Preliminary Histological Study of the Integument and its Glands in the Zigzag Salamander, *Plethodon dorsalis*

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ABSTRACT

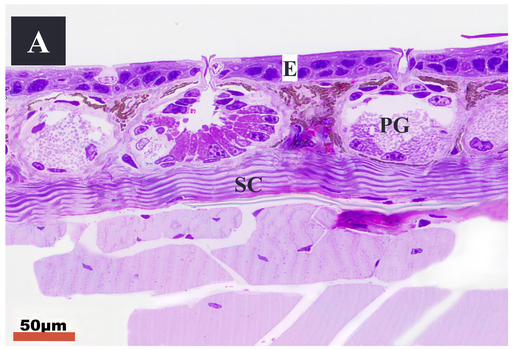
Our histological research involves the Northern Zigzag Salamander, *Plethodon dorsalis*. Zigzag Salamanders are restricted to the unglaciated regions of Indiana, they are primarily found in west-central, south-central, and southeastern parts of Indiana. In our research, we embedded dorsal and ventral trunk skin samples in Embed812 plastic for light and eventually electron microscopy. We used a microtome to create semi-thin sections to initially provide a histological description of the glands within the dermis of this salamander. Little is known about the histology of the skin and its glands in *Plethodon* outside of studies on courtship and mental glands involved with reproduction. We provide in this study the first description of the granular and mucous glands of the skin of the trunk in zigzag salamanders. The dorsal skin has many more granular vs. mucosal glands in zigzags. The mucous glands are much smaller than the granular glands and the granular glands are compartmentalized into a gland proper and a granular demilune. Their granular and mucous glands appear similar to the limited number of studies done on other species within *Plethodon*. The next step is to explore both gland types under the electron microscope to provide ultrastructural details of organelle makeup within both gland types.

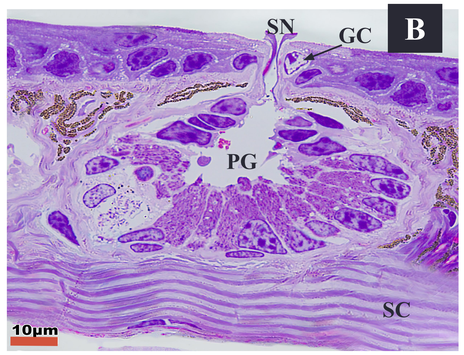
INTRODUCTION

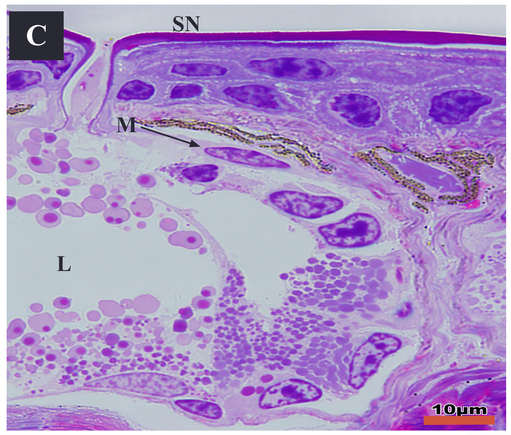
The Northern Zigzag salamander, *Plethodon dorsalis*, belongs to the Plethodontidae family and is found in Indiana often on wooded hillsides and ravines, under moist leaf litter and flat rocks. *Plethodon dorsalis* prefer a moist habitat to serve as a buffer from weather extremes due to their unique possession of neither lungs nor gills. Since this salamander lacks lungs they breathe through their skin and the mucous membrane in the mouth and throat, so these surfaces must remain moist at all times in order to absorb oxygen. The purpose of this project is to examine the skin glands within *Plethodon dorsalis* in a histological study to add data to the overall integumental and glandular information within lungless salamanders. There has been little previous research on the skin within *Plethodon dorsalis*, so the conclusions and observations made from the analysis of the glands will benefit and aid in further research on this specific type of salamander. These observations and conclusions can then be compared to the glandular system in previously studied salamanders, such as *Plethodon shermani*, followed by future research to provide greater detail to the function and ultrastructural organization of the glands that is missing in this preliminary studies.

