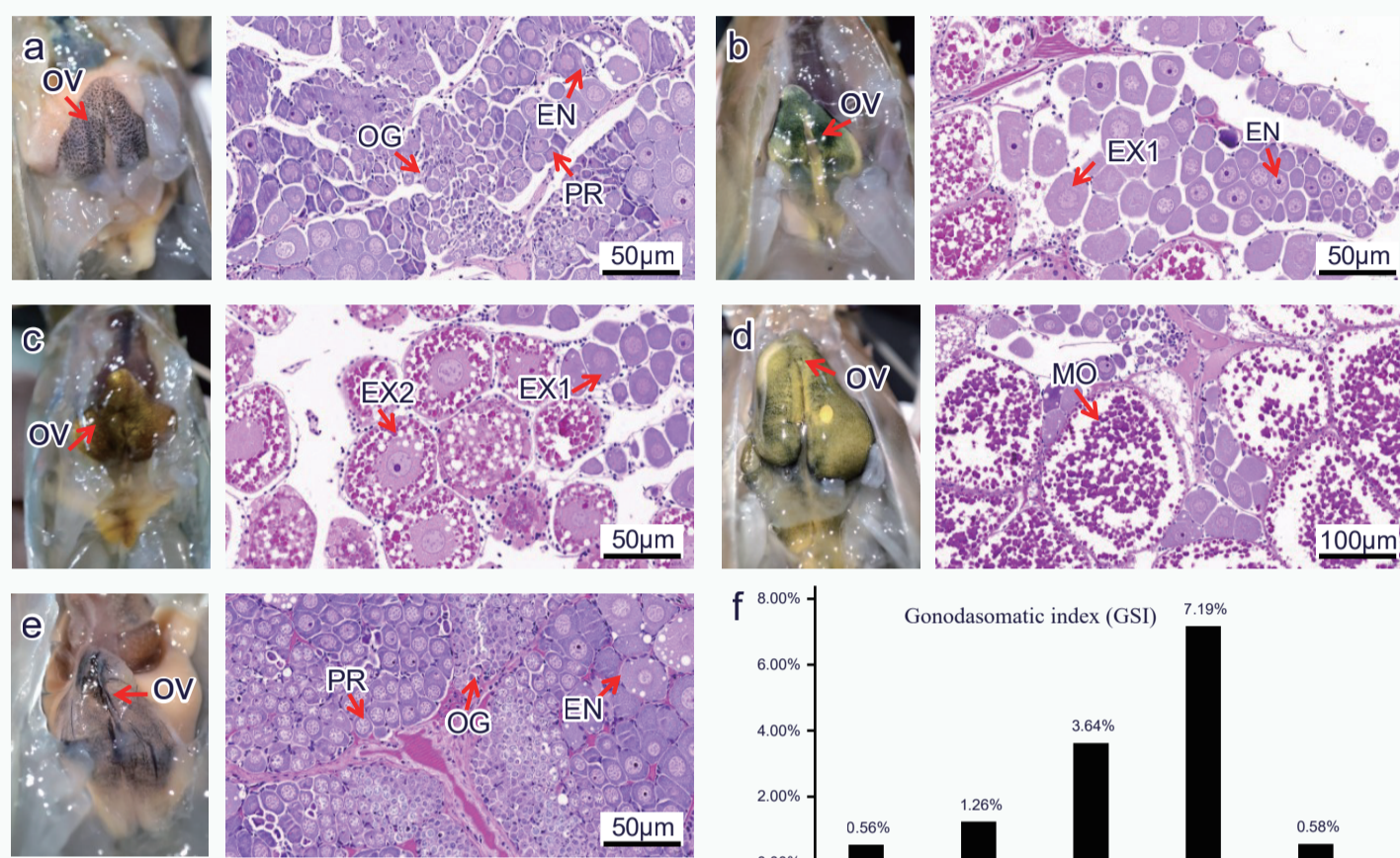


# Studies on the patterns of ovary re-development and screening the key genes and signaling pathways initiating ovary re-development of *Macrobrachium rosenbergii*

