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Use of otolithic morphometrics and ultrastructure for comparing between three goatfish species (family: Mullidae) from the northern Red Sea, Hurghada, Egypt

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Abstract

This work highlights the role of otolithic morphometrics, shape indices and ultrastructure in the identification of three Mullidae species from the Red Sea. Differences in otolithic measurements were detectable in all three-goatfish species. The statistical analysis of otolithic morphometric parameters showed that otolithic measurements are good indicators of fish size. For all three species, the correlation between fish length and different otolithic variables was statistically significant. The coefficient of determination (r^2) ranged from 0.83 to 0.92 in the three species, being higher for *M. vanicolensis* in all cases. The otolith area of *M. vanicolensis* was most strongly related to fish length, with a high correlation between otolith area and fish length (r^2=0.92) being observed in this species. The mean values of the six examined shape indices of the otoliths were considerably different among the three species, and the high degree of differentiation of these indices among species makes them useful for other researchers who wish to use otoliths in fish identification and classification. Remarkable variations in the morphological characteristics of fish otoliths were recorded among the studied species via scanning electron microscopy, including variations in the ornamentation of the ostium, cauda, and column of the otolith. These differences in otolithic characteristics and morphology might be useful for fisheries, biologists, archaeologists and geologists in discriminating *Mulloidichthys flavolineatus*, *M. vanicolensis*, and *Parupeneus forsskali*. This work contributes to the bioecological knowledge regarding commercially important fishes and provides key information for studying the trophic ecology of fish-eating species and fishery management.

Keywords: Goatfish species, Otoliths, Morphometrics, Scanning electron microscope**,** Red Sea

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Introduction

Family Mullidae (Goatfishes) is widely distributed globally, especially in the Pacific and Indo-Pacific regions, representing important food-chain components in coastal ecosystems (Pavlov *et al.*, 2015). The family consists of six genera and approximately 62 species (Nelson, 2006). Goatfishes are one of the most economically and commercially important groups inhabiting the northern Egyptian Red Sea sector (Sabrah, 2015). Three common goatfish genera are present along the Egyptian Red Sea coast: Mulloidichthys, Parupeneus and Upeneus (Kuronuma and Abe, 1986).

Otoliths are calcareous structures found in the inner ear of fishes (Campana, 2004). There are three pairs of otoliths (sagittae, asterisci and lapilli) in the otic sacs (Popper and Lu, 2000), and they function as mechanoreceptors that are involved in balance and hearing (Popper *et al.*, 2005). In most species, the sagittal otoliths (the largest of the three otolith pairs) are most commonly used to estimate age and growth, movement and habitat, population structure, and trophic ecology (Campana and Casselman, 1993; Rooker *et al.*, 2008). Otoliths are often collected in routine fisheries investigations for age determination as well as from predator stomachs (Nielsen and Andersen, 2001), allowing precise information on the length, weight, age, and quantity of individual fish prey to be obtained in many cases. Furthermore, otoliths are generally considered to serve as taxonomical and biological archives, as they reflect species' growth and development. Most importantly for the current study, otolithic morphology is species specific (Popper *et al.*, 2005). Otolithic morphology ranges from ellipsoidal to more complex shapes, with protrusions and invaginations (Campana, 2004), and has been used in many studies for species identification (Tuset *et al.*, 2006; Lord *et al.*, 2012; Bani *et al.*, 2013; Sadighzadeh *et al.*, 2014). However, for accurate species identification, a reference collection of otoliths from known species must be produced to generate sufficient comparative material to produce accurate descriptions of morphological characteristics.

To our knowledge, there is no available information concerning the identification of goatfish from the Egyptian Red Sea using otolithic morphometrics and/or ultrastructure. Therefore, this study had three objectives: 1) to determine the possibility of readily discriminating common goatfish species based on the shape and ultrastructure of their otoliths; 2) to quantify otolith length, width, and height relationships with respect to fish length; and 3) to identify characteristics and ultrastructural features allowing otoliths from common Mullidae species to be distinguished from each other.

Materials and methods

Sampling

Three goatfish species (Family: Mullidae) were collected (n=275) from the northern part of the Red Sea

Province at Hurghada (latitudes 27° 10' N–27° 33' N and longitudes 33° 70' E–33° 85' E) (Fig. 1) using artisanal fishing gear (gill net and trammel net) from September 2015 to August 2016. These species included *Mulloidichthys flavolineatus* (n=152), *Mulloidichthys vanicolensis* (n=21), and *Parupeneus forsskali* (n=102). For all individuals, total length (TL) was measured to the nearest millimeter and weight (W) to the nearest gram. The sagittal otoliths (Fig. 2) of the fish were removed, washed and dried and then stored in plastic vials until being examined and photographed.

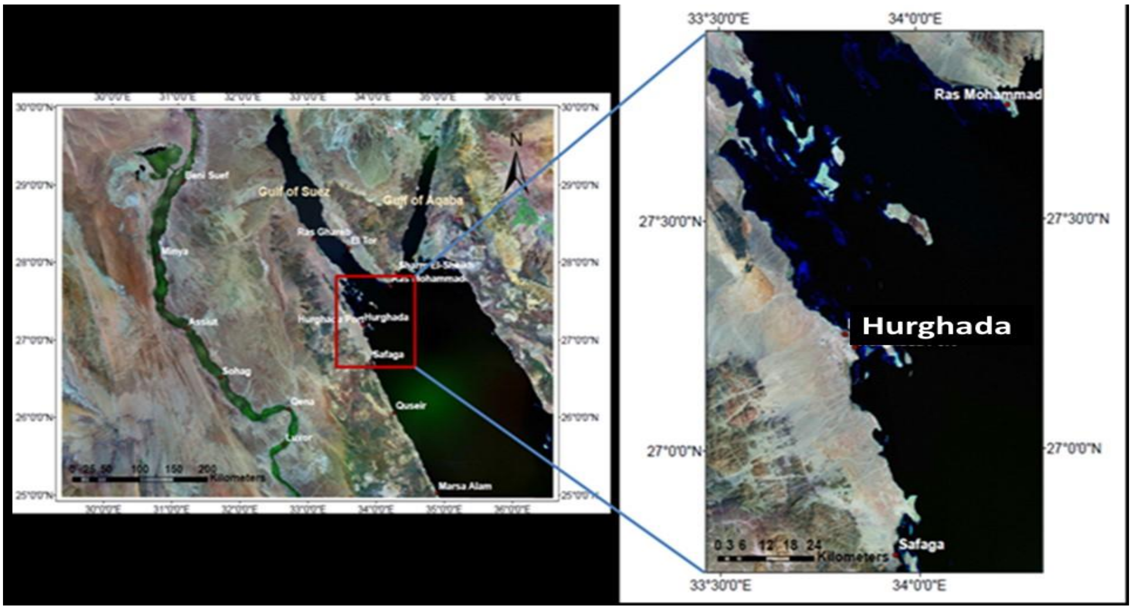

Figure 1: Map showing the study area at Hurghada, Egypt.

