

Genome-Wide Epigenetic Analysis of CpG Methylation (5mC) and Gene Regulation in a Eusocial Marine Shrimp (*Synalpheus chacei*)

DNA Methylation Functions Primarily as a Genome Defense Mechanism
Rather Than a Regulator of Social Phenotypes

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Abstract

Synalpheus chacei is a eusocial snapping shrimp with reproductive division of labor, making it a rare marine model for studying epigenetic regulation. We performed assembly-wide CpG methylation (5mC) analysis to test whether promoter methylation regulates social behavior genes. Of 6,848 predicted genes, 1,103 (16%) exhibited highly methylated promoters ($\geq 50\%$ methylation, $\geq 30\times$ coverage). Instead, functional annotation (80% success) and GO enrichment identified these methylated genes as predominantly transposable elements (77% annotated as TEs). 46 of the 47 enriched GO terms ($p < 0.05$) localized exclusively to the methylated set, with top functions including DNA binding ($p = 3.9 \times 10^{-13}$) and RNA-directed DNA polymerase ($p = 6.3 \times 10^{-11}$). These results identify promoter methylation in *S. chacei* as a primary genome defense mechanism, silencing transposable elements rather than regulating social behavior genes. This conserved role likely provides the genomic stability necessary for long-lived reproductive castes.

Introduction

Unique Marine Model: *Synalpheus* snapping shrimps are the only marine lineage to have evolved eusociality, characterized by overlapping generations and reproductive division of labor (Duffy, 1996)

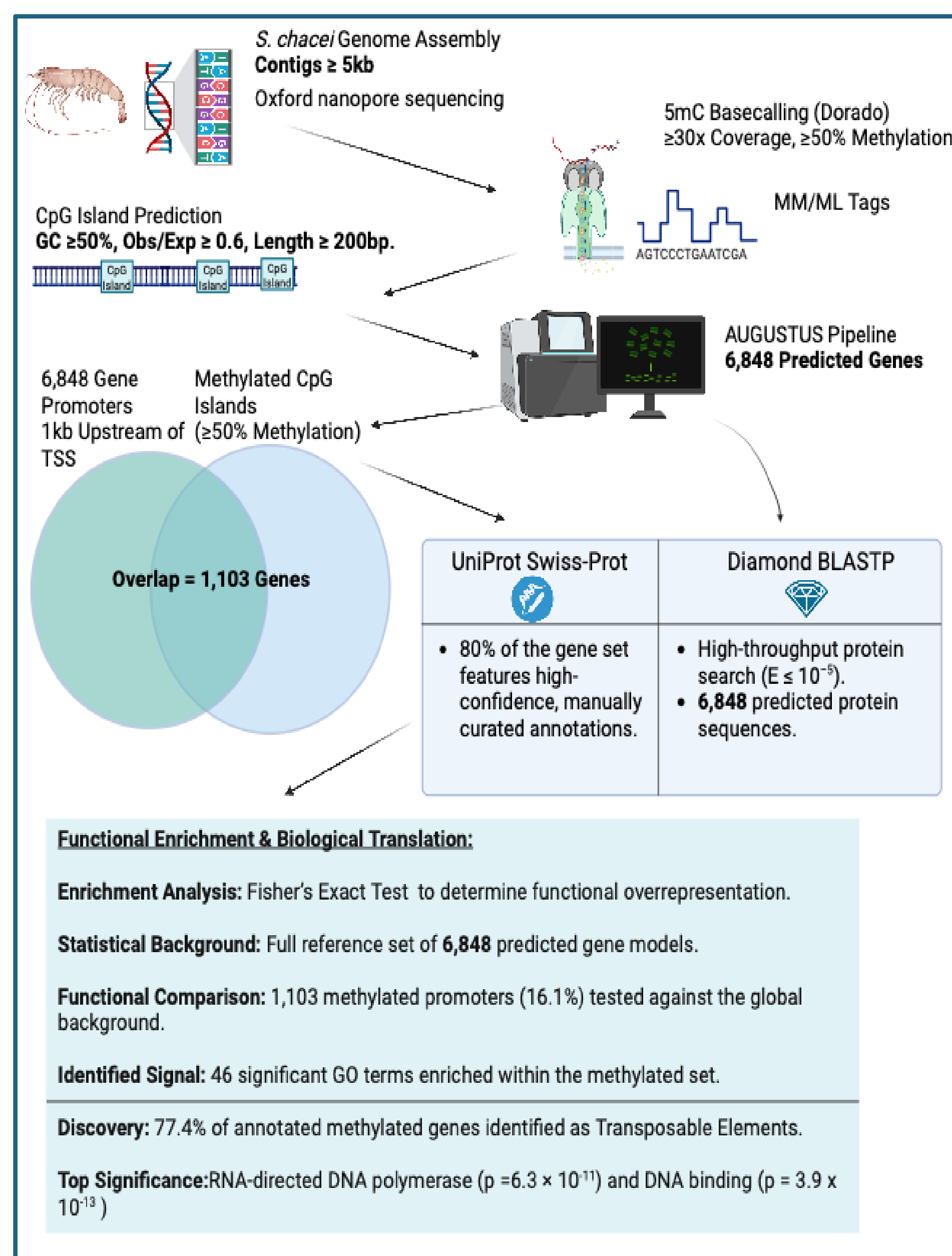
Genomic Cost of Sociality: (Chak et al., 2021) established that eusocial species in the *Synalpheus* genus possess significantly higher transposable element (TE) loads than solitary relatives, potentially impacting genome stability.

