

Full Length Research paper

Comparative analysis of the miRNA profiles from *Taenia solium* and *Taenia asiatica* adult

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Taenia solium and *Taenia asiatica* are zoonotic parasites which are transmitted between pigs and humans, with pigs acting as the intermediate host and humans as the definitive host. The aim of the present study was to compare the microRNA (miRNA) profiles between *T. solium* and *T. asiatica* by Solexa deep sequencing and bioinformatic analyses. A total of 18.26 and 15.24 million reads with high quality and three new miRNAs were obtained in each species, respectively. The two cestodes shared nearly the same level of total sRNA, but the known miRNAs were in a very different manner. Nucleotide guanine (G) and uracil (U) were the most frequently used first nucleotide in both *T. solium* and *T. asiatica*, respectively. Furthermore, it was also found that there were great differences in the kinds and copy numbers of miRNAs among the two *Taenia* species, which might indicate the different evolutions in the two parasites. To our knowledge, this is the first report on the characterization of the miRNA profiles for the two *Taenia* cestodes, and it would lay a foundation for further functional studies of miRNAs of *T. solium* and *T. asiatica*.

Key words: *Taenia solium*, *Taenia asiatica*, microRNA profiles, human, pig.

INTRODUCTION

Human taeniasis is caused by intestinal infection of adult *Taenia* spp., with *Taenia saginata*, *Taenia asiatica* and *Taenia solium* as the most common causative agents (Anantaphruti et al., 2007; Ito et al., 2011). Due to the special eating habits of local people in Asian countries, pork and pig visceral organs including the liver, omentum, serosa, and lung have been the main dishes in daily food. Moreover, in some rural areas, people are likely to consume uncooked or raw meat (Eom and Rim, 1993; Ito et al., 2004; Murell, 2005). This eating habit has led to the high occurrence of taeniasis, especially those caused by *T. solium* and *T. asiatica*.

T. solium can be transmitted between humans and pigs (O'Neal et al., 2011). In humans, the larval stage can

parasitize in the central nervous system, leading to neurocysticercosis with symptom of epilepsy or even death (Garcia et al., 2005a, b). This disease causes serious public health problem (Afonso et al., 2008; Sorvillo et al., 2011).

T. asiatica is morphologically and phylogenetically close to *T. saginata* and *T. solium* and pig is the intermediate host (Eom and Rim, 1993). *T. asiatica* is an important human parasite in Asian countries including Korea, China, Taiwan, Thailand, Indonesia, Vietnam, Japan and the Philippines (Nakao et al., 2002). This species has *T. saginata*-like morphology, but has a *T. solium*-like lifecycle (Bowles and McManus, 1994; Hoberg et al., 2000). Currently, it is still not clear whether

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T. asiatica causes human cysticercosis, and whether this parasite is also distributed out of Asia (Jeon et al., 2005; Jeon and Eom, 2007).

Due to their similarities in morphology, identification and differentiation between *T. asiatica* and *T. solium* can be difficult (Beveridge and Gregory et al., 1976; Eom et al., 2002; Proctor, 1972). Furthermore, if the samples are from different hosts or various developing stages, morphology will not be enough for identification and differentiation. With the development of molecular technologies, PCR-based techniques have been developed for the identification and differentiation of *Taenia* species, including *T. solium* or *T. asiatica* (Al-Sabi and Kapel, 2011; Jeon et al., 2011a, b). However, there are currently no published data about the microRNA (miRNA) profiles of *T. solium* or *T. asiatica*. Herein we developed an integrative approach combining Solexa deep sequencing with bioinformatic analysis to comparatively study the miRNA profiles between the two *Taenia* species.

MATERIALS AND METHODS

Samples

Adult *Taenia* tapeworms were collected from stools of two patients of Miao nationality in Guizhou province, China. The two samples were identified by morphology. *T. solium*: Adult measures 2 to 10 m and contains 1000 proglottids. The scolex bears a rostellum with two crowns of hooks (Beaver et al., 1984). The gravid segment measures about 12 by 6 mm and the lateral uterine branches, visible by the mass of eggs. Gravid segments are less mobile and usually expelled with the stools, separately or in groups of three to six proglottids (WHO, 1983). *T. asiatica*: Adults are large sized tapeworms (mean 341 cm long and 9.5 mm wide) with on average 712 segments. The scolex is spheroidal, with a cuspidal rostellum. The cervical swelling is distinct. Proglottids are rectangular. Anterior proglottids are wide and short; the posterior proglottids are long and narrow.

Free proglottids bear a posterior protuberance. Mature proglottids have two ovary lobes that are unequal in size. The vaginal sphincter is round to oval (Eom and Rim, 1993). The uterus has numerous lateral branches, between 16-32. The way of leaving the host is as a single proglottid and spontaneously, independent from defaecation as for *T. saginata* (Fan, 1988; Ito et al., 2003) and polymerase (PCR) (Jeon et al., 2011a, b). Gravid proglottids of the two species were respectively incubated in physiological saline for 3 h at 37°C individually and washed for 3 times to get rid of contamination from the host. The parasites were then stored at -80°C until use.