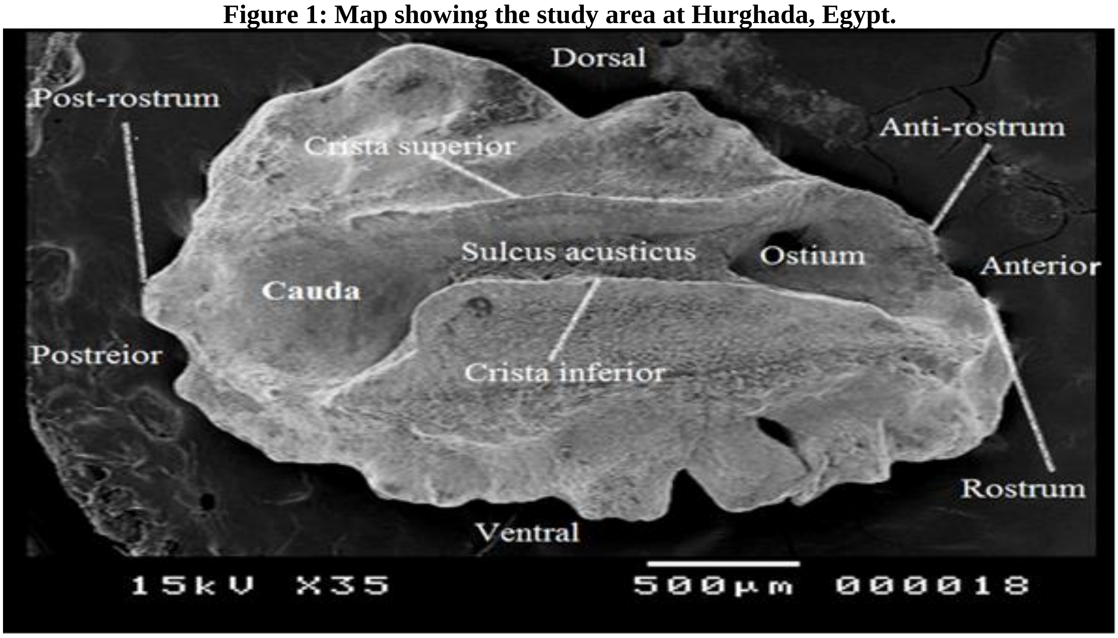

Figure 2: Proximal view of the left sagitta of common goatfish species from the Red Sea, Egypt.

Morphometric and shape analysis of otoliths

For morphometric analysis, the otoliths from the left side of the fish were oriented with the inner side (sulcus acusticus) upwards and the rostrum to the right, for digitization using a stereomicroscope linked to a digital camera (Optica 2.1) (Fig. 2). Then, otolith length (OL, mm), otolith width (OWid, mm), otolith area (OA, mm^2), and otolith perimeter (OP, mm) were measured using ImageJ software. Otolith weight (OW, mg) was measured using an AS220 kL^{-1} model balance. The relationships between fish total length (TL) and otolith variables were estimated using the power equation (otolith variables=aTL^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.

To describe otolith shape, six dimensionless shape factors (aspect ratio (AS), compactness (CO), form factor (FF), rectangularity (RE), roundness (RO), and ellipticity (EL) were obtained by combining size parameters (Russ, 1990; Tuset *et al.*, 2003b; Pinkerton, 2015) (Table 1).

Table 1: Otoliths shape indices.

Index	**Formula**
Aspect ratio (AS)	(OWid / OL)
Compactness (CO)	(OP^2 / OA)
Form factor (FF)	4π OA / Op^2
Rectangularity (RE)	OA / (L * l)
Roundness (RO)	4 OA / πL^2
Ellipticity (EL)	(OL - OWid) / (OL + OWid)

OL, otolith length, OWid-otolith width, OA- otolith area, OP- otolith perimeter.

Scanning electron microscopy

The otoliths were physically cleaned by carefully removing any adhering tissue and debris without damaging the scale surface. Then, they were immersed in a solution of sodium hypochlorite for several minutes to soften adhering tissues for further cleaning. For scanning electron microscopy (SEM) examination, the otoliths were fixed on a specimen holder using sticker tape and coated with a 30-nm layer of gold. Electron micrographs were produced on a GAOL, GSMS 400 LV scanning electron microscope in back-scattering mode and on a Stereo Scan Cambridge Mark 2A (15 KV) at the Assiut University Electron Microscope Center, Assiut, Egypt. The morphological descriptions of the otoliths were based on the terminology proposed by Tuset *et al.*, (2008) (Fig. 2).

Statistical analysis

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 goatfish from the Red Sea was conducted using SPSS.

A paired sample t-test was employed to compare the left and right otoliths (SPSS, Version 18). Summaries of the descriptive statistics for the otolith shape indices of common species of family Mullidae were performed using SPSS. A power equation was applied to estimate the interaction between fish length and otolithic measurements.

Results

The summarized results for the left and right otolithic measurements as well as descriptive statistics (minimum, maximum, mean, standard error and standard deviation) and paired-t tests are shown in Table 2. An insignificant difference (*p*>0.05) in the weight, length, width, area, and perimeter of the otoliths was observed between the left and right sagittal pairs for each goatfish species (Table 2). Therefore, the left otoliths were selected for the recording of other measurements and statistics.

Table 2: Summary of descriptive statistics and paired t-test results for left and right sagitta otoliths of three goatfish (*Mulloidichthys flavolineatus, M. vanicolensis and Parupeneus forsskali*) from the Red Sea, Egypt.

