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The presence of identical deformed wing virus sequence variants in co-occurring *Apis* species in Northern Thailand may represent a potential epidemiological threat to native honey bees of Southeast Asia

Thunyarat Chantaphanwattana [a,b], Hassan Shafiey [c,d], Patcharin Phokasem [a], Terd Disayathanoowat [a,e,*], Robert J. Paxton [c,d,*]

[a] Bee Protection Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[b] Graduate School, Chiang Mai University, Chiang Mai 50200, Thailand
[c] General Zoology, Institute for Biology, Martin Luther University Halle-Wittenberg, Hoher Weg 8, 06120 Halle, (Saale), Germany
[d] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Puschstrasse 4, 04103 Leipzig, Germany
[e] Research Center in Deep Technology Associated with Beekeeping and Bee Products for Sustainable Development Goals, Chiang Mai University, Chiang Mai 50200, Thailand

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ABSTRACT

Widespread native honey bee species in South and East Asia (*Apis cerana*, *Apis dorsata* and *Apis florea*) and the imported western honey bee (*Apis mellifera*) share habitats and potentially also share pathogens. Chief among the threats facing *A. mellifera* in Europe and North America is deformed wing virus (DWV), including its two principal genotypes: A and B (DWV-A and DWV-B respectively). Though DWV-A has been recorded in Asia's native *Apis* species, it is not known if DWV-B, or both DWV-A and DWV-B, are currently widespread in Asia and, if so, whether viral transmission is primarily intraspecific or interspecific. This study aims to fill these knowledge gaps by (i) determining the DWV genotype in four co-occurring *Apis* host species using qPCR and (ii) inferring viral transmission between them using nucleotide sequences of DWV from *Apis* host species collected at three independent localities in Northern Thailand. We found DWV-A and -B in all four *Apis* species, the exotic *A. mellifera* and the native *A. cerana*, *A. dorsata* and *A. florea*. That DWV-A sequences were identical across *Apis* species at the same locality, with a similar pattern for DWV-B sequences, suggests that DWV's epidemiology is largely driven by ongoing interspecific transmission (spillover) of DWV across co-occurring native and exotic *Apis* species. Both genotypes of DWV represent a serious threat to Asia's exotic and native honey bee species.

1. Introduction

The western honey bees, *Apis mellifera*, is the most important insect pollinator for a great number of crops, agricultural products and wild plants worldwide (Klein et al., 2007) yet it is vulnerable to infection by many parasites and pathogens (McMenamin and Flenniken, 2018). Chief among its pathogens are viruses that have been considered a key threat for colony health (Levin et al., 2019; Remnant et al., 2017). Though over 32 viruses are known to infect *A. mellifera*, deformed wing virus (DWV) in particular is considered a leading cause of elevated colony losses (McMahon et al., 2016; Yañez et al., 2020).

DWV has been driven to high prevalence in *A. mellifera* populations because of its transmission by the exotic and invasive *Varroa destructor* (varroa), an ectoparasitic mite that is a major vector of DWV in *A. mellifera* (Martin et al., 2012; Mondet et al., 2014; Möckel et al., 2011). *Varroa destructor*, which originated as a harmless ectoparasite of the South and East Asian eastern honey bee *Apis cerana*, has achieved worldwide distribution as an ectoparasite of *A. mellifera* (Traynor et al., 2020). It is likely that, following the introduction of *V. destructor* from South and East Asia to Europe, varroa either brought DWV with it (Hasegawa et al., 2023) or varroa picked up DWV from *A. mellifera* in Europe (Wilfert et al., 2016). Then, as varroa mites were further distributed from Europe across the world with trade in *A. mellifera*, so too was DWV spread worldwide in populations of *A. mellifera* (Wilfert et al., 2016).

Pollinating insects are globally in decline (Zattara and Aizen, 2020)

* Corresponding authors at: Bee Protection Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand (T. Disayathanoowat). General Zoology, Institute for Biology, Martin Luther University Halle-Wittenberg, Hoher Weg 8, 06120 Halle, (Saale), Germany (R.J. Paxton).
E-mail addresses: terd.dis@cmu.ac.th (T. Disayathanoowat), robert.paxton@zoologie.uni-halle.de (R.J. Paxton).

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and, among them, native honey bee species have gained considerable attention (Panziera et al., 2022); pathogens, including viruses, are also thought to play a role in their decline (Dicks et al., 2021; Potts et al., 2016; Vanbergen et al., 2013). So-called honey bee viruses (strictly speaking: *A. mellifera* viruses) such as DWV are found in other wild bee species e.g. DWV has been found in bumble bees (*Bombus* spp.) (Fürst et al., 2014; Genersch et al., 2006; Gisder & Genersch 2017; McMahon et al., 2015) as well as in the Asiatic honey bee species *A. cerana*, *Apis dorsata* and *Apis florea* (Forsgren et al., 2015; Li et al., 2012; Li et al., 2023; Yañez et al., 2015; Zhang et al., 2012). The rise in prevalence of DWV in *A. mellifera* due to varroa parasitism of this honey bee species may account for why DWV is thought to spill over into other bee species (Manley et al., 2019), potentially accounting for its presence in *A. cerana* (Forsgren et al., 2015; Li et al., 2012; Yañez et al., 2015; Zhang et al., 2012).

