# Aethina tumida (Coleoptera: Nitidulidae) and Oplostomus haroldi (Coleoptera: Scarabaeidae): Occurrence in Kenya, Distribution Within Honey Bee Colonies, and Responses to Host Odors

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ABSTRACT Aethina tumida Murray (Coleoptera: Nitidulidae) is considered a minor parasitic pest of African honey bee, Apis mellifera L. (Hymenoptera: Apidae), colonies, but little information is available on other coleopteran pests. We surveyed for A. tumida and other beetles in honey bee colonies at four major beekeeping locations: Watamu, Chawia-Taita, Matuu, and Nairobi in Kenya and compared their distribution within the colonies. The presence of A. tumida was confirmed in all the colonies surveyed, whereas Oplostomus haroldi Witte (Coleoptera: Scarabaeidae) was found for the first time to be associated with honey bee colonies in varying numbers at all the sites, except that none were found in colonies in Nairobi. More than 90% of A. tumida and O. haroldi were found in Watamu and Chawia, located within the coastal province of Kenya. Although A. tumida was found mostly on the bottom board of the hives, consistent with previous reports, O. haroldi was found on the frames. Laboratory bioassays using a two-choice olfactometer showed that both beetle species were significantly attracted to worker honey bee volatiles and commercial pollen dough inoculated with the yeast Kodamaea ohmeri associated with A. tumida. Based on these findings, we report for the first time O. haroldi as a pest of African honey bee colonies in Kenya. We propose that differences in their densities recorded in the colonies may be due to dissimilarities in the colony environments in the areas surveyed and that odor-baited traps that have been successfully been used to manage populations of A. tumida also will be suitable for use against O. haroldi.

KEY WORDS Aethina tumida, Oplostomus haroldi, honey bee, Kodamaea ohmeri, Kenya

The honey bee, Apis mellifera L. (Hymenoptera: Apidae), is widely distributed in Africa, where adaptation to different geographical areas has given rise to various races (Ruttner 1988, Michener 2000). The honey bee is important for its honey production (Krochmal 1985; Crane 1990, 1999); for the pollination services it renders to food production and the stability of nature (Crane and Walker 1984, Borneck and Merle 1989, Delaplane and Mayer 2000); and for hive products, including beeswax, propolis, pollen, royal jelly, bee brood, and bee venom (Crane 1990, 1999, Michener, 2000). Honey bees, like other economically important arthropods, are challenged and constrained by pests. In Africa, the pest composition of honey bees is similar to that documented for the European honey bee. These include the wax moth, Galleria mellonella L. (Lepidoptera: Pyralidae); bee lice (*Braula* spp.) (Diptera: Braulidae), various ant species (Hymenoptera: Formicidae), and the beetle Aethina tumida Murray (Coleoptera: Nitidulidae) (Lundie 1940, Hepburn and Radloff 1998). *Oplostomus fuligineus* Olivier (Coleoptera: Scarabaeidae) (Donaldson 1989) has been reported only in southern Africa. The mite *Varroa destructor* Anderson (Parasitiformes: Varroidae) has been reported in the northern and southern parts of the continent (De Jong et al. 1982, Hepburn and Radloff 1998, Crane 1990, FAO 2006) and more recently in East Africa (Frazier et al. 2010).

Of the two hive beetles, *A. tumida* is the most widely studied, although it has been described as an insignificant pest in honey bee colonies in its native host range in sub-Saharan Africa (Lundie 1940, Schmolke 1974). Over a decade ago, it was reported as an invasive pest of European honey bee colonies in the United States (Sanford 1998); thereafter, it was detected in Australia (Neumann and Elzen 2004). Following the earlier documentation of its biology by Lundie (1940), huge strides have been made in the last decade to understand its behavior (Elzen et al. 2002, Neumann and Elzen 2004, Ellis et al. 2004), chemical ecology (Suazo et al. 2003; Torto et al. 2005, 2007a,b; Benda et al. 2008), and management (Ellis et al. 2003; Hood and Miller 2005; Arbogast et al. 2007, 2009a).

In Kenya, beekeeping is increasingly becoming an important income generating activity to fight poverty,

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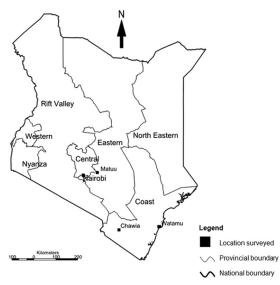


Fig. 1. Map of Kenya showing the locations surveyed used to sample for *A. tumida* and *O. haroldi* hive beetles in June 2009 and January 2010.

hunger, and unemployment (Raina 2006). Despite this, guidelines for the movement of hives across boundaries, restrictions of movement, sanitary requirements and control of pests are lacking. Furthermore, unconfirmed reports from beekeepers suggest the presence of various coleopteran pests in honey bee colonies which may be contributing to the absconding of honey bees in certain localities. Therefore, there is an urgent need to document arthropods associated with honey bee colonies in the country and to investigate their interactions with bees to fill in this gap. This study had two objectives. The first was to carry out) a survey of honey bee colonies for A. tumida and other beetle pests in selected beekeeping areas in Kenya. The second was to compare the responses of these beetles to honey bee odors and to a lure consisting of pollen dough inoculated with the yeast Kodamea ohmeri (NRRL Y-30722), which is associated with the small hive beetle (Torto et al. 2007a). This lure has been used previously to trap this beetle in the United States (Torto et al. 2007b; Arbogast et al. 2007, 2009a).

## Materials and Methods

**Study Sites.** Five beekeeping sites in different parts of Kenya (Coastal, Eastern, and Nairobi provinces) were used to compare the occurrence and distribution of beetles in honey bee colonies (Fig. 1). The apiaries surveyed were selected because the beekeepers at these sites kept bees in Langstroth hives, similar to those kept at the International Centre of Insect Physiology and Ecology (*icipe*) site in Nairobi. The surveys were carried out in June 2009 and January 2010.

**Coast Province.** Two beekeeping sites in this province were surveyed: Watamu and Chawia-Taita, ≈170 km from each other. At the Watamu site, two apiaries, A and B,  $\approx 2$  km apart were surveyed. Apiary A was located within the compound of one of Kenya's national museums (Arabuko-Sokoke) (03° 18' 24.3" S,  $040^{\circ} 1' 4.4'' E$ ),  $\approx 12 \text{ m above sea level (ASL)}$ . There were 15 honey bee colonies located at this site within a secondary forest consisting of African baobab, figs, false mahogany, neem, and mango trees, which provided full shade to the hives. All but one colony possessed supers. Monkeys were the most prominent animals within the museum compound. Apiary B, located at Maganganyi village (03° 18′ 29″ S, 039° 59′ 56.2″ E), at ≈11 m ASL, had four honey bee colonies, and these colonies were placed under an open thatched house, which provided shade to the colonies. Two of these colonies were single chamber hives, whereas the other two hives had supers. Mixed farming consisting of growing various cereals, tubers, legumes and mangoes and keeping goats and poultry was the dominant activity in the area. Also, fishing formed an integral part of the daily activities at this location.

Apiaries C and D were located at Chawia-Taita township (03° 28′ 30.7″ S, 038° 20′ 17.9″ E), ≈1,500 m ASL, and they were located within 1 km of one another. Apiary C, located on the slopes of a hill, with very little vegetation cover, contained nine colonies. A single palm tree provided shade to the colonies. Seven of these colonies had supers, whereas the other two had no supers. Apiary D, located in a small forest of eucalyptus trees, contained three colonies, one of which had no super. Six months later, each apiary had been reduced to a single hive without a super after attacks by honey badgers, which caused the bees to abscond. The main human activities in this village community were mixed cropping, consisting mainly of growing cereals, legumes, and a few mango trees and keeping cattle and other ruminants.

Eastern Province. The apiary surveyed was located at Ndalani (01° 5′ 6.3″ S, 37° 28′ 13.1″ E), a village within the town of Matuu, which is a semiarid area. This apiary,  $\approx$ 1,100 m ASL, was situated by the side of a stream and had 12 colonies. Sparsely distributed acacia, siamese senna, a few mango trees, aloe, and ocimum were the dominant plants in the area. During the second inspection of this apiary, only five hives were occupied by honey bees, the others having absconded probably due to poor management. The principal human activities within this village community were mixed cropping of cereals, legumes and a bit of animal husbandry.

Nairobi Province. The apiaries surveyed in Nairobi were located at Kasarani (site E) and Kamiti (site F). The apiaries were within 5 km apart. The beeyard at site E was on the main campus of *icipe* (01° 13' 25.3" S, 36° 53' 49.2" E),  $\approx$ 1,600 m ASL. It had 17 honey bee colonies, placed on a gentle slope that ran down toward a small stream that separated the site from the campus buildings. Hives were either partially or fully shaded by different tree types present within the beeyard; these trees included acacias, bottlebrush, neem, and avocado. Other plants found within this area included cactus, black jack, striga, and wild grasses. The main human activities here involved the

cultivation of legumes, cereals, pumpkins, sunflowers, and various vegetables.

Site F was located within the Kamiti prison compound (01° 11′ 25.6″ S, 36° 53′ 35.1″ E)  $\approx$ 1,600 m ASL. It contained >14 Langstroth box hives, but only four were occupied by honey bees in June 2009. Two months later, all the colonies had been sold, and the apiary no longer existed. Colonies were partially shaded by avocado and eucalyptus trees scattered within the compound. Groundcover in the apiary consisted mostly of overgrown grasses. Cereals and legumes were the main crops, and these plants were grown in small patches within the compound.

Survey of Beetles. At all of the sites, honey bee colonies were chosen at random within each apiary, and only those without honey bee combs constructed across the frames were surveyed for beetles. Beetles found on the top board, bottom board, frames, and inside walls of the hives were counted. An aspirator was used in counting small hive beetles. Frames were removed and carefully checked for beetles on both sides after which they were transferred into an empty box. After all the frames had been removed, the inside of the hive box and the bottom box were checked for beetles. After counting, the hive was reassembled. During the survey, beetles were removed from the hive as they were counted to avoid duplicating counts. After counting, all A. tumida beetles were returned to the hive, whereas O. haroldi were kept for identification. Specimens of these beetles were identified as Oplostomus (Macromoides) haroldi Witte (Coleoptera: Scarabaeidae) by Dr. Mike Thomas (Florida Department of Agriculture and Consumer Affairs, Division of Plant Industry, Gainesville, FL). The beetles were sexed based on the morphology of their abdominal sterna; males possess a groove on the second to fifth abdominal sterna, which is absent in females (Donaldson 1989).

Counts of both *A. tumida* and *O. haroldi* were pooled across the different apiaries and were subjected to logistic regression analysis using SAS procedure GENMOD and pairwise orthogonal comparisons (SAS Institute 2003). Within each apiary site, beetle counts for the different hive sections were again subjected to logistic regression analysis and pairwise orthogonal comparisons carried out in locations where significant differences in beetle numbers were observed. The total numbers of the different sexes of the large hive beetle at each site were compared using a chi-square ( $\chi^2$ ) one-sample test ( $H_0$ : equal numbers of each sex occurred in all locations). All analyses were carried out at an  $\alpha$  level of 0.05.

**Rearing of Beetles.** Adult *A. tumida* were collected from honey bee colonies at the *icipe* apiary, Nairobi to start a laboratory colony. The beetles were transferred into Mason jars (800 ml) containing a vial of water with a wick and fed on commercial pollen dough (4% pollen with sugar, soy, yeast, and water) (Global Patties, Airdrie, AB, Canada). Eggs were laid within the pollen dough substrate and hatched into larvae that were reared on the same substrate until they reached the wandering larval stage. Two hundred wandering larvae were then removed from the Mason jar and transferred for pupation to moist autoclaved red laterite soil held within rectangular plastic bowls (19 by 14 by 10 cm) with perforated lids. F1 adult beetles emerged  $\approx 20$  d later and were sexed using a microscope slide which was placed on the anterior ventral side of the abdomen and pressed slightly to force out the genitalia of the beetle which were observed under a light microscope. Males and females were then placed in separate Mason jars and fed with commercial pollen dough for a 10–14-d period. *A. tumida* were kept at  $26 \pm 2^{\circ}$ C,  $50 \pm 5\%$  RH, and a photoperiod of 10:14 (L:D) h.

Attempts to establish a laboratory colony of O. haroldi for subsequent assays failed, so field-collected beetles were used for the olfactometer assays. The beetles (350) were collected from honey bee colonies on the coast of Kenya and were kept in the laboratory for 21 d on a substrate composed of sifted soil and crushed cow dung mixed in a ratio of 3:1 by volume and moistened with distilled water to habituate them before being used in olfactometer assays. Females and males were kept separately from each other in plastic rectangular bowls (26 by 19 by 14 cm) with lids made of perforated Styrofoam and wrapped all round with mosquito nets to prevent the beetles from creating exit holes in the lids. Males were differentiated from females by the presence of a grove running along the midsection of the second to fifth abdominal sternite. Beetles were provided with water by placing moist cotton wool balls on the lids of 8.5-mm petri dishes on top of the substrate within the rectangular bowls and kept at conditions as previously described above for the small hive beetle.

