

# Variation in Larval Polychaete Morphology and SEM Imaging

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Marine Invertebrate Zoology Summer A 2025 FHL 432

Polychaete Larvae, SEM Microscopy, Marine Plankton

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## Abstract

This study examines the morphological variation in larval polychaetes collected during different times of day around San Juan Island, Washington. Focusing on the use of scanning electron microscopy (SEM), the project documents polychaete morphotypes collected from plankton tows and compares their form across time points. The work builds on an ongoing multi-year project investigating marine invertebrate diversity through both manual sorting and eDNA analysis. This contribution emphasizes microscopy preparation and imaging, with the goal of supporting future taxonomic identification and sequence validation.

## Introduction

Polychaete larvae represent a critical component of marine plankton communities and play essential roles in trophic dynamics and dispersal. Their larval stages, often morphologically distinct from adults, exhibit diel vertical migration behaviors that can cause variation in community composition by time of day. This project, conducted as part of the Marine Invertebrate Zoology course at Friday Harbor Labs, contributes to an ongoing research effort by Dr. Megan Schwartz, Amanda Schupner, and Mallori Standley examining larval marine invertebrate diversity through manual sorting and environmental DNA (eDNA). My focus was the documentation of polychaete larvae using SEM to help distinguish morphotypes and contribute to a broader taxonomic and genetic catalog.

## Hypothesis and Objectives

We hypothesized that larval polychaete abundance and diversity would vary by time of day due to diel vertical migration, with more forms observed at night. Additionally, we predicted that SEM imaging would reveal distinct morphological characters to assist in distinguishing larval types. The main goals were: (1) to conduct plankton tows at

different time points, (2) sort and preserve polychaete larvae, and (3) document fine-scale morphological traits via SEM.

## Methods

Three plankton tows were conducted from inside Friday Harbor Cove to the end of the FHL dock on San Juan Island: June 28 at 1:00 PM and 11:00 PM, and July 10 at 4:00 AM. Each tow lasted approximately 11 minutes using a ~155  $\mu\text{m}$  mesh plankton net. Samples were sorted under a dissecting microscope, and polychaete larvae were isolated and preserved in formalin. These specimens were then transitioned to ethanol via 10 sequential 50% volume swaps with 95% EtOH, followed by five 50% swaps with 100% EtOH. Due to interaction with residual seawater, a precipitate formed immediately on the larvae, particularly on the chaetae, which was gradually broken up by gentle tube inversion.

For SEM preparation, larvae were transferred to 12 mm, 0.45  $\mu\text{m}$  filter paper 'hats,' placed into a 96-well plate, and treated with 1–3 drops of hexamethyldisilazane (HMDS). They were left to soak overnight and air-dried for 45 minutes. Specimens were mounted on SEM stubs using adhesive; some were transferred directly, others were mounted on the paper itself. Samples were coated with gold-platinum using a Cressington 108 sputter coater and imaged on a JEOL Neoscope JCM-5000 at Friday Harbor Labs.

## Results and Conclusion

Larval polychaetes were more diverse and abundant during the night and pre-dawn tows, consistent with expected diel vertical migration patterns. Four morphotypes were identified, including one likely mitraria larva (possibly Oweniidae), a spionid-like larva with visible chaetae, one intermediate form, and one larger, unidentified form. SEM imaging revealed fine-scale features of the prostomium, chaetae, and body shape, supporting morphotype distinction. Each morphotype was preserved for future sequencing. This work contributes new SEM images to the FHL larval archive and provides a morphological framework for ongoing taxonomic and eDNA validation work.

## Works Cited

Kozloff, E. N. (1996). *Marine Invertebrates of the Pacific Northwest*. University of Washington Press.

Shanks, A. L. (2001). *An Identification Guide to the Larval Marine Invertebrates of the Pacific Northwest*. Oregon State University Press.



# From Rowboats to Resolution: Imaging Polychaete Larvae

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Course: Marine Invertebrate Zoology (FHL 432)

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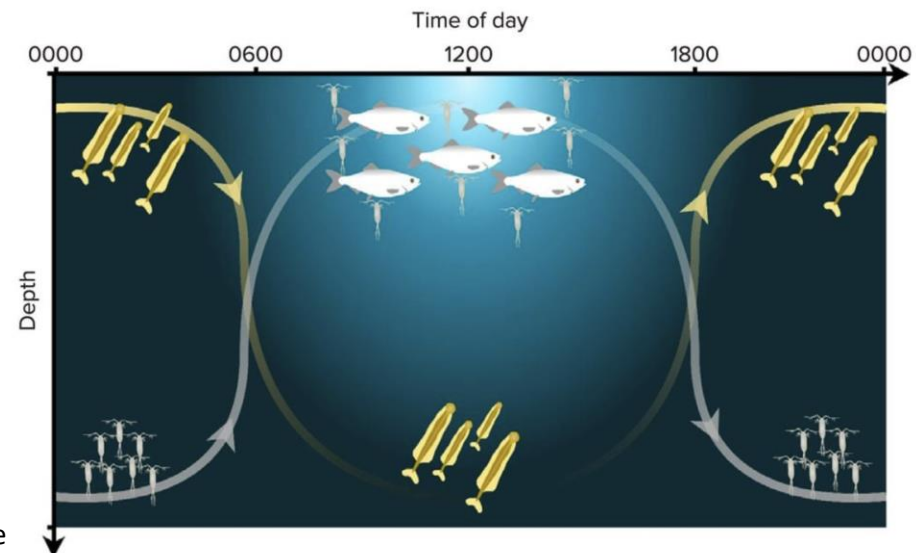
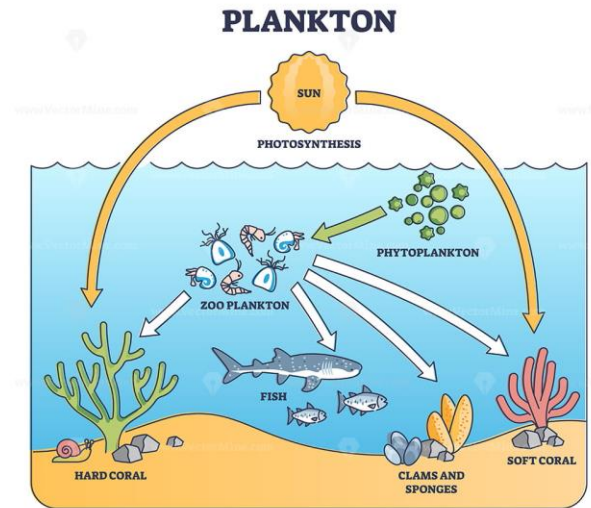
Keywords: Polychaete Larvae, SEM Microscopy, Marine Plankton

