



# The Development of the Neural Crest and peripheral glia in a Holocephali, the Elephant shark



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## Introduction

The neural crest is composed of potent cells that emerge after delamination from the dorsal neural tube. This gives rise to neural stem cell- like populations that migrate extensively throughout the development of embryonic vertebrates. For instance, these progenitor cells give rise to a variety of cranial and facial features, and to the development of electro sensory receptors

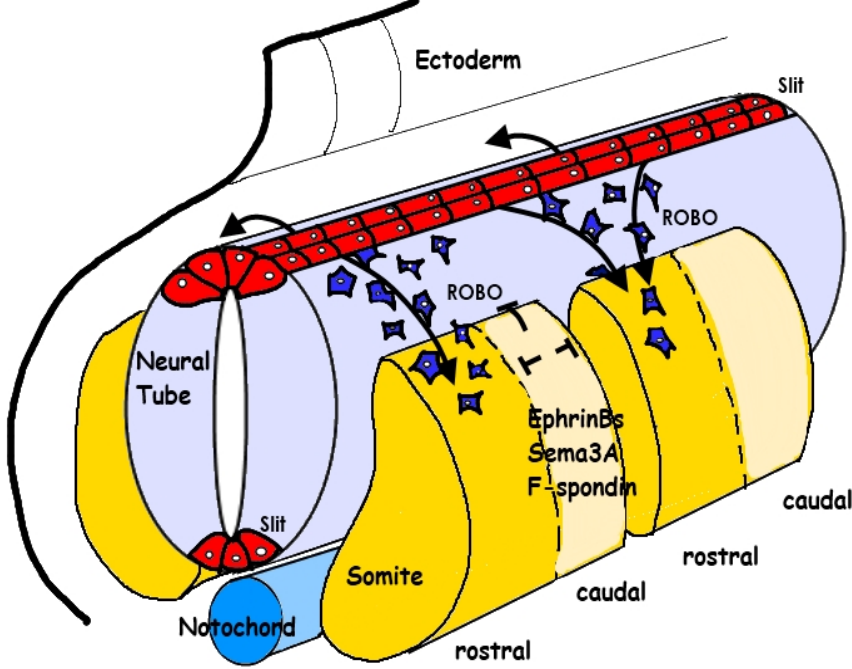


Figure 1. This shows the formation of neural crest. The neural crest arises at the border of the neural plate and the epidermis. The neural tube invaginates within itself in a stereotypical form.

Chondrichthyes make-up two subclasses. The sharks and rays belong to the subclass Elasmobranchii and the chimaeras (also known as ratfishes) to the subclass Holocephali. This study focuses on understanding the detailed development of the peripheral nervous system in Chondrichthyes *milli*. In order to identify the migrating neural crest cells, we use *In Situ Hybridization* and antibody staining procedures with a SOX 9 probe (via *In Situ*) and TuJ1 (neural marker) as an antibody staining. We found that chimaera Sox 9 positive cells labeled a thin migratory population and a disseminated group of cells under the skin, possibly melanocytes. From these studies, we are beginning to see some slight differences between shark and rat fish NS embryos.

