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Original Article

Ultramicroscopy of structures involved in the posterior region of scales in two flathead fishes (Teleostei: Perciformes)

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Abstract: Morphology of structures involved in the posterior region of scales in two flathead fish species viz. *Platycephalus indicus* and *Grammoplites suppositus* was studied using ultramicroscopy. The fish individuals were divided into three size groups based on their standard lengths and their scales were removed from four body regions. The microscopic observations indicated that the scales of both species were ctenoid. The posterior margin of all scales was formed by two rows of ctenii. Typically, the shape of the posterior region of scales in *P. indicus* was crescent, while it was triangular in *G. suppositus*. The number of ctenii in the scales of *P. indicus* was minimum 12 and maximum 60, while in *G. suppositus* it was minimum 6 and maximum 38. Moreover, the results indicated that the number of ctenii was increased during fish development because the smaller fishes have fewer ctenii in their scales than the adults, while, their general morphology has not been changed properly. This developmental change was significantly higher in *P. indicus* than *G. suppositus*. The increase of ctenii during fish development allows greater flexibility in movement. In conclusion, modification in the ornamentations of the posterior region has a hydrodynamic function and they are subject to modification during the fish development. The ctenii varying considerably in the number and could be easily counted, therefore, could be used as an appropriate taxonomic character at least in flathead fishes or even other fish groups.

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Introduction

The flatheads of Perciformes are one of the members of small to medium fish species with a notably flat head distributing in the marine waters of tropical Indo-West Pacific regions (Mastrototaro et al., 2007). From this order, the family Platycephalidae includes about 20 genera (Imamura, 1996) and 85 valid species (Froese and Pauly, 2015; Fricke et al., 2020). The members of this family are characterized by having an elongated body, dorso-ventrally depressed head, and a large-mouth. Usually, the lower jaw is longer than the upper one (Imamura and McGrouther, 2008). These fishes are benthic in nature, frequently found on the sandy and muddy bottoms at depths of 10 to 300 m, more often in shallower than 100 m (Mastrototaro et al., 2007).

The flathead fishes of the family Platycephalidae are particularly very interesting owing to the dramatic ornamentation that exists in the posterior region of their scales from the microscopic view (Hughes, 1981; Motamedi et al., 2020). Nevertheless, little information is available on the microscopic characterization of the posterior region in the scales of these fishes (Hughes, 1981; Roberts, 1993; Wonsettler and Webb, 1997). The only comprehensive study on the scales of the family Platycephalidae has been done by Hughes (1981). He described the formation, development, and resorption of ctenii in the scales of flathead fishes of family Platycephalidae.

In the present study, the posterior region of the scales in two species of the family Platycephalidae was investigated from the ontogenetic view using the Scanning Electron Microscopy (SEM). To do this objective, the scales from four body regions of each species were removed and the microscopic ornamentations in their posterior regions were described.

Materials and Methods

Studied fishes and sampling: The studied materials

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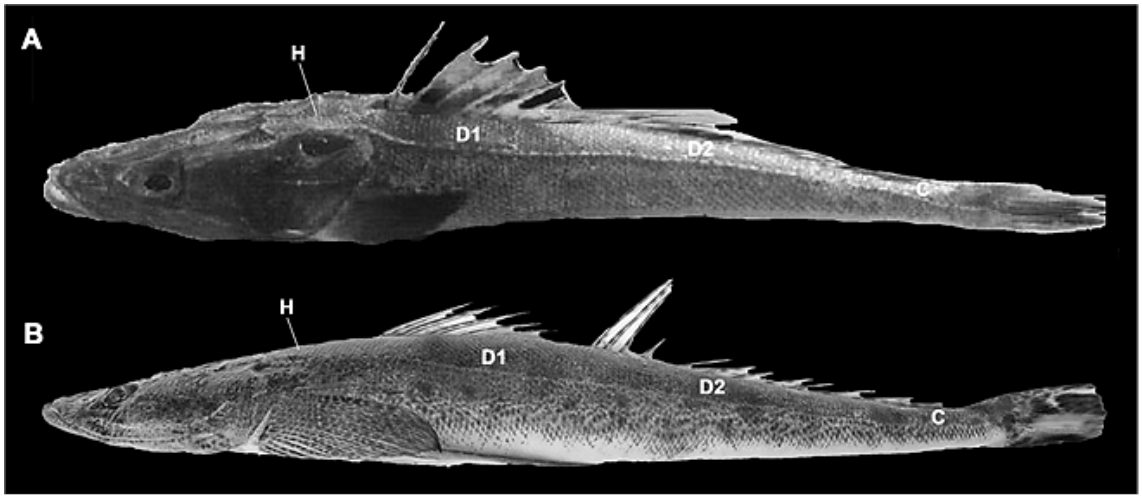

