

Molecular Identification of *Aedes atlanticus* and *Aedes tormentor*

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Overview

- Background (*Ae. atlanticus* and *Ae. tormentor*)
- Morphological Characters (Harrison)
- Molecular Methods
- Sequence Analysis
- Next Steps

Background

- *Aedes atlanticus* and *Ae. tormentor* are almost indistinguishable as adult females
- Novel morphological characters have been discovered by Dr. B. Harrison
- Isolated Viruses:
 - West Nile
 - Eastern Equine Encephalomyelitis
 - Keystone



Photo Credit: A. Anderson



Division of Vector-Borne Infectious Diseases

West Nile Virus



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- PDF (254 KB/77 pages)

Entomology

[Identification guide to medically important mosquitoes of Louisiana](#)

64 Mosquito species have been found in West Nile positive* mosquito pools in the United States since 1999.

* from which West Nile virus isolated, West Nile RNA detected, or West Nile antigen detected using a variety of diagnostic tests.

Data were obtained by CDC field investigations or were reported by state surveillance programs to ArboNET as of 04/09/2009.

Mosquito Species producing WNV positives by year

[1999](#) [2000](#) [2001](#) [2002](#) [2003](#) [2004](#) [2005](#) [2006](#) [2007](#) [2008](#)

Total list - all years

Mosquito Species producing WNV positives

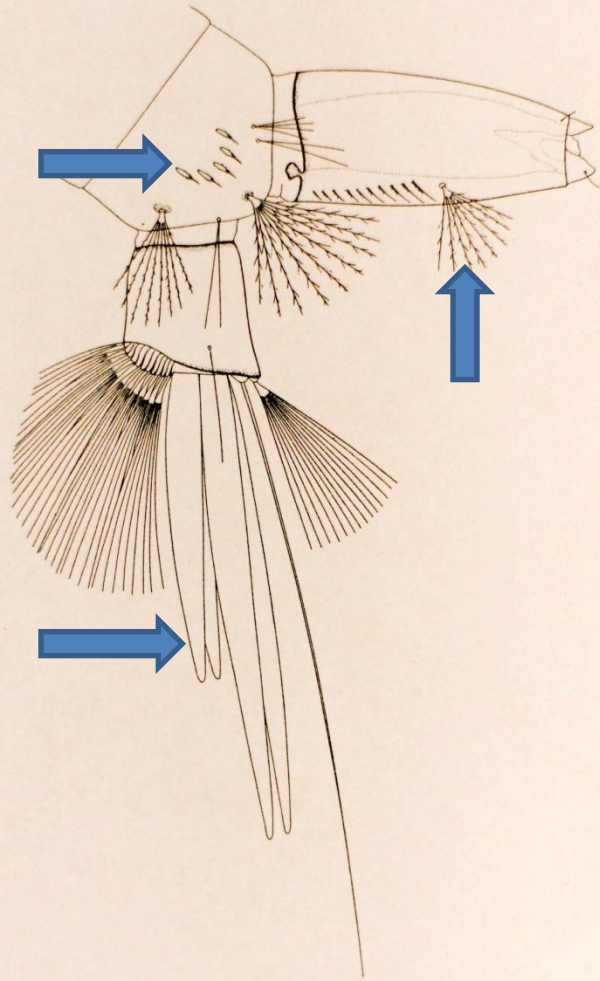
1		Aedes aegypti
2		Aedes albopictus
3		Aedes atlanticus/tormentor
4		Aedes atropalpus
5		Aedes canadensis
6		Aedes cantator
7		Aedes cinereus
8		Aedes condolecens*
9		Aedes dorsalis
10		Aedes dupreei
11		Aedes fitchii
12		Aedes fulvus pallens
13		Aedes grossbecki
14		Aedes infirmatus
15		Aedes japonicus
16		Aedes melanimon
17		Aedes nigromaculis
18		Aedes provocans
19		Aedes sollicitans
20		Aedes squamiger
21		Aedes sticticus

