



## Histological Structures of the Pseudobranch in Adult Mosquito Fish (*Gambusia holbrooki* Girard 1859)

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### Abstract

This paper deals with the histological structure of the pseudobranch of mosquito fish (*Gambusia holbrooki*), which lives in an Iraqi environment. The laboratory placed the adult fish, both male and female, in a fishbowl. The head is fixed in solution Bouin's aqueous; using the routine method that is colored with H&E stains. The microscopic slide observations show the pseudobranch in the adult is located in the cranial region on both sides of the head, It is a submerged type. The fish features some oval parts that are connected to each other by a thick connective tissue, forming a triangular cavity in the middle, and also features an irregular dashed line of melanophore. It bears resemblance to the respiratory gills, but differs from them by losing the gill arches and their appendices, as well as by the arrangement of their components and function. This difference explains their connection to the ocular placenta and its oxygenated blood supply.

**Keywords:** Histological, Pseudobranch, mosquito fish (*Gambusia holbrooki*).

### 1. Introduction

In this research, we dealt with the study of the histological structure of the pseudo-gills, which have other names such as (pseudobranch, rudimentary gill, opercula, or hyoid hemibranchs); because they're supplied with oxygenated blood from the vessels of the first gill arch, and it does not contribute to the breathing process [1]. Furthermore, their structure bore a striking resemblance to that of respiratory gills, leading to their naming [2]. Pseudobranchs, which distinguish bony fishes, are absent in some fish species, including eels and similar species, catfishes (Siluroidae), and featherbacks (Notopteridae) [3]. They are also missing in some non-bony fish, such as cyclostomes. Holocephali and Dipnoi, [4]. Elasmobranchs, also known as spiracular pseudobranchs [5], have similar structures but are much smaller, located within the respiratory orifice. These structures are part of the maxillary arch located in the cranial region on both sides, in the basal part of the inner operculum [5-10]. The composition of the pseudobranch forms varies; for example, some types have separate filaments and lamellae, known as the free type; others have free filaments but joined lamellae, known as the covered type, or semi-free [11]. A thin connective



tissue surrounds the filaments and lamellae in one of the two simple types of embedded pseudobranch, known as the submerged type, simple form [12]. It can become more complex when dense connective tissue surrounds it, leading to the term "submerged type." a more complex form [2].

The structure and cellular components of the epithelium in the pseudobranch vary depending on the species of fish. This distinguishes two types of epithelium with respect to their position in relation to the external medium [5]. Many mucous pseudocytes and taste cells may be present in the epithelium, either absent or replaced by pseudocysts. The differences between pseudocysts and chloride cells relate to mitochondria, tubules, apical vesicles, apical fossa, innervation, and vascular compartment [2, 4].

## 2. Materials and methods

Adult fish (male, female) were collected from two sources: the spinning market in Baghdad and from the schedules of the University of Baghdad in Jadiriya. The laboratory placed the fish in ponds for fish farming. The fish were drugged by inserting the needle into the dorsal side of the back of the head, then placed on a plate Wax and beheaded behind the area of the Operculum. Bouin's aqueous solution completely fixes the head in place. The routine method is then used to wash the blocks, and a microtome rotary at 325 HM was used to cut the sections into transverse and longitudinal (sagittal arrow and frontal) sections with a thickness of 5 mm. The next step involved coloring the Eocene with H&E [13].

## 3. Results

The results of the current study showed that the pseudobranch in the adult mosquito fish is located between the respiratory gills and the eye in the cranial region on both sides of the head. That's a submerged type, more complex form. The lamellae and filaments are fused, covered with a layer of connective tissue thick, embedded under the lining of the inner ventral basal part of the glandular cover, and extended along its bottom, so it could not be studied visually (**Figure 1**). It attaches from the front to the first gill arch, a crucial location for supplying the placenta with the oxygenated blood required for nourishment.

The mosquito fish has some oval parts connected to each other by thick connective tissue, forming a triangular cavity in the middle that looks like a grape leaf, as shown in **Figure 1**.

