Tropical Biomedicine 37(4): 947–962 (2020)

microRNAs expression profiles in *Schistosoma japonicum* of different sex 14 and 28 days post-infection

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Received 8 June 2020; received in revised form 14 September 2020; accepted 16 September 2020

Abstract. Different miRNAs are involved in the life cycles of *Schistosoma japonicum*. The aim of this study was to examine the expression profile of miRNAs in individual *S. japonicum* of different sex before and after pairing (18 and 24 dpi). The majority of differential expressed miRNAs were highly abundant at 14 dpi, except for sja-miR-125b and sja-miR-3505, in both male and female. Moreover, it was estimated that sja-miR-125b and sja-miR-3505 might be related to laying eggs. sja-miR-2a-5p and sja-miR-3484-5p were expressed at 14 dpi in males and were significantly clustered in DNA topoisomerase III, Rap guanine nucleotide exchange factor 1 and L-serine/L-threonine ammonia-lyase. Target genes of sja-miR-2d-5p, sja-miR-31-5p and sja-miR-125a, which were expressed at 14 dpi in males but particularly females, were clustered in kelch-like protein 12, fructose-bisphosphate aldolase, class I, and heat shock protein 90 kDa beta. Predicted target genes of sja-miR-3483-3p (expressed at 28 dpi in females but not in males) were clustered in 26S proteasome regulatory subunit N1, ATP-dependent RNA helicase DDX17. Predicted target genes of sja-miR-219-5p, which were differentially expressed at 28 dpi in females but particularly males, were clustered in DNA excision repair protein ERCC-6, protein phosphatase 1D, and ATPase family AAA domain-containing protein 3A/B. Moreover, at 28 dpi, eight miRNAs were significantly up-regulated in females compared to males. The predicted target genes of these miRNAs were significantly clustered in heat shock protein 90 kDa beta, 26S proteasome regulatory subunit N1, and protein arginine N-methyltransferase 1. To sum up, differentially expressed miRNAs may have an essential role and provide necessary information on clarifying this trematode's growth, development, maturation, and infection ability to mammalian hosts in its complex life cycle, and may be helpful for developing new drug targets and vaccine candidates for schistosomiasis.

BACKGROUND

Schistosomiasis, which is caused by the genus *Schistosoma*, is one of the most serious and debilitating parasitic diseases worldwide (Song *et al*., 2016). It has been reported that approximately 250 million people in 78 countries endemic with this disease, while nearly 800 million are at the risk of being infected (Gray *et al*., 2010; The Carter Center, 2014; WHO, 2016). In China, *Schiatosomiasis japonica* represents a serious public health problem (Li *et al*., 2014; Song *et al*., 2016; Sun *et al*., 2017).

Currently, vaccines for schistosomiasis prevention are not available (Tebeje *et al.*, 2016), and the only useful drug, i.e., praziquantel has an essential the role in treatment of schistosomiasis (Siqueira *et al.*, 2017). However, the drug is ineffective against young worms (Wang *et al.*, 2012). The life cycle of *S. japonicum* is complicated, with males and females pairing and eventually developing into adult worms in hosts. Given this condition, *S. japonicum* goes through remarkable morphological and molecular changes throughout its life cycle (Walker *et al.*, 2011; Mourão *et al.*, 2012; Sun *et al.*, 2013). Previous studies have relied on genomic analysis technology to explain biology of *S. japonicum* and its interactions with hosts (*S. japonicum* Genome Sequencing and Functional Analysis Consortium, 2009), thus increasing our knowledge of microRNAs (miRNAs) and their evolutionary characteristics, developmental genetic switches, and novel biomarkers for *S. japonicum*. Consequently, a deeper understanding of the mechanisms of schistosome development and the pathogenesis of schistosomiasis may help developing novel strategies to control this disease.

Currently, different schistosome life cycle stages have been associated with different categories of miRNAs, suggesting that miRNAs may be involved in the regulation of development (Xue *et al.*, 2008; Huang *et al.*, 2009; Chen *et al.*, 2010; Hao *et al.*, 2010; Cai *et al.*, 2011). Several lines of evidence have also indirectly implied that miRNAs may regulate the pathogenic schistosomiasis (Cheng *et al.*, 2013). During the development of *S. japonicum*, males and females begin to pair approximately 14 days post-infection (14 dpi), and the female begins to lay eggs approximately 28 days post-infection (28 dpi) (He *et al.*, 1980). Moreover, Sun *et al.* (2014) have reported a total of 2258 miRNAs identified in *S. japonicum* collected from single and double-sex infected mice; however, the authors did not provide information about the individual miRNAs before and after pairing (female at 14 dpi and 28 dpi, male at 14 dpi and 28 dpi).

In this study, the expression profiles of miRNAs in individual *S. japonicum* of different sex before and after pairing (18 dpi and 24 dpi) were investigated. Moreover, based on the analysis of the predicted targets, the different and specific functional requirements before and after pairing were determined based on the novel miRNA profiles.

MATERIAL AND METHODS

Parasites

Cercariae of *S. japonicum* were collected from the lab-infected snail *O. hupensis hupensis*, as well as hatched miracidia provided by the National Institute of Parasitic Disease, Chinese Center for Disease Control and Prevention (NIPD). Hepatic schistosomula were isolated from the portal system and from mesenteric veins of infected mice at 14 dpi. Adult worms of *S. japonicum* were isolated from the hepatic portal system and mesenteric veins of infected mice at 28 dpi. In addition, male and female adult worms were manually separated under a light microscope.

All animals were handled in strict accordance with the guidelines defined by the relevant national and/or local animal welfare bodies, and all animal experiments were approved by the ethics committee of NIPD (No. IPD-2018-16).

RNA isolation

According to the manufacturer's protocol, total RNAs from samples in female and male *S. japonicum* (schistosomulum and adult worm) at 14 dpi and 28 dpi were extracted using Trizol reagent (Invitrogen, Carlsbad, CA). RNA concentration and purity were photometrically measured at 260 nm and 280 nm using a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE) and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The extracted RNA samples were then stored at -80°C.

Small RNA isolation and library preparation

The cloning and analysis of small RNAs were performed according to the previous description (Ai *et al*., 2012; Xu *et al*., 2012). A 10 µg total RNA was separated by using a Novex 15% TBE-Urea gel (Invitrogen Co. Ltd). Then, 18-30 nt RNA was purified and the 5' and 3' adaptors (Illumina) were added to each end of the fragments. After each adaptor ligation, the small RNA was purified by using 6% TBE PAGE gel. Complementary DNA (cDNA) was obtained by a reverse transcription PCR (RT-PCR) kit (Invitrogen) for reverse transcription. Finally, the RT-PCR product was purified by using a 6% TBE PAGE gel (Invitrogen) and sequenced with an Illumina-Solexa sequencer. Image and signal processing was completed using Solexa Genome Analysis System (Illumina) A file containing the trace, "base-calling" and quality score data were generated for subsequent bioinformatics analysis.

