CHAPTER III

Results

3.1 DNA extraction

The quantity of isolated genomic DNA of *T. clareae* and *T. koenigerum* could not be spectrophotometrically measured due to the limited amount of DNA obtained. Electrophoretic analysis revealed that extracted total DNA was greatly degraded, therefore the DNA concentration of each sample could not be compared with the standard DNA marker (Fig 3.1).

3.2 Testing range of DNA template for PCR amplification of the ITS region

To overcome difficulties to quantify the DNA template, two fold increases in volume (from 1-10 µl) of extracted DNA was served as the DNA template in the PCR amplification of the ITS region as described in Fig.3.2. The ITS PCR product approximately 600 bp in size was initailly observed at 1 µl DNA template. The resulting product was gradually increased until 4 µl of DNA . At the concentration of template greater than this, smeared PCR product was usually observed. As a result, amplification of the ITS region was basically carried out using 4 µl of extracted *T. clareae* and *T. koenigerum* DNA. If smeared PCk product was observed after electrophoresis, the amount of DNA template used in the amplification reactions was then reduced.

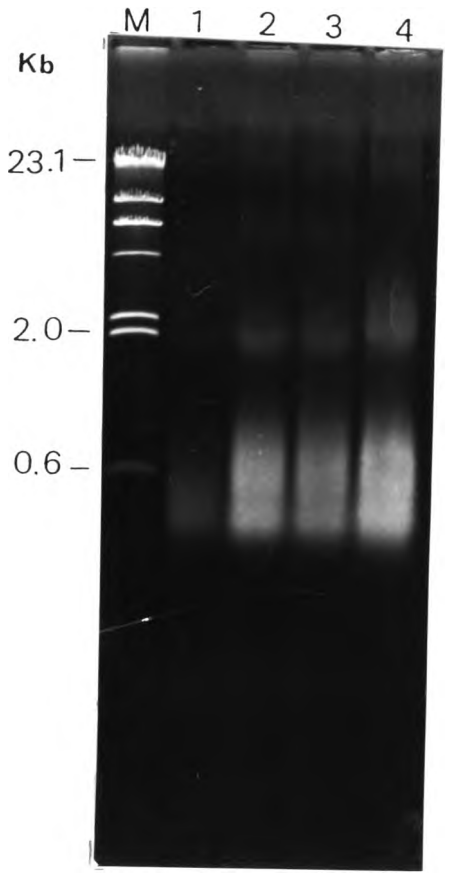

Fig. 3.1 Agarose gel electrophoresis showing the quality of DNA extracted from *Tropilealaps* mites.

lane M = DNA marker (λ/HindIII)

lanes 1-4 = Total DNA from 1, 3, 5 and 10 mite individuals, respectively.

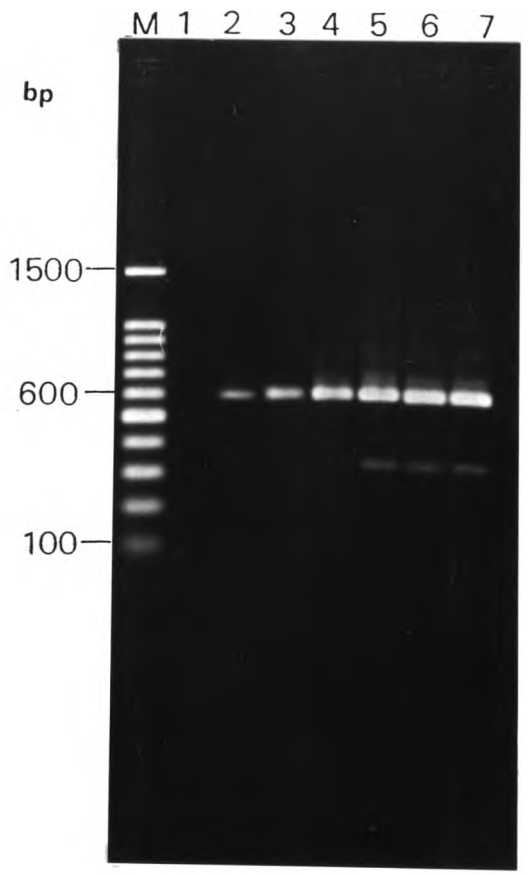

Fig. 3.2 Agarose gel electrophoresis showing the PCR-amplified ITS product (600bp) using different amount of the DNA template at a constant $MgCl_2$ (3.5 mM) and primer concentration (0.10 μM).

lane M = A 100 bp DNA ladder

lanes 1-7 = The resulting ITS product using 0, 1, 2, 4, 6 and 8 μl of DNA template, respectively.

3.3 Optimization of PCR condition for amplification of the ITS region in *T. clareae* and *T. koenigerum* .

To evaluate the most appropriate amount of $MgCl_2$ for amplification of the ITS region from *T. clareae*, a series of 0-4 mM $MgCl_2$ concentration with a 0.5 mM increment was included in the PCR reaction at a primer concentration of 0.2 μM. As can be seen from Fig.3.3, the amplified product was firstly appeared at 0.5 mM $MgCl_2$. An increase of $MgCl_2$ concentration to be higher than 1.5 mM did not yield significantly higher amount of the amplified product, therefore an optimal $MgCl_2$ concentration was chosen at this concentration. The most optimal primer concentration was examined in the same manner, the concentration of primer was varied in the range of 0-0.35 μM, with a 0.5 μM increment. The amplified product was firstly visualized at 0.05 μM of each primer and dramatically increased when the primer were adjusted to 0.10 μM. Higher concentrations of the primers did not resulted in elevating of the PCR product (Fig.3.4). The optimal primer concentration was chosen at 0.10 μM. Therefore, an amplification of the ITS region in *T. clareae* was routinely carried out with the existence of 1.5 mM $MgCl_2$ and 0.10 μM each of the primers.

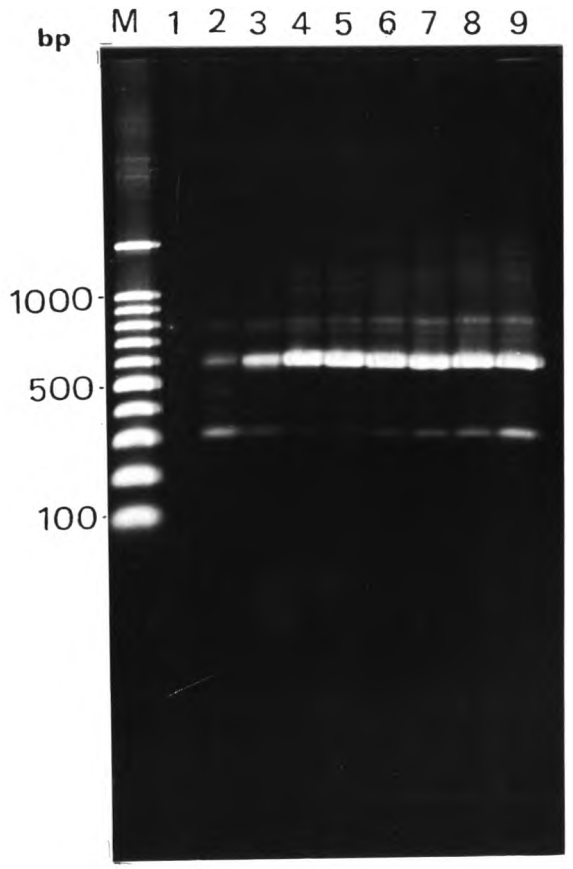

Fig. 3.3 Agarose gel electrophoresis showing the results from optimization of $MgCl_2$ concentration used for amplification of the ITS region at a constant primer concentration of 0.2 μM.

lane M = A 100 bp DNA ladder

lanes 1-9 = The PCR-amplified ITS product resulted from using 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0,3.5 and 4.0 mM of $MgCl_2$ in the PCR, respectively.

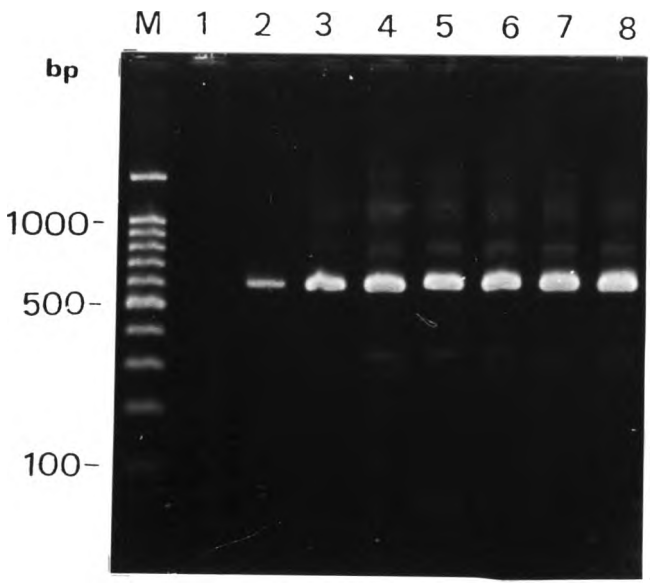

Fig 3.4 Agarose gel electrophoresis showing the results from optimization of primer concentrations used for amplification of the ITS region at 1.5 mM $MgCl_2$ concentration.

lane M = A 100 bp ladder

lanes 1-8 = The PCR-amplified ITS product when 0, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 μM of primers was included in the reaction.

