CHAPTER I

Introduction

1.1 Biology of Bee Mites

All bee mites are large obligate external parasites fed and reproduced in the honey bee brood, principally during late larval and pupal stages of the hosts inside the sealed brood cells. Newly developed adults, along with the maternal mites, leave the cells with the emerging bees. Although the hosts survive, they are weaker leading to their shorter life span (De Jong, 1990).

Several honey bee associated mite species are found in Asia. These are composed of members of Varroidae including *Varroa jacobsoni*, *V. underwoodi*, *Euvarroa sinhai* and *E. wongsirii*, and Laelapidae including *Tropilaelaps clareae* and *T. koenigerum*. At present, these parasites associate with six *Apis* taxa (Table 1.1). Although horizontal infection of these mites can occurred in the European honey bee (*A. mellifera*), more host/parasite specific infection was observed in the native honey bee species (*A. dorsata*, *A. cerana*, *A. koshevnikovi*, *A. mellifera*, *A. florea*, *A. andreniformis* and *A. laboriosa*) (Wongsiri et al., 1995).

Among all bee mites, *V. jacobsoni* and *T. clareae* are possibly the most important parasitic taxa as they are more destructive than others. The values loss from these parasitic species are significant to beekeepers, as they severely attack *A. mellifera* brood.

Table 1.1 Honey bee mites and host associations.

Mite Species	Host Species	Host Ecology & Behavior
Varroidae		
V. jacobsoni	*Apis cerana*	Construct multi-comb nests inside
	A. koschevnikovi	cavities in trees or caves.
	A. mellifera	*A. cerana* can be kept in man-
		made hives; colonies are smaller in
V. underwoodi	*A. cerana*	size; has distinct shorter drone
		production seasons
E. sinhai	*A. florea*	Build single-comb nests attached
E. wongsirii	*A. andreniformis*	to small tree branches or twigs in
		dense vegetation; small colonies;
		frequently short distance
		vegetation and migration; high
		swarming rates; drone cells larger
		than workers.
Laelapidae		
T. clareae	*A. dorsata*	Build single-comb nests in the
	A. laboriosa	open; combs suspended under
	(*A. mellifera*)	large branches of tall trees, roofs
		of buildings, or rock overhangs,
T. koenigerum	*A. dorsata*	large colonies, obligatory seasonal
	A. laboriosa	migration over long distance.

(Delfinado-Baker and Peng, 1995)

Tropilaelaps is an old world tropical genus of the family Laelapidae. It is proposed that *T. clareae* and *T. koenigerum*, have evolved from their presumbly ancestor within the Hypoaspidinae and become parasites of the honey bee brood (Eickwork, 1988). Previously, biology of *T. clareae* was not well understood. After *A. mellifera* was introduced to Asia for commercial honey production purposes, it was found that *T. clareae* could also infect this honey bee species. Unfortunately, *A. mellifera* is highly susceptible to *T. clareae* resulting in a significant loss of the honey production (De Jong, 1990).

Therefore, *T. clareae* is one of the most serious pest of cultured honey bee in Asia (Burgett, Akratanakul and Morse, 1983; Delfinado-Baker, Underwood and Baker, 1985). This parasite was firstly described in the Phillipines by Delfinado and Baker (1961). It firmly attached to dead larvae, pupae and adults of *A. mellifera* (Bhardwaj, 1968; Krantz and Kitprasert, 1990). Subsequently, it was also found to be a parasite on *A. dorsata* (Bharadwaj, 1968) and found in *A. mellifera* only in the geographic areas where *A. dorsata* is overlapping distributed to. Based on the basic information that *T. clareae* distributes in tropical and subtropical areas, *A. dorsata* rather than *A. mellifera* should be a native host for this parasite (Burgett, Akratanakul and Morse, 1983; Kumar, Kumar and Bhala, 1993). Interestingly, *T. clareae* is more harmful to the exotic species like *A. mellifera* than the native host, *A. dorsata* (Eickwork, 1988).

Tropilaelaps spp., can be morphologically classified as follows, (Krantz, 1978).

Phylum Arthropoda

Class Arachnida

Subclass Acari

Order Parasitiformes

Suborder Gamasida

Family Laelapidae

Genus *Tropilaelaps*

species *clareae*

koenigerum

Scientific name : *Tropilaelaps clareae* Delfinado and Baker, 1961

Tropilaelaps koenigerum Delfinado-Baker and Baker, 1982

Morphologically, the external features of the respiratory system or more specifically, the peritremes and stigmata, have been taxonomically used for classification and systematics in the subclass Acari (Krantz, 1978). Thus, the middle position of the stigmata, which dorsolaterally open near coxae III or IV (Fig 1.1), is called gamasid which is the morphologically specific markers for members of suborder Gamasida. In most gamasid mites, each stigma has an elongated peritreme formed by sclerotized groove usually extends forward along

