Preliminary Histological Description of the Stomach and Esophagus within the Northern Cricket Frog, *Acris crepitans*

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ABSTRACT

The current study investigates the histological characteristics of the stomach and esophagus of *Acris Crepitans*, commonly known as Northern Cricket Frogs. This frog can be found in areas north of Mexico, east of the Rocky Mountains, and in areas near Ontario, Canada. Deemed opportunistic eaters, their eating habits differ to what is available nearby. Tissue samples were dehydrated, infiltrated, and embedded in Embed812 plastic. A microtome was used to slice thin sections, which were then stained with toluidine and basic fuchsin and examined under light microscopy. The esophagus sections revealed the following layers: the mucosa, lamina propria, submucosa, and muscularis externa. The esophagus also contained goblet cells, esophageal glands, and a regenerative layer. Differing with previous research the esophagus has pseudostratified columnar epithelium, and an adventitia was not observed. The sections of the stomach revealed 5 layers: the mucosa, muscularis mucosa, submucosa, muscularis externa, and the serosa. The stomach oxyntic and mucous neck cells, along with gastric gland, were revealed in the mucosa. Our results show consistency with previous literature in finding the same layers within the esophagus and the stomach. The findings of this study are preliminary in nature as further studies using TEM and differential staining are needed to further describe structures found in the stomach and esophagus of cricket frogs.

INTRODUCTION

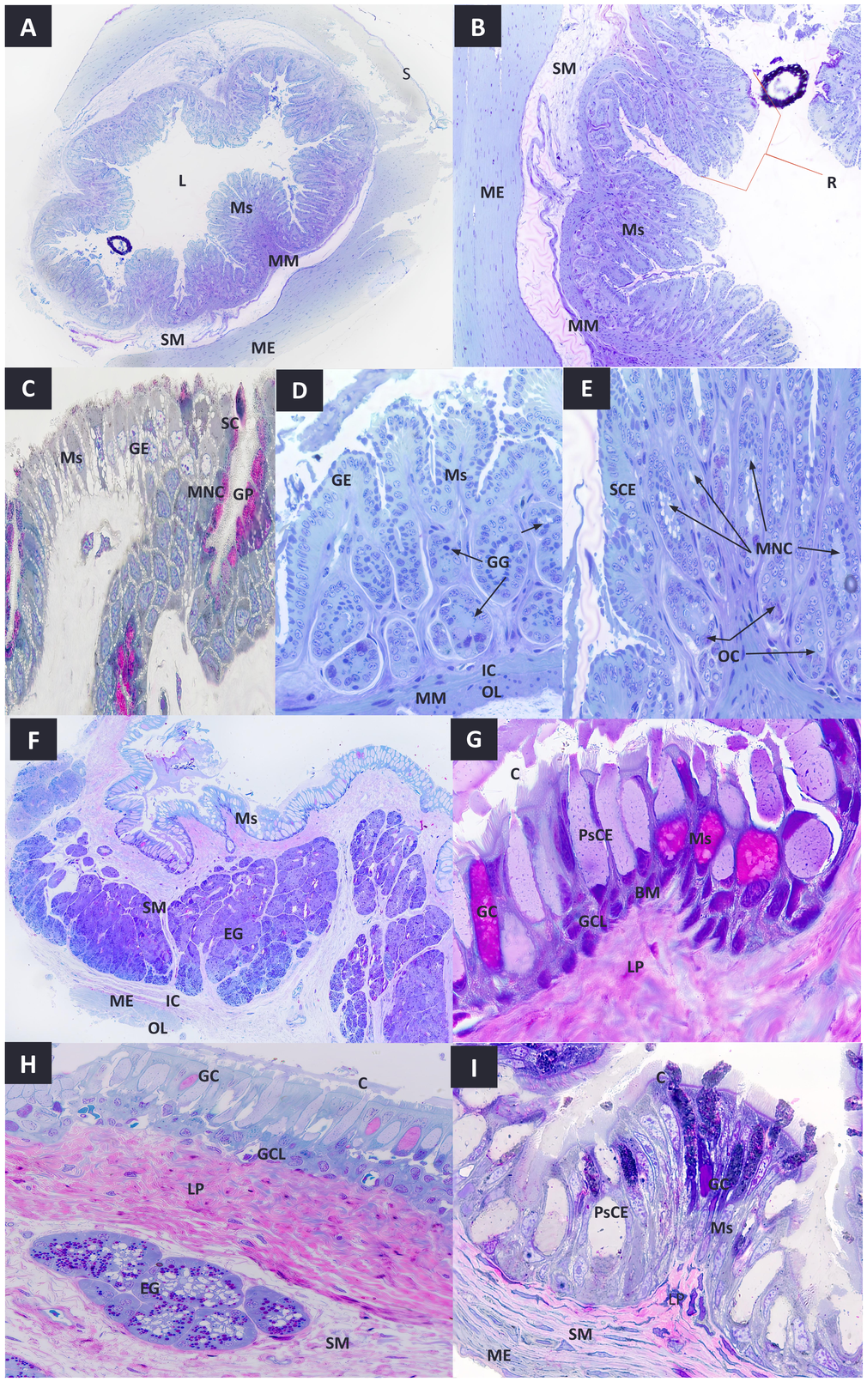
Acris crepitans are commonly known as Northern cricket frogs and belong to class Amphibia; order Anura; family Hylidae. Cricket frogs are widespread across North America to Mexico, east to the Rocky Mountains, and in areas near Ontario, Canada. Although they can survive under many environmental conditions, cricket frogs prefer permanent bodies of water where they eat what is available (Suter, 2022). Cricket frogs are often variable in color ranging from tan, greenish, and reddish brown. This species is active from late March to early November with a short lifespan of 3-5 years.

