MOLECULAR EVOLUTIONARY RELATIONSHIPS OF VIETNAMESE AND GLOBAL PULMONARY *PARAGONIMUS* SPECIES IN THE FAMILY PARAGONIMIDAE AND SUBORDER XIPHIDIATA (PLATYHELMINTHES: TREMATODA)

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SUMMARY

Paragonimiasis, caused by *Paragonimus* species belonging to the family Paragonimidae (Platyhelminthes: Trematoda), often occurs in poor, upland, ethnic minorities, in Vietnam and the world. Asian *Paragonimus* species are distributed from Japan, South Korea, along with North and Southeast China, North-West and Central Vietnam, the Philippines, Thailand, Bangladesh, India, and Sri Lanka. There are various genetic variants, strains, and genotypes forming different complexes and evolutionary lineages. The 18S, 28S rDNA sequences and the intergenic transcribed spacer regions (ITS-1, ITS-2) of nuclear ribosomal transcription units are commonly used as molecular markers in genetic studies and phylogenetic analyses. We obtained a portion of 28S rDNA (domains D1–D3) of *Paragonimus* spp. including *P. heterotremus* (from Vietnam), *P. ohirai* (Japan), *P. iloktsuenensis* (Japan), and *P. westermani* (India and Vietnam) and conducted phylogenetic analysis for molecular evolutionary studies. The results showed that the family Paragonimidae formed the biggest cluster in a phylogenetic tree, which comprises of 46 sequences of 11 species belonging to 11 subgroups, among which the *P. westermani* complex of strains originating from China, Korea, Japan, India, Philippines, Malaysia, and Vietnam is present. *P. westermani* complex is arranged in a position of "sister" (sister group) with the subgroup *P. siamensis*. The *P. heterotremus* and *P. ohirai* complexes, and the *P. miyazakii*, *P. harinasutai*, *P. mexicanus*, *P. kelicotti*, and *P. macrorchis* complexes are clustered in a common population. *P. westermani* of Vietnam is in close proximity to the East Asian strains, as of which has been previously reported. *P. ohirai* and *P. iloktsuenensis* are considered “sibling” species, sharing the same clade. Phylogenetic analysis using the 28S rDNA sequences directly presented species position and their molecular evolutionary relationships in the families Paragonimidae, Troglopetmatidae, Nanophyetidae, and Collyriclidae. Evolutionary analysis has also clarified a number of complex delineation problems and made a clear nomenclature for *Paragonimus* sp. of Vietnam, in particular, which has scientific grounds merited to recognize as that it is really the *P. westermani* species.

**Keywords:** 28S rDNA/rRNA, ribosomal transcription unit, *Paragonimus* spp., *Paragonimus westermani*, phylogenetic, complex, evolution, Vietnam

INTRODUCTION

The genus *Paragonimus* of the epidemiologically, numerous species of Paragonimidae family, suborder Xiphidata veterinary and medical importance due to the
paragonimiasis disease, which often occurs in poor, upland, ethnic minorities in the remote mountainous areas, in Vietnam and the world. Infection rate is often very high in primary and secondary school children, as they have a habit of eating uncooked/undercooked infected crabs (Le et al., 2006; Blair et al., 2014; Yoshida et al., 2019). Paragonimiasis is one of the most common neglected, re-emerging diseases in many countries in Asia, Africa, and America. About 23 million people are infected and 292 millions are at risk of infection (Blair, 2014; Blair et al., 2016). Over 50 species are recognized as valid species, of which, about 10 species cause serious diseases in humans and are transmitted from animals, including P. westermani, P. heterotremus, P. ohirai, P. miyazakii, P. skrjabini, P. iloktsuenensis, P. kellicoti, and P. mexicanus. They are divided into the complexes of P. westermani, P. heterotremus, P. ohirai, P. skrjabini etc…, of which each complex has many different genotypes and strains (Blair, 2008; 2014; Blair et al., 2016). Paragonimiasis is also divided into groups according to geographical distribution: Asian group, African group, and American group (Procop, 2009; Blair, 2014; Blair et al., 2016; Cumberlidge et al., 2018; Le et al., 2019). Asian Paragonimus species have many different and complicated strains, distributed from Japan, Korea, along with North and Southeast China, North-west and Central Vietnam, the Philippines, Thailand, Bangladesh, India and Sri Lanka (Le et al., 2006; Blair, 2014; Sanpool et al., 2013; 2015; Blair et al., 2016; Doanh et al., 2013; Yoshida et al., 2019).

