GENETIC ANALYSIS OF *INDOPLANORBIS* AND *LYMNAEA* AND THEIR TREMATODE PARASITES IN THAILAND

ABDULHAKAM DUMIDAE

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy in Parasitology - (Type 1.1)
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Thesis entitled "Genetic analysis of *Indoplanorbis* and *Lymnaea* and their trematode parasites in Thailand"

By Abdulhakam Dumidae

has been approved by the Graduate School as partial fulfillment of the requirements

for the Doctor of Philosophy in Parasitology - (Type 1.1) of Naresuan University

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ABSTRACT

Indoplanorbis exustus and lymnaeid snails, a group of freshwater pulmonate snails, are widely distributed in tropical and subtropical zones. They play a significant role as the first intermediate host of trematode parasites that affect both human and livestock health. A full understanding of the genetic relationship of hosts and parasites is of paramount importance for effective parasite management. The goals of this study were to investigate the current transmission status of trematode cercariae in *I. exustus* and lymnaeid snails in Thailand and to examine the genetic diversity, genetic structure, and demographic history of these snails. In this study, 575 *Indoplanorbis* and 672 lymnaeid snails were collected from 56 locations in 27 provinces in six regions of Thailand. Subsequently, cercarial infection in the snails was observed by using the shedding method. *I. exustus* and lymnaeid snails released 5 types of trematode cercariae, namely, xiphidiocercariae, echinostome cercariae I, echinostome cercariae II, furcocercous cercariae, and strigea cercariae. The overall infection rate of cercariae in snails was 1.76% (22/1,247). The phylogenetic analysis based on ITS2 and 28S rDNA sequences revealed 5 cercaria types assigned to four trematode families, of which two belong to the group of human intestinal flukes. By incorporating shell morphology and sequence analysis of the mitochondrial COI and 16S rDNA genes, the lymnaeid snails were classified into two species, *Radix rubiginosa* and *Orientogalba viridis*.

Haplotype analysis of *R. rubiginosa* and *O. viridis* revealed only a few haplotypes were infected with cercariae. The genetic diversity and genetic structure revealed that *R. rubiginosa* and *O. viridis* experienced a bottleneck phenomenon and limited gene flow between populations. Population demographic history analyses revealed that *R. rubiginosa* and *O. viridis* experienced population reductions followed by recent population expansion. Meanwhile, phylogenetic and network analyses of *I. exustus* haplotypes based on sampled sequences from Thailand and a publicly accessible database of snails from other countries using the COI, 16S rDNA, and ITS1 genes demonstrated four main clades. Only snails in clade A were distributed in all regions of Thailand and harbored trematode cercariae. The level of genetic diversity of *I. exustus* in Thailand was relatively high, but most populations were not genetically different, thus suggesting the appearance of gene flow within the *I. exustus* populations. Overall, the haplotype network was star-shaped, thus suggesting the recent demographic expansion of populations. This result was also supported by the unimodal mode of the mismatch distribution graph and the large negative values of the neutrality tests. Therefore, the *I. exustus* snail was likely another freshwater snail of the invasive species in Thailand. These findings may improve our understanding of parasite-snails evolutionary relationships, as well as the underlying molecular genetic basis, which is information that can be used for further effective control of the spread of trematode disease.

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Abdulhakam Dumidae

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CHAPTER I

INTRODUCTION

Background and significance of the study

Trematodes, commonly referred to as flukes, constitute a group of parasitic flatworms that infest various hosts, including fishes, birds, wildlife, mammals, and humans (Schell, 1970). Trematodes are extensively distributed in numerous regions across the world, with a notable prevalence in Asian countries such as Korea, Laos, Cambodia, Myanmar, Vietnam, and Thailand. These parasites persist as significant public health issues in the region (Anucherngchai et al., 2016; Chontananarth et al., 2014; Doanh & Nawa, 2016; Hung et al., 2015; Sohn et al., 2014; Wongsawad & Wongsawad, 2010). Trematode infections can kill or impair the health of their vertebrate hosts, including humans (Suwannatrai et al., 2018).

Numerous trematode species have been documented as parasitic agents of human diseases, contributing to significant morbidities and mortalities in various regions worldwide (Chai, 2009, 2019; Hung et al., 2013). Especially, the foodborne trematode infections, i.e., *Paragonimus* spp., *Opisthorchis* spp., *Clonorchis* spp., *Fasciola* spp., *Fasciolopsis buski*, *Metagonimus* spp., and *Heterophyes* spp., are the cause of sick people with globally estimated to be 2.02 million (Torgerson et al., 2015). Moreover, a high prevalence of intestinal and liver flukes was reported in the local people in Southeast Asian countries (Chai et al., 2005; Chai et al., 2013; Krailas et al., 2014; Wongratanacheewin et al., 2001). Infections caused by liver flukes, especially *Opisthorchis viverrini* have a significant impact on public health and veterinary medicine. Infection occurs when humans or domestic animals consume raw, pickled, salted, or smoked fish carrying the infective stage, metacercariae (*Opisthorchis*), or raw aquatic plants infected with the infective metacercariae (*Fasciola* and *Fasciolopsis*) (Rohela et al., 2005; Sriamporn et al., 2004; Suwannahitatorn et al., 2019).

Fish-borne trematode infections are frequently found in people in rural areas of northern and northeastern Thailand (Pungpak et al., 1998; Radomyos et al., 1998). Additionally, *O. viverrini* or a liver fluke, poses a significant public health concern which associate with bile duct cancer (cholangiocarcinoma or CCA) in the country

(Sripa et al., 2011; Suwannatrai et al., 2018). In 2010, approximately 6 million cases in Thailand are reported to be infected with *O. viverrini*, leading to diagnoses of hepatobiliary diseases and cholangiocarcinoma due to chronic infection (Shin et al., 2010). *Haplorchis taichui* is among the intestinal trematodes identified as the predominant species in the northeast and north of Thailand (Radomyos et al., 1998; Srisawangwong & Tesana, 1997). This worm is associated with heterophyiasis, leading to significantly high rates of eosinophilia, diarrhea, and abdominal pain (Kumchoo et al., 2005). Additionally, *H. taichui* infection can result in ulceration, mucosal hemorrhages, chronic inflammation, and potentially serve as for the cause of irritable bowel syndrome (IBS)-like symptoms (Sukontason et al., 2005; Watthanakulpanich et al., 2010). Human infections with *H. taichui* occur through eating of raw or improperly cooked cyprinoid fish harboring the infective metacercaria of this intestinal fluke. Cyprinoid fish, in particular, have been significantly recognized as the intermediate hosts for this fluke (Sukontason et al., 1999; Wongsawad et al., 2000). Presently, many Thai individuals continue to enjoy the consumption of traditional Thai dishes made from raw cyprinoid fish (Chuboon et al., 2005). Consequently, the infection rate of trematodiasis continues to be a persistent issue in Thailand (Krailas et al., 2014). In addition to its impact on humans, there are relatively high rates of echinostome trematode infections found in domestic ducks in central, northeastern, and northern Thailand. Free-range ducks in the rural communities of the country are an important role for local economy, contributing to egg and meat production (Saijuntha et al., 2013). Furthermore, the infective larval stage of blood flukes, cercaria have been reported in Thailand including *Schistosoma spindale*, *S. incognitum*, and *Orientobilharzia harinasutai*, which have been known as an important blood fluke of domestic mammals and cause dermatitis in humans (Harinasuta & Kruatracrue, 1967; Harinasuta & Sornmani, 1965; Kruatrachue et al., 1965).

Trematodes undergo a complex life cycle that typically involves the utilization of two intermediate hosts for maturation and the completion of their life cycle. Trematodes necessitate the invertebrate animals (snails) as the intermediate host for the asexual reproduction of larval stages and a vertebrate animal as the definitive host for the sexual reproduction of adult trematodes (Poulin & Cribb, 2002). The majority of species follow a heteroxenous life cycle, utilizing mollusks as the first intermediate

host. The adult trematodes are discovered in a variety of vertebrate definitive hosts, including mammals, birds, reptiles, amphibians, and fishes (Hechinger & Lafferty, 2005). In brief, adult worms usually produce eggs after sexual reproduction in the definitive hosts. Eggs are released via feces, urine or sputum and reach freshwater. The miracidium stage hatches from the eggs and seeks out the mollusks as the first intermediate host, typically the fauna of freshwater snails (Bogitsh et al., 2019a; Jones & Cappello, 2004). In the snails, the parasite processes an asexual reproduction leading to a thousand number of larval trematodes, which then develop into cercaria stage. The cercaria release from the snail and penetrate second intermediate hosts and encyst develop to metacercariae infective stage. In contrast, in the schistosomes were found cercaria penetrate the definitive hosts directly (Keiser & Utzinger, 2009; Toledo & Fried, 2014).

The prevalence of trematodes is contingent on the existence of susceptible intermediate hosts, and the eating behavior of the local residents (Radomyos et al., 1998). The transmission of trematodes is facilitated by favorable ecological conditions, often involving specific water sources such as irrigation canals for agricultural lands, and rivers (Anucherngchai et al., 2017). In Thailand, several freshwater snails have been identified as the first intermediate hosts for several trematode parasites. For instance, *Bithynia siamensis* is an intermediate host of Lecithodendriidae and Strigeidae, and *Melanoides tuberculata* is an intermediate host of Philophthalmidae and Heterophyidae (Anucherngchai et al., 2016; Chontananarth et al., 2017). Thus, larval flukes shed by snails could be used to assess environmental effects. However, only a few research have been concerned with the cercaria infectious potential in Thailand. For instances, Dechruksa et al. (2007) have identified 2 cercaria-types (parapleurolophocercous cercariae and xiphidiocercariae) in thiarid snails collected in Phitsanulok province, with the prevalence of 0.9%. Moreover, Chontananarth & Wongsawad (2013) have identified 9 cercarial types in the freshwater snails collected in Chiang Mail province with a prevalence of 17.27%. Recently, Dunghungzin & Chontananarth (2020) documented the presence of five types of cercariae in snails from three provinces in the central region of Thailand, with an overall prevalence of 2.45%.

Detection and identification of cercaria by conventional methods have traditionally focused only on morphological characteristics. Morphological taxonomy

of cercariae into species level can be challenging, given that cercariae are small, and their morphologies often resemble each other closely (Pearson & Ow-Yang, 1982). Thus, molecular analyses are the most reliable tools for identifying parasitic pathogens (Anucherngchai et al., 2016; Barber et al., 2000; Chontananarth et al., 2017; Sripalwit et al., 2015; Wongsawad et al., 2017). Sequencing and genetic studies of trematodes, utilizing genes in the mitochondria and nuclear have been employed to categorize, explore the evolution, and investigate the dispersal of these parasites. Recently, cytochrome c oxidase subunit I (COI) (Sanpool et al., 2015), cytochrome b (cytb) (Dao et al., 2017), small subunit (SSU) ribosomal RNA (18S rRNA and 28S rRNA) (Park, 2007), and ribosomal internal transcribed spacer 2 (ITS2) (Sanpool et al., 2015) have been utilized as the markers to examine the genetic variations of several trematodes. Of particular interest, ITS2 region is likely to be of value in the identification of various stages of trematodes at the species level (Morgan & Blair, 1995). In comparison, there have been only limited studies attempting to characterize cercariae in Thailand. For instance, (Anucherngchai et al., 2016) used the ITS2 region to study the phylogenetic relationship of cercarial stage trematodes from the Chao-Phraya Basin, which results in showing the phylogenetic tree of all cercarial stage trematodes separated into five groups. Subsequently, Chontananarth et al. (2017) revealed six families of the cercarial stage in Nakhon Nayok province using the same target nucleotide region, which included families Echinostomatidae, Philophthalmidae, Heterophyidae, Lecithodendriidae, Prothogonimidae, and Cyathocytylidae. Recently, Wiroonpan et al. (2021) reported the phylogenetic tree of cercarial trematodes isolated from freshwater snails from Bangkok, which revealed eight cercaria types in ten different families.

Thailand comprises several distinct geographic regions and encompasses diverse ecosystems (Kiguchi et al., 2021). Land use in several regions mainly for agriculture comprises rice grow cultivation and grows other crops such as vegetables and maize. Consequently, numerous canals were constructed to retain water, with several areas almost entirely traversed by canals branching off from the main river. Irrigation development in various regions has result contained several different kinds of water resources, including rice paddies, canals, dams, and rivers. These bodies of water provide appropriate environments for the growth of intermediate host snails (Anucherngchai et al., 2016; Chantima et al., 2013). Therefore, environmental changes

following agricultural activities lead to the abundance of intermediate hosts and the wide spread of parasites between the areas (Dunghungzin et al., 2017). In terms of snail families and their involvement in transmission trematodes, *Lymnaeidae* and *Planorbidae* hold the first two positions among the top ten (Monzon et al., 1993). Among these families have two genera known in Thailand including *Radix* spp. (syn. *Lymnaea*) (Lymnaeidae) and *Indoplanorbis exustus* (Planorbidae), which can be found widely distributed in Thailand. These snails typically attach themselves to aquatic plants in small pools, shallow reservoirs, and wetlands, including small canals and rice fields. Moreover, in the dry season, they can survive by burying themselves in mud (Liu et al., 2010). In Thailand, lymnaeid and *I. exustus* snails are significantly recognized as the first intermediate hosts for transmitting of blood flukes *Schistosoma incognitum* and *S. spindale,* as well as other medium intestinal or liver flukes such as *Echinostoma* spp., *Fasciola* spp., respectively (Anucherngchai et al., 2016; Bunnag et al., 1983; Papasarathorn et al., 1963; Srihakim & Pholpark, 1991). The *S. incognitum* and *S. spindale* cause intestinal schistosomiasis in mammals, especially cows, buffaloes, pigs, goats, and sheep (Agrawal & Southgate, 2000; Bunnag et al., 1983). In addition, lymnaeid and *I. exustus* snails have been associated with outbreaks of dermatitis caused by cercarial schistosome in human in Thailand (Bunnag et al., 1983; Kullavanijaya & Wongwaisayawan, 1993), Laos (Ditrich et al., 1992), and Malaysia (Palmieri et al., 1977).

In recent years, analysis of the nucleotide (DNA) sequencing has been employed to examine and elucidate the evolution of organisms with closely related on morphology (Mouahid et al., 2018; Standley et al., 2014; Zeng et al., 2017). Use of DNA sequence data for identifying snails could prove valuable for their taxonomic status (Ali et al., 2015; Colgan et al., 2007). Recently, phylogenetic and molecular taxonomic approaches have been utilized to elucidate the relationships between lymnaeid species and accurate identification (Hunova et al., 2012; Schniebs et al., 2011). Al-Asadi (2021) conducted a bioinformatics analysis using nucleotide regions in cytochrome c oxidase subunit I (COI) in the mitochondria of the cell. The study uncovered that *Radix auricularia* snails in the AL-Sewaib River in Basrah province of Iraq are more closely related to Iranian *R. auricularia* snails than to those found in Russia and European countries. The research also demonstrated genetic diversity in

R. auricularia snails gathered from the AL-Sewaib River, revealing six distinct forms. These forms exhibited variations in their morphological characteristics as well. For *I. exustus*, the phylogeny based on COI and 16S rDNA genes of this snail have been reported from the Middle East, Asia, and South-East Asia (Devkota et al., 2015; Gauffre-Autelin et al., 2017; Liu et al., 2010). All research studies showed four genetically distinct clades, indicating a notable genetic divergence among populations.

Molecular population studies provide insights into specific genetic structures, population formations, and genetic variations (Avise, 2000). Additionally, they can assist in identifying the impacts of various factors on a population, encompassing ecological, climate, geographical, environmental, and human activities (Bohonak, 1999; Byrne, 2008; Jin et al., 2008). While genetic analyses of snails serving as intermediates for trematodes have been extensively studied in other regions of the world, there is limited information available in Thailand. This is noteworthy, given that snail intermediate hosts are commonly distributed in the region and have significant impacts on human health. Furthermore, no research on the genetic relationship between trematode cercariae and snail intermediate hosts has been reported in the country. Hence, the main goals of the current research are to investigate the prevalence, analyze the phylogenetic tree of cercariae isolated from lymnaeid and *I. exustus*, and assess the genetic diversity of these snails in Thailand.

Purposes of the study

1. To survey cercariae in *Indoplanorbis exustus* and lymnaeid snails collected from Thailand.

2. To analyze nucleotide sequences and to construct phylogenetic tree based on the internal transcribed spacer 2 (ITS2) region and 28S ribosomal DNA gene (28S rDNA) of cercariae.

3. To analyze nucleotide sequences, phylogenetic tree, genetic diversity of the mitochondrial genes (COI and 16S rDNA), nuclear genes (18S rDNA and 28S rDNA), and nuclear ribosomal DNA internal transcribed spacer 1 (ITS1) of lymnaeid and *I. exustus*.

Scope of the study

This research is an experimental study that begins with the collection of intermediate host snails of trematodes including lymnaeid and *I. exustus* in each region of Thailand. The experiment was performed in the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand. The collected snails were preliminary identified based on external morphology of their shells. The individual freshwater snail samples were inspected for cercarial infections using the shedding method. Genomic DNA from snails and cercariae were extracted and subsequently amplified by polymerase chain reaction (PCR). The ITS2 and 28S rDNA from cercariae were sequenced and analyzed by bioinformatics software. Meanwhile, the mitochondrial COI and 16S rDNA genes and nuclear ribosomal genes (18S rDNA, 28S rDNA, and ITS1) were amplified for lymnaeid and *I. exustus* snails. Furthermore, analyses for the phylogeny, genetic diversity, haplotype network, and genetic structure of snail population were conducted to assess the molecular diversity of lymnaeid and *I. exustus* snails in Thailand.

CHAPTER II

RELATED WORKS AND STUDIES

Freshwater snails of medical and veterinary importance

Thailand is one of the countries that has a diversity of freshwater and brackish water mollusks. In total, approximately 170 and 96 species of mollusk species in freshwater and brackish water, respectively, belonging to 75 genera, 23 families, and 6 orders have been documented in several regions of the country. These are distributed across three subclasses: Prosobranchia, Opisthobranchia, and Pulmonata. Several species in eight families of freshwater snails act as the intermediate hosts for trematode parasites with over 30 species of medical and veterinary significance (Anucherngchai et al., 2016, 2017; Chontananarth & Wongsawad, 2013; Chuboon & Wongsawad, 2009; Dechruksa et al., 2007; Krailas et al., 2014; Kulsantiwong et al., 2015; Namsanor et al., 2015; Ukong et al., 2007; Veeravechsukij et al., 2018).

1. **Family Viviparidae**

Genus *Filopaludina*

The morphology of this genus is the shell comparatively small with rather thin. The color of the shell is green. It has a green periderm and strong brown colour bands on the upper whorls. The operculum is thin and has the color brownish (Brandt, 1974).

The *Filopaludina* is commonly found in the shallow water along edges of canals, reservoirs, lakes, marshes, irrigation works, rice fields, which often in great numbers (Piyatiratitivorakul & Boonchamoi, 2008). In Thailand, there are 2 species, which of medical and veterinary importance including *Filopaludina martensi* and *F. sumatrensis*, which are found in many regions of Thailand. These snails act as both first and secondary intermediate hosts of trematodes, especially intestinal flukes *Echinostoma* (Chai et al., 2011; Chantima et al., 2013; Noikong et al., 2014).

2. Family Ampullariidae

Genus *Pila*

The morphology of the shell is subglobose, surface smooth with strong greenish or olive brown. The operculum is flat and calcareous plate with a nacreous inner surface (Brandt, 1974).

The *Pila* is often found in freshwater marshes or moderately flowing runoffs. These snails serve as secondary intermediate hosts of trematodes, especially *Echinostoma malayanum* and *E. ilocanum* (Pariyanonda & Tesna, 1990; Sri-Aroon, 2011).

3. Family Bithyniidae

Genus *Bithynia*

The shell morphology is conic or ovate-conoidal and brownish in color with a wide umbilicus. The operculum is concentric with the spiral nucleus (Brandt, 1974; Chitramvong, 1992).

The *Bithynia* habitats in Thailand are generally found in rice fields, shallow, marshes, and temporary ponds (Ngern-klun et al., 2006). Two subspecies of *Bithynia* snails found in the country include *Bithynia siamensis goniomphalos* and *Bithynia siamensis siamensis*, which serve as the first intermediate hosts of an important liver fluke, *O. viverrini* (Brandt, 1974; Wykoff et al., 1965).

4. Family Pomatiopsidae

Genus *Tricula*

The shell morphology is glassy with an elongated and smooth surface. The umbilicus is closed or a narrow chink and thin operculum. A perture triangular with broadly rounded edges. There are tentacles elongated and broadly rounded at their tips (Brandt, 1974).

The genus *Tricula* is commonly found in mountain streamlets, primarily in densely vegetated and shaded areas (Gittenberger et al., 2020). Certain species of *Tricula* act as intermediate hosts for schistosomes, which have the potential to infect humans and other mammals. In Thailand, Kruatrachue et al. (1983) was found *Schistosoma sinensium* from the snail *Tricula bollingi* in northwest Thailand.

5. Family Nassariidae

Genus *Anentome*

The morphology of the shell is elongated ovate-conoidal, sometimes somewhat fusiform, straw-coloured or olive-brown; unicoloured or with 1-3 dark brown spiral band, one below the suture, one at the periphery and one on the base of the body whorl; rather solid, not translucent, with strong axial ribs (Brandt, 1974).

The habitat of snails is found in lakes and ponds (Brandt, 1974). In Thailand, only one species, *Anentome helena*, is important as the first intermediate host of the parasite of fish (cotylomicrocercous cercaria), blood parasite of vertebrate animals (furcocercous cercaria), and intestinal parasite of birds and amphibians (Virgulate cercaria) (Haruay & Piratae, 2019).

6. Family Thiaridae

Genus *Tarebia*

The morphology of the shell is greenish or brownish colour and slightly fusiform with elongate ovate-conoidal or turreted. The shell has strong axial ribs which are dissolved into two or three spiral rows of tubercle, and with distinct spiral grooves and ridges. The axial sculpture is obsolete on the lower half of the body whorl (Brandt, 1974).

The snail inhabits diverse environments, ranging from lakes, rivers, and irrigation canals (Appleton et al., 2009). In Thailand, only one species of snail in this family, *Tarebia granifera*, is important as the first intermediate host of several species of the intestinal trematodes, i.e., *Centrocestus formosanus*, *Acanthatrium hitaense*, *Loxogenes liberum*, *Loxogenoides bicolor*, *Maritreminoides caridinae*, *M. obstipus*, *Stictodora tridactyla*, *Haematoloechus similis*, *Haplorchis taichui*, and *H. pumilio* (Anucherngchai et al., 2017; Dechruksa et al., 2007; Veeravechsukij et al., 2018).

Genus *Melanoides*

The morphology of the shell is elongate turreted with many whorls. An operculum is present with an oval in shape. The spire is long and consists of many whorls which increase slowly in diameter. The shell is sculptured with strong spiral grooves and axial ribs. The colour is brownish or olive and the shell is often ornated by brown flames and spiral bands (Brandt, 1974).

The snail can be found in a variety of habitats, including rivers, streams, ponds, and marshes, as well as anthropogenized aquatic settings including garden ponds, irrigation systems, and artificial lakes (Murray, 1975). In Thailand, only one species, *Melanoides tuberculata*, is clinically important as the first intermediate host of *Haplorchis taichui* and *H. pumilio* (Anucherngchai et al., 2017).

7. Family Lymnaeidae

Genus *Radix*

The shell of the genus *Radix* is thin, short, and ovate or ovoidal-conic in shape, which contains no operculum. The aperture is large, elliptical, and the peristome is thin and sharp (Brandt, 1974) (Figure 1). The snail is commonly habitat in freshwater lakes, ponds, and slow-moving rivers characterized by mud bottoms. *Radix* can live on vegetation or boulders, and they can of tolerating anoxic environments (Clarke, 1981; Jokinen, 1992; Sytsma, 2004). In Thailand, there are three species of medical and veterinary importance, namely *Radix rubiginosa* (syn. *Lymnaea rubiginosa*), *Radix swinhoei* (syn. *Lymnaea swinhoei*), *and Orientogalba viridis* (syn. *Austropeplea viridis*) (Brandt, 1974; Kaset et al., 2010). *Radix rubiginosa*, a small freshwater snail, has been reported as the first intermediate host for *Fasciola gigantica*, *Schistosoma incognitum*, *Trichobilharzia* sp., and *Echinostoma* (Bunnag et al., 1983; Japa et al., 2021; Kaset et al., 2010). Meanwhile, *R. swinhoei* and *O. viridis* have been documented as intermediate hosts for *Fasciola* spp. in neighboring countries such as China, Taiwan, Japan, and Vietnam (Dung et al., 2013; Itagaki et al., 1988; Li et al., 2004).

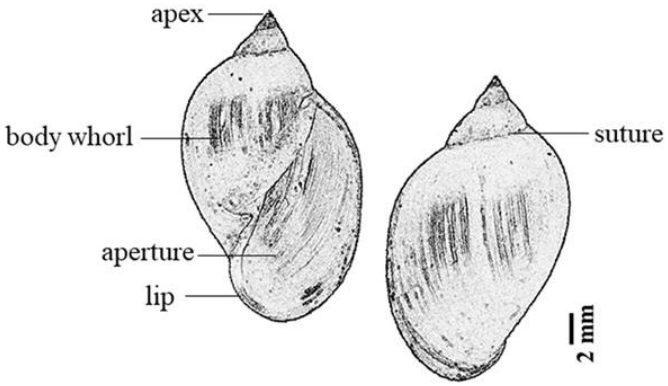

Figure 1 The external shell morphology of *Radix* spp.

Kaset et al. (2010) employed the 16S rDNA gene for molecular analysis of Lymnaeidae collected from various locations throughout Thailand. A phylogenetic tree of the lymnaeid 16S rDNA sequences were performed using Bayesian phylogenetic analysis. According to the findings, the Lymnaeidae family in Thailand may be divided into three clades: *Radix rubiginosa*, *R. swinhoei*, and *O. viridis*. Nevertheless, *R. swinhoei* clustered with European *Radix* and exhibited lower sequence conservation, with less than 80% identity. Hence, relying only on the analysis of 16S rDNA proved insufficient for accurately determining its taxonomic classification (Figure 2).

Hunova et al. (2012) in the Czech Republic analyzed the genetics of *Radix* based on sequencing of the ITS2 region. Sequence analysis of ITS2 region revealed that *Radix* snails clustered into four different groups including *R. auricularia*, *R. lagotis*, *R. labiate*, and *R. peregra*. Genetics with highest similarity was observed between *R. peregra* and *R. lagotis*, reaching 0.034. In contrast, *R. auricularia* has the most genetic differences between species.

Dung et al. (2013) documented the identification of *Radix* snails in Vietnam by employing a dual approach that incorporated both morphological and molecular methods. The use of the nucleotide sequences of the ITS2 region for molecular analyses divided this snail into three morphological types according to the ITS2 sequence lengths (type 1, 450 bp; type 2, 470 bp, and type 3, 451 bp). Type 1 exhibited a high similarity of 99% with *R. rubiginosa* according to BlASTn results, confirming its species identification. Type 2 was closest to *R. auricularia* with high identity with 99%. Similarly, the ITS2 sequence of type 3 showed the similarity 99% with sequences of *O. viridis*. In the phylogenetic tree based on this nucleotide sequence, types 1-3 from Vietnam were grouped with *R. rubiginosa*, *R. auricularia*, and *O. viridis*, respectively, supporting confirm their identity (Figure 3).

Lawton et al. (2015) used COI and ITS2 sequences to identify *R. auricularia* populations in the United Kingdom. The analysis of the COI and ITS2 revealed that both genetic markers can be differentiated species, but COI had more molecular diversity and higher phylogenetic resolution. When comparing COI and ITS2, the COI gene exhibited higher values for variable sites, nucleotide diversity, pairwise divergence, and specific substitutions. Therefore, COI sequences are useful for studies of the evolutionary relationships and genetic diversity of *Radix* populations.

Phylogenetic analysis of COI revealed that *R. auricularia* was segregated into three major groups, with clade 1 comprising *Radix* from mainland Europe and the United Kingdom. A second clade encompassed individuals from Tajikistan and Russia, while a final third clade consisted of a sequence from Armenia. For haplotype network analysis was found the United Kingdom snails were separated into two clades, which clade 1 is closely related to haplotype from Europe including Albania, Armenia, Croatia, France, Greece, Montenegro, Russia, and Spain. Clade 2 contained only all the United Kingdom haplotypes. Furthermore, haplotype network analysis indicated that the Russian and Tajikistan samples formed a distinct group separate from the European samples. These findings showed that *R. auricularia* had invaded the United Kingdom several times from mainland Europe.

Aksenova et al. (2017) reported molecular genetic analysis of *R. auricularia* in Lake Baikal regions in Siberia, as well as from other sites around Baikal Lake. This analysis utilized mitochondrial COI and nuclear ITS2 markers. Haplotype analysis unveiled sixteen haplotypes of *R. auricularia*, including a unity COI haplotype and two unique ITS2 haplotypes among ten samples collected from the Khakusy spring. Two COI haplotypes from the Khakusy spring were grouped with the Asian clade that included some haplotypes from the Lake Baikal region, as well as other from Siberia and the Russian Far East. Simultaneously, the average of the COI p-distance between the haplotype from the Khakusy spring sample and other haplotypes is 1.31 ± 0.35%.

Recently, Al-Asadi (2021) analyzed the bioinformatics of *R. auricularia* in the AL-Sewaib river in Basrah province of Iraq. Based on COI sequences between *R. auricularia* from Basrah and different countries, *Radix* sequences from Basrah showed 96-99% similarity with *R. auricularia* from Iran. On the other hand, COI sequences of *Radix* from Basrah showed low similarity (86-88%) with *R. auricularia* from European countries and Russia. The phylogenetic analysis uncovered a sole group closely associated with *R. auricularia* from Iran. This study suggests that *R. auricularia* specimens from Basrah, Iraq, exhibit a closer genetic relationship to those from Iran than to those from European countries and Russia.

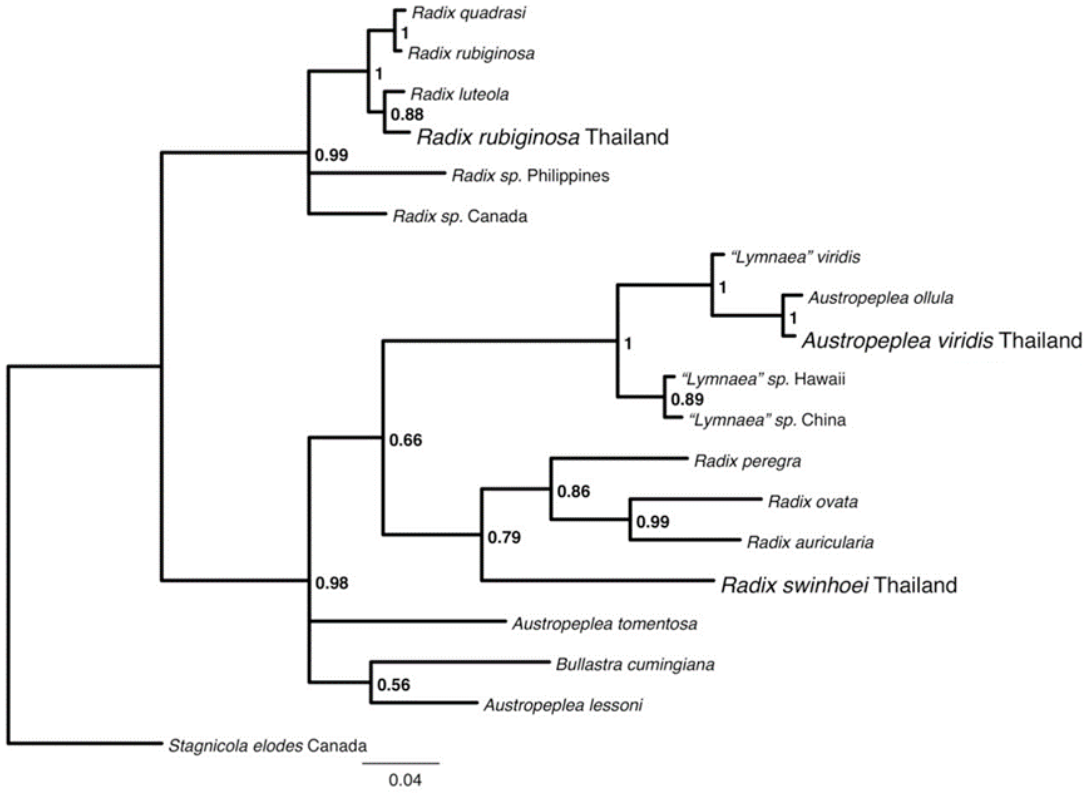

Figure 2 Phylogenetic tree of lymnaeid snails, constructed using Bayesian phylogenetic analysis with MrBayes, based on 16S rDNA sequences.

Source: Kaset et al., 2010

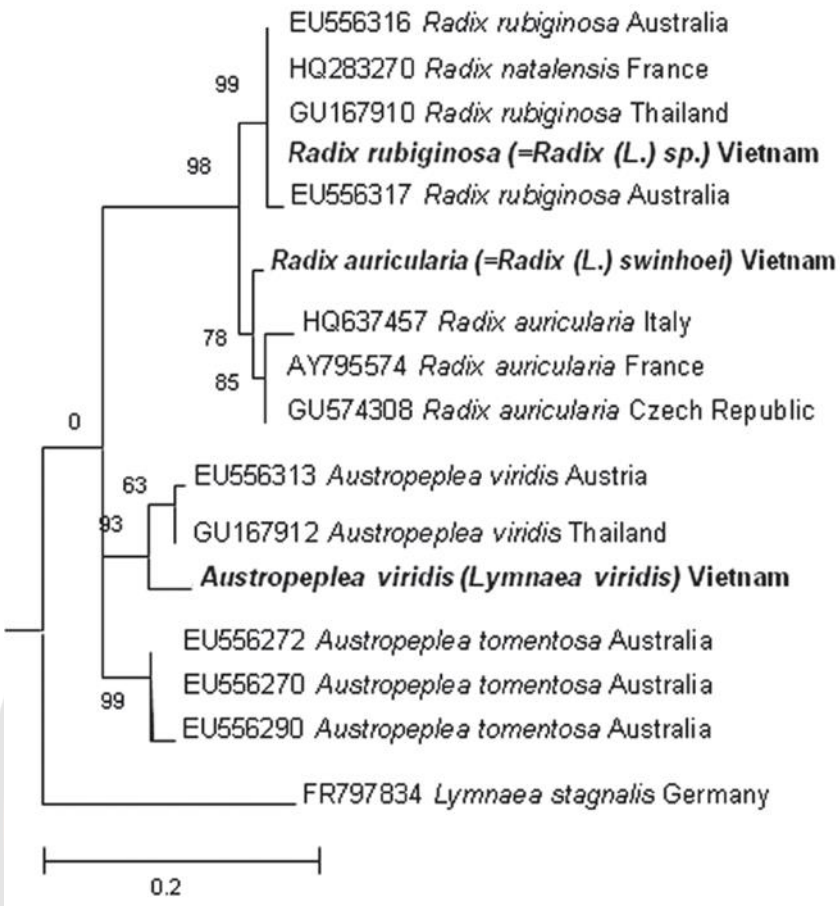

Figure 3 Phylogenetic tree of lymnaeid snails, constructed using maximum likelihood phylogenetic analysis with MEGA 5.2.2, based on ITS2 sequences.

Source: Dung et al., 2013

8. Family Planorbidae

Genus *Gyraulus*

The general morphology of the genus *Gyraulus*, the shell is rather small with few horizontally coiled whorls which are either regularly rounded and carinated at the periphery. All whorls can be seen from both sides. The shell surface with or without spiral lines (Brandt, 1974).

The habitat of snails lives on water plants in freshwater. In Thailand, only one species, *Gyraulus convexiusculus*, is important as the first intermediate host of the intestinal flukes *Echinostoma ilocanum* and *Paramphistoma* sp. (Brandt, 1974).

Genus *Indoplanorbis*

Indoplanorbis exustus is the sole species within the *Indoplanorbis* genus that has been formally categorized. The morphology of this species was found the shell is discoid in shape, dorso-ventrally flat with rapidly increasing whorls. Each whorl exhibits a height greater than its width (Figure 4) (Brandt, 1974; Brown, 1994; Kristensen & Ogunnowof, 1987).

The habitat of snails is found attached to water plants in paddy fields, pools, lakes, ponds as well as stagnant pools of water in reservoirs (Liu et al., 2010). Additionally, they may be present in pools formed during flooding in field areas, where they can endure dry seasons by burying themselves in mud. As a result, the dispersion may occur in mud clumps attached to the bodies of cattle or transport by birds (Bera, 2019; Liu et al., 2010). In Thailand, *I. exustus* is an important first intermediate host for several trematode species. It's known to harbor the larvae of *Schistosoma spindale*, a blood fluke that can cause cercarial dermatitis (Papasarathorn et al., 1963). Therefore, this snail is referred to as "hoy kunn" (itchy snail) by the rural populace in Thailand, as is *Lymnaea* (Brandt, 1974). It also acts as the first and second intermediate hosts for several flukes in family Echinostomatidae (Srisawangwong et al., 2004; Wiroonpan et al., 2021).

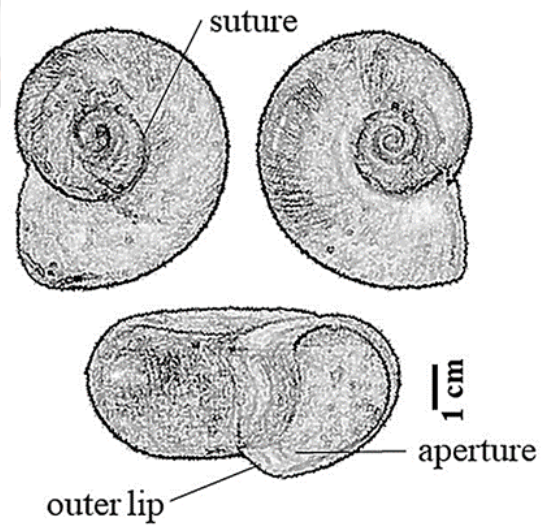

Figure 4 The external shell morphology of *I. exustus*.

Liu et al. (2010) documented the phylogeography of *I. exustus* across ten Asian countries, utilizing the COI and 16S rDNA genes. The results suggested that the spread of *I. exustus* began in the late Miocene, characterized by the split of an ancestral bulinine lineage into Assam and peninsular India clades. During the Late Pliocene, the clade of the Southeast Asian diverged from the clade of peninsular India, and subsequently, this clade spread at a significantly faster rate, leading to the colonization of a range of *Indoplanorbis* snails in the mid-Pleistocene. The estimated timescale for the expansion implies that the dispersal of *I. exustus* to Southeast Asia was probably aided by paleogeographical events and global warming.

Gauffre-Autelin et al. (2017) investigated the geographical distribution patterns and evolutionary history of *I. exustus* collected in Southeastern and Southern Asia, utilizing the COI gene. The phylogenetic tree revealed five distinct clades, which showed genetic distances between different clades ranging from 4.4 to 13.9%. The basal clade A comprises the snail samples from Nepal and clade B consists of samples from Nepal and Northern Myanmar. Clades C and D were geographically distributed in southern Laos and subcontinent of the Northern India, encompassing North India, Nepal, and Bangladesh, respectively. Lastly, clade E displays a wide distribution in the Indo-Malayan region, extending from the Indian subcontinent (Bangladesh, Nepal, India, and Sri Lanka) to Southeast Asia (Thailand, Philippines, Malaysia, Myanmar, and the Indonesian archipelago). The study findings propose that *I. exustus* probably started in the moist subtropical plains of Nepal or nearby southern regions during the Early Miocene. The divergence of the five clades of *I. exustus* is attributed to significant cladogenetic events and recurrent climate changes resulting from climatic oscillations.

Mouahid et al. (2018) reported genetic divergence of *I. exustus* snails collected from Africa and Guadeloupe (Frech West Indies) using five molecular markers, namely, COI, 16S rDNA, ITS1, ITS2, and 5.8S. The results of the COI analysis from 70 samples revealed 14 haplotypes and the phylogenetic tree indicated four distinct clades (A, B, C, and D) of *I. exustus* (Figure 5). Among the 14 haplotypes, several sequences were grouped in clade D, except for five specimens from Bangladesh that revealed five haplotypes and belonged to clade C. Meanwhile, the samples from South-East Asia were grouped to clade D, except the samples from Laos that were belonged to clades C and D. The snail samples from Oman were found three haplotypes,

and two of which were found in the same waterbody and one in two different water bodies. The samples from the Guinea Gulf (Gabon, Benin, and Ivory Coast) had a shared haplotype with the Batu Kajak and Borneo of Malaysia. For the snail samples from Guadeloupean were found two haplotypes, which were closely related to the haplotype from Sri Lanka. On the other hand, the samples from Nepal were very diverse and it was widely found dispersed to four clades of *I. exustus* snail. The nucleotide diversity of the COI sequences indicates that the percentages of intraclade diversity (0-5.33%) were lower than interclade diversity (7.39-14.09%), suggesting that these four clades represent distinct evolutionary groups. They highlighted that the strains identified in Africa and Guadeloupe all originate from Asia, belonging to a single clade that is widely distributed globally.

Recently, Saijuntha et al. (2021) documented the genetic diversity of *I. exustus* snail in South and Southeast Asia, utilizing the COI gene. Haplotype analysis of snails from 43 sites in South and Southeast Asia identified 42 haplotypes (Ie1-Ie42), with Ie1 being the most prevalent and widely distributed in Southeast Asia. Additionally, another frequently occurring haplotype was Ie4, identified in various areas in Thailand, while Ie7 haplotype was specifically located in Ayutthaya and Chiang Rai provinces. Other haplotypes, including Ie3 found exclusively in Phichit province, Ie21 and Ie22 identified in Satun province, and Ie24 and Ie42 observed in Cambodia and the Philippines, respectively. Haplotypes Ie28-Ie32 were exclusive to Bangladesh, and Ie33-Ie41 haplotypes were specifically identified in Sri Lanka within the South Asian region. Moreover, the haplotype network analysis can be divided into five haplogroups. Haplogroup I contained twenty-seven haplotypes (Ie1-Ie23, Ie25-Ie27, and Ie42) derived from samples across Southeast Asia, and haplogroup II consisted of nine haplotypes (Ie33-Ie41) derived from samples in Sri Lanka. Haplogroup III included only haplotype Ie24 found in Cambodia, and haplogroup IV comprised three haplotypes (Ie29, Ie31, and Ie32) originating from Bangladesh. Last group, haplogroup V contained two haplotypes (Ie28 and Ie30) found in the samples from Bangladesh. Also, the phylogenetic tree of this snail showed five major clades (A-E). Clade E is composed of two subclades (E1 and E2), with subclade E1 being the most widely distributed globally. Clade A, B, and C were specifically found only in South Asia, while clade D was found only in Southeast Asia. The distinction of

subclades and haplotypes of *I. exustus* snail indicates that this snail is a species complex with a global distribution.

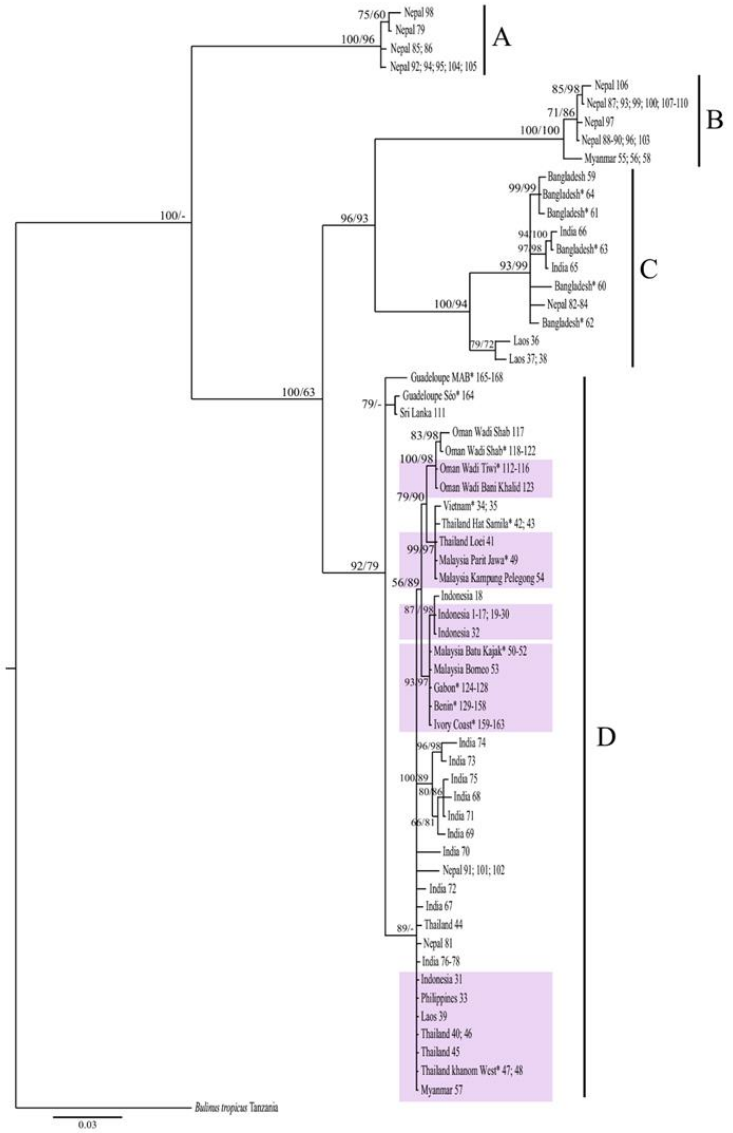

Figure 5 Bayesian tree of *I. exustus* constructed based on several COI sequences (582 bp). Node supports are denoted by bootstrap values of the maximum-likelihood method and Bayesian posterior probabilities.

Source: Mouahid et al., 2018

General characteristics of the trematodes

The trematodes belong to the phylum Platyhelminthes, class Trematoda and subclass Digenea (Keiser & Utzinger, 2009). The general characteristics of adult trematodes are dorsoventral, flattened, and bilaterally symmetrical body. The trematodes average size varies according to the species (Keiser & Utzinger, 2009). The tegumental surface of trematodes comprises a tough syncytial tegument, which tegument implicated has sensory functions and is involved with absorption of nutrient, secretion, synthesis, and osmoregulation (Cox, 1979; Halton, 2004; Pappas, 1975). Their most distinctive external feature is the presence of two muscular suckers (oral and ventral suckers). There are no circulatory and respiratory systems (Smith, 1976; Whitfield, 1982). The reproductive system is always hermaphrodites, having both male and female organs, with the exception of blood flukes (Cox, 1993). The life cycle of trematodes needs two main hosts, 1) a snail as the intermediate host and 2) a vertebrate animal as the definitive host. These trematode parasites have complication of their life cycles involving sexual reproduction, which occurs in the definitive hosts and asexual reproduction, which takes place in the snails (Jones & Cappello, 2004; Saari et al., 2019).

1. Trematode classification

Trematodes in the phylum Platyhelminthes are categorized into four mainly distinct groups based on their habitat inside the infected organism. These are blood, liver, lung, and intestinal flukes (Bogitsh et al., 2019a).

1.1 Blood flukes

Blood flukes cause the tropical disease schistosomiasis or bilharzia (Moné et al., 2010). The common species of blood flukes found in human is *S. mansoni* with commonly prevalent in Africa, South America, and the Arabian Peninsula. *Schistosoma japonicum* was usually found in Southeast Asia, and *S. haematobium* was found in Africa and the Arabian Peninsula (King, 2009). Infection occurs through contact with water hosting the snail vector, whether during work activities or while bathing (Gryseels et al., 2006). The cercariae aggressively seek people and pierce the host skin (Gryseels et al., 2006). *Schistosoma haematobium* produces urinary system inflammation and blockage, whereas the other two species induce hepatosplenic and intestinal inflammation (Gryseels et al., 2006).

Schistosomiasis is widespread in 74 countries of the globe. The majority of these countries fall into the developing category. Also, a population with inadequate medical conditions could effect in a heightened risk of disease transmission (Chitsulo et al., 2000).

1.2 Liver flukes

Liver flukes are one of the causes of food-borne diseases that result caused by ingesting infectious food. Among the most prevalent are the following such as *O. viverrini*, *Clonorchis sinensis*, and *Fasciola hepatica* (Fried et al., 2004). In south-east Asia, both *O. viverrini* and *C. sinensis* are most common, that cause infection for these two parasites by eating uncooked freshwater fish that contain metacercariae. The major symptoms of the illness include fever, loose stool, general malaise, loss of appetite, epigastric pain, as well as an increased risk of cholangiocarcinoma (CCA) or a cancer in bile duct (Hong & Fang, 2012; Sripa et al., 2012). Similarly, *F. hepatica* is a cause of fascioliasis, a disease that was long considered to be primarily a veterinary problem but is now recognized as a serious human illness, with the highest prevalence in poor nations (Mas-Coma et al., 2005; Mas-Coma et al., 1999).

1.3 Lung flukes

Lung flukes are foodborne diseases resulting from the consumption of contaminated food and are prevalent in tropical and subtropical regions (Mahanty et al., 2011). Among the most common *Paragonimus* genus, this more than 50 species, only a few were found identified as human pathogens, especially *Paragonimus westermani* and *P. heterotremus* (Blair et al., 1999). *Paragonimus* is found to be widely distributed in the Americas, Africa, and Southeast Asia. Crabs and crayfish, among other freshwater crustaceans, act as intermediate hosts, and infection occurs when they are consumed raw or lightly cooked (Blair, 2014; Singh et al., 2012; Zhen & Zhou, 2019).

1.4 Intestinal flukes

Intestinal flukes are digenean trematodes that are spread by food and can infect a variety of definitive hosts. Around 74 species have been identified as colonizing the human intestine (Chai et al., 2009), but only a handful are known to cause infection (Furst et al., 2012). The majority of infected humans are concentrated

in Southeast Asia, encompassing countries such as China, Korea, India, Vietnam, the Philippines, Lao PDR, Indonesia, and Thailand (Chai, 2007; Chai et al., 2005; Chai & Lee, 2002; Chai et al., 2009; Fried et al., 2004; Yu & Mott, 1994). The most common human intestinal fluke is *Fasciolopsis buski*, and the other major intestinal flukes are *Echinostoma* spp., *Metagonimus yokogawai*, and *Heterophyes heterophyes* (Furst et al., 2012). *Echinostoma ilocanum* is the most frequent organism in the genus *Echinostoma* that causes infection in humans (Bogitsh et al., 2019b). *Metagonimus yokogawai* and *H. heterophyes* are less commonly encountered agents responsible for human intestinal fluke infections. Other minute intestinal flukes that infrequently cause disease in human such as *Phaneropsolus bonnei* and *Prosthodendrium molenkampi* (Furst et al., 2012).

2. Life cycle of trematodes

The life cycle of trematodes is complex. However, a general feature consists of vertebrate as definitive hosts and freshwater snails as intermediate hosts (Jones & Cappello, 2004). Trematode life cycles are varied and have species-specific features. Adult worms usually produce eggs after sexual reproduction in the definitive hosts, which can be humans or a variety of animals. Eggs are discharged through feces, urine, or sputum and reach freshwater. After the eggs reach water, the egg completes its development, and a miracidium develops within the eggs. Later, the miracidium hatch from eggs and free-swimming. Within 24 hours, the miracidium finds and penetrates suitable snails as first intermediate host (Bogitsh et al., 2019b; Jones & Cappello, 2004). Much importance of the first intermediate hosts snails is *Lymnaea* spp., *Physella* spp., *Melanoides* spp., *Bithynia* spp., and *I. exustus* (Anucherngchai et al., 2016; Chontananarth et al., 2017; Dodangeh et al., 2019).

In the snails, the trematode parasite reproduces asexual, forming the first sporocysts and then further daughter sporocysts or redia. This allows one miracidium to develop into many trematode larvae, which then mature into cercaria stages. The cercaria release from the snail, penetrate second intermediate hosts (such as fishes, frogs, snails, tadpoles, and water plants), and encyst develop to metacercaria infective stage (Keiser & Utzinger, 2009; Toledo & Fried, 2014). When humans and vertebrates eat raw second intermediate hosts contaminated with the metacercariae. This led to becoming infected. In contrast with other trematodes, the schistosomes or blood fluke

were found cercaria penetrate the definitive hosts directly. Upon ingestion, the metacercariae undergo excystation in the small intestine, migrate to various organs, and subsequently mature into the adult stage within the host (Figure 6) (Bogitsh et al., 2019b; Jones & Cappello, 2004).

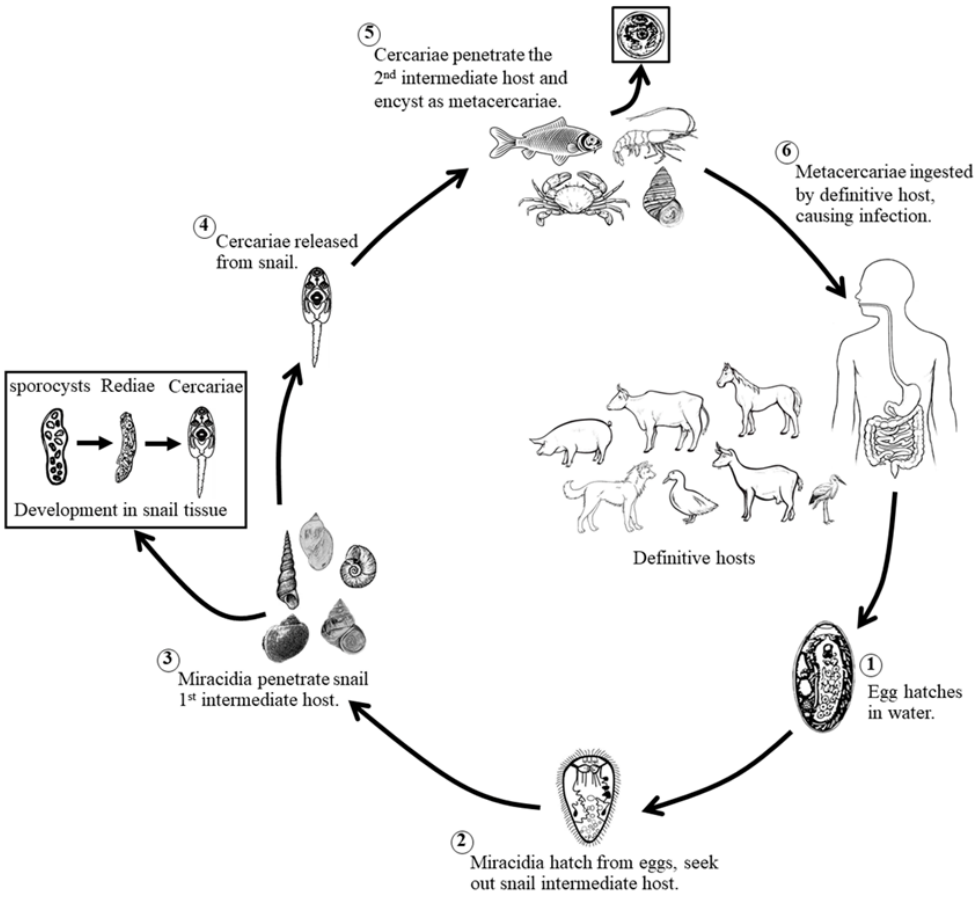

Figure 6 General life cycle of trematodes.

Morphological and biological characteristics of cercarial type

Luhe (1909) was the first to classify cercariae types based on exterior characteristics such as sucker, collar spines, stylet, tail, etc. Subsequently, Faust (1924) identified cercariae by looking at the morphology of a flame cell in the body, resulting in a more detailed group categorization. Currently, a diverse array of cercariae types has been identified, and they can be classified at various taxonomic levels, including superfamilies, families, or genera. For example, echinostome cercariae have been specifically identified as trematodes belonging to the family Echinostomatidae

(Chontananarth & Wongsawad, 2013), while monostome cercariae have been identified as Nolocotylidae. Virgulate xiphidiocercariae have been elucidated as the trematode in Family Lecithodendriidae (Frandsen & Christensen, 1984). At present, several morphotypes of cercariae have been reported from the globe.

1. Megarulous cercariae

The cercaria exhibits an elongated body with granules and lacks eyespots. It features a subterminal oral sucker positioned at the anterior terminal, adjacent to the pharynx. The ventral sucker is situated in the central portion of the body, and a bifurcate esophagus is present between the ventral sucker and the pharynx. The tail is slender and shorter in length compared to the body. Adhesive gland cells are located at the tip of the cercaria's tail (Figure 7) (Anucherngchai et al., 2016, 2017).

The cercariae encyst after developing within rediae (Veeravechsukij et al., 2018). The metacercariae were found in the conjunctival sac in the eyes of various species of birds, including birds of the orders Anseriformes and Galliformes (Díaz et al., 2002). The type of megarulous cercariae has developed into the families Philopthalmidae (Anucherngchai et al., 2016, 2017; Veeravechsukij et al., 2018). Several species of Philopthalmidae were reported in snails of the genus *Melanoides* and *Tarebia* (Anucherngchai et al., 2016, 2017).

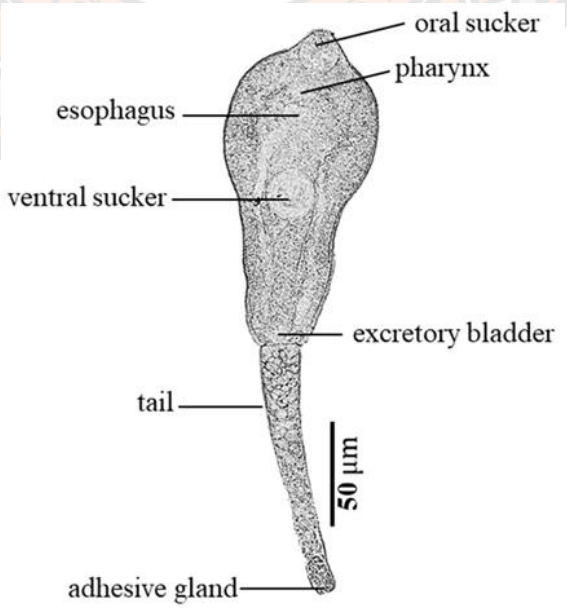

Figure 7 Morphology of megarulous cercariae.

2. Echinostome cercariae

The body of this cercaria type is elongated and oval, characterized by white pigment granules, and lacks eyespots. The oral sucker is circular in shape, positioned at the sub-terminal end of the body, and is surrounded by collar spines. The tail of this cercaria is long and almost a certain length (Figure 8) (Anucherngchai et al., 2016; Dunghungzin & Chontananarth, 2020; Frandsen & Christensen, 1984; Jayawardena et al., 2011).

