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Transcriptomic identification of skin-enriched genes reveals the molecular basis of skin functional specialization in the coral hind (Cephalopholis miniata)

Bingcong Ye
Chinese Academy of Fishery Sciences
Yanfeng Yue
yyf8502@163.com

Chinese Academy of Fishery Sciences
Jing Liang
Yuanhao Ren
Chinese Academy of Fishery Sciences
Liguo Yang
Chinese Academy of Fishery Sciences
Xuefeng Song
Chinese Academy of Fishery Sciences
Bianbian Zhang
Chinese Academy of Fishery Sciences

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Abstract

Fish skin is a multifunctional tissue that serves as a primary interface between the organism and its aquatic environment, integrating physical barrier, innate immune defense, metabolic homeostasis, and pigmentation functions. However, the molecular basis underlying skin specialization in coral reef-associated serranid fishes remains poorly understood. Here, we performed comparative transcriptomic analysis of five tissues—skin, gill, liver, muscle, and intestine—from healthy wild-caught coral hind (*Cephalopholis miniata*) to characterize the tissue-specific molecular features of skin. Transcriptome profiling revealed clear tissue-dependent expression patterns, with skin forming a distinct transcriptional cluster from the other tissues. Comparative analysis identified 1,495 genes consistently up-regulated in skin relative to gill, liver, muscle, and intestine, representing a core skin-enriched gene set. Functional enrichment analyses demonstrated that these genes were predominantly associated with extracellular matrix organization, cell adhesion, epithelial structure and barrier formation, innate immune defense, glutathione metabolism, arachidonic acid metabolism, and pigmentation-related processes. Representative genes involved in extracellular matrix and epithelial integrity, including COL11A1, COL7A1, COL6A2, SPARC, LAMB4, ITGA6, and ITGB4, showed strong skin-biased expression, suggesting an enhanced structural barrier system. Concurrent enrichment of complement components, defensin-, lysozyme-, and chemokine-related genes indicated a constitutive innate immune surveillance program. Genes associated with glutathione metabolism, such as GPX7, GPX8, and GSS, together with arachidonic acid metabolism-related genes including ALOX5, ALOX5B, and PTGDSB, further suggested active redox and lipid-mediated homeostatic regulation. Notably, pigmentation-associated genes, including MITFA, TYRP1B, PMELA, melanoregulin, and Wnt-family genes, were also enriched in the skin transcriptome, indicating that pigment-cell-related processes are an intrinsic component of skin molecular specialization. Overall, our results demonstrate that *C. miniata* skin possesses a coordinated transcriptional program integrating epithelial barrier integrity, innate immunity, metabolic homeostasis, and pigmentation. The identified skin-enriched genes provide a candidate molecular framework for understanding the specialized ecological and physiological functions of serranid fish skin and provide valuable targets for future functional and spatial validation.

1. Introduction

The skin is one of the largest and most functionally diverse organs in teleost fishes and represents the primary interface between the animal and its surrounding aquatic environment. Unlike the highly keratinized epidermis of terrestrial vertebrates, teleost skin is characterized by a living epidermis covered by a mucus layer, which forms a dynamic first line of defense against mechanical injury, environmental stressors, and microbial invasion. This mucosal surface contains a wide range of antimicrobial and immune-related molecules, including antimicrobial peptides, lysozymes, lectins, complement components, mucins, and other soluble defense factors, thereby integrating physical protection with innate immune surveillance (Díaz-Puertas et al. 2023; Doyle et al. 2022; Salinas et al. 2021). In addition to pathogen defense, fish skin contributes to wound repair, osmoregulation, ion transport, and

environmental adaptation, highlighting its role as an active physiological organ rather than merely a passive external barrier (Esteban 2024).

The barrier function of teleost skin depends on the coordinated organization of extracellular matrix (ECM), epithelial cytoskeleton, cell–cell adhesion, and tight-junction complexes. In particular, claudins and other junction-associated proteins regulate paracellular permeability and contribute to the structural integrity of epithelial tissues in teleosts (Doyle et al. 2022). Meanwhile, the skin-associated immune system contains both constitutive and inducible defense mechanisms. Complement proteins can mediate pathogen opsonization and lysis, whereas antimicrobial peptides such as defensins, together with lysozyme and chemokines, contribute to pathogen restriction and immune-cell recruitment (Li and Zhang 2022). These mechanisms are particularly important in aquatic environments, where the skin is continuously exposed to microorganisms and fluctuating physicochemical conditions. Consequently, coordinated regulation of epithelial integrity, innate immunity, inflammatory signaling, and redox homeostasis is expected to be fundamental for maintaining skin function in marine teleosts (Song et al. 2021).

Fish skin is the largest external organ and serves as the primary barrier protecting fish from environmental stressors and pathogen invasion. In addition to its structural function, fish skin plays indispensable roles in innate immunity, mucus secretion, wound healing, osmoregulation, and body pigmentation (Xu and Liu 2024). Therefore, understanding the molecular characteristics of skin is of considerable importance for elucidating disease resistance, environmental adaptation, and coloration mechanisms in marine fishes.

Skin function is further complicated by its role in pigmentation. Teleost skin contains multiple chromatophore lineages, including melanophores, xanthophores, erythrophores, and iridophores, whose differentiation, distribution, and intracellular pigment organization collectively determine body coloration (Parichy 2021). Pigment-cell development and melanogenesis are regulated by conserved transcriptional and signaling networks. Among these, microphthalmia-associated transcription factor (MITF) represents a central regulator of melanocyte differentiation and pigment synthesis, whereas downstream genes such as TYR, TYRP1, DCT, and PMEL participate in melanin biosynthesis and melanosome formation (Murisier and Beermann 2006; Widlund and Fisher 2003). Wnt/β-catenin signaling is also closely linked to pigment-cell specification and differentiation through regulation of MITF, providing an important connection between extracellular signaling and pigment-cell development (Dorsky et al. 2000; Takeda et al. 2000). Importantly, pigmentation is not an isolated process but is influenced by cellular metabolism, oxidative status, lipid metabolism, and environmental stimuli. Thus, transcriptomic characterization of skin may provide insights not only into pigmentation but also into the broader physiological mechanisms that maintain skin homeostasis.

Oxidative stress and lipid-derived signaling pathways may represent additional links between environmental adaptation, immunity, and pigmentation. Glutathione and glutathione-dependent enzymes constitute an important antioxidant defense system in fish and participate in the regulation of reactive

oxygen species (ROS) and cellular redox balance (Santana et al. 2022). In parallel, arachidonic acid metabolism generates a broad spectrum of lipid mediators that regulate inflammatory responses, cellular signaling, and tissue homeostasis (Xu et al. 2022). These metabolic pathways are particularly relevant to the skin because the tissue is directly exposed to environmental stressors and must simultaneously maintain barrier integrity, immune responsiveness, and cellular homeostasis. Therefore, the identification of genes associated with glutathione metabolism, arachidonic acid metabolism, and related lipid-mediated signaling may provide additional molecular evidence for the functional specialization of fish skin.