The ribosomal transcription unit (rTU) or simply rDNA positioned in the nuclear genome, they are of 7–10 kb for each in parasites, which contains three ribosomal coding genes (18S, 5.8S, and 28S rRNA genes) separated by two intergenic regions (internal transcribed spacer 1 and 2, that ITS-1 is between 18S and 5.8S; and ITS-2 between 5.8S and 28S rDNA, respectively). The rTUs are up to hundreds of units connected in arrays by nucleotide sequences containing many repetitive structures, called IGS (non-transcribed intergenic spacer). Each unit forms a characteristic structural frame: IGS-18S-ITS1-5.8S-ITS2-28S-IGS, and arranged in series. In the human genome, rTU is located in the secondary constriction, or NOR (nucleolar organizing region), in chromosomes 13, 14, 15, 21, and 22 (McStay, 2016). The ribosomal coding genes including 18S, 28S rRNA genes or/and the intergenic regions (ITS-1, ITS-2) are commonly used in the taxonomic analyses, taxonomic relationships, and species originality studies (Weider et al., 2005; Blair, 2006; Blair, 2014; 2016). The rTU molecular markers are also used in species identification and paternity identification for independent species, either for "exotic species" or "hybrid or introgressive hybridization" (Blair, 2014; Blair et al., 2016).

Up to now, there is not enough data of the entire ribosomal transcription unit (rDNA/rTU) for species in the family Paragonimidae and suborder Xiphidiata, especially for Paragonimus and some recently reported species in Vietnam (Doanh et al., 2009). For other members in Troglo tremata/Xiphidiata as well, there are limited complete rDNA data, although several distinct ribosomal genes and regions (18S, 28S or ITS regions) have been captured and used in diagnostic and epidemiological studies (Doanh et al., 2013). All of these sequences (18S, 28S, ITS-1, ITS-2) were used as molecular markers in the analysis of phylogeny, classification, and molecular evolutionary relationships between species (Weider et al., 2005; Blair, 2006; Pérez-Ponce de León, Hernández-Mena, 2019).

In this paper, we present the acquisition of partial 28S rDNA (domains D1–D3) of Paragonimus spp. including: P. heterotremus (Vietnam), P. ohirai (Japan), P. iloktsuenensis (Japan) and P. westermani (India and Vietnam) and some other species, including P. westermani (sample discovered in Vietnam); and used to analyze phylogenetic relationships between species to assess their molecular evolution of pulmonary flukes in the family Paragonimidae and suborder Xiphidiata.
MATERIALS AND METHODS

Parasite samples of species in the family Paragonimidae

The samples in this study were the adult pulmonary flukes and metacercariae which were identified by morphology and verified by molecular analysis. Samples were freshly frozen forms, either 70% ethanol preserved or genomic DNA, store at −20°C. Nine strains of 5 species of Paragonimus spp. in this study (Table 1), they are: 1) *Paragonimus heterotremus* (Vietnam) including 3 strains: strain LC, designated as Phete-LC-VN; strain D2YB, designated as Phete-D2YB-VN; strain D3YB, symbol Phete-D3YB-VN; 2) *Paragonimus westermani* including 3 strains: strain QT2 (Vietnam), designated as Pwest-QT2-VN; strain Meghalaya(2n) (India), designated as Pwest-Meghalaya(2n)-IN; strain Bogil(3n) (Korea), designated as Pwest-Bogil(3n)-KR; 3) *Paragonimus ohirai* (Japan) including 2 strains: strain Nagoya, designated as Pohir-Nagoya-JP; and strain Kochi, designated as Pohir-Kochi-JP; 4) *Paragonimus miyazakii* (Japan), strain OkuST1, designated as Pmiya-OkuST1-JP; 5) *Paragonimus iloktsuenensis* (Japan), strain Amami, designated as Pilok-Amami-JP.

