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Discrimination of Five Gobid Species (Family: Gobiidae) based on Otolith Morphometry

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ABSTRACT

Background: The fishes of family Gobiidae are one of the least studied fishes, especially for otolith structure. The otoliths were possess species specific features. Hence, traits of sagittal otolith of gobid species studied.

Methods: Five gobid species *Odontamblyopus roseus* (Valenciennes, 1837), *Trypauchen vagina* (Bloch and Schneider, 1801), *Glossogobius giuris* (Hamilton, 1822), *Parachaeturichthys polynema* (Bleeker, 1853) and *Boleophthalmus dussumieri* (Valenciennes, 1837) were investigated by three methods including morphological, shape indices and step wise discriminant function analysis (SDFA).

Result: Interrelationship between shape indices investigated, at 95% level of confidence (P<0.05), revealed that perimeter of *P. polynema* and area of *T. vagina* have isometric growth with their length (b = 3.0071, 2.90, respectively) and otolith area of *B. dussumieri* have positive allometric growth (b = 4.23077). SDFA, based on otolith morphometry, discriminated species up with 97.18% accuracy. Hence, the results of present investigation can be used for discrimination of the species and as a tool in predicting fish size from the otoliths and in calculating the biomass of these less studied fish species.

Key words: Allometric growth, Investigation, Sagitta**,** Shape indices, Taxonomy.

INTRODUCTION

Taxonomic study of fishes plays an important role in conservation of fisheries resources by discriminating and identifying species (Fischer, 2014) and also to know the evolutionary relationship among a group of species (Bani *et al.* 2013). The methods, used for identifying species and discriminating them, are often based on meristic count, morphological attribute, morphometric characters and molecular tools (Waessle and Millesi, 2013). However, the morphological features of hard parts, such as otolith and bone, also possess significant capability to discriminate species (Aguirre and Lombarte, 1999; Tuset *et al.* 2003a, Ponton, 2006), at the same time, the molecular technique is costly and time consuming.

The otoliths are formed during early stages of development and grow continuously throughout the life till death (Vikas *et al.*, 2019) and the elements are deposit on the otolith by acellular process that reflect no reabsorption of element or metabolically inertness, even during unfavourable condition (Campana and Neilson, 1985). The otoliths, also known as ear bone, are embedded in endolymph of the internal ear and are constituents of calcium carbonate matrix (carbon, oxygen and calcium) and impurities (Campana, 1999; Dove and Kingsford, 1998; Milton and Chenery, 2001). The impurities of otolith are composed of protein and trace elements. Further, the otoliths are made of 33 trace element including two radioactive elements (radium and thorium) (Friedrich and Halden, 2008, 2010, Vandermyde and Whitledge, 2008). Fishes contain three types of otoliths and generally, sagitta is larger than other otoliths, except in catfishes.

Due to high irregularity but species specific shape of otolith, it is used for identification, biological study, food and feeding behaviour, pray and predator relationship, stock identification (Tuset *et al.* 2003a, b, Pothin *et al.* 2006; Duarte-Neto *et al.* 2008), archaeology (Reichenbacher *et al.* 2007), trophic ecology (H¨ark¨onen, 1986; Tuset *et al.* 1996) paleoichthyology (Nolf, 1985) and functional morphology (Ladich, 2000; Parmentier *et al.* 2001).

Further, the digitization of otolith image, extraction of data, mathematical analysis of shape indices and Fourier series (Tuset *et al.* 2006) provide an opportunity to discriminate species with accuracy.

The family Gobiidae is one of the largest family of the group Acanthomorph, comprising of more than 1763 species, under 170 genera. Gobies are found worldwide, in all three marine, estuarine and freshwater habitats (Thacker and Roje, 2011; Daraji *et al.* 2017). Taxonomic ambiguity is a one of the major reasons for lack of biological study on these species (Bani *et al.* 2013) where, taxonomic ambiguity is reported to be result of similarity in morphological and meristic traits. Therefore, the present study was to characterize sagittal otoliths of the five species of the family

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Gobiidae based on morphometric characters, in association with shape indices of otolith that can be used to adjudicate the ambiguity in these species.

