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Secreted microRNA data from the parasitic filarial nematode *Acanthocheilonema viteae*

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Data Article

Secreted microRNA data from the parasitic filarial nematode *Acanthocheilonema viteae*



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ABSTRACT

microRNAs (miRNAs) are an abundant class of non-coding RNA species with important regulatory roles in gene expression at the posttranscriptional level. The helminth *Acanthocheilonema viteae* serves as model organism for research on parasitic filarial nematodes.

Total RNA secreted or excreted *in vitro* by 1500 adult female and male *A. viteae* over 3 weeks was isolated from culture media previously processed by differential ultracentrifugation. miRNA sequencing revealed the presence of 360 unique miRNA candidates released by adult *A. viteae in vitro*. Among them, 74 high-confidence unique miRNAs, as well as several potential novel miRNA candidates were discovered. A large proportion of the sequenced miRNA candidates appeared differentially expressed between the male and female samples based on normalized copy count.

The presence of extracellular vesicles, often rich in miRNAs, could not be confirmed unambiguously by transmission electron microscopy.

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Specifications Table

Subject	Immunology and Microbiology: Parasitology
Specific subject area	microRNA sequencing
Type of data	Table
How data were acquired	Illumina HiSeq 2500
Data format	Raw (FASTQ) and processed data (excel)
Parameters for data collection	<i>A. viteae</i> adult females and males
Description of data collection	Female and male <i>A. viteae</i> secreted miRNAs extracted from ultracentrifuged culture media were sequenced and analyzed.
Data source location	Institute of Parasitology, University of Zurich Zurich Switzerland
Data accessibility	Repository name: Gene Expression Omnibus (NCBI) Data identification number: GSE143793 Direct URL to data: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE143793

Value of the Data

- Excretory/secretory miRNAs from the model helminth *Acanthocheilonema viteae* could serve as potential biomarkers of infection with filarial nematodes and in comparative studies of host-parasite specificity.
- Excretory/secretory miRNAs from *A. viteae*, described here for the first time, may represent a useful tool for helminth research.
- miRNAs may be species-specific or similar in sequence and function to those of their hosts. Hence, they may be a result of the evolutionary arms races underlying all host-helminth associations and contribute to the establishment of a successful infection.

1. Data description

Approximately 1500 adult worms were collected; males and females were separated and cultured *in vitro* for up to 3 weeks. Total RNA was used for library preparation, for female and male culture media, respectively. Analyzed data can be found in [Supplementary Table 1](#). Across both samples, a total of 360 unique putative mature miRNAs were sequenced, including 74 high-confidence unique mature miRNAs (assigned to groups 1 and 2a, see methods; the top 10 can be found in [Table 1](#)). Another 25 sequences mapped to the *A. viteae* genome as well as non-nematode miRNAs in miRBase (group 2b). Another 221 mature sequences mapped to miRBase entries, but not to the current *A. viteae* genome (group 3). Finally, 41 predicted candidates mapped to the *A. viteae* genome but to no other miRNA registered in miRBase and hence, represent novel miRNA candidates (group 4). A total of 192 sequences appeared differentially expressed between the female and male sample, \log_2 fold change ≥ 1 or -1 , p -value < 0.05 ([Supplementary Table 2](#)). We failed to identify EVs unambiguously in *A. viteae* culture supernatants using transmission electron microscopy.

2. Experimental design, materials, and methods*2.1. Animal infections*

Ethical clearance was obtained from the Cantonal Veterinary Office of Basel (permit number 2802). All experiments were conducted in accordance with the Swiss cantonal and national regulations on animal experimentation. Briefly, 30 male Syrian golden hamsters (*Mesocricetus auratus*) were inoculated subcutaneously with 250 *A. viteae* third stage larvae obtained from infected ticks (*Ornithodoros moubata*), as described [1]. Hamsters were euthanized after 8 weeks and adult *A. viteae* were collected from the subcutaneous tissue of the back and neck region.

Table 1

Top 10 high confidence miRNA candidates in secretions from *A. viteae* females and males. High confidence miRNAs are those sequences assigned to groups 1 and 2a (see methods).

Rank	<i>A. viteae</i> females		<i>A. viteae</i> males	
	Name	Normalized counts	Name	Normalized counts
1	bma-miR-100a_R+1	66,324	bma-miR-36c-3p_R-1_1ss20GA	4842
2	bma-miR-100d_R+1	61,458	bma-miR-100a_R+1	4550
3	bma-miR-36c-3p_R-1_1ss20GA	45,502	bma-miR-100d_R+1	4051
4	asu-miR-81a	11,637	bma-miR-279_R+1	2286
5	bma-miR-100b_R+1	11,596	asu-miR-81a	1726
6	asu-miR-86-5p	8020	asu-lin-4-5p_1ss19TC	1622
7	bma-miR-100c_R+2_1ss12CT	7865	bma-miR-71_R+4	1172
8	bma-miR-279_R+1	6178	bma-miR-2c_R+1_1ss1CT	1145
9	bma-miR-71_R+4	5456	asu-let-7-5p	1084
10	bma-miR-36a_R+1_1ss20TC	4955	bma-miR-100b_R+1	1082

