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APPENDICES

APPENDIX A
MOLECULAR METHODS

1. SAMPLES

Adult workers of *T. pagdeni* were corrected from north (n = 15), northeast (n = 24), central (n = 61) and peninsular (n = 45) of Thailand. Other species of stingless bees; *T. collina* (n = 111), *T. minor* (n = 23), *T. fuscobalteata* (n = 15), *T. terminata* (n = 14), *T. doipaensis* (n = 1), *T. apicalis* (n = 13), *T. sp* (unknown, n = 3), *T. itama* (n = 5), *T. canifrons* (n = 1), *T. fimbriata* (n = 2), *T. melanoleuca* (n = 1), *T. thoracica* (n = 4), *T. melina* (n = 1), *Lisotrigona furva* (n = 3), *T. laeviceps* (n = 12), were included in the experiment (Figure A.1, Table A.1 and APPENDIX B). These speciments were used to isolate species-specific AFLP markers, to study genetic diversity and population structure of *T. pagdeni* in Thailand using nuclear DNA and mitochondrial DNA polymorphisms, and to determine the partial mitochondrial DNA sequence. The sepciments were identified morphologically according to Sakagami (1978) and Sakagami *et al.* (1983), and verified by Professor Charles D. Michener (University of Kansas).

Figure A.1 Collection sites of stingless bees in Thailand. See Table A.1 for locality names; N = North, NE = Northeast, C = Central and P = Peninsular Thailand.

Table A.1 Collection sites, populations, map abbreviation (Figure A.1) and sample sizes of stingless bees used in this study.

Localities	Population	Map Abbreviations
Chaing Mai	North	CM
Nan	North	NA
Phrae	North	PR
Uttaradit	North	UT
Kamphaengpetch	North	KP
Phitsanulok	North	PL
Phichit	North	PC
Sukhothai	North	ST
Udon Thani	Northeast	UD
Khon Kaen	Northeast	KK
Ubon Ratchathani	Northeast	UB
Si Sa Ket	Northeast	SS
Sakon Nakorn	Northeast	SN
Mukdahan	Northeast	MD
Roi Et	Northeast	RE
Surin	Northeast	SU
Chaiyaphum	Northeast	CY
Buri Ram	Northeast	BR
Maha Sarakham	Northeast	MK
Nakhon Ratchasima	Northeast	NR
Nonthaburi	Central	NB
Lop Buri	Central	LB
Suphan Buri	Central	SP
Sing Buri	Central	SI
Nakhon Nayok	Central	NY
Bangkok	Central	BK
Ayutthaya	Central	AY
Phetchaburi	Central	PB
Kanchanaburi	Central	KB
Ratchaburi	Central	RB
Prachuap Khiri Khan	Central	PK
Trat	Central	TR
Chanthaburi	Central	CT
Chon Buri	Central	CB
Chumphon	Peninsular	CP
Ranong	Peninsular	RN
Krabi	Peninsular	KA

Table A. 1 (continued)

Localities	Population	Map Abbreviations
Nakhon Si Thammarat	Peninsular	NT
Surat Thani	Peninsular	SR
Phatthalung	Peninsular	PT
Songkhla	Peninsular	SK

2. DNA preparation

2.1 Standard phenol-chloroform-SDS method (Smith and Hagen, 1996)

One entire stingless bee per nest was ground in 1.5 ml sterilized microcentrifuge tubes. Genomic DNA was isolated from each frozen bee using phenol-chloroform extraction (Smith and Hagen, 1996). Five hundred microliters of STE extraction buffer (100 mM NaCl, 50 mM Tris-HCl pH 8.0 and 1 mM EDTA), fifteen micrograms and ten micrograms of proteniase K (20 mg/ml) and RNase A (10 mg/ml) were added to the ground tissue and incubated at 65°C for 3 hours. After that, 10 µl of RNase A (10 mg/ml) was added and incubated at 37°C for 1 hour. Then, the sample was extracted twice with an equal volume of phenol: chloroform (1:1) gently. The extracted solution was mixed for 15 minutes and centrifuged at 10,000xg at room temperature for five minutes. The supernatant were extracted with an equal volume of chloroform to remove phenol and centrifuged again at 10,000xg at room temperature for 5 minutes. The resultant supernatant were mixed with a double volume of 95% ethanol and incubated at -20°C overnight to precipitate the DNA. The DNA was centrifuged at 10,000xg at 4°C for 25 minutes. The DNA pellet was dried at 37°C and dissolved with 1XTE buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA). DNA concentration was estimated by comparison the intensity of DNA-ethidium bromide complex with a known quantity of digested lambda DNA on a mini-gel (Sambrook and Russell, 2001). Genomic DNA was stored at -4°C until use.

2.2 Measuring nucleic acid concentration using spectrophotometry

The extracted DNA concentration of individual bee from each colony was estimated by the optical density at 260 nanometre (OD_{260}). An OD_{260} of 1.0 present concentration of 50 µg/mg double stranded DNA (Sambrook *et al.*, 2001). Therefore, the DNA concentration was estimated in µg/ml by using the formula,

$$[DNA] = OD_{260} \times \text{dilution factor} \times 50.$$

The purity of DNA samples were evaluated by comparison a ratio of OD_{260}/OD_{280}. The ratio of the purified DNA was approximately 1.8 (Sambrook *et al.*, 2001).

3. Development of *T. pagdeni* specific marker using AFLP (Amplified Fragment Length Polymorphism) and SSCP (Single Strand Conformational Polymorphism) procedure

3.1 Restriction enzyme digestion and adaptor ligation

The AFLP procedure was performed following the protocol of Vos *et al.* (1995) with a few modifications. Each genomic DNA (250 ng) of different *Trigona* species was simultaneously digested with 2.5 units of *Pst*I in a 25 µl reaction mixture consisted of 1XO-Phor-Ail buffer (10 mM Tris-acetate, pH 7.5, 10 mM magnesium acetate, and 50 mM potassium acetate) at 37°C for approximately 3 hours. Then, the *Pst*I digested reaction was inactivated at 65°C for 15 minutes. After that, *Tru*9I (3 units) was added in a final volume of 40 µl which contained 1xO-Phor-All buffer, and incubated at 65°C for approximately 3 hours. After that, the *Pst*I and *Mse*I double stranded adaptors (Table A.2) were ligated to the restriction fragments at 12°C for overnight by adding 10 µl of the adaptor ligation solution, which consisted of *Pst*I and *Mse*I adaptors, 0.4 mM ATP, 10 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate and 50 mM potassium acetate, and 1 unit of T4 DNA ligase.

3.2 Pre-amlification

This ligated DNA was used as a DNA template. The preamplification reaction was performed by using 30 ng of P+A (5'-GACTGCGTACATGCAG**A**-3') and M+C (5'-GATGAGTCCTGAGTAA**C**-3') primers, which are specific to the adaptors, containing a single sclective nucleotide which described in Table A.2 at 3' end in a 25 µl reaction volume including 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 0.1 % Triton X-100, 200 µM each dNTP, 1.5 mM $MgCl_2$, 1.5 units of DyNAzyme TM II DNA polymerase (Finzymes) and 1 µl of the ligation product. The PCR was processed by a Perkin Elmer 9700 thermocycler containing 20 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 1 minute and extension at 72°C for a minute. The final extention was 72°C for 5 minutes.

3.3 Selective amplification

The pre-amplification product is diluted for 12.5 fold with sterile deionized water and selectively amplified with primers containing three selective bases shown in Table 2 at the 3' terminus of each primer. Each 25 μl of selective amplification reaction contained 10 mM Tris-HCl, pH 8.8, 50 mM KCl 0.1% Triton X-100, 200 μM of each dNTP, 1.5 mM $MgCl_2$, 30 ng of combinations of P_{+3} and M_{+3} primers, 1.5 units of DyNAzyme TM II DNA polymerase (Finzymes) and 1 μl of the diluted preamplification product. The following cycle was consisted of denaturation at 94°C for 30 seconds, annealing at 65°C for 45 seconds and extension at 72°C for 90 seconds for 2 cycles. And then, it was followed by 12 cycles of a touch down phase with lowering of the annealing temperature for 0.7°C in every cycle. The amplification was further carried out for another 28 cycles of 94°C for 30 seconds, 53°C for 45 seconds and 72°C for 90 seconds. The final extension was performed at 72°C for 5 minutes.

3.4 Agarose gel electrophoresis

The desired amount of agarose powder was weighed and mixed with an appropriate volume of 1XTBE (89 mM Tris-HCl, 89 mM boric acid and 2.5 mM EDTA, pH 8.3). The gel slurry was heated in a microwave oven until completely melted. After cooling the solution to about 60°C, it was poured into the gel tray containing a comb and allowed to solidify at room temperature. When required, the comb was carefully removed. The agarose gel, still in its plastic tray, was inserted into the electrophoresis chamber and covered with an enough amount of 1XTBE buffer for approximately 0.5 cm. DNA samples or PCR products were then mixed with the one-fifth volume of the 10x loading dye (0.25% bromophenol blue and 25% Ficoll in water) and pipeted into the well. A 100 bp DNA ladder or λ /*Hin*dIII was used as the standard marker. Electrophoresis was performed in 1XTBE buffer at 100 volts until migration of the bromphenol blue dye monitoring approximately one half of the gel. The electrophoresis gel was then stained with ethidium bromide solution (0.5 μg/ml) for 5-15 minutes and destained by soaking in the deionized water to remove unbound ethidium bromide from the gel. To visualized DNA fragments, the gel was placed on an ultraviolet transilluminator and photographed.

Table A.2 AFLP primers and their sequences used for the PCR amplification and ligation procedure

Primer	Sequences
Adaptor sequences	
*Pst*I adaptor	5'-CTCGTAGACTGCGTACATGCA-3'
	5'-TGTACACAGTCTAC-3'
*Mse*I adaptor	5'-GAC GAT GAG TCC TGA G-3'
	5'-TAC TCA GGA CTC AT-3'
Preamplification primers	
P_{+A}	5'-GACTGCGTACATGCAGA-3'
M_{+C}	5'-GAT GAG TCC TGA GTA AC-3'
Selective amplification primers	
P_{+3}-1	P_{+A}-AC
P_{+3}-2	P_{+A}-AG
P_{+3}-3	P_{+A}-CA
P_{+3}-4	P_{+A}-CT
P_{+3}-5	P_{+A}-CC
P_{+3}-6	P_{+A}-CG
P_{+3}-7	P_{+A}-GC
P_{+3}-8	P_{+A}-GG
P_{+3}-9	P_{+A}-GT
P_{+3}-10	P_{+A}-GA
P_{+3}-11	P_{+A}-TG
P_{+3}-12	P_{+A}-TC
P_{+3}-13	P_{+A}-TA
P_{+3}-14	P_{+A}-TT
P_{+3}-15	P_{+A}-AA
P_{+3}-16	P_{+A}-AT
M_{+3}-1	M_{+C}-A A
M_{+3}-2	M_{+C}-AC
M_{+3}-3	M_{+C}-AG
M_{+3}-4	M_{+C}-AT
M_{+3}-5	M_{+C}-TA
M_{+3}-6	M_{+C}-TC
M_{+3}-7	M_{+C}-TG
M_{+3}-8	M_{+C}-TT
M_{+3}-9	M_{+C}-GA
M_{+3}-10	M_{+C}-GT
M_{+3}-11	M_{+C}-GC
M_{+3}-12	M_{+C}-GG
M_{+3}-13	M_{+C}-CA
M_{+3}-14	M_{+C}-CT
M_{+3}-15	M_{+C}-CG
M_{+3}-16	M_{+C}-CC

3.5 Preparation of glass plate for polyacrylamide gel electrophoresis

Two glass plates; the long and the short glass plates, were cleaned with de-ionized water to eliminate impurities, finally washed with 2 ml of 95% commercial grad ethanol in one plane of glass and allow the alcohol to dry. This cleaning step was then repeated twice. Afterwards, the long glass plate was completely covered with 1 ml of freshly prepared binding solution containing 4 µl of bind silane (Pharmacia, USA), 995 µl of ethanol and 5 µl of glacial acetic acid, and left for approximately 10-15 minutes. The coated long glass was further cleaned with 95% ethanol for 3 times to eliminate excess binding solution. For the short glass plate, it was also treated as the long one which referred above, except for coating step. The short glass was covered by the Repel silane (2% dimethyldichlorosilane in octamethylcyclotetrasitoxone). The cleaned glass plates were assembled to each other with a pair of spacer in between. The bottom and both sides of assembled glass plates were sealed with the plastic tape.

3.6 Preparation of denaturing polyacrylamide gel electrophoresis

The products of AFLP were separated on the denaturing polyacrylamide gel. The denaturing polyacrylamide gel was provided by the combination of 19% of acrylamide, 1% bisacrylamide, 7 M urea and 10XTBE (0.89 M Tris, 0.89 M boric acid, 0.025 M EDTA, pH 8.3) in final volume of 40 µl. The acrylamide solution was gently swirled and degassed for 20 minutes. Before it was poured into the assembled plates using a 50 ml syringe, the acrylamide solution was then added with 240 µl of freshly prepared 10% ammonium persulphate and 24 µl of TEMED, and gently swirled approximately 1 minute. The flat edge of the shark-tooth comb was then inserted. The filled plate sandwich was allowed to polymerize at room temperature in the horizontal position for 1 hour. After the gel had polymerized, it was covered by water-soaked tissue paper and hold by the spring clips. The polymerized gel was left at room temperature for 4 hours (or overnight) for complete polymerization. When all was done, the spring clips and the sealing tape were carefully removed. The shark-tooth comb was rinsed with water.

3.7 Electrophoresis

The assembled gel was placed in the vertical sequencing apparatus with the short glass plate inward, and carefully clamped with integral gel clamps along with the sides of the sequencing apparatus. The upper and lower buffer chambers were carefully filled with approximately 300 ml of 1XTBE. The shark-toothed comb was then gently reinserted into the gel until the teeth just touched the surface of the gel. A 6% denaturing polyacrylamide gel (19% acrylamide, 1% bisacrylamide, 7 M urea and 10XTBE; 0.89 M Tris, 0.89 M boric acid, 0.025 M EDTA, pH 8.3) was prerun at 35 W for 15 minutes.

The amplification products (6 µl) was mixed with 3 µl of the loading buffer (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol) and denatured at 95°C for 5 minutes, quickly cooled on ice. Six microlites of DNA samples was carefully loaded to each well. Electrophoresis was performed in 1XTBE for 2 hours at 35 W (Blears, 1998).

3.8 Silver staining

The assembled gel plates were carefully separated by a plastic wedge. The long glass plate with the gel was placed in a plastic tray, containing 2 litres of the fix/stop solution, and then was well shaken for 30 minutes. The gel was soaked in the deionized water and shaken for 3 minutes three times. The gel was taken out of the tray, and was then incubated in a mixture of 0.1% silver nitrate and 0.15% formaldehyde (1.5 liters) with agitation at room temperature for 30 minutes. After incubation, the gel was placed in deionized water (1.5 liters) and shaken with agitation. This step should take no longer than 10-15 seconds to soak the gel in the deionized water, and immediately immersed in 1.5 liters of the chilled developing solution (3% sodium carbonate, 0.15% formaldehyde, 0.02% sodium thiosulphate) and shaken until first band was observed. The gel was then transferred to another chilled developer (1.5 liters) and shaken until the bands become visualized (usually 2-3 minutes). When the bands reached desirable intensity, 10% acetic acid (2 liters) was directly added to stop the development and shaken continuously for 3

minutes. The stained gel was rinsed with deionized water at least 3 minutes. The gel was left at room temperature (for SSCP gels) or at 80° C for 2-3 hours (for AFLP gels).

3.9 Cloning and characterization of candidate species-specific AFLP fragment

3.9.1 Elution of DNA from polyacrylamide gels

AFLP fragments represented in only *Trigona pagdeni* were considered. Candidate species-specific AFLP fragment were rinsed gently with deionized water and excised from the gel using a steriled razor blade. The gel fragment was then washed with 500 µl of sterile deionized water 2 times for 2 hours each at room temperature. After that, twenty microlitres of water was added, and incubated at 50°C for 30 minutes and overnight at 30°C. Reamplification of the target fragment was performed by the original primer pairs used in the selective amplification process according to the same PCR recipes, with the exception that 60 ng of each primer and 5 µl of the eluted AFLP product were used. The PCR profiles were predenatured at 94°C for 3 minutes. Then, the amplification reaction was carried out by 5 cycles of 94°C for 30 seconds, 42°C for 45 seconds and 72°C for 1 minute, followed by additional 35 cycles at higher stringent annealing temperature at 50°C. The final extension was performed at 72°C for 7 minutes. The reamplified product was electrophoretically analyzed through a 1.5% agarose gel at 100 volt for approximately 40 minutes.

3.9.2 Elution of DNA from agarose gels

The required DNA fragment was elute out from 1.5% agarose gel without contamination from ethidium bromide (EtBr) and UV damage by dividing through agarose gels in triplication. The 100 bp -DNA marker was run in first well. Second one was loaded with 5 µl of the DNA fragment into the distal well of the gel, and another loaded side by side with the remained DNA fragment. After electrophoresis, both lanes, the DNA standard one and its proximal DNA sample one, were cut and strained with ethidium bromide for 1 minute. The markers and the DNA sample were visualized by UV light and marked on the EtBr-stained fragment by a razor blade. Position of the

markers and the EtBr-stained fragment were used to regulate the position of the non-stained target DNA fragment.

The excised DNA fragment (unstained) was transferred into a microcentrifug tube and weighed. DNA was recovered from the agarose gels using a Qiaqiuck gel extraction kit (QIAGEN), according to the conditions recommended by the manufactures. The purified sample was stored at -20°C until required.

3.9.3 Ligation of PCR product to vector

The ligation reaction was processed in the total volume of 10 µl. The reaction mixture included 3 µl of the gel eluted PCR product, 25 ng of pGEM® -T easy vector, 5 ul of 2XRapid ligation buffer (60 mM Tris-HCl pH 7.8, 2.0 mM DTT, 2 mM ATP and 10% PEG 80000) and 3 units of T4 DNA ligase. The reaction compound was carefully mixed by pipetting and then was incubated at 4°C overnight.

3.9.4 Preparation of competent host cells

A colony of *E. coli* stain JM109 was cultured as the starter in 50 mL of LB broth (1% bactrotryptone, 0.5% bacto yeast extract and 0.5% NaCl), and shaken at 37°C overnight. A half of the starter were combined in 250 ml of LB broth medium, and then cultured at 37°C with vigorous shake for 3-4 hours (OD 260~0.5-0.8). When it reached desirable growth, the culture was then placed on ice for 15-30 minutes and centrifuged in a cold rotor (Beckman J2-21, USA) at 4,000xg for 15 minutes. After that, the supernatant was removed, pellet was cleaned by resuspending in a 250 ml of cold sterile deionized water and then it was centrifuged again. The pellet was resuspended gently with 30 ml of cold sterile $MgCl_2$-$CaCl_2$ (20 mM $CaCl_2$ and 80 mM $MgCl_2$) and centrifuged again. Finally, the pellet was further resuspended gently to a final volume of 2 ml of 0.1 mM cold sterile $CaCl_2$, aliquoted (100 µl) into 1.5 ml microcentrifuge tubes and stored at -80°C until required.

3.9.5 Transformation of the ligation product to *E. coli* host cells

The portion of ligation mixture was transformed into competent cell *E. coli* strain JM109 by $CaCl_2$ procedure (Sambrook and Russell, 2001). One hundred microliters of the cell suspension were gently placed on ice. Then, 10 µl of ligation mixture was added and mixed gently. The cell suspension was left on ice for 30 minutes. After that, the mixture of cells and DNA was shocked by heating at 42°C for 90 seconds and set on ice rapidly approximately 1-2 minutes. The end of this process, the cells were immediately resuspended with 800 µl of LB medium (1% bactrotryptone, 0.5% bacto yeast extract and 0.5% NaCl), and incubated with shaking at 37°C for 1 hour. After incubation period, the cultured cell suspension was centrifuged at 10,000xg for 30 seconds at room temperature.

The pellet was gently resuspended in 100 µl of LB and spread on the LB agar plates, which included 50 µg/ml of amplicillin, 25 µg/ml of IPTG and 20 µg/ml of X-gal. Finally, it was incubated at 37°C overnight. As a result, the recombinant clones, containing the target insert, are white, where those without the inserted DNA are blue.

3.9.6 Detection of recombinant clone by colony PCR

The recombinant clones containing the inserted DNA were identified by colony PCR using universal primers. The colony PCR was carried out in a 25 µl reaction volume composted of 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 0.1% Triton X-100, 200 each of dATP, dTTP, dGTP and dTTP, 1.5 mM $MgCl_2$, 0.2 µM of SP6 (5' ATTTAGGTGACACTATAGAA-3') and T7 (5'TAATACGACTCACTATAGGG-3') primers and 1 unit of DyNazyme™II DNA Polymerase. White positive colonies are picked up and mixed well by micropipette tip in the amplification reaction. The PCR reaction was predenatured at 94°C for 3 minutes and followed by 35 cycles of 94°C for 30 seconds, 50°C for 60 seconds and 72°C for 60 seconds. The final extension was performed at 72°C for 7 minutes. The PCR products were electrophoretically analyzed through 1.5% agarose gel.

3.9.7 Plasmid extraction

A recombinant colony was picked up into 3 ml of LB broth containing 50 μg/ml of amplicillin, and cultured with constant shaking at 250 rpm overnight at 37°C. The end of this period, the cell culture was collected in 1.5 ml microcentrifuge tube and centrifuged at 10,000xg for 1 minute. Recombinant plasmid DNA was isolated by using a QIAprep spin miniprep kit (QIAGEN). A 250 μl of the P1 buffer was used to resuspend the cell pellets. Then, the cell suspension was added with a 250 μl of the P2 buffer and gently inverted 4-6 times, and centrifuged at 10,000xg for 10 minutes. The supernatant was carefully transferred to the QIAprep column and centrifuged at 10,000xg for 1 minute. The flow-through solution was discarded. The QIAprep spin column was placed back into the collection tube, and washed by adding 500 μl of the PB buffer and centrifuged again at 10,000xg for 1 minute. The flow-through solution was discarded. The column matrix was dried by centrifuge again at 10,000xg for 3 minutes. The collection tube containing the flow-through solution was removed. The QIAquick column was then placed into a sterile 1.5 ml microcentrifuge tube. Plasmid DNA was eluted out by adding 15-50 μl of the EB buffer (10 mM Tris-HCl, pH 8.5) in the center of the QIAprep column matrix, and then was let stand for 2 minutes until the elution buffer is absorbed by the matrix. After that, the purified plasmid DNA was collected by centrifuge at 10,000xg for 1 minute and electrophoretically analyzed through 0.7% agarose gel. The concentration of eluted plasmid DNA was spectrophotometrically measure.

3.10 DNA sequencing and primer design

Determining the DNA sequence is useful in several researches and provides highly reproducible and genetic informative analysis (Weising *et al.*, 1995). In the past years, DNA sequencing has been carried out with 2 methods; the chain-termination method developed by Sanger *et al.* in 1977 and chemical cleavage method according to Maxam and Gilbert in 1977. Due to their technical complexity, these sequencing procedures require extensive use of hazardous chemicals, and difficulties with scale-up.