Aedes



Aedes atlanticus/tormentor

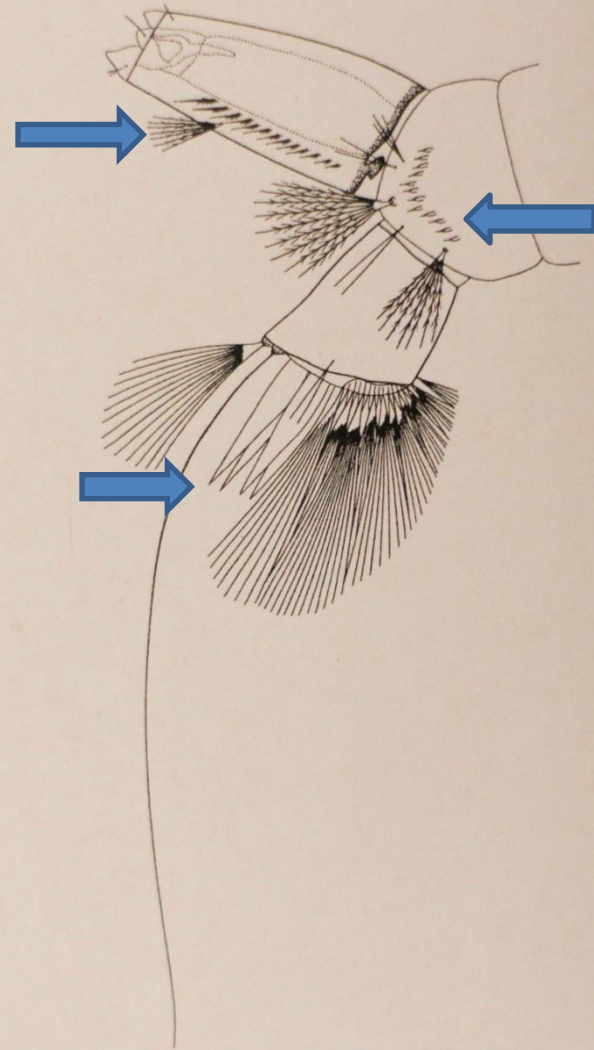
Oc. atlanticus



Carpenter & LaCasse 1955

Jung Kim

Oc. tormentor



Carpenter & LaCasse 1955

Jung Kim

Dr. Bruce Harrison's Work

- Studied these two species for >40 years
- Found morphological characters to ID the adults



Ae. tormentor



Ae. atlanticus

Research Goals

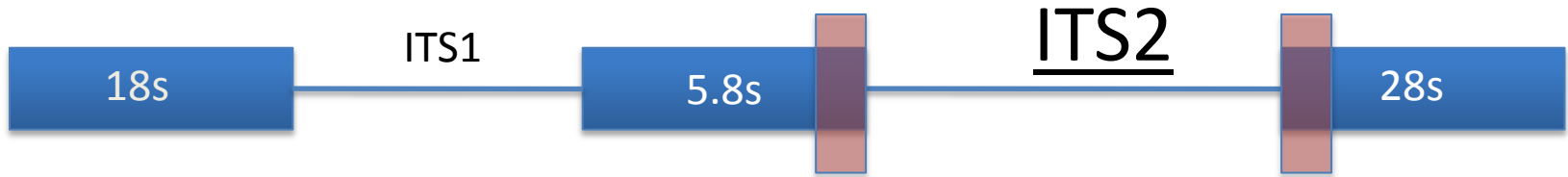
- To develop a useful molecular method to distinguish the two species (at any life stage)
- Molecular confirmation of morphological characteristics
- Strategy Considerations:
 - Conserved Primers with PCR Size Polymorphism
 - Sequencing
 - Restriction Enzyme Digest or Species Specific Primers

Methods

- Extracted gDNA
 - Larval Specimens (Known ID) Reared to Adults
- PCR amplification with conserved primers
 - ITS2 Gene (CP1-1A and CP1-1B primers)
- PCR products cloned into TOPO-TA Vector
- Plasmids Sequenced
- Sequence Analysis

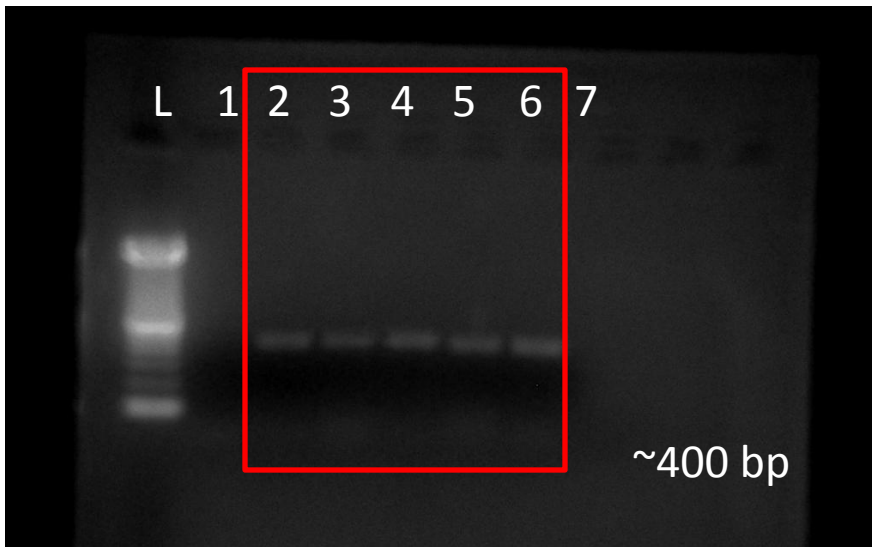
What is the ITS2?

