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Morphological and Genetic Relationship of two Closely-Related Species of Snappers (Family: Lutjanidae) from Egyptian Red Sea

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Abstract: The present study is the first attempt to study the morphological and genetic relationship of two species of family: Lutjanidae in Red Sea using meristic and morphometric characteristics as well as SDS-PAGE for muscle proteins. Specimens of *Lutjanus quinqelineatus* and *Lutjanus ehrenbergii* (Bloch, 1790) were collected from Hurghada fishing harbour, Egypt and immediately brought to the laboratory in an insulated ice box. The results showed that the meristic and morphometric measurements among the species of *L. quinquelineaatus* and *L. ehrenbergii* did show some variations, however, these variations were not significantly different ($p>0.05$) enough to make a submission that the species were morphologically different. In addition, *L. quinquelineatus* and *L. ehernbergii* had the same number of protein bands (20 bands). In the other hand, *L. quinquelineatus* and *L. ehernbergii* muscle proteins were separated into several bands, these bands were differed in quantitative parameters. Electrophoretic pattern of *L. quinquelineatus* showed unique bands (MW., 266.43, 215, 119.82, 30.51 and 27.8 kD) while *L. ehernbergii* had another unique bands (MW., 75.97, 50.81, 30.86, 30.07, 27.18 and 16.6 kD). Based on the results, the two species analyzed are more or less closely related to each other. Future studies using biochemical-genetic markers and DNA barcoding hopefully will establish new ventures in the field of stock management and conservation of snappers.

Keywords: Red Sea, snapper, meristic, morphometric SDS-PAGE, genetic analysis.

1 Introduction

Fishes of the family Lutjanidae is one of the largest in the order perciformes and comprises 4 subfamilies, 17 genera and 112 species, mainly found on coral reefs in tropical and subtropical regions of the Atlantic and Indo-Pacific [1].

Morphometric and meristic characters of fishes were found to be of taxonomic importance in sex, race and species identification by many investigators [2]. Morphometric is the empirical fusion of geometry with biology [3]. Patterns of morphometric variation in fishes indicate differences in growth and maturation rates because body form is a product of ontogeny [4]. Phenotypic plasticity of fish allows them to respond adaptively to environmental changes by modification in their physiology and behavior which leads to changes in their morphology, reproduction or survival that mitigate the effects of environmental variation [5]. The meristic characters were also found to be valid in race and species identification and in turn in stock identification for fishery purposes [6]. The electrophoresis of proteins is an effective procedure for creating systematic data from macromolecules. SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a strategy broadly utilized in biochemistry, criminology, genetics and molecular biology to separate proteins according to their electrophoretic mobility [7]. Electrophoresis of sarcoplasmic proteins, serum proteins, liver proteins and a number of enzymes regularly has been utilized by some researchers as a guide in the species identification of fish [8-13]. Soluble proteins of muscle sarcoplasm are among the most effortless to extract and highly a rich reservoir of species specific and biochemical genetic markers. The highly water-soluble sarcoplasmic proteins comprising of glycolytic enzymes, myoglobin and other proteins present in intracellular fluid of muscle were often used for specific identification [7].

So far, no attempt has been made to analyze the genetic structure of snappers from Red sea [14, 15]. The present study investigate the feasibility of using meristic and

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morphometric characteristics as well as analyses of protein profiles (SDS-PAGE) for identification of two snapper species presented in the catches of Red sea. Precise species determination of wild snappers has a significant value for sustainable management and conservation of its stocks.

2 Materials and method

2.1 Study area

The Hurghada City (Fig.1) Lies at the northern part of the Red Sea proper between Latitudes 27:10 N - 27. 33 N and Longitudes 33.70 E - 33.85 E. Hurghada is considered as one of the productive fishing grounds along the Egyptian coasts of Red Sea.

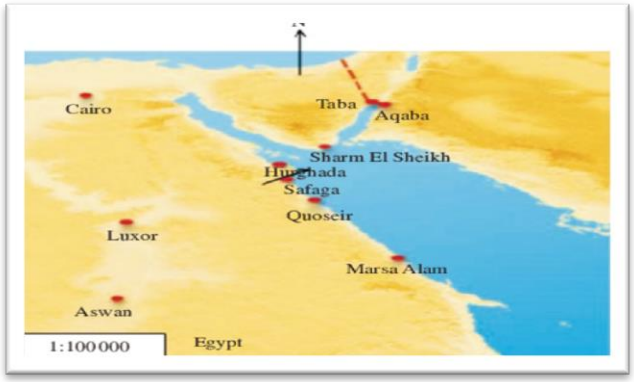

Fig. 1: Map of Red Sea showing the main fishing landing sites along the Red Sea, Egypt.