Table 1. List and information of 56 strains/species providing the 28S rDNA sequences (D1–D3) used to construct a phylogenetic tree for analyzing the species relationship, determining the taxonomic position and molecular evolution of species in the suborder Xiphidiata (Trematoda: Platyhelminthes).

| No | Family/Species       | Abbreviation | Sequence designation | Country of isolation | Genbank accession No |
|----|----------------------|--------------|----------------------|----------------------|----------------------|
| 1  | *Collyriclum faba*   | Cfab         | Cfab-Orlicke-CZ       | Czech                | JQ231122             |
| 2  | *Paragonimus harinasutai* | Phari         | Phari-Nakorn-TH       | Thailand             | HM172616             |
| 3  | *Paragonimus heterotremus* | Phete         | Phete-(egg)-IN       | India                | DQ836249             |
| 4  | *Paragonimus westermani* | Phete         | Phete-(egg5)-IN      | India                | HM172615             |
| 5  | *Paragonimus miyazakii* | Phete         | Phete-D2YB-VN        | Vietnam              | This study           |
| 6  | *Paragonimus westermani* | Phete         | Phete-D3YB-VN        | Vietnam              | This study           |
| 7  | *Paragonimus westermani* | Phete         | Phete-sp2uz2017-VN   | Vietnam              | MK828944             |
| 8  | *Paragonimus westermani* | Phete         | Phete-L2017-VN       | Vietnam              | MK817556             |
| 9  | *Paragonimus westermani* | Phete         | Phete-LC-VN          | Vietnam              | This study           |
| 10 | *Paragonimus westermani* | Phete         | Phete-Manipur-IN     | India                | KF781294             |
| 11 | *Paragonimus westermani* | Phete         | Phete-PheteroChi-CN  | China                | HM172617             |
| 12 | *Paragonimus iloktsuenensis* | Pilok       | Pilok-CN              | Japan                | AY116875             |
| 13 | *Paragonimus westermani* | Pilok         | Pilok-Amami-JP       | Japan                | This study           |
| 14 | *Paragonimus kellicotti* | Pkell       | Pkell-Missouri-US     | United States        | HQ900670             |
| 15 | *Paragonimus macrorchis* | Pmacr        | Pmacr-Chanta-TH      | Thailand             | HM172618             |
| 16 | *Paragonimus mexicanus*  | Pmexi        | Pmexi-Concordia-EC   | Ecuador              | HM172619             |
| 17 | *Paragonimus miyazakii*  | Pmiya        | Pmiya-Kochi-JP       | Japan                | HM172620             |
| 18 | *Paragonimus miyazakii*  | Pmiya        | Pmiya-OkuST1-JP      | Japan                | This study           |
| 19 | *Paragonimus ohirai*    | Pohir        | Pohir-Kinosaki-JP    | Japan                | HM172621             |
| 20 | *Paragonimus miyazakii*  | Pohir        | Pohir-Nagoya-JP      | Japan                | This study           |
| 21 | *Paragonimus ohirai*    | Pohir        | Pohir-Kochi-JP       | Japan                | This study           |
|   | Species/Strain            | Accession  | Country   |   |
|---|--------------------------|------------|-----------|---|
| 22 | *Paragonimus pseudoheterotremus* | Ppseuhet   | Ppseuhet-(Llam)-TH | Thailand | HM004189 |
| 23 | *Paragonimus siamensis* | Psiam      | Psiam-m1Assam-IN | India   | JQ322628 |
| 24 | *Paragonimus siamensis* | Psiam      | Psiam-m2Assam-IN | India   | JQ322629 |
| 25 | *Paragonimus siamensis* | Psiam      | Psiam-m3Assam-IN | India   | JQ322630 |
| 26 | *Paragonimus siamensis* | Psiam      | Psiam-PspSLan12-LK | Sri Lanka | HM172624 |
| 27 | *Paragonimus westermani* | Pwest      | Pwest-Manipur-IN | India   | KF781290 |
| 28 | *Paragonimus westermani* | Pwest      | Pwest-Meghalaya(2n)-IN | India | This study |
| 29 | *Paragonimus westermani* | Pwest      | Pwest-Meghalaya(2n)-IN | India | DQ836244 |
| 30 | *Paragonimus westermani* | Pwest      | Pwest-mp18Pradesh-IN | India | JN656181 |
| 31 | *Paragonimus westermani* | Pwest      | Pwest-mp23Pradesh-IN | India | JN656180 |
| 32 | *Paragonimus westermani* | Pwest      | Pwest-mp27Pradesh-IN | India | JN656179 |
| 33 | *Paragonimus westermani* | Pwest      | Pwest-mt1Assam-IN | India   | JN656176 |
| 34 | *Paragonimus westermani* | Pwest      | Pwest-mt3Assam-IN | India   | JN656175 |
| 35 | *Paragonimus westermani* | Pwest      | Pwest-mt4Assam-IN | India   | JN656174 |
| 36 | *Paragonimus westermani* | Pwest      | Pwest-mt10Assam-IN | India   | JN656177 |
| 37 | *Paragonimus westermani* | Pwest      | Pwest-mt1Assam-IN | India   | JN656178 |
| 38 | *Paragonimus westermani* | Pwest      | Pwest-mt4Assam-IN | India   | JN656173 |
| 39 | *Paragonimus westermani* | Pwest      | Pwest-Pradesh-IN | India   | DQ836247 |
| 40 | *Paragonimus westermani* | Pwest      | Pwest-PwJpnMie3-JP | Japan   | HM172626 |
| 41 | *Paragonimus westermani* | Pwest      | Pwest-PwKor1(3n)-SR | South Korea | HM172627 |
| 42 | *Paragonimus westermani* | Pwest      | Pwest-PwLiguhe-CN | China   | HM172628 |
| 43 | *Paragonimus westermani* | Pwest      | Pwest-PwSorso4-PH | Philippines | HM172629 |
| 44 | *Paragonimus westermani* | Pwest      | Pwest-PwUruLan1-MY | Malaysia | HM172630 |
| 45 | *Paragonimus westermani* | Pwest      | Pwest-PwXigu4n-CN | China   | HM172631 |
| 46 | *Paragonimus westermani* | Pwest      | Pwest-QT2-VN | Vietnam | This study |
| 47 | *Paragonimus westermani* | Pwest      | Pwest-QT2-VN | Vietnam | This study |

