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10-5-2024

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# Histological Characterization of Larch Mountain Salamander (*Plethodon larselli*) Head Glands



*Plethodon larselli* - Photo by John Clare

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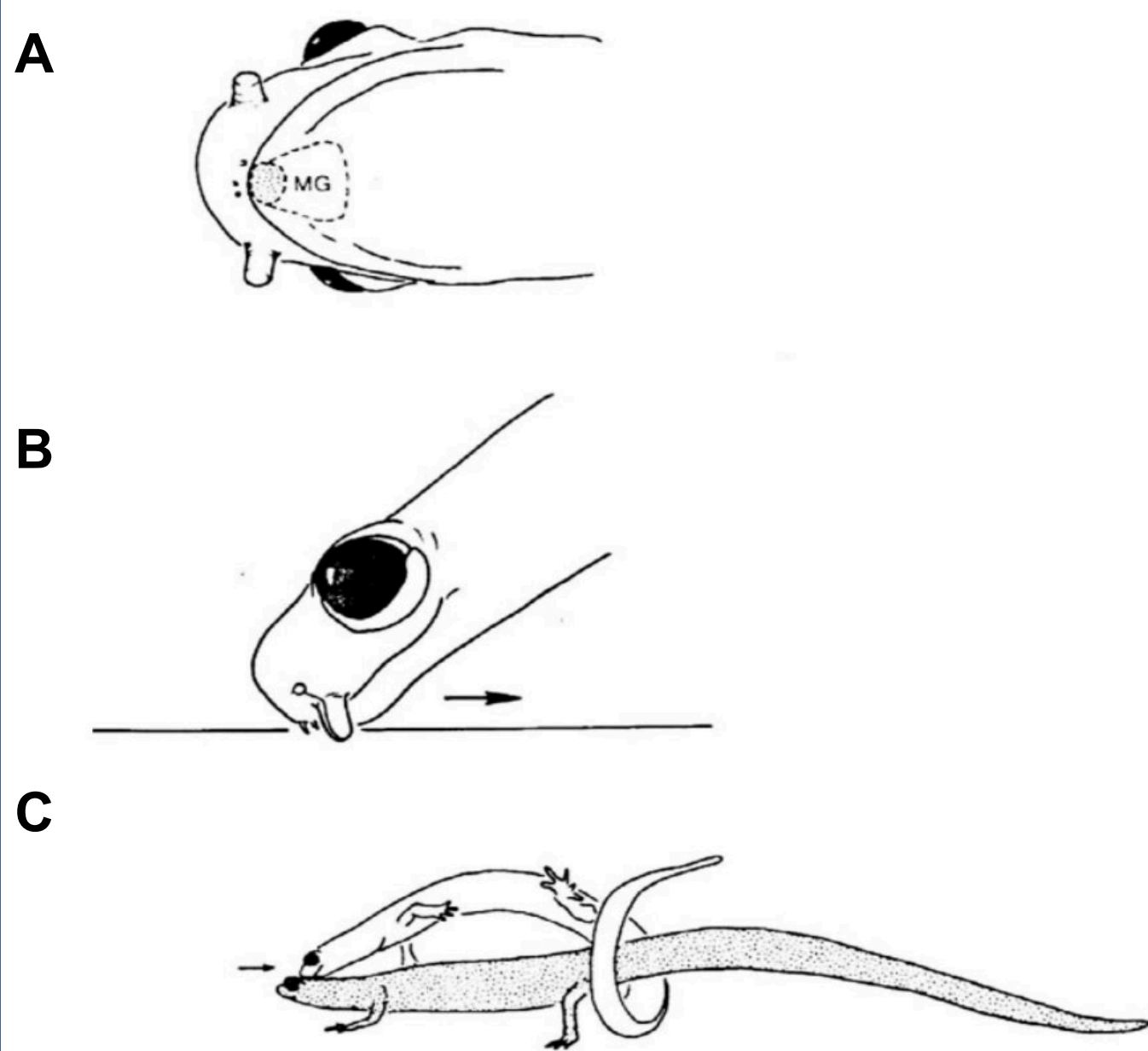


*Plethodon larselli* - Photo by Gary Nafis

## INTRODUCTION

In salamanders, successful reproduction requires communication. Most male salamanders communicate to the female during courtship via a gland on their chin, called the mental gland. The sexually dimorphic mental gland in the dermis of the lower jaw is typical in males of species in the family Plethodontidae and is considered ancestral to the family (Sever et al. 2016). The mental gland consists of an aggregate of modified granular glands (MGGs) that produce pheromones (Houck and Sever, 1994; Rupp and Sever, 2018). During courtship, pheromones secreted from mental glands are delivered to the female through head rubbing and biting (Houck and Sever, 1994; Picard, 2005; Arnold et al. 2017).

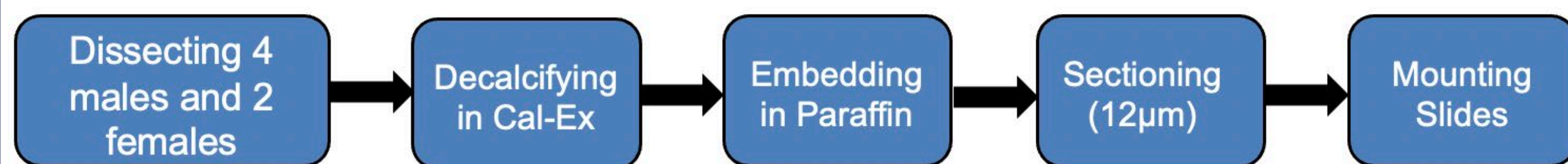
These pheromones increase female receptivity and are an integral part of courtship (Houck and Reagan, 1990; Fontana et al. 2007). Interestingly, males in some clades of Plethodontidae, including *Plethodon larselli* (Larch Mountain Salamander), lack mental glands (Crisafulli, 2008). This is an evolutionary puzzle—why would a fitness enhancing gland be lost? No research has explored the courtship behavior of *P. larselli*, and no histological studies have characterized their mental gland region. In this project, Periodic acid Schiff differential staining (Floyd, 1990; Rollins and Staub, 2017) was used to confirm the absence of mental courtship glands in *P. larselli* and explore the hypothesis that pheromone secreting tissue is located elsewhere on the head of *P. larselli*.



