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Scale microstructure study of Bluering angelfish, *Pomacanthus annularis* (Bloch, 1787) from Veraval fishing harbour, Gujarat, India

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Abstract

Despite one of the important species of coral reef environment, information regarding scale morphology of Pomacanthids is rare. In present study, first attempt was made to document various scale characteristics of Bluering angelfish, *Pomacanthus annularis* (Bloch, 1787) from Veraval fishing harbour, Gujarat, India. For detailed study, scales from different body area: head scale, belly scale and caudal scale were observed and measured with the application of Scanning Electron Microscopy (SEM). The contemporary analysis of scale morphology recorded spinoid type cycloid scale in all over body regions in considered species. Besides scale type some consistent scale characteristics from different body regions such as; semi-circular shaped and posteriorly located focus, a striate form of interradianal circuli, rectangular shaped and round ended lepidonts on circuli of anterior field, elongated and triangular shaped spinous structure on the posterior field were found distinctive traits of studied species. Based on the obtained result, the study concluded that certain features on a scale can be potentially employed as reliable taxonomic tools. Moreover, morphometric indices (J-indices, radii count, scale size) were additionally considered as an effectual tool for species discrimination, even in the case of closely related species.

Keywords:

Fish scale, J-Indices, Lepidonts, SEM, Spinoid

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Introduction

Marine angelfishes of the family Pomacanthidae are typically tropical coral reef inhabitant widespread in the Atlantic, Indian and Pacific oceans, which contain 8 genera and 82 species worldwide (Chung and Woo, 1999; Rajeswari *et al.*, 2014). Among them, over 80% of the Pomacanthidae found along Indo-Pacific region (Chung, 1996). They play an important role in coral reef environment and economically important as high-priced ornamental fishes. Most species mainly feed on algae and sponges, small amounts of zoantharians, gorgonians and hydroids (Chung, 1996; Chung and Woo, 1999).

Scale structure has been utilized as versatile research material in the ichthyological field. The importance of scale morphology in systematic studies of fishes increased with the introduction and development of scanning electron microscopy (SEM) (Lippitsch, 1990; Jawad, 2005; Teimori, 2016). Prior studies strongly recommend that the detailed study of fish scale microstructure can be useful in the identification of fishes up to major groups and species level, phylogeny, age and growth studies, migration, past environment experience by fish, pollution status of water body, paleontological and genetic studies, sexual dimorphism and in determining the diet of piscivorous predators

(Lagler, 1947; Lippitsch, 1990; Tandon and Johal, 1996; Jawad, 2005; Esmaeili et al., 2014). Many authors have demonstrated the worth of scale morphology and ultrastructural characteristics for fish taxonomy and classification (Roberts, 1993; Jawad, 2005; Dey et al., 2014; Brager and Moritz, 2016; Al-Awadhi et al., 2017; Azab and Khalaf-Allah et al., 2019). Few studies have been reported to use the morphometric characteristics of scales from different body regions in fish systematics (Jawad and Al-Jufaili, 2007; Harabawy et al., 2012; Al-Awadhi et al., 2017; Razak and Vauzia, 2019).

Various aspect of pomacanthids biology, habitat, diversity, feeding, growth and behaviour patterns (Enrich, 1975; Chung, 1996; Chung and Woo, 1999; Bariche, 2010; Alwany, 2009) were studied, but information regarding scale morphology is rare (Roberts, 1993). Despite its prominent importance among the coral habitat, the scale morphology of the Pomacanthidae has not been studied along Indian coast in great detail. Therefore, the present work aimed to documenting valuable scale characteristics of Pomacanthidae species, *P. annularis* for future systematics studies.

