

Short Communication:

Morphological study of the scales of *Barbodes cf. binotatus* (Cyprinidae) from Toba Lake, Indonesia by using Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS)

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Abstract. Lubis K, Sudibyo M, Siregar EH, Laili N. 2021. Short Communication: Morphological study of the scales of *Barbodes cf. binotatus* (Cyprinidae) from Toba Lake, Indonesia by using Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS). *Biodiversitas* 22: 3821-3828. Spotted barb (*Barbodes cf. binotatus*) is a freshwater fish of Sumatra Island, especially found in the Toba. The study of morphological characteristics of the scales of Spotted barb (*B. cf. binotatus*) is necessary in fish taxonomy. Therefore, the present study investigated the morphology and the chemical elements of the scales located between the dorsal fin and lateral line and those on the lateral line using SEM-EDS (Scanning Electron Microscope (SEM)- Energy Dispersive X-ray Spectroscopy (EDS)). We found that the scales of *Spotted barb* (*B. cf. binotatus*) have the general morphological characteristics of cycloid scales. The morphologies of the scales between the dorsal fin and lateral line and those on the lateral line are slightly different. Lateral line scales of *Spotted barb* (*B. cf. binotatus*) have no focus area, replaced by a lateral line canal located along the anterior-posterior axis of the scales with two openings. The anterior opening is bigger and wider in compared to the posterior opening. The circuli of the dorsal fin scale is orientated towards the focus. However, the circuli of the lateral scale faces the lateral line canal. The scales contain some elements such as Ca, K, O, Mg, Na, and P.

Keywords: *Barbodes cf. binotatus*, cycloid scale, scale, SEM-EDS, Toba Lake

INTRODUCTION

Several methods can be used for identification of animal species such as image recognition systems (IRSs), interactive electronic keys, computer-based morphometric identification (IPEz), genetic methods, using of local (folk) expertise, scales, otoliths and hydroacoustics (Omer 2017). The scale morphological characteristics play an important role in the taxonomy field. The macro- and micro structures of fish scales have proved to be a useful tool in fish classification since the early 1900s (Motamedi et al. 2020). In the fishery and taxonomy fields, scales of fish are valuable since they can be used in estimating fish age (Busst 2016), identification (Ibáñez and O'Higgins 2011), and phylogenetic information (Khemiri et al. 2001). Fish scales have potential for providing information in environmental studies such as conservation plans and fishery assessment and management (Omer 2017). Fish scales can also be used as a substrate to produce alkaline protease and amino acid rich aqua hydrolyzate by *Bacillus altitudinis* GVC11, which is suitable for nutritional supplements and organic fertilizer (Harikrishna et al. 2017). Another study reported that fish scales composed of collagen covered with calcium salts have potential for cosmetic, pharmaceutical and biomedical applications (Sionkowska and Kozłowski 2014).

Most of bony fishes have scales, where the functions of scale are described such as to protect the skin of fish from being getting injured, to give the color of the fish's body and as a sensory receptor with lateral line on the scale which enable the fish to detect wave vibrations in the water (Cartmell 2017). Spotted barb (*B. cf. binotatus*) is originated from Sumatra with a habitat in fresh water at an altitude of 0 to 2000 meters above sea level (Froese and Pauly 2019). In Sumatra Island, Spotted barb (*B. cf. binotatus*) can be found in many places, namely: Maninjau Lake, Toba Lake (Kartamihardja 2019), and Gunung Tujuh Lake (Roesma et al. 2019). Spotted barb (*B. cf. binotatus*) has several local names in Bahasa Indonesia, including wader cacuk (Central and East Java), beunteur (West Java), tewaring in Samarinda (Pratama et al. 2018), keperas in Batang Toru, South Tapanuli and pora-pora in Toba Lake village. Spotted barb (*B. cf. binotatus*) is a synonym of *Puntius binotatus* (Eschmeyer et al. 2018) with the following characteristic: has four barbels; one large blotch at anterior base of dorsal fin and a round spot in the middle of caudal peduncle; color from silvery gray to greenish-gray; darker dorsally; paler or nearly white on throat and belly. It also presents in 10 other countries, namely: Brunei Darussalam, Cambodia, Laos, Myanmar, Malaysia, Philippines, Singapore, Thailand, Vietnam, and Palau, but in Singapore and Palau, they are introduced, not native species (Froese and Pauly 2019).

