Morphological variation within the apple snail *Pomacea paludosa* as a predictor of parasite infection

Alexandria Sand

Lori Tolley-Jordan

Brian Helms

Apple Snails as invasive species

- *Pomacea* species are a rising concern as invaders, specifically *P. maculata* and *P. canaliculata*
- Native to South America and are now found throughout southeast Asia, Australia, and North America through the facilitation of aquarium trade
- Traits of a successful invader
  - Dual respiratory system adaptation has aided their continued dispersal and introduction into novel ecosystems
  - High fecundity

Invasive apple snails and associated parasites

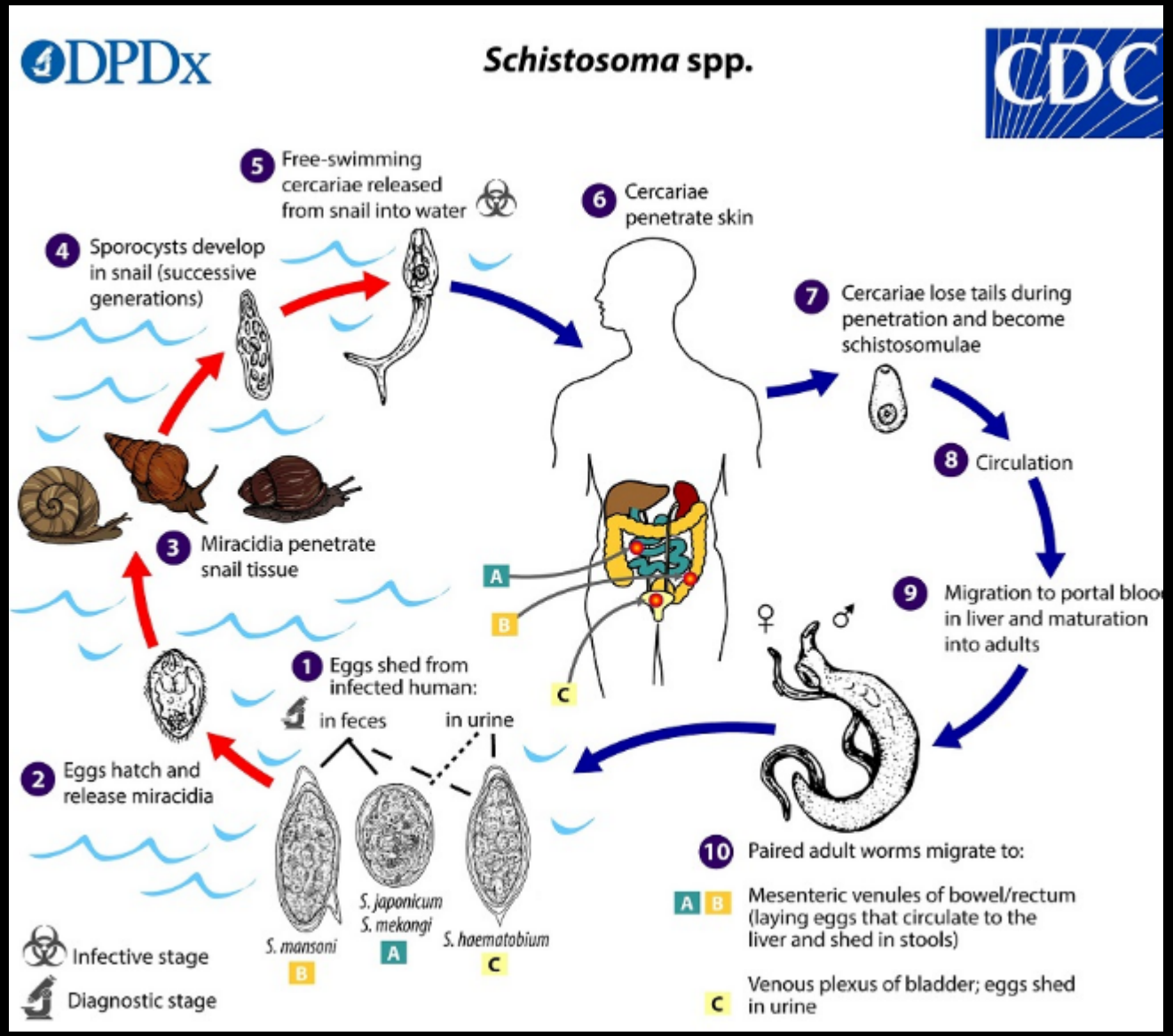
Serve as intermediate host to a vast number of parasites

