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Original article

Prevalence and behavioral bioassays of *Platybolium alvearium* (Coleoptera: Tenebrionidae) in colonies of honeybees (*Apis* : Hymenoptera: Apidae) in northern Vietnam

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Abstract – *Platybolium alvearium* beetles occur in hives occupied by colonies of *Apis cerana* in Asia. We quantified beetle abundance in 180 *A. cerana* and 30 *Apis mellifera* hives and made behavioral observations in northern Vietnam. Although sometimes common, the beetles were uncommon in *A. cerana* hives ($n = 180$) during hive inspections in May/June 2015. In contrast, none were observed in *A. mellifera* hives. Beetle abundance was greater in more populous *A. cerana* colonies, a pattern atypical in previous studies of other honeybee associates (i.e., *Aethina tumida*). *A. cerana* worker bees did not react to adult beetles upon contact within their colonies. In bioassays, individual beetles preferred (i) comb of endemic *A. cerana* over comb of introduced *A. mellifera* and (ii) *A. cerana* beeswax over paraffin. Our results suggest that *P. alvearium* beetles are moderately integrated commensals of *A. cerana* that do not harm their host colonies in Vietnam.

Apis cerana **/** ***Apis mellifera*** **/ commensal / colony integration / defensive behavior / honeybee associate**

1. INTRODUCTION

A wide variety of insect species have evolved symbioses with social insects and exhibit varying degrees of nest and colony integration (Wilson 1971; Atkinson and Ellis 2011). Close relationships are relatively common between insects of diverse taxa and colonies of ants and termites; in contrast, species specialized to live with flying bees and wasps are less frequent (Wilson 1971; Kistner 1982). Honeybees (Hymenoptera: Apidae: *Apis*), in particular, coexist with relatively few symbionts. An exception is the beetles (Coleoptera): several species regularly inhabit honeybee nests, differ in prevalence and abundance, and exhibit a wide range of nest adaptations (Wilson 1971; Atkinson and Ellis 2011).

Atkinson and Ellis (2011) noted for beetles that the degree of colony integration by honeybee associates appears correlated to the degree of damage they cause and the level of defensive response exhibited by bees within their colonies.

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Some highly integrated species are extremely destructive, such as the small hive beetle (Coleoptera: Nitidulidae: *Aethina tumida* Murray), a specialized invasive pest of *Apis mellifera* L. *Aethina tumida* larvae cause extensive damage to colonies of European honeybees and elicit elaborate defensive responses from the bees (Ellis 2005; Ellis and Hepburn 2006). Other destructive honeybee pests such as the large hive beetle, *Oplostomus fuligineus* Oliveri (Coleoptera: Scarabaeidae), and the morphologically similar *Oplostomus haroldi* Witte (Coleoptera: Scarabaeidae) of Africa are known invaders that cause modest damage by consuming brood, pollen, and honey stores (Donaldson 1989; Torto et al. 2010). Weakly integrated associates, such as *Cryptophagus hexagonalis* Tournier (Coleoptera: Cryptophagidae; Haddad et al. 2008), *Cychramus luteus* Fabricius (Coleoptera: Nitidulidae; Neumann and Ritter 2004), and *Glischrochilus fasciatus* Olivier (Coleoptera: Nitidulidae; Ellis et al. 2008), seem to be accidental associates of honeybees and are generally ignored by bees in hives.

Recently, several species of darkling beetles (Tenebrionidae) have been documented to be regularly associated with colonies of honeybees, in particular *Apis cerana* F. in Asia. Pande et al. (2015) reported on *Platybolium alvearium* Blair in northeastern India, where it sometimes reaches high abundance in honeybee colonies (details of their study are summarized below). *P. alvearium* was also detected in surveys of bee hives in Himachal Pradesh, northern India (Chandra and Mattu 2017). Li et al. (2016) described instances of *Alphitobius diaperinus* Panzer in *Ap. cerana* colonies in southeast China. These beetles were found over a narrow geographic range but consistently occurred in high numbers in infested hives. While there is no evidence that they harm the bee colonies, Li et al. (2016) showed that *Al. diaperinus* can act as a reservoir of bee diseases and thus this species may be of concern to beekeepers. Furthermore, Maitip et al. (2016) described occurrences of the black fungus beetle, *Alphitobius laevigatus* Fabricius (Tenebrionidae), in Vietnam and China. *Al. laevigatus* occurred in over half of the colonies they sampled but caused no noticeable damage to them (Maitip et al. 2016).

The objective of our research is to better define the relationship between *Platybolium alvearium* and honeybees. During visits to northern Vietnam from 2009 to 2013, one of us (GWO) frequently observed small beetles inside colonies of *Ap. cerana.* Specimens were identified by PB as *Platybolium alvearium* (Tenebrionidae) (Fig. 1), initially reported to be a scavenger in honeybee hives (Cherian and Mahadevan 1940).[1] These beetles were