The coral hind, *Cephalopholis miniata*, is a commercially and ecologically important marine fish belonging to the family Serranidae. Which body coloration is an important phenotypic trait associated with ecological adaptation, market value, and aquaculture performance. Previous transcriptomic studies in leopard coral grouper (*Plectropomus leopardus*) have demonstrated that skin coloration is associated with extensive transcriptional changes involving melanogenesis, tyrosine metabolism, Wnt signaling, MAPK signaling, energy metabolism, and cytoskeletal regulation (Song et al. 2022; Wen et al. 2022). In particular, transcriptomic and proteomic analyses have implicated Wnt, calcium, MAPK, and metabolic pathways in the differentiation of red and black skin, while subsequent studies have identified pigmentation-associated genes including MITF, TYR, TYRP1, DCT, and PMEL (Song et al. 2022). Recent work has further suggested that antioxidant and lipid-metabolic pathways contribute to red skin coloration in *P. leopardus*, highlighting the potential interaction between pigmentation, oxidative homeostasis, and lipid metabolism (Liu et al. 2025; Pang et al. 2026). These studies provide an important comparative framework for investigating the molecular basis of skin specialization in other closely related serranid fishes.

However, compared with *P. leopardus*, the molecular characteristics of skin in the coral hind *C. miniata* remain poorly characterized. Existing studies of this species have largely focused on taxonomy, ecological distribution, population resources, and reproductive biology, whereas comprehensive transcriptomic information describing tissue-specific functional differentiation is still limited. The lack of a systematic comparison of skin with other major organs has consequently restricted the identification of genes and molecular pathways underlying the specialized functions of *C. miniata* skin. In particular, it remains unclear whether the skin transcriptome is characterized by coordinated enrichment of structural barrier components, innate immune effectors, antioxidant pathways, lipid-mediated signaling, and pigmentation-associated genes.

To address this knowledge gap, we performed comparative transcriptomic profiling of five major tissues —skin, gill, liver, muscle, and intestine—from healthy wild *C. miniata*. RNA-seq was used to establish a comprehensive transcriptomic resource, followed by functional annotation, principal component analysis, hierarchical clustering, differential expression analysis, and GO/KEGG enrichment analyses. By comparing the skin transcriptome with those of four internal and external tissues, we aimed to identify genes showing consistent skin-enriched expression and to characterize the functional modules underlying skin specialization. Particular attention was given to genes involved in epithelial and ECM

organization, innate immunity, chemokine-mediated signaling, antioxidant defense, arachidonic acid metabolism, and pigmentation. This integrative approach provides a molecular framework for understanding the multifunctional nature of *C. miniata* skin and establishes candidate genes for subsequent functional validation, disease-resistance studies, and pigmentation-related research.

2. Materials and Methods

2.1. Animals and Sampling

This study obtained 3 individuals sourced from Qionghai City, Hainan province, China. The methods of fish cultivation and sampling used in this study were based on previous report. A total of 15 samples similar individuals of each group with 0.5 ± 0.05g per sample) were collected during the different groups after ice anesthesia; these ovary samples were washed in 1 x PBS buffer and quick-frozen in liquid nitrogen for RNA extraction.

2.2. Library Constructing and Sequencing

Total RNA was extracted from each tissue sample using TRIzol Reagent (Invitrogen). RNA quality was assessed by agarose gel electrophoresis (agarose: 2%; TBE: 0.4 MTris base, 0.4 M boric acid, 0.5 M EDTA; 10x loading buffer: 0.25 M bromophenolblue, 0.4 M sucrose; Dye: Ethidium bromide, EtBr) and Nanodrop 2000 (ThermoScientific, CA, USA). According to previous studies, sequencing libraries were constructed using the Illumina Truseq TM RNA sample prep Kit (San Diego, CA, USA),and the libraries were polled and then sequenced on an Illumina Novaseq 6000 platform with 2 x 150 paired ends.

2.3. De Novo Assembly, Clustering Analysis, and Functional Annotation

High-quality raw data was obtained using the Illumina Novaseq 6000 platform.The quality of raw reads was visually evaluated by fastx tookit software version 0.0.14.The sequence adapters and low-quality bases (Quality score < 20) were filtered out, and short length reads (< 35 nt) were removed by fastp software version 0.19.5. Subsequent procedures, including assembly, clustering, and functional annotation, were performed based on previous reports. The major procedures as following: the high-quality raw data were assembled into transcripts by Trinity software version v2.8.5, the assembled transcripts were filtered out and de-redundant using TransRate software version v1.0.3and CD-HIT software version v4.5.7, and gene functional annotations were separately performed according to the following databases: NR (NCBI non-redundant proteins sequences), COG (Clusters of Orthologous Groups of proteins), Swiss-Prot (A manually annotated and reviewed protein sequence database), Pfam (Protein family),GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes).

2.4. Analysis of DEGs and Functional Enrichment

To measure expression levels, the raw data was aligned to unigenes using bowtie2 and RSEM software and reported as transcripts per kilobase of exon model per million mapped reads (TPM). The

identification of DEGs between different groups was carried out using edgeR software with thresholds set at [Log2FC| > 4.0 and p-adjust < 0.001. Functional enrichment analysis of DEGs (GO and KEGG) was performed using the Goatlools software version 0.6.5 with a Fisher exact test on the Majorbio platform (https://cloud.majorbio.com/ (accessed on)), [M7.1]and the corrected p-adjust < 0.05 was considered as significant enrichment.

3. Results

3.1 Sequencing and de novo assembly

To obtain a comprehensive transcriptomic resource for Cephalopholis miniata, fifteen cDNA libraries derived from the skin, gill, liver, muscle, and intestine (three biological replicates per tissue) were sequenced using the Illumina NovaSeq 6000 platform. After removing adaptor sequences, low-quality reads, and ambiguous reads, a total of 104.14 Gb of high-quality clean data were generated. Each library yielded more than 6.25 Gb of clean data, and the percentage of bases with a quality score of Q30 exceeded 96.99%, indicating that the sequencing data were of high quality and suitable for subsequent analyses.

The clean reads from all libraries were pooled and assembled de novo using Trinity. After assembly optimization and redundancy removal, a total of 81,285 transcripts and 58,804 unigenes were obtained. The average N50 length of the assembled sequences was 2,834 bp, demonstrating a high-quality transcriptome assembly.

To further evaluate the reliability of the assembled transcriptome, clean reads from each library were mapped back to the assembled reference sequences. The mapping rates ranged from 87.00% to 93.01%, suggesting that the assembled transcriptome exhibited good completeness and accurately represented the expressed transcripts of *C. miniata*. A total of 58,416 expressed unigenes and 80,783 expressed transcripts were detected across all samples. Among them, unigenes represented the longest transcript of each gene and therefore were used for subsequent functional annotation and downstream analyses. Detailed sequencing and assembly statistics are summarized in Table 1.

Table 1
Assessment of the optimized transcriptome assembly

Type	Unigene	Transcript
N50 length (bp)	2834	3019
Average length (bp)	1514.61	1662.36
Fragment mapped percent (%)		89.97
GC percent (%)	44.90	45.15
BUSCO score	C:94.9%[S:88.7%;D:6.2%]	C:98.7%[S:76.5%;D:22.2%]

3.2. Overall functional annotation

All assembled unigenes were functionally annotated against six public databases, including NR, Swiss-Prot, Pfam, eggNOG, GO, and KEGG, using BLASTx with an E-value threshold of $< 10^{-5}$. Among the 58,804 unigenes, 29,291 (49.81%), 21,596 (36.73%), 21,181 (36.02%), 24,486 (41.64%), 15,019 (25.54%), and 20,357 (34.62%) unigenes were annotated in the NR, Swiss-Prot, Pfam, eggNOG, GO, and KEGG databases, respectively. Overall, 29,605 unigenes (50.35%) were assigned functional annotations in at least one of the six databases (Fig. 2A).

