

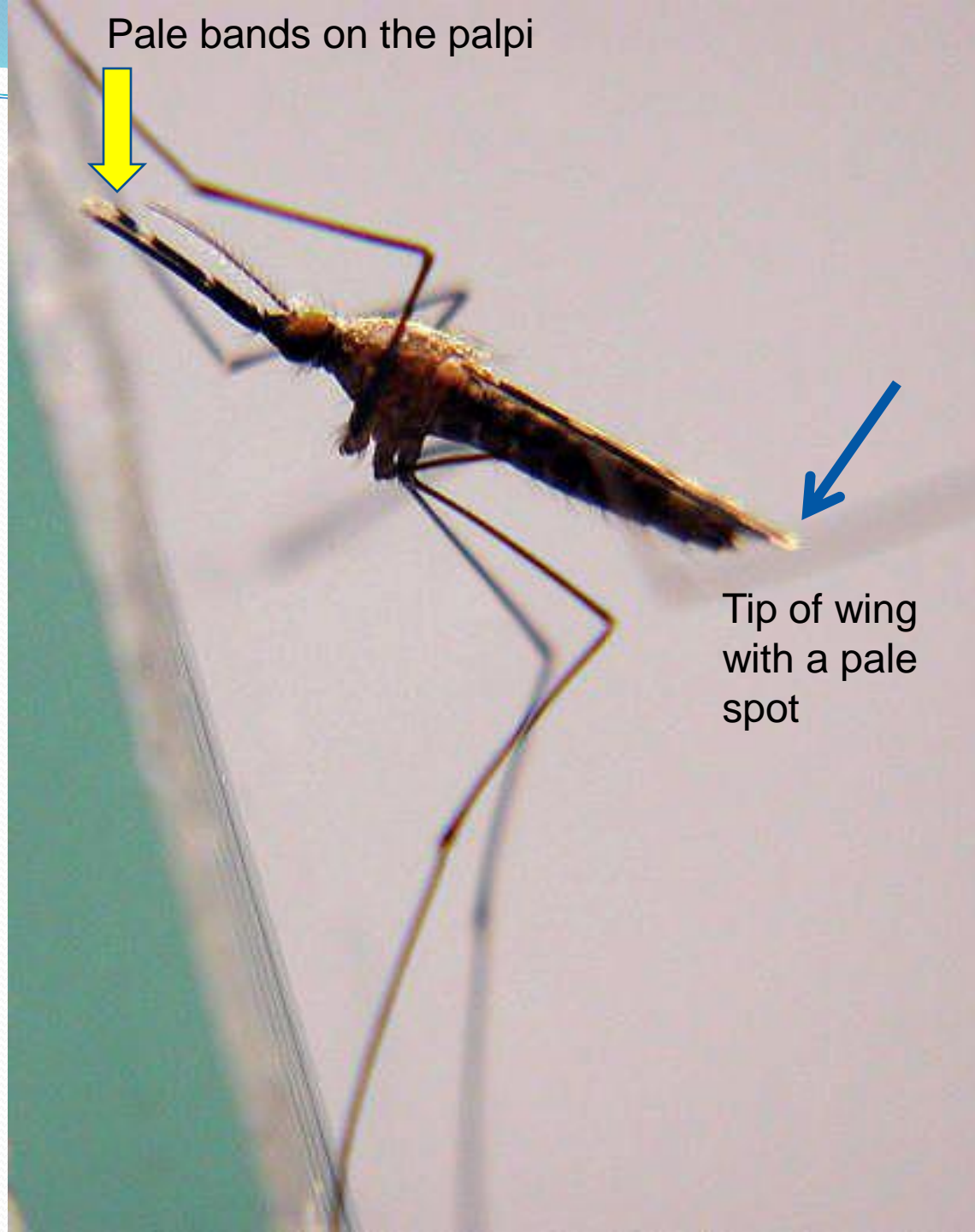
The *Anopheles crucians* Complex and *Anopheles georgianus*

**Bruce A. Harrison, PhD
PHPM, NC DENR, Winston-Salem, NC**

What is it,
Anopheles
crucians?

WRONG!

It is the
**Crucians
Complex**



Historical Information

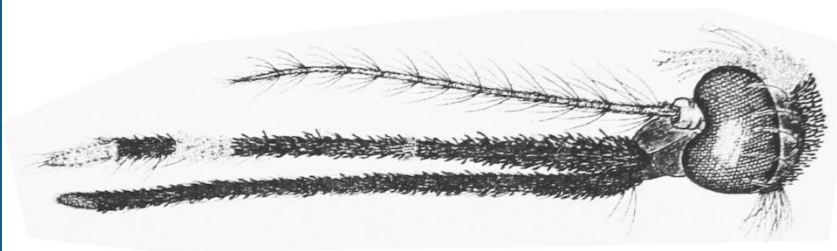
- Anopheles crucians*** was described in 1828 from the New Orleans area and is the 8th oldest *Anopheles* species.
- Anopheles bradleyi*** was described as a subspecies by King (1939) from Brevard County, Florida and elevated to species status in 1941. It is a brackish water species that occurs along or near the coast from New England to Texas.
- Anopheles georgianus*** was described as a subspecies by King (1939) from Brooks County, Georgia and elevated to species status in 1941. It is an rare/uncommon freshwater species that occurs from North Carolina to Louisiana.
- These were considered the *An. crucians* group until 1993.

