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SHORT COMMUNICATION **OPEN ACCESS**

Modified Staining Technique for the Osteological Analysis of Teleost Fishes

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In ichthyology, comparative anatomical observations of the bones and cartilages are rare since there is no established technique for preparing the specimens that display all the bones and cartilages. This is a protocol for single staining of clearing and staining teleosts that was standardized from the existing protocol of Taylor and Van Dyke [2],and Dingerkus and Uhler [1] especially for flatfishes of the order Pleuronectiformes.In particular, the following method is critical for determining differences in skeletal patterning. The major procedures are simplified below.

Depending on the size, the specimens were fixed in 10% formalin for 1–2 days.Fixed specimens were soaked in distilled water for around 24-48 hours subsequently changing the water every half an hour.Then the samples were moved to two changes of 95% ethanol for 4 to 5 hours, and then through the successive solution of 75% and 50% ethanol for 4 to 5 hours each and finally immersed in distilled water for 12-24 hours.The specimens were placed in trypsin solution to accelerate the clearing process. (Trypsin solution should contain saturated sodium borate solution and distilled water. Here 140 ml distilled water was taken off and 70 ml distilled water was mixed with saturated sodium borate and 70 ml distilled water was mixed with 2g trypsin).

Subsequently, those specimens were transferred to potassium hydroxide and alizarin red stain solution (Measurement of alizarin red should be done according to the size of the specimen. In this protocol 0.02mg of alizarin red was used).Specimens were kept in alizarin red solution for 24 hours or more based on the size of the specimen.Finally, the stained specimens were treated with three baths of KOH, Hydrogen peroxide, and glycerine solution until it becomes visible and clear for 5 -7 days (All the measurements of chemicals should be standardized according to the size of the fish). The cleared and transparent samples were permanently stored in pure glycerine solution to which a few thymol crystals have been added to prevent decomposition. Pictures of the stained specimen were taken using a digital camera.

The above protocol yields excellent, reproducible results that can be used for all teleosts fishes, and vertebrates.

Figure 1. Specimen of flatfish before staining

Figure 2. Photograph of a cleared and stained whole specimen of flatfish

REFERENCES

1. Dingerkus, G. and Uhler, L.D., 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain technology*, *52*(4), pp.229-232.https://doi.org/10.3109/10520297709116780
2. Taylor, W.R. and Van Dyke, G.C., 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium (Paris)*, *9*(2), pp.107-119.

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