## Phylogeny Tree

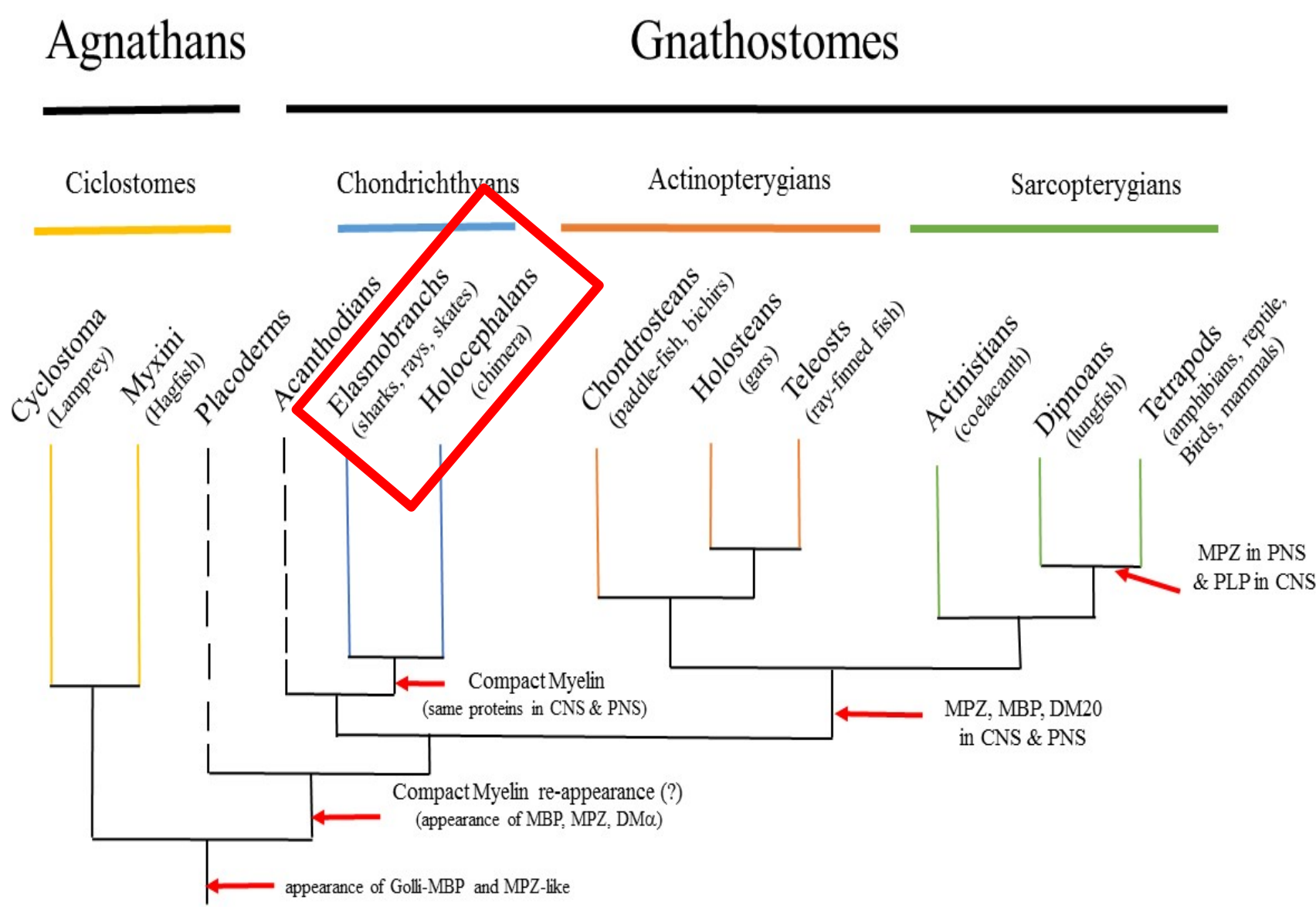


Figure 2. : The Elasmobranchs and the Holocephals share a common ancestor.

