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Original article

Mites from debris and sealed brood cells of *Apis dorsata* colonies in Sabah (Borneo) Malaysia, including a new haplotype of *Varroa jacobsoni*

Gudrun KOENIGER[a]*, Nikolaus KOENIGER[a], Denis L. ANDERSON[b], Chariya LEKPRAYOON[c], Salim TINGEK[d]

[a] Institut für Bienenkunde, Universität Frankfurt, Karl-von-Frisch-Weg 2, 61440 Oberursel, Germany
[b] CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia
[c] Bee Research Unit, Chulalongkorn University, Bangkok 10330, Thailand
[d] Agricultural Research Station Tenom, Peti Surat 197, Tenom 89908, Sabah, Malaysia

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Abstract – In the debris of five *Apis dorsata* colonies at a single site in Sabah, Borneo we found the mites *Tropilaelaps clareae, Tropilaelaps koenigerum, Varroa rindereri, Varroa jacobsoni* and *Euvarroa wongsirii*. Most were *T. clareae*, but *T. koenigerum* were also quite common. The *V. rindereri* specimens belonged to the same haplotype as described previously from *A. koschevnikovi* from Borneo. However, the *V. jacobsoni* belonged to a new haplotype, which we named the 'Borneo 2 haplotype of *V. jacobsoni*'. Of the mites detected in the debris, 84% of the *T. clareae* and 57% of the *T. koenigerum* were damaged. Inspection of 1673 brood cells of two *A. dorsata* colonies at the same site resulted in adult *T. clareae* and *T. koenigerum* together with their offspring (nymphs). The percentage of infested drone and worker cells did not differ, nor did the number of mites per cell: 6.0 ± 1.6 in worker brood and 6.1 ± 1.9 in drone brood (n = 10). We found no *Varroa* mites in the inspected brood cells, suggesting that the mites do not reproduce in *A. dorsata* and indicating that interspecific mite transfer occurs between sympatric *Apis* species in Borneo.

***Apis dorsata* / Borneo 2 haplotype of *V. jacobsoni* / *V. rindereri* / debris / *Tropilaelaps* species**

1. INTRODUCTION

The recent and still progressing introduction of *Apis mellifera* L. into many parts of Asia has had a major impact on the indigenous honeybee species (Verma, 1993). Among several other effects, the natural bee host-mite parasite balance may be severely altered. Brood parasitic mite species, formerly restricted to a single indigenous

* Correspondence and reprints
E-mail: Gudrun.Koeniger@em.uni-frankfurt.de

Apis species, successfully invaded the introduced *A. mellifera* colonies and have multiplied exponentially.

Aggarwal (1988) reviewed the incidences of *Tropilaelaps clareae* Delfinado & Baker in colonies of three *Apis* species in India, *Apis dorsata* Fabr., *A. mellifera* and *Apis florea* Fabr. In the *A. dorsata* and *A. mellifera* colonies a "reproductive continuum" was found, while infestations in the *A. florea* colonies were always very low and no mites were found in brood cells. Rath et al. (1991) also studied the debris of two *A. dorsata* colonies in Chiang Mai, Thailand. Within 32 and 27 days, respectively, more than 3000 *T. clareae* mites were counted, but no other mites were detected.

The exploration of the host-parasite relationship of brood parasitic mites and their natural honey bee host species has become an urgent matter because there are now only a few regions left in Asia into which *A. mellifera* has not been introduced. The relationship in Sri Lanka was studied in 1983, when no *A. mellifera* were present. Brood cells and adult bees were examined in the three bee species, *A. dorsata*, *Apis cerana* Fabr. and *A. florea*. Only one brood parasitic mite species was found on each honey bee species: *Tropilaelaps koenigerum, Varroa jacobsoni* Oudemans and *Euvarroa sinhai* Delfinado & Baker, respectively (Koeniger et al., 1983). More recent studies resulted in a more complex picture. Closely related *Apis* species may host the same mite species and also closely related mite species can parasite the same *Apis* species, they may occur even in the same colony. So, the natural distribution of the mites corresponds well to the systematic position (Koeniger, 1990).

In this study, we collected the debris of five *A. dorsata* colonies in Tenom, Sabah, Malaysia, for a period of about four weeks. In addition, we inspected worker and drone brood cells of two other *A. dorsata* colonies at this site to check for the presence and reproduction of mites. Three other indigenous honeybee species were nesting nearby: *A. andreniformis* F. Smith, *A. cerana* and *Apis koschevnikovi* v. Buttel-Reepen. No *A. mellifera* colonies had ever been introduced into this part of Borneo.

2. MATERIALS AND METHODS

In February and March 2000, nine colonies of *A. dorsata* were nesting naturally under the roof of the bee museum in the Agricultural Research Station at Tenom, Sabah, Malaysia. We selected four colonies for our studies. Colony 9 was small, measuring about 50 × 30 cm, colony 8 measured 100 × 80 cm, and colonies 3 and 2 each measured about 130 × 90 cm. Colony 2 had been present at the site for more than a year, while the other colonies had settled 3–4 months before the start of the studies. During the course of the studies, colony 2 produced several reproductive swarms, and all colonies had drone brood cells present. In February 2001, an *A. dorsata* swarm settled at the same site and it became part of our studies.

2.1. Bottom boards

Bottom boards of 200 × 80 cm with a 10 cm high rim along the sides were constructed, painted white, and placed on stands directly under each of the five *A. dorsata* colonies. Removable plastic sheets to collect *A. dorsata* nest debris were fixed to the bottom boards. The legs of each stand were placed in a pot of detergent water, to prevent intrusion of ants. The distance between bottom board and colony was about 3.5 m.

2.2. Records

Every morning between 08:00 and 09:00 from 11 February to 23 March 2000, debris that had fallen from each nest onto

the plastic sheets was collected with a soft brush and examined for mites with a dissecting microscope (magnification × 10 to × 20). On some days, heavy rains prevented the collection of debris. Mites were separated from the debris for identification. The daily mite fall was monitored for 22 consecutive days from colonies 2, 3, 8 and 9. On four occasions, the debris samples were pooled for 1 to 4 days before examination with the dissecting microscope. The debris of the swarm was sampled daily for 17 days, from 24 February to 13 March 2001, and then only once a week from 20 March to 28 April 2001.

2.3. Collection of brood and inspection of capped brood cells

During night, smoke was applied to colony 4 to chase the bees away from the lower part of the comb. About 1000 sealed brood cells were cut from the exposed comb and immediately frozen. The comb was later thawed, the cells opened and brood removed. The brood and opened cells were then examined for mites using a dissecting microscope at × 12 magnification, with the aid of a glass fiber illuminator. Brood cells from another *A. dorsata* colony in a tree nearby were also examined after climbing the tree with a rope and removing the whole comb.

2.4. Identification of mites in the *A. dorsata* nest debris

Adult female *T. clareae, T. koenigerum* and *E. wongsirii* were identified in the field from their distinct morphological characteristics. The precise identity of *Varroa* mites was determined using DNA sequencing techniques. For these studies, 32 of 48 *Varroa* mites that were collected from the plastic sheets were placed in 96% methanol and shipped to Canberra, Australia. There, they were immediately frozen at –20 °C. DNA preparations were subsequently obtained from each mite and a region of the mitochondrial DNA (mtDNA) cytochrome oxidase-I (CO-I) coding gene was amplified and sequenced as described by Anderson and Fuchs (1998). The sequences were then compared with known sequences of other *Varroa* mites to obtain a positive identity.

