Molecular Identification of Aedes bahamensis (Diptera: Culicidae)

Authors: Byrd, Brian D., Gymburch, Erin E., O'Meara, George F., and Wesson, Dawn M.

Source: Florida Entomologist, 94(4) : 1057-1059

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.094.0446

MOLECULAR IDENTIFICATION OF *AEDES BAHAMENSIS* (DIPTERA: CULICIDAE)

BRIAN D. BYRD[1], ERIN E. GYMBURCH[1], GEORGE F. O'MEARA[2] AND DAWN M. WESSON[3]
[1]Western Carolina University, Environmental Health Sciences Program, College of Health and Human Sciences, Cullowhee, NC 28723, USA

[2]University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory, Vero Beach, FL 32962, USA

[3]Tulane University, School of Public Health and Tropical Medicine, Department of Tropical Medicine, New Orleans, LA 70112, USA

Aedes (Howardina) bahamensis Berlin (or *Howardina bahamensis* of Reinert et al. 2004) is an exotic species first detected in the United States in 1986 from eggs and adult females collected in Miami-Dade and Broward counties, Florida (Berlin 1969; Pafume et al. 1988; O'Meara et al. 1989). In the U.S., immature *Ae. bahamensis* are chiefly found in artificial containers (e.g., abandoned tires, cemetery vases, etc.) and exotic tank bromeliads (O'Meara et al. 1995a). Although *Ae. bahamensis* readily feeds on humans, it is not a major pest or disease vector. However, the immature stages are frequently found in habitats that are also known to harbor 2 medically important mosquito species: *Ae. albopictus* (Skuse) and *Ae. aegypti* (L.) (O'Meara et al. 1995b; Lounibos et al. 2010). Morphological differences between these 3 species provide useful characters that readily distinguish the egg, larval, and adult life stages (Linley 1989; Darsie & Ward 2005). Nevertheless, a molecular method to identify *Ae. bahamensis* would be useful to confirm the identity of damaged adult specimens collected in fan-based traps and would allow rapid identification of the species at any life stage. Here we report the rDNA second internal transcribed spacer (ITS2) sequence and a species-specific PCR primer for the identification of *Ae. bahamensis*.

Specimens of *Aedes bahamensis* were obtained from colony material maintained by GFO at the Florida Medical Entomology Laboratory (Vero Beach, Florida) and field samples collected by BDB. Field collected specimens were identified using key characters described by Darsie (1992). Total DNA was obtained from adult specimens (8 colony and 14 field) using the Qiagen DNeasy kit (Qiagen, Valencia, California) or the DNAzol reagent (Molecular Research Center, Inc., Cincinnati, Ohio) per the manufacturers' instructions. The resulting extractions were PCR amplified in 50 µL reactions using the Invitrogen PCR Supermix (Invitrogen, Carsbad, California). Each reaction mixture contained 3 µLs of DNA template (4-35 ng/µL), 1 µL of each forward and reverse primer (200 nM final concentration), and 45 µL of the PCR supermix. Amplification cycling conditions were 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, 54 °C for 30 s and 72 °C for 1 min. The CP-P1A/P1B primer pair was used to amplify the complete ITS2 (Fig. 1) (Wesson et al. 1992). A negative control (H_2O *en lieu* of DNA template) was included in each run. PCR amplicons were visualized on a 1.5% agarose gel. There were no obvious intraspecific amplicon size polymorphisms. The PCR products were gel purified with the Qiaquick Gel Extraction kit (Qiagen) and subsequently cloned into the pCR 2.1 TOPO vector (Invitrogen). Purified plasmids were obtained using the Promega Wizard Plus SV miniprep kit (Promega, Madison, WI) and then sequenced (n = 10) using the Applied Biosystems (Carlsbad, CA) Big Dye Terminator V3.0 chemistry by the Davis Sequencing Facility, University of California (Davis, California). The sequences were verified as ITS2 after evaluating the results of an NCBI BLAST query, secondary structure analysis, and the identification of specific sequence motifs known to exist on the ITS2 of mosquitoes (Coleman 2007). Novel ITS2 sequences, partial 5.8S, and partial 28S sequences for *Aedes bahamensis* were annotated and representative samples were submitted to the NCBI GenBank (Accession numbers: JN020552, JN020553) (Keller et al. 2009).

