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ORIGINAL ARTICLE

THE PSEUDOBRANCH DEVELOPMENT IN EASTERN MOSQUITO FISH *GAMBUSIA HOLBROOKI*, GIRARD 1859 (CYPRINODONTIFORMES, POECILIIDAE) FROM JADIRIYAH LAKE, BAGHDAD, IRAQ

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ABSTRACT

The aim of the current investigation is to study the embryogenesis of the pseudobranchia (PB) in the Eastern mosquito fish *Gambusia holbrooki*, Girard, 1859 (Cyprinodontiformes, Poeciliidae). Histological sections from 2-4 mm long embryos revealed the presumable PB as a cell mass surrounded by a row of squamous cells in the operculum, as well as the formation of cartilage and blood vessels, and the beginning of the formation of its lamellae. In 5-7.75 mm long embryos, a prominent rise appeared in the mass of cells and PB lamellae, along with an increase in melanin deposition around the blood vessels. Moreover, a row of squamous cells encircled the rise from the front and back. At this stage, the parallel arrangement of the cartilage-attached lamellae correlated with increased melanin concentration around the blood vessels. In newborns, the differentiated cells increased similar to that appearing in the adult's stages.

Keywords: Embryo, Fish, *Gambusia*, Histology, Pseudobranchia, Teleost.

INTRODUCTION

The Eastern mosquito fish *Gambusia holbrooki* Girard, 1859 (Cyprinodontiformes, Poeciliidae), is an exotic teleost fish that lives in shallow waters, springs, and rivers (Coad, 2010). Pseudobranchia (PB) are modified gills represented by half a gill arch in most families of bony fishes. They are located inside a closed type of gill cover, where a layer of epithelium covers them to prevent them from functioning like true gills. Usually, they develop from the posterior part of the gill cover (Stoskopf, 1993). PBs have been described in almost all fish species except those in the genera *Gymnarchus* Cuvier, 1829 and *Cobitis* Linnaeus, 1758 and some species of Anguilliformes, suborder Siluroidei (Roy *et al*., 1997). However, they vary in size, shape, and location. Many activities of PBs have been documented in previous studies,

including ocular oxygen secretion (Waser and Heisler, 2004), sensory or chemical receptors (Laurent and Dunel-Erb, 1984), respiration, vision, osmoregulation, and endocrine functions (Quinn *et al*., 2003). Moreover, the production of carbonic anhydrase and maintenance of the blood's acid-base balance are both facilitated by pseudo-glial cells (PSCs) (Wittenberg and Haedrich, 1974; Rahim *et al*., 2014; Mokhtar *et al*., 2023). There is a lack of information about PB embryonic development; and therefore, the present paper aims to examine the development of the pseudobranchiat in the exotic Eastern mosquito fish *G. holbrooki*, Girard, 1859.

MATERIALS AND METHODS

Adult males and females of Eastern mosquitofish *Gambusia holbrooki* Girard, 1859 were collected from Jadiriyah Lake (33°16'23"N 44°22'24"E) at the University of Baghdad, Iraq. The fish were kept under laboratory conditions. Pregnant females were placed on a wax plate, and the dorsal part of the back of the head was pierced with a needle to administer anesthesia, and then the fish was dissected (Billet and Wild, 1975). Using an Olympus-style microscope, fine thread, and a millimeter ruler, the lengths of the embryos were measured. Immediately after measurement, the largest embryo was fixed in Smith's fixative. Smith's fixative was prepared by mixing solutions A and B and left for 24 hours. Solution A consists of 0.5 g of potassium dichromate and 87 ml of distilled water; solution B consists of 2.5 ml of glacial acetic acid and 10 ml of concentrated formalin (Rugh, 1968). Histological sections (5-7 mm thick) were prepared using a 325-HM rotary microtome then mounted in slides and finally stained with Haematoxylin-Eosin stains (Bancroft and Steven, 1982). Fish taxonomy following Coad (2010).