# Background & Collaborative Framework

- Part of ongoing project led by Dr. Megan Schwartz
- Current students: Amanda Schupner & Mallori Standley
- Focus: manual sorting + eDNA; my angle: polychaete morphology via SEM

# Why Polychaetes & Plankton Matter

- Key to marine food webs and recruitment processes
- Larval stages are ecologically rich and morphologically diverse
- Polychaetes undergo diel vertical migration
- Time of day affects presence/abundance



# Research Questions & Scope

- Does plankton abundance/diversity vary by time of day?
- Can SEM clarify larval morphotypes missed under light microscopy?
- Longer-term: compare sorting + SEM to eDNA

# Sampling Methods

- Three sampling events:
  - June 28 1pm (full light)
  - June 28 11pm (full dark)
  - July 10 4am (dawn)



- Same location: inside Friday Harbor Cove to FHL dock (~11 min)

# Tow Gear & Setup

- Net mesh: 155  $\mu\text{m}$ ; towed horizontally
- Tow duration ~11 min each; surface skim
- Sorted immediately post-tow under dissecting scope



# Sorting & Fixation



# Sorting & Fixation

- Focused on isolating polychaete larvae
- Preserved in formalin
- Pooled specimens post-sorting

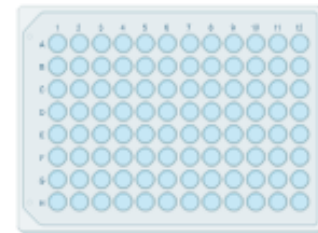
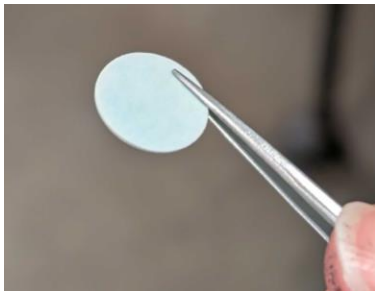


# Ethanol Transition & Precipitate

- 10x 50% swaps with 95% EtOH
- Precipitate formed immediately; began inverting at ~3rd round
- Followed by 5x 50% swaps with 100% EtOH
- All steps in 1.5 mL tubes; specimens stayed submerged

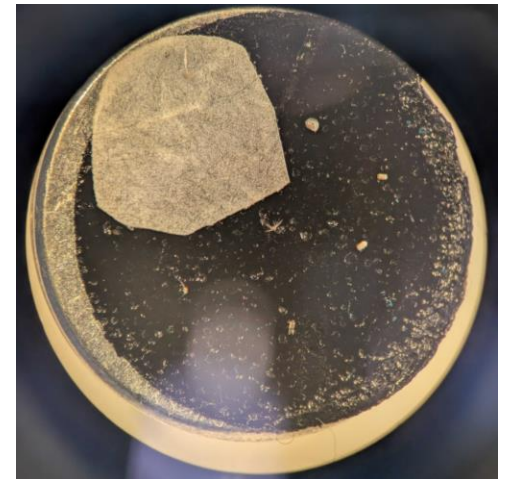
# HMDS Drying & Filter Paper Hats™

- Created 'hats' with 12 mm, 0.45  $\mu\text{m}$  filter paper (quartered)
- Placed larvae on paper; added to 96-well plate
- 1–3 drops HMDS per well, soaked overnight
- Air dried for ~45 min under hood next day



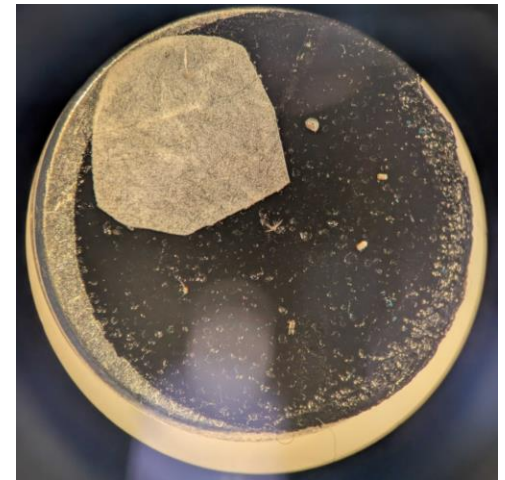
# Stub Mounting & SEM

- Lost 2 specimens removing paper (springboard effect)
- Some larvae mounted directly, others on full paper
- Mounted on adhesive SEM stubs
- Coated using Cressington 108 sputter coater (platinum)
- Imaged at FHL using JEOL Neoscope JCM-5000