Olfactometer. Bioassays were conducted in a dual choice olfactometer (100 by 31 by 31 cm) constructed from glass and aluminum (Fig. 2). Air from a compressed air tank was first purified by passing it through activated charcoal and then it was delivered into each arm of the olfactometer at 0.35 liter/min. A vacuum line powered by a fan pulled air from the center of the olfactometer at 0.71 liter/min. Two 40-W fluorescent light bulbs placed above the center of the olfactometer illuminated the test arena evenly.

Comparison of Beetle Responses to Odors. We determined the responses of 25 male and 25 female (7-14-d-old) A. tumida or O. haroldi to a choice between one of two odor sources and a control in the olfactometer. One odor source, provided by 300-400 worker honey bees collected from hive frames, was tested against air (control). The second odor source was pollen dough (15 g) inoculated with the yeast K. ohmeri and was compared with noninnoculated pollen dough (control). Test beetles were released singly into the center of the olfactometer and after 10 min the odor to which the beetle responded was recorded. Beetles were used only once and then discarded, and the positions of the odor sources were reversed between replicates to minimize positional bias. The proportion of beetles of both sexes responding to the different odors sources were subjected to a chi-square

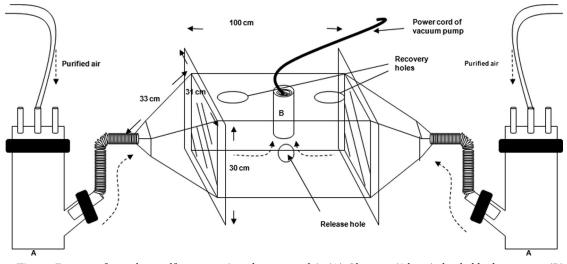


Fig. 2. Diagram of two choice olfactometer (not drawn to scale). (A) Glass jars (3 liters) that hold odor sources. (B) Vacuum pump; broken arrows represent the direction of flow of charcoal filtered air.

one-sample test at  $\alpha = 0.05$ , with H<sub>0</sub>: the proportion of beetles responding to odor sources are not different.

## **Results and Discussion**

Survey. In total, 1,947 and 1,379 adult *A. tumida* were counted during the June 2009 and January 2010 surveys respectively. In June, the highest count was at Watamu, sites A + B (1,514), followed by Nairobi, sites E + F (210); Chawia-Taita, sites C + D (197); and Matuu (26) (Fig. 3A). A similar trend was found in January 2010, with the highest number counted at Watamu (1,242), followed Nairobi (86), Matuu (34), and Chawia (17) (Fig. 3B). No *A. tumida* larvae were found in any of the inspected colonies. There were

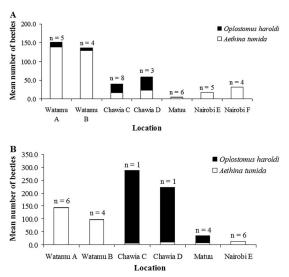


Fig. 3. Mean number of A. *tumida* and O. *haroldi* recorded at each location during the survey (n, number of surveyed hives). (A) June 2009. (B) January 2010.

significant differences in the distribution of the small hive beetle within the hive (Table 1). Significantly more beetles (38–86% depending upon the hive and location) were found on the bottom board of the hive. Multiple pairwise comparisons of the data confirmed that significantly more of the beetles occurred at the bottom of the hive (Tables 3 and 4).

In the survey for O. haroldi, 394 and 626 adult beetles in total were collected during the June 2009 and January 2010 surveys, respectively. The highest number was recorded in the June 2009 survey at Chawia-Taita (292), followed by Watamu (97), and the least in Matuu (5) (Fig. 3A). A similar trend was observed in January 2010, with more beetles obtained at Chawia-Taita (493) and Matuu (108) and less at Watamu (25). No O. haroldi was detected in any of the honey bee colonies surveyed in Nairobi. Most O. haroldi were found on the frames (Table 2). At all locations during both surveys, except at Matuu in June 2009, both sexes of O. haroldi were collected, with more males than females occurring in the honey bee colonies (Fig. 4A and B). The number of males was significantly higher than those of females; Watamu A  $(\chi^2 = 17.29, P < 0.001)$ , Chawia C  $(\chi^2 = 180.19, P < 0.001)$ 0.001), apiary in Chawia D ( $\chi^2 = 100.0, P < 0.001$ ) and Matuu ( $\chi^2 = 7.0, P = 0.008$ ) but not at Watamu B ( $\chi^2 =$ 1.88, P = 0.17) in June 2009 (Fig. 4A). Similarly, a significantly higher proportion of males occurred at each site; Watamu A ( $\chi^2 = 12.25, P < 0.001$ ), Watamu B ( $\chi^2 = 9.00, P = 0.003$ ), Chawa C ( $\chi^2 = 81.14, P <$ 0.001), Chawia D ( $\chi^2 = 4.83$ , P = 0.028), and Matuu ( $\chi^2 = 7.26$ , P = 0.007) in January 2010 (Fig. 4B).