The application of Scanning Electron Microscopy (SEM) in fish taxonomy especially to identify surface micro-structure of fish scales has been widely used such as for the fishes of the Genus *Channa* (Dey et al. 2014; Farinordin et al. 2017) and fish families (like Cyprinidae, Channidae, Nandidae) (Farinordin et al. 2017). However, no study on scale morphologies of Spotted barb (*B. cf binotatus*) from Toba Lake using SEM has ever been reported. Hence, the present research aims to describe the scales morphologies between dorsal-fin base and lateral-line row, and lateral line of *B. cf binotatus* from Toba Lake, North Sumatra, Indonesia using SEM and to determine chemical elements of the scales. Identification of the chemical elements in fish scales is important because fish scales can be a reliable biogeochemical tag for tracing the origin of fishes.

MATERIALS AND METHODS

Ethical approval

This experiment was performed on the basis of approval by the research institute and community service of ethics committee of Medan State University, Indonesia, with the ethical approval number: ic220181/ic2rse/lppm/2020.

Scales collection

The fishes were collected from a natural pond in Pananggangan II Village (Toba Lake) (Figure 1) from July to September 2020 using landing fly fishing net. Ten female fishes of the same size were euthanized for taking the scales. The scales were gently removed with fine forceps from the left side of the body between dorsal-fin base and lateral-line row (Figure 2), and also from the

lateral line following the methods of Esmaeili and Gholami (2011).

SEM analysis

The scales were rinsed with triple distilled water and then treated briefly with 10% potassium hydroxide solution. The cleaned, scales were dehydrated in 30, 50, 70, and 90% of ethanol. Furthermore, the scales were examined by a Zeiss Discovery V12 Stereo Zoom Microscope. Then, the scales were mounted on the metallic stubs by double adhesive tape and coated with a thick layer of colloidal graphite (AGG303 (Aquadag 500 gr). The observation using SEM-EDS followed the method of Lippitsch (1990).

RESULTS AND DISCUSSION

Description of the scales between dorsal-fin base and lateral-line row

There are two types of scales between dorsal fin base-lateral line row and lateral-line scale due to differences in the center part (Figure 3a and 3b), the radii of the first type have no pattern (Figure 4a), whereas the second type has radii with an irregular mosaic pattern (Figure 4b) containing granules of various shapes and sizes. Radii were found on the anterior/rostral, lateral and posterior of the scale. The anterior/rostral side of both types of scales has interradial tongues that are wavy and notched (Figure 4c), consisting of the circuli, intercircular space, and lepidonts. However, the posterior side contains a tubercle (Figure 4c). Each circuli is separated from the other by the intercircular space (Figure 4d). Lepidonts can be found on the circuli, which are presented in a variety of shapes and sizes (Figure 4d).

