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**Original article**

# Genetic subpopulations of *Varroa* mites and their *Apis cerana* hosts in Thailand[1]

Natapot WARRIT[a], Deborah Roan SMITH[a]*, Chariya LEKPRAYOON[b]

[a] Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045, USA
[b] Department of Biology, Faculty of Sciences, Chulalongkorn University, 10330, Thailand

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**Abstract** – Thailand is the only place known where large populations of Mainland and Sundaland *Apis cerana* come into contact and where native strains of both *Varroa destructor* and *V. jacobsoni* occur. This provides a unique opportunity to investigate occurrence of *Varroa* species and mitochondrial lineages on different genetic lineages of *A. cerana* in a natural setting. We sampled Thai *Varroa* and *A. cerana* on a north to south transect, and identified mite and bee mtDNA haplotypes by RFLPs and COI sequence. Ranges of Mainland and Sundaland *A. cerana* meet at the Kra ecotone, between 10°34'N and 11°24'N. *Varroa jacobsoni* was found on both Sundaland and Mainland *A. cerana*; south of Kra ecotone (on Sundaland *A. cerana*) mites had the Malaysia haplotype, while north of Kra ecotone (on Mainland *A. cerana*) mites had the NorthThai1 or NorthThai2 haplotype. *Varroa destructor* was only found in Chiang Mai and Chiang Rai Provinces (18°36'N, 98°48'E) at altitudes above 1000 m. We found no evidence of *V. destructor* with so-called Japan-Thailand haplotype on Thai *A. cerana*.

***Apis cerana* / *Varroa* / coevolution / mitochondrial DNA / Thailand**

## 1. INTRODUCTION

The native hosts of *Varroa* are Asian species of cavity-nesting honeybees, including *Apis cerana* Fabr. and *A. nigrocincta* F. Smith (de Guzman and Rinderer, 1999). *Varroa* cause relatively little damage on their original hosts, but in colonies of *A. mellifera* L., *Varroa* cause serious damage by feeding on hemolymph of larvae, pupae and adults, and by transmitting or activating viral diseases (Bowen et al., 1999; Brødsgaard et al., 2000; Doebler, 2000; Sammataro et al., 2000; Martin, 2001). They are responsible for a reduction in the number of commercial and feral *A. mellifera* colonies in most parts of the world except Australia and some parts of Africa (Beetsma, 1994; Oldroyd, 1999; Sammataro et al., 2000; Zhang, 2000).

Until recently, the culprit was believed to be a single mite species, *V. jacobsoni*. Recent genetic, morphological, and behavioral studies (Anderson, 1994; Kraus and Hunt, 1995; Anderson and Sukarsih, 1996; de Guzman et al., 1997; Anderson and Fuchs, 1998; de Guzman et al., 1998; de Guzman and Rinderer, 1999; Anderson, 2000; Anderson and Trueman, 2000; Fuchs et al., 2000) have revealed that this is actually a complex of at least two and probably five species. The original Indonesian mite described as *V. jacobsoni* Oudemans (Oudemans, 1904) cannot reproduce on *A. mellifera*, a species which is not native to southeast Asia (Anderson, 1994; Anderson and Sukarsih, 1996; Anderson and Fuchs, 1998). The mite that infests the non-native host, *A. mellifera,* is recognized as a separate species, named *V. destructor* Anderson and Trueman (2000).

* Corresponding author: debsmith@ku.edu
[1] Manuscript editor: Stefan Fuchs

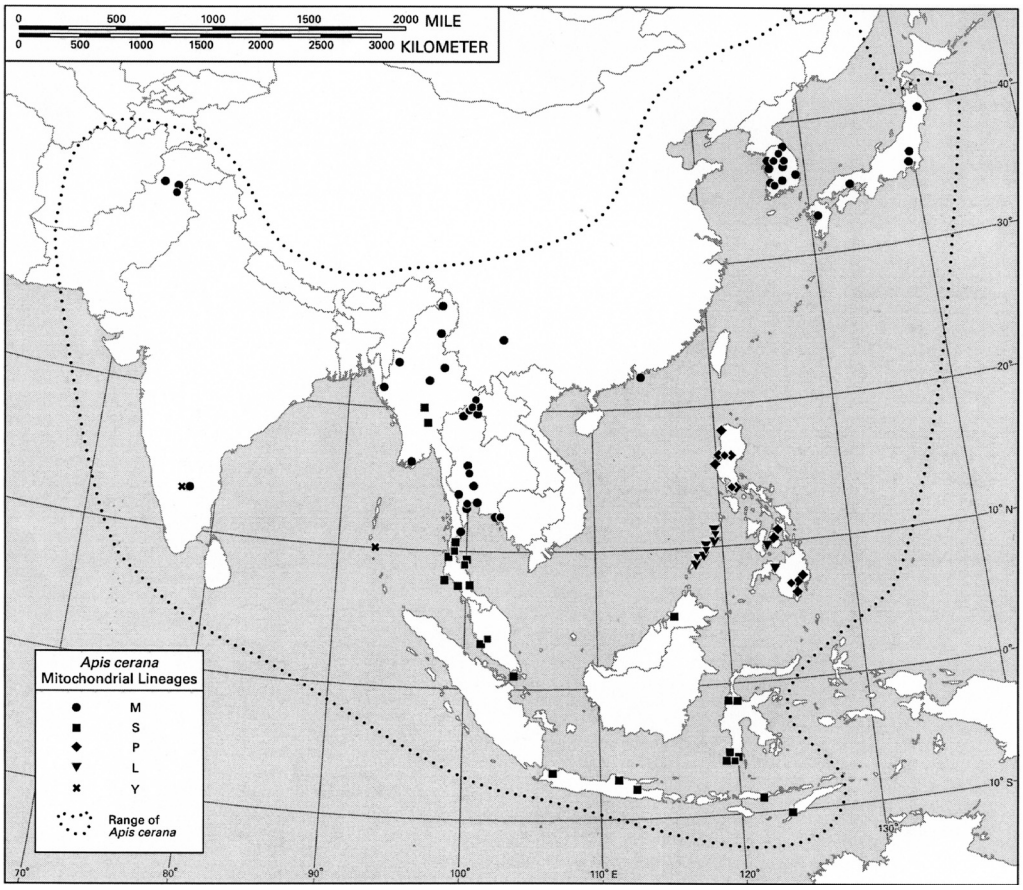


**Figure 1.** Approximate range of *Apis cerana*, showing major mitochondrial lineages (after de la Rua et al., 2000; Hepburn et al., 2001; Smith and Hagen, 1996, 1999; Smith et al., 2000). Circle = Mainland Asia, square = Sundaland, diamond = Oceanic Philippine islands, inverted triangle = Palawan, cross = "yellow plains bees".

The geographic distributions of *V. jacobsoni* Oudemans, *V. destructor* Anderson and Trueman, and other *Varroa* correspond broadly to the distributions of major mitochondrial DNA (mtDNA) lineages of *A. cerana*, their natural hosts (Smith and Hagen, 1996, 1999; Anderson and Trueman, 2000; de la Rua et al., 2000; Smith et al., 2000; Hepburn et al., 2001). The work of Anderson and Trueman (2000) indicated that *V. destructor* occurs on northern or "Mainland Asian" *A. cerana*, *V. jacobsoni* on southern or "Sundaland" *A. cerana*, and as yet unnamed Philippine *Varroa* occur on Philippine *A. cerana* (Fig. 1). These *Varroa* species are distinguished by morphology and by sequence differences in the mitochondrial Cytochrome Oxidase I (COI) gene (Anderson and Trueman, 2000).

On a finer scale, both *V. destructor* and *V. jacobsoni* show intraspecific, geographic variation in COI sequences. Anderson and Trueman (2000) reported nine mitochondrial COI haplotypes (mitochondrial genotypes) from *V. jacobsoni* and seven haplotypes from *V. destructor*. These haplotypes may be correlated with differences in the mites ability to utilize specific host populations and species. All *V. destructor* known to have colonized *A. mellifera* have one of two mtDNA haplotypes, reported in mites from Korea (Korean haplotype), Japan and Thailand (Japan-Thailand haplotype) (Anderson and Trueman, 2000).

*Varroa destructor* with other haplotypes, as well as *V. jacobsoni*, are apparently unable to reproduce on *A. mellifera*. There is also evidence that at least some populations of *V. destructor* – distinguished by different mitochondrial haplotypes – are best adapted to reproduce on particular populations of mainland Asian *A. cerana*. For example, native *A. cerana* in Vietnam harbor *V. destructor* with the "Vietnam" COI haplotype, while introduced *A. mellifera* harbor *V. destructor* with the "Korea" COI haplotype. No evidence was found of Korean *V. destructor* reproducing on nearby Vietnamese *A. cerana*, nor of Vietnamese *V. destructor* on *A. mellifera* (Fuchs et al., 2000).

The Thai-Malay peninsula presents a unique opportunity to investigate these host-parasite relationships in detail because of the variety of *A. cerana* lineages and *Varroa* species that occur there naturally. Morphological (Limbipichai, 1990), mtDNA (Deowanish et al., 1996, 1998; Smith and Hagen, 1996; Sihanuntavong et al., 1999; Smith and Hagen, 1999; Smith et al., 2000; Hepburn et al., 2001) and microsatellite (Sittipraneed et al., 2001) studies have shown that the Mainland and Sundaland *A. cerana* populations come into contact at a sharp boundary in the Isthmus of Kra, at a biogeographic transition area known as the Kra Ecotone (Whitmore, 1984) (Figs. 1, 2). *Varroa jacobsoni* has been reported from *A. cerana* with Sundaland lineage mtDNA in peninsular Malaysia (south of the Kra ecotone), and *V. destructor* with the Japan-Thailand haplotype was reported from *A. cerana* in Bangkok, north of the ecotone (Anderson and Trueman, 2000), although as we show this report is probably erroneous.

Thus, Thailand is the only place known where large populations of Mainland and Sundaland *A. cerana* and both *V. destructor* and *V. jacobsoni* naturally co-occur. This enables us to address host-specificity under natural conditions. We investigated the distribution of *Varroa* species and mitochondrial lineages in Thailand to evaluate three alternatives: (1) *V. destructor* and *V. jacobsoni* are tightly linked to Mainland and Sundaland *A. cerana* hosts, respectively, or (2) the close proximity of southern and northern host lineages permits the two mite species to "introgress" onto different hosts, or (3) there is a match of host and parasite that does not correspond to the boundaries between *A. cerana* mitochondrial lineages and *Varroa* species.

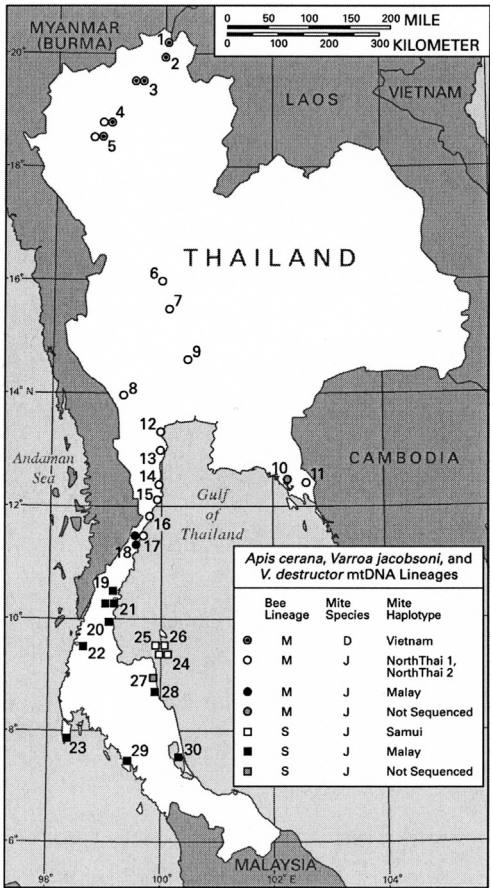


**Figure 2.** *Apis cerana* and *Varroa* collection sites in Thailand. Numbers correspond to those in Table I. Symbol shape and shading indicate *A. cerana* mitochondrial DNA (mtDNA) lineage and species and mtDNA haplotype of *Varroa* found in the bees' nests.