GO classification assigned the annotated unigenes to three major functional categories, including biological process, cellular component, and molecular function (Fig. 2B). Within the biological process category, cellular process, biological regulation, developmental process, response to stimulus, and localization were the predominant terms. For cellular component, cellular anatomical structure and protein-containing complex were the major categories. In the molecular function category, binding and catalytic activity accounted for the largest proportions, followed by transcription regulator activity, molecular function regulator activity, and transporter activity. KEGG annotation further assigned the unigenes to diverse pathways covering metabolism, environmental information processing, cellular processes, organismal systems, and human disease-related pathways (Fig. 2C). Among the annotated pathways, signal transduction represented the largest category, followed by immune system, endocrine system, cell motility, signaling molecules and interaction, and cellular community. Metabolism-related pathways, including lipid metabolism and metabolism of cofactors and vitamins, were also represented. These annotations indicated that the assembled transcriptome contained genes involved in diverse biological processes, particularly those associated with signal transduction, immune regulation, metabolism, and cellular functions.

Collectively, the GO and KEGG annotation results demonstrated the functional diversity of the assembled transcriptome and provided a foundation for subsequent analysis of tissue-specific gene expression and the molecular characteristics of skin in *C. argus*.

3.3.Transcriptomic profiling and tissue-specific expression patterns

To characterize the transcriptional landscape of the skin and identify genes associated with tissue-specific functions, RNA-seq was performed on five tissues, including skin (S), gill (G), liver (L), muscle (Mu), and intestine (In), with three biological replicates for each tissue. The sequencing data showed consistently high quality across all 15 libraries, with Q20 values exceeding 99% and Q30 values generally above 96%, supporting the reliability of the transcriptomic datasets for subsequent comparative analyses. Unsupervised clustering analysis revealed a clear tissue-dependent organization of the transcriptomic profiles. Samples from the same tissue clustered closely with each other, whereas samples from different tissues were clearly separated, indicating strong tissue-specific transcriptional signatures (Fig. 3A). Importantly, the three biological replicates of skin showed highly similar expression

patterns, demonstrating good within-tissue reproducibility. The sample correlation heatmap further supported this observation, with replicates from each tissue displaying consistently high correlations and forming distinct tissue-associated clusters (Fig. 3B).

Principal component analysis (PCA) provided further evidence for the strong tissue-specificity of gene expression. The first two principal components explained 33.06% and 26.57% of the total transcriptomic variance, respectively, accounting for 59.63% of the overall variation (Fig. 3C). Biological replicates from the same tissue clustered tightly together, while the five tissues occupied distinct regions of the PCA space. Skin samples formed a reproducible cluster that was clearly separated from liver, muscle, intestine, and gill, demonstrating that tissue identity represents a major source of transcriptional variation in Cromileptes altivelis. Together, these results indicate that the skin possesses a distinct and reproducible transcriptional profile compared with the major internal and respiratory tissues examined here, providing a robust basis for subsequent identification of skin-enriched genes and characterization of their potential biological functions.

3.4.Identification of differentially expressed genes between skin and other tissues

Differential expression analysis was conducted to investigate transcriptional differences between skin and other tissues, including gill, liver, muscle, and intestine. A total of four pairwise comparisons were performed, including Cm_S vs Cm_G, Cm_S vs Cm_L, Cm_S vs Cm_Mu, and Cm_S vs Cm_In. A large number of differentially expressed genes (DEGs) were identified between skin and the other tissues (Fig. 4). Compared with gill, 17,706 DEGs were detected in skin, including 7,603 up-regulated and 10,103 down-regulated genes. The comparison between skin and liver exhibited the highest number of DEGs, with 32,372 genes, including 27,823 up-regulated and 4,549 down-regulated genes in skin. Similarly, 29,843 DEGs were identified between skin and muscle, consisting of 25,548 up-regulated and 4,295 down-regulated genes. Compared with intestine, 26,827 DEGs were detected, including 16,237 up-regulated and 10,590 down-regulated genes.

Among the four comparisons, the largest transcriptional divergence was observed between skin and liver, followed by skin and muscle, suggesting substantial functional specialization between skin and these internal tissues. In contrast, the relatively lower number of DEGs between skin and gill indicated a closer transcriptional relationship between the two external tissues exposed to aquatic environments. Overall, these results demonstrated that skin exhibited distinct gene expression profiles compared with other tissues, reflecting its specialized physiological roles. The identified DEGs provided a basis for further screening of skin-enriched genes and exploring the molecular mechanisms associated with skin barrier function, immune defense, and environmental adaptation in *C. miniata*.

3.5.Functional characterization of skin-enriched genes

To investigate the molecular basis of skin functional specialization in Cephalopholis miniata, genes consistently up-regulated in skin relative to the gill, liver, muscle, and intestine were identified from the

four pairwise comparisons. A total of 1,495 genes were commonly up-regulated in skin across all four comparisons and were therefore defined as skin-enriched genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses revealed that these genes were predominantly associated with extracellular matrix organization, tissue structure, cell adhesion, innate immune defense, redox regulation, lipid metabolism, and pigmentation (Fig. 5A-C).

GO enrichment analysis showed a strong representation of terms related to the extracellular matrix and tissue organization, including extracellular matrix, collagen-containing extracellular matrix, external encapsulating structure, extracellular matrix organization, extracellular structure organization, and tissue development. Terms related to plasma membrane, calcium ion binding, and extracellular matrix structural constituent were also significantly enriched. Consistent with these findings, KEGG analysis identified ECM–receptor interaction, focal adhesion, and cell adhesion molecules among the major enriched pathways (Fig. 5D-E). Several collagen- and ECM-associated genes, including COL7A1, COL6A2, COL11A1, SPARC, FRAS1, ITGA6, ITGB4, and LAMB4, were identified in the skin-enriched gene set. In addition, epithelial and cell-junction-related genes, including CLDN4, CLDN8, CLDN11B, DSP, JUP, CDH11, KRT15, and KRT19, were also represented. Together, these results indicate that skin-enriched genes are strongly associated with extracellular matrix organization, epithelial integrity, and cell–cell and cell–matrix adhesion, highlighting the structural specialization of skin.

The skin-enriched gene set also showed a prominent innate immune signature. KEGG enrichment included leukocyte transendothelial migration and cell adhesion molecules, while multiple complement-related genes, including C3, C7, C8B, C9, and CFI, were identified among the skin-enriched genes. Several genes associated with immune signaling and antimicrobial defense, including CCL27, CCL44, IL17RD, an IL-17A/F-related gene, an NLR-related gene, β-defensin-like 1, and C-type lysozyme, were also detected. The coordinated enrichment of complement components, chemokines, cytokine-related genes, and antimicrobial factors suggests that the skin possesses a transcriptionally active innate immune defense system, in addition to its structural barrier function.