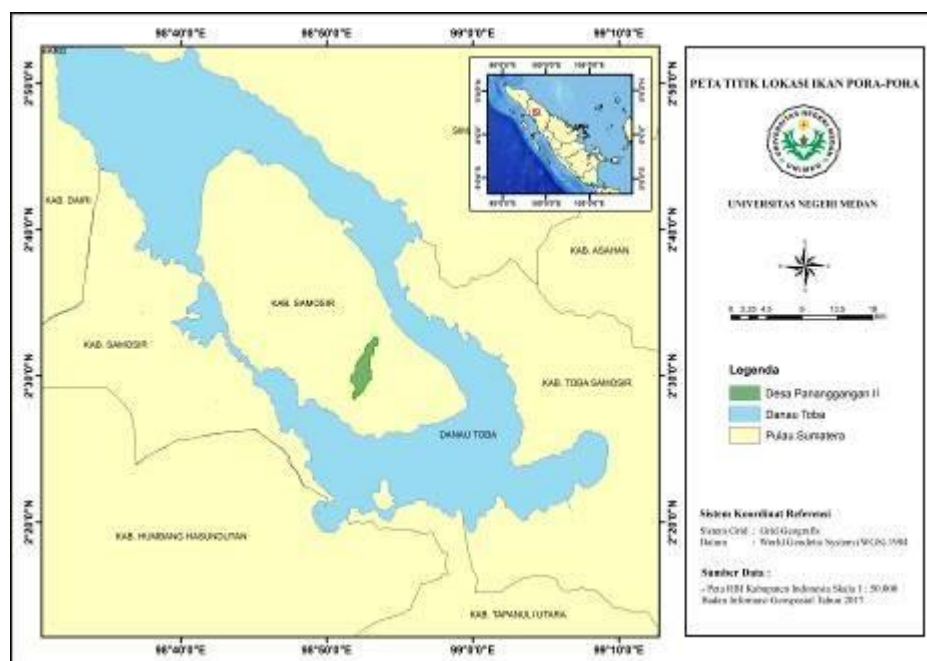
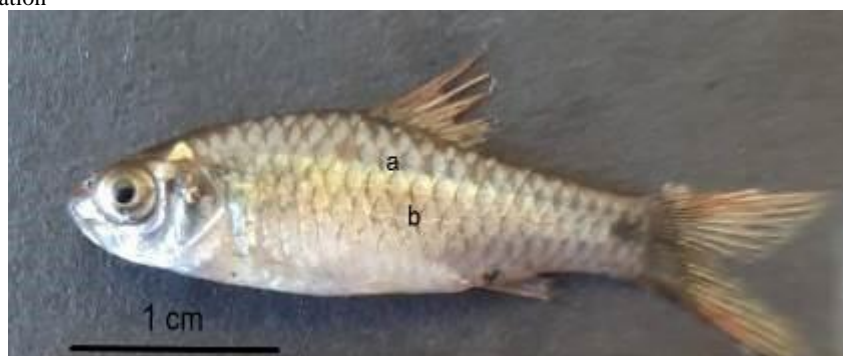
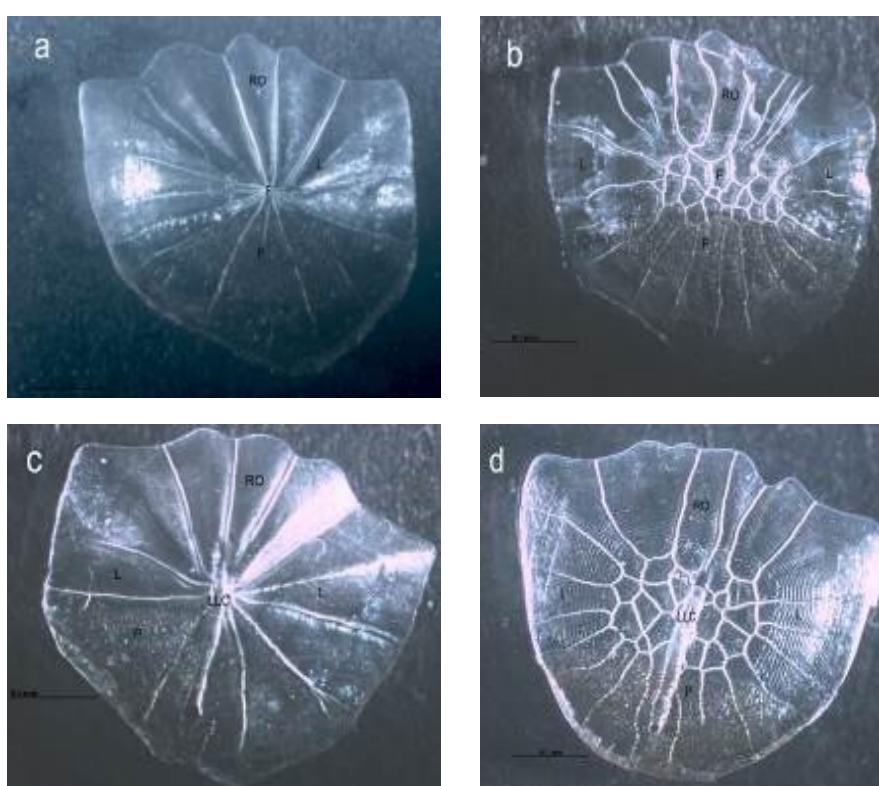


