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REVIEW ARTICLE

Standard methods for *Tropilaelaps* mites research

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Summary

Mites in the genus *Tropilaelaps* are native brood parasites of the non-domesticated giant Asian honey bees, *Apis dorsata*, *A. breviligula* and *A. laboriosa*. They spread onto the managed European honey bee (*A. mellifera*) some time after humans introduced that bee into Asia. Nowadays, *A. mellifera* is kept for beekeeping throughout Asia and *Tropilaelaps* mites are one of its most damaging pests. At present, these mites remain confined to Asia and bordering areas but are recognized as emerging threats to world apiculture. In spite of their important pest-status, *Tropilaelaps* mites remain poorly studied and much remains to be learned about them. The methods reviewed here are intended to assist future research efforts to better understand these parasites. The review begins with an introduction that clarifies why *Tropilaelaps* mites are worthy of immediate research. It is followed by an outline of the mites' taxonomy and descriptions of various methods (including their pros and cons) by which mites can be collected, identified and aspects of their life history and behaviour studied. The role that microbial pathogens play in the pathogenicity of *Tropilaelaps* mites is briefly discussed and a list of future research priorities is suggested.

Métodos estándar para la investigación de ácaros *Tropilaelaps*

Resumen

Los ácaros del género *Tropilaelaps* son parásitos originales de la cría de las abejas de la miel gigantes asiáticas no domesticadas *Apis dorsata*, *A. breviligula* y *A. laboriosa*. Se propagaron hacia la abeja de la miel europea (*A. mellifera*) en algún momento después de que el ser humano introdujera esta abeja en Asia. Hoy día, *A. mellifera* es utilizada para la apicultura en toda Asia y el ácaro *Tropilaelaps* es una de sus plagas más dañiinas. Actualmente estos ácaros se mantienen confinados en Asia y sus zonas fronterizas pero están reconocidos como una amenaza emergente para el mundo de la apicultura. A pesar de su importante estatus como plaga, el ácaro *Tropilaelaps* permanece escasamente estudiado y aún hay mucho que aprender sobre él. Los métodos revisados aquí son un intento para ayudar en los futuros esfuerzos de la investigación para entender mejor este parásito. La revisión comienza con una introducción que aclara porqué el ácaro *Tropilaelaps* debe ser tenido en cuenta para una investigación inmediata. Continúa con un esquema sobre la taxonomía del ácaro y la descripción de varios métodos (incluyendo pros y contras) para su colecta, la identificación y aspectos sobre la vida y comportamiento ya estudiados. Se discute brevemente el papel que juegan los microbios patógenos en la patogenicidad de *Tropilaelaps* y se sugiere una lista de prioridades para futuras investigaciones.

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小蜂螨研究的标准方法

摘要

Tropilaelaps 属的螨是一类幼体寄生虫，原始寄主为野生大型亚洲蜜蜂 *Apis dorsata*, *A. breviligula* 和 *A. laboriosa*。在亚洲引入欧洲蜜蜂（*A. mellifera*）之后的某个时间，该蜂螨传播至饲养的欧洲蜜蜂。现如今，*A. mellifera*的饲养已遍及亚洲，小蜂螨是其危害性最大的害虫之一。目前，这些螨仍仅限于亚洲及其周边地区，但已被公认为世界养蜂业新出现的威胁因素。虽然*Tropilaelaps* 是十分重要的虫害，但关于它的研究甚少，诸多方面有待进一步了解。本章综述了相关方法，旨在有助于将来对其更深入的探索。本综述首先在前言部分阐明为何小蜂螨值得当前研究，其次概述了该螨的分类，描述了蜂螨采集、鉴定、生活史和行为研究的不同方法（包括它们的优缺点）。简要探讨了微生物致病体在小蜂螨致病性中发挥的作用，并列举了一些未来需要重点研究的内容。

Keywords: COLOSS, *BEEBOOK*, honey bee, *Tropilaelaps mercedesae*, *Tropilaelaps clareae*, *Tropilaelaps koenigerum*, *Tropilaelaps thaii*, *Apis mellifera*, giant Asian honey bees, research method, protocols, identification, taxonomy

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1. Introduction

Mites in the genus *Tropilaelaps* are native brood parasites of the non-domesticated giant Asian honey bees, *Apis dorsata*, *A. breviligula* and *A. laboriosa*. They spread onto the managed European honey bee (*A. mellifera*) some time after humans introduced that bee into Asia. Nowadays, *A. mellifera* is kept for beekeeping throughout Asia and *Tropilaelaps* mites are one of its most damaging pests (Burgett *et al.*, 1983; Woyke, 1985a; Anderson and Morgan, 2007; Dainat *et al.*, 2009). At present, these mites remain confined to Asia and bordering areas but are recognized as emerging threats to world apiculture.

A question that is constantly asked of *Tropilaelaps* mites, the answer to which justifies or counters the need to direct scarce resources to study them, is: can they become a serious global pest of *A. mellifera*, like *Varroa destructor*? The answer is emphatically yes and it lies in how the mites feed and reproduce on their bee hosts, how they disperse, how they spread among bee colonies and how they might survive on *A. mellifera* in temperate zones outside of Asia.

The breeding cycle of *Tropilaelaps* mites on their honey bee hosts superficially resembles that of *Varroa* mites, in that a mature mated female enters a bee brood cell that contains a developing bee larva that is in the process of being capped by worker bees with a wax covering. Safely concealed within the sealed cell, the mother mite produces several offspring that all feed on blood (haemolymph) of the developing bee. Eventually the mites are released from the cell when the developing bee (which by now may or may not be physically damaged) chews its way out of the cell through the wax capping (Woyke, 1987; 1994). Unlike *Varroa* mites, the survival of *Tropilaelaps* mites depends solely on them having regular access to bee brood (larvae or pupae) on which to feed, as their mouthparts and body shape do not allow them to feed on adult bees, as do *Varroa* mites. The mites can only survive for a few days in the absence of bee brood (Woyke, 1984; Koeniger and Musaffar, 1988; Rinderer *et al.*, 1994). This limited food source restricts their ability to disperse, as they can only disperse on adult bees on which they cannot feed.

The ever-increasing global trade of live honey bees, which provides a potential pathway for *Tropilaelaps* mites to disperse out of Asia, has not yet contributed to any increase in their geographical range. This is probably because live bee trade involves movements of adult bees (in the form of 'package bees') and live adult queen bees, on which *Tropilaelaps* mites cannot feed or survive for more than about 74 hours (Wilde, 2000). Because the mites cannot feed on adult bees, very few are found on them at any one time, even in heavily infested bee colonies (Woyke, 1984; 1985b). Those mites that do venture onto adult bees can spread to neighbouring bee colonies by various means, such as on swarms, on worker bees that rob resources from other colonies, on foraging bees that become disorientated and enter the wrong colony or simply by moving between forager bees from different colonies that visit the same flowers. In the Philippines, it has been suggested that *Tropilaelaps* mites spread between *A. breviligula* (their natural host) and *A. mellifera* colonies by interspecific robbing (Laigo and Morse, 1969).

As *Tropilaelaps* mites venture onto adult bees at some stage of their life, then live bee exports from Asia could potentially carry them, albeit at low numbers, and therefore aid their dispersion. However, for mites to survive this pathway, the exported bees would need to be moved quickly to their destination country and, on arrival, come in contact with local brood-right bee colonies into which mites could disperse and feed on brood before they starve to death. Another new potential pathway for the mites' spread out of Asia was recently uncovered in Australia, when *A. dorsata* worker bees were detected

on air cargo that arrived at an international airport from Malaysia. These bees were probably night-foragers that had become disoriented by airport lights in Malaysia and rested and became stranded on cargo that was being loaded into an airplane bound for Australia.

Even given these and other potential pathways for *Tropilaelaps* mites to spread, they nevertheless still remain restricted to Asia and bordering areas. They currently occur as far west as Afghanistan-Pakistan and as far east as the large Melanesian island of New Guinea, where they were introduced in the 1980s in brood-right hived colonies of *A. mellifera* imported from Java (Delfinado and Aggarwal, 1987; Anderson, 1994; Baker *et al*., 2005). They were also reported from Kenya during the early 1990s (Kumar *et al*., 1993; Matheson, 1997), but this report has not been verified and it may have resulted from a false identification, as recent testing in Australia of mites that had been collected from honey bees in Kenya, and assumed to be *Tropilaelaps*, were found to be plant mites (Anderson, unpublished data).

