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Morphometric Shape Analysis of Otolith from Selected Goby Fishes

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ABSTRACT Four selected fish otolith of Goby species, *Glossogobius celebius, Glossogobius giuris, Awaous ocellaries*, and *Awaous melanocephalus* were compared in this study using Elliptic Fourier SHAPE analysis. Kruskal-Wallis and Discriminant Function Analysis of shape data show significant differences in otolith shapes between species. The observed otolith shapes of the gobies provide a good taxonomic tool to separate the species of this genus. The diversity and complexity in otolith shapes may also be considered important for fisheries scientists, archeologists and for the discrimination of other species of this complex genus of fish.

KEYWORDS: Otolith, Morphometrics, Elliptic Fourier, SHAPE

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INTRODUCTION

Worldwide, the most diverse group of freshwater fishes are gobies with no less than 2,117 species. Previous studies on gobies were focused on its life history (Manacop 1953), fishery, biology, ecology, conservation and management (Blanco 1956; Herre 1927; Manacop 1953; Montilla 1931). However, studies on otolith morphology of the goby species are few. The endolymphatic infilling in the fish known as otolith is a complex structure in the saccule and utricle of the inner ear. The calcium carbonate that the otolith is made up of is primarily acquired from the water and is providing information on shape conservation, coexisting crystal morphs, and continuing changes in crystal morph. Morphological characteristics of fish otoliths are highly variable between and within species (Chilton and Bleamish, 1982). The uniqueness of fish otoliths was first observed by Aristotle, in the third century BC (Stinton 1975) and later was for their taxonomic utility (Cuvier & Valenciennes 1836; Nolf 1985; Hecht 1987; Smale *et al* 1995). Studies have also shown that analysis of otolith shapes is not only a way of providing information on species (Schmidt, 1969; Nolf, 1985), phylogeny (Lombarte and Castellon, 1991), their ecobiological specification (Platt and Popper, 1981; Morales-Nin, 2000) and geographic origin (Castonguay *et al.*, 1991; Campana and Casselman, 1993) as this gives important information on the biology of the species. This study was therefore, aimed to assess the shape variations of otolith obtained from four species of goby fishes namely, *Glossogobius celebius*, *Glossogobius giuris*, *Awaous ocellaris*, and *Awaous* melanocephalus. Biological tools such as Geometric Morphometrics and SHAPE analysis were developed to investigate morphological variations of an organisms. Existing studies convinced that morphometrics can measure a character of evolutionary importance and their ontogeny or evolutionary relationship by identifying the difference in the shape of organisms. Elliptic Fourier Analysis with the development of image analysis software SHAPE (Iwata and Ukai, 2002) is utilized for this study. Elliptic Fourier analysis is considered to be the most objective and powerful shape analysis technique for capturing the entire shape variation in the otolith outline (Campana and Casselman, 1993).

