Journal of Biotechnology **18**(4): 643-652, 2020

PHYLOGENETIC ANALYSES OF RIBOSOMAL TRANSCRIPTION UNITS FROM *HAPLORCHIS TAICHUI* AND *H. PUMILIO* SPECIES OF HETEROPHYIDAE (PLATYHELMINTHES: OPISTHORCHIATA)

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Ngày nhận bài: 26.4.2020
Ngày nhận đăng: 10.6.2020

SUMMARY

Heterophyidiasis caused by minute intestinal flukes becomes of public concern in many countries worldwide. *Haplorchis taichui* and *H. pumilio*, belonging to the family Heterophyidae (Platyhelminthes: Trematoda) are two of many infecting humans and commonly found in Vietnam. Sequence study of these two small intestinal flukes is still very limited, hence we need more prospective markers for taxonomic identification and classification. This study provides complete coding sequence of the ribosomal transcription units (rTU) from *H. taichui* and *H. pumilio* (Vietnamese samples) and demonstrates the use of complete 28S rDNA sequences for phylogenetic analysis.The complete coding sequence of the rTU (from 5' 18S to 3' 28S), consisting of complete 18S, ITS-1, 5.8S, ITS2 and complete 28S rRNA genes and spacers, from *H. taichui* (7,268 bp) and *H. pumilio* (7,416 bp) from human hosts in Vietnam, were determined and annotated. The 18S and 5.8S genes of both species were of the same length (1,992 bp/18S, 160 bp/5.8S), but 28S genes differed (3,875 bp/*H. taichui* and 3,870 bp/*H. pumilio*). ITS-1 in *H. taichui* (797 bp) and ITS-2 in *H. pumilio* (280 bp) do not contain tandem repeat units (TRUs), while ITS-1 in *H. pumilio* (1,106 bp) contains 3 TRUs of 136 bp/each and 2 TRUs of 116 bp/each and ITS-2 in *H. taichui* (444 bp) contain 3 TRUs (83–85 bp/each). A phylogenetic tree inferred from the alignment of complete 28S rDNA sequences of 32 trematode strains/species, including 2 Vietnamese *Haplorchis* spp. and 24 species of 8 families in the suborders Xiphidiata (families Nanophyetidae, Paragonimidae, Collyriclidae), Opisthorchiata (Heterophyidae, Opisthorchiidae), and Echinostomata (Echinostomatidae, Fasciolidae). and *Schistosoma japonicum* of the family Schistosomatidae is used as an outgroup. The topology of the phylogenetic tree clearly confirmed the status of the Vietnamese *H. taichui* and *H. pumilio* species. These species gathered in a group (in the family Heterophyidae) clearly identified in the position of "sister" group to those in the family Opisthorchiidae (suborder Opisthorchiata, superfamily Opisthorchioidea).

Keywords: minute intestinal flukes, *Haplorchis pumilio*, *Haplorchis taichui*, phylogeny, ribosomal transcription unit, rTU, Vietnam.

INTRODUCTION

The superfamily Opisthorchioidea Looss, 1899 (Digenea) comprises a group of minute intestinal flukes of more than 60 common species globally distributed in dozens of countries around the world. Of epidemiological importance are the small intestinal trematodes

(heterophyids) in the genera *Haplorchis*, *Metagonimus*, *Stellantchasmus*, *Procerovum* and *Centrocestus* (Chai *et al.*, 2009; Chai, 2019). Genus *Haplorchis* of the family Heterophyidae Odhner, 1914 consists of 10 species including 3 species of the most influential human pathogens, *Haplorchis taichui*, *H. pumilio*, and *H. yokogawai* (Chai, Jung, 2017; 2020; Chai, 2019; Santos, Borges, 2020). Most infected people live in Asian countries, including Korea, China, Taiwan, Vietnam, Laos, Thailand, Malaysia, Indonesia, the Philippines, and India (Thaenkham *et al.*, 2011; Chai, Jung, 2020). Heterophyid species in Vietnam have well been described epidemiologically and morphologically, but molecular data useful for diagnosis and identification, as well as taxonomy, are still limited (Dung *et al.*, 2007; Van *et al.*, 2009; Le *et al.*, 2017).