MATERIALS AND METHODS

Samples of gobid fishes *O. roseus* (Valenciennes, 1837), *T. vagina* (Bloch and Schneider, 1801), *G. giuris* (Hamilton, 1822), *P. polynema* (Bleeker, 1853) and *B. dussumieri* Valenciennes, 1837 were collected from the Dol net landings at Karanja (Dharamtar estuary, Maharashtra), East coast of Arabian Sea (Fig 1). The specimens were transported to laboratory in ice, washed and photographed with Canon EOS 1300D. Samples were identified up to species level using available standard identification keys (Murdy and Shibukawa, 2001; Murdy, 1989). After morphological study of fish (meristic and morphometric), the saccular otolith (sagitta) of both side were extracted (213) by making cut in otic capsule of neuro cranium. The collected otoliths were cleaned with distilled water and diluted nitric acid, air dried and stored in plastic vials for further study.

Otolith were weighed with six digit Sartorius GD 603 weighing balance (accuracy 0.000001gm) and photographed using calibrated Leica stereo-zoom microscope SZX16 placing the rostrum to the right and the convex side kept upwards. Analysis of general otolith morphology was performed following Tuset *et al.* (2006). The measured otolith dimensions are otolith length (Le)- the longest distance between tip of rostrum and anti-rostrum, otolith width (Wi)- the longest distance between dorsal to ventral rim of otolith at right angles to the otolith length through the core point of the otolith (Škeljo and Ferri, 2012, Hunt, 1992), elongation or aspect ratio (El) ratio of otolith length (Le) to otolith width (Wi), Area quantity that expresses the extent of otolith in the plane, Perimeter length of line that encompasses an otolith shape, roundness condition of otolith circular line wherein all points on the periphery of an otolith are at equidistant from a common centre point, circularity ratio of the area of an otolith to the area of circle having the same perimeter of the otolith, ellipticity degree of deviation from a circle, rectangularity a measure of the normalised discrepancies between the areas of the rectangle and the form factor counterbalance for irregularity in the shape of an otolith which is the ratio of the otolith area to the square of the otolith length (Russ, 2016). For the extraction of Shape indices following formula (Table 1), were used.

RESULTS AND DISCUSSION

The traditional gobid taxonomy is dependent on morphological and meristic characters with varying degree of accuracy. The morphological characteristic of otolith is a rigid and species specific and not affected by external factors Geffen (1983). Sometimes identification of species based on morphological characters of body leads to ambiguity; Hence the present study is based on otolith morphometric features. The left and rights are very similar, hence only right side otoliths used for comparison.

Table 1: Size parameters and shape indices of otolith.

Size parameters	Shape indices
Area (Ae)	Circularity (Ci) = $(Pe^2)/Ae$
Perimeter (Pe)	Ellipticity (Ell) = (Le-We)/(Le+We)
Width (Wi)	Rectangularity (Re) = Ae/(Le*We)
Length (Le)	Form Factor (FF) = $(4\pi Ae)/Pe^2$
	Aspect ratio or Elongation (El) = (Le/Wi)

