

Prevalence and reproduction of *Tropilaelaps mercedesae* and *Varroa destructor* in concurrently infested *Apis mellifera* colonies

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Abstract – The prevalence of *Tropilaelaps mercedesae* and *Varroa destructor* in concurrently infested *A. mellifera* colonies in Thailand was monitored. We also assessed the fecundity of *T. mercedesae* and *V. destructor* in naturally infested brood and in brood cells deliberately infested with both mite genera. Results showed that the natural co-infestation of an individual brood cell by both mite genera was rare (<0.1 %). Overall, *T. mercedesae* was the more dominant brood parasite of *A. mellifera* than *V. destructor*. In naturally infested brood, the proportion of nonreproductive *Tropilaelaps* (29.8±3.9 %) was lower than that of *Varroa* (49.6±5.9 %). Both mites produced similar numbers of progeny (*T. mercedesae* = 1.48±0.05; *V. destructor* = 1.69±0.14). The two mite genera also reproduced normally when they were deliberately introduced into the same brood cells. In two separate assessments, the average worker brood infestations of *T. mercedesae* (19.9 %) were significantly higher than that of *V. destructor* (0.7 %). Our results on the higher prevalence and reproductive ability of *T. mercedesae* in concurrently infested colonies reaffirm *Tropilaelaps*' competitive advantage over *V. destructor* and their reported negative impact to *A. mellifera* colonies.

Apis mellifera / *Tropilaelaps mercedesae* / *Varroa destructor* / concurrent infestation / seasonal abundance

1. INTRODUCTION

Varroa destructor and *Tropilaelaps* have been co-infesting *A. mellifera* colonies for about 50 years in Asia (Delfinado 1963). However, infestations of *T. clareae* (likely referring to *T. mercedesae*) were higher than those of *V. jacobsoni* (likely referring to *V. destructor*) in Thailand (Burgett et al. 1983). Similar trends were observed in Afghanistan and Vietnam (Woyke 1987a, 1989). However, in the Philippines, those

A. mellifera colonies that had higher infestations of *T. clareae* than *Varroa* in April had higher *Varroa* than *T. clareae* infestations in September (Fajardo and Cervancia 2004). In Northern Thailand, Kavinseskan et al. (2003) monitored *T. clareae* (probably referring to *T. mercedesae*) infestations in mite-inoculated colonies of Primorsky bees (=Russian honey bees, RHB) and Thai *A. mellifera*. The author found that RHB colonies (mean=18.5 %) supported higher brood infestation than the local bees (mean=11.4 %) with the highest infestations observed in May (RHB=33 %, Thai *A. mellifera* =21 %). Factors that influence population fluctuations of both mites in concurrently infested colonies have not been studied.

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Although both *V. destructor* and *T. mercedesae* are observed in infesting colonies, *T. mercedesae* is considered to be a more serious problem of *A. mellifera* colonies than *Varroa* mites in Northern Thailand (Burgett et al. 1983; Anderson and Morgan 2007). This discrepancy in severity may be due to differences in their abilities to compete for honey bee hosts and reproduce within brood cells. In this study, we monitored the build-up and synchronization in the populations of both *V. destructor* and *T. mercedesae* in concurrently infested *A. mellifera* colonies. Reproduction was also assessed in naturally infested brood and in brood cells deliberately infested with both mite genera to determine if variation in reproduction exists. Knowledge on differential reproduction may help explain population fluctuations, competitive advantage, or virulence of one mite species.

2. MATERIALS AND METHODS

2.1. Experiment 1: brood infestations of *V. destructor* and *T. mercedesae* in concurrently infested *A. mellifera* colonies

Observations were conducted using 16 colonies housed in 10-frame Langstroth hives from September 2011 to September 2012. No acaricidal treatments were applied to the colonies during the course of the study. All queens were hybrids based on an Italian honey bee (*A. m. ligustica*). The brood area (cm²) was determined by visual estimation of comb area covered by capped brood (Rogers et al. 1983). Mite infestation parameters were determined by randomly examining 50–100 worker brood cells from each colony on a monthly schedule (de Guzman et al. 2007). Stages of mite progeny were differentiated and recorded.