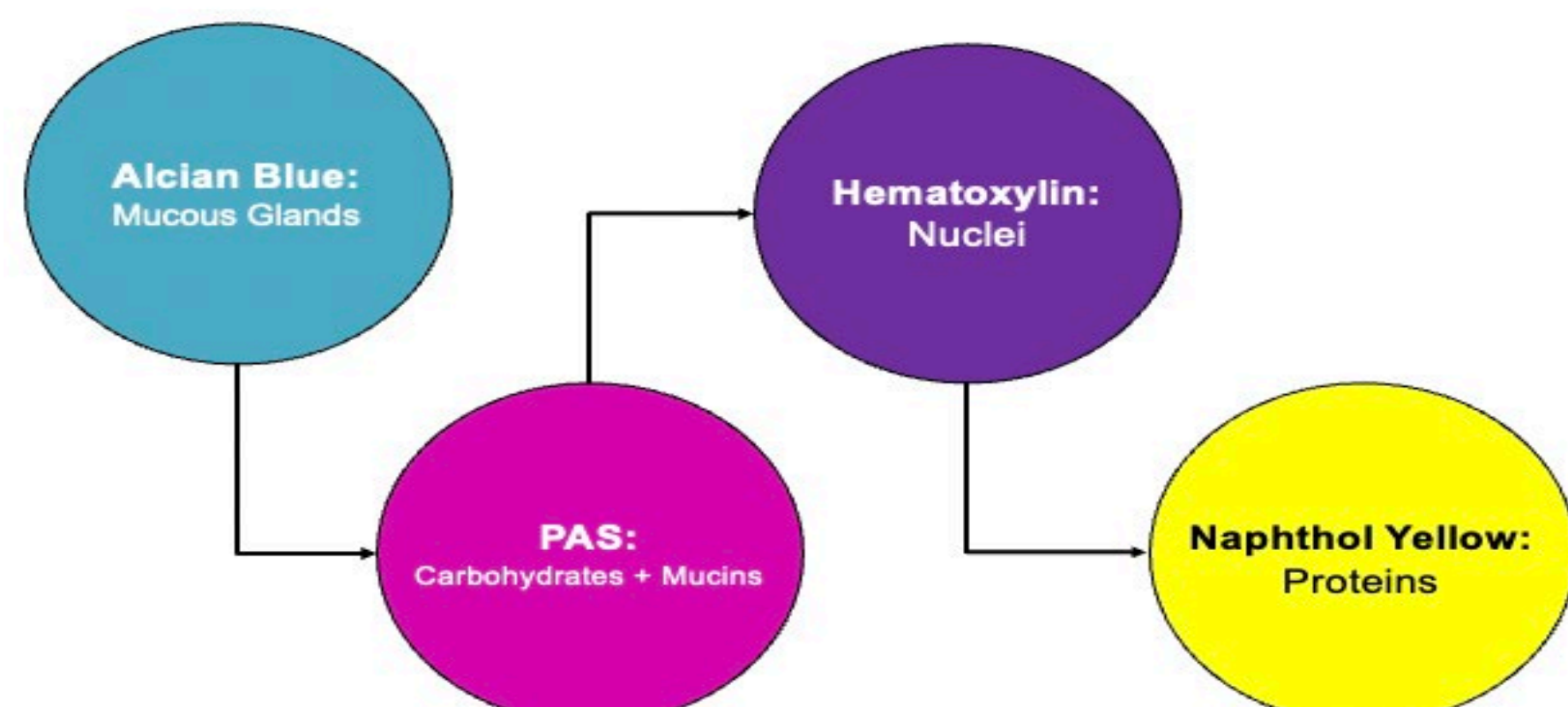
**Figure 1:** The mechanism of transdermal pheromone delivery in *Eurycea wilderae*, a Plethodontid, during courtship. (A) Ventral view of mental gland tissue in *E. wilderae*. (B) The "scraping" motion of the male's head used to abrade the female's epidermis with his premaxillary teeth. This delivers pheromones directly into the bloodstream. (C) Male (unshaded) position during the contact sub-module of courtship; head and cheek rubbing is also observed. Figure adapted from Arnold et al. 2017.

