Developing mosquito-based detection methods for the brown treesnake in Guam

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2. University of Guam, Combosch Island Evolution Lab
3. USDA Wildlife Services, National Wildlife Research Center

Chagasia bathana
COSTA RICA: Heredia
La Selva Biological Station

Mariana Islands

Guam, USA

Data NOAA
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Data LDEO-Columbia, NSF, NOAA
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Fauna of Guam

Coconut crab, Cocos

Native reptiles

- Six species of gecko
- Six species of skink
- One monitor species
- No native snakes, terrestrial turtles, or amphibians

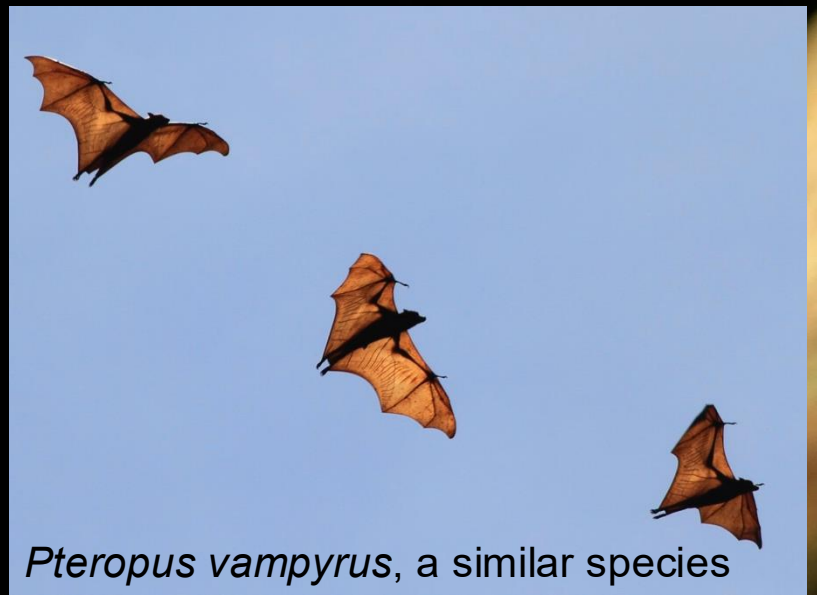
Oceanic gecko, *Gehyra oceanica*

Mariana monitor

- Likely native and endemic
- Member of the mangrove monitor complex

Mariana fruit bat

- Endemic to Mariana and Caroline Islands
- Federally endangered



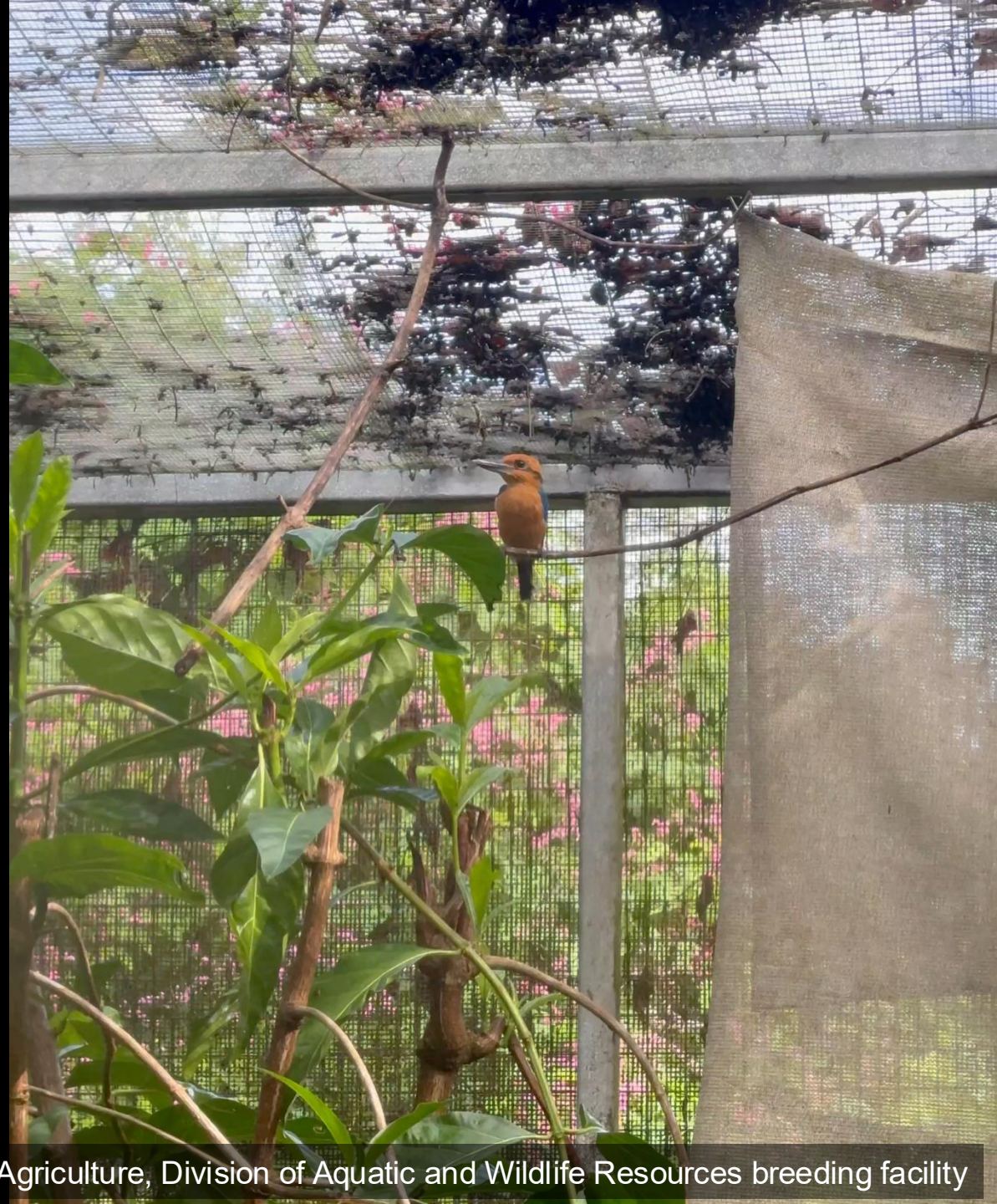
Pteropus vampyrus, a similar species

Guam Rail, Guam Dept. of Agriculture, Division of Aquatic and Wildlife Resources breeding facility

Guam kingfisher

- Endemic to Guam
- Extinct in the wild since 1986
- Global population: <200

Image: CC BY-SA 2.0 MostlyDross



Guam Kingfisher, Guam Dept. of Agriculture, Division of Aquatic and Wildlife Resources breeding facility

Bridled white-eye
Guam subspecies extinct
Last seen 1983

Mariana crow
Critically endangered
Extirpated from Guam

Guam flycatcher
Extinct
Last seen 1983

Micronesian rufous fantail
Guam subspecies extinct
Last seen 1984

Mariana fruit dove
Extirpated from Guam
Last seen 1984

Micronesian honeyeater
Extirpated from Guam
Last seen 1986

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Brown treesnake

Boiga irregularis

- Mildly venomous colubrid from northern Australia and Papua New Guinea
- Introduced to Guam shortly after WWII, likely from Papua New Guinea
- First detection in the early 1950s, island-wide by 1968
- No natural predators, abundant, naïve prey species
- Extreme densities : >13,000 snakes per sq. mile, exceeding carrying capacity
- Extirpation of most of Guam's forest vertebrates, extinction of 12 native birds
- Widespread power outages; ~150 per year due to snakes
- Brown tree snake populations currently decreasing on Guam

BROWN TREESNAKE CONTROL
USDA
Caution: Pesticide
(Acetaminophen)

Image: USDA

LIVE
ANIMALS
Alaska
AIRCARGO
WeatherTech

4400LB/2000 KG
PRO

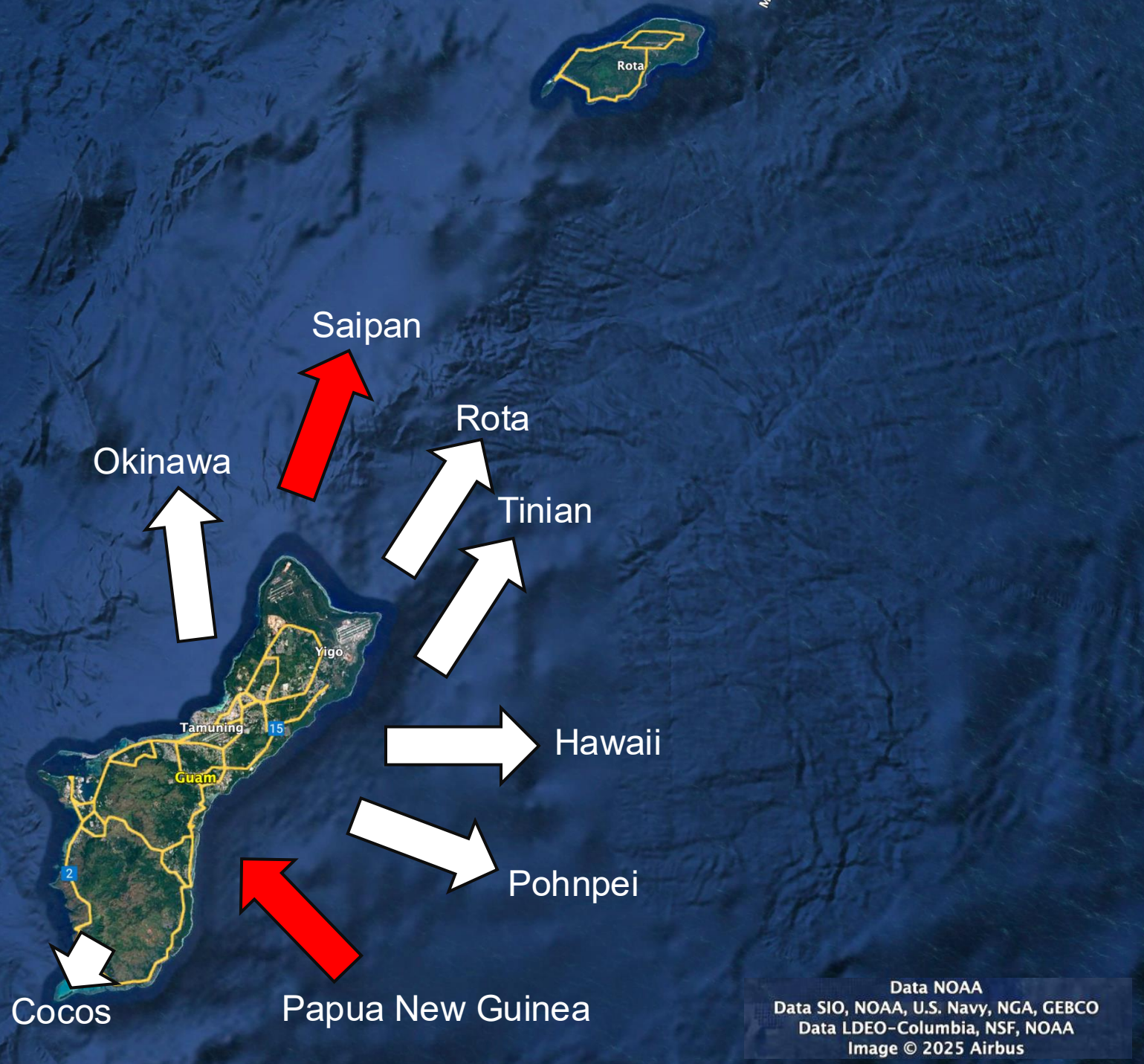
K
WILDLIFE SERVICES
SNAKE SLAYER 6

CAMERA BATTERY BOX

Dispersal of snakes from Guam

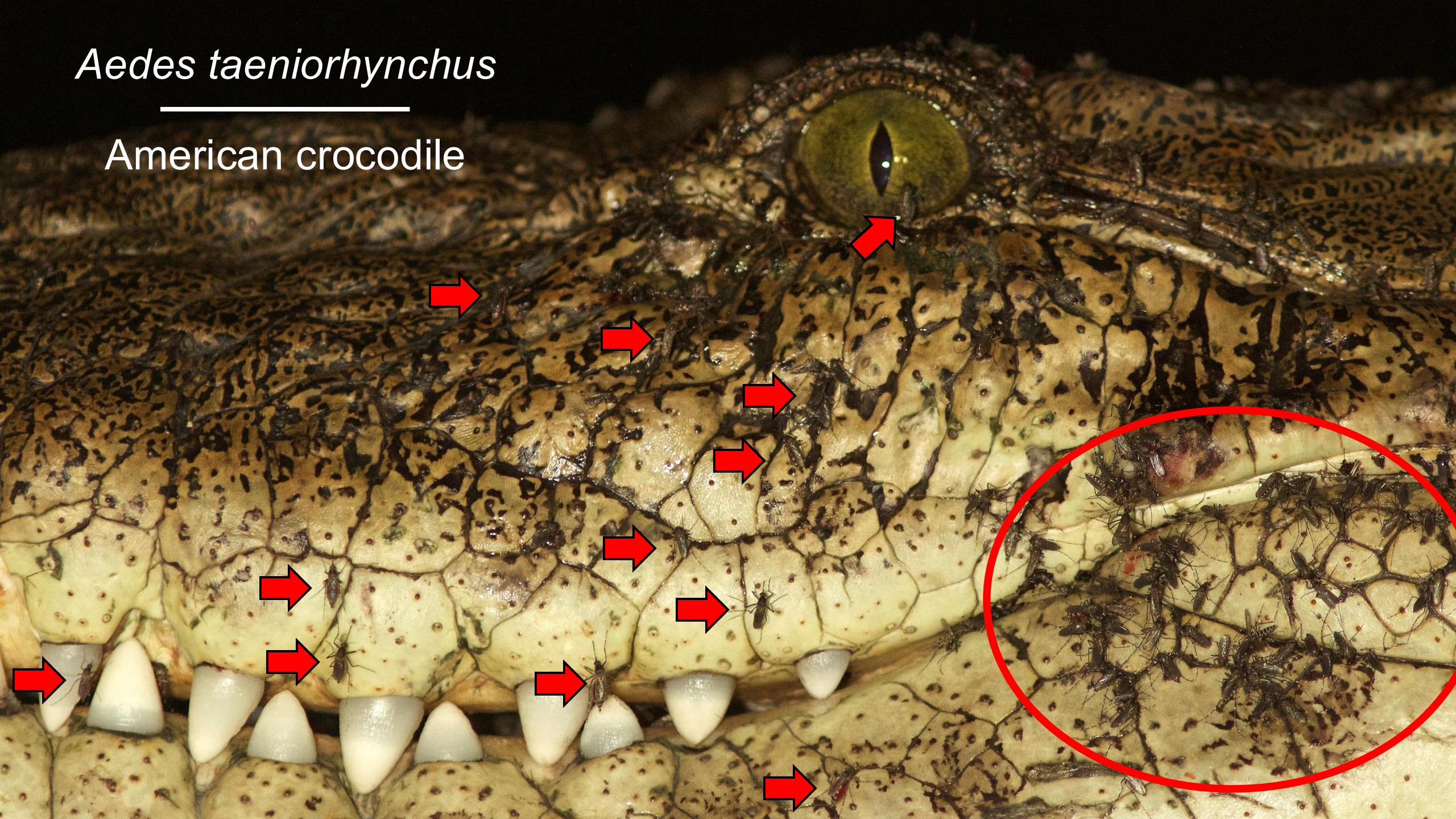
Destination	Number intercepted (2009)
Rota	4
Saipan	78
Tinian	8
Diego Garcia	1
Federated States of Micronesia	1
Palau	1
United States Mainland	3
Hawaii	14

Perry and Vice 2009



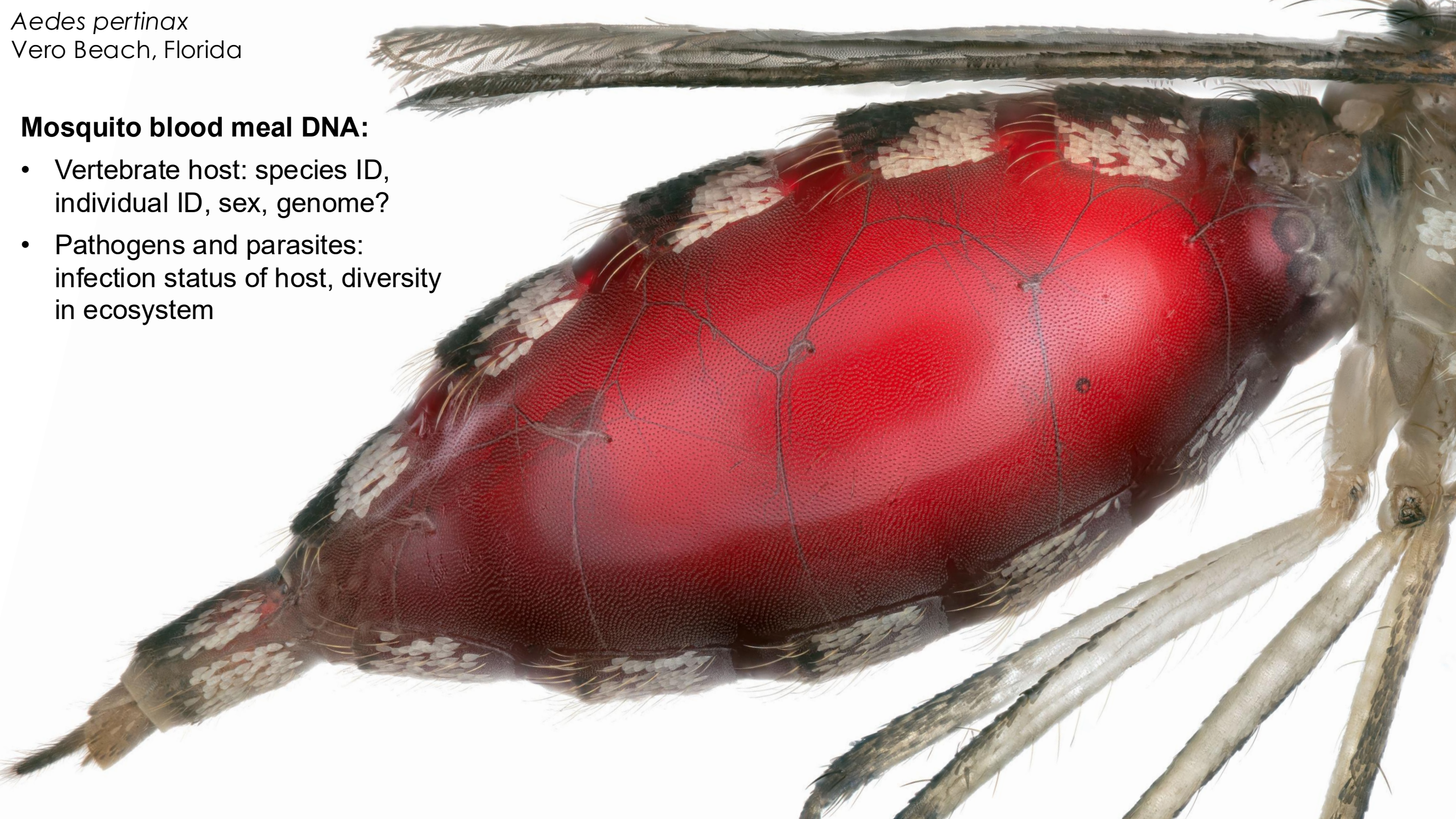
How can we detect incipient populations of brown treesnakes in new locations?