Since the results of the 13-month observation showed rare co-infestations of both *Tropilaelaps* and *Varroa* mites, we decided to confirm our observation by examining three additional concurrently infested *A. mellifera* colonies. For each colony, different stages of worker and drone brood were examined for the presence or absence of these two parasitic mites. Adult bee infestation was also determined by sampling about 400–500 bees per colony and washed with soapy water to remove mites (Rinderer et al. 2004). The mites were collected and then differentiated according to species.

2.2. Experiment 2: comparative reproduction of *V. destructor* and *T. mercedesae* in artificially inoculated brood

During the conduct of experiment 1, we rarely observed brood cells that were infested with both *Tropilaelaps* and *Varroa*. This experiment sought to investigate mite reproduction when both *Tropilaelaps* and *Varroa* were deliberately introduced into the same brood cells. To provide colonies as free of mites as possible, eight previously acaricide-treated *A. mellifera* colonies were used in this study (taufuvalinate was used as acaricide in the bee colonies). To obtain brood of the same age, each queen was caged over an empty comb for 24 h by using a push-in cage (8 mesh screen) providing a brood area of about 400 brood cells. On the eighth day when brood cells were capped, one foundress *T. mercedesae* and one *V. destructor* were introduced into the same brood cell. All inoculum foundress mites (dark in color) were collected from tan-bodied pupae of highly infested *A. mellifera* colonies. Inoculum *Tropilaelaps* were first examined under a dissecting microscope to exclude males. To inoculate newly sealed larvae, the mite transfer technique was used (Garrido and Rosenkranz 2003; Kirrane et al. 2011; Khongphinitbunjong et al. 2013). Nine days following mite inoculation, the brood cells containing tan-bodied pupae were opened to assess mite reproduction. All stages of mites were differentiated.

2.3. Mite reproductive status

For experiment 1, reproductive foundress *Tropilaelaps* and *Varroa* mites were those that had at least one progeny. Experiment 2 used two criteria to assess the proportions of nonreproductive foundress mites in order to compare the results of previous studies (de Guzman et al. 2007; Khongphinitbunjong et al. 2013). For criteria 1, reproductive foundress *Varroa* mites were those that produced an adult male and young daughter or viable offspring (de Guzman et al. 2008). Since *Tropilaelaps* mites have shorter life cycle when compared to *Varroa*, it is possible that *Tropilaelaps* foundress may lay more eggs which can develop to adult offspring by the time of the bee emergence (Sihag 1988; Sammatore et al. 2000). Woyke (1987a) reported that *Tropilaelaps* was also able to copulate outside the natal cell. Thus, reproductive foundress

Tropilaelaps mites were those that had at least one progeny (Khongphinitbunjong et al. 2013). For criteria 2, regardless of the mites' mating behavior, reproductive foundress *Varroa* or *Tropilaelaps* were those that produced ≥ 1 progeny.

2.4. Data analyses

For experiment 1, only brood cells infested with either *T. mercedesae* or *V. destructor* were considered for statistical analyses. Prior to analyses, data on the percentage infestation and percentage nonreproduction (NR) were transformed using an arcsine square-root transformation. A repeated measures analysis of variance (ANOVA) with observation dates and mite type as the main effects was performed to determine differences in infestations of both mite types through time. A *z*-test for proportions was used to compare the overall nonreproductive status for both mite species. A one-way ANOVA was used to determine infestation trends of *V. destructor* and *T. mercedesae* and the amount of brood in the colony through time.

For experiment 2, a *z*-test for two proportions was used to compare reproduction success between the two mite genera. Differences in the reproductive status for each trial were compared using the Marascuillo procedure for multiple proportions (<http://www.itl.nist.gov/div898/handbook/prc/section4/prc474.htm>). A paired sample *t* test was used to compare differences in the number of progeny produced by foundress *Tropilaelaps* and *Varroa* mites.

3. RESULTS

3.1. Experiment 1: infestations of *V. destructor* and *T. mercedesae* in concurrently infested *A. mellifera* colonies

The test colonies reared brood continuously during the experimental period. The highest numbers of sealed brood cells were recorded in March to June 2012 (Figure 1) ($F_{11,178}=4.63$, $P<0.0001$). Of the 18,250 worker brood cells examined throughout this experiment, only 13 brood cells ($<0.1\%$) were found to be concurrently infested with both *V. destructor* and *T. mercedesae*. Of the 970 infested brood cells, 24% were infested with *Varroa*, while 76% were

infested by *Tropilaelaps*. Only nine colonies (out of 16 colonies) produced drone brood during the experiment. In total, 506 drone brood cells were produced throughout the experiment, and all were examined. Only 13 drone cells (2.6%) were infested with *Tropilaelaps*, and 78 (15.4%) were infested with *Varroa* mites.