## Methods

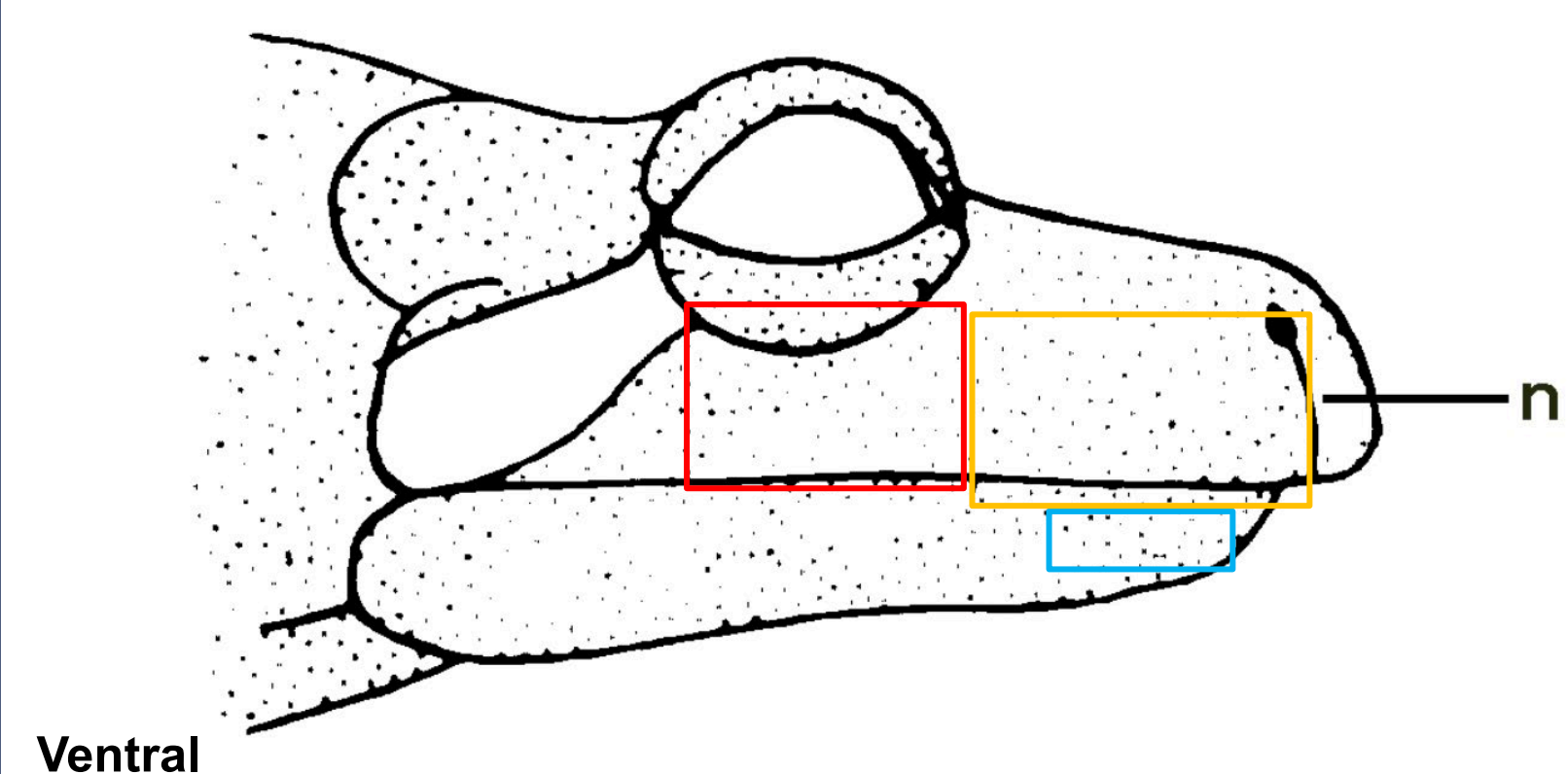
### Standard Histological Procedure



### Quad Stain



### Head Regions Examined



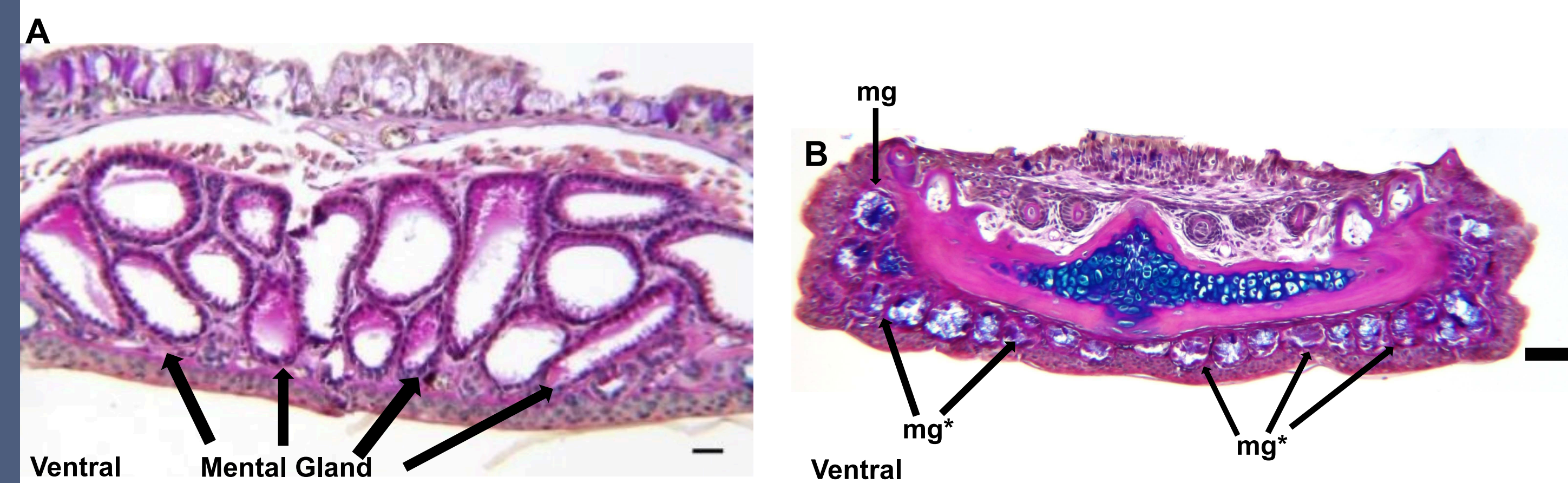
**Figure 2:** Sagittal view of salamander head. Highlighted regions were examined for potential pheromone producing glands. n = nasolabial groove; blue = mental gland region; yellow = nasolabial/external nasal gland region; red = orbital gland region. Sketch adapted from INHS Prairie Research Institute.