	Min		Max		Mean		SE		SD		*p* value
M. flavolineatus	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right-Left
Weight	0.0013	0.0012	0.0086	0.0086	0.004	0.004	0.0001	0.0001	0.0012	0.0012	**0.741**
Length	2.38	2.4	4.77	4.76	3.62	3.61	0.03	0.03	0.37	0.37	**0.687**
Width	1.77	1.71	3.54	3.43	2.5	2.42	0.02	0.02	0.26	0.25	**0.000**
Area	2.85	2.54	11.12	10.28	6.3	5.88	0.1	0.1	1.28	1.2	**0.001**
Perimeter	3.21	6.55	16.21	14.98	11.1	10.58	0.15	0.12	1.8	1.45	**0.002**
M. Vanicolensis											
Weight	0.0035	0.0035	0.0146	0.0146	0.0077	0.0078	0.0007	0.0007	0.0033	0.0034	**0.386**
Length	3.54	3.59	4.89	4.87	4.37	4.35	0.1	0.09	0.44	0.4	**0.219**
Width	2.39	2.32	3.81	3.68	3.06	2.99	0.09	0.09	0.42	0.41	**0.01**
Area	6.1	5.97	12.13	10.85	9.16	8.75	0.42	0.38	1.9	1.73	**0.000**
Perimeter	11.11	11.03	17.58	18	14.84	14.49	0.48	0.43	2.21	1.96	**0.127**
P. forsskali											
Weight	0.0012	0.0013	0.0054	0.0055	0.0025	0.0025	0.0001	0.0001	0.0008	0.0009	**0.827**
Length	2.51	2.62	4.16	4.11	3.1	3.11	0.03	0.03	0.33	0.31	**0.519**
Width	1.61	1.58	2.88	2.84	2	1.96	0.02	0.02	0.24	0.24	**0.000**
Area	2.03	2.83	6.64	6.62	3.8	4.14	0.09	0.08	0.95	0.81	**0.001**
Perimeter	3.51	7.14	11.73	12.15	7.64	8.94	0.13	0.1	1.35	0.98	**00.000**

The morphometric measurements of the fish and otoliths are shown in Table 3, with statistical descriptions of each species. Fish total length (TL) ranged from 110–334 mm, 174–287 and 144–271 for *M. flavolineatus*, *M. vanicolensis* and *P. forsskali*, respectively. The weight of the fish ranged from 11.85–453.26 g, 52.8–297.9 g and 32.13–237.37 g for *M. flavolineatus*, *M. vanicolensis* and *P. forsskali*, respectively. Individual maximum otolith length (OL) ranged from 4.1 mm (*P. forsskali*) to 4.8 mm (*M. vanicolensis*). The otoliths of *M. vanicolensis* were the largest, reaching up to 4.8 mm. The otoliths of this species also exhibited the greatest perimeter (ranging from 11.03 to 18). The otoliths of *P. forsskali* presented the smallest values for all examined parameters. Overall individual otolith weight (OW) ranged from 0.005 (*P. forsskali*) to 0.01 g (*M. vanicolensis*). *M. vanicolensis* displayed the heaviest otoliths, with an average OW of 0.0077±0.0007 g, as well as the widest otoliths and the greatest surface area. The overall otolithic measurements of *M. flavolineatus* occupied an intermediate position between those of *M. vanicolensis* and *P. forsskali.*

Table 3: Statistical Descriptions of fish weight (FW), fish total length (TL), otolith weight (OW), otolith length (OL), otolith width (OWid), otolith perimeter (OP), and otolith area (OA) of three goatfish (*Mulloidichthys flavolineatus, M. vanicolensis and Parupeneus forsskali*) from the Red Sea, Egypt.

Species	Variables	Minimum	Maximum	Mean	SE	SD
M. flavolineatus	**FW**	11.85	453.26	96.64	5.23	64.47
	TL	110	334	207.4	2.75	33.92
	OW	0.0012	0.0086	0.0039	0.000098	0.00122
	OL	2.4	4.76	3.61	0.03	0.37
	OWid	1.71	3.43	2.42	0.02	0.25
	OP	6.55	14.98	10.58	0.12	1.45
	OA	3.54	10.28	5.92	0.09	1.12
	No.			152		
M. vanicolensis	**FW**	52.8	297.9	190.14	19.69	90.22
	TL	174	287	246.91	9.06	41.50
	OW	0.0035	0.015	0.008	0.00078	0.0034
	OL	3.59	4.87	4.35	0.089	0.40
	OWid	2.32	3.68	2.99	0.09	0.41
	OP	11.03	18	14.49	0.43	1.96
	OA	5.97	10.85	8.7493	0.38	1.73
	No.			21		
P. forsskali	**FW**	32.13	237.37	76.94	3.94	39.75
	TL	144	271	185.30	2.63	26.53
	OW	0.0013	0.006	0.0025	0.00009	0.0009
	OL	2.62	4.11	3.11	0.031	0.31
	OWid	1.58	2.84	1.96	0.024	0.24
	OP	7.14	12.15	8.94	0.097	0.98
	OA	2.83	6.62	4.12	0.08	0.81
	No.			102		

According to the power equation (otolith variables=aTL^b), all morphometric measurements of the three selected species' otoliths exhibited a good relationship with fish total length (Table 4). The maximum length, weight, width, area and perimeter of the otoliths were linearly related to total fish length for the studied fish species. All regressions were highly significant, and the analysis of otolith morphometric parameters versus TL indicated that the regression models explained nearly all of the variance that best fit TL. The coefficients of determination (r^2) ranged from 0.78 to 0.81 in *M. flavolineatus*, from 0.83 to 0.92 in *M. vanicolensis*, and from 0.78 to 0.85 in *P. forsskali*, being higher for *M. vanicolensis* in all cases. The otolith area of *M. vanicolensis* was highly correlated with fish length (r^2=0.92) and was most strongly related to fish length. The statistical analysis of otolith morphometric parameters showed that the otolithic measurements were good indicators of fish size.

Table 4: Power relationships between fish length and otolith variables for the three-goatfish species (*Mulloidichthys flavolineatus, M. vanicolensis and Parupeneus forsskali*) from the Red Sea. OW, otolith weight; TL, total length; OL, otolith length; OWid, otolith width; OA, otolith area; OP, otolith perimeter.

