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Merging Mycology and Microbial Ecology: Investigating the Relationship Between a Prominent Trichomycete Fungus (*Zancudomyces culisetae*) and the Gut Microbiome of Its Mosquito Host

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Abstract

Trichomycetes is an ecological group of microfungi that colonize the digestive tract of certain Arthropoda. *Zancudomyces culisetae*, a prominent member of this group, inhabits the hindguts of multiple dipteran larval hosts, including the Yellow Fever Mosquito *Aedes aegypti*. Recently, a vast community of microbes has been discovered in the digestive tract of larval and adult *A. aegypti*. This microbiome affects host fitness, and is required for larval development. I propose laboratory investigations that will explore the potential impact that colonization of *Z. culisetae* has on the microbial community composition and structure of the larval and adult *A. aegypti* gut microbiome.

Merging Mycology and Microbial Ecology: Investigating the Relationship Between a Prominent Trichomycete Fungus (*Zancudomyces culisetae*) and the Gut Microbiome of its Mosquito Host



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Introduction

Trichomycetes is an ecological group of microfungi that colonize the digestive tract of certain Arthropoda. *Zancudomyces culisetae*, a prominent member of this group, colonizes and inhabits the hindguts of multiple dipteran larval hosts. *Aedes aegypti*, the Yellow Fever Mosquito, is a host for *Z. culisetae* in the wild while in its immature, aquatic stage (Pereira et al., 2005), and is a major vector for Yellow, Dengue, and Zika Fevers. Recent studies have discovered vast communities of microbes inhabiting the digestive tract of *A. aegypti* larvae (Coon et al., 2014), and adults (Gusmao et al., 2010, Coon et al., 2014, Chandler et al., 2015).

It has been shown that the gut microbiome is required for larval survival, and that adult *A. aegypti* may transstadially inherit a subset of bacterial community members from the larval gut microbiome during and after pupation (Coon et al., 2014). Recent studies have neither accounted for the presence of fungi, nor investigated whether fungal infection of the host gut alters the bacterial community of the host gut microbiome. Investigating these questions is vital to expand our understating of the factors that influence *A. aegypti* fitness, and to assess potential interactions that bacteria and fungi have within the mosquito gut microecosystem. To address these concepts, I plan to conduct laboratory investigations utilizing established fungal inoculation techniques of *A. aegypti* larvae (Williams and Lichtwardt, 1972), polymerase chain reactions (PCR) targeting the bacterial 16S gene, next generation sequencing (NGS), and microbiome community composition and structure bioinformatic analyses.

Goals/Objectives

- 1) Contribute to the current knowledge of the microbiome composition and structure in the digestive tract of the *A. aegypti* larval and adult gut
- 2) Modify and implement established protocols to allow for regulated inoculation of larvae with *Z. culisetae* spores, and for the sterile harvest of adults after pupation.
- 3) Assess potential larval gut microbiome community shifts in response to infection by *Z. culisetae* at the fourth larval developmental instar.
- 4) Contribute to the current knowledge of transstadial transmission of the gut microbiome from *A. aegypti* larvae to adults.
- 5) Investigate whether fungal infection of the larval gut has an effect on the adult gut microbiome.

