Ultrastructure and morphometry of the sagittal otolith as confirmatory identification in three carangid species from the northern Red Sea, Egypt

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Abstract. Otoliths morphometric indices and ultrastructure are very useful structures for identification of fishes for various studies. This investigation aimed to investigate the ultrastructure and compare the morphometric indices between the right and left otoliths of three carangid species from Hurghada, Red Sea, Egypt in order to distinguish and confirm their identification. The statistical analysis of otolithic morphometric parameters showed that otolithic measurements are good indicators of fish size. For *Carangoides ferdau*, *Carangoides malabaricus* and *Gnathanodon speciosus* the correlation between fish length and different otolithic variables was statistically significant. The coefficient of determination (r^2) ranged from 0.72 to 0.96 in the three species, being higher for *C. ferdau* in all cases. According to the results from independent sample t-test value, significant differences were found in length, width, and area of otoliths of *C. ferdau* and *C. malabaricus,* whereas no significant differences were found for *G. speciosus*. Furthermore, no significant differences were found in the mean values of the six examined shape indices of the otoliths including: circularity (C), rectangularity (RE), form-factor (FF), roundness (RD), ellipticity (EL) and aspect ratio (AR) between the right and left otoliths were considerably different among the three species. The results suggest that the length and weight of sagittal otoliths are likely suitable indicators for fish total length in all studied species. The ultrastructure scanning by electron microscopy showed a remarkable variation in the morphological characteristics of fish otoliths, including variations in the ornamentation of the ostium, cauda, and column of the otolith. These differences in otolithic characteristics were supportive for discriminating *C. ferdau, C. malabaricus* and *G. speciosus.* This work contributes to the bio ecological knowledge regarding commercially important fishes and provides key information for fisheries, biologists, archaeologists and geologists in discriminating different species. These differences in otolithic characteristics and morphology might be useful for fisheries, biologists, archaeologists and geologists in discriminating *C. ferdau, C. malabaricus* and *G. speciosus*. This work contributes to the bio ecological knowledge regarding commercially important fishes and provides key information for studying the trophic ecology of fish-eating species and fishery management.
Key Words: Carangid species, Hurghada, morphometrics, otoliths, Red Sea, scanning electron microscope.

Introduction. Fish hard parts including scales, otoliths, spines, vertebrae and others are of great importance in different scientific fields such as taxonomy, aging, fisheries management, abnormality (Osman 2000; Mohammad 2016; Farrag et al 2016; Osman et al 2020). Otoliths have been used to study the morphometric parameters of fishes (Agüera & Brophy 2011; See et al 2016; Fashandi et al 2019). They have also being used as indirect tools for studying the fish populations and assessing the relationship between the fishes and their environment (Lord et al 2012; Zengin et al 2015), and in analysis of otolith chemical properties to distinguish fish life histories; diet for cetacean, birds, pinnipeds and piscivorous fishes and archaeological studies (Campana 2004; Tournois et al 2013; Wenzel et al 2013). Morphometric method based on otoliths was more reliable than the ones that use external morphometric traits, because otoliths are not affected by short-term variations in fish physiological state or by standard tissue preservation techniques, and their appearance and shape often differ geographically (Farias et al 2009). Otoliths are calcareous structures found in the inner ear of bony fishes (Campana 2004; Osman et al 2020).

Teleost fish have three pairs of otoliths (sagittae, asterisci and lapilli) in the otic sacs (Popper & Lu 2000; Osman et al 2020), and they play a function as mechanoreceptors that are involved in balance and hearing (Popper et al 2005). The sagittae is the largest otolith in most teleosts and shows variable morphological and topographical properties among the species. Fish otoliths have various features on different levels including outer shapes and ultrastructure. In addition to that there are some differences between left and right ones which gave more importance to study these hard parts in fishes. Accordingly, otoliths shape analysis is widely used in identification of different groups such as mature and juvenile specimens, populations, spawning aggregations and genders (Tyagun et al 2013; Mehanna et al 2018; Mohammad et al 2020).

The family Carangidae is one of the major families of bony fishes, with a worldwide distribution, and about 140 species belonging to 32 genera (Abdussamad et al 2013; Fashandi et al 2019). At least 10 carangid species were recorded in the Egyptian sector of the Shalateen fishing area of Red Sea (Mohammad 2016). Due to the importance of these species in the fisheries of the Red Sea companied with similarities between several species, this study aimed to investigate the morphometrics and ultrastructure indices in both right and left otoliths of three carangid species from Hurghada, Red Sea, Egypt to support our knowledge about these species and to put a key for correct identification used in future for fisheries and conservation.

Material and Method

Study area and samples collection. The investigated area is located in the northern part of Red Sea Province at Hurghada city (latitudes 26°55' N - 27°30' N and longitudes 33°58' E - 33°35' E) as shown in Figure 1. Specimens of the three species were collected monthly from Hurghada fishing Port, Red Sea, Egypt during the time from December 2018 to November 2019 and measured in a standard length as *Carangoides ferdau* (105 collected specimens; 140-538 mm in SL); *Carangoides malabaricus* (173 collected specimens; 145-460 mm in SL), and *Gnathanodon speciosus* (130 collected specimens, 125-503 mm in SL). Hook and line is the dominant fishing method in the study area and it has been used as the sampling approach exclusively in the present study.