The following abbreviations were used in the current study: AF-Anal Fin, Br- Brain, BV-Blood Vessels, Ca-Cartilage, CF- Caudal Fin, CO-Connective Tissue, DF- Dorsal Fin, E- Eye, G-Gill, GA-Gill Arch, GFR-Gill Formation Region, GGRF1-First Gill Arch, GRF2-Second Gill Arch, Grg- Gill Region, HA-Hyoid Arch, Mp Melanophores, O- Operculum, OL-Optic Lobes, OV–Otic Vesicles, P-Primordial, PB- Pseudobranch, PBC Pseudobranchial Cell PBL-Pseudobranchial Lamellae, PF- Pectoral Fin, S-Squamous, T-Trunk, UCs- Undifferentiated cells Y-Yolk.

RESULTS AND DISCUSSION

The embryonic development of the mosquito fish *Gambusia holbrooki* Girard, 1859 was examined until day 9 (total length: 7.3 ± 0.6 mm). We observed the formation of the head, trunk, and tail region, as well as the formation of body segments, eye, and brain, in a 2 mm long embryo (Plate1), which is similar to that of a 26–30 hour-old embryo of the Nile tilapia fish *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae), where the body segments appear at 31 hours. All organs were observed to be developing, including the PB (Carol *et al*., 2001). After the fourth day following fertilization, the PB appears in the zebrafish *Danio rerio* (Hamilton, 1822) as a small bud located behind the eye (Thiruppathy *et al*., 2022).

Plate (1) show that at the base of the eye region, the gill cover begins as a mass of body wall mesodermal cells, and at the top, the presumptive PB forms as a mass of elongated cells

originating from the ectoderm, surrounded by a row of spindle cells. While in cartilaginous fish the PB arises beside the mandible arch, it begins at stage S29 as a thickened epithelial tissue (Hirschberger and Gillis, 2022). In 2.75–3.25 mm long embryos, the cells grow and merge with those forming the gill cover (Pls. 2, 3), and a small bulge may appear. After 100 hours post- hatching, the PB of *O. niloticus* was found on the anterior wall of the gill cavity (Carol *et al.*, 2001). In frontal sections, the PB first appears in a 4 mm-long embryo, where the cells from an oval cell mass surrounded by a row of squamous cells, which is partially connected to the first-gill arch and providing a blood vessel. In Plate (4), shows the beginning of cartilage growth, the formation of blood vessels, the beginning of PBL development, and the differentiation of its cells. Plate (5) displays PB cells from a 5.25 mm long embryos. The frontal section of a 5.5 mm long embryo in Plate (6) shows an increase in the number of PBL and their parallel agglutination, interspersed with a growing number of its differentiated cells having a dark-coloured nuclei and light-cytoplasm. This stage marks the initial appearance of melanin's around blood vessels.

The beginning of cartilage development in the spiracular cavity and the appearance of PBL extending into the cavity are associated with 31S stage (Hirschberger and Gillis, 2022). Further lamellar differentiation occurs at the 32S stage. A thicker mass with blood vessels (Primordial PB) also appears at stage 31S. According to Furgała-Selezniow *et al.*, (2016), the onset of the PB filament in *Leuciscus aspius* (Linnaeus, 1758) can be seen at 4 days post-hatch (dph) in the abdominal cavity of the gill cover behind the eye area. The PB filament consists of one filament per side.

The frontal section of a 6.25-6.50 mm-long embryo's shows PB buds forming in front of the first-gill arch, along with an increase in mass and cartilage differentiation. In plates 7 and 8, illustrate PB surrounded by a row of squamous cells at the back and another row of squamous cells extending from the first-gill arch at the front. Plates (7, 8) show how the concentration of melanocytic cells increases around the blood vessels and the formation of an oval-shaped PB in a 6.25 mm long embryo. This is accompanied by the development of a cell bud on the opposite side of the gill cover. It is seen that the central part of the PB contains undifferentiated cells whereas along its length-parallel to blood vessels-cartilage formation increases. At 169–172 days and 6.3 ± 0.7 inches long, the PB appeared in the *Oreochromis niloticus* consists of several rows of lamellae covered in epithelial tissue, in addition to the appearance of columnar cells between capillaries (Carol *et al.*, 2001). PB filaments in zebrafish appear at 17 days post-fertilization and complete development by 90 days post-fertilization after hatching (Thiruppathy *et al.*, 2022).