| 48 | *Nanophyetus japonensis* | Njapo      | Njapo-NJ142-JP | Japan | LT796170 |
| 49 | *Nanophyetus japonensis* | Njapo      | Njapo-NJ161-JP | Japan | LT796169 |
| 50 | *Nanophyetus salminicola* | Nsalm      | Nsalm-Oregon-US | United States | AY116873 |
| 51 | *Nanophyetus salminicola* | Nsalm      | Nsalm-OK42-US | United States | MG806919 |
| 52 | *Nanophyetus salminicola* | Nsalm      | Nsalm-Karp55-RU | United States | MG806919 |
| 53 | *Nanophyetus schikhobalowi* | Nschi      | Nschi-03Karp1442-RU | Russia | LN871820 |
| 54 | *Nepthotrema truncatum* | Ntrun      | Ntrun-(adult) | N/A | AF151936 |
| 55 | *Skrjabinophyetus neomidis* | Sneom      | Sneom-UA | Ukraine | AF184252 |

| 56 | *Schistosoma haematobium* | Shaem      | Shaem-N10-ML* | Mali | AY157263 |

**Note:** Species/strain: Those in parentheses () are the numbers of species in a family; after the slash (/) is the number of strains providing nucleotide sequences for phylogenetic analysis in this study. *Outgroup sequence (from *Schistosoma haematobium*). N/A: not available.
**Total genomic DNA extraction**

Total genomic DNA was extracted from a ~10mg section of an adult worm using the GeneJET™ Genomic DNA Purification Kit (Thermo Scientific Inc., MA, USA) as instructed, eluted in 100 μL, and stored at −20°C until use. The concentration of DNA was estimated using a GBC UV/visible 911A spectrophotometer (GBC Scientific Equipment Pty. Ltd., Australia). A working concentration of 50 ng/μL was prepared and 2 μL of this used as a template for PCR in a 50 μL reaction volume.

