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THE COMPLEXITY OF GILL RAKERS EPITHELIA IN *GOBIO CARPATHICUS* VLADYKOV, 1925 – A MORPHOLOGICAL AND HISTOCHEMICAL DESCRIPTION

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Abstract

Gill rakers represent the inner surface of the gill arch, composed of rows of stiff strainers that serve to sort and position large food particles in the oesophagus. The histological structure reveals a central axe composed of cartilage, covered by an epithelium. However, due to their complex role combined with their anatomical disposition, the gill rakers epithelia displays a high histological complexity. Accordingly, the aim of the present study was to provide a throughout morphological and histochemical description of the gill rakers epithelia harvested from common omnivorous fish. Paired gills were harvested from Carpathian gudgeon fish Gobio carpathicus Vladykov, 1925 and fixed in 10% buffered formalin. The samples were processed with the paraffin embedding technique and subsequently stained with Goldner's trichrome method, PAS, Alcian Blue and combined PAS-AA techniques. The obtained results show that the gill rakers are covered by a stratified epithelium composed of several layers of cells, including basal cells and mucus-producing cells, together with taste buds. The histochemical assessment revealed mucous cells able to produce complex mucins, that are both PAS and AA- positive. However, even if the gill rakers are part of the gills, the covering epithelium reveals some features similar to the described structure of the anterior pharynx, but also the gill rakers epithelia display common features with the epidermal structure.

Key words*: fish epithelia, gills, histochemical reaction, mucus-producing cells.*

INTRODUCTION

Gobio carpaticus Vladykov, 1925, popularly called Carpathian gudgeon, from the Cypriniformes order, Gobionidae family is a common omnivorous fish found in the Danube drainage and in the Tisza catchment (Bănărescu, 1964). According to more recent molecular data, it seems that the specimens found in Mures and Tisza rivers may represent a distinct lineage (Kottelat and Freyhof, 2007), that may be at risk because of invasive species like the racer goby (*Babka gymnotrachelus* (Kessler, 1857)) or monkey goby (*Neogobius fluviatilis* (Pallas, 1814)) (Muntean et al., 2022).

The feeding mechanism of this common omnivorous fish implies several structures, the gills being one of the first that get in contact with the ingested food (Genten et al., 2009; Mokhtar, 2021). Gill rakers represent the inner surface of the gill arch, composed of rows of stiff strainers that serve to sort and position large food particles in the oesophagus and then in the stomach and intestines, being a good indicator for the fish diet (Genten et al., 2009).

The histological findings describe the gill raker as a dense core (cartilage or bone) which is covered by connective tissue and a stratified epithelium, similar to the one from the pharynx (Genten et al., 2009; Mokhtar, 2021).

This stratified epithelium found in the teleost gill raker is very different from the pharyngeal epithelium of mammals, which makes it almost unique and histologically diverse. It presents common features with the teleost skin epithelia due to their common capacity to secrete mucus (Genten et al., 2009). Its feature is extremely important, since it is involved in several functions like immune defence, lubrication of the ingested particles or the pre-gastric chemical digestion of foods (Genten et al., 2009; Tibbetts, 1997). Because the aquatic environment is rich in pathogenic organisms and substances, the capacity of the mucous cells to produce a secretion rich in lysozymes, antibacterial peptides, IgM, lectins or pentraxins is a great

way for a primary biodefence (Jung et al., 2002; Genten et al., 2009).
Accordingly, the histological complexity of the secretory epithelium from the rakers, corroborated with the rich function of the mucus shaped the aim of the present study that provide a thoroughly histological and histochemical description of the gill raker epithelia in Carpathian gudgeon.

MATERIALS AND METHODS

Paired gills were harvested from the Carpathian gudgeons (*Gobio carpathicus* Vladykov, 1925) caught in natural waters (Mureș reservoir, Romania), during recreational fishing. The samples were fixed with 10% buffered formalin for 5 days and subsequently demineralised with trichloroacetic acid 5% for 7 days. Following, the gills were processed with the usual paraffin embedding technique and sectioned at 5 µm thickness. The mounted sections were stained with Goldner's trichrome staining method (to underline the histological morphology of the gill rakers) and with PAS, Alcian Blue (pH 2.5) and combined PAS-AA techniques (to highlight the mucin-producing cells) (Suvarna et al., 2019). The microscopical assessment was achieved with an Olympus BX41 microscope equipped with a Olympus SC 180 digital camera (Olympus, Japan).

