CHAPTER 2

Literature review

2.1 Honey bees

The family Apidae houses the nine recognized species of true honey bees in the genus *Apis*, and is within the order Hymenoptera. All honey bee species are classified as highly eusocial insects, which live together in highly organized colonies, frequently known as "super-organisms" (Seeley, 1989; Fuchs and Moritz, 1998). Honey bee colonies are comprised of two castes: queens, workers and two sexes, males and females (Figure 2.1). Each caste and sex has its own morphological characteristics.

A honey bee queen possesses a large and long tapered abdomen. Her primary functions are to lay eggs and to secrete pheromones that help maintain colony cohesion. The queen's average life span ranges from one to four years. The majority of the hive's population is comprised of worker bees (sterile females), with 15,000 to 60,000 workers per colony. They have the smallest body size. Depending upon their age or colony demand, worker bees carry out a variety of duties. Their responsibilities include: rearing brood, receiving nectar, nest hygiene, secreting wax, building comb, colony defense from predators, and foraging. The average worker life is span is about 30 days. Drones are males. They are recognized by their larger eyes and shorter tongues than workers (Dade, 1977). They arise from unfertilized eggs. Drones function for just one duty, mating (Winston, 1987).

Figure 2.1 The two castes and two sexes of adult honey bees (*Apis mellifera*). Queen *ca*. 1.8-2.0 cm. in length (top left), drone *ca*. 1.4-1.5 cm. in length (top right) and worker *ca*. 1.2-1.3 cm. in length (below the queen and drone).
Photos by N. Buawangpong.

2.2. Life cycle of the honey bees

Honey bees undergo a holometabolous development or complete metamorphosis; egg, larva, and pupa emerging as adult bees (Figure 2.2). Queens and workers develop from fertilized eggs. However drones develop from unfertilized eggs (Henderson, 1991). The egg stage lasts three days. The wormlike grub is the larva, the active feeding stage which last takes ca. 6 days for worker bee larvae. Then, larva becomes a pupa, during which time develops antennae, head, thorax, legs and abdomen. This last developmental stage lasts approximately 12 days after which the adult worker bee emerges. The total developmental time for *A. mellifera* is 16, 21 and 24 days for queen, worker and drone, respectively. Condition, nutrition, and honey bee lineage have an effect on the emerged adult bee development time (Winston, 1987).

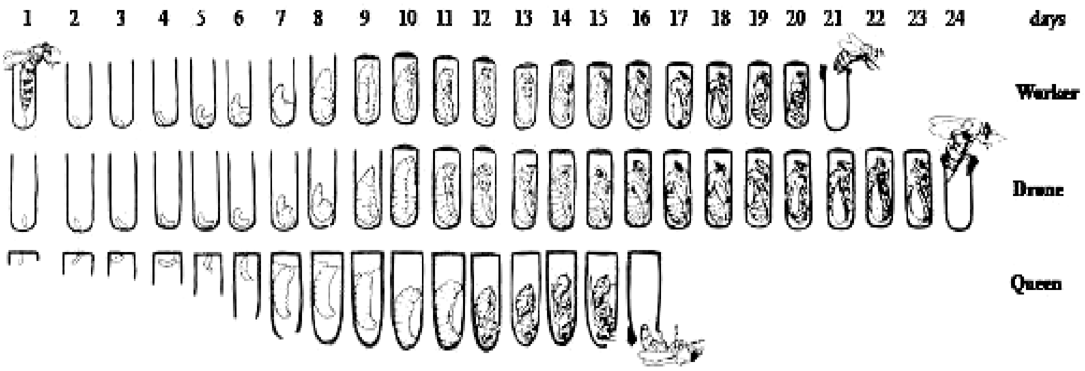

Figure 2.2 Average developmental periods and stages for three honey bee castes: worker, drone, and queen. (Winston, 1987)

2.3 Honey bee species in Thailand

Thailand is home to five honey bee species: the black dwarf honey bee (*A. andreniformis*, Figure 2.3), the red dwarf honey bee (*A. florea*, Figure 2.3), the giant honey bee (*A. dorsata*, Figure 2.4), eastern honey bee (*A. cerana*, Figure 2.5), and the European honey bee or western honey bee, *A. mellifera*, which an introduced species to Southeast Asia (Figure 2.6).

A. mellifera was introduced into Southeast Asia in the late 20th century for commercial purposes (Crane, 1988). Upon entry to Southeast Asia, the indigenous brood mite parasites *Varroa* and *Tropilaelaps* 'discovered' *A. mellifera* as a competent alternate, non-adapted host.