Molecular genetic markers have greatly contributed to applied research in the fields of diagnosis, classification, phylogeny, evolution, epidemiology and population genetics (Hoelzer *et al.*, 2018). DNA sequences of the mitochondrial genome (mtDNA) and the nuclear ribosomal transcription unit (rTU or rDNA) are the source to provide the molecular markers for the above listed fields in parasitology research (Le *et al.* al., 2002; 2020; Heneberg, 2013; Hu, Gasser, 2006; Crampton-Platt, 2016). There is a great need, as well, to apply molecular approaches, especially to clarify the species/genus/family/inter-family and taxonomic relationships, intra and interspecific variation and polymorphism, especially for“sibling”, “synonymy”, "sister" and “adaptive” and “introgressive” hybrid species (Weider *et al*., 2005; Blair, 2006).

There are hundreds of ribosomal transcription units (rTU or rDNA) in a nuclear genome in animal cells (McStay, 2016). In trematodes, each unit is about 7–10 kb in length, consists of three coding regions, the 18S, 5.8S and 28S rDNA genes, and are separated by two internal transcribed spacer regions, ITS-1 and ITS-2. Sequentially repeated units arranged into hundreds of copies with the 28S gene being followed by a further non-transcribed intergenic spacer region (IGS) which connects one rTU to another, with the typical structure of the rTU as 5′ 18S-ITS1-5.8S-ITS2-28S-IGS 3′ (Zhao *et al.*, 2011; Cerqueira, Lemos, 2019; Qiu *et al.*, 2019).

In this study, we provide the sequence of near-complete ribosomal transcription units from *Haplorchis pumilio* and *H. taichui* (samples of Vietnam). We have determined the structural arrangement of the rTU and provide a detailed account of the characteristics of each ribosomal gene and the intergenic regions. We also provide a detailed comparative phylogenetic analysis of the complete 28S rDNA sequences, emphasizing their utility as molecular markers for molecular evolutionary studies of the family Heterophyidae in the suborder Opisthorchiata and the superfamily Opisthorchioidea.

Table 1. List and information of 32 strains/species providing the complete 28S rDNA sequences used for construction of phylogenetic tree to assess the relationship of species in the suborder Opisthorchiata (Platyhelminthes: Opisthorchioidea)

No	Family/Species	Length (bp)	Sequence designation	Country of isolation	Genbank accession Number
	Heterophyidae				
1	*Haplorchistaichui*	3875	Htai-QT3-VN	Vietnam	This study
2	*Haplorchispumilio*	3870	Hpum-HPU8-VN	Vietnam	This study
	Opisthorchiidae				
3	*Clonorchis sinensis*	3877	Csin-NH-VN	Vietnam	Not published

