A NEW SPECIES OF *VARROA* (ACARI: VARROIDAE) ASSOCIATED WITH *APIS KOSCHEVNIKOVI* (APIDAE: HYMENOPTERA) IN BORNEO

L. I. de Guzman[1] and M. Delfinado-Baker[2]

1. USDA/ARS, Honey-Bee Breeding, Genetics and Physiology Research Laboratory, 1157 Ben Hur Road, Baton Rouge, LA 70820. 2. USDA/ARS, Systematic Entomology Laboratory, Bldg. 046, Rm. 107, BARC-West, Beltsville, MD 20705

ABSTRACT - The general morphology and RAPD banding pattern of adult female *Varroa rindereri*, n. sp. collected from drone brood of *Apis koschevnikovi* Buttel-Reepen, a cavity-nesting bee in Borneo, is described and illustrated. This is the third known species of *Varroa* recorded from cavity nesting bees in Asia, the others being *V. jacobsoni* Oudemans and *V. underwoodi* Delfinado-Baker & Aggarwal. The new species resembles *V. jacobsoni*. Morphological and molecular differences between the two species are discussed.

INTRODUCTION

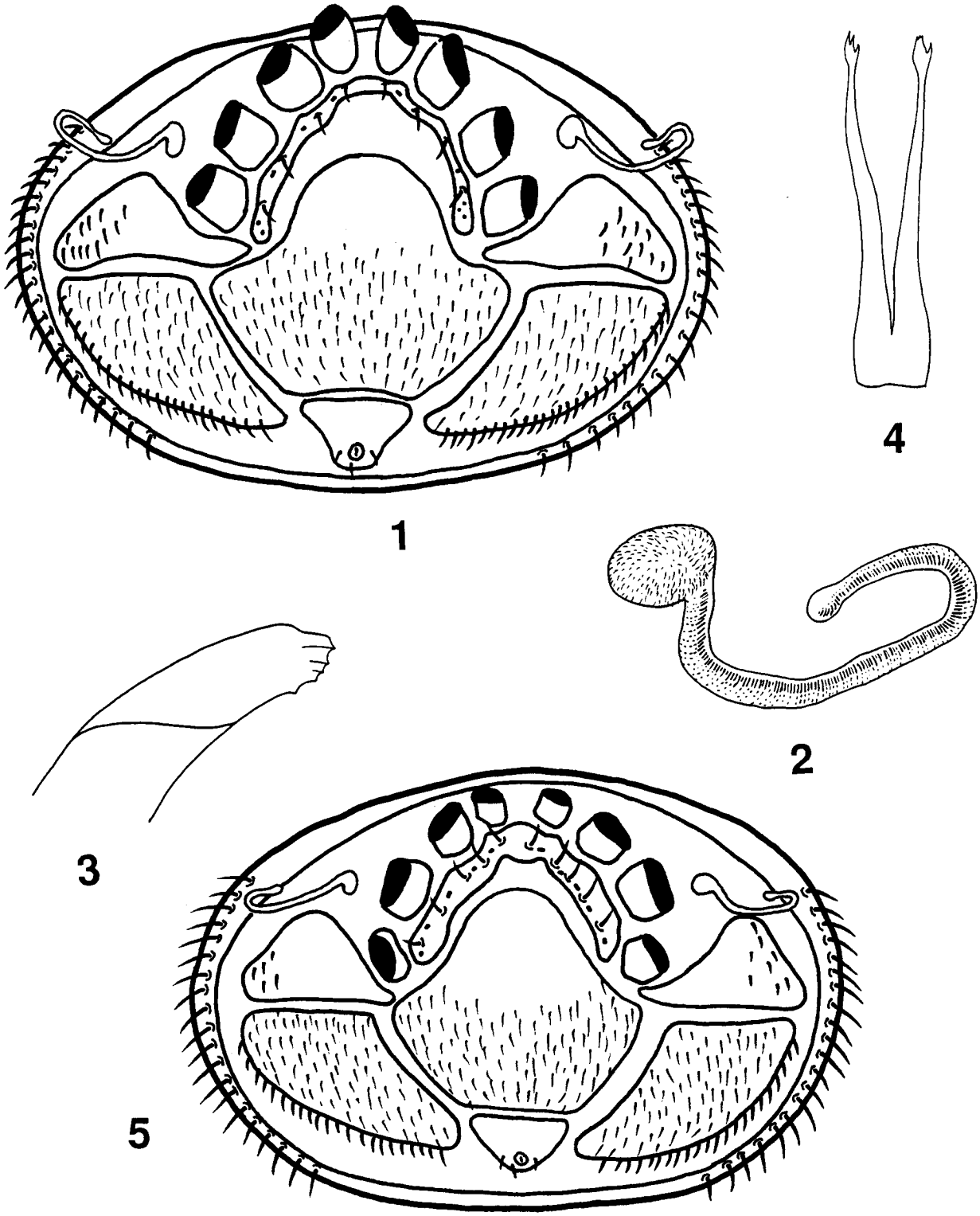
Examination of *Varroa* mite samples recently collected from honey bees in Borneo by T. E. Rinderer has resulted in the recognition of a new species in the genus *Varroa*. Specimens of the new species were found in drone brood of *A. koschevnikovi* Buttel-Reepen, a cavity-nesting bee of Borneo and Sumatra. The new mite is the third species in the genus *Varroa* associated with cavity-nesting bees in Asia. The other species, *V. jacobsoni* Oudemans (1904) and *V. underwoodi* Delfinado-Baker & Aggarwal (1987), are from a cavity nesting bee, *Apis cerana* F. from Java and Nepal, respectively. The new species resembles *V. jacobsoni* but may be separated from it by a set of characters discussed below. Morphological and molecular data were compared with those of *V. jacobsoni* collected simultaneously from *A. cerana* in Borneo. Morphological measurements were taken using a digitizer with data input into a computerized program and analyzed using student's t-test. All measurements are given in micrometers (μm).