# Stub Mounting SEM

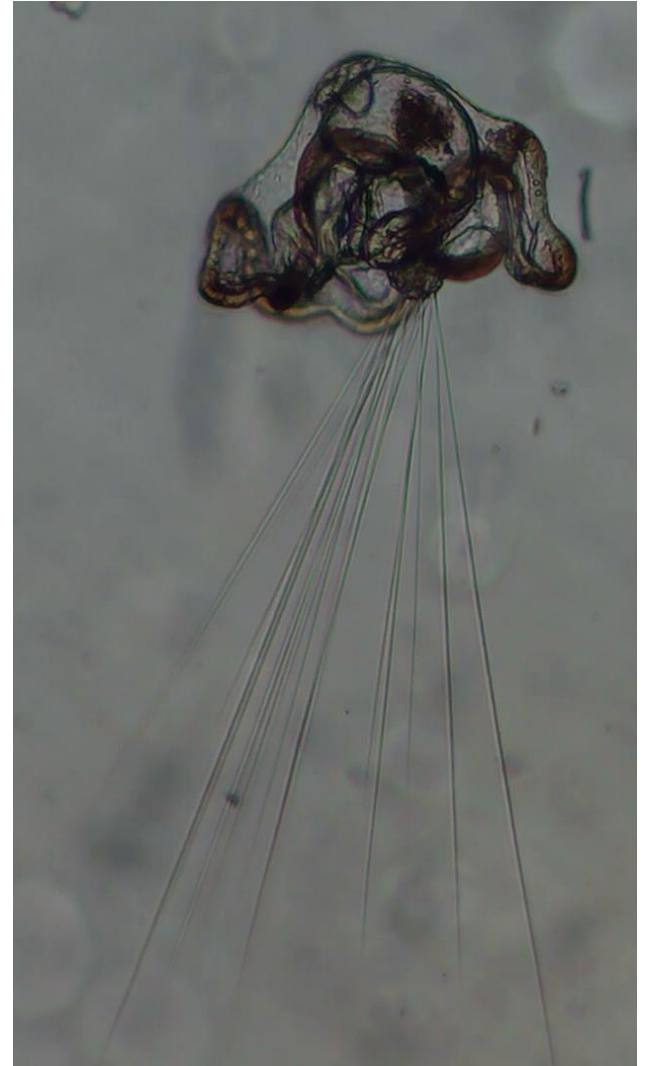
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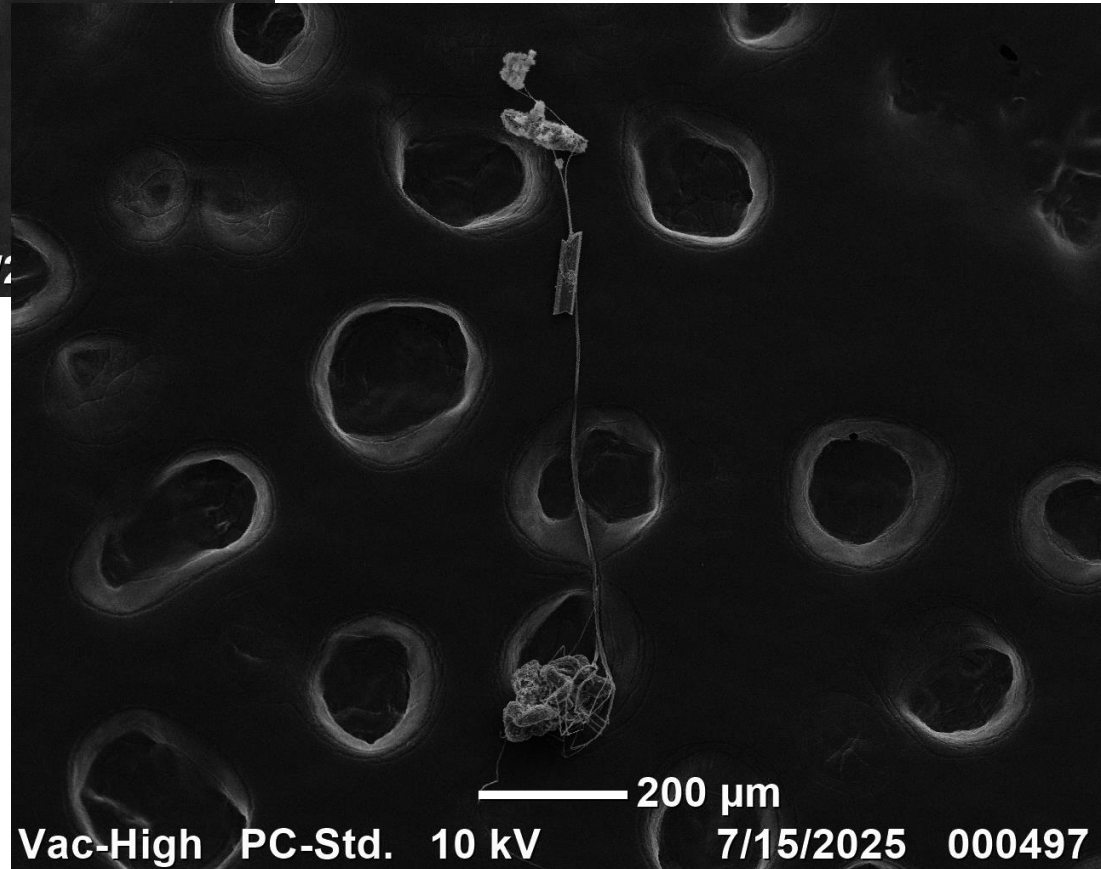
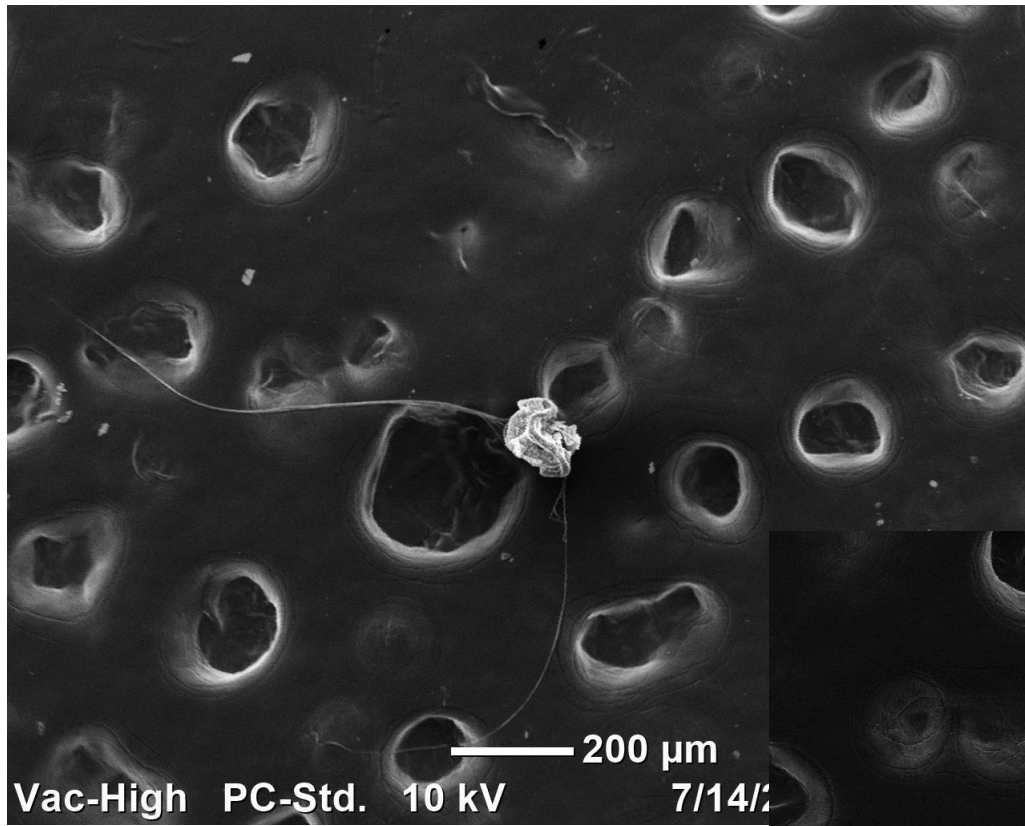
# Larval Morphotypes