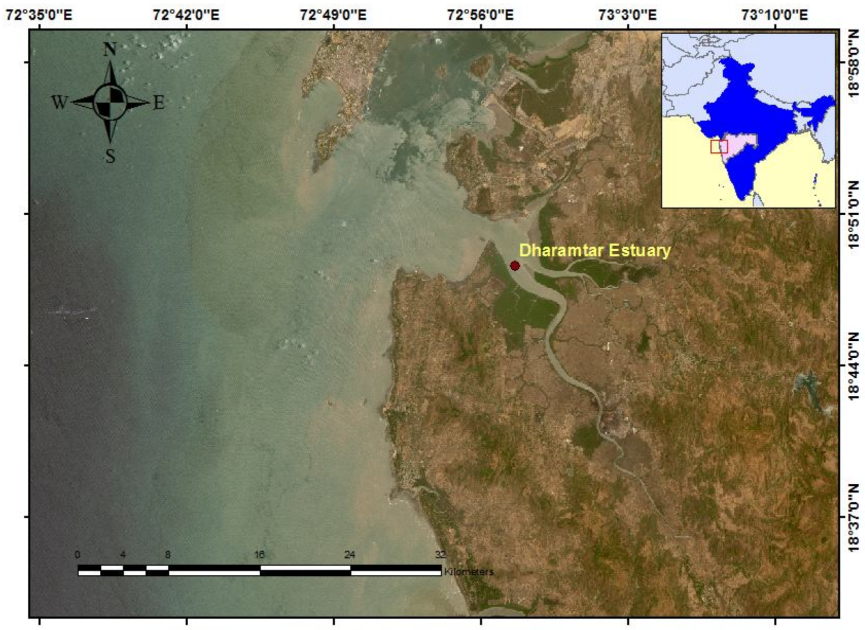

Fig 1: Sampling location in Dharamtar estuary, Maharashtra, Eastern Arabian Sea.

Otolith morphology

Morphological characteristics were studied in five gobid fishes (Fig 2) and the characters are described below -

B. dussumieri

Otolith cuneiform in shape, notch present on anterior side, crista superior larger than crista inferior, ostium and cauda not separated well, the dorsal side of otolith with groove, periphery of otolith rough on dorsal and smooth on ventral side.

T. vagina

Kidney shaped otolith, the posterior part broader than anterior, periphery smooth, a small notch observed on anterior side, external outgrowth on posterior side, core point thick, structure on otolith not clear.

O. roseus

Otolith bowl shaped, two flat notch on anterior side, three small grooves on ventral side, few annuli clearly visible near periphery on ventral side, core point thicker than periphery, peripheral margin smooth, anterior side broader than posterior.

P. polynema

Otolith oval in shape, clear notch on anterior side, rostrum larger than anti rostrum, few redial suture on ventral side, anterior side broader than posterior, otolith more or less elongated, rostrum on ventral side with few grooves.

G. giuris

Otolith squared in shape, pointed extension on anterior side, some irregular precipitation of calcium on posterior side, otolith with moderate thickness, the periphery smooth.

In the present study, mean values of nine morphometric characters for the five species were compared (Table 2). Otolith of *G. giuris* species have maximum otolith area (9.352 mm^2), length (4.276 mm) and ellipticity (3.358). Maximum elongation (1.425), form factor (0.697) and rectangularity (0.717) was observed in *P. polynema*, maximum perimeter length (14.645 mm) in *B. dussumieri*, while maximum width (2.753 mm) and circularity (25.541) in *T. vagina* (Table 2).

Interrelationship between otolith characters

The regression of otolith parameters with length, stabilised on 95% level of confidence, showed isometric growth (2.994) of the otolith area of *O. roseus* with their length with lower p- value (2.128E-17) and higher goodness of fit (R^2 = 0.8012). The perimeter, width, ellipticity, rectangularity and elongation also fitted well, with significant relationship. The perimeter of *P. polynema* shows isometric growth (b = 3.0071) with least P value (P = 7.251E-31); apart from this area, width and ellipticity also showed good relationship with the otolith length at lower P- value and higher goodness of fit.

The otolith area of *T. vagina* revealed close to isometric growth (b = 2.90) with their length at lower P- value (7.637E-10), while perimeter showed positive correlation. The otolith area of *B. dussumieri* revealed higher regression coefficient 'b' (4.23077) at lower P- value of coefficient b (2.901E-07) and higher goodness of fit (R^2 = 0.8557). The otolith of *G. giuris* showed positive allometric correlation of area (4.4937) and perimeter (3.71202) with length, at lower P- value 1.816E-42 and 7.672E-28, respectively, indicating higher goodness of fit (R^2) 0.7946 and 0.9151, respectively. The width and