Nowadays, automated DNA sequencing (DNA sequencers) was developed to support large genomics projects and the need to increase productivity (Figure A.2). The current methods can directly sequence in a single reaction and are able to sequence as many as 384 fluoresecently labeled samples in a run and carry out as many as 24 runs a day. Thus, this current method is appropriate for population genetic studies and systematic studies of various organisms.

The recombinant plasmid was sequenced using the M13 reverse or M13 forward primers under BigDyeTM terminator cycling conditions on an automatic sequencer 3730xl (sequencing service, Macrogen Inc; Korea). All sequences were analyzed and compared by the homology search to confirm the correct fragments using Blast*N* (nucleotide similarity) and Blast*X* (translated protein similarity) available at http:// www. ncbi.nlm.nih.gov.. A pair of PCR primers is designed from each fragment using Fast PCR program version 5.2.21 (Kalenda R., 2007) (Table A.3).

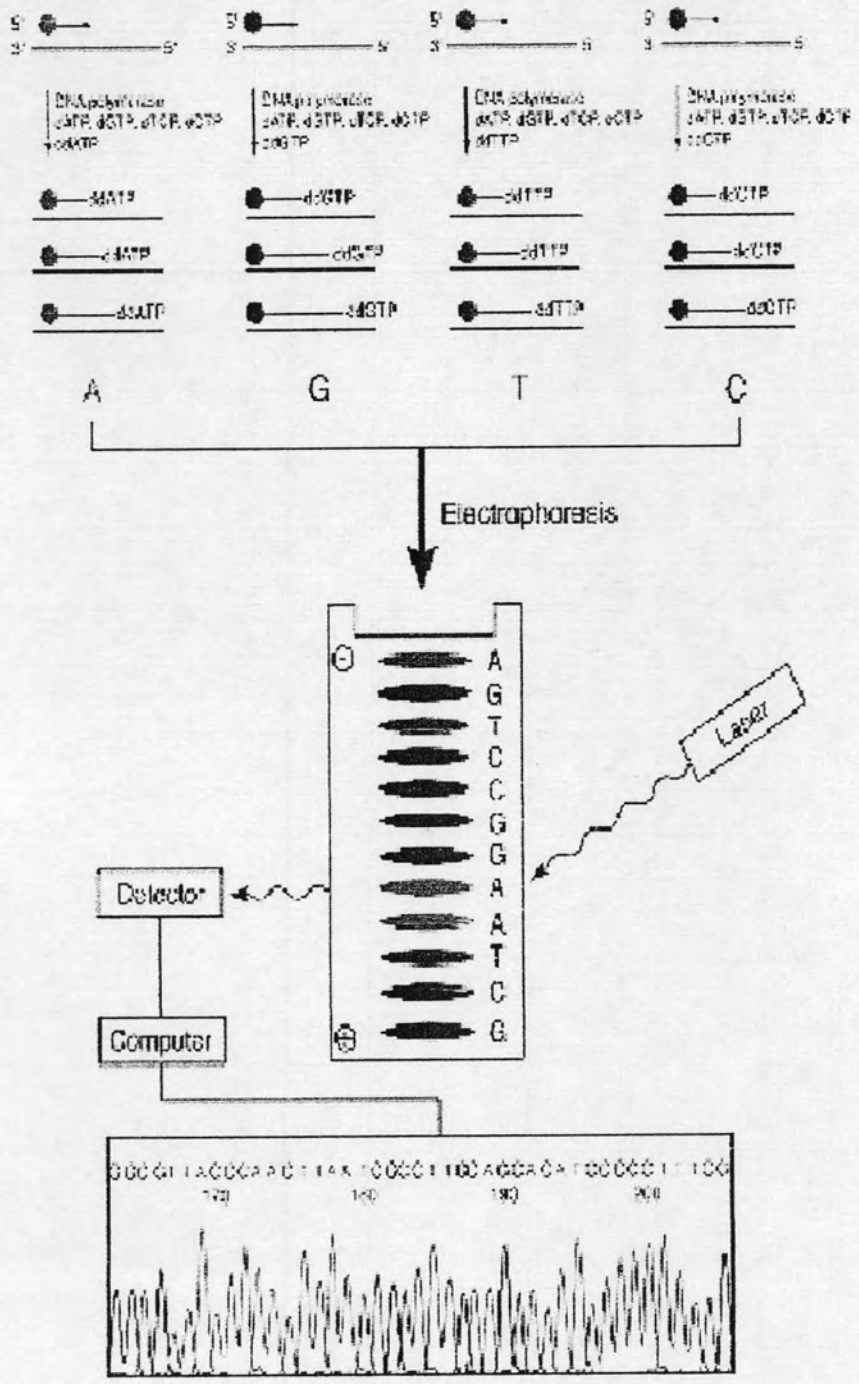


Figure A.2 Automated DNA sequencing

3.11 Polymerase chain reaction and species-specific test

PCR amplification of the extracted DNA were carried out in a 25 μl reaction volume which usually contained 10 mM Tris-HCl (pH 8.8), 50 mM KCl and 0.1% Triton X-100, 2.0 mM $MgCl_2$, 100 μM dNTPs, 1 unit of DyNAzyme™DNA polymerase, 0.1 μM of each primer (Table A.3) and 50 ng of genomic DNA. The amplification reaction was performed as described in Table A.4. For species-specific test, the primer pairs are used to investigate the presence of the expected PCR product size in representative individuals of *T. pagdeni* and other *Trigona* species. The amplification product was analyzed through 1.6% agarose gel electrophoresis at 7.5 V/cm and visualized under a UV transilluminator after ethidium bromide staining (Sambrook and Russell, 2001). The positive PCR products are electrophoretically analyzed and purified by a Qiaqiuck gel extraction kit (QIAGEN), as described previously. After that, the purified amplification products were further characterized following single strand conformational polymorphism (SSCP) to determine polymorphisms of the amplification products.

Table A.3 Primers designed from a candidate species-specific AFLP marker of *T. Pagdeni*

Primer	Sequence	Tm (°C)	Expected size (bp)
CUTPTL1-F	5'-CAT CTG GTT GTC GGT CTG TAA-3'	55.5	163
CUTPTL1-R	5'-TTC CTT CTC CTA ATC TTT GCG G-3'	55.7	

Table A.4 PCR profiles and composition for specificity test of a SCAR marker derived from the candidate species-specific AFLP marker of *T. pagdeni*

Primer	MgCl2 (mM)	Primer (μM)	PCR condition
CUTPTL1	2.0	0.1	-94° C for 3 minutes -35 cycles of 94°C for 30 seconds 56°C for 50 seconds 72°C for 30 -72°C for 7 minutes

3.12 Non-denaturing polyacrylamide gel electrophoresis

Non-denaturing polyacrylamide gel was used for fractionation of single-stranded DNA secondary structure conformation. The glass plates (PROTIN II xi Cell) were cleaned and prepared as described above (Topic 3.5). A 40% stock solution (37.5:1 crosslink) was diluted to prepare 17.5% polyacrylamide solution. The acrylamide gel solution (30-40 ml) was mixed with 1XTBE (dilution of 10XTBE; 0.89 M Tris, 0.89 M boric acid, 0.025 M EDTA, pH 8.3), as well as 300 µl of 10% ammonium persulfate and 30 µl of TEMED. The analytical comb was inserted into the prepared gel, and left to polymerize at least for 4 hours.

3.13 SSCP (Single Strand Conformational Polymorphism) analysis

For SSCP experiments, a volume (8 µl) of the purified PCR products from AFLP-derived fragments was mixed with 32 µl of the formamide loading dye (95% formamide, 0.25% bromophenol blue, 0.25% xylene cyanol and 10 mM NaOH), denatured at 95°C in a hot plate for 5 minutes and immediately chilled on ice for 3 minutes. The denatured samples were then loaded into the slots of the native polyacrylamide gels, run in 1XTBE at 250-300 volts for 16-18 hours at 4°C. The gels were then silver stained, as described earlier (Topic 3.8), and sealed with plastic films.

4. Genetic diversity and population structure of *Trigona pagdeni* Schwarz in Thailand by using TE-AFLP (Three Enzymes Amplified Fragment Length Polymorphism)

4.1 TE-AFLP was performed according to protocol of van der Wurff *et al.* (2000).

4.1.1 Restriction enzyme digestion and adaptor ligation

Approximately 30 ng of genomic DNA extracted from each bee was added to digestion-ligation combination in a final volume of 20 µl containing 1XDigestion-ligation buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT,

0.5mM ATP), 4 picomol of both *Xba*I and *Bam*HI adaptors (Table A.5), 0.5 unites T4 DNA ligase, 6 units *Xba*I and 1.25 units *Bam*HI and 1 unit *Rsa*I and incubated at 30°C for approximately 1.5 hours.

4.1.2 PCR amplification

The digestion-ligation mixure of 0.5 µl was used as template for PCR amplification, total volume of 12.75 µl PCR reaction containing 2.5 picomol each of unlabelled *Xba*I- primer and ^{32}P-labelled *Bam*HI primer, 1XPCR buffer (10 mM Tris-HCl, pH 7.6, 50 mM KCl, 0.1% Triton X-100 and 1.5 mM $MgCl_2$), 50 µM of each dNTP (Promega), and 0.6 units *Taq* DNA polymerase (Promega). The *Xba*I and *Bam*HI primer sequences (van der Wurff *et al.*, 2000) are identical to one strand of the *Xba*I and *Bam*HI linkers, respectively, with arbitrary base extensions. We used a labeled *Bam*HI primer with the arbitrary extension "C" or "G", and paired it with two different *Xba*I primers: X_{+AC}, X_{+CC}, X_{+CT} and X_{+TG} (Table A.5). The polymerase chain reaction was performed on a thermal cycler (TC-412; TECHNE) using 2.5 minutes denaturation at 95°C followed by: 10 cycles of 30 seconds denaturation at 95°C, 30 seconds annealing at 70°C, 60 seconds elongation at 72°C, and 40 cycles of 30 seconds denaturation at 95°C, 30 seconds annealing at 60°C and 60 seconds elongation at 72°C, with a final 20 minutes at 72°C (van der Wurff *et al.*, 2000).

For analysis, the PCR product was mixed with 3 µl loading dye (formamide containing 5 mg/ml blue dextran and 2.4 µl of sterile double-distilled water) and denatured for 5 minutes at 95°C. Three µl was loaded on an 8% denaturing polyacrylamide gel with 0.6XTBE electrophoresis buffer and electrophoresed for 5-6 hours at 500 V. The dried gel was used to expose Kodak X-Omat film.

4.1.3 Scoring TE-AFLP variation

Bands generated by each primer combination were scored by eye. Presence of a TE-AFLP band was scored as 1, absence as 0. Thus each bee was characterized by a multi-locus phenotype.

4.2 Data analysis

Individuals were grouped into four populations based on collection site: North, Northeast, Central and Peninsular of Thailand (Figure A.1, Table A.1). Genetic diversity, genetic distance among populations, and population structure statistics were calculated using Genetic Analysis in Excel (GenAlEx6; Peakall and Smouse, 2006).

Genetic diversity was estimated as the proportion of polymorphic loci (P), and expected heterozygosity (H_e). A band was considered polymorphic if our samples showed any variation for presence or absence, though "singletons" (bands present or absent in only a single individual) and other rare variants have little effect on assessment of population structure. Estimation of H_e followed the method of Lynch and Milligan (1994), which considers each band position as a different locus with two alleles, band amplified (dominant) and band not amplified (recessive); absence of a band indicates a recessive homozygote. Populations were assumed to be outbreeding or completely inbreeding. At each locus, the frequency of the recessive allele (q) was estimated from the frequency of putative recessive homozygotes (q^2), and the frequency of the dominant allele is estimated as: $p = 1 - q$. Expected heterozygosity at each locus = $h = 1 - \Sigma x_i^2$, where x_i is the frequency of the *i*th allele, or in this case, $1 - (p^2 + q^2)$. Expected heterozygosity averaged over all loci was calculated as:

$$H_e = 1 - 1/m \sum_{y=1}^{m} \Sigma x_i^2$$

where y represents loci or bands 1 through m.

Analysis of Molecular Variance (AMOVA; Excoffier *et al.*, 1992) implemented in GenAlEx (Peakall and Smouse, 2006) was used to assess the patterns of observed genetic variation. AMOVA can be used with binary or dominant markers such as TE-AFLPs to calculate Φ statistics, which are analogous to Wright's F-statistics for co-dominant data (Wright, 1951; 1965). Φ_{PT}, which can take values from 0-1, measures the similarity of pairs of individuals drawn at random from the same population, relative to pairs of individuals drawn from the entire collection, and is analogous to Wright's F_{ST}.

Significance testing was performed by comparing observed statistics to the distributions generated by 999 random permutations of the data into populations of the same size.

In order to search the data set for geographic patterns, we examined (1) differentiation among the four geographic populations and (2) differentiation between Peninsular samples and those from north of the Isthmus of Kra. Because mean expected heterozygosity and pairwise genetic distances among the four populations indicated the Northeast population differed from the others, we also examined (3) differentiation between Northeast samples and the others. In each case, significance testing was performed by comparing observed statistics to the distributions generated by 999 random permutations of the data into populations of the same size.

5. Genetic diversity and population structure of *Trigona pagdeni* Schwarz in Thailand by using mtDNA polymorphisms

5.1 PCR amplification and development of primers

The primers, shown in Table A.6, were successfully developed to amplify mitochondrial genes in stingless bees. A portion of cytochrome b (cyt b) mitochondrial DNA gene was known from (GenBank accession no. AY575080). In the other hand, mitochondrial DNA (mtDNA) gene segments of cytochrome c oxidase I (COI), large ribosomal RNA (16S) and ATP synthase subunit 6 and subunit 8 (ATPase(6,8)) and cytochrome c oxidase III (COIII) were obtained from PCR amplification using primers are described in Table A.6 respectively. These three portions were amplified by PCR conditions described in Table A.7. Then, the PCR products were purified by using a Qiaqiuck gel extraction kit (QIAGEN) according the protocol provided above (Topic 3.9.2). After purification, the samples were sequenced by using the M13 reverse or M13 forward primers which referred above. The cyt b, 16S rRNA, and COI segment of mtDNA sequences obtained were used to design internal primers using Fast PCR program version 5.2.21 (Kalenda, 2007) for the inverse PCR and genome walking technique.

Table A.5 TE-AFLP primers and their sequences used for the PCR amplification and ligation procedure

Primer	Sequences
Adaptor sequences	
BamH I adaptor	5'-ACG AAG TCC CGC GCC AGC AA-3'
	5'-GAT CTT GCT GGC GCG GG-3'
Xba I adaptor	5'-ACG TTG TGG CGG CGT CGA GA-3'
	5'-CTA GTC TCG ACG CCG CC-3'
Selective amplification primers	
B_{+G}	5'-GTT TCG CGC CAG CAA GAT CCG-3'
B_{+C}	5'-GTT TCG CGC CAG CAA GAT CCC-3'
X_{+AC}	5'-GGC GTC GAG ACT AGA AC-3'
X_{+CC}	5'-GGC GTC GAG ACT AGA CC-3'
X_{+CT}	5'-GGC GTC GAG ACT AGA CT-3'
X_{+TG}	5'-GGC GTC GAG ACT AGA TG-3'

Table A.6 Pairs of primers used to amplify mtDNA regions of *T. pagdeni*

Primer	Sequence	T_m (°C)	Main genes	Expected size (bp)	Reference
COI-2191	5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3'	50	COI	500	Hall&Smith,1991
COI-1718	5'-GGA GGA TTT GGA AAT TGA TTA GTT CC-3'	55.2			
LR13943F	5'–CAC CTG TTT ATC AAA AAC AT–3'	48.2	16S rRNA	550	Costa et al., 2003
LR13392R	5'–CGT CGA TTT GAA CTC AAA TC–3'	51			
MtD19	5'-GAA ATT TGT GGA GCA AAT CAT AG-3'	51.9	ATPase(6, 8), COIII	1700	Simon et al., 1994
MtD22	5'-TCA ACA AAG TGT CAG TAT CA-3'	50.1			

Table A.7 PCR conditions used for amplification of mtDNA gene of *T. pagdeni* using primers in Table A.6

Primer	**dNTPs (μM)**	**$MgCl_2$ (mM)**	**Primer (μM)**	**PCR condition**
COI-2191 COI-1718	250	2.5	0.1	-94°C for 1 minute -35 cycles of 94°C for 30 seconds 40°C for 30 seconds 72°C for 1 minute for - 72°C for 10 minutes
LR13943F LR13392R	200	2.5	0.1	-94°C for 3 minutes - 5 cycles of 94°C for 30 seconds 40°C for 60 seconds 72°C for 50 seconds -35 cycles of 94°C for 30 seconds 56°C for 60 seconds 72°C for 50seconds -72°C for 7 minutes
MtD19 MtD22	250	2.0	0.1	-94°C for 1 minute -35 cycles 94°C for 60 seconds 42°C for 80 seconds 72°Cfor 1 minute - 72°C for 10 minutes

5.2 Inverse PCR

The inverse polymerase chain reaction (Ochman *et al.*, 1988) is an application of the conventional PCR amplification used to obtain flanking regions of unknown sequences (Figure A.3). DNA is digested with a restriction enzyme that does not cut the template DNA, and ligated under optimal conditions to make a circular DNA. PCR primers pointing away from the known sequences are used to amplify the flanking sequences. This method has been generally applied in several studies and can be used to analyze the regions adjacent to a known sequence, or to proceed along a stretch of uncharacterized DNA (Bensch *et al.*, 2002).

The mtDNA sequence of COI gene obtained with such primers (COI-2191/ COI-1718) revealed in Table A.6, was used to screen for cut sites of restriction enzymes by using NEBcutter program version 2 (http://tools.neb.com/NEBcutter2/index.php). Then, genomic DNA from one *Trigona pagdeni* was treated in three separated tube in a total volume of 100 µl, each containing one of these enzymes; *Nco*I, *Hind*III and *Hinf*I; Table A.8. The restriction reactions were incubated at 37°C for 3 hours. Then, the digested DNA was purified by phenol-chloroform and precipitated with 95% ethanol as referred earlier. This was followed by a step of self-ligation, by adding 5 units of ligase and 1XLigase buffer (0.4 mM ATP, 10 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate and 50 mM potassium acetate) and holding overnight at 16°C. The self-ligated DNA mixture was purified again by phenol-chloroform and precipitated with 95% ethanol, and used as the template for the inverse PCR with the primers Inverse COI-1 (5'-CCATTTTTTGATCCTACAGAAGGAGATCC-3') and Inverse COI-1 R (5'-GTGGAAATGCTATATCCGGTGATCCTAATA-3') (annealing temperature 50°C and extension at 72°C for 3 minutes). The PCR combination containing 0.5 units Taq DNA polymerase, 1XPCR buffer (10 mM Tris-HCl (pH 8.8), 50 mM KCl and 0.1% Triton X-100), 1.1 mM $MgCl_2$, 200 µM dNTP and 0.2 µM of each primer. The PCR profile was performed with 94°C for 3 minutes, followed by 94°C for 30 seconds, 50°C for 60 seconds and 72°C for 3 minutes for 35 cycles and the final extension was 72°C for 10 minutes. When checked on a 1% agarose gel, it showed that the template treated with

*Hinf*I resulted in an amplified fragment of approximately 600 bp. This fragment was purified by using a Qiaqiuck gel extraction kit (QIAGEN) according the protocol described earlier (Topic 3.9.2), then sequenced from both ends with the original primers and aligned with the original COI sequence (approximately 424 included primer sites). The analysis revealed that the *Hinf*I cut site was located at 390 bp and 174 bp away from 5' and 3' of the ends of the original COI fragment, respectively. Thus the total length between the *Hinf*I cut sites was 988 bp. Two new primers, COI-F (5'-ATAATTATTGTTGCTGATGTA-3') and COI-R (5'-CTATTCATATAACTGGAATTTC-3'), were designed (pointing inwards) on each side of the original fragment, to amplify a 500 bp fragment (including primers).

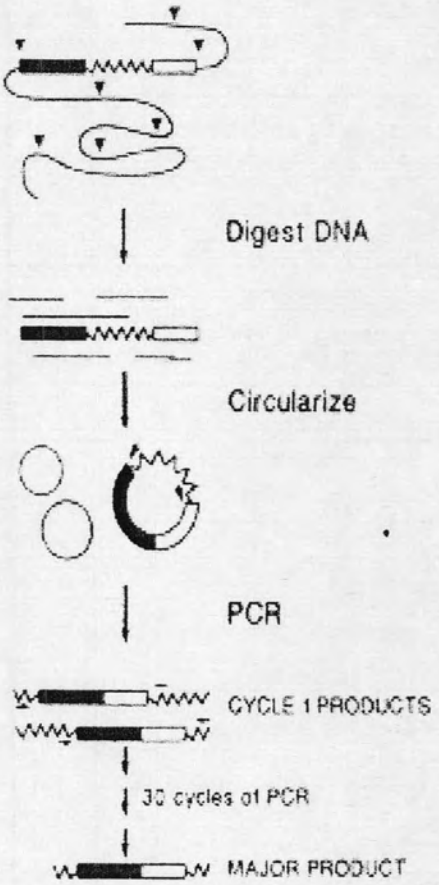


Figure A.3 A flow chart of the inverse PCR procedure (Ochman *et al.*, 1988)

Table A.8 Recognition sites of restriction enzymes for inverse PCR

restriction enzyme	Recognition sites	Buffer and temperature for digestion
*Nco*I	5'C / C A T G G 3'	1X NE Buffer no. 3 Incubate at 37°C.
*Hin*dIII	5' A / A G C T T 3'	1X NE Buffer no.2 Incubate at 37°C
*Hinf*I	5' G / A N T C 3'	1X NE Buffer no.2 Incubate at 37°C.

5.3 Genome walking

Genome walking is an efficient and reliable method that enables the characterization of unknown DNA sequences flanking know regions based on restriction digestion and PCR amplification. Genomic DNA is digested with restriction enzymes and ligated to adaptor. The ligated product is used as a template for amplifying flanking regions using PCR amplification (Figure A.4). The Genome walking procedure allows identification and isolation of 3' and 5' adjacent to a known region of interesting genes. Thus, genome walking is a relatively fast, reliable and procedure, approach to sequence or clone DNA adjacent to a known region without the need to construct DNA libraries. Moreover, it requires little information about sequences to be amplified

The 16S rRNA sequences obtained using primers (LR13943F/LR13392R) as listed in Table 6 and known cyt b sequence from GenBank accession no. AY575080, were used to screen for cut sites of restriction enzymes by using NEBcutter program version 2 (http://tools.neb.com/NEBcutter2/index.php). After screening, the sequence adjacent to the specific mtDNA fragment was obtained by using the Genome Walker protocol (Clonetech, Palo Alto, CA) with a few modifications. Five restriction enzymes (blunt-end generating enzymes; *Dra*I, *Alu*I, *Ssp*I, *Rsa*I and *Eco*RV) (Table A.9) were individually used for digestion in a total volume of 100 µl. The restriction reaction was incubated at 37°C for 3 hours. Then, the restricted DNA was purified by phenol-chloroform and precipitated with 95% ethanol as mention earlier (Topic 2.1).

After digestion, the ligation of the Genome Walker Adapter primer (F 5'-GTAATACGACTCACTTAGGGCACGC-3'; R 5' -GCGTGCCCTAAGTGAGTCGT ATTAC-3') at the end of the restriction sites of DNA fragment was performed in a 10 µl volume. The ligation combination, contained with 3 units of T4 DNA ligase, 1X Ligation buffer (0.4 mM ATP, 10 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate and 50 mM potassium acetate), 5% PEG (polyethylene glycol), 9 µM Genome walker adaptor and 100 ng of the restricted DNA, was incubated overnight at 16°C. After that, the

ligated DNA was purified again by phenol-chloroform and precipitated with 95% ethanol and used as a template for PCR amplification.