2.2. Sample Collection

Specimens of *L. quinqelineatus* (n= 120, TL (14-31.7 cm)) and *L. ehrenbergii* (n= 120, TL (12-30 cm)) (Bloch, 1790) were collected from Hurghada fishing harbour, Egypt and immediately brought to the laboratory in an insulated ice box.

2.3. Morphometric and Meristic characteristics

2.3.A. Morphometrics

For each Fish, 18 morphometric measurements were measured on the specimens of *L. quinqelineatus* and *L. ehrenbergii* according to [2], they are diagrammatically represented in (Fig. 2): The numbers in the Figure correspond with those given below:

1. Total length (**TL**)
2. Standard length (**SL**)
3. Body depth (**BD**)
4. Caudal peduncle depth (**CPD**)
5. Head length (**H**)
6. Predorsal fin length (**PRDFL**)
7. Head depth (**HD**)
8. Preventral fin length (**PRVFL**)
9. Distance between ventral and dorsal fins origin (**VDOL**)
10. Distance between anal and dorsal fin ends (**ADFEL**)
11. Dorsal fin base length (**DFBL**)
12. Distance between the ventral fin origin and the end of anal fin (**VOAEFL**)
13. Distance between the first spine of the dorsal fin and the end of anal fin (**SPDAEFL**)
14. Distance between dorsal fin end and ventral fin origin (**DEVOFL**)
15. Distance between the ventral fin end and the anal fin origin (**VEAOFL**)
16. Distance between dorsal fin end and dorsal caudal fin origin (**DEDCF**)
17. Distance between anal fin end and ventral of caudal fin origin (**AEVCFL**)
18. Eye diameter (**ED**)

2.3.B. Meristic studies

The following meristic counts were recorded:

1. Number of the dorsal fin spines (**DFS**)
2. Number of the dorsal fin soft rays (**DFSR**)
3. Number of the pectoral fin rays (**PFSR**)
4. Number of the ventral fin rays (**VFR**)
5. Number of the ventral fin spines (**VFS**)
6. Number of the anal fin rays (**AFR**)
7. Number of the anal spines (**AFS**)
8. Number of the caudal fin rays (**CFR**)
9. Total number of gill rakers (**TGR**)

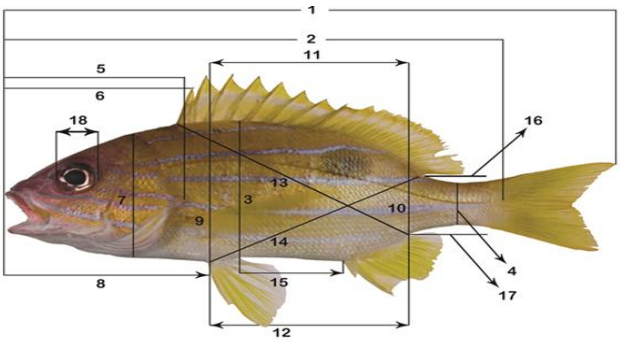

Fig. 2: Schematic illustrations of measurements taken on the body of two *Lutjanus* species from the Red Sea, Hurghada, Egypt.

2.4. Protein Extraction and SDS-PAGE

A piece of muscle tissue (125 mg) was homogenized with 1 ml of chilled extraction buffer, and the sample was centrifuged at 10,000 rpm at 4°C for one hour. Supernatant was collected and used as protein source. Equal amount of 20 µl of tissue extracts was used for determination of total protein (mg/dl). Methodology for protein extraction, casting of gel was performed according to [16]. After running gel was stained and the position of the protein band in the gel was expressed to compare with standard protein markers with known molecular weight. The banding pattern obtained was subjected to cluster analysis using XLSTAT software.

2.5 Statistical analyses

Statistical analyses for morphometric and meristic data were performed using the SPSS software package version 16 (SPSS, 1998) and Excel (Microsoft office, 2010).

3 Results

3.1. Profile of body shape and coloration

L. quinquelineatus: Dorsal profile of head steeply sloped. Preorbital width usually less than eye diameter. Preopercular notch and knob well developed. Scale rows on back rising obliquely above lateral line. Generally bright yellow, including fins, with a series of blue stripes on the side. A round black spot, about the size of the eye or larger, is below the anterior most soft dorsal rays, touching the lateral line but mostly above it. Body depth 2.3-2.9 in SL (Fig. 3A).