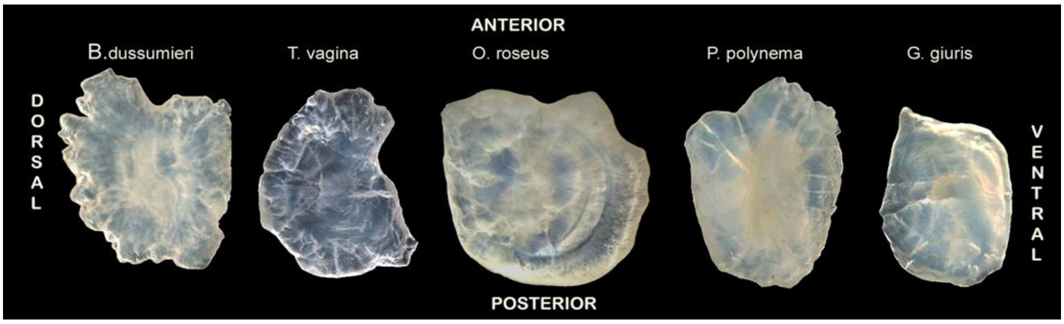

Fig 2: Otolith of five gobid fishes.

Table 2: Indices of otolith morphometry.

	T. vagina n = 27	*O. roseus* n = 47	*B. dussumieri* n = 16	*P. polynema* n = 45	*G. giuris* n = 78
Area (Ae)	5.916	5.479	9.222	1.911	9.352
Perimeter (Pe)	12.071	10.452	14.645	5.804	13.432
Length (Le)	3.309	2.934	4.174	1.919	4.276
Width (We)	2.753	2.751	3.392	1.341	3.106
Circularity	25.541	19.739	24.136	18.214	20.160
Ellipticity	0.097	0.032	0.103	0.175	3.358
Rectangularity	4.932	5.142	0.647	0.717	0.673
Form factor	0.507	0.643	0.534	0.697	0.633
Elongation	1.220	1.067	1.231	1.425	1.355

ellipticity also showed good correlation at low P- value and high goodness of fit (Table 3).

SDFA

After factor analysis seven morphometric variables were sorted and subjected to Stepwise Discriminant Function Analysis (SDFA) to find out relevant characteristics for their discrimination power. The factor analysis showed that SDFA incorporated seven out of nine fed characters in the model. Comparative importance of these mentioned variables in the SDFA model, is indicated by their loading value on the function (Roots). The factor loading for perimeter, area and length on both root emphasises on its higher separating power in the comparison of characters (Table 4).

The factor structure matrix showed higher contribution of ellipticity and rectangularity to Root 1 and rectangularity elongation to Root 2 which stress their significance in discriminating the different species (Table 5).

A classification matrix, generated by SDFA, revealed a 97.173% correct classification. Out of 213 specimens, only 3 specimens of *T. vagina* were found with overlapping characters with *O. roseus.* Rest of the specimens were correctly (100%) classified (Table 6). An instance of misclassification between *T. vagina* and *O. roseus* showed

Table 3: ANOVA of morphometric parameters of otolith.

		LexAe	LexPe	LexWe	LexCi	LexEll	LexRe	LexFF	LexEl
O. roseus	b	2.99	1.75	0.74	-3.1	0.03	2.46	0.07	0.07
	SE (b)	0.222	0.833	0.083	2.655	0.015	0.338	0.098	0.033
	P (b)	2.18E-17	0.04064	1.70E-11	0.243	0.02489	3.85E-09	0.47635	0.02532
	R^2	0.801	0.089	0.637	0.03	0.106	0.541	0.011	0.106
G. giuris	b	4.493	3.712	0.856	-0.46	0.845	-0.004	0.011	-0.009
	SE (b)	0.156	0.216	0.04	0.371	0.037	0.004	0.011	0.006
	P (b)	1.81E-42	7.67E-28	9.47E-34	0.2142	1.09E-35	0.9242	0.2957	0.1671
	R^2	0.915	0.794	0.856	0.02	0.872	0.001	0.013	0.024
B. dussumieri	b	4.23	1.37	0.75	-6.31	0.009	-0.01	0.099	0.024
	SE (b)	0.46	0.91	0.09	3.51	0.01	0.01	0.09	0.03
	P (b)	2.90E-07	0.15328	1.01E-06	0.093	0.47829	0.92774	0.30563	0.4869
	R^2	0.855	0.14	0.827	0.187	0.036	0.006	0.074	0.035
P. polynema	b	1.83	3	0.6	1.97	0.03	-0.02	-0.07	0.09
	SE (b)	0.043	0.098	0.016	0.612	0.006	0.009	0.02	0.018
	P (b)	1.50E-36	7.20E-31	1.50E-07	0.0023	4.80E-06	0.0156	0.0008	3.90E-06
	R^2	0.976	0.956	0.967	0.195	0.388	0.128	0.231	0.394
T. vagina	b	2.9	3.37	1.75	3.32	0.02	1.84	-0.09	0.01
	SE (b)	0.3	0.94	0.94	4.53	0.01	0.45	0.11	0.04
	P (b)	7.60E-10	0.0014	0.0156	0.46	0.2396	0.0003	0.4138	0.7854
	R^2	0.785	0.338	0.128	0.021	0.054	0.4	0.026	0.003

