Scanning electron microscopy of scales in cyprinid fish, *Alburnoides bipunctatus* (Blotch, 1782)

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Abstract

The normal and lateral line cycloid scales of a cyprinid fish; *Alburnoides bipunctatus* (Blotch, 1782) have been subjected to SEM to study their detailed structure. It shows the general architectural pattern of a cycloid cyprinid scale. The focus of the scale is clear and sharp located in the anterior field. Circuli are observed in all fields. Primary radii which originate from very near the focus divide the posterior field of scale into compartments. Originating far away from the focus, the secondary radii are seen. But the tertiary radii are few. The anterior radii are absent. Lepidonts (teeth-like structures) are absent or are very week on the circuli. Tubercles (granules) at the posterior filed of scale are not well developed. Many resorbtion regions are seen on the scale. Presence of a long, straight, S-shape or J-shape central canal originating from the upper margin of anterior region which in most cases extending down to the posterior margin is characteristic feature of lateral line scale. The architectural specification of scales such as focus shape and position, circuli, chromatophore, lepidonts and lateral line canal might be used as important taxonomic tools.

Keywords: scale, scanning electron microscopy, lepidont, *Alburnoides bipunctatus*

Introduction

Scales, the dermal derivatives of fish body are important structures used as a versatile research material (Kaur and Dua, 2004). Scale morphology has proved to be a useful tool in fish classification, determining the diet of piscivorous predators (Lekuona et al., 1998; Campos et al., 2002) or in the paleontological analysis (Meunier and Poplin, 1995; Jawad and Al-Jufail, 2007). It could provide complete knowledge of life history, age at recruitment, growth rates, age at first maturity and average life span of fishes (Tzeng et al., 1994). Detailed structures of the fish scale can be helpful in identification of fishes up to major group (Lagler, 1947; Van Oosten, 1957; Norman, 1957) or species level (Chu, 1935; Das, 1959; Lanzing and Higginbotham, 1974). It could be also used in study of fish phylogeny (Kobayashi, 1951; Kobayashi, 1952), sexual dimorphism (Esmaeili, 2001), past environment experienced by fish, discriminating between hatchery reared and wild populations, migration and pathology of fish scale (Kaur and Dua, 2004; Campos et al., 2002; Lekuon et al., 1998; Esmaeili, 2001). So the role of hard parts, especially scale cannot be over-looked for effective fishery management's practices (Johal and Sawhney, 1999; Johal and Bansal, 2000). The importance of scale morphology used in classification was strengthened with the introduction and development of SEM (Scanning Electron Microscopy) (De lamator and courtenay, 1973, 1974; Jawad, 2005 a,b; Jawad and Al-Jufail, 2007). During the perusal of literature it has been found that the age and growth studies on cyprinid native fishes of Iran are rarely opted and some taxa are completely ignored. So an attempt has been made here to study the ultrastructure of the scale of a widely distributed cyprinid freshwater fish, *A. bipunctuatus* by using scanning electron microscopy. In Iran it is called under different names such as tailoress fish, possibly from the lateral line pattern like stitches, lapak, parak, sima, kuli, shebeh zury (Coad, 2008). Due to its small size, this fish has no fisheries value but it has aesthetics value because of pretty colors and pattern on the body.

Material and Methods

To study the ultrastructure of the scale of *A. bipunctatus*, the fishes were collected by the authors from Sarab-e- Beyza spring stream (29° 57′ 41.8″ N, 52° 21′ 11.1″ E), Kor River basin, southwest Iran (Figure 1) using dipnet and electrofishing device in 2007. The scales of fishes were gently removed with fine forceps from the left side of body below the dorsal fin preferably the

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third or forth row and also from lateral line from the middle of the lateral line (Figure 2). Immediately, the scales were washed thoroughly with water by gently rubbing them between the tips of the fingers and cleared with 3% potassium hydroxide solution too. The cleaned scales were dehydrated in 30, 50, 70 and 90% ethanol for 30 minutes respectively and dried on filter paper to avoid curling (Lippitsch, 1990). Then the scales were kept between the two microslides for 2-3 days. The scales were not put in absolute alcohol as 100% ethanol curls the scale margins (Esmaeili, 2001). The cleaned and dried scaled were mounted on metallic stubbs by double adhesive tape with dorsal surface upward and ventral surface sticking to the tape and coated with a 100˚A thick layer of gold in a vacuum in a gold coating unit (SC7640 SPUTTER COATER, Model: FISONS) (Esmaeili, 2001). The scales were viewed under vacuum in a Leica Cambridge scanning electron microscope at an accelerating voltage of 20kv at low probe current. Various images of the scales were taken and were saved in the computer attached to the scanning electron microscope. When gold-coated scales were not being viewed, the stubbs were stored in a desiccator to avoid moisture.