4	*Clonorchis sinensis*	3877	Csin-CSD-CN	China	MK450526
5	*Metorchis orientalis*	3878	Mori-MOB-CN	China	MK482052
6	*Metorchis orientalis*	3878	Mori-MOE-CN-28S	China	MK482055
7	*Opisthorchis felineus*	3877	Ofel-UstTula-RU	Russia	Not published
8	*Opisthorchis parageminus*	3877	Opar-PC6-VN	Vietnam	Not published
9	*Opisthorchis viverrini*	3877	Oviv-PY2-VN	Vietnam	Not published
	Collyriclidae				
10	*Collyriclum faba*	3867	Cfab-Orlicke-CZ	Czech	JQ231122
	Paragonimidae				
11	*Paragonimus heterotremus*	3881	Phet-LC-VN	Vietnam	Not published
12	*Paragonimus ohirai*	3881	Pohi-Kino-JP	Japan	Not published
13	*Paragonimus iloktsuenensis*	3881	Pilo-Amami-JP	Japan	Not published
14	*Paragonimus miyazakii*	3881	Pmiy-OkuST1-JP	Japan	Not published
15	*Paragonimus westermani*	3881	Pwes-Meghalaya(2n)-IN	India	Not published
16	*Paragonimus westermani*	3881	Pwes-Bogil(3n)-KR	South Korea	Not published
	Nanophyetidae				
17	*Nanophyetus japonensis*	3885	Njap-NJ142-JP	Japan	LT796170
18	*Nanophyetus japonensis*	3885	Njap-NJ161-JP	Japan	LT796169
19	*Nanophyetus salminicola*	3874	Nsal-Karp51-RU	Russia	LN871822
20	*Nanophyetus salminicola*	3874	Nsal-Karp55-RU	Russia	LN871823
21	*Nanophyetus schikhobalowi*	3886	Nsch-03Karp1442-RU	Russia	LN871820
	Echinostomatidae				
20	*Echinostoma malayanum*	3863	Emal-EMI3-TH	Thailand	Not published
21	*Echinostoma miyagawai*	3861	Emiy-RED11-TH	Thailand	Not published
22	*Echinostoma miyagawai*	3861	Emiy-HLJ-CN	China	Not published
23	*Echinostoma revolutum*	3863	Erev-MSD15-TH	Thailand	Not published
24	*Hypoderaeum conoideum*	3863	Hcon-RED42-TH	Thailand	Not published
25	*Isthmiophora hortensis*	3862	Ihor-Waka-JP	Japan	AB189982
	Fasciolidae				
26	*Fasciola gigantica*	3863	Fgig-NB-VN	Vietnam	MN970009
27	*Fasciola gigantica*	3863	Fgig-T4V-VN	Vietnam	MN970010
28	*Fasciola hepatica*	3863	Fhep-Geelong-AU	Australia	MN970007
29	*Fasciola* sp. (hybrid)	3863	Fsp(hyb)-DL11-VN	Vietnam	MN970008
30	*Fasciola (Fascioloides) jacksoni*	3863	Fjac-Madu-LK	Sri Lanka	MN970006
31	*Fasciolopsis buski*	3862	Fbus-HT-VN	Vietnam	MN970005
	Schistosomatidae				
32	*Schistosoma japonicum*	3924	Sjap-S15-PH*	Philippines	AY157607

Note: *Outgroup sequence (from *Schistosoma japonicum* (Schistosomatidae)).

MATERIALS AND METHODS

The samples of small intestinal flukes collected from patients in Quang Tri (Vietnam), including *H. taichui*, strain QT3, designated as Htai-QT3-VN, and *H. pumilio*, strain HPU8, designated as Hpum-HPU8- VN were used to sequence the ribosomal transcription unit. Samples were freshly frozen, or preserved in 70% alcohol, and stored at –20 °C until use. They were provided by Dr. Do Trung Dung (National Institute of Malariology Parasitology and Entomology) identified based on morphological characteristics (Dung *et al.*, 2007; 2013; De, Le, 2011) and confirmed by molecular sequence and phylogenetic analysis using *cox*1 to determine species relationships (Le *et al.*, 2017).

Genomic DNA extraction and primers

Total genomic DNA was extracted from individual cercariae, metacercariae or adult specimens using the GeneJET™ Genomic DNA Purification Kit (Thermo Fisher Scientific Inc., MA, USA), according to the manufacturer's instructions. Genomic DNA was eluted in 50 µL of the elution buffer provided in the kit and stored at −20 °C. The DNA concentration was estimated using a GBC UV/visible 911A spectrophotometer (GBC Scientific Equipment Pty. Ltd., Braeside VIC, Australia) and diluted to a working 50 ng/µL: 2 µL were used as template in a PCR of 50 µL volume.

All rTU-universal primers, used both for amplification and sequencing the rTU of H. pumilio and *H. taichui*, are listed in Table 2. Primers UD18SF/U3SR amplified the 18S and ITS-1 region and U3SF/1500R amplified the ITS-2 and 28S region. The primer pairs U18SF/U18SR and U28SF/U28SR, were used for obtaining major fragments of ribosomal 18S or 28S, respectively. These primers were also used as sequencing primers, as were additional internal primers (Table 2).

PCR amplification

PCR reactions of 50 µL were prepared using 25 µL of DreamTaq PCR Master Mix (2×) (Thermo Fisher Scientific Inc., MA, USA) and 2 µL DNA template (50 ng/µL), 2 µL of each primer (10 pmol/µL), 2 µL DMSO (dimethyl sulfoxide) and 17 µL H2O. All PCRs were performed in a MJ PTC-100 thermal cycler with initiation at 94 °C for 5 min, followed by 35 cycles consisting of denaturation for 30 s at 94 °C, annealing at 56 °C for 30 s, extension at 72 °C for 6 min; and a final extension at 72 °C for 10 min. The PCR products (10 µL of each) were examined on a 1% agarose gel, stained with ethidium bromide, and visualized under UV light (Wealtec, Sparks, NV, USA).

Sequencing and sequence analysis

The amplicons were eluted from the gel and subjected to direct sequencing by primer-walking in both directions. For the DNA fragment inserted into the recombinant plasmid, universal primers were used including M13F (5´GTAAAACGACGGCCAG 3´) and M13R (5´CAGGAAACAGCTATGAC3´), or internal designed primers for sequencing. The final sequences were obtained using GENEDOC2.7 and MEGA X (Kumar *et al.*, 2018). The entire rDNA sequence for each *Haplorchis* species was obtained after editing chromatograms (Chromas 2.6.6; http://technelysium.com.au/wp/chromas/) and 18S, 5.8S and 28S rRNA genes were determined by using the previously published reference sequences and those available in GenBank. These included *Clonorchis sinensis* (five isolates, GenBank: MK450523−MK450527; Qiu *et al.*, 2019) and *Metorchis orientalis* (five isolates, MK482051−MK482055; Qiu *et al.*, 2019). *Eurytrema pancreaticum* (5 isolates, GenBank: KY490000−KY490004; Su *et al.*, 2018). For intergenic regions, ITS-1 was recognized as located between 18S and 5.8S; ITS-2 as between 5.8S and 28S; and the IGS as between 3′ 28S and 5′ 18S sequences, respectively. Repeat units (RUs) were detected in the ITS-1 or ITS-2 or in IGS using the Tandem Repeat Finder v3.01 (Benson, 1999).

Phylogenetic construction

An alignment of 32 complete 28S rDNA sequences including 2 sequences from *H. taichui* (3,875 bp) and *H. pumilio* (3,780 bp),

respectively, and 29 strains of 23 species representing 8 families in the suborders Opisthorchiata (families Heterophyidae, Opisthorchiidae), Xiphidiata (Nanophyetidae, Paragonimidae, Collyriclidae), and Echinostomata (Echinostomatidae, Fasciolidae), (Table 1), was conducted using GENEDOC 2.7 (available at: http://iubio.bio.indiana.edu/soft/molbio/ibmpc/genedoc-readme.html). Also included in the alignment was *Schistosoma haematobium* (Schistosomatidae) as an outgroup. The alignment was trimmed to the length of the shortest sequence, saved in FASTA format and imported into the MEGA X software. To examine the phylogenetic position of the Vietnamese *Haplorchis* spp. relative to other trematodes, a phylogenetic tree was reconstructed (see list of sequences in Table 1) using Neighbor-Joining (NJ) analysis with the general time reversible (GTR) + G+ I model (gamma rate heterogeneity and a proportion of invariant sites). This model was given the best Bayesian information criterion score by MEGA. Confidence in each node was assessed using 1,000 bootstrap resamplings (Kumar *et al.*, 2018).

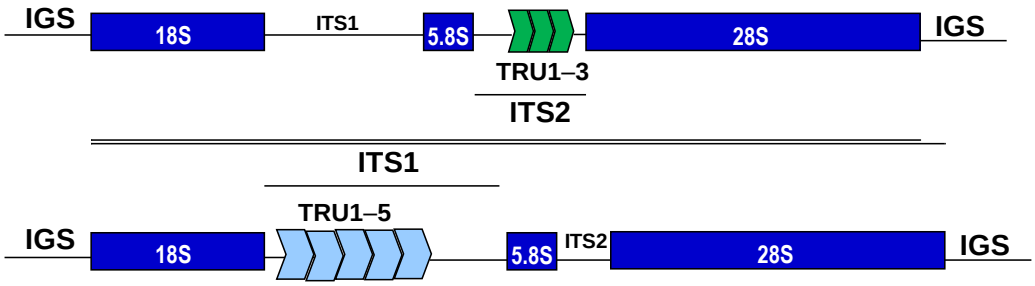
RESUTLS

Structural organization and characteristics of the ribosomal transcription unit of *Haplorchis pumilio* and *H. taichui*

Complete coding region of the ribosomal transcription units (rTU) from *H. taichui* and *H. pumilio* were determined. The coding region of rTU is 7,268 bp nucleotides in length for *H. taichui* (Htai-QT3-VN), and 7,416 bp nucleotides for *H. pumilio* (Hpum-HPU8-VN). These sequences have been deposited in GenBank under accession nos. KX815126 and KX815125, respectively. The IGS was not sequenced for both due to the highly repetitive sequences included in this region. The five regions of the rTU are: 18S, ITS-1, 5.8S, ITS-2 and 28S, structurally organized as usually seen in the ribosomal DNA operon of metazoans (Fig. 1).