So the question remains: does the current restricted distribution of *Tropilaelaps* mites reflect their inability to survive outside of Asia in temperate zones in the absence of their native bee hosts? The successful establishment of *Tropilaelaps* mites on *A. mellifera* in New Guinea suggests the answer is no, and that they can survive in temperate zones given an important proviso.

New Guinea is located to the north of Australia and it contains no native *Apis* species. Humans introduced colonies of *A. mellifera* to the island last century and their descendants have since thrived in the cool temperate-like highland regions, but not so well in the hotter humid tropical lowland regions (Clinch, 1979). Since their introduction to New Guinea in the 1980's, *Tropilaelaps* mites have become an endemic damaging pest of *A. mellifera* in the western half of the island (Irian Jaya). Their success is thought to be due to the unbroken year-round production of brood by the *A. mellifera* colonies, which provides a continuous food source for the mites and an ideal environment for their reproduction.

Hence, the New Guinea situation confirms that *Tropilaelaps* mites can survive and become an endemic pest of *A. mellifera* in temperate zones in the absence of their native hosts provided they have access to *A. mellifera* brood on a year-round basis. Such conditions are found in many temperate countries, such as parts of the USA, Australia and Europe. It has also been suggested that, in coming years, temperate areas in which *A. mellifera* can produce brood all year round may increase, as colder regions become warmer, due to the effects of climate change (Le Conte and Navajas, 2008).

This all adds up to a situation where it appears that good fortune has played a major role in restricting the distribution of *Tropilaelaps* mites and that it may be only a matter of time until they spread outside of Asia to cause hardship for temperate zone beekeepers. It is therefore no surprise that the mites are currently recognized as emerging threats to world apiculture (OIE, 2004) and hence deserve immediate attention from the global research community. The methods presented here should assist those future research efforts.

2. Taxonomy and host-specificity

2.1. Taxonomy

The first species in the genus was described more than 50 years ago and the most recent in 2007. The taxonomy of the genus has recently been revised and it currently stands as follows (Lindquist *et al*., 2009; Anderson and Morgan, 2007):

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Arachnida
Subclass:	Acari
Superorder:	Parasitiformes
Order:	Mesostigmata
Family:	Laelapidae
Genus:	*Tropilaelaps*
Species:	*T. clareae* Delfinado and Baker (1961)
	T. koenigerum Delfinado-Baker and Baker (1982)
	T. mercedesae Anderson and Morgan (2007)
	T. thaii Anderson and Morgan (2007)

2.2. Host specificity

Some behavioural and morphological features of *Tropilaelaps* mites, such as their fast movement and 'pincer-shaped' chelicerae, suggests they may have only recently adopted a parasitic life-style on honey bees and that they may not be very host-specific. Indeed, when they were first discovered in the Philippines in the 1960's they were found inside an *A. mellifera* colony and on field rats nesting nearby (Delfinado and Baker, 1961). It is likely that those rats picked up the mites after they entered the *A. mellifera* colonies, which rats often do in the tropics, because evidence gathered since that initial discovery indicates that *Tropilaelaps* mites are highly specialized parasites of honey bees.

Tropilaelaps mites are now recognized as common natural parasites of giant honey bees distributed throughout Asia. They have not colonized any other host organism, other than *A. mellifera*. Molecular studies have confirmed that *T. clareae* is native to *A. breviligula* in the Philippines (except on Palawan Island), *T. mercedesae* and *T. koenigerum* to *A. dorsata* and *A. laboriosa* in other parts of Asia (including Palawan Island) and *T. thaii* to *A. laboriosa,* in mountainous region of Mainland Asia (Laigo and Morse, 1969; Delfinado-Baker and Baker, 1982; Tangjingjai *et al*., 2003; Anderson and Morgan, 2007).

Tropilaelaps mercedesae and *T. clareae* colonized *A. mellifera* after humans introduced that bee into Asia. However, in contrast with *Varroa* mites, of which only a few genotypes have switched-host to *A. mellifera*, many different genotypes of *T. mercedesae* and *T. clareae* now utilize *A. mellifera* as a host. In this respect, *Tropilaelaps* mites are less host-specific than *Varroa* mites but they are still relatively host-specific compared to some mites, such as the water mite *Protzia eximia* that parasitizes a wide variety of insect hosts (Walter and Proctor, 1999). Evidence suggests that *T. koenigerum* and *T. thaii* are restricted to their Asian bee hosts and are harmless to *A. mellifera* (Anderson and Morgan, 2007).

Very occasionally, *Tropilaelaps* mites are found in *A. cerana* and *A. florea* colonies in Asia, but in none of these instances have the mites been found to be producing offspring (Otis and Kralj, 2001). The exception is a report of a single female *T. mercedesae* found parasitizing and producing offspring on *A. cerana* brood in Thailand (Anderson and Morgan, 2007). The authors, when commenting on this find, stated that their observation was the exception rather the rule, as *T. mercedesae* mites are rarely found in *A. cerana* colonies and, when they are, they are not found inside brood cells or with offspring. Obviously, other factors, other than the ability to produce offspring on that bee, are responsible for preventing *T. mercedesae* from colonising *A. cerana*.

Fig. 1. A gravid *T. mercedesae* adult female feeding on an *A. mellifera* pupa. Note the first pair of legs of the mite is held upright, resembling antennae. Photo: Denis Anderson.

Fig. 2. Comparisons of a female *T. mercedesae* (left) with two female *V. jacobsoni* (right) on an *A. mellifera* larva. Photo: Denis Anderson.

3. Sample collection

3.1. Mite appearance

Adult *Tropilaelaps* mites are small (< 1 mm long), light brown, and hold their first pair of legs upright, resembling antennae (Fig. 1). They can often be seen moving quickly over the surface of combs in infested colonies. Their body shape is quite different from that of *Varroa* mites, being much longer than it is wider (Fig. 2).

There are clear morphological differences between the sexes of the different species. Males of *T. thaii* have not yet been discovered. Males of *T. mercedesae*, *T. clareae* and *T. koenigerum* are slightly smaller than their female counterparts and their epigynial thoracic plates are also shorter and sharply pointed toward their posterior end (Fig. 3). In any collection of *Tropilaelaps* mites, males are usually much less common than females (Rath *et al.*, 1991; Anderson and Morgan, 2007). Males can be easily identified in the field using a magnifying glass to observe the chela spermatodactyl (sperm transfer organ), which in *T. mercedesae* and *T. clareae* is long with a spirally coiled apex and in *T. koenigerum* is short with a 'pig-tail' loop at its apex (Fig. 4).

The nymph stages of *Tropilaelaps* are brilliant white and are easily observed with the naked eye (Fig. 5).

3.2. Where to find mites

Adult females are most easily found inside of capped worker and drone bee brood cells of infested colonies, where they reproduce. They do not appear to markedly favour either cell type for their reproduction. This is also the only place where nymphal stages can be found. To find a mother mite and her nymph offspring, simply uncap a bee brood cell of an infested colony and remove the developing bee inside. Tilt the entire comb so that the ambient light will be directed into a cell. Any mites present will be easily seen with the naked eye in the bottom of the cell or on the cell wall.

The presence of adult mites with offspring inside bee brood cells is clear evidence that they have reproduced and have not simply entered the bee cell after being transported (say on robbing bees) from another colony of a sympatric bee species, which might confuse host-specificity attributed to them. Male mites are best found inside of capped cells in which the developing bee is about to emerge or else in random collections of adult mites found moving on the surfaces of combs.

