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Tropilaelaps species identification and viral load evaluation of *Tropilaelaps* and *Varroa* mites and their *Apis mellifera* hosts in Palawan, Philippines

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ABSTRACT

Apis mellifera pupae and their parasites *Tropilaelaps* and *Varroa destructor* were collected from honey bee hives in Palawan, Philippines for species identification of the *Tropilaelaps* and viral analyses. Genetic analysis identified *Tropilaelaps mercedesae* infesting *A. mellifera* on the island. Viral analyses showed that all pupae and their infesting *Tropilaelaps* or *Varroa* shared the same Deformed Wing Virus (DWV) variant infections with DWV-B being more prevalent than DWV-A. Pupae infested with either *Varroa* or *Tropilaelaps* had higher levels of both DWV variants than uninfested pupae. Vigilance is needed to prevent the spread of *Tropilaelaps clareae* into Palawan and *T. mercedesae* and DWV variants from Palawan to other provinces.

Apis mellifera colonies in Asia have been facing serious problems caused by the mites *Tropilaelaps* spp. and *Varroa destructor* since the 1960s (Delfinado, 1963). *Tropilaelaps* is more abundant than *Varroa* and, like *Varroa*, *Tropilaelaps mercedesae* transmits Deformed Wing Virus (DWV) and other viruses (Chanpanitkitchote et al., 2018; Dainat et al., 2009; Forsgren et al., 2009). *Tropilaelaps clareae* was previously identified as the *Tropilaelaps* species causing mortality of *A. mellifera* colonies in the Philippines (Anderson and Morgan, 2007). However, our recent nationwide survey revealed that *V. destructor* is the major problem for *A. mellifera* beekeeping in the country, and *Tropilaelaps* was detected only in the archipelagic province of Palawan (Cervancia et al., 2016).

Pupae with yellow thorax (~8 days post-capping) and their infesting mites were collected from two *A. mellifera* colonies in Palawan for mite species identification and viral analyses. One colony was co-infested by *Varroa* and *Tropilaelaps*. Of 100 pupae examined in the first colony, three were infested by *Varroa* only (mean = 4.0 ± 0.6 *Varroa*/infested cell), 15 were infested with *Tropilaelaps* only (mean = 2.6 ± 0.3 *Tropilaelaps*/infested cell), and five were concurrently infested with both mite species (mean = 6.6 ± 1 *Varroa*; 3.2 ± 1.1 *Tropilaelaps*/infested cell). The overall infestation rate was 23%. The second colony was infested with *Tropilaelaps* only with an infestation rate of 50% (n = 100) and an average of 4.8 ± 0.4 mites per infested cell. The maximum mite load recorded was 14 *Tropilaelaps* on a single pupa. Each pupa and their infesting mites were placed in a vial with 70% ethanol and stored at −80 °C until processing. Species identification for eight female *Tropilaelaps* was conducted via examination of mtDNA CO-I and nuclear ITS1-5.8S-ITS2 gene sequences using restriction enzyme digests (BsrI, BstYI, SwaI, BaeGI [Bme1580I]) as described by (Anderson and Morgan, 2007). We identified the *Tropilaelaps* infesting *A. mellifera* in Palawan as *T. mercedesae*. This *Tropilaelaps* species was also observed infesting *Apis dorsata*, which is only found on Palawan (Anderson and Morgan, 2007). *T. clareae* was not detected. Therefore, it is likely that the spread of *T. mercedesae* to *A. mellifera* on Palawan resulted from migration from *A. dorsata* colonies and not from introductions of mites from other islands where *T. clareae* is thought to be the prevalent species.

Knowledge of the incidence of DWV and its variants in honey bee colonies in the Philippines is lacking. Thus, we quantified viral levels in uninfested pupae (n = 16), pupae and their infesting *Tropilaelaps* (n = 9), pupae with *Varroa* alone (n = 3) and pupae infested with both mites (n = 3). The DWV sequence regions amplified were based on the following primers: GAG ATT GAA GCG CAT GAA CA (Forward)/TGA ATT CAG TGT CGC CCA TA (Reverse) for DWV-A (Boncristiani et al., 2012), and CTG TAG TTA AGC GGT TAT TAG AA (Forward)/GGT GCT TCT GGA ACA GCG GAA (Reverse) were utilized for DWV-B (Ryabov et al., 2017). Virus quantification was achieved via qRT-PCR using plasmid clones with viral sequences specific to these primer pairs following standard and established protocols (de Guzman et al., 2019). The presence or absence of other common honey bee viruses was also

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Table 1
Virus infection rate (%) in honey bee pupae and their infesting *Varroa destructor* and *Tropilaelaps mercedesae*.

