IDENTIFICATION OF GERREID SPECIES (ACTINOPTERYGII: PERCIFORMES: GERREIDAE) FROM THE PACIFIC COAST OF MEXICO BASED ON SAGITTAL OTOLITH MORPHOLOGY ANALYSIS

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Background. Although the species of the family Gerreidae have been subjected to many studies, their taxonomy at specific- and sometimes generic levels is still confusing. To contribute to the clarification of the taxonomy of the Mexican Pacific mojarras, the morphology of sagittal otoliths among six species of mojarras (*Diapterus brevirostris*, *Eugerres lineatus*, *Eucinostomus dowii*, *E entomelas*, *E. currani*, and *Gerres cinereus*) was compared using size and shape descriptors. Otolith shape has long been known to be species-specific but this has not been tested in species of the family Gerreidae. Therefore, our goal was to explore the effectiveness of otolith descriptors for identifying gerreid fish at species level.

Materials and methods. Gerreid fish were captured between January 2009 and January 2010, off the Pacific coast of Mexico. The right- and left sagittae of 160 individuals were extracted for analysis. Size and shape descriptors considered in the presently reported analysis were: surface area, perimeter, length, width, rectangularity, ellipticity, roundness, circularity, aspect ratio, form-factor, Feret length, Feret width, Feret maximum, Feret minimum, diameter maximum, diameter minimum, diameter mean, ratio maximum, and ratio minimum. Other measurements were made in the otolith region of sulcus acusticus, such as: cauda length, ostium length, ostium width, sulcus length, and rostrum width. Canonical discriminant analysis on otoliths morphology was used to identify differences between species.

Results. Rectangularity, roundness, otolith length, and Feret length were the main otolith descriptors that explain the inter-specific variability. Significant differences (Wilks' lambdas (λ), $P < 0.001$), high canonical correlation coefficients, and also a high classification success (overall mean >90%) allowed the separation of the species by using discriminant functions. Results from both *G*-test and Cohen's kappa procedure confirmed the high rates of classification success obtained by the discriminant analysis.

Conclusion. These results suggest the usefulness of otolith morphology for differentiation of Gerreidae species from Mexican Pacific waters, thereby demonstrating that otolith shape is species-specific. Otolith morphology descriptions provided in this study is presented for the first time for the species included.

Keywords: Gerreidae, canonical discriminant analysis, otolith morphology, Mexico

INTRODUCTION

Fish family Gerreidae, commonly known as mojarras or silver-biddies is one of the most representative groups in aquatic systems of tropical and subtropical areas of the world (Yáñez-Arancibia 1980, Matheson and McEachran 1984). Gerreidae comprises some 100 nominal species grouped in eight genera (Eschmeyer 2012), two of which are monotypic: *Pentaprion* and *Ulaema*. Currently, two species are considered valid in *Parequula*, eight in *Eugerres*, six in *Diapterus*, 10 in *Eucinostomus*, and 28 in *Gerres* (Froese and Pauly 2012). Although, since the 19th century, the mojarras have been subject of many studies, mostly focused on ecological issues, their taxonomy at specific- and sometimes generic levels is still confusing (De La Cruz-Agüero and Galvan-Magaña 1993, Chen et al. 2007). At present, the taxonomic status of more than 50 nominal species, mainly included in the last three mentioned genera, has not recently been revised and some gerreid taxa still have recognition problems and their taxonomic validity is questionable, e.g.: *Ulaema lefroyi* (Goode, 1874); *Eucinostomus havana* (Nichols, 1912); *Diapterus aureolus* (Jordan et Gilbert, 1882); *Eugerres periche* (Evermann et Radcliffe, 1917).

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Although gerreid fish have an important value for human consumption in tropical and subtropical regions, separate catch statistics are not reported for the majority of species and the landings are often summarized as mojarras. This lack of species-specific data is partly due to the difficulty in distinguishing many of the species (De La Cruz-Agüero and Galvan-Magaña 1993). Traditionally, identification of the Gerreidae taxa has been difficult and time consuming, because of the subjectivity of characteristics and overlap of meristic and morphometric characters provided in taxonomic keys (Bussing 1995). Other contributions to this taxonomic problem in the family are the inadequate or incomplete descriptions of type materials, their poor current condition, loss of some of them, and lack of published taxonomic reviews (Yáñez-Arancibia 1980).

Otoliths, the calcium carbonate structures located in fish inner ear, have been used as an auxiliary characteristic for identification of fishes in paleoichthyology (Nolf 1995) and stock recognition in fisheries management (Begg and Brown 2000, Tuset et al. 2003a, Neves et al. 2011), as well as a useful morphological character in taxonomy (Torres et al. 2000a, Tuset et al. 2006), systematics (Lombarte et al. 1991, Smale et al. 1995, Volpedo and Echeverría 2000), and food habits analysis (Radhakrishnan et al. 2010). Otolith shape has long been known to be species-specific (L'Abée-Lund 1988) and less variable in growth than body growth, presumably because otoliths are internal organs contributing to audition and balance (Lord et al. 2012), although its morphology can often vary geographically within a species (Lombarte and Lleonart 1993). In the majority of teleost species, sagittal otoliths are the largest pair, so they are the structures most frequently used in comparative studies because of the relative ease with which this structure can be reached (Nolf 1995). In addition, it has been established that from the three otolith pairs, sagittae is the pair with highest interspecific variation (Tuset et al. 2003b).

Comparison of otolith morphology using size parameters and shape indices of six Gerreidae species that inhabit the west Pacific coast of Mexico, including the Gulf of California was performed in the frames of the presently reported study. These species are important in small-scale artisanal fisheries of the country (Aguirre-León and Yáñez-Arancibia 1986): the short-beaked mojarra, *Diapterus brevirostris* (Sauvage, 1879); streaked mojarra, *Eugerres lineatus* (Humboldt, 1821); Dow's mojarra, *Eucinostomus dowii* (Gill, 1863); dark-spot mojarra, *Eucinostomus entomelas* Zahuranec in Yáñez-Arancibia, 1980; Pacific flagfin mojarra, *E. currani* Zahuranec *in* Yáñez-Arancibia, 1980; and yellow fin mojarra, *Gerres cinereus* (Walbaum, 1792). Our main objective was to assess otolith morphology variation to test the hypothesis that otolith morphology distinguishes gerreid fish at species level and therefore, to contribute in highlighting mojarras taxonomy in the Pacific coast of Mexico.