The CP-P1A/P1B primer pair produces a 380 base pair (bp) amplicon for *Ae. bahamensis* that differs from the amplicons of *Ae. albopictus* (600 bp), *Ae. aegypti* (360 bp), and *Ae. triseriatus* (Say)

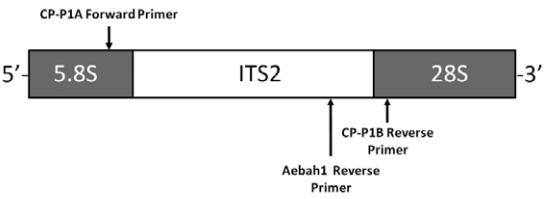

Fig. 1. *Aedes bahamensis* partial 5.8S, complete ITS2, and partial 28S structure and primer annealing positions. The CP-P1A primer (5'- gtggatcctgtgaactgcaggacacatg-3') anneals in the 5.8S region, the CP-P1B reverse primer (5'- gtgtcgacatgcttaaatttaggggta-3') anneals in the 28S region, and the species-specific reverse primer (5'-aacatagccacggtggtatc-3') anneals within the ITS2.

(385 bp) using the same primer pairs (Fig. 2). These four species are sympatric in southern Florida where the distribution of *Ae. bahamensis* remains limited to Miami-Dade and Broward counties. Because the CP-P1A/P1B size polymorphisms may not be readily distinguished between *Ae. bahamensis* and *Ae. aegypti* or *Ae. triseriatus*, a species-specific reverse primer was designed. In order to identify a suitable region to design an *Ae. bahamensis* specific primer, a multiple sequence alignment was created using Culicidae rDNA ITS2 sequences obtained from GenBank and the novel sequences obtained in this study. A species-specific primer (Aebah1: 5'-aacatagccacggtggtatc-3') was then designed using Primer3 to produce a 300 bp amplicon when used with the CP-P1A forward primer (Fig. 2) (Rozen & Skaletsky 2000). The PCR amplification cycling conditions for this primer pair are identical to the CP-P1A/P1B conditions reported above. The CP-P1A/Aebah1 primer pair will not amplify the sympatric container-inhabiting *Aedes* (i.e., *Ae. albopictus*, *Ae. aegypti*, or *Ae. triseriatus*) (Fig. 2) or other container inhabiting *Aedes* mosquitoes [e.g., *Ae. hendersoni* (Cockerell), *Ae. atropalpus* (Coquillett) and *Ae. japonicus* (Theobald)] found in some areas of the southeastern United States (data not shown).

To our knowledge, the work described here represents the first PCR based method for the rapid molecular identification of *Aedes bahamensis*. Such assays are particularly useful to confirm the identity of a specimen when key morphological characters become damaged. PCR based assays may be used on genomic DNA obtained from an organism at any life stage. Similarly, species-specific PCR assays may be used to validate the integrity of pooled mosquito specimens and confirm the presence of heterospecies contamination (Gerhardt et al. 2001).

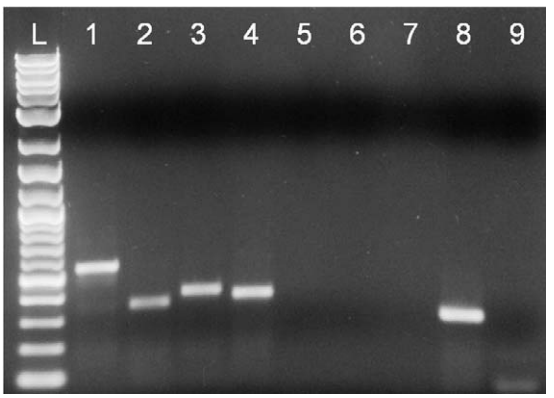

Fig. 2. Gel electrophoresis image of CP-P1A/P1B amplicons for *Ae. albopictus* [Lane 1: 600 bp], *Ae. aegypti* [Lane 2: 365 bp], *Ae. triseriatus* [Lane 3: 385 bp], and *Ae. bahamensis* [Lane 4: 380 bp]. CP-P1A/Aebah1 primer pair does not amplify *Ae. albopictus* [Lane 5], *Ae. aegypti* [Lane 6] or *Ae. triseriatus* [Lane 7]. The CP1-1A/Aebah1 primer pair only amplifies *Ae. bahamensis* [Lane 8: 300 bp]. Negative control [Lane 9]. The molecular standard (L) is the 2-log DNA ladder (New England Biolabs, Ipswich, MA).