Echinostomes develop in rediae and encyst in vertebrates (amphibians and fishes) and invertebrates (such as snails) (Dawes, 1946; Frandsen & Christensen, 1984; Olsen, 1974). The type of echinostome cercariae has developed into trematodes in the Echinostomatidae family, which are intestinal parasites of mammals, birds, reptiles, and fishes (Chontananarth & Wongsawad, 2013; Dawes, 1946; Frandsen & Christensen, 1984; Jayawardena et al., 2011; Olsen, 1974). Some Echinostomatidae species may be of veterinary importance (Dawes, 1946; Frandsen & Christensen, 1984; Olsen, 1974) e.g., *Hypoderaeum conoideum*, *Echinostoma revolutum*, and *Echinoparyphium recurvatum* are the most pathogenic and frequent. Pathogens inhabit the intestines of wild waterfowl, marsh birds, geese, and domestic ducks, lead to their exhaustion and death (Galat & Yatusevich, 2015; Huffman & Fried, 1990; Lunaschi et al., 2018). Snails of the genus *Bulinus*, *Biomphalaria*, *Gyraulus*, *Ceratophallus*, *Lymnaea*, *Lentorbis*, *Segmentorbis*, and *Pila* were reported infected with echinostome cercariae (Abdel-Ghani, 1974; Frandsen & Christensen, 1984; Rysavy et al., 1973; Salem et al., 1993).

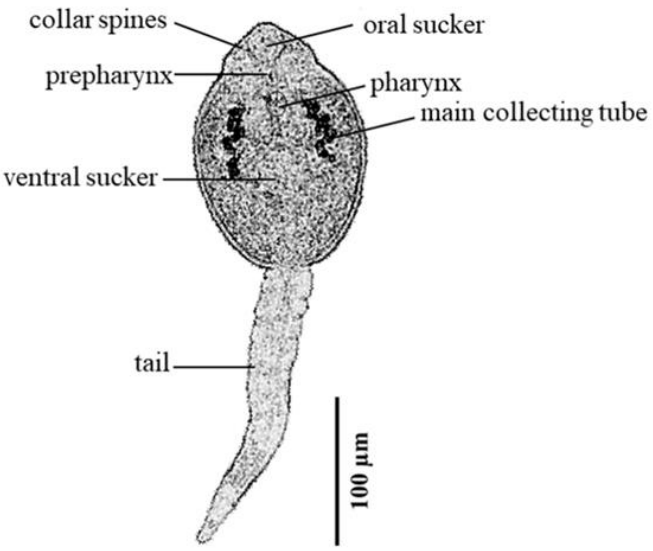

Figure 8 Morphology of echinostome cercariae.

3. Furcocercous cercariae

The cercariae body is long, slender, and oval in shape. It has a pair of pigmented eyespots with a globular shape, presented on the anterior position near the pharynx. The oral sucker is pear-shaped, and the ventral sucker is present at three-fourths down of body length. The tail possesses a distinctive structure, dividing into two furcae (Anucherngchai et al., 2016, 2017). Cercariae of the furcocercous are classified into five subgroups (Figure 9) (Frandsen & Christensen, 1984; Miller, 1926).

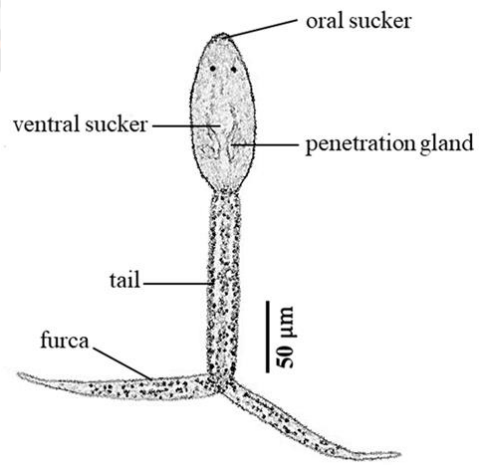

Figure 9 Morphology of furcocercous cercariae.

3.1 Longifurcate-pharyngeate distome cercariae

The furcal finfolds are not present. It has a pharynx, as well as oral and ventral suckers. It has one type of penetration gland cells. The excretory pores are found on the furcae sides. Caudal bodies are present in the tail stem. This cercaria is featured with the presence of a longifurcate tail (Frandsen & Christensen, 1984; Lotfy et al., 2017; Martin & Cabrera, 2018).

3.2 Brevifurcate-apharyngeate distome cercariae

These cercariae were distinguished by the presence of a brevifurcate tail. The oral and ventral suckers of this cercaria are present and lack the pharynx. There are two types of penetration gland cells. Furcal finfolds and eyespots are occasionally seen (Frandsen & Christensen, 1984; Lotfy et al., 2017).

3.3 Lophocercous-apharyngeate cercariae

The body of the cercariae is in the resting position above the tail. Some species have a dorsal finfold that extends the full length of the body. It has no eyespots and pharynx, and absence of the oral sucker and the vestigial or missing ventral sucker. It has one type of penetration gland cells. These cercariae are distinguished by the presence of a brevifurcate tail (Frandsen & Christensen, 1984; Lotfy et al., 2017).

3.4 Longifurcate-pharyngeate monostome cercariae

This cercariae has pharynx and oral sucker, but ventral sucker is vestigial or absent. It has one type of penetration gland cells. The body finfold is not present, but the furcal finfolds are occasionally present. The excretory pores are found at the furcae tips, caudal bodies in the tail-stem are not present (Frandsen & Christensen, 1984; Lotfy et al., 2017).

3.5 Brevifurcate-apharyngeate monostome cercariae

The body of the cercaria is in the resting position below the tail. It has a body finfold. Sometimes the eyespots appear. The oral sucker is present, but the ventral sucker and pharynx are absent. It has one type of penetration gland cells. The tail is a brevifurcate tail (Frandsen & Christensen, 1984; Lotfy et al., 2017).

The furcocercous cercariae can penetrate the definitive host without encystment (blood flukes) or encyst in vertebrates. The longifurcate pharyngeate distome type develops in sporocysts and encysts in fishes, snails, reptiles, and tadpoles.

The brevifurcate apharyngeate distome develops in sporocysts and penetrates the definitive host directly. The lophocercous apharyngeate develops in sporocysts and penetrates the definitive host directly. The longifurcate pharyngeate monostome develops in sporocysts and encysts in fishes. The brevifurcate apharyngeate monostome type develops in rediae and encysts in fishes (Frandsen & Christensen, 1984; Miller, 1926).

The longifurcate-pharyngeate distome cercariae group has evolved into the Diplostomatidae and Strigeidae families, which are intestinal parasites of mammals and birds. The Brevifurcate-apharyngeate distome cercariae have evolved into species of the families Schistosomatidae (blood parasites of birds and mammals) and Spirorchiidae (blood parasites of reptiles). The lophocercous-apharvngeate cercariae have developed into the Sanguinicolidae family (blood parasites of fish). The longifurcate-pharyngeate monostome cercariae have developed into the Cyathocotylidae family (intestinal parasites of reptiles, birds, and mammals). The brevifurcate-apharyngeate monostome cercariae have developed into the family Clinostomatidae, which are mouth and oesophagus parasites of birds (Frandsen & Christensen, 1984; Martin & Cabrera, 2018).

The species in the family Schistosomatidae is very important in medical and veterinary. Some species in the Sanguinicolidae are of great veterinary and economical importance. Snails of the genus *Lymnaea*, *Bithynia, Bulinus, Biomphalaria*, *Gyraulus*, *Ceratophallus*, *Cleopatra*, *Gabbiella*, *Melanoides*, *Melanopsis*, and *Segmentorbis* have been documented as the intermediate hosts for the furcocercous cercariae (Frandsen & Christensen, 1984; Leiper, 1915; Rysavy et al., 1975).

4. Gymnocephalous cercariae

The body of this cercaria is oval-shaped and encompassed with spines. It has a large number of cystogenous glands in the body. This cercaria has no eyespots. The oral sucker is approximately the same size as the ventral sucker. The ventral sucker is situated on the mid-ventral surface of its body. The tail is longer than its body with groups of 3-5 different pigment granules (Figure 10) (Dawes, 1946; Frandsen & Christensen, 1984; Hechinger, 2012; Jayawardena et al., 2011; Veeravechsukij et al., 2018).

These cercariae developed in rediae and encyst in fishes (Frandsen & Christensen, 1984). The gymnocephalous cercariae have developed into the family Fasciolidae, which are intestinal and liver parasites in herbivorous mammals (Frandsen & Christensen, 1984). Species of the family Fasciolidae are important in veterinary (Frandsen & Christensen, 1984). Intermediate hosts of the gymnocephalous cercariae include snails belonging to the genera *Bithynia, Bulinus, Biomphalaria, Gabbiella, Ceratophallus, Gyraulus, Melanoides,* and *Lymnaea* (Frandsen & Christensen, 1984; Rysavy et al., 1975).

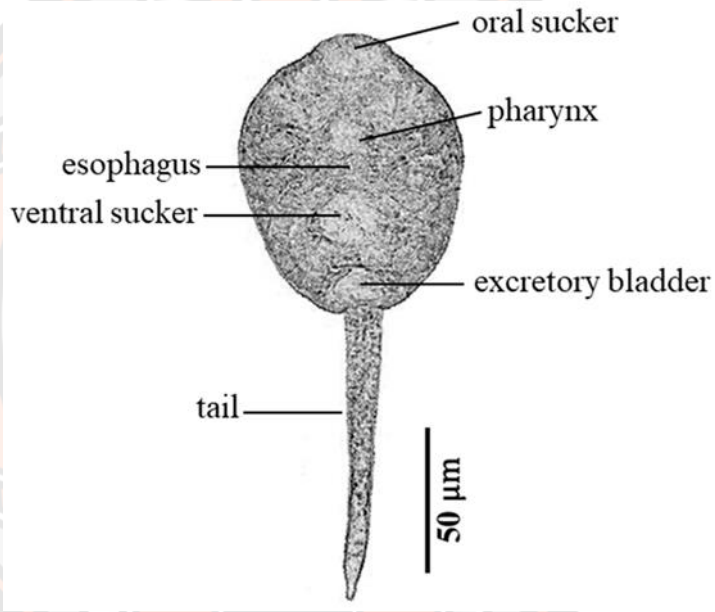

Figure 10 Morphology of gymnocephalous cercariae.

5. Monostome cercariae

The body of this monostome cercaria is transparent and oval. The esophagus is bifurcated with dark-brown pigment. A circular oral sucker of this cercaria is located in the sub-terminal portion of the body. The tail of the monostome cercaria is thick and shorter than the overall length of its body. They are classified into two subtypes; urbanensis cercariae, which have two eyespots (diocellate), and ephemera cercariae, which have three eyespots (triocellate) (Figure 11) (Anucherngchai et al., 2016; Dawes, 1946; Frandsen & Christensen, 1984).

Monostome develops in a redia and encyst on external substrates (Frandsen & Christensen, 1984). The monostome cercariae have developed into the families

Microscaphidiidae (digestive tracts parasites of marine and freshwater teleosts and chelonians) (Blair, 2005a), Mesometridae (digestive tract parasites of marine teleost fishes) (Jones & Blair, 2005), Pronocephalidae (intestines parasites of aquatic reptiles and marine fishes) (Blair, 2005b), and Notocotylidae (digestive tract parasites of mammals and birds) (Barton & Blair, 2005). Monostomes are of no economical, medical, and veterinary significance (Dawes, 1946; Frandsen & Christensen, 1984; Olsen, 1974). Snails of the genus *Lymnaea*, *Biomphalaria*, *Bellamya*, *Bithynia*, *Cleopatra*, *Melanoides*, *Potamides*, and *Planorbis* have been reported intermediate hosts of the monostome cercariae (Diab, 1993; Fahmy et al., 1977; Frandsen & Christensen, 1984; Taima, 2002; Wanas, 1993).

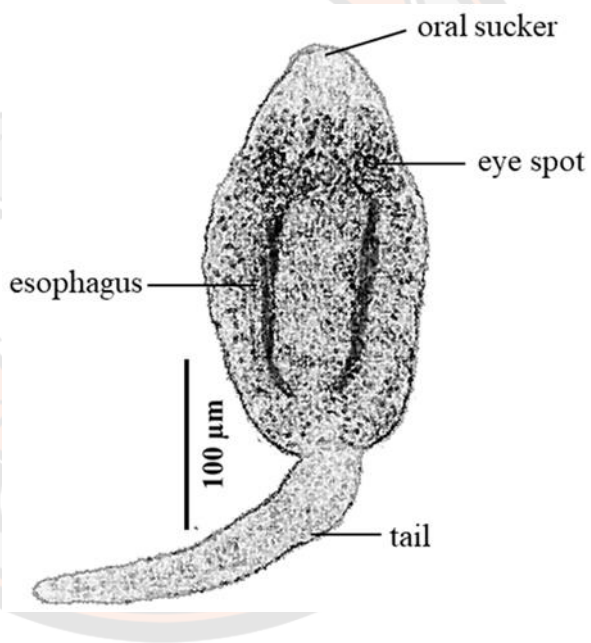

Figure 11 Morphology of monostome cercariae.

6. Opisthorchioid cercariae

The cercaria lacks a ventral sucker. Two eye spots are located close to the end of the lower third of its body. None of adhesive organs are found at the posterior end of the body. This cercarial type has a few numbers of the cystogenous glands in its body (Dawes, 1946; Frandsen & Christensen, 1984; Olsen, 1974). They are classified into two subtypes; pleurolophocercous, in which the tail has dorso-ventral finfolds (Figure 12), and parapleurolophocercous cercariae, in which the tail possesses lateral

finfolds and a dorso-ventral finfolds at two-thirds of the length of the tail (Figure 13) (Anucherngchai et al., 2016; Chontananarth & Wongsawad, 2013; Frandsen & Christensen, 1984; Kumar, 1999).

The opisthorchioid develops in redia in prosobranch snails and encyst in amphibians and fishes. The adult flukes are parasitic in the gallbladder, bile-ducts, and liver of mammals and birds. In addition, some species are digestive tracts parasites of teleosts and reptiles (Bray et al., 2008; Dawes, 1946; Frandsen & Christensen, 1984; Olsen, 1974). The pleurolophocercous cercariae have developed into the families Opisthorchiidae, Cryptogonimidae, and Heterophyidae. In contrast, the parapleurolophocercous cercariae have developed into only the family Heterophyidae (Bray et al., 2008; Dawes, 1946; Frandsen & Christensen, 1984; Olsen, 1974). The species of Opisthorchiidae and Heterophyidae are important in medical such as *Opisthorchis viverrini*, *O. felineus*, *Heterophyes heterophyes*, and *Haplorchis taichui* (Dawes, 1946; Frandsen & Christensen, 1984; Lotfy, 2010; Olsen, 1974). Snails of the genus *Bithynia*, *Gabbiella*, *Ceratophallus*, *PirineIla*, *Melanoides*, and *Lymnaea* have been reported intermediate hosts of the Opisthorchiidae cercariae (Frandsen & Christensen, 1984; Taima, 2002; Tesana et al., 2014).

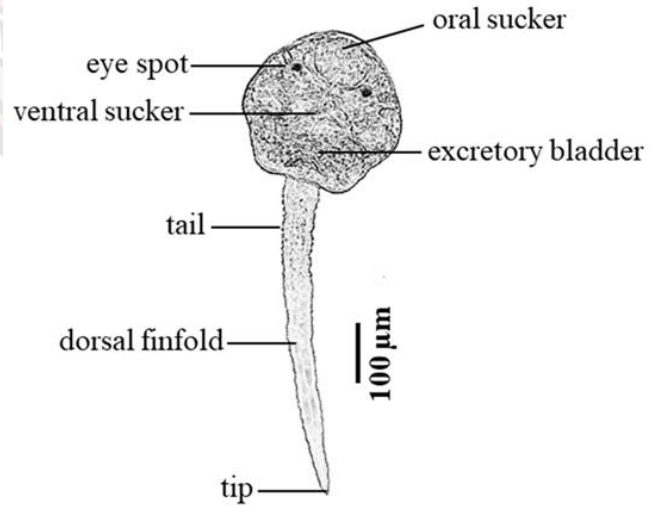

Figure 12 Morphology of pleurolophocercous cercariae.

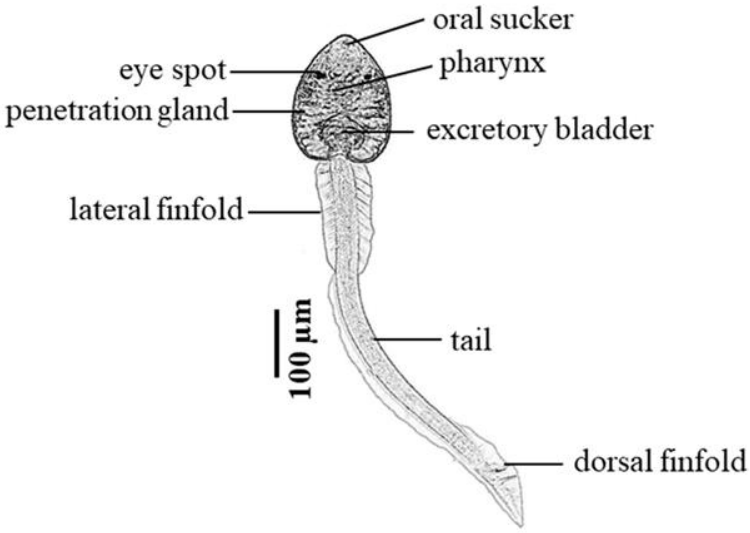

Figure 13 Morphology of parapleurolophocercous cercariae.

7. Paramphistome cercariae

The body of this type of cercaria is large and ovate in shape. Most areas of the body are smooth surfaces and heavily pigmented. A pair of conical eye spots is in antero-posterior direction at a level between the buccal pouches and the intestinal bifurcation. It has an oral sucker that is equal size to the ventral sucker that is located at the posterior end of its body. The tail is singular and inserted into the posterior end of the body (Figure 14) (Frandsen & Christensen, 1984; Krailas et al., 2014; Willey, 1936). This type of cercariae can be classified into three subtypes.

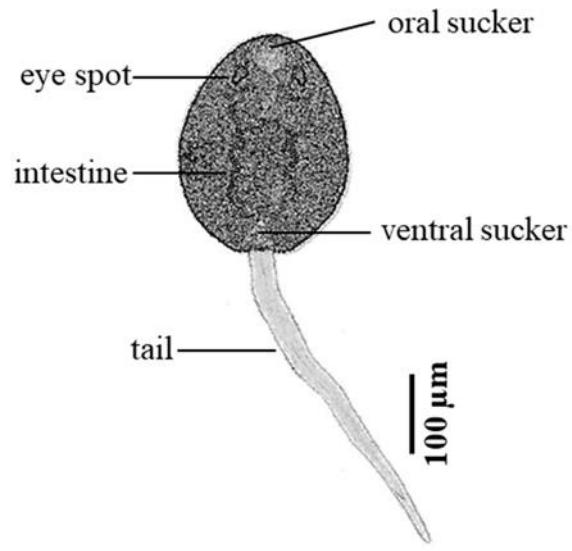

Figure 14 Morphology of paramphistome cercariae.

7.1 Diplocotylea cercariae

The body of this cercariae is without pigmented and mediolateral branches of the ascending main excretory tubes. The posterior of the body has a few cystogenous glands which appear as rounded cells with oval or rod-like granules. There are pharyngeal appendages and an acetabulum that is significantly bigger than the oral sucker (Frandsen & Christensen, 1984; Sey, 1991).

7.2 Pigmentata cercariae

The body of this cercarial type is pigmented and mediolateral branches of the ascending main excretory tubes. Numerous cystogenous glands are present in the posterior part of its body. It has an oral sucker larger than the acetabulurn and without pharyngeal appendages (Frandsen & Christensen, 1984; Sey, 1991).

7.3 Intermedia cercariae

The body of this cercarial type with or without pigment. The excretory tubes have anterolateral and posterolateral diverticula. It has pharyngeal appendages (Sey, 1991).

The paramphistome develop in rediae and encyst on objects in water or the skin of tadpoles (Dawes, 1946; Frandsen & Christensen, 1984; Sey, 1991). The paramphistome cercariae have developed into the family Paramphistomatidae. The type of paramphistome is intestinal parasites of mammals, amphibians, reptiles, fishes, birds, including man (Frandsen & Christensen, 1984; Jones, 2005). The pigmentata subtype is an intestinal parasite that infects mammals, particularly ruminants. Some species are extremely important in the veterinary (Frandsen & Christensen, 1984). Several species of paramphistome cercariae were found in freshwater snails of the genus *Bulinus*, *Gyraulus*, *Ceratophallus*, *Segmentorbis*, *Lentorbis*, and *Biomphalaria* (Barton & Blair, 2005; Frandsen & Christensen, 1984).

8. Xiphidiocercariae (Stylet cercariae)

The cercaria has an elongated, oval-shaped, and colorless body. It's an oval-shaped oral sucker with a unique stylet at the anterior end of its body. The tail of this cercaria is slender and shorter than its body (Figure 15). This type of cercariae can be classified into four subtypes (Dawes, 1946; Frandsen & Christensen, 1984; Olsen, 1974; Schell, 1985).

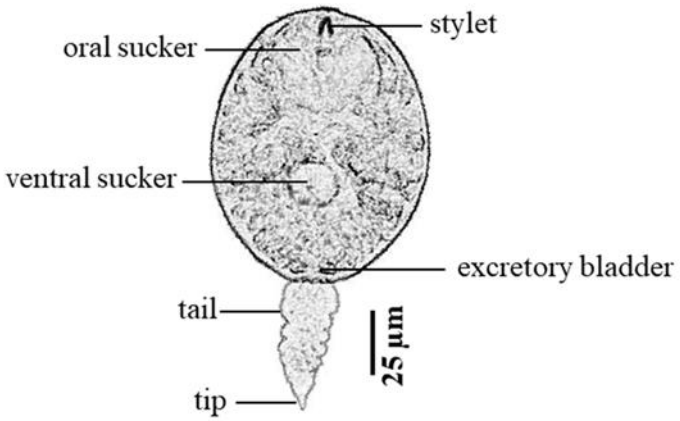

Figure 15 Morphological characteristics of xiphidiocercariae.

8.1 Armatae cercariae

The oral and ventral suckers of this cercariae are the same size, or the ventral sucker is bigger. The tail is without a dorso-ventral finfold and the virgula organ is absent (Frandsen & Christensen, 1984; Lotfy et al., 2017).

8.2 Ubiquita cercariae

The ventral sucker of this cercaria is vestigial or not present. The tail is without a dorso-ventral finfold and the virgula organ is absent (Lotfy et al., 2017).

8.3 Virgulate cercariae

The oral sucker contains a bilobed or pyriform virgula organ. The oral sucker is larger than the ventral sucker. The tail of this cercaria is without a dorso-ventral finfold (Frandsen & Christensen, 1984; Lotfy et al., 2017).

8.4 Ornatae cercariae

The oral sucker of this cercarial type is bigger than the ventral sucker. The tail provided with a dorso-ventral finfold and the virgula organ is not present (Frandsen & Christensen, 1984; Lotfy et al., 2017).

The xiphidiocercariae develop in sporocysts and encyst in amphibians, reptiles, and invertebrates (Dawes, 1946; Frandsen & Christensen, 1984; Olsen, 1974). The armatae cercariae has developed into trematodes in the families Telorchiidae, Reniferidae, Plagiorchiidae, Cephalogonimidae, and Auridistomidae. Telorchiids are parasitic in the intestines of amphibians and reptiles. Reniferidae are parasitic in Nearctic and Neotropical snakes. Plagiorchiidae is parasitic in the intestines of

vertebrates. Cephalogonimids are parasitic in the gastro-intestinal tract of reptiles, amphibians, and fishes. Auridistomidae is parasitic in the intestines of turtles in North American, European, and African. The ubiquita cercariae have developed into families Eumegacetidae and Microphallidae, which are intestinal parasites of birds. The virgulate cercariae have developed into the families Pleurogenidae, Gyrabascidae, and Lecithodendriidae. Pleurogenidae are parasitic mammals and amphibians. Gyrabascidae is parasitic of rodents and bats. Lecithodendriidae is an intestinal parasite of amphibians, birds, and bats. The ornatae cercariae has developed into trematodes in the families Macroderoididae and Haematoloechidae. Macroderoididae is intestinal parasites of amphibians and fishes. Haematoloechidae is lung parasites of amphibians (Bray et al., 2008; Dawes, 1946; Frandsen & Christensen, 1984; Olsen, 1974; Schell, 1985).

The xiphidiocercariae has no veterinary or medical significance (Dawes, 1946; Frandsen & Christensen, 1984; Olsen, 1974). Several species of xiphidiocercariae were found in freshwater snails of the genus *Bithynia*, *Segmentorbis*, *Melanoides*, *Lymnaea*, *Gabbiella*, *Ceratophallus*, *Bulinus*, *Biomphalaria*, and *Bellamya* (El-Gindy & Hanna, 1963; Frandsen & Christensen, 1984; Rysavy et al., 1975).

Natural intermediate host of trematodes

Many trematodes have two intermediate hosts, which are referred to as the first and second intermediate hosts. Roughly 350 snail species globally are recognized for their potential medical or veterinary significance. The genus *Bulinus*, *Biomphalaria*, and *Oncomelania* are significant intermediate hosts of trematodes in the transmission of human schistosomes (Dodangeh et al., 2019). Numerous snail species have been documented as the first intermediate hosts for trematodes (Table 1). In addition, the most important first intermediate host of trematodes are members of the genus *Lymnaea* and *Indoplanorbis* is the commonest in many countries, including Thailand (Anucherngchai et al., 2016; Devkota et al., 2011; Dodangeh et al., 2019).

The second intermediate host of the trematodes harbour metacercariae infective to definitive hosts, including humans, and have an important role as the source of transmission. They include molluscs, crustaceans, fishes, crabs, insects, amphibians, snakes, and edible water plants (Table 2) (Hong et al., 1983; Li, 1991; McMullen, 1937; Sithithaworn et al., 2012).

Table 1 The first intermediate hosts for trematodes from several countries.

Country/Geographical areas	**1st Intermediate host**	**References**
Thailand	*Bithynia siamensis*, *Tarebia granifera*, *T. scabra*, *Filopaludina martensi*, *F. filose*, *F. sumatrensis*, *Wattebledia* sp., *Radiix auricularia*, *Melanoides tuberculata*, *Cerithidia cinculata*, *Indoplanorbis exustus*, *Pila diffusa*, *Brotia costula*, *Eyriesia eyriesi*	(Anucherngchai et al., 2016; Chontananarth & Wongsawad, 2013; Dunghungzin & Chontananarth, 2020)
Iran	*R. auricularia*, *R. palutris*, *R. truncatula*, *R. stagnalis*, *R. gedrosiana*, *Physa gyrina* spp., *Planorbis planorbis*, *Bulinus truncates*, *Viviparus bengalensis*, *M. tuberculata*, *Melanopsis* spp.	(Dodangeh et al., 2019; Imani-Baran et al., 2013)
Nepal	*T. granifera*, *Gabbia orcula*, *Gyraulus euphraticus*, *I. exustus*, *Bellamya bengalensis*, *L. luteola*	(Devkota et al., 2011)

Table 1 (Cont.)

Country/Geographical areas	1st Intermediate host	References
Kenya	*R. natalensis*, *Biomphalaria pfeifferi*, *B. sudanica*	(Owiny et al., 2019)
Nigeria	*Bulinus globosus*, *B. truncatus*, *B. pfeifferi*, *R. natalensis*	(Hailegebriel et al., 2020; Luka & Mbaya, 2015)
Tanzania	*Bimphalaria sudanica*, *B. choanomphala*, *B. pfeifferi*	(Hailegebriel et al., 2020)
Egypt	*Bulinus forskalii*, *B. truncatus*, *Bithynia* sp., *Gyraulus ehrenbergi*, *P. planorbis*, *M. tuberculata*, *R. natalensis*, *Biomphalaria globosus*, *Bulinus nasutus*	(Lotfy et al., 2017)
Uganda	*Biomphalaria stanleyi*, *B. sudanica*, *B. choanomphala*, *B. pfeifferi*, *Biomphalaria* spp.	(Hailegebriel et al., 2020)
Ethiopia	*B. pfeifferi*, *B. globosus*, *B. forskalii*, *R. natalensis*	(Mereta et al., 2019)
Vietnam	*Triculinae* sp., *Sulcospira quangtriensis*, *Parafossarulus striatulus*, *M. tuberculata*, *Bithynia fuchsiana*	(Doanh et al., 2018; Nguyen et al., 2021)

Table 1 (Cont.)

Country/Geographical areas	1st Intermediate host	References
Philippines	*M. tuberculate*, *R. rubiginosa*, *T. granifera*, *Radix* spp., *Pomacea canaliculata*	(Fornillos et al., 2019; Paller et al., 2019)
China	*R. auricularia*	(Sheng, 2004)
Denmark	*R. stagnalis*, *R. auricularia*, *R. peregra*	(Christiansen et al., 2016)
Kuwait	*Clypeomorus bifasciata*, *Cerithidea cingulate*	(Abdul-Salam et al., 1997; Al-Kandari et al., 2000)
Bulgaria	*Planorbarius corneus*, *Physa fontinalis*, *R. peregra*, *R. palustris*, *R. truncatula*, *R. stagnalis*, *P. planorbis*, *Biomphalaria alexandrina*, *Bulinus truncatus*	(McCarthy & Kanev, 1990)
Germany	*R. stagnalis*	(Loy & Haas, 2001)
Pakistan	*I. exustus*, *Physa* spp., *Bellamya* spp., *Gyraulus* spp., *Radix* spp., *Oncomelania* spp., *Bulinus* spp.	(Niaz et al., 2013)

Table 2 The second intermediate host for trematodes from several countries.

Trematodes	**Geographic occurrences (humans)**	**2nd intermediate host**	**References**
Opisthorchis viverrini	Cambodia, Laos, Thailand, Vietnam	freshwater fish	(Sithithaworn et al., 2012; Yong et al., 2012)
Opisthorchis felineus	Spain, Italy, France, Switzerland, Germany, Russia, Turkey	freshwater fish	(Chai et al., 2005; De Liberato et al., 2011; Traverso et al., 2012)
Paragonimus spp.	Worldwide	freshwater crabs, crayfish	(Blair et al., 1999; Waikagul, 1986; Yokogawa et al., 1962)
Phaneropsolus spp.	Indonesia, Laos, Thailand	naiads of dragon fly	(Lie, 1951; Radomyos et al., 1984, 1989)
Plagiorchis spp.	Korea, Thailand, Philippines	nymphs of stone fly	(McMullen, 1937)
Prosthodendrium molenkampi	Indonesia, Laos, Thailand	naiads of dragon fly	(Radomyos et al., 1984, 1989)
Watsonius watsoni	Africa, eastern Asia	aquatic plants	(Li, 1991; Yu & Mott, 1994)
Neodiplostomum soulense	Korea	frog tadpoles, grass snakes	(Hong et al., 1983; Seo et al., 1988)

Table 2 (Cont.)

Trematodes	**Geographic occurrences (humans)**	**2nd intermediate host**	**References**
Nanophyetus salmincola	United States, Siberia	salmon, steelhead trout	(Eastburn et al., 1987; Millemann & Knapp, 1970; Skrjabin & Podjapolskaja, 1931)
Metorchis spp.	Canada, China, Russia	freshwater fish	(Lin et al., 2001; Mordvinov et al., 2012; Watson et al., 1979)
Haplorchis spp.	Asia	freshwater fish	(Kumchoo et al., 2005)
Gastrodiscoides hominis	Asia, Russia, Africa	aquatic plants, crustaceans, molluscs, amphibians	(Fried et al., 2004; Kumar, 1980; Surinthrangkul et al., 1965)
Fischoederius elongates	China	aquatic plants	(Li, 1991; Yu & Mott, 1994)
Fasciolopsis buski	Asia	chestnut, hyacinth, morning glory	(Weng, 1989)
Fasciola spp.	Worldwide	aquatic plants	(Boray, 1969; Mas-Coma & Bargues, 1997)
Echinostoma spp.	Asia	freshwater snails, clams	(Chai, 2009)

Table 2 (Cont.)

Trematodes	**Geographic occurrences (humans)**	**2nd intermediate host**	**References**
Eurytrema pancreaticum	Asia	grasshoppers	(Basch, 1965)
Echinochasmus spp.	Asia	freshwater fish	(Chai, 2009)
Dicrocoelium dendriticum	Worldwide	ants	(Krull & Mapes, 1953)
Clonorchis sinensis	Thailand, China, Japan, Korea, Russia, Taiwan, North Vietnam	freshwater fish	(Figurnov VA et al., 2002; Traub et al., 2009)
Clinostoma complanatum	Japan, Israel, India, Korea, Thailand	freshwater fish	(Kim et al., 2009; Tiewchaloern et al., 1999; Witenberg, 1944)
Carneophallus breviceca	Philippines	freshwater shrimp	(Velasquez, 1975)
Amphimerus pseudofelineus	Ecuador	freshwater fish	(Artigas & Perez, 1962; Rodriguez et al., 1949)
Alaria americana	North America	amphibians, reptiles, raccoons, opossums	(Freeman et al., 1976)
Achillurbainia recondita	Honduras	freshwater crabs	(Beaver et al., 1977)
Achillurbainia nouvelli	China, Thailand	freshwater crabs	(Chen, 1965; Tesjaroen et al., 1989)

Prevalence of cercariae in snails

To complete the life cycle, several species of trematodes need freshwater snails as an essential intermediate host. It serves as an intermediary for the development of larvae and the increase in the number of larval stages to contact definitive hosts which are humans and other animals. Nearly all trematode families require snails as the first intermediate hosts, and they are the keystone animals in the trematode life cycles. Approximately 30 snail species have been documented in association with the life cycle of trematodes in Thailand (Table 3). The country has numerous agricultural regions distributed across all areas, contributing significantly to the production of rice intended for export (Anucherngchai et al., 2016; Dunghungzin & Chontananarth, 2020). Consequently, farmers in this region possibly will generate and release fecal material containing egg/larval trematodes into various water sources, such as irrigation canals, reservoirs, and rivers. This contributes to the widespread distribution of many trematodes in Thailand, as well as a high incidence of cercarial infection (Chantima et al., 2013; Chontananarth & Wongsawad, 2013; Chontananarth et al., 2014; Krailas et al., 2012; Kumchoo et al., 2005; Wongsawad et al., 2009).

In Thailand, fifteen morphological types of trematodes cercariae have been reported, such as xiphidiocercariae, pleurolophocercous cercariae, parapleurolo phocercous cercariae, and gymnocephalous cercariae, which are identified as the human intestinal fluke family (Chai, 2009, 2019; Hung et al., 2013). In addition, cercaria-stage of trematodes were found in nine families of brackish-water snails that were reported from six provinces (Chachoengsao, Samut Prakan, Chon Buri, Chanthaburi, Rayong, and Trat) (Sri-Aroon et al., 2004). Meanwhile, Anucherngchai et al. (2016) reported nine morphological types of cercaria in freshwater snails across ten provinces in the Chao-Phraya Basin. These are virgulate cercariae, xiphidiocercariae, pleurolophocercous cercariae, parapleurolophocercous cercariae, megarulous cercariae, monostome cercariae, echinostome cercariae, furcocercous cercariae, and cercariae.

Table 3 Prevalence of cercariae infection in the snail's first intermediate host.

Type of cercaria	Snail species	Families of trematodes	Species of trematodes	References
Echinostome cercaria	*I. exustus* *R. auricularia* *B. siamensis* *F. sumatrensis* *F. martensi* *Melanoides tuberculata* *Cerithidea djadjariensis* *C. cingulata*	Echinostomatidae	*Echinostoma malayanum* *E. revolutum* *Echinochasmus pelecani* *Hypoderaeum conoideum*	(Anucherngchai et al., 2016; Dunghungzin & Chontananarth, 2020; Dunghungzin et al., 2017; Krailas et al., 2014; Sri-Aroon, 2011; Sritongtae et al., 2015)
		Himasthlidae	*Himasthla interrupta*	
Megalurous cercaria	*Tarebia granifera* *M. tuberculata* *F. sumatrensis* *C. cingulata* *C. alata*	Philopthalmidae	*Philophthalmus gralli* *Philophthalmus* sp. *Cloacitrema philippinum* *Parorchis acanthus*	(Dunghungzin & Chontananarth, 2020; Dunghungzin et al., 2017; Krailas et al., 2014; Sritongtae et al., 2015)

Table 3 (Cont.)

Type of cercaria	Snail species	Families of trematodes	Species of trematodes	References
	C. quadrata *C. djadjariensis*			
Paramphistome cercaria	*M. tuberculata*	Paramphistomatidae	*Gastrothylax crumenifer*	(Krailas et al., 2014)
Xiphidiocercariae	*M. tuberculata* *T. granifera* *Assiminea brevicula* *Clithon peguensis* *Thiara scabra* *Bithynia siamensis*	Lecithodendriidae	*Acanthatrium hitaense* *Loxogenoides bicolor* *Lecithodendrium linstowi* *Ganeo tigrinus* *Lecithodendrium* sp. *Loxogenes liberum* *Mehraorchis ranarum*	(Anucherngchai et al., 2016; Dunghungzin & Chontananarth, 2020; Krailas et al., 2014; Sritongtae et al., 2015; Ukong et al., 2007; Veeravechsukij et al., 2018)

Table 3 (Cont.)

Type of cercaria	Snail species	Families of trematodes	Species of trematodes	References
		Plagiorchidae	*Haematoloechus similis*	
		Microphallidae	*Maritreminoides caridinae* *M. obstipus* *Ascorhytis charadriformis*	
Parapleurophocercous cercariae	*M. tuberculata* *M. jugicostis* *M. tuberculata* *T. granifera* *R. auricularia* *B. siamensis*	Heterophyidae	*Haplorchis pumilio* *H. taichui* *Acanthotrema tridactyla* *Centrocestus formosanus*	(Anucherngchai et al., 2016; Dechruksa et al., 2007; Krailas et al., 2014; Ukong et al., 2007)
Pleurophocercous cercaria	*M. tuberculata* *M. jugicostis* *Bithynia siamensis* *F. filosa*	Heterophyidae	*C. formosanus* *H. pumilio* *A. tridactyla*	(Anucherngchai et al., 2016; Krailas et al., 2014; Ukong et al., 2007)

Table 3 (Cont.)

Type of cercaria	Snail species	Families of trematodes	Species of trematodes	References
Furcocercous cercaria	*M. tuberculata* *M. jugicostis* *B. siamensis* *T. granifera*	Aporocotylidae	*Cardicola alseae*	(Anucherngchai et al., 2016; Krailas et al., 2014; Ukong et al., 2007; Veeravechsukij et al., 2018)
		Diplostomidae	*Alaria mustelae*	
		Strigeidae	*Apatemon gracilis*	
		Transversotrematidae	*Transversotrema laruei*	
		Cyathocotylidae	*Mesostephanus appendiculatus*	
Monostome cercaria	*B. siamensis* *I. exustus* *Pila diffusa* *T. granifera* *Wattebledia* sp.	Notocotylidae	*Cyclocoelum mutabile*	(Anucherngchai et al., 2016; Chontananarth & Wongsawad, 2013; Dunghungzin et al., 2017; Wiroonpan et al., 2021)

Table 3 (Cont.)

Type of cercaria	Snail species	Families of trematodes	Species of trematodes	References
Cercariaeum cercaria	*B. siamensis* *Anentome helena* *Gyraulus siamensis* *T. granifera* *F. martensi* *F. sumatrensis*	Cyclocoelidae	*Cyclocoelum mutabile*	(Dunghungzin & Chontananarth, 2020; Wiroonpan et al., 2021)
	M. tuberculata	Allocreadioidea	*Podocotyle lepomis*	(Krailas et al., 2014)
Renicolid cercaria	*M. tuberculata*	unclassified	*Cercaria caribbea*	(Krailas et al., 2014)

Molecular characterization of cercariae

The conventional techniques for investigating cercarial infections in freshwater snails include shedding (Rajanna et al., 2018) or crushing (Caron et al., 2008). However, these procedures are time-consuming and demand a considerable level of expertise. Furthermore, these methods rely solely on morphological criteria for cercaria identification. However, difficulties arise because cercariae are very similar and small in the larval stage. Moreover, the cercaria stage exhibits limited stable morphological characteristics and is susceptible to variations induced by the host. (Graczyk, 1991). Thus, these approaches make identifying the cercarial type at the species level difficult. As a result, molecular genetic techniques have been recognized as the most reliable tools for identifying a variety of parasites, including trematodes (Anucherngchai et al., 2016; Barber et al., 2000; Chontananarth et al., 2017; Sripalwit et al., 2015; Wongsawad et al., 2017). Molecular techniques have been frequently used in the study to identify and geographical distribution for numerous trematode species, for instance *Calicophoron calicophorum*, *Paramphistomum epiclitum*, *Explanatum explanatum* (Chamuah et al., 2016), *Fasciolopsis buski*, *Fasciola gigantica*, *Paragonimus westermani*, *Haplorchis taichui*, and *Philophthalmus gralli* (Prasad et al., 2011; Veeravechsukij et al., 2018). In addition, molecular methods were also utilized to identify cercarial stages into more specific levels such as echinostome cercaria or Echinostomatidae, megalurous cercaria or Philopthalmidae, cercariaeum cercariae or Cyclocoelidae, xiphidiocercariae or Lecithodendriidae, and parapleurolophocercous cercaria or Heterophyidae (Dunghungzin & Chontananarth, 2020) (Figure 16).

Several nucleotide regions or genes especially the nuclear ribosomal RNA gene were used to identify species of trematodes from different stages including cercariae, metacercariae, and adults (Anucherngchai et al., 2016, 2017; Davies et al., 2015; Prasad et al., 2011; Skov et al., 2009). In the previous study, nucleotide regions in the internal transcribed spacer 2 (ITS2) of the 18S rDNA gene have been used as a potential marker for the identification of trematodes at the species level (Sahu et al., 2016). This region can be used for the detection of various stages of minute intestinal trematode infections in the intermediate hosts (Dzikowski et al., 2004; Krailas et al., 2016; Thaenkham et al., 2010) (Figures 17, 18). Similarly, Barber et al. (2000) reported

the species identification, phylogenetic relationships, and geographical distribution of *Schistosoma bovis* and *S. haematobium* using the same target gene.

In another studies, Sato et al. (2009) used ITS regions in ribosomal DNA to identify and differentiate species among liver (*O. viverrini* and *Clonorchis sinensis*) and intestinal (*Haplorchis pumilio* and *H. taichui*) flukes. The ITS1 region is successfully used to differentiate four species, while the ITS2 region is useful for distinguishing *H. taichui* from the other three species. Later, Saijuntha et al. (2011) employed the ITS1 sequence for genetic analysis and investigated the phylogenetic relationships of *Echinoparyphium recurvatum* and *Echinostoma revolutum*, comparing an isolate from Thailand with other isolates in the GenBank database. Nucleotide variations in the ITS1 sequence between *E. recurvatum* and *E. revolutum* were identified at 3% (6/203 alignment positions). The phylogenetic analysis indicated that *E. recurvatum* from Thailand forms a sister taxa cluster with *E. revolutum* (Figure 19).

Al-Kandari et al. (2015) studied the phylogeny of intestinal fluke *Stictodora tridactyla* in the family Heterophyidae isolated from the snail *Cerithidea cingulata*. Based on the analysis of ITS1 and COI sequences, *S. tridactyla* from Kuwait Bay clustered with the cercariae batillariae with forming a well-supported monophyletic clade. In addition, the analyses based on ITS2 showed *S. tridactyla* clustered with *Procerovum cheni*, *Haplorchis yokogawai*, *H. popelkae*, *H. taichui*, and *H. pumilio*. This study represents the investigation of *S. tridactyla* larval stages using ITS1, ITS2, and COI sequences, as well as the phylogenetic associations of *S. tridactyla* with other heterophyid flukes.

Sanpool et al. (2015) used the ITS2 region and COI gene to study morphology and molecular identification of *Paragonimus macrorchis* in Lao PDR. The study revealed that ITS2 sequences from all *P. macrorchis* specimens showed a 99-100% similarity with sequences of *P. macrorchis* in the GenBank DNA database. Additionally, the COI sequences identified a similarity of 94% compared to the *P. macrorchis* sequence from Thailand. The phylogenetic analyses demonstrated that *P. macrorchis* constitutes a distinct cluster separate from other *Paragonimus* species in Asia. This investigation indicated that *P. macrorchis* from Lao PDR and Thailand exhibited relatively low similarities in COI sequences, despite the high similarity observed in their ITS2 sequences.

Anucherngchai et al. (2016) used the ITS2 region to study the phylogenetic relationship of cercarial stage trematodes in the Chao-Phraya Basin of Thailand. The phylogenetic tree of all cercarial sequences revealed a monophyletic cluster. Five monophyletic groups comprised of Echinostomatidae (echinostome cercaria), Heterophyidae (parapleurolophocercus cercaria), Lecithodendriidae (xiphidiocercaria), Philophthalmidae (megarulous cercaria), and Strigeidae (furcocercous cercaria). The sequencing data indicated that the Philophthalmidae and Echinostomatidae families were closely related, and that these two families were separated from the other groups (Figure 20). In addition, they also reported a phylogenetic tree of the ITS2 sequences of the cercarial stage from Ratchaburi province in 2017. The phylogenetic tree of all identified cercarial sequences branched into five groups, encompassing parapleurolo-phocercous cercaria (Heterophyidae), xiphidiocercaria (Lecithodendriidae), megarulous cercaria (Philophthalmidae), furcocercous cercaria (strigeids), and transversotrema cercaria (Transversotrematidae). In addition, the parapleurolophocer-cous cercaria samples were distinguished and categorized into *Haplorchis taichui* and *H. pumilio*, while the megarulous cercaria was identified as genus *Philophthalmus* (Figure 21). This study demonstrates that the nucleotide sequences of the ITS2 region can serve as a worthy tool for exploring the phylogenetic relationships of trematodes at the family level (Anucherngchai et al., 2017).

Chontananarth et al. (2017) used ITS2 sequences to identify cercariae released by freshwater snails collected in Nakhon Nayok province of Thailand. This ITS2 sequence from each cercaria trematode was utilized to reconstruct a neighbor-joining phylogenetic tree. Cercarial sequences revealed 6 distinct phylogenetic clades including Echinostomatidae (echinostome cercariae), Philophthalmidae (megarulous cercariae), Heterophyidae (parapleurolophocercus cercariae), Lecithodendriidae (xiphidiocercaria and virgulate cercaria), Prothogonimidae (xiphidiocercaria), and Cyathocytylidae (furcocercous cercaria) (Figure 22). Hence, this research substantiates the utility of the ITS2 region for examining phylogenetic relationships or classifying species at the family level.

In a recent study, Wiroonpan et al. (2021) unveiled a phylogenetic tree based on ITS2 sequences of trematode cercariae released by freshwater snails collected in Bangkok, central Thailand. The phylogenetic tree illustrated eight distinct cercaria

types distributed among at least nine families, with five of these families (Cyathocotylidae, Diplostomidae, Echinostomatidae, Heterophyidae, and Lecithodendriidae) being associated with human intestinal fluke groups. The sequence of the monostome cercaria was discovered to be closely associated with the species *Catatropis vietnamensis*. The brevifurcate-pharyngeate-clinostomatoid cercaria was classified within the family Clinostomidae, showing a close relationship with *Clinostomum phalacrocoracis*. Moreover, the strigea cercaria belongs to the family Diplostomidae, showing no close relative to any specific species. Likewise, the vivax cercaria forms a group within the Cyathocotylidae family, but no species with close relationships were identified (Figure 23).

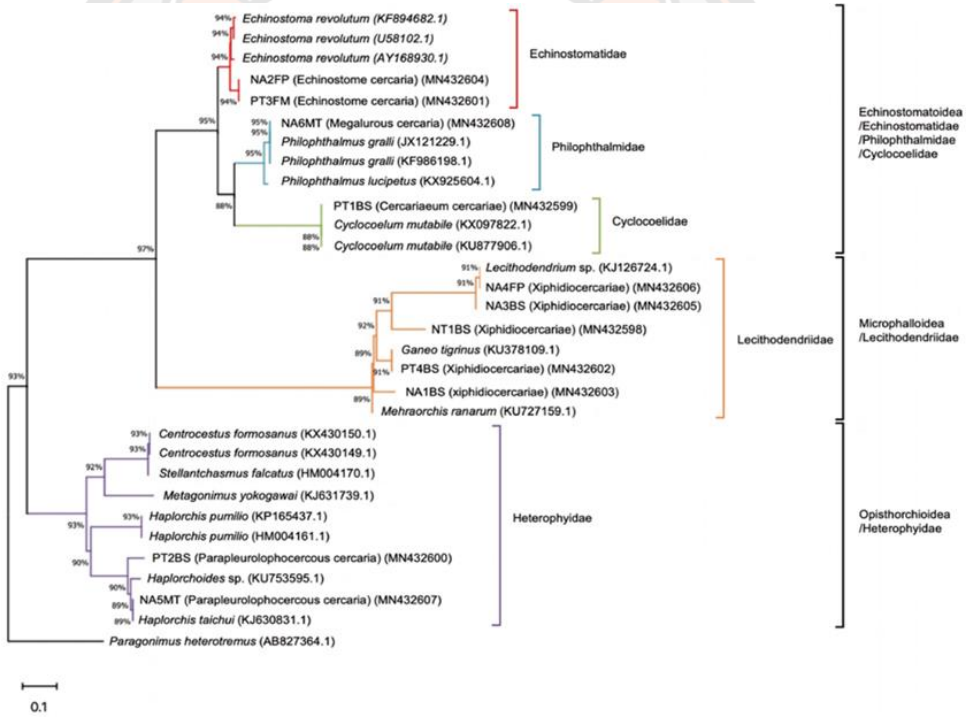

Figure 16 The rooted phylogenetic relationships were determined for each cercarial type through the maximum likelihood (ML) method, employing the Kimura two-parameter with discrete gamma distribution (K2+G) model on partial ITS2 sequences. Bootstrap values were independently computed through 10,000 replicates.

Source: Dunghungzin & Chontananarth, 2020

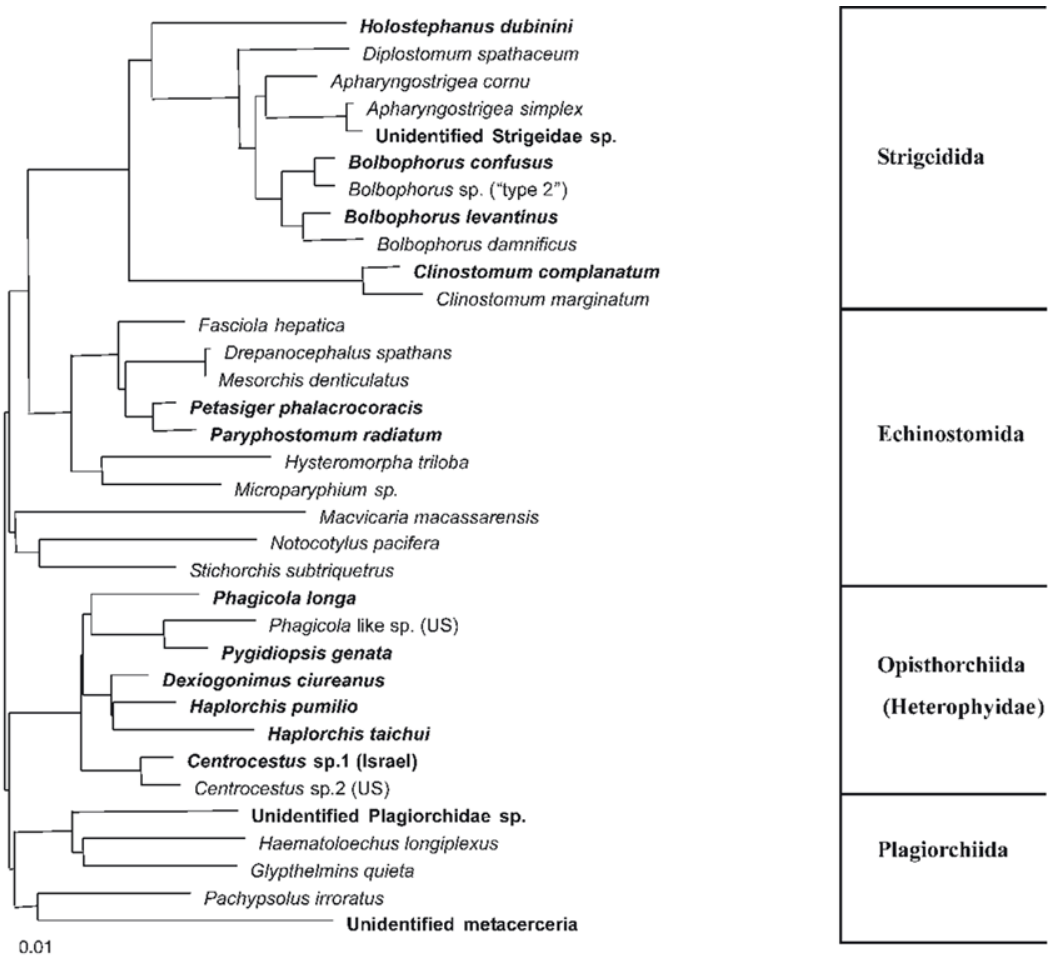

Figure 17 Phylogenetic analysis of heterophyid species and their relationships with other major groups of trematodes was carried out utilizing the alignment of the 18S rDNA gene. Species sequenced in the current study are highlighted in bold. The scale bar denotes a 1% estimated difference in nucleotide sequence positions.

Source: Dzikowski et al., 2004)

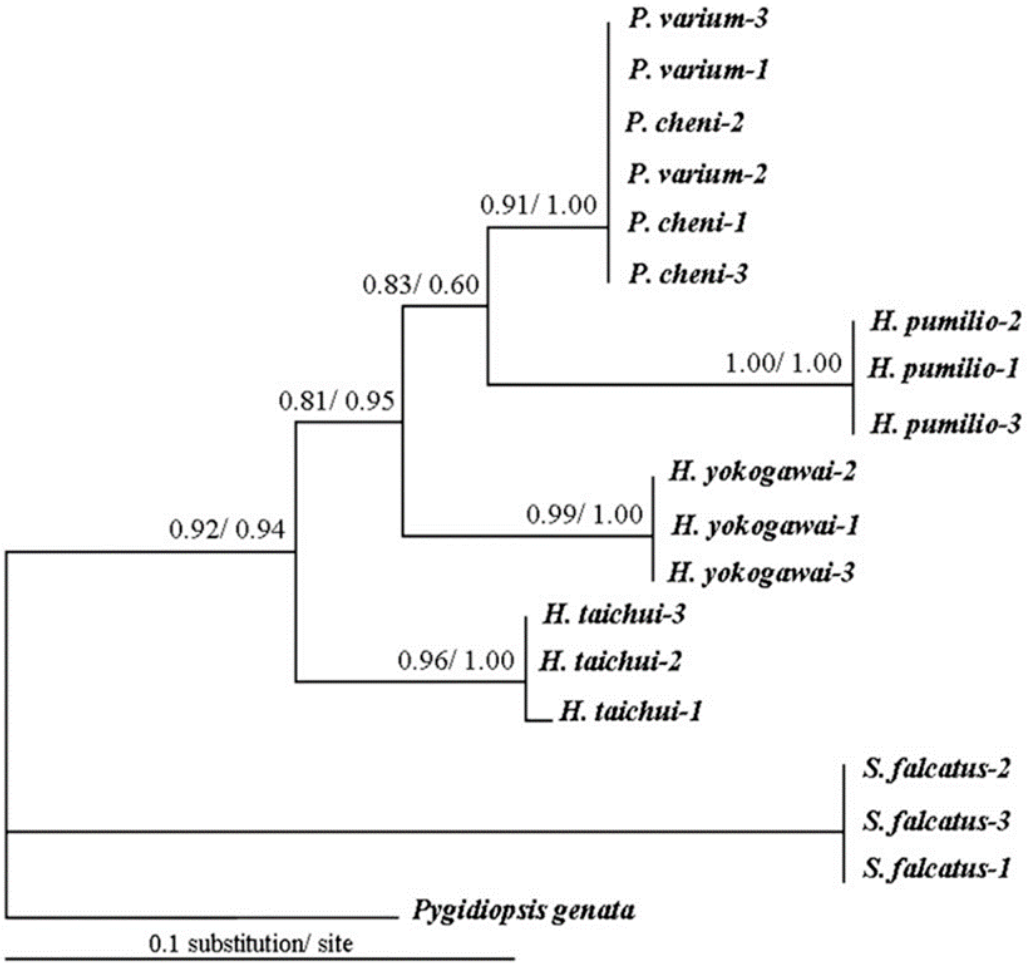

Figure 18 Phylogenetic connections among six species of the heterophyid flukes in the subfamily Haplorchiinae were reformed based on ITS2 nucleotide sequences. P-values of the approximate likelihood ratios (aLTR) from the SH-test and Bayesian posterior probability values (BPP) indicate on each node (aLTR/BPP).

Source: Thaenkham et al., 2010

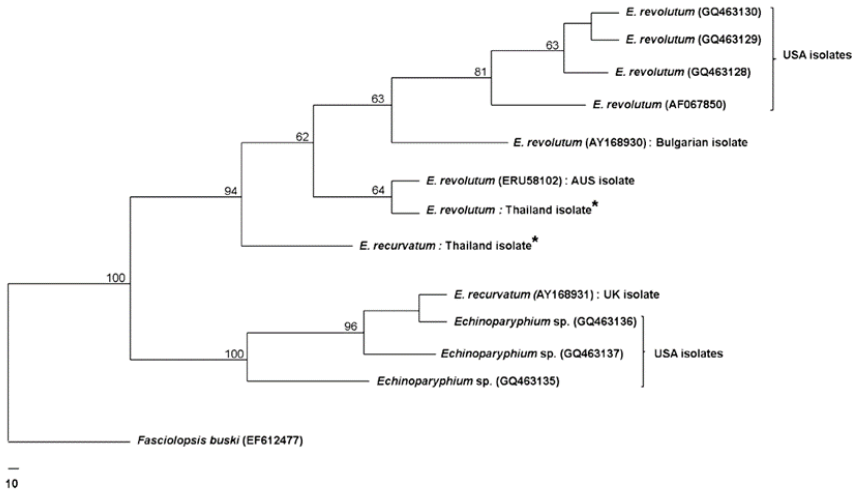

Figure 19 Neighbor-joining phylogenetic tree depicts the relationships among intestinal flukes of *E. revolutum, E. recurvatum*, and *Echinoparyphium* sp., utilizing ITS1 sequences. Bootstrap values (>50%) are indicated.

