Profiling the Antimicrobial Peptidome of Nematodes Highlights a New Source of Antimicrobial Diversity

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ANTIMICROBIAL PEPTIDES (AMPs) are key facets of innate immunity possessing broad spectrum antimicrobial activities. AMPs are critical to the invertebrate immune response and are abundant within arthropods and molluscs [1]. Our knowledge of parasitic nematode AMPs at present is limited and is primarily drawn from our understanding of those derived from *Caenorhabditis elegans* [2]. Identifying the role that endogenous nematode-derived AMPs play in nematode biology is key to our understanding of nematode immunity and critical to unravelling how nematode parasites establish themselves in microbe-rich hazardous host environments.

Project aim: Characterise AMP diversity across phylum Nematoda and explore AMP function to nematode biology

1) NEMATODES POSSESS A RICH REPERTOIRE OF AMPs

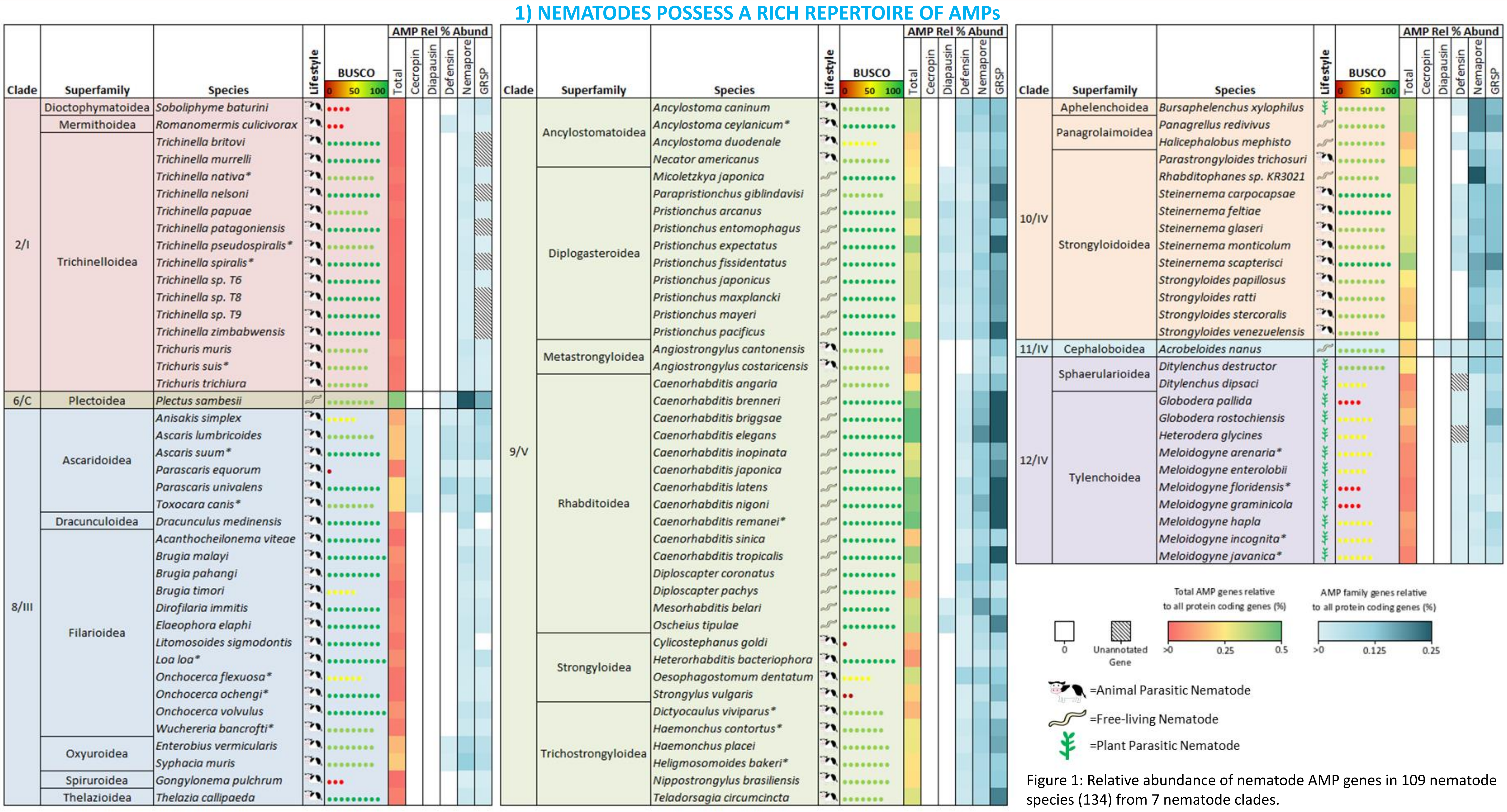
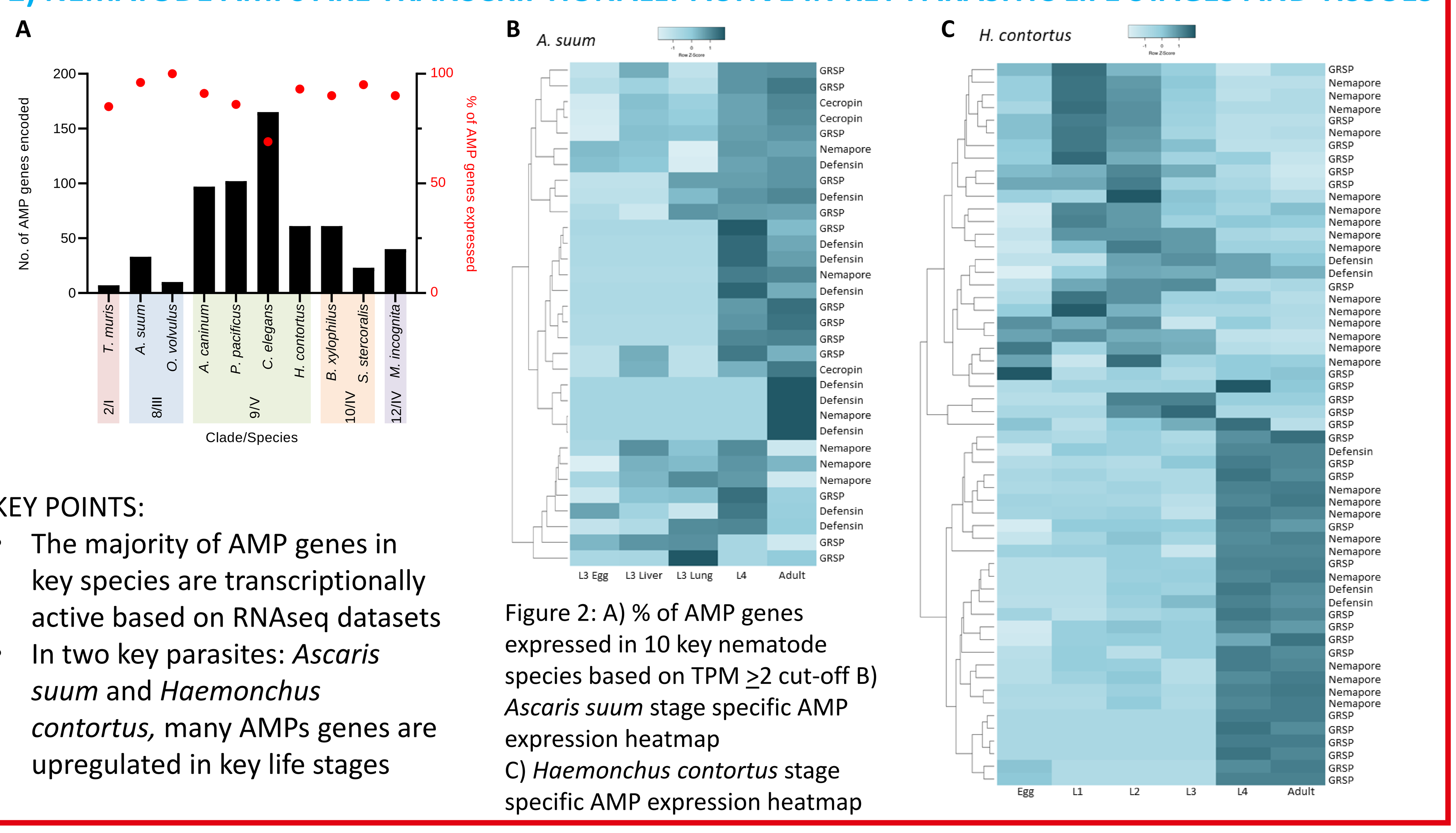


Figure 1: Relative abundance of nematode AMP genes in 109 nematode species (134) from 7 nematode clades.