A 6.75 embryo's transverse section resembles a plant leaf, containing cartilaginous rods and blood vessels distributed throughout it. Its shape appears renal in the longitudinal section. The section also shows connections between blood vessels coming from the true gills and those of the PB and inner vessel of the gill cover. These blood vessels are then transmitted to the brain and eye from the opposite side, as seen in Plate (9). The dashed line of melanocytes in embryo measuring 6.5-7.75 mm in length develops, signifying the lamellae attached to the cartilage,

as observed in Plates (8-10) illustrate this structure around the blood vessels, as they arrange in parallel like grape leaves, where melanin concentration of melanin is higher.

Plate (11) presents histological sections of newborns (24 hours -1 day after birth), where adipose tissue surrounds the now-enlarged PB. The differentiation of columnar cells between the capillaries becomes more pronounced, resembling that of the adult. Although *Dentex dentex* L. appears in the study's data of Santamaría *et al*. (2004) by two pairs of filaments and the initial formation of secondary lamellae are observed by on the sixth day post- hatching, marking the end of the second stage S2, and which marks the start of the PB appearance as a double structure located in the anterior region of the gill cavity. In stage 3, a third filament appears, and in stage 4, the number and length of filaments gradually increase. The lamellae of *Leuciscus aspius* are freely distributed and parallel to the PB filaments by day 9 post hatch (dph) stage. They are connected along their length by a thin connective tissue, and the (PBC) surrounds a network of blood vessels. At 14 dph stage, both pillar and epithelial are also distinguishable, and continue to develop through the end of stages 24-36. The PB of mosquito fish has mucous and chloride cells (Furgała-Selezniow *et al*., 2016).

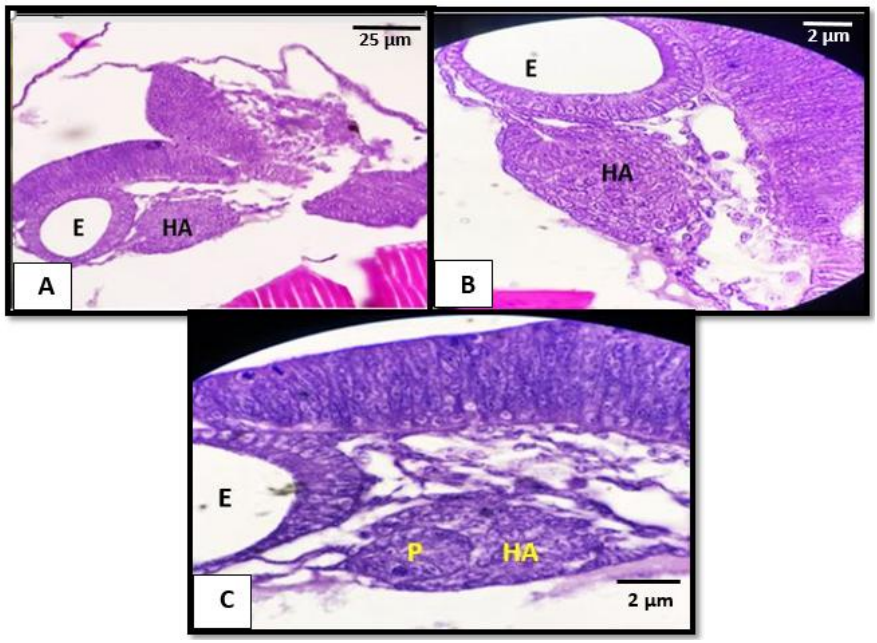

Plate (1): (A, B, C) A frontal section of 2 mm long embryo showing the beginning of the gill cover, pierced in the upper part by a mass of cells surrounded by a row of squamous cells.

