

Geometric morphometric analysis of fish scales for identifying genera, species, and local populations within the Mugilidae

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Abstract: Geometric morphometric methods (GMMs) were used to determine if scale morphology can discriminate between genera, species, geographic variants, and stocks of mullet (Mugilidae). GMMs were used because they allow standard multivariate analyses while preserving information about scale shape, which is important in making biological interpretations of results. The method was tested on ctenoid scales from mullets collected from different areas of the Gulf of Mexico and Aegean Sea. Scales were submitted to generalised procrustes analysis, followed by principal components analysis of resulting shape coordinates. Principal component scores were submitted to cross-validated discriminant analysis to determine the efficacy of scale landmarks in discriminating by taxon and population. Fish scale form was least effective in discriminating populations from nearby areas, better when populations are more geographically dispersed, and best between species and genera. Scale form variations reflected previous genetic studies that differentiated congeneric *Mugil cephalus* and *Mugil curema*, which are distinct from other Mugilidae. The method is nondestructive, quick, and less costly than genetic analysis, thus allowing many individuals to be screened.

Résumé : Nous utilisons des méthodes morphométriques géométriques (« GMMs ») pour vérifier si la morphologie de l'écaille permet de distinguer les genres, les espèces, les variantes géographiques et les stocks chez les mulets (Mugilidae). Nous avons choisi les GMMs parce qu'elles permettent les analyses multidimensionnelles habituelles, tout en conservant l'information sur la forme des écailles qui est importante dans l'interprétation biologique des résultats. Nous testons la méthode sur les écailles cténoïdes de mulets récoltés dans diverses régions du golfe du Mexique et de la mer Égée. Les écailles ont été soumises à l'analyse généralisée du logiciel procrustes, puis les coordonnées de forme obtenues à l'analyse en composantes principales. Les positions des composantes principales ont été soumises à une analyse discriminante à validation croisée afin de déterminer l'efficacité des repères sur l'écaille pour distinguer les taxons et les populations. La forme de l'écaille de poisson permet de discriminer moins bien entre les populations de régions adjacentes, mieux entre les populations plus éloignées géographiquement et le mieux entre les espèces et les genres. Les variations de forme des écailles reflètent les résultats d'études génétiques antérieures qui distinguent les congénères *Mugil cephalus* et *Mugil curema* qui sont différents des autres Mugilidae. La méthode ne requiert pas la destruction de l'échantillon, elle est rapide et moins coûteuse qu'une analyse génétique, ce qui permet de trier un nombre élevé d'individus.

[Traduit par la Rédaction]

Introduction

Species identification and population discrimination are important in the conservation of biodiversity, natural resources, and fisheries management. It is also often necessary to identify individual specimens correctly to population when investigating biological traits such as growth, mortality, fecundity, trophic relationships, parasite relationships, and historical and paleontological events.

Traditionally, identification of species membership has relied on morphological and meristic characteristics (Casselman et al. 1981; Ihssen et al. 1981; Cadrin 2000), but these

methods often encounter problems with reliability (e.g., Jerry and Cairns 1998; Swain and Foote 1999; Murta 2000). More recently, genetic methods have been used (e.g., Hutchinson et al. 2001; Mariani et al. 2005), but they are costly and not readily available in the field. Alternatively, Fourier analysis of fish scales has been used to discriminate between stocks (e.g., Jarvis et al. 1978; Richards and Esteves 1997; Poulet et al. 2005). More recently, Watkinson and Gillis (2005) used wavelet analysis as an alternative to Fourier analysis to improve discrimination. Alternative methods of shape analysis, based on landmark data (and geometric morphometric methods (GMMs)), have also found wide ap-

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plicability in biology (Chen et al. 2005; Crews and Hedin 2006; Recasens et al. 2006) because of the natural links between homologies and measurements (the landmark coordinates; cf. Fourier and wavelet approaches), the statistical properties of the resulting shape spaces (Dryden and Mardia 1998), and good statistical power. These GMMs preserve geometry throughout the analysis and, like Fourier and wavelet analyses, provide graphical visualisations of the statistical findings that can aid biological interpretation (Walker 1996; O'Higgins 2000). Different measurement methods (e.g., Fourier, outline curvatures, wavelets) result in varying assessments of the differences between specimens to the extent that the choice of method is arbitrary and the resulting distance matrices (the elements of which are the relative distances between specimens) will also be arbitrary. The landmark-based methods differ fundamentally from others in that they utilise biological equivalences between structures, matching or "homologous" landmarks, and to this extent, the resulting metrics are founded in biology. Having said this, in practical terms, the Fourier and other "landmark-free" approaches work well and offer the immediate advantage of capturing the subtleties of outline detail. GMMs have advanced to incorporate these outline features (e.g., Bookstein et al. 2005) and it is expected that these approaches will, in time, offer all of the advantages of outline measurement methods while incorporating all available homology information. However, the extent to which outline variations between landmarks are informative depends greatly on the features of the structure being measured, the adequacy of sampling of form that can be achieