



Egg Identification Guide for *Aedes albopictus* in the Northeast, USA

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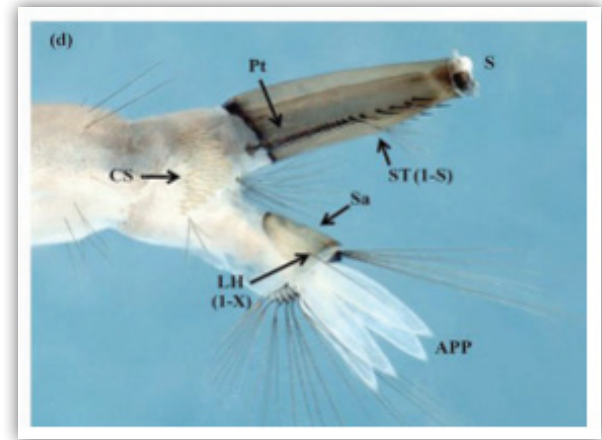
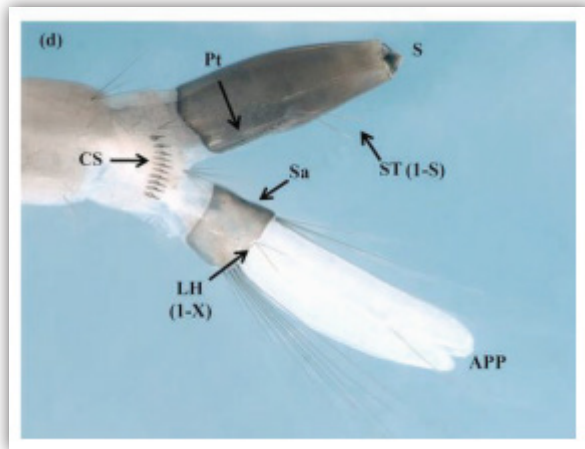


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Current Methods of Ovitrap Surveillance

1. Hatching and rearing eggs to identify as larvae

Larval identification depends on anatomical features that can only be seen under a microscope, as show in the example below:



Key characteristics used to differentiate *Ae. albopictus* (left) vs. *Ae. japonicus* (right)
from Farajollahi, Ary, and Dana C. Price, 2013

2. Hatching and rearing eggs to identify as adults

3. qPCR of eggs (quantitative polymerase chain reaction)

- This method extracts genetic material from the eggs, which can be identified to species.
- The relative amount of genetic material detected from each species can be used to estimate the number of eggs in the trap belonging to each species.

Why do egg identification?

Egg identification can distinguish *Aedes albopictus* eggs from other urban *Aedes* species while avoiding the issues of other methods.

Rearing and identifying as larvae or adults

- Slow process
- Requires the eggs to be in good condition
- Requires the eggs to be out of diapause
- Takes a lot of space
- Some data is lost due to embryo mortality

Identifying through qPCR

- Expensive
- Requires very specialized equipment
- Destroys the eggs, so no further experiments can be conducted
- Imprecise

Egg Collection & Identification:

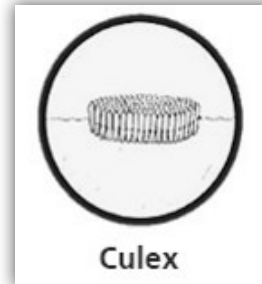
We are Looking at Species that Lay Eggs Above the Water Line in Containers

Mosquitoes lay eggs in three ways:

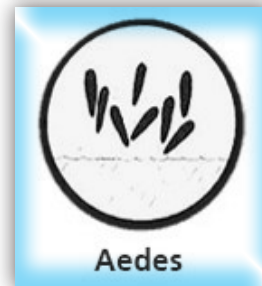
1. Individually, on the water. This method of egg laying applies to *Anopheles* mosquito species.



2. In rafts, on the water. This method of egg laying applies to *Culex* mosquito species.



3. Individually, pasted above the water line. This method of egg laying applies to *Aedes* mosquito species.



This egg identification guide is looking at *Aedes* mosquitoes, which lay eggs above the water line in containers.

- Traditionally, a paddle (stick) is placed in a partially-filled water container for *Aedes* mosquitoes to lay eggs on.
- Paper lining can be a better option, as it provides more surface area.



Container Species that Lay Above the Water Line in the Northeast USA

We have three *Aedes* species that we regularly collect in ovitraps in the Northeast:

1. *Aedes japonicus*
(Asian bush mosquito)



Judy Gallagher; <https://www.flickr.com/photos/52450054@N04/1408470561/>

2. *Aedes triseriatus*
(Eastern tree hole mosquito)



<http://www.lawrencereeves.com/mosquitoes/>

3. *Aedes albopictus*
(Asian tiger mosquito)



Ilustração; <http://www.imprensadopovo.com.br>

Aedes japonicus: Asian bush mosquito

- Thought to be a generalist mammalian biter, not an aggressive human biter
- Colonizes urban and forested habitat
- Lays eggs in tree holes and artificial containers
- Invasive species to the USA. Native to Asia and introduced in the mid- to late-1990s
- Thought to be a **moderate** disease vector
 - Has been implicated in outbreaks of Japanese encephalitis in Asia
 - Has been found infected with West Nile virus and La Crosse virus in the USA
 - Laboratory competent in several studies for other encephalitic diseases



Judy Gallagher
<https://www.flickr.com/photos/52450054@N04/14084705611/>



Ary Farajollahi; Bugwood.org

Aedes japonicus Eggs

- Matte finish
- Uneven, rough appearance
- No distinct, regular geometry of the surface ridges



Untouched photograph. Image taken at 40x using specialized equipment (Macropod Micro Kit) at the Cornell University Insect Collection.

