Research in Marine Sciences
Volume 6, Issue 2, 2021
Pages 899 - 914

Assessing sexual dimorphism and systematic with the biometry and scale characteristics of Black *pomfret Parastromateus* niger (Bloch, 1795)

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Received: 2020-12-12 *Accepted: 2021-04-18*

Abstract

The knowledge about biometric differences is essential for the description of sexual differentiation of any particular species and also has numerous implementations in fish biology. A total of 100 samples (50 males and 50 females) of *Parastromateus niger* (Bloch, 1795) commonly called 'black pomfret' were gathered from Quetta fish market of Balochistan to observe first time biometrically by calculating the morphometric, meristic and scale characteristics for examining the sexual dimorphism and phylogenetic relationship. The overall results revealed that highly strong and significant correlations ($r>70$; $p<0.05$) were observed between morphometric characteristics and total body length (TL), except diameter of eye (ED) for males, females and combined sexes, respectively. Whereas, week and insignificant correlations ($r<0.50$; $p>0.05$) were found between meristic characters and TL respectively. Moreover, significant variations (t-test; $p<0.05$) were found in morphological characteristics between sexes, except ED, snout length (SL) and body depth (DB) and anal fin-rays count (AFC), which reveals insignificant variation (t-test; $p>0.05$), respectively. However, detail microstructures of scale exhibited no structural variations between males and females; however, could be valuable in systematic study of this species. Thus, it had been concluded that variations in morphometric characteristics could be consider as valuable tools that displays the sexual dimorphism of black pomfret.

Keywords: Dimorphism; Meristic; Microstructures of scale; Morphometric; *Parastromateus niger;* Sexual phylogeny.

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1. Introduction

Family Carangidae is composed of diverse groups of economically important coastal pelagic fishes and include 32 genera and 140 species, which are commonly called as, scads, black pomfrets, pampanos, jacks, queen fish, and amberjacks (Nelson, 2006). *Parastromateus niger* belongs to this family is commonly known as “Black Pomfret” commonly abundant in Pacific Ocean and Indian ocean. It found near the muddy bottom and mainly feed on small invertebrates and eggs and larvae of fishes or sometimes shows seasonal variations in their diets (Dadzie, 2007). This species is a rich source of essential protein for human consumption. Spawning season extends from July to October. Body shape was laterally compress and elongated with dark grey colored fins. Pectoral fins are sickled-shape, while both dorsal and anal fins are wedged-shape, whereas caudal fin is forked shape. The size of female is comparatively larger than males and reaches up to 30cm in TL (Tan, 2009). Whole body is covered with both cycloid and ctenoid scales. As several microstructures of scale including its size, width, shapes, and types, and also the various shapes of ctenii on ctenoid scale, different position of focus, and arrangements of radii and circulii on scale had also been used by several workers like Lagler (1947); Vernerey and Barthelat (2010), Esmaeili and Gholami (2011), Ibanez *et al*. (2011), Zubia and Rehana (2011), Esmaeli *et al.,* (2012) Ambareen *et al*. (2015), and Zubia *et al*. (2015a), which had also demonstrated that scale characters can be used as viable tools in observing the phylogenetic relationships between various fish groups, and also for sexual dimorphism. Likewise, Matondo *et al.* (2010), had observed sexual dimorphism between sexes of *Upeneus vittatus* on the basis of its scale shapes.

Morphological characters can be categorized into two types, (i) morphometric characters, and (ii) meristic characters (Honebrink, 2000). Information’s regarding to morphometric and meristic variables play a vital role in analysis of fish taxonomy and their phylogeny. Mostly morphometric characters can be classified into (i) genetically (Narrow range), (ii) intermediate (Moderate range), and (iii) environmentally (Vast range) controlled characters that also show’s a great potential value in systematic, fisheries management and assessment of sexual dimorphism (Quist *et al.,* 2012; Fatnassi *et al.,* 2017; Ukenye *et al.,* 2019; Famoofo and Abdul, 2020). These characters are now used to identify the fish body shapes as previously described by Cabral and Murta, (2002) and Palma and Andrade (2002). Mostly, fish exhibit great variances in their external morphology, even within same and different species or sometimes also varied among many populations belong to single species (Hossain *et al*., 2015). Morphometric characters therefore can be considered as important tools in identification of species, genus or stocks of fish (Murta *et al.,* 2008; Imam *et al.,* 2011). As fish species identification is quiet essential practices in fisheries conservation and management; therefore, traditionally, most works usually used only external morphological characteristics for it (Zubia *et al.,* 2015b); however, sometimes these methods had been proved unreliable. Therefore, further analysis on microstructures of fish scale has also been considered to observe their significance in systematic studies of various species, as well as for sexual dimorphism. Presently, no published literature was available on biometric data as

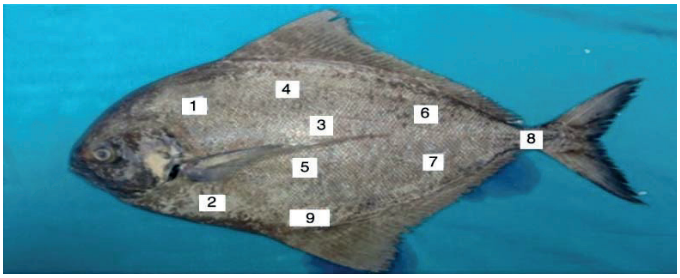

Figure 1. Showing the eight body regions for scale collection from *Parastromateus niger*

well as the microstructures of scales of the *Parastromateus niger*.

The objective of this study will be a first approach to describe morphological and scale characters for observing their basic importance in sexual dimorphism and phylogeny of this species.