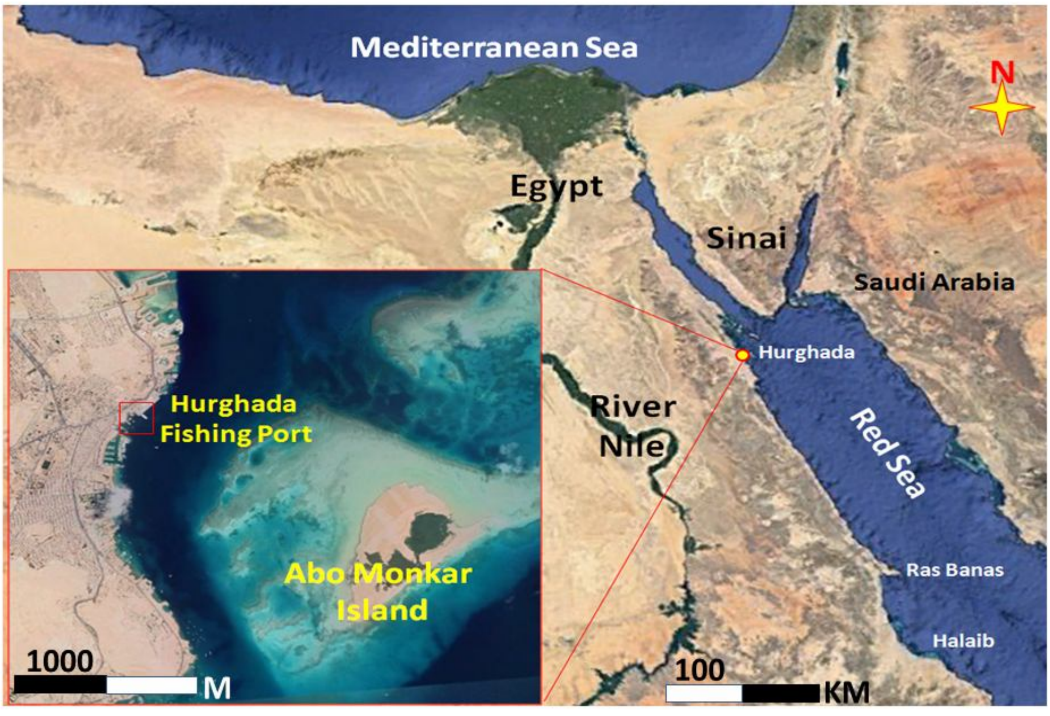

Figure 1. Google earth map of sampling sites in Hurghada, Red Sea, Egypt.

Otolith analysis. Sagittal otoliths of fish specimens were removed and cleaned with ethanol 70%. Left and right sagitta were dried and preserved in an Eppendorf tube. Then, the otolith weight was measured to the nearest 0.0001 g. Its morphometrics were taken using stereoscopic binocular microscope (ZTX-3E) slides linked to a digital camera (Optica 2.1), which was utilized for observation and photography (the rostrum to the left and sulcus acusticus upward) (Figure 2).

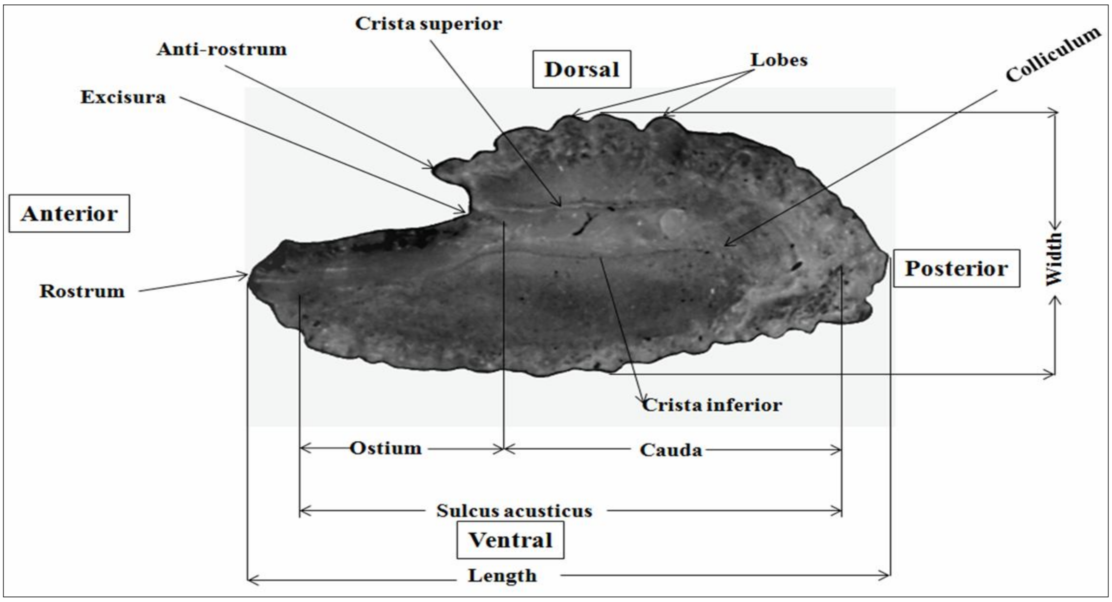

Figure 2. Proximal surface of sagittal otolith of species *C. malabaricus* from Hurghada, Red Sea, Egypt (example of the otolith shape description).

Digital otolith images were taken by a CCD and saved to a PC with a digital resolution 150 dpi (10 Mega pixels) in “jpg” format. The images format is used by the camera related program which can be used to get the angle of the pictures we need to rotate each to align in a unique form. In order to compare the left and right sagittae, the morphometric parameters including otolith length (L, mm), otolith width (W, mm), otolith area (A, mm^2), otolith perimeter (P, mm) were measured using ImageJ software and otolith weight (Wt, g) was measured using an AS220 kL-1 model balance.

The relationships between fish standard length (SL) and otolith variables were estimated using the linear equation (Otolith variables = a*SL±b), followed by log transformation to estimate (a) and (b) via simple linear regression, in which (a) is the angular coefficient characterizing the otolith’s growth rate and (b) is a constant specific to the species.

Other measurements allowed the calculation to describe otolith shape, six dimensionless shape factors: circularity or compactness (C), rectangularity (RE), form factor (FF), roundness (RD), ellipticity (EL) and aspect ratio (AR) were obtained by combining size parameters according to Tuset et al (2003) and Ponton (2006) (Table 1).