Figure 1. Map of Iran and Fars province showing fish collection site.

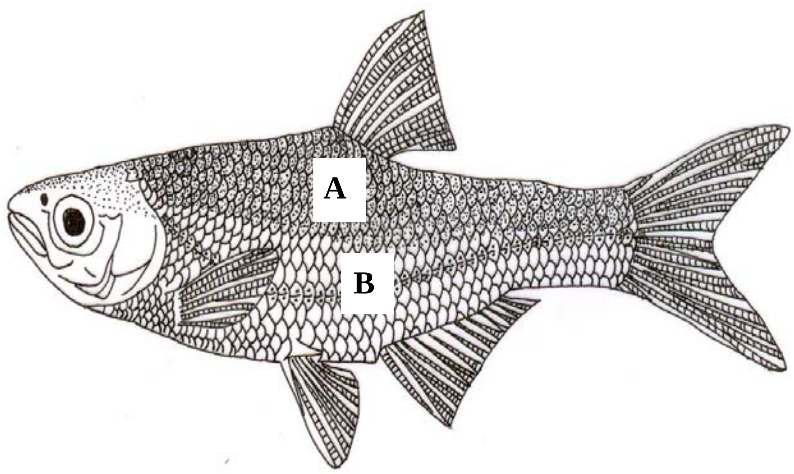


Figure 2. Schematic drawing of *Alburnoides bipunctatus* (total length, 52.8 mm) showing location of key scales using for scanning electron microscopy. A, scale below dorsal fin; B, lateral line scale.

Results

The general structure of normal and lateral line scales of hypothetical fish and that of *A. bipunctatus* is given in Figure 3. Like most of the cyprinid scales, there is no cteni at the posterior part of the scale of *Alburnoides bipunctatus* (Blotch, 1782) hence it is a cycloid scale. The scales maintain the same morphological proportions located on the different parts of the body. The scales of the lateral line and below the dorsal fin are largest respectively. The scales on the other parts of fish are smaller in size. As the scales below dorsal fin depict all the features, these scales have been designated as key scales. The dorsal part of scale is rough, convex and has distinct structures, which consists of ridges, grooves and granules (tubercles) and the ventral part of scale is shiny and smooth. Each scale has a focus. Focus is the first part of the scale to be formed during ontogenesis and has different locations in different species (Kaur and Dua, 2004; Esmaeili, 2001) (Figure 3). In this species, the focus is distinct and lies in the anterior part of the scale divides the scale into anterior, rostral or cephalic (A), posterior or caudal (P) and lateral fields (L). The shape of focus is oval or round (Figures 3a, b, 4b). Few mocus pores are found in the focus region. From the focus lines of growth (the ridges) start appearing which are named as circuli (Figures 3, 4). The space between circuli are called inter circular space. Circuli (the lines of growth) are the elevated ridges present on the surface of scale and show differences with regard to thickness, arrangement and relative spacing (Kaur and Dua, 2004). The intercirculus space is maximum in the posterior part and minimum in the anterior part. It is due to anterior location of the focus on the scale. Intercirculus space is intermediate in the lateral parts (Figure 3a, b). Each circuli is wedge shaped, having broad base and pointed upper part (Figure 4). The anterior part is overlapped by the posterior part of the preceding scale (Johal and Bansal, 2000; Esmaeili, 2001). Hence the anterior part is soft and uncoated whereas the posterior part of the scale is covered with a thick layer of epidermis. The posterior part occupies more than 70% of the total surface area of the scale. Presence of lepidonts, the scale denticles or tooth-like process is another characteristic feature of some circuli of this fish (Figure 4c, d, e). There is very minute lepidonts on the circuli of the posterior part of the scale under high magnification (Figure 4d). The majority of circuli is without lepidonts and smooth in considered species (Figure 4i, j). Distinct breaks in the circuli points towards the formation of annuli are observed in scale of *A. bipunctatus*. The annuli indicate the fish age. Annuli clearly marked in rostral and lateral fields by alternate wide light and dark, narrow spaces that correspond to summer and winter growth in many fishes (Jawad and Al-Jufail, 2007).