2. Materials and methods

2.1. Fish sampling

A total of 100 samples of *Parastomateus niger* (50 females and 50 males) purchased from Fish market of district Quetta of Balochistan from June 2018 to July 2019. Then 11 morphometric and 2 meristic characters calculated by the procedures previously followed by Manimegalai *et al*. (2010), and Zubia *et al.* (2015b) by using measuring board with meter scale and Vernier calliper.

2.2. Morphometric measurements and Meristic counts

Morphometric measurements include, total body length (TL), standard length (STL), fork length (FL), head length (HL), snout length (SL), eye diameter (ED), post-orbital length (POL), pectoral fin length (PFL), anal fin length (AFL), dorsal fin length (DL), body depth (BD); while Meristic characters includes, dorsal fin-rays count (DFC) and anal fin-rays count (AFC), respectively.

2.3. Scale sampling and Scanning Electron Microscopy (SEM)

In this study, scale samples collected from eight body regions of fishes includes, i.e., **1:** head region, **2:** Pectoral region, **3:** Abdominal region, **4:** Region below dorsal fin, **5:** Mid-dorsal region, **6:** Caudal peduncle region, **7:** Region below anal fin, **8:** Base of caudal fin **9:** Region above pelvic fin by followed the method of Zsuzsanna (2016). Then washed Scales in warm water at 60°C for 3 to 4 hours for removing mucous or dust particles from scale surface. Scales were further cleaned by soaked in 5% KOH solution for 10 minutes and dried on filter paper, and finally kept between two microscopic slides for prevents them for curling after air dry. These dried scales were mounted by using aluminum stubs for Scanning Electron Microscopy (Drill Japan JSM-6380A) analysis and finally take several images of the scales as shown in Figure 1. The images were then photographed by digital attached camera, as shown in Figures 2, 3, 4.1-4.8, 5.1-5.8 respectively.

2.4. Statistics

All Statistical analysis of data was carried out using MS Excel and SPSS statistical package program for Windows Ver. 26.

2.4.1 Linear Regression relationships between Morphometric and meristic characters with total body length (TL) of fish

Linear regression equation was also adopted to detect the proportional relationships between all above mentioned morphological characteristics of this species with TL as described by Rimzhim and Goswami (2015) as mention below:

$$Y = a+bX \qquad (1)$$

where, 'X' represents TL of fish, and 'Y' represents morphometric and meristic characters; 'a' shows intercept, and 'b' represents the regression slope (Zubia *et al.*, 2015b).

2.4.2 Correlation Coefficient (r)

In this study, the values of correlation coefficient (r) were also calculated between all these morphometric and meristic characters with TL at 5% significance ($p<0.05$) for observing the strength of relationship between two variables i.e.
i. $r<0.50$ represents weak correlations between two TL versus morphological character
ii. $r> 0.60$ shows moderate type of relationship TL versus morphological character
iii. $r>0.70$ shows stong relationships between two variables TL versus morphological character
whereas, variations between all these morphometric and meristic variables of male and female sexes of black pomfret were observe by using two sample t-tests at 95% confidence interval (CI).

2.4.3 t-test analysis

For conduct a 2-sample t-test, male and female populations of Black pomfet were considered independent. The 2-sample t-test was used to analyzes the difference between means of each morphological characters of male and female populations of this species is statistically significant at $p<0.05$ or insignificant when $p>0.05$. The hypotheses of a two-tailed test would be:

- H_0: $\mu_1 - \mu_2 = \delta_0$ (means of each morphological character of two populations are equal)
- H1: $\mu_1 - \mu_2 \neq \delta_0$ (means of each morphological character of two populations are different)

where, μ_1 and μ_2 are the means values and δ_0 is the hypothesized difference between the two population means. If the test's p-value is less than 0.05, it is considered to be highly significance level.

3. Results

3.1. Morphometric measurements and meristic counts

The TL calculated for combined sexes of this species was ranging between 12.4 to 33.5 cm in TL, while females ranged from 14.2 to 33.5 cm in TL, whereas males were found in TL ranged between 12.4 and 25.0 cm, respectively.

3.2. Linear relationship between total body length (TL) vs. Morphometric and meristic characteristics of males and females Parastromateus niger

In this study, eleven morphometric and two meristic characters were calculated and their relationships were observed with TL, as presented in Table 1, respectively. The correlation coefficient (r) values calculated for females were found in ranged from 0.88 to 0.98, while for male ranged from 0.85 to 0.97, respectively.

Table 1. Linear Regression relationships between TL and various morphological characteristics of *Parastromateus niger*

