Investigations of Morphological Differences Between *Aedes triseriatus* and *Ae. hendersoni*

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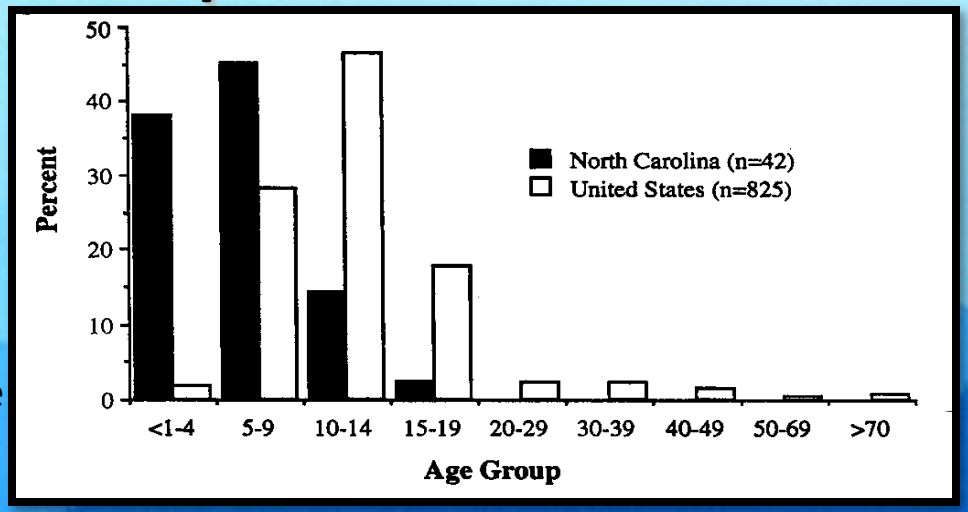
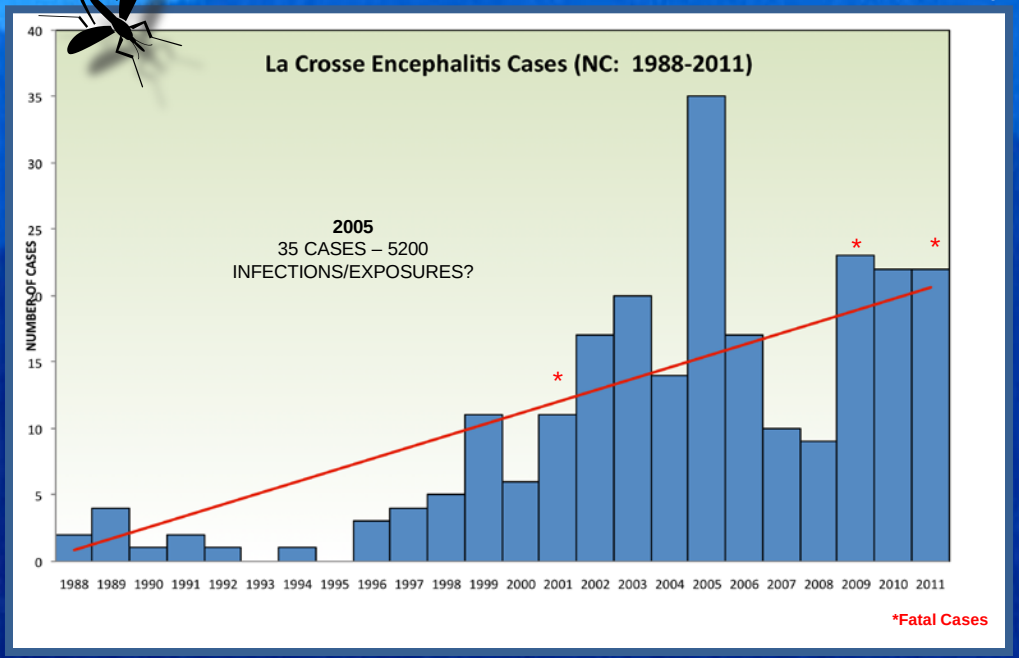
Vector-Borne Infectious Disease Lab

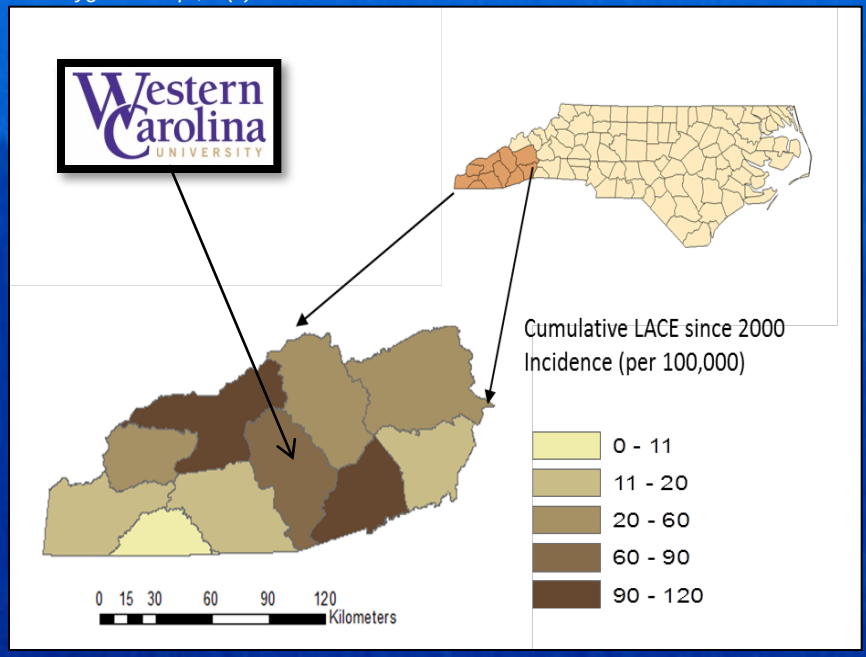
La Crosse Encephalitis

- Most common human arboviral disease in NC
- Recognized in WNC since 1964
- Infections greatly under-recognized (**1:150-300**)
- Disease most prevalent in children **(<15 yrs**)
- Western NC counties have the largest burden of LACE
- Primary research focus of the WCU Vector-borne Infectious Disease Lab



Szumlas *et al* Seroepidemiology of La Crosse virus infection in humans in western North Carolina. *Am J Trop Med Hyg. 1996 Apr;54(4):332-7*





Epidemic GIS Maps created by David Rollick

Background

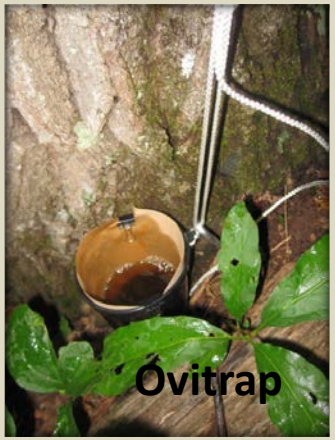
Aedes triseriatus

LACV Primary "natural" vector:
Eastern Tree-hole Mosquito
Sister species: *Aedes hendersoni*

- *Aedes triseriatus* is the primary vector of LACv
- *Aedes hendersoni* is mostly incompetent for LACv transmission
- Identification of *Aedes triseriatus* and *Aedes hendersoni* is difficult
- Accurate identification is required for proper surveillance and public health efforts
- Paradigm of vertical distribution
- These two species are known to hybridize!