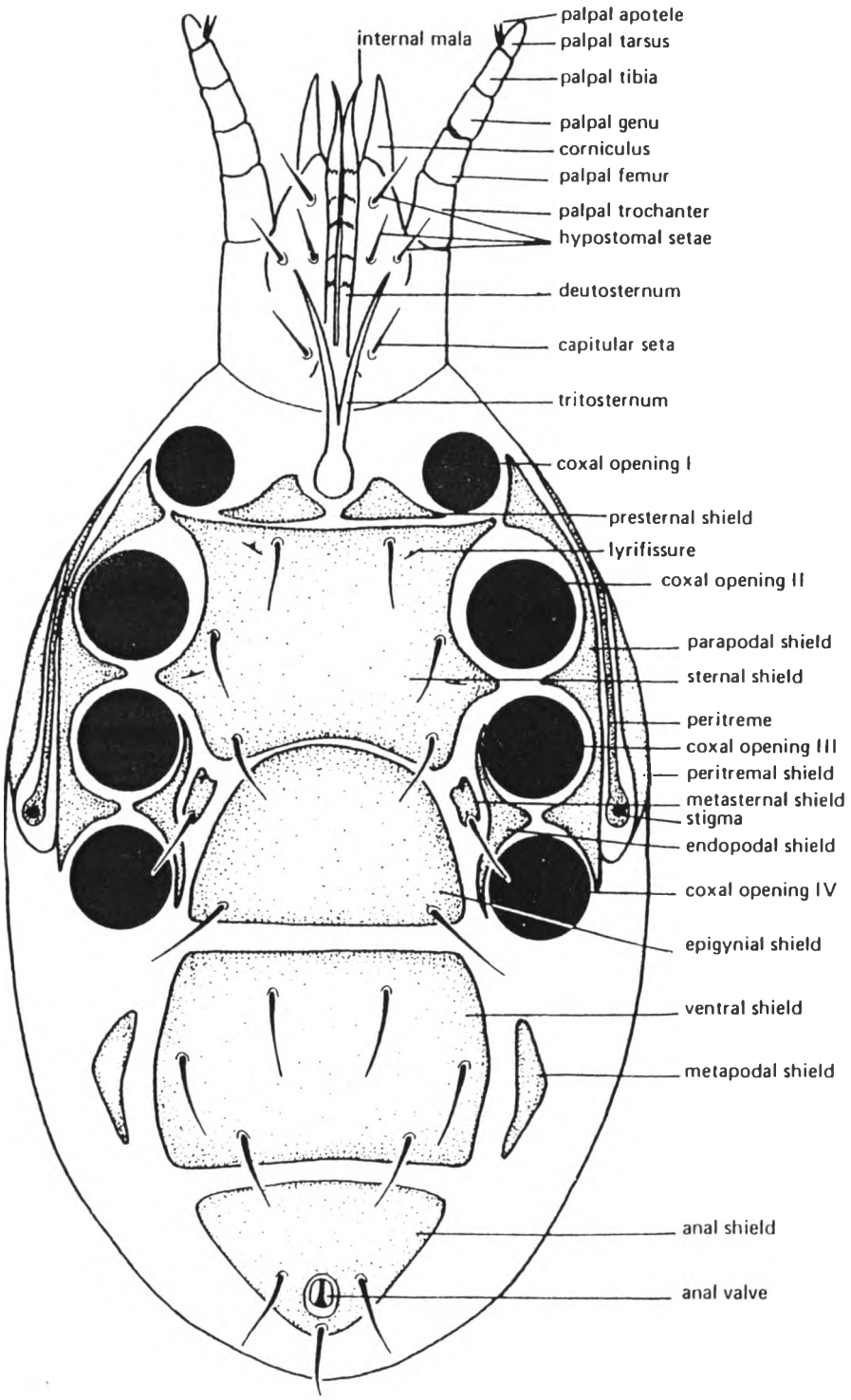

Fig. 1.1 General morphology of gamasid mites (ventral view) after Kantz, 1978

the lateral margin and occasionally reaches to or slightly beyond coxae I. The peritremes are long, more or less straight and associated with peritrematic shields that are useful for species identification. The peritremes of *Tropilaelaps* spp., are long and extended forward to coxae I, and are typical characteristic features of gamasid mites. These features are not considerate adaptations to live in their hosts, such as the giant honey bee *A. dorsata* and *A. laboriosa* (Bruce, Delfinado-Baker and Vincent, 1997).

T. clareae is fairly large (adult female is 976-1083 μm in length and 528-581 μm in width while adult male is 940-1054 μm long and 501-552 μm wide), elongated and light-reddish brown. (Delfinado-Baker and Baker, 1982; De Jong, 1990). Under low magnification of light microscope, an anal plate appears as a horseshoe shape in female which is different from that in male (Delfinado - Baker and Baker, 1982). Basically, *T. koenigerum* is smaller than *T. clareae*. Female adults are about 684-713 μm in length and 433-436 μm in width which is more oval in shape than *T. clareae*. The color of this species are lighter than that of the other. Unlike *T. clareae*, females are bigger than males (about 570 μm in length and 364 μm in width). The ventral plate and a pear-shaped anal plate of *T. koenigerum* are similar in both sexes. The female epigynial plate has declivus sides and a bluntly pointed apex, while the male movable chela-spermatodactyl has a pigtail like loop figured towards the apex (Figure 1.2) (Delfinado-Baker and Baker, 1982).