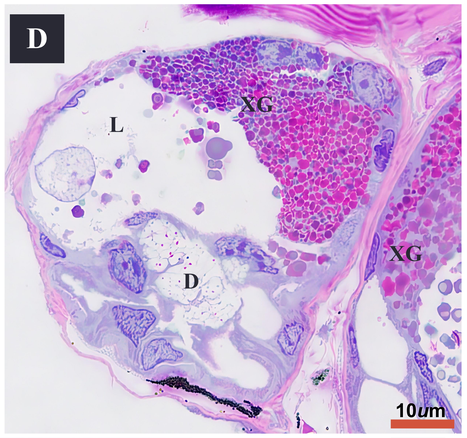
MATERIALS and METHODS

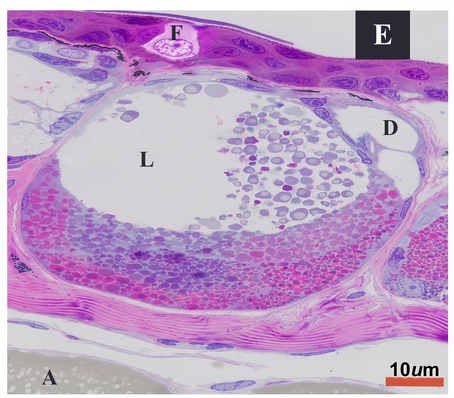
- **Animal Collection:** Two male and two female *Plethodon dorsalis* were collected in Monroe Ct, Indiana on October 22nd, 2021.
- **Tissue Preparation:** The salamanders were sacrificed using MS222 in accordance with UIndy IACUC policies. The salamanders were dissected and skin was removed from the dorsal and ventral surfaces and fixed in Trumps fixative. The sections of skin were approximately cut into squares of 3mm x 3mm in preparation for dehydration and embedding of the samples. Tissues were removed from the fixative and were cut into small pieces then rinsed in Cacodylate buffer (or PBS) with four 5-minute washes. Following this the tissues were post-fixed with 2% osmium tetroxide for two hours and then underwent another set of four 5-minute buffer rinses. The tissue is then dehydrated with a 50% ethanol wash for 15 minutes before being stored in 70% ethanol solution overnight. The next day, Embed-812, DDSA, and NSA were warmed and slowly mixed to avoid bubbles for two hours then degassed under vacuum and set aside for filtration. Dehydration of the tissue continued with increasing ethanol concentrations (95% for 15 minutes, then two 20-minute washes with 100% ethanol), followed by two 15-minute propylene oxide washes under a hood while spinning. The tissue was infiltrate progressively with increasing concentrations of plastic resin mixed with propylene oxide, eventually sitting in pure plastic while spinning overnight. On the third day, fresh plastic resin was mixed, degassed, and used to infiltrate tissue for 2-4 hours while spinning. Flat beam capsules were prepared by drying in an oven and labels for plastic blocks were created. The tissue samples were then placed in fresh plastic resin in the proper orientation in embedding molds with labels and placed in the vacuum oven for 2 days at 60 degrees celsius.
- **Microscopic Analysis:** The tissue samples were sectioned using a Leica ultramicrotome and a diamond knife. The sections of skin were heat fixed to a microscope slide and stained with Epon Tissue Stain to visualize the ultrastructure of the glands via light microscopy.