3. RESULTS

3.1. Species and number of mites in the *A. dorsata* nest debris

Several mite species were found in the *A. dorsata* nest debris: *T. clareae, T. koenigerum, V. rindereri, V. jacobsoni* and *E. wongsirii* (Tabs. I–III). Most of the mites were *T. clareae*, although *T. koenigerum* also was quite common. *Varroa* mites were found in low numbers, being detected at a level of less than one mite per colony per day (Tab. IV). Only one *Euvarroa* mite was found. Altogether, we found 48 *Varroa* mites (36 from colonies 2, 3, 8 and 9, eight in the pooled samples collected from these colonies, and four from the newly settled swarm), more than a thousand *T. clareae,* and several hundred *T. koenigerum*.

Adult female *T. clareae, T. koenigerum*, and *Varroa* mites were found in the debris of all five colonies, but *E. wongsirii* was found only under the newly settled swarm. The large colony 2, which issued several reproductive swarms, had the highest number of mites in the debris: 540 *T. clareae* and 20 *Varroa* mites.

Of the 32 female *Varroa* mites that were identified using mtDNA sequencing techniques, three were so deteriorated they could not be positively identified. Of the remaining 29 mites, 21 were *V. rindereri* and eight were *V. jacobsoni*. The mtDNA sequences obtained from each of the female *V. rindereri* were identical to that reported for *V. rindereri* from Borneo by Anderson and Trueman (2000) (GenBank accession number

Table I. Numbers and types of mites detected in the debris of four *A. dorsata* colonies within 22 days.

Colony	*T. clareae*	*T. koenigerum*	*V. rindereri* + *V. jacobsoni*
2	598	90	17
3	246	37	6
8	120	10	7
9	120	16	6
Totals	1 084	153	36

Table II. Numbers and types of mites detected in pooled samples of the four colonies shown in Table I in periods between one and three days.

Sample	*T. clareae*	*T. koenigerum*	*V. rindereri* + *V. jacobsoni*
1	142	20	2
2	233	20	4
3	117	16	1
4	81	13	1
Totals	573	69	8

Table III. Numbers and types of mites detected in the settled swarm (period before emerging brood: 1–17 days. Period with emerging brood: 20 to 48 days).

Period (days)	*T. clareae*	*T. koenigerum*	*V. rindereri* + *V. jacobsoni*	*Euvarroa wongsirii*
1–17	4	0	3	1
20–48	208	37	1	0

Table IV. Average number and mite species per colony per day.

	T. clareae	*T. koenigerum*	*V. rindereri* + *V. jacobsoni*
Col. 2	27.18 ±10.5	4.01 ±2.3	0.77 ±0.8
Col. 3	11.18 ± 6.7	1.68 ±1.9	0.27 ±0.6
Col. 8	5.45 ± 4.7	0.46 ±0.9	0.32 ±0.5
Col. 9	5.45 ± 5.0	0.73 ±1.1	0.27 ±0.5
Swarm (20–48 days after settling)	7.4	1.3	0.04

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  1 ATTTATTTTGATTTTTTGGGCATCCAGAAGTTTATATTTTAATTTTACCT 50    Borneo
    |||||||||||||||||| ||||||||||||||||||||||||||||||
  1 ATTTATTTTGATTTTTTGGACATCCAGAAGTTTATATTTTAATTTTACCT 50    Borneo 2


 51 GGATTTGGAATTATTTCTCATGTAATTTGTATACAAAGAGGGAAAAAGCA 100   Borneo
    ||||||||||||||||||||||||||||||||||||||||||||||||||
 51 GGATTTGGAATTATTTCTCATGTAATTTGTATACAAAGAGGGAAAAAGCA 100   Borneo 2


           .         .         .         .         .
101 ACCTTTTGGTAATTTAGGGATAATTTATGCTATAATAACTATTGGTATTT 150   Borneo
    ||||||||||||||||||||||||||||||||||||||||||||||||||
101 ACCTTTTGGTAATTTAGGGATAATTTATGCTATAATAACTATTGGTATTT 150   Borneo 2