- Echinostomatidae
- Amphistomatidae
- Schistosomatidae
- Paramphistomidae

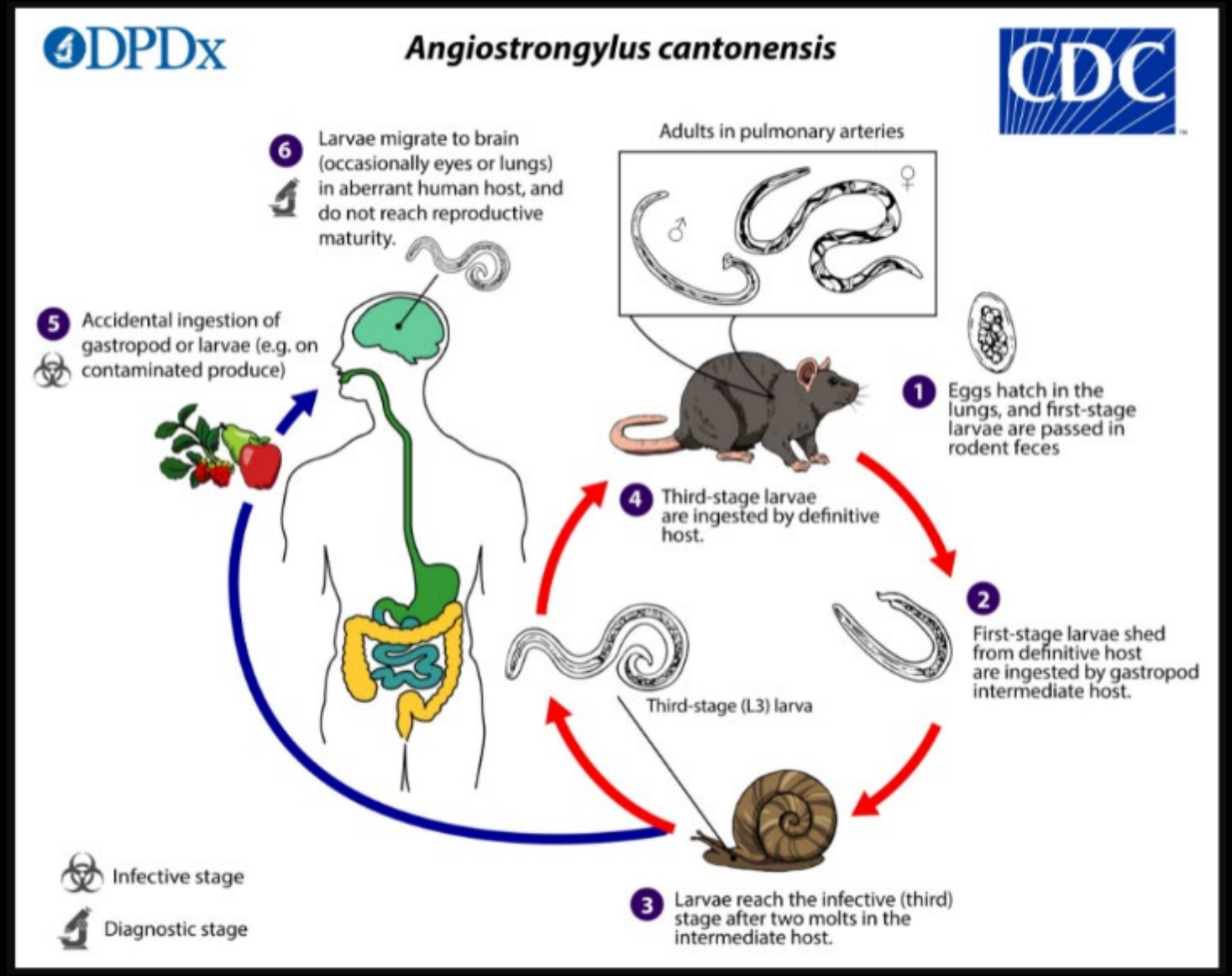
Posing threats to humans and surround wildlife