Table 1

Size parameters and otoliths shape indices with calculation formulas

Size parameters	*Otolith based shape indices*	*Formula*
Otolith length = (L) mm	Circularity or compactness (C)	$C = P^2/A$
Otolith width = (W) mm	Rectangularity (RE)	$RE = A/(L*W)$
Otolith weight = (Wt) mg	Form-factor (FF)	$FF = (4\pi*A)/P^2$
Otolith area = (A)	Roundness (RD)	$RD = 4A/(\pi*L^2)$
Otolith perimeter = (P)	Ellipticity (EL)	$EL = (L-W)/(L+W)$
	Aspect ratio (AR)	$AR = L/W$

Statistical analysis. Statistical analyses were performed using SPSS V18.0 software. A Kolmogorov-Smirnov test was used to check the normality of the data distributions and variance homogeneity. Statistical description of the weight, length and otolith size of *C. ferdau*, *C. malabaricus* and *G. speciosus* were conducted using SPSS. Differences between left and right otoliths were tested using a paired t-test (SPSS, Version V18). Summaries of the descriptive statistics for the otolith shape indices of *C. ferdau*, *C. malabaricus* and *G. speciosus* were performed using SPSS. A linear equation was applied to estimate the interaction between fish length and otolith measurements.

Scanning electron microscopy. Scanning electron microscopy (SEM) examination for the otoliths was applied after fixation of specimen on holder using sticker tape and coated with a 30 nm layer of gold at the Electron Microscope Center, Assiut Univ., Egypt. The morphological descriptions of the otoliths were based on the terminology proposed by Tuset et al (2008) (Figure 3).

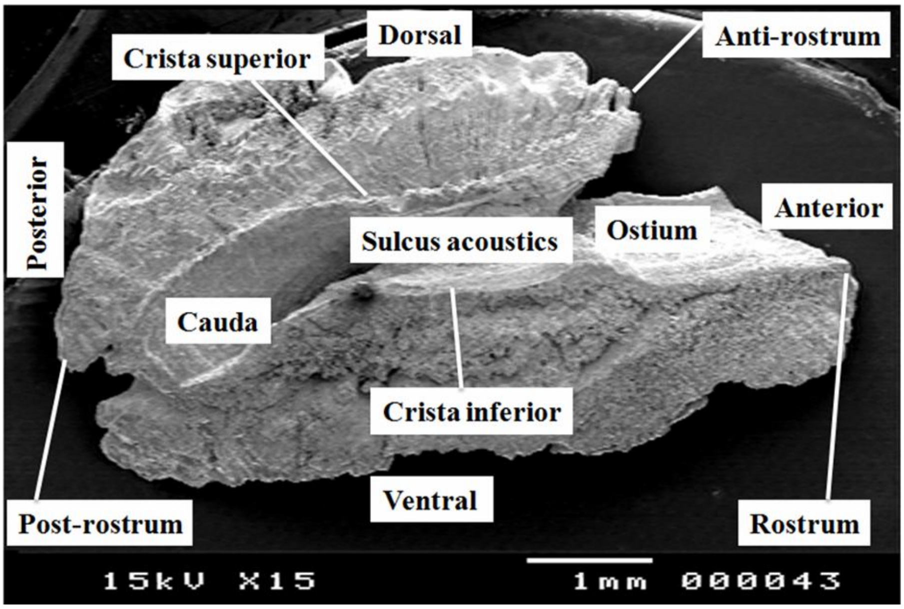

Figure 3. Proximal view of the sagittal otoliths of common carangid from Hurghada, Red Sea, Egypt.

Results. The summarized results for the left and right otolith measurements as well as descriptive statistics (minimum, maximum, mean, standard error and standard deviation) and paired-t tests are shown in Table 2. No statistically significant differences ($p > 0.05$) for the weight, length, width, area, and perimeter of the otolith were observed between the left and right sagittal pairs for *C. ferdau*, *C. malabaricus* and *G. speciosus* (Table 2).

Table 2

Summary of descriptive statistics and paired t-test results for left and right sagitta otolith (length (L), width (W), weight (Wt), area (A), and perimeter (P)) for *C. ferdau*, *C. malabaricus* and *G. speciosus* from Hurghada, Red Sea, Egypt

Parameter	*Min*		*Max*		*Mean*		*SE*		*P. value*
	Right	*Left*	*Right*	*Left*	*Right±SD*	*Left±SD*	*Right*	*Left*	
					C. ferdau				
L	3.46	3.29	11.23	11.22	6.07±2.49	6.05±2.55	0.242	0.252	0.789
W	1.56	1.55	11.00	10.95	4.74±2.70	4.74±2.75	0.364	0.272	0.641
Wt	0.003	0.002	0.24	0.24	0.08±0.08	0.08±0.08	0.007	0.008	0.727
A	4.77	4.77	18.43	18.43	11.38±3.77	11.36±3.79	0.367	0.376	0.658
P	9.44	9.44	28.89	28.89	17.36±5.76	17.36±5.82	0.562	0.576	0.678
					C. malabaricus				
L	2.18	2.11	7.65	7.66	4.88±1.22	4.916±1.22	0.0927	0.9242	0.001
W	1.87	1.92	3.80	3.80	2.76±0.53	2.757±0.53	0.0401	0.0400	0.180
Wt	0.001	0.001	0.06	0.05	0.02±0.01	0.015±0.01	0.0005	0.0005	0.08
A	5.38	6.77	16.48	16.45	9.85±3.04	9.848±3.04	0.2308	0.2297	0.696
P	12.56	12.76	21.70	21.50	15.75±2.71	15.735±2.71	0.2061	0.2057	0.023

Parameter	Min		Max		Mean		SE		P. value
	Right	Left	Right	Left	Right±SD	Left±SD	Right	Left	
					G. speciosus				
L	2.63	2.63	11.67	11.67	6.432±2.56	6.422±2.56	0.224	0.225	2.978
W	2.32	2.31	11.05	11.11	5.461±2.65	5.430±2.64	0.232	0.232	5.100
Wt	0.01	0.01	0.29	0.29	0.087±0.08	0.084±0.08	0.007	0.007	8.740
A	4.99	4.99	19.05	19.05	11.847± 3.71	11.836±3.71	0.325	0.325	0.076
P	9.93	9.93	28.89	28.89	18.322± 5.05	18.318±5.05	0.443	0.443	1.080