3.4 Preparation of DNA template and direct sequencing

Amplification of an ITS region of twenty *T. clareae* individuals originating from different geographic locations and host species (*A. dorsata* or *A. mellifera*) and 5 individuals of *T. koenigerum* from geographically distinct samples were performed. The size of PCR-amplified ITS region of both *T. clareae* and *T. koenigerum* were about 600 bp long (Fig. 3.5). Length polymorphisms at both intraspecific and interspecific levels were not observed. After electrophoresis, the amplified ITS fragment was purified with approximately 80% yield (Fig. 3.6). This double stranded DNA template was subjected to cycle-sequencing. No sequence polymorphisms were found for all *T. clareae* investigated both from *A. dorsata* and from *A. mellifera* hosts. Fig. 3.7 showed the partial ITS sequences obtained from the internal primer, inITS4. No sequence polymorphisms were observed between geographically different and host-different *T. clareae*. This circumstance was also observed in all *T. koenigerum* investigated. Fig. 3.8 showed an autoradiogram illustrating interspecific polymorphisms of an amplified ITS region of *T. clareae* and *T. koenigerum.*

The average base compositions for an ITS region of both species were 25%A, 30%T, 25%G and 20%C. A sequence from each species (519 bp for *T. clareae* and 520 bp from *T. koenigerum*) was aligned by using Clustal W (Fig 3.9). The sequence similarity of the ITS region was 94.2 %. A total of 19 point mutations was illustrated between these sequences. There were 10 transitional mutations (5 of A↔G and T↔C) and 9 transversional mutations. Differences due to insertions/deletions was also observed (number of gaps = 7). Interestingly, the ITS region of *T. koenigerum* consistently contained a sequence of TTCTC which

was not found in that of *T. clareae*. The genetic distance (sequence divergence) between these two taxa calculated using Kimura's two parameter approach was 3.79 %.

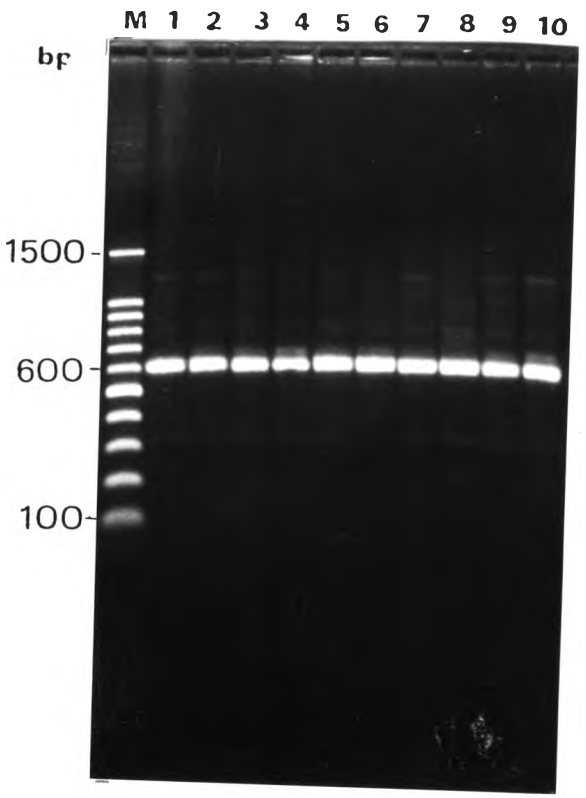

Fig 3.5 The ITS region amplified by PCR and electrophoresed through a 1.5% agarose gel.

lane M = A 100 bp DNA ladder

lanes 1-5 = The PCR-amplified ITS region of *T. clareae*

lanes 6-10 = The PCR-amplified ITS region of *T. koenigerum*

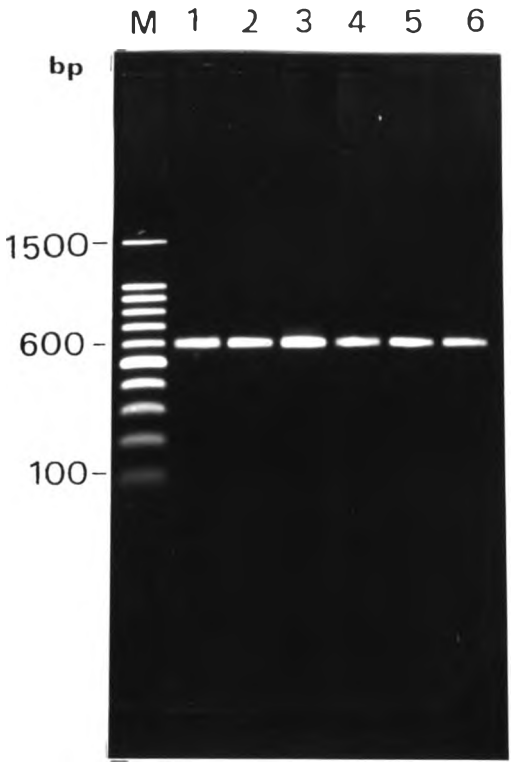

Fig 3.6 Five microliters of the PCR products from each individual of *T. clareae* or *T. koenigerum* was electrophoretically size-fractionated through a 1.5% agarose gel. The purified ITS DNA was recovered using a GeneClean II Kit (Bio101).

lane M = A 100 bp DNA ladder

lanes 1-3 = The ITS DNA amplified through PCR

lanes 4-6 = The ITS DNA recovered using GeneClean.

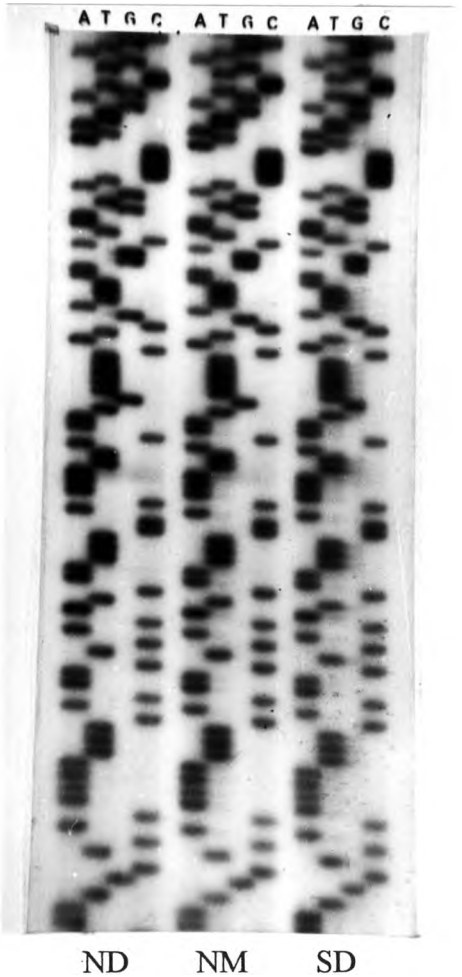

Fig 3.7 An autoradiogram showing partial ITS sequence obtained from using the primer inITS4. No sequence polymorphisms were observed between geographically different (ND and SD) and host-different *T. clareae*

Abbreviation ND = Geographic origin from the North, *A. dorsata* is the host.

NM = Geographic origin from the North, *A. mellifera* is the host.

SD = Geographic origin from the South, *A. dorsata* is the host.

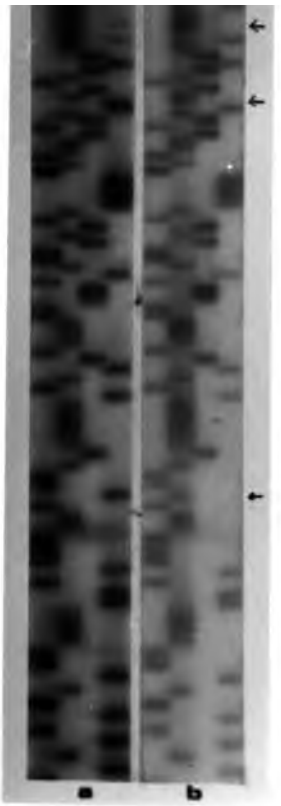

Fig. 3.8 An autoradiogram illustrating interspecific polymorphisms (arrows) of an amplified ITS region of *T. clareae* (a) and *T. koenigerum* (b).

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                                                                        60
T. clareae     CGTAGTGAAC CTGCGGAAGG ATCATTACTG TCGCAAAGTC CATTCACTCC GTCGGCGAGC
T. koenigerum ..........  ..........  ..........  ....G.....  ..........  ..........
                                                                       120
               GAGTGGTGCT CGAATGATGT TCTAACCCTC TC-----CGC GGAGGCGACG GGAGAGGCAT
               ..........  .........C  ..........  ..TTCTC...  .......G..  ..........
                                                                       180
               CTGTGCCCAG TATCGTATGT ATTCCATTCG TATTGCGATC TGACTTCGGC TGTGAAGTTA
               .........C  ..........  ....G.....  ...C.....A .A.........  ..........
                                                                       240
               GGCGCGCGTC GCCGGTGCGT CCGGTTTGAC ATGCTTTTCC ATTTAACTCG TGCTATGGAG
               .........-  .......T..  ..........-  -..........  C..C......  ......C...
                                                                       300
               AAAAGAAGAA CGCATCAGGA CTCAATATGG GGGATCACTT AGTCCTTAAA TCGATGAAAA
               ...--.....  .......A..  ..........  ..........  ..........  ..........
                                                                       360
               ACATTGTAAT TTGTGGAAAT TGATGTGAGT TGTGAAATTT TGTGAGCATT GTGTTTTTGA
               .....A....  ..........  ..........  ..........  ..........  ..........
                                                                       420
               ATGAAAATTT CAGCATGGAT GCCTTGTGTC TATGCTACAC TTGTTTCAGT ATATAACTCG
               ..........  ..........  ..........  ..........  ..........  ..........
                                                                       480
               TAGTATATGT ACTTACTATT GCCGT-ACGC AATGGTATAA AATCTCCACG GTCACGAGAG
               ..C....A..  ...G......  .....T.T..  ..........  ......G...  ..........
                                                                   525
               TGATGCTGCC TGCTCAAGTT GACGTGTATC TGAAATCAAG TGTGA
               ..........  ..........  ..........  ..........  .....
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Fig. 3.9 Sequence for ITS from *T. clareae* and *T. koenigerum* after alignment. Dots indicate identities, and dashes (---) are gaps introduced to located homologous nucleotide position. An insertion of 5 bp (TTCTC) at the nucleotide position 93-97 is *T. koenigerum*-specific. Two sites of GTAC sequence in *T. clareae* (starting from nucleotide sites 428 and 444) whereas in *T. koenigerum* found one site (starting from nucleotide site 444 that same in *T. clareae*) that are recognition site of *Rsa*I.