[1] Distribution and identification of the wax beetle *Platybolium alvearium*

The new genus and species *Platybolium alvearium* were described by Blair (1938) based on specimens originating from Sri Lanka, China, and India (where some specimens were collected in excrements of the honeycomb moth *Galleria mellonella* (Linnaeus, 1758) [Lepidoptera: Pyralidae] or observed "attacking honeycomb"). That wax beetle species, reported from Vietnam for the first time in this paper, is currently classified in the tribe Triboliini whereas the other two tenebrionids previously associated with *Apis cerana* hives (the black fungus beetle *Alphitobius laevigatus* and the lesser mealworm *Alphitobius diaperinus*) belong to the tribe Alphitobiini (Löbl et al. 2008; see Matthews and Bouchard 2008 for diagnostic morphological characters of the two tribes). *P. alvearium* can be readily separated from species in *Alphitobius* in having a narrow longitudinal ridge between each elytral stria (see arrows in Figure 1). Morphological characters to separate *Al. laevigatus* from *Al. diaperinus* were recently published by Maitip et al. (2016). In order to assist future molecular identifications, we provide the 658-base-pair DNA barcode sequence for *Platybolium alvearium* for the first time (Appendix 1; <dx.doi.org/10.5883/DS-PLATYBOL>). Identification of *Platybolium alvearium* was first performed using morphological data. Two specimens (one adult and one larva) of *P. alvearium* and two of *A. diaperinus* (for comparison) were subsequently submitted to the Canadian Centre for DNA Barcoding (Guelph, Canada) for amplification of the DNA barcode region of cytochrome c oxidase subunit 1. Sequences from the four samples were then compared against the species sequence library in the Barcode of Life Data System (BOLD). The two specimens of *A. diaperinus* matched at 99.75 and 100 %, respectively, with other sequences of that species in BOLD. The molecular identification of the *two Platybolium* specimens was inconclusive (i.e., their closest matches were 87.06 and 87.31% to other darkling beetles in the subfamily Tenebrioninae), indicating that they represented a new taxon in the BOLD reference library (Public records can be found at www.boldsystems.org/index.php/Public_SearchTerms?query=DS-PLATYBOL. Voucher beetle specimens are housed in the Canadian National Collection of Insects, Arachnids and Nematodes (Ottawa, Canada).

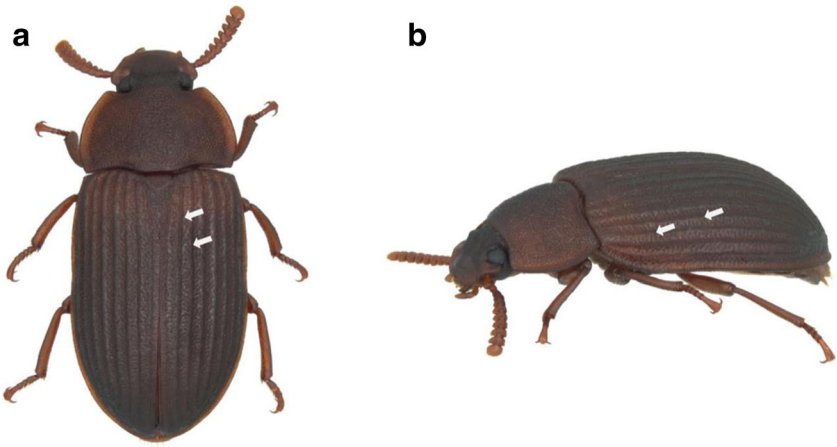

Figure 1. a Dorsal and **b** lateral views of *Platybolium alvearium*. Davies © Her Majesty the Queen in Right of Canada.

regularly observed on the bottom boards of hives, but also where frames rest on the hive and in the spaces between frames (Pham Thi Huyen and GWO, unpubl. data). They seemed to be ignored by adult honeybees. In striking contrast, this same species was recently described as a threat to *Ap. cerana* colonies in northeastern India by Pande et al. (2015). There, they observed active aggression by bees towards the beetles and significant damage to colonies due to beetle presence, as well as absconding of one or more colonies with heavy beetle infestations. Moreover, they reported that larvae eat wax flakes and adults consumed both comb and beeswax flakes (Pande et al. 2015). Digestion of beeswax has not been confirmed experimentally in any beetle species, including *P. alvearium*. Metabolic breakdown of beeswax is a rare physiological ability that has been confirmed in only a few animals with highly specialized relationships with bee colonies, specifically greater and lesser honey-guide birds of the family Indicatoridae (Friedmann and Kern 1956; Diamond and Place 1988; Downs et al. 2002) and the greater wax moth, *Galleria mellonella* (Pyralidae; Dadd 1966), which has been described as "a lipid specialist" (Dadd 1973).

With current concerns over highly integrated bee associates that can cause significant damage to bee colonies (i.e., *Ae. tumida*), it is of interest to understand the behavior and ecology of other beetle associates of honeybees such as the poorly known species *Platybolium alvearium.* Such research may provide broader understanding of the varied relationships between beetles and honeybees. To that end, we studied the relationship between *P. alvearium* and honeybees in northern Vietnam by (1) quantifying the prevalence and abundance of the beetles in hives of *Ap. cerana* and *Ap. mellifera*; (2) evaluating relationships between *Ap. cerana* colony populations, environmental factors, and prevalence/abundance of beetles; and (3) quantifying beetle preferences in behavioral two-choice bioassays to determine their behaviors towards various bee products (i.e., comb, beeswax, honey).

2. MATERIAL AND METHODS

2.1. Beetle prevalence and abundance within bee hives

To quantify *P. alvearium* prevalence and abundance in hives, we surveyed 10 managed colonies at each of 18 *Ap. cerana* apiaries ($n = 180$ colonies) and 3 *Ap. mellifera* apiaries ($n = 30$ colonies) within Hưng Yên and Hòa Bình provinces in northern Vietnam. All sampling was conducted from 25 May to 8 June, 2015. Within surveyed

Table I.. Dimensions and weight of each material used in the four two-choice bioassays.

Test	Material 1	Mass (g)	Dimensions (cm)	Material 2	Mass (g)	Dimensions (cm)
1	*Ap. mellifera* comb	4.6	2.3 × 1.5 × 2.0	*Ap. cerana* comb	4.6	2.5 × 1.5 × 2.0
2	Paraffin	6.8	1.0 × 2.0 × 2.0	Beeswax	6.8	1.0 × 2.0 × 2.0
3	Beeswax	6.8	1.0 × 2.0 × 2.0	Empty *Ap. cerana* comb	4.6	2.5 × 1.5 × 2.0
4	*Ap. cerana c* omb with honey	4.7	2.5 × 1.5 × 2.0	Empty *Ap. cerana* comb	4.6	2.5 × 1.5 × 2.0

hives, beetle abundance was recorded on the frame rests, around the hive edges when the lid was first removed, and on the sides and bottom boards of hives after all combs had been removed and inspected.