MATERIALS AND METHODS

The right and left sagittae of 160 individuals were extracted for analysis, although sometimes it was not possible to obtain the complete pairs (230 sagittae in total). The gerreid fish were captured between January 2009 and January 2010, from the Pacific, off three Mexican states (Fig. 1): Baja California Sur (La Paz Bay, Magdalena Bay, and Espiritu Santo Island), Sinaloa (Mazatlan har-

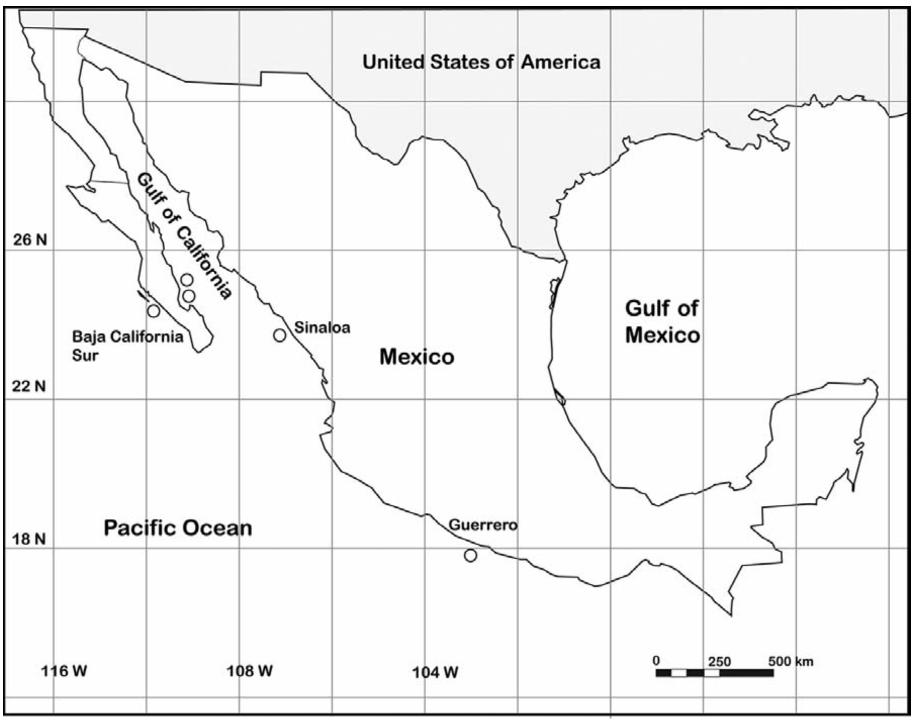

Fig. 1. Sampling locations (hollow circles) of species of the fish family Gerreidae in the states of Baja California Sur, Sinaloa, and Guerrero in the west coast of Mexico (January 2009 and January 2010)

bour), and Guerrero (Acapulco harbour). These specimens came from artisanal catches of local fisheries and occasional samples by the authors. Each fish was measured to the nearest mm (standard length, SL). Otoliths were placed in distilled water just after extraction to clean them and stored in labelled glass vials. All fish and sagittae (hereafter called otoliths) were deposited and catalogued in the fish collection of the Centro Interdisciplinario de Ciencias Marinas (CICIMAR-IPN) at La Paz, B.C.S., Mexico.

Considering all sampled fish, body size varied from 55 mm SL in *D. brevirostris* and 245 mm SL in *G. cinereus* (Table 1). The sex was not considered separately because gerreid fish do not have sexual dimorphism (De La Cruz Agüero and Galvan-Magaña 1993). Digital images of each pair of otoliths by species were taken with an Olympus® stereo-microscope model SZ61 fitted with an adapter for digital camera Olympus® SP-320 with 3× optical zoom and a light source Olympus® SZ2-LGBST to highlight edges of the structures. The otoliths were positioned systematically with the sulcus acusticus up and the rostrum pointing to the left before capture as bright objects on dark background.

Digitized images of each otolith were analyzed using the Image Pro Plus® program (version 6.0) to measure surface area (Ao; units in mm^2), perimeter (Po), length (Lo), width (Wo), diameter maximum (Dmx), diameter minimum (Dmn), Diameter mean (Dm), ratio maximum (Rmx) and ratio minimum (Rmn)—all of them taken to the nearest 0.01 mm. Such measurements were used to calculate dimensionless shape indices (Tuset et al. 2003b, Ponton 2006, Pothin et al. 2006) which are independent from differences in otolith size (Tuset et al. 2006, Lord et al. 2012), which include (Fig. 2, Table 2): rectangularity, roundness, circularity, form-factor, ellipticity, and aspect ratio. Previously, to calculate roundness, rectangularity, and ellipticity it was necessary to obtain Feret length (Fl), Feret width (Fw), and its two components: Feret maximum (Fmx) and Feret minimum (Fmn) (see Pertusa 2003). Other measurements were made in the otolith region that is associated with the sensory macula: sulcus acusticus (e.g., sulcus length (Sl), cauda length (CL), ostium length (OL), ostium width (OW), and rostrum width (RW).

We used a Friedman's Q test (FQT), a non-parametric test used to compare repeated observations on the same subjects belonging to three or more paired groups, to detect differences between sulcus acusticus ratios by species. The FQT makes no assumptions for data distribution (e.g., normality or homoscedasticity of variance) (Zar 2009).

Canonical discriminant analysis (CDA) was run for all fish with both otoliths pooled together as recommended by Morat et al. (2012), and used to assess whether species can be distinguished between each other based on otolith descriptors or not. Percentage of correct assignment of individuals, calculated within classification matrix, can then be used as a morphometric measurement of similarity between taxa and as an evidence for the a-priori established taxonomic structure (e.g., conventional identification produced by taxonomic keys). Quadratic Mahalanobis distances (MD) and their approximations to the *F*-statistics were used to assess significant differences between species centroids in the multivariate space. Also a dendrogram was constructed by hierarchical cluster analysis (UPGMA), based on the Euclidian distance of MD values to assess the degree of similarity between Gerreidae species by otolith descriptors.

