

INTRASPECIFIC RELATIONSHIPS AND GENETIC STRUCTURE OF
A LUNG FLUKE, *PARAGONIMUS WESTERMANI*



A THESIS
BY
KOWIT HENGPHASATPORN

Presented in Partial Fulfillment of the Requirements for the
Master of Education Degree in Biology
at Srinakharinwirot University
April 2015

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Paragonimus westermani, the type species of the genus, is an important causative agent of paragonimiasis in mammals. It is widely distributed in Asia and forms a species complex. In order to study intraspecific relationships and genetic structure of *P. westermani*, 63 samples from 12 localities were used for DNA extraction and amplification of the ITS2 and COI regions. In addition, seven sequences of both loci, each representing a different locality, were obtained from GenBank. A total of 70 sequences was employed for the alignment of each locus, and subjected to phylogenetic tree reconstruction using MP and ML analyses. Based on both types of analysis, five monophyletic groups were generated, with *P. westermani* from Sri Lanka as an outgroup. The population from South Asia formed a basal group, while those from East Asia were more advanced. The COI sequence data as well as a combined data set of the ITS2 and COI regions revealed identical tree topologies. The COI-based network showed 18 haplotypes which demonstrated a geographical structure of genetic variation of *P. westermani* species complex. This analysis also indicated a high level of haplotype diversity, but rather low genetic variability, especially in the East Asian populations.

ความสัมพันธ์ภายในสปีชีส์ และโครงสร้างทางพันธุกรรมของพยาธิใบไม้ปอด
Paragonimus westermani



เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา
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รศ.ดร.อัจฉริยา รังษิรุจิ และ ดร.สิทธิพร ปานเม่น

พยาธิใบไม้ปอด (*Paragonimus westermani*) เป็นสปีชีส์ต้นแบบของสกุล *Paragonimus* ซึ่งก่อให้เกิดโรคพยาธิใบไม้ปอดในสัตว์เลี้ยงลูกด้วยนม พยาธิสปีชีส์นี้พบแพร่กระจายทั่วไปในทวีปเอเชีย และจัดเป็นกลุ่มสปีชีส์ที่มีความซับซ้อน ในการศึกษาความสัมพันธ์ภายในสปีชีส์ และโครงสร้างทางพันธุกรรมของ *P. westermani* ผู้วิจัยใช้ตัวอย่างพยาธิจำนวน 63 ตัวอย่าง ที่เก็บได้จาก 12 แหล่งในทวีปเอเชีย เพื่อสกัดดีเอ็นเอ และเพิ่มปริมาณดีเอ็นเอบริเวณ ITS2 และ COI นอกจากนี้ยังได้นำลำดับนิวคลีโอไทด์ของบริเวณ ITS2 และ COI อย่างละ 7 ตัวอย่างที่ได้มาจากต่างแหล่งใน GenBank มาวิเคราะห์ร่วมด้วย การเทียบเคียง 70 ลำดับนิวคลีโอไทด์ของดีเอ็นเอแต่ละบริเวณ และการวิเคราะห์ความสัมพันธ์เชิงวิวัฒนาการของ *P. westermani* โดยใช้ตัวอย่างจากประเทศศรีลังกาเป็นบรรพบุรุษ พบว่าทั้งวิธี MP และ ML ให้ผลเป็นแผนภาพต้นไม้แห่งวิวัฒนาการที่สอดคล้องกัน ซึ่งประกอบด้วย 5 กลุ่มที่มีบรรพบุรุษร่วมสุดท้ายเดียวกัน โดยประชากรของ *P. westermani* จากเอเชียใต้ จัดเป็นกลุ่มที่มีความโบราณ ในขณะที่ประชากรจากเอเชียตะวันออกเฉียงใต้มีวิวัฒนาการที่สูงที่สุด นอกจากนี้ยังพบว่ารูปแบบของแผนภาพต้นไม้แห่งวิวัฒนาการที่ได้จากข้อมูลของ COI และข้อมูลของ ITS2 และ COI นั้นมีความเหมือนกันทุกประการ และจากการวิเคราะห์โครงข่ายแฮพโลไทป์โดยใช้บริเวณ COI พบว่าประชากรของ *P. westermani* ทั้งหมดแบ่งได้เป็น 18 แฮพโลไทป์ ซึ่งมีความแปรผันทางพันธุกรรมตามโครงสร้างทางภูมิศาสตร์ ผลการศึกษาแสดงให้เห็นว่าประชากรมีความหลากหลายของแฮพโลไทป์สูง แต่มีความผันแปรทางพันธุกรรมต่ำ โดยเฉพาะในกลุ่มประชากรจากเอเชียตะวันออกเฉียง

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Paragonimus westermani ”

by
Kowit Hengphasatporn

has been approved by the Graduate School as partial fulfillment of the requirements for the
Master of Education degree in Biology of Srinakharinwirot University.

..... Dean of Graduate School

(Assoc. Prof. Dr. Somchai Santiwatanakul)

Thesis Committee

Oral Defense Committee

..... Major-advisor Chair

(Assoc. Prof. Dr. Achariya Rangsiruji) (Asst. Prof. Dr. Somkiat Phornphisutthimas)

..... Co-advisor Committee

(Dr. Sittiporn Parnmen)

(Assoc. Prof. Dr. Achariya Rangsiruji)

..... Committee

(Dr. Sittiporn Parnmen)

..... Committee

(Asst. Prof. Dr. Duangjai Boonkusol)

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CHAPTER ONE

INTRODUCTION

Background

Paragonimiasis is a disease caused by lung flukes belonging to the genus *Paragonimus*. Almost 50 species of *Paragonimus* have been described, most of which are found in carnivorous mammalian hosts. Of these, 9 species have been reported to infect humans (Castilla; *et al.* 2003). The most common species is *P. westermani* (Kerbert, 1878) Braun, 1899. The parasite was discovered from a Bengal tiger of an Amsterdam zoo and named after the zoo's director (Yokogawa. 1965). *Paragonimus westermani* is an important pathogen in East Asia (Liu; *et al.* 2008). In Thailand however, there were also reports of paragonimiasis, particularly in Saraburi and Nakhon Nayok provinces where *P. heterotremus* is a well known causative agent (Yoonuan; *et al.* 2008).

Classification and morphology of *Paragonimus westermani*

Paragonimus westermani is the type species of the genus and has been regarded as most infectious to humans. The first case was found in human lung tissue in 1879 by Ringer (Muller. 1996). This species is widely distributed in the Far East, especially in China, Korea, Japan, Taiwan, the Philippines, India as well as Thailand. The genus belongs to the class Trematoda, phylum Platyhelminthes (Figure 1).

Paragonimus species have many stages in the life cycle. The morphology of an embryonated egg, metacercaria and adult stages of *P. westermani* are shown in Figure 2. The embryonated egg is yellowish-brown in color, thick-shelled with large operculum, and measured 80 to 120 μm $\times$ 50 to 65 μm . The metacercaria is circular with an average diameter of 352 μm . It has a double cyst wall; thick inner cyst wall and thin outer cyst wall (Higo; & Ishii. 1987). Adult *P. westermani* is oval in shape and reddish-brown in color. The integument is spiny. The adult worm is 7 to 16 mm in length, 4 to 8 mm in width and 5 mm thick. Both oral and ventral suckers are equal in size (Guerrant; Walker; & Weller. 1999).

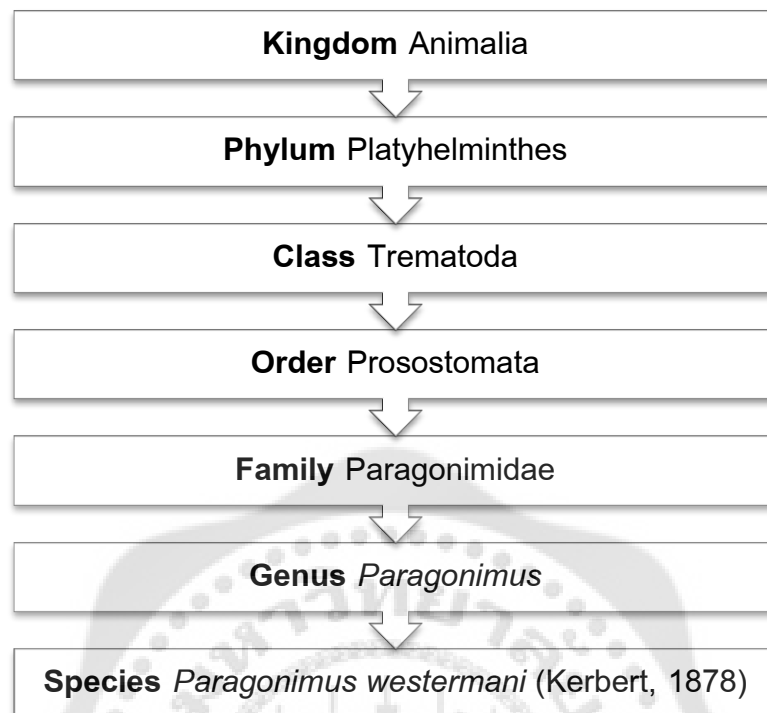


Figure 1 Classification of *Paragonimus westermani*.

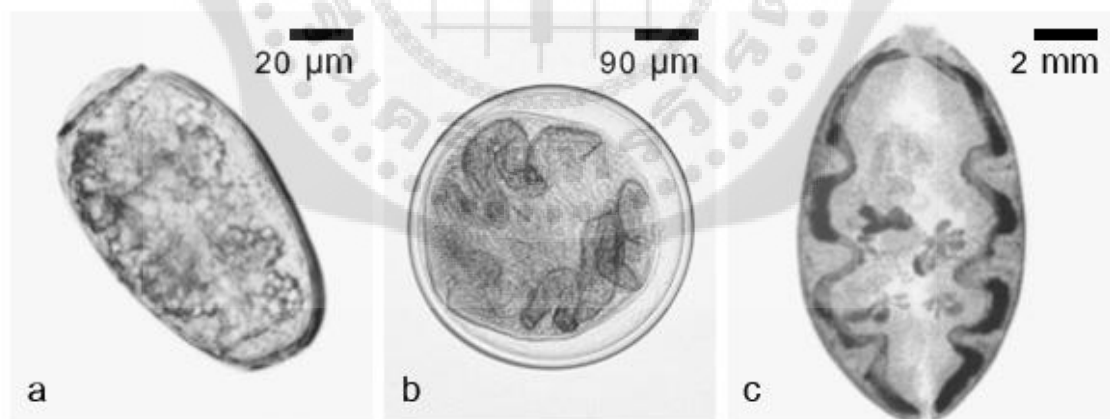


Figure 2 *Paragonimus westermani* a: embryonated egg, b: metacercaria, c: adult.

Sources: Figure 2a and b: Modified from Orihel TC; & Ash LR. (1995). *Parasites in Human Tissues*. p. 311.

Figure 2c: <http://workforce.calu.edu/Buckelew/Paragonimus%20westermani%20metacercariae.htm>. Retrieved date: 13 April 2014.

Life cycle of *Paragonimus*

The life cycle of *Paragonimus* was reviewed in Fürst; *et al.* (2012). After fertilization occurs, embryonated eggs are released to the environment with sputum or may be released with feces if the host swallows the sputum. For about 3 weeks the eggs begin to hatch and develop into miracidia, which then seek the first intermediate host; a snail and penetrate its soft tissue. Miracidia then go through several stages of development, including sporocysts, rediae and cercariae by having mitotic cell division. The cercariae leave the snail and penetrate the second intermediate host; freshwater crustaceans such as crabs or crayfish. They encyst and become metacercariae. This stage of the parasite is infective. After a mammal which is a definitive host feeds on raw or undercooked crustaceans the metacercariae excyst in duodenum and penetrate through peritoneum into lungs. They encapsulate and grow into adult worms within 6 to 8 weeks. Figure 3 shows the life cycle of *Paragonimus*.

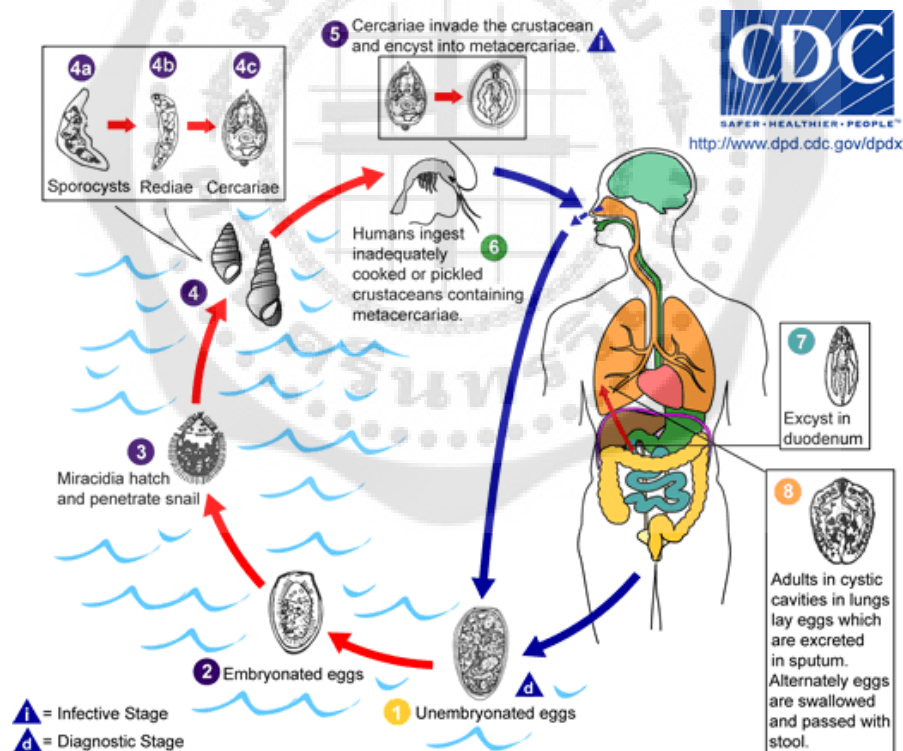


Figure 3 Life cycle of *Paragonimus*.

Source: <http://www.cdc.gov/parasites/paragonimus/biology.html> Retrieved date: 23 July 2014.

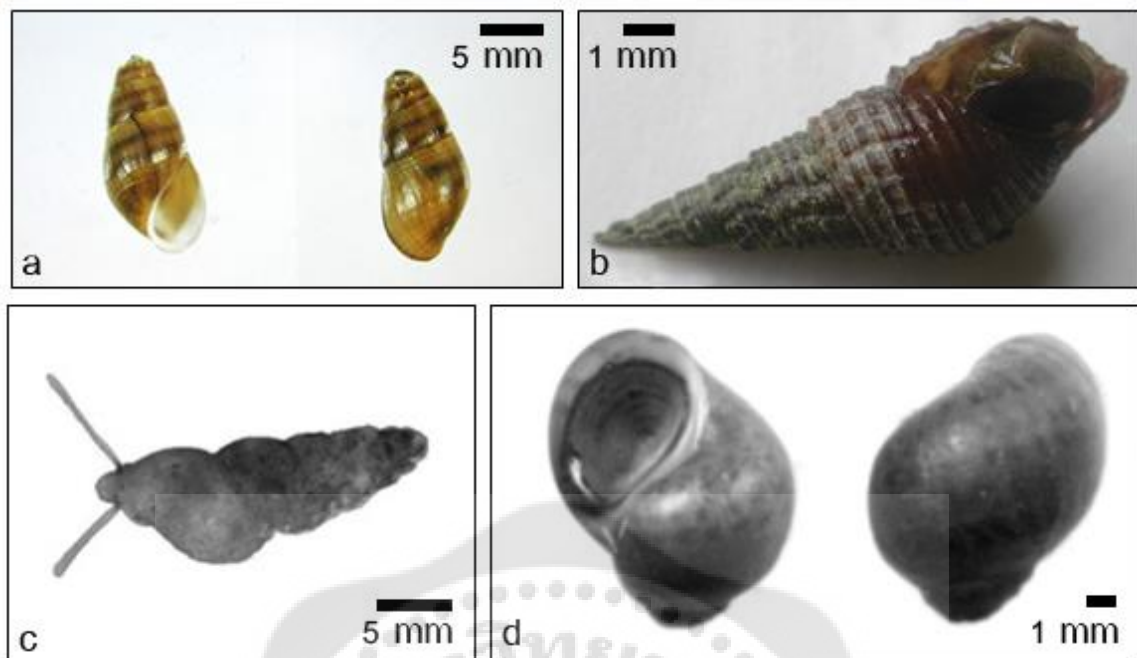


Figure 5 First intermediate host of *P. westermani* a: *Semisulcospira* sp., b: *Tarebia* sp., c: *Aroapyrgus* sp., d: *Paludomus* sp.

Sources: Figure 5a: http://atlas.or.kr/atlas/alphabet_view.php?my_codeName=Paragonimus%20westermani. Retrieved date: 13 April 2014.

Figure 5b: Moslemi; *et al.* (2012). Impacts of an Invasive Snail (*Tarebia granifera*) on Nutrient Cycling in Tropical Streams: the Role of Riparian Deforestation in Trinidad, West Indies. *Plos One*. 7(6): 3.

Figure 5c: Arias; *et al.* (2011). *Paragonimus* sp. in Crabs and Awareness of the Educational Community to Aquatic Ecosystems in La Miel and La Clara, Caldas, Antioquia. *Biomedica*. 31(2): 209-215.

Figure 5d: Iwagami; *et al.* (2009). The First Intermediate Host of *P. westermani* in Sri Lanka. *Acta Tropica*. 109(1): 27-29.

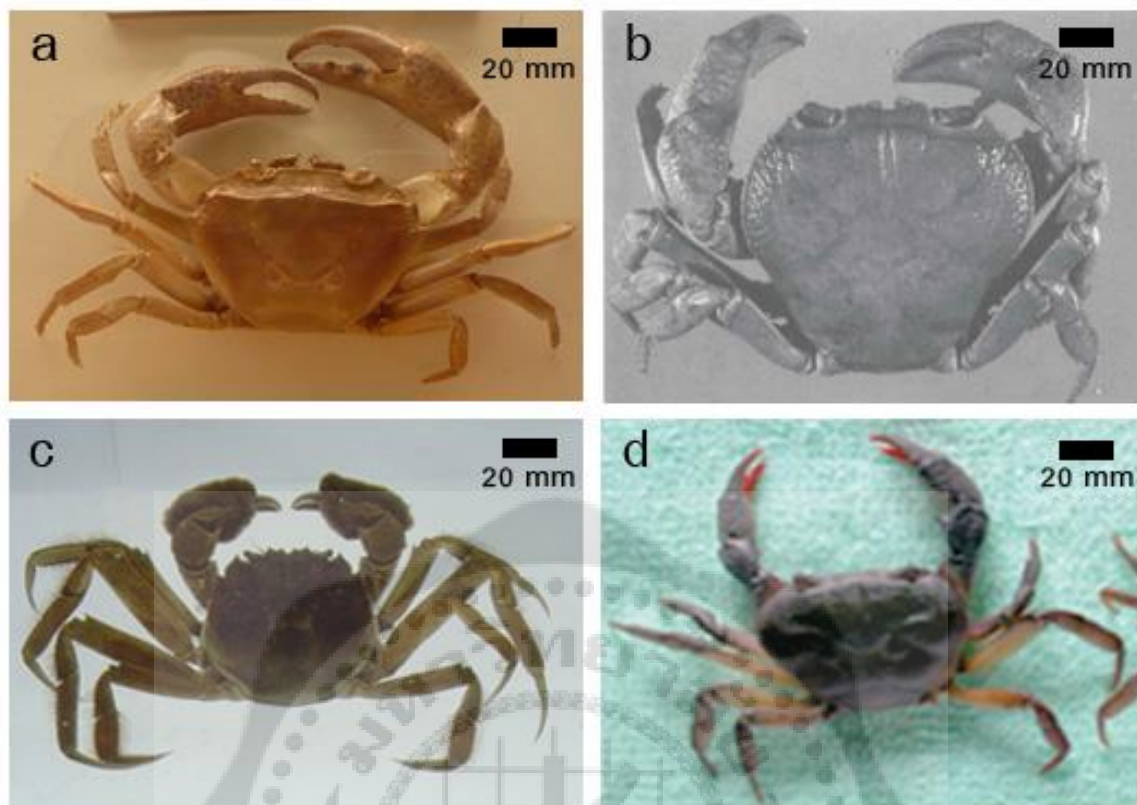


Figure 6 Second intermediate host of *P. westermani* **a:** *Parathelphusa* sp., **b:** *Potamon* sp., **c:** *Eriocheir* sp., **d:** *Indochinamon* sp.

Sources: Figure 6a: https://www.flickr.com/photos/hai_ren/4592416936/. Retrieved date 13 April 2014.

Figure 6b: Habe; *et al.* (2013). *Paragonimus paishuihoensis* Metacercariae in Freshwater Crabs, *Potamon lipkei*, in Vientiane Province, Lao PDR. *Korean Journal of Parasitology*. 51(6): 683-687.

Figure 6c: <http://online.sfsu.edu/bholzman/courses/Fall02%20projects/mitten>. Retrieved date: 13 April 2014.

Figure 6d: Sohn; *et al.* (2009). *Indochinamon ou* (crustacea: Potamidae) as a New Second Intermediate Host for *Paragonimus harinasutai* in Luang Prabang Province, Lao PDR. *Korean Journal of Parasitology*. 47(1): 25-29.

Paragonimiasis

The symptoms of paragonimiasis can be classified into three main forms: pulmonary, extrapulmonary and pleuropulmonary. Pulmonary paragonimiasis is the most common form which can be divided into acute and chronic phases. The acute phase involves diarrhea, chest pain, abdominal pain, fever, cough, skin rash, raised and itchy bumps which appear after an incubation period about 2 to 15 days (Chung; *et al.* 1981). The chronic phase on the other hand, shows hemoptysis (rust-color sputum), chest radiographic abnormalities, chronic cough and fibrotic encapsulation in the lung parenchyma. These symptoms are similar to those of pulmonary tuberculosis (Procop. 2009). Extrapulmonary paragonimiasis can occur when the flukes migrate to ectopic sites such as brain, spinal cord, liver and intestinal wall (Singh; Sugiyama; & Rangsiruji. 2012). The third form, pleuropulmonary paragonimiasis occurs in both the pleural cavity and lungs (Yokogawa. 1965).