1. Mitraria (Oweniidae?)
  2. Intermediate morphotype
  3. Spionid-like (chaetae visible)
  4. Large mystery larva (1 specimen)
- ~1-2 per morphotype sent for sequencing

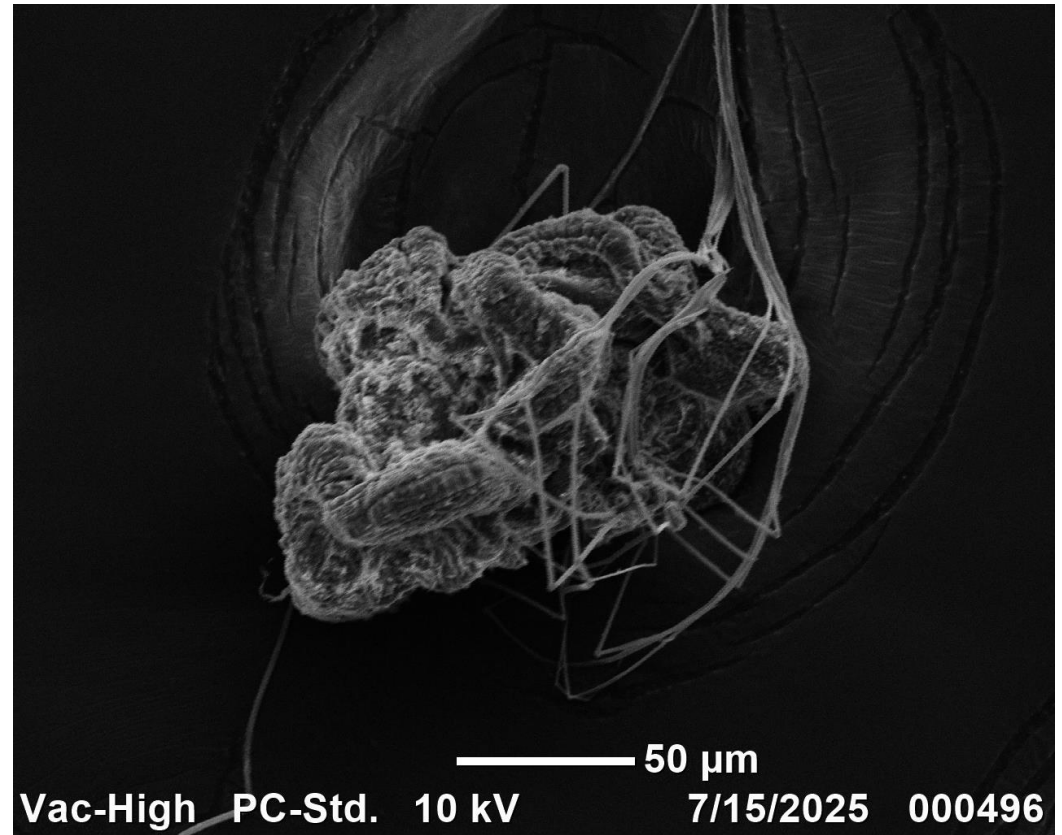
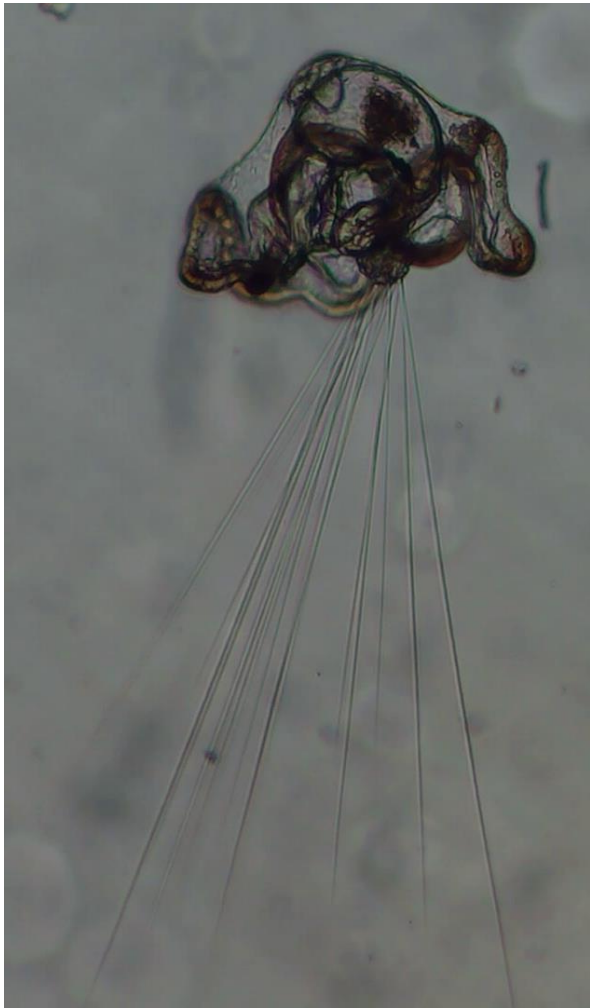
# 1. Mitraria