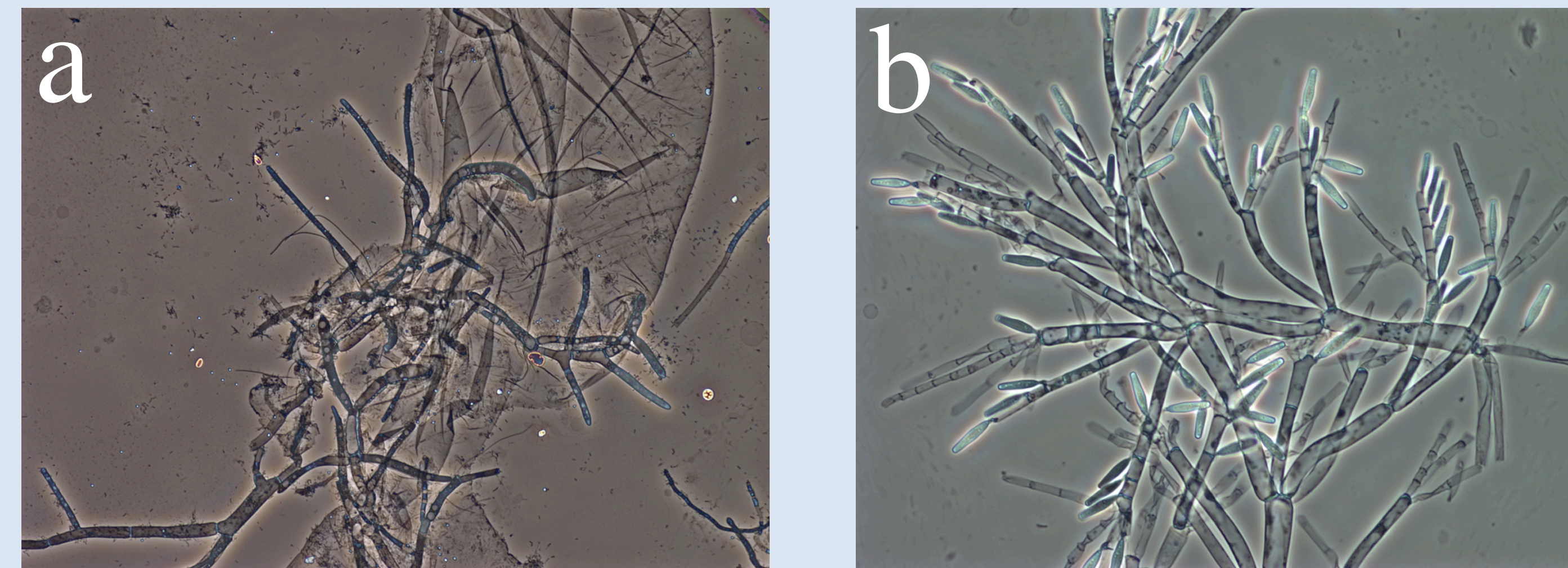


Figure 1. *Zancudomyces culisetae* isolated from mosquito digestive tracts. a) *Z. culisetae* thalli directly attached to a mosquito hindgut. b) Mature *Z. culisetae* producing and releasing spores (highlighted structures). Photo by: Michael McCormick, White Research Laboratory, Boise State University



Figure 2. Female *Aedes aegypti* consuming a blood meal. Females consume blood prior to egg production. Photo by: James Gathany, Center for Disease Control and Prevention