## Abstract

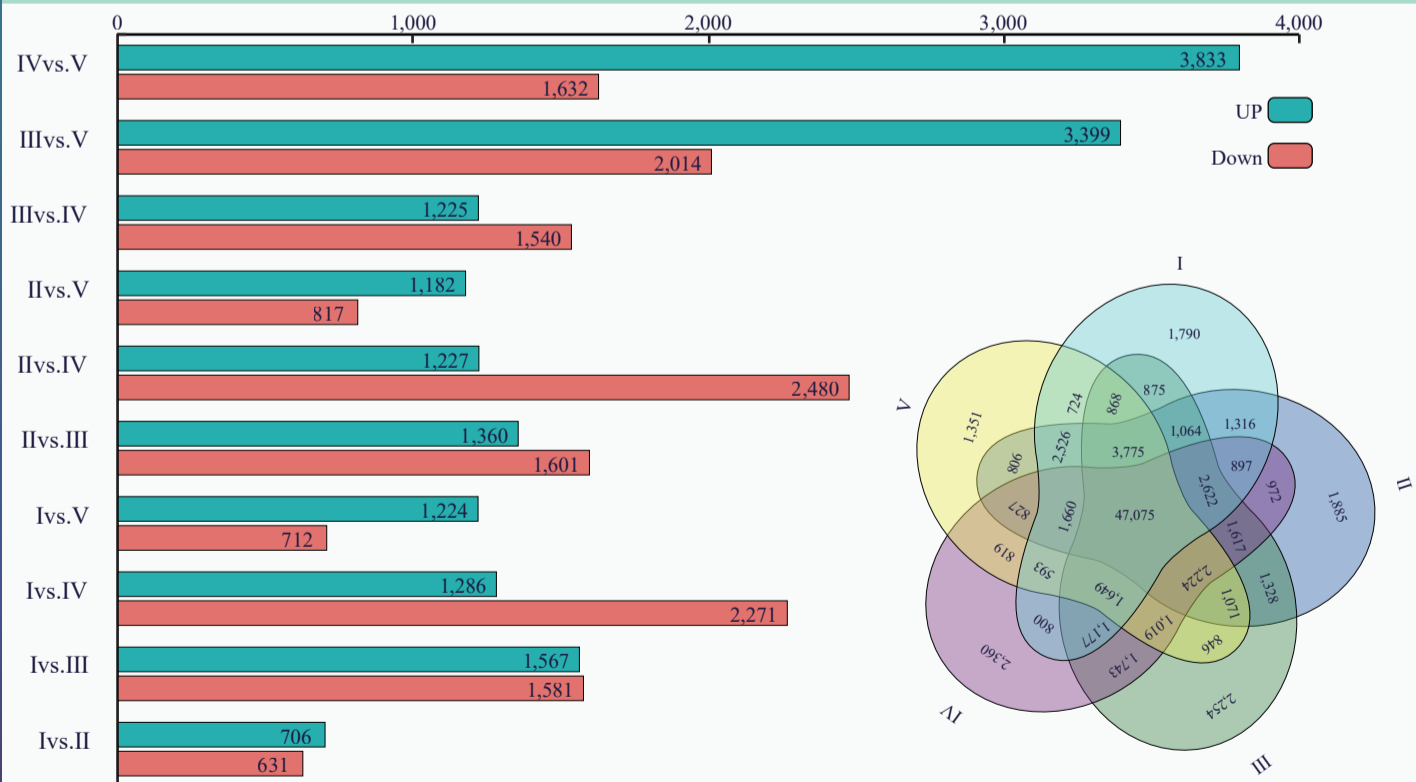
Under optimal temperature conditions, *Macrobrachium rosenbergii* can spawn year-round, which is often used to increase economic efficiency. This study examined the dynamics and key genes involved in ovary re-development through external morphology, histology, and transcriptomics. Ovary re-development cycle was classified into five stages: oogonia accumulation, oocyte formation, oocyte maturation, re-spawning and recovery. Transcriptome sequencing generated 99.3 Gb of data, annotating 89,614 unigenes and identifying 32,288 differentially expressed genes. Both WGCNA and PPI analyses highlighted the importance of ribosome and proteasome pathways, with KEGG analysis further confirming the significant enrichment. Ribosomal gene families like *RPL24* and proteasomal gene families such as *PSMC2* were identified as key genes in initiating ovary re-development. These findings lay a theoretical foundation for revealing the molecular regulatory mechanisms of ovary re-development.