The restriction-ligation mixture was amplified with internal primer (based on the sequence of the mtDNA fragment) (Table A.10) and a first adapter primer (AP1; 5'–GTAATACGACTCACTATAGGGC–3') in combination of 0.5 units *Taq* DNA polymerase, 1XPCR buffer (10 mM Tris-HCl pH 8.8, 50 mM KCl and 0.1% Triton X-100), 1.1 mM $MgCl_2$, 200 µM dNTP and 0.2 µM of each primer. The PCR amplification was carried out at 94°C for 25 seconds, 65°C for 3 minutes; 35 cycle followed by 94°C for 25 seconds, 55°C for 3 minutes, and 60°C for 10 minutes; 1 cycles. The PCR products were checked on a 1.5% agarose gel to verify that a unique fragment was obtained. The template treated with each restriction enzyme; *Dra*I, *Rsa*I, *Alu*I and *Ssp*I resulted in an amplified fragment of approximately 350, 250, 200 and 200 bp, respectively (Table A.10). The PCR products were purified by using a Qiaqiuck gel extraction kit (QIAGEN) according the protocol described previously (Topic 3.9.2), and used for sequencing according to the method referred above (Topic 3.9.2). The flanking sequence information was used to design new primer pairs; cyt b-F (5'-TTC ACT ATA TTA TAA AAG ATG TAA GTT C-3') and cyt b-R (5'-GGC AAA AAG AAA ATA TCA TTC AGG-3'), LR13107-F (5'-TGG CTG CAG TAT AAC TGA CTG TAC AAA GG-3') and (5'-GAA ACC AAT CTG ACT TAC GTC GAT TTG A-3') that amplified an approximately 600 and 500 bp fragment of cyt b and 16S rRNA gene, respectively.

5.4 PCR amplification of the mtDNA gene segments

A pair of primers designed from the mtDNA gene segments (approximately 500-700 bp in length) which revealed in Table A.11, was tested against genomic DNA of representative individual *T. pagdeni*. The amplification reaction was performed by PCR condition as described in Table A.12. The presence of PCR products were checked on a 1.5% agarose gel for DNA profiles. All PCR products from genomic DNA of each *T. pagdeni* were then determined on a native polyacrylamide gel by SSCP analysis. An amplified gene region showing high polymorphic level was selected and subjected to population genetic studies.

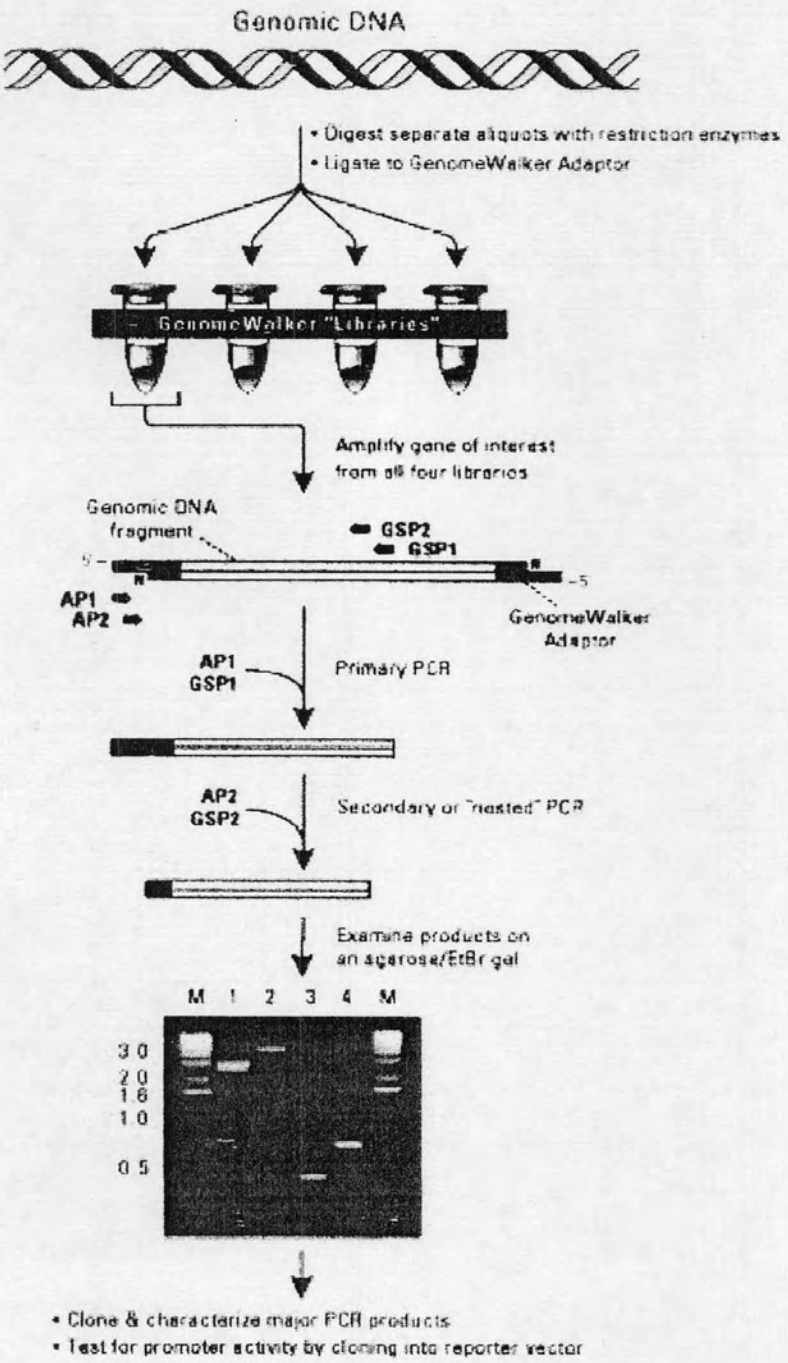


Figure A.4 Flow chart of genome walking protocol. AP1 and AP2 are adaptor primers, and GSP1 and GSP2 are gene specific primers.

Table A.9 Recognition sites of restriction enzymes for Genome walking

restriction enzyme	Recognition sites	Buffer and temperature for digestion
*Dra*I	5' T T T / A A A 3'	1X NE Buffer no. 4 Incubate at 37°C.
*Alu*I	5' A G / C T 3'	1X NE Buffer no. 2 Incubate at 37°C.
*Ssp*I	5' A A T / A T T 3'	1X NE Buffer SspI Incubate at 37°C.
*Rsa*I	5' G T / A C 3'	1X NE Buffer no.1 Incubate at 37°C.
*Eco*RV	5' G A T / A T C 3'	1X NE Buffer no.3 Supplemented with 100 µg/ml Bovine Serum Albumin Incubate at 37°C.

Table A.10 Sequences of primers, expected size amplifiled using each primer pair based on genome walking technique

mtDNA gene	restriction enzyme	Primer	Sequence	Tm (°C)	Expected size (bp)
cyt b	*DraI*	cytb-1R	5'-GTA GAG GAT TTG AAG AAC CGG-3'	54.2	350
		AP1	5'–GTA ATA CGA CTC ACT ATA GGG C–3'	53	
cyt b	*SspI*	cytb-2R	5'-AGC TCC TCA AAA TGA TAT TTG TCC TCA TGG-3'	59.2	200
		AP1	5'–GTA ATA CGA CTC ACT ATA GGG C–3'	53	
16S rRNA	*AluI*	LR-1F	5'-TTA CCT TAG GGA TAA CAG CG-3'	52.6	200
		AP1	5'–GTA ATA CGA CTC ACT ATA GGG C–3'	53	
16S rRNA	*RsaI*	LR-2F	5'-ATG TTT TTG ATA AAC AGG TG-3'	48	250
		AP1	5'–GTA ATA CGA CTC ACT ATA GGG C–3'	53	

Table A.11 Sequences, melting temperature of primers and size of the expected amplification products of mtDNA segments in *T. pagdeni*.

mtDNA region	Primer	Sequence	Tm (°c)	Expected size (bp)
ATPase(6,8)	ATPS6-F	5'-AAG ATA TAT GGA AAT AAG CT-3'	44.7	500
	tRNA-ASP-R	5'-ATA AAA TAA CGT CAA AAT GTC A-3'	47.6	
COI	COI-F	5'- ATA ATT ATT GTT GCT GAT GTA-3'	46.2	500
	COI-R	5'-CTA TTC ATA TAA CTG GAA TTT C-3'	45.6	
cyt b	cyt b-F	5'-TTC ACT ATA TTA TAA AAG ATG TAA GTT C-3'	48.3	500
	cyt b-R	5'-GGC AAA AAG AAA ATA TCA TTC AGG-3'	52.3	
16S rRNA	LR13107-F	5'-TGG CTG CAG TAT AAC TGA CTG TAC AAA GG-3'	60.5	600
	LR12647-R	5'-GAA ACC AAT CTG ACT TAC GTC GAT TTG A-3'	57.7	

Table A.12 PCR conditions used for amplification of mitochondrial DNA gene of *T. pagdeni* using primers in Table A.11

Main genes	**dNTPs (μM)**	**$MgCl_2$ (mM)**	**Primer (μM)**	**PCR condition**
ATPase(6, 8)	200	2.5	0.1	-94°C for 3 minutes -5 cycles of 94°C for 30 seconds 40°C for 60 seconds 72°C for 50 seconds -35 cycles of 94°C for 30 seconds 56°C for 60 seconds 72°C for 50 seconds - 72°C for 7 minutes
COI	200	2.5	0.1	-94°C for 3 minutes -5 cycles of 94°C for 30 seconds 40°C for 60 seconds 72°C for 50 seconds -35 cycles of 94°C for 30 seconds 56°C for 60 seconds 72°C or 50 seconds -72°C for 7 minutes

Table A.12 (continued)

Main genes	dNTPs (μM)	$MgCl_2$ (mM)	Primer (μM)	PCR condition
cyt b	200	2.5	0.1	-94°C for 3 minutes -5 cycles of 94°C for 30 seconds 40°C for 60 seconds 72°C for 60 for 5 cycles and -35 cycles of 94°C for 30 seconds 58°C for 60 seconds 72°C for 60 seconds - 72°C for 7 minutes
16S rRNA	200	2.5	0.1	-94°C for 3 minutes -5 cycles of 94°C for 30 seconds 40°C for 60 seconds 72°C for 50 seconds -35 cycles of 94°C for 40 seconds 62°C for 60 seconds 72°C for 50 seconds - 72°C for 7 minutes

5.5 SSCP (Single Strand Conformational Polymorphism) analysis

5.5.1 Non-denaturing polyacrylamide gel electrophoresis

Non-denaturing polyacrylamide gel and the glass plates (PROTIN II xi Cell) were prepared as described in Topic 3.5, 3.12 and 3.13, with the exception for the concentration of acrylamide gel and crosslink ratio. A volume (7 µl) of the PCR products from each mtDNA region was mixed with 28 µl of the formamide loading dye (95% formamide, 0.25% bromophenol blue, 0.25% xylene cyanol and 10 mM NaOH), denatured at 95°C for 5 minutes and immediately placed on ice for 3 minutes. The denatured samples were then loaded into the slots of the 11% native polyacrylamide gels, run in 1XTBE at 200-250 volts for 15-16 hours at 4°C. The gels were then silver stained, as described earlier (Topic 3.8), and sealed with plastic films.

5.5.2 Scoring SSCP variation

The bands visible in each lane in SSCP gels were observed by eyes. After that, the bands for each amplified mtDNA region (COI, Cytb, ATPase(6,8) and 16S rRNA) were scored to generate 0/1 matrix.

5.6 Data analysis

Genetic Analysis in Excel (GenAlEx6; Peakall and Smouse, 2006) was conducted to analysis genetic diversity, genetic distance among populations, and population structure statistics. Genetic differentiation between the populations was estimated by Φ statistics which were defined above, using AMOVA implemented in GenAlEx (Peakall and Smouse, 2006). Then, the significance was tested by comparing observed statistics to the distributions generated by 999 random permutations of the data into populations of the same size. For geographic patterns, *T. pagdeni* samples were divided into six populations (North, Northeast, Central, Prachuap Khiri Khan, Chumphon and Peninsular populations). We examined (1) differentiation among the six geographic populations, and (2) differentiation between samples corrected from north and south of the Isthmus of Kra. Samples from populations North, Northeast, Central and Prachuap Khiri Khan were

allocated to the Asian mainland group (north of the Isthmus of Kra) whereas samples from Chumphon and Peninsular population were allocated to the Sundaland group (south of the Isthmus of Kra).

6. Determination of the partial mitochondrial DNA sequences of *T. pagdeni*

6.1 Long PCR amplification

Almost the entire mitochondrial genome of *T. pagdeni* was amplified using a long PCR technique (Cheng *et al.*, 1994; Miya and Nishida, 1999). On the basis of the four partial sequences (COI, cyt b, 16S rRNA, and ATPase(6,8)+COIII genes) as described above (5.4), three sets of primer pairs(LR12647-R+COI2494, LR12677+cytb10729 and COIII9821+cytb5031, respectively; Table A.13) were designed on each gene to amplified three long PCR products; the 16S/COI, 16S/cytb and cytb/COIII regions, respectively. Long PCR was done in a Model PTC-200 Peltier thermal cycler (MJ research Inc.), and the reactions were carried out with 30 cycles of a 25-µl reaction volume containing 4.75 µl of sterile distilled H2O, 2.5 µl of 10×LA PCR buffer II (Takara), 4.0 µl of dNTP (2.5 mM), 5.0 µl of each primer (0.4 µM), 0.25 µl of 1.25 -unit Takara LA Taq™ (Takara Bio, Otsu, Shiga, Japan), and 1.0 µl of template containing approximately 5 ng DNA. The thermal cycle profile was performed with denaturation at 98°C for 10 seconds and annealing and extension combined at the same temperature (60°C) as revealed in Table A.14. Long-PCR products were electrophoresed on a 1.0% agarose gel and later stained with ethidium bromide for band characterization via ultraviolet transillumination. Long-PCR products from *T. pagdeni* were purified by using a Qiaqiuck gel extraction kit (QIAGEN), and cloned into pGEM® -T easy vector according the protocol described earlier (Topic 3.9.2).

Table A.13 Long PCR primer sequences and size of the expected amplification product of the mtDNA segment in *T. pagdeni*

mtDNA region	Primer	Sequences	Tm (°C)	Expected size (bp)
COI, COII, ATPase8, ATPase6, COIII, ND3, 12S, 16S rRNA	LR12647-R	5'-GAAACCAATCTGACTTACGTCGATTTGA-3'	57.7	5855
	COI2494	5'-CGAGCATATTTTACATCAGCAACAAT-3'	55.2	
cyt b, ND6, ND5, ND4, ND4L	LR12677	5'-GTTCAAATCGACGTAAGTCAGATTGGTTTC-3'	58.6	5089
	cytb10729	5'-AGCTCCTCAAAATGATATTTGTCCTCATGG-3'	59.2	
COIII, ND3, 12S, 16S rRNA, ND1, cyt b	COIII9821	5'-TTAACGATAGAGTTTACGGGTCAAT-3'	54.4	4879
	cytb5031	5'-AGCTACAGCATTTCTTGGGTATGTAC-3'	57.5	
COIII, ND3, 12S, 16S rRNA, ND1, cyt b, ND6, ND5, ND4, ND4L	ND4L-F	5'-CTGTATGTGAAGGAATTTTAGGA-3'	51.4	10129
	COIII-4W	5'-GTATGGCCTCCAAAAATAGT-3'	51.4	
16S rRNA, ND1, cyt b, ND6, ND5, ND4	ND4-3W	5'-TAAGGGTATATGGAAGATTC-3'	47.2	6563
	LR-3W	5'-CTTATATGTCGATTTTAGAA-3'	43.6	
COI, COII, ATPase8, ATPase6, COIII, ND3, 12S, 16S rRNA	LR-1W	5'-TGAGACAGTGTATATTTCAT-3'	47.0	4943
	COI-1W	5'-GAAAGACTCTTATCTAAACG-3'	46.8	
COII, ATPase8, ATPase6, COIII, ND3, 12S, 16S rRNA	COII-2w	5'-CAGCTCAACCTAATACCTAC-3'	51.1	3414
	LR-2W	5'-ATCGTTACTTGAACTTTTCC-3'	49.5	

Table A.13 (continued)

mtDNA region	Primer	Sequences	Tm (°C)	Expected size (bp)
ND6, ND5, ND4	ND4-2W	5'-TCTATTCCATTGGATTGATC-3'	48.4	3380
	UN-2W	5'-CATCGGTTTTGTAAATTGAT-3'	48.0	
COIII, ND3, 12S, 16S rRNA	tRNA-Leu-3W	5'-GCACTGATCTGCCAAAATAA-3'	52.4	3288
	COIII-4W	5'-GTATGGCCTCCAAAAATAGT-3'	51.4	
12S, 16S rRNA, ND1	SR-2W	5'-AGATACCCTATTATTCTAGG-3'	46.1	2532
	ND1-2W	5'-GATTCTCCTTCTACTAGATC-3'	47.5	
ND5, ND4	ND4-2W	5'-TCTATTCCATTGGATTGATC-3'	48.4	2450
	ND5-3W	5'-TACGAATCTTATAATATCCG-3'	45.0	
ND5, ND4	ND4-3W	5'-TAAGGGTATATGGAAGATTC-3'	47.2	1739
	ND5-3W	5'-TACGAATCTTATAATATCCG-3'	45.0	
ND5, ND4	ND4-3W	5'-TAAGGGTATATGGAAGATTC-3'	47.2	1100
	ND5-4W	5'-TGCTATTGGTAGTCATACAA-3'	49.4	

Table A.14 PCR conditions used for long PCR amplification of mitochondrial DNA gene of *T. pagdeni* using primers in Table A.13.

Primer	dNTPs (μM)	$MgCl_2$ (mM)	Primer (μM)	PCR condition
LR12647-R COI2494	2.5 mM each	2.5	0.4	-94°C for 1 minute - 30 cycles of 98°C for 10 seconds 60°C for 15 minutes - 72°C for 10 minutes
LR12677 cytb10729	2.5 mM each	2.5	0.4	-94°C for 1 minute - 30 cycles of 98°C for 10 seconds 60°C for 15 minutes -72°C for 10 minutes
COIII9821 cytb5031	2.5 mM each	2.5	0.4	-94°C for 1 minute -30 cycles of 98°C for 10 seconds 60°C for 15 minutes -72°C for 10 minutes
ND4L-F COIII-4W	2.5 mM each	2.5	0.4	-94°C for 1 minute; -30 cycles of 98°C for 10 seconds 60°C for 15 minutes -72°C for 10 minutes
LR-3W ND4-3W	2.5 mM each	2.5	0.4	94°C for 1 minutes - 30 cycles 98°C for 10 seconds 60°C for 15 minuts -72°C for 10 minuts

Table A.14 (continued)

Primer	dNTPs (μM)	$MgCl_2$ (mM)	Primer (μM)	PCR condition
LR-1W COI-1W	2.5 mM each	2.5	0.4	-94°C for 1 minute - 30 cycles of 98°C for 10 seconds 60°C for 15 minutes -72°C for 10 minutes
COII-2w LR-2W	2.5 mM each	2.5	0.4	-94°C for 1 minute -30 cycles of 98°C for 10 seconds 60°C for 10 minutes -72°C for 10 minutes
ND-2W UN-2W	2.5 mM each	2.5	0.4	-94°C for 1 minute -30 cycles of 98°C for 10 seconds 60°C for 10 minutes -72°C for 10 minutes
$tRNA^{Leu}$-3W COIII-4W	2.5 mM each	2.5	0.4	-94°C for 1 minute -30 cycles of 98°C for 10 seconds 60°C for 10 minutes -72°C for 10 minutes
SR-2W ND1-2W	2.5 mM each	2.5	0.4	-94°C for 1 minute -30 cycles of 98°C for 10 seconds 60°C for 10 minutes -72°C for 10 minutes

Table A.14 (continued)

Primer	dNTPs (μM)	$MgCl_2$ (mM)	Primer (μM)	PCR condition
ND4-2W ND5-3W	2.5 mM each	2.5	0.4	-94°C for 1 minute -30 cycles of 98°C for 10 seconds 60°C for 10 minutes -72°C for 10 minutes
ND5-3W ND4-3W	2.5 mM each	2.5	0.4	-94°C for 1 minute -30 cycles of 98°C for 10 seconds 60°C for 4 minutes -72°C for 10 minutes
ND5-4W ND4-3W	2.5 mM each	2.5	0.4	-94°C for 1 minute -30 cycles of 98°C for 10 seconds 60°C for 4 minutes -72°C for 10 minutes

6.2 PCR and sequencing

Recombinant plasmids from cloning three long PCR amplification products of *T. pagdeni*, were extracted by using a QIAprep spin miniprep kit (QIAGEN) according to the protocol described previously (Topic 3.9.2). After that, internal sequencing primers which listed in Table A.15 were applied for sequencing by primer walking. DNA sequencing was performed under BigDyeTM terminator cycling conditions on an automatic sequencer 3730xl (sequencing service, Macrogen Inc;Korea). All sequences were analyzed and compared by the homology search to assure the correct fragments using Blast*N* (nucleotide similarity) available at http:// www. ncbi.nlm.nih.gov..

Coding regions were identified using searches for open reading frames, including start and stop codons, and by amino acid alignment with *Melipona bicolor* using the BLAST*X* algorithm (NCBI). Transfer RNAs were identified with the help of the tRNA-scan program in BioEdit version 7.0.9. (Hall, 1999). Ribosomal RNAs were identified by alignment with the 16S and 12S rRNAs genes of *Melipona bicolor*.

The known sequences of each fragment (16S/COI, 16S/cytb and cytb/COIII regions), were overlapped and analysed. To verify gene order on the overlapped fragment, ten internal primer pairs (ND4L-F/COIII-4W, ND4-3W/LR-3W, LR-1W/COI-1W, COII-2w/LR-2W, ND4-2W/UN-2W, $tRNA^{Leu}$-3W/COIII-4W, SR-2W/ND1-2W, ND4-2W/ND5-3W, ND4-3W/ND5-3W and ND4-3W/ND5-4W; Table A.13,Figure A.5), were used to generate PCR products using Takara LA Taq^{TM} (Takara Bio, Otsu, Shiga, Japan) by PCR. The PCR conditions were provided in Table A.14.

