CHAPTER III

RESULT

3.1 DNA extraction

Genomic DNA was extracted from each stingless bee individual using the extraction protocol described in 2.7.1. High molecular weight DNA obtained was at least 23.1 kb. The DNA concentration was estimated by comparison the intensity of EtBr-DNA complex with a known amount of λ/*Hin*dIII marker in 0.8% agarose gel electrophoresis (Figure 3.1). The extracted DNA was used in subsequent analysis.

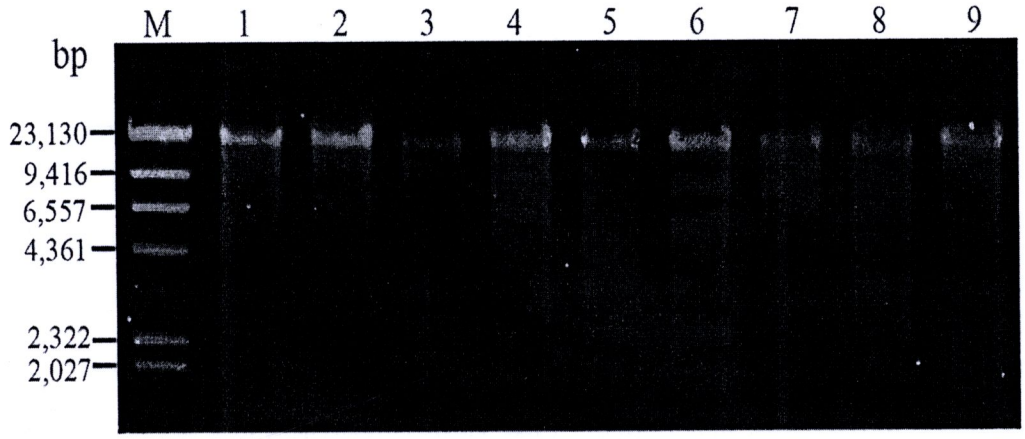


Figure 3.1 High molecular weight DNA of *Tetragonilla collina* worker extracted from one bee per nest. Lane M is λ/*Hin*dIII markers.

3.2 Development of *T. collina*-specific marker using AFLP (Amplified Fragment Length Polymorphism) and SSCP (Single Strand Conformational Polymorphism) analysis

3.2.1 AFLP analysis

The AFLP analysis consisted of several steps: genomic DNA digestion, adaptor ligation, preamplification, and selective amplification of the digested/ligated fragments. One primer combination (*Pst*I$_{+A}$ and *Mse*I$_{+C}$ primer) was used in the preamplification step. The preamplified products were then amplified with selective primers having three selective bases at the 3′ end of each primer. There were 64 primer combinations which were used to test against genomic DNA of 11 stingless bee species including *Tetragonilla collina*. The products of selective amplification showed different band patterns in each stingless bee species using the same primer combination (Figure 3.2). The products were then size-fractionated through denaturing polyacrylamide gel. The primer combinations provided a low level of polymorphism in *Tetragonilla collina* and different band patterns from other species were screened to search species-specific bands in *T. collina*. The *Pst*I$_{+AGT}$ and *Mse*I$_{+CAG}$ primer combination provided a 316 bp fragment found only in *T. collina* (Figure 3.3).

3.2.2 Cloning and characterization of a species-specific AFLP fragment

A 316 bp fragment only found in *T. collina* was successfully reamplified (Figure 3.4). The purified product was cloned. Colony PCR was performed to verify the inserted fragment of 316 bp (Figure 3.5). The recombinant plasmid was sequenced in both directions. The nucleotide sequence (Figure 3.6) did not match any sequence in the GenBank (*E*-value >1e-04) and was regarded as an anonymous DNA segment.

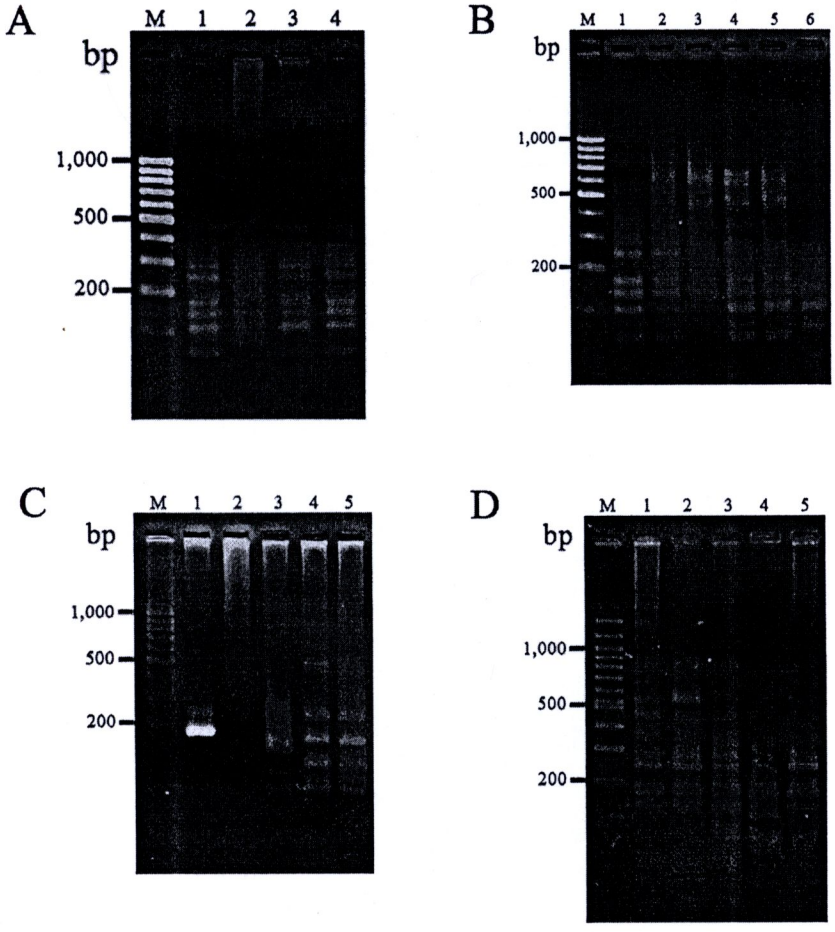


Figure 3.2 The selective amplification products of each stingless bee species on agarose gel; *Tetragonilla collina* (lanes 1-4 A and lanes 1-6 B), *Heterotrigona itama* (lane 1C), *Tetrigona apicalis* (lane 2C), *Lophotrigona canifrons* (lane 3C), *Tetragonula fuscobalteata* (lane 4C), *Tetragonula pagdeni* (lane 5C), *Tetragonula minor* (lane 1D), *Lepidotrigona terminata* (lane 2D), *Geniotrigona thoracica* (lane 3D), *Homotrigona fimbriata* (lane 4D), and *Tetragonula melina* (lane 5D) amplified by *Pst*I$_{+AGT}$/*Mse*I$_{+CAG}$. Lanes M (A, B, C, and D) are a 100 bp DNA ladder.

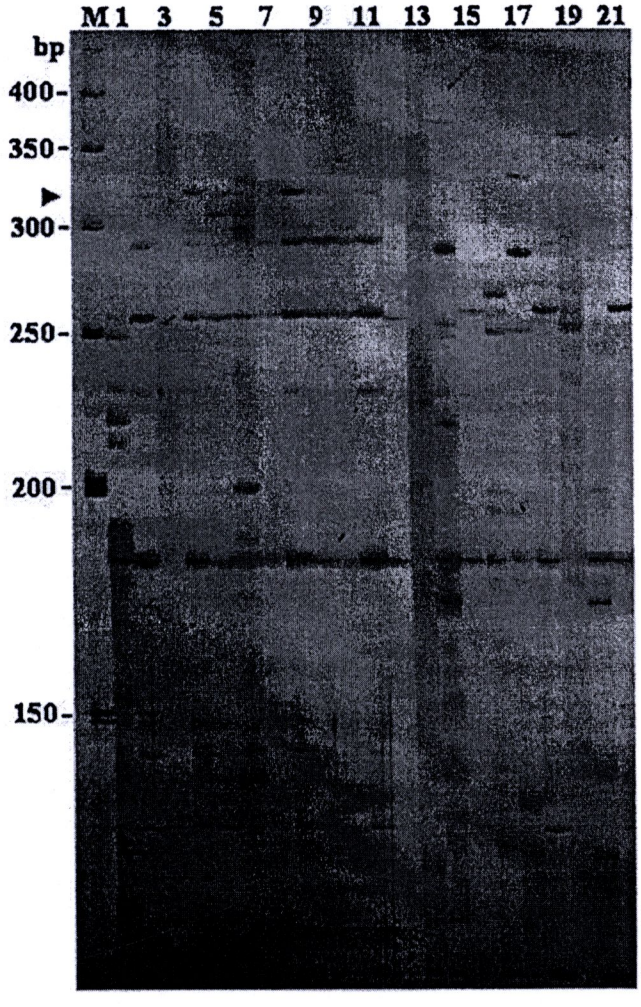


Figure 3.3 AFLP patterns of various stingless bees; *Heterotrigona itama* (lanes 1 and 19), *Tetragonilla collina* (lanes 2-11), *Tetragonula pagdeni* (lane 12), *Tetrigona apicalis* (lane 13), *Lophotrigona caniforns* (lane 14), *Tetragonula minor* (lane 15), *Lepidotrigona doipaensis* (lane 16), *Geniotrigona thoracica* (lane 17), *Tetragonula fuscobalteata* (lane 18), *Homotrigona fimbriata* (lane 20), *Tetragonula melina* (lane 21) genotyped by *Pst*I$_{+AGT}$/*Mse*I$_{+CAG}$. Lane M is a 50 bp DNA ladder. An arrow indicates an AFLP band of 316 bp found only in *T. collina*.

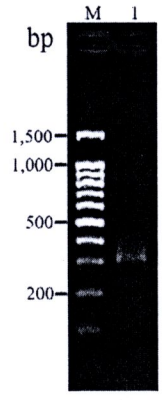


Figure 3.4 Reamplification of the species-specific marker of *T. collina*. Lame M is a 100 bp ladder

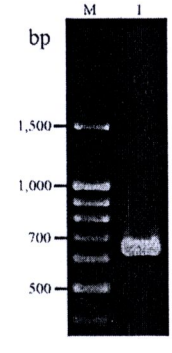


Figure 3.5 Colony PCR product of the recombinant clone containing the targeted insert (the species-specific AFLP marker in *T. collina*). Lame M is a 100 bp DNA ladder

5′GACTGCGTAC ATGCAGAGTG **GTTCGGATTT GGTTGGCATT G**GAACCCTGA
TCAGCGCGAA TTCTCACTGG AGTTCCCAGC ACACGGCGGT CGGAGAGTGA
TCAAATCTCA AGTCGTTGGC CGTCTGAGGG CCGCAGTGTC GGAGCACTAC
ATGTCCGTGC TTATCGCGAT ATCAGACCAG GGCAAGGTCT TCGATGCGAT
CTCTCGCCAT AAGGCGAGCA ACTACTTCAT GCGGACGGGT CAGTACCTAC
GCTTGGTCGA **CTGGCGCTTC GTACACCG**CG CGCGCCTAGA CGTGCTCCTG
TTACTCAGGA CTCATC 3′

Figure 3.6 Nucleotide sequence of a *T. collina*-specific AFLP fragment (316 bp). The locations and sequences of a forward primer (CUTc1-F) and those complementary to a reverse primer (CUTc1-R) for species-diagnostic SCAR marker (CUTc1) are illustrated in boldface and underlined.

3.2.3 Development of species-diagnostic SCAR (sequence-characterized amplified region) marker in *T. collina*

A pair of primers (CUTc1-F and CUTc1-R) was developed from the nucleotide sequences of candidate *T. collina*-specific AFLP fragment (Figure 3.6). The developed SCAR marker (hereafter called CUTc1) was tested against one individual per nest of *T. collina* (134 nests) and 14 other stingless bee species (Figure 3.7). The expected amplification product (259 bp) was found in all *Tetragonilla collina* individuals (134/134 nests accounting for 100% of investigated specimens) but not in the other genus and species of investigated specimens, *Tetrigona apicalis*, *Lophotrigona canifrons*, *Lepidotrigona doipaensis*, *Homotrigona fimbriata*, *Tetragonula fuscobalteata*, *Heterotrigona itama*, *Tetragonula laeviceps*, *Tetrigona melanoleuca*, *Tetragonula melina*, *Tetragonula minor*, *Geniotrigona thoracica*, *Lepidotrigona terminata* and *Lisotrigona furva*. Nevertheless, cross-species amplification was found in *Tetragonula pagdeni* (43/51 nests, 84.3%). It indicated that species-specific PCR of CUTc1 marker unsuccessfully discriminated *Tetragonilla collina* from *Tetragonula pagdeni*.