Furthermore, glutathione metabolism and arachidonic acid metabolism were significantly enriched in the KEGG analysis. Genes such as GPX7, GPX8, and GSS were associated with glutathione metabolism, indicating the involvement of the skin-enriched gene set in antioxidant and redox-related processes. Genes including ALOX5, ALOX5B, and PTGDS were associated with arachidonic acid metabolism, suggesting that lipid-mediated regulatory processes are also represented in the skin transcriptome. These metabolic signatures, together with the structural and immune-related pathways, indicate that skin specialization involves multiple coordinated biological processes rather than a single functional pathway. Notably, several pigmentation-associated genes were identified among the 1,495 skin-enriched genes, including MITF, PMEL, TYRP1, and melanoregulin, as well as WNT7A, WNT7B, WNT10A, and WNT10B. These genes are associated with pigment-cell development, melanosome formation, and pigmentation-related signaling, providing a distinct molecular signature associated with the pigmentation characteristics of *C. miniata* skin. Overall, the functional enrichment analyses demonstrate that the skin-enriched transcriptome is characterized by coordinated signatures related to structural

organization and barrier integrity, innate immune defense, antioxidant and lipid metabolism, and pigmentation. These findings provide a molecular basis for understanding the functional specialization of *C. miniata* skin and identify candidate genes potentially underlying its structural, defensive, environmental-response, and pigmentation-related properties(Fig. 6).

4. Discussion

The present study provides a comparative transcriptomic framework for elucidating the molecular specialization of the skin in the wild coral hind, *C. miniata.* The distinct clustering of biological replicates, strong separation of skin from gill, liver, muscle, and intestine in PCA, and the large number of differentially expressed genes collectively demonstrate that skin possesses a highly specialized transcriptional profile rather than representing a simple extension of other tissues. Notably, 1,495 genes were consistently up-regulated in skin relative to all four comparison tissues, providing a stringent gene set for characterizing the molecular features of skin specialization. These genes were predominantly associated with extracellular matrix organization, epithelial integrity, innate immunity, redox homeostasis, arachidonic acid metabolism, and pigmentation. Importantly, the independent TPM analysis further supported the biological relevance of these candidates, as representative genes from these functional modules showed substantially higher expression in skin than in the other tissues. The first major feature of the skin-enriched transcriptome was the strong representation of extracellular matrix and epithelial barrier functions. GO enrichment was dominated by extracellular matrix, collagen-containing extracellular matrix, extracellular matrix organization, tissue development, and extracellular structure organization, whereas KEGG analysis highlighted ECM-receptor interaction, focal adhesion, and cell adhesion molecules. Consistent with these pathway-level results, COL11A1, COL7A1, COL6A2, SPARC, LAMB4, ITGA6, and ITGB4 were identified among the conserved skin-enriched genes. Several of these genes also showed clear enrichment in the TPM dataset; for example, SPARC displayed a mean skin TPM of approximately 477.9 compared with 79.1 in the other tissues, while LAMB4 showed a more than five-fold difference in skin expression. These findings are consistent with the established view that teleost skin is a structurally complex interface in which the epidermis, dermal extracellular matrix, cytoskeleton, and cell-cell and cell-matrix adhesion systems collectively maintain tissue integrity. The extracellular matrix not only provides mechanical support but also contributes to epithelial organization, tissue remodeling, and cellular signaling, thereby forming an important structural foundation for barrier function in fish skin (Doyle et al. 2022; Esteban 2024).

The particularly strong enrichment of tight-junction and epithelial adhesion genes further suggests that the skin of *C. miniata* possesses a highly developed epithelial barrier. CLDN4, CLDN8, and CLDN11B, together with DSP, JUP, KRT15, and KRT19, were consistently up-regulated in skin. Among them, CLDN11B showed one of the strongest tissue biases in the TPM dataset, with a mean skin expression of approximately 44.9 TPM compared with only 0.006 TPM in the other tissues, corresponding to an approximately 8.7-fold log2 difference. DSP and CLDN4 also exhibited pronounced skin enrichment. Claudins are integral components of epithelial tight junctions and regulate paracellular permeability, ion movement, and epithelial barrier properties in teleosts (Doyle et al. 2022). Therefore, the coordinated

elevation of ECM, laminin-integrin, tight-junction, desmosomal, and keratin-associated genes indicates that the skin-enriched transcriptome reflects an integrated structural barrier system rather than isolated expression of individual structural genes. This is particularly relevant for marine fishes because the skin is continuously exposed to microorganisms, mechanical disturbance, and changes in the surrounding aquatic environment.

In addition to its physical barrier function, the skin-enriched gene set revealed a pronounced innate immune signature. Complement components C3, C7, C8B, and C9, together with CFI, were consistently elevated in skin, while immune-related genes such as CCL27A, CCL44, IL17RD, IL17A/F3, NLR-related genes, β-defensin-like 1, and C-type lysozyme were also identified. This coordinated enrichment is important because teleost skin represents a major mucosal immune surface where constitutive innate defense must operate continuously in direct contact with the aquatic environment (Li and Zhang 2022; Xu and Liu 2024; Zhou et al. 2026). Complement components can mediate pathogen recognition, opsonization, inflammatory signaling, and membrane attack complex formation, and the presence of multiple complement components in the skin suggests that complement-mediated defense may constitute an important local effector mechanism in *C. miniata* skin (Li and Zhang 2022). The simultaneous enrichment of C3, C7, C8B, and C9 is particularly noteworthy because these components participate at different stages of complement activation and terminal complex formation, suggesting that the skin may maintain a locally competent complement cascade rather than relying exclusively on systemic immune organs.

The antimicrobial and chemotactic components identified in the present study further support this interpretation. β-defensin-like 1 and C-type lysozyme showed consistent skin enrichment, with β-defensin-like 1 reaching approximately 20.9 TPM in skin compared with 2.72 TPM in the other tissues. Fish epidermal mucus contains a diverse repertoire of antimicrobial molecules, including antimicrobial peptides, lysozyme, lectins, proteases, and complement-associated factors, which collectively constitute the first line of defense against invading microorganisms (Song et al. 2021; Xu and Liu 2024; Zhou et al. 2026). The identification of CCL27A and CCL44 is also of particular interest because chemokines coordinate leukocyte trafficking and immune-cell recruitment. In the present dataset, CCL27A displayed a mean skin expression of approximately 122.5 TPM compared with 7.33 TPM in the other tissues, corresponding to a strong skin-biased expression pattern. Teleost chemokine systems are highly diversified and participate in both immune surveillance and inflammatory recruitment, although the precise functions of individual chemokines can differ among fish lineages (Xu and Liu 2024). Thus, the concurrent enrichment of complement components, antimicrobial peptides, lysozyme, chemokines, and IL-17-related signaling genes suggests that *C. miniata* skin functions as an active immunological interface in which physical barrier formation and immune surveillance are tightly integrated.

Another important feature of the skin-enriched transcriptome was the activation of antioxidant and lipid-mediated metabolic pathways. KEGG analysis identified glutathione metabolism and arachidonic acid metabolism as significantly enriched pathways, with GPX7, GPX8, and GSS associated with glutathione-related processes and ALOX5, ALOX5B, and PTGDSB associated with arachidonic acid metabolism. The

TPM data further strengthened this observation. For example, GPX8 showed a mean skin expression of 24.72 TPM versus 3.98 TPM in the other tissues, whereas ALOX5 exhibited a particularly strong skin bias, with 2.18 TPM in skin compared with only 0.029 TPM in the other tissues. PTGDSB, despite its high expression across tissues, was also strongly enriched in skin, reaching approximately 1040.7 TPM. Glutathione and glutathione-dependent enzymes represent an important antioxidant system that protects fish tissues against reactive oxygen species and environmental oxidative stress (Santana et al. 2022). For an externally exposed tissue such as skin, the enrichment of glutathione-related genes may therefore reflect an intrinsic requirement to maintain redox balance under continuous environmental challenge.