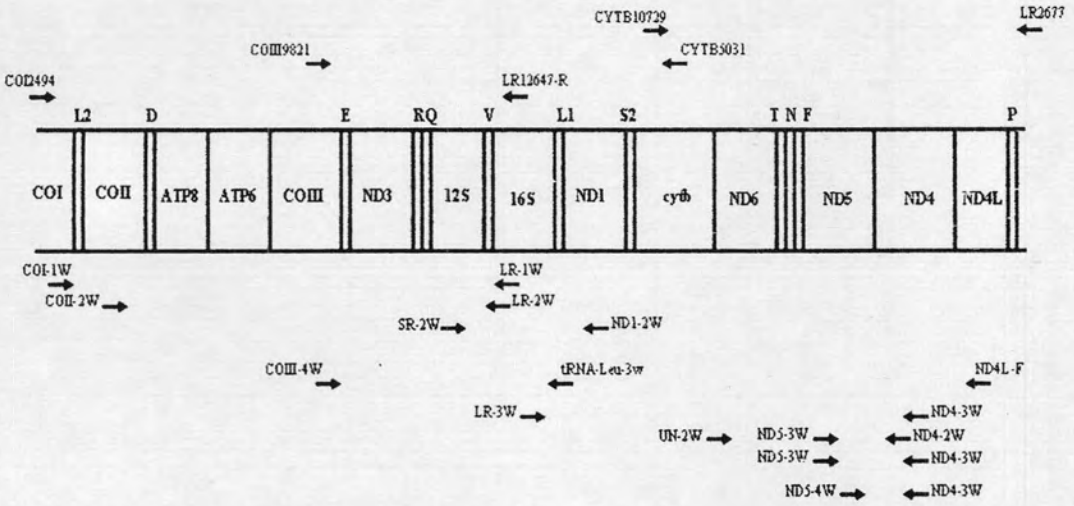


Figure A.5 Gene organization and positions of ten internal primer pairs; ND4L-F/COIII-4W, ND4-3W/LR-3W, LR-1W/COI-1W, COII-2W/LR-2W, ND4-2W/UN-2W, tRNA-Leu-3W/COIII-4W, SR-2W/ND1-2W, ND4-2W/ND5-3W, ND4-3W/ND5-3W and ND4-3W/ND5-4W, including three primer pairs; LR12647-R+COI2494, LR12677+ Cytb10729 and COIII9821+Cytb5031 of the three overlapping segments. Transfer RNA (tRNA) genes were designated by single-letter amino acid codes.

Table A.15 Primer sequences and melting temperature used to sequence the long PCR amplification products of *T. pagdeni*

Primer no.	Primer	Sequence	Tm (°C)
1	LR-1W	5'-TGAGACAGTGTATATTTCAT-3'	47.0
2	COI-1W	5'-GAAAGACTCTTATCTAAACG-3'	46.8
3	COII-2w	5'-CAGCTCAACCTAATACCTAC-3'	51.1
4	LR-2W	5'-ATCGTTACTTGAACTTTTCC-3'	49.5
5	ATP6-3W	5'-ACTCAATTATTACAAACCCG-3'	49.3
6	SR-3W	5'-CCGCTATTGCTGGCACTTTA-3'	56.7
7	COIII-4W	5'-GTATGGCCTCCAAAAATAGT-3'	51.4
8	ND3-4W	5'-GGACGAAACAGCAATGGCAA-3'	57.7
9	UN-1W	5'-GGCTTCTAACCTTTAAAATA-3'	46.6
10	ND6-1W	5'-CTTGATTTCGAAATATTTTA-3'	42.8
11	UN-2W	5'-CATCGGTTTTGTAAATTGAT-3'	48.0
12	ND4L-F	5'-CTGTATGTGAAGGAATTTTAGGA-3'	51.4
13	ND4-2W	5'-TCTATTCCATTGGATTGATC-3'	48.4
14	ND4-3W	5'-TAAGGGTATATGGAAGATTC-3'	47.2
15	ND5-3W	5'-TACGAATCTTATAATATCCG-3'	43.5
16	ND5-4W	5'-TGCTATTGGTAGTCATACAA-3'	49.4
17	cytb-1W	5'-GCTAGGAGGAGTAATTATAC-3'	47.8
18	ND3-1W	5'-CTTCCTTATCTTTGACATTG-3'	47.8
19	SR-2W	5'-AGATACCCTATTATTCTAGG-3'	46.1
20	ND1-2W	5'-GATTCTCCTTCTACTAGATC-3'	47.5
21	$tRNA^{Leu}$-3W	5'-GCACTGATCTGCCAAAATAA-3'	52.4
22	LR-3W	5'-CTTATATGTCGATTTTAGAA-3'	43.6
23	LR-4W	5'-GAAAGAGACTCATAATTCT-3'	45.1
24	mtD7-W	5'-AAAGAGGTAAGAAAGAAGTC-3'	48.3

APPENDIX B

SAMPLE COLLECTIONS

1. Total of 145 *T. pagdeni* were selected to investigate in this study

Code	Species	Population	Province	Locality
TLCMN1	*T. pagdeni*	North	Chiang Mai	Hang Dong district
TLCMN2	*T. pagdeni*	North	Chiang Mai	Hang Dong district
TLCMN3	*T. pagdeni*	North	Chiang Mai	Hang Dong district
TLCMN14	*T. pagdeni*	North	Chiang Mai	San Sai district
TLNAN24	*T. pagdeni*	North	Nan	Muang district
TLNAN25	*T. pagdeni*	North	Nan	Muang district
TLUTN16	*T. pagdeni*	North	Uttaradit	Laplae district
TLKPN23	*T. pagdeni*	North	Kamphaeng Phet	Muang district
TLPLN1	*T. pagdeni*	North	Phitsanulok	Muang district
TLPLN2	*T. pagdeni*	North	Phitsanulok	Muang district
TLSTN26	*T. pagdeni*	North	Sukhothai	Si Satchanalai district
TLSTN27	*T. pagdeni*	North	Sukhothai	Muang district
TLSTN28	*T. pagdeni*	North	Sukhothai	Muang district
TLSTN30	*T. pagdeni*	North	Sukhothai	Si Samrong district
TLSTN31	*T. pagdeni*	North	Sukhothai	Si Samrong district
TLUDNE1	*T. pagdeni*	Northeast	Udon Thani	Kut Chap district
TLKKNE17	*T. pagdeni*	Northeast	Khon Kaen	Phra Yuen district
TLKKNE18	*T. pagdeni*	Northeast	Khon Kaen	Phra Yuen district
TLKKNE19	*T. pagdeni*	Northeast	Khon Kaen	Nong Wang district
TLUBNE3	*T. pagdeni*	Northeast	Ubon Ratchathani	Muang district
TLUBNE2	*T. pagdeni*	Northeast	Ubon Ratchathani	Muang district
TLUBNE5	*T. pagdeni*	Northeast	Ubon Ratchathani	Sirinthon district
TLUBNE8	*T. pagdeni*	Northeast	Ubon Ratchathani	Chong Meg district
TLSSNE9	*T. pagdeni*	Northeast	Si Sa Ket	Muang district
TLRENE11	*T. pagdeni*	Northeast	Roi Et	Suwannaphum district
TLRENE12	*T. pagdeni*	Northeast	Roi Et	Suwannaphum district
TLRENE13	*T. pagdeni*	Northeast	Roi Et	Kaset Wisai district
TLRENE22	*T. pagdeni*	Northeast	Roi Et	Muang district
TLRENE23	*T. pagdeni*	Northeast	Roi Et	Jaturapakpiman district
TLRENE24	*T. pagdeni*	Northeast	Roi Et	Jaturapakpiman district
TLSUNE25	*T. pagdeni*	Northeast	Surin	Jom Phra district
TLSUNE14	*T. pagdeni*	Northeast	Surin	Kra Pho district
TLSUNE26	*T. pagdeni*	Northeast	Surin	Kra Pho district
TLSUNE27	*T. pagdeni*	Northeast	Surin	Tha Tum district
TLBRNE15	*T. pagdeni*	Northeast	Buri Ram	Chorakaemak district
TLBRNE16	*T. pagdeni*	Northeast	Buri Ram	Chorakaemak district
TLMKNE10	*T. pagdeni*	Northeast	Maha Sarakham	Phayakapumpisai district

Code	Species	Population	Province	Locality
TLMKNE20	*T. pagdeni*	Northeast	Maha Sarakham	Wapi Pathum district
TLMKNE21	*T. pagdeni*	Northeast	Maha Sarakham	Wapi Pathum district
TLNBC1	*T. pagdeni*	Central	Nonthaburi	Pak Kret district
TLNBC3	*T. pagdeni*	Central	Nonthaburi	Pak Kret district
TLLBC5	*T. pagdeni*	Central	Lop Buri	Muang district
TLLBC7	*T. pagdeni*	Central	Lop Buri	Muang district
TLLBC9	*T. pagdeni*	Central	Lop Buri	Muang district
TLSPC10	*T. pagdeni*	Central	Suphan Buri	Muang district
TLSIC13	*T. pagdeni*	Central	Sing Buri	Muang district
TLSIC14	*T. pagdeni*	Central	Sing Buri	Muang district
TLNYC15	*T. pagdeni*	Central	Nakhon Nayok	Banna district
TLAYC45	*T. pagdeni*	Central	Ayutthaya	Muang district
TLAYC46	*T. pagdeni*	Central	Ayutthaya	Muang district
TLAYC47	*T. pagdeni*	Central	Ayutthaya	Muang district
TLAYC48	*T. pagdeni*	Central	Ayutthaya	Muang district
TLBKC16	*T. pagdeni*	Central	Bangkok	Pathumwan district
TLBKC17	*T. pagdeni*	Central	Bangkok	Chatuchak district
TLBKC22	*T. pagdeni*	Central	Bangkok	Chatuchak district
TLBKC23	*T. pagdeni*	Central	Bangkok	Chatuchak district
TLPBC26	*T. pagdeni*	Central	Phetchaburi	Banlad district
TLPBC25	*T. pagdeni*	Central	Phetchaburi	Banlad district
TLPBC27	*T. pagdeni*	Central	Phetchaburi	Banlad district
TLPBC28	*T. pagdeni*	Central	Phetchaburi	Banlad district
TLKBC50	*T. pagdeni*	Central	Kanchanaburi	Sai Yok district
TLKBC51	*T. pagdeni*	Central	Kanchanaburi	Sai Yok district
TLKBC52	*T. pagdeni*	Central	Kanchanaburi	Sangklaburi district
TLKBC53	*T. pagdeni*	Central	Kanchanaburi	Sangklaburi district
TLKBC54	*T. pagdeni*	Central	Kanchanaburi	Sangklaburi district
TLKBC56	*T. pagdeni*	Central	Kanchanaburi	Sai Yok district
TLKBC57	*T. pagdeni*	Central	Kanchanaburi	Sai Yok district
TLKBC58	*T. pagdeni*	Central	Kanchanaburi	Muang district
TLKBC59	*T. pagdeni*	Central	Kanchanaburi	Muang district
TLKBC60	*T. pagdeni*	Central	Kanchanaburi	Thong Pha Phum district
TLPKC42	*T. pagdeni*	Central	Prachuap Khiri Khan	Muang district
TLPKC43	*T. pagdeni*	Central	Prachuap Khiri Khan	Muang district
TLPKC29	*T. pagdeni*	Central	Prachuap Khiri Khan	Muang district
TLPKC30	*T. pagdeni*	Central	Prachuap Khiri Khan	Muang district
TLPKC31	*T. pagdeni*	Central	Prachuap Khiri Khan	Amphoe Kui Buri
TLPKC32	*T. pagdeni*	Central	Prachuap Khiri Khan	Amphoe Kui Buri
TLPKC33	*T. pagdeni*	Central	Prachuap Khiri Khan	Amphoe Kui Buri
TLPKC35	*T. pagdeni*	Central	Prachuap Khiri Khan	Amphoe Kui Buri
TLPKC36	*T. pagdeni*	Central	Prachuap Khiri Khan	Amphoe Kui Buri
TLPKC41	*T. pagdeni*	Central	Prachuap Khiri Khan	Amphoe Muang

Code	Species	Population	Province	Locality
TLPKC44	*T. pagdeni*	Central	Prachuap Khiri Khan	Sira Loi district
TLPKC39	*T. pagdeni*	Central	Prachuap Khiri Khan	Sila Loi district
TLPKC38	*T. pagdeni*	Central	Prachuap Khiri Khan	Sila Loi district
TLPKC40	*T. pagdeni*	Central	Prachuap Khiri Khan	Muang district
TLPKC61	*T. pagdeni*	Central	Prachuap Khiri Khan	Thub Sakae district
TLPKC64	*T. pagdeni*	Central	Prachuap Khiri Khan	Bang Saphan district
TLPKC65	*T. pagdeni*	Central	Prachuap Khiri Khan	Bang Saphan district
TLPKC66	*T. pagdeni*	Central	Prachuap Khiri Khan	Bang Saphan district
TLPKC67	*T. pagdeni*	Central	Prachuap Khiri Khan	Thub Sakae district
TLPKC69	*T. pagdeni*	Central	Prachuap Khiri Khan	Bang Saphan district
TLPKC62	*T. pagdeni*	Central	Prachuap Khiri Khan	Muang district
TLPKC68	*T. pagdeni*	Central	Prachuap Khiri Khan	Bang Saphan district
TLTRC3	*T. pagdeni*	Central	Trat	Khao Saming district
TLCTC5	*T. pagdeni*	Central	Trat	Laem Sing district
TLCTC9	*T. pagdeni*	Central	Chanthaburi	Khlung district
TLCTC12	*T. pagdeni*	Central	Chanthaburi	Makham district
TLCTC10	*T. pagdeni*	Central	Chanthaburi	Khlung district
TLTRC4	*T. pagdeni*	Central	Chanthaburi	Borai district
TLCBC15	*T. pagdeni*	Central	Chon Buri	Muang district
TLCBC16	*T. pagdeni*	Central	Chon Buri	Si Racha district
TLCPPT27	*T. pagdeni*	Peninsular	Chumphon	Muang district
TLCPPT28	*T. pagdeni*	Peninsular	Chumphon	Muang district
TLCPPT29	*T. pagdeni*	Peninsular	Chumphon	Muang district
TLCPPT33	*T. pagdeni*	Peninsular	Chumphon	Tha Sae district
TLCPPT34	*T. pagdeni*	Peninsular	Chumphon	Tha Sae district
TLCPPT85	*T. pagdeni*	Peninsular	Chumphon	Lang Suan district
TLCPPT86	*T. pagdeni*	Peninsular	Chumphon	Lang Suan district
TLCPPT89	*T. pagdeni*	Peninsular	Chumphon	Thung Tako district
TLCPPT90	*T. pagdeni*	Peninsular	Chumphon	Thung Tako district
TLCPPT91	*T. pagdeni*	Peninsular	Chumphon	Thung Tako district
TLCPPT92	*T. pagdeni*	Peninsular	Chumphon	Thung Tako district
TLCPPT93	*T. pagdeni*	Peninsular	Chumphon	Thung Tako district
TLCPPT94	*T. pagdeni*	Peninsular	Chumphon	Thung Tako district
TLCPPT98	*T. pagdeni*	Peninsular	Chumphon	Sawi district
TLCPPT99	*T. pagdeni*	Peninsular	Chumphon	Sawi district
TLCPPT100	*T. pagdeni*	Peninsular	Chumphon	Sawi district
TLCPPT103	*T. pagdeni*	Peninsular	Chumphon	Muang district
TLCPPT104	*T. pagdeni*	Peninsular	Chumphon	Muang district
TLCPPT105	*T. pagdeni*	Peninsular	Chumphon	Muang district
TLRNPT106	*T. pagdeni*	Peninsular	Ranong	Muang district
TLKAPT107	*T. pagdeni*	Peninsular	Krabi	Khao Phanom district
TLKAPT 108	*T. pagdeni*	Peninsular	Krabi	Khao Phanom district
TLNTPT37	*T. pagdeni*	Peninsular	Nakhon Si Thammarat	Chang Klang district

Code	Species	Population	Province	Locality
TLSRPT69	*T. pagdeni*	Peninsular	Surat Thani	Wiang Sa district
TLSRPT70	*T. pagdeni*	Peninsular	Surat Thani	Wiang Sa district
TLSRPT71	*T. pagdeni*	Peninsular	Surat Thani	Chaiya district
TLSRPT72	*T. pagdeni*	Peninsular	Surat Thani	Chaiya district
TLSRPT77	*T. pagdeni*	Peninsular	Surat Thani	Tha Chang district
TLSRPT84	*T. pagdeni*	Peninsular	Surat Thani	Wiang Sa district
TLPTPT38	*T. pagdeni*	Peninsular	Phatthalung	Muang district
TLPTPT39	*T. pagdeni*	Peninsular	Phatthalung	Muang district
TLPTPT40	*T. pagdeni*	Peninsular	Phatthalung	Muang district
TLPTPT41	*T. pagdeni*	Peninsular	Phatthalung	Muang district
TLSKPT43	*T. pagdeni*	Peninsular	Songkhla	Muang district
TLSKPT48	*T. pagdeni*	Peninsular	Songkhla	Muang district
TLSKPT54	*T. pagdeni*	Peninsular	Songkhla	Muang district
TLSKPT 50	*T. pagdeni*	Peninsular	Songkhla	Muang district
TLSKPT56	*T. pagdeni*	Peninsular	Songkhla	Muang district
TLSKPT61	*T. pagdeni*	Peninsular	Songkhla	Muang district
TLSKPT63	*T. pagdeni*	Peninsular	Songkhla	Hat Yai district
TLSKPT65	*T. pagdeni*	Peninsular	Songkhla	Muang district
TLSKPT66	*T. pagdeni*	Peninsular	Songkhla	Hat Yai district
TLSKPT78	*T. pagdeni*	Peninsular	Songkhla	Hat Yai district
TLSKPT79	*T. pagdeni*	Peninsular	Songkhla	Hat Yai district
TLSKPT80	*T. pagdeni*	Peninsular	Songkhla	Singhanakhon district

2. Total of 209 other stingless bees were used in this study

Sample	Speices	Population	Province	Locality
COL2N	*T. collina*	North	Nan	Pua district
COL3N	*T. collina*	North	Nan	Pua district
COL5N	*T. collina*	North	Phrae	Muang district
COL8N	*T. collina*	North	Phrae	Muang district
COL9N	*T. collina*	North	Uttaradit	Laplae district
COL10N	*T. collina*	North	Uttaradit	Laplae district
COL11N	*T. collina*	North	Uthai Thani	Nong Chang district
COL12N	*T. collina*	North	Uthai Thani	Nong Chang district
COL14N	*T. collina*	North	Uthai Thani	Nong Chang district
COL23N	*T. collina*	North	Uthai Thani	Lan Sak district
COL24N	*T. collina*	North	Uthai Thani	Lan Sak district
COL26N	*T. collina*	North	Kamphaeng Phet	Muang district
COL27N	*T. collina*	North	Kamphaeng Phet	Muang district
COL30N	*T. collina*	North	Chiang Mai	San Sai district
COL31N	*T. collina*	North	Phichit	Muang district
COL32N	*T. collina*	North	Phichit	Muang district
COL33N	*T. collina*	North	Phichit	Pho Thale district
COL34N	*T. collina*	North	Phichit	Pho Thale district
COL1NE	*T. collina*	Northeast	Khon Kaen	Nong Song Hong district
COL2NE	*T. collina*	Northeast	Khon Kaen	Phra Yuen district
COL3NE	*T. collina*	Northeast	Khon Kaen	Phra Yuen district
COL9NE	*T. collina*	Northeast	Khon Kaen	Phra Yuen district
COL10NE	*T. collina*	Northeast	Khon Kaen	Phra Yuen district
COL13NE	*T. collina*	Northeast	Maha Sarakham	Wapi Pathum district
COL14NE	*T. collina*	Northeast	Maha Sarakham	Wapi Pathum district
COL17NE	*T. collina*	Northeast	Roi Et	Muang district
COL19NE	*T. collina*	Northeast	Roi Et	Chaturakphak Phiman district

Sample	Speices	Population	Province	Locality
COL20NE	*T. collina*	Northeast	Si Sa Ket	Kanthararom district
COL26NE	*T. collina*	Northeast	Si Sa Ket	Kanthararom district
COL31NE	*T. collina*	Northeast	Si Sa Ket	Kanthararom district
COL35NE	*T. collina*	Northeast	Surin	Tha Tum district
COL43NE	*T. collina*	Northeast	Buri Ram	Nang Rong
COL49NE	*T. collina*	Northeast	Buri Ram	Nang Rong
COL51NE	*T. collina*	Northeast	Sakhon Nakhon	Phanna Nikhom district
COL52NE	*T. collina*	Northeast	Sakhon Nakhon	Phanna Nikhom district
COL56NE	*T. collina*	Northeast	Chaiyaphum	Phu Khiao district
COL58NE	*T. collina*	Northeast	Chaiyaphum	Kaeng Khro district
COL59NE	*T. collina*	Northeast	Udon Thani	Kut Chap district
COL61NE	*T. collina*	Northeast	Udon Thani	Kut Chap district
COL64NE	*T. collina*	Northeast	Ubon Ratchathani	Phibun Mangsahan district
COL65NE	*T. collina*	Northeast	Ubon Ratchathani	Phibun Mangsahan district
COL69NE	*T. collina*	Northeast	Mukdahan	Loeng Nok Tha district
COL70NE	*T. collina*	Northeast	Ubon Ratchathani	Sirinthon district
COL71NE	*T. collina*	Northeast	Khon Kaen	Muang district
COL1C	*T. collina*	Central	Ratchaburi	Muang district
COL3C	*T. collina*	Central	Kanchanaburi	Sai Yok district
COL4C	*T. collina*	Central	Kanchanaburi	Sai Yok district
COL5C	*T. collina*	Central	Kanchanaburi	Sai Yok district
COL6C	*T. collina*	Central	Kanchanaburi	Sai Yok district
COL10C	*T. collina*	Central	Kanchanaburi	Thong Pha Phum district
COL11C	*T. collina*	Central	Kanchanaburi	Thong Pha Phum district
COL12C	*T. collina*	Central	Kanchanaburi	Thong Pha Phum district
COL13C	*T. collina*	Central	Kanchanaburi	Thong Pha Phum district
COL14C	*T. collina*	Central	Kanchanaburi	Thong Pha Phum district
COL15C	*T. collina*	Central	Kanchanaburi	Thong Pha Phum district
COL16C	*T. collina*	Central	Kanchanaburi	Thong Pha Phum district
COL17C	*T. collina*	Central	Kanchanaburi	Thong Pha Phum district

Sample	Speices	Population	Province	Locality
COL18C	*T. collina*	Central	Kanchanaburi	Thong Pha Phum district
COL20C	*T. collina*	Central	Kanchanaburi	Thong Pha Phum district
COL21C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan Noi district
COL22C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan Noi district
COL23C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan Noi district
COL24C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan Noi district
COL25C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan Noi district
COL26C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan Noi district
COL27C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan Noi district
COL28C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan Noi district
COL29C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan Noi district
COL30C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan Noi district
COL31C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan district
COL32C	*T. collina*	Central	Prachuap Khiri Khan	Bang Saphan district
COL33C	*T. collina*	Central	Chanthaburi	Makham district
COL34C	*T. collina*	Central	Chanthaburi	Makham district
COL5S	*T. collina*	Peninsular	Surat Thani	Kanchanadit
COL11S	*T. collina*	Peninsular	Surat Thani	Tha Chang district
COL16S	*T. collina*	Peninsular	Ranong	Muang district
COL17S	*T. collina*	Peninsular	Ranong	Muang district
COL18S	*T. collina*	Peninsular	Chumphon	Muang district
COL20S	*T. collina*	Peninsular	Chumphon	Muang district
COL22S	*T. collina*	Peninsular	Chumphon	Lang Suan district
COL26S	*T. collina*	Peninsular	Chumphon	Lang Suan district
COL27S	*T. collina*	Peninsular	Chunphon	Thung Tako district
COL28S	*T. collina*	Peninsular	Chumphon	Sawi district
COL30S	*T. collina*	Peninsular	Chumphon	Sawi district
COL32S	*T. collina*	Peninsular	Chumphon	Wat Khao chun Toa
COL35S	*T. collina*	Peninsular	Chumphon	Phato district
COL36S	*T. collina*	Peninsular	Ranong	Muang district