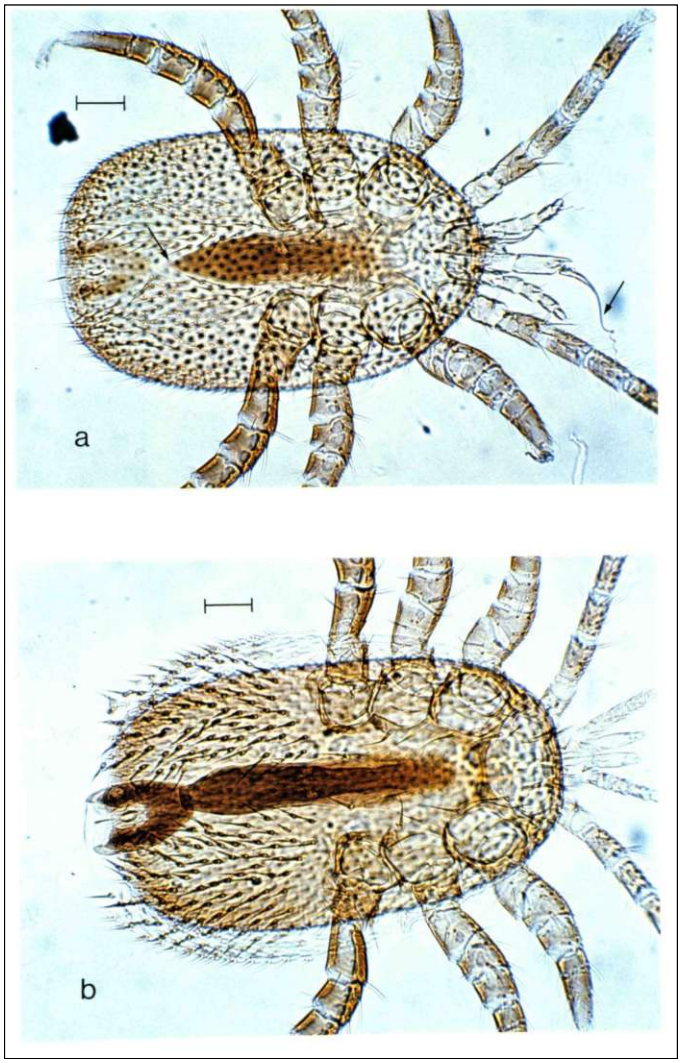

Fig. 3. Comparison of mounted specimens of a *T. mercedesae* male (top) and female (bottom). Anterior arrow on male points to the corkscrew like spermodactyl (sperm transfer organ) and posterior arrow points at the non-overlapping epigynial thoracic plate. Bars = 0.1 mm. Photo: Denis Anderson.

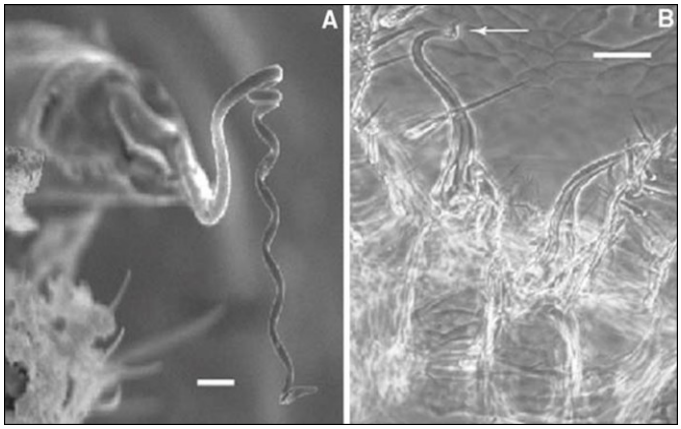

Fig. 4. Scanning electron micrographs of the sperm transfer organs of male (A) *T. clareae* and (B) *T. koenigerum*. Bar = 10 µm. Photo: Denis Anderson.

Fig. 5. A family of mites (*T. mercedesae*) with mother mite (light brown) and different stages of offspring (white) at the bottom of a cell from which the honey bee pupa was removed. Photo: Denis Anderson.

Adult *Tropilaelaps* mites are much more mobile than adult *Varroa* mites and can be seen moving quickly across the surface of infested brood combs. In this situation they are hard to collect. When they are not moving, they also become well camouflaged against the background colour of the wax combs and are hard to see. When the brood comb is lifted out of the colony into the open light mites also quickly enter open cells and remain still on the cell walls, where again, they are hard to see.

Adults of both sexes can also be found on the bodies of adult bees, but only in extremely low numbers, even in heavily infested colonies. This is probably because mites avoid adult bees as much as possible because they cannot feed on them and hence can only survive for a few days (Woyke, 1984; Wilde, 2000). Nevertheless, at some stage they have to move on to adult bees in order to disperse from the colony.

3.3. Collecting mites from bee brood

Phoretic mites that are moving on the surfaces of bee combs are relatively hard to collect with forceps. In these situations it is best to collect them with a fine bristle bush slightly wetted with honey, water or alcohol (not human spittle as it will contaminate the sample with human DNA) or a small mouth aspirator.

When large numbers of mites are required, it is best to collect them from infested capped bee brood cells. This can be done in the field, or if large numbers of colonies needs to be sampled, combs can be collected and sampled later in the comfort of the laboratory. To do this:

1. Remove the wax cappings from a large number of bee brood cells on one side of a comb all at once.
2. Remove the developing bee brood from these cells.

3. Invert the comb over a sheet of white paper and tap it relatively hard on its upper surface to dislodge mites from the cells onto the paper.
4. Collect the mite from the paper into small vials containing 70% ethyl alcohol, using a fine paint brush wetted in honey, alcohol or water (not human spittle) or with a fine pair of tweezers, as shown for *Varroa* mites in Dietemann *et al*. (2013). *Tropilaelaps* mites (similar to *Varroa* mites) will immediately sink to the bottom of a container when immersed in ethyl alcohol. The white paper onto which mites fall can also be substituted with brown paper if mite collection is targeted at nymphal stages.

There are benefits in sometimes collecting live mites into hot water before transferring them into alcohol for storage (Section 4.1 below).

Pros: Mites collected into ethyl alcohol can be subsequently tested in the laboratory in a range of different tests, such as morphometric and DNA analyses.

Cons: Mites used in inoculation or behavioural studies cannot be placed into ethyl alcohol after collection. But even then, mites collected by this method are of little use for those types of studies, as the development stage of the collected mites cannot be ascertained with certain. Live mites to be used in those kinds of studies are best collected from very recently capped bee brood cells that contain late larval or prepupal stages. This ensures that the mites collected are mature adults that are at a specific stage of development – that is, at the pre-reproductive stage. These mites can be kept alive until needed by keeping them in a small Petri dish or a glass bottle with late stage bee larvae.

3.4. Collecting mites from adult bees

As mentioned, adult *Tropilaelaps* mites are usually only present in low numbers on adult bees in any given bee colony. Hence, it is usually a waste of time trying to manually find them on individual bees. A simple method for collecting them from adult bees is:

1. Collect a sample of 200 or so adult bees from an infested colony into a transparent container (such as a plastic bottle) that contains 70% ethyl alcohol.
2. Secure the lid on the container and shake it vigorously for about 1 minute.
3. Collect mites from the bottom of the container into containers containing fresh 70% ethyl alcohol, as described for *Varroa* mites in Dietemann *et al*. (2013).

Mites can also be removed from adult bees by washing the bees in soapy water or by treating them by the 'sugar-shake' method as also described for *Varroa* mites in Dietemann *et al*. (2013). Also, an alternative method to placing adult bees into ethanol, soapy water or sugar prior to shaking to dislodge mites, is to place the bees into plastic bags, label accordingly and freeze until the bees can be visually examined. However, this is much slower than the shaking methods.

Pros: A quick and simple method for finding mites on adult bees in any given infested bee colony.

Cons: Bee colonies need to be relatively highly infested with mites for this method to succeed. To increase the chances of collecting mites from adult bees, one can select a frame with emerging brood to collect mites emerging with the bees. However, it should be remembered that some of those mites will be new adults and may not be mated or fully mature.

3.5. Storage of collected mites

Methods for storing *Tropilaelaps* mites are identical for those described for *Varroa* mites in Dietemann *et al*. (2013).

3.6. Shipping collected mites

Methods for shipping *Tropilaelaps* mites are identical to those also described for *Varroa* mites in Dietemann *et al*. (2013).

4. Methods for identifying mites

4.1. Morphological methods

Morphological analyses are best carried out on mite specimens that have been cleared of their body tissue and mounted on glass microscope slides. Methods for clearing and mounting *Tropilaelaps* mites are the same as those described for *Varroa* mites (Dietemann *et al*., 2013). Generally these specimens have been previously collected and stored in alcohol. However, sometimes it helps to collect specimens that are destined for morphological analyses into hot water before they are transferred in alcohol. This relaxes their internal body tissues and exposed hard-to-see organs, such as chelicerae, which are normally hard to see in mounted specimens that have simply been collected into alcohol.

