



## Otoliths as taxonomic tool to identify catfishes of the genus *Mystus* (Teleostei: Bagridae) from India

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### ABSTRACT

To contribute to the clarification of taxonomy of bagrid catfishes belonging to the genus *Mystus*, the lapillus otoliths of five species (*Mystus armatus*, *M. malabaricus*, *M. cavasius*, *M. gulio* and *M. tengara*), using various otolith shape indices such as circularity, ellipticity, rectangularity and form factor were compared. Among the indices studied, rectangularity and form factor bestowed maximum F-ratio of 49.223 and 30.621 respectively, contributing maximum to species discrimination. The F-ratio for circularity, ellipticity, rectangularity, form factor and roundness were found to be 4.154, 28.735, 30.621, 49.223 and 14.58 respectively. The shape indices studied varied significantly among the five species. Cross validation by discriminant analysis of otolith shape data explained 100% variability by the first four functions. The jack-knifed classification matrix identified that the original groups are correctly classified to an extent of 68.6%. The description provided in this article will serve as a baseline database for bagrid otolith of the genus *Mystus*.

Keywords: Freshwater fish, *Mystus*, Shape indices, Siluriform, Species identification

### Introduction

Otoliths are paired calcified structures, which aid in balance as well as hearing ability in fishes (Campana, 1999; Campana and Thorrold, 2001). They are primarily made up of calcium carbonate matrix comprising of calcium, oxygen and carbon (Campana, 1999) and are the first calcified tissues which develop in the embryonic stage of fishes (Radtke and Dean, 1981). Otolith science has wide applications in fish ecomorphology (Volpedo and Echevernia, 2003), paleontology (Nolf and Brzobohaty, 2009), age determination (Ilkayaz *et al.*, 2011), daily and annual growth (Fowler, 1990) and stock delineation (Burke *et al.*, 2008; Aguera and Brophy, 2011; Legua *et al.*, 2013; Avigliano *et al.*, 2017; Lacroix *et al.*, 2017; Song *et al.*, 2018). As otolith size and shape vary between species (Campana and Thorrold, 2001), it can be utilised as a taxonomical character for species identification (Afanasyev *et al.*, 2017) as well as in evolution and phylogeny (Lombarte *et al.*, 2010; Nolf, 2013).

Otoliths act as a natural tag as they can detect changes in habitat conditions (Volk *et al.* 1999), based on the study of trace element composition (Wells *et al.*, 2003). The otolith elemental composition and its accumulation

are highly influenced by the elemental concentration in the surrounding waterbody (Khan *et al.*, 2012). The uptake of elements is subsequently influenced by temperature (Elsdon and Gillanders, 2004, Fowler *et al.*, 1995, Walther *et al.*, 2010), salinity (Fowler *et al.*, 1995), dissolved oxygen and pH (Mayer *et al.*, 1994), water chemistry (Bath *et al.*, 2000), growth rate (Kalish, 1989), diet (Buckel *et al.*, 2004), ontogeny (Walther *et al.*, 2010) and physiology (Kalish, 1989) of the fish. Several studies have been carried out to identify fish species using otoliths in different parts of the world (Mollo, 1981; Martinez and Gonzo, 1988; 1991; Tuset *et al.*, 2008). In India, a country with high level of siluriform diversity, no information is available on description and use of catfish otoliths for species identification, except for a study by Tilak (1963) who described morphology of catfish otolith. The present communication aims to fill this knowledge gap by characterising the morphological variation in otoliths of five species of the genus *Mystus* Scopoli, 1777.

### Materials and methods

#### Sampling

Samples of five species of *Mystus* were collected from different parts of India during 2015-2018 (Fig. 1). Since,

the lapillus is the largest among the three otoliths (lapillus, sagittal and astericus) in catfishes, this was used for taxonomic identification. A total of 152 otoliths of different species, comprising *Mystus gulio* (30) and *M. cavasius* (32) collected from West Bengal; *M. tengara* (30) collected from Assam and Maharashtra and *M. malabaricus* (30) and *M. armatus* (30) collected from Kerala were extracted after recording total length (mm) of each specimen.

Otoliths were collected from the lower side of the brain inside the cranial cavity where the entire structure of internal ear can be observed (Campana, 1999) and were removed by turning ventral side of the fish upwards to allow removal of the lower jaw, gills and the hypobranchial apparatus in order to expose the base of skull. The otic capsule, present on both left and right sides, were broken using a sharp scalpel and the pair of otoliths was removed using fine forceps. The extracted otoliths were cleaned by brushing with a soft bristled brush (Secor and Dean, 1992) and soaked in ultra-pure water to remove all adhering biological residues. To dissolve the remaining biological residues, otoliths were soaked in 3% hydrogen peroxide for 5 min, followed by immersion in 1% nitric acid for 5 min, to remove surface contaminants. Further, to remove the acid, otoliths were flooded with ultra-pure water for 5 min. Finally, the sampled otoliths were dried under a laminar flow-hood. The undamaged and decontaminated

otoliths were stored in acid-washed, dry polypropylene vials with reference numbers and sealed, for further examination and analysis.