Currently, DWV has been recognised as comprising four genotypes: DWV-A, DWV-B, DWV-C, and DWV-D (de Miranda et al., 2022; Lanzi et al., 2006; Mordecai et al., 2016; Ongus et al., 2004). Of these genotypes, DWV-A and DWV-B are the most common variants found in *A. mellifera* (McMahon et al., 2016), with DWV-B rising in prevalence and potentially replacing DWV-A across much of DWV's distribution (Paxton et al., 2022). This is of particular concern because DWV-B has been shown to be more virulent in adult *A. mellifera* than DWV-A (McMahon et al., 2016). DWV-A has been the dominant variant in *A. mellifera* (Wilfert et al., 2016), and is the DWV variant formerly detected in Asiatic *Apis* species (Zhang et al., 2012). Over the past two decades since its first identification, DWV-B has spread across much of the world in *A. mellifera*, including where it is an exotic species in South and East Asia; for example, it was first detected in *A. mellifera* in China and Philippines in 2015 (Paxton et al., 2022). A recent study of Chinese honey bees by Diao et al. (2019) showed that DWV-A was widespread in both the non-native *A. mellifera* and native *A. cerana* though DWV-B was only detected in *A. mellifera*. The distribution of DWV-C is not well defined. According to Li et al. (2023), DWV-C is rare in Southeast Asia, whilst previous studies have only reported it in the UK, Hawaii, and Brazil (Brettell et al., 2019; de Souza et al., 2019; Kevill et al., 2019). DWV-D has not been detected since its first discovery in Egyptian honey bees in 1977 (de Miranda et al., 2022).

We currently lack information on the occurrence of DWV-A or DWV-B in *A. dorsata* and *A. florea* of South and East Asia and on their patterns of transmission within and between these and other *Apis* host species, which may have a direct impact on the epidemiology of these viruses in their respective host species. To address these knowledge gaps, we compared the prevalence and titres of DWV-A and DWV-B in both exotic, imported (*A. mellifera*) and native honey bees (*A. cerana*, *A. dorsata* and *A. florea*). We also constructed phylogenies of DWV-A and DWV-B and compared them across naturally infected *Apis* species to infer patterns of viral transmission. This information is important to inform on routes of viral transmission and the threat to which exotic and native honey bee species are exposed.

Our study focused on four *Apis* spp. that are widespread in South and East Asia: the exotic *A. mellifera* and the native and widespread *A. cerana*, *A. dorsata* and *A. florea* (Smith, 1991). Thailand is a biodiversity-rich country of SE Asia with four native honey bee species (*Apis cerana*, *A. florea*, *A. andreniformis*, and *A. dorsata*), though *A. andreniformis* is rarely found (Wongsiri et al., 2000). In the early 1940s, *A. mellifera* was introduced to Thailand from Europe for research purposes and is now the main species used in commercial Thai beekeeping operations (Wongsiri, 1989, 2000). Thailand is therefore an ideal study location to understand DWV's prevalence and transmission among *Apis* species, in particular in relation to the exotic *A. mellifera*.

2. Materials and methods

2.1. Honey bee samples

A total of 60 honey bee colonies comprising wild *A. cerana* (n = 17), wild *A. dorsata* (n = 15) wild *A. florea* (n = 13) and managed *A. mellifera* (n = 15) were sampled from three locations in Chiang Mai and Lampang provinces of Northern Thailand during November 2017; locations were a minimum of 30 km from each other (Fig. 1; Table S1). From each location, honey bee samples were collected within a maximum 10 km radius (mean distance between colonies within a location was 5.4 km). Approximately 50 adult bees per colony were collected as a bulk sample and each bulk sample stored in a 50 ml conical tube containing RNA-Later. Samples were transported to the laboratory on ice and subsequently stored at −80 °C until RNA extraction.

2.2. RNA extraction and virus detection by qPCR

To extract RNA so as to detect viruses by qPCR, 20 bees from one colony were crushed under liquid nitrogen using a sterile mortar and pestle. Total RNA was extracted from the crushed material using TRIzol (Invitrogen, Schwerte, Germany) according to the manufacturer's recommendations and following a standard protocol (de Miranda et al., 2013), and eluted in 30 μl RNase-free water. Then 800 ng RNA was converted to cDNA using Oligo-dT oligonucleotides (Thermo Scientific, Schwerte, Germany) and reverse transcriptase (M-MLV and Revertase, Promega, Mannheim, Germany) according to the manufacturer's instructions, and subsequently diluted 1:10 in RNase-free water for use in qPCRs. Thereafter, methods for qPCR followed those given in Tehel et al. (2019).