## 2. MATERIALS AND METHODS

### 2.1. Collections

Seventy-seven feral and semi-domesticated *A. cerana* colonies and their resident *Varroa* were collected from 30 locations in Thailand along a transect from Chiang San, Chiang Rai Province (20°16'N, 100°00'E) to Krasay Sin, Songklha Province (7°35'N, 100°17'E) during June–August 2001 (Fig. 2, Tab. I). *Varroa* were collected mostly from *A. cerana*

**Table I.** *Apis cerana* and *Varroa* collections in the Thai-Malay Peninsula. Locality numbers correspond to those in Figure 2. Geographic regions indicate groupings used in Analysis of Molecular Variance of *Varroa* populations (see Tab. III): N = northwest mountain, C = central, P = peninsular Thailand, S = Samui Island. Latitude and longitude measurements were determined using Garmin Model GPS 12 geographic positioning device. *Apis cerana* mtDNA lineage: M = mainland Asia lineage, S = Sundaland lineage. D = *V. destructor*, J = *V. jacobsoni*. *Varroa* haplotype names follow Anderson and Trueman (2000) and Warrit (2002).

| (Geographic region) and Locality | Latitude | Longitude | *A. cerana* lineage | *A. cerana* Haplotype[1] | *Varroa* species[2] | *Varroa* Haplotype[3] |
|---|---|---|---|---|---|---|
| 1. (N) Chiang San, Chiang Rai | 20°16'N | 100°00'E | M | ThaiN1 (2) | D (2) | Vietnam (2) |
| 2. (N) Mueang, Chiang Rai | 20°00'N | 99°57'E | M | ThaiN1 (2) | D (1) | Vietnam (1) |
| 3. (N) Mae Sruy, Chiang Rai | 19°35'N | 99°28'E | M | ThaiN1 (1) | D (1) | not sequenced |
| | | | | ThaiN2 (1) | D (1) | Vietnam (1) |
| 4. (N) Chang Kein, Chiang Mai | 18°50'N | 98°53'E | M | ThaiN1 (3) | D (1) | not sequenced |
| | | | | | J (2) | NThai2 (2) |
| 5. (N) San Pa Tong, Chiang Mai | 18°36'N | 98°48'E | M | ThaiN1 (2) | D (1) | not sequenced |
| | | | | | J (1) | NThai2 (1) |
| 6. (C) Bang Raka, Phitsanulok | 16°44'N | 100°10'E | M | ThaiN1 (1) | J (1) | NThai1 (1) |
| 7. (C) Kork Pra, Nakorn Sawan | 15°33'N | 100°04'E | M | ThaiN1 (2) | J (2) | NThai2 (1) |
| 8. (C) Sai Yok, Karnjana Buri | 14°02'N | 99°15'E | M | ThaiN1 (1) | J (1) | NThai1 (1) |
| 9. (C) Po Thong, Ang Thong | 14°40'N | 100°25'E | M | ThaiN1 (2) | J (2) | NThai1 (1) |
| 10. (C) Khlung, Chanta Buri | 12°27'N | 102°14'E | M | ThaiN1 (1) | J (1) | not sequenced |
| 11. (C) Kow Sa Ming, Trat | 12°29'N | 102°30'E | M | ThaiN1(4) | J (4) | NThai1 (1) |
| 12. (C) Amphawa, Samut Songkram | 13°22'N | 99°57'E | M | ThaiN1 (3) | J (2)(1)$^+$ | NThai1 (1), (1)$^+$ |
| 13. (C) Ban Lard, Phet Buri | 13°02'N | 99°56'E | M | ThaiN1 (2) | J (2) | NThai1 (2) |
| 14. (C) Pran Buri, Prachup Kiri Kan | 12°27'N | 99°58'E | M | ThaiN1 (2) | J (2) | NThai1 (2) |
| 15. (C) Sam Roi Yot, Prachup Kiri Kan | 12°11'N | 99°56'E | M | ThaiN1 (1) | J (1) | NThai1 (1) |
| 16. (C) Meung, Prachup Kiri Kahn | 11°53'N | 99°47'E | M | ThaiN1 (5) | J (4)(1)$^+$ | NThai1 (3)(1)$^+$ |
| 17. (P) Tup Sa Kae, Prachup Kiri Kan | 11°31'N | 99°35'E | M | ThaiN1 (2) | J (2) | NThai1 (1) |
| | | | | | | Malaysia1 (1) |
| | | | | ThaiN3 (1) | J (1) | Malaysia1 (1) |
| 18. (P) Bang Sapan, Prachup Kiri Kan | 11°24'N | 99°31'E | M | ThaiN1 (2) | J (2) | Malaysia1 (1) |
| 19. (P) Tha Sae, Chum Phon | 10°34'N | 99°06'E | S | Malay1 (2) | J (2) | Malaysia1 (1) |
| 20. (P) Suan Pheng, Chumphon | 10°01'N | 99°03'E | S | Malay1 (2) | J (2) | Malaysia1 (2) |
| 21. (P) Sawee, Chumphon | 10°20'N | 99°04'E | S | Malay1 (8) | J (7)(3)$^+$ | Malaysia1 (1) |
| | | | | ThaiS1 (2) | | Malaysia1 (1) |
| 22. (P) Ka Poe, Ranong | 09°35'N | 98°36'E | S | Malay1 (2) | J (2) | Malaysia1 (1) |
| 23. (P) Kra Tu, Phuket Island | 07°54'N | 98°21'E | S | Malay1 (3) | J (1), (2)$^+$ | Malaysia1 (1)$^+$ |
| 24. (S) Thaling Ngam, Ko Samui, | 09°28'N | 99°57'E | S | Malay1 (2) | J (2), (1)$^+$ | Samui1 (1) |
| Surat Thani | | | | KoSamui1 (1) | | Samui1 (1) |
| 25. (S) Lipa Yai, Ko Samui, Surat Thani | 09°31'N | 99°56'E | S | Malay1 (1) | J (1) | not sequenced |
| 26. (S) Wat Lard Varnon, Ko Samui, Surat Thani | 09°29'N | 99°57'E | S | Malay1 (2) | J (2) | Samui1 (1) |
| 27. (P) Sichon, Nakhonsri Thammarat | 09°02'N | 99°53'E | S | Malay1 (4) | J (4) | not sequenced |
| 28. (P) Thasala, Nakhonsri Thammarat | 08°46'N | 99°55'E | S | Malay1 (5) | J (5) | Malaysia1 (1) |
| 29. (P) Si Kao, Trang | 07°31'N | 99°24'E | S | Malay1 (2) | J (2) | Malaysia1 (1) |
| 30. (P) Krasay Sin, Songkla | 07°35'N | 100°17'E | S | Malay1 (1) | J (1) | Malaysia1 (1) |