#### Measurement of otolith shape indices

Digital images of each otolith were recorded with a DP72 digital camera fitted to an SZX16 stereozoom microscope (Olympus Inc., Tokyo). The microscope magnification was adjusted to the size of the otolith to ensure the highest resolution possible, varying between 3X to 5X. The digitised images were analysed using Sigma Scan Pro Version 5.0.0 image analysis software to measure its Area (Ao), Perimeter (Po), Maximum length (Lo) and Maximum width (Wo). Otolith shape indices including circularity, ellipticity, rectangularity and form factor were calculated following Russ (1990) and Tuset *et al.* (2003) (Table 1) and were used for canonical discriminant analysis (CDA).

Table 1. Size parameters and size descriptors used for identification of *Mystus* spp. collected in the present study

| Size parameter | Shape indices                       |
|----------------|-------------------------------------|
| Area (Ae)      | Circularity = $(Pe^2)/Ae$           |
| Perimeter (Pe) | Ellipticity = $(Le - We)/(Le + We)$ |
| Width (We)     | Rectangularity = $Ae/(Le * We)$     |
| Length (Le)    | Form factor = $(4\pi Ae)/Pe^2$      |
|                | Roundness = $(4Ae)/(\pi Le^2)$      |



Fig. 1. Sampling sites of *Mystus* spp. collected during the present study

### Data analysis

Shape indices could be linked to the fish sizes and therefore, to remove allometric effects of body size in shape analysis, the theoretical equation outlined below were used to scale the data (Lleonart *et al.*, 2000):

$$Y_i^* = Y_i [(X_0/X_i)^b]$$

where  $Y_i^*$  is a transformed measurement (it is a theoretical value that would measure  $Y_i$  if the standard length was  $X_0$ );  $X_i$  = Standard length of individual  $i$ ;  $X_0$  = Species-wise mean of standard length and  $b$  = Within group slope regressions of the  $\log Y_i$  vs  $\log X_i$

The transformed variables obtained from the above equation were then used for the analyses. Differences in shape indices between species were tested by one-way analysis of variance (ANOVA) followed by *ad hoc* comparisons (Duncan's multiple range test). Canonical discriminant analysis (CDA) was performed with shape indices, to compare otolith shape among the species (SPSS, 1999).

### Results

The otoliths of all five species of the genus *Mystus* studied are kidney shaped in general (Fig. 2), but the shape indices indicated significant variation in otolith among the species ( $p < 0.05$ ). Results of ANOVA of five otolith shape indices revealed maximum F-ratio of 49.223 and 30.621 in form factor and rectangularity, respectively, contributing maximum to species discrimination (Table 2). The mean values of circularity index of all the studied species were found to be close, except *M. tengara*. Similarly, the means of ellipticity of *M. cavasius* and *M. armatus* were found to be very close. The mean value of rectangularity index of *M. cavasius* was found to overlap with *M. malabaricus* and that of *M. gulio* with *M. armatus*. The mean values of roundness of *M. cavasius* indicated closeness with *M. gulio* and that of *M. malabaricus* with *M. tengara*. On the contrary, the mean values of form factor in all the species differed significantly from each other. The overall examination of the various shape indices indicated significant differences between species, however, a slight overlapping of values between *M. malabaricus* and *M. tengara* was recorded (Fig. 3). The first four functions of CDA based on shape indices explained 100% variability, where the first two discriminant functions of CDA accounted for 93.31% variance (Table 3). The output of jack-knifed classification validates most of the patterns detected in the CDA analysis, which displayed successful classification of the five species. The classification ranged from 14.28 to 89.47% (Table 4) and the original groups are 68.6% correctly classified. In cross-validation, each case is classified by the functions derived from all cases other than that case and 66.7% of cross-validated grouped cases were found to be correctly classified.