## Figure 1



Note: a, Oogonia accumulation stage ovary,  $\times 30$ ; b, Oocyte formation stage ovary,  $\times 30$ ; c, Oocyte maturation stage ovary,  $\times 30$ ; d, Re-spawning stage ovary,  $\times 15$ ; e, Recovery stage ovary,  $\times 30$ ; f, Gonadosomatic index (%); OV, Ovary; OG, Oogonia; PR, Pre-vitellogenic oocyte; EN, Endogenous vitellogenic oocyte; EX1, Early exogenous vitellogenic oocyte; EX2, Mid-to-late exogenous vitellogenic oocyte; MO, Mature oocyte; I, Oogonia accumulation stage; II, Oocyte formation stage; III, Oocyte maturation stage; IV, Re-spawning stage; V, Recovery stage.

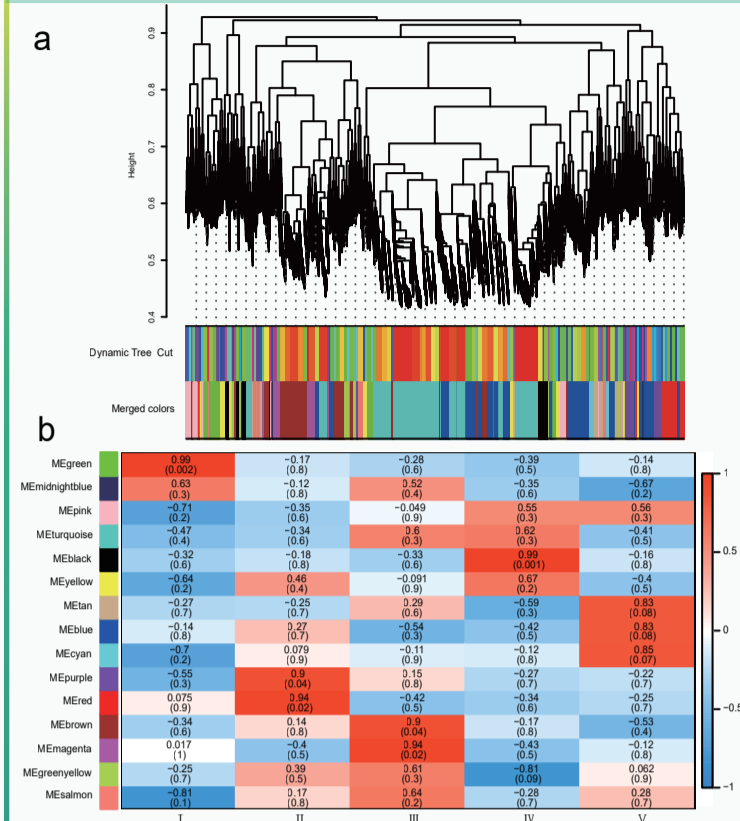
## Figure 2



Transcriptomic data from the five ovarian redevelopment stages were compared pairwise to analyze DEGs. The results revealed a total of 32,288 DEGs across all comparison groups, comprising 17,009 upregulated genes and 15,279 downregulated genes. The comparison between Stage IV and Stage V identified the highest number of DEGs, with 5,465 in total, including 3,833 upregulated and 1,632 downregulated genes. Venn diagram analysis showed that a total of 47,075 genes were identified across the five distinct ovarian redevelopment stages.