Sample	Speices	Population	Province	Locality
COL37S	*T. collina*	Peninsular	Ranong	Muang district
COL38S	*T. collina*	Peninsular	Ranong	Kra Buri district
COL39S	*T. collina*	Peninsular	Chumphon	Muang district
COL40S	*T. collina*	Peninsular	Chumphon	Muang district
COL41S	*T. collina*	Peninsular	Chumphon	Tha sea district
COL42S	*T. collina*	Peninsular	Surat Thani	Chaiya district
COL43S	*T. collina*	Peninsular	Surat Thani	Chaiya district
COL45S	*T. collina*	Peninsular	Surat Thani	Kanchanadit district
COL47S	*T. collina*	Peninsular	Surat Thani	Phon Phin district
COL48S	*T. collina*	Peninsular	Surat Thani	Phon Phin district
COL49S	*T. collina*	Peninsular	Surat Thani	Khiri Rat Nikhom district
COL50S	*T. collina*	Peninsular	Surat Thani	Khiri Rat Nikhom district
COL52S	*T. collina*	Peninsular	Surat Thani	Vibhavadi district
COL53S	*T. collina*	Peninsular	Surat Thani	Vibhavadi district
COL55S	*T. collina*	Peninsular	Surat Thani	Vibhavadi district
COL57S	*T. collina*	Peninsular	Surat Thani	Vibhavadi district
COL58S	*T. collina*	Peninsular	Surat Thani	Tha Chana district
COL59S	*T. collina*	Peninsular	Nakhon Si Thammarat	Sichon district
COL60S	*T. collina*	Peninsular	Nakhon Si Thammarat	Sichon district
COL61S	*T. collina*	Peninsular	Nakhon Si Thammarat	Muang district
COL62S	*T. collina*	Peninsular	Nakhon Si Thammarat	Muang district
COL66S	*T. collina*	Peninsular	Nakhon Si Thammarat	Thung Song district
COL67S	*T. collina*	Peninsular	Nakhon Si Thammarat	Thung Song district
COL68S	*T. collina*	Peninsular	Nakhon Si Thammarat	Thung Song district
MIN01S	*T. minor*	Peninsular	Songkhla	Muang district
MIN02N	*T. minor*	North	Nan	Pua district
MIN03N	*T. minor*	North	Nan	Pua district
MINO4N	*T. minor*	North	Uttaradit	Laplae district
MIN05N	*T. minor*	North	Uttaradit	Laplae district
MIN06N	*T. minor*	North	Uttaradit	Laplae district
MIN07N	*T. minor*	North	Uttaradit	Laplae district
MIN08N	*T. minor*	North	Uttaradit	Laplae district
MIN09NE	*T. minor*	Northeast	Roi Et	Muang district

Sample	Speices	Population	Province	Locality
MIN10NE	*T. minor*	Northeast	Ubon Ratchathani	Muang district
MIN11NE	*T. minor*	Northeast	Ubon Ratchathani	Muang district
MIN12NE	*T. minor*	Northeast	Si Sa Ket	Huai Thap Than district
MIN13NE	*T. minor*	Northeast	Si Sa Ket	Huai Thap Than district
MIN14NE	*T. minor*	Northeast	Si Sa Ket	Huai Thap Than district
MIN15NE	*T. minor*	Northeast	Si Sa Ket	Huai Thap Than district
MIN16NE	*T. minor*	Northeast	Si Sa Ket	Huai Thap Than district
MIN17NE	*T. minor*	Northeast	Si Sa Ket	Huai Thap Than district
MIN18NE	*T. minor*	Northeast	Si Sa Ket	Huai Thap Than district
MIN19E	*T. minor*	Northeast	Chanthaburi	Khlung district
MIN20E	*T. minor*	Northeast	Chanthaburi	Khlung district
MIN21E	*T. minor*	Northeast	Trat	Borai district
MIN22E	*T. minor*	Northeast	Trat	Khao Saming district
MIN23E	*T. minor*	Northeast	Chumphon	Muang district
FUS02C	*T. fuscobalteata*	Central	Kanchanaburi	Sai Yok district
FUS03C	*T. fuscobalteata*	Central	Kanchanaburi	Sai Yok district
FUS04C	*T. fuscobalteata*	Central	Kanchanaburi	Sai Yok district
FUS05C	*T. fuscobalteata*	Central	Kanchanaburi	Sai Yok district
FUS06C	*T. fuscobalteata*	Central	Kanchanaburi	Sai Yok district
FUS07C	*T. fuscobalteata*	Central	Kanchanaburi	Sai Yok district
FUS08C	*T. fuscobalteata*	Central	Kanchanaburi	Sai Yok district
FUS14E	*T. fuscobalteata*	Central	Trat	Khao Saming district
FUS01N	*T. fuscobalteata*	North	Uttaradit	Laplae district
FUS09N	*T. fuscobalteata*	North	Chiang Mai	Mae Rim district
FUS10N	*T. fuscobalteata*	North	Chiang Mai	Mae Rim district
FUS11N	*T. fuscobalteata*	North	Chiang Mai	Mae Rim district
FUS12N	*T. fuscobalteata*	North	Chiang Mai	Mae Rim district
FUS13N	*T. fuscobalteata*	North	Chiang Mai	Mae Rim district
FUS15N	*T. fuscobalteata*	North	Phrae	Wang Chin district
TER01N	*T. terminata*	North	Uttaradit	Laplae district
TER02NE	*T. terminata*	Northeast	Roi Et	Chaturaphak Phiman district
TER03NE	*T. terminata*	Northeast	Roi Et	Chaturaphak Phiman district
TER04NE	*T. terminata*	Northeast	Roi Et	Chaturaphak Phiman district
TER05NE	*T. terminata*	Northeast	Ubon Ratchathani	Muang district
TER06NE	*T. terminata*	Northeast	Ubon Ratchathani	Muang district
TER07NE	*T. terminata*	Northeast	Ubon Ratchathani	Muang district
TER08NE	*T. terminata*	Northeast	Ubon Ratchathani	Muang district
TER09C	*T. terminata*	Central	Ratchaburi	Chom Bueng district
TER10C	*T. terminata*	Central	Ratchaburi	Muang district
TER11N	*T. terminata*	North	Lampang	Soem Ngam district
TER12N	*T. terminata*	North	Lampang	Soem Ngam district
TER13N	*T. terminata*	North	Lampang	Soem Ngam district

Sample	Speices	Population	Province	Locality
TER15N	*T. terminata*	North	Chiang Mai	Mae Rim district
DOI14N	*T. doipaensis*	North	Chiang Mai	Mae Rim district
API02N	*T. apicalis*	North	Chiang Mai	Hang Dong district
API03N	*T. apicalis*	North	Chiang Mai	Muang district
API09N	*T. apicalis*	North	Chiang Mai	Mae Rim district
API04C	*T. apicalis*	Central	Ratchaburi	Muang district
API05C	*T. apicalis*	Central	Ratchaburi	Chom Bueng district
API06C	*T. apicalis*	Central	Ratchaburi	Chom Bueng district
API07C	*T. apicalis*	Central	Ratchaburi	Chom Bueng district
API08C	*T. apicalis*	Central	Ratchaburi	Suan Phueng district
API01S	*T. apicalis*	Peninsula	Nakhon Si Thammarat	Chang Klang district
API10S	*T. apicalis*	Peninsula	Chumphon	Sawi district
API11S	*T. apicalis*	Peninsula	Chumphon	Sawi district
API12S	*T. apicalis*	Peninsula	Chumphon	Muang district
API13S	*T. apicalis*	Peninsula	Chumphon	Muang district
T. sp 1C	Unknown	Central	Kanchanaburi	Sangklaburi district
T. sp 2C	Unknown	Central	Kanchanaburi	Thong Pha Phum district
T. sp 3N	Unknown	North	Chiang Mai	Mae Rim district
ITM01S	*T. itama*	Peninsula	Songkhla	Hat Yai district
ITM02S	*T. itama*	Peninsula	Songkhla	Hat Yai district
ITM03S	*T. itama*	Peninsula	Songkhla	Singhanakhon district
ITM04S	*T. itama*	Peninsula	Surat Thani	Chaiya district
ITM05S	*T. itama*	Peninsula	Surat Thani	Chaiya district
CAN01S	*T. canifrons*	Peninsula	Surat Thani	Chaiya district
FIM01N	*T. fimbriata*	North	Chiang Mai	Muang district
FIM02NE	*T. fimbriata*	Northeast	Si Sa Ket	Muang district
MELA01N	*T. melanoleuca*	North	Chiang Mai	Muang district
THO01S	*T. thoracica*	Peninsula	Songkhla	Hat Yai district
THO02S	*T. thoracica*	Peninsula	Surat Thani	Chaiya district
THO03NE	*T. thoracica*	Northeast	Nong Khai	Tha Bo district
THO 03C	*T. thoracica*	Central	Ratchaburi	Muang district
MEL01S	*T. melina*	Peninsula	Surat Thani	Kanchanadit district
Lfur01NE	*Lisotrigona furva*	Northeast	Surin	Kra Pho district
Lfur02NE	*Lisotrigona furva*	Northeast	Nakhon Ratchasima	Chaloem Phra Kiat district
Lfur03C	*Lisotrigona furva*	Central	Ratchaburi	Suan Phueng district
L03N	*T. laeviceps*	North	Phitsanulok	Muang district
L04N	*T. laeviceps*	North	Phitsanulok	Muang district
L06N	*T. laeviceps*	North	Phichit	Muang district
L07N	*T. laeviceps*	North	Phichit	Muang district
L08N	*T. laeviceps*	North	Phichit	Muang district
L09N	*T. laeviceps*	North	Phichit	Muang district
L012N	*T. laeviceps*	North	Phichit	Po Talae district

Sample	Speices	Population	Province	Locality
L018N	*T. laeviceps*	North	Phrae	Wang Chin district
L019N	*T. laeviceps*	North	Phrae	Wang Chin district
L020N	*T. laeviceps*	North	Phrae	Wang Chin district
L021N	*T. laeviceps*	North	Phrae	Wang Chin district
L022N	*T. laeviceps*	North	Phrae	Wang Chin district

APPENDIX C

LURIA-BERTANI MEDIUM (LB MEDIUM)

Liquid media, composition per 1 litre

Bacto tryptone	10 g
NaCl	10 g
Yeast extract	5 g

Solid media, composition per 1 litre

Bacto tryptone	10 g
NaCl	10 g
Yeast extracts	5 g
Agar	15 g

All compositions were dissolved together with 800 ml of distilled water; and then the mixture was adjusted to pH of 7.0 with 6 M NaOH. The total volume of solution was then adjusted to 1 litre with deionized water. The medium was sterilized by autoclaving at 15 Ib/in2 for 15 minute.

APPENDIX D

REAGENTS FOR ALKALINE LYSIS

1. **Solution I (100 ml)**

   5.0 ml 1.0 M Glucose

   2.5 ml 1.0 M Tris-HCl, pH 8.0

   2.0 ml 0.5 M EDTA, pH 8.0

   After autoclave 20 μg/ml of RNase was added and stored at 4°C

2. **Solution II (25 ml)**

   0.5 ml 10 M NaOH

   1.25 ml 20% SDS

3. **Solution III (500 ml)**

   147 g potassium acetate

   57.5 ml glacial acetate

   Autoclave and store at 4°C

4. **TE buffer (500 ml)**

   5 ml 1 M Tris-HCl, pH 8.0

   1 ml 0.5 M EDTA

   Autoclave and store at room temperature

APPENDIX E

CHEMICALS FOR PREPARATION OF POLYACRYLAMIDE GEL AND SILVER STAINING

1. 4.5% Denature acrylamide solution (crosslink =19:1), 500ml

Acrylamide	21.375 g
N,N'-methylene-bis-acrylamide	1.125 g
7 M Urea	210 g

2. 40% acrylamide solution (crosslink = 37.5:1), 500 ml

Acrylamide	194.80 g
N,N'-methylene-bis-acrylamide	5.19 g

3. 40% acrylamide solution (75:1), 500 ml

Acrylamide	197.37 g
N,N'-methylene-bis-acrylamide	2.63 g

4. Bind silane

95% ethanol	995 µl
Bind silane	4 µl
Acetic acid	5 µl

5. Fix/stop solution (10% glacial acetic acid), 2 liters

Glacial acetic acid	200 µl
Deionized water	1800 ml

6. Staining solution, 1.5 liters

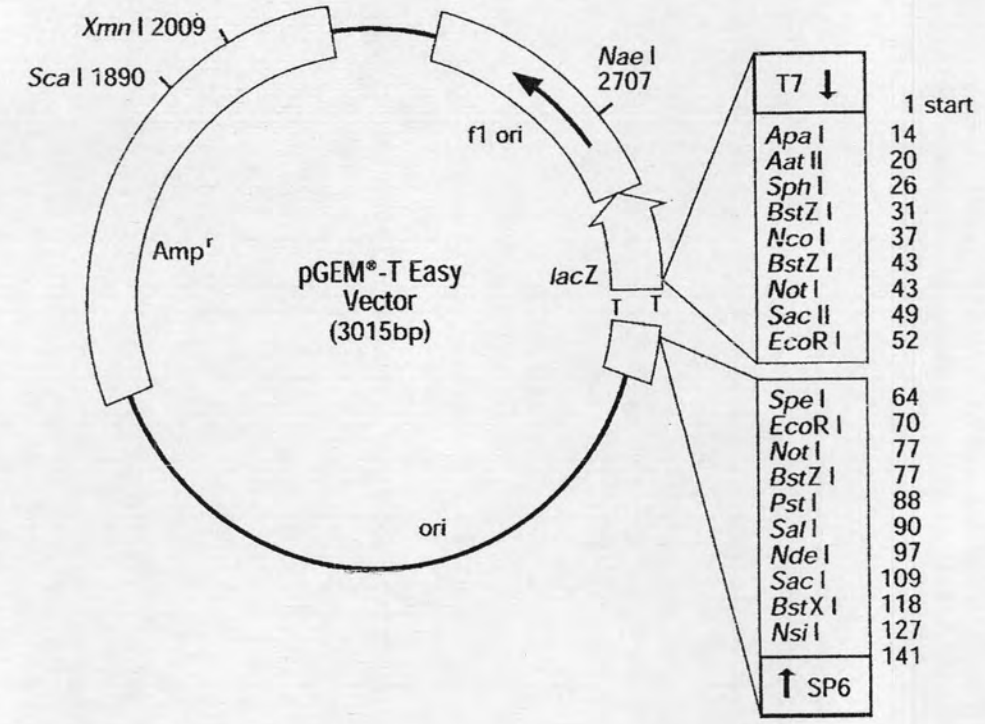
$AgNO_3$	1.5 g
37% formaldehyde	2.25 ml

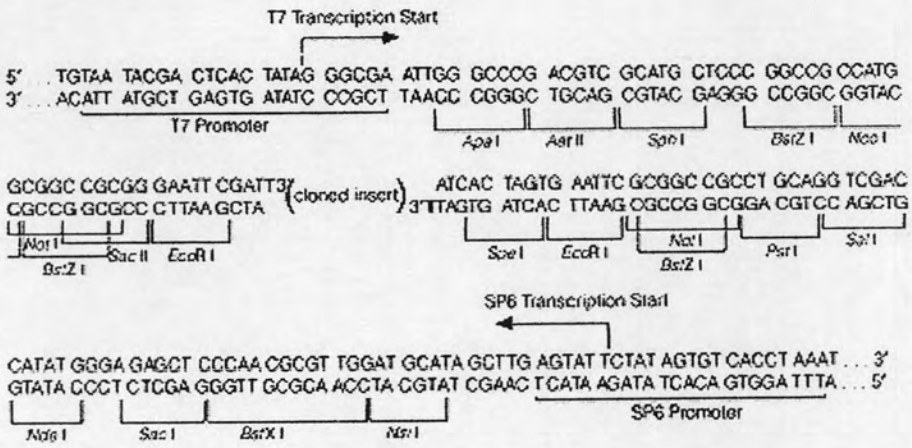
7. Developing solution, 3 liters

$NaCO_3$	90 g
37% formaldehyde	5 ml
Sodium thiosulfate (10 mg/ml)	600 µl

APPENDIX F

RESTRICTION MAPPING OF pGEM®-T EASY VECTOR





APPENDIX G

TE-AFLP AND SSCP HAPLOTYPES

1. Eighty-seven TE-AFLP haplotypes for the primer pair *Bam*HI-C and *Xba*I-CC

Sample	Population	Haplotype code	Haplotype
TLCMN14	North	4	1001000110001001101000011000h
TLNAN24	North	8	1001000110001001101010011011h
TLNAN25	North	20	1001000110001101101111111000h
TLUTN16	North	29	1001000110101001101010011000h
TLKPN23	North	7	1001000110001001101010011010h
TLPLN1	North	9	1001000110001001101010111000h
TLPLN2	North	71	1101000110001101101010111001h
TLSTN26	North	30	1001000111001001101010011011h
TLSTN27	North	38	1001000111001101101010111011h
TLSTN28	North	76	1101000111001001101010111000h
TLSTN30	North	33	1001000111001001101010111010h
TLSTN31	North	10	1001000110001001101010111001h
TLUDNE1	Northeast	59	1010100110001111101010011100h
TLKKNE17	Northeast	76	1101000111001001101010111000h
TLKKNE18	Northeast	67	1101000110001001101010111101h
TLKKNE19	Northeast	87	1110101110101011101010111011h
TLUBNE2	Northeast	56	1010100010011001101010011001h
TLUBNE5	Northeast	1	0010101100101011101010111001h
TLUBNE8	Northeast	86	1110101110101011101010111001h
TLSSNE9	Northeast	66	1101000110001001101010111011h
TLRENE11	Northeast	83	1110100111101011101110110001h
TLRENE12	Northeast	58	1010100110001010101110111011h
TLRENE13	Northeast	11	1001000110001001111100111001h
TLRENE22	Northeast	87	1110101110101011101010111011h
TLRENE23	Northeast	72	1101000110001101101010111011h
TLRENE24	Northeast	65	1101000110001001101010111001h
TLSUNE25	Northeast	62	1101000110001001101010011000h
TLSUNE14	Northeast	77	1101000111001001101010111001h
TLSUNE26	Northeast	64	1101000110001001101010111000h
TLSUNE27	Northeast	84	1110101110101011101010011000h
TLBRNE15	Northeast	76	1101000111001001101010111000h
TLBRNE16	Northeast	85	1110101110101011101010111000h
TLMKNE10	Northeast	73	1101000110011001101010011001h
TLMKNE20	Northeast	31	1001000111001001101010111000h
TLMKNE21	Northeast	85	1110101110101011101010111000h

Sample	Population	Haplotype code	Haplotype
TLNBC1	Central	64	1101000110001001101010111000h
TLLBC5	Central	66	1101000110001001101010111011h
TLLBC7	Central	72	1101000110001101101010111011h
TLSPC10	Central	65	1101000110001001101010111001h
TLSIC13	Central	65	1101000110001001101010111001h
TLSIC14	Central	71	1101000110001101101010111001h
TLNYC15	Central	78	1101001110001001101010111011h
TLBKC16	Central	65	1101000110001001101010111001h
TLAYC45	Central	71	1101000110001101101010111001h
TLAYC46	Central	65	1101000110001001101010111001h
TLAYC47	Central	24	1001000110011001101010111000h
TLPBC26	Central	16	1001000110001101101010111001h
TLKBC50	Central	16	1001000110001101101010111001h
TLKBC51	Central	26	1001000110011101101010111001h
TLKBC52	Central	27	1001000110011101101010111101h
TLKBC53	Central	27	1001000110011101101010111101h
TLKBC54	Central	25	1001000110011101000010111101h
TLKBC56	Central	28	1001000110011101101010111111h
TLKBC57	Central	17	1001000110001101101010111011h
TLKBC58	Central	19	1001000110001101101011111111h
TLKBC59	Central	14	1001000110001101101010011011h
TLKBC60	Central	18	1001000110001101101011111101h
TLPKC29	Central	74	1101000110011011101010011001h
TLPKC31	Central	23	1001000110011001101010011011h
TLPKC32	Central	66	1101000110001001101010111011h
TLPKC41	Central	34	1001000111001001101010111011h
TLPKC44	Central	46	1001010110001101101110111011h
TLPKC39	Central	22	1001000110011001101010011010h
TLPKC40	Central	23	1001000110011001101010011011h
TLPKC61	Central	40	1001000111011101101010111011h
TLPKC64	Central	54	1001010111001101101010111011h
TLPKC65	Central	48	1001010111001001101010111001h
TLPKC66	Central	48	1001010111001001101010111001h
TLPKC67	Central	55	1001010111001101101011111101h
TLPKC69	Central	36	1001000111001011101010111001h
TLPKC62	Central	44	1001010110001101101010111001h
TLPKC68	Central	39	1001000111001101101010111111h
TLTRC3	Central	64	1101000110001001101010111000h
TLTRC4	Central	69	1101000110001011101010111011h
TLCTC5	Central	79	1101001110001011101010111001h
TLCTC9	Central	65	1101000110001001101010111001h
TLCTC12	Central	65	1101000110001001101010111001h
TLCBC15	Central	65	1101000110001001101010111001h

Sample	Population	Haplotype code	Haplotype
TLCBC16	Central	21	1001000110011001101010011001h
TLCPPT27	Peninsular	6	1001000110001001101010011001h
TLCPPT28	Peninsular	32	1001000111001001101010111001h
TLCPPT29	Peninsular	53	1001010111001101101010111001h
TLCPPT33	Peninsular	60	1101000110000001101010011001h
TLCPPT34	Peninsular	50	1001010111001011101010111001h
TLCPPT85	Peninsular	42	1001010110001001101010011001h
TLCPPT86	Peninsular	51	1001010111001101101010011001h
TLCPPT89	Peninsular	6	1001000110001001101010011001h
TLCPPT90	Peninsular	13	1001000110001101101010011001h
TLCPPT91	Peninsular	8	1001000110001001101010011011h
TLCPPT92	Peninsular	41	1001010110000001101010111001h
TLCPPT93	Peninsular	3	1001000110000001101010111001h
TLCPPT99	Peninsular	37	1001000111001101101010111001h
TLCPPT100	Peninsular	45	1001010110001101101010111011h
TLCPPT103	Peninsular	81	1101010110001001101010111001h
TLCPPT104	Peninsular	34	1001000111001001101010111011h
TLCPPT105	Peninsular	54	1001010111001101101010111011h
TLRNPT106	Peninsular	49	1001010111001001101010111011h
TLNTPT37	Peninsular	52	1001010111001101101010111000h
TLSRPT69	Peninsular	35	1001000111001001101110111110h
TLSRPT70	Peninsular	43	1001010110001001101010111010h
TLSRPT71	Peninsular	5	1001000110001001101010011000h
TLSRPT72	Peninsular	80	1101010110001001101010111000h
TLSRPT77	Peninsular	42	1001010110001001101010011001h
TLSRPT84	Peninsular	47	1001010111001001101010111000h
TLPTPT38	Peninsular	66	1101000110001001101010111011h
TLPTPT39	Peninsular	57	1010100110000101101010111010h
TLPTPT40	Peninsular	15	1001000110001101101010101011h
TLPTPT41	Peninsular	70	1101000110001101101010101011h
TLSKPT43	Peninsular	61	1101000110000001101010111011h
TLSKPT48	Peninsular	68	1101000110001011101000101011h
TLSKPT54	Peninsular	12	1001000110001011101000111001h
TLSKPT56	Peninsular	2	1000000110001011101000111000h
TLSKPT63	Peninsular	82	1101010110001001101010111011h
TLSKPT65	Peninsular	80	1101010110001001101010111000h
TLSKPT66	Peninsular	80	1101010110001001101010111000h
TLSKPT78	Peninsular	75	1101000111001001101010011011h
TLSKPT80	Peninsular	63	1101000110001001101010011011h

