

A Comparative Study on the Distribution of Some Cytokeratins in Specific Parts of the Digestive System Epithelium in Three Lower Vertebrate Species

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Abstract

We have studied the distribution of some cytokeratins in the epithelial cells in some parts of the digestive systems in three lower vertebrate species: *Scyliorhinus canicula* (Chondrichthyes), *Sparus aurata* (Osteichthyes), and *Salamandra salamandra* (Amphibia). The aim was to compare the evolution of animal tissues by the presence/absence and distribution extent of the cytokeratins. We have used three types of cytokeratins: cocktail, 7, 20.

The result was positive with cytokeratin cocktail in all studied parts of the three species. This emphasizes the presence of cytokeratin in the digestive epithelium of lower vertebrates.

Keywords - Vertebrates, Chondrichthyes, Osteichthyes, Amphibia, Cytokeratin.

I. INTRODUCTION

Cytokeratins are fibroblasts, which are intermediate filaments of epithelial cell cytoplasm, provide structural support to cellular structures and play a role in various cellular functions (such as differentiation), consisting of polypeptide chains, which help cells resist mechanical stress. The expression of these cytokeratins within the epithelial cells is specific to certain organs or tissues. Thus, it is clinically used to determine cell origin of various human tumors [1].

There are two types (I and II) of cytokeratins: basic or moderate and acidic. Within each category, cytokeratins are numbered by decreasing size, from low molecular weight (LMWCKs) to high molecular weight (HMWCKs). Cytokeratin compounds are usually found in heterogeneous pairs of acidic and basic subunits of similar size [2].

Ck7 of type II is known as Sarcolectin (SCL) which appears particularly in the simple and simple granular epitheliums that line the digestive tract, glands and blood vessels (simple epithelium) and is often associated with ck20.

Ck20 pattern I of primary cellular proteins in mature intestinal cells and calyx cells is found mainly in the mucous membranes of the stomach and intestines [3].

It is often used with ck7 to differentiate between different types of adenomas [4].

Ck7,20 of low molecular weight cytokeratins are generally distributed in the epithelium and tumors [5]. The development of expression of the second type of cytokeratins in the spinal cord of different adult vertebrates was studied using immunohistochemical anti-CK technique. Antimicrobial antibiotic chemistry as the expression of the second cytokeratin pattern was stronger in lower vertebrates, particularly amphibians, than in higher vertebrates [6].

Intermediate protein filaments were isolated from the liver of different vertebrates by electron microscopy, biochemical and immunochemical methods. It has been shown that the method of isolation from rat liver filaments can be applied efficiently to all other groups of vertebrates. The studied intermediate threads also contain the same microscopic form, which cannot be easily distinguished [7].

Keratins are not only complex in terms of their specific expression patterns within a particular animal species but also in their vertebrate evolution, and are excellent molecular and pathological indicators.

Immunological studies of lamprey, shark, trout, and zebra fish (Osteichthyes and Chondrichthyes) have shown that fish have a complex expression system for keratinocytes [8]. The researcher [9] has conducted a chemical/immunological study on the placement of keratins in the skin of fish and amphibians using anti-keratin and confirmed that, in Chondrichthyes and Osteichthyes, the coloration of acid keratins AE1 and base AE3 was positive and strong and the pattern of distribution was uniform in all layers of the skin. In frogs, AE1 was found mainly in the base layers while AE3 dyes the skin completely.

Reference [10] tracked evolutionary-like keratinocytes to lower eukaryotes and confirmed that these proteins evolved to form the intermediate strands that form the skin cell structure of vertebrates. Two types of keratins were identified in skin cells of vertebrates (from fish to humans) at the levels of protein and DNA. The two groups of erythrocytes, I and II, were

co-ordinated during the evolution of vertebrates; indicating the main role played by both keratin types. The researchers [11] have studied ck7 distribution in the *Xenopus* frog and shark, and observed a strong coloration in the following parts: epithelial epithelium of the gastrointestinal tract, mesentery, and some spinal cord cells.

II. MATERIALS AND METHODS

Animal individuals were collected alive in order to ensure that the epithelium was not destroyed. The parts of the digestive system to be studied were fixed in formalin 10%, transferred to ascending concentrations of alcohol and then to xylol and dyed with hematoxylin eosin. The tissue was then prepared for treatment with immunoglobulin, the cytokeratin: cocktail, 7, 20 [13]. The cocktail is AE1: ck 10,15,16,17,18,19, AE31,2,3,4,5,6,7,8.

III. RESULTS AND DISCUSSION

Salamander esophagus in (Fig.1) showed a positive reaction when treated with cytokeratin cocktail while the negative coloration occurred with each of the cytokeratin 7 and 20.

The salamander stomach in (Fig.2) showed positive signs when treated with cytokeratin cocktail and the coloration was clear in the basal pole of the surface cells. The general coloration of the adenoblast cells was negative with cytokeratin 7 and very positive with cytokeratin 20 in the apical pole of the surface cells.

As for the intestine of salamander (Fig.3) it was treated positively with cytokeratin cocktail which coloured the surroundings of epithelial cell and its upper part. The coloration was very mild for the entire epithelial cells in the ck 20 and negative in the cytokeratin 7.

This corresponds to the study of [12] who showed that cytokeratin 7 was present in simple epithelium and usually associated with cytokeratin 20 [4]. Since mucosa of esophagus and intestine of salamander were pseudo stratified, it is normal to have negative cytokeratin coloured with ck 7, but a question comes from the lack of discoloration of the stomach with simple epithelium and cytokeratin 7. We also disagree with the researchers [11], who studied the distribution of ck7 in the *Xenopus* tadpole, and observed strong coloration in the epithelium of the gastrointestinal tract.

Esophagus of *S. aurata* (Fig.4) showed high positive coloration with ck cocktail and good coloration for ck 7, 20.

The epithelial tissue of the *S. aurata* stomach (Fig.5) was well treated with ck cocktail in the basal cell pole only, and the coloration was mild for cytokeratin 7 at the top of the superficial cells and absent in the gland cells. The epithelium was also

positive for ck 20 at the top of the superficial cells and at the base of gland cells with the main cells secreting to the pepsinogen.

The sections in (Fig.6) show a high positive interaction with the intestine of *S. aurata* with ck cocktail. A complete coloration of the surface cells was observed, while the coloration was only in the upper pole of the surface cells in the cytokeratin 7 without any coloration of goblet cells. In addition, the coloration of the ck20 was positively high in the whole surface cells of the epithelium with slight coloration of the calyx cells.

Esophagus of *Scyliorhinus canicula* in (Fig.7) was normal positive for ck cocktail with the cytoplasm of the epithelial cells. Again, it was mild for ck 7, and normal for ck 20, where the base of epithelial cells was more colored than its apical.

The *S. canicula* stomach (Fig.8) showed normal coloration for cytokeratin cocktail on the apical pole of the superficial cells and in some gland cells. The coloration was also normal for cytokeratin 7 on the apical pole of the surface cells and glands. The coloration was good for cytokeratin 20 on the apical surface of the cells.

As shown in (Fig.9) *S. canicula* intestine showed a positive acceptable and complete coloration of epithelial cells for the cytokeratin cocktail, and the result was the same when dealing with cytokeratin 7, 20.

The results showed that cytokeratin is present in the epithelium of the Osteichthyes and Chondrichthyes, but these two groups of fish differ in the presence of ck7: these fishes have a false applied epithelium, which characterizes the simple epithelium [12] but here we should make sure that the [8] study of immunology on some Osteichthyes and Chondrichthyes had confirmed that fish have a complex expression system for keratins. As for stomach and intestines, the presence of cytokeratin 7 is normal, as its epithelium is simple.

TABLE I: Distribution of cytokeratins in the studied parts of the digestive systems.

	CK cocktail	CK 7	CK 20
<i>S.salamandra</i> esophagus	+++	-	-
<i>S. aurata</i> esophagus	++++	+++	+++
<i>S.canicula</i> esophagus	++	+	++
<i>S.salamandra</i> stomach	++	-	+
<i>S. aurata</i> stomach	++	+	+
<i>S.canicula</i> stomach	+	+	++
<i>S.salamandra</i>	+	-	+

intestine			
<i>S. aurata</i> intestine	++	+	++
<i>S. canicula</i> intestine	+	+	+

Cytokeratin 7 is present in the simple epithelium, and it is normal that we do not see it in the

Pseudostratified epithelium of the salamander's esophagus and intestine, but it may be present in the esophagus of the stratified epithelium of shark and *S. aurata*, because it is the cytokeratin of the second type that can be found particularly in the lower vertebrates [6].

