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Varroa jacobsoni (Acari: Varroidae) is more than one species

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Abstract. *Varroa jacobsoni* was first described as a natural ectoparasitic mite of the Eastern honeybee (*Apis cerana*) throughout Asia. It later switched host to the Western honeybee (*A. mellifera*) and has now become a serious pest of that bee worldwide. The studies reported here on genotypic, phenotypic and reproductive variation among *V. jacobsoni* infesting *A. cerana* throughout Asia demonstrate that *V. jacobsoni* is a complex of at least two different species. In a new classification *V. jacobsoni* is here redefined as encompassing nine haplotypes (mites with distinct mtDNA CO-I gene sequences) that infest *A. cerana* in the Malaysia–Indonesia region. Included is a Java haplotype, specimens of which were used to first describe *V. jacobsoni* at the beginning of this century. A new name, *V. destructor* n. sp., is given to six haplotypes that infest *A. cerana* on mainland Asia. Adult females of *V. destructor* are significantly larger and less spherical in shape than females of *V. jacobsoni* and they are also reproductively isolated from females of *V. jacobsoni*. The taxonomic positions of a further three unique haplotypes that infest *A. cerana* in the Philippines is uncertain and requires further study.

Other studies reported here also show that only two of the 18 different haplotypes concealed within the complex of mites infesting *A. cerana* have become pests of *A. mellifera* worldwide. Both belong to *V. destructor*, and they are not *V. jacobsoni*. The most common is a Korea haplotype, so-called because it was also found parasitizing *A. cerana* in South Korea. It was identified on *A. mellifera* in Europe, the Middle East, Africa, Asia, and the Americas. Less common is a Japan/Thailand haplotype, so-called because it was also found parasitizing *A. cerana* in Japan and Thailand. It was identified on *A. mellifera* in Japan, Thailand and the Americas.

Our results imply that the findings of past research on *V. jacobsoni* are applicable mostly to *V. destructor*. Our results will also influence quarantine protocols for bee mites, and may present new strategies for mite control.

Key words: *Varroa jacobsoni*, *Varroa destructor*, mtDNA CO-I gene sequence, genetic variation

Introduction

The genus *Varroa* (Acari: Varroidae) is currently represented by three highly-specialized species of obligate ectoparasitic mites that feed on the haemolymph of social cavity-nesting bees (*Apis* spp.) in Asia. *Varroa jacobsoni* Oudemans was first described from *Apis cerana* Fabricius in Java, Indonesia (Oudemans, 1904a), *V.*

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underwoodi Delfinado-Baker and Aggarwal from *A. cerana* in Nepal (Delfinado-Baker and Aggarwal, 1987a), and *V. rindereri* De Guzman and Delfinado-Baker from *A. koschevnikovi* Buttel-Reepen in Borneo (De Guzman and Delfinado-Baker, 1996).

Of the three described species, *V. jacobsoni* has the widest distribution. It parasitizes *A. cerana* throughout Asia (Koeniger *et al*., 1981) and *A. nigrocincta* Smith in Indonesia (Hadisoesilo and Otis, 1998; results from the present study). About 30 years ago it also began parasitizing the European honey bee, *A. mellifera* Linnaeus, when that bee was introduced into Asia. Details of this host-shift and the subsequent movement of *V. jacobsoni*-infested *A. mellifera* colonies between countries are unclear, but provided a means for the mite to spread from Asia (Akratanakul and Burgett, 1975; Crane, 1978; De Jong *et al*., 1982). Today, *V. jacobsoni* is almost cosmopolitan, although notably not yet found in Australia, New Zealand, Hawaii and parts of Africa (Matheson, 1996; De Guzman and Rinderer, 1999). It is recognized as the most serious pest of *A. mellifera* worldwide.

Female *V. jacobsoni* from different populations show remarkable consistency in phenotypic characters, except for body size. In general, females infesting *A. cerana* are smaller than those infesting *A. mellifera* (Delfinado-Baker, 1988) and have been regarded as a distinct biotype (Delfinado-Baker and Houck, 1989). However, even though mites from different populations are physically alike, their virulence toward *A. mellifera* is not uniform (Moritz and Haenel, 1984; Camazine, 1986; Ritter *et al*., 1990; Moretto *et al*., 1991; Anderson, 1994; Eguaras *et al*., 1995; De Jong and Soares, 1997). The greatest variation is associated with *V. jacobsoni* of Javanese origin, which are mites from which the species was first described (Oudemans, 1904a). These mites completely lack the ability to reproduce on *A. mellifera* (Anderson, 1994; Anderson and Sukarsih, 1996) and their mitochondrial DNA (mtDNA) cytochrome oxydase I (CO-I) gene sequences differ by 6.7% from those of phenotypically similar mites that reproduce on *A. mellifera* in Europe (Anderson and Fuchs, 1998). This, and other reports of variation among *V. jacobsoni* populations (Kraus and Hunt, 1995; De Guzman *et al*., 1997, 1998, 1999), suggest that *V. jacobsoni* may be more than one species, and demonstrates the need for a comprehensive comparative study.

In this paper, genetic variation in *V. jacobsoni* was first examined by comparing and analysing mtDNA sequences of a region of the CO-I coding gene of adult female mites collected from populations of *A. cerana* distributed throughout Asia. Previous studies using this gene region have shown sequence variation between, but not within, two different *V. jacobsoni* populations (Anderson and Fuchs, 1998). *V. jacobsoni* were also collected from populations of *A. mellifera* worldwide, and their CO-I gene sequences compared with those from mites collected from *A. cerana*. Mite body sizes and shapes were measured and the differences compared with

relationships calculated from the DNA sequences. Other studies provided information on the ability of sympatric mites of different haplotypes to interbreed with one another.

Methods

Mite isolates

Female *V. jacobsoni* isolates were collected in 70% ethanol from *A. cerana* populations throughout Asia, from *A. mellifera* populations worldwide, and from *A. nigrocincta* colonies in Indonesia (Tables 1 and 2, Figure 1). Female *V. underwoodi* and female *V. rindereri* were also used in our studies. The *V. underwoodi* females were collected from an *A. cerana* colony in Papua New Guinea, and the *V. rindereri* females from Malaysian Borneo were kindly donated by Dr G.W. Otis, University of Guelph, Ontario, Canada. All collected mites were subsequently stored in 70% ethanol at −20°C until needed for further analyses.

DNA extraction, amplification, cloning and sequencing

Crude DNA preparations obtained from leg tissue dissected from individual mites were used to amplify a region of the mtDNA CO-I coding gene by polymerase chain reaction (PCR) (Saiki, 1990) using the COXF and COXRa primers developed by Anderson and Fuchs (1998), and following the methods described by Anderson and Fuchs (1998) and Anderson *et al*. (1998). Amplified mtDNA fragments from 1 mite of each *V. jacobsoni* isolate (listed in Tables 1 and 2) were cloned into the pBlueScript SK vector at the *Sma* I restriction endonuclease site and sequenced using a Model 373A DNA Sequencing System (Applied Biosystems) in conjunction with the M13 forward primer 5′-GTAAAACGACGGCCAGT-3′ and reverse primer 5′-AACAGCTATGACCATG-3′ using a DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions. Both DNA strands of each cloned DNA were sequenced at least five times to confirm the accuracy of the sequence data. Further mtDNA fragments were amplified by PCR from varying numbers of mites of most *V. jacobsoni* isolates (see Tables 1 and 2), and from *V. rindereri* and *V. underwoodi*, and these were sequenced directly without cloning. To confirm whether DNA sequences were invariate within individual mite populations, mtDNA fragments, that had been amplified by PCR from varying numbers of *V. jacobsoni* isolates from Canada, Germany, Indonesia, Japan, New Guinea, Russia, South Korea, and Thailand (see Tables 1 and 2), were digested with combinations of the *Xho*I and *Sac*I restriction enzymes and the fragments visualized as bands in 2% agarose gels, as described by Anderson and Fuchs (1998). Other isolates from the Philippine islands of Luzon and Mindanao (see Tables 1 and 2)

Table 1. Details of the *Varroa* isolates from Asian bee hosts.