Identification of specimens to the species level using morphology can be troublesome. Hence, the starting-point for identifying them should always be from information known about the location and host bee from which they were collected. Mites found on either giant Asian honey bees or European honey bees in the Philippines (except on Palawan Island) will in all probability be *T. clareae*, whereas those found on European honey bees in other parts of Asia (and Melanesia) will be *T. mercedesae.* The two other species, *T. koenigerum* and *T. thaii,* have been found only on their native Asian bee hosts, *A. dorsata, and A. laboriosa* respectively.

Table 1. Key to identification of *Tropilaelaps* mites. Males of *T. thaii* have not been discovered, but this species is restricted to *A. laboriosa*.

1 a. Collection sites: The Philippines (except Palawan Island) and Sulawesi Island (in Indonesia).	*Tropilaelaps clareae* Parasitizes *Apis breviligula* and *Apis mellifera* in the Philippines and *Apis dorsata binghami* and *Apis mellifera* in Sulawesi.
1 b. Collection sites: All other localities (including Palawan Island).	
2 a. Body length: ≤ 700 µm.	*Tropilaelaps koenigerum* Primarily found parasitizing *A dorsata dorsata* in mainland Asia.
2 b. Body length: ≥ 840 µm	
3 a. Female chelicerae: No apical tooth.	*Tropilaelaps thaii* Parasitizes *Apis laboriosa* in mountainous regions of mainland Asia.
3 b. Female chelicerae: One apical tooth present	*Tropilaelaps mercedesae* Primarily parasitizes *Apis dorsata dorsata* and *Apis mellifera* in mainland and southeast Asia.

Many of the physical characters used to identify mites (such as their body size, sensilli and structures on body plates) are highly variable within the genus and even among member of the same species (Anderson and Morgan 2007). A key for identifying the different species of *Tropilaelaps* is given in Table 1.

The 4 main physical characters most useful for identifying *Tropilaelaps* mites to the species levels are:

1. Body length.
   T. koenigerum is the smallest member of the genus with a body length of < 700 µm for females and ~575 µm for males. Female *T. mercedesae*, *T. clareae* and *T. thaii* are much longer at ~ 950-990 µm, ~870-885 µm and ~ 890 µm respectively, while the body lengths of male *T. mercedesae* and *T. clareae* are slightly smaller than their respective females at 907-927 µm and 852-858 µm, respectively. Males of *T. thaii* have yet to be discovered.
2. Shape of the anal plate.
   Male and female *T. koenigerum* have a distinct pear-shaped anal plate, female *T. Thaii* have a bell-shaped anal plate, while the anal plates of male and female *T. mercedesae* and *T. clareae* are highly variable and of little use as a taxonomic reference.
3. Structure of the male sperm transfer organ (chela spermatodactyl or spermadactyl).
   Males of *T. koenigerum* have a 'pig-tail' loop at the apex of the chela spermatodactyl, while the apex of the chela spermatodactyls of male *T. mercedesae* and *T. clareae* are long cork-screw-like structures (Fig. 4).
4. Placement and shape of teeth on female chelicerae.
   Female *T. koenigerum* have a single sub apical tooth on the chelicerae with a characteristic groove near its anterior base, whereas *T. mercedesae* and *T. clareae* females also have the sub apical tooth, but without the groove. Female *T. thaii* lack the sub apical tooth (Fig. 6).

4.2. Molecular methods and systematics

Molecular technology was first used in *Tropilaelaps* research in the 1990s to examine genetic variation between so-called *T. clareae* and *T. koenigerum* (Tangjingjai *et al*., 2003). It has since proved very useful to examine genetic variation within the genus and to help re-define known species and describe new species and new types within species (Anderson and Morgan, 2007).

DNA sequence obtained from a 538 base-pair fragment of *Tropilaelaps* mtDNA *cox1* gene, as well as from the entire nuclear ITS1-5.8s-ITS2 genes, provides a useful means for identifying mites to the species level. The *cox1* sequence is also useful for looking at genetic variation within a species.

Methods for extracting, amplifying and sequencing *Tropilaelaps* DNA are the same as those described for *Varroa* in Dietemann *et al*. (2013). The DNA primers used to amplify the *cox1* and ITS1-5.8s-ITS2 gene regions are shown in Table 2.

Table 2. Forward (F) and reverse (R) primer sequences (and their names) used in *Tropilaelaps* research to amplify fragments (base pairs) of specific genes.

Gene region	**Fragment size (bp)**	**Primer sequences (5'-3')**	**Primer name**
CoxI	538	(F) CTATCCTCAATTATTGAAATAGGAAC	TCF1
		(R) TAGCGGCTGTGAAATAGGCTCG	TCR2
ITS1-5.8S-ITS2	522-526	(F) GGAAGTAAAAGTCGTAACAAGG	ITS5
		(R) TCCTCCGCTTATTGATATGC	ITS4

Once a DNA sequence is obtained from a particular mite specimen, it is compared to other sequences of the same region that have been deposited in the GenBank database (Dietemann *et al*., 2013). The ITS region shows no genetic variation within a particular species of *Tropilaelaps*, whereas *cox1* sequence shows from 1-4% variation within species and from 11-15% between species. Mites of particular

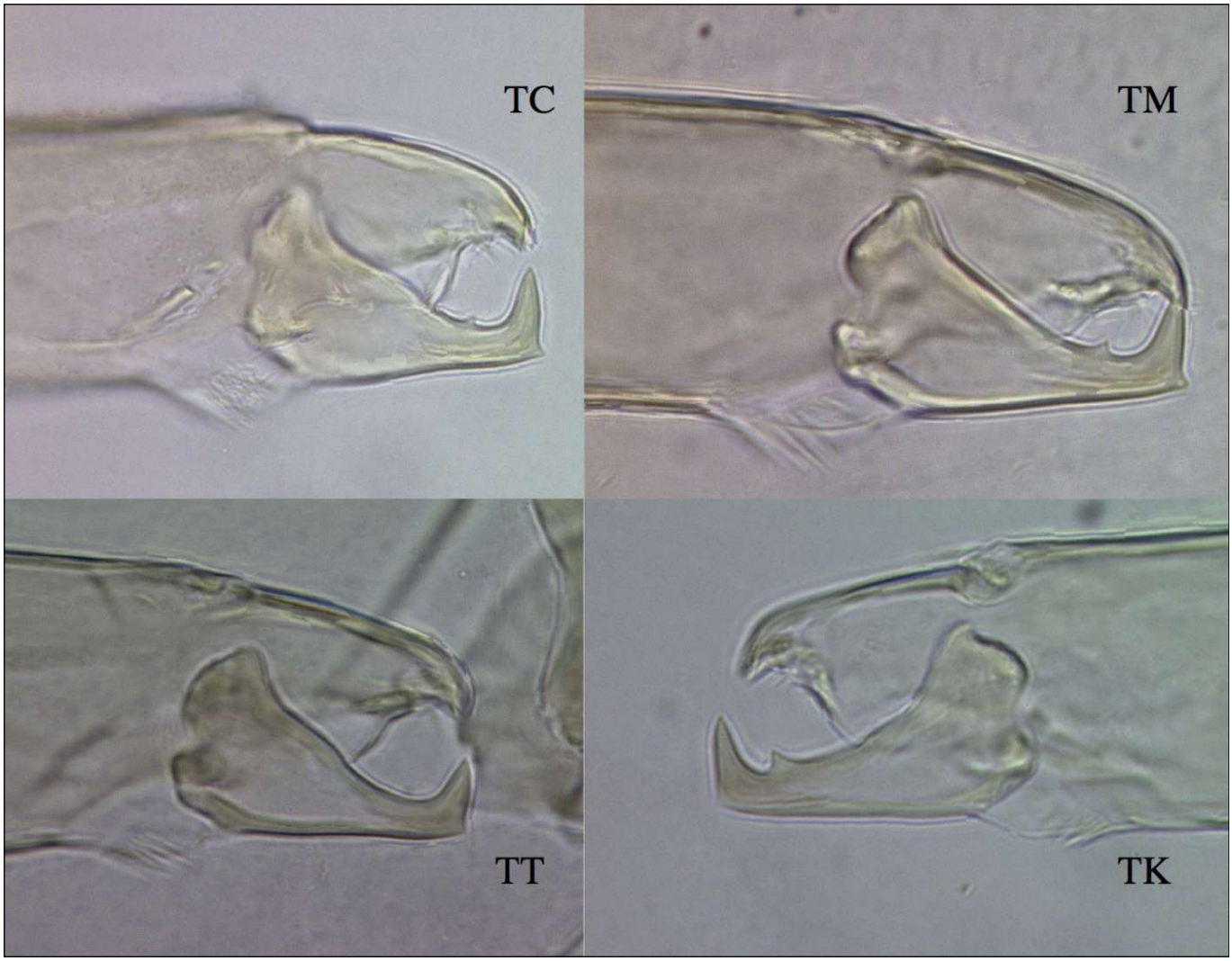

Fig. 6. Comparisons of female chelicerae of *T. clareae* (TC), *T. mercedesae* TM), *T. koenigerum* (TK) and *T. thaii* (TT) (light microscopy, x800). Photo: Denis Anderson.