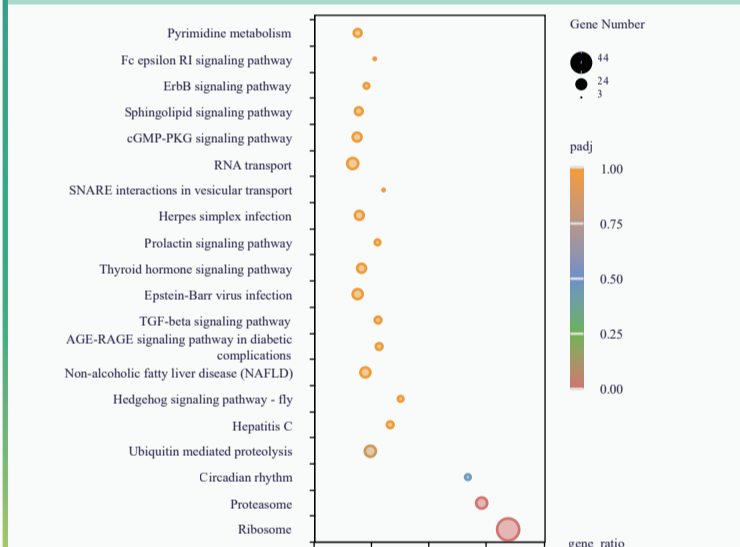
## Figure 3

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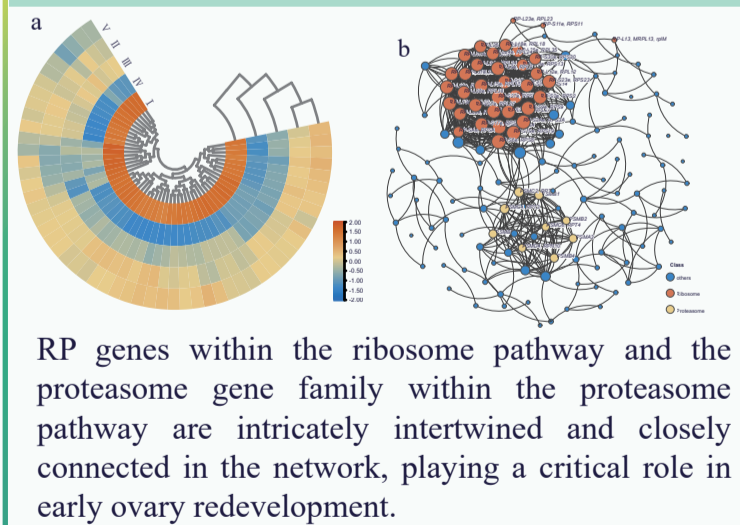
The MEgreen module exhibited the highest correlation with Stage I ovaries, as did the MEblack module with Stage IV ovaries, both with a correlation coefficient of 0.99 and a significance level of  $P < 0.01$ , indicating a highly significant correlation.

## Figure 4



Ribosome and proteasome pathways were significantly enriched ( $P_{\text{adjust}} < 0.05$ ).

## Figure 5



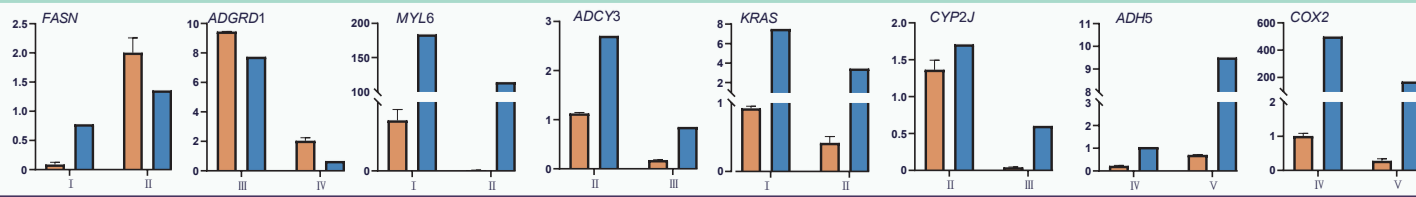
RP genes within the ribosome pathway and the proteasome gene family within the proteasome pathway are intricately intertwined and closely connected in the network, playing a critical role in early ovary redevelopment.

## Summary

Ribosome and proteasome signaling pathways play key roles in early ovary redevelopment of *Macrobrachium rosenbergii*.

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## Figure 6



The qRT-PCR validation results were consistent with the expression patterns of the same genes in the transcriptome, confirming the reliability of the transcriptome sequencing data.