3.2.4 Characterization of the SCAR marker using SSCP analysis

SSCP analysis was performed to characterize the amplified CUTc1 marker found in *Tetragonilla collina* and *Tetragonula pagdeni*. Non-overlapping SSCP patterns between *T. collina* and *T. pagdeni* were observed (Figure 3.8A). Nucleotide sequences of representative individuals of these species were different, owing to a 15-bp indel (CGGCCGCCAAGCGGC) and several single nucleotide polymorphisms (SNPs). In addition, within species SNPs were also observed in *T. pagdeni* (Figure 3.8B). Moreover, the polymorphic SSCP patterns of CUTc1 marker in *T. collina* were observed (Figure 3.9A). An AA (259/259 bp) genotype was found in all *T. collina* from north (21 nests) and northeast (32 nests), and 23/28 nests from central region, whereas a BB (253/253 bp) genotype was observed in most individuals from peninsular Thailand (42/53 nests). In addition, heterozygotes exhibiting the AB (253/259 bp) genotype were observed in some individuals from central region (Prachuap Khiri Khan, 5/28 nests) and those from peninsular Thailand (Chumphon, Ranong, Surat Thani, and Nakon Si Thammarat, 11/53 nests). Genotypic differences between these specimens were consistent when the amplified product of CUTc1 from

representative individuals carrying AA, AB and BB genotypes were re-examined by denaturing gel electrophoresis (Figure 3.9B). Nucleotide sequences of stingless bees carrying different homozygotic genotypes indicated allelic polymorphism owing to a 6-bp indel (GACCAG) present in AA but absent in BB genotypes (Figure 3.9C).

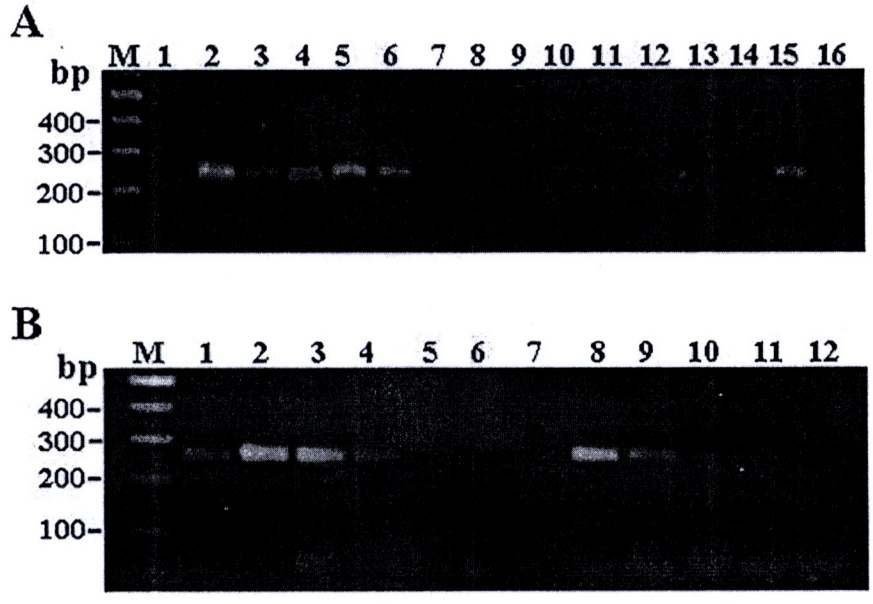
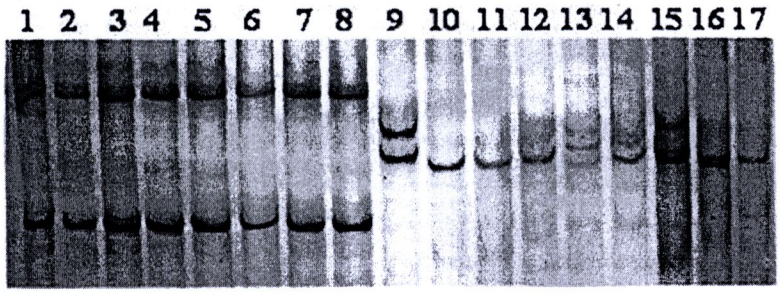


Figure 3.7 Amplification results of species-diagnostic SCAR marker CUTc1 against genomic DNA of *Tetragonilla collina* (lanes 2-6, A and 1-4, B), *Tetrigona apicalis* (lanes 7-9, A), *Lepidotrigona doipaensis* (lane 10, A), *Homotrigona fimbriata* (lane 11, A), *Heterotrigona itama* (lane 12, A), *Tetragonula minor* (lane 13, A), *Tetragonula fuscobalteata* (lane 14, A), *Tetragonula pagdeni* (lanes 15, A and 8-9, B), *Tetrigona melanoleuca* (lane 16, A), *Tetragonula laeviceps* (lane 5, B), *Lisotrigona furva* (lane 6, B), *Tetragonula melina* (lane 7, B), *Lepidotrigona terminata* (lane 10, B), *Geniotrigona thoracica* (lane 11, B), and *Lophotrigona canifrons* (lane 12, B). Lanes M (A and B) and 1 (A) are a 100 bp DNA ladder and the negative control (without genomic DNA template), respectively.

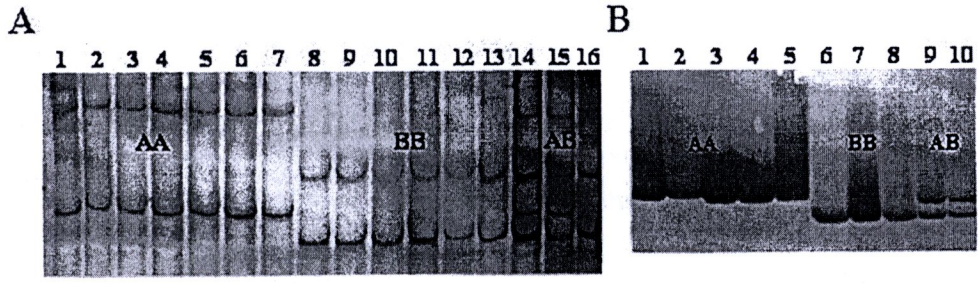
A



B

```
T. pagdeni-C1   GGTTCGGATTTGGTTGGCATTGGAACCCTGATCAGTACGAACTCTCACTGGAGTGCCCAG 60
T. pagdeni-C2   GGTTCGGATTTGGTTGGCATTGGAACCCTGATCAGTGCGAATTCTCACTGGAGTGCCCAG 60
T. collina-N    GGTTCGGATTTGGTTGGCATTGGAACCCTGATCAGCGCGAATTCTCACTGGAGTTCCCAG 60
T. collina-NE   GGTTCGGATTTGGTTGGCATTGGAACCCTGATCAGCGCGAATTCTCACTGGAGTTCCCAG 60
                ********************************  **** ************ *****

T. pagdeni-C1   CACGTGGCGGTCGGAGAGTGATCATACCTCCGGCCGCCAAGCGGCAAGTCGTTGGACATC 120
T. pagdeni-C2   CACGCGGCGGTCGGAGAGTGATCATACCTCCGGCCGCCAAGCGGCAAGTCGTTGGACATC 120
T. collina-N    CACACGGCGGTCGGAGAGTGATCAAAATCTC--------------AAGTCGTTGGCCGTC 105
T. collina-NE   CACACGGCGGTCGGAGAGTGATCAAAATCTC--------------AAGTCGTTGGCCGTC 105
                ***  ****************** * ***               ********** * **

T. pagdeni-C1   TGAGGGCCGCAGTGTCGGAGCACTACATGTCCGTGCTTATCGCGAAGCCGGACCAGGGCA 180
T. pagdeni-C2   TGAGGGCCGCAGTGTCGGAGCACTACATGTCCGTGCTTATCGCGAAGCCGGACCAGGGCA 180
T. collina-N    TGAGGGCCGCAGTGTCGGAGCACTACATGTCCGTGCTTATCGCGAAATCGGACCAGGGCA 165
T. collina-NE   TGAGGGCCGCAGTGTCGGAGCACTACATGTCCGTGCTTATCGCGAAATCGGACCAGGGCA 165
                ************************************************  ************

T. pagdeni-C1   AGGTCTTCGATGCGACCTTCCGTCATGGAGCGAGTAATCGCTTCATACGGACAGGTCAGT 240
T. pagdeni-C2   AGGTCTTCGATGCGACCTTCCGTCATGGAGCGAGTAATCGCTTCATACGGACAGGTCAGT 240
T. collina-N    AGGTCTTCGATGCGATCTGTCCGCATAAGGCGAGCAACTACTTCATGCGGACGGGTCAGT 225
T. collina-NE   AGGTCTTCGATGCGATCTGTCCGCATAAGGCGAGCAACTACTTCATGCGGACGGGTCAGT 225
                *************** **  ** ***   ***** **   ****** ***** *******

T. pagdeni-C1   ACCTGCGCTTTGCCGACTGGCGCTTCGTACACCG 274
T. pagdeni-C2   ACCTGCGCTTTGCCGACTGGCGCTTCGTACACCG 274
T. collina-N    ACCTACGCTTGGTCGACTGGCGCTTCGTACACCG 259
T. collina-NE   ACCTACGCTTGGTCGACTGGCGCTTCGTACACCG 259
                **** ***** * **********************
```

Figure 3.8 SSCP patterns of the amplified CUTc1 of *Tetragonilla collina* (lanes 1-8, A) and *Tetragonula pagdeni* (lanes 9-17, A) and nucleotide sequences of CUTc1 (B) in representative individuals of *Tetragonilla collina* originating from the north (N) and northeast (NE) and *Tetragonula pagdeni* originating from the central region (C), respectively.



C

```
T. collina-C1(AA)   GGTTCGGATTTGGTTGGCATTGGAACCCTGATCAGCGCGAATTCTCACTGGAGTTCCC
T. collina-C2(AA)   GGTTCGGATTTGGTTGGCATTGGAACCCTGATCAGCGCGAATTCTCACTGGAGTTCCC
T. collina-NE(AA)   GGTTCGGATTTGGTTGGCATTGGAACCCTGATCAGCGCGAATTCTCACTGGAGTTCCC
T. collina-S (BB)   GGTTCGGATTTGGTTGGCATTGGAACCCTGATCAGCGCGAATTCTCACTGGAGTTCCC
T. collina-C3(BB)   GGTTCGGATTTGGTTGGCATTGGAACCCTGATCAGCGCGAATTCTCACTGGAGTTCCC
                    ************************************************************

T. collina-C1(AA)   CACACGGCGGTCGGAGAGTGATCAAATCTCAAGTCGTTGGCCGTCTGAGGGCCGCAGT
T. collina-C2(AA)   CACACGGCGGTCGGAGAGTGATCAAATCTCAAGTCGTTGGCCGTCTGAGGGCCGCAGT
T. collina-NE(AA)   CACACGGCGGTCGGAGAGTGATCAAATCTCAAGTCGTTGGCCGTCTGAGGGCCGCAGT
T. collina-S (BB)   CACACGGCGGTCGGAGAGTGATCAAATCTCAAGTCGTTGGCCGTCTGAGGGCCGCAGT
T. collina-C3(BB)   CACACGGCGGTCGGAGAGTGATCAAATCTCAAGTCGTTGGCCGTCTGAGGGCCGCAGT
                    ************************************************************

T. collina-C1(AA)   CGGAGCACTACATGTCCGTGCTTATCGCGAAATCGGACCAGGGCAAGGTCTTCGATGC
T. collina-C2(AA)   CGGAGCACTACATGTCCGTGCTTATCGCGAAATCGGACCAGGGCAAGGTCTTCGATGC
T. collina-NE(AA)   CGGAGCACTACATGTCCGTGCTTATCGCGAAATCGGACCAGGGCAAGGTCTTCGATGC
T. collina-S (BB)   CGGAGCACTACATGTCCGTGCTTATCGCGAAATCG------GGCAAGGTCTTCGATGC
T. collina-C3(BB)   CGGAGCACTACATGTCCGTGCTTATCGCGAAATCG------GGCAAGGTCTTCGATGC
                    ********************************      ********************

T. collina-C1(AA)   TCTCTCGCCATAAGGCGAGCAACTACTTCATGCGGACGGGTCAGTACCTACGCTTGGT
T. collina-C2(AA)   TCTCTCGCCATAAGGCGAGCAACTACTTCATGCGGACGGGTCAGTACCTACGCTTGGT
T. collina-NE(AA)   TCTCTCGCCATAAGGCGAGCAACTACTTCATGCGGACGGGTCAGTACCTACGCTTGGT
T. collina-S (BB)   TCTCTCGCCATAAGGCGAGCAACTACTTCATGCGGACGGGTCAGTACCTACGCTTGGT
T. collina-C3(BB)   TCTCTCGCCATAAGGCGAGCAACTACTTCATGCGGACGGGTCAGTACCTACGCTTGGT
                    ************************************************************

T. collina-C1(AA)   ACTGGCGCTTCGTACACCG 259
T. collina-C2(AA)   ACTGGCGCTTCGTACACCG 259
T. collina-NE(AA)   ACTGGCGCTTCGTACACCG 259
T. collina-S (BB)   ACTGGCGCTTCGTACACCG 253
T. collina-C3(BB)   ACTGGCGCTTCGTACACCG 253
                    ********************
```

Figure 3.9 The amplified CUTc1 against genomic DNA of *T. collina* fractionated in 12.5% non-denaturing polyacrylamide (SSCP) gel electrophoresis (A) and 6% denaturing (B). Three genotypes; AA (lanes 1-7, A and 1-5, B), BB (lanes 8-13, A and 6-8, B) and AB (lanes 14-16, A and 9-10, B) were observed. Nucleotide sequence of CUTc1 in *T. collina* possessing genotype AA (259/259 bp alleles; *T. collina*-C1, *T. collina*-C2 and *T. collina*-NE) and BB (253/253 bp alleles; *T. collina*-S and *T. collina*-C3) are illustrated (C).