Materials and methods

The specimens of *P. annularis* were collected from Veraval fishing harbor, Gujarat during the period of September, 2018 to November, 2019. Morphometric measurements and meristic counts were recorded for identification of specimen after collection. Standard identification key is used to identify fish specimens (Day, 1878; Fischer and Bianchi, 1984). Additionally, for a detailed study of scale microstructure of considered species, head scale (HS), belly scale (BS) and caudal scales (CS) from 2nd/3rd row of the scale below dorsal fin were gently removed with fine forceps. The scales were washed thoroughly with water and cleaned with 2% potassium hydroxide solution for a few minutes. Afterwards, the cleaned

scales were dehydrated with ascending series (30%, 50%, 60% and 70%) of ethanol. Immediately, the scales were kept between the two microslides for 72 hours. The cleaned and dried scales were coated with a thick layer of gold in Ion sputter and observed under SEM at 15 kV. Various SEM micrographs were recorded and measured for detailed study. The relative J-indices for scale length [$(J_{sl} = \text{length of scale} / \text{fish standard length} \times 100)$] and width ($J_{sw} = \text{width of scale} / \text{fish standard length} \times 100$) were calculated (Esmaeili et al., 2014; Brager and Moritz, 2016). Other scale morphometrics and meristic characters were also recorded. The statistical analysis was performed with using IBM: SPSS v.20 statistical packages.

Results and discussion

Scale microstructure

Spinoid cycloid type scale with rough dorsal and smooth ventral surface was found on body regions of the fish. The scale maintains the same morphological properties located on different parts of the body. The length of the scale was greater than width, which indicates anon-circular shape of the scale. The scale surface was divided into an anterior or rostral, lateral and posterior or caudal field. The anterior field of the scale is embedded into the skin and overlapped by the posterior side of the accompanying scale. The dorsal surface of scale shows characteristics ornamentation, which comprises of ridges, grooves and granules forming circular to near-circular rings around a centrum called the focus.

Focus

The first developing part of the scale during ontogenesis known as focus. In *P. annularis*, semi-circular shaped and posterior located focus recorded on all over (Fig 1). The focus on the scale was located on the posterior field. The circuli that surrounds the focus appears to be incomplete.

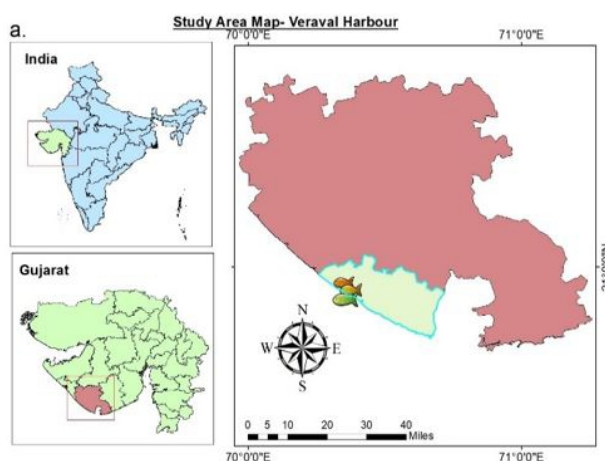


Plate 1. Sampling site



Plate 2. Lateral view of Pomacanthus annularis

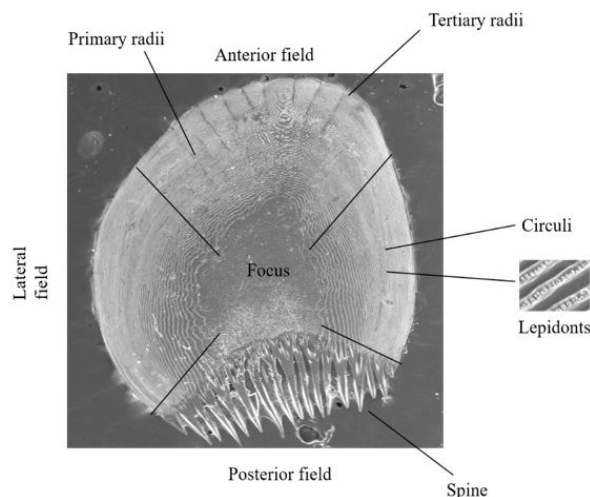


Plate 3. Scale terminology

Circuli

The elevated (growth) ridges present on the outer surface of the scale, which found contrasts with regard to width, spacing, shape and arrangement. Many growth ridges found on anterolateral field of the scale. Circuli were distinct, densely separated in anterior field and widely separated in lateral part observed. Absence of circuli on posterior field observed due to development of the spine. The first interradial circuli

was straight and without denticles observed (Fig 2). The interradial circuli on anterolateral were almost straight, but slight concave circuli also observed. Space between circuli is known as inter circular space. A detailed variation recorded in width, groove and inter-circular space of anterolateral circuli. The maximum width and inter circular space were observed in lateral circuli as shown in Table.1.