Bee host[1]	Collection details: COUNTRY (island) [locality]	Number of isolates/Year(s) collected[2]	Number of mites: SD/RE/BM[3]	Identity of mites: Haplotype/species[4]
AC	CHINA [Guangzhou]	1/1996	4/0/4	China/*V. destructor*
AC	INDONESIA (Ambon) [Ambon]	2/1997	3/0/20	Ambon/*V. jacobsoni*
AC	INDONESIA (Ambon) [Paso]	1/1997	3/0/0	Ambon/*V. jacobsoni*
AC	INDONESIA (Bali) [Ubud]	2/1998	6/0/11	Bali/*V. jacobsoni*
AC	INDONESIA (Biak) [Biak]	3/1995+96+97	10/33/20	Java/*V. jacobsoni*
AC	INDONESIA (Flores) [Labuhan Bajo]	2/1998	1/0/2	Flores/*V. jacobsoni*
AC	INDONESIA (Java) [Parung Panjang]	4/1991+95+96+98	8/50/0	Java/*V. jacobsoni*
AC	INDONESIA (Java) [Malang]	2/1996+98	3/50/20	Java/*V. jacobsoni*
AC	INDONESIA (Lombok) [Kuta]	1/1998	3/0/20	Lombok/*V. jacobsoni*
AC	INDONESIA (Lombok) [Mataram]	2/1998	2/0/4	Lombok/*V. jacobsoni*
AC	INDONESIA (Sulawesi) [Palu]	2/1996	6/10/19	Java/*V. jacobsoni*
AC	INDONESIA (Sulawesi) [Ujung Pandang]	1/1997	2/0/0	Java/*V. jacobsoni*
AC	INDONESIA (Sumatra) [Medan]	2/1997	3/0/20	Sumatra/*V. jacobsoni*
AC	INDONESIA (Sumatra) [Padang]	1/1997	4/0/20	Sumatra/*V. jacobsoni*
AC	INDONESIA (Sumbawa) [Sumbawa]	1/1998	1/0/1	Sumbawa/*V. jacobsoni*
AC	INDONESIA (Timor) [Loli]	2/1998	3/0/20	Java/*V. jacobsoni*
AC	INDONESIA (Yapen) [Serui]	4/1995+96+97+98	5/102/30	Java/*V. jacobsoni*
AC	JAPAN (Honshu) [Tamagawa]	2/1994	7/0/40	Japan-Thailand/*V. destructor*
AC	MALAYSIA (Borneo) [Sabah]	1/1993	3/0/20	Borneo/*V. jacobsoni*
AC	MALAYSIA [Kluang]	2/1995	4/0/16	Malaysia/*V. jacobsoni*
AC	MALAYSIA [UPM, Serdang]	2/1989+95	6/0/0	Malaysia/*V. jacobsoni*
AC	NEPAL [Katmandu]	1/1997	0/0/0	Nepal/*V. destructor*
AC	NEW GUINEA[5]	6/1993+95(×3)+98(×2)	15/20/131	Java/*V. jacobsoni*
AC	PHILIPPINES (Luzon) [Batangas]	1/1996	5/0/5	Luzon 1/unresolved
AC	PHILIPPINES (Luzon) [San Fernando][6]	4/1997+98(×3)	5/50/21	Luzon 2/unresolved
AC	PHILIPPINES (Mindanao) [Davao]	3/1998	0/13/14	Mindanao/unresolved
AC	SOUTH KOREA [Kwangju]	1/1996	3/0/4	Korea/*V. destructor*
AC	SOUTH KOREA [Suwon]	2/1996+97	3/10/0	Korea/*V. destructor*
AC	SRI LANKA [Horana]	1/1995	0/0/0	Sri Lanka/*V. destructor*?
AC	THAILAND [Bangkok]	2/1997	2/10/14	Japan-Thailand/*V. destructor*
AC	VIETNAM [Phjong, Ninh Binh Prov.]	2/1996	5/0/2	Vietnam/*V. destructor*
AN	INDONESIA (Sulawesi) [Palu]	2/1996	3/10/5	Java/*V. jacobsoni*
AN	INDONESIA (Sulawesi) [Ujung Pandang]	1/1997	4/10/20	Java/*V. jacobsoni*

[1] AC = *Apis cerana*; AN = *Apis nigrocincta*.

[2] More than one isolate was collected in some years (as shown in parentheses).

[3] SD = MtDNA CO-I gene sequenced directly without cloning; RE = haplotype confirmed by PCR-restriction enzyme analyses; BM = body length and width measured. Note that the mtDNA CO-I gene from 1 mite of each isolate was also cloned and sequenced. See text for experimental detail.

[4] As resolved by the mtDNA sequence analysis. Some haplotypes were unresolved by the mtDNA analysis, while the Sri Lanka haplotype (?) is regarded as conspecific with *V. destructor* pending further data. See Results and Discussion for details.

[5] New Guinea comprises Papua New Guinea (PNG) and Irian Jaya. Isolates examined came from Aiyura, Goroka, Lae, Oksapmin and Vanimo in PNG and from Koya Timor in Irian Jaya.

[6] San Fernando, La Union Province, Luzon, the Philippines.

Table 2. Details of the reproducing female *Varroa* isolates from *Apis mellifera*.

Country	Number of isolates/Year(s) collected[1]	Number of mites: SD/RE/BM[2]	Identity of mites: Haplotype/species[3]
ARGENTINA	2/1995	6/0/20	Korea/*V. destructor*
BELGIUM	1/1996	2/0/0	Korea/*V. destructor*
BRAZIL[4]	3/1989+93+97	6/20/12	Japan-Thailand+Korea/*V. destructor*
CANADA	3/1996+97(×2)	7/20/27	Japan-Thailand+Korea/*V. destructor*
CHINA	1/1996	4/0/0	Korea/*V. destructor*
COSTA RICA	1/1998	4/0/0	Korea/*V. destructor*
DENMARK	1/1996	2/0/0	Korea/*V. destructor*
EGYPT	1/1996	2/0/0	Korea/*V. destructor*
FRANCE	1/1996	3/0/12	Korea/*V. destructor*
GERMANY	1/1996	4/12/20	Korea/*V. destructor*
GREECE	1/1996	2/0/4	Korea/*V. destructor*
INDONESIA	4/1994+96+97(×2)	10/50/70	Korea/*V. destructor*
ISRAEL	1/1996	2/0/0	Korea/*V. destructor*
ITALY	3/1992+96(×2)	6/0/2	Korea/*V. destructor*
JAPAN	4/1995+96(×3)	9/10/13	Japan-Thailand/*V. destructor*
MEXICO	2/1995	5/15/40	Korea/*V. destructor*
NETHERLANDS	1/1997	2/0/3	Korea/*V. destructor*
PUERTO RICO	1/1996	3/0/0	Korea/*V. destructor*
PHILIPPINES	4/1997+98(×3)	11/220/31	Korea/*V. destructor*
PORTUGAL	1/1991	2/0/6	Korea/*V. destructor*
RUSSIA	1/1995	3/10/20	Korea/*V. destructor*
SOUTH AFRICA	2/1998	4/0/0	Korea/*V. destructor*
SOUTH KOREA	2/1996	2/20/20	Korea/*V. destructor*
SPAIN	1/1991	2/0/1	Korea/*V. destructor*
SWITZERLAND	1/1997	2/0/0	Korea/*V. destructor*
THAILAND	2/1992+96	6/20/4	Japan-Thailand+Korea/*V. destructor*
UKRAINE	1/1997	3/0/3	Korea/*V. destructor*
UNITED KINGDOM	2/1997	5/20/27	Korea/*V. destructor*
UNITED STATES[5]	8/1990+97(5)+98(×2)	16/0/79	Japan-Thailand/Korea/*V. destructor*
URUGUAY	1/1994	2/0/20	Korea/*V. destructor*
VIETNAM	4/1996	12/0/32	Korea/*V. destructor*
YUGOSLAVIA	1/1996	2/0/3	Korea/*V. destructor*

[1] More than one isolate was collected in some years (as shown in parentheses).

[2] SD = MtDNA CO-I gene sequenced directly without cloning; RE = haplotype confirmed by PCR-restriction enzyme analyses; BM = body length and width measured. Note that the mtDNA CO-I gene from 1 mite of each isolate was also cloned and sequenced. See text for experimental detail.

[3] As resolved by the mtDNA sequence analysis. See Results and Discussion for details.

[4] All mites examined in the 1989 and 1993 collections were Japan/Thailand haplotypes, and the mites examined in the 1997 collection were mixtures of Japan/Thailand and Korea haplotypes with Japan/Thailand haplotypes being most common.

[5] All mites examined in the 1990 collection were Korea haplotypes, and the mites examined in the 1997–98 collections were mixtures of Korea and Japan/Thailand haplotypes with the Korea haplotypes being most common.

were similarly treated using combinations of the *Apo*I, *Xho*I and *Sac*I restriction enzymes. The recognition sites targeted by the restriction enzymes were identified from the DNA sequence data.