Varroa rindereri n. sp. (Figs. 1-4)

Female - General morphology and chaetotaxy similar to *V. jacobsoni* (Fig. 5). Body length 1144, width 1713, holotype (mean: 1180 x 1698) transversely oval, brown.

Dorsum - Dorsal shield well sclerotized, convex dorsally, flattened ventrally; dorsal shield covered with varying lengths of barbed setae, those on the posterior and lateral about twice as long as those located centrally; with a variable number (19-25, mean: 23) of lanceolate setae on each lateral margin of shield.

Venter (Fig. 1) - Sternal shield well sclerotized, with 8 setae (mean: 9), and 10 pores (mean: 11) arranged asymmetrically. About 4-6 pores situated laterally and below seta 4 or in some cases seta 5. Metasternal shield fused with sternal shield. Genital shield large, with lateral projections deep and angular as compared to shallow and rounded in *V. jacobsoni* and *underwoodi*. Anal shield triangular as opposed to semicircular in *V. jacobsoni*; with 3 setae, postanal seta shorter than perianals on both side of terminal anal opening. Endopodal shields enlarged, extending laterad of coxae IV, bearing 13-16 setae (mean: 12) on its expanded area. Metapodal shields greatly enlarged, broadly triangular, surface covered with numerous setae, with 21 long setae (mean: 23) along posterior margins of shields. Exopodals well sclerotized, fused anteriorly, forming a sclerotized framework along anterior ventral border of dorsal shield. Stigmata and peritremes ventro-lateral; stigmata located in region of coxae III-IV. Peritremes long measuring 587 (mean: 582) protruding beyond the lateral margins and looped portion can be visible dorsally; wide-looped distally, distance measuring



Figs. 1-4. *Varroa rindereri* n. sp. (female) - 1. ventral aspect, 2. peritreme, 3. corniculi, 4. tritosternum, 5. *Varroa jacobsoni* Oudemans (female) - ventral aspect.