[1] The number in parentheses indicates the number of colonies collected at each locality.
[2] The number in parentheses indicates the number of mites tested by restriction enzyme digest.
[3] The number in parentheses indicates the number of mites whose DNA was sequenced.
$^+$ = *Varroa* collected from drone brood cells of *A. cerana*.

worker brood, though some were collected from drone brood. Specimens were preserved in the field in 95% ethanol before being transported to the University of Kansas, USA for molecular analysis.

### 2.2. Identification of species and mitochondrial lineages

A non-coding mtDNA sequence in *A. cerana* (Cornuet et al., 1991; Smith and Hagen, 1996; Smith et al., 2000) was used to identify bees as members of either the Mainland or Sundaland mitochondrial lineage. A section of the COI gene was used to identify mites as *V. destructor* or *V. jacobsoni* and to identify their particular mtDNA haplotypes.

*DNA Extraction*: *Apis cerana* and *Varroa* DNAs were extracted according to Qiagen's DNEasy protocol for animal tissue utilizing DNA binding columns (Qiagen, Valencia, Ca.). One bee and one mite per nest were analyzed.

*PCR*: Amplification of the non-coding region of *A. cerana* mtDNA followed methods of Smith and Hagen (1996) and Smith et al. (2000). Amplification of a portion of the *Varroa* COI gene followed the methods of Warrit (2002), using a new pair of primers (V51: 5´-GTAATTTGTATACAAAGAGGG-3´ and V1400: 5´-CAATATCAATAGAAGAATT-AGC-3´) located inside the 458 base-pair portion of the COI gene originally surveyed by Anderson and Trueman (2000).

*Sequencing*: PCR amplified fragments were sequenced for all samples of *A. cerana*, and for a subset of 41 *Varroa* samples. PCR products were prepared for sequencing by agarose gel purification and Qiagen's Qiaquick Spin for gel extraction (Qiagen, Valencia, Ca.). Sequencing reactions were performed using USB's (Cleveland OH) Thermo Sequenase Radiolabeled Terminator manual cycle sequencing kit and appropriate primers (an internal primer described in Smith and Hagen (1996) for non-coding region of *A. cerana*, and primer V51 for the COI fragment of *Varroa*). New sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov) under accession numbers DQ061190, DQ061189, DQ061188, DQ064572, DQ064571, DQ064570.

*Restriction enzyme digests*: Sequencing showed that the particular *V. destructor* and *V. jacobsoni* haplotypes found in Thailand could be distinguished by means of restriction enzyme digests of the amplified COI fragment, and this procedure was used to identify the remaining *Varroa* samples to species. Each 15 µL reaction contained 10 µL of the amplified partial COI gene, 5 µL sterile distilled water and approximately 1 unit of one of the two enzymes, *Xho*I or *Sac*I, and was incubated at 37 °C for 3 h. Digestion products were separated by electrophoresis through 1% agarose TBE gels (0.089 M Tris, 0.089 M boric acid, 0.001 M EDTA) containing ethidium bromide for 1.5 h at 95 V, and visualized using UV illumination. Restriction digests of *Varroa* were scored as *V. destructor* (presence of both restriction sites) or *V. jacobsoni* (presence of *Sac*I restriction site, a positive control for successful restriction digestion).

### 2.3. Data analysis

Sequence divergences among *A. cerana* non-coding sequences were estimated using p-distance (proportion of nucleotide sites at which two sequences differ), and divergence among *Varroa* COI sequences were estimated using Kimura's 2-parameter model (Kimura, 1980) as implemented in MEGA version 2.1 (Kumar et al., 1994). We combined data on *Varroa* sequences observed in Thailand with published sequences of *V. destructor* Japan/Thailand haplotype, *V. underwoodi* and *V. rindereri* (Anderson and Trueman, 2000) to construct most-parsimonious trees of *Varroa* haplotypes using branch and bound search (Swofford, 1998) with equal weighting of all bases.

An analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was calculated using AMOVA version 1.50 for both *A. cerana* and *Varroa* sequences, to quantify genetic differentiation among geographic populations of the bees and the mites.