3.3 Genetic diversity and population structure of *T. collina* using TE-AFLP (Three Enzymes-Amplified Fragment Length Polymorphism) and TE-AFLP derived markers

3.3.1 TE-AFLP analysis

Genomic DNA of individuals representing 98 nests of *T. collina* was carried out by TE-AFLP procedure: genomic DNA digestion, adaptor ligation, and amplification of the digested/ligated fragments. Two sets of primer combinations (*Xba*I-CC/*Bam*HI-C and *Xba*I-CT/*Bam*HI-C) were used for generating fingerprints of each specimen. The *Bam*HI-C primer was end labeled with ^{32}P. The fingerprint patterns were visualized by autoradiography (Figure 3.10).

3.3.2 Scoring TE-AFLP variation

A total of 53 bands were scored from analysis of *T. collina* using TE-AFLP with two sets of primers (*Xba*I-CC/*Bam*HI-C and *Xba*I-CT/*Bam*HI-C). Thirty bands (57%) were variable (absent in at least one individual), while the rest 23 bands were present in all 98 individuals. We observed 47 unique banding patterns or phenotypes for the primer pair *Xba*I-CC/*Bam*HI-C, and 79 phenotypes for the primer pair *Xba*I-CT/*Bam*HI-C (appendix B). Relatively high genetic diversity was observed in all geographic samples (Table 3.1). The percentage number of polymorphic bands and H_e for each region varied from 42-55% and 0.090-0.141, respectively. The greatest level of within region diversity was found in the Central region.

The unbiased genetic distance between pairs of geographic regions was 0.022-0.094. Significant geographic heterogeneity was observed between all pairwise comparisons of regions analyzed by F_{ST}-based statistics, Φ_{PT} ($P < 0.001$, Table 3.2). The exact test revealed significant genetic differences in most comparisons except between *T. collina* from Central and Peninsular Thailand ($P = 0.6202$) and between North and Northeast ($P = 0.1875$). The results indicated stronger degrees of differentiation between North+Northeast and Peninsular Thailand ($\Phi_{PT} = 0.334$ and 0.359) than other comparisons ($\Phi_{PT} = 0.076$-0.242). Lower (but significant) levels of geographic differentiation were observed between the Central and each of the other populations ($\Phi_{PT} = 0.076$-0.199).

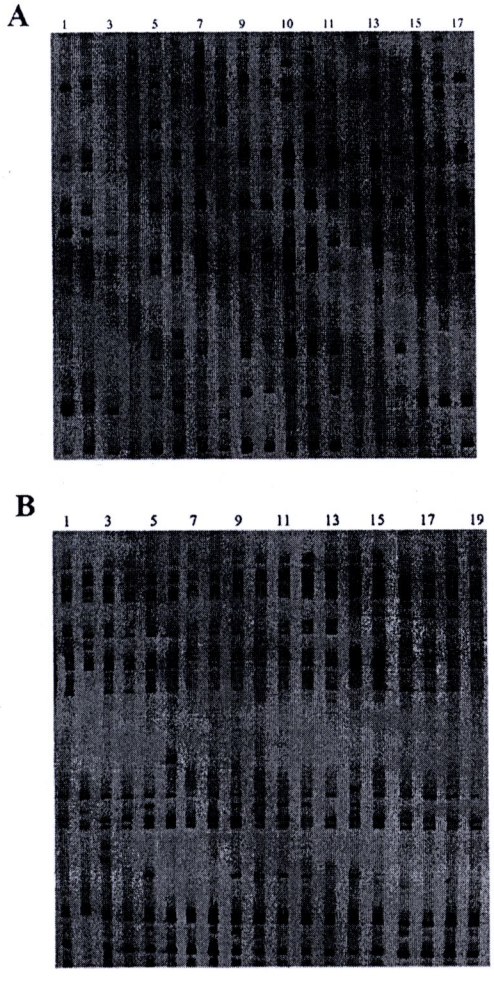


Figure 3.10 Autoradiogram of some TE-AFLP patterns generated by two sets of primer pairs; *Xba*I-CC and *Bam*HI-C (panel A), and *Xba*I-CT and *Bam*HI-C (panel B). The amplification was obtained by *T. collina* DNA from each of four geographic regions (Central, lanes 1-4 A, 1-6 B; North, lanes 5-7 A, 7-12 B; Northeast, lanes 8-14 A, lanes 13-16 B; and Peninsular Thailand, lanes 15-17 A, 17-19 B).

Table 3.1 Comparision of TE-AFLP bands and the expected heterozygosity (H_e) generated by *Bam*HI-C and *Xba*I-CC, and *Bam*HI-C and *Xba*I-CT primer pairs in *T. collina* from 4 geographic regions in Thailand

TE-AFLP bands (*N* = 98)	**Central** (*N* = 20)	**North** (*N* = 18)	**Northeast** (*N* = 23)	**Peninsular** (*N* = 37)
Number scored	51	43	45	48
Number fixed	23	25	24	23
Number absent	2	10	8	5
# polymorphic	28	18	21	25
% polymorphic	55% of 51* (53% of 53)**	42% of 43 (34% of 53)	47% of 45 (40% of 53)	52% of 48 (47% of 53)
Mean H_e (± SD)	0.141 (0.025)	0.095 (0.021)	0.090 (0.020)	0.139 (0.026)

*Total number of TE-AFLP bands in a particular regions; ** Total number of scorable TE-AFLP bands across all regions.

Table 3.2 Pairwise F_{ST}-based statistics (Φ_{PT}, below diagonal), Nei's (1972) genetic distance (in brackets) and the exact test (above diagonal) among populations of *T. collina* in Thailand

	Central	**North**	**Northeast**	**Peninsular**
Central ($N = 20$)	-	$P = 0.0104$	$P < 0.0001$	$P = 0.6202^{ns}$
North ($N = 18$)	0.199* (0.050)	-	$P = 0.1835^{ns}$	$P < 0.0001$
Northeast ($N = 23$)	0.242* (0.059)	0.242* (0.046)	-	$P < 0.0001$
Peninsular ($N = 37$)	0.076* (0.022)	0.334* (0.087)	0.359* (0.094)	-

*significant differences at $P < 0.05$; ns, not significant

AMOVA illustrated significant variance components among the four geographic regions, North, Central, Northeast, and Peninsular Thailand (Φ_{PT} = 0.258, P = 0.001), between North-to-Central and Peninsular Thailand (Φ_{PT} = 0.207, P = 0.001) and between Northeast and other populations (North, Central and Peninsular Thailand; Φ_{PT} = 0.172, P = 0.001, Table 3.3).

Table 3.3 AMOVA for genetic differentiation of *T. collina* among four geographic regions (A), between the North-to-Central and Peninsular populations (B), and between Northeast and other populations (C)

A. Four geographic regions

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	3	1.253	26	0.258	0.001
Within population	94	3.603	74		

B. North-to-Central (North+Northeast+Central) vs. Peninsular Thailand

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	1	1.052	21	0.207	0.001
Within population	96	4.023	79		

C. Northeast vs. other populations

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	1	0.871	17	0.172	0.001
Within population	96	4.207	83		

3.3.3 Cloning and characterization of a TE-AFLP derived fragment in *T. collina*

A polymorphic AFLP band (420 bp) that was variably found in investigated *T. collina* was successfully reamplified by *Bam*HI-C and *Xba*I-CT primer pairs (Figure 3.11). The purified product was cloned. The inserted fragment was verified by colony PCR. The recombinant plasmid was sequenced in both directions. The nucleotide sequence (Figure 3.12) did not match any sequence in the GenBank (*E*-value >1e-04) and was regarded as an anonymous DNA segment.

3.3.4 Development and characterization of TE-AFLP derived SCAR marker using SSCP analysis

A pair of primers (TECU-F and TECU-R) was developed from the nucleotide sequences of polymorphic AFLP fragment (Figure 3.12). The developed SCAR marker (hereafter called TECU) was tested against one individual per nest of *T. collina* (Figure 3.13). The expected amplification product (222 bp) was found in all individuals representing 96 nests of *T. collina.*

SSCP analysis was performed to characterize the amplified TECU marker in 96 investigated specimens. Three SSCP patterns (pattern I, II, III) were observed (Figure 3.14). The nucleotide sequences of representative individuals of each SSCP pattern (Figure 3.15) were different due to a 4-bp indel (GACA) and 6 single nucleotide polymorphisms (SNPs). The pattern I was commonly distributed in all *T. collina* from North (15 nests), Central (12 nests), and Northeast (23 nests) while the pattern II was found in *T. collina* from Prachuap Khiri Khan (3/8 nests, 37.5%), Chumphon (10/13 nests, 77%), and Peninsular Thailand (20/25 nests, 80%). In addition, the pattern III was also observed in those from Prachuap Khiri Khan (5/8 nests, 62.5%), Chumphon (3/13 nests, 23%), and Peninsular Thailand (5/25 nests, 20%). The phylogeographic pattern among 3 patterns based on number of mutation was illustrated in Figure 3.16. Seven mutation steps were found between pattern I and III. The pattern II was an intermediate pattern between pattern I and III.

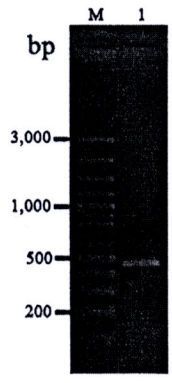


Figure 3.11 Reamplification of the TE-AFLP derived marker of *T. collina.* Lane M is a 100 bp ladder.

```
5'GTTTCGCGCC AGCAAGATCC CACAGTTTTT TATCGATACT GGGATATTTC
  TTTAATCCTC TGTGCAAGCA GCGATGGTTG CTCGATAACG ACGTATCAGT
  GTCGTTCATG GCAATGAAGA GGACTTCTTT TTTTTTTTCT TTATCGAAAT
  ATCACCGAAC AACACAATTA CGAGACATCT TGCAAAATAC AAAGTGTAGT
  TTACACTTTC ACACCATCAT AGACCACGAA CAGTCTTCAT TATGGCACAG
  CTAAATATAT CTTCTCGCAT CCTTTTGCAT TTACACGATA TCAATCGAGA
  TTCCACGCGC TCGAGAAGTC AATAGTTCGT CAAAGTTATT AACGAGAAAC
  AAAAGATATT CGCTTGGAGA AAAGTGAAAA CTCGCGGCTC ACGTTTCTTC
  TCGAGTCTAG TCTCGACGCC 3'
```

Figure 3.12 Nucleotide sequence of the TE-AFLP derived fragment (420 bp). The locations and sequences of a forward primer (TECU-F) and those complementary to a reverse primer (TECU-R) for developed SCAR marker (TECU) are illustrated in boldface and underlined.

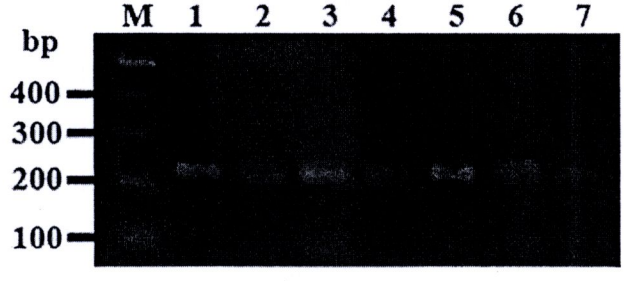


Figure 3.13 Amplification results of TECU marker against genomic DNA of *T. collina.* Lane M is a 100 bp DNA ladder.

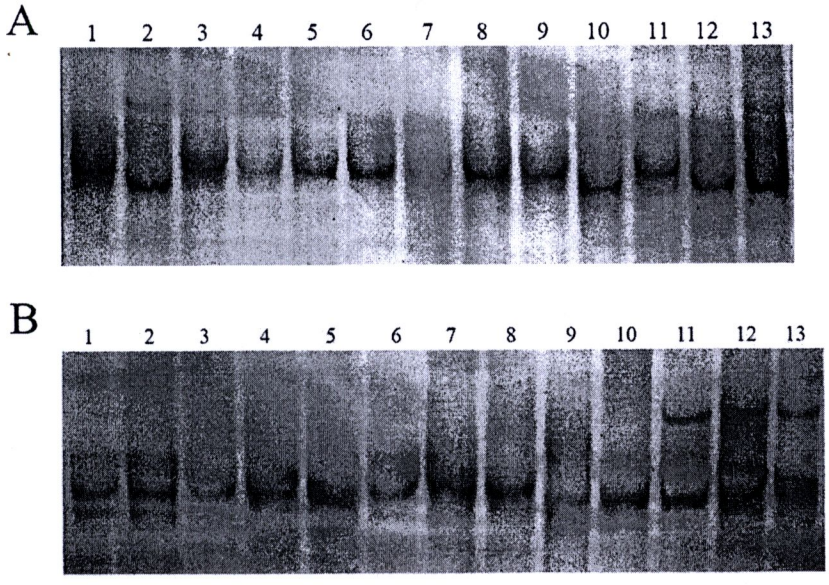


Figure 3.14 The SSCP pattern of the amplified TECU marker of *T. collina* from different geographic regions in Thailand. Three patterns; pattern I (lanes 1, 3-9, 11, A and lanes 1-8, B), pattern II (lanes 10, 12, A and lanes 9, 10, B), and pattern III (lanes 2, 13, A and lanes 11-13, B) were observed.