X	Y	Mean±S.D	Min	Max.	A	b	r	p-value	CT
Combined sexes (N=100)									
TL	M/C								
	TL	23.7±4.9	12.4	33.5					
	FL	23.3±5.2	11.2	29.4	0.14	1.1	0.98	0.00	***
	STL	20.4±4.9	9.5	27.0	1.17	1.2	0.97	0.00	***
	HL	6.1±1.3	3.3	8.0	-1.8	4.6	0.93	0.00	***
	SL	2.1±0.5	1.0	2.9	5.12	9.5	0.80	0.00	***
	ED	1.1±0.2	0.7	1.4	0.45	22	0.64	0.00	**
	POL	2.8±0.6	1.5	3.9	0.65	8.1	0.93	0.00	***
	PFL	8.3±1.6	4.0	11.0	-0.7	3.3	0.94	0.00	***
	DFL	13.4±2.7	7.0	16.5	-0.8	2.0	0.91	0.00	***
	AFL	12.2±2.6	6.3	15.1	-0.5	2.1	0.93	0.00	***
	BD	11.9±2.3	5.9	15.1	-3.3	2.4	0.90	0.00	***
	DFC	43.5±1.6	41.0	46.0	-61	2.0	0.31	0.33	*
	AFC	38.9±0.5	35.0	40.0	-59	2.1	0.25	0.14	*
Female (N=50)									
TL	M/C								
	TL	26.5±6.0	14.2	33.5					
	FL	23.3±5.3	12.0	29.4	-0.08	0.88	0.98	0.00	***
	STL	20.4±5.0	10.2	27.0	-1.49	0.83	0.97	0.00	***
	HL	6.1±1.3	4.0	8.0	0.52	0.21	0.94	0.00	***
	SL	2.1±0.5	1.0	2.9	-0.24	0.09	0.92	0.00	***
	ED	1.1±0.2	0.7	1.4	0.19	0.03	0.88	0.00	***
	POL	3.1±0.6	1.7	3.9	0.14	0.11	0.94	0.00	***
	PFL	8.3±1.7	5.2	11.0	1.20	0.27	0.91	0.00	***
	DFL	13.4±2.8	7.6	16.5	1.40	0.45	0.94	0.00	***
	AFL	12.2±2.7	6.4	15.1	0.61	0.44	0.96	0.00	***
	BD	11.9±2.4	6.8	15.1	1.66	0.39	0.96	0.00	***
	DFC	43.5±1.7	42.0	46.0	38.77	0.18	0.41	0.33	*
	AFC	38.9±0.5	38.0	40.0	37.97	0.04	0.16	0.22	*
Male (N=50)									
TL	M/C								
	TL	21.0±3.9	12.4	25.0					
	FL	18.6±3.5	11.2	22.0	0.24	0.87	0.97	0.0	***
	STL	16.4±3.2	9.5	19.2	-0.33	0.80	0.96	0.0	***
	HL	5.1±0.9	3.3	6.5	0.86	0.20	0.85	0.0	***
	SL	1.8±0.5	1.0	2.5	-0.54	0.11	0.75	0.0	***
	ED	1.0±0.2	0.7	1.3	0.29	0.03	0.47	0.02	*
	POL	2.6±0.6	1.5	3.0	-0.36	0.14	0.93	0.0	***
	PFL	6.6±1.2	4.0	7.5	0.33	0.30	0.95	0.0	***
	DFL	11.2±2.3	7.0	15.0	-0.05	0.54	0.86	0.0	***
	AFL	10.4±2.2	6.3	13.2	-1.00	0.54	0.93	0.0	***
	BD	10.6±2.0	5.9	13.2	0.82	0.46	0.88	0.0	***
	DFC	42.0±1.1	41.0	44.0	42.76	-0.04	0.17	0.32	*
	AFC	38.4±1.8	35.0	40.0	31.33	0.34	0.54	0.12	*

Note: S.D=standard deviation; a=intercept; b=regression slope; r=correlation coefficient; *** shows strong correlation (r>0.70), ** shows moderate correlation (r >0.60; * shows weak correlation (r<0.50), 2-sample t-test value significant if P<0.05; whereas α shows 2 sample t=test values insignificant if P>0.05. Total Length and all other measurements were in cm. M/C represents Morphological characteristics.

Table 2. Analysis of 2-sample t-test at 5% Confidence level (Cl)

Two samples t-test and CI				
x	Y	t-test	p-value	CI
	M/C			
TL	TL	2.90	0.01	(1.71, 10.88)
	FL	-2.80	0.01	(-9.45,-1.33)
	STL	-2.61	0.02	(-8.51,-0.90)
	HL	-2.56	0.02	(-2.21,-0.21)
	SL	-1.69	0.11	(-0.84,0.08)
	ED	-1.23	0.23	(-0.28,0.07)
	POL	-2.43	0.03	(-1.22,-0.09)
	PFL	-3.13	0.01	(-3.26,-0.64)
	DFL	-2.41	0.03	(-4.85,-0.34)
	AFL	-2.13	0.05	(-4.37,-0.04)
	BD	-1.95	**0.07α**	(-3.83,0.13)
	DFC	-2.61	0.02	(-2.80,-0.29)
	AFC	-1.22	**0.24α**	(-1.87,0.52)

Note: 2-sample t-test value significant if P<0.05; whereas α shows 2-sample t=test values insignificant if P>0.05.

3.3. Sexual dimorphism

Moreover, significant variations (t-test, p<0.05) were found between males and females of *Parastromateus niger,* except the snout length (SL), ED and body depth (DB), dorsal-fin rays count (DFC), and anal-fin rays counts (AFC) that revealed insignificant variations (t-test, p>0.05) between sexes of this species, as shown in Table 2.

3.4. Scale characteristics

Moreover, scale microstructures includes i.e., scale shapes, size, number of radii, and position of focus were also observed from head region, pectoral region, abdominal region, region below dorsal fin, mid-dorsal region, region below anal fin, base of the caudal fin and caudal peduncle region of black pomfret fishes of this study inorder to observe the variations in morphology of scales collected from different body region and their significance in systematic study of this species, as shown in Figures 2, 3, 4.1-4.8, 5.1-5.8 respectively.

4. Discussion

4.1. Relationship of morphological characters with TL

The results of this study showed that strong and significant correlations (p<0.05) were found between TL and morphometric characters of males, females and combined sexes of this species, except eye diameter (ED) of males and combined sexes showed weak (r<0.50) or moderate (r=0.60), but significant correlations (p<0.05) with TL, respectively. Our present result was in agreements with Dadzie (2007), Shakir (2008), Zubia *et al.,* (2015b), and Smith (2018), who also found the similar results of morphometric studies.

