**Supplementary material**

***Varroa destructor*: A complex parasite, crippling honey bees worldwide**

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**Box S1. Varroa species identification**

The presence of varroa mites on their hosts and into newly introduced areas has been notified in different ways. As the global invasion by varroa mites became a rapidly growing problem, developing methods to accurately identify the culprit behind Western honey bee colony losses became essential. Given that varroa mites form a cryptic species complex [S1], an on-field glance diagnosis made by a non-taxonomist expert is challenging or almost impossible. Since the 1970s and with the help of beekeeping movements, what was described as “*V. jacobsoni*”, expanded its range out of Asia by conquering several countries each year [S2]. Varroa is an animal notifiable by the OIE. The development of molecular markers was a game-changer and allowed a major taxonomic revision in 2000. As its name indicates, “*V. destructor*” is the real identity of the cosmopolitan Western honey bee nightmare while *V. jacobsoni* remained unable to reproduce on this host. Despite its status as notifiable infectious disease, *V. destructor’s* presence is not systematically reported nor confirmed in the OIE database even when confirmed by molecular approaches

When its presence is reported, some still prefer the rapidity and affordability of morphometrics to report *V. destructor*: the slightly larger and wider in body size than *V. jacobsoni*. Nonetheless, the mtDNA barcoding of the COX1 gene slowly grew as standard method to report the novel presence of Varroa [S3]. One consequence of the later availability of molecular tools in Varroa invasion is that all observations of “*V. jacobsoni*” made before the 2000 research milestone may be difficult to confirm and more so in Asia where several species coexist. Another problem is that once the parasite settled in a new area, varroa population species and strains composition are not systematically checked if so, it is rare that the process is repeated over time. Considering that varroa populations in a region remained the same over the course of the invasion could be a big bias in mite control as i) more jumps than expected occurred in the varroa genus onto *A. mellifera* [S4-S6], and that ii) honey bee-varroa is a dynamic co-evolutive system in which arm races for survival shape both host and parasite populations [S7]. As a striking case, varroa populations in South America [S8], North America [S9, S10], and Japan [S4, S11] have been experiencing a rather quick turnover as the less “virulent” Japanese *V. destructor* was replaced by the more “virulent” Korean one.

**Box S2. Classification of haplotypes and haplogroups**

The concept of varroa haplotype has changed almost every decade as different mitochondrial markers were adopted to study their genetic variability in native and invasive populations. Following the taxonomic revision in 2000, haplotype for Varroa mites was considered to correspond to the 458-nucleotide identity of the partial COX1 mtDNA sequence (except 426-nt for *V. rindereri* AF107261) [S1]. A total of 18 haplotypes were named by the geographical location they were first obtained and depending on their phylogenetic relationship: “LOCATION”. Building on that basis is a novel haplotype with at least one SNP difference was found and the country name already used, then a number was simply added as “LOCATION+NUMBER” (e.g., China 2 AY372063 [S12] and Borneo 2 AY037890 [S13]). This unspoken rule was respected until 2010, where haplotype meaning changed to the 2696 nucleotide identity of partial COX1, COX3, ATP6 and CYTB mtDNA genes concatenated sequences. These new haplotypes were supposedly building on the basis of previous COX1 458-nt identity and if variation was detected in other mtDNA genes then sub named with “LOCATION+NUMBER+SUB-NUMBER” (e.g., Japan J1-1, J1-2, J1-3, J1-4, J1-5 and J1-6). Additionally, Navajas et al. [S4] defined that “mites with identical COX1 sequences were regarded as members of the same ‘haplogroup’ and, mites of the same haplogroup that showed variation within their concatenated sequences were regarded as variants of a particular haplogroup.”

However, by aligning all sequences from these studies, we found some possibly undetected confusion regarding this naming rule that could be problematic when referring in the future to one haplotype. First, *V. destructor* Korean K1-1 and K1-2 sequences are 100% identical and should be considered as K1-1/2. Haplotypes K1-1, K1-2, K1-3 and K1-4 were claimed identical on the 458 bp of the mitochondrial COX1 gene and to be part of the same K1 haplogroup [S4]. Yet, contrary to other haplogroups like J1 or we found that K1-4 differed from one transition in position 1125 (A > G) from other K1 haplotypes. Other confusion arises with Chinese haplogroup C2 (GQ379067) [S4] which could naively be considered the former described China 2 (AY372063) [S12]. To help clarify this, we proposed to redefine the haplogroups and give some advice for future naming (underlying supplementary data is all available via the interactive map weblink – see bottom of Box S3):

1. Mites with identical COX1 sequence based on the region chosen by Anderson and Trueman (2000) (AJ493124.2 COX1 positions 698 to 1155, included) should be considered as part of the same haplogroup.