The aims of research

1. To study intraspecific relationships of *P. westermani* in Asia using nucleotide sequences of the ITS2 and COI regions.
2. To investigate the genetic structure of *P. westermani* in Asia based on a haplotype network analysis of the COI sequence data.

CHAPTER TWO

LITERATURE REVIEW

***Paragonimus westermani* and its genetic diversity**

Paragonimus westermani, the type species of the genus, is widely distributed in Asia. The species is genetically variable as it contains different sets of chromosomes and forms a species complex. According to Blair; *et al.* (1997) it was divided into two groups based on molecular data. First, the northern group consisted of the samples from Japan, Korea, China and Taiwan. Second, the southern group consisted of the samples from Malaysia, Thailand and the Philippines. In addition, *P. westermani* isolates were recently reported from Sri Lanka (Iwagami; *et al.* 2008), Vietnam (Doanh; *et al.* 2009) and India (Devi; *et al.* 2010). Analyses of these isolates provided new insights on molecular phylogenetic relationships of this species complex.

As mentioned earlier, *P. westermani* is well known for its diversity of ploidy levels with different chromosome numbers, including diploid (2n) and triploid (3n) (reviewed in Dreyfuss; & Rondelaud. 2008). Moreover, the tetraploid (4n) form of *P. westermani* was also reported in China (Terasaki; *et al.* 1995). *Paragonimus* is a causative agent of paragonimiasis. In china, the largest number of *Paragonimus* species has been reported and there are at least 9 species which are pathogenic to humans (Yang; *et al.* 2000). Among these, *P. westermani* and *P. skrjabini* are the most important ones (Liu; *et al.* 2008). A previous study by Lou; *et al.* (2011) revealed the existence of *P. westermani* in several areas of China, including Fujian, Guangdong, Sichuan, Yunnan and Jinhua.

Molecular approaches for *P. westermani* studies

Internal transcribed spacer (ITS) regions are parts of the nuclear ribosomal DNA which are organized in tandem repeating units. Each unit contains three genes (5.8S, 18S and 28S), coding for ribosomal RNA and two spacers (ITS1 and ITS2) (Figure 7) (White; *et al.* 1990). Due to their faster rates of evolution than the coding regions, ITS1 and ITS2 are usually employed for phylogenetic studies at the interspecific level (van Herwerden; Blair; & Agatsuma. 1999). Moreover, for *Paragonimus* species there are sufficient ITS data generated and submitted to the public databases (DDBJ/EMBL/GenBank) (Kans; & Ouellette. 2001).

Mitochondrial and ribosomal RNA genes have the advantage of being present in high copy numbers in each cell, facilitating recovery and amplification. However, the ribosomal RNA genes may not exhibit enough sequence diversity to enable discrimination below the species level. Blair; *et al.* (1997) found that base differences among the ITS2 sequences of *P. westermani* were rather few. These sequences were more conserved compared to the COI sequences (reviewed in Iwagami; *et al.* 2000). In trematodes, variation within the ITS2 sequences is minimal or non-existent. This is because they accumulate base substitutions at a lower rate than the COI sequences (Vilas; Criscione; & Blouin. 2005). Evolution in the mitochondrial genes on the other hand, is relatively rapid and these genes are therefore, good candidates for phylogenetic analyses at the intraspecific level (Herbert; *et al.* 2003).

The evolutionary history of a group of organisms can be demonstrated in a branching diagram called a phylogenetic tree. The tree can be reconstructed by using either morphological data or molecular data (Willis; *et al.* 2010). For *Paragonimus*, adult worms exhibit relatively few morphological characters. These include shapes and degree of branching of sexual organs, arrangement of spines on integument and relative sizes of suckers. The characters of the metacercaria cyst are even more highly conserved than those of the adults (Blair; *et al.* 1999). Due to these limitations of the morphological characters, molecular data are now being used more frequently to generate phylogenetic trees.

Blair; *et al.* (1998) reconstructed the phylogenetic trees based on the ITS2 and COI sequence data which showed relationships among the species and isolates of *Paragonimus* (Figure 9). Based on these molecular data, the samples can be divided into two groups; group I consisted of *P. westermani* and *P. siamensis*, while group II consisted of all other species. The tree topology of the ITS2 data was slightly different from that of the COI data. The COI tree was more resolved, thus it could further classify *P. westermani* at the intraspecific level.

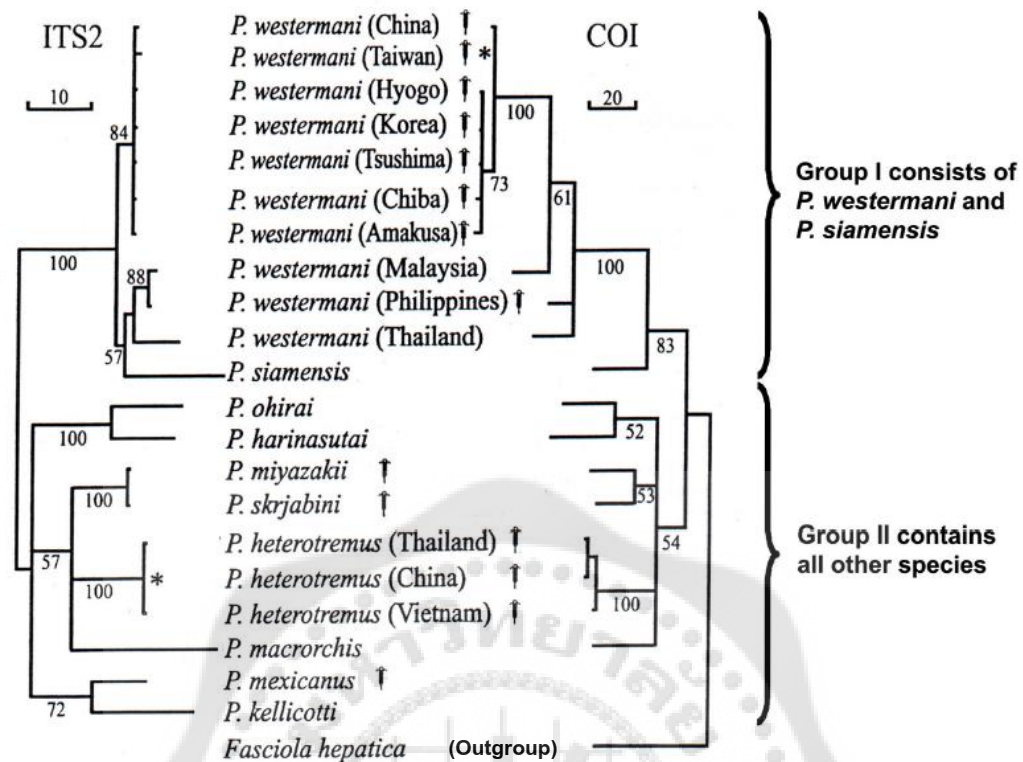


Figure 9 Phylogenetic relationships among *Paragonimus* species based on the ITS2 and COI sequences. Numbers on branches indicate bootstrap values.

Source: Blair; *et al.* (1998). Phylogenetic Relationships among the Thai Species of *Paragonimus* Inferred from DNA Sequences. In *Proceedings of the 9th International Congress of Parasitology*. Monduzzi International, Bologna. p. 645.

In 2000, Blair investigated the phylogenetic relationships among *Paragonimus* species as well as different *P. westermani* strains using the COI sequences and allozyme data. Although the two sources of data yielded similar trees, the COI tree was more resolved and complete (Figure 10).

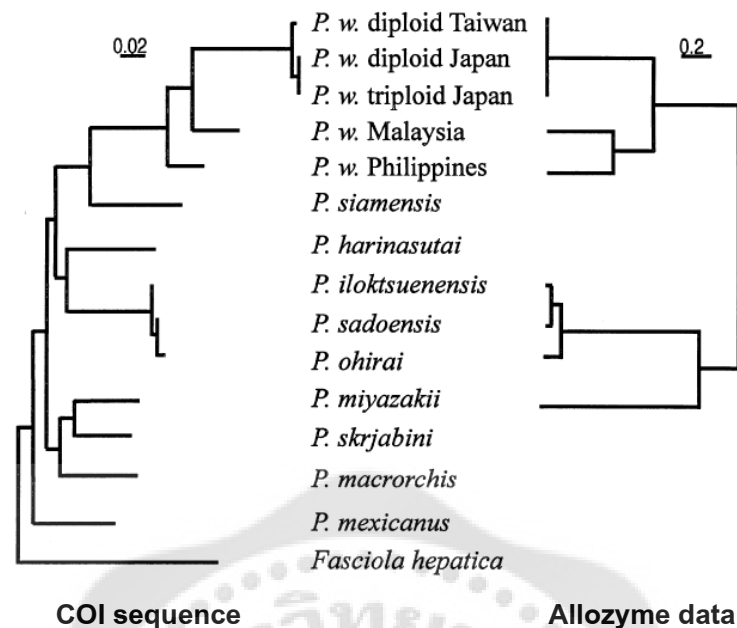


Figure 10 Phylogenetic relationships among species and strains of *Paragonimus* based on the COI sequences and allozyme data.

Source: Blair. (2000). Genomes of *Paragonimus westermani* and Related Species: Current State of Knowledge. *Parasitology*. 30: 423.

In addition, a phylogenetic analysis based on a combined data set of the ITS2 and COI sequences by Iwagami; *et al.* (2008) revealed the ancient divergence of *P. westermani* in Sri Lanka. It was also observed that *P. westermani* is closely related to *P. siamensis* (Figure 11). Later, Doanh; *et al.* (2009) reported a discovery of *P. westermani* in Vietnam and its phylogenetic status in *P. westermani* complex (Figure 12). Based on the ITS2 sequence data, it was found that *P. westermani* isolates were divided into four groups according to their geographical origins. The results also supported that within the *P. westermani* complex the Sri Lankan isolates formed a basal group.

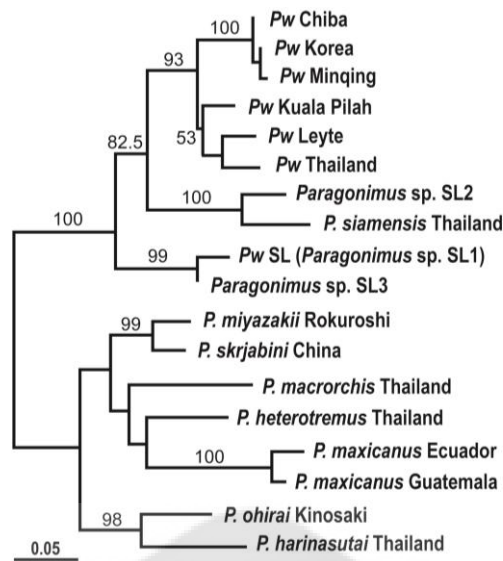


Figure 11 Phylogenetic tree of *Paragonimus* species based on a combined data set of the ITS2 and COI nucleotide sequences. Numbers on branches indicate bootstrap values.

Source: Iwagami; *et al.* (2008). Ancient Divergence of *Paragonimus westermani* in Sri Lanka. *Parasitology Research*. 102(5): 851.

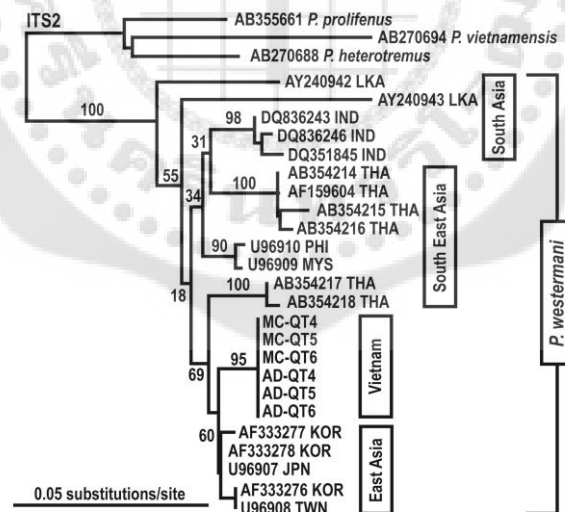


Figure 12 Phylogenetic tree of *Paragonimus* species reconstructed from the ITS2 sequence data. Numbers on branches indicate bootstrap values.

Source: Doanh; *et al.* (2009). Discovery of *Paragonimus westermani* in Vietnam and Its Molecular Phylogenetic Status in *P. westermani* Complex. *Parasitology Research*. 104(5): 1152.

Prasad; *et al.* (2009) carried out the phylogenetic analyses of *Paragonimus* species using ITS2 secondary structures (Figure 13). The results revealed a high resolution among the species studied. Moreover, the comparison of the ITS2 sequences indicated that a high species-specific homogeneity did exist. Thus, the ITS2 sequence data could be used as one of the species markers. The ITS2 secondary structures can be considered a promising tool for *Paragonimus* species identification as they contain more molecular information than usual primary sequence alignments.

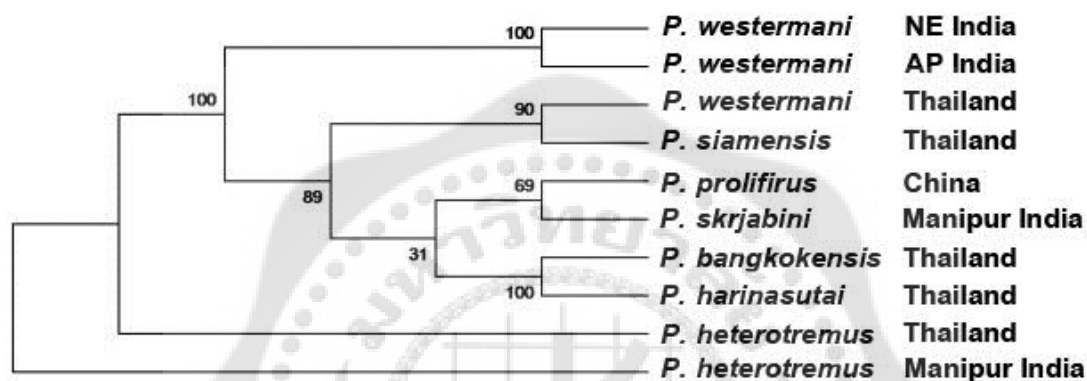


Figure 13 Phylogenetic tree of *Paragonimus* species based on secondary structures of the ITS2 region. Numbers on branches indicate bootstrap values.

Source: Prasad; *et al.* (2009). Phylogenetic Reconstruction Using Secondary Structures and Sequence Motifs of ITS2 rDNA of *Paragonimus westermani* (Kerbert, 1878) Braun, 1899 (Digenea: Paragonimidae) and Related Species. *BMC Genomics*. 3(25): 4.

Singh; Sugiyama; & Rangsiruji (2012) carried out a study on *Paragonimus* and paragonimiasis in India. Based on the ITS2 sequence data, it was shown that *P. westermani* samples from Manipur and Arunachal Pradesh were in the same clade as *P. westermani* and *P. siamensis* from Thailand (Figure 14). By using the ITS2 sequences, Devi; *et al.* (2013) found three distinct genotypes within *P. westermani* complex in northeastern India. Two genotypes were classified as *P. westermani*, while the third genotype was identified as *P. siamensis*. The samples of *P. siamensis* from northeastern India were closely related to those from Sri Lanka and Thailand (Figure 15).

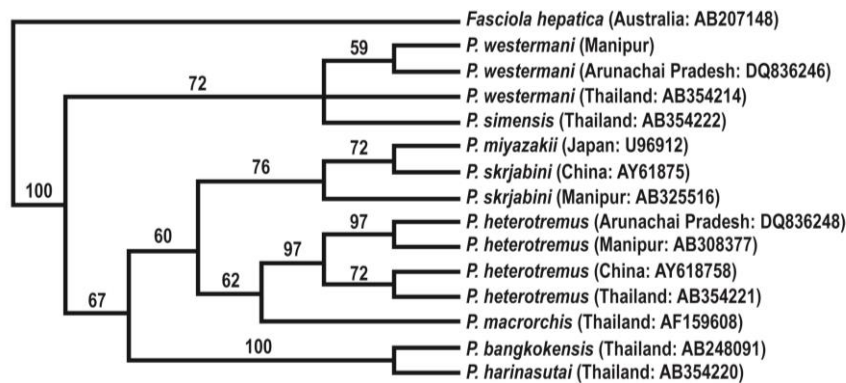


Figure 14 Phylogenetic relationships of *Paragonimus* species in India and other countries based on the ITS2 sequence data. Numbers on branches indicate bootstrap values.

Source: Singh; Sugiyama; & Rangsiruji. (2012). *Paragonimus* & Paragonimiasis in India. *Indian Journal of Medical Research*. 136(2): 195.

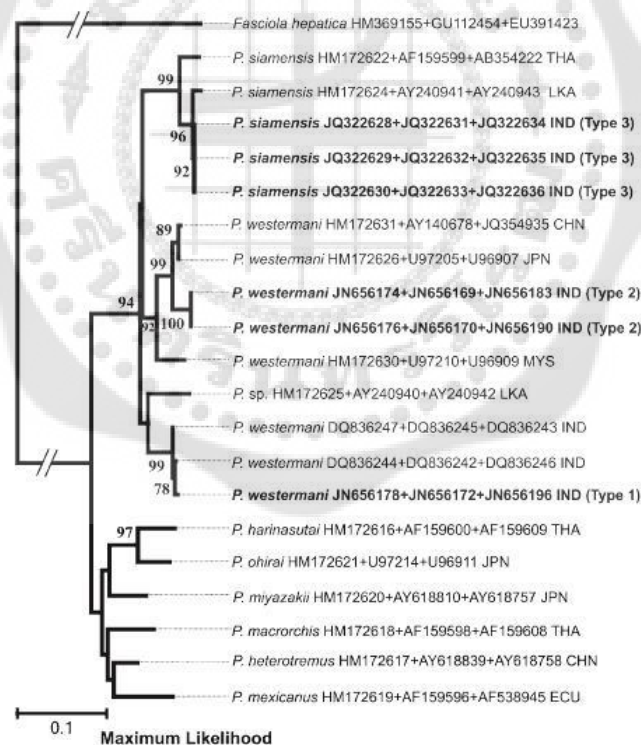


Figure 15 Phylogenetic tree of *Paragonimus* species based on the ITS2 sequence data. Numbers on branches indicate bootstrap values.

Source: Devi; *et al.* (2013). Presence of Three Distinct Genotypes within the *P. westermani* Complex in Northeastern India. *Parasitology*. 140(1): 85.

In addition to the studies of molecular phylogenetics, haplotype network analyses are also essential. Haplotype networks show population sizes of different genetic types and steps of mutation. According to Chaichanasak; *et al.* (2012), the haplotype network based on the NADH dehydrogenase subunit 1 (ND1) sequences of *Fasciola* species yielded 30 haplotypes of the specimens from Thailand (Figure 16). These haplotypes were separated into two groups based on spermatogenesis. Of 30 haplotypes, only one (TH1) was aspermic, and it was therefore, assumed as a common aspermic ancestor. The network analysis indicated that the ancestral haplotype of the spermic parasite might be TH19 because basic branches of other haplotypes were connected with this haplotype.

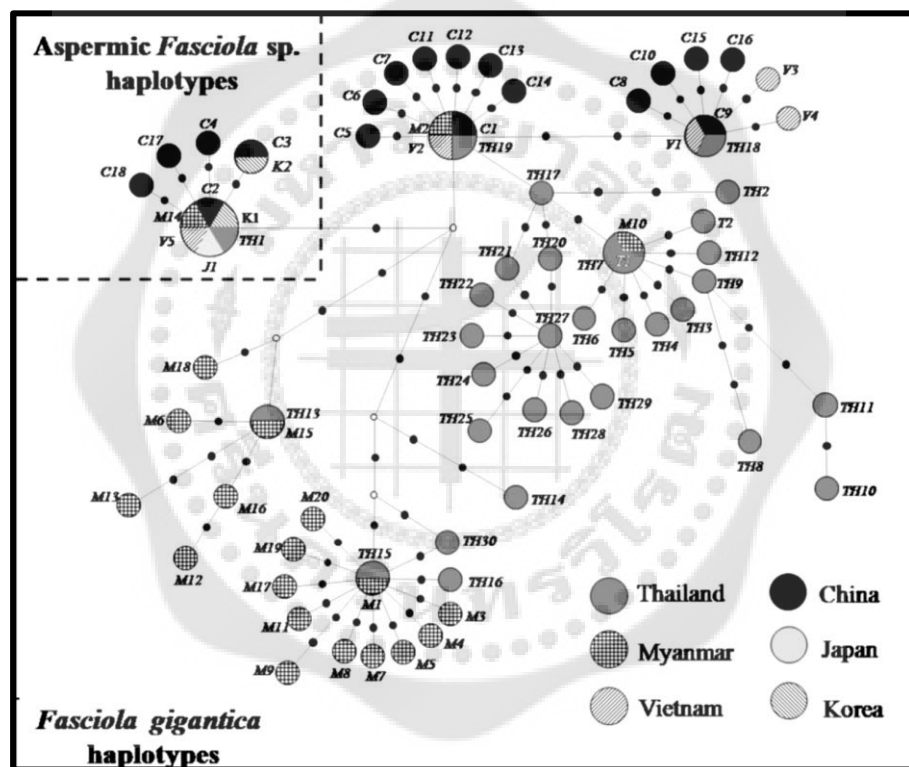


Figure 16 Median-joining network for ND1 haplotypes of *Fasciola* species from Thailand and other countries. The lines in the haplotype networks are the branches linking between the haplotype groups and black dots on these lines are steps of mutation. White dots are missing links of haplotypes.