Source: Saijuntha et al., 2011

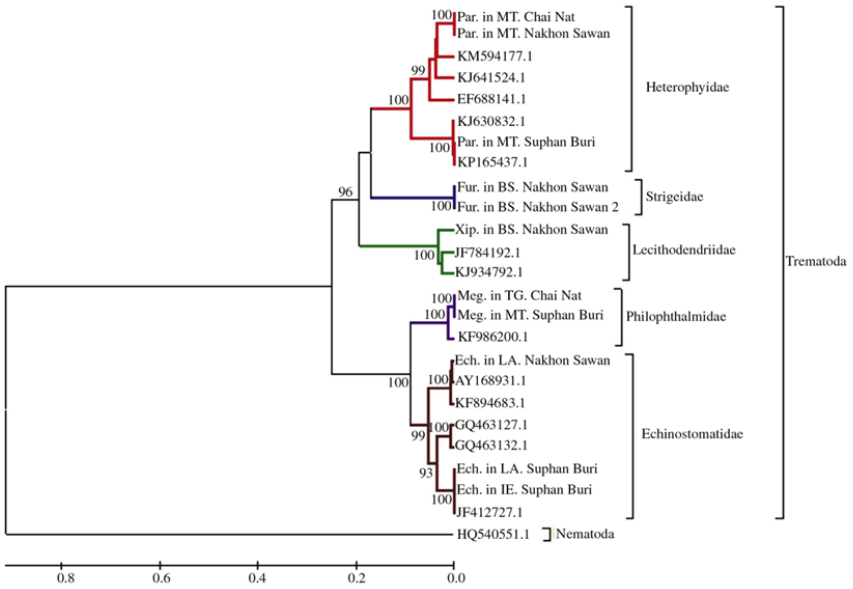

Figure 20 Phylogenetic relationship among various cercarial infections within freshwater snails. MT: *M. tuberculata*; BS: *B. siamensis*; TG: *T. granifera*; LA: *R. auricularia* and IE: *I. exustus*.

Source: Anucherngchai et al., 2016

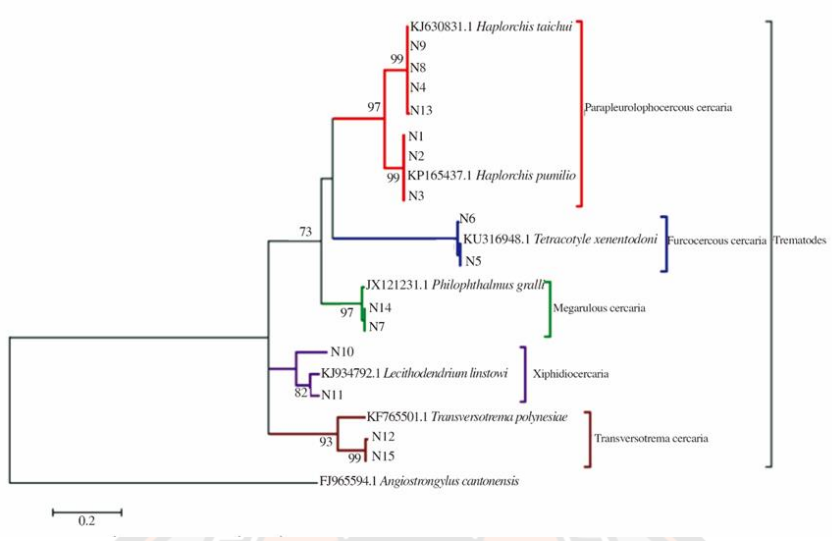

Figure 21 Phylogeny of cercariae released by freshwater snails.

Source: Anucherngchai et al., 2017

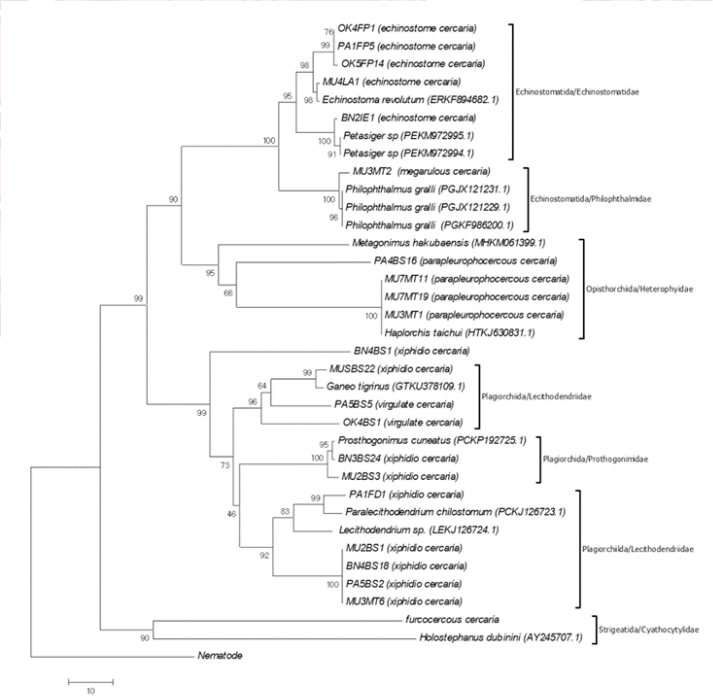

Figure 22 Neighbor-joining phylogenetic tree of each cercarial type based on ITS2 sequences. Bootstrap values were independently computed through 10,000 replicates.

Source: Chontananarth et al., 2017

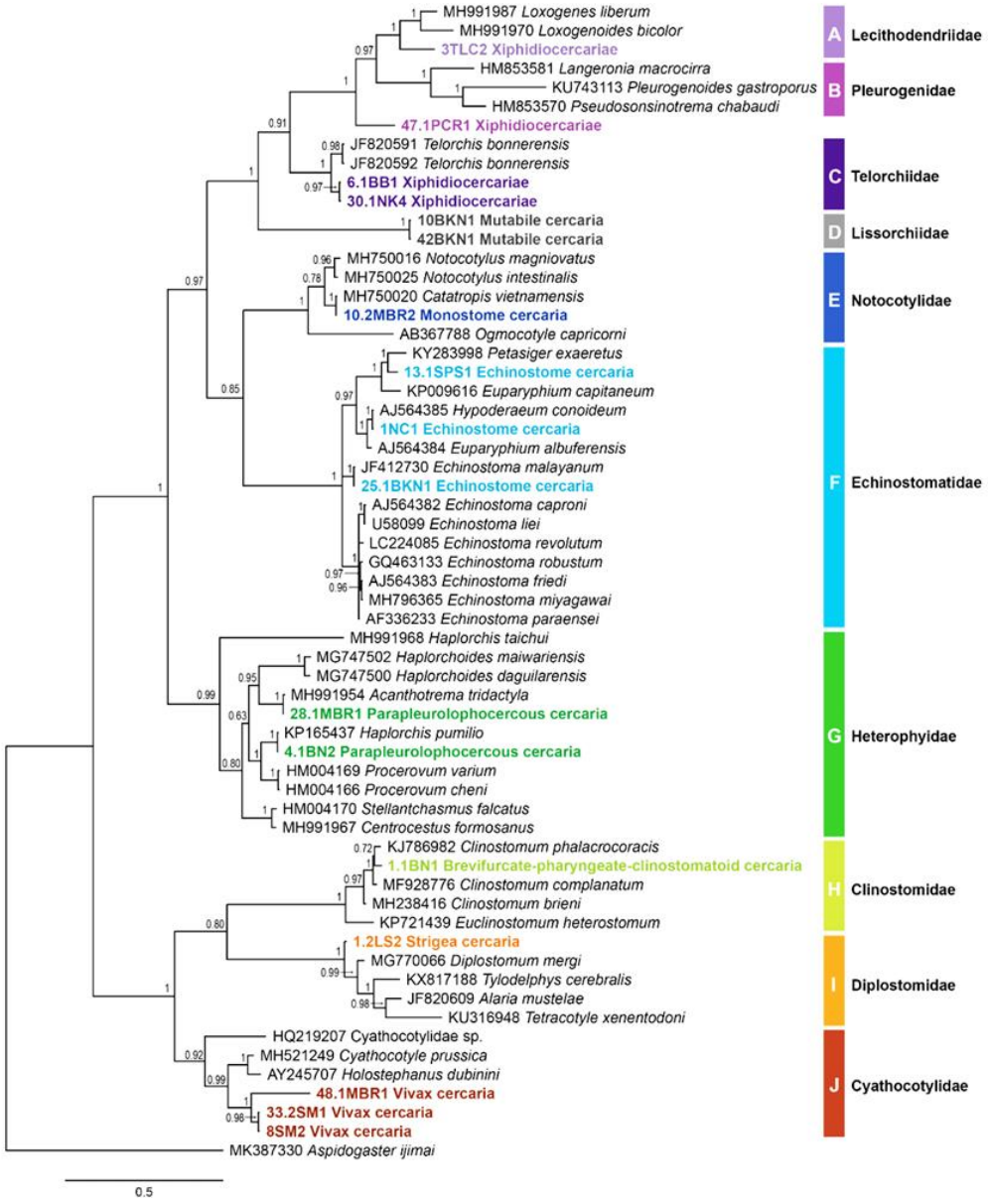

Figure 23 Bayesian Inference (BI) phylogenetic tree among cercariae and related trematodes based on the nucleotide sequences of the ITS2 region. At each node of the tree, the support value of posterior probability was indicated. Alphabets from A to J within the colored boxes corresponded to clades representing each family of digenetic trematodes.

Source: Wiroonpan et al., 2021

CHAPTER III

RESEARCH PROCEDURES OF THE STUDY

Sample size of snails

The present study collected the snail samples of the *Indoplanorbis exustus* and *Lymnaea*. The sample size was determined through sample size calculation (Ngowi et al., 2017) as follows:

$$n = z^2pq/d^2$$

n = minimum sample size

p = estimated prevalence (percentage prevalence of cercarial infection in snail)

q = 1.0-p

z = confidence interval (for a level of confidence of 95%, z = 1.96)

d = allowable error in estimating prevalence (margin of error) (0.04)

Number of samples of *Indoplanorbis exustus*

$$n = z^2pq/d^2$$

n = minimum sample size

p = estimated prevalence is 0.055 or 5.50% (Anucherngchai et al., 2016)

q = 1.0-p (0.945)

z = confidence interval (for a level of confidence of 95%, z = 1.96)

d = allowable error in estimating prevalence (margin of error) (0.04)

$$n = \frac{1.96^2 \times 0.055 \times 0.945}{0.04^2} = 125$$

Minimum sample size is 125 snails.

Number of samples of *Lymnaea*

$$n = z^2pq/d^2$$

n = minimum sample size

p = estimated prevalence is 0.096 or 9.60% (Anucherngchai et al., 2016)

q = 1.0-p (0.904)

z = confidence interval (for a level of confidence of 95%, z = 1.96)

d = allowable error in estimating prevalence (margin of error) (0.04)

$$n = \frac{1.96^2 \times 0.096 \times 0.904}{0.04^2} = 208$$

Minimum sample size is 208 snails.

Collection of snail samples

In this study, *I. exustus* and lymnaeid snails were randomly collected from six distinct geographical regions across Thailand, encompassing the central, east, north, northeast, south, and west regions. Snails were gathered from various habitats, such as paddy fields, canals, and rivers, using a sieve. They were then placed in plastic boxes (sized 20 x 24 x 29 cm) with water and air ventilation. Each plastic box housed between 20-30 snails. Transportation of the snail samples to the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand performed with aeration at ambient temperature. All snails underwent a cleaning process with tap water and were classified based on previously documented shell morphologies characteristic of *I. exustus* and lymnaeids (Brandt, 1974).

Isolation of cercaria from snails

The individual snail samples were investigated for cercarial infections using the shedding method (Caron et al., 2008). The snails were rinsed to eliminate mud and plant materials, using dechlorinated tap water. After washing and identification of species, snails were immediately placed individually into 50 ml (4 cm in diameter and 6.5 cm in high) capacity containers containing 20 ml of dechlorinated tap water at room temperature. Each container was sealed with perforated plastic to prevent snails from escaping, ensuring sufficient aeration. The containers were then kept at room temperature for 24 hours. (Chu & Dawood, 1970; Frandsen & Christensen, 1984; Ramitha & Vasandakumar, 2015). During the daytime, each snail was exposed to natural light for a period of 3 to 5 hours to stimulate the shedding of cercariae from the infected snails (Sharif et al., 2010). During the nighttime, containers of snails were placed in darkness overnight. After one day, the water in each container was poured

into Petri dish and examined under a stereomicroscope for the presence of cercariae. Cercariae presence was determined through the examination of morphological characteristics, movement behaviour, and resting position under the stereomicroscope and light microscope according to previously describe (Anucherngchai et al., 2016, 2017; Chontananarth & Wongsawad, 2013; Dunghungzin & Chontananarth, 2020). Under a stereomicroscope, liberated cercariae from snails were collected by using a sterile Pasteur pipette. Then carefully transferred to a sterile 1.5 ml microcentrifuge tube and saved at -20°C for extraction of the genomic DNA. Furthermore, the soft bodies of the snails were separated from the shells. Approximately 25 mg of the foot muscle tissue was carefully excised from each specimen and subsequently preserved at -20^{o}C for molecular analysis.

Identification of snails and cercariae

1. **DNA extraction of snails and cercariae**

Individual snails and cercariae were subjected to genomic DNA extraction using the NucleoSpin® Tissue Kit (Macherey-Nagel, Duren, Germany) in accordance with the manufacturer's instructions. The snail tissue or cercariae were separately transferred to a microcentrifuge tube (1.5 ml in size) containing 180 µl of the tissue lysis (T1) buffer. Then, 25 µl of a 30 mg/ml of proteinase K solution was added to the tube. To homogenize the sample, tissue was grinded with a sterile pipette tip of 1,000 µl for 5 minutes. Following this, the microcentrifuge tube containing homogenized samples was incubated in a water bath at 56°C for overnight. The following day, 200 µl of sample lysis (B3) buffer was added to the microcentrifuge tube. The tube was then vortexed vigorously and placed in a water bath at 70°C for 10 minutes. Subsequently, 210 µl of 100% ethanol was added to the tube for enhancing DNA-binding conditions. The mixture solution was then transferred to a NucleoSpin® Tissue Column placed in a collecting tube. Then the tube was centrifuged at 11,000 × g for 1 minute. The flowthrough was discarded, and the column was then placed in a new collecting microcentrifuge tube. Consequently, 500 µl of BW buffer was added to the tube, which was then centrifuged at 11,000 × g for 1 minute. Then, 600 µl of B5 buffer was added to the tube, which was centrifuged at 11,000 × g for 1 minute. The resulting solution of the flow-through was discarded. Then, the column was repositioned into the collection

tube, which was centrifuged at 11,000 × g for 1 minute to certify the drying of the silica membrane. The column was put into a 1.5 ml microcentrifuge tube. An approximately 30-80 µl of Buffer BE was added on the tube, which was then incubated at 25-28°C (room temperature) for 1 minute followed by centrifugation at 11,000 × g for minute. The extracted genomic DNA was dissolved in Buffer BE. To check the quality of genomic DNA, the solution was assessed by electrophoresis on a 0.8% agarose gel in 1× TBE (Tris base-boric acid-EDTA) buffer at current constancy of 100 V. Then, the gel was stained with ethidium bromide solution (10 mg/ml), destained with distilled water, and photographed under ultraviolet light. The genomic DNA solution was then preserved in refrigerator at -20°C for future use in the PCR analysis.

2. Polymerase chain reaction (PCR) of snails and cercariae

The fragments of nucleotide in the mitochondria (COI and 16S rDNA) and ribosomal nuclear (18S rDNA, 28S rDNA, and ITS1) of *I. exustus* and lymnaeids were amplified via PCR by using specific primer pairs. Cercariae was amplified using PCR for the selected nucleotide regions, including 28S rDNA and ITS2. The primers utilized in the current study are shown in Table 4. All the polymerase chain reactions (PCRs) were achieved in a Biometra TOne Thermal Cycler (Analytik Jena AG, Jena, Germany). Thirty µl of PCR components in a final reaction volume included 15 µl of Quick Taq™ HS DyeMix (Toyobo, Shanghai Biotech, China), 1.5 µl of each primer at 5 µM (0.25 µM), 9 µl of distilled water, and 3 µl (20-200 ng) of the genomic DNA of the samples. The details of the PCR parameters that were used in each targeted nucleotide region are shown in Table 4. The PCR products were assessed through electrophoresis on 1.2% agarose gel in 1× TBE buffer, running with current constancy at 100 V for 35 minutes. After completion of the electrophoresis, the agarose gel was stained with ethidium bromide (10 mg/ml) and visualized using an ultraviolet transilluminator. Successful amplicons of the targeted genes were cleaned by using the NucleoSpin® Gel and PCR Clean-Up Kit from Macherey-Nagel, Germany, following the manufacturer's instructions.

Table 4 Summary of PCR amplification primers used.

Gene or region	Primer sequence/(Reference)	PCR (bp)	PCR condition	Target organism
COI	LCO1490_forward 5'-GGTCAACAAATCATAAAGATATTGG-3' HCO2198_reverse 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al., 1994)	710	94°C/5 min; 94°C/30 sec, 47°C/1 min, 72°C/1 min, 40 cycles; 72°C/10 min	*I. exustus* and *Lymnaea*
16S rDNA	16Sar_forward 5'-CGCCTGTTTATCAAAAACAT-3' 16Sbr_reverse 5'-CCGGTCTGAACTCAGATCACGT-3' (Kessing et al., 1989)	500		
18S rDNA	18SLYMFOR_forward 5′-GCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCA-3′ 18SLYMREV_reverse 5′-TGCGCGCCTCTGCCTTCCTTGGATGTGGTAGCCCT-3′ (Stothard et al., 2000)	500	94°C/4 min; 94°C/30 sec, 61°C/40 sec, 72°C/2 min, 25 cycles; 72°C/7 min	*I. exustus*

Table 4 (Cont.)

Gene or region	Primer sequence/(Reference)	PCR (bp)	PCR condition	Target organism
28S rDNA	28SFmod_forward 5′-ACCCGCTGAATTTAAGCATAT-3′ 28SRmod_reverse 5′-GCTATCCTGACGGAAACTTC-3′ {Van Bocxlaer, 2017 #369}	1,135	94°C/10 min; 94°C/30 sec, 54°C/2 min, 72°C/1 min, 30 cycles; 72°C/7 min	
ITS1	ITS1-S_forward 5’-CCATGAACGAGGAATTCCCAG-3’ 5.8S-AS_reverse 5’-TTAGCAAACCGACCCTCAGAC-3’ (Ebbs et al., 2018)	802	94°C/10 min; 94°C/30 sec, 53°C/1 min, 72°C/1 min, 25 cycles; 72°C/7 min	
ITS2	ITS3_forward 5′-GCATCGATGAAGAACGCAGC-3′ ITS4_reverse 5′-TCCTCCGCTTATTGATATGC-3′ (Barber et al., 2000)	480	94°C/5 min; 94°C/1 min, 56°C/1 min, 72°C/30 sec, 35 cycles; 72°C/10 min	cercariae

Table 4 (Cont.)

Gene or region	Primer sequence/(Reference)	PCR (bp)	PCR condition	Target organism
28S rDNA	CF1_forward 5′-GAGTTGAACTGCAAGCTCTGG-3′ CR2_reverse 5′-TTCGCCCCTATACTCACGTTAT-3′ (Carranza et al., 2006)	877	94°C/5 min; 94°C/30 sec, 50°C/1 min, 72°C/1 min, 35 cycles; 72°C/10 min	

3. DNA purification and sequencing for snails and cercariae

The purified PCR amplicons were obtained using the NucleoSpin® Gel and PCR Clean-Up Kit (Macherey-Nagel, Duren, Germany). A total of 56 µl of NTI buffer was added to the microcentrifuge tube containing 28 µl of PCR product solution. Then the resulting mixture was transferred onto a NucleoSpin® Gel and PCR Clean-Up Kit Column. The tube containing a column was centrifuged at 11,000 × g for 30 seconds. Subsequently, 700 µl of NT3 Buffer (was added to the tube, and the mixture was centrifuged at 11,000 × g for 30 second. Then, the column was moved to a new 1.5 ml microcentrifuge tube, and distilled water (15 µl) was added, followed by incubation at room temperature (25-28°C) for 1 minute. The tube was then centrifuged at 11,000 × g for 1 minute. The cleaned PCR products were checked by running it on a 1.2% agarose gel at current constancy of 100 V in 1× TBE buffer. The gel containing the bands of PCR products was stained with ethidium bromide (10 mg/ml) for 10 minutes, subsequently destained with cleaned distilled water for 20 minutes. Photography of the DNA bands was performed under UV light. Nucleotide sequencing for both forward and reverse directions was conducted at Macrogen Inc., Seoul, Korea.

Sequence and phylogenetic analyses

Electropherograms of each sequence was manually checked and edited using SeqMan II (DNASTAR, Madison, WI, USA). Alignment of all nucleotide sequences in the present study and reference sequences retrieved from the NCBI database were performed using ClustalW software. Trimming of the sequences was carried out using the Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 (Kumar et al., 2016). In this study, similarity of a nucleotide region in the sequences was performed by the Basic Local Alignment Search Tool (BLAST) in GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The nucleotide sequences of the COI and 16S rDNA genes of snails were concatenated using the MEGA X program (Kumar et al., 2018). Sequence divergence based on the Kimura 2-parameter model (Kimura, 1980) was computed using the MEGA version 7.0.

The ML phylogenetics was reconstructed by Tamura 3-parameter model (Tamura, 1992) for the COI and 16S rDNA sequences of the lymnaeid snails. Similarly, the nucleotide sequences of the 18S and 28S rDNA regions of *I. exustus* were analyzed the phylogenetic relationships by ML tree. For analysis of the ITS2 and 28S rDNA sequences in cercariae, ML phylogeny was reconstructed as well. Additionally, the Tamura-Nei model (Tamura & Nei, 1993) was applied to COI, 16S rDNA, and ITS1 in *I. exustus*. Meanwhile, the NJ phylogenetic tree was constructed by using the Kimura 2-parameter model (Kimura, 1980). The bootstrap support of each phylogenetic tree was calculated based on 1,000 replications through the MEGA Version 7.0 program. The p-distances between haplotypes were estimated using MEGA version 7.0 software to judge the level of genetic variation.

Network analysis

The number of haplotypes and polymorphic sites were calculated by using DnaSP version 5 (Librado & Rozas, 2009) and ARLEQUIN version 3.5.1.2 (Excoffier and Lischer, 2010). The haplotype relationships of each COI, 16S rDNA, 28S rDNA, ITS1 region, and the combined mtDNA of *I. exustus* and lymnaeid snails were estimated by using the median-joining algorithm (Bandelt et al., 1999) in the PopART v1.7 (Leigh & Bryant, 2015) and Network 5.0.1.1 (http://www.fluxus-engineering.com).

Population genetic structure analyses

To determine the genetic diversity within the *I. exustus* and lymnaeids population, haplotype frequencies, haplotype diversity (h) and nucleotide diversity (π) were calculated using the DnaSP version 5 (Librado & Rozas, 2009) and ARLEQUIN version 3.5.1.2 (Excoffier & Lischer, 2010).

To detect genetic differentiation among the population, pairwise F-statistics (F_{ST}) was calculated in ARLEQUIN version 3.5.1.2 (Excoffier & Lischer, 2010). Due to the small sampling size, populations having one sample were excluded for the F_{ST} analysis.

Neutrality and demographic history

The demographic history of expansion of the *I. exustus* and lymnaeid snails was analyzed by mismatch distribution. The sudden expansion model (Rogers & Harpending, 1992) revealed by sum-of-squares deviation (SSD) and Harpending's raggedness index (Harpending, 1994). Historical demographic expansions were also evaluated by the neutrality test conducted using two methods, Fu's Fs test (Fu, 1997) and Tajima's D (Tajima, 1989) statistical tests were used as an indication of recent population expansion and population equilibrium. Mismatch distribution analysis and neutrality tests were estimated by using ARLEQUIN version 3.5.1.2. (Excoffier & Lischer, 2010).

CHAPTER IV

RESULTS

Morphological identification of the snails

In this research, 1,247 samples were collected, with 575 originating from *Indoplanorbis* and 672 from lymnaeid snails. These samples were obtained from 56 locations spread across 27 provinces in six regions of Thailand (Figure 24). The morphology of the snail samples was consistent with *I. exustus* and lymnaeids. The morphology of *I. exustus* is characterized by a discoid shell with dorso-ventrally flat shapes and a rapid increase in whorls. Each whorl exhibits a greater height than width. The aperture expanded and the peristome was sharp without lip (Figure 25). All lymnaeid snails had ovate or ovoidal-conic shell form with without an operculum. The shell was typically dextral containing four whorls with a short conic spire and the aperture was moderately expanded. According to morphological examination, 360 snails from 24 locations in 17 provinces fitted the morphology of *R. rubiginosa* with a shell thin and translucent, columellar part shows a slightly twisted fold, a high conical spire and weakly-inflated body whorl are the most characteristics of shell traits of *R. rubiginosa* (Figure 26A). All remaining snails were at first identified 312 as *O. viridis* for 19 locations from 9 provinces of Thailand. The general characteristic of the *O. viridis* included shell is smaller than the *R. rubiginosa*. The whorls are well-rounded, with relatively wide and short spires, and the outer margin of columella twisted (Figure 26B).

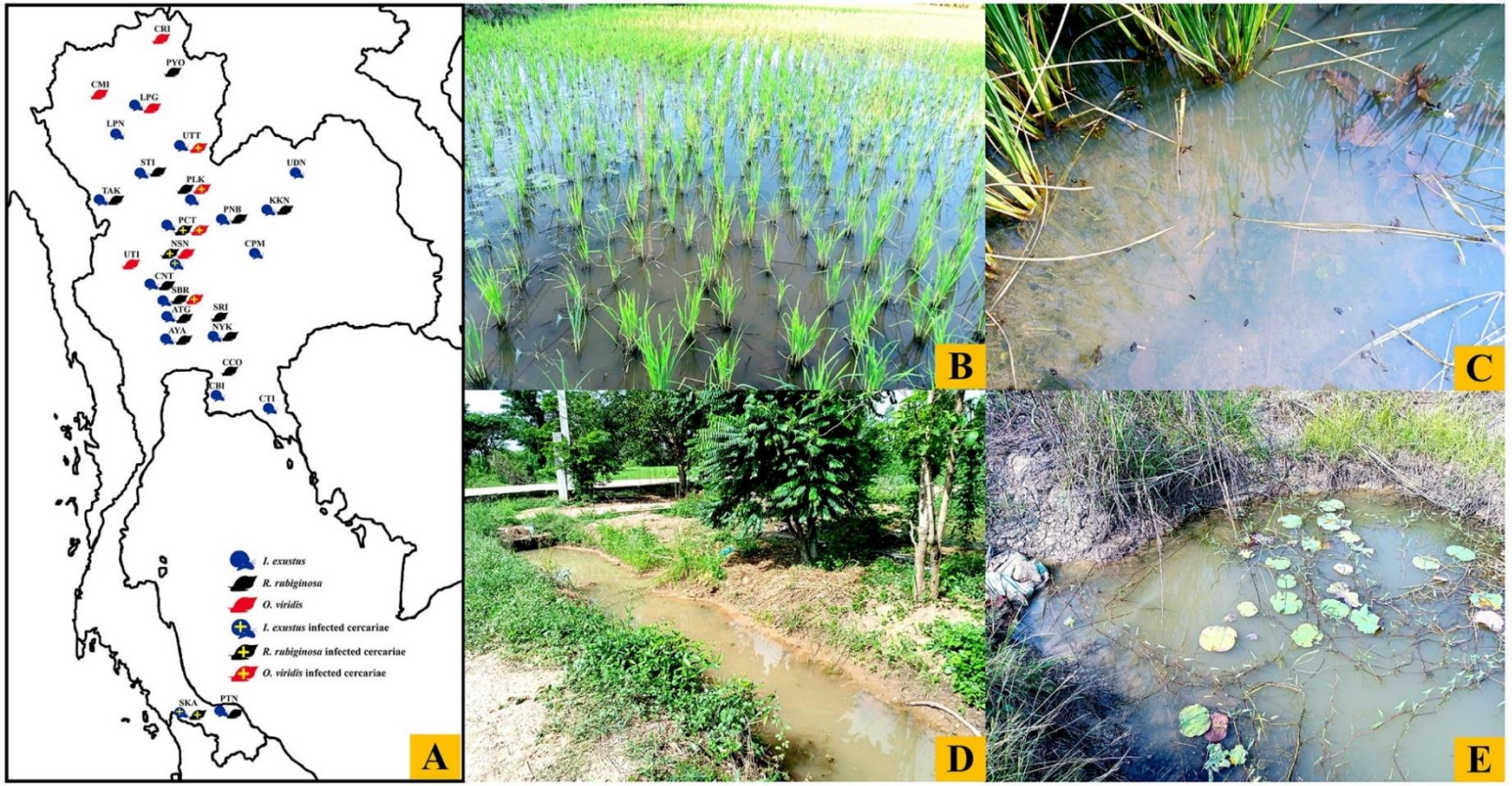

Figure 24 Map of Thailand illustrating the 27 sampling sites for snails employed in this study (A). An environment of collecting site of snails from different areas of Thailand (B-E). Specifics regarding the sampling sites are provided in Table 27.

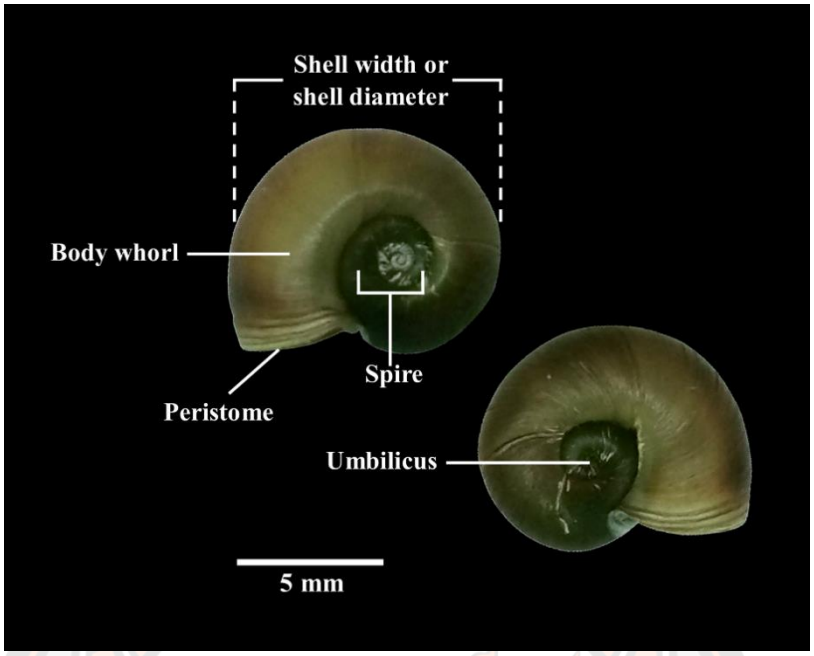

Figure 25 Shell morphology of *I. exustus* in the present study.

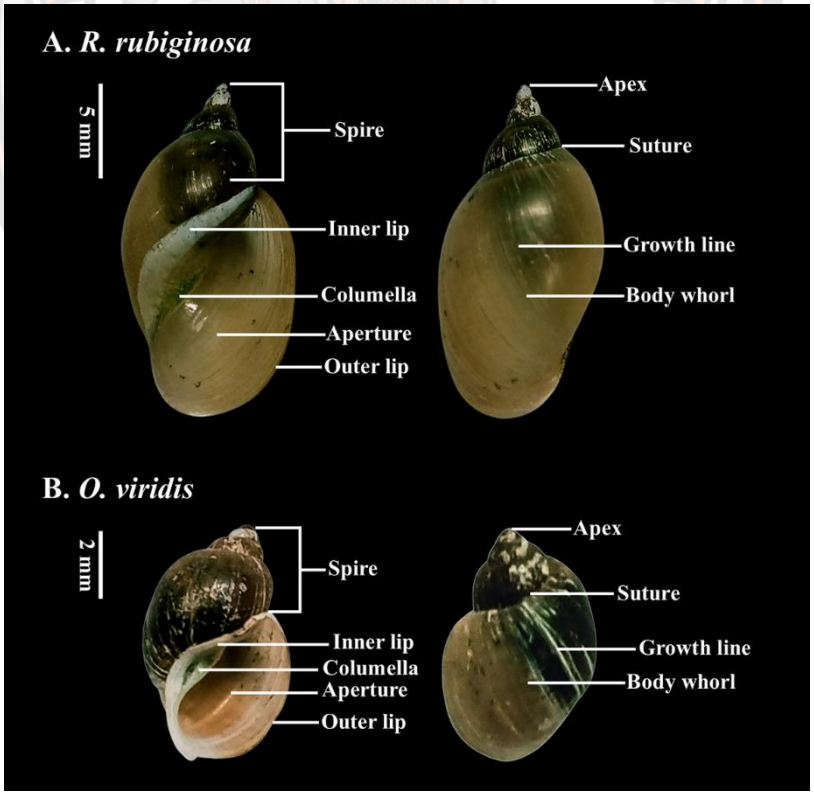

Figure 26 Shell morphology of *R. rubiginosa* (A) and *O. viridis* (B) in the present study.

Diversity and prevalence of cercaria in snails

A total of 1,247 snail specimens were collected, with 575 identified as *I. exustus*, 360 as *R. rubiginosa*, and 312 as *O. viridis*. The overall prevalence of the cercariae in collected snails was 1.76% (22 out of 1,247). Cercarial infections in snails were detected at seven locations spread across six provinces in Thailand (Figure 24). According to these results, the prevalence ratio for each snail species revealed that *O. viridis* had the highest level of prevalence (4.49%, 14/312) compared to *R. rubiginosa* (1.67%, 6/360) and *I. exustus* (0.35%, 2/575). The Uttaradit province had the highest prevalence of cercarial infection (21.88%, 7/32), followed by Phichit (5.56%, 3/54), Sing Buri (3.18%, 4/126), Nakhon Sawan (2.54%, 3/118), Songkhla (1.82%, 4/220), and Phitsanulok provinces (0.44%, 1/225), respectively.

Based on the morphological characteristics, the cercariae identified in this study can be classified into five different types, including xiphidiocercaria, echinostome cercaria I, echinostome cercaria II, furcocercous cercaria, and strigea cercaria. The xiphidiocercaria was the predominant cercarial type infecting snails at 59.10% (13/22), followed by furcocercous cercaria (18.18%, 4/22), echinostome cercaria I (13.64%, 3/22), and echinostome cercaria II and strigea cercaria with prevalence values each type of 4.55% (1/22). *Orientogalba viridis* was infected by xiphidiocercaria, echinostome cercaria I, and echinostome cercaria II with prevalence values of 85.71% (12/14), 7.14% (1/14), and 7.14% (1/14), respectively. In *R. rubiginosa*, the infection of each cercaria type consisted of furcocercous cercaria (50%, 3/6), echinostome cercaria I (33.33%, 2/6), and strigea cercaria (16.66%, 1/6), respectively. Meanwhile, *I. exustus* exhibited infections in both xiphidiocercaria and furcocercous cercaria, each with a prevalence value of 50% (1/2). The information about cercarial types found in individual snail species is provided in Table 5.

Table 5 The number of snail samples and cercarial types in each snail species.

Snail species	No. of snails	No. of snails infected with cercarial types					Total
		Xip	Ech I	Ech II	Fur	Str	
Indoplanorbis exustus	575	1	-	-	1	-	2
Radix rubiginosa	360	-	2	-	3	1	6
Orientogalba viridis	312	12	1	1	-	-	14
Total	1247	13	3	1	4	1	22

Note: Xip = xiphidiocercaria, Ech I = echinostome cercaria I, Ech II = echinostome cercaria II, Fur = furcocercous cercaria, and Str = strigea cercaria.

Morphological description of cercariae

The classification of cercariae in this study was conducted by considering their general morphological features and organ characteristics, aligning with previously established morphological descriptions. The morphological classification of five cercaria types relied on common criteria, including ventral and suckers, stylet, collar spines, eyespots, finfold, and tail. The following provides a description of the five distinct morphological cercarial types.

1. Xiphidiocercaria

The cercaria body is characterized by a small size and elongated oval structure. The round oral sucker with small size is positioned at the front end of the body. It has a stylet located in the center of the oral sucker, which are the characteristics dominant of this cercarial type. The ventral sucker of this cercarial type is located at its central body. Its acetabulum or ventral sucker of this cercarial type is roundish and smaller than its oral sucker. Its tail of this cercaria is slender and shorter than overall body length (Figure 27A).

2. Echinostome cercaria I

This cercaria exhibits an ovate-shaped body. The rounded oral sucker adjacent the front end of the body. Its oral sucker is surrounded by spines that called “collar spines”. The globular ventral sucker of this cercarial type is much bigger than its oral sucker. Its ventral sucker is situated at two-thirds of the body length, which

measured from the anterior part. The bifurcated esophagus is long and situated between the ventral sucker and the pharynx. Their collecting tubes are notably positioned along the body's sides, extending from its pharynx to the acetabulum (ventral sucker). Within these collecting tubes, substantial granules with dark borders are present. The tail of this cercarial type is longer and slenderer than the body (Figure 27B).

3. Echinostome cercaria II

The echinostome cercaria II exhibits an oval to elongated-oval body shape. The rounded oral region, positioned in the subterminal section of the body, and its oral sucker is encircled by a collared spine. Additionally, the rounded ventral sucker is large when compared to its oral sucker. The esophagus is elongated and present with a branched (bifurcated) intestine extending to the back end of the body. The tail of this cercaria has a tubular shape and is relatively long compared to the body, featuring finfolds along the tail stem (Figure 27C).

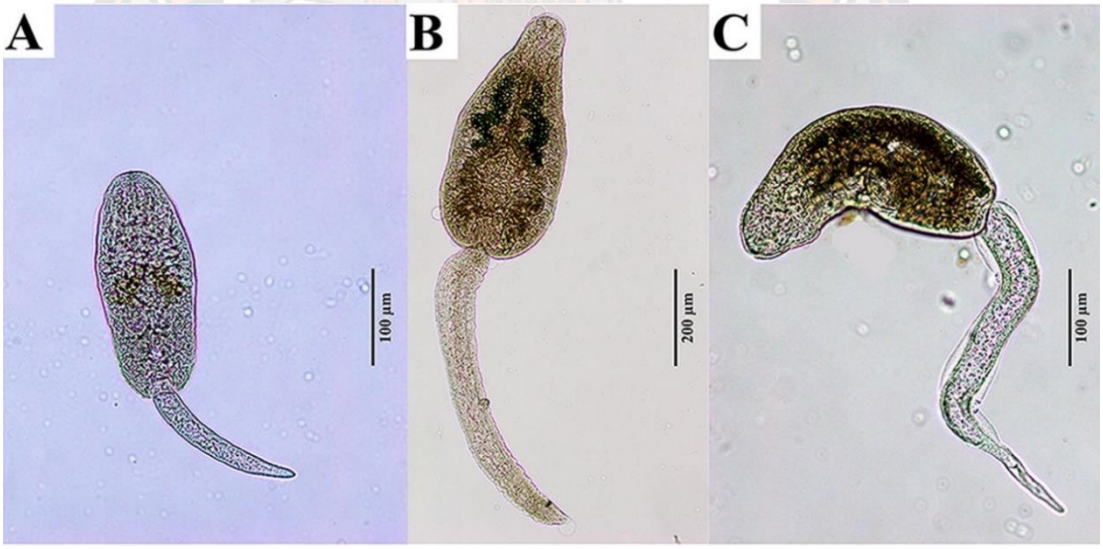

Figure 27 The illustrations of cercariae infected in *I. exustus, R. rubiginosa,* and *O. viridis* snails collected in this study, including (A) xiphidiocercaria, (B) echinostome cercaria I, and (C) echinostome cercaria II.

4. Furcocercous cercaria

The body of cercarial type has an oval-elongated appearance, with a length shorter than its tail. Its body contains a pair of eye spots with blackened pigmentation. Its rounded oral sucker is situated at the anterior terminal portion of the body. Additionally, the finfold lies on dorso-median along the longitudinal center of its body. A tail with two furcae is longer than the body. The tail furca is lesser length compared to stem of the tail. A lateral finfold is evident along the border of the furcae (Figure 28A).

5. Strigea cercaria

This type of cercaria exhibits an oval shape to elongated body. Its oral sucker is a circular structure that is positioned at the front terminal end of the body. The ventral sucker is arranged at the body's midpoint and is of the same size with its oral sucker. The tail of this cercaria divides into two furcae and exceeds the length of the body. The furcal tails appear to be either equal in length to the tail stem (Figure 28B).

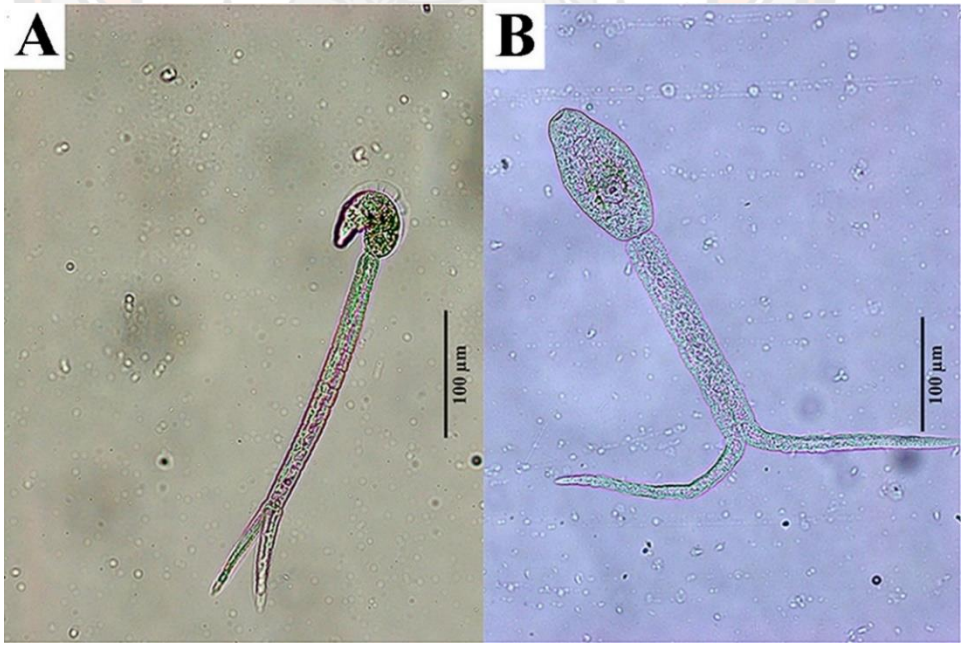

Figure 28 The illustrations of cercariae infected in the *I. exustus* and *R. rubiginosa* snails from the present study, including (A) furcocercous cercaria and (B) strigea cercaria.

Molecular identification of cercariae

In comparison with structural classification, which could only identify a cercaria into the group level, the use of ITS2 and 28S rDNA sequences in this study allowed for identifying cercariae up to the species level. In the current study, examination was conducted on 97 samples, which represented five morphological types of cercariae. Out of these, ITS2 amplification and sequencing were successfully performed for 80 samples, while 17 samples were sequenced for 28S rDNA. The ITS2 sequences of the xiphidiocercaria (69 samples), echinostome cercaria I (6 samples), and echinostome cercaria II (5 samples) had a length of approximately 348-357 base pairs. All sequences were compared known sequences in NCBI by BLASTn search. Sixty-eight samples of xiphidiocercaria (GenBank accession nos. OQ975594-OQ975661) were classified as *Plagiorchis* sp., exhibiting the highest similarity (98.58-100%) with GenBank accession no. KX781392. Meanwhile, a single xiphidiocercaria sample (GenBank accession no. OP586621) exhibited 99% similarity to *Xiphidiocercariae* sp. (GenBank accession no. MW020045). The other four sequences of echinostome cercaria I in this study (GenBank accession nos. OQ975459-OQ975462) demonstrated similarities ranging from 99.43% to 100% with *Petasiger* sp. (GenBank accession no. KM972995). Additionally, two sequences of echinostome cercaria I (GenBank accession nos. OQ975463 and OQ975464) displayed similarities of 98.02% to 98.30% with *Pegosomum asperum* (GenBank accession no. KX097824). Moreover, the examination of ITS2 sequences for echinostome cercaria II from five sequences (GenBank accession nos. OQ975452-OQ975456) indicated a similarity ranging from 99.72% to 100% with identified sequences of *Echinostoma revolutum* (GenBank accession no. MZ964325).

Based on 28S rDNA sequences (653-660 bp) from 11 samples of furcocercous cercaria (GenBank accession nos. OP600054-OP600058 and OQ975753-OQ975758) from this study exhibited high similarity (99.70-99.85%) with identified sequences of *Euclinostomum* sp. (GenBank accession no. MW604803), whereas the 6 samples (GenBank accession nos. OQ975678-OQ975683) of strigea cercaria were identified as *Neodiplostomum banghami* with 97.24-98.16% similarity of the GenBank accession no. OL799105 (Table 6).

Table 6 Similarity of 97 cercariae sequences in the present study after BLASTn search in GenBank.

Nucleotide region	Type of cercariae	Code	Accession number	Maximum identity to (GenBank Accession number)	Similarity (%)
ITS2	Xiphidiocercaria	CX1I532	OP586621	*Xiphidiocercariae* sp. (MW020045)	99.43
		CC1Ov444	OQ975594	*Plagiorchis* sp. (KX781392)	100
		CC2Ov 444	OQ975595		100
		CC3Ov444	OQ975596		100
		CC4Ov444	OQ975597		100
		CC5Ov444	OQ975598		100
		CC9Ov444	OQ975599		100
		CC10Ov444	OQ975600		99.15
		CC11Ov444	OQ975601		100
		CC12Ov444	OQ975602		99.43
		CD1Ov445	OQ975603		100
		CD2Ov445	OQ975604		100
		CD3Ov445	OQ975605		100
		CD4Ov445	OQ975606		100

Table 6 (Cont.)

Nucleotide region	Type of cercariae	Code	Accession number	Maximum identity to (GenBank Accession number)	Similarity (%)
		CD5Ov445	OQ975607		99.72
		CD6Ov445	OQ975608		100
		CD7Ov445	OQ975609		100
		CD8Ov445	OQ975610		100
		CD9Ov445	OQ975611		99.71
		CD10Ov445	OQ975612		100
		CD11Ov445	OQ975613		100
		CF2Ov635	OQ975614		99.71
		CF3Ov635	OQ975615		99.71
		CF4Ov635	OQ975616		99.71
		CF5Ov635	OQ975617		99.43
		CF6Ov635	OQ975618		99.71
		CF7Ov635	OQ975619		98.58
		CF8Ov635	OQ975620		100
		CG1Ov636	OQ975621		99.71

Table 6 (Cont.)

Nucleotide region	Type of cercariae	Code	Accession number	Maximum identity to (GenBank Accession number)	Similarity (%)
		CG2Ov636	OQ975622		99.71
		CG3Ov636	OQ975623		100
		CG4Ov636	OQ975624		100
		CG5Ov636	OQ975625		99.71
		CH1Ov637	OQ975626		100
		CH3Ov637	OQ975627		100
		CH4Ov637	OQ975628		100
		CH5Ov637	OQ975629		99.43
		CH6Ov637	OQ975630		99.71
		CI1Ov638	OQ975631		99.71
		CI2Ov638	OQ975632		99.72
		CI3Ov638	OQ975633		100
		CI4Ov638	OQ975634		100
		CI5Ov638	OQ975635		99.71
		CJ1Ov639	OQ975636		99.71

Table 6 (Cont.)

Nucleotide region	Type of cercariae	Code	Accession number	Maximum identity to (GenBank Accession number)	Similarity (%)
		CJ2Ov639	OQ975637		99.71
		CJ3Ov639	OQ975638		100
		CJ4Ov639	OQ975639		99.71
		CJ5Ov639	OQ975640		99.71
		CL1Ov641	OQ975641		99.72
		CL2Ov641	OQ975642		99.71
		CL3Ov641	OQ975643		99.71
		CL4Ov641	OQ975644		99.71
		CL5Ov641	OQ975645		99.71
		PA1Ov1687	OQ975646		99.71
		PA2Ov1687	OQ975647		100
		PA4Ov1687	OQ975648		99.43
		PA5Ov1687	OQ975649		99.71
		PB1Ov1688	OQ975650		100
		PB2Ov1688	OQ975651		99.43

Table 6 (Cont.)

Nucleotide region	Type of cercariae	Code	Accession number	Maximum identity to (GenBank Accession number)	Similarity (%)
		PB3Ov1688	OQ975652		99.71
		PB5Ov1688	OQ975653		99.71
		PC1Ov1689	OQ975654		99.71
		PC2Ov1689	OQ975655		99.43
		PC5Ov1689	OQ975656		99.43
		PD1Ov1690	OQ975657		99.43
		PD2Ov1690	OQ975658		98.58
		PD3Ov1690	OQ975659		100
		PD4Ov1690	OQ975660		99.71
		PD5Ov1690	OQ975661		99.71
	Echinostome cercaria I	CB3Ov304	OQ975459	*Petasiger* sp. (KM972995)	100
		CB4Ov304	OQ975460		100
		CB5Ov304	OQ975461		99.72
		CB9Ov304	OQ975462		99.43

Table 6 (Cont.)

Nucleotide region	Type of cercariae	Code	Accession number	Maximum identity to (GenBank Accession number)	Similarity (%)
		CP1Rb1520	OQ975463	*Pegosomum asperum* (KX097824)	98.30
		CS3Rb1547	OQ975464		98.02
	Echinostome cercaria II	CK1Ov640	OQ975452	*Echinostoma revolutum* (MZ964325)	100
		CK2Ov640	OQ975453		100
		CK3Ov640	OQ975454		100
		CK4Ov640	OQ975455		100
		CK5Ov640	OQ975456		99.72
28S rDNA	Furcocercous cercaria	CA1I410	OP600054	*Euclinostomum* sp. (MW604803)	99.85
		CA2I410	OP600055		99.70
		CA3I410	OP600056		99.85
		CA4I410	OP600057		99.70
		CA5I410	OP600058		99.85
		CM2Rb1324	OQ975753		99.70

Table 6 (Cont.)

Nucleotide region	Type of cercariae	Code	Accession number	Maximum identity to (GenBank Accession number)	Similarity (%)
		CN3Rb1325	OQ975754		99.70
		CN4Rb1325	OQ975755		99.70
		CT1Rb1560	OQ975756		99.85
		CT4Rb1560	OQ975757		99.70
		CT9Rb1560	OQ975758		99.85
	Strigea cercaria	CE1Rb452	OQ975678	*Neodiplostomum banghami*	98.16
		CE2Rb452	OQ975679	(OL799105)	98.16
		CE3Rb452	OQ975680		98.16
		CE4Rb452	OQ975681		97.24
		CE5Rb452	OQ975682		98.16
		CE7Rb452	OQ975683		98.01

Phylogeny of cercariae

A phylogenetic tree of cercariae based on ITS2 sequences was established employing the ML and NJ methods. Both approaches revealed consistent topologies. The phylogenetic tree of the 103 ITS2 sequences (80 from this study and 23 downloaded from GenBank) revealed two different families of digenean trematodes, supported by bootstrap values of 70% for the ML tree and 74% for the NJ tree (Figure 29). Sixty-eight xiphidiocercaria sequences (released from *O. viridis*) were included within a clade of the family Plagiorchiidae, which was grouped together with *Plagiorchis* sp. (GenBank accession nos. KX160474, KX160475, and KX781392), and the genetic divergence among sequences was 0.04%. Conversely, one xiphidiocercaria sequence (released from *I. exustus*) was found to be intimately related to the *Xiphidiocercariae* sp. and closely related to a clade of the family Plagiorchiidae, with a genetic divergence among sequences of 0.57%. Regarding echinostome cercaria I, four sequences (released from *O. viridis*) were clustered with a clade of *Petasiger* sp. (GenBank accession nos. KM972994 and KM972995) and two sequences (released from *R. rubiginosa*) were grouped with *Pegosomum asperum* (GenBank accession nos. KX097824 and KX097826) in the family Echinostomatidae, with the genetic divergence between sequences were 0.14% and 1.84% respectively. Meanwhile, five sequences of echinostome cercaria II (released from *O. viridis*) were closely related to *Echinostoma revolutum* (GenBank accession nos. AY168930 and MZ964325) in the family Echinostomatidae, and the genetic divergence among sequences was 0.0%.

The ML tree generated from the 28S rDNA gene, incorporating data from 17 samples of furcocercous cercaria and strigea cercaria in this work along with 27 sequences taken from the database of GenBank, unveiled the presence of two main families of digenean trematodes (Figure 30). All samples of furcocercous cercaria (released from *R. rubiginosa* and *I. exustus*) fell in the family Clonostomidae, which was closely related to *Euclinostomum* sp. (GenBank accession nos. MW604803 and MW604806), and the genetic divergence among the sequences was 0.21%. In contrast, 6 strigea cercaria (released from *R. rubiginosa*) were grouped with *Neodiplostomum banghami* (GenBank accession nos. OL799104 and OL799105) in the family Diplostomidae, with the genetic divergence among sequences was 2.02%.

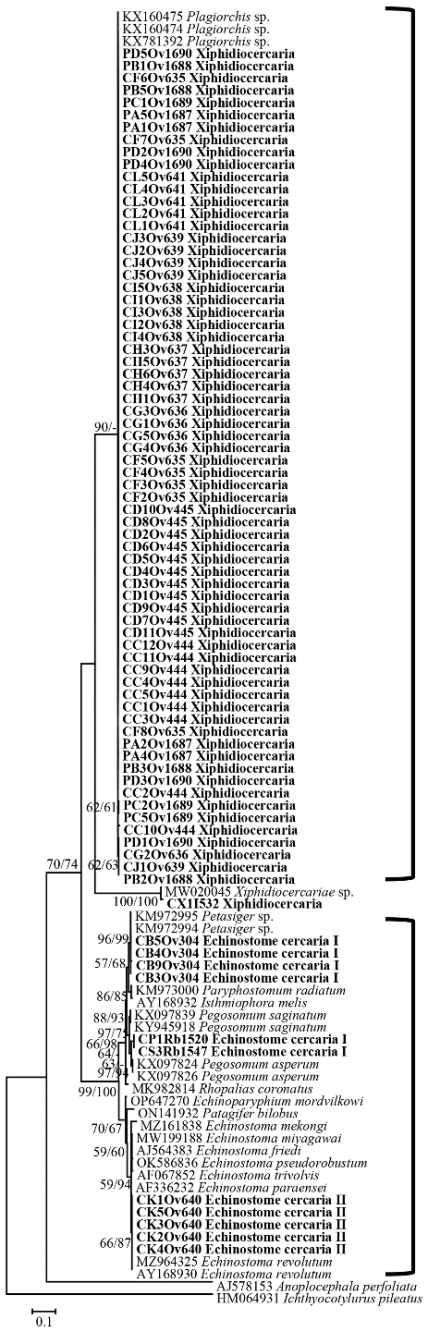

Figure 29 Maximum likelihood phylogenetic tree was created using ITS2 sequences of cercarial types collected from *I. exustus, R. rubiginosa,* and *O. viridis* in Thailand, alongside other published sequences from GenBank. The ML (left) and NJ (right) bootstrap values ≥50% are indicated at the branch points. Bold letters highlight sequences from the current study.

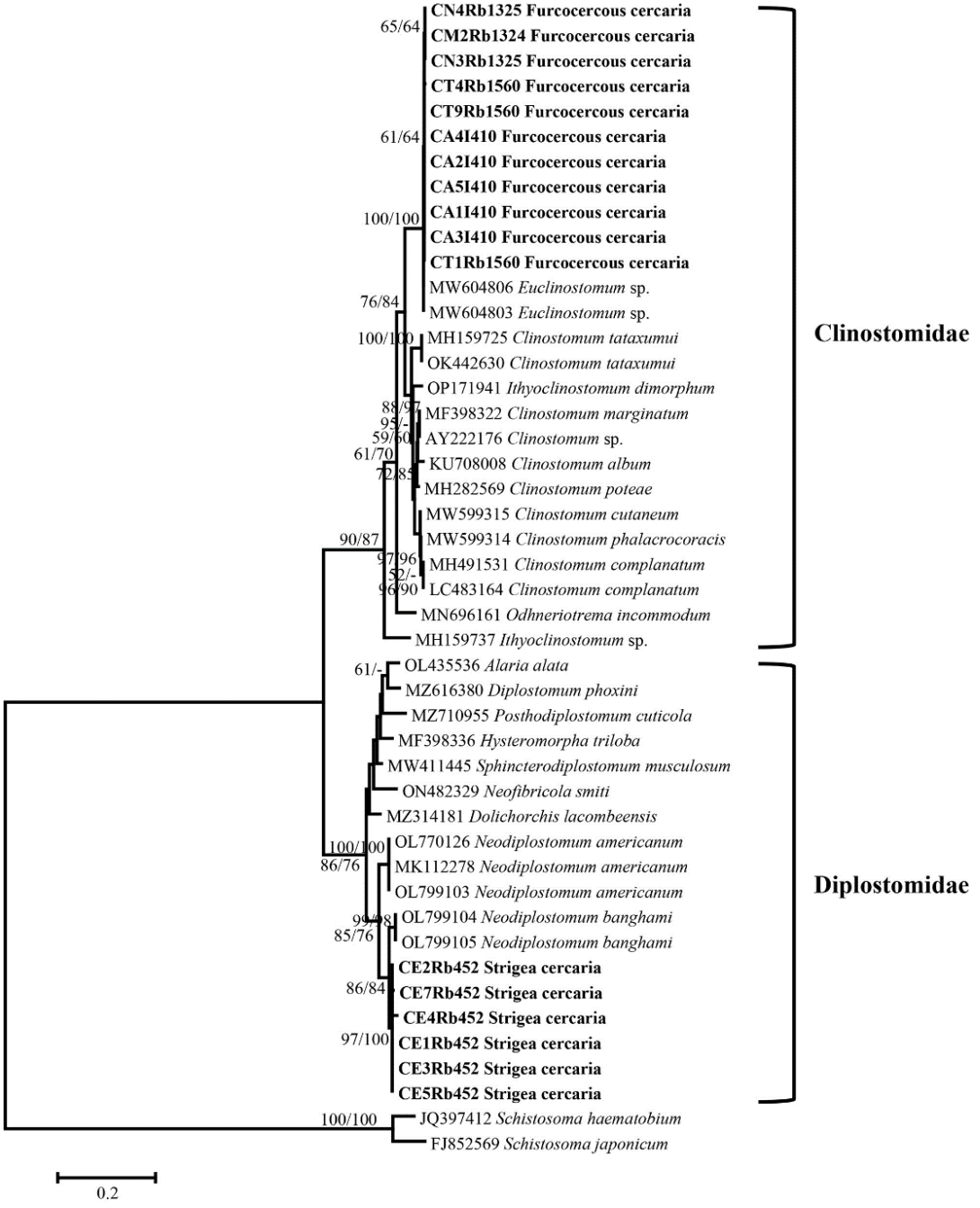

Figure 30 Maximum likelihood phylogenetic tree was created using 28S rDNA sequences of cercarial types collected from *I. exustus* and *R. rubiginosa* in Thailand, alongside other published sequences from GenBank. The ML (left) and NJ (right) bootstrap values ≥50% are indicated at the branch points. Bold letters highlight sequences from the current study.