151 TAGGTTTTATTGTATGAGCTCATCATATATTTACAGTAGGTATAGATATT 200   Borneo
    ||||||||||||||||||||||||||||||||||||||||||||||||||
151 TAGGTTTTATTGTATGAGCTCATCATATATTTACAGTAGGTATAGATATT 200   Borneo 2


201 GATACTCGGGCTTATTTTACTGCGGCTACAATGATTATTGCGGTTCCCAC 250   Borneo
    ||||||||||||||||||||||||||||||||||||||||||||||||||
201 GATACTCGGGCTTATTTTACTGCGGCTACAATGATTATTGCGGTTCCCAC 250   Borneo 2


251 TGGTATTAAAATTTTTTCTTGATTAGCAACAATTCATGGCTCTATAGTAA 300   Borneo
    |||||||||||||||||||| ||||||||||||||||||||||||||||||
251 TGGTATTAAAATTTTTTCTTGGTTAGCAACAATTCATGGCTCTATAGTAA 300   Borneo 2


301 AATTAGATGTTCCAATAATCTGATCCTTGGGTTTTATTTTTTTATTTACT 350   Borneo
    ||||||||||||||||||||||||||||||||||||||||||||||||||
301 AATTAGATGTTCCAATAATCTGATCCTTGGGTTTTATTTTTTTATTTACT 350   Borneo 2


351 TTAGGGGGAATTACTGGTGTGATTTTAGCTAATTCTTCTATTGATATTGT 400   Borneo
    ||||||||||||||||||||||||||||||||||||||||||||||||||
351 TTAGGGGGAATTACTGGTGTGATTTTAGCTAATTCTTCTATTGATATTGT 400   Borneo 2


401 TTTACATGATACTTATTATGTAGTAGCACATTTTCATTATGTATTAAGTA 450   Borneo
    ||||||||||||||||||||||||||||||||||||||||||||||||||.|
401 TTTACATGATACTTATTATGTAGTAGCACATTTTCATTATGTATTAAGAA 450   Borneo 2


451 TAGGAGCT 458                                           Borneo
    ||||||||
451 TAGGAGCT 458                                           Borneo 2
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Figure 1. Original Borneo haplotype of *V. jacobsoni* and Borneo 2 haplotype of *V. jacobsoni*.

AF107261, http: //www.ncbi.nlm.nih.gov/Genbank/index.html). The sequences obtained from each of the female *V. jacobsoni* were all identical and were most similar to that of the Borneo haplotype of *V. jacobsoni* also described by Anderson and Trueman (2000), but differed by three base-pairs from that sequence. This is therefore the second haplotype of *V. jacobsoni* that has been detected in Borneo and is assigned the name of the 'Borneo 2 haplotype of *V. jacobsoni*'. Its unique mtDNA sequence has been placed in the GenBank database under the accession number AY037890 (http: //www.ncbi.nlm.nih.gov/Genbank/index.html). The mtDNA sequence of the Borneo 2 haplotype is shown for comparison with that of the original Borneo haplotype in Figure 1.

3.2. Ratio of damaged mites

The numbers of damaged and undamaged *Tropilaelaps* mites were counted, and the extent of the damage recorded in four samples each containing more than a hundred mites (Tab. V). Similar studies were also conducted on the 48 *Varroa* mites and on the single *Euvarroa* mite.

Among the *T. clareae* mites, only 16% were without visible damage, 60% had a leg (or several) missing, and 24% were badly cut or damaged. Among the *T. koenigerum*, we found 43% undamaged, 40% with injured legs and 17% with mutilated bodies.

The numbers of damaged *Varroa* mites were fewer and the extent to which they were damaged were much less than observed among the *Tropilaelaps* mites. Of the 44 *Varroa* mites examined from colonies 2, 3, 8 and 9, four were still alive and 34 of the dead were undamaged. Only six mites had damaged legs, and one of the six also had a damaged body. Thus 86.4% of the *Varroa* mites remained intact. The four *Varroa* and one *Euvarroa* mites collected from under the newly settled swarm were not damaged.