To analyze the relationship between beetle prevalence/abundance and environmental factors in the *Ap. cerana* hives, we recorded hive cleanliness, hive wetness, and bee colony size within each hive. Hive cleanliness was ranked on a three-point scale ranging from (1) excessive dirt and detritus (dirt or detritus covering > 50% of the inside of the hive), (2) some dirt and detritus (dirt and detritus covering 10–50% of the inside of the hive), and (3) mostly clean (little or no dirt and detritus covering < 10% of the inside of the hive). Hive wetness was ranked as either (1) mostly dry or (2) damp wood or pooling of water. Finally, colony size was estimated by removing each comb and visually estimating the percentage of each frame surface covered by bees, multiplying that percentage by the maximum size of a comb within a frame (673 cm^2), and then summing the values for all comb surfaces to obtain an estimate for the total comb area covered by bees in each colony (Delaplane et al. 2013). One person (SJD) evaluated all of the environmental factors to keep estimations of the variables consistent. Additionally, at each apiary, the total number of hives was recorded.

We recorded general behaviors of the beetles and beetle-bee interactions, such as the nature of contacts and evidence of aggression towards beetles, during casual inspections of *Apis cerana* hives between 2009 and 2013 and our detailed inspections in 2015.

2.2. Bioassays to determine beetle preferences for hive products

We performed bioassays to test beetle preferences to different substances based on concepts and methods of Graham et al. (2010). To do so, we collected beetles from the surveyed hives for use in bioassays and stored them individually in 3 × 9 × 3 cm plastic containers with a freshly picked leaf to reduce desiccation. During storage, the temperature ranged from 25 to 30°C. Each beetle was initially tested within 48 h of collection. After being tested, we returned beetles individually to their labeled containers to ensure they would not be used again in the same bioassay. Some beetles were used for more than one bioassay but never twice in comparisons involving the same two materials.

We performed four two-choice bioassays to examine beetle preference for different substances (Table I) at 25 °C in a laboratory at the National Institute of Animal Sciences, Thuy Phuong, Tu Liem North, Hanoi, Vietnam. We did not have access to an olfactometer such as the one used by Graham et al. (2010), so we constructed bioassay arenas from 15 × 25 × 5 cm clear plastic containers with a removable lid (Fig. 2a). To prevent the mixing of odors of different substances in the middle of the arena, we created a grid of ventilation holes along the sides and top of the arena. The grid area was constructed by punching 1.5 mm diameter holes approximately 3 mm apart in a band 10.5 mm in width in the center of the sides and top of the container, for a total of 45 holes on each side and 135 holes on the top (Fig. 2a). A grid with 15 5 × 5 cm squares was

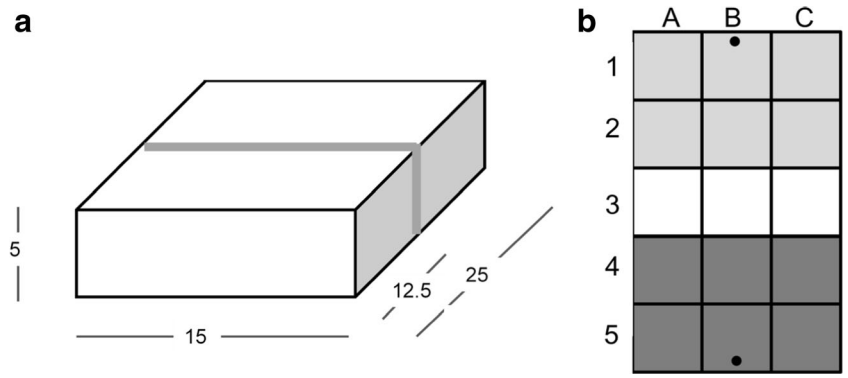

Figure 2. a Bioassay arena measurements (cm). Dark gray lines indicate the location of ventilation holes. **b** Illustration of the grid on the bottom of the arena used for choice tests (bioassays). Black dots represent the location of the two materials being competed. Material was suspended 1 cm above the bottom of the arena. Areas that we considered as close to one of the two products are shaded light gray and dark gray.

placed under the arena (Fig. 2b). During preliminary trials with substances placed directly on the floor of the arena, the beetles moved beneath the first material they contacted and remained there. To avoid this in the bioassays, all test materials were suspended from the lid with the lower surface of the substances 1 cm from the floor of the arena.