**Responses to Host Odors.** For both species of beetles, there were significant differences in the proportion of test beetles that responded to worker honey bee volatiles and to pollen dough innoculated with *K. ohmeri* compared with the controls (P < 0.05; Figs. 5 and 6). However, the percentage of males and females

Apiary location	Top	Bottom	Frame	Box	$\chi^2$ value	df	Р
June 2009							
Watamu (site A)	$34.2 \pm 13.7$	$72.6 \pm 19.6$	$11.8 \pm 5.1$	$19.8 \pm 16.1$	5.78	3, 16	0.1230
Watamu (site B)	$34.5 \pm 11.9$	$77.6 \pm 7.6$	$10.0 \pm 6.9$	$6.3 \pm 1.4$	13.55	3, 12	0.0036*
Chawia-Taita (site C)	$0.1 \pm 0.1$	$11.1 \pm 3.0$	$4.5 \pm 1.2$	$0.5 \pm 0.3$	29.96	3, 28	< 0.0001*
Chawia-Taita (site D)		$16.7 \pm 3.3$	$5.7 \pm 3.2$		4.45	1, 4	0.0348*
Matuu		$3.0 \pm 0.8$		$1.3 \pm 1.3$	1.08	1, 10	0.2980
Nairobi (site E)	$0.8\pm0.6$	$8.4 \pm 5.2$	$2.0 \pm 0.8$	$6.0 \pm 1.3$	7.71	3, 16	0.0524
Nairobi (site F)	$1.0 \pm 0.7$	$13.8 \pm 3.0$	$1.5\pm0.9$	$14.8 \pm 4.6$	19.48	3, 12	0.0002*
Jan. 2010							
Watamu (site A)	$42.3 \pm 4.5$	$63.5 \pm 13.0$	$2.3 \pm 2.0$	$30.7 \pm 9.8$	25.49	3, 20	< 0.0001*
Watamu (site B)	$33.5 \pm 13.4$	$37.8 \pm 13.2$	$1.0 \pm 1.0$	$24.8 \pm 10.1$	13.07	3, 12	0.0045*
Chawia-Taita (site C)		$2.0 \pm 0.0$	$2.0 \pm 0.0$	$2.0 \pm 0.0$	n.d.		
Chawia-Taita (site D)		$7.0 \pm 0.0$		$3.0 \pm 0.0$	n.d.		
Matuu	$0.3 \pm 0.3$	$2.8 \pm 1.5$	$0.8\pm0.8$	$4.8 \pm 3.0$	5.97	3, 12	0.1129
Nairobi (site E)	$0.3 \pm 0.3$	$11.3 \pm 4.9$	$1.2 \pm 1.0$	$1.5 \pm 1.1$	11.53	3, 20	0.0092*

Table 1. Mean  $\pm$  SE and logistic regression of the number of A. tumida adults recorded in the different hive sections during surveys carried out in different locations in Kenya in June 2009 and January 2010

n.d., not done (Sites for which no logistic regression was not done because only single hives were available for inspection). \*Statistically significant at P = 0.05.

