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Source: Florida Entomologist, 101(1):109-112.
Published By: Florida Entomological Society
https://doi.org/10.1653/024.101.0119
URL: http://www.bioone.org/doi/full/10.1653/024.101.0119

First record of *Blattella vaga* (Blattodea: Ectobiidae) from southern Alabama

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Abstract

The field cockroach, *Blattella vaga* Hebard (Blattodea: Ectobiidae), is native to central Asia including Afghanistan, India, Iran, Pakistan, and Sri Lanka. It was described first in 1935; however, from specimens collected in Arizona and California. Since then, the distribution of *B. vaga* has slowly increased along the southern United States and Mexican border, apparently following major interstate highways. We report the first record of *B. vaga* from Mobile, Alabama, and suggest that this species will spread to Florida and possibly northward into Georgia and South Carolina. The identification was confirmed using morphological, chemical, and molecular methods. We suggest that when possible, multiple independent methods should be used to confirm species identifications.

Key Words: Distribution; cuticular hydrocarbon profile; mitochondrial gene sequence

Resumen

La cucaracha de campo, *Blattella vaga* Hebard (Blattodea: Ectobiidae), proviene de Asia central, incluyendo Afganistán, India, Irán, Pakistán y Sri Lanka. Sin embargo, fue descrita por primera vez en 1935 a partir de especímenes recolectados en Arizona y California. Desde entonces, la distribución de *B. vaga* ha extendido lentamente a lo largo del sur de los Estados Unidos y México, aparentemente siguiendo las principales carreteras interestatales. Reportamos el primer registro de *B. vaga* en la ciudad de Mobile, Alabama y sugerimos que esta especie se extenderá a la Florida y posiblemente hacia el norte a Georgia y Carolina del Sur. La identificación se confirmó utilizando métodos morfológicos, químicos y moleculares. Sugerimos que, cuando sea posible, se utilicen múltiples métodos independientes para confirmar las identificaciones de especies.

Palabras Clave: Distribución; perfil de hidrocarburo cuticular; secuencia del gen mitocondrial

The field cockroach, *Blattella vaga* Hebard (Blattodea: Ectobiidae), was described in 1935 by Morgan Hebard from specimens collected in Arizona and California (Hebard 1935). This species resembles the other established *Blattella* spp. in North America: the Asian cockroach, *B. asahinai* Mizukubo, and the German cockroach, *B. germanica* (L.) (Atkinson et al. 1991; Appel 1995). Adults of these species are similar in length, with a pair of longitudinal stripes on their pronotum. Both *B. asahinai* and *B. vaga* live outdoors, fly to lights, and can become peridomestic pests (Helfer 1987; Atkinson et al. 1991). In contrast, *B. germanica* is almost exclusively (but see Appel & Tucker 1986) a domiciliary pest in apartments, homes, and food preparation areas (Ebeling 1978). Unlike *B. asahinai* and *B. vaga*, *B. germanica* does not fly and is repelled by light (Ebeling et al. 1966).

Since its description in 1935 from specimens collected in 1933, *B. vaga* has been reported periodically from the southern tier of the United States (Hogue 1993; Drees & Jackman 1998). Atkinson et al. (1991) reported the distribution of *B. vaga* to include the southern regions of the contiguous states of California, Nevada, New Mexico, Arizona, Texas, and Louisiana. There have been no reports of *B. vaga* from Alabama or Mississippi (Dakin & Hays 1970; Pratt 1988; GBIF Secretariat 2017). The distribution pattern of this species appears to overlap major interstate highways such as I-10 (east from southern California through Arizona, New Mexico, Texas, and Louisiana) and I-40 (east from southern California through Arizona, New Mexico, and Texas) (GBIF Secretariat 2017). Because there are many distribution records along I-10, it would not be surprising to find *B. vaga* near that interstate considering the proximity of its eastern-most record in Louisiana to Mobile, Alabama. Austin et al. (2007) reported infestations of the closely related *B. asahinai* in Texas also near major highways. Similarly, Snoddy and Appel (2008) concluded that distributions of *B. asahinai* in Alabama and Georgia followed major interstates northward from Florida.

The objective of this study was to determine the identity of cockroaches from Alabama that we hypothesized could be *B. vaga*, and a range extension for this species. We used several independent methods to determine the identification of this species and offer several predictions of new distributions.

