Histological identification and characterization of the skin of the Slimy Salamander, *Plethodon glutinosus*

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ABSTRACT

This preliminary study sought to identify and characterize structures found in the northern slimy salamander (*Plethodon glutinosus*). This study mainly focused on identifying and characterizing structures located on the dorsal and ventral sides of the skin in these salamanders. Due to their production of sticky mucus while threatened, the glands were evaluated to determine their part in helping produce this secretory defense. Samples were collected and fixed then placed in resin to section for microscopic analysis. These samples were then electively stained and examined. Through this, Gribbins Et Al were able to pinpoint defining features of the glands and integument regions of the Plethodon. This information will be cross-referenced with amphibians with similar secretory abilities similar to the *Plethodon Glutinosus* for glandular comparison.

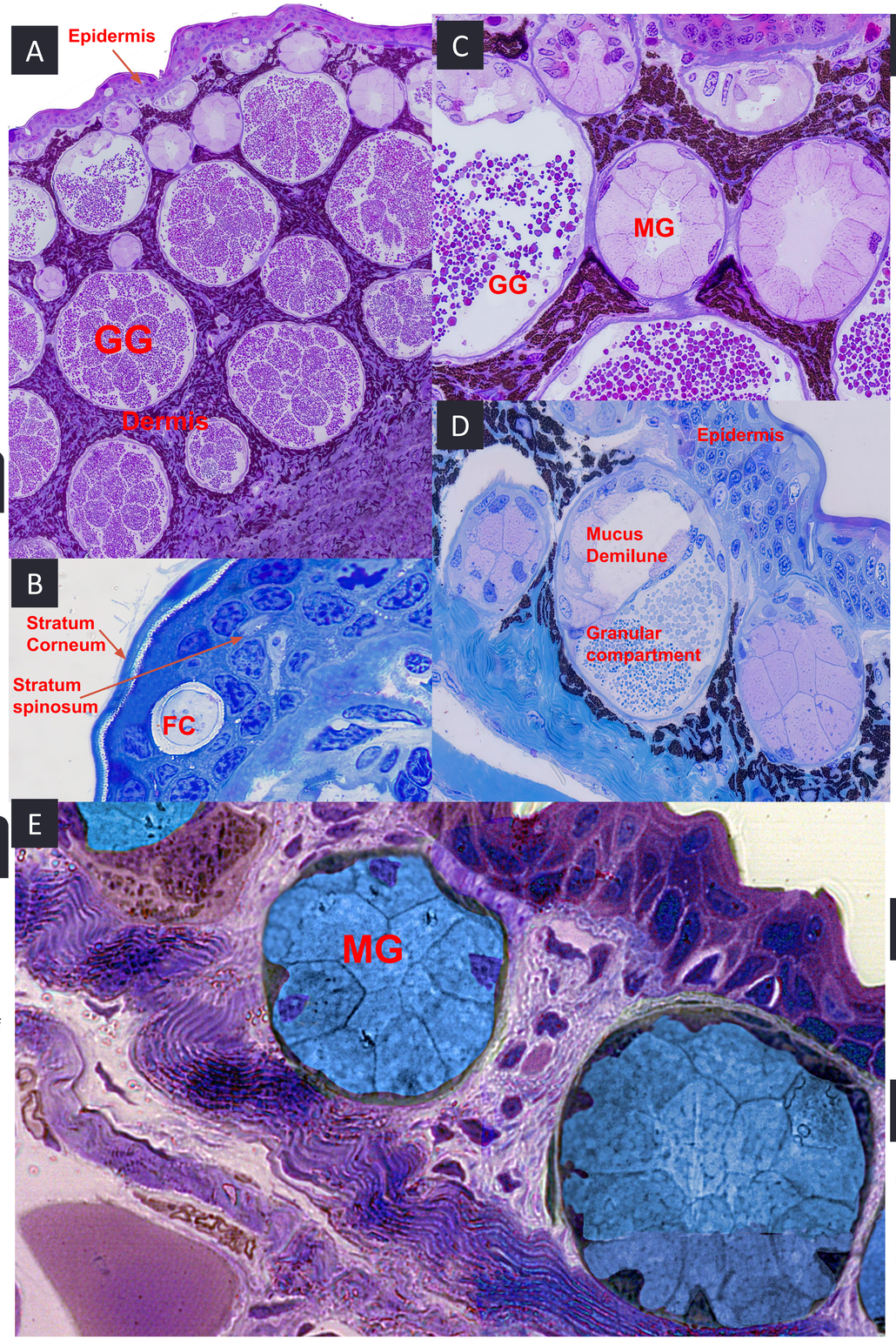
INTRODUCTION

Plethodon glutinosus, otherwise known as the northern slimy salamander are a species of salamander commonly found in North America. They are found widely across the United States and often live in moist environments located in woodlands and near streams. They can often be found under fallen trees, rocks, and other debris of the forest floor.

These salamanders commonly have a defense mechanism of producing an adhesive mucous when threatened or provoked during handling. These salamanders commonly have a black body with small white dots speckled across their abdomen and back. The aim of this preliminary study is to describe the glands of the integument that produce this adhesive defense.

MATERIALS and METHODS

Specimen collection:
Tissue preparation:
Tissues were collected from the ventral and dorsal sides of the specimens and put in Trump's fixative for 2 days before being washed in cacodylate buffer (3 x 10min). Samples were then postfixed in 2% osmium tetroxide(1 x 2hr) and then were dehydrated in a series of ethanol solutions. Tissues then were washed in a solution of propylene oxide(2 x 20min). Tissue samples were then placed in a 1-part epoxy resin to 2-parts propylene oxide solution (1 hr), then a following solution of 2-parts epoxy resin to 1-part propylene oxide (1 hr). Tissues samples were then placed in a 100% epoxy resin solution overnight for infiltration. Tissue samples were then infiltrated with new 100% epoxy resin for 2 hours. Following this, samples were placed in beam capsule and flat molds with new epoxy resin and placed in a vacuum oven at 65° Celsius at 25 pa for 2 days.
Microscopy:
