

The rumen fluke, *Calicophoron daubneyi*, expresses an expanded repertoire of pattern-recognition receptors

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Introduction

The rumen fluke, *Calicophoron daubneyi*, is a parasite of livestock that has increased in prevalence throughout Europe in recent years. Adult flukes attach to the rumen epithelium via a posterior sucker (Figure 1) and thus make intimate contact with rumen microbes. Whilst the extracellular vesicles secreted by *C. daubneyi* modulate rumen microbial species diversity [1] we know little about the innate immune molecules expressed/secreted by the parasite that help establish infection in this microbiome-rich environment. Using multi-omics resources, we describe the expansion of putative pattern recognition receptor (PRR) and antimicrobial gene families in *C. daubneyi*.



Figure 1. (A) Adult *C. daubneyi* within the rumen. (B) H&E-stained section of an adult fluke attached to the rumen epithelium via its posterior sucker.

Methodology

- The *C. daubneyi* draft genome (Robinson, unpublished) was mined for putative innate immune molecules:
 - Saposin-like proteins (SAPLIPs)
 - Peptidoglycan-recognition proteins (PGRPs)
 - DM9 domain-containing proteins (DM9CPs)
- Phylum-wide BLAST to assess gene expansion in *C. daubneyi*
- Identification of conserved domains using InterPro

- Temporal expression patterns determined using TPM values from RNAseq datasets from 4 developmental stages [2]
- Phylogenetic analysis using MEGA X