2.2. In vitro culture, media processing, and extraction of total RNA

Females and males were separated and cultured for up to 3 weeks at 1–2 worms per ml RPMI-1640 containing 200 U/ml penicillin, 200 µg streptomycin, and 4 mM L-glutamine, at 37 °C, 5% CO₂. Media from the first 24 h served as initial ‘wash’ and were discarded. Worms were checked for viability (movement) and transferred to fresh media every 1–2 days. Those showing poor or no motility were removed. Collected culture media were spun at 1000×g for 30 min at 4 °C, filtered through 0.22 µm pores, and frozen at –20 °C for several weeks. Upon thawing, media were concentrated over a 3 kDa ultrafiltration cellulose membrane (Millipore PLBC06210). Concentrated media were further spun 45 min at 12,000×g and ultracentrifuged in a Beckman system (Optima L-80 XP), using a SW40Ti rotor and Ultra-Clear thinwall tubes (14 × 95 mm, Beckman 344060) as follows: 2 h at 100,000×g, 4 °C; the pellet was washed once with 12 ml ice cold sterile PBS (Gibco), and centrifuged again for 2h at 100,000×g, 4 °C, as described [2,3].

Total RNA was extracted from the ‘pellet’ in 0.5 ml PBS using the blood protocol of a total RNA purification kit (Norgen Biotek 17200) and shipped to LC Sciences (Houston, TX, USA) for miRNA sequencing and analysis.

2.3. miRNA sequencing and analysis

The total RNA quality and quantity were determined using a Bioanalyzer 2100 (Agilent, CA, USA) with RIN number >7.0. The library was prepared following Illumina’s TruSeq small-RNA-sample preparation protocols. Single-end sequencing with 50 bp reads was performed on Illumina’s HiSeq 2500 sequencing system. Raw reads were subjected to LC Sciences’ proprietary program, ACGT101-miR (LC Sciences, Houston, Texas, USA) to remove adapter dimers, junk, low complexity, common RNA families (rRNA, tRNA, snRNA, snoRNA) and repeats. Unique sequences in length of 18–26 nucleotides were mapped to the *A. viteae* genome (https://parasite.wormbase.org/Acanthocheilonema_viteae_prjeb1697/Info/Index) by BLAST search and to nematode and all miRNAs/pre-miRNAs in miRBase v22.0 [4] to identify known miRNAs and novel 3p- and 5p-derived miRNAs. Length variation at both 3’ and 5’ ends and one mismatch inside of the sequence were allowed in the alignment. Sequences were mapped to the nematode and all miRNA precursors collection in miRBase v22.0 by BLAST search, and the mapped pre-miRNAs were further BLASTed to determine their genomic locations. The unmapped sequences were BLASTed against the *A. viteae* genome, and the hairpin RNA structures containing sequences were predicted from the flanking 80 nt sequences using RNAfold (<http://rma.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>). The criteria for secondary structure prediction were: (1) number of nucleotides in one bulge in stem region (≤12), (2) number of base pairs in the stem region of the predicted hairpin (≥16), (3) free energy cutoff (kCal/mol ≤ -15), (4) length of hairpin (up and down stems + terminal loop ≥50) (5) length of hairpin loop (≤20), (6) number of nucleotides in one bulge in mature region (≤8), (7) number of biased errors in one bulge in mature region (≤4), (8) number

of biased bulges in mature region (≤ 2), (9) number of errors in mature region (≤ 7), (10) number of base pairs in the mature region of the predicted hairpin (≥ 12), (11) percent of mature in stem (≥ 80). Unique reads were further analyzed in order to identify conserved and novel *A. viteae* miRNAs. Briefly, reads which mapped to miRNAs/pre-miRNAs of specific species in miRBase and for which pre-miRNAs further mapped to the *A. viteae* genome and EST were assigned to group 1. Reads from group 2a were mapped to miRNAs/pre-miRNAs of selected species in miRBase and the mapped pre-miRNAs were not further mapped to the genome, but the reads (and the miRNAs of the pre-miRNAs) were mapped to genome; the extended genome sequences from the genome loci may form hairpins. In group 2b, reads were mapped to miRNAs/pre-miRNAs of selected species in miRBase and the mapped pre-miRNAs were not further mapped to the *A. viteae* genome, but the reads (and the miRNAs of the pre-miRNAs) were mapped to the genome; the extended genome sequences from the genome loci may not form hairpins. Those reads which were (i) mapped to miRNAs/pre-miRNAs of selected species in miRBase, (ii) for which the mapped pre-miRNAs were not further mapped to the *A. viteae* genome, and (iii) failed to map to the genome, were classified as group 3. Reads in group 4 were not mapped to pre-miRNAs of selected species in miRBase, but the reads were mapped to the *A. viteae* genome and the extended genome sequences may form hairpins. Normalization of sequence counts in each sample was achieved by dividing the counts by a library size parameter of the corresponding sample, as in Ref. [5].

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dib.2020.105334>.

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