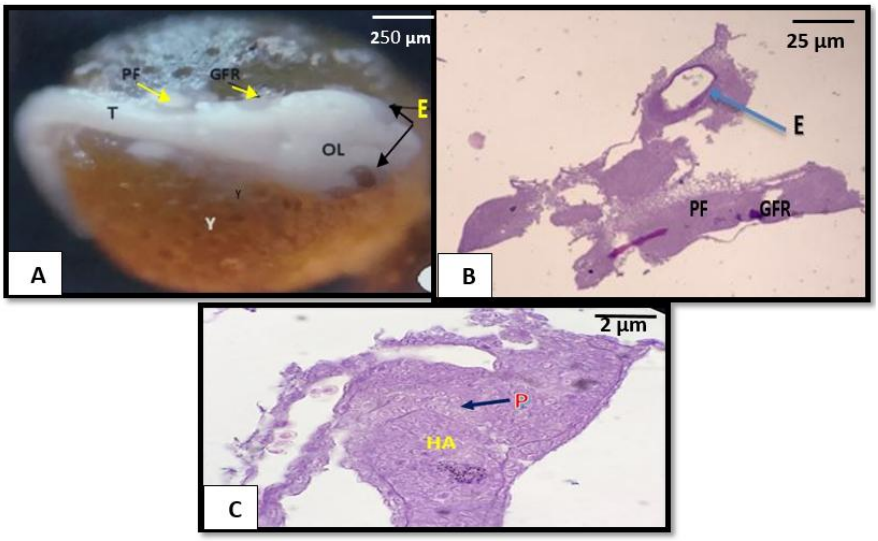

Plate (2): (A) Embryo of 2.75 mm in length, (B, C) A frontal section showing the beginning of the gill cover, merging with it, and penetrated in the upper part by a mass of cells respectively.

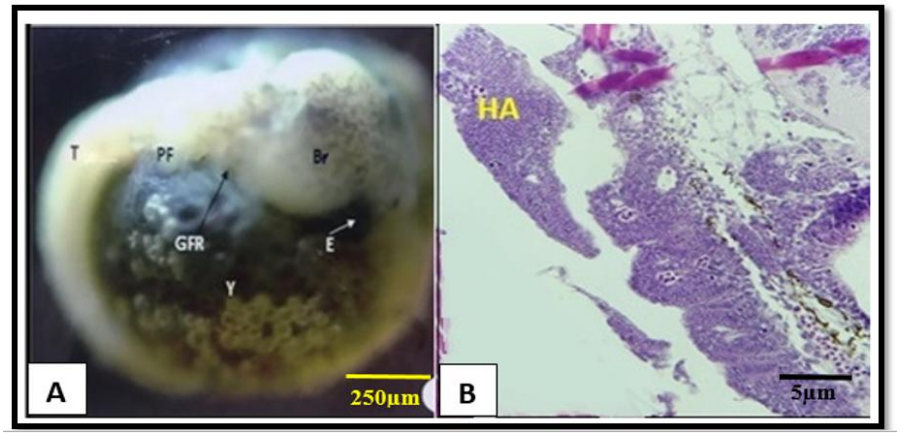

Plate (3): (A) Embryo of 3.25 mm in length, (B) A frontal section showing the beginning of the gill cover, with the fusion of cell mass, H& E.

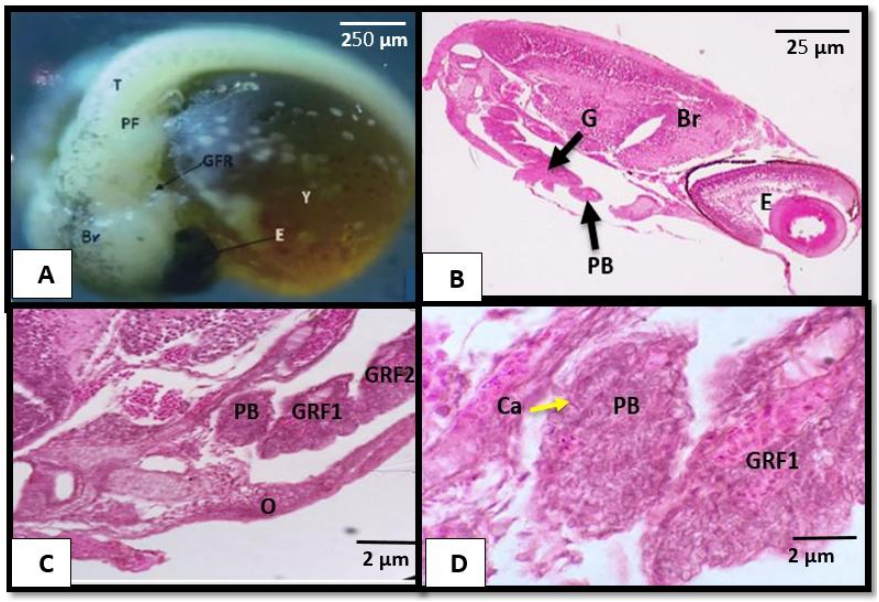

Plate (4): (A) Embryo of 4 mm in length, (B) A cross-section showing a cellular mass representing the PB, 100X, (C, D) A frontal section showing the formation of a cell mass representing the PB. H & E.