RESULTS AND DISCUSSIONS

The gill arch inner surface of the Carpathian gudgeon presented several comb-like structures, relatively short and thick, termed gill rakers (Figure 1). From a histological perspective, each raker presents a dense and hard core formed by hyaline cartilage, covered by a stratified epithelium and abundant connective tissue in between (*lamina propria*). However, the microscopic morphology of the epithelium displays differences at the level of the rakers when compared with the inter-raker region of the gill arch (Figure 1 A), the latter presenting a slightly lower density of mucus-producing cells and a slightly higher size in thickness. Despite the region addressed, the covering epithelium at the level of the gill arch was sustained by a lamina propria composed of both fibroblasts and fibrocytes, together with connective fibres. Therefore, the common aspect of the gill raker epithelium frames it in the buccopharyngeal epithelium type for the teleost fish (Walker et al., 1981; Tibbetts, 1997). Accordingly, several layers of cells, displayed on a very thick, mild wavy basal membrane can be observed. Moreover, taste buds are identified as intra-epithelial sensorial structures, with an onion-like aspect, usually on the apical part of the raker (Figure 1B).
From a histochemical perspective, both PAS reaction and Alcian blue staining highlighted mucus secreting cells. Few of them seem to be present in the intermediate part of the multi-layered epithelium but the cells appear to be displayed predominantly on the last epithelial layer, releasing their secretion product at the surface, forming a mucous coat at the apical part of the epithelium. However, on both histochemical stainings can be observed that the mucous coat is discontinuous, being absent (or very thin) at the level of the intra-epithelial taste-buds (Figure 1 C, D).
The histological architectonics of the epithelium in the case of Carpathian gudgeons was similar with the previously reported findings in fathead minnow (*Pimephales promelas*)(Walker et al., 1981) and other teleosts fish (Tibbetts, 1997; Vigliano et al., 2006; Bassuoni et al., 2021; Grau et al., 1992).
The high-magnification light microscopy revealed a few differences in terms of cytoplasmic details of the mucous cells. Accordingly, on all three histochemical stainings it was observed that at the level of the raker epithelium, the cells display a foamy aspect of the cytoplasm, due to the vacuoles with a small diameter (Figure 2 A, D, G). Nevertheless, in the inter-raker region, the mucous cells tend to be more spherical containing a cytoplasm with a vacuolated aspect (Figure 2 C, F, I). When it comes to mucous cells in teleost fish epithelia, an applied classification was made available. Therefore, the cells in the pharyngeal mucosa, together with the ones from the oral, oesophageal and rectal mucosae, are considered to be type-A saccular cells with rounded vacuoles and flattened nuclei disposed at the basal pole. The type-B cells represent the more known Goblet cells that are representative of the intestinal tract mucosa. They may display a PAS positive reaction, Alcian-blue positive

stain or both (Tibbetts, 1997). In the pharyngeal region, they are present in a stratified epithelium that displays a slightly similar morphology (to a certain extent) with the transitional stratified epithelium, being placed themselves in several layers (Tibbetts, 1997). The same pattern was observed also in the gill arch epithelia of the present study, where the mucus-producing cells were arranged in both apical and intermediate thirds of the epithelium.

In terms of tinctorial affinity, the mucous cells keep the same pattern in the two described regions. On the other hand, the mucus granules display a higher PAS positive reaction compared with the Alcian blue stain, suggesting that the biochemical componence may be predominantly composed of neutral glycoproteins, instead of acidic mucins (Tibbetts, 1997). It also should be mentioned that the decalcification step does not affect significantly the tinctorial affinity for those histochemical reactions (Gona, 1979). However, the positivity obtained using the combined staining method suggests a mixture of mucins in the cytoplasm (Figure 2 G, H, I).

It is known that the gill rakers may act like a sieve (Genten et al., 2009; Mokhtar, 2021). Considering this feature, they are in constant contact with water and particles that are coming from the environment, so the high amount of mucus here may have a multipurpose function. On the first hand, the mucus coat may act as a lubricant for food particles, facilitating an easy movement towards the pharyngeal cavity, but also in the pregastric digestion (Bassuoni et al., 2021; Tibbetts, 1997).