Statistical significance of CDA was assessed by Wilks' lambda (λ) test. Values close to zero indicate a good model fit and values ≈ 1 indicate lower discrimination power (Klecka 1980, Lord et al. 2012). Another way to judge the relevance of a discriminant function is by examining the canonical correlation coefficient (r^*; Klecka 1980). If the groups are no very different on the variables being analyzed, then all of the correlations will be low. Classification efficiency (e.g., estimate error rates in CDA) was cross-validate according to Lachenbruch and Mickey (1968). The bias of the classification was determined with the Cohen's kappa (κ) coefficient, which estimates the improvement over chance of the percent corrected classification rates (Tuset et al. 2003b). Values of κ range from 0 to 1, with zero indicating that the discriminant analysis yielded no improvement over chance, while a κ of 1 occurs only with perfect agreement (Titus et al. 1984). The κ values were scaled up to a percentage, and the prior probability of classification was equal for all groups. Finally, a *G*-test of independence was used to test agreement between observed classification rates and expected classification rates (Tuset et al. 2003a). All statistical analyses were performed in XLStat® 2009, a statistical plug-in for MS Excel® 2007 spreadsheet program. Significance level was set at 0.05 for all statistical tests used.

RESULTS

The gross morphology and morphometry of the otolith are described by species according to Tuset et al. (2003a) and Volpedo et al. (2008), and all ratios are expressed as the average (see also Fig. 2 and Table 2):

Diapterus brevirostris. Otolith shape: oblong or semicir-

Table 1

Details of samples of the fish family Gerreidae used in the otolith morphology analysis for species identification

Species	*N*	*n*	Standard length [mm]	
			Mean	Range
Diapterus brevirostris	22	37	156.8	55.0–170.0
Eugerres lineatus	27	36	166.0	79.0–200.0
Eucinostomus dowii	12	21	162.0	120.0–168.0
Eucinostomus entomelas	36	43	178.0	83.0–195.0
Eucinostomus currani	28	45	140.3	60.0–170.0
Gerres cinereus	35	48	223.0	110.0–245.0
Total	160	230		

N = number of individuals, *n* = number of otoliths (sagittae) examined.

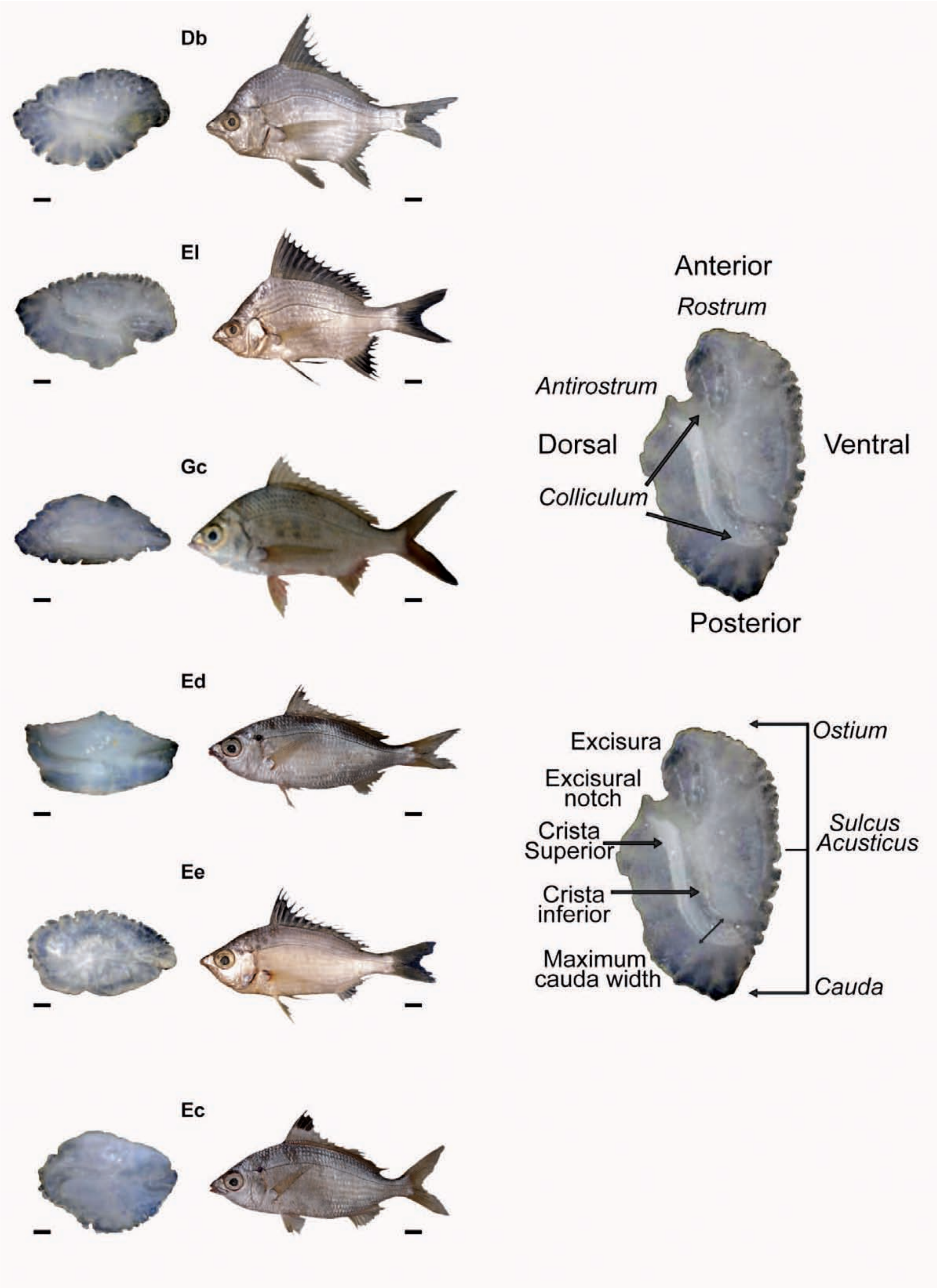

Fig. 2. Morphology of sagittae in six species of the fish family Gerreidae, indicating terminology used in the study; The sulcus acusticus is facing the observer (right sagittae per species; the left sagitta with terminology, modified from Tuset et al. 2003a); Db = *Diapterus brevirostris*, El = *Eugerres lineatus*, Gc = *Gerres cinereus*, Ed = *Eucinostomus dowii*, Ee = *Eucinostomus entomelas*, Ec = *Eucinostomus currani*; Scale bar for otoliths = 1 mm, and 1 cm for fish

cular. Sulcus acusticus: ostial to medium. Ostium: rectangular. Cauda: slightly curved. Anterior region: angled. Proximal margin: lobed. Dorsal margin: serrate. Summary of ratios: cauda length / ostium length (CL / OL) = 1.313; ostium length / ostium width (OL / OW) = 1.229; and cauda length / rostrum width (CL / RW) = 1.607.