Table 2. Pairwise comparison of adult A. tumida distribution
in different sections of the honey bee hive at different locations in
Kenya in June 2009 and January 2010

June 2009	
Watamu (site B)	
Top vs. bottom	$\chi^2 = 2.00, P = 0.1569$
Top vs. frame	$\chi^2 = 4.37, P = 0.0367^*$
Top vs. box	$\chi^2 = 7.97, P = 0.0048^*$
Bottom vs. frame	$\chi^2 = 12.11, P = 0.0005^*$
Bottom vs. box	$\chi^2 = 17.54, P < 0.0001^*$
Frame vs. box	$\chi^2 = 0.58, P < 0.4482$
Chawia-Taita (site C)	A
Top vs. bottom	$\chi^2 = 17.16, P < 0.0001^*$
Top vs. frame	$\chi^2 = 10.78, P = 0.0010^*$
Top vs. box	$\chi^2 = 1.36, P = 0.2435$
Bottom vs. frame	$\chi^2 = 4.06, P = 0.0440^*$
Chawia-Taita (site D)	
Bottom vs. box	$\chi^2 = 22.69, P < 0.0001^*$
Frame vs. box	$\chi^2 = 10.95, P < 0.0009^*$
Bottom vs. frames	$\chi^2 = 7.07, P = 0.0079^*$
Nairobi (site F)	
Top vs. bottom	$\chi^2_{-} = 17.60, P < 0.0000*$
Top vs. frame	$\chi^2 = 0.31, P = 0.5807$
Top vs. box	$\chi^2 = 18.61, P < 0.0001^*$
Bottom vs. frame	$\chi^2 = 15.99, P < 0.0001^*$
Bottom vs. box	$\chi^2 = 0.03, P = 0.8595$
Frame vs. box	$\chi^2 = 17.08, P = 0.0005^*$
Jan. 2010	
Watamu (site A)	
Top vs. bottom	$\chi^2 = 3.98, P = 0.0460^*$
Top vs. frame	$\chi^2 = 54.84, P < 0.0001^*$
Top vs. box	$\chi^2 = 1.25, P = 0.2635$
Bottom vs. frame	$\chi^2 = 32.88, P < 0.0001^*$
Bottom vs. box	$\chi^2 = 0.77, P = 0.3791$
Frame vs. box	$\chi^2 = 41.93, P < 0.0001^*$
Watamu (site B)	
Top vs. bottom	$\chi^2 = 0.47, P = 0.4917$
Top vs. frame	$\chi^2 = 21.38, P < 0.0001^*$
Top vs. box	$\chi^2 = 0.04, P = 0.8452$
Bottom vs. frame	$\chi^2 = 16.60, P < 0.0001^*$ $\chi^2 = 0.24, P = 0.6223^*$
Bottom vs. box	$\chi^2 = 0.24, P = 0.6223^*$
Frame vs. box	$\chi^2 = 19.97, P < 0.0001^*$
Nairobi (site E)	
Top vs. bottom	$\chi^2 = 5.76, P = 0.0164^*$
Top vs. frame	$\chi^2 = 6.97, P = 0.0083^*$
Top vs. box	$\chi^2 = 11.31, P = 0.0008^*$
Bottom vs. frame	$\chi^2 = 0.08, P = 0.7837$ $\chi^2 = 1.80, P = 0.1600$
Bottom vs. box Frame vs. box	$\chi^2 = 1.89, P = 0.1690$ $\chi^2 = 1.28, P = 0.2582$
Frame VS. DOX	$\chi^2 = 1.28, P = 0.2582$

\*Statistically significant at P = 0.05.

responding to each of these odors were not significantly different.

Results of the current study showed that *A. tumida* and *O. haroldi* occur at all the beekeeping sites selected for this study, except in Nairobi where *O. haroldi* was not detected. Of the total number of beetles recorded from the three provinces,  $\approx 90\%$  were *A. tumida* but in the coastal province, 99% were *O. haroldi*. These differences in the numbers of the beetles may be associated with differences in the environmental conditions in these areas. The coastal province, is the major fruit-growing area in Kenya (Griesbach 2003, Rwomushana et al. 2008), whereas the Eastern and Nairobi provinces are semiarid, with the former at

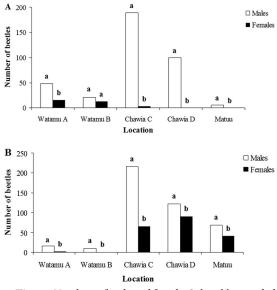


Fig. 4. Numbers of male and female *O. haroldi* recorded from five apiaries in three provinces in Kenya. For each apiary, bars with the same letter are not significantly different (chi-square one-sample test, P < 0.01). (A) June 2009. (B) January 2010.