- Schistosomiasis
- Cercarial Dermatitis
- Eosinophilic meningitis

Generalized Trematode Life Cycle



Generalized Nematode Life Cycle

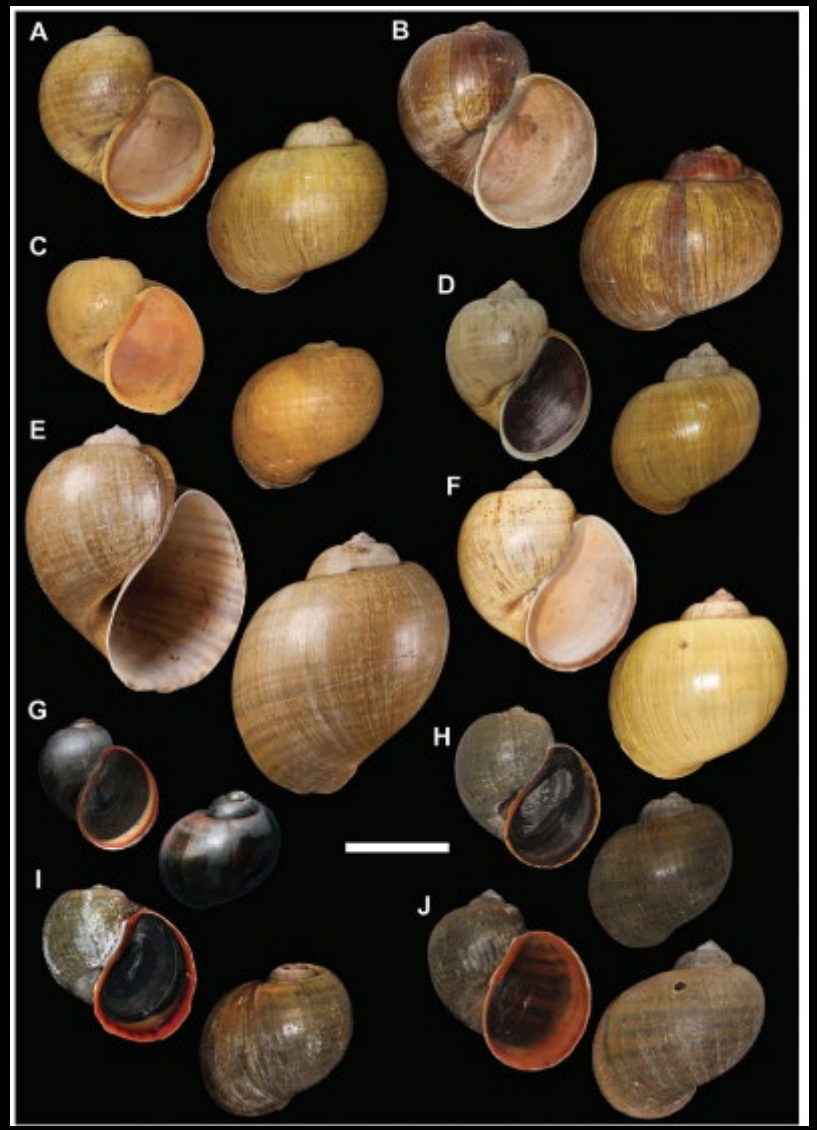


Morphological variations

Can be found within snail species

Can be indicative of environmental conditions, genetic differentiation, sexual dimorphism, and population isolation

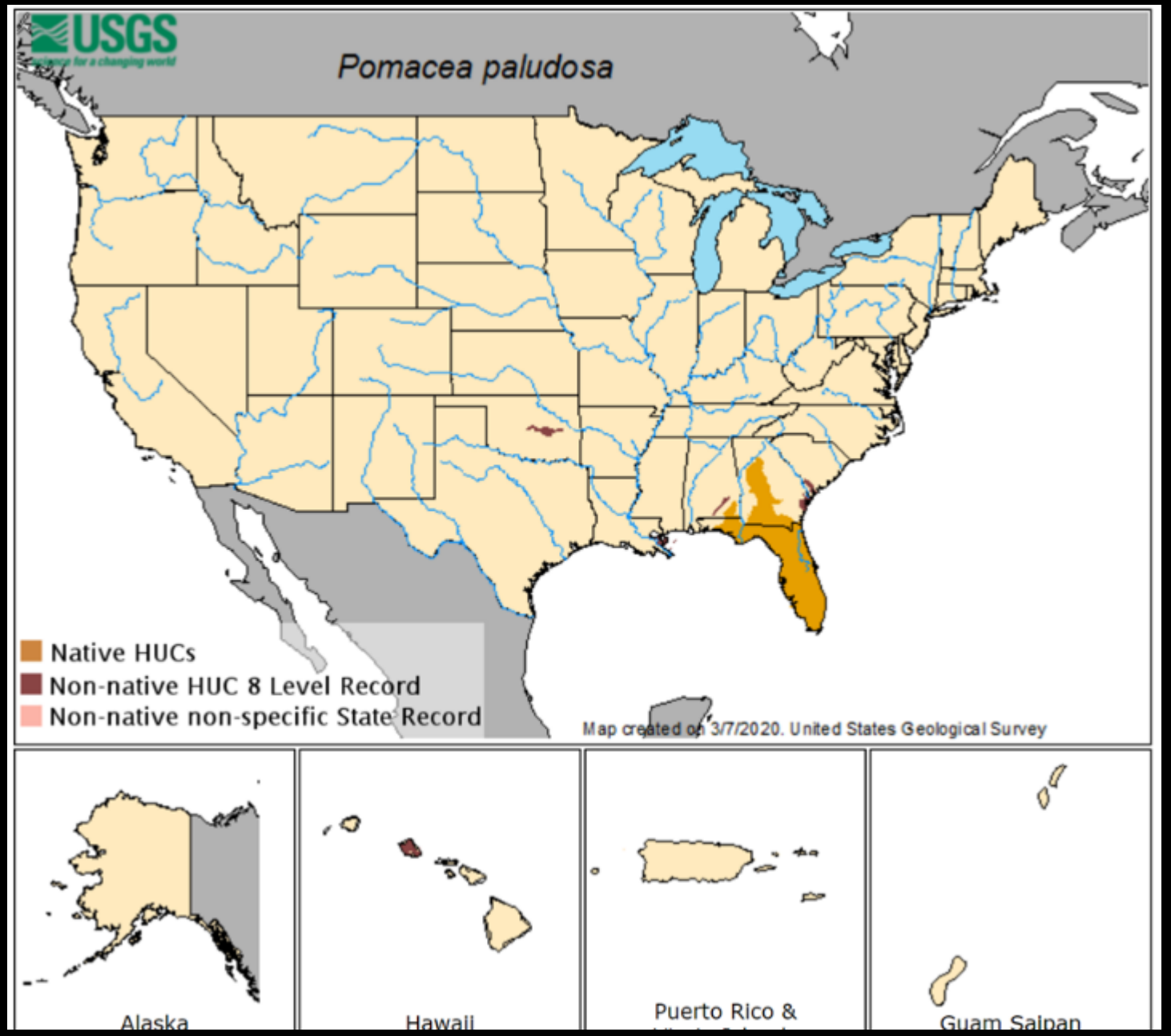
It is not known if these morphological variations can be used to predict parasitism in apple snails



Morphological variations between *P. maculata* (Hayes et al., 2012)

The Florida Applesnail (*P. paludosa*)

- The only native snail within the United States
- Shares morphological and behavioral traits with invasive apple snail species
- Has successfully populated areas in Alabama



Objectives

1) To quantify environmental and habitat correlates of *P. paludosa* density

2) To quantify nematode and trematode loads and infection rates in *P. paludosa*

3) To quantify environmental, habitat and biological correlates of parasite infections in *P. paludosa*