The sections also display an irregular dashed line of black melanophore, indicating collections of melanophore, as depicted in **Figure 1-Figure 7**.

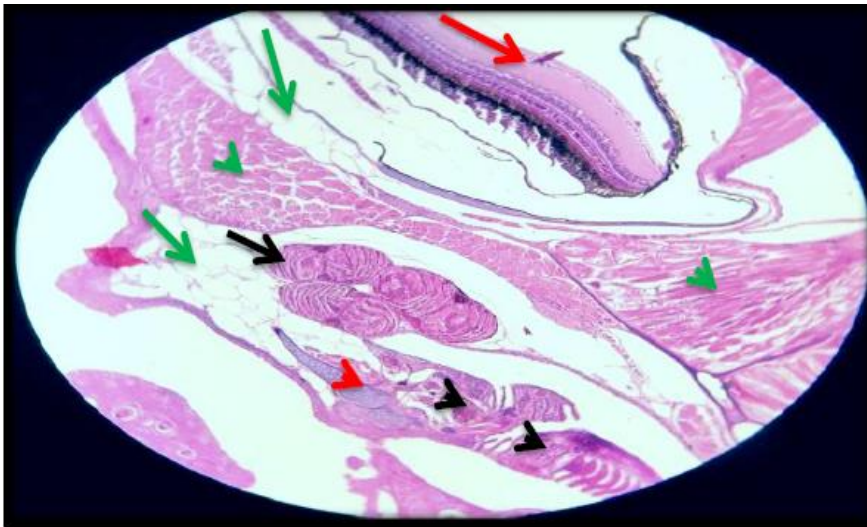
In general, the pseudobranch is similar to respiratory gills in terms of the presence of filaments, secondary lamellae, and blood vessels, but they differ from them by the loss of the gill arches and their appendices, as well as the arrangement of their components and their function.

There are several rows of thin plates in the pseudobranch that are arranged in a way that makes them look like feathers (**Figures 5- Figure 10 and Figure 12**). These plates are joined together along their length by a thin layer of connective tissue, and the filaments are all surrounded by a dense connective membrane (**Figure 11 and Figure 12**) that is held up by a parabrachial cartilaginous rod (**Figure 9 and Figure 10**).

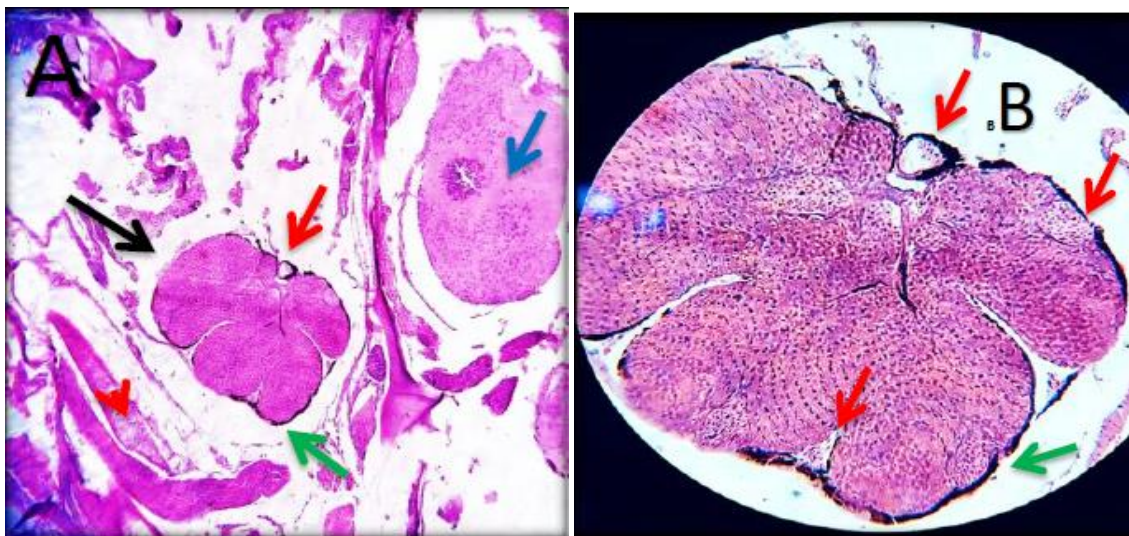
There were goblet cells and taste buds on the non-keratinized stratified squamous epithelium on the connective tissue (**Figures 11 and Figure 12**). The superficial surface of the epithelium contains goblet cells, while alarm cells have existed in the middle to deeper layers of the epithelium covered.