3.5 Determination of inter- and intraspecific genetic variation of *T. clareae* and *T. koenigerum* using RAPD analysis.

Since the intraspecific diversity of *T. clareae* or *T. koenigerum* could not be detected by sequencing of the ITS region, an alternative method based on RAPD approach was then carried out. A total of 20 octanucleotide primers were screened whether they could be used to examine the genetic variation within and between these species. Based on the sequencing results, these taxa were closely related so only *T. clareae* was initially used during screening for the suitable RAPD primers. It was found that only OPA08 did not yield the successful amplification results (Fig. 3.10). Three primers (OPA07, OPA11 and OPA12) were selected for further studies on genetic variation of *T. clareae* and *T. koenigerum* on the basis of consistent, repeatable and easily scorable results of these primers.

The most optimal $MgCl_2$ concentration for each random primer was carefully examined as described previously. The most optimal $MgCl_2$ concentration for all selected primers was 2.0 mM (Fig. 3.11). This concentration resulted in consistent and repeatable results across investigated individual.

Analysis of sixteen and two colonies (principally 8 individuals per colony) of *T. clareae* and *T. koenigerum* using OPA07, OPA11 and OPA12 indicated both inter- and intraspecific polymorphisms. Examples of RAPD banding patterns generated by OPA07, OPA11 and OPA12 are shown in Fig. 3.12-3.17. A total of 86 fragment were unambiguously scored (265-2200 bp in size). Of these, fifty-one (36 polymorphic bands) and twenty-two fragments (3 polymorphic bands)

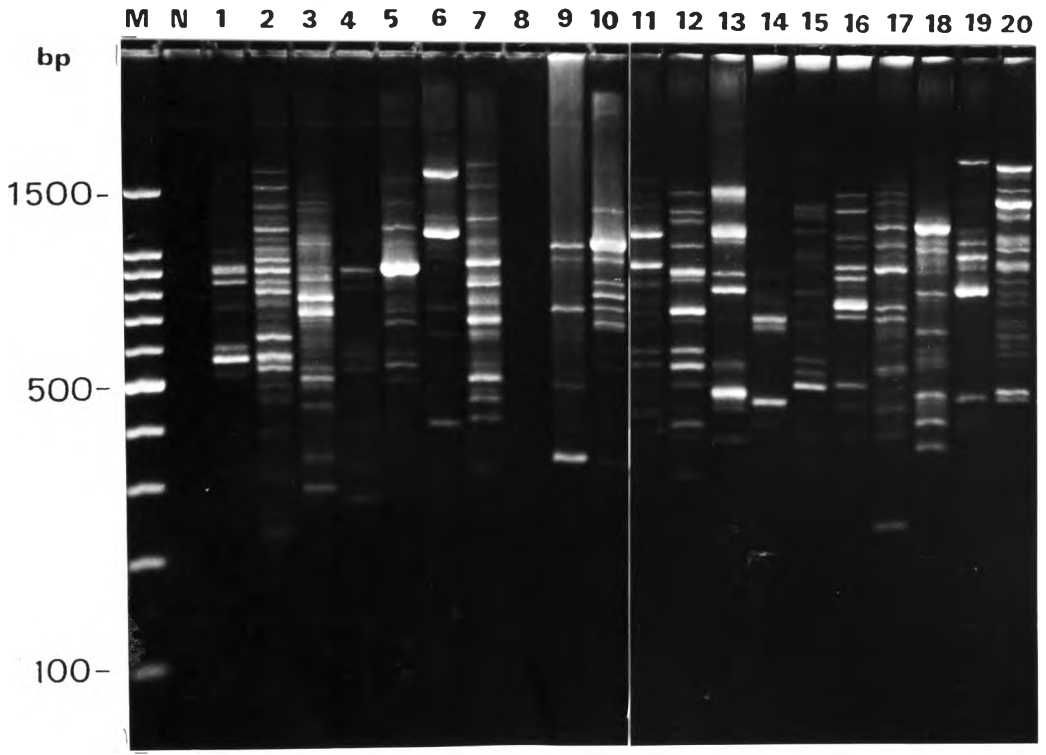

Fig 3.10 Ten microliters of each RAPD reaction was electrophoretically separated through a 1.8% Metaphor agarose gel.

lane M = A 100 bp DNA ladder

lane N = Negative control

lanes 1-20 = Banding patterns of *T. clareae* resulted from RAPD-PCR using primer OPA01-OPA20, respectively.

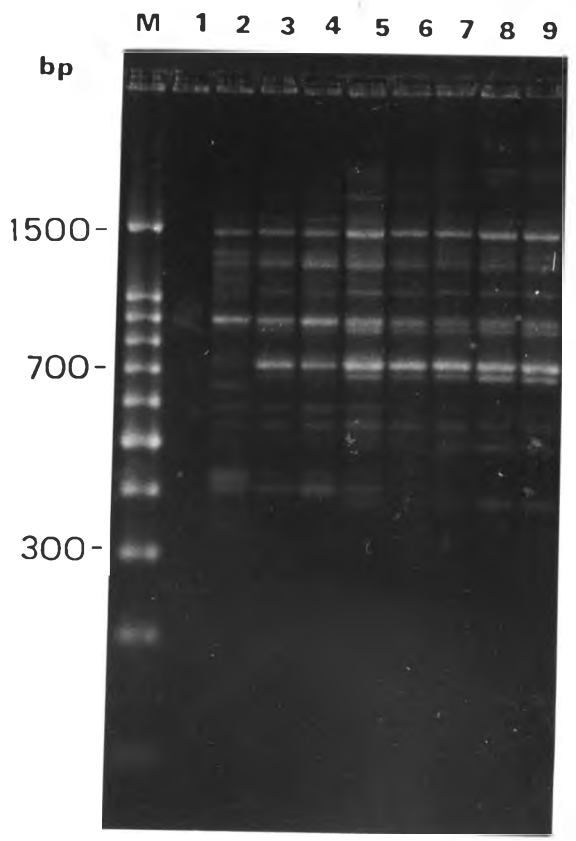

Fig 3.11 Optimization of $MgCl_2$ concentration for RAPD-PCR assay using the primer OPA07 (at 0.4 μM primer concentration).

lane M = A 100 bp DNA ladder

lane 1-9 = Amplification patterns resulted from including of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mM $MgCl_2$ in the RAPD reaction, respectively

were found in *T. clareae* and *T. konigerum*, respectively. Only thirteen fragments were shared by both species (Table 3.1 and 3.2).

The RAPD patterns of all investigated individuals are shown in Appendix A. Only reproducible bands were scored for presence (1) or absence (0). RAPD fragments having band intensity more than 2-fold difference did not score and omitted from the subsequent analysis. One hundred fifty-two RAPD genotypes were observed from OPA07, OPA11 and OPA12, respectively.

RAPD analysis using OPA07 primer

In the present study, a polymorphic band was classified to be polymorphic if it is possessed by less than 95% of overall investigated specimens. The percentage of monomorphic and polymorphic bands in *T. clareae* were found 8.8% and 50%, respectively.Eigth monomorphic bands were found in *T. koenigerum* (Table 3.2). As can be seen from Fig. 3.12 and 3.13, inter and intraspecific polymorphic were clearly observed. In this study, a band carried by individuals of all colony in a given species but disappeared from the other was regarded as a species-specific band. For *T. clareae*, 4 fragments (1980, 1210, 850 and 640 bp) were species-specific while a total of 8 species-specific fragments were observed in *T. koenigerum* (2040, 2000, 1550, 1375, 1195, 1110, 610 and 430 bp). The 950 bp, 880 bp and 760 bp fragments were found in both taxa and may be useful as genus specific RAPD markers. A 1360 bp fragment was only observed in the C1D sample. The percentage of RAPD bands observed in each sample (colony) of *T. clareae* and *T. koenigerum* can be illustrated by Table 3.3 (A).

A total of 60 detected RAPD genotypes was observed when *T. careae* and *T. koenigerum* individuals were amplified using OPA07. Genotypes distribution frequencies resulted from RAPD banding patterns are shown in Table 3.4 (A). Interestingly, no shared genotypes were observed at interspecific level.

RAPD analysis using OPA11 primer

Five species-specific bands (1650 bp, 1140 bp, 920 bp,540 bp and 430 bp fragments) were found in *T. clareae* whereas four specific RAPD fragments (1490, 1440, 1300 and 810 bp) were observed in *T. koenigerum* (Fig. 3.14 and 3.15). The RAPD fragment found in all groups was not observed. The 910 bp and 640 bp fragments were found in *T. koenigerum* but observed in relatively low frequencies in *T. clareae* (0.8 % and 19.2 % of investigated individuals). The percentage of RAPD bands observed in each sample (colony) of *T. clareae* and *T. koenigerum* can be illustrated by Table 3.3 (B). Fifty-eight genotypes were observed from 141 specimens investigated (Table3.4B). The interspecifically shared genotype was not observed between these taxa. Interestingly, a 1210 bp appeared in almost all *T. clareae* samples in *A. mellifera* host (83.9% of all individuals) but it was found in 25.4% of *T. clareae* sample in *A. dorsata* host (Table 3.3B).

RAPD analysis using OPA12 primer

The results from this primer clearly indicated interspecific differences between *T. clareae* and *T. koenigerum* (Fig. 3.16 and 3.17). A total of seven fragment (1490 bp, 1360 bp, 1250 bp, 900 bp, 720 bp 595 bp and 545 bp) was specific to the former. Four of these (900 bp, 720 bp, 595 bp and 545 bp)

were fixed and found in all investigated *T. clareae* individuals (Table 3.4C). Eight *T. koenigerum*-specific fragments were completely fixed. Therefore these and four RAPD fragments from *T. clareae* can be used for unambiguous dissociation of these two species easily. A 1250 bp RAPD fragment was fixed in *T. koenigerum* and found in almost all of the *T. clareae* mite. Therefore, it may be served with relatively high confidence as a genus-specific RAPD marker.