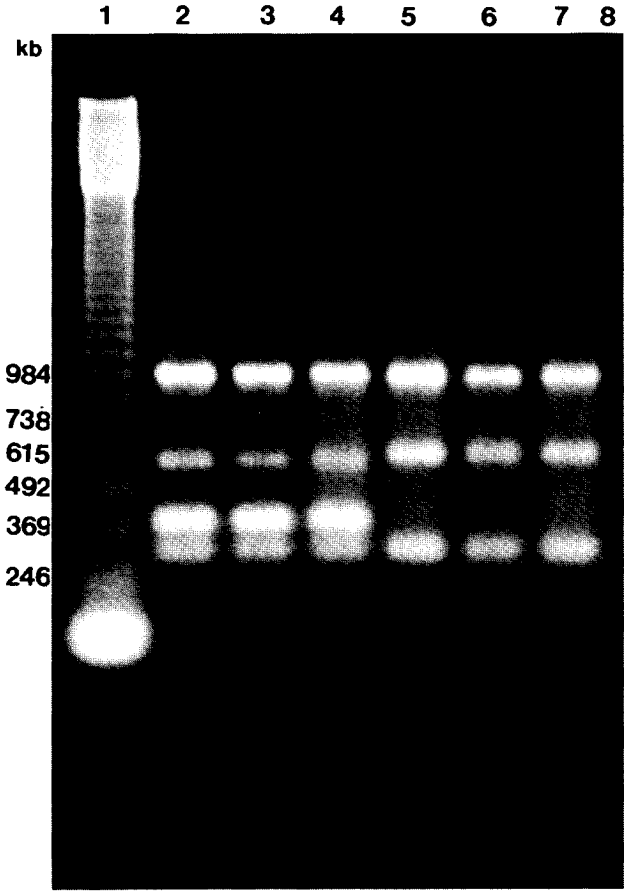


Fig. 6. RAPD banding patterns of three *V. rindereri* from *A. koschevnikovi* (lanes 2-4) and three *V. jacobsoni* from *A. cerana* (lanes 5-7) in Borneo, Malaysia. Lane 8 was the control (without template DNA) and lane 1 was 123 kb ladder.

43 (mean: 49) (Fig. 2) as compared to short and sharp-looped in *V. jacobsoni* and *underwoodi* in which the peritreme can only be viewed ventrally. Legs robust; ambulacra sucker-like, with sclerites bound by membranes. Leg chaetotaxy on coxa, trochanter, femur, genu, tibia, and tarsus as follows:

Leg I - 2,5,10-11,10-12,10-13,26-29
Leg II - 2,5,9-11,10-12,10-12,15
Leg III - 2,5,9,9-11,10-13,15
Leg IV - 1,6,7,9-10,10-11,15

Gnathosoma - Small, located antero-ventrally, completely concealed under dorsal shield between coxae I. Chelicerae lacking fixed chela, movable chela bidentate, blade-like, broad and flat in profile, not tapered distally as in *V. jacobsoni* or *V. underwoodi*; deutosternum with small denticles; tectum smooth, with straight margin, concave or rounded in other species; corniculi large, blade-like, flat in profile with serrations subapically (Fig. 3); three pairs of gnathosomal setae arranged in longitudinal row, with hypostomal setae 1 and 2 closer to each other than seta 2 to posterior capitular seta 3. Palpal apotele claw-like, with a short tine at base; trochanter lacking a seta; palpal chaetotaxy as follows: trochanter 0, genu 2, femur 2, tibia 7, tarsus 10. Tritosternum bifid, nearly smooth, laciniae with flat, pilose tips (Fig. 4).

COLLECTION DATA

Thirty five adult females were collected from drone brood of three different colonies of *A. koschevnikovi*. However, only 10 mites (eight from one colony and one mite each from the other two colonies) were used in the

Table 1. Comparison of morphological characters between *Varroa rindereri* from *Apis koschevnikovi* and *Varroa jacobsoni* from *Apis cerana* in Borneo, Malaysia (Mean ± standard error).