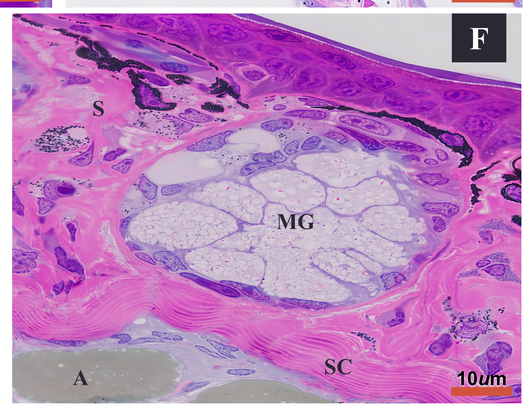


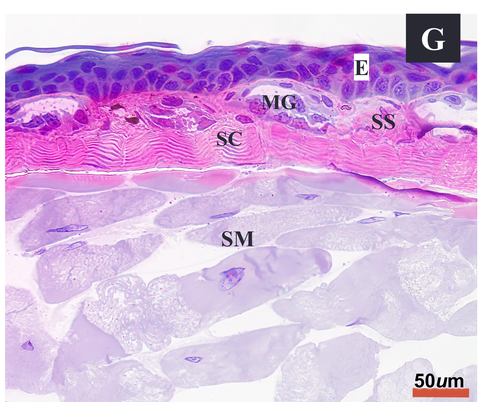


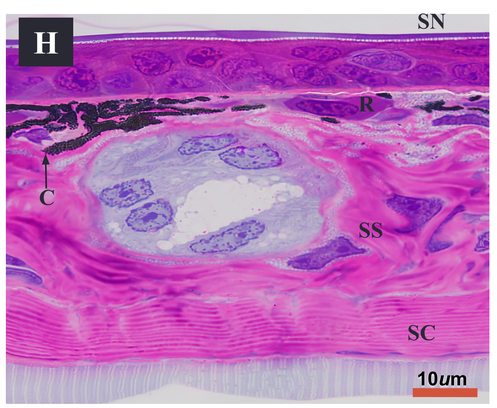












RESULTS & DISCUSSION

Figure A:
This cross sectional image shows dorsal skin at 40X magnification. Numerous granular poison glands lie above the stratum compactum and there is a noticeable presence of the stratum compactum along the dorsal-most region of the epidermis. These glands show typical structural orientation, with granule-rich secretory cells. Notably, the epidermis appears to be only two cells thick, which is potentially an adaptation for cutaneous respiration.
Figure B:
This light microscope image features a dorsal skin poison gland at 100X magnification. The acinar poison gland is anchored by the stratum compactum. The stratum corneum, although torn, extends into the duct. A nearby guard cell is visible, while its counterpart is missing due to sectioning plane. Secretory blebs protruding into the lumen of the gland suggest apocrine secretion.
Figure C:
This figure exhibits a mixed gland on the dorsal skin at 100X magnification. These mixed glands include poison and mucus with mixed densities, suggesting different protein combinations. The stratum corneum extends into the gland duct, potentially regulating secretion. Myoepithelial cells near the duct suggest a contractile function in glandular ejection, warranting further investigation of the duct-valve dynamic.
Figure D:
This shows a dorsal skin mixed gland at 100X magnification. This more mature gland resembles the glands in Figures A and B but is larger, featuring two adjacent mixed glands separated by collagen. The presence of myoepithelial cells and uniform granules further support their classification as apocrine mixed glands, secreting both mucus and poison granules.
Figure E:
This figure depicts an empty mucus demilune at 100X magnification. Flask-shaped epithelial cells are observed, which may be mitochondria-rich cells and will be explored further through TEM. Nerves and their respective axons are present, supporting the idea of neural regulation of secretion.
Figure F:
This light microscope image presents a pure dorsal skin mucous gland at 100X magnification. The spongiosum consists of loose collagen, providing flexibility and support. Uniform staining and lack of dense granules confirm this gland's specialization in mucus production.
Figure G:
This figure shows mucosal glands in the ventral skin at 40X magnification. Organized epidermal layers are shown, with smaller glands within the stratum spongiosum. The stratum compactum provides structural integrity. Fewer glands are observed and they are much smaller in diameter than those of the dorsal skin, likely because the ventral side is less exposed to predators than the dorsal side.
Figure H:
This cross sectional figure shows a small mucous gland in the ventral skin at 100X magnification. A thin white line beneath the stratum corneum, likely containing desmosomes for strong cell to cell adhesion, will be evaluated further with TEM. Pigment-rich chromatophores are present.
Legend Abbreviations:
poison gland (PG), mucous gland (MG), mixed gland (XG), epidermis (E), stratum compactum (SC), stratum corneum (SN), chromatophore (C), spongiosum (S), skeletal muscle (SM), stratum spinosum (SS), lumen (L), demilune (D), myoepithelial cell (M), flask cell (F), axon (A), guard cell (GC), RBC (R)
Future Research:
Future studies should examine the ways in which the two cell thick epidermis facilitates cutaneous respiration in *Plethodontidae*. Transmission electron microscopy (TEM) could provide insight into further structure and function of the flask shaped epidermal cells and the stratum corneum. The roles of guard and myoepithelial cells in secretion should also be further explored. Additionally, the thin white layer beneath the stratum corneum remains unidentified and should be further characterized.

ACKNOWLEDGEMENTS

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