Molecular identification of snails

For *Indoplanorbis* species identification, genetic investigations were conducted on a randomly selected group of 162 individual snails from Thailand. Based on 569 bp of the COI gene, all 162 sequences (GenBank accession nos. OP588466-OP588627) in the present study demonstrated the highest identity (98-100%) with COI sequences of *I. exustus* (GenBank accession nos. MH037077, MH037081, and MT274331). Similarity, the 16S rDNA gene (381 bp) from 162 samples (GenBank accession nos. OP585918-OP586079) displayed 99-100% similarity with *I. exustus* (GenBank accession no. MH037103). Additionally, a BLASTn search using 600 bp of the ITS1 sequences (GenBank accession nos. OP586437-OP586598) revealed 99-100% identity to *I. exustus* (GenBank accession no. MH037127). Examining the 339 bp of the 18S rDNA gene (GenBank accession nos. OQ975759-OQ975802) in 44 sequences revealed the highest similarity (100%) with the known sequence of *I. exustus* from Thailand (GenBank accession no. AY282598). Additionally, the 28S rDNA sequence (1036 bp) of the *I. exustus* (GenBank accession nos. OQ975465-OQ975508) had a similarity of 100% with *I. exustus* in Thailand (GenBank accession no. AF435662). This robust similarity provides confirmation that the taxa identified in our study belong to *I. exustus*.

To identify the Lymnaeid snails, a total of 116 *R. rubiginosa* and 84 *O. viridis* were randomly for genetic analyses. The genetic analysis included the amplification of nucleotide regions (COI and 16S rDNA) with PCR followed by sequencing. Additionally, identification was confirmed through a BLASTN search. All the sequences generated from the samples in the current study have been deposited to the GenBank database under the accession numbers OQ974570-OQ974685 for the COI of *R. rubiginosa* and OQ974825-OQ974908 for *O. viridis*. Additionally, 16S rDNA sequences have been submitted with accession numbers OQ975324-OQ975439 for *R. rubiginosa* and OQ975510-OQ975593 for *O. viridis*. Based on the 520 bp of the mitochondrial COI gene, 116 sequences of *R. rubiginosa* in this current study had 98.08-100% similarities with *R. rubiginosa* from Thailand (GenBank accession nos. KX056255 and KM067685). The 16S rDNA sequences (367-371 bp) obtained from 116 samples of *R. rubiginosa* in this study demonstrated the highest similarity (99.18-100%) with documented sequences of *R. rubiginosa* in Thailand (GenBank accession

no. GU451749). In addition, 84 samples of *O. viridis* displayed 98.92-100% similarity to *O. viridis* from Thailand and Australia (GenBank accession nos. GU167909 and AF485642) following a BLASTn search by the 16S rDNA gene. The molecular identification of both *R. rubiginosa* and *O. viridis*, based on a partially nucleotide region of two genes (16S rDNA and COI), in the mitochondria, concurred with the morphological characterization.

Comparison of genetic variability of snails among genetic markers

Genetic variation of *I. exustus*, utilizing both in the mitochondria (16S rDNA and COI genes) and nuclear (ITS1, 18S, and 28S rDNA genes). A total of 162 sequences of the COI gene were included in data analyses. The intraspecific distances among 162 samples varied from 0% to 5.82%, with an average of 0.19%. In contrast, the 16S rDNA gene exhibited minimal intraspecific genetic divergence in *I. exustus*, ranging from 0 to 0.53% (mean 0.01%), unlike the COI marker. When considering the combined dataset, the genetic divergence among the 162 samples varied from 0% to 3.49%, with an overall divergence of 0.12%. Furthermore, the analysis of nuclear 28S rDNA from 44 individual *I. exustus* samples revealed the highest intraspecific genetic divergence among the nuclear markers. The divergence within the 28S rDNA ranged from 0% to 0.78%, with an average of 0.09%. In contrast, the 18S rDNA gene showed no variation, indicating a pairwise genetic distance of 0%. Consequently, the genetic variability assessment of *I. exustus* in Thailand indicated that the mitochondrial COI marker exhibited higher genetic variation compared to the nuclear markers (Table 7).

In the analysis of genetic variability among genetic markers in *R. rubiginosa* and *O. viridis*, datasets for the 16S rDNA, COI, and their combination were examined. The intraspecific distances observed in these datasets suggest that these genetically mitochondrial markers, whether employed individually or in grouping, reliably distinguished *R. rubiginosa* from *O. viridis*. According to the COI data, the highest of the intraspecific genetic variance was found in *R. rubiginosa* snail, which was ranged from 0 to 3.36%, with a mean of 1.00%. In contrast, the COI gene showed very low genetic divergence in the *O. viridis*, with ranges from 0 to 0.97% and an average was 0.26%. The same set of samples used for COI was also amplified for the 16S rDNA gene. The mitochondrial 16S rDNA marker demonstrated minimal intraspecific genetic

divergence in *R. rubiginosa*, ranging from 0 to 1.39%, with an average of 0.37%, in contrast to the findings observed with the mitochondrial COI marker. For the mitochondrial 16S rDNA marker of *O. viridis*, the intraspecific genetic distances exhibited from 0% to 1.64% with a mean of 0.17%, displayed somewhat greater variability compared to the findings from the COI dataset. The combination of the mtDNA dataset (COI+16S rDNA) exhibited higher genetic variability within *R. rubiginosa* (0%-2.41%) than the *O. viridis* (0%-1.13%). These indicated the mitochondrial COI and 16S rDNA genes had a sufficient intraspecific genetic variation to assess genetic variability and the structure of *R. rubiginosa* and *O. viridis* populations of. Details of a comparison of genetic divergences among these genetic markers are shown in Table 8.

Table 7 Genetic variations of *I. exustus* from Thailand based on mitochondrial and nuclear genes.

Gene	Sequence length (bp)	Number of samples	Variation sites	Percent genetic divergences (mean)
COI	569	162	60	0-5.82 (0.19)
16S rDNA	381	162	3	0-0.53 (0.01)
Combined mtDNA	950	162	63	0-3.49 (0.12)
ITS1	600	162	9	0-0.67 (0.04)
18S rDNA	339	44	0	0 (0)
28S rDNA	1036	44	17	0-0.78 (0.09)

Table 8 Genetic variations of *R. rubiginosa* and *O. viridis* from Thailand based on COI, 16S rDNA, and combined dataset.

Gene	Sequence length (bp)	Number of samples		Variable sites		Percent genetic divergences (mean)	
		R. rubiginosa	*O. viridis*	*R. rubiginosa*	*O. viridis*	*R. rubiginosa*	*O. viridis*
COI	520	116	84	41	9	0-3.36 (1.00)	0-0.97 (0.26)
16S rDNA	371	116	84	17	13	0-1.39 (0.37)	0-1.64 (0.17)
Combined mtDNA	891	116	84	58	22	0-2.41 (0.74)	0-1.13 (0.22)

Genetic diversity of snails

1. *Indoplanorbis exustus*

Cytochrome oxidase subunit I gene or COI (569 bp), 16S ribosomal DNA gene or 16S rDNA (381 bp), a combined dataset of two mitochondrial DNA or mtDNA regions (950 bp), internal transcribed spacer I or ITS1 (600 bp), nuclear 18S (339 bp) and 28S (1036 bp) ribosomal DNA were genetically analyzed from 162 individual *I. exustus* samples, which represented 21 populations from Thailand, along with sequences from other geographical regions in GenBank.

Mitochondrial COI analysis was performed on 206 samples, revealing 48 haplotypes (I1-I48) with 170 variable nucleotide sites. Among these, 45 haplotypes were unique, while three (I1, I13, and I25) were shared across multiple populations. Notably, haplotype I25 was the most shared among populations in Benin, Gabon, Ivory Coast, and Malaysia. Moreover, the variation in haplotype diversity within individual populations varied from 0 in Benin to 1.0000 in Bangladesh and France (French West Indies), averaging at 0.5803. In terms of nucleotide diversity, the range within each population varied from 0 in Benin to 0.0816 in Nepal, with an overall mean of 0.0224 (Table 9). Additionally, among the 162 samples in the current study, a total of 23 haplotypes (I1-I23) were identified. Only the I1 and I4 haplotypes of *I. exustus* were infected with furcocercous cercaria and xiphidiocercariae, respectively. Haplotype I1 was widely distributed in 20 populations encompassing six geographic regions of Thailand (north, northeast, central, west, east, and south), accounting for 79.0%. The other frequent haplotypes included I2 and I4 (accounting for 2.47% each) and C8 and C16 (accounting for 1.85% each). The diversity of each haplotype in snail population in Thailand varied from 0 in Khon Kaen, Sukhothai, Ang Thong, Nakhon Nayok, and Tak to 0.7167 in Sing Buri, with an average of 0.3756. Furthermore, the diversity of the nucleotide in snail within each population showed variability, ranging from 0 in Khon Kaen, Sukhothai, Ang Thong, Nakhon Nayok, and Tak to 0.0046 in Sing Buri, with an overall mean of 0.0021 (Table 10).

Table 9 Diversity indices of the COI sequences in the *I. exustus* populations from Thailand and various geographical regions.

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Thailand	162	60	23	2	21	0.3752 ± 0.0497	0.0021 ± 0.0014
Bangladesh	6	18	6	0	6	1.0000 ± 0.0962	0.0118 ± 0.0075
Benin	5	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
France	2	10	2	0	2	1.0000 ± 0.5000	0.0176 ± 0.0184
Gabon	1	0	1	1	0	NA	NA
India	1	0	1	0	1	NA	NA
Indonesia	1	0	1	0	1	NA	NA
Ivory Coast	1	0	1	1	0	NA	NA
Laos	1	0	1	1	0	NA	NA
Malaysia	4	5	2	2	0	0.6667 ± 0.2041	0.0058 ± 0.0045
Nepal	15	119	9	0	9	0.9048 ± 0.0544	0.0816 ± 0.0421
Oman	4	3	3	0	3	0.8333 ± 0.2224	0.0029 ± 0.0025
Philippines	1	0	1	1	0	NA	NA

Table 9 (Cont.)

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Sri Lanka	1	0	1	0	1	NA	NA
Vietnam	1	0	1	0	1	NA	NA
Total	**206**	**170**	**48**	**3**	**45**	**0.5803 ± 0.0431**	**0.0224 ± 0.0112**

Note: NA = not calculated due to the constraints of a small sample size.

Table 10 Diversity indices of COI sequences in the *I. exustus* populations from 21 populations of Thailand.

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Uttaradit	1	0	1	1	0	NA	NA
Lamphun	6	5	2	2	0	0.3333 ± 0.2152	0.0029 ± 0.0023
Lampang	1	0	1	1	0	NA	NA

Table 10 (Cont.)

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Chaiyaphum	8	9	2	1	1	0.2500 ± 0.1802	0.0039 ± 0.0027
Khon Kaen	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Udon Thani	6	1	2	2	0	0.3333 ± 0.2152	0.0005 ± 0.0007
Phitsanulok	29	27	6	2	4	0.4729 ± 0.1098	0.0043 ± 0.0027
Sukhothai	3	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phichit	11	1	2	1	1	0.1818 ± 0.1436	0.0003 ± 0.0005
Phetchabun	1	0	1	1	0	NA	NA
Chai Nat	18	11	6	2	4	0.5621 ± 0.1342	0.0023 ± 0.0016
Sing Buri	16	11	6	2	4	0.7167 ± 0.0988	0.0046 ± 0.0029
Nakhon Sawan	9	1	2	1	1	0.2222 ± 0.1662	0.0003 ± 0.0005
Ang Thong	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Ayuthaya	1	0	1	1	0	NA	NA
Nakhon Nayok	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Tak	15	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000

Table 10 (Cont.)

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Chanthaburi	1	0	1	0	1	NA	NA
Chon Buri	10	4	4	2	2	0.5333 ± 0.1801	0.0014 ± 0.0012
Pattani	3	1	2	1	1	0.6667 ± 0.3143	0.0011 ± 0.0014
Songkhla	17	1	2	2	0	0.2206 ± 0.1208	0.0003 ± 0.0005
Total	**162**	**60**	**23**	**4**	**19**	**0.3756 ± 0.0505**	**0.0021 ± 0.0015**

Note: NA = not calculated due to the constraints of a small sample size.

Analysis of the 16S rDNA (206 sequences) identified 73 variable nucleotide sites, which were identified and classified into 18 haplotypes (I1-I18). Seventeen haplotypes were unique, and one (I1) was shared by multiple populations. Haplotype I1 was found in populations in Thailand, Benin, Gabon, Indonesia, Ivory Coast, Laos, Malaysia, Nepal, Oman, Philippines, and Vietnam. Moreover, the haplotype diversity within each population of snails varied from 0 in Benin, France, Oman, and Malaysia to 0.9333 in Bangladesh, with an overall mean of 0.2954. Nucleotide diversity within each snail population ranged from 0 in Benin, France, Oman, and Malaysia to 0.0718 in Nepal, with an overall mean of 0.0136 (Table 11). Among the *I. exustus* (162 sequences) from Thailand, 4 haplotypes (I1-I4) were identified. Only the I1 haplotype was infected with furcocercous cercaria and xiphidiocercariae. Haplotype diversity within each population in Thailand varied from 0 in Lamphun, Chaiyaphum, Khon Kaen, Udon Thani, Phitsanulok, Sukhothai, Phichit, Chai Nat, Nakhon Sawan, Nakhon Nayok, Tak, Chon Buri, and Pattani to 1.0000 in Ang Thong, with an average of 0.1214. Meanwhile, nucleotide diversity within each snail population spanned from 0 in Lamphun, Chaiyaphum, Khon Kaen, Udon Thani, Phitsanulok, Sukhothai, Phichit, Chai Nat, Nakhon Sawan, Nakhon Nayok, Tak, Chon Buri, and Pattani to 0.0026 in Ang Thong, with an average of 0.0003 (Table 12).

Table 11 Diversity indices of 16S rDNA sequences in the *I. exustus* populations from Thailand and various geographical regions.

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Thailand	162	3	4	1	3	0.1179 ± 0.0340	0.0003 ± 0.0005
Bangladesh	6	42	5	0	5	0.9333 ± 0.1217	0.0384 ± 0.0232
Benin	5	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
France	2	0	1	0	1	0.0000 ± 0.0000	0.0000 ± 0.0000
Gabon	1	0	1	1	0	NA	NA
India	1	0	1	0	1	NA	NA
Indonesia	1	0	1	1	0	NA	NA
Ivory Coast	1	0	1	1	0	NA	NA
Laos	1	0	1	1	0	NA	NA
Malaysia	4	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Nepal	15	64	7	1	6	0.8476 ± 0.0648	0.0718 ± 0.0373
Oman	4	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Philippines	1	0	1	1	0	NA	NA

Table 11 (Cont.)

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Sri Lanka	1	0	1	0	1	NA	NA
Vietnam	1	0	1	1	0	NA	NA
Total	**206**	**73**	**18**	**1**	**17**	**0.2954 ± 0.0429**	**0.0136 ± 0.0073**

Note: NA = not calculated due to the constraints of a small sample size.

Table 12 Diversity indices of 16S rDNA sequences in the *I. exustus* populations from 21 populations of Thailand.

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Uttaradit	1	0	1	1	0	NA	NA
Lamphun	6	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Lampang	1	0	1	1	0	NA	NA

Table 12 (Cont.)

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Chaiyaphum	8	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Khon Kaen	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Udon Thani	6	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phitsanulok	29	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Sukhothai	3	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phichit	11	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phetchabun	1	0	1	1	0	NA	NA
Chai Nat	18	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Sing Buri	16	1	2	1	1	0.1250 ± 0.1064	0.0003 ± 0.0005
Nakhon Sawan	9	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Ang Thong	2	1	2	1	1	1.0000 ± 0.5000	0.0026 ± 0.0037
Ayuthaya	1	0	1	1	0	NA	NA

Table 12 (Cont.)

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Nakhon Nayok	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Tak	15	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Chanthaburi	1	0	1	1	0	NA	NA
Chon Buri	10	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Pattani	3	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Songkhla	17	1	2	1	1	0.5294 ± 0.0450	0.0013 ± 0.0013
Total	**162**	**3**	**4**	**1**	**3**	**0.1214 ± 0.0349**	**0.0003 ± 0.0005**

Note: NA = not calculated due to the constraints of a small sample size.

Regarding the analysis of 950 bp of combined mtDNA sequences (COI+ 16S rDNA) that were obtained from 206 individuals, a total of 53 haplotypes (I1-I53) were identified with 243 variable nucleotide sites. Out of these, 50 haplotypes of snails were unique, and three (I1, I13, and I28) were shared among multiple populations. Haplotype I28 exhibited the highest level of sharing among populations in Benin, Gabon, Ivory Coast, and Malaysia. Moreover, haplotype diversity of snails varied from 0 in Benin to 1.0000 in Bangladesh and France, with an average of 0.6419, and nucleotide diversity varied from 0 in Benin to 0.0780 in Nepal, with an overall mean of 0.0189 (Table 13). Among the samples from Thailand, 26 haplotypes (I1-I26) were identified. Only I1 and I4 haplotypes were infected with furcocercous cercaria and xiphidiocercariae, respectively. Additionally, the I1 haplotype had the highest frequency and was dispersed in all populations (except for Chanthaburi province), accounting for 72.80%. Haplotype diversity in Thailand varied from 0 in Khon Kaen, Sukhothai, Nakhon Nayok, and Tak to 1.0000 in Ang Thong, with an overall average of 0.4701, and nucleotide diversity of snail ranged from 0 in Khon Kaen, Sukhothai, Nakhon Nayok, and Tak to 0.0029 in Sing Buri, with an overall mean of 0.0014 (Table 14).

Table 13 Diversity indices of the combined mtDNA in the *I. exustus* populations from Thailand and various geographical regions.

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Thailand	162	63	26	2	24	0.4670 ± 0.0496	0.0013 ± 0.0009
Bangladesh	6	61	6	0	6	1.0000 ± 0.0962	0.0231 ± 0.0137
Benin	5	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
France	2	10	2	0	2	1.0000 ± 0.5000	0.0105 ± 0.0111
Gabon	1	0	1	1	0	NA	NA
India	1	0	1	0	1	NA	NA
Indonesia	1	0	1	0	1	NA	NA
Ivory Coast	1	0	1	1	0	NA	NA
Laos	1	0	1	1	0	NA	NA
Malaysia	4	5	2	2	0	0.6667 ± 0.2041	0.0035 ± 0.0027
Nepal	15	183	11	0	11	0.9429 ± 0.0454	0.0780 ± 0.0399
Oman	4	3	3	0	3	0.8333 ± 0.2224	0.0017 ± 0.0015
Philippines	1	0	1	1	0	NA	NA

Table 13 (Cont.)

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Sri Lanka	1	0	1	0	1	NA	NA
Vietnam	1	0	1	0	1	NA	NA
Total	**206**	**243**	**53**	**3**	**50**	**0.6419 ± 0.0406**	**0.0189 ± 0.0093**

Note: NA = not calculated due to the constraints of a small sample size.

Table 14 Diversity indices of the combined mtDNA in the *I. exustus* populations from 21 provinces of Thailand.

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Uttaradit	1	0	1	1	0	NA	NA
Lamphun	6	5	2	2	0	0.3333 ± 0.2152	0.0017 ± 0.0013
Lampang	1	0	1	1	0	NA	NA

Table 14 (Cont.)

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Chaiyaphum	8	9	2	1	1	0.2500 ± 0.1802	0.0023 ± 0.0016
Khon Kaen	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Udon Thani	6	1	2	2	0	0.3333 ± 0.2152	0.0003 ± 0.0004
Phitsanulok	29	27	6	2	4	0.4729 ± 0.1098	0.0026 ± 0.0016
Sukhothai	3	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phichit	11	1	2	1	1	0.1818 ± 0.1436	0.0001 ± 0.0003
Phetchabun	1	0	1	1	0	NA	NA
Chai Nat	18	11	6	2	4	0.5621 ± 0.1342	0.0013 ± 0.0010
Sing Buri	16	12	7	2	5	0.7750 ± 0.0876	0.0029 ± 0.0018
Nakhon Sawan	9	1	2	1	1	0.2222 ± 0.1662	0.0002 ± 0.0003
Ang Thong	2	1	2	1	1	1.0000 ± 0.5000	0.0011 ± 0.0014
Ayuthaya	1	0	1	1	0	NA	NA
Nakhon Nayok	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Tak	15	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000

Table 14 (Cont.)

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Chanthaburi	1	0	1	0	1	NA	NA
Chon Buri	10	4	4	2	2	0.5333 ± 0.1801	0.0008 ± 0.0007
Pattani	3	1	2	1	1	0.6667 ± 0.3143	0.0007 ± 0.0008
Songkhla	17	2	3	2	1	0.6324 ± 0.0661	0.0008 ± 0.0006
Total	**162**	**63**	**26**	**4**	**22**	**0.4701 ± 0.0503**	**0.0014 ± 0.0009**

Note: NA = not calculated due to the constraints of a small sample size.

The sequence alignment of 194 *I. exustus* samples based on the ITS1 region showed 131 variable nucleotide sites. These nucleotide samples were classified into 22 haplotypes (I1-I22), of which there were twenty unique haplotypes and two shared haplotypes (I1 and I13). Haplotype I1 was the most shared among the populations found in Thailand, Benin, Gabon, Ivory Coast, Malaysia, Oman, and Vietnam. Moreover, haplotype diversity varied from 0 in Benin, Oman, and Malaysia to 0.9000 in Bangladesh, with an overall mean of 0.3487, and nucleotide diversity of snails ranged from 0 in Benin, Oman, and Malaysia to 0.0815 in Nepal, with an average of 0.0122 (Table 15). Among the *I. exustus* in Thailand, 10 haplotypes (I1-I10) were identified. Only the I1 haplotype was infected with furcocercous cercaria and xiphidiocercariae. Haplotype I1 was dispersed in all populations (except Sukhothai province), accounting for 89.50% of the haplotypes. Furthermore, haplotype diversity for Thailand ranged from 0 in Khon Kaen, Udon Thani, Sukhothai, Phichit, Chai Nat, Ang Thong, Chon Buri, and Songkhla to 1.0000 in Nakhon Nayok, with an overall mean of 0.2040, and nucleotide diversity of snails ranged from 0 in Khon Kaen, Udon Thani, Sukhothai, Phichit, Chai Nat, Ang Thong, Chon Buri, and Songkhla to 0.0033 in Nakhon Nayok, with an overall mean of 0.0003 (Table 16).

Table 15 Diversity indices of ITS1 sequences in the *I. exustus* populations from Thailand and various geographical regions.

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Thailand	162	9	10	1	9	0.1981 ± 0.0422	0.0003 ± 0.0005
Bangladesh	5	25	4	1	3	0.9000 ± 0.1610	0.0201 ± 0.0128
Benin	5	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
France	3	2	2	0	2	0.6667 ± 0.3143	0.0022 ± 0.0022
Gabon	1	0	1	1	0	NA	NA
Ivory Coast	1	0	1	1	0	NA	NA
Malaysia	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Nepal	12	116	7	1	6	0.7727 ± 0.1276	0.0815 ± 0.0427
Oman	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Vietnam	1	0	1	1	0	NA	NA
Total	**194**	**131**	**22**	**2**	**20**	**0.3487 ± 0.0453**	**0.0122 ± 0.0063**

Note: NA = not calculated due to the constraints of a small sample size.

Table 16 Diversity indices of ITS1 sequences in the *I. exustus* populations from 21 provinces of Thailand.

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Uttaradit	1	0	1	1	0	NA	NA
Lamphun	6	1	2	1	1	0.3333 ± 0.2152	0.0005 ± 0.0007
Lampang	1	0	1	1	0	NA	NA
Chaiyaphum	8	2	2	1	1	0.2500 ± 0.1802	0.0008 ± 0.0008
Khon Kaen	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Udon Thani	6	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phitsanulok	29	3	4	2	2	0.2586 ± 0.1042	0.0004 ± 0.0005
Sukhothai	3	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phichit	11	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phetchabun	1	0	1	1	0	NA	NA
Chai Nat	18	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Sing Buri	16	1	2	2	0	0.2333 ± 0.1256	0.0003 ± 0.0005
Nakhon Sawan	9	2	3	2	1	0.4167 ± 0.1907	0.0007 ± 0.0008

Table 16 (Cont.)

Location	No. of *I. exustus* examined	No. of variable sites	No. of haplotypes	Shared haplotypes	Unique haplotypes	Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
Ang Thong	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Ayuthaya	1	0	1	1	0	NA	NA
Nakhon Nayok	2	2	2	1	1	1.0000 ± 0.5000	0.0033 ± 0.0041
Tak	15	1	2	2	0	0.1333 ± 0.1123	0.0002 ± 0.0003
Chanthaburi	1	0	1	1	0	NA	NA
Chon Buri	10	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Pattani	3	1	2	1	1	0.6667 ± 0.3143	0.0011 ± 0.0013
Songkhla	17	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Total	**162**	**9**	**10**	**3**	**7**	**0.2040 ± 0.0433**	**0.0003 ± 0.0005**

Note: NA = not calculated due to the constraints of a small sample size.

The 18S (339 bp) and 28S rDNA (1036 bp) were genetically analyzed from the 44 individual *I. exustus* samples, which represented 21 provinces from Thailand. The 28S rDNA data revealed that *I. exustus* displayed the highest intraspecific genetic variance, ranging from 0% to 0.78%. On the contrary, the 18S rDNA gene exhibited no variation, with a pairwise genetic distance of 0%, signifying the existence of a sole haplotype. The haplotype analysis based on 28S rDNA from 44 *I. exustus* sequences exposed the presence of 8 different haplotypes (I1-I8) with 17 variable sites (Table 17). The genetic distances among haplotypes exhibited variation, ranging from 0.001 to 0.010 (Table 18). Out of these, 6 haplotypes (I2, I3, I4, I6, I7, and I8) were unique, and 2 haplotypes (I1 and I5) were shared by at least two regions. Haplotype I1 was the most widely distribution covering all regions of Thailand including north, northeast, central, west, east, and south, which accounted for 81.82% of all samples and 100% of all populations. In contrast, the haplotype I5 was mainly distributed along the south and central of Thailand, which made up 4.55% of all samples and 33.33% of all populations. The genetic divergence within each population varied, ranging from 0% in the west and east regions to 0.24% in the north region, with an overall mean of 0.09% (Table 19).

Table 17 Comparative analysis of nucleotide sequence variation within the 28S rDNA gene among the 8 haplotypes of *I. exustus*.

Haplotype	Nucleotide positions															
	3	**5**	**20**	**28**	**29**	**93**	**96**	**69**	**70**	**76**	**77**	**88**	**95**	**130**	**132**	**133**	**134**
I1	C	A	G	C	C	G	G	A	G	A	C	G	G	G	A	T	A
I2	C	A	G	C	C	G	A	C	A	A	C	A	A	G	A	T	A
I3	A	A	G	C	C	G	G	A	A	A	C	G	G	G	A	A	G
I4	C	G	G	C	C	G	G	A	A	A	C	G	G	G	A	T	A
I5	C	A	G	C	C	C	G	A	A	A	C	G	G	G	A	T	A
I6	C	A	C	G	C	G	G	A	A	T	A	G	G	G	A	T	G
I7	C	A	G	C	G	G	G	A	A	A	C	G	G	G	A	T	A
I8	C	A	G	C	C	G	G	A	A	A	C	G	G	T	T	T	T

Table 18 Genetic distance between haplotypes of *I. exustus* using 28S rDNA sequences.

Haplotypes	I1	I2	I3	I4	I5	I6	I7	I8
I1	-							
I2	0.005	-						
I3	0.003	0.008	-					
I4	0.001	0.006	0.004	-				
I5	0.001	0.006	0.004	0.002	-			
I6	0.005	0.010	0.006	0.006	0.006	-		
I7	0.001	0.006	0.004	0.002	0.002	0.006	-	
I8	0.003	0.008	0.005	0.004	0.004	0.007	0.004	-

Table 19 Genetic divergence and haplotype distribution of *I. exustus* from Thailand based on 28S rDNA sequences.

Population	No. sequences	Genetic divergence (%)	No. haplotypes	Haplotype frequencies							
				I1	I2	I3	I4	I5	I6	I7	I8
North	4	0.24	2	3	1	0	0	0	0	0	0
Northeast	6	0.19	3	4	0	0	0	0	1	1	0
Central	23	0.03	3	21	0	0	0	1	0	0	1
West	2	0	1	2	0	0	0	0	0	0	0
East	3	0	1	3	0	0	0	0	0	0	0
South	6	0.16	4	3	0	1	1	1	0	0	0
Total	**44**	**0.09**	**8**	**36**	**1**	**1**	**1**	**2**	**1**	**1**	**1**

2. *Radix rubiginosa*

Cytochrome oxidase subunit I (520 bp), 16S rDNA (371 bp), and combined dataset of two mitochondrial DNA (mtDNA) regions (891 bp) were genetically analyzed from 116 individual *R. rubiginosa* samples, which represented 17 populations from Thailand, along with sequences from other geographical regions in GenBank.

Mitochondrial COI analysis was performed on 116 samples, revealing 23 haplotypes (R1-R23) with 41 variable nucleotide sites. Of these, 18 haplotypes (78%) were specific to individual populations, while five (22%) were shared among multiple populations. Eight populations (47%) comprised multiple haplotypes, whereas nine had a single haplotype. Haplotype R1 was the most common, found in 23 individuals (19.8% of all samples) across five populations (29.4% of all populations), predominantly in central and southern Thailand. The observed average haplotype diversity (h) of 0.8085 and nucleotide diversity (π) of 0.0097 indicated high haplotype diversity but relatively low nucleotide diversity. Details of the genetic diversity indices for snails across all provinces are provided in Table 20.

Analyzing 371 bp of the 16S rDNA sequences from 116 individuals revealed the identification of 15 haplotypes (R1-R15) with 17 variable nucleotide sites. Out of these, 10 haplotypes (67%) were exclusive, and five haplotypes (33%) were shared by multiple populations. Nine populations (53%) consisted of multiple haplotypes, and eight populations (47%) had a single haplotype. Haplotype R1 was the most shared among the populations, found in 54 individuals (46.6% of all samples) across eight populations (47% of all populations) in five regions (central, east, north, south, and west) of Thailand. The haplotype diversity of snails ranged from 0 to 0.8000, averaging at 0.7214, while the nucleotide diversity of snail varied from 0 to 0.0108, with an overall mean of 0.0068 (Table 21).

The analysis of 891 bp of combined mtDNA sequences (COI+16S rDNA) that were obtained from 116 individuals, which revealed 32 different haplotypes (R1-R32) with 58 variable sites. Among these haplotypes, 25 haplotypes (72%) were private to their specific populations, while 7 haplotypes (22%) were shared by at least two localities. Eight (47%) populations comprised multiple haplotypes, and nine populations had a single haplotype. Haplotype R1 was the most common, found in 18 individuals (15.5% of all samples) across 5 populations (29.4% of all populations), mainly in the central and southern parts of Thailand. There is average haplotype diversity (h) of 0.9084 and nucleotide diversity (π) of 0.0085. The genetic diversity indices of snails from all provinces are provided in Table 22.

Table 20 Diversity indices of COI sequences in the *R. rubiginosa* populations from 17 provinces of Thailand.

Location	No. of sequences	Segregation sites	Haplotype			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
			No. of haplotypes	Shared haplotypes	Unique haplotypes		
Ang Thong	1	0	1	1	0	NA	NA
Ayutthaya	1	0	1	1	0	NA	NA
Chai Nat	13	19	7	3	4	0.8462 ± 0.0854	0.0121 ± 0.0068
Nakhon Sawan	12	15	6	2	4	0.8182 ± 0.0957	0.0108 ± 0.0063
Nakhon Nayok	12	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phichit	10	12	3	1	2	0.3778 ± 0.1813	0.0049 ± 0.0033
Phitsanulok	6	7	2	2	0	0.5333 ± 0.1721	0.0072 ± 0.0049
Phetchabun	12	5	3	1	2	0.6212 ± 0.0867	0.0045 ± 0.0029
Sing Buri	1	0	1	0	1	NA	NA
Saraburi	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Sukhothai	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Chachoengsao	5	6	2	0	2	0.6000 ± 0.1753	0.0069 ± 0.0049
Phayao	10	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000

Table 20 (Cont.)

Location	No. of sequences	Segregation sites	Haplotype			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
			No. of haplotypes	Shared haplotypes	Unique haplotypes		
Khon Kaen	3	6	2	1	1	0.6667 ± 0.3143	0.0077 ± 0.0065
Pattani	11	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Songkhla	12	11	3	2	1	0.3182 ± 0.1637	0.0035 ± 0.0025
Tak	3	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Total	**116**	**41**	**23**	**5**	**18**	**0.8085 ± 0.0129**	**0.0097 ± 0.0053**

Note: NA = not calculated due to the constraints of a small sample size.

Table 21 Diversity indices of 16S rDNA sequences in the *R. rubiginosa* populations from 17 provinces of Thailand.

Location	No. of sequences	Segregation sites	Haplotype			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
			No. of haplotypes	Shared haplotypes	Unique haplotypes		
Ang Thong	1	0	1	1	0	NA	NA
Ayutthaya	1	0	1	0	1	NA	NA
Chai Nat	13	9	5	3	2	0.7564 ± 0.0974	0.0098 ± 0.0059
Nakhon Sawan	12	6	4	4	0	0.7727 ± 0.0825	0.0077 ± 0.0049
Nakhon Nayok	12	4	2	1	1	0.3030 ± 0.1475	0.0033 ± 0.0025
Phichit	10	1	2	1	1	0.2000 ± 0.1541	0.0005 ± 0.0008
Phitsanulok	6	7	4	2	2	0.8000 ± 0.1721	0.0101 ± 0.0069
Phetchabun	12	2	3	2	1	0.3182 ± 0.1637	0.0009 ± 0.0011
Sing Buri	1	0	1	1	0	NA	NA
Saraburi	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Sukhothai	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Chachoengsao	5	1	2	2	0	0.6000 ± 0.1753	0.0016 ± 0.0018
Phayao	10	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000

Table 21 (Cont.)

Location	No. of sequences	Segregation sites	Haplotype			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
			No. of haplotypes	Shared haplotypes	Unique haplotypes		
Khon Kaen	3	6	2	1	1	0.6667 ± 0.3143	0.0108 ± 0.0092
Pattani	11	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Songkhla	12	4	3	2	1	0.3182 ± 0.1637	0.0029 ± 0.0023
Tak	3	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Total	**116**	**17**	**15**	**5**	**10**	**0.7214 ± 0.0364**	**0.0068 ± 0.0041**

Note: NA = not calculated due to the constraints of a small sample size.

Table 22 Diversity indices of combined mtDNA sequences in the *R. rubiginosa* populations from 17 provinces of Thailand.

Location	No. of sequences	Segregation sites	Haplotype			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
			No. of haplotypes	Shared haplotypes	Unique haplotypes		
Ang Thong	1	0	1	1	0	NA	NA
Ayutthaya	1	0	1	0	1	NA	NA
Chai Nat	13	28	8	3	5	0.8590 ± 0.0886	0.0111 ± 0.0061
Nakhon Sawan	12	21	7	3	4	0.9091 ± 0.0562	0.0095 ± 0.0053
Nakhon Nayok	12	4	2	1	1	0.3030 ± 0.1475	0.0014 ± 0.0011
Phichit	10	13	4	1	3	0.5333 ± 0.1801	0.0031 ± 0.0020
Phitsanulok	6	14	4	2	2	0.8000 ± 0.1721	0.0083 ± 0.0053
Phetchabun	12	7	5	2	3	0.7576 ± 0.0927	0.0030 ± 0.0019
Sing Buri	1	0	1	0	1	NA	NA
Saraburi	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Sukhothai	2	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Chachoengsao	5	7	2	0	2	0.6000 ± 0.1753	0.0047 ± 0.0033
Phayao	10	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000

Table 22 (Cont.)

Location	No. of sequences	Segregation sites	Haplotype			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
			No. of haplotypes	Shared haplotypes	Unique haplotypes		
Khon Kaen	3	12	2	1	1	0.6667 ± 0.3143	0.0089 ± 0.0072
Pattani	11	0	1	0	1	0.0000 ± 0.0000	0.0000 ± 0.0000
Songkhla	12	15	3	2	1	0.3182 ± 0.1637	0.0033 ± 0.0021
Tak	3	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Total	**116**	**58**	**32**	**7**	**25**	**0.9084 ± 0.0102**	**0.0085 ± 0.0044**

Note: NA = not calculated due to the constraints of a small sample size.

3. *Orientogalba viridis*

Genetic analysis was conducted on 84 individual *O. viridis* samples from nine populations in Thailand, utilizing sequences for COI (520 bp), 16S rDNA (371 bp), and a combined dataset of two mitochondrial DNA (mtDNA) regions (891 bp). These sequences were complemented with data from other geographical regions sourced from GenBank.

The analysis of 520 bp of COI sequences, obtained from 84 individuals, identified 8 distinct haplotypes (O1-O8) with 9 variable sites. Among these haplotypes, 4 haplotypes (50%) were exclusive to specific populations, while the remaining four (50%) were shared among at least two localities. Five (56%) populations comprised multiple haplotypes, and four populations had a single haplotype. Haplotype O1 was the most common, found in 35 individuals (41.7% of all samples) across 6 populations (66.7% of all populations) in the central and north regions of Thailand. The haplotype diversity, with an average of 0.7105, suggested haplotype diversity with a high level, while the nucleotide diversity of this snail, at 0.0026, indicated a comparatively lower level of nucleotide diversity. The genetic diversity indices of this snail from all provinces are provided in Table 23.

Regarding the analysis of 371 bp of the 16S rDNA sequences illustrated 8 haplotypes (O1-O8) with 13 variable nucleotide sites. Out of these, 3 haplotypes of the snail (37%) were unique, and five haplotypes (63%) were shared among multiple populations. Six populations (67%) composed multiple haplotypes, and three populations (33%) had a single haplotype. Haplotype O1 was the most shared among the populations, found in 35 individuals (41.7% of all samples) across eight populations (67% of all populations) in the northern and central areas of Thailand. The range for haplotype diversity was 0 to 0.7229, averaging at 0.6591, while nucleotide diversity varied from 0 to 0.0046, with an overall mean of 0.0031 (Table 24).

Among the 84 sequences of *O. viridis* from 9 populations and analyzed using combined mtDNA sequences, fifteen distinct haplotypes (O1-O15) were identified, revealing 22 nucleotide variation sites. Of these, 5 haplotypes (constituting 33.3% of all haplotypes) were shared across multiple localities, while the remaining ten haplotypes were unique (66.7% of all haplotypes). The distribution pattern showed that six populations (66.7%) contained multiple haplotypes, while three populations exhibited a single haplotype. Notably, the most widespread haplotype, OV2, was present in samples from both the central and northern regions of Thailand, accounting for 36.9% of all samples and found in 55.6% of all populations. The average haplotype diversity was notably high at 0.7535, contrasting with the relatively low nucleotide diversity, averaging at 0.0028. Details of genetic diversity are shown in Table 25.

Table 23 Diversity indices of COI sequences in the *O. viridis* populations from 9 provinces of Thailand.

Location	No. of sequences	Segregation sites	Haplotype			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
			No. of haplotypes	Shared haplotypes	Unique haplotypes		
Nakhon Sawan	1	0	1	1	0	NA	NA
Phichit	11	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phitsanulok	22	6	4	3	1	0.7229 ± 0.0416	0.0045 ± 0.0028
Sing Buri	11	4	3	3	0	0.4727 ± 0.1617	0.0017 ± 0.0014
Uthai Thani	4	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Chiang Mai	10	2	3	2	1	0.6444 ± 0.1012	0.0015 ± 0.0013
Chiang Rai	3	1	2	1	1	0.6667 ± 0.3143	0.0013 ± 0.0016
Lampang	10	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Uttaradit	12	2	3	2	1	0.4394 ± 0.1581	0.0009 ± 0.0009
Total	**84**	**9**	**8**	**4**	**4**	**0.7105 ± 0.0295**	**0.0026 ± 0.0018**

Note: NA = not calculated due to the constraints of a small sample size.

Table 24 Diversity indices of 16S rDNA sequences in the *O. viridi*s populations from 9 provinces of Thailand.

Location	No. of sequences	Segregation sites	Haplotype			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
			No. of haplotypes	Shared haplotypes	Unique haplotypes		
Nakhon Sawan	1	0	1	1	0	NA	NA
Phichit	11	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phitsanulok	22	6	4	3	1	0.7229 ± 0.0416	0.0046 ± 0.0031
Sing Buri	11	3	3	3	0	0.4727 ± 0.1617	0.0019 ± 0.0017
Uthai Thani	4	1	2	2	0	0.5000 ± 0.2652	0.0014 ± 0.0017
Chiang Mai	10	7	5	3	2	0.6667 ± 0.1633	0.0038 ± 0.0029
Chiang Rai	3	1	2	2	0	0.6667 ± 0.3143	0.0018 ± 0.0022
Lampang	10	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Uttaradit	12	1	2	2	0	0.3030 ± 0.1475	0.0008 ± 0.0010
Total	**84**	**13**	**8**	**5**	**3**	**0.6591 ± 0.0300**	**0.0031 ± 0.0023**

Note: NA = not calculated due to the constraints of a small sample size.

Table 25 Diversity indices of combined mtDNA in the *O. viridis* populations from 9 provinces of Thailand.

Location	No. of sequences	Segregation sites	Haplotype			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
			No. of haplotypes	Shared haplotypes	Unique haplotypes		
Nakhon Sawan	1	0	1	1	0	NA	NA
Phichit	11	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Phitsanulok	22	12	6	3	3	0.7792 ± 0.0459	0.0046 ± 0.0026
Sing Buri	11	7	3	3	0	0.4727 ± 0.1617	0.0018 ± 0.0013
Uthai Thani	4	1	2	2	0	0.5000 ± 0.2652	0.0006 ± 0.0007
Chiang Mai	10	9	6	2	4	0.7778 ± 0.1374	0.0024 ± 0.0017
Chiang Rai	3	2	3	1	2	1.0000 ± 0.2722	0.0015 ± 0.0015
Lampang	10	0	1	1	0	0.0000 ± 0.0000	0.0000 ± 0.0000
Uttaradit	12	3	3	2	1	0.4394 ± 0.1581	0.0009 ± 0.0008
Total	**84**	**22**	**15**	**5**	**10**	**0.7535 ± 0.0310**	**0.0028 ± 0.0017**

Note: NA = not calculated due to the constraints of a small sample size.

Phylogenetic and network analysis of snails

1. *Indoplanorbis exustus*

The phylogenetic tree and haplotype network constructed from COI, 16S rDNA, combined mtDNA, and ITS1 sequences demonstrated consistent results, which can be divided into four clades (A to D) (Figures 31-34). *Indoplanorbis exustus* clade A was the largest group and the most widely distributed in many regions throughout the world. Furthermore, only clade A of *I. exustus* was infected with furcocercous cercaria and xiphidiocercariae. In addition, the phylogenetic analysis of *I. exustus* based on 18S and 28S rDNA sequences revealed consistent results. The topology of phylogenetic tree showed that the group of *I. exustus* was clearly separated from other snail genera in the family Planorbidae. Both phylogenetic tree construction methods (ML and NJ) based on the 44 sequences of 18S rDNA (339 bp) from the current study, along with three sequences obtained from GenBank, revealed only one group. This group exhibited a close relationship to *I. exustus* from India (GenBank accession no. AY577492), the Philippines (GenBank accession no. HM756308), and Thailand (GenBank accession no. AY282598), with the highest bootstrap values supported for 99% for both phylogenetic trees reconstructed by ML and NJ methods (Figure 35). Meanwhile, the phylogenetic analysis conducted on the 28S rDNA sequences (1,036 bp) from the 45 *I. exustus* samples (comprising 44 sequences from the present study and one from GenBank) revealed only one group. This group showed a close relationship exclusively with *I. exustus* from Thailand (GenBank accession no. AF435662), with branch support values of 99% for both ML and NJ methods (Figure 36). These results showed that there is only one group in the genetic structure of *I. exustus* in Thailand, based on the 18S and 28S rDNA sequence analysis.

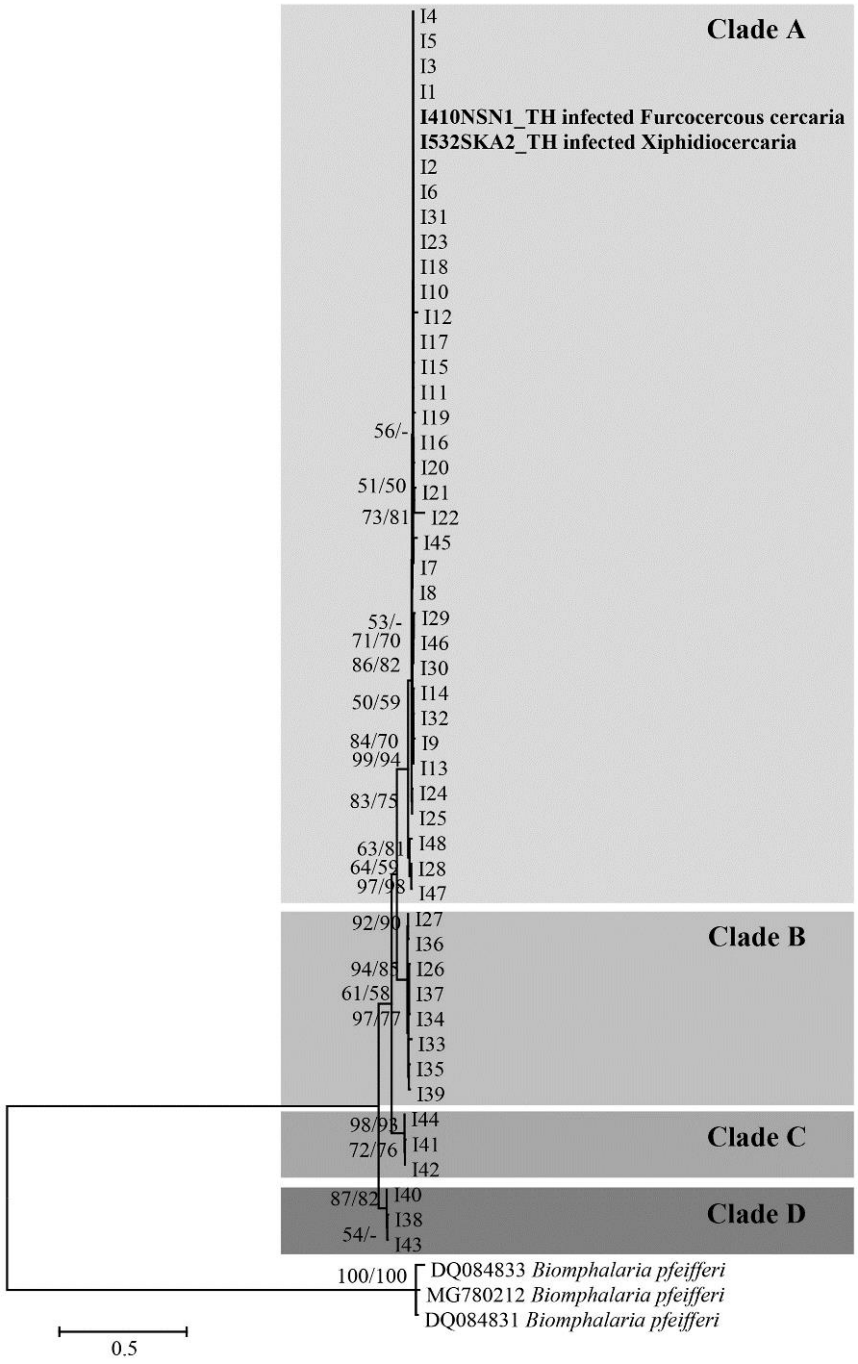

Figure 31 Maximum likelihood tree of COI sequences (569 bp) of 48 haplotypes generated from 206 sequences of *I. exustus* (162 sequences from several regions of Thailand and 44 sequences from various other geographical regions). The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Samples in bold indicate infected with trematode cercariae.

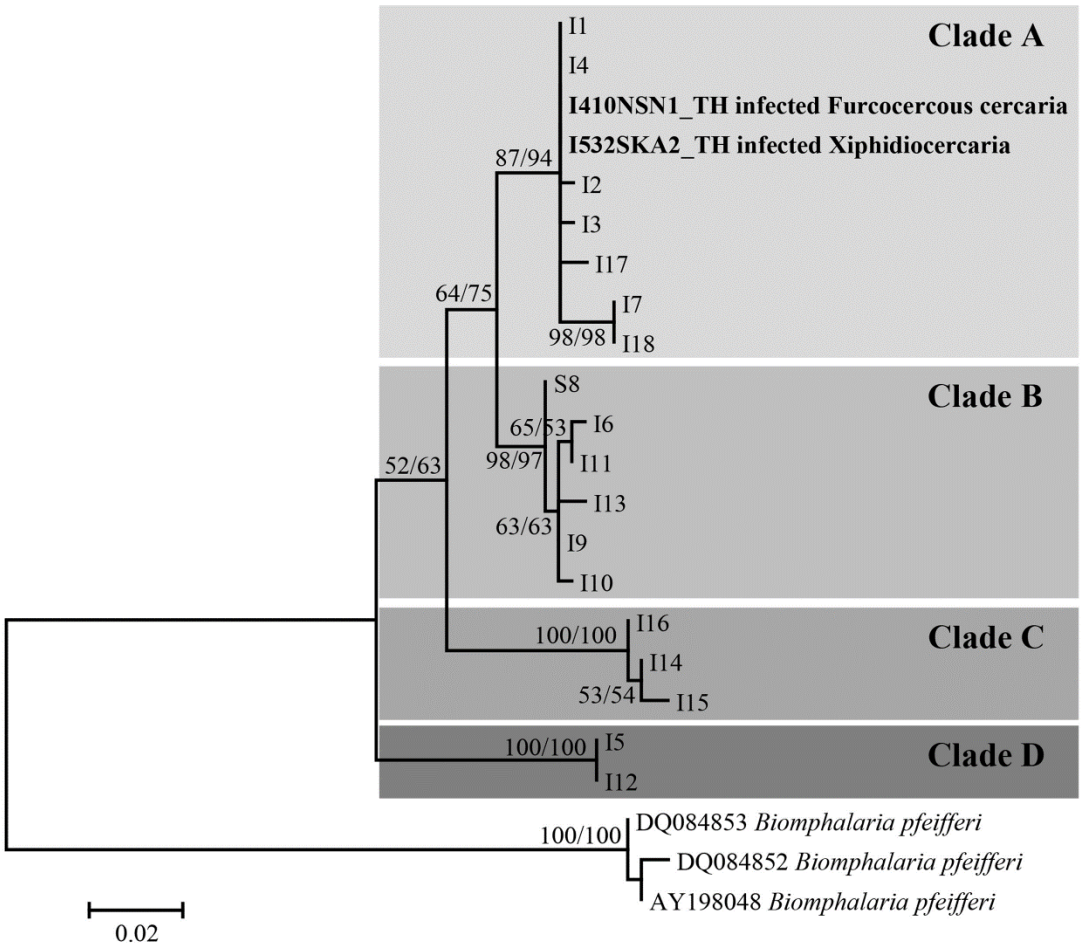

Figure 32 Maximum likelihood tree of 16S rDNA sequences (389 bp) of 18 haplotypes generated from 206 sequences of *I. exustus* (162 sequences from several regions of Thailand and 44 sequences from various other geographical regions). The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Samples in bold indicate infected with trematode cercariae.

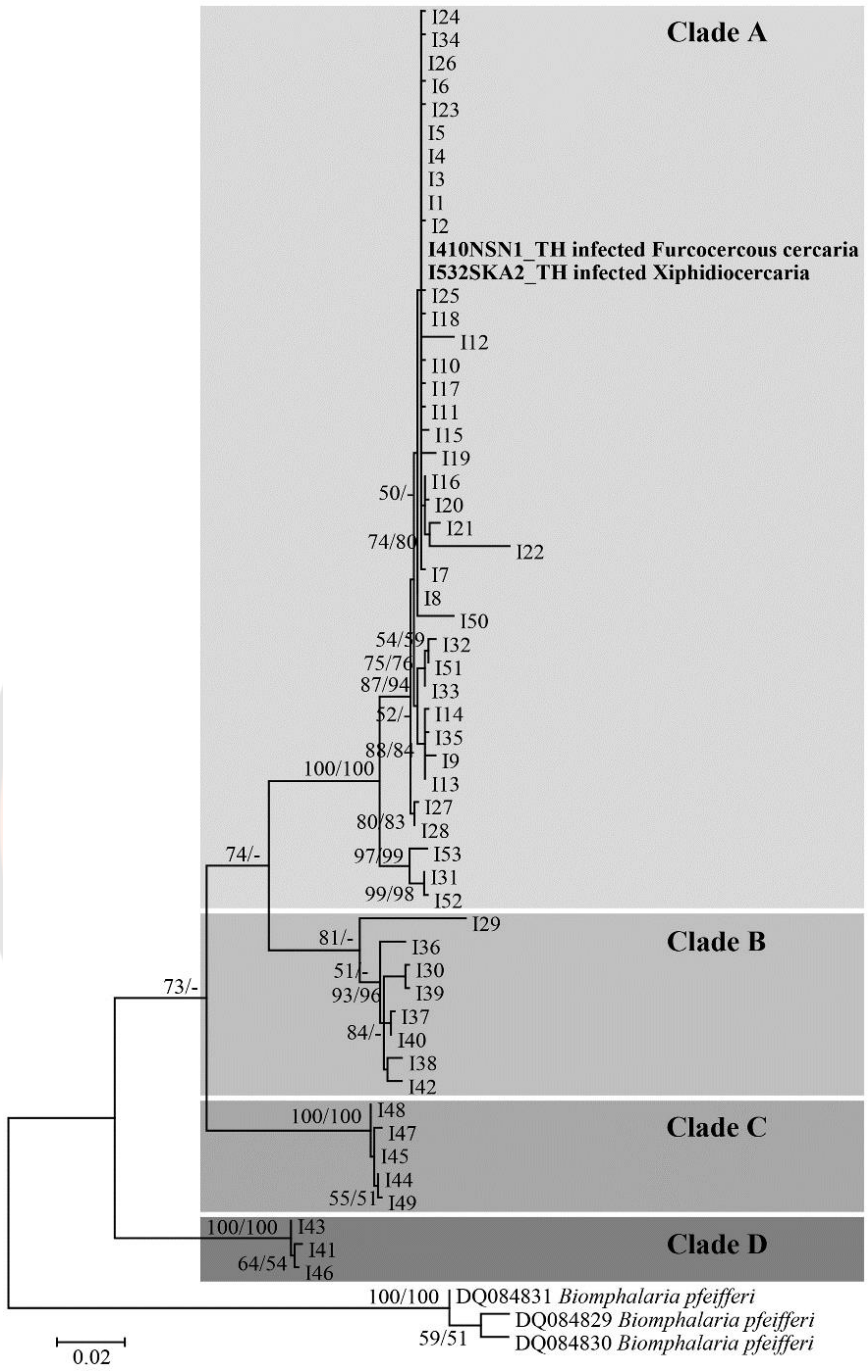

Figure 33 Maximum likelihood tree of the combined mitochondrial genes (950 bp) of 53 haplotypes generated from 206 sequences of *I. exustus* (162 sequences from several regions of Thailand and 44 sequences from various other geographical regions). The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Samples in bold indicate infected with trematode cercariae.

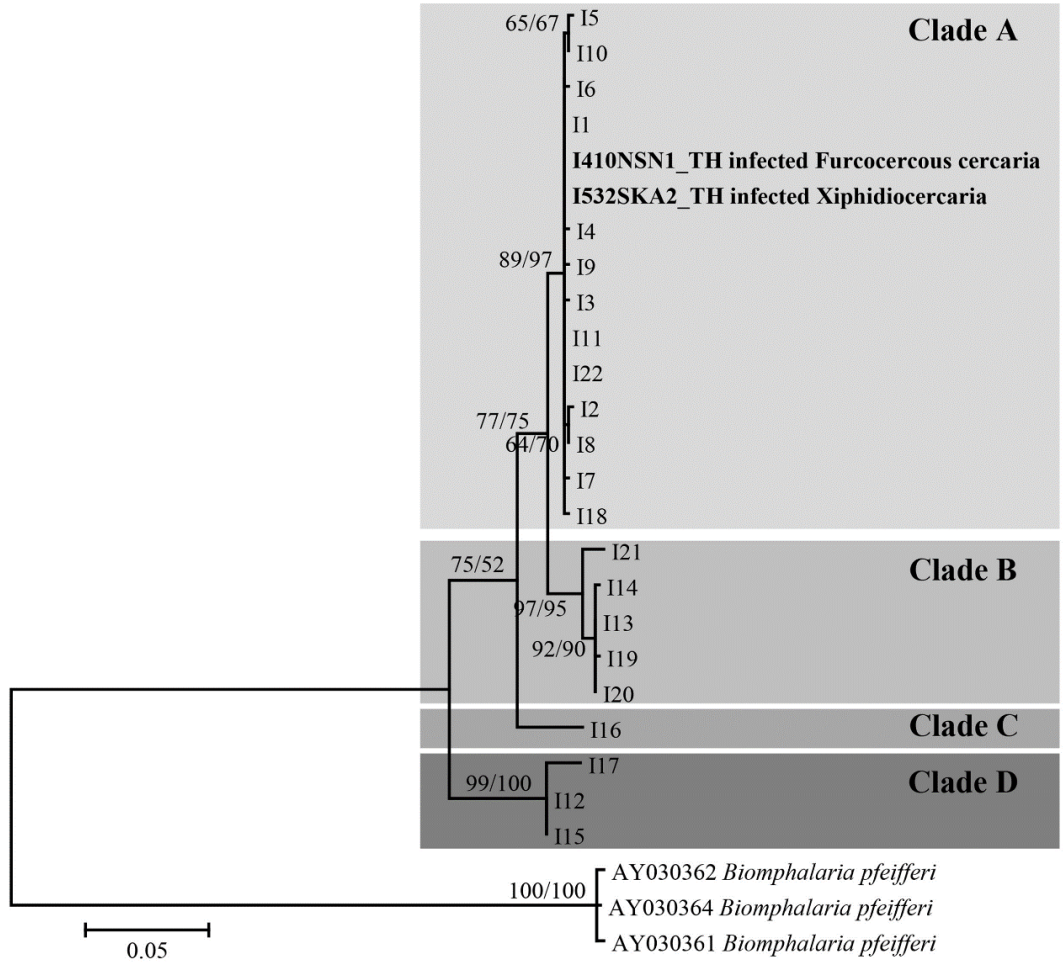

Figure 34 Maximum likelihood tree of ITS1 sequences (600 bp) of 22 haplotypes generated from 194 sequences of *I. exustus* (162 sequences from several regions of Thailand and 32 sequences from various other geographical regions). The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Samples in bold indicate infected with trematode cercariae.