Medical Importance of the *An. crucians* group

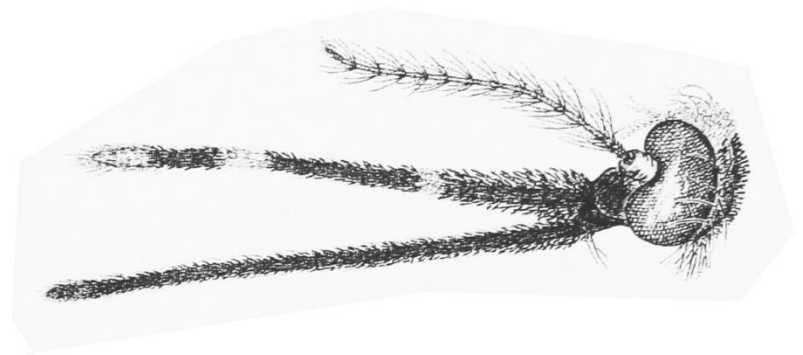
- ❑ *An. crucians* was found a good laboratory vector of both falciparum and vivax malaria in the U.S. in the 1920s.
- ❑ EEE virus was first isolated from pools of “*An. crucians*” collected in Louisiana and Georgia in the 1950s.
- ❑ South Carolina, Georgia, Florida, Louisiana, and Alabama have periodically isolated EEE from a number of pools of “*An. crucians*.” However, North Carolina has never found this virus in “*An. crucians*” although thousands have been pooled, even during active EEE periods.
- ❑ “*An. crucians*” has also been found positive for LaCrosse, Tensaw, Trivittatus, and West Nile viruses.



bradleyi

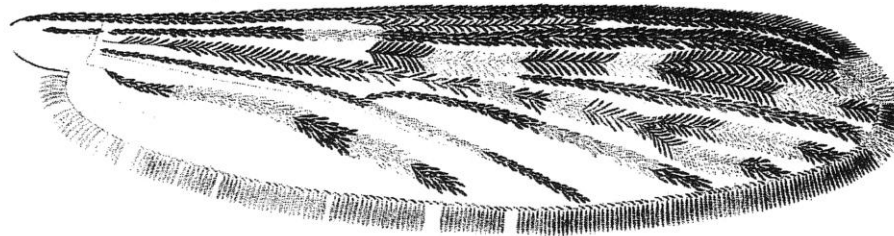
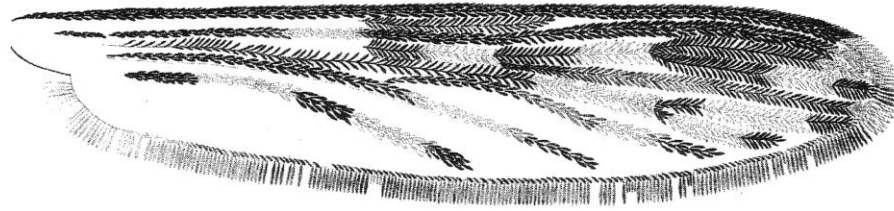
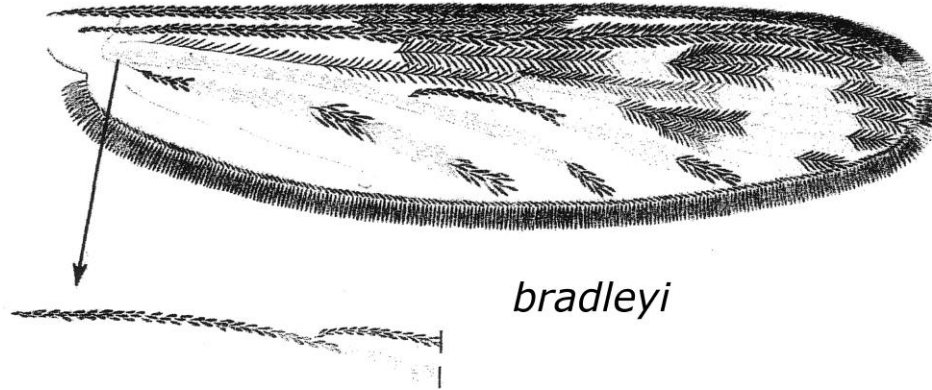