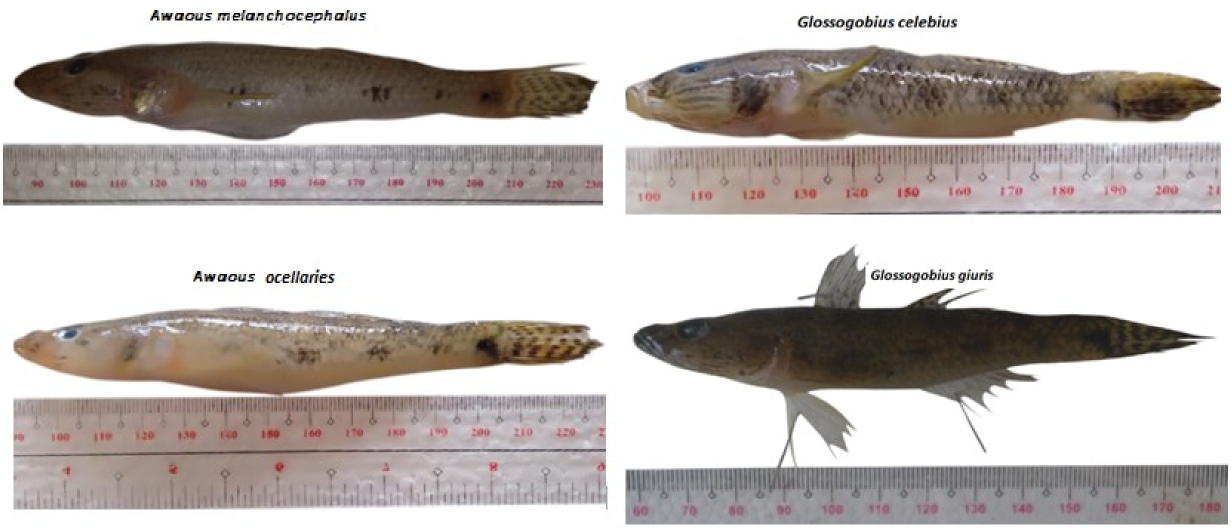

Fig. 1. Selected goby fishes from Balo-i Lake.

MATERIALS AND METHODS

Collection of samples

Ten adult-sized of each specimen were collected from Balo-i Lake in Lanao del Norte. Samples obtained were brought to the laboratory for dissection. The samples were washed thoroughly with running water and then placed it in the dissecting pan.

Processing of the fish otolith

Otolith samples of each species were carefully identified (Figure 2) using a magnifying lens because the extraction is daunting and the otolith is too small to be pulled out. Afterwards, the extracted otolith was then placed in the screw cap vial for image processing. Finally, the samples were photographed under the microscope at 40x high magnification using Sony Xperia Z1 camera. Captured images were then converted to bitmap files for SHAPE analysis software that was used in this study.

Statistical and SHAPE analysis

The Chaincode is a coding system for describing geometrical information about contours. This will extract the contour determination of objects from an image file and record them as chaincode (Freeman, 1974). It also alters a full-color image to a binary (black and white color) image, reduces noise, traces the contours of objects and describes the contour information as chain code. Chain coder outputs chaincode file, which is analyzed by the program Che2Nef (Iwata *et al*., 2000). Normalization EFDs index was performed by Che2Nef procedure as suggested by Kuhl and Giardina (1982). This program calculates the normalized EFDs from the chain code information and can perform two types of normalization. The first type is based on the first harmonic ellipse that correlates to the first Fourier approximation to the contour determination wherein the size and orientation of the contour is standardized. This integrates the size and alignment of the major axis. The second type of normalization is performed in conformity with the direction and absolute size of the vector from the center point.

The Princomp program performs a principal component analysis obtained from the normalized EFDs using the Che2Nef. This described the contour shape in the first 20 harmonics of Fourier coefficients. But according to Rohlf and Archie 1984, the principal components analysis can efficiently summarize the information contained in these coefficients. Also, the principal component In this study, the principal component values were used to analyze the variance-covariance of the coefficients wherein small values are generally important for explaining the observed morphological variations of the otolith.

To justify the results whether significantly different (*p* same value < 0.05) between groups, Kruskal-Wallis Test was done using the PAST software version 2.0. This test uses ranks of ordinal data to perform an analysis of variance to determine whether multiple groups are similar to each other. To observe the results graphically, boxplot and XY graph were visibly presented. These will examine the variations observed between different groups and the distribution of the population with respect to the mean shape. Finally, the means for the significant discriminant functions are analyzed in order to identify which groups the respective functions seem to discriminate.

RESULTS AND DISCUSSION

Figure 2 shows the otoliths from *G. celebius, G. giuris, A. melanocephalus* and *A. ocellaris.* Qualitative inspection of the figure shows the shapes vary within and between species. Comparison between species within a genus show *G. celebius* and *G. giuris* (B) show a curvaceous triangle-like shape, the rostrum is narrow and pointed. *A. melanocephalus* (C) and *A. ocellaries* (D) showed irregular circular-form shapes with sinuate margins. Figures 3 illustrates the comparison of contour points between species of a genus. Table 1 shows the list of significant PC components obtained from principal components analysis between *G. celebius* and *G. giuris* and *A. ocellaries* and *A. melanocephalus.*