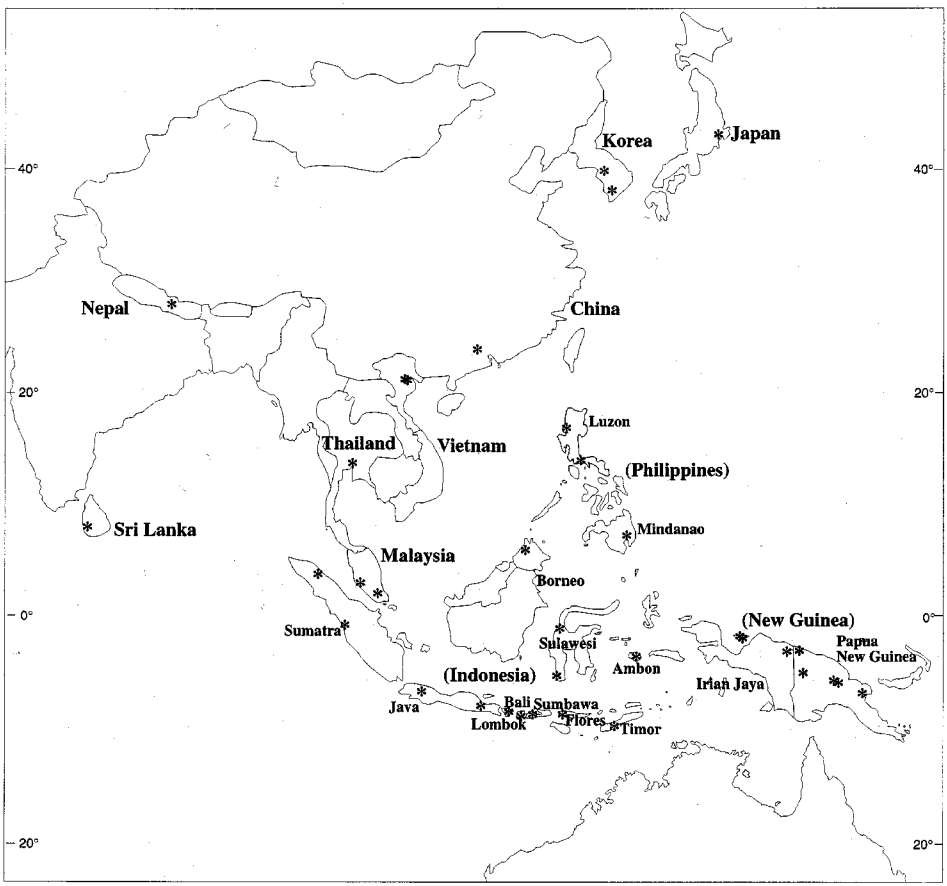

Figure 1. Localities (*) from which *Varroa jacobsoni* were collected from *Apis cerana* for the mtDNA analysis.

Sequence analysis methods

To confirm the identity and relatedness of DNA sequences, searches of the combined GenBank, EMBL and DDBJ database were made using the FASTA program via the networked facilities of the Australian National Genomic Information Service. Phylogenetic trees were estimated by both parsimony and likelihood algorithms using PAUP*4.0d64 (Swofford, 1998). The quality of the resulting tree estimates was assessed by comparing results across a range of plausible character transformation and evolutionary process models. Only those parts of the phylogeny not sensitive to process model assumptions were accepted. Statistical support for relationships was assessed using conventional, nonparametric bootstrap tests (Felsenstein, 1985).

Morphological analyses

Previous studies (Delfinado-Baker, 1988; Belfinado-Baker and Houck, 1989) were unable to identify distinct phenotypic characters (other than body size) that would allow so-called *V. jacobsoni* specimens to be sorted into separate groups. With the assistance of Dr R.B. Halliday, CSIRO Entomology, Canberra, Australia, we carried out careful comparative morphological studies on a range of specimens of *Varroa* from populations that our mtDNA analysis had shown were genetically distinct. We used conventional light microscopy techniques and cryo-scanning electron microscopy (Craig and Beaton, 1996) to study the structure and setation of the dorsal shield, the structure and chaetotaxy of the sternal, epigynial, anal, and metapodal shields, the peritreme, tritosternum and hypostome, and the number, arrangement and morphology of setae on the legs and palps.

Although Delfinado-Baker (1988) and Delfinado-Baker and Houck (1989) distinguished differences in the body size of female *Varroa* collected from *A. cerana* and *A. mellifera*, the size differences overlapped. In our studies we measured the body length and width of up to 20 unmounted female specimens from most *V. jacobsoni* isolates (see Tables 1 and 2) using a dissecting microscope fitted with an ocular micrometer. Differences in body size and shape within and between populations, that we had determined were genetically distinct from our mtDNA analyses, were tested by ANOVA (analysis of variance) using the Genstat 5 computer program. In addition to the above measurements, body measurements were also obtained from each of 20 adult non-reproducing female *V. jacobsoni* collected from single *A. mellifera* colonies at Mount Hagen and Telefomin in Papua New Guinea. These measurements were compared with those taken from females collected from *A. cerana* colonies in New Guinea (see Table 1) to test whether there was a statistically significant difference in the size of mites of the same haplotype collected from different hosts.

Genetic isolation of artificially sympatric mite haplotypes

In Java, Indonesia, female *V. jacobsoni*, which parasitize and reproduce on drone brood of *A. cerana*, are totally incapable of reproducing on either worker or drone brood of *A. mellifera* (Anderson, 1994; Anderson and Sukarsih, 1996). They are also genetically distinct from phenotypically larger female *V. jacobsoni* that were introduced to Java during or shortly before 1993 and which can readily reproduce on worker and drone *A. mellifera* broods (Anderson and Fuchs, 1998).

To determine whether the recently introduced mites could reproduce naturally on *A. cerana* in Java, large numbers of adult females and nymphs of *V. jacobsoni* were collected from capped drone and worker cells of 6 hived *A. cerana* colonies at a single location in West Java in August 1997 and from capped worker and drone cells of 38 hived *A. cerana* colonies distributed throughout East, Central and West Java during October 1997. The identity of each mite in a representative subsample of

adults and nymphs was determined by PCR-restriction enzyme analyses of their mtDNA, as described earlier. Similar collections were made from the same sites in June 1998, and mites in a subsample taken from these collections were likewise identified. During these collections, a total of 38 adult female mites were collected from the bodies of adult bees from 10 hived *A. cerana* colonies by placing a single acaricide-impregnated plastic strip (Apistan®) between brood combs of each colony, and 1 hour later collecting the mites that had fallen onto sheets of white paper placed on the hive bottom-boards. These mites were also identified by PCR-restriction enzyme analyses of their mtDNA.

Results

Sequence analysis

The COXF and COXRa primers amplified a 458 base-pair (bp) DNA fragment from the crude DNA prepared from each individual mite. This was four bases longer than the fragments previously reported to have been amplified from *V. jacobsoni* DNA preparations using the same primers (Anderson and Fuchs, 1998); comparisons indicated that they had inadvertently omitted a 4-bp region from the beginning of the 5′ end of their published sequences.

(a) Sequences from mites collected from A. cerana *in Asia*

Eighteen different haplotypes (mites with unique mtDNA sequences) were detected among the *V. jacobsoni* isolates collected from *A. cerana* throughout Asia. The numbers of mites from which mtDNA sequences were obtained are shown in Table 1. For all, except the Sri Lanka and Nepal isolates (1 mite from 1 location), these samples are of an adequate size and of sufficiently wide geographical distribution to capture some within-country sequence variation, should it occur. These data, together with restriction enzyme digest data on the identity of more mites of each isolate (Table 1), confirmed that only one mite haplotype was present in any one country or isolated island. The exception was on the northern Philippines island of Luzon where two distinct haplotypes were detected, one from Batanges (Luzon 1 haplotype) and the other from San Fernando in La Union Province (Luzon 2 haplotype).

Searches of international databases showed that the DNA sequence obtained from mites on *A. cerana* in Java was identical to the mtDNA CO-I gene sequence obtained from mites collected from the same bee by Anderson and Fuchs (1998) (accession number AF010478). This mite sequence also showed 75.3% similarity with the CO-I gene fragment of the phytophagous spider mite *Tetranychus urticae* Koch (accession number X74571). Hence, that sequence was chosen as the outgroup for our phylogenetic analysis. MtDNA CO-I gene sequences obtained in the present study

from *V. underwoodi* and *V. rindereri* (GenBank accession numbers AF107260 and AF107261) were used to represent those species already described as morphologically distinct. All sequences used, except that of *V. rindereri*, were of the same length and were aligned without the need for numerical algorithms. The first 26 and last six positions of the *V. rindereri* sequence were not available, and so this sequence was aligned to the others by coding those positions as missing.