Figure 2.3 The differentiation between *A. florea* and *A. andreniformis*. (A) *A. florea*, single exposed comb on a tree branch. (B) *A. andreniformis,* single exposed comb on a tree branch. (C) The mature *A. florea* workers have reddish tinge on the first abdominal tergite. (D) The mature *A. andreniformis* workers have a black color on the first abdominal tergite. Photos by N. Buawangpong.

2.3.1 Thai commercial bee species

The managed honey bees of Thailand consist of two species of cavity nesting bees that are cultured and kept in man-made hives *viz*. the European or western honey bee (*A. mellifera*) and the cavity nesting eastern honey bee (*A. cerana*) (Winston, 1987).

2.3.2 Honey bee species associated with *Varroa* and *Tropilaelaps* mites

While *Varroa* has become the prominent acarine parasite of *A. mellifera* with nearly a world-wide distribution, the indigenous hosts for *Varroa* are the eastern honey bee *Apis cerana*. The first description of *Tropilaelaps* was from a colony of *A. mellifera* in the Philippines (Delfinado and Baker, 1961) but it was later realized that the giant honey bee, *A. dorsata*, is the natural host of *Tropilaelaps* (Laigo and Morse, 1968).

Previous studies have reported *Tropilaelaps* infesting *A. cerana*, and the dwarf honeybee, *A. florea* (Delfinado-Baker, 1982; Kapil and Aggarwal, 1987; Aggarwal, 1988; Delfinado-Baker et al., 1989). In these cases, the mite's presence is considered incidental as *Tropilaelaps* is known only to reproduce on competent hosts, e.g., *A. dorsata* and *A. laboriosa* (Woyke et al., 2004). These reports are recognized as incidental infestations and only once has *Tropilaelaps* been found to be reproductive on a bee host other than *A. dorsata* or *A. mellifera* (Anderson and Morgan, 2007). This latter report found just one female *Tropilaelaps* offspring in *A. cerana* brood in Thailand.

Table 2.1 enumerates the four species of *Tropilaelaps* and *Varroa* with their indigenous honey bee host species.

Table 2.1 Mesostigmatid mites (Family *Laelapidae* and *Varroidae*) parasitizing honey bee (*Apis*) species.

Mite species	**Adapted bee host species**
Family *Laelapidae*	
Genus *Tropilaelaps*, Delfinado and Baker (1961)	
T. clareae, Delfinado and Baker (1961)	*A. dorsata*
T. koenigerum, Delfinado and Baker (1982)	*A. dorsata,* and *A. laboriosa*
T. mercedesae, Anderson and Morgan (2007)	*A. dorsata,* and *A. laboriosa*
T. thaii, Anderson and Morgan (2007)	*A. laboriosa*
Family *Varroidae*	
Genus *Varroa*, Oudemans (1904)	
V. jacobsoni, Oudemans (1904)	*A. cerana*
V. underwoodi, Delfinado-Baker and Aggarwal (1987)	*A. cerana*
V. rindereri, de Guzman and Delfinado-Baker (1996)	*A. koschevnikovi*
V. destructor, Anderson and Trueman (2000)	*A. cerana,* and *A. nigrocincta*

2.3.3 Giant Asian honey bees

The giant Asian honey bees, *A. laboriosa* and *A. dorsata*, are known to be the largest honey bees in terms of both the nest combs and body size (Ruttner, 1988). *A. laboriosa* is found at higher altitudes frequently nesting on cliffs especially the Himalayas. *A. dorsata* is found throughout South and Southeast Asia. A mature giant honey bee colony will possess 50,000 worker bees (Morse and Laigo, 1969). This species is very aggressive and ferocious. They build a single comb nest, which is usually located on tall tree branches, under cliff overhangs or buildings (Figure 2.4).

Figure 2.4 Colonies of *A. dorsata* in Thailand. (A) Multiple colonies on image of Kruba Sri Wichai, Lamphun Province. (B) Colony aggregation, Chiang Mai Province. Photos by N. Buawangpong.

2.3.4. Cavity nesting Asian honey bees

The cavity nesting Asian honey bees are medium-sized bees. Four species have been described: *A. cerana*, *A. koschevnikovi*, *A. nuluensis* and *A. nigrocincta*. Oldroyd and Wongsiri (2006) reported that Thailand has two species of eastern honey bees: *A. cerana* and *A. koschevnikovi*. *A. cerana* is found throughout Thailand while *A. koschevnikovi* is restricted to southern Thailand. *A. cerana,* like her three sibling species, builds multiple combs in dark cavities such as tree hollows or man-made structure similar to *A. mellifera* (Figure 2.5). *A. cerana* is a domesticated honey bee species that can be managed. *A. cerana* is known for its aggressive behavior and has evolved behavioral mechanisms (auto- and allo-grooming and brood nest hygiene) against their acarine brood parasites.