- Ribosomal target located between 5.8s and 28s



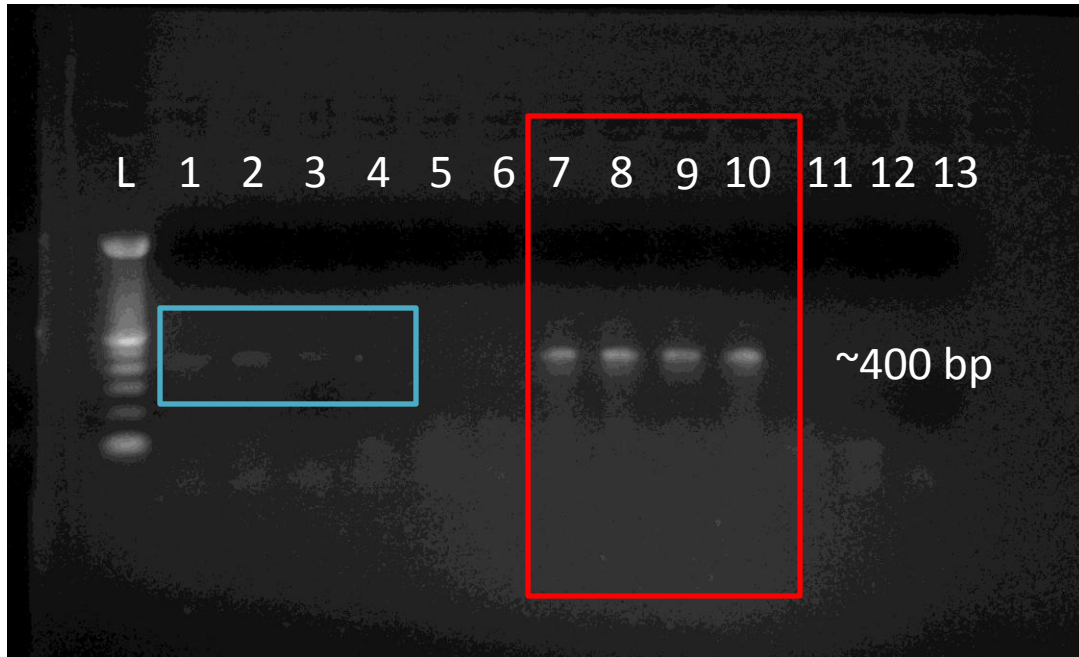
- High variability between species
- Area of rapid mutation
- Unique sequence for each species
- Bordered by highly conserved regions

Ae. atlanticus ITS2 PCR



Lane	Product
L	100 bp Ladder
1	A-9 UNDL (no amplification)
2	A-9 (1:10)
3	A-15 UNDL
4	A-15 (1:10)
5	A-19 UNDL
6	A-19 (1:10)
7	Negative Control (H ₂ O)

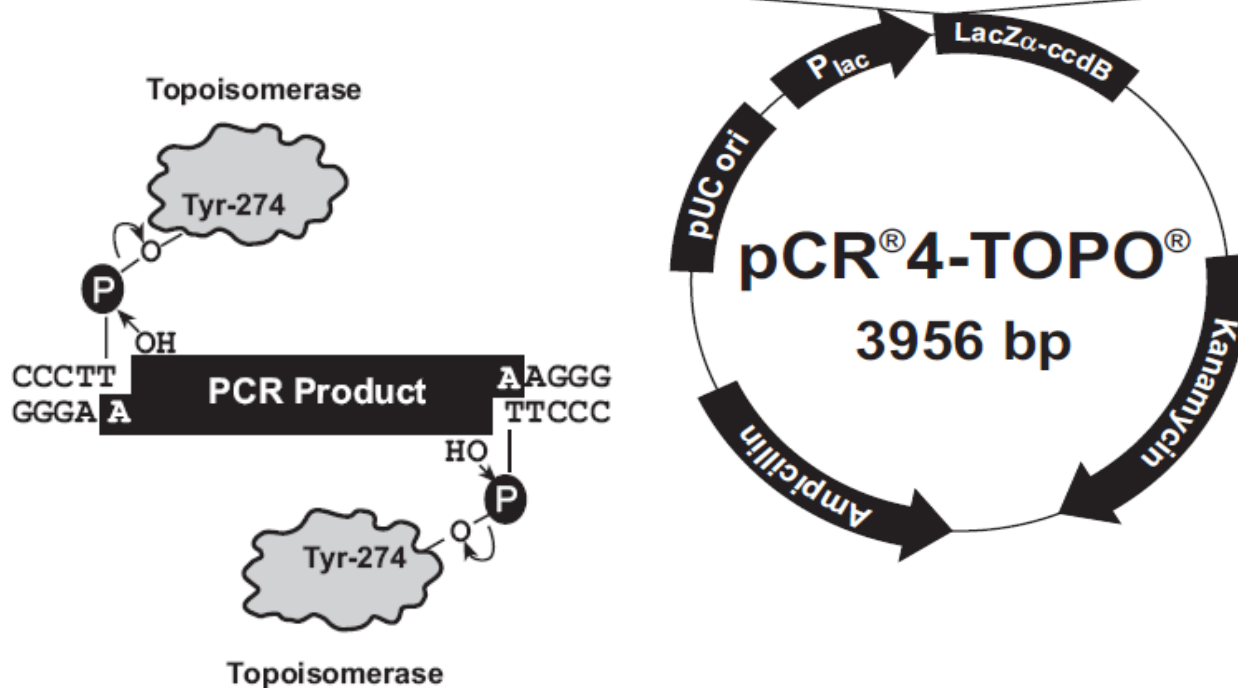
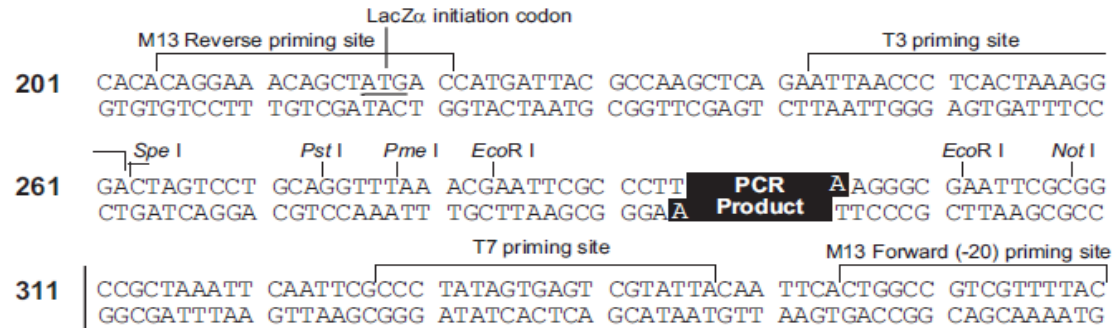
Ae. tormentor ITS2 PCR



