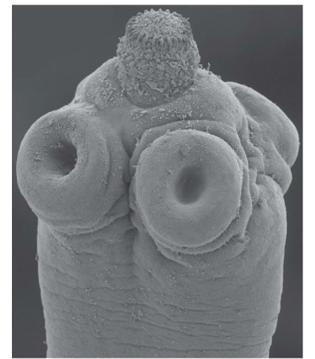


Investigating germline markers in the mouse bile-duct tapeworm, *Hymenolepis microstoma*: The search for Vasa, Piwi and Nanos



Catherine Seed (MSc Student), Francesca Jarero (PhD Student) and Peter D. Olson (Supervisor)
Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD

Introduction

Pluripotency is maintained in germ cells and other stem cells through the action of a small number of highly conserved proteins. Free-living and parasitic flatworms (phylum Platyhelminthes) are unique in relying entirely on stem cells, called 'neoblasts' for growth, tissue turnover and regeneration. Recent research suggests that the same regulatory network functions to maintain stemness in free-living flatworms (i.e. planarians). However, little is known about neoblast regulation in parasitic flatworms which rely upon them not for regeneration, but for their complex life cycles. Using the model tapeworm *Hymenolepis microstoma*, we are investigating the components and roles of three well known germline makers:

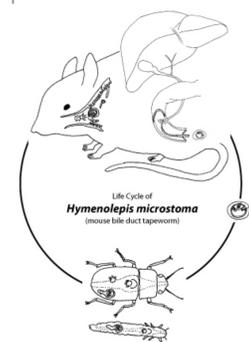


Figure 1 *Hymenolepis microstoma* has a complex lifecycle, where a larval stage must be completed in an intermediate beetle host, while progression to sexually reproductive adult stage occurs in the mouse definitive host.

- **Vasa** is an ATP-dependent DEAD-box helicase, which plays a role in both the separation of the germline and soma (Pfister et al., 2008), as well as regeneration in flatworms (Rebscher et al., 2007).
- **Piwi** plays a role in gene silencing by binding antisense piRNAs (Palakodeti et al., 2008). This protein has emerged as a vital component for germline specification, and survival (Nakagawa, 2012), playing a role both in protecting germline integrity and epigenetic gene regulation of germline fate (Palakodeti et al 2008).
- **Nanos** is a posterior group gene which is widely associated with the germline in a variety of taxa including vertebrates, nematodes, planarians and cnidarians (Handberg and Salo, 2007) (Agee et al., 2006)

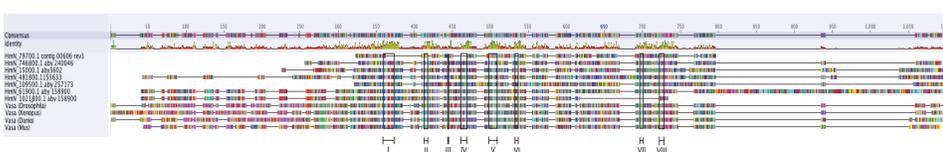
Methods

The BLAST searching tool was used to search the *Hymenolepis* genome, gene model database and transcriptome. Sequences with similarity to Vasa, Piwi and Nanos were obtained using sequences from evolutionarily distant species, as well as the more closely related flatworms (*Schistosoma japonicum*, *Schistosoma mansoni*) and other lophotrochozoans (*Helobdella robusta*) as search enquiries.

Sequences were analysed using the NCBI conserved domain database and Interproscan protein signature recognition software to analyse for functional domains. Phylogenetic analyses were performed using a Bayesian algorithm in order to assess the grouping of the identified sequences with closely related protein clades.

Results

Seven sequences were identified as showing similarity to Vasa-like proteins, each displaying variations in combinations of the eight characteristic motifs found in Vasa homologs (Figure 2). One sequence contained multiple stop codons, and was discarded. Phylogenetic analysis showed that no identified sequence grouped structurally with known Vasa proteins. Two of the sequences grouped with the related PL10 sub-family (Figure 3), while another grouped with the eIF4A sub-family (Figure 3).