**Primers used for acquisition of rDNA unit**

Each rDNA unit (5' 18S-ITS1-5.8S-ITS2-28S-IGS 3') has a length of about 7–8 to 9–10 kb, depending on each trematode species. There are some samples for which only the entire coding region, not the IGS region were obtained. The majority of the rTU universal primers used were previously reported and some were added for use in this study (Le et al., 2017; 2020; Le Thanh Hoa et al., 2019). PCR is applied with the combination of alternative primers to obtain different long and short DNA fragments.

**PCR amplification and sequencing**

PCR reactions of 50 μL were prepared using 25 μL of DreamTaq PCR Master Mix (2x) (Thermo Fisher Scientific Inc., MA, USA) and 2 μL of DNA template (50 ng/μL), 2 μL of each primer (10 pmol/μL), 2 μL DMSO (dimethyl sulphoxide) and 17 μL of water, performed in an MJ PTC-100 Thermal Cycler. Initiation was at 94°C for 5 min, followed by 35 cycles consisting of denaturation for 30 sec at 94°C, annealing at 52°C for 30 sec, 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). The amplicons were purified by GeneJET PCR Purification Kit; or the right band of the expected size was eluted by GeneJET Gel Extraction Kit (Thermo Fisher Scientific Inc.) if multiple bands present. The PCR products were sent to a service company for direct sequencing or primer-walking in both directions until the complete sequence for the whole fragment was obtained.

**Sequence and data processing, identifying rDNA characteristics of species**

The entire rDNA sequence for each species/isolate 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. The majority of the PCR products were directly sequenced; the overlapped sequences were compared and connected to obtain the entire rDNA coding sequence. For some species, only a fragment of 28S rDNA region (D1–D3 domains) of about 1,200–1,250 bp was obtained and used for comparative analysis and constructing phylogenetic tree.

**Phylogenetic analyses**

Fifty-six 28S rDNA sequences of ~1100 bp, comprising of 9 sequences from 5 Paragonimus species in this study including *P. westermani* of Vietnam (strain QT2, designated as *P.westernai QT2-VN*) and 46 sequences from species in the families of Collyriclididae, Paragonimidae, Nanophyetidae (Troglotrematidae) of the suborder Xiphidiata, and a sequence of *S. haematobium* as an outgroup, were aligned and used to perform phylogenetic analyses (Table 1). The final 28S rDNA alignment was composed of 56 species/isolates and MEGA X was used to perform Neighbor-Joining (NJ), Maximum Composite Likelihood replacement model phylogenetic reconstruction with 1000 bootstrap resamplings. MEGA X identified the general time-reversible GTR + G + I model (γ rate heterogeneity and a proportion of invariant sites) as the most appropriate model for phylogenetic reconstruction based on the lowest Bayesian information criterion score (Kumar et al., 2018).
RESULTS AND DISCUSSION

Taxonomic position and species relationship of *Paragonimus* species of Vietnam and the world, including strains of 5 species: *P. heterotremus* (Phete-LC-VN; Phete-D2YB-VN; and Phete-D3YB-VN); *P. westermani* (Pwest-QT2-VN and Pwest-Meghalaya(2n)-IN); *P.
ohirai (Pohir-Nagoya-JP and Pohir-Kochi-JP); P. miyazakii (Pniya-OkuST1-JP); and P. iloktsuenensis (Pilok-Amami-JP) have been identified in the phylogenetic tree well-positioned in the family Paragonimidae and suborder Xiphidiata (Fig. 1).

The tree in Fig. 1, besides the outgroup, clearly distinguished are 4 included families, Paragonimidae, Troglotrematidae, Nanophyetidae, and Collyriclidae, with clustered species for each family.

1. The largest one is the group of species of the family Paragonimidae, consisting of 46 sequences of 11 species in 11 subgroups, one of which is the P. westermani complex which includes strains originating from different countries, ie., China, Korea, Japan, India, Philippines, Malaysia, and Vietnam. The P. westermani complex arranged in the position "sister" group with the subgroup P. siamensis, including strains from India and Sri Lanka. The P. heterotremus complex and the P. ohirai complex and the P. miyazakii, P. harinasutai, P. mexicanus, P. kellicotti, and P. macrorchis complexes were arranged together. The P. westermani species (strain QT2) of Vietnam is close to the East Asian strains of P. westermani, as previously noted when analyzed by other investigators (Doanh et al., 2009). P. ohirai and P. iloktsuenensis are considered “sibling” species (Blair et al., 2016), they share the same branch in taxonomic position in the phylogenetic tree.