Identification of non-coding RNA

Clean reads were analyzed as previously described (Xu *et al*., 2010). Firstly, in order to obtain clean reads, adaptors and low-quality sequences were trimmed from the raw data file, as well as reads smaller than 18 nt were removed. Then, the clean reads were searched against GenBank and Rfam database (version 10.1) to remove non-coding RNAs (rRNA, tRNA, snRNA, snoRNA) and other ncRNAs (Gardner *et al*., 2011). Repetitive sequences in the clean reads were eliminated by Repeat Masker (Bergman & Quesneville, 2007) (http://www.repeatmasker.org). The Sanger miRBase (Kozomara A & Griffiths-Jones, 2011) (version 16.0) was used to identify conserved miRNAs (Mount, 2007). The same miRNA family was judged according to reads showing high similarity to conserved miRNAs from other organisms with mismatches ≤ 2 (Wei *et al*., 2009). The expression and coding characters of miRNAs were statistically analyzed by family distribution and nucleotide bias. "Unannotated" reads were those, which could not be matched using any of the databases. The genome of *S. japonicum* (http://lifecenter.sgst.cn/schistosoma/cn) was used as the reference genome for short read alignment using the program Short Oligonucleotide Analysis Package (SOAP) (SOAP, http://soap.genomics.org.cn). The prediction of novel miRNAs was analyzed by Mfold (Zuker, 2008) and then evaluated with MirCheck (http://web.wi.mit.edu/bartel/pub/software.html). The free energy hybridization threshold of a stem-loop hairpin was set lower than -18 kcal/mol.

Target prediction and function analysis

Algorithms Miranda was used to understand the molecular function of differentially expressed miRNAs. Moreover, TargetScan (Lewis *et al*., 2003) was used to get the putative target sites of miRNA candidates. The result of Miranda was filtered using the Energy of miRNA_target sites: -20, Score of miRNA_target sites: 150. Sequences predicted by both software were considered as miRNA targets.

To better understand miRNA target function and classification, all predicted target proteins with an E value of 1e-5 were identified by BLASTX searching against the Interpro and KEGG database (version 76). The best hits were used to validate the target gene function and metabolic pathway regulated by miRNAs. We also established the biological process, cellular component, and molecular function of target genes using the Interpro database the same way as we used the GO database.

Analysis of miRNA expression

In order to analyze novel miRNA expression at 14 dpi and 28 dpi of *S. japonicum*, RT-PCR with SYBR Green (TOYOBO) was used. RT-PCR was performed using an ABI PRISM® 7300 Sequence Detection System and SYBR Green PCR Master Mix (TOYOBO) in a 20 µl reaction. The PCR mix contained 5 µl cDNA for each miRNA (in 1: 20 dilution), 5 µM forward and reverse primers, and 10 µl 2× SYBR Green PCR Master Mix. The *S. japonicum* 18S rRNA gene (GenBank accession number L06668) was used as the endogenous control. The primer pairs for the RT-PCR are shown in Table 1. The PCR conditions were set at 95°C 10 min, followed

by 40 cycles of 95°C for 15s, 65°C for 30s, and 72°C for 30s. The threshold cycle (Ct) was defined as the cycle number at which the fluorescence intensity passed a predetermined threshold value.

Confirmation of miRNA expression by the reverse transcriptase-polymerase chain reaction

To confirm the miRNA differential expressions at 14 dpi and 28 dpi of *S. japonicum*, stem-loop real-time reverse transcriptase-polymerase chain reaction (RT-PCR) with SYBR Green was applied. Real-time quantitative PCR was performed using an ABI PRISM® 7300 Sequence Detection System and SYBR Green PCR Master Mix (TOYOBO) in a 20 µl reaction. The PCR mix contained 5 µl cDNA for each miRNA (in 1: 20 dilution), 5 µM forward and reverse primers, 10 µl 2× SYBR Green PCR Master Mix. The *S. japonicum* 18S rRNA gene (GenBank accession number L06668) was served as the endogenous control. The primers are shown in Table 1. The PCR recycle conditions were set at 95°C 6 min, followed by 50 cycles of 95°C for 10s, 55°C for 10s, and 72°C for 30 s. The threshold cycle (Ct) was defined as the cycle number at which the fluorescence intensity passed a predetermined threshold value.

Statistical analysis

At least three independent experiments were performed. Results were presented as mean ± standard deviation. One-way ANOVA and Student's t-test were used to perform statistical analyses. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Profile character of short RNAs from male and female Schistosomulum (14 dpi)

Totally, 32,820,190 and 33,445,677 reads were obtained with Solexa deep sequencing from the female and the male samples of schistosomulum (14 dpi), respectively. After filtering low-quality tags, including 52 and 32 adaptors and contamination formed by adaptor-adaptor ligation, a total of 32,047,967 and 32,302,846 high-quality reads were obtained. Length distribution analysis showed that most reads were distributed among 20-23 nt in both genders. The highest percentage was 29.17% with reads that were 20 nt long, which was followed by 23.08% of 23 nt reads in male (Fig. 1A), and 27.56% with 22 nt long reads, followed by 26.29% of 20 nt reads in female (Fig.1B).

Among the clean reads, 2.59% and 2% were identified as noncoding sRNA, including tRNA, rRNA, siRNA, snRNA and snoRNA (Table 2) in male and female, respectively. Allowing the maximum mismatches of 2 nt, 4,155 (0.48%) tRNAs, 15,905 (1.83%) rRNAs, 241 (0.03%) snoRNAs, and 2,156 (0.25%) snRNA were recovered in Unique sRNAs of the male. In addition, 3,527 (0.42%) tRNAs, 11,068 (1.33%) rRNAs, 188 (0.02%) snoRNAs, and 1,935 (0.23%) snRNA were recovered in

Table 1. The primers used in identified novel miRNA expression at 14 dpi and 28 dpi of *Schistosoma japonicum*

Name of primers	Sequences (5' to 3')
1.sja-miR-125b-RT	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGCAATT
1.sja-miR-125b-F	TTCCCTGAGACTGATAATTG
2.sja-miR-10-5p-RT	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAAACTC
2.sja-miR-10-5pF	AACCCTGTAGACCCGAGT
3.sja-miR-36-3p-RT	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGCGAAT
3.sja-miR-36-3p-F	CACCGGGTAGACATTCATT
4.sja-miR-71a-RT	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACCATCTC
4.sja-miR-71a-F	GAAAGACGATGGTAGTGAG
5.sja-miR-3505-RT	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAGCTAC
5.sja-miR-3505-F	TGACTGTCTGGACTCAGTA
6.sja-miR-let7-RT	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACACCACA
6.sja-miR-let7-F	GGAGGTAGTTCGTTGTGT
MiRNA primer-R	TGCGTGTCGTGGAGTCG