Figure 2.5 Colonies of *A. cerana* in traditional log hives, Chiang Mai province, Thailand. Photo by N. Buawangpong.

2.3.5 European or Western honey bee

A. mellifera or the European or western honey bee, is the most commonly managed honey bee species in the world. The European honey bee was first introduced to North America starting in the early 16th century. Introductions to the remainder of the western Hemisphere quickly followed. *A. mellifera* beekeeping now prospers in North and South America, New Zealand, Australia, Japan, China, and the Pacific Islands (Morse, 1990). Larger scale introductions of *A. mellifera* in Thailand commenced in the 1980s. Presently there are ca. 300,000 *A. mellifera* colonies in Thailand which are located primarily in northern Thailand (Wongsiri et al., 2000). *A. mellifera*, likes the species of eastern honey bees, builds multiple, parallel combs in natural cavities as well as man-made hive structures (Figure 2.6). When compared to the eastern honey bee species in tropical and sub-tropical zones, *A. mellifera* is considered a gentle and a good honey producer.

Figure 2.6 Standard Langstroth hives of *A. mellifera* located, Chiang Mai, Thailand. Photo by N. Buawangpong.

2.4 Biology of *Varroa* sp.

Varroa is presently considered as the most serious honey bee parasite in a worldwide arena. The four species in the genus *Varroa* (Family Varroidae) are all indigenous to Asia. The four recognized species are: *V. jacobsoni* Oudemans, *V. destructor* Anderson and Trueman, *V. underwoodi* Delfinado-Baker and Aggarwal, and *V. rindereri* de Guzman and Delfinado-Baker. The first description of *Varroa* was in 1904 from specimens from the Indonesian state of Java. Named *V. jacobsoni* Oudemans, it was described as an acarine parasite of the eastern honey bee *A. cerana*.

For nearly 100 years it was thought that *V. jacobsoni* was the singular species in the genus. In the early 21st century however, further research revealed the genus *Varroa* possessed more than a single species. Anderson and Trueman (2000) reported findings based on genetic variation, that *V. jacobsoni* is indeed a complex of at least two species, *V. jacobsoni sensu stricto* and *V. destructor*.

V. destructor is now the most common and geographically widespread of all *Varroa* species. This species, *via* anthropogenic introductions, is now worldwide and the most devastating pest of *A. mellifera* (Moritz and Haenel, 1984; Camazine, 1986; Delfinado-Baker, 1988; Delfinado-Baker and Houck, 1989; Ritter et al., 1990; Moretto et al., 1991; Anderson, 1994; Eugaras et al., 1995; Kraus and Hunt, 1995; Anderson and Sukarsih, 1996; De Jong and Soares, 1997; de Guzman et al., 1997, 1998; Anderson and Fuchs, 1998).

2.4.1 Morphology of *Varroa* spp.

Oudemans (1904) first described the characteristics of mites under the genus *Varroa*. The fixed cheliceral digit of is absent and there are only 2 pairs of hypostomatic setae. Peritremes of the female are short, looped medially or apically and confined to the level of coxae III or coxae III-IV while the peritremes of the male are very short or vestigial. *Varroa* mites are easily seen by naked eyes (Krantz et al., 2009). *Varroa* adults are darkish-red to bright red in color, with a broadly elliptical and flattened dorso-ventral body. Males are more circular in body shape than the female. The *Varroa* nymphal stages (protonymph and deutonymph) are white or cream in colour (Figure 2.7). *Varroa* is known to feed on both immature stadia as well as adult bees (Figure 2.8). When parasitizing adult bee hosts they are capable of attaching themselves beneath the abdominal sclerites as well as the propodeum where they can resist the host’s auto-grooming defenses (Yoder et al., 1999). Because *A. cerana* is the indigenous host of *Varroa*, this bee species has evolved effective defenses against the parasite which incorporate both auto- and allo-grooming behaviors (Peng et al., 1987; Büchler et al., 1992; Fries et al., 1996; Boot et al., 1997; Rath, 1999).