Lepidonts

Circuli bears small denticles or tooth-like structure termed as lepidonts. In studied species, numerous, moderately spaced and round ended lepidonts found on the anterolateral circuli (Fig 3). Lepidonts on the circuli of the anterior field observed with consistent shape and arrangement in scales from different body regions. Small, rectangular as well as irregularly shaped lepidont found on circuli of lateral field (Fig 4).

Radii

On scale surface, circuli interrupted at right angel by deep grooves called radii. Radii were found only on the anterior field of scale. Three types of radii were recorded and categorized based on their point of origin on the scale of *P. annularis*: primary radii, extend from the focus to the margin; secondary radii, extends

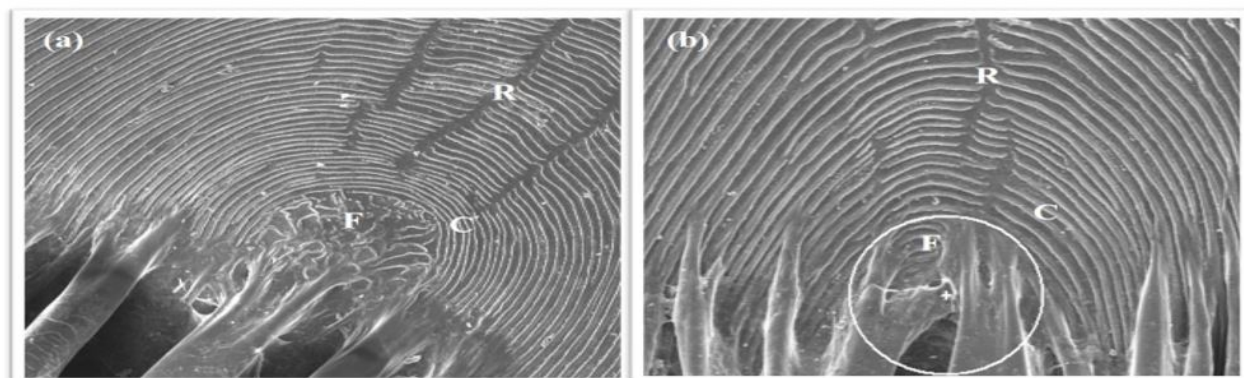


Fig 1. Scanning electron micrographs in the focus region of scales obtained from the different body regions of *P. annularis* i.e., shows semi-circular shaped focus and surround with incomplete circuli in BS and CS. F: Focus, C: Circuli and R: Radii.