A parsimony tree estimation model, with transversions given twice the weight of transitions, yielded 27 trees (length 378, CI = 0.5534, RI = 0.7070). The strict consensus of these trees is shown in Figure 2a. The 18 different taxa divided into two main clades, the Mindanao-Vietnam and Flores-Java clades, with *V. underwoodi* and *V. rindereri* at the base. An unweighted parsimony analysis yielded 63 trees (length 250, CI = 0.5411, RI = 0.7100). The consensus of these trees differed from the weighted consensus tree only in that (i) *V. rindereri* was placed in an unresolved polytomy with the two main ingroup clades and the Mindanao haplotype; and (ii) the final four taxa as shown in Figure 2a were resolved as Malaysia (Borneo (Ambon, Bali, Java)). Differential downweighting of third-codon positions had no effect on

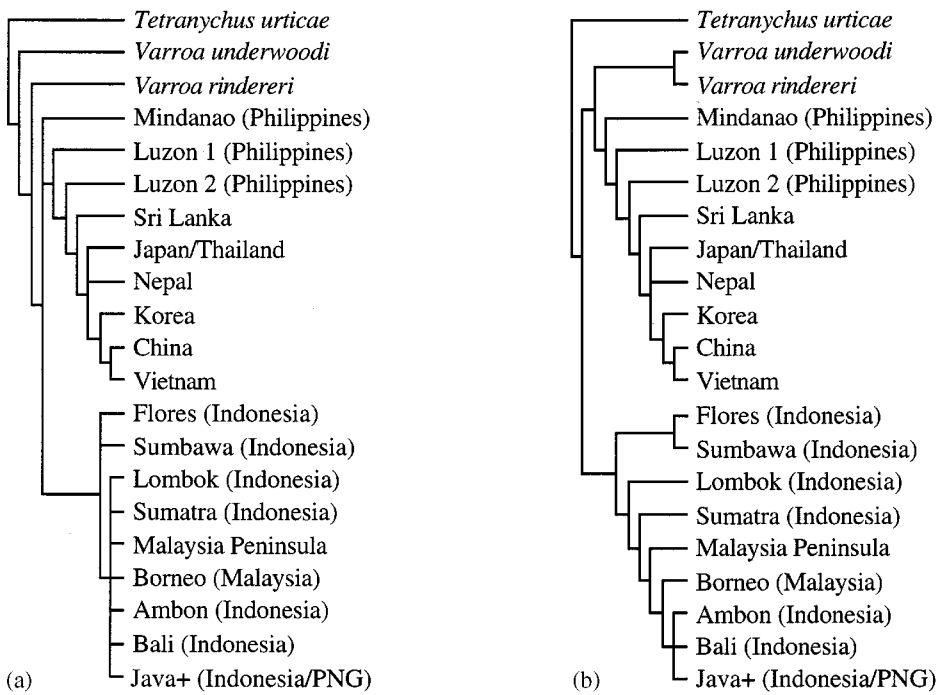

Figure 2. (a) Strict consensus of 27 trees from parsimony analysis with transversions weighted 2× transitions; (b) strict consensus of nine maximum likelihood trees. Luzon 1 = Batanges, Luzon, Philippines; Luzon 2 = San Fernando, La Union, Luzon, Philippines; Java+ = Java, Biak Is., Yapen Is., Timor, Sulawesi and Irian Jaya (Indonesia) and Papua New Guinea.

the parsimony result as far as they affected the consensus tree. Likelihood analysis using empirically observed base frequencies and a 2-parameter HKY85 model (Hasegawa *et al*., 1985; Swofford *et al*., 1998) yielded nine equally-likely trees (log-likelihood = −1816.07995). The strict consensus of these trees (Figure 2b) showed the same two ingroup clades (Mindanao-Vietnam and Flores-Java) as found by parsimony analysis. These were separated by a clade comprising *V. rindereri* and *V. underwoodi*.

The branch which connects to the outgroup taxon *T. urticae* is significantly longer than any other branch on these trees. It accounts for 47% of the total tree length under transversion-weighted parsimony. Long branches can force tree-topology errors under any method of tree estimation (Felsenstein, 1978; Swofford *et al*., 1996; Siddall, 1998). However, the deletion of *T. urticae* left the parsimony consensus tree for ingroup taxa unchanged, while likelihood analysis yielded the same nine trees as before when *T. urticae* was excluded. We conclude that there is no evidence for this long branch having biased the tree estimate in this case.

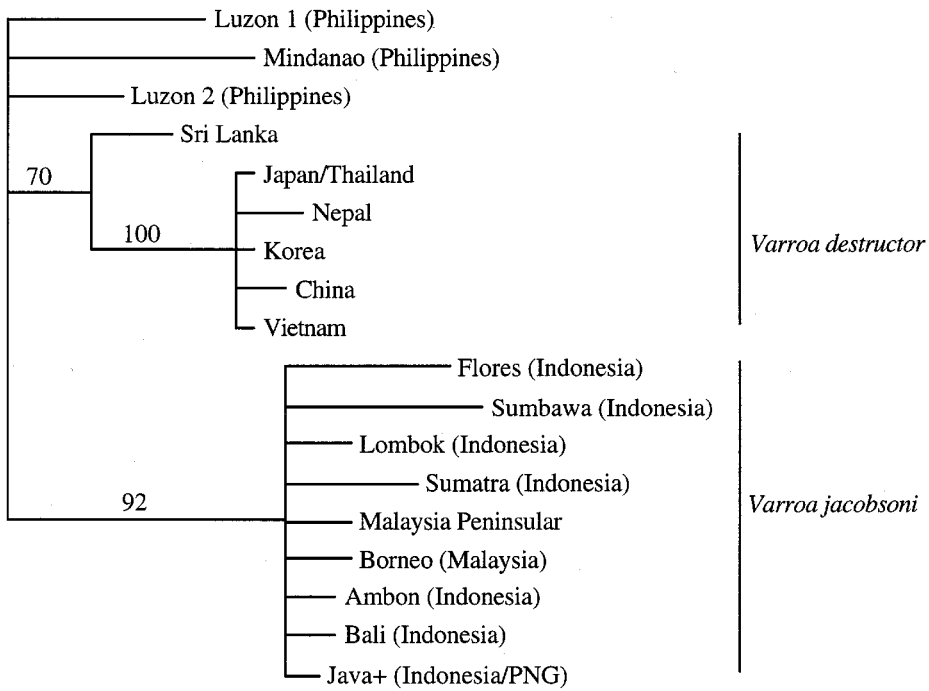

Figure 3. Majority rule consensus tree from transversion-weighted parsimony bootstrap (1000 pseudo-replicates). Branches with support less than 60% are collapsed. Branch lengths are drawn in proportion to the number of weighted changes under an ACCTRAN reconstruction of characters on the consensus tree. *V. rindereri* and *V. underwoodi* were included in the analysis but for clarity are not shown on the tree as drawn. They attach to the basal polytomy at bootstrap score 100%. Note that Java+ = Java, Biak Is., Yapen Is., Timor, Sulawesi and Irian Jaya (Indonesia) and Papua New Guinea.

Table 3. Matrix of pairwise distances (percent) between the main taxa resolved in the sequence analysis, corrected for multiple hits using the HKY85 model (Hasegawa *et al*., 1985). Where a comparison is between two individual sequences we report that pairwise distance. Where it is between an individual and a clade, or a clade and a clade, we report the mean of all individual pairwise distances involved in the comparison not the distance between the centroids of the clades. The distance between centroids will differ because the correction for multiple hits is non-linear. If a constant molecular clock can be applied, the values in this table will be in proportion to the times which have elapsed since the lineages diverged.

	1	2	3	4	5	6	7	8
1. Mindanao haplotype	–							
2. Luzon 1 haplotype	5.1	–						
3. Luzon 2 haplotype	5.1	3.4	–					
4. Sri Lanka haplotype	4.8	5.1	3.9	–				
5. Flores-Java clade	5.2	6.3	4.3	5.6	–			
6. Japan/Thai.-Vietnam clade	6.3	4.7	4.1	3.4	6.6	–		
7. *V. rindereri*	7.5	8.6	6.7	8.3	6.7	8.8	–	
8. *V. underwoodi*	10.0	9.2	8.4	8.2	9.7	9.8	8.8	–

Bootstrap analyses (1000 replicates, heuristic search) using parsimony confirmed that the Flores-Java clade and a Japan/Thailand-Vietnam clade were well supported by these data (Figure 3). The bootstrap test failed to support the relationships indicated in Figure 2 among Mindanao, Luzon 1 and Luzon 2 (bootstrap scores $<$50%). The bootstrap score for a sister relationship between a Sri Lanka isolate and the Japan/Thailand-Vietnam clade varied from 64–73%, depending on the model. The branching order within each well-supported clade is sensitive to variation in the model and/or tree estimation technique used, and cannot be considered reliable.

The mean pairwise distances (uncorrected) among haplotypes within the Japan/Thailand-Vietnam clade was 0.8%, and within the Flores-Java clade 1.8%. The mean haplotype-haplotype pairwise distance between these two clades was 6.2%. Mean pairwise distances from each clade to the morphologically distinguishable *V. rindereri* were 8.2% for the Japan/Thailand-Vietnam clade and 6.4% for the Flores-Java clade. The corresponding distances to *V. underwoodi* were 9.1% and 9.0%. Hence, the differences between the Japan/Thailand-Vietnam and Flores-Java clades are large enough to indicate species-level separation, whereas distances within the clades are small enough to indicate within population-level variation. Mean pairwise distances (corrected for multiple hits) among all the identified clades and haplotypes are shown in Table 3.

(b) MtDNA sequences from mites collected from A. nigrocincta *in Indonesia*

Sequences obtained from *V. jacobsoni* collected from *A. nigrocincta* colonies in Sulawesi, Indonesia (Table 1) were identical to those of the Java haplotype obtained from *A. cerana* in Java (Figure 3).

