MATERIAL AND TECHNIQUES
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*Mollienisia sphenops* (Cuv. & Val.) is a small exotic aquarium fish which has been introduced in India from its native place, that is, Central America and the West Indies. It measures 5 cms. to 10 cms. in length and the mature individuals are easily distinguished from the immature ones by their dark black colour. Sexual dimorphism is quite evident in the fish. Females are comparatively larger in size than the males which are characterised by the possession of an elongated gonopodium or intermittent anal fin. Moreover, the abdomen of a gravid female is swollen.

*Mollienisia sphenops* (Cuv. & Val.) is a viviparous fish in which the embryo develops within the follicle of its original egg and remains there for the entire period of gestation. The egg follicle is vascularised, dilated and fluid filled. The fish breeds monthly and in a litter gives birth to 4-10 young ones which are released one after another. The newly born young ones measure about 10.0 mm. in length.

For the study of development and morphology of the chondrocranium, adult females were collected from the tank of Botanical Garden, University of Saugar and were dissected to obtain the embryos from the follicles. Freshly born young ones were collected with the help of a close-meshed net from
the aquaria in which the fishes were reared. The embryos and young ones were directly transferred to the fixatives. A mixture of Corrosive-formol with Acetic acid, aqueous Bouin's fluid, 90% Ethyl alcohol and 5% Formalin were used as fixatives. However, the best result was obtained with the aqueous Bouin's fluid which was prepared as follows:

Picric Acid (saturated aqueous solution) 75 parts
Formalin 40% ... 25 parts
Glacial Acetic Acid ... 5 parts

Embryos and young ones were fixed for 24 hours in aqueous Bouin's fluid and washed thoroughly in water to remove the fixative. These were then separated according to their size and stored in 70% alcohol. Embryos measuring more than 11 mm. in length were decalcified either by 3% Nitric acid in 70% alcohol or by the electrolytic method of Richman et al. (1947) as modified by Bhargava (1968) who used 10% acetic acid instead of the formic acid in the electrolytic mixture. An F.M.F. of approximately 7.5 volts was applied to the object at anode. Decalcification took place in 12 to 14 hours. The embryos were first washed thoroughly with water to remove all traces of Hydrochloric acid and Acetic acid. The embryos of selected length were dehydrated in alcohol series, cleared in xylene and embedded in paraffin wax (M.P. 53°- 60°C).

Transverse serial sections were cut at 8 micra thick
on a Beck's Rotary Microtome. The sections were stained either
with Delafield's haematoxylin and eosin or Mallory's triple stain.
The Mallory's triple stain was prepared according to the following
formula:

<table>
<thead>
<tr>
<th>Stain</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Acid Fuchsin (Aqueous solution)</td>
<td>1%</td>
</tr>
<tr>
<td>B</td>
<td>Aniline Blue</td>
<td>0.5 gm.</td>
</tr>
<tr>
<td></td>
<td>Orange G.</td>
<td>2.0 gms.</td>
</tr>
<tr>
<td></td>
<td>Phosphotungstic acid</td>
<td>1.0 gm.</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>100 mls.</td>
</tr>
</tbody>
</table>

The sections were first stained in stain A. for
4 minutes and then transferred directly to stain B. in which
they were kept for 10 minutes. These were then washed thoroughly
with water and differentiated in 90% alcohol. It has been
found out that the Mallory's triple stain gave the best result
and differentiated the tissues as follows:

The nuclei were stained red, cartilage - light blue,
bone - dark blue, fibrous connective tissue - sky-blue,
nerve - bluish violet, muscles - brick red, erythrocyte and
keratin - orange.

The development of the chondrocranium has been studied
from serial sections by the graphical reconstruction method as
described by Woodworth (1897).

The observations incorporated in the thesis were made
with the help of a Meopta Research Microscope. The photomicrographs given in the present work were made on ORWO-DK 5, 35 mm. Documenten film by using a Rectaflex (junior) camera with Spencer photo-micrographic attachment.