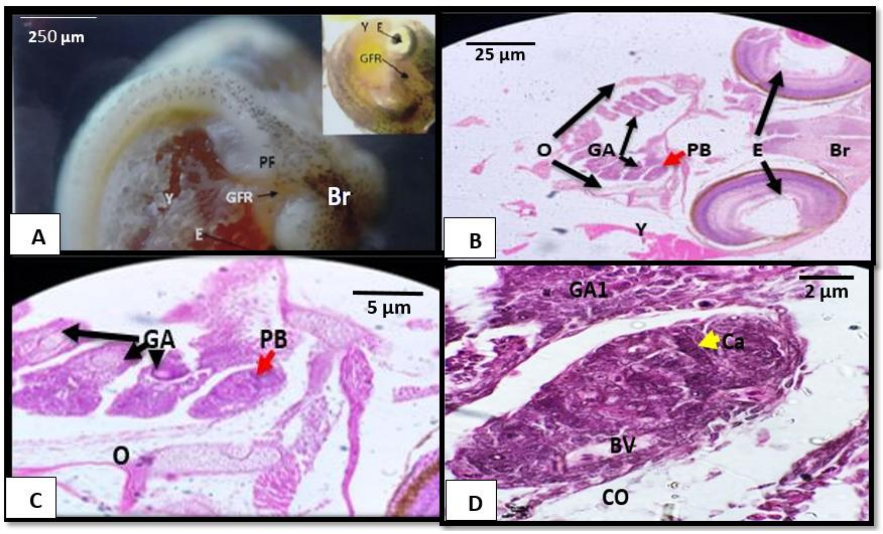

Plate (5): (A) Embryo of 5.25 mm in length, (B) A frontal section showing the beginning of the PB with the gill arches and the gill cover, (C, D) A frontal section showing the beginning of the PB.

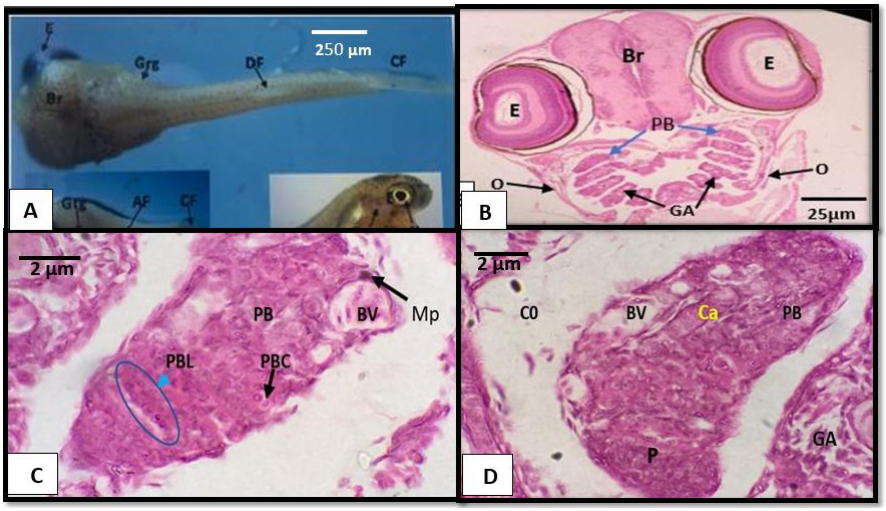

Plate (6): (A) A frontal section of an embryo of 5.5 mm in length, showing the beginning of the pseudobranchia, 100X, (B, C) A frontal section showing the beginning of the pseudobranchia, surrounded by connective tissue from the top on the side of the gill cover It shows blood vessels and specialized cells are clearer with the formation of lamellae lets H &E.

