

# 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

83	1) NEMATODES POSSESS A RICH REPERTOIRE OF AMPs																										
	Clade	Superfamily	Species	BUSCO 50 100	Total W	Diapausin B	Defensin %	GRSP pu	Clade	Superfamily	Species	Lifestyle	BUSCO 0 50 100	Total W	Diapausin Ba	Nemapore ndy	ade	Superfamily	Species	Lifestyle	BUSCO 0 50 100	Total A	Rel uisnedeid	Defensin %	GRSP pu		
		Dioctophymatoidea	Soboliphyme baturini								Ancylostoma caninum	31						Aphelenchoidea	Bursaphelenchus xylophilus	444	•••••						
		Mermithoidea	Romanomermis culicivorax	···								Ancylostomatoidea	Ancylostoma ceylanicum*	·····						Panagrolaimoidea	Panagrellus redivivus	S					
			Trichinella britovi								Ancylostoma duodenale	~						0	Halicephalobus mephisto	S							
			Trichinella murrelli	<b>**</b>							Necator americanus	31							Parastrongyloides trichosuri		*******						
- 1			Trichinella nativa*	71							Micoletzkya janonica	Se .							Rhahditonhanes sn KR3021	SP							

		menulena maciva	Concellant of the			L		
		Trichinella nelsoni	31					
		Trichinella papuae	31					
		Trichinella patagoniensis	31					
2/1		Trichinella pseudospiralis*	31					
	Trichinelloidea	Trichinella spiralis*	31					
		Trichinella sp. T6	31				ľ	
		Trichinella sp. T8	31					
		Trichinella sp. T9	31					
		Trichinella zimbabwensis	31					
		Trichuris muris	31				ľ	
		Trichuris suis*	31					
		Trichuris trichiura	31					
6/C	Plectoidea	Plectus sambesii	S	*******				
		Anisakis simplex	31	*****				
		Ascaris lumbricoides	31					
	Ascaridoidea	Ascaris suum*	31	•••••				
	Ascandoluca	Parascaris equorum	31	•				
		Parascaris univalens	30					
		Toxocara canis*	31					
	Dracunculoidea	Dracunculus medinensis	31	•••••				
		Acanthocheilonema viteae	31					
		Brugia malayi	31	•••••				
		Brugia pahangi	30					
		Brugia timori	30					
8/111		Dirofilaria immitis	30	•••••				
	Filarioidea	Elaeophora elaphi	30					
	rianoidea	Litomosoides sigmodontis	31	•••••				
		Loa loa*	31	•••••				
		Onchocerca flexuosa*	30	*****				
		Onchocerca ochengi*	31					
		Onchocerca volvulus	30					
		Wuchereria bancrofti*	31					
	Ovvuroidea	Enterobius vermicularis	31					
	Oxydroidea	Syphacia muris	31					
	Coincraidee	Canaulanama autobaum	70					

	wilcoletzkyu jupolitu	2007		
	Parapristionchus giblindavisi	S		
	Pristionchus arcanus	S		
	Pristionchus entomophagus	S		
Diplogastoroidoa	Pristionchus expectatus	S		
Diplogasteroidea	Pristionchus fissidentatus	2		
	Pristionchus japonicus	S		
	Pristionchus maxplancki	S		
	Pristionchus mayeri	S		
	Pristionchus pacificus	S		
Vetastronguloidea	Angiostrongylus cantonensis	31		
vietastrongyloidea	Angiostrongylus costaricensis	31		
	Caenorhabditis angaria	20		
	Caenorhabditis brenneri	2		
	Caenorhabditis briggsae	S		
	Caenorhabditis elegans	S		
	Caenorhabditis inopinata	5		
	Caenorhabditis japonica	S		
	Caenorhabditis latens	S		
Rhabditoidea	Caenorhabditis nigoni	S		
	Caenorhabditis remanei*	S		
	Caenorhabditis sinica	S		
	Caenorhabditis tropicalis	5		
	Diploscapter coronatus	S		
	Diploscapter pachys	S		
	Mesorhabditis belari	5		
	Oscheius tipulae	S		
	Cylicostephanus goldi	31	•	
Strongulaidaa	Heterorhabditis bacteriophora	31		
Strongyloidea	Oesophagostomum dentatum	31		
	Strongylus vulgaris	31	••	
	Dictyocaulus viviparus*	31		
	Haemonchus contortus*	31		
rich octron mulai da a	Haemonchus placei	31		
nchostrongyioidea	Heligmosomoides bakeri*	31		
	Nippostronavlus brasiliensis	31		

			ninaballopinanes spi ninsozz	and the second				
			Steinernema carpocapsae	30				
	10/11/		Steinernema feltiae	31				
	10/10		Steinernema glaseri	31				
		Strongyloidoidea	Steinernema monticolum	3				
			Steinernema scapterisci	31				
			Strongyloides papillosus	31				
			Strongyloides ratti	31				
			Strongyloides stercoralis	3				
			Strongyloides venezuelensis	31				
	11/IV	Cephaloboidea	Acrobeloides nanus	S	*******			
			Ditylenchus destructor	¥				
		Sphaerularioidea	Ditylenchus dipsaci	¥				
1			Globodera pallida	¥				
			Globodera rostochiensis	¥				
			Heterodera alycines	¥				
			Meloidogyne arenaria*	¥				
	12/IV	2010/01/01/01	Meloidoavne enterolobii	¥				
-		Tylenchoidea	Meloidoavne floridensis*	¥				
			Meloidoavne araminicola	¥				
			Meloidoavne hapla	¥				
			Meloidoavne incoanita*	¥				
			Meloidoavne javanica*	¥				
			weiolaogyne javanica	4		-	-	_
			Total AMP genes relative	AN	1P family genes	relativ	æ	
			to all protein coding genes (%)	to all	protein coding	genes	(%)	
		0 Unannotated	>0 0.25 0.5	>0	0.125	-	0.25	
		Gene						
		-Animal Da	rasitic Nematode					
		Turning -Allindi Pa	asilie meritaloue					
	0	Service - Free-living	s Nematode					
		=Plant Para	sitic Nematode					
		4						
			· · · · · · · · · · · · · · · · · · ·		- ·	4.04	<b>`</b>	



Teladorsagia circumcincta

lemapor

Nemapore

Defensi Defensi

Nemapor Nemapor 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

**C** *H. contortus* 

• 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

9/V





### 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

suum and Haemonchus contortus, many AMPs genes are upregulated in key life stages Ascaris suum stage specific AMP expression heatmap C) Haemonchus contortus stage specific AMP expression heatmap



#### 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

## not belong to the known AMP families and may only be shared by closely related species

#### **FUTURE WORK**

Determine functional role of nematode AMPs

- $\rightarrow$  Ascaris suum RNA interference
- $\rightarrow$  In-situ hybridisation
- → Antimicrobial Assays —





#### References:

[1]: Gómez EA, Giraldo P, Orduz S. InverPep: A database of invertebrate antimicrobial peptides. Journal of global antimicrobial resistance. 2017 Mar 1;8:13-7.
 [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.