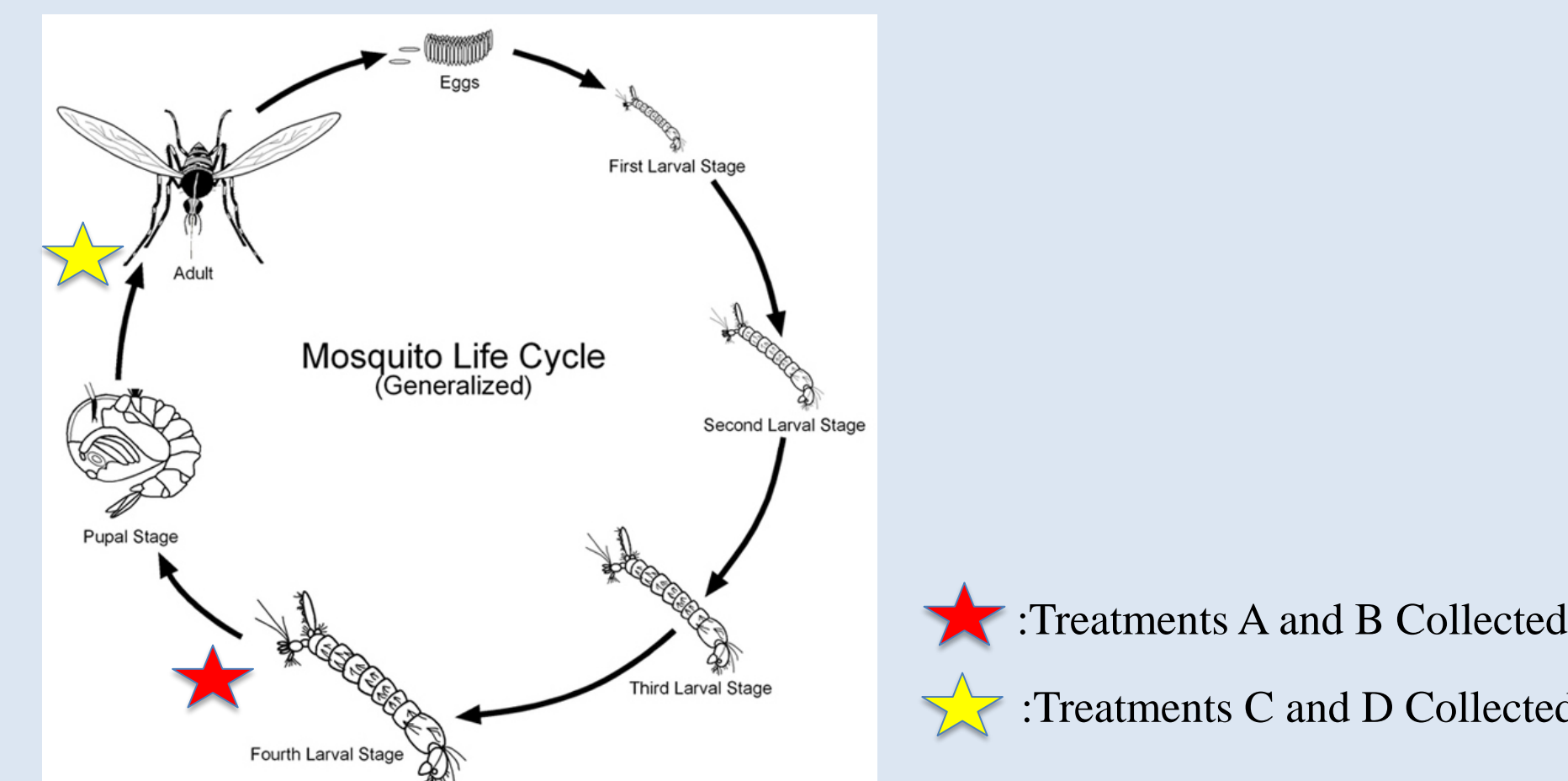


Figure 3. Generalized diagram of the mosquito life cycle. Eggs deposited on water surface. Larvae develop in water, and periodically molt and increase in size. Fourth instar larvae form pupae at the water surface. Adults emerge from pupae. Illustration by: Scott Charlesworth, Purdue University

Materials and Methods

Treatment Groups (with replicates):
A) No fungal inoculation, larvae collected at fourth instar
B) Fungal inoculation, larvae collected at fourth instar
C) No fungal inoculation, adults harvested immediately following pupation
D) Fungal inoculation, adults harvested immediately following pupation

Aedes aegypti eggs will be hatched in separate rearing containers, and larvae will be exposed to their relative treatments. Treatment groups A and C will not be inoculated with *Zancudomyces culisetae* spores, while groups B and D will be inoculated. Larvae will be reared in a growth chamber to control for photoperiod, temperature, humidity, and other abiotic factors. Once the larvae have matured to the fourth developmental instar, individuals from groups A and B will be collected. Individuals from groups C and D will be collected immediately after pupation. Bacterial DNA from each individual will be extracted using microbial DNA extraction kits. PCR targeting the bacterial 16S gene will be carried out on the microbial DNA. The amplicons generated will be sequenced on the Illumina Miseq platform. Once sequencing data have been gathered, bioinformatic analyses will be conducted to estimate the gut microbiome composition and structure of the individuals from each treatment group.

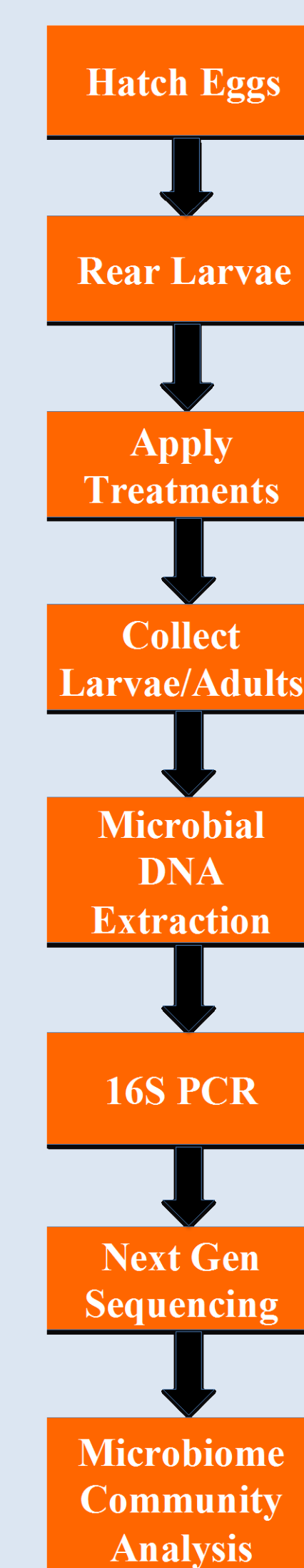


Figure 4. Flowchart Summarizing the Materials and Methods

Significance of Research

Recent studies have discovered that the mosquito gut microbiome is essential to ensure proper larval development (Coon et al., 2014), and can affect adult fitness as well (Minard et al., 2013). Research suggests that a subset of bacterial community members from the larval gut is transstadially transmitted to adults during pupation (Coon et al., 2014). It is paramount that we study factors that may alter the composition and structure of the mosquito gut microbiome. If the infection of the host gut by a fungus impacts the gut microbiome, future microbiome research could benefit from analyses that account for the role that fungi play within microbial communities. It is possible that new findings in this field could pave the way for developing future methods of mosquito pest control utilizing knowledge acquired from studying fungi in the gut microecosystem.

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