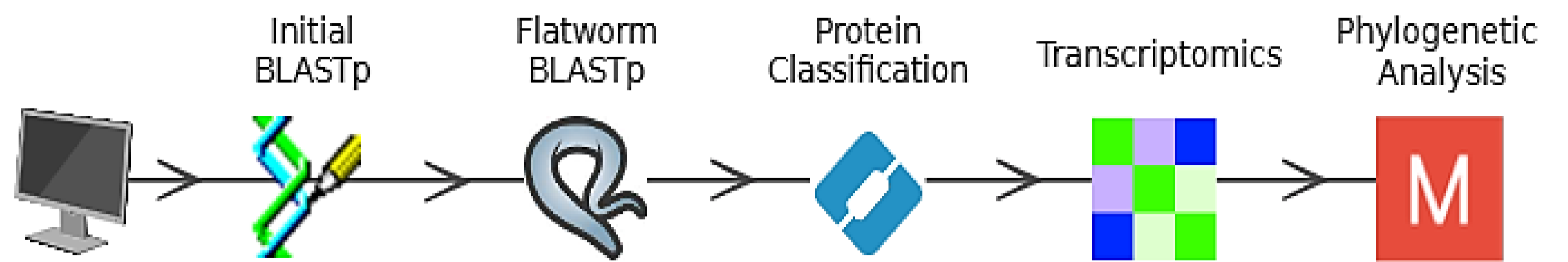


Figure 2. Bioinformatics pipeline

Results

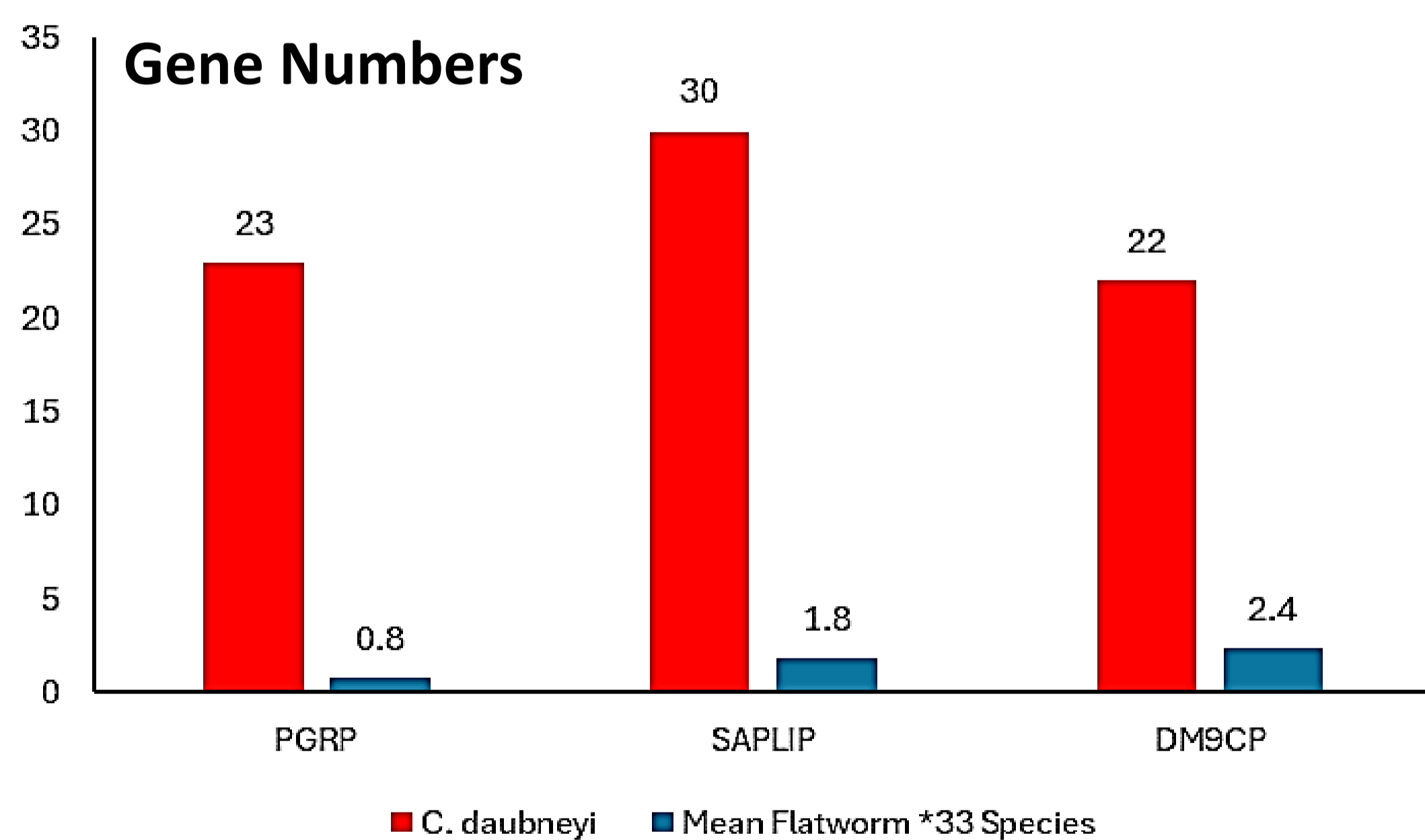


Figure 3. *C. daubneyi* has increased numbers of PGRPs, SAPLIPs, and DM9CPs when compared to other flatworm species.



Figure 5. *C. daubneyi* life stages within the duodenum and rumen.