crucians

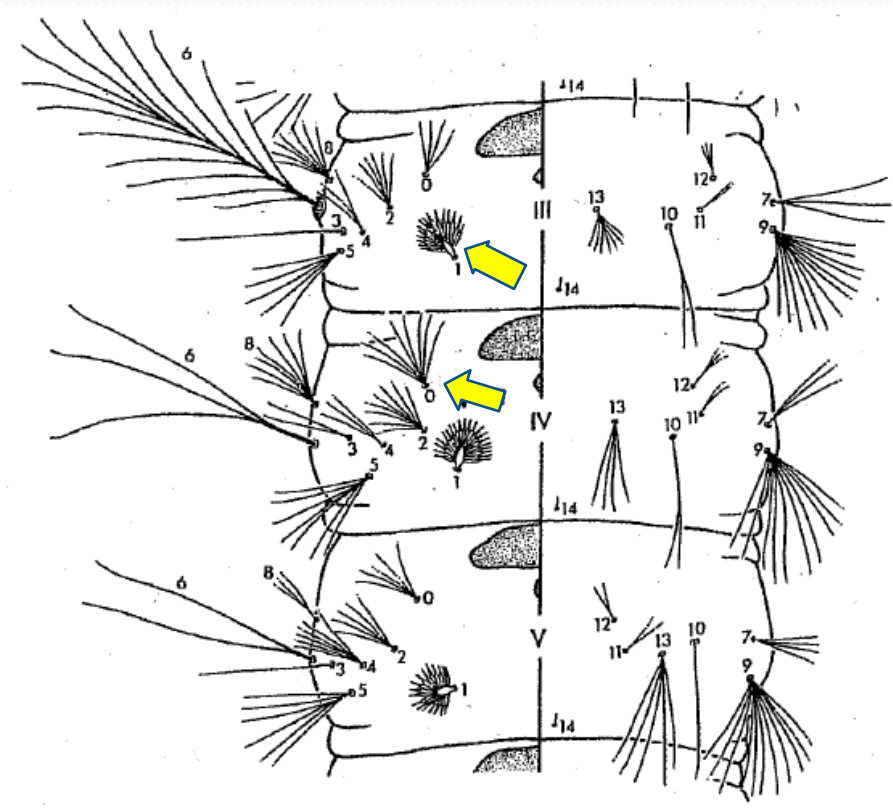


georgianus

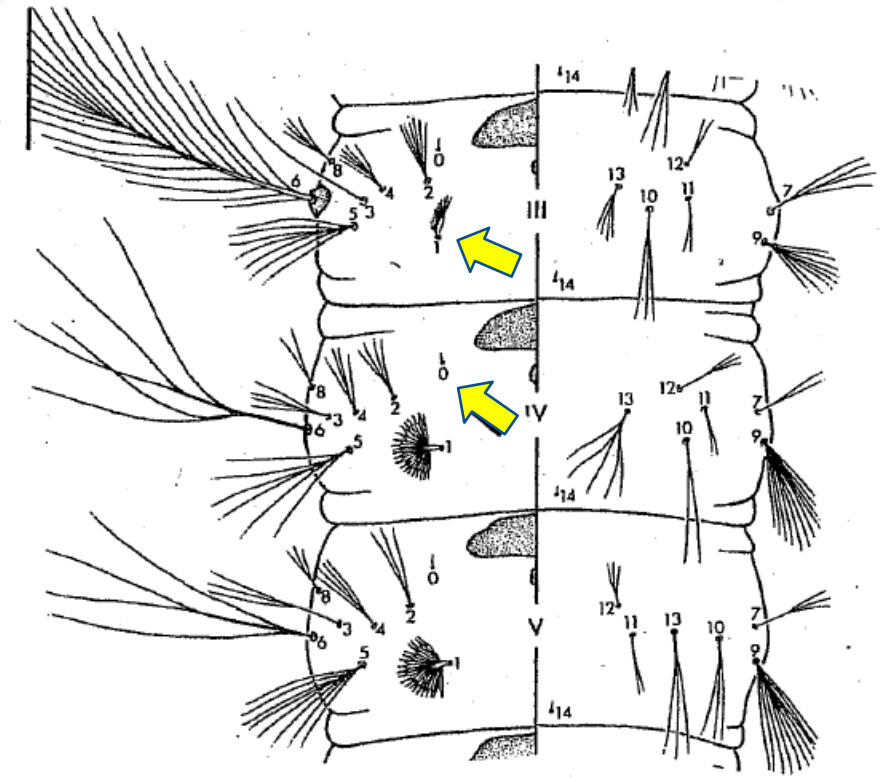
Wing Differences ?



An. crucians versus *An. georgianus*



crucians 4th instar

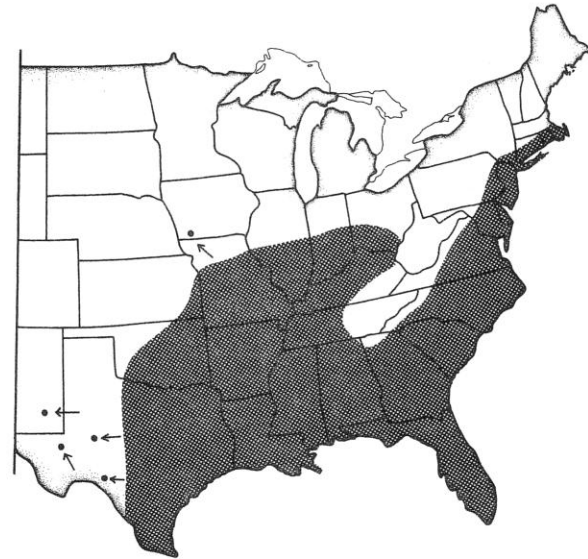


georgianus 4th instar

Distributions



bradleyi



crucians



georgianus

So everything is great, we have 3 species we can ID by distribution, biological differences and larval characters.