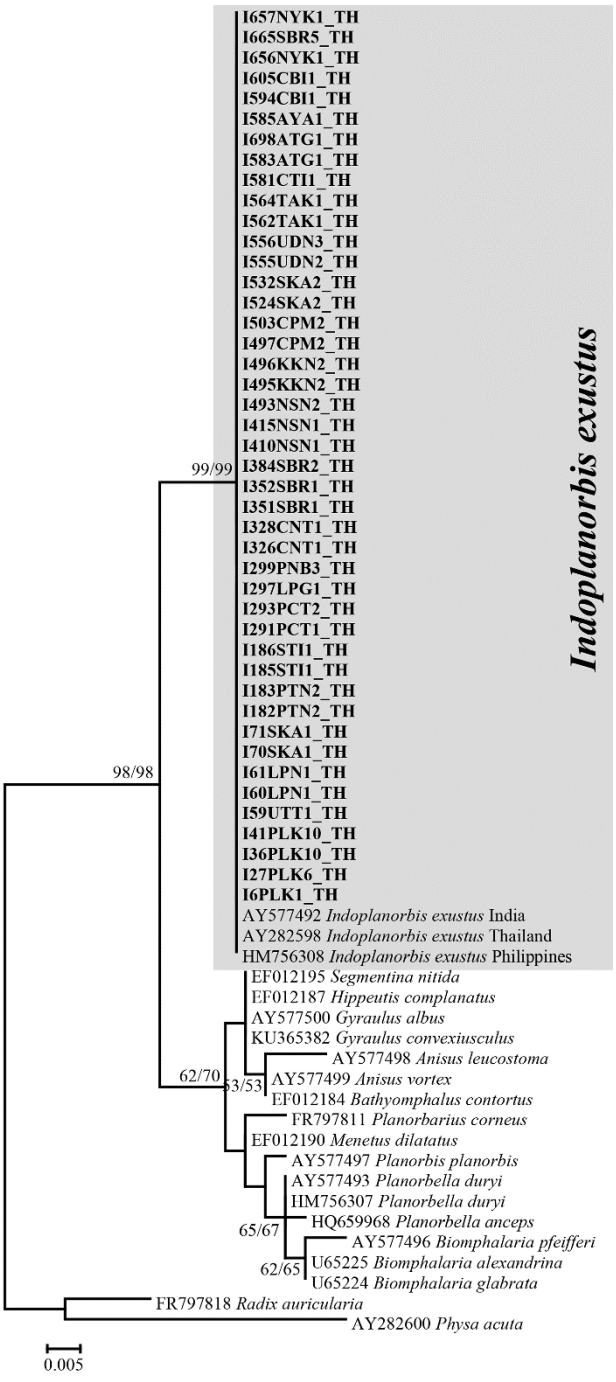

Figure 35 Maximum likelihood phylogenetic tree of *I. exustus* constructed using a partial 18S rDNA sequence (339 bp). The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Sequences obtained in the current study are highlighted with bold letters.

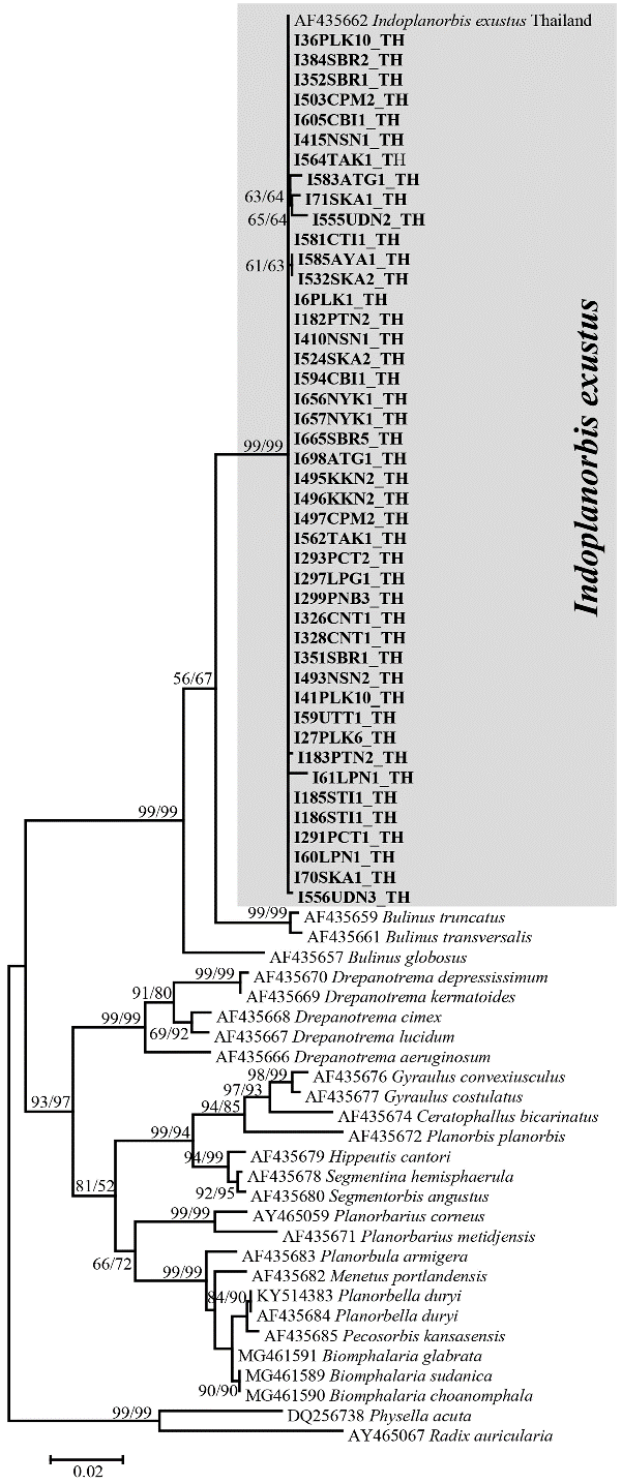

Figure 36 Maximum likelihood phylogenetic tree of *I. exustus* constructed using a partial 28S rDNA sequence (1036 bp). The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Sequences obtained in the current study are highlighted with bold letters.

The MJ network mitochondrial COI sequences (569 bp) were obtained from 162 sequences from individual *I. exustus* in Thailand and 44 COI sequences from other geographical regions in GenBank. Clade A was the largest group (186 sequences, 34 haplotypes) and contained all of the haplotypes from Thailand (I1-I23) together with haplotypes from Southwest Asia (Oman), Southeast Asia (Malaysia, Indonesia, Laos, Vietnam, and Philippines), South Asia (Sri Lanka and Nepal), West Africa (Benin and Ivory Coast), Central Africa (Gabon), and the Caribbean region (French West Indies). Clade B contained all the haplotypes (10 sequences, 8 haplotypes) from South Asia (Bangladesh, India, and Nepal), whereas clades C and D consisted of only samples from Nepal in South Asia, which contained 7 sequences (3 haplotypes) and 3 sequences (3 haplotypes), respectively (Figure 37). The estimates of evolutionary divergence indicate that the percentages "within clade" range from 0% to 7.05%, which are lower than the percentages "among clade," ranging from 8.11% to 16.93%. This observation suggests that the four identified clades represent distinct phylogenetic groups (Table 26).

The 16S rDNA sequences (381 bp) demonstrated topologies like those observed in the COI gene. Analyzing the 16S rDNA sequences, four genetically divergent clades were identified within the 162 individuals of *I. exustus* collected from several provinces of Thailand and 44 sequences originating from various other geographical regions. Levels of genetic differentiation among the four clades were much higher than those within clades. The genetic divergence of the 16S rDNA within clades was 0-1.84%, and that among clades was 3.16-10.05% (Table 26). Moreover, clade A included 7 haplotypes (186 sequences), with four haplotypes (I1-I4) from Thailand together with 3 other haplotypes from Southwest Asia (Oman), Southeast Asia (Malaysia, Indonesia, Laos, Vietnam, and Philippines), South Asia (Sri Lanka and Nepal), West Africa (Benin and Ivory Coast), Central Africa (Gabon), and the Caribbean region (French West Indies). Clade B consisted of 6 haplotypes (9 sequences) from South Asia (Bangladesh, India, and Nepal). Moreover, clade C contained 3 haplotypes (7 sequences) from only South Asia (Nepal). Clade D comprised 2 haplotypes (4 sequences) from Bangladesh and Nepal in South Asia (Figure 38).

The MJ network of combined mtDNA sequences (950 bp) revealed four genetically distinct clades, consistent with the patterns observed in both mitochondrial COI and 16S rDNA sequences. Clade A consisted of 37 haplotypes (186 sequences)

with haplotypes I1-I26 from Thailand and was closely related to haplotypes from South Asia, Southeast Asia, Southwest Asia (Oman), West Africa, Central Africa, and the Caribbean region. Clades B, C, and D contained haplotypes from only South Asia (Figure 39). The estimates of evolutionary divergence within clades and among clades are shown in Table 26.

The MJ network constructed using ITS1 sequences (600 bp) revealed four distinct clades which exhibited similarities to those observed in COI, 16S rDNA, and the combined mitochondrial DNA dataset (Figure 40). The estimates of evolutionary divergence within clades and among clades are shown in Table 26.

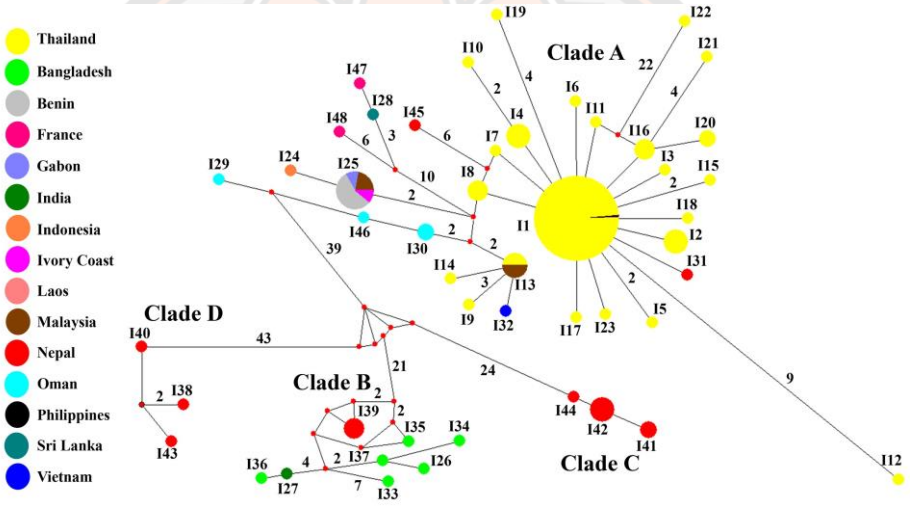

Figure 37 Median-joining network of *I. exustus* from Thailand and various geographical regions, constructed using COI sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small red dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. Numbers on each branch denote the mutational step, and a branch without a number denotes a single mutation step.

Table 26 Estimates of genetic differences (%) within (bold) and among clades of *I. exustus* for COI, 16S rDNA, combined mtDNA, and ITS1 sequences.

Gene or region	Clade	A	B	C	D
COI	A	**0-7.05**			
	B	8.11-13.93	**0-2.12**		
	C	10.58-15.52	8.99-10.05	**0-0.35**	
	D	11.99-16.93	11.99-13.23	11.29-11.99	**0.35-0.53**
16S rDNA	A	**0-1.84**			
	B	3.16-4.49	**0-1.30**		
	C	6.87-7.69	6.61-7.67	**0-0.79**	
	D	8.73-10.05	8.17-9.23	9.76-10.05	**0.00**
Combined	A	**0-4.86**			
mtDNA	B	6.34-11.42	**0-4.43**		
	C	9.31-12.16	8.45-10.38	**0-0.42**	
	D	10.99-13.74	7.58-11.51	10.78-11.21	**0.21-0.31**
ITS1	A	**0-0.66**			
	B	2.38-3.22	**0-1.53**		
	C	4.35-4.71	4.79-5.28	**0.00**	
	D	6.91-8.66	6.45-8.01	7.62-9.01	**0-1.35**

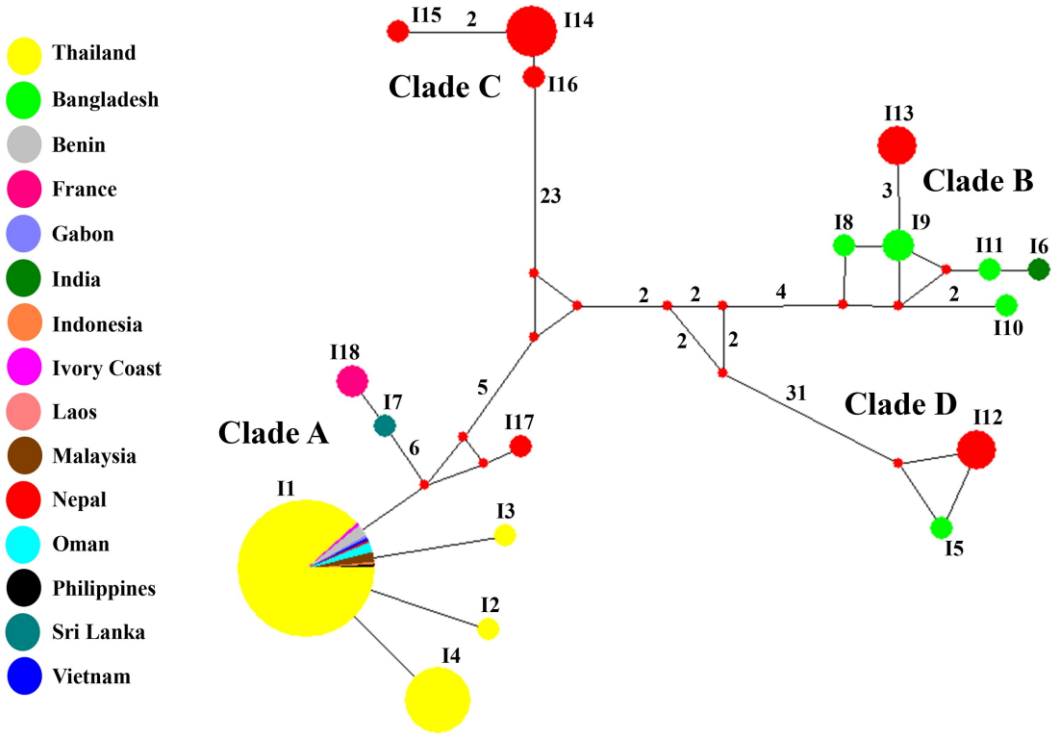

Figure 38 **Median-joining network of *I. exustus* from Thailand and various geographical regions, constructed using 16S rDNA sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small red dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. Numbers on each branch denote the mutational step, and a branch without a number denotes a single mutation step.**

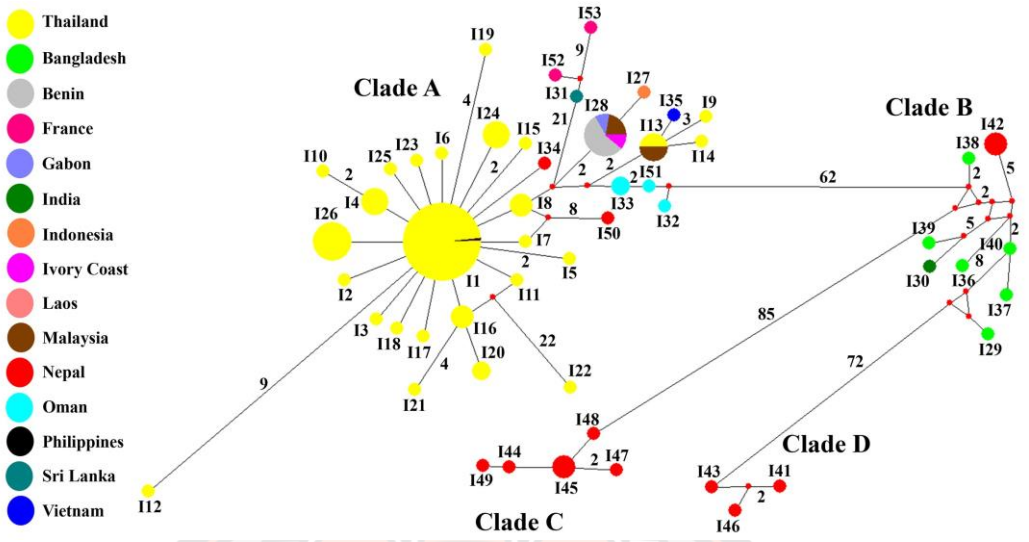

Figure 39 Median-joining network of *I. exustus* from Thailand and various geographical regions, constructed using the combined mitochondrial sequences of COI and 16S rDNA. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small red dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. Numbers on each branch denote the mutational step, and a branch without a number denotes a single mutation step.

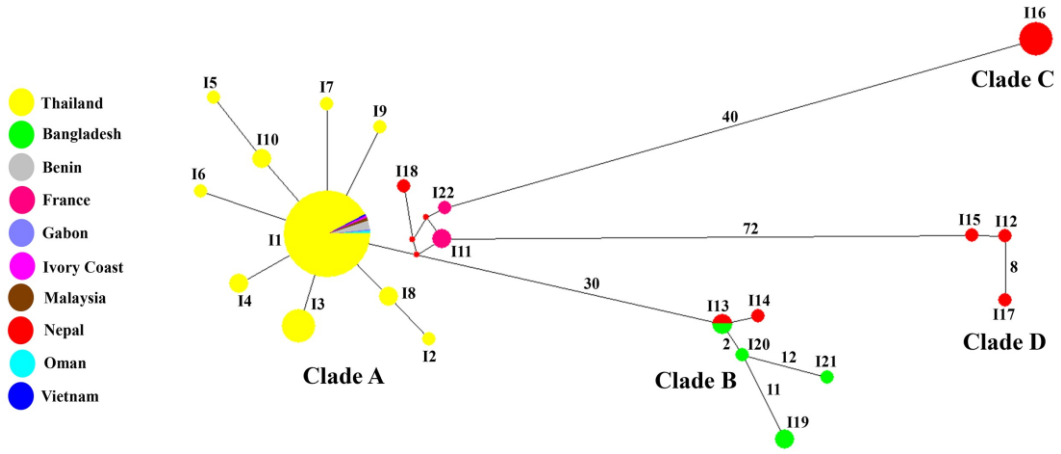

Figure 40 Median-joining network of *I. exustus* from Thailand and various geographical regions, constructed using ITS1 sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small red dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. Numbers on each branch denote the mutational step, and a branch without a number denotes a single mutation step.

The structure of haplotype network of *I. exustus* in Thailand represented a star-like phylogeny, with the most common haplotypes in the star's center. In the MJ network for Thailand, haplotype I1 emerges as the most frequent and is located at the center of the network. Analysis of COI sequences indicates that haplotype I1 comprises 128 *I. exustus* sequences, exhibiting a widespread distribution across 20 provinces in six regions of Thailand (Figure 41). Similarly, examination of 16S rDNA sequences reveals that haplotype I1, encompassing 152 sequences, is distributed across 21 provinces in six regions (Figure 42). Furthermore, the combined analysis of mtDNA and ITS1 sequences demonstrates that haplotype I1 consists of 118 and 145 sequences, respectively, and is widely distributed in 20 provinces (Figures 43, 44). Additionally, the analysis of 28S rDNA highlights haplotype I1, comprising 36 sequences, as the most widely distributed, covering all regions of Thailand (Figure 45).

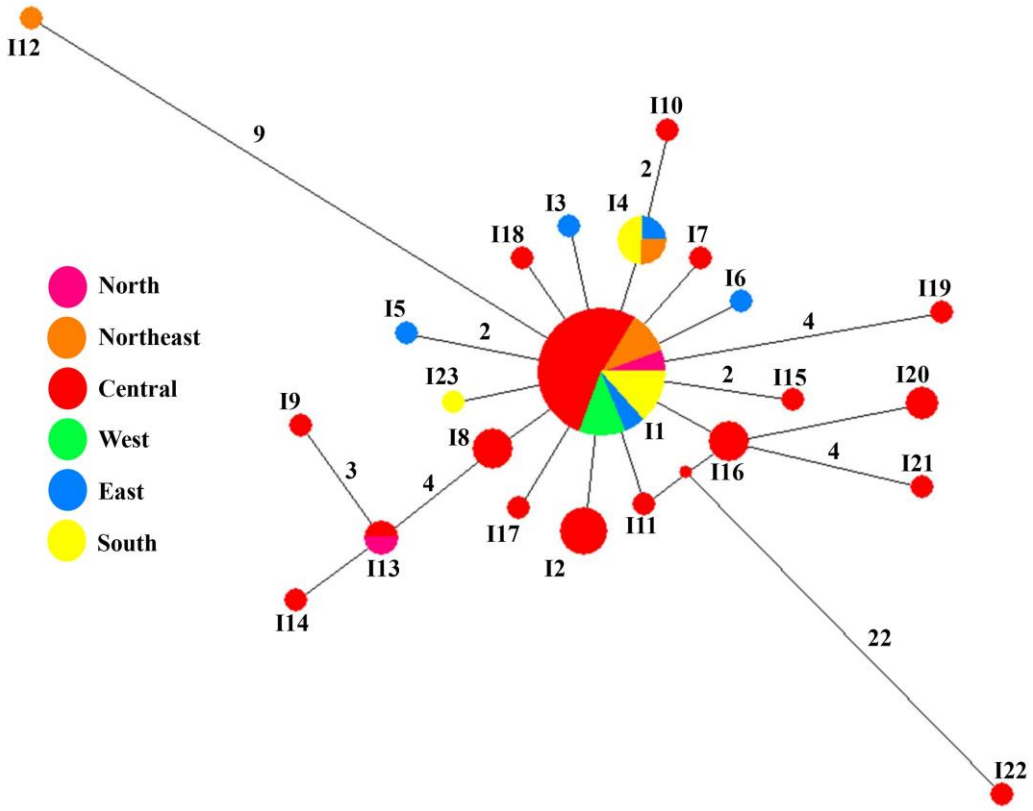

Figure 41 Median-joining network of *I. exustus* from Thailand based on COI sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small red dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. Numbers on each branch denote the mutational step, and a branch without a number denotes a single mutation step.

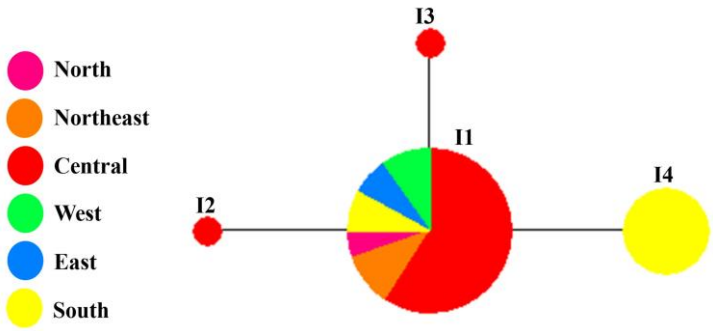

Figure 42 **Median-joining network of *I. exustus* from Thailand based on 16S rDNA sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles.**

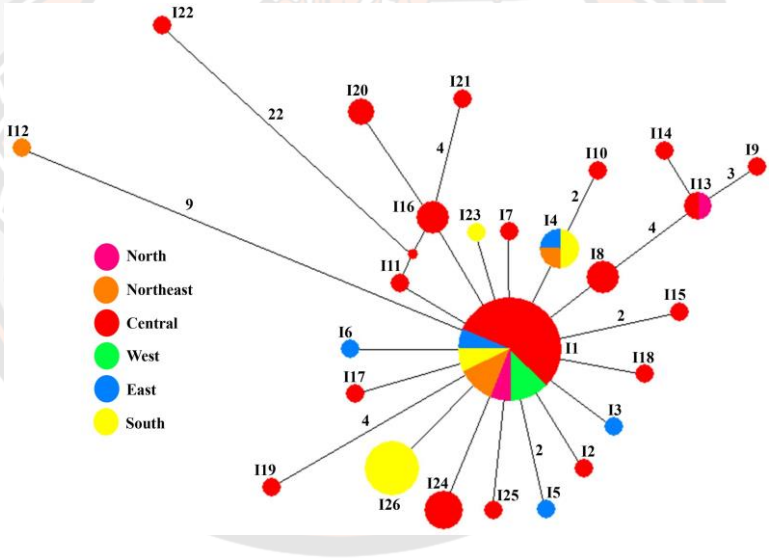

Figure 43 **Median-joining network of *I. exustus* from Thailand based on combined mitochondrial sequences of the COI and 16S rDNA genes. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small red dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. Numbers on each branch denote the mutational step, and a branch without a number denotes a single mutation step.**

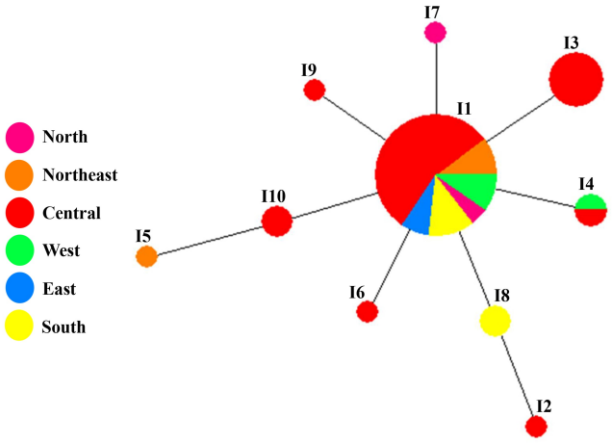

Figure 44 Median-joining network of *I. exustus* from Thailand based on ITS1 sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles.

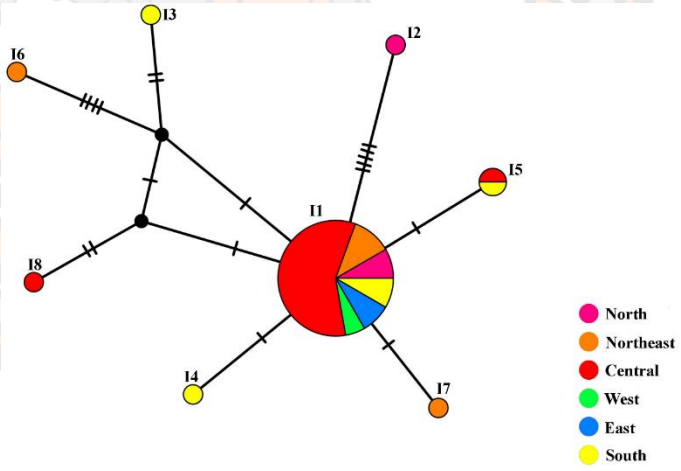

Figure 45 Median-joining network of *I. exustus* from Thailand based on 28S rDNA sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small black dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. A bar represents each mutation between haplotypes.

2. *Radix rubiginosa* and *Orientogalba viridis*

The genetic relationships of 116 *R. rubiginosa* and 84 *O. viridis* representatives in Thailand were assessed through the construction of a phylogenetic tree and haplotype network using COI (520 bp), 16S rDNA (371 bp), and combined mtDNA (891 bp) sequences. The phylogenetic trees and haplotype networks for both *R. rubiginosa* (Figures 46-51) and *O. viridis* (Figures 52-57) from various provinces of Thailand revealed no distinct pattern of population genetic structure. This observation was supported by moderate to high bootstrap values.

Considering the haplotype diversity of *R. rubiginosa* and *O. viridis* infected with cercariae, PCR successfully amplified COI, 16S rDNA and mtDNA (COI and 16S rDNA) in 18 out of 20 cercaria-infected snails (5 *R. rubiginosa* and 13 *O. viridis*). Within the dataset comprising 23 COI *R. rubiginosa* haplotypes (R1-R23), the R2 haplotype (3 individuals) displayed infections with echinostome cercariae I and furcocercous cercariae, while the R14 (1 individual) and R15 (1 individual) haplotypes were infected with only furcocercous cercariae (Figure 46). Examining the dataset of 15 16S rDNA *R. rubiginosa* haplotypes, the R1 haplotype (2 individuals) exhibited exclusive infection with furcocercous cercariae, whereas the R2 haplotype (3 individuals) showed infections with echinostome cercariae I and furcocercous cercariae (Figure 47). In the dataset of 32 combined mtDNA *R. rubiginosa* haplotypes (R1-R32), the R1 haplotype (3 individuals) demonstrated infections with echinostome cercariae I and furcocercous cercariae, while the R19 (1 individual) and R20 (1 individual) haplotypes were infected with only furcocercous cercariae (Figure 48). For *O. viridis*, 4 out of 8 haplotypes (O1-O8) based on the COI sequence exhibited cercariae infection. Specifically, haplotype O1 (8 individuals) showed infection with both xiphidiocercariae and echinostome cercariae II; O5 (2 individuals) exhibited infection with xiphidiocercariae and echinostome cercariae I; and O2 (2 individuals) and O8 (1 individual) were infected only with xiphidiocercariae (Figure 52). Additionally, analysis of 16S rDNA sequences revealed that 3 out of the 8 haplotypes (O1-O8) showed infection with cercariae. Among these, haplotype O2 (9 individuals) showed infection with xiphidiocercariae and echinostome cercariae II; O4 (2 individuals) displayed infection with xiphidiocercariae and echinostome cercariae I; and O1 (2 individuals) was infected only with xiphidiocercariae (Figure 53).

Furthermore, examination of the combined mtDNA sequences for 4 out of 15 haplotypes (O1-O15) indicated cercariae infection. Specifically, haplotype O1 (8 individuals) exhibited infection with both xiphidiocercariae and echinostome cercariae II; O7 (2 individuals) showed infection with xiphidiocercariae and echinostome cercariae I; and O2 (2 individuals) and O13 (1 individual) were infected only with xiphidiocercariae (Figure 54).

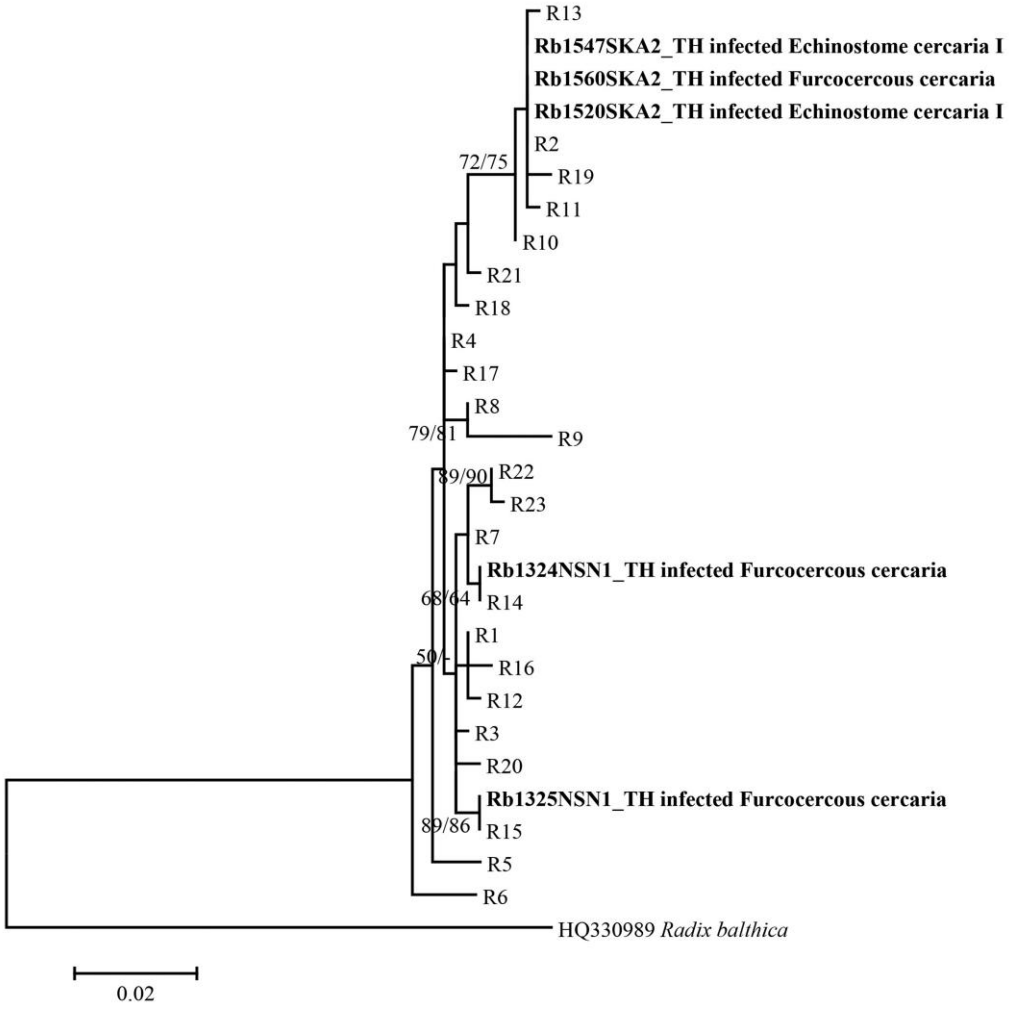

Figure 46 Maximum likelihood tree of COI sequences (520 bp) of 23 haplotypes generated from 116 sequences of *R. rubiginosa* from Thailand. The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Samples in bold indicate infected with trematode cercariae.

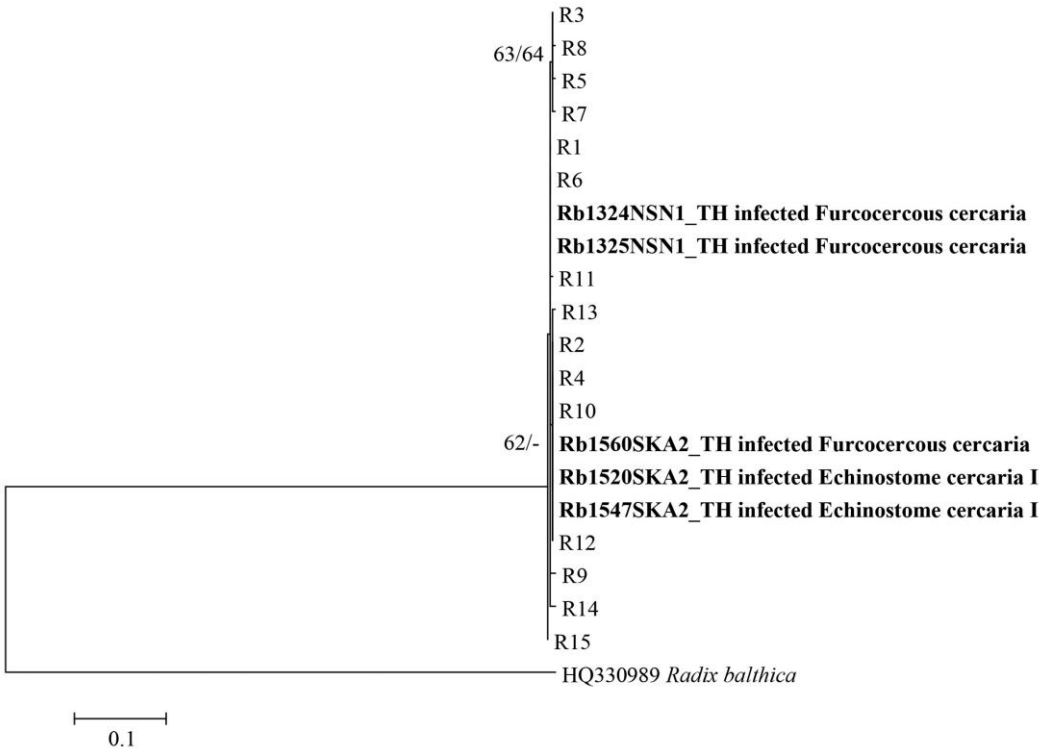

Figure 47 Maximum likelihood tree of 16S rDNA sequences (371 bp) of 15 haplotypes generated from 116 sequences of *R. rubiginosa* from Thailand. The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Samples in bold indicate infected with trematode cercariae.

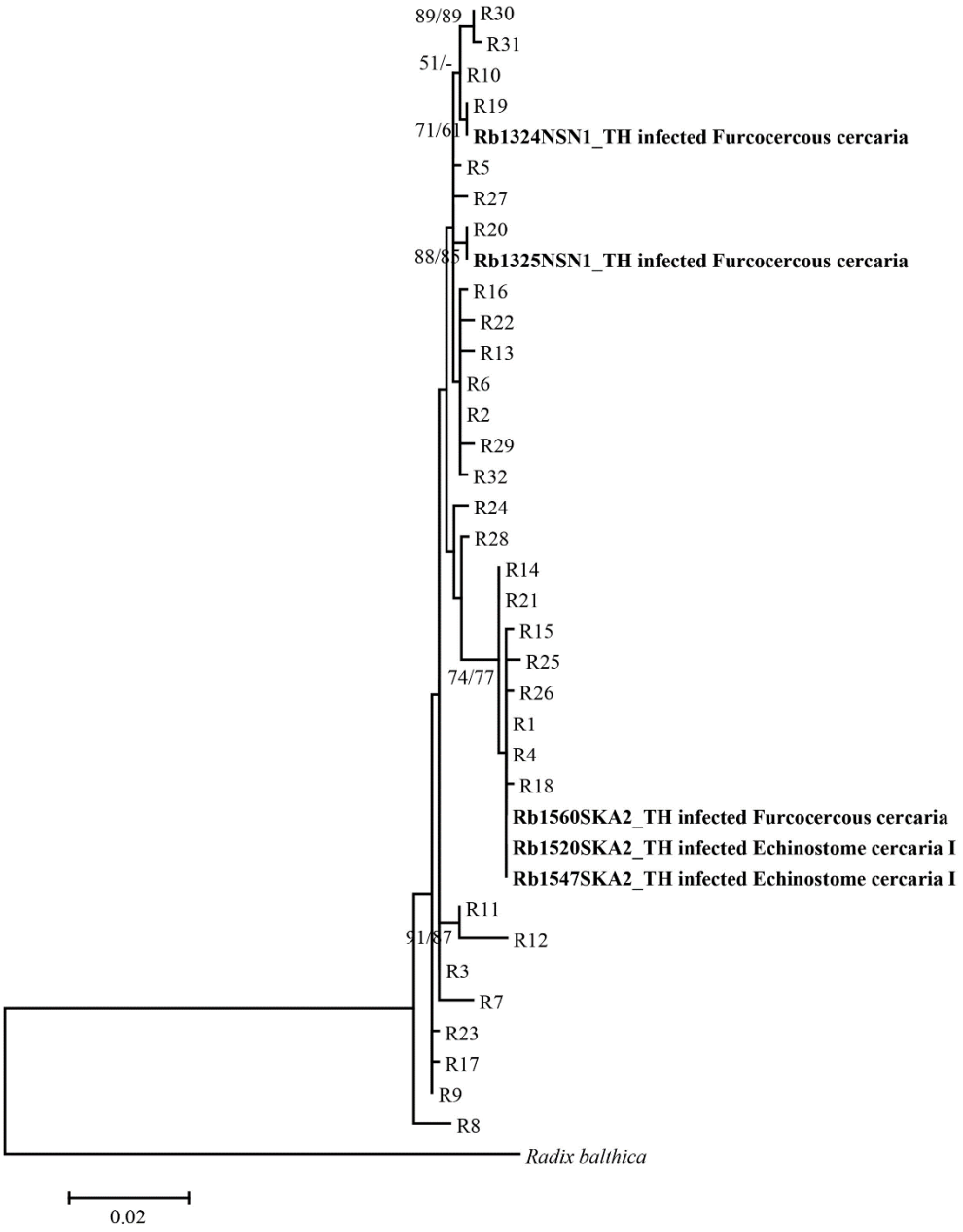

Figure 48 Maximum likelihood tree of combined mtDNA sequences (891 bp) of 32 haplotypes generated from 116 sequences of *R. rubiginosa* from Thailand. The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Samples in bold indicate infected with trematode cercariae.

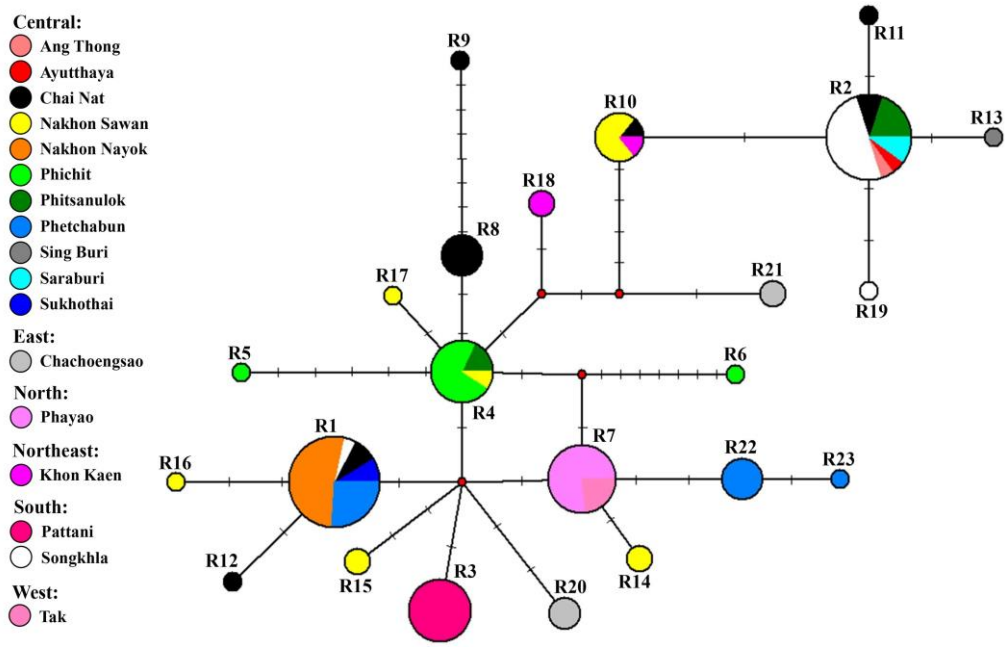

Figure 49 Median-joining network of *R. rubiginosa* from Thailand based on COI sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small red dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. A bar represents each mutation between haplotypes.

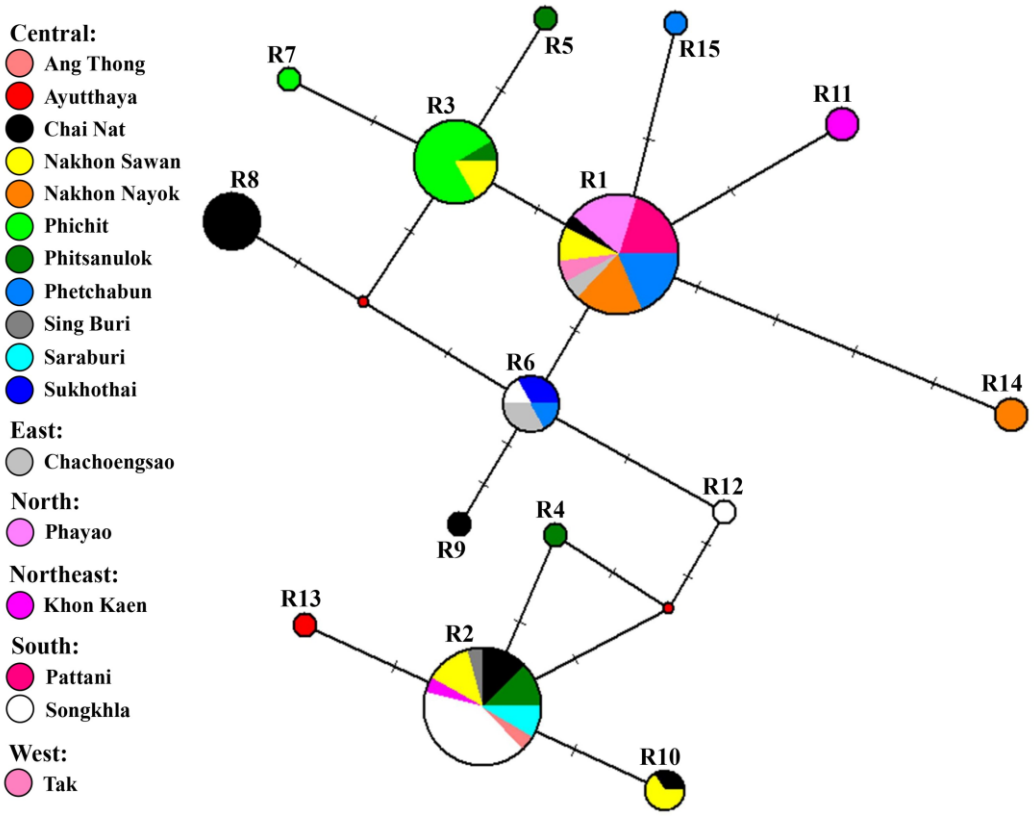

Figure 50 Median-joining network of *R. rubiginosa* from Thailand based on 16S rDNA sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small red dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. A bar represents each mutation between haplotypes.

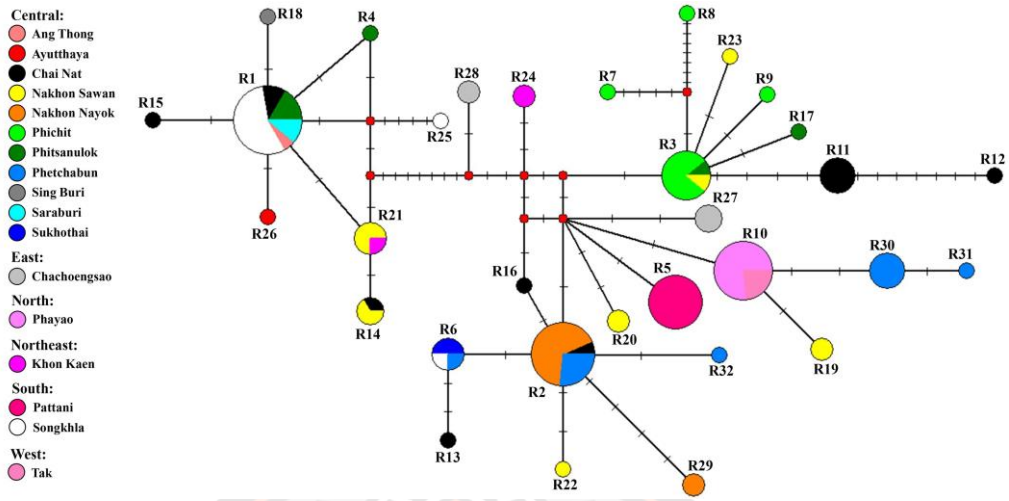

Figure 51 Median-joining network of *R. rubiginosa* from Thailand based on combined mtDNA sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small red dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. A bar represents each mutation between haplotypes.

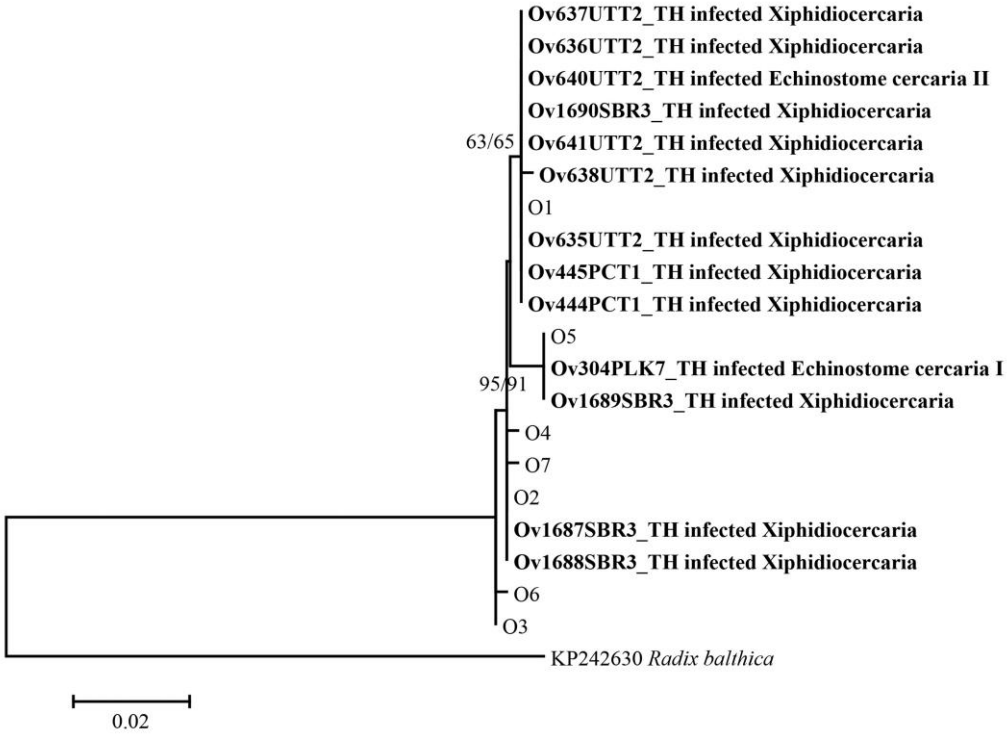

Figure 52 Maximum likelihood tree of COI sequences (520 bp) of 8 haplotypes generated from 84 sequences of *O. viridis* from Thailand. The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Samples in bold indicate infected with trematode cercariae.

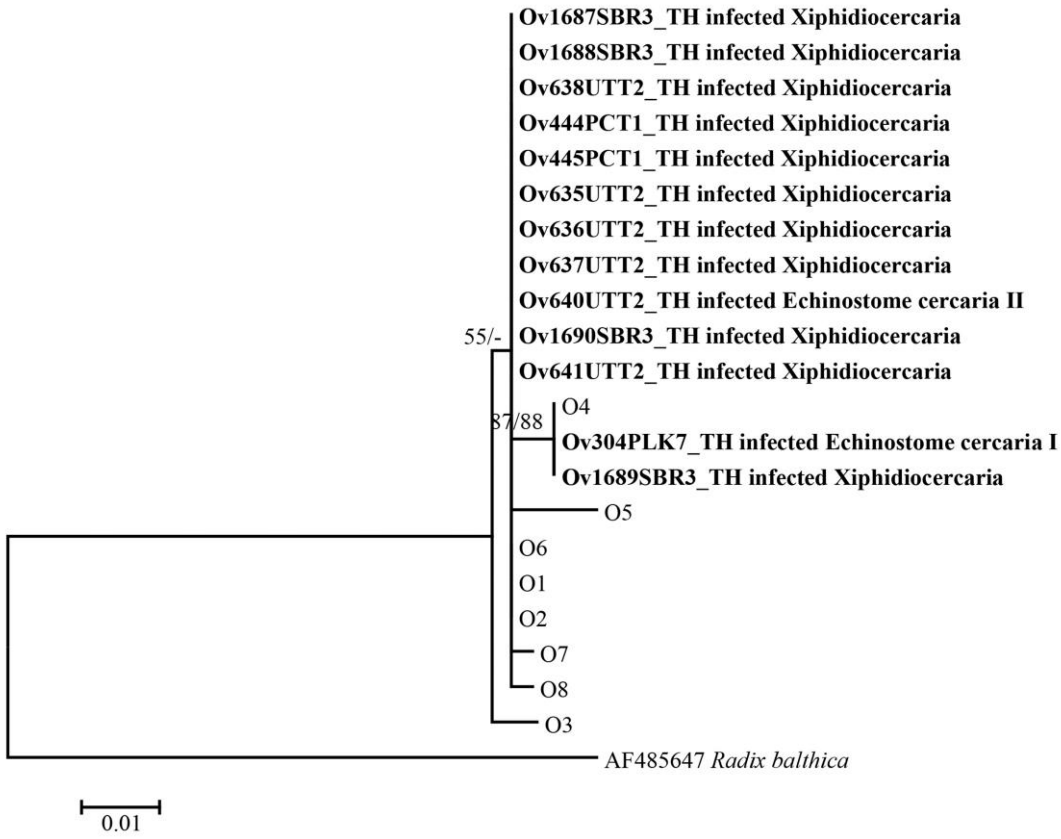

Figure 53 Maximum likelihood tree of 16S rDNA sequences (371 bp) of 8 haplotypes generated from 84 sequences of *O. viridis* from Thailand. The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Samples in bold indicate infected with trematode cercariae.

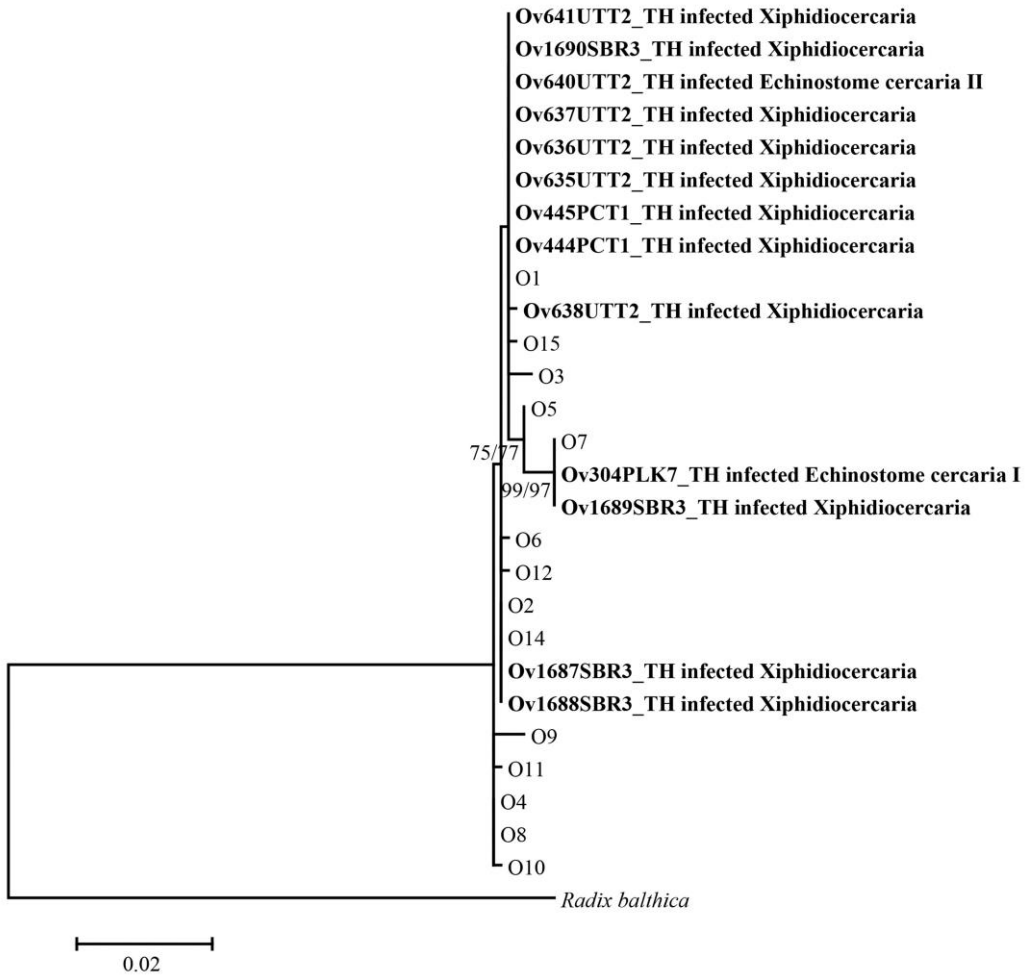

Figure 54 Maximum likelihood tree of combined mtDNA sequences (891 bp) of 15 haplotypes generated from 84 sequences of *O. viridis* from Thailand. The ML (left) and NJ (right) bootstrap values ≥50% are shown at the branch points. Samples in bold indicate infected with trematode cercariae.

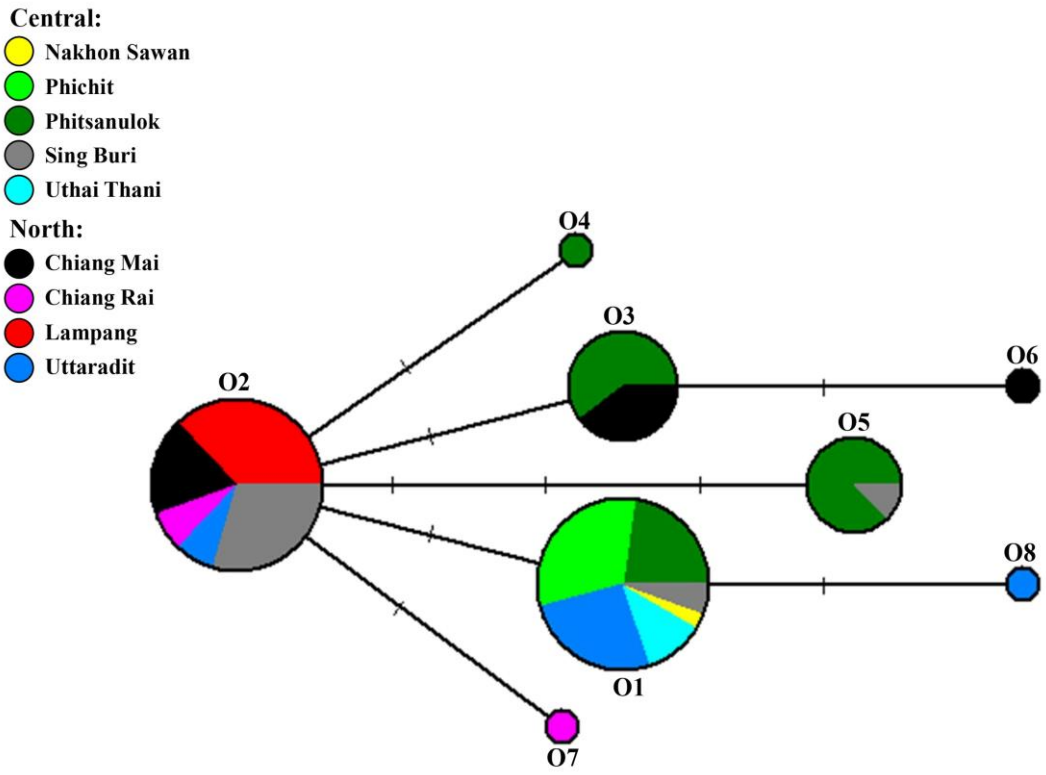

Figure 55 Median-joining network of *O. viridis* from Thailand based on COI sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. A bar represents each mutation between haplotypes.

