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CONFIRMATION OF ASIAN COCKROACH *BLATELLA ASAHINAI* (BLATTODEA: BLATTELIDAE) INTRODUCTION TO TEXAS BASED ON GENETICS, MORPHOLOGY, AND BEHAVIOR

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Thought to be native to Japan based on the original description of specimens collected from Okinawa, Japan (Brenner et al. 1988), the Asian cockroach *Blattella asahinai* Mizukubo is now found in several southeastern states. Among these are Florida, portions of southern Georgia, southwestern Alabama, and Charleston County, South Carolina (Hu et al. 2005). The potential for the spread of this species to other southern U.S. States has been proposed (Atkinson et al. 1991). Interstate highways are now proving to provide major introduction routes throughout the southeast U.S. (E. Snoddy, pers. comm.). While populations of the Asian Cockroach have been proposed to occur in western Harris County, Texas (Tucker 2006) there has been no confirmation of this in literature nor have any samples been submitted for confirmation to Texas A&M University for independent verification. Furthermore, because of the close similarity with other blattelid species from the region species confirmation is crucial. Some populations have been described as occurring near the Barker Reservoir (in Western Harris County) in both residential and undeveloped areas. Additional populations were discovered by pest control technicians in a residential area (Hunters Creek Village subdivision of Houston, ~20 km east of the Barker Reservoir area) just west of Loop 610 Freeway near Voss Road. These populations were located in and near homes adjacent to Buffalo Bayou along the I-10 corridor, a possible introduction pathway to these communities. This is the first attempt to confirm species identity of this newly established exotic cockroach to Texas applying molecular, morphological, and ethological characters. Confirmation of this species in Texas demonstrates yet another invasive species which may have negative consequences for the areas where they have become established.

Introduction of exotic Asian cockroach *Blattella asahinai* Mizukubo to the United States was first described in Lakeland, Florida in 1986 (Brenner et al. 1988; Roth 1986). Because of their overall similarity, original populations were thought to be German cockroaches *Blattella germanica* (L.). Likewise, another congener of Asian origin, the field cockroach *Blattella vega* Hebard is sometimes confused with *B. asahinai* and *B. germanica*. When the ability to observe these species in nature is eliminated, it can be difficult to discern them from preserved specimens, and identification has been based on cuticular hydrocarbon methods (Carlson & Brenner 1988) and molecular diagnostics (Pachamuthu et al. 2000). Herein, we apply ethological, morphological, and genetic approaches to confirm the presence of *B. asahinai* in Harris County, Texas.

Field collected samples were preserved in 100% ethanol and voucher specimens were deposited at the Center for Urban & Structural Entomology at Texas A&M University. Geographic positions were recorded for samples subjected to DNA analysis with a Garmin Vista C GPS unit (Garmin International, Inc., Olathe, Kansas). Coordinates for both locations in Hunter's Creek Village were 29°46'11.06"N, 95°28'21.22"W near Point Broad Oaks Circle, and 29°46'01.12"N, 95°28'00.82"W near Bayou Timber. Additional sites have been found west of these locations and more are being evaluated at the present time. Morphological and ethological identification through comparisons of *B. asahinai* with other *Blattella* congeners was evaluated in accordance with Lawless (1999), Brenner et al. (1988), Ross & Mullins (1988), and Richman (2000). Extraction and purification of genomic DNA was performed with Qiagen DNEasy tissue kits (Qiagen, Inc., Valencia, CA) according to manufacturers' recommendations. PCR amplification of the nuclear rRNA ITS1 region was performed following Szalanski & Owens (2003) by using the primers rDNA2 (5'-TTGATTACGTCCCTGCCCTTT-3') described by Vrain et al. (1992), and primer rDNA1.58s (5'-GCCACCTAGTGAGCCGAGCA-3') by Cherry et al. (1997). These primers amplify a 3' portion of the 18S gene, the entire ITS1 region, and a 5' section of the 5.8S gene. The ITS1 PCR protocol was 40 cycles at 94°C for 45 s, 54°C for 45 s and 72°C for 60 s. Amplified DNA from individual cockroaches was purified and concentrated with minicolumns according to the manufacturers instructions (Wizard PCRpreps, Promega, Madison, WI). Samples were sent to the University of Arkansas Medical School DNA Sequencing Facility, Little Rock, AR, for direct sequencing in both directions. Consensus sequences

for each sample were obtained and aligned with Clustal W (Thompson et al. 1994) with Bioedit 5.09 software (Hall 1999). One genotype of *B. asahinai* was found and the sequence was submitted to GenBank (Accession number EF583620). A BLAST (Biological Local Alignment Tool) (Altschul et al. 1990) search for similar nucleotide sequences or BLASTN (version 2.2.16, Mar 25 2007) was conducted to compare our sequence to GenBank DNA sequences to confirm species identity. From a 776-bp fragment of the ITS1 nuclear rRNA marker, 98% sequence similarity (Max score = 1358, Query coverage = 100%, E-value = 0.0) was obtained from a *B. asahinai* partial sequence (Accession number AF321253) previously submitted by Mukha et al. (2002) from lab cultures maintained at North Carolina State University. While the relationship to *B. germanica* is close (see GenBank accessions AF005243 and AF321244), the overall total score (1358 bits or 765/780 = 98%) is most similar to *B. asahinai*, and as such provides genetic confirmation of its identity.