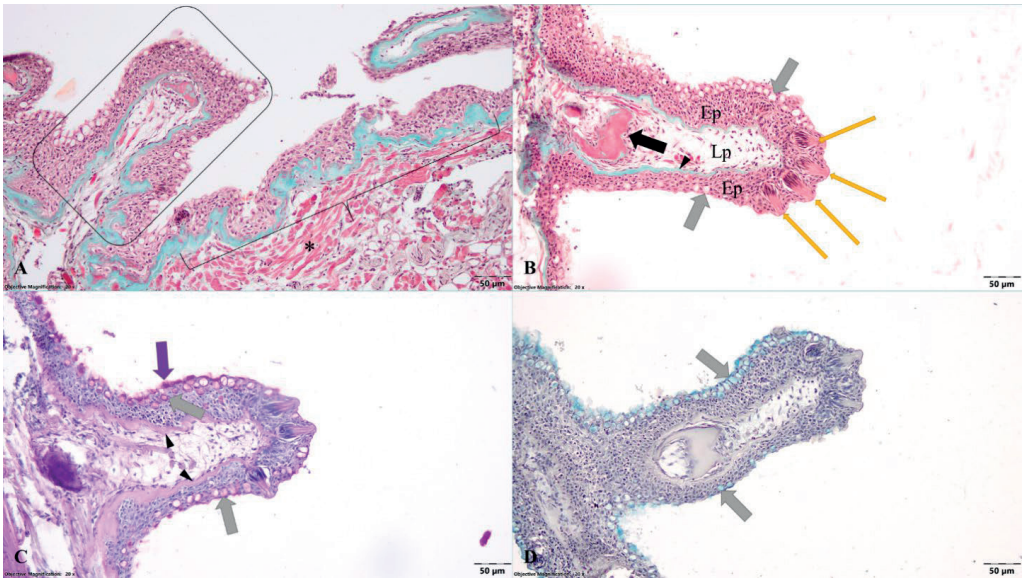

Figure 1. Gill arch of Carpathian gudgeon fish *Gobio carpathicus* Vladykov, 1925; A - Pharyngeal region of the gill arch presenting comb-like projections termed gill rakers (black square) and the inter-raker region (accolade), together with the underneath muscle tissue (*) - Goldner's trichrome stain; B - One gill raker formed by a mineralized tissue that serves as sustaining core (black arrow), covered by a stratified epithelium (Ep) with mucus-producing cells (grey arrows), intraepithelial sensorial structures - taste buds (long yellow arrows), a thick basement membrane (arrowhead) and lamina propria (Lp) - Goldner's trichrome stain; C - PAS positive mucus-secreting cells (grey arrows) in gill raker epithelium which release their secretion at the surface, forming a discontinuous mucous covering layer (purple arrow), PAS positive basement membrane with mild waves (arrowheads) - Periodic acid-Schiff reaction; D - mucous cells with a faintly positive reaction for Alcian Blue stain (grey arrows)