L. ehrenbergii: This species is distinguished by the following characters: body moderately deep; greatest depth 2.5-3.0 in SL; preopercular notch and knob poorly developed; vomerine tooth patch triangular, with a medial posterior extension; gill rakers of first gill arch 6-7 + 10-14 - 16-21; caudal fin truncate to slightly emarginate; scale rows on back parallel to lateral line. Colour of back and upper sides dark brown, lower sides and belly whitish with a silver sheen; usually a series of 4-5 narrow yellow stripes on the sides below the lateral line; a distinct round, black spot on the back below the posterior part of the spinous portion of the dorsal fin (Fig. 3B).

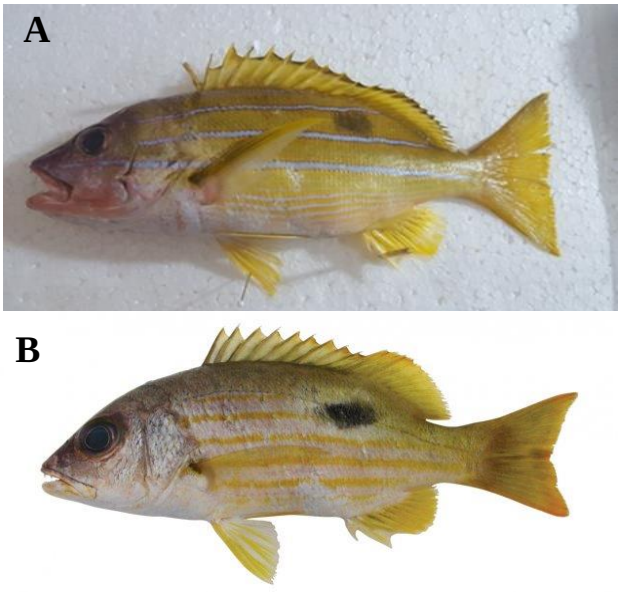

Fig. 3: Photographs of *L. quinquelineatus* (A) and *L. ehrenbergii* (B) from the Red Sea, Hurghada, Egypt.

3.2. Meristic and Morphometric characteristics

Morphometric characteristics: Descriptive data for the morphometric characters that are calculated as the percentage of standard length in the sampled specimens are given Table (1). the morphometric characters differences between *L. quinquelineatus* and *L. ehrenbergii* were statistically insignificant ($p>0.05$).

Meristic characteristics: Meristic counts for the specimens examined are given in Table (2). Overlaps occurred in all characteristics between the two species. The comparisons showed that all meristic characters were found to be insignificantly different at 5% level (Table 2). There were no differences in any of the meristic characters observed between *L. quinquelineatus* and *L. ehrenbergii*.

Table 1: Morphometric indices (relative to TL) of *L. quinquelineatus* and *L. ehrenbergii* from Hurghada, Red Sea, Egypt.

Morphometric index	*L. quinqelineatus*	*L. ehrenbergii*
TL/SL	1.27±0.003	1.26±0.003
TL/BD	3.16±0.02	3.39±0.02
TL/CPD	10. 62±0.15	9.85±0.003
TL/HL	3.24±0.01	3.38±0.01
TL/PRDFL	3.08±0.003	3.19±0.01
TL/HD	3.08±0.02	5.5±0.06
TL/PRVFL	5.09±0.07	3.22±0.01
TL/VDOL	3.34±0.02	3.56±0.02
TL/ADFEL	7.42±0.003	7.42±0.07
TL/DFBL	2.09±0.01	2.35±0.01
TL/VOAEFL	2.38±0.01	2.33±0.02
TL/SPDAEFL	1.93±0.01	2.04±0.01
TL/DEVOFL	2.05±0.01	2.01±0.01
TL/VEADFL	9.66±0.12	8.08±0.1
TL/DEDCF	9.46±0.1	8.27±0.1
TL/AEVCFL	7.95±0.1	7.90-±0.1
TL/ED	14.78±0.003	14.60±0.2

Table 2: Meristic indices of *L. quinquelineatus* and *L. ehrenbergii* from Hurghada, Red Sea, Egypt.