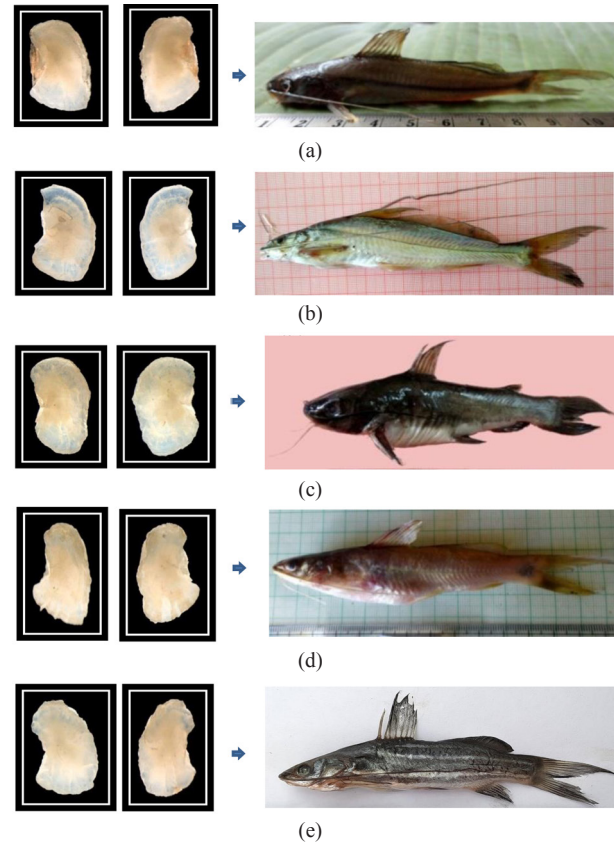


Fig. 2. Left and right otoliths of (a) *M. armatus*; (b) *M. cavasius*; (c) *M. gulio*; (d) *M. malabaricus* and (e) *M. tengara* collected in the present study

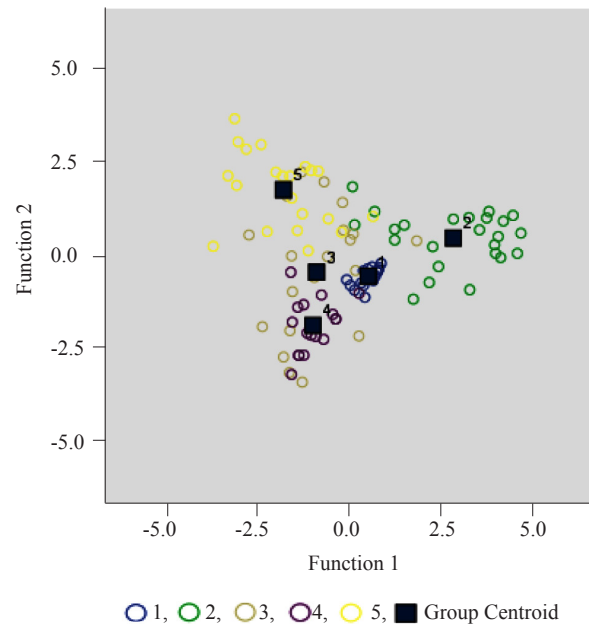


Fig. 3. Scatter plot on canonical discriminant analysis of otolith of (1) *M. cavasius*; (2) *M. gulio*; (3) *M. malabaricus*; (4) *M. tengara* and (5) *M. armatus*

Table 2. Results of *ad hoc* comparisons of various otolith shape indices between five species of the genus *Mystus*