Therefore, the left otoliths were selected for the recording of other measurements and statistics, shown in Table 3. The otolith length (L) ranged from a minimum of 2.145 mm for *C. malabaricus* to a maximum of 11.67 mm for *G. speciosus*. *G. speciosus* displayed the widest otolith, with an average of 2.31-11.08 mm W, as well as the heaviest otoliths, with an average of 0.01-0.29 g Wt. The minimum otolith area and perimeter were 4.77 A and 9.44 P for *C. ferdau*, while the maximum otolith area was 19.05 A for *G. speciosus* and the maximum otolith perimeter was 28.89 P for *C. ferdau* and *G. speciosus.*

Table 3

Statistical description of fish length (TL), weight (TW) and fish otolith (Length (L), width (W), weight (Wt), area (A), and perimeter (P) of *C. ferdau, C. malabaricus* and *G. speciosus* from Hurghada, Red Sea, Egypt

Species	Variables	Minimum	Maximum	Mean±SD	Std. error
C. ferdau N = 105	SL (mm)	140	538	311.03 ± 118.724	10.413
	TW (gm)	74.12	2800.43	780.86 ± 757.750	73.939
	L	3.75	11.22	6.06 ± 2.519	0.247
	W	1.55	10.97	4.74 ± 2.726	0.318
	Wt	0.0025	0.24	0.08 ± 0.079	0.0075
	A	4.77	18.43	11.37 ± 3.779	0.371
	P	9.44	28.89	17.36 ± 5.789	0.569
C. malabaricus N = 173	SL (mm)	145	460	275.12 ± 80.177	6.096
	TW (gm)	106.05	1808.98	629.57 ± 447.867	34.051
	L	2.145	7.655	4.89 ± 1.217	0.5084
	W	1.895	3.800	2.75 ± 0.527	0.0400
	Wt	0.001	0.055	0.02 ± 0.006	0.0005
	A	6.075	16.465	9.84 ± 3.028	0.2302
	P	12.66	21.600	15.74 ± 2.708	0.2059
G. speciosus N = 130	SL (mm)	125	503	305.446 ± 106.417	9.333
	TW (gm)	58.00	2680.45	879.56 ± 760.45	66.696
	L	2.63	11.67	6.42 ± 2.56	0.224
	W	2.31	11.08	5.44 ± 2.64	0.232
	Wt	0.01	0.29	0.085 ± 0.08	0.007
	A	4.99	19.05	11.84 ± 3.71	0.325
	P	9.93	28.89	18.32 ± 5.05	0.443

According to the linear equation all morphometric measurements of otoliths for three species exhibited a good relationship with fish standard length (SL) (Table 4). The length, weight, width, area and perimeter of the otoliths were linearly related to SL of fishes for the studied fish species. According to Table 4 all regressions were highly significant. The coefficients of determination (r^2) ranged from 0.91 to 0.96 in *C. ferdau*, from 0.83 to 0.94 in *C. malabaricus*, and from 0.82 to 0.95 in *G. speciosus*.

Table 4

Regression equations between fish length (SL) and otolith (length (L), width (W), weight (Wt), area (A) and perimeter (P) for *C. ferdau, C. malabaricus* and *G. speciosus* from Hurghada, Red Sea, Egypt

C. ferdau		*C. malabaricus*		*G. speciosus*	
L=0.020 SL+0.183	R^2=0.91	L=0.014 SL+1.132	R^2=0.83	L=0.023 SL-0.731	R^2=0.95
W=0.023 SL-1.794	R^2=0.95	W=0.006 SL+1.085	R^2=0.86	W=0.024 SL-1.841	R^2=0.92
Wt=0.001 SL-0.109	R^2=0.93	Wt=6.51E^{-5} SL-0.003	R^2=0.90	Wt=0.001 SL-0.130	R^2=0.82
A=0.032 SL+2.248	R^2=0.96	A=0.036 SL–0.185	R^2=0.94	A=0.034 SL+1.533	R^2=0.94
P=0.048 SL+3.437	R^2=0.95	P=0.033 SL+6.775	R^2=0.93	P=0.046 SL+4.415	R^2=0.92

The mean values of the six examined shape indices (C, RE, FF, RD, EL and AR) of the three carangid species otoliths are shown in Table 5. The highest mean values of RE, RD, EL and AR were recorded in *C. malabaricus* and the highest mean values of C in *G. speciosus*, and FF in *C. ferdau*. The lowest mean values of RE, FF, RD, EL and AR were observed in *G. speciosus*, whereas the lowest value of C was recorded in *C. malabaricus* (Table 5). The Kolmogorov-Smirnov Z test confirmed a normal distribution ($p > 0.05$; non-significant) for all examined shape indices measurements.

Table 5

Minimum, maximum, mean, standard error, and standard deviation of otoliths six shape descriptors (C, RE, FF, RD, EL and AR) for *C. ferdau, C. malabaricus* and *G. speciosus* from Hurghada, Red Sea, Egypt

Species	*Variables*	*Minimum*	*Maximum*	*Mean±SD*	*Std. error*
C. ferdau NO. 105	C	18.01	48.43	26.61 ± 9.11	0.889
	RE	0.15	0.86	0.527 ± 0.225	0.219
	FF	0.26	0.70	0.52 ± 0.14	0.013
	RD	0.18	0.79	0.47 ± 0.18	0.017
	EL	0.01	0.39	0.15 ± 0.99	0.009
	AR	1.02	2.27	1.403 ± 0.293	0.028
C. malabaricus No. 173	C	20.51	34.13	25.644 ± 1.88	0.144
	RE	0.49	1.64	0.765 ± 0.156	0.014
	FF	0.37	0.61	0.493 ± 0.03	0.002
	RD	0.34	1.85	0.556 ± 0.19	0.015
	EL	0.05	0.36	0.272 ± 0.05	0.004
	AR	1.00	2.19	1.770 ± 0.178	0.013
G. speciosus No. 130	C	17.09	46.26	28.55 ± 7.24	0.635
	RE	0.15	1.04	0.44 ± 0.238	0.209
	FF	0.27	0.74	0.463 ± 0.09	0.008
	RD	0.18	1.13	0.469 ± 0.25	0.225
	EL	0.001	0.23	0.095 ± 0.05	0.005
	AR	1.00	1.60	1.22 ± 0.144	0.126

Figure 4 shows the relationship of the left otolith length (L) with EL and AR being determined as linear relationship, while the relationship between L with C, RE, FF and RD being determined as a nonlinear relationship. As fish otolith length (L) increased, the values of C were generally increased. While the RE and RD were decreased with L otolith length L increased. Pearson’s correlation coefficients between the examined shape factors and otolith length (L) are presented in Table 6, all of variables for *C. ferdau*, *C. malabaricus* and *G. speciosus* were significantly correlated with otolith length.