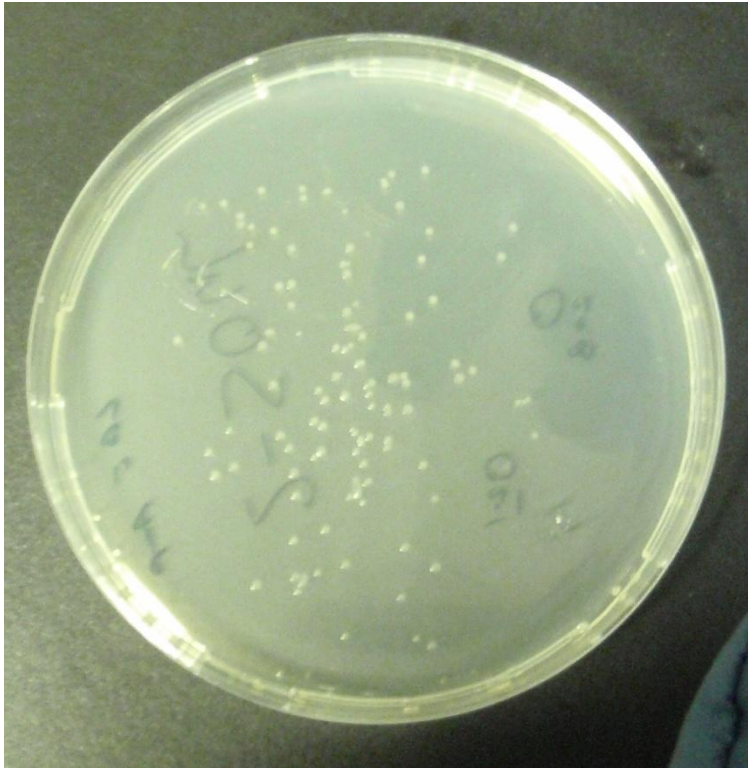
Lane	Product
L	100 bp Ladder
1	T-9 (1:5)
2	T-9 (1:5)
3	T-17 (1:5)
4	T-17 (1:5)
5	T-18 (1:5)
6	T-18 (1:5)
7	T-9 (PCR 1:10)
8	T-9 (PCR 1:10)
9	T-17 (PCR 1:10)
10	T-17 (PCR 1:10)
11	T-18 (PCR 1:10)
12	T-18 (PCR 1:10)
13	Negative Control (H ₂ O)



