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**The Origins and Diversity of Mosquito Larval Habitats**

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**Abstract**

**Significance**

**Introduction**

Mosquitoes occupy a wide diversity of aquatic habitats ranging from the smallest bamboo internodes to intertidal rock pools ( ). This diversity of distinct habitats is accompanied by an array of unique behaviors and morphologies within different mosquito genera and subgenera often reflecting the ecological niches in which their constituent species are best adapted ( ).

A diversity of distinct habitat preferences, behaviors, and morphologies are exhibited and correlated with different mosquito genera and subgenera which reflect the ecological niches to which their constituent species are best adapted ( ). A diverse spectrum of ecological behaviors and unique morphological adaptations accompany these niche preferences including intraguild predation ( ), predator avoidance ( ),

Mosquito larvae obtain nutrients which ensure fitness in the adult life stage ( ).

Many previous studies classifying mosquito larval habitats placed emphasis a specific region (e.g., Laird, 1956; Kühlhorn, 1965; Happold, 1965; Hayes *et al.*,1985; Stein *et al.*,2011) or taxonomic group (e.g., Minakawa *et al.*, 2004; Lima Bersot *et al.*, 2023), but rarely had a global emphasis (e.g., Bates 1949, Laird 1988). Of the regional and global classification schemes, certain habitat elements are common throughout all schemes: the separation between container type habitats and ground pool habitats, saline versus freshwater, and the separation of container habitats based on phytotelmata versus non-phytotelmata. The Laird (1988) classification scheme attempted to merge these different schemes into a single, comprehensive classification based on previous work and expert knowledge. Habitats were broadly grouped into three major categories and eleven subcategories ranging from flowing streams to artificial containers. However, due to issues connecting different habitat descriptions together there is no globally comprehensive list of species-level habitat usage, thus making community level comparisons across mosquito family difficult.

**Methods**

Larval Habitat Database Construction

Mosquito larval habitat descriptions were harvested from taxonomic reviews (Zavortink, 1971), books (Belkin, 1962), and peer-reviewed academic literature ( ). Mosquito literature has a long history of including a “bionomics” section in species descriptions. This section includes relevant observational, ecological information like larval habitats, blood hosts, and other interesting ecological information about a species. As such, nearly all species (if the larvae have been collected) have at least some information pertaining to larval habitats. Keywords or phrases describing the larval habitat for each recorded mosquito species were entered and linked with the original publication for the record. All data and subsequent scripts used in this study can be found at <https://github.com/cbsither/Larval_Habitat_Pub> GitHub repository.

Habitat Classifications

Given the multitude of unique ways in which similar larval habitats are described throughout the literature, we developed a methodology for classification to compare across diverse descriptions. To accomplish this, the phytotelmata and freshwater/brackish water habitat descriptions were obtained from Greeney (2001) and European Nature Information System freshwater classification system (Davies *et al.*, 2004). This provided a means to group each habitat phrase to a discrete bin. Phytotelmata (e.g., bromeliads, tree holes, *Nepenthes*) are easier to recognize and classify as compared to “ground pool” or “stream margin” and other general terminologies used throughout the mosquito literature. A comprehensive list of all unique terms and their respective bin category can be found in Appendix A. In circumstances where phrases could represent multiple habitat categories or seemed ambiguous, we went back to the original habitat description to identify and use any additional information provided in the original description to bin the larval habitat(s) for a mosquito species. Ultimately, this approach creates many unique habitat descriptors, but not all habitats can be considered natural groups or are uniquely defined by their species assemblages.

To ensure all habitats can be uniquely identified,

Habitat Diversity and Community Assemblages

Ancestral State Reconstruction

Not all mosquito species in the larval habitat dataset are represented in the phylogenetic tree from Soghigian *et al.* (2023). To make use of all larval habitat observations we generated 1,000 TACT (Taxonomic Addition for Complete Trees) trees. The TACT program (Chang *et al.*,2019) stochastically places tips on a fixed backbone phylogeny based on the taxonomy of the tip being placed and the tips in the tree. Thus, if the taxonomy reflects the likely relationships among taxa in the tree, then a set TACT tree contains the uncertainty in branch lengths and relationships among those taxa. By generating a large population of TACT trees, then the uncertainty is reflected in the population of trees and in subsequent analyses. This allows for the inclusion of taxa that are not represented in the initial molecular phylogeny without having to make strong assumptions about the relationships or branch lengths of that missing tip in a tree.