KEY POINTS:

- Identification of >5000 nematode AMP genes through homology directed approaches reflects that the nematode antimicrobial peptidome is varied and appears to be highly specialised
- Some AMP families are restricted; Cecropins are restricted to the Ascarids, Diapausins are restricted to specific free-living nematodes whereas Defensins, Nemapores and Glycine Rich Secreted Peptides (GRSPs) are broadly distributed across the phylum
- Specific expansions of AMP genes in clades 9/V and 10/IV, whereas Clades 2/I, 8/III and 12/IV appear less AMP rich

2) NEMATODE AMPs ARE TRANSCRIPTIONALLY ACTIVE IN KEY PARASITIC LIFE STAGES AND TISSUES



KEY POINTS:

- The majority of AMP genes in key species are transcriptionally active based on RNAseq datasets
- In two key parasites: *Ascaris suum* and *Haemonchus contortus,* many AMPs genes are upregulated in key life stages

Figure 2: A) % of AMP genes expressed in 10 key nematode species based on TPM >2 cut-off B) *Ascaris suum* stage specific AMP expression heatmap C) *Haemonchus contortus* stage specific AMP expression heatmap

3) NOVEL NEMATODE AMPs ARE POTENT ANTIBACTERIAL AGENTS IN VITRO

KEY POINTS

- Novel nematode AMPs were also identified through a non homology directed approach using machine learning AMP prediction tools
- This identified 337 additional 'high confidence' AMPs found in multiple species which lack homology to known AMP families
- Characterisation of these novel AMPs is ongoing but initial peptide screening against key bacterial species has uncovered a number of potent peptides with selective antibacterial activities including:
  - -A *Meloidogyne spp.* specific peptide with selective activity against gram positive bacteria (*Staphylococcus aureus* & *Enterococcus faecalis*)
  - -A *Trichuris spp.* specific peptide with gram negative activity (*Eschericheria coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*)
  - - A peptide specific to *Heligmosomoides bakeri* with gram negative activity
- These data indicate that nematodes also possess specific AMPs which do not belong to the known AMP families and may only be shared by closely related species

CONCLUSIONS

- **Nematodes are AMP rich possessing highly specialised AMP profiles**
- **Within known AMP families, there is a high level of peptide diversity indicating that AMP family members have functionally diversified in different species to combat differing microbial niches**
- **Nematode AMPs are transcriptionally active with many upregulated in key parasitic life stages indicating that they play a key role to nematode biology**
- **Nematode AMPs which lack homology to known AMP families are bioactive and possess selective antibacterial activities**
- **These data highlight nematodes as a novel source of antimicrobial diversity that could be exploited for antiworm and antimicrobial therapies**

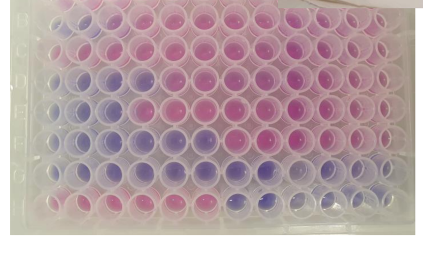
FUTURE WORK

Determine functional role of nematode AMPs

→ Ascaris suum RNA interference

→ In-situ hybridisation

→ Antimicrobial Assays



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[2]: Bruno R, Maresca M, Canaan S, Cavalier JF, Mabrouk K, Boidin-Wichlacz C, Olleik H, Zeppilli D, Brodin P, Massol F, Jollivet D. Worms' antimicrobial peptides. Marine drugs. 2019 Sep;17(9):512.

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