Hardened samples were faced and sectioned using an ultramicrotome using glass knives and mounted on to slides for microscopic viewing. The samples were then stained with PAS and Toluidine Blue stains to visualize microscopic structures. Alcian Blue was also used to show mucus glands are positive for mucin.



RESULTS & DISCUSSION

Figure A:
A close up of both granular and mucous glands located in the dorsal dermis of the skin. Note there are several layers of glands under the skin. And the granular glands are most common on the dorsal surface. The epidermis is quite thin (3-5 layers) with many blood vessels in juxtaposition to the epidermis for gas exchange.

Figure B:
This figure depicts a flask cell (FC), otherwise known as a mitochondrial rich cell. These cells are often hypothesized for the ion transport in and out of the body along with water and osmolarity regulation. These are located in the epithelial skin layer and are quite large compared to other salamanders studied to date. We hypothesize the size difference is likely because of the more terrestrial life style of this *Plethodon*. The figure also shows nicely the stratum corneum and the spinosal layer of the epidermis.

Figure :C
This figure depicts a large and numerous Granular glands (GG) and smaller, less abundant mucous glands (MG) at higher power. These glands are suspected in aiding in the production of the mucous based on the abundance of them within the skin and their dorsal location.

Figure D:
Possible mixed glands can be seen throughout the dorsal side of the skin. This particular image depicts granular compartment along with the mucous demilune. The size and mixing power of these glands are most likely involved in the thickness of secretion. Especially the mucus producing portion which appears eosinophilic and made up of glycoproteins that would attract water and thicken secretions.

Figure E:
This image depicts stained mucous glands that are alcian blue positive. This visualizes an acidic type of mucin within the mucous gland which could be lower in pH and uncomfortable for the mucous membranes of a predator.

Preliminary examination revealed the presence of granular, mucous, and mixed glands distributed throughout the epidermis and dermis. Additionally, flask cells were observed in the epidermis, while chromatophores and dermally located blood vessels were consistently present. The mucous glands exhibited eosinophilic properties and tested positive for periodic acid-Schiff (PAS) and Alcian Blue (AB) staining. Notably, the mixed glands located dorsally were significantly larger than those on the ventral surface. The high density of mixed and granular glands, combined with an apparent apocrine secretory mechanism, likely contributes to the production of the thick, adhesive mucus observed in this species. Future research will be done with TEM to describe the ultrastructure of the glands cells, secretion type, and the flask cells.

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