Materials and Methods

SPECIMEN COLLECTION AND REARING

Cockroaches were collected on 15 Mar 2016 near Cedar Point Fishing Pier, Mobile County, Mobile, Alabama (30.3103°N, 88.1383°W). A total of 11 specimens (third instar through terminal instar) were collected by hand from under wood and in surrounding grass; the soil was a moist

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dark clay. Specimens were reared at room temperature and ambient photoperiod in a 0.47 L jar with moist Eco Earth™ (Zoo Med Laboratories, Inc., San Luis Obispo, California, USA) shredded coconut fiber substrate and leaves from various deciduous trees. Cockroaches were fed dry Purina Dog Chow® (Nestlé Purina, St. Louis, Missouri, USA) and pieces of fresh carrot. The jar was lightly misted with water weekly.

A colony of *B. vaga* that had been obtained from the University of California, Riverside in 1985 and reared continuously at Auburn University was used to compare with wild-caught specimens. The Auburn colony was reared in 3.8 L glass jars at 28 ± 2 °C, 40-55% RH, and a photoperiod of 12:12 h (L:D). Colonies were provided corrugated cardboard for cockroach harborage, dry dog food, and clean water. Colonies were fed, supplied water, and cleaned weekly.

MORPHOLOGICAL EXAMINATION

Adult male specimens were killed by a brief exposure to hydrogen cyanide gas. Tergal glands and genitalia were prepared for examination as described by Roth (1985). The abdomens were removed from the rest of the body briefly and placed in 10% KOH at 37 °C for 24 h to loosen and remove internal tissues. Abdomens then were washed in water, dehydrated in a series of increasingly concentrated ethyl alcohol solutions, and cleared in xylol. Abdomens were dissected to remove the tergites with tergal glands and to separate the genitalia. Tergal glands, subgenital and supraanal plates, and the genitalia were mounted on glass slides with Permount™ (Fisher Scientific, Fair Lawn, New Jersey, USA).

Intact male cockroaches and slide-mounted material were examined with a dissecting microscope at 10–100X. Morphological characters were used to follow the key to *Blattella* spp. presented in Roth (1985).

CUTICULAR HYDROCARBON ANALYSIS

Adult male cockroaches were killed by freezing at –20 °C for 2 h. Cuticular hydrocarbons were extracted by immersing a single dead, thawed male cockroach in 0.5 ml of hexane under laboratory conditions for 15 min. Hexane extracts were evaporated to dryness under a gentle steam of nitrogen and re-dissolved in 250 µL of hexane solvent to achieve equivalent concentration of cuticular hydrocarbons among the samples. Extracts then were analyzed using an Agilent 7890A Gas Chromatograph (Agilent Technology, Santa Clara, California, USA) coupled to a 5975C Mass Selective Detector, with an HP-5ms capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Mass spectra were obtained using electron impact (EI, 70 eV). One µL of each extract was injected into the gas chromatograph-mass selective detector in splitless injection mode. The gas chromatograph oven temperature was programmed from 40 °C for 1 min to 280 °C at 20 °C per min, and then the temperature was ramped at 1 °C per min to 320 °C and held for 1 min. The injector and transfer line temperatures were set at 300 and 320 °C, respectively. The patterns of cuticular hydrocarbon components of wild (unknown) specimens were compared with a laboratory colony of *B. vaga* (known) using their mass spectra, retention indices (Kováts index) (NIST 2005 library search software version 2.0, National Institute of Standards and Technology, Gaithersburg, Maryland, USA), and the mass spectra previously reported for *B. vaga* (Carlson & Brenner 1988).

DNA EXTRACTION AND PCR AMPLIFICATION

Blattella vaga DNA was isolated from fresh tissue using EZNA SP Plant DNA kit (OMEGA Bio-tek, Norcross, Georgia, USA) following the manufacturer's protocol. The extraction process included homogenizing several freshly removed legs in 200 µl of lysis buffer (100 mM NaCl, 100 mM EDTA, 100 mM Tris, and 0.5% SDS, at pH 7.0) followed by incubation steps after addition of proteinase K (4 ml of 20 mg per ml) at 55 °C for 3 h followed by RNAase (2 ml of 10 mg per ml) at 37 °C for 20 min. DNA pellets were precipitated in 100% ethanol and stored overnight at –20 °C. Finally, the DNA was washed in 70% ethanol, centrifuged, and vacuum dried. DNA then was suspended in 100 ml of TE buffer (10 mM Tris, pH 8.0, and 1 mM EDTA) and stored at –20 °C.