From the first gill arch artery, an arterial vascular apparatus comes into the pseudobranch and spreads out as a capillary apparatus in the middle of the filaments. This apparatus then branches between the lamellae. The efferent and afferent hyphae arteries, as well as the lamellar blood flow through filaments and secondary lamellae, make up a structure that looks like a network. Large secretory cells called pseudobranchial cells surround it. The space between the lamellae is frequently filled with lacuna tissue. Fatty tissue encircles the pseudobranchs; epithelial tissue contains numerous mucous and squamous cells; a layer of loose connective tissue forms beneath it. (Figure 4- Figure8).

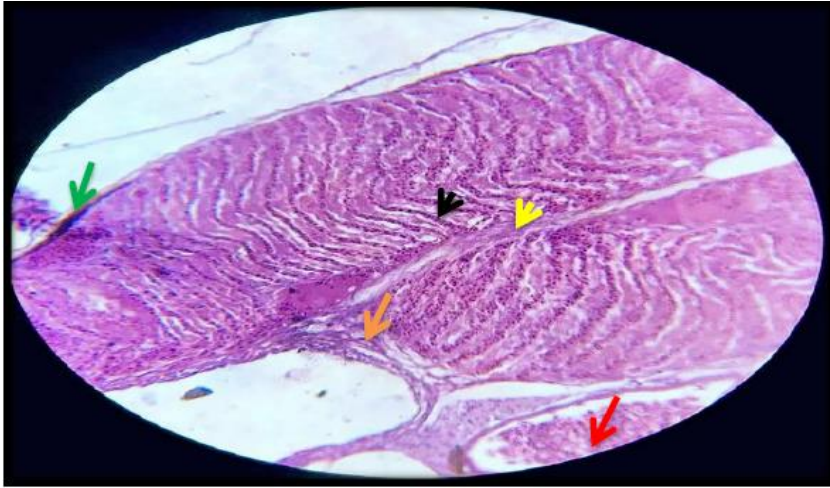
Observations revealed the presence of pillar cells, visceral cells, glandular pseudocytes, blood cells in blood vessels, and epithelial cells within the pseudobranch structure. The choroid vessels, composed of numerous capillaries interspersed with rows of cells resembling fibroblasts, directly connect to the pseudobranch vessels (Figure 1).



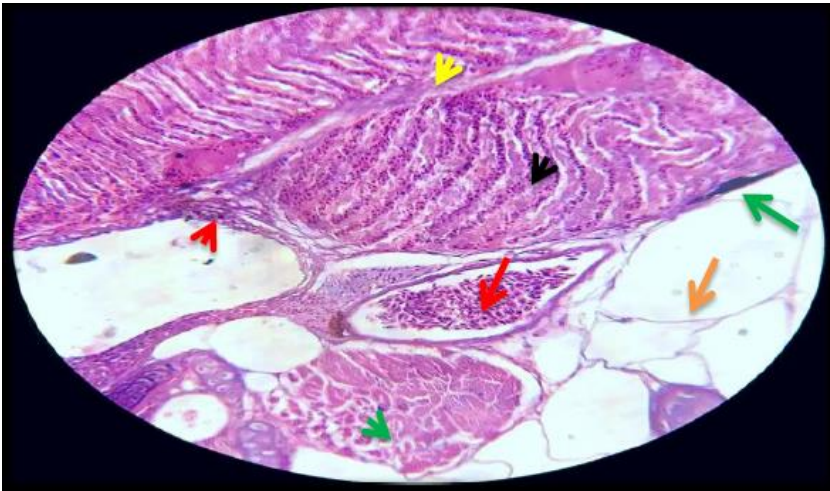
**Figure 1.** A photograph showing the histological sections of pseudobranch in mosquito fish (black arrow), gill (black head arrow), operculum (red head arrow), eye (red arrow), muscle (green head arrow), adipose tissue (green arrow), longitudinal section (10x).



