Nationwide Surveillance Plan for *Culicoides* species

Purpose

Oropouche virus (OROV) was first detected in 1955 in Trinidad and Tobago and has been found most frequently in the Amazon basin. In 2024, human cases of OROV were reported outside of this historic region, to include outbreaks in Cuba. In addition, travel-associated cases have been identified in many more countries, including the United States and Europe. *Culicoides paraensis* is widely considered to be the primary vector for OROV throughout the Amazon basin. *Culicoides paraensis* occurs throughout much of the eastern and central U.S.; however, targeted surveillance for this species has been lacking and its importance as a potential OROV vector in the U.S. is poorly understood.

With the emergence of travel related OROV cases, there has been a need to define the distribution of potential *Culicoides* vectors more accurately within the continental United States. AMCA, as a partner with the Centers for Disease Control and Prevention (CDC), has proposed to help fill this urgent need with your assistance. Because our members are recognized leaders in the field of vector surveillance, we are asking that you assist in refining the national map of *Culicoides* vectors, with focus on *C. paraensis*, in the U.S.

Trapping Protocol and Frequency

Surveillance of adult populations has proven to be a quick and informative method for determining biting midge distributions. Although there are a variety of trap types available for the collection of *Culicoides* midges, we are requesting programs use a modified CDC Miniature Light Trap *(see design protocol below)* to collect adult midges. These traps are lightweight, portable, and battery operated and should be baited with dry ice (or a similar source of CO_2) to increase their appeal to host-seeking midges. By using the same trap types and trapping methodology, we can ensure comparable data is collected throughout the country.

We are asking that approximately 5-6 traps be placed throughout your surveillance jurisdiction in habitat conducive to produce *Culicoides paraensis (see habitat description below).* Ideally, each trap is set once per week in the same location all summer.

We would request that the traps be set in the mid-afternoon, allowed to run overnight, and be collected the following morning. In this manner, one trap site would consist of a single trap night each week.

Please record the latitude and longitude of each trap location, trap location name, the date each trap is set. Trapping best practices and *Culicoides* life history information gathered from surveillance work conducted in your region is also welcomed and encouraged.

There is no need to sort the specimens or attempt counting of the specimens collected. However, if your agency has trained staff that can preliminarily sort trap collections by separating *Culicoides* from other trap material (bycatch), that would help expedite the identification process. Basic identification materials and training can be provided upon request. Vials of collected specimens should be stored and ultimately shipped to the CDC (or another partner agency) for thorough sorting and identification. ***Addresses to be provided.***

Habitat Preference for Trapping

Culicoides adults are most abundant near productive larval habitats but will disperse to mate and feed. Females will readily fly a mile or more from their larval habitat sources in search of bloodmeals. The adults can live a several weeks under normal conditions.

Similar to mosquitoes, both males and females feed on nectar, but the females require blood for their eggs to mature. The females will blood-feed primarily around dawn and dusk; however, there are some species that prefer to feed during the day. Larvae require water, air and food and are not strictly aquatic or terrestrial.

Almost all *Culicoides* require moisture-rich habitats for development of egg, larval and pupal life stages and the availability of these environments is key to their distribution, abundance and seasonality. However, they are not strictly aquatic. *Culicoides paraensis* prefer dry to moist tree hole environments. Their larval development sites do not need to retain standing water for complete larval development. Although wooded areas are more likely to have tree holes, they are also highly adaptable and can occur in urban environments as well.

Based on information gleaned from the literature, additional ecological, phenological, and surveillance data for *C. paraensis* is summarized below and can be used to help determine where and when to set traps in your region (modified from Dunford *et al*., CDC, in prep):

Culicoides paraensis have been taken in light traps from late April to November in southern states such as Louisiana and Alabama and from July to August in Mid-Atlantic states such as Virginia. *Culicoides paraensis* were collected in Tennessee using CDC Miniature Light Traps from April to September. Seasonal adult activity appears to follow similar peak activity patterns of many mosquito species, with longer seasons (April to November or longer) in southern states and peak activity from June to August in more northern states. *Culicoides paraensis* is mainly found in tree holes and moist wood debris, with reports of it being reared from sap and rotten vegetation.