PCR amplification was performed with a programmable thermal controller (SureCycler 8800, Agilent Technology, Santa Clara, California, USA). Composition of the reaction mixture (50 µl) used for amplification was 28.5 µl water, 5µl 10X PCR buffer II, 800 mM dNTP mix, 0.8 mM of each primer, 2.5 U AmpliTaq DNA polymerase, 2 µl DNA, and 3 mM $MgCl_2$.

The central portion of the mitochondrial CO1 gene was amplified using the primer pairs C1-J-1718 (5′-GGAGGATTTGGAAATTGATTAGTTCC-3′) and C1-N-2191 (5′-CCCGGTAAAATTAAAATATAAACTTC-3′) (Pachamuthu et al. 2000). Amplification was performed using the following temperature profile: 94 °C for 2 min, 35 cycles of 94 °C for 45 s, 48 °C for 1 min, and 72 °C for 1.5 min. After a final extension step at 72 °C for 5 min, PCR products were stored at 4 °C.

PCR products were isolated by loading the PCR product onto a 1.0% agarose TAE gel containing ethidium bromide (0.6 mg per ml), and DNA fragments were purified by E.Z.N.A. Gel Extraction Kit (OMEGA Bio-tek, Norcross, Georgia, USA) using the manufacturer's protocol.

Capillary sequencing was conducted using an Applied Biosystems 3130 Genetic Analyzer (Fisher Scientific, Fair Lawn, New Jersey, USA) at the Genomics and Sequencing Laboratory, Auburn University, Alabama. Sequences were aligned using NCBI's nucleotide blast program.

Results

All adult males were unambiguously identified as *B. vaga* (couplet 16) using Roth's species key to the genus *Blattella* (Roth 1985). Diagnostic characteristics of adult males included similar size non-spine-like styles on the subgenital plate (Fig. 1A), only abdominal tergite 7 uniquely modified (Fig. 1B), and genital phallomere L2d present and flattened (Fig. 1C) (Roth 1985). Additional adult characteristics included wide longitudinal stripes on the pronotum and the area between the eyes darkly colored (Buxton & Freeman 1968). Nymphal characteristics included an overall yellowish coloration with longitudinal brown stripes. All morphological characters of the field-collected and reared specimens matched those of colony specimens.

Cuticular hydrocarbon analysis showed almost identical chromatograms for field-collected specimens and the laboratory colony of *B. vaga* (Fig. 2). The individual retention times of all 14 major cuticular hydrocarbon components were the same for the field-collected specimens and the laboratory colony, indicating a probable match.

Using GenBank data Sequence ID: AF228735.1 as a comparison, there was 98% similarity (Fig. 3) between the partial sequence of the mitochondrial cytochrome c oxidase subunit I-like protein gene from the field-collected cockroach and the published sequence for *B. vaga*, indicating a very high probability of a match.

Discussion

Based on morphological, molecular, and cuticular hydrocarbon analysis, we have identified the cockroaches collected from Mobile, Alabama, as the field cockroach, *Blattella vaga* Hebard. To the best of our knowledge, this is the first report of *B. vaga* collected from Alabama. As with most, if not all, reports of range extensions, it is possible