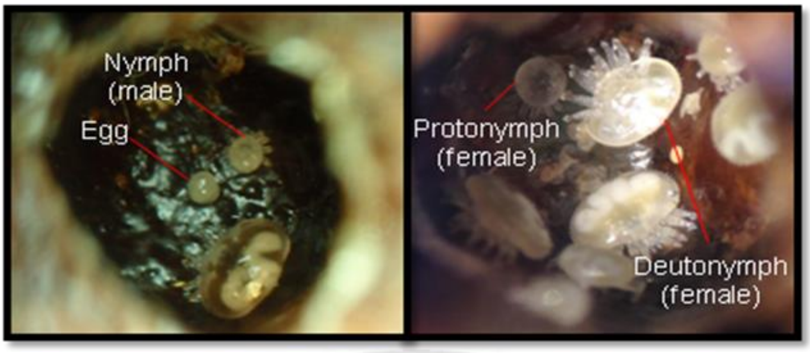

Figure 2.7 Egg and nymphal stages of *V. destructor*.
Photos by N. Buawangpong.

Figure 2.8 Adult *V. destructor* on *A. mellifera* worker adult (left)
and larva (right). Photos by N. Buawangpong.

The two species, *V. destructor* and *V. jacobsoni*, are morphologically similar, except in body size and shape. *V. jacobsoni* is more circular in shape and for most geographical locations smaller than *V. destructor* (Figure 2.9). However, *V. jacobsoni* collected on *A. cerana* in Laos are larger than *V. jacobsoni* from other locations (Anderson and Trueman, 2000).

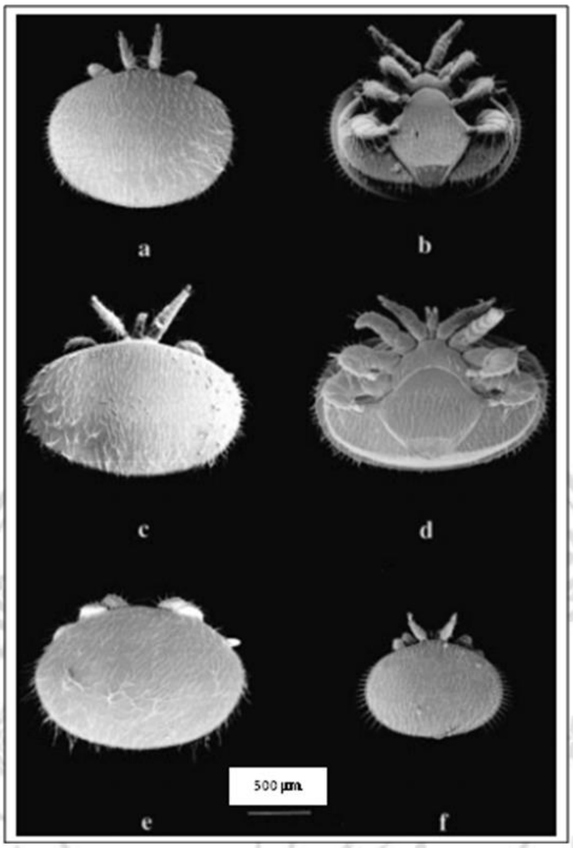

Figure 2.9 Dorsal and ventral aspects of adult females:
(a, b) *V. jacobsoni* (c, d) *V. destructor* (e) *V. rindereri*
(f) *V. underwoodi* (Anderson and Trueman, 2000)

2.4.2 Life cycle of *Varroa* spp.

The two phases of *Varroa*'s life cycle consist of a phoretic (non-reproductive phase, the time spent on adult bees) and the parasitic phase (reproductive phase) (Oldroyd and Wongsiri, 2006). For the phoretic phase, the mites spend time feeding on adult bees. In the parasitic phase, 1 or 2 days before the host brood cell is capped, a gravid female enters the brood cell of a 5th instar larva and then embeds herself in the brood food beneath the host. After consuming the residual brood food the female mite commences to ingest host hemolymph. The mite lays her first egg 60 h after cell capping. Around 1-6 eggs are laid per host (Figure 2.10). The first egg develops to be a male. The remaining 3 to 4 mite eggs are female and they are laid at approximately 24 h. intervals (Dietemann et al., 2013).