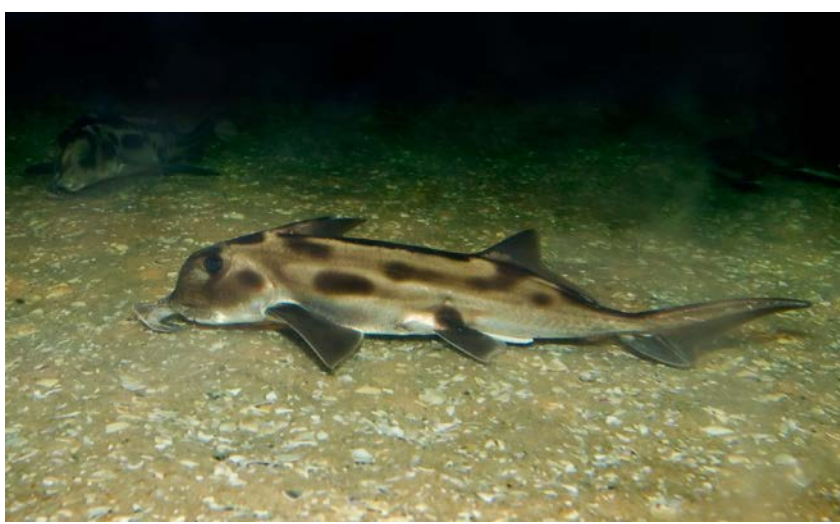


Figure 3. *Callorhynchus milli* Image from <http://Eol.org>



Figure 4. Elephant shark. Image: <https://Sharktrust.org>

## Sox9 in situ for St. 29 Chimera

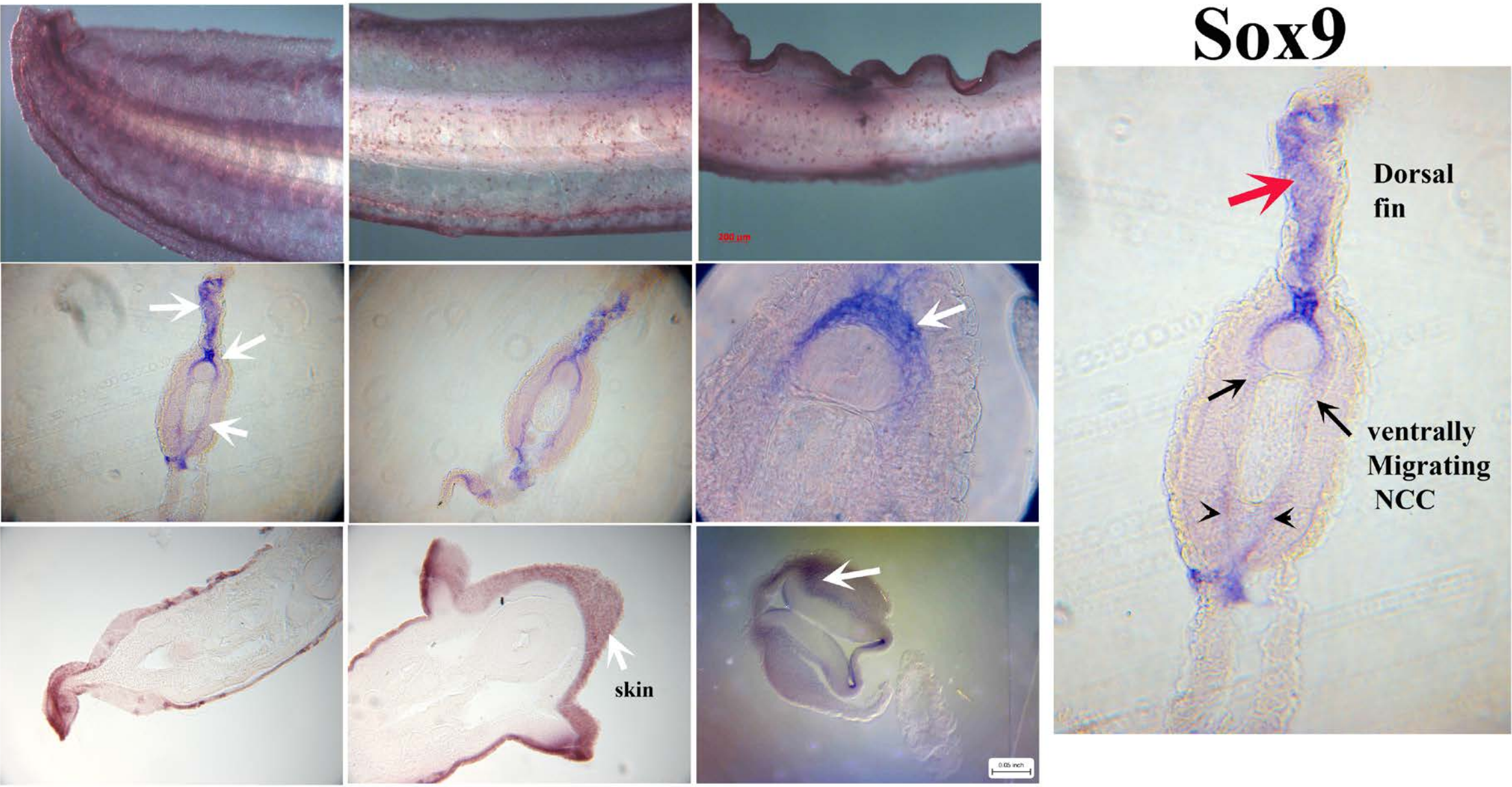


Figure 4. Sox9 expression in *Callorhynchus milli*. In situ hybridization was carried out using a *Chiloscyllium punctatum* CpSox9 probe. Embryo was vibratomed after taking pictures. In the whole embryo we observed a punctuated, surface staining for Sox9 that may correspond to melanocytes or future dermal denticles. Sections through the embryo showed that Sox9 labeled the migrating neural crest along the trunk and cranial region.