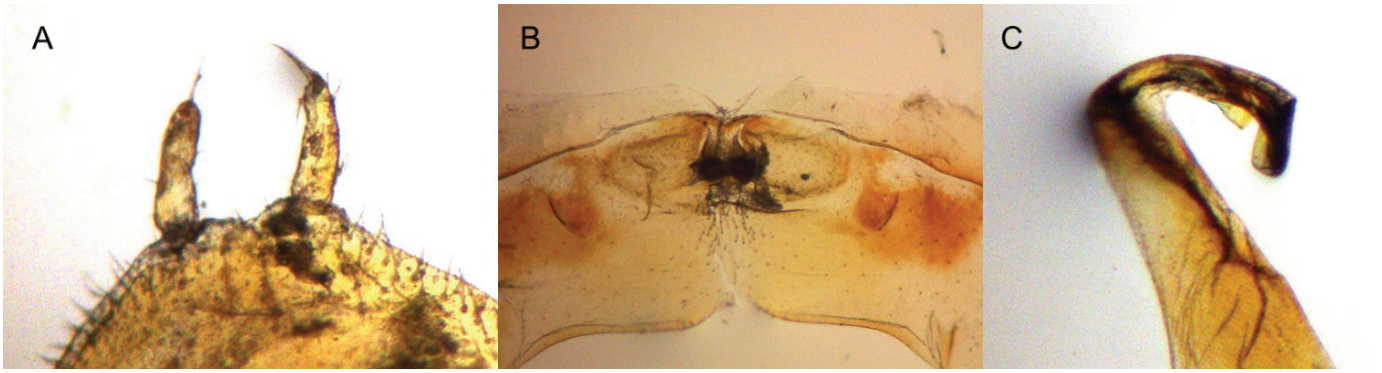

Fig. 1. Adult male *Blattella vaga* from field-collected colony. (A) Subgenital plate showing 2 non-spinelike styles; (B) abdominal tergite 7 with modification; (C) genital phallomere L2d.

those populations of *B. vaga* have existed in Alabama before 2016 and that populations may be distributed widely in southern Alabama.

There was a very high (98%) similarity in the partial gene sequence between the field-collected and published sequence for *B. vaga*. Even though the sequence similarity was not 100%, it is likely that differences in individual bases are not significant and result in coding for the same amino acids and ultimately proteins (Griffiths et al. 2008). There is no generally agreed upon percentage DNA sequence similarity for separation of insect species (Cognato 2006), although the small difference (2%) is certainly suggestive of a positive match.

Cuticular hydrocarbons are important for resistance to desiccation (Hadley 1981) and have been identified as pheromones (Blomquist 2010). Cuticular hydrocarbon analysis showed identical retention times for the 14 major components (Fig. 2), indicating the presence of the same hydrocarbons from field-collected and laboratory-reared *B. vaga*. Furthermore, our results are consistent with gas chromatographic profiles and mass spectra previously reported for *B. vaga* (Carlson & Brenner 1988).There were, however, quantitative differences between the 2 groups. Several studies have demonstrated quantitative differences in cuticular hydrocarbon profiles due to abiotic and biotic factors. Exposure to different temperatures (Gibbs et al. 1998) and consumption of different diets (Liang & Silverman 2000) can affect cuticular hydrocarbon profiles.

Several factors probably facilitated movement of *B. vaga* from Arizona and southern California to Alabama. Adults of both sexes readily fly during the photophase and are not repelled by light, behaviors

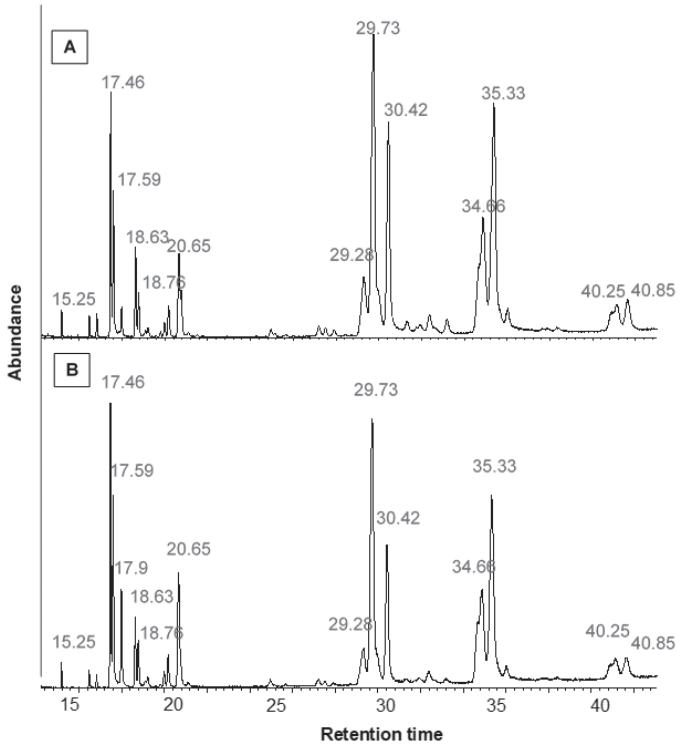

Fig. 2. Gas chromatography traces of cuticular hydrocarbons of (A) a male unknown field-collected cockroach, and (B) a male *Blattella vaga* from a laboratory colony. Numbers near peaks represent retention times.