The data concerning life cycle of *T. clareae* is only available on *A. mellifera* host and are unfortunately incomplete. Although no data for

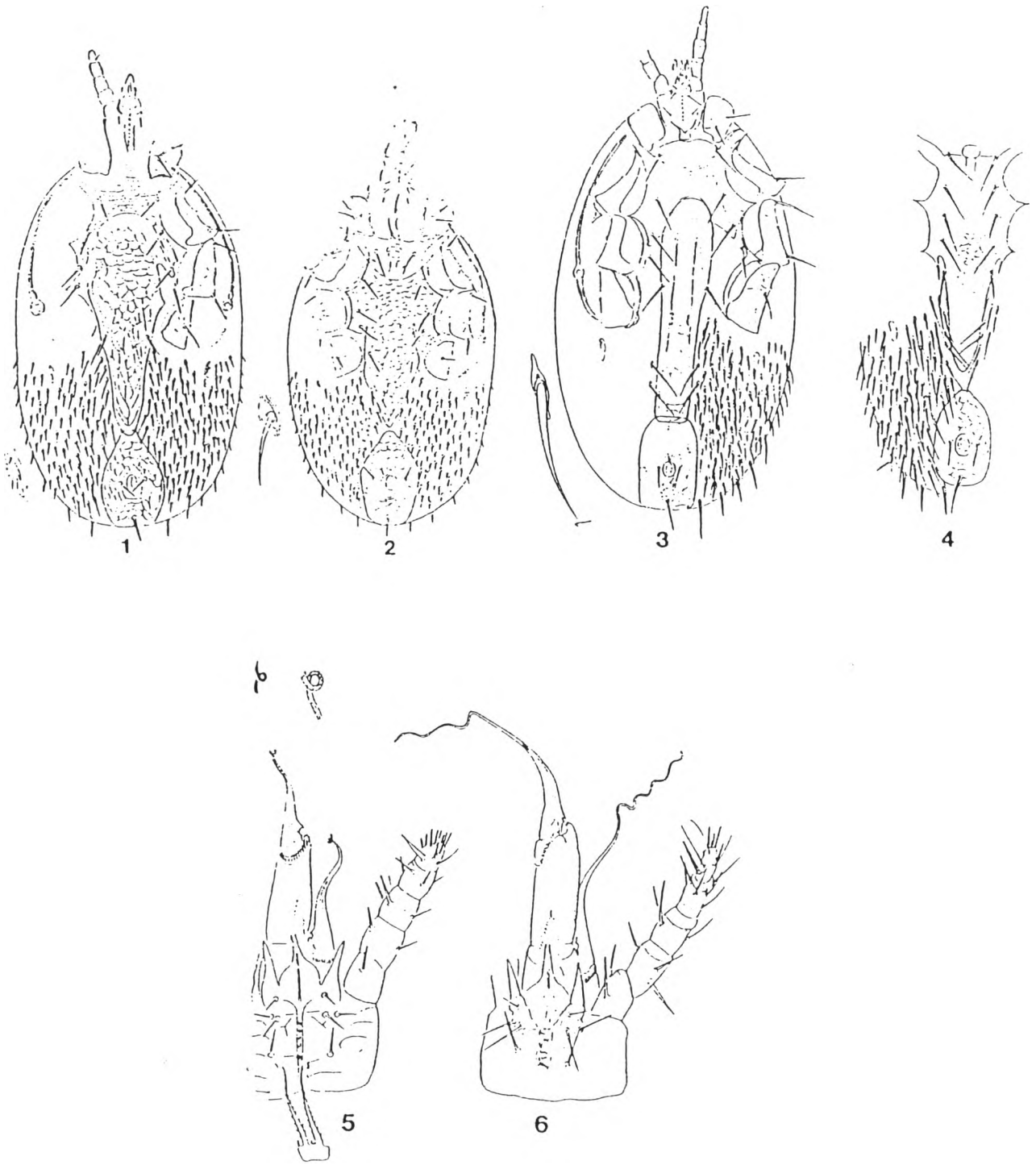

Fig 1.2 *T. koenigerum* :(1) female venter, (2) male venter, (5) male gnathosoma and close-up of movable chela-spermatodactyl ; *T. clareae* : (3) female venter, (4) male venter, (6) male gnathosoma showing movable chela-spermatodactyl (Delfinado-Baker and Baker, 1982).

developmental biology of male *T. clareae* has been reported, the female mites of this species exhibit a typical life cycle of this group of parasites. After hatching, it is developed to larval, protonymph, deutonymph and adult stages. A summary of *T. clareae* life cycle is shown by Fig. 1.3. The female *T. clareae* adults enter the brood cells shortly before capping and start laying eggs at 40-48 hours afterwards. Each female individual give an average of 4 eggs. All of which develop to the adult stage within 6 days (Delfinado-Baker and Peng, 1995). The first offspring is usually female, while the second onwards is male (Rath et al., 1991; Ritter and Ritter, 1988; Wei, 1992 all cited in Delfinado-Baker and Peng, 1995). *T. clareae* mates by podospermy and can be occurred inside or outside the brood cells. Multiple mating is observed in males whereas such a circumstance was not found in female (Rath et al., 1990 cited in Delfinado-Baker and Peng, 1995).