UNTIL 1993 !

In 1993 researchers at the USDA laboratory in Gainesville, FL, discovered by mitochondrial DNA tests that there were two other members of the group, one like *crucians*, and the other like *bradleyi*. But, *georgianus* was not found. This makes 5 species.

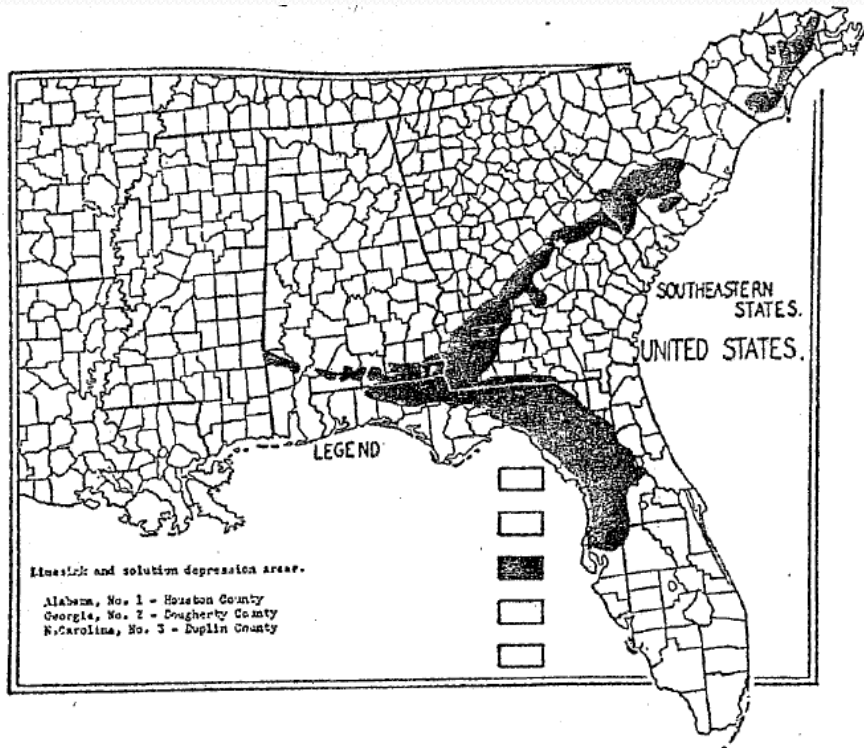
IN 2004 MORE TAXONOMIC COMPLICATIONS!

In 2004 morphological representatives of the 5 supposed species (above) were sent to the Smithsonian, and molecular systematists determined by rDNA ITS2 sequences that there were 6 not 5 species. This time only *bradleyi* was identifiable by morphology, and the other 5 species were designated A, B, C, D, and E, while *crucians* and *georgianus* could not be identified. Five of the species were collected at one site in central Florida.

ENOUGH!!

- In early 2007 I decided to quit butting my head against a wall with the other species and focus on finding the real *An. georgianus*. This species could not be identified in the adult stage, but its larval characters were supposed to be unique. This became a personal quest because I grew up in Brooks County!
- The last confirmed specimens of this species were collected in 1951, and it may no longer exist. However, there are special circumstances that can explain the absence of confirmed records.
- I reviewed the old literature to find descriptions of the larval habitats for this species. In other words, you must target the right habitat to find a rare species.