Ck20 type I of the main cellular proteins in mature intestinal cells and calyx cells is found mainly in the mucosa of the stomach and intestines [12]. We have observed that it is present in the stomach and

intestines of the three studied species. It is not present in salamander esophagus but is present in fish esophagus.

The parts of the digestive system of the three species have been positively mixed with the cytokeratin cocktail which confirms the presence of cytokeratin in the lower vertebrate epithelium. The results of our study add to the results of the researchers [9], who conducted a chemical immunological study on the placement of keratins in the skins of fish and amphibians using anti-keratin. These results confirm those carried out on Chondrichthyes and Osteichthyes. AE1 and AE3 were positively, strongly and uniformly distributed throughout the epidermis. In frogs, AE1 was found mainly in the base layers while AE3 dyes the skin completely.

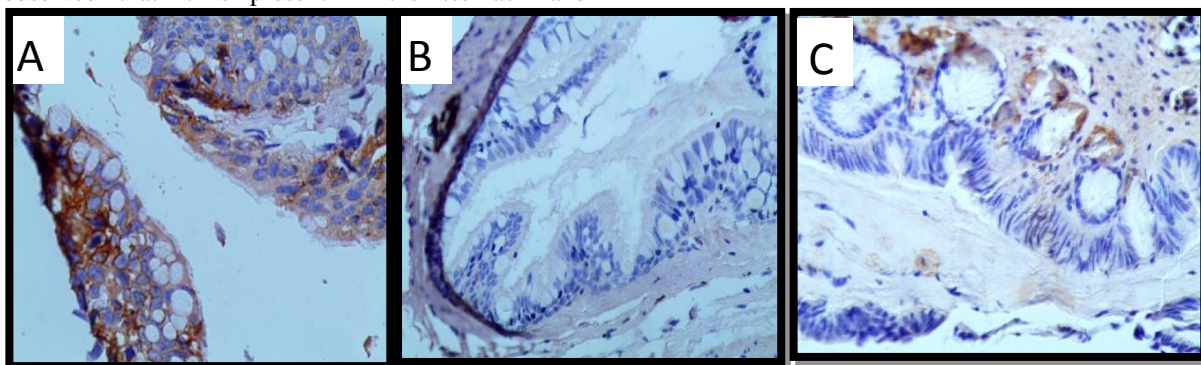


Fig 1: Coloration of salamander esophagus with cytokeratins A cocktail, B ck7, Ck20.

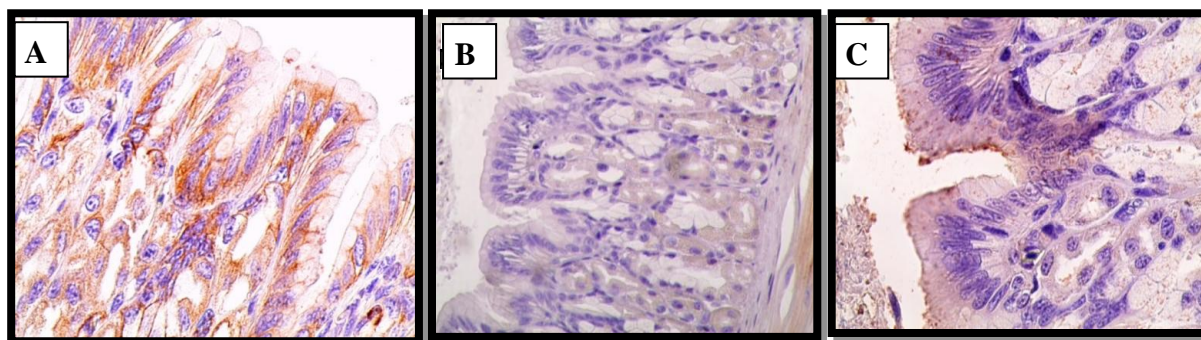


Fig 2: Coloration of salamander stomach with cytokeratins A cocktail, B ck7, Ck20.