species that vary in the *cox1* gene sequence are referred to as 'haplotypes'. The concept of 'haplogroup', as described for *Varroa* mites in Dietemann *et al*. (2013), has not been adopted for *Tropilaelaps* mites, as there is much more variation in the *cox1* gene of *Tropilaelaps* than in *Varroa* (Anderson and Trueman, 2000).

Tropilaelaps mites can also be identified to the species level by digesting amplified fragments of their *cox1* gene with a combination of the *Fau*I *Bsr*I *Bst*YI and *Swa*I restriction enzymes, without the need for sequencing the fragments. Products produced from these digestions are visualized as bands in 2% agarose gels (Anderson and Morgan, 2007). The results of using these restriction enzymes to digest the cox1 gene fragment of the 4 known species are shown in Fig. 7. In summary, *Fau*1 only digests *coxI* fragments obtained from *T. koenigerum*, *Bsr*I only digests fragments from *T. koenigerum* and *T. mercedesae* (2 bands are produced from each species, but the smaller band produced from *T. mercedesae* is larger than that of *T. koenigerum*), *Bst*YI only digests fragments from *T. clareae*, while *Swa*I only digests fragments from *T. thaii*.

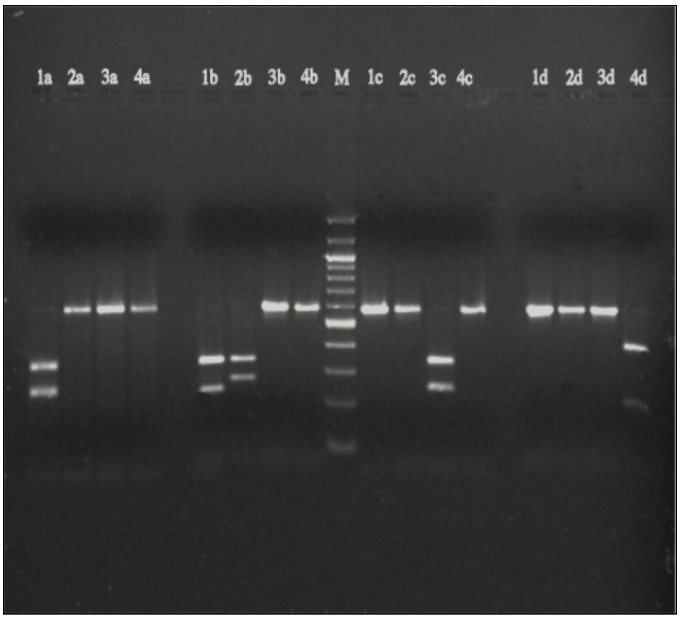

Fig. 7. Mites can be identified by digesting fragments of their cox1 gene with *Fau*I, *Bsr*I, *Bst*YI and *Swa*I restriction enzymes (labeled a–d respectively). The numbers 1-4 represent: *T. koenigerum*, *T. mercedesae*, *T. clareae* and *Thaii* respectively. M = 100 bp DNA Ladder. Photo: Denis Anderson.

As *cox1* gene sequence can resolve *Tropilaelaps* mites to the species level it is useful in phylogenetic studies. Methods used to carry out phylogenic analyses on *Tropilaelaps cox1* gene sequence are the same as those used for *V. destructor* and other species, described in Dietemann *et al*. (2013). A phylogenetic tree of all the currently known and published *Tropilaelaps* haplotypes is shown in Fig. 8.

5. Life cycle and rearing

Much remains to be learned about the life cycle of *Tropilaelaps* mites. Reports published to date refer to the life cycle of *T. clareae* on *A. mellifera*, but with the recent taxonomic revision of the genus, which separated *T. clareae* into 2 species (Anderson and Morgan, 2007), it is now clear that those reports refer to *T. mercedesae* on *A. mellifera*. There are no reports of the life cycles of *T. clareae*, *T, koenigerum* or *T. thaii*, although they are thought to be very similar to that of *T. mercedesae* on *A. mellifera*. Obviously, this is an area ripe for research.

5.1. Life cycle of *T. mercedesae* on *A. mellifera*

As mentioned, to begin their reproduction, mated female mites enter *A. mellifera* worker or drone brood cells that are in the process of being capped (Burgett *et al*., 1983; Ritter and Schneider-Ritter, 1986). There is no marked difference in the type of cell type that female mites choose to reproduce in. However, much variation has been reported in the timing of different events during the reproduction phase. After entering a cell a single female mite lays from 1-4 eggs (but typically 3-4) about 1 day apart. A comparison of the time in hours after cell capping that the first egg, larva, protonymph, deutonymph, and young adult mite appear in a cell, as reported by different authors, is shown in Table 3.

At the end of the reproduction phase, the mother mite and her offspring exit the cell when the developing bee chews its way out of the cell through the wax capping. They then enter a brief phoretic phase, in which they move about the comb, probably mate (as recently emerged male and female mites have been observed mating in glass test tubes (Woyke, 1994)) and spend time on adult bees, before they commence a new reproduction phase. The phoretic phase of *T. mercedesae* mites is much shorter than that of *Varroa* mites, and may be as short as 1-2 days. This means that *T. mercedesae* mites have quicker reproductive cycles than *Varroa* mites and hence their population buildup within a bee colony is much faster than that of *Varroa* mites, said by a UK Government source to be in the order of about 25:1 in favour of *Tropilaelaps* (DEFRA, 2005). A diagram that summarizes data on the life cycle of *T. mercedesae* on *A. mellifera* in New Guinea, reported by Saleu (1994), is shown in Fig. 9.

5.2. Methods for studying mite development on *A. mellifera*

No artificial media has been described for rearing *Tropilaelaps* mites and hence aspects of their reproduction must be studied within a bee colony. To study their life cycle in an *A. mellifera* colony one first needs to select a colony that is already reasonably infested with mites. The general method is as follows:

1. Determine mite infestation levels in a number of *A. mellifera* colonies by removing the cell cappings from 300 capped cells/ colony and determining how many contain mites.
2. Select a colony with the highest mite infestation and use this for further studies.
3. Remove 2 brood combs from the middle of the brood area of the selected colony and replace them with 2 brood combs from a non-infested colony that contains larvae that are 2-3 days away from being capped (Note: these brood combs can be obtained from other nearby colonies in which queen bees have been restrained to combs with a queen excluder for 24 hours. The queens will fill the cells of the combs with eggs, meaning that all brood that develops for the eggs with be within 24 hours of the same age).
4. Once the 2 brood combs have been capped, remove 1 at regular intervals to the laboratory (usually every 12 or 24 hours is best).
5. In the laboratory uncap a number of cells, remove the bee brood and determine the number of adult mites, eggs and mite nymphs within each cell.
6. Once all cells on this comb have been inspected, move on to the second comb.
7. Collate the recorded data. There are many methods by which this can be done but a simple method is simply to produce a diagram of the capping phase of the bee and plot the data recorded directly onto it.

Pros: This method is relatively simple and will show the progression of mite development, from egg to adult.

Cons: The method requires access to both infested and no-infested bee colonies.