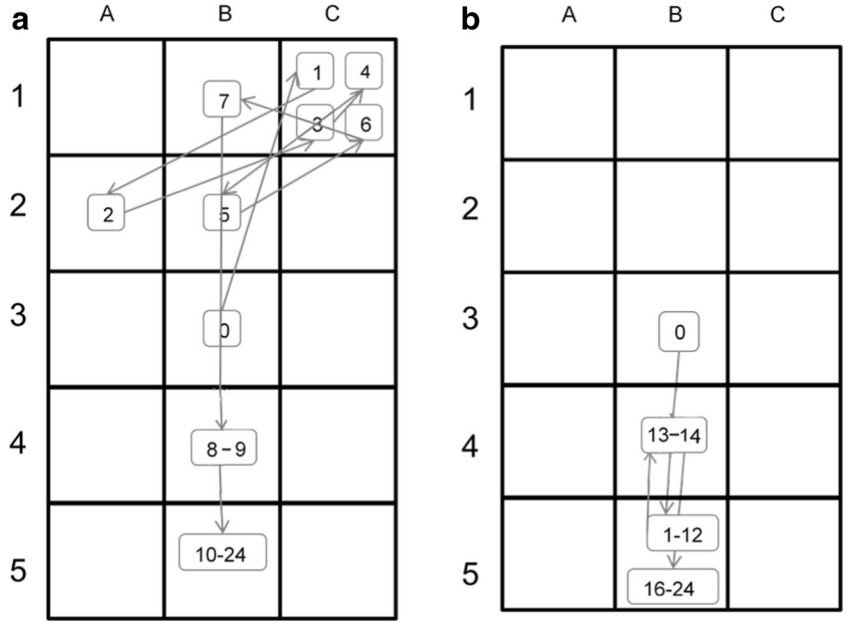

Figure 3. Depictions of *P. alvearium* movement during two trials from the bioassay comparing two types of comb. A single beetle was placed in the center of the arena at point and time "0," equidistant from empty *Ap. cerana* comb on the right side versus empty *Ap. mellifera* comb on the left side. Beetle position (grid point) was recorded every 5 min for 2 h. Numbers display the 24 sequential locations of a beetle over time. **a** The beetle moved around at the end of the arena with the *Ap. mellifera* comb for 35 min, then moved quickly to the other end where it remained close to the *Ap. cerana* comb for the last 70 min of the trial. **b** The beetle moved immediately to the *Ap. cerana* comb (grid B5), but in the middle of the observation period moved to two adjacent grids before returning to B5.

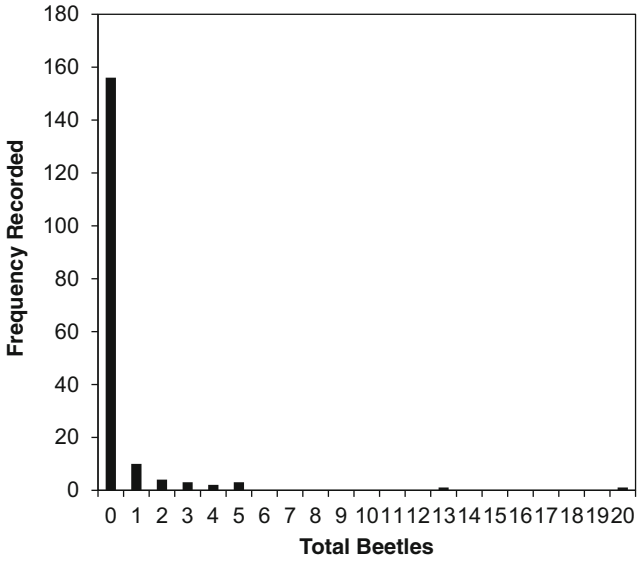

Figure 4. Frequency distribution of the total number of *P. alvearium* beetles per active *Ap. cerana* hive in northern Vietnam. Most hives sampled contained no beetles. In hives that contained beetles, they usually occurred in low numbers ($n < 5$).

To begin a trial, a beetle was placed in the center of an arena under an inverted glass vial and allowed to come to rest. After 10 min, the glass vial was gently removed so as not to disturb the beetle. Subsequently, at 5-min intervals over the next 2 h, we recorded the beetle's position as the grid square it occupied (i.e., 24 observations per beetle; $n = 30$ beetles per bioassay). Between reuse, the arenas were cleaned with water, 90% ethanol (C_2H_6O), 99% isopropyl alcohol (C_3H_8O), and 98% hexane (C_6H_{14}) sequentially, to eliminate beetle odors from previous trials. The

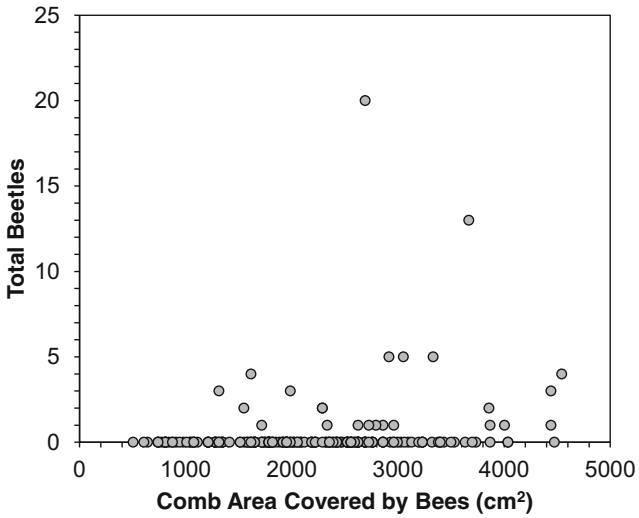

Figure 5. Positive relationship between *Ap. cerana* colony population and number of *P. alvearium* beetles in hives (zero-inflated regression model test; $z = 3.54$, $df = 179$; $p < 0.01$).

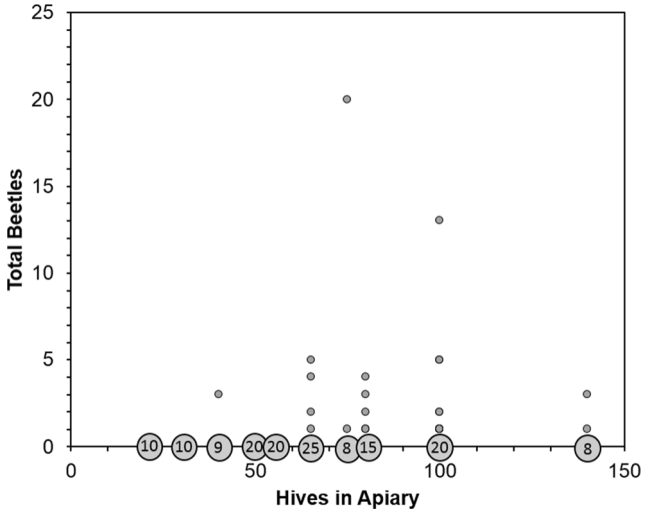

Figure 6. Positive relationship between the number of hives in an apiary and total beetles recorded in the hives surveyed in that apiary (zero-inflated regression model; $z = -1.99$, $df = 179$, $p = 0.047$). Larger points on the x-axis display the total number of zero values.

positions of the two substances being compared were alternated from one trial to the next to eliminate positional effects.