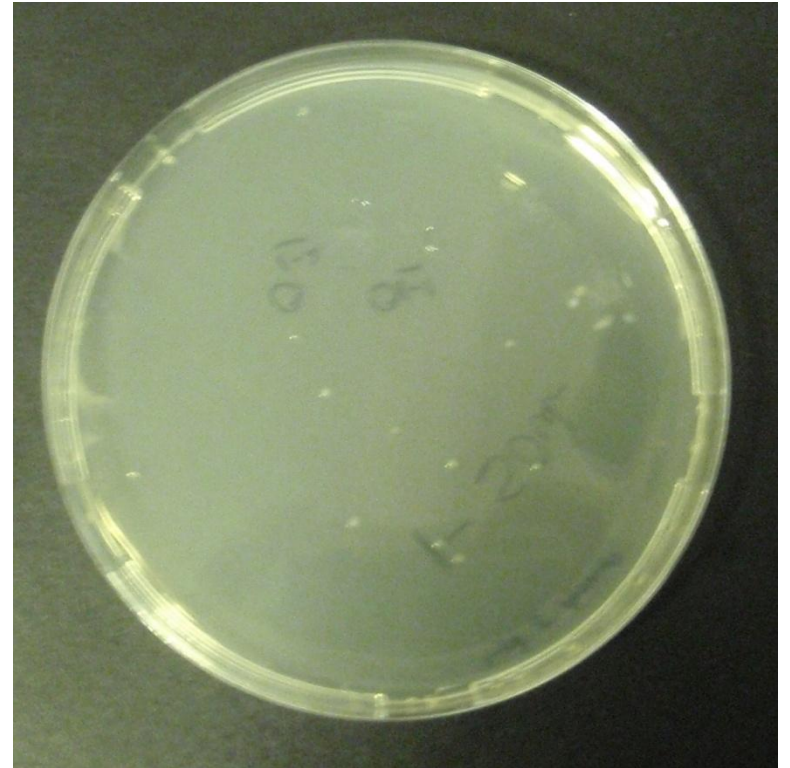
TOPO-TA Cloning



Cloning Results



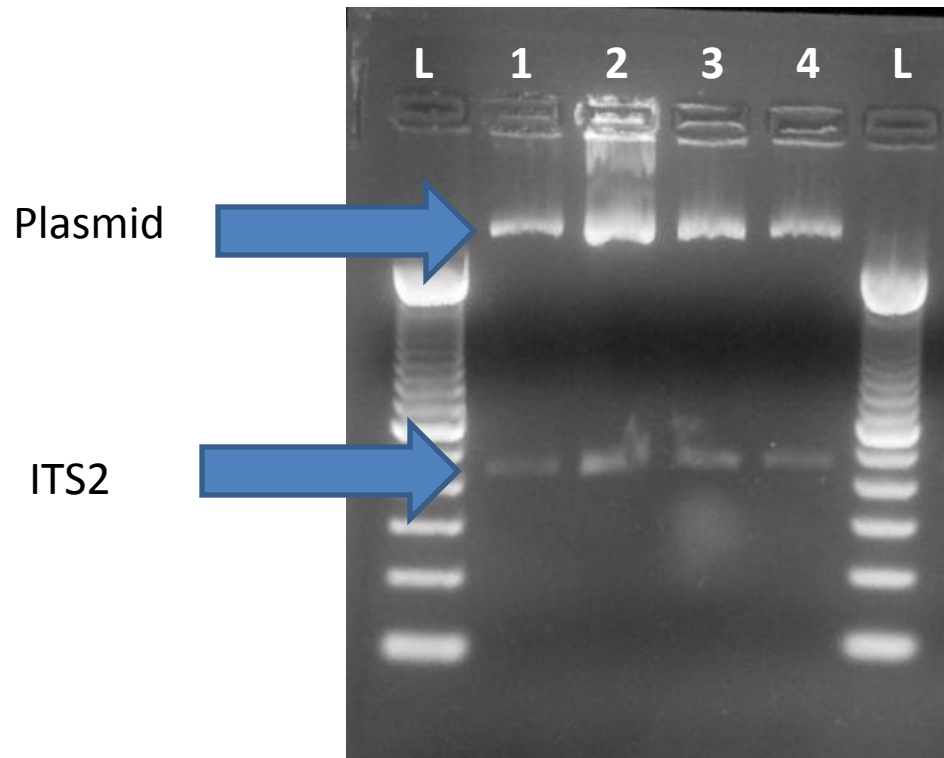
Ae. atlanticus (A-15)



Ae. tormentor (T-17)

Confirmation of ITS2 cloning

- EcoR1 Digest Confirms Insertion of PCR Products
- Visualized by Gel Electrophoresis
- Sequenced



Lane	Product
L	Ladder
1	T-17 Plasmid 1-1
2	T-17 Plasmid 1-2
3	A-15 Plasmid 2-1
4	A-15 Plasmid 2-2

Sequence Analysis

```
atlanticus      GTGGATCCTGTGAACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACATC
tormentor      GTGGATCCTGTGAACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACATC
*****

atlanticus      GTACAACAGTACGATGTACACATTTTTGAGTGCCTATATTTATCCATTCAACTATACGTG
tormentor      GTACAACAGTACGATGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTATACGTG
*****

atlanticus      TGTGCGCGTACCATTGTTCCGGGTGGACAGGCGCACGGCCCATAGCACGTATGCGGCGTGAT
tormentor      TGTGCGCGTACCACGTTCCGGGTGGACAGGCGCACGGCCCATAGCACGTATGCGGCGTGAT
*****

atlanticus      GTTTTCCCGACCCGT TCGGT AAAACATTGAAGATAGTCAGGCGCGTCCCACC CGCCC CGG
tormentor      GTTTTCCCGACCCGT TCGGA AAAACATTGAAGATAGTCAGGCGCGTCCCACC GACCC --G
*****

atlanticus      TGTGGACGTGTTGATGAATACATCCCATATGCCAG TCCGA TTGGCTATGTTGTGTTCCA
tormentor      TGTGGACGTGTTGATGAATACATCCCATATGCCAG TCCGA TTGGCTATGTTGTGTTCCA
*****

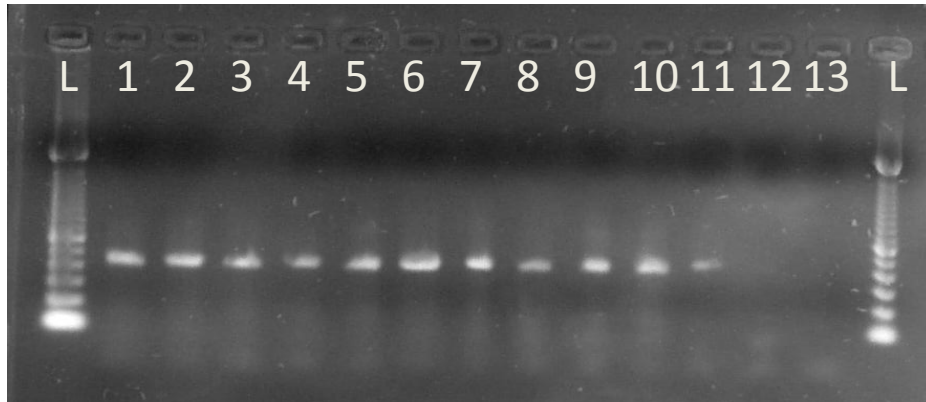
atlanticus      TCAGCCGAGATCGGCGTGTGTACCTACCTGTGCGCCCCCGATCCCCCCCTTTATCTCA
tormentor      TCAGC--GAGCTCGG---TGTACCTACCTGTG----CCCGATCTCCCTTTTATCTC-CA
*****

atlanticus      CC CAGTAGGCCTCAAATAATGTGTGACTACCCCTAAATTTAAGCATGTCGACAC
tormentor      CT CAGTAGGCCTCAAATAATGTGTGACTACCCCTAAATTTAAGCATGTCGACAC
* *****
```