Source: Chaichanasak; *et al.* (2012). Identification of *Fasciola* Flukes in Thailand based on Their Spermatogenesis and Nuclear Ribosomal DNA, and Their Intraspecific Relationships based on Mitochondrial DNA. *Parasitology International*. 61: 547.

In 2013, Mladineo; *et al.* studied a monogenean parasite (*Furnestinia echeneis*) that transferred between wild and reared fish populations. The results in Figure 17 showed a COI-based network with most of the unique haplotypes closely related to the central haplotype (H1). This central and common haplotype was likely an ancestor, from which one or a few stepwise nucleotide substitutions could explain all other independent haplotypes. Although haplotype diversity was high, low nucleotide diversity values indicated only small differences between the haplotypes.

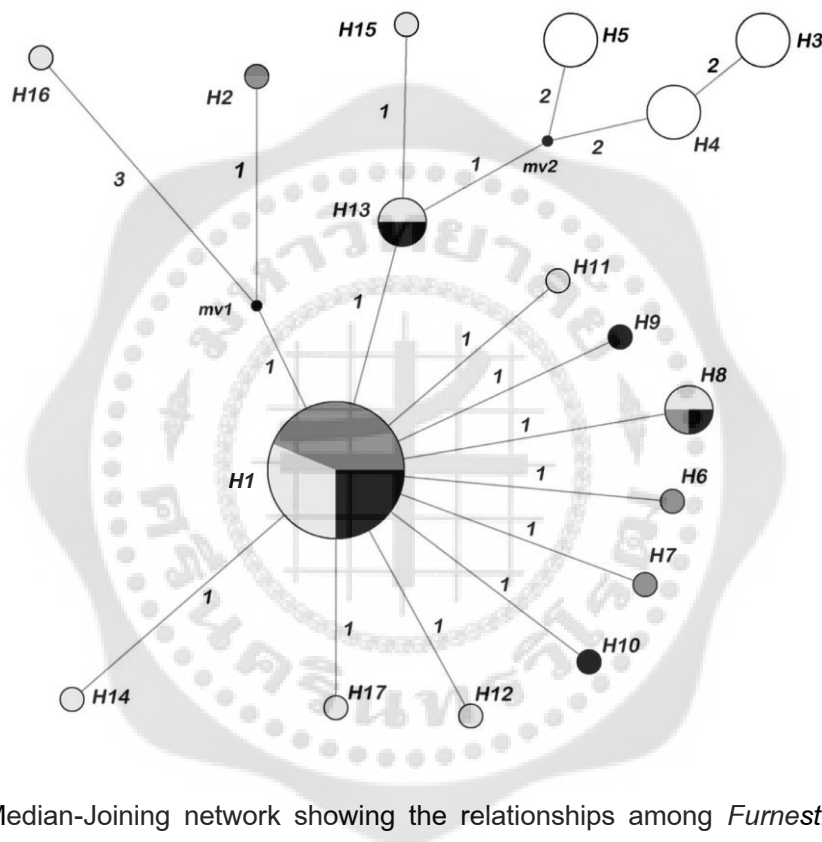


Figure 17 Median-Joining network showing the relationships among *Furnestinia echeneis* haplotypes defined by COI sequence variation. The number on each branch refers to the number of base substitution among haplotypes. The sizes of circles are proportional to the frequency of individual haplotypes. Four colors indicate different fish population as follows: black (Adriatic wild sea bream), grey (Adriatic cage sea bream adults), white-grey (Adriatic cage sea bream fingerlings) and white (western Mediterranean wild sea bream).

Source: Mladineo; *et al.* (2013). Morphological Plasticity and Phylogeny in a Monogenean Parasite Transferring between Wild and Reared Fish Populations. *Plos One*. 8(4): 7.

Recently, Sun; *et al.* (2013) described the genetic structure of *Clonorchis sinensis*, a human liver fluke, in China using a multilocus haplotype analysis. In Figure 18, the haplotype network was constructed based on the ITS1 sequences. All haplotypes were separated into three clusters; ancestral, central China and Henan. The super linkage populations defined the haplotypes with the highest ancestral probability. These results also indicated that the genetic expansion of the populations of *C. sinensis* most likely originated from central China.

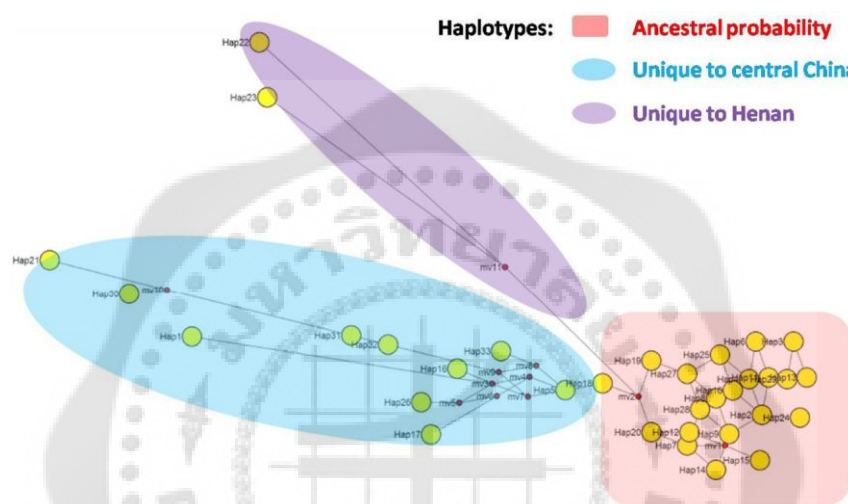


Figure 18 Haplotype network of *Clonorchis sinensis* based on the ITS1 sequence data.

The yellow circles represent haplotypes. The red dots are missing links between haplotypes.

Source: Sun; *et al.* (2013). Low Divergence of *C. sinensis* in China based on Multilocus Analysis. *Plos One*. 8(6): 10.

Brusentsov; *et al.* (2013) investigated the genetic diversity in *Opisthorchis felineus* (Eurasian liver fluke) populations from eastern Europe, northern Asia and central Asia. The haplotype analyses were based on the COI, COIII and ITS1 sequence data. All haplotype networks displayed star-like structures (Figure 19), each contained one major haplotype and many rare variants. This is the first population genetic analysis of *O. felineus* which revealed its low genetic diversity across the whole territory studied. In terms of degree of variability, COIII showed the highest genetic diversity, followed by COI and ITS1.

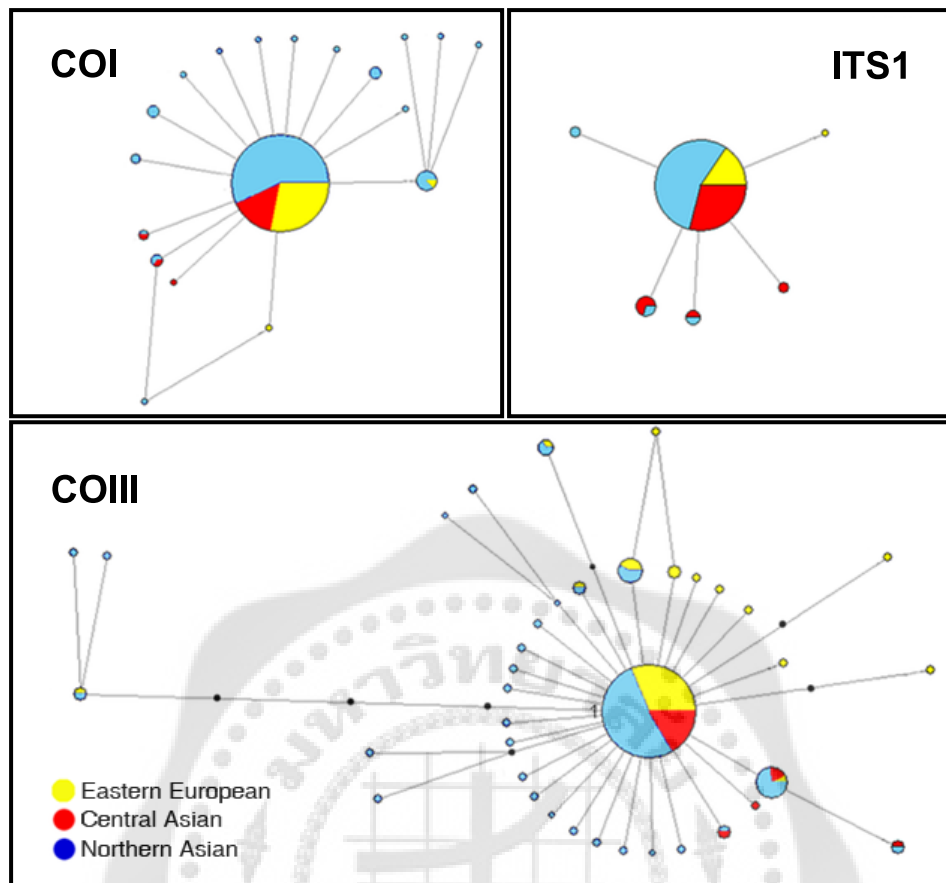


Figure 19 Haplotype networks of *Opisthorchis felineus* based on the COI, COIII and ITS1 sequence data. The sizes of haplotypes are proportional to the number of samples. Black dots indicate un-sampled or extinct haplotypes.

Source: Modified from Brusentsov; *et al.* (2013). Low Genetic Diversity in Wide-Spread Eurasian Liver Fluke *O. felineus* Suggests Special Demographic History of This Trematode Species. *Plos One*. 8(4): 5.

Doanh; *et al.* (2015) conducted a study on molecular phylogenetics and haplotype network of *Paragonimus heterotremus* complex (Figure20). The analyses involved *P. heterotremus* and *P. pseudoheterotremus* isolates from different countries in Asia, including China, Vietnam, India and Thailand. The haplotype network showed that all *P. heterotremus* and *P. pseudoheterotremus* could be separated into three groups; group A: *P. heterotremus* from India, group B: *P. heterotremus* from China, Thailand and Vietnam, and group C: *P. pseudoheterotremus* from Thailand. *Paragonimus heterotremus* from India was suggested as the root of the clusters of haplotype network.

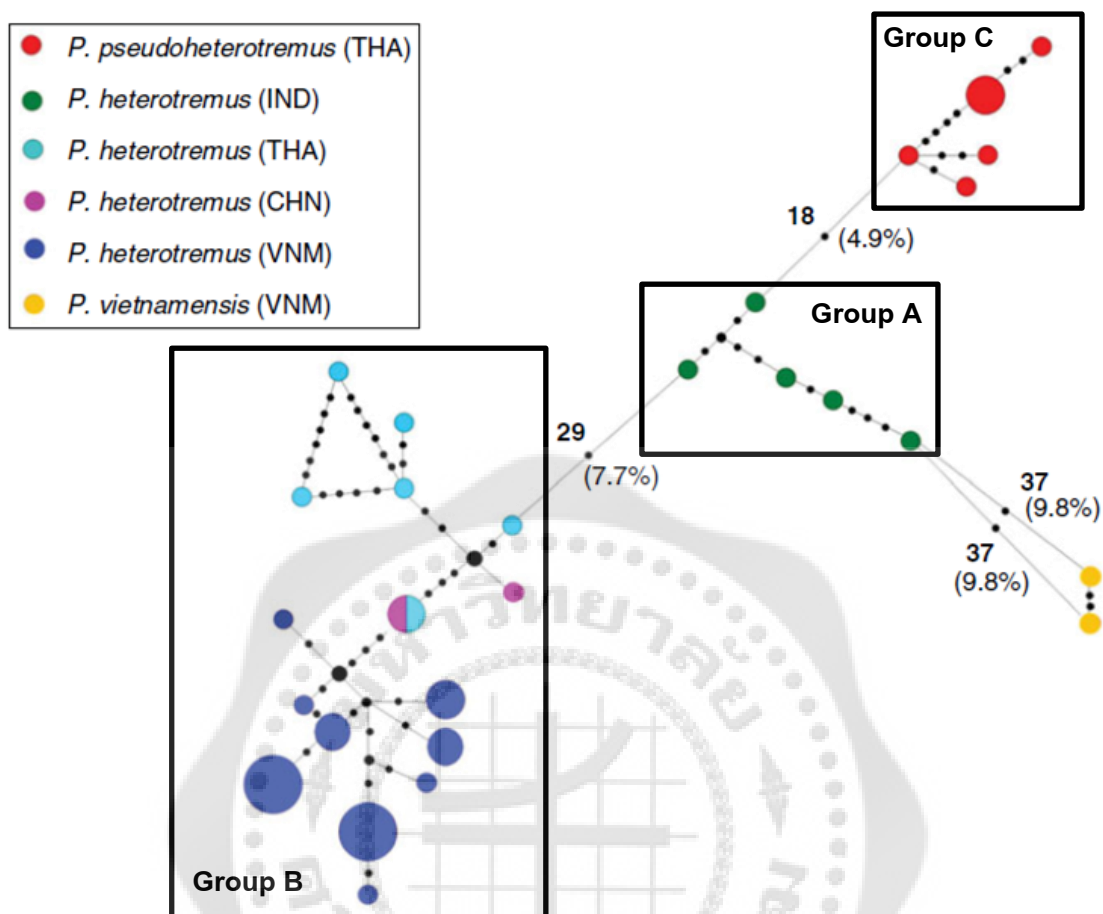


Figure 20 Median-joining network of *P. heterotremus* species complex determined by the COI sequences. The percentage on each branch reveals genetic distance. Black dots are steps of mutation.

Source: Doanh; *et al.* (2015). Metacercarial Polymorphism and Genetic Variation of *Paragonimus heterotremus* (Digenea: Paragonimidae), and a Re-appraisal of the Taxonomic Status of *Paragonimus pseudoheterotremus*. *Journal of Helminthology*. 89(2): 187.

CHAPTER THREE

MATERIALS AND METHODS

Materials

Chemicals and reagents

2-Log DNA Ladder
6X gel loading dye blue
Agarose
Boric acid
DNeasy Blood and Tissue Kit
dNTP mix
Ethanol
Ethidium bromide
Ethylenediamine tetraacetic acid (EDTA)
Hydrochloric acid (HCl)
Isopropanol
Nuclease-free water
Proteinase K
QIAquick Gel Extraction Kit
QIAquick PCR Purification Kit
RNase A
Sodium acetate ($C_2H_3NaO_2$)
Sodium chloride (NaCl)
TopTaq DNA polymerase
Tris base

Brands

New England BioLabs
New England BioLabs
Vivantis
Merck
Qiagen
New England BioLabs
Merck
Bio-Rad
Sigma
Merck
Merck
Qiagen
Qiagen
Qiagen
Qiagen
Carlo Erba
Univar
Qiagen
Research Organics

Laboratory instruments

-20°C freezer
Force 1418 microcentrifuge
Gel electrophoresis apparatus
Hybridization water bath
Mastercycler gradient 5331
Photodocumentation system DP-001.FDC

Brands

Panasonic
Select BioProducts
Bio-Rad
New Brunswick Scientific
Eppendorf
Vilber Lourmat

Parasite specimens

The parasite used in this study was *P. westermani* at the adult stage. The specimens were obtained from Dr. Hiromu Sugiyama (Department of Parasitology, National Institute of Infectious Diseases, Japan) under a joint research collaboration. Collecting sites and details of the specimens were shown in Figure 21 and Table 1.



Figure 21 Collecting sites of *P. westermani*.

Table 1 Localities and numbers of *P. westermani* specimens used under study.

Locality of specimen		ITS2	COI	No. of specimen
1. China	1.1 Erlongdu	*	*	5
	1.2 Guafangzi	*	*	5
	1.3 Minqing	*	*	4
	1.4 Taipingshao	*	*	5
	1.5 Wudaolingzi	*	*	5
	1.6 Xiguhetai	*	*	5
	1.7 Xigutai	*	*	5
2. India	2.1 Arunachal Pradesh	DQ836246	DQ836245	1
	2.2 Meghalaya	DQ836243	DQ836242	1
3. Japan	Mie	*	*	4
4. Korea		*	*	5
5. Malaysia	Sungai Wa	U96909	U97210	1
6. the Philippines	Leyte	*	*	4
7. Sri Lanka		AY240942	AY240940	1
8. Taiwan	Karapai	U96908	AY140673	1
9. Thailand	9.1 Saraburi	*	*	7
	9.2 Surat Thani	*	*	4
	9.3 Surat Thani	AB354218	AB354225	1
	(Thailand second type)	*	*	5
10. Vietnam	Quangtri	FJ434982	FJ434988	1
Total				70

Note: * = Sequences obtained from the specimens used in this study.

Methods

1. DNA extraction, amplification and gel electrophoresis

1.1 DNA extraction

Genomic DNA was extracted from *P. westermani* adult worms individually, using DNeasy Blood and Tissue Kit (Qiagen). DNA extraction method followed the instruction

manual provided by the company with slight modifications. Each adult fluke was placed in a 2 mL tube, then 180 µL of lysis buffer and 20 µL of Proteinase K were added. It was incubated at 56°C in a water bath until the tissue was completely lysed. Then 4 µL of RNase A was added, mixed and incubated for 2 min at room temperature, followed by 200 µL of buffer AL and 200 µL of ethanol. The mixture was transferred to a DNeasy Mini spin column placed in a 2 mL tube, centrifuged for 1 min at 5,225 *xg* and the flow-through was discarded. The column was placed in a new 2 mL tube and 500 µL of buffer AW1 was added and centrifuged for 1 min at 5,225 *xg*. The column was washed again with 500 µL of buffer AW2 and centrifuged for 3 min at 13,800 *xg*. The genomic DNA was eluted by adding 70 µL of buffer AE directly on to the DNeasy membrane. It was incubated at room temperature for 1 min and then centrifuged for 1 min at 5,225 *xg*. The DNA was kept at -20°C until use.

1.2 DNA amplification

Each sample for Polymerase Chain Reaction (PCR) with a volume of 25 µL was prepared in a 0.2 mL microcentrifuge tube. Each reaction included 5 µL of 5X Q-solution, 2.5 µL of 10X TopTaq buffer, 2.2 µL each of forward and reverse primers for ITS2 or COI, 1.5 µL of MgCl₂, 1.5 µL of dNTP mix, 0.2 µL of TopTaq DNA polymerase, 1 µL of DNA template and 8.9 µL of nuclease-free water. Details of the primers used for DNA amplification were shown in Table 2. A PCR amplification profile consisted of the following steps: preheating for 1 min at 98°C, 30 cycles of denaturation for 1 min at 98°C, annealing for 1 min at 50°C, extension for 1 min at 72°C and final extension for 2 min at 72°C.

Table 2 Details of the primers used for DNA amplification.

Primer	Primer sequence (5'- 3' orientation)	Amplified region	Source
3S (forward)	GGTACCGGTGGATCACTCGGCTCGTG	ITS2	Bowles; <i>et al.</i> 1995
A28 (reverse)	GGGATCCTGGTTAGTTTCTTTTCCTCCGC	ITS2	Blair; <i>et al.</i> 1997
JB3 (forward)	TTTTTTGGGCATCCTGAGGTTTAT	COI	Bowles; <i>et al.</i> 1995
JB4.5 (reverse)	TAAAGAAAGAACATAATGAAAATG	COI	Bowles; <i>et al.</i> 1995

1.3 Gel electrophoresis

PCR products were analyzed by gel electrophoresis on 1% (w/v) agarose gel in 1X TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA). When the gel was set, 5 μ L of 2-Log DNA Ladder was added to the first lane, while other lanes contained 5 μ L of individual PCR products which were mixed with 1 μ L of loading dye. The gel was stained with ethidium bromide, visualized and photographed under ultraviolet light.

2. Purification of PCR products

2.1 Using QIAquick PCR Purification Kit

The procedure was slightly modified from the instruction manual provided by the company. Each PCR product with a volume of 20 μ L which contained a single band was transferred to a 1.5 mL tube. Then, 250 μ L of buffer PB was added, and centrifuged for 30 sec at 5,225 g . The PCR product was transferred to a QIAquick spin column, incubated for 1 min at room temperature and centrifuged for 1 min at 13,800 g . The substance in the column was poured out, and 750 μ L of buffer PE was added. It was incubated for 5 min at room temperature and centrifuged for 1 min at 13,800 g . The flow-through was discarded and the column was centrifuged for 1 min at 13,800 g . The column was then placed in a clean 1.5 mL tube and 35 μ L of nuclease-free water was added to the center of spin column membrane. It was incubated for 2 min at room temperature and centrifuged for 1 min at 13,800 g . Each purified PCR product was examined by gel electrophoresis.

2.2 Using QIAquick Gel Extraction Kit

In the case of PCR products with multiple bands shown on the agarose gel, only required bands were excised, placed separately in 1.5 mL tubes and weighed. Three volumes of buffer QG were added to 1 volume of the gel and incubated at 50°C until the gel slice was completely dissolved. Then, 10 μ L of 3 M sodium acetate was added and mixed. A volume of isopropanol with an equal amount to the gel slice was also added to the sample and mixed. The mixture was transferred to a QIAquick spin column, incubated for 1 min at room temperature and centrifuged for 2 min. The flow-through was discarded and the column was placed back in the same collection tube. Buffer QG with a volume of 500 μ L was added to the column, incubated for 1 min and centrifuged for 1 min. To wash the column, 750 μ L of buffer PE was added, incubated for 5 min and centrifuged for

1 min. The flow-through was discarded and the column was centrifuged for 1 min and placed in a clean 1.5 mL tube. Nuclease-free water with a volume of 35 μ L was added to the center of spin column membrane. The column was incubated for 1 min at room temperature and centrifuged for 1 min. All centrifugation steps were carried out at 13,800 $\times g$. The products were visualized by gel electrophoresis.

3. DNA sequencing

All PCR products containing the ITS2 and COI regions and their respective primers were sent to the Macrogen, Inc. in South Korea for DNA sequencing.