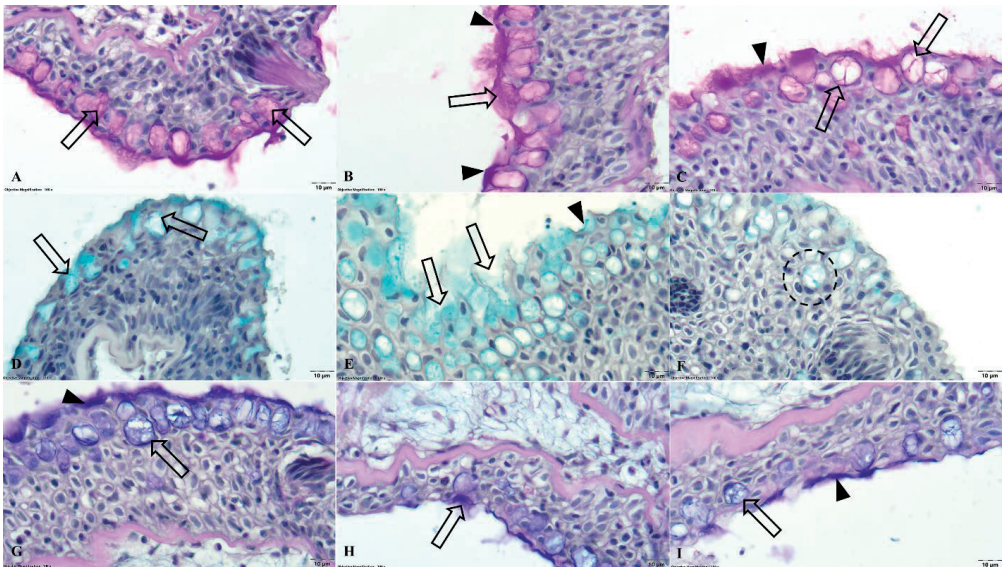

Figure 2 Histochemical details of mucus-producing cells from the gill arch of Carpathian gudgeon fish *Gobio carpathicus* Vladykov, 1925 (A, B, C - PAS stain; D, E, F - Alcian blue stain; G, H, I - PAS-Alcian combined stain; 100x magnification degree); A - Apical tip of the gill raker epithelium, displaying a high density of cells with saccular aspect, basal flattened picnotic nuclei and intracytoplasmic small and abundant vacuoles that give a foamy aspect; the vacuoles contain PAS positive mucus granules (empty arrows); B - PAS positive mucus coat at the surface of the gill raker epithelium (arrowheads) released through the apical pole of the cells (empty arrow); C - gill arch epithelium in the inter-rakers region displaying a slightly different feature of cells shape (more spherical - empty arrows) and composition (intracytoplamatic vacuoles with a bigger diameter that gives the cytoplasm a vacuolated aspect, with PAS positive mucous granules - arrowhead); D - mucus-producing cells faintly positive for Alcian blue stain, from the gill raker epithelium apical region, that present a granular cytoplasm (empty arrows); E - weakly positive mucus coat for Alcian blue stain highlighted at the surface of the gill raker epithelium (arrowhead) and the apparent releasing mechanism from the producing cells (empty arrows); F - Mucus secreting cells from the inter-raker region of the epithelium, presenting a basal picnotic and flattened nucleus and vacuolated cytoplasm faintly positive for Alcian blue stain (dashed circle); G, I - mucus producing cells positive for the double PAS and Alcian blue staining, maintaining the previously described morphology (G - raker epithelium; I - inter-raker region epithelium) (empty arrows) and the mucus coat at the surface of the epithelium (arrowheads); H - a mucous cell presumably intercepted during the mucus release phenomenon (empty arrow)

On the other hand, a thicker or less thicker mucus layer at the surface epithelium may protect it against mechanical abrasions (Bassuoni et al., 2021) and also acts like a chemical barrier, participating in innate or acquired immunity (Alexander et al., 1992; Jung et al., 2002).

Another interesting finding is related with the secretion mechanism of the mucus. In general, mucous cells are secreting their product through the merocrine secretion (Song et al., 2023; Specian et al., 1980). Exceptionally, the mucus can be released by the apocrine pathway, but only in some specialised glands, while the holocrine secretion is not common for mucus-secreting cells (Lodish et al., 2000; Kierszenbaum et al., 2015; Alberts et al., 2015). The latter mentioned mechanism implies that the whole cell undergoes apoptosis, releasing its entire content, this forming the secretion product (Kierszenbaum et al., 2015). In Carpathian gudgeon (but not limited to it), the unicellular glands disposed in the last layer of the epithelium seem to release their mucous product by a slightly different pattern than the common ones. If analysed thoroughly, it can be observed in several cells that the bulging apical pole of mucous cells presents a shattered appearance and the mucus release seems to be the consequence of an “explosion” (outburst) at the cellular surface (Figure 2 B, E). Moreover, those cells display also either karyopyknosis or karyolysis, suggesting features that are specific for the apoptotic cells (Häcker, 2000). However,

further research is needed in order to provide a thorough explanation about the detailed secretion mechanism.

CONCLUSIONS

The gill arch, including the rakers, displays a complex morphologic diversity. In *Gobio carpathicus*, a common omnivorous fish, a stratified epithelium with several secretory cells covers the whole gill arch, with a slightly different morphology in between the rakers and inter-rakers regions. The mucus secretion was released through the apical pole of the type A saccular cells, which are intensely PAS positive and faintly Alcian Blue positive. Thus, taking into consideration all the histochemical and morphological aspects described, the epithelium from the gill rakers is a stratified, mucus secreting structure, with an important involvement in several processes, vital for the fish.

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