In the posterior and lateral parts, the circuli are partitioned by deep and narrow grooves that run radially towards the focus. They are called radii which are categorized into three types depending upon their point of origin on the scale including: Primary radii, originating from the focus, reaching the margin of the scale; secondary radii, originating midway between focus and margin and tertiary radii originating between midway and margin (Figure 3a, b). The radii cut the circuli and annuli at right angle. Primary radii are present on posterior and lateral fields of the scale. They are absent in the anterior part of the scale. The relative number of primary and secondary radii is more as compared to the tertiary radii. Some of scales are without radii. These are called simple scales, the term which has been used by Lippitsch (1990). The scales with well developed radii are here called “sectioned”. The posterior part of the scale confers color to the fish body due to the presence of chromatophore which lies on the tubercles. Tubercles are not clear in this species. Scales of *Alburnoides bipunctatus* (Blotch 1782) shows severe signs of resorbtion (Figure 4, f and h). Numerous cavities were present on the calcified layer surface. Schematic drawing of lateral line scale is showed in (Figure 3b).

Lateral line scale of this fish also is divided into anterior (rostral) and posterior (caudal) parts. Scanning electron microscopy of lateral line scale of *A.bipunctatus* showed presence of a long, straight, S-shape or J-shape central canal originating from the upper margin of anterior region or focus in most cases extending down to the posterior margin. The canal opening is open and round in two sides in all cases. The posterior opening of the lateral line canal is wider than the anterior opening (Figure 5h, i). Anterior, posterior and lateral parts of lateral line have several mucus pores (Figure 5h). In the posterior part, the circuli are spaced (Figure 5f). There is no clear granulation on the posterior portion of lateral line scale. Another character of lateral line canal is the presence of a few accessory pores in its wall in considered species (Figure 5a). We observed no obvious difference between male and female in scale structures.

Discussion

This paper describes the scale morphology of a cyprinid fish, *Alburnoides bipunctatus* (Blotch, 1782) from Iran. It shows the general architectural pattern of a cycloid cyprinid scale having focus, circuli and radii. The focus of the scale is clear and sharp located in the anterior field and is the first part of the scale to be formed during ontogenesis. Circuli are observed in all fields. The arrangement of the circuli corresponds to the scale shape (Esmaeili, 2001).The circuli formation is due to the excess calcium salts secreted by the skin and their subsequent deposition on the scale and distance between circuli indicates fast and slow growth period. Lepidonts (teeth-like structures) are absent or are very week on the circuli. Lepidonts are important structures known to support species distinctness (Kaur and Dua, 2004; Jawad and Al-Jufail, 2007; Esmaeili, 2001). The taxa usually differ with regard to shape, texture, attachment and orientation of lepidonts on the crest of circuli (Kaur and Dua, 2004). Lepidnots of different size and shape have been reported in many fish species (Lippitsch, 1990; Delmater and Courtenay, 1974; Jawad and Jufaili, 2007). They might characterize genera and may even distinguish some taxa at the specific level (Delmater and Courtenay, 1974). Lepidonts are not homologous to breeding tubercles and contact organs (Delmater and Courtenay, 1974). Radii are present on the lateral and posterior parts of scales of *A. bipunctatus*. There is no significant relationship between number of radii and scale size, as the numbers of radii depend on location of the scale on the fish body. However, in some other teleotes such as *Mullus surmuletus* L., 1758 and *M. barbatus* L., 1758, the number of radii is correlated to fish size (Jawad and Jufaili, 2007). The presence of primary and secondary radii is a growth phenomenon and obviously only weakly influenced by genetic factors (Lippitsch, 1990). The radii formation is considered to be related to the accommodation power of the large surface area of the anterior and lateral parts of the scale in the lesser space as these two parts of the scale are overlapped by the posterior part of the preceding scale. The higher number of radii is correlated with the better nutritive conditions of the fish (Johal et al., 1984; Tandon and Johal, 1996). Radii represent the line of scale flexibility.