In both *H. taichui* and *H. pumilio*, the 18S rRNA gene was 1,992 bp in length, and the 5.8S gene was 160 bp long; however, the 28S rRNA gene was determined as 3,875 bp from *H. taichui*, and 3,875 bp from *H. pumilio* (Table 2).

***Haplorchis taichui* (coding region, 7,268 bp)**



***Haplorchis pumilio* (coding region, 7,416 bp)**

Figure 1. Structural organization of the complete coding reion of the ribosomal transcription units for *Haplorchis taichui* and *H. pumilio*. TRU1-3 are the tandem repeat units in the ITS-2 region of *H. taichui*; TRU1-5 are the repeats in *H. pumilio.*

On the other hand, the length of ITS in each species is different. In *H. taichui*, the length of ITS-1 was 797 bp long, containing no tandem repeat unit, while in *H. pumilio* the length of ITS-1 was 1,106 bp containing 5 tandem repeat units (TRU) including 3 TRUs (TRU1–3) of 136 bp/each and 2 TRU (TRU4–5) of 116 bp/each.

The ITS-2 in *H. taichui* has a length of 444 bp containing tandem repeat units, of which 2 TRU (TRU1 and 3) have length of 85 bp/each, while TRU2 has only 83 bp. Additionally, in *H. pumilio*, ITS-2 of 280 bp does not contain any repeat.

Table 2. Position of ribosomal genes and internal transcribed spacers in the coding region of the transcription unit of *Haplorchis taichui* (Htai-QT3-VN) and *H. pumilio* (Hpum-HPU8-VN).

Gene/ Intergenic region	Position (5'->3')	Repeat	Size (bp)	Intergenic spacer (bp)	Note
***H. taichui* (7,268 bp)**					GenBank: KX815126
18S	1–1992		1992	0	rRNA gene
ITS-1	1993–2779		797	0	No tandem repeat
5.8S	2790–2949		160	0	rRNA gene
ITS-2	2950–3393		444	+121	121 bp to TR1
	3071–3155	TRU1	85	0	Tandem repeat
	3156–3238	TRU2	83	0	Tandem repeat
	3239–3323	TRU3	85	+70	Tandem repeat
28S	3394–7268		3875		rRNA gene
IGS	//		//		Not obtained
***H. pumilio* (7,416 bp)**					GenBank: KX815125
18S	1–1992		1992	0	Gen rRNA
ITS-1	1993–3098		1106	+66	66 bp to TRA1
	2059–2194	TRU1	136	0	Tandem repeat
	2195–2330	TRU2	136	0	Tandem repeat
	2331–2466	TRU3	136	0	Tandem repeat
	2467–2582	TRU4	116	+7	Tandem repeat
	2590–2705	TRU5	116	0	Tandem repeat
	2706–3098	Int seq.	393	0	Intergenic sequence
5.8S	3099–3258		160	0	rRNA gene
ITS-2	3259–3546		280	0	No tandem repeat
28S	3547–7416		3870		Gen rRNA
IGS	//		//		Not obtained

Note: TRU: tandem repeat unit.