	AQTGGSGKT /AXXXGKT Motif I	PTRELA Motif II	GG Motif III	TPGR Motif IV	DEAD Motif V	SAT Motif VI	ARGVD/ARGXD Motif VII	HRRIGTR /HRRGR Motif VIII
Aby_155633	✓	✓	✓	✓	✓	✓	✓	✓
Aby_156031	✓	✓	✓	✓	✓	✓	✓	✓
Aby_158900	✓	✓	✓	✓	✓	✓	✓	✓
Aby_257173	✓	✓	✓	✓	✓	✓	✓	✓
Aby_3602	✓	✓	✓	✓	✓	✓	✓	✓
Aby_241046	✓	✓	✓	✓	✓	✓	✓	✓
Contig00660R	✓	✓	✓	✓	✓	✓	✓	✓

Figure 2 a) Multiple alignment of identified gene models with Vasa from *Drosophila melanogaster*, *Xenopus laevis*, *Danio rario* and *Mus musculus*. The eight conserved motifs are marked. b) Table showing the presence of Vasa conserved motifs in the identified transcript data.

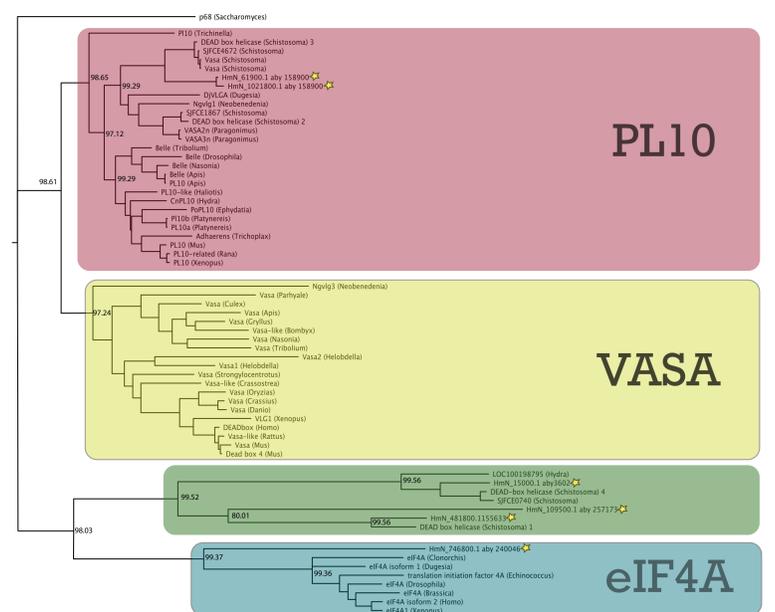


Figure 3. Vasa-like genes in *Hymenolepis*: Phylogenetic tree of representative DEAD box helicase proteins constructed using Bayesian inference analysis of amino acid sequences, using p68 as an outgroup. Gene models from *Hymenolepis* are indicated by the prefix HmN, and are followed by the accession information for the corresponding transcript data, as indicated by the prefix Aby or Contig.

