A Preliminary Histological Study on the Integument and its Structures within the Northern Cricket Frog (*Acris crepitans*)

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INTRODUCTION

In our preliminary study, we evaluated the dorsal and ventral skin of the Northern Cricket Frog, *Acris crepitans*. The Northern Cricket Frog is native to the United States and Northeast Mexico, living in multiple habitats including arid regions, forests, and grasslands. The most suitable habitat for Northern Cricket Frogs is in the southeastern region of the United States near permanent bodies of water. Our methods involved dehydration, embedding, and infiltration of our skin tissue samples. Light microscopy showed the presence of flask cells within the ventral and dorsal dermis and poison and mucous glands were located within the dorsal skin. In this preliminary study, we examined the structures within the dorsal and ventral skin of the Northern Cricket Frog and compared these structures to previously studied amphibians: *Hyla arborea arborea* (European Tree Frog) and *Phyllomedusinae* (Leaf Frog). We hypothesized that although we would find similar structures with similar functions between the three species of frogs, the Northern Cricket Frog would be most similar to the European Tree Frog because of their similar environments.

MATERIALS & METHODS

Dorsal and ventral samples were taken from the Northern Cricket Frog, *Acris crepitans*. Over a three-day process, the samples were dehydrated, infiltrated, and embedded. After the embedding process, a Leica Ultracut Microtome was used to cut thin slices from a tissue block averaging 3-10 microns thick. Sectioning was completed using a glass knife and cut samples were placed on a microscope slide and stained with Methylene Blue/Toluidine Blue for approximately 45 seconds each. Each sample was then evaluated under an Olympus CX41 light microscope.

Day 1

- Rinse tissue samples three times for 15 minutes with Cacodylate buffer/PBS (0.2M) and let tissue sit in Osmium tetroxide for two hours
- After the two hours, rinse three times for 10 minutes with Cacodylate buffer/PBS (0.2M)
- Samples sit in 70% ethanol alcohol overnight

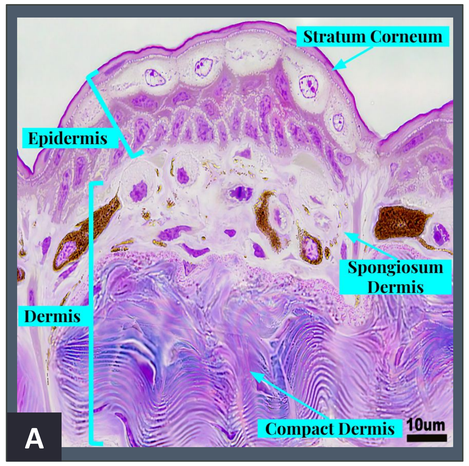
Day 2

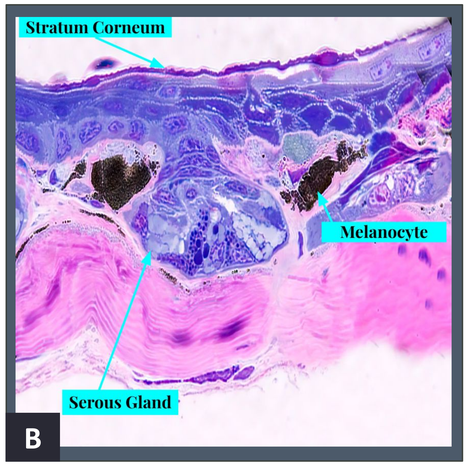
- 20mL Embed-812 resin, 16mL DDSA, and 8mL NMA anhydrides
- Add 1.2mL BDMA to stirring plastic and let stir for 2 hours
- Dehydrate with 50% ethanol for 15 minutes, 85% ethanol for 20 minutes, and then 95% ethanol for 20 minutes
- On rotator, dehydrate with 100% ethanol for 20 minutes (x2) and then Propylene Oxide for 20 minutes (x2)
- Infiltrate with 1 part plastic, 1 part propylene for 1 hour, followed by 2-part plastic, 1 part propylene for 1 hour, then pure plastic and let spin overnight