Our results showed significant effects of both mite type ($F_{1,13}=42.75$, $P<0.0001$) and date of observations ($F_{12,137}=5.80$, $P<0.0001$) for the prevalence of both mite genera. Since there was a significant interaction between mite type and date of observation ($F_{12,136}=4.39$, $P<0.0001$), the differences in infestation rates between mite genera were determined for each date of observation. Initially in September 2011, the colonies began with a significantly higher infestation of *Tropilaelaps* ($10.6\pm 2.8\%$) than *Varroa* ($2.1\pm 1.0\%$) ($F_{1136}=20.94$, $P<0.0001$) (Figure 2). Thereafter, infestation levels of both mite genera decreased significantly although *Tropilaelaps* infestations remained higher than those of *Varroa* from October, November, and December 2011 ($F_{1136}=23.55$, $P<0.0001$; $F_{1136}=8.76$, $P=0.0036$; $F_{1136}=4.74$, $P=0.0311$, respectively). Infestation by *Tropilaelaps* significantly decreased in January 2012, slightly increased in February 2012 with a small peak in March 2012, a gradual decrease in April 2012 and a steep decline in May 2012. However, infestations by *Tropilaelaps* and *Varroa* were similar during these months (January, $F_{1136}=0.22$, $P=0.6374$; February, $F_{1136}=0.41$, $P=0.5253$; April, $F_{1136}=3.06$, $P=0.0823$; May, $F_{1136}=0.72$, $P=0.3987$) except in March 2012 when *Tropilaelaps* had higher infestation than *Varroa* mites ($F_{1136}=4.24$, $P=0.0413$). Infestations by both mite species remained similarly low in June 2012 ($F_{1136}=5.26$, $P=0.0233$). At this time, only four of the 15 surviving colonies were infested. Although infestation by *Varroa* increased in July 2012, no difference in the rates of infestation between the mite species was observed ($F_{1136}=2.54$, $P=0.1135$). Infestation by both mite species similarly decreased in August ($F_{1136}=2.82$, $P=0.0957$) when only 11 colonies were sampled because several colonies were too weak to sample. Infestations increased again in September 2012 with *Tropilaelaps* having a higher rate of infestation

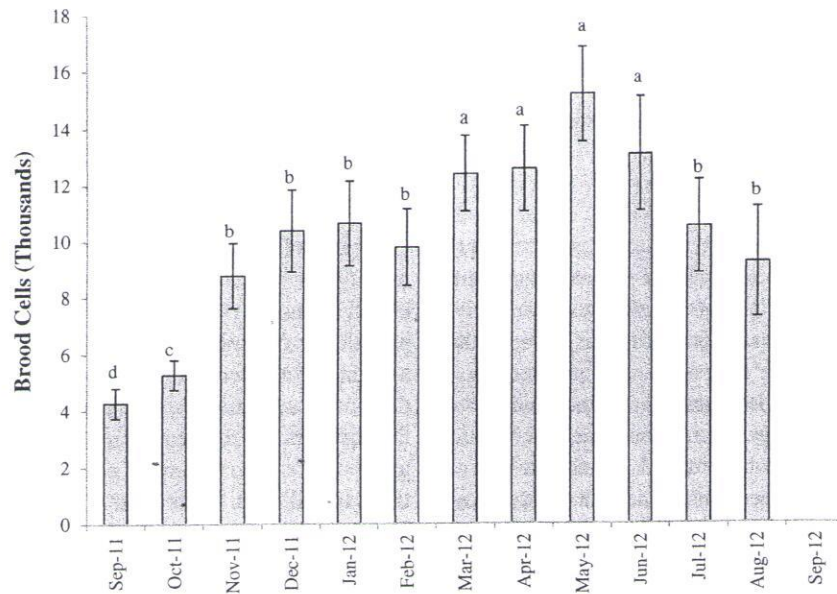


Figure 1. The average number (mean \pm SE) of sealed worker brood cells for the 16 colonies monitored for 13 months.

than *Varroa* ($F_{1136}=15.21$, $P=0.0002$). However, there were only four colonies remained alive or strong enough to sample at this time. There was a significant negative correlation between brood

area (cm^2) and *Tropilaelaps* infestation ($r = -0.248$; $P=0.0007$). No correlation between brood area and *Varroa* infestation was detected ($r=0.023$; $P=0.752$).