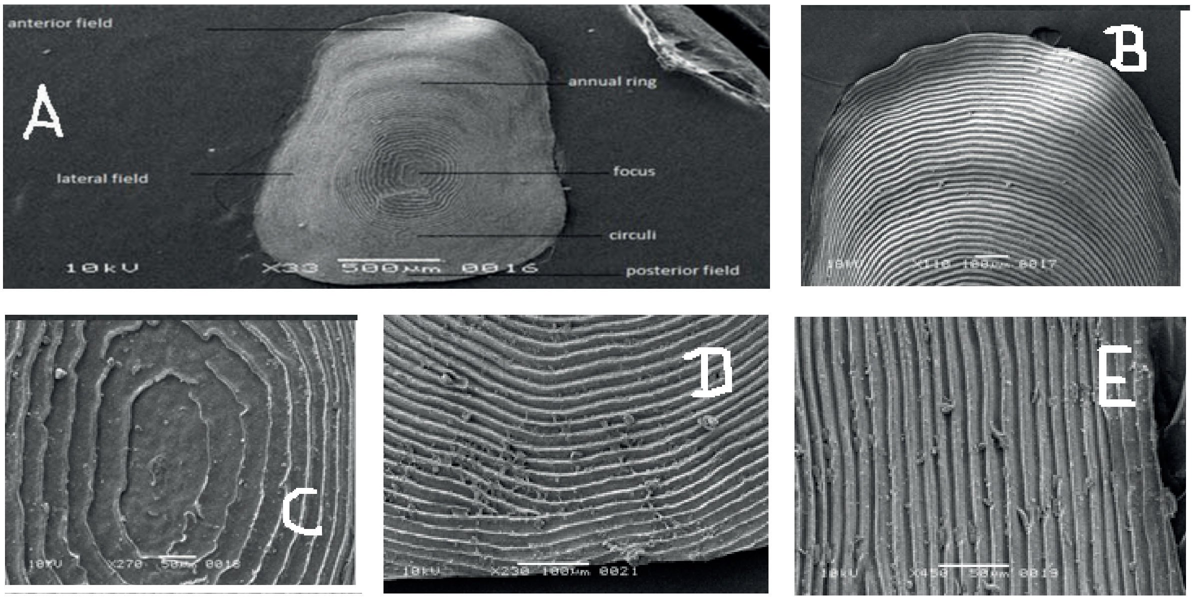

Figure 2. SEM microphotographs of scale of *Parastromateus niger* (A) complete scale from pectoral region (B) anterior field with circuli (C) Focus (D) Posterior field (E) Lateral field

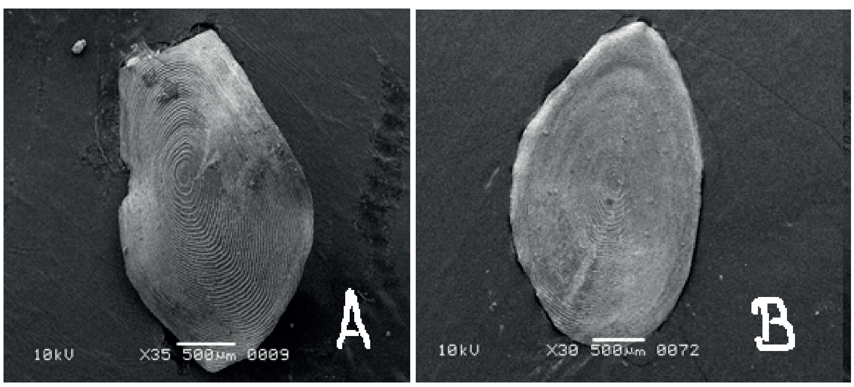

Figure 3. SEM microphotograph of *Parastromateus niger*. (A) Scale of male fish from head region, (B) Scale of female fish from head region

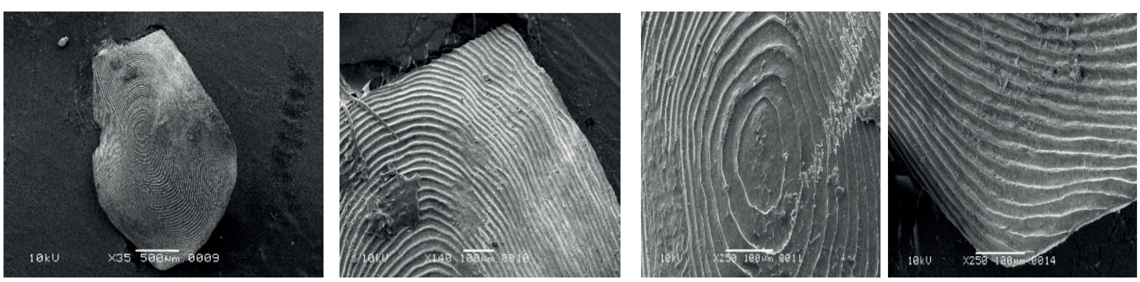

Figure 4.1. SEM of region 1 (head) showing anterior field, focus and posterior field

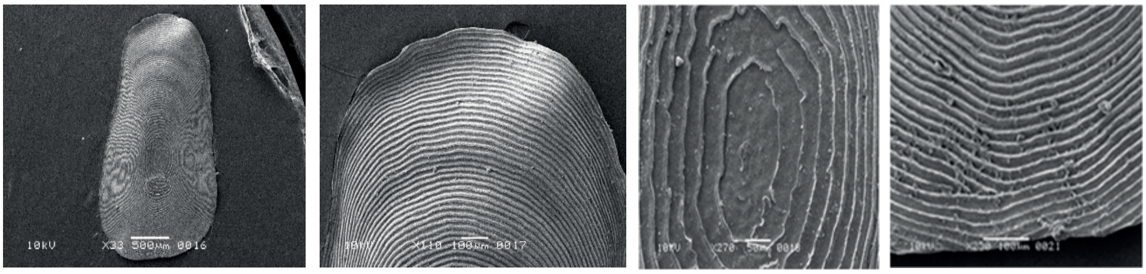

Figure 4.2. SEM of region 2 (pectoral) showing anterior field, focus and posterior field