The enrichment of arachidonic acid metabolism provides a complementary metabolic dimension to this antioxidant response. Arachidonic acid-derived lipid mediators participate in inflammatory regulation, immune signaling, stress responses, and tissue homeostasis in fish (Xu et al. 2022). The elevated expression of ALOX5 and ALOX5B suggests that the skin may possess enhanced capacity for generating oxygenated lipid mediators, while the high expression of PTGDSB indicates active prostaglandin-related metabolism. These pathways may provide a molecular link between barrier maintenance, innate immunity, inflammation, and environmental adaptation. Importantly, this interpretation does not imply that the identified genes directly cause a particular immune or physiological phenotype; rather, their coordinated expression indicates that lipid-mediated signaling is an integral component of the skin transcriptional program. A particularly interesting finding was the coexistence of antioxidant, lipid-metabolic, and pigmentation-associated signatures. The skin-enriched gene set contained MITFA, TYRP1B, PMELA, and melanoregulin, together with WNT7A, WNT7B, WNT10A, and WNT10B. These genes showed clear skin-biased expression, including approximately 4.7-fold and 4.5-fold log2 differences for TYRP1B and PMELA, respectively. The coordinated enrichment of these genes provides stronger evidence for an active pigment-cell-associated molecular program in *C. miniata* skin than would be inferred from pathway enrichment alone.

The biological relevance of this pigmentation signature is supported by studies of closely related serranid fishes. In *P. leopardus*, transcriptomic analyses have demonstrated that skin-color variation involves melanogenesis, tyrosine metabolism, Wnt signaling, cAMP signaling, and PI3K/Akt signaling, with MITF, TYR, TYRP1, DCT, and WNT1 implicated in melanin production and pigment-cell regulation (Song et al. 2022). More recently, comparative transcriptomic analysis during *P. leopardus* morphogenesis identified coordinated changes in carotenoid metabolism, melanin synthesis, and pigment-cell development, including TYR, TYRP1, DCT, PMEL, WNT, EDNRB, SOX10, and related genes (Liu et al. 2025). Thus, the identification of MITFA, TYRP1B, PMELA, and Wnt-family genes in *C. miniata* provides evidence that conserved pigment-cell regulatory mechanisms may operate across closely related serranid species. The observed combination of Wnt-family genes and MITFA is particularly informative. In zebrafish, Wnt signaling directly activates the MITF homolog nacre and promotes pigment-cell specification (Dorsky et al. 2000), while Wnt3a has been shown to stimulate MITF transcription through β-catenin/LEF1-dependent regulation (Takeda et al. 2000). Therefore, the simultaneous skin enrichment of WNT7A, WNT7B, WNT10A, WNT10B, and MITFA in the present study is

consistent with a conserved signaling framework in which Wnt-associated developmental signals may contribute to pigment-cell differentiation and pigmentation-related gene expression. However, because the present study examined tissue-level transcriptomes rather than isolated chromatophore populations, these findings should be interpreted as evidence of a pigmentation-associated transcriptional program rather than direct proof of Wnt-mediated regulation of MITFA in *C. miniata*.

The relationship between pigmentation and metabolic homeostasis may also be biologically relevant in *C. miniata*. Recent multi-omics work in *P. leopardus* demonstrated that red versus black skin coloration was associated not only with melanogenesis-related genes such as PMEL, TYR, TYRP1, DCT, and WNT1, but also with antioxidant and lipid-metabolism pathways, including fatty acid metabolism and unsaturated fatty acid biosynthesis (Liu et al. 2025). The coexistence of glutathione metabolism, arachidonic acid metabolism, and pigmentation-related genes in our 1,495-gene skin-enriched set therefore raises the possibility that pigmentation in *C. miniata* is embedded within a broader metabolic and stress-response network rather than functioning as an isolated pathway. This interpretation is consistent with the recent understanding that pigment-cell differentiation and pigment synthesis are influenced by cellular redox state, lipid metabolism, and environmental conditions.

Taken together, the results support a model in which *C. miniata* skin is characterized by coordinated transcriptional specialization across several interconnected functional modules. The ECM and epithelial junction network provides mechanical and paracellular barrier integrity; complement, antimicrobial peptides, lysozyme, chemokines, and IL-17-related genes provide constitutive immune surveillance; glutathione-associated enzymes contribute to redox protection; arachidonic acid metabolism provides lipid-derived signaling capacity; and the MITF–TYRP1–PMEL/Wnt-associated module provides a molecular basis for pigmentation and pigment-cell-associated functions. Rather than representing independent pathways, these modules are likely functionally interconnected. For example, epithelial integrity determines the physical interface with the environment, immune signaling responds to perturbations of this interface, antioxidant mechanisms limit the oxidative costs of environmental and inflammatory responses, and lipid mediators may coordinate inflammatory and tissue-homeostatic processes. The pigmentation machinery may operate within the same metabolic environment, providing a potential molecular connection between coloration, environmental adaptation, and cellular homeostasis.

5. Conclusions

Overall, this study establishes a molecular framework for understanding the multifunctional specialization of *C. miniata* skin. The integration of comparative transcriptomics, four-way intersection analysis, functional enrichment, and tissue-level TPM expression identifies a coherent set of candidate genes associated with structural barrier formation, innate immune defense, redox regulation, lipid-mediated signaling, and pigmentation. In particular, the coordinated enrichment of ECM/junctional genes, complement and antimicrobial factors, glutathione- and arachidonic acid-related genes, and the MITF–TYRP1–PMEL/Wnt-associated pigmentation module suggests that *C. miniata* skin functions as an

integrated barrier–immune–metabolic–pigmentation organ. These findings provide a foundation for future investigations of disease resistance, environmental adaptation, and body-color regulation in coral reef-associated serranid fishes.

Declarations

Author Contributions: B.Y. (Bingcong Ye): Data curation, Formal analysis, Investigation, Software, Visualization, Writing—original draft; Y.Y. (Bingcong Yue): Conceptualization, Methodology, Investigation, Software, Visualization, Writing—original draft. J.L. (Jin Liang): Formal analysis, Validation; Y.R. (Yuanhao Ren): Investigation, Methodology. L.Y. (Liguo Yang): Formal analysis and data curation. X.S. (Xuefeng Song): Formal analysis, Investigation, Data curation, Writing—review and editing; B.Z. (Bianbian Zhang): Conceptualization, Methodology, Resources, Visualization, Supervision, Writing—review & editing, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: *Cephalopholis miniata* is neither an endangered nor protected species. All experiments in this study were conducted according to national and institutional guidelines. The animal study protocol was approved by the Laboratory Animal Ethic Committee of the East China Sea Fisheries Research Institute (protocol code: CAFS LAECECSFRI-2025-08-20-1 and approval date: 10 July 2025).