How can we detect incipient populations of brown treesnakes in new locations?


Aedes taeniorhynchus
American crocodile

Aedes taeniorhynchus
American crocodile

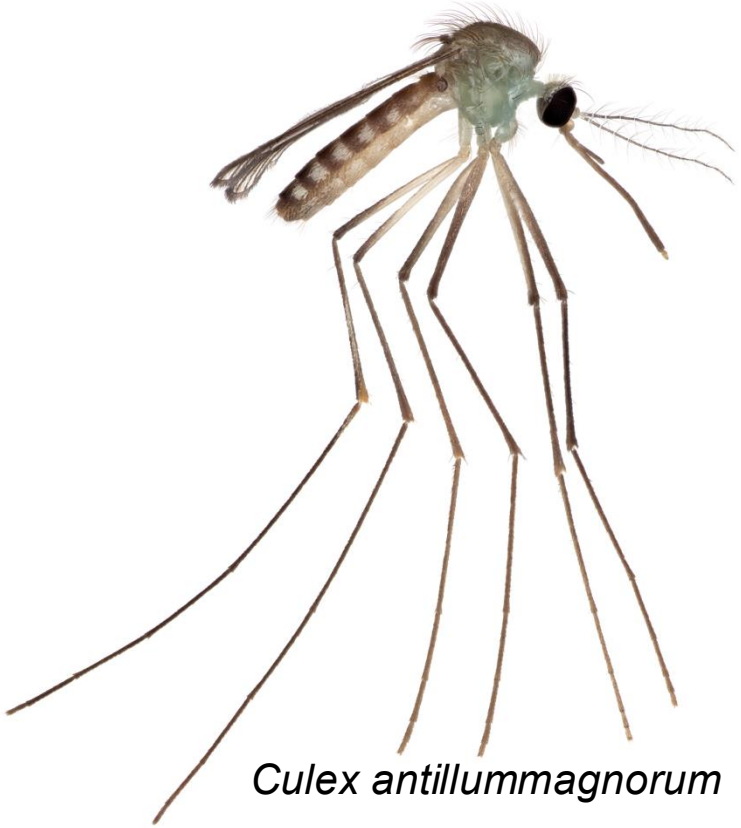
Aedes pertinax
Vero Beach, Florida



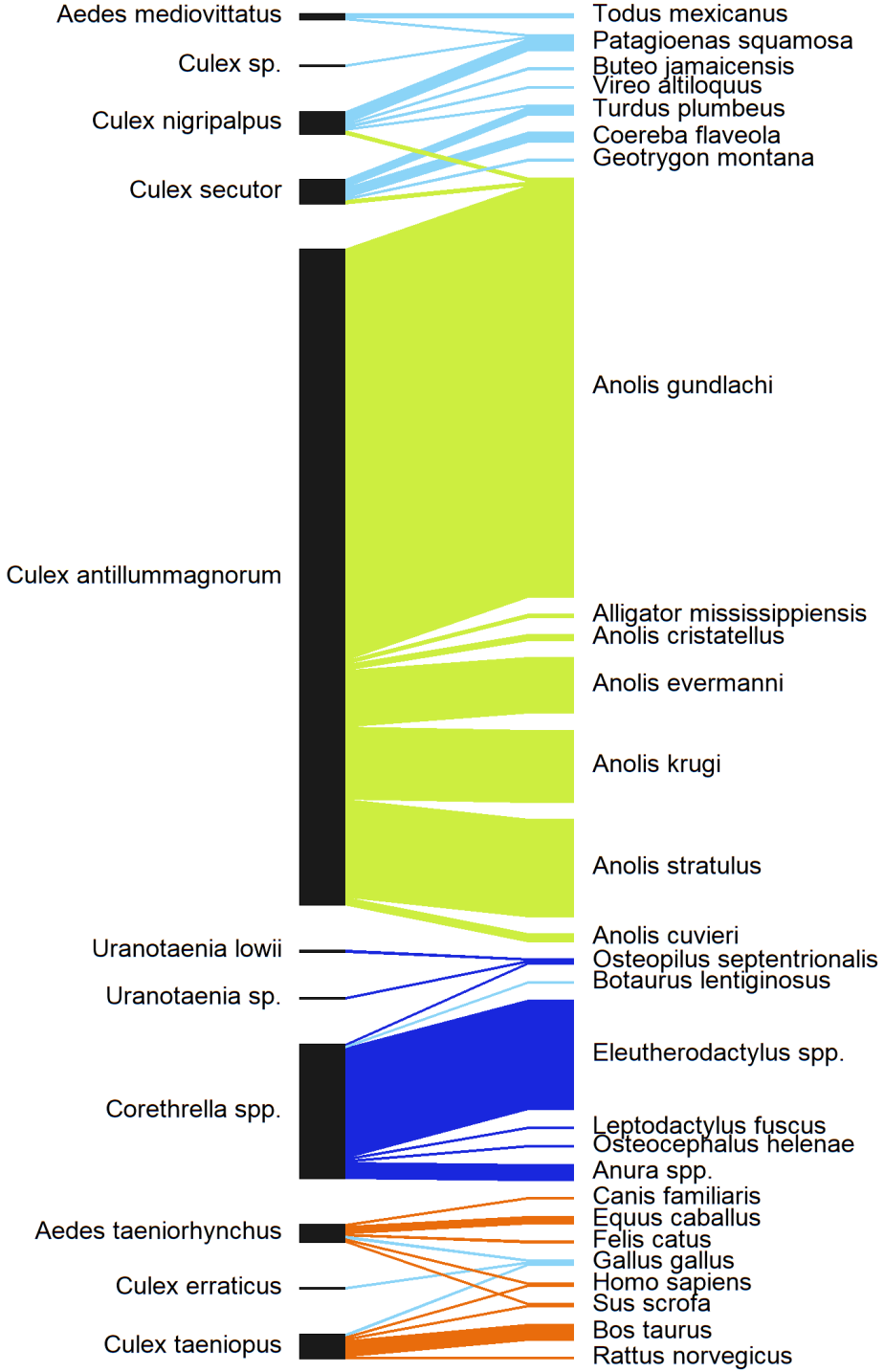
Mosquito blood meal DNA:

- Vertebrate host: species ID, individual ID, sex, genome?
- Pathogens and parasites: infection status of host, diversity in ecosystem

Plasmodium diversity

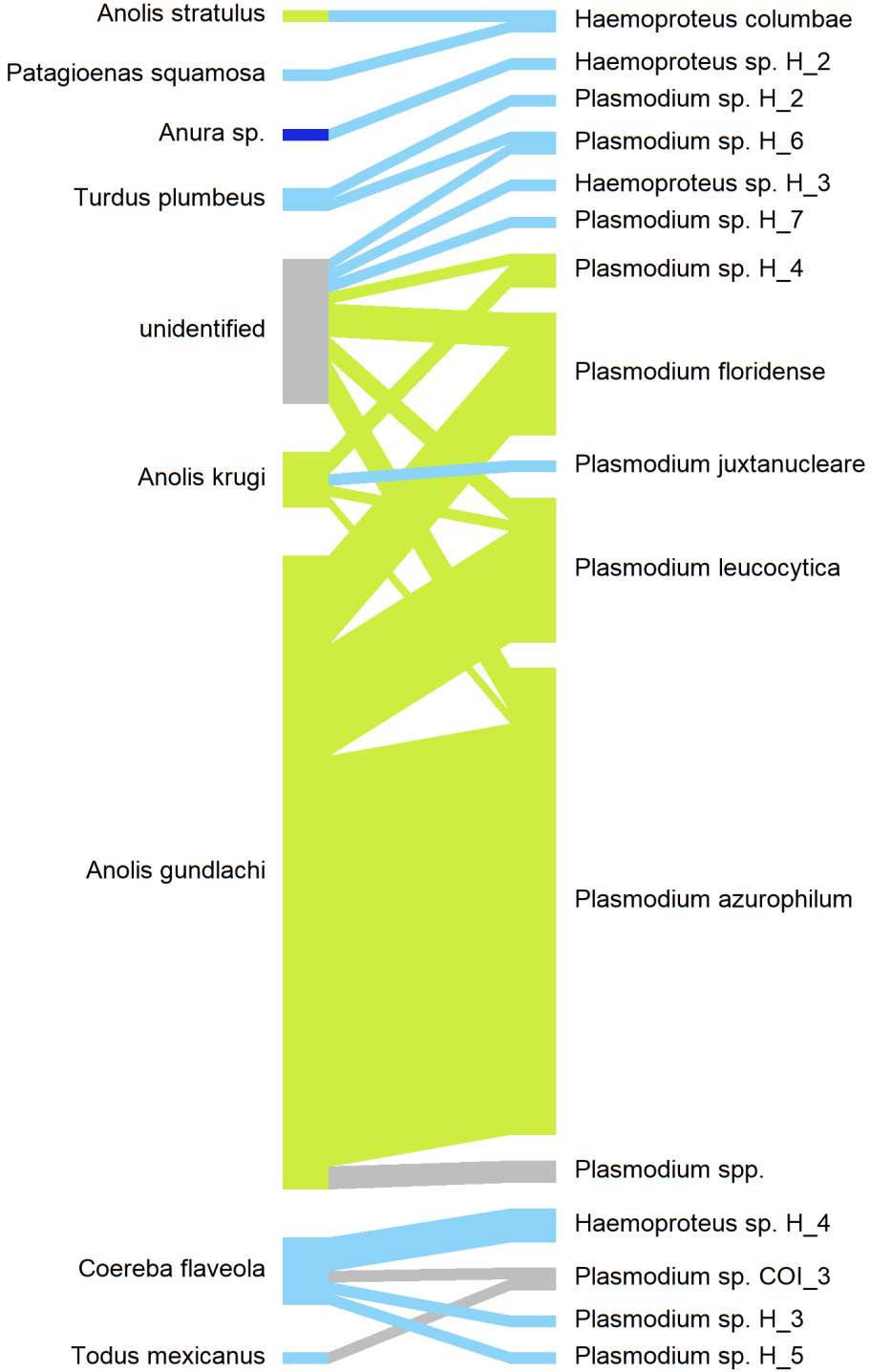


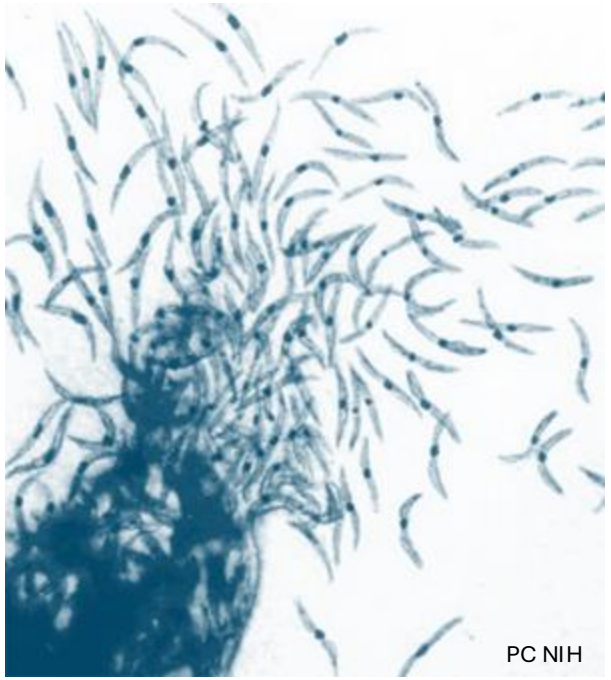
Culex antillummagnorum



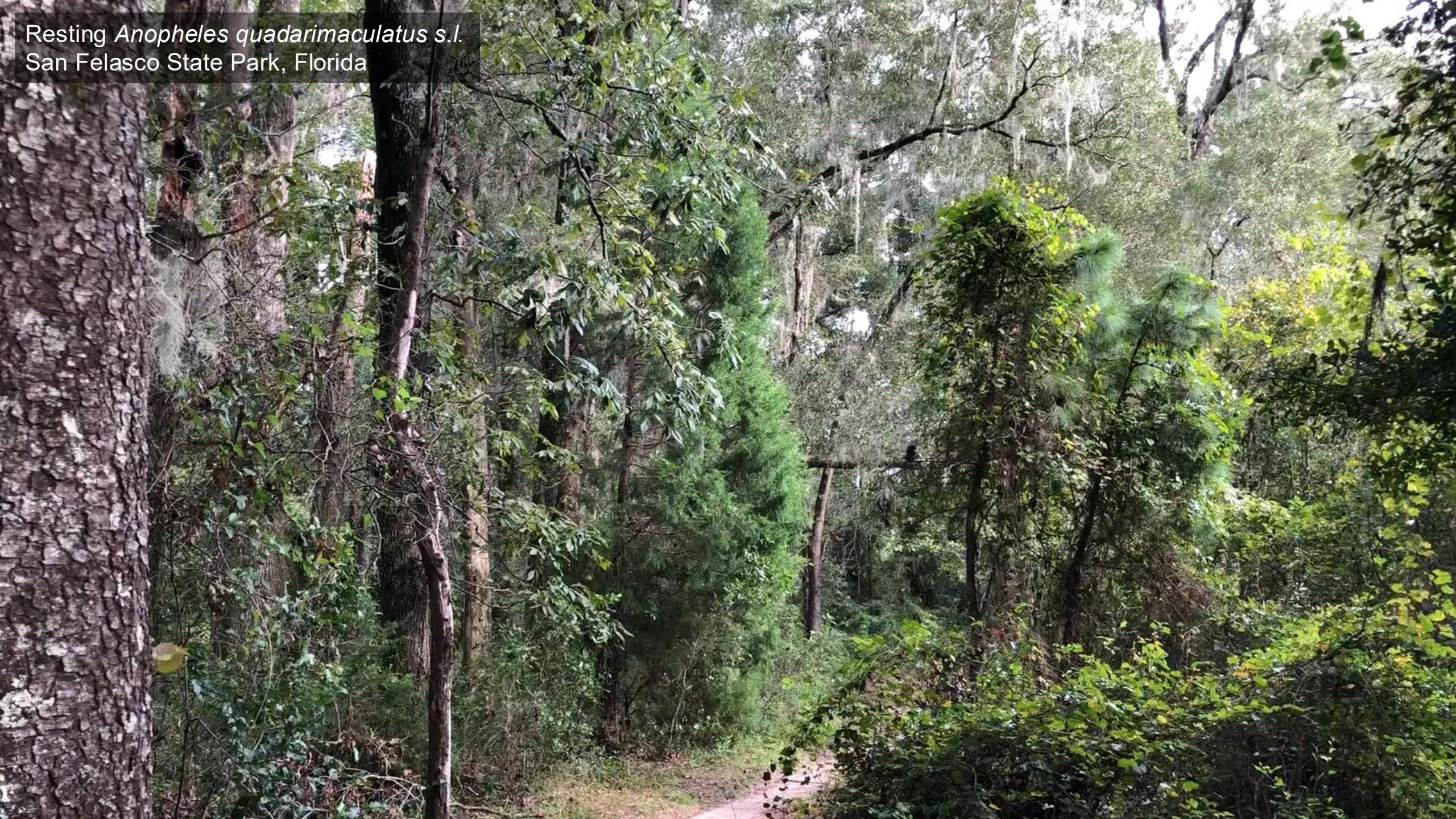
Bipartite network of dipteran-vertebrate interactions uncovered by blood meal analysis of mosquitoes and corethrellid midges collected from El Verde Field Station, El Yunque National Forest, Puerto Rico. Vertebrates are color coded by class (Amphibia = dark blue, Aves = light blue, Mammalia = orange, Reptilia = green).