Mulloidichthys flavolineatus		***Mulloidichthys vanicolensis***		***Parupeneus forsskali***	
$OL=0.1501TL^{0.597}$	r^2= 0.84	$OL=0.299TL^{0.486}$	r^2= 0.85	$OL=0.0996TL^{0.659}$	r^2= 0.85
$OWid=0.1058TL^{0.58\wedge}$	r^2= 0.80	$OWid=0.058TL^{0.716}$	r^2= 0.83	$OWid=0.0346TL^{0.773}$	r^2= 0.83
$OW=8E\text{-}07TL^{1.602}$	r^2= 0.82	$OW=1E\text{-}08TL^{2.431}$	r^2= 0.87	$OW=2E\text{-}08TL^{2.237}$	r^2= 0.83
$OA=0.0237TL^{1.033}$	r^2= 0.78	$OA=0.0168TL^{1.134}$	r^2= 0.92	$OA=0.00647TL^{1.236}$	r^2= 0.83
$OP=0.1646TL^{0.780}$	$r^{2=}$ 0.78	$OP=0.8502TL^{0.717}$	$r^{2=}$ 0.85	$OP=0.2275TL^{0.703}$	r^2= 0.78

The mean values of the six examined shape indices of the three species' otoliths are shown in Table 5. They were considerably different among the three species. The highest mean values of AS, CO, and RO were recorded in *M. vanicolensis*. The lowest mean values of AS and RO were observed in *P. forsskali*, whereas the lowest value of COM was recorded in *M. flavolineatus*. The highest FF was recorded in *M. flavolineatus* and the lowest in *M. vanicolensis*. All three species exhibited nearly the same value of RE. The highest ellipticity was observed in *P. forsskali* (Table 5). The Kolmogorov-Smirnov Z test confirmed a normal distribution (p>0.05; non-significant) for nearly all measurements (excluding the weight and width of the otoliths of *M. flavilineatus*). According to the present results, the aspect ratio gradually increased as fish otolith length increased.

The relationship between otolith length and the aspect ratio was close to 1; thus, the otoliths of the three species were elongated (Fig. 3). Roundness and ellipticity values increased as otolith length increased. Form factor and rectangularity values decreased as otolith length decreased. Pearson's correlation coefficients between the examined shape factors and maximal otolith length are presented in Table 6, and all of the variables for *M. flavolineatus* were significantly correlated with otolith length. Nearly all of the variables for *M. vanicolensis* were also significantly correlated with otolith length (excluding AS and EL). For *M. vanicolensis*, only FF and RO were significantly correlated with otolith length.

Table 5: Minimum, maximum, mean, standard error, and standard deviation of otoliths six shape descriptors (AS, CO, FF, RO, RE, and EL) of three goatfish species (*Mulloidichthys flavolineatus, M. vanicolensis and Parupeneus forsskali*) from the Red Sea, Egypt.

	Variables	N	Minimum	Maximum	Mean	SE	SD
M. flavolineatus	**AS**	152	0.58	0.77	0.67	0.003	0.036
	CO	152	11.71	26.04	19.03	0.212	2.616
	FF	152	0.48	1.07	0.67	0.008	0.100
	RO	152	25.98	286.78	103.09	3.612	44.53
	RE	152	0.57	0.90	0.67	0.004	0.049
	EL	152	0.13	0.26	0.19	0.002	0.025
M. vanicolensis	**AS**	21	0.62	0.80	0.69	0.011	0.049
	CO	21	20.38	32.58	24.18	0.579	2.653
	FF	21	0.39	0.62	0.52	0.011	0.052
	RO	21	98.03	314.34	219.27	16.715	76.599
	RE	21	0.60	0.72	0.67	0.006	0.029
	EL	21	0.11	0.24	0.19	0.007	0.034
P. forsskali	**AS**	102	0.56	0.72	0.63	0.003	0.035
	CO	102	16.13	24.36	19.52	0.169	1.704
	FF	102	0.52	0.78	0.65	0.006	0.057
	RO	102	27.41	142.39	53.31	2.240	22.57
	RE	102	0.57	0.79	0.68	0.0042	0.042
	EL	102	0.16	0.28	0.23	0.003	0.026

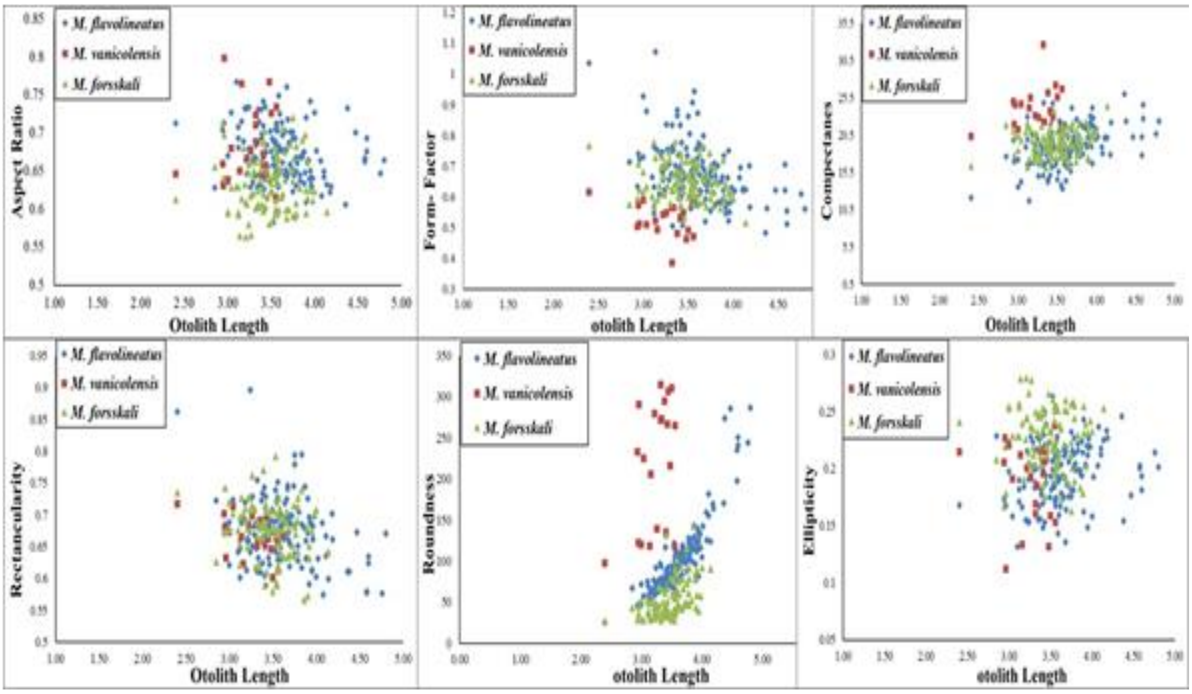

Figure 3: Relationship between the shape indices (Aspect ratio, Compactness, Form factor, Rectangularity, Roundness and Ellipticity) of the left otolith and otolith length in three goatfish species (*Mulloidichthys flavolineatus, M. vanicolensis and Parupeneus forsskali*) from the Red Sea, Egypt.