The main morphological differences between the Asian and German cockroaches are the shape of a groove in one segment (eighth) of the abdomen, and a small tergal gland in the males (Roth 1986). Structural differences in adults of the 2 species were observed, as outlined by Mizukubo (1981) that noted differences in the male and female genitalia, male tergites VII and VIII, subgenital plate, and the length of the last abdominal segment, and differences in the measurements of the forewings (Ross 1990). The wings of the Asian cockroach are usually longer and narrower than those of the German cockroach. They extend beyond the tip of the abdomen and cover the egg capsule in the females. The Asian cockroach has a fairly comparable reproductive capacity to German cockroaches (Ross & Mullins 1988; Atkinson et al. 1991). Asian cockroaches are generally lighter in color than most German cockroaches. Similarly, it is the color and size of *Blattella vega* which allow one to distinguish them in the field from either *B. asahinai* or *B. germanica*. Flock (1941) states that they are differentiated from *B. germanica* "by the blackish/brown area on the face from the mouthparts to between the eyes… that the species is slightly smaller and olivaceous in coloration than the German cockroach." Additional morphological differences have been described between the species (Lawless 1999) with 5 morphological differences between 4 body parts in the adults of the 2 species. These include setal patterns on the ventral surface of the left mandible of adult females (continuous line of setae underneath the teeth on the ventral surface in ♀ *B. germanica versus* discontinuous line of setae for the same character for ♀ *B. asahinai*), vein coloration of the right tegmen (*B. germanica* ♀ right tegmen dark colored lower 3rd of the tegmen along the median edge *versus B. asahinai* ♀ dark colored lower half of the tegmen along the median edge), wing size (*B. germanica* hind-wing width is 4.00-5.40 mm at widest part of wing versus 5.70-6.79 mm for *B. asahinai*), wing venation (*B. germanica* ♂ hindwing with 16 veins (range 16-18) in the lower half of the wing *versus* 22 veins (range 20-22) for *B. asahinai* ♂), and setal differences in the marginal bristles on the cerci (2 setae of the marginal bristles of the ♀ cerci of *B. asahinai* are more delicate and shorter than those of *B. germanica*).

Ethologically, *B. asahinai* is active both at day and night and is an accomplished flier, living in outdoor habitats, both feral and peridomestic, preferring leaf litter and grassy areas. Asian cockroaches are strong fliers and are attracted to light (Benson & Zungoli 1997), known to fly up to 120 feet (or more), and establish population densities ranging from 30,000 to 250,000 per acre (Hu et al. 2005). In contrast, the German cockroach is a poor flier and lives primarily indoors; *B. vega*, while living primarily outdoors is not a good flier, not gregarious, rarely enters buildings, and actually avoids others of its own species in behavioral assays (Rust & Appel 1985). In our collection of these specimens, *B. asahinai* were observed flying around exterior lights and purposefully entering homes, newly constructed and well-established, that backed up to the Buffalo Bayou. They were present in large numbers (hundreds of roaches) around windows and doors and observed flying away when attempts were made to power spray the exterior of these residences for control (G. J. Glenn, pers. observation).

A discrete species, occupying a basal monophyletic cluster (or clade) which is diagnosably distinct and irreducible, demonstrating a parental pattern of ancestry and descent (Cracraft 1989; Wheeler & Nixon 1990), is the basis of our cladistic species identification in this occurrence. Application of the nuclear rRNA ITS region has been useful in determining the phylogeny of *Blattella* species (Mukha et al. 2002). Our evaluations of ITS1 DNA sequences confirm the presence of *B. asahinai* in Harris County, Texas, whereas Tucker (2006) did not independently confirm species identity to known voucher material. The BLAST search revealed 98% sequence similarity between our *B. asahinai* samples and a *B. asahinai* GenBank sequence (Accession number AF321253). We positively confirm the identity of this species to Texas on the basis of ethological, morphological, and genetic evaluations. It is unclear what ecological impact this species will have in Texas at this point or whether it will spread to adjacent counties, but given its establishment success, it will probably be considered a pest species in the future.

Summary

Introduction of exotic Asian cockroach *Blattella asahinai* Mizukubo to Texas was discovered in Harris County, Texas in May 2006 around the

Barker Reservoir (in western Harris County) in both urban and undeveloped areas and later in Hunter's Creek Village subdivision of Houston. Morphological, ethological, and genetic comparisons confirm the identity of the species which is frequently misidentified as either the German cockroach *Blattella germanica* (L.) or the field cockroach *Blattella vega* Hebard. Evaluation of ITS1 DNA gene sequences resulted in BLAST scores >98% sequence similarity with *Blattella asahinai*. Ethological and morphological characteristics of this species should allow its identification by pest management professionals (PMPs) in the field. Given the ability of this pest to fly up to 120 feet and establish population densities ranging from 30,000 to 250,000 per acre, this species will likely become a new pest that Texas PMPs will have to consider when developing exterior preventive measures for their customers. It is unclear what ecological impact this species will have in Texas as it continues to spread to adjacent counties.

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