Plasmodium diversity





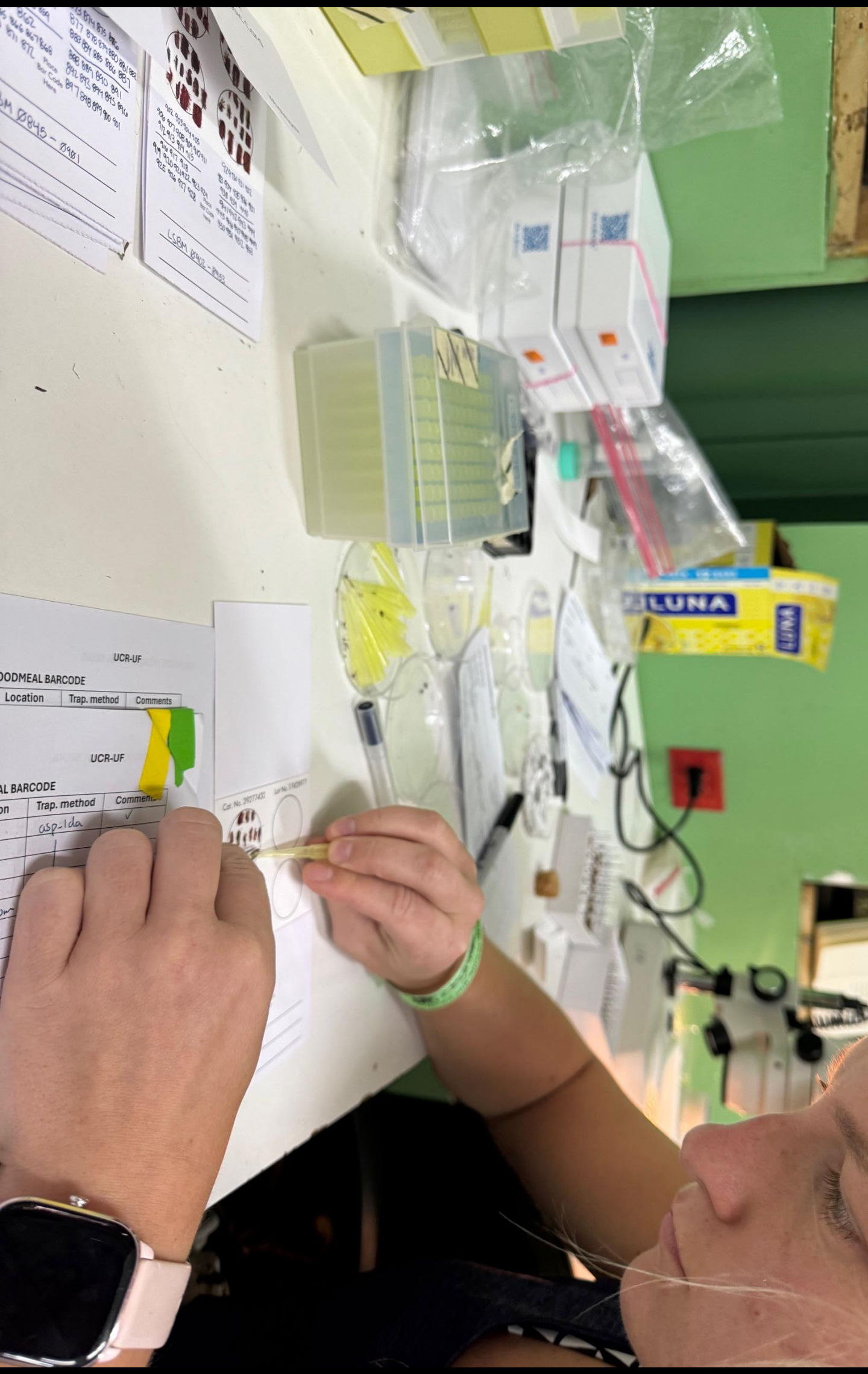
Bipartite network of vertebrate-parasite interactions uncovered by haemosporidian-targeting blood meal analysis of mosquitoes and corethrellid midges collected from El Verde Field Station, El Yunque National Forest, Puerto Rico. Vertebrates are color coded by class and parasite species/OTUs are color coded by class of host (Amphibia = dark blue, Aves = light blue, Mammalia = orange, Reptilia = green, unknown = grey).


Resting *Anopheles quadarimaculatus s.l.*
San Felasco State Park, Florida

More information:
Burkett-Cadena et al. 2019. Human-powered pop-up resting shelter for sampling cavity-resting mosquitoes. Acta Tropica 190: 288-292.

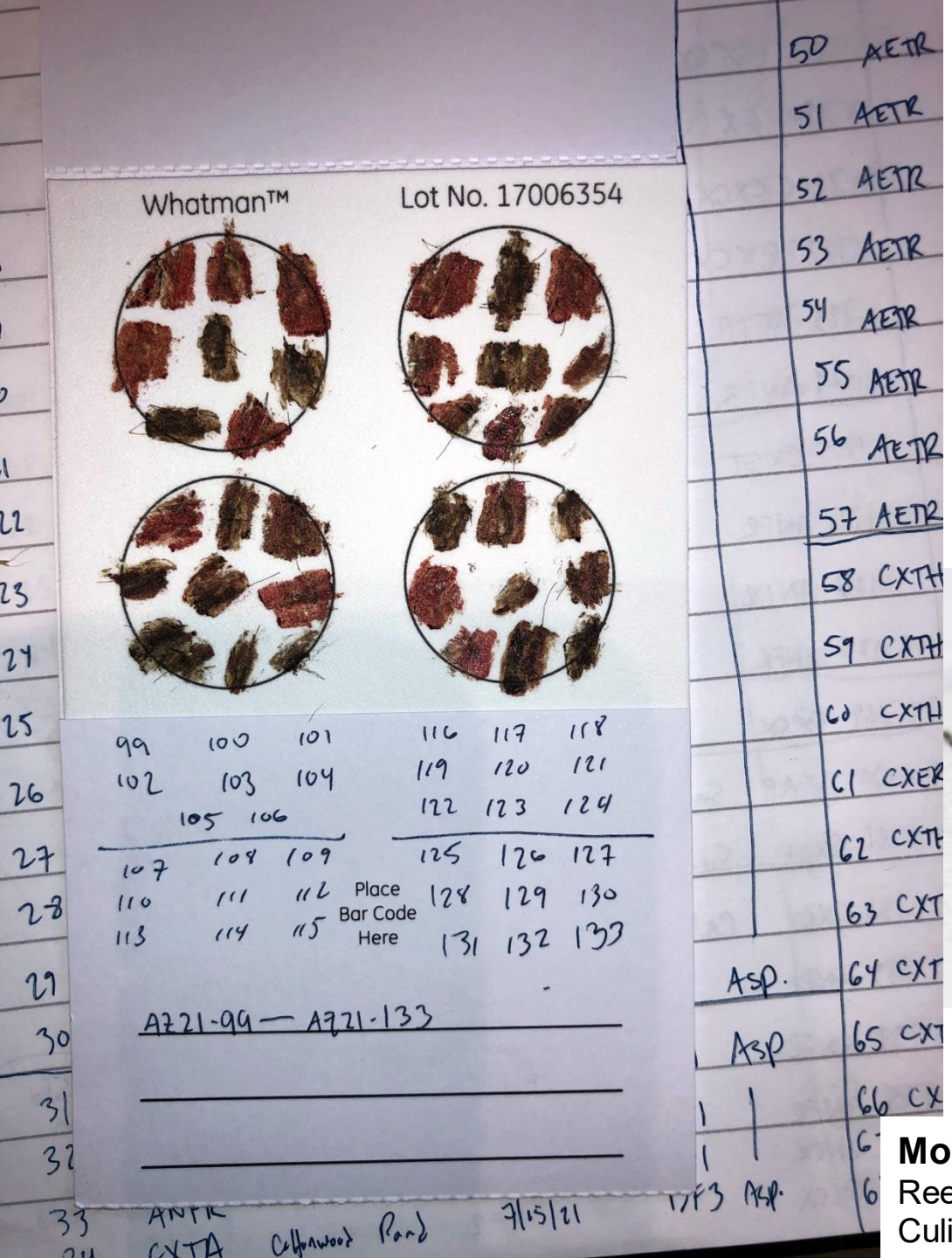
Large-diameter aspirator
Santa Catalina Mountains, Arizona

Leica
FTA™
FTA™
Classic Card
Whatman
Rcby
7122/21


UCR-UF
OODMEAL BARCODE
Location
Trap. method
Comments
UCR-UF
AL BARCODE
Trap. method
asp-1da
LUNA

Field preservation of mosquito blood meals on Whatman FTA Classic Cards

- Effective DNA preservation for extended duration
- Room temperature storage
- No specialized equipment necessary
- RNA expected to be preserved as well



More information:

Reeves et al. 2016. Maintenance of host DNA integrity in field-preserved mosquito (Diptera: Culicidae) blood meals for identification by DNA barcoding. Parasites and Vectors 9: 503.

Blood Meal Analysis:

A collection of molecular techniques designed to taxonomically identify the source of an invertebrate's blood meal

- Serological assays (ELISA, precipitin test)
- DNA-based: PCR, PCR + Sanger sequencing
- Some next gen sequencing approaches

DNA barcoding workflow

Specimen collection

- Fresh specimens
- Preserved specimens
- Museum specimens
- **Mosquito blood meals**

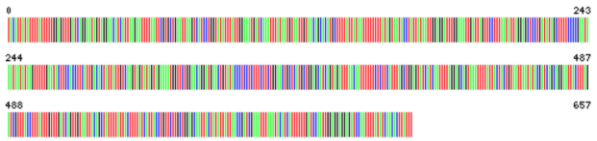
Laboratory processing

DNA extraction

PCR amplification

Sanger sequencing

Sequence Identification



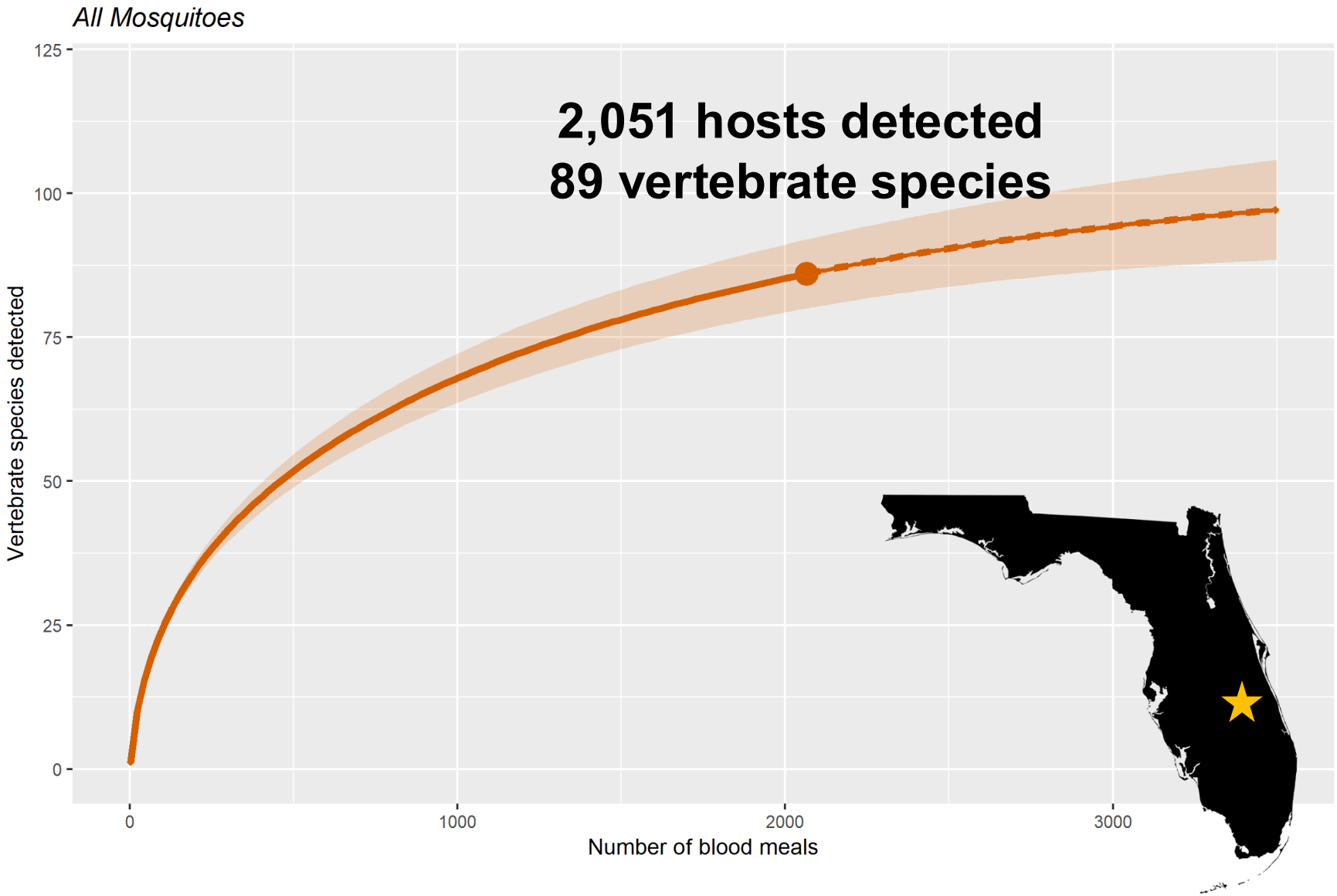
More information:
Reeves et al. 2016. Maintenance of host DNA integrity in field-preserved mosquito (Diptera: Culicidae) blood meals for identification by DNA barcoding. Parasites and Vectors 9: 503.

More information:
Reeves et al. 2018. Barcoding blood meals: New vertebrate-specific primer sets for assigning taxonomic identities to host DNA from mosquito blood meals. PLOS Negl. Trop. Dis. 12: e0006767.