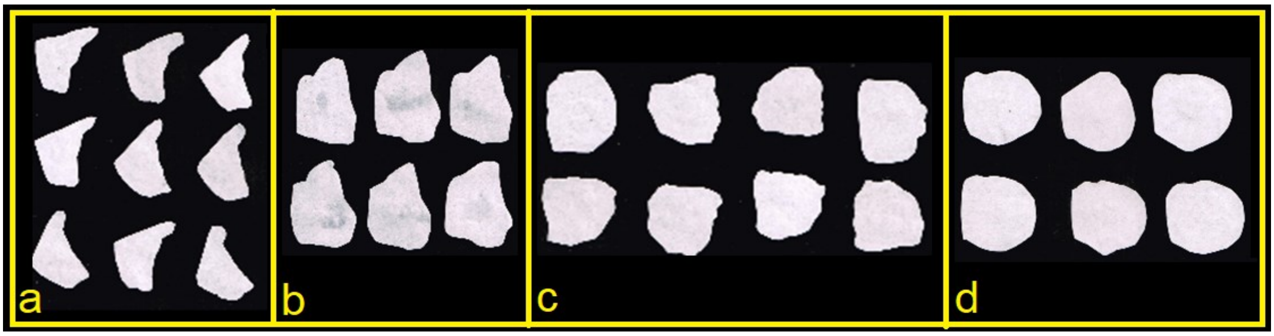

Figure 2. Otolith shapes of (A) *G. celebius*, (B) *G. giuris*, (C) *A. melanocephalus* and (D) *A. ocellaries*

For *G. celebius* and *G. giuris,* Normalized EFDs shows only 1 significant PC (89.7575%) that significantly decribes the variations between the 2 species of *Glossogobius*. This component described the variations where the 2 species vary in the overall shapes of their otolith (length, width, shape of each field and etc. (Fig. 2b)

Between *A. melanocephlus* and *A. ocellaries,* the results generated by EFDs normalization showed the shapes of the otolith were variable within and between the two species based on contour determination. These variations are graphically presented based on the four significant PC values (Fig. 3 and Table 1).

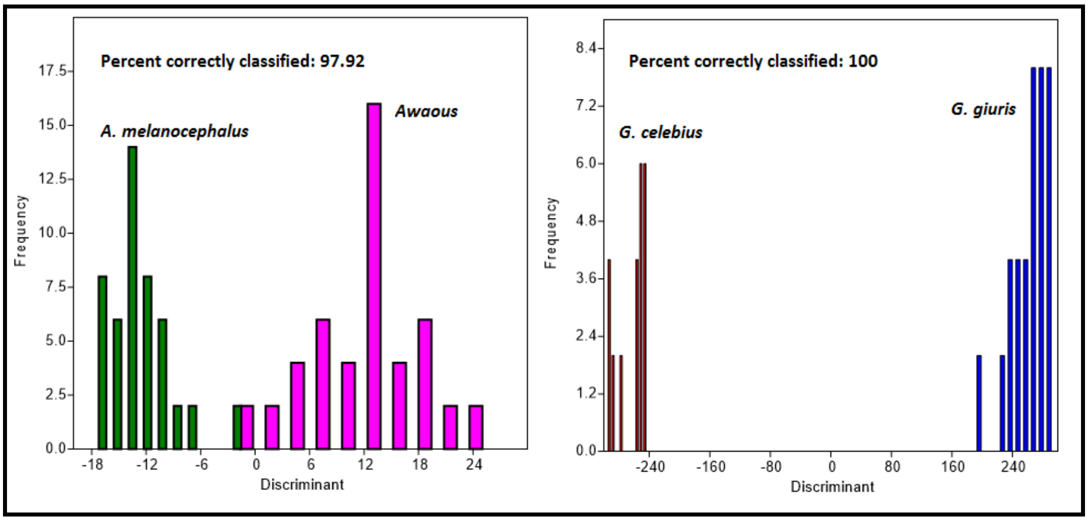

Figure 3. Discriminant Function Analysis between *G. celebius* and *G. giuris* and between *Awaous ocellaries* and *A. melanocephalus.*

Table 1. List of Effective Principal Components with its Corresponding Eigenvalue and % Variance.