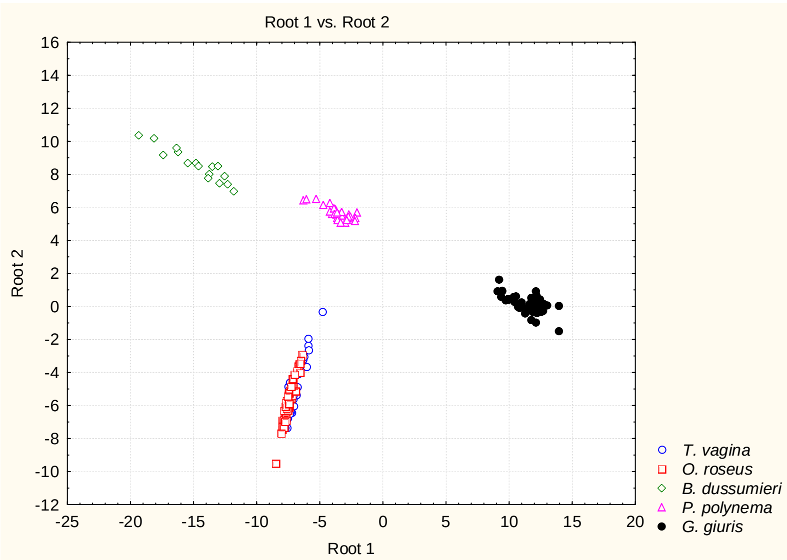

Fig 3: Scatter plot between Root 1 and Root 2.

Table 4: Factor loading of morphometric variables.

Variables	Factor Loadings (Varimax raw) Extraction: Principal components (Marked loadings are >.700000)	
	Factor 1	Factor 2
Area	0.970407	-0.069158
Perimeter	0.965102	0.211408
Length	0.979201	-0.067401
Width	0.930947	0.188627
Circularity	0.254866	0.680378
Ellipticity	0.749863	-0.532995
Rectangularity	-0.172528	0.794358
Form factor	-0.285143	-0.668629
Elogation	-0.028682	-0.765339
Expl. Var	**4.437755**	**2.500410**
Prp. Totl	**0.493084**	**0.277823**

Table 5: Factor structure matrix of variables.

Variable	Factor structure matrix correlations variables - Canonical roots (Pooled-within-groups correlations)			
	Root 1	Root 2	Root 3	Root 4
Rectangularity	-0.304585	-0.903799	-0.101636	0.064897
Ellipticity	0.336910	0.025245	0.383156	0.110845
Width	0.040599	-0.108250	0.503303	0.194077
Elogation	0.138556	0.364003	-0.359165	0.776179
Area	0.065529	-0.034650	0.391196	0.193262
Length	0.094308	-0.046213	0.531638	0.411386
Perimeter	0.048141	-0.069449	0.460379	0.411917

the morphometric proximity of the species that was again affirmed by the less square Mahalanobis distance (9.022). Maximum squared mahalanobis distance in the morphometric term have been observed between *G. giuris* and *B. dussumieri* followed by *O. roseus and G. giuris* pair (Table 7).

The adequacy of the variables in species differentiation and classification power of the SDFA model is also indicated by the scatterplot, plotted based on canonical scores in different cases of same species, where the values of the species were aggregated and well-spaced from other species of the group (Fig 3).