Figure 1. Photographs of (A) *Grammoplites suppositus* and (B) *Platycephalus indicus* showing four studied body regions where scales were removed. H, head region; D1, the region below the first dorsal fin; D2, the region below the second dorsal fin; C, caudal peduncle region.

are two flathead fishes, including Bartail flathead *Platycephalus indicus* (Linnaeus, 1758) and Spotfin flathead *Grammoplites suppositus* (Troschel, 1840) were collected from the Iranian coastal waters of the Persian Gulf (Fig. 1). A total of 42 specimens (21 per each species) were collected by the Trawl net and transferred to the laboratory for further examinations. After anesthesia by 1% clove solution, fishes were fixed in 5% formaldehyde and later stored in 70% (for the study of scale morphology). All fish materials and the examined scales were deposited at the Zoological Museum of Shahid Bahonar University of Kerman (ZM-SBUK).

Scale preparation: The fish specimens were divided into three range sizes based on their standard lengths (seven specimens per size class) and their scales were extracted from four body regions for microscopic examination. To do this objective, the left-hand side of the fish was divided into four regions along the longitudinal axes of the body as follow: region H (head), region D1 (from the 3rd to 4th rows below the dorsal fin or above the lateral line), region D2 (the region below the second dorsal fin) and region C (caudal peduncle region) (Fig. 1). Scales were immediately rinsed in distilled water, cleaned mechanically to remove irrelevant matter using a fine brush, and transferred into a 1% KOH solution for 40 min to remove soft tissues from the surface.

Ultramicroscopy and scale terminology: The cleaned scales were dehydrated through an ascending ethanol series (30, 50, 70, and 90%) at 30 min intervals (Lippitsch, 1990), dried on Whatman filter paper, kept for several hours between two glass slides to avoid curling of the margins of the scales. Scanning Electron Microscopy images from the posterior region of scales were prepared for each size class. Scales were mounted dorsal-up on SEM stubs. SEM images were captured with a CamScan MV2300 SEM at Shahid Bahonar University of Kerman (SBUK). For defining the posterior region and its structure types and distribution in the posterior region, we follow Hughes (1981). To determine the appearance of cetnii in the most posterior region of scales we follow Hughes (1981) and Bräger and Moritz (2016). Concerning the number of ctenii, three types were considered as follows: the number of 0-9 ctenii (as few), the number of 10-30 ctenii (as moderate), and the number of ctenii>30 (as many).

Results

Typically, the posterior region of body scale in the studied flathead fishes is covered by comb-like (or tooth-like) structures so-called ctenii (or the spine-like ornamentations in the posterior margin of scale). Generally, ctenii are defined as structures consisting of a base and a spine. These tooth-like structures that ossify separately are more or less detached from the main body of the scale. The ctenii appear in one or more rows marginally or sub-marginally at the posterior field, and in the studied fishes, they were

Table 1. Morphological description of the microstructures involved in the posterior region of two studied flathead species in three size classes. The description is presented for the scales from four body regions.