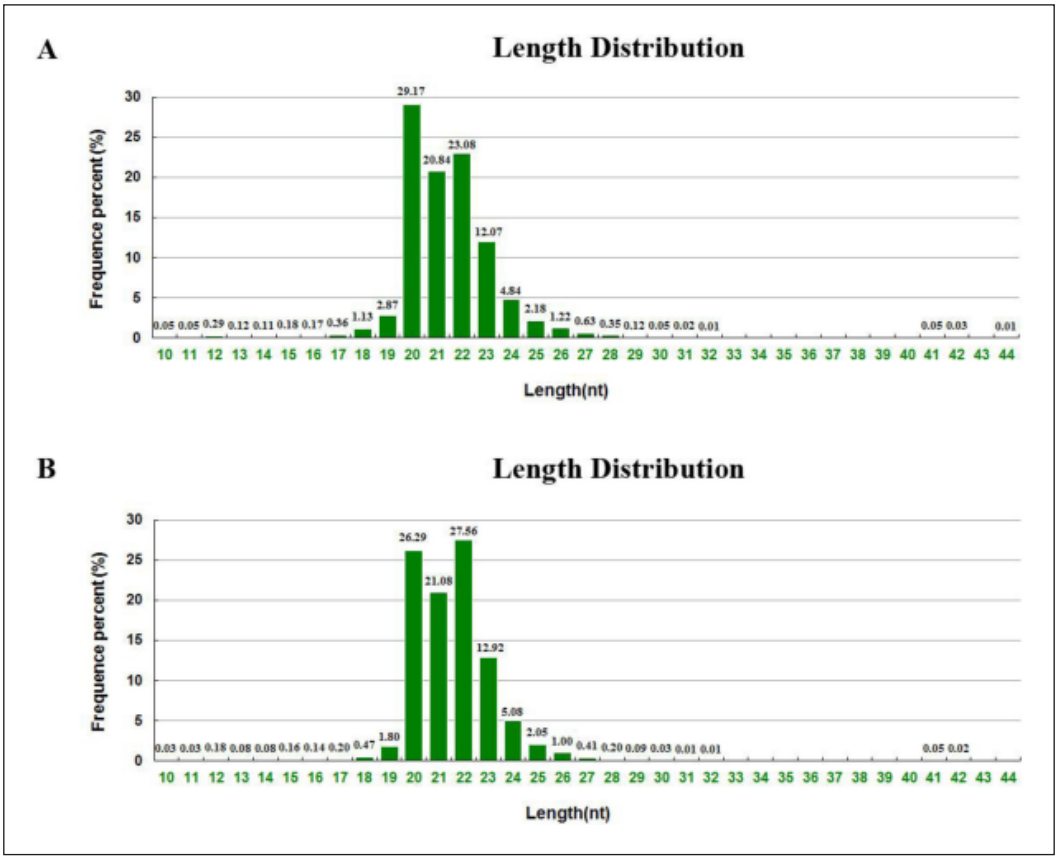

Figure 1. Length distribution analysis of small RNAs of male and female Schistosomulum (14 dpi). A resprents length distribution analysis of small RNAs of male Schistosomulum (14 dpi); B resprents length distribution analysis of small RNAs of female Schistosomulum (14 dpi).

Table 2. Small RNA annotation of 14 dpi male and female Schistosomulum

Category	14 dpi Male				14 dpi Female			
	Unique sRNAs	Percent (%)	Total sRNAs	Percent (%)	Unique sRNAs	Percent (%)	Total sRNAs	Percent (%)
Total	867552	100.00	29868057	100.00	831314	100.00	29711020	100.00
exon_antisense	6067	0.70	384121	1.29	5515	0.66	357092	1.2
exon_sense	10522	1.21	562133	1.88	8628	1.04	417787	1.41
intron_antisense	8562	0.99	568474	1.9	8003	0.96	559974	1.88
intron_sense	30872	3.56	1414260	4.74	26754	3.22	1059441	3.57
miRNA	677	0.08	6173140	20.67	690	0.08	8024446	27.01
rRNA	15905	1.83	1159093	3.88	11068	1.33	454410	1.53
repeat	170547	19.66	6985139	23.39	154586	18.6	6350150	21.37
snRNA	2156	0.25	40353	0.14	1935	0.23	28837	0.10
snoRNA	241	0.03	2688	0.01	188	0.02	2111	0.01
tRNA	4155	0.48	145993	0.49	3527	0.42	111156	0.37
unann	617848	71.22	12432663	41.63	610420	73.43	12345616	41.55

Unique sRNAs of the female. Except for the ncRNA mentioned above, 617,848 (71.22%) and 610,420 (73.43%) of total reads had no match to the public database and were marked as un-annotated reads (Table 2).

The level of the total percentage of ncRNA repressed in the schistosomulum of different sex (14 dpi) showed the following results: in males, 20,064,794 (67.18%%) sequences were perfectly mapped to the *S. japonicum* genome, which included 293,674 (33.85%) unique sequences; in females, there were 19,615,952 (66.02%) sRNAs mapping to the target genome, which contained 259,398 (31.2% %) unique sequences.

Profile character of short RNAs from male and female adult worm of *S. japonicum* (28 dpi)

Totally, 31,842,941 and 35,875,743 reads were obtained with solexa deep sequencing from the male and the female adult samples of *S. japonicum* (28 dpi), respectively. After filtering low-quality tags, including 52 and 32 adaptors and contamination formed by adaptor-adaptor ligation, a total of 31,636,846 and 35,680,733 high-quality reads were obtained. Length distribution analysis showed that most reads were distributed among 20-23 nt in both genders. The highest percentage was 42.69% with reads of 22 nt long and followed by 18.12% of 21 nt reads in male (Fig. 2A), and it was 36.10% with reads of 22 nt long and followed by 21.40% of 20 nt reads in female (Fig. 2B).

Among the clean reads, 5.28% and 2.71% were identified as noncoding sRNA, including tRNA, rRNA, siRNA, snRNA and snoRNA (Table 3) in male and female, respectively. Allowing the maximum mismatches of 2 nt, 6,358 (1.21%) tRNAs, 19,130 (3.65%) rRNAs, 174 (0.03%) snoRNAs, and 2,025 (0.39%) snRNA were recovered in Unique sRNAs of the male. In addition, 5,403 (0.63%) tRNAs, 15,744 (1.82%) rRNAs, 168 (0.02%) snoRNAs, and 2,072 (0.24%) snRNA were recovered in

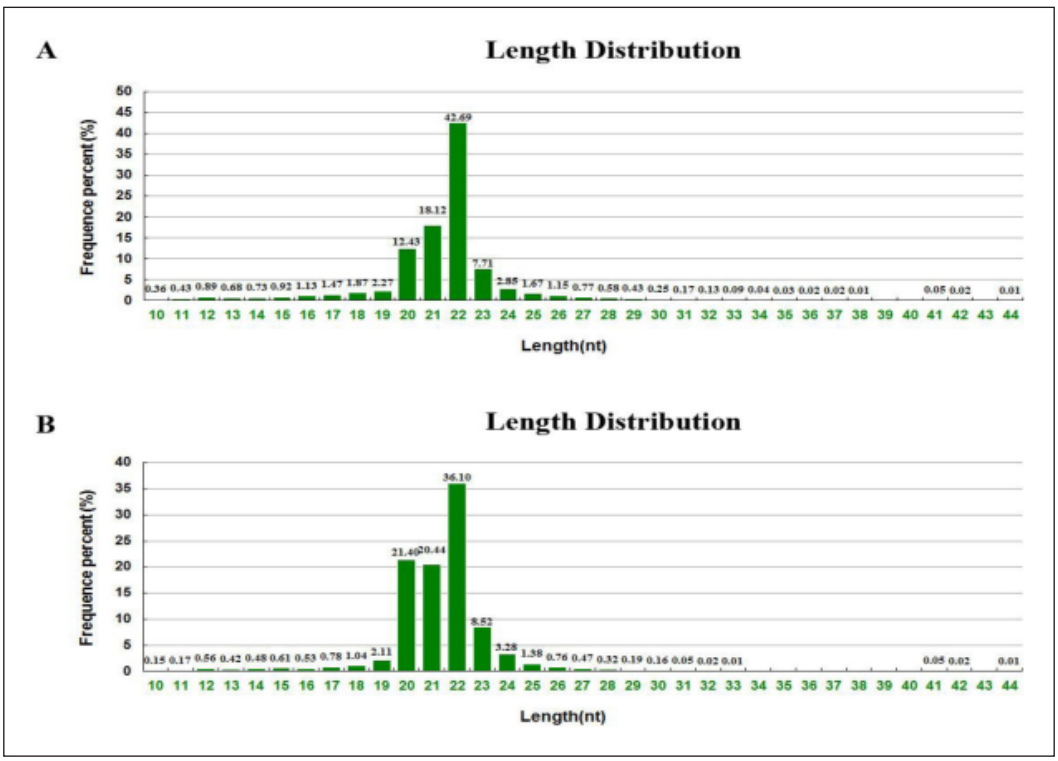

Figure 2. Length distribution analysis of small RNAs of male and female adult worm of *Schistosoma japonicum* (28 dpi). A resprents length distribution analysis of small RNAs of male adult worm of *S. japonicum* (28 dpi); B resprents length distribution analysis of small RNAs of female adult worm of *S. japonicum* (28 dpi).