Tubercles are not clear in this species. No earlier attempt has been made to study the importance of tubercles in species specificity. The shape of tubercles in other species varies from round to oval, semi-oval and oblong structure. Tubercule are formed by the aggregation of epithelial layer of the skin which covers the posterior part of the scale. They impart specific color to fish as they contain chromotophores in the outer surface. Presence of chromatophore on the posterior part of scale is a characteristic feature of the cycloid and ctenoid scales of carps and perches respectively (Tandon and Johal, 1996; Johal et al., 1984; Johal et al., 1996; Johal and Agarwal, 1997).

Severe signs of resorbtion were found on the scale of this cyprinid fish. This resorbtion in teleost scales occurs under various physiological and experimental conditions and has been suggested to be initiated during periods of increased calcium demand such as during sexual maturation when estradiols induce vitellogenesis (Crichton, 1935; Jarvi and Menzies, 1936; Van Someren, 1937; Takagi, 1990; Persson et al., 1995, 1998) and lack of food (Esmaeili, 2001).

Presence of a long, straight, S-shape or J-shape central canal originating from the upper margin of anterior region which in most cases extending down to the posterior margin is characteristic feature of lateral line scale of *A. bipunctatus*. Lateral line scales prove their potential in fish classification and taxonomy. Number, position of canal, its alignment viz. straight or oblique, perforation in anterior, posterior or lateral are important features for fish classification (Kaur and Dua, 2004). According to Delmater & Courtenay (1973) scanning electron microscopy of lateral line scales of teleost fishes demonstrates a wide range of structural variation of lateral line canal from a simple direct or slightly oblique perforation to an extended canal with or without simple to highly complex cantilevered extensions covering the anterior opening.

The above observations regarding the architectural specification of scales such as focus shape and position, circuli, chromatophore, lepidonts and lateral line canal might be used as important taxonomic tools.