2.3. Statistical analyses

Regression analyses were performed to determine the relationship between beetle abundance and (1) colony population and the environmental factors of (2) hive cleanliness, (3) hive wetness, and (4) number of hives in the apiary. We estimated the dispersion parameter using a Quasi-Poisson regression model. This model allowed us to account for over-dispersion and a large number of zero beetle counts, under the assumption that zero-counts

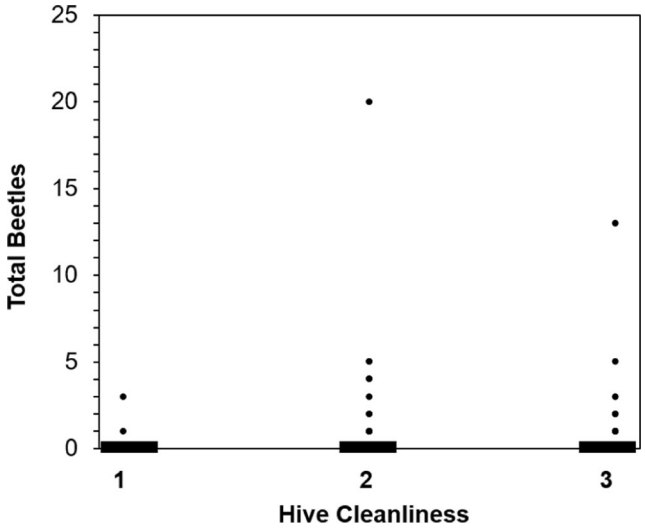

Figure 7. Boxplot of the Total number of *P. alvearium* beetles recorded in *Ap. cerana* hives with respect to **a** hive cleanliness ranked on a scale ranging from (1) excessive dirt and detritus, (2) some detritus and dirt, and (3) mostly clean ($z = 1.70$, $df = 179$, $p = 0.09$).

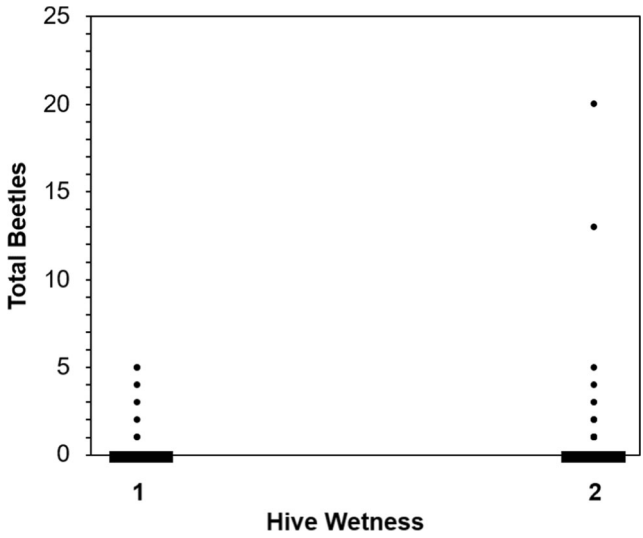

Figure 8. Frequency of *P. alvearium* beetles in *Ap. cerana* colonies with respect to hive wetness ranked as either (1) mostly dry or (2) damp wood or pooling of water ($z = 0.227$, $df = 179$, $p = 0.820$).

follow a binomial distribution. Based on the obtained estimated value of the dispersion parameter, we used a zero-inflated regression model. The statistical analyses were performed using R version 3.3.1 with the significance level set at $P < 0.05$ (R Core Team 2013).

We evaluated bioassay results in two ways. Preference was evaluated first using a chi-square test that compared the number of trials within a bioassay that the beetles were close to the two materials being tested (i.e., not in the center row of the arena). However, this approach did not consider the actual scores obtained within each trial (i.e., to what degree an individual beetle within a trial spent more time near one material than the other; for example, compare the beetle paths depicted in Fig 3a, b). We therefore additionally used paired *t* tests to evaluate the strength of beetle responses by comparing the scores (number of squares each beetle had occupied at each end of the arena) for each substance during each trial of a bioassay.

Figures were created in Microsoft Excel (2013) and Microsoft PowerPoint (2013).

Figure 9. The corner of an active *Ap. cerana* hive in Vietnam, with 2 *P. alvearium* beetles located in the crevices of the hive, indicated with a red circle.

3. RESULTS

In preliminary observations in *Apis cerana* hives (2009–2013), larvae were observed only on bottom boards among moist leaves and detritus. Adults were regularly observed on the frame rests of hives and in other cracks and crevices within the hives, but rarely on the combs. In one instance, GWO observed a beetle as it walked on largely empty comb among several worker bees that expressed no obvious reaction to it. On one

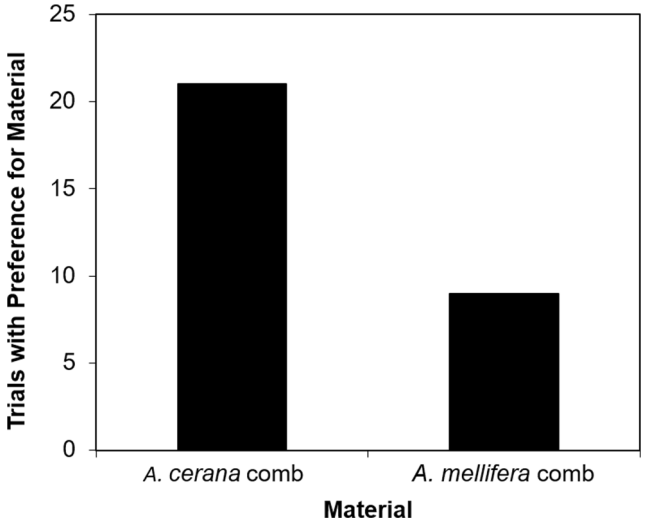

Figure 10. Results of two-choice bioassay comparing *P. alvearium* preference for *Ap. cerana* vs. *Ap. mellifera* comb. The bars indicate the number of trials in which a beetle spent more time close to *Ap. cerana* comb ($n = 21$) compared to more time close to *Ap. mellifera* comb ($n = 9$).

occasion, two pairs of beetles, likely mating pairs, occupied separate cells of an empty comb that had been placed in a bush. In an apiary in Hà Tĩnh Province in October 2011, hive beetles were encountered in half of 40 colonies inspected, sometimes with more than 20 beetles counted per hive (Pham Thi Huyen and Gard Otis, unpubl. data.). Similarly, in Cúc Phương, Ninh Bình province, in early May of 2013, beetles were moderately abundant (> 30) in a few hives and not observed in others.