4. Phylogenetic and haplotype network analyses

4.1 Sequence alignment

A biological classification involves grouping of organisms with the same characters. When using the genetic data, nucleotides are considered as characters for grouping. In this study, nucleotide sequences of the ITS2 and COI regions were obtained from the specimens as well as from the GenBank database and put in a text file with FastA format. These sequences were aligned using Clustal W in BioEdit program (Larkin; *et al.* 2007) in order to identify regions with similarities that could be related with evolutionary relationships between the aligned sequences. The alignments were exported in Nexus and Phylip formats. The information derived from the alignments was used to reconstruct phylogenetic trees.

4.2 Phylogenetic analyses

Different approaches, including distance-based and character-based methods are usually employed to infer phylogenetic relationships. These methods include neighbor joining (NJ), maximum parsimony (MP) and maximum likelihood (ML). In this study, only the character-based methods (MP and ML) were used in PAUP* version 4.0b program (Swofford. 1998) and RAxML software (Stamatakis. 2014) for phylogenetic tree reconstruction of the ITS2 and COI regions. To confirm the robustness of each clade within the trees obtained, bootstrap analyses with 1,000 replicates were carried out.

4.2.1 Maximum parsimony (MP)

Maximum parsimony (or parsimony) is evaluated based on changes of character states. It is used to find a phylogenetic tree that explains the data, with as few evolutionary changes as possible. In this study, aligned sequence data in Nexus format were employed in PAUP* version 4.0b program and the commands used for the MP analysis are shown below.

- set criterion=parsimony;
- outgroup *Paragonimus westermani* (Sri Lanka);
- hsearch;
- showtrees;
- savetrees file=trees name.tre;
- contrees;
- bootstrap nreps=1000;

4.2.2 Maximum likelihood (ML)

Maximum likelihood uses explicit models of evolution (Figure 22) to calculate the likelihood or the probability of the data. It is used to find a phylogenetic tree that maximizes the probability of the genetic data given the tree. Several commands used in this study are given as follows:

- Using an internet browser to access <https://www.phylo.org>.
- A set of aligned sequence data in Phylip format was submitted.
- ML analysis was performed in the RAxML-HPC2 on XSEDE 8.1.11 on the Cipres Science Gateway server: <https://www.phylo.org/portal2/login!input.action> (Miller; *et al.*, 2010; Stamatakis. 2014), with 1,000 replicates for bootstrap analysis. The universal GTR-Gamma model was chosen for the analysis.
- Output data was obtained as RAxML_bipartitions.result and ML tree with branch lengths was viewed using Figtree program.

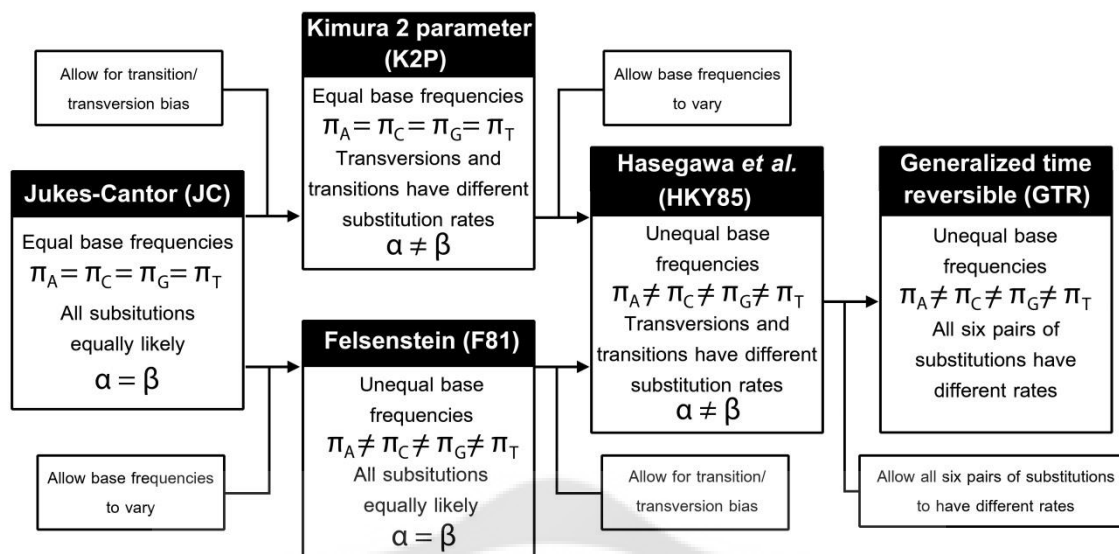


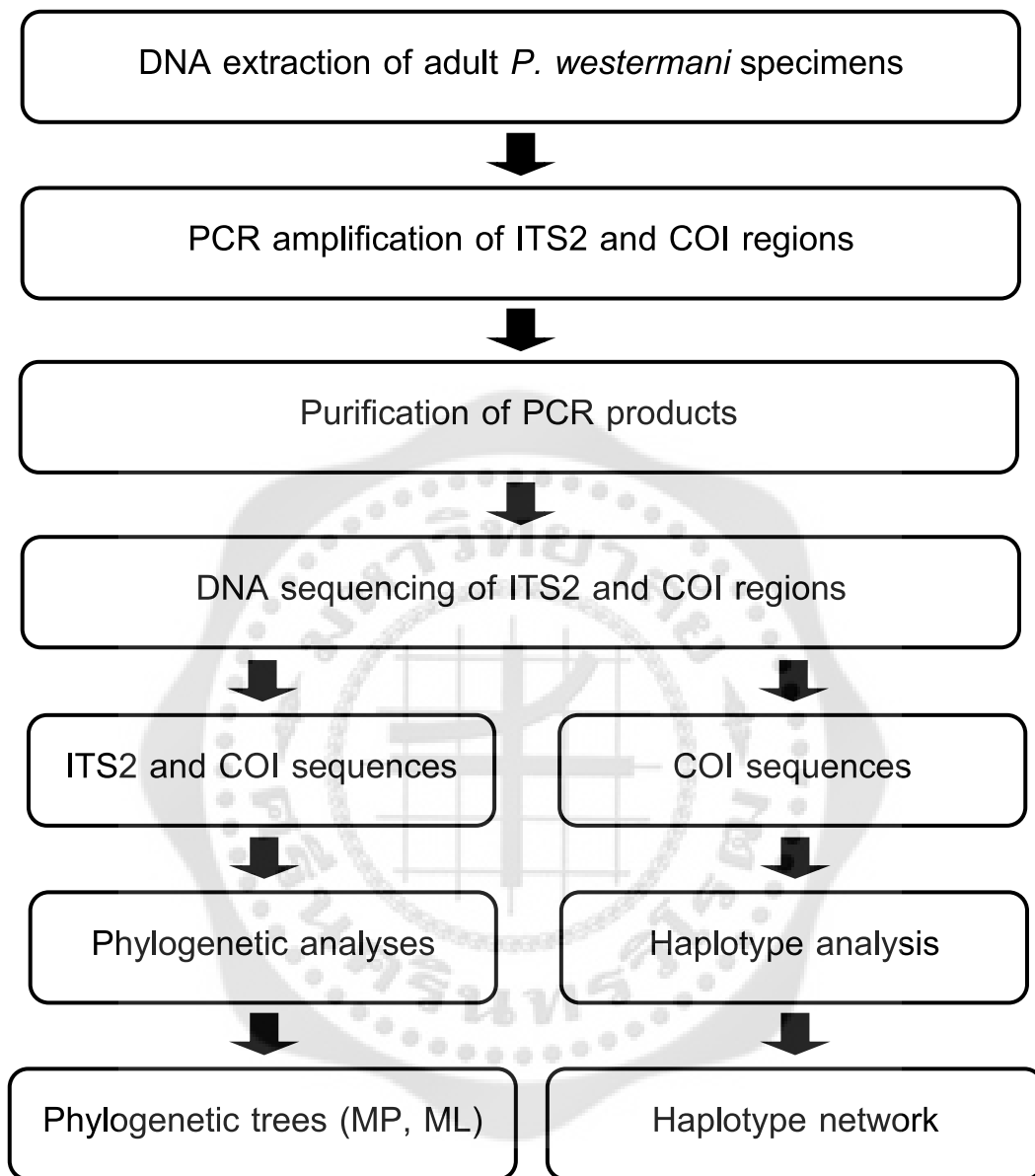
Figure 22 Models of evolution.

Source: Modified from Page, RDM; & Holmes, EC. (1998). *Molecular Evolution: a Phylogenetic Approach*. p. 153.

4.3 Haplotype network analysis

In the present study, a haplotype analysis based on the COI sequences was conducted using the TCS 1.21 program (Clement; Posada; & Crandall. 2000). This program is used for estimating homogeneous genetic data within a population. It is the parsimony method for connecting existing haplotypes to yield a network with certain mutational steps. In this study, Nexus file was converted to Phylip file in PAUP* version 4.0b program. A haplotype network was constructed by TCS version 1.21 program using Phylip file with default parameters.

An overview of this study.



CHAPTER FOUR

RESULTS

1. DNA extraction, amplification and gel electrophoresis

Sixty-three DNA samples were extracted from *P. westermani* adults and used for PCR amplification. The PCR products obtained for the ITS2 as well as COI regions are shown in Figure 23 with the sizes of ca. 500 base pairs (bp) and ca. 450 bp, respectively.

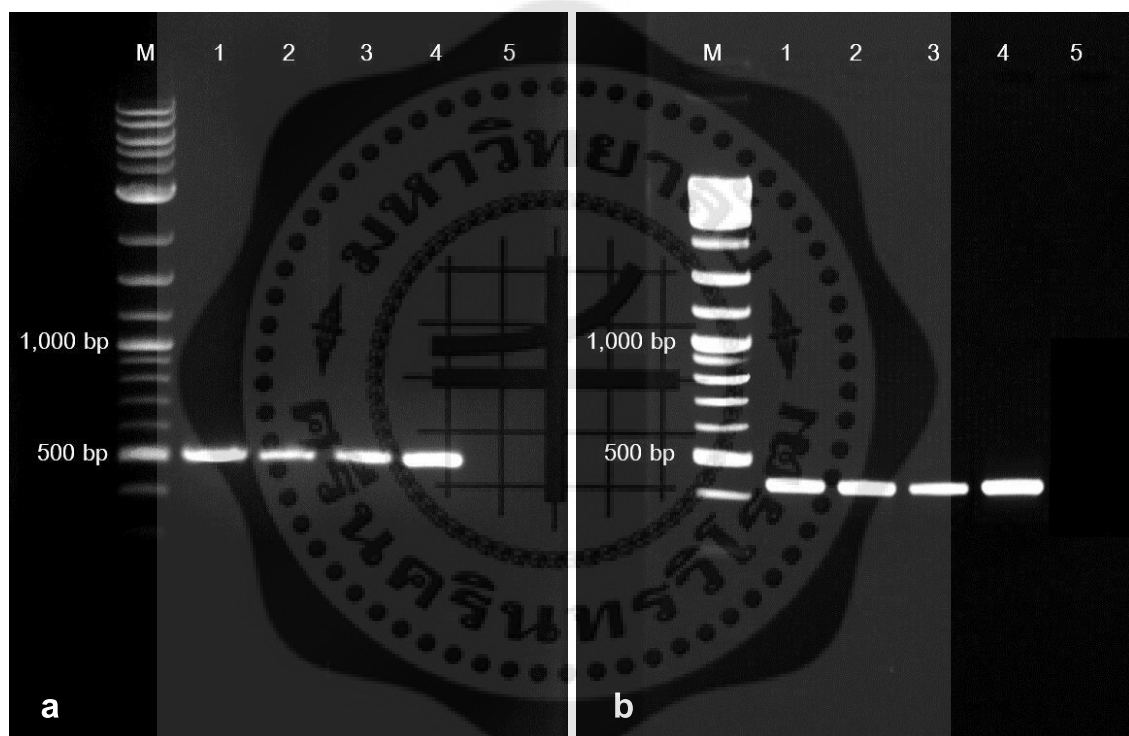


Figure 23 Lanes 1-4 are representative bands of the PCR products of *P. westermani*: (a) ITS2 region and (b) COI region. Lane 5 is a negative control and M denotes 2-Log DNA Ladder.

2. Sequence alignment, sequence characteristics and phylogenetic analyses of the ITS2 region

The total alignment of the ITS2 sequences of 70 specimens of *P. westermanni* was 360 bp in length (Figure 24). Of these 360 characters, 323 (89.72%) characters were constant, while 15 (4.17%) variable characters were parsimony-uninformative and 22 (6.11%) variable characters were parsimony-informative. The GC content of all sequences ranged from 54.70 to 57.22%. The ITS2 sequence characteristics are summarized in Table 3.

The phylogenetic trees of the ITS2 sequences were reconstructed using both the MP and ML methods. Based on the MP analysis, 8 most parsimonious trees were obtained with the tree length of 43 steps. The resulting consensus MP tree (Figure 25) revealed five major clades with moderate to high bootstrap support (BS). Clade I (BS = 97%) contained the samples from India. Clade II (BS = 98%) included the samples from the Philippines. Clade III (BS = 97%) contained the samples from central as well as southern Thailand. Clade IV (BS = 98%) comprised the members from southern Thailand (second type). Clade V (BS = 60%) contained the sample from Vietnam and the members mainly from East Asia, including China, Japan, Korea and Taiwan. In addition, the ML analysis also yielded five strongly supported clades (BS = 89-100%) (Figure 26). The tree topologies based on both MP and ML methods were identical.

Specimen	10	20	30	40	50
China (Erlongdu 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Erlongdu 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Erlongdu 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Erlongdu 4)	ATATTGCGGC	CACGGGTTAA	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Erlongdu 5)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Guafangzi 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Guafangzi 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Guafangzi 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Guafangzi 4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Guafangzi 5)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Minqing 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Minqing 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Minqing 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Minqing 4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Taipingshao 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Taipingshao 2)	ATATTGCGGC	CACGGGTTAA	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Taipingshao 3)	ATATTGCGGC	CACGGGTTAA	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Taipingshao 4)	ATATTGCGGC	CACGGGTTAA	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Taipingshao 5)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Wudaolingzi 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Wudaolingzi 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Wudaolingzi 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Wudaolingzi 4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Wudaolingzi 5)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Xiguhetai 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Xiguhetai 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Xiguhetai 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Xiguhetai 4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Xiguhetai 5)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Xigutai 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Xigutai 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Xigutai 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Xigutai 4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
China (Xigutai 5)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Japan (Mie 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Japan (Mie 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Japan (Mie 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Japan (Mie 4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Korea (1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Korea (2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Korea (3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Korea (4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Korea (5)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Philippines (Leyte 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Philippines (Leyte 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Philippines (Leyte 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Philippines (Leyte 4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Saraburi 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Saraburi 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Saraburi 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Saraburi 4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Saraburi 5)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Saraburi 6)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Saraburi 7)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Surat Thani 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Surat Thani 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Surat Thani 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (Surat Thani 4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (second type 1)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (second type 2)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (second type 3)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (second type 4)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (second type 5)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Thailand (second type 6)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Vietnam (Quangtri)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Taiwan (Karapai)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Malaysia (Sungai Wa)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
India (Meghalaya)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
India (Arunachal Pradesh)	ATATTGCGGC	CACGGGTTAG	CCTGTGGCCA	CGCCTGTCCG	AGGGTCGGCT
Sri Lanka	-----	-----	-----	-----	-----

Figure 24 Sequence data matrix of the aligned ITS2 region of nuclear ribosomal DNA for 70 specimens of *P. westermanni*. Hyphens denote alignment gaps and numbers indicate alignment length (bp).

Specimen	60	70	80	90	100
China (Erlongdu 1)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Erlongdu 2)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Erlongdu 3)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Erlongdu 4)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Erlongdu 5)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Guafangzi 1)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Guafangzi 2)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Guafangzi 3)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Guafangzi 4)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Guafangzi 5)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Minqing 1)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Minqing 2)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Minqing 3)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Minqing 4)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Taipingshao 1)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Taipingshao 2)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Taipingshao 3)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Taipingshao 4)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Taipingshao 5)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Wudaolingzi 1)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Wudaolingzi 2)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Wudaolingzi 3)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Wudaolingzi 4)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Wudaolingzi 5)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Xiguhetai 1)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Xiguhetai 2)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Xiguhetai 3)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Xiguhetai 4)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Xiguhetai 5)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Xigutai 1)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Xigutai 2)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Xigutai 3)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Xigutai 4)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
China (Xigutai 5)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Japan (Mie 1)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Japan (Mie 2)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Japan (Mie 3)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Japan (Mie 4)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Korea (1)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Korea (2)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Korea (3)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Korea (4)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Korea (5)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Philippines (Leyte 1)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Philippines (Leyte 2)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Philippines (Leyte 3)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Philippines (Leyte 4)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Saraburi 1)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Saraburi 2)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Saraburi 3)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Saraburi 4)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Saraburi 5)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Saraburi 6)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Saraburi 7)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Surat Thani 1)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Surat Thani 2)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Surat Thani 3)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (Surat Thani 4)	TATAAACCAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (second type 1)	TATGAACAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (second type 2)	TATGAACAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (second type 3)	TATGAACAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (second type 4)	TATGAACAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (second type 5)	TATGAACAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Thailand (second type 6)	TATGAATAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Vietnam (Quangtri)	TATAAACAAAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGCT	TTGCCAGCTG
Taiwan (Karapai)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Malaysia (Sungai Wa)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
India (Meghalaya)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
India (Arunachal Pradesh)	TATAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG
Sri Lanka	--TAAACTAT	CGCGACGCCC	AAAAAGTCGC	GGCTTGGGTT	TTGCCAGCTG

Figure 24 (continued).

Specimen	110	120	130	140	150
China (Erlongdu 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Erlongdu 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Erlongdu 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Erlongdu 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Erlongdu 5)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Guafangzi 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Guafangzi 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Guafangzi 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Guafangzi 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Guafangzi 5)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Minqing 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Minqing 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Minqing 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Minqing 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Taipingshao 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Taipingshao 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Taipingshao 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Taipingshao 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Taipingshao 5)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Wudaolingzi 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Wudaolingzi 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Wudaolingzi 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Wudaolingzi 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Wudaolingzi 5)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Xiguhetai 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Xiguhetai 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Xiguhetai 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Xiguhetai 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Xiguhetai 5)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Xigutai 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Xigutai 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Xigutai 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Xigutai 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
China (Xigutai 5)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Japan (Mie 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Japan (Mie 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Japan (Mie 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Japan (Mie 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Korea (1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Korea (2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Korea (3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Korea (4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Korea (5)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Philippines (Leyte 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCAATGG
Philippines (Leyte 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCAATGG
Philippines (Leyte 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCAATGG
Philippines (Leyte 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCAATGG
Thailand (Saraburi 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (Saraburi 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (Saraburi 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (Saraburi 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (Saraburi 5)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (Saraburi 6)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (Saraburi 7)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (Surat Thani 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (Surat Thani 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (Surat Thani 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (Surat Thani 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Thailand (second type 1)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Thailand (second type 2)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Thailand (second type 3)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Thailand (second type 4)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Thailand (second type 5)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Thailand (second type 6)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Vietnam (Quangtri)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Taiwan (Karapai)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTGTGG
Malaysia (Sungai Wa)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
India (Meghalaya)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
India (Arunachal Pradesh)	GCGTGATCTC	CCCAATCTGG	TCTTGTGCCT	GTGGGGTGCC	AGATCTATGG
Sri Lanka	GCGTGATTTT	CCCAACCTGG	TCCTGTGCCA	GTGGGGTGCC	AGATCTATGG

Figure 24 (continued).

Specimen	160	170	180	190	200
China (Erlongdu 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Erlongdu 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Erlongdu 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Erlongdu 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Erlongdu 5)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Guafangzi 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Guafangzi 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Guafangzi 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Guafangzi 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Guafangzi 5)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Minqing 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Minqing 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Minqing 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Minqing 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Taipingshao 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Taipingshao 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Taipingshao 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Taipingshao 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Taipingshao 5)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Wudaolingzi 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Wudaolingzi 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Wudaolingzi 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Wudaolingzi 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Wudaolingzi 5)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Xiguhetai 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Xiguhetai 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Xiguhetai 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Xiguhetai 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Xiguhetai 5)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Xigutai 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Xigutai 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Xigutai 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Xigutai 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
China (Xigutai 5)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Japan (Mie 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Japan (Mie 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Japan (Mie 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Japan (Mie 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Korea (1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Korea (2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Korea (3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Korea (4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Korea (5)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Philippines (Leyte 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Philippines (Leyte 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Philippines (Leyte 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Philippines (Leyte 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Saraburi 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Saraburi 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Saraburi 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Saraburi 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Saraburi 5)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Saraburi 6)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Saraburi 7)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Surat Thani 1)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Surat Thani 2)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Surat Thani 3)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (Surat Thani 4)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (second type 1)	CGTCTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (second type 2)	CGTCTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (second type 3)	CGTCTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (second type 4)	CGTCTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (second type 5)	CGTCTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Thailand (second type 6)	CGTCTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Vietnam (Quangtri)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Taiwan (Karapai)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Malaysia (Sungai Wa)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
India (Meghalaya)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
India (Arunachal Pradesh)	CGTTTCCCTA	ACATACTCGG	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA
Sri Lanka	CGTTTCCCTA	ACATGCTCGT	GCGCACCAC	GTTGCGGCTG	AAAGCCTTGA

Figure 24 (continued).