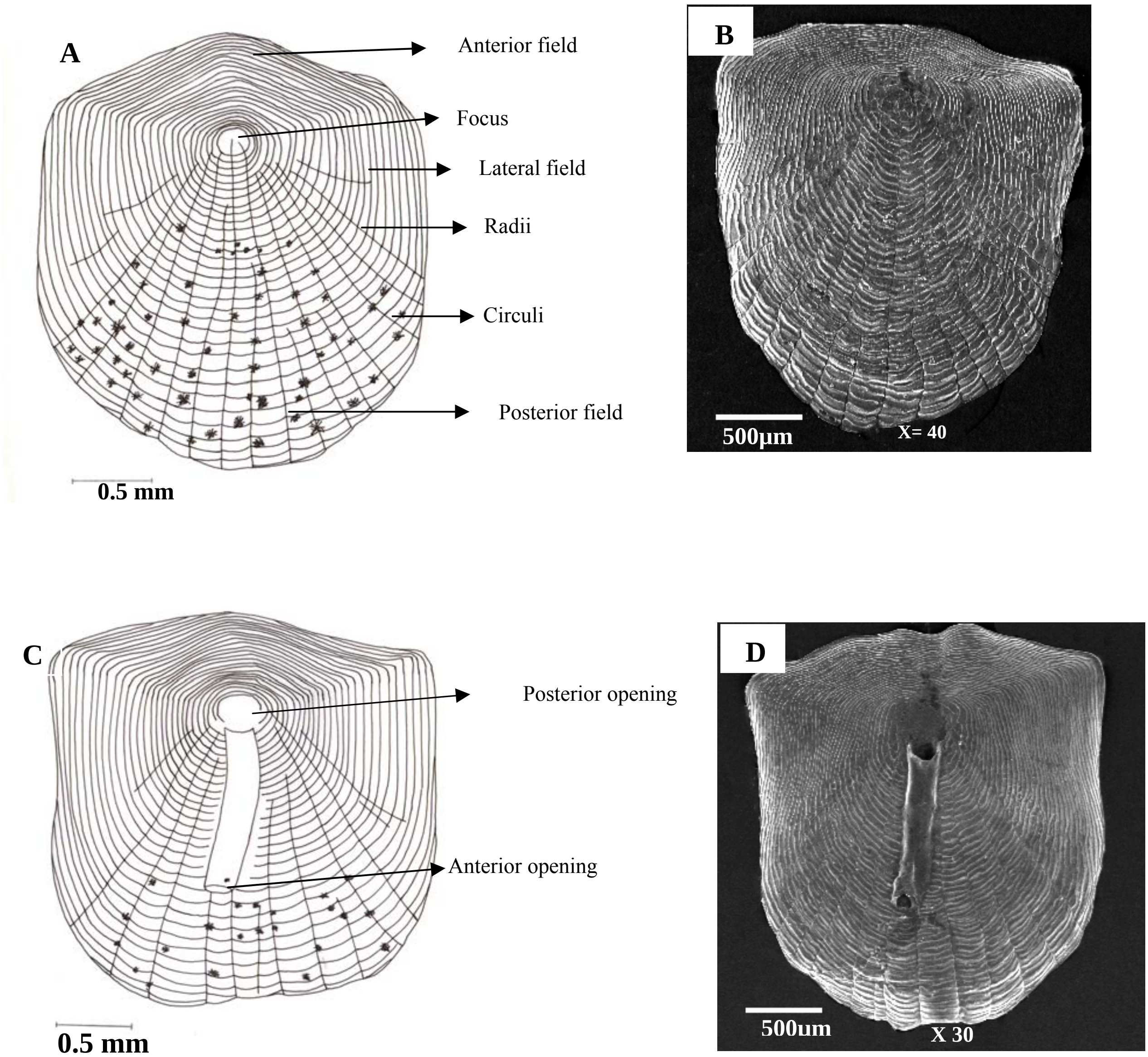


Figure 3. A, Schematic drawing of a sectioned cyprinid scale. B, SEM microphotograph of a normal *A.bipunctatus* scale. C, schematic drawing of a sectioned cyprinid lateral line scale. D, SEM micrograph of *A.bipunctatus* lateral line scale.