The two patients were volunteers accepting the treatment by pumpkin seed and betel nut in order to get live adult worms (Feng, 1956), and all work was approved by the Chinese Center for Disease Control and Prevention committees.

Total RNA isolation and small RNA preparation

Samples were weighted and grounded into fine powder with mortar in liquid nitrogen. Total RNA was isolated with Trizol reagent (Invitrogen) according to the manufacturer's protocol. Then total RNA was examined on 1% agarose gel, and the concentration was determined using a BioPhotometer (Eppendorf). The purified total RNA was stored at -80°C until use.

Novex 15% TBE-Urea gel (Invitrogen) was used for small RNA isolation according to the protocols reported previously (Lau et al., 2001; Chen et al., 2010a, b). The RNA fragments of 20-30 bases were reverse transcribed with a RT-PCR kit (Invitrogen) and purified with a 6% TBE PAGE gel. Then the purified small cDNA fragments were stored at -80°C until use.

High-throughput sequencing and computational analysis

High-throughput sequencing was performed with a Solexa sequencer at Huada Genomics Institute Co. Ltd, China. After removing adaptors, contamination of adaptor-adaptor, low quality sequences and reads smaller than 18 nt, total reads of the small cDNA fragments were obtained. Then non-coding RNAs were removed by search against GenBank and Rfam database (version 9.1) (<http://www.sanger.ac.uk/software/Rfam/mirna>). Next, conserved miRNAs were analyzed by using the Sanger miRBase (version 16.0) (Altschul et al., 1990; Chen et al., 2011a, b).

The genome of *Schistosoma japonicum* (<http://lifecenter.sgst.cn/schistosoma/cn>) was used as reference genome to analyze potential novel miRNAs. Clean reads were mapped to the reference genome using Short Oligo nucleotide Analysis Package (SOAP). The structures of novel miRNA candidates were predicted with Mfold (<http://www.bioinfo.rpi.edu/applications/mfold>) (Xu et al., 2010).

The copy numbers of conserved miRNAs of the two *Taenia* species were statistically analyzed to compare the expression difference. The value of fold change was obtained with the formula: $\log_2(\text{copy number of } T. \text{ solium} / \text{copy number of } T. \text{ asiatica})$; and P-value was calculated as described previously (Allen et al., 2005; Schwab et al., 2005; Xu et al., 2010).

RESULTS

Profile differences of short RNAs from the two cestodes

After Solexa deep sequencing and removing of 5' and 3' adaptors, as well as contamination formed by adaptor-adaptor ligation and low quality tags was removed, 18.59 and 15.54 million reads were obtained for *T. solium* and *T. asiatica*, respectively, with a total of 18.26 and 15.24 million high quality reads. Analysis of length distribution showed that there were some differences in the length distribution of miRNAs between *T. solium* and *T. asiatica*. In *T. solium*, the most abundant reads was 22 nt long with a percentage of 21.42%, and it was 16.86%, 14.14%, 13.71% and 7.53%, respectively for reads of 21, 20, 23 and 24 nt long (Figure 1A). While in *T. asiatica*, reads were significantly focused on 22 and 21 nt with percentages of 28.81% and 19.55% (Figure 1B).

After getting rid of reads smaller than 18 nt, there were 17.51 and 14.90 million clean reads for *T. solium* and *T. asiatica*, with 12.28 and 14.72 million unique reads, respectively. Among the clean reads, 78.63% and 85.79% (mapped to *S. japonicum*) were identified as non-coding sRNA. The total percentage of ncRNA was at nearly the same level between the two species. The known miRNAs were 1,931,115 (11.02%) in *T. solium* and 1,131,646 (7.06%) *T. asiatica*, with 140 (0.01%) and 135 (0.01%) unique reads, respectively (Table 1). However, from Table