Figure 1. Sampling location**Figure 2.** The location of fish scale collection**Figure 3 (a-d).** Photograph of scales between dorsal-fin base and lateral-line row (a-b) and lateral line scale (c-d). Rostral/anterior (RO), lateral (L), posterior (P), focus (F), lateral line canal (LLC) by using Zeiss Discovery V12 Stereo Zoom Microscope

The lateral part has circuli and lepidonts (4e). The circuli of scales between dorsal-fin base and lateral-line row faces the focus. The separation between the lateral and posterior parts is characterized by a reduction in the number of circuli and widening of the intercirculi, while the posterior area consists of the tubercles and mucous pore (Figure 4e). Posteriorly, we found many tubercles with varying shapes.

Description of the lateral-line scale row

There are two types of lateral line scales due to variations at the center part (Figure 3c and 3d). Therefore, the radii structures of the two types of lateral line scales are

also different. The radii of the first type of focus scale do not have a pattern (5a and 5b), while those of the second type of scale have an irregular mosaic pattern (Figure 5c), granulated with various shapes and sizes (Figure 5d). The focus area of the lateral line scale is replaced by a lateral line canal. The ends of the anterior and posterior parts of the lateral line canal have openings called the anterior opening line canal and the posterior opening line canal respectively. The anterior opening is bigger and wider than the posterior opening, while the posterior opening is more circular (Figure 5e and 5f). The circuli were oriented towards the lateral line canal, containing lepidonts of blunt

tooth shape and various sizes (Figure 5g and 5h). Tubercles and mucous pore can be seen posteriorly (Figure 5i-5j).