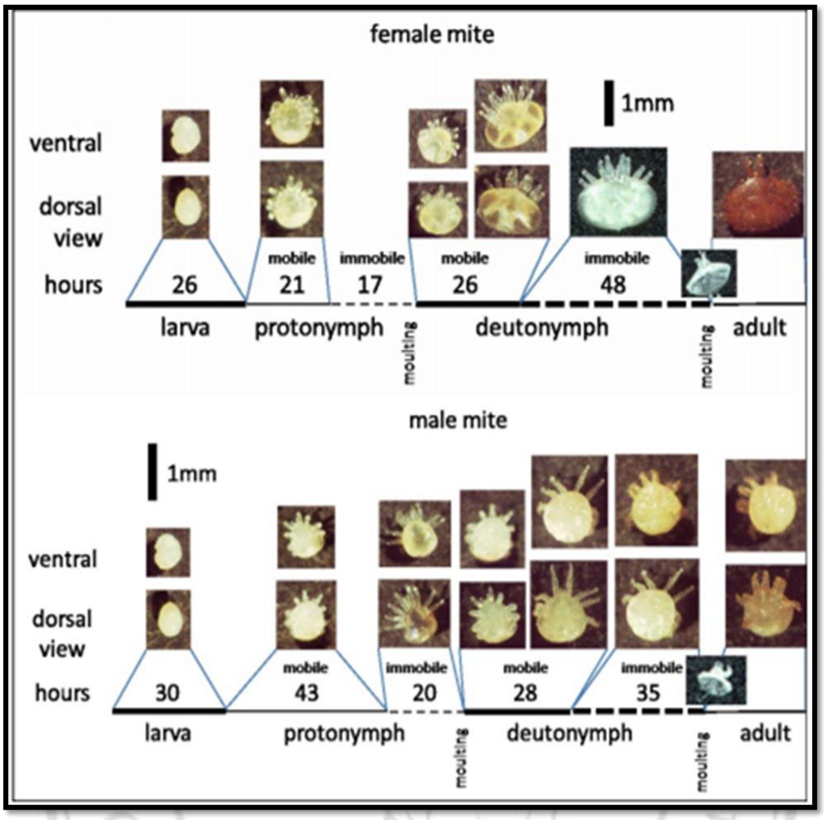

Figure 2.10 Approximate developmental time of *V. destructor* on *A. mellifera* (Dietemann et al., 2013)

2.4.3 Non-reproductive *Varroa*

Not all *Varroa* female mites that parasitize honey bee brood become reproductive. The causes for non-reproductive mites are several (Harbo and Harris, 2001; de Guzman et al., 2007; 2008, Kirrane et al., 2011). Gravid *Varroa* that produces an adult male and daughter or viable offspring are considered fully reproductive. Harbo and Harris (2001) considered *Varroa* non-reproductive when the mites enter a host cell but: (a) produces no progeny, (b) produces only males, (c) produces progeny that are late to mature, or (d) the mother mite dies before producing egg.

2.4.4 Identification of *Varroa* spp.

Four species have been recorded (Table 2.2): *V. jacobsoni, V. destructor, V. underwoodi* and *V. rinderi*. These initial identifications were based largely on morphometrics, *e.g.*, size and shape of peritremes and body size ratio (Figure 2.9)

(Anderson and Trueman, 2000; Oldroyd and Wongsiri, 2006). Recent DNA analysis has confirmed the validity of *Varroa* classification (Anderson and Trueman, 2000; Warrit et al., 2004; 2006).

2.5 Biology of *Tropilaelaps* spp.

Tropilaelaps mites are haemophagic therefore they feed on the haemolymph of capped brood; late instar larva, pre-pupa and pupa of their honey bee hosts (Figure 2.11). They are in the family *Laelapidae*. The indigenous hosts of *Tropilaelaps* are the two giant Asian honey bee species, *A. dorsata* and *A. laboriosa* (Laigo and Morse, 1968). *Tropilaelaps* was first described in 1961 as *T. clareae* Delfinado and Baker. The initial discovery was from an *A. mellifera* colony in the Philippines (Delfinado and Baker, 1961). It was later realized that the natural host for *T. clareae* was the giant honey bee *A. dorsata* (Laigo and Morse, 1968). Since its initial discovery in the Philippines, it has been shown to naturally occur in all regions where giant honey bee species are known to be endemic. A singular exception was the anthropogenic introduction of *Tropilaelaps* into South Korea (Woo and Lee, 1993) a region where giant honey bees are not native.

In 2007, Anderson and Morgan reported that mites in the genus *Tropilaelaps* are comprised of at least four distinct species; *T. mercedesae*, *T. clareae*, *T. koenigerum* and *T. thaii*.

Figure 2.11 Adult *T. mercedesae* infesting an *A. mellifera* drone larva. Photo by N. Buawangpong.