Genotype distribution frequencies of RAPD patterns using OPA12 are shown in Table 3.4C. The lowest number of genotype (35) was found from this primer compared to 60 and 58 patterns resulted from OPA07 and OPA11, respectively.

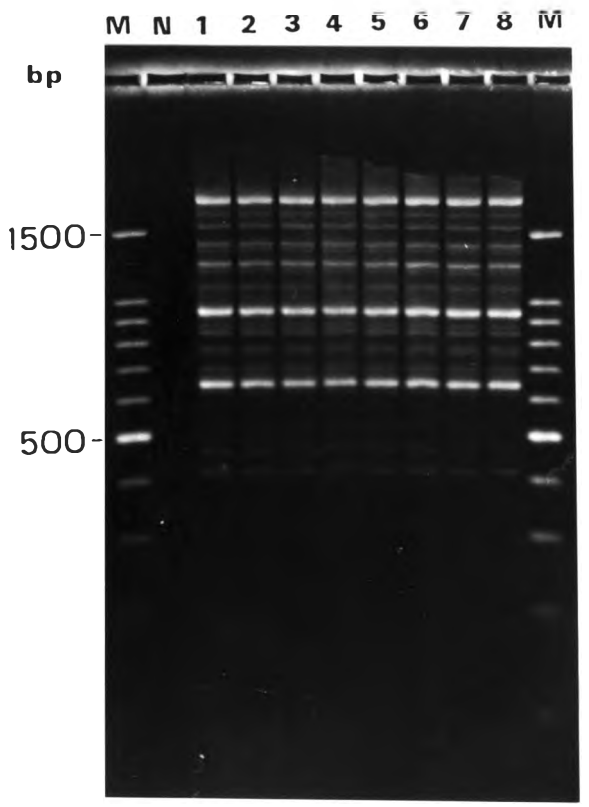

Fig. 3.12 RAPD patterns of *T. clareae* generated from OPA07. Ten microliters of the PCR products was electrophoretically separated through a 1.8% Metaphor agarose gel.

lane M = A 100 bp DNA ladder

lane N = Negative control

lanes 1-8 = RAPD banding patterns of *T. clareae* individuals from the same colony.

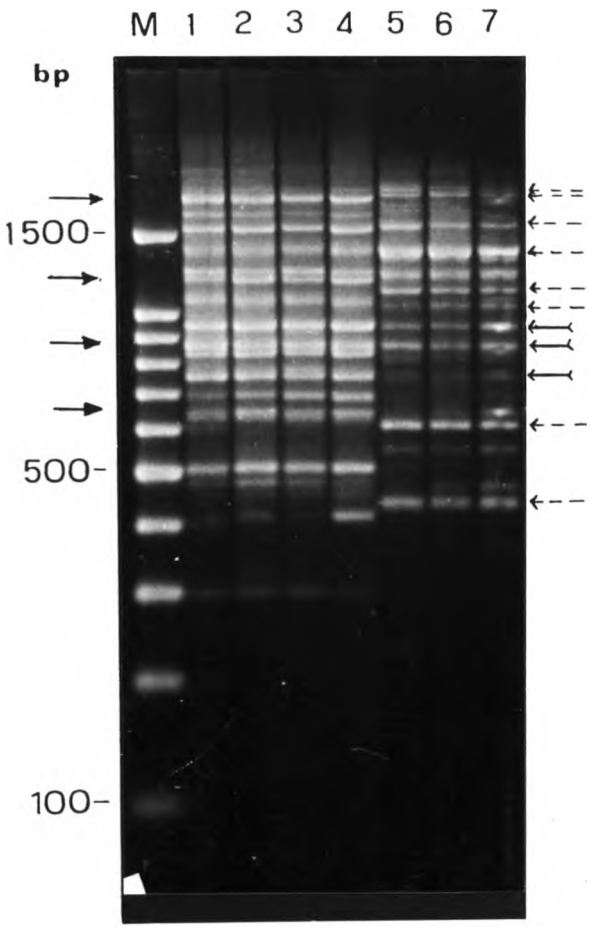

Fig 3.13 Interspecifically different RAPD banding patterns between *T. clareae* and *T. koenigerum* were observed. Ten microliters of RAPD-amplified products using OPA07 was loaded into a 1.8% Metaphor agarose gel and electrophoretically analyzed.

lane M = A 100 bp DNA ladder

lanes 1-4 = RAPD banding patterns of four *T. clareae* individuals

lanes 5-7 = RAPD banding patterns of three *T. koenigeum* individuals.

⟶ = showing species specific band of *T. clareae*,

- - → = showing species specific band of *T. koenigerum*

⟼ = showing genus specific band .

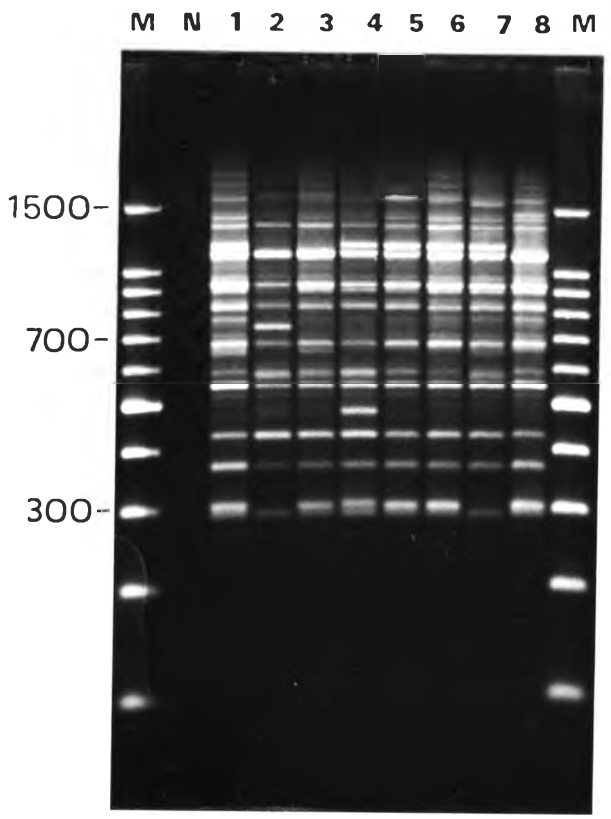

Fig. 3.14 RAPD patterns of *T. clareae* generated from OPA11. Ten microliters of the resulting product were loaded into a 1.8 % Metaphor agarose gel and electrophoretically analyzed.

lane M = A 100 bp DNA ladder

lane N = Negative control

lanes 1-8 = RAPD banding patterns of eight individuals of *T. clareae* originating from the same colony.

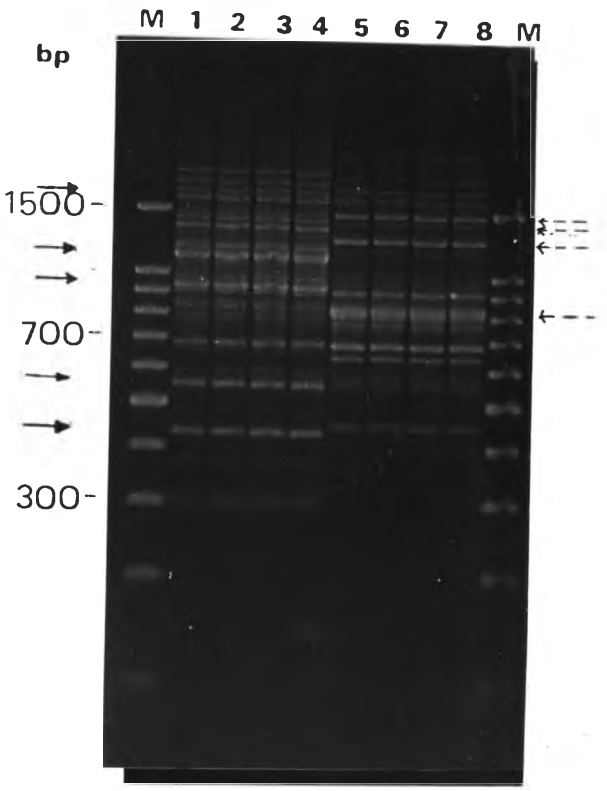

Fig 3.15 Interspecifically different RAPD banding patterns between *T. clareae* and *T. koenigerum* were observed. Ten microliters of RAPD-amplified products using OPA11 was loaded into a 1.8% Metaphor agarose gel and electrophoretically analyzed.

lane M = A 100 bp DNA ladder

lanes 1-4 = RAPD banding patterns of 4 representatives of *T. clareae*

lanes 5-8 = RAPD banding patterns of 4 representatives of *T. koenigerum*

→ = showing species specific band of *T. clareae*,

- - → = showing species specific band of *T. koenigerum*

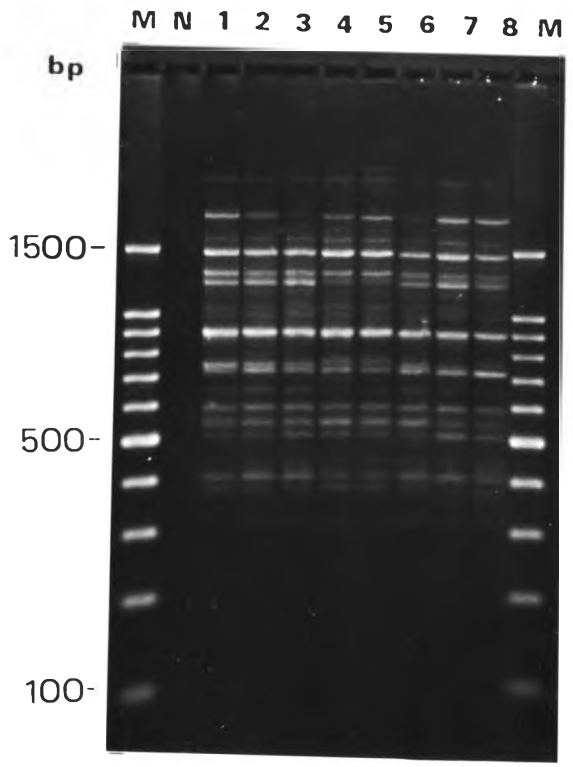

Fig. 3.16 RAPD banding patterns of *T. clareae* generated from OPA12. Ten microliters of the resulting product were loaded into a 1.8 % Metaphor agarose gel and electrophoretically analyzed.

lane M = A 100 bp DNA ladder

lane N = Negative control

lanes 1-8 = RAPD banding patterns of eight individuals of *T. clareae* originating from the same colony.