We screened the cDNA for DWV-A and DWV-B plus six other, common honey bee viral targets, namely acute bee paralysis virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), Kashmir bee virus (KBV), sacbrood virus (SBV) and slow bee paralysis virus (SBPV) by qPCR in a Bio-Rad C1000 thermal cycler (Bio-Rad, Munich, Germany) using a SensiFAST™ SYBR® No-ROX Kit (Bioline, Luckenwalde, Germany) following the manufacturer's recommendations. qPCR-specific primers are listed in Table S2. The PCR conditions consisted of one cycle at 95 °C for 5 min followed by 40 cycles of 95 °C for 15 *sec*, 57 °C (for BQCV, CBPV, DWV-A, DWV-B, KBV, SBV and SBPV) or 53 °C (for ABPV) for 30 *sec*, 72 °C for 30 *sec* and data collection. Thereafter, a melting profile was generated by one cycle of 95 °C for 1 min and 50 °C for 1 min followed by 50 °C to 95 °C at 0.5 °C per second increments to ensure the correct fragment had been amplified i.e. a clear melt curve profile peaking at the correct dissociation temperature.

We performed a number of quality controls in amplifications. qPCRs were run in triplicate on 96-well PCR plates and the Cq value averaged for each sample. Samples were defined as positive for a virus using a Cq threshold of 35 cycles ($Cq < 35$) and negative for a virus as $Cq \geq 35$ (de Miranda et al., 2013). As a further quality control, the host honey bee *β*-actin gene was amplified in each sample as an internal check on RNA integrity and successful cDNA synthesis. Furthermore, positive and template-free (negative) controls were run on each plate.

2.3. Viral quantification

To quantify the DWV titre in different *Apis* species, qPCRs were run with a calibration (standard) curve covering eight orders of magnitude (10^1-10^8), generated using a viral PCR product with a known RNA copy number (genome equivalent, GE). The quantity of each virus was calculated from the standard curve (de Miranda et al., 2013).

2.4. Sanger sequencing of DWV variants

DWV-A and DWV-B positive samples were selected for PCR amplification of a 452 bp partial sequence of the RNA-dependent RNA

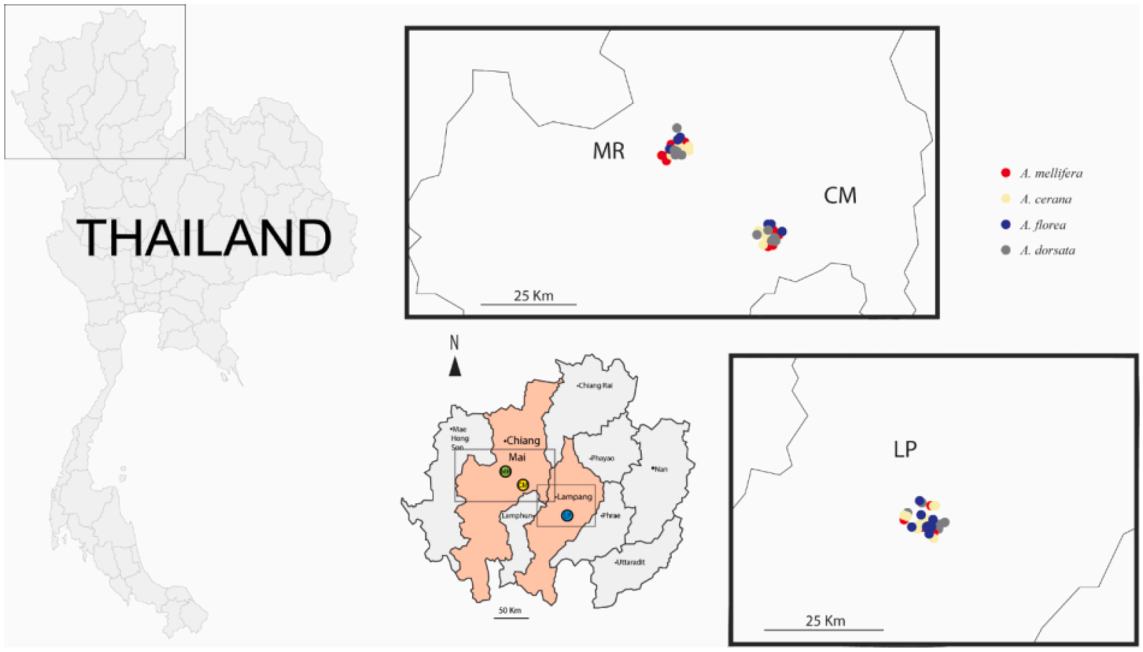

Fig. 1. The independent sampling locations in Chiang Mai and Lampang provinces, Northern Thailand. MR: Mae Rim district, Chiang Mai; CM: Muang district, Chiang Mai; LP: Muang district, Lampang.

polymerase (RdRp) gene using primers F15 (5′-TCC ATC AGG TTC TCC AAT AAC GGA-3′) and B23 (5′-CCA CCC AAA TGC TAA CTC TAA GCG-3′) (Yue and Genersch, 2005). The PCR conditions were: 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 *sec*, 54.3 °C for 1 min and at 72 °C for 30 *sec*, with a final extension step at 72 °C for 5 min.