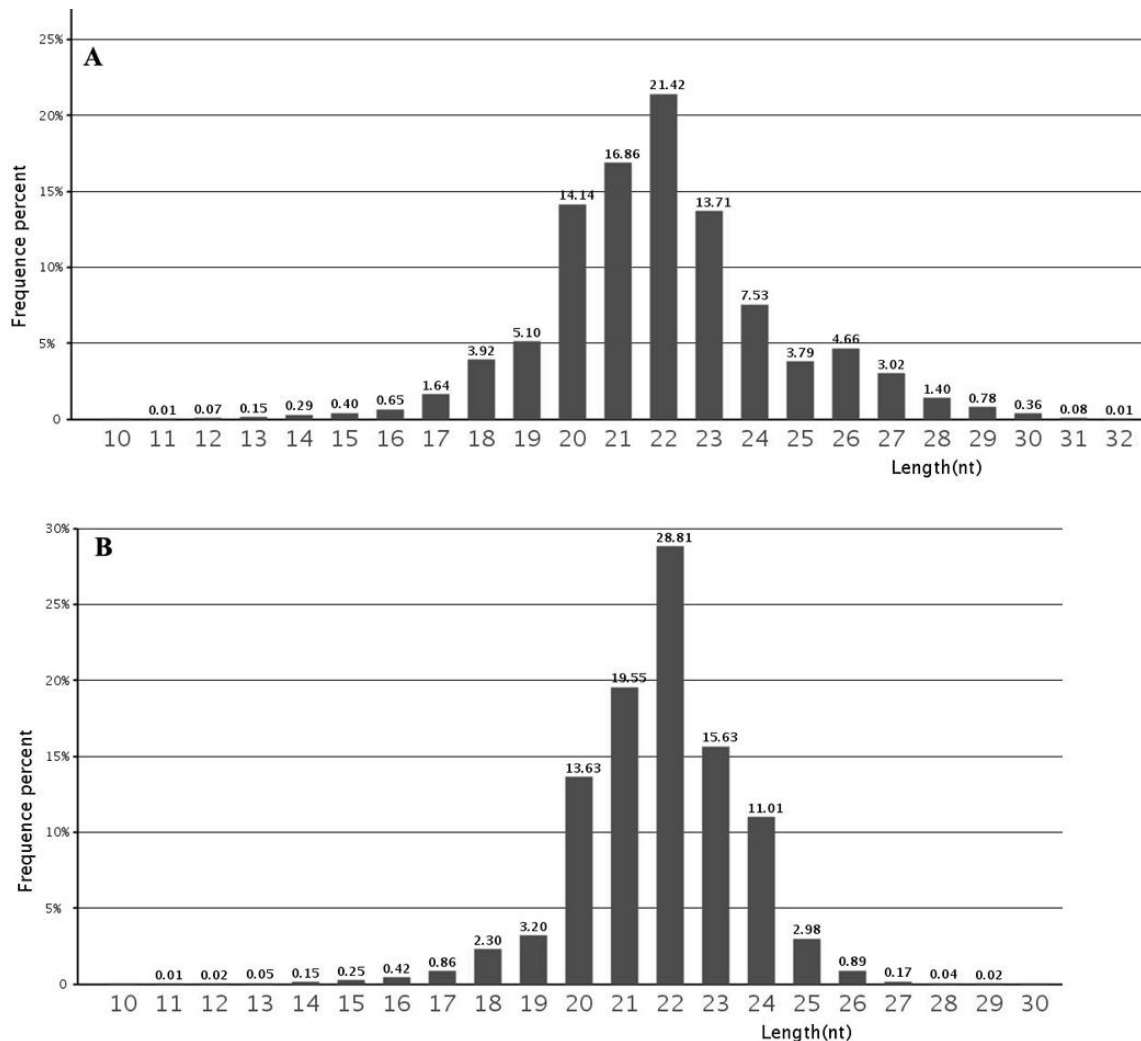


Figure 1. Length distribution of small RNA from *T. solium* and *T. asicatia* by Solexa deep sequencing (A: Length distribution of small RNA from *T. solium*; B: Length distribution of small RNA from *T. asicatia*).

1, the ratio of unique reads between them was the same, but there was big difference of total sRNAs. On the other hand, it indicated that *T. asicatia* is an independent *Taenia* species with *T. solium* except for miRNAs, the total percentage of other ncRNA in *T. solium* (8.97%) was nearly two fold than that in *T. asicatia* (4.97%), including rRNA (4.41%), snRNA (0.09%), snoRNA (0.00%) and tRNA (4.47%) in *T. solium* and 0.65%, 0.02% and 4.30% in *T. asicatia*, respectively. Except for ncRNAs mentioned above, there were 1.38 (78.63%) and 1.28 (85.79%) million of total reads not matched to the public database in each species, respectively, which were marked as unannotated reads (Table 1).

sRNAs distribution between *T. solium* and *T. asicatia*

A total of 32.41 million total reads for both *T. solium* and *T. asicatia* was analyzed. It was found that these two

cestodes shared 21.78 million sRNAs in common with a percentage of 67.21%, and only 14.47% (4.69 million reads) and 18.33% (5.94 million reads) were *T. solium* and *T. asicatia* specific, respectively (Table 2 and Figure 2A).

Out of 32.41 million total reads, 2.59 million reads was unique ones. Among the unique reads, only 4.35% (0.11 million unique reads) was shared between the two cestodes. Among the 2.59 million unique reads, 1.12 million was specific for *T. solium* with a percentage of 43.11%, while 1.36 million was specific for *T. asicatia* with a percentage of 52.54%. The unique ncRNA of *T. asicatia* specific was higher than that of *T. solium* specific (Table 2 and Figure 2B).