## 3. RESULTS

### 3.1. Apis cerana *subpopulations in Thailand*

Six 96 base pair non-coding sequences were found among the 77 samples of *A. cerana*. The haplotypes Thai1 or ThaiN1 (n = 37), Malay1 (n = 34), and KoSamui1 (n = 1) were previously reported from Thailand and Malaysia (Smith and Hagen, 1996). Three new rarer haplotypes were discovered and given the names ThaiN2 (n = 1), ThaiN3 (n = 1), and ThaiS1 (n = 2). The ThaiN2 and ThaiN3 haplotypes differ from the ThaiN1 haplotype by single base-pair substitutions (one transversion in ThaiN2 haplotype and one transition in ThaiN3 haplotype). The ThaiS1 sequence differs from Malay1 sequence by one transition. The sequences place ThaiN1, ThaiN2, and ThaiN3 haplotypes in the Mainland mitochondrial lineage, and Malay1, Kosamui1, and ThaiS1 haplotypes in the Sundaland mitochondrial lineage. Sequence divergence between Mainland Asia and Sundaland *A. cerana* in Thailand was greater than that

within each population: between group mean p-distance = 0.066, within group mean p-distances = 0.01. All *A. cerana* found north of 10°34'N had Mainland Asian mtDNA, while all those south of this point had only Sundaland mtDNA ($\phi_{ST} = 0.975$, $P < 0.0001$). *Apis cerana* with the Sundland haplotype "KoSamui1" were only found on the island of Samui (Ko Samui).

### 3.2. *Varroa* species and populations in Thailand

*Varroa* were collected from 76 of the 77 *A. cerana* nests. The fragment of the *Varroa* COI gene amplified by our primers was 328 base-pairs long, 130 base-pairs shorter than the fragment sequenced by Anderson and Trueman (2000). However, the shorter sequence contains enough informative sites to determine mites' species and haplotype. Cytochrome oxidase I sequences from 36 *Varroa* were identified as *V. jacobsoni* and four as *V. destructor,* based on comparison with published COI sequences (Anderson and Trueman, 2000). The *V. destructor* sequences matched the equivalent portions of the Vietnam haplotype reported by Anderson and Trueman (2000); no evidence was found of the *V. destructor* Japan/Thailand haplotype previously reported from Thai *A. cerana* by these authors. Four mitochondrial haplotypes were found among the Thai *V. jacobsoni*. The Malaysia haplotype (n = 13) was reported previously (Anderson and Trueman, 2000). Three new haplotypes were discovered and given the names NorthThai1 (n = 16), NorthThai2 (n = 4), and Samui1 (n = 3). The mean sequence divergence among Thai *V. jacobsoni* haplotypes was 0.012 (standard error 0.005), while the mean sequence divergence between Thai *V. jacobsoni* and *V. destructor* (Vietnam haplotype) was 0.077 (s.e. 0.017).

Results of sequencing showed that the *V. destructor* and *V. jacobsoni* COI haplotypes found in Thailand can be distinguished by an *Xho*I restriction site in the amplified portion of the COI gene which was present in *V. destructor* and absent in *V. jacobsoni*. This polymorphism was used to identify the remaining *Varroa* samples to species. In total eight samples had both *Xho*I and *Sac*I restriction sites indicating they were *V. destructor*. The remaining 68 had only the *Sac*I restriction site, indicating they were *V. jacobsoni*.

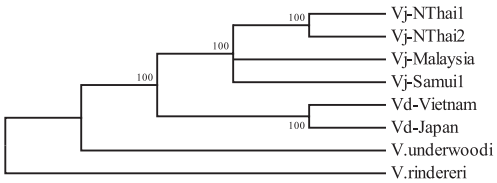


**Figure 3.** Relationship of *Varroa destructor* and *V. jacobsoni* mitochondrial haplotypes found in Thailand (*V. destructor* with Japan/Thailand not observed in this study); *Varroa rindereri* and *V. underwoodi* included as outgroups. Vj = *V. jacobsoni,* Vd = *V. destructor.* Tree search by branch-and-bound method (in Kumar et al., 1994). Strict consensus of three equally parsimonious trees of length 64 (29 parsimony informative characters, CI for informative sites = 0.8947, RI for informative sites = 0.8134).

A branch and bound search using 29 informative characters from the *Varroa* COI data set yielded a single most-parsimonious tree of 64 steps (Fig. 3), which supported grouping the Thai *Varroa* into two clades corresponding to *V. jacobsoni* and *V. destructor*.

### 3.3. Correspondence between bee and mite populations.

Figure 2 and Tables I and II show the geographical distributions of *A. cerana* and *Varroa* mtDNA haplotypes in western and southern Thailand. All eight *V. destructor* were found on *A. cerana* with Mainland mtDNA, while *V. jacobsoni* was found on both Mainland (31 samples) and Sundaland (37 samples) *A. cerana*.

Mitochondrial sequence data revealed four main combinations of bee and mite haplotype, each in a different geographic location (Fig. 2, Tab. IIIA). These locations are characterized by the following haplotype combinations: **(1) Mountain population** (Chiang Mai and Chiang Rai provinces in northwest Thailand): Only *A. cerana* with Mainland Asian mtDNA were detected here (ThaiN1 and ThaiN2 haplotypes). Both *V. destructor* with Vietnam haplotype (n = 4) and *V. jacobsoni* with NThai2 haplotype (n = 2) were found in these colonies. **(2) Central population** (from the Kra ecotone north to the mountains of the northwest): Only *A. cerana* with Mainland Asian mtDNA was

**Table II.** Summary of associations between *Apis cerana* mitochondrial lineages (Mainland and Sundaland) and haplotypes and *Varroa* species and mitochondrial haplotypes in Thailand. *Varroa* samples that were not sequenced were identified to species using a restriction site polymorphism in Cytochrome Oxidase I.

| | *V. destructor* | ----------*V. jacobsoni*---------- | | | | | Totals |
|---|---|---|---|---|---|---|---|
| *A. cerana* mtDNA | Vietnam | NorthThai1 | NorthThai2 | Malaysia | Samui1 | V. jacobsoni not sequenced | |
| Mainland ThaiN1 | 7 (3 sequenced) | 16 | 4 | 2 "mismatch" | 0 | 8 | 37 |
| Mainland ThaiN2 | 1 (1 sequenced) | 0 | 0 | 0 | 0 | 0 | 1 |
| Mainland ThaiN3 | 0 | 0 | 0 | 1 "mismatch" | 0 | 0 | 1 |
| Sundaland Malay1 | 0 | 0 | 0 | 9 | 2 | 23 | 34 |
| Sundaland ThaiS1 | 0 | 0 | 0 | 1 | 0 | 1 | 2 |
| Sundaland KoSamui1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Totals | 8 (4 sequenced) | 16 | 4 | 13 | 3 | 32 | 76 |

**Table III.** Genetic differentiation among *Varroa* mites in four geographic regions in Thailand: Northwestern Mountains, Central Thailand, Peninsular Thailand, and Samui Island. Analysis of Molecular Variance and $\phi$-statistics (Excoffier et al., 1992) compare observed distribution of *Varroa* species and mitochondrial haplotypes to 2000 permutations in which individuals are randomly assigned to four populations of the same size.