Detailed surveys within apiaries in northern Vietnam in 2015 demonstrated that beetle abundance was low in that region at that time of year. In the 180 *Ap. cerana* hives sampled, we detected 83 beetles (mean abundance 0.46

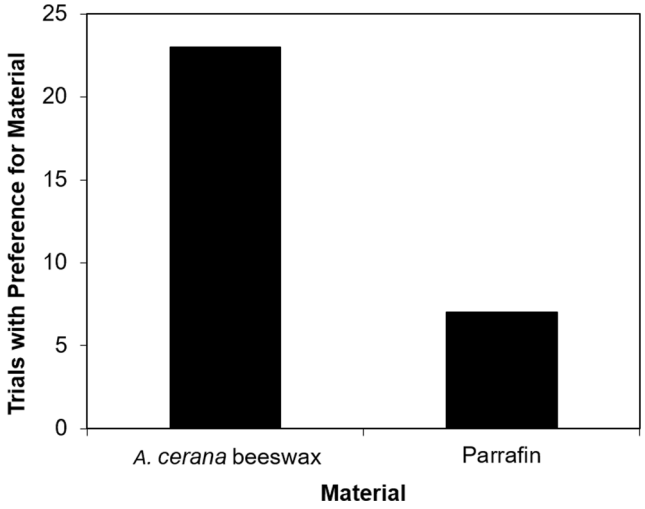

Figure 11. Results of two-choice bioassay comparing *P. alvearium* preference for *Ap. cerana* beeswax vs. paraffin. The bars indicate the number of trials in which a beetle spent more time close to *Ap. cerana* wax ($n = 23$) compared to more time close to paraffin ($n = 7$).

beetles/hive). They were observed in only 23 hives (prevalence 13% of hives infested) (Fig. 4). Beetle abundance was positively and significantly related to colony population ($z = 3.54$, $p < 0.001$; Fig. 5) and with the number of hives in the apiary ($z = -1.99$, $p = 0.047$; Fig. 6). There was no significant association between hive cleanliness and beetle abundance ($z = 1.70$, $p = 0.09$; Fig. 7), but in general, beetles were more frequently observed in hives that had some dirt and detritus on the bottom board. We found no relationship between beetles and hive wetness ($z = 0.227$, $p = 0.820$; Fig. 8). Of the 30 *Ap. mellifera* hives sampled, we observed no beetles in any of them.

During our detailed surveys in 2015, we never saw *Apis cerana* act aggressively towards *P. alvearium*. In fact, no interactions were ever observed between the bees and beetles. When disturbed or exposed, the beetles moved rapidly at a rate of approximately 1.5 cm/s and quickly hid in crevices within the hive. *P. alvearium* was most frequently seen in cracks between the boards of the hive lid and between the hive frame rests, and sometimes on the bottom boards of the hives. Figure 9 depicts a beetle in location that was frequently occupied. In 2015, we never observed beetles on combs or close to pollen, nectar, or honey.

During the bioassays, beetle response was highly variable. Some beetles moved to one end and then became inactive. More frequently, beetles would either move continuously but spend most of the 2-h study period at one end of the arena (Fig. 3a), or they would stop moving and remain at one end for a long period of time (Fig. 3b). Of the four bioassays performed, we found that beetles preferred to be in the vicinity of *Ap. cerana* comb compared to *Ap. mellifera* comb (chi-square test: $X^2 = 4.8$, $df = 1$, $p < 0.05$, Fig. 10; paired t test: $t = 3.03$, $df = 29$, $p = 0.005$). They also preferred to be near *Ap. cerana* beeswax compared to paraffin (chi-square test: $X^2 = 8.53$, $df = 1$, $p < 0.01$, Fig. 11; paired t test: $t = 3.41$, $df = 29$, $p = 0.002$). Evaluation of "*cerana* comb with honey" versus "empty *cerana* comb" (chi-square test: $X^2 = 0$, $df = 1$, $p > 0.05$; paired t test: $t = 0.92$, $df = 29$, $p = 0.36$) and "*cerana* beeswax" versus empty *cerana* comb (chi-square test: $X^2 = 0.53$, $df = 1$, $p > 0.05$; paired t test: $t = 1.42$, $df = 29$, $p = 0.16$) indicated that the beetles did not prefer either product in those two bioassays (Fig. 12).

4. DISCUSSION

We examined the abundance, prevalence, and behavior of the honeybee associate *Platybolium*

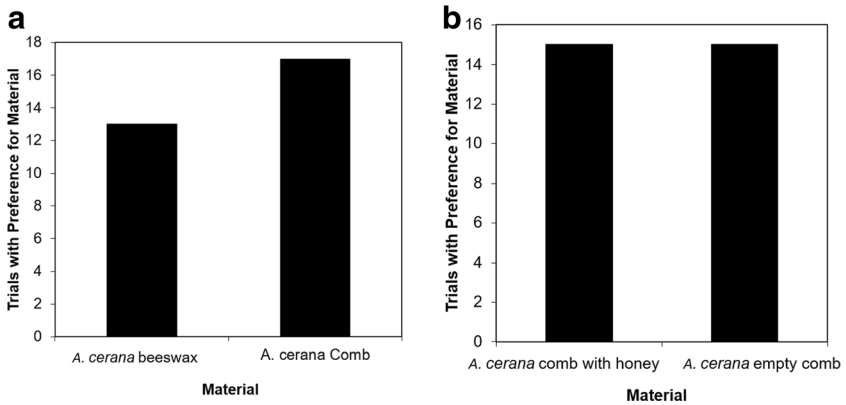

Figure 12. Two-choice bioassay results showing the number of trials where *P. alvearium* spent **a** more time close to *Ap. cerana* comb ($n = 17$) than *Ap. cerana* beeswax and **b** more time close to empty *Ap. cerana* comb ($n = 15$) than *Ap. cerana* comb containing honey ($n = 15$).