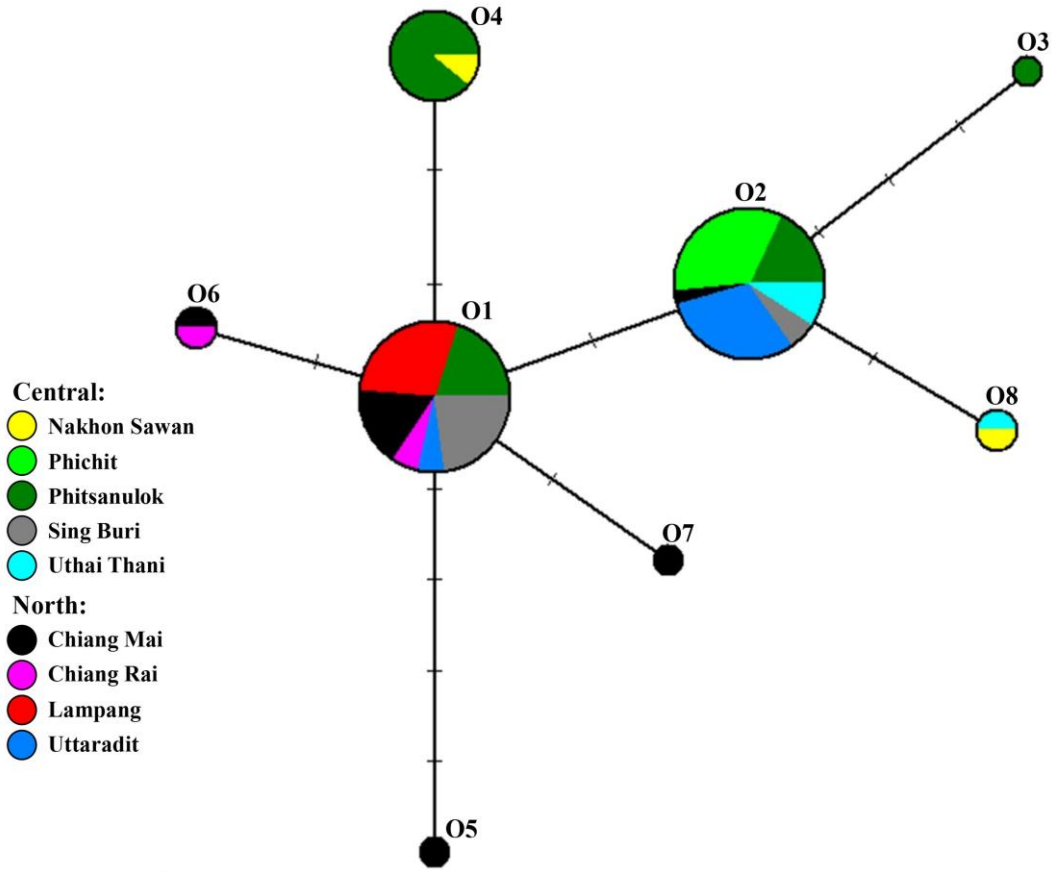

Figure 56 Median-joining network of *O. viridis* from Thailand based on 16S rDNA sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. A bar represents each mutation between haplotypes.

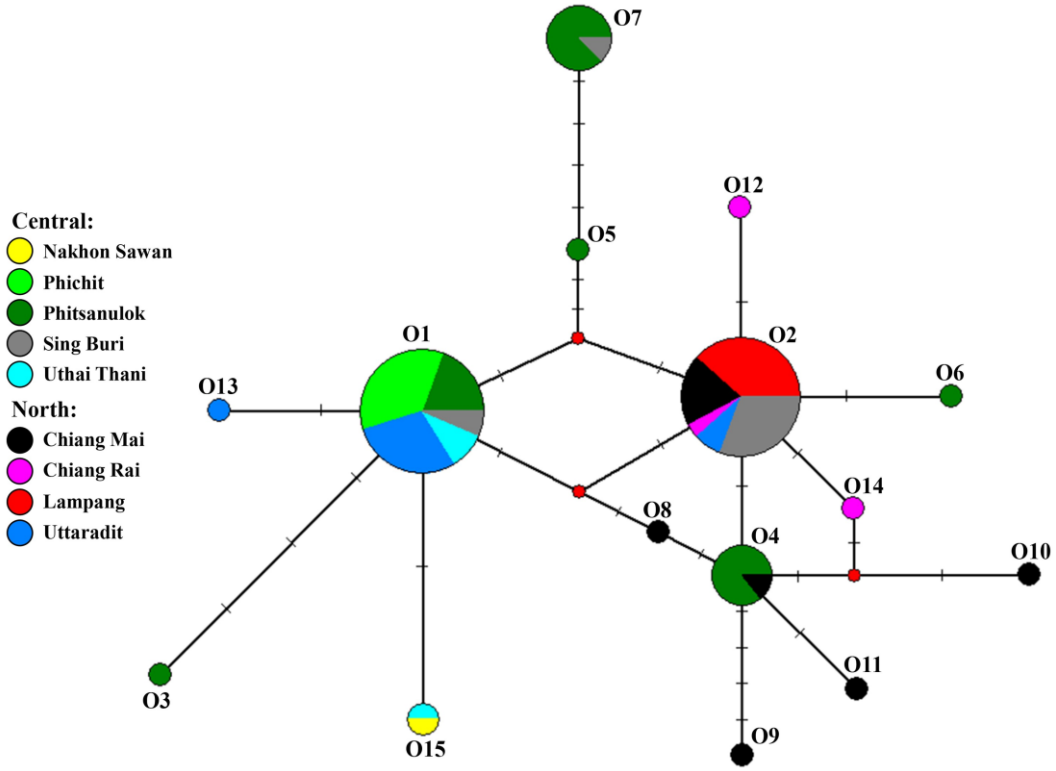

Figure 57 Median-joining network of *O. viridis* from Thailand based on combined mtDNA sequences. Each circle in the network represents a distinct haplotype, with circle sizes proportional to the frequency of each haplotype. Geographic origins of the haplotypes are represented by the colors assigned to the circles. Median vectors, indicated by small red dots, signify ancestral haplotypes that are either unsampled or absent within the dataset. A bar represents each mutation between haplotypes.

Population genetic structure of snails

1. *Indoplanorbis exustus*

Genetic variation among *I. exustus* populations (F_{ST})was analyzed based on COI, 16S rDNA, combined mtDNA, and ITS1. Pairwise F_{ST} values of 16 *I. exustus* populations in Thailand for the COI sequences demonstrated that most populations were not genetically significantly different (Figure 58), except between the populations in Sing Buri and Phitsanulok, Phichit, Chai Nat, Tak, Chon Buri, and Songkhla, which showed statistically significant differentiation ($P < 0.05$). The examination of pairwise F_{ST} values of snail populations using 16S rDNA sequences indicated that many populations

showed no significant genetic differences (Figure 59). An exception was observed in the distinction between Songkhla and other populations, wherein nearly all the F_{ST} values were statistically significant differences, as well as in the results of the combined mtDNA sequences (Figure 60). Moreover, the population pairwise F_{ST} values of the ITS1 sequences demonstrated that most populations between Sukhothai and the other populations were significantly different (Figure 61).

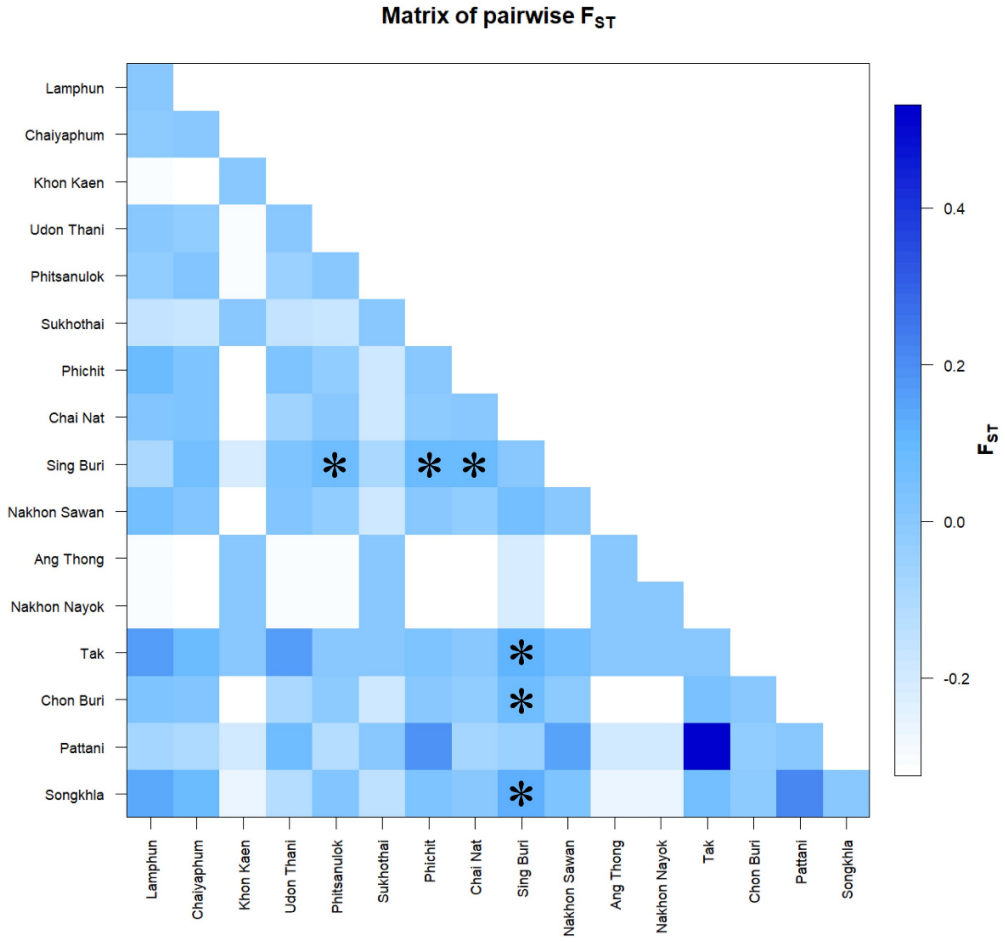

Figure 58 Graph of pairwise F_{ST} distance matrices between populations of *I. exustus* in Thailand based on COI sequences. Darker blue indicates a higher pairwise F_{ST} value and lighter blue indicates a lower value. Asterisks (*) indicate F_{ST} values with statistically significant distinctions ($P < 0.05$).

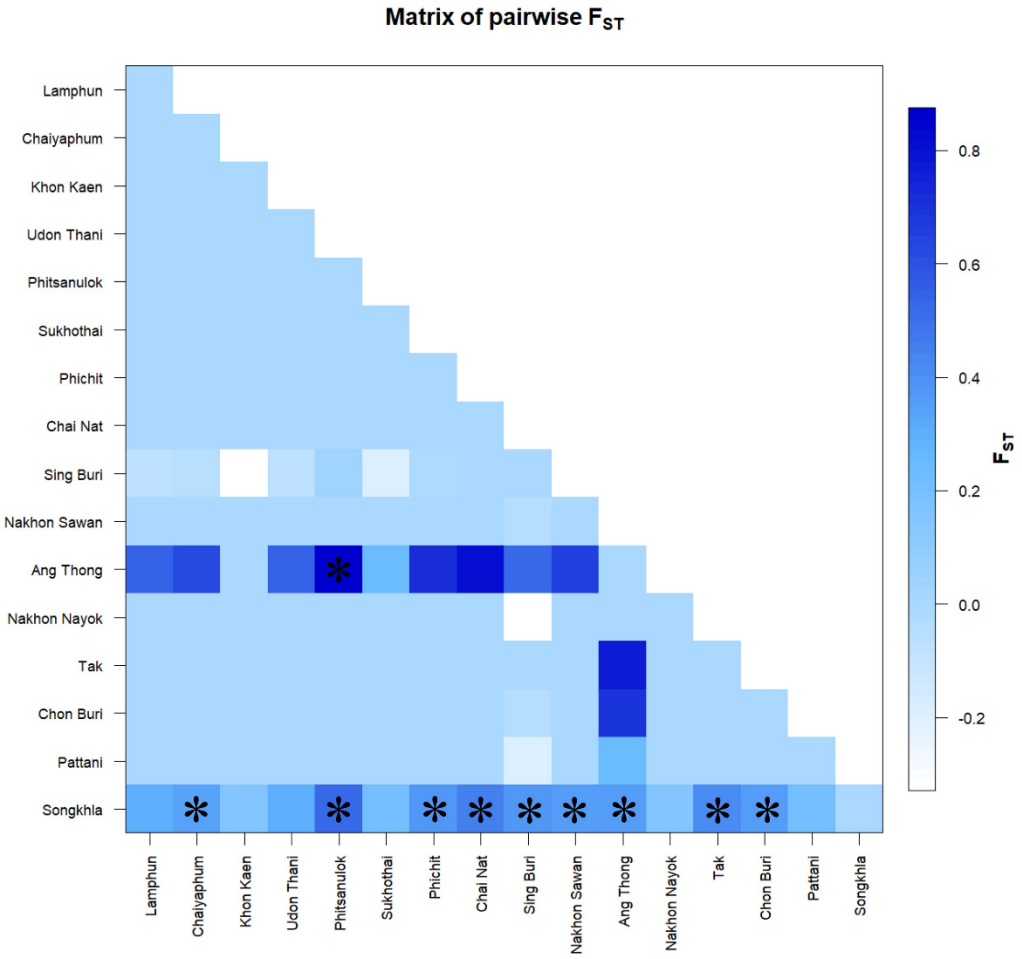

Figure 59 Graph of pairwise F_{ST} distance matrices between populations of *I. exustus* in Thailand based on 16S rDNA sequences. Darker blue indicates a higher pairwise F_{ST} value and lighter blue indicates a lower value. Asterisks (*) indicate F_{ST} values with statistically significant distinctions ($P < 0.05$).

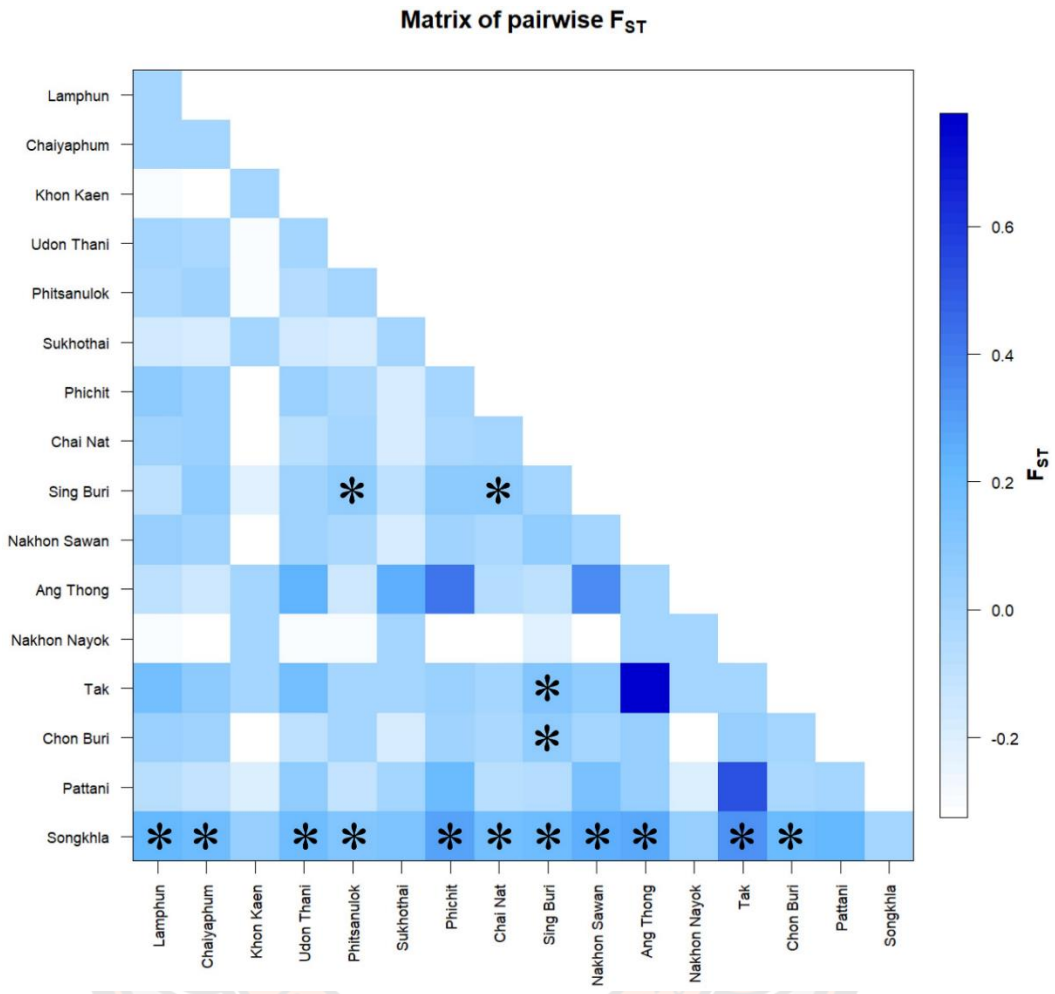

Figure 60 Graph of pairwise F_{ST} distance matrices between populations of *I. exustus* in Thailand based on combined mtDNA sequences. Darker blue indicates a higher pairwise F_{ST} value and lighter blue indicates a lower value. Asterisks (*) indicate F_{ST} values with statistically significant distinctions ($P < 0.05$).

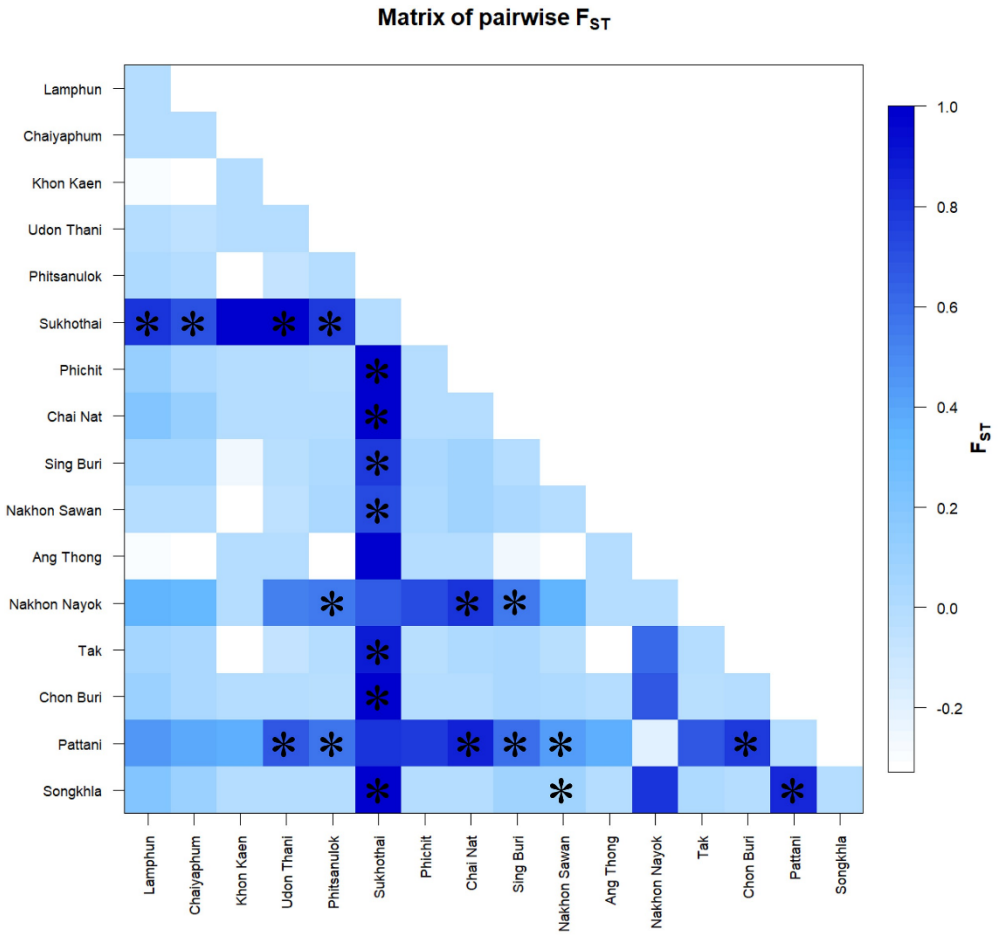

Figure 61 Graph of pairwise F_{ST} distance matrices between populations of *I. exustus* in Thailand based on ITS1 sequences. Darker blue indicates a higher pairwise F_{ST} value and lighter blue indicates a lower value. Asterisks (*) indicate F_{ST} values with statistically significant distinctions (P < 0.05).

2. *Radix rubiginosa* and *Orientogalba viridis*

Genetic differentiation among populations (F_{ST}) of *R. rubiginosa* and *O. viridis* was analyzed based on COI, 16S rDNA, and combined mtDNA sequences. For the mitochondrial COI sequences of *R. rubiginosa* populations, the analysis of pairwise F_{ST} values exhibited that most 66% (60 of 91) were significantly different, with a P value < 0.05. The overall population genetic variance (pairwise F_{ST} values) showed values ranging from 0.000 to 1.000. The snail populations from Sukhothai and Nakhon Nayok, as well as Tak and Phayao provinces, displayed the lowest pairwise F_{ST} values (0.000). In contrast, the highest pairwise F_{ST} values (1.000) were identified

in 13 pairs of snail populations. (Figure 62). Similarly, analysis based on 16S rDNA indicated significant differentiation in 54% (49 of 91) of the populations. The overall population genetic variance ranged from 0.000 to 1.000. The lowest pairwise F_{ST} values (0.000) were observed among snail populations from Pattani and Phayao, Tak and Phayao, Tak and Pattani provinces, while the highest pairwise F_{ST} values (1.000) were observed in 7 pairs of snail populations (Figure 63). Furthermore, pairwise F_{ST} analysis of the combined mtDNA revealed significant differentiation in 70% (64 of 91) of the populations. The overall population genetic variance ranged from -0.297 to 1.000. The lowest pairwise F_{ST} values (-0.297) were recorded among snail populations from Songkhla and Saraburi provinces, while the highest pairwise F_{ST} values (1.000) were found in 9 pairs of snail populations (Figure 64).

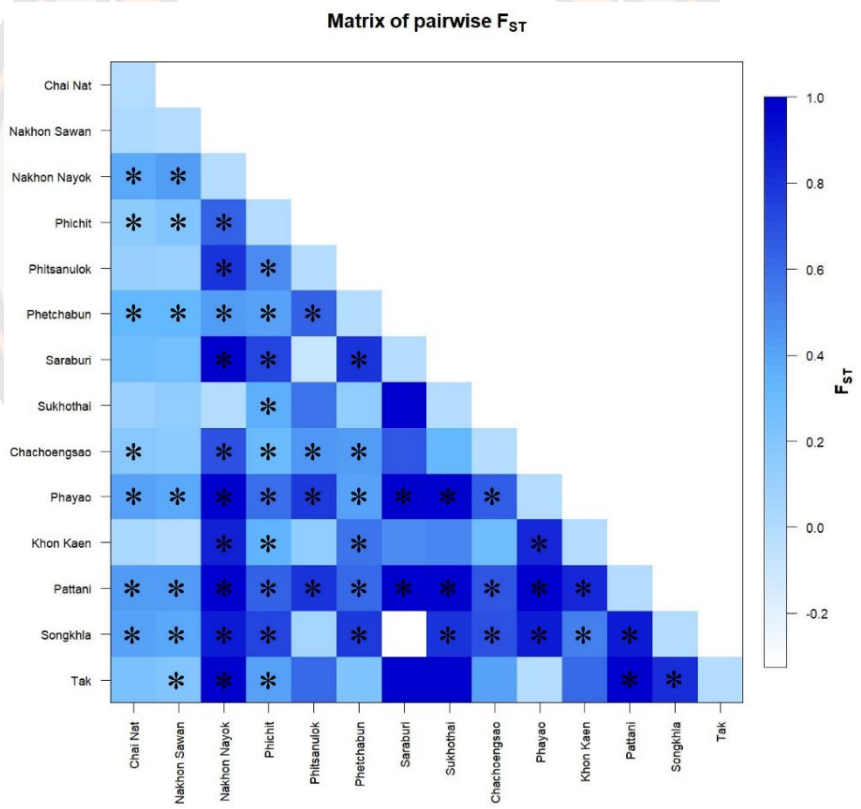

Figure 62 Graph of pairwise F_{ST} distance matrices between populations of *R. rubiginosa* in Thailand based on COI sequences. Darker blue indicates a higher pairwise F_{ST} value and lighter blue indicates a lower value. Asterisks (*) indicate F_{ST} values with statistically significant distinctions ($P < 0.05$).

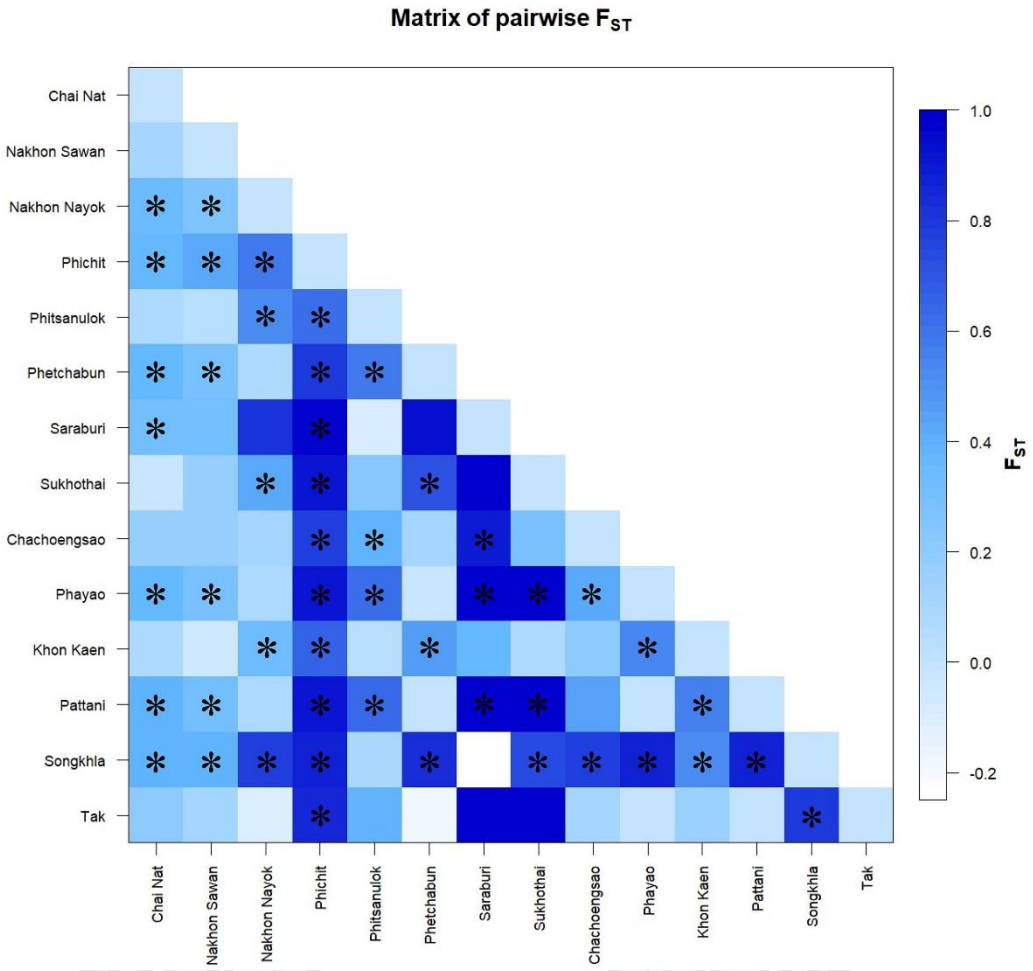

Figure 63 Graph of pairwise F_{ST} distance matrices between populations of *R. rubiginosa* in Thailand based on 16S rDNA sequences. Darker blue indicates a higher pairwise F_{ST} value and lighter blue indicates a lower value. Asterisks (*) indicate F_{ST} values with statistically significant distinctions ($P < 0.05$).

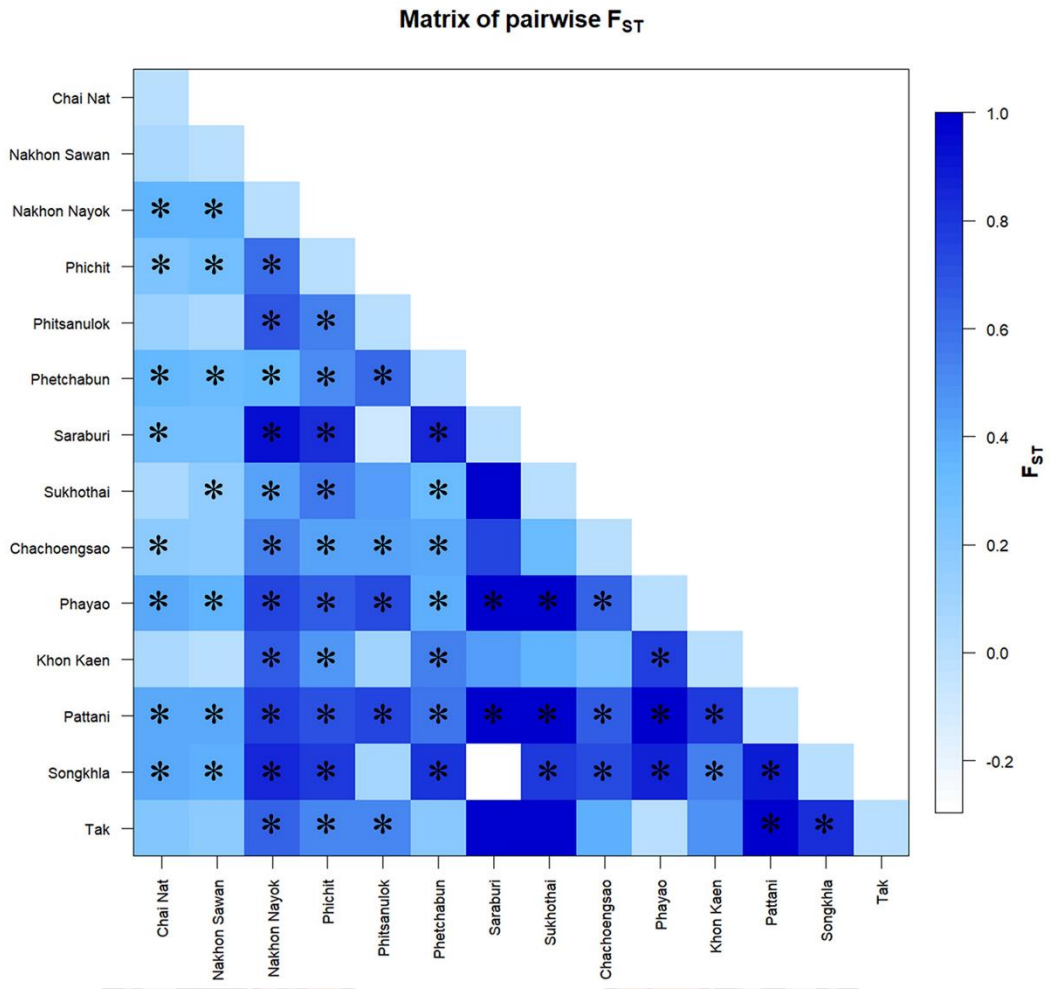

Figure 64 Graph of pairwise F_{ST} distance matrices between populations of *R. rubiginosa* in Thailand based on combined mtDNA sequences. Darker blue indicates a higher pairwise F_{ST} value and lighter blue indicates a lower value. Asterisks (*) indicate F_{ST} values with statistically significant distinctions ($P < 0.05$).

The pairwise F_{ST} analysis for *O. viridis*, based on COI sequences, revealed that 16 out of 28 F_{ST} values (57%) were statistically significant ($P < 0.05$). The overall population genetic variance showed values ranging from 0.000 between the populations in Uthai Thani and Phichit provinces to 1.000 between the populations from Lampang with Phichit and Uthai Thani provinces (Figure 65). For 16S rDNA sequences, 61% (17 out of 28) of pairwise F_{ST} values were significant, with population genetic variance values ranging from 0.000 (between populations in Lampang and Chiang Mai

provinces) to 1.000 (between populations from Lampang and Phichit provinces) (Figure 66). Additionally, the combined mtDNA sequences analysis for *O. viridis* indicated that 19 (68%) out of 28 F_{ST} values were statistically significant. The overall population genetic variance ranged from 0.004 between the populations in Chiang Rai and Sing Buri provinces to 1.000 between the populations from Lampang and Phichit provinces (Figure 67).

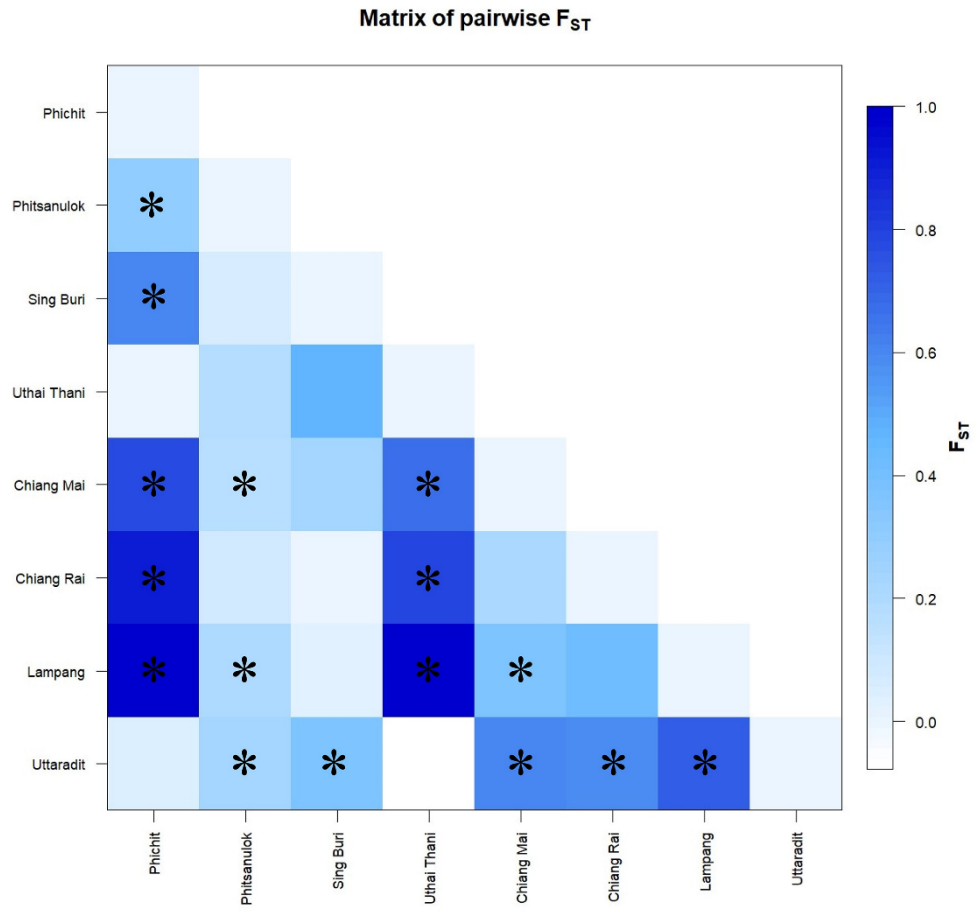

Figure 65 Graph of pairwise F_{ST} distance matrices between populations of *O. viridis* in Thailand based on COI sequences. Darker blue indicates a higher pairwise F_{ST} value and lighter blue indicates a lower value. Asterisks (*) indicate F_{ST} values with statistically significant distinctions ($P < 0.05$).

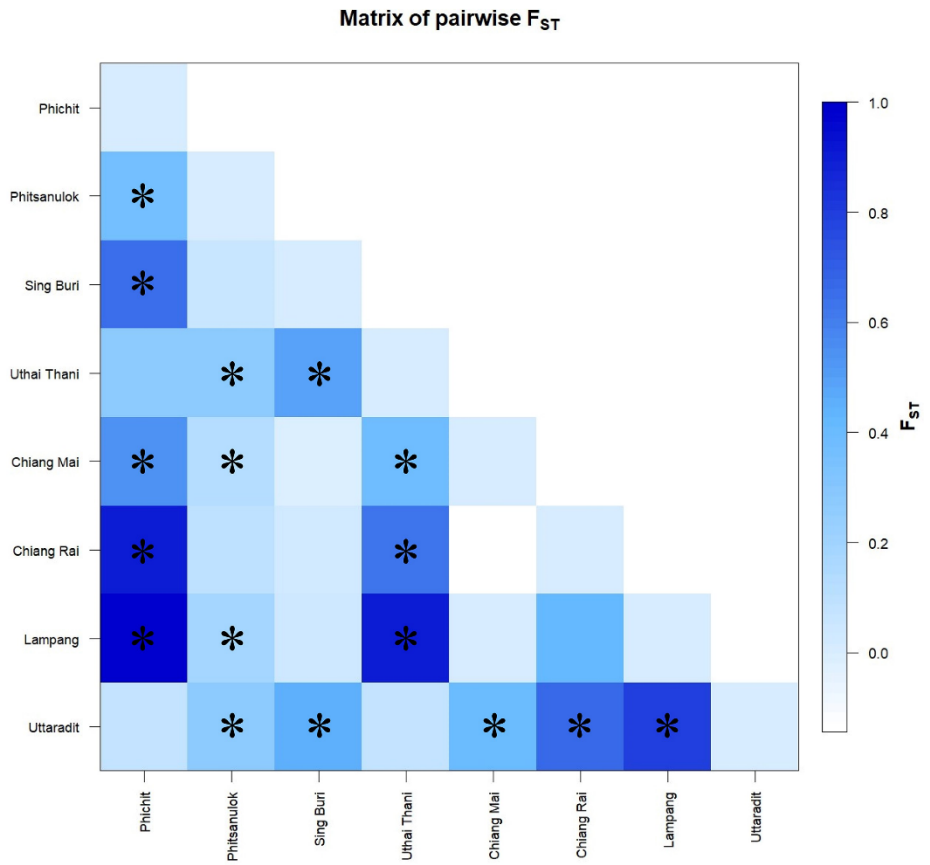

Figure 66 Graph of pairwise F_{ST} distance matrices between populations of *O. viridis* in Thailand based on 16S rDNA sequences. Darker blue indicates a higher pairwise F_{ST} value and lighter blue indicates a lower value. Asterisks (*) indicate F_{ST} values with statistically significant distinctions ($P < 0.05$).

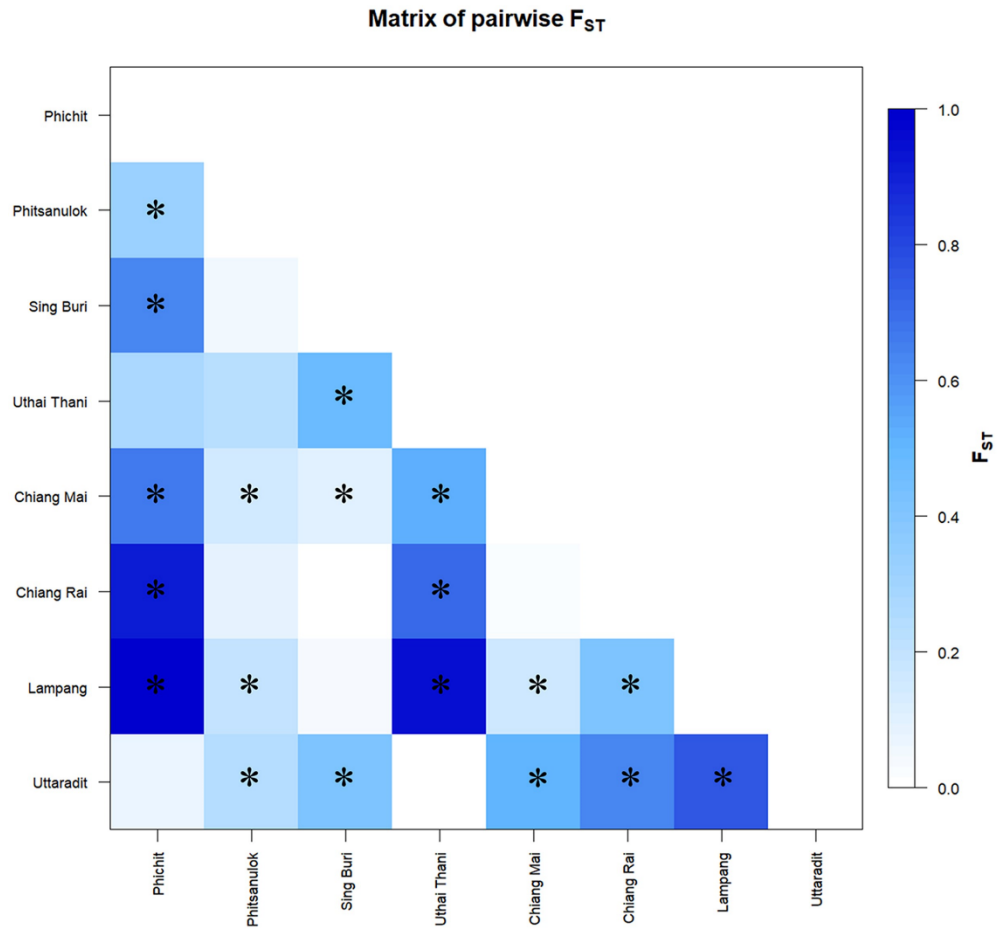

Figure 67 Graph of pairwise F_{ST} distance matrices between populations of *O. viridis* in Thailand based on combined mtDNA sequences. Darker blue indicates a higher pairwise F_{ST} value and lighter blue indicates a lower value. Asterisks (*) indicate F_{ST} values with statistically significant distinctions (P < 0.05).

Demographic history of snails

1. *Indoplanorbis exustus*

Mismatch distribution analysis of *I. exustus* based on combined mtDNA sequences demonstrated a unimodal graph (Figure 68), indicative of a recent population expansion. Both the sum-of-squares deviation (SSD) and Harpending's raggedness index (HRI) were not significantly different from the simulated data under the sudden population expansion model, with SSD showing a value of 0.0034 (P = 0.3980) and HRI a value of 0.1121 (P = 0.7920). Additionally, both Fu's Fs (-28.6235, P < 0.001) and

Tajima's D (-2.7292, P < 0.001) tests reveal highly significant negative values, providing additional confirmation of the sudden expansion of population.

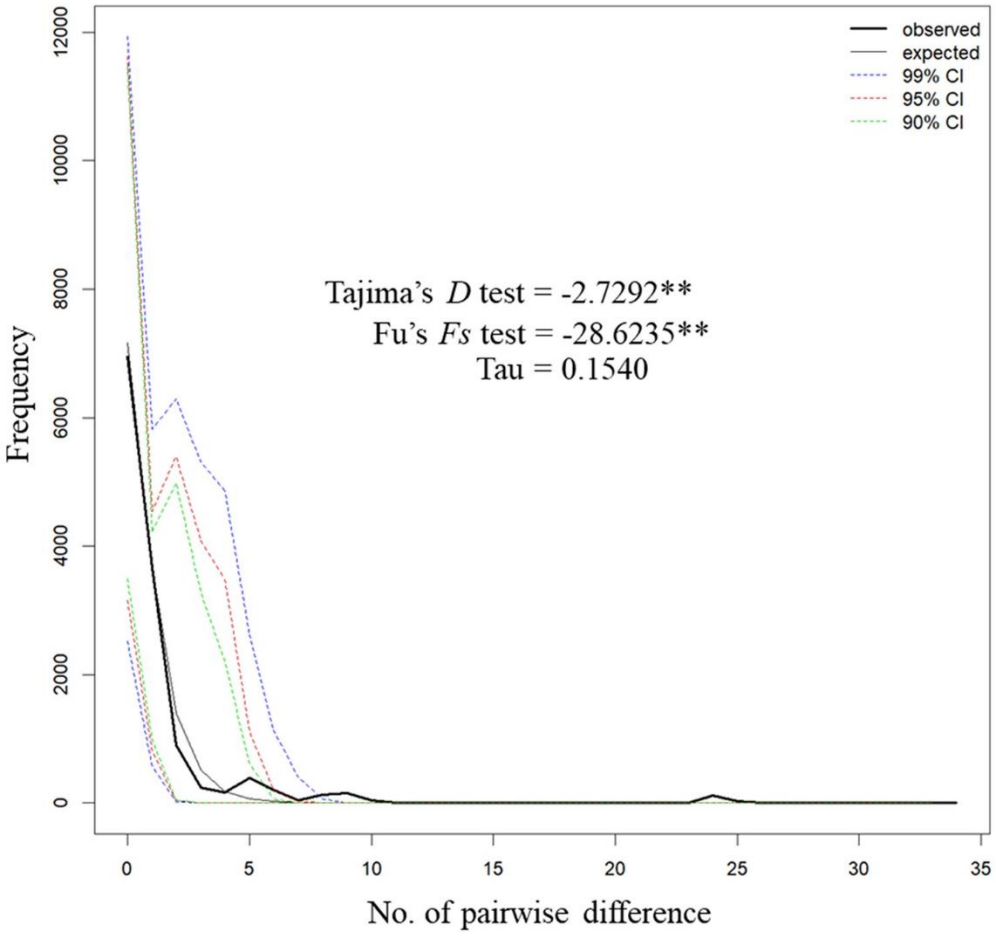

Figure 68 Mismatch distribution of *I. exustus* from Thailand based on combined mtDNA sequences. The mismatch distributions of *I. exustus* align with the sudden population expansion model, as both the sum-of-square deviation (SSD) and Harpending's raggedness index (HRI) exhibit no significant differences between the observed values and the expected values from the simulation. Asterisks indicate values showing statistical significance (P < 0.001).**

2. *Radix rubiginosa* and *Orientogalba viridis*

Neutrality tests and mismatch distribution analyses were conducted using combined mtDNA sequences to elucidate the demographic history of both *R. rubiginosa* and O. *viridis*. The Tajima's D value of *R. rubiginosa* was negative with a nonsignificant P value (-1.0894, P = 0.1230), suggesting deviation from evolutionary neutrality. In contrast, Fu's Fs test revealed significant negative values (-24.7336, P = 0.0000). Following the result of Fu's Fs test, the null hypothesis of neutral evolution was rejected. The analysis of mismatch distribution indicated a bimodal curve in *R. rubiginosa* populations (Figure 69). Both the sum-of-square deviation (SSD = 0.0264, P = 0.1530) and Harpending's raggedness index (HRI = 0.0214, P = 0.2230) showed no significant differences from the simulated data under the sudden population expansion model.

For *O. viridis*, the values of Tajima's D (-1.5024, P = 0.0412) and Fu's Fs (-26.8608, P = 0.0000) were significant and negative. The analysis of the mismatch distribution revealed multimodal curves (Figure 70); however, neither the sum-of-square deviation (SSD = 0.0214, P = 0.4710) nor Harpending's raggedness index (HRI = 0.0879, P = 0.5230) exhibited significant differences from the simulated data under the sudden population expansion model.

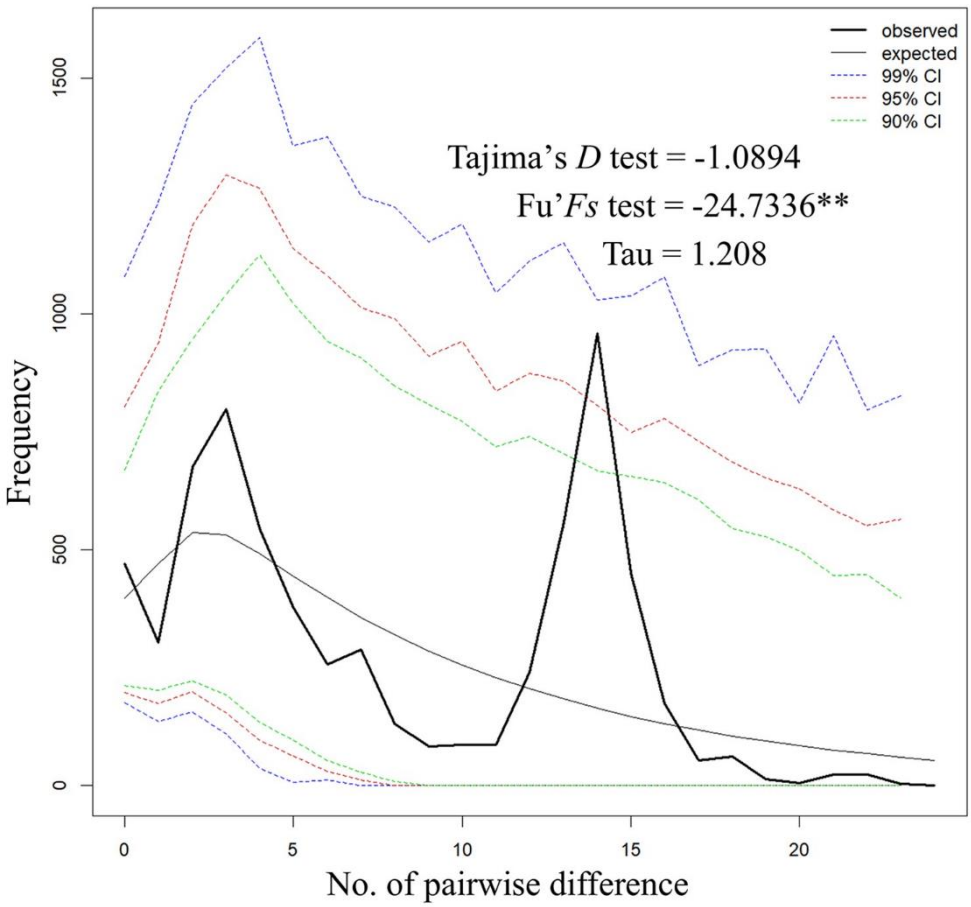

Figure 69 Mismatch distribution of *R. rubiginosa* from Thailand based on combined mtDNA sequences. The mismatch distributions of *R. rubiginosa* align with the sudden population expansion model, as both the sum-of-square deviation (SSD) and Harpending's raggedness index (HRI) exhibit no significant differences between the observed values and the expected values from the simulation. Asterisks indicate values showing statistical significance (P < 0.001).**

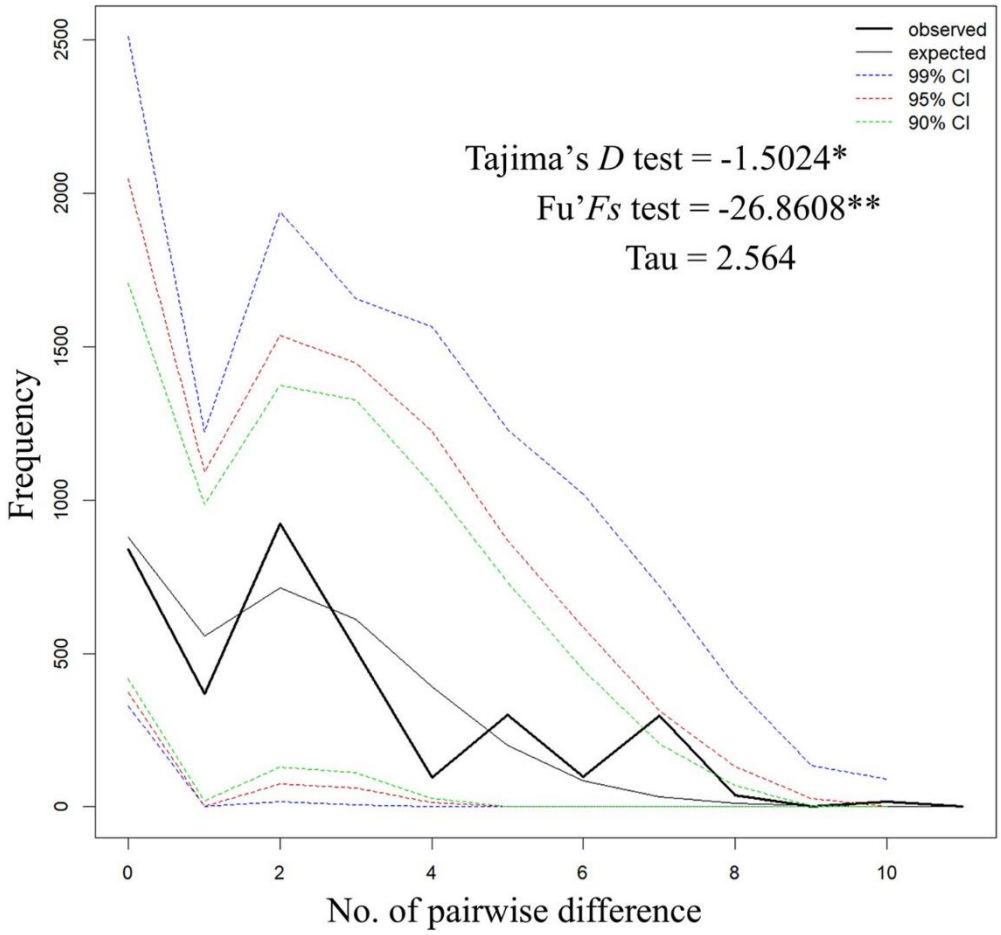

Figure 70 Mismatch distribution of *O. viridis* from Thailand based on combined mtDNA sequences. The mismatch distributions of *O. viridis* align with the sudden population expansion model, as both the sum-of-square deviation (SSD) and Harpending's raggedness index (HRI) exhibit no significant differences between the observed values and the expected values from the simulation. Asterisks indicate values showing statistical significance (*P < 0.05; **P < 0.001).

CHAPTER V

DISCUSSION

In the present study, *I. exustus*, *R. rubiginosa* and *O. viridis* were widely distributed with a rather erratic pattern in Thailand. The snail species occur in somewhat different habitats. *Indoplanorbis exustus* and *R. rubiginosa* were common in all regions of Thailand. Consistent with previous findings that found *I. exustus* and *R. rubiginosa* were widely distributed in many provinces spanning all six regions of Thailand (Kaset et al., 2010; Saijuntha et al., 2021), whereas *O. viridis* was spread only in northern Thailand. The variation in snail diversity in each area might be influenced by agricultural activities, especially the application of agrochemicals, including herbicides and fertilizers, some of which could have molluscicidal activity (Dung et al., 2010; Halstead et al., 2018). Most snail species in this study were collected from water bodies located within agricultural land.

Our current study showed that the overall prevalent rate of cercaria infection within 3 snails (*I. exustus*, *R. rubiginosa*, and *O. viridis*) was 1.76% (22/1,247). This figure contrasts with earlier reports on cercarial infection in snails collected from Thailand, where cercarial infection rates were notably higher. In previous investigations, prevalence studies on cercariae indicated infection rates of nearly 20% (Chontananarth & Wongsawad, 2013; Krailas et al., 2014). These estimates of infection were reduced in later studies to 10.46% (Veeravechsukij et al., 2018). Recently, multiple surveys of cercariae in freshwater snails were carried out across diverse provinces in Thailand, uncovering infection rates that varied between 3.31% and 5.57% (Krailas et al., 2022; Tapdara et al., 2022; Wiroonpan et al., 2021). The low infection rate is related to snail number and environmental factors (Mereta et al., 2023). Prior reports indicate that the incidence of larval trematode infection in freshwater snails could be influenced by human activities and the diversity of specific fauna within the surveyed regions (Chontananarth & Wongsawad, 2013). The observed lower infection rate in the conducted research could be attributed to the limited diversity of the animal hosts, including second intermediate and definitive hosts, within the sampling locations. Additionally, reductions in human behaviors, such as urination,

open-field defecation, farming, and livestock grazing, have been strongly correlated with lower rates of parasitic trematode infection in freshwater snails (Mereta et al., 2019). During sample collection, it was frequently observed that *I. exustus*, *R. rubiginosa*, and *O. viridis* commonly inhabited the water surface level, displaying an attaching behavior to floating water plants or other floating objects. This phenomenon may make snail-miracidia contact a rare occurrence. Therefore, these factors make the degree of infection vary in freshwater snails.

Interestingly, the results revealed a higher prevalence of cercarial infection in *O. viridis* (4.49%, 14/312) compared to *R. rubiginosa* (1.67%, 6/360) and *I. exustus* (0.35%, 2/575). Our findings suggested that *O. viridis* is more susceptible to trematode cercariae infection than *R. rubiginosa* and *I. exustus*. It is difficult to explain why *O. viridis* is more susceptible to trematode cercariae infection because of the limited amount of research on *O. viridis*. In a prior investigation, in a study by Lee et al. (1995), the susceptibility of *O. viridis* to trematode (*Fasciola hepatica*) infection was investigated, revealing a high susceptibility to infection at the miracidia stage in this snail species. A similar phenomenon has been documented in other freshwater snails, as reported by Chanawong & Waikagul (1991). Their study indicated that *Bithynia siamensis siamensis* in Thailand exhibits a higher susceptibility to fluke infection when compared to *Bithynia siamensis goniomphalos*. Nevertheless, a previous study examined trematode (*Fasciola* spp.) infection in *O. viridis* and found a low prevalence (Dung et al., 2013). Consequently, the susceptibility of *O. viridis* to trematode infection remains uncertain.