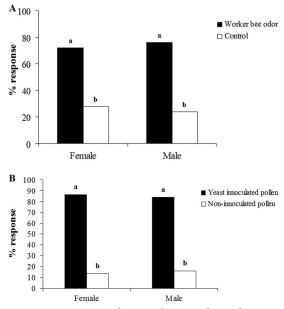


Fig. 5. Responses of *A. tumida* to two hive odors. (A) Worker honey bee volatiles versus air. (B) *K. ohmeri*-inoculated pollen dough versus pollen dough. Pairs of open and closed bars with the same letter are not significantly different (chi-square one-sample test, P < 0.01).

an elevation of 1,100 m and the latter at 1,600 m; they are the coldest of the three provinces. The average environmental conditions in these areas also vary

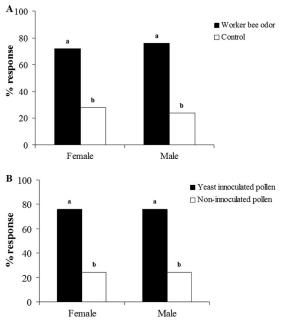


Fig. 6. Responses of *O. haroldi* to two hive odors. (A) Worker honey bee volatiles versus air. (B) Inoculated pollen dough versus pollen dough. Pairs of open and closed bars with the same letter are not significantly different (chi-square one-sample test, P < 0.01).

monthly; ≈160 mm of rainfall and 25.0°C at Watamu to 9 mm and 20.6°C at Chawia-Taita, 8 mm and 18.7°C at Matuu, and 46 mm and 16.5°C at Nairobi in June; and 11 mm of rainfall and 26.6°C at Watamu; 37 mm and 22.3°C at Chawia-Taita, 50 mm and 20.2°C at Matuu, and 40.3 mm and 20.2°C for Nairobi in January (Griesbach 2003). Previous research has shown that A. tumida can complete its life cycle on certain fruit, which may serve as potential alternative hosts for the beetles (Arbogast et al. 2009b). They also develop more rapidly in warmer than colder climates (De Guzman and Frake 2007). Therefore, it is possible that the combined effects of the warmer climate and wide-scale availability of potential alternate hosts in the Coastal province than in the other provinces may contribute to the different numbers of beetles infesting honey bee colonies at these different sites. A comprehensive survey in other provinces over an extended period should provide a full picture of the occurrence of both beetle species in the country.

More A. tumida were found in honey bee colonies located in Watamu, but more O. haroldi were recorded in the colonies at the sites in Chawia, which is located  $\approx 175$  km from Watamu. A. tumida has been reported to prefer shady rather than sunny areas (Arbogast et al. 2007). Our results suggest that the Arabuko-Sokoke forest in Watamu may provide the requisite shade and moisture levels for increased A. *tumida* activity at this site compared with the sites in Chawia. However, the higher number of O. haroldi recorded in Chawia may be due to the preference of females to lay their eggs in moist soil beneath cow dung pads (Donaldson 1989), which was found to be readily available at the sites in Chawia, where mixed farming was the dominant activity practiced by the community in the area.

The congregation of *A. tumida* mainly at the bottom board of Langstroth hives has been reported previously (Lundie 1940), an observation attributed to honey bee defensive behavior and the beetle's scavenging behavior (Lundie 1940, Torto et al. 2007b). In Kenva, apart from the Langstroth hive, honey bees are also kept in traditional logs and the Kenya top bar hive (KTBH). A survey of a few of the KTHBs (three) showed that adult A. tumida beetles were mainly confined to the corners rather than the bottom board of the hive. A study of the occurrence, distribution, and economic impact of A. tumida in different hive types used in Kenya is therefore warranted. The detection of no A. tumida larvae in any of the honey bee colonies surveyed suggests a strong hygienic behavior of the honey bees, which corroborates our earlier suggestion of the possible use of alternate hosts for oviposition and development by A. tumida. Studies are needed to confirm this.

The presence of larger numbers of *O. haroldi* on the frames compared with other sections of the hive could be attributed to their size, larger than the bees, which allows the beetles to access food resources far beyond the bottom board where *A. tumida* mainly occur. However, the fact that at both sampling times (June and January), that is 6 mo apart, both *A. tumida* and