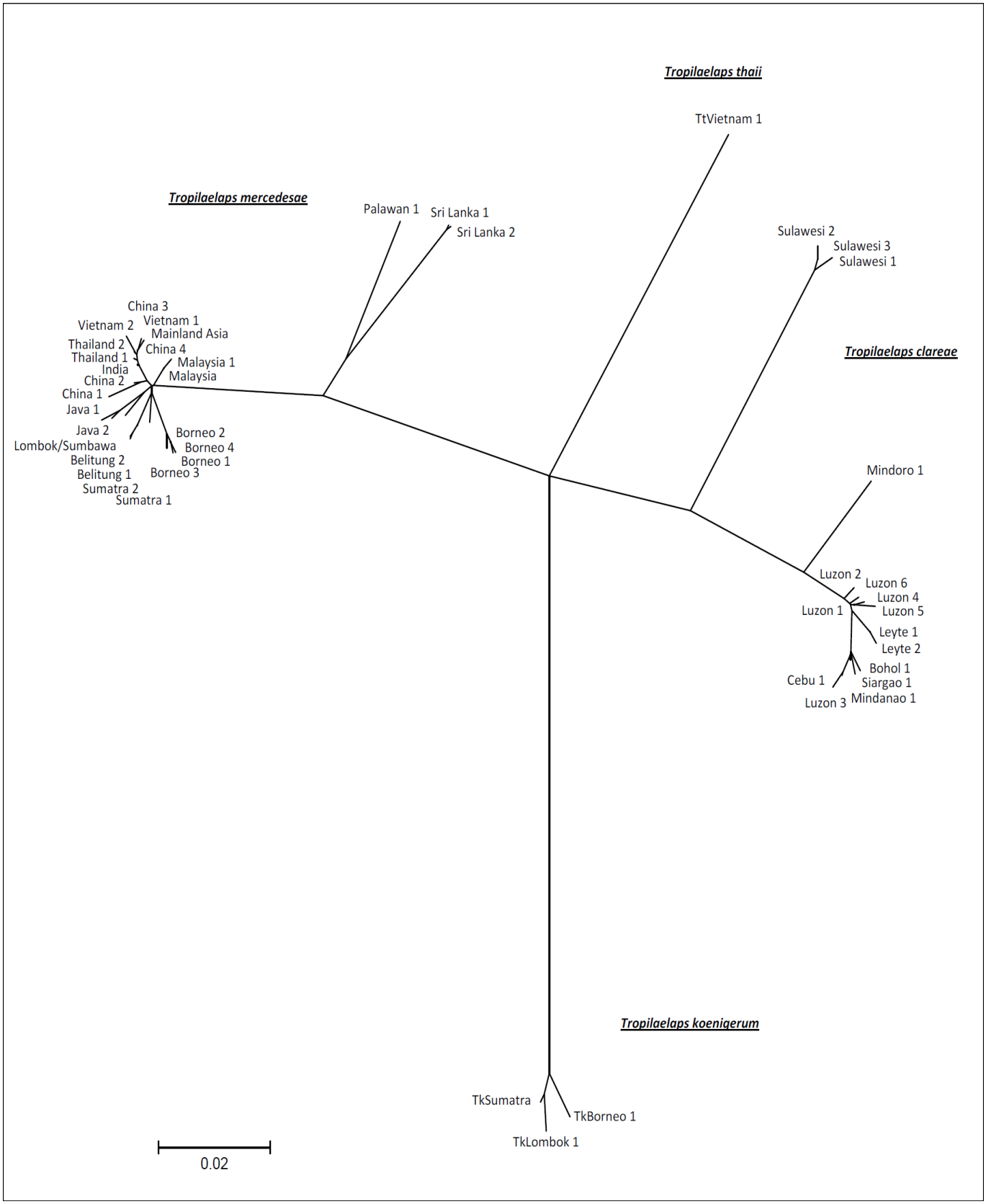

Fig. 8. A phylogenetic tree of all the currently known and published haplotypes of *Tropilaelaps*. Photo Denis Anderson and John Roberts.

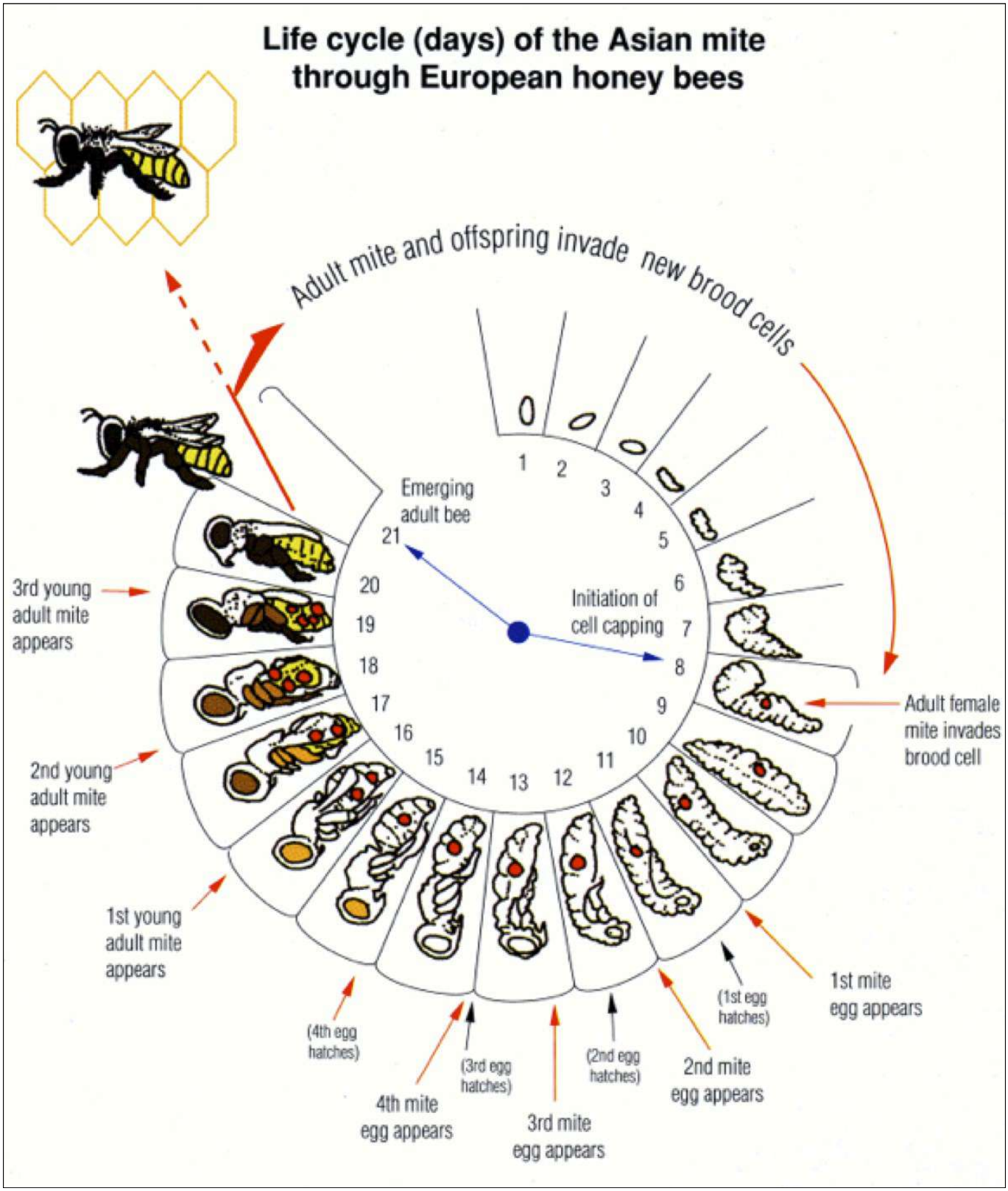

Fig. 9. Life cycle of *T. mercedesae* on *A. mellifera*. Diagram was constructed from data reported by Saleu (1994). Photo: Denis Anderson.

Table 3. Comparison of time (in hours) after bee brood cell capping of the first appearance of different developmental stages of *T. mercedesae*, as reported in 4 different studies: Saleu, 1994 (New Guinea); Kiprasert, 1984 (Thailand); Woyke 1984, 1985a (Afghanistan and Vietnam); Kumar et al., 1993 (India). The numbers in brackets represent honey bee brood development time in days.