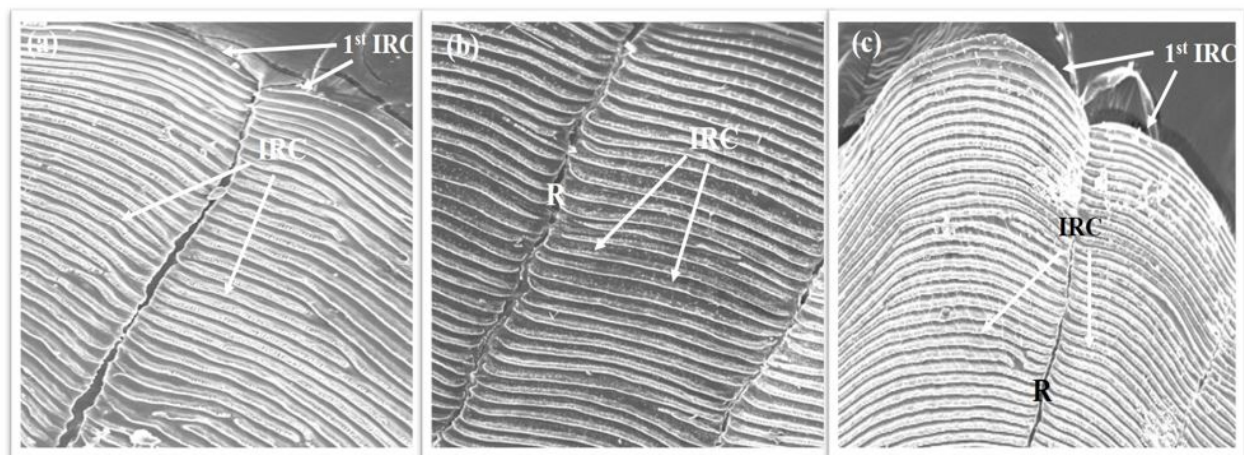


Fig 2. Scanning electron micrographs in the anterior circuli of scale from the different regions of *P. annularis* i.e., recorded striate shaped 1st Inter-Radial circuli (1st IRC) and Inter Radial circuli (IRC) in the anterior circuli of HS, BS and CS.

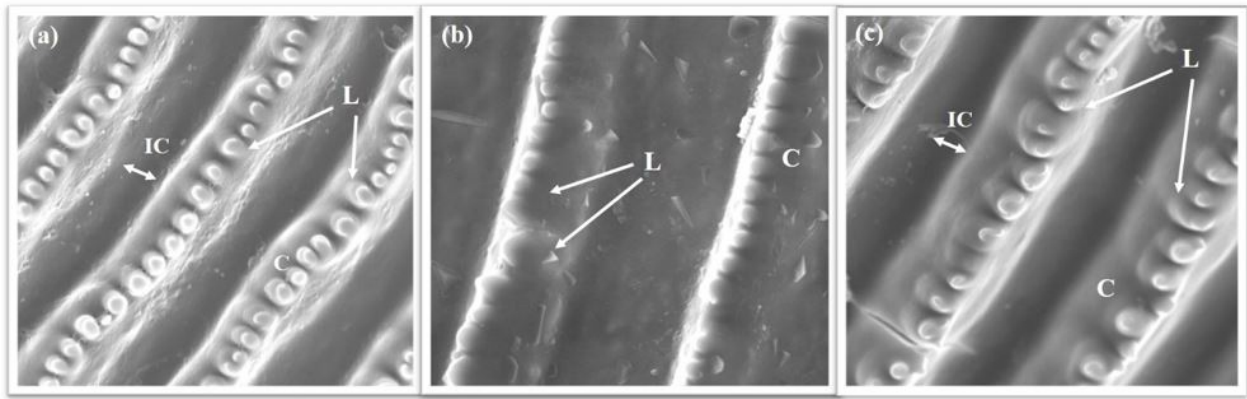


Fig 3. Scanning electron micrographs in the anterior circuli of scales obtained from the different body regions of *P. annularis* i.e., recorded round ended lepidonts (L) and inter-circular space (IC) in (a) HS (b) BS and (c) CS.