**A. Observed distribution of *Varroa* species and mitochondrial haplotypes in four geographic regions. The Peninsula region includes the three cases of bee-mite "mismatch" – *V. jacobsoni* with Malaysia haplotype in nests of Mainland *A. cerana*.**

| Groups | *V. destructor* | ----------*V. jacobsoni*---------- | | | |
|---|---|---|---|---|---|
| | | NorthThai1 | NorthThai2 | Samui1 | Malaysia |
| Northwest Mountain | 7 | 0 | 3 | 0 | 0 |
| Central | 0 | 15 | 1 | 0 | 0 |
| Samui Island | 0 | 0 | 0 | 3 | 0 |
| Peninsula | 0 | 1 | 0 | 0 | 14 |

**B. AMOVA analysis of genetic differentiation within and among *Varroa* groups occurring in four geographic regions. Both *V. destructor* (Vietnam haplotype, n = 4) and *V. jacobsoni* (four haplotypes: NorthThai1, n = 16; NorthThai2, n = 4; Samui, n = 3; and Malaysia, n = 13) included.**

| Component | df | Variance | % of total component | $\phi_{ST}$-statistics variance | *P*-value |
|---|---|---|---|---|---|
| Among groups | 3 | 0.0135 | 73.66 | 0.736 | <0.0005 |
| Within groups | 40 | 0.0048 | 26.95 | | |

**C. AMOVA analysis of genetic differentiation within and among groups of *V. jacobsoni* occurring in four geographic regions.**

| Component | df | Variance | % of total component | $\phi_{ST}$-statistics variance | *P*-value |
|---|---|---|---|---|---|
| Among groups | 3 | 0.0064 | 91.13 | 0.9113 | <0.0005 |
| Within groups | 33 | 0.0006 | 8.87 | | |

detected here (ThaiN1, n = 24). Only *V. jacobsoni* was found in these colonies (n = 24, 16 sequenced), and all of the mites sequenced had the NThai1 (n = 15) or NThai2 (n = 1) haplotype. **(3) Peninsula population** (South of the Kra ecotone): Only *A. cerana* with Sundaland mtDNA was detected here (Malay1 haplotype, n = 29, and ThaiS1 haplotype, n = 2). Only *V. jacobsoni* was found in these nests (n = 31, 17), and all of the mites sequenced had the Malaysia haplotype (n = 17). **(4) Samui Island population** (off the eastern coast of peninsular Thailand): Only *A. cerana* with Sundaland mtDNA was found here (Malay1 haplotype, n = 5, and KoSamui1 haplotype, n = 1). Only *V. jacobsoni* was found in these nests (n = 6), and all of the mites sequenced had the Samui1 haplotype (n = 3). AMOVA showed significant geographic partitioning of mtDNA variation among these four *Varroa* populations ($\phi_{ST} = 0.74$, $P < 0.0005$, for *V. destructor* and *V. jacobsoni* populations, $\phi_{ST} = 0.91$, $P < 0.0005$, for *V. jacobsoni* populations alone, Table IIIB, C).

In the Kra ecotone (at Tup Sa Kae, Prachup Kiri Kan, 11°31'N, 99°35'E, and Bang Sapan, Prachup Kiri Kan, 11°24'N, 99°31'E) where Mainland and Sundaland *A. cerana* meet, we found three nests with an apparent "mismatch" of bee and mite haplotypes – the southern *V. jacobsoni* with Malaysia mtDNA haplotype was found on the northern type of *A. cerana* with Mainland mtDNA. However, since these mites were found in worker brood cells, it is quite possible that they were "visitors" from the southern population – feeding, but unable to reproduce in the northern *A. cerana* colonies.

## 4. DISCUSSION

We found no evidence of *V. destructor* on *A. cerana* near the Bangkok region of Thailand, where Anderson and Trueman's Thai samples originated (Anderson and Trueman, 2000), and no evidence of the so-called Japan-Thailand haplotype in any *V. destructor* collected from any Thai *A. cerana*. It is likely (Anderson, pers. comm.) that the *V. destructor* samples provided from Bangkok were actually taken from imported *A. mellifera* colonies. This is a pleasing result, because it means that the mites that have colonized *A. mellifera* possess mtDNA haplotypes originating in a discrete geographic region that includes Korea and Japan – though the complete extent of its range is not known.

As previously reported, northern and southern mitochondrial lineages of *A. cerana* – Mainland *A. cerana* (n = 39) and Sundaland *A. cerana* (n = 37) – came into contact at the Isthmus of Kra at latitudes 10°34'N to 11°24'N. This corresponds to the Kra ecotone, a biogeographical boundary where the forest changes from semi-seasonal rainforest to non-seasonal rainforest (Fig. 2). The distribution of the northern lineage reached its southern limit at Bang Sapan, Prachup Kiri Khan Province (11°24'N and 99°31'E), and the distribution of the southern, Sundaland lineage reached the farthest north at Tha Sae, Chumphon Province (10°34'N and 99°06'E). We found no overlap in the ranges of these two *A. cerana* mitochondrial lineages. Within each lineage, Mainland and Sundaland, mitochondrial diversity was surprisingly low. Prior to this study, only three mitochondrial non-coding sequences were known from Thai *A. cerana*: ThaiN1 from bees north of the Isthmus of Kra, Malay1 from bees south of the Isthmus of Kra, and KoSamui1 from Samui island. Our extensive survey revealed little additional variation. In the populations north of the Isthmus of Kra we detected two new, rare haplotypes, ThaiN2 (1 colony) and ThaiN3 (1 colony), each differing from the common ThaiN1 by only 1 base substitution. In the populations south of the Isthmus of Kra we detected one new haplotype, ThaiS1 (2 colonies), in addition to the common Malay1 haplotype. On the island of Samui, we found two haplotypes, KoSamui1 and Malay1.