Table 3. Small RNA annotation of 28 dpi male and female adult worm of *Schistosoma japonicum*

Category	28 dpi Male				28 dpi Female			
	Unique sRNAs	Percent (%)	Total sRNAs	Percent (%)	Unique sRNAs	Percent (%)	Total sRNAs	Percent (%)
Total	524099	100.00	27150428	100.00	864237	100	32402321	100
exon_antisense	2945	0.56	142862	1.29	5304	0.61	284633	0.88
exon_sense	8560	1.63	336029	1.88	10156	1.18	478988	1.48
intron_antisense	4285	0.82	260818	1.9	8195	0.95	575156	1.78
intron_sense	16955	3.24	667169	4.74	27132	3.14	1003491	3.1
miRNA	442	0.08	13782722	20.67	585	0.07	12197289	37.64
rRNA	19130	3.65	1041006	3.88	15744	1.82	630786	1.95
repeat	73833	14.09	2240278	23.39	146829	16.99	5535202	17.08
snRNA	2025	0.39	36906	0.14	2072	0.24	27792	0.09
snoRNA	174	0.03	2483	0.01	168	0.02	1608	0
tRNA	6358	1.21	275372	0.49	5403	0.63	286893	0.89
unann	389392	74.3	8364783	41.63	642649	74.36	11380483	35.12

Unique sRNAs of the female. Except for the ncRNA mentioned above, 389,392 (74.3%) and 836,4783 (41.63%) of total reads had no match to the public database and were marked as un-annotated reads (Table 3).

The level of the total percentage of ncRNA repressed in different gender of adult *S. japonicum* (28 dpi) showed the following results: in males, 19,682,739 (72.5%) sequences were perfectly mapped to the *S. japonicum* genome, which included 143,968 (27.47%) unique sequences; in females, there were 22,860,844 (70.55%) sRNAs mapping to the target genome, which contained 254,308 (29.43%) unique sequences.

Common and specific short RNAs sequences in schistosomulum (14 dpi) and adult worm of *S. japonicum* (28 dpi)

Among the high-quality reads, 94.89%, 90.62%, 96.90%, and 91.56% of the total reads were shared by females at 14 dpi and 28 dpi, males at 14 dpi and 28 dpi, males at 14 dpi and females at 14 dpi, and females and males at 28 dpi, respectively (Table 4). In the comparison between females at 14 dpi and 28 dpi, there were 1.39 million (2.25%) and 1.77 million (2.86%) specific sRNAs expressed, respectively. Meanwhile, the comparison between males at 14 dpi and 28 dpi, males and females at 14 dpi, as well as females and males at 28 dpi, the specific sRNAs in each sample successively was 3.20 (5.61%), 2.15 (3.77%), 0.98 (1.65%), 0.86 (1.45%), 2.06 (4.98%) and 2.97 (3.45%) million.

Known microRNA in schistosomulum (14 dpi) and adult worm of *S. japonicum* (28 dpi)

The known miRNAs expressions in schistosomulum (14 dpi) and adult worm of *S. japonicum* (28 dpi) were shown in Table 5. There were 30, 31, 25, and 27 known miRNAs in males and females at 14 dpi, and in males and females at 28 dpi, respectively. The number of miRNA precursors was 51, 51, 45, and 49 in each sample, respectively. Moreover, there was no miRNA star (miRNA*) found in the above four specimens (Table 5).

Novel miRNA precursor candidates of schistosomulum (14 dpi) and adult worm of *S. japonicum* (28 dpi)

The total number of novel miRNA precursor candidates of shistosomulum (14 dpi) and adult worm of *S. japonicum* (28 dpi) were 47477, 58541, 22617 and 33979 (in males at 14 dpi, females at 14 dpi, females and males at 28 dpi), respectively (Table 4). Moreover, the number of unique miRNA candidates were 464, 480, 454, and 336 in males at 14 dpi, females at 14 dpi, females and males at 28 dpi), respectively (Table 6).

Table 4. Common and specific sequence between 14 dpi female and 28 dpi female, 14 dpi male and 28 dpi male, 14 dpi male and 14 dpi female, 28 dpi male and 28 dpi female

Class	Unique sRNAS	Percent (%)	Total sRNAS	Percent (%)	Class	Unique sRNAS	Percent (%)	Total sRNAS	Percent (%)
Total_sRNAs	1264794	100.00	62113341	100.00	Total_sRNA	1116947	100.00	57018485	100.00
SJ28Female & SJ14Female	430757	34.06	58941610	94.89	SJ14Male & SJ28Male	274704	24.59	51667473	90.62
SJ28Female specific	433480	34.27	1777190	2.86	SJ14Male specific	592848	53.08	3200022	5.61
SJ14Female specific	400557	31.67	1394541	2.25	SJ28Male specific	249395	22.33	2150990	3.77
Class	Unique sRNAS	Percent (%)	Total sRNAS	Percent (%)	Class	Unique sRNAS	Percent (%)	Total sRNAS	Percent (%)
Total_sRNAs	1184819	100.00	59579077	100.00	Total_sRNAs	1122825	100.00	59552749	100.00
SJ14Male & SJ14Female	514047	43.39	57733410	96.90	SJ28Female & SJ28Male	265511	23.65	54529103	91.56
SJ14Male specific	353505	29.84	983612	1.65	SJ28Female specific	598726	53.32	2968010	4.98
SJ14Female specific	317267	26.78	862055	1.45	SJ28Male specific	258588	23.03	2055636	3.45

Table 5. Summary of known miRNA in 14 dpi male and female Schistosomulum, 28 dpi male and female worm of *Schistosoma japonicum*

	miRNA	miRNA*	miRNA-5p	miRNA-3p	miRNA precursors	unique sRNAs matched to miRNA precursors	total sRNAs matched to miRNA precursors
Known miRNA in miRbase	32	0	23	23	55	–	–
SJ14Female	31	0	17	17	51	693	8024453
SJ14Male	30	0	19	17	51	679	6173144
SJ28Female	27	0	17	19	49	588	12197307
SJ28Male	25	0	17	16	45	445	13782730

Table 6. Novel miRNA precursor candidates of each sample

Sample	Number of unique miRNA candidates	Number of total miRNA candidates
SJ14Female	464	47477
SJ14Male	480	58541
SJ28Female	454	22617
SJ28Male	336	33979

Differential expression analysis of schistosomulum (14 dpi) and adult worm of *S. japonicum* (28 dpi)

There were 5 and 9 significantly differentially expressed miRNAs between mals and females at 14 dpi and 28 dpi, respectively. At 14 dpi, sja-miR-2a-5p and sja-miR-3484-5p were expressed only in male, while three miRNAs (sja-miR-2d-5p, sja-miR-31-5p and sja-miR-125a) showed high expression in females (Table 7). In females and males at 28 dpi male, except for sja-miR-219, other eight miRNAs (sja-miR-2c-5p, sja-miR-3483-3p, sja-miR-3489, sja-miR-3492, sja-miR-3495, sja-miR-3499, sja-miR-3502, and sja-miR-3505) were highly expressed in females, and 9 miRNAs were differentially compared with those at 14 dpi.