Amphibians have been used as the standard model to study the physiological process in the body (Akat et al., 2014). Limited research can be found on histological analysis on the stomach and esophagus of the *Acris crepitans*. Our approach is to compare the tissues of closely related species with that of *Acris Crepitans*. The studies on *R. catesbeianalX. laevis* (Yoshida, 2001), *Rhinella icterica* (Santos et al., 2014), *Boana albopunctata/Boana raniceps* (Valverde et al., 2019), *Triturus carnifex* (Liquori et al., 2007), and *Hyla orientalis* (Akat et al., 2014) have helped identify key cellular and tissue features of the esophagus and stomach. Describing the layers and components of the stomach and esophagus as well as the effects of the changing environment and the effect on the esophageal structures resulting from temperature fluctuation (Rahman, 2014) are interesting. Our study will provide foundational comparative data that will allow for such studies to occur across frog taxa.

MATERIALS and METHODS

- **Tissue preparation:** Tissue samples were prepared in a three day period.
  - Day 1: Tissues were cut into small samples and rinsed three times with Cacodylate buffer. They were then placed in osmium tetroxide for two hours. After this period, samples were rinsed again with cacodylate buffer three times. The samples were left in 70% ethanol overnight.
  - Day 2: Plastic mixture was prepared combining 20mL of Embed-812, 16mL of DDSA, 8mL of NSA ensuring the absence of bubbles. Then 1.2mL of BDMA was added, the mixture was then left to sit for two hours.
    - Samples were dehydrated in ethanol as follows: 50% for 15 min; 85% for 20 min; 95% for 20 min.
  - A rotator was used in the following steps.
    - While on the rotator, samples were dehydrated with 100% ethanol twice, 20 minutes each and spun. Samples were then dehydrated with Propylene Oxide twice, for 20 minutes each and spun. Next samples were infiltrated with 1:1 mixture of plastic and propylene oxide (estimated by eye and spun for 1 hour. The infiltration process followed with 2:1 ratio of plastic and propylene oxide (estimated by eyel), and spun for 1 hour. Finally samples were infiltrated with pure plastic and samples were spun overnight.
  - Day 3: Fresh plastic was prepared and samples were spun for 2 hrs ensuring no bubbles formed. Sufficient plastic was made to incubate all tissues for another 2 hrs and embed the beam capsules. The tissues were then infiltrated with fresh plastic, spun for 2 hrs, and prepared for embedding. Beam capsules were prepared and placed in oven to remove and residual moisture before embedding
  - Embedding: Each capsule was filled halfway with plastic, followed by the tissue sample. The capsules were then filled to the top with plastic, and labeled. Samples were then left in the vacuum oven at 60-65°C for 48 hrs.

- **Microscopic analysis:** Tissue samples were faced using a glass knife, mounted, and stained with a PAS solution to observe the general histology of the stomach and esophagus. For further light microscopic analysis, thin sections were cut using a diatome diamond knife and an ultra microtome. These sections were then stained with fuchsin and toluidine blue and examined under a light microscope. Photographs were taken, analyzed, and edited using Adobe photoshop.



RESULTS & DISCUSSION

Figure A: 10x Transverse view of the stomach. Light microscopy analysis showed similarities between the *R. icteria* [3] and *Acris crepitans,* particularly in the presence of five distinct layers: mucosa (Ms), muscularis mucosa (MM), submucosa (SM), muscularis externa (ME), and Serosa (S). However, this structural organization differs in other amphibians, such as *B. albopunctata and B. raniceps* [6] where no serosa was shown. In contrast the serosa is *H. orientalis* [1] the serosa is visible. While the *A. crepitans* does have a muscularis mucosa, as seen in B. albopunctata, it is absent in the *B. raniceps* [6] and the *H. Orientalis* [1].

