A Histological Analysis of the Skin and Glands of *Dendrobates auratus* Adult and Juvenile Specimens

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Introduction

The diurnal poison dart frog species *Dendrobates auratus* can be found in Central and South America. They are often found on the floor of rainforests near small streams or pools of water.

Figure 1: *Dendrobates auratus* adult frogs

There are many color variations for poison dart frogs, with their bright colorings and markings used to warn predators of their toxicity. Poison dart frog toxicity comes from their diets, mainly consisting of ants and other arthropods which contain high concentrations of alkaloids. These alkaloids are absorbed, transported, and then stored in the poison glands of the frogs. In our study, we sought to provide a histological analysis of the skin and glands, namely the poison and mucous glands, of *Dendrobates auratus* adults and juveniles.

Materials & Methods

For the embedding process of our samples the first day involved several steps. First, three rinses were completed in Cacodylate buffer/PBS (0.2M) for 15 minutes. Under a fume hood, tissue was stained with 2% Osmium tetroxide (OsO4) created with a one to one mixture of 4% OsO and DI water. The sample then sat for approximately 2 hours and three more rinses were completed in Cacodylate buffer/PBS (0.2M) for 10 minutes. The sample sat in 70% Ethanol overnight. To make the plastic on day two, the 20ml Embed-812 resin, 16ml DDSA, and 8ml NMA anhydrides were warmed at 60°C. The components were than mixed very slowly for 20 minutes so there were no air bubbles or layers in the beaker. Once blended, 1.2ml BDMA was added to the plastic while stirring and was left to stir slowly for 2 hours covered with airtight parafilm. On the rotator, the sample was dehydrated for 15 minutes with 50% ethanol, then for 20 minutes with 85% ethanol, then for 20 minutes with 95% ethanol, then for 20 minutes 2 times with 100% ethanol while spinning, and then dehydrated for 20 minutes 2 times with Propylene Oxide in the fume hood while spinning After dehydration, the sample was infiltrated with 1 part plastic and 1 part propylene for 1 hour in the fume hood, while spinning. Then the sample was infiltrated with 2 part plastic and 1 part propylene for 1 hour in the fume hood, while spinning. Finally, the sample was infiltrated with pure plastic and left to spin overnight. On the third day, the plastic was spun with a stir bar on low and covered for 2 hours. The tissue was infiltrated with new plastic, while being spun for 2 hours. The flat bean capsules were prepared and placed in the oven until the time of embedding, with the oven at 60-65°C. Labels were then made for the plastic blocks. To embed the tissue the capsules were filled halfway with new plastic, tissue was put into the plastic capsules and pushed to the bottom so they could be oriented. Plastic was then filled to the top and tissue labels were inserted. The capsules were then placed in the oven at 60-65°C for two days.

Juvenile and adult *Dendrobates auratus* ventral and dorsal skin samples were used. A microtome was used to cut the plastic capsules containing tissue samples. Thin slices cut by the microtome and containing samples were put onto microscope slides for further analysis. The slides were heated, stain was applied, they were then rinsed and ready for microscopic examination. The slides were then examined using light microscopy and transmission electron microscopy (TEM). The TEM images were taken by Dr. Kevin Gribbins.

Results

Figure Key: M = mucous gland, P = poison gland, SS = stratum spongiosum, SC = stratum compactum, E = epithelium, SM = skeletal muscle, Ch = chromatophores, C = cartilage