(c) MtDNA sequences from mites collected from A. mellifera *worldwide*

Sequences obtained from reproducing female *V. jacobsoni* collected from *A. mellifera* in 32 different countries (Table 2) were either those of the Korea haplotype or the Japan/Thailand haplotype, which were also detected on *A. cerana* in Korea and Japan/Thailand respectively (Figure 3). The particular haplotypes infesting *A. mellifera* at the various collection sites are listed in Table 2. The Korea haplotype was most common, and was found infesting *A. mellifera* in Africa, Europe, the Middle East, Asia and the Americas. The Japan/Thailand haplotype was less common, and was found infesting *A. mellifera* in Japan, Thailand and the Americas.

Morphological analyses

We were unable to identify any morphological characters (except body size) that would distinguish female mites belonging to each of the two main genetically distinct groups of mites resolved by our mtDNA analysis, or between them and each of the three unique haplotypes from the Philippines. Hence, the new species we describe below (which is also indicated in Figure 3) cannot be distinguished from *V. jacobsoni* using the morphological characters we examined (see methods).

The GENSTAT analysis of mite body length and width measurements showed no significant differences in body sizes (log width + log length) of mites of the same haplotype at any one location; of mites of the same haplotype in different locations (within countries or on islands within countries); of mites of different haplotypes within the Flores-Java clade or within the Japan/Thailand-Vietnam clade; or of mites of the same haplotype collected from different bee hosts. However, mites of the Flores-Java clade and the Japan/Thailand-Vietnam clade were clearly distinct, with no overlap in size values (Figure 4) ($F = 1937.89$). The size of mites of the Mindanao haplotype overlapped that of mites in the Flores-Java and Japan/Thailand-Vietnam clades, although the mean values were significantly different from those mites. The size of mites of the Luzon 1 and Luzon 2 haplotypes were indistinguishable from each other and from those mites in the Flores-Java clade. Mean body sizes (and standard deviations) are given in Table 4.

The difference in body shape (log width − log length) between mites of the different genetic groups shown in Figure 3 was barely significant ($F = 2.80$ $P < 0.05$). However, female mites of the Japan/Thailand-Vietnam clade appeared less spherical in shape than mites of the Flores-Java clade (Figure 5).

Genetic isolation of artificially sympatric mite haplotypes

In a subsample of 550 adult females and 183 female nymphs that were identified from large numbers of *V. jacobsoni* collected from capped brood cells of *A. cerana* colonies in Java during 1997 and 1998, all were identified as the Java haplotype, as

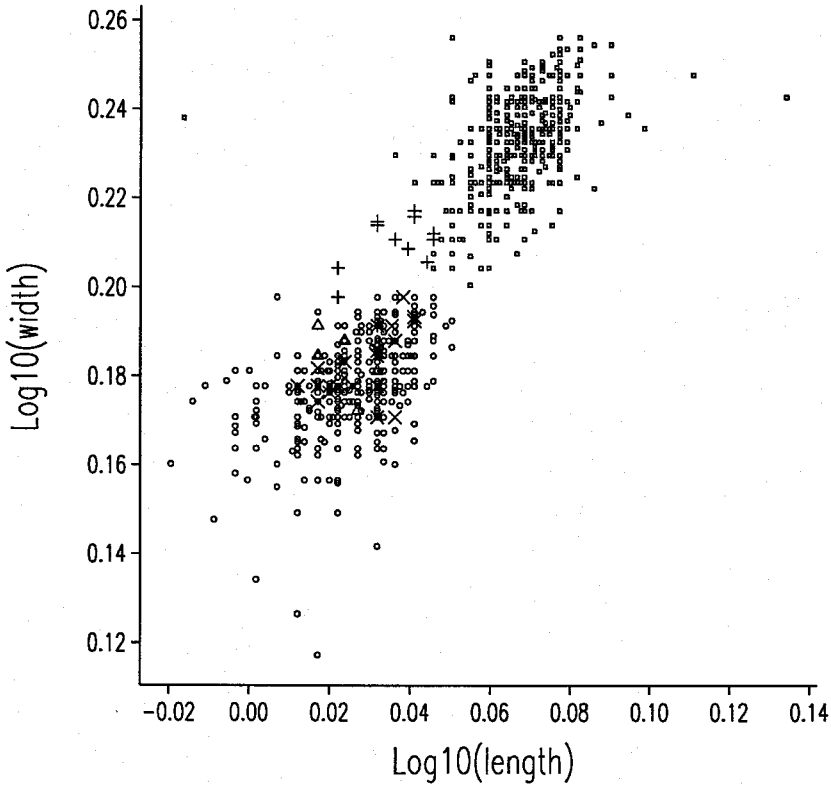

Figure 4. Plot of the body sizes (μm) of different isolates of the *Varroa* taxon groups resolved by the mtDNA analysis. Body width has been plotted against body length, and hence body size (log width + log length) and body shape (log width − log length) are correlated with the direction of the diagonal. □ = *V. destructor*; △ = Luzon 1 haplotype; ○ = *V. jacobsoni*; × = Luzon 2 haplotype and + = mindanao haplotype.

Table 4. Body lengths and widths of female mites in the different *Varroa* taxon groups.

Taxon group* (*species*)	Body length (μm)	SD	Body width (μm)	SD	*n*
Flores-Java (*V. jacobsoni*)	1063.0	26.4	1506.8	36.0	439
Japan/Thai.-Vietnam (*V. destructor*)	1167.3	26.8	1708.9	41.2	533
Luzon 1 haplotype	1055.2	15.6	1526.2	25.7	5
Luzon 2 haplotype	1067.4	21.4	1524.0	27.8	21
Mindanao	1086.0	22.4	1616.5	26.7	14

* = Refer to Figure 3.
SD = standard deviation.
n = number of mites examined.

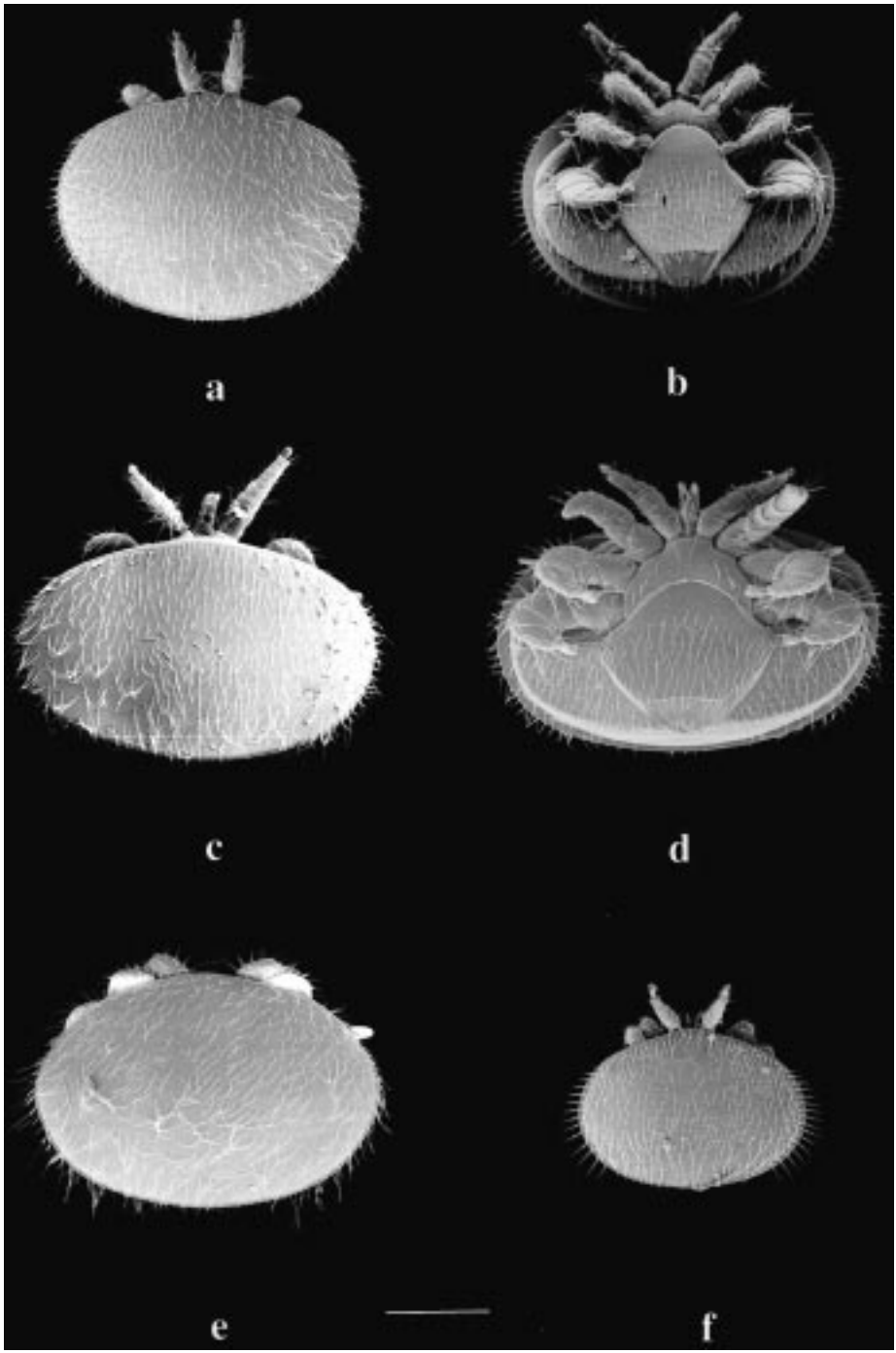

Figure 5. Appearances of the dorsal and ventral surfaces of adult females of *V. jacobsoni* (Java haplotype) (a & b) and *V. destructor* (Korea haplotype) (c & d). Also shown for comparisons are the dorsal surfaces of *V. rindereri* (e) and *V. underwoodi* (f). Bar = approx. 500 μm.