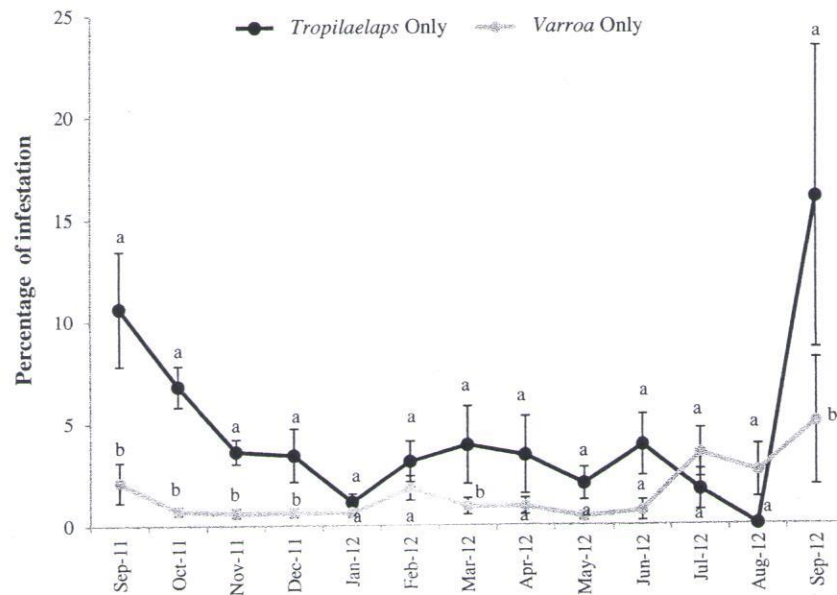


Figure 2. Prevalence (mean \pm SE) of *T. mercedesae* and *V. destructor* in worker brood cells of concurrently infested *A. mellifera* colonies through time.

A separate examination of three concurrently infested colonies showed a similar trend. Out of the 1230 worker brood cells examined, only four cells (0.3 %) were concurrently infested. *Tropilaelaps* was the more predominant mite species than *Varroa* mites in worker brood cells with an average infestation of 19.9 % (*Tropilaelaps*) and 0.7 % (*Varroa*). *Varroa* (2.5 %) infestation was numerically higher than that of *Tropilaelaps* (1.9 %), and no concurrent infestation was observed in the drone brood ($n=481$ cells). Adult bee infestation was also low: *Tropilaelaps* = 0.31 % and *Varroa* = 0.16 %.

Proportion of nonreproductive mites ANOVA revealed significant mite type ($F_{1, 13}=15.05$; $P=0.0019$) and date of observation ($F_{12, 95}=3.29$; $P=0.0005$), but no two-way interaction ($F_{11, 24}=1.41$; $P=0.2311$) for the proportion of NR foundresses was detected (Figure 3). Regardless of mite type, the highest proportion of NR foundresses were observed in January 2012 and the lowest NR in November 2011. Overall, there

were more *Varroa* mites that did not reproduce as compared to *Tropilaelaps*. Further, both mites produced similar number of progeny when observed in purple-eyed and tan-bodied pupae (*Tropilaelaps* = 1.48 ± 0.05 ; *Varroa* = 1.69 ± 0.14 progeny per foundress) ($t=0.88$, $P=0.381$).