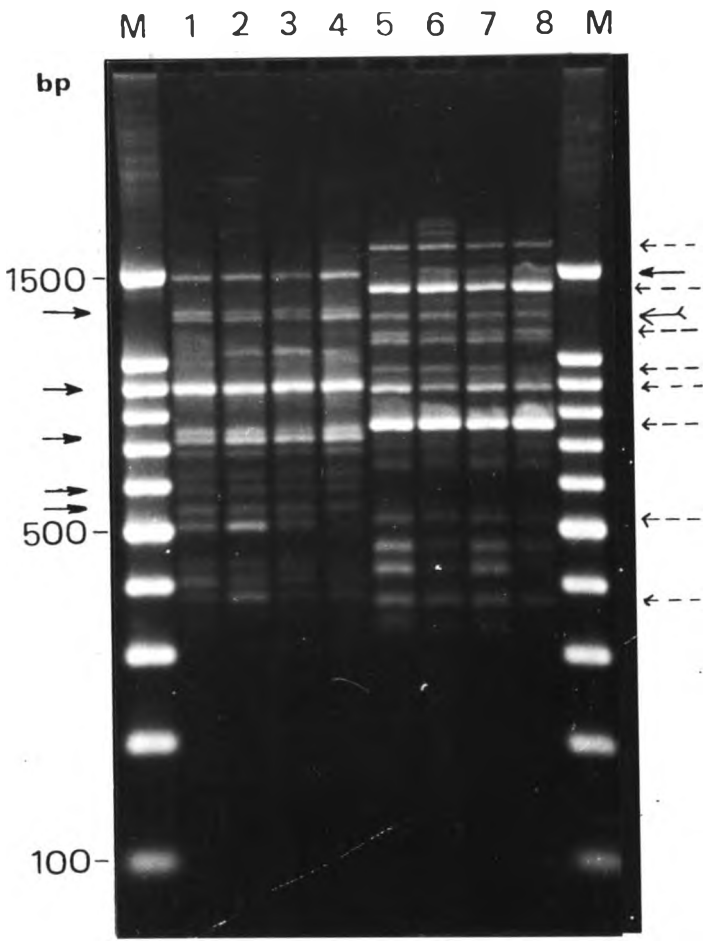

Fig 3.17 Interspecifically different RAPD patterns between *T. clareae* and *T. koenigerum* were observed. Ten microliters of RAPD-amplified products using OPA12 was loaded into a 1.8% Metaphor agarose gel and electrophoretically analyzed.

lane M = A 100 bp DNA ladder

lanes 1-4 = RAPD banding patterns of 4 representatives of *T. clareae*

lanes 5-8 = RAPD banding patterns of 4 representatives of *T. koenigeum*

→ = showing species specific band of *T. clareae*,

--→ = showing species specific band of *T. koenigerum*

⟩→ = showing genus specific band .

Table 3.1 Number of amplified bands in RAPD analysis of *Tropilaelaps clareae* and *T. koenigeum*

Primer No.	No. of amplified bands	No. of bands found in *T. clareae*	No. of bands found in *T. koenigerum*	No. of bands found in both species	No. of species - specific bands of *T. clareae*	No. of species-specific bands of *T. koenigerum*	No. of specific bands of both species
OPA07	34	20	8	6	4	8	3
OPA11	26	18	5	3	5	4	-
OPA12	26	13	9	4	7	8	1

Table 3.2 Total number of bands, percentage of polymorphic and monomorphic bands (in brackets) found in *T. clareae* and *T. koenigerum*

Primer	No. of amplified bands	No. of bands found in *T. clareae*		No. of bands found in *T. koenigerum*		No. of bands found in both species	
		No. of monomorphic bands	No. of polymorphic bands	No. of monomorphic bands	No. of polymorphic bands	No. of monomorphic bands	No. of polymorphic bands
OPA07	34	3 (8.8%)	17 (50%)	8 (23.5%)	-	3 (8.8%)	3 (8.8%)
OPA11	26	6 (23%)	16 (46.2%)	3 (11.5%)	2 (7.7%)	-	3 (11.5%)
OPA12	26	5 (19.2%)	8 (30.8%)	1 (3.8%)	1 (3.8%)	1 (3.8%)	3 (11.5%)

Table 3.3 The percentage of RAPD fragments (in base pairs) within each colony (n=8 except C2D, n=7 and C1M, n=6) of *T. clareae* and *T. koenigerum*.

A. Primer OPA07

	C1D	C2D	C3D	E1D	E2D	N1D	S1D	S2D	N1M	N2M	NE1M	NE2M	C1M	C2M	E1M	S1M	TKC	TKE
2040	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
2020	0	0	0	100	12.5	0	0	0	0	0	0	0	50	0	0	50	0	0
2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
1980	100	100	100	100	100	100	100	100	100	75	100	50	100	100	100	50	0	0
1710	100	87.5	0	100	12.5	0	100	100	12.5	0	0	0	100	100	100	62.5	0	0
1550	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
1530	100	100	0	100	12.5	0	100	100	87.5	0	100	0	100	100	62.5	50	0	0
1390	100	100	100	100	100	100	100	87.5	100	87.5	100	50	100	0	100	87.5	0	0
1375	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
1360	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1210	100	100	100	100	100	100	100	100	25	100	100	100	100	100	100	100	0	0
1195	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
1180	0	0	25	62.5	87.5	87.5	100	87.5	75	87.5	100	100	100	87.5	0	75	0	0
1160	0	0	0	12.5	25	12.5	0	0	0	0	0	0	0	12.5	0	25	0	0
1110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
1070	100	100	0	100	100	0	100	100	0	0	0	0	100	50	0	25	0	0
1050	0	0	0	0	12.5	0	0	0	0	0	0	0	0	0	0	0	100	100
950	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	62.5	100
880	100	100	100	100	100	87.5	100	100	87.5	87.5	100	100	100	100	100	100	100	100
850	100	100	100	100	37.5	75	100	100	100	37.5	100	100	100	100	100	100	0	0
780	0	0	0	0	0	0	0	12.5	0	0	0	0	0	0	0	0	0	0
760	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
690	100	100	100	100	0	12.5	100	100	100	87.5	100	0	100	100	100	100	0	0
640	100	87.5	100	100	100	100	100	75	100	100	100	100	100	100	100	100	0	0
620	0	0	0	62.5	0	0	100	62.5	0	0	25	0	83.33	25	0	0	0	0
610	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
590	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	12.5	0	0
550	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	12.5	100	100
510	100	100	100	100	0	0	100	100	0	0	0	0	100	100	100	50	0	0
480	0	0	0	87.5	0	0	100	12.5	87.5	25	0	0	16.67	12.5	0	0	0	12.5
460	100	100	100	12.5	100	100	50	0	100	100	100	100	83.33	100	100	100	0	0
430	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
410	100	0	0	0	0	0	0	25	87.5	0	0	100	100	100	0	87.5	0	0
405	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.5	0	0

Table 3.3 (continue)
B. Primer OPA11

	C1D	C2D	C3D	E1D	E2D	N1D	S1D	S2D	N1M	N2M	NE1M	NE2M	C1M	C2M	E1M	S1M	TKC	TKE
1650	75	100	87.5	100	100	100	100	100	100	87.5	37.5	75	100	100	50	100	0	0
1490	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	100
1440	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	87.5	100
1300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
1210	0	28.75	12.5	87.5	75	0	0	0	87.5	62.5	100	75	100	100	62.5	87.5	0	0
1140	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
920	100	100	100	100	100	100	100	100	100	100	100	87.5	100	100	100	100	0	0
910	0	0	0	0	0	0	0	0	0	0	0	12.5	0	0	0	0	100	100
820	100	100	0	100	100	0	100	100	100	100	100	75	100	100	100	100	0	0
810	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
770	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	0
740	0	0	0	0	0	0	0	0	100	0	37.5	12.5	100	0	12.5	0	0	0
720	0	0	0	0	0	0	0	0	0	62.5	12.5	12.5	0	0	0	0	0	0
680	100	100	100	100	100	100	100	100	75	100	87.5	87.5	0	100	100	100	0	0
640	12.5	28.57	0	0	0	100	0	37.5	100	0	12.5	12.5	0	0	0	0	100	100
590	87.5	0	0	0	37.5	100	100	100	87.5	100	87.5	87.5	100	100	100	37.5	0	0
580	0	0	25	0	0	0	0	0	12.5	0	12.5	25	0	12.5	0	0	0	0
540	100	100	100	100	100	100	100	100	100	100	87.5	100	100	100	100	100	0	0
480	25	0	0	0	0	0	0	0	75	62.5	50	37.5	100	100	12.5	0	0	0
450	0	0	0	0	0	0	0	0	0	100	12.5	12.5	0	0	0	0	75	100
430	100	100	100	100	100	100	100	100	100	87.5	100	100	100	87.5	100	87.5	0	0
395	0	0	0	0	0	0	0	0	87.5	0	0	0	0	0	0	0	0	0
370	100	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
305	100	100	37.5	87.5	75	87.5	100	50	0	0	50	37.5	33.33	75	75	50	0	0
290	87.5	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
265	0	0	0	0	0	0	0	0	0	100	0	0	100	100	0	0	0	0

Table 3.3 (continue)

C. OPA12 Primer

	C1D	C2D	C3D	E1D	E2D	N1D	S1D	S2D	N1M	N2M	NE1M	NE2M	C1M	C2M	E1M	S1M	TKC	TKE
1670	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
1490	100	100	75	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
1420	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
1360	100	100	75	100	100	100	87.5	100	100	50	100	100	66.67	100	75	100	0	0
1250	100	100	75	100	100	100	87.5	100	100	75	100	100	100	100	75	100	100	100
1140	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
1120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	100	100
1050	100	0	100	12.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
970	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
900	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
895	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
760	0	12.5	0	0	0	0	0	0	25	0	0	0	16.67	25	0	0	100	100
740	100	87.5	0	100	37.5	75	0	0	62.5	62.5	100	75	100	100	0	100	0	0
720	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
680	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
660	0	100	0	100	100	0	0	0	62.5	0	75	50	0	100	0	100	0	0
595	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
545	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
510	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
505	0	87.5	25	62.5	87.5	100	0	0	100	100	100	50	100	100	100	100	0	0
480	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
420	0	0	0	0	0	0	0	0	0	50	0	0	33.33	62.5	100	0	100	100
410	0	100	100	100	100	100	100	100	100	75	100	100	100	100	100	100	0	0
390	100	0	100	0	0	0	0	0	100	75	0	0	100	100	25	100	0	0
385	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
310	0	0	100	0	0	0	0	0	100	62.5	0	0	0	0	100	75	0	0

Table 3.4 Genotype distribution frequencies of RAPD patterns of *T. clareae* and *T. koenigerum*.