As F15/B23 primers did not generate DWV-B partial sequences from DWV-A / DWV-B mixed samples, we amplified a 1150 bp long partial sequence of the RdRp gene of DWV-B using the primers VDV1-Pol-8910-F (5′-GCG TCG TAC CGT GCC GCT A-3′) and VDV1-Pol-10060-R (5′-GAC GCA TTG CCG TTA TGC-3′) (Ryabov et al., 2017) that overlapped with the fragments generated by primers F15/B23. PCR amplification was performed using the following conditions: 94 °C for 2 min, followed by 35 cycles of 94 °C for 45 *sec*, 55.9 °C for 45 *sec* and 72 °C for 90 *sec*, with a final extension step at 72 °C for 5 min.

The PCR products were resolved on a 1% agarose gel and visualised under UV light. PCR products of the primer pair F15/B23 were purified using a QIAquick PCR purification kit (QIAGEN) following the manufacturer's recommendation. The 1150 bp positive samples were purified using 0.1 μl FastAP and 0.5 μl Exonuclease I (Thermo Scientific).

The purified PCR products from all positive samples were cloned into a pGEM-T Easy vector (Promega, Mannheim, Germany) following the manufacturer's instructions and used to transform JM109 *E. coli* competent cells (Promega, Mannheim, Germany) that were then plated out on agar containing X-gal and ampicillin following the manufacturer's recommendations and scored blue/white. White colonies were PCRed with M13 primers: forward (5′-GTT TTC CCA GTC ACG AC-3′) and reverse (5′-CAG GAA ACA GCT ATG AC-3′). The PCR conditions were: 94 °C for 2 min, followed by 35 cycles of 94 °C for 15 *sec*, 57 °C for 30 *sec* and 72 °C 30 *sec*, with a final extension at 72 °C for 5 min. PCR products were purified using 0.1 μl FastAP and 0.5 μl Exonuclease I (Thermo Scientific) and commercially Sanger sequenced.

2.5. Sequence analysis

Nucleotide sequences were compared using the Basic Local Alignment Search Tool (BLAST) against the National Centre for Biotechnology Information (NCBI) database. Viral partial sequences representing a total of 24 independent samples (one DWV-A or one DWV-B sequence per colony) derived from the four *Apis* species were aligned, assembled and manually corrected using BioEdit (Hall, 1999) then deposited on NCBI (Table S3). Maximum likelihood was then used to construct a phylogenetic tree of viral sequences using PhyML v.3.0 online (https://www.atgc-montpellier.fr/phyml/) (Guindon et al., 2010) with 10,000 bootstraps and using the best substitution model (GTR with gamma rate variation), as estimated using smart model selection (SMS) (Lefort et al., 2017). The phylogenetic tree was inferred with Georgia and Kyrgyzstan DWV-A and the United Kingdom DWV-B sequences obtained from NCBI (Table S3) and visualized with FigTree v.1.4.4 (Rambaut, 2012).

To investigate whether viral sequences reflected a preponderance of interspecific versus intraspecific DWV transmission, we used a Bayesian statistical approach to test for the effect of geographic location (reflecting a preponderance of interspecific transmission) or *Apis* host species (reflecting a preponderance of intraspecific transmission) on viral sharing for both DWV-A and DWV-B. To do so, we used the MrBayes v3.2.7 plug-in for Geneious (Huelsenbeck and Ronquist, 2001) to test whether the variation in viral sequences could be best explained by location (L, reflecting interspecific transmission) or host species (H, reflecting intraspecific transmission). Model testing was performed with the GTR model with gamma rate variation using stepping stone (SS) sampling. The model with the highest marginal likelihood score was selected as the null hypothesis. A Bayes Factor (BF) score was then computed to show the relative likelihood of the phylogenies constrained by location (L) or host (H). We sampled the posterior distributions for 100 steps (each) of 100,000 generations for two independent parallel runs, discarding the first 25% of generations as burn-in. The low average standard deviation of the split frequencies across steps (0.0170) indicated an acceptable convergence in the sampling process. The BF interpretation was guided by Kass and Raftery (1995), whereby a threshold of 2 ln (BF) > 10 was considered as decisive support for the null against the alternative hypothesis.

2.6. Statistical analysis

Statistical analyses were conducted in R v.4.1.2 (R Core Team, 2021). Significance was defined as $p < 0.05$.

Prevalence and its 95% confidence intervals of the five detected viruses were calculated using the R package *epiR*, version 2.0.52 assuming

a Poisson distribution (Stevenson et al., 2018). To test if viral prevalence varied across host species, we used generalized linear mixed models (GLMMs) with binomial error structure. Honey bee and virus identity were used as fixed factors and sampling location was included as a random factor. The analysis was performed using the function *glmer* of the R package *lme4* (Bates et al., 2014), followed by Tukey's HSD *post hoc* pairwise comparisons. To compare viral titres of infected colonies between host species, we used a linear mixed-effects model (LMM), with *Apis* species as a fixed factor and location as a random factor; normality and homogeneity of variances of the data conformed to model assumptions. The LMM was performed using the *lmer* function of the *lme4* package (Bates et al., 2014) and titres were $\log_{10}$ transformed to approach normality. The full model was compared with a null model using a Maximum Likelihood ratio test (ML). To evaluate differences in viral titers, means were compared as a type III ANOVA, followed by Tukey's HSD *post hoc* comparison.