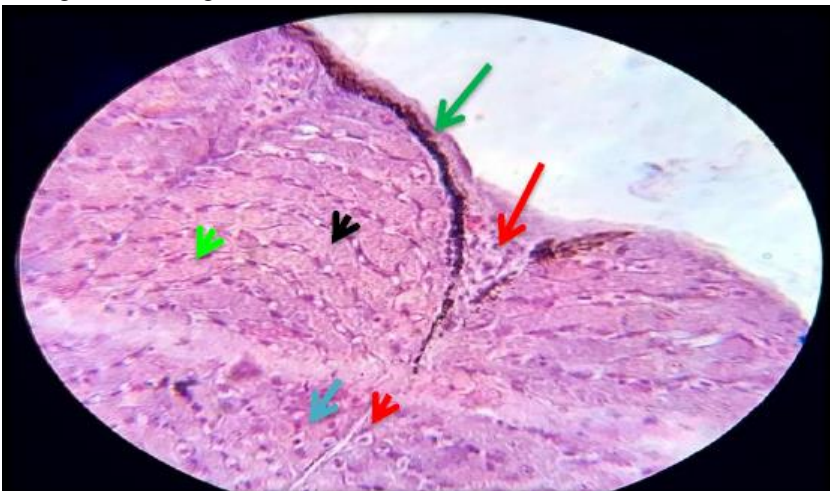
**Figure 2.** A photograph showing the histological sections of pseudobranch in mosquito fish (black arrow), blood vessels (red arrow), the sections also show the presence of an irregular dashed line of black melanophore (green arrow), operculum (red head arrow), part of brain (blue arrow) cross section (A, 40X, B, 10x).



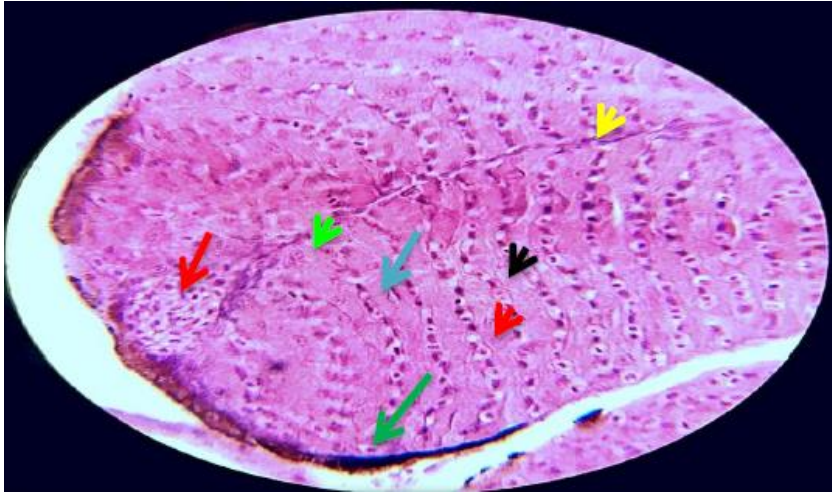
**Figure 3.** A photograph showing the histological sections of pseudobranch in mosquito fish in which the filaments are clearly visible with the lamella (black head arrow), and blood vessels (red arrow), connective tissue (orange arrow), the outer borders are shown surrounded by a dashed black line composed of melanin cells (green arrow), undifferentiated cells (yellow head arrow), connective tissue (orange arrow) longitudinal section 40x.