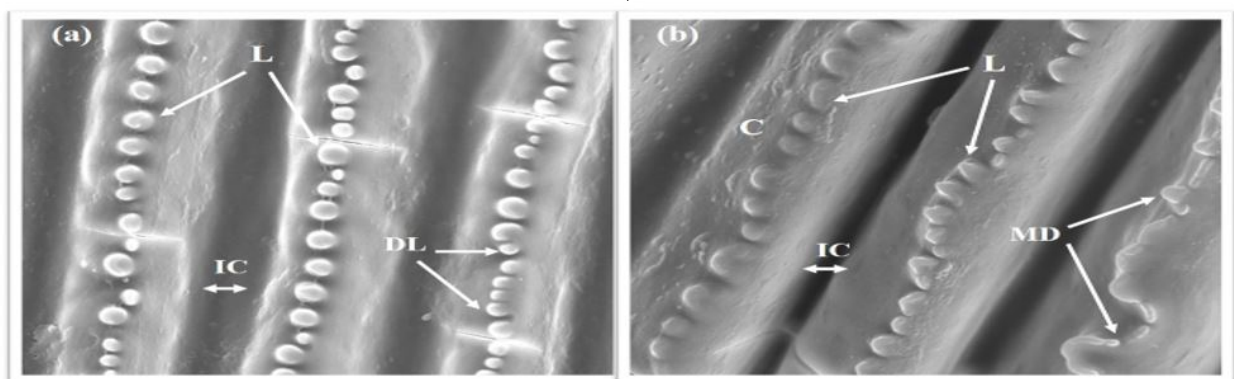


Fig 4. Scanning electron micrographs in the lateral circuli of scales obtained from the different body regions of *P. annularis* i.e., recorded small, round ended lepidonts (L) on lateral circuli of (a) BS and (c) CS. Developing lepidonts on lateral circuli of (a) BS. Minute dentition on lateral circuli of (b) CS. Developing lepidonts (DL), Minute dentition (MD) and Inter-circular space (IC).

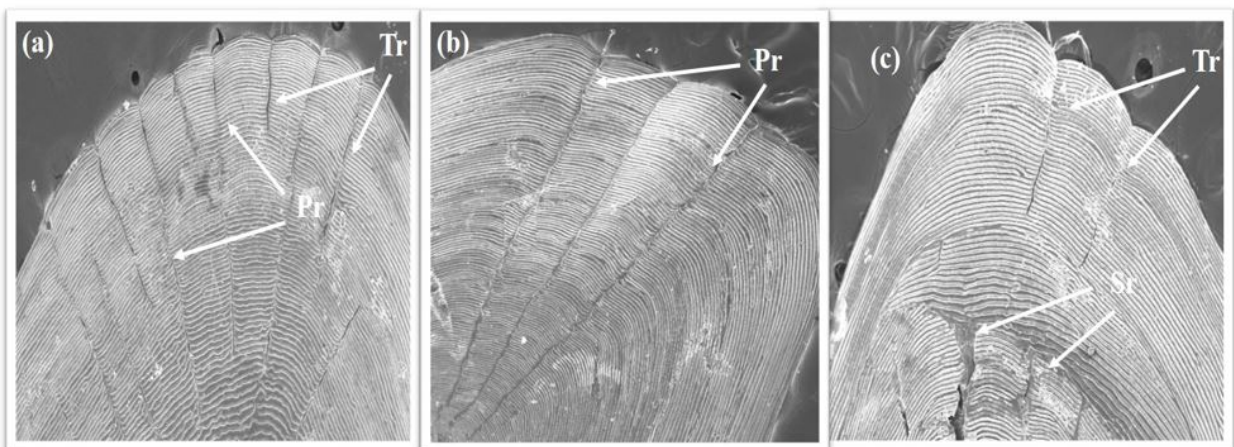


Fig 5. Scanning electron micrographs shows three types of radii in anterior region of scale from different region of *P. annularis*; (a) primary radii (Pr) and tertiary radii (Tr) recorded in HS, (b) primary radii (Pr) recorded in BS (c) primary radii and secondary radii (Sr) recorded in CS.