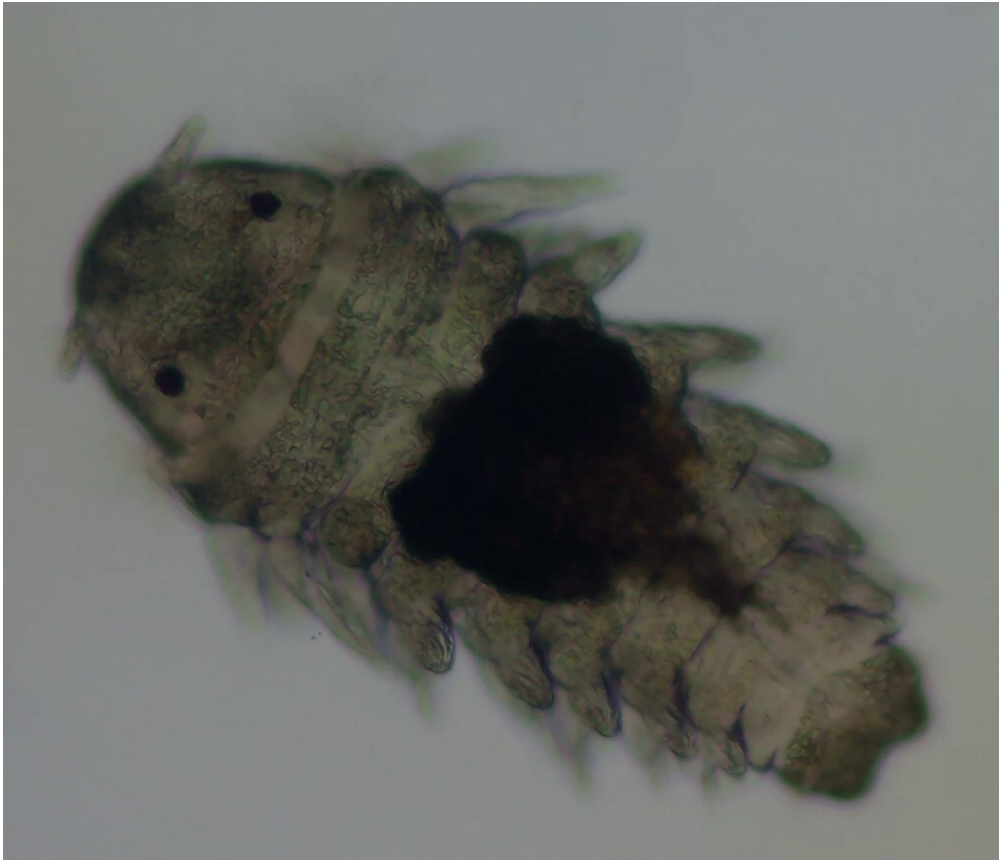
# 1. Mitraria



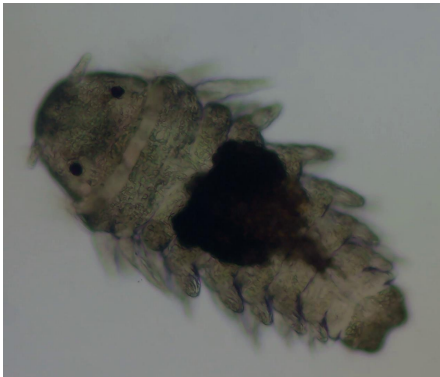
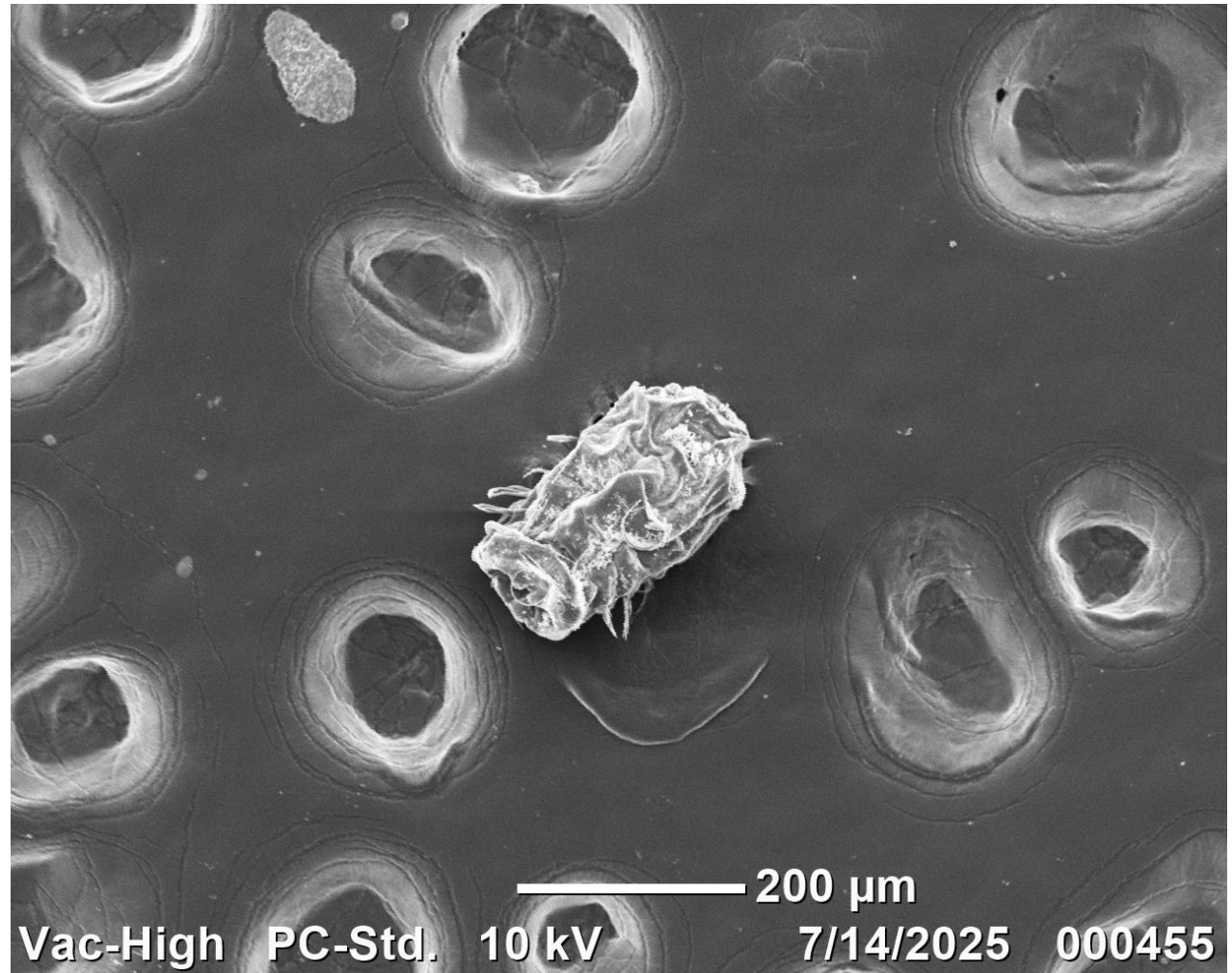
# 1. Mitraria