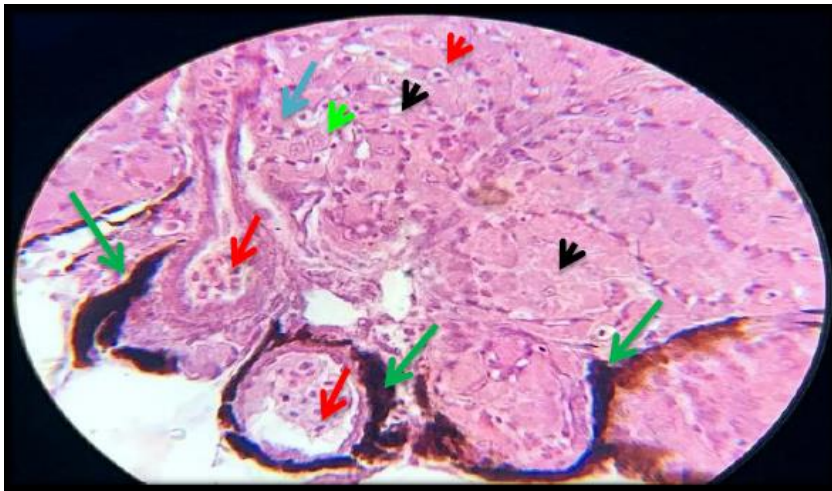
**Figure 4.** A photograph showing the histological sections of pseudobranch in mosquito fish in which the filaments are clearly visible with the lamella (black head arrow), blood vessels (red arrow), and the outer borders are shown surrounded by a dashed black line composed of melanin cells (green arrow), outer layer of simple squamous epithelial tissue containing goblet mucosa cells (red head arrow), undifferentiated cells (yellow head arrow), connective tissue (orange arrow) longitudinal section 40x.



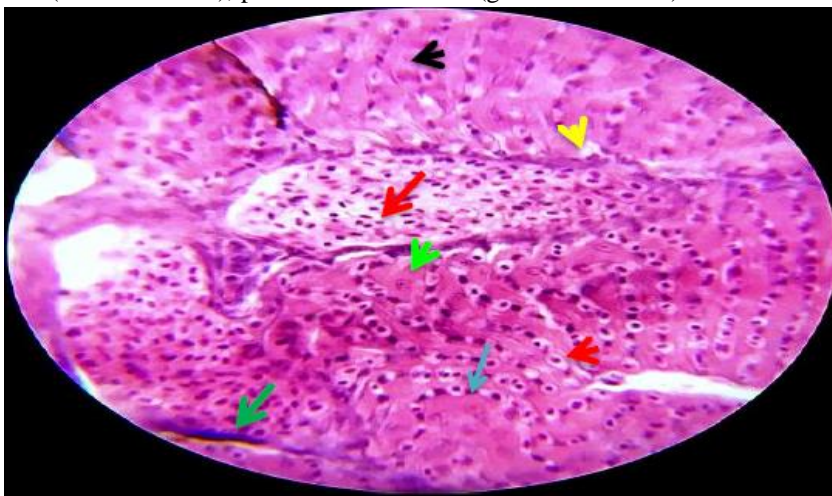
**Figure 5.** A photograph showing the histological sections of pseudobranch in mosquito fish in which the filaments are clearly visible with the lamella (black head arrow), and the outer borders are shown surrounded by a dashed black line composed of melanin cells (green arrow), blood vessels (red arrow), pillar cell (blue arrow), blood capillaries cell (red head arrow), pseudobranchial cells (green head arrow) cross section 40x.



**Figure 6.** A photograph showing the histological sections of pseudobranch in mosquito fish in which the filaments are clearly visible with the lamella (black head arrow), blood vessels (red arrow), and the outer borders are shown surrounded by a dashed black line composed of melanin cells (green arrow), undifferentiated cells (yellow head arrow), pillar cell (blue arrow), blood capillaries cell (red head arrow), pseudobranchial cells (green head arrow) cross section 40x.