Expression difference of known miRNAs

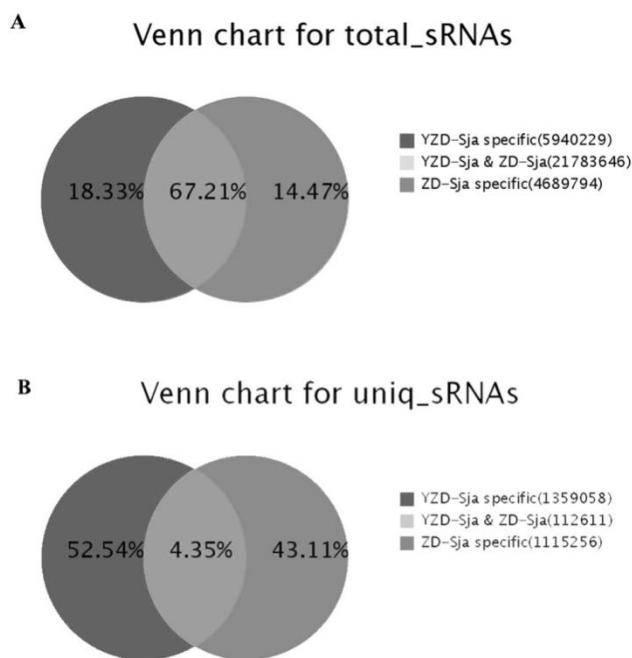
The expression level of known miRNAs in *T. solium* and

Table 1. Detailed classification of reads of *T. solium* and *T. asiatica*.

Classification	<i>T. solium</i>		<i>T. asiatica</i>	
	Total sRNAs	Unique sRNAs	Total sRNAs	Unique sRNAs
Total	17516306 (100%)	1227867 (100%)	14897363 (100%)	1471669 (100%)
miRNA	1931115 (11.02%)	140 (0.01%)	1131646 (7.60%)	135 (0.01%)
rRNA	772303 (4.41%)	38128 (3.11%)	96096 (0.65%)	16141 (1.10%)
snRNA	16471 (0.09%)	2510 (0.20%)	3424 (0.02%)	1410 (0.10%)
snoRNA	246 (0.00%)	169 (0.01%)	473 (0.00%)	275 (0.02%)
tRNA	783826 (4.47%)	27201 (2.22%)	639930 (4.30%)	16331 (1.11%)
unann	13773804 (78.63%)	1157298 (94.25%)	12781053 (85.79%)	1435737 (97.56%)

Table 2. Common and species-specific reads of *Taenia solium* and *Taenia asiatica*.

Classification	Unique sRNAs	Percentage (%)	Total sRNAs	Percentage (%)
Total sRNAs	2586925	100	32413669	100
Common reads	112611	4.35	21783646	67.21
<i>T. solium</i> specific	1115256	43.11	4689794	14.47
<i>T. asiatica</i> specific	1359058	52.54	5940229	18.33

**Figure 2.** Coverage of sRNA in *T. solium* and *T. asiatica* by Solexa sequencing (A: Coverage of sRNA in *T. solium*; B: Coverage of sRNA in *T. asiatica*).

T. asiatica was compared and the scatter plot figure is shown in Figure 3. When compared with *T. asiatica*, *miR-124-3p* was down-expressed (with green), and *miR-10-5p*, *miR-219-5p*, and *miR-7-5p* were up-expressed (with red) in *T. solium*. While *miR-219-5p*, *miR-277*, *miR-307*

and *miR-71* were expressed in a nearly equal level (with blue) in both species (Figure 3).

Among all known miRNAs of both species, 5 of them were expressed with copy numbers higher than 1000 (*miR-7-5p*, *miR-71*, *miR-277*, *miR-219-5p* and *miR-2b-3p*). In the miRNAs expression in *T. solium*, *miR-10-5p* was expressed with copy number higher than 100; *miR-307* and *miR-124-3p* were expressed with copy number fewer than 100. There were some differences in miRNAs of *T. asiatica*, *miR-307* and *miR-10-5p* have copy numbers fewer than 100. The copy number of *miR-124-3p* was higher than 100 in *T. asiatica*, which was only half of the number in *T. solium* (Table 3).

Among the differentially expressed miRNAs, 3 miRNAs (*miR-71*, *miR-7-5p* and *miR-219-5p*) were the most abundant miRNAs expressed in *T. solium*, with copy numbers higher than 100,000. Whereas, only 2 miRNAs of these miRNAs (*miR-71* and *miR-219-5p*) had copy numbers higher than 100,000 in *T. asiatica*.

Just as the phenomenon of *miR-124-3p*, the differential expressed miRNAs of the two parasites. In *T. asiatica*, this miRNA expressed higher than *T. solium*, which may indicate this miRNA will play more essential activity in the life cycle of *T. asiatica*.