Characters	Fish body region (see Fig. 1)											
	Head (H)			Dorsal 1 (D1)			Dorsal 2 (D2)			Caudal peduncle (C)		
	Class I	Class II	Class III	Class I	Class II	Class III	Class I	Class II	Class III	Class I	Class II	Class III
	Platycephalus indicus											
Cteni in posterior region	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni
Shape of cteni	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed
No. of cteni	Moderate (17-20)	Moderate (17-25)	Many (47-51)	Moderate (12-16)	Many (36-46)	Many (54-58)	Moderate (26-27)	Many (37-38)	Many (38-45)	Moderate (17-19)	Many (34-36)	Many (45-60)
The region covered by cteni	Covered the whole posterior region	Covered the whole posterior region	Covered the whole posterior region	Covered almost the whole posterior region	Covered the whole posterior region	Covered the whole posterior region	Covered the whole posterior region	Covered the whole posterior region	Covered the whole posterior region	Covered half of the posterior region	Covered the whole posterior region	Covered the whole posterior region
	Grammoplites suppositus											
Cteni in posterior region	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Present/ Transforming cteni	Not observed	Present/ Transforming cteni
Shape of cteni	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/truncate	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	Comb-like/pointed	——	Comb-like/pointed
No. of cteni	Few (6-7)	Moderate (10-13)	Many (33-35)	Moderate (13-15)	Moderate 16-19	Moderate 19-23	Few (8-9)	Moderate 25-28	Many (36-38)	Moderate (10-12)	——	Many (35-37)
The region covered by cteni	Restricted to the middle region	Restricted to the middle region	Covered the whole posterior region	Covered the whole posterior region	Restricted to middle part	Covered the most part of posterior region	Covered almost the whole posterior region	Covered the whole posterior region	Covered the whole posterior region	Covered almost the whole posterior region	——	Covered the whole posterior region