Specimen	210	220	230	240	250
China (Erlongdu 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Erlongdu 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Erlongdu 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Erlongdu 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Erlongdu 5)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Guafangzi 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Guafangzi 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Guafangzi 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Guafangzi 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Guafangzi 5)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Minqing 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Minqing 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Minqing 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Minqing 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Taipingshao 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Taipingshao 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Taipingshao 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Taipingshao 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Taipingshao 5)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Wudaolingzi 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Wudaolingzi 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Wudaolingzi 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Wudaolingzi 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Wudaolingzi 5)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Xiguhetai 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Xiguhetai 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Xiguhetai 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Xiguhetai 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Xiguhetai 5)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Xigutai 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Xigutai 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Xigutai 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Xigutai 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
China (Xigutai 5)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Japan (Mie 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Japan (Mie 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Japan (Mie 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Japan (Mie 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Korea (1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Korea (2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Korea (3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Korea (4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Korea (5)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Philippines (Leyte 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Philippines (Leyte 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Philippines (Leyte 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Philippines (Leyte 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Saraburi 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Saraburi 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Saraburi 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Saraburi 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Saraburi 5)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Saraburi 6)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Saraburi 7)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Surat Thani 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Surat Thani 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Surat Thani 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (Surat Thani 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Thailand (second type 1)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGATTGGTTT	ATGTGCGCGT
Thailand (second type 2)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGATTGGTTT	ATGTGCGCGT
Thailand (second type 3)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGATTGGTTT	ATGTGCGCGT
Thailand (second type 4)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGATTGGTTT	ATGTGCGCGT
Thailand (second type 5)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGATTGGTTT	ATGTGCGCGT
Thailand (second type 6)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGATTGGTTT	ATGTGCGCGT
Vietnam (Quangtri)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT
Taiwan (Karapai)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAA	TGAATGATTT	ATGTGCGCGT
Malaysia (Sungai Wa)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
India (Meghalaya)	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
India (Arunachal Pradesh)	CGGGGATGTG	GCNACGGAAT	CGTGGCTCAG	TAAATGATTT	ATGTGCGCGT
Sri Lanka	CGGGGATGTG	GCAACGGAAT	CGTGGCTCAG	TGAATGATTT	ATGTGCGCGT

Figure 24 (continued).

Specimen	260	270	280	290	300
China (Erlongdu 1)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Erlongdu 2)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Erlongdu 3)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Erlongdu 4)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Erlongdu 5)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Guafangzi 1)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Guafangzi 2)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Guafangzi 3)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Guafangzi 4)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Guafangzi 5)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Minqing 1)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Minqing 2)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Minqing 3)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Minqing 4)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Taipingshao 1)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Taipingshao 2)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Taipingshao 3)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Taipingshao 4)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Taipingshao 5)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Wudaolingzi 1)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Wudaolingzi 2)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Wudaolingzi 3)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Wudaolingzi 4)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Wudaolingzi 5)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Xiguhetai 1)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Xiguhetai 2)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Xiguhetai 3)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Xiguhetai 4)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Xiguhetai 5)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Xigutai 1)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Xigutai 2)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Xigutai 3)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Xigutai 4)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
China (Xigutai 5)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Japan (Mie 1)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Japan (Mie 2)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Japan (Mie 3)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Japan (Mie 4)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Korea (1)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Korea (2)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Korea (3)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Korea (4)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Korea (5)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Philippines (Leyte 1)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGTGTTT
Philippines (Leyte 2)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGTGTTT
Philippines (Leyte 3)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGTGTTT
Philippines (Leyte 4)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGTGTTT
Thailand (Saraburi 1)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (Saraburi 2)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (Saraburi 3)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (Saraburi 4)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (Saraburi 5)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (Saraburi 6)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (Saraburi 7)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (Surat Thani 1)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (Surat Thani 2)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (Surat Thani 3)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (Surat Thani 4)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (second type 1)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (second type 2)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (second type 3)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (second type 4)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (second type 5)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Thailand (second type 6)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Vietnam (Quangtri)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Taiwan (Karapai)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCTTTC
Malaysia (Sungai Wa)	TTCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGATC
India (Meghalaya)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
India (Arunachal Pradesh)	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC
Sri Lanka	TCCGCTGTCC	TGTCTTCATC	TGTGGTTTAT	GTTGCGCGTG	GTCTGCGTTC

Figure 24 (continued).

Specimen	310	320	330	340	350
China (Erlongdu 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Erlongdu 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Erlongdu 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Erlongdu 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Erlongdu 5)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Guafangzi 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Guafangzi 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Guafangzi 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Guafangzi 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Guafangzi 5)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Minqing 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Minqing 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Minqing 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Minqing 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Taipingshao 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Taipingshao 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Taipingshao 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Taipingshao 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Taipingshao 5)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Wudaolingzi 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Wudaolingzi 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Wudaolingzi 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Wudaolingzi 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Wudaolingzi 5)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Xiguhetai 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Xiguhetai 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Xiguhetai 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Xiguhetai 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Xiguhetai 5)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Xigutai 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Xigutai 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Xigutai 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Xigutai 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
China (Xigutai 5)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Japan (Mie 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Japan (Mie 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Japan (Mie 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Japan (Mie 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Korea (1)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Korea (2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Korea (3)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Korea (4)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Korea (5)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Philippines (Leyte 1)	GATGCTGACC	TACGTTTGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Philippines (Leyte 2)	GATGCTGACC	TACGTTTGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Philippines (Leyte 3)	GATGCTGACC	TACGTTTGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Philippines (Leyte 4)	GATGCTGACC	TACGTTTGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Thailand (Saraburi 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTC	CATTCTTCTG	ACCTCGGATT
Thailand (Saraburi 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTTCTG	ACCTCGGATT
Thailand (Saraburi 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTC	CATTCTTCTG	ACCTCGGATT
Thailand (Saraburi 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTC	CATTCTTCTG	ACCTCGGATT
Thailand (Saraburi 5)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTTCTG	ACCTCGGATT
Thailand (Saraburi 6)	GATGCTGACC	TACGTATGTG	CCATGTGGTC	CATTCTTCTG	ACCTCGGATT
Thailand (Saraburi 7)	GATGCTGACC	TACGTATGTG	CCATGTGGTC	CATTCTTCTG	ACCTCGGATT
Thailand (Surat Thani 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTC	CATTCTTCTG	ACCTCGGATT
Thailand (Surat Thani 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTC	CATTCTTCTG	ACCTCGGATT
Thailand (Surat Thani 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTC	CATTCTTCTG	ACCTCGGATT
Thailand (Surat Thani 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTC	CATTCTTCTG	ACCTCGGATT
Thailand (second type 1)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Thailand (second type 2)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Thailand (second type 3)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Thailand (second type 4)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Thailand (second type 5)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Thailand (second type 6)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Vietnam (Quangtri)	GATGCTGACC	TACGTTTGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Taiwan (Karapai)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
Malaysia (Sungai Wa)	GATGCTGACC	TACGTATGTG	CCATGTGGTT	CATTCTCCTG	ACCTCGGATC
India (Meghalaya)	GATGCTGACC	TATGTATGTG	CCATGTGGCT	CATTCTCCTG	ACCTCGGATC
India (Arunachal Pradesh)	GATGCTGACC	TATGTATGTG	CCATGTGGCT	CATTCTCCTG	ACCTCGGATC
Sri Lanka	GATGTTGACC	TACGTATGTG	CCATTTGGTT	CATTCTCCT-	-----

Figure 24 (continued).

Specimen	360
	I
China (Erlongdu 1)	AGACGTGAGT
China (Erlongdu 2)	AGACGTGAGT
China (Erlongdu 3)	AGACGTGAGT
China (Erlongdu 4)	AGACGTGAGT
China (Erlongdu 5)	AGACGTGAGT
China (Guafangzi 1)	AGACGTGAGT
China (Guafangzi 2)	AGACGTGAGT
China (Guafangzi 3)	AGACGTGAGT
China (Guafangzi 4)	AGACGTGAGT
China (Guafangzi 5)	AGACGTGAGT
China (Minqing 1)	AGACGTGAGT
China (Minqing 2)	AGACGTGAGT
China (Minqing 3)	AGACGTGAGT
China (Minqing 4)	AGACGTGAGT
China (Taipingshao 1)	AGACGTGAGT
China (Taipingshao 2)	AGACGTGAGT
China (Taipingshao 3)	AGACGTGAGT
China (Taipingshao 4)	AGACGTGAGT
China (Taipingshao 5)	AGACGTGAGT
China (Wudaolingzi 1)	AGACGTGAGT
China (Wudaolingzi 2)	AGACGTGAGT
China (Wudaolingzi 3)	AGACGTGAGT
China (Wudaolingzi 4)	AGACGTGAGT
China (Wudaolingzi 5)	AGACGTGAGT
China (Xiguhetai 1)	AGACGTGAGT
China (Xiguhetai 2)	AGACGTGAGT
China (Xiguhetai 3)	AGACGTGAGT
China (Xiguhetai 4)	AGACGTGAGT
China (Xiguhetai 5)	AGACGTGAGT
China (Xigutai 1)	AGACGTGAGT
China (Xigutai 2)	AGACGTGAGT
China (Xigutai 3)	AGACGTGAGT
China (Xigutai 4)	AGACGTGAGT
China (Xigutai 5)	AGACGTGAGT
Japan (Mie 1)	AGACGTGAGT
Japan (Mie 2)	AGACGTGAGT
Japan (Mie 3)	AGACGTGAGT
Japan (Mie 4)	AGACGTGAGT
Korea (1)	AGACGTGAGT
Korea (2)	AGACGTGAGT
Korea (3)	AGACGTGAGT
Korea (4)	AGACGTGAGT
Korea (5)	AGACGTGAGT
Philippines (Leyte 1)	AGACGTGAGT
Philippines (Leyte 2)	AGACGTGAGT
Philippines (Leyte 3)	AGACGTGAGT
Philippines (Leyte 4)	AGACGTGAGT
Thailand (Saraburi 1)	AGACGTGAGT
Thailand (Saraburi 2)	AGACGTGAGT
Thailand (Saraburi 3)	AGACGTGAGT
Thailand (Saraburi 4)	AGACGTGAGT
Thailand (Saraburi 5)	AGACGTGAGT
Thailand (Saraburi 6)	AGACGTGAGT
Thailand (Saraburi 7)	AGACGTGAGT
Thailand (Surat Thani 1)	AGACGTGAGT
Thailand (Surat Thani 2)	AGACGTGAGT
Thailand (Surat Thani 3)	AGACGTGAGT
Thailand (Surat Thani 4)	AGACGTGAGT
Thailand (second type 1)	AGACGTGAGT
Thailand (second type 2)	AGACGTGAGT
Thailand (second type 3)	AGACGTGAGT
Thailand (second type 4)	AGACGTGAGT
Thailand (second type 5)	AGACGTGAGT
Thailand (second type 6)	AGACGTGAGT
Vietnam (Quangtri)	AGACGTGAGT
Taiwan (Karapai)	AGACGTGAGT
Malaysia (Sungai Wa)	AGACGTGAGT
India (Meghalaya)	AGACGTGAGT
India (Arunachal Pradesh)	AGACGTGAGT
Sri Lanka	-----

Figure 24 (continued).

Table 3 Sequence characteristics of the ITS2 region of *P. westermanni* under study.

Sequence characteristics	ITS2
Length range (total) (bp)	287-360
Length mean (total) (bp)	358.96
Length ingroup (bp)	360
Length outgroup (bp)	287
Aligned length (bp)	360
GC content range (%)	54.70-57.22
GC content mean (%)	56.04
Number of constant sites (%)	323 (89.72%)
Number of variable sites (%)	37 (10.28%)
Number of informative sites (%)	22 (6.11%)
Number of uninformative sites (%)	15 (4.17%)
Transition/transversion ratio (ts/tv ratio)	2.18
Heterogeneity rate (α)	0.36

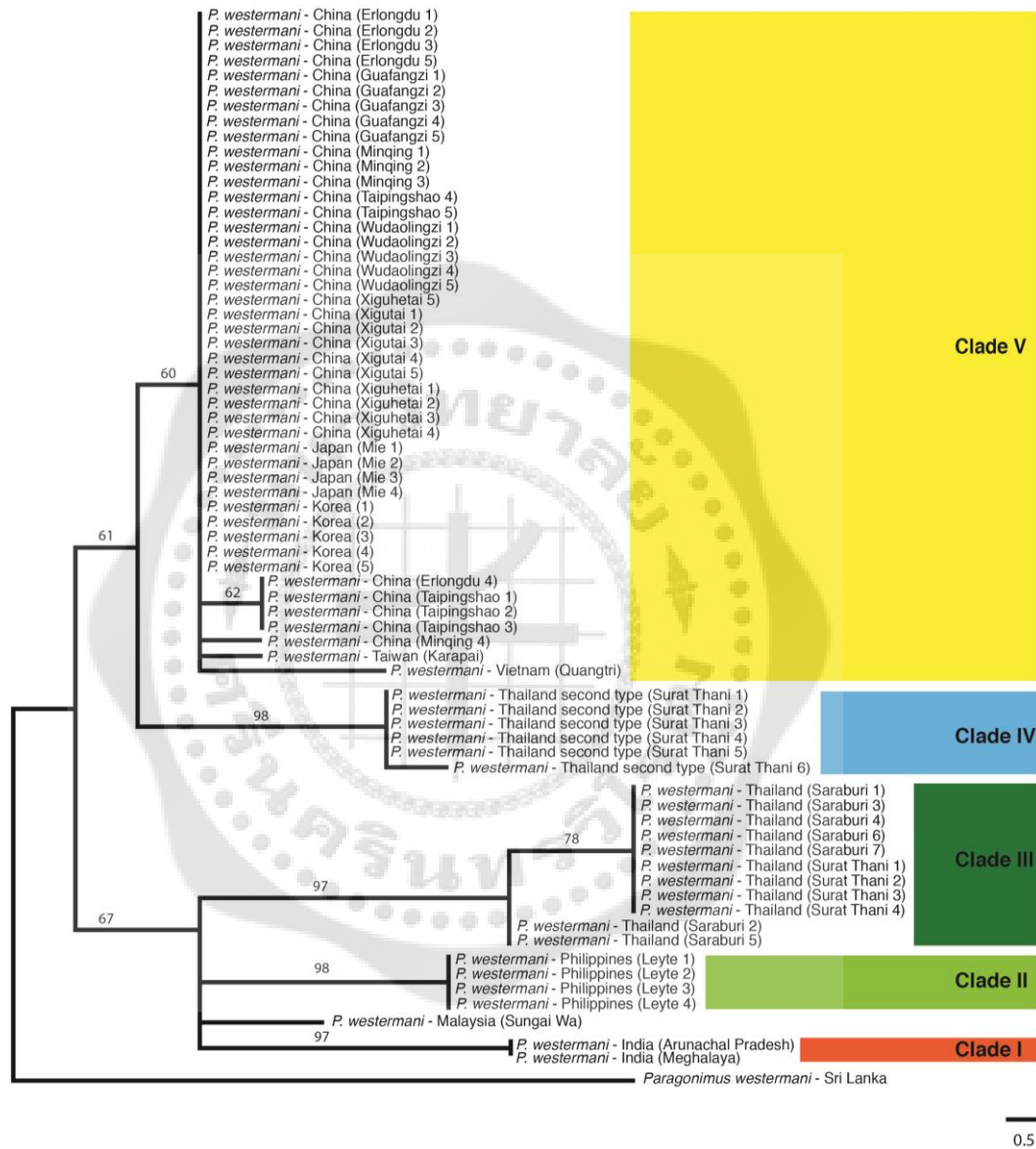


Figure 25 Strict consensus tree depicting intraspecific relationships of *P. westermanni* based on ITS2 sequence data and reconstructed using MP analysis. Bootstrap values of 1,000 replicates are given in numbers above branches.

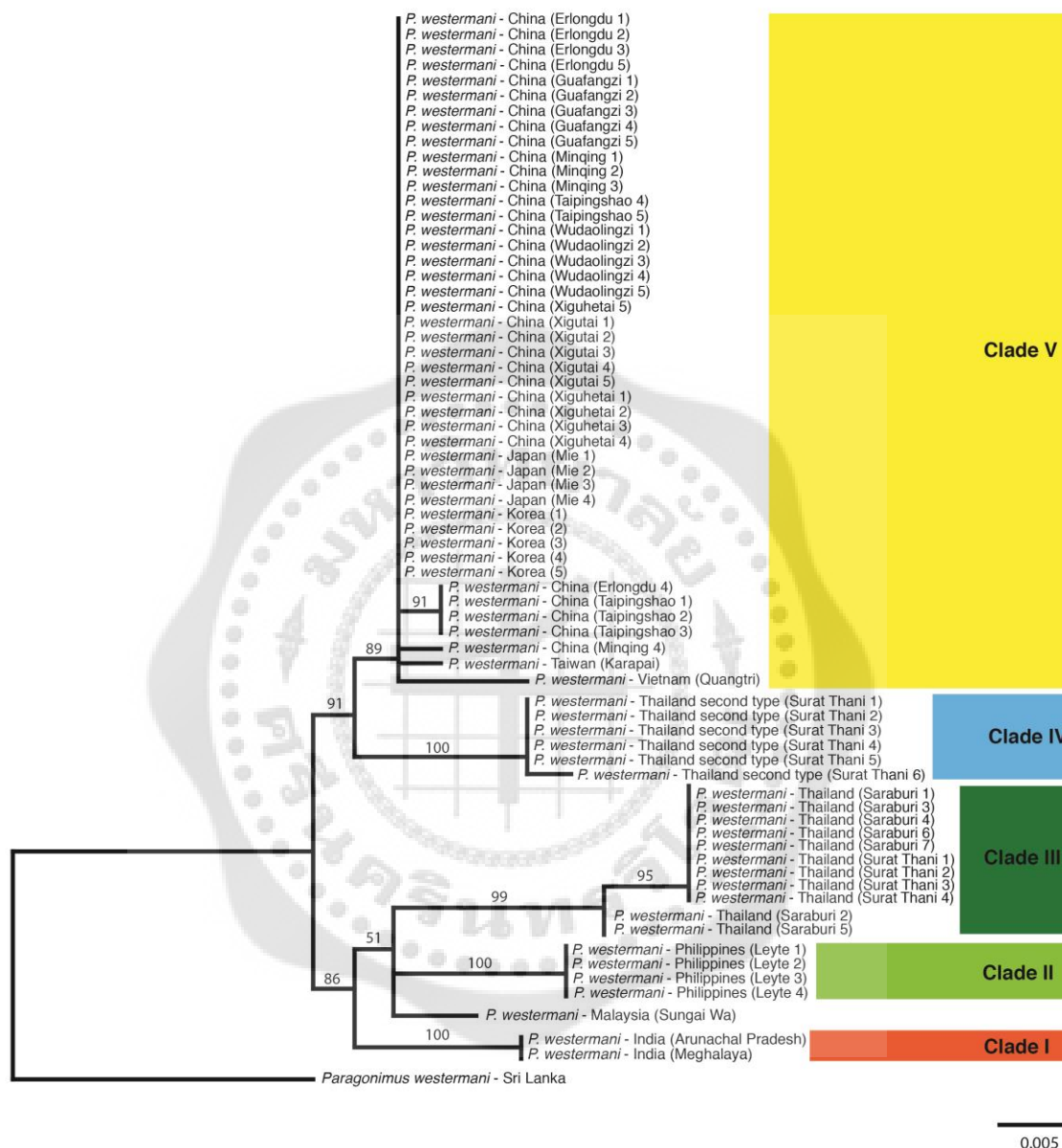


Figure 26 ML tree showing intraspecific relationships of *P. westermanni* based on ITS2 sequence data under the GTR model. Bootstrap values of 1,000 replicates are given in numbers above branches.

3. Sequence alignment, sequence characteristics and phylogenetic analyses of the COI region

The aligned length of the COI sequence data of 70 specimens of *P. westermanni* was 322 bp (Figure 27). Out of 322 characters, 233 (72.36%) characters were constant, and 89 (27.64%) characters are variable. The number of parsimony-informative sites was 72 (22.36%). The GC content of all sequences was in the range of 42.55 to 49.38%. The COI sequence characteristics are shown in Table 4.

The phylogenetic analysis of the COI region based on the MP method yielded 20 most parsimonious trees with 151 steps. The strict consensus tree (Figure 28) revealed five strongly supported clades. Clade I (BS = 100%) contained the samples from India. Clade II (BS = 99%) included the samples from the Philippines. Clade III (BS = 100%) contained the samples from central and southern Thailand. Clade IV (BS = 100%) comprised the members from southern Thailand (second type). Clade V (BS = 100%) included the sample from Vietnam and the members from East Asia, including China, Japan, Korea and Taiwan. For the ML analysis, the phylogenetic tree (Figure 29) obtained was similar to that of the MP analysis. Five monophyletic groups were supported with high bootstrap values (BS = 86-100%).