This study identified five distinct types of cercariae by discerning morphological characteristics: xiphidiocercariae, echinostome cercariae I, echinostome cercariae II, furcocercous cercariae, and strigea cercariae. Their morphological characters were similar to those previously described (Anucherngchai et al., 2016, 2017; Chontananarth & Wongsawad, 2013; Dunghungzin & Chontananarth, 2020; Krailas et al., 2022; Pantoja et al., 2021). In Thailand, the xiphidiocercaria type has been recorded in several species of snails belonging to the families Bithyniidae (*Bithynia siamensis siamensis* and *Hydrobioides nassa*), Planorbidae (*Gyraulus siamensis*), Lymnaeidae (*Radix auricularia*), Thiaridae (*Melanoides tuberculata* and *Tarebia granifera*), Physidae (*Physa acuta*), and Viviparidae (*Filopaludina*

sumatrensis polygramma and *F. martensi martensi*) (Anucherngchai et al., 2017; Dunghungzin & Chontananarth, 2020; Krailas et al., 2022; Tapdara et al., 2022; Wiroonpan et al., 2021). Meanwhile, echinostome cercariae were released from several species of snails in the families Bithyniidae (*B. s. siamensis*), Lymnaeidae (*R. auricularia*), Nassariidae (*Anentome helena*), Planorbidae (*I. exustus* and *G. siamensis*), Thiaridae (*T. granifera*) and Viviparidae (*F. martensi* and *F. s. polygramma*) (Dunghungzin & Chontananarth, 2020; Krailas et al., 2022; Wiroonpan et al., 2021). Furcocercous cercariae were found to be released from snails in the families Bithyniidae (*B. s. siamensis*), Lymnaeidae (*R. auricularia*), Planorbidae (*I. exustus*), and Thiaridae (*M. tuberculata*) (Anucherngchai et al., 2017; Krailas et al., 2022; Wiroonpan et al., 2021). Meanwhile, the strigea cercaria type was found in snails of the families Lymnaeidae (*R. auricularia*) and Viviparidae (*F. s. polygramma*) (Wiroonpan et al., 2021). In this investigation, xiphidiocercariae showed the highest prevalence of infection, equivalent to 13 out of 22 snails infected with cercariae. This cercaria type usually requires birds or mammals as definitive hosts for its development into adult-stage intestinal flukes (Tkach, 2008; Tkach et al., 2000). Hence, the elevated prevalence of xiphidiocercariae recognized in the current study might be attributed to the coexistence of their definitive and intermediate hosts for this cercaria type within the same ecosystem, resulting in the completion of the parasite's life cycle.

In this research, the presence of *Plagiorchis* and *Echinostoma revolutum* was identified, both being trematodes of public health significance and playing a crucial role as intestinal flukes (Guk, 2007; Chai et al., 2012). *Plagiorchis* spp. are intestinal trematodes of birds, reptiles, and mammals, including humans (Bodell et al., 2014; Guk et al., 2007), and their metacercariae, the infective stage, are found in arthropods, freshwater snails, and freshwater fish (Chai & Lee, 2002; Gordy et al., 2016; Guk et al., 2007). *Plagiorchis* has been considered as the cause of intestinal diseases in patients in Indonesia, Japan, Korea, the Philippines, and Thailand (Ahn et al., 1998; Asada et al., 1962; Eduardo & Lee, 2006; Guk et al., 2007). In Thailand, Radomyos et al. (1989) reported 4 human cases of plagiorchiasis, and all the patients resided in the northeast region, including Khon Kaen, Udon Thani, and Ubon Ratchathani provinces. Meanwhile, *E. revolutum* is known as an intestinal trematode with notable importance in both medical and veterinary contexts (Nagataki et al., 2015; Sohn et al.,

2011). This species is currently recognized to have a broad distribution encompassing the Americas, Europe, Africa, Oceania, and Asia (Chai et al., 2009). Within Southeast Asia, instances of *E. revolutum* infection in humans have been documented in Thailand, Indonesia, Lao PDR, and Cambodia (Chai et al., 2009; Chai et al., 2012; Sohn et al., 2011). The most common cause of infection is ingesting raw or undercooked freshwater snails (second intermediate host) that contain the metacercarial stage (Noikong et al., 2014). Clinical manifestations associated with intestinal echinostomiasis in patients include abdominal pain, looseness of the bowels, and, in severe cases, potential intestinal perforations (Chai et al., 2012; Toledo & Esteban, 2016). Apart from its detrimental impact on humans, this species has been identified as infecting free-grazing ducks in northeastern, central, and northern Thailand, exhibiting a high infection rate of 33.3% (Saijuntha et al., 2013). Duck infection with *E. revolutum* may cause severe symptoms of emaciation and catarrhal enteritis and lead to death (Yousuf et al., 2009). These ducks hold significant economic importance in Thailand due to their contributions to the production of eggs and meat. Our findings suggested the role of *I. exustus*, *R. rubiginosa*, and *O. viridis* as the first intermediate hosts of flukes that can then infect second intermediate hosts, and the presence of these snails can increase the risks to humans in the studied area.

Regarding the genetic variation in *R. rubiginosa* and *O. viridis*, this study employed shell morphological characteristics for the identification of *R. rubiginosa* and *O. viridis,* subsequently confirming the species through DNA sequence data. *Radix rubiginosa* and *O. viridis* had similar morphologies, and the predominant difference between these two species was that *R. rubiginosa* had a higher conical spire and inflated body whorl than *O. viridis*. However, both snail species belong to a group of snails in which the phenotypic plasticity of shell shape results in shell shape differences according to environmental conditions (Dung et al., 2013; Pfenninger et al., 2006; Vinarski et al., 2020; Whelan, 2021). Therefore, differentiating between *R. rubiginosa* and *O. viridis* based on shell morphology can be challenging without expertise in conchology. Molecular sequence data enable swift identification of snail species, even for investigators without expertise in malacology. In this study, the confirmation of *R. rubiginosa* and *O. viridis* identification was established through BLASTn searches, revealing sequence identities ranging from 98% to 100%. This suggested that

R. rubiginosa and *O. viridis* can be identified based on COI and 16S rDNA gene markers in addition to traditional methods that rely on morphology. These findings were in concordance with those of a previous study (Aksenova et al., 2017; Kaset et al., 2010; Remigio, 2002). In addition, this study assessed the intraspecific genetic variations of the mitochondrial 16S rDNA and COI markers to compare the genetic divergences of these two genetic markers. In our study, the COI data revealed that *R. rubiginosa* displayed the highest intraspecific genetic divergence. In contrast, the COI gene showed very low genetic divergence in *O. viridis*. Conversely, the mitochondrial 16S rDNA marker exhibited minimal intraspecific genetic divergence in *R. rubiginosa*, contrasting with the findings from the COI marker. Genetic variations between *R. rubiginosa* and *O. viridis* could be attributed to either the smaller sample sizes of *O. viridis* or variations inherent in the two mitochondrial genes. The COI gene is recognized as a universal marker due to its rapid rate of evolution across a broad range of diverse invertebrates (Folmer et al., 1994; Knowlton & Weigt, 1998), providing a greater depth of genetic information for evolutionary analysis compared to other mitochondrial genes (Bunchom et al., 2021; Mouahid et al., 2018). Genetic data from the COI gene exhibit an evolutionary rate approximately three times faster than that observed in 16S rDNA in some invertebrate groups (Knowlton & Weigt, 1998). The genetic variation in the COI gene may arise from faster evolution in the COI gene, a characteristic not apparent in the 16S rDNA gene (Dumidae et al., 2021; Feng et al., 2011). However, for *O. viridis*, the intraspecific distance of the 16S rDNA exhibited more variation than that of the COI gene, and a similar result has been reported in the freshwater snail *Pomacea maculata* (Kannan et al., 2020). Therefore, the mitochondrial 16S rDNA gene holds promise as a potential marker for investigating the genetic divergence of lymnaeid snails, specifically *O. viridis*.

In the exploration of the genetic variability of *I. exustus*, both mitochondrial and nuclear genes were utilized. Intraspecific distances, based on COI sequences, ranged from 0% to 5.82%, with an average of 0.19%. In contrast, the 16S rDNA gene exhibited minimal intraspecific genetic divergence in *I. exustus*, ranging from 0% to 0.53% (mean 0.01%). Additionally, the analysis of nuclear 28S rDNA in *I. exustus* samples revealed the highest intraspecific genetic divergence among the nuclear markers. Divergence within the 28S rDNA ranged from 0% to 0.78%, with an average

of 0.09%. Conversely, the 18S rDNA gene showed no variation, indicating a pairwise genetic distance of 0%. This indicates that the 18S rDNA gene has a comparatively slow evolutionary rate and lower variability in comparison to the 28S rDNA gene (Bargues & Mas-Coma, 1997; Matsuda et al., 2014). These findings align with a prior study on the genetic variation of the 18S and 28S rDNA genes in a freshwater snail from the genus *Bulinus*, a sister group of *I. exustus* (Jørgensen et al., 2011). Comparatively, when examining genetic divergence among the genetic markers in *I. exustus* in previous reports, the intraspecific genetic distance values of the COI, 16S rDNA, ITS1, and ITS2 sequences were 0-5.33%, 0-2.34%, 0-2.21%, and 0-1.57%, respectively (Mouahid et al., 2018). These results indicated that the 28S rDNA had intraspecific genetic distance values that were closer to those of the nuclear ITS2 region than those of the nuclear ITS1 region and the mitochondrial COI and 16S rDNA genes. Generally, mitochondrial genes evolve faster than nuclear rDNA genes, accumulating a higher degree of sequence variation. In contrast, nuclear rDNA genes are more conserved than mitochondrial genes. This high intraspecies conservation makes nuclear rDNA genes helpful markers for resolving higher taxonomic levels or for use in biodiversity studies (Choudhary et al., 2015; Hwang & Kim, 1999; Patwardhan et al., 2014; Pawlowski et al., 2012). Our findings showed that nuclear 18S and 28S rDNA had lower intraspecies discrimination power than mitochondrial COI and 16S rDNA markers (Matumba et al., 2020). However, the mitochondrial genes both of COI and 16S rDNA and the nuclear genes including ITS1, 18S, and 28S rDNA are considered reliable genetic markers to identify *I. exustus* (Jørgensen et al., 2011; Mouahid et al., 2018).

In our investigation, genetic characterization was conducted by sequencing the COI, 16S rDNA, ITS1, and combined mtDNA genes of *I. exustus* collected from across Thailand was performed with sequences from other geographical regions in GenBank. These genetic markers provided congruent results from both phylogenetic tree and haplotype network, which divided *I. exustus* into four different clades (A to D). By phylogenetic tree and haplotype network of the COI sequences, clade A was the largest group that contained several haplotypes from all samples of Thailand together with Southwest Asia (Oman), Southeast Asia (Philippines, Malaysia, Indonesia, Vietnam, and Laos), South Asia (Sri Lanka and Nepal), West Africa (Benin

and Ivory Coast), Central Africa (Gabon), and Caribbean region (French West Indies). Clade B contained all of the haplotype samples from South Asia, whereas clades C and D consisted of only samples from Nepal in South Asia. These results indicated that *I. exustus* clade A is the most widely distributed clade in many regions throughout the world. The genetic groups classified in our study were consistent with those observed in all prior studies (Devkota et al., 2015; Gauffre-Autelin et al., 2017; Mouahid et al., 2018). Mouahid et al. (2018) revealed a phylogenetic tree of *I. exustus* divided into four clades (A-D). Clade A was specifically found in South Asia (Nepal), clade B occurred in Southeast Asia (Myanmar) and South Asia (Nepal), clade C occurred in Southeast Asia (Laos) and South Asia (India, Bangladesh, and Nepal), and clade D spanned a wide geographical distribution, including South Asia (Nepal, India, and Sri Lanka), Southeast Asia (Malaysia, Vietnam, Indonesia, Laos, Myanmar, Thailand, and the Philippines), Southwest Asia (Oman), West Africa (Benin and Ivory Coast), Central Africa (Gabon), and the Caribbean region (French West Indies). The genetic divergence among the four clades exhibited notably high levels, ranging from 8.11% to 16.93% for COI, 3.16% to 10.05% for 16S rDNA, 6.34% to 13.74% for combined mtDNA, and 2.38% to 9.01% for ITS1. *Indoplanorbis exustus* was likely a complex of cryptic species, which was proposed by Devkota et al. (2015); Gauffre-Autelin et al. (2017); Saijuntha et al. (2021).

Regarding the genetic variation of *I. exustus*, the study explored the genetic diversity across 31 localities in 21 provinces spanning six regions of Thailand. *Indoplanorbis exustus* in Thailand exhibited high genetic divergence with 23 haplotypes identified through the COI sequence, consistent with the findings of a previous study by Saijuntha et al. (2021). These scholars documented a high genetic divergence with 21 haplotypes of *I. exustus* in Thailand, also based on COI sequences. In addition, our results demonstrated that all the haplotypes of *I. exustus* in Thailand (based on COI, 16S rDNA, ITS1, and combined mtDNA) belonged to clade A, the predominant genetic group that distributed globally. However, more than a few of unique haplotypes were found in specific localities, resulting in genetic distinctions among the different localities. The high rate of self-fertilization in *I. exustus* (Bony et al., 2013; Escobar et al., 2011) may lead to levels of population genetic differences

because self-fertility allows for a single individual to rapidly establish populations in new habitats (Ryland & Bishop, 1990).

Simultaneously, the haplotype analysis revealed a varied range of haplotypes within populations for both species of lymnaeid snails. *Radix rubiginosa* showed 23 different haplotypes (R1-R23) based on COI sequences, of which 18 (78%) haplotypes were matchless and 5 (22%) were mutual among at least two localities. Data for *O. viridis* revealed 8 haplotypes (O1-O8). Of these, 4 haplotypes (50%) were exclusive to specific populations, while the remaining four (50%) were shared among at least two localities. The presence of shared haplotypes observed in this study indicated gene flow or migration between distantly distributed populations (Koopman et al., 2007). Despite the low dispersal ability of this snail, anthropogenic ecological transformations, hydrological connectivity, and flash floods have facilitated the expansion of these snails, leading to their establishment in new areas (Gu et al., 2015; Martin et al., 2020). In addition, stochastic transfer by waterfowl can spread snails over large distances and across natural barriers (Kopp et al., 2012). Previous research revealed that freshwater snails can become attached to mallards' bodies and feathers (van Leeuwen & van der Velde, 2012). Several previous studies revealed relatively high haplotype diversity in snails of the family Lymnaeidae. (Bolotov et al., 2017) reported the genetic diversity of COI sequences in *Radix balthica*, revealing 50 different haplotypes in Iceland and 479 haplotypes in mainland Europe and on islands of the United Kingdom. Many haplotypes (29 COI haplotypes) were also found in *R. euphratica* from the Middle East and Central Asia (Mirfendereski et al., 2021) The elevated mutation rate of mitochondrial DNA contributed, in part, to the extensive diversity of haplotypes (Vandewoestijne et al., 2004). The elevated mutation rate was corroborated by the median-joining network analysis, demonstrating that the haplotype had undergone at least one mutation step before transitioning into a new haplotype.

Remarkably, our study found that only a few haplotypes of *R. rubiginosa* and *O. viridis* were infected with trematode cercariae. Similarly, it was observed that trematode cercariae infections were exclusive to *I. exustus* clade A, the most widely distributed clade found in numerous regions worldwide. The association between host and trematode infection was previously reported in the snail *I. exustus* (Devkota et al., 2015), which reported the existence of a snail-host complex and classified *I. exustus*

into four different lineages. Lineage I included *I. exustus* infected with echinostome and *Schistosoma* sp. Lineage II comprised snails infected with xiphidiocercariae, strigeids, and sanguinicolid cercariae. Lineage III consisted of snails positive for *S. nasale*, and lineage IV comprised snails infected with strigeids, sanguinicolids, and xiphidiocercariae, *S. nasale*, *S. spindale*, *S. indicum*, and *Schistosoma* sp. The establishment success of parasites in a particular area may be significantly influenced by the genetic composition of intermediate snail hosts (Van den Broeck et al., 2015). This situation corresponds to the hypothesis of the matching phenotypic model at the molecular level. According to this model, the outcome of the infection is determined by the interactions that occur during its initial stages, involving the interplay between antigens in the parasite and immune receptors in the host (Mitta et al., 2012). Snails compatible with parasites have little or no immunopathic responses upon infection, thus allowing parasites to successfully establish and develop (Richards et al., 1992). However, it's worth noting that Hammoud et al. (2022) found that snail haplotype did not influence trematode infection status. In contrast, Schmid-Hempel & Stauffer (1998) emphasized that host-parasite susceptibility can increase with the loss of genetic variation. This underscores the pivotal role of *I. exustus*, *R. rubiginosa*, and *O. viridis* in facilitating the spread of trematode parasites in Thailand.

Population genetic structure analysis of *I. exustus* in Thailand demonstrated that the majority of comparisons did not exhibit significant genetic differences. This suggests that the observed genetic uniformity in the *I. exustus* population in Thailand may be attributed to gene flow (Szalanski et al., 2008). Moreover, the MJ network analysis indicated that most specimens shared a central haplotype, and the other haplotypes displayed short branch lengths, closely linking them to this central haplotype. The overall structure exhibits a star-like shape, which suggests a recent population expansion (Slatkin & Hudson, 1991). This phenomenon was further supported by the unimodal pattern observed in the mismatch distribution graph, accompanied by highly negative values derived from both Tajima's D and Fu's Fs tests. Therefore, it is probable that the *I. exustus* snail is another freshwater snail invasive species in Thailand.

Our study was in concordance with the previous studies of Saijuntha et al. (2021), which revealed that *I. exustus* in Thailand was commonly found in paddy fields, canals, and swamps, which were widely distributed in more than 30 provinces covering six regions of Thailand. This study also demonstrated that *I. exustus* in Thailand shared haplotypes throughout multiple populations, despite some areas being geographically distant localities. This suggests the presence of gene flow within the *I. exustus* population in Thailand (Saijuntha et al., 2021). The occurrence of *I. exustus* in distant locations from its "native" geographic area is likely associated with human activities involving the trade of aquatic plants to which *I. exustus* can easily attach. For example, *Pomacea* spp. originated in South and Central America and subsequently spread to various regions globally, spanning Asia, Europe, North America, and the Pacific Islands (Hayes et al., 2015; Hayes et al., 2008; Rawlings et al., 2007; Saijuntha et al., 2021). Additionally, *Helisoma duryi*, with its origin in North America, has expanded its distribution into South America and Africa (Pointier et al., 2005).

Our results revealed a low level of genetic variation within both *R. rubiginosa* and *O. viridis* populations. The observed results could be associated with severe repeated or long periods of population bottlenecks, which are a result of natural environmental changes, especially seasonal changes. Seasonal variations in the availability of aquatic habitats are the main factor affecting the fluctuations of snails. According to Suhardono & Copeman (2000), *R. rubiginosa* in dry fallow rice fields died during the dry season after four weeks without rain, but those in persistent aquatic refuges such as streams and springs were able to survive. Populations that survive a bottleneck may undergo genetic drift (Nyström et al., 2006). Population bottlenecks lead to a diminished population size and losses in genetic variation because of random genetic drift, which may result in reduced genetic variability in the population (Birungi & Munstermann, 2002; Shirk et al., 2014; Weber et al., 2004). This phenomenon has been previously observed in other freshwater snails, such as *R. balthica*, *R. truncatula*, *Pomacea* spp., and *Bulinus* spp. (Aksenova et al., 2017; Dumidae et al., 2021; Mutsaka-Makuvaza et al., 2020; Meunier et al., 2004). Besides, *R. rubiginosa* and *O. viridis* had high haplotype diversity (0.9084 and 0.7535, respectively), which indicates a rapid expansion after population decrease (Grant & Bowen, 1998). In addition, it also

indicates adaptations enabling the colonization of a wide range of habitats (Liu et al., 2019).

Grant & Bowen (1998) categorized the genetic diversity of the population into four primary types using mtDNA markers: (I) low haplotype (h) and nucleotide diversities (π), (II) high haplotype (h) and low nucleotide diversities (π), (III) low haplotype (h) and high nucleotide diversities (π), and (IV) high haplotype and nucleotide diversities. Our results fell into the second type which was considered by high haplotype diversity and low nucleotide diversity. This model suggests that it may be linked to population expansion after an effective small size of the population, followed by a phase of rapid growth of the population. This phenomenon boosts the persistence of newly emerged mutations within the population (Avise, 2000; Grant & Bowen, 1998; Rogers & Harpending, 1992).

In the analysis of population genetic structure (pairwise F_{ST}) of *R. rubiginosa* and *O. viridis*, most comparisons showed a significant genetic difference. These results indicate limited gene flow or low migration between the populations of *R. rubiginosa* and *O. viridis*. Many factors can limit migration between populations, such as geographic barriers, climate, habitat type, the presence of predators, and interspecific competition (Fernandez et al., 2010; Saito et al., 2021). Furthermore, geographical isolation is an important factor that affects the distribution of populations (Bunchom et al., 2021). Lymnaeid snails often live in clear, shallow, and gently flowing fresh water (Tookhy et al., 2023), where the water habitat is relatively isolated. Water habitat separation may affect genetic differences because the water flow between catchments is limited. This would subsequently lead to limited migration between populations in various areas (Bunchom et al., 2021). This phenomenon has been previously documented in other freshwater snails in Thailand, including *B. siamensis* and *Hydrobioides nassa* (Bunchom et al., 2021a, 2021b). Another aspect is that Lymnaeid snails have a relatively low dispersal ability, and their activity range is limited (Haun et al., 2012), which may lead to a gradual decrease in gene flow and result in genetic differentiation between populations in a given area.

Tajima's D of *R. rubiginosa* was negative nonsignificant. Conversely, Fu's Fs test showed significant negative values. For *O. viridis*, both Tajima's D and Fu's Fs values were significant and negative. Tajima's D is particularly responsive to recent instances of population expansion or bottlenecks, while Fu's Fs is indicative of population expansion, typically yielding substantial negative values (Fu, 1997; Tajima, 1989). Therefore, our results suggest that the populations of *R. rubiginosa* and *O. viridis* may have undergone historical growth, but this expansion might have been confined to specific regions. This result was supported by the limited gene flow revealed by the pairwise F_{ST} analysis. In addition, the examination of mismatch distribution exposed bimodal characteristics within the population of *R. rubiginosa*, whereas *O. viridis* exhibited multimodal characteristics of population differentiation. In general, bimodal and multimodal graphs indicate decreasing population sizes and a ragged distribution, which indicates that the population was widely distributed (Rogers, 1995; Rogers & Harpending, 1992). Meanwhile, the sum-of-square deviation and Harpending's raggedness index for both *R. rubiginosa* and *O. viridis* demonstrated nonsignificant deviation from the model of simulated sudden demographic expansion, which indicates that the data best fit a model of population demographic expansion. Our findings suggested the possibility that *R. rubiginosa* and *O. viridis* experienced a population reduction followed by an expansion.

Snails introduced into new areas led to host-parasite associations and to the development of life cycles. In the past, *I. exustus*, *R. rubiginosa*, and *O. viridis* may have acted as potential hosts for several trematode parasites, such as *Fasciola gigantica*, *F. hepatica*, *Echinostoma* spp., *Plagiorchis* spp., *Trichobilharzia regenti*, *Schistosoma indicum*, *S. nasale*, and *S. spindale* (Devkota et al., 2015; Japa et al., 2021; Krailas et al., 2022; Lee et al., 1995; Tookhy et al., 2023). *Indoplanorbis exustus*, acknowledged for its high fecundity, is a hermaphroditic invasive snail species renowned for its elevated reproductive capacity. In just one year after its introduction, this snail species demonstrates a remarkable ability to colonize habitats that already harbor well-established populations of other freshwater snails (Pointier et al., 2005). The species' capacity for high fecundity with self-fertilization is likely a key factor contributing to its invasive potential (Raut et al., 1992). These traits may play a substantial role in influencing the distribution of the parasites into new locations.

Invasive snails impact the transmission of trematode parasites in at least two ways. Firstly, the invasion of snails serves as a primary facilitator in establishing the life cycle of trematode parasite, thereby increasing the likelihood of rapid infection of secondary intermediate and definitive hosts (Lu et al., 2018). Secondly, the invasion of snails accelerates the parasite spread by rapidly expanding their range, completing the parasite life cycle in nonendemic areas (Lv et al., 2009). The presence of the parasitosis vector in a geographical area raises the potential for disease emergence if the parasite is introduced (Boissier et al., 2016). Consequently, *I. exustus*, *R. rubiginosa*, and *O. viridis* are important for maintaining the life cycles of trematode parasites and can facilitate parasite spread into new areas.

In summary, our study offers comprehensive data on cercarial infection and the distribution of *Indoplanorbis* and lymnaeid snails in Thailand. Notably, *O. viridis* exhibited a higher prevalence of cercarial infection than *R. rubiginosa* and *I. exustus*. The cercariae found could be classified into five different types based on their morphology, and molecular analysis of the ITS2 region and 28S rDNA gene revealed four trematode families. This underscores the crucial role played by *I. exustus* and lymnaeid snails in the successful spread of digenean trematodes. Subsequently, samples of *I. exustus*, *R. rubiginosa*, and *O. viridis* from six regions of Thailand were randomly selected for genetic analysis. The genetic analysis of *I. exustus* demonstrated high genetic diversity due to the existence of numerous different haplotypes. Additionally, the phylogenetic and network analyses of haplotypes of *I. exustus* based on sequences from Thailand and other countries available in GenBank, revealed four distinct clades (A to D). Clade A was the largest group and contained haplotypes from all samples of Thailand. The haplotype network structure of *I. exustus* of Thailand has a star-like phylogeny, which is indicative of the population's recent demographic expansion. The shape of the mismatch distribution graph and the strongly negative results of the Tajima's D and Fu's Fs tests offer additional support for this scenario. Therefore, it is probable that the *I. exustus* snail is another freshwater snail invasive species in Thailand. Conversely, analyses of the genetic diversity and structure of the *R. rubiginosa* and *O. viridis* populations indicated that they have experienced a bottleneck phenomenon and limited migration between populations. Furthermore, demographic event history analysis revealed population reductions followed by

subsequent expansions in both *R. rubiginosa* and *O. viridis*. This information has potential applications in the management and surveillance of public health, particularly in areas susceptible to the spread of trematode parasites.

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APPENDIX

APPENDIX A SNAIL SAMPLE INFORMATION

Table 27 Demographic information of the *I. exustus, R. rubiginosa* and *O. viridis* samples in the present study.

Collection site	Code	Latitude/ Longitude	Region	Habitat	No. of snails collected	No. of snails used for the shedding method (cercaria positive)			No. of snails used for genetic analysis		
						I. exustus	*R. rubiginosa*	*O. viridis*	*I. exustus*	*R. rubiginosa*	*O. viridis*
Ban Kaeng, Tron District, Uttaradit Province	UTT1	17.4582/ 100.1674	North	Paddy field	1	1 (0)	0	0	1	0	0
Nam Ang, Tron District, Uttaradit Province	UTT2	17.4576/ 100.2178	North	Paddy field	31	0	0	31 (7)	0	0	12
Tha Sop Sao, Mae Tha District, Lamphun Province	LPN1	18.4382/ 99.0969	North	Irrigation canal	9	9 (0)	0	0	6	0	0
Mae Tha, Mae Tha District, Lampang Province	LPG1	18.1725/ 99.5615	North	Paddy field	17	1 (0)	0	16 (0)	1	0	10
Nong Ha, San Sai District, Chiang Mai Province	CMI1	18.8938/ 98.9944	North	Paddy field	7	0	0	7 (0)	0	0	0

Table 27 (Cont.)

Collection site	Code	Latitude/ Longitude	Region	Habitat	No. of snails collected	No. of snails used for the shedding method (cercaria positive)			No. of snails used for genetic analysis		
						I. exustus	*R. rubiginosa*	*O. viridis*	*I. exustus*	*R. rubiginosa*	*O. viridis*
Mueang Kaeo, Mae Rim District, Chiang Mai Province	CMI2	18.8882/ 98.9855	North	Paddy field	22	0	0	22 (0)	0	0	10
Pa Ko Dam, Mae Lao District, Chiang Rai Province	CRI1	19.7681/ 99.7369	North	Paddy field	3	0	0	3 (0)	0	0	3
Wiang, Mueang Phayao, Phayao Province	PYO1	19.1721/ 99.8943	North	Lotus pond	66	0	66 (0)	0	0	10	0
Thung Lui Lai, Khon San District, Chaiyaphum Province	CPM2	16.6007/ 101.7428	Northeast	Wetland pond	17	17 (0)	0	0	8	0	0
Na Fai, Phu Phaman District, Khon Kaen Province	KKN2	16.7320/ 101.8440	Northeast	Wetland pond	5	2 (0)	3 (0)	0	2	3	0

Table 27 (Cont.)

Collection site	Code	Latitude/ Longitude	Region	Habitat	No. of snails collected	No. of snails used for the shedding method (cercaria positive)			No. of snails used for genetic analysis		
						I. exustus	*R. rubiginosa*	*O. viridis*	*I. exustus*	*R. rubiginosa*	*O. viridis*
Kut Chap, Kut Chap District, Udon Thani Province	UDN2	17.3901/ 102.4995	Northeast	Wetland pond	1	1 (0)	0	0	1	0	0
Nong Wua So, Nong Wua So District, Udon Thani Province	UDN3	17.1837/ 102.4293	Northeast	Wetland pond	6	6 (0)	0	0	5	0	0
Tha Pho, Mueang Phitsanulok District, Phitsanulok Province	PLK1	16.7118/ 100.1977	Central	Pond	19	19 (0)	0	0	8	0	0
	PLK2	16.7069/ 100.1978		Paddy field	1	0	0	1 (0)	0	0	1
	PLK3	16.7030/ 100.2127		Paddy field	2	0	0	2 (0)	0	0	2
	PLK4	16.7044/ 100.2156		Paddy field	20	0	0	20 (0)	0	0	5
	PLK5	16.6936/ 100.2268		Paddy field	2	0	1 (0)	1 (0)	0	0	1

Table 27 (Cont.)

Collection site	Code	Latitude/ Longitude	Region	Habitat	No. of snails collected	No. of snails used for the shedding method (cercaria positive)			No. of snails used for genetic analysis		
						I. exustus	*R. rubiginosa*	*O. viridis*	*I. exustus*	*R. rubiginosa*	*O. viridis*
	PLK6	16.6926/ 100.2249		Paddy field	9	9 (0)	0	0	5	0	0
	PLK7	16.7249/ 100.1795		Paddy field	15	0	2 (0)	13 (1)	0	2	5
	PLK8	16.7194/ 100.1872		Wetland pond	1	0	1 (0)	0	0	1	0
	PLK9	16.7191/ 100.1879		Paddy field	23	4 (0)	4 (0)	15 (0)	4	3	3
	PLK10	16.7090/ 100.2034		Paddy field	23	23 (0)	0	0	10	0	0
	PLK11	16.6824/ 100.2303		Paddy field	106	3 (0)	0	103 (0)	2	0	4
	PLK12	16.6778/ 100.2387		Paddy field	3	0	0	3 (0)	0	0	0
Wat Phrik, Mueang Phitsanulok District, Phitsanulok Province	PLK13	16.7017/ 100.2551	Central	Paddy field	1	0	0	1 (0)	0	0	1

Table 27 (Cont.)

Collection site	Code	Latitude/ Longitude	Region	Habitat	No. of snails collected	No. of snails used for the shedding method (cercaria positive)			No. of snails used for genetic analysis		
						I. exustus	*R. rubiginosa*	*O. viridis*	*I. exustus*	*R. rubiginosa*	*O. viridis*
Khlong Maphlap, Si Nakhon District, Sukhothai Province	STI1	17.3623/ 99.9892	Central	Lotus pond	5	3 (0)	2 (0)	0	3	2	0
Ban Na, Wachirabarami District, Phichit Province	PCT1	16.5127/ 100.1519	Central	Paddy field	20	3 (0)	0	17 (2)	1	0	11
Sam Ngam, Sam Ngam District, Phichit Province	PCT2	16.5287/ 100.2189	Central	Paddy field	7	4 (0)	3 (1)	0	4	2	0
Pho Sai Ngam, Bueng Na Rang District, Phichit Province	PCT3	16.1187/ 100.1266	Central	Lotus pond	27	14 (0)	13 (0)	0	6	8	0
Nam Ko, Lom Sak, District, Phetchabun Province	PNB3	16.7769/ 101.1922	Central	Lotus pond	5	5 (0)	0	0	1	0	0

Table 27 (Cont.)

Collection site	Code	Latitude/ Longitude	Region	Habitat	No. of snails collected	No. of snails used for the shedding method (cercaria positive)			No. of snails used for genetic analysis		
						I. exustus	*R. rubiginosa*	*O. viridis*	*I. exustus*	*R. rubiginosa*	*O. viridis*
Bo Rang, Wichian Buri District, Phetchabun Province	PNB4	15.5994/ 101.1461	Central	Lotus pond	28	0	28 (0)	0	0	12	0
Tha Chanuan, Manorom District, Chai Nat Province	CNT1	15.3717/ 100.1546	Central	Paddy field	44	18 (0)	26 (0)	0	6	6	0
	CNT2	15.3413/ 100.1677		Paddy field	26	14 (0)	12 (0)	0	6	6	0
Hang Nam Sakhon, Manorom District, Chai Nat Province	CNT3	15.3111/ 100.1842	Central	Irrigation canal	23	22 (0)	1 (0)	0	6	1	0
Chi Nam Rai, In Buri District, Sing Buri Province	SBR1	15.0609/ 100.3247	Central	Paddy field	31	31 (0)	0	0	5	0	0
	SBR2	15.07644/ 100.3115		Irrigation canal	8	7 (0)	1 (0)	0	6	1	0
Tha Ngam, In Buri District, Sing Buri Province	SBR3	15.0370/ 100.3361	Central	Paddy field	44	0	0	44 (4)	0	0	10

Table 27 (Cont.)

Collection site	Code	Latitude/ Longitude	Region	Habitat	No. of snails collected	No. of snails used for the shedding method (cercaria positive)			No. of snails used for genetic analysis		
						I. exustus	*R. rubiginosa*	*O. viridis*	*I. exustus*	*R. rubiginosa*	*O. viridis*
Namtan, In Buri District, Sing Buri Province	SBR4	14.9897/ 100.3530	Central	Paddy field	2	0	0	2 (0)	0	0	1
	SBR5	14.9625/ 100.3717		Paddy field	41	41 (0)	0	0	5	0	0
Nam Song, Phayuha Khiri District, Nakhon Sawan Province	NSN1	15.4261/ 100.1180	Central	Irrigation canal	109	83 (1)	26 (2)	0	7	8	0
Nong Krot, Banphot Phisai District, Nakhon Sawan Province	NSN2	16.0211/ 100.1140	Central	Paddy field	9	2 (0)	6 (0)	1 (0)	2	4	1
Chorakhe Rong, Chaiyo District, Ang Thong Province	ATG1	14.6493/ 100.4764	Central	Paddy field	4	3 (0)	1 (0)	0	2	1	0

Table 27 (Cont.)

Collection site	Code	Latitude/ Longitude	Region	Habitat	No. of snails collected	No. of snails used for the shedding method (cercaria positive)			No. of snails used for genetic analysis		
						I. exustus	*R. rubiginosa*	*O. viridis*	*I. exustus*	*R. rubiginosa*	*O. viridis*
Ban Len, Bang Pa-in District, Ayutthaya Province	AYA1	14.2282/ 100.6114	Central	Paddy field	2	1 (0)	1 (0)	0	1	1	0
Thong Lang, Ban Na District, Nakhon Nayok Province	NYK1	14.1862/ 101.0387	Central	Paddy field	2	2 (0)	0	0	2	0	0
Pa Kha, Ban Na District, Nakhon Nayok Province	NYK2	14.2801/ 101.0501	Central	Lotus pond	46	0	46 (0)	0	0	12	0
Phu Khae, Chaloem Phra Kiat District, Saraburi Province	SRI1	14.6728/ 100.8850	Central	Irrigation canal	2	0	2 (0)	0	0	2	0
Hat Thanong, Mueang Uthai Thani District, Uthai Thani Province	UTI1	15.4198/ 100.0977	Central	Paddy field	10	0	0	10 (0)	0	0	4

Table 27 (Cont.)

Collection site	Code	Latitude/ Longitude	Region	Habitat	No. of snails collected	No. of snails used for the shedding method (cercaria positive)			No. of snails used for genetic analysis		
						I. exustus	*R. rubiginosa*	*O. viridis*	*I. exustus*	*R. rubiginosa*	*O. viridis*
Nam Ruem, Mueang Tak District, Tak Province	TAK1	16.8900/ 99.2211	West	Irrigation canal	15	15 (0)	0	0	12	0	0
Mae Tho, Muang Tak District, Tak Province	TAK4	16.8196/ 99.0708	West	Wetland pond	5	0	5 (0)	0	0	3	0
Wang Prachop, Muang Tak District, Tak Province	TAK5	16.9145/ 99.3335	West	Paddy field	3	3 (0)	0	0	3	0	0
Phlio, Laem Sing District, Chanthaburi Province	CTI1	12.5146/ 102.1597	East	Lotus pond	1	1 (0)	0	0	1	0	0
Saen Suk, Mueang Chon Buri District, Chon Buri Province	CBI1	13.2803/ 100.9268	East	Lotus pond	74	74 (0)	0	0	10	0	0

Table 27 (Cont.)

Collection site	Code	Latitude/ Longitude	Region	Habitat	No. of snails collected	No. of snails used for the shedding method (cercaria positive)			No. of snails used for genetic analysis		
						I. exustus	*R. rubiginosa*	*O. viridis*	*I. exustus*	*R. rubiginosa*	*O. viridis*
Khlong Nakhon Nueang Khet, Mueang Chachoengsao District, Chachoengsao Province	CCO1	13.7562/ 101.0551	East	Paddy field	8	0	8 (0)	0	0	5	0
Sai Khao, Khok Pho District, Pattani Province	PTN2	6.6784/ 101.0842	South	Paddy field	16	4 (0)	12 (0)	0	3	11	0
Kho Hong, Hat Yai District, Songkhla Province	SKA1	7.0113/ 100.4987	South	Lotus pond	89	89 (0)	0	0	8	0	0
Phawong, Mueang Songkhla District, Songkhla Province	SKA2	7.1542/ 100.5762	South	Lotus pond	131	41 (1)	90 (3)	0	9	12	0
Total					**1,247**	**575 (2)**	**360 (6)**	**312 (14)**	162	116	84

APPENDIX B GENBANK ACCESSION NUMBERS OF SNAILS

Table 28 List of the GenBank accession numbers of *I. exustus* in this study.

Sample code	GenBank accession numbers				
	COI	**16S rDNA**	**ITS1**	**18S rDNA**	**28S rDNA**
I1PLK1_TH	OP588466	OP585918	OP586437	-	-
I2PLK1_TH	OP588467	OP585919	OP586438	-	-
I3PLK1_TH	OP588468	OP585920	OP586439	-	-
I6PLK1_TH	OP588469	OP585921	OP586440	OQ975759	OQ975465
I7PLK1_TH	OP588470	OP585922	OP586441	-	-
I12PLK1_TH	OP588471	OP585923	OP586442	-	-
I15PLK1_TH	OP588472	OP585924	OP586443	-	-
I16PLK1_TH	OP588473	OP585925	OP586444	-	-
I24PLK6_TH	OP588474	OP585926	OP586445	-	-
I25PLK6_TH	OP588475	OP585927	OP586446	-	-
I26PLK6_TH	OP588476	OP585928	OP586447	-	-
I27PLK6_TH	OP588477	OP585929	OP586448	OQ975760	OQ975466
I28PLK6_TH	OP588478	OP585930	OP586449	-	-
I29PLK9_TH	OP588479	OP585931	OP586450	-	-
I30PLK9_TH	OP588480	OP585932	OP586451	-	-
I31PLK9_TH	OP588481	OP585933	OP586452	-	-
I32PLK9_TH	OP588482	OP585934	OP586453	-	-
I34PLK10_TH	OP588483	OP585935	OP586454	-	-
I36PLK10_TH	OP588484	OP585936	OP586455	OQ975761	OQ975467
I37PLK10_TH	OP588485	OP585937	OP586456	-	-
I38PLK10_TH	OP588486	OP585938	OP586457	-	-
I39PLK10_TH	OP588487	OP585939	OP586458	-	-
I40PLK10_TH	OP588488	OP585940	OP586459	-	-
I41PLK10_TH	OP588489	OP585941	OP586460	OQ975762	OQ975468
I44PLK10_TH	OP588490	OP585942	OP586461	-	-

Table 28 (Cont.)

Sample code	GenBank accession numbers				
	COI	**16S rDNA**	**ITS1**	**18S rDNA**	**28S rDNA**
I45PLK10_TH	OP588491	OP585943	OP586462	-	-
I46PLK10_TH	OP588492	OP585944	OP586463	-	-
I57PLK11_TH	OP588493	OP585945	OP586464	-	-
I58PLK11_TH	OP588494	OP585946	OP586465	-	-
I59UTT1_TH	OP588495	OP585947	OP586466	OQ975763	OQ975469
I60LPN1_TH	OP588496	OP585948	OP586467	OQ975764	OQ975470
I61LPN1_TH	OP588497	OP585949	OP586468	OQ975765	OQ975471
I62LPN1_TH	OP588498	OP585950	OP586469	-	-
I63LPN1_TH	OP588499	OP585951	OP586470	-	-
I64LPN1_TH	OP588500	OP585952	OP586471	-	-
I65LPN1_TH	OP588501	OP585953	OP586472	-	-
I70SKA1_TH	OP588502	OP585954	OP586473	OQ975766	OQ975472
I71SKA1_TH	OP588503	OP585955	OP586474	OQ975767	OQ975473
I72SKA1_TH	OP588504	OP585956	OP586475	-	-
I73SKA1_TH	OP588505	OP585957	OP586476	-	-
I74SKA1_TH	OP588506	OP585958	OP586477	-	-
I75SKA1_TH	OP588507	OP585959	OP586478	-	-
I76SKA1_TH	OP588508	OP585960	OP586479	-	-
I77SKA1_TH	OP588509	OP585961	OP586480	-	-
I182PTN2_TH	OP588510	OP585962	OP586481	OQ975768	OQ975474
I183PTN2_TH	OP588511	OP585963	OP586482	OQ975769	OQ975475
I184PTN2_TH	OP588512	OP585964	OP586483	-	-
I185STI1_TH	OP588513	OP585965	OP586484	OQ975770	OQ975476
I186STI1_TH	OP588514	OP585966	OP586485	OQ975771	OQ975477
I187STI1_TH	OP588515	OP585967	OP586486	-	-
I291PCT1_TH	OP588516	OP585968	OP586487	OQ975772	OQ975478
I293PCT2_TH	OP588517	OP585969	OP586488	OQ975773	OQ975479
I294PCT2_TH	OP588518	OP585970	OP586489	-	-

Table 28 (Cont.)

Sample code	GenBank accession numbers				
	COI	**16S rDNA**	**ITS1**	**18S rDNA**	**28S rDNA**
I295PCT2_TH	OP588519	OP585971	OP586490	-	-
I296PCT2_TH	OP588520	OP585972	OP586491	-	-
I297LPG1_TH	OP588521	OP585973	OP586492	OQ975774	OQ975480
I299PNB3_TH	OP588522	OP585974	OP586493	OQ975775	OQ975481
I304PCT3_TH	OP588523	OP585975	OP586494	-	-
I305PCT3_TH	OP588524	OP585976	OP586495	-	-
I306PCT3_TH	OP588525	OP585977	OP586496	-	-
I307PCT3_TH	OP588526	OP585978	OP586497	-	-
I308PCT3_TH	OP588527	OP585979	OP586498	-	-
I309PCT3_TH	OP588528	OP585980	OP586499	-	-
I326CNT1_TH	OP588529	OP585981	OP586500	OQ975776	OQ975482
I328CNT1_TH	OP588530	OP585982	OP586501	OQ975777	OQ975483
I330CNT1_TH	OP588531	OP585983	OP586502	-	-
I332CNT1_TH	OP588532	OP585984	OP586503	-	-
I334CNT1_TH	OP588533	OP585985	OP586504	-	-
I335CNT1_TH	OP588534	OP585986	OP586505	-	-
I338CNT2_TH	OP588535	OP585987	OP586506	-	-
I340CNT2_TH	OP588536	OP585988	OP586507	-	-
I342CNT2_TH	OP588537	OP585989	OP586508	-	-
I344CNT2_TH	OP588538	OP585990	OP586509	-	-
I346CNT2_TH	OP588539	OP585991	OP586510	-	-
I348CNT2_TH	OP588540	OP585992	OP586511	-	-
I350SBR1_TH	OP588541	OP585993	OP586512	-	-
I351SBR1_TH	OP588542	OP585994	OP586513	OQ975778	OQ975484
I352SBR1_TH	OP588543	OP585995	OP586514	OQ975779	OQ975485
I353SBR1_TH	OP588544	OP585996	OP586515	-	-
I355SBR1_TH	OP588545	OP585997	OP586516	-	-
I381SBR2_TH	OP588546	OP585998	OP586517	-	-

Table 28 (Cont.)

Sample code	GenBank accession numbers				
	COI	**16S rDNA**	**ITS1**	**18S rDNA**	**28S rDNA**
I382SBR2_TH	OP588547	OP585999	OP586518	-	-
I383SBR2_TH	OP588548	OP586000	OP586519	-	-
I384SBR2_TH	OP588549	OP586001	OP586520	OQ975780	OQ975486
I385SBR2_TH	OP588550	OP586002	OP586521	-	-
I386SBR2_TH	OP588551	OP586003	OP586522	-	-
I388CNT3_TH	OP588552	OP586004	OP586523	-	-
I389CNT3_TH	OP588553	OP586005	OP586524	-	-
I390CNT3_TH	OP588554	OP586006	OP586525	-	-
I391CNT3_TH	OP588555	OP586007	OP586526	-	-
I392CNT3_TH	OP588556	OP586008	OP586527	-	-
I393CNT3_TH	OP588557	OP586009	OP586528	-	-
I410NSN1_TH	OP588558	OP586010	OP586529	OQ975781	OQ975487
I411NSN1_TH	OP588559	OP586011	OP586530	-	-
I412NSN1_TH	OP588560	OP586012	OP586531	-	-
I413NSN1_TH	OP588561	OP586013	OP586532	-	-
I414NSN1_TH	OP588562	OP586014	OP586533	-	-
I415NSN1_TH	OP588563	OP586015	OP586534	OQ975782	OQ975488
I416NSN1_TH	OP588564	OP586016	OP586535	-	-
I493NSN2_TH	OP588565	OP586017	OP586536	OQ975783	OQ975489
I494NSN2_TH	OP588566	OP586018	OP586537	-	-
I495KKN2_TH	OP588567	OP586019	OP586538	OQ975784	OQ975490
I496KKN2_TH	OP588568	OP586020	OP586539	OQ975785	OQ975491
I497CPM2_TH	OP588569	OP586021	OP586540	OQ975786	OQ975492
I498CPM2_TH	OP588570	OP586022	OP586541	-	-
I499CPM2_TH	OP588571	OP586023	OP586542	-	-
I500CPM2_TH	OP588572	OP586024	OP586543	-	-
I501CPM2_TH	OP588573	OP586025	OP586544	-	-
I502CPM2_TH	OP588574	OP586026	OP586545	-	-

Table 28 (Cont.)

Sample code	GenBank accession numbers				
	COI	**16S rDNA**	**ITS1**	**18S rDNA**	**28S rDNA**
I503CPM2_TH	OP588575	OP586027	OP586546	OQ975787	OQ975493
I510CPM2_TH	OP588576	OP586028	OP586547	-	-
I515SKA2_TH	OP588577	OP586029	OP586548	-	-
I517SKA2_TH	OP588578	OP586030	OP586549	-	-
I518SKA2_TH	OP588579	OP586031	OP586550	-	-
I519SKA2_TH	OP588580	OP586032	OP586551	-	-
I524SKA2_TH	OP588581	OP586033	OP586552	OQ975788	OQ975494
I532SKA2_TH	OP588582	OP586034	OP586553	OQ975789	OQ975495
I533SKA2_TH	OP588583	OP586035	OP586554	-	-
I547SKA2_TH	OP588584	OP586036	OP586555	-	-
I549SKA2_TH	OP588585	OP586037	OP586556	-	-
I555UDN2_TH	OP588586	OP586038	OP586557	OQ975790	OQ975496
I556UDN3_TH	OP588587	OP586039	OP586558	OQ975791	OQ975497
I557UDN3_TH	OP588588	OP586040	OP586559	-	-
I558UDN3_TH	OP588589	OP586041	OP586560	-	-
I559UDN3_TH	OP588590	OP586042	OP586561	-	-
I560UDN3_TH	OP588591	OP586043	OP586562	-	-
I562TAK1_TH	OP588592	OP586044	OP586563	OQ975792	OQ975498
I564TAK1_TH	OP588593	OP586045	OP586564	OQ975793	OQ975499
I565TAK1_TH	OP588594	OP586046	OP586565	-	-
I566TAK1_TH	OP588595	OP586047	OP586566	-	-
I567TAK1_TH	OP588596	OP586048	OP586567	-	-
I569TAK1_TH	OP588597	OP586049	OP586568	-	-
I571TAK1_TH	OP588598	OP586050	OP586569	-	-
I572TAK1_TH	OP588599	OP586051	OP586570	-	-
I573TAK1_TH	OP588600	OP586052	OP586571	-	-
I574TAK1_TH	OP588601	OP586053	OP586572	-	-
I575TAK1_TH	OP588602	OP586054	OP586573	-	-

Table 28 (Cont.)

Sample code	GenBank accession numbers				
	COI	**16S rDNA**	**ITS1**	**18S rDNA**	**28S rDNA**
I576TAK1_TH	OP588603	OP586055	OP586574	-	-
I577TAK5_TH	OP588604	OP586056	OP586575	-	-
I578TAK5_TH	OP588605	OP586057	OP586576	-	-
I579TAK5_TH	OP588606	OP586058	OP586577	-	-
I581CTI1_TH	OP588607	OP586059	OP586578	OQ975794	OQ975500
I583ATG1_TH	OP588608	OP586060	OP586579	OQ975795	OQ975501
I698ATG1_TH	OP588627	OP586079	OP586598	OQ975802	OQ975508
I585AYA1_TH	OP588609	OP586061	OP586580	OQ975796	OQ975502
I594CBI1_TH	OP588610	OP586062	OP586581	OQ975797	OQ975503
I595CBI1_TH	OP588611	OP586063	OP586582	-	-
I598CBI1_TH	OP588612	OP586064	OP586583	-	-
I599CBI1_TH	OP588613	OP586065	OP586584	-	-
I600CBI1_TH	OP588614	OP586066	OP586585	-	-
I601CBI1_TH	OP588615	OP586067	OP586586	-	-
I602CBI1_TH	OP588616	OP586068	OP586587	-	-
I603CBI1_TH	OP588617	OP586069	OP586588	-	-
I604CBI1_TH	OP588618	OP586070	OP586589	-	-
I605CBI1_TH	OP588619	OP586071	OP586590	OQ975798	OQ975504
I656NYK1_TH	OP588620	OP586072	OP586591	OQ975799	OQ975505
I657NYK1_TH	OP588621	OP586073	OP586592	OQ975800	OQ975506
I662SBR5_TH	OP588622	OP586074	OP586593	-	-
I665SBR5_TH	OP588623	OP586075	OP586594	OQ975801	OQ975507
I668SBR5_TH	OP588624	OP586076	OP586595	-	-
I669SBR5_TH	OP588625	OP586077	OP586596	-	-
I670SBR5_TH	OP588626	OP586078	OP586597	-	-

Table 29 List of the GenBank accession numbers of *R. rubiginosa* in this study.

Code	GenBank accession numbers	
	COI	**16S rDNA**
Rb36PLK7_TH	OQ974570	OQ975324
Rb57PLK7_TH	OQ974571	OQ975325
Rb76PLK8_TH	OQ974572	OQ975326
Rb77PLK9_TH	OQ974573	OQ975327
Rb110PLK9_TH	OQ974574	OQ975328
Rb117PLK9_TH	OQ974575	OQ975329
Rb383PTN2_TH	OQ974576	OQ975330
Rb384PTN2_TH	OQ974577	OQ975331
Rb385PTN2_TH	OQ974578	OQ975332
Rb386PTN2_TH	OQ974579	OQ975333
Rb387PTN2_TH	OQ974580	OQ975334
Rb388PTN2_TH	OQ974581	OQ975335
Rb389PTN2_TH	OQ974582	OQ975336
Rb390PTN2_TH	OQ974583	OQ975337
Rb391PTN2_TH	OQ974584	OQ975338
Rb392PTN2_TH	OQ974585	OQ975339
Rb394PTN2_TH	OQ974586	OQ975340
Rb395STI1_TH	OQ974587	OQ975341
Rb396STI1_TH	OQ974588	OQ975342
Rb446PCT2_TH	OQ974589	OQ975343
Rb448PCT2_TH	OQ974590	OQ975344
Rb577PYO1_TH	OQ974591	OQ975345
Rb578PYO1_TH	OQ974592	OQ975346
Rb579PYO1_TH	OQ974593	OQ975347
Rb580PYO1_TH	OQ974594	OQ975348
Rb581PYO1_TH	OQ974595	OQ975349
Rb582PYO1_TH	OQ974596	OQ975350
Rb583PYO1_TH	OQ974597	OQ975351

Table 29 (Cont.)

Code	GenBank accession numbers	
	COI	**16S rDNA**
Rb584PYO1_TH	OQ974598	OQ975352
Rb585PYO1_TH	OQ974599	OQ975353
Rb586PYO1_TH	OQ974600	OQ975354
Rb703PCT3_TH	OQ974601	OQ975355
Rb704PCT3_TH	OQ974602	OQ975356
Rb705PCT3_TH	OQ974603	OQ975357
Rb706PCT3_TH	OQ974604	OQ975358
Rb707PCT3_TH	OQ974605	OQ975359
Rb708PCT3_TH	OQ974606	OQ975360
Rb710PCT3_TH	OQ974607	OQ975361
Rb711PCT3_TH	OQ974608	OQ975362
Rb731CNT1_TH	OQ974609	OQ975363
Rb732CNT1_TH	OQ974610	OQ975364
Rb733CNT1_TH	OQ974611	OQ975365
Rb734CNT1_TH	OQ974612	OQ975366
Rb735CNT1_TH	OQ974613	OQ975367
Rb736CNT1_TH	OQ974614	OQ975368
Rb846CNT2_TH	OQ974615	OQ975369
Rb847CNT2_TH	OQ974616	OQ975370
Rb848CNT2_TH	OQ974617	OQ975371
Rb849CNT2_TH	OQ974618	OQ975372
Rb850CNT2_TH	OQ974619	OQ975373
Rb851CNT2_TH	OQ974620	OQ975374
Rb1307SBR2_TH	OQ974621	OQ975375
Rb1308CNT3_TH	OQ974622	OQ975376
Rb1324NSN1_TH	OQ974623	OQ975377
Rb1325NSN1_TH	OQ974624	OQ975378
Rb1326NSN1_TH	OQ974625	OQ975379

Table 29 (Cont.)

Code	GenBank accession numbers	
	COI	**16S rDNA**
Rb1327NSN1_TH	OQ974626	OQ975380
Rb1328NSN1_TH	OQ974627	OQ975381
Rb1329NSN1_TH	OQ974628	OQ975382
Rb1330NSN1_TH	OQ974629	OQ975383
Rb1331NSN1_TH	OQ974630	OQ975384
Rb1354NSN2_TH	OQ974631	OQ975385
Rb1355NSN2_TH	OQ974632	OQ975386
Rb1356NSN2_TH	OQ974633	OQ975387
Rb1357NSN2_TH	OQ974634	OQ975388
Rb1469KKN2_TH	OQ974635	OQ975389
Rb1470KKN2_TH	OQ974636	OQ975390
Rb1471KKN2_TH	OQ974637	OQ975391
Rb1472SKA2_TH	OQ974638	OQ975392
Rb1479SKA2_TH	OQ974639	OQ975393
Rb1480SKA2_TH	OQ974640	OQ975394
Rb1481SKA2_TH	OQ974641	OQ975395
Rb1482SKA2_TH	OQ974642	OQ975396
Rb1485SKA2_TH	OQ974643	OQ975397
Rb1486SKA2_TH	OQ974644	OQ975398
Rb1520SKA2_TH	OQ974645	OQ975399
Rb1529SKA2_TH	OQ974646	OQ975400
Rb1547SKA2_TH	OQ974647	OQ975401
Rb1560SKA2_TH	OQ974648	OQ975402
Rb1569SKA2_TH	OQ974649	OQ975403
Rb1576TAK4_TH	OQ974650	OQ975404
Rb1577TAK4_TH	OQ974651	OQ975405
Rb1578TAK4_TH	OQ974652	OQ975406
Rb1731ATG1_TH	OQ974653	OQ975407

Table 29 (Cont.)

Code	GenBank accession numbers	
	COI	**16S rDNA**
Rb1732AYA1_TH	OQ974654	OQ975408
Rb1753CCO1_TH	OQ974655	OQ975409
Rb1754CCO1_TH	OQ974656	OQ975410
Rb1756CCO1_TH	OQ974657	OQ975411
Rb1757CCO1_TH	OQ974658	OQ975412
Rb1758CCO1_TH	OQ974659	OQ975413
Rb1895NYK2_TH	OQ974660	OQ975414
Rb1896NYK2_TH	OQ974661	OQ975415
Rb1897NYK2_TH	OQ974662	OQ975416
Rb1898NYK2_TH	OQ974663	OQ975417
Rb1899NYK2_TH	OQ974664	OQ975418
Rb1900NYK2_TH	OQ974665	OQ975419
Rb1901NYK2_TH	OQ974666	OQ975420
Rb1902NYK2_TH	OQ974667	OQ975421
Rb1903NYK2_TH	OQ974668	OQ975422
Rb1906NYK2_TH	OQ974669	OQ975423
Rb1907NYK2_TH	OQ974670	OQ975424
Rb1908NYK2_TH	OQ974671	OQ975425
Rb2002SRI1_TH	OQ974672	OQ975426
Rb2003SRI1_TH	OQ974673	OQ975427
Rb2037PNB4_TH	OQ974674	OQ975428
Rb2038PNB4_TH	OQ974675	OQ975429
Rb2039PNB4_TH	OQ974676	OQ975430
Rb2040PNB4_TH	OQ974677	OQ975431
Rb2041PNB4_TH	OQ974678	OQ975432
Rb2042PNB4_TH	OQ974679	OQ975433
Rb2043PNB4_TH	OQ974680	OQ975434
Rb2044PNB4_TH	OQ974681	OQ975435

Table 29 (Cont.)

Code	GenBank accession numbers	
	COI	**16S rDNA**
Rb2045PNB4_TH	OQ974682	OQ975436
Rb2046PNB4_TH	OQ974683	OQ975437
Rb2047PNB4_TH	OQ974684	OQ975438
Rb2048PNB4_TH	OQ974685	OQ975439

Table 30 List of the GenBank accession numbers of *O. viridis* in this study.

Code	GenBank accession numbers	
	COI	**16S rDNA**
Ov1PLK2_TH	OQ974825	OQ975510
Ov5PLK3_TH	OQ974826	OQ975511
Ov6PLK3_TH	OQ974827	OQ975512
Ov7PLK4_TH	OQ974828	OQ975513
Ov13PLK4_TH	OQ974829	OQ975514
Ov18PLK4_TH	OQ974830	OQ975515
Ov24PLK4_TH	OQ974831	OQ975516
Ov25PLK4_TH	OQ974832	OQ975517
Ov28PLK5_TH	OQ974833	OQ975518
Ov39PLK7_TH	OQ974834	OQ975519
Ov45PLK7_TH	OQ974835	OQ975520
Ov48PLK7_TH	OQ974836	OQ975521
Ov56PLK7_TH	OQ974837	OQ975522
Ov86PLK9_TH	OQ974838	OQ975523
Ov91PLK9_TH	OQ974839	OQ975524
Ov109PLK9_TH	OQ974840	OQ975525
Ov182PLK11_TH	OQ974841	OQ975526
Ov183PLK11_TH	OQ974842	OQ975527
Ov184PLK11_TH	OQ974843	OQ975528

Table 30 (Cont.)