Table 6

Correlation coefficients of Pearson (R) between shape indices of the left otoliths and otoliths length for *C. ferdau, C. malabaricus* and *G. speciosus* from Hurghada, the Red Sea, Egypt

Index	*C. ferdau*		*C. malabaricus*		*G. speciosus*	
	R	*P*	*R*	*P*	*R*	*P*
C	0.976**	0.0001	0.564**	0.0001	0.885**	0.000
RE	-0.947**	0.0001	-0.775**	0.147	-0.946**	0.000
FF	-0.931**	0.0001	-0.546**	0.000	-0.879**	0.000
RD	-0.882**	0.0001	-0.694**	0.000	-0.887**	0.000
EL	-0.557**	0.0001	0.649**	0.000	-0.470**	0.000
AR	-0.566**	0.0001	0.665**	0.000	-0.458**	0.000

Note: ** Correlation is significant at the 0.05 level

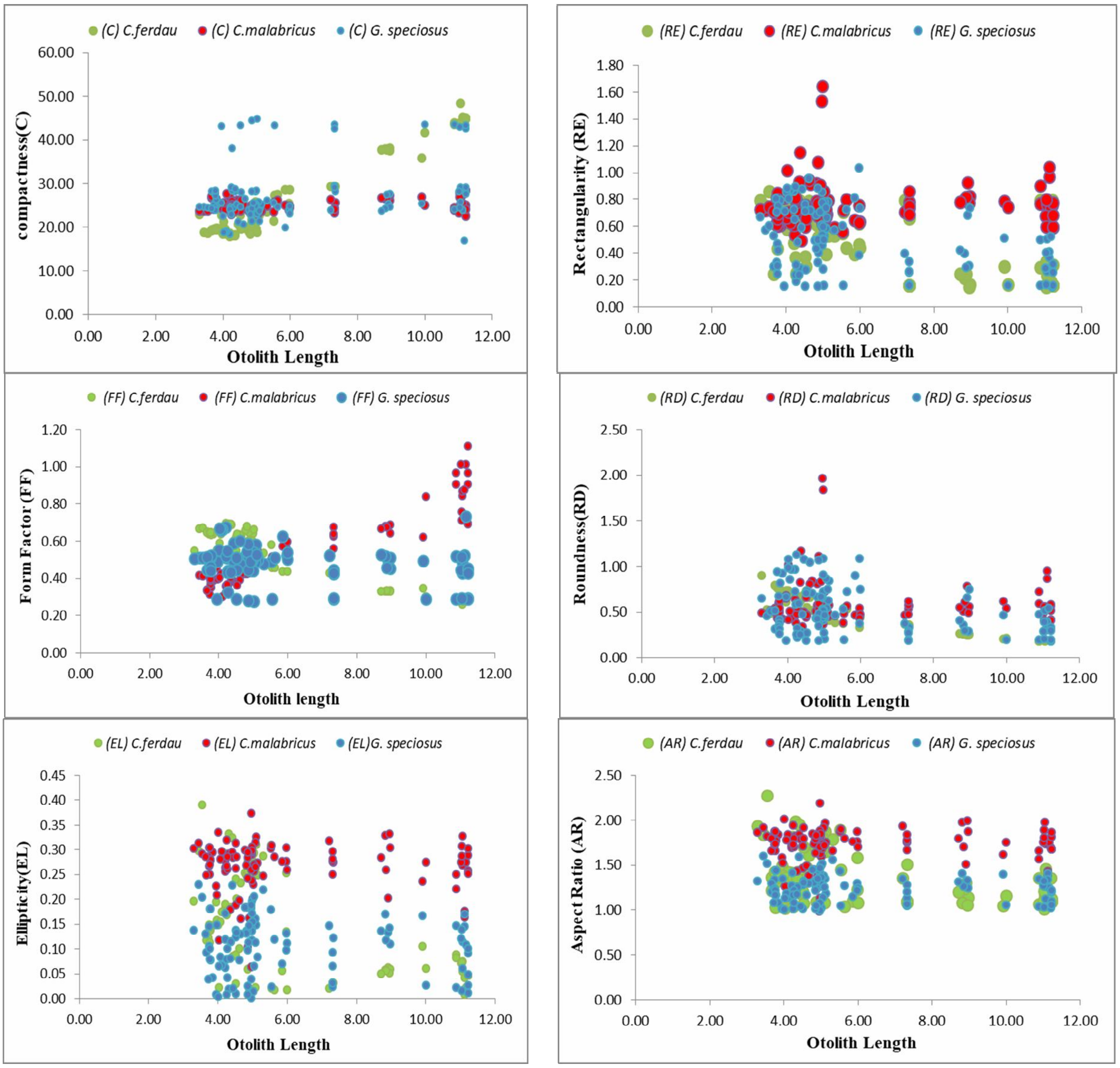

Figure 4. Relationships between otolith length (L) and shape indices (C, RE, FF, RD, EL and AR) of *C. ferdau, C. malabaricus* and *G. speciosus* from Hurghada, Red Sea, and Egypt.

Scanning electron microscopy analysis of otolith topography. The left sagitta otoliths of *C. ferdau*, *C. malabaricus* and *G. speciosus* were examined using scanning electron microscope (Table 7, SEM; Figures 5, 6, 7 and 8) for the first time to these species in our region.