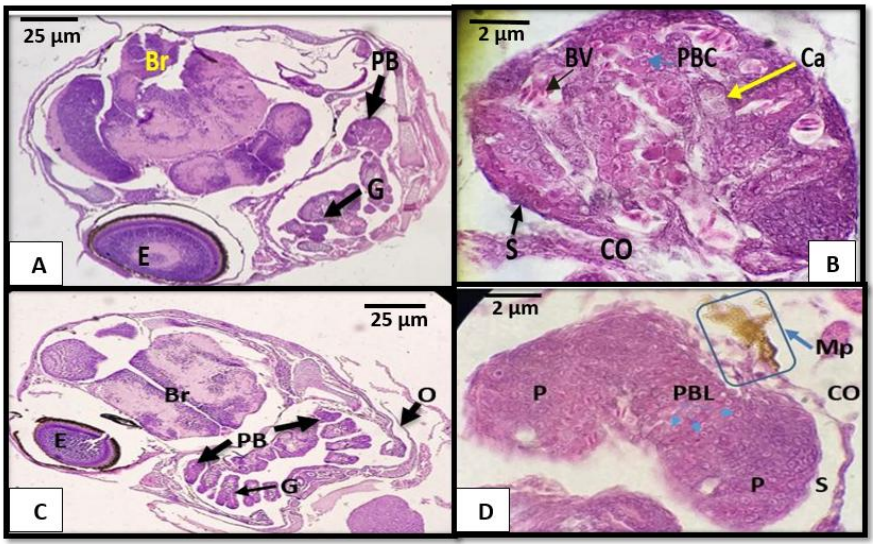

Plate (7): (A) Embryo of 6.25 mm in length, (B) A frontal section showing the PB, surrounded by connective tissue from above on the side of the gill cover. The upper side of the gill cover shows blood vessels surrounded by melanin, an increase in the number of specialized cells, lamellae, and cartilage differentiation, (C, D) A frontal section shows the beginning of the false gills.

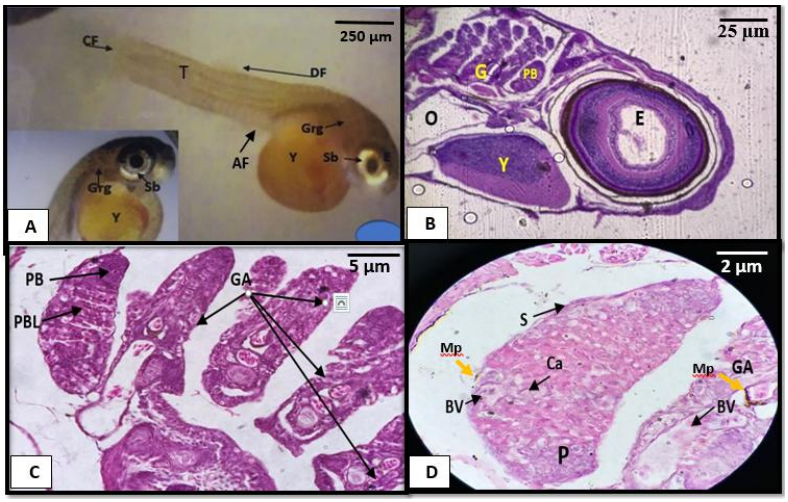

Plate (8): (A) A frontal section of an embryo, 6.5 mm in length, showing the beginning of the PB, (B, C, D) A frontal section showing the beginning of the PB, surrounded by connective tissue from the top on the side of the gill cover and the bottom opposite the first-gill arch showing blood vessels and specialized cells clearer with the formation of lamellae, and the differentiation of cartilage. H & E.