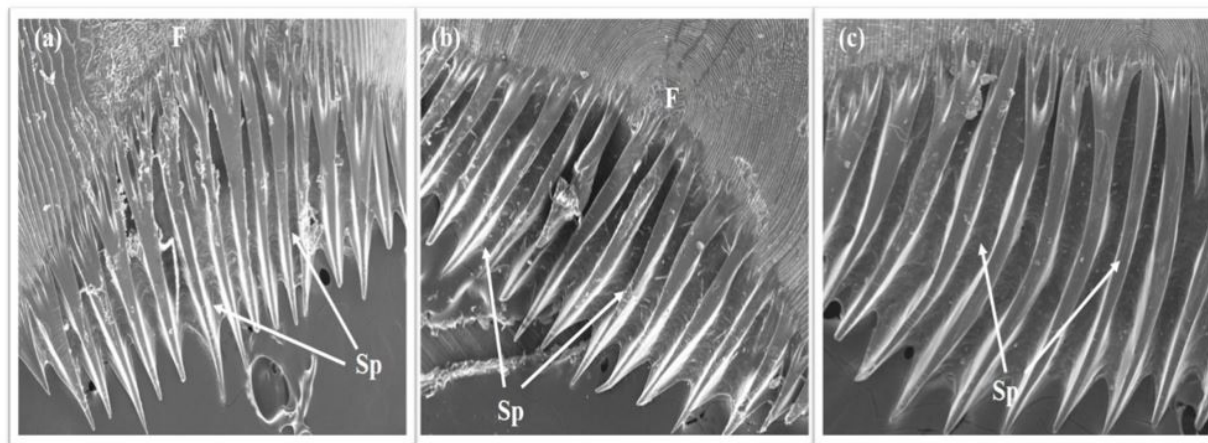


Fig 6. Scanning electron micrographs in the posterior field of scales from the different body regions of *P. annularis* i.e., recorded long, triangular shaped spine on posterior field of (a) HS (b) BS and (c) CS. Spine (Sp), Focus (F).

between focus and midway; and tertiary radii extend between midway and margin (Fig.5). The radii number varies between 3-7 on the scale of studied species. In the present study, no significant relationship between the number of radii and scale size observed.

Posterior field

The posterior field in the scale of *P. annularis*, characterized by the presence of elongated spine in linear rows (Fig.6). Average number of the spine, width and length shown in Table2.

Table 1. Morphological observation of *P. annularis*

Morphometric characteristic (cm)

Total length (cm)	19.40
Standard length	15.80
Pre –anal length	8.00
Anal fin base length	7.40
Anal fin length	Second spine
length- 2 cm	
Largest ray length- 2.6 cm	
Pre dorsal length	3.70
Dorsal fin base length	12.30
Dorsal fin length	Largest spine
length- 1.60 cm	
Largest ray length- 7.00 cm	
Pre pelvic length	4.40
Pelvic fin length	5.50
Pre pectoral length	4.40
Pectoral fin length	4.70
Body depth	11.10
Pre-orbital length	1.50
Orbital length	1.20
Post-orbital length	1.90
Head length	4.60
Caudal peduncle length	1.30
Caudal peduncle width	2.40
Caudal fin length	3.60

Meristic characteristic

D: XIII/20, A:III/20, P: 18-19, V: I/5, C:15, Ll: 63, Lt.r.: 14/35

Table 2. Structural details of some scale microstructure in *P. annularis*

Sr. No.	Characters	Measurement
1	Scale width	2.93 ± 0.52
2	Scale length	3.66 ± 0.83
3	Focus	
	Position	Postero-central
	Location (Distance from posterior margin)	1.357 ± 0.49
4	Anterior circuli	
	Average width	7 ± 0.45
	Average inter-circular space	2.07 ± 0.32
5	Lateral circuli	
	Average width	8.76 ± 2.19
	Average inter-circular space	2.46 ± 0.33
6	Lepidonts	
	Shape	Round ended
	Breadth	1.59 ± 0.08
7	Radii	
	No.	5.33 ± 2.08
	Inter-radial distance	8.65 ± 1.46
8	Spine	
	No.	17.6 ± 1.15
	Shape	Triangular
	Length	566.94 ± 49.10
	Breadth	57.08 ± 7.13

In present study, general scale morphology of *P. annularis* elucidates various scale characters including radii, shape and position of focus, striate type circuli and width, round ended lepidonts and elongated spine. The investigation recorded square shaped, spinoid type cycloid scale inmost part of the body. All spined scales classified into three categories (a) crenate: with simple marginal indentations and projections, (b) spinoid: with spines persistent with the main body of the scale, and (c) ctenoid, with spine developed as discrete ossifications rather from the main body of the scale (Roberts, 1993; Esmaeili et al., 2014). The presence of spinoid scale has been reported in