Epigenetic Hypothesis: In social insects, cytosine methylation (5mC) regulates caste-specific social genes (Kucharski et al., 2008). We hypothesized that 5mC methylation similarly targets behavioral pathways in *S. chacei*

Genome Defense Theory: Alternatively, DNA methylation may function as a “genome defense” mechanism (Yoder et al., 1997), specifically silencing high TE loads to protect chromosomal integrity of long-lived reproductive individuals (queens).

Study Objective: First assembly-wide characterization of the *S. chacei* methylome and identify the functional targets of promoter-proximal CpG methylation across 6,848 predicted genes.

Methods



Results

Assembly-Wide Methylation Landscape:

- 15,270 contigs analyzed ($\geq 5\text{kb}$)
- 6,848 protein-coding genes predicted
- 2.6M CpG sites: 440,356 highly methylated (8%)
- 1,103 genes with methylated promoters (16%)
- 5,745 genes with unmethylated promoters (84%)

Promoter Methylation Distribution

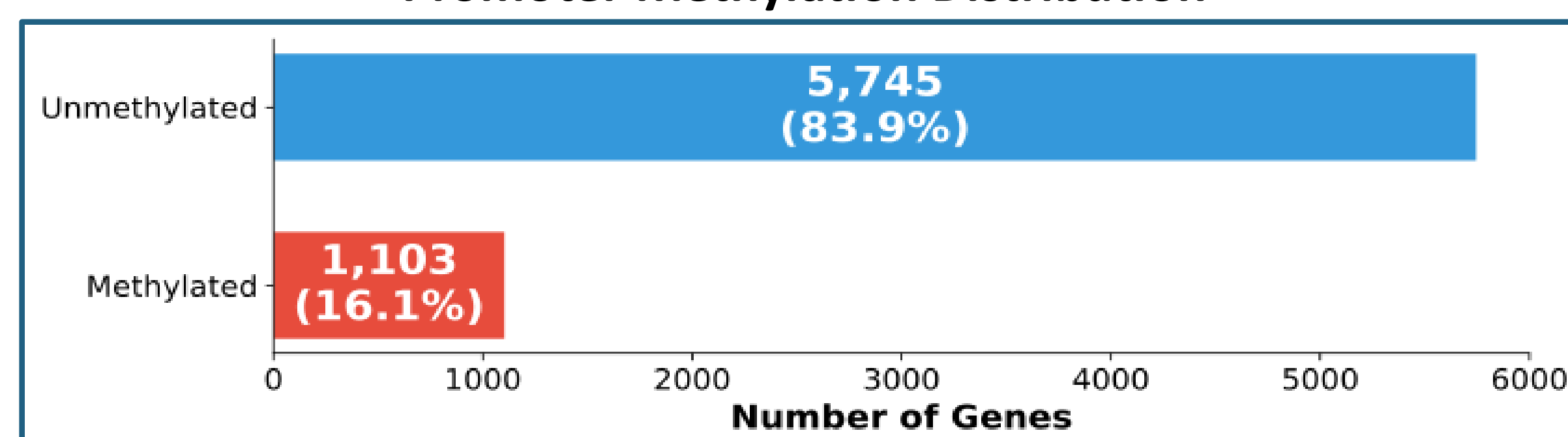


Figure 1. Promoter Methylation Profile.

Distribution of genes with methylated (16%, $n=1,103$) and unmethylated (84%, $n=5,745$) promoters. Methylation status determined by $\geq 25\%$ reciprocal overlap between CpG islands and 1kb upstream of TSS.

Annotation Success Rates:

- Total Annotated:** 5,504/6,848 (80%)
- Unmethylated: 93.8% (5,388/5,745)
- Methylated: 2.8% (31/1,103)

TE Enrichment:

- 77% of annotated methylated genes are TEs (24/31)
- 46/47 GO terms enriched in methylated set ($p < 0.05$, 97.87%)
- Top enriched terms:
 - DNA binding ($p = 3.9 \times 10^{-13}$)
 - RNA-directed DNA polymerase ($p = 6.3 \times 10^{-11}$)
- Structural Evidence: AUGUSTUS detected TE domains (integrase, reverse transcriptase) in methylated promoters.