A. Primer OPA07

sample	Genotype																																
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI
C1D	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C2D	0	5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C3D	0	0	0	0	6	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2	0	0	0	0	0
C2M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	3	1	
E1D	0	0	0	0	0	0	1	1	1	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E2D	0	0	0	0	0	0	0	0	0	0	0	5	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N2M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NE1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NE2M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	1	1	1	0	0	0	0	0	0	0	0
S1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TKC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TKE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3.4 (continue)

Primer OPA 07 (continue)

sample	Genotype																							
	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH
C1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C2D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C3D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C2M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E2D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E1M	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N1M	0	0	4	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N2M	0	0	0	0	0	0	0	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NE1M	0	0	0	0	0	0	0	0	0	0	0	2	6	0	0	0	0	0	0	0	0	0	0	0	0
NE2M	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	0	0	0	0	0	0	0	0
S1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	2	0	0	0
TKC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	5	0
TKE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	1

Table 3.4 (continue)

B. Primer OPA11

sample	Genotype																																	
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI
C1D	5	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C2D	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C3D	0	0	0	0	0	0	0	0	1	3	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2	0	0	0	0	0	0	0	0	0	0	0
C2M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	4	1	0	0	0	0	0	0	0
E1D	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E2D	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E1M	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0
N1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	
N2M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NE1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NE2M	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1D	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2D	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
S1M	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TKC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TKE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3.4 (continue)
B. Primer OPA11 (continue)

sample	Genotype																					
	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AX	BA	BB	BC	BD	BE	BF
C1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C2D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C3D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C2M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E2D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N1M	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N2M	0	0	0	1	1	2	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NE1M	0	0	0	0	0	0	0	0	0	2	2	2	1	0	0	0	0	0	0	0	0	0	0
NE2M	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	1	1	0	0	0	0	0	0
S1D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1M	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
TKC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	1	1
TKE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0

Table 3.4 (continue)
C. Primer OPA12

sample	Genotype																																
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI
C1D	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C2D	0	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C3D	0	0	0	5	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C2M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0
E1D	0	4	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E2D	0	3	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	2	0	0	0	0	0	0	0	0	0	0
N1D	0	0	0	0	0	0	0	0	0	0	2	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	2	1	0	0	0	0	0	0	0	0
N2M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	1	2	1	1	0	0	0
NE1M	0	6	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NE2M	0	2	1	0	0	0	0	0	0	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
S1D	0	0	0	0	0	0	0	0	0	0	0	0	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2D	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0
TKC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0
TKE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8

Estimation of genetic relatedness within and between *Tropilaelaps* species was carried out. Presumably, individuals from the same colony was regarded as the same group, therefore, one hundred and twenty-five investigated individuals were grouped to 16 samples of *T. clareae* and 2 samples of *T. koenigerum*. For *T. clareae*, the similarity index within each group was between 0.8055-1.000 (OPA07), 0.8359 - 1.000 (OPA11) and 0.8251 - 1.000 (OPA12) (Table 3.5). The average similarity within each group was 0.8717-0.9823. Individuals from S1D possessed the highest genetic similarity within group whereas those from N2M showed the lowest within-group similarity. Apparently, the mean similarity index of *T. clareae* in the *A. dorsata* host was higher than that from the *A. mellifera* host (0.9567 in the former compared to 0.9241 in the latter). Genetic polymorphisms were not observed in *T. koenigerum* when OPA12 was employed. Nevertheless, lower similarity indices were observed with other primers. The mean similarity within each group was 0.9559 and 0.9969 for TKC and TKE, respectively.

Similarity index between group of *T. clareae* from OPA07 was 0.5829-0.9870 whereas that between a pair of TKC and TKE was 0.9600. Interspecific similarity ranged from 0.1749-0.2875. These values were dramatically lower than between group similarity of each species.

The similarity index both within and between species resulted from RAPD analysis using OPA11 is shown by Table 3.6B. The interspecific similarity between a pair of representative groups of *T. clareae* and *T. koenigerum* was 0.0000 to 0.1113. Within each species, the similarity between pairs of samples was

0.5835-0.9582 in *T. clareae* while that between TKC and TKE was 0.9023. Regarding the host species, *T. clareae* from *A. dorsata* showed higher similarity index (0.9582-0.9870) than did *T. clareae* from *A. mellifera* (0.7747-0.9018).

Like OPA07 and OPA11, large similarity values resulted from the primer OPA12 were observed intraspecifically (0.6543-0.9820 for *T. clareae* and 0.9600 for *T. koenigerum*) whereas significant lower similarity was found at interspecific level (0.0621-0.1515). Considering the host species, the ranges of similarity of *T. clareae* were not largely different (0.6943-0.9821 and 0.7736-0.9489) for A. *dorsata* and *A. mellifera* host species, respectively.

The mean similarity index between pairs of samples across all primers was illustrated by Table 3.6D. Higher intraspecific similarity levels were typically observed than interspecific similarity level. Genetic distance was converted from similarity values and showed the same trend (Dij = 1-Sij).

In *T. clareae*, the mean genetic distance between pairs of samples across all investigated primer ranged from 0.0181 to 0.2013 while that between TKC and TKE was 0.0289. Considering the host species, genetic distance of *T. clareae* within *A. mellifera* was 0.0538-0.1446.

Table 3.5 Within colony similarity of sixteen and two of *T. clareae* and *T. koenigerum*

Samples	OPA07	OPA11	OPA12	average all primers
C1D	1.0000	0.9231	1.0000	0.9744
C2D	0.9787	0.9516	0.9625	0.9643
C3D	0.9814	0.9054	0.9140	0.9336
C1M	0.9538	0.9802	0.9382	0.9574
C2M	0.9423	0.9661	0.9626	0.9570
E1D	0.9367	0.9760	0.9636	0.9588
E2D	0.9061	0.9355	0.9614	0.9343
E1M	0.9786	0.9081	0.9340	0.9402
N1D	0.9255	0.9881	0.9774	0.9637
N1M	0.9249	0.9273	0.9392	0.9305
N2M	0.8740	0.9159	0.8251	0.8717
NE1M	0.9829	0.8359	0.9796	0.9328
NE2M	0.9429	0.8359	0.9182	0 8990
S1D	0.9827	1.0000	0.9643	0.9823
S2D	0.9175	0.9080	1.0000	0.9418
S1M	0.8055	0.9239	0.9829	0.9041
TKC	0.9781	0.8895	1.0000	0.9559
TKE	0.9907	1.0000	1.0000	0.9969

Table 3.6 Similarity index with a correction of within-colony similarity effect (upper diagonal) and genetic distance(below diagonal) for each pairwise comparison of *T. clareae* and *T koenigerum* samples.