We reconstructed the ancestral states of mosquito larval habitat preferences over the evolutionary history of Culicidae using the *ace* function in the *Ape* R package v 5.0 (Paradis and Schliep, 2019). We used a reduced larval habitat classification scheme of all phytotelmata (fallen plant parts [FPP], modified leaves [ML], tree holes [TH], leaf axils [LA], bamboo [BAM], fruit husks [FH], and flowering parts [FP]) and all other habitats collectively termed “ground pools”. Species which are reported in both habitats (phytotelmata and ground pools) had larval habitats states indicated as ‘uncertain’ allowing the program to integrate over both states for a particular species. This approach reduces model complexity and interpretability rather than adding an additional state into the model representing species being present in both habitats. Model selection was conducted during several preliminary analyses where three different continuous time *k*-state Markov models (equal rates, symmetric rates, and all-rates-different [ARD]) were compared with Akaike Information Criterion (Akaike, 1998) (AIC). The ARD model was the best model during all preliminary analyses and was used for all subsequent analysis. The *ace* function has a power function parameter kappa (*k*) that exponentially scales the branch lengths of the tree. When *k*=1 the full branch lengths and speciation process on a tree are considered. If *k*=0 then all the branch lengths equal 1, and only the speciation process on the tree is represented in the ancestral state probabilities. Often *k*=0 is interpreted to represent punctuated equilibrium evolution of a character state, and if *k*=1 then phyletic gradualism (Nunn, 2011). With no prior knowledge about what *k* should be or how larval habitat characters evolve, we calculated the ancestral states for larval habitats with different values of *k* from 0 to 1 with 0.01 increasing increments on each of the 1,000 TACT trees. The result is the probability of an ancestral state at a node given uncertainty of taxonomic placement in the TACT tree and the uncertainty in the evolutionary process of the character state (controlled by *k*). Instances where the probability of a particular ancestral state is favored across all values of *k* then that is the most likely ancestral larval habitat for an internal node. Marginal probabilities for each internal node in all trees are calculated with respect to the descendant tips and descending nodes.

**Results**

Data Acquisition and Classification

We assembled a dataset of 1,998 mosquito species (approximately 53% of all known diversity) and their respective habitat descriptions. A total of XX unique habitat phrases and descriptions exist throughout the scientific literature. All 11 mosquito tribes, 39 of the 42 mosquito genera, and 313 of the 390 mosquito subgenera are represented in the dataset (Supplemental Figure XX). Many habitat types are represented by small sample sizes which can subsequently confound variance estimates between habitat types. To account for this, we cleaned the dataset by removing habitat categories with sample sizes containing less than 10 observations. This removed 41 habitat categories and incidentally 10 mosquito species. The cleaned larval habitat dataset

Habitat Uniqueness across the Mosquito Family

1. Bray-Curtis Dissimilarity Metric
2. Habitat Occupancy
   1. Mosquitoes found single or multiple habitats
   2. Relationship between total publications and total habitats

Timing and Patterns of Phylotemata Inhabiting Mosquitoes

**Discussion**

We hope this study highlights a critical and pervasive issue impacting all comparative analysis studies merging phylogenetic and ecological data: simply knowing the present day shared common ancestry cannot alone resolve questions anchored in the past. Alternative data in the form of prior information is necessary when attempting to address questions involving data that has been censored or removed from evolutionary processes like extinction. Our approach in this paper utilizes one possible approach when attempting to account for extinction by outlining all known hypotheses, then systematically addressing the outcomes given the data. This resulted in two distinct hypotheses that hinge on the belief of whether the phytotelmata habitats existing prior to origin of angiosperms provided enough niche stability for phytotelmata specialist mosquitoes to exist. This is not a belief state that we can currently resolve with modern scientific methods outside of developing more hypotheses from other comparative methods like niche modeling using present day ecological data.

**References**

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