**Figure 7.** A photograph showing the histological sections of pseudobranch in mosquito fish in which the filaments are clearly visible with the lamella (black head arrow) and blood vessels (red arrow), and the outer borders are shown surrounded by a dashed black line composed of melanin cells (green arrow), pillar cell (blue arrow), blood capillaries cell (red head arrow), pseudobranchial cells (green head arrow) cross section 40x.

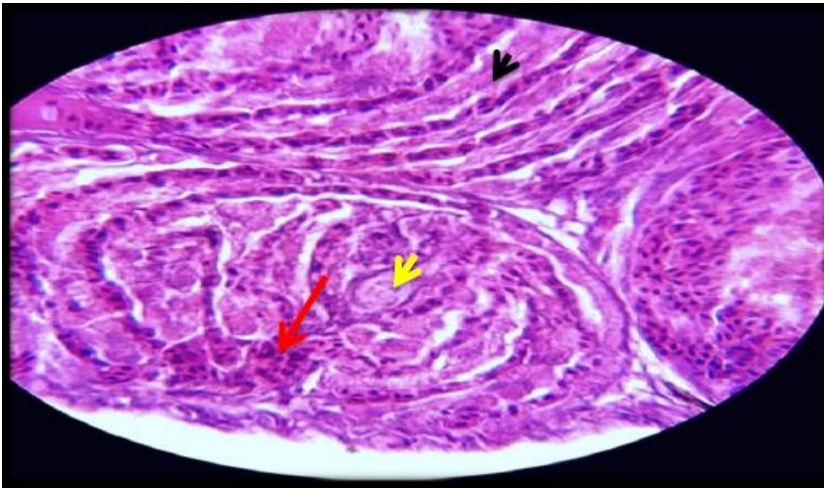


**Figure 8.** A photograph showing the histological sections of pseudobranch in mosquito fish in which the filaments are clearly visible with the lamella (black head arrow) and blood vessels (red arrow), and shown a dashed black line

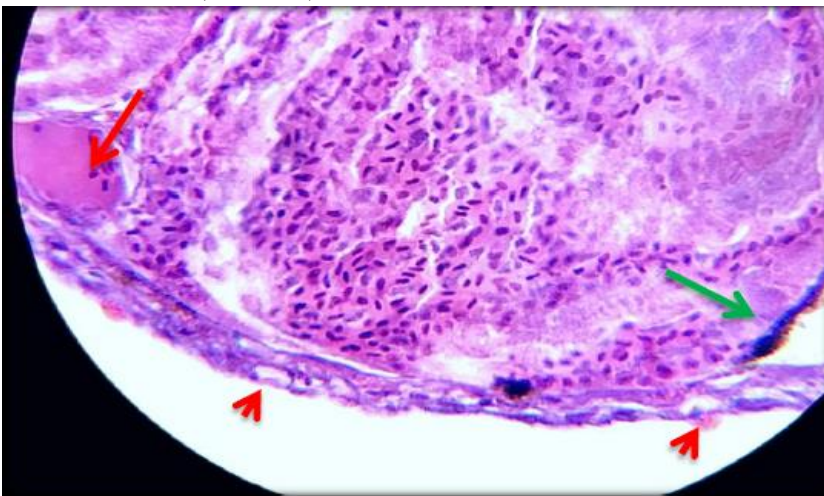
composed of melanin cells (green arrow), undifferentiated cells (yellow head arrow), pillar cell (blue arrow), blood capillaries cell (red head arrow), pseudobranchial cells (green head arrow) cross section 40x.