A. Primer OPA07

	C1D7	C2D7	C3D7	C1M7	C2M7	E1D7	E2D7	E1M7	N1D7	N1M7	N2M7	NE1M7	NE2M7	S1D7	S2D7	S1M7	TKC7	TKE7
C1D7	-	0.9288	0.8075	0.8907	0.8746	0.8158	0.7113	0.8818	0.6782	0.7168	0.6746	0.7789	0.6904	0.8305	0.8403	0.8050	0.1829	0.2060
C2D7	0.0665	-	0.8700	0.8732	0.8543	0.8502	0.7643	0.9363	0.7319	0.7026	0.7277	0.8365	0.6601	0.8741	0.8728	0.7918	0.1988	0.2237
C3D7	0.1831	0.1100	-	0.7760	0.7828	0.7414	0.7736	0.9221	0.8520	0.7208	0.8464	0.8723	0.7733	0.7741	0.7601	0.7980	0.2192	0.2463
C1M7	0.0863	0.0931	0.1915	-	0.9870	0.8797	0.7326	0.8322	0.6981	0.7323	0.6946	0.8146	0.7186	0.9111	0.8940	0.8235	0.1749	0.1984
C2M7	0.0965	0.1062	0.1790	0.0510	-	0.7935	0.6881	0.8458	0.6925	0.7279	0.7000	0.8074	0.7546	0.8439	0.8427	0.8152	0.1905	0.2155
E1D7	0.1526	0.1075	0.2176	0.0656	0.1460	-	0.6849	0.8072	0.6417	0.6719	0.6525	0.7568	0.5829	0.9200	0.8780	0.7420	0.1799	0.2098
E2D7	0.2417	0.1781	0.1710	0.1974	0.2362	0.2366	-	0.7250	0.8658	0.6719	0.8070	0.8067	0.7847	0.6603	0.7021	0.7102	0.2315	0.2587
E1M7	0.1075	0.0424	0.0578	0.1340	0.1147	0.1505	0.2174	-	0.7808	0.7187	0.7762	0.8541	0.7505	0.8319	0.8249	0.8102	0.2073	0.2331
N1D7	0.2846	0.2202	0.1015	0.2415	0.2424	0.2894	0.0501	0.1713	-	0.7005	0.8678	0.8738	0.8567	0.6935	0.6657	0.7401	0.2220	0.2509
N1M7	0.2456	0.2492	0.2324	0.2071	0.2057	0.2590	0.2590	0.2331	0.2248	-	0.7126	0.7994	0.7072	0.7204	0.6633	0.7368	0.2570	0.2875
N2M7	0.2624	0.1986	0.0813	0.2174	0.2081	0.2528	0.0830	0.1501	0.0320	0.1868	-	0.8670	0.7933	0.7072	0.6647	0.7451	0.2206	0.2519
NE1M7	0.2125	0.1443	0.1099	0.1538	0.1552	0.2012	0.1378	0.1266	0.0804	0.1545	0.0614	-	0.8069	0.8168	0.7923	0.8073	0.2104	0.2366
NE2M7	0.2811	0.3006	0.1889	0.2297	0.1880	0.3569	0.1398	0.2557	0.0775	0.2267	0.1151	0.1560	-	0.6391	0.6315	0.7761	0.2317	0.2600
S1D7	0.1608	0.1066	0.2079	0.0571	0.1186	0.0397	0.2190	0.1487	0.2606	0.2334	0.2211	0.1660	0.3237	-	0.9157	0.7500	0.1798	0.2108
S2D7	0.1184	0.0752	0.1893	0.0416	0.0871	0.0491	0.2106	0.1231	0.2558	0.2579	0.2310	0.1579	0.2987	0.0344	-	0.7473	0.1921	0.2174
S1M7	0.0978	0.1003	0.0954	0.0561	0.0587	0.1291	0.1629	0.0818	0.1254	0.1284	0.0946	0.0869	0.0980	0.1440	0.1142	-	0.2074	0.2319
TKC7	0.8064	0.7799	0.7607	0.7913	0.7699	0.7778	0.7109	0.7712	0.7300	0.6948	0.7057	0.7703	0.7290	0.8008	0.7559	0.6864	-	0.9804
TKE7	0.7893	0.7611	0.7398	0.7738	0.7510	0.7539	0.6897	0.7515	0.7073	0.6703	0.6804	0.7502	0.7068	0.7759	0.7367	0.6662	0.0043	-

Table 3.6 (continue)

B. Primer OPA11

	C1D11	C2D11	C3D11	C1M11	C2M11	E1D11	E2D11	E1M11	N1D11	N1M11	N2M11	NE1M11	NE2M11	S1D11	S2D11	S1M11	TKC11	TKE11
C1D11	-	0.8608	0.7450	0.7477	0.8403	0.8777	0.8901	0.8989	0.8721	0.7712	0.8023	0.8190	0.8197	0.9582	0.9183	0.8645	0.0155	0.0156
C2D11	0.0765	-	0.7309	0.6644	0.7635	0.8979	0.8771	0.8141	0.7977	0.7014	0.7159	0.7420	0.7472	0.8761	0.8399	0.8531	0.0325	0.0327
C3D11	0.1693	0.1976	-	0.5835	0.6710	0.7949	0.7837	0.7246	0.7783	0.6480	0.6654	0.6610	0.7011	0.7779	0.7594	0.7714	0.0000	0.0000
C1M11	0.2039	0.3015	0.3592	-	0.8953	0.7607	0.7735	0.7859	0.6846	0.8654	0.8476	0.8072	0.7753	0.7667	0.7539	0.7747	0.0000	0.0000
C2M11	0.1043	0.1953	0.2647	0.0778	-	0.8409	0.8568	0.8581	0.7737	0.8240	0.9018	0.8419	0.8263	0.8583	0.8284	0.8515	0.0000	0.0000
E1D11	0.0719	0.0659	0.1458	0.2174	0.1221	-	0.9536	0.8861	0.8104	0.7933	0.7966	0.8358	0.8300	0.9080	0.8680	0.9424	0.0000	0.0000
E2D11	0.0391	0.0664	0.1367	0.1844	0.0940	0.0021	-	0.8989	0.8293	0.8069	0.8173	0.8434	0.8418	0.9247	0.8911	0.9339	0.0000	0.0000
E1M11	0.0167	0.1158	0.1822	0.1583	0.0790	0.0561	0.0229	-	0.8319	0.8108	0.8243	0.8643	0.8455	0.9263	0.8929	0.8873	0.0000	0.0000
N1D11	0.0832	0.1722	0.1684	0.2995	0.2034	0.1716	0.1325	0.1162	-	0.7125	0.7462	0.7543	0.7897	0.9026	0.9070	0.8016	0.1113	0.1119
N1M11	0.1539	0.2381	0.2683	0.0883	0.1227	0.1584	0.1244	0.1069	0.2425	-	0.8024	0.8215	0.7992	0.7941	0.7951	0.8140	0.0000	0.0000
N2M11	0.1172	0.2197	0.2453	0.1005	0.0392	0.1494	0.1084	0.0877	0.2058	0.1192	-	0.8064	0.8035	0.8269	0.8280	0.8233	0.0000	0.0000
NE1M11	0.0606	0.1518	0.2097	0.1009	0.0591	0.0702	0.0423	0.0078	0.1577	0.0601	0.0696	-	0.8206	0.8326	0.8171	0.8420	0.0217	0.0250
NE2M11	0.0380	0.1248	0.1478	0.1110	0.0529	0.0542	0.0221	0.0047	0.1006	0.0607	0.0506	0.0000	-	0.8447	0.8341	0.8398	0.0360	0.0395
S1D11	0.0033	0.0997	0.1748	0.2235	0.1248	0.0800	0.0430	0.0278	0.0915	0.1695	0.1311	0.0853	0.0514	-	0.9545	0.8998	0.0000	0.0000
S2D11	0.0172	0.1099	0.1673	0.2102	0.1287	0.0940	0.0506	0.0351	0.0610	0.1425	0.1040	0.0748	0.0361	0.0195	-	0.8779	0.0531	0.0534
S1M11	0.0590	0.0847	0.1433	0.1774	0.0935	0.0076	0.0000	0.0288	0.1499	0.1116	0.0967	0.0379	0.0183	0.0622	0.0580	-	0.0000	0.0000
TKC11	0.8906	0.8880	0.8974	0.9349	0.9278	0.9327	0.9125	0.8988	0.8275	0.9084	0.9084	0.8410	0.8050	0.9447	0.8657	0.9067	-	0.9023
TKE11	0.9459	0.9431	0.9527	0.9901	0.9830	0.9880	0.9677	0.9540	0.8821	0.9636	0.9586	0.8930	0.8567	1.0000	0.9206	0.9620	0.0424	-

Table 3.6 (continue)

C. Primer OPA12

	C1D12	C2D12	C3D12	C1M12	C2M12	E1D12	E2D12	E1M12	N1D12	N1M12	N2M12	NE1M12	NE2M12	S1D12	S2D12	S1M12	TKC12	TKE12
C1D12	-	0.7548	0.8003	0.8193	0.7874	0.7832	0.7278	0.6543	0.7842	0.7662	0.7337	0.7714	0.7851	0.7587	0.7587	0.7915	0.0870	0.0909
C2D12	0.2278	-	0.6943	0.8564	0.9082	0.9637	0.9547	0.7679	0.9236	0.8608	0.7772	0.9705	0.9284	0.8309	0.8480	0.9102	0.0942	0.0983
C3D12	0.1567	0.2453	-	0.7587	0.7240	0.7039	0.7156	0.7898	0.7361	0.8234	0.7548	0.7016	0.7246	0.7679	0.7780	0.7917	0.0621	0.0648
C1M12	0.1498	0.0953	0.1674	-	0.9080	0.8479	0.8320	0.8096	0.9005	0.8731	0.8567	0.8830	0.8517	0.7880	0.8005	0.8927	0.1224	0.1276
C2M12	0.1939	0.0557	0.2143	0.0424	-	0.8997	0.8862	0.7968	0.8621	0.8908	0.8221	0.9103	0.8604	0.7508	0.7671	0.9374	0.1439	0.1497
E1D12	0.1986	0.0007	0.2349	0.1030	0.0634	-	0.9440	0.7489	0.9137	0.8475	0.7647	0.9644	0.9315	0.8373	0.8544	0.9043	0.0843	0.0880
E2D12	0.2529	0.0086	0.2220	0.1178	0.0758	0.0185	-	0.7910	0.9149	0.8547	0.7717	0.9509	0.9202	0.8606	0.8779	0.8907	0.0861	0.0900
E1M12	0.3127	0.1817	0.1342	0.1265	0.1515	0.1999	0.1567	-	0.8227	0.8427	0.8493	0.7846	0.7736	0.7826	0.7923	0.8114	0.1452	0.1515
N1D12	0.2045	0.0477	0.2096	0.0573	0.1080	0.0568	0.0545	0.1330	-	0.8509	0.8257	0.9512	0.9283	0.8845	0.9020	0.8666	0.0879	0.0920
N1M12	0.2034	0.0914	0.1[illegible]3	0.0656	0.0601	0.1039	0.0959	0.0938	0.1074	-	0.8368	0.8669	0.8322	0.7650	0.7815	0.9489	0.0985	0.1025
N2M12	0.1788	0.1180	0.1148	0.0250	0.0718	0.1297	0.1215	0.0303	0.0756	0.0454	-	0.8012	0.7750	0.7414	0.7474	0.8337	0.1105	0.1152
NE1M12	0.2184	0.0019	0.2452	0.0759	0.0608	0.0073	0.0196	0.1722	0.0273	0.0925	0.1012	-	0.9369	0.8367	0.8538	0.9148	0.0842	0.0879
NE2M12	0.1740	0.0133	0.1915	0.0765	0.0801	0.0094	0.0196	0.1525	0.0195	0.0965	0.0966	0.0120	-	0.8867	0.9042	0.8649	0.0881	0.0921
S1D12	0.2235	0.1339	0.1713	0.1632	0.2127	0.1267	0.1022	0.1666	0.0863	0.1867	0.1534	0.1352	0.0545	-	0.9821	0.7550	0.0833	0.0875
S2D12	0.2235	0.1347	0.1790	0.1686	0.2142	0.1274	0.1028	0.1747	0.0868	0.1881	0.1652	0.1360	0.0550	0.0000	-	0.7714	0.0952	0.1000
S1M12	0.1999	0.0638	0.1567	0.0678	0.0354	0.0690	0.0814	0.1470	0.1136	0.0121	0.0703	0.0664	0.0857	0.2185	0.2200	-	0.0777	0.0808
TKC12	0.9130	0.8884	0.8949	0.8467	0.8374	0.8975	0.8964	0.8218	0.9008	0.8711	0.8020	0.9056	0.8710	0.8988	0.9048	0.9137	-	0.9600
TKE12	0.9091	0.8843	0.8922	0.8415	0.8316	0.8938	0.8907	0.8155	0.8967	0.8670	0.7974	0.9019	0.8670	0.8964	0.9000	0.9106	0.0400	-