Nucleotide bias analysis of miRNA profiles

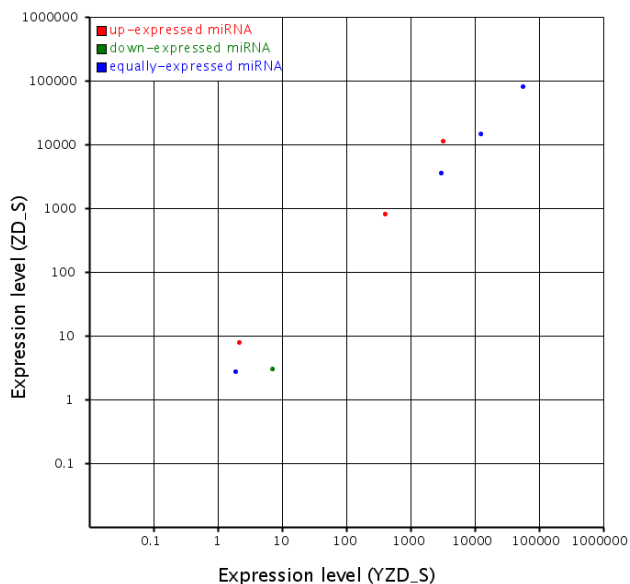
The two *Taenia* species had different characteristics in the first nucleotide. The nucleotide guanine (G) was the most frequently used first nucleotide in *T. solium* with a percentage of 57.40%, followed by U (27.97%) and A (11.30%), while C was seldomly used as the first

Table 3. Comparison of differential expressed miRNA in *Taenia solium* and *Taenia asiatica*.

miR name	<i>T. solium</i> express	<i>T. asiatica</i> express	<i>T. solium</i> std	<i>T. asiatica</i> std	Fold-change (log2 Ts/Ta)	P-value	Sig-label
miR-307	48	28	2.7403	1.8795	0.5439849	0.113507	
miR-7-5p	196737	48358	11231.649	3246.078	1.7908003	0	**
miR-71	1403680	844843	80135.62	56710.91	0.4988173	0	
miR-10-5p	138	32	7.8784	2.148	1.8749087	1.48E-13	**
miR-124-3p	52	107	2.9687	7.1825	-1.2746548	6.38E-08	**
miR-277	62523	44802	3569.4170	3007.3779	0.2471823	5.19E-170	
miR-219-5p	253761	187482	14487.13	12584.91	0.2030767	0	
miR-2b-3p	14162	5982	808.5038	401.5476	1.0096835	0	**

std: Normalized expression level of miRNA in a sample; Sig-label: significance label; **: fold change (log2)>1 or fold change (log2)<-1, and p-value <0.01).

Scatter plot (control:x | treatment:y)

**Figure 3.** Expression difference in known miRNAs between *T. solium* (ZD) and *T. asiatica* (YZD).

nucleotide (3.30%) (Appendix 1). For *T. asiatica*, the nucleotide uracil (U) was the most frequently used first nucleotide with a percentage of 46.84% followed by C (20.67%) and G (19.46%), while A was seldom used as the first nucleotide (13.03%) (Appendix 1).

The nucleotide bias for each position of known miRNAs was also different between *T. solium* and *T. asiatica* (Appendix 1). In *T. solium*, nucleotide A mainly was distributed at the 8-14th, 22th, and 23th positions. Nucleotide U was distributed with the highest percentages at the 16th (82.31%) and 4th positions (80.27%), and did not appear at the end of miRNAs. Altogether, (G+C) possessed a predominant percentage

at the middle of known miRNAs than (A+U), especially at the 3th and 8-11th positions which reached a percentage as high as more than 80%. Another interesting phenomenon was that the percentage of C was very low at all of the positions (1-24) with the percentage ranging from 0-9.30%, except at position 21 (26.89%). For *T. asiatica*, nucleotide A was mainly distributed at the end positions (18-24). Nucleotide U was distributed with the highest percentages at the 4th (70.87%) and 16th positions (70.86%). (A+U) possessed a predominant percentage at the end of known miRNAs than (C+G), especially at the positions of 22-24 nucleotides which reached a percentage as high as 100%.

Novel miRNAs in *T. solium* and *T. asiatica*

Due to the fact that no genome sequences of *Taenia* spp. are available at public database at present, the genome of the most related species *S. japonicum* was used as reference genome. A total of 2.90 (16.56%) and 1.71 (11.48%) million reads for *T. solium* and *T. asiatica*, respectively, were perfectly mapped, including 19,120 and 10,468 unique reads, respectively. The un-annotated unique reads that cannot match onto the *S. japonicum* genome were marked as potential novel candidates and used for novel miRNA prediction. Altogether, three novel miRNAs was found in *T. solium* and *T. asiatica*, respectively (Appendix 2 and 3).