3.3. Infestation and reproduction of mites in the brood cells

Of the 1069 brood cells inspected in colony 2 and the 604 in the colony in a nearby

Table V. Details of the injuries observed among the *Tropilaelaps* mites.

	Tropilaelaps clareae	*Tropilaelaps koenigerum*
Complete	N = 89–16% min 14.2%, max 19.8%	N = 30–43% min 38.5%, max 50%
Leg injuries	N = 336–60% min 53.8%, max 66.9%	N = 27–40% min 30%, max 46.2%
Body cuts	N = 135–24% min 18.3%, max 29.9%	N = 12–17% min 12.5%, max 30%

Table VI. Numbers of *A. dorsata* brood cells infested with *Tropilaelaps* mites.

Origin of comb	Worker brood cells inspected	Worker brood cells infested	Drone brood cells inspected	Drone brood cells infested
Colony 2 settled < 12 month	1 043	289 (27.7%)	26	7 (26.9%)
Colony in tree settled 3–4 months	137	17 (12.4%)	367	54 (14.7%)

tree (which was identified as an *Albizia* spp.), both adult *T. clareae* and *T. koenigerum* were detected, along with their offspring (nymphs). The percentage of infested drone and worker brood cells did not differ, nor did the number of mites per cell (Tab. V, VI). The number of *Tropilaelap*s mites per cell was 6.0 ± 1.6 in worker brood and 6.1 ± 1.9 in drone brood (n = 10).

No *Varroa* mites were detected in the more than 1500 *A. dorsata* brood cells inspected.

4. DISCUSSION

This is the first report of *V. rindereri* and *V. jacobsoni* occurring naturally in the debris of *A. dorsata* colonies. The mites were present in the debris collected from all five colonies studied, but the total number found (44) was very small compared to the more than 2000 *Tropilaelaps* mites found in the same debris. Only one *Euvarroa* mite was found in the debris. Hence, as *Euvarroa* and *Varroa* mites are not natural parasites of *A. dorsata*, and as we also found no evidence that these mites were utilizing the *A. dorsata* colonies for their reproduction, our results indicate that *A. dorsata* colonies in Tenom are exposed to all the honeybee brood mites which occur in the vicinity of the nest. Our results also indicate that such interspecific exposure is not only restricted to *A. mellifera* colonies that are introduced into areas of Asia that contain mite-infested indigenous honey bee species, as reported from India (Aggarwal, 1988). During other observations on the debris collected from two *A. dorsata* colonies in Thailand (Rath et al., 1991), no mites other than *T. clareae* were found. The number of fallen mites in their samples was higher than in our studies. For example, 1442 *T. clareae* fell from colony 1 in 32 days (45 per day) in the Thailand study, while 598 mites fell in 22 days (27 per day) from the biggest colony at Tenom, which produced several swarms. From another colony in the Thailand study, 1941 *Tropilaelaps* fell within the 8 days before it absconded (242.6 *T. clareae* per day). In yet another study in Malaysia and Thailand, two *A. dorsata* combs were collected only hours after the colonies absconded and before ants could destroy them. There were 475 capped brood cells on one comb (Malaysia). The infestation rate was 63% and cells were infested with 25 to 28 *T. clareae* each. Thus, the colony left more than 1000 mites behind when it absconded (unpublished data). In the comb collected in Thailand, the *T. clareae* infestation rate per cell was similar to that observed in the Malaysian comb. Such high infestation levels may play an important role in absconding of *A. dorsata* and may represent a mechanism of mite elimination (Koeniger et al., 1993).