Eugerres lineatus. Otolith shape: oval. Sulcus acusticus: ostial to medium. Ostium: rectangular. Cauda: slightly curved. Anterior region: angled. Proximal margin: lobed. Dorsal margin: dentate. Remarks: Some specimens showed deformations in the posterior region. Summary of ratios: cauda length / ostium length (CL / OL) = 1.413; ostium length / ostium width (OL / OW) = 1.208; and cauda length / rostrum width (CL / RW) = 1.684.

Eucinostomus dowii. Otolith shape: semicircular. Sulcus acusticus: ostial to medium. Ostium: funnel-like. Cauda: strongly curved. Anterior region: truncated. Proximal margin: sinuous. Dorsal margin: sinuous to serrated. Summary of ratios: cauda length / ostium length (CL / OL) = 2.488; ostium length / ostium width (OL / OW) = 1.415; and cauda length / rostrum width (CL / RW) = 3.474.

Eucinostomus entomelas. Otolith shape: semicircular. Sulcus acusticus: ostial to medium. Ostium: rectangular. Cauda: slightly curved. Anterior region: angled. Proximal margin: lobed. Dorsal margin: serrated. Summary of ratios: cauda length / ostium length (CL / OL) = 3.348; ostium length / ostium width (OL / OW) = 1.159; and cauda length / rostrum width (CL / RW) = 3.832.

Eucinostomus currani. Otolith shape: oval. Sulcus acusticus: ostial to medium. Ostium: rectangular. Cauda: slightly curved. Anterior region: angled. Proximal margin: lobed. Dorsal margin: dentate. Comments: dorsal margin serrations were observed in specimens larger than 40 mm SL. Summary of ratios: cauda length / ostium length (CL / OL) = 2.528; ostium length / ostium width (OL / OW) = 1.251; and cauda length / rostrum width (CL / RW) = 3.049.

Gerres cinereus. Otolith shape: oblong. Sulcus acusticus: ostial to medium. Ostium: funnel-like. Cauda: strongly curved. Anterior region: angulated. Proximal margin: sinuous. Dorsal margin: sinuous. Remarks: proximal margin presents a projection that is directed toward the external face of the otolith, like a flap. Summary of ratios: cauda length / ostium length (CL / OL) = 2.118; ostium length / ostium width (OL / OW) = 1.261; and cauda length / rostrum width (CL / RW) = 2.664.

FQT was used to compare the data set for each of species (n = 6) based on all sulcus proportions. The Friedman's Q statistics ($P < 0.159$, $\alpha = 0.05$; DF = 5) suggests that there was no difference among sulcus acusticus proportions per species (e.g., samples were not significantly different).

To apply the CDA, and assure independence among otolith descriptors (e.g., size parameters and shape indices) we performed a preliminary pair wise correlation analysis among all variables. Thus, strongly correlated variables ($r > 0.9$, $P < 0.05$) were excluded from the analysis to avoid redundancy. The results showed that of the twenty three descriptors only ten are not correlated at 5% significance level: diameter maximum (Dmx), ratio maximum (Rmx), rectangularity, otolith length (Lo), area (Ao), perimeter (Po), roundness, Feret length (Fl), Feret maximum (Fmx), and form-factor (Table 3).

Five discriminant functions produced were significant for discriminating the six gerreid species using ten descriptors of otoliths. The first three functions account for 93% of the variation (e.g., F1 (40%): $\lambda = 0.006$, $P < 0.001$; $r^* = 0.905$; F2 (33%): $\lambda = 0.034$, $P < 0.001$; $r^* = 0.885$; F3 (20%): $\lambda = 0.156$, $P < 0.001$); $r^* = 0.831$, while the discriminant functions F4 and F5 made a negligible contribution and therefore, were not considered ($r^* = 0.612$ and $r^* = 0.443$, respectively). Interspecific discriminant analysis of otolith descriptors demonstrated that 90% (207) from total of sagittae (230) were correctly classified, ranging from 100% in *E. currani* to 76% in *D. brevirostris* (Table 4). Thus, misclassification rates varied between 0% (*E. currani*) and 24% (*D. brevirostris*). The Cohen's kappa indicates that a classification efficiency of 88% better than would have occurred by chance alone ($\kappa = 0.879$; standard error = 0.024; 95% confidence interval = 0.832 to 0.926; $Z = 36.6$, $P < 0.001$). Also the G-test indicated that otoliths were assigned to the correct species at rates significantly greater than would be expected by chance ($G = 669.811$; DF = 25, $P < 0.000$).

Score plots for the first three discriminant functions show a separation between the six species although some overlapping can be noticed, all quadratic Mahalanobis distances and species centroids were significant ($P < 0.001$) (Fig. 3). Among all descriptors, only diameter maximum and ratio maximum were not significant ($P < 0.57$ and $P < 0.31$, respectively). Otolith descriptors with highest contribution to eigenvalues were (standardized coefficients in parentheses): rectangularity (–24.7), otolith length (–17.9), and roundness (–8.9) for F1; rectangularity (–21.1), Feret length (–13.97.1), and roundness (5.8) for F2, and rectangularity (–20.6), Feret length (–13.9), and roundness (–8.5) for F3.

DISCUSSION

We found no studies for otoliths of the family Gerreidae, although they were synthetically treated in a study for selected west Atlantic species (Lemos et al. 1993). This family has historically been characterized by the complexity of its

Table 2

Otolith shape indices established from morphometric measurements taken on the mesial surface of each sagittae by species (see Tuset et al. 2003b)

Shape indices	Formulae
*Roundness	$(4Ao)(\pi Fl^2)^{-1}$
Circularity	Po^2Ao^{-1}
Ellipticity	$(Fl - Fw)(Fl + Fw)^{-1}$
*Rectangularity	$Ao(FlFw)^{-1}$
*Form factor	$(4\pi Ao)Po^{-2}$

*Not correlated (P < 0.05).