Ae. atlanticus has 415 bps

Ae. tormentor has 403 bps

Blind Specimens



Samples provided by Dr. Bruce Harrison

Lane	Product
L	Ladder
1	V-1
2	V-3
3	V-5
4	V-6
5	V-10
6	M-3
7	M-4
8	OT-1
9	OT-2
10	OT-3
11	A-15
12	T-17
13	Neg. Control
L	Ladder

```

*****
seq_009_E01      GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
seq_004_B02      GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
seq_011_F01      GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
seq_002_A02      GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
TORMENTOR-CBS-1-2_M13F  GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
TORMENTOR-CBS-1-1_M13F  GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
ATLANTICUS-CBS-2-1_M13F  GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
ATLANTICUS-CBS-2-2_M13F  GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
seq_015_H01      GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
seq_005_C01      GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
seq_003_B01      GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
seq_013_G01      GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
seq_001_A01      GGTGGGACGCGCCTGACTATCTTCAATGTTTTTCCGAACGGGTGGGAAAACATCACGCCGCATACGTGCTATGG 75
1.....10.....20.....30.....40.....50.....60.....70.....

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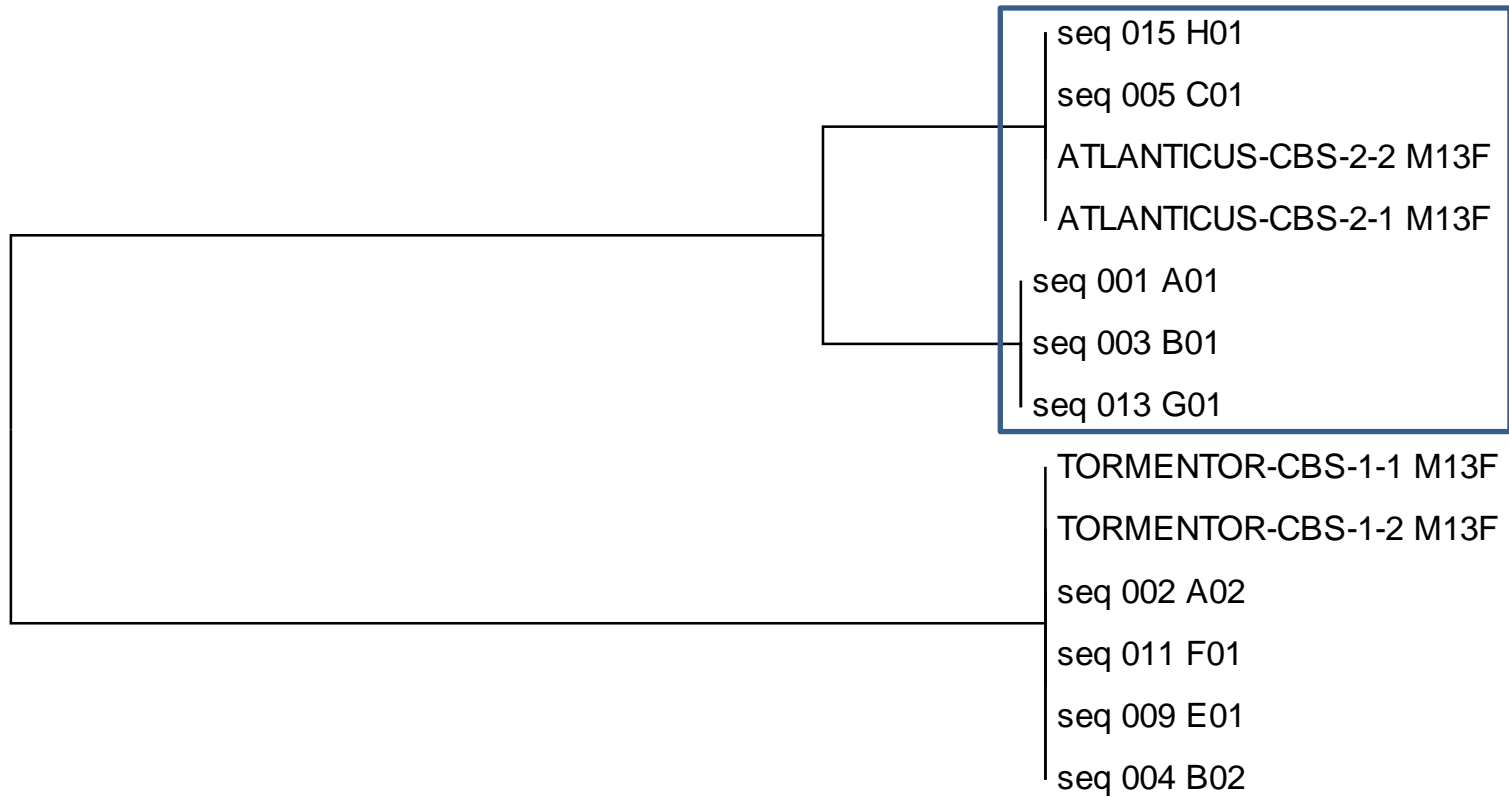
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*****
seq_009_E01      GCCGTGCGCCTGTCCACCCGGACGTGGTACGCGCACACACGTATAGTTGAATAGATAAAATATAGGCACTCAAAAA 150
seq_004_B02      GCCGTGCGCCTGTCCACCCGGACGTGGTACGCGCACACACGTATAGTTGAATAGATAAAATATAGGCACTCAAAAA 150
seq_011_F01      GCCGTGCGCCTGTCCACCCGGACGTGGTACGCGCACACACGTATAGTTGAATAGATAAAATATAGGCACTCAAAAA 150
seq_002_A02      GCCGTGCGCCTGTCCACCCGGACGTGGTACGCGCACACACGTATAGTTGAATAGATAAAATATAGGCACTCAAAAA 150
TORMENTOR-CBS-1-2_M13F  GCCGTGCGCCTGTCCACCCGGACGTGGTACGCGCACACACGTATAGTTGAATAGATAAAATATAGGCACTCAAAAA 150
TORMENTOR-CBS-1-1_M13F  GCCGTGCGCCTGTCCACCCGGACGTGGTACGCGCACACACGTATAGTTGAATAGATAAAATATAGGCACTCAAAAA 150
ATLANTICUS-CBS-2-1_M13F  GCCGTGCGCCTGTCCACCCGAACATGGTACGCGCACACACGTATAGTTGAATGGATAAAATATAGGCACTCAAAAA 150
ATLANTICUS-CBS-2-2_M13F  GCCGTGCGCCTGTCCACCCGAACATGGTACGCGCACACACGTATAGTTGAATGGATAAAATATAGGCACTCAAAAA 150
seq_015_H01      GCCGTGCGCCTGTCCACCCGAACATGGTACGCGCACACACGTATAGTTGAATGGATAAAATATAGGCACTCAAAAA 150
seq_005_C01      GCCGTGCGCCTGTCCACCCGAACATGGTACGCGCACACACGTATAGTTGAATGGATAAAATATAGGCACTCAAAAA 150
seq_003_B01      GCCGTGCGCCTGTCCACCCGAACATGGTACGCGCACACACGTATAGTTGAATAGATAAAATATAGGCACTCAAAAA 150
seq_013_G01      GCCGTGCGCCTGTCCACCCGAACATGGTACGCGCACACACGTATAGTTGAATAGATAAAATATAGGCACTCAAAAA 150
seq_001_A01      GCCGTGCGCCTGTCCACCCGAACATGGTACGCGCACACACGTATAGTTGAATAGATAAAATATAGGCACTCAAAAA 150
...80.....90.....100.....110.....120.....130.....140.....150