Compared with both females and males at 28 dpi, the majority of differential expressed miRNAs were highly abundant at 14 dpi, except for sja-miR-125b and sja-miR-3505, which may be related to laying eggs process. These data suggested that more miRNAs have been actively expressed at 14 dpi in both male and female (Mark: begin to paired). In addition, there were 3 female-biased expressions and 15 male-biased expressions in miRNAs at 14 dpi and 28 dpi (Table 7 and Table 8). We found that sja-let-7 was highly-expressed at 14 dpi in both males and females. Moreover, sja-bantam was expressed at different stages in males and females; its highest expression was observed at 28dpi in females.

Schistosomulum and adult worm of *S. japonicum* genes targeted by *S. japonicum* miRNAs

The application of KEGG pathway analysis was used to investigate the characteristics of the biochemical and metabolic processes involved in schistosomulum and adult worm of *S. japonicum* genes targeted by *S. japonicum* miRNAs. There was a significant difference in the expression of miRNAs, probably due to the function of sexual differentiation. We found the predicted target genes of sja-miR-2a-5p and sja-miR-3484-5p, which were expressed at 14 dpi in males and were clustered in DNA topoisomerase III, Rap guanine nucleotide exchange factor 1 and L-serine/L-threonine ammonia-lyase. Target genes of sja-miR-2d-5p, sja-miR-31-5p and sja-miR-125a, which were expressed at 14 dpi in male and female (mainly females) were clustered in kelch-like protein 12, fructose-bisphosphate aldolase, class I, and heat shock protein 90kDa beta. Predicted target genes of sja-miR-3483-3p (expressed at 28dpi in females without in the 28 dpi male) were clustered in 26S proteasome regulatory subunit N1, ATP-dependent RNA helicase DDX17. Predicted target genes of sja-miR-219-5p, which were differentially expressed at 28 dpi in male and female (mainly males) were clustered in DNA excision repair protein ERCC-6, protein phosphatase 1D, and ATPase family AAA domain-containing protein 3A/B. At 28 dpi, eight miRNAs were significantly up-regulated in females compared to males, and the predicted target genes of these miRNAs were significantly clustered in heat shock protein 90kDa beta, 26S proteasome regulatory subunit N1, and protein arginine N-methyltransferase 1.

Predicted target genes of these miRNAs, which revealed significantly high expression in females at both 14 dpi and 28 dpi were clustered in heat shock protein 90kDa beta (HSP90).

Based on the sequence similarity, clean reads but not the ncRNA or repeat sequences were blasted against known mature miRNAs dataset of *S. japonicum* in the public database of miRBase (Release 12.0) with the BLAST software (Altschul *et al.*, 1990). Two co-expression and two differential expression miRNAs were identified. To verify the results of RNA-seq, the modified stem-loop RT-PCR was performed to examine the miRNA sja-miR-125b (the highest co-expression of female and male in 14 days), sja-miR-10-5p

Table 7. Differential expression analysis of male and female Schistosomulum (14 dpi)

pairwise	miR-name	SJ14Female-std	SJ14Male-std	fold-change (log2 SJ14Female/ SJ14Male)	p-value	sig-lable
SJ14Female.vs.SJ14Male	sja-bantam	1332.25299	2070.62251	-0.63619649	0.110738	
SJ14Female.vs.SJ14Male	sja-let-7	573.452912	515.836902	0.15276004	0.4158519	
SJ14Female.vs.SJ14Male	sja-miR-1	12.8869329	13.9616181	-0.11555722	0.766251	
SJ14Female.vs.SJ14Male	sja-miR-10-3p	93.8281046	71.2940278	0.39623889	0.00683229	**
SJ14Female.vs.SJ14Male	sja-miR-10-5p	99531.0873	55267.7309	0.84870982	0.00585114	**
SJ14Female.vs.SJ14Male	sja-miR-124-3p	99.2262629	89.8372855	0.14340768	0.2648972	
SJ14Female.vs.SJ14Male	sja-miR-124-5p	13.8230297	8.38935368	0.72044228	0.04411403	*
SJ14Female.vs.SJ14Male	sja-miR-125a	252.309296	125.406907	1.00857656	0.00157489	**
SJ14Female.vs.SJ14Male	sja-miR-125b	83842.6662	75990.3632	0.14186812	0.5467651	
SJ14Female.vs.SJ14Male	sja-miR-133	11.3267715	8.63700988	0.39113286	0.1073099	
SJ14Female.vs.SJ14Male	sja-miR-190-3p	848.727784	605.302703	0.48764511	0.1136886	
SJ14Female.vs.SJ14Male	sja-miR-190-5p	50.0187734	51.6053601	-0.04505124	0.8458558	
SJ14Female.vs.SJ14Male	sja-miR-2162-3p	1820.08425	2018.15035	-0.14902843	0.2065674	
SJ14Female.vs.SJ14Male	sja-miR-2162-5p	55.9473866	40.120304	0.47973821	0.00118765	**