Surveillance of LACv in Endemic Areas

- Ground-level oviposition surveillance efforts do not collect only for *Aedes triseriatus*
- Proper identification of vectors is crucial as not to inflate the risk of transmission
- Possible hybrids also may be observed


Ovitrap

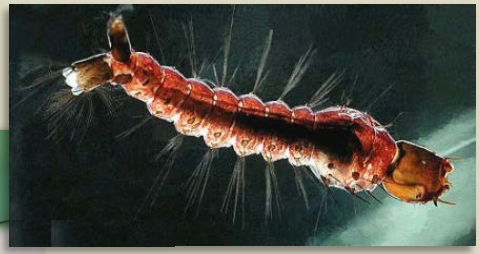

Aedes eggs

Other vectors are observed:


Aedes japonicus

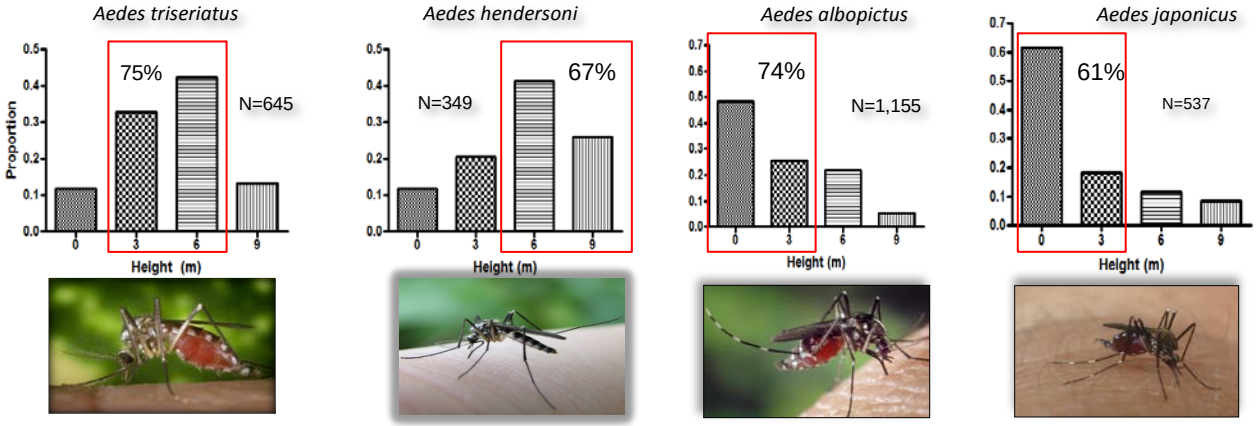

Aedes albopictus

Non-vectors observed occasionally:


Toxorynchites rutilus


Aedes hendersoni

Vertical Distribution '11: A Pilot Study



2011 Native vs Invasive Vertical Distribution

Aedes triseriatus: 75% of the total eggs identified were oviposited at 3 or 6 meters

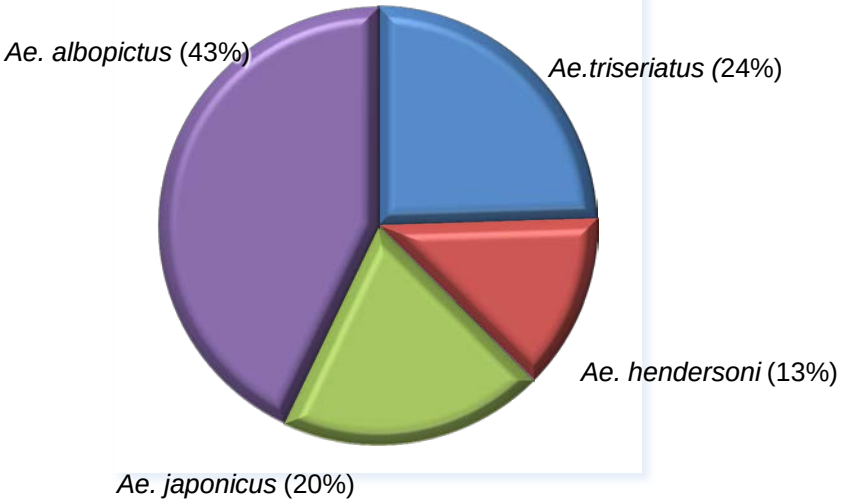
Aedes hendersoni: 67% of the total eggs identified were oviposited at 6 or 9 meters

Aedes albopictus: 74% of the total eggs were oviposited at 3 meters or below (ground level)

Aedes japonicus: 61% of the total eggs were oviposited at 3 meters or below (ground level)

Photo Credit: CDC: J.Gathany (2002)

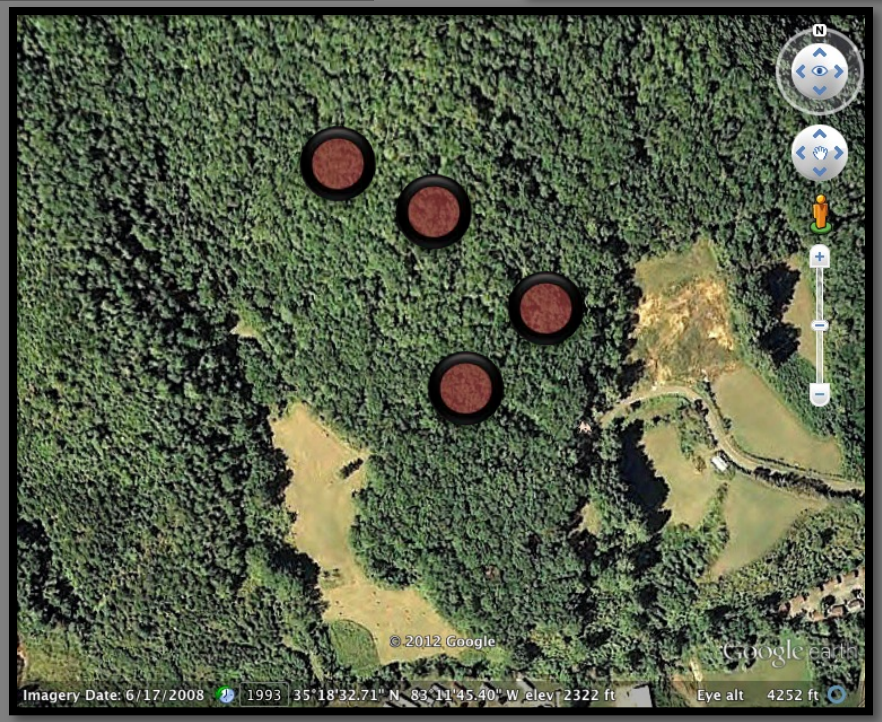
Relative Abundance 2011



Pilot Study conducted in 2011 on WCU Campus
~6,500 *Aedes* Eggs Collected--41% hatch rate
2,686 reared larvae identified to species
63% of identified larvae were invasive species
21% of all *Ae. triseriatus* unable to confidently ID