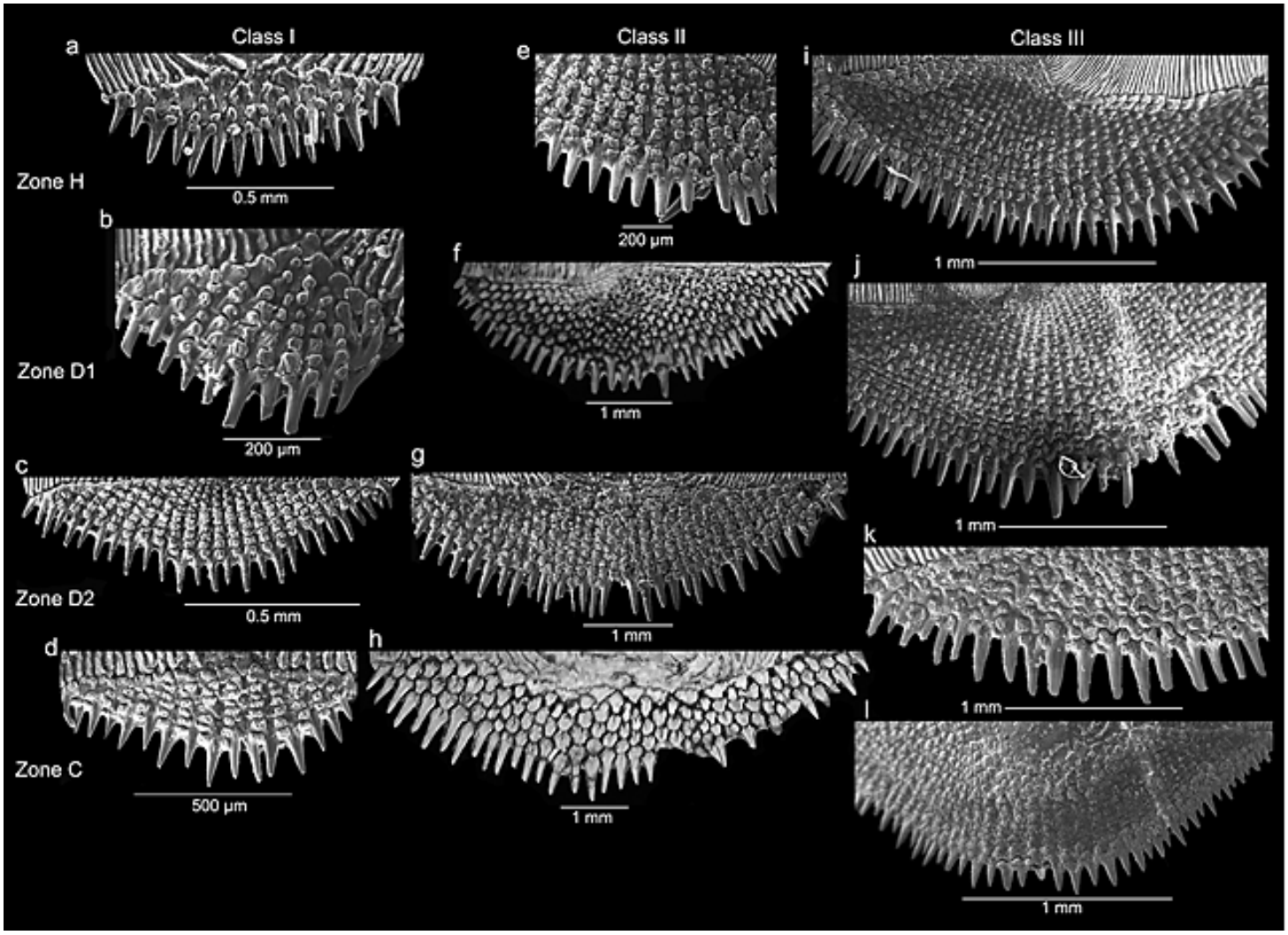

Figure 2. SEM images of the posterior region in the scales of *Platycephalus indicus* and pattern of changes in ctenii during fish growth.

found to be in transforming ctenii type i.e., separate ossifications that arise as whole spines in two or more alternating rows marginally and transform into truncated spines sub-marginally.

Characteristics of the posterior region of scales: The SEM images of the posterior region for the scales of two studied species are shown in Figures 2 and 3, and the details for the posterior region of the scales are summarized in Table 1. The most common characteristic for the scales of the studied flathead fishes is the presence of transforming ctenii in their posterior part (Figs. 2, 3). Also, the SEM imaging indicated that the posterior region of the scales in the fishes from all three size classes in both species was covered by ctenii. The only exception, however, was the scales from the caudal peduncle region (region C) in young individuals (class II) of *G. suppositus* that had not ctenii in the posterior region (Fig. 3h).

Generally, the shape of the posterior region of the scales in *P. indicus* was crescent (Fig. 2), while it is typically triangular in *G. suppositus* (Fig. 3). The number of ctenii in the posterior region of the scales in *P. indicus* was minimum 12 (in class I of region D1) and maximum 60 (in class III of region C), while in *G. suppositus* it was minimum 6 (in class I region H) and maximum 38 (in class III region D2) (Table 1). The posterior margin of all ctenoid scales examined in this study was formed by two rows of ctenii, while the three complete rows of ctenii were not observed in any of the examined scales. The most frequent state, however, was the two incomplete rows with one complete row between them. The ctenii of both rows were similar in morphology (Figs. 2, 3).