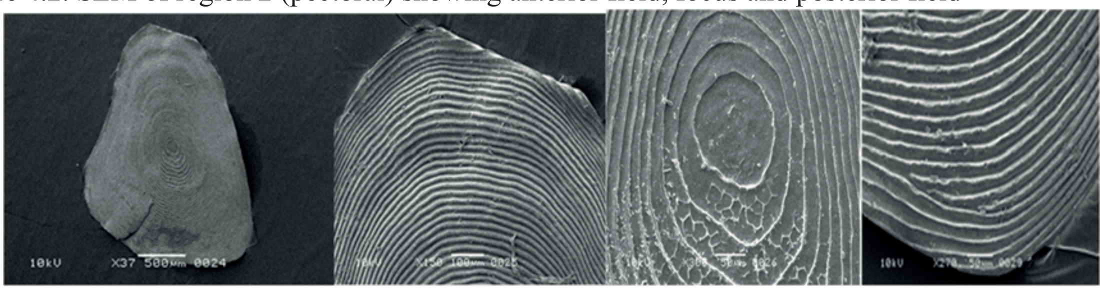

Figure 4.3. SEM of region 3 (abdominal region) showing anterior field, focus and posterior field

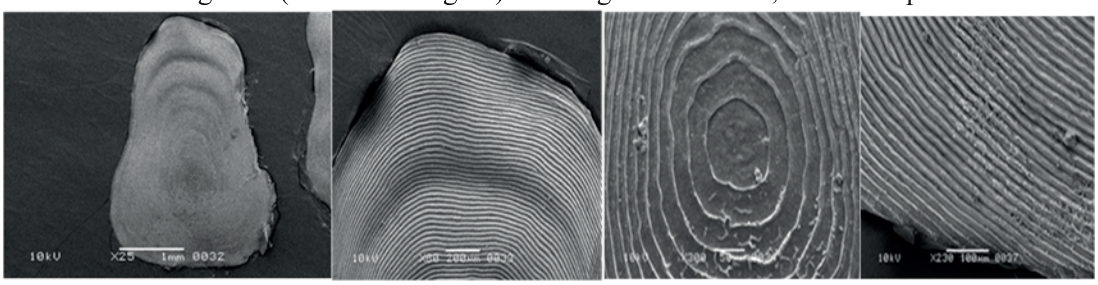

Figure 4.4. SEM of region 4 (below dorsal fin) showing anterior field, focus and posterior field Scales of Male fish of *Parastromateus niger*

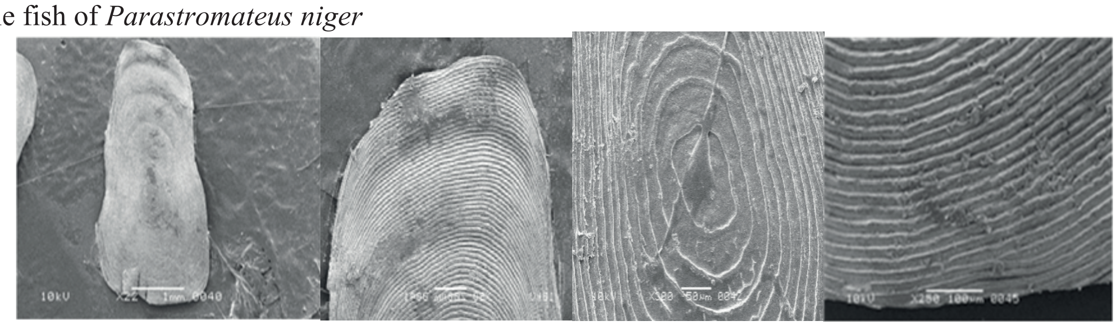

Figure 4.5. SEM of region 5 (Mid dorsal region) showing anterior field, focus and posterior field

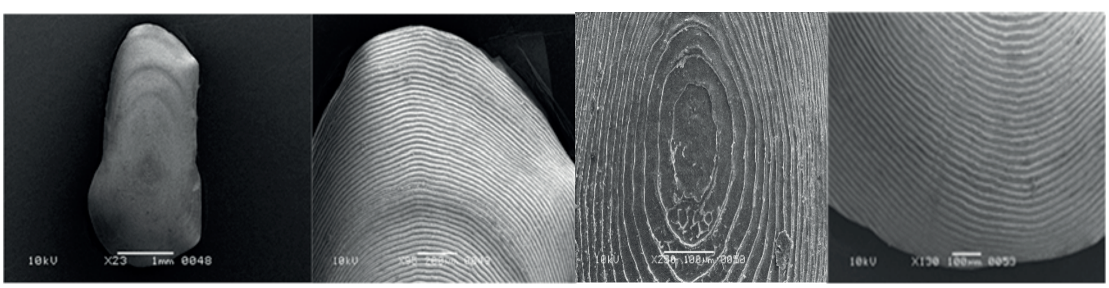

Figure 4.6. SEM of region 6 (Caudal peduncle) showing anterior field, focus and posterior field

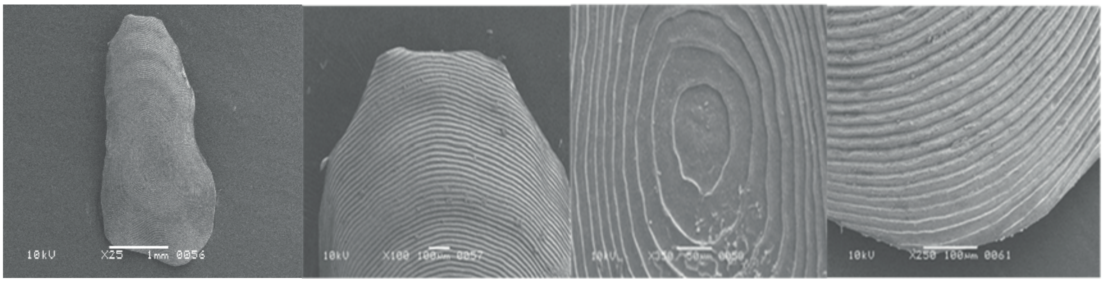

Figure 4.7. SEM of region 7 (below anal region) showing anterior field, focus and posterior field

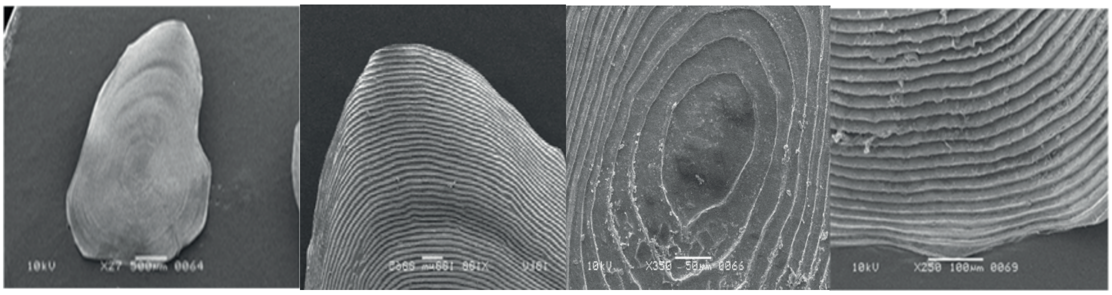

Figure 4.8. SEM of region 8 (Caudal region) showing anterior field, focus and posterior field Scales of Male fish of *Parastromateus niger*

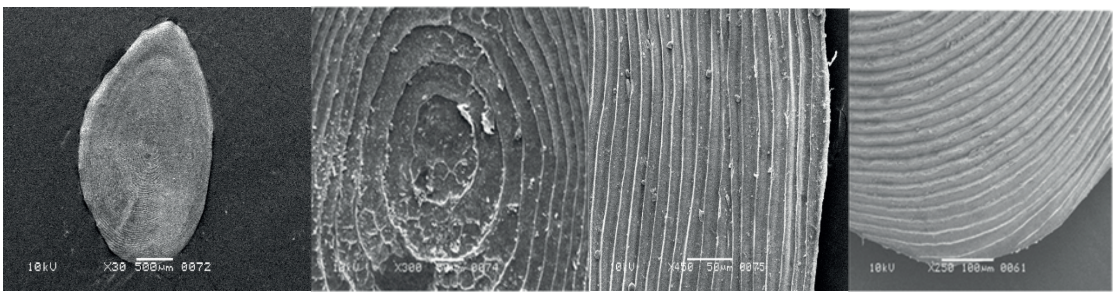

Figure 5.1. SEM of region 1 (head region) showing focus, Lateral field and posterior field

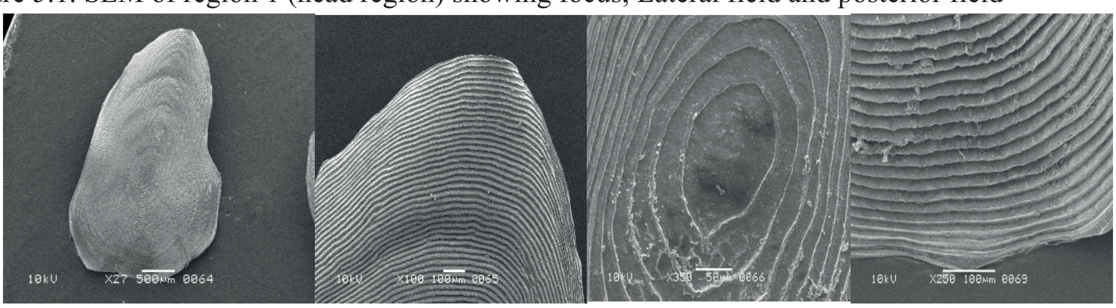

Figure 5.2. SEM of region 2 (pectoral) showing anterior field, focus and posterior field

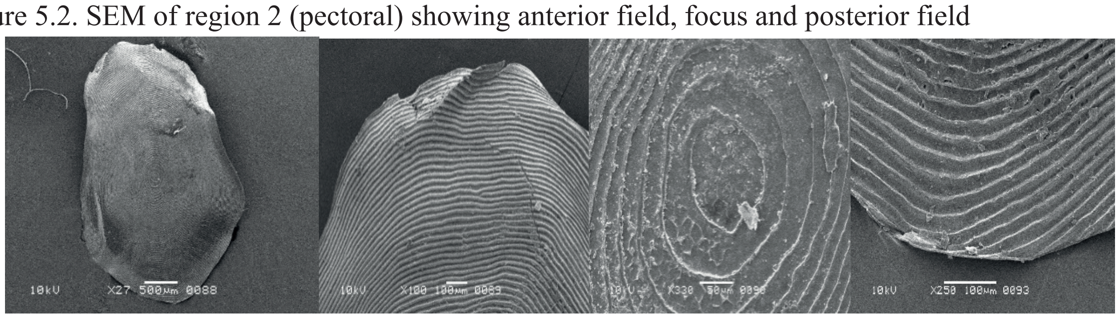

Figure 5.3. SEM of region 3 (abdominal region) showing anterior field, focus and posterior field

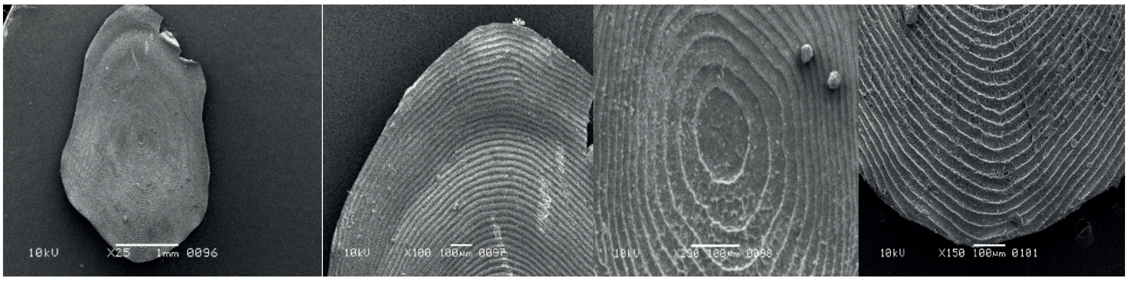

Figure 5.4. SEM of region 4 (below dorsal fin) showing anterior field, focus and posterior field Scales of Female fish of Parastromateus niger