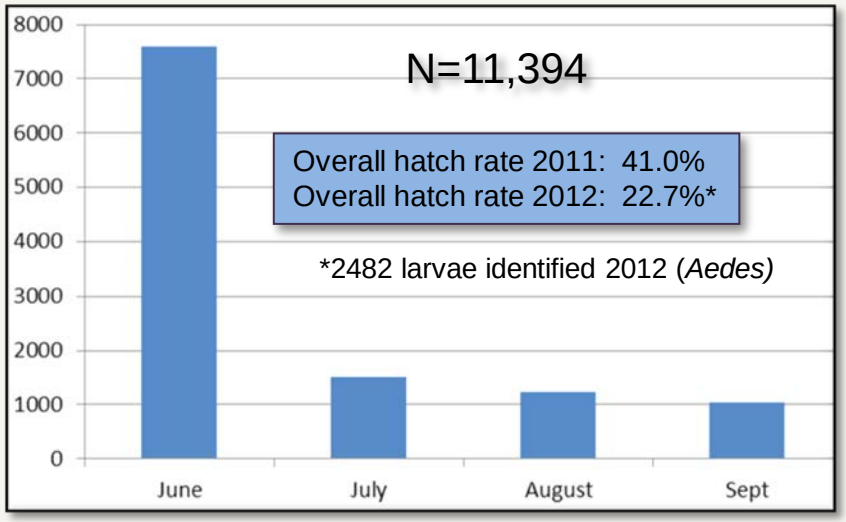
A
Elev. 2674 feet
100+ years

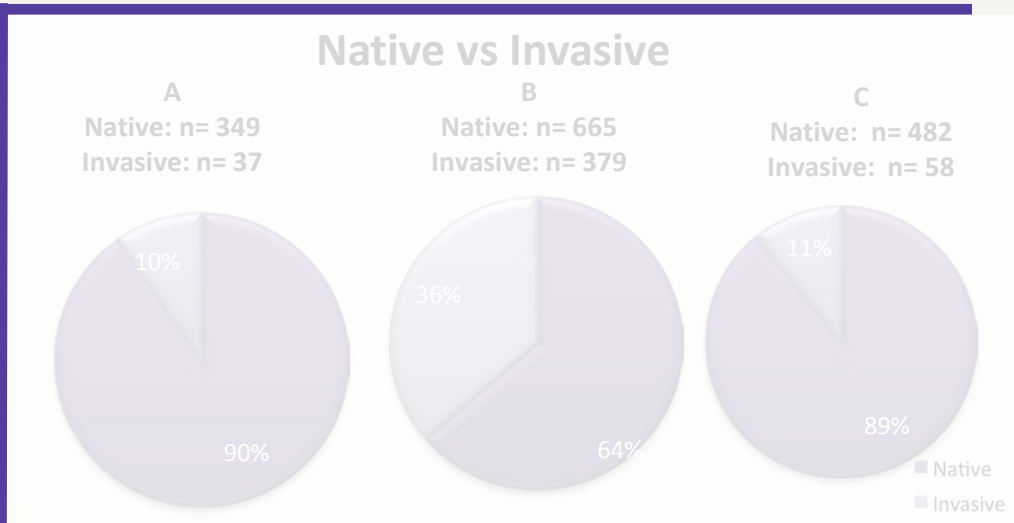
B
Elev. 2227 feet
80-100 years



C
Elev. 2322 feet
30-60+ years

Vertical Distribution '12: A Comparative Approach





49% of all *Ae. triseriatus* identified found above 0 meters!
33% of all *Ae. hendersoni* identified found at 0 meters!!

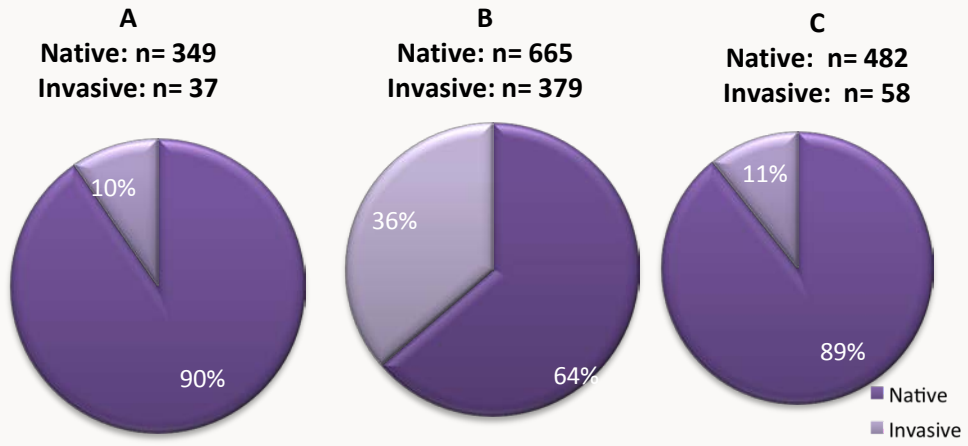
There appears to be site specific differences!

Site	Ground Level Percent (95% CI)
A	36.4 (26.5-47.5)
B	26.5 (21.2-32.4)
C	61.2 (47.2-73.5)

Overall, 33% of *Aedes hendersoni* were collected at ground level in 2012 across three different sites

Vertical Distribution '12: A Comparative Approach

Native vs Invasive



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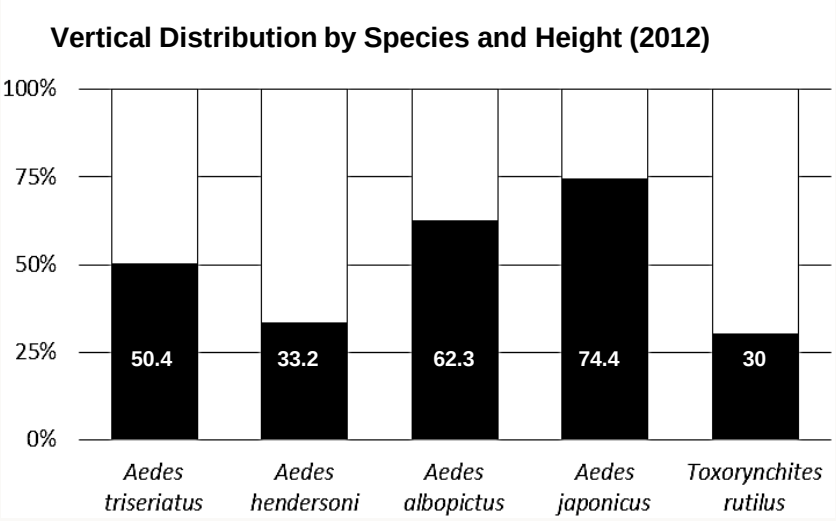

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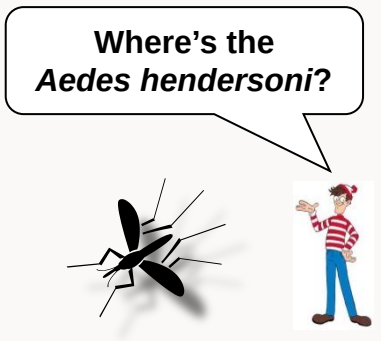

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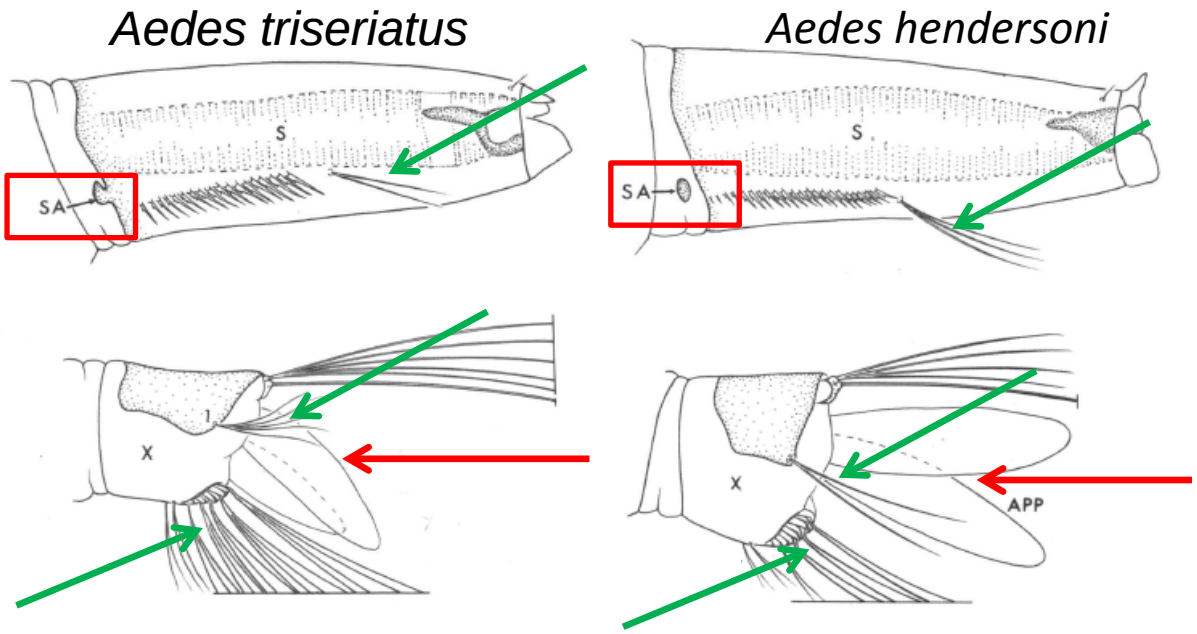
Purpose of Study

- To investigate morphological differences between the larvae of *Aedes triseriatus* and *Aedes hendersoni* and their hybrids
- To determine the validity (sensitivity and specificity) of 2° characters
- Correlate morphological data with DNA identities (In Progress)
- To determine the potential for verifying hybridization between the sister species

Darsie &Ward (1°) vs. Lunt (2°)

Traditional morphological differences (e.g., acus and anal papillae; Darsie and Ward, 2005) were used to identify *Ae. triseriatus* and *Ae. hendersoni*.