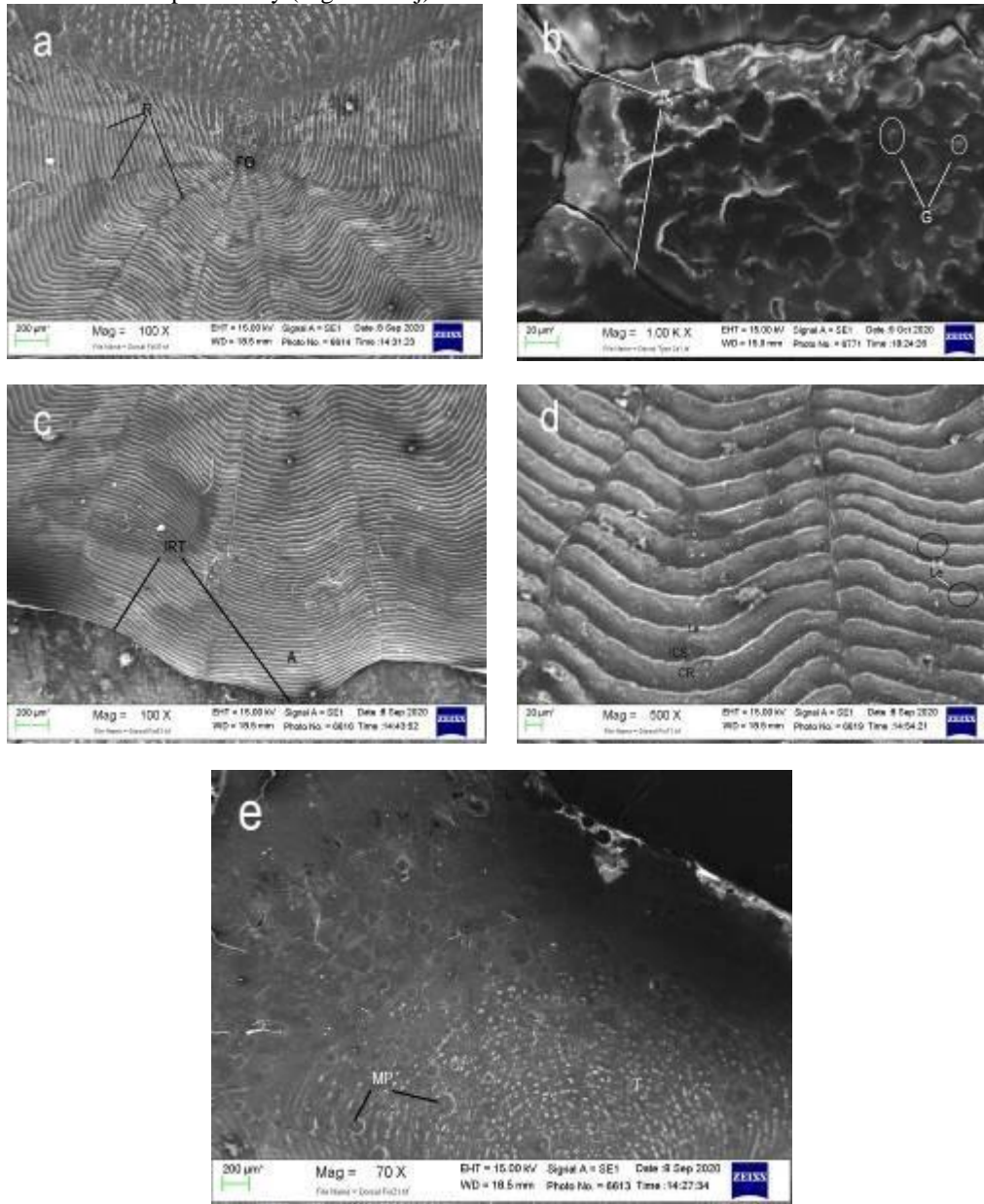


Figure 4 (a-e). SEM morphology of scales between dorsal-fin base and lateral-line row of Spotted barb (*B. cf. binotatus*). Radii (R), granule (G), interradial tongues (IRT), circuli (CR), anterior (A), intercirculi space (ICS), interradial (IRC), lepidont (Le), focus (FO), mucous pore (MP), tubercle (T), posterior (P)