Mite development Stage	Study 1	Study 2	Study 3	Study 4
Egg:	72 (10-11)	96 (12)	24-48 (9-10)	96 (12)
Larva:	96 (11-12)	96 (12)	24-48 (9-10)	96 (12)
Protonymph:	96 (11-12)	192 (16)	72-96 (11-12)	192 (16)
Deutonymph:	168 (14-15)	216 (17)	120 (13)	192 (16)
Young Adult:	216 (16-17)	312 (22)	192 (16)	298 20)

5.3. Methods for studying mite development on giant Asian honey bees

There are virtually no reported studies on the life cycle of different species of *Tropilaelaps* on their native Asian giant bee hosts. This is a pity, as mites of the different species do not typically cause as much harm to their giant honey bee hosts as they do to *A. mellifera*. Hence studies on the life cycles of mites on the giant honey bees could provide clues as to how to control them on *A. mellifera*.

Studies on the life cycle of *Tropilaelaps* mites on their giant honey bee hosts in Asia can be done as follows:

1. First, and most importantly, locate a local 'honey-hunter' to gain access to a nest, as virtually all giant honey bee nests in the wild in Asia are 'owned' by a local hunter and accessing them without permission (or help) can lead to serious problems.
2. Once a nest is located, smoke the bees off the single comb (typically most of the bees will fly off the comb when it is smoked, but they will return later).
3. Remove a section of the comb that contains capped brood using a sharp knife (in this way the comb will not be significantly damaged and the bees will soon return to it and resume their normal activities).
4. Transport the comb to the comfort of the laboratory for examination.
5. In the laboratory open each capped cell and remove the developing bee noting the approximate age of the bee (prepupa, white –eyed pupa, pink-eyed pupa, and so on).
6. Record how many individuals of the various mite stage are present.
7. Collate the recorded data.

Pros: This method is relatively simple and will show the progression of mite development, from egg to adult.

Cons: It is often difficult to gain access to wild nests.

5.4. Methods for studying mite mating behaviour

Mating behaviour can be studied by placing a single adult male mite and female mite inside a clear plastic Petri dish (or glass bottle) that contains a bee larva on which the mites can feed. Mites are kept alive in the dish by continually replacing the bee larva.

Pros: This method allows for mating behaviour to be observed and videoed.

Cons: Behaviours of mites *in-vitro*, such as those shown in plastic or glass containers, may not necessarily be those shown naturally inside the bee colony.

6. Pathogenicity, control and association with pathogens

Tropilaelaps mercedesae (formerly known as *T. clareae*) may infest as much as 90% of the brood in *A. mellifera* colonies (Kiprasert, 1984), but smaller brood infestation levels of 3 to 6% have been consistently reported from *A. dorsata* colonies (Underwood, 1986) and adult *A. dorsata* and *A. cerana* workers show greater resistance to the mite than *A. mellifera* workers (Khongphinitbunjong *et al.*, 2012). High infestations of *A. mellifera* brood by this species often results in callow adult bees with deformed wings (De Jong *et al.*, 1982; Burgett *et al.*, 1983) and reduced body weights (Kiprasert, 1984) (see Fig. 10). Untreated infestations rapidly increase to high levels and invariably lead to the death of entire colonies (Atwal & Goyal, 1971; Ritter, 1988; Woyke, 1985a, 1985b).

Fig. 10. Damage caused by *T. mercedesae* to *A. mellifera* brood. Photo: Denis Anderson.

The control of *Tropilaelaps* mites in *A. mellifera* colonies has been reviewed by De Jong *et al*. (1982) and Ritter and Sneider-Ritter (1988), but is in need of revision. Many of the various synthetic chemical acaricides used to control *Varroa* mites are also effective against *Tropilaelaps* mites (Pichai, *et al*., 2008). Sulphur, formic acid and thymol have also proved satisfactory (Atwal and Goyal, 1971; Raffique *et al*., 2012). Non-chemical means of controlling *T. mercedesae* in *A. mellifera* colonies have been achieved by interrupting the brood cycle of the bees. For example, Woyke (1984; 1985a & b) controlled *T. mercedesae* in *A. mellifera* colonies by removing all brood for 2 days, but removal for 5 days is recommended in order to kill all mites. Such methods would probably not be viable for commercial beekeepers that manage large numbers of colonies and they are very time-consuming.

There have been few studies on the pathogens associated with *Tropilaelaps*. However, like *Varroa* mites, *Tropilaelaps* mites have been associated with spread and infection of deformed wing virus in *A. mellifera* colonies (Dainat *et al*., 2009; Forsgren *et al*., 2009). Methods involved with studying bee viruses can be found in Dietemann *et al*. (2013).

7. Future research priorities

Much remains to be learnt about *Tropilaelaps* mites. The taxonomy of the genus is one area that has been relatively well studied, and it now seems to be well resolved. Given that each species is closely associated with a particular giant Asian bee species or sub-species, and that most of these bees have been examined for mites, it is unlikely that further species will be discovered. Nevertheless, more haplotypes of each species will undoubtedly be found, as has recently been demonstrated in China (Luo *et al*., 2011). With this in mind, the follow list highlights areas that warrant immediate research effort.

1. Morphological descriptions of the different life stages of all species.
2. Life habits and reproductive behaviour of all species on both their native and adopted hosts.
3. Control of *T. mercedesae* and *T. clareae* on *A. mellifera*.
4. Associations of microbial pathogens with *T. mercedesae* and *T. clareae* infestations on *A. mellifera*.
5. Resistance (or tolerance) mechanisms of Asian bees to *Tropilaelaps* mites.
6. Genomic sequence information on *T. mercedesae* and *T. clareae*.

8. Concluding comments

Tropilaelaps mites, particularly *T. mercedesae* and *T. clareae*, present a serious threat to world beekeeping and hence they deserve the immediate attention of the global research community. Of the two species, *T. mercedesae* is the more likely to spread out of Asia, as it is found throughout mainland Asia and South East Asia, while *T. clareae* is confined just to the Philippines.

Evidence suggests that while these two species may be as, if not more, pathogenic to *A. mellifera* as *V. destructor*, they may be easier to control for small-scale beekeepers. However, large-scale commercial beekeepers that manage hundreds and thousands of bee colonies, and cannot afford the time to keep them broodless for even short periods, will find these mites as difficult to control as *V. destructor*.

Pre-emptive research carried out on *Tropilaelaps* mites before they spread globally is far more desirable than reactive research carried out once the mites have spread, as it reduces potential future losses and hardship for beekeepers.

9. Acknowledgements

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10. References

ANDERSON, D L (1994) Non-reproduction of *Varroa jacobsoni* in *Apis mellifera* colonies in Papua New Guinea and Indonesia. *Apidologie* 25: 412-421.

ANDERSON, D L; MORGAN, M J (2007) Genetic and morphological variation of bee-parasitic *Tropilaelaps* mites (Acari: Laelapidae): new and re-defined species. *Experimental and Applied Acarology* 43: 1-24.

ANDERSON, D L; TRUEMAN, J W H (2000) *Varroa jacobsoni* is more than one species. *Experimental and Applied Acarology* 24: 165-189.

ATWAL, A A; GOYAL, N P (1971) Infestation of honey bee colonies with *Tropilaelaps*, and its control. *Journal of Apicultural Research* 10: 137-142.

BAKER, A B; HICK, A; CHMIELEWSKI, W (2005) Aspects of the history and biogeography of the bee mites *Tropilaelaps clareae* and *T. koenigerum*. *Journal of Apicultural Science* 49: 13-19.

BURGETT, M D; AKRATANAKUL, P; MORSE, R A (1983) *Tropilaelaps clareae*: a parasite of honey bees in South-East Asia. *Bee World* 64: 25-28.

CLINCH, P (1979) *Nosema apis* and mites in honey bee colonies in Papua New Guinea. *Journal of Apicultural Research* 18: 298-301.

DAINAT, B; KEN, T; BERTHOUD, H; NEUMANN, P (2009) The ectoparasitic mite *Tropilaelaps mercedesae* (Acari, Laelapidae) as a vector of honey bee viruses. *Insectes Sociaux* 56: 40-43. http://dx.doi.org/10.1007/s00040-008-1030-s

DEFRA (2005) Tropilaelaps: *parasitic mites of honey bees*. UK Department for environment, Food and Rural Affairs; London, UK. 14 pp.