Table 7

The differences between left sagitta otoliths for *C. ferdau*, *C. malabaricus* and *G. speciosus* were examined using scanning electron microscope, from Hurghada, Red Sea, Egypt

Otoliths	*C. ferdau*	*C. malabaricus*	*G. speciosus*
General view	Otoliths are thin and fragile, generally discoidal with crenate margins with smooth and entirely convex distal surface (Figure 5-A).	Otoliths are thin and fragile, generally discoidal with crenate margins with smooth and entirely convex distal surface (Figure 5- B).	Otoliths are thick, with serrate margins, while the otolith distal side has undulated surface and is entirely convex. The proximal side has irregular surface and is entirely concave (Figure 5-C).
Dorsal rime	Was lobed and sinuate (Figure 5-D).	Was lobed and sinuate (Figure 5-E).	Was sinuate and emarginated (Figure 5-F).
Ventral rime	Regular lobes and exhibited extending from the rostrum anterior to the post rostrum posteriorly (Figure 5-A).	Regular lobes and exhibited extending from the rostrum anterior to the post rostrum posteriorly (Figure 5- B).	Sinuate and emarginated (Figure 5-C).
Ventral rime	The crista superior and inferior were smooth (Figure 5-A).	The crista superior and inferior were smooth (Figure 5-B).	The crista superior and inferior were rough and crushed (Figure 5-C).
Ventral rime	The sulcus acustics was rounded, with curved terminal end (Figure 5-A).	The sulcus acustics was rounded, with curved terminal end (Figure 5-A).	The sulcus acustics is rounded, with curved terminal end (Figure 5-C).
Ostium	Ostium small pore, rounded and fine ornamentation was observed toward the edge, transforming into a more coarsely textured surface or crystalline structure toward the inner side (Figure 6-A).	Ostium small pore, rounded, the edge had ornamentation sand-like shaped and the inner side had rectangular stone shaped (Figure 6-B).	Ostium large pore, irregularly shaped, the edge had ornamentation fine sandy and projecting lath-like crystals internally (Figure 6-C).
Column	Column’s ornamentation consisted of triangular and square stone-like shapes was long, narrow, and deep, with straight crista superior and inferior (Figure 7-A).	Column’s ornamentation consisted of rectangular and sedimentary rocks form shapes was long and narrow with straight crista superior and inferior (Figure 7-B).	Column’s was took irregular projections form, short and shallow with curved crista superior and inferior (Figure 7-C).
Cauda	Was funnel shaped and sand-like crystals (Figure 8-A).	Was low magnification, the surface appeared relatively smooth (Figure 8-B).	Was crucible shaped and initially straight, with curves then occurring distally toward the inner rim and presented the same type of ornamentation, in the form of projecting lath-like crystals (Figure 8-C).

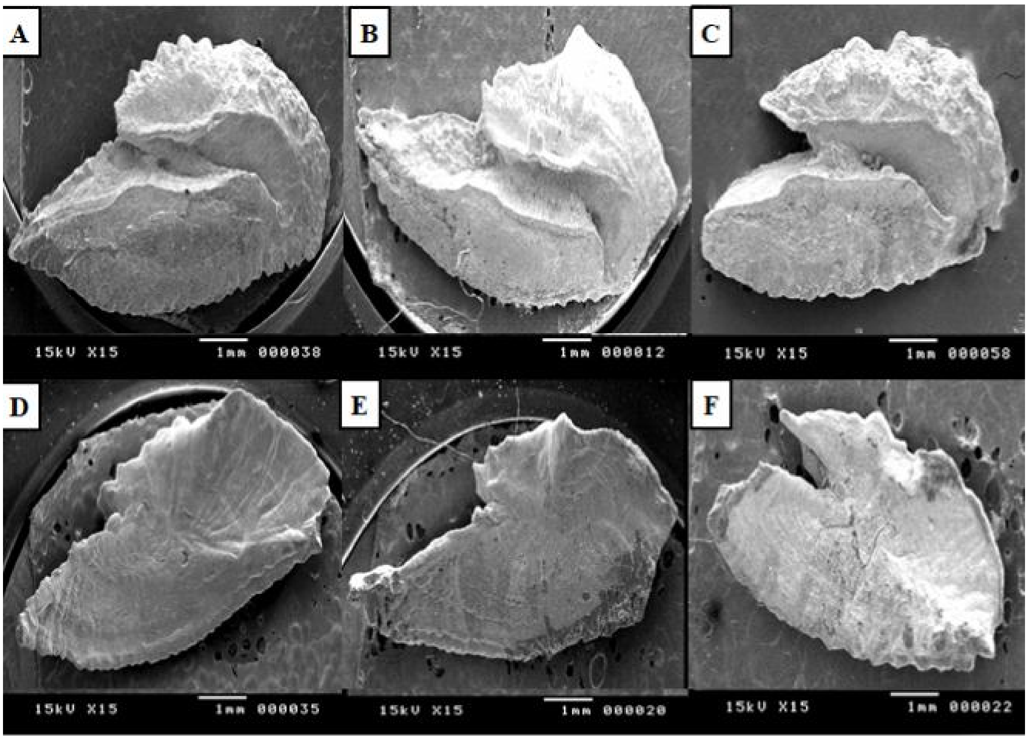

Figure 5. Scanning electron microscope photographs of the proximal (A, B, C) and distal (D, E, F) views of the left sagitta of the three carangid species (A and D - *C. ferdau*; B and E - *C. malabaricus*; C and F - *G. speciosus*) from Hurghada, Red Sea, Egypt.