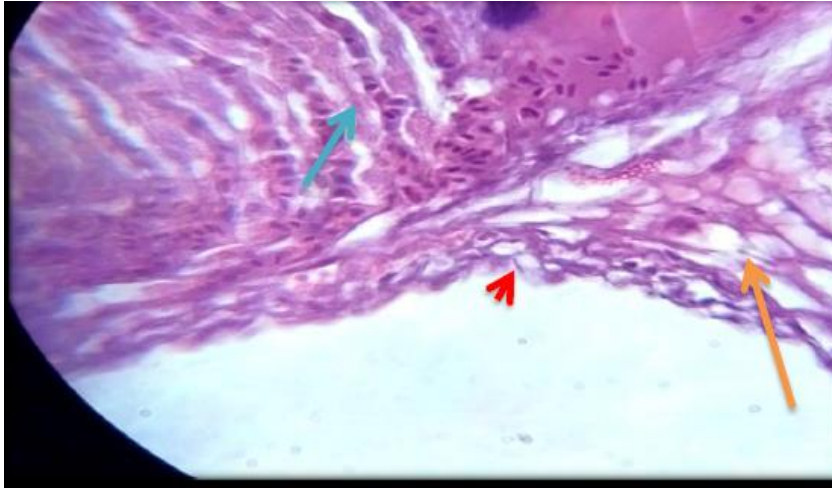
**Figure 9.** A photograph showing the histological sections of pseudobranch in mosquito fish and a thin bone plate (yellow arrow), undifferentiated cells (yellow head arrow), pillar cell (blue arrow), blood capillaries cell (red head arrow), pseudobranchial cells (green head arrow) longitudinal section 40x.



**Figure 10.** A photograph showing the histological sections of pseudobranch in mosquito fish in which the filaments are clearly visible with the lamella (black head arrow), The cartilage a weak structure appears. (yellow head arrow) and blood vessels (red arrow) cross section 40x.



**Figure 11.** A photograph showing the histological sections of pseudobranch in mosquito fish shows the tissue surrounding the pseudo-glands consisting of loose connective tissue and an outer layer of simple squamous epithelial tissue containing goblet mucosa cells (green arrow), blood vessels (red arrow), and an outer layer of simple squamous epithelial tissue containing goblet mucosa cells (red head arrow) cross section 40x.



**Figure 12.** A photograph showing the histological sections of pseudobranch in mosquito fish representing sections shows the tissue surrounding the pseudo-glands consisting of loose connective tissue and an outer layer of simple squamous epithelial tissue containing goblet mucosa cells (red head arrow), pillar cell (blue arrow), connective tissue (orange arrow) longitudinal section 100X

#### 4. Discussion

The results of the current study showed that the pseudobranch in the adult mosquito fish is located between the respiratory gills and the eye in the cranial region on both sides of the head. That's a submerged type, more complex form; The lamellae and filaments are fused and covered with a layer of connective tissue thick, embedded under the lining of the inner ventral basal part of the glandular cover, and extended along its bottom, so it could not be studied visually (**Figures 1 and Figure 2**). It is attached from the front to the first gill arch, and this location is important for supplying the placenta with the oxygenated blood needed to nourish it; This result is similar to the study [2, 6-9, 14].

The mosquito fish has some oval parts connected to each other by a thick connective tissue, forming in the middle a triangular cavity that looks like a grape leaf, as in **Figures 1 and 2**. This is similar to the pseudobranch composition in *Pelvicachromis pulcher* fish, according to a study [14], and euryhaline fish such as minnows, rainbow trout, and carp; In several marine fish, such as the gadoid *Trisopterus minutus*, the lamellae and filaments of the codfish pseudobranch are fused and coated with a layer of connective tissue [2], as it showed similar results to the results of the current study. But the pseudobranch of the euryhaline milkfish includes a pair of gill-like structures located near the first gill arch, which contains a row of parallel filaments with free lamellae attached to the epithelium of the opercular membrane just behind the eyes [11, 15]. In hilsa (*Tenualosa ilisha*), a pseudobranch comprises a row of parallel filaments bearing numerous leaf-like lamellae arranged on both sides throughout its length [11, 16].