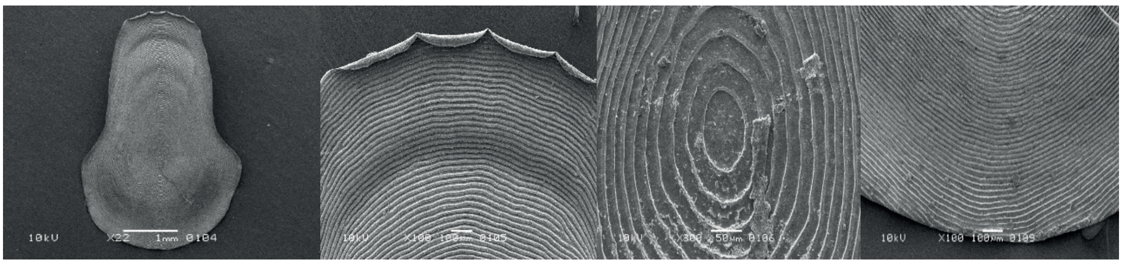

Figure 5.5. SEM of region 5 (Mid dorsal region) showing anterior field, focus and posterior field

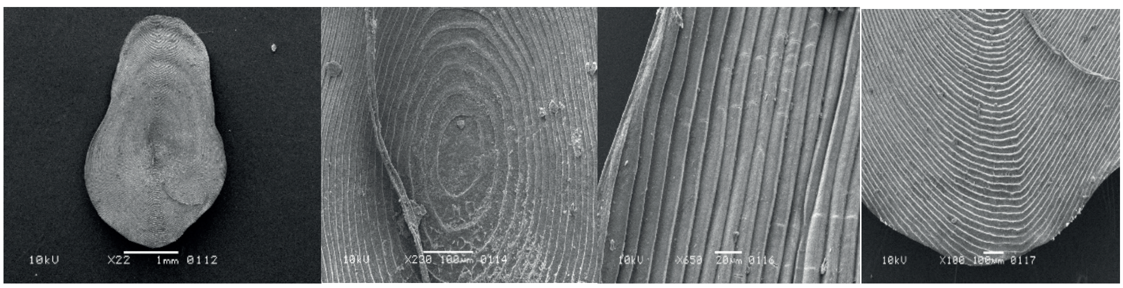

Figure 5.6. SEM of region 6 (Caudal peduncle) showing focus, Lateral field and posterior field

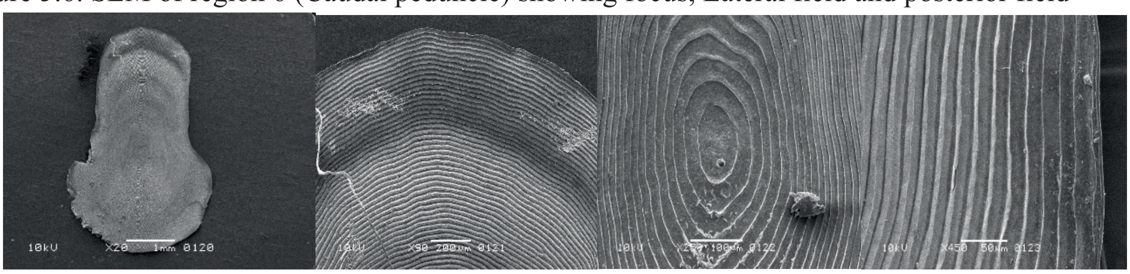

Figure 5.7. SEM of region 7 (below anal region) showing anterior field, focus and Lateral field

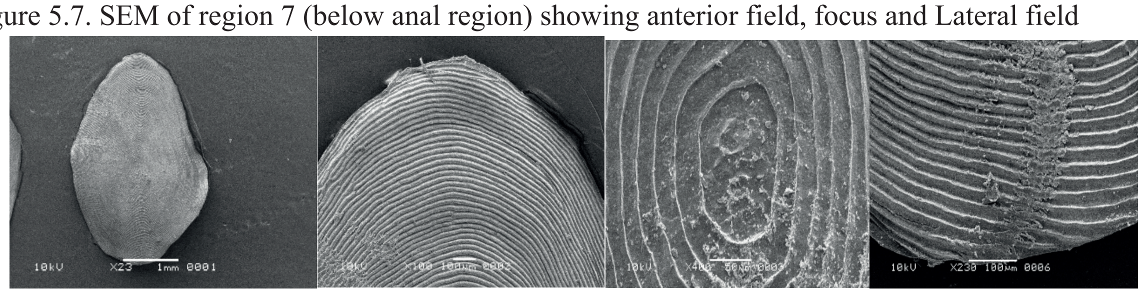

Figure 5.8. SEM of region 8 (Caudal region) showing anterior field, focus and posterior field Scales of Female fish of Parastromateus niger

But meristic characteristics of males, females and combined sexes were showing only weak and insignificant correlations ($r<0.50$; $p>0.05$) with total body length (TL). Meristic characters were fixed for males and females of this species throughout the life history. Hence, it had been proved that sexual dimorphism occurs in *Parastromateus niger* based on all these morphometric characteristics of this study. Therefore, our present investigation was in agreements with Zhan and Wang (2012), Rathipriya *et al.* (2018), Famoofo and Abdul (2020). All such morphometric variations between sexes might be because of changes in several environmental conditions of habitat, availability of food as well as breeding season (Hossain, 2010). Hence, morphometric variables are considered good tools, which help in assessment of sexual dimorphism of this species (Fatnassi *et al.,* 2017). Though, fish is much sensitive vertebrate to the environmental changes; however, it has great ability to quickly adapt them by producing variations in its morphometric variables (Hossain, 2010). Ghasemzadeh and Ivantsoff (2004) observed that variations in external morphological characters are recurrently using as a primary way of generating taxonomic differentiation between different families or genera or species. According to Narejo (2010), the information of morphometric variables of fish along with their statistical association is quite necessary for authentic systematic investigation.