Data Availability Statement: Data from this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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Figures



Figure 1

Sequence length distribution

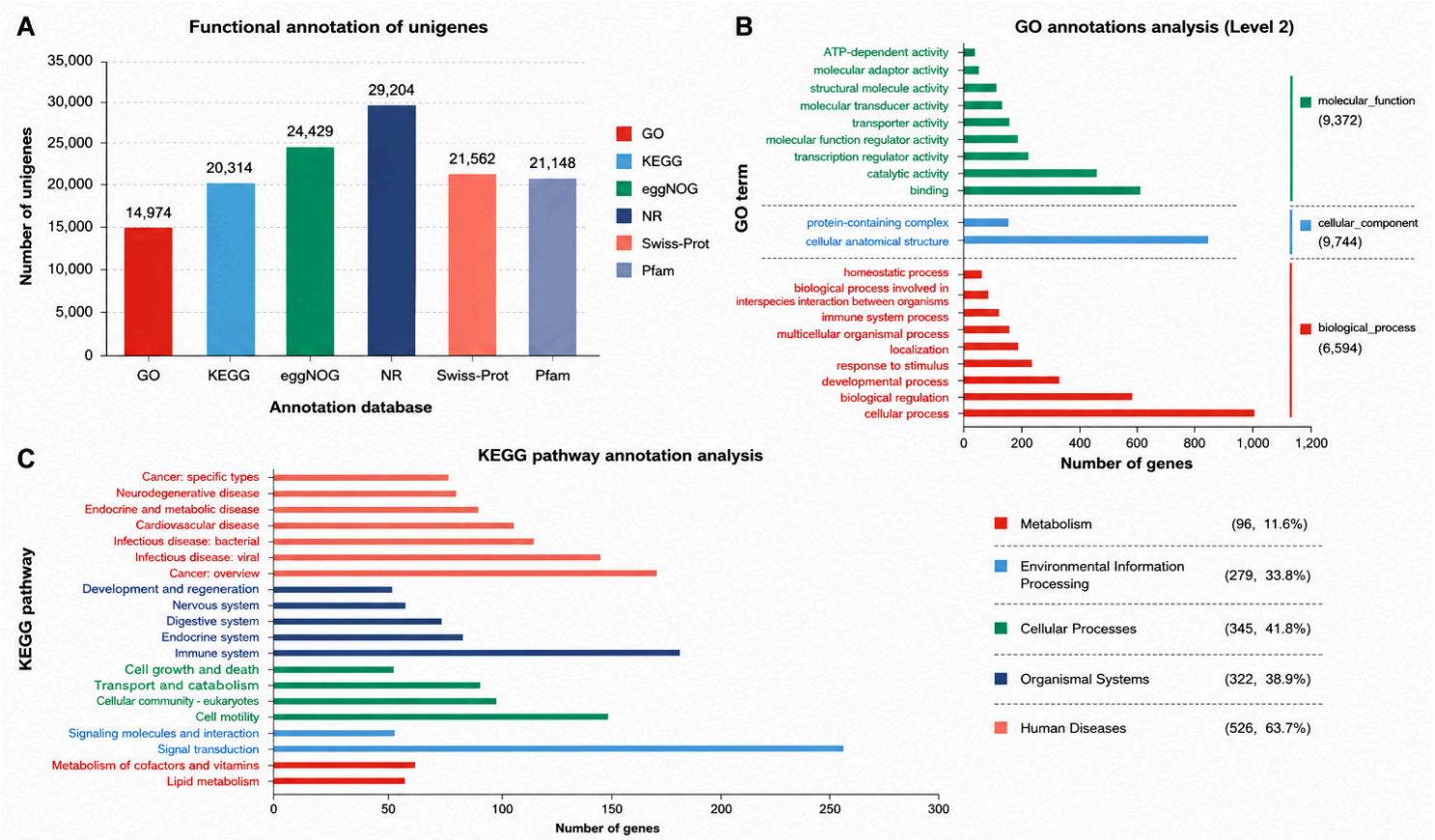


Fig. 2. Functional annotation of the transcriptome of *Cephalopholis miniata*.
(A) Number of unigenes annotated in six public databases: GO, KEGG, eggNOG, NR, Swiss-Prot and Pfam.
(B) GO annotation of unigenes at Level 2, categorized into molecular function, cellular component and biological process.
(C) KEGG pathway classification of the annotated unigenes. The numbers in parentheses indicate the number of genes in each category, and the percentages indicate the proportion of genes in the total annotated unigenes.

Figure 2

See image above for figure legend.

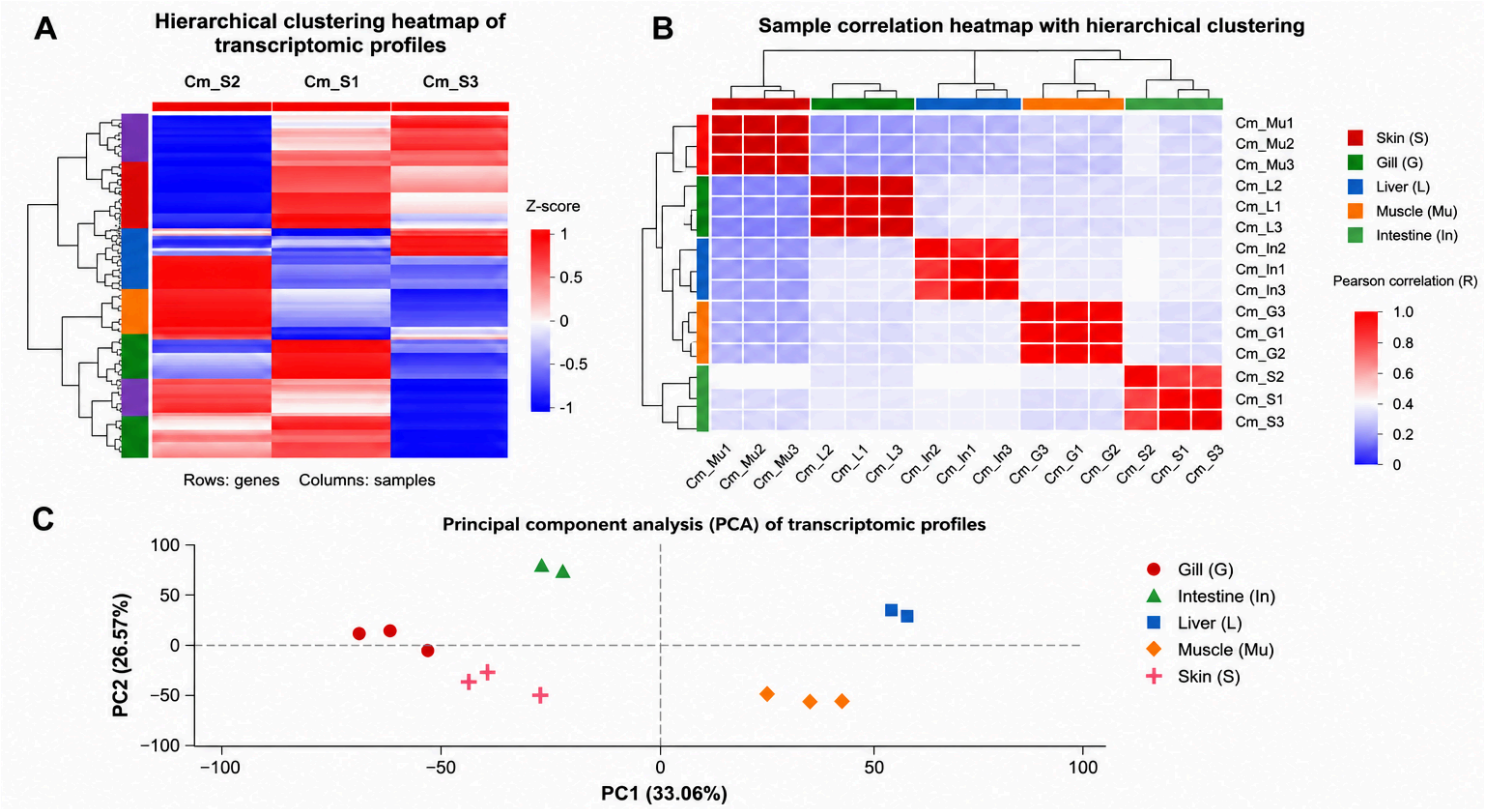


Fig. 3. Transcriptomic profiling and tissue-specific expression patterns across five tissues of *Cephalopholis miniata*.