| Shape indices       | Species code          | Mean $\pm$ SD (mm)              | F ratio | p value |
|---------------------|-----------------------|---------------------------------|---------|---------|
| CR (Circularity)    | <i>M. cavasius</i>    | 18.046 $\pm$ 0.567 <sup>a</sup> | 4.154   | <0.05   |
|                     | <i>M. gulio</i>       | 18.395 $\pm$ 0.688 <sup>a</sup> |         |         |
|                     | <i>M. malabaricus</i> | 18.348 $\pm$ 1.124 <sup>a</sup> |         |         |
|                     | <i>M. tengara</i>     | 19.021 $\pm$ 0.438 <sup>b</sup> |         |         |
|                     | <i>M. armatus</i>     | 18.208 $\pm$ 0.767 <sup>a</sup> |         |         |
| EL (Ellipticity)    | <i>M. cavasius</i>    | 0.204 $\pm$ 0.006 <sup>c</sup>  | 28.735  | <0.05   |
|                     | <i>M. gulio</i>       | 0.244 $\pm$ 0.029 <sup>d</sup>  |         |         |
|                     | <i>M. malabaricus</i> | 0.188 $\pm$ 0.029 <sup>b</sup>  |         |         |
|                     | <i>M. tengara</i>     | 0.167 $\pm$ 0.017 <sup>a</sup>  |         |         |
|                     | <i>M. armatus</i>     | 0.207 $\pm$ 0.026 <sup>c</sup>  |         |         |
| RE (Rectangularity) | <i>M. cavasius</i>    | 0.720 $\pm$ 0.007 <sup>b</sup>  | 30.621  | <0.05   |
|                     | <i>M. gulio</i>       | 0.771 $\pm$ 0.032 <sup>c</sup>  |         |         |
|                     | <i>M. malabaricus</i> | 0.720 $\pm$ 0.038 <sup>b</sup>  |         |         |
|                     | <i>M. tengara</i>     | 0.692 $\pm$ 0.010 <sup>a</sup>  |         |         |
|                     | <i>M. armatus</i>     | 0.770 $\pm$ 0.031 <sup>c</sup>  |         |         |
| FO (Form factor)    | <i>M. cavasius</i>    | 18.379 $\pm$ 0.247 <sup>c</sup> | 49.223  | <0.05   |
|                     | <i>M. gulio</i>       | 22.393 $\pm$ 3.309 <sup>d</sup> |         |         |
|                     | <i>M. malabaricus</i> | 16.558 $\pm$ 2.377 <sup>b</sup> |         |         |
|                     | <i>M. tengara</i>     | 18.188 $\pm$ 0.399 <sup>c</sup> |         |         |
|                     | <i>M. armatus</i>     | 13.716 $\pm$ 1.904 <sup>a</sup> |         |         |
| RO (Roundness)      | <i>M. cavasius</i>    | 0.606 $\pm$ 0.009 <sup>a</sup>  | 14.580  | <0.05   |
|                     | <i>M. gulio</i>       | 0.596 $\pm$ 0.025 <sup>a</sup>  |         |         |
|                     | <i>M. malabaricus</i> | 0.625 $\pm$ 0.029 <sup>b</sup>  |         |         |
|                     | <i>M. tengara</i>     | 0.629 $\pm$ 0.020 <sup>b</sup>  |         |         |
|                     | <i>M. armatus</i>     | 0.644 $\pm$ 0.024 <sup>c</sup>  |         |         |

<sup>a-d</sup>Values bearing different superscripts indicate significant differences between species ( $p < 0.05$ ).

Table 3. Eigen value and proportion of discriminant functions in the canonical discriminant analysis (CDA) for shape indices

| Discriminant function | Eigen value        | Variability explained (%) | Cumulative variability explained (%) |
|-----------------------|--------------------|---------------------------|--------------------------------------|
| 1                     | 2.960 <sup>a</sup> | 62.89                     | 62.89                                |
| 2                     | 1.431 <sup>a</sup> | 30.41                     | 93.31                                |
| 3                     | 0.313 <sup>a</sup> | 6.65                      | 99.97                                |
| 4                     | 0.001 <sup>a</sup> | 0.027                     | 100                                  |

<sup>a</sup>Means of first four canonical discriminant functions were used in the analysis

Table 4. Jack-knifed classification matrix of the discriminant analysis between five species of the genus *Mystus* from India

| Species               | <i>M. cavasius</i> | <i>M. gulio</i> | <i>M. malabaricus</i> | <i>M. tengara</i> | <i>M. armatus</i> | Total |
|-----------------------|--------------------|-----------------|-----------------------|-------------------|-------------------|-------|
| <i>M. cavasius</i>    | 89.47              | 0               | 10.52                 | 0                 | 0                 | 100   |
| <i>M. gulio</i>       | 29.16              | 66.66           | 0                     | 0                 | 4.16              | 100   |
| <i>M. malabaricus</i> | 19.04              | 0               | 14.28                 | 38.09             | 28.57             | 100   |
| <i>M. tengara</i>     | 5.88               | 0               | 5.88                  | 88.23             | 0                 | 100   |
| <i>M. armatus</i>     | 9.52               | 0               | 9.52                  | 0                 | 80.95             | 100   |

## Discussion

The present work represents the first study on otoliths of the genus *Mystus*. Ahmed and Attia (2016) suggested that each species of fish has characteristic otolith shape. The morphological characteristics of fish otoliths vary between different species, from a simple disc in flatfishes to irregular shape as in red fish (Hunt, 1992).

The landmark morphological features of otoliths are used for species identification owing to their large size and inter-specific variation (Nolf, 1985). Information on bagrid otolith is scanty, whereas several reports on sagittal otolith have been published, indicating claviform shape in Loricaridae (Martinez and Gonzo, 1991), ovoid and flat in *Oreochromis niloticus*, elongated in *Mugil cephalus*

and triangular rosette shape in *Clarias lazera* (Ahmed and Attia, 2016). Tilak (1963) and Mollo (1981) studied the astericus, the lagenar otolith of some taxa of catfishes that was substantiated by Kumar (2014) on the otolith of ariid catfishes who also found lapillus as the largest one among the three otoliths. In the present study, lapillus otolith of five species of *Mystus* were extracted and found to be kidney shaped and helpful in species discrimination (Fig. 2). The left and right otoliths are mirror images of each other (Hunt, 1979).