Both *V. destructor* and *V. jacobsoni* were found in Thailand, but unlike the two mitochondrial lineages of *A. cerana*, their ranges did not meet near the Kra ecotone. Instead, *V. jacobsoni* was found on both Sundaland *A. cerana* south of the Isthmus of Kra, and on Mainland *A. cerana* north of the Isthmus of Kra (Fig. 2). *Varroa destructor* was only found in Chiang Mai and Chiang Rai Provinces, between Chang Kein and San Pa Tong (18°36'N, 98°48'E), at altitudes greater than 1000 m.

It was initially puzzling to find *V. jacobsoni* on Mainland *A. cerana*, since Anderson and Trueman (2000) had found this species only on Sundaland populations of *A. cerana*. However, examination of the mtDNA COI sequences of *V. jacobsoni* revealed a subtle match of host

and parasite at the level of the *Varroa* mitochondrial haplotype (Fig. 2). With the exception of the three nests located in the Kra ecotone, *V. jacobsoni* south of the Isthmus of Kra (on Sundaland *A. cerana*) possessed the Malaysia or Samui1 haplotype, while *V. jacobsoni* north of the Isthmus of Kra (on Mainland *A. cerana*) had the NorthThai1 or NorthThai2 haplotype. *Varroa destructor*, with the Vietnam haplotype, was found only on Mainland *A. cerana* in the mountains of northwest Thailand.

Although most of our mite samples came from worker brood, we are confident that the bee and *V. jacobsoni* combinations we found in the Central region north of the Kra ecotone and in the Peninsula region south of the Kra ecotone are indicative of breeding populations of mites on their *A. cerana* hosts, since we detected no other bee or mite strains in these regions.

We observed three cases of host and parasite "mismatch" – the southern mitochondrial type of *V. jacobsoni* on the northern mitochondrial lineage of *A. cerana*. These three *A. cerana* nests were the southernmost of the Mainland nests collected, at the Kra ecotone where the northern and southern lineages of bee, and northern and southern strain of *V. jacobsoni* are in close proximity. These three nests could be either instances of mites occupying a nest without reproduction, or mites reproducing on "inappropriate" hosts. Only further examination of such colonies during drone production season can resolve this.

In Chiang Mai and Chiang Rai, both *V. destructor* and *V. jacobsoni* (with northern mtDNA type) were found in nests of *A. cerana*. It is not clear if both species are able to reproduce in the nests of these bees, nor is it clear if there are differences in parasite susceptibility among *A. cerana* colonies in this location. Again, studies during drone production season are needed to answer these questions.

Previous studies (Anderson and Trueman, 2000) indicated that *V. destructo*r is found on *A. cerana* that occur on the Asian mainland and have Mainland mtDNA haplotypes, *V. jacobsoni* is found on *A. cerana* that occur in the Indonesian Archipelago and have Sundaland mtDNA haplotypes, and unique (unnamed) *Varroa* are found on Philippine *A. cerana* with unique Philippine mtDNA haplotypes. This broad geographical congruence of *A. cerana* genetic lineages and *Varroa* species suggested coevolution of host and parasite as a result of vicariance: geographic isolation of host populations, followed by genetic divergence of both host and resident parasite populations.

Although historical geological events were probably important in determining the broad geographic distribution of host and parasites, spatial heterogeneity in host populations, parasite populations and environment are also likely to be important (Gandon and Van Zandt, 1998). Proximal factors likely to be involved in the *Apis-Varroa* interactions observed today include climate (De Jong et al., 1984; Moretto et al., 1991; Thomas and Blanford, 2003), the ability of the mite to detect appropriate host brood cells (Le Conte et al., 1989; Trouiller et al., 1992; Kraus, 1994; Kuenen and Calderone, 1998; Calderone and Kuenen, 2001; Aumeier et al., 2002), ability of a female mite to begin egg-laying after feeding on the larva of a potential host, and the ability of the hosts to detect and destroy mites (Peng et al., 1987; Spivak, 1996; Rath, 1999). Most *Varroa* living in the islands of Indonesia have no access to Mainland Asian bees, and *Varroa* living in mainland Asia have no access to Sundaland bees. The Thai-Malay region is unique in that it is the only place where large populations of the two major mitochondrial lineages of *A. cerana* – the Mainland and Sundaland lineages – naturally come into contact. It is also the only place where native strains of both *V. destructor* and *V. jacobsoni* have been shown to occur. This presents *V. jacobsoni* and *V. destructor* with the opportunity to "colonize" new hosts. Our study showed that *Varroa* species boundaries are not congruent with the boundary between Mainland and Sundaland *A. cerana* mitochondrial lineages in Thailand. *Varroa jacobsoni* occurs on both Sundaland and Mainland *A. cerana* in Thailand, but the *V. jacobsoni* populations on each *A. cerana* lineage have different mitochondrial haplotypes. This suggests that populations of *Varroa* on different host populations are genetically differentiated, and may be adapted to specific characteristics of their local host populations.