GO Terms Enriched in Methylated Gene Promoters

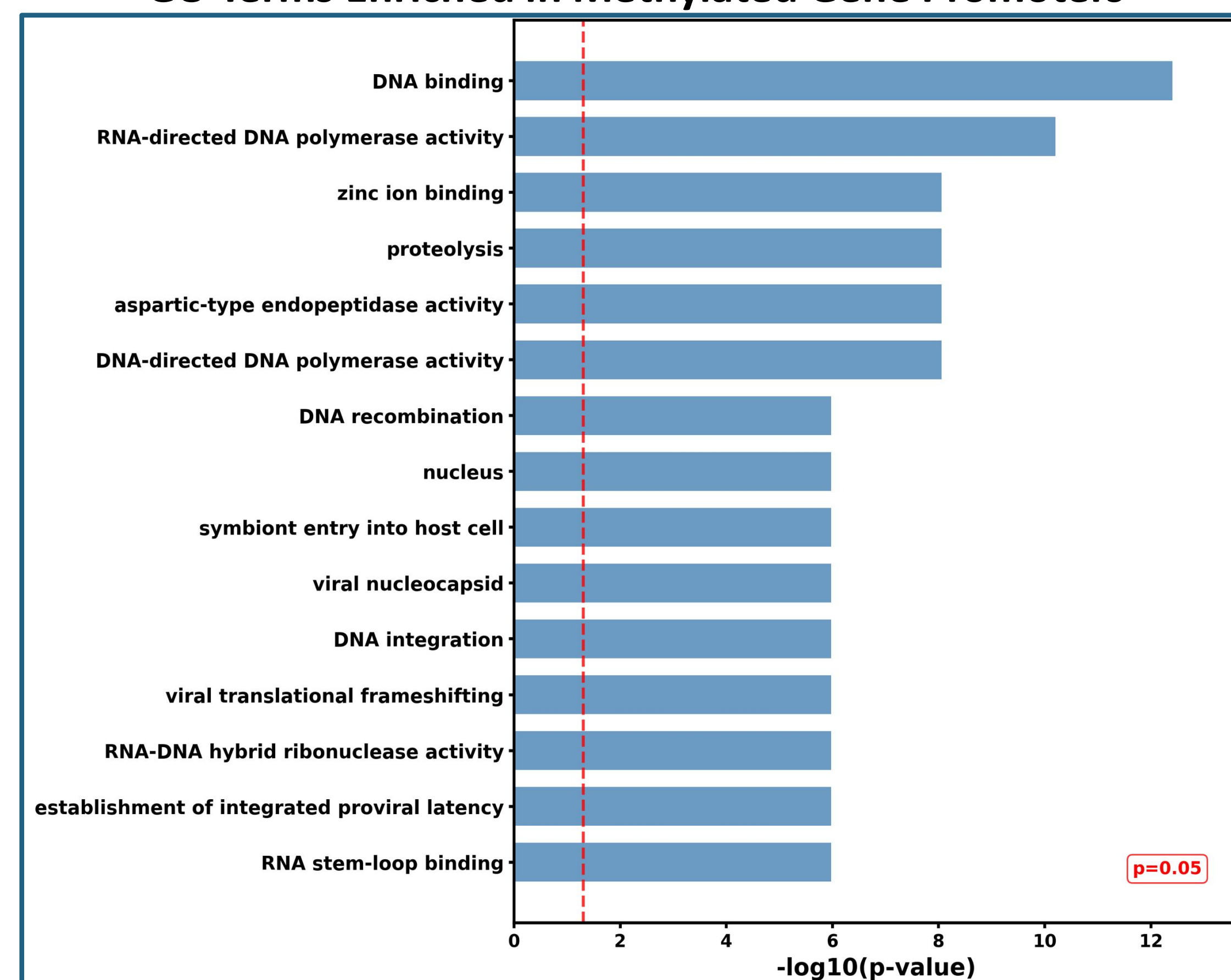


Figure 2. Gene Ontology Enrichment Analysis.

Top 15 significantly enriched GO terms in methylated gene promoters. All 46 enriched terms ($p < 0.05$) localized exclusively to the methylated set ($n=31$ annotated genes). Red dashed line indicates significance threshold ($p=0.05$).

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Discussion and Conclusion

Discussion:

Genome-wide analysis reveals that promoter methylation in *Synalpheus chacei* functions primarily as a genome defense mechanism rather than a regulator of social behavioral genes. Enrichment of transposable elements (TEs) among methylated genes (77%, $p = 3.9 \times 10^{-13}$) contrasts sharply with the caste-specific gene regulation observed in social insects (Kucharski et al., 2008), suggesting fundamental differences in epigenetic architecture between terrestrial and marine eusocial lineages. The 33-fold annotation disparity between unmethylated (93.8%) and methylated (2.8%) genes reflects low-complexity TE sequences resistant to characterization (Slotkin & Martienssen, 2007). This pattern, combined with GO enrichment in methylated promoters for TE-associated functions including DNA binding and RNA-directed DNA polymerase activity, demonstrates targeted TE silencing. The conservation of this genome defense role across diverse taxa (Yoder et al., 1997; Zemach et al., 2010) may be particularly critical in eusocial *Synalpheus*, which carry elevated TE loads compared to solitary relatives (Chak et al., 2021). Methylation-mediated TE suppression likely provides the genomic stability necessary for long-lived reproductive queens, where unchecked transposition would accumulate deleterious mutations over extended lifespans characteristic of eusocial species (Korb & Thorne, 2017).

Conclusion:

Promoter methylation in *Synalpheus chacei* targets transposable elements (77% of annotated methylated genes) rather than social behavior genes, challenging assumptions that marine eusocial species use methylation-based caste determination like terrestrial social insects. This pattern suggests epigenetic regulation of eusociality evolved independently in marine and terrestrial lineages through distinct mechanisms. Future comparative methylome analyses across solitary and eusocial *Synalpheus* species will determine whether alternative epigenetic pathways or methylation-independent mechanisms regulate marine caste differentiation.