2. Seventy-seven TE-AFLP haplotypes for the primer pair *Bam*HI-C and *Xba*I-AC

Sample	Population	Haplotype code	Haplotype
TLCMN14	North	1	0001010001101 00h
TLNAN24	North	23	1010011001100 10h
TLNAN25	North	26	1010011001110 10h
TLUTN16	North	56	1011011010101 00h
TLKPN23	North	2	0011010001101 0h
TLPLN1	North	13	1010000010100 00h
TLPLN2	North	19	1010010010100 00h
TLSTN26	North	9	1001011001101 00h
TLSTN27	North	25	1010011001101 10h
TLSTN28	North	43	1011010001101 10h
TLSTN30	North	67	1110010001101 10h
TLSTN31	North	41	1011010001101 00h
TLUDNE1	Northeast	37	1011000011100 00h
TLKKNE17	Northeast	38	1011000110100 00h
TLKKNE18	Northeast	60	1011100110100 00h
TLKKNE19	Northeast	14	1010001010101 00h
TLUBNE2	Northeast	76	1111110011100 00h
TLUBNE5	Northeast	45	1011010011100 00h
TLUBNE8	Northeast	45	1011010011100 00h
TLSSNE9	Northeast	49	1011010111100 00h
TLRENE11	Northeast	63	1011111000100 00h
TLRENE12	Northeast	62	1011110101100 00h
TLRENE13	Northeast	34	1010111001101 00h
TLRENE22	Northeast	30	1010101011101 10h
TLRENE23	Northeast	12	1001011011101 10h
TLRENE24	Northeast	31	1010110001101 10h
TLSUNE25	Northeast	32	1010110011101 00h
TLSUNE14	Northeast	77	1111111010101 00h
TLSUNE26	Northeast	61	1011110010100 00h
TLSUNE27	Northeast	32	1010110011101 00h
TLBRNE15	Northeast	64	1011111001101 00h
TLBRNE16	Northeast	65	1011111010101 0h
TLMKNE10	Northeast	33	1010111000100 00h
TLMKNE20	Northeast	29	1010101010101 0h
TLMKNE21	Northeast	28	1010101010101 0h
TLNBC1	Central	55	1011011010100 00h
TLLBC5	Central	59	1011011011101 00h

Sample	Population	Haplotype code	Haplotype
TLLBC7	Central	58	1011011011110000h
TLSPC10	Central	55	101101101010000h
TLSIC13	Central	55	101101101010000h
TLSIC14	Central	75	111101101110100h
TLNYC15	Central	59	101101101110100h
TLBKC16	Central	27	101001101110100h
TLAYC45	Central	10	100101101010100h
TLAYC46	Central	10	100101101010100h
TLAYC47	Central	10	100101101010100h
TLPBC26	Central	22	101001100110001h
TLKBC50	Central	10	100101101010100h
TLKBC51	Central	7	100101001010100h
TLKBC52	Central	3	100101000100100h
TLKBC53	Central	6	100101000110100h
TLKBC54	Central	68	111001010011100h
TLKBC55	Central	15	101001000100100h
TLKBC56	Central	15	101001000100100h
TLKBC57	Central	15	101001000100100h
TLKBC58	Central	18	101001000110100h
TLKBC59	Central	18	101001000110100h
TLPKC29	Central	53	101101100110100h
TLPKC31	Central	24	101001100110101h
TLPKC32	Central	17	101001000110001h
TLPKC41	Central	24	101001100110101h
TLPKC44	Central	17	101001000110001h
TLPKC39	Central	66	111001000110100h
TLPKC40	Central	41	101101000110100h
TLPKC61	Central	74	111101100110100h
TLPKC64	Central	41	101101000110100h
TLPKC65	Central	41	101101000110100h
TLPKC66	Central	47	101101010110100h
TLPKC67	Central	42	101101000110101h
TLPKC69	Central	54	101101100110101h
TLPKC62	Central	41	101101000110100h
TLPKC68	Central	42	101101000110101h
TLTRC3	Central	48	101101011010101h
TLTRC4	Central	44	101101001010101h
TLCTC5	Central	57	101101101010101h
TLCTC9	Central	20	101001011110101h
TLCTC12	Central	44	101101001010101h
TLCBC15	Central	57	101101101010101h
TLCBC16	Central	11	100101101010101h
TLCPPT27	Peninsular	39	101101000110000h

Sample	Population	Haplotype code	Haplotype
TLCPPT28	Peninsular	46	101101010110001h
TLCPPT29	Peninsular	17	101001000110001h
TLCPPT33	Peninsular	40	101101000110001h
TLCPPT34	Peninsular	71	111101010110000h
TLCPPT85	Peninsular	51	101101100110000h
TLCPPT86	Peninsular	54	101101100110101h
TLCPPT89	Peninsular	39	101101000110000h
TLCPPT90	Peninsular	18	101001000110100h
TLCPPT91	Peninsular	42	101101000110101h
TLCPPT92	Peninsular	16	101001000110000h
TLCPPT93	Peninsular	39	101101000110000h
TLCPPT99	Peninsular	40	101101000110001h
TLCPPT100	Peninsular	40	101101000110001h
TLCPPT103	Peninsular	21	101001100110000h
TLCPPT104	Peninsular	72	111101100110000h
TLCPPT105	Peninsular	52	101101100110001h
TLRNPT106	Peninsular	50	101101100010001h
TLNTPT37	Peninsular	70	111101000110001h
TLSRPT69	Peninsular	17	101001000110001h
TLSRPT70	Peninsular	17	101001000110001h
TLSRPT71	Peninsular	73	111101100110001h
TLSRPT72	Peninsular	69	111101000110000h
TLSRPT77	Peninsular	22	101001100110001h
TLSRPT84	Peninsular	40	101101000110001h
TLPLPT38	Peninsular	51	101101100110000h
TLPLPT39	Peninsular	39	101101000110000h
TLPLPT40	Peninsular	4	100101000110000h
TLPLPT41	Peninsular	4	100101000110000h
TLSKPT43	Peninsular	39	101101000110000h
TLSKPT48	Peninsular	19	101001001010000h
TLSKPT54	Peninsular	16	101001000110000h
TLSKPT56	Peninsular	16	101001000110000h
TLSKPT63	Peninsular	36	101100000110001h
TLSKPT65	Peninsular	35	101100000110000h
TLSKPT66	Peninsular	40	101101000110001h
TLSKPT78	Peninsular	8	100101100110001h
TLSKPT80	Peninsular	5	100101000110001h

3. Forty-seven SSCP haplotypes for the cyt b gene

Sample	Population	Haplotype code	Haplotype
TLCM1N	North	16	0000010001000001h
TLCM2N	North	16	0000010001000001h
TLCM3N	North	16	0000010001000001h
TLCMN14	North	16	0000010001000001h
TLNAN24	North	17	0000011000010000h
TLNAN25	North	17	0000011000010000h
TLPLN1	North	2	0000000000101001h
TLPLN2	North	2	0000000000101001h
TLSTN26	North	19	0000100000010000h
TLSTN27	North	2	0000000000101001h
TLSTN28	North	2	0000000000101001h
TLSTN30	North	1	0000000000010001h
TLSTN31	North	15	0000010000010001h
TLUDNE1	Northest	13	0000001010000000h
TLKKNE17	Northest	9	0000000110000000h
TLKKNE18	Northest	23	0000100100001000h
TLKKNE19	Northest	10	0000000110001000h
TLUBNE2	Northest	25	0001001010000000h
TLUBNE3	Northest	29	0010001000000000h
TLUBNE5	Northest	8	0000000010010100h
TLUBNE8	Northest	8	0000000010010100h
TLSSNE9	Northest	43	0110100000000000h
TLRENE11	Northest	38	0100001010000000h
TLRENE12	Northest	37	0100000110000000h
TLRENE13	Northest	21	0000100010000000h
TLRENE22	Northest	9	0000000110000000h
TLRENE23	Northest	14	0000001110000000h
TLRENE24	Northest	21	0000100010000000h
TLSUNE25	Northest	10	0000000110001000h
TLSUNE14	Northest	9	0000000110000000h
TLSUNE26	Northest	20	0000100000010010h
TLSUNE27	Northest	10	0000000110001000h
TLBRNE15	Northest	14	0000001110000000h
TLBRNE16	Northest	7	0000000010001010h
TLMKNE10	Northest	9	0000000110000000h
TLMKNE20	Northest	14	0000001110000000h
TLMKNE21	Northest	24	0000100110000000h
TLNBC1	Central	42	0110001000000000h
TLNBC3	Central	18	0000100000001000h
TLLBC5	Central	13	0000001010000000h

Sample	Population	Haplotype code	Haplotype
TLLBC7	Central	11	0000001000000001h
TLSPC10	Central	10	0000000110001000h
TLSIC13	Central	6	0000000010001001h
TLSIC14	Central	6	0000000010001001h
TLNYC15	Central	46	1000100000000010h
TLAYC47	Central	36	0100000010000000h
TLAYC48	Central	22	0000100100000000h
TLBKC16	Central	42	0110001000000000h
TLBKC17	Central	36	0100000010000000h
TLBKC22	Central	36	0100000010000000h
TLBKC23	Central	36	0100000010000000h
TLPBC25	Central	43	0110100000000000h
TLPBC26	Central	43	0110100000000000h
TLPBC27	Central	43	0110100000000000h
TLKBC50	Central	39	0100100000000100h
TLKBC51	Central	39	0100100000000100h
TLKBC52	Central	26	0001100000000100h
TLKBC53	Central	46	1000100000000010h
TLKBC54	Central	46	1000100000000010h
TLKBC56	Central	46	1000100000000010h
TLKBC57	Central	46	1000100000000010h
TLKBC58	Central	46	1000100000000010h
TLKBC59	Central	46	1000100000000010h
TLKBC60	Central	46	1000100000000010h
TLTRC3	Central	12	0000001000010000h
TLTRC4	Central	9	0000000110000000h
TLCTC5	Central	19	0000100000010000h
TLCTC9	Central	27	0010000000011000h
TLCTC12	Central	19	0000100000010000h
TLCBC16	Central	45	1000000000000100h
TLPKC31	Prachuap Khiri Khan	47	1010100000000000h
TLPKC32	Prachuap Khiri Khan	47	1010100000000000h
TLPKC33	Prachuap Khiri Khan	47	1010100000000000h
TLPKC35	Prachuap Khiri Khan	47	1010100000000000h
TLPKC36	Prachuap Khiri Khan	47	1010100000000000h
TLPKC41	Prachuap Khiri Khan	47	1010100000000000h
TLPKC42	Prachuap Khiri Khan	47	1010100000000000h
TLPKC43	Prachuap Khiri Khan	32	0010100001000000h
TLPKC39	Prachuap Khiri Khan	43	0110100000000000h
TLPKC38	Prachuap Khiri Khan	43	0110100000000000h
TLPKC40	Prachuap Khiri Khan	43	0110100000000000h
TLPKC61	Prachuap Khiri Khan	3	0000000001000100h
TLPKC64	Prachuap Khiri Khan	22	0000100100000000h

Sample	Population	Haplotype code	Haplotype
TLPKC65	Prachuap Khiri Khan	22	0000100100000000h
TLPKC66	Prachuap Khiri Khan	13	0000001010000000h
TLPKC67	Prachuap Khiri Khan	30	0010010000000100h
TLPKC69	Prachuap Khiri Khan	28	0010000110000000h
TLPKC62	Prachuap Khiri Khan	34	0011000100000000h
TLPKC68	Prachuap Khiri Khan	13	0000001010000000h
TLCPPT27	Chumphon	35	0011100000000000h
TLCPPT28	Chumphon	35	0011100000000000h
TLCPPT29	Chumphon	35	0011100000000000h
TLCPPT85	Chumphon	47	1010100000000000h
TLCPPT86	Chumphon	33	0011000000000001h
TLCPPT89	Chumphon	35	0011100000000000h
TLCPPT90	Chumphon	35	0011100000000000h
TLCPPT92	Chumphon	35	0011100000000000h
TLCPPT93	Chumphon	35	0011100000000000h
TLCPPT94	Chumphon	35	0011100000000000h
TLCPPT99	Chumphon	35	0011100000000000h
TLCPPT100	Chumphon	35	0011100000000000h
TLCPPT103	Chumphon	35	0011100000000000h
TLCPPT104	Chumphon	35	0011100000000000h
TLCPPT105	Chumphon	35	0011100000000000h
TLRNPT106	Chumphon	31	0010100000000000h
TLKPT107	Peninsular	40	0101000000000000h
TLKPT 108	Peninsular	40	0101000000000000h
TLNTPT37	Peninsular	40	0101000000000000h
TLSRPT69	Peninsular	40	0101000000000000h
TLSRPT72	Peninsular	3	0000000001000100h
TLSRPT77	Peninsular	44	0111000000000000h
TLSRPT84	Peninsular	41	0101000000000001h
TLPTPT38	Peninsular	4	0000000001011000h
TLPTPT40	Peninsular	4	0000000001011000h
TLSKPT43	Peninsular	5	0000000001011100h
TLSKPT48	Peninsular	5	0000000001011100h
TLSKPT54	Peninsular	5	0000000001011100h
TLSKPT56	Peninsular	5	0000000001011100h
TLSKPT63	Peninsular	5	0000000001011100h
TLSKPT65	Peninsular	5	0000000001011100h
TLSKPT79	Peninsular	5	0000000001011100h
TLSKPT80	Peninsular	5	0000000001011100h

4. Forty-one SSCP haplotypes for the ATP(6, 8) gene

Sample	Population	Haplotype code	Haplotype
TLNAN24	North	4	0000000000000100001h
TLNAN25	North	4	0000000000000100001h
TLPLN1	North	9	0000000001000000010h
TLPLN2	North	9	0000000001000000010h
TLSTN26	North	5	0000000000001000001h
TLSTN27	North	22	0010000000000000001h
TLSTN28	North	22	0010000000000000001h
TLSTN30	North	5	0000000000001000001h
TLSTN31	North	22	0010000000000000001h
TLUDNE1	Northest	30	0100000000100000000h
TLKKNE17	Northest	33	1000000000000001000h
TLKKNE18	Northest	2	0000000000000010001h
TLKKNE19	Northest	2	0000000000000010001h
TLUBNE2	Northest	39	1100000000000000000h
TLUBNE3	Northest	39	1100000000000000000h
TLUBNE5	Northest	3	0000000000000011000h
TLUBNE8	Northest	3	0000000000000011000h
TLRENE11	Northest	3	0000000000000011000h
TLRENE12	Northest	3	0000000000000011000h
TLRENE13	Northest	39	1100000000000000000h
TLRENE22	Northest	34	1000000000000010000h
TLRENE23	Northest	35	1000000000100000000h
TLRENE24	Northest	39	1100000000000000000h
TLSUNE25	Northest	8	0000000000100001000h
TLSUNE14	Northest	30	0100000000100000000h
TLSUNE26	Northest	1	0000000000000001100h
TLSUNE27	Northest	8	0000000000100001000h
TLBRNE15	Northest	30	0100000000100000000h
TLBRNE16	Northest	1	0000000000000001100h
TLMKNE10	Northest	33	1000000000000001000h
TLMKNE20	Northest	39	1100000000000000000h
TLMKNE21	Northest	39	1100000000000000000h
TLNBC1	Central	12	0000000010000000010h
TLAYC47	Central	26	0011000000000000000h
TLAYC48	Central	36	1000000010000000000h
TLLBC5	Central	19	0001000000000000100h
TLLBC7	Central	6	0000000000010100000h
TLLBC9C	Central	6	0000000000010100000h
TLSPC10	Central	23	0010000001000000000h
TLSIC13	Central	36	1000000010000000000h
TLSIC14	Central	13	0000000010000100000h

Sample	Population	Haplotype code	Haplotype
TLNYC15	Central	10	0000000001000001000h
TLBKC16	Central	9	0000000001000000010h
TLBKC17	Central	29	0100000000000001000h
TLBKC22	Central	29	0100000000000001000h
TLBKC23	Central	26	0011000000000000000h
TLPBC25	Central	28	0100000000000000100h
TLPBC26	Central	28	0100000000000000100h
TLPBC27	Central	28	0100000000000000100h
TLKBC52	Central	41	1110000000000000000h
TLKBC53	Central	39	1100000000000000000h
TLKBC54	Central	39	1100000000000000000h
TLKBC57	Central	40	1101000000000000000h
TLKBC58	Central	39	1100000000000000000h
TLKBC59	Central	39	1100000000000000000h
TLKBC60	Central	37	1001000000000000000h
TLKBC29	Central	15	0000001000000100000h
TLTRC3	Central	31	0100000001000000000h
TLCTC5	Central	23	0010000001000000000h
TLCTC12	Central	18	0001000000000000010h
TLCBC16	Central	11	0000000001000000010h
TLPKC31	Prachuap Khiri Khan	17	0000100000000000010h
TLPKC32	Prachuap Khiri Khan	17	0000100000000000010h
TLPKC33	Prachuap Khiri Khan	17	0000100000000000010h
TLPKC35	Prachuap Khiri Khan	17	0000100000000000010h
TLPKC36	Prachuap Khiri Khan	27	0100000000000000001h
TLPKC38	Prachuap Khiri Khan	26	0011000000000000000h
TLPKC39	Prachuap Khiri Khan	26	0011000000000000000h
TLPKC40	Prachuap Khiri Khan	22	0010000000000000001h
TLPKC41	Prachuap Khiri Khan	22	0010000000000000001h
TLPKC42	Prachuap Khiri Khan	22	0010000000000000001h
TLPKC43	Prachuap Khiri Khan	22	0010000000000000001h
TLPKC61	Prachuap Khiri Khan	7	0000000000011000000h
TLPKC64	Prachuap Khiri Khan	25	0010100000000000000h
TLPKC65	Prachuap Khiri Khan	25	0010100000000000000h
TLPKC66	Prachuap Khiri Khan	38	1010000000000000000h
TLPKC69	Prachuap Khiri Khan	25	0010100000000000000h
TLPKC62	Prachuap Khiri Khan	7	0000000000011000000h
TLPKC68	Prachuap Khiri Khan	39	1100000000000000000h
TLCPPT27	Chumphon	24	0010001000000000000h
TLCPPT28	Chumphon	24	0010001000000000000h
TLCPPT29	Chumphon	24	0010001000000000000h
TLCPPT85	Chumphon	24	0010001000000000000h
TLCPPT89	Chumphon	21	0001001000000000000h

Sample	Population	Haplotype code	Haplotype
TLCPPT92	Chumphon	24	0010001000000000000h
TLCPPT93	Chumphon	24	0010001000000000000h
TLCPPT94	Chumphon	32	0100001000000000000h
TLCPPT99	Chumphon	21	0001001000000000000h
TLCPPT100	Chumphon	21	0001001000000000000h
TLCPPT103	Chumphon	24	0010001000000000000h
TLCPPT104	Chumphon	24	0010001000000000000h
TLCPPT105	Chumphon	24	0010001000000000000h
TLRNPT106	Chumphon	16	0000010100000000000h
TLKPT107	Peninsular	14	0000000110000000000h
TLKPT 108	Peninsular	14	0000000110000000000h
TLNTPT37	Peninsular	20	0001000100000000000h
TLSRPT69	Peninsular	16	0000010100000000000h
TLSRPT70	Peninsular	16	0000010100000000000h

5. Thirty-nine SSCP haplotypes for the 16S rRNA gene

Sample	Population	Haplotype code	Haplotype
TLCM1N	North	8	00000001000000100h
TLCM2N	North	8	00000001000000100h
TLCM3N	North	8	00000001000000100h
TLCMN14	North	8	00000001000000100h
TLNAN24	North	39	1100000000000000h
TLNAN25	North	39	1100000000000000h
TLSTN26	North	34	1000000000001000h
TLSTN27	North	39	1100000000000000h
TLSTN28	North	39	1100000000000000h
TLSTN30	North	39	1100000000000000h
TLSTN31	North	39	1100000000000000h
TLPLN1	North	31	100000000000001h
TLPLN2	North	31	100000000000001h
TLUDNE1	Northeast	34	1000000000001000h
TLKKNE17	Northeast	34	1000000000001000h
TLKKNE18	Northeast	34	1000000000001000h
TLKKNE19	Northeast	34	1000000000001000h
TLUBNE2	Northeast	21	0001010000000000h
TLUBNE3	Northeast	21	0001010000000000h
TLUBNE5	Northeast	27	0011000000000000h
TLUBNE8	Northeast	27	0011000000000000h
TLSSNE9	Northeast	36	1000001000000000h
TLRENE11	Northeast	19	0001000000100000h
TLRENE12	Northeast	25	0010000010000000h
TLRENE13	Northeast	14	0000100000100000h
TLRENE22	Northeast	15	0000100001000000h
TLRENE23	Northeast	15	0000100001000000h
TLRENE24	Northeast	15	0000100001000000h
TLSUNE25	Northeast	37	1000100000000000h
TLSUNE14	Northeast	18	0001000000001000h
TLSUNE26	Northeast	22	0001100000000000h
TLSUNE27	Northeast	15	0000100001000000h
TLBRNE15	Northeast	21	0001010000000000h
TLBRNE16	Northeast	15	0000100001000000h
TLMKNE10	Northeast	34	1000000000001000h
TLMKNE20	Northeast	15	0000100001000000h
TLMKNE21	Northeast	15	0000100001000000h
TLNBC1	Central	11	0000010010000000h
TLNBC3	Central	2	0000000000101000h
TLLBC5	Central	34	1000000000001000h
TLLBC7	Central	34	1000000000001000h

Sample	Population	Haplotype code	Haplotype
TLLBC9C	Central	39	11000000000000000h
TLSPC10	Central	38	10100000000000000h
TLSIC13	Central	9	00000001001000000h
TLSIC14	Central	9	00000001001000000h
TLBKC16	Central	11	00000100100000000h
TLBKC17C	Central	12	00000110000000000h
TLBKC22C	Central	12	00000110000000000h
TLBKC23	Central	12	00000110000000000h
TLAYC47	Central	2	00000000000101000h
TLPBC26	Central	20	00010000100000000h
TLPBC25	Central	20	00010000100000000h
TLPBC27	Central	20	00010000100000000h
TLPBC28C	Central	20	00010000100000000h
TLKBC50	Central	32	10000000000000010h
TLKBC51	Central	33	10000000000000100h
TLKBC52	Central	23	00100000000000100h
TLKBC53	Central	26	00101000000000000h
TLKBC54	Central	35	10000000000100000h
TLKBC56	Central	26	00101000000000000h
TLKBC57	Central	26	00101000000000000h
TLKBC58	Central	26	00101000000000000h
TLKBC59	Central	26	00101000000000000h
TLKBC60	Central	26	00101000000000000h
TLTRC3	Central	17	00010000000000001h
TLCTC9	Central	34	10000000000001000h
TLCTC12	Central	35	10000000000100000h
TLCTC10	Central	35	10000000000100000h
TLCBC16	Central	29	01000000001000000h
TLPKC29	Prachuap Khiri Khan	24	00100000001000000h
TLPKC30	Prachuap Khiri Khan	25	00100000010000000h
TLPKC31	Prachuap Khiri Khan	10	00000001100000000h
TLPKC32	Prachuap Khiri Khan	10	00000001100000000h
TLPKC33	Prachuap Khiri Khan	10	00000001100000000h
TLPKC35	Prachuap Khiri Khan	16	00001000100000000h
TLPKC36	Prachuap Khiri Khan	16	00001000100000000h
TLPKC41	Prachuap Khiri Khan	11	00000100100000000h
TLPKC42	Prachuap Khiri Khan	11	00000100100000000h
TLPKC43	Prachuap Khiri Khan	11	00000100100000000h
TLPKC44	Prachuap Khiri Khan	13	00001000000100000h
TLPKC39	Prachuap Khiri Khan	6	00000000100000010h