Table 6: Correlation coefficients of Pearson r between shape indices of the left otoliths and otoliths length in the three-goatfish species (*Mulloidichthys flavolineatus, M. vanicolensis and Parupeneus forsskali*) from the Red Sea, Egypt.

Index	*M. flavolineatus*		*M. vaniclensis*		*M. forsskali*	
	r	***p***	**r**	***p***	**r**	***p***
AS	-0.243**	0.003	0.401	0.072	0.098	0.325
Com	0.513**	0.000	0.433	0.50	0.239*	0.015
FF	-0.517**	0.000	-0.480*	0.028	-0.235*	0.018
RO	0.953**	0.000	0.992**	0.000	0.972**	0.000
RE	-0.402**	0.000	-0.302	0.183	-0.432**	0.000
ELL	0.242**	0.003	-0.409	0.066	-0.095	0.341

Correlation is significant at the 0.01 level

Scanning electron microscopy analysis of goatfish otolithic topography

Images of the otoliths of the three examined goatfish species are presented in Fig. 4. The sagittal shape was oval in *M. flavolineatus* (Fig. 4 A and D) and oblong in *M. vanicolensis* (Fig. 4 B and E) and *P. forsskali* (Fig. 4 C and F). The rostrum occupied the anterior margin in all three goatfish species. The posterior margin was rounded in all three species but was also equipped with projections in *M. vanicolensis* and *P. forsskali* (Fig. 4 B, C). All three sagittae exhibited scalloped or crenulated ventral and dorsal rims. The dorsal rim of *M. flavolineatus* was lobed and sinuate (Fig. 4 A). In the case of *M. vanicolensis* and *P. forsskali*, the dorsal rim was sinuate and emarginated (Fig. 4 B, C). The ventral rim of *M. flavolineatus* presented regular lobes only in the most dorsal portion (Fig. 4 A). In *M. vanicolensis*, the ventral lobes occurred in a single row and were irregular, extending from the rostrum anteriorly to the post rostrum posteriorly (Fig. 4 B). *P. forsskali* exhibited many rows of irregular dense lobes covering the entire ventral rim, extending from the rostrum anteriorly to the post rostrum posteriorly (Fig. 4 C).

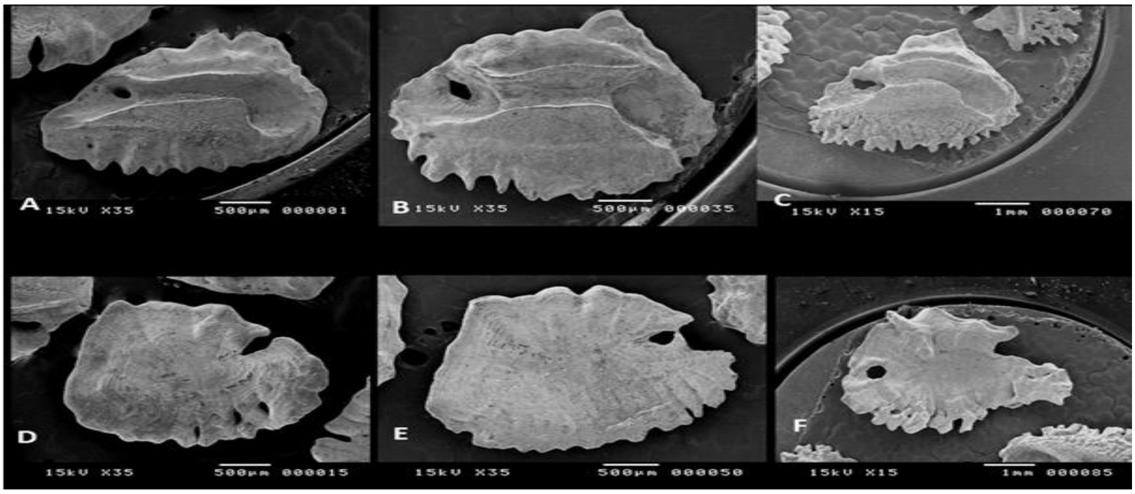

Figure 4: The scanning electron microscope photographs of the proximal (A, B, C) and distal (D, E, F) views of the left sagitta of the three-goatfish species from the Red Sea (A and D, *Mulloidichthys flavolineatus*; B and E, *Mulloidichthys vanicolensis*; C and F, *Parupeneus forsskali*).

The rostrum of *M. flavolineatus* was elongated, free of projections and did not exhibit an antirostrum (Fig. 4 A). In contrast, the rostra of *M. vanicolensis* and *P. forsskali* were nearly round, with projections and antirostra (Fig. 4 B, C). The proximal face of the sagittal otolith displayed a sulcus acusticus lined dorsally by crista superior and ventrally by crista inferior. The sulcus was ostial and homosulcoid. The crista superior of *M. flavolineatus* (Fig. 4 A) was smooth, while those of *M. vanicolensis* and *P. forsskali* were rough and crushed (Fig. 4 B, C). The sagittae presented depressions in dorsal and ventral areas, oriented parallel to the sulcus acusticus. These depressions were shallow in the case of *M. flavolineatus* (Fig. 4 A) and deep in the case of *M. vanicolensis* and *P. forsskali* (Fig. 4 B, C). The sulcus acusticus was open anteriorly and posteriorly and divided into the ostium, column, and cauda (Fig. 4). The ostium was provided with a pore in all three species. This pore was small and rounded in *M. flavolineatus* (Fig. 4 A), while it was large and irregularly shaped in both *M. vanicolensis* and *P. forsskali* (Fig. 4 B, C). The cauda of *M. flavolineatus* was funnel shaped (Fig. 4 A), while those of both *M. vanicolensis* and *P. forsskali* were crucible shaped and were initially straight, with curves then occurring distally toward the inner rim (Fig. 4 B, C). The column of the *M. flavolineatus* was long, narrow, and deep, with straight crista superior and inferior (Fig. 4 A). The columns of *M. vanicolensis* and *P. forsskali* were short and shallow, with curved crista superior and inferior (Fig. 4 B, C).

Ornamentations in the sulcus acusticus

At low magnification, the surface appeared relatively smooth, but a remarkable difference was observed in the ornamentation of the ostium, cauda, and column.