(A) Hierarchical clustering heatmap of gene expression in the three skin replicates (Cm_S1–Cm_S3). Rows represent genes, and columns represent samples. Colors indicate row Z-score values.

(B) Heatmap of pairwise Pearson correlation coefficients between all 15 samples. Hierarchical clustering was performed based on 1–Pearson correlation.

(C) Principal component analysis (PCA) of transcriptomic profiles. Each point represents one sample, and samples are colored by tissue type. The percentages in parentheses indicate the proportion of variance explained by each principal component.

S, skin; G, gill; L, liver; Mu, muscle; In, intestine. Numbers (1–3) indicate biological replicates.

Figure 3

See image above for figure legend.

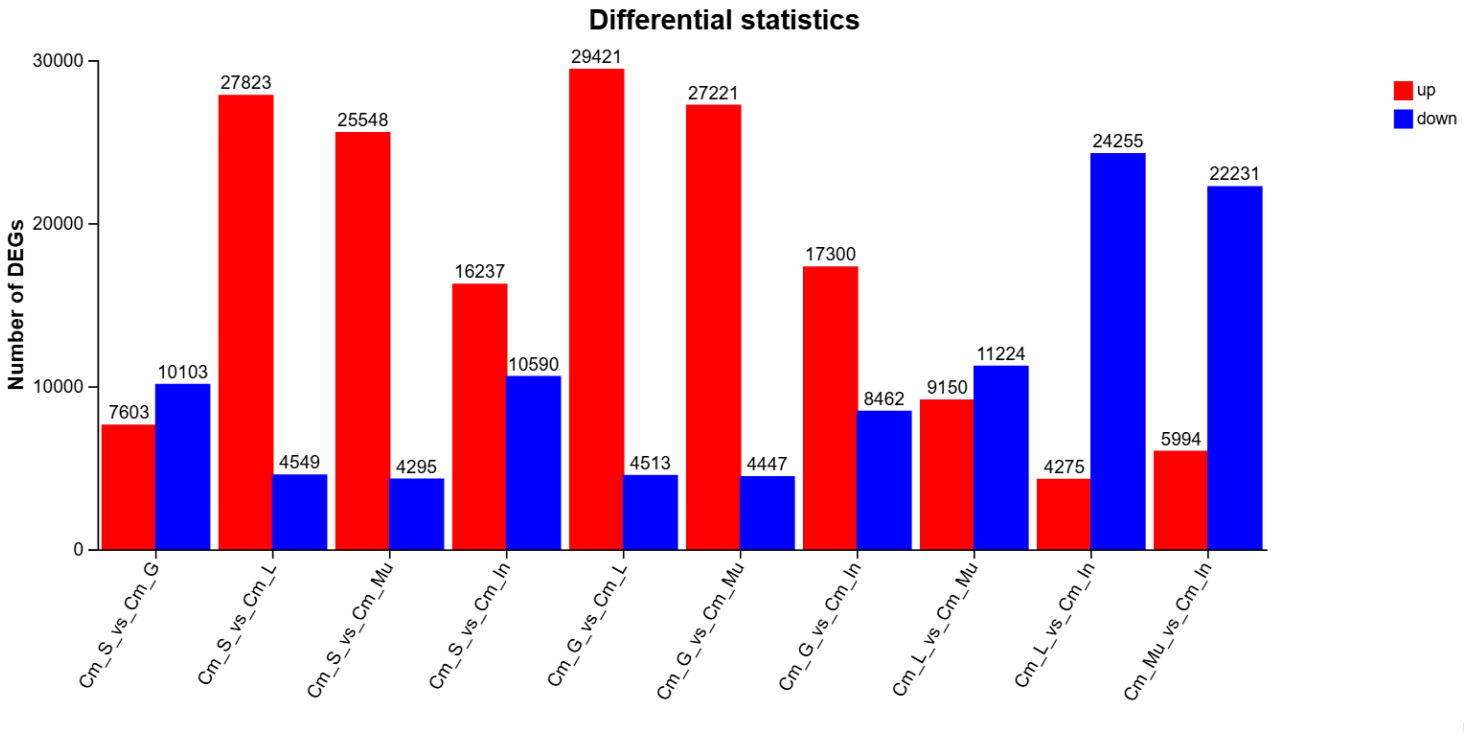


Figure 4

Number of DEGs.