Male and female *T. clareae* leave their natal cells with the newly hatching honey bees, and are dispersed within a short period, usually 2-3 days (Definado-Baker and Peng, 1995); however, there have been no reports on the reproductive period and mortality rate of juvenile based on different sexes. The infection of *T. clareae* to *A. dorsata* and *A. laboriosa* are presumably for dispersion. Nevertheless, both reproduction and dispersion are happened when *A. mellifera* is infected with *T. clareae* resulting in outbreaks of this parasite over vast geographic areas (Wongsiri et al., 1995; Delfinado-Baker, Rath and Boecking, 1992). In *T. koenigerum* there have been no reports on the life cycle of this taxon.

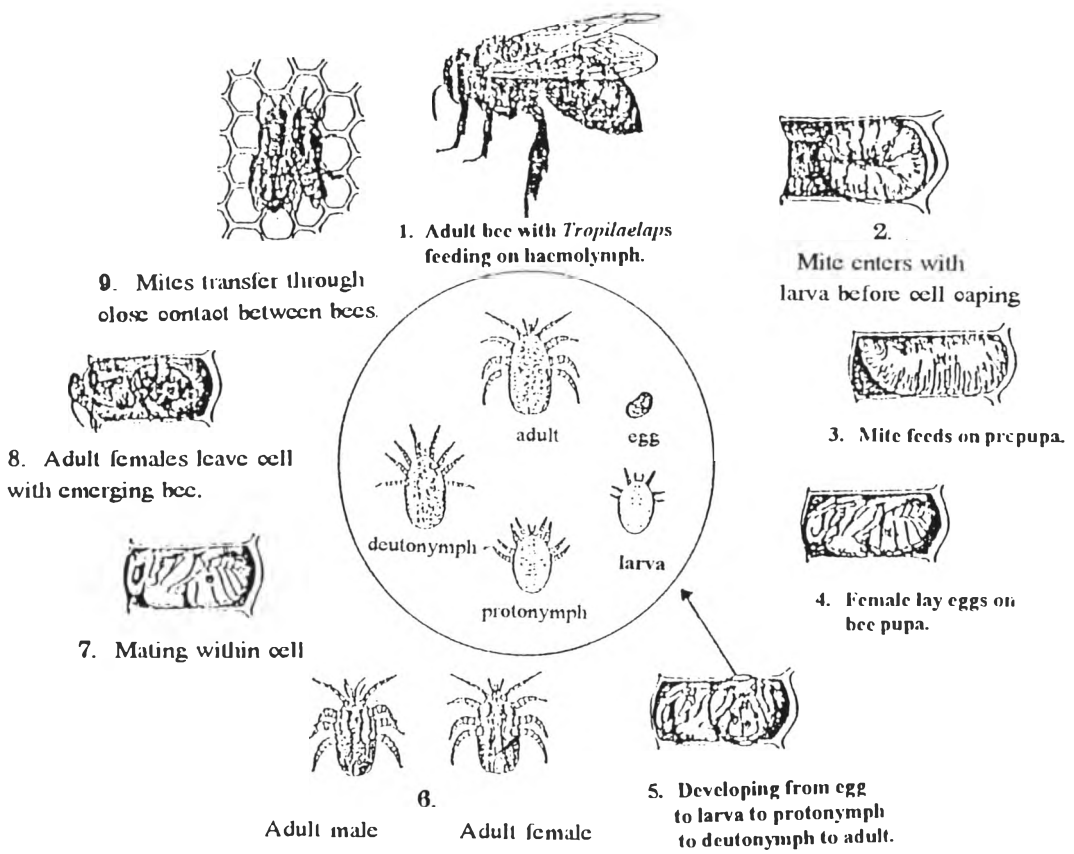

Fig 1.3 Life cycle of *T. clareae* (Wongsiri, 1989).

Distribution of *T. clareae* is associated with that of its natural host, *A. dorsata*. Historically, repeated introductions of *A. mellifera* to Asia have allowed consistently contact between these bee species. In 1961, it was reported that *A. mellifera* in the Philippines were seriously infested by *T. clareae*. Currently, infestion of *T. clareae* to *A. dorsata* is reported from many countries including those in Asia and Europe corresponding with the natural geographic range of the host (Fig. 1.4) (Matheson, 1993).

1.2 Classical methods for genetic variation studies

Classification, variation and evolution of various species have been investigated by several different biological methods such as morphology, life history, behavior and ecology. For example, morphometric studies of *Psoroptes* spp. mites from bighorn sheep, deer, cattle and rabbits utilized 9 morphological characters for unambiguously discrimination (Boyce et al., 1990). However, this method has several limitations. Systematic studies based on morphometric analysis require a large number of samples and experienced scientists to decide whether investigated characters are informative (Rinderer, 1986). More importantly, some morphometric characters are often environmentally influenced therefore populations of a particular species may be misclassified due to ecological variants. Accordingly, the classical method has been increasingly confirmed by molecular techniques, based on protein or DNA polymorphisms (Weising et al., 1995).