Table3.6 (continue)

D. overall primer

	C1D	C2D	C3D	C1M	C2M	E1D	E2D	E1M	N1D	N1M	N2M	NE1M	NE2M	S1D	S2D	S1M	TKC	TKE
C1D	-	0.8481	0.7843	0.8192	0.8341	0.8256	0.7764	0.8117	0.7783	0.7514	0.7369	0.7898	0.7651	0.8491	0.8391	0.8203	0.0951	0.1042
C2D	0.1236	-	0.7651	0.7980	0.8420	0.9039	0.8654	0.8394	0.8177	0.7549	0.7403	0.8497	0.7786	0.8604	0.8536	0.8517	0.1085	0.1182
C3D	0.1697	0.1843	-	0.7061	0.7259	0.7466	0.7576	0.8122	0.7888	0.7307	0.7555	0.7450	0.7330	0.7733	0.7658	0.7870	0.0938	0.1037
C1M	0.1467	0.1633	0.2314	-	0.9301	0.8294	0.7794	0.8092	0.7611	0.8236	0.7996	0.8349	0.7819	0.8219	0.8161	0.8303	0.0991	0.1087
C2M	0.1216	0.1191	0.2193	0.0571	-	0.8447	0.8104	0.8336	0.7761	0.8142	0.8080	0.8532	0.8138	0.8177	0.8127	0.8680	0.1115	0.1217
E1D	0.1410	0.0580	0.1994	0.1287	0.1105	-	0.8608	0.8141	0.7886	0.7709	0.7379	0.8523	0.7815	0.8884	0.8668	0.8629	0.0881	0.0993
E2D	0.1779	0.0840	0.1763	0.1665	0.1353	0.0857	-	0.8050	0.8700	0.7778	0.7987	0.8670	0.8489	0.8152	0.8237	0.8449	0.1059	0.1162
E1M	0.1456	0.1133	0.1247	0.1396	0.1151	0.1355	0.1323	-	0.8118	0.7907	0.8166	0.8343	0.7899	0.8469	0.8367	0.8363	0.1175	0.1282
N1D	0.1908	0.1467	0.1598	0.1994	0.1843	0.1726	0.0790	0.1402	-	0.7546	0.8132	0.8597	0.8576	0.8269	0.8249	0.8028	0.1410	0.1516
N1M	0.2010	0.1929	0.2013	0.1203	0.1295	0.1739	0.1598	0.1446	0.1916	-	0.7839	0.8293	0.7795	0.7598	0.7466	0.8332	0.1185	0.1300
N2M	0.1861	0.1788	0.1471	0.1143	0.1064	0.1773	0.1043	0.0894	0.1045	0.1171	-	0.8243	0.7906	0.7585	0.7467	0.8007	0.1104	0.1224
NE1M	0.1638	0.0993	0.1883	0.1102	0.0917	0.0929	0.0666	0.1022	0.0885	0.1024	0.0774	-	0.8548	0.8257	0.8211	0.8547	0.1054	0.1165
NE2M	0.1644	0.1462	0.1761	0.1391	0.1070	0.1402	0.0605	0.1376	0.0659	0.1280	0.0874	0.0538	-	0.7902	0.7899	0.8269	0.1186	0.1305
S1D	0.1292	0.1134	0.1847	0.1479	0.1520	0.0821	0.1214	0.1144	0.1461	0.1965	0.1685	0.1288	0.1432	-	0.9508	0.8016	0.0877	0.0994
S2D	0.1197	0.1066	0.1785	0.1401	0.1433	0.0902	0.1213	0.1110	0.1345	0.1962	0.1667	0.1229	0.1299	0.0181	-	0.7989	0.1135	0.1236
S1M	0.1189	0.0829	0.1318	0.1004	0.0625	0.0686	0.0814	0.0859	0.1290	0.0840	0.0870	0.0637	0.0673	0.1416	0.1307	-	0.0950	0.1042
TKC	0.8700	0.8521	0.8510	0.8576	0.8450	0.8693	0.8399	0.8306	0.8194	0.8248	0.8052	0.8390	0.8017	0.8814	0.8421	0.8356	-	0.9476
TKE	0.8814	0.8628	0.8613	0.8685	0.8552	0.8786	0.8494	0.8403	0.8287	0.8336	0.8121	0.8484	0.8102	0.8908	0.8524	0.8463	0.0289	-

The mean genetic distance of all pairwise comparisons was used for phylogenetic reconstruction using unweighted pair-group method using an arithmetic average (UPGMA). The dendrogram indicate large separation of congeneric species like *T. clareae* and *T. koenigerum* (Fig.3.18). Nevertheless, phylogeographic separation was not observed among 16 samples of *T. clareae* (Fig.3.19-3.20). Based on this dendrogram, two lineages of *T. clareae* was observed. The first group contained all samples in the *A. mellifera* host and those of E1D and N1D (from *A. dorsata*). The other group consisted of all remaining *T. clareae* that uses *A. dorsata* as a host. This indicated closer relationships of such a parasite from the same rather than from different host species.

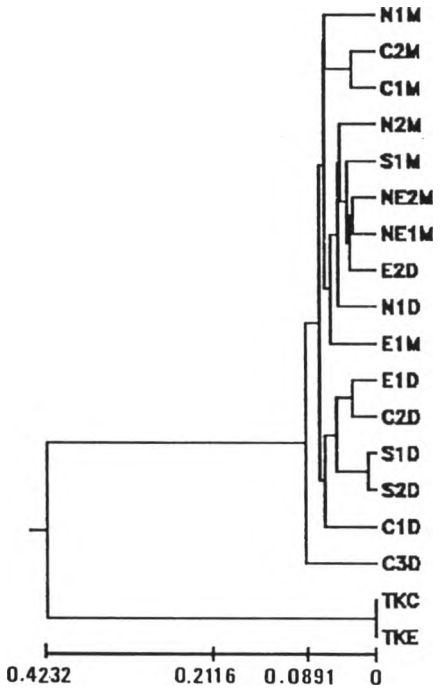

Fig 3.18 UPGMA dendrogram showing relationships among 16 groups of *T. clareae* and 2 groups of *T. koenigerum* based on genetic distance shown in Table 3.6 D.

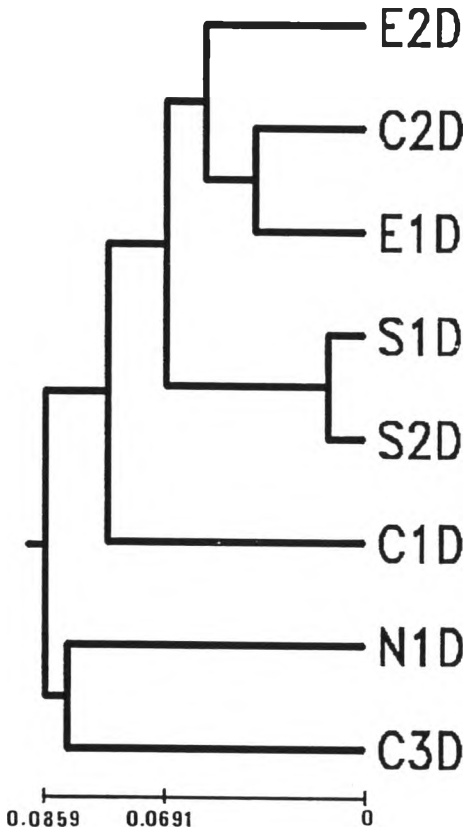

Fig 3.19 UPGMA dendrogram showing the relationships among 8 groups of *T. clareae* having *A. dorsata* host.

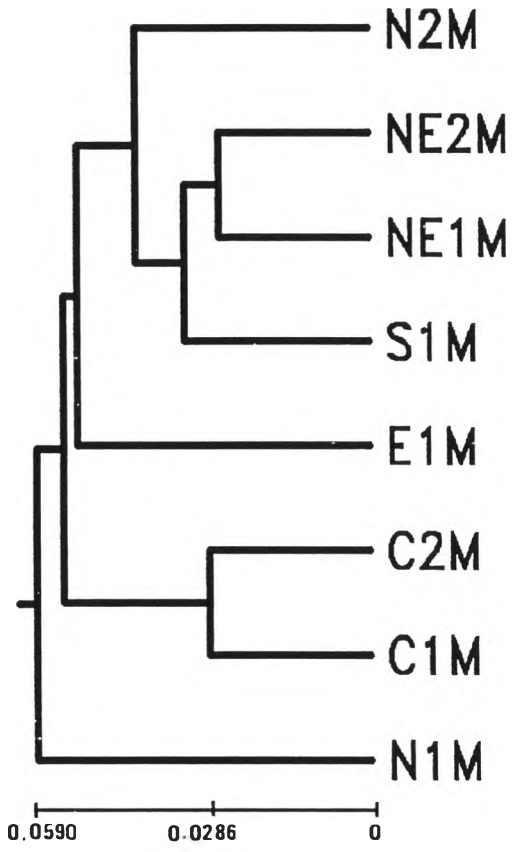

Fig 3.20 UPGMA dendrogram showing the relationships among 8 groups of *T. clareae* having *A. mellifera* host.