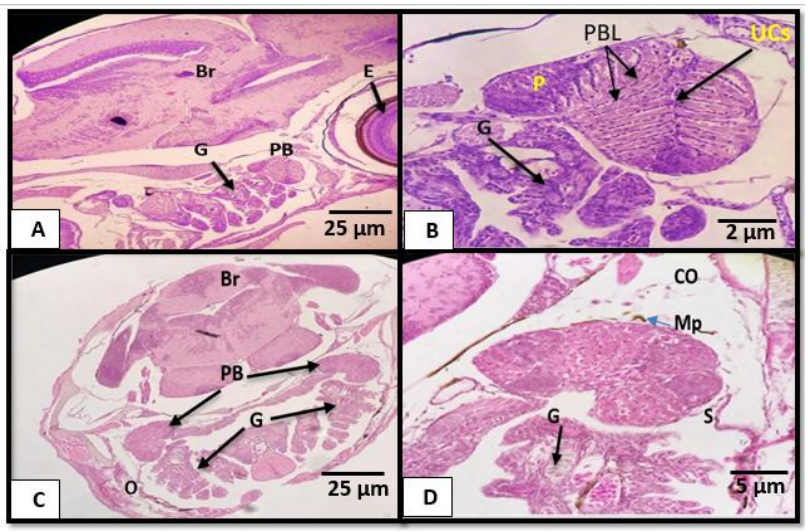

Plate (9): (A) A cross-section of an embryo 6.75 mm in length showing the PB, (B) A cross-section showing the PB, surrounded by connective tissue and an increase in thickness from the bottom opposite the first-gill arch showing blood vessels and specialized cells are clearer with the formation of lamellae, and differentiation of cartilage, (C, D) A frontal section showing the PB, specialized cells more visible with lamellae let formation, and cartilage differentiation.

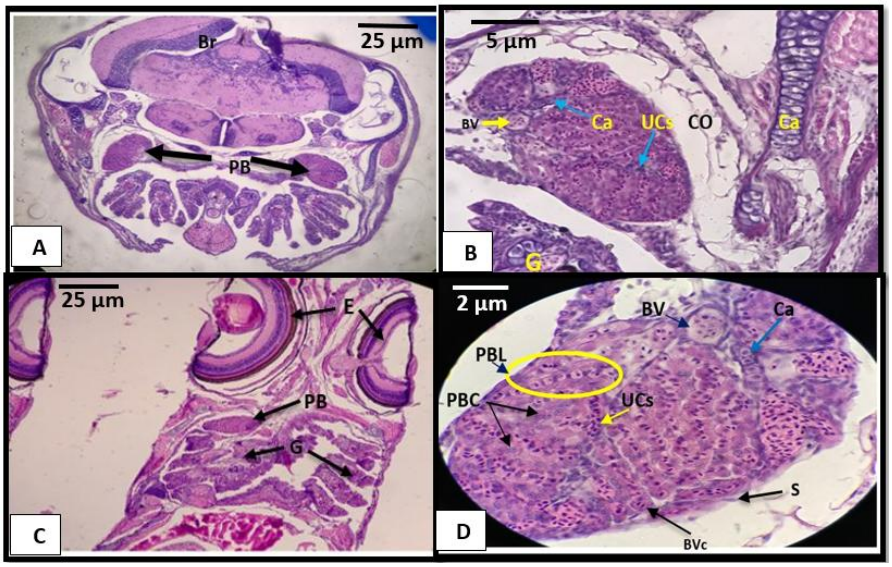

Plate (10): (A) Embryo of 7.75 mm in length, (B, C, D) A frontal section showing the beginning of the PB, surrounded by connective tissue from the top on the side of the gill cover and an increase in thickness from the bottom opposite the first gill arch showing part of melanin, specialized cells clearer with the formation of lamellae, and the differentiation of cartilage.