## Sox9 in situ in St.27 Chimera

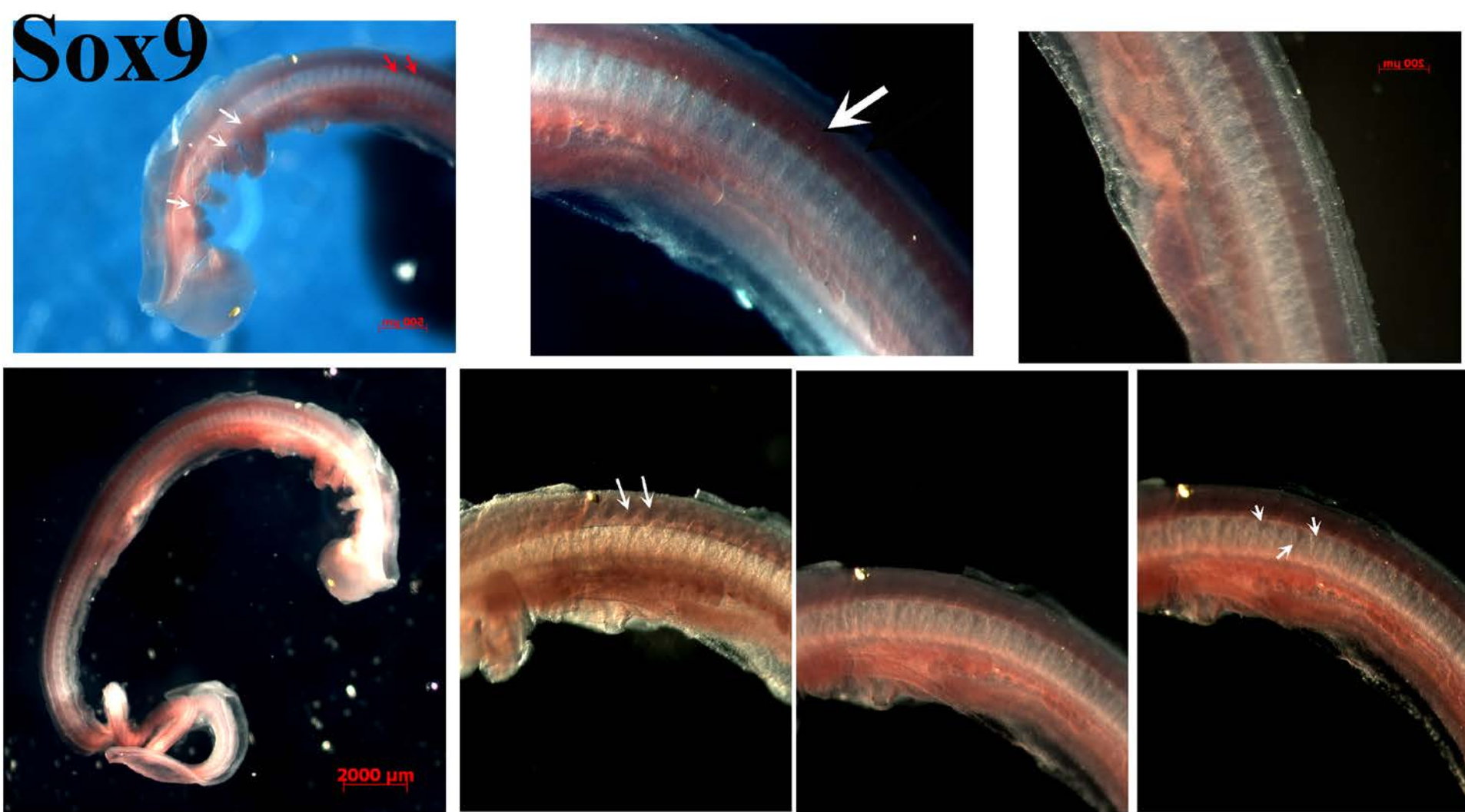


Figure 5. Sox9 expression in *Callorhynchus milli*. In situ hybridization with CpSox9 probe labeled trunk neural crest and ventral stream of these migrating cells.