3. Results

3.1. Virus prevalence in Apis populations

In total, five viral targets (ABPV, BQCV, DWV-A, DWV-B and SBV) were detected in all honey bee species whilst CBPV, KBV and SBPV were not detected in any host species (Table S4). DWV-A and DWV-B were detected in *A. mellifera* across all sampling locations and in all native Thai *Apis* species across at least 2 of the 3 locations per species (Fig. 2; Table S5). To our knowledge, this is the first report of DWV-B in *A. florea* and *A. dorsata*.

The most prevalent virus we detected in the exotic *A. mellifera* was DWV-A (67% of colonies averaged across locations), followed by DWV-B (60%) (Table S2). The prevalence of other viruses in *A. mellifera* was lower: BQCV (47%), ABPV (40%) and SBV (7%), and their prevalence was lower still in all Thai native *Apis* species except SBV; for further details, see the legend to Table S2).

Apis mellifera had overall the highest prevalence of both DWV-A and DWV-B compared to native Thai honey bees (Fig. 2; Table S2), significantly so for DWV-B (GLMM: *A. cerana* (prevalence: 18%) $z = 2.731$, Tukey's HSD $p < 0.05$; *A. florea* (prevalence: 15%) $z = 2.105$, Tukey's HSD $p < 0.05$) though not significantly so for DWV-A ($p = 0.073$, Fig. 2; Table S2).

3.2. Virus titres across honey bee samples

A total of 42 of 60 honey bee colonies were qPCR positive for either DWV-A, DWV-B or both. When analysed at the colony-level and disregarding co-infection status, infected colonies of *A. mellifera* harboured a higher DWV-A titre than those of the native Thai species but DWV-B titres did not differ across the four *Apis* species (Fig. S1).

When taking coinfection status into account, DWV titres of infected colonies differed not only between *Apis* species (LMM: estimate ± s.e. = 1.431 ± 0.664, df = 3, $p = 0.038$) but also between infection by a single genotype or both genotypes (co-infection) (LMM: estimate ± s.e. = 1.467 ± 0.388, df = 1, $p = 0.001$). When DWV-A alone was present in a colony, its titre was higher in *A. mellifera* than *A. cerana* (*post hoc* comparisons, $p = 0.012$), *A. florea* ($p = 0.005$) and *A. dorsata* ($p = 0.032$). In addition, we found a significantly higher DWV-A titre when an *A. mellifera* colony was infected by only genotype DWV-A versus when co-infected by DWV-A and DWV-B ($p = 0.005$; Fig. 3). In contrast, DWV-B did not differ significantly among *Apis* species and when represented as a single infection (DWV-B alone) versus as a co-infection with DWV-A ($p > 0.05$; Fig. 3).

3.3. Phylogenetic trees of DWV-A and DWV-B found in Apis species

The best maximum likelihood phylogenetic tree based on a 452 basepair fragment of the RNA-dependent RNA polymerase (RdRp) region of DWV-A and DWV-B (14 and 10 sequences respectively; Table S3) revealed the presence of two clusters, represented by DWV-A and DWV-B, each with high (100%) bootstrap support (Fig. 4).

Within DWV-A, Thai isolates were separate from Georgia and Kyrgystan reference sequences isolated from *A. mellifera*, suggesting geographic variation in DWV-A sequence across Asia. Within the same location (MR) in Thailand, sequences from *A. mellifera* (MR2AM3) were identical to those from *A. cerana* (MR2AC1) and *A. florea* (MR3AF1). Moreover, DWV-A from *A. florea* (LP5AF1 and LP6AF1) had the same DWV-A sequence as *A. dorsata* (LP1AD2) from the same location, LP (Fig. 4).

All Thai isolates of DWV-B sequences also clustered together, and were separate from the DWV-B reference sequences from the United Kingdom (Fig. 4), suggesting genetic structuring in DWV over a broad geographic scale. Within location LP in Thailand, *A. mellifera*, *A. cerana*, and *A. florea* isolates of DWV-B were very similar in sequence. Furthermore, all DWV-B sequences from location CM, including isolates from *A. mellifera*, *A. cerana* and *A. dorsata*, clustered closely together