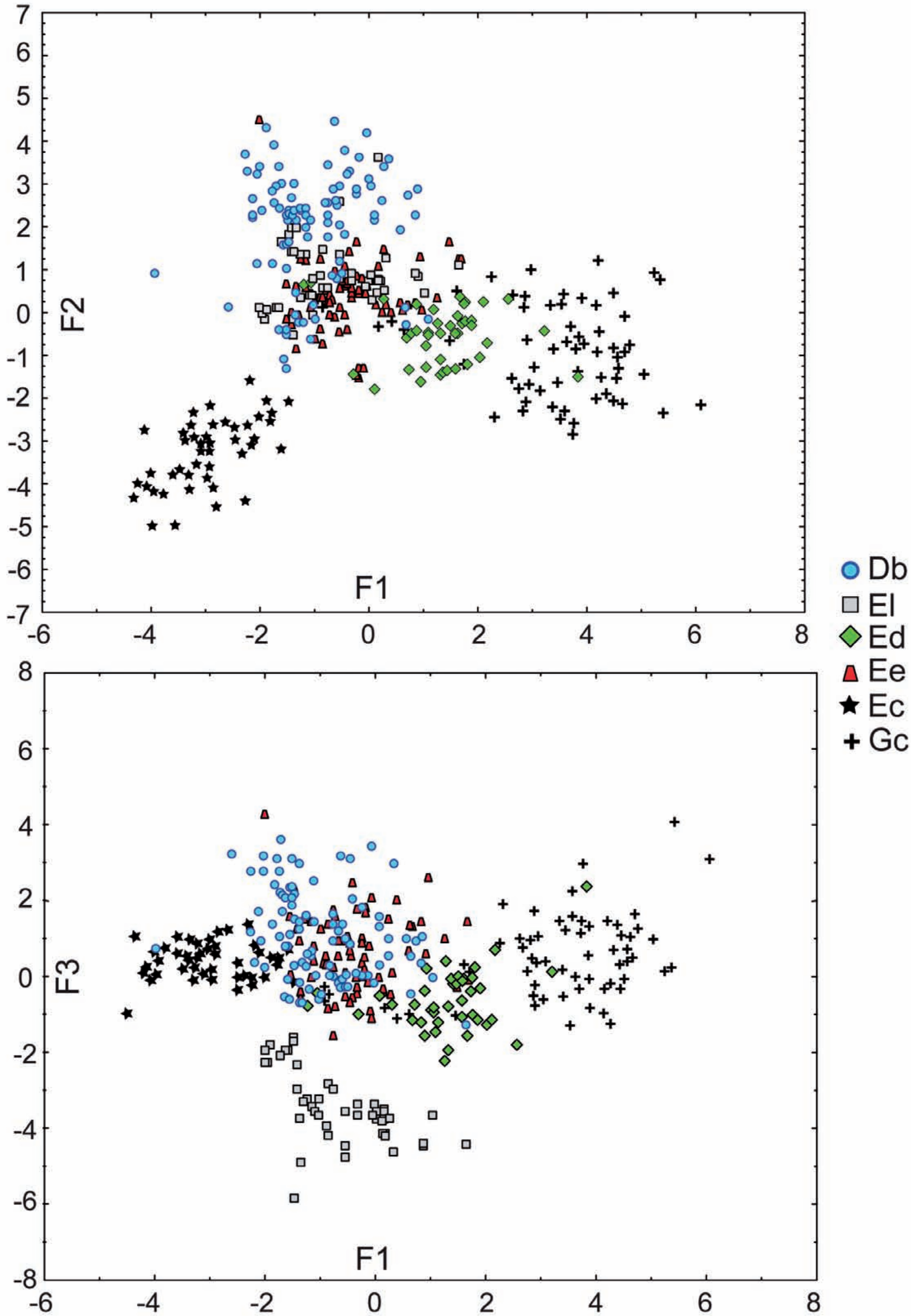

Fig. 3. Scatter plots of first (F1) and second (F2), and first (F1) and third (F3) discriminant functions; Canonical discriminant analysis using ten otolith shape and size descriptors of the six species of Gerreidae; The first discriminant axis (F1) explains 40%, the second axis (F2) 33%, and the third axis (F3) 20% of the variation; Db = *Diapterus brevirostris*, El = *Eugerres lineatus*, Ed = *Eucinostomus dowii*, Ee = *Eucinostomus entomelas*, Ec = *Eucinostomus currani*, Gc = *Gerres cinereus*

taxonomy, both at generic and specific levels (Matheson and McEachran 1984), primarily due to morphological plasticity in some taxa and to uncertain definitions of valid genera. Hence, currently taxonomy within the family Gerreidae is under considerable debate. Another consideration to be mentioned is that the majority of taxonomic reviews (as graduate thesis) about Gerreidae genera have not been published (Curran unpublished[*], Zahuranec unpublished[**], Deckert unpublished[***] (partially), Matheson unpublished[****] (partially), De La Cruz-Agüero unpublished[*****], Burnes-Romo unpublished[******]), although none of them have studied otoliths.

The majority of descriptive studies on fish otolith provide subjective descriptions without an objective framework for comparisons. These descriptions are not standardized (e.g., not related to universal forms of recognition, as could be geometrical shapes: square, rectangle, circle, and so on). Otolith morphology descriptions provided in this study is presented for the first time for the six included Gerreidae species, following a standardized terminology (Volpedo and Echeverría 2000). Usefulness of the employed descriptors is shown in the classification rate of CDA.

Moreover, morphometric properties of sulcus acusticus produced no significant results when comparing mean proportions among gerreid species, despite the demonstrated interspecific differences in other fish species (Torres et al. 2000b, Tuset et al. 2003a), which may be adaptations of the different sizes of fish to environmental conditions occurring at different depths (Lombarte 1992). This is not the case for Gerreidae species in present research, possibly due to the size range used and shallowness of commercial catches. However, there are certain patterns in proportions CL / OL and CL / RW of taxa that should be investigated: higher values in genus *Eucinostomus*, intermediate values in *G. cinereus*, and lower values in species of *Diapterus* and *Eugerres*. This morphometric pattern corresponds, to some extent, with the taxonomic and phylogenetic arrangements (as discussed below).