## Tuj1 labels neurons in the St.27 Chimera

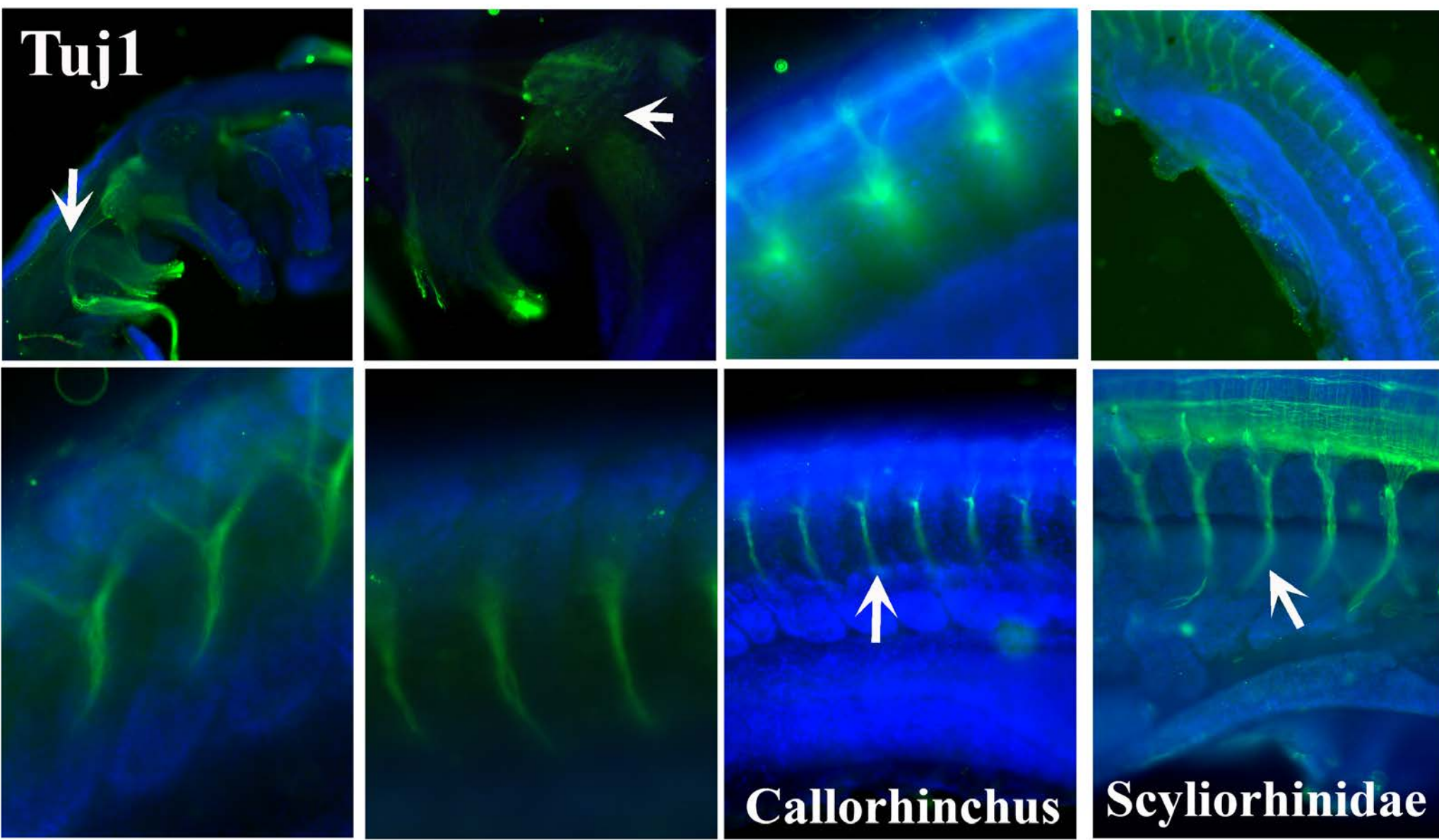


Figure 6. Tuj1/beta-III tubulin neuronal labeling in St. 27 *Callorhynchus milli*. We immunolabeled the embryo with Tuj1 (1:200 dilution) and a secondary Alexa-488 (green fluorescence). The antibody labeled strongly peripheral axons, DRG and cranial ganglia. The peripheral ganglia and spinal nerves of *Callorhynchus* are smaller than for another chondrichthyan, *Scyliorhinus*.