```



150 bp region of the rDNA ITS2



0.005

Bruce's ID (BLINDED ID #)	SEQ ID	Species ID Call
V-14	seq_011_F01	<i>Ae. tormentor</i>
V-11	seq_002_A02	<i>Ae. tormentor</i>
V-12	seq_004_B02	<i>Ae. tormentor</i>
V-13	seq_009_E01	<i>Ae. tormentor</i>
M-1	seq_015_H01	<i>Ae. atlanticus</i>
V-7	seq_005_C01	<i>Ae. atlanticus</i>
M-6	seq_013_G01	<i>Ae. atlanticus</i>
V-4	seq_003_B01	<i>Ae. atlanticus</i>
V-2	seq_001_A01	<i>Ae. atlanticus</i>

Aedes atlanticus ITS2 *Hpy*188I Restriction Digest

20 40 60
 GTGGATCCTGTGAACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACATCGTACAAC
 CACCTAGGACACTTGACGTCTGTGTACTTGTGGCTGTTCAACTTGCGTATAACGTGTAGCATGTTG

80 100 120
 AGTACGATGTACACATTTTTGAGTGCCCTATATTTATCCATTCAACTATACGTGTGTGCGCGTACCAT
 TCATGCTACATGTGTA AAAACTCACGGATATAAATAGGTAAGTTGATATGCACACACGCGCATGGTA

140 160 180 200
 GTTCGGGTGGACAGGCGCACGGCCCATAGCACGTATGCGGCGTGATGTTTTCCCGACCCGTTCCGGTA
 CAAGCCACCTGTCCGCGTGCCGGGTATCGTGCATACGCCGCACTACAAAAGGGCTGGGCAAGCCAT