The results of the present study show that three methods (morphology, shape indices and step wise discriminant function analysis), applied for classifying sagittal otoliths, provide potential power for identification and discrimination of five species of family Gobiidae. The results of present study also show that otolith characteristics are species specific that can be applied for discrimination of species and, otolith can play a vital role in the study of species ecology (Nazir and Khan 2021, Reichenbacher *et al.* 2007; Volpedo and Echevarría, 2003; Paxton, 2000; Aguirre and Lombarte, 1999). The factors such as sound, hearing, depth, habitat, behaviour, swimming activity of species affect the shape of otolith (Wilson, 1985; Lombarte *et al.* 2003; Volpedo and Echeverría, 2003; Sadighzadeh *et al.* 2014; Tuset *et al.* 2015; Cruz and Lombarte, 2004). The data was fitted well to the regression model (R e" 0.90) for area of all species, for perimeter for *G.giuris* and *P. polynema*, for width *G.giuris*, *P. polynema* and *B. dussumieri*. The outcome of regression analysis revealed more than 95% level of confidence (P<.05) for area and width of all the species;

Table 6: Classification matrix of five gobid species.

Group	Classification Matrix Rows: Observed classifications Columns: Predicted classifications					
	Per cent correct	*T. vagina* P=.12676	*O. roseus* P=.2206	*B. dussumier* P=.07512	*P. polynema* P=.21127	*G. giuris* P=.36620
T. vagina	88.8889	24	3	0	0	0
O. roseus	93.6170	3	44	0	0	0
B. dussumieri	100.0000	0	0	16	0	0
P. polynema	100.0000	0	0	0	45	0
G.giuris	100.0000	0	0	0	0	78
Total	**97.1831**	**27**	**47**	**16**	**45**	**78**

Table 7: Square Mahalanobis distance between five gobid species.

Measurement	Squared Mahalanobis Distances				
	T. vagina	*O. roseus*	*B. dussumieri*	*P. polynema*	*G.giuris*
T. vagina	0.0000	9.0228	288.3034	131.0143	377.5301
O. roseus	9.0228	0.0000	286.8584	152.4117	400.2591
B. dussumieri	288.3034	286.8584	0.0000	220.2540	801.6234
P. polynema	131.0143	152.4117	220.2540	0.0000	282.2125
G.giuris	377.5301	400.2591	801.6234	282.2125	0.0000

perimeter of all species except *B. dussumieri*, rectangularity of all species except *B. dussumieri* and *G. giuris*, circularity of *B. dussumieri* and *P. polynema*. ellipticity of *O. roseus*, *P. polynema* and *G. giuris*, form factor of *P. polynema* and elongation of *O. roseus* and *P. polynema*. The area and perimeter of all the species and rectangularity of *T. vagina* and *O. roseus* showed positive correlation with the otolith length and out of these. area of *O. roseus*, *T. vagina* and perimeter of *P. polynema* showed isometric growth, while area of *G. giuris* and *B. dussumieri* and perimeter of *G. giuris* and *T. vagina* showed positive allometric growth.

In the present study, differences were observed in almost all morphometric parameters (shape indices). The morphological variation in otolith may be due the depth of inhabitation, skull size and somatic growth pattern (Aguirre and Lombarte, 1999; Tuset *et al.* 2006).

The SDFA performed to find correct classification of all five gobid species, based on sagittal otolith shape, revealed 97.1831% accuracy. Lombarte and Lleonart (1993) reported around 97% accuracy in salmodis from NW Atlantic. Bani *et al.* (2013) reported 94.7% accuracy in discrimination of gobid species from Anzali coast, Guilan, Iran. There was no significant difference between left and right otolith in all five species, but both otoliths were not mirror image as also reported by Harvey *et al.* (2000); Hunt (1979); Waessle *et al.* (2003).

Results encourage the use of these relationships as a tool in predicting fish size from the otoliths and in calculating the biomass during feeding studies and also these data can be used as baseline information for taxonomic discrimination of the species.

CONCLUSION

In the present investigation, shape estimation of otolith of five species of family Gobiidae, were analysed using - morphology, morphometric indices and SDFA. The features of otolith were found to be species specific, hence can be important in species identification, biological study and species conservation. The morphometric indices were fitted well for regression (P<0.05) and SDFA discriminated 97.18% based on otolith morphometry. The outcome of present study will be useful for identification of species and in turn, management of gobid resources.

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