SJ14Female.vs.SJ14Male	sja-miR-219-5p	82.0020814	90.1158988	-0.13612113	0.4504001	
SJ14Female.vs.SJ14Male	sja-miR-277	767.942628	920.414257	-0.2612848	0.3615372	
SJ14Female.vs.SJ14Male	sja-miR-2a-3p	4703.57449	3923.95766	0.26144808	0.00182198	**
SJ14Female.vs.SJ14Male	sja-miR-2a-5p	0.01	0.3714843	-5.21522932	0.01619183	*
SJ14Female.vs.SJ14Male	sja-miR-2b-3p	99.9751404	119.679857	-0.25953905	0.2787144	
SJ14Female.vs.SJ14Male	sja-miR-2b-5p	276.273375	212.612845	0.37786777	0.09236782	
SJ14Female.vs.SJ14Male	sja-miR-2c-3p	847.074013	857.354798	-0.01740433	0.862755	
SJ14Female.vs.SJ14Male	sja-miR-2c-5p	6038.26133	4450.13421	0.44028435	0.1365844	
SJ14Female.vs.SJ14Male	sja-miR-2d-3p	2136.57859	1731.9217	0.30292867	0.00016955	**
SJ14Female.vs.SJ14Male	sja-miR-2d-5p	1.31053555	0.18574215	2.81878336	0.00781897	**
SJ14Female.vs.SJ14Male	sja-miR-2e-3p	55.3857285	50.9862196	0.11940691	0.213092	
SJ14Female.vs.SJ14Male	sja-miR-2e-5p	2.30903882	1.98124958	0.2208818	0.6192086	
SJ14Female.vs.SJ14Male	sja-miR-307	374.313915	376.74699	-0.0093473	0.9683188	
SJ14Female.vs.SJ14Male	sja-miR-310	0.49925164	0.74296859	-0.57353406	0.3013142	
SJ14Female.vs.SJ14Male	sja-miR-31-5p	1.80978719	0.27861322	2.69948443	0.01016709	*
SJ14Female.vs.SJ14Male	sja-miR-3479-3p	236.052415	254.219086	-0.10696511	0.4600628	
SJ14Female.vs.SJ14Male	sja-miR-3479-5p	0.3432355	0.2476562	0.47086006	0.7114215	
SJ14Female.vs.SJ14Male	sja-miR-3481-3p	1.06090973	1.05253884	0.01142844	0.9493682	
SJ14Female.vs.SJ14Male	sja-miR-3481-5p	1.34173878	2.22890578	-0.73223181	0.07201406	
SJ14Female.vs.SJ14Male	sja-miR-3482-3p	1.87219364	2.97187437	-0.66664347	0.1958045	
SJ14Female.vs.SJ14Male	sja-miR-3482-5p	3.40115178	3.83867106	-0.17458355	0.5745236	
SJ14Female.vs.SJ14Male	sja-miR-3483-3p	0.3432355	0.2476562	0.47086006	0.7832769	
SJ14Female.vs.SJ14Male	sja-miR-3484-5p	0.01	0.34052727	-5.08969844	0.02426888	*
SJ14Female.vs.SJ14Male	sja-miR-3486-5p	0.43684518	0.71201157	-0.7047786	0.1490595	
SJ14Female.vs.SJ14Male	sja-miR-3487	6.0534261	5.13886609	0.23630185	0.1615179	
SJ14Female.vs.SJ14Male	sja-miR-3488	0.21842259	0.1238281	0.81878336	0.7242039	
SJ14Female.vs.SJ14Male	sja-miR-3489	46.4928087	70.5820162	-0.60229306	0.09538728	
SJ14Female.vs.SJ14Male	sja-miR-3490	0.12481291	0.2476562	-0.98857156	0.49663	
SJ14Female.vs.SJ14Male	sja-miR-3491	0.21842259	0.40244132	-0.88165636	0.3103331	
SJ14Female.vs.SJ14Male	sja-miR-3492	224.257595	229.855908	-0.0355729	0.8688266	
SJ14Female.vs.SJ14Male	sja-miR-3493	0.01	0.06191405	-2.63026682	0.373901	
SJ14Female.vs.SJ14Male	sja-miR-3494	0.40564196	0.65009752	-0.68044927	0.5681987	
SJ14Female.vs.SJ14Male	sja-miR-3495	31.7336822	31.8547784	-0.00549487	0.8822446	
SJ14Female.vs.SJ14Male	sja-miR-3496	0.56165809	0.89775371	-0.67662756	0.484287	
SJ14Female.vs.SJ14Male	sja-miR-3497	0.06240645	0.01	2.64169525	0.373901	
SJ14Female.vs.SJ14Male	sja-miR-3498	0.62406455	0.61914049	0.01142844	0.9240003	
SJ14Female.vs.SJ14Male	sja-miR-3499	18.6283267	21.0507768	-0.17637538	0.3421255	
SJ14Female.vs.SJ14Male	sja-miR-3500	0.56165809	0.52626942	0.0938906	0.9445722	
SJ14Female.vs.SJ14Male	sja-miR-3501	0.06240645	0.18574215	-1.57353406	0.09287917	
SJ14Female.vs.SJ14Male	sja-miR-3502	2.43385173	2.69326114	-0.14611284	0.741526	
SJ14Female.vs.SJ14Male	sja-miR-3503	0.06240645	0.01	2.64169525	0.373901	
SJ14Female.vs.SJ14Male	sja-miR-3504	1.15451941	1.85742148	-0.68600879	0.1269994	
SJ14Female.vs.SJ14Male	sja-miR-3505	1.77858396	1.42402313	0.32075649	0.3260326	
SJ14Female.vs.SJ14Male	sja-miR-3506	2.09061623	1.70263636	0.29615791	0.3108077	
SJ14Female.vs.SJ14Male	sja-miR-3507	4.58687442	4.58163965	0.00164742	0.9711712	
SJ14Female.vs.SJ14Male	sja-miR-36-3p	26208.9948	23184.6135	0.17689434	0.1669596	
SJ14Female.vs.SJ14Male	sja-miR-36-5p	31.4216499	22.3819288	0.48942457	0.0569673	
SJ14Female.vs.SJ14Male	sja-miR-61	651.273761	617.71647	0.07631931	0.212293	
SJ14Female.vs.SJ14Male	sja-miR-71a	13751.1687	12708.9421	0.11371029	0.2048831	
SJ14Female.vs.SJ14Male	sja-miR-71b-3p	13.8854362	12.3828099	0.16523377	0.6965346	
SJ14Female.vs.SJ14Male	sja-miR-71b-5p	3515.76123	3120.00373	0.17228934	0.182484	
SJ14Female.vs.SJ14Male	sja-miR-7-3p	4.11882601	6.16044791	-0.58080206	0.1706581	
SJ14Female.vs.SJ14Male	sja-miR-7-5p	0.3432355	0.80488264	-1.22957966	0.2568139	
SJ14Female.vs.SJ14Male	sja-miR-8-3p	134.860349	139.058955	-0.04423042	0.864476	