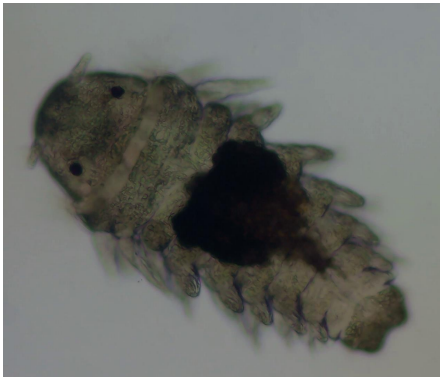
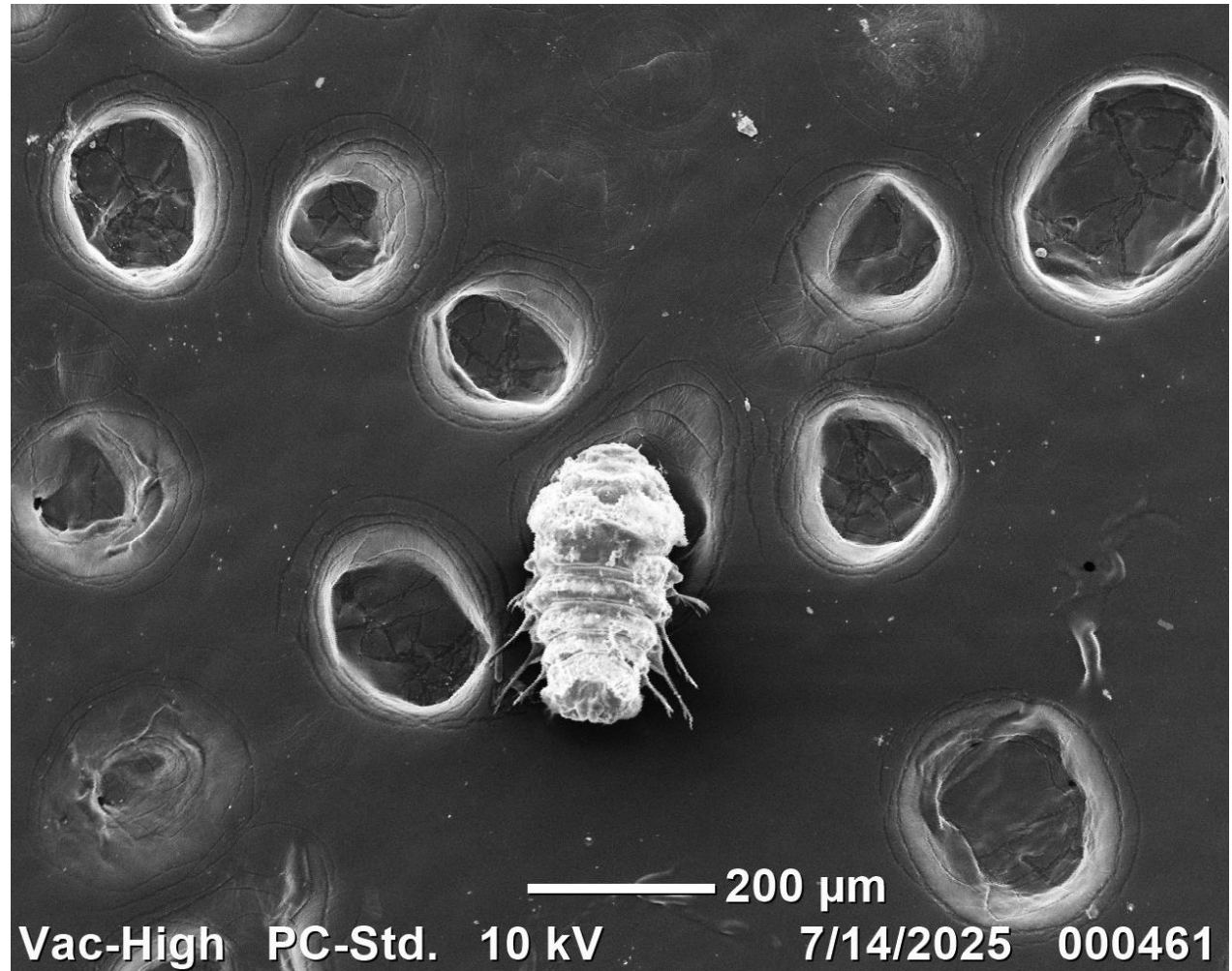
## 2. Intermediate morphotype