alvearium in northern Vietnam to further define its relationship to honeybees. We found *P. alvearium* to regularly occur in colonies of *Apis cerana*. During our casual observations in Hà Tĩnh Province (2009–2013) and Cúc Phương (early May 2013), beetles were frequently encountered in active *Ap. cerana* colonies but they were never abundant. They were less commonly recorded in the more detailed hive surveys in Hưng Yên and Hòa Bình provinces in late May–early June 2015. It is noteworthy that we detected no beetles in the 30 *Ap. mellifera* hives we surveyed.

In bioassays, the *Platybolium* beetles oriented towards and remained closer to *Ap. cerana* comb than *Ap. mellifera* comb and preferred *Ap. cerana* beeswax more than paraffin. On one occasion, we found two pairs of beetles thought to be mating in empty cells of a comb left outside a hive. In India, this species is sometimes referred to as the "wax beetle" (Singh 1962). Collectively, these observations suggest a fairly close association between the beetles and *Ap. cerana*. In our observations in northern Vietnam, however, *Platybolium* beetles caused no damage to the combs or the bees and elicited no absconding, biting, stinging, or confinement behavior. Beekeepers who manage *Apis cerana* colonies in Vietnam never report damage caused by *Platybolium* beetles. The absence of beetles in our sample of 30 *Ap. mellifera* colonies suggests that they may be specifically associated with *Ap. cerana*. Despite the seemingly close association with *Ap. cerana*, we did not see the beetles eat any bee products (i.e., honey, pollen, beeswax), or fruits placed in their holding containers. Unfortunately, we did not have time to elucidate their feeding preferences and have no information on their occurrence elsewhere in the environment.

Our observations differed markedly from the findings from northeastern India by Pande et al. (2015). They reported very large populations of *P. alvearium*, as well as active aggression towards adult beetles and attempts to sting the beetles. They observed groups of eggs that had been laid on bottom boards as well as the ends of brood and honey frames. Moreover, colony absconding may have been stimulated by large beetle populations at the end of winter. The differences in *Ap. cerana* responses in northeastern India and in northern Vietnam are difficult to resolve. They may be related to geographic differences in the behavior and/or ecology of the bees, the beetles, or both. Geographic differences in colony defense by bees to invaders have been noted previously in different races of *Ap. mellifera* (Eischen et al. 1986; Elzen et al. 2001; Kandemir et al. 2012).

P. alvearium beetles in our study, although never common, were more abundant in *Ap. cerana* hives that were more populous. This result contradicts the observations of Cherian and Mahadevan (1940) and Pande et al. (2015) who stated that weaker colonies in India were more susceptible to *P. alvearium* infestation. Pande et al. (2015) credited this finding to weaker colonies having exposed wax comb and thus being more desirable to beetles because larvae feed on wax flakes. Similarly, Ellis and Hepburn (2006) reported that weaker *Ap. mellifera* colonies are typically correlated with larger populations of other beetle associates of honeybees such as *Ae. tumida.* Our positive correlation may have been influenced by the generally low numbers of beetles in our study. Further study of *Platybolium* in various locations in Asia is warranted to further elucidate the relationship between beetle abundance and bee populations.

Beetle abundance was also higher in *Ap. cerana* apiaries that contained many hives. Kistner (1982) stated that, in general, social insect colonies that are located close together have more symbionts than those that are more dispersed. In our study, *P. alvearium* abundance may be higher in larger apiaries because the beetles are attracted to semiochemicals emitted from the colonies (c.f. Fombong et al. 2016) and larger numbers of colonies emit more chemicals. We have shown that the beetles are attracted to more odorous *Apis cerana* beeswax compared to paraffin in our bioassays; this attraction provides a potential mechanism for the positive relationship between beetle abundance and hive population.

Even after accounting for the effects of bee colony populations and apiary size, however, there was still substantial variation among colonies in the prevalence and abundance of *P. alvearium.* This variation is likely due to many other biotic and abiotic factors that we did not measure. We suspect that abundance of the beetles varies seasonally. Previous observations have shown regular occurrences of > 20 beetles per hive in October (Pham Thi Huyen and GWO, unpubl. data), but they were never as abundant as reported by Pande et al. (2015). Further research should focus on understanding within-hive and ambient environmental factors that influence *P. alvearium* abundance in different locations.

Bioassays can indicate which colony a bee-associate chooses to infest in nature based on what volatiles it is attracted to, or repelled by, in a controlled setting (Graham et al. 2010). In our two-choice bioassays, *P. alvearium* preferred to move towards and remain near *Ap. cerana* comb rather than *Ap. mellifera* comb. Moreover, we failed to find any beetles in the 30 *Ap. mellifera* colonies we sampled. The apiaries of *Ap. mellifera* ($n = 3$) and *Ap. cerana* ($n = 18$) were all in close proximity to each other so we assume that *P. alvearium* beetles had the opportunity to inhabit *Ap. mellifera* colonies but failed to do so. In contrast, in northern India, *P. alvearium* beetles were more common in *Ap. mellifera* colonies than in the native *Ap. cerana* colonies (Agni Chandra, pers. comm.). A. Chandra and V. K. Mattu collected *Platybolium* beetles from 274 (33.3%) of 822 *Ap. mellifera* hives in 15 apiaries and 4 (7.5%) of 53 *Ap. cerana* hives in 15 apiaries surveyed in Himachel Pradesh.. The mechanisms underlying these contrasting findings are unclear. This result may be related to the much larger sizes of the *Ap. mellifera* apiaries in the northern India study. Clearly, *P. alvearium* does inhabit *Ap. mellifera* hives in some situations.