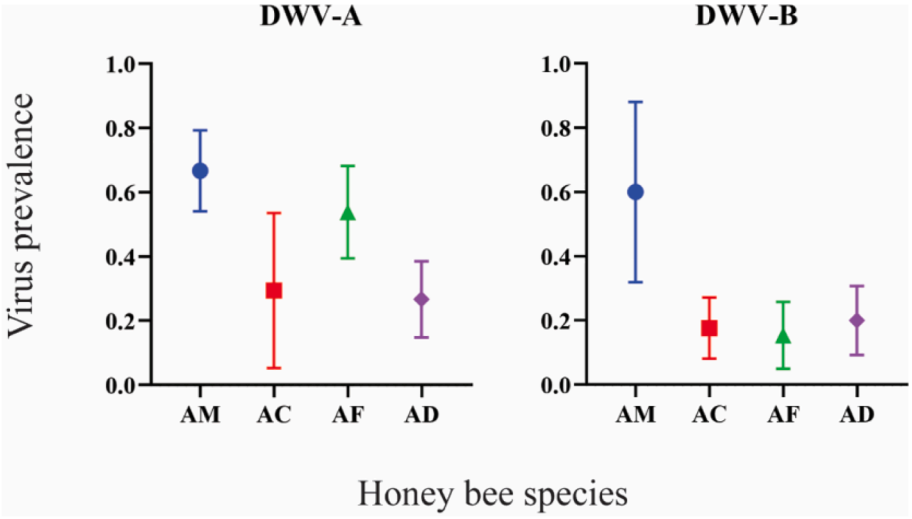

Fig. 2. Prevalence of DWV-A and DWV-B in *A. mellifera* (AM, n = 15 colonies), *A. cerana* (AC, n = 17), *A. florea* (AF, n = 13) and *A. dorsata* (AD, n = 15), with 95% confidence intervals calculated with *epiR*.

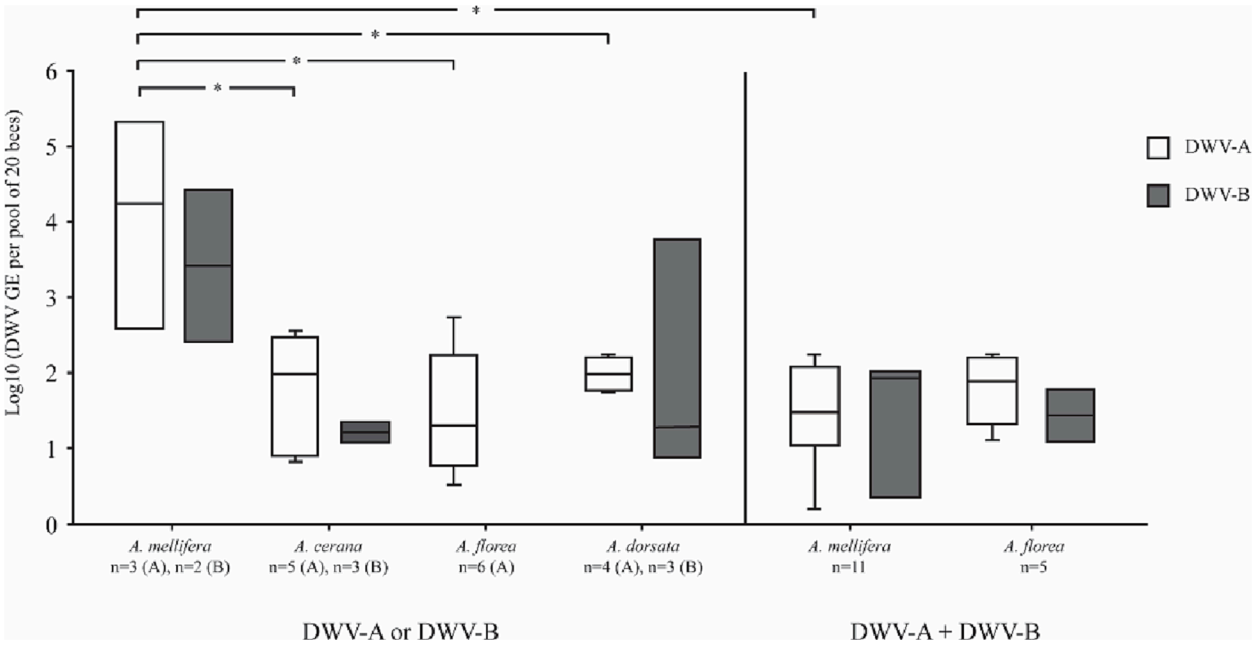

Fig. 3. DWV-A and DWV-B titres (in genome equivalents: GEs) in infected colonies of four *Apis* species analyzed by qPCR. A linear mixed model (LMM) was used to estimate the effects of honey bee species and genotype coinfection on viral infection. The box and whiskers plots give the median (central line), upper and lower interquartiles (bar) and range (whiskers). (*, Tukey post hoc pairwise comparison, $p < 0.05$).

(Fig. 4).

Marginal log likelihoods estimated by stepping stone sampling (ss) of a phylogeny based on only the Thai sequences revealed that DWV-A and DWV-B sequences isolated from different *Apis* host species were more similar within a location than they were to isolates from the same host species sampled across different locations in Thailand (2 ln BF = 14.44; Table S6). These data strongly support ongoing pathogen transmission among *Apis* species that inhabit the same field site.

4. Discussion

A total of 60 honey bee colonies was investigated for viral prevalence, viral titre, and genetic (sequence) identity of DWV-A and DWV-B in exotic and native honey bees at 3 independent locations in Thailand, SE Asia. Both viral genotypes were detected in all *Apis* species, along with three other viruses: ABPV, BQCV and SBV. DWV-A and DWV-B had the highest prevalence, and DWV-A and DWV-B sequences suggest that transmission of DWV among the community of *Apis* species is predominantly interspecific, among all *Apis* species living within close proximity.