Phylogenetic analysis

Complete 28S rDNA sequences obtained from Vietnamese *H. taichui* and *H. pumilio* were aligned with other 30 28S rDNA sequences representing 23 species in trematode in 8 families Heterophyidae, Opisthorchiidae, Nanophyetidae, Paragonimidae, Collyriclidae, Echinostomatidae, and Fasciolidae (Table 1). The alignment used was ~3,870 bp in length. The phylogenetic tree shown in Fig. 2 is based on the Neighbor-Joining (NJ) analysis with Maximum Composite Likelihood parameter. Bootstrap values are shown at relevant nodes. The alignment produced a well-supported phylogeny of eight families, illustrating that besides the outgroup (*S. japonicum*, family Schistosomatidae), there are 7 clusters placed in 3 suborders: i) Suborder Xiphidiata (families Paragonimidae, Nanophyetidae, Collyriclidae); ii) Suborder Opisthorchiata (families Opisthorchiidae, Heterophyidae): iii) Suborder Echinostomata (families Echinostomatidae, Fasciolidae). *H. taichui* and *H. pumilio* were in a group positioned as a "sister" monophyly with the opisthorchiids of the family Opisthorchiidae in the superfamily Opisthorchioidea (Fig. 2).

DISCUSSION

In this study, we have presented the coding sequence of the ribosomal transcription units (rTUs) for two common species of the family Heterophyidae, *Haplorchis taichui* and *H. pumilio* (3,785 bp and 3,780 bp, respectively), which infect humans in Vietnam; and used these complete 28S rDNA sequences for phylogenetic analysis to examine the taxonomic and family relationships among trematodes in the suborders Opisthorchiata, Xiphidiata, and Echinostomata. The obtained complete coding rDNA sequences encompass virtually the complete 18S gene (1,992 bp) and complete 28S gene (typical length about 3.78 to 3.785 kb). Also obtained were the complete ITS-1, 5.8S gene and ITS-1 and ITS-2 sequences for these species.

We have found tandem repetitive sequences arranged in the ITS-1 of *H. pumilio* and in the ITS-2 of *H. taichui*. ITS sequences of both species have been reported from Israel (Dzikowski *et al.*, 2004). The ITS-1 sequences differed substantially in length between Vietnamese and Israeli individuals of the same species, 797 versus 582 bp in *H. taichui* and 1,106 versus 640 bp in *H. pumilio*; due to differences in numbers of tandem repeats. The ITS-1 of the Vietnamese sequences contained five complete repeats, in strong contrast to the Israeli *H. pumilio* which possessed only two short tandem repeats (30 bp) in their ITS-1. These indicate intraspecific polymorphism found in geographical isolates as reported commonly in trematodes (Dzikowski *et al.*, 2004; Zheng *et al.*, Van *et al.*, 2009). Likewise ITS-2 of *H. taichui* also showed repetitive sequences. The presence ofrepeats in the internal transcribed spacers of trematodes has been reported for several taxa, including those in Fasciolidae, Heterophyidae, Opisthorchiidae, Paramphistomatidae, Schistosomatidae and others (Dzikowski *et al.*, 2004; Zheng *et al.*, Sato *et al.*, 2010; Tatonova *et al.*, 2012; Le *et al.*, 2020). The presence of repeats, variation in length and sequence variation, within and between species, all contribute to difficulties when trying to align ITS regions and this particularly is not suitable for deep-level phylogenetic analysis of species (Blair, 2006). In contrast, the alignment of the 18S and 28S rDNA sequences is generally straightforward, even among distantly related species, they are of considerable value for species identification, phylogenetic and evolutionary studies (Olson *et al.*, 2003; Thaenkham *et al.*, 2011; Tkach *et al.*, 2016; Le *et al.*, 2020)

The phylogenetic analyses of the complete 28S sequences indicate that the superfamily Opisthorchioidea presents clear systematic and taxonomic relationships for the families Heterophyidae and Opisthorchiidae, and well agreed with previous findings (Olson *et al.*, 2003; Thaenkham *et al.*, 2011; Tkach *et al.*, 2016; Le *et al.*, 2020). However, more species need to be examined using complete 28S rDNA combined with other markers. The Heterophyidae is too large and not a monophyletic and the entire superfamily Opisthorchioidea presents broad systematic and taxonomic challenges to be met in the future using combined morphological and molecular approaches (Thaenkham *et al.*, 2011; Le *et al.*, 2017).