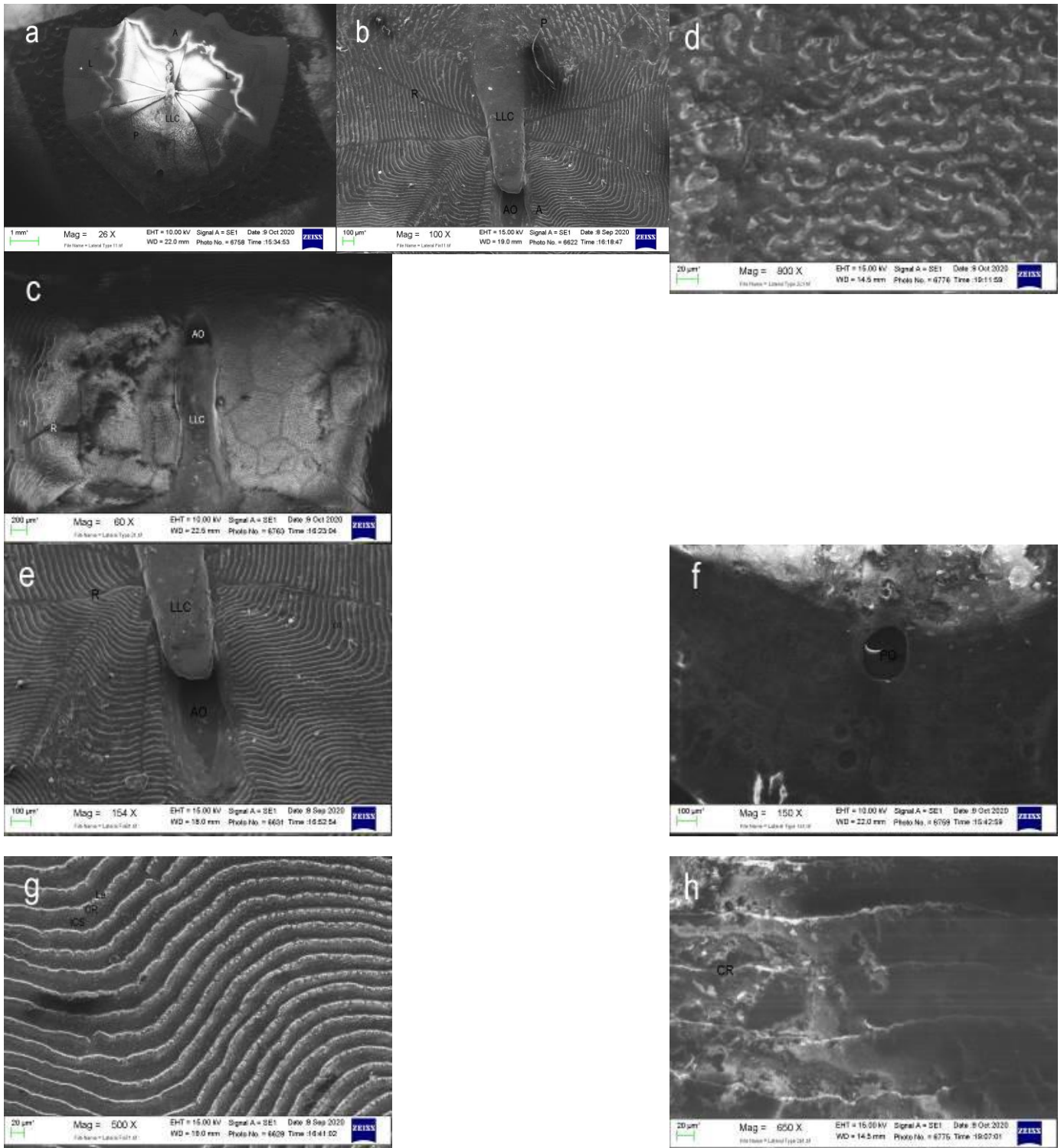




Figure 5 (a-j). SEM morphology of lateral line scale of Spotted barb (*B. cf binotatus*). Circuli (CR), anterior (A), radii (R), intercirculi space (ICS), lepidont (Le), fokus area (FO), lateral line canal (LLC), anterior opening (AO), posterior opening (PO), tubercle (T), posterior (P)

SEM-EDS analysis

The result of SEM-EDS analysis revealed that the scales between dorsal-fin base and lateral-line row, and lateral line scales contain several elements (Figure 6a and 6b). The lateral line scales of Spotted barb (*B. cf binotatus*) consist of calcium (Ca), potassium (K), oxygen (O), magnesium (Mg), sodium (Na) and phosphorus (P), whereas Na is unavailable in scales between dorsal-fin base and lateral-line row. The carbon of both scales might be from the coating process of SEM.