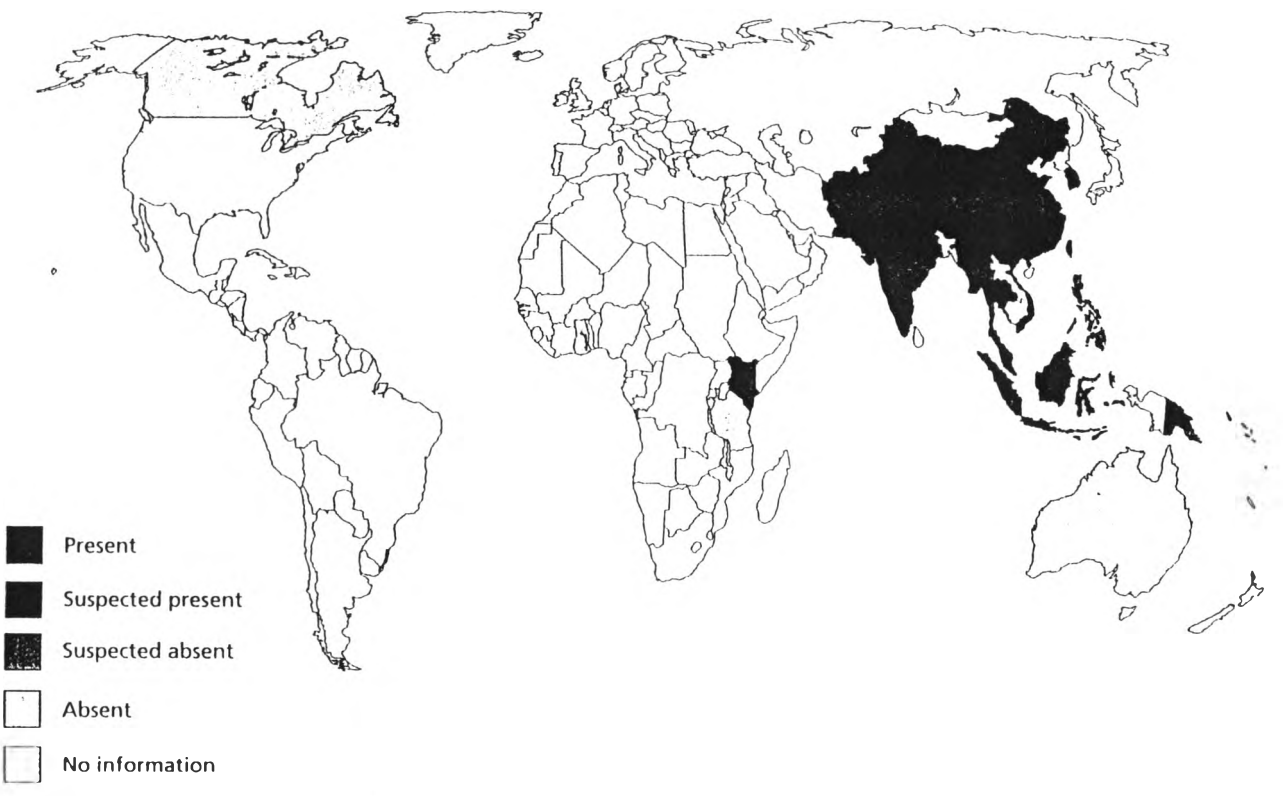

Fig. 1.4 Worldwide distribution of *Tropilaelaps* (Matheson, 1993).

1.3 Molecular techniques for genetic variation studies

1.3.1 Allozyme technique

Molecular markers based on protein polymorphisms generally developed through allozyme analysis. This can be carried out by electrophoretic separation of proteins (usually enzymes) followed by specific staining of the electrophoresed products. Although some studies use seed protein patterns as the markers in plants, the majority of protein markers are represented by allozymes. Allozyme electrophoresis has been successfully applied to many organisms from bacteria to animals and plants since the 1960s (May, 1992). This approach is relatively straightforward and easy to carry out. A tissue extract is prepared and electrophoresed on the supporting media (usually starch or polyacrylamide gels) according to the net charges and sizes. The protein bands can then be visualized by specific histochemical stains of investigated enzymes. Once the electrophoresed gel are stained, the status of homo- or heterozygosity at such a locus can be examined. The number of band is reflected from configuration of the enzyme molecule (mono, di or tetramers coupling with homo or heterozygotic states). The positions of polymorphic bands are genetically informative (Weising et al., 1995).

Analysis of protein polymorphisms in mites have been mainly reported in studies of inter- and intraspecific levels. For example, genetic divergence between the green and red forms of the two-spotted spider mite was examined using malate dehydrogenase (*MDH**) and further analyzed by polyacrylaminde gel electrophoresis. The *MDH** specific allele for each forms of the mites was identified (Goka et al., 1996). Theoretically, the allozyme approach is a

reasonably powerful technique as a large number of individuals can be determined in a limited period of time. However, it has some limitations. For instance, synonymous mutation is not able to be detected. Likewise, nucleotide substitutions changing one non-polar amino acid to another do not alter the electrophoretic mobility of the protein. Lacks of allozyme variability (17 gene loci, from 14 allozyme systems) among populations of *V. jacobsoni* composing of 12 apiaries from European countries and one from China were reported (Biasiolo, 1992). As described previously, allozymes underestimate levels of genetic variation due to its low ability to detect polymorphic loci. Therefore, allozyme analysis may not be an appropriate technique for evaluation of genetic variability in *T. clareae* and *T. koenigerum*.

1.3.2 DNA-based techniques

Analysis of polymorphisms at the DNA level is the direct approach to study genetic variation at both inter- and intraspecific levels. Theoretically, various DNA-based techniques, having different sensitivity of detection are available but the most important factor is to select the most appropriate technique (e.g. reasonable sensitivity, cost-effective, less time consuming) to answer a particular problem.