Typically, a ctenus in the posterior part of the

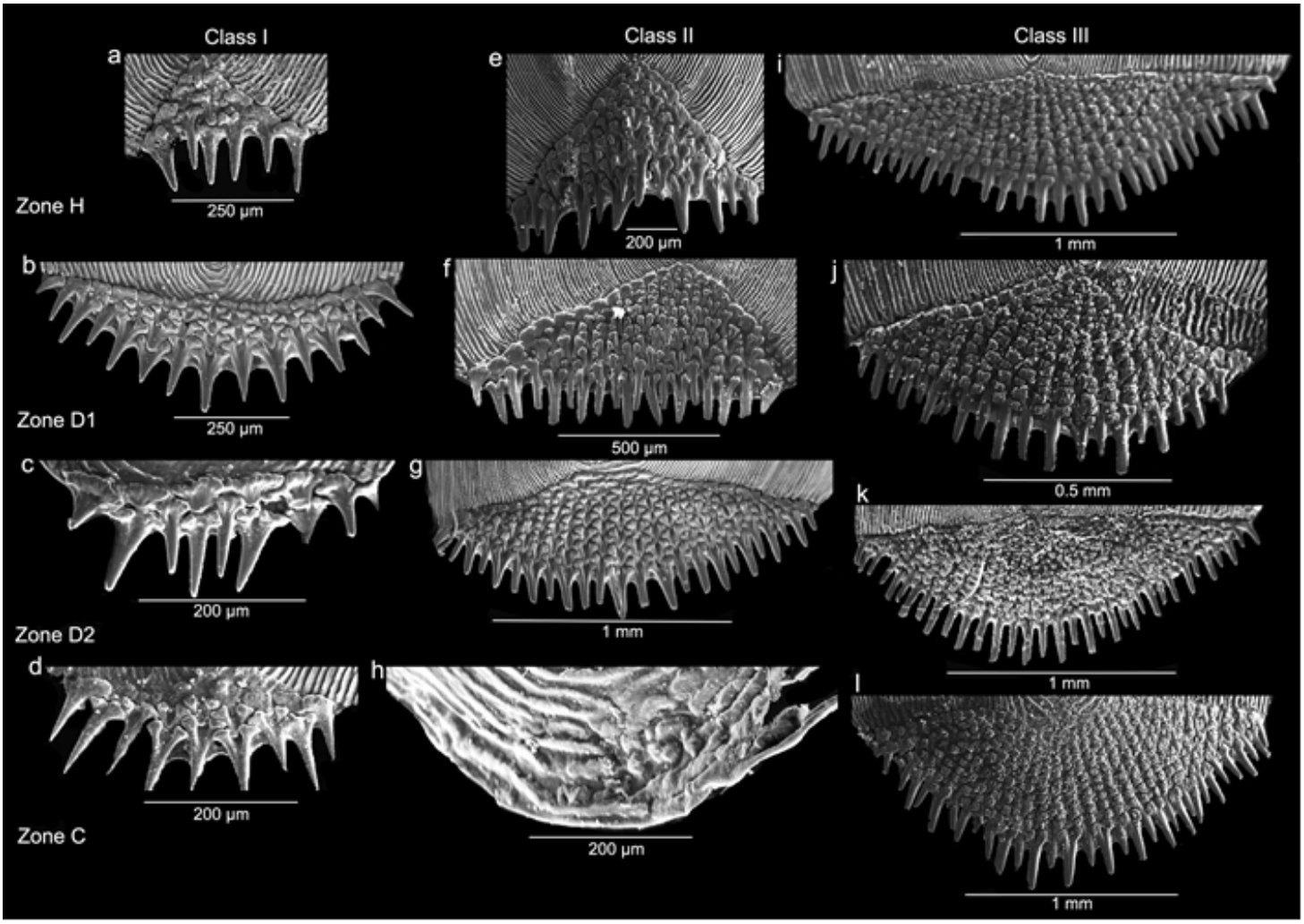

Figure 3. SEM images of the posterior region in the scales of *Grammoplites suppositus* and pattern of changes in ctenii during fish growth.

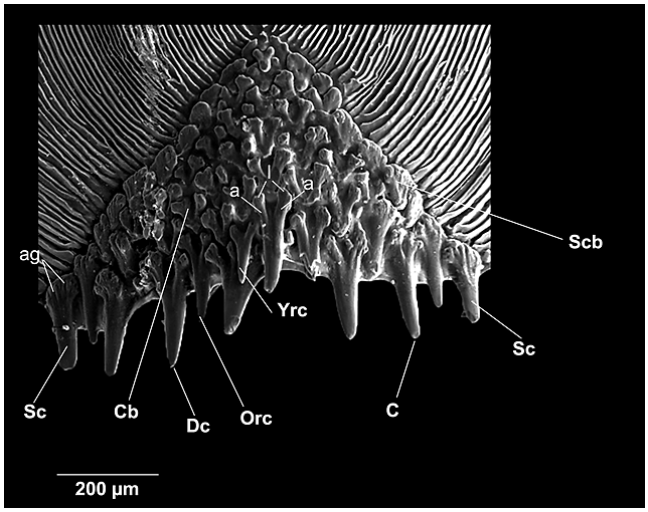

Figure 4. Microstrutures involved in the posterior region of scale in *Grammoplites suppostius*. Structures in the posterior field of scale in *G. suppositus*. Ctenii (c), subctenii (sc), cteniial base (cb), subcteniial base (scb). (a) Degenerating ctenus, (b) older row ctenus and, (c) younger row ctenus.

studied scales consists of a long, narrowing spine with generally a pointed or a truncate end, bifurcating anteriorly into two arms (Fig. 4). The arms and lobes of the base of a ctenus were joined and formed the sides of an arch. The lobes were sculptured to form articulation grooves which provide a suitable tool of interlocking with nearby ctenii (Fig. 4). Also, several pores were observed on the surface of the base of a ctenus (Fig. 4). The ctenii in the posterior region of scales in both species and three classes were generally similar, with a bit variation regarding space between two lobes at the base of each ctenus.