We thank Bruce Harrison from the Public Health Pest Management Program, North Carolina Department of Environment and Natural Resources for his thoughtful review and comments. This work was partially supported by the Tulane University Research Enhancement Fund and the Centers for Disease Control Cooperative Agreement T01/CCT622308. Ms. Erin Gymburch is an undergraduate research student supported by the Western Carolina University College of Health and Human Sciences Summer Research Program.

Summary

Here we report novel rDNA ITS2 sequences for *Aedes bahamensis* and a species-specific PCR primer that produces a diagnostic 300 bp PCR product. This technique may be used to confirm the identity of damaged or degraded specimens that may not be readily identifiable using morphological characteristics.

References Cited

Berlin, O. G. W. 1969. Mosquito studies (Diptera, Culicidae) XII. A revision of the neotropical subgenus *Howardina* of *Aedes*. Contrib. Am. Entomol. Inst. (Ann Arbor) 4 (2): 1-190.

Coleman, A. W. 2007. Pan-eukaryote ITS2 homologies revealed by RNA secondary structure. Nucleic Acids Res. 35(10): 3322-9.

Darsie, R. F., Jr. 1992. Key characters for identifying *Aedes bahamensis* and *Aedes albopictus* in North America, north of Mexico. J. Am. Mosq. Control Assoc. 8(3): 323-4.

Darsie, R. F., Jr., and Ward, R. A. 2005. Identification and Geographic Distribution of the Mosquitoes of North America, North of Mexico. University Press of Florida, Gainesville. 384 pp.

Gerhardt, R. R., Gottfried, K. L., Apperson, C. S., Davis, B. S., Erwin, P. C., Smith, A. B., Panella, N. A., Powell, E. E., and Nasci, R. S. 2001. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. Emerg. Infect. Dis. 7(5): 807-11.

Keller, A., Schleicher, T., Schultz, J., Muller, T., Dandekar, T., and Wolf, M. 2009. 5.8S-28S rRNA interaction and HMM-based ITS2 annotation. Gene 430(1-2): 50-7.

Linley, J. R. 1989. Comparative fine structure of the eggs of *Aedes albopictus*, *Ae. aegypti*, and *Ae. bahamensis* (Diptera: Culicidae). J. Med. Entomol. 26(6): 510-21.

Lounibos, L. P., O'Meara, G. F., Juliano, S. A., Nishimura, N., Escher, R. L., Reiskind, M. H., Cutwa, M., and Greene, K. 2010. Differential survivorship of invasive mosquito species in south florida cemeteries: Do site-specific microclimates explain pat-

terns of coexistence and exclusion? Ann. Entomol. Soc. Am. 103(5): 757-770.

O'MEARA, G. F., EVANS, L. F., JR., GETTMAN, A. D., AND CUDA, J. P. 1995b. Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera: Culicidae) in Florida. J. Med. Entomol. 32(4): 554-62.

O'MEARA, G. F., EVANS, L. F., JR., GETTMAN, A. D., AND PATTESON, A. W. 1995a. Exotic Tank Bromeliads Harboring Immature *Aedes albopictus* and *Aedes bahamensis* (Diptera: Culicidae) in Florida. J. Vector Ecol. 20(2): 216-224.

O'MEARA, G. F., LARSON, V. L., MOOK, D. H., AND LATHAM, M. D. 1989. *Aedes bahamensis*: its invasion of south Florida and association with *Aedes aegypti*. J. Am. Mosq. Control Assoc. 5(1): 1-5.

PAFUME, B. A., CAMPOS, E. G., FRANCY, D. B., PEYTON, E. L., DAVIS, A. N., AND NELMS, M. 1988. Discovery of *Aedes (Howardina) bahamensis* in the United States. J. Am. Mosq. Control Assoc. 4(3): 380.

REINERT, J. F., HARBACH, R. E., AND KITCHING, I. J. 2004. Phylogeny and classification of the Aedini (Diptera: Culicidae) based on morphological characters of all life stages. Zool. J. Linn. Soc. 142: 289-368.

ROZEN, S., AND SKALETSKY, H. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. 132: 365-86.

WESSON, D. M., PORTER, C. H., AND COLLINS, F. H. 1992. Sequence and secondary structure comparisons of ITS rDNA in mosquitoes (Diptera: Culicidae). Mol. Phylogenet. Evol. 1(4): 253-69.