Myctophidae, Pomatomidae, Cheatodontidae and Macrouridae (Roberts, 1993; Brager and Moritz, 2016). A few authors reported to mistaken continuous spinous structure from the main body as ctenii (Chung and Woo, 1999; Razak and Vauzia, 2019). Each scale with semi-circular shaped focus, positioned at posterior field observed in studied species as shown in Fig.1. The similar type of focus position observed in *Chaetodon falcula* (Bloch, 1795) by Razak and Vauzia (2019). Earlier workers formidably revealed that the position of focus on the scale remains the same throughout life (Jawad and Al-Jufaili, 2007; Esmaeili et al., 2007). Diverse focus shape and position recorded in different species and also within species (Kaur and Dua, 2004; Esmaeili et al., 2014). The antero-lateral field marked with growth ridges called circuli. The circuli formation is due to the overabundance calcium salts emitted by the skin and their subsequent deposition on the scale, so the distance between circuli indicates fast or slow growth period. Circuli arrangement was also responsible for scale shape (Esmaeili et al., 2007). According to Kaur and Dua (2004), the arrangement of circuli around the focus is important features for species specificity. The present study noted that the shape of the first interradii circuli was straight, which is another exclusive characteristic within species (Fig.2). Some other shape (convex, concave) recorded in cichlid and cyprinids fishes (Lippitsch, 1990; Esmaeili et al., 2007).

In present work, it was evident that lepidonts on circuli of the anterior field have a consistent rounded end shape in scales from various body regions. The present outcome agreed with the results obtained by Razak and Vauzia (2019) in *C. falcula* (Bloch, 1795). Similar trend also observed on scale of *Saurida tumbil* (Bloch, 1795) (Jawad and Al-Jufaili, 2007). Lepidonts on circuli were significant structures known to support species distinctness (Kaur and Dua, 2004). They additionally play a vital role in anchoring and water circulation around the scale surface (Jawad and Al-Jufaili, 2007). Variation also observed in size and arrangement of lepidont on circuli of the anterior and lateral circuli (Table.1). Small size lepidonts recorded on lateral field, due to less significant of anchorage on lateral field (Jawad and Al-Jufaili, 2007). Differences in the shape, size, and arrangement of lepidonts proved worthwhile in distinguish genera and even some taxa at the specific level (Hollander, 1986; Lippitsch, 1990; DeLamater and Courtney, 1974; Esmaeili et al., 2014; Harabawy et al., 2012; Azab and Khalaf-Allah, 2019). The presence of primary, secondary and tertiary radii on the anterior field of the scale shows growth phenomenon in considered species (Fig.5). Average 3 to 7 numbers of radii recorded on the scale of considered species. Analogous observation recorded in scale of *P. imperator* (Bloch, 1787) with 3-9 numbers of radii (Chung and Woo, 1999). There was no noteworthy correlation between radii number and scale size recorded in studied species. On the other hand, a significant relationship between radii number and

scale size recorded in *Mullus surmuletus* (Linnaeus, 1758) and *M. barbatus* (Linnaeus, 1758) (Esmaeili and Gholami, 2009).

Several authors recommended that the higher number of radii may correlate with the better nutritive conditions and flexibility of the fish body (Tandon and Johal, 1996; Jawad and Al-Jufaili, 2007; Esmaeili et al., 2014). The present work documented the presence of elongated and triangular shaped spine on the posterior field of scale of *P. annularis*. A comparable spinoid structure on posterior field has been recorded in scale of *P. semicirculatus* (Cuvier, 1831) (Roberts, 1993). Several past studies have been successfully demonstrated the potential of granulation pattern on the posterior field of scale as a characteristic feature of the cycloid and ctenoid scale of cyprinids and percoids (Lippitsch, 1990; Johal and Agarwal, 1997; Esmaeili et al., 2014). Prior study confirmed the worth of apical (posterior) margin of scale in differentiate between Chaetodontids and Pomacanthids (Cockerell, 1915; Razak and Vauzia, 2019). The present work strongly emphasized the presence of spinous structures on scales as distinctive characteristics of studied species. According to Roberts (1993), the presence of spined scales can be a valuable tool not only in systematics studies but also accommodating in dealing with teleost evolution.

Conclusion

The present study reported some consistent scale characteristics of *P. annularis* such as position and shape of focus (semi-circular), circuli arrangement, lepidonts shape on circuli (round ended) and shape of the spinous structure (triangular), which effectively highlight as specific species characteristics. Furthermore, the quantitative characters of scales also helpful to provide adequate information regarded to species distinctness. Based on obtain result, the present work affirms the worth of scale morphology in systematics study of considered species.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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