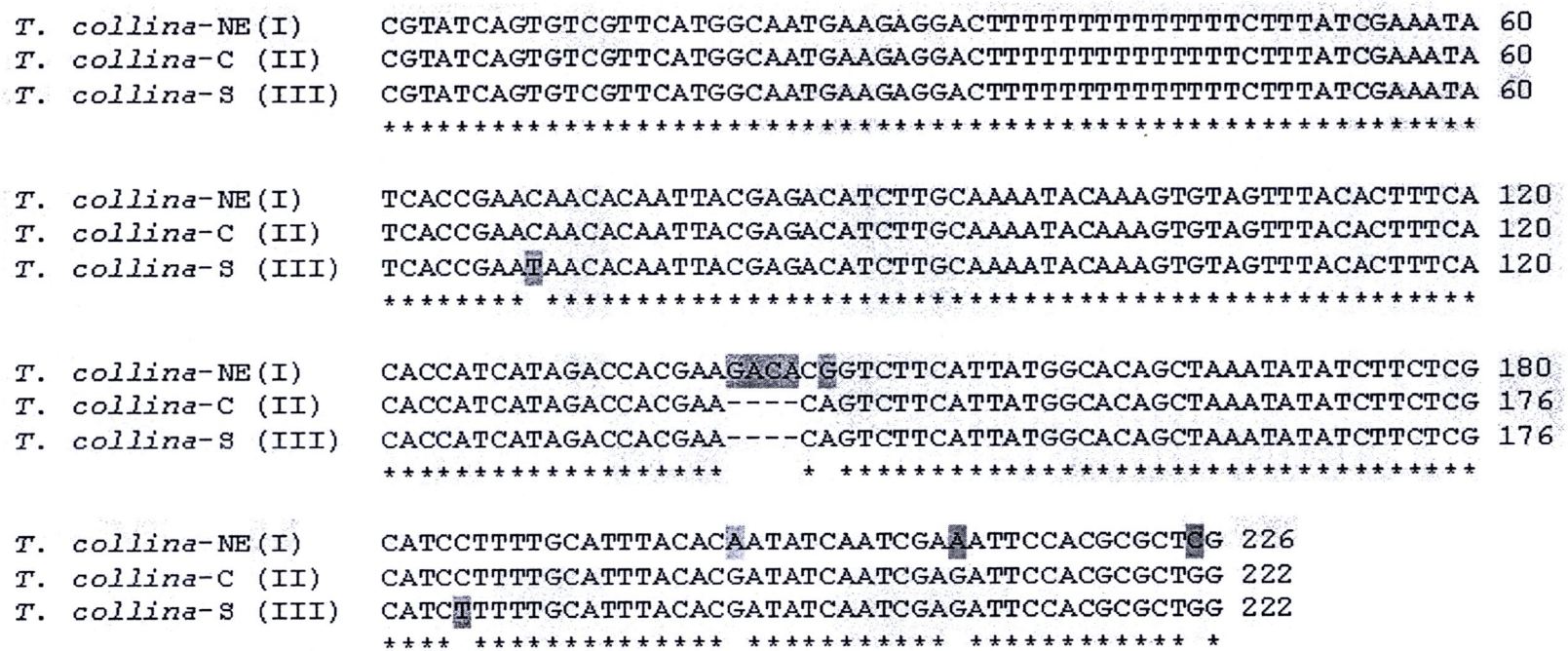


Figure 3.15 Nucleotide sequences of TECU marker in representative individuals of *T. collina* possessing the pattern I (*T. collina*-NE), pattern II (*T. collina*-C), and pattern III (*T. collina*-S).

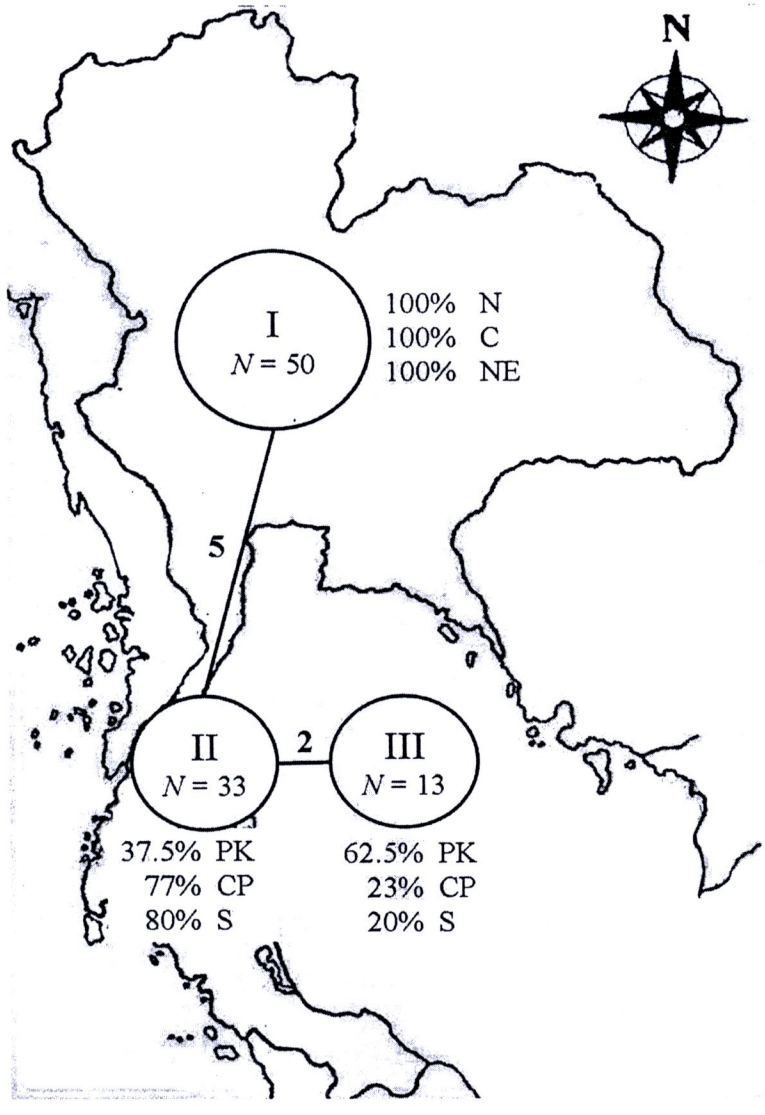


Figure 3.16 Phylogeographic pattern deduced from TECU marker (pattern I, II, and III). Numbers along connected lines indicated inferred mutation steps. Letter *N* represented the amount of *T. collina* possessing each SSCP pattern. The percentages were calculated from the total number of *T. collina* in a particular region; North (15 nests), Central (12 nests), Northeast (23 nests), Prachuap Khiri Khan (8 nests), Chumphon (13 nests), and Peninsular Thailand (25 nests). Capital letters behind percentage were abbreviations of Central (C), Northeast (NE), Prachuap Khiri Khan (PK), Chumphon (CP), and Peninsular Thailand (S).

A total of 3 bands were scored from SSCP analysis of TECU marker in *T. collina* and all bands were variable (absent in at least one individual).Genetic diversity was only observed in Prachuap Khiri Khan, Chumphon, and Peninsular Thailand populations (Table 3.4). The percentage of polymorphic bands for these 3 regions was 50% while the mean expected heterozygosity, H_e varied from 0.063 – 0.158. No polymorphic bands and H_e were observed in North, Central, and Northeast populations.

The unbiased genetic distance between pairs of geographic regions was 0.000-1.994. Significant geographic heterogeneity was observed in most pairwise comparisons of regions analyzed by F_{ST}-based statistics, Φ_{PT} ($P < 0.05$) and the exact test ($P < 0.0001$) except the pairwise comparison of North, Central, and Northeast and the pairwise comparison of Chumphon versus either Prachuap Khiri Khan or Peninsular Thailand and (Table 3.5). The results indicated no degrees of differentiation between North, Central, and Northeast and between Chumphon and Peninsular Thailand ($\Phi_{PT} = 0.000$) while the strong degrees of differentiation were observed in other comparisons ($\Phi_{PT} = 0.899$-0.948).

AMOVA analysis revealed significant molecular variability among 6 population groupings; North, Central, Northeast, Prachuap Khiri Khan, Chumphon, and Peninsular Thailand (88%, $\Phi_{PT} = 0.877$, $P = 0.001$). Higher variance component was observed among 4 populations; North-to-central, Prachuap Khiri Khan, Chumphon, and Peninsular Thailand (90%, $\Phi_{PT} = 0.903$, $P = 0.001$, Table 3.6).

Table 3.4 Comparison of SSCP bands and the expected heterozygosity (H_e) of TECU marker from 6 populations of *T. collina* in Thailand

SSCP Bands (*N* = 96)	**Central** (*N* = 12)	**North** (*N* = 15)	**Northeast** (*N* = 23)	**Peninsular Thailand** (*N* = 25)	**Prachuap Khiri Khan** (*N* = 8)	**Chumphon** (*N* = 13)
Number scored	1	1	1	2	2	2
Number fixed	1	1	1	1	1	1
Number absent	2	2	2	0	0	0
# polymorphic	0	0	0	1	1	1
% polymorphic	0% of 1* (0% of 3)**	0% of 1 (0% of 3)	0% of 1 (0% of 3)	50% of 2 (33.3% of 3)	50% of 2 (33.3% of 3)	50% of 2 (33.3% of 3)
Mean H_e (± SD)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.063 (0.063)	0.158 (0.158)	0.072 (0.072)

*Total number of scorable SSCP bands in a particular regions; ** Total number of scorable SSCP bands across all region

Table 3.5 Pairwise F_{ST}-based statistics (Φ_{PT}, below diagonal), Nei's (1972) genetic distance (in brackets) and the exact test (above diagonal) of TECU marker among populations of *T. collina* in Thailand

	Central	**North**	**Northeast**	**Peninsular Thailand**	**Prachuap Khiri Khan**	**Chumphon**
Central (N = 12)	-	P = 1.0000	P = 1.0000	P < 0.0001	P < 0.0001	P < 0.0001
North (N = 15)	0.000^{ns} (0.000)	-	P = 1.0000	P < 0.0001	P < 0.0001	P < 0.0001
Northeast (N = 23)	0.000^{ns} (0.000	0.000^{ns} (0.000)	-	P < 0.0001	P < 0.0001	P < 0.0001
Peninsular Thailand (N = 25)	0.899* (1.265)	0.906* (1.265)	0.921* (1.265)	-	P = 0.3529	P = 1.0000
Prachuap Khiri Khan (N = 8)	0.919* (1.994)	0.930* (1.994)	0.948* (1.994)	0.283* (0.071)	-	P = 0.7298
Chumphon (N = 13)	0.910* (1.298)	0.920* (1.298)	0.938* (1.298)	0.000^{ns} (0.000)	0.201^{ns} (0.062)	-

*significant differences at P < 0.05; ns, not significant

Table 3.6 Analysis of Molecular Variance (AMOVA) of TECU marker for genetic differentiation of *T. collina* among 6 population groupings; North, Central, Northeast, Prachuap Khiri Khan, Chumphon, and Peninsular Thailand (A), and among 4 populations; North-to-central (North+Central+Northeast), Prachuap Khiri Khan, Chumphon, and Peninsular Thailand (B)

A. Six population groupings

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	5	0.650	88	0.877	0.001
Within population	90	0.091	12		

B. Four population groupings

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	3	0.831	90	0.903	0.001
Within population	92	0.089	10		

3.4 Mitochondrial DNA diversity of *T. collina* using PCR-SSCP

The three mitochondrial DNA gene segments; large subunit ribosomal RNA (16S rRNA), cytochrome oxidase I (COI), and cytochrome b (cytb), were used for genetic diversity and population structure studies of *T. collina*. The specific primer pairs of these 3 genes were successfully developed and amplified against 103 individual colonies of *T. collina*. The amplified products were then characterized by SSCP analysis to examine mtDNA polymorphisms.

3.4.1 Analysis of 16S ribosomal RNA (16S rRNA) gene polymorphism

The expected products (478 bp) were found in all investigated *T. collina* (103/103 nests accounting for 100% of investigated specimens). The products were identified on the 11% non-denaturing polyacrylamide gel (75:1 crosslink) to characterize the 16S rRNA gene polymorphisms (Figure 3.17). Seventeen SSCP phenotypes were observed (appendix B). The nucleotide sequences of 6 common phenotypes (pattern A, B, C, D, E, and F, Figure 3.18), which observed in at least 4 individuals, were different due to a 1-bp indel (T) and several SNPs. The phylogeographic pattern of 6 haplotypes was shown in Figure 3.19. The pattern A was found in all *T. collina* from Prachuap Khiri Khan (12/12 nests, 100%) and most individuals from Central (16/19 nests, 84.2%). The pattern B, C, and D were observed in samples from Northeast (6/25 nests, 24%; 5/25 nests, 20%; 4/25 nests, 16%, respectively). The pattern E, which was different from pattern F by 3 point mutations was distributed in all individuals from Chumphon (11/11 nests, 100%) and some individuals from Peninsular Thailand (5/25 nests, 20%) whereas most of them (20/25 nests, 80%) were observed in pattern F.

Twenty-nine scored fragments were considered polymorphic. Gene diversity was observed in North, Central, Northeast, and Peninsular Thailand (Table 3.7). All scorable bands of each population were polymorphic bands except those from Prachuap Khiri Khan and Chumphon had only fixed bands (present in all samples of particular region). The mean expected heterozygosity (H_e) varied from 0.043-0.065 in North, Central, Northeast, and Peninsular Thailand.