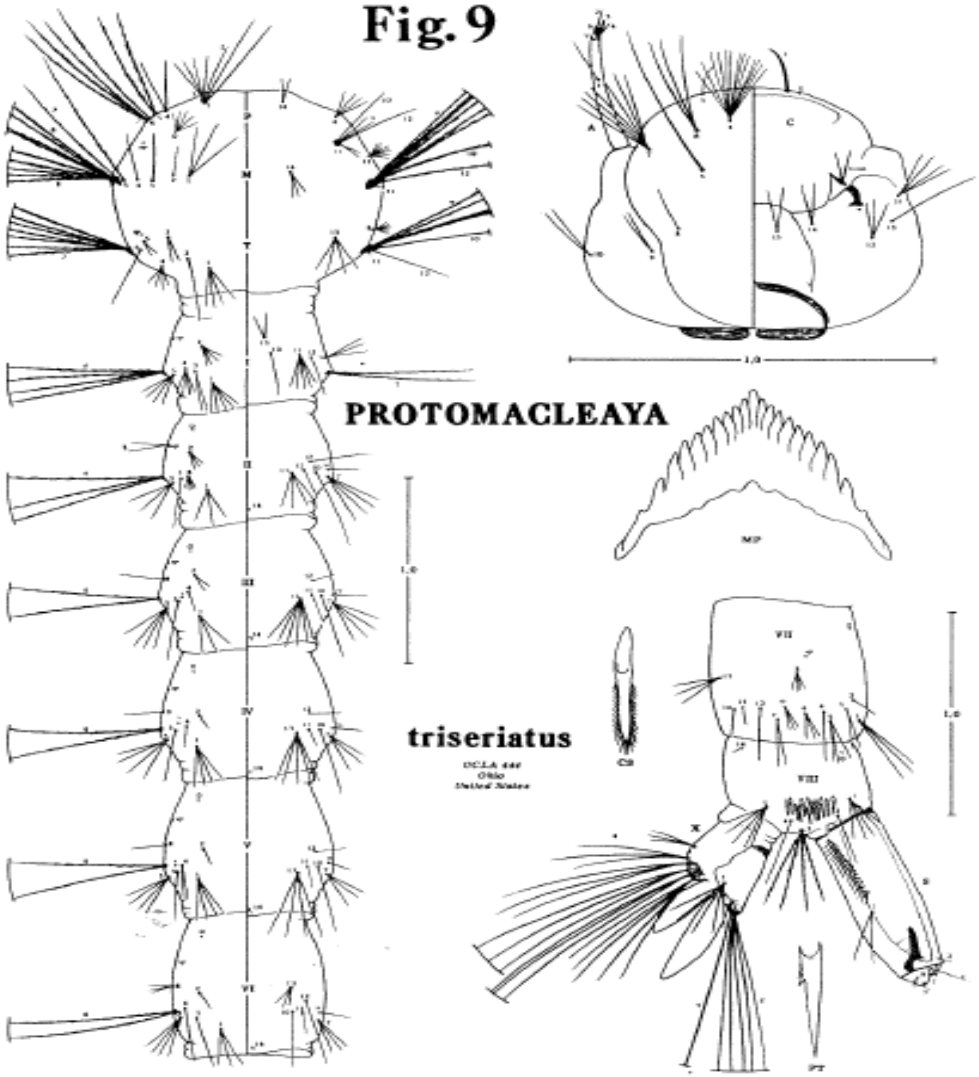
Secondary morphologic characters (setae 1-S, setae 4-X and setae 1-X; Lunt et al., 1977) were used to describe differences between *Ae. triseriatus* and *Ae. hendersoni*.

- **2011**: Unable to identify 21% (n= 209)
- Issues with 1° characters not always present for use in ID
- Observed 'weird' species/ questionable
- Investigated the utility of previously described 2° characters (Lunt)
- Investigation of potential novel 2° characters
- 209 specimens. +14 character states = 2926 obs.

- **2012**: Unable to identify 11% (n= 74)
- Issues with 1° characters not always present for use in ID
- Observed 'weird' species/ questionable
- Implementation utilizing previously described 2° characters plus potential novel 2° characters showed reduction in amount identified!
- 74 specimens. + 14 character states= 1036 obs

14 Character States Investigated

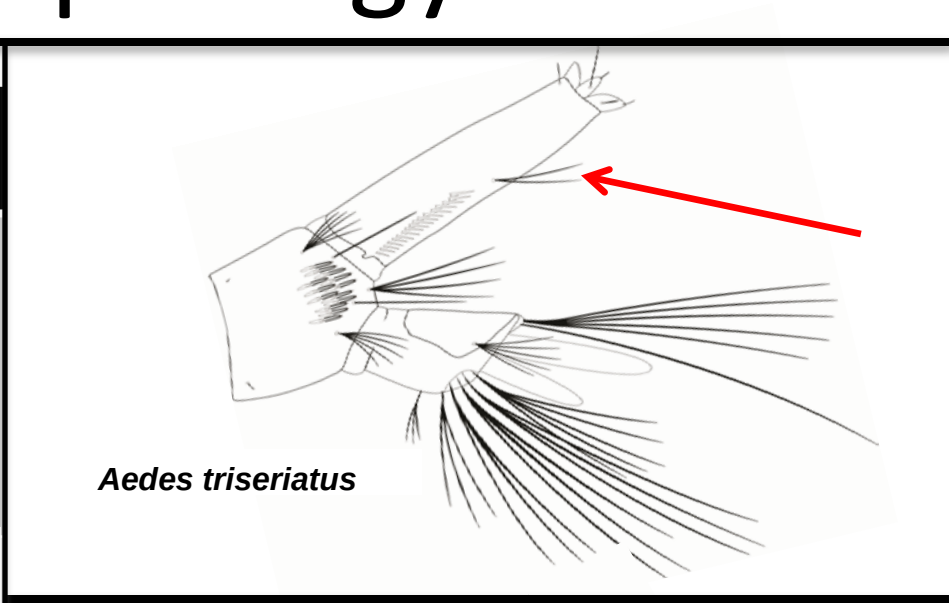
- 1-S setae Branch #’s
- 1-S setae length ratio
- 4-X # of branch pairs(ANT: 1,2,3 of branches)
- 1-X Branch #, Branch Equality (noted insertion)
- 1-X Saddle Ratio (Saddle Length vs. Setae Length)
- 6-I Branch #’s
- 3,4M Length Ratio
- 8-P Branch #’s
- 7-C Branch #’s
- 6,4-C Length Ratio