The percentage of mutilated *Varroa* mites in the debris was very low (13.6%). This may indicate that *A. dorsata* cannot injure *Varroa* effectively. Our brood inspection did not result in any *Varroa* mites. However, the sample was small (less than 5% of the brood) and we cannot rule out that perhaps a small amount of *Varroa* reproduction may have been present. On the other hand, there are no reports of *Varroa* mites in brood cells of *A. dorsata* or on adults (Koeniger et al., 1983). We have frequently observed robbing *Apis cerana and A. koschevnikovi* bees landing on the *A. dorsata* curtain, which might be one transfer mechanism of mites.

The highest impact of mites in all five *A. dorsata* colonies studied resulted from *T. clareae*. The percentage of mutilated mites were as high as 86%. It is likely that this is a result of active mite defense by the bees, as described by Koeniger and Muzaffar (1988). Also, of the *T. koenigerum* observed in the debris, more than half were damaged. At the same time the percentage of infested brood cells with *Tropilaelaps* mites was high (about 17 and 27% respectively). Perhaps without a defense mechanism of severely

damaging the mites, a colony may not survive such an infestation. On the other hand, when considering the high number of mites (7.4 per day) falling from the newly settled *A. dorsata* colony in our study, *Tropilaelaps* mites seem to have a counter-mechanism which enables them to survive even the initial absconding and migration of a swarm.

There are only few reports on the infestation level and reproduction of *T. clareae* in worker brood of *A. dorsata* (Aggarwal, 1988), and there are no reports of the infestation rate in *A. dorsata* drone brood. Aggarwal (1988) gives similar numbers to our results (four eggs + two larvae on average). The even distribution of mites in drone and worker brood in our studies contrasts to the results for *Varroa* mites. *Varroa* can reproduce in *A. cerana* colonies only on drone brood (Koeniger et al., 1981, 1983; Tewarson et al., 1992; Anderson & Sukarsih, 1996; Boot et al., 1997; Rath 1999). Also in *A. mellifera* colonies, *Varroa* mites infest a higher percentage of drone brood and have more offspring in drone brood than in worker brood (Fuchs, 1990).

According to our studies, an infestation by several brood mite species can occur even when *A. mellifera* is not introduced into the region. Obviously the density of colonies of different species must be high. In ARS Tenom we found several nests of the four indigenous species within a radius of only 200 m. In Sri Lanka our studies were done in the semi-arid region around Anuradhapura where the density of honey bee colonies was low.

Our detection of a second haplotype of *V. jacobsoni* in Borneo warrants further comment. This haplotype, designated the Borneo 2 haplotype of *V. jacobsoni*, is most closely related to the original Borneo haplotype of *V. jacobsoni* that was described by Anderson and Trueman (2000) (see Fig. 1). The original Borneo haplotype was collected from an *A. cerana* colony at Sabah in 1993. Hence, it is possible that the local *A. cerana* at Sabah may carry two different haplotypes of *V. jacobsoni*. However, a *Varroa* mite that was collected from an *A. koschevnikovi* colony at Sabah in Tenom 2000 was also later identified as the Borneo 2 haplotype of *V. jacobsoni* (Anderson, unpublished data). Hence, further studies are needed in Borneo to determine the natural host of the Borneo 2 haplotype of *V. jacobsoni*.