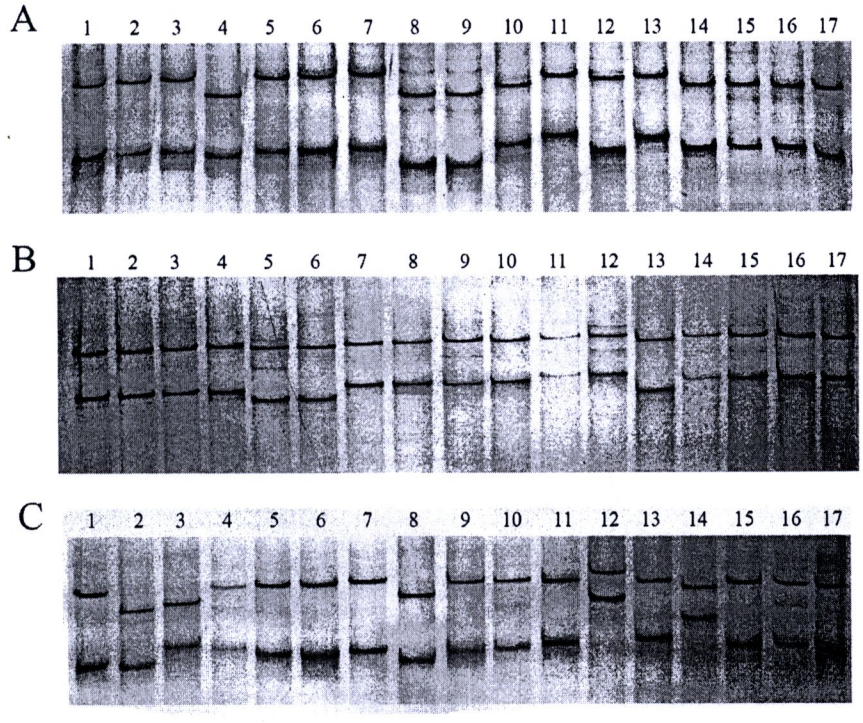


Figure 3.17 The SSCP pattern of the amplified 16S rRNA gene of *T. collina* from different geographic regions in Thailand; North population (lanes 6-9 A, 5-9 B, and 2-3, 7 C), Central population (lanes 2-5 A, 4 B, and 5-6 C), Northeast population (lanes 10-13 A, 10-13 B, and 4, 8-14 C), Peninsular population (lanes 14-17 A and 14-15 B), Prachuap Khiri Khan population (lanes 1 A, 1-3 B, and 1 C), and Chumphon population (lanes 16-17 B and 15-17 C).

```
Pattern A    ATGGCTGCAGTATAACTGACTGTACAAAGGTAGCATAATCAATTGATTTTTAAATGAAAT 60
Pattern B    ATGGCTGCAGTATAACTGACTGTACAAAGGTAGCATAATCAATTGATTTTTAAATGAAAT 60
Pattern C    ATGGCTGCAGTATAACTGACTGTACAAAGGTAGCATAATCAATTGATTTTTAAATGAAAT 60
Pattern D    ATGGCTGCAGTATAACTGACTGTACAAAGGTAGCATAATCAATTGGTTTTTAAATGAAAT 60
Pattern E    ATGGCTGCAGTATAACTGACTGTACAAAGGTAGCATAATCAATTGGTTTTTAAATGAAAT 60
Pattern F    ATGGCTGCAGTATAACTGACTGTACAAAGGTAGCATAATCAATTGGTTTTTAAATGAAAT 60
             ************************************************ **************

Pattern A    CTGGAATGAAAGGATTAATGAAATATATACTGTCTCATGTATATAAAGTGAATTTAAAAT 120
Pattern B    CTGGAATGAAAGGATTAATGAAATATATACTGTCTCATGTATATAAAATGAACTTAAAAT 120
Pattern C    CTGGAATGAAAGGATTAATGAAATATATACTGTCTCATGTATATAAAATGAACTTAAAAT 120
Pattern D    CTGGAATGAAAGGATTAATGAAATATATACTGTCTCATGTATATAGAGTGAATTTAAAAT 120
Pattern E    CTGGAATGAAAGGATTAATGAAATATATACTGTCTCATGTATATAAAATGAACTTAAAAT 120
Pattern F    CTGGAATGAAAGGATTAATGAAATATATACTGTCTCATGTATATAAAATGAACTTAAAAT 120
             ************************************************ * **** *******

Pattern A    TTTAATTAAAATGTTAAAATTTTTTTATGGGACGATAAGACCCTATAGAATTTTATATTG 180
Pattern B    TTTAATTAAAATGTTAAAATTTTTTTATGGGACGATAAGACCCTATAGAATTTTATATTG 180
Pattern C    TTTAATTAAAATGTTAAAATTTTTTTATGGGACGATAAGACCCTATAGAATTTTATATTG 180
Pattern D    TTTAATTAAAATGTTAAAATTTTTTTATGGGACGATAAGACCCTATAGAATTTTATATTG 180
Pattern E    TTTAATTAAAATGTTAAAATTTTTTTATGGGACGATAAGACCCTATAGAATTTTATATTG 180
Pattern F    TTTAATTAAAATGTTAAAATTTTTTTATGGGACGATAAGACCCTATAGAATTTTATATTG 180
             ************************************************************

Pattern A    CTACATTTTAATACTTAATATAAAATATGTAGTAATATTTGGTTGGGAGGACTATTAAAT 240
Pattern B    CTACATTTTAATACTTAATATAAAATATGTAGTAATATTTGGTTGGGAGGACTATTAAAT 240
Pattern C    CTACATTTTAATACTTAATATAAAATATGTAGTAATATTTGGTTGGGAGGACTATTAAAT 240
Pattern D    CTACATTTTAATACTTAATATAAAATATGTAGTGATATTTGGTTGGGAGGACTATTAAAT 240
Pattern E    CTACATTTTAATACTTAATATAAAATATGTAGTAATATTTGGTTGGGAGGACTATTAAAT 240
Pattern F    CTGCATTTTAATACTTAATATAAAATATGTAGTAATATTTGGTTGGGAGGACTATTAAAT 240
             ** ****************************** ***************************

Pattern A    TTGATTAACTTTAATTTTT-GTTTACTTTGATTTAAGAAAGTACAATGATCTTTAAATTA 299
Pattern B    TTGACTAACTTTAATTTTT-GATTACTTTAATTTAAGAAAATACAATGATCTTTAAATTA 299
Pattern C    TTGACTAACTTTAATTTTT-GATTACTTTAATTTAAGAAAATACAATGATCTTTAAATTA 299
Pattern D    TTGACTAACTTTAATTTTT-GATTACTTTGATTTAAGAAAATACAATGATCTTTAAATTA 299
Pattern E    TTGACTAACTTTAATTTTT-GATTACTTTGATTAAAGAAAATACAGTGATCTTTAAATTA 299
Pattern F    TTGACTAACTTTAATTTTTTGATTACTTTGATTAAAGAAAATACAATGATCTTTAAATTA 300
             **** ************** * ******* *** ****** **** **************

Pattern A    AAATATCTAGATTAAATTACCTTAGGGATAACAGCGTAATATTTTTTTATAGATCGTATA 359
Pattern B    AAATATCTAGATTAAATTACCTTAGGGATAACAGCGTAATATTTTTTTATAGATCGTATA 359
Pattern C    AAATAGCTAGATTAAATTACCTTAGGGATAACAGCGTAATATTTTTTTATAGATCGTATA 359
Pattern D    AAATATCTAGACTAAATTACCTTAGGGATAACAGCGTAATATTTTTTTATAGATCGTATA 359
Pattern E    AAATATCTAGATTAAATTACCTTAGGGATAACAGCGTAATATTTTTTTATAGATCGTATA 359
Pattern F    AAATATCTAGATTAAATTACCTTAGGGATAACAGCGTAATATTTTTTTATAGATCGTATA 360
             ***** ***** ************************************************
```

Figure 3.18 Alignment of 6 patterns of 16S rRNA gene from *T. collina* in Thailand. Asterisk indicated the same nucleotides among 6 patterns.

```
Pattern A    GAAAAAAATGATTGCGACCTCGATGTTGAATTAAGATAAATTTTAAACGCAGGAGTTTAA 419
Pattern B    GAAAAAAATGATTGCGACCTCGATGTTGAATTAAGATAAATTTTAAACGCAGGAGTTTAA 419
Pattern C    GAAAAAAATGATTGCGACCTCGATGTTGAATTAAGATAAATTTTAAACGCAGGAGTTTAA 419
Pattern D    GAAAAAAATGATTGCGACCTCGATGTTGAATTAAGATAAATTTTAAACGCAGGAGTTTAA 419
Pattern E    GAAAAAAATGATTGCGACCTCGATGTTGAATTAAGATAAATTTTAAACGCAGGAGTTTAA 419
Pattern F    GAAAAAAATGATTGCGACCTCGATGTTGAATTAAGATAAATTTTAAACGCAGGAGTTTAA 420
             ************************************************************

Pattern A    TGATTAAGTCTGTTCGACTTTTAAAATCTTACATGATTTGAGTTCAAATCGACGTAAGT 478
Pattern B    TGATTAAGTCTGTTCGACTTTTAAAATCTTACATGATTTGAGTTCAAATCGACGTAAGT 478
Pattern C    TGATTAAGTCTGTTCGACTTTTAAAATCTTACATGATTTGAGTTCAAATCGACGTAAGT 478
Pattern D    TGATTAAGTCTGTTCGACTTTTAAAATCTTACATGATTTGAGTTCAAATCGACGTAAGT 478
Pattern E    TGATTAAGTCTGTTCGACTTTTAAAATCTTACATGATTTGAGTTCAAATCGACGTAAGT 478
Pattern F    TGATTAAGTCTGTTCGACTTTTAAAATCTTACATGATTTGAGTTCAAATCGACGTAAGT 479
             ************************************************************
```

Figure 3.18 continue

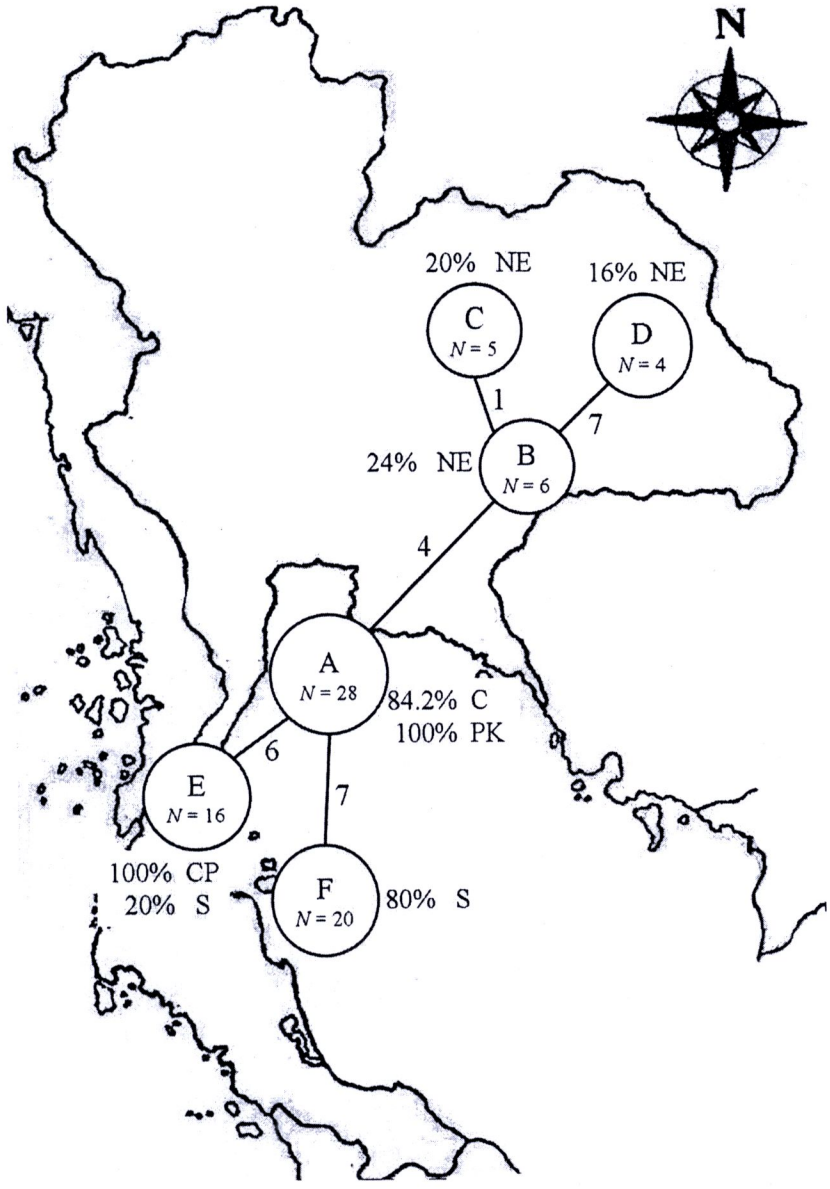


Figure 3.19 Phylogeographic pattern deduced from 16S rRNA gene haplotypes of *T. collina* (pattern A, B, C, D, E, and F). Numbers along connected lines indicated inferred mutation steps. Letter *N* represented the amount of *T. collina* possessing each pattern. The percentages were calculated from the total number of *T. collina* in a particular region; North (11 nests), Central (19 nests), Northeast (25 nests), Prachuap Khiri Khan (12 nests), Chumphon (11 nests), and Peninsular Thailand (25 nests). Capital letters behind percentage were abbreviations of Central (C), Northeast (NE), Prachuap Khiri Khan (PK), Chumphon (CP), and Peninsular Thailand (S).