Characters	*Varroa rindereri* (n = 10)	*Varroa jacobsoni* (n = 11)	P > |T|
Body length	1,180 ± 11[a]	1,077 ± 6[b]	0.0001**
Body width	1,698 ± 14[a]	1,596 ± 10[b]	0.0001**
Peritreme length	582 ± 13[a]	426 ± 9[b]	0.0001**
Loop distance	49 ± 2[a]	18 ± 2[b]	0.0001**
No. of marginal setae	23 ± 0.5[a]	19 ± 0.4[b]	0.0001**
No. of endopodal setae	12 ± 0.5[a]	7 ± 0.3[b]	0.0001**
No. of sternal setae	9 ± 0.3[b]	10 ± 0.3[a]	0.0011**
No. of sternal pores	9 ± 0.4[b]	11 ± 0.4[a]	0.0253*
No. of metapodal setae	23 ± 0.8[a]	22 ± 0.5[a]	0.28[ns]

*Significant at P = 0.05 ** Significant at P = 0.01

Row means with different letters differ significantly (student's t-test, P=0.05).

measurement of different morphological characters. Samples of *V. jacobsoni* (75 mites) were collected from drone brood of five *A. cerana* colonies. Eleven mites from two of these colonies were used for comparison. All source colonies (both bee species) were located in an apiary at Tenom Agricultural Park, Borneo, Malaysia. Additional samples, three females previously identified as *V. jacobsoni* by M. Delfinado-Baker (Otis 1991) that were collected from *A. koschevnikovi*, Tenom, Sabah, Borneo by G. W. Otis on May 7, 1989, were also examined. Holotype female and 4 female paratypes deposited at the U. S. National Museum Collection at the USDA/ARS, Systematic Entomology Laboratory, ARS, USDA at Beltsville, Maryland, and 5 female paratypes USDA/ARS, Honey-Bee Breeding, Genetics and Physiology Research Laboratory, Baton Rouge, Louisiana.

DNA ANALYSIS

V. rindereri and *V. jacobsoni* were also compared using random amplification of polymorphic DNA (RAPD). For both species, DNA from three mites was individually extracted using a Chelex extraction method (Walsh et al.1991). Each mite was collected from a different colony.The primer used was D01 from Operon Inc., which is 10 nucleotides long and approximately 60% G/C. DNA amplification was conducted as described by Kraus and Hunt (1995). *V. rindereri* showed two specific bands that were not present in *V. jacobsoni* from Borneo while one band present in *V. jacobsoni* was not found in *V. rindereri* (Fig. 6). There were two bands that were shared between the two mite species. The banding pattern for each species was found in every representative sample.

DISCUSSION

The genus *Varroa* previously included two species, *jacobsoni* and *underwoodi*, from a cavity-nesting honey bee, *A. cerana* in Asia. *V. jacobsoni* subsequently shifted from its original host to another species of cavity-nesting honey bee, *A. mellifera* L., which is widely distributed while *underwoodi* is still restricted to *A. cerana* in Nepal and Korea. The new *Varroa* species, *rindereri*, was obtained from a third species of cavity nesting honey bee, *A. koschevnikovi*, which is sympatric with *A. cerana* in Borneo. *Apis mellifera* is not present in Borneo. Although adult females of *V. rindereri* are similar to females of *V. jacobsoni*, the difference in the overall body size between the two species in Malaysia is statistically significant (Table 1). *Varroa rindereri* is larger than *V. jacobsoni* from Indonesia (mean: 1065 x 1575) (Oudemans 1904), Nepal (mean: 1125 x 1646), Europe (mean: 1117 x 1677) or SW Asia (mean: 1108 x 1660) (Delfinado-Baker & Houck 1989). Although *V. rindereri* is larger than *V. jacobsoni*, the numbers of setae and pores on the sternal shield of *V. rindereri* are fewer.