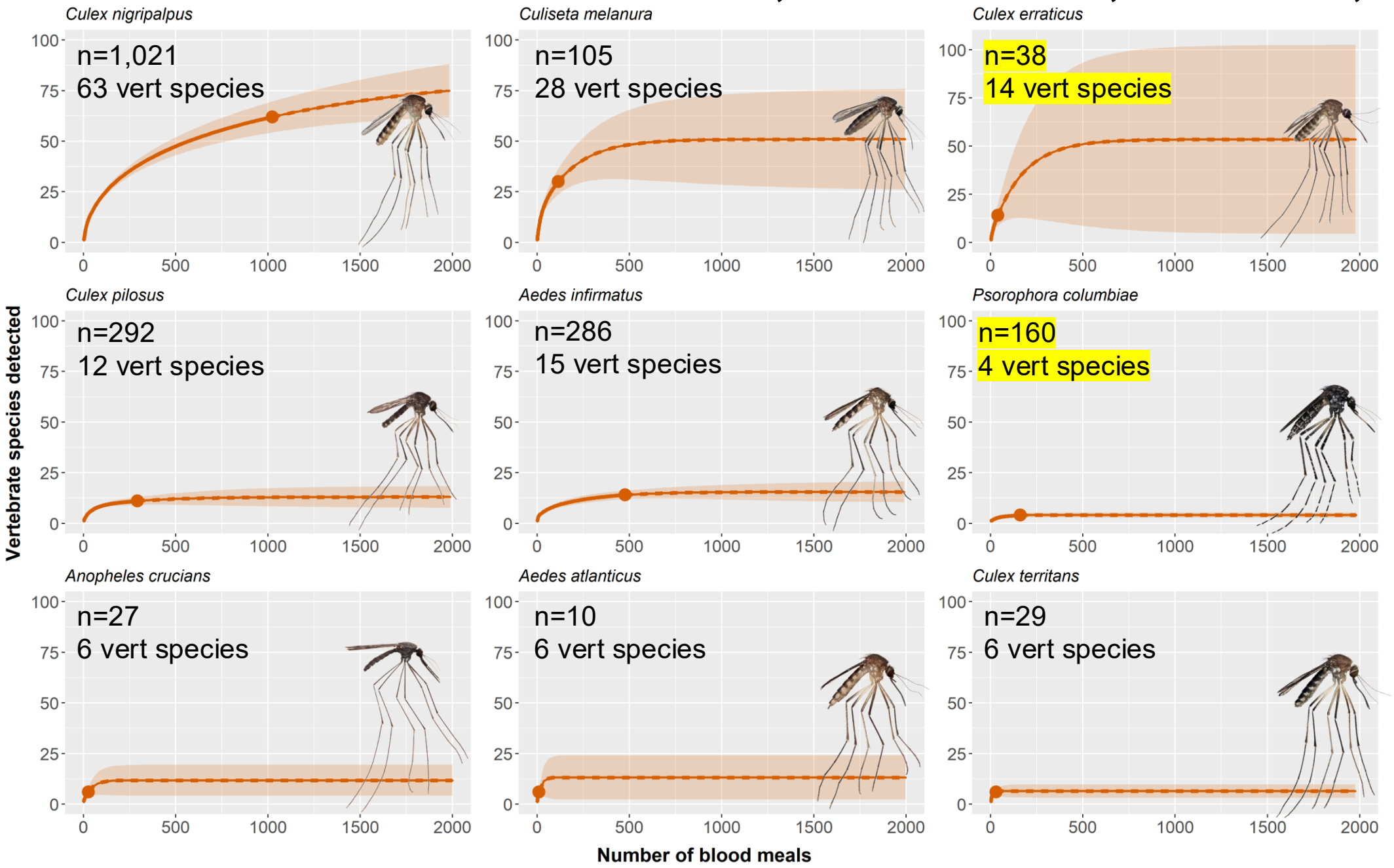
Mosquito blood meal-based vertebrate detection, DeLuca Preserve, Osceola Co., Florida

Hannah Atsma

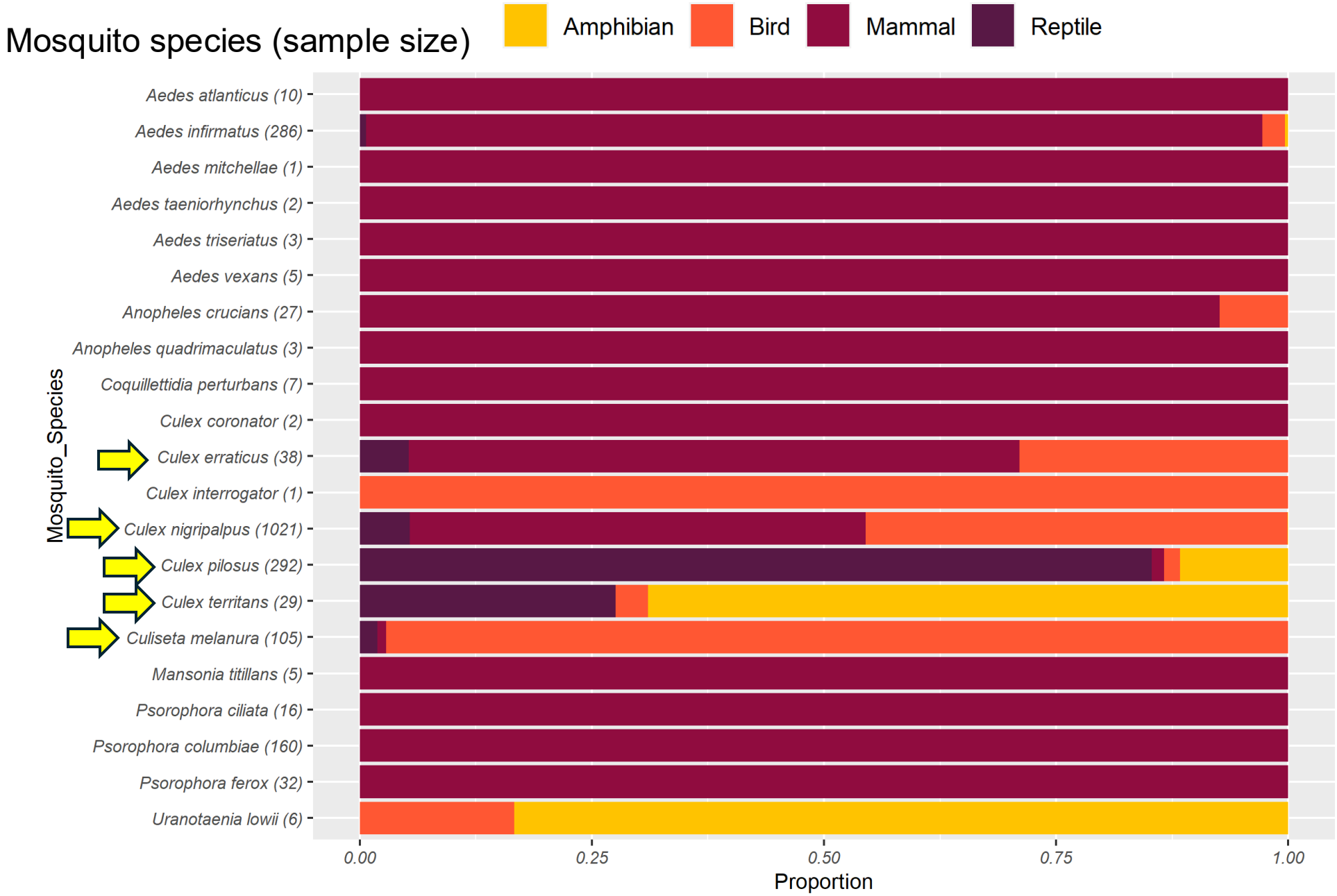
- Mosquitoes collected across nine months
- 54,637 mosquitoes collected
- 3,508 blood meals
- 21 mosquito species



Mosquito blood meal-based vertebrate detection, DeLuca Preserve, Osceola Co., Florida



Mosquito blood meal-based vertebrate detection, DeLuca Preserve, Osceola Co., Florida



5/21 mosquito species fed on reptilian hosts

Goal: Develop mosquito blood meal-based methods for detecting brown tree snakes in Guam and the Marianas

Objectives:

1. Collect blood fed mosquitoes and identify species likely to feed on reptiles in the Marianas
2. Develop resources for mosquito identification and recognition of reptile-feeding species
3. Train local personnel on methods of collecting blood fed mosquitoes and processing mosquito blood meals

Mosquito fauna of Guam

Native, endemic species

1. *Aedes* (*Stegomyia*) guamensis

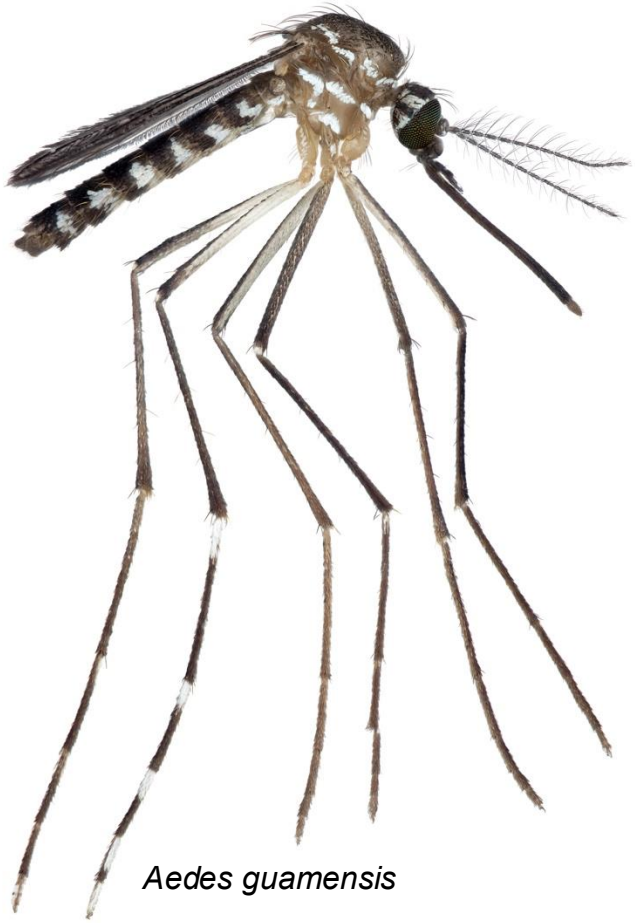
Pandani Group

2. *Aedes (Stegomyia) pandani*
3. *Aedes (Stegomyia) rotanus*
4. *Aedes (Stegomyia) saipanensis*

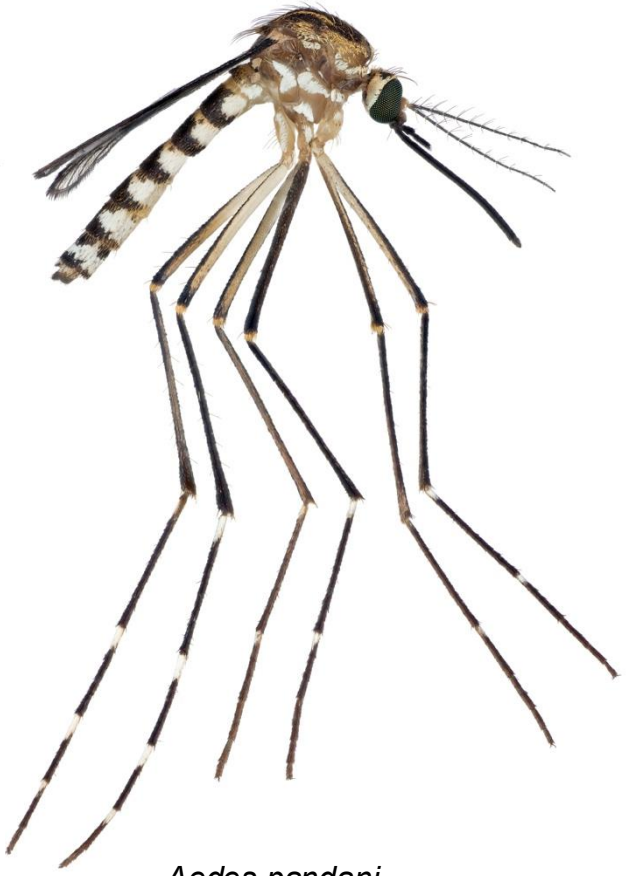
5. *Aedes (Aedimorphus) oakleyi*

Genus *Culex*

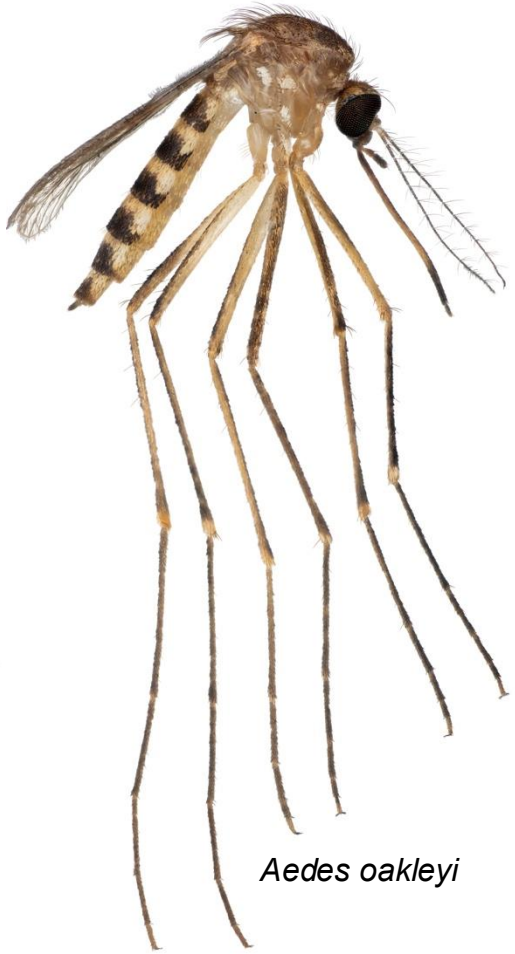
6. *Culex* (*Culex*) *marianae*
7. *Culex* (*Culex*) *litoralis*