Table 3.7 Comparison of genetic diversity in six populations of 16S rRNA gene in *T. collina* in Thailand

SSCP Bands ($N = 103$)	**Central** (19)	**North** (11)	**Northeast** (25)	**Peninsular Thailand** (25)	**Prachuap Khiri Khan** (12)	**Chumphon** (11)
Number scored	4	7	15	4	2	2
Number private	2	6	15	2	0	0
Number fixed	0	0	0	0	2	2
Number absent	25	22	14	25	27	27
# polymorphic	4	7	15	4	0	0
% polymorphic	100% of 4* (14% of 29)**	100% of 7 (24% of 29)	100% of 15 (52% of 29)	100% of 4 (14% of 29)	0% of 2 (0% of 29)	0% of 2 (0% of 29)
Mean H_e (± SD)	0.043 (0.024)	0.061 (0.023)	0.065 (0.016)	0.047 (0.025)	0.000 (0.000)	0.000 (0.000)

*Total number of scorable SSCP bands in a particular regions; ** Total number of scorable SSCP bands across all region

The unbiased genetic distance between pairs of populations was 0.003-0.148 (Table 3.8). Significant geographic heterogeneity was observed in most pairwise comparisons of regions analyzed by F_{ST}-based statistics, Φ_{PT} ($P < 0.05$). The exact test revealed significant genetic differences in comparison between Peninsular Thailand and each population. The results indicated strong degree of differentiation in each comparison (Φ_{PT} = 0.070-0.826) whereas no degrees of differentiation was found between Prachuap Khiri Khan and Chumphon.

AMOVA analysis showed high molecular variance components among 6 populations; North, Central, Northeast, Prachuap Khiri Khan, Chumphon and Peninsular Thailand (56%, Φ_{PT} = 0.563, P = 0.001), among 5 population groupings; North, Central, Northeast, Prachuap Khiri Khan+Chumphon, and Peninsular Thailand (44%, Φ_{PT} = 0.437, P = 0.001), and between North and South of Isthmus of Kra (33%, Φ_{PT} = 0.334, P = 0.001, Table 3.9).

3.4.2 Analysis of cytochrome oxidase I (COI) gene polymorphism

The expected products (497 bp) were found in 73 nests from total 103 investigated *T. collina*, accounting for 71% of investigated specimens. To characterize the polymorphisms, the products were run on the 11% non-denaturing polyacrylamide gel (Figure 3.20). Four common SSCP patterns (pattern A, B, C, and D) of all 34 patterns were sequenced (Figure 3.21, appendix B). Their nucleotide sequences were unlike by several SNPs. The pattern A was found in samples from Prachuap Khiri Khan (6/10 nests) and one individual from Central (1/10 nest). Both pattern B and C were observed in samples from Chumphon (2/11 nests for B, 2/11 nests for C) and Peninsular Thailand (6/22 nests for B, 3/22 nests for C) while pattern D was distributed in those from Peninsular Thailand (6/22 nests).

Fifty-seven SSCP bands were scored as polymorphic. Gene diversity was observed in all 6 populations, North, Central, Northeast, Prachuap Khiri Khan, Chumphon and Peninsular Thailand (Table 3.10). No fixed bands were present in all populations of *T. collina*. The percentage of polymorphic bands for all populations was 100%. The mean expected heterozygosity (H_e) for 6 populations was in the vicinity (H_e = 0.029-0.039).

Table 3.8 Pairwise F_{ST}-based statistics (Φ_{PT}, below diagonal), Nei's (1972) genetic distance (in brackets) and the exact test (above diagonal) of 16S rRNA gene among six populations of *T. collina* in Thailand

	Central	**North**	**Northeast**	**Peninsular Thailand**	**Prachuap Khiri Khan**	**Chumphon**
Central (*N* = 19)	-	$P = 0.0015$	$P < 0.0001$	$P < 0.0001$	$P = 1.0000$	$P = 0.0001$
North (*N* = 11)	0.527* (0.078)	-	$P = 0.0153$	$P < 0.0001$	$P = 0.0075$	$P = 0.0821$
Northeast (*N* = 25)	0.419* (0.071)	0.219* (0.043)	-	$P < 0.0001$	$P = 0.0304$	$P = 0.0321$
Peninsular Thailand (*N* = 25)	0.690* (0.107)	0.528* (0.080)	0.412* (0.067)	-	$P < 0.0001$	$P = 0.0008$
Prachuap Khiri Khan (*N* = 12)	0.070 (0.003)	0.646* (0.095)	0.492* (0.089)	0.783* (0.126)	-	$P = 0.0005$
Chumphon (*N* = 11)	0.826* (0.130)	0.650* (0.102)	0.483* (0.089)	0.726* (0.094)	0.000 ns (0.148)	-

*significant differences at $P < 0.05$; ns, not significant

Table 3.9 Analysis of Molecular Variance (AMOVA) of 16S rRNA gene for genetic differentiation of *T. collina* among 6 population groupings; North, Central, Northeast, Prachuap Khiri Khan, Chumphon, and Peninsular Thailand (A), among 5 populations; North, Central, Northeast, Prachuap Khiri Khan+Chumphon, and Peninsular Thailand (B), and between North (North+Central+Northeast+Prachuap Khiri Khan) and South (Chumphon and Peninsular Thailand) of Isthmus of Kra (C)

A. Six population groupings

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	5	1.069	56	0.563	0.001
Within population	97	0.831	44		

B. Five population groupings

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	4	0.819	44	0.437	0.001
Within population	98	1.057	56		

C. North and South of Isthmus of Kra

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	1	0.696	33	0.334	0.001
Within population	101	1.388	67		

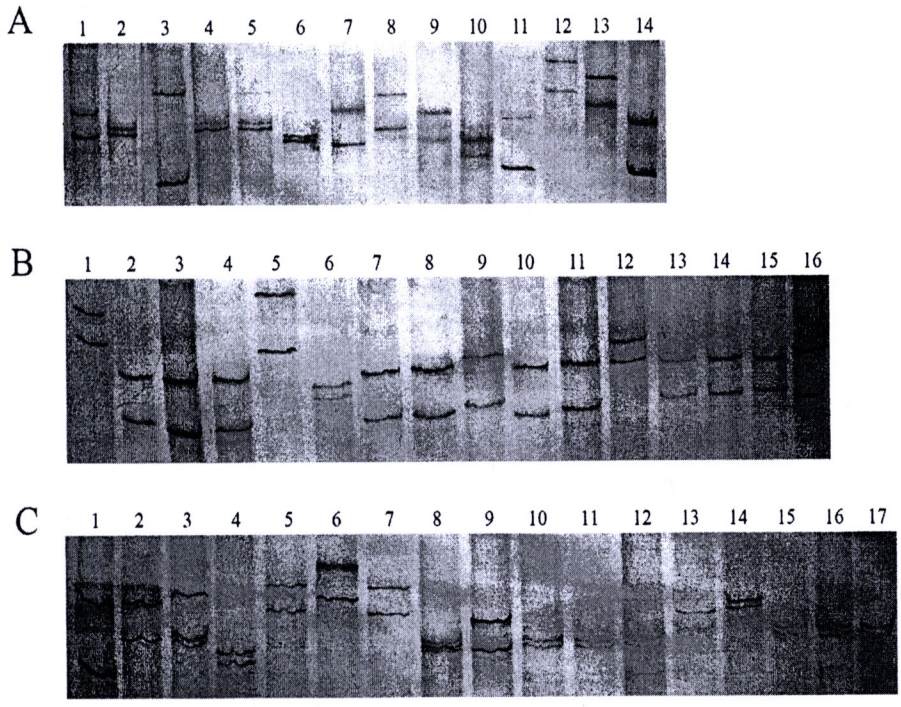


Figure 3.20 The SSCP pattern of the amplified COI gene of *T. collina* from different geographic regions in Thailand; North population (lanes 8-10 A and 1-7 C), Central population (lanes 11-13 A and 1, 4-6 B), Northeast population (lanes 6-7 A and 8-17 C), Peninsular population (lanes 2-5 A and 10, 16 B), Prachuap Khiri Khan population (lanes 14 A and 2-3, 7 B), and Chumphon population (lanes 1 A and 8-9, 11-15 B).

```
Pattern A    CATTCATCTCCTTCTGTTGATTTTACTATTTTTTCAATTCATATGACTGGGGTTTCATCT 60
Pattern B    CATTCATCTCCTTCTGTTGATTTTACTATTTTTTCAATTCATATGACTGGGGTTTCATCT 60
Pattern C    CATTCATCTCCTTCTGTTGATTTTACTATTTTTTCAATTCATATGACTGGGGTTTCATCT 60
Pattern D    CATTCATCTCCTTCTGTTGATTTTACTATTTTTTCAATTCATATGACTGGGGTTTCATCT 60
             ************************************************************

Pattern A    ATTTTAGGATCACTAAATTTTATTGTAACAATTTTTATAATAAAAAATTTTTCAATTAGG 120
Pattern B    ATTTTAGGGTCACTAAATTTTATTGTAACAATTTTTATAATAAAAAATTTTTCAATTAGC 120
Pattern C    ATTTTAGGGTCACTAAATTTCATTGTAACAATTTTTATAATAAAAAATTTTTCAATTAAC 120
Pattern D    ATTTTAGGGTCACTAAATTTTATTGTAACAATTTTTATAATAAAAAATTTTTCAATTAAC 120
             ******** *********** ************************************

Pattern A    TATGATCAAATTACACTATTTTCTTGATCTATTTCAATTACTGTAATTCTTTTAATCATC 180
Pattern B    TATGATCAAATTACACTATTTTCTTGATCTATTTCAATTACTGTAATTCTTTTAATTGTC 180
Pattern C    TATGATCAAATTACACTATTTTCTTGGTCTATTTCAATTACTGTAATTCTTTTAATCATC 180
Pattern D    TATGATCAAATTACACTATTTTCGTGATCTATTTCAATTACTGTAATTCTTTTAATCATT 180
             ********************** ** ****************************  *

Pattern A    TCTCTTCCTGTTTTGGCTGGAGCTATTACAATACTTTTATTTGATCGAAACTTTAATACT 240
Pattern B    TCTCTTCCTGTCTTGGCTGGAGCTATTACAATACTTTTATTTGATCGAAATTTTAATACT 240
Pattern C    TCTCTTCCTGTTTTGGCTGGAGCTATTACAATACTTTTATTTGATCGAAACTTCAATACT 240
Pattern D    TCTCTTCCTGTTTTAGCTGGAGCTATTACAATACTTTTATTTGATCGAAATTTTAATACT 240
             *********** ** ********************************** ** ******

Pattern A    TCATTTTTTGATCCGGTAGGAGGAGGTGATCCAATTCTTTACCAACATCTATTTTGATTC 300
Pattern B    TCATTTTTTGATCCAGTAGGAGGAGGTGATCCAATTCTTTACCAACATCTATTTTGATTC 300
Pattern C    TCATTTTTTGATCCGGTGGGAGGAGGTGATCCAATTCTTTACCAACATCTATTTTGATTC 300
Pattern D    TCATTTTTTGATCCAGTGGGAGGGGGTGATCCAATTCTTTACCAACATCTATTTTGATTC 300
             ************** ** ***** ************************************

Pattern A    TTTGGACATCCTGAAGTTTATATTCTTATTCTTCCTGGATTTGGATTAATTTCCCAGATT 360
Pattern B    TTTGGACATCCTGAAGTTTATATTCTTATTCTTCCTGGATTTGGATTAATTTCCCAGATT 360
Pattern C    TTTGGGCATCCTGAAGTTTATATTCTCATTCTTCCTGGATTTGGATTAATTTCCCAGATT 360
Pattern D    TTTGGACATCCTGAAGTTTATATTCTTATTCTTCCTGGATTTGGATTAATTTCTCAGATT 360
             ***** ******************** ************************** ******

Pattern A    ATTATGAATGAAAGGGGAAAGAAGGAGGTTTTTGGGAATTTAAGAATAATTTATGCAATA 420
Pattern B    ATTATAAATGAAAGGGGAAAGAAGGAGGTTTTTGGGAATTTAAGAATAATTTATGCAATA 420
Pattern C    ATTATAAATGAAAGAGGAAAGAAGGAGGTTTTCGGAAATTTAAGAATAATTTACGCAATA 420
Pattern D    ATTATAAATGAAAGGGGGAAGAAGGAGGTTTTTGGAAATTTGAGAATAATTTATGCAATG 420
             ***** ******** ** ************** ** ***** *********** *****

Pattern A    ATTGGTATTGGATTTCTTGGTTTTATTGTTTGAGCTCATCATATATTTACTGTAGGATTA 480
Pattern B    ATTGGTATTGGATTTCTTGGTTTTATTGTTTGAGCTCATCATATATTTACTGTAGGATTA 480
Pattern C    ATTGGTATTGGATTTCTTGGTTTTATTGTTTGAGCTCATCATATATTTACTGTGGGATTA 480
Pattern D    ATTGGTATTGGATTTCTTGGTTTTATTGTTTGAGCTCATCATATATTTACTGTGGGATTA 480
             ************************************************** ******

Pattern A    GATATTGATACACGAGC 497
Pattern B    GATATTGATACACGAGC 497
Pattern C    GATATTGATACACGAGC 497
Pattern D    GATATTGATACACGAGC 497
             ****************
```

Figure 3.21 Alignment of 4 patterns of COI gene from *T. collina* in Thailand. Asterisk indicated the same nucleotides among 4 patterns.