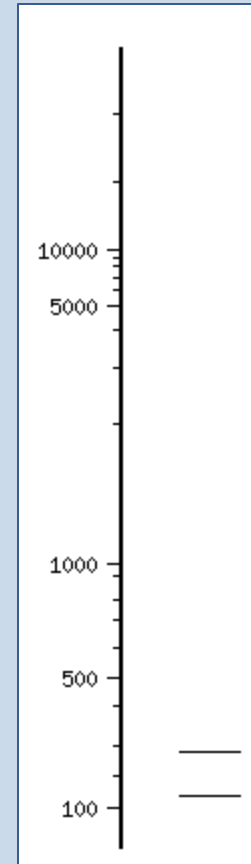
220 240 260
 AAACATTGAAGATAGTCAGGCGCGTCCCACCCGCCCGGTGTGGACGTGGTTGATGAATACATCCCA
 TTTGTA ACTTCTATCAGTCCGCGCAGGGTGGGCGGGGCCACACCTGCACCACTACTTATGTAGGGT

280 300 320
 TATGCCAGTCCGATTGGCTATGTTGTGTTCATCAGCGCGAGATCGGCGTGTGTACCTACCTGTGCG
 ATACGGTCAAGCTA ACCGATAACAACAAGGTAGTCGCGCTCTAGCCGCACACATGGATGGACACGC

340 360 380 400
 CCCCCGATCCCCCCTTTATCTCACCCAGTAGGCCTCAAATAATGTGTGACTACCCCTAAATTTA
 GGGGGCTAGGGGGGGAAATAGAGTGGGTCATCCGGAGTTTATTACACACTGATGGGGGATTTAAAT

AGCATGTCGACAC
 TCGTACAGCTGTG

*Hpy*188I



Product Sizes:
 279
 136

Aedes tormentor ITS2 *Hpy188I* Restriction Digest

20 40 60
 GTGGATCCTGTGAACTGCAGGACACATGAACACCGACAAGTTGAACGCATATTGCACATCGTACAA
 CACCTAGGACACTTGACGTCCTGTGTACTTGTGGCTGTTCAACTTGCGTATAACGTGTAGCATGTT

80 100 120
 CAGTACGATGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTATACGTGTGTGCGCGTACC
 GTCATGCTACATGTGTAAAACTCACGGATATAAATAGATAAGTTGATATGCACACACGCGCATGG

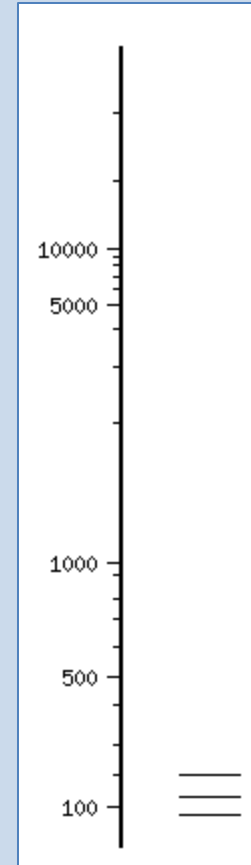
140 160 180 *Hpy188I*
 ACGTCCGGGTGGACAGGCGCACGGCCCATAGCACGTATGCGGCGTGATGTTTTCCCGACCCGTTCCG
 TGCAGGCCACCTGTCCGCGTGCCGGGTATCGTGCATACGCCGCACTACAAAAGGGCTGGGCAAGC

Hpy188I
 200 220 240 260
 GAAAAACATTGAAGATAGTCAGGCGCGTCCCACCGACCCGTGTGGACGTGGTTGATGAATACATCC
 CTTTTGTAACCTCTATCAGTCCGCGCAGGGTGGCTGGGCACACCTGCACCAACTACTTATGTAGG

Hpy188I
 280 300 320
 CATATGCCAGTCCGATTGGCTATGTTGTGTTCCATCAGCGAGCTCGGTGTACCTACCTGTGCCCCGA
 GTATACGGTCAGGCTAACCGATACAACACAAGGTAGTCGCTCGAGCCACATGGATGGACACGGGCT

340 360 380
 TCTCCCCTTTATCTCCACTCAGTAGGCCTCAAATAATGTGTGACTACCCCTAAATTTAAGCATGT
 AGAGGGGAAATAGAGGTGAGTCATCCGGAGTTTATTACACACTGATGGGGGATTAAATTCGTACA

400
 CGACAC
 GCTGTG



Product Sizes:
 198
 125
 79

Results (To Date)

- Sequence results confirm the morphological characters (Harrison)
- Novel Sequences
 - 94% Sequence Similarity (3% from Indels)
 - Useful Heterogeneity (Hpy 188I RE)?
 - Not Enough Difference for PCR Specific Primers

Next Steps

- Restriction Enzyme Digest
 - Work in Progress
- Sequencing of Samples Across a Broader Geographic Range
 - In progress

References

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- Mullen, Gary, and Lance Durden. *Medical and Veterinary Entomology*. 1st ed. San Diego, California: Academic Press, 2002. 238-239. Print.
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Acknowledgements

- MAMCA
- Robert Collins



Questions?