V. rindereri differs in many ways from *V. jacobsoni* or *V. underwoodi* especially by the characters of the fixed chela of the chelicera, laciniae of the tritosternum, margin of tectum and corniculi. The trochanter of the palpus is smooth, lacking a seta; a seta is always present on palpal trochanters of *V. jacobsoni* and *V. underwoodi*.

The recent association of *V. jacobsoni* with the widely distributed Western cavity-nesting bee, *A. mellifera*, is known to have had drastic effects on honey bee populations. Milani (1994), Akimov *et al.* (1988) and Delfinado-Baker & Peng (1995) have summarized much of what we know about *Varroa*. The biology of *V. rindereri* is unknown. However, this mite species may be specific to its host, *A. koschevnikovi*. Subsequent examination of mite samples showed no mite contamination or cross infestation between species despite the observed interspecific colony invasions by foragers (T.E. Rinderer, pers. comm.). All mites collected from the three *A. koschevnikovi* colonies were of the *rindereri* type while those mites collected from the five *A. cerana* colonies at the same apiary were all of the *jacobsoni* species. In addition, T.E. Rinderer observed differences in the phoretic behavior of the two mite species during the collection. When pupae were removed from their cells, *V. jacobsoni* tended to hang onto the pupal hosts while *V. rindereri* remained inside the cells. The recent collection from honey bees in Asia indicates the need for further collections and studies to assess possible pest potential of the new mites not only on other Asian bees but also on *A. mellifera*.

ACKNOWLEDGMENTS

We thank T.E. Rinderer, USDA/ARS, Honey-Bee Breeding, Genetics and Physiology Research Laboratory, Baton Rouge, Louisiana, in whose honor we name the new mite, for the opportunity to study his mite collection from Borneo. We also thank Salim Tingek and the Saban Ministry of Agriculture, who graciously provided access to colonies of honey bees in apiaries of the Tenom Agricultural Research Station. The primer was provided by Ed Smith, Department of Animal Genetics, Tuskegee University, Tuskegee, Alabama. This manuscript is completed in cooperation with Louisiana Agricultural Experiment Station.

REFERENCES

Akimov, I. E., S. Starovir and A. T. Yastrebtsov. 1988. (The *Varroa* mite. The causative agent of varroatosis of bees. A morphological outline). Klesch *Varroa* vozbuditel varroatozoa pchel. Morfologicheskii Ocherk. Naukova Dumka, Kiev. UKRSSR. 120 pp.

Delfinado-Baker, M. and K. Aggarwal. 1987. A new *Varroa* (Acari: Varroidae) from the nest of *Apis cerana* (Apidae). Internat. J. Acarol. 13(4): 233-237.

Delfinado-Baker, M. and M. Houck. 1989. Geographic variation in *Varroa jacobsoni* (Acari, Varroidae):

Application of multivariate morphometric techniques. Apidologie 20: 345-357.

Delfinado-Baker, M. and C. Peng. 1995. *Varroa jacobsoni* and *Tropilaelaps clareae*: A perspective of life history and why Asian bee-mites preferred European honey bees. Am. Bee J. 135(6): 415-420.

Kraus, B. and G. Hunt. 1995. Differentiation of *Varroa jacobsoni* Oud populations by random amplification of polymorphic DNA (RAPD). Apidologie 26: 283-290.

Milani, N. 1994. Analytical bibliography on *Varroa jacobsoni* Oud. and related species. Apicoltura 8 (1992-93): 1-147.

Otis, G. 1991. A review of the diversity of species within *Apis*. In: Diversity in the Genus *Apis*. D. R. Smith (ed). Westview Press, Oxford, 265 pp.

Oudemans, A. C. 1904. On a new genus and species of parasitic Acari. Notes Leyden Mus. 24: 197-204.

Walsh, P.S., D.A. Metzger and R. Higuchi. 1991. Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10(4): 506-513.

*****