Ostium

Two types of ornamentation were observed on the ostium of each species (Fig. 5). Fine ornamentation was observed toward the edge of the ostium, transforming into a more coarsely textured surface or a coarser crystalline structure toward the inner side of the ostium in both *M. flavolineatus* and *M. vanicolensis* (Fig. 5 A, B). In contrast, the ostium of *P. forsskali* exhibited nearly the same textured surface on the edge and the inner side (Fig. 5 C). In *M. flavolineatus*, the ornamentation of the edge of the ostium had a sand-like appearance, whereas that of the inner side of the ostium had the appearance of rectangular stones (Fig. 5 A). The ostium of *M. vanicolensis* presented fine sandy ornamentation externally and projecting lath-like crystals internally (Fig. 5 B). Both the outer and the inner projections of *P. forsskali* consisted of projecting lath-like crystals (Fig. 5 C).

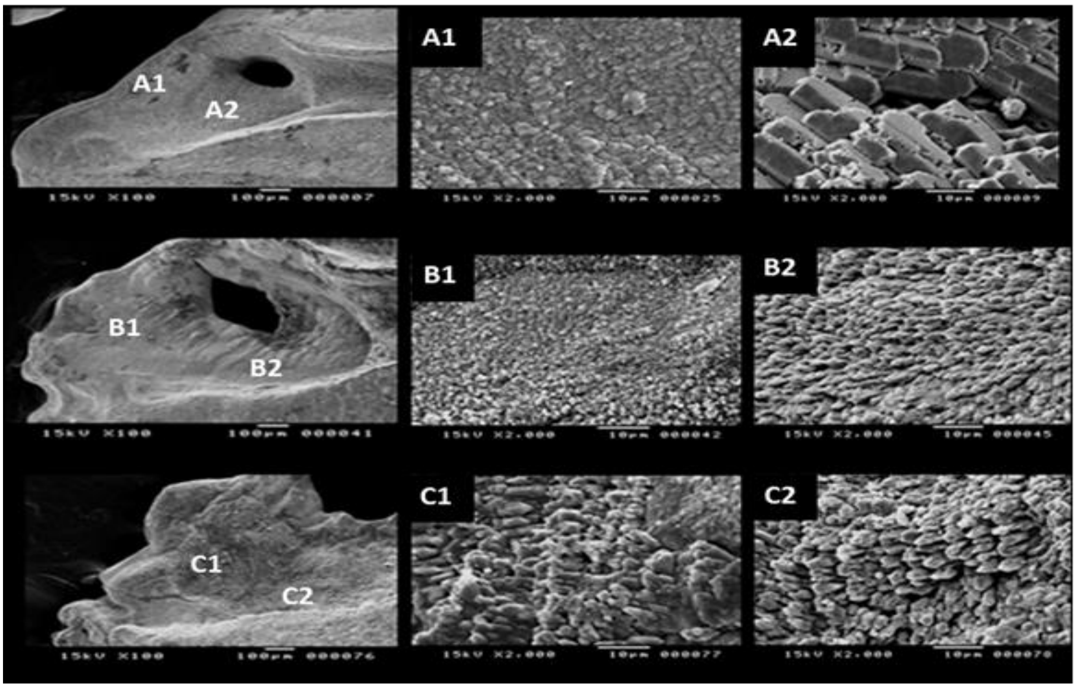

Figure 5: Scanning electron microscope photographs of the ostium of the left sagitta of the three-goatfish species from the Red Sea (A, *Mulloidichthys flavolineatus*; B, *Mulloidichthys vanicolensis*; C, *Parupeneus forsskali*).

Cauda

Each species exhibited only one type of ornamentation on the cauda. *M. flavolineatus* presented sand-like crystals (Fig. 6 A), whereas *M. vanicolensis* and *P. forsskali* presented the same type of ornamentation, in the form of projecting lath-like crystals (Fig. 6 B, C).

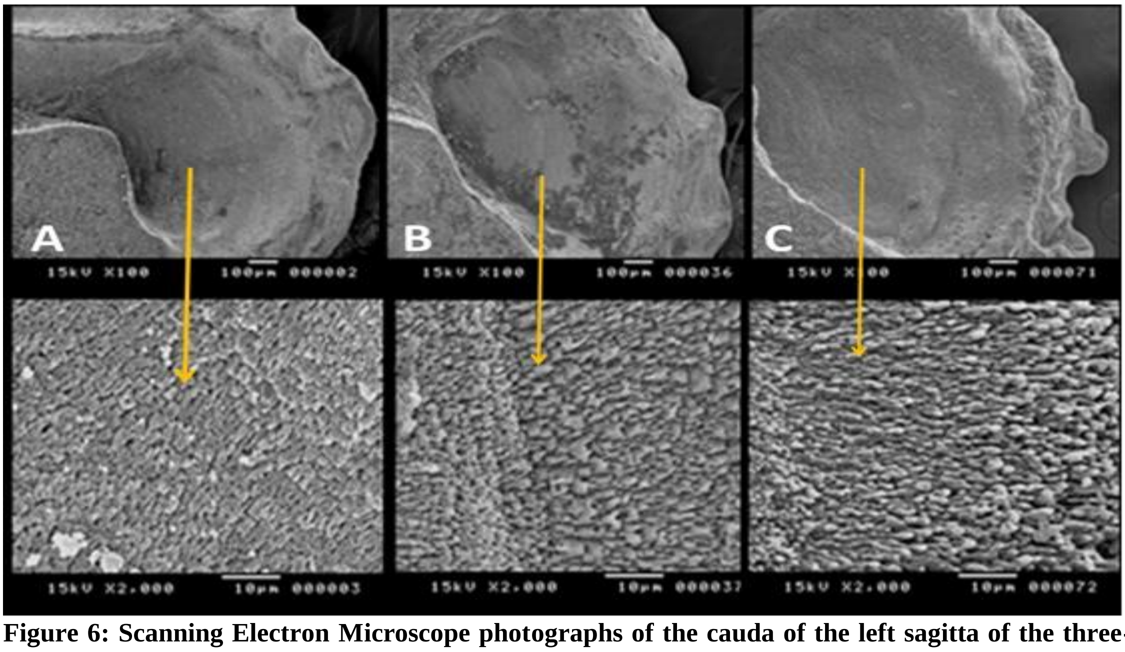

Figure 6: Scanning Electron Microscope photographs of the cauda of the left sagitta of the three-goatfish species from the Red Sea (A, *Mulloidichthys flavolineatus*; B, *Mulloidichthys vanicolensis*; C, *Parupeneus forsskali*).