CONCLUSION

In conclusion, the present study determined and annotated the coding sequence of the ribosomal transcription unit (rTU), consisting of complete 18S, ITS-1, 5,8S, ITS-2 and complete 28S rRNA genes and spacers, from *H. taichui* and *H. pumilio* in Vietnam. The ITS-2 in *H. taichui* and ITS-1 in *H. pumilio* contained tandem repeats. The 28S rDNA sequences are conserved among individuals within a species but variable between species and genera. Based on complete 28S rDNA, the sequence analysis of 32 sequences representing 24 trematode species in 8 families were clearly resolved. The family Heterophyidae containing *H. taichui* and *H. pumilio* in the phylogenetic tree is associated with Opisthorchiidae in a “sister” monophyletic position. The entire coding sequences of the rTU

provided here can be used for the diagnosis of heterophyid species in human and animal infections.

Acknowledgments. *This research is funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 108.02-2017.09 (PI: Thanh Hoa Le) and the PhD candidate (Le Thi Viet Ha) was the scientist participated in the research implementation.*

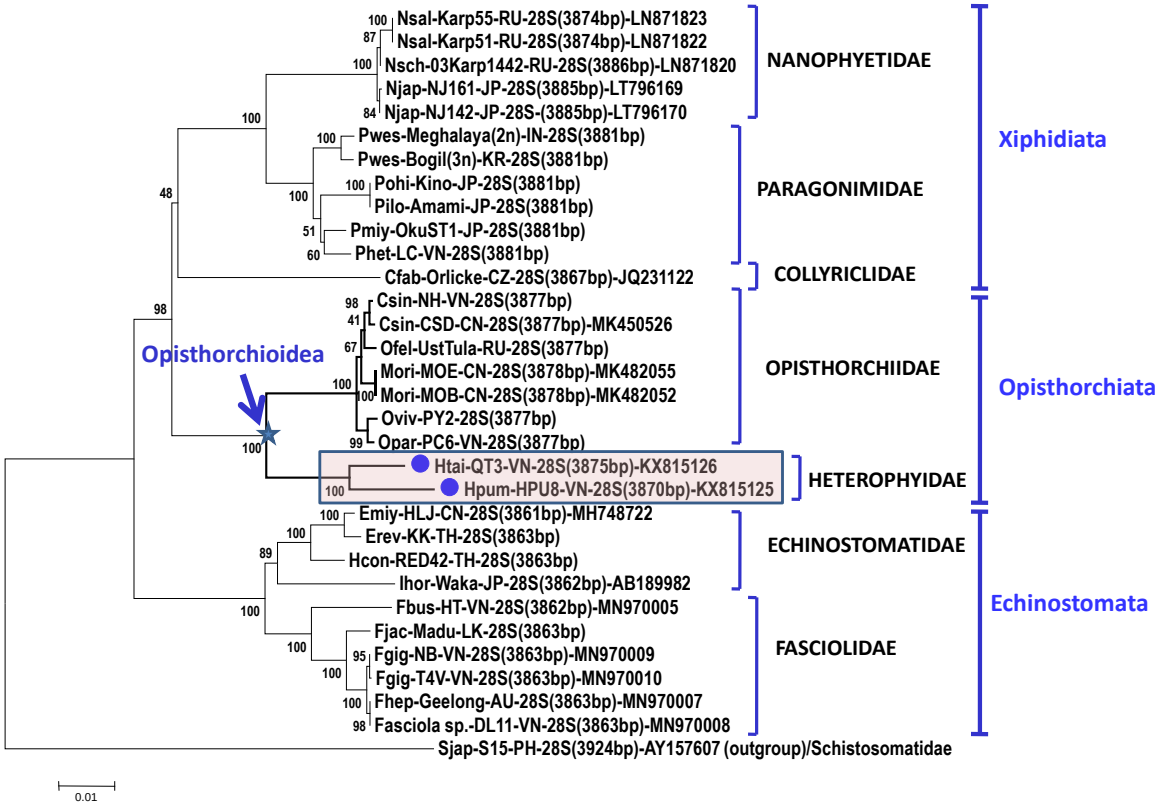


Figure 2. Phylogenetic tree showing the position of *Haplorchis taichui* and *H. pumilio* within the Heterophyidae and other trematodes based on the analysis of complete 28S rDNA sequences (~3.8–3.9 kb). *Schistosoma japonicum* (Schistosomatidae) was used as the outgroup taxon. The tree depicted was inferred using Neighbor-Joining (NJ) analysis with the general time reversible (GTR) + G + I model (gamma rate heterogeneity and a proportion of invariant sites) in the MEGA X package. Support for each node was evaluated using 1,000 bootstrap resamplings (Kumar *et al*., 2018). The Heterophyidae group (squared) formed from *H. taichui* and *H. pumilio* (indicated by a solid circle) is separately represented within the superfamily Opisthorchioidea (shown by a star and arrow at the basal node). Designated names of each species, followed by abbreviations/designation of strains, where available and country origin are provided. The 28S gene and its length (in bracket) are gien in the between and accession numbers (where available) at the end of each sequence. The scale-bar indicates the number of substitutions per site.