3.2. Experiment 2: reproduction of *V. destructor* and *T. mercedesae* in deliberately infested worker brood

Our results showed that the reproduction of *Tropilaelaps* and *Varroa* was similar in brood cells ($n=254$ tan-bodied pupae) deliberately infested with both mite species ($z=1.84$, $P<0.01$). In addition, 45 % (criteria 1: *Varroa* mites had one adult male and daughter, while *Tropilaelaps* mites had at least one progeny) or 52 % (criteria 2: *Varroa* or *Tropilaelaps* had those which produced ≥ 1 progeny) supported reproduction of both mites (Table I). Only 15 % of the inoculated brood cells had both mites that did not

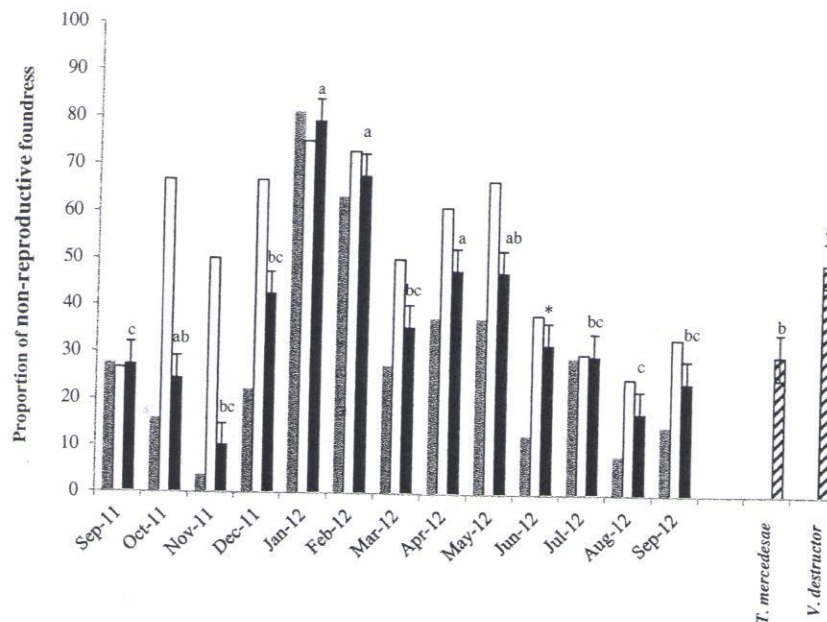


Figure 3. Proportion (mean \pm SE) of nonreproductive foundress (did not produce any progeny) in naturally infested brood cells. Black bars indicate the proportions of nonreproductive (NR) foundress regardless of mite species, gray bars for NR *T. mercedesae*, and white bars for NR *V. destructor* for each month of observation. Striped bars represent the average NR for *T. mercedesae* and *V. destructor* (infested colonies = 16). June was nonestimable in the means comparison because of low sample size (infested colonies = 4) (asterisk).

Table I. Reproduction of *T. mercedesae* (T) and *V. destructor* (V) when co-inhabiting single host pupa ($n=254$ brood cells that were deliberately infested).

Reproductive status	Criteria 1	Criteria 2
VNR, TNR	18.1 % ($n=46$)	15.0 % ($n=38$)
VNR, TR	24.0 % ($n=61$)	17.3 % ($n=44$)
VR, TNR	13.0 % ($n=33$)	16.1 % ($n=41$)
VR, TR	44.9 % ($n=114$)	51.6 % ($n=131$)

Criteria 1 =reproductive *Varroa* produced adult male and young daughter; reproductive *Tropilaelaps* had ≥ 1 progeny. Criteria 2 = reproductive *Varroa* and *Tropilaelaps* s produced ≥ 1 progeny

R reproductive; NR nonreproductive; VNR, TNR both *V. destructor* and *T. mercedesae* foundress are nonreproductive; VNR, TR *V. destructor* foundress is nonreproductive but *T. mercedesae* is reproductive; VR, TNR *V. destructor* foundress is reproductive but *T. mercedesae* is nonreproductive; VR, TR foundress of both mite species are reproductive

produce any progeny. *V. destructor* produced more progeny per foundress (2.2 ± 0.1) than did *T. mercedesae* (1.5 ± 0.1) ($t=5.31$, $P < 0.0001$) in concurrently infested hosts.