Number of the dorsal fin soft rays (DFSR)+ X Spines						
Counts	N	14	15	0	0	Mean± SD
L. quinqelineatus	120	99	21	0	0	14.5±0.7
L. ehrenbergii	120	1	82	37	0	14±1.0
Number of the pectoral fin rays (PFSR)+ 0 Spines						
Counts	N	13	14	15	16	Mean± SD
L. quinqelineatus	120	10	80	28	2	14.5±1.3
L. ehrenbergii	120	1	14	102	3	13.5±1.3
Number of the caudal fin rays (CFR) +0 Spines						
Counts	N	16	17	18	0	Mean± SD
L. quinqelineatus	120	63	46	11	0	17±1.0
L. ehrenbergii	120	1	86	23	10	16.5±1.3
Total number of gill rakers (TGR)						
Counts	N	13	14	15	16	Mean± SD
L. quinqelineatus	120	15	19	29	57	14.5±1.3
L. ehrenbergii	120	1	16	18	85	14.5±1.3

3.3. Electrophoretic Protein Patterns

Eelectrophoretic pattern of muscle proteins in *L. quinquelineatus* and *L. ehernbergii* were shown in (Fig. 4). *L. quinquelineatus* and *L. ehernbergii* had the same number of protein bands (20 bands) (Fig. 4 and Table 3). In the other hand, *L. quinquelineatus* and *L. ehernbergii* muscle proteins were separated into several bands, these bands were differed in quantitative parameters (Table 3). *L. quinquelineatus* had unique bands (MW., 266.43, 215, 119.82, 30.51 and 27.8 kD) while *L. ehernbergii* had another unique bands (MW., 75.97, 50.81, 30.86, 30.07,27.18 and 16.6 kD) (Table 3).

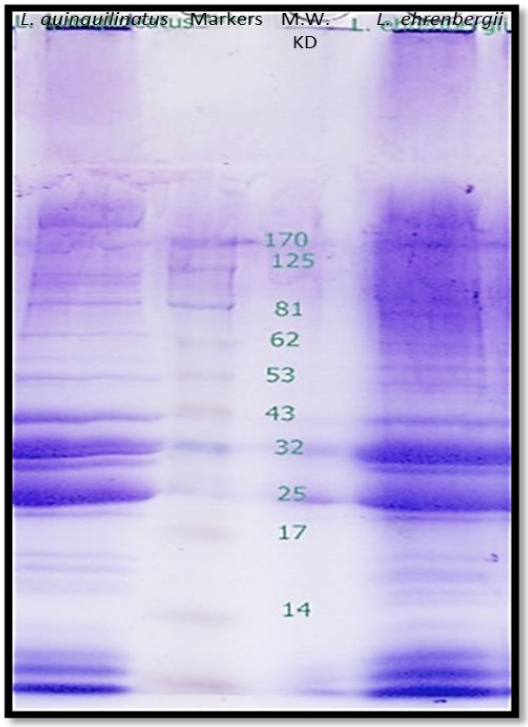

Fig. 4: Comparison of sarcoplasmic proteins of *L. quinquelineatus* and *L. ehernbergii*.

Table 3: Molecular weights of sarcoplasmic protein bands of **L. quinquelineatus** and **L. ehernbergii**.

Marker (kD)	*L.quinquelineatus*	*L. ehernbergii*
-	**266.43**	-
-	**215**	-
-	**190.89**	**190.89**
170	**160.36**	**160.36**
125	**119.82**	-
-	**109.47**	**109.47**
81	**84.88**	**90.06**
-	-	**75.97**
62	**67.03**	**62**
53	**52.38**	**55.32**
-	-	**50.81**
43	**41**	**39.67**
32	**31.91**	-
-	**31.47**	**31.21**
-	-	**30.86**
-	**30.51**	-
-	-	**30.07**
-	**27.8**	-
-	-	**27.18**
-	**26.66**	**26.23**
25	**22.95**	**22.85**
-	**21.62**	**20.90**
-	**19.67**	**18.64**
17	-	**16.6**
-	**15.2**	**15.2**
14	**14.65**	**14.45**

4 Discussions

Morphometric indices of traditional characters were used for identification of fish races and species [17], although such indices were frequently used by fish taxonomists, they were subjected to different criticisms since they were found to vary according to individual factors such as size and sex [18].