also were 38 adult female mites collected from the bodies of adult *A. cerana* in Java in 1998. Hence, these results show that individual mites of the Korea haplotype, which are the mites that reproduce in the *A. mellifera* colonies in Java (Anderson and Fuchs, 1998; also see results above), do not enter *A. cerana* colonies in Java. Therefore, because mites of the Java haplotype occasionally enter *A. mellifera* colonies in Java but cannot reproduce (Anderson, 1994; Anderson and Fuchs, 1998), it is clear that artificially sympatric mites of the Java and Korea haplotypes are reproductively isolated from one another.

Discussion

Varroa rindereri and *V. underwoodi* have been described from their physical characteristics as unique species (Delfinado-Baker and Aggarwal, 1987a; De Guzman and Delfinado-Baker, 1996). Our studies indicate that the third member of the genus, long referred to as *V. jacobsoni*, should be redefined, as it currently includes individuals that clearly belong to at least two different species. Our comparisons and analyses of mtDNA sequences obtained from mites that were called *V. jacobsoni*, and collected from widespread populations of *A. cerana*, recovered 18 distinct haplotypes which resolved into two well-separated clades (Japan/Thailand-Vietnam, and Flores-Java) plus three separate haplotypes (Mindanao, Luzon 1 and Luzon 2) (Figure 3).

Mean pairwise genetic distances within each of the two well defined clades were below 2%. The genetic distance (uncorrected) between these clades (6.2%) was almost as large as to the morphologically distinguishable *V. rindereri* (6.4% from the Flores-Java clade to *V. rindereri* and 8.2% from the Japan/Thailand Vietnam clade to *V. rindereri*) and *V. underwoodi* (9.0% and 9.1% respectively). *V. rindereri* and *V. underwoodi* themselves are separated by 8.2% on this measure. Similar patterns of pairwise distances have been observed for this molecule in other Acari. Navajas *et al*. (1994) reported 0–2.1% (uncorrected) nucleotide pairwise differences between different isolates of the cassava green mite *Mononychellus progresivus* Doreste, 5% between the close but distinct species *Tetranychus urticae* and *T. turkestani* Ugarov and Nikolskii, and 11% between *M. progresivus* and *T. urticae*, which belong to different genera. We conclude that the within-clade differences which we observed are sufficiently small to represent differences between populations of the same species, while the between-clade differences are sufficiently large to represent differences between species.

The phylogenetic analysis of mtDNA data uncovered a statistically supported hierarchic pattern. The 18 different haplotypes divided into a Japan/Thailand-Vietnam clade with five haplotypes and a Flores-Java clade with nine haplotypes. Three isolated sequences (Luzon 1, Luzon 2 and Mindanao) may possibly be more closely related to the Japan/Thailand-Vietnam clade than to the Flores-Java clade, while on the basis of a limited sample another sequence (Sri Lanka) is most probably

sister to the Japan/Thailand-Vietnam clade. Likelihood analysis, although not parsimony, placed *V. rindereri* and *V. underwoodi* as a sister-species pair, which separated the Flores-Java clade from the remaining taxa. Nonparametric bootstrap strongly supported the separate existence of two main clades but does not support any firm conclusion about relationships at the base of the tree. These basal relationships were resolved differently by parsimony and likelihood (Figure 2). On the parsimony bootstrap consensus tree (Figure 3) they were unresolved.

Female mites in the Japan/Thailand-Vietnam clade and the Mindanao sample were significantly larger than the others, with the Japan/Thailand-Vietnam clade not overlapping in size with the Flores-Java clade (Figure 4). Furthermore, representative mites of the Korea haplotype of the Japan/Thailand-Vietnam clade (which are mites that parasitize *A. mellifera* worldwide) were shown to be reproductively isolated from mites of the Java haplotype of the Flores-Java clade.

Hence, we have clearly demonstrated that *V. jacobsoni* is a species complex. Our evidence shows that it comprises two distinct sibling species. One is *V. jacobsoni*, redefined as encompassing mites of the Flores-Java clade shown in Figure 3. The other is a new species which we name *V. destructor* n. sp. In this proposed classification *V. jacobsoni* includes the Java haplotype, specimens of which were used to describe *V. jacobsoni* at the beginning of this century (Oudemans, 1904a). *V. destructor* is represented by mites of the Japan/Thailand-Vietnam clade shown in Figure 3. We regard the Sri Lanka haplotype as being conspecific with *V. destructor* pending further data, while three single haplotypes from the Philippines will require further studies to determine their taxonomic positions (discussed below). The appearances of adult females of the two species are compared with each other and with those of females of *V. rindereri* and *V. underwoodi* in Figure 5. A brief description of the two species follows.

Varroa jacobsoni Oudemans. (Figure 5, a & b).
Varroa jacobsoni Oudemans 1904a: 156.
Varroa jacobsonii Oudemans 1904b: 216.

Material examined: *Holotype*: 1 adult female, INDONESIA, Java, Semarang, ii.1904, in nest of *A. indica*, E. JACOBSON coll., specimen held at Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands (RMNH). *Paratype*: 1 adult female, same data as holotype. *Other specimens examined*: MALAYSIA: 15 adult females, Selangor, 29.i.1989, in nest of *A. cerana*, G.W. OTIS coll. (3 specimens deposited with the Australian National Insect Collection, Canberra, Australia (ANIC), 3 with the British Museum of Natural History, London, UK (BMNH), 3 with the United States National Museum, Washington DC, USA (USNM), 3 with the Museum National d'Histoire Naturelle, Paris, France (MNHM) and 3 with RMNH); 13 adult females, Borneo, Sabah, 9.v.1989, in nest of *A. cerana*, G.W. OTIS coll. (3 specimens deposited with ANIC, 3 with BMNH, 3 with USNM, 2 with MNHN and

2 with RMNH). INDONESIA: 16 adult females, Java, Sukabumi, 12.vi.1998, in nest of *A. cerana*, D.L. ANDERSON coll. (4 specimens deposited with ANIC, 3 with BMNH, 3 with USNM, 3 with MNHN and 3 with RMNH); 1 female Sumbawa, Sumbawabesar, 20.vi.1998, in nest of *A. cerana*, D.L. ANDERSON coll. (specimen deposited with ANIC); 1 female, Flores, Labuan Bajo, 24.vi.1998, in nest of *A. cerana*, D.L. ANDERSON coll. (specimen deposited with ANIC); 16 adult females, Sumatra, Padang, 9.viii.1997, in nest of *A. cerana*, D.L. ANDERSON coll. (4 specimens deposited with ANIC, 3 with BMNH, 3 with USNM, 3 with MNHN and 3 with RMNH); 14 adult females, Irian Jaya, Koya Timor, 4.iii.1996, in nest of *A. cerana*, D.L. ANDERSON coll. (specimens deposited with ANIC); 4 adult females, Irian Jaya, Wamena, 29.iii.1995, in nest of *A. cerana*, D.L. ANDERSON coll. (specimens deposited with ANIC). PAPUA NEW GUINEA: 19 adult females, Telefomin, 6.iv.1995, in nest of *A. mellifera*, D.L. ANDERSON coll. (specimens deposited with ANIC); 4 adult females, Mount Hagen, 12.iv.1995 in nest of *A. cerana*, D.L. ANDERSON coll. (specimens deposited with ANIC); 20 adult females, Lae, 15.iv.1995, in nest of *A. cerana*, D.L. ANDERSON coll. (specimens deposited with ANIC). AUSTRALIA: 22 adult females, Dauan Island, Torres Strait, 25.xi.1994, in nest of *A. cerana*, J. GRIMSHAW coll. (specimens deposited with ANIC).

Female: A brief description of the adult female is given by Oudemans (1904a), and a more complete description with 10 accompanying figures is given by Oudemans (1904b). The adult female can be readily distinguished from other females of different haplotypes within the species and from females of *V. destructor* and of unresolved *Varroa* haplotypes that infest *A. cerana* in the Philippines by its mtDNA CO-I gene sequence, shown in Figure 6.

Male: Not presently described.