4.2. Scale characteristics

In this study, scale shows structural pattern of true cycloid type scale, which is generally ovoid to oblong in shapes. Microstructures includes anterior, posterior and lateral fields, circuli, focus lies in center or more towards anterior field of scale, while radii were completely absent on them. Anterior field was inserted in skin, whereas posterior field was exposed. The scales from the pectoral region represent all characteristic features of a true cycloid scale; therefore, could be designed as key type scales of this species that could be valuable in taxonomy. Scales found in head and caudal regions were smaller in size. Focus was mostly oval or round shaped, while its position was also found to be different in all scales when collected from several body regions of fish. Focus lies more towards the center of all scales, except the head scales of male fish, in which focus was lies more slightly near the anterior field. From focus, lines-of-growth were emerging out, and are known as 'circuli' (Figure 2), which contain space between them are called inter circular space. These circuli were chiefly many elevated ridges occurs on the scale surface and showed great variances with regard to their thickness, arrangement or relative spacing between them. For example, the posterior field of scale contains comparatively large inter-circulur space that the anterior field. Such minimum inter-circulur space occurs in anterior field of scale might be due to the presence of focus on it. The circuli were found as wedged shape with broad base and pointed from their upper region. Posterior portion of scale was enclosed with thick layered epidermis, while anterior portion was soft, and epidermis was lacking on it. Distinct annuli were also observed in anterior and lateral fields of scale, which indicates the fish age. These annual rings were apparently visible as dark or light bands in alternative forms as presented in the Figures 2, 3, 4.1-4.8, 5.1-5.8 of this study.

As yet no previous literature was published to explain the structure of black pomfret scales, therefore, literature published on other fish species was considered in this study. Lagler (1947) observed that the scale size, shapes, types and number of various countable structures like ctenii, radii, circuli and position of focus on fish scales could be considered as least usable characters that can used for the identifications of higher taxonomic levels (families), as well as species or geographical variant populations. Likewise, Batts (1964) had also described similar key-based microstructures of fish scale that have been proved as valuable tools in identification of various flat fish species. Sire *et al.* (2009) had described that fish scale can be considered as most important structure of fish integumentary system, which shows great variations in their ontogeny, histology and morphology during various ichthyological studies of various fish groups. Therefore, the use of scale morphology in systematic classifications of various fish groups can be seen about one hundred years ago by Agassiz (1833), who described the classification of various fish groups based on their scale types, however with development of scanning electron microscopy (SEM), detail studies of microstructure's of a fish scale has now considered as an important key characters that can be valuable in systematic and phylogeny of different groups of fishes as reported by several workers including Roberts (1993), Esmaeili *et al.* (2012), Zubia *et al.* (2015a), Teimori *et al.* (2017), and Alcaraz and Gholami (2019). Earliest researchers like Dapar *et al.* (2012), Ganzon *et al.* (2012) and Ibáñez *et al.* (2012) also revealed that the ancient lifetimes of fish, variations in habitat condition and various other environmental factors can produce great variations in scale morphology. For example, study of scale morphology among six Iranian endemic *Aphanius* species by Teimori *et al.* (2017) observed that the general structure of scale in these species was true cycloid, except in *Aphanius ginaonis,* which possess though cycloid scales but also with some kind of spinous structures that are found on their posterior edge that could be used as a valuable key character for reviewing the phylogenetic relationships of this species.

Moreover, the detail studies of all microstructures of true cycloid scales of *Parastromateus niger* by scanning electron microscopy (SEM) of the present study was in agreement with Hilton *et al.* (2010), Negi and Negi (2015), and Zsuzsanna (2016), who also reported the similar results for scale morphology in *Molva molva, Barilius barna* and *Merluccius merluccius*. The position of focus lies in focus more towards the anterior field was also in accordance with Esmaeili *et al.,* (2012), who also stated the same position of focus in the scales of *Garra rossica.* As the inter circular space lies among the circuli is actually indicates the fast and slow growth rates in fishes, because during both winter and autumn seasons, the growth of whole fish and also its scales becomes retarded due to less availability of food, and thus reduce the space between the circuli, and therefore responsible for the formation of dark bands or annuli on scales. The position of circuli round the focus can also play an important role in identification of any fish species (Esmaeili *et al.* 2007). Hence, according to Fisher and Pearcy (2005), circuli are those microstructures of scale that can be considered a useful indicator of seasonal changes, spawning season, feeding rate, pollution, migration from one aquatic environment to other (Helfman *et al.,* 2009). In addition, the development of radii on fish scale was also connected with types of aquatic habitat, scale flexibility, nourishment, and movement of fishes as previously described by Johal *et al.* (1984), Tandon and Johal (1996), and Esmaeili *et al*. (2007).

Conclusion

As a result, this study was a first approach to describe morphological and scale characters for observing their basic importance in sexual dimorphism and phylogeny of Black pomfret *Parastromateus niger* (Bloch, 1795). These results of this species will shed light on the next researchers. Thus, from the obtained results, it was concluded that biometry and scale characteristics could also provide key characters in fish taxonomy, variations in the populations belong to same species as well as in sexual dimorphism.

Acknowledgements

I express my gratitude to Mr. Yusuf, Technical engineer in centralized lab of University of Karachi for his assistance in scale pictures from scanning electron microscopy (SEM).

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