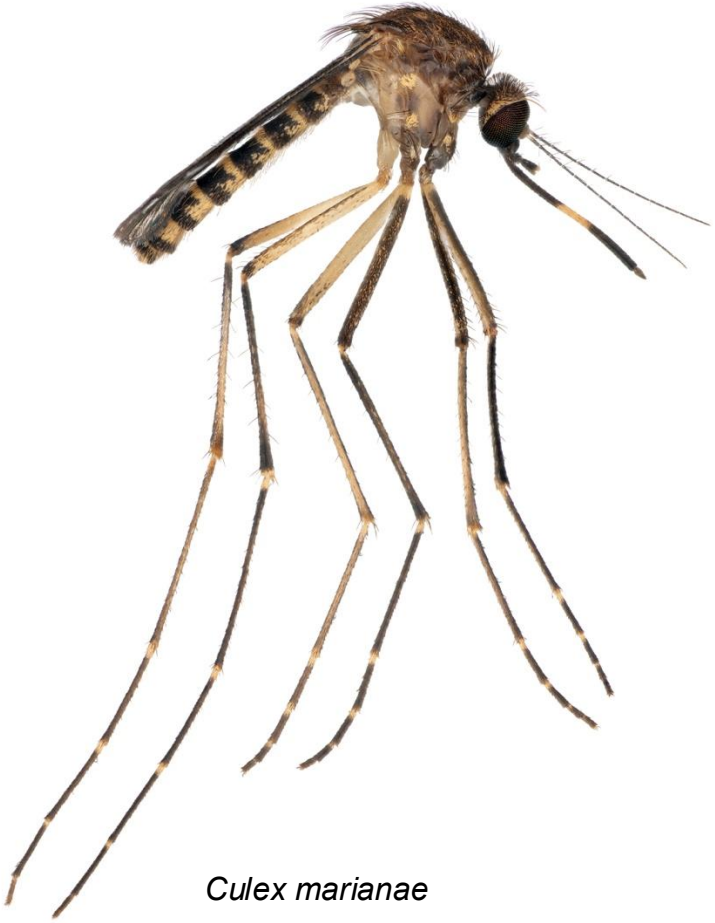
Aedes guamensis



Aedes pandani



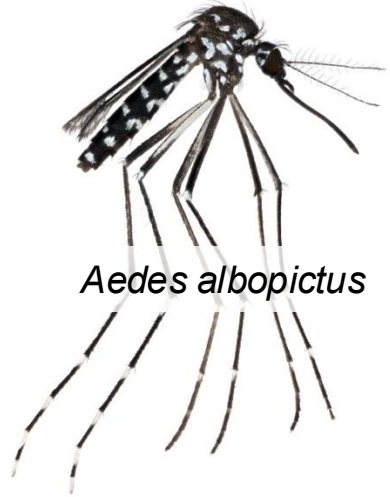
Aedes oakleyi



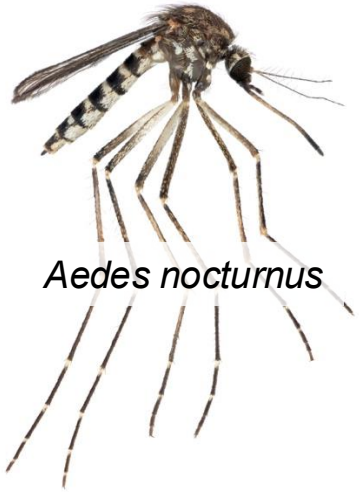
Culex marianae

Mosquito fauna of Guam

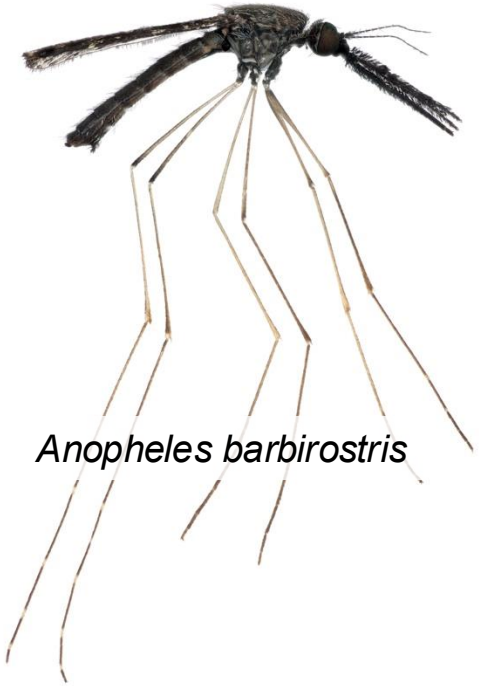
Nonnative and invasive species



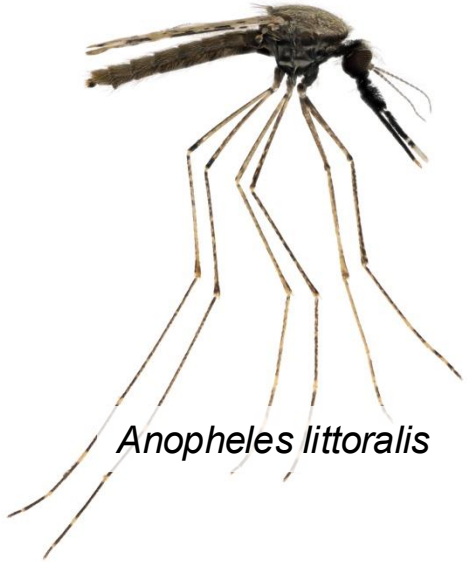
Aedes albopictus



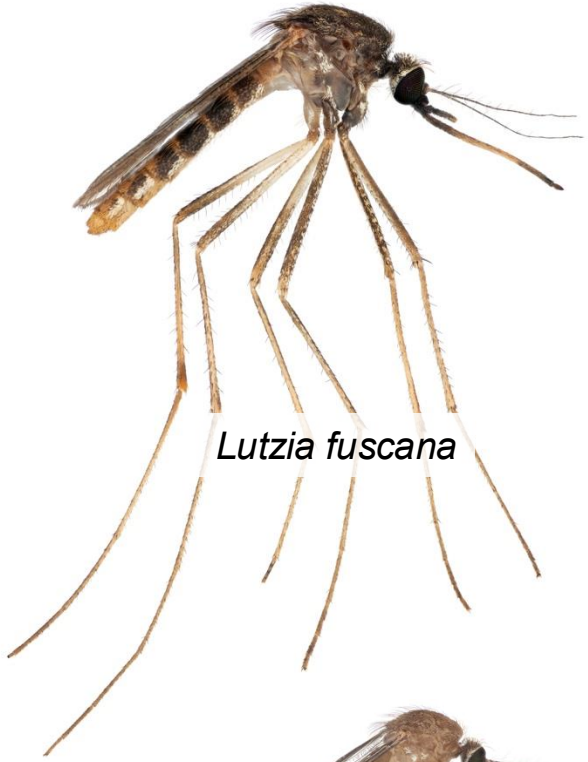
Aedes nocturnus



Anopheles barbirostris

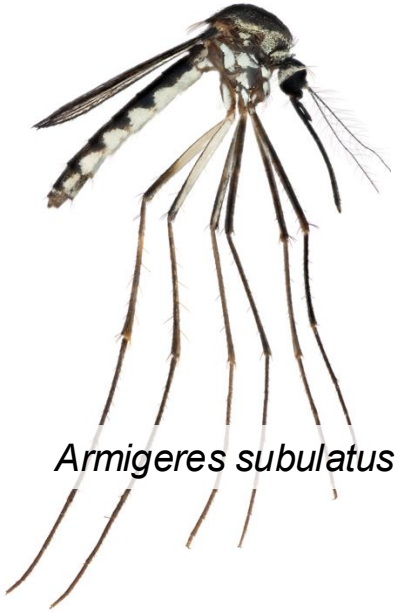


Anopheles littoralis

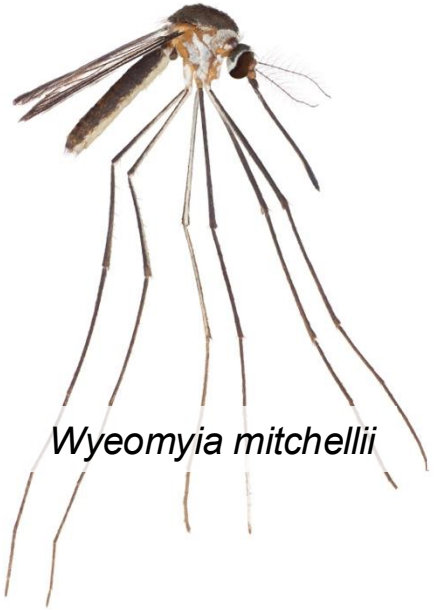


Lutzia fuscana

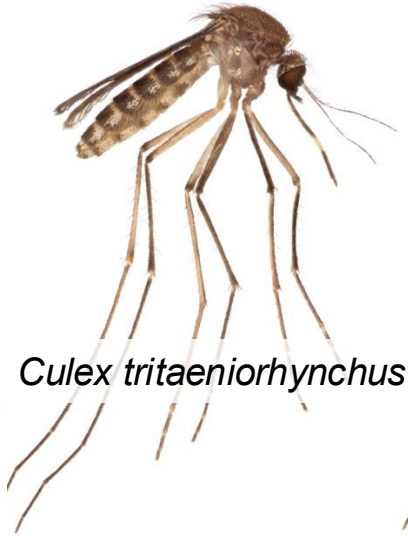
Mansonia uniformis



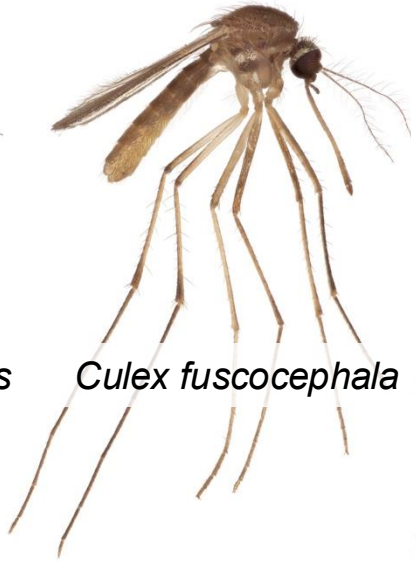
Armigeres subulatus



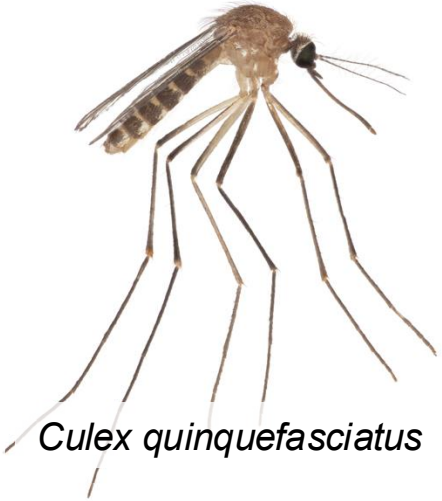
Wyeomyia mitchellii



Culex tritaeniorhynchus



Culex fuscocephala

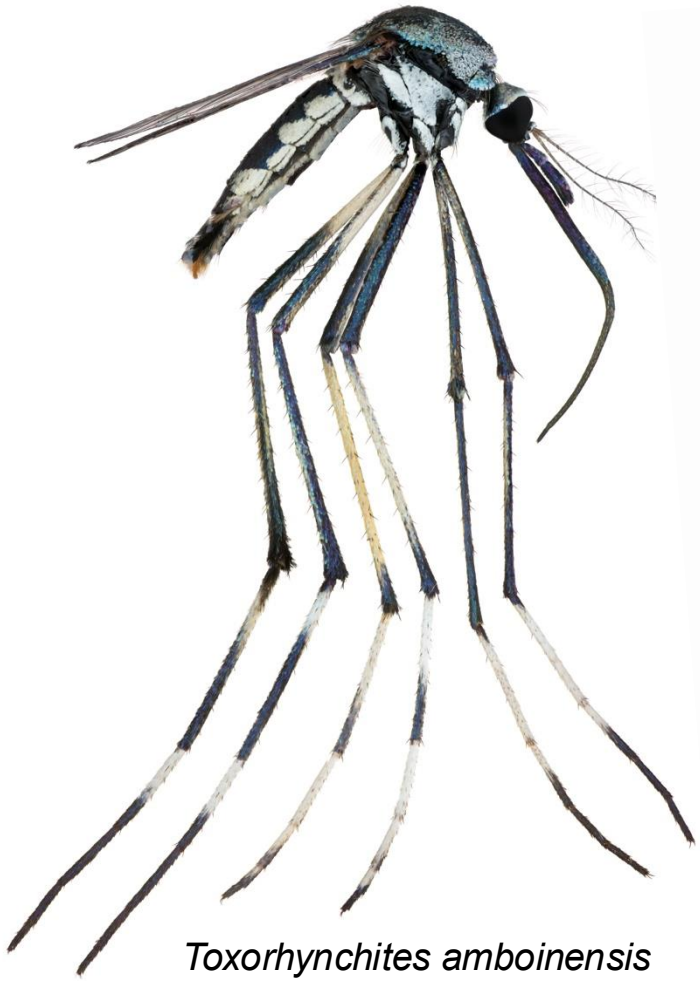


Culex quinquefasciatus

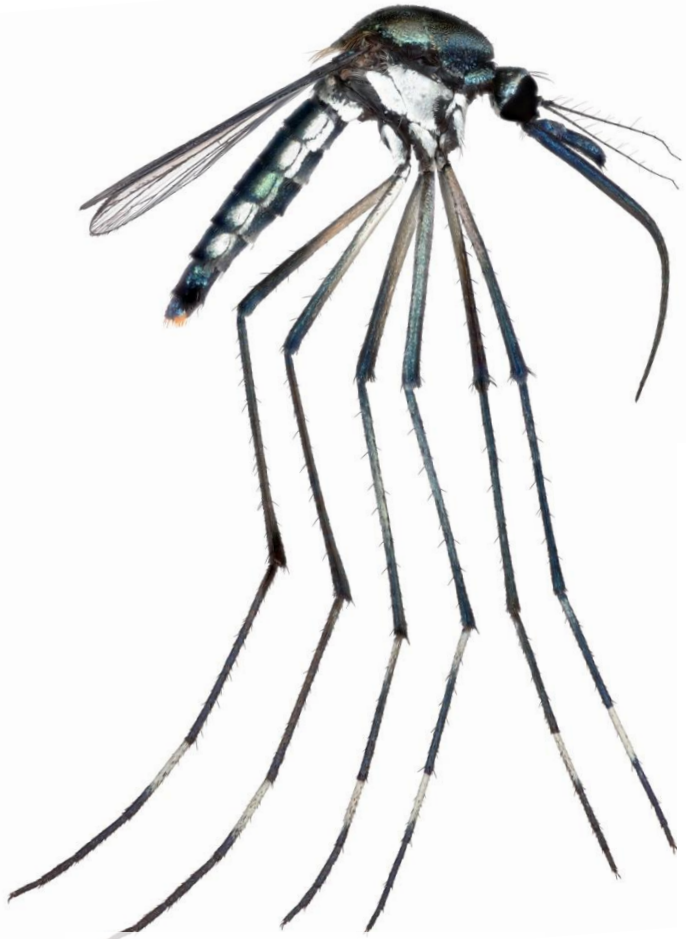
Aedeomyia catasticta

Mosquito fauna of Guam

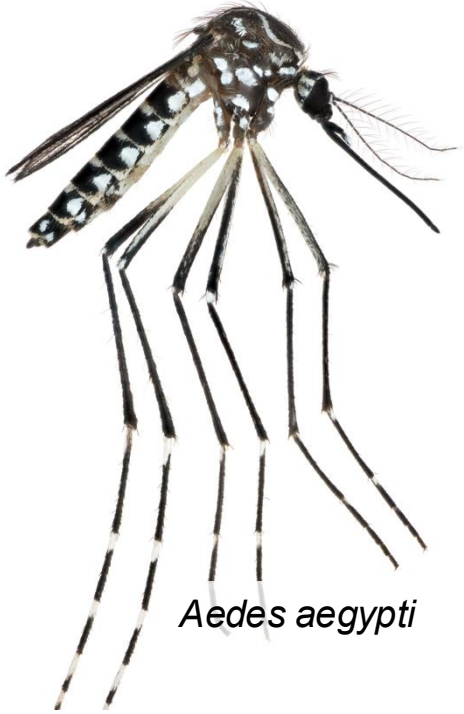
Detected, introduced, and extirpated



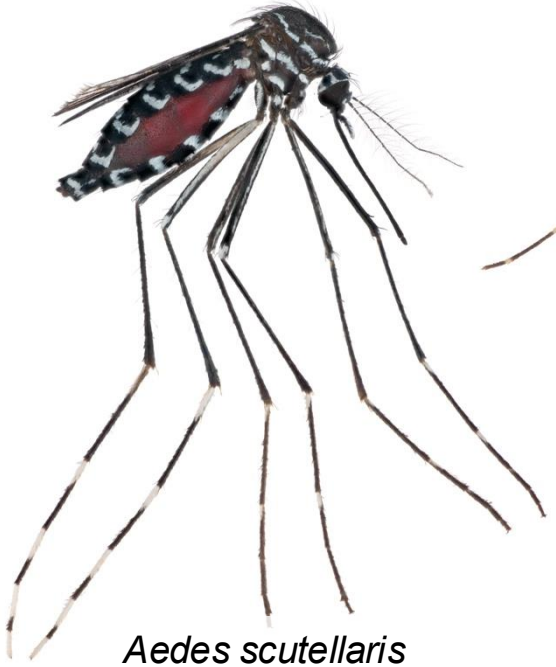
Toxorhynchites amboinensis



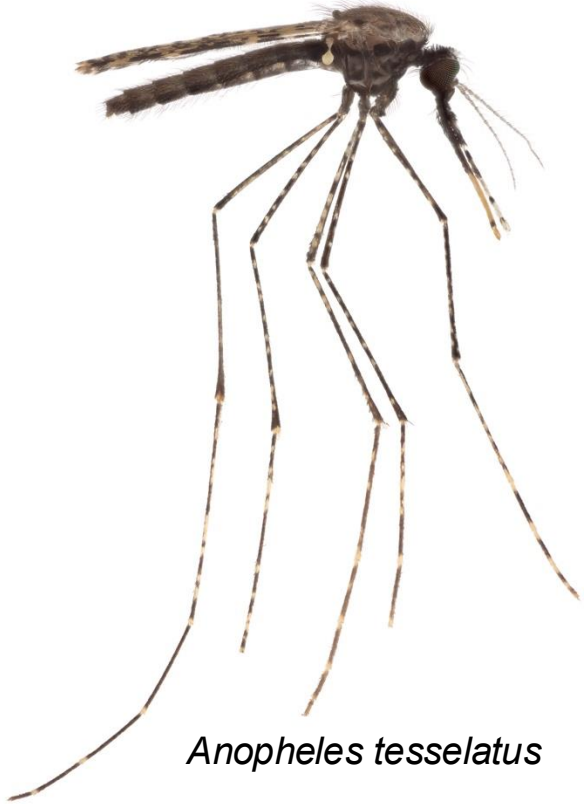
Toxorhynchites brevipalpis



Aedes aegypti



Aedes scutellaris



Anopheles tesselatus

Mosquito fauna of Guam

43

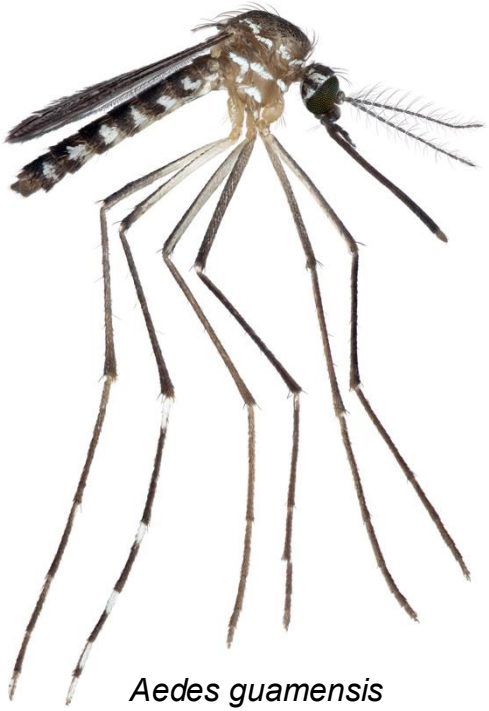
mosquito species recorded in Guam

7

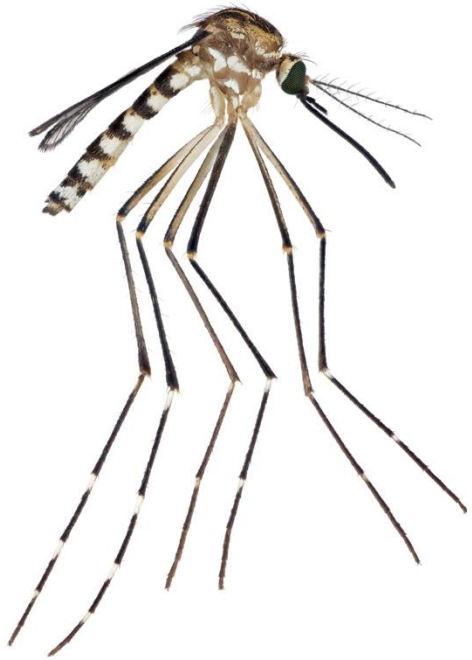
mosquito species native to Guam

16

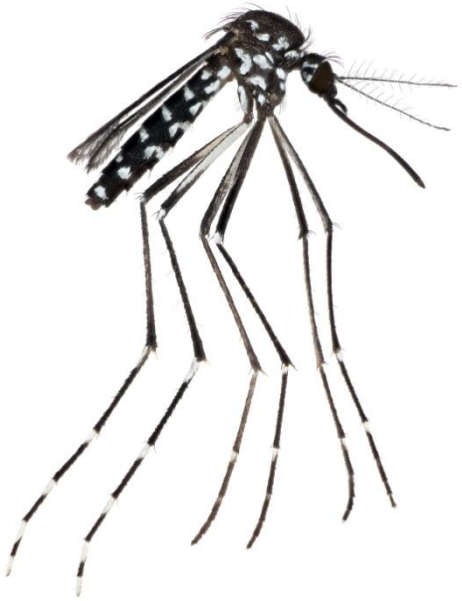
nonnative species established in Guam



Aedes guamensis



Aedes pandani



Aedes albopictus

Host associations

- Mosquito host associations have never been assessed in wild mosquitoes in Guam
- Reeves and Rudnick 1951 assessed human feeding with indoor collections of four species
- Host associations of native species, others, unknown