CDA results show that this set of morphological variables of otoliths produced a good discrimination among all considered species. Besides, morphological affinity between species corresponds to the current taxonomic arrangement, except for *E. currani*, as shown in the phenogram based on quadratic Mahalanobis distances (Euclidean distance = 75; Fig. 4). This morphological affinity is also close to the two proposed phylogenetic clades based on molecular data for the American gerreid genera: clade 1: *Eucinostomus* / *Gerres* and clade 2: *Diapterus* / *Eugerres* (see Ruiz-Carus and Uribe-Alcocer 2003, Chen et al. 2007), and agreeing with the same two morphological assemblages in taxonomic keys, characterized by the by the shape of preopercle and preorbital bone (Bussing 1995).

Actual separation rate in CDA with otoliths (93% considering the first three discriminant functions) is similar to several CDA's reports for gerreid species although using variables of body morphology: 97% for *Eucinostomus* spp. in the Pacific coast (De La Cruz Agüero and Galvan-

Table 3

Mean values of the main otolith descriptors (not correlated at 5% significance level) obtained from morphometric measurements of the fish family Gerreidae taken on the lateral view of each sagittae (standard deviation in parentheses)

Descriptor	Species					
	Db	El	Ed	Ee	Ec	Gc
Dmx	8.11 (0.69)	7.27 (0.84)	6.95 (0.38)	5.30 (0.35)	6.81 (0.61)	6.96 (0.81)
Rmx	4.14 (0.86)	3.76 (0.43)	3.64 (0.21)	2.73 (0.19)	3.48 (0.40)	3.65 (0.42)
Po	24.4 (2.41)	22.9 (2.95)	19.7 (1.21)	16.3 (1.44)	20.4 (2.10)	20.2 (2.02)
Lo	8.06 (0.70)	7.29 (0.83)	7.0 (0.37)	5.30 (0.35)	6.8 (0.60)	7.1 (0.82)
Ao	28.8 (4.24)	21.9 (3.42)	19.5 (1.82)	14.4 (1.71)	20.1 (3.10)	16.9 (3.82)
Fmx	8.14 (0.70)	7.36 (0.83)	7.03 (0.38)	5.32 (0.34)	6.88 (0.60)	7.10 (0.82)
Fl	6.58 (0.51)	5.88 (0.51)	5.59 (0.25)	4.54 (0.28)	5.50 (0.40)	5.34 (0.58)
Ff	1.99 (0.19)	1.66 (0.15)	2.13 (0.19)	1.67 (0.19)	1.87 (0.19)	2.53 (0.28)
Rd	1.66 (0.20)	1.92 (0.35)	1.58 (0.10)	1.47 (0.16)	1.69 (0.21)	1.96 (0.24)
Rc	0.71 (0.02)	0.68 (0.03)	0.72 (0.02)	0.70 (0.02)	0.69 (0.02)	0.70 (0.02)

Acronyms for species: Db = *Diapterus brevirostris*, El = *Eugerres lineatus*, Ed = *Eucinostomus dowii*, Ee = *Eucinostomus entomelas*, Ec = *Eucinostomus currani*, Gc = *Gerres cinereus*; Dmx = diameter maximum, Rmx = ratio maximum, Rc = rectangularity, Lo = otolith length, Ao = area, Po = perimeter, Rd = roundness, Fl = Feret length, Fmx = Feret maximum, Ff = form-factor.

[*] Curran H.W. 1942. A systematic revision of the gerreid fishes referred to the genus *Eucinostomus*, with a discussion of their distribution and speciation. PhD Dissertation. University of Michigan, Ann Arbor, MI, USA.

[**] Zahuranec B.J. 1967. The Gerried fishes of the genus *Eucinostomus* in the eastern Pacific. MSc Dissertation, University of California, San Diego, CA, USA.

[***] Deckert G.D. 1973. A systematic revision of the genera *Diapterus* and *Eugerres*: with the description of a new genus, *Schizopterus* (Pisces: Gerreidae). MSc Dissertation. Northern Illinois University, DeKalb, IL, USA.

[****] Matheson R.E. 1983. Taxonomic studies of the *Eucinostomus argenteus* complex (Pisces: Gerreidae). PhD Dissertation. Texas A&M University, College Station, TX, USA.

[*****] De La Cruz-Agüero J. 2001. Sistemática y Biogeografía del género *Eucinostomus* (Teleostei: Gerreidae). PhD Dissertation, Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, La Paz, Baja California Sur, México.

[******] Burnes-Romo L.A. 2009. Revision del estatus taxonómico de *Gerres cinereus* (Walbaum, 1972) (Teleostei: Gerreidae). MSc Dissertation. Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, La Paz, Baja California Sur, México.

Magaña 1993) and 96% and 93% for Pacific and Atlantic species of *Eugerres*, respectively (González Acosta et al. 2005, 2007). In other studies the classification accuracy obtained using otolith descriptors has varied between 70% and 95% (Tuset et al. 2006), indicating that otolith morphology can be used for identifying and separating Gerreidae species and it is likely that an individual is better characterized by its two otoliths rather than by only one of them (see Morat et al. 2012).

Although it has been established that otolith shape is markedly species-specific (L'Abée-Lund 1988, L'Abée-Lund and Jensen 1993), it is also known that there are external driving forces acting on otoliths shape, whatever the species (Lord et al. 2012) including: age, sex, size, food availability, substrate, water temperature, as well as other biological and environmental factors, linked to the species and geographical sites (Tuset et al. 2003a). In the presently reported study, considering that some of these factors are related to sample locations along the Pacific coast of Mexico, and that our analyses were made regardless of sex and minimizing size effect considering mostly adult specimens, there may be a confounding effect increasing the misclassification rate in some species (e.g., *D. brevirostris*: 24% and *G. cinereus*: 21%); Table 4). Another possibility could have been an inadequate sample size in that species; according to the criterion that relates the number of variables with the number of individuals (Junquera and Peréz-Gándaras 1993).