4. DISCUSSION

V. destructor and *Tropilaelaps* have been co-infesting *A. mellifera* colonies for about 50 years in Asia (Delfinado 1963). Our investigation demonstrates that *T. mercedesae* is competitively superior to *V. destructor* mites in concurrently infested *A. mellifera* colonies in Northern Thailand. The infestation rates by both mite genera fluctuated throughout the study and showed almost identical patterns. Based on our two assessments, the abundance of *Tropilaelaps* than *Varroa* observed in this study agreed with previous observations stating that *Tropilaelaps* mites outcompete *Varroa* mites in *A. mellifera* colonies (Burgett et al. 1983; Pettis et al. 2012). However, our observation is in contrast to what has been shown in South Korea where *V. destructor* infestation rates are greater than *Tropilaelaps* infestation (Lee et al. 2005). It is also possible that the dramatically different climatic conditions for Korea (temperate) compared to Thailand (tropical) are largely responsible for this discrepancy in prevalence. For example, the environmental conditions in Pakistan allow continuous rearing of brood and thus survival of *Tropilaelaps*

(Waghchoure-Camphor and Martin 2009). According to these authors, *T. clareae* (likely referring to *T. mercedesae* based on species distribution reported by Anderson and Morgan 2007) infestations coincide with the increase in brood production (April to May). In our study, we found a negative correlation between the amount of brood and *Tropilaelaps* infestation, which corroborated the findings of Kavinseksan et al. (2004)). This decrease in infestations with the increase in brood production may reflect the "dilution" effect observed in the case of *Acarapis* mites (de Guzman and Burgett 1991). These authors explained that the parasite infestation rate cannot increase at the same rate as the host population.

The dominance of *Tropilaelaps* over *Varroa* may also be influenced by their ability to reproduce. Overall, both mites produced similar numbers of progeny on average. However, higher proportion of *Tropilaelaps* (70 %) than *Varroa* (50 %) produced at least one progeny. This ability to reproduce even just one progeny may increase the population of *Tropilaelaps* faster than *Varroa* mites.

Varroa is known to prefer drone brood about three to eight times more than worker brood (Fuchs and Langenbach 1989). Thus, the overall low infestation of *Varroa* mites may be associated with the minimal production of drone brood during this study. In contrast, *T. clareae* infests

worker brood about 1.5 times more than drone brood (Woyke 1987b). When infesting its indigenous host, *A. dorsata*, *T. mercedesae* did not exhibit host sex preference, i.e., drone and worker brood experienced similar infestation rates (Buawangpong et al. 2013). In this study, only a few of the colonies produced drone brood (total=506) throughout the experiment. Nevertheless, the infestation rate of drone brood by *Varroa* mites was 3.6 times more than worker brood. For *Tropilaelaps* mites, the infestation rate of worker brood was 9.4 times greater than that of drone brood. However, we cannot conclude whether or not *Tropilaelaps* prefer worker over drone brood because of limited production of drone brood.

The co-infestation of a single host by *Tropilaelaps* and *Varroa* is rare, an observation also reported by Ritter and Schneider-Ritter (1988) and Burgett et al. (1989) with the *Acarapis* species complex. In general, insect frass and its volatile components provide cues in habitat location (Weiss 2006). In this study, avoidance of an infested cell may be one of the reasons for such a low mixed-genera infestation. It is possible that a blend of chemicals or volatiles produced by the resident *Tropilaelaps* itself or from their feces and wounds of honey bee hosts deters *Varroa* mites from invading. For *Varroa* mites, they submerge in the larval food of a L4 larvae after invasion. Thus, we are unsure if they too are able to produce these volatiles while being submerged. It is also unlikely that the mites are competing for food or space since infestations by both mites were generally low and that brood was available for infestation. We found that both mite species reproduced similarly when introduced together in the same brood cell. This reproductive fecundity of *T. mercedesae* may contribute to their higher prevalence, an indication of increased virulence of this mite species for *A. mellifera* colonies in Thailand. Also, possible infections from other pathogens vectored by *Tropilaelaps*, e.g., DWV virus (Dainat et al. 2009; Khongphinitbunjong et al. 2015), that can have synergistic effects on the overall

health of infested colonies should be of consideration for further studies.

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Prévalence et reproduction de *Tropilaelaps mercedesae* et *Varroa destructor* dans des colonies d'*Apis mellifera* infestées simultanément

Apidae / abeilles / acariens / infestation simultanée / abondance saisonnière / Thaïlande

Verbreitung und Fortpflanzung von *Tropilaelaps mercedesae* und *Varroa destructor* in gleichzeitig befallenen Völkern von *Apis mellifera*

Apidae / Honigbiene / Milben / gleichzeitiger Befall / saisonale Häufigkeit / Thailand

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