Deformed wing virus (DWV) is thought to be a highly prevalent virus in *A. mellifera* infected with varroa mites (Martin et al., 2012; Traynor et al., 2020) throughout the world (Paxton et al., 2022; Wilfert et al., 2016). We also found DWV-A and DWV-B to be highly prevalent in our Thai samples, including in three native Thai *Apis* species. That the prevalence of DWV-A and DWV-B in *A. mellifera* was overall higher than in native Thai *Apis* species suggests that *A. mellifera* may be DWV's reservoir host.

Many viruses of *A. mellifera*, including DWV, have been detected in other bee species (Nanetti et al., 2021; Tehel et al., 2016), with evidence in some cases pointing to viral replication i.e. active infection in those bee species (Alger et al., 2019; Radzevičiūtė et al., 2017; Tehel et al., 2016). Our phylogenetic analysis of DWV-A and DWV-B sequences supports the idea that both genotypes are also readily transmitted among exotic *A. mellifera* and native honey bee species at the same location in SE Asia. Given the close phylogenetic relationship between *Apis* species, it may not be surprising that a virus from one honey bee species can be found, and presumably also replicate, in another *Apis* species.

It might be expected that migratory beekeeping leads to transmission of virus and homogenization of the viral gene pool across our study sites. *Apis mellifera* has been imported into Thailand since the 1980's, especially to the north of the country (Chiang Mai province) (Sanpa and Chantawannakul, 2009). Moreover, *A. mellifera* colonies are regularly migrated to Chiang Mai from elsewhere in Thailand for honey production or pollination (Phankaew, 2016). Our results, however, support the idea that there is geographic structure to the viral population, with the same viral variant shared among host *Apis* species at any one location. In other words, our results are consistent with the view that horizontal, interspecific transmission of DWV-A and DWV-B among syntopic *Apis* species predominates over intraspecific transmission. The epidemiology of DWV therefore seems to be primarily influenced by ongoing host switching. Though viral sequences alone do not allow the directionality or dynamics of viral transmission to be determined, the higher prevalence of DWV-A and DWV-B in *A. mellifera* suggests that it may be the reservoir host for both viral genotypes and the principal source host for viral transmission. Phylogenies of DWV-A from *A. mellifera*, *A. florea* and *A. dorsata* in southern China also support the idea that DWV-A is readily transmitted among *Apis* host species living in close proximity (Zhang et al., 2012). Moreover, the similarity of viral sequences from co-occurring *A. mellifera* and *A. cerana* in China support ongoing interspecific transmission of SBV (Ai et al., 2012), suggesting that viral sharing is widespread among these two host species.

Exactly how viral transmission occurs between *Apis* species is not clear. Virus could be transmitted at flowers visited by two or more *Apis* species, in the same way that *Bombus* spp. are thought to acquire DWV from honey bees (Alger et al., 2019). However, it is well known that varroa mites can harbor numerous bee viruses, including DWV-A and DWV-B (Bradford et al., 2017), and which they efficiently vector (Gisder and Genersch, 2021). Movement of mites between individuals and colonies of different honey bee species may be a plausible and efficient means by which both genotypes of DWV are able to be transmitted across *Apis* species, leading to identical viral sequences in co-occurring *Apis* species.

Interestingly, we found that the titre of DWV-A in DWV-A/DWV-B co-infections of *A. mellifera* was significantly lower than when the