2.5.1 The current distribution of *Tropilaelaps*

In 1985, Woyke claimed that *Tropilaelaps* would be biogeographically limited to the tropical distribution of the two giant honey bee species primarily due to the parasite's requirement for the continual presence of host brood, *e.g.*, tropical regions. Unfortunately such has not proven to be the case as was shown by the introduction of *Tropilaelaps* into South Korea in 1993 (Woo and Lee, 1993). This was obviously an anthropogenic introduction as the Korean peninsula possesses no giant honey bee species and is temperate in climate. The presence of *Tropilaelaps* in Kenya is also an obvious anthropogenic introduction (Ellis and Munn, 2005).

Thirty years ago, Burgett and Akratanakul (1985) hypothesized *Tropilaelaps* could become a major problem for commercial beekeeping on a world-wide basis. Presently, *Tropilaelaps* is known in Kenya, Afghanistan, Bhutan, India, Nepal, Pakistan, China, Hong Kong, Indonesia, South Korea, Malaysia, Myanmar, the Philippines, Taiwan, Thailand, Vietnam, Papua New Guinea (Matheson, 1993), and Bangladesh (Mangum, 2003).

After the introduction of *A. mellifera* into Southeast Asia, *Tropilaelaps* soon infested this non-adapted honey host species, which quickly caused serious losses (Burgett et al., 1983).

2.5.2 Morphology of *Tropilaelaps* spp.

Adult *Tropilaelaps* posses a light reddish-brown body color, and an elongated body. The adult mites display fast movement, and usually hold their first pair of legs upright. Its body is covered with numerous, short, spine-like setae. *Tropilaelaps* is smaller than *Varroa* but still can be seen without magnification (Figure 2.12). Because the gross morphology differs little between males and females, the anal plates must be observed to distinguish gender. Female mites have horseshoe-shaped while males have pear-shaped (Delfinado-Baker and Baker, 1982).

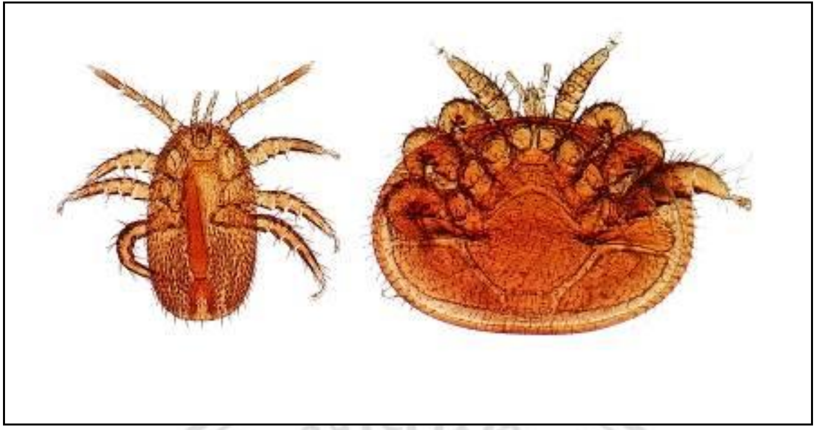

Figure 2.12 Adult female *Tropilaelaps mercedesae* (left) and adult female *Varroa destructor* (right). Photo by N. Buawangpong

2.5.3 Life cycle of *Tropilaelaps* spp.

The life cycle of *Tropilaelaps* as a brood parasite of *A. mellifera* is similar to that of *Varroa*, although there are slight differences. A mated female mite enters an *A. mellifera* brood cell prior to capping (Kapil and Aggarwal 1987, 1989). The gravid female mite feeds on larval haemolymph before she lays her first egg shortly after the brood cell is sealed (Ritter and Schneider-Ritter, 1988). Typically, a single foundress can lay a total of four eggs within a brood cell (Woyke, 1987a). A six-legged larvae ecloses from the egg and develops through the nymphal stages (protonymph and deutonymph) before becoming adult (Figure 2.13). Previously Rath et al. (1991) and Ritter and Schneider-Ritter (1988) found that the first eggs of *Tropilaelaps* are usually females, the eggs after are males. The development from egg to the adult stage takes approximately 6 days (Woyke, 1987b).

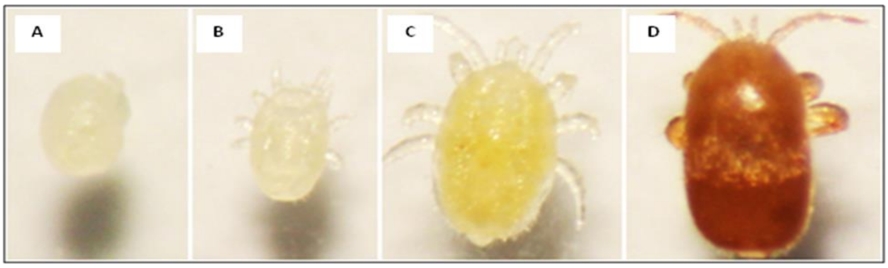

Figure 2.13 Developmental stages (dorsal view) of *Tropilaelaps mercedesae.* (A) *T. mercedesae* larva, (B) protonymph, (C) deutonymph, (D) mature adult. Photos by K. Khongphinitbunjong.