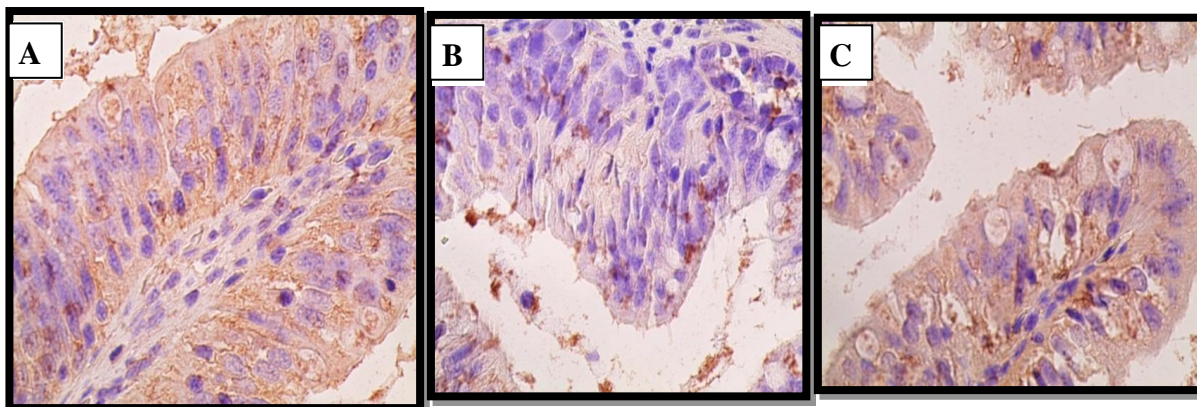


Fig 3: Coloration of salamander intestine with cytokeratins A cocktail, B Ck7, Ck20.

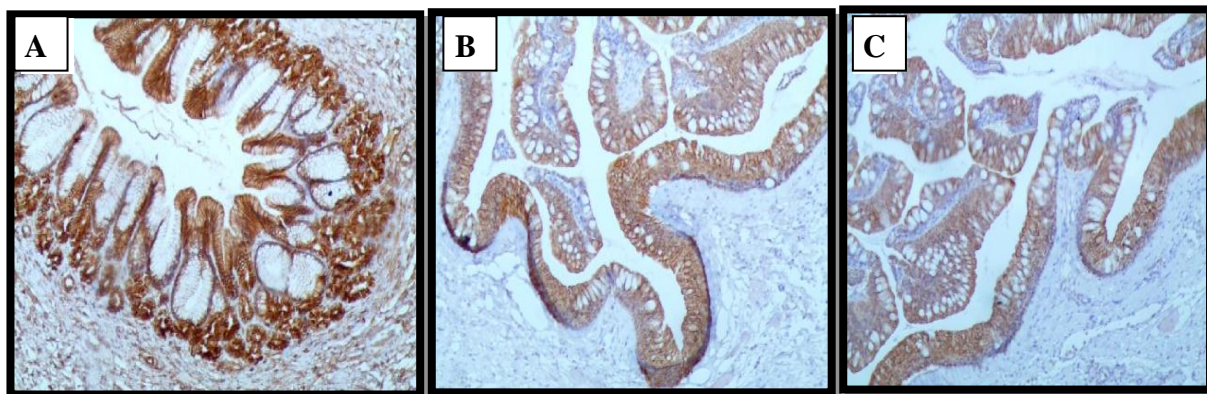


Fig 4: Coloration of *S. aurata* esophagus with cytokeratins A cocktail, B ck7, Ck20.

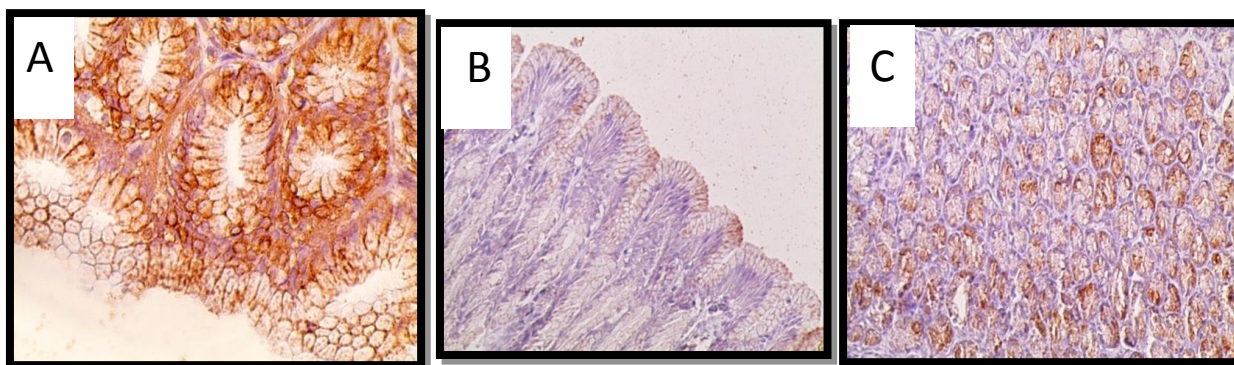


Fig 5: Coloration of *S. aurata* stomach with cytokeratins A cocktail, B ck7, Ck20.