Specimen	10	20	30	40	50
China (Erlongdu 1)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Erlongdu 2)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Erlongdu 3)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Erlongdu 4)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Erlongdu 5)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Guafangzi 1)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Guafangzi 2)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Guafangzi 3)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Guafangzi 4)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Guafangzi 5)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Minqing 1)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Minqing 2)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Minqing 3)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Minqing 4)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Taipingshao 1)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Taipingshao 2)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Taipingshao 3)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Taipingshao 4)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Taipingshao 5)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Wudaolingzi 1)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Wudaolingzi 2)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Wudaolingzi 3)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Wudaolingzi 4)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Wudaolingzi 5)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Xiguhetai 1)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Xiguhetai 2)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Xiguhetai 3)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Xiguhetai 4)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Xiguhetai 5)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Xigutai 1)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Xigutai 2)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Xigutai 3)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Xigutai 4)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
China (Xigutai 5)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Japan (Mie 1)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Japan (Mie 2)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Japan (Mie 3)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Japan (Mie 4)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Korea (1)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Korea (2)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Korea (3)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Korea (4)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Korea (5)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Philippines (Leyte 1)	GTTTGGGTAT	TATGGGTTGG	TGTTTGCGAT	GGGGGCTATT	GTGTGTCTGG
Philippines (Leyte 2)	GTTTGGGTAT	TATGGGTTGG	TGTTTGCGAT	GGGGGCTATT	GTGTGTCTGG
Philippines (Leyte 3)	GTTTGGGTAT	TATGGGTTGG	TGTTTGCGAT	GGGGGCTATT	GTGTGTCTGG
Philippines (Leyte 4)	GTTTGGGTAT	TATGGGTTGG	TGTTTGCGAT	GGGGGCTATT	GTGTGTCTGG
Thailand (Saraburi 1)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (Saraburi 2)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (Saraburi 3)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (Saraburi 4)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (Saraburi 5)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (Saraburi 6)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (Saraburi 7)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (Surat Thani 1)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (Surat Thani 2)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (Surat Thani 3)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (Surat Thani 4)	GTTTGGGTAT	TACGGGTTAG	TGTTTGCGAT	GGGGGCCATC	GTGTGCTTGG
Thailand (second type 1)	GTTTGGGTAT	TATGGGTTGG	TGTTTCGCGAT	GGGGGCTATC	GTATGTTTGG
Thailand (second type 2)	GTTTGGGTAT	TATGGGTTGG	TGTTTCGCGAT	GGGGGCTATC	GTATGTTTGG
Thailand (second type 3)	GTTTGGGTAT	TATGGGTTGG	TGTTTCGCGAT	GGGGGCTATC	GTATGTTTGG
Thailand (second type 4)	GTTTGGGTAT	TATGGGTTGG	TGTTTCGCGAT	GGGGGCTATC	GTATGTTTGG
Thailand (second type 5)	GTTTGGGTAT	TATGGGTTGG	TGTTTCGCGAT	GGGGGCTATC	GTATGTTTGG
Thailand (second type 6)	GTTTGGGTAT	TATGGGTTGG	TGTTTCGCGAT	GGGGGCTATC	GTATGTTTGG
Vietnam (Quangtri)	GTTTGGGTAT	TACGGGTTGG	TGTTTGCGAT	GGGGGCCATC	GTGTGTCTGG
Taiwan (Karapai)	GTTCGGGTAC	TACGGGCTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
Malaysia (Sungai Wa)	GTTTGGGTAT	TACGGGTTGG	TGTTTGCGAT	GGGGGCCATT	GTGTGTCTGG
India (Meghalaya)	GTTTGGGTAT	TATGGGTTGG	TGTTTGCGAT	GGGGGCTATT	GTGTGTCTGG
India (Arunachal Pradesh)	GTTTGGGTAT	TATGGGTTGG	TGTTTGCGAT	GGGGGCTATT	GTGTGTCTGG
Sri Lanka	GTTTGGGTAT	TATGGGTTGG	TGTTTGCTAT	GGGGGCTATT	GTGTGTTTGG

Figure 27 Sequence data matrix of the aligned COI region of mitochondrial DNA for 70 specimens of *P. westermanni*. A hyphen denotes an alignment gap and numbers indicate alignment length (bp).

Specimen	60	70	80	90	100
China (Erlongdu 1)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Erlongdu 2)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Erlongdu 3)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Erlongdu 4)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Erlongdu 5)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Guafangzi 1)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Guafangzi 2)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Guafangzi 3)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Guafangzi 4)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Guafangzi 5)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Minqing 1)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Minqing 2)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Minqing 3)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Minqing 4)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Taipingshao 1)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Taipingshao 2)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Taipingshao 3)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Taipingshao 4)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Taipingshao 5)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Wudaolingzi 1)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Wudaolingzi 2)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Wudaolingzi 3)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Wudaolingzi 4)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Wudaolingzi 5)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Xiguhetai 1)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Xiguhetai 2)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Xiguhetai 3)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Xiguhetai 4)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Xiguhetai 5)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Xigutai 1)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Xigutai 2)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Xigutai 3)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Xigutai 4)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
China (Xigutai 5)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Japan (Mie 1)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Japan (Mie 2)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Japan (Mie 3)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Japan (Mie 4)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Korea (1)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Korea (2)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Korea (3)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Korea (4)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Korea (5)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Philippines (Leyte 1)	GCAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTAAAG
Philippines (Leyte 2)	GCAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTAAAG
Philippines (Leyte 3)	GCAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTAAAG
Philippines (Leyte 4)	GCAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTAAAG
Thailand (Saraburi 1)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTTA	TGGTTGGTTT	GGATGTAAAG
Thailand (Saraburi 2)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTTA	TGGTTGGTTT	GGATGTAAAG
Thailand (Saraburi 3)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTTA	TGGTTGGTTT	GGATGTAAAG
Thailand (Saraburi 4)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTTA	TGGTTGGTTT	GGATGTAAAG
Thailand (Saraburi 5)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTTA	TGGTTGGTTT	GGATGTAAAG
Thailand (Saraburi 6)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTTA	TGGTTGGTTT	GGATGTAAAG
Thailand (Saraburi 7)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTTA	TGGTTGGTTT	GGATGTAAAG
Thailand (Surat Thani 1)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTTA	TGGTTGGTTT	GGATGTAAAG
Thailand (Surat Thani 2)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTTA	TGGTTGGTTT	GGATGTAAAG
Thailand (Surat Thani 3)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTCA	TGGTTGGTTT	GGATGTAAAG
Thailand (Surat Thani 4)	GCAGTGTGTG	GTGGGCGCAC	CACATGTTTA	TGGTTGGTTT	GGATGTAAAG
Thailand (second type 1)	GTAGTGTGTG	GTGAGCGCAC	CATATGTTCA	TGGTTGGTTT	GGATGTAAAG
Thailand (second type 2)	GTAGTGTGTG	GTGAGCGCAC	CATATGTTCA	TGGTTGGTTT	GGATGTAAAG
Thailand (second type 3)	GTAGTGTGTG	GTGAGCGCAC	CATATGTTCA	TGGTTGGTTT	GGATGTAAAG
Thailand (second type 4)	GTAGTGTGTG	GTGAGCGCAC	CATATGTTCA	TGGTTGGTTT	GGATGTAAAG
Thailand (second type 5)	GTAGTGTGTG	GTGAGCGCAC	CATATGTTCA	TGGTTGGTTT	GGATGTAAAG
Thailand (second type 6)	GTAGTGTGTG	GTGAGCGCAC	CATATGTTCA	TGGTTGGTTT	GGATGTAAAG
Vietnam (Quangtri)	GCAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Taiwan (Karapai)	GTAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTTT	GGATGTCAAG
Malaysia (Sungai Wa)	GCAGTGTGTG	GTGGGCGCAT	CACATGTTCA	TGGTTGGTCT	AGATGTCAAG
India (Meghalaya)	GGAGCGTTGT	GTGGGCGCAC	CACATGTTTA	TGGTTGGTCT	GGATGTAAAG
India (Arunachal Pradesh)	GGAGCGTTGT	GTGGGCGCAC	CACATGTTTA	TGGTTGGTCT	GGATGTAAAG
Sri Lanka	GAAGTGTGTG	GTGGGCTCAC	CACATGTTTA	TGGTTGGTCT	GGATGTAAAG

Figure 27 (continued).

Specimen	110	120	130	140	150
China (Erlongdu 1)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Erlongdu 2)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Erlongdu 3)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Erlongdu 4)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Erlongdu 5)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Guafangzi 1)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Guafangzi 2)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Guafangzi 3)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Guafangzi 4)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Guafangzi 5)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Minqing 1)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Minqing 2)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Minqing 3)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Minqing 4)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Taipingshao 1)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Taipingshao 2)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Taipingshao 3)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Taipingshao 4)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Taipingshao 5)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Wudaolingzi 1)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Wudaolingzi 2)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Wudaolingzi 3)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Wudaolingzi 4)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Wudaolingzi 5)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Xiguhetai 1)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Xiguhetai 2)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Xiguhetai 3)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Xiguhetai 4)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Xiguhetai 5)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Xigutai 1)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Xigutai 2)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Xigutai 3)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Xigutai 4)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
China (Xigutai 5)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Japan (Mie 1)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Japan (Mie 2)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Japan (Mie 3)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Japan (Mie 4)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Korea (1)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Korea (2)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Korea (3)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Korea (4)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Korea (5)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Philippines (Leyte 1)	ACTGCTGTTT	TTTTTAGTTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Philippines (Leyte 2)	ACTGCTGTTT	TTTTTAGTTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Philippines (Leyte 3)	ACTGCTGTTT	TTTTTAGTTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Philippines (Leyte 4)	ACTGCTGTTT	TTTTTAGTTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Saraburi 1)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Saraburi 2)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Saraburi 3)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Saraburi 4)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Saraburi 5)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Saraburi 6)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Saraburi 7)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Surat Thani 1)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Surat Thani 2)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Surat Thani 3)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (Surat Thani 4)	ACTGCTGTCT	TTTTTAGCTC	TGTCACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (second type 1)	ACTGCTGTTT	TTTTTAGTTC	TGTTACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (second type 2)	ACTGCTGTTT	TTTTTAGTTC	TGTTACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (second type 3)	ACTGCTGTTT	TTTTTAGTTC	TGTTACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (second type 4)	ACTGCTGTTT	TTTTTAGTTC	TGTTACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (second type 5)	ACTGCTGTTT	TTTTTAGTTC	TGTTACGGGG	GTGATTGGGA	TACCTACGGG
Thailand (second type 6)	ACTGCTGTTT	TTTTTAGTTC	TGTTACGGGG	GTGATTGGGA	TACCTACGGG
Vietnam (Quangtri)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGG	GTGATTGGGA	TACCCACGGG
Taiwan (Karapai)	ACTGCTGTTT	TCTTTAGTTC	TGTCACGGGA	GTGATTGGGA	TACCCACGGG
Malaysia (Sungai Wa)	ACCGCTGTCT	TTTTTCACTC	TGTTACGGGG	GTGATTGGGA	TACCTACGGG
India (Meghalaya)	ACCGCTGTGT	TCTTTAGTTC	TGTTACGGGA	GTGATTGGGA	TTCCTACGGG
India (Arunachal Pradesh)	ACCGCTGTGT	TCTTTAGTTC	TGTTACGGGA	GTGATTGGGA	TTCCTACGGG
Sri Lanka	ACTGCTGTGT	TTTTTAGTTC	CGTTACGGGG	GTGATTGGGA	TTCCTACGGG

Figure 27 (continued).

Specimen	160	170	180	190	200
China (Erlongdu 1)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Erlongdu 2)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Erlongdu 3)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Erlongdu 4)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Erlongdu 5)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Guafangzi 1)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Guafangzi 2)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Guafangzi 3)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Guafangzi 4)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Guafangzi 5)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Minqing 1)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Minqing 2)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Minqing 3)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Minqing 4)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Taipingshao 1)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Taipingshao 2)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Taipingshao 3)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Taipingshao 4)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Taipingshao 5)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Wudaolingzi 1)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Wudaolingzi 2)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Wudaolingzi 3)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Wudaolingzi 4)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Wudaolingzi 5)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Xiguhetai 1)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Xiguhetai 2)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Xiguhetai 3)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Xiguhetai 4)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Xiguhetai 5)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Xigutai 1)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Xigutai 2)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Xigutai 3)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Xigutai 4)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
China (Xigutai 5)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Japan (Mie 1)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Japan (Mie 2)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Japan (Mie 3)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Japan (Mie 4)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Korea (1)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Korea (2)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Korea (3)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Korea (4)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Korea (5)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Philippines (Leyte 1)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGATCT	CGTTTGCGAT
Philippines (Leyte 2)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGATCT	CGTTTGCGAT
Philippines (Leyte 3)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGATCT	CGTTTGCGAT
Philippines (Leyte 4)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGATCT	CGTTTGCGAT
Thailand (Saraburi 1)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (Saraburi 2)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (Saraburi 3)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (Saraburi 4)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (Saraburi 5)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (Saraburi 6)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (Saraburi 7)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (Surat Thani 1)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (Surat Thani 2)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (Surat Thani 3)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (Surat Thani 4)	GATTAAGGTC	TTCTCTTGGT	TGTTTCATGTT	GGGTGGATCT	CGTTTACGAT
Thailand (second type 1)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGAAC	CGTTTGCGGT
Thailand (second type 2)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGAAC	CGTTTGCGGT
Thailand (second type 3)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGAAC	CGTTTGCGGT
Thailand (second type 4)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGAAC	CGTTTGCGGT
Thailand (second type 5)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGAAC	CGTTTGCGGT
Thailand (second type 6)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGAAC	CGTTTGCGGT
Vietnam (Quangtri)	GATTAAGGTT	TTTTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Taiwan (Karapai)	GATTAAGGTT	TTCTCTTGGT	TGTTTCATGCT	GGGTGGAAC	CGTTTGCGGT
Malaysia (Sungai Wa)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGAAC	CGTTTGCGGT
India (Meghalaya)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGAAC	CGTTTGCGGT
India (Arunachal Pradesh)	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	GGGTGGAAC	CGTTTGCGGT
Sri Lanka	GATTAAGGTT	TTTTCTTGGT	TGTTTATGTT	AGGTGGTGCC	CGTTTGCGGT

Figure 27 (continued).

Specimen	210	220	230	240	250
China (Erlongdu 1)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Erlongdu 2)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Erlongdu 3)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Erlongdu 4)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Erlongdu 5)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Guafangzi 1)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Guafangzi 2)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Guafangzi 3)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Guafangzi 4)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Guafangzi 5)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Minqing 1)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Minqing 2)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Minqing 3)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Minqing 4)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Taipingshao 1)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Taipingshao 2)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Taipingshao 3)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Taipingshao 4)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Taipingshao 5)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Wudaolingzi 1)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Wudaolingzi 2)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Wudaolingzi 3)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Wudaolingzi 4)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Wudaolingzi 5)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Xiguhetai 1)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Xiguhetai 2)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Xiguhetai 3)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Xiguhetai 4)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Xiguhetai 5)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Xigutai 1)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Xigutai 2)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Xigutai 3)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Xigutai 4)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
China (Xigutai 5)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Japan (Mie 1)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Japan (Mie 2)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Japan (Mie 3)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Japan (Mie 4)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Korea (1)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Korea (2)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Korea (3)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Korea (4)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Korea (5)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Philippines (Leyte 1)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Philippines (Leyte 2)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Philippines (Leyte 3)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Philippines (Leyte 4)	TCTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Saraburi 1)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Saraburi 2)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Saraburi 3)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Saraburi 4)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Saraburi 5)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Saraburi 6)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Saraburi 7)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Surat Thani 1)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Surat Thani 2)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Surat Thani 3)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (Surat Thani 4)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TCTGTTCAAC
Thailand (second type 1)	TTTGAGATCC	TGTTTTGTGG	TGGATACTCG	GGTTTATCTT	TCTGTTCAAC
Thailand (second type 2)	TTTGAGATCC	TGTTTTGTGG	TGGATACTCG	GGTTTATCTT	TCTGTTCAAC
Thailand (second type 3)	TTTGAGATCC	TGTTTTGTGG	TGGATACTCG	GGTTTATCTT	TCTGTTCAAC
Thailand (second type 4)	TTTGAGATCC	TGTTTTGTGG	TGGATACTCG	GGTTTATCTT	TCTGTTCAAC
Thailand (second type 5)	TTTGAGATCC	TGTTTTGTGG	TGGATACTCG	GGTTTATCTT	TCTGTTCAAC
Thailand (second type 6)	TTTGAGATCC	TGTTTTGTGG	TGGATACTCG	GGTTTATCTT	TCTGTTCAAC
Vietnam (Quangtri)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Taiwan (Karapai)	TTTGGGATCC	TGTTTTGTGG	TGAATTCCTG	GGTTTATCTT	CCTGTTCAAC
Malaysia (Sungai Wa)	TTTGAGATCC	TGTTTTGTGG	TGGATCCTTG	GGTTTATCTT	TTTGTTCAAC
India (Meghalaya)	TTTGGGATCC	CGTTTTGTGG	TGAATACTCG	GGTTTATCTT	TCTGTTTACG
India (Arunachal Pradesh)	TTTGAGATCC	CGTTTTGTGG	TGAATACTCG	GGTTTATCTT	TCTGTTTACG
Sri Lanka	TTTGGGATCC	TGTTTTGTGA	TGGATACTTG	GATTCATTTT	TTTGTTTACG

Figure 27 (continued).

Specimen	260	270	280	290	300
China (Erlongdu 1)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Erlongdu 2)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Erlongdu 3)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Erlongdu 4)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Erlongdu 5)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Guafangzi 1)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Guafangzi 2)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAATCT
China (Guafangzi 3)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Guafangzi 4)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Guafangzi 5)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Minqing 1)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Minqing 2)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Minqing 3)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Minqing 4)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Taipingshao 1)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Taipingshao 2)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Taipingshao 3)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Taipingshao 4)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Taipingshao 5)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Wudaolingzi 1)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Wudaolingzi 2)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Wudaolingzi 3)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Wudaolingzi 4)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Wudaolingzi 5)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Xiguhetai 1)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAATCT
China (Xiguhetai 2)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAATCT
China (Xiguhetai 3)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAATCT
China (Xiguhetai 4)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAATCT
China (Xiguhetai 5)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Xigutai 1)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Xigutai 2)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Xigutai 3)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
China (Xigutai 4)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAATCT
China (Xigutai 5)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
Japan (Mie 1)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
Japan (Mie 2)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
Japan (Mie 3)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAATCT
Japan (Mie 4)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
Korea (1)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
Korea (2)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
Korea (3)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
Korea (4)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAATCT
Korea (5)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
Philippines (Leyte 1)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCCTCTATTC	TGGACAGTCT
Philippines (Leyte 2)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCCTCTATTC	TGGACAGTCT
Philippines (Leyte 3)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCCTCTATTC	TGGACAGTCT
Philippines (Leyte 4)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCCTCTATTC	TGGACAGTCT
Thailand (Saraburi 1)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (Saraburi 2)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (Saraburi 3)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (Saraburi 4)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (Saraburi 5)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (Saraburi 6)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (Saraburi 7)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (Surat Thani 1)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (Surat Thani 2)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (Surat Thani 3)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (Surat Thani 4)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCTTCCATCC	TGGATAGTCT
Thailand (second type 1)	ATAGGTGGTG	TGACTGGCAT	TATTTTGTCT	TCTTCCATAT	TGGATAGCCT
Thailand (second type 2)	ATAGGTGGTG	TGACTGGCAT	TATTTTGTCT	TCTTCCATAT	TGGATAGCCT
Thailand (second type 3)	ATAGGTGGTG	TGACTGGCAT	TATTTTGTCT	TCTTCCATAT	TGGATAGCCT
Thailand (second type 4)	ATAGGTGGTG	TGACTGGCAT	TATTTTGTCT	TCTTCCATAT	TGGATAGCCT
Thailand (second type 5)	ATAGGTGGTG	TGACTGGCAT	TATTTTGTCT	TCTTCCATAT	TGGATAGCCT
Thailand (second type 6)	ATAGGTGGTG	TGACTGGCAT	TATTTTGTCT	TCTTCCATAT	TGGATAGCCT
Vietnam (Quangtri)	ATAGGTGGTG	TGACCGGCAT	CATCCTGTCT	TCCTCCATAC	TGGATAGTCT
Taiwan (Karapai)	ATAGGTGGTG	TGACCGGCAT	CATTCTGTCC	TCCTCCATAC	TGGATAGTCT
Malaysia (Sungai Wa)	ATAGGGGGTG	TGACTGGCAT	CATTTTGTCT	TCCTCCATAC	TGGATAGTCT
India (Meghalaya)	ATTGGAGGTG	TGACGGGTAT	CATTCTCTCT	TCCTCTATCC	TTGACAGCTT
India (Arunachal Pradesh)	ATTGGAGGTG	TGACGGGTAT	CATTCTCTCT	TCCTCTATCC	TTGACAGCTT
Sri Lanka	ATAGGTGGTG	TGACGGGTAT	TATTCTCTCC	TCTTCCATAT	TGGATAGTCT

Figure 27 (continued).