Sample	Population	Haplotype code	Haplotype
TLPKC38	Prachuap Khiri Khan	16	00001000100000000h
TLPKC40	Prachuap Khiri Khan	11	00000100100000000h
TLPKC61	Prachuap Khiri Khan	25	00100000010000000h
TLPKC66	Prachuap Khiri Khan	29	01000000001000000h
TLPKC67	Prachuap Khiri Khan	28	01000000000100000h
TLPKC69	Prachuap Khiri Khan	29	01000000001000000h
TLPKC62	Prachuap Khiri Khan	25	00100000010000000h
TLPKC68	Prachuap Khiri Khan	28	01000000000100000h
TLCPPT27	Chumphon	30	01000000100000000h
TLCPPT28	Chumphon	30	01000000100000000h
TLCPPT29	Chumphon	30	01000000100000000h
TLCPPT33	Chumphon	7	00000000100001000h
TLCPPT34	Chumphon	30	01000000100000000h
TLCPPT85	Chumphon	30	01000000100000000h
TLCPPT86	Chumphon	30	01000000100000000h
TLCPPT89	Chumphon	30	01000000100000000h
TLCPPT90	Chumphon	30	01000000100000000h
TLCPPT91	Chumphon	30	01000000100000000h
TLCPPT92	Chumphon	30	01000000100000000h
TLCPPT93	Chumphon	30	01000000100000000h
TLCPPT99	Chumphon	30	01000000100000000h
TLCPPT100	Chumphon	30	01000000100000000h
TLCPPT104	Chumphon	30	01000000100000000h
TLCPPT105	Chumphon	30	01000000100000000h
TLRNPT106	Chumphon	5	00000000100000001h
TLKPT107	Peninsular	5	00000000100000001h
TLKPT108	Peninsular	5	00000000100000001h
TLNTPT37	Peninsular	5	00000000100000001h
TLSRPT69	Peninsular	5	00000000100000001h
TLSRPT70	Peninsular	4	00000000001000001h
TLSRPT71	Peninsular	30	01000000100000000h
TLSRPT72	Peninsular	30	01000000100000000h
TLSRPT77	Peninsular	30	01000000100000000h
TLSRPT84	Peninsular	1	00000000000100001h
TLPTPT38	Peninsular	3	00000000000110000h
TLPTPT39	Peninsular	3	00000000000110000h
TLPTPT40	Peninsular	3	00000000000110000h
TLPTPT41	Peninsular	3	00000000000110000h
TLSKPT43	Peninsular	3	00000000000110000h
TLSKPT48	Peninsular	3	00000000000110000h
TLSKPT54	Peninsular	3	00000000000110000h

Sample	Population	Haplotype code	Haplotype
TLSKPT56	Peninsular	3	00000000000110000h
TLSKPT63	Peninsular	3	00000000000110000h
TLSKPT61	Peninsular	3	00000000000110000h
TLSKPT65	Peninsular	3	00000000000110000h
TLSKPT66	Peninsular	3	00000000000110000h
TLSKPT78	Peninsular	3	00000000000110000h
TLSKPT79	Peninsular	3	00000000000110000h

APPENDIX H
MITOCHONDRIAL DNA POLYMORPHISMS

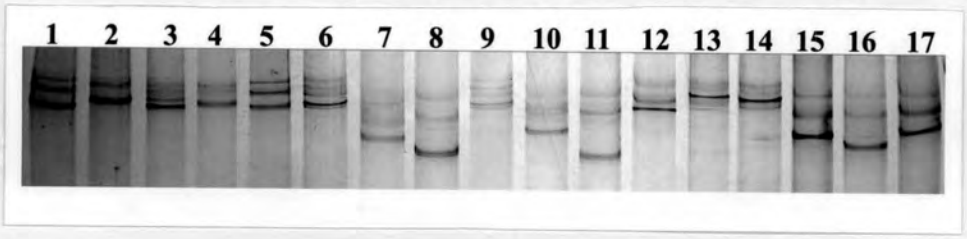


Figure H.1 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals (lanes 1 = TLCMN1, 2 = TLNAN24, 3 = TLSTN30, 4 = TLLBC7, 5 = TLSPC10, 6 = TLSIC13, 7 = TLPKC29, 8 = TLPKC31, 9 = TLAYC48, 10 = TLPKC67, 11 = TLSSNE9, 12 = TLBRNE15, 13 = TLMKNE21, 14 = TLRENE23, 15 = TLCPPT29, 16 = TLCPPT85 and 17 = TLCPPT104). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

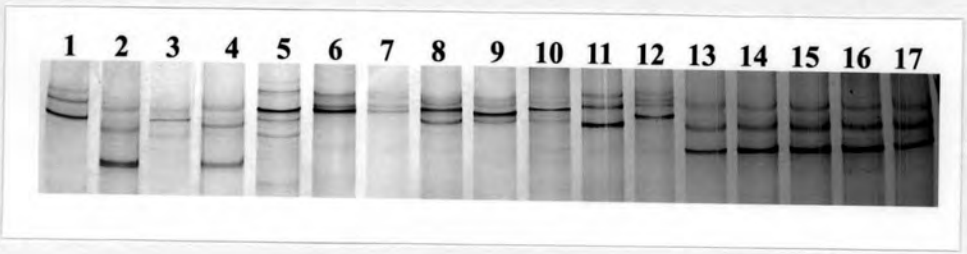


Figure H.2 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals (lanes 1 = TLSTN27, 2 = TLPKC31, 3 = TLPKC69, 4 = TLNYC15, 5 = TLNBC3, 6 = TLCTC5, 7 = TLCTC12, 8 = TLMKNE10, 9 = TLRENE23, 10 = TLKKNE17, 11 = TLSUNE26, 12 = TLSUNE25, 13 = TLCPPT93, 14 = TLCPPT94, 15 = TLCPPT100, 16 = TLCPPT103 and 17 = TLCPPT105). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

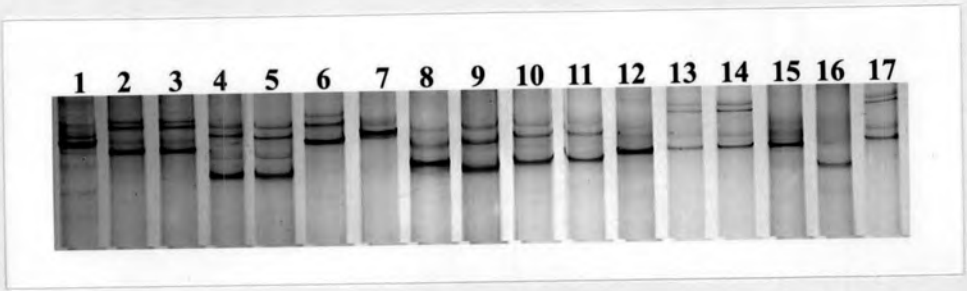


Figure H.3 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals (lanes 1 = TLSTN31, 2 = TLUBNE5, 3 = TLUBNE8, 4 = TLRENE11, 5 = TLRENE12, 6 = TLBRNE16, 7 = TLRENE23, 8 = TLPKC31, 9 = TLKBC50, 10 = TLKBC57, 11 = TLKBC59, 12 = TLPKC61, 13 = TLSKPT63, 14 = TLSKPT65, 15 = TLSRPT72, 16 = TLSRPT77 and 17= TLSKPT80). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

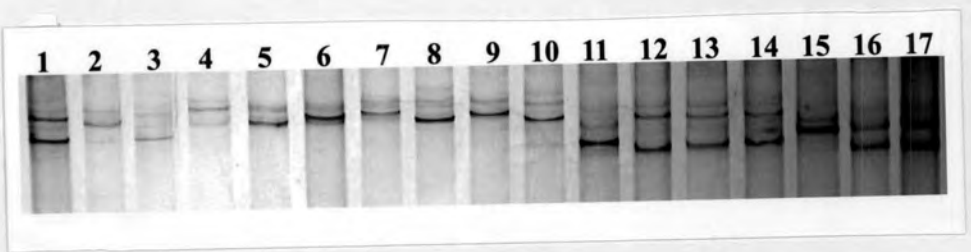


Figure H.4 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals (lanes 1 = TLCMN14, 2 = TLUDNE1, 3 = TLUBNE3, 4 = TLKKNE19, 5 = TLRENE22, 6 = TLRENE23, 7 = TLRENE24, 8 = TLTRC4, 9 = TLLBC5, 10 = TLSPC10, 11 = TLPKC31, 12 = TLKBC53, 13 = TLKBC54, 14 = TLKBC58, 15 = TLCPPT86, 16 = TLKAPT107 and 17 = TLKAPT108). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

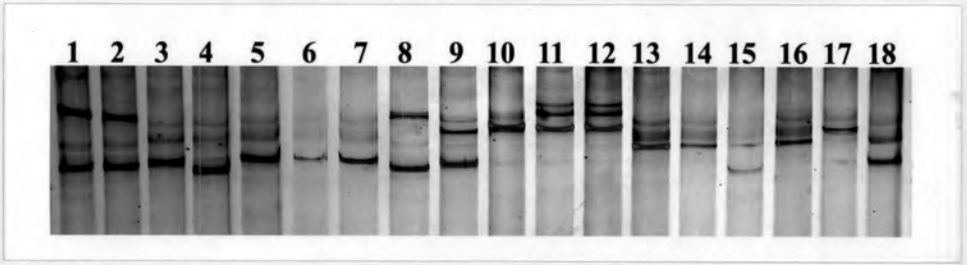


Figure H.5 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals (lanes 1 = TLBKC17, 2 = TLBKC22, 3 = TLPKC31, 4 = TLPKC39, 5 = TLPKC40, 6 = TLPKC41, 7 = TLPKC42, 8 = TLAYC47, 9 = TLKBC53, 10 = TLRENE23, 11 = TLSUNE25, 12 = TLSUNE27, 13 = TLCPPT27, 14 = TLCPPT28, 15 = TLSRPT77, 16 = TLCPPT98, 17 = TLRNPT106 and 18 = TLKAPT107). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

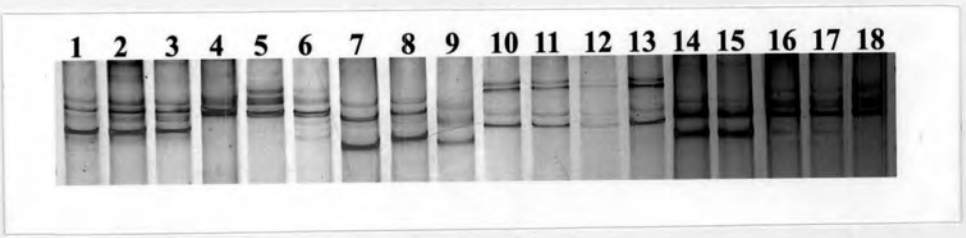


Figure H.6 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals (lanes 1 = TLCMN1, 2 = TLCMN2, 3 = TLCMN3, 4 = TLRENE23, 5 = TLSIC13, 6 = TLSIC14, 7 = TLKBC50, 8 = TLKBC57, 9 = TLNTPT37, 10 = TLSKPT43, 11 = TLSKPT48, 12 = TLSKPT54, 13 = TLSKPT56, 14 = TLSRPT69, 15 = TLSRPT84, 16 = TLCPPT90, 17 = TLCPPT94 and 18 = TLCPPT104). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

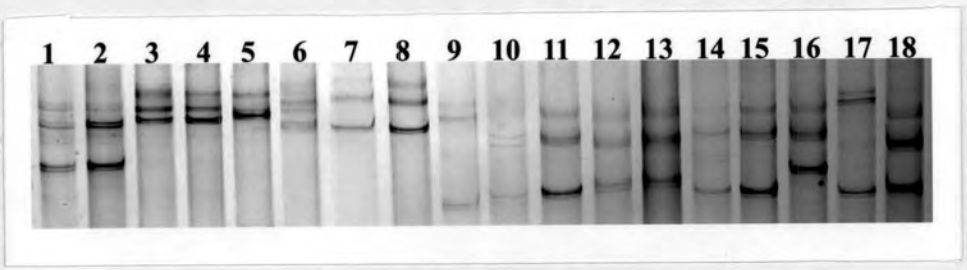


Figure H.7 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals (lanes 1 = TLCMN3, 2 = TLCMN14, 3 = TLNAN24, 4 = TLNAN25, 5 = TLSTN26, 6 = TLSTN31, 7 = TLPLN1, 8 = TLPLN2, 9 = TLBKC23, 10 = TLPBC25, 11 = TLPKC33, 12 = TLPKC35, 13 = TLPKC36, 14 = TLPKC38, 15 = TLPKC39, 16 = TLPKC40, 17 = TLAYC47 and 18 = TLKAPT108). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

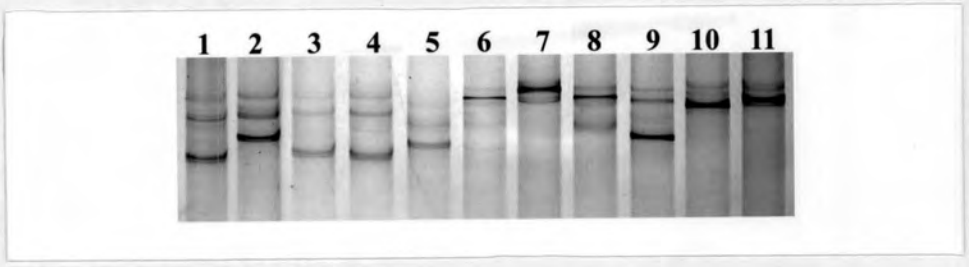


Figure H.8 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals (lanes 1 = TLKBC51, 2 = TLKBC52, 3 = TLKBC56, 4 = TLKBC60, 5 = TLPKC62, 6 = TLPKC66, 7 = TLPKC68, 8 = TLPKC69, 9 = TLUBNE2, 10 = TLSUNE14 and 11 = TLMKNE20). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

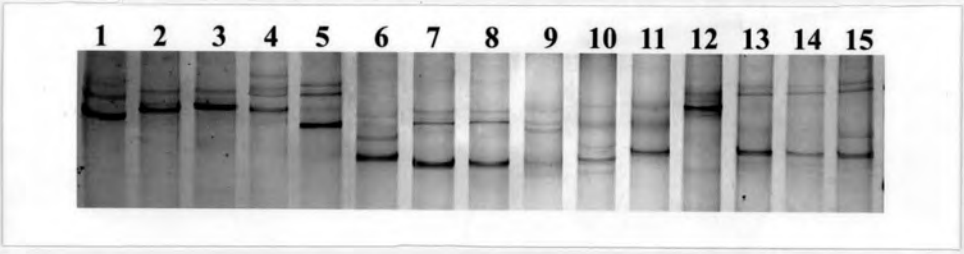


Figure H.9 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals (lanes 1 = TLPLN2, 2 = TLNAN25, 3 = TLSTN26, 4 = TLTRC3, 5 = TLCTC9, 6 = TLCBC16, 7= TLNBC1, 8 = TLBKC16, 9 = TLPBC26, 10 = TLPKC32, 11 = TLPKC43, 12= TLRENE13, 13 = TLPTPT38, 14 = TLPTPT40 and 15 = TLSKPT79). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

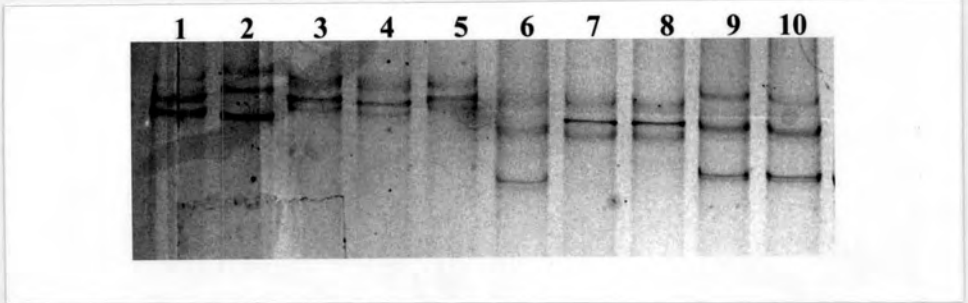


Figure H.10 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals (lanes 1 = TLSTN31, 2 = TLSTN28, 3 = TLRENE13, 4 = TLRENE23, 5 = TLRENE24, 6 = TLPKC36, 7 = TLPKC64, 8 = TLPKC65, 9 = TLCPPT89 and 10 = TLCPPT99). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

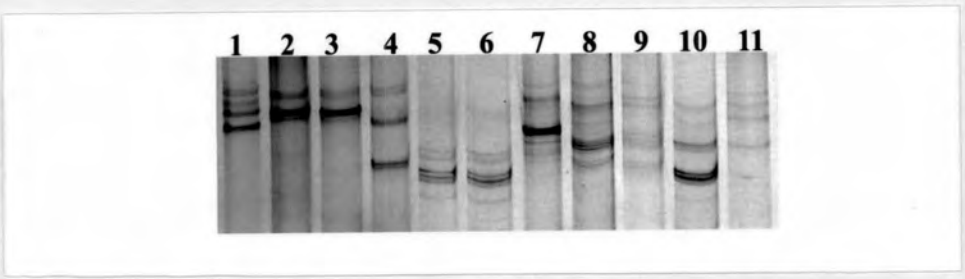


Figure H.11 SSCP patterns of the amplified cyt b gene of *T. pagdeni* individuals and *T. fuscobalteata* (lanes 1 = TLSTN30, 2 = TLMKNE20, 3 = TLRENE23, 4 = TLCPPT93, 5= FUS10N, 6 = FUS13N, 7 = FUS14E, 8 = L03N, 9 = L07N, 10 = L012N and 11 = L020N). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

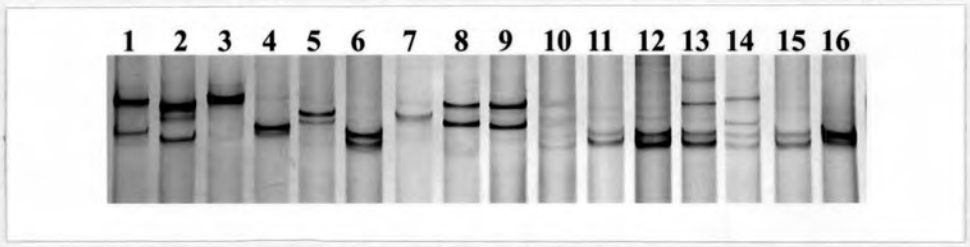


Figure H.12 SSCP patterns of the amplified ATP(6, 8) gene of *T. pagdeni* individuals (lanes 1 = TLTRC3, 2 = TLCTC12, 3 = TLCBC16, 4 = TLBRNE15, 5 = TLBRNE16, 6 = TLRENE24, 7 = TLLBC9, 8 = TLPKC39, 9 = TLAYC47, 10 = TLKBC52, 11 = TLKBC53, 12 = TLKBC54, 13 = TLKBC57, 14 = TLKBC58, 15 = TLKBC59 and 16 = TLPKC68). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

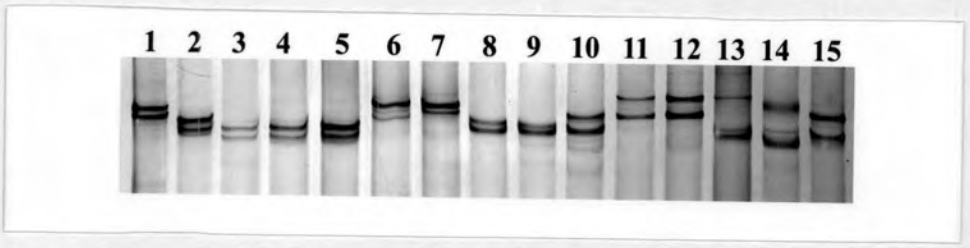


Figure H.13 SSCP patterns of the amplified ATP(6, 8) gene of *T. pagdeni* individuals (lanes 1= TLSTN27, 2 = TLUDNE1, 3 = TLUBNE2, 4 = TLMKNE21, 5 = TLRENE24, 6 = TLSUNE25, 7 = TLSUNE26, 8 = TLPKC32, 9 = TLPKC33, 10 = TLPKC35, 11 = TLPKC38, 12 = TLPKC39, 13 = TLKBC60, 14 = TLPKC62 and 15 = TLPKC66). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

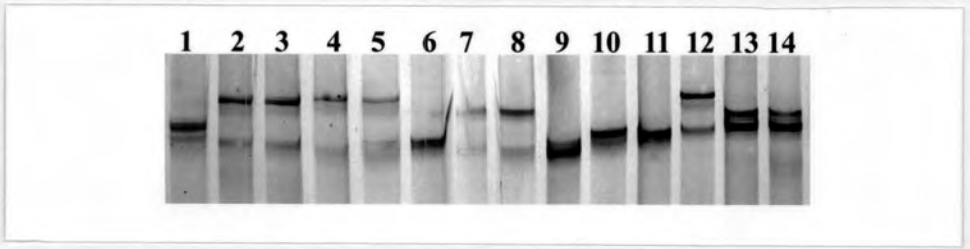


Figure H.14 SSCP patterns of the amplified ATP(6, 8) gene of *T. pagdeni* individuals (lanes 1 = TLUBNE3, 2 = TLUBNE5, 3 = TLUBNE8, 4 = TLRENE11, 5 = TLRENE12, 6 = TLRENE13, 7 = TLKKNE18, 8 = TLKKNE19, 9 = TLRENE22, 10 = TLRENE23, 11 = TLRENE24, 12 = TLBKC22, 13 = TLPBC25 and 14 = TLPBC27). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

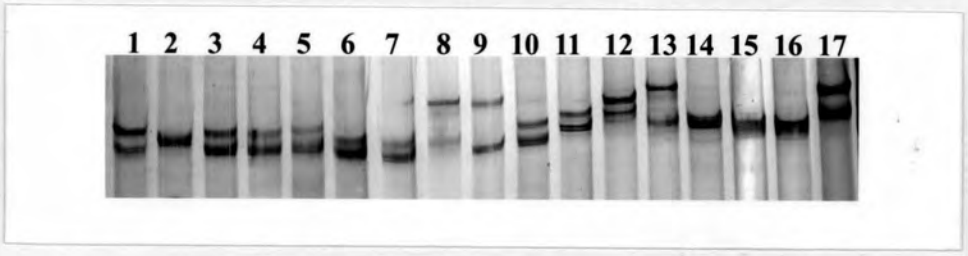


Figure H.15 SSCP patterns of the amplified ATP(6, 8) gene of *T. pagdeni* individuals (lanes 1= TLSTN31, 2= TLPKC33, 3= TLPKC40, 4= TLPKC42, 5= TLPKC43, 6= TLPKC69, 7= TLPKC66, 8= TLBKC23, 9= TLPKC39, 10= TLPKC41, 11= TLPBC26, 12= TLLBC5, 13= TLBKC17, 14= TLPKC35, 15= TLRENE13, 16= TLRENE24 and 17= TLCPPT99). The PCR product was denature and size-fractionated through an 11% native polyacrylamide gel (75:1).