Table 8. Differential expression analysis of adult male and female worm of *Schistosoma japonicum* (28 dpi)

pairwise	miR-name	SJ28Female-std	SJ28Male-std	fold-change (log2 SJ28Female/ SJ28Male)	p-value	sig-lable
SJ28Female.vs.SJ28Male	sja-bantam	7975.124764	691.571486	3.527556797	0.06601843	
SJ28Female.vs.SJ28Male	sja-let-7	155.5085	158.3551713	-0.02617054	0.96442	
SJ28Female.vs.SJ28Male	sja-miR-1	2.826356683	3.262467871	-0.20702015	0.9203389	
SJ28Female.vs.SJ28Male	sja-miR-10-3p	34.65231058	25.20346058	0.459329715	0.5284895	
SJ28Female.vs.SJ28Male	sja-miR-10-5p	87257.78602	128402.7405	-0.55732022	0.1662015	
SJ28Female.vs.SJ28Male	sja-miR-124-3p	23.22911899	27.13943053	-0.22445601	0.8436504	
SJ28Female.vs.SJ28Male	sja-miR-124-5p	0.765471602	1.649159583	-1.10731025	0.3728084	
SJ28Female.vs.SJ28Male	sja-miR-125a	435.6711062	637.7228404	-0.54969012	0.1893544	
SJ28Female.vs.SJ28Male	sja-miR-125b	245686.9135	349279.5234	-0.50756105	0.3214888	
SJ28Female.vs.SJ28Male	sja-miR-133	2.855797898	2.473739374	0.207200375	0.7575477	
SJ28Female.vs.SJ28Male	sja-miR-190-3p	90.35509021	153.586949	-0.76537785	0.06505089	
SJ28Female.vs.SJ28Male	sja-miR-190-5p	44.27958803	27.85645643	0.668630053	0.254794	
SJ28Female.vs.SJ28Male	sja-miR-2162-3p	489.8723838	450.1130122	0.122118691	0.6795604	
SJ28Female.vs.SJ28Male	sja-miR-2162-5p	15.83937391	6.919299989	1.194817315	0.1198851	
SJ28Female.vs.SJ28Male	sja-miR-219-5p	44.19126439	99.95341124	-1.17749459	0.01181171	*
SJ28Female.vs.SJ28Male	sja-miR-277	568.7454	446.4203288	0.349380246	0.5862748	
SJ28Female.vs.SJ28Male	sja-miR-2a-3p	928.5170527	443.8748869	1.064775319	0.1705301	
SJ28Female.vs.SJ28Male	sja-miR-2a-5p	0.01	0.071702591	-2.84202524	0.373901	
SJ28Female.vs.SJ28Male	sja-miR-2b-3p	48.46024062	24.3071782	0.995419147	0.3208904	
SJ28Female.vs.SJ28Male	sja-miR-2b-5p	232.7033669	113.4334983	1.036645336	0.1362916	
SJ28Female.vs.SJ28Male	sja-miR-2c-3p	104.8401682	15.88212381	2.722715821	0.07153119	
SJ28Female.vs.SJ28Male	sja-miR-2c-5p	517.6943324	128.9929604	2.004808189	0.03397996	*
SJ28Female.vs.SJ28Male	sja-miR-2d-3p	266.6196471	102.2478941	1.382711966	0.0820081	
SJ28Female.vs.SJ28Male	sja-miR-2d-5p	0.058882431	0.01	2.557837233	0.373901	
SJ28Female.vs.SJ28Male	sja-miR-2e-3p	19.60784949	13.26497925	0.563808896	0.489697	
SJ28Female.vs.SJ28Male	sja-miR-2e-5p	0.471059447	0.286810362	0.715811989	0.3491035	
SJ28Female.vs.SJ28Male	sja-miR-307	107.3426715	93.75113716	0.195315604	0.7681738	
SJ28Female.vs.SJ28Male	sja-miR-310	0.147206077	0.107553886	0.452777583	0.6618122	
SJ28Female.vs.SJ28Male	sja-miR-31-5p	0.588824309	0.107553886	2.452777583	0.2401411	
SJ28Female.vs.SJ28Male	sja-miR-3479-3p	239.4748464	161.4742339	0.568570153	0.2895729	
SJ28Female.vs.SJ28Male	sja-miR-3479-5p	0.117764862	0.01	3.557837233	0.1169925	
SJ28Female.vs.SJ28Male	sja-miR-3480-3p	0.088323646	0.01	3.142799733	0.373901	
SJ28Female.vs.SJ28Male	sja-miR-3481-3p	1.177648618	0.932133677	0.337300366	0.6149557	
SJ28Female.vs.SJ28Male	sja-miR-3481-5p	0.883236463	0.143405181	2.622702585	0.0791651	
SJ28Female.vs.SJ28Male	sja-miR-3482-3p	0.97156011	1.541605697	-0.66605865	0.5668704	
SJ28Female.vs.SJ28Male	sja-miR-3482-5p	0.294412154	2.652995851	-3.17171328	0.1948922	
SJ28Female.vs.SJ28Male	sja-miR-3483-3p	0.32385337	0.01	5.017268851	0.000232496	**
SJ28Female.vs.SJ28Male	sja-miR-3484-5p	0.01	0.143405181	-3.84202524	0.373901	
SJ28Female.vs.SJ28Male	sja-miR-3485-3p	0.058882431	0.01	2.557837233	0.373901	
SJ28Female.vs.SJ28Male	sja-miR-3486-5p	0.382735801	0.071702591	2.416251707	0.369566	
SJ28Female.vs.SJ28Male	sja-miR-3487	7.772480878	5.879612426	0.402654104	0.8020005	
SJ28Female.vs.SJ28Male	sja-miR-3488	0.529941878	0.143405181	1.885736991	0.06665169	
SJ28Female.vs.SJ28Male	sja-miR-3489	43.21970428	3.692683414	3.548947715	0.005475989	**
SJ28Female.vs.SJ28Male	sja-miR-3490	0.117764862	0.01	3.557837233	0.1169925	
SJ28Female.vs.SJ28Male	sja-miR-3491	0.294412154	0.01	4.879765328	0.1325751	
SJ28Female.vs.SJ28Male	sja-miR-3492	120.6206597	24.55813727	2.296203989	0.001458799	**
SJ28Female.vs.SJ28Male	sja-miR-3494	0.206088508	0.179256476	0.201238816	0.8846557	
SJ28Female.vs.SJ28Male	sja-miR-3495	37.39034362	8.138244029	2.199876284	0.02123222	*
SJ28Female.vs.SJ28Male	sja-miR-3496	0.588824309	0.143405181	2.037740084	0.1606805	
SJ28Female.vs.SJ28Male	sja-miR-3499	27.58641887	1.147241449	4.587717227	0.002872435	**
SJ28Female.vs.SJ28Male	sja-miR-3500	0.294412154	0.143405181	1.037740084	0.4626249	
SJ28Female.vs.SJ28Male	sja-miR-3502	2.060885081	0.430215543	2.260132505	0.0262687	*
SJ28Female.vs.SJ28Male	sja-miR-3503	0.01	0.179256476	-4.16395334	0.1210549	
SJ28Female.vs.SJ28Male	sja-miR-3504	0.824354032	0.358512953	1.201238816	0.1076292	
SJ28Female.vs.SJ28Male	sja-miR-3505	10.83436728	0.322661658	5.069448944	0.02599001	*
SJ28Female.vs.SJ28Male	sja-miR-3506	1.001001325	0.01	6.645300074	0.1337789	
SJ28Female.vs.SJ28Male	sja-miR-3507	2.355297236	2.294482898	0.037740084	0.9490775	
SJ28Female.vs.SJ28Male	sja-miR-36-3p	9682.509171	9620.551683	0.009261336	0.986818	
SJ28Female.vs.SJ28Male	sja-miR-36-5p	6.064890382	2.868103622	1.080384422	0.1626144	
SJ28Female.vs.SJ28Male	sja-miR-61	197.1678198	351.9163145	-0.83580829	0.1942248	
SJ28Female.vs.SJ28Male	sja-miR-71a	2544.398163	2175.743408	0.225816025	0.6163959	
SJ28Female.vs.SJ28Male	sja-miR-71b-3p	2.679150606	3.513426937	-0.39110321	0.6704208	
SJ28Female.vs.SJ28Male	sja-miR-71b-5p	586.0568347	178.0375324	1.718859171	0.1803544	
SJ28Female.vs.SJ28Male	sja-miR-7-3p	0.235529724	0.932133677	-1.98462773	0.3297433	
SJ28Female.vs.SJ28Male	sja-miR-7-5p	0.235529724	0.358512953	-0.60611611	0.8476834	
SJ28Female.vs.SJ28Male	sja-miR-8-3p	33.18024981	96.87019985	-1.54572817	0.166735	

(the highest co-expression of female and male in 28 days), sja-miR-36-3p (the highest differential expression of female and male in 14 days), as well as sja-miR-3505 (the highest differential expression of female and male in 28 days). The sequences, as well as the melt curves of the four miRNAs, are presented in Table 9 and Figure 3. The novel miRNAs identified in the present study may clarify the development of *S. japonicum*, thus furthering the understanding of the biology of this trematode.

Table 9. Sequences in Real-time quantitative PCR verification of co-expression and differential miRNA in male and female adult worm of Schistosomulum and *Schistosoma japonicum*

miRNA	Sequence	Expression stage
sja-miR-125b	TCCCTGAGACTGATAATTGC	the highest co-expression of female and male in 14 days
sja-miR-10-5p	AACCCTGTAGACCCGAGTTT	the highest co-expression of female and male in 28 days
sja-miR-36-3p	CACCGGGTAGACATTCATTCGC	the highest differential expression of female and male in 14 days
sja-miR-3505	TGACTGTCTGGACTCAGTAGCT	the highest differential expression of female and male in 28 days