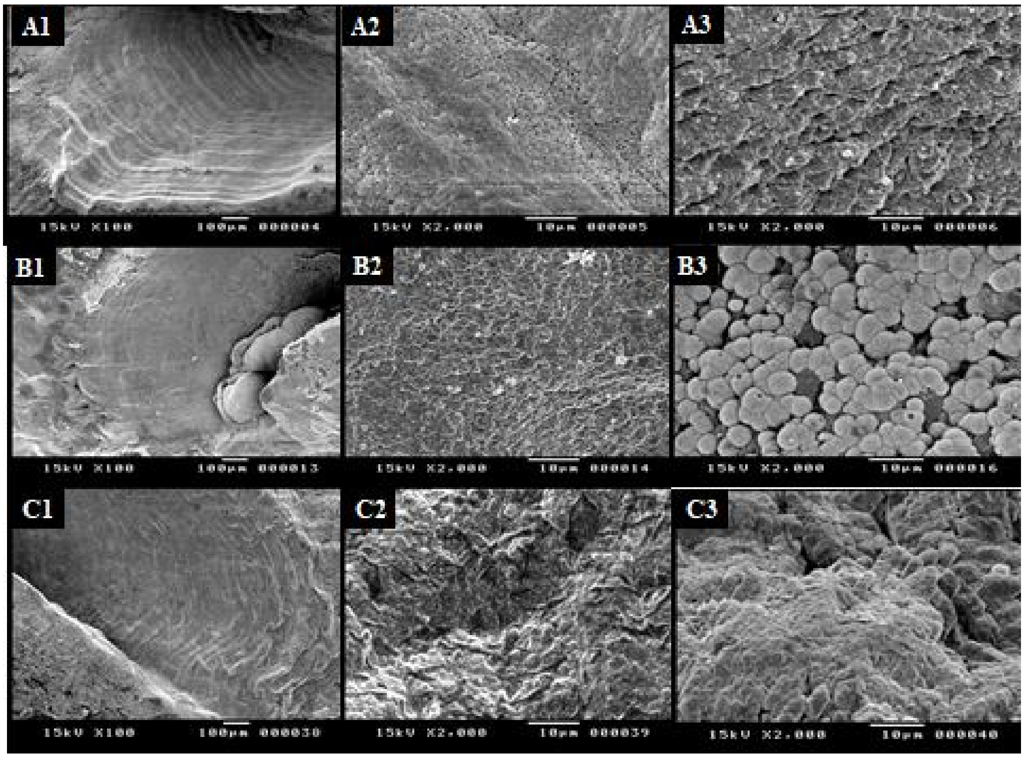

Figure 6. Scanning electron microscope photographs of ostium for the left sagitta of the three carangid species (A - *C. ferdau*; B - *C. malabaricus*; C - *G. speciosus*) from Hurghada, Red Sea, Egypt.

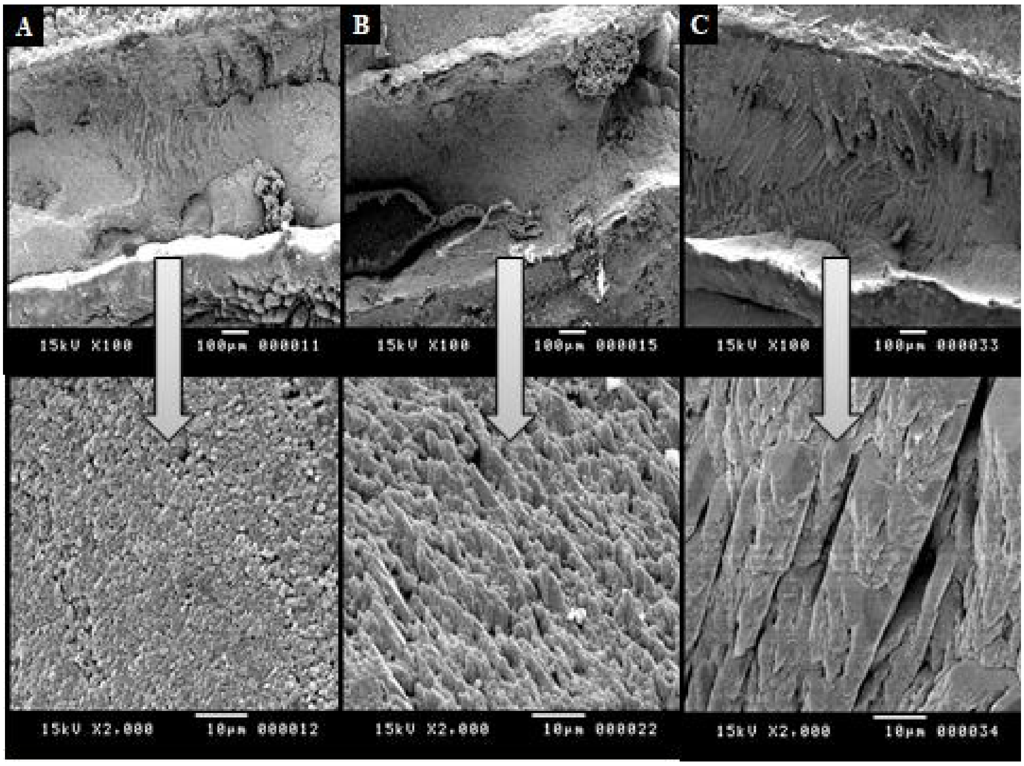

Figure 7. Scanning electron microscope photographs of column for the left sagitta of the three carangid species (A - *C. ferdau*; B - *C. malabaricus*; C - *G. speciosus*) from Hurghada, Red Sea, Egypt.