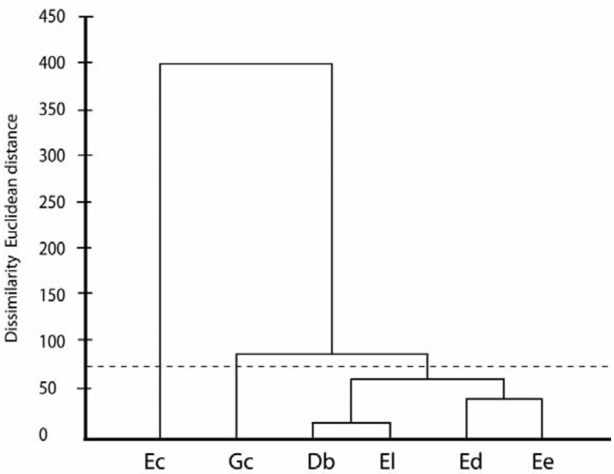

Fig. 4. Dendrogram indicating the phenotypic relationship from the otolith morphology (shape and size data) for Gerreidae species: Ec = *Eucinostomus currani*, Gc = *Gerres cinereus* Db = *Diapterus brevirostris*, El = *Eugerres lineatus*, Ed = *Eucinostomus dowii*, Ee = *Eucinostomus entomelas*; Dissimilarity based on the Euclidian distance of Mahalanobis distance values, grouping by hierarchical cluster analysis (UPGMA)

Nevertheless, we can conclude that otolith morphology can be used for separation of gerreid species through CDA. Otoliths descriptors here studied (e.g., rectangularity, roundness, otolith length, Feret length, and CL / OL and CL / RW ratios) can also be used as additional features to those already used for proper taxonomic identification, with possible implications of phylogenetic value. For further research on otolith morphology of Gerreidae, would be desirable increasing the number of sagittae per species, and the use of geometric morphometry (e.g., contour analysis) because it might increase the discrimination power and precision of species classifications (Chen et al. 2011).

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Table 4

Classification matrix as a result of canonical discriminant analysis testing for differences between analyzed species of the fish family Gerreidae based on ten sagittal otolith shape and size descriptors

Actual group	Predicted group						SBS	CC [%]
	Db	El	Ed	Ee	Ec	Gc		
Db	<u>28</u>	0	0	9	0	0	(37)	75.67
El	1	<u>35</u>	0	0	0	0	(36)	97.22
Ed	0	0	<u>20</u>	0	0	1	(21)	95.232
Ee	1	0	1	<u>41</u>	0	0	(43)	95.34
Ec	0	0	0	0	<u>45</u>	0	(45)	100
Gc	0	0	5	5	0	<u>38</u>	(48)	79.16
Total	30	35	26	55	45	39	(230)	90.43

Acronyms for species: Db = *Diapterus brevirostris*, El = *Eugerres lineatus*, Ed = *Eucinostomus dowii*, Ee = *Eucinostomus entomelas*, Ec = *Eucinostomus currani*, Gc = *Gerres cinereus*; SBS = sagittae by species; CC = correct classification percentage by species; Underlined: quantity of correctly classified otoliths for each species; sample sizes in parentheses; Overall classification success of 90.43%; Cohen's kappa = 0.879, $P < 0.001$; G-test = 669.8, $P < 0.000$.

REFERENCES

Aguirre-León A., Yáñez-Arancibia A. 1986. Las mojarras de la Laguna de Términos: taxonomía, biología, ecología y dinámica trófica (Pisces: Gerreidae). Anales del Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México **13** (1): 369–444.

Begg G.A., Brown W. 2000. Stock identification of haddock *Melanogrammus aeglefinus* on Georges Bank based on otolith shape analysis. Transactions of the American Fisheries Society **129** (4): 935–945. DOI: 10.1577/1548-8659(2000)129<0935:SIOHMA>2.3.CO;2

Bussing W.A. 1995. Gerreidae. Pp. 1114–1128. *In*: Fisher W., Krupp F., Schneider W., Sommer C., Carpenter K.E., Niem V.H. (eds.) Guía para la identificación de especies para los fines de la pesca. Pacífico Centro-Oriental. FAO, Roma.

Chen W., Al-Husaini M., Beech M., Al-Enezi K., Rajab S., Husain H. 2011. Discriminant analysis as a tool to identify catfish (Ariidae) species of the excavated archaeological otoliths. Environmental Biology of Fishes **90** (3): 287–299. DOI: 10.1007/s10641-010-9742-6

Chen W.-J., Ruiz Carus R., Ortí G. 2007. Relationships among four genera of mojarras (Teleostei: Perciformes: Gerreidae) from the western Atlantic and their tentative placement among percomorph fishes. Journal of Fish Biology **70** (Suppl. sb): 202–218. DOI: 10.1111/j.1095-8649.2007.01395.x

De La Cruz-Agüero J., Galvan-Magaña F. 1993. Morphological discrimination of *Eucinostomus* spp. from the Pacific coast of Mexico. Bulletin of Marine Science **52** (2): 819–824.

Eschmeyer W.N. (ed.) 2012. Catalog of Fishes. California Academy of Sciences. (http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp) Electronic version accessed on 10 May 2012.

Froese R., Pauly D. (Eds) 2012. FishBase. World Wide Web electronic publication. www.fishbase.org, version (10/2012).

González-Acosta A.F., De La Cruz-Agüero J., Castro-Aguirre J.L. 2005. A review of eastern Pacific species of the genus *Eugerres* (Perciformes: Gerreidae). Bulletin of Marine Science **76** (3): 661–673.

González-Acosta A.F., De La Cruz-Agüero J., Castro-Aguirre J.L. 2007. A review of the marine western Atlantic species of the genus *Eugerres* (Perciformes: Gerreidae). Bulletin of Marine Science **80** (1): 109–124.

Junquera S., Pérez-Gándaras G. 1993. Population diversity in Bay of Biscay anchovy (*Engraulis encrasicolus* L. 1758) as revealed by multivariate analysis of morphometric and meristic characters. ICES Journal of Marine Science **50** (4): 383–391. DOI: 10.1006/jmsc.1993.1043

Klecka W.R. 1980. Discriminant analysis. Sage University Papers, Series on Quan- titative Applications in the Social Sciences, Beverly Hills, CA, USA.

L'Abée-Lund J.H. 1988. Otolith shape discriminates between juvenile Atlantic salmon, *Salmo salar* L., and brown trout, *Salmo trutta* L. Journal of Fish Biology **33** (6): 899–903. DOI: 10.1111/j.1095-8649.1988.tb05538.x

L'Abée-Lund J.H., Jensen A.J. 1993. Otoliths as natural tags in the systematics of salmonids. Environmental Biology of Fishes **36** (4): 389–393. DOI: 10.1007/BF00012418

Lachenbruch P.A., Mickey M.R. 1968. Estimation of error rates in discriminant analysis. Technometrics **10** (1): 1–11. DOI: 10.1080/00401706.1968.10490530

Lemos B., Correa M., Abilhoa V. 1993. Catálogo de otólitos de Gerreidae (Osteichthyes-Perciformes) do Litoral do Estado do Paraná. Neritica **7**: 109–117.