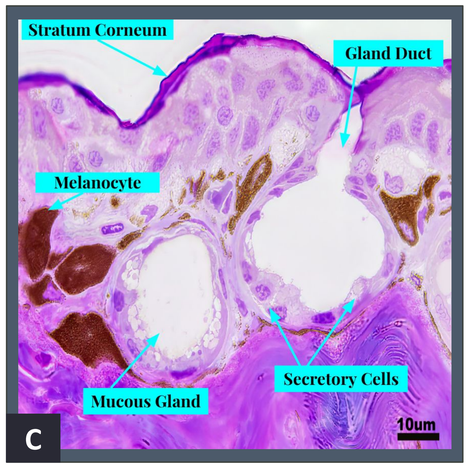
Day 3

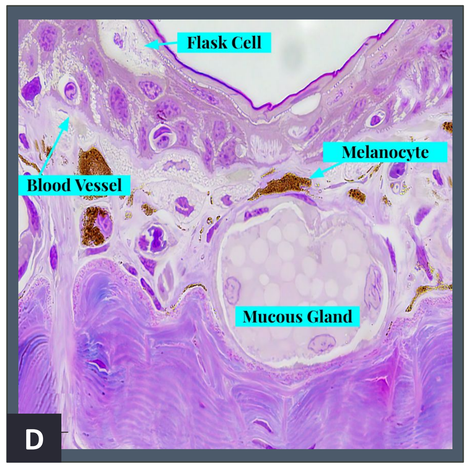
- Infiltrate tissue with new plastic and spin for 2 hours
- Prepare flat beam capsules and place in oven until the time of embedding
- Set oven to 60-65 degrees Celsius and place capsules in oven and place the tissues under vacuum (20-25Pa) and leave at 60-65°C for 2 days

Dorsal Skin

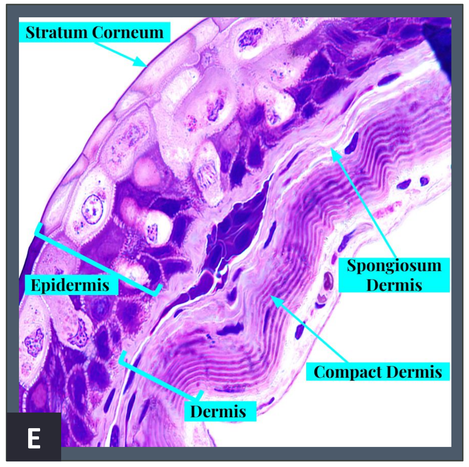


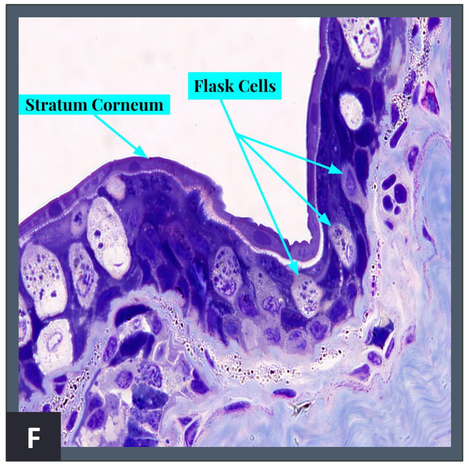






Ventral Skin





RESULTS & DISCUSSION

We identified three layers of skin: **epidermis, spongiosum dermis**, and **compact dermis** (Figures A & E). Superficial to deep, the epidermis is layered as follows: stratum corneum, stratum granulosum, stratum spinosum, and stratum germinativum. The stratum corneum in the dorsal skin of *Acris crepitans* is notably thicker than that of the ventral skin. The epidermal layers in *Acris crepitans* are similar to *Hyla arborea arborea* and *Phyllomedusinae* as all three have the same organization.

Granular and glandular glands were identified in the dermis of the dorsal and ventral skin samples. Two types of granular glands were identified: **serous glands (type I)** (Figure B) and **poison glands (type II)** in the dorsal samples. In *Acris crepitans*, the granular glands are located primarily in the dorsal skin compared to the number of glands present in the ventral skin. Similarly, serous glands, both type I and II, are found in the dorsal skin of *Hyla arborea arborea* and *Phyllomedusinae*; however, *Hyla arborea arborea* and *Phyllomedusinae* have serous glands present in the ventral skin unlike *Acris crepitans*. **Mucous glands** (Figures C & D) were found in both dorsal and ventral samples of *Acris crepitans* which matches the structures within *Hyla arborea arborea*.

Flask cells (Figure F) were found in the ventral and dorsal skin samples. Research has not shown the presence of flask cells in the dorsal skin of *Hyla arborea arborea* or *Phyllomedusinae*. **Keratinocytes** were observed in both the ventral and dorsal samples of the Northern Cricket Frog specifically in the outermost layer of the stratum corneum. The flask cells work together with the keratinocytes to provide protection to the skin while allowing moisture retention and gas exchange. Flask cells have also been hypothesized to be important in osmolarity regulation which could contribute to the functionality of the skin in *Acris crepitans* since they have a terrestrial lifestyle, allowing cricket frogs to spend time away from water in warmer months.

Lipid glands were not found in the dorsal or ventral skin of the Northern Cricket Frog in this study. The lipid glands were not likely found due to differential environment conditions. The Northern Cricket Frog, similar to the European Tree Frog, tends to live near permanent bodies of water. Due to their close proximity to bodies of water, Northern Cricket frogs likely do not require the lipids glands to aid in moisture retention. This is unlike the *Phyllomedusinae* which lives in high canopies of tropical rainforest trees where bodies of water are not easily accessible. The lipid glands are necessary for Phyllomedusinae to maintain moisture retention. *Acris crepitans* is most similar to *Hyla arborea arborea* between the species, supporting our hypothesis.

Future Research:

- Use of transmission electron microscope to examine structures in the epidermis
- Evaluate the structural and functional differences of keratinocytes, flask cells, and lipid glands between the three species

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