Bee status/Mite species	n	DWV-A	DWV-B	AKI	CBPV	LSV-U	BQCV	# of detections
Infested bee	15	100.0	100.0	80.0	6.7	0.0	0.0	2.87 ± 0.09[a]
Uninfested bee	16	56.3	100.0	25.0	37.5	0.0	0.0	2.19 ± 0.19[b]
V. destructor	6	100.0	100.0	66.7	16.7	0.0	0.0	2.83 ± 0.31[a]
T. mercedesae	12	100.0	100.0	58.3	16.7	8.3	0.0	2.83 ± 0.21[a]

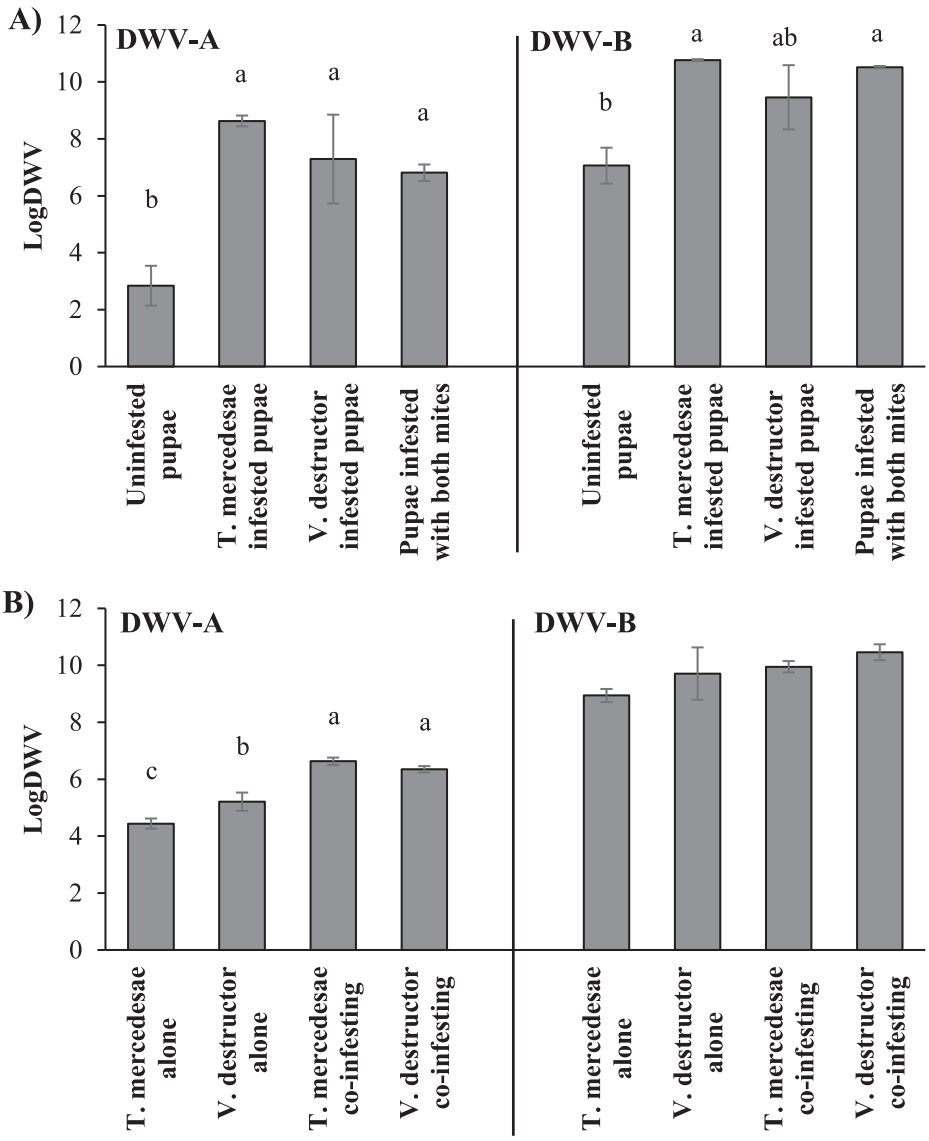

Fig. 1. (A) Levels of DWV-A and DWV-B in uninfested pupae and pupae infested with *Varroa destructor*, *Tropilaelaps mercedesae* or both. (B) Levels of DWV-A and DWV-B in *V. destructor* or *T. mercedesae* collected from either singly infested or co-infested cells. For each group, bars with different letters are significantly different ($P < 0.05$, one-way analysis of variance); unlabeled bars do not differ significantly ($P > 0.05$).