2. The second group of species belongs to the family Troglotrematidae, including 2 species of Skrjabinophytus neomidis and Nephrotrema truncatum. Troglotrematidae is a family of controversial grouping and their nomenclature is not yet to be elucidated (Blair, 2008).

3. The third group, recently named Nanophyetidae, seems to have all species of the genus Nanophyetus including strains N. japonensis, N. salminicola and N. schikhabalowi.

4. The fourth group is the Collyriclidae family, has only one species available for comparison, Collyriclum faba with 28S rDNA sequence in GenBank (JQ231122, Czech). Some of the sequences of Collyriclum spp. registered in Genbank are 18S rDNA sequences, not 28S rDNA.

The reference group selected for the tree as outgroup is Schistosoma haematobium (family: Schistosomatidae), completely separate, which supports the more precise classification of the Paragonimidae family on the phylogenetic tree of the suborder Xiphidiata (Fig. 1).

SOME DISCUSSION

Paragonimus spp. draw out attention with high diversity in morphology and genetics, forming relatively controversial complexes, so far to some extent, the exact taxonomic criteria have not been determined (Blair et al., 2016). In the family tree in Fig. 1, the family Paragonimidae is a collection of many separate groups formed from strains/species with wide and far geographical distribution. The 28S rDNA analysis has ensured a relatively accurate and reliable arrangement when handled by the method of "Neighbor-Joining" (NJ) or "Maximum Likelihood" (ML) in the MEGA X program with a bootstrap of 1000 resamplings (Kumar et al., 2018).

Species placement in the phylogenetic tree formed from analysis of 55 28S rDNA sequences of 17 species of 4 families in the suborder Xiphidiata in this study, excluding S. haematobium (outgroup), basically agreed with some studies of morphology classification and recent molecular analysis. Paragonimidae, previously imported in the family Troglotrematidae (Tkach et al., 2000; Olson et al., 2003), has recently been proposed to convert into an independent family of Paragonimus spp. in the taxonomic clarification; and likewise, the family Nanophyetidae comprising all the Nanophyetus spp. Such rearrangement of species and families have made a proposal of the change for suborder Troglotrema to suborder Xiphidiata (Blair et al., 2008; 2016; Ponce de
In this study, the family Paragonimidae is divided into different complexes including *P. westermani* complex, *P. heterotremus* and *P. ohirai* that they were recognized and identified in the same groups as indicated in the phylogenetic tree (Fig. 1). However, the suborder Xiphidiata replacing the suborder Trogloctrematidae might make some jumping positions for species since the suborder Xiphidiata is too large to cover morphologically and genetically distinct species (Ponce de León, Hernández-Mena, 2019). Categorization and taxonomic classification for taxa and subfamilies related to pulmonary flukes still have many issues to consider, but at least, the use of the nuclear ribosomal markers (18S and 28S rDNA) contributes to an increasingly clear identification of species and families, as is the case with species of the family Paragonimidae and other families in the suborder Xiphidiata.

Thus, the 28S rDNA marker extracted from the ribosomal transcription unit (rTU) data has been effectively utilized in molecular classification, in species relationship determination, and molecular evolution. It also provides scientific evidence for the nomenclature and reclassification of species/genera and families Paragonimidae, Trogloctrematidae, Nanophyetidae, and Collyriclidae in suborder Xiphidiata (Tkach et al., 2000; Olson et al., 2003; Ponce de León, Hernández-Mena, 2019). Especially, this study once again established a clear taxonomic position of *P. westermani* species of Vietnam (collected metacercaria samples from crabs in Quang Tri) together with the valid *P. westermani* species in the taxonomic classification system.

