Evaluating the Necessity of DNA Barcoding: A Comparative Analysis of *Anopheles bradleyi* and *Anopheles crucians* in Suffolk County

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Introduction

Anopheles bradleyi and Anopheles crucians are two different species of mosquito. While in adolescence, as pupae and larvae, they are easier to distinguish between due to differences in setae (Floore 1976), as they mature the two become virtually indistinguishable (Figures 1-4). The struggle to distinguish between the two species has become one of the most challenging issues in modern entomology. The two belong to what is known as the Anopheles crucians complex, a cryptic species group that contains at least six genetically distinct species (Wilkerson 2004). Cryptic species are groups of organisms that are morphologically indistinguishable from one another (Davinack 2024).

Anopheles bradleyi exist primarily in water marshes or salt water sources (The Biology of Anopheles quadrimaculatus 1992) while *Anopheles crucians* prefer inland freshwater sources (Anopheles crucians Complex date unknown). This habitat difference can be a way to distinguish between the two mosquito species.

This study sought to distinguish between two sample pools, one of which is assumed to be *Anopheles bradleyi* and the other assumed to be *Anopheles crucians*. Staff at the Suffolk County Department of Health Services identified the samples so, in theory our results should conclude similarly to assumption.

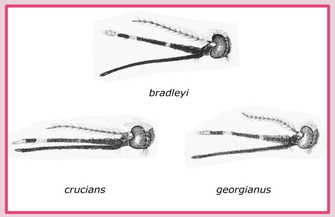

Figure 1. Morphological differences in *Anopheles* subgroups in the United States.

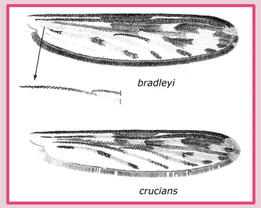

Figure 2. Wing differences between *Anopheles bradleyi* and *Anopheles crucians*.

Figure 3: *Anopheles crucians'* larvae setae (has more branches on their setae than *Anopheles bradleyi*).

Figure 4. *Anopheles bradleyi* larvae setae.

Abstract

Anopheles bradleyi and *Anopheles crucians* are two species of mosquito that look virtually identical in adulthood, therefore it can be hard to identify species based purely on morphology. Of the twenty samples collected by the Suffolk County Department of Health Services, based on purely morphological classification and observed by entomologists, we predicted that samples 001-005 were *Anopheles bradleyi* and that samples 006-020 were *Anopheles crucians*. DNA barcoding was used to assess the accuracy of assumed species identification and test if DNA Barcoding is truly necessary when distinguishing between two physically similar species. After using the Chelex protocol we amplified the COI region using PCR; confirmed successful amplification using gel electrophoresis, and analyzed sequences on DNA Subway. All samples were successfully sequenced and determined to be *Anopheles crucians*. While our initial morphological classification had assumed that samples 001-005 were *Anopheles bradleyi* this was not the case and shows that morphological identification cannot be reliably used to tell these species apart.

Figure 5: Samples 001-003

Figure 6: Samples 007-0009

Materials and Methods

Samples were provided by the Suffolk County Department of Health Services and these samples were obtained from the locations as followed: BH Southaven- samples 11 and 19, BNMuncy- sample 18 (2), EHThreeMileHarbor- sample 14 (1), ISGardinerPk- samples 2, 7, 9, and 20 (4), ISHeckscher- sample 4 (1), ISLake- sample 13 (1), ISOrowacSump- sample 16 (1), RHManorville- sample 12 (1), SMSteuben- samples 6, 10, 15, and 17 (4) , BHFIWatchHillWest- samples 3 and 8 (2), BHFIPines- samples 1 and 5 (2).

Mosquitoes were observed under the dissection microscope and photographed, using a metric ruler for reference (Figures 5 and 6). Images and metadata were then uploaded to the Sample Database. Samples 001-005 were speculated to be *Anopheles bradleyi* and samples 006-020 were speculated to be *Anopheles crucians*. Mosquito DNA was isolated using the Chelex protocol, and the COI region was amplified using PCR.

Successful amplification was determined using gel electrophoresis (Figures 7 and 8). All samples showed a bright band at 600-700 bp, indicating the COI region was successfully amplified. Samples were sent out for sequencing and analyzed using BLAST in DNA Subway.

Discussion

Relying on morphological identification to differentiate between *Anopheles bradleyi* and *Anopheles crucians* was found to be ineffective. While a seasoned entomologist identified five samples of our twenty as *Anopheles bradleyi* using morphology, barcoding revealed that all twenty samples were *Anopheles crucians*. Indeed the main benefit of DNA sequencing in this instance is to identify mosquitos that look identical to the naked eye, and it improves biodiversity studies and disease-vector monitoring (Beebe 2018).

In addition, this gives question to whether *Anopheles crucians* has a higher ecological tolerance than previously thought due to the implication that they were found in areas that *Anopheles bradleyi* are found more traditionally. Future studies should include a survey of biodiversity on areas that *Anopheles crucians* populates versus the areas *Anopheles bradleyi* populates due to the inference that areas *Anopheles crucians* may be expanding their range.
DNA markers help scientists study mosquito evolution and population genetics in the species as well as supports disease prevention by identifying vector species correctly (Wei 2026). Though we analyzed the COI region, there may be other promising genetic markers. *Anopheles bradleyi* has a unique ITS2 DNA sequence length when compared to *Anopheles crucians* (Wilkerson 2004) and other promising molecular markers to distinguish cryptic species of mosquitoes include ribosomal DNA, the ITS region, and random amplified polymorphic DNA (Wei 2026).

Results

Following our successful results from gel electrophoresis and the analysis from DNA Subway it was concluded that all of the samples (001-020) are *Anopheles crucians* commonly known as acid water swamp mosquitos.

References

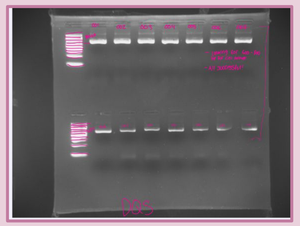

Figure 7: Samples 001-014

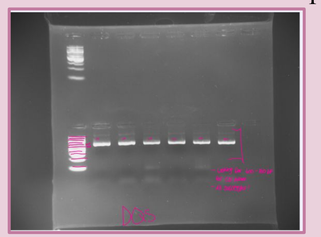

Figure 8 Samples 015-020

Acknowledgements

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