Methods

Study Area

- Conecuh River Covington Co., AL
- Two major dams and associated reservoirs
  - Point A
  - Gantt
- Point A is a 600-acre focal reservoir near Andalusia, AL
  - Created in 1926, rebuilt in 1929
- *Pomacea paludosa* was first reported in both reservoirs in 1962 (Hubricht, 1962)

Population Estimates

- 50m transects at three sites (4 total transects)
- 1-m^2 quadrant used to measure all snails in 10 systematic quadrants within each transect
- Assess Habitat
  - Estimate percentage cover of organic and inorganic substrates
  - Quantify depth, physicochemisty, benthic organic matter (ash-free dry mass) and periphyton biomass (chlorophyll *a*)
- Transects will be augmented with trapping to sample inaccessible habitats
- Individual snails will be collected from each quadrant for subsequent lab analyses


Map data ©2021 Imagery ©2021 , Maxar Technologies, USDA Farm Service Agency
Boat Landing Rd
Boat Landing Rd
Site A Transect 1
Site B Transect 2
Site C Transect 3
Site B Transect 4
Place of Trap Deployments
Map created 11/8/2021. Google Maps

Parasites

- Snails dissected within 48 hours
  - Determine wet weight, sex, and photograph
- Representative tissue samples of the reproductive tissue smeared onto a wet mount and inspected
  - 20x (trematodes), 40x (nematodes)
  - three random 50µm² quadrants used for parasite counts
  - Approximate average number of parasites per infected specimen determined

Male

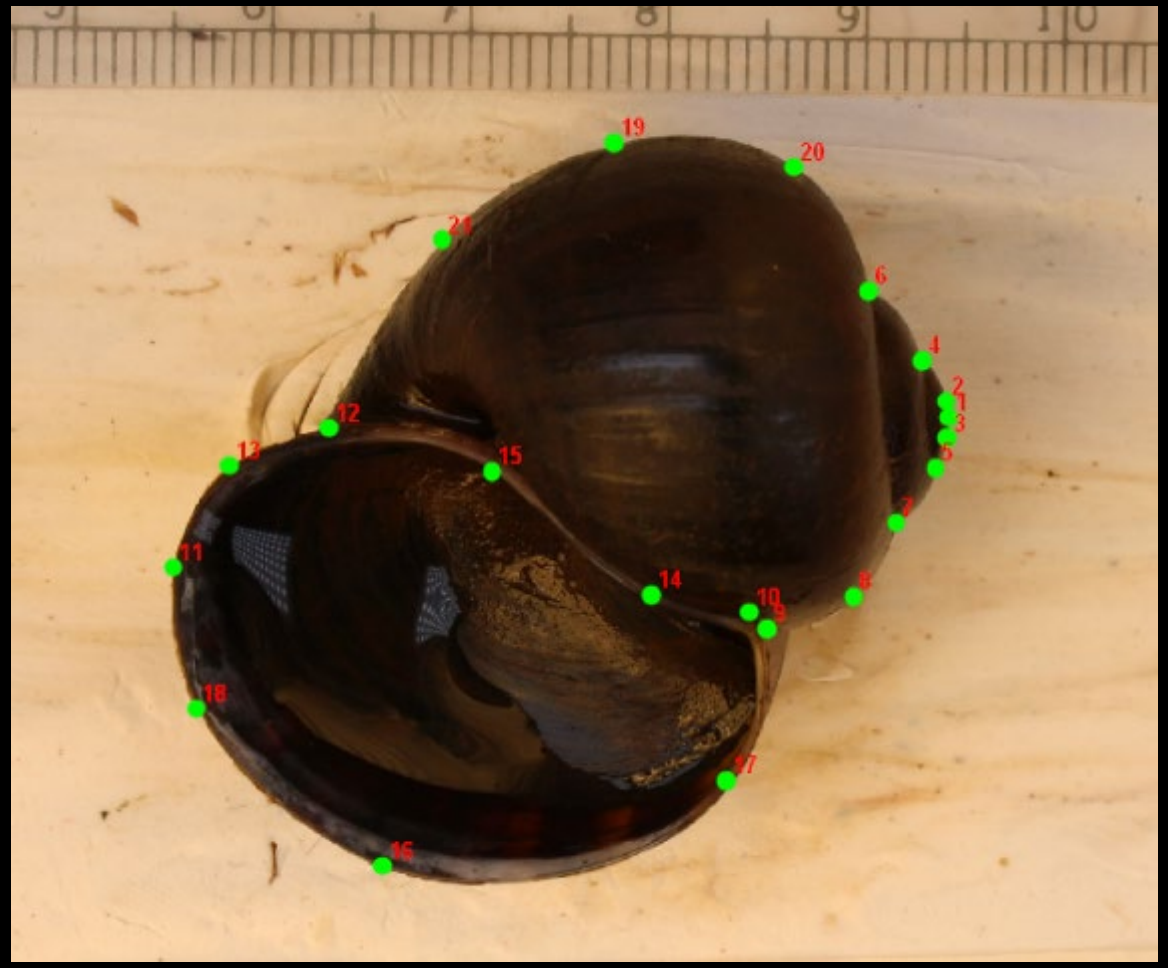
Female

Morphology

- Collected snails digitized
  - Camera mounted 15cm above snail specimen
- Two views highlighting ventral and dorsal orientations
  - Each picture will include identification and scale