determined. One adult female mite per infested cell was used in the analysis. Our results showed that all bee and mite samples were negative for Black queen cell virus (BQCV). AKI (the complex of Acute bee paralysis virus, Kashmir virus and Israeli acute paralysis virus), Chronic bee paralysis virus (CBPV), and DWV were detected in both *Varroa* and *Tropilaelaps* and in infested and uninfested bees. Lake Sinai Virus (LSV-U) was detected only in a single *Tropilaelaps* (Table 1). There were more virus types detected in parasitized bees and their infesting *Varroa* or *Tropilaelaps* than in uninfested pupae ($F = 3.79$; $P = 0.012$).

Overall, DWV-B was more prevalent than DWV-A in both pupae and infesting mites. Mite-infested pupae (singly infested with *Varroa* or *Tropilaelaps* or co-infested) had significantly higher levels of DWV-A ($F = 14.46$, $P < 0.0001$) or DWV-B ($F = 24.51$, $P < 0.0001$) than uninfested pupae (Fig. 1A). However, pupae infested with *Varroa* alone had comparable levels of DWV-B levels to that of uninfested pupae. While co-infestation of a single pupa by both mite species is rarely observed (Buawangpong et al., 2015), co-infestation did not lead to increased DWV-A or DWV-B levels in the pupal hosts (Fig. 1A). For both mite species, however, co-infestation resulted in increased DWV-A levels only as compared to mites collected from singly infested pupae ($F = 23.28$, $P < 0.0001$; Fig. 1B). DWV-B levels were equally high in all mites collected from either singly or co-infested cells ($F = 3.08$,

$P < 0.0619$). This similarly high infection of pupae and their infesting mites may be an indicative of a maximum threshold in viral replication as suggested by (Wu et al., 2017). Of the 16 uninfested pupae, nine had mixed DWV-A and -B infections, with DWV-B levels higher than DWV-A. Seven pupae were infected only with DWV-B, while none were infected only with DWV-A. This differential representation of one viral variant can be attributed to several factors including varied prevalence rates and virulence properties, as seen in other geographic areas, particularly regarding the spread of DWV-B (Kevill et al., 2019; McMahon et al., 2016; Mordecai et al., 2016; Ryabov et al., 2017). While viruses can be transmitted horizontally or vertically (Chen et al., 2006), feeding by *Tropilaelaps* during the larval stages of the bees can play a major role in DWV infection in bees that are not infested as pupae (Phokasem et al., 2019). This mite feeding during early bee development may promote viral proliferation, as the bees are exposed to the virus longer before emergence, in addition to the impact of stress on susceptibility to viral infection (Nazzi and Pennacchio, 2018; Prisco et al., 2011). Also, becoming re-infested during the pupal stage (as repeat hosts) may significantly elevate viral load as demonstrated by the high levels of both DWV variants compared to their infesting *Tropilaelaps* mites (Fig. 1). Such indiscriminate feeding causes multiple wounds on host bees (Phokasem et al., 2019) and feeding transmits multiple variants of DWV (Wu et al., 2017). This behavior and virus transmission are the reasons that *T. mercedesae* is considered the most serious parasite of *A. mellifera* in much of Asia. Since our survey, the beekeepers we visited in Palawan lost all the colonies, possibly due to the high levels of mite infestation and DWV infection we observed in this study.

The presence of *T. mercedesae* and *A. dorsata* in only in the Province of Palawan is probably due to the island's unique geological history. Palawan is believed to have been connected to Borneo, where *A. dorsata* and *T. mercedesae* are also found, in the mid Pleistocene (Smith et al., 2000), which accounts for the similarity in flora and fauna. As far as we know, all *A. mellifera* colonies in the Palawan islands were obtained from a bee program established in 2005 that has since collapsed. The colonies owned by the program were sourced from other parts of the country where *T. clareae* has been detected. Nevertheless, the originating colonies may have been infested with *Varroa* only as no *T. clareae* was identified from the samples analyzed in this study. With the identification of *T. mercedesae* infesting *A. mellifera* colonies in Palawan and the high levels of mite-borne viruses detected, vigilance is needed to prevent the spread of *Tropilaelaps clareae* into Palawan and *T. mercedesae* and DWV variants from Palawan to other provinces. If infestation by a single mite species can be devastating to *A. mellifera* colonies in the Philippines, the potential synergistic effects of two species of *Tropilaelaps*, *V. destructor* and mite-borne viruses may end an industry that is already barely surviving.

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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.2020.107324.

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