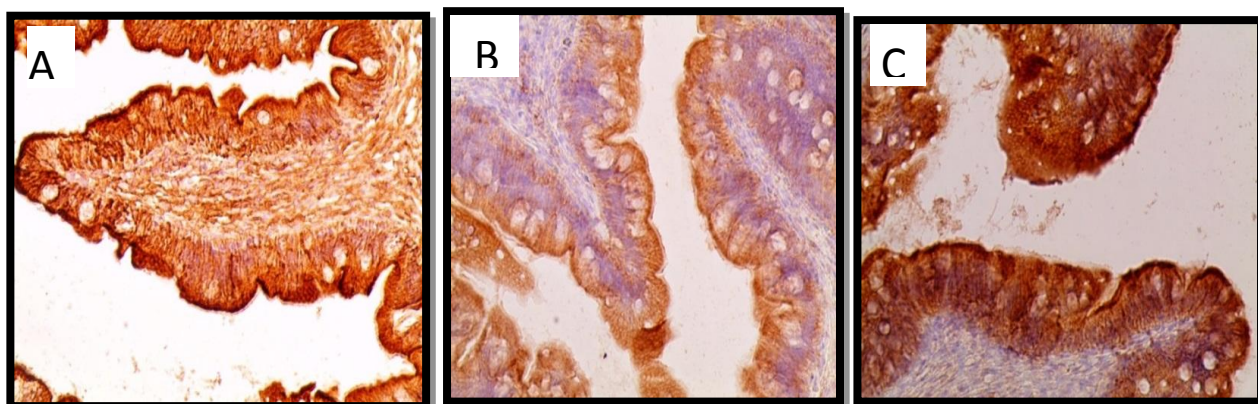


Fig 6: Coloration of *S. aurata* intestine with cytokeratins A cocktail, B ck7, Ck20.

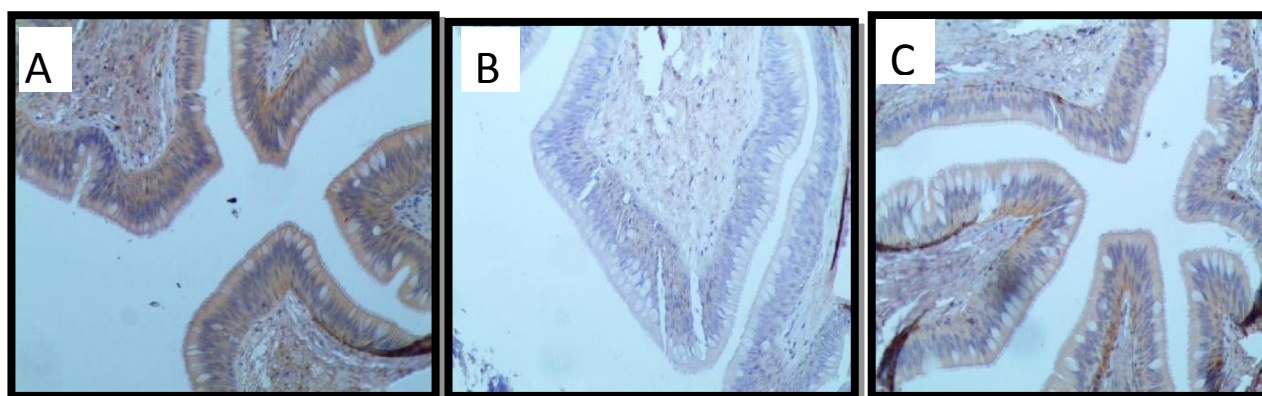


Fig 7: Coloration of *Scyliorhinus canicula* esophagus with cytokeratins A cocktail, B ck7, Ck20.