## RESULTS

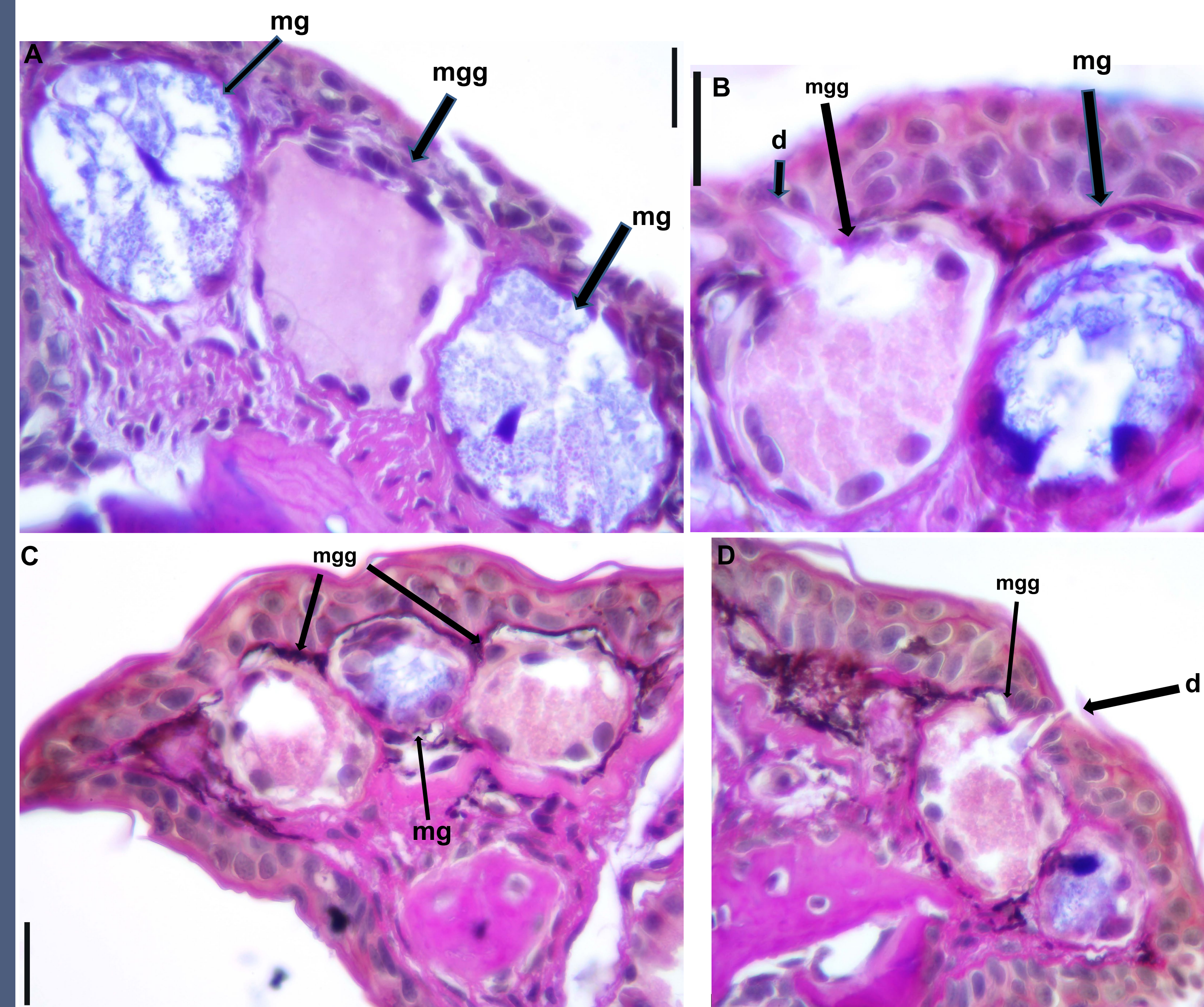
**Result 1:** Male and female *P. larselli* salamanders do not exhibit mental glands in their mental region. Interestingly, there are scattered modified mucous glands throughout the head (mgs\*) (Figs. 3, 5).

**Result 2:** Male and female *P. larselli* salamanders exhibit scattered PAS+ modified granular glands (miggs) that secrete their contents through a duct in the epidermis. The highest concentration is in the orbital gland region on the upper jaw (Fig. 4).

