the reactions to diverse environments, such as alterations in seawater temperature or salinity or the extreme condition like deep sea. This data will facilitate

the examination of genes implicated in the interactions of *C. hirotai* with environmental changes, and help prepare for forthcoming alterations in benthic ecosystems. Additionally, when comparing the gene expression profiles between coral larvae and mature individuals, our findings will help researchers recognize important elements and genes associated with the key biological processes during development, such as metamorphosis.

## Data Availability

The information of this project were deposited in Bioproject of PRJNA1062078 and the sample information were deposited in Biosample of SAMN39287690. The transcriptomic read data were deposited in the Sequence Read Archive under the accession number SRR28002316.

## Author Contribution

KL, TL, and SH collected the samples, conducted the experiments, and collected the data. KL, SY and SW analyzed the data and prepared the manuscript.

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