CONCLUSION

Phylogenetic tree and taxonomic position of 9 *Paragonimus* strains of 5 species, *P. heterotremus*, *P. westermani*, *P. ohirai*, *P. miyazakii* and *P. iloktsuenensis*, through analysis of 28S ribosomal markers and phylogeny along with 46 sequences of 17 species of the family Paragonimidae, Trogloctrematidae, Nanophyetidae and Collyriclidae in the suborder Xiphidiata were identified. Phylogenetic analysis using the 28S rDNA sequences showed clearly molecular evolutionary relationships of species in the family Paragonimidae, Trogloctrematidae, Nanophyetidae, and Collyriclidae and clarified a number of complex identification and nomenclature problems of *P. westermani* of Vietnam to recognize as that it is really *P. westermani* species.

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**QUAN HỆ TIÊN HÓA PHÂN TỬ SÁN LÁ PHỐI PARAGONIMUS VIỆT NAM VÀ THẾ GIỚI TRONG HỘ PARAGONIMIDAE VÀ PHẦN BỘ XIPHIDIATA (PLATYHELMINTHES: TREMATODA)**

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TỔM TÁT

Bệnh sán lá phổi (paragonimiasis) do *Paragonimus*, họ Paragonimidae, thuộc phân bộ Xiphidiata
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(Platyhelminthes: Trematoda) gây ra, thường xẩy ra trong cộng đồng nghèo, vùng cao, dân tộc thiểu số, ở Việt Nam và thế giới. Các loài *Paragonimus* của châu Á phân bố từ Nhật Bản, Hàn Quốc, dọc theo Bạc và Đông-Nam Trung Quốc, Tây-Bắc và miền Trung Việt Nam, Philippin, Thái Lan, Bangladesh, Ấn Độ và Sri Lanka. Sàn là phổ biến chúng di truyền, phát tán và tạo nên các phức hệ và đồng tiến hóa khác nhau. Các chuỗi gen 18S, 28S và vùng giao gen (ITS-1, ITS-2) trong đơn vị sao chép ribosome được sử dụng làm chỉ thị phân từ trong nghiên cứu di truyền và phân tích phát họ. Chúng tôi thu nhận một phân chuỗi gen 28S rRNA (vùng D1–D3) của các loài *Paragonimus* spp. gồm: *P. heterotremus* (Việt Nam), *P. ohirai* (Nhật Bản), *P. iloktsuenensis* (Nhật Bản) và *P. westermani* (Ấn Độ và Việt Nam) và xác lập cây phát họ phân tích tiến hóa phân từ. Kết quả cho thấy, trên cây phát họ tập hợp lóm nhất là các loài thuộc họ Paragonimidae, bao gồm 46 chuỗi của 11 loài thuộc 11 phân nhóm, trong đó phức hệ *P. westermani* gồm các chúng có nguồn gốc Trung Quốc, Hàn Quốc, Nhật Bản, Ấn Độ, Philippin, Malaysia và Việt Nam. Phức hệ *P. westermani* sắp xếp ở vị trí “chị em” (sister group) với phân nhóm *P. siamensis*. Phức hệ *P. heterotremus* và phức hệ *P. ohirai* và các loại *P. miyazakii*, *P. harinasutai*, *P. mexicanus*, *P. kellicotti*, *P. macrorchis* nằm trong một tập hợp chung, *P. westermani* của Việt Nam ở vị trí gần với các chúng Đông Á, như trước đây đã được ghi nhận. Hai loài *P. ohirai* và *P. iloktsuenensis* được coi là “đồng hình” (sibling species), chia sẻ cùng nền phân phát họ. Phân tích phát họ sử dụng chỉ thị 28S rDNA đã cho thấy mối quan hệ về loại và tiến hóa phân từ trong họ Paragonimidae, Troglocrematidae, Nanophyetidae và Collyriidae. Nhận diện chúng đã làm sáng tỏ một số vấn đề phân định phát họ và danh pháp của *P. westermani* của Việt Nam và có cơ sở để công nhận đó thực sự chính là loại *P. westermani*.

Từ khóa: 28S rDNA/rrRNA, đơn vị sao chép ribosome, *Paragonimus*, *Paragonimus westermani*, phát họ, phức hệ, tiến hóa, Việt Nam