Mosquito collections, Oct. 2024

BIOLOGIST

INSECTS OF MICRONESIA
Diptera: Culicidae*

By RICHARD M. BOHART
University of California, Davis

INTRODUCTION

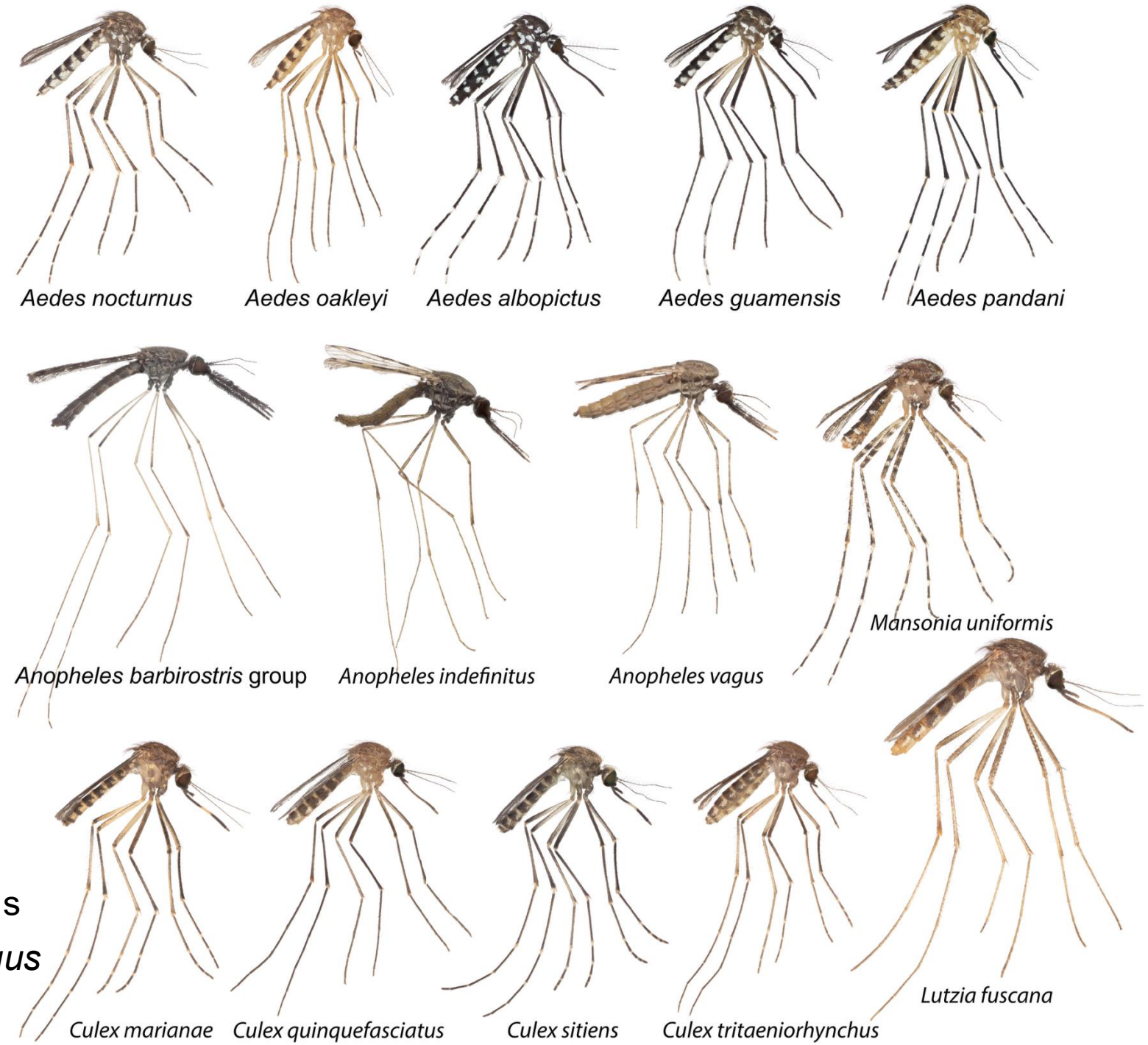
The only extensive study previously made on the mosquitoes of Micronesia was that of Bohart and Ingram (1946).[1] More recently the faunas of individual islands have been revised by Knight and Hurlbut (1949) for Ponape, and by Yamaguti and La Casse (1950) for Guam.

Bohart and Ingram treated 21 named species and four unnamed ones from Micronesia and the Volcano Islands. The present list of 45 species from these areas and the Bonins is nearly double that previously known. It probably includes most of those occurring in the area, except in the Palaus where additional forms are certain to be found.

The most obvious and most interesting feature of island faunas is the high percentage of endemism. Table 1 outlines the known geographical distribution of Micronesian mosquitoes. It can be seen that of the 45 species listed, 28 (62.2 percent) are apparently endemic to the area and these are endemic to particular island groups. In the Gilb
the Caroline atolls, Marcus and Wake Islands, and
are no species peculiar to these particular
area the percentage of endemism is
Yap, 25.0 in the Bonins, 28.6
Kusaie, 33.3 in Palau
number of end
(four)

14

mosquito species collected

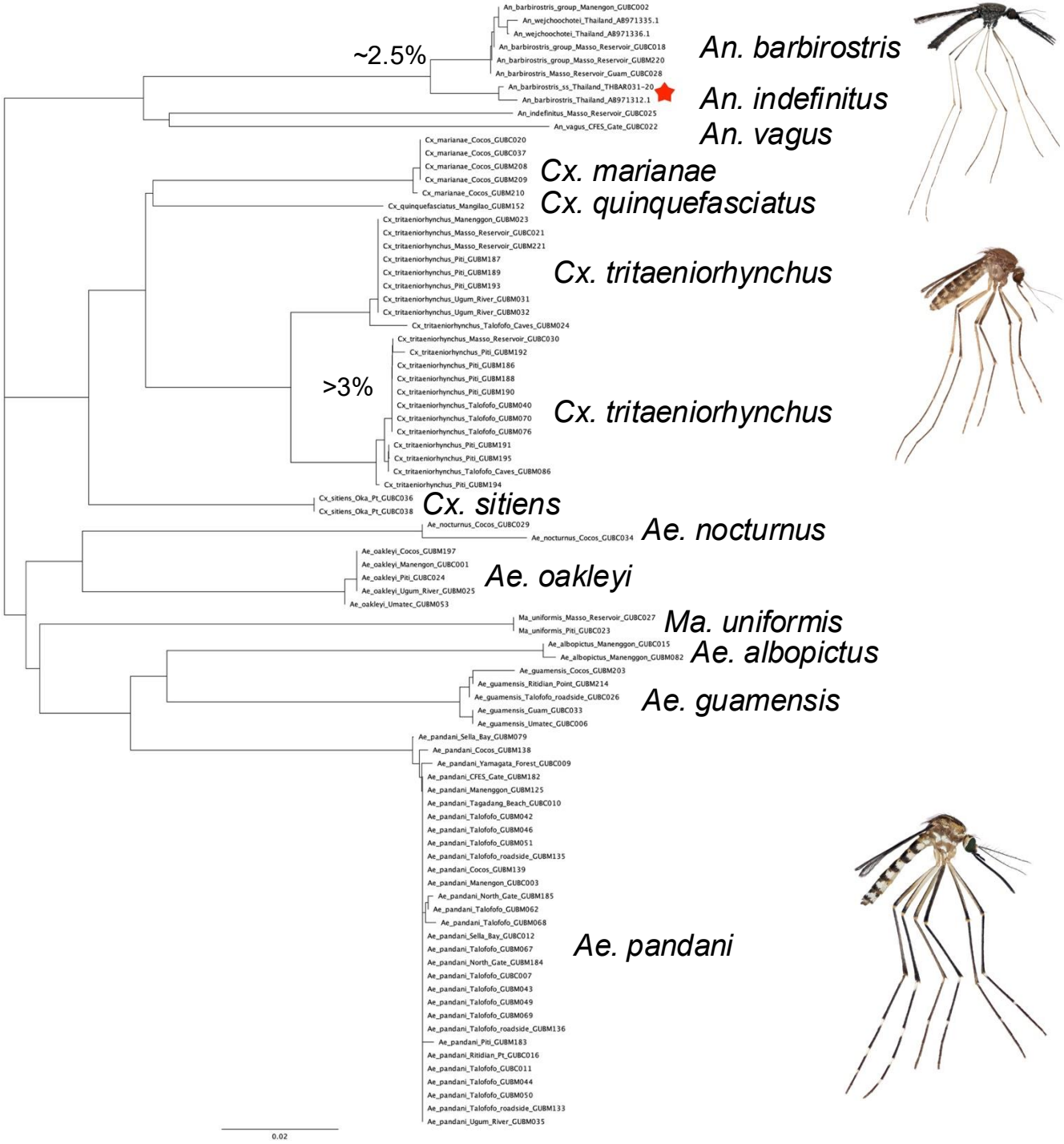


**Culex sitiens* only found in larval collections

***Anopheles indefinitus* and *Anopheles vagus* only collected in CDC light traps

14

mosquito species collected



**Culex sitiens* only found in larval collections

***Anopheles indefinitus* and *Anopheles vagus* only collected in CDC light traps

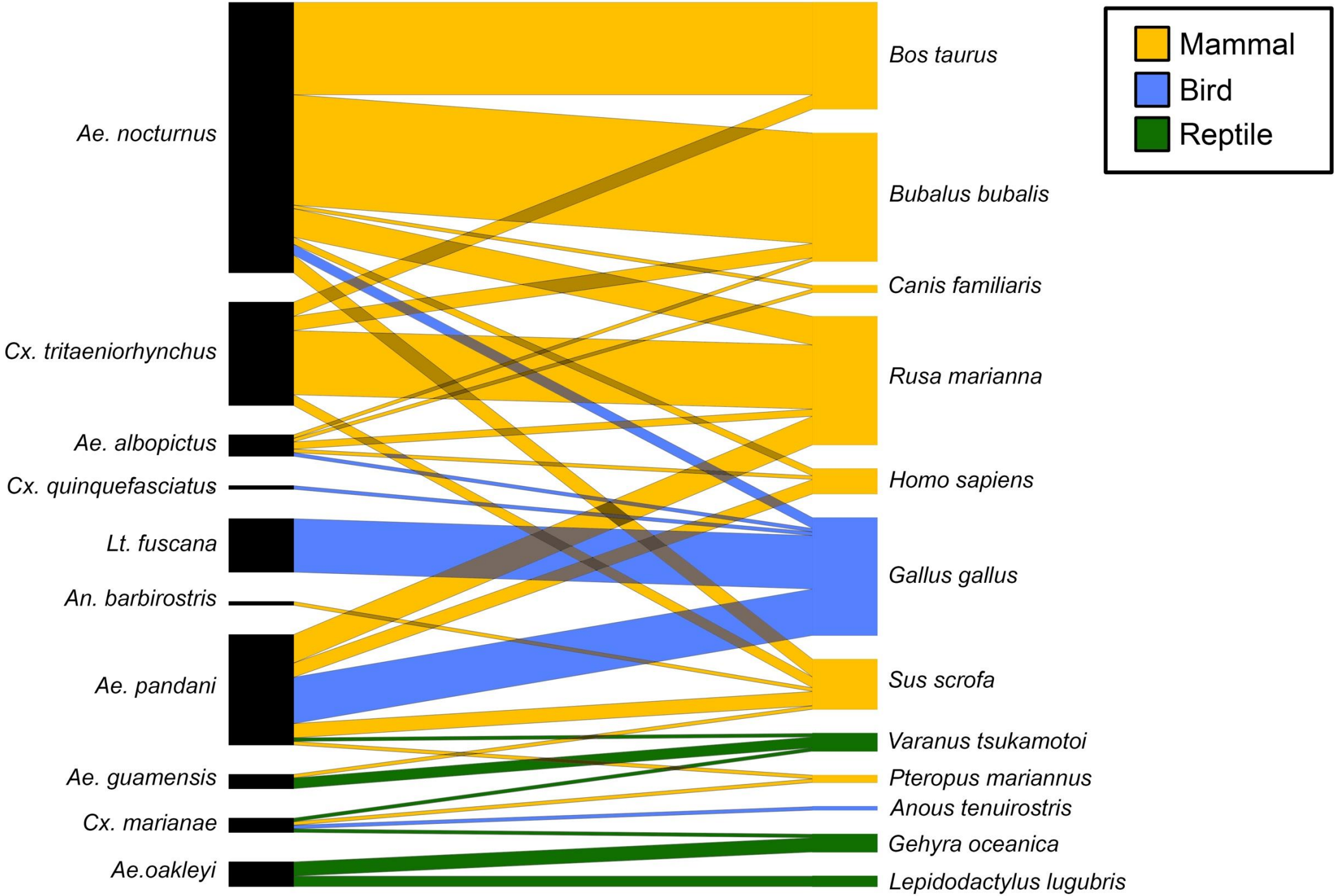
Mosquito blood meals collected:

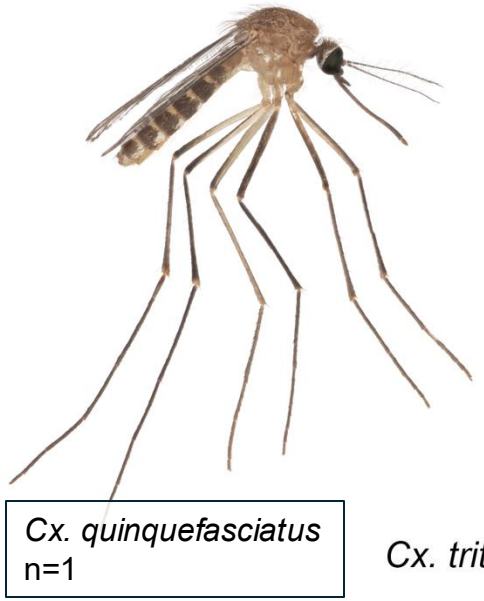
221

Mosquito blood meals identified:

174 (79%)

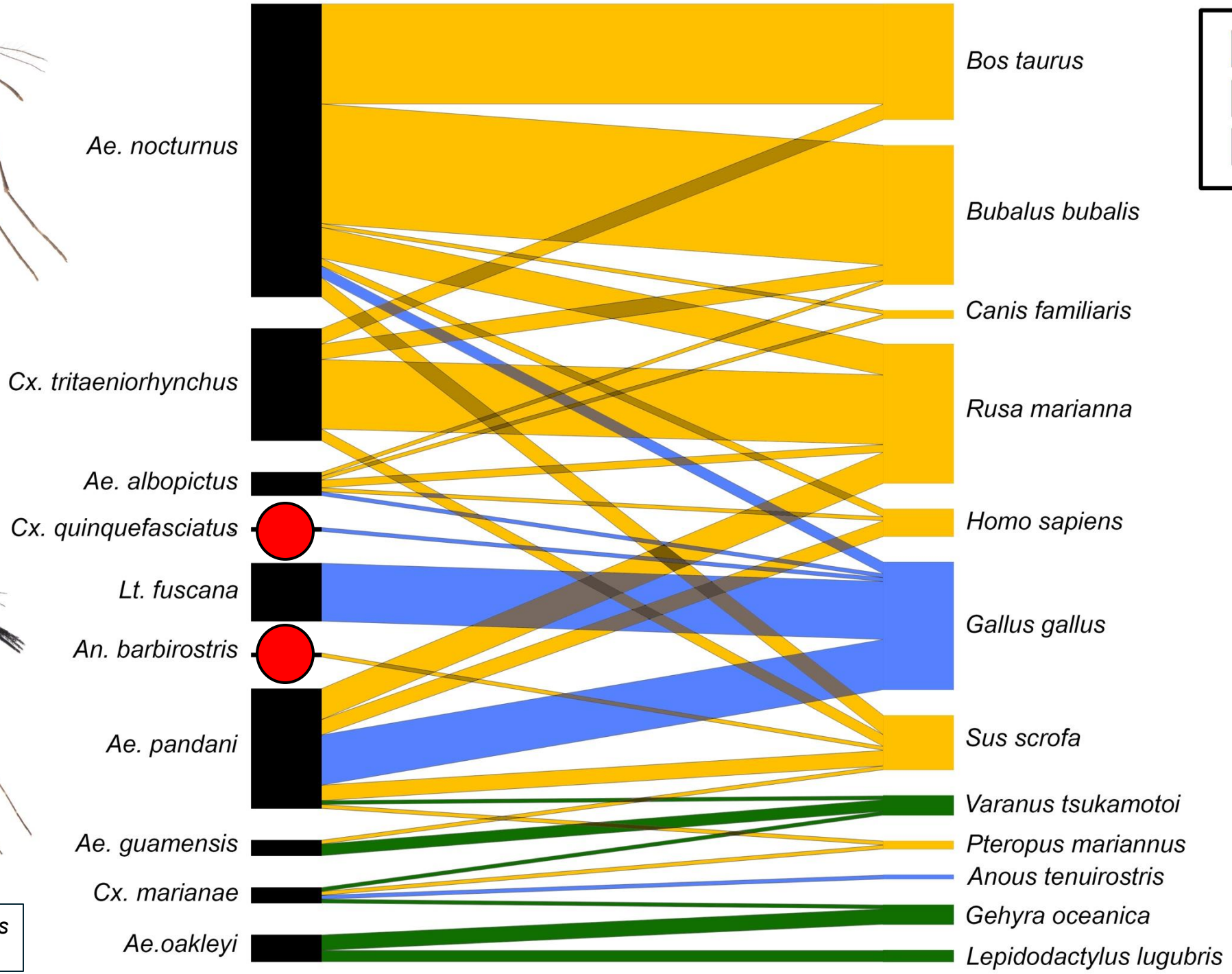
Mosquito species	Number of blood meals	Number identified (%)
Aedes (*Stegomyia*) *albopictus*	7	6 (86%)
Aedes (*Stegomyia*) *guamensis*	5	4 (80%)
Aedes (*Stegomyia*) Pandani Group	42	31 (74%)
Aedes (*Aedimorphus*) *nocturnus*	83	76 (92%)
Aedes (*Aedimorphus*) *oakleyi*	14	7 (50%)
Anopheles (*Anopheles*) *barbirostris*	1	1 (100%)
Culex (*Culex*) *quinquefasciatus*	2	1 (50%)
Culex (*Culex*) *tritaeniorhynchus*	38	29 (76%)
Culex (*Culex*) *marianae*	6	4 (67%)
Lutzia (*Metalutzia*) *fuscana*	23	15 (65%)