## Holocephali skin

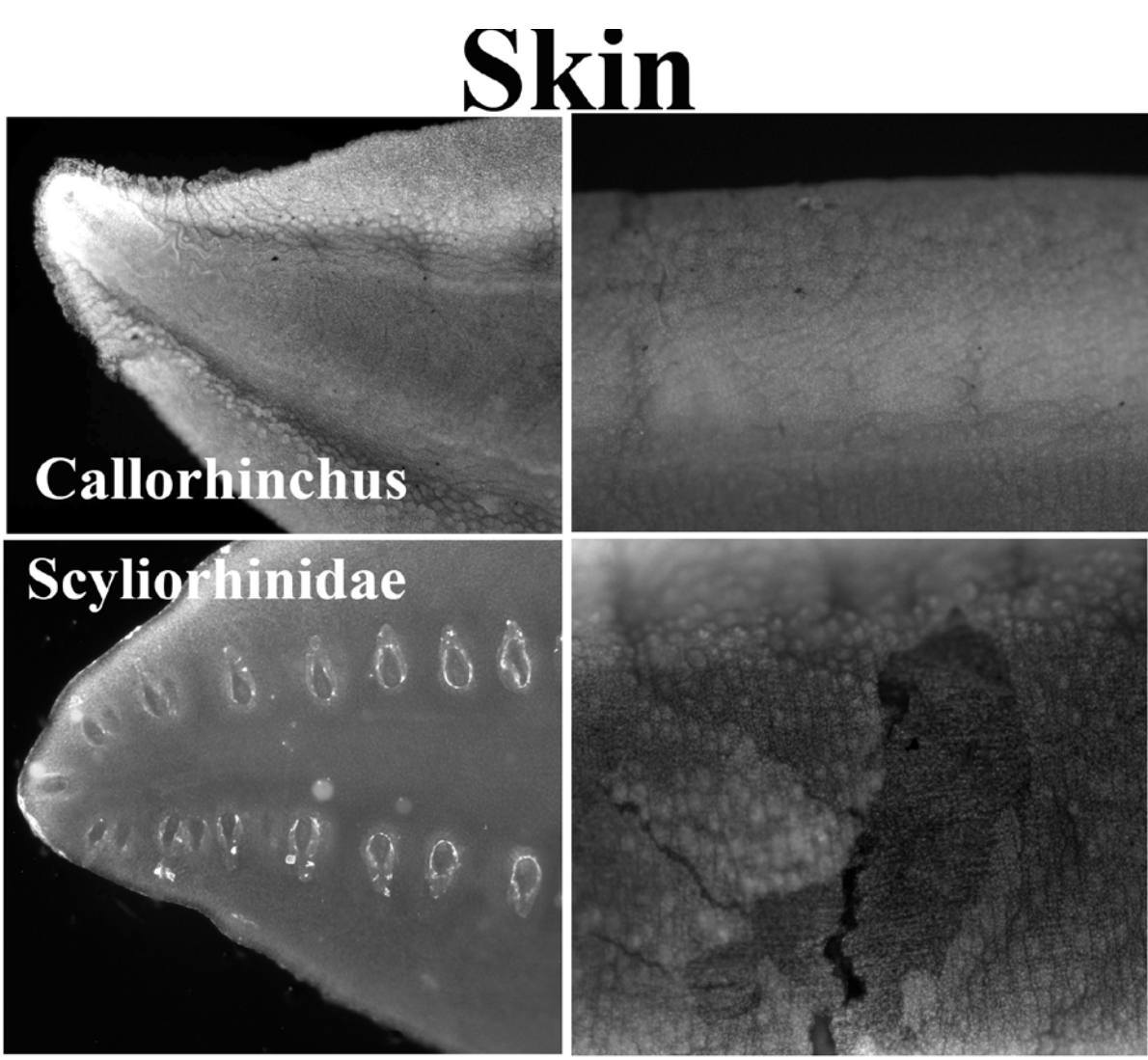


Figure 7. Tail end and Skin comparison between *Callorhynchus* and *Scyliorhinus* using DAPI nuclear staining.