Limestone and Solution Depression Areas

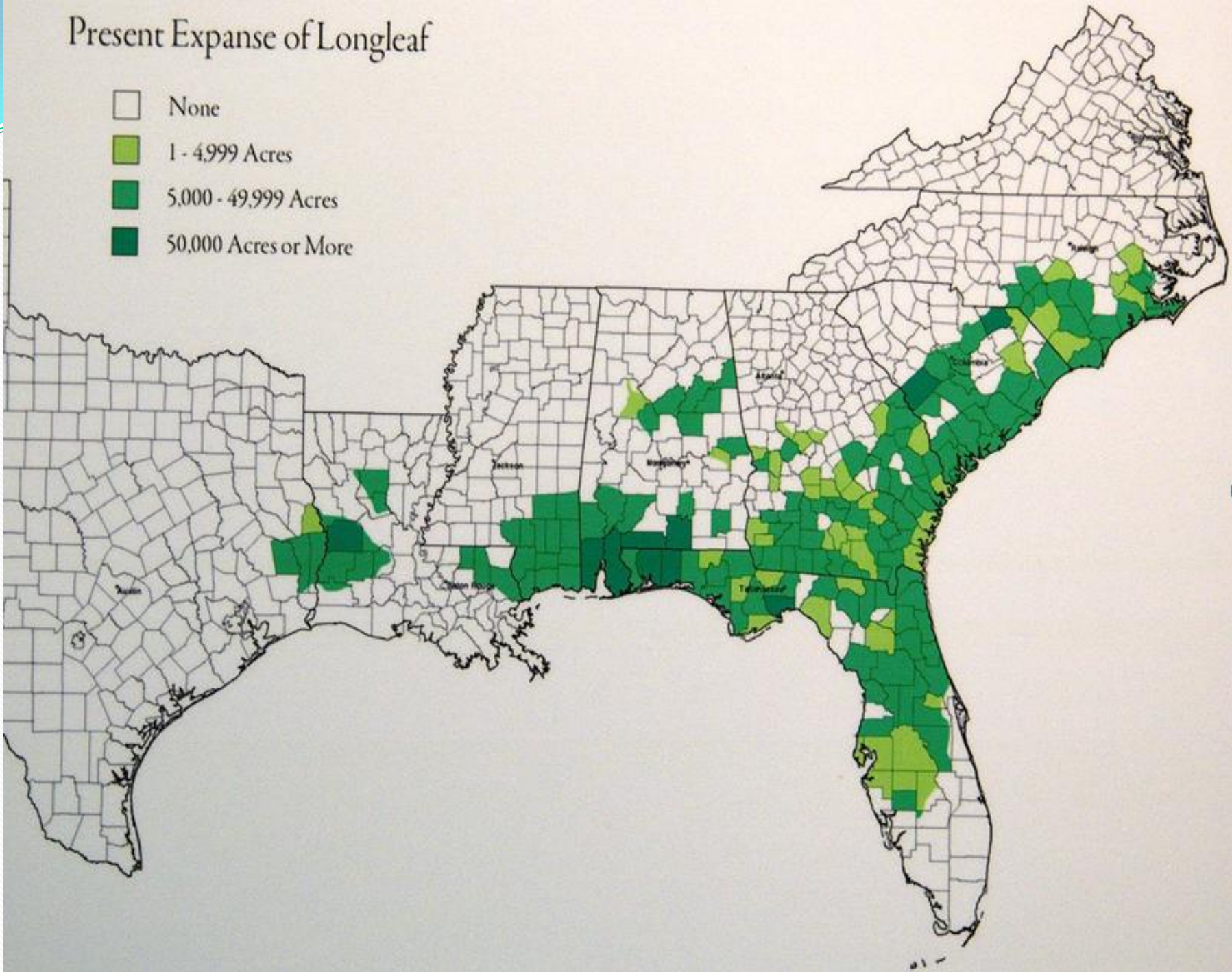


Floore, Harrison & Eldridge (1976)

FIG. 2. Limesink and solution depression areas in the southeastern U.S. (Boyd and Ponton 1933)

Present Expanse of Longleaf

- None
- 1 - 4,999 Acres
- 5,000 - 49,999 Acres
- 50,000 Acres or More

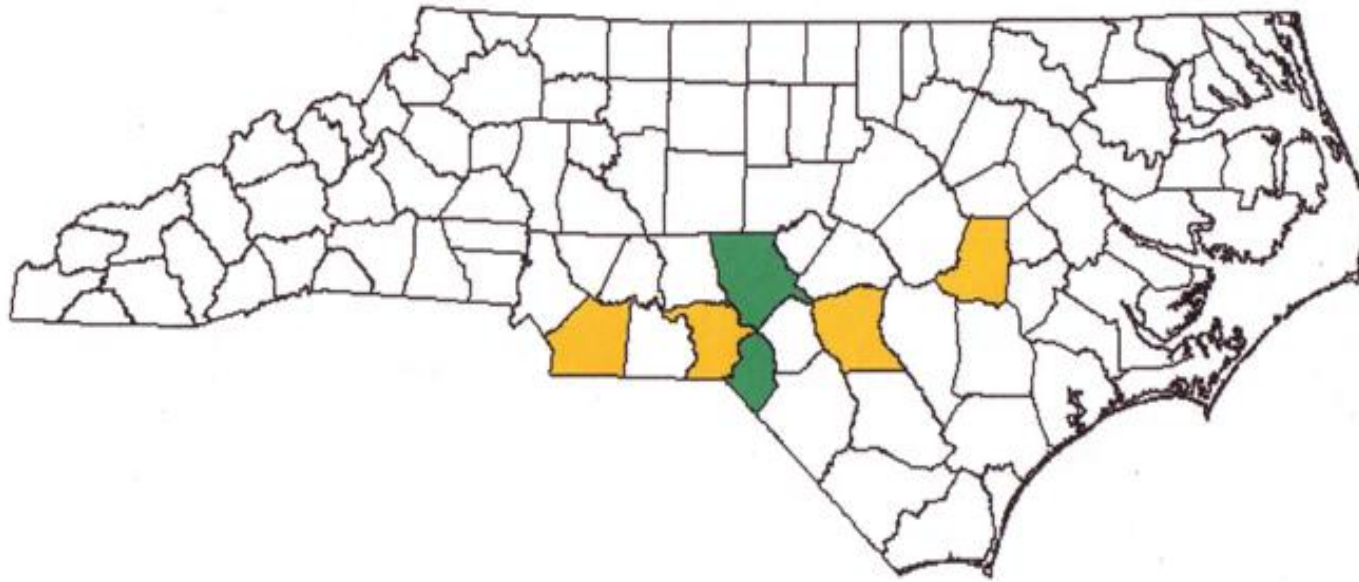


Must find the right habitat



An. georgianus King in North Carolina

(May 2007)