Our bioassays also indicated that *P. alvearium* has no preference for *Ap. cerana* comb with honey over empty comb. In contrast, Pande et al. (2015), in their study of beetle longevity under different feeding treatments, reported that beetles preferred to rest on wax flakes with honey and fed on them more than was the case with wax flakes without honey. Cherian and Mahadevan (1940) and Singh (1962) described this beetle species as a scavenger of debris on bottom boards of weak hives that may nibble old empty comb. Thakur (1991) referred to it thriving in non-hygienic hives. Consumption of beeswax and comb by larval and adult *Platybolium*, and more importantly their digestion of beeswax, should be studied in more detail. If confirmed that they obtain nutrition from beeswax, it would strongly suggest that *P. alvearium* is highly integrated into colonies of *Ap. cerana* since the ability of animals to digest beeswax is very rare, having been documented only in honey-guide birds (Friedmann and Kern 1956; Diamond and Place 1988; Downs et al. 2002) and the greater wax moth (Dadd 1966).

Finally, we have shown that *P. alvearium* has a preference for beeswax over paraffin. This may indicate that the beetles prefer bee products over non-bee products or *Ap. cerana* beeswax specifically. Alternatively, it may simply indicate that they orient towards more odorous materials.

Our research has uncovered some interesting aspects of the relationship of *P. alvearium* and *Ap. cerana* in Vietnam. However, there is clearly more to learn, especially relative to the differing reports of the abundance of the beetles and the damage they cause from different regions of Asia (cf., this study; Pande et al. 2015). If further research demonstrates adaptations of *P. alvearium* to live in colonies of *Ap. cerana* (i.e., a high degree of integration), then our observations of *P. alvearium* will contradict the suggestions of Atkinson and Ellis (2011) about highly integrated beetle associates being more harmful to their hosts.

At present, however, *P. alvearium* in northern Vietnam seems to be a moderately integrated species within *Apis cerana* colonies that causes no harm and does not provoke a defensive response from honeybees. Maitip et al. (2016) made similar observations of *Al. laevigatus*, showing that it, like *P. alvearium*, is often abundant in colonies, yet they too seem

to cause no harm to bee colonies. We additionally found that contrary to many bee pests, *P. alvearium* is more abundant in stronger, not weaker, honeybee colonies, although this result may reflect the general scarcity of the beetles at the time of our hive surveys. Our observations, coupled with the highly contrasting results of *P. alvearium* in northeastern India (Pande et al. 2015) and reports of two *Alphitobius* beetle species associated with *Apis cerana* in Asia (Li et al. 2016, Maitip et al. 2016), suggest that additional investigations in Asia may alter our understanding of beetle symbionts of honeybees.

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AUTHOR CONTRIBUTIONS

SJD, HDP, LTPN, and GWO designed research. SJD, HDP, and LTPN executed the fieldwork and data collection. PB identified the beetles and provided the high-resolution photographs. TP and GWO statistically analyzed the data. SJD, PB, and GWO interpreted data and shared in the writing of the manuscript. All of the authors were involved in the manuscript revisions and approved the manuscript prior to submission.Funding information

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest The authors declare that they have no conflict of interest.

APPENDIX 1. BARCODE REGION (CYTOCHROME C OXIDASE SUBUNIT I) OF THE MITOCHONDRIAL DNA OF *PLATYBOLIUM* ALVEARIUM (PUBLISHED TO GENBANK: <DX.DOI.ORG/10.5883/DS-PLATYBOL>)

AACACTTTATTTTATCTTTGGCGCATGATCAG
GAATAATTGGCACATCCCTTAGACTCTTAA
TCCGAGCAGAACTTGGAAACCCAGGCTCTT
TAATTGGTGACGATCAAATTTATAATGTAA
TCGTCACAGCCCATGCATTTATCATAATTTTCTTTA
TAGTTATACCAATCATAATTGGAGGCTTCG
GAAATTGACTAGTTCCCCTAATACTAGGAG
CCCCGGATATAGCCTTCCCCCGAATAAACA
ATATAAGATTCTGACTACTTCCACCTTCAT
TAACACTTCTGTTAATAAGAAGAATTGTTG
AAAGAGGAGCGGGTACAGGATGAACAGTGT
ACCCCCCACTTTCATCCAATATCGCACACG
GAGGATCCTCCGTTGATTTAGCAATTTTTA
GATTACATTTAGCAGGAATTTCTTCCATCC
TAGGAGCTGTTAACTTCATTACTACAGTAA
TTAATATACGTCCTCAAGGAATATCATTCG
ATCGAATACCTTTATTTGTATGAGCAGTAGTAATTA
CTGCCGTACTTCTTCTTCTTTCTCTTCCCGTACTAG
CCGGAGCAATCACTATACTCCTAACAGACC
GAAATATTAATACATCCTTCTTTGATCCTG
CAGGAGGAGGAGATCCTATTCTTTACCAAC
ACCTATTC

Essais biologiques de prévalence et de comportement de *Platybolium alvearium* (Coleoptera: Tenebrionidae) dans des colonies d'abeilles domestiques (*Apis*: Hymenoptera: Apidae) dans le nord du Vietnam

***Apis cerana, Apis mellifera* / commensal / intégration de colonies / comportement défensif / associé aux abeilles /**

Prävalenz und Verhaltens-Biotests von *Platybolium alvearium* (Coleoptera: Tenebrionidae) in Bienenvölkern (*Apis*: Hymenoptera: Apidae) in Nordvietnam.

***Apis cerana, Apis mellifera* / Kommensalen / Abwehrverhalten / Bienenvolk-Eingliederung**

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