## Sox9 expression in eye

### Eye

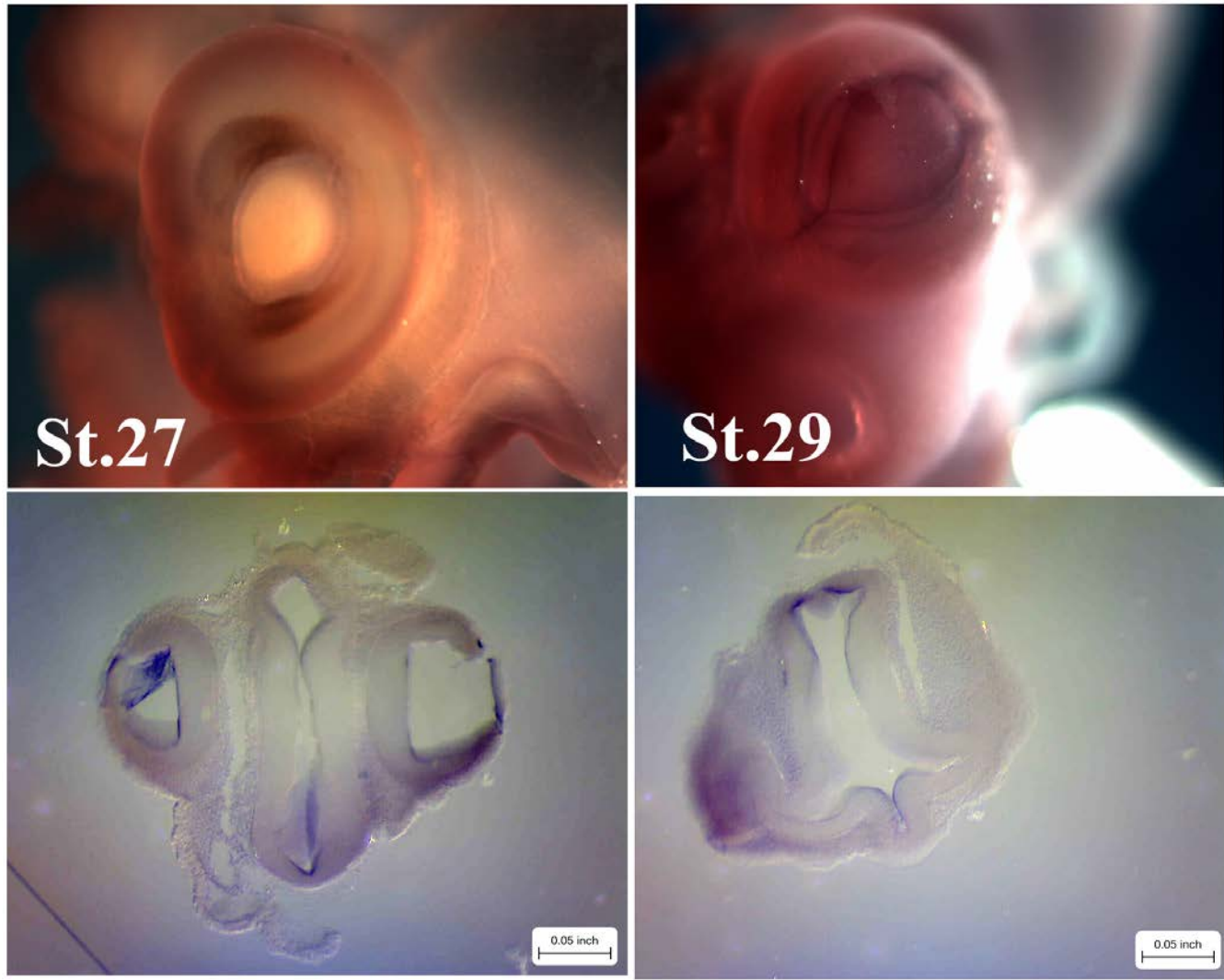


Figure 8. Sox9 expression in *Callorhynchus milli* eye. In situ hybridization with CpSox9 probe labeled corneal neural crest.

## Conclusions

- The *Callorhynchus milli* tail shows solid expression of Sox9 in migrating neural crest. We observed large number of cells entering dorsal and ventral fins.
- Sox9 also labeled a set of cells in the skin of *Callorhynchus milli*, we are trying to determine if these cells are also Sox10 and FoxD3-positive.
- Labeling with Tuj1 shows development of DRGs and neurons, our images suggest that the shark embryos have small PNS ganglia compared with other Osteichthyes because of very thin DRG and spinal nerves.

## Acknowledgment

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