Tropilaelaps, like *Varroa*, exhibits both a phoretic phase on adult bee hosts and a parasitic phase feeding on immature bee stadia. In the phoretic stage, adult *Tropilaelaps* spend fewer days on adult bees. According to previous research, *Tropilaelaps* mouthparts prevent the mites from feeding on adult bees, and they can survive no more than three days without feeding (Koeniger and Muzaffar, 1988; Rinderer et al., 1994; Woyke, 1987a). The shorter phoretic phase of *Tropilaelaps* (in contrast to *Varroa*) assures that *Tropilaelaps* populations can increase faster than *Varroa* (Woyke, 1994).

2.5.4 Non-reproductive *Tropilaelaps.*

Foundress mites that do not produce viable progeny are considered as non-reproductive (NR) (de Guzman et al., 2007) or when a foundress mite fails to produce any progeny (Harbo and Harris, 1999). Khongphinitbunjong et al. (2013) considered *Tropilaelaps* foundresses NR by similar criteria to those used for non-reproductive *Varroa*. The *Tropilaelaps* foundress was determine NR when: (a) a foundress produced no progeny, (b) a foundress with a young daughter but without adult son, (c) a foundress with an adult son without any young daughter, or (d) a foundress with progeny too young to mature before the host bee emerged. Non-reproductive mites fail to contribute to overall mite population growth (Harbo and Harris, 2005). In *A. dorsata*, the adapted host, a NR rate of 65% was reported by Kavinseksan (2004) while a lower NR has been recorded in *A. mellifera* colonies: 18.3% in Vietnam and 7.3% in Afghanistan (Woyke, 1990) and 27% in Thailand (Ritter and Schneider-Ritter, 1988). The specific criteria for NR used in these studies are unknown.

2.5.5 Identification of *Tropilaelaps* spp.

Both morphometric and genetic finger printing can be used to differentiate *Tropilaelaps* species. Anderson and Morgan (2007) expanded the number of *Tropilaelaps* species using both morphological and molecular identification. Table 2.1 also provides a key for species identification for *Tropilaelaps* females which include body size, structure of chelicerae (Figure 2.14), and ventral plate shape (Figure 2.15). For males, the configuration of the apex of male chela spermatodactyl is a distinguishing character. DNA sequencing, RFLPs profiles of the mtDNA COI and nuclear ITS1-5.8S-ITS2 gene sequences (Anderson and Morgan, 2007) are proper markers for differentiating the mite species.

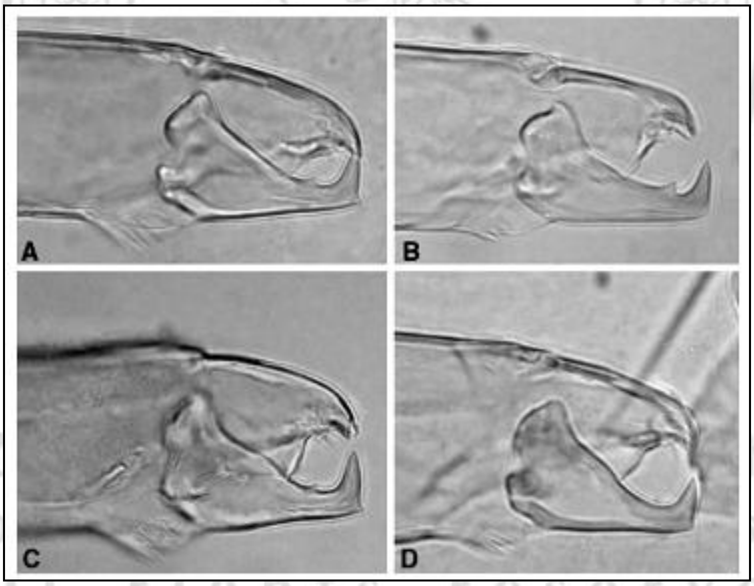

Figure 2.14 *Tropilaelaps* adult female chelicerae (Anderson and Morgan, 2007), under light microscopy 800X. (A) *T. clareae* (B) *T. koenigerum* (C) *T. mercedesae* (D) *T. thaii*