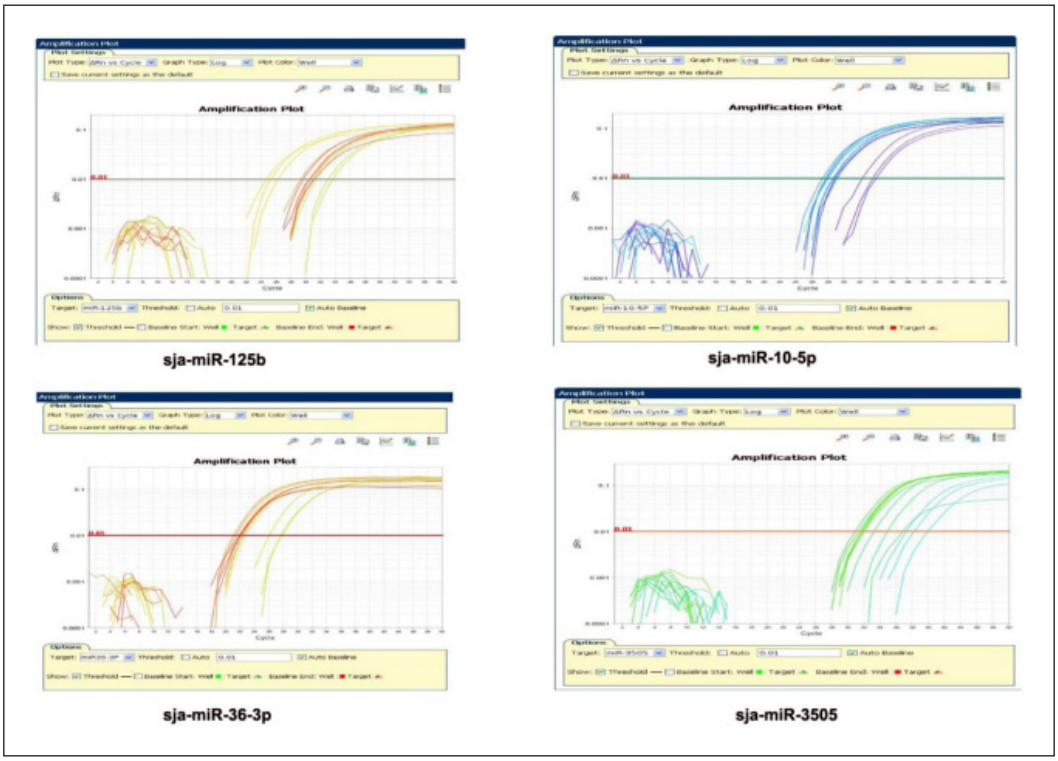

Figure 3. Real-time quantitative PCR verification of co-expression and differential miRNA in male and female adult worm of Schistosomulum and *Schistosoma japonicum*. Melt curves of four miRNAs and endogenous control (18S) is shown.

DISCUSSION

MicroRNAs are a class of small non-coding RNAs that have an important role in the transcriptional and post-transcriptional regulation of genes in both plants and animals (Liu *et al.*, 2017; Masuda *et al.*, 2017; Zhao *et al.*, 2018). Over the last decade, an increasing number of miRNAs have been identified in *S. japonicum*, some of which regulate cellular differentiation, sexual maturation and metabolism of the trematode (Cai *et al.*, 2011, 2013; Sun *et al.*, 2014; Zhu *et al.*, 2016)

During the development of *S. japonicum*, the sexual differentiation starts approximately 14 dpi infection, while egg-laying starts approximately 28 days post-infection (He & Yang, 1980). In terms of the published criteria for distinguishing bilaterian miRNAs from other types of small RNAs (Ruby *et al.*, 2006, 2007; Grimson *et al.*, 2008), it can provide insight into the mechanism underlying *S. japonicum* development, differentiation and maturation by comparing the miRNA profiles in female and male worms at 14 dpi and 28 dpi.

In this study, we examined the expression profile of miRNAs in individual *S. japonicum* of different sex before and after pairing (18 and 24 dpi). The majority of differential expressed miRNAs were highly abundant at 14 dpi, except for sja-miR-125b and sja-miR-3505, in both male and female (Mark: begin to be paired). Moreover, it was estimated that sja-miR-125b and sja-miR-3505 might be related to laying eggs. Besides, we found three female-biased expressions and 15 male-biased expressions in miRNAs at 14 dpi and 28 dpi; the function of 3 female-biased expressed miRNA was related to eggs laying, while 15 male-biased expressions were associated with sexual maturity.

In previous studies, let-7 has shown to be essential for the proliferation, differentiation, and apoptosis in cells (Crippa *et al.*, 2012; Shimono *et al.*, 2015; Jin *et al.*, 2016). Studies suggested that sja-let-7 take part in the transformation from miracidium to sporocyst in the snail intermediate host (Huang *et al.*, 2009). Moreover, sja-let-7 participates in chromosome transmission fidelity protein 18, large subunit ribosomal protein L21e and N-acetyltransferase 10 (Sun *et al.*, 2014). In this study, we found that sja-let-7 was highly-expressed at 14 dpi in both males and females, which suggested that this miRNA has an important role in sexual maturation of *S. japonicum*.

Bantam are known as regulators for both proliferation and apoptosis that target the proapoptotic gene hid (Banerjee *et al.*, 2017; Wu *et al.*, 2017; Brennecke *et al.*, 2003). Moreover, predicted target genes of sja-bantam participate in ATP-dependent RNA helicase DDX17, ATP-dependent DNA helicase PIF1 and chromodomain-helicase-DNA-binding protein 7 (Sun *et al.*, 2015). In this study, sja-bantam was expressed at different stages in males and females. At the same time, the highest expression levels were observed at 28 dpi in females as the report description on the function in ovary development (Zhu *et al.*, 2016).

Using a KEGG pathway analysis, we found a significant difference in the expression of miRNAs. We found that the predicted target genes of sja-miR-2a-5p and sja-miR-3484-5p, which were expressed at 14 dpi in males, were significantly clustered in DNA topoisomerase III, Rap guanine nucleotide exchange factor 1 and L-serine/L-threonine ammonia-lyase. Target genes of sja-miR-2d-5p, sja-miR-31-5p and sja-miR-125a, which were expressed at 14 dpi in males and particularly females, were clustered in kelch-like protein 12, fructose-bisphosphate aldolase, class I, and heat shock protein 90kDa beta. Liu *et al.* (2009) have reported that HSP90 was related to reproductive development. Predicted target genes of sja-miR-3483-3p (expressed at 28 dpi in female without in the 28 dpi male), were significantly clustered in 26S proteasome regulatory subunit N1, ATP-dependent RNA helicase DDX17. Predicted target genes of sja-miR-219-5p, which were differentially expressed at 28 dpi in females and particularly males, were clustered in DNA excision repair protein ERCC-6, protein phosphatase 1D, and ATPase family AAA domain-containing protein 3A/B. At 28 dpi, eight miRNAs were significantly up-regulated in females compared to males, and the predicted target genes of these miRNAs were

significantly clustered in heat shock protein 90 kDa beta, 26S proteasome regulatory subunit N1, and protein arginine N-methyltransferase 1.

CONCLUSIONS

The miRNAs research on *S. japonicum* may have an essential role in clarifying this trematode's growth, development, maturation and infection ability to mammalian hosts in its complex life cycle. These data may be helpful for developing new drug targets and vaccine candidates for schistosomiasis.

Conflicts of interest

The authors declare no conflicts of interest with regards to this study or the manuscript prepared for publication.

Acknowledgments. This study was supported by the General Program Shanghai Municipal Commission of Health and Family Planning of China (Grant No. 201840286 and 201640278), the Program for the Shanghai Natural Science Foundation of China (Grant No. 18ZR1443500), the Youth Science Foundation of Chinese Center for Disease Control and Prevention (Grant No. 2018A105), the Shenzhen San-Ming Project for prevention and research on vector-borne diseases (SZSM201611064), the National Sharing Service Platform for Parasite Resources (TDRC-22), the Program for National Key Research and Development Program of China (Grant No. 2016YFC1202000, 2016YFC1202005, and 2016YFC1202700), the Program for the National Science and Technology Major Program (Grant No. 2012ZX10004-220 and 2018ZX10734-404), the Chinese Special Program for Scientific Research of Public Health (Grant No. 201502021 and 201202019), the National Key Technology R&D Program (Grant No. 2008BAI56B03), the Fourth Round of Three-Year Public Health Action Plan of Shanghai, China (Grant No. 15GWZK0101), the Open project of Key Laboratory of Tropical Disease Control (Sun Yat-sen University), Ministry of Education (Grant No. 2019kfkt02), and the Parasitic and Tropical Diseases Resource Center Project of National Science and Technology Basic Conditions Platform Program, Key Laboratory of Echinococcosis Prevention and Control (Project No. 2020WZK2002)

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