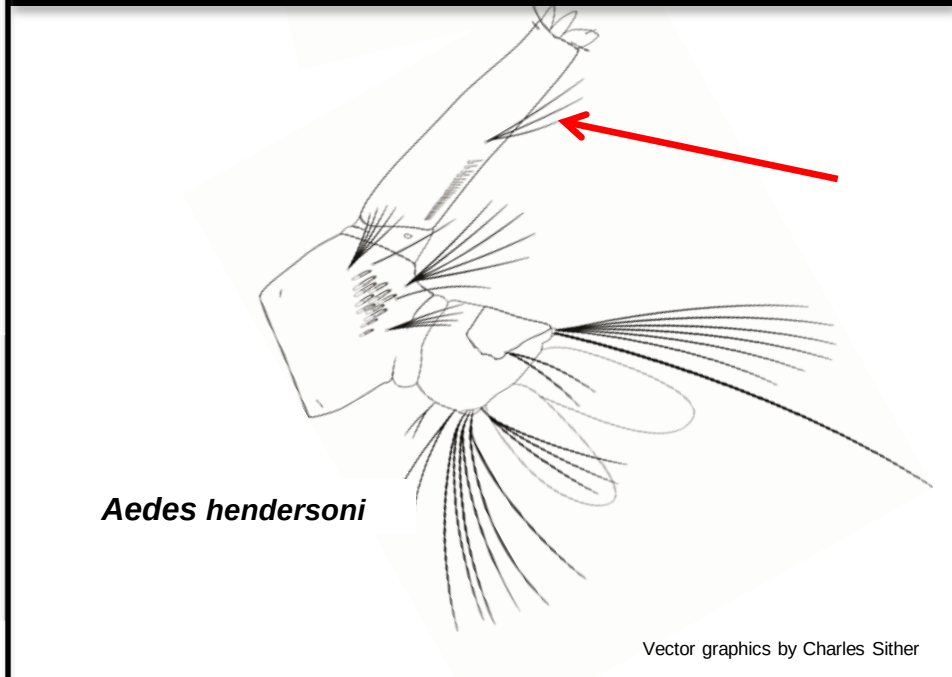
Zavortink, 1972

Distal Morphology

Characters: 2°	*Ae. triseriatus*	*Ae. hendersoni*
Setae 1-S	**2 branches**	**3 branches**
Setae 1-X	4-5 branches Setae/Saddle Ratio ≤1	2-3 branches Setae/Saddle Ratio >1
Setae 4-X	5 Setae (origin within grid) Setae with 3-4 branches	4 Setae (origin within grid) Setae with 2-3 branches



Aedes triseriatus



Aedes hendersoni

Vector graphics by Charles Sither

Distal Morphology

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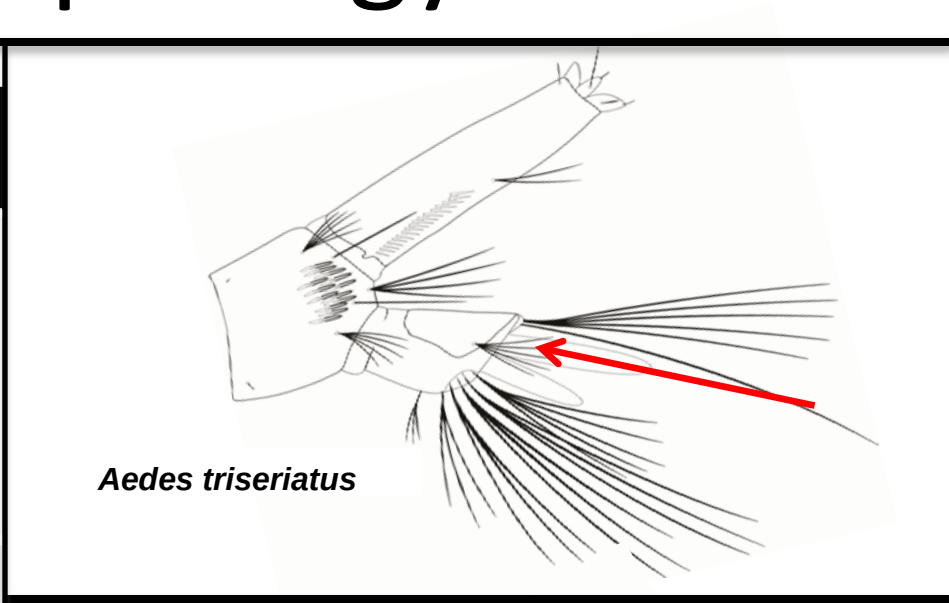
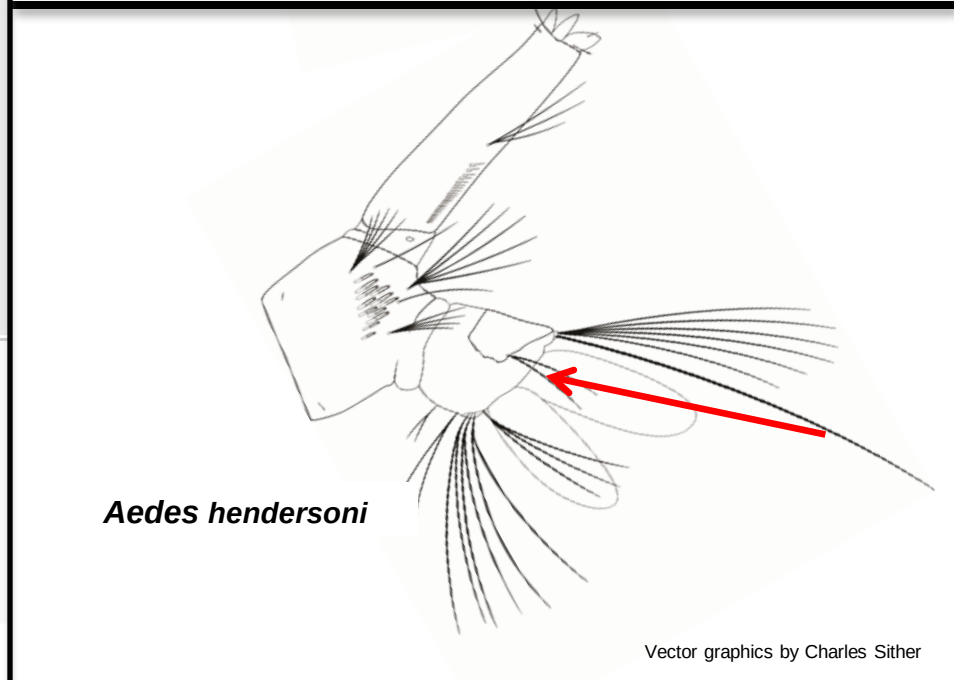
Setae 1-X	**4-5 branches Setae/Saddle Ratio ≤1**	**2-3 branches Setae/Saddle Ratio >1**
Setae 4-X	5 Setae (origin within grid) Setae with 3-4 branches	4 Setae (origin within grid) Setae with 2-3 branches



Aedes triseriatus



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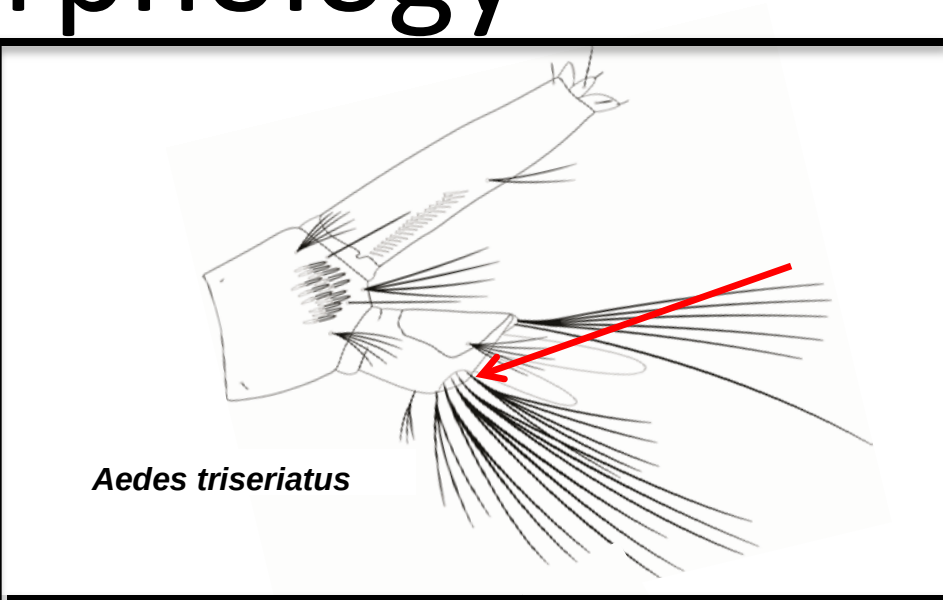
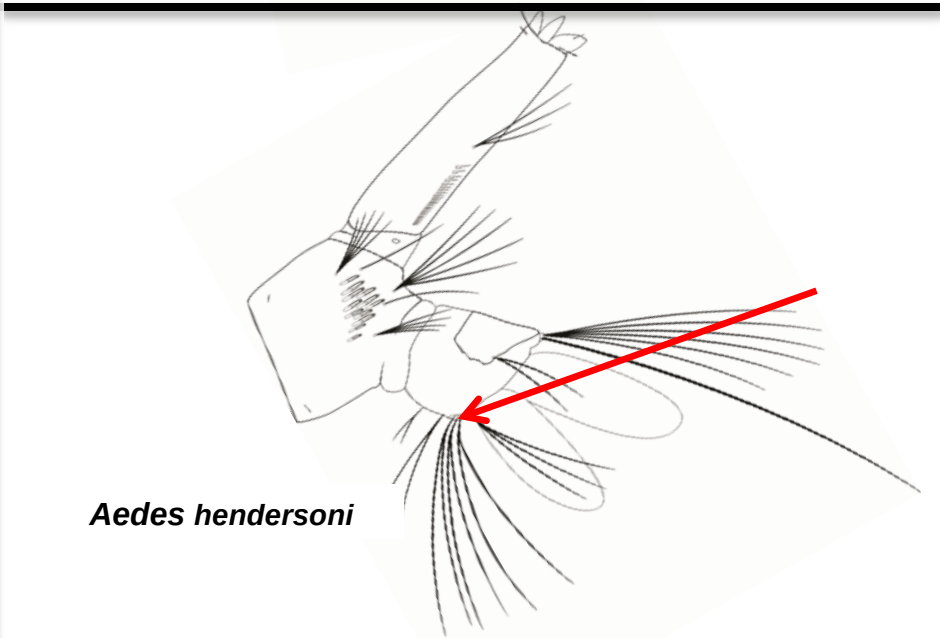