Specimen	310	320	
China (Erlongdu 1)	GTTGCATGAT	ACGTGGTTCG	TC
China (Erlongdu 2)	GTTGCATGAT	ACGTGGTTCG	TC
China (Erlongdu 3)	GTTGCATGAT	ACGTGGTTCG	TC
China (Erlongdu 4)	GTTGCATGAT	ACGTGGTTCG	TC
China (Erlongdu 5)	GTTGCATGAT	ACGTGGTTCG	TC
China (Guafangzi 1)	GTTGCATGAT	ACGTGGTTCG	TC
China (Guafangzi 2)	GTTGCATGAT	ACGTGGTTCG	TC
China (Guafangzi 3)	GTTGCATGAT	ACGTGGTTCG	TC
China (Guafangzi 4)	GTTGCATGAT	ACGTGGTTCG	TC
China (Guafangzi 5)	GTTGCATGAT	ACGTGGTTCG	TC
China (Minqing 1)	GTTGCATGAT	ACGTGGTTCG	TC
China (Minqing 2)	GTTGCATGAT	ACGTGGTTCG	TC
China (Minqing 3)	GTTGCATGAT	ACGTGGTTCG	TC
China (Minqing 4)	GTTGCATGAT	ACGTGGTTCG	TC
China (Taipingshao 1)	GTTGCATGAT	ACGTGGTTCG	TC
China (Taipingshao 2)	GTTGCATGAT	ACGTGGTTCG	TC
China (Taipingshao 3)	GTTGCATGAT	ACGTGGTTCG	TC
China (Taipingshao 4)	GTTGCATGAT	ACGTGGTTCG	TC
China (Taipingshao 5)	GTTGCATGAT	ACGTGGTTCG	TC
China (Wudaolingzi 1)	GTTGCATGAT	ACGTGGTTCG	TC
China (Wudaolingzi 2)	GTTGCATGAT	ACGTGGTTCG	TC
China (Wudaolingzi 3)	GTTGCATGAT	ACGTGGTTCG	TC
China (Wudaolingzi 4)	GTTGCATGAT	ACGTGGTTCG	TC
China (Wudaolingzi 5)	GTTGCATGAT	ACGTGGTTCG	TC
China (Xiguhetai 1)	GTTGCATGAT	ACGTGGTTCG	TC
China (Xiguhetai 2)	GTTGCATGAT	ACATGGTTCG	TC
China (Xiguhetai 3)	GTTGCATGAT	ACGTGGTTCG	TC
China (Xiguhetai 4)	GTTGCATGAT	ACATGGTTCG	TC
China (Xiguhetai 5)	GTTGCATGAT	ACGTGGTTCG	TC
China (Xigutai 1)	GTTGCATGAT	ACGTGGTTCG	TC
China (Xigutai 2)	GTTGCATGAT	ACGTGGTTCG	TC
China (Xigutai 3)	GTTGCATGAT	ACGTGGTTCG	TC
China (Xigutai 4)	GTTGCATGAT	ACGTGGTTCG	TC
China (Xigutai 5)	GTTGCATGAT	ACGTGGTTCG	TC
Japan (Mie 1)	GTTGCATGAT	ACGTGGTTCG	TC
Japan (Mie 2)	GTTGCATGAT	ACGTGGTTCG	TC
Japan (Mie 3)	GTTGCATGAT	ACGTGGTTCG	TC
Japan (Mie 4)	GTTGCATGAT	ACGTGGTTCG	TC
Korea (1)	GTTGCATGAT	ACGTGGTTCG	TC
Korea (2)	GTTGCATGAT	ACGTGGTTCG	TC
Korea (3)	GTTGCATGAT	ACGTGGTTCG	TC
Korea (4)	GTTGCATGAT	ACATGGTTCG	TC
Korea (5)	GTTGCATGAT	ACGTGGTTCG	TC
Philippines (Leyte 1)	GTTGCATGAC	ACGTGGTTTG	TT
Philippines (Leyte 2)	GTTGCATGAC	ACGTGGTTTG	TT
Philippines (Leyte 3)	GTTGCATGAC	ACGTGGTTTG	TT
Philippines (Leyte 4)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Saraburi 1)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Saraburi 2)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Saraburi 3)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Saraburi 4)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Saraburi 5)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Saraburi 6)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Saraburi 7)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Surat Thani 1)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Surat Thani 2)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Surat Thani 3)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (Surat Thani 4)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (second type 1)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (second type 2)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (second type 3)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (second type 4)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (second type 5)	GTTGCATGAC	ACGTGGTTTG	TT
Thailand (second type 6)	GTTGCATGAC	ACGTGGTTTG	TT
Vietnam (Quangtri)	GCTGCATGAT	ACGTGGTTCG	TT
Taiwan (Karapai)	GTTGCATGAT	ACGTGGTTCG	TC
Malaysia (Sungai Wa)	GTTGCATGAT	ACGTGGTTTG	TT
India (Meghalaya)	GTTGCATGAT	ACGTGGTTTG	TT
India (Arunachal Pradesh)	GTTGCATGAT	ACGTGGTTTG	TT
Sri Lanka	GTTGCATGAC	ACATGATTG	TT

Figure 27 (continued).

Table 4 Sequence characteristics of the COI region of *P. westermanni* under study.

Sequence characteristics	COI
Length range (total) (bp)	321-322
Length mean (total) (bp)	321.99
Length range ingroup (bp)	321-322
Length outgroup (bp)	322
Aligned length (bp)	322
GC content range (%)	42.55-49.38
GC content mean (%)	48.02
Number of constant sites (%)	233 (72.36%)
Number of variable sites (%)	89 (27.64%)
Number of informative sites (%)	72 (22.36%)
Number of uninformative sites (%)	17 (5.28%)
Transition/transversion ratio (ts/tv ratio)	4.84
Heterogeneity rate (α)	0.21

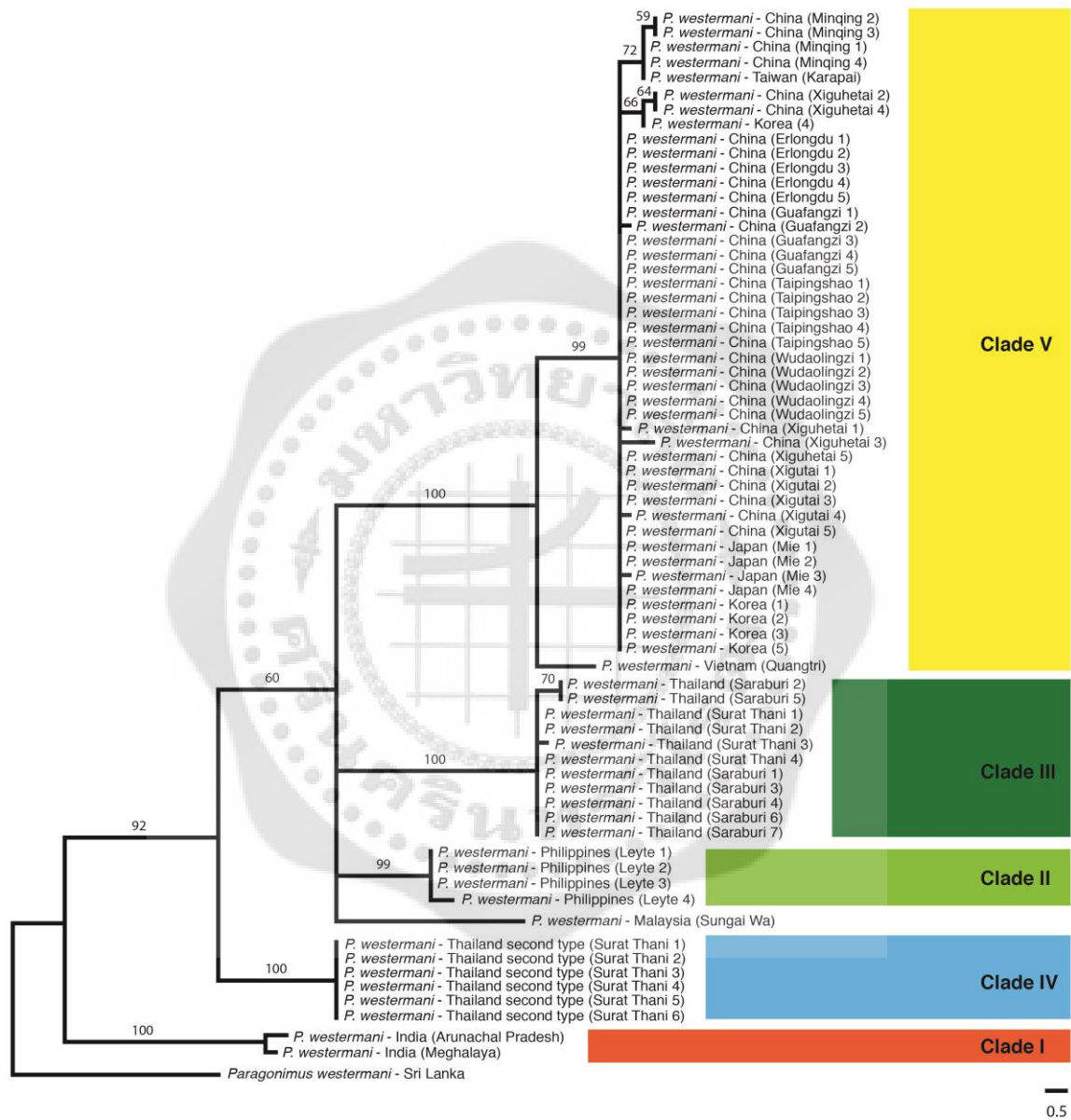


Figure 28 Strict consensus tree revealing intraspecific relationships of *P. westermanni* based on COI sequence data and constructed using MP analysis. Bootstrap values of 1,000 replicates are given in numbers above branches.

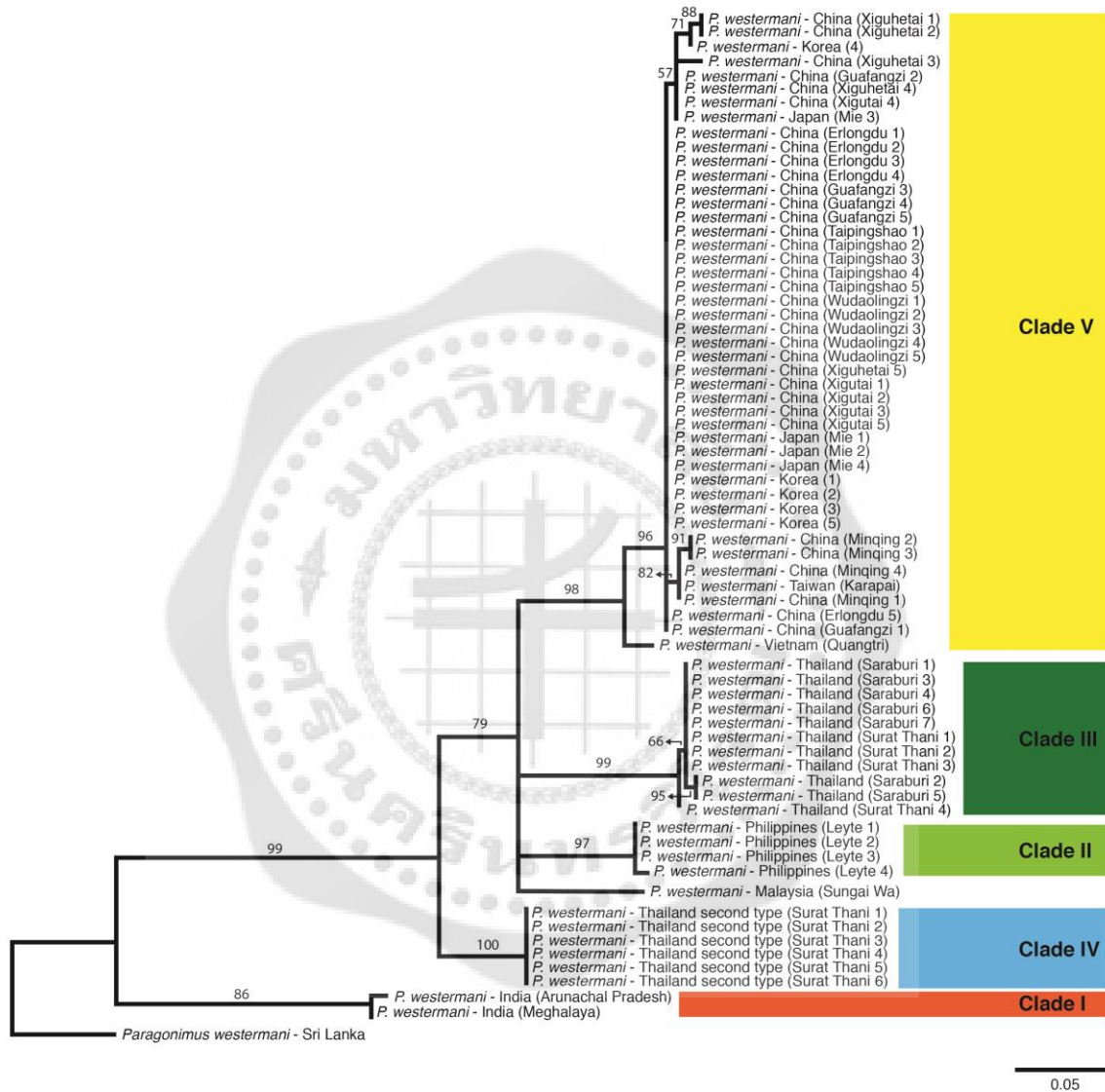


Figure 29 ML tree depicting intraspecific relationships of *P. westermanni* based on COI sequence data using the GTR model. Bootstrap values of 1,000 replicates are given in numbers above branches.

4. Phylogenetic analyses of the combined ITS2 and COI sequence data

A sequence alignment of a combined data set of the ITS2 and COI regions for 70 specimens of *P. westermanni* revealed a total of 682 characters. The phylogenetic analyses using both MP and ML methods yielded completely resolved trees with congruent topologies (Figures 30 and 31). They resembled the COI trees, with highly supported clades (BS = 99-100%).

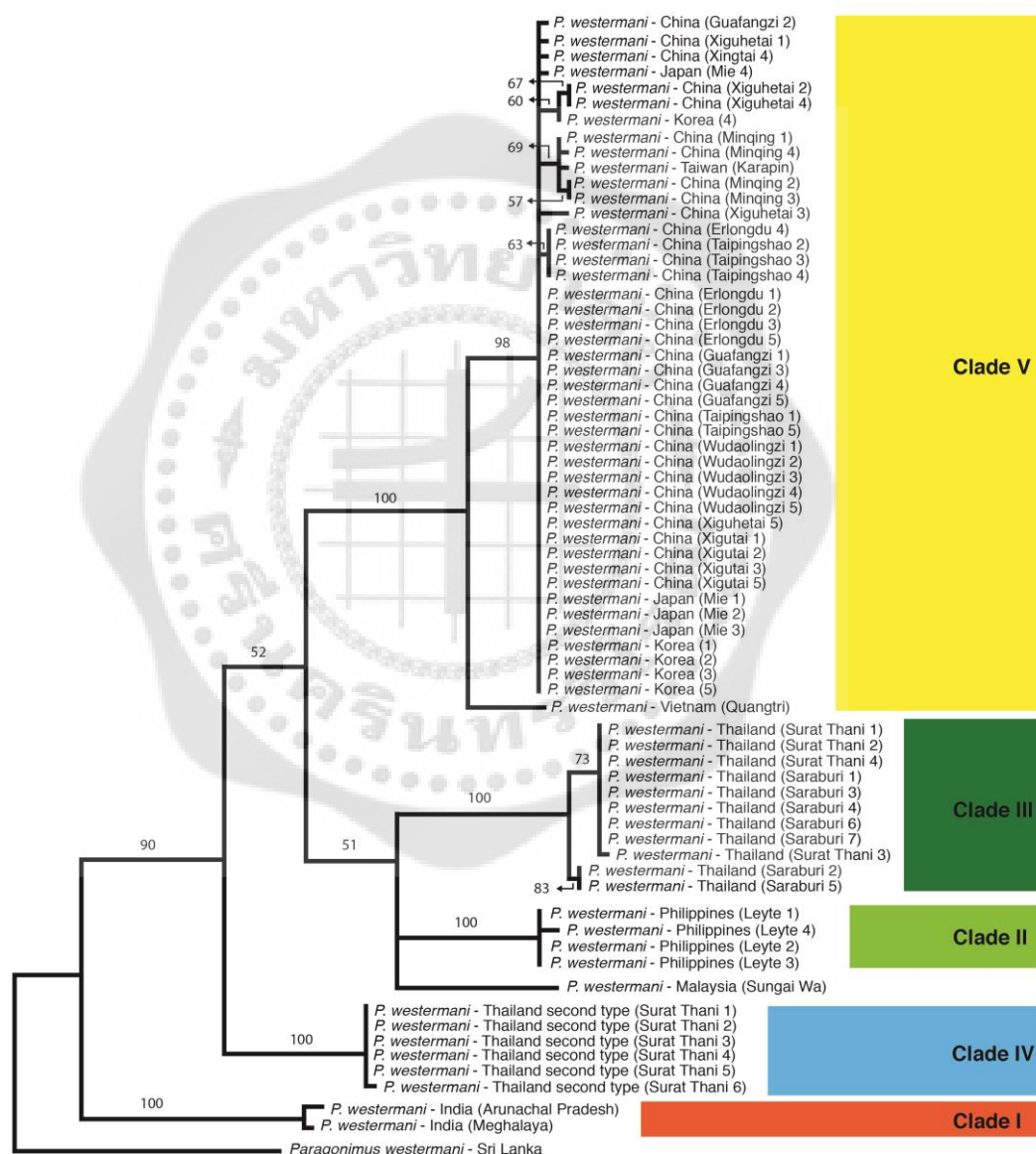


Figure 30 Strict consensus tree of 10 most parsimonious trees (198 steps) of *P. westermanni* based on a combined data set of the ITS2 and COI regions. Bootstrap values of 1,000 replicates are given in numbers above branches.

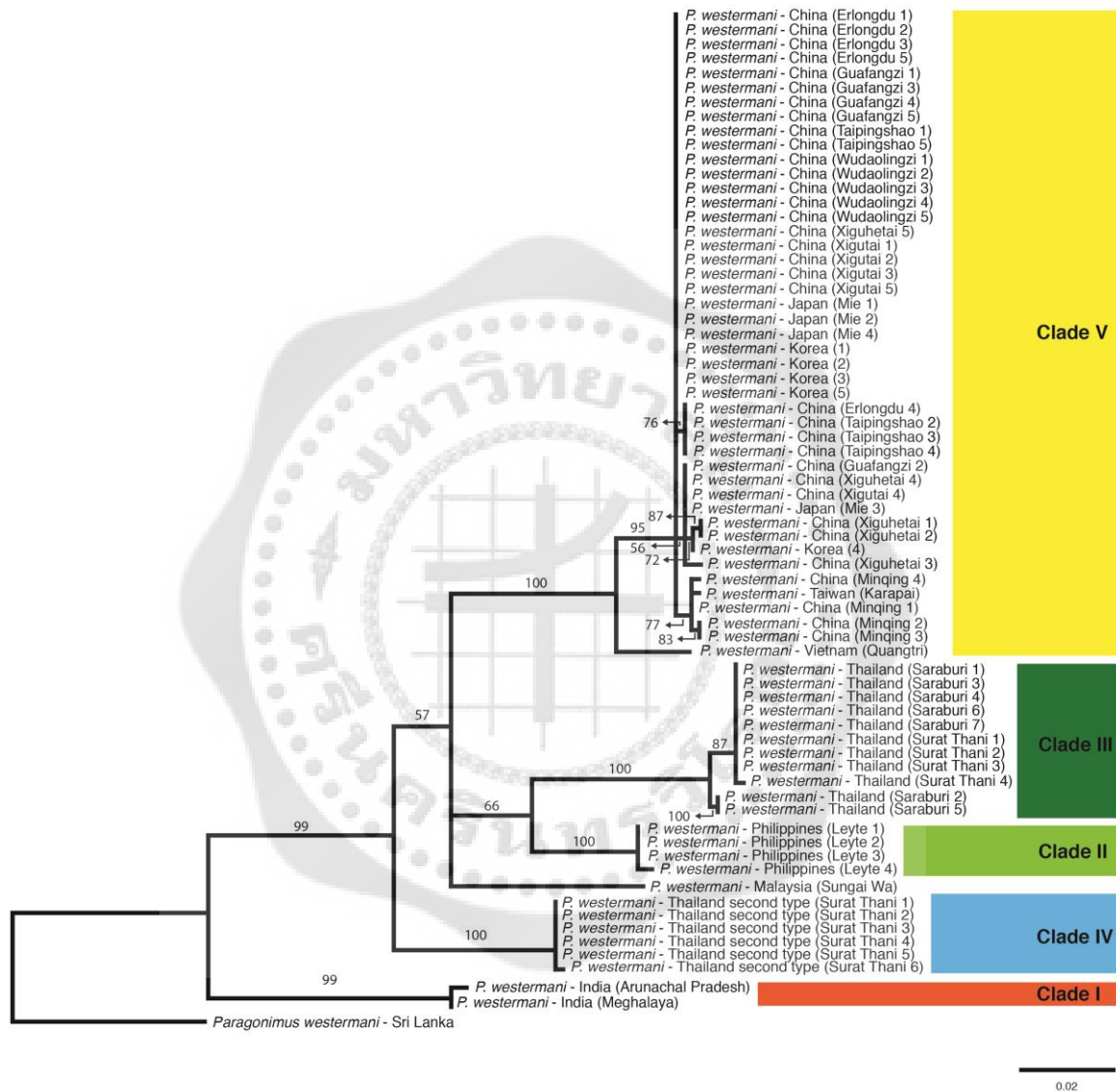


Figure 31 ML tree showing intraspecific relationships of *P. westermanni* based on combined sequence data of the ITS2 and COI regions under the GTR model. Numbers on branches represent bootstrap values of 1,000 replicates.

5. Haplotype analysis

The haplotype network constructed from the 70 COI sequences of *P. westermanni* showed 18 haplotypes (Figure 32). These included six haplotypes from China (H12 - H15 and H17 - H18), four haplotypes from Thailand (H4 and H6-H8), two haplotypes each from India (H2 - H3), the Philippines (H9 and H10), Japan (H12 and H15) and Korea (H12 and H16) and single haplotypes from Sri Lanka (H1), Malaysia (H5), Vietnam (H11) and Taiwan (H13). Almost all haplotypes were unique, except three haplotypes that possessed shared alleles. These included H12 (China, Korea and Japan), H13 (China and Taiwan) and H15 (China and Japan). H12 is the largest haplotype that contained 31 sequences of *P. westermanni* from East Asia, including China (77.42%), Korea (12.90%) and Japan (9.68%). Details of all 18 haplotypes and their components are shown in Table 5.

Table 5 Haplotype analysis of *P. westermanni* based on COI sequence data.