Nuclear DNA, mitochondrial DNA, and nuclear ribosomal DNA have been commonly employed in genetic variation studies. The following discussion describes some of the attributes of mitochondrial and nuclear ribosomal DNA.

A. Mitochondrial DNA

Genetic polymorphisms using analysis of mitochondrial DNA (mtDNA) have been employed. Since 1979 in most cases, it is more powerful than that of allozymes for determination of population structure, biogeogrophy and phylogenetic relationships (Avise et al., 1987). Due mainly to its small genome size, rapid rate of evolutionary changes and maternal inheritance, mtDNA is also suitable for examining history and evolution among closely related taxa (Gray, 1989; Lansman et al., 1981; Simon et al., 1991 all cited in Hoy, 1994). Studies of mtDNA polymorphisms have been reported using RFLP and/or DNA sequencing (Navajas et al, 1996). The former can be carried out from the entire mtDNA or alternatively, from PCR-amplified mtDNA segments followed by restriction analysis of such amplification band . The latter is suitable for small fragments which can be amplified by DNA cloning or by PCR followed by DNA sequencing (Satta and Tukahara 1996; Pashley and Ke 1992 all cited in Hoy, 1994).

mtDNA has a number of positive properties which is suitable for evolutionary and systematic studies. These are (1) maternal inheritance, (2) general conservation of gene order and composition within the same phylum, (3) a rapid rate of sequence divergence, and (4) small size and abundance resulting in its easy isolation (Hoy, 1994). Analysis of mtDNA in various taxa causes better understanding of this extrachromosomal DNA. It was subsequently found that the universal genetic code system is not valid for some genetic codes. Navajas et al. (1996) investigated genetic variation of phytophagous mites using sequence analysis of cytochrome oxidase subunit I (CO I, 340 bp) in twenty phytophagous

mite species. High genetic variation levels of this A+T rich region were observed which occurred through synonymous transitions. Moreover, it was also found that the genetic codes in phylophagous mites were apparently similar to those in insects.

B. Nuclear ribosomal DNA.

Ribosomes are a major component of cells involving in translation of messenger RNAs into proteins. Ribosomes consist of ribosomal RNA (rRNA) and proteins and can be dissociated into a large and a small subunits. The ribosomal DNA is frequently used to examine interspecifically evolutionary relationships among various taxa because they are universally present in all organisms having the protein synthesizing system. The rDNA is versatile to be used for detection of polymorphisms at different levels because this moderately repettitive region contains both conserved (e.g. 18S and 28S) and more variable regions (e.g. ITS, IGS).

In eukaryotes, the nuclear ribosomal genes encoding the 18S (small subunit) and 28S (large subunit) rRNAs are clustered as arrays of tandem repeats located in the nucleolar organizing regions of the chromosomes (Fig. 1.5). There are approximately 100 to 500 copies of rDNA repeated transcription units found in most animals (Hoy, 1994). The repeated transcription unit is composed of part of the promoter region, external transcribed spacer (ETS), an 18S rDNA coding region, an internal noncoding transcribed spacer (ITS), a 28S rRNA coding region, and an intergenic nontranscribed spacer (IGS).

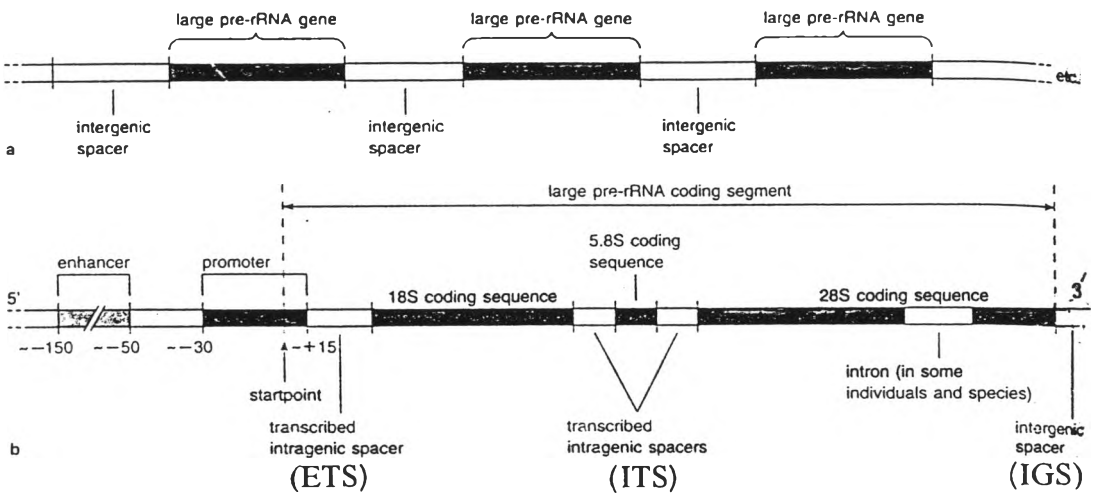

Fig. 1.5 Large pre- rRNA genes and their spacer: (a) The pattern of tandemly repetitive repeats of the large pre-rRNA genes, (b) the arrangement of coding sequences and internal transcribed spacer (Wolfe, 1993).