In the present study, the morphometric measurements among the species of *L. quinquelineaatus* and *L. ehrenbergii* from the two sampling stations did show some variations, however, these variations were not significantly different. The meristic characters i.e numbers of dorsal spine (10) and dorsal fins (14 rays) and anal spine (3), anal fins (8 rays) were not significantly different in both species of *L. quinquelineaatus* and *L. ehrenbergii*. The scale count in *L. quinquelineaatus* ranges between 36 – 60 cm while in *L. ehrenbergii* ranges between 36 – 58 cm, these shows there were no significant difference in scale count between the 2 species of fish from [19]. Although high genetic divergence has been detected between the two groups of *L. reissneri*, their morphometric and meristic characters are quite similar [20]. Diedhiou *et al* [21] found differences concerning morphometric and meristic features recognized from the juvenile stage onward: the height of the caudal peduncle is larger in *P. isidori* than in *P. adspersus*. Quist *et al* [22] stated that native bluehead suckers and flannel mouth suckers, nonnative white suckers, and hybrids of these species can be accurately identified by a few, easily measured meristic and morphometric characteristics. Narejo *et al*. (2008) [23] revealed significant intertype differences in six morphometric measurements (total length, standard length, fork length, head length, eye diameter and girth) and seven meristic characters (total number of scutes, pre pelvic scutes, post pelvic scutes, dorsal fin rays, pectoral fin rays, pelvic fin rays and anal fin rays). A simple yet useful criterion based on external markings and/or number of dorsal spines is currently used to differentiate two congeneric archer fish species *Toxotes chatareus* and *Toxotes jaculatrix*. Overall, meristic traits were more useful than morphometrics in differentiating the two

species; nevertheless, meristics and morphometrics together provide information about the morphological differentiation between these two closely related archer fishes [24]. Kumari et al. [25] indicated that simply two morphometric and meristic characters are sufficient to differentiate these two closely related species (*Otolithes cuvieri*, Trewavas, 1974 and *Otolithes ruber*, (Schneider, 1801)).

As an aid to traditional taxonomic characters, biochemical methods have been used in systematics. The application of separation and structural studies of proteins to solve taxonomic problems has been discussed by (Alston and Turner [26]; Tsuyuki *et al*.[27]) in biochemical systematics. Studies on genetic variation at protein level led to major contributions in diverse arrays of biologically oriented disciplines [28]. Proteins are considered as gene products and electrophoretic mobilities of different proteins in closely related species or in different populations can be genetically interpreted [29]. Different electrophoretic techniques have been used to identify the differences among fish species and muscle protein is commonly used to assess the polymorphism among fish species [(30-32].

The current study, *L. quinquelineatus* and *L. ehernbergii* had the same number of protein bands (20 bands). In other hand, *L. quinquelineatus* and *L. ehernbergii* muscle proteins were separated into several bands, these bands were differed in quantitative parameters. Chow and Patrick [33] studied fourteen snapper species belonging to the three genera (*Lutjanus* and two monotypic genera *Ocyurus* and *Rhomboplites*) of the subfamily Lutjaninae in the western Atlantic. Cluster and additive tree analyses based on the genetic distance indicated that the lane snapper (*L. synagris*) has a closer relationship with the red snapper group (*L. analis* and *L. vivanus*) than with the gray snapper group (*L. apodus* and *L. griseus*). Also, Richards *et al*. [34] added three genera and four species to the list of known snappers. The electropherogram generated by SDS-PAGE showed difference both in the number of bands and the molecular weight of the sarcoplasmic proteins between two species *Orthriasinsignis euphyraticus* and *Cyprinion macrostomus* [35]. Isoelectric focusing (IEF), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and two-dimensional (2-D) gel electrophoresis for species identification of red snapper (*Lutjanus campechanus*) was reported by Huang *et al*.[36]. Vasconcellos et al. [37] based on morphometric, allozymes and mitorchondiral DNA (Control region) analysis identified a single Brazilian stock, revealing significant levels of genetic sub-structuring between populations from Belize and Brazil. Sulaiman et al. [38] investigated the *L. malabaricus* genetic population structure of red snapper and groupers species in Brunei. Klangnurak et al. [39] demonstrated potential population genetics differences that may imply the existence of hitherto unsuspected barriers between the Gulf of Thailand and populations within the Andaman Sea, which has important consequences for stock management of a vital food fish. The protein banding pattern in three genera *Lutjanus*, *Pinjalo*, *Pristipomoides*, shown much variation, but overall, of the three genera appear to exhibit similar protein banding [12]. Mohammed et al. [40] indicated the effectiveness of RAPD markers in detecting the ratio of polymorphism, monomorphism and estimating genetic distance among *Alestes baremoze*, *Alestes dentex*, *Brycinus nurse*, and *Brycinus macrolepidotus* from Kreima at the River Nile, Sudan.

4 Conclusions

Based on the morphometric and meristic as well as the protein band pattern, the two species analyzed are more or less closely related to each other. Future studies using biochemical-genetic markers and DNA barcoding hopefully will establish new ventures in the field of stock management and conservation of snappers.

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