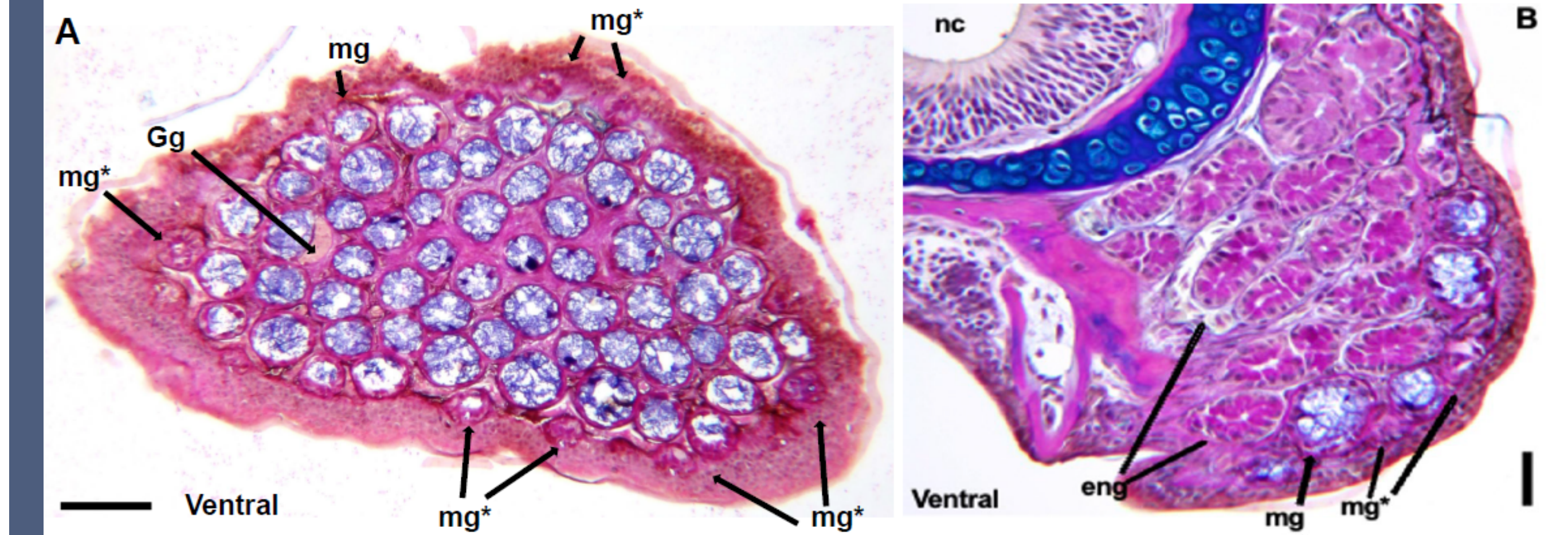
**Result 3:** Male and female *P. larselli* exhibit PAS+ nasolabial glands (nlgs) and external nasal glands (engs) in the nasolabial/external nasal gland region (Fig. 5).



**Figure 3:** Photomicrographs of transverse sections of the area posterior to and at the mandibular symphysis, the mental gland region. Male *P. larselli* do not have a traditional mental gland but do have modified mucous glands in this region (blue region in Fig. 2). (A) Male *E. wilderae* with a traditional mental gland (adapted from Pierson et al. 2024). (B) Male *P. larselli*. mg = mucous gland; mg\* = modified mucous gland. Scale bars, 100µm.



**Figure 4:** Photomicrographs of transverse sections of the area ventral and lateral to the orbital glands on the upper jaw (red region in Fig. 2). Both sexes of *P. larselli* have PAS+ modified granular glands in the orbital region. migg = modified granular gland; mg = mucous gland; d = duct; Scale bars, 100µm.



**Figure 5:** Photomicrographs of transverse sections of the tip of the snout and the nasolabial/external nasal gland region lateral to the nasal cavity (yellow region in Fig. 2). (A-B) Male *P. larselli*. mg = mucous gland; mg\* = modified mucous gland; eng = external nasal gland; Gg = granular gland; nc = nasal cavity. Scale bars, ~

## Discussion

- We confirmed the absence of a mental gland in *Plethodon larselli*. This is unusual because most Plethodontids have a mental gland, the major pheromone producing gland for courtship.
- We discovered glands in both sexes that resemble modified granular glands chemically and morphologically, which we hypothesize are secreting pheromones.
  - The potential modified granular glands secrete their contents through epidermal ducts; this supports a potential role in courtship during cheek rubbing behavior (typical of many Plethodontids).
- The PAS+ nasolabial/external nasal glands secrete into the external nares and have potential role in courtship/signaling (Trame et al. 2022).
- We discovered a gland in both sexes that resembles a modified mucous gland type with PAS+ and alcian blue staining.
- Further work is needed to identify the contents of the potential PAS+ modified granular, modified mucous, and nasolabial/external nasal glands. These glands support our hypothesis that *P. larselli*, despite not having a mental gland, has pheromone producing glands elsewhere in the snout.
- We predict that future behavioral work describing *Plethodon larselli* courtship behavior will identify cheek and head rubbing behavior in both males and females.

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## ACKNOWLEDGMENTS

- Dr. Siegel
- Angie Hinz and GSRP
- Charles R. Conner Museum (WSU)
- Department of Biology, Gonzaga University