yellow = historical

green = 2007

Adults reared from larvae that fit the characters of *An. georgianus* were collected in 2007 in NC and submitted to the Smithsonian. They were determined by rDNA ITS2 to be a new member of the complex, species F. Now there are **7 members in the complex**. However, we are convinced that species 7 is actually *An. georgianus*. The search for new sites for *An. georgianus* continues.

Carolina Sandhills National Wildlife Refuge, Chesterfield Co., McBee, SC, 2008

Tiny shallow pools
and deer tracks
containing running
seepage water



P. Whitt

Sloping hillside with endangered plants and
seepage water containing *An. georgianus*



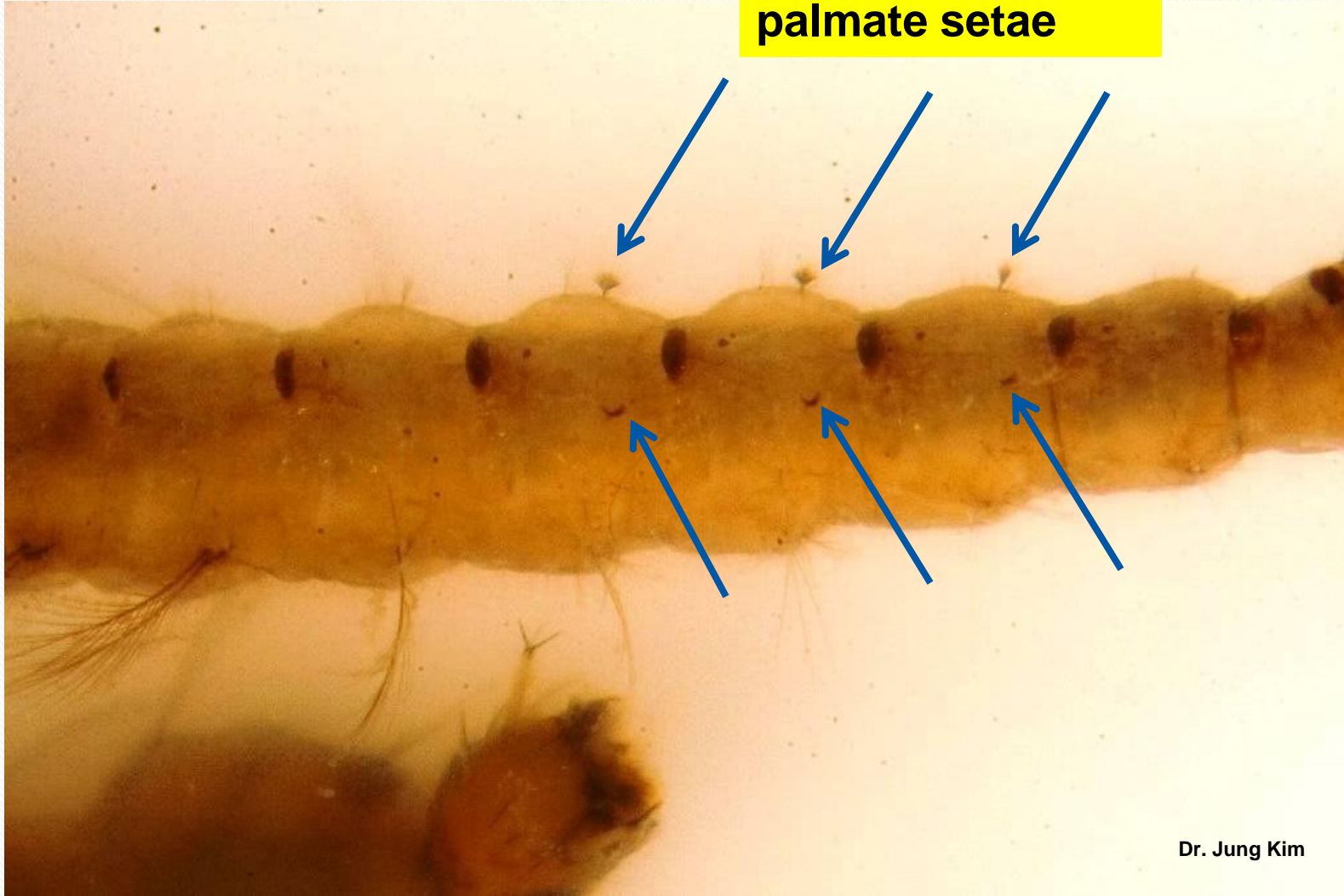
P. Whitt

I even went to Ichauway Preserve in 2009 to search for *An. georgianus* with some crazy people I don't know to collect in a partially collapsed sink hole.