Culicoides paraenesis is considered a mid to low elevation (<1000m), tree hole inhabiting species. In Tennessee, *C. paraenesis* was found to have a distinct canopy preference (i.e., 9-22m), being one of the dominant diurnal feeders from April to October. Movement was observed upward along the tree trunk and spreading horizontally within the canopy, perhaps a function of larval/resting site preferences and access to birds. It has been recorded at a much lower preference (5m) in Wisconsin hardwood forests. Several living tree species have been identified as hosting *C. paraenesis*, in addition to stumps and standing dead trees. Tree hole collections of *C. paraenesis* have occurred across a pH range of 4.1-9.4, with the greatest number of collections occurring in greater than 8.7 range in a variety of tree species (i.e., *Liquidamber styraciflua* (sweet gum), *Magnolia grandiflora* (magnolia), *Quercus imbricaria* (shingle oak), *Q. nigra* (water oak), and *Q. virginiana* (southern live oak). Though not sampled directly, several species of mixed hardwoods have been present when *C. paraensis* collections were made to include sweet gum, eastern cottonwood (*Populus deltoides*), American sycamore (*Platanus occidentalis*), silver maple (*Acer saccharinum*) and box elder (*Acer negundo*) and can be reasonably presumed that they also serve as larval habitat. *Culicoides paraensis* has also been reported along coastal Georgia barrier islands in maritime forests (likely to be in primarily live oak forests) from late August-September, and in hardwood forests and ravine habitats along coastal plains in Georgia.

Culicoides paraensis has been found to readily feed on both cattle and white-tailed deer in the early morning hours of 0530-0800 hours from April to October from direct collection in southern states such as Alabama. They have been readily collected feeding on white tailed deer doe- and calf-baited traps in Great Smoky Mountains National Park (GSMNP); it has also been reported feeding on humans and a feral cat in GSMNP. Several studies that reported *C. paraenesis* noted that they were either reared from tree-hole collections, collected in animal-baited traps, collected feeding on deer, collected in deer breeding facilities/farms, or collected “biting man”; additional collection records include feeding on rabbits and galliform birds such as turkeys and chickens in poultry houses. More recent light trap collections have been taken in zoo settings suggesting this species will also feed on various other animal fauna. There are many reports of females relentlessly biting humans, and it has been reported feeding on humans during the day, before intensifying biting activity at dusk. The literature also noted that *C. paraensis* was a diurnal feeder in Virginia and has been “collected extensively on man”.

Modified CDC Miniature Light Trap Design

Please refer to the included *"Modifying CDC Light Trap Chambers for Mosquito and No-see-um Collection"* protocol[1] as provided by Nathan Burkett-Cadena, PhD at the University of Florida, Florida Medical Entomology Laboratory (file name: Mosquito Trap No-see-um modification_Burkett-Cadena FMEL_2025.pdf). This protocol will detail precisely how to modify a standard CDC light trap for the collection of *Culicoides*.

AMCA has limited funding to support to Member agencies capable of setting traps and collecting specimens for identification, if needed.

Please reach out to Dan Markowski (amca.ta@mosquito.org) if you need assistance, trapping supplies or have additional questions.

[1] Burkett-Cadena's protocol calls for adding 70% isopropyl alcohol to the 50ml vial for collection and storage. This technique can be difficult for taxonomists. If you have access to dry ice, you do not need to include any alcohol solution in the 50ml vials, but you would need to quickly cap the vials and freeze the samples upon collection.

Protocol Summary
for
Collection/Shipment of Biting Midge Specimens for Identification (and PCR analyses/Pathogen Screening if available)

1. Utilize modified CDC Miniature Light Traps. Please refer to the included *"Modifying CDC Light Trap Chambers for Mosquito and No-see-um Collection"* protocol as provided by Nathan Burkett-Cadena, PhD at the University of Florida, Florida Medical Entomology Laboratory.

2. Service CDC miniature light traps daily if possible, weekly at a minimum.

3. A cooler with ice packs or dry ice can be used in the field, if available, to transport collection cups back to a location for sorting.

4. Separate biting midges (and smaller dipterans) from other non-dipteran, larger insects in the collection cups. Specimens may be kept frozen, at 4 °C, and/or on chill tables during the sorting process. Separate *Culicoides* specimens if presorting and preliminary identification can occur at your agency.

5. Process trap samples as quickly as possible and store vialed samples in a freezer. Because storage in some alcohol solutions can cause wing pattern fading and make identification difficult, we strongly suggest you only use 70% isopropyl alcohol in the collection or storage of any specimens. If you have dry ice available, you can immediately freeze the specimens upon collection to keep them in the vials.

6. Specimen vials should include labelling containing latitude and longitude of each trap location, trap location name, the date each trap is set.