The sections also show the presence of an irregular dashed line of black melanophore, which are collections of melanophore, as in **Figure 1- Figure 7**. To the extent of the researcher's knowledge, there is no study that has clarified these structures, so the importance of the presence of these groups in the pseudobranch may be important for the release of a metabolite that helps in the expansion and extension of the capillary vessels [17]. Also, it is believed that melanin is a binding material for oxygen [18], and from my point of view as a researcher, both of these beliefs are accepted for the presence of these structures in the pseudobranch; in addition to that, because they provide the eyes with oxygenated blood, the carriers of melanin appear in a greater quantity than what is found in the respiratory gills studied by [19, 20].

The pseudobranch are similar in general to respiratory gills in terms of the presence of filaments, secondary lamellae, and blood vessels, but they differ from them by their loss of the gill arches and their appendices as well as the arrangement of their components and their function [20].

The filaments in the pseudobranch consist of several rows of thin plates arranged in the form of parallel rows that appear similar to the feathery shape, and are fused along their length by a thin connective tissue, while the filaments are all surrounded by a dense connective membrane (**Figure 1- Figure 3**) and (**Figure10-Figure12**), that supported by pseudobranchial cartilaginous rod (**Figure 9 and Figure10**) [5-10, 20]. The goblet cells, and taste buds were seen in the non-keratinized stratified squamous epithelium that situated on the connective tissue (**Figure 11 and Figure12**) The superficial surface of epithelium contained goblet cells while alarm cells were existed in middle to deeper layers of epithelium covered.

The pseudobranch is provided with an arterial vascular apparatus derived from the first gill arch artery, as it enters into it and is distributed as a capillary apparatus located in the center of the filaments, which in turn branches between the lamellae. The efferent and afferent hyphae arteries and the lamellar blood transfusion with filaments and secondary lamellae form a network-like structure surrounded by large secretory cells called pseudobranchial cells. As for the space between the lamellae, it is often filled with the lacunae tissue. The pseudobranch is surrounded by fatty tissue, epithelial tissue has many mucous cells, squamous cells and below it is a layer formed by loose connective tissue. This result is similar to other studies [2, 9] but it's different with presence the taste-buds.

Along the parallel lamellae located the spherical pseudobranchial cells (PSCs) beneath the pavement cells (PVC) and were associated with the blood compartments separated by pillar cells. Between the PVC and PSCs was an empty space that was not observed in the gills [15, 20]. The pseudobranchial cells (PSCs) have clear cytoplasm with abundant acidophilic granules and a large euchromatic nucleus in center of cytoplasm and a prominent nucleolus [9].

As for the cells in the structure of the pseudobranch, it was observed that there were pillar cells, visceral cells, glandular pseudocytes, blood cells in blood vessels, and epithelial cells. The pseudobranch vessels connect directly with the choroid vessels, which consist of a large number of capillaries interchanged with rows of fibroblast-like cells. Some fish lack the presence of the placental reticulum, and the loss of pseudobranch has been observed in these fish, such as some Siluridae, Ictaluridae, Notopteridae, Cobitidae, Anguillidae, etc. This supports the pseudobranch relationship to supplying the retina with oxygenated blood, but its function has not yet been fully determined [14, 20]. When the pseudobranch is removed, the fish becomes dark, so the pseudobranch appears to be involved in the control of chromatophores in the skin. This may occur through the choroid gland of the eye, which receives blood from the pseudobranch and degenerates after it is removed.

## 5. Conclusions

The pseudobranch in the adult mosquito fish *Gambusia holbrooki* is located in the cranial region on both sides of the head, between the respiratory gills and the eye. It is Submerged type more complex form covered with Layer of connective tissue thick. The mosquito fish has three oval parts connected to each other by a thick connective tissue, forming in the middle a triangular cavity, they are also have an irregular dashed line of black melanophore. It was found that the pseudobranch in the mosquito fish are generally similar to the respiratory gills in terms of the presence of strings, lamellae and blood vessels, but they differ from it by losing the gill arches and their appendices as well as the arrangement of their components and function, it was found that



they contain high quantities of melanin carriers; this explains their connection to the ocular placenta and its supply of oxygenated blood.

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### Conflict of Interest

There is no conflict of interest.

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