Table 3.10 Comparison of genetic diversity in six populations of COI gene in *T. collina* in Thailand

SSCP Bands ($N = 73$)	**Central** (10)	**North** (10)	**Northeast** (10)	**Peninsular Thailand** (22)	**Prachuap Khiri Khan** (10)	**Chumphon** (11)
Number scored	17	13	10	13	7	12
Number private	10	11	9	7	1	6
Number fixed	0	0	0	0	0	0
Number absent	40	44	47	44	50	45
# polymorphic	17	13	10	13	7	12
% polymorphic	100% of 17* (30% of 57)**	100% of 13 (23% of 57)	100% of 10 (18% of 57)	100% of 13 (23% of 57)	100% of 7 (12% of 57)	100% of 12 (21% of 57)
Mean H_e (± SD)	0.034 (0.008)	0.039 (0.012)	0.030 (0.012)	0.034 (0.010)	0.029 (0.013)	0.033 (0.010)

*Total number of scorable SSCP bands in a particular regions; ** Total number of scorable SSCP bands across all region

The unbiased genetic distance between pairs of populations was 0.009-0.033 (Table 3.11). Significant geographic heterogeneity was observed in almost all pairwise comparisons of regions analyzed by F_{ST}-based statistics, Φ_{PT} ($P < 0.05$) except between Chumphon and Peninsular Thailand. The degree of differentiation in each comparison varied from 0.062 (between Chumphon and Peninsular Thailand) to 0.378 (between Northeast and Prachuap Khiri Khan).

AMOVA results revealed significance molecular variance components among 6 populations; North, Central, Northeast, Prachuap Khiri Khan, Chumphon and Peninsular Thailand (20%, $\Phi_{PT} = 0.204$, $P = 0.001$), among 5 population groupings; North, Central, Northeast, Prachuap Khiri Khan, and Chumphon+Peninsular Thailand (21%, $\Phi_{PT} = 0.208$, $P = 0.001$), and between North and South of Isthmus of Kra (11%, $\Phi_{PT} = 0.106$, $P = 0.001$, Table 3.12).

3.4.3 Analysis of cytochrome b (cytb) gene polymorphism

The expected products (316 bp) were found in 91 investigated *T. collina* (91/103 nests accounting for 88% of investigated specimens). Thirty-four SSCP phenotypes were obtained when the products were characterized on the 12.5% non-denaturing polyacrylamide gel (Figure 3.22, appendix B). The nucleotide sequences of 8 common SSCP phenotypes (pattern A, B, C, D, E, F, G, and H) were dissimilar by several SNPs (Figure 3.23). The phylogeographic pattern of them was shown in Figure 3.24. Both pattern A and B were found in specimens from Central (10/15 nests, 66.7% for A, 4/15 nests, 26.7% for B). Pattern C was observed in those from Prachuap Khiri Khan (6/8 nests, 75%). The pattern D and E, which were different by 1 point mutation were observed in Northeast population (5/24 nests, 20.8% and 7/24 nests, 29%, respectively). Pattern F, G, and H were distributed in individuals from Peninsular Thailand (7/23 nests, 30.4%, 10/23 nests, 43.5%, 4/23 nests, 17.4%, respectively). In addition, one individual from Chumphon (1/11 nests, 12.5%) was found in pattern F.

Table 3.11 Pairwise F_{ST}-based statistics (Φ_{PT}, below diagonal), Nei's (1972) genetic distance (in brackets) and the exact test (above diagonal) of COI gene among six populations of *T. collina* in Thailand

	Central	**North**	**Northeast**	**Peninsular Thailand**	**Prachuap Khiri Khan**	**Chumphon**
Central ($N = 10$)	-	$P = 1.0000$	$P = 1.0000$	$P = 0.9998$	$P = 1.0000$	$P = 1.0000$
North ($N = 10$)	0.144* (0.019)	-	$P = 1.0000$	$P = 0.9729$	$P = 1.0000$	$P = 1.0000$
Northeast ($N = 10$)	0.189* (0.020)	0.257* (0.027)	-	$P = 0.9976$	$P = 1.0000$	$P = 1.0000$
Peninsular Thailand ($N = 22$)	0.110* (0.013)	0.217* (0.023)	0.246* (0.024)	-	$P = 0.9947$	$P = 1.0000$
Prachuap Khiri Khan ($N = 10$)	0.153* (0.016)	0.313* (0.032)	0.378* (0.033)	0.281* (0.027)	-	$P = 1.0000$
Chumphon ($N = 11$)	0.083* (0.013)	0.190* (0.023)	0.233* (0.023)	0.062^{ns} (0.009)	0.276* (0.026)	-

*significant differences at $P < 0.05$; ns, not significant

Table 3.12 Analysis of Molecular Variance (AMOVA) of COI gene for genetic differentiation of *T. collina* among 6 population groupings; North, Central, Northeast, Prachuap Khiri Khan, Chumphon, and Peninsular Thailand (A), among 5 populations; North, Central, Northeast, Prachuap Khiri Khan, and Chumphon+Peninsular Thailand (B), and between North (North+Central+Northeast+Prachuap Khiri Khan) and South (Chumphon and Peninsular Thailand) of Isthmus of Kra (C)

A. Six population groupings

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	5	0.419	20	0.204	0.001
Within population	67	1.640	80		

B. Five population groupings

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	4	0.437	21	0.208	0.001
Within population	68	1.666	79		

C. North and South of Isthmus of Kra

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	1	0.223	11	0.106	0.001
Within population	71	1.873	89		

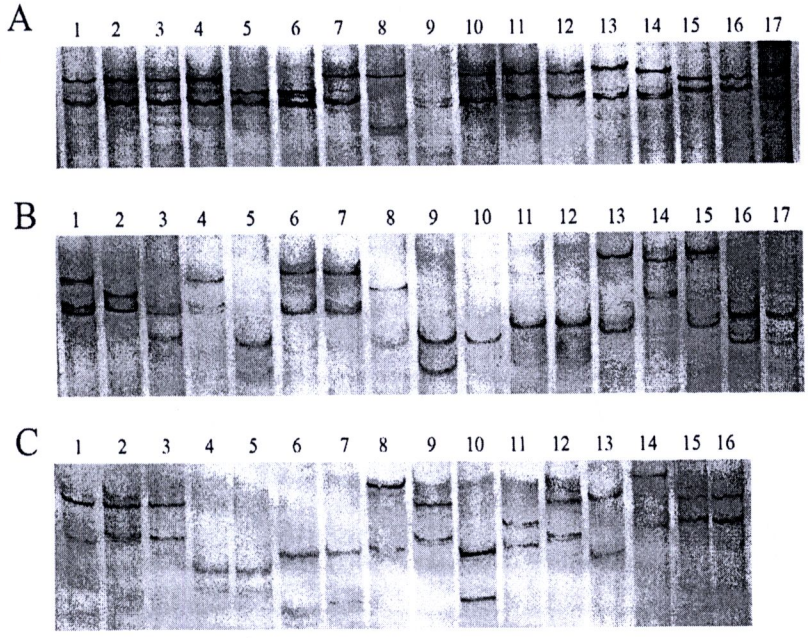


Figure 3.22 The SSCP pattern of the amplified cytb gene of *T. collina* from different geographic regions in Thailand; North population (lanes 5-8 A, 13-17 B, and 6-8 C), Central population (lanes 1-4 A, 6-7, 11-12 B, and 1-3 C), Northeast population (lanes 9-12 A, and 9-12 C), Peninsular population (lanes 13-16 A, 1-2, 4 B, and 16 C), Prachuap Khiri Khan population (lanes 5, 8-10 B and 4-5 C), and Chumphon population (lanes 17 A, 3 B, and 13-15 C).

```
Pattern A    TTGTAGAGTGATTATGAGGAGGATTTTCTATTAATAATTCTACTCTTAATCGATTTTTTT 60
Pattern B    TTGTAGAGTGATTATGAGGAGGATTTTCTATTAATAATTCTACTCTTAATCGATTTTTTT 60
Pattern C    TTGTAGAGTGATTATGAGGAGGATTTTCTATTAATAATTCTACTCTTAATCGATTTTTTT 60
Pattern D    TTGTAGAGTGATTATGAGGAGGATTCTCAATTAATAATTCTACTCTTAATCGATTTTTTT 60
Pattern E    TTGTAGAGTGATTATGAGGAGGATTCTCAATTAATAATTCTACTCTTAACCGATTTTTTT 60
Pattern F    TTGTAGAGTGATTATGAGGAGGATTTTCTATTAATAATTCTACTCTTAACCGATTTTTTT 60
Pattern G    TTGTAGAGTGATTATGAGGAGGATTTTCTATTAATAATTCTACTCTTAATCGATTCTTTT 60
Pattern H    TTGTAGAGTGATTATGAGGAGGATTTTCTATTAATAATTCTACTCTTAATCGATTCTTTT 60
             ************************ ** ******************** ***** ****

Pattern A    CACTACATTTTATTTTACCATTTATTATTCTTATTTTAGTATTTATTCATATTATAATAT 120
Pattern B    CACTACATTTTATTTTACCATTTATTATTCTTATTTTAGTATTTATTCATATTATAATAT 120
Pattern C    CATTACATTTTATTTTACCATTTATTATTCTTATTTTAGTATTTATTCATATCATAGTAT 120
Pattern D    CATTACATTTTATTTTACCATTTATTATTCTTATTTTAGTATTTATTCATATTATAATAT 120
Pattern E    CATTACATTTTATTTTACCATTTATTATTCTTATTTTAGTATTTATTCATATTATAATAT 120
Pattern F    CATTACATTTTATTTTACCATTTATTATTCTTATTTTAGTATTTATTCATATCATAGTAT 120
Pattern G    CATTACATTTTATTTTACCATTTATTATTCTTATTTTAGTATTTATTCATATCATAGTAT 120
Pattern H    CGTTACATTTTATTTTACCATTTATTATTCTTATTTTAGTATTTATTCATATTATAGTAT 120
             *  ************************************************* *** ***

Pattern A    TGCATACATCTGGATCTTCAAATCCAATCCACTCAAAAATAAATATCTATAAAATTTCAT 180
Pattern B    TGCATACATCTGGATCTTCAAATCCAATCCATTCAAAAATAAATATCTATAAAATTTCAT 180
Pattern C    TGCATATATCTGGATCTTCAAACCCAATTCATTCTAAAATAAATATTTATAAAATTTCAT 180
Pattern D    TACACATATCTGGATCTTCAAATCCAATTCATTCAAAAATAAATATTTACAAAATTTCAT 180
Pattern E    TACACATATCTGGATCTTCAAATCCAATTCATTCAAAAATAAATATTTACAAAATTTCAT 180
Pattern F    TGCATATATCCGGATCTTCAAATCCAATTCATTCTAAAATAAATATTTATAAAATTTCAT 180
Pattern G    TGCATATATCTGGATCTTCAAACCCAATTCATTCTAAAATAAATATTTATAAAATTTCAT 180
Pattern H    TGCATATATCTGGATCTTCAAATCCAATTCATTCTAAAATAAATATTTATAAAATTTCAT 180
             * ** * *** *********** ***** ** ** *********** ** **********

Pattern A    TTTATCCATATTTTGTAATTAAAGATATAGTAACTATTTTATTTACTATTTTATTATTTA 240
Pattern B    TTCATCCATATTTTGTAATTAAAGATATAGTAACTATTTTATTTACTATTTTATTATTTA 240
Pattern C    TTTACCCGTACTTTGCAATTAAAGATATAGTAACTATTTTGTTTATTATTTTATTATTTA 240
Pattern D    TTTATCCATATTTTATAATTAAAGATATAGTAACTATTTTATTTACTATCTTATTATTTA 240
Pattern E    TTTATCCATATTTTATAATTAAAGATATAGTAACTATTTTATTTACTATCTTATTATTTA 240
Pattern F    TTTATCCATACTTCGCAATTAAAGATATAGTAACTATTTTGTTTACTATTTTATTATTTA 240
Pattern G    TTTATCCATACTTCGCAATTAAAGATATAGTAACTATTTTGTTTACTATTTTATTATTTA 240
Pattern H    TTTATCCATACTTTGCAATTAAAGATATAGTAACTATTTTGTTTACTATTTTATTATTTA 240
             ** * ** ** **   ************************ **** *** **********

Pattern A    TACTTTTAAATTTTCAGATACCTTATTTTTTAAGAGATCCTGATAATTTTAAAATAGCTG 300
Pattern B    TACTTTTAAATTTTCAGATACCTTATTTTTTAAGAGATCCTGATAATTTTAAAATAGCTG 300
Pattern C    TATTTTTAAATCTTCAGGCACCCTATATTTTAAGTGATCCAGATAATTTTAAAATAGCTG 300
Pattern D    TACTTTTAAATTTTCAGATACCTTATTTTTTAAGAGATCCTGATAATTTTAAAATAGCTG 300
Pattern E    TACTTTTAAATTTTCAGATACCTTATTTTTTAAGAGATCCTGATAATTTTAAAATAGCTG 300
Pattern F    TATTTTTAAATTTTCAGGCACCCTATATTTTAAGTGATCCAGATAATTTTAAAATAGCTG 300
Pattern G    TATTTTTAAATTTTCAGGCACCCTATATTCTAAGTGATCCAGATAATTTTAAAATAGCTG 300
Pattern H    TATTTTTAAATTTTCAGGCACCCTATATTTTAAGTGATCCAGACAATTTTAAAATAGCTG 300
             ** ******** *****  *** *** ** **** ***** ** ****************

Pattern A    ATCCTATAGTTACTCC 316
Pattern B    ATCCTATAGTTACTCC 316
Pattern C    ATCCTATAGTTACTCC 316
Pattern D    ATCCTATAGTTACTCC 316
Pattern E    ATCCTATAGTTACTCC 316
Pattern F    ATCCTATAGTTACTCC 316
Pattern G    ATCCTATAGTTACTCC 316
Pattern H    ATCCTATAGTTACTCC 316
             ****************
```

Figure 3.23 Alignment of 8 patterns of cytb gene from *T. collina* in Thailand. Asterisk indicated the same nucleotides among 8 patterns.