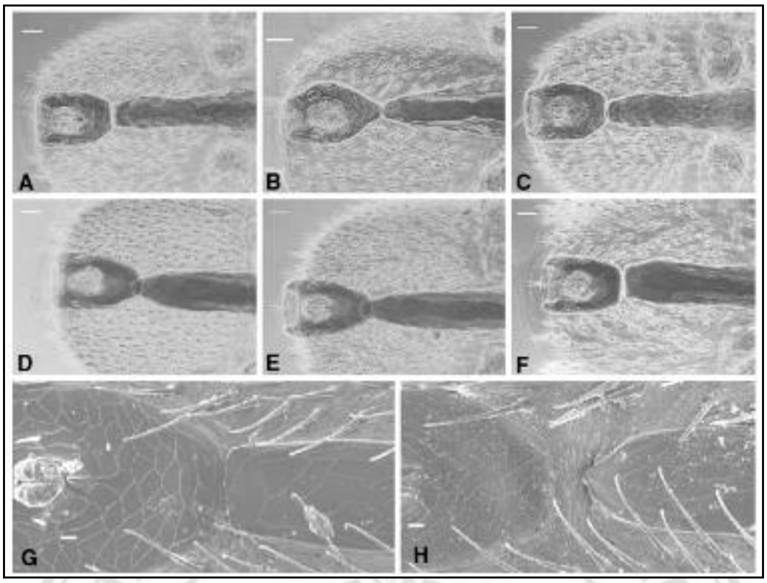

Figure 2.15 The ventral plates of *Tropilaelaps* adult females (Anderson and Morgan, 2007). (A) Bluntly pointed apex of epigynial plate (*T. clareae*). (B) Pear-shaped anal plate (*T. koenigerum*). (C) Slightly bell-shaped anal plate (*T. thaii*). (D-F) Variation in the shape of apex of the epigynial plate, from pointed to blunt, of the anal and epigynial plate (*T. mercedesae*).

2.6 Pathogenic effects on honey bees

Infestations by either *Varroa* or *Tropilaelaps* often result in irregular brood patterns, dead or malformed wingless callow honey bees (De Jong, 1997; Shimanuki et al., 1994). Akratanakul (1987) also found that adult bees infested by *Tropilaelaps* during the pupal stage, have a shorter lifespan relative to uninfested bees. Without chemical intervention, *Tropilaelaps* can kill colonies of *A. mellifera* within a few months (Crane, 1990). Additionally, *Varroa* and *Tropilaelaps* are viral vectors i.e., acute bee paralysis virus (ABPV) and deformed wing virus (DWV) (Dietz and Hermann, 1988; Fries et al., 2003; Dainat et al., 2009; Forsgren et al., 2009; Khongphinitbunjong et al., 2013). The virulence of the viruses associated with *Varroa* and *Tropilaelaps* can hasten the collapse of the host *A. mellifera* colony (Benjamin and McCallum, 2008).

Table 2.2 An identification key of *Varroa* and *Tropilaelaps* female mite species. (Modified from Oldroyd and Wongsiri, 2006; Warrit and Lekprayoon, 2011).

Morphological characters	Mite species
(1) (a) Body elongated, longer than wide	Genus *Tropilaelaps* (2)
(b) Body broadly elliptical, wider than long	Genus *Varroa* (5)
(2) (a) Anal plate rectangular	(3)
(b) Anal plate pear-shaped	(4)
(3) (a) Apex of epigynial plate varies, from female body size: length 978.8 ± 31.5 µm and width 542.5 ± 23.6 µm; male body size: length 920.9 ± 19.5 µm and width 523.2 ± 19.2 µm	*T. mercedesae*
(b) Apex of the epigynial plate always bluntly pointed; female body size: length 881.9 ± 24.1 µm and width 484.4 ± 14.5 µm; male body size: length 856.6 ± 19.2 µm and width 500.9 ± 9.8 µm	*T. clareae*
(4) (a) Subapical tooth on moveable chela present	*T. koenigerum*
(b) Subapical tooth on moveable chela absent	*T. thaii*
(5) (a) Peritremes long looping up from ventral side and extending beyond and thus sometimes visible from dorsal surface	*V. rindereri*
(b) Peritremes not extending beyond lateral margin and not visible from dorsal surface	(6)
(6) (a) Setae of the lateral margin long and slender	*V. underwoodi*
(b) Setae shorter and stout	(7)
(7) (a) Body size ratio (width to length) 1.2-1.3:1	*V. jacobsoni*
(b) Body size ratio at least 1.4:1	*V. destructor*