Different portions of the rDNA repeated unit evolve with significantly different rates (Kuperus and Chapco 1994; Navajas et al. 1995; Sappal et al. 1995). Thus, evolutionary studies may employ different segments of the unit depending on the taxonomic levels of organisms under investigation. In general, a high degree of polymorphism has been found in the noncoding segments of the repeat unit (ETS, ITS, IGS). Thus, population and species diagnostic markers in sibling species have been studied using these segments (McLain et al., 1995; Norris et al., 1996; Porter and Collins 1991).

1.3.2.2 Molecular techniques based on DNA analysis

A. Restriction fragment length polymorphism analysis (RFLP)

Restriction fragment length polymorphisms (RFLP) is one of several techniques used to determine DNA variation based on the assumption that digested DNA fragments illustrating identical length are similar in sequences and are from the same evolutionary origin. Technically, the target DNA digested with restriction endonucleases are size-fractionated by agarose gel electrophoresis and transferred onto a membrane. The investigated fragment(s) is identified by hybridization with the specific radiolabeled probe (Davis, Batteg and Kuehl, 1994). An example of RFLPs in mite is the identification of species-diagnostic markers between three sibling species of genus *Panonychus* (*P. mori*, *P. citri* and *P. ulmi*) and *Tetranychus urticae* through Southern hybridization with rDNA probes (Osakabe and Sakagami, 1994).

B. Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is a new approach applied for population genetic and systematic studies. This technique allows short DNA fragment (usually smaller than 2 kb) to be amplified *in vitro*. To amplify a particular DNA fragment, two single-stranded complementary primers are designed to a specific motif of the DNA template. The activity of a thermostable DNA polymerase in the suitable buffer system and thermo-cycling (denaturation, annealing and polymerization steps) results in exponential amplification of a given DNA fragment between the primer sites (Weising et al., 1995).

The ability to amplify interested DNA (RT-PCR for RNAs) through PCR opens the new approach for various biological disciplines (e.g. systematics, evolution, ecology). For instance, studies of bee mites using RFLP had been formerly limited by the amount of required DNA as typical RFLP needs at least 2 μg of genomic DNA for each analysis. Nevertheless, approximately 10-50 ng of genomic DNA is more than sufficient to be used as the template for the amplification reaction. This makes several molecular biological studies possible in tiny organisms like *T. clareae* and *T. koenigerum*.

C. PCR-RFLPs

This technique has been, and still is, the most common approach used for determination of genetic diversity at various taxonomic levels. After the DNA of interest is amplified by PCR, an aliquot of the amplification reaction is then simply digested with each restriction endonuclease. The resulting DNA fragments are electrophoresed through the appropriate gel medium (agarose or polyacrylamide) and visualized under the UV light after ethidium bromide staining. The most important advantage of this technique is that hybridization of labeled DNA probes to the target restricted DNA is obviated. Furthermore, the technique *per se* is much simpler than conventional RFLP approach.

D. Randomly amplified polymorphic DNA (RAPD) PCR.

RAPD-PCR was developed by Williams et al. (1990) who demonstrated that genomic DNA from a distantly related group of organisms could be amplified using a single short primer (9 or 10 nucleotides long) composed of an arbitrary

oligonucleotide sequence. The primer can be randomly designed without any prior knowledge of the sequence information of organisms under investigation. The only limitation is that the primers should have at least 50 % G+C content and should not contain palindromic sequences. Different random primers use with the same genomic DNA produce different numbers and sizes of DNA fragments (Ellsworth et al., 1993; Kernodle et al., 1993). After amplification, the amplified DNA patterns can be conveniently determined by agarose gel electrophoresis.

In insect, RAPD-PCR has been increasingly used for several applications. These include identification of molecular markers in populations (Tasanakajorn et al., 1995; Rao, Bhat and Toey 1996), determination of paternity in dragonflies (Hardrys et al., 1993), and communally breeding beetles (Scoot et al., 1992; Scoot and Williams, 1993), an analysis of population genetic structure and genetic variation (Richner et al., 1997; Shankaranarayanan et al., 1997 and Swoboda et al., 1997).

Based on various applications described above, RAPD-PCR may be useful for determination of genetic differences in *T. clareae* and *T. koenigerum* at individual, population or species levels because the number of primers used are unlimited and the primers which are suitable for such different taxonomic levels can be chosen. RAPD-PCR is particularly useful for species having limited genetic information or for organisms which have not been genetically investigated before.

de Guzman et al. (1997) examined genetic variation in *V. jacobsoni* from the United States, Russia, Morocco, Germany, Italy, Spain, Portugal, Japan, Brazil and Puerto Rico using RAPD analysis. The results showed that all *V. jacobsoni*

collected from the United States had the same RAPD pattern to that of those originated from Russia, Morocco, Germany, Italy, Spain, and Portugal (called the Russian pattern). Nevertheless, this pattern was different from that found in Japan, Brazil, and Puerto Rico (called the Japanese pattern). The OPP-03 and OPP-07 generated 422 bp and 766 bp fragments which common in samples carrying the Russian pattern but were not observed in those of the Japanese pattern. Two bands located at 675 bp and 412 bp were found in all individuals from the United States and Europe. These results suggested that *V. jacobsoni* of the United State was historically originated from Russia through Europe, while that of Brazil and Puerto Rico are probably originated from Japan. The two different patterns were widespread over vast geographic areas by introduction of the host species.