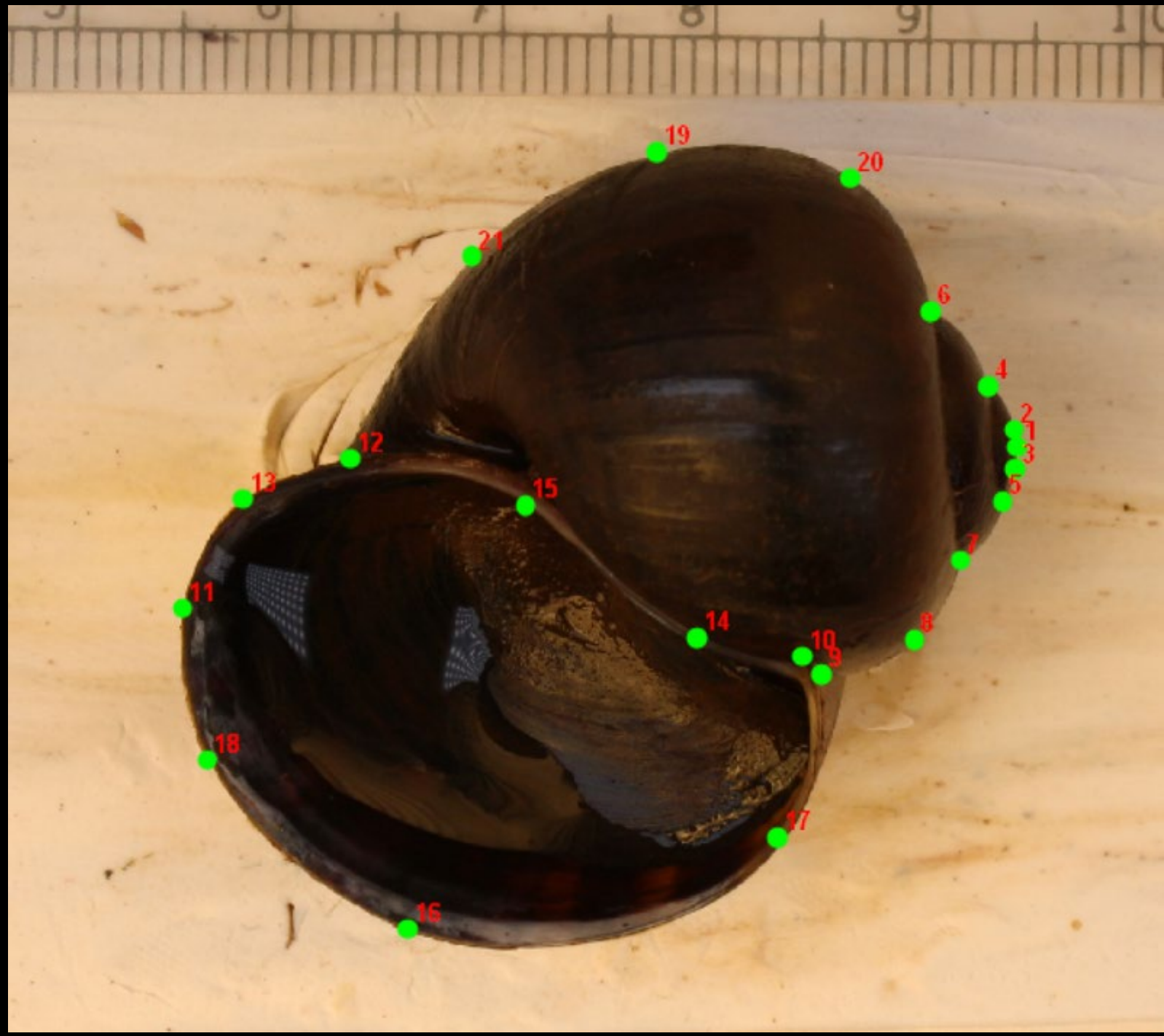
Geometric morphometric analysis (GMM)

- Landmark-based technique used to quantify shape
- Landmarks are used to capture the shape in mv space
  - Fixed – homologous structures
  - Sliding – curvature capture
- Advantageous compared to traditional morphological approaches
  - Able to capture subtle shape change
  - Allows for rigorous statistical analysis