Column

The column's ornamentation differed among the three species. The ornamentation of *M. flavolineatus* consisted of triangular and square stone-like shapes (Fig. 7 A), while that of *M. vanicolensis* took the form of irregular projections. In the case of *P. forsskali*, the ornamentation was rectangular and stone-like (Fig. 7 B, C).

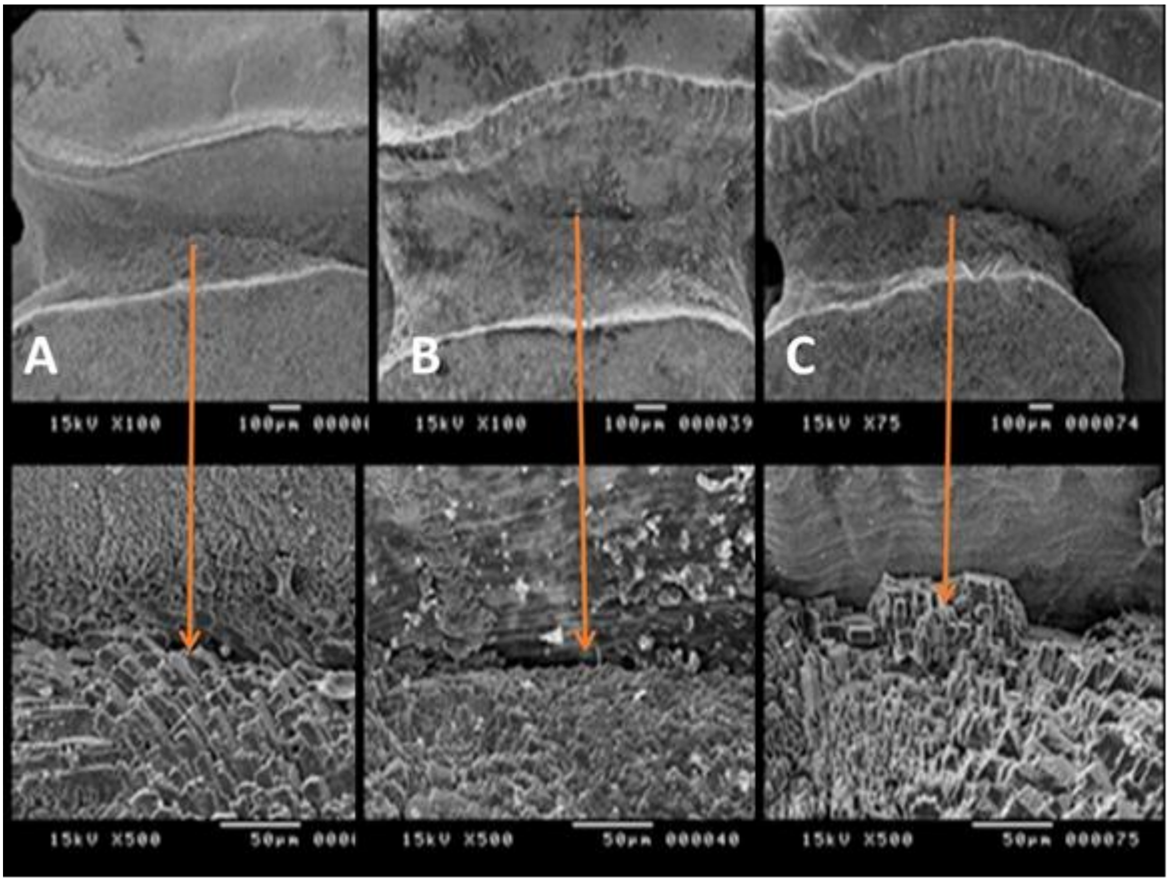

Figure 7: Scanning Electron Microscope photographs of the column of the left sagitta of the three-goatfish species from the Red Sea (A, *Mulloidichthys flavolineatus*; B, *Mulloidichthys vanicolensis*; C, *Parupeneus forsskali*).

Discussion

Goatfish represent one of the most economically important fisheries in Egyptian waters, in both the Red and Mediterranean Seas. The fishery status of any fish stock needs to be identified correctly. The fishes of this group (family Mullidae) are teleost fishes comprising many species with some shape similarities; hence, these species need to be clearly distinguished. Many studies have been performed on the fisheries and biology of goatfishes in Egypt (Golani and Ritte, 1999; Sabrah, 2015), with little attention being paid to goatfish taxonomy and phylogeny. Recently, stock identification was achieved using otolithic features (Lombarte *et al.*, 2006; Mehanna *et al.*, 2016). The morphological characteristics of fish otoliths are the most widely used tools in species identification and comparative taxonomy of fishes, due to the large size and inter-specific variability of fish otoliths (Battaglia *et al.*, 2010). To our knowledge, the otolithic shape indices and measurements of goatfish from the Red Sea have never been estimated previously. Thus, the present study may be the first to discriminate goatfish species using otolithic indices and their relationships with fish size and otolithic ultrastructure. In the present work, sagittal otoliths were employed because they are easily accessible structures and exhibit a distinctive degree of interspecific variation in their form, weight, and growth, in accordance with Nolf (1985). The morphometric parameters of sagittal otoliths have been used in earlier studies to identify species in other groups of teleosts (Tuset *et al.*, 2003a; Ponton, 2006; Short *et al.*, 2006; Tuset *et al.*, 2006). Our results suggest that differences in otolithic measurements are detectable in all three goatfish species examined in this work.

The results of the present study indicate no significant morphometric differences between the measurements of the left and right otoliths. Remarkable differences are not usually observed between left and right otolithic measurements (for many fish species) (Morat *et al.*, 2008; Jawad *et al.*, 2011a; Yilmaz *et al.*, 2014). This finding is in agreement with previous work (Valinassab *et al.*, 2012) on clupeids from the Persian Gulf. In another previous study, Hunt, (1992) studied eight species from the Atlantic Ocean, and no statistically significant difference was observed between the left and right otoliths. Similarly, Harvey *et al.*, (2000) observed no significant differences between the left and right otoliths of 63 species collected from the Eastern North Pacific Ocean. However, significant differences between left and right otolithic variables have been recorded for some other fish species (Mérigot *et al.*, 2007).