Edwards et al. (1997) employed RAPD-PCR to discriminate three *Typlodromalus* spp. (*T. limonicus*, *T. manihoti* and *T. tenuiscutus*). Five of eight RAPD-PCR primers could be used to distinguish these three species unambiguously. The genetic distances within-species (0.072-0.186) were much lower than that of between-species (0.407-0.656) illustrating the effective ability to identify cryptic mite species using this approach.

E. DNA Sequencing

The most direct method for determination of polymorphisms at the DNA level is sequencing of the interested orthologous DNA fragment of related organisms. DNA sequencing provides highly informative data and can be used for different discriminatory power by choosing appropriate regions of the genome.

Theoretically, DNA sequencing can be carried out using either chemical or enzymatic methods. The enzymatic method is currently more popular and is based on the ability of DNA polymerase to extend the primer until a chain-terminating nucleotide (each of ddNTPs) is incorporated to the newly synthesizing chain. The sequencing reaction is performed as a set of four separate reactions, each of which contains all four deoxyribonucleoside triphosphates (dNTPs) supplemented with a limiting amount of each dideoxyribonucleoside triphosphate (ddNTP) per reaction. Because ddNTPs lack the 3′ OH group necessary for chain elongation, the growing oligonucleotide is selectively terminated at G, A, T or C, depending on the respective dideoxy analog in the reaction. The resulting fragments are separated according to size by high resolution denaturing polyacrylamide gel electrophoresis (Sanger et al., 1977).

More recently, the sequencing approach has been greatly facilitated by PCR. The investigated DNA segment was amplified using a pair of primers. The PCR product can then be sequence directly or alternatively after cloning (Hoelzel and Green, 1992). In various taxa, the ITS of nuclear rDNA sequence are useful for evolutionary and systematics studies (Tang et al., 1996; Paskewitz, Wesson and Collins 1993; Vogler and DeSalle 1994; Kollipara et al., 1997). The rDNA primers are available in several organisms and seem to be universal for example a pair of primers originally developed from the fungal ITS also worked well in insects (White et al., 1990).

A phylogenetic relationship among members of *Ixodes* sepecies in the family Acari was recently reported based on sequence divergence of the ITS

region of rDNA (Wesson et al., 1993). Morphologically, *Ixodes pucificus* and *I. dammine* are taxonomically complicated and are not able to unambiguously distinguish. As a result, direct sequencing of PCR-amplified ITS segment was carried out in three *Ixodes* species including *I. pucificus* (from California and Arizona), *I. scapularis* (from Georgia and North Carolina), and *I. dammini* (from Maryland, Massachusetts, New Jersey, New York and Wisconsin). Variation of nucleotide sequences was observed in these taxa at intra-individuals within a population, inter-individuals from different geographic origin of a species and interspecific levels. DNA polymorphisms resulted from small deletions and insertions were typical in the ITS region. Homogenization of rDNA multigene arrays for sequence variants by concerted evolution seemed to occur at a relatively rapid rate. It was also found that numerous polymorphic sites found in *I. pacificus* and *I. dammine*, facilitating a possible use of these sequences to assess relationship among sibling species.

Based on the maximum parsimony and two distance methods (unweighted pair-group with arithmetic averages and neighbor-joining), sequence variation in ITS1 and ITS2 suggested that *I. scapularis* and *I. dammini* are closely related species reflected from high similarly in ITS sequences of individuals from geographically isolated locations. Evidently, individuals from geographically separated samples of *I. pacificus* were intraspecifically less related and clearly different genetically to those of *I. scapularis/dammini*. Likewise, McLain et al. (1995) reported population genetic structure of *I. scapularis* from eastern seaboard of the United States by determination of the ITS sequence. Twenty sequences were obtained from individuals originating from different localities; 10

of which were from the southeast (Georgia and Florida), seven were from the middle east (North Carolina and Maryland) and the rest were from the northeast (Massachusetts, New Jersey and New York). Phylogeny based on neighbor-joining and the maximum parsimony methods allocated most of the Southeastern and the middle Eastern individual together but fail to cluster those from the northeast.

Fenton at al. (1997) studied genetic variation in eriophyid mites by analysis of DNA sequences of PCR-amplified ITS. A total of 7 species composing of *Cecidophyopsis ribis*, *C. grossulariae*, *C. spicata*, *C. alpina*, *C. aurea*, *C. grossulariea* and *Phylocoptes gracillis* were investigated. It was found that 92-99 % of ITS1 sequences from different *Cecidophyopsis* spp. was similar. Inter-specific differences between *Cecidophyopsis* were found in seventeen simple sequence repeats (SSRs), fourteen point mutations and two deletions. No intra-specific variation in SSRs was observed.

1.4 Aims of this thesis

Prior to the present research, there have been no reports on genetic variation studies concerning two species of *Tropilaelaps* (*T. clareae* and *T. koenigerum*). Thus, the objectives of this thesis are to discriminate these two species by sequencing the ITS_1- ITS_2 region and to determine intraspecific genetic variation of *T. clareae* from different geographic locations within a species and between different hosts using RAPD approach.