Furthermore, four types of distinct, bony structures, each with a specific distribution in the posterior field of the scales of studied flathead fishes were recognized (Fig. 4). The first structure was recognized as ctenii. The ctenii from the more posterior row were

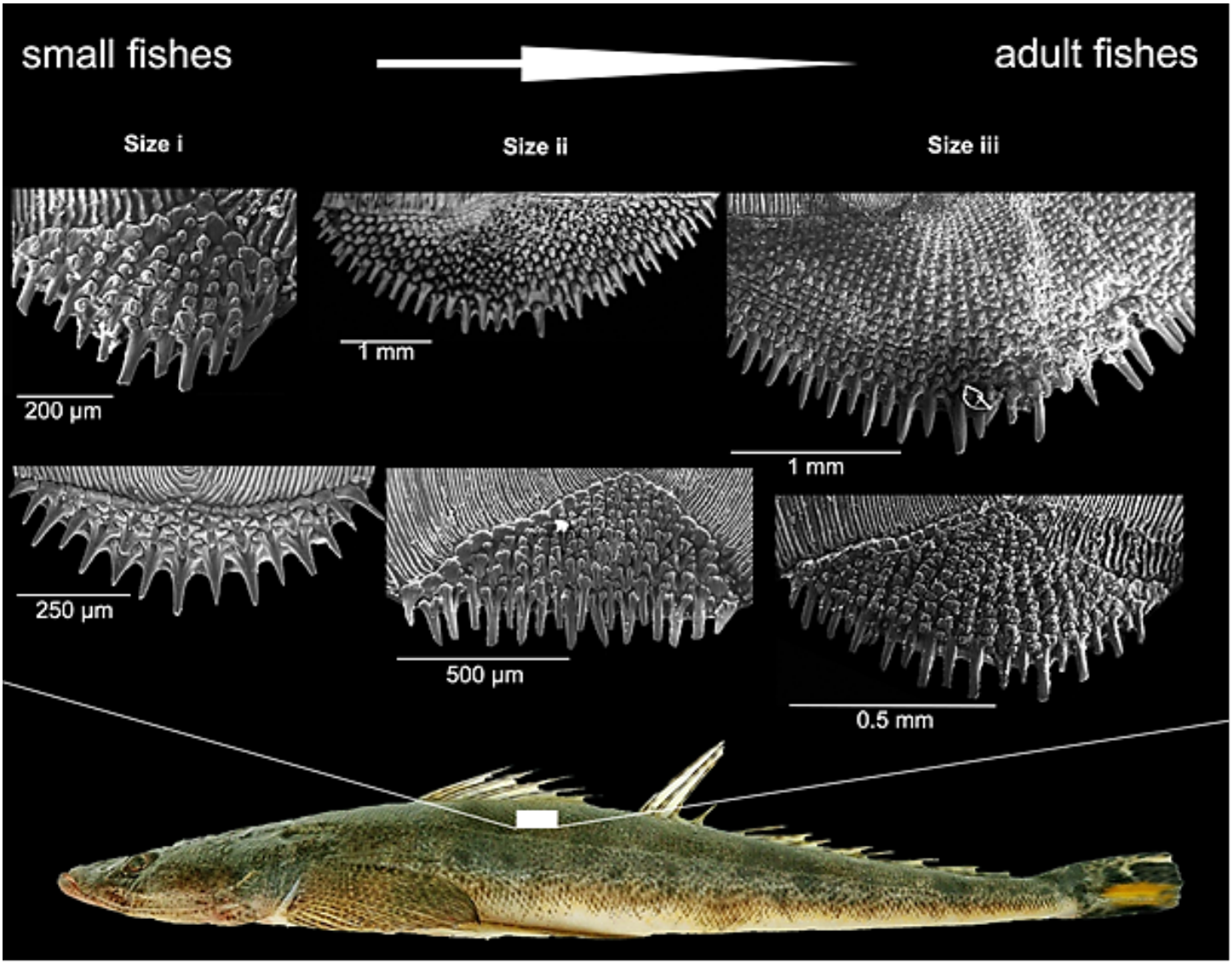

Figure 5. Comparison of the posterior region of the scales from region II, among the three size classes of *Platycephalus indicus*.