2. If at least one SNP appears on the 458 nucleotide COX1 fragment, then the novel haplotype should be named “LOCATION+NUMBER”.

3. If additional non-described variation is found in COX3, ATP6 or CYTB standard markers is found, then the new haplotype should be named as “LOCATION+NUMBER+SUB-NUMBER” following the previous existing order.

In the near future, the availability of two varroa reference genomes will offer huge opportunities to get genome-wide and population informative markers as diagnostic tools. We advise that this nomenclature is followed as much as possible to allow temporal tracking the evolution Varroa population genetic diversity and structure.

**Box S3. Host distribution of haplotypes**

To better understand the temporal dynamic of *V. destructor* populations during the worldwide invasion, we visually reported the distribution of species and strains/lineages only confirmed by mtDNA COX1 sequencing. Such an approach has previously provided a distribution map emphasizing the supposed parapatry trend [S14] between the two sister species *V. destructor* and *V. jacobsoni* found on their original host and sympatry with the related *V. underwoodi* [S15].

For this, we reviewed 68 articles from 1995 to 2020 using either RAPD, mtDNA analysis (PCR-RFLP, sequencing) and/or nuclear microsatellites on varroa mites or environmental honey DNA [S16]. We collected distribution data about varroa species identity, mtDNA haplogroup, date of sampling, geographical localization, honey bee host. For geographical localization, three cases occurred: a) exact coordinates were available, b) city or locality was available and c) no localization was available outside of the country level. In the second case, we approximated the geographical position as the center of the city/locality or placed it to the nearest Agricultural Institute or Academic Center/University as some past sampling was known to be carried in experimental research apiaries (Supplementary Data 2).

In addition, 485 mitochondrial sequences were downloaded from NCBI Genbank (last update on the 01 February 2020) for which for the same information if not included in the previous papers list. We obtained three mitogenomes, 387 partial COX1 sequences (length ranging from 188 to 1088bp), 36 partial COX3 sequences (ranging from 323 to 436 bp), 36 partial ATP6 sequences (ranging from 287 to 339 bp), 23 partial CYTB sequences (ranging from 899 to 985 bp). All COX1 sequences were blasted and aligned to 44 reference mtDNA haplotypes (Supplementary Data 3) [S1, S4, S6, S12, S13, S17-S19]. Despite highly variable length and inconsistent sequence overlapping due to the usage of variable primers and high sequences trimming, we expanded the known COX1 haplogroups (Supplementary Data 2). Following the nomenclature rules, we found that *V. destructor* is the most diverse with 31 haplogroups (including 22 K-like), followed by *V. jacobsoni* with 19, five for *V. underwoodi*, for undetermined *Varroa* sp. and one for *V. rindereri*. We use these haplogroups to build an interactive distribution map of the varroa mites on their honey bee hosts mikheyevlab.github.io/varroa-mtDNA-world-distrib/.

The R code for these interactive maps are freely available on the GitHub link with the data tables used to build the points and country layers.

**Box S4**. **How can we ethically cull colonies?**

The decision to kill a bee colony is never easy, nor taken lightly. It is done out of necessity, to protect other colonies or to comply with the law. However, once a decision has been made to euthanize a colony, the most ethical approach is to make this as quick, painless and thorough as possible. The methods used vary by region and country. We advocate that individuals check with their apiary inspection service or veterinary institutions for the legal and most humane ways to eliminate the colony. Some will eliminate the entire colony. Others will sacrifice the queen, treat the remaining bees and brood with a miticide and then recombine with healthy colonies or add a new queen, depending on the appropriate situation and the health of the bees. Recombining can inadvertently allow the virulent virus to continue to exist even though varroa infestation is reduced, and risks perpetuating miticide-resistant varroa. Hence, long term it may be wiser to eliminate the entire colony, though not all beekeepers are comfortable with euthanization. These types of choices are never easy, as you weigh the benefits and drawbacks of protecting bees from individual colonies versus the entire apiary population.

**Table S1. Landmark discoveries in varroa research**

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| **Year** | **Reference** |
| 1995 | Kraus, B. & Hunt, G. Differentiation of Varroa jacobsoni Oud populations by random amplification of polymorphic DNA (RAPD). *Apidologie* **26**, 283–290 (1995) |
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