Shape analysis is the skeleton of otolith study, which is based on the concept that shape varies with geographical location, even within a species (Campana and Casselman, 1993). Thus, the shape indices data perform a key role in discriminating individuals into different stocks of the single species. In the present study, all the shape indices studied viz., circularity, ellipticity, rectangularity, form factor and roundness were found to be efficient in successful differentiation of the five species of the genus *Mystus*. The higher F-ratio of form factor and rectangularity indicated maximum contribution for species discrimination. The scatter plot based on CDA of otolith indicates variation in the five species and also displays separate cluster formed by different species except the overlap between *M. tengara* and *M. malabaricus*. The jack-knifed classification tests validated the observed pattern in CDA.

Factors inducing changes in specific otolith shape indices are not clearly understood (Burke *et al.*, 2008). It should be assumed that the shape of otolith may be alike for fishes dwelling in similar environmental circumstances but might vary with difference in habitat (Parmentier *et al.*, 2001). Among the five shape indices studied, circularity was found to be highest for *M. tengara* (19.021), but its values for the other four species were more or less similar, ranging from 18.046 to 18.395. Similarly, circularity and form factor were found to be higher for *M. gulio* when compared with other species. This shows a correlation between form factor and circularity as suggested by Reist (1985) and Stransky (2005). This may be the probable reason for formation of a clear cluster of *M. gulio* in the scatterplot. But the readings of rectangularity overlapped between *M. gulio* and *M. armatus* (0.771 vs 0.770). The value of roundness of *M. tengara* closely matched with that of *M. malabaricus* (0.629 vs 0.625). The form factor index ranged between 13.70 and 22.39, for all the *Mystus* species studied and was found to overlap in the case of *M. cavasius* and *M. tengara* (18.379 vs 18.188). However, combination of the five shape indices is useful for understanding variation of otolith morphometry in these five species. A significant difference ( $p < 0.05$ ) was observed in the shape indices of the species when *ad hoc* comparison (Duncan's multiple range test) was applied.

Santificetur *et al.* (2017) reported different F-values of circularity (14.07) and rectangularity (0.70) for *Genidus barbatus* and circularity (14.26) and rectangularity (0.69) for *Genidus genidus* as differentiating traits. Similarly, Lacroix *et al.* (2017) utilised F-value for roundness (117.07), ellipticity (120.286), rectangularity (194.259), form factor (20.256) and circularity (360.67) in stock discrimination of cusk eel. However, F-ratio for roundness (14.58), ellipticity (28.735), form factor (49.223) and circularity (4.154), recorded in the present study were lower than those reported in other species, except rectangularity (30.621) (Lacroix *et al.*, 2017; Santificetur *et al.*, 2017). The difference recorded in otolith morphometric variables among various species (Table 1) may be attributed to the environmental condition, demography (Hoff and Fuiman, 1993; Bostanci *et al.*, 2015), variation in fish growth rate (Campana and Casselman, 1993), food availability and type, niches, river flow, current speed (Vignon and Morat, 2010), biological and behavioural characteristics like swimming activity (Aguirre and Lombarte, 1999) and ontogenetic and genetic factors (Lord *et al.*, 2012; Hussy *et al.*, 2016). Biotic interactions also have an effect on fish metabolism and thus can cause stress, which further influence the shape of otolith (Allemand *et al.*, 2007). The morphology of otolith may not only connect with common ancestry, but also depends on the anatomy relating to sound perception (Nolf, 1985; 1993). In this study, the variation of otolith shape observed within the genus could be attributed to a combination of various factors like habitat, food uptake, environmental conditions and genetics. The otolith microstructure and chemistry in different spatial and temporal scales is also influenced by environmental factors as well as growth (Miyan *et al.*, 2016). Hence, each *Mystus* sp. collected from different parts of India has its own innate data in its spatial and temporal spectrum. Nolf (1985; 1993) opined that there exists different degrees of morphological convergence among evolutionary distant taxa and divergence among closed ones and thus, otolith alone cannot perform well in taxonomy or phylogenetic study. Otolith characteristics of significant relevance supplemented by fish morphometry can be utilised for phylogenetic studies (Adams, 1940). The present investigation provides baseline database of otolith shape indices of *Mystus* species and in combination with other taxonomic tools, it can create a good basis for species and stock identification.

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