Aedes triseriatus

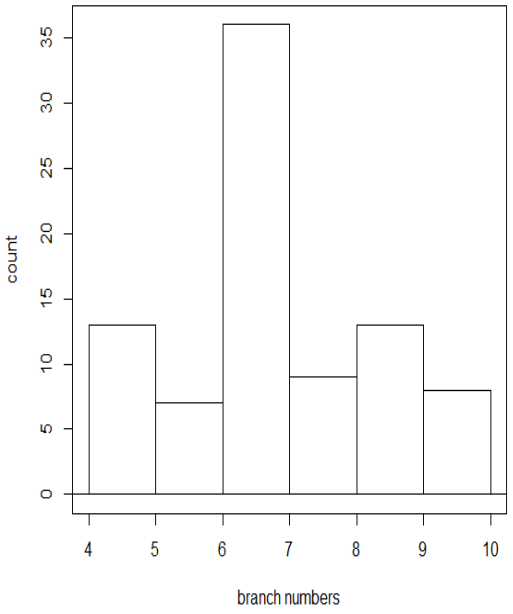


Aedes hendersoni

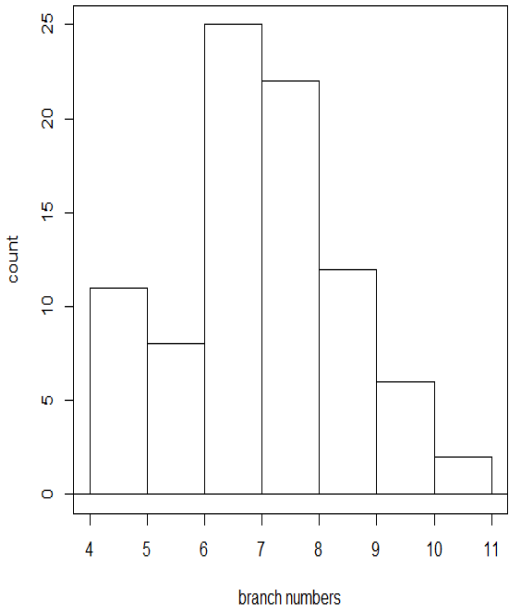
Vector graphics by Charles Sither

Setae 1-X (Unknowns)