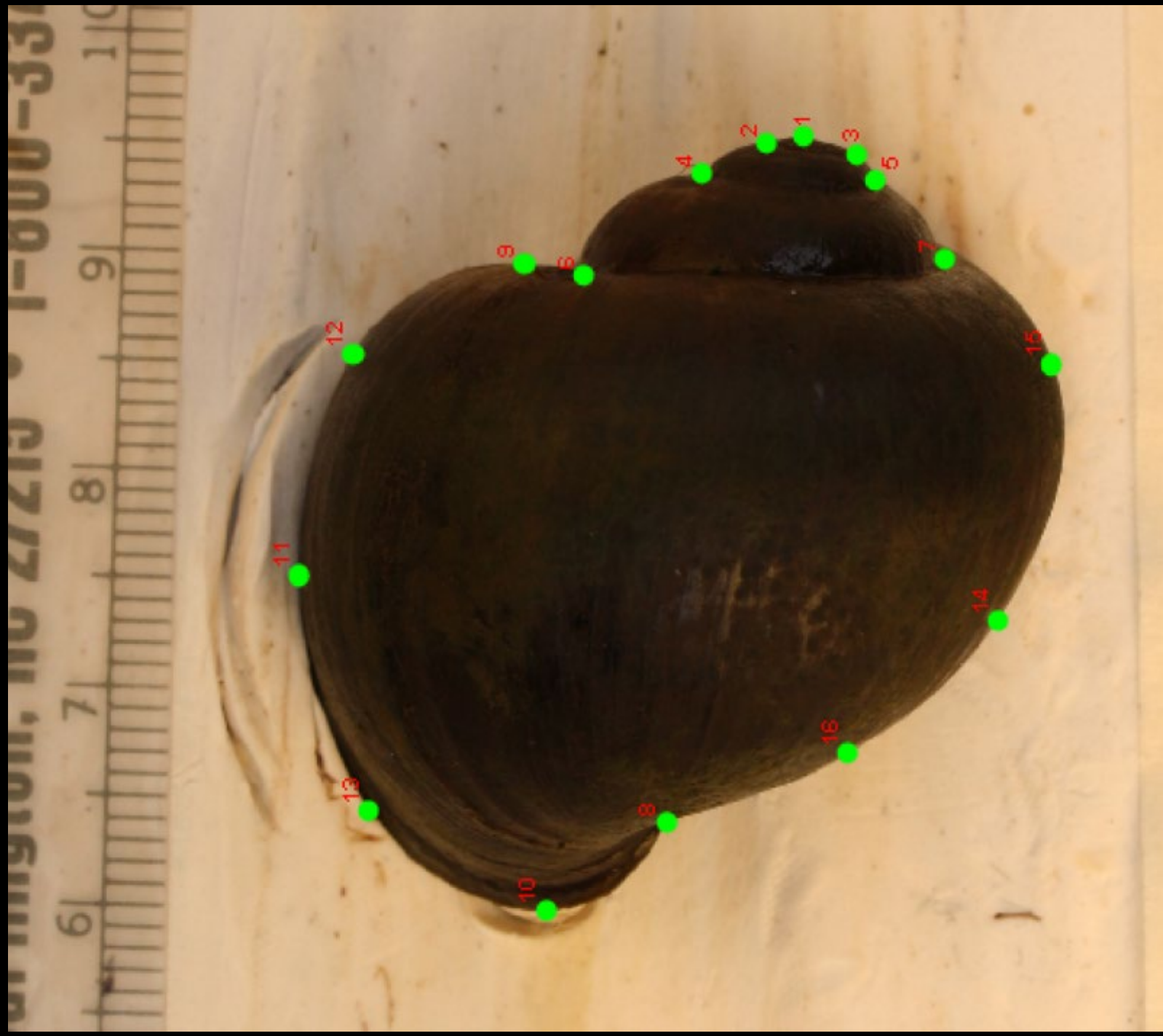
Landmark-based methodology

- Ventral Orientation will include 21 landmarks for GMM analysis
  - 1-11 (Fixed)
  - 12-21 (Semi/Sliding)



Landmark-based methodology

- Dorsal Orientation will include 16 landmarks for GMM analysis
  - 1-10 (Fixed)
  - 11-16 (Semi/Sliding)