Code	GenBank accession numbers	
	COI	**16S rDNA**
Ov211PLK11_TH	OQ974844	OQ975529
Ov298PLK13_TH	OQ974845	OQ975530
Ov304PLK7_TH	OQ974846	OQ975531
Ov429PCT1_TH	OQ974847	OQ975532
Ov430PCT1_TH	OQ974848	OQ975533
Ov431PCT1_TH	OQ974849	OQ975534
Ov432PCT1_TH	OQ974850	OQ975535
Ov433PCT1_TH	OQ974851	OQ975536
Ov434PCT1_TH	OQ974852	OQ975537
Ov435PCT1_TH	OQ974853	OQ975538
Ov436PCT1_TH	OQ974854	OQ975539
Ov437PCT1_TH	OQ974855	OQ975540
Ov444PCT1_TH	OQ974856	OQ975541
Ov445PCT1_TH	OQ974857	OQ975542
Ov454LPG1_TH	OQ974858	OQ975543
Ov455LPG1_TH	OQ974859	OQ975544
Ov456LPG1_TH	OQ974860	OQ975545
Ov457LPG1_TH	OQ974861	OQ975546
Ov458LPG1_TH	OQ974862	OQ975547
Ov459LPG1_TH	OQ974863	OQ975548
Ov460LPG1_TH	OQ974864	OQ975549
Ov461LPG1_TH	OQ974865	OQ975550
Ov462LPG1_TH	OQ974866	OQ975551
Ov463LPG1_TH	OQ974867	OQ975552
Ov517CMI2_TH	OQ974868	OQ975553
Ov518CMI2_TH	OQ974869	OQ975554
Ov529CMI2_TH	OQ974870	OQ975555
Ov531CMI2_TH	OQ974871	OQ975556

Table 30 (Cont.)

Code	GenBank accession numbers	
	COI	**16S rDNA**
Ov533CMI2_TH	OQ974872	OQ975557
Ov539CMI2_TH	OQ974873	OQ975558
Ov540CMI2_TH	OQ974874	OQ975559
Ov542CMI2_TH	OQ974875	OQ975560
Ov543CMI2_TH	OQ974876	OQ975561
Ov545CMI2_TH	OQ974877	OQ975562
Ov571CRI1_TH	OQ974878	OQ975563
Ov572CRI1_TH	OQ974879	OQ975564
Ov574CRI1_TH	OQ974880	OQ975565
Ov635UTT2_TH	OQ974881	OQ975566
Ov636UTT2_TH	OQ974882	OQ975567
Ov637UTT2_TH	OQ974883	OQ975568
Ov638UTT2_TH	OQ974884	OQ975569
Ov640UTT2_TH	OQ974885	OQ975570
Ov641UTT2_TH	OQ974886	OQ975571
Ov645UTT2_TH	OQ974887	OQ975572
Ov646UTT2_TH	OQ974888	OQ975573
Ov647UTT2_TH	OQ974889	OQ975574
Ov650UTT2_TH	OQ974890	OQ975575
Ov652UTT2_TH	OQ974891	OQ975576
Ov653UTT2_TH	OQ974892	OQ975577
Ov1311UTI1_TH	OQ974893	OQ975578
Ov1312UTI1_TH	OQ974894	OQ975579
Ov1313UTI1_TH	OQ974895	OQ975580
Ov1314UTI1_TH	OQ974896	OQ975581
Ov1358NSN2_TH	OQ974897	OQ975582
Ov1687SBR3_TH	OQ974898	OQ975583
Ov1688SBR3_TH	OQ974899	OQ975584

Table 30 (Cont.)

Code	GenBank accession numbers	
	COI	**16S rDNA**
Ov1689SBR3_TH	OQ974900	OQ975585
Ov1690SBR3_TH	OQ974901	OQ975586
Ov1691SBR3_TH	OQ974902	OQ975587
Ov1692SBR3_TH	OQ974903	OQ975588
Ov1693SBR3_TH	OQ974904	OQ975589
Ov1694SBR3_TH	OQ974905	OQ975590
Ov1695SBR3_TH	OQ974906	OQ975591
Ov1706SBR3_TH	OQ974907	OQ975592
Ov2034SBR4_TH	OQ974908	OQ975593

APPENDIX C GENETIC DIVERSITY OF SNAILS

Table 31 List of the haplotypes identified in the *I. exustus* samples from Thailand based on COI, 16S rDNA, combined mtDNA, ITS1, and 28S rDNA analyses.

Sample code	Haplotype				
	COI	16S rDNA	Combined mtDNA	ITS1	28S rDNA
I1PLK1_TH	I1	I1	I1	I1	-
I2PLK1_TH	I1	I1	I1	I1	-
I3PLK1_TH	I1	I1	I1	I1	-
I6PLK1_TH	I16	I1	I16	I1	I1
I7PLK1_TH	I8	I1	I8	I1	-
I12PLK1_TH	I22	I1	I22	I1	-
I15PLK1_TH	I8	I1	I8	I3	-
I16PLK1_TH	I21	I1	I21	I1	-
I24PLK6_TH	I1	I1	I1	I10	-
I25PLK6_TH	I1	I1	I1	I1	-
I26PLK6_TH	I1	I1	I1	I1	-
I27PLK6_TH	I1	I1	I1	I1	I1
I28PLK6_TH	I1	I1	I1	I1	-
I29PLK9_TH	I1	I1	I1	I9	-
I30PLK9_TH	I1	I1	I1	I10	-
I31PLK9_TH	I1	I1	I1	I1	-
I32PLK9_TH	I1	I1	I1	I1	-
I34PLK10_TH	I20	I1	I20	I1	-
I36PLK10_TH	I1	I1	I1	I1	I1
I37PLK10_TH	I20	I1	I20	I1	-
I38PLK10_TH	I1	I1	I1	I1	-
I39PLK10_TH	I1	I1	I1	I1	-

Table 31 (Cont.)

Sample code	Haplotype				
	COI	**16S rDNA**	**Combined mtDNA**	**ITS1**	**28S rDNA**
I40PLK10_TH	I1	I1	I1	I1	-
I41PLK10_TH	I1	I1	I1	I1	I1
I44PLK10_TH	I1	I1	I1	I1	-
I45PLK10_TH	I1	I1	I1	I1	-
I46PLK10_TH	I1	I1	I1	I1	-
I57PLK11_TH	I1	I1	I1	I1	-
I58PLK11_TH	I8	I1	I8	I1	-
I59UTT1_TH	I1	I1	I1	I1	I1
I60LPN1_TH	I1	I1	I1	I1	I1
I61LPN1_TH	I1	I1	I1	I1	I2
I62LPN1_TH	I1	I1	I1	I1	-
I63LPN1_TH	I1	I1	I1	I1	-
I64LPN1_TH	I1	I1	I1	I1	-
I65LPN1_TH	I13	I1	I13	I7	-
I70SKA1_TH	I1	I4	I26	I1	I1
I71SKA1_TH	I1	I4	I26	I1	I3
I72SKA1_TH	I1	I4	I26	I1	-
I73SKA1_TH	I1	I4	I26	I1	-
I74SKA1_TH	I1	I4	I26	I1	-
I75SKA1_TH	I1	I4	I26	I1	-
I76SKA1_TH	I1	I4	I26	I1	-
I77SKA1_TH	I1	I4	I26	I1	-
I182PTN2_TH	I1	I1	I1	I8	I1
I183PTN2_TH	I1	I1	I1	I8	I4
I184PTN2_TH	I23	I1	I23	I1	-
I185STI1_TH	I1	I1	I1	I3	I1
I186STI1_TH	I1	I1	I1	I3	I1

Table 31 (Cont.)

Sample code	Haplotype				
	COI	**16S rDNA**	**Combined mtDNA**	**ITS1**	**28S rDNA**
I187STI1_TH	I1	I1	I1	I3	-
I291PCT1_TH	I1	I1	I1	I1	I1
I293PCT2_TH	I1	I1	I1	I1	I1
I294PCT2_TH	I1	I1	I1	I1	-
I295PCT2_TH	I1	I1	I1	I1	-
I296PCT2_TH	I1	I1	I1	I1	-
I297LPG1_TH	I1	I1	I1	I1	I1
I299PNB3_TH	I1	I1	I1	I1	I1
I304PCT3_TH	I11	I1	I11	I1	-
I305PCT3_TH	I1	I1	I1	I1	-
I306PCT3_TH	I1	I1	I1	I1	-
I307PCT3_TH	I1	I1	I1	I1	-
I308PCT3_TH	I1	I1	I1	I1	-
I309PCT3_TH	I1	I1	I1	I1	-
I326CNT1_TH	I16	I1	I16	I1	I1
I328CNT1_TH	I1	I1	I1	I1	I1
I330CNT1_TH	I1	I1	I1	I1	-
I332CNT1_TH	I16	I1	I16	I1	-
I334CNT1_TH	I1	I1	I1	I1	-
I335CNT1_TH	I1	I1	I1	I1	-
I338CNT2_TH	I19	I1	I19	I1	-
I340CNT2_TH	I1	I1	I1	I1	-
I342CNT2_TH	I1	I1	I1	I1	-
I344CNT2_TH	I1	I1	I1	I1	-
I346CNT2_TH	I1	I1	I1	I1	-
I348CNT2_TH	I1	I1	I1	I1	-
I350SBR1_TH	I1	I1	I1	I3	-

Table 31 (Cont.)

Sample code	Haplotype				
	COI	**16S rDNA**	**Combined mtDNA**	**ITS1**	**28S rDNA**
I351SBR1_TH	I1	I1	I1	I1	I1
I352SBR1_TH	I7	I1	I7	I1	I1
I353SBR1_TH	I1	I1	I1	I1	-
I355SBR1_TH	I1	I1	I1	I1	-
I381SBR2_TH	I1	I1	I1	I1	-
I382SBR2_TH	I1	I1	I1	I3	-
I383SBR2_TH	I13	I1	I13	I1	-
I384SBR2_TH	I14	I1	I14	I1	I1
I385SBR2_TH	I9	I1	I9	I1	-
I386SBR2_TH	I1	I2	I2	I1	-
I388CNT3_TH	I1	I1	I1	I1	-
I389CNT3_TH	I1	I1	I1	I1	-
I390CNT3_TH	I1	I1	I1	I1	-
I391CNT3_TH	I17	I1	I17	I1	-
I392CNT3_TH	I10	I1	I10	I1	-
I393CNT3_TH	I15	I1	I15	I1	-
I410NSN1_TH	I1	I1	I1	I1	I1
I411NSN1_TH	I1	I1	I1	I1	-
I412NSN1_TH	I18	I1	I18	I1	-
I413NSN1_TH	I1	I1	I1	I1	-
I414NSN1_TH	I1	I1	I1	I4	-
I415NSN1_TH	I1	I1	I1	I1	I1
I416NSN1_TH	I1	I1	I1	I6	-
I493NSN2_TH	I1	I1	I1	I1	I1
I494NSN2_TH	I1	I1	I1	I1	-
I495KKN2_TH	I1	I1	I1	I1	I1
I496KKN2_TH	I1	I1	I1	I1	I1

Table 31 (Cont.)

Sample code	Haplotype				
	COI	**16S rDNA**	**Combined mtDNA**	**ITS1**	**28S rDNA**
I497CPM2_TH	I1	I1	I1	I1	I1
I498CPM2_TH	I1	I1	I1	I1	-
I499CPM2_TH	I12	I1	I12	I1	-
I500CPM2_TH	I1	I1	I1	I1	-
I501CPM2_TH	I1	I1	I1	I1	-
I502CPM2_TH	I1	I1	I1	I1	-
I503CPM2_TH	I1	I1	I1	I5	I1
I510CPM2_TH	I1	I1	I1	I1	-
I515SKA2_TH	I1	I1	I1	I1	-
I517SKA2_TH	I1	I1	I1	I1	-
I518SKA2_TH	I1	I1	I1	I1	-
I519SKA2_TH	I1	I1	I1	I1	-
I524SKA2_TH	I4	I1	I4	I1	I1
I532SKA2_TH	I4	I1	I4	I1	I5
I533SKA2_TH	I1	I1	I1	I1	-
I547SKA2_TH	I1	I1	I1	I1	-
I549SKA2_TH	I1	I1	I1	I1	-
I555UDN2_TH	I1	I1	I1	I1	I6
I556UDN3_TH	I1	I1	I1	I1	I7
I557UDN3_TH	I1	I1	I1	I1	-
I558UDN3_TH	I1	I1	I1	I1	-
I559UDN3_TH	I1	I1	I1	I1	-
I560UDN3_TH	I4	I1	I4	I1	-
I562TAK1_TH	I1	I1	I1	I1	I1
I564TAK1_TH	I1	I1	I1	I1	I1
I565TAK1_TH	I1	I1	I1	I4	-
I566TAK1_TH	I1	I1	I1	I1	-

Table 31 (Cont.)

Sample code	Haplotype				
	COI	**16S rDNA**	**Combined mtDNA**	**ITS1**	**28S rDNA**
I567TAK1_TH	I1	I1	I1	I1	-
I569TAK1_TH	I1	I1	I1	I1	-
I571TAK1_TH	I1	I1	I1	I1	-
I572TAK1_TH	I1	I1	I1	I1	-
I573TAK1_TH	I1	I1	I1	I1	-
I574TAK1_TH	I1	I1	I1	I1	-
I575TAK1_TH	I1	I1	I1	I1	-
I576TAK1_TH	I1	I1	I1	I1	-
I577TAK5_TH	I1	I1	I1	I1	-
I578TAK5_TH	I1	I1	I1	I1	-
I579TAK5_TH	I1	I1	I1	I1	-
I581CTI1_TH	I6	I1	I6	I1	I1
I583ATG1_TH	I1	I3	I25	I1	I8
I698ATG1_TH	I1	I1	I1	I1	I1
I585AYA1_TH	I1	I1	I1	I1	I5
I594CBI1_TH	I1	I1	I1	I1	I1
I595CBI1_TH	I1	I1	I1	I1	-
I598CBI1_TH	I1	I1	I1	I1	-
I599CBI1_TH	I3	I1	I3	I1	-
I600CBI1_TH	I1	I1	I1	I1	-
I601CBI1_TH	I1	I1	I1	I1	-
I602CBI1_TH	I1	I1	I1	I1	-
I603CBI1_TH	I4	I1	I4	I1	-
I604CBI1_TH	I5	I1	I5	I1	-
I605CBI1_TH	I1	I1	I1	I1	I1
I656NYK1_TH	I1	I1	I1	I2	I1
I657NYK1_TH	I1	I1	I1	I1	I1

Table 31 (Cont.)

Sample code	Haplotype				
	COI	**16S rDNA**	**Combined mtDNA**	**ITS1**	**28S rDNA**
I662SBR5_TH	I2	I1	I24	I1	-
I665SBR5_TH	I1	I1	I1	I1	I1
I668SBR5_TH	I2	I1	I24	I1	-
I669SBR5_TH	I2	I1	I24	I1	-
I670SBR5_TH	I2	I1	I24	I1	-

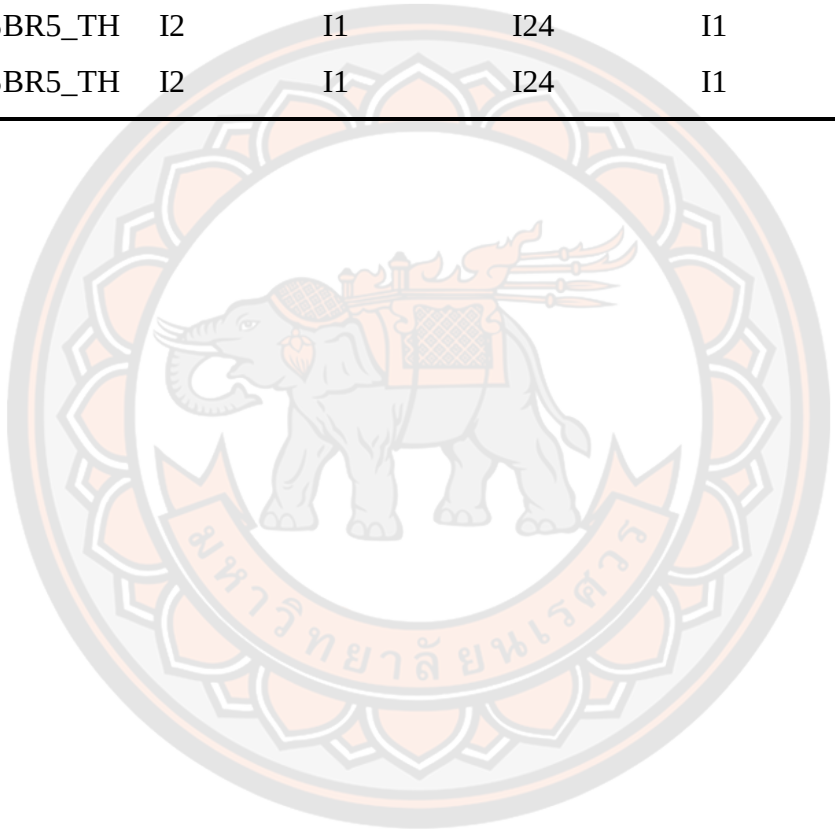

Table 32 Haplotype frequency of *I. exustus* based on COI sequences in each population in Thailand.

Haplotype	Population code																					Haplotype
	UTT	LPN	LPG	CPM	KKN	UDN	PLK	STI	PCT	PNB	CNT	SBR	NSN	ATG	AYA	NYK	TAK	CTI	CBI	PTN	SKA	frequencies (%)
I1	1	5	1	7	2	5	21	3	10	1	12	8	8	2	1	2	15		7	2	15	79.00
I2												4										2.47
I3																			1			0.62
I4						1													1		2	2.47
I5																			1			0.62
I6																		1				0.62
I7												1										0.62
I8							3															1.85
I9												1										0.62
I10											1											0.62
I11									1													0.62
I12				1																		0.62
I13		1										1										1.23
I14												1										0.62
I15											1											0.62

Table 32 (Cont.)

Haplotype	Population code																				Haplotype
	UTT	LPN	LPG	CPM	KKN	UDN	PLK	STI	PCT	PNB	CNT	SBR	NSN	ATG	AYA	NYK	TAK	CTI	CBI	PTN	SKA	frequencies (%)
I16							1				2											1.85
I17											1											0.62
I18													1									0.62
I19											1											0.62
I20							2															1.23
I21							1															0.62
I22							1															0.62
I23																				1		0.62
Total	**1**	**6**	**1**	**8**	**2**	**6**	**29**	**3**	**11**	**1**	**18**	**16**	**9**	**2**	**1**	**2**	**15**	**1**	**10**	**3**	**17**	**100**

Table 33 Haplotype frequency of *I. exustus* based on 16S rDNA sequences in each population in Thailand.

Haplotype	Population code																				Haplotype
	UTT	LPN	LPG	CPM	KKN	UDN	PLK	STI	PCT	PNB	CNT	SBR	NSN	ATG	AYA	NYK	TAK	CTI	CBI	PTN	SKA	frequencies (%)
I1	1	6	1	8	2	6	29	3	11	1	18	15	9	1	1	2	15	1	10	3	9	93.8
I2												1										0.62
I3														1								0.62
I4																					8	4.94
Total	**1**	**6**	**1**	**8**	**2**	**6**	**29**	**3**	**11**	**1**	**18**	**16**	**9**	**2**	**1**	**2**	**15**	**1**	**10**	**3**	**17**	**100**

Table 34 Haplotype frequency of *I. exustus* based on combined mt DNA sequences in each population in Thailand.

Haplotype	Population code																						Haplotype
	UTT	LPN	LPG	CPM	KKN	UDN	PLK	STI	PCT	PNB	CNT	SBR	NSN	ATG	AYA	NYK	TAK	CTI	CBI	PTN	SKA	frequencies (%)
I1	1	5	1	7	2	5	21	3	10	1	12	7	8	1	1	2	15		7	2	7	72.80
I2												1										0.62
I3																			1			0.62
I4						1													1		2	2.47
I5																			1			0.62
I6																		1				0.62
I7												1										0.62
I8							3															1.85
I9												1										0.62
I10											1											0.62
I11									1													0.62
I12				1																		0.62
I13		1										1										1.23
I14												1										0.62
I15											1											0.62

Table 34 (Cont.)

Haplotype	Population code																					Haplotype
	UTT	LPN	LPG	CPM	KKN	UDN	PLK	STI	PCT	PNB	CNT	SBR	NSN	ATG	AYA	NYK	TAK	CTI	CBI	PTN	SKA	frequencies (%)
I16							1				2											1.85
I17											1											0.62
I18													1									0.62
I19											1											0.62
I20							2															1.23
I21							1															0.62
I22							1															0.62
I23																				1		0.62
I24												4										2.47
I25														1								0.62
I26																					8	4.94
Total	**1**	**6**	**1**	**8**	**2**	**6**	**29**	**3**	**11**	**1**	**18**	**16**	**9**	**2**	**1**	**2**	**15**	**1**	**10**	**3**	**17**	**100**

Table 35 Haplotype frequency of *I. exustus* based on ITS1 sequences in each population in Thailand.

Haplotype	Population code																					Haplotype
	UTT	LPN	LPG	CPM	KKN	UDN	PLK	STI	PCT	PNB	CNT	SBR	NSN	ATG	AYA	NYK	TAK	CTI	CBI	PTN	SKA	frequencies (%)
I1	1	5	1	7	2	6	25		11	1	18	14	7	2	1	1	14	1	10	1	17	89.50
I2																1						0.62
I3							1	3				2										3.70
I4													1				1					1.23
I5				1																		0.62
I6													1									0.62
I7		1																				0.62
I8																				2		1.23
I9							1															0.62
I10							2															1.23
Total	**1**	**6**	**1**	**8**	**2**	**6**	**29**	**3**	**11**	**1**	**18**	**16**	**9**	**2**	**1**	**2**	**15**	**1**	**10**	**3**	**17**	**100**

Table 36 Haplotype frequency of *I. exustus* based on 28S rDNA sequences in each population in Thailand.

Haplotype	Population code																						Haplotype frequencies (%)
	UTT	LPN	LPG	CPM	KKN	UDN	PLK	STI	PCT	PNB	CNT	SBR	NSN	ATG	AYA	NYK	TAK	CTI	CBI	PTN	SKA	
I1	1	1	1	2	2		4	2	2	1	2	4	3	1		2	2	1	2	1	2	81.82
I2		1																				2.27
I3																					1	2.27
I4																				1		2.27
I5															1						1	4.55
I6						1																2.27
I7						1																2.27
I8														1								2.27
Total	**1**	**2**	**1**	**2**	**2**	**2**	**4**	**2**	**2**	**1**	**2**	**4**	**3**	**2**	**1**	**2**	**2**	**1**	**2**	**2**	**4**	**100**

Table 37 Population pairwise F_{ST} between 16 populations of *I. exustus* based on mitochondrial cytochrome c oxidase subunit I sequences.

Populations	LPN	CPM	KKN	UDN	PLK	STI	PCT	CNT	SBR	NSN	ATG	NYK	TAK	CBI	PTN	SKA
LPN	0.000															
CPM	-0.006	0.000														
KKN	-0.304	-0.317	0.000													
UDN	0.000	-0.030	-0.304	0.000												
PLK	-0.021	0.011	-0.303	-0.052	0.000											
STI	-0.153	-0.174	0.000	-0.153	-0.174	0.000										
PCT	0.081	0.034	-0.325	0.025	-0.018	-0.187	0.000									
CNT	0.018	0.024	-0.321	-0.067	-0.002	-0.187	-0.016	0.000								
SBR	-0.086	0.060	-0.215	0.022	0.064*	-0.096	0.076*	0.087*	0.000							
NSN	0.052	0.012	-0.321	0.012	-0.025	-0.180	0.002	-0.026	0.059	0.000						
ATG	-0.304	-0.317	0.000	-0.304	-0.303	0.000	-0.325	-0.321	-0.215	-0.321	0.000					
NYK	-0.304	-0.317	0.000	-0.304	-0.303	0.000	-0.325	-0.321	-0.215	-0.321	0.000	0.000				
TAK	0.166	0.083	0.000	0.166	-0.001	0.000	0.029	-0.001	0.109*	0.060	0.000	0.000	0.000			
CBI	0.027	0.013	-0.323	-0.085	-0.008	-0.184	0.006	-0.024	0.066*	-0.006	-0.323	-0.323	0.042	0.000		
PTN	-0.074	-0.106	-0.200	0.068	-0.113	0.000	0.195	-0.076	-0.047	0.148	-0.200	-0.200	0.531	-0.022	0.000	
SKA	0.136	0.084	-0.270	-0.114	0.011	-0.143	0.031	-0.001	0.118*	0.032	-0.270	-0.270	0.052	-0.008	0.210	0.000

Note: asterisks (*) indicate statistical significance of $P < 0.05$.

Table 38 Population pairwise F_{ST} between 16 populations of *I. exustus* based on mitochondrial 16S rDNA sequences.

Populations	LPN	CPM	KKN	UDN	PLK	STI	PCT	CNT	SBR	NSN	ATG	NYK	TAK	CBI	PTN	SKA
LPN	0.000															
CPM	0.000	0.000														
KKN	0.000	0.000	0.000													
UDN	0.000	0.000	0.000	0.000												
PLK	0.000	0.000	0.000	0.000	0.000											
STI	0.000	0.000	0.000	0.000	0.000	0.000										
PCT	0.000	0.000	0.000	0.000	0.000	0.000	0.000									
CNT	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000								
SBR	-0.078	-0.050	-0.329	-0.078	0.039	-0.194	-0.025	0.007	0.000							
NSN	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.040	0.000						
ATG	0.538	0.627	0.000	0.538	0.874*	0.250	0.710	0.808	0.530	0.660	0.000					
NYK	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.329	0.000	0.000	0.000				
TAK	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.004	0.000	0.776	0.000	0.000			
CBI	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.032	0.000	0.687	0.000	0.000	0.000		
PTN	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.194	0.000	0.250	0.000	0.000	0.000	0.000	
SKA	0.303	0.337*	0.141	0.303	0.524*	0.215	0.376*	0.446*	0.380*	0.351*	0.361*	0.141	0.419*	0.364*	0.215	0.000

Note: asterisks (*) indicate statistical significance of $P < 0.05$.

Table 39 Population pairwise F_{ST} between 16 populations of *I. exustus* of the combined mtDNA sequences.

Populations	LPN	CPM	KKN	UDN	PLK	STI	PCT	CNT	SBR	NSN	ATG	NYK	TAK	CBI	PTN	SKA
LPN	0.000															
CPM	-0.006	0.000														
KKN	-0.304	-0.317	0.000													
UDN	0.000	-0.030	-0.304	0.000												
PLK	-0.021	0.011	-0.303	-0.052	0.000											
STI	-0.153	-0.174	0.000	-0.153	-0.174	0.000										
PCT	0.081	0.034	-0.325	0.025	-0.018	-0.187	0.000									
CNT	0.018	0.024	-0.321	-0.067	-0.002	-0.187	-0.016	0.000								
SBR	-0.086	0.057	-0.220	0.018	0.063*	-0.100	0.072	0.085*	0.000							
NSN	0.052	0.012	-0.321	0.012	-0.025	-0.180	0.002	-0.026	0.055	0.000						
ATG	-0.090	-0.144	0.000	0.225	-0.149	0.250	0.416	-0.055	-0.097	0.352	0.000					
NYK	-0.304	-0.317	0.000	-0.304	-0.303	0.000	-0.325	-0.321	-0.220	-0.321	0.000	0.000				
TAK	0.166	0.083	0.000	0.166	-0.001	0.000	0.029	-0.001	0.105*	0.060	0.776	0.000	0.000			
CBI	0.027	0.013	-0.323	-0.085	-0.008	-0.184	0.006	-0.024	0.062*	-0.006	0.050	-0.323	0.042	0.000		
PTN	-0.074	-0.106	-0.200	0.068	-0.113	0.000	0.195	-0.076	-0.053	0.148	0.045	-0.200	0.531	-0.022	0.000	
SKA	0.214*	0.180*	0.051	0.187*	0.111*	0.135	0.277*	0.164*	0.184*	0.257*	0.262*	0.051	0.344*	0.198*	0.213	0.000

Note: asterisks (*) indicate statistical significance of $P < 0.05$.

Table 40 Population pairwise F_{ST} between 16 populations of *I. exustus* based on ITS1 sequences.

Populations	LPN	CPM	KKN	UDN	PLK	STI	PCT	CNT	SBR	NSN	ATG	NYK	TAK	CBI	PTN	SKA
LPN	0.000															
CPM	-0.008	0.000														
KKN	-0.304	-0.317	0.000													
UDN	0.000	-0.040	0.000	0.000												
PLK	0.023	-0.013	-0.315	-0.074	0.000											
STI	0.802*	0.707*	1.000	1.000*	0.779*	0.000										
PCT	0.108	0.041	0.000	0.000	-0.027	1.000*	0.000									
CNT	0.203	0.110	0.000	0.000	-0.002	1.000*	0.000	0.000								
SBR	0.050	0.050	-0.265	-0.025	0.006	0.778*	0.033	0.077	0.000							
NSN	-0.008	0.001	-0.321	-0.051	0.029	0.726*	0.023	0.082	0.042	0.000						
ATG	-0.304	-0.317	0.000	0.000	-0.315	1.000	0.000	0.000	-0.265	-0.321	0.000					
NYK	0.346	0.315	0.000	0.538	0.558*	0.647	0.710	0.808*	0.549*	0.352	0.000	0.000				
TAK	0.053	0.042	-0.329	-0.076	0.001	0.893*	-0.022	0.012	0.042	-0.031	-0.329	0.625	0.000			
CBI	0.090	0.029	0.000	0.000	-0.033	1.000*	0.000	0.000	0.025	0.012	0.000	0.687	-0.029	0.000		
PTN	0.460	0.396	0.368	0.675*	0.571*	0.800	0.791	0.859*	0.586*	0.424*	0.368	-0.200	0.671	0.775*	0.000	
SKA	0.191	0.101	0.000	0.000	-0.005	1.000*	0.000	0.000	0.072	0.075*	0.000	0.798	0.008	0.000	0.853*	0.000

Note: asterisks (*) indicate statistical significance of $P < 0.05$.

Table 41 List of the haplotypes identified in the *R. rubiginosa* samples from Thailand based on COI, 16S rDNA, and combined mtDNA analyses.

Code	Haplotype		
	COI	16S rDNA	Combined mtDNA
Rb36PLK7_TH	R2	R2	R1
Rb57PLK7_TH	R4	R5	R17
Rb76PLK8_TH	R2	R2	R1
Rb77PLK9_TH	R4	R3	R3
Rb110PLK9_TH	R2	R2	R1
Rb117PLK9_TH	R2	R4	R4
Rb383PTN2_TH	R3	R1	R5
Rb384PTN2_TH	R3	R1	R5
Rb385PTN2_TH	R3	R1	R5
Rb386PTN2_TH	R3	R1	R5
Rb387PTN2_TH	R3	R1	R5
Rb388PTN2_TH	R3	R1	R5
Rb389PTN2_TH	R3	R1	R5
Rb390PTN2_TH	R3	R1	R5
Rb391PTN2_TH	R3	R1	R5
Rb392PTN2_TH	R3	R1	R5
Rb394PTN2_TH	R3	R1	R5
Rb395STI1_TH	R1	R6	R6
Rb396STI1_TH	R1	R6	R6
Rb446PCT2_TH	R4	R3	R3
Rb448PCT2_TH	R4	R3	R3
Rb577PYO1_TH	R7	R1	R10
Rb578PYO1_TH	R7	R1	R10
Rb579PYO1_TH	R7	R1	R10
Rb580PYO1_TH	R7	R1	R10
Rb581PYO1_TH	R7	R1	R10

Table 41 (Cont.)

Code	Haplotype		
	COI	**16S rDNA**	**Combined mtDNA**
Rb582PYO1_TH	R7	R1	R10
Rb583PYO1_TH	R7	R1	R10
Rb584PYO1_TH	R7	R1	R10
Rb585PYO1_TH	R7	R1	R10
Rb586PYO1_TH	R7	R1	R10
Rb703PCT3_TH	R4	R3	R3
Rb704PCT3_TH	R4	R3	R3
Rb705PCT3_TH	R5	R3	R7
Rb706PCT3_TH	R6	R3	R8
Rb707PCT3_TH	R4	R3	R3
Rb708PCT3_TH	R4	R3	R3
Rb710PCT3_TH	R4	R3	R3
Rb711PCT3_TH	R4	R7	R9
Rb731CNT1_TH	R8	R8	R11
Rb732CNT1_TH	R9	R8	R12
Rb733CNT1_TH	R1	R9	R13
Rb734CNT1_TH	R8	R8	R11
Rb735CNT1_TH	R10	R10	R14
Rb736CNT1_TH	R8	R8	R11
Rb846CNT2_TH	R11	R2	R15
Rb847CNT2_TH	R2	R2	R1
Rb848CNT2_TH	R12	R1	R16
Rb849CNT2_TH	R8	R8	R11
Rb850CNT2_TH	R8	R8	R11
Rb851CNT2_TH	R1	R1	R2
Rb1307SBR2_TH	R13	R2	R18
Rb1308CNT3_TH	R2	R2	R1
Rb1324NSN1_TH	R14	R1	R19

Table 41 (Cont.)

Code	Haplotype		
	COI	**16S rDNA**	**Combined mtDNA**
Rb1325NSN1_TH	R15	R1	R20
Rb1326NSN1_TH	R14	R1	R19
Rb1327NSN1_TH	R15	R1	R20
Rb1328NSN1_TH	R4	R3	R3
Rb1329NSN1_TH	R10	R2	R21
Rb1330NSN1_TH	R10	R2	R21
Rb1331NSN1_TH	R10	R2	R21
Rb1354NSN2_TH	R16	R1	R22
Rb1355NSN2_TH	R10	R10	R14
Rb1356NSN2_TH	R17	R3	R23
Rb1357NSN2_TH	R10	R10	R14
Rb1469KKN2_TH	R18	R11	R24
Rb1470KKN2_TH	R10	R2	R21
Rb1471KKN2_TH	R18	R11	R24
Rb1472SKA2_TH	R19	R12	R25
Rb1479SKA2_TH	R2	R2	R1
Rb1480SKA2_TH	R2	R2	R1
Rb1481SKA2_TH	R2	R2	R1
Rb1482SKA2_TH	R2	R2	R1
Rb1485SKA2_TH	R2	R2	R1
Rb1486SKA2_TH	R2	R2	R1
Rb1520SKA2_TH	R2	R2	R1
Rb1529SKA2_TH	R2	R2	R1
Rb1547SKA2_TH	R2	R2	R1
Rb1560SKA2_TH	R2	R2	R1
Rb1569SKA2_TH	R1	R6	R6
Rb1576TAK4_TH	R7	R1	R10
Rb1577TAK4_TH	R7	R1	R10

Table 41 (Cont.)

Code	Haplotype		
	COI	**16S rDNA**	**Combined mtDNA**
Rb1578TAK4_TH	R7	R1	R10
Rb1731ATG1_TH	R2	R2	R1
Rb1732AYA1_TH	R2	R13	R26
Rb1753CCO1_TH	R20	R1	R27
Rb1754CCO1_TH	R21	R6	R28
Rb1756CCO1_TH	R20	R1	R27
Rb1757CCO1_TH	R20	R1	R27
Rb1758CCO1_TH	R21	R6	R28
Rb1895NYK2_TH	R1	R14	R29
Rb1896NYK2_TH	R1	R1	R2
Rb1897NYK2_TH	R1	R1	R2
Rb1898NYK2_TH	R1	R14	R29
Rb1899NYK2_TH	R1	R1	R2
Rb1900NYK2_TH	R1	R1	R2
Rb1901NYK2_TH	R1	R1	R2
Rb1902NYK2_TH	R1	R1	R2
Rb1903NYK2_TH	R1	R1	R2
Rb1906NYK2_TH	R1	R1	R2
Rb1907NYK2_TH	R1	R1	R2
Rb1908NYK2_TH	R1	R1	R2
Rb2002SRI1_TH	R2	R2	R1
Rb2003SRI1_TH	R2	R2	R1
Rb2037PNB4_TH	R1	R1	R2
Rb2038PNB4_TH	R22	R1	R30
Rb2039PNB4_TH	R22	R1	R30
Rb2040PNB4_TH	R22	R1	R30
Rb2041PNB4_TH	R23	R1	R31
Rb2042PNB4_TH	R1	R1	R2

Table 41 (Cont.)

Code	Haplotype		
	COI	**16S rDNA**	**Combined mtDNA**
Rb2043PNB4_TH	R22	R1	R30
Rb2044PNB4_TH	R1	R15	R32
Rb2045PNB4_TH	R1	R6	R6
Rb2046PNB4_TH	R1	R1	R2
Rb2047PNB4_TH	R22	R1	R30
Rb2048PNB4_TH	R1	R1	R2

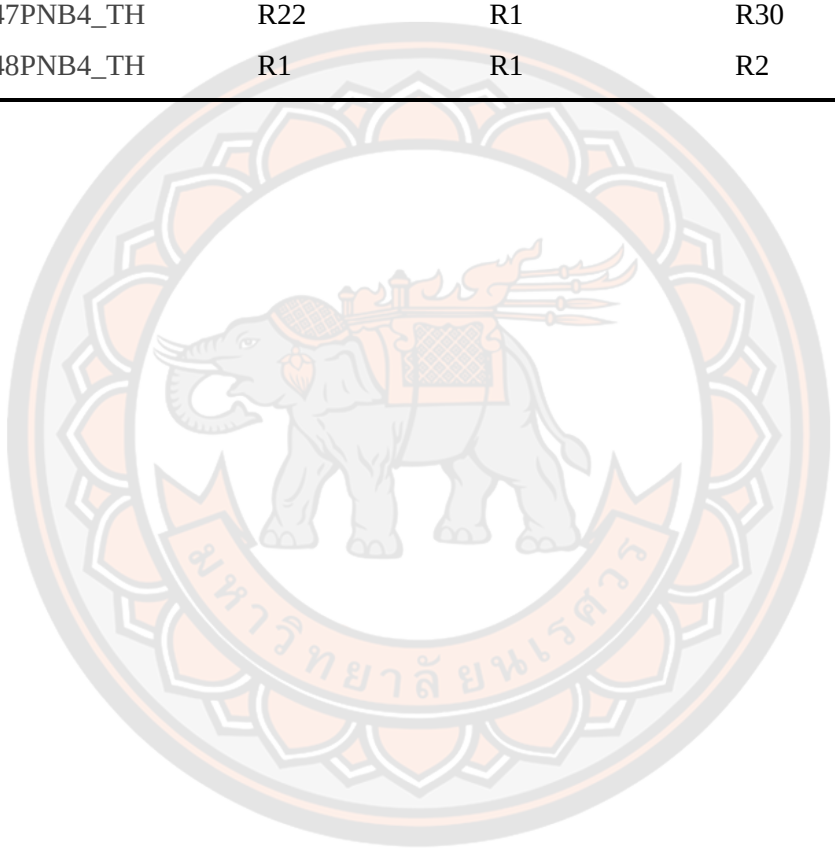

Table 42 Haplotype frequency based on COI sequences of *R. rubiginosa* in each population in Thailand.

Haplotype	Population code																	Haplotype frequencies (%)
	ATG	AYA	CNT	NSN	NYK	PCT	PLK	PNB	SBR	SRI	STI	CCO	PYO	KKN	PTN	SKA	TAK	
R1			2		12			6			2					1		23 (19.8)
R2	1	1	2				4			2						10		20 (17.2)
R3															11			11 (9.48)
R4				1		8	2											11 (9.48)
R5						1												1 (0.86)
R6						1												1 (0.86)
R7													10				3	13 (11.2)
R8			5															5 (4.31)
R9			1															1 (0.86)
R10			1	5										1				7 (6.03)
R11			1															1 (0.86)
R12			1															1 (0.86)
R13									1									1 (0.86)

Table 42 (Cont.)

Haplotype	Population code																	Haplotype frequencies (%)
	ATG	AYA	CNT	NSN	NYK	PCT	PLK	PNB	SBR	SRI	STI	CCO	PYO	KKN	PTN	SKA	TAK	
R14				2														2 (1.72)
R15				2														2 (1.72)
R16				1														1 (0.86)
R17				1														1 (0.86)
R18														2				2 (1.72)
R19																1		1 (0.86)
R20												3						3 (2.59)
R21												2						2 (1.72)
R22								5										5 (4.31)
R23								1										1 (0.86)
Total	**1**	**1**	**13**	**12**	**12**	**10**	**6**	**12**	**1**	**2**	**2**	**5**	**10**	**3**	**11**	**12**	**3**	**116 (100)**

Table 43 Haplotype frequency based on 16S rDNA sequences of *R. rubiginosa* in each population in Thailand.

Haplotype	Population code																	Haplotype frequencies (%)
	ATG	AYA	CNT	NSN	NYK	PCT	PLK	PNB	SBR	SRI	STI	CCO	PYO	KKN	PTN	SKA	TAK	
R1			2	5	10			10				3	10		11		3	54 (46.6)
R2	1		3	3			3		1	2				1		10		24 (20.7)
R3				2		9	1											12 (10.3)
R4							1											1 (0.86)
R5							1											1 (0.86)
R6								1			2	2				1		6 (5.17)
R7						1												1 (0.86)
R8			6															6 (5.17)
R9			1															1 (0.86)
R10			1	2														3 (2.59)
R11														2				2 (1.72)
R12																1		1 (0.86)
R13		1																1 (0.86)
R14					2													2 (1.72)

Table 43 (Cont.)

Haplotype	Population code																		Haplotype frequencies (%)
	ATG	AYA	CNT	NSN	NYK	PCT	PLK	PNB	SBR	SRI	STI	CCO	PYO	KKN	PTN	SKA	TAK		
R15								1											1 (0.86)
Total	**1**	**1**	**13**	**12**	**12**	**10**	**6**	**12**	**1**	**2**	**2**	**5**	**10**	**3**	**11**	**12**	**3**		**116 (100)**

Table 44 Haplotype frequency based on combined mt DNA sequences of *R. rubiginosa* in each population in Thailand.

Haplotype	Population code																		Haplotype frequencies (%)
	ATG	AYA	CNT	NSN	NYK	PCT	PLK	PNB	SBR	SRI	STI	CCO	PYO	KKN	PTN	SKA	TAK		
R1	1		2				3			2						10			18 (15.5)
R2			1		10			4											15 (12.9)
R3				1		7	1												9 (7.76)
R4							1												1 (0.86)
R5															11				11 (9.48)
R6								1			2					1			4 (3.45)

Table 44 (Cont.)

Haplotype	Population code																	Haplotype frequencies (%)
	ATG	AYA	CNT	NSN	NYK	PCT	PLK	PNB	SBR	SRI	STI	CCO	PYO	KKN	PTN	SKA	TAK	
R7						1												1 (0.86)
R8						1												1 (0.86)
R9						1												1 (0.86)
R10													10				3	13 (11.2)
R11			5															5 (4.31)
R12			1															1 (0.86)
R13			1															1 (0.86)
R14			1	2														3 (2.59)
R15			1															1 (0.86)
R16			1															1 (0.86)
R17							1											1 (0.86)
R18									1									1 (0.86)
R19				2														2 (1.72)
R20				2														2 (1.72)

Table 44 (Cont.)

Haplotype	Population code																	Haplotype frequencies (%)
	ATG	AYA	CNT	NSN	NYK	PCT	PLK	PNB	SBR	SRI	STI	CCO	PYO	KKN	PTN	SKA	TAK	
R21				3										1				4 (3.45)
R22				1														1 (0.86)
R23				1														1 (0.86)
R24														2				2 (1.72)
R25																1		1 (0.86)
R26		1																1 (0.86)
R27												3						3 (2.59)
R28												2						2 (1.72)
R29					2													2 (1.72)
R30								5										5 (4.31)
R31								1										1 (0.86)
R32								1										1 (0.86)
Total	**1**	**1**	**13**	**12**	**12**	**10**	**6**	**12**	**1**	**2**	**2**	**5**	**10**	**3**	**11**	**12**	**3**	**116 (100)**

Table 45 Population pairwise F_{ST} between 14 populations of *R. rubiginosa* based on COI sequences.

Populations	CNT	NSN	NYK	PCT	PLK	PNB	SRI	STI	CCO	PYO	KKN	PTN	SKA	TAK
CNT	0.000													
NSN	0.022	0.000												
NYK	0.386*	0.431*	0.000											
PCT	0.165*	0.202*	0.628*	0.000										
PLK	0.129	0.091	0.809*	0.490*	0.000									
PNB	0.334*	0.329*	0.436*	0.415*	0.631*	0.000								
SRI	0.281	0.266	1.000*	0.737*	-0.090	0.795*	0.000							
STI	0.101	0.143	0.000	0.371*	0.567	0.149	1.000	0.000						
CCO	0.176*	0.169	0.698*	0.307*	0.443*	0.425*	0.673	0.319	0.000					
PYO	0.408*	0.382*	1.000*	0.587*	0.787*	0.407*	1.000*	1.000*	0.664*	0.000				
KKN	0.038	0.003	0.862*	0.353*	0.136	0.571*	0.489	0.520	0.286	0.840*	0.000			
PTN	0.422*	0.436*	1.000*	0.626*	0.799*	0.606*	1.000*	1.000*	0.682*	1.000*	0.852*	0.000		
SKA	0.402*	0.380*	0.891*	0.730*	0.062	0.781*	-0.326	0.808*	0.707*	0.883*	0.542*	0.888*	0.000	
TAK	0.238	0.202*	1.000*	0.417*	0.625	0.229	1.000	1.000	0.417	0.000	0.625	1.000*	0.827*	0.000

Note: asterisks (*) indicate statistical significance of $P < 0.05$.

Table 46 Population pairwise F_{ST} between 14 populations of *R. rubiginosa* based on 16S rDNA sequences.

Populations	CNT	NSN	NYK	PCT	PLK	PNB	SRI	STI	CCO	PYO	KKN	PTN	SKA	TAK
CNT	0.000													
NSN	0.102	0.000												
NYK	0.348*	0.264*	0.000											
PCT	0.366*	0.426*	0.581*	0.000										
PLK	0.080	0.023	0.516*	0.610*	0.000									
PNB	0.355*	0.283*	0.072	0.785*	0.589*	0.000								
SRI	0.302*	0.314	0.811	0.969*	-0.090	0.939	0.000							
STI	-0.028	0.168	0.414*	0.915*	0.225	0.712*	1.000	0.000						
CCO	0.162	0.160	0.104	0.776*	0.386*	0.109	0.897*	0.285	0.000					
PYO	0.372*	0.291*	0.069	0.909*	0.611*	-0.016	1.000*	1.000*	0.417*	0.000				
KKN	0.080	-0.041	0.319*	0.662*	0.040	0.462*	0.368	0.076	0.199	0.536*	0.000			
PTN	0.386*	0.306*	0.080	0.913*	0.629*	-0.007	1.000*	1.000*	0.440	0.000	0.562*	0.000		
SKA	0.384*	0.380*	0.774*	0.873*	0.100	0.840*	-0.250	0.727*	0.768*	0.867*	0.521*	0.873*	0.000	
TAK	0.199	0.106	-0.111	0.856*	0.376	-0.189	1.000	1.000	0.117	0.000	0.142	0.000	0.804*	0.000

Note: asterisks (*) indicate statistical significance of $P < 0.05$.

Table 47 Population pairwise F_{ST} between 14 populations of *R. rubiginosa* based on combined mtDNA sequences.

Populations	CNT	NSN	NYK	PCT	PLK	PNB	SRI	STI	CCO	PYO	KKN	PTN	SKA	TAK
CNT	0.000													
NSN	0.052	0.000												
NYK	0.371*	0.372*	0.000											
PCT	0.241*	0.282*	0.611*	0.000										
PLK	0.110	0.067	0.692*	0.535*	0.000									
PNB	0.342*	0.317*	0.333*	0.502*	0.619*	0.000								
SRI	0.289*	0.283	0.925*	0.831*	-0.091	0.842*	0.000							
STI	0.058	0.152*	0.415*	0.572*	0.445	0.316*	1.000	0.000						
CCO	0.172*	0.167	0.539*	0.428*	0.423*	0.398*	0.751	0.315	0.000					
PYO	0.396*	0.355*	0.755*	0.672*	0.725*	0.375*	1.000*	1.000*	0.642*	0.000				
KKN	0.055	-0.012	0.673*	0.470*	0.091	0.548*	0.435	0.368	0.261	0.763*	0.000			
PTN	0.409*	0.398*	0.764*	0.699*	0.739*	0.575*	1.000*	1.000*	0.662*	1.000*	0.779*	0.000		
SKA	0.396*	0.380*	0.847*	0.788*	0.079	0.800*	-0.297	0.785*	0.728*	0.878*	0.534*	0.883*	0.000	
TAK	0.225	0.173	0.649*	0.523*	0.532*	0.195	1.000	1.000	0.388	0.000	0.478	1.000*	0.819*	0.000

Note: asterisks (*) indicate statistical significance of $P < 0.05$.

Table 48 List of the haplotypes identified in the *O. viridis* samples from Thailand based on COI, 16S rDNA, and combined mtDNA analyses.

Code	Haplotype		
	COI	**16S rDNA**	**Combined mtDNA**
Ov1PLK2_TH	O5	O4	O7
Ov5PLK3_TH	O5	O4	O7
Ov6PLK3_TH	O1	O2	O1
Ov7PLK4_TH	O1	O2	O1
Ov13PLK4_TH	O1	O2	O1
Ov18PLK4_TH	O1	O3	O3
Ov24PLK4_TH	O1	O2	O1
Ov25PLK4_TH	O1	O2	O1
Ov28PLK5_TH	O1	O2	O1
Ov39PLK7_TH	O5	O4	O7
Ov45PLK7_TH	O3	O1	O4
Ov48PLK7_TH	O1	O4	O5
Ov56PLK7_TH	O3	O1	O4
Ov86PLK9_TH	O3	O1	O4
Ov91PLK9_TH	O5	O4	O7
Ov109PLK9_TH	O5	O4	O7
Ov182PLK11_TH	O4	O1	O6
Ov183PLK11_TH	O5	O4	O7
Ov184PLK11_TH	O3	O1	O4
Ov211PLK11_TH	O3	O1	O4
Ov298PLK13_TH	O3	O1	O4
Ov304PLK7_TH	O5	O4	O7
Ov429PCT1_TH	O1	O2	O1
Ov430PCT1_TH	O1	O2	O1
Ov431PCT1_TH	O1	O2	O1
Ov432PCT1_TH	O1	O2	O1

Table 48 (Cont.)

Code	Haplotype		
	COI	**16S rDNA**	**Combined mtDNA**
Ov433PCT1_TH	O1	O2	O1
Ov434PCT1_TH	O1	O2	O1
Ov435PCT1_TH	O1	O2	O1
Ov436PCT1_TH	O1	O2	O1
Ov437PCT1_TH	O1	O2	O1
Ov444PCT1_TH	O1	O2	O1
Ov445PCT1_TH	O1	O2	O1
Ov454LPG1_TH	O2	O1	O2
Ov455LPG1_TH	O2	O1	O2
Ov456LPG1_TH	O2	O1	O2
Ov457LPG1_TH	O2	O1	O2
Ov458LPG1_TH	O2	O1	O2
Ov459LPG1_TH	O2	O1	O2
Ov460LPG1_TH	O2	O1	O2
Ov461LPG1_TH	O2	O1	O2
Ov462LPG1_TH	O2	O1	O2
Ov463LPG1_TH	O2	O1	O2
Ov517CMI2_TH	O2	O1	O2
Ov518CMI2_TH	O3	O1	O4
Ov529CMI2_TH	O3	O2	O8
Ov531CMI2_TH	O2	O1	O2
Ov533CMI2_TH	O3	O5	O9
Ov539CMI2_TH	O2	O1	O2
Ov540CMI2_TH	O2	O1	O2
Ov542CMI2_TH	O6	O6	O10
Ov543CMI2_TH	O2	O1	O2
Ov545CMI2_TH	O3	O7	O11

Table 48 (Cont.)

Code	Haplotype		
	COI	**16S rDNA**	**Combined mtDNA**
Ov571CRI1_TH	O2	O1	O2
Ov572CRI1_TH	O7	O1	O12
Ov574CRI1_TH	O2	O6	O14
Ov635UTT2_TH	O1	O2	O1
Ov636UTT2_TH	O1	O2	O1
Ov637UTT2_TH	O1	O2	O1
Ov638UTT2_TH	O8	O2	O13
Ov640UTT2_TH	O1	O2	O1
Ov641UTT2_TH	O1	O2	O1
Ov645UTT2_TH	O1	O2	O1
Ov646UTT2_TH	O1	O2	O1
Ov647UTT2_TH	O2	O1	O2
Ov650UTT2_TH	O1	O2	O1
Ov652UTT2_TH	O1	O2	O1
Ov653UTT2_TH	O2	O1	O2
Ov1311UTI1_TH	O1	O2	O1
Ov1312UTI1_TH	O1	O2	O1
Ov1313UTI1_TH	O1	O2	O1
Ov1314UTI1_TH	O1	O8	O15
Ov1358NSN2_TH	O1	O8	O15
Ov1687SBR3_TH	O2	O1	O2
Ov1688SBR3_TH	O2	O1	O2
Ov1689SBR3_TH	O5	O4	O7
Ov1690SBR3_TH	O1	O2	O1
Ov1691SBR3_TH	O2	O1	O2
Ov1692SBR3_TH	O2	O1	O2
Ov1693SBR3_TH	O2	O1	O2

Table 48 (Cont.)

Code	Haplotype		
	COI	**16S rDNA**	**Combined mtDNA**
Ov1694SBR3_TH	O2	O1	O2
Ov1695SBR3_TH	O2	O1	O2
Ov1706SBR3_TH	O1	O2	O1
Ov2034SBR4_TH	O2	O1	O2

Table 49 Haplotype frequency based on COI sequences of *O. viridis* in each population in Thailand.

Haplotype	Population code									Haplotype frequencies (%)
	NSN	PCT	PLK	SBR	UTI	CMI	CRI	LPG	UTT	
O1	1	11	8	2	4				9	35 (41.7)
O2				8		5	2	10	2	27 (32.1)
O3			6			4				10 (11.9)
O4			1							1 (1.19)
O5			7	1						8 (9.52)
O6						1				1 (1.19)
O7							1			1 (1.19)
O8									1	1 (1.19)
Total	**1**	**11**	**22**	**11**	**4**	**10**	**3**	**10**	**12**	**84 (100)**

Table 50 Haplotype frequency based on 16S rDNA sequences of *O. viridis* in each population in Thailand.

Haplotype	Population code									Haplotype
	NSN	PCT	PLK	SBR	UTI	CMI	CRI	LPG	UTT	frequencies (%)
O1			7	8		6	2	10	2	35 (41.7)
O2		11	6	2	3	1			10	33 (39.3)
O3			1							1 (1.19)
O4			8	1						9 (10.7)
O5						1				1 (1.19)
O6						1	1			2 (2.38)
O7						1				1 (1.19)
O8	1				1					2 (2.38)
Total	**1**	**11**	**22**	**11**	**4**	**10**	**3**	**10**	**12**	**84 (100)**

Table 51 Haplotype frequency based on combined mt DNA sequences of *O. viridis* in each population in Thailand.

Haplotype	Population code									Haplotype
	NSN	PCT	PLK	SBR	UTI	CMI	CRI	LPG	UTT	frequencies (%)
O1		11	6	2	3				9	31 (36.9)
O2				8		5	1	10	2	26 (31)
O3			1							1 (1.19)
O4			6			1				7 (8.33)
O5			1							1 (1.19)
O6			1							1 (1.19)
O7			7	1						8 (9.52)
OV8						1				1 (1.19)
OV9						1				1 (1.19)
OV10						1				1 (1.19)
OV11						1				1 (1.19)
OV12							1			1 (1.19)
OV13									1	1 (1.19)
OV14							1			1 (1.19)
OV15	1				1					2 (2.38)
Total	**1**	**11**	**22**	**11**	**4**	**10**	**3**	**10**	**12**	**84 (100)**

Table 52 Population pairwise F_{ST} between 8 populations of *O. viridis* based on COI sequences.

Populations	PCT	PLK	SBR	UTI	CMI	CRI	LPG	UTT
PCT	0.000							
PLK	0.297*	0.000						
SBR	0.600*	0.067	0.000					
UTI	0.000	0.189	0.463	0.000				
CMI	0.774*	0.162*	0.226	0.671*	0.000			
CRI	0.907*	0.085	-0.010	0.794*	0.221	0.000		
LPG	1.000*	0.192*	0.029	1.000*	0.370*	0.411	0.000	
UTT	0.051	0.225*	0.375*	-0.078	0.602*	0.586*	0.724*	0.000

Note: asterisks (*) indicate statistical significance of $P < 0.05$.

Table 53 Population pairwise F_{ST} between 8 populations of *O. viridis* based on 16S rDNA sequences.

Populations	PCT	PLK	SBR	UTI	CMI	CRI	LPG	UTT
PCT	0.000							
PLK	0.363*	0.000						
SBR	0.654*	0.055	0.000					
UTI	0.270	0.269*	0.496*	0.000				
CMI	0.548*	0.139*	-0.014	0.382*	0.000			
CRI	0.907*	0.099	0.021	0.640*	-0.144	0.000		
LPG	1.000*	0.193*	0.039	0.892*	0.000	0.411	0.000	
UTT	0.080	0.273*	0.449*	0.086	0.397*	0.670*	0.802*	0.000

Note: asterisks (*) indicate statistical significance of $P < 0.05$.

Table 54 Population pairwise F_{ST} between 8 populations of *O. viridis* based on combined mtDNA sequences.

Populations	PCT	PLK	SBR	UTI	CMI	CRI	LPG	UTT
PCT	0.000							
PLK	0.327*	0.000						
SBR	0.626*	0.063	0.000					
UTI	0.271	0.226	0.480*	0.000				
CMI	0.666*	0.152*	0.109*	0.520*	0.000			
CRI	0.907*	0.091	0.004	0.710*	0.025	0.000		
LPG	1.000*	0.193*	0.034	0.942*	0.171*	0.412*	0.000	
UTT	0.063	0.246*	0.409*	0.008	0.506*	0.626*	0.762*	0.000

Note: asterisks (*) indicate statistical significance of $P < 0.05$.