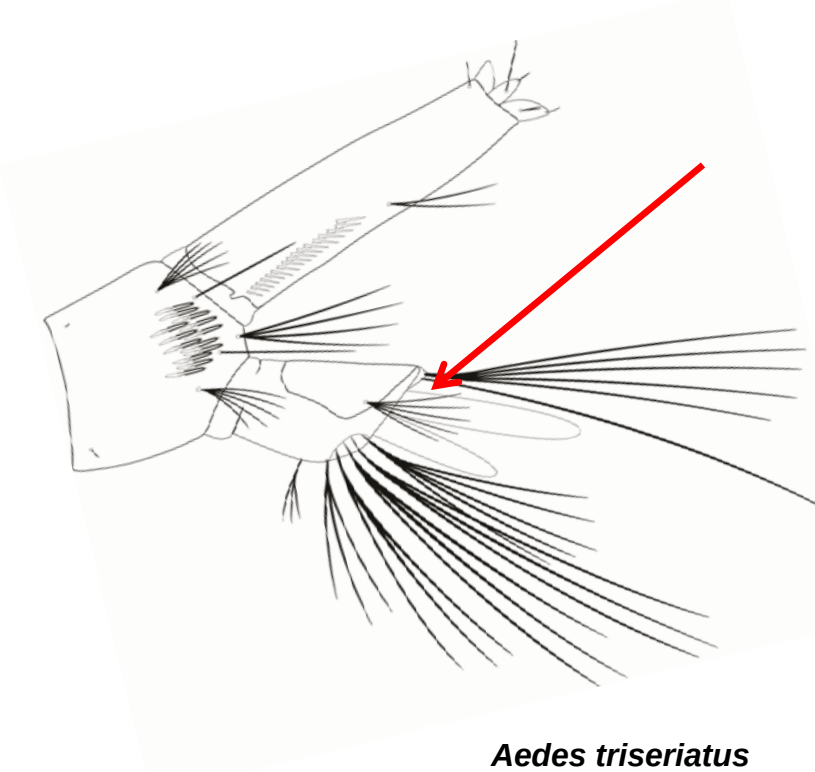
Number of Branches



L



R



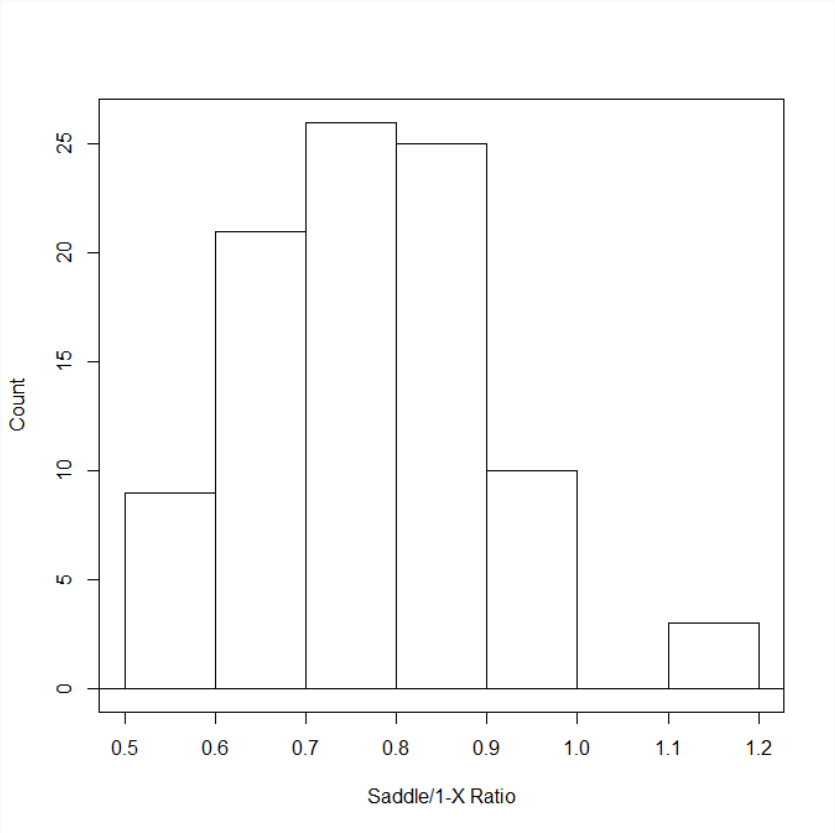
Aedes triseriatus

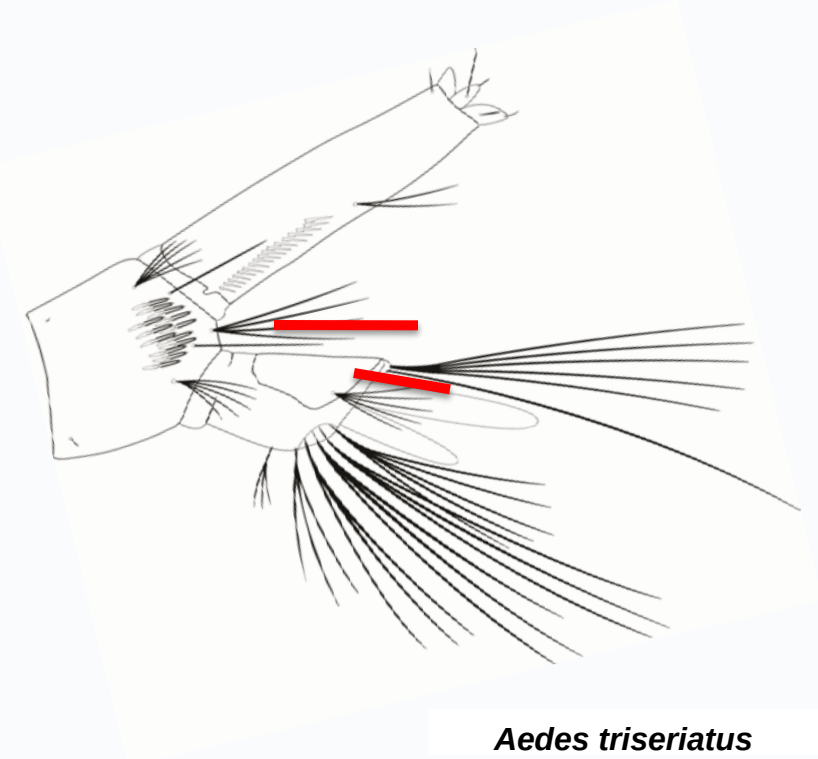
All unknowns have 4 or more branches on 1-X

Consistent with Lunt (1977).

Setae 1-X (Unknowns)

1-X Saddle Ratio





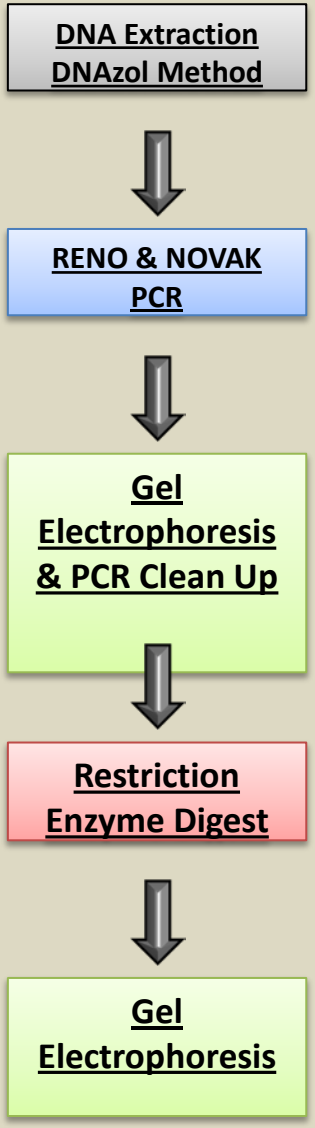
Aedes triseriatus

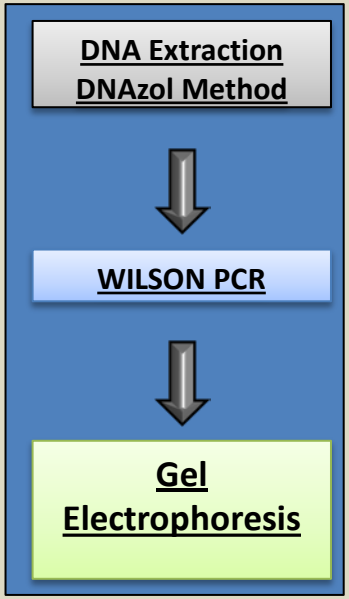
86% of unknowns have saddle ratio of less than 1.0
Suggestive for ID……..

Novel Species Specific PCR

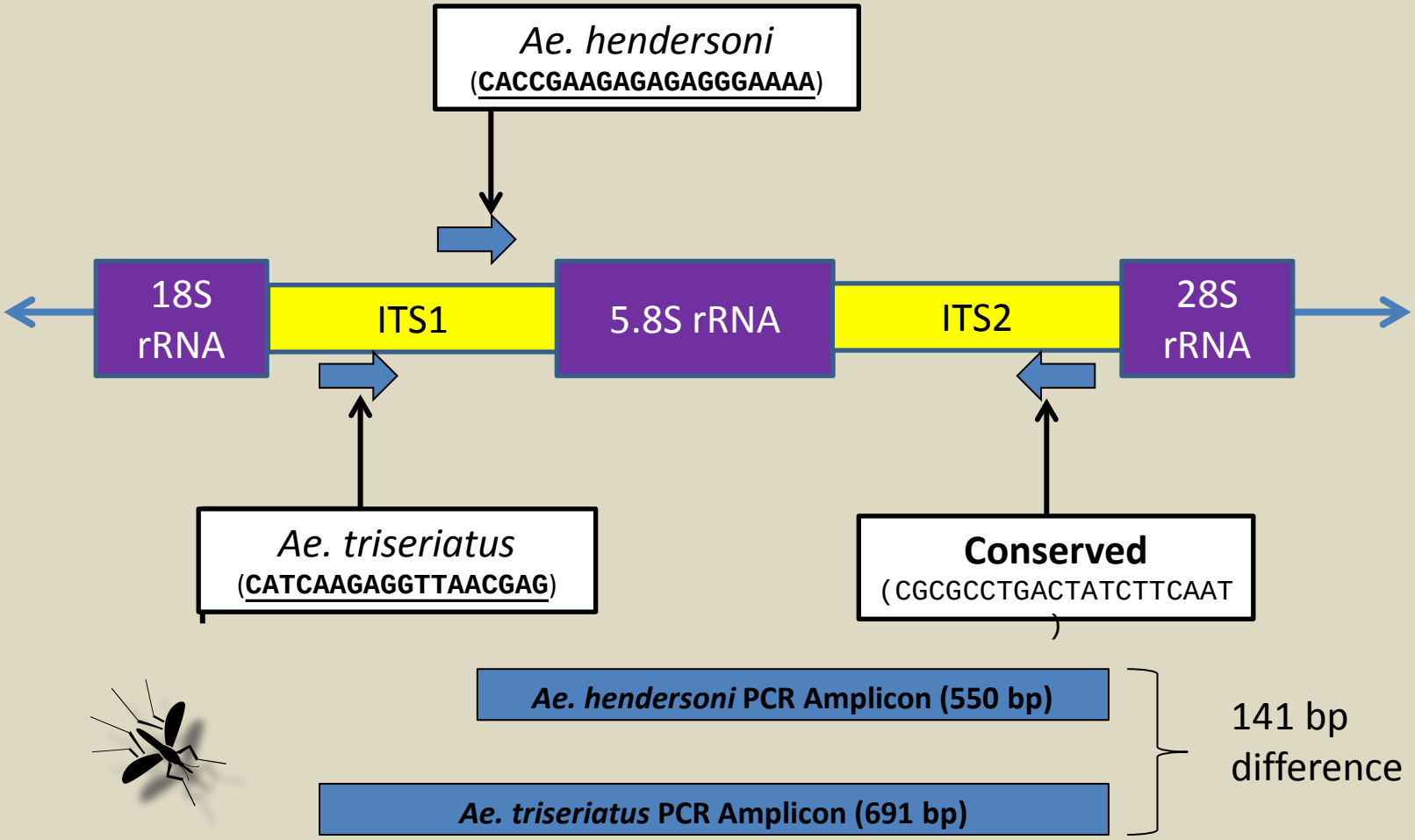
- Quicker (hours vs. days)
- Simpler (less steps)
- Easier to interpret
- Less expensive
- Fewer opportunities for operator error
- Less chance of contamination
- Potentially useful to determine hybrid species