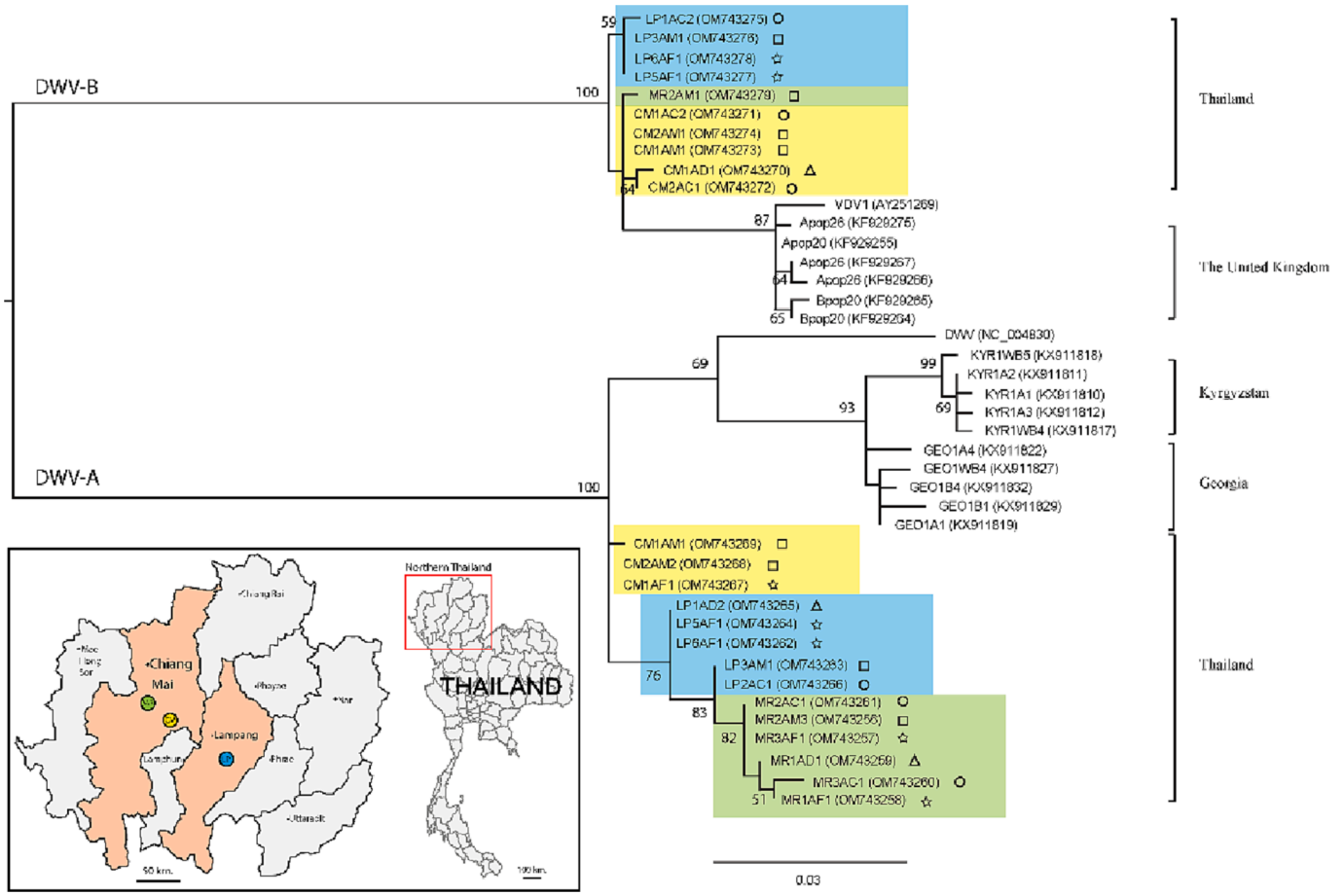

Fig. 4. Maximum likelihood phylogeny of DWV based on the RNA-dependent RNA polymerases (RdRp) region of DWV-A and DWV-B found in *Apis* species in Thailand. Shown is the phylogenetic tree with the highest log likelihood, constructed using PhyML 3.0, and bootstrap support (>50%) of nodes (10,000 bootstrap replicates). Non-coloured (unshaded) isolates represent reference sequences from NCBI. The coloured shading indicates the three Thai sampling locations (green = Mae Rim, Chiang Mai province, Thailand (MR); blue = Muang, Lampang province, Thailand (LP); yellow = Muang, Chiang Mai province, Thailand (CM). Symbols give the different host *Apis* species (square = *A. mellifera*, circle = *A. cerana*, star = *A. florea*, triangle = *A. dorsata*) from which viral sequences were obtained. The scale bar gives the number of substitutions per site (as a % of the 452 bp sequence). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

colony was infected by only DWV-A. This might be due to viral competition or interference, as may be brought on by recombinational meltdown between DWV-A and DWV-B when both genotypes infect the same host individual (Paxton et al., 2022). In contrast to our observational results, previous experimental studies have found that infection by DWV-A reduced the growth of DWV-B in coinfected hosts (Norton et al., 2020), though DWV-B nevertheless predominated (achieved higher titre) in coinfected hosts and colonies (Norton et al., 2021). Differences between our and Norton et al.'s (2020, 2021) studies may lie in the origin of the honey bees, the viral variants or in climate; Thailand has a tropical climate where DWV-A may be favoured whereas Norton et al.'s (2021) experiments were undertaken in the temperate Netherlands, where DWV-B may be favoured. Experiments using the same viral variants and host honey bees undertaken in different climatic conditions are needed to differentiate among these hypotheses.

In our study, we reported viral titres at the colony level from a pool of analysed honey bees. Higher titres could arise from either higher viral loads in individual honey bees or a large number of infected honey bees within the pool of sampled bees. Further studies are needed reveal viral loads of individual honey bees and how inter-individual variance in load contributes to variation in viral titres observed at the colony level.

5. Conclusions

DWV-A and DWV-B are multi-*Apis* species pathogens that reach high prevalence and titre, and appear to be transmitted readily between co-occurring honey bee species in SE Asia, possibly aided by vector mite transmission within and between colonies and species. As both DWV-A and DWV-B are virulent killers of *A. mellifera* in its native range (McMahon et al., 2016), their seemingly ready transmission to other *Apis* species is a considerable potential threat to South and East Asian native honey bees. Control of these virulent pathogens will benefit from a bee community-level approach.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2023.107957.

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