## 2. Intermediate morphotype



## 2. Intermediate morphotype



# 3. Spionids



# 3. Spionids



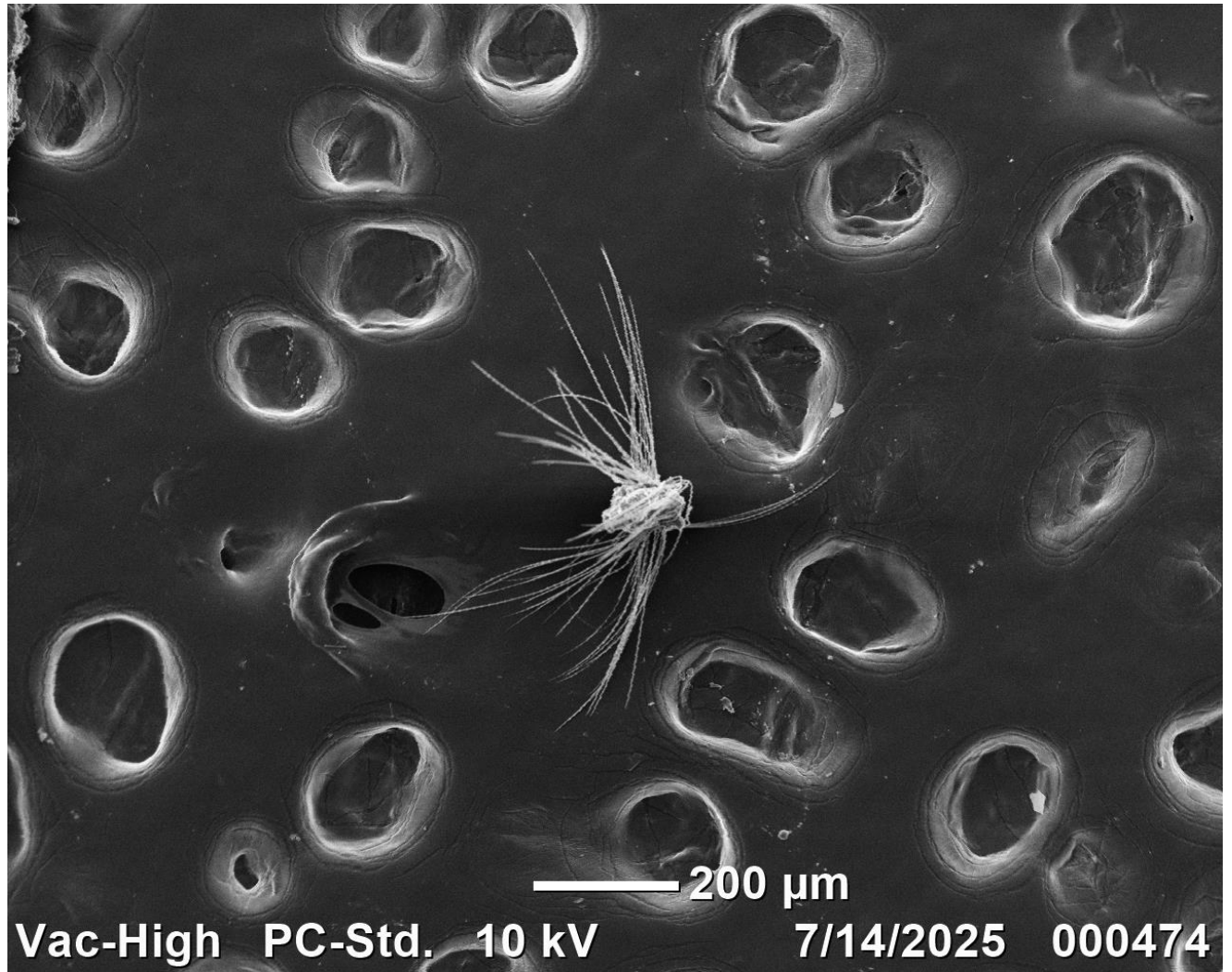
## 4. Large mystery larva



# 3. Spionids



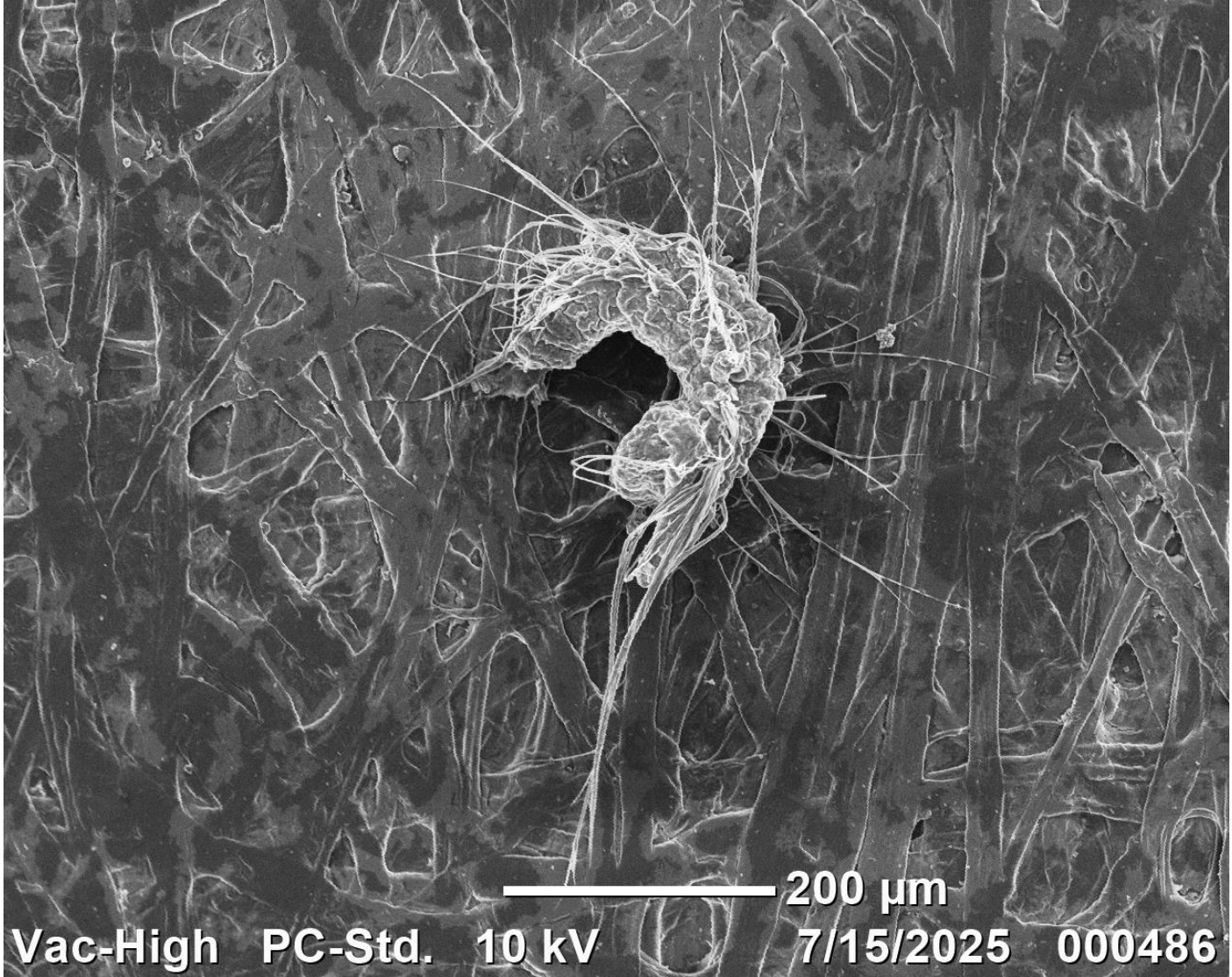
# 3. Spionids



# 3. Spionids



# 3. Spionids



# Reflections & Future Work

- Does plankton abundance/diversity vary by time of day?
  - Most diversity observed during night/pre-dawn
- Can SEM clarify larval morphotypes?
  - Manual sorting + SEM = strong combo for morphology
  - Supports larval archive for FHL
- Next: finalize IDs, confirm with sequence/eDNA

A night scene of a fireworks display over a body of water. The fireworks are bright green and orange, exploding in the dark sky. The water is dark, reflecting the lights from the fireworks and the boats. Several boats are visible on the water, some with lights on. In the foreground, the silhouettes of people are visible, sitting on a grassy bank and watching the fireworks. The background shows a dark shoreline with some lights and trees.

# Thank you!

Dr. Megan Schwartz

Dr. Rebecca Varney

Amanda Schupner

Mallori Standley

My classmates and the FHL community

Shanks (2001), Kozloff (1996), and others