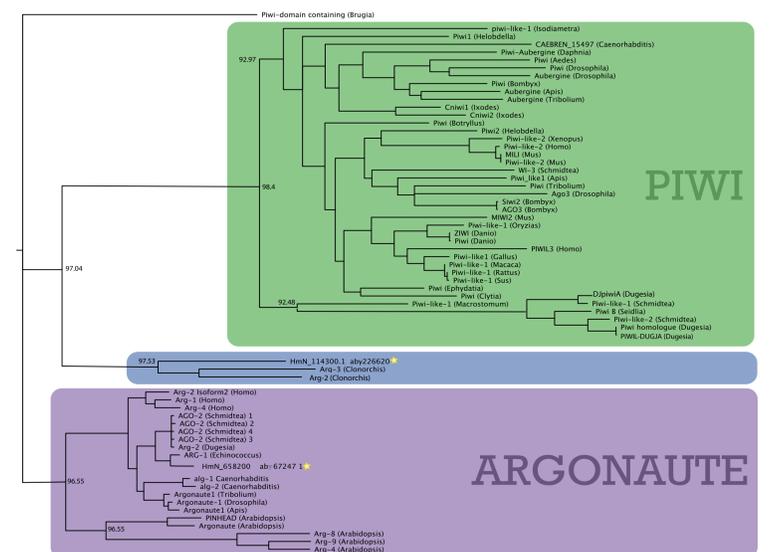


Figure 4. Piwi-like genes in *Hymenolepis*: Phylogenetic tree of representative Argonaute proteins constructed using Bayesian inference analysis of amino acid sequences, using Piwi-domain containing protein from *Brugia* as an outgroup. Gene models from *Hymenolepis* are indicated by the prefix HmN, and are followed by the accession information for the corresponding transcript data, as indicated by the prefix Aby or Contig.

Domain analysis revealed two sequences containing both PAZ and piwi-like domains found in piwi proteins. However, these sequences grouped independently of the piwi clade in phylogenetic analysis (Figure 4). Sequences with potential homology to Nanos have not yet been verified, however, Nanos-like genes are known from other flatworms.

Conclusions and Future Research

Readily identifiable orthologs of these germline markers are known from free-living flatworms; however, it appears that parasitic flatworms have fewer and more divergent orthologs that are difficult to classify.

Future research aims to characterise the spatial expression of the identified relatives of these proteins via whole mount in-situ hybridisation in order to determine whether these DEAD-box helicases and Argonaute family proteins play a role in the germline of *Hymenolepis*. This search will also include characterisation of other prospective germline markers.

As no other Argonaute proteins associate with piRNAs, this raises questions about the importance of piRNAs in other flatworm species. These data also support the relevance of studying stem cell regulation in *Hymenolepis* in order to contextualise the role that they play during regeneration in free-living flatworms.

References
Agee, S.J. Lyons, D.C. Weisblat, A.W. (2006) Maternal expression of a NANOS homolog is required for early development of the leech *Helobdella robusta*. *Developmental Biology*. 298:1-11
Cunningham, L.J. Olson, P.D. (2010) Description of *Hymenolepis microstoma* (Nottingham strain): a classical tapeworm model for research in the genomic era. *Parasites and Vectors* 3: 12
Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2012) Geneious v5.6. Available from <http://www.geneious.com>.
Handberg-Thorsager, M. Saló, E. (2007) The planarian nanos-like gene SMednos is expressed in germline and eye precursors during development and regeneration. *Dev Genes Evol*. 217:403-411
Nakagawa, H. Ishizu, H. Hasegawa, R. Kobayashi, K. Matsumoto, M. (2012) Drpiwi-1 is essential for germline cell formation during sexualisation of the planarian *Dugesia ryukyuensis*. *Developmental Biology*. 361: 167-176
Palakodeti, S. Smielewska, M. Lu, Y.C. (2008) The PIWI proteins SMEDWI-2 and SMEDWI-3 are required for stem cell function and piRNA expression in planarians. *RNA*. 14: 1174-1186
Pfister, D. De Mulder, K. Hartenstein, V. Kulaes, G. Borgonie, G. Marx, F. Morris, J. Laduma, P. (2008) Flatworm stem cells and the germ line: developmental and evolutionary implications of mcvasa expression in *Macrostomum lignano*. *Developmental Biology*. 319 (1): 146-159
Rebscher, N. Zelada-Gonzalez, F. Banisch, T.U. Raible, D. Arendt, D. (2007) Vasa unveils a common origin of germ cells and of somatic stem cells from the posterior growth zone in the polychaete *Platynereis dumerilii*. *Developmental Biology* 306: 599-611