Discussion

Micro-structural components of fish scales, such as the focus, chromatophores, radii, circuli, lepidonts, and the region below focus, are considered to be of taxonomic

significance (Dey et al. 2014). In our study, we found two different types of patterns at the center of each the dorsal fin and lateral line scales. The center of the scales is between dorsal-fin base and lateral-line row, which is called as the focus. The first type of the scales between dorsal-fin base and lateral-line row has radii directly faces the focus, such as forming a central point (without a pattern). Meanwhile, the second type has radii that lead to the focus, forming an irregular mosaic pattern. Previous study also reported that the scales between dorsal-fin base and lateral-line row type of Spotted barb (*B. cf binotatus*), *Puntius lateristriga*, and *Hampala macrolepidota* (Hampala Barb) from Malaysia with different patterns on the focus (Farah-ayuni 2016).

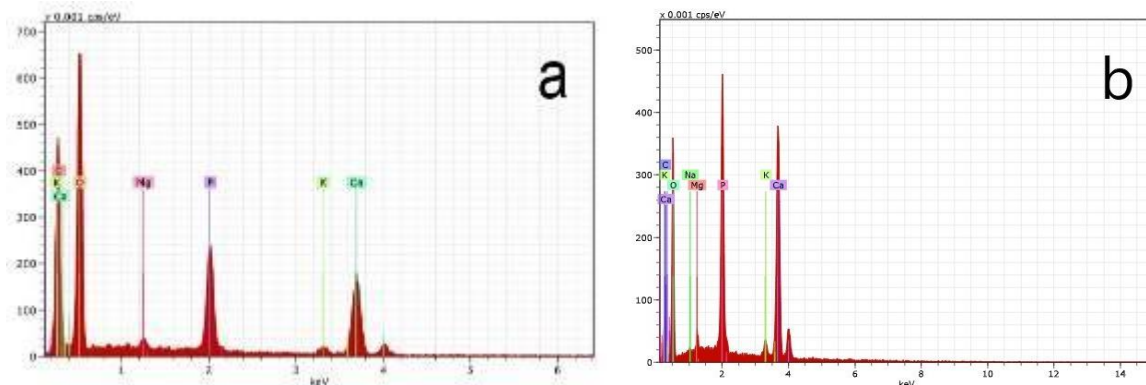


Figure 6. SEM-EDS Image of the scales between dorsal-fin base (a) and and lateral-line row (b)

The second type of Spotted barb (*B. cf binotatus*) scales which are from Toba Lake, showing both the scales between dorsal-fin base and lateral-line row, and lateral line scales with irregular mosaic patterns (irregular mosaic). Fish scales with irregular mosaic patterns were also found in *Sparisoma cretense* (Parrotfish) and *Thalassoma pavo* (Brager and Moritz 2016). Another study reported that an irregular mosaic pattern of fish scale is a

characteristic of regenerating scales (Kabir 2021). Reported in the lateral line scale of *Neolissochilus hexagonolepis* and *Neolissochilus hexastichus* (Nongrum and Bhuyan 2020). Morphological variations of scales on the body of a fish are often found not only in Spotted barb (*B. cf binotatus*), but also in other cyprinid species such as *Cyprinus carpio*, *Barbonymus gonionotus*, *Barbonymus schwanefeldii*, *Labeo rohita*, *Cyclocheilichthys apogon* (Farinordin et al.

2017), and *Scardinius racovitzai* (Şerban and Grigoras 2018). The differences in scales' morphological characteristics in each part of the fish body are very important for taxonomic identification in each species (Kontas et al. 2020). Spotted barb (*B. cf binotatus*) scales that we found were similar to Farah-ayuni (2016) study, namely: cycloid type; radii cover at anterior, posterior and lateral, radii arrangement is uniform; interradii tounge structure is irregular and serrated; focus position is in the middle of scale.