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PHÂN TÍCH PHẢ HỆ CỦA CÁC ĐƠN VỊ MÃ HÓA RIBOSOME TỪ CÁC LOÀI SÁN LÁ RUỘT NHỎ *HAPLORCHIS TAICHUI* VÀ *H. PUMILIO* THUỘC HỌ HETEROPHYIDAE (PLATYHELMINTHES: OPISTHORCHIATA)

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TÓM TẮT

Bệnh sán lá ruột nhỏ (heterophyidiasis) đang trở thành mối quan tâm của cộng đồng ở nhiều nước trên thế giới. *Haplorchis taichui* và *H. pumilio*, thuộc họ Heterophyidae (Trematoda: Platyhelminthes) là hai trong số nhiều loài lây nhiễm ở người và thường gặp ở Việt Nam. Nghiên cứu giải trình tự sán lá ruột nhỏ còn rất hạn chế, trong đó có hai loài phổ biến là *H. taichui* và *H. pumilio*. Vì vậy cần có thêm các chỉ thị di truyền phân tử tiềm năng để xác định và phân loại loài. Chúng tôi đã thu nhận toàn bộ phần mã hóa của đơn vị mã hóa ribosome (rTU hay rDNA) của các loài *H. taichui* và *H. pumilio* (mẫu Việt Nam) và phân tích phả hệ. Phần mã hóa của đơn vị mã hóa ribosome (từ 5’ 18S đến 3’ 28S) gồm 18S rDNA, ITS-1, 5.8S rDNA, ITS-2, 28S rDNA và các vùng giao gen, đã được xác định từ *H. taichui* (7.268 bp) và *H. pumilio* (7.416 bp) từ vật chủ người ở Việt Nam và chú thích. Các gen 18S và 5.8S của cả 2 loài có độ dài như nhau (1.992 bp/18S, 160 bp/5.8S), nhưng gen 28S có độ dài khác nhau (3.875 bp/*H. taichui* và 3.870 bp/*H. pumilio*). ITS-1 ở *H. taichui* (797 bp) và ITS-2 ở *H. pumilio* (280 bp) không chứa cấu trúc lặp, trong khi đó ITS-1 ở *H. pumilio* (1.106 bp) chứa 3 cấu trúc lặp liền kề TRU (136 bp/mỗi đơn vị) và 2 TRU (116 bp/mỗi đơn vị) và ITS-2 ở *H. taichui* (444 bp) chứa 3 TRU (83–85 bp/cấu trúc). Chuỗi nucleotide toàn phần của *H. taichui* và *H. pumilio* của Việt Nam được sắp xếp so sánh với 32 chuỗi 28S rDNA của 24 loài thuộc 8 họ trong các phân bộ Xiphidiata (Nanophyetidae, Paragonimidae, Collyriclidae), phân bộ Opisthorchiata (các họ Heterophyidae, Opisthorchiidae), phân bộ Echinostomata (các họ Echinostomatidae, Fasciolidae) và *Schistosoma japonicum* thuộc họ Schistosomatidae được sử dụng làm nhóm ngoại hợp. Cây phả hệ xác nhận rõ vị trí của các loài *H. taichui* và *H. pumilio* Việt Nam được tập hợp vào một nhóm (họ Heterophyidae) phân định rõ rệt ở vị trí “*chị em*” (sister group) với các loài ở họ Opisthorchiidae (phânbộ Opisthorchiata, liên họ Opisthorchioidea).

Từ khóa: Đơn vị mã hóa ribosome, *Haplorchis pumilio*, *Haplorchis taichui*, rTU, sán lá ruột nhỏ, phả hệ, Việt Nam.