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**Résumé – Sous-populations génétiques d'acariens *Varroa* et de leur hôte *Apis cerana* en Thaïlande.** La répartition géographique des espèces de *Varroa* correspond en gros à la répartition des lignées d'ADN mitochondrial (ADNmt) de leur hôte, *Apis cerana* Fabr. : *V. destructor* est présent sur *A. cerana* en Asie continentale, *V. jacobsoni* sur *A. cerana* du Sundaland (Indonésie, Malaisie, et Brunéi) et l'espèce sans nom des Philippines sur *A. cerana* des Philippines (Fig. 1). La péninsule thaïmalaisienne présente une occasion unique d'étudier les relations hôte-parasite puisque qu'on y rencontre deux lignées *d'A. cerana* (Asie continentale et Sundaland) et deux espèces de *Varroa* (*V. jacobsoni* et *V. destructor*). On a prélevé dans 77 colonies sauvages et semi-domestiquées d'*A. cerana* réparties en 30 localités de Thaïlande (Fig. 2 et Tab. I) des abeilles et leurs acariens parasites *Varroa*. Les lignées d'*A. cerana* ont été déterminées par séquençage d'une région non codante de l'ADNmt. L'espèce et la lignée mitochondriale de *Varroa* ont été déterminées par le séquençage d'une portion du gène de la cytochrome oxydase I (COI) et par l'absence ou la présence d'un site de restriction caractéristique dans COI. *A. cerana* d'Asie continentale (n = 40) et du Sundaland (n = 37) viennent en contact au niveau de l'isthme de Kra (latitude 10°34'N environ) (Figs. 1 et 2). *V. destructor* (n = 8), ni *V. jacobsoni* (n = 68) n'ont été trouvés en Thaïlande, mais leurs aires de répartition ne se rencontrent pas près de l'écotone (frontière entre deux écosystèmes) de Kra. Comme attendu, nous avons trouvé *V. jacobsoni* sur *A. cerana* du Sundaland au sud de l'écotone de Kra. Par contre nous l'avons trouvé également bien au nord de l'écotone de Kra sur *A. cerana* du continent asiatique. A quelques exceptions près, *V. jacobsoni* sur *A. cerana* du Sundaland possédait le génotype NorthThai1 ou NorthThai 2. *Varroa destructor* n'a été trouvé que dans les provinces de Chiang Mai et de Chiang Rai, au-dessus de 1000 m d'altitude ; seul l'haplotype du Vietnam de *V. destructor* a été détecté. Nous n'avons pas trouvé de preuve de l'existence de l'haplotype « Japon-Thaïlande » de *V. destructor* sur *A. cerana* en Thaïlande, et aucune preuve d'aucun type de *V. destructor* sur *A. cerana* dans la région de Bangkok. Les mentions antérieures de l'haplotype « Japon-Thaïlande » de *V. destructor* sur *A. cerana* en Thaïlande étaient probablement des erreurs ; ces acariens ont probablement été prélevés sur des abeilles *A. mellifera* importées.

***Apis cerana* / *Varroa* / co-évolution / ADN mitochondrial / Thaïlande**

**Zusammenfassung – Subpopulationen von *Varroa* Milben und ihre *Apis cerana* Wirte in Thailand.** Die geographische Verbreitung der *Varroa* Arten stimmt weitgehend mit der durch mitochondriale DNA (mtDNA) bestimmten Verbreitung der Subpopulationen ihrer Wirte *Apis cerana* überein: *V. destructor* kommt bei *A. cerana* des asiatischen Festlands vor, *V. jacobsoni* bei *A. cerana* des Sundaland, und die noch nicht benannten philippinischen Arten von *Varroa* bei den philippinischen Linien von *A. cerana* (Abb. 1). Die thailändisch-malayische Halbinsel bot eine einzigartige Gelegenheit, die Wirt/Parasit Beziehungen zu untersuchen, weil dort 2 Linien von *A. cerana* (Festland und Sundaland) und zwei Arten von *Varroa* (*V. jacobsoni* und *V. destructor*) vorkommen. Siebenundsiebzig wilde und halb-domestizierte *A. cerana* Völker wurden mitsamt ihren *Varroa* Milben in 30 Gebieten von Thailand gesammelt (Abb. 2 and Tab. I). *Apis cerana* Linien wurden durch Sequenzierung einer nicht kodierenden Region der mtDNA bestimmt. Die Arten und mitochondrialen Linien der *Varroa* Milben wurden durch Sequenzierung eines Abschnitts des Cytochrome Oxidase I (COI) Gens und auf Grund der Präsenz oder des Fehlens einer typischen Restriktionsstelle bei COI bestimmt. *A. cerana* vom Festland (n = 40) und Sundaland (n = 37) hatten am Isthmus von Kra (etwa beim Breitengrad 10°34'N) Kontakt miteinander (Abb. 1, 2). Beide Arten *V. destructor* (n = 8) und *V. jacobsoni* (n = 68) wurden in Thailand gefunden, aber der Übergang lag nicht in der Nähe des Isthmus von Kra. Wie erwartet fanden wir *V. jacobsoni* auf der Sundaland-*A. cerana* südlich von der Kra Übergangszone. Unerwartet fanden wir *V. jacobsoni* aber auch weit nördlich der Kra-Zone auf der Festland-*A. cerana*. Mit einigen Ausnahmen hatten *V. jacobsoni* auf *A. cerana* von Sundaland den malaysischen mitochondrialen Haplotyp. *V. jacobsoni* der Festland *A. cerana* hatten den NordThai1 oder NordThai2 Genotyp. *Varroa destructor* wurde nur in Chiang Mai und in den Chiang Rai Provinzen gefunden, und zwar in Höhen über 1000 m; es wurde nur der Vietnam Haplotyp von *V. destructor* entdeckt. Wir fanden keinen Hinweis auf den „Japan-Thailand" Haplotypen von *V. destructor* auf *A. cerana* in Thailand, und außerdem kein Anzeichen von *V. destructor* auf irgendeinem Typ von *A. cerana* im Gebiet von Bangkok. Frühere Meldungen von einem „Japan-Thailand" Haplotyp von *V. destructor* auf *A. cerana* in Thailand waren wahrscheinlich ein Irrtum; diese Milben wurden wahrscheinlich von importierten *A. mellifera* Völkern gesammelt.

***Apis cerana* / *Varroa* / Coevolution / mitochondriale DNA / Thailand**

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