different from those of the more anterior row. The second type of structure exists at the ends of each row of ctenii was distinguished as a subctenus. The third type of structure was termed as ctenial base and makes up the bulk of the posterior field. The fourth type of structure is similar to, but usually distinct from, a ctenial base. It formed the anterior border of the posterior field and represented the base of a subctenus. This structure was termed as a subctenial base (Fig. 4).

Discussions

Like other members of the flathead fishes, the two studied species have rough and ctenoid scales. It should also be noted that we found some scales from the caudal peduncle region in young individuals of *G. suppositus*, which had not ctenii in the posterior region. This exception is more likely because of the deformed and regenerated scales, which have been examined from this region.

The ctenii in the scales of studied flathead fishes were of transforming type, which arises as whole spines in two or three alternating rows marginally and transforms into truncated spines submarginally (Johnson, 1984). The two other types of ctenii in fish scales are "peripheral ctenii", which occur as whole spines in one row at the scale margin (e.g. in the gobioid *Gobiomorus*) (Figure 3B in Roberts, 1993, p. 67), and the "whole ctenii", which has separate whole spines marginally and submarginally (e.g. in the scale of the black cardinal fish *Epigonus telescopus,* see also

Figure 20A in Roberts, 1993, p. 86).

In further examination, we compared the scales from region II, among the three size classes because usually, scales from this region are the most grown scales (see also Fig. 5). Our observations indicated that the smaller fishes had fewer ctenii in their scales than the adults. Therefore, it is likely that the number of ctenii is increased during fish development of these fishes (Table 1, Fig. 5). However, the general morphology of ctenii had not been changed properly during development (Fig. 5). The developmental change in the number of ctenii was found to be significantly higher in *P. indicus* than *G. suppositus*. In *P. indicus*, the number of ctenii in the fish scales of class I was moderate (12-27), while they were increased to many ctenii (38-60) in the fishes of class III. In *G. suppositus*, the number of ctenii in the fish scales of class I were few (6-9) or moderate (12-27), while they were increased to moderate (19-23) or many ctenii (33-38) in the fish scales of class III.

It has been documented that the growth of the posterior region of scale is happened by adding new ctenii to the posterior margin (Hughes, 1981). However, our microscopic observations revealed that the developing ctenii were morphologically similar to mature ctenii but were relatively smaller in size. By growing a new ctenus, the spine of the ctenus behind which it is growing is degenerating. Therefore, the spine of the older ctenus is replaced by the new growing ctenus. Hughes (1981) concluded that the spinal degeneration of older ctenii when new ctenii develop behind them is a mechanism for conserving and recycling scale material.

Functionally, ctenii in the posterior region of scales may improve hydrodynamic efficiency of swimming by affecting the profile of the overlying epidermis, and thus assisting in breaking vortices caused by the swimming fish and thereby reducing drug (Burdak, 1969; Sire, 1986). As a result, increasing the number of ctenii in the posterior margin of scales in the studied fishes during their development may allow greater flexibility in fish movement. However, the number of ctenii in the scales of small and large fishes in *G. suppositus* was found to be obviously lower than *P. indicus*. This means that *G. suppositus* is not probably very active in swimming as *P. indicus*. Therefore, it can be assumed that modification in the ornamentations of the posterior region has a hydrodynamic function and they are subject to modification during the growth of the fish (Burdak, 1986; Sire, 1986; Sire and Arnulf, 2000). Besides, variation in the number of ctenii in the posterior region of the scales in the adult flathead fishes was found to be suitable for discrimination of the flathead species. Takagi (1953) has also been accentuated that ctenii are the most conspicuous structures of the posterior field of fish scales. These structures varying considerably in the number and could be easily counted, therefore, could be used as an appropriate taxonomic character at least in flathead fishes or even other fish groups.

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