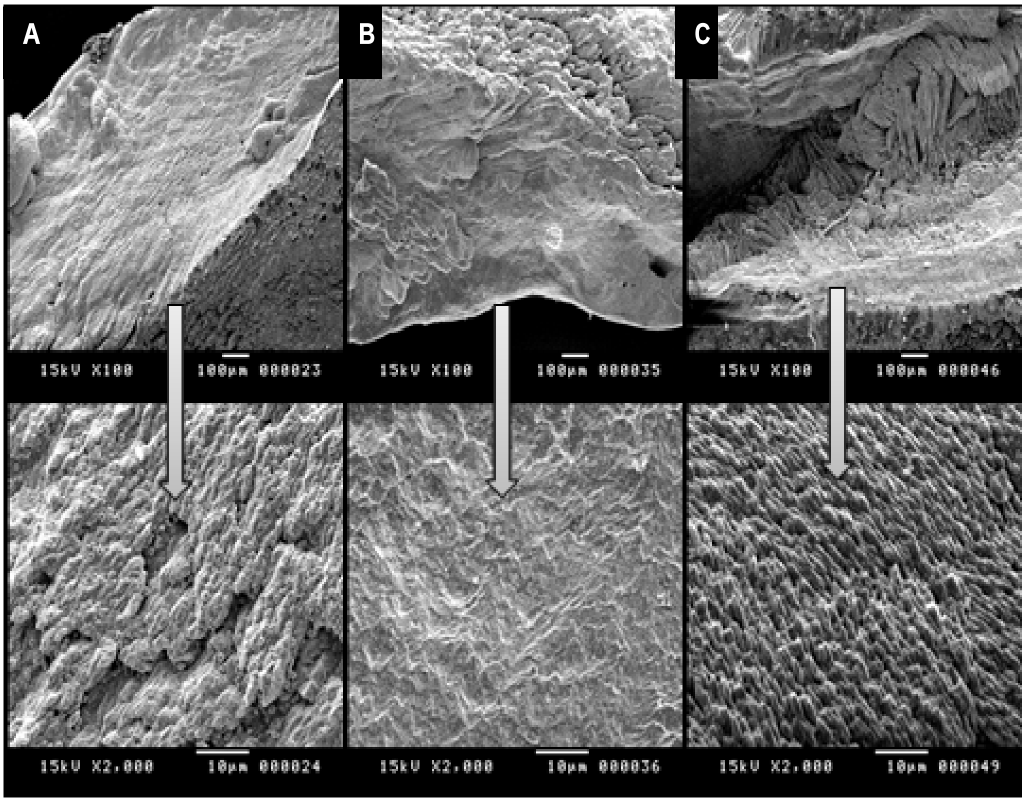

Figure 8. Scanning electron microscope photographs of cauda for the left sagitta of the three Carangid species (A - *C. ferdau*; B - *C. malabaricus*; C - *G. speciosus)* from Hurghada, Red Sea, Egypt.

Discussion. Sagitta otolith has been used as a taxonomic tool for identifying fishes due to their large size and degree of interspecific differences (Rivera Felix et al 2013). Sagitta otolith form, weight, growth, consistency and chemical composition have a distinctive degree of interspecific variation; and they are easily accessible structures (Zorica et al 2010; Fashandi et al 2019).

The comparisons of otolith morphometric parameters between left and right otoliths were performed frequently for differentiation of fish species in many previous studies (Sadighzadeh et al 2012; Rivera Felix et al 2013; Yilmaz et al 2014; Kontas & Bostanci 2015; Kurucu & Bostanci 2018; Fashandi et al 2019; Osman et al 2020). In the present study no statistically significant differences ($p > 0.05$) for the weight, length, width, area, and perimeter of the otolith were observed between the left and right sagittal pairs for *C. ferdau*, *C. malabaricus* and *G. speciosus*. Similar results were reported by Megalofonou (2006), Rivera Felix et al (2013), and Osman et al (2020).

In the present study the relationships between the morphological parameters (length, width, weight, area and perimeter,) of the otoliths and fish body length (SL) in the three carangid species were examined using linear equation. The relationships between otolith size (L, W, Wt, A and P) and total body length (TL) have also been estimated in other fish species using Power functions. The relationships between otolith size and TL have been estimated in other fish species using linear functions (Morat et al 2008; Pavlov 2016; Osman et al 2020).

Comparison of possible differences between the left and right otoliths of fishes was a major aspect of studies conducted on fish otoliths. In the peresent study significant differences ($p > 0.05$) for weight, length, width, area, and perimeter of the otolith were observed between the left and right sagittal pairs for *C. ferdau* and *C. malabaricus* whereas no significant differences were found for *G. speciosus*. No significant differences were found in the otolith length, width, weight, perimeter and area between left and right otoliths of two species *C. crysophrys* and *C. malabaricus* (Fashandi et al 2019), similar finding were reported by Kontas & Bostanci (2015). Based on the lack of statistically significant differences in the left and right otoliths parameters, all other statistical analysis can be performed using only the amounts of left otoliths parameters in order to avoid redundant analysis (See et al 2016; Fashandi et al 2019).

Valinassab et al (2012) studied 10 species of clupeids and did not find any significant differences in morphometric parameters between left and right otoliths. Similar results were reported by Megalofonou (2006) on *Thunnus thynnus* from the Mediterranean Sea coasts of Greece and Italy. Rivera Felix et al (2013) also did not find any significant differences in sagittal otolith length and width between the left and right otoliths of their males and females' samples. In the current study we showed that there were increasing relationships between otolith length with compactness, aspect ratio and ellipticity as a linear relationship and a non-linear relationship between the otolith length and rectangularity, roundness and form factor. Similar trends have been reported on *Carangoides crysophrys and Carangoides malabaricus* by Fashandi et al (2019). Zorica et al (2010) revealed that three shape indices including form factor, roundness and aspect ratio were evaluated for five pelagic fish species from the Adriatic Sea (Croatia).

The current study also compared otolith shape among the three carangidae species using SEM, to observe variations in otolith morphology. In *C. ferdau* and *C. malabaricus* the sagittal shape was found to be thin semi oval, with regular lobes on the dorsal and ventral rims. In *G. speciosus*, the otolith shape is oblong, with irregularly lobed dorsal and ventral rims. Remarkable variations in the morphological characteristics of fish otoliths were recorded between the studied species, including variations in the rostrum, sulcus acusticus, ostium, column and cauda. Remarkable variations were also recorded in the ornamentation of the ostium, cauda, and column. These differences in otolith characteristics might be important to fishery biologists, archaeologists and geologists, who can use them to distinguish *C. ferdau*, *C. malabaricus* and *G. speciosus*.

Conclusions. This work contributes to the bio-ecological knowledge regarding commercially important fishes and provides key information for studying the trophic ecology of fish-eating species and fisheries management. Otolith external outline has shown the importance of otolith morphology study for discriminating between the carangid species and local populations. Further studies are needed to examine the otolith morphology for other marine and freshwater species.

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