Score	Expect	Identities	Gaps
809 bits(438)	0.0	460/470(98%)	5/470(1%)

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Query  8    CCAGATATAGCATTTCCCCGAATAAATAATATAAGTTTTCTCCGACTATTACCACCTTCA  67
            ||||||||||||||||||||||||||||||||||||||||||| ||  ||||||||||||||||||
Sbjct  1    CCAGATATAGCATTTCCCCGAATAAATAATATAAGTTT-CT--GACTATTACCACCTTCA  57

Query  68   TTATCACTTTTATTGGCTAGTAGTCTGGTTGAAAGAGGTGCTGGAACAGGATGAACTGTA  127
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  58   TTATCACTTTTATTGGCTAGTAGTCTGGTTGAAAGAGGTGCTGGAACAGGATGAACTGTA  117

Query  128  TATCCACCTCTAGCAAGAGGTATTGCTCATGCTGGTGCATCAGTTGATTTAGCAATTTTT  187
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  118  TATCCACCTCTAGCAAGAGGTATTGCTCATGCTGGTGCATCAGTTGATTTAGCAATTTTT  177

Query  188  TCCTTACATTTAGCAGGAGTTTCATCAATTTTAGGTGCTGTTAATTTTATTTCAACAATT  247
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  178  TCCTTACATTTAGCAGGAGTTTCATCAATTTTAGGTGCTGTTAATTTTATTTCAACAATT  237

Query  248  ATTAATATAAAACCTATTAACATAAAACCTGAACGGATTCCCCTTTTTGTATGATCTGTA  307
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  238  ATTAATATAAAACCTATTAACATAAAACCTGAACGGATTCCCCTTTTTGTATGATCTGTA  297

Query  308  GGTATTACAGCTTTATTACTTTTATTATCATTACCTGTATTAGCAGGGACGCTATTACAA  367
            ||||||||||||||||||||||||||||||||||||||||||||||||||  ||||||||||
Sbjct  298  GGTATTACAGCTTTATTACTTTTATTATCATTACCTGTATTAGCAGGG--GCTATTACAA  355

Query  368  TATTATTAACTGATCGTAATTTAAATACATCATTCTTTGATCCTGCTGGTGGTGGTGATC  427
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  356  TATTATTAACTGATCGTAATTTAAATACATCATTCTTTGATCCTGCTGGTGGTGGTGATC  415

Query  428  CAATTTTATATCAACATTTATTCTGATTCTTTGGTCATCCAGAAGTTTAT  477
            |||||||||||||||||||||||||||  |||||||||| |  |||| |||
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Fig. 3. *Blattella vaga* cytochrome c oxidase subunit I-like protein gene, partial sequence; mitochondrial gene for mitochondrial product. Using GenBank data Sequence ID: AF228735.1, Length: 1,235, there was one 98% match confirming that the sequence for the wild-caught male cockroach matched the published sequence for *B. vaga*.

similar to *B. asahinai*, an invasive species that also has extended its range from 1 city in Florida to the entire state and northward into at least Alabama, Georgia, North Carolina, and South Carolina (Snoddy & Appel 2008). Rather than long directional flights, both *B. asahinai* and *B. vaga* tend to take short (0.5–5 m) flights and change direction from 1 flight to the next (Appel & Snoddy personal observations). We believe that these behaviors contribute to *B. vaga* flying onto vehicles and being transported to new locations. In addition, it is likely that *B. vaga*-infested potted plants, sod, waste, or other materials could be transported along interstate highways by commercial trucking.

Physiologically, *B. vaga* is relatively resistant to desiccation (Appel et al. 1983), which would allow it to survive exposure to hot and dry climates, as well as desiccation from exposure to moving air. Like *B. asahinai* (Snoddy 2007), this species avoids extreme conditions (hot and dry, and cold) by burrowing into a substrate.

In conclusion, we have used 3 independent methods (morphological, molecular, and chemical) that confirmed the identification of *B. vaga* from specimens collected in Mobile, Alabama. Because of its behavior, physiology, and distribution pattern along major interstate highways, it is likely this species will become established elsewhere in the southeastern US.

Acknowledgments

We thank Xing Ping Hu, Nannan Liu, and Charles H. Ray (Auburn University) for reviewing the manuscript and Marla J. Eva for technical support. This work was supported in part by the USDA National Institute of Food and Agriculture, AAES Hatch/Multistate Grants ALA-08-058 to AGA and ALA-08-044 to HYF.

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