7. Specimens should be stored/shipped at one of the following conditions:
   - Dry, frozen at -80 or -20 °C (if dry ice/cold chain materials are available)
   - With 70% Isopropyl alcohol, stored at 4 °C (if dry ice/cold chain material are not available)

If specimens are stored in 70% isopropyl alcohol, the protocol below is not needed. Vials can be packed, topped off with alcohol, taped around the cap edge, and shipped directly to CDC. Address to be provided.

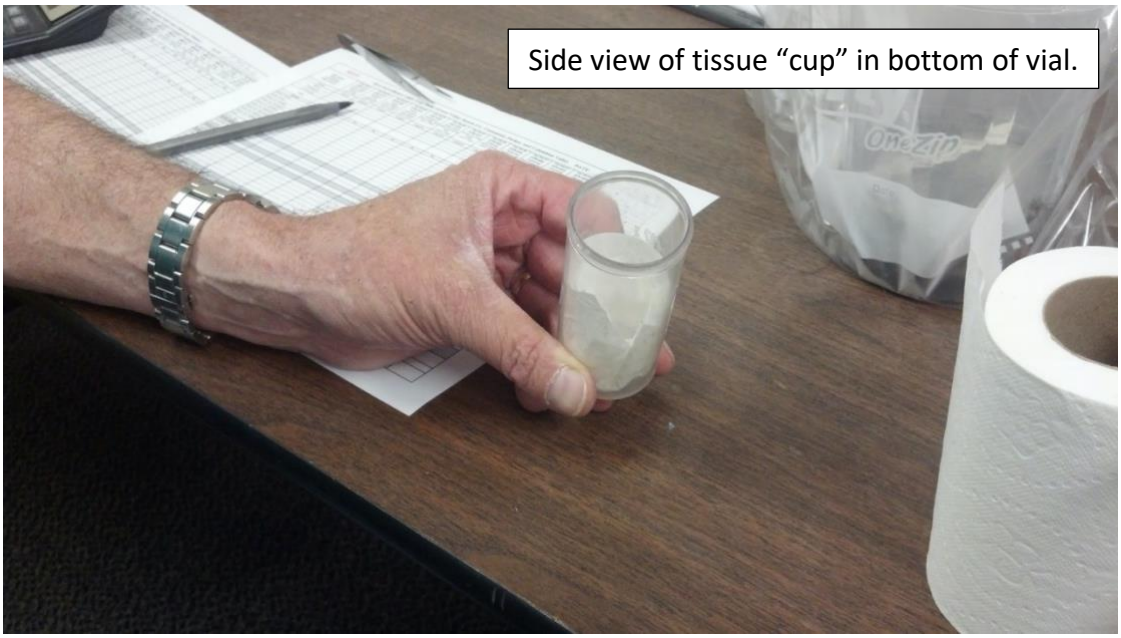
Shipping of Specimens

Contact Dan Markowski (amca.ta@mosquito.org) for address to ship specimens for identification.

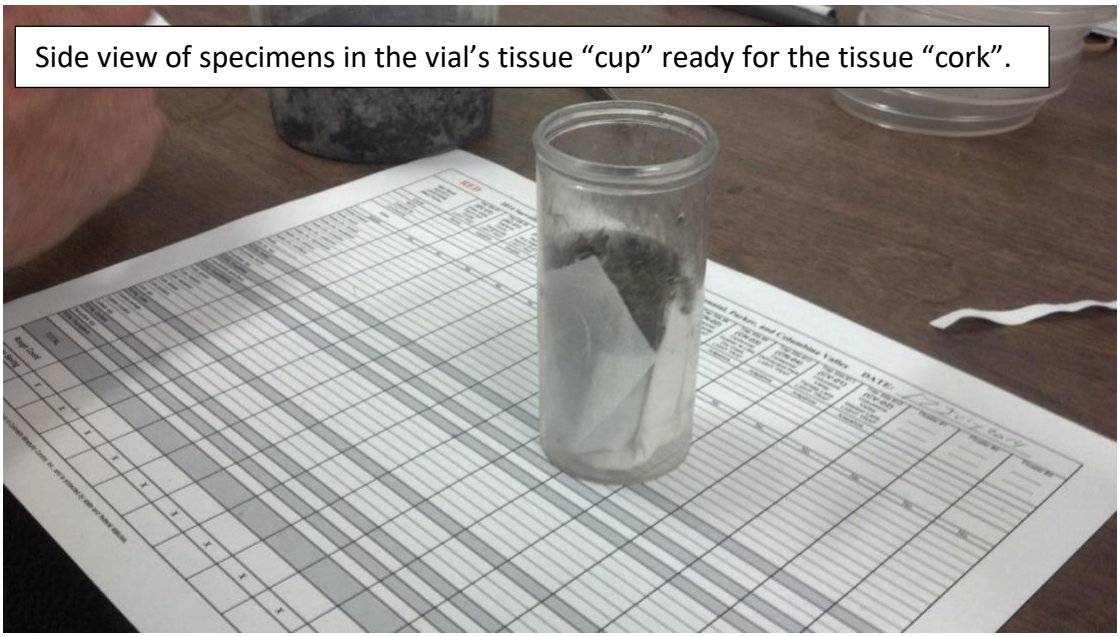
Dry Sample Vial Preparation – cushioning samples for shipment (adapted protocol from Dr. Michael Weissmann, VDCI Entomologist):