DEJONG, D; MORSE, E W; EICKWORT, G C (1982) Mite pests of honey bees. *Annual Review of Entomology* 27: 229-252.

DELFINADO, M D; BAKER, E W (1961) *Tropilaelaps*, a new species of mite from the Philippines (Laelapidae [s. lat]: Acarina). *Fieldiana Zoology* 44: 53-56.

DELFINADO-BAKER, M; AGGARWAL, K (1987) Infestations of *Tropilaelaps clareae* and *Varroa jacobsoni* in *Apis mellifera* colonies in Papua New Guinea. *American Bee Journal* 127: 443.

DELFINADO-BAKER, M D; BAKER, E W (1982) A new species of *Tropilaelaps* parasitic on honey bees. *American Bee Journal* 122: 416-417.

DIETEMANN, V; NAZZI, F; MARTIN, S J; ANDERSON, D; LOCKE, B; DELAPLANE, K S; WAUQUIEZ, Q; TANNAHILL, C; FREY, E; ZIEGELMANN, B; ROSENKRANZ, P; ELLIS, J D (2013) Standard methods for varroa research. In *V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS* BEEBOOK*, Volume II: standard methods for* Apis mellifera *pest and pathogen research. Journal of Apicultural Research* 52(1): http://dx.doi.org/10.3896/IBRA.1.52.1.09

FORSGREN, E; DE MIRANDA, J R; ISAKSSON, M; WEI, S; FRIES, I (2009) Deformed wing virus associated with *Tropilaelaps mercedesae* infesting European honey bees (*Apis mellifera*). *Experimental and Applied Acarology* 47: 87-97. http://dx.doi.org/10.1007/s10493-008-9204-4

KHONGPHINITBUNJONG, K; DE GUZMAN, L I; BURGETT, M D; RINDERER, T E; CHANTAWANNAKUL, P (2012) Behavioural responses underpinning resistance and susceptibility of honey bees to *Tropilaelaps mercedesae*. *Apidologie* 43: 590-599. http://dx.doi.org/10.1007/s13592-012-0129-x

KIPRASERT, C (1984) Biology and systematics of the parasitic bee mite *Tropilaelaps clareae* Delfinado and Baker (Acarina: Laelapidae). MSc thesis, Kasetsart University; Bangkok, Thailand.

KOENIGER, N; MUSAFFAR, N (1988) Lifespan of the parasitic honey bee mite *Tropilaelaps clareae* on *Apis cerana*, *dorsata* and *mellifera*. *Journal of Apicultural Research* 27: 207-212.

KUMAR, N R; KUMAR, R; MBAYA, J; MWANGI, R W (1993) *Tropilaelaps clareae* found on *Apis mellifera* in Africa. *Bee World* 74: 101-102.

LAIGO, F M; MORSE, R A (1969) Control of the bee mites *Varroa jacobsoni* Oudemans and *Tropilaelaps clareae* Delfinado and Baker with chlorobenzilate. *Philippine Entomologist* 1: 144-148.

LE CONTE, Y; NAVAJAS, M (2008) Climate change: impact on bee populations and their illnesses. *Revue Scientifique Et Technique – Office International Des Epizooties* 27: 485-497.

LINDQUIST, E E; KRANTZ, G W; WALTER, D E (2009) Order Mesostigmata. In *G W Krantz; D E Walter (Eds). A manual of acarology.* Texas Technical University Press. pp 124-232.

LUO, Q H; ZHOU, T; WANG, Q; DAI, P L; WU, Y Y; SONG, H L (2011) Identification of *Tropilaelaps* mites (Acari, Laelapidae) infesting *Apis mellifera* in China. *Apidologie* 42: 485-498. http://dx.doi.org10.1007/s13592-011-0028-x

MATHESON, A (1997) Country records for honey bee diseases, parasites and pests. Appendix 11. In *R M Morse; K Flottum (Eds). Honey bee pests, predators and diseases (3rd Ed.).* A I Root Co., Medina OH, USA. pp 586-602.

OIE (2004) *Tropilaelaps* infestation of honey bees (*Tropilaelaps clareae*, *T. koenigerum*). In *Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees) (5th Ed.).* Office International des Epizooties; Paris, France.

OTIS, G W; KRALJ, J (2001) Parasitic brood mites not present in North America. In *T C Webster; K S Delaplane (Eds). Mites of the honey bee.* Dadant and Sons; Illinois, USA. pp 251-272.

PICHAI, K; POLGAR, G; HEINE, J (2008) The efficacy of Bayvarol® and CheckMite+® in the control of *Tropilaelaps mercedesae* in the European honey bee (*Apis mellifera*) in Thailand. *Apiacta* 43: 12-16.

RATH, W; DELFINADO-BAKER, M; DRESCHER, W (1991) Observations on the mating behaviour, sex ratio, phoresy and dispersal of *Tropilaelaps clareae* (Acari: Laelapidae). *International Journal of Acarology* 17: 201-208.

RAFFIQUE, M K; MAHMOOD, R; ASLAM, M; SARWAR, G (2012) Control of *Tropilaelaps clareae* mite by using formic acid and thymol in honey bee *Apis mellifera* colonies. *Pakistan Journal of Zoology* 44: 1129-1135.

RINDERER, T E; OLDROYD, B P; LEKPRAYOON, C; WONGSIRI, S; THAPA, R (1994) Extended survival of the parasitic mite *Tropilaelaps clareae* on adult workers of *Apis mellifera* and *Apis dorsata*. *Journal of Apicultural Research* 33: 171-174.

RITTER, W (1988) *Varroa jacobsoni* in Europe the tropics and subtropics. In *G R Needham; R E Page, Jr; M Delfinado-Baker; C E Bow (Eds). Africanized honey bees and bee mites.* Halsted Press; Chichester, UK. pp 349-359.

RITTER, W; SCHNEIDER-RITTER, U (1986) *Varroa jacobsoni* und *Tropilaelaps clareae* in bienenvolkern von *Apis mellifera* in Thailand. *Apidologie* 17: 384-386.

RITTER, W; SCHNEIDER-RITTER, U (1988) Differences in biology and means of controlling *Varroa jacobsoni* and *Tropilaelaps clareae*, two novel parasitic mites of *Apis mellifera*. In *G R Needham; R E Page, Jr; M Delfinado-Baker; C E Bow (Eds). Africanized honey bees and bee mites.* Halsted Press; Chichester, UK. pp 378-395.

SALEU, L (1994) Studies of the parasitic mite *Tropilaelaps clareae* in *Apis mellifera* colonies in Papua New Guinea. MSc thesis, The Australian National University; Canberra, Australia 96 pp.

TANGJINGJAI, W; VERAKALASA, P; SITTIPRANEED, S, LEKPRAYOON, C (2003) Genetic differences between *Tropilaelaps clareae* and *Tropilaelaps koenigerum* in Thailand based on ITS and RAPD analyses. *Apidologie* 34: 514-524. http://dx.doi.org/10.1051/apido:2003042

UNDERWOOD, B A (1986) The natural history of *Apis laboriosa* Smith in Nepal. MSc thesis, Cornell University; Ithaca, NY, USA.

WALTER, D E; PROCTOR, H C (1999) *Mites: ecology, evolution and behaviour*. UNSW Press. 322 pp.

WILDE, J (2000) Is it possible to introduce *Tropilaelaps clareae* together with imported honey bee queens to Europe? *Pszczeln. Zesz. Nauk*. 44: 155-162.

WOYKE, J (1984) Survival and prophylactic control of *Tropilaelaps clareae* infesting *Apis mellifera* colonies in Afghanistan. *Apidologie* 15: 421–434.

WOYKE, J (1985a) *Tropilaelaps clareae*, a serious pest of *Apis mellifera* in the tropics, but not dangerous for apiculture in temperate zones. *American Bee Journal* 125: 497–499.

WOYKE, J (1985b) Further investigation into control of the parasitic bee mite *Tropilaelaps clareae* without medication. *Journal of Apicultural Research* 24: 250–254.

WOYKE, J (1987) Length of successive stages in the development of the mite *Tropilaelaps clareae* in relation to honey bee brood age. *Journal of Apicultural Research* 26: 110–114.

WOYKE, J (1994) Mating behaviour of the parasitic honey bee mite *Tropilaelaps clareae*. *Experimental and Applied Acarology* 18: 723 –733.