Lombarte A.J. 1992. Changes in otolith area: sensory area ratio with body size and depth. Environmental Biology of Fishes **33** (4): 405–410. DOI: 10.1007/BF00010955

Lombarte A.J., Lleonart J. 1993. Otolith size changes related with body growth, habitat depth and temperature. Environmental Biology of Fishes **37** (3): 297–306. DOI: 10.1007/BF00004637

Lombarte A., Rucabado J., Matallanas J., Lloris D. 1991. Taxonomía numérica de Nototheniidae en base a la forma de los otolitos. Scientia Marina **55** (2): 413–418.

Lord C., Morat F., Lecomte-Finiger R., Keith P. 2012. Otolith shape analysis for three *Sicyopterus* (Teleostei: Gobioidei: Sicydiinae) species from New Caledonia and Vanuatu. Environmental Biology of Fishes **93** (2): 209–222. DOI: 10.1007/s10641-011-9907-y

Matheson R.E.jr., McEachran J.D. 1984. Taxonomic studies of the *Eucinostomus argenteus* complex (Pisces: Gerreidae): preliminary studies of external morphology. Copeia **1984** (4): 893–902.

Morat F., Letourneur Y., Nérini D., Banaru D., Batjakas I.E. 2012. Discrimination of red mullet populations (Teleostean, Mullidae) along multi-spatial and ontogenetic scales within the Mediterranean basin on the basis of otolith shape analysis. Aquatic Living Resources **25** (1): 27–39. DOI: 10.1051/alr/2011151

Neves A., Sequeira V., Farias I., Vieira A.R., Paiva R., Serrano Gordo L. 2011. Discriminating bluemouth, *Helicolenus dactylopterus* (Pisces: Sebastidae), stocks in Portuguese waters by means of otolith shape analysis. Journal of the Marine Biological Association of the United Kingdom **91** (Special issue 06): 1237–1242. DOI: 10.1017/S002531541000189X

Nolf D. 1995. Studies on fossil otoliths. The state of the art. Pp. 513–544. *In*: Secor D.H., Dean J.M., Campana S.E. (eds.) Recent developments in fish otolith research. University of South Carolina Press, Columbia, SC, USA.

Pertusa J.F. 2003. Técnicas de análisis de imagen: Aplicaciones en biología. Universitat de Valéncia, Aldaia, Spain.

Ponton D. 2006. Is geometric morphometrics efficient for comparing otolith shape of different fish species? Journal of Morphology **267** (6): 750–757. DOI: 10.1002/jmor.10439

Pothin K., González-Salas C., Chabanet P., Lecomte-Finiger R. 2006. Distinction between *Mulloidichthys flavolineatus* juveniles from Reunion Island and Mauritius Island (south-west Indian Ocean) based on otolith morphometrics. Journal of Fish Biology **69** (1): 38–53. DOI: 10.1111/j.1095-8649.2006.01047.x

Radhakrishnan K.V., Liu M., He W., Murphy B.R., Xie S. 2010. Otolith retrieval from faeces and reconstruction of preyfish size for Great Cormorant (*Phalacrocorax carbo*) wintering at the East Dongting Lake National Nature Reserve, China. Environmental Biology of Fishes **89** (3–4): 505–512. DOI: 10.1007/s10641-010-9685-y

Ruiz-Carus R., Uribe-Alcocer M. 2003. Phylogenetic assessment of *Eucinostomus gula*, *Eugerres plumieri*, and *Diapterus auratus* (Pisces; Gerreidae) based on allozyme and mtDNA analyses. Caribbean Journal of Science **39** (1): 109–115.

Smale M.J., Watson G., Hecht T. 1995. Otolith atlas of southern African marine fishes. Ichthyological Monographs of the J.L.B. Smith Institute of Ichthyology, Grahamstown, Republic of South Africa.

Titus K., Mosher J.A., Williams B.K. 1984. Chance-corrected classification for use in discriminant analysis: ecological applications. American Midland Naturalist **111** (1): 1–7.

Torres G.J., Lombarte A.J., Morales-Nin B. 2000a. Sagittal otolith size and shape variability to identify geographical intraspecific differences in three species of the genus *Merluccius.* Journal of the Marine Biological Association of the United Kingdom **80** (2): 333–342. DOI: 10.1017/S0025315499001915

Torres G.J., Lombarte A.J., Morales-Nin B. 2000b. Variability of the sulcus acusticus in the sagittal otolith of the genus *Merluccius* (Merluciidae). Fisheries Research **46** (1–3): 5–13. DOI: 10.1016/S0165-7836(00)00128-4

Tuset V.M., Lombarte A., González J.A., Pertusa J.F., Lorente MaJ. 2003a. Comparative morphology of the sagittal otolith in *Serranus* spp. Journal of Fish Biology **63** (3): 1491–1504. DOI: 10.1111/j.1095-8649.2003.00262.x

Tuset V.M., Lozano I.J., González J.A., Pertusa J.F., García-Díaz M.M. 2003b. Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). Journal of Applied Ichthyology **19** (2): 88–93. DOI: 10.1046/j.1439-0426.2003.00344.x

Tuset V.M., Rosin P.L., Lombarte A.J. 2006. Sagittal otolith shape used in the identification of fishes of the genus *Serranus*. Fisheries Research **81** (2–3): 316–325. DOI: 10.1016/j.fishres.2006.06.020

Volpedo A.V., Echeverría D.D. 2000. Catálogo y claves de otolitos para la identificación de peces del Mar Argentino. Editorial Dunken, Buenos Aires, Argentina.

Volpedo A.V., Tombari A.D., Echeverría D.D. 2008. Eco-morphological patterns of the sagitta of Antarctic fish. Polar Biology **31** (5): 635–640. DOI: 10.1007/s00300-007-0400-1

Yáñez-Arancibia A. 1980. Taxonomía, ecología y estructura de las comunidades de peces en lagunas costeras con bocas efímeras del Pacifico de México. Anales del Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México **2**: 1– 306.

Zar J.H. 2009. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, NJ, USA.

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