Haplotype	Locality	No. of specimen	Haplotype	Locality	No. of specimen
H1	Sri Lanka	1	H12	China (Xigutai)	4
H2	India	1		China (Wudaolingzi)	5
	(Arunachal Pradesh)			China (Xiguhetai)	1
H3	India (Meghalaya)	1		Japan (Mie)	3
H4	Thailand second type	6		Korea	4
	(Surat Thani)		H13	China (Minqing)	2
H5	Malaysia (Sungai Wa)	1		Taiwan (Karapai)	1
H6	Thailand (Surat Thani)	1	H14	China (Minqing)	2
H7	Thailand (Saraburi)	5	H15	China (Guafangzi)	1
	Thailand (Surat Thani)	3		China (Xiguhetai)	1
H8	Thailand (Saraburi)	2		China (Xigutai)	1
H9-10	The Philippines (Leyte)	4		Japan (Mie)	1
H11	Vietnam (Quangtri)	1	H16	Korea	1
H12	China (Erlongdu)	5	H17	China (Xiguhetai)	2
	China (Guafangzi)	4	H18	China (Xiguhetai)	1
	China (Taipingshao)	5			

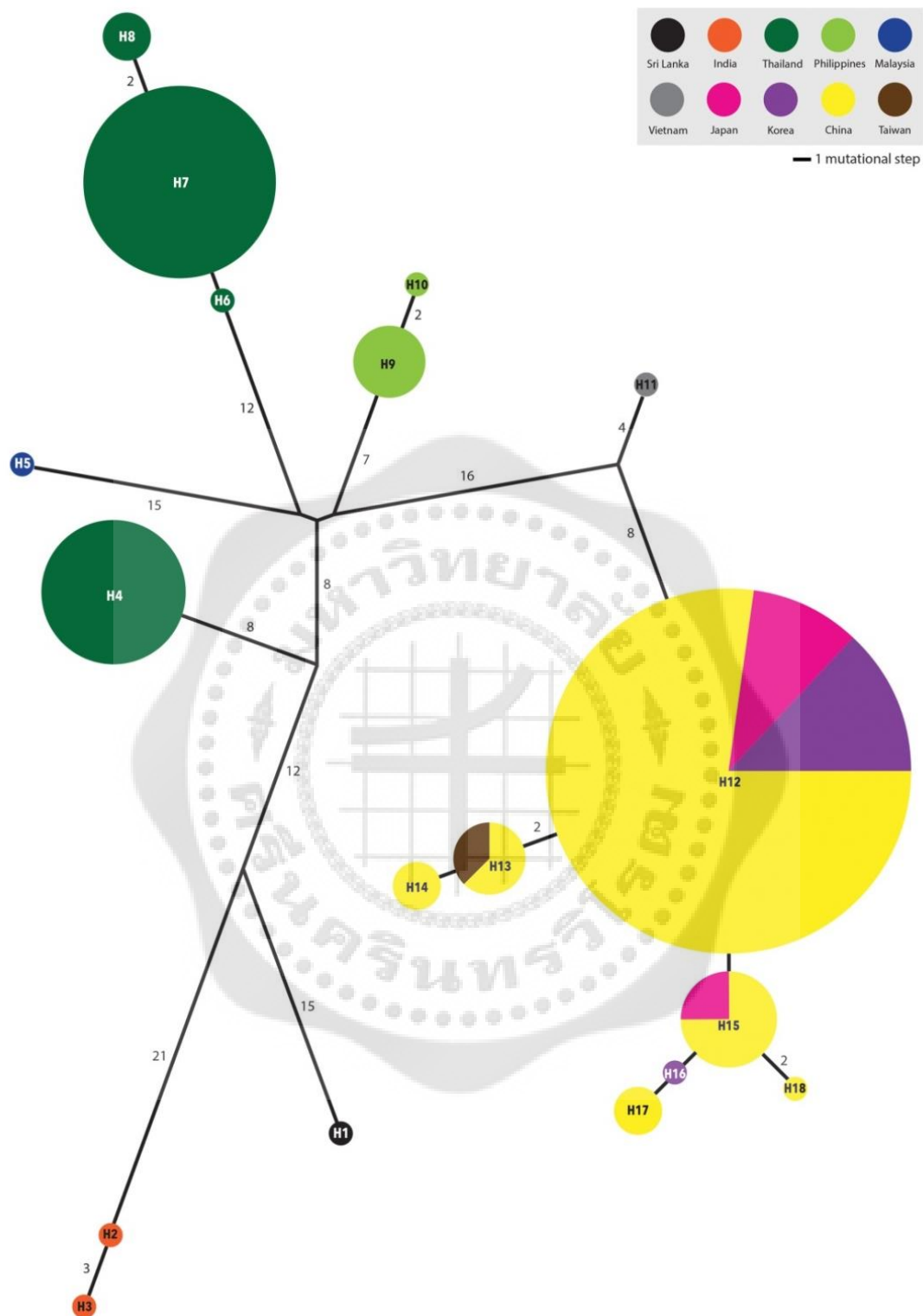


Figure 32 Haplotype network based on COI sequence data of *P. westermanni*. Circles represent haplotypes from H1 to H18. Color codes indicate the localities of specimens. The size of the circle is proportional to the number of specimens. Numbers on the lines represent mutational steps between haplotypes.

CHAPTER FIVE

DISCUSSION

This study indicated that both ITS2 and COI sequence data were capable of resolving the phylogenetic tree of *P. westermanni* to the intraspecific level. Based on the MP and ML analyses, both loci generated the phylogenetic trees consisting of five distinct clades of *P. westermanni* populations. The ITS2-based and COI-based trees were almost similar, except the position of clade IV which included the samples from southern Thailand (second type). When the combined data sets of the ITS2 and COI regions were employed the phylogenetic trees obtained were identical to these of the COI trees. This was because the COI sequences possessed more parsimony-informative sites (ITS2 = 6.11%, COI = 22.36%). Moreover, a higher transition/transversion ratio and a lower heterogeneity rate (α) was observed for the COI sequences (ts/tv: ITS2 = 2.18%, COI = 4.84% and α : ITS2 = 0.36, COI = 0.21). Other studies also showed that transition was favored over transversion in *Paragonimus* (Binchai; *et al.* 2007, Thaenkham. & Waikagul. 2008). Unlike nuclear genes, mitochondrial genes are maternally inherited, lack of introns and exhibit limited recombination (Hebert; *et al.* 2003). Thus, the COI sequence data have been widely used as a universal marker for systematics and barcoding of animal phyla (Hebert; Ratnasingham; & deWaard. 2003, Hebert; *et al.* 2003).

Since five strongly supported monophyletic groups were obtained from both loci it indicated that there was no conflict with the species trees. However, the different position of *P. westermanni* (second type) in clade IV demonstrated two incongruent gene trees. This suggested a conflict between nuclear and mitochondrial DNA phylogenies. The discrepancy of the ITS2-based and COI-based trees may arise due to several modes of molecular drive such as nucleotide substitutions, duplication and loss of DNA sequences. Under a selective pressure, a particular type of the ITS2 sequences could have been selected and fixed for the samples of *P. westermanni* (second type) and then undergone concerted evolution. Based on COI sequence data the population of

P. westermani (second type) was closely related to other basal groups from India and Sri Lanka. This suggested that the entire population of *P. westermani* (second type) has retained the characteristic of the COI sequences of the ancestral populations.

According to Sugiyama; *et al.* (2007), both *P. westermani* and *P. westermani* (second type) were simultaneously detected in that same crab host, *Phricotelphusa aedes* found in Surat Thani. The metacercariae of the latter were similar to those of the former, but were much smaller. At the adult stage however, both *P. westermani* and *P. westermani* (second type) were identical. Interesting, the susceptibility of feline hosts to *P. westermani* (second type) was different from that of *P. westermani*. Experimental infection revealed that in *P. westermani* worms were detected only the lung or pleural cavity of cat (Binchai; *et al* 2007), but in *P. westermani* (second type) juvenile worms lodged predominantly in the liver and some matured into adults in the pleural cavity or lung (Sugiyama; *et al.* 2007). Since the phylogenetic analyses of the COI sequences showed that *P. westermani* (second type) possessed a primitive type of mtDNA, this indicated that Thailand (Surat Thani) might be the center of diversity with sympatric populations of *P. westermani* and *P. westermani* (second type).

The haplotype network based on COI sequence data showed 18 haplotypes. Of these, 15 haplotypes were independent the other three were shared. This network revealed a geographical structure of genetic variation of *P. westermani* species complex. According to Iwagami; *et al.* (2008) *P. westermani* from Sri Lanka was indicated as an ancestor. An evolutionary scenario of *P. westermani* probably started from Sri Lanka (H1), and then the population was distributed to northeastern India (H2 - H3). Some individuals migrate to southern Thailand, establishing *P. westermani* (second type) (H4). Due to its restricted host range and lower rate of infection, *P. westermani* (second type) has become endemic to Surat Thani. Apparently, a sympatric *P. westermani* which was co-infected with *P. westermani* (second type) seemed to be naturally selected and began its adaptive radiation. The population of *P. westermani* from Surat Thani diverged to Malaysia (H5), the Philippines (H9 - H10) and Vietnam (H11) and several countries in East Asia

(H12 - 18). From this study, it was obvious that there was a high level of haplotype diversity among *P. westermani* populations. However, low genetic variability was observed, especially in the East Asian populations. This indicated a rapid and recent divergence of *P. westermani* within these groups. Similarly, Doanh; *et al* (2015) reported that the COI-haplotype network of the *P. heterotremus* complex revealed that the population of *P. heterotremus* from India was basal to all other *P. heterotremus* and *P. pseudoheterotremus* populations. The haplotype network also showed that *P. pseudoheterotremus* could not be considered a distinct species based on metacercarial polymorphism and genetic variation, but it should be regarded as a geographically isolated population of the *P. heterotremus* complex.

In conclusion, this study of the molecular analyses of *P. westermani* based on the ITS2 and COI sequence data showed different clusters that formed a species complex. The combined data set of both loci, rooted with Sri Lankan *P. westermani*, yielded five strongly supported clades. A further analysis of the COI-haplotype network revealed 18 haplotypes in which a possible evolutionary scenario of *P. westermani* in Asia could be inferred.



BIBIOGRAPHY

BIBIOGRAPHY

- Arias SM SL, Casas E, Henao A, Velásquez LE. *Paragonimus* sp. in crabs and awareness of the educational community to aquatic ecosystems in La Miel and La Clara, Caldas, Antioquia. Biomedical. 2011; 31(2): 209-15.
- Binchai S, Rangsiruji A, Ketudat P, Morishima Y, Sugiyama H. Molecular systematics of a new form of *Paragonimus westermani* discovered in Thailand. Southeast Asian J Trop Med Publ Health. 2007; 38:92–6.
- Birky CW. The inheritance of genes in mitochondria and chloroplasts: laws, mechanism and models. Annu Rev Genet. 2001; 35:125-48.
- Blair D, Agatsuma T, Watanabe T, Okamoto M, Ito A. Geographical genetic structure within the human lung fluke, *Paragonimus westermani*, detected from DNA sequences. Parasitology. 1997 Oct; 115(4):411-7.
- Blair D, Waikagul J, Honzako Y, Agatsuma T. Phylogenetic relationships among the Thai species of *Paragonimus* inferred from DNA sequences. In: Tada I, Kojima S, Tsuji M, editors. Proceedings of the 9th International Congress of Parasitology; 1998 August 24-28; Chiba, Japan. Monduzzi International, Bologna; 1998. p. 643-7.
- Blair D, Wu B, Chang ZS, Gong X, Agatsuma T, Zhang YN, et al. A molecular perspective on the genera *Paragonimus* Braun, *Euparagonimus* Chen and *Pagumogonimus* Chen. J Helminthol. 1999 Dec; 73(4):295-9.
- Blair D. Genomes of *Paragonimus westermani* and related species: current state of knowledge. Int J Parasitol. 2000 Apr; 30(4):421-6.
- Bowles J, Blair D, McManus DP. A molecular phylogeny of the human schistosomes. Mol Phylogenet Evol. 1995 Jun; 4(2):103-9.
- Brown WM, George MJ, Wilson AA. Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA. 1979 Apr; 76(4):1967-71.
- Brusentsov I, Katokhin AV, Brusentsova IV, Shekhovtsov SV, Borovikov SN, Goncharenko GG, et al. Low genetic diversity in wide-spread eurasian liver fluke *Opisthorchis felineus* suggests special demographic history of this trematode species. Plos One. 2013 Apr; 8(4):e62453. doi: 10.1371/journal.pone.0062453.
- Castilla EA, Jessen R, Sheck DN, Procop GW. Cavitary mass lesion and recurrent pneumothoraces due to *Paragonimus kellicotti* infection: North American paragonimiasis. Am J Surg Pathol. 2003 Aug; 27(8):1157-60.

- Chaichanasak P, Ichikawa M, Sobhon P, Itagaki T. Identification of *Fasciola* flukes in Thailand based on their spermatogenesis and nuclear ribosomal DNA, and their intraspecific relationships based on mitochondrial DNA. *Parasitol Int.* 2012 Dec; 61(4):545-9.
- Chung HL, Ho LY, Hsu CP, Ts'ao WJ. Recent progress in studies of *Paragonimus* and paragonimiasis control in China. *Chin Med J (Engl).* 1981 Aug; 94(8):483-94.
- Clement M, Posada D, Crandall KA. TCS: a computer program to estimate gene genealogies. *Mol Ecol.* 2000 Oct; 9(10):1657-9.
- Devi KR, Narain K, Agatsuma T, Blair D, Nagataki M, Wickramasinghe S, et al. Morphological and molecular characterization of *Paragonimus westermani* in northeastern India. *Acta Trop.* 2010 Oct; 116(1):31-8.
- Devi KR, Narain K, Mahanta J, Nirmolia T, Blair D, Saikia SP, et al. Presence of three distinct genotypes within the *Paragonimus westermani* complex in northeastern India. *Parasitology.* 2013 Jan; 140(1):76-86.
- Doanh PN, Shinohara A, Horii Y, Habe S, Nawa Y. Discovery of *Paragonimus westermani* in Vietnam and its molecular phylogenetic status in *P. westermani* complex. *Parasitol Res.* 2009 Apr; 104(5):1149-55.
- Doanh PN, Thaenkham U, An PT, Hien HV, Horii Y, Nawa Y. Metacercarial polymorphism and genetic variation of *Paragonimus heterotremus* (Digenea: Paragonimidae), and a re-appraisal of the taxonomic status of *Paragonimus pseudoheterotremus*. *J Helminthol.* 2015 Mar; 89(2): 182-8.
- Dreyfuss G, Rondelaud D. Biodiversity of flukes. *Parasite.* 2008 Sep; 15(3):282-5.
- Fürst T, Duthaler U, Sripa B, Utzinger J, Keiser J. Trematode infections: liver and lung flukes. *Infect Dis Clin North Am.* 2012 Jun; 26(2):399-419.
- Guerrant R, Walker D, Weller P. Tropical infectious diseases: principles, pathogens, and practice. Churchill Livingstone; 1999.
- Habe S, Doanh PN, Yahiro S, Vannavong N, Barennes H, Odermatt P, et al. *Paragonimus paishuihoensis* metacercariae in freshwater crabs, *Potamon lipkei*, in Vientiane Province, Lao PDR. *Korean J Parasitol.* 2013 Dec; 51(6):683-7.
- Hebert P, Cywinska A, Ball S, DeWaard J. Biological identifications through DNA barcodes. *Proc Biol Sci.* 2003 Feb; 270(1512):313-21.
- Higo H, Ishii Y. Comparative studies on surface ultrastructure of newly excysted metacercariae of Japanese lung flukes. *Parasitol Res.* 1987; 73(6):541-9.

- Iwagami M, Ho LY, Su K, Lai P, Fukushima M, Nakano M, et al. Molecular phylogeographic studies on *Paragonimus westermani* in Asia. J Helminthol. 2000 Dec; 74(4):315-22.
- Iwagami M, Rajapakse R, Paranagama W, Okada T, Kano S, Agatsuma T. Ancient divergence of *Paragonimus westermani* in Sri Lanka. Parasitol Res. 2008 Apr; 102(5):845-52.
- Iwagami M, Rajapakse R, Yatawara L, Kano S, Agatsuma T. The first intermediate host of *Paragonimus westermani* in Sri Lanka. Acta Trop. 2009 Jan; 109(1):27-9.
- Iwagami M. *Potamiscus cognatus* (Roux, 1936), a new crab host for *Paragonimus westermani* in Malaysia. J Parasitol. 1969 Apr; 55(2):459.
- Kans JA, Ouellette BFF. Submitting DNA sequences to the databases. In: Baxevanis AD, Ouellette BFF, editors. Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, volume 43, 2nd ed. John Wiley & Sons, Inc. 2001. p. 65-81.
- Kuntz RB. Biology of *Paragonimus westermani* (Kerbert, 1878) Braun, 1899: infection in the crab host (*Eriocheir japonicus* de Haan) on Taiwan. Trans Am Microsc Soc. 1969; 88(1):118-126.
- Larkin M, Blackshields G, Brown N, Chenna R, McGettigan P, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007 Nov; 23(21):2947-8.
- Liat L, Betterton C. *Paragonimus westermani* in Malaysian *Felidae* and *Viverridae*: probable modes of transmission in relation to host feeding habits. J Helminthol. 1977; 51(4):295-9.
- Liu Q, Wei F, Liu W, Yang S, Zhang X. Paragonimiasis: an important food-borne zoonosis in China. Trends Parasitol. 2008 Jul; 24(7):318-23.
- Lou HQ, Hu Y, Zhang L, Zhang YJ, Tu PG, Yu XT, et al. Epidemiological survey and phylogenetic analysis of *Paragonimus westermani* isolates in Jinhua, China. Afr J Biotechnol. 2011; 10(65):14286-92.
- Miller MA, Pfeiffer W, Schwartz T. "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA 1-8.

- Mladineo I, Šegvic-Bubic T, Stanic R, Desdevises Y. Morphological plasticity and phylogeny in a monogenean parasite transferring between wild and reared fish populations. *Plos One*. 2013 Apr; 8(4):e62011. doi: 10.1371/journal.pone.0062011.
- Moslemi JM, Snider SB, MacNeill K, Gilliam JF, Flecker AS. Impacts of an invasive snail (*Tarebia granifera*) on nutrient cycling in tropical streams: the role of riparian deforestation in Trinidad, West Indies. *Plos One*. 2012; 7(6):e38806. doi: 10.1371/journal.pone.0038806.
- Muller R. Liver and lung flukes. In: Cox FEG, editor. The Wellcome Trust illustrated history of tropical diseases. The Wellcome Trust, London, United Kingdom; 1996. p. 274-85.
- Orihel TC, Ash LR. Parasites in Human Tissues. ASCP Press; 1995.
- Page RDM, Holmes EC. Molecular evolution: a phylogenetic approach. Blackwell Publishing Ltd.; 1998.
- Prasad PK, Tandon V, Biswal DK, Goswami LM, Chatterjee A. Phylogenetic reconstruction using secondary structures and sequence motifs of ITS2 rDNA of *Paragonimus westermani* (Kerbert, 1878) Braun, 1899 (Digenea: Paragonimidae) and related species. *BMC Genomics*. 2009; 10(3):S25. doi:10.1186/1471-2164-10-S3-S25.
- Procop G. North American paragonimiasis (caused by *Paragonimus kellicotti*) in the context of global paragonimiasis. *Clin Microbiol Rev*. Jul 2009; 22(3):415-446.
- Sato Y, Iwagami M, Iwashita J, Abe T, Yukawa M, Blas LB, et al. Phylogenetic status of a lung fluke in the Philippines based on mitochondrial genome. *J Trop Med*. 2003; 31(1):1-3.
- Singh TS, Sugiyama H, Rangsiruji A. *Paragonimus* & paragonimiasis in India. *Indian J Med Res*. 2012 August; 136(2):192-204.
- Sohn WM, Ryu JS, Min DY, Song HO, Rim HJ, Vonghachack Y, et al. *Indochinamon ou* (Crustacea: Potamidae) as a new second intermediate host for *Paragonimus harinasutai* in Luang Prabang Province, Lao PDR. *Korean J Parasitol*. 2009 Mar; 47(1):25-9.
- Stamatakis, A. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 2014 January; doi: 10.1093/bioinformatics/btu033.

- Sun J, Huang Y, Huang H, Liang P, Wang X, Mao Q, et al. Low divergence of *Clonorchis sinensis* in China based on multilocus analysis. Plos One. Jun 2013; 8(6):e67006. doi: 10.1371/journal.pone.006700.
- Swofford DL. PAUP*: Phylogenetic analysis using parsimony (* and other methods). Version 4. Sunderland, MA, Sinauer Associates; 1998.
- Terasaki K, Habe S, Ho L, Jian H, Agatsuma T, Shibahara T, et al. Tetraploids of the lung fluke *Paragonimus westermani* found in China. Parasitol Res. 1995; 81(7): 627-30.
- Thaenkham U, Waikagul J. Molecular phylogenetic relationship of *Paragonimus pseudoheterotremus*. Southeast Asian J Trop Med Public Health 2008; 39:217.
- van Herwerden L, Blair D, Agatsuma T. Intra- and Inter- individual variation in ITS1 of *Paragonimus westermani* (Trematoda: Digenea) and related species: implications for phylogenetic studies. Mol Phylogenet Evol. 1999 Jun; 12(1): 67-73.
- Vilas R, Criscione CD, Blouin MS. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. Parasitology. 2005 Dec; 131(6):839-46.
- White TJ, Bruns TD, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: Innis MA, Gelfand DH, Sninsky JJ, White TJ editors. PCR protocols, a guide to methods and applications. San Diego, California: Academic Press; 1990.
- Willis CG, Halina M, Lehman C, Reich PB, Keen A, McCarthy S, et al. Phylogenetic community structure in Minnesota oak savanna is influenced by spatial extent and environmental variation. Ecography. 2010 June; 33(3):565-577.
- World Health Organization. Control of food borne trematode infections. WHO technical report series no. 849. Geneva. Organization. 1995. p. 1-157.
- Yang JS, Chen MG, Feng Z, Blair D. *Paragonimus* and paragonimiasis in China: a review of the literature. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2000; 18(special issue):1-78.
- Yokogawa M. *Paragonimus* and paragonimiasis. In: Dawes B, editor. Advance in Parasitology volume 3, 1965. p. 99-158.

Yoonuan T, Vanvanitchai Y, Dekumyoy P, Komalamisra C, Kojima S, Waikagul J.

Paragonimiasis prevalences in Saraburi Province, Thailand, measured 20 years apart. Southeast Asian J Trop Med Public Health. 2008 Jul; 39(4):593-600.





VITAE

Name : KOWIT HENGPHASATPORN

Date of Birth : 25 July 1988

Place of Birth : Bangkok, Thailand

Address : 222/56 Ramindra 14, Ladprao sub-district,
Ladprao district, Bangkok 10230, Thailand

Telephone : 08-1988-3444

E-mail address : Heng.kowit@gmail.com

Education :

2005	High School from Satriwitthaya 2 School
2011	Bachelor of Science (Zoology) from Chiang Mai University
2015	Master of Education (Biology) from Srinakharinwirot University