Apiary location	Top	Bottom	Frame	Box	$\chi^2$ value	df	Р
June 2009							
Watamu (site A)		$3.8 \pm 1.2$	$8.0 \pm 3.9$	$0.8\pm0.4$	6.65	2, 12	0.0360*
Watamu (site B)	$1.5 \pm 1.5$	$1.3 \pm 0.6$	$5.0 \pm 2.0$	$0.8\pm0.5$	4.39	3, 15	0.2222
Chawia-Taita (site C)	$0.6 \pm 0.4$	$5.1 \pm 1.5$	$17.1 \pm 5.4$	$1.1 \pm 0.5$	21.93	3, 15	< 0.0001*
Chawia-Taita (site D)		$6.0 \pm 1.5$	$27.0 \pm 5.1$	$0.3 \pm 0.3$	22.46	2, 12	< 0.0001*
Matuu			$0.5\pm0.5$	$0.3 \pm 0.2$	0.10	1, 10	0.7491
Watamu (site A)		$0.5 \pm 0.3$	$1.7 \pm 0.7$	$0.5 \pm 0.3$	3.44	2, 15	0.1791
Jan. 2010							
Watamu (site A)		$0.5 \pm 0.3$	$1.7 \pm 0.7$	$0.5 \pm 0.3$	3.44	2, 15	0.1791
Watamu (site B)			$2.3 \pm 1.7$		n.d		
Chawia-Taita (site C)	$1.0 \pm 0.0$	$6.0 \pm 0.0$	$242.0 \pm 0.0$	$32.0 \pm 0.0$	n.d		
Chawia-Taita (site D)	$6.0 \pm 0.0$	$17.0 \pm 0.0$	$146.0 \pm 0.0$	$43.0. \pm 0.0$	n.d		
Matuu			$22.5 \pm 14.4$	$4.5 \pm 3.6$	1.25	1,6	0.2626

Table 3. Mean  $\pm$  SE and logistic regression analysis of counts of *O*. *haroldi* adults recorded in the different hive sections during surveys in different locations throughout Kenya in June 2009 and January 2010

n.d., sites for which logistic regression was not carried out.

\*Statistically significant at P = 0.05.

*O. haroldi* were detected in the honey bee colonies at most sites surveyed suggests that there is strong association between these beetles and honey bees, which may require further research. Interestingly, more *O. haroldi* males ( $\approx$ 60–100%) than females ( $\approx$ 0–40%) were found in all the colonies surveyed, which suggests that males may remain in the hive to seek females for mating, whereas after mating, the females depart to seek an oviposition site.

In our olfactometer assays, both species of beetles were attracted to volatiles released from worker honey bees and pollen dough inoculated with the yeast *K. ohmeri* associated with *A. tumida*. The composition of these volatiles have been reported in previous studies to comprise fermentation-related products, floral volatiles and honey bee alarm pheromone and their mimics (Torto et al. 2005, 2007a,b), which are known to attract nitidulids. Because *O. haroldi* responded to these volatiles, they may play a role in its host location, and thus can be exploited for use in its management, as has been done for *A. tumida* (Torto et al. 2007b; Arbogast et al. 2007, 2009ab).

In summary, we report for the first time the occurrence and distribution of *A. tumida* in honey bee colonies in three provinces of Kenya and the detection

Table 4. Pairwise comparison of *O. haroldi* adults recorded in the different hive sections of honey bee hives at different locations in Kenya in June 2009

Watamu (site A)	
Bottom vs. frame	$\chi^2 = 1.25, P = 0.2641$
Bottom vs. box	$\chi^2 = 3.63, P = 0.0569$
Frame vs. box	$\chi^2 = 8.26, P = 0.0040^*$
Chawia-Taita (site D)	
Bottom vs. frame	$\chi^2 = 25.47, P < 0.0001^*$
Bottom vs. box	$\chi^2 = 7.76, P = 0.0053^*$
Frame vs. box	$\chi^2 = 8.26, P < 0.0001^*$
Chawia-Taita (site C)	
Top vs. bottom	$\chi^2 = 9.51, P = 0.0020^*$
Top vs. frame	$\chi^2 = 24.43, P < 0.0001^*$
Top vs. box	$\chi^2 = 0.63, P = 0.4290$
Bottom vs. frame	$\chi^2 = 5.33, P = 0.0209^*$
Bottom vs. box	$\chi^2 = 6.10, P = 0.0135^*$
Frame vs. box	$\chi^2 = 20.61, P < 0.0001^*$

\*Statistically significant at P = 0.05.

of *O. haroldi* in these colonies. Both species of the beetles were strongly attracted to worker honey bee volatiles and to pollen dough inoculated with *K. ohmeri*. The information generated in this study could be used in setting up guidelines for the movement of hives across boundaries and for the management of beetles in highly infested areas in Kenya.

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