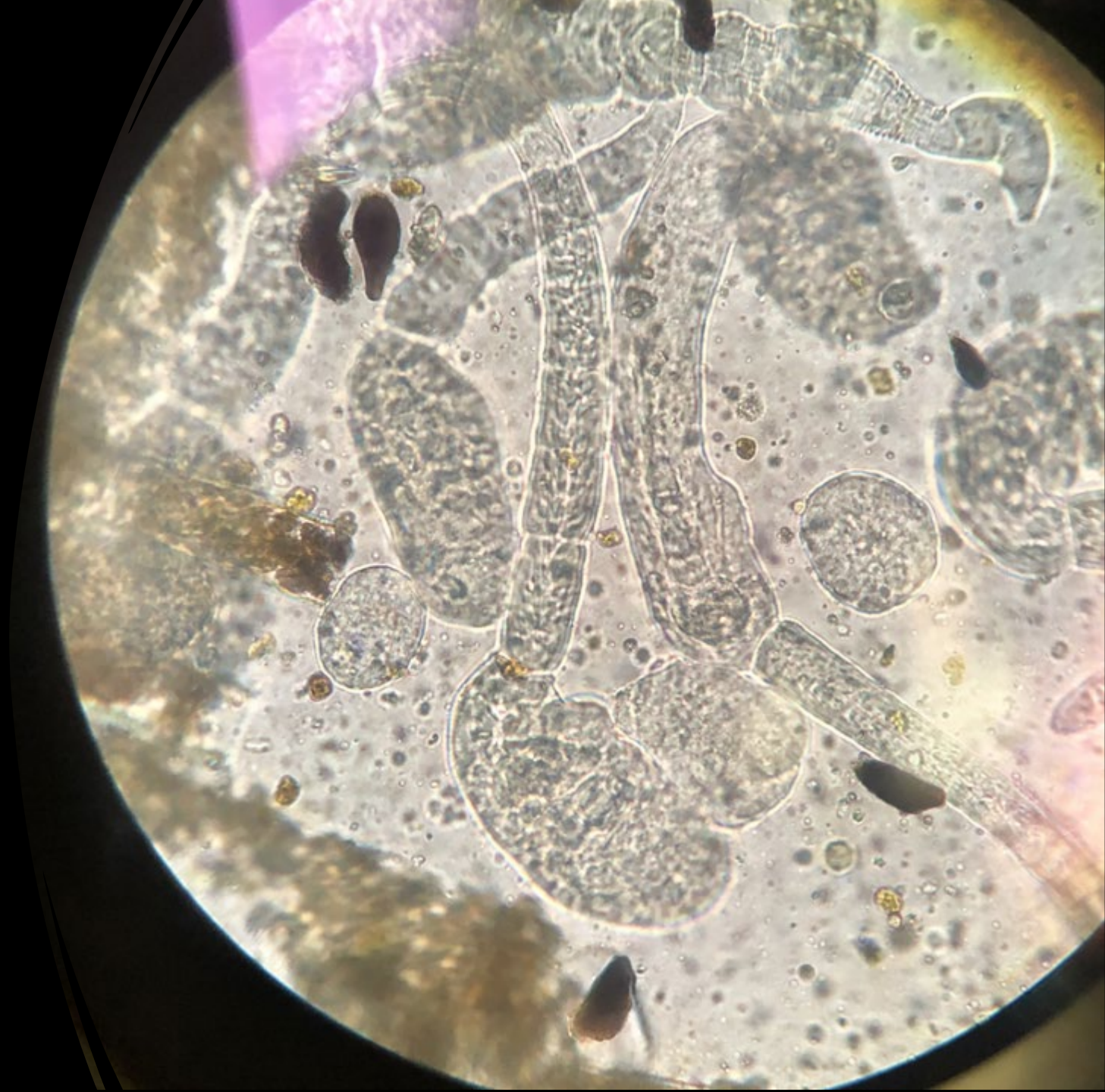

1
3
5
7
8
17
6
13
20
16
14
15
18
11
12
0

Population Analyses

- Response variables
  - Snail abundance per quadrant
- Environmental predictors
  - Abiotic over percentage
  - Biotic cover percentage
  - Depth
- Generalized Linear Mix Model (GLMM)
  - Environmental variables fixed effects
  - Transect random effect
- Densities from snail traps will be estimated by calculating catch per unit effort (# snails per trap (CPUE)), and analyzed in a similar fashion

Parasites

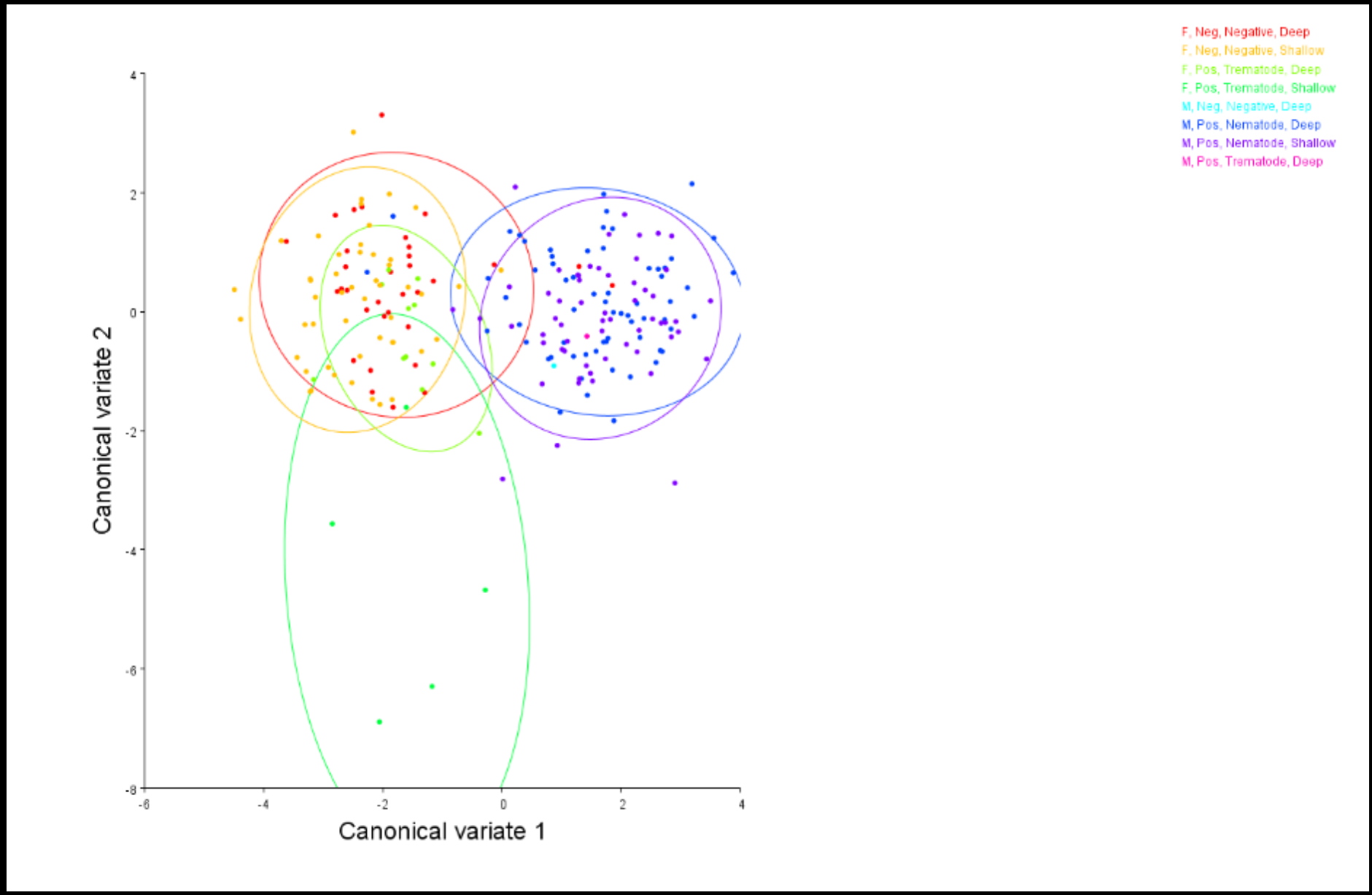
- Parasite load size determined by averaging nematode and trematode counts
  - 3 random 50µm² quadrants per specimen
- Estimated parasite load sizes will then be modeled to habitat variables, snail sex, and wet mass using GLM with Poisson distribution