Figure B: 40x view of the stomach wall. 4 distinct layers are seen the mucosa,submucosa, muscularis mucosa, and Muscularis externa. The rugae (R) are folds found in the walls of the stomach that increase the surface area and play a role in protection and digestion.

Figure C: View of the gastric epithelium (GE) lining shows mucous neck cells (MNC) and the surface cells (SC) found in the *A. crepitans* which display similarities to the fundic glands of the *T. Carnifex*. [2]

Figure D: Microscopic analysis of the *A. crepitans* mucosa (Ms), reveals a simple columnar epithelium (SCE) observed similarly in the *T. Carnifex [2], R icteria* [3]. However, it differs from *B. albopunctata/B.raniceps* [6], which exhibit a pseudostratified columnar epithelium. The muscularis mucosa is (MM) demonstrated, it is composed of an outer longitudinal layer (OL) and inner circular (IC) muscle layer, similarly seen in *B. albopunctata/ B. raniceps.* Although a muscularis mucosa is found in the tissues of other amphibians it is not specifically described.[7]

Figure E: Focusing on the basal layer of the mucosa (Ms), simple columnar epithelium (SCE) lines the mucosa, forming gastric pits. Gastric glands (GG) are visible (**Figure D**). *A. crepitans* shares similarities with *B. albopunctata/B. raniceps* [6], *R. icteria* [3], *and T. carnifex* [2] in the type of gastric cells found within in the gastric glands. Mucus neck cell (MNC), with flat nuclei are visible, along with oxyntic cells (OC) which function in absorption which have a more basal rounded nuclei. [6]

Figure F: a 10x view of the esophagus reveals that the histological structure of *A. crepitans* shows some similarity to previously documented species such as the *Rana catesbeiana*, and *Xenopus laevis*.[7] The Microscopic analysis identified esophageal layers: Mucosa (Ms); Submucosa (SM); Muscularis externa (ME) with both outer and inner longitudinal muscle layers. *A. crepitans* showed no presence of adventitia, and displayed esophageal glands (EG) which were not depicted in research studies mentioned but have been described in other families such as bufonidae and ranidae [6].

Figure G: A 100X view of the mucosa (Ms) reveals Histological structures similar to the esophagus of the *B. albopunctata/ B. raniceps.*[6] The type of epithelium is comparable, consisting of a ciliated pseudostratified columnar epithelium (PsCE). Goblet cells (GC) are demonstrated are comparable to those founds in other described species such as the *H. orientalis* [1] *and the B. albopunctata/B. raniceps* [6], these cells function in mucous production. Other layers observed in the *B. albopunctata/B. raniceps* [6], that are comparable include the regenerative germ cell layer (GCL), basement membrane (BM), and a lamina propria (LP) beneath it.

Figure H: Expanded view of the mucosa (Ms) revelas visible components of ciliated (C) pseudostratified columnar epithelium (PsCE), along with goblet Cells (GC). The germ cell layer (GCL) is also visible contributing to the regenerative capacity of the epithelium. Additionally esophageal glands, also known as pluricellular glands and are present and function in mucus production.

Figure I: Microscopic analysis of the juvenile *A. crepitans* esophagus reveals the presence of key layers: mucosa (Ms), lamina propria (LP), muscularis externa (ME), and submucosa (SM). The epithelium is composed of ciliated pseudostratified columnar epithelium (PsCE), with visible goblet cells (GC) that function in mucus production. Despite the presence of these layers, the juvenile still shows developmental immaturity, with certain components and distinguishing features absent, which will likely develop as the organism matures.

Discussion: *Acris Crepitans* shares many features including layers and cellular components, with other amphibians from the same or closely related families. The most notable features comparative importance in the stomach include the type of epithelium, the 5 distinct layers of the stomach, and the presence of mucous neck cells and oxyntic cells. In the esophagus, variations were observed in the type of epithelium, as well as the presence of esophageal glands. Additionally, the study highlighted differences in the developmental progression of juvenile to adult *A. crepitans* demonstrating the structural changes that occur with maturation. While the study contributes to the body of knowledge of the histology of the *A. crepitans* stomach and esophagus, it is preliminary in nature. Further research will be conducted using transmission electron microscope (TEM) to provide a more detailed ultrastructural analysis of cells and the tissue components.

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