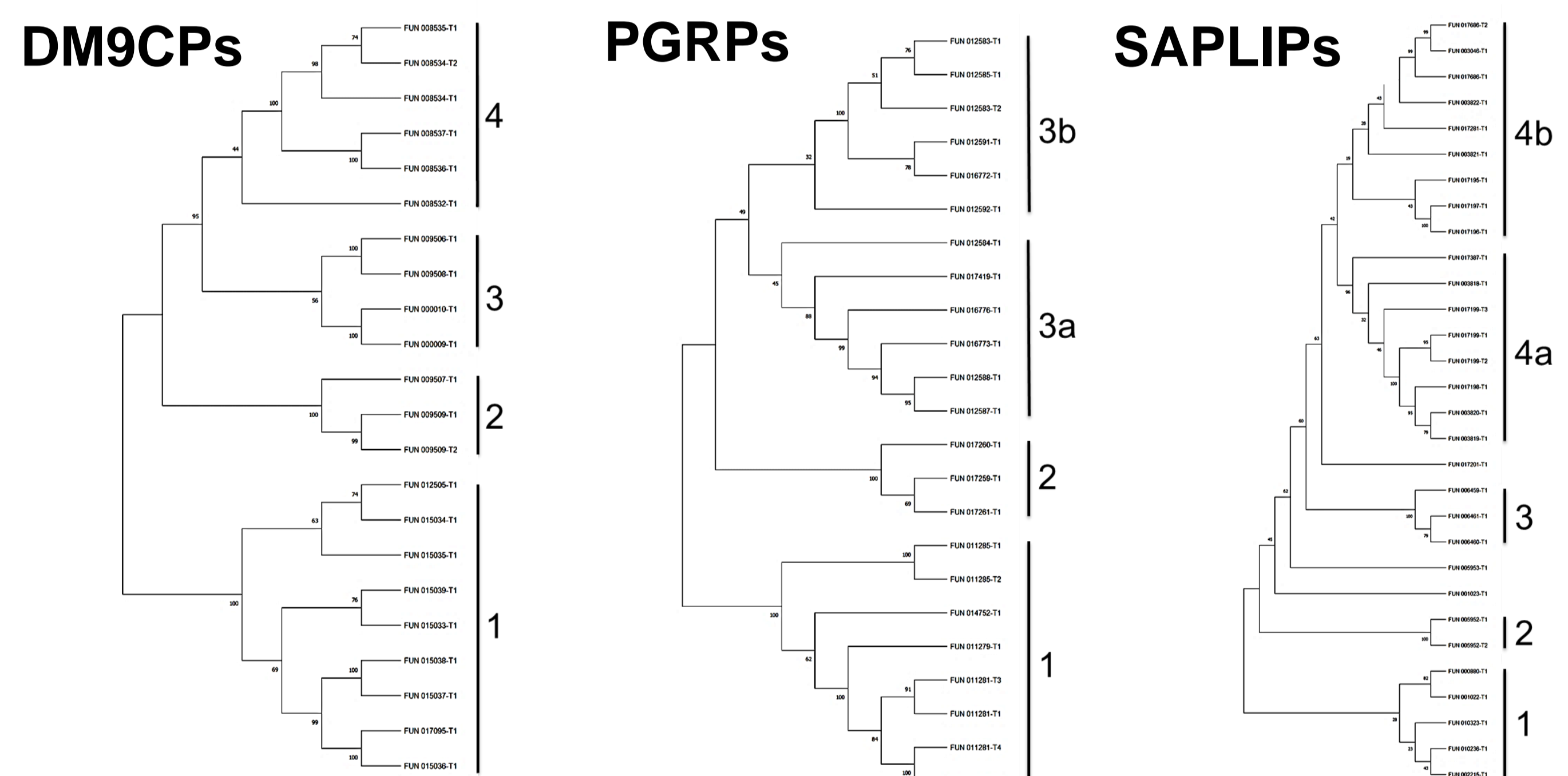


Figure 4. Neighbour-joining trees showing the expansion of the *C. daubneyi* gene families.

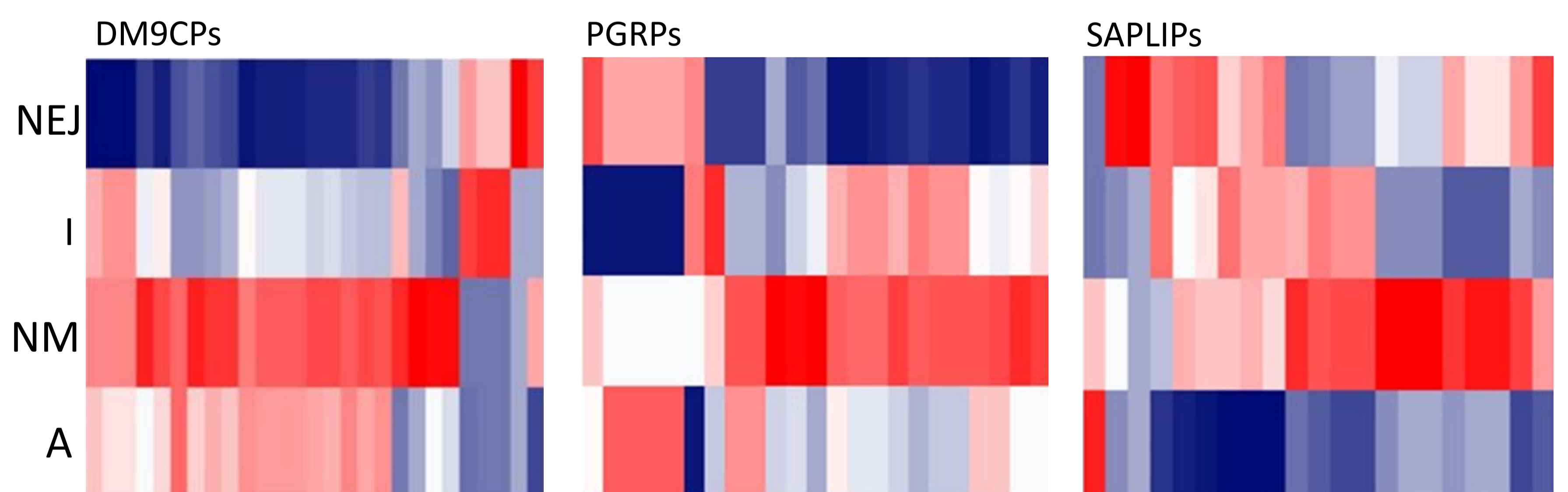


Figure 6. Differential expression of *C. daubneyi* DM9CPs, PGRPs, and SAPLIPs. Life stages listed include newly excysted juvenile (NEJ), immature (I), newly migrated juvenile (NM), and adult (A).

Conclusions

Our data suggest that the SAPLIP, DM9CP and PGRP gene families have undergone significant expansion in *C. daubneyi* compared to other platyhelminth species and that they are typically up-regulated upon arrival of the migratory flukes in the rumen. This supports the hypothesis that rumen fluke have evolved a complex innate immune system due to the unique selection pressures associated with life in the microbiome-rich rumen. Future work will focus on investigating the functional roles of these molecules in *C. daubneyi*-microbial interactions.

References

- [1] Allen et al. (2021). *Front Cell Infect Microbiol*, 11, 661830.
 [2] Huson et al. (2021). *Mol Cell Proteomics*, 20, 100055

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