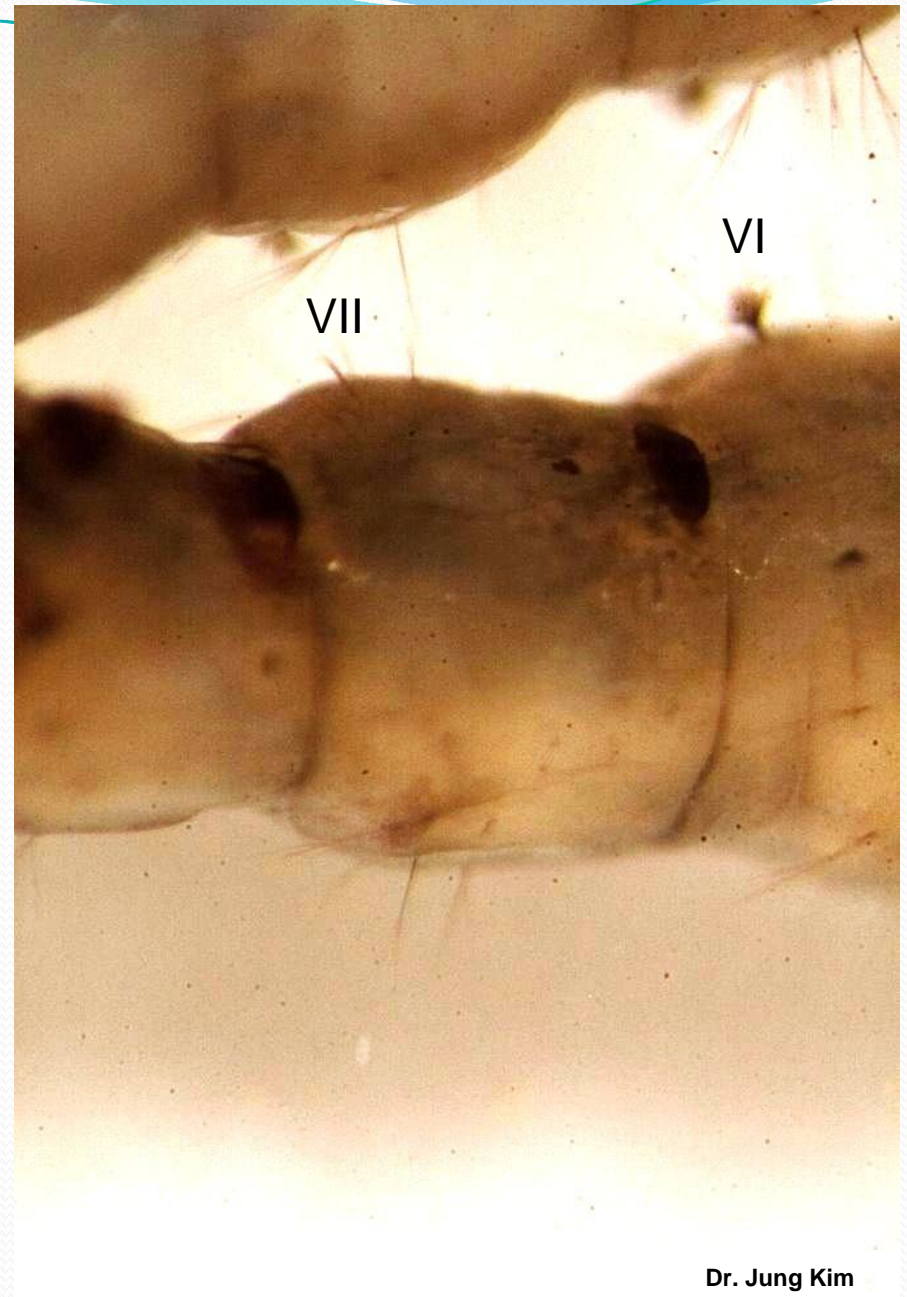
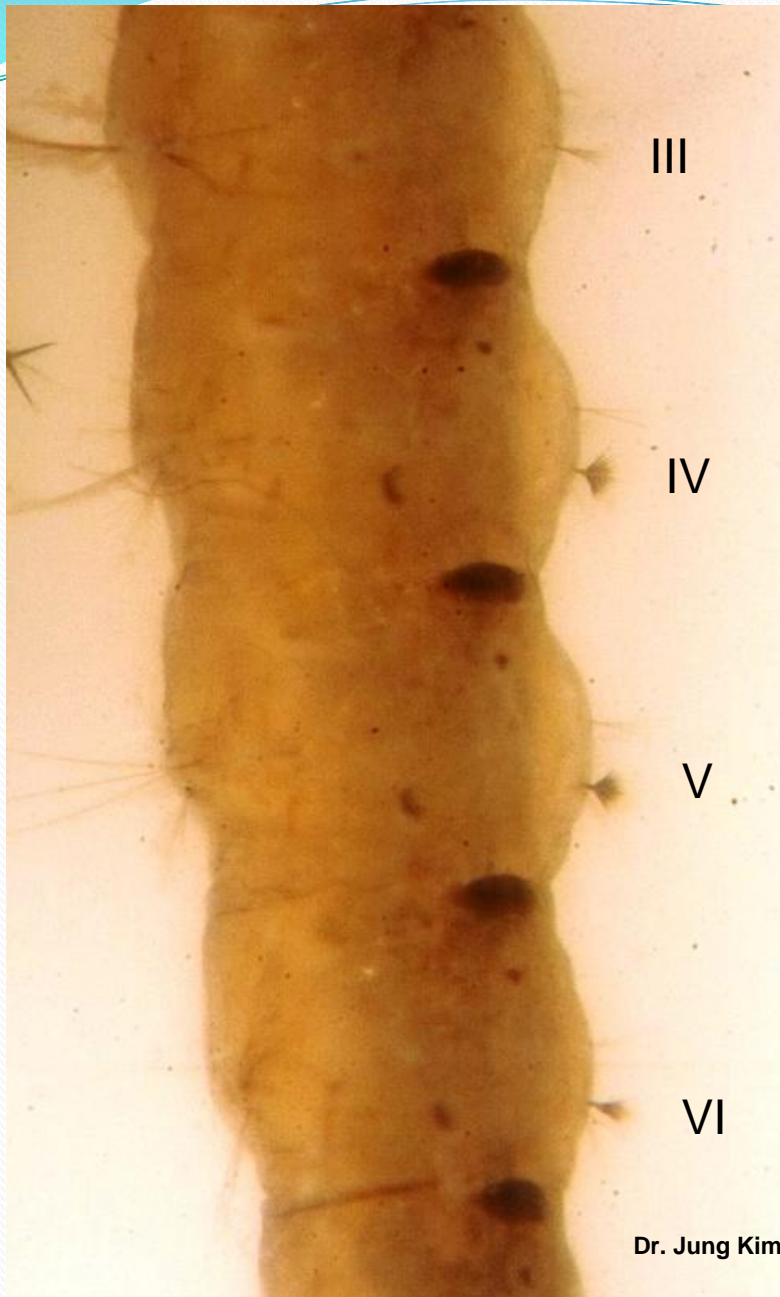


An. georgianus larvae are easy to spot

Only three pairs
of fully developed
palmate setae



Palmate setae on *An. georgianus*



Lateral view of 4th instar *An. georgianus*



Dr. Jung Kim

An. georgianus larvae are adapted to live in flowing water (seepage)

Seta 3-X branches on the last segment of the larva have curved stout tips used for anchoring the larva in flowing water



BIOLOGICAL OBSERVATIONS FOR *AN. GEORGIANUS*

- 1. Disturbed larvae play “possum” for at least a minute on the bottom of your dipper. They rest in an angular V-shape or angular S-shape and look like a tiny broken twig.**
- 2. Fourth-instar larvae appear black to the naked eye, but under a dissection microscope they are either dark brown or dark green with scattered tiny gray spots.**
- 3. Abdominal segment III on the larva normally does not have a large dorsal pale spot like many specimens of other *Anopheles*.**
- 4. They prefer very shallow clear flowing seepage water, but can occur in clear water pools with emergent grass. We did not find them in heavily tea-stained water.**

Summary Information

1. There are now 7 members in the Crucians Complex: *bradleyi*, *georgianus*, and *crucians* A, B, C, D, and E. Additional species may be found in the future. Eventually someone will find out which of the 5 lettered species is really *crucians* and the other 4 lettered species (which are new species) will need to be described in a publication to meet the naming requirements of the International Commission of Zoological Nomenclature (ICZN).
2. In North Carolina we now have records for *bradleyi*, *georgianus*, and *crucians* A, D, and E.
3. Because of the above situation it is best to identify all adults as the Crucians Complex.

Summary Information (Cont.)

- 4. *Anopheles georgianus* has been found again, after 59 years with no confirmed collections. It still must be identified by the larval stage or by molecular assays. It requires very specific and uncommon larval habitats and is an uncommon species.**
- 5. Over 100 adults of *An. georgianus* have been reared with associated larval and pupal exuviae from three counties in North and South Carolina.**
- 5. *An. georgianus* is not known to be of medical importance, but historically it has been recorded biting people.**
- 6. The search for new sites for *An. georgianus* continues, as does the search for other morphological characters that will identify this species.**

Counties in Georgia with confirmed records for *An. georgianus*

1. Bibb
2. Baker
3. Brooks
4. Colquitt
5. Liberty
6. Lowndes
7. Muscogee
8. Richmond
9. Sumter
10. Terrell
11. Thomas

My title did not fit what was on the agenda, but I did bring *An. georgianus* home to you.

Now it is your turn to find it again!

ACKNOWLEDGMENTS

- 1. Parker Whitt, PHPM, NC DENR, Winston-Salem, NC.**
- 2. Dr. Chris Evans, DHEC, Columbia, SC**
- 3. Dr. Fredi Ruiz, WRBU, Smithsonian Institution, Washington, DC**
- 4. Dr. Jung Kim, PHPM, NC DENR, Raleigh, NC**