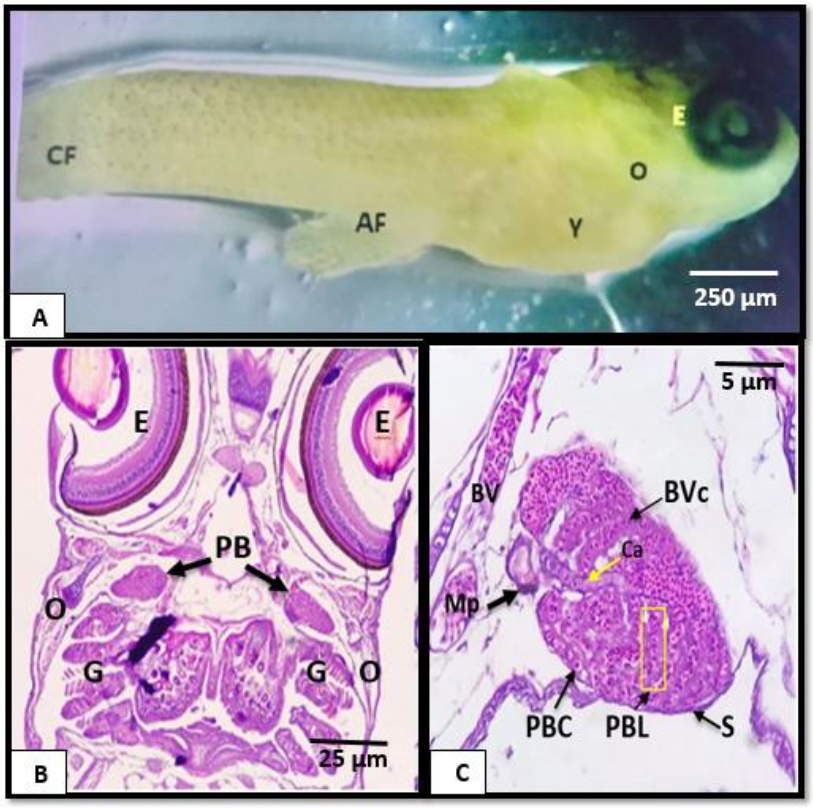

Plate (11): (A) A photograph representing a visual newborn (120X), (B, C) A Frontal section of a one-day-old newborn showing the beginning of the Pseudobranch, surrounded by connective tissue from the top on the side of the gill cover, and its thickness increasing from the bottom opposite the first gill arch showing part of melanin, Specialized cells are more evident with Lamellae formation, (D, E) Cartilage differentiation.

CONCLUSIONS

The PB in the Eastern mosquito fish embryo is located in the cranial region on both sides of the head, between the respiratory gills and the eyes. By examining of different histological sections from 2-4 mm embryos, revealed the formation of the primordial PB behind the eyes within the tissue of the operculum in front of the gills region. The cells aggregate to form an oval cell mass, surrounded by a row of squamous cells, along with the formation of cartilage, blood vessels and the early development of its lamellae. In 5.25-8 mm embryos, differentiation of PBC, an increase in the number of its lamellae, PBC, and the initial appearance of melanin around the blood vessels were noted. Moreover, the PB was surrounded from both the back and front by rows of squamous cells. At this stage the lamellae attached to the cartilage became arranged in parallel, leading to an increased concentration of melanin around the blood vessels in addition to its concentration in the outer part. In

newborns increased cellular differentiation was observed, resembling that of adult fish. As a result, the current study highlights the histological structure of the pseudobranchia in the embryo of mosquito fish. Further functional and molecular investigations are needed to confirm its potential role in the immune system.

CONFLICT OF INTERNESTS STATEMENT

"The authors declare there is no conflict of interests related to this work."

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التكوين الجنيني للغلاصم الكاذبة في سمكة البعوض *Gambusia holbrooki* Girard, 1859 (Cyprinodontiformes, Poeciliidae) في بحيرة الجادرية بغداد، العراق

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الخلاصة

الهدف من البحث الحالي هو دراسة التكوين الجنيني للغلاصم الكاذبة في أسماك البعوض الشرقي (*Gambusia holbrooki* Girard, 1859)، حيث أوضحت المقاطع النسيجية للغلاصم الكاذبة للأجنة بطول 4-2 ملم بأنها ظهرت بهيئة كتلة بيضوية محاطة بصف من الخلايا الحرشفية في الغطاء الغلصمي، بالإضافة إلى تكوين الغضاريف والأوعية الدموية وبداية تكوين الصفائح الغلصمية الكاذبة. اما في الاجنة التي يبلغ طولها 5- 25, 8ملم، فقد ظهر ازدياد في كتلة الخلايا والصفائح الغلصمية الكاذبة فضلا عن زيادة في ظهور الميلانين حول الأوعية الدموية، كما ظهر صف الخلايا الحرشفية بأنه يطوق الارتفاع في كتلة الخلايا من الأمام والخلف. كما يؤدي الترتيب المتوازي للصفائح المرتبطة بالغضاريف في هذه المرحلة إلى زيادة تركيز الميلانين حول الأوعية الدموية، وفي الأجنة حديثة الولادة تتزايد الخلايا المتمايزة على غرار ما يظهر في الأسماك البالغة.