Previously published molecular identification method (Reno and Novak, 2000) is more complicated and time consuming

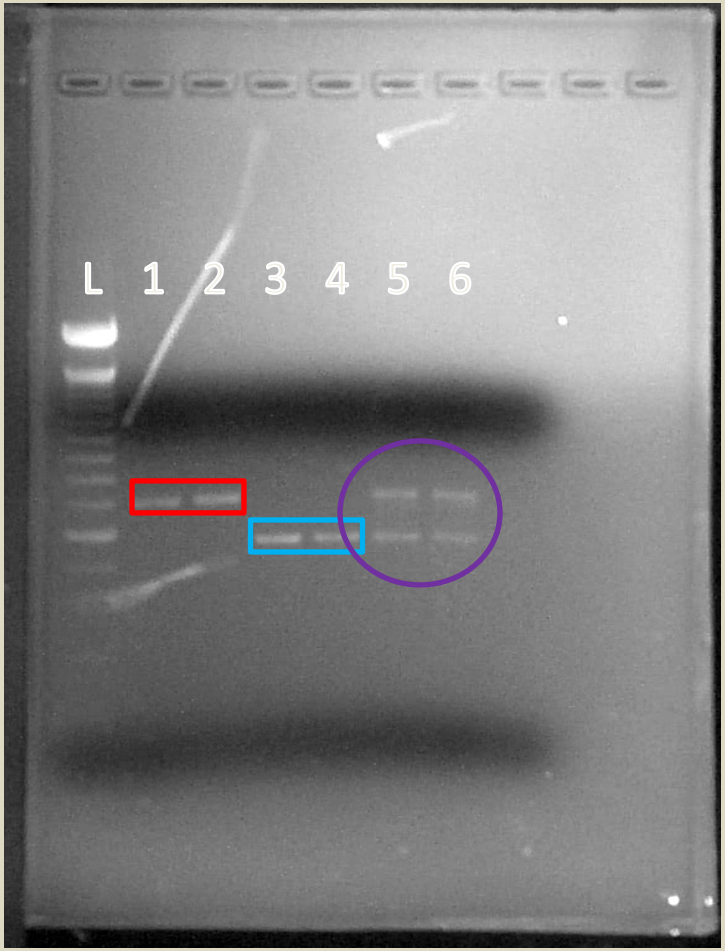




Species Specific Primer Design (rDNA)

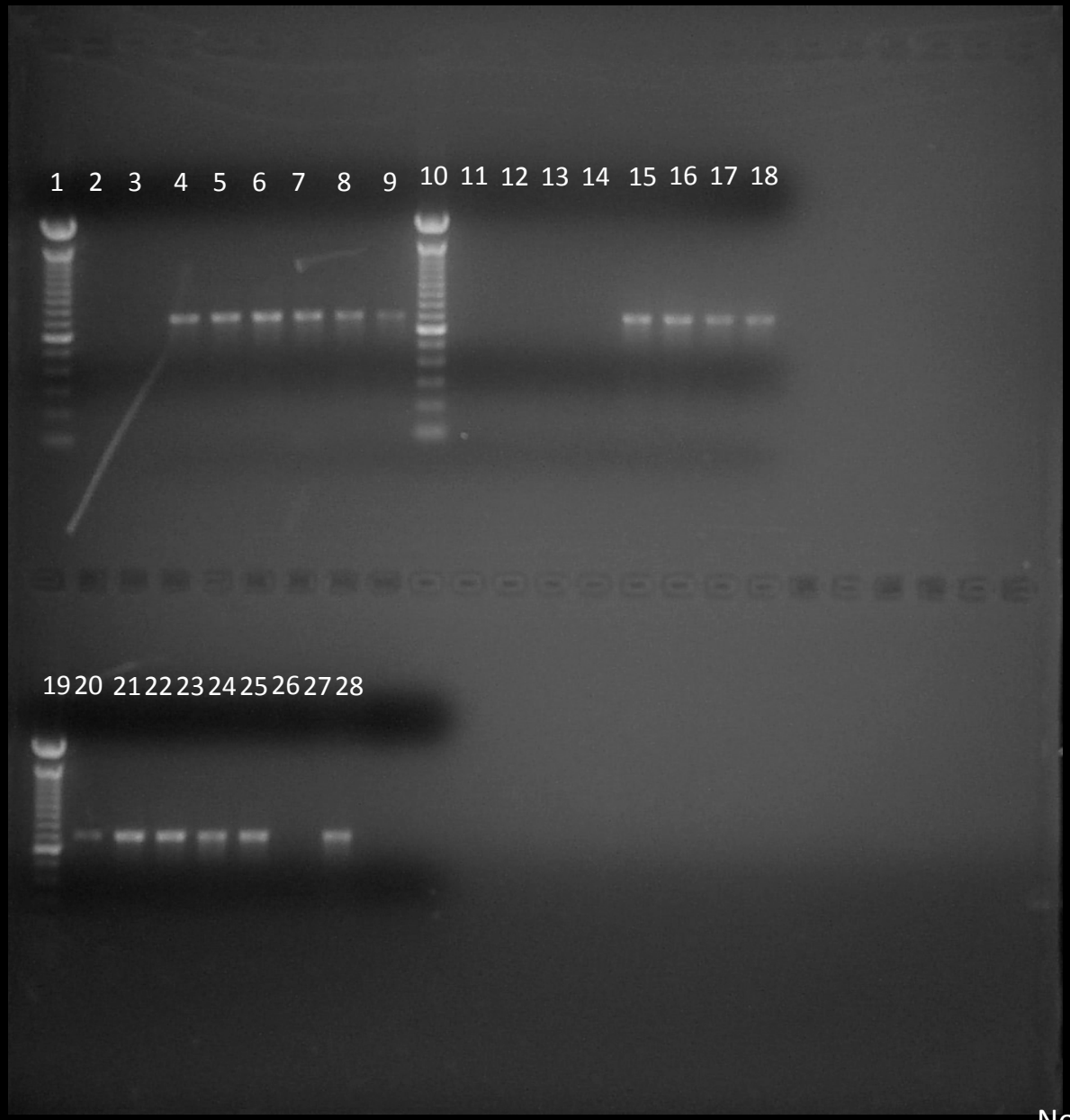


Novel Assay



LANE	SAMPLE
L	100 bp ladder (DNA Standard)
1-2	*Ae. triseriatus* (691 bp) amplicon
3-4	*Ae. hendersoni* (550 bp) amplicon
5-6	DNA from both

RH PCR 14 (21 June 2013) [ID using Aetris Primers only)



Lanes:

1: 100 bp Ladder
2: #34
3: #34
4: #37
5: #37
6: #39
7: #39
8: #45
9: #45
10: 100 bp Ladder
11: #48
12: #48
13: #50
14: #50
15: #64
16: #64
17: #69
18: #69
19: 100 bp Ladder
20: #70
21: #70
22: #71
23: #71
24:#72
25: #72
26: #73
27: #73
28: Negative Control

PCR Conditions: 40 Cycles, (95-54s, 54-30s, 72-45s)

Notes: PCR Amplification for all samples except #s 34, 48, and 50. No primer-dimer. No evidence of contamination.

Conclusions

- ***Ae. hendersoni* collected NOT only in the canopy!**
- ***Ae. japonicus* oviposits mostly at ground level**
- **Use of secondary characters increases ID success**
  - We like character setae 1-X!!
- **Novel PCR assay is useful for verification**
  - On going work

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Questions?

Western Carolina University

Vector-Borne Infectious Disease Facility