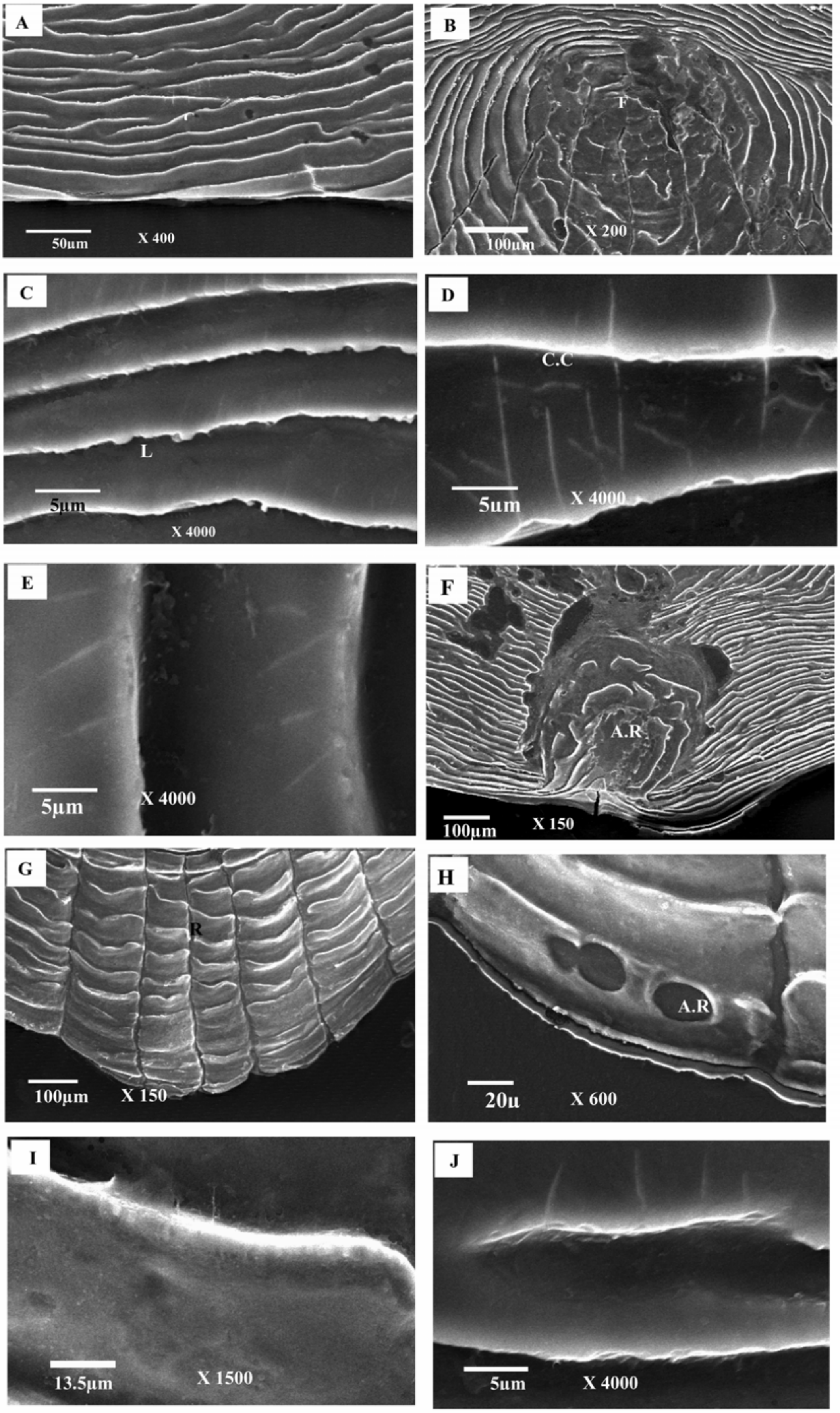
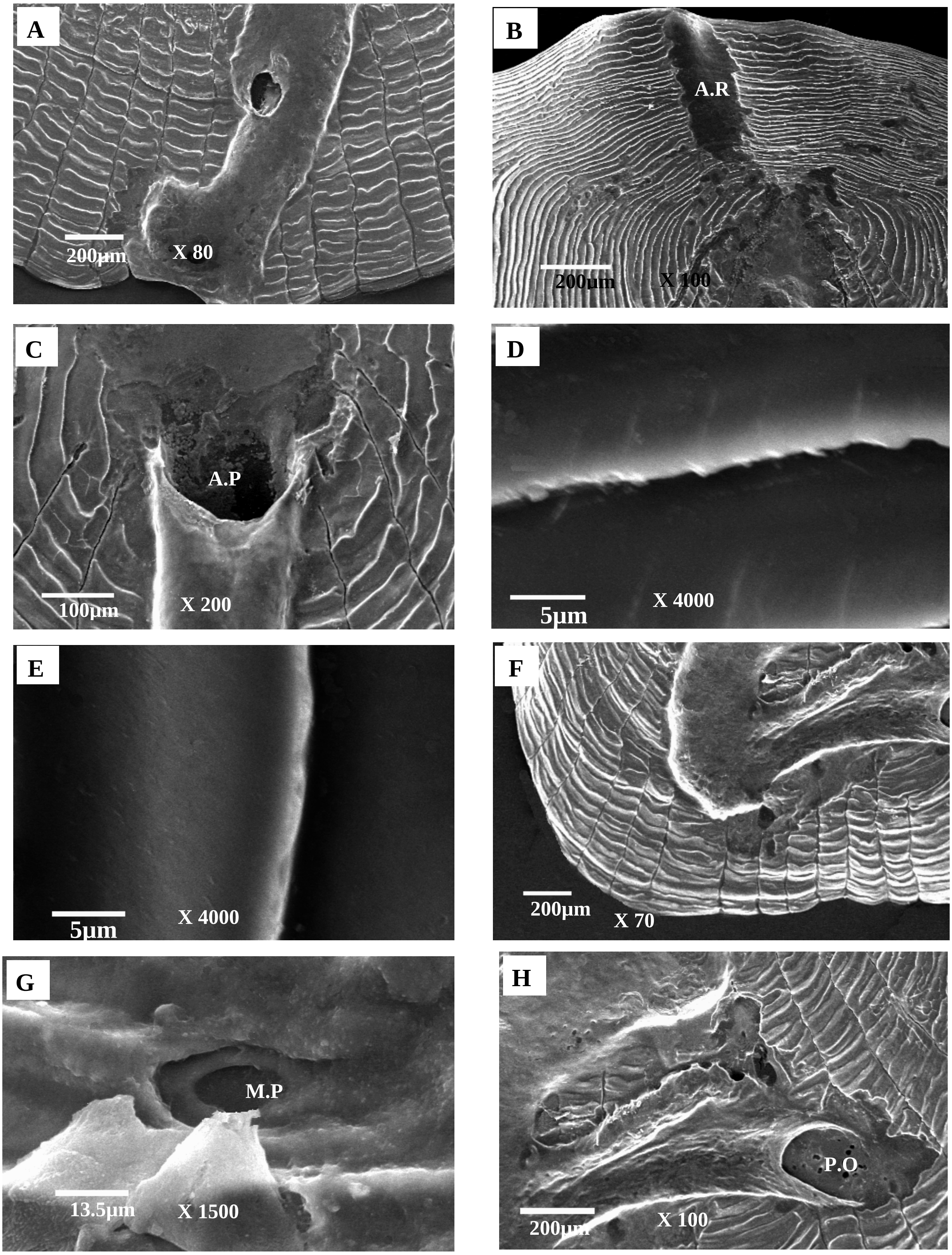