DISCUSSION

In previous studies, genetic markers such as the first and second internal transcribed spacers (ITS-1, ITS-2) and mitochondrial cytochrome c oxidase subunit (*cox1*) have been used to reveal genetic differentiation between *T. solium* and *T. asiatica* (Jeon et al., 2011a, b; Nkouawa et al., 2009). Due to their sufficient differences in reproductive biology, host preference and gene sequences,

these two *Taenia* taxa are considered as separated species (Jeri et al., 2004; Jeon et al., 2011a, b; Nkouawa et al., 2009). *T. solium* and *T. asiatica* have complex life cycles and can be transmitted between pigs and humans. The present study compared the miRNA profiles between adult *T. solium* and *T. asiatica*. In different developmental stages of their life cycle, *Taenia* may express various miRNAs to regulate their gene expression, which warrants further studies.

The present study revealed that the two *Taenia* cestodes shared a very high percentage of total reads (67.21%), while with a very small percentage of unique reads (4.35%). This is possibly due to a high redundant expression of a few kinds of sRNAs, and these sRNAs might be essential for their fundamental metabolism. On the other hand, the total percentage of ncRNA was nearly at the same level in the two cestodes (19.99% in *T. solium* and 12.57% in *T. asiatica*).

Although, the expression of the predominant miRNAs (such as *miR-7-5p*, *miR-71*, *miR-277*, *miR-219-5p* and *miR-2b-3p*) were at the same level in the two *Taenia* taxa, which occupy the most copy numbers, some miRNAs were expressed differently between *T. solium* and *T. asiatica*, such as *miR-10-5p* in *T. solium* and *miR-124-3p* in *T. asiatica*. This phenomenon indicated that these two closely-related *Taenia* species are different in gene expression patterns. The two species also differ in the nucleotides in different positions of miRNAs.

Conclusions

The study represents the first characterization of *T. solium* and *T. asiatica* miRNAs, which will help to better understand, explore the complex biology of this zoonotic parasite and provide effective control methods for diseases caused by these important parasites. The reported data of *T. solium* and *T. asiatica* miRNAs should provide valuable references for further miRNA studies.

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REFERENCES

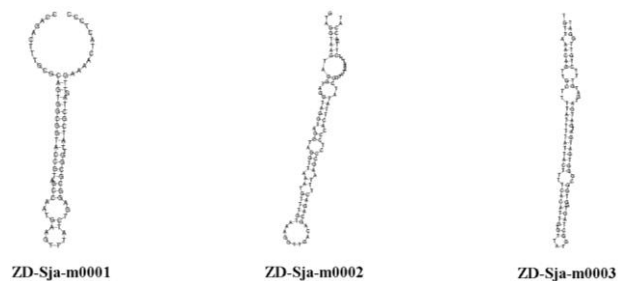
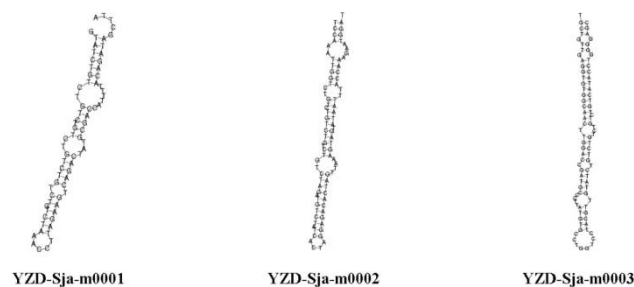
Afonso SM, Vaz Y, Neves L, Pondja A, Dias G, Willingham AL, Vilhena