The Scanning Electron Microscopy results in our study showed that Spotted barb (*B. cf binotatus*) have the general morphological structure of a cycloid scale type, i.e. presence of focus, circuli, intercirculi, radii, tubercles, and lepidont, which is similar to *Garra rossica* (Esmaeili et al. 2012). Base of scales between dorsal-fin base and lateral-line row, and lateral line scales of *B. cf binotatus* in our study has pentagonal shaped with curved and wavy at anterior sides, more or less the similar to the both of sexes of Spotted barb (*B. cf binotatus*) from Salug Valley River of Zamboanga del Sur, Philippines, which is a cycloid scale with elliptic, oblong, pentagonal, rectangular, square, and triangular shapes on the base of their scales (Ganzon 2012). The scales between dorsal-fin base and lateral-line row have circuli which is grooved, tight and form a convex line and also forms a straight transverse line starting from the rostral edge of the focus area. Circuli on the scales are clearly visible anteriorly and laterally and not present at posterior. The appearance of circuli is as a result of the accumulation of calcium released by the skin. Therefore, the distance between circuli reflects the fast and slow periods of growth (Ganzon 2012). Lepidonts are also found on the scales of Spotted barb (*B. cf binotatus*). The structures of lepidonts on the scales of Spotted barb (*B. cf binotatus*) like denticles or blunt teeth, located in the anterior part of the circuli that have various shapes and sizes. Radii on the scales between dorsal-fin base and lateral-line row, and lateral line scales are found on the anterior, lateral and posterior, which are shaped like a straight line that leads radially to the focus area. Radii serve as a separator between each circuli. Radii consists of several types, namely primary radii, secondary radii and tertiary radii, which can provide information about the growing conditions of fish (Esmaeili et al. 2012). In addition, tubercles can be seen on the posterior side of Spotted barb (*B. cf binotatus*) scales with various shapes. The tubercles are formed due to an aggregation of the epithelial layer of the skin that covers the posterior part of the scales, which results in the appearance of a specific color in the fish since it contains chromatophores on its outer surface (Esmaeili et al. 2012).

Contrary to the center of scales between dorsal-fin base and lateral-line row, the center of a lateral line scales consists of canal, which is located along the anterior and posterior axes of the scales. The anterior and posterior ends of the line canal are composed of two holes that are called as the anterior opening line canal and the posterior opening line canal, functioning as mechano-sensory. The mechano-sensory system can provide information about the existence and identity of prey and predators and as a means of

receiving communication signals between fish (Edgley and Genner 2019). Differences in the center of the scales between dorsal-fin base and lateral-line row, and lateral line scales are common. Some studies reported that the scale varying morphologies of cyprinids and other fish species between body regions and between species can be occurred (Kontas et al. 2020).

As described in the results, the scales between dorsal-fin base and lateral-line row, and lateral line scales of *B. cf binotatus* that were observed using SEM-EDS contained some elements, namely: O, Ca, P, Mg, K, and Na. However, Na element did not present in the dorsal fin scale. Different species contain different elements of scales, for example, the scales of *Barilius bendelensis*, which of the same family as Spotted barb (*B. cf binotatus*), exhibit different elements compared to the scales of Spotted barb (*B. cf binotatus*) which are Ca, P, Mg, C (carbon), Na (sodium), Fe (iron), Cl (chlorine) and S (sulfur) (Thomas 2019). Thomas (2019) also reported that the elements of the various parts of these scales can help in differentiating population habitat conditions, determining fish life history, and sexual dimorphism.

We conclude that Spotted barb (*B. cf binotatus*) from Toba Lake is of the cycloid type with two different patterns at the center of both the scales between dorsal-fin base and lateral-line row, and lateral line scales. The elements of Ca, K, O, Mg, Na and P were found in both scales, except Na element undetected in the scales between dorsal-fin base and lateral-line row. Based on these results, SEM images of the scales of Spotted barb (*B. cf binotatus*) are a reliable tool in fish scales morphological description, which is useful in taxonomy study and also can be used to observe the breakage of the circuli.

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