The relationships between the morphological parameters (length, width, perimeter, and area) of the otoliths and fish body length (TL) in the three goatfish species were examined using power equations. The relationships between otolith size and body length (TL) have also been estimated in other fish species using linear functions (Morat *et al.*, 2008; Pavlov, 2016). Previous studies have focused mainly on the relationship between otolith length and width and fish size (Jawad *et al.*, 2011b). This work supplies additional information (compared with other similar studies) by considering six otolithic measurements (OL, OH, OW, Owid, OA, and OP). Thus, our findings are more reliable than those relying on a single equation, since the tip of the otolithic rostrum may be damaged, making it impossible to measure OL or OW (Jawad *et al.*, 2011b; Yilmaz *et al.*, 2014), influencing the reliability of subsequent calculations. Presenting the six models ($OL=aTL^b$, $OH=aTL^b$, $OW=aTL^b$, $OWid=aTL^b$, $OA=aTL^b$ and $OP=aTL^b$) for each species helps mitigate this potential problem. It is appropriate to use the functions indicated in this paper within the range of the fish size examined in the present work. Further studies on fish size–otolithic variable relationships involving a larger sample size, a wider range of fish lengths, and different growth phases would help to support the results presented herein. The ability to identify species using morphometric parameters of the sagittal otoliths has been demonstrated for other groups of teleosts (Tuset *et al.*, 2003b; Ponton, 2006; Short *et al.*, 2006; Tuset *et al.*, 2006). The differences in the morphological characteristics of the sagittal otoliths among these species may be associated with the size of the skull (Bani *et al.*, 2013). Although relative skull size was not recorded in this study, personal observations indicate that the greatest head length belongs to *M. vanicolensis*, which also exhibits the longest otoliths.

The power relationship between sagittal otoliths and fish length was described by a negative allometric relationship (b less than 3). Similar results were previously recorded in the freckled goatfish, *Upeneus tragula* (Pavlov *et al.*, 2015). Several studies have shown a linear relationship between body length and otolith length in fish (Harvey *et al.*, 2000; Fossen *et al.*, 2003; Lychakov *et al.*, 2006). Linear otolith growth might represent a common relationship between otoliths and body length in the juvenile stage (Huang and Chiu, 1997). In the present study, power equations were applied and showed a strong correlation between the variables, indicating that otolithic measurements are good indicators of fish size. For all three species, the correlation between fish length and different otolithic variables was statistically significant (0.005), with otolith length showing the highest (0.70–0.96) Pearson's correlation coefficient, compromised by high variability in otolith shape. This result could be explained by the fact that otolithic length is most sensitive to variations in the growth rate and most closely related to changes in fish metabolism (Pawson, 1990; Flecher, 1991). In the present study, the coefficient of determination (r^2) ranged from 0.83 to 0.92 in the three species, being higher for *M. vanicolensis* in all cases. The otolith area of *M. vanicolensis* was most strongly related to fish length, with a high correlation between otolith area and fish length (r^2=0.92) being observed in this species. Concerning findings of this study, the observed variability of otolithic shape encourages further research to verify the potential role of otolithic morphometric measurements in fish identification.

Otolithic shape indices such as the aspect ratio, compactness or circularity, form factor, roundness, rectangularity, and ellipticity have been described for many fish species (Tuset *et al.*, 2003b; Pavlov *et al.*, 2015), confirming identifications performed based on morphometric variables (Tuset *et al.*, 2003a; Tuset *et al.*, 2003b). The results of this study show that the shape indices differ significantly in the analyzed species. Such differences were observed for almost all shape indices of the three selected goatfish species.

Among the shape indices, AS, COM, RO, and FF were found to be more efficient than other factors in the studied species. The highest mean values of AS, COM, and RO were recorded in *M. vanicolensis*, whereas the lowest mean values of AS and RO were found in *P. forsskali*, although the lowest value of COM was recorded in *M. flavolineatus*. Thus, in the last species, otolith shape is more circular than in *M. vanicolensis*, indicating that all of the otoliths of *M. flavolineatus* grow equally in terms of length and height. The numeric values of the FF show that the sagittal shape is geometrically irregular in the three species. The highest FF was recorded in *M. flavolineatus*, and the lowest was observed in *M. vanicolensis*, which presented the most irregular otolithic shape among the studied species. The shape analyses revealed rectangular shapes in the otoliths of the three species, as they exhibited nearly the same

Although the study of shape indices is complicated, it can confirm identifications made based on the differentiation of fish species (Špiranec and Banek Zorica, 2010; Sadighzadeh *et al.*, 2012) and populations (Mérigot *et al.*, 2007; Duarte-Neto *et al.*, 2008; Pavlov, 2016). In the present study, the shape indices were compromised by their high differentiation; thus, the results of this study will be useful for other researchers in verifying the role of the otolith in fish identification and classification. These findings were supported by previous results (Tuset *et al.*, 2008). The obtained ellipticity values show that the shape of the three species is irregular, with the lowest value being found in *M. vanicolensis* and the highest in *P. forsskali*. According to the Kolmogorov-Smirnov Z test, a normal distribution was confirmed for nearly all otolithic measurements in each of the examined fish species. Otolith area, perimeter and shape indices have been suggested as an easier means of discriminating stocks compared with other methods (Bolles and Begg, 2000; Tuset *et al.*, 2003b). The aspect ratio and ellipticity were found to be directionally proportional to otolith length, while the form factor (FF) and roundness (RD) were inversely proportional to this parameter. Such similarity in otolithic shape indices may be derived from the fact that all of the studied goatfish species occupy the same ecological niche. Fish occupying the same ecological niche show similarities in otolithic shape variables (Parmentier *et al.*, 2001). The correlation between shape factors and otolith length were strong and significant in the case of *M. flavolineatus*. Excluding AS and El, all other shape factors in *M. forsskali* exhibited significant correlations with otolith length. In the case of *M. vanicolensis*, only FF and RO were significantly correlated with otolith length. The results of this study show that the shape indices significantly differ from species to species, although they indicate a similar otolithic pattern. These results correlate with those of Tuset *et al.* (2008), who postulated that otoliths are the most widely employed tool for the discrimination of fish species because of their form, weight, growth, consistency and chemical composition.

The current study also compared otolithic shape among the three goatfish species using SEM, to observe variations in otolithic morphology. In *M. flavolineatus*, the sagittal shape was found to be oval, with regular lobes on the dorsal and ventral rims. In *M. vanicolensis* and *P. forsskali*, the otolithic 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 otolithic characteristics might be important to fishery biologists, archaeologists and geologists, who can use them to distinguish *M. flavolineatus*, *M. vanicolensis* and *P. forsskali*. This work contributes to the bioecological knowledge regarding commercially important fishes and provides key information for studying the trophic ecology of fish-eating species and fisheries management.

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