Preliminary Histological Observations on the Proximal Intestines of Cricket Frog, *Acris crepitans*

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Introduction

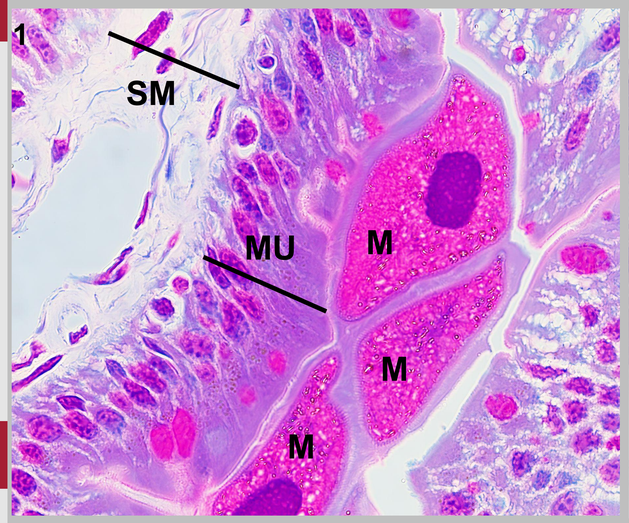
The northern cricket frog, *Acris crepitans*, is a member of the family Hylidae and is native to the United States and Northeast Mexico. Very few studies have been done on the normal intestinal morphology of frogs, especially few focusing on A. crepitans. The northern cricket frog typically lives around permanent water sources like ponds, creeks, lakes, and rivers and feeds on insects. This study is a morphological assessment of the small intestine histology of *Acris crepitans* in order to better understand Anuran digestive system anatomy, and compare to previous research.

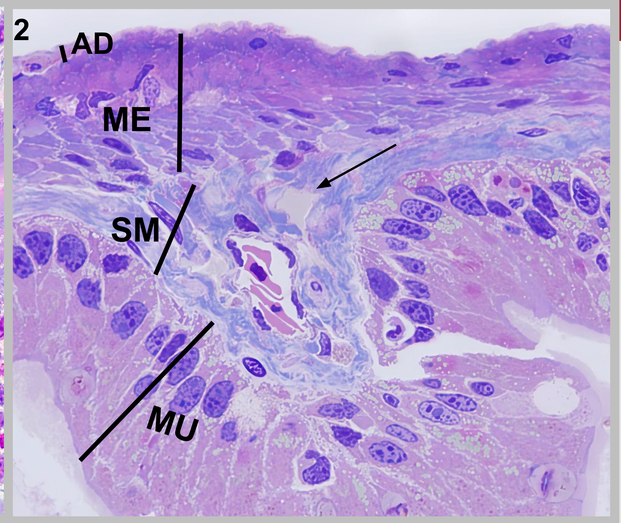
Background

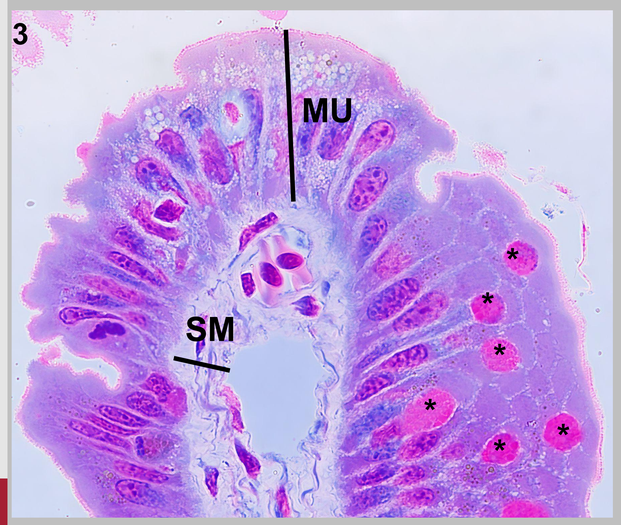
While previous research on *Acris crepitans* has mainly focused on conservation efforts, reproductive and behavioral studies, there has been several studies that involve the digestive histology of frog species within the same order. One such study by Valverde et al. 2019 found that Anura species often lack a muscularis mucosa layer, though it is unclear why. In addition, other studies have investigated changes in intestinal morphology during times of stress, like during temperature fluctuations and aestivation. Rahman et al. 2014 found that the muscosal muscularis layer is highly dependent on temperature, being absent in lower temperatures and the thickest sublayer in high temperatures. By using these studies as comparative models, our goal is to better understand *Acris crepitans'* intestinal morphology and how that relates to other Anuran species. This research was performed during our graduate level histology class in which we learned the histological techniques necessary for research in the field.