	Between *G. celebius* and *G. giuris*.		
Principal Components	**Eigenvalue**	**Proportion (%)**	**Cumulative (%)**
Prin Comp 1	9.904255E-003	89.7575	89.7575
	Between *A. melanocephalus* and *A. ocellaries*		
Prin Comp 1	5.428224E-003	48.471	48.715
Prin Comp 2	1.945811E-003	17.3752	65.8466
Prin Comp 3	1.740738E-003	15.5440	81.3906
Prin Comp 4	7.037242E-004	6.2839	87.6745

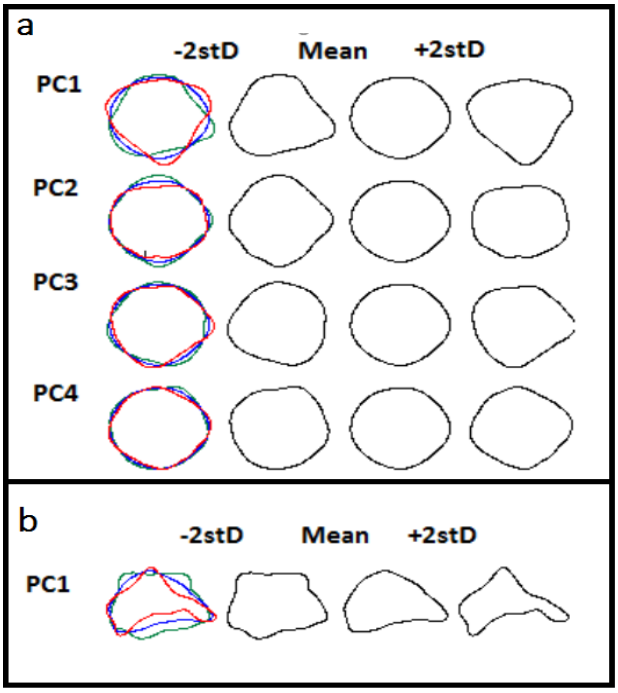

Figure 4. Contour shape analysis between (a) A. *melanocephalus* and *Awaous ocellaries* (b) *G. celebius* and *G. giuris*

Table 2. Kruskal – Wallis Test Results and its corresponding *p* (same) value.

Species	Kruskal-Wallis Test p(same)
Between *A. melancephalus* and *Awaous ocellaries*	0.006327
Between *G. celebius* and *G. giuris*	0.01869

Comparison between species within the genus show significant differences between *A. melanocephalus* and *A. ocellaries* and also species between *G. celebius* and *G. giuris* (Table 2, Fig. 3) based on the over-all shape, including the shape of the posterior margins where the most variations in the fish otolith were observed.

Results of this study clearly show the otoliths examined have distinct shapes which is characteristic of the fish species examined. Variations within species however were clearly observed and this is in conformity with Messieh and MacDougall 1989 study that within species some population components may have significant differences in otolith morphometrics. These variations could be influenced by many factors, such as seasonal variations, temperature, habitat and diet (Campana, 2001). While there are variations in morphological characteristics of otolith shapes within the species these are still useful to discriminate the species (Marrow, 1979; Harkonen, 1986; Hecht 1987; Smale *et al* 1995; Furlani *et al* 2007). Fish biologists as well as taxonomists and archaeologist, often rely on the otolith shapes aside from looking at size of preserved or undigested otoliths to reorganize the species and size composition of the diet of fish predators.

Since the otolith is correlated to the interspecific variation of lifestyles, motor activities and hearing skills of the animals including fishes (Gauldie 1998; Lychakov 1990, 1992; Lychakov and Rebane 1993; Platt & Pooper 1981; Popper & Coombs 1982), understanding more of the causes of variability within the species should be further explored. The use of biological tools such as Elliptic Fourier SHAPE analysis can be of good use to describe populations of fishes affected by both abiotic and biotic factors such as those population structures at different seasonal variations, temperature, habitat and diet. Campana and Casselman (1993) in their study on the shape of the otolith concluded that otolith shape varies among some stocks appeared to be environmentally lured rather than genetically convinced thus otolith investigations can be of use in stock studies.

CONCLUSION

This study shows that variability within and between species of gobies based on the shape outline of the otolith. *Glossogobius* species share a curvaceous triangle-like shape with the narrow and pointed rostrum while *A. melanocephalus* and *Awaous ocellaries,* show an irregular circular-form otolith shape with sinuate margins. These characters provide a good taxonomic tool to separate the species of the two genera of gobies from each other. However, the observed diversity and complexity in otolith characteristics within the four species might be considered important not only for taxonomic purposes but also for fisheries scientists and managers especially in the determination of fish stocks.

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