Remarks: *V. jacobsoni* is redefined here as represented by several haplotypes that parasitize *A. cerana* colonies in the Malaysia-Indonesia-New Guinea region. These are the Flores, Sumbawa, Lombok, Sumatra, Malaysia, Borneo, Ambon, Bali and Java haplotypes shown in Figure 3. They can be distinguished from haplotypes of *V. destructor*, and of unresolved *Varroa* haplotypes that infest *A. cerana* in the Philippines, by their mtDNA CO-I gene sequences, which have been deposited in the GenBank database under the accession numbers given in Table 5. Female mites of the Java haplotype lack the ability to reproduce on *A. mellifera* and are significantly smaller than females of *V. destructor*. In our studies, the mean body length and width of representative unmounted females was 1063.0 μm (±26.4 μm) and 1506.8 μm (±36.0 μm) respectively (Table 4).

Varroa destructor n. sp., (Figure 5, c & d).

Material examined: *Holotype*: 1 adult female, SOUTH KOREA, Jungup, 20.x.1996, in colony of *A. mellifera*, D.L. ANDERSON coll., specimen deposited with ANIC.

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V. jacobsoni :  ATTTATTTTGATTTTTTGGGCATCCAGAAGTTTATATTTTAATTTTACCT  :   50
V. destructor:  ..................A..C.....................G...  :
Luzon 2      :  ..................A..C..G.....A.................  :
Luzon 1      :  ..................A..C.........................  :
Mindanao     :  .................................................  :

V. jacobsoni :  GGATTCGGAATTATTTCTCATGTAATTTGTATACAAAGAGGGAAAAAGCA  :  100
V. destructor:  ..T..T..T.....................................G.....  :
Luzon 2      :  ..T..T..G.....................................  :
Luzon 1      :  ..T..T........C.....................A..G.....  :
Mindanao     :  .....T.....................................A..G.....  :

V. jacobsoni :  ACCTTTTGGTAATTTAGGGATAATTTATGCTATAATAACTATTGGTATTT  :  150
V. destructor:  G........A.................C..............C.......  :
Luzon 2      :  .........A.....................................  :
Luzon 1      :  .........A.................C.....................  :
Mindanao     :  .................................................  :

V. jacobsoni :  TAGGTTTTATTGTATGAGCTCATCATATATTTACAGTAGGGATAGATATT  :  200
V. destructor:  ................G.....................A.........  :
Luzon 2      :  .....................................A.........  :
Luzon 1      :  ....G................................A.........  :
Mindanao     :  .....................................A.........  :

V. jacobsoni :  GATACTCGGGCTTATTTTACTGCGGCTACAATGATTATTGCGGTTCCCAC  :  250
V. destructor:  ........A..A..........A........A..............T..  :
Luzon 2      :  ......................A.......................T..  :
Luzon 1      :  ......................A.......................T..  :
Mindanao     :  ................C.....A................A.....T..  :

V. jacobsoni :  TGGTATTAAAATTTTTTCTTGATTAGCGACAATTCATGGTTCTATAGTAA  :  300
V. destructor:  ............................A...................T.  :
Luzon 2      :  ....................G.....T..............A.....T.  :
Luzon 1      :  ....................G.....T..............A.....T.  :
Mindanao     :  .........G.................T...................T.  :

V. jacobsoni :  AATTAGATGTTCCAATAATCTGATCCTTGGGTTTTATTTTTTTATTTACT  :  350
V. destructor:  ..........C..G.....T.....T..A.....................  :
Luzon 2      :  ..........A........T.....T..A.....................  :
Luzon 1      :  ..................T.....T..A..A..................  :
Mindanao     :  ..................T.....T..A..A..................  :

V. jacobsoni :  TTGGGGGGAATTACTGGTGTGATTTTAGCTAATTCTTCTATTGATATTGT  :  400
V. destructor:  ..A.....T...........A.............................  :
Luzon 2      :  ..A.....T...........A.............................  :
Luzon 1      :  ........T...........A...........C.................  :
Mindanao     :  ..A..A..T...........A.............................  :

V. jacobsoni :  TTTACATGATACTTATTATGTAGTAGCACATTTTCATTATGTATTAAGTA  :  450
V. destructor:  ....................................C...........A.  :
Luzon 2      :  .................................................A.  :
Luzon 1      :  C..G.............................C...........A.  :
Mindanao     :  A..............C........G.....................A.  :

