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

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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 Echinostomatida (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, superfamly 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, Procercovum* 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, Metagonimus pumilio,* and *Stellantchasmus yokogawai* (Chai et al., 2009; 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., 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 Opisthoriata 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 Opisthoriata (Platyhelminthes: Opisthorchioidea)

| No | Family/Species           | Length (bp) | Sequence designation | Country of isolation | Genbank accession Number |
|----|--------------------------|-------------|----------------------|----------------------|----------------------------|
| 1  | *Haplorchistaichui*      | 3875        | Htai-QT3-VN          | Vietnam              | This study                 |
| 2  | *Haplorchispumilio*      | 3870        | Hpum-HPU8-VN         | Vietnam              | This study                 |
| 3  | *Clonorchis sinensis*    | 3877        | Csin-NH-VN           | Vietnam              | Not published              |
|   | Genus                      | Accession   | Country  | Location          |
|---|---------------------------|-------------|----------|-------------------|
| 4 | Clonorchis sinensis       | Csin-CSD-CN | China    | MK450526          |
| 5 | Metorchis orientalis      | Mori-MOB-CN | China    | MK482052          |
| 6 | Metorchis orientalis      | Mori-MOE-CN-28S | China    | MK482055          |
| 7 | Opisthorchis felineus     | Ofel-UstTula-RU | Russia  | Not published    |
| 8 | Opisthorchis parageminus  | Opar-PC6-VN | Vietnam  | Not published    |
| 9 | Opisthorchis viverrini    | Oviv-PY2-VN | Vietnam  | Not published    |
| 10| Collyriclidae             |             |          |                   |
| 11| Paragonimus heterotremus  | Phet-LC-VN  | Vietnam  | Not published    |
| 12| Paragonimus ohirai        | Pohi-Kino-JP | Japan   | Not published    |
| 13| Paragonimus iloktsuensis  | Pilo-Amami-JP | Japan   | Not published    |
| 14| Paragonimus miyazakii     | Pmiy-OkuST1-JP | Japan   | Not published    |
| 15| Paragonimus westermani    | Pwes-Meghalaya(2n)-IN | India  | Not published    |
| 16| Paragonimus westermani    | Pwes-Bogil(3n)-KR | South Korea | Not published |
| 17| Nanophyetidae             |             |          |                   |
| 18| Nanophyetus japonensis    | Njap-NJ142-JP | Japan   | LT796170          |
| 19| Nanophyetus japonensis    | Njap-NJ161-JP | Japan   | LT796169          |
| 20| Nanophyetus salminicola   | Nsal-Karp51-RU | Russia  | LN871822          |
| 21| Nanophyetus salminicola   | Nsal-Karp55-RU | Russia  | LN871823          |
| 22| Nanophyetus schikhobalowi| Nsch-03Karp1442-RU | Russia | LN871820          |
| 23| Echinostomatidae          |             |          |                   |
| 24| Echinostoma malayanum     | Emal-EMI3-TH | Thailand | Not published    |
| 25| Echinostoma miyagawai     | Emiy-RED11-TH | Thailand | Not published    |
| 26| Echinostoma miyagawai     | Emiy-HLJ-CN | China    | Not published    |
| 27| Echinostoma revolutum     | Erev-MSD15-TH | Thailand | Not published    |
| 28| Hypoderaeum conoideum     | Hcon-RED42-TH | Thailand | Not published    |
| 29| Isthmiophora hortensis    | Ihor-Waka-JP | Japan    | AB189982          |
| 30| Fasciolidae               |             |          |                   |
| 31| Fasciola gigantica        | Fgig-NB-VN  | Vietnam  | MN970009          |
| 32| Fasciola gigantica        | Fgig-T4V-VN | Vietnam  | MN970010          |
| 33| Fasciola hepatica         | Fhep-Geelong-AU | Australia | MN970007      |
| 34| Fasciola sp. (hybrid)     | Fsp(hyb)-DL11-VN | Vietnam  | MN970008          |
| 35| Fasciola (Fascioloides) jacksoni | Fjac-Madu-LK | Sri Lanka | MN970006          |
| 36| Fasciolopsis buski        | Fbus-HT-VN  | Vietnam  | MN970005          |
| 37| Schistosomatidae          |             |          |                   |
| 38| Schistosoma japonicum     | 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 *cox1* 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’CAGGAACACAGCTATGAC3’), 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).

RESULTS

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 (Hta-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).

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* (Htaiq-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) |                  |        |           |                        |      |
| 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        | Tandem repeat          |      |
|                        | 3156–3238        | TRU2   | 83        | Tandem repeat          |      |
|                        | 3239–3323        | TRU3   | 85        | Tandem repeat          |      |
| 28S                    | 3394–7268        | 3875   |           | rRNA gene              |      |
| IGS                    |                  |        |           |                        |      |

| **H. pumilio** (7,416 bp) |                  |        |           |                        |      |
| 18S                    | 1–1992           | 1992   | 0         | rRNA gene             |      |
| ITS-1                  | 1993–3098        | 1106   | +66       | 66 bp to TR1          |      |
|                        | 2059–2194        | TRU1   | 136       | Tandem repeat         |      |
|                        | 2195–2330        | TRU2   | 136       | Tandem repeat         |      |
|                        | 2331–2466        | TRU3   | 136       | Tandem repeat         |      |
|                        | 2467–2582        | TRU4   | 116       | Tandem repeat         |      |
|                        | 2590–2705        | TRU5   | 116       | Tandem repeat         |      |
|                        | 2706–3098        | Int seq.| 393      | Intergenic sequence   |      |
| 5.8S                   | 3099–3258        | 160    | 0         | rRNA gene             |      |
| ITS-2                  | 3259–3546        | 280    | 0         | No tandem repeat       |      |
| 28S                    | 3547–7416        | 3870   |           | rRNA gene             |      |
| IGS                    |                  |        |           |                        |      |

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 opisthorchids 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 of repeats 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.

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**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 given at the end of each sequence. The scale-bar indicates the number of substitutions per site.
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PHÂN TÍCH PHÁ HỆ CỦA CÁC ĐƠN VỊ MÃ HÓA RIBOSOME TỪ CÁC LOÀI SẢN LÀ RUỘT NHÓ HAPLORCHIS TAICHUI VÀ H. PUMILIO THUỘC HỘ HETEROXYDE (PLATYHEMINTHES: OPISTHORCHIATA)

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2Viện Công nghệ sinh học, Viện Hàn lâm Khoa học và Công nghệ Việt Nam
3Học viện Khoa học Kỹ thuật, Viện Hàn lâm Khoa học và Công nghệ Việt Nam

TÓM TÁT

Bệnh sán là ruột nhọ (heterophyidiasis) dang 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 gây nhiễm ở người và thú ở Việt Nam. Nghiên cứu giải trình tự sán là ruột nhọ còn rất hạn chế, trong đó có hai loài phổ biến là H. taichui và H. pumilio. Việc cần có thể giúp nghiên cứu phân tử để xác định và phân loại loài. Chúng tôi đã thu nhận toàn bộ phân tử của đơn vị mã hóa ribosome (rTU hay rDNA) của các loài H. taichui và H. pumilio (mẫu Việt Nam) và phân tích phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử. Phân tử 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 phân tử.

Từ khóa: Đơn vị mã hóa ribosome, Haplorchis pumilio, Haplorchis taichui, rTU, sản là ruột nhọ, pha thể, Việt Nam.