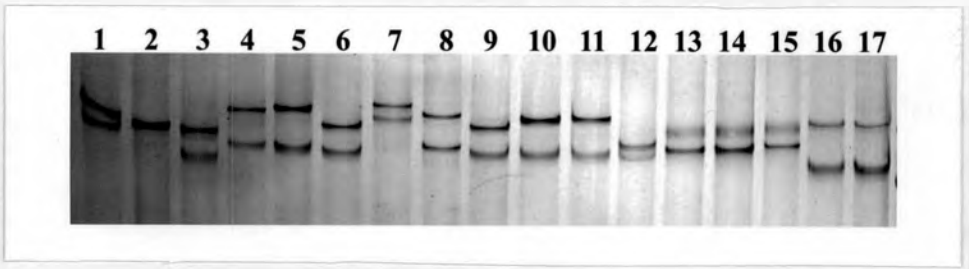


Figure H.16 SSCP patterns of the amplified ATP(6, 8) gene of *T. pagdeni* individuals (lanes 1 = TLNAN25, 2 = TLSTN27, 3 = TLSTN30, 4 = TLUBNE5, 5 = TLSPC10, 6 = TLSIC13, 7 = TLNYC15, 8 = TLPKC39, 9 = TLAYC48, 10 = TLMKNE10, 11 = TLKKNE17, 12 = TLRENE24, 13 = TLCPPT92, 14 = TLCPPT93, 15 = TLCPPT94, 16 = TLKAPT107 and 17 = TLKAPT108). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

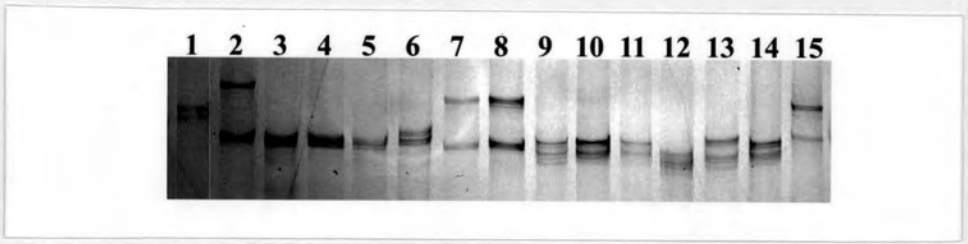


Figure H.17 SSCP patterns of the amplified ATP(6, 8) gene of *T. pagdeni* individuals (lanes 1 = TLLBC9, 2 = TLBKC16, 3 = TLPKC31, 4 = TLPKC32, 5 = TLPKC33, 6= TLPKC35, 7 = TLPKC38, 8 = TLPKC39, 9 = TLRENE13, 10 = TLMKNE20, 11 = TLMKNE21, 12 = TLRENE22, 13 = TLRENE23, 14 = TLRENE24 and 15 = TLSUNE25). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

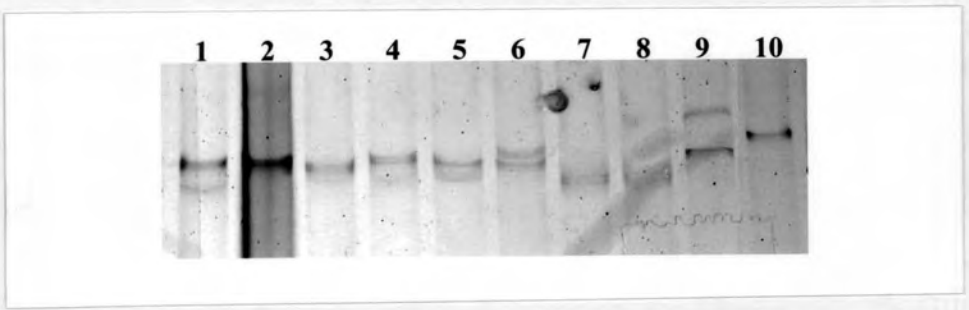


Figure H.18 SSCP patterns of the amplified ATP(6, 8) gene of *T. pagdeni* individuals (lanes 1 = TLSTN31, 2 = TLSTN28, 3 = TLRENE13, 4 = TLRENE23, 5 = TLRENE24, 6 = TLPKC36, 7 = TLPKC64, 8 = TLPKC65, 9 = TLCPPT86 and 10 = TLCPPT99). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

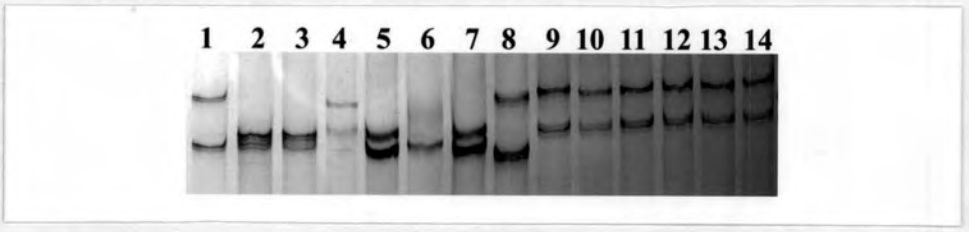


Figure H.19 SSCP patterns of the amplified ATP(6, 8) gene of *T. pagdeni* and *T. fuscobalteata* individuals (lanes 1 = TLPKC39, 2 = TLMKNE20, 3 = TLRENE24, 4 = TLSUNE27, 5 = TLCPPT28, 6 = TLCPPT90, 7 = TLCPPT105, 8 = TLRNPT106, 9 = FUS02C, 10 = FUS03C, 11 = FUS04C, 12 = FUS05C, 13 = FUS06C and 14 = FUS08C). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

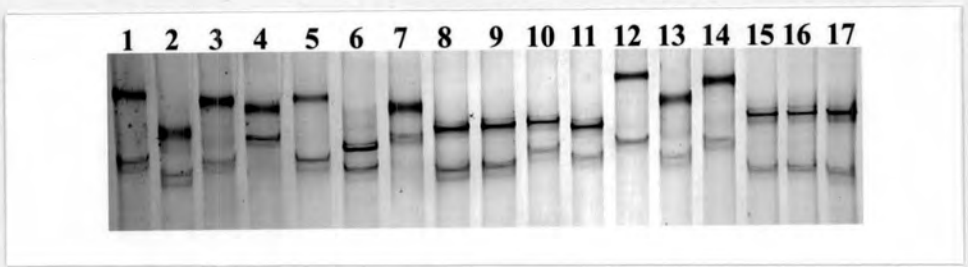


Figure H.20 SSCP patterns of the amplified 16S rRNA gene of *T. pagdeni* individuals (lanes 1 = TLNAN24, 2 = TLTRC3, 3 = TLCTC9, 4 = TLNBC1, 5 = TLLBC7, 6 = TLNYC15, 7 = TLBKC16, 8 = TLUBNE5, 9 = TLUBNE8, 10 = TLSSNE9, 11 = TLRENE12, 12 = TLRENE13, 13 = TLBRNE15, 14 = TLKKNE17, 15 = TLPTPT40, 16 = TLPTPT41 and 17 = TLSKPT54). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

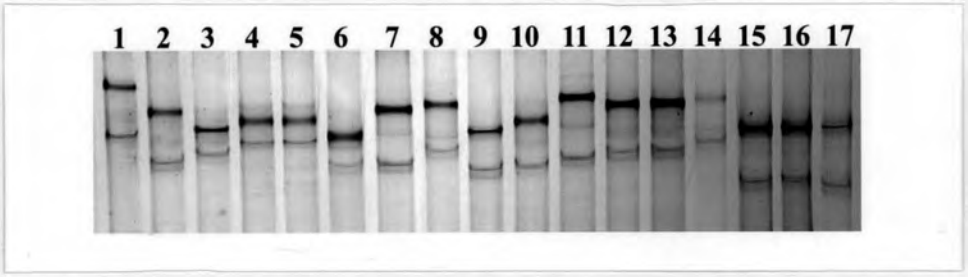


Figure H.21 SSCP patterns of the amplified 16S rRNA gene of *T. pagdeni* (lanes 1 = TLCMN1, 2 = TLNAN24, 3 = TLSIC13, 4 = TLBKC17, 5 = TLBKC22, 6 = TLCTC10, 7 = TLCTC12, 8 = TLCBC16, 9 = TLUBNE5, 10 = TLSUNE14, 11 = TLBRNE16, 12 = TLKKNE18, 13 = TLKKNE19, 14 = TLCPPT34, 15 = TLSKPT61, 16 = TLSKPT63 and 17= TLSKPT65). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

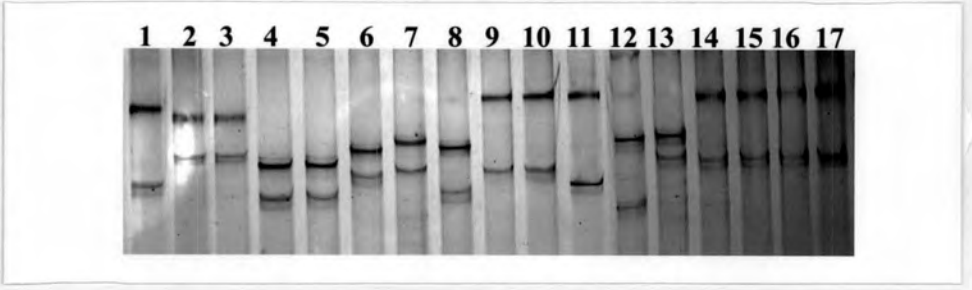


Figure H.22 SSCP patterns of the amplified 16S rRNA gene of *T. pagdeni* individuals (lanes 1 = TLNAN24, 2 = TLBKC22, 3 = TLBKC23, 4 = TLPBC26, 5 = TLPBC27, 6 = TLPKC30, 7 = TLPKC32, 8 = TLUBNE5, 9 = TLMKNE21, 10 = TLRENE23, 11 = TLSUNE26, 12 = TLSKPT66, 13 = TLSRPT70, 14 = TLSRPT71, 15 = TLCPPT92, 16 = TLCPPT99 and 17 = TLCPPT100). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

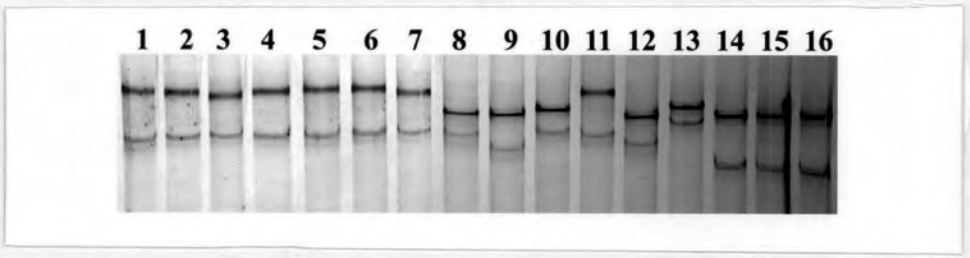


Figure H.23 SSCP patterns of the amplified 16S rRNA gene of *T. pagdeni* individuals (lanes 1 = TLNAN24, 2 = TLNAN25, 3 = TLSTN26, 4 = TLSTN28, 5 = TLSTN30, 6 = TLSTN31, 7 = TLLBC5, 8 = TLSPC10, 9 = TLUBNE5, 10 = TLSSNE9, 11 = TLMKNE10, 12 = TLRENE11, 13 = TLNTPT37, 14 = TLPTPT38, 15 = TLPTPT39 and 16 = TLSKPT43). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

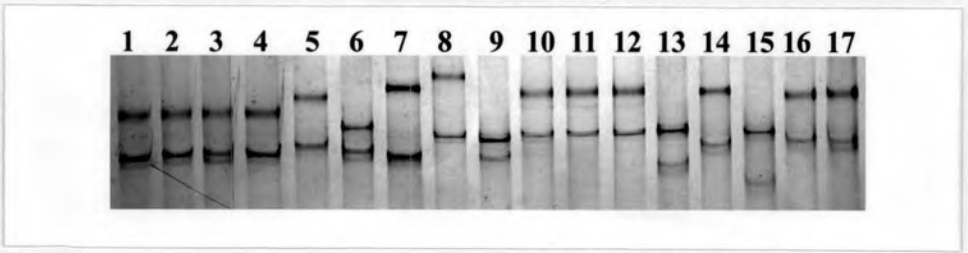


Figure H.24 SSCP patterns of the amplified 16S rRNA gene of *T. pagdeni* individuals (lanes 1 = TLPKC40, 2 = TLPKC41, 3 = TLPKC42, 4 = TLPKC43, 5 = TLPKC44, 6 = TLAYC48, 7 = TLKBC50, 8 = TLKBC52, 9 = TLKBC54, 10 = TLKBC57, 11 = TLKBC58, 12 = TLKBC59, 13 = TLUBNE5, 14 = TLRENE24, 15 = TLSKPT66, 16 = TLCPPT90 and 17 = TLCPPT93). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

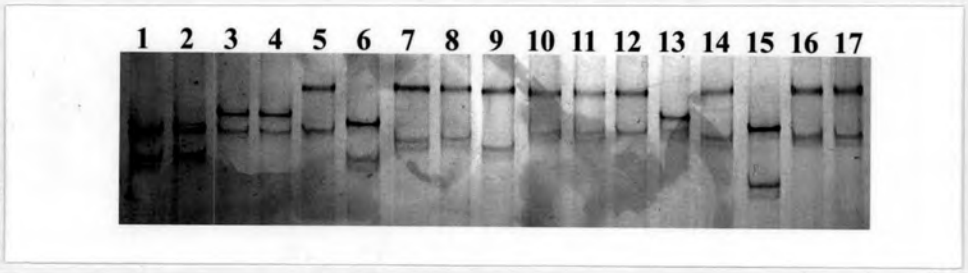


Figure H.25 SSCP patterns of the amplified 16S rRNA gene of *T. pagdeni* individuals (lanes 1 = TLPBC25, 2 = TLPBC28, 3 = TLPKC31, 4 = TLPKC33, 5 = TLPKC38, 6 = TLUBNE5, 7 = TLRENE22, 8 = TLRENE24, 9 = TLSUNE25, 10 = TLCPPT27, 11 = TLCPPT28, 12 = TLCPPT29, 13 = TLSRPT69, 14 = TLSRPT72, 15 = TLSKPT78, 16 = TLCPPT104 and 17 = TLCPPT105). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

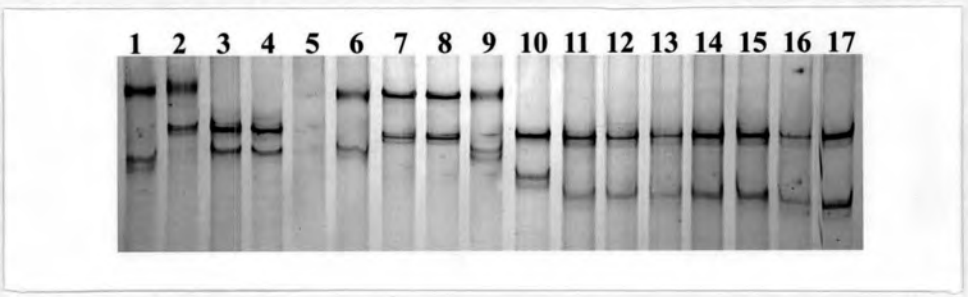


Figure H.26 SSCP patterns of the amplified 16S rRNA gene of *T. pagdeni* individuals (lanes 1 = TLNAN24, 2 = TLKBC60, 3 = TLPKC61, 4 = TLPKC62, 6 = TLPKC66, 7 = TLPKC67, 8 = TLPKC69, 9 = TLUBNE5, 10 = TLPTPT40, 11 = TLPTPT41, 12 = TLSKPT48, 13 = TLSKPT54, 14 = TLSKPT56, 15 = TLSKPT65 and 16 = TLSKPT79). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

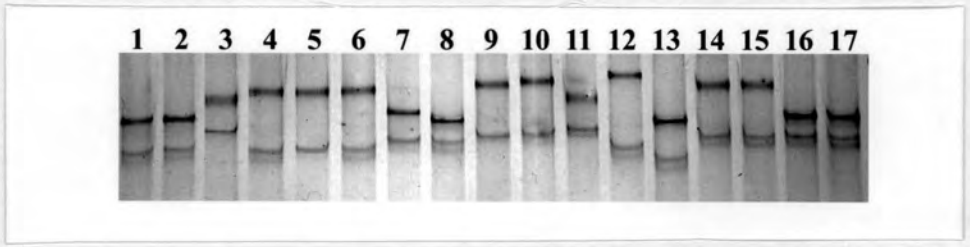


Figure H.27 SSCP patterns of the amplified 16S rRNA gene of *T. pagdeni* individuals (lanes 1 = TLPLN1, 2 = TLPLN2, 3 = TLNBC3, 4 = TLNAN24, 5 = TLSTN27, 6 = TLLBC9, 7 = TLSIC14, 8 = TLPKC29, 9 = TLPKC36, 10 = TLPKC39, 11 = TLAYC47, 12 = TLKBC51, 13 = TLUBNE5, 14 = TLCPPT92, 15 = TLCPPT104, = TLRNPT106 and 17= TLKAPT108). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

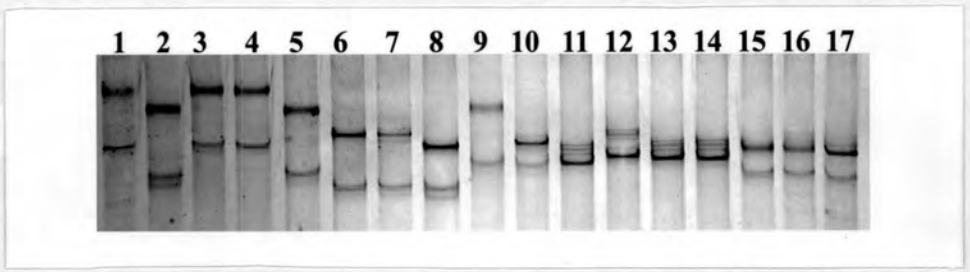


Figure H.28 SSCP patterns of the amplified 16S rRNA gene of *T. pagdeni* individuals (lanes 1 = TLCMN14, 2 = TLNAN24, 3 = TLCMN2, 4 = TLCMN3, 5 = TLUDNE1, 6 = TLUBNE2, 7 = TLUBNE3, 8 = TLUBNE5, 9 = TLCPPT33, 10 = TLNTPT37, 11 = FUS01N, 12 = FUS02C, 13 = FUS03C, 14 = FUS04C, 15 = L03N, 16 = L04N and 17 = L06N). The PCR product was denatured and size-fractionated through an 11% native polyacrylamide gel (75:1).

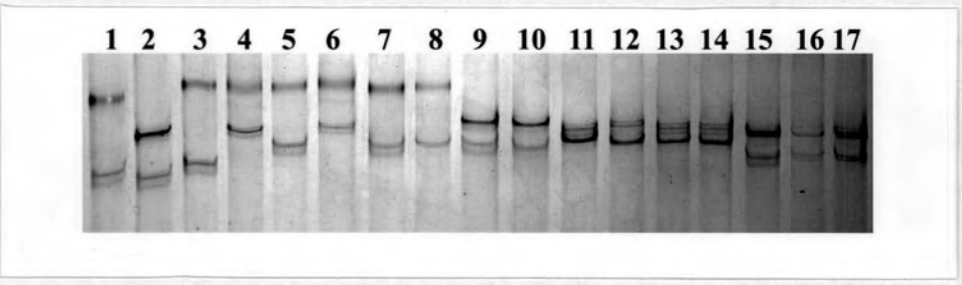


Figure H.29 SSCP patterns of the amplified 16S rRNA gene of *T. pagdeni* and *T. fuscobalteata* individuals (lanes 1 = TLNAN24, 2 = TLUBNE5, 3 = TLSUNE27, 4 = TLKBC53, 5 = TLPKC35, 6 = TLSKPT56, 7 = TLCPPT85, 8 = TLCPPT86, 9 = TLKAPT107, 10 = TLNTPT37, 11 = FUS08C, 12 = FUS09N, 13 = FUS10N, 14 = FUS11N, 15 = L07N, 16 = L08N and 17= L09N). The PCR product was denatured and size-fractionated through a 11% native polyacrylamide gel (75:1).

APPENDIX I
PERSONAL INFORMATION

FIELD OF RESEARCH INTEREST

My dissertation focused on molecular marker and population genetic studies of stingless bees *Trigona pagdeni* in Thailand. Methodologically, I am familiar with biochemical laboratory techniques such as genomic DNA extraction, PCR, Inverse PCR technique, Genome walking technique, DNA based marker (AFLP, TE-AFLP, SCAR marker and SSCP analysis), DNA cloning as well as population genetic analysis program (AMOVA analysis, MEGA program, GenALEx, Winclada, and POPGEN program) and Microsoft Office and Photoshop Adobe.

SCHOLARSHIPS

June 2003 - May 2008

Academic scholarship from Thailand Research Fund to the Royal Golden Jubilee Ph.D. program, Bangkok, Thailand

ACADEMIC EXPERIENCES

1. The 17th FAOBMB Symposium/2nd IUBMB Special Meeting/7th A-IMBN Conference Genomics and Health in the 21st Century at The Imperial Queen's Park Hotel, Bangkok, Thailand 22-26 November 2004. (Secretary general)
2. The 15th IUSSI World Congress, at the Omni Shoreham Hotel in Washington D.C., USA July 30-Aug. 6, 2006 (Proceeding) "Development of species-specific Markers for identification of *Trigona laeviceps* Smith in Thailand " (Poster presentation)
3. RGJ-Ph.D. Congress VIII, Jomtein Palm Beach Resort Pattaya, Chon Buri. April 20-22, 2007. (Proceeding) "Genetic diversity and population structure of the stingless bee *Trigona laeviceps* Smith in Thailand''(Oral presentation).
4. The 12th Biological Sciences Graduate Congress (Science Empowering life). Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia December

17-19, 2007 (Proceeding) "Genetic diversity and population structure of the stingless bee *Trigona pagdeni* in Thailand".(Oral presentation)

PUBLICATIONS

1. **Thummajitsakul, S.**, Klinbunga, S., Smith, D. and Sittipraneed, S. 2008. Genetic diversity and population structure of *Trigona pagdeni* Schwarz in Thailand, Apidologie 39: 446-455.

REFERENCES

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Associate Professor Deborah Smith, Ph.D. Department of Ecology and Evolutionary Biology, Entomology Program, Haworth Hall, University of Kansas, Lawrence, Kansas 66045, USA

e-mail: debsmith@ku.edu

BIOGRAPHY

Miss Sirikul Thummajitsakul was born on Aug 26, 1980 in Saraburi province, Thailand. She graduated with a Bachelor of Science degree in Biochemistry, Faculty of Science, Chulalongkorn University in 2003. She has further studied for the Doctor of Philosophy (Ph.D.) degree in Biochemistry, Faculty of Science, Chulalongkorn University since 2003.