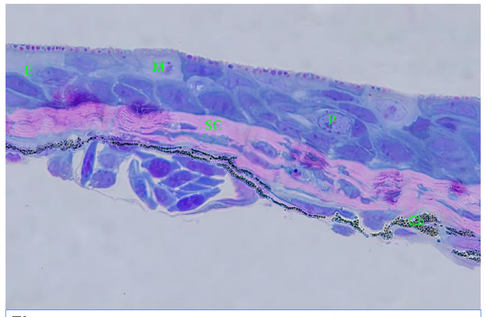

Figure 2: *Dendrobates auratus* juvenile ventral skin

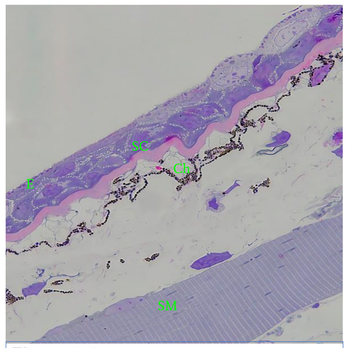

Figure 3: *Dendrobates auratus* juvenile ventral skin

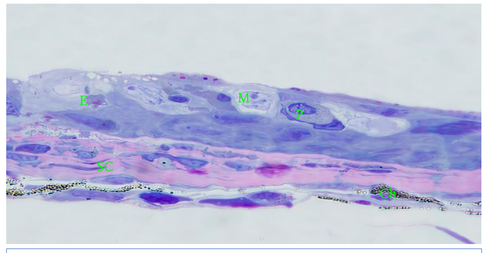

Figure 4: *Dendrobates auratus* juvenile dorsal skin

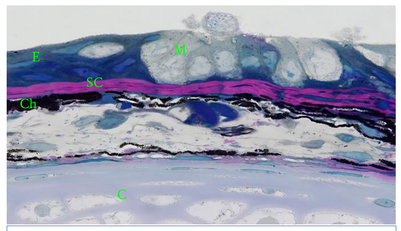

Figure 5: *Dendrobates auratus* juvenile dorsal skin

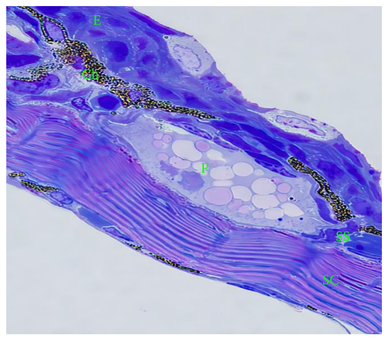

Figure 6: *Dendrobates auratus* adult ventral skin

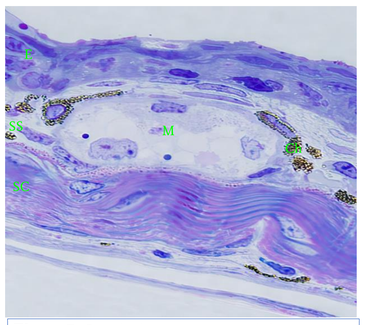

Figure 7: *Dendrobates auratus* adult ventral skin

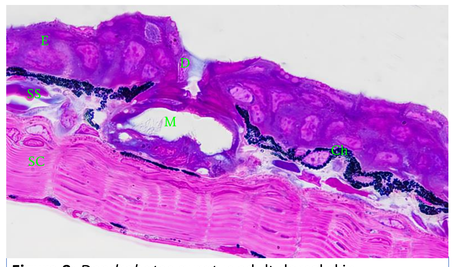

Figure 8: *Dendrobates auratus* adult dorsal skin

Figure 9: *Dendrobates auratus* adult dorsal skin

Discussion

When examining the skin of tadpole *D. auratus*, we were able to confirm the epidermal origin of both poison and mucous glands. Interestingly, the stratum compactum layer of the dermis was above a layer of chromatophores, and there were no chromatophores developed above this layer like in adults. When examining the skin of adult *D. auratus*, poison and mucous glands were both present in the dermis. Additionally, excretory ducts were visible for both gland types. In general, the ventral skin layers were thinner than the dorsal skin layers, as expected. When compared to previous studies, the tadpole poison glands did not appear like smaller versions of adult poison glands, as stated by Angel et al. 2002. Adult poison glands appeared to be surrounded by a myoepithelial layer, as supported by Prates et al. 2012

For Future Studies

In future studies we would like to explore the processes and mechanisms for alkaloid absorption and storage in poison dart frogs. Further research on the secretory glands of poison dart frogs would include examining the biochemical properties of the poison and mucous secretions. A future investigation of the secretory cells within the epithelium of the juvenile dorsal skin, which seem to be absent from the adult samples, may be worth looking at for studies on dart frog gland development. In the future, an examination of the location of the stratum compactum in juveniles compared to adults and the migration mechanism of the glands to get to the dermis, could be helpful in identifying structures.

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