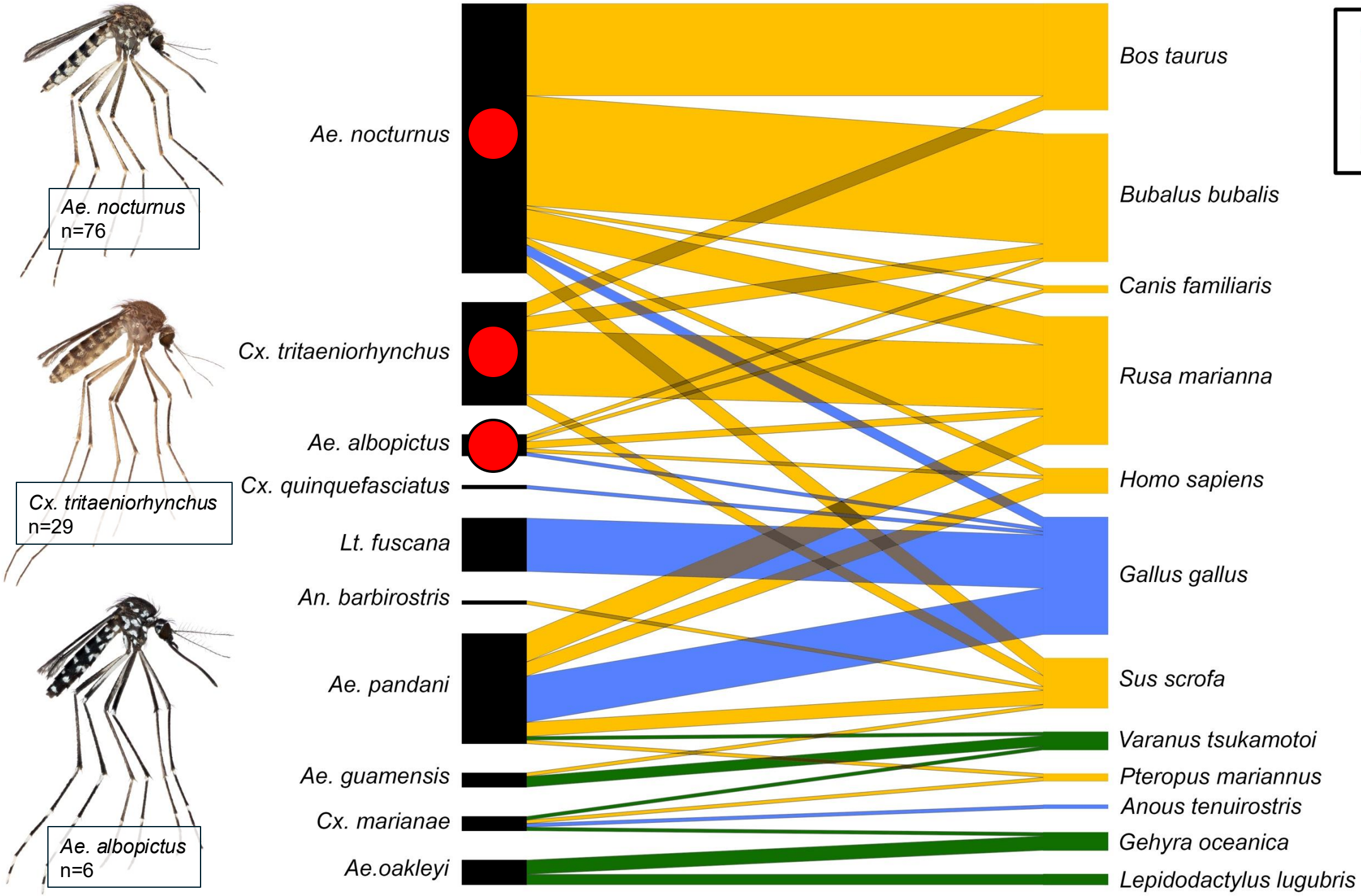

Mammal
Bird
Reptile
Ae. nocturnus
Cx. tritaeniorhynchus
Ae. albopictus
Cx. quinquefasciatus
Lt. fuscana
An. barbirostris
Ae. pandani
Ae. guamensis
Cx. marianae
Ae.oakleyi
Bos taurus
Bubalus bubalis
Canis familiaris
Rusa marianna
Homo sapiens
Gallus gallus
Sus scrofa
Varanus tsukamotoi
Pteropus mariannus
Anous tenuirostris
Gehyra oceanica
Lepidodactylus lugubris

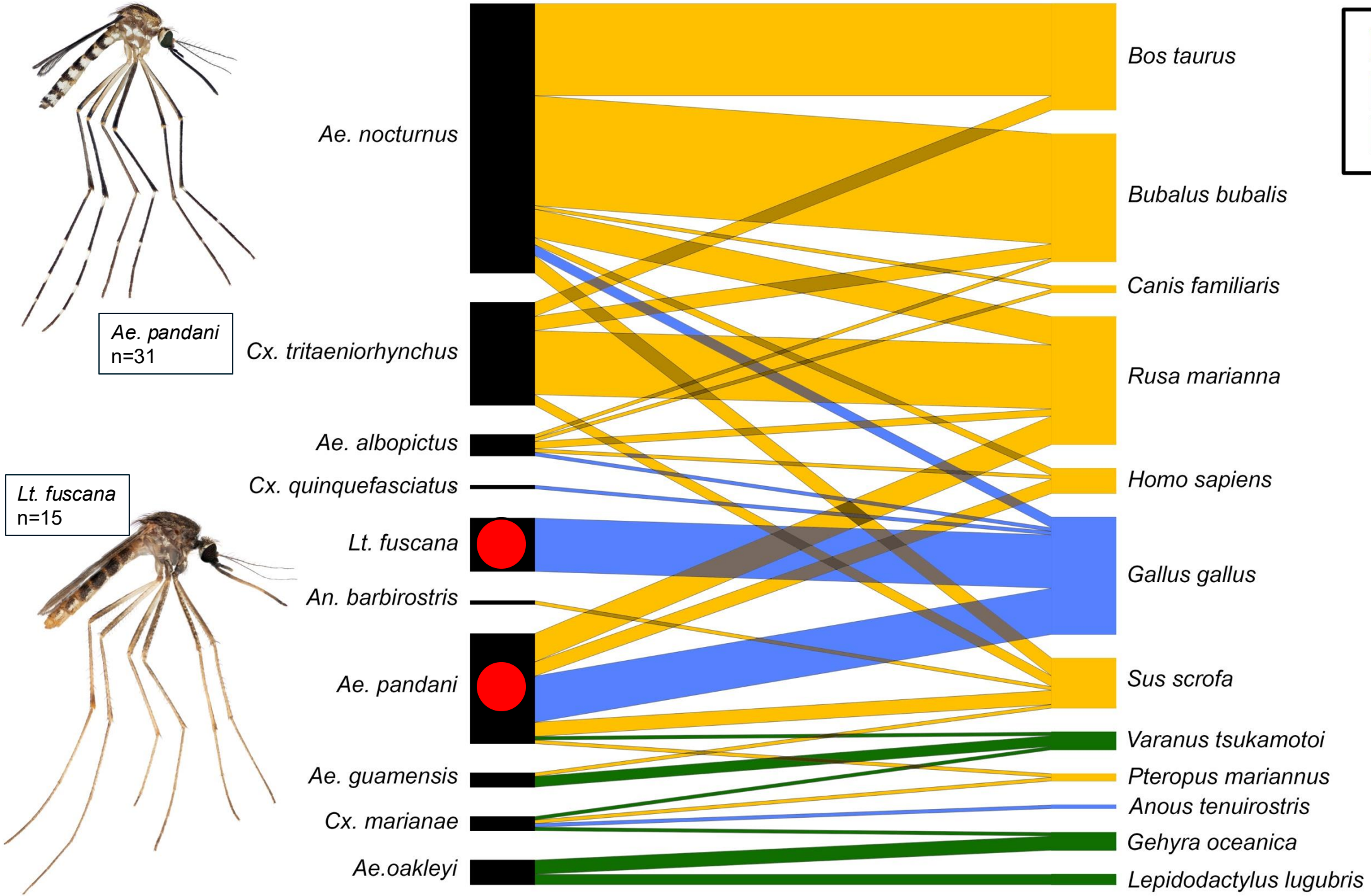


Cx. quinquefasciatus
n=1

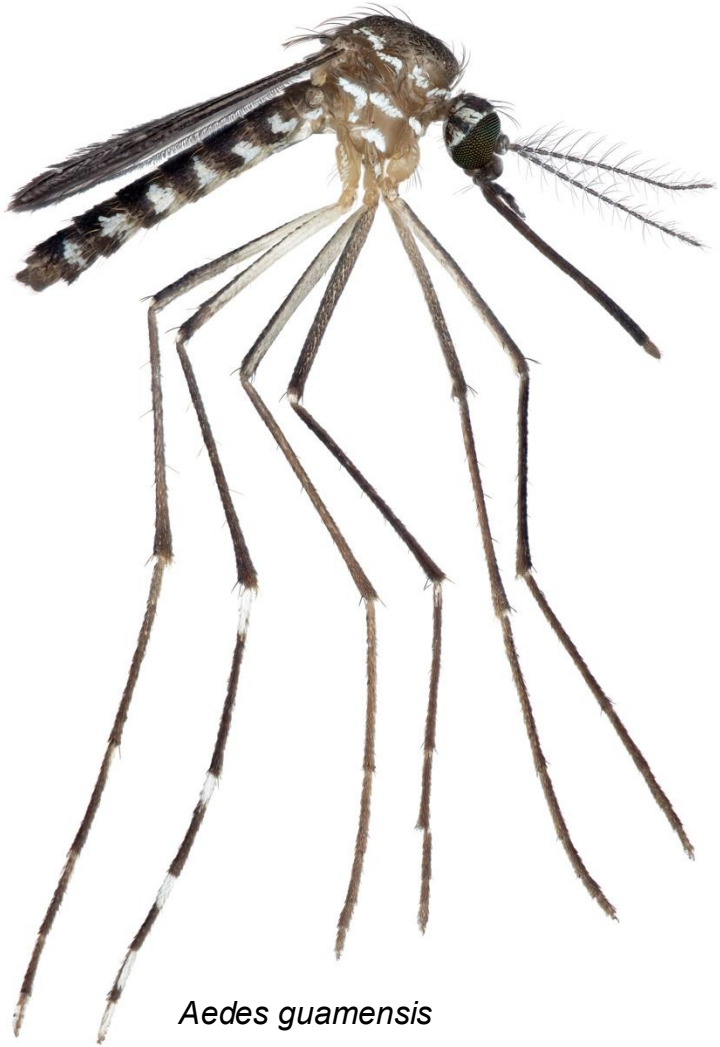
An. barbirostris
n=1




Ae. nocturnus
n=76
Cx. tritaeniorhynchus
n=29
Ae. albopictus
n=6
Ae. nocturnus
Cx. tritaeniorhynchus
Ae. albopictus
Cx. quinquefasciatus
Lt. fuscana
An. barbirostris
Ae. pandani
Ae. guamensis
Cx. marianae
Ae.oakleyi
Bos taurus
Bubalus bubalis
Canis familiaris
Rusa marianna
Homo sapiens
Gallus gallus
Sus scrofa
Varanus tsukamotoi
Pteropus mariannus
Anous tenuirostris
Gehyra oceanica
Lepidodactylus lugubris
Mammal
Bird
Reptile


Ae. pandani
n=31
Lt. fuscana
n=15
Ae. nocturnus
Cx. tritaeniorhynchus
Ae. albopictus
Cx. quinquefasciatus
Lt. fuscana
An. barbirostris
Ae. pandani
Ae. guamensis
Cx. marianae
Ae.oakleyi
Bos taurus
Bubalus bubalis
Canis familiaris
Rusa marianna
Homo sapiens
Gallus gallus
Sus scrofa
Varanus tsukamotoi
Pteropus mariannus
Anous tenuirostris
Gehyra oceanica
Lepidodactylus lugubris
Mammal
Bird
Reptile

Aedes (*Stegomyia*) *guamensis*

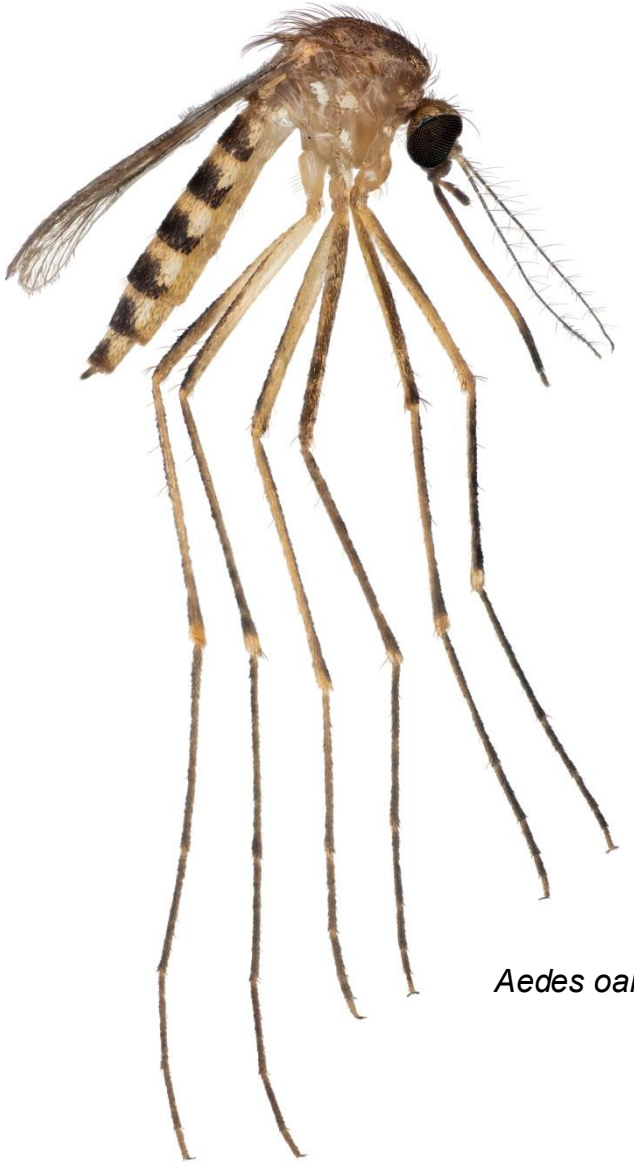


Aedes guamensis

- Member of subgenus *Stegomyia*, like *Ae. aegypti* and *Ae. albopictus*
- Native, endemic to Mariana Archipelago
- Dominant tree hole mosquito prior to arrival of *Ae. albopictus*
- Larvae in natural and artificial containers
- Host associations previously unknown, does not bite humans
- Strong association with reptiles (3/4 bood meals from monitor lizards)