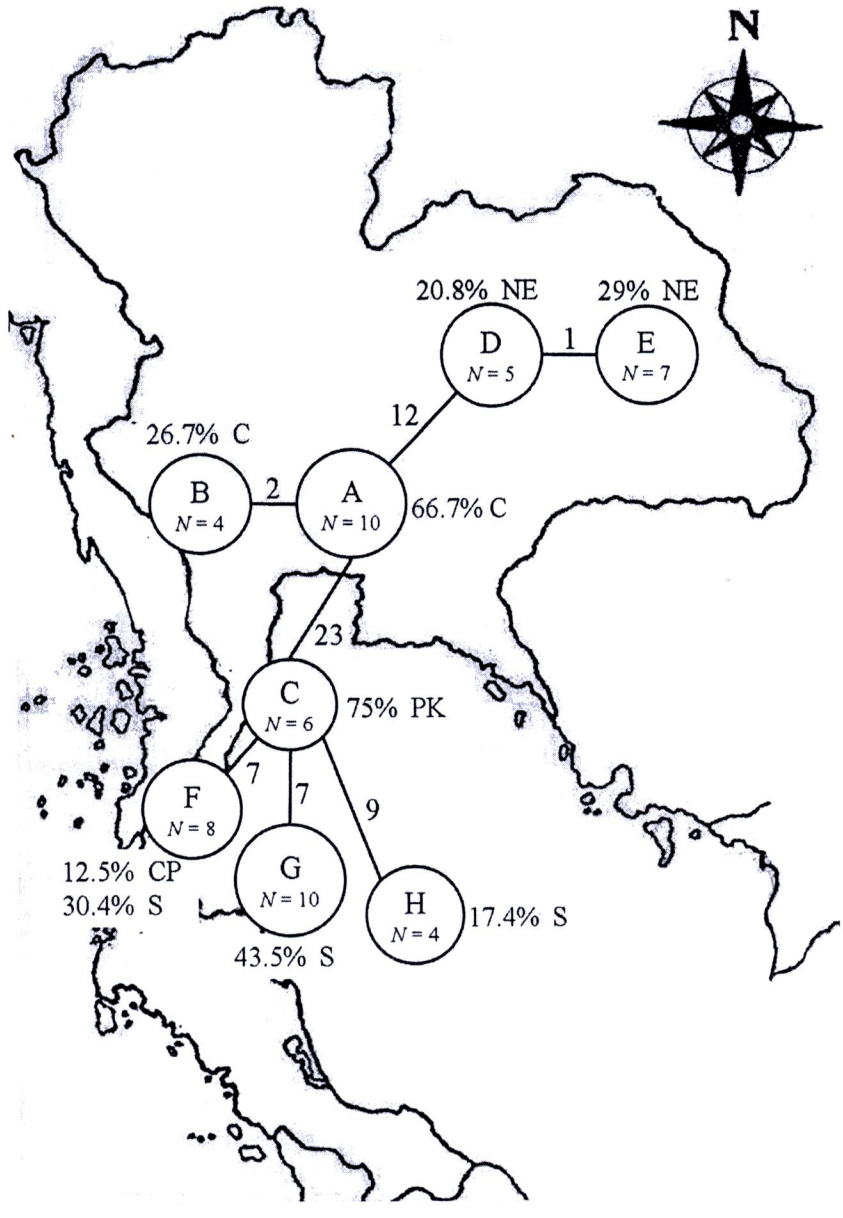


Figure 3.24 Phylogeographic pattern deduced from cytb gene of *T. collina* (pattern A, B, C, D, E, F, G, and H). Numbers along connected lines indicated inferred mutation steps. Letter *N* represented the amount of *T. collina* possessing each pattern. The percentages were calculated from the total number of *T. collina* in a particular region; North (10 nests), Central (15 nests), Northeast (24 nests), Prachuap Khiri Khan (8 nests), Chumphon (11 nests), and Peninsular Thailand (23 nests). Capital letters behind percentage were abbreviations of Central (C), Northeast (NE), Prachuap Khiri Khan (PK), Chumphon (CP), and Peninsular Thailand (S).

A total of 38 scored SSCP bands were considered polymorphic. Genetic diversity was observed in all 6 populations, North, Central, Northeast, Prachuap Khiri Khan, Chumphon and Peninsular Thailand (Table 3.13). The percentage of polymorphic bands for all populations was 100% and the mean expected heterozygosity (H_e) varied from 0.033-0.050.

The unbiased genetic distance between pairs of populations was 0.015-0.072 (Table 3.14). Significant geographic heterogeneity was observed in all pairwise comparisons of regions analyzed by F_{ST}-based statistics, Φ_{PT} ($P < 0.05$). The results indicated high degree of differentiation in each comparison ranged from 0.079 (between Northeast and Chumphon) to 0.627 (between Central and Prachuap Khiri Khan).

AMOVA analysis showed high molecular variance components among 6 populations; North, Central, Northeast, Prachuap Khiri Khan, Chumphon and Peninsular Thailand (29%, $\Phi_{PT} = 0.294$, $P = 0.001$), and between North and South of Isthmus of Kra (13%, ΦPT = 0.133, $P = 0.001$, Table 3.15).

Table 3.13 Comparison of genetic diversity in six populations of cytb gene in *T. collina* in Thailand

SSCP Bands ($N = 91$)	**Central** (15)	**North** (10)	**Northeast** (24)	**Peninsular Thailand** (23)	**Prachuap Khiri Khan** (8)	**Chumphon** (11)
Number scored	5	13	14	8	4	14
Number private	1	6	6	3	1	6
Number fixed	0	0	0	0	0	0
Number absent	33	25	24	30	34	24
# polymorphic	5	13	14	8	4	14
% polymorphic	100% of 5* (13% of 38)**	100% of 13 (34% of 38)	100% of 14 (37% of 38)	100% of 8 (21% of 38)	100% of 4 (11% of 38)	100% of 14 (37% of 38)
Mean H_e (± SD)	0.033 (0.017)	0.050 (0.013)	0.049 (0.013)	0.042 (0.018)	0.039 (0.020)	0.050 (0.012)

*Total number of scorable SSCP bands in a particular regions; ** Total number of scorable SSCP bands across all region

Table 3.14 Pairwise F_{ST}-based statistics (Φ_{PT}, below diagonal), Nei's (1972) genetic distance (in brackets) and the exact test (above diagonal) of cytb gene among six populations of *T. collina* in Thailand

	Central	North	Northeast	Peninsular Thailand	Prachuap Khiri Khan	Chumphon
Central ($N = 15$)	-	$P = 0.5389$	$P = 0.6540$	$P = 0.2885$	$P = 0.5338$	$P = 0.6770$
North ($N = 10$)	0.411* (0.048)	-	$P = 0.8613$	$P = 0.1845$	$P = 1.0000$	$P = 0.9992$
Northeast ($N = 24$)	0.286* (0.031)	0.111* (0.019)	-	$P = 0.0288$	$P = 0.9038$	$P = 0.8864$
Peninsular Thailand ($N = 23$)	0.431* (0.041)	0.304* (0.037)	0.284* (0.034)	-	$P = 0.3312$	$P = 0.8609$
Prachuap Khiri Khan ($N = 8$)	0.627* (0.070)	0.281* (0.039)	0.305* (0.042)	0.497* (0.061)	-	$P = 0.9979$
Chumphon ($N = 11$)	0.346* (0.038)	0.082* (0.019)	0.079* (0.015)	0.229* (0.027)	0.289* (0.040)	-

*significant differences at $P < 0.05$

Table 3.15 Analysis of Molecular Variance (AMOVA) of cytb gene for genetic differentiation of *T. collina* among 6 population groupings; North, Central, Northeast, Prachuap Khiri Khan, Chumphon, and Peninsular Thailand (A), and between North (North+Central+Northeast+Prachuap Khiri Khan) and South (Chumphon and Peninsular Thailand) of Isthmus of Kra (B)

A. Six population groupings

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	5	0.547	29	0.294	0.001
Within population	85	1.311	71		

B. North and South of Isthmus of Kra

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	1	0.252	13	0.133	0.001
Within population	89	1.637	87		

3.4.4 Analysis of geographic population structure based on all 3 mtDNA gene polymorphisms

Although the specimens used to examine the data were the same sample set but the number of amplified specimens was inconsistent in each mtDNA gene. Therefore, the positively amplified specimens by the primers of 3 mtDNA genes were used to analyze (61 nests).

A total of 113 SSCP bands were generated from the data of 3 mtDNA genes. Genetic diversity was observed in all 6 populations, North, Central, Northeast, Prachuap Khiri Khan, Chumphon and Peninsular Thailand (Table 3.16). No fixed bands were present in samples from North, Central, Northeast, and Peninsular Thailand except those from Prachuap Khiri Khan and Chumphon. The mean expected heterozygosity (H_e) varied from 0.030-0.052.

The unbiased genetic distance between pairs of populations was 0.029-0.067 (Table 3.17). Significant geographic heterogeneity was observed in all pairwise comparisons of regions analyzed by F_{ST}-based statistics, Φ_{PT} ($P < 0.05$). The degree of differentiation in each comparison varied from 0.231 (between North and Northeast) to 0.515 (between Prachuap Khiri Khan and Peninsular Thailand).

AMOVA analysis showed significance molecular variance components among 6 populations; North, Central, Northeast, Prachuap Khiri Khan, Chumphon and Peninsular Thailand (37%, $\Phi_{PT} = 0.369$, $P = 0.001$, Table 3.18).

Table 3.16 Comparison of genetic diversity in six populations of 16S rRNA, COI, and cytb gene in *T. collina* in Thailand

SSCP Bands	**Central**	**North**	**Northeast**	**Peninsular Thailand**	**Prachuap Khiri Khan**	**Chumphon**
($N = 61$)	**(6)**	**(8)**	**(10)**	**(20)**	**(6)**	**(11)**
Number scored	20	30	31	25	13	28
Number private	10	21	25	12	3	13
Number fixed	0	0	0	0	2	2
Number absent	93	83	82	88	100	85
# polymorphic	20	30	31	25	11	26
% polymorphic	100% of 20*	100% of 30	100% of 31	100% of 25	85% of 13	93% of 28
	(18% of 113)**	(26.5% of 113)	(27% of 113)	(22% of 113)	(10% of 113)	(23% of 113)
Mean H_e	0.043	0.052	0.047	0.040	0.030	0.033
(± SD)	(0.010)	(0.010)	(0.009)	(0.009)	(0.009)	(0.007)

*Total number of scorable SSCP bands in a particular regions; ** Total number of scorable SSCP bands across all region

Table 3.17 Pairwise F_{ST}-based statistics (Φ_{PT}, below diagonal), Nei's (1972) genetic distance (in brackets) and the exact test (above diagonal) of 16S rRNA, COI, and cytb gene among six populations of *T. collina* in Thailand

	Central	**North**	**Northeast**	**Peninsular Thailand**	**Prachuap Khiri Khan**	**Chumphon**
Central ($N = 6$)	-	$P = 1.0000$	$P = 1.0000$	$P = 1.0000$	$P = 1.0000$	$P = 1.0000$
North ($N = 8$)	0.264* (0.045)	-	$P = 1.0000$	$P = 0.7046$	$P = 1.0000$	$P = 1.0000$
Northeast ($N = 10$)	0.244* (0.038)	0.231* (0.039)	-	$P = 0.2239$	$P = 1.0000$	$P = 1.0000$
Peninsular Thailand ($N = 20$)	0.399* (0.048)	0.396* (0.052)	0.389* (0.049)	-	$P = 0.9907$	$P = 0.9645$
Prachuap Khiri Khan ($N = 6$)	0.234* (0.029)	0.341* (0.052)	0.371* (0.054)	0.515* (0.067)	-	$P = 1.0000$
Chumphon ($N = 11$)	0.363* (0.049)	0.324* (0.048)	0.309* (0.042)	0.402* (0.046)	0.462* (0.062)	-

*significant differences at $P < 0.05$

Table 3.18 Analysis of Molecular Variance (AMOVA) of 16S rRNA, COI, and cytb gene for genetic differentiation of *T. collina* among 6 population groupings; North, Central, Northeast, Prachuap Khiri Khan, Chumphon, and Peninsular Thailand

Component	df	Estimate Variance	% of total variance	Φ_{PT}	*P*-value
Among population	5	2.157	37	0.369	0.001
Within population	55	3.696	63		