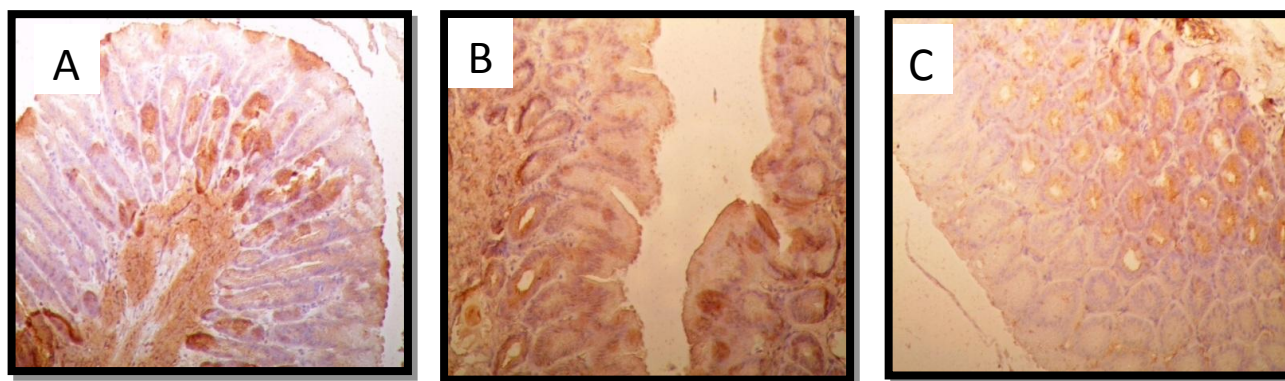


Fig 8: Coloration of *Scyliorhinus canicula* stomach with cytokeratins A cocktail, B ck7, Ck20.

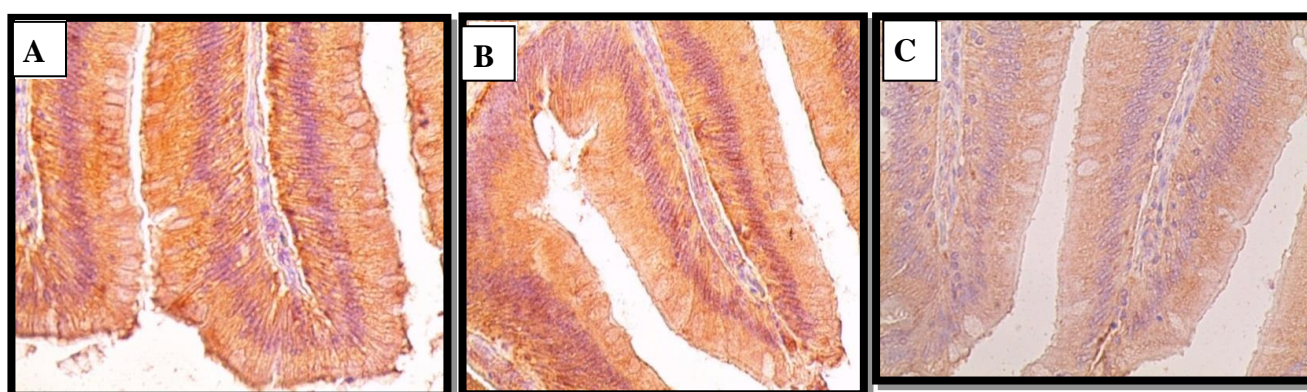


Fig 9: Coloration of *Scyliorhinus canicula* intestine with cytokeratins A cocktail, B ck7, Ck20.

From our study and previous studies, we confirm the presence of cytokeratin in lower vertebrates (fish and amphibians).

From the comparison of the evolution of these vertebrates according to the distribution of these keratins, it remains difficult to determine which species is more developed, although we suggest that, through this study, Salamander is the least developed according to the distribution of Cytokeratin 7,20 in its epithelium. References had confirmed that cytokeratin 7 is the distinctive feature of the simple epithelium. These studies have been done on vertebrates and humans in particular: In *S. aurata* and shark, Cytokeratin 7 was positive in all parts of the digestive system and associated with ck20. Again, it was positive in the glandular glands of shark and negative in the glandular glands of *S. aurata*. Thus, it is likely that shark is the most advanced among the three studied species

ck20, is a characteristic feature of the gastrointestinal cells of the digestive system epithelium. It is combined in the stomachs and intestines of the three studied species and, thus, our results are consistent with [12]. Ck7 - type II is known as Sarcolectin (SCL) which appears particularly in the simple and simple granular epitheliums. It lines the digestive tract, glands and blood vessels (simple epithelium) and is often associated with ck20. Ck20-type I of the main cellular proteins in mature intestinal

cells and goblet cells is found particularly in the gastrointestinal mucosa.

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