Larval habitat of *Aedes guamensis*, Cocos

Aedes (*Aedimorphus*) *oakleyi*

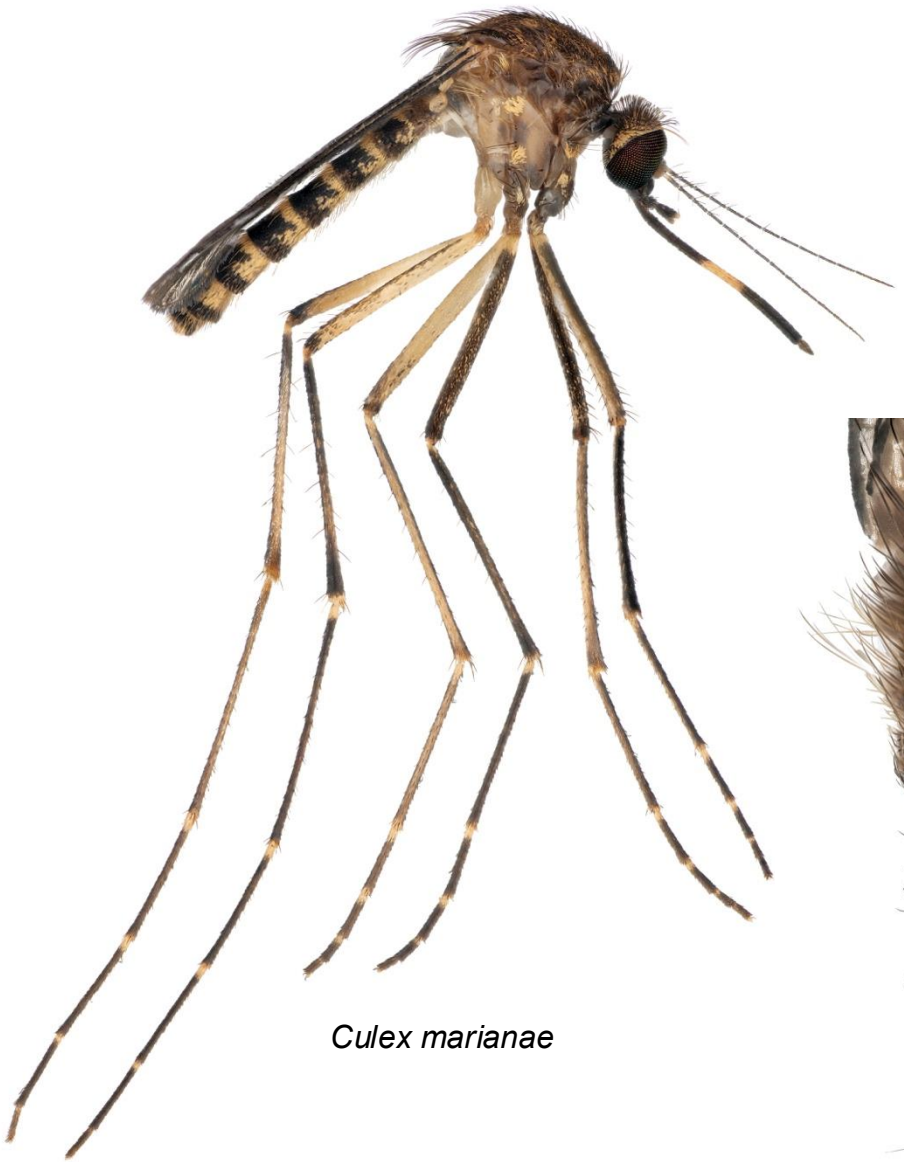


Aedes oakleyi

- Member of subgenus *Aedimorphus*, like *Ae. vexans*
- Native, endemic to Mariana Archipelago
- Host associations previously unknown, does not bite humans
- Larvae in natural and artificial containers
- Poorly known, resting sites unstudied
- Strong association with reptilian hosts (7/7 blood meals from geckos), likely a specialist of reptiles

Gehyra oceanica

Culex (*Culex*) *marianae*



Culex marianae

- Member of subgenus *Culex*, like *Cx. quinquefasciatus*
- Previously a subspecies of *Cx. annulirostris*
- Native, endemic to Mariana Archipelago
- Host associations previously unknown, not strongly attracted to human hosts
- Larvae in fresh to brackish ground pools, containers, vegetated water
- Small sample size, but appears to be a generalist

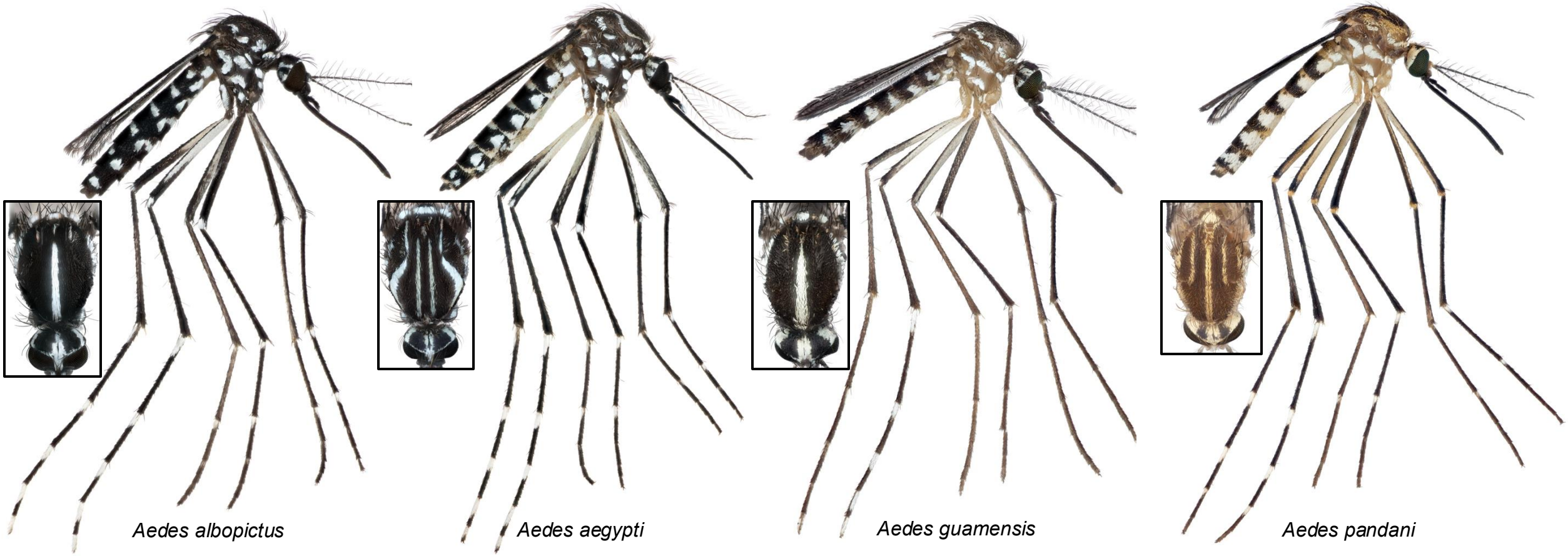
Lesser noddy

Oceanic gecko

Mariana monitor

Mariana fruit bat

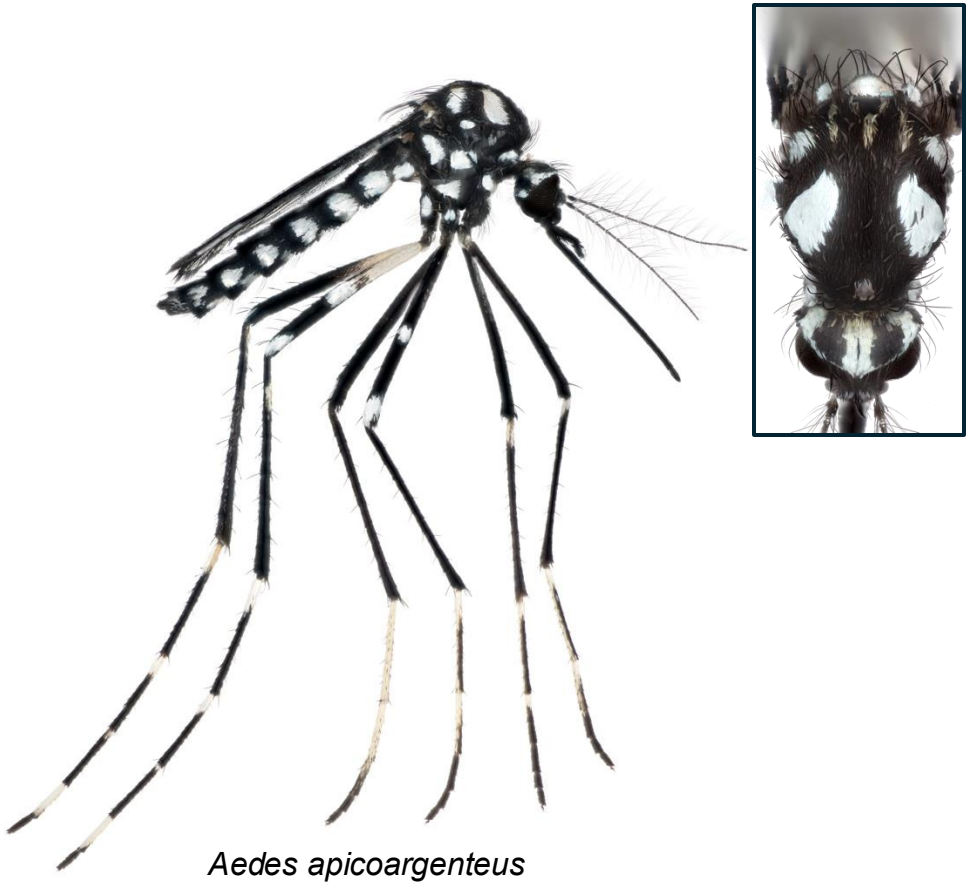
Aedes subgenus *Stegomyia*



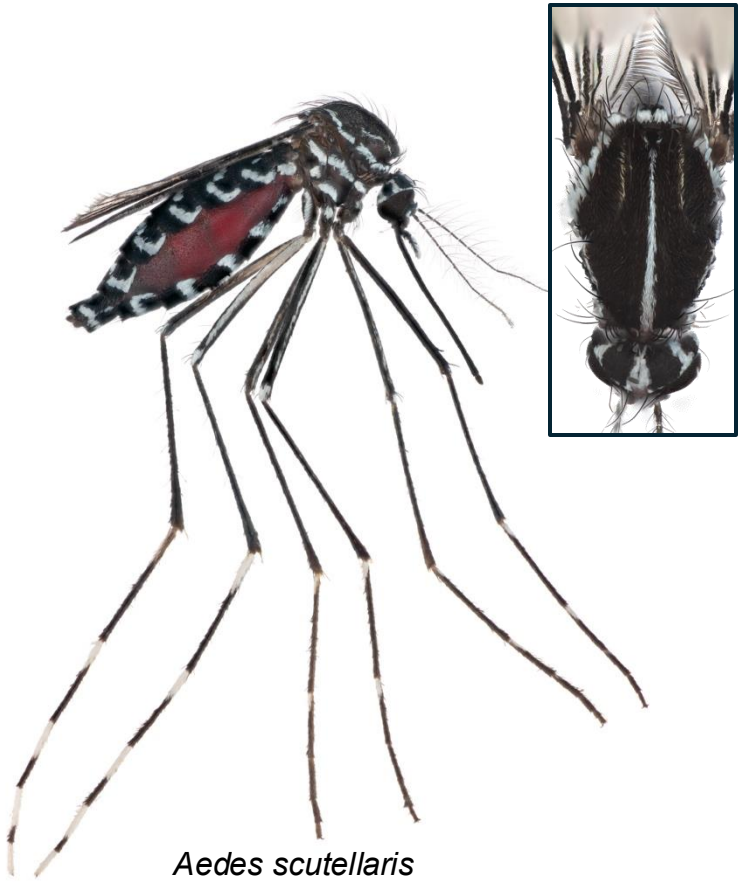
Widespread/cosmopolitan, globally important vectors

Poorly known, Mariana Archipelago endemics

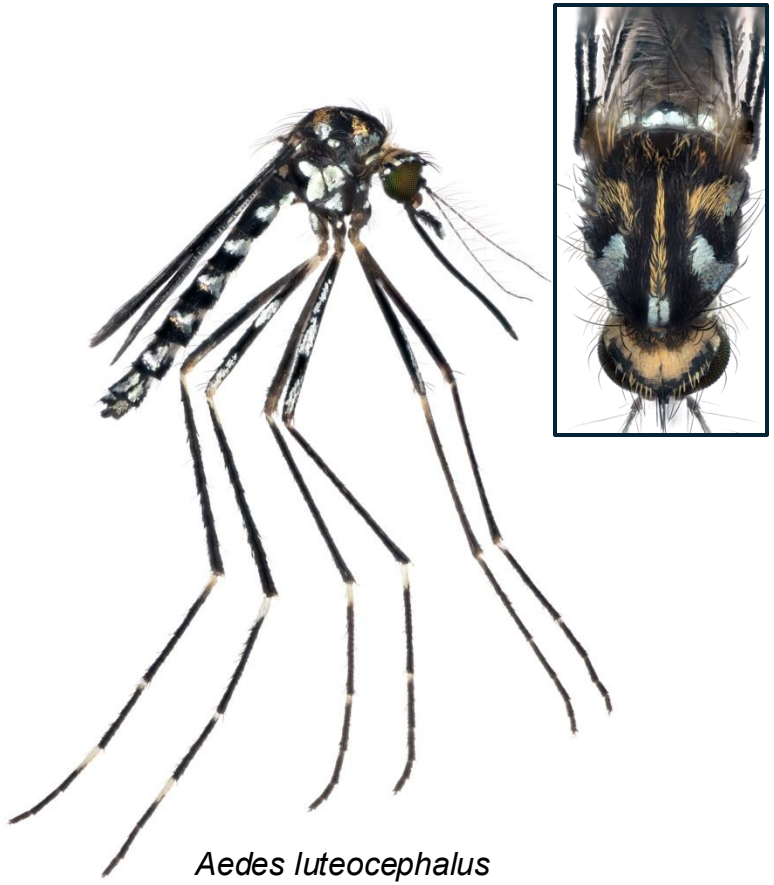
Aedes subgenus *Stegomyia* – 133 species, globally



Aedes apicoargenteus



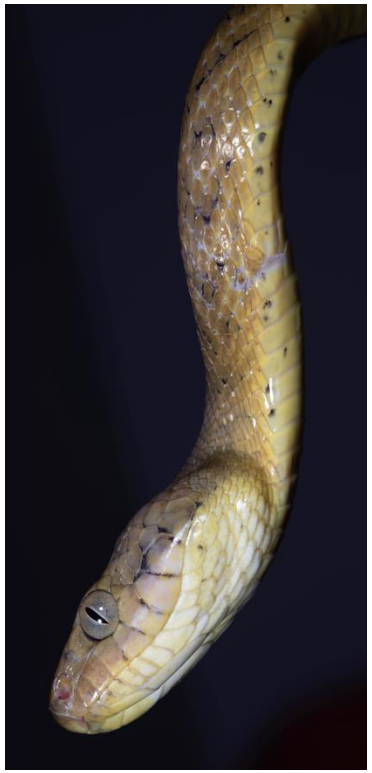
Aedes scutellaris



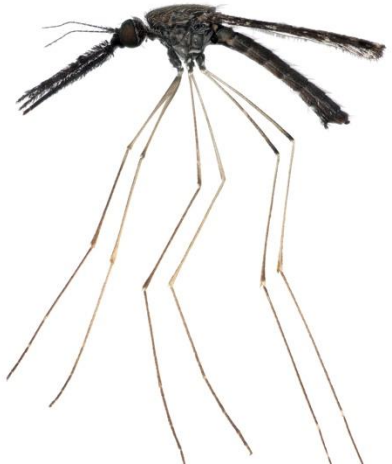
Aedes luteocephalus

Conclusions

- *Aedes guamensis* and *Ae. oakleyi* are likely specialists of reptilian hosts, but sample size was limited
- *Culex marianae* is likely a generalist that will feed from reptiles, but sample size was limited
- *Aedes nocturnus, Ae. pandani*, and *Cx. tritaeniorhynchus* are abundant, but feed from mammals or birds (*Ae. pandani*)
- *Aedes guamensis*, *Aedes oakleyi*, *Culex marianae* are candidate species for maximizing return on investment in future surveys for brown treesnakes on Guam



- Cryptic diversity in *Culex tritaeniorhynchus* in Guam
- COI sequences from Guam *Anopheles barbirostris* do not match *An. barbirostris s.s.*; likely *An. wejchoochotei*



Acknowledgements

- Julie Siegel, NWRC Grants Specialist, and NWRC leadership for expediting the cooperative agreement
- Dr. Aubrey Moore, University of Guam (retired) for project inspiration
- Alex Urlaub, Jen Espineira, Ray Gellner for molecular lab support
- This work was supported by funding provided by the Department of the Navy, Joint Region Marianas, under IAA# 19-1740-1063-IA. Project management provided by NAVFAC Marianas

Aedes atlanticus and eastern diamondback rattlesnake, Liberty Co., Florida

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