- M, Duarte PC, Jost CC, Noormahomed EV (2011), Human and porcine *Taenia solium* infections in Mozambique: identifying research priorities. *Anim. Health Res. Rev.* 12: 123-129.
- Allen E, Xie Z, Gustafson AM, Carrington JC (2005). MicroRNA-directed phasing during trans-acting siRNA biogenesis in plants. *Cell* 121:207-221.
- Al-Sabi MN, Kapel CM (2011). Multiplex PCR identification of *Taenia* spp. in rodents and carnivores. *Parasitol. Res.* 109:1293-1298.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990), Basic local alignment search tool. *J. Mol. Biol.* 215:403-410.
- Anantaphruti MT, Yamasaki H, Nakao M, Waikagul J, Watthanakulpanich D, Nuamtanong S, Maipanich W, Pubampen S, Sanguankiat S, Muennoo C, Nakaya K, Sato MO, Sako Y, Okamoto M, Ito A (2007). Sympatric occurrence of *Taenia solium*, *T. saginata*, and *T. asiatica*, Thailand. *Emerg. Infect. Dis.* 13:1413-1416.
- Beaver PC, Jung RC, Cupp EW (1984), *Clinical Parasitology*. 9th edition. Lea & Febiger. Philadelphia.
- Beveridge I, Gregory GG (1976). The identification of *Taenia* species from Australian carnivores. *Aust. Vet. J.* 52:369-373.
- Bowles J, McManus DP (1994). Genetic characterization of the Asian *Taenia*, a newly described Taeniid cestode of Humans. *Am. J. Trop. Med. Hyg.* 50:33-44.
- Chen MX, Ai L, Xu MJ, Chen SH, Zhang YN, Guo J, Cai YC, Tian LG, Zhang LL, Zhu XQ, Chen JX (2011). Identification and characterization of microRNAs in *Trichinella spiralis* by comparison with *Brugia malayi* and *Caenorhabditis elegans*. *Parasitol. Res.* 109:553-558.
- Chen MX, Ai L, Xu MJ, Zhang RL, Chen SH, Zhang YN, Guo J, Cai YC, Tian LG, Zhang LL, Zhu XQ, Chen JX (2011). *Angiostrongylus cantonensis*: identification and characterization of microRNAs in male and female adults. *Exp. Parasitol.* 128:116-120.
- Eom KS, Jeon HK, Kong Y, Hwang UW, Yang Y, Li X, Xu L, Feng Z, Pawlowski ZS, Rim HJ (2002). Identification of *Taenia asiatica* in China: molecular, morphological, and epidemiological analysis of a Luzhai isolate. *J. Parasitol.* 88:758-764.
- Eom KS, Rim HJ (1993). Morphologic descriptions of *Taenia asiatica* sp. n. Korean. *J. Parasitol.* 31:1-6.
- Fan PC (1988), Taiwan taenia and taeniasis. *Parasitol. Today* 4:86-88.
- Feng LZ (1956), The research of using composition of pumpkin seed and betel nut to treat taeniasis. *Chinese Medical Journal*, 42:138-139 (Chinese).
- Garcia HH, Del Brutto OH, Cysticercosis Working Group in Peru (2005b). Neurocysticercosis: updated concepts about an old disease. *Lancet. Neurol.* 4:653-661.
- Garcia HH, Del Brutto OH, Nash TE, White AC Jr, Tsang VC, Gilman RH (2005a). New concepts in the diagnosis and management of neurocysticercosis (*Taenia solium*). *Am. J. Trop. Med. Hyg.* 72: 3-9.
- Hoberg EP, Jones A, Rausch RL, Eom KS, Gardner SL (2000), A phylogenetic hypothesis for species of the genus *Taenia* (Eucestoda: Taeniidae). *J. Parasitol.* 86:89-98.
- Ito A, Nakao N, Wandra T (2003). Human taeniasis and cysticercosis in Asia. *The Lancet* 362:1918-1920.
- Ito A, Okamoto M, Li T, Wandra T, Dharmawan NS, Swastika KI, Dekumyoy P, Kusolsuk T, Davvajav A, Davaasuren A, Dorjsuren T, Mekonnen SM, Negasi ZH, Yanagida T, Sako Y, Nakao M, Nakaya K, Lavikainen AJ, Nkouawa A, Mohammadzadeh T (2011). The first workshop towards the control of cestode zoonoses in Asia and Africa. *Parasit. Vectors* 4:114.
- Ito A, Wandra T, Yamasaki H, Nakao M, Sako Y, Nakaya K, Margono SS, Suroso T, Gauci C, Lightowers MW (2004). Cysticercosis/taeniasis in Asia and the Pacific. *Vector. Borne. Zoonotic. Dis.* 4:95-107.
- Jeon HK, Eom KS (2007). Complete sequence of the mitochondrial genome of *Taenia saginata*: comparison with *T. solium* and *T. asiatica*. *Parasitol. Int.* 56:243-246.
- Jeon HK, Kim KH, Eom KS (2011b), Molecular identification of *Taenia* specimens after long-term preservation in formalin. *Parasitol. Int.* 60:203-205.
- Jeon HK, Lee KH, Kim KH, Hwang UW, Eom KS (2005). Complete sequence and structure of mitochondrial genome of the human tapeworm, *Taenia asiatica* (Platyhelminthes; Cestoda). *Parasitol.* 130:717-726.