V. jacobsoni :  TAGGAGCT  :  458
V. destructor:  ....G...  :
Luzon 2      :  ....G...  :
Luzon 1      :  ....G...  :
Mindanao     :  .G......  :
```

Figure 6. Alignment of the 5′ 458 nucleotides of the mtDNA CO-I genes of the holotype of *V. jacobsoni* (Java haplotype), the holotype of *V. destructor* (Korea haplotype) and each of the unresolved haplotypes from the Philippines (Luzon 2, Luzon 1 and Mindanao).

Table 5. GenBank accession numbers for the mtDNA CO-I gene sequences obtained from *Varroa* mites collected from *Apis cerana* in Asia.

Varroa species	Haplotype	GenBank accession numbers
Varroa destructor	China	AF106900
Varroa destructor	Korea	AF106899
Varroa destructor	Japan/Thailand	AF106897
Varroa destructor	Nepal	AF106898
Varroa destructor	Vietnam	AF106901
*Varroa destructor (tentative)**	Sri Lanka	AF106896
Varroa jacobsoni	Ambon	AF106908
Varroa jacobsoni	Bali	AF106909
Varroa jacobsoni	Borneo	AF106907
Varroa jacobsoni	Flores	AF106902
Varroa jacobsoni	Java	AF106910
Varroa jacobsoni	Lombok	AF106904
Varroa jacobsoni	Malaysia	AF106906
Varroa jacobsoni	Sumatra	AF106905
Varroa jacobsoni	Sumbawa	AF106903
Currently unresolved*	Luzon 1	AF106894
Currently unresolved*	Luzon 2	AF106895
Currently unresolved*	Mindanao	AF106893

* See text for experimental detail.

Paratypes: 13 adult females, same data as holotype (2 specimens deposited with ANIC, 3 with BMNH, 3 with USNM, 3 with MNHN and 2 with RMNH); 14 adult females, USA, Beltsville, Maryland, 17.vii.1997, in colony of *A. mellifera*, D.L. ANDERSON coll. (3 specimens deposited with ANIC, 3 with BMNH, 3 with USNM, 3 with MNHN and 2 with RMNH); 4 adult females, CANADA, Simcoe, Ontario, 26.v.1996, in colony of *A. mellifera*, G.W. OTIS coll. (specimens deposited with ANIC); 9 adult females JAPAN, Tamagawa, 22.x.1996, in colony of *A. mellifera*, D.L. ANDERSON coll. (2 specimens deposited with ANIC, 2 with BMNH, 2 with USNM, 2 with MNHN and 1 with RMNH); 16 adult females, GERMANY, Oberursel, vii.1996, in colony of *A. mellifera*, S. FUCHS coll. (4 specimens deposited with ANIC, 3 with BMNH, 3 with USNM, 3 with MNHN and 3 with RMNH); 10 adult females, UNITED KINGDOM, York, 28.viii.1997, in colony of *A. mellifera*, S. MARTIN coll. (2 specimens deposited at ANIC, 2 with BMNH, 2 with USNM, 2 with MNHN and 2 with RMNH); 15 adult females, BRAZIL, Sao Paulo, 6.viii.1997, in colony of Africanized *A. mellifera*, A.L. MERLO coll. (3 specimens deposited with ANIC, 3 with BMNH, 3 with USNM, 3 with MNHN and 3 with RMNH); 13 adult females, SOUTH AFRICA, Stellenbosch, iii.1998, in colony of *A. mellifera*, M. ALLSOP coll. (3 specimens deposited with ANIC, 3 with BMNH, 3 with USNM, 2 with MNHN and 2 with RMNH); 9 adult females, RUSSIA, Vladivostok, vi.1995, in colony of *A. mellifera*, G. DE LATTE and R. DANKA coll. (2 specimens deposited with ANIC, 2 with BMNH, 2 with

USNM, 2 with MNHN and 1 with RMNH); 25 adult females, INDONESIA, Biak Island, Irian Jaya, 23.iii.1995, in colony of *A. mellifera*, D.L. ANDERSON coll. (specimens deposited with ANIC); 20 adult females, INDONESIA, Pati, Java, vi.1994, in colony of *A. mellifera*, D.L. ANDERSON coll. (specimens deposited with ANIC).

Female: Morphologically similar to *V. jacobsoni*, but larger. Mean body length 1167.3 μm (±26.8 μm), mean body width 1708.9 μm (±41.2 μm) (Table 4). The adult female can be readily distinguished from other females of different haplotypes within the species and from females of *V. jacobsoni* and of unresolved *Varroa* haplotypes that infest *A. cerana* in the Philippines by its mtDNA CO-I gene sequence, shown in Figure 6.

Male: A detailed description of the male of *V. jacobsoni* by Delfinado-Baker (1984) that was taken from *V. jacobsoni* specimens collected from Burma, Brazil and Tunisia, is probably that of the male of *V. destructor*. However, further studies are needed to confirm this.

Remarks: *V. destructor* is represented by several haplotypes that infest *A. cerana* in mainland Asia (Figure 3). These are the Japan/Thailand, Nepal, Korea, China and Vietnam haplotypes. A Sri Lanka haplotype is regarded as being conspecific with *V. destructor* pending further data. These haplotypes can be distinguished from haplotypes of *V. jacobsoni* by their larger body size and mtDNA gene sequences. These sequences have been deposited in the GenBank database under the accession numbers given in Table 5. Most of what has been published to date about *V. jacobsoni* on *A. mellifera* is now applicable to *V. destructor*, particularly to the Korea haplotype. This haplotype infests and reproduces on *A. mellifera* worldwide. A Japanese/Thailand haplotype of *V. destructor* also infests and reproduces on *A. mellifera*, but it has a more restricted distribution than the Korea haplotype (Table 2).

The unresolved Varroa *haplotypes that infest* A. cerana *in the Philippines*

Due to the limited number of mite samples we analysed from *A. cerana* in the Philippines, further studies are needed to determine the taxonomic positions of the three unique *Varroa* haplotypes that we detected on those bees. In our phylogenetic analyses these haplotypes (Mindanao, Luzon 1 and Luzon 2) were widely separated from each other and from *V. jacobsoni* and *V. destructor* on the trees (Figure 3), but their phylogenetic relationships were uncertain. For these reasons, they appear to stand alone as distinct species. There was also evidence that each of these haplotypes was genetically isolated from the Korea haplotype of *V. destructor*, as the Korea haplotype was the only haplotype detected in *A. mellifera* colonies in the Philippines, whereas the Mindanao, Luzon 1 and Luzon 2 haplotypes were each only detected on local indigenous strains of *A. cerana*. The mtDNA CO-I gene sequences from each of these Philippine haplotypes have been deposited in the GenBank database under the accession numbers given in Table 5, and they are also shown in Figure 6.

Correlation of the Varroa *haplotypes with the biogeography of* A. cerana

The biogeography of the *Varroa* haplotypes discovered in our studies correlates closely with the biogeography of haplotypes of *A. cerana* in Asia. Comparisons by Smith and Hagen (1996) of mtDNA sequences obtained from adult *A. cerana* collected throughout Asia showed that *A. cerana* haplotypes belong to an 'Asian Mainland' group (India, Nepal, Thailand, Korea, Japan and Taiwan), a 'Sundaland' group (Peninsular Malaysia, Borneo, Java, Bali, Lombok, Flores, Timor and Sulawesi) and a 'Philippine' group (Luzon 1 and 2, Mindanao and Sangihe). The phylogenetic relationships among these groups was not determined. Nevertheless, the remarkably close correlation of their biogeography with the biogeography of the mite species and haplotypes described here reflects the natural host-parasite relationship that has long existed between *A. cerana* and *Varroa* mites, and suggests that further discoveries of variants of *A. cerana* will probably lead to discoveries of new variants of *Varroa*. Given that the Indian *A. cerana* differs from *A. cerana* in Sri Lanka and Nepal, we predict that new variants of *Varroa* await discovery in India.

Size and host correlations

Our comparisons of the body sizes of female mites belonging to the different haplotypes and species resolved by our mtDNA analysis clarify the results obtained from an earlier morphological study of *V. jacobsoni* by Delfinado-Baker and Houck (1989). Those workers reported that the size of adult female *V. jacobsoni* collected from *A. mellifera* was significantly different from that of adult female *V. jacobsoni* collected from *A. cerana*, even though there was overlap in the body sizes of mites collected from each bee species. Those workers also reported a correlation between the size of a female *V. jacobsoni* and the size of the bee species from which it was collected, with the relatively larger bee (*A. mellifera*) tending to be infested with large mites and the smaller bee (*A. cerana*) being infested with mostly small mites. However, the female mites examined in their studies had been collected from widespread populations of *A. cerana*, then pooled and examined. This was also the case for the female mites collected from *A. mellifera*. As our studies indicate, these pooled samples would have contained mixtures of different species of mite. Hence, those mites examined from *A. mellifera* would have been mostly of the physically larger *V. destructor*, the exception being the smaller mites collected from *A. mellifera* in Papua New Guinea, which would have been non-reproducing mites of the Java haplotype of *V. jacobsoni*. Similarly, the mites they examined from *A. cerana* on mainland Asia would have been haplotypes of the larger *V. destructor*, while those collected from *A. cerana* in South East Asia would have been haplotypes of the smaller *V. jacobsoni*, and of the currently unresolved Philippine haplotypes. In our studies, we compared the body sizes of female mites after taking into account their taxonomic status, biogeography and host distribution. This approach clearly showed

statistically significant differences in the size of mites from some of the different genetic groups. It also showed that the size of a bee host did not influence the body size of its resident *Varroa* mite. For instance, in our studies the body sizes of female *V. destructor* collected from the relatively small bee *A. cerana* were indistinguishable from those of female *V. destructor* collected from the larger *A. mellifera*. Likewise, the body sizes of female *V. jacobsoni* that we collected from *A. cerana* and *A. mellifera* in New Guinea were indistinguishable.

Correlations with other studies on variation in V. jacobsoni

Our results are consistent with reports of genetic differences among *V. jacobsoni* infesting *A. mellifera* in Brazil and the United States (US) (De Guzman *et al*., 1997), among *V. jacobsoni* infesting *A. mellifera* in South America and Europe (Issa, 1989) and among *V. jacobsoni* infesting *A. mellifera* in the US and *A. cerana* in Malaysia (Kraus and Hunt, 1995). They are also consistent with reports of insignificant genetic variability within and between populations of *V. jacobsoni* affecting *A. mellifera* in Europe (Biasiolo, 1992; De Guzman *et al*., 1999). It remains to be seen whether there are allozyme differences between the two *Varroa* species resolved in our studies.

Correlations with artificial movements of bee colonies

Our results are consistent with reports on the movement of *Varroa*-infested populations of *A. mellifera* between countries. For example, our detection of the Japan/Thailand haplotype of *V. destructor* in Brazil is consistent with a report that *V. jacobsoni* was first introduced to Brazil on *A. mellifera* imported from Japan via Paraguay in 1972 (De Jong *et al*., 1982). Our detection of the Java haplotype of *V. jacobsoni* in New Guinea is consistent with reports that *Varroa* mites were first introduced to New Guinea on *A. cerana* imported from Java during the 1970s (Delfinado-Baker and Aggarwal, 1987b; Anderson, 1994).

The artificial spread of V. destructor

The findings we have reported here illustrate the remarkable amount of genetic variation that exists among *Varroa* populations infesting *A. cerana* in Asia. This level of variation is, however, not mirrored among *Varroa* populations infesting *A. mellifera*. In all, only three of the 18 haplotypes we detected infesting *A. cerana* have so far been detected on *A. mellifera*. One of these, the Java haplotype of *V. jacobsoni* (Figure 3), cannot utilize *A. mellifera* for its reproduction, and hence is only a temporary inhabitant of *A. mellifera* colonies (Anderson, 1994; Anderson and Sukarsih, 1996; Anderson and Fuchs, 1998). The other two haplotypes, the Japan/Thailand and Korea haplotypes of *V. destructor*, can utilise *A. mellifera* for their reproduction. Unlike their behaviour on *A. cerana* where they can only reproduce on

capped drone brood (Koeniger *et al*., 1981), these haplotypes can reproduce on both capped drone and worker brood of *A. mellifera*. The fact that these haplotypes have spread from *A. cerana* to *A. mellifera* and become independent parasites of that bee is also remarkable, considering; (i) that other haplotypes of *V. destructor* have not achieved this and (ii) the Korea haplotype lacks the ability to spread into colonies of *A. cerana* in Java after being introduced on *A. mellifera* (results from this study; Anderson and Sukarsih, 1996). Nonetheless, the ability of these haplotypes to spread to and reproduce on *A. mellifera* allowed for their spread out of Asia. Of the two haplotypes, the Korea haplotype, which has also been referred to elsewhere as the Russian or R genotype and also as the GER genotype (De Guzman *et al*., 1997, 1998; Anderson and Fuchs, 1998), now has the largest geographical range (Table 2). It also appears more pathogenic to *A. mellifera* than the less widely distributed Japan/Thailand haplotype, which has also been referred to elsewhere as the Japan or J genotype (De Guzman *et al*., 1998, 1999). As our results indicate that the Korea haplotype is the only haplotype present in Europe, it is therefore this mite which is developing resistance to the chemicals used to control it on *A. mellifera* in Europe (Lodesani *et al*., 1995). New methods for controlling this mite are urgently needed and could well come from identifying and understanding those factors which prevent the majority of *Varroa* haplotypes from exploiting *A. mellifera* as an alternative host.

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