Résumé – Les acariens dans les débris et les cellules de couvain operculé des colonies *Apis dorsata* à Sabah (Bornéo), y compris un nouvel haplotype de *Varroa jacobsoni*. L'introduction d'*Apis mellifera* L. dans de nombreuses régions de l'Asie a eu et a encore une influence sur les populations indigènes d'abeilles. En dehors de quelques autres effets, l'équilibre naturel entre les espèces d'abeilles du genre *Apis* et leurs acariens parasites a été sérieusement menacé. L'exploration de la relation hôte-parasite des acariens du couvain et de leurs hôtes, les abeilles, dans les conditions originelles est de plus en plus urgente, parce qu'il ne reste en Asie que peu de régions où *A. mellifera* n'a pas encore été importée.
Nous avons étudié les débris de cinq colonies d'*Apis dorsata* Fabr. à Tenom (Sabah, Bornéo), dans une région où aucune colonie d'*A. mellifera* n'a été installée. Quelques colonies d'*Apis cerana* Fabr., *Apis koschevnikovi* v. Buttel-Reepen et *Apis andreniformis* F. Smith nichaient naturellement dans la zone d'étude. Nous avons installé des plateaux à une distance d'environ 3,5 m sous les cinq colonies d'*A. dorsata*, les pieds des plateaux étant placés dans un récipient d'eau contre l'invasion par les fourmis. Chaque matin nous avons récolté les débris tombés des colonies.
Dans ces débris, nous avons trouvé les espèces d'acariens *Tropilaelaps clareae* Delfinado and Baker, *T. koenigerum, Varroa rinderi, Varroa jacobsoni* Oudemans et *Euvarroa wongsirii* Lekprayoon & Tangkanasing (Tabs. I–III). *T. clareae* était l'espèce la plus fréquente (n = 1869), mais

T. koenigerum était aussi régulièrement présent (n = 241). Un séquençage de l'ADN mitochondrial a été effectué sur 29 des 48 individus du genre *Varroa* trouvés. Nous avons identifié 21 *V. rinderi* qui appartenaient au même haplotype, déjà trouvé et décrit chez *A. koschevnikovi* à Bornéo. Mais les huit acariens de l'espèce *V. jacobsoni* appartenaient à un nouvel haplotype, que nous avons nommé "haplotype Bornéo 2 de *V. jacobsoni*" (Genbank nº d'accès AY037890). La séquence de l'ADNmt du gène de la cytochrome oxydase I (COI) du nouvel haplotype est très semblable à celle de l'haplotype de *V. jacobsoni* déjà décrit à Bornéo (Anderson & Trueman, 2000) (Fig. 1). Elle ne s'en différencie que par trois paires de base et il est à remarquer que ce premier haplotype Bornéo avait été trouvé dans une colonie d'*A. cerana* à Sabah en 1993.

Parmi les *T. clareae* trouvés dans les débris, 84 % d'entre eux étaient mutilés : 60 % avaient une ou plusieurs pattes manquantes et 24 % des lésions à la carapace. Chez *T. koenigerum* 40 % avaient des pattes abîmées et 17 % des corps mutilés (Tab. V).

Nous avons aussi inspecté 1673 cellules de couvain dans deux colonies d'*A. dorsata* situées au même endroit et trouvé à la fois des adultes de *T. clareae* et *T. koenigerum* et leur descendance (Tab. VI). Il n'y avait pas de différence entre le pourcentage d'infestation des cellules de mâles et des cellules d'ouvrières, ni entre le nombre d'acariens par cellule (6,0 ± 1,6 dans le couvain d'ouvrière et 6,1 ± 1,9 dans le couvain de mâles). Lors de l'examen des 1673 cellules de couvain aucun acarien du genre *Varroa* n'a été trouvé, ce qui suggère que ces acariens ne se reproduisent pas dans les colonies d'*A. dorsata*. La présence d'acariens du genre *Varroa* semble plutôt indiquer un transfert naturel d'acariens entre espèces sympatriques d'abeilles.

Apis dorsata* / haplotype Bornéo 2 de *V. jacobsoni* / débris de la colonie / espèces de *Tropilaelaps* / espèces de *Varroa

Zusammenfassung – Milben im Abfall und in Brutzellen von *Apis dorsata* Völkern in Sabah (Borneo), Malaysia, einschließlich eines neuen Haplotyps von *Varroa jacobsoni*. Die Einführung von *Apis mellifera* L. in viele Teile von Asien hatte und hat einen Einfluss auf die einheimische Bienenpopulation. Neben einigen anderen Effekten könnte das natürliche Gleichgewicht zwischen Honigbienenarten und ihren parasitischen Milben ernsthaft gefährdet werden. Die Exploration des Wirt – Parasit – Verhältnis von Brutmilben und ihren Wirtstieren, den Honigbienen, unter ursprünglichen Verhältnissen wird immer dringender, weil es in Asien nur noch wenige Gebiete gibt, in die *A. mellifera* noch nicht importiert wurde.