- Jeon HK, Yong TS, Sohn WM, Chai JY, Hong SJ, Han ET, Jeong HG, Chhakda T, Sinuon M, Socheat D, Eom KS (2011a). Molecular identification of taenia tapeworms by *cox1* gene in koh Kong, cambodia. *Korean J. Parasitol.* 49:195-197.
- Jeri C, Gilman RH, Lescano AG, Mayta H, Ramirez ME, Gonzalez AE, Nazerali R, Garcia HH (2004). Species identification after treatment for human taeniasis. *Lancet*, 363:949-950.
- Lau NC, Lim LP, Weinstein EG, Bartel DP (2001). An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 294:858-862.
- Murell KD (2005). Epidemiology of taeniasis and cysticercosis, In K. D. Murell (ed.), WHO/FAO/OIE guidelines for the surveillance, prevention and control of taeniasis/cysticercosis. OIE, Paris, France. 32-44.
- Nakao M, Okamoto M, Sako Y, Yamasaki H, Nakaya K, Ito A (2002). A phylogenetic hypothesis for the distribution of two genotypes of the pig tapeworm *Taenia solium* worldwide. *Parasitology*, 124: 657-662.
- Nkouawa A, Sako Y, Nakao M, Nakaya K, Ito A (2009). Loop-mediated isothermal amplification method for differentiation and rapid detection of *Taenia* species. *J. Clin. Microbiol.* 47:168-174.
- O'Neal S, Noh J, Wilkins P, Keene W, Lambert W, Anderson J, Compton Luman J, Townes J (2011), *Taenia solium* Tapeworm Infection, Oregon, 2006-2009. *Emerg. Infect. Dis.* 17:1030-1036.
- Proctor BM (1972). Identification of tapeworms. *South Afric. Med. J.* 46:234-238.
- Schwab R, Palatnik JF, Riester M, Schommer C, Schmid M, Weigel D (2005). Specific effects of microRNAs on the plant transcriptome. *Dev. Cell* 8:517-527.
- Sorvillo F, Wilkins P, Shafir S, Eberhard M (2011), Public health implications of cysticercosis acquired in the United States. *Emerg. Infect. Dis.* 17:1-6.
- WHO 1983. Guidelines for surveillance prevention and control of taeniasis/cysticercosis.
- Xu MJ, Liu Q, Nisbet AJ, Cai XQ, Yan C, Lin RQ, Yuan ZG, Song HQ, He XH, Zhu XQ (2010). Identification and characterization of microRNAs in *Clonorchis sinensis* of human health significance. *BMC. Genomics* 11:521.

Appendix 1. Nucleotide bias analysis of miRNA profiles of adult *T. solium* and *T. asicatia*.

	A%	U%	C%	G%	(A+U)%	(G+C)%
1	76.42/0	5.9/29.76	7.26/27.76	10.43/42.48	82.32/29.76	17.69/70.24
2	0/0	0/70.24	0/29.13	100/0.62	0/70.24	100/29.75
3	0/0	10.43/29.13	7.26/40.86	82.31/30.01	10.43/29.13	89.57/70.87
4	7.26/0	80.27/70.87	2.04/0	10.43/29.13	87.53/70.87	12.47/29.13
5	14.97/0	0/29.13	0/29.38	80.27/41.48	14.97/29.13	80.27/70.86
6	0/1.62	17.69/68.62	2.04/29.13	80.27/0.64	17.69/70.24	82.31/29.77
7	0/0	75.74/29.76	0/42.48	24.26/27.76	75.74/29.76	24.26/70.24
8	10.43/1.62	4.54/68.62	0/0	85.03/29.76	14.97/70.24	85.03/29.76
9	4.54/0	12.47/30.75	7.26/27.76	75.74/41.48	17.01/30.75	83.00/69.24
10	0/0	77.78/68.62	0/31.38	22.22/0	77.78/68.62	22.22/31.38
11	12.47/0	0/29.13	0/40.86	87.53/30.01	12.47/29.13	87.53/70.87
12	0/0	24.26/68.62	0/0	75.74/31.38	24.26/68.62	75.74/31.38
13	11.79/0	77.78/31.38	0/27.76	10.43/40.86	89.57/31.38	10.43/68.62
14	10.43/1.62	0/68.62	7.26/29.13	82.31/0.62	10.43/70.24	89.57/29.75
15	0/0	10.43/29.13	9.30/42.48	80.27/28.38	10.43/29.13	89.57/79.86
16	0/0	82.31/70.86	0/0	17.69/29.13	82.31/70.86	17.69/29.13
17	15.87/0	8.39/28.32	0/28.57	75.74/43.11	24.26/28.32	75.74/71.68
18	7.26/19.46	10.43/52.53	0/27.38	82.31/0.62	17.69/71.99	82.31/28.00
19	0/2.00	77.78/48.85	0/40.86	22.22/8.30	77.78/50.85	22.22/49.16
20	1.50/39.80	15.87/51.47	7.26/1.19	75.74/7.55	17.37/91.27	83.00/8.74
21	0/63.41	51.26/32.95	26.89/0	5.90/3.64	51.26/96.36	32.79/3.64
22	43.48/85.48	0/14.52	0/0	56.52/0	43.48/100	56.52/0
23	43.48/24.04	0/75.96	0/0	56.52/0	43.48/100	56.52/0
24	-/100	-/0	-/0	-/0	-/100	-/0

**Appendix 2.** Novel miRNA predicted in *T. solium*. The predicted stem-loop structures for the miRNA precursors of *T. solium*.**Appendix 3.** Novel miRNA predicted *T. asicatia*. The predicted stem-loop structures for the miRNA precursors of *T. asicatia*.