1) Place a sheet or 2 of toilet paper or cheap tissue on the bottom of the vial to form a "cup" to hold the specimens. *(Note: The specimens should be shipped in 50ml conical vials, not the vials pictured. All other protocol parameters remain the same.)*

View looking into the vial at "cup" of tissue.



2) Add specimens.

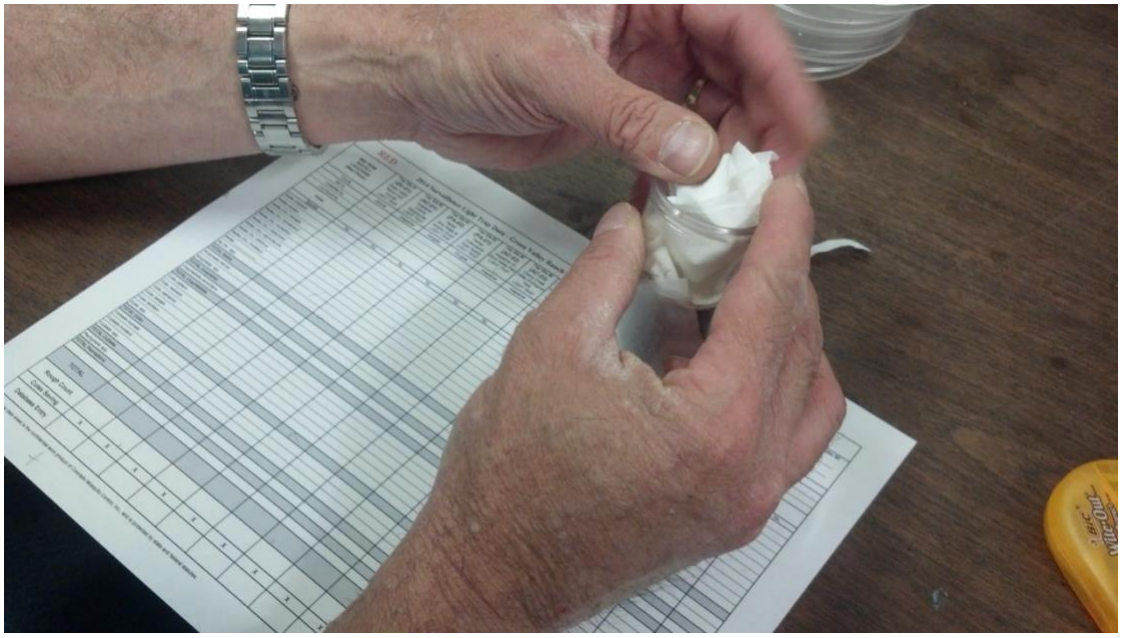


Side view of specimens in the vial's tissue "cup" ready for the tissue "cork".

3) Add top tissue "cork" – place it on top of the specimens so as to hold them in place but **not enough to squish them**.

Side view of vial with tissue “cork” in place.

4) Add additional tissue to fill the vial to the top to keep the sample from moving – again, enough to hold the sample in place **but not to squish them**. Insert trap label, if you have one, on top of tissue stating location and date of sample, if applicable.



5) Cap it – make sure cap is completely closed so as not to come off during transit. Add a vial label, if you have one, or clearly label the vial with an appropriate sample number connected to a datasheet with Date, Location, and Notes (rough count and/or trap issues, etc.).

6) Package all labeled vials, along with datasheets containing all relevant collection data, and mail to the address provided by AMCA. *Address TBD.*

Happy hunting.