Figure 4. SEM microphotographs of *A.bipunctatus* (R), circuli (C), lepidont (L), focus (F), crest of circuli (C.C), Absorbtion Region(A.R).


A
200μm
X 80
B
A.R
200μm
X 100
C
A.P
100μm
X 200
D
5μm
X 4000
E
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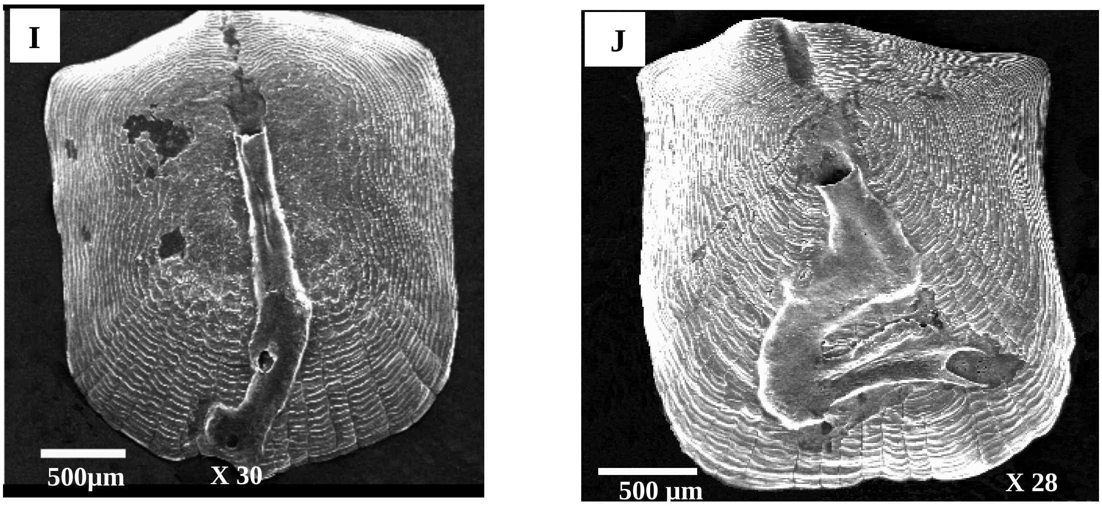

Figure 5. SEM microphotographs of lateral line scale of *A.*bipunctatus circuli (C), crest of circuli (C.C), circular groove (C. lateral circuli (l. C), primery radii (P. R), secondary radii (S. R), line grown (L. G). Anterior pore (A. P), Posterior pore (P. O), Tubercle (T) and Mocus pore (M. P).

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