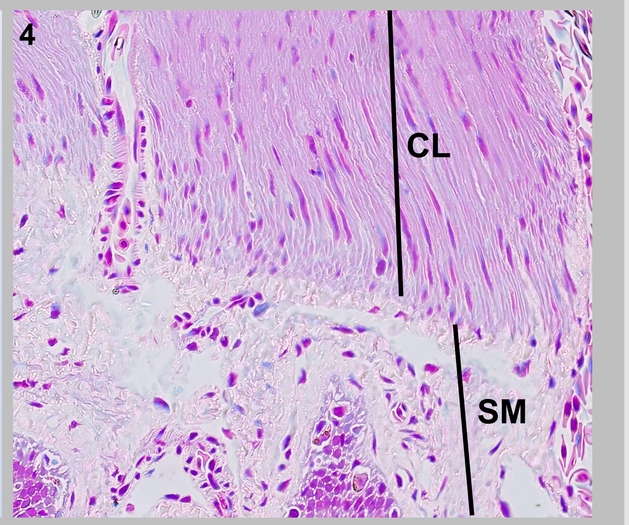
Materials and Methods

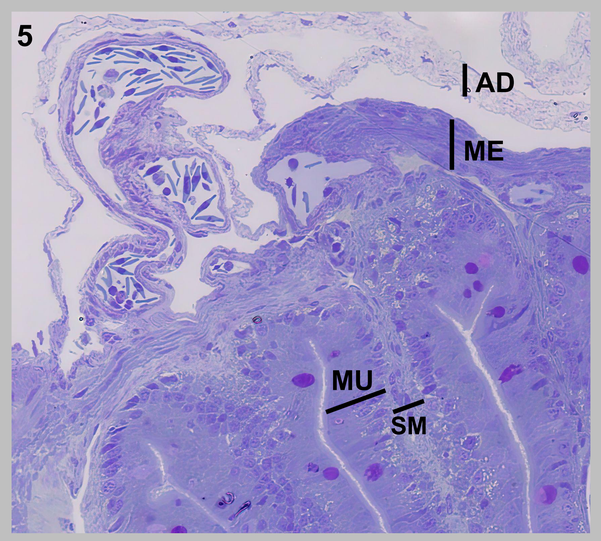
- Cricket frogs were euthanized and samples were taken from proximal small intestine
- Tissue stabilization
  - Samples are rinsed three times with Cacodylate buffer/PBS [0.2M] (15 minutes)
- Tissue Staining
  - 2% OsO4 (Osmium Tetroxide) for 2 hours
- Rinsed three times with Cacodylate buffer/PBS [0.2M] (10 minutes)
- Sit overnight with 70% Ethanol (dehydration)
- Samples are dehydrated with an ethanol series
  - Gradually replaces tissues water with ethanol through ascending concentrations
- Infiltrate samples with plastic and propylene slowly, then with pure plastic
  - Embed-812 Resin, DDSA, and NMA anhydrides
- Embed tissue with plastic and place in oven for two days
- After plastic sets, samples are sectioned using microtome and stained in preparation for analysis

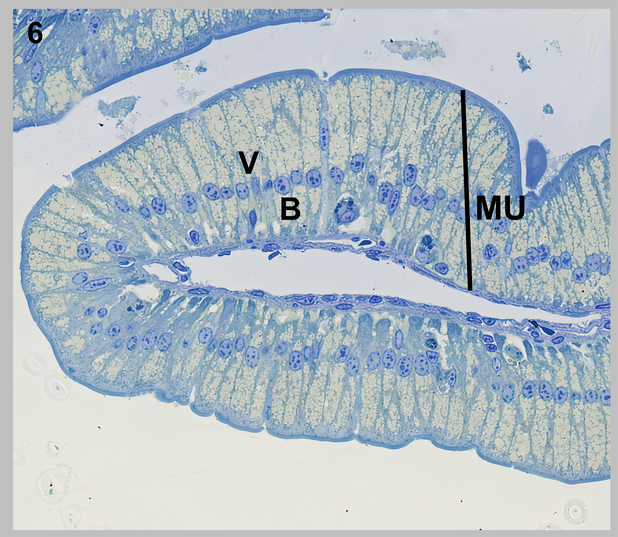












Results and Discussion

1.Mast cells (**M**) present on the apical/lumen surface of intestines. Mast cells produce an immune response by secreting inflammatory mediators and chemicals like histamine that produce an inflammatory response. Stained with Periodic Acid-Schiff.

2. Lacteal vessel present in the connective tissue layer of the villus (labeled with arrow). Lacteals are responsible for absorbing dietary lipids within the small intestine. Stained with Basic Fuchsin & Toluidine Blue.

3. Apex of an intestinal villi. Enterocyte nuclei are stained a dark magenta and goblet cells (*) are present on the right side of the villi. Stained with Toluidine Blue and Basic Fuchsin.

4. Submucosal (**SM**) and circular muscular layer (**CL**) of the small intestine. Note apparent lack of muscularis mucosa layer. Stained with Periodic Acid-Schiff.

5. Dark purple staining goblet cells as well as intestinal crypts and presence of RBCs and WBCs within bloodstream. Stained with Toluidine Blue/Methylene Blue.

6. Villi showing light staining vesicles (**V**) in apical portion of the enterocyte and basal nuclei (**B**) of simple columnar epithelium. The frog was fed within 1-2 days before euthanization, which makes these vesicles possibly endocytotic, bringing nutrients into the cells from the newly digested food. Presence of numerous vesicles and light staining basal nuclei indicates the frog sampled was active and feeding close to the time of sampling. Also note the long epithelial height and prominent brush border, both indicative of a recently fed organism. Stained with Toluidine Blue.

Tissue layers: Mucosal (**MU**), Submuscosa (**SM**), Circular layer (**CL**), Muscularis Externa (**ME**), Adventitia (**AD**)

While Valverde et al 2019 found there is often a lack of the muscularis mucosa (**MM**) layer, findings from Rahman et al 2014 may explain the apparent lack of the **MM** as a result of the temperature in the habitat at the time of collection. The **MM** functions to provide movement in keeping the mucosa in flux, which may be less important in organisms with shorter intestinal systems

Future Research

Future studies should include further differentiation between cells and tissues and further differentiation of the layers present to determine whether the cricket frog does lack the muscularis mucosa layer. Vesicles in Figure 6. should be studied further to determine their true function as either endocytotic or exocytotic. This can be done through the use of Transmission Electron Microscope (TEM) images that will allow for closer visualization of the small intestine tissues. In addition, more attention to the temperature of the habitats could explain the possible lack of muscularis mucosa.

Acknowledgements & References

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