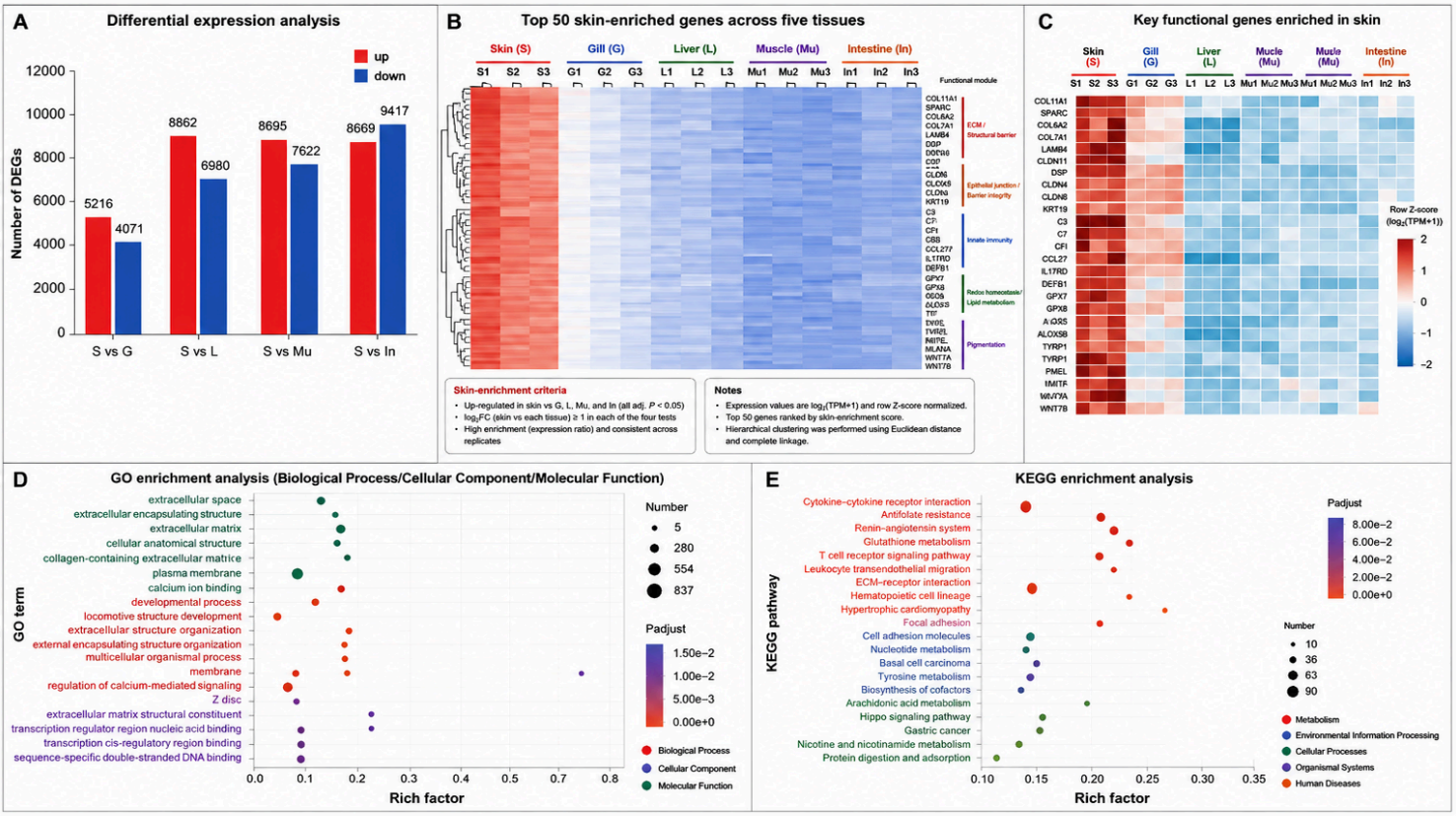


Fig. 5. Identification and functional characterization of skin-enriched genes in *Cephalopholis miniata*.

(A) Numbers of up-regulated (red) and down-regulated (blue) DEGs in skin vs gill (S vs G), liver (S vs L), muscle (S vs Mu) and intestine (S vs In).

(B) Heatmap of the top 50 skin-enriched genes across five tissues. Genes are clustered by expression pattern. Functional modules are indicated.

(C) Heatmap of representative key functional genes enriched in skin.

(D) GO enrichment analysis of skin-enriched genes. The top 20 terms in the three GO categories are shown. Dot size represents the number of genes, and color indicates the adjusted *P* value.

(E) KEGG enrichment analysis of skin-enriched genes. The top 20 pathways are shown. Dot size represents the number of genes, and color indicates the adjusted *P* value.

Figure 5

See image above for figure legend.

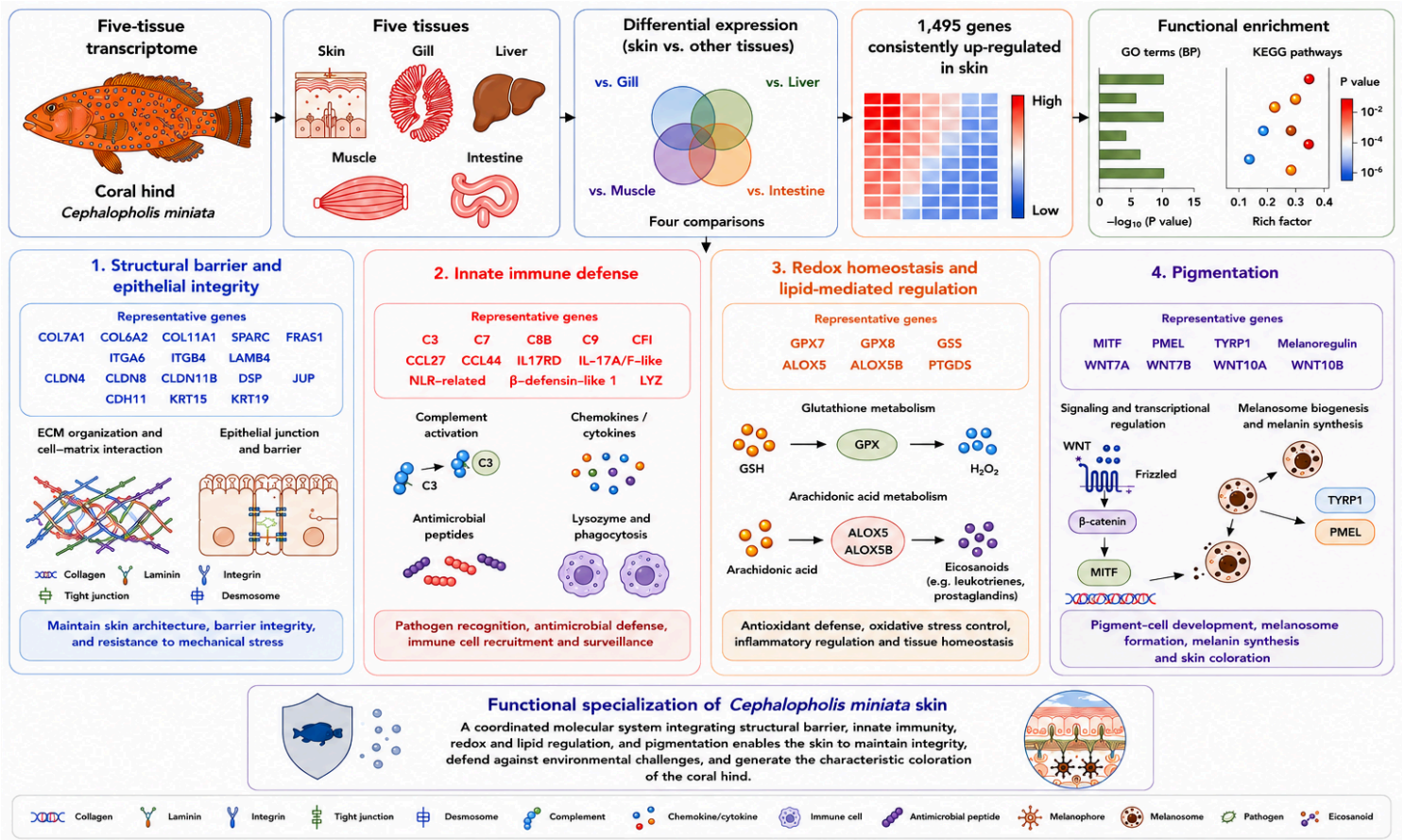


Figure 6. A transcriptomic framework for the functional specialization of *Cephalopholis miniata* skin. Five tissues (skin, gill, liver, muscle, and intestine) were subjected to RNA sequencing. Comparative analysis identified 1,495 genes consistently up-regulated in skin across four comparisons (vs. gill, liver, muscle, and intestine). Functional enrichment highlighted four major biological themes: (1) structural barrier and epithelial integrity, (2) innate immune defense, (3) redox homeostasis and lipid-mediated regulation, and (4) pigmentation. Representative genes and biological processes are shown in each module. This coordinated molecular system enables the skin to maintain barrier integrity, resist pathogens and environmental stress, and generate the characteristic coloration of the coral hind.

Figure 6

See image above for figure legend.