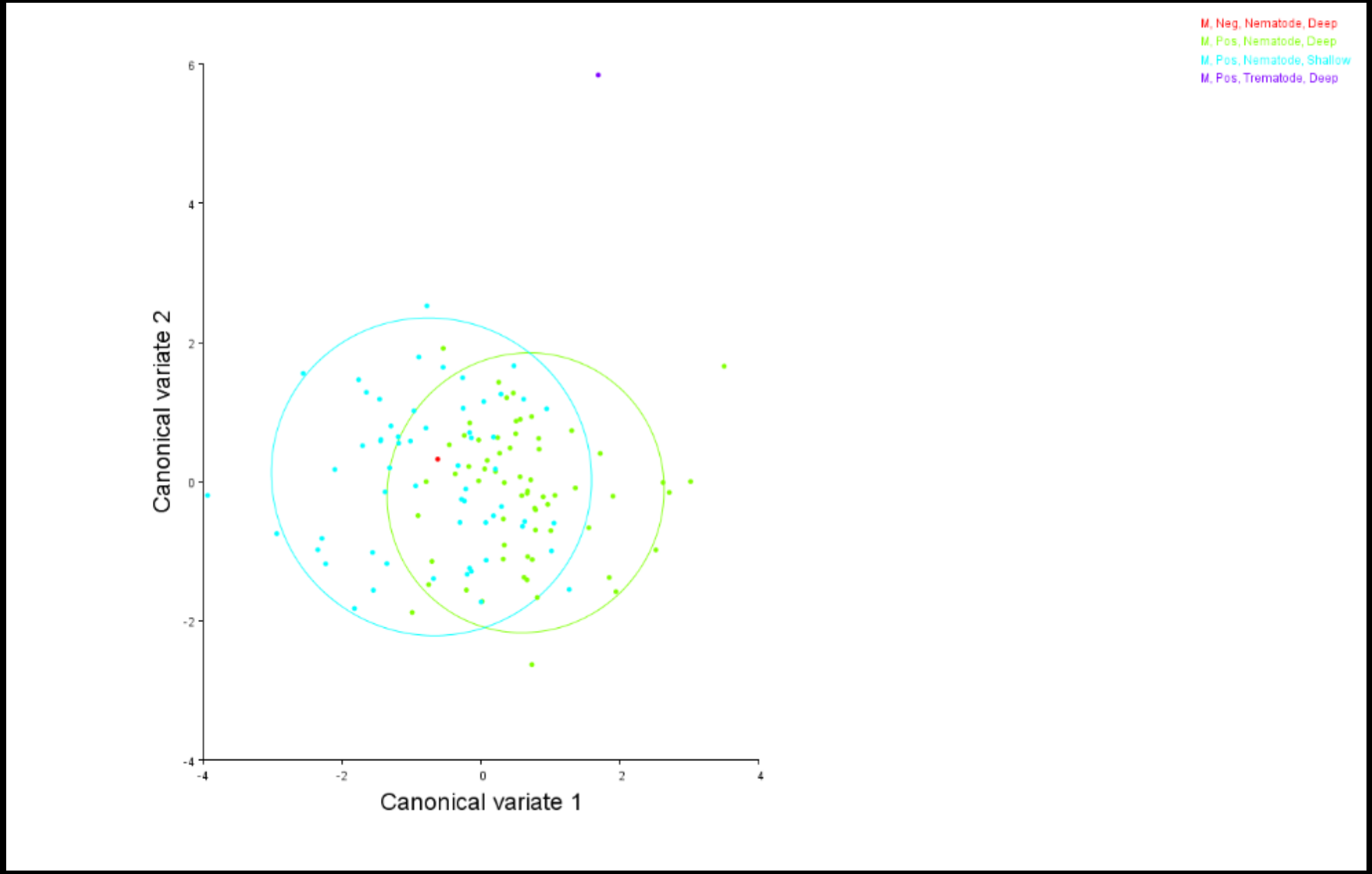
Morphology

- Digitized landmarked photographs will be analyzed by a Procrustes superimposition and relative warp analysis
  - Using tpsRelw (Rohlf, 2021)
  - Accounts for orientation or size discrepancies
- Relative and partial warp scores will be used in a canonical variate analysis (CVA)
  - Using MorphoJ (Klingenberg, 2011)
- CVA used to determine any shell shape differences
  - Sexes
  - Parasitized and non-parasitized

All animals


F, Neg, Negative, Deep
F, Neg, Negative, Shallow
F, Pos, Trematode, Deep
F, Pos, Trematode, Shallow
M, Neg, Negative, Deep
M, Pos, Nematode, Deep
M, Pos, Nematode, Shallow
M, Pos, Trematode, Deep
Canonical variate 2
Canonical variate 1
4
2
0
-2
-4
-6
-8
-6
-4
-2
0
2
4

Males Only


M, Neg, Nematode, Deep
M, Pos, Nematode, Deep
M, Pos, Nematode, Shallow
M, Pos, Trematode, Deep
Canonical variate 2
Canonical variate 1
6
4
2
0
-2
-4
-4
-2
0
2
4

Females Only