Wir untersuchten den Totenfall bei 5 *Apis dorsata* Fabr. Völkern in Tenom (Sabah), Borneo, in einer Region in der keine *A. mellifera* Völker angesiedelt wurden. Einige Völker der *Apis cerana* Fabr., *Apis koschevnikovi* v. Buttel-Reepen und *Apis andreniformis* F. Smith nisteten natürlicherweise im Untersuchungsareal. Wir stellten eine Unterlage etwa 3,5 m unterhalb der *A. dorsata* Völker auf, die gegen einen Belauf durch Ameisen geschützt war. Jeden Morgen sammelten wir den Abfall unter den Völkern eine.

Wir fanden die Milbenarten *Tropilaelaps clareae* Delfinado & Baker, *T. koenigerum, Varroa rindereri, V. jacobsoni* Oudemans and *Euvarrao wongsirii* Lekprayoon & Tangkanasing (Tab. I–III). *T. clareae* waren am häufigsten (n = 1869), aber auch *T. koenigerum* wurden regelmäßig gefunden (n = 241). Von den 48 aufgefundenen *Varroa* Milben wurde bei 29 eine mtDNA Sequenzierung durchgeführt. Wir identifizierten 21 *V. rindereri* die zum gleichen Haplotyp gehörte, der bereits bei *A. koschevnikovi* in Borneo gefunden und beschrieben wurde. Aber die 8 Milben der Art *V. jacobsoni* gehörten zu einem neuen Haplotyp, den wir als 'Borneo 2 Haplotyp von *V. jacobsoni*' bezeichneten (Genbank accession Nummer AY037890). Die Sequenz

der mitochrondrialen DNA des Cytochrom Oxidase I Gens des neuen Haplotyps ist dem bereits von Borneo beschriebenen Haplotyp von *V. jacobsoni* (Anderson and Trueman, 2000) (Abb. 1) am ähnlichsten, unterschied sich jedoch von dessen Sequenz in 3 Basenpaaren. Bei der Entdeckung eines 2. Haplotyps von *V. jacobsoni* unter *A. dorsata* Völkern in Borneo muss angemerkt werden, dass der erste Borneo Haplotyp von einem *A. cerana* Volk 1993 in Sabah gefunden wurde.

Von den im Totenfall gefundenen *T. clareae* waren 84 % beschädigt; 60 % hatten verletzte Beine und 24 % hatten einen beschädigten Panzer. Bei *T. koenigerum* fanden wir 40 % mit abgebissenen Beinen und 17 % mit angebissenem oder sonst beschädigtem Panzer (Tab. V).

Zusätzlich inspizierten wir 1673 Brutzellen von 2 *A. dorsata* Völkern vom selben Ort und fanden adulte *T. clareae* und *T. koenigerum* zusammen mit ihren Nachkommen (Tab. VI). Der Prozentsatz der befallenen Drohnen- und Arbeiterinnenzellen unterschied sich nicht, das gleiche gilt für die Anzahl der Milben pro Zelle: 6,0 ± 1,6 in Arbeiterinnenbrut und 6,1 ± 1,9 in Drohnenbrut (n = 10). Bei der Untersuchung der 1673 Brutzellen wurden keine *Varroa* Milben entdeckt, was gegen eine Reproduktion im *A. dorsata* Volk spricht. Das Vorkommen von Varroa Milben scheint vielmehr anzuzeigen, dass ein Transfer von Milben zwischen den sympatrischen Bienenarten natürlicherweise vorkommt.

***Apis dorsata* / Borneo 2 Haplotyp von *V. jacobsoni* / Totenfall / *Tropilaelaps* species / *Varroa* species**

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