Aedes triseriatus: Eastern tree hole mosquito

- Thought to be a generalist biter, but will feed on people
- Colonizes forested habitat and occasional urban habitat
- Lays eggs primarily in tree holes and artificial containers
- Native species to the USA
- Thought to be a **moderate** disease vector
 - Has been implicated in La Crosse virus transmission
 - Field isolates of West Nile virus have been documented
 - Laboratory competent for several other viral diseases



Susan Ellis; Bugwood.org



<http://www.lawrencereeves.com/mosquitoes/>

Aedes triseriatus Eggs

- Very similar to *Aedes japonicus* eggs
- Matte finish
- Uneven, rough appearance
- No distinct, regular geometry of surface ridges



Untouched photograph. Image taken at 40x using specialized equipment (Macropod Micro Kit) at the Cornell University Insect Collection.

Aedes albopictus: Asian tiger mosquito

- Aggressive human biter, will feed on other species as well
- Colonizes urban habitat
- Lays eggs in artificial containers
- Invasive species to the USA. Native to Asia.
- Transmits multiple diseases globally
 - Has been implicated in outbreaks of dengue, chikungunya, Zika
 - Has been found infected with West Nile virus, EEE, and La Crosse viruses in USA
 - Laboratory competent for over 20 arboviruses
- Has not been implicated in transmission in the Northeast USA



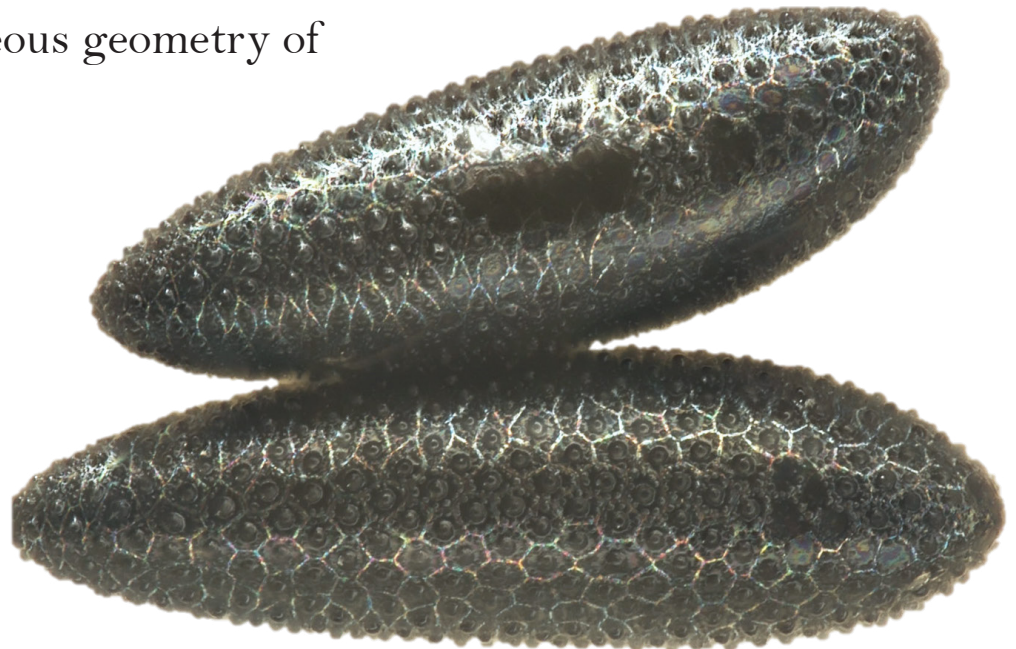
Ary Farajollahi; Bugwood.org



Windsor-Essex County Health Unit; www.wechu.org

Aedes albopictus Eggs

- Shiny finish
- Spherical, evenly spaced horns
- Regular, homogeneous geometry of surface ridges



Untouched photograph. Image taken at 40x using specialized equipment (Macropod Micro Kit) at the Cornell University Insect Collection.

Aedes japonicus Eggs



TOO SIMILAR TO TELL
APART



Aedes triseriatus Eggs

Aedes albopictus Eggs



Steps to Identify Eggs

1. Set and collect eggs from ovitraps

- For instructions on making your own ovitraps out of plastic 2-liter soda bottles, read the Mosquito Egg Trap Instructions document located at <https://blogs.cornell.edu/harrington/tigernet>

2. Remove oviposition papers and transport to the laboratory in a plastic bag containing a moist sponge or paper towel to maintain humidity

3. Affix the portion of the oviposition paper containing eggs to a microscope slide using double-sided tape

- If the number of eggs on the oviposition paper is low, all eggs can be examined.
- If the number of eggs on the oviposition paper is high, a sample of eggs can be tested. We removed the left-most 25x25mm, the central 25x25mm, and the right-most 25x25mm sections of the papers containing eggs for our samples.

4. Examine the microscope slide under a compound microscope using a light source that illuminates from above.

- It is helpful to toggle between 10x and 40x when viewing the eggs.

This is How it Looks Under a Compound Microscope

Aedes albopictus Eggs



Aedes japonicus/triseriatus Eggs

Careful! *Ae. japonicus/triseriatus* eggs can have distinct, isolated ridges. BUT, these ridges are never regular, semi-spheres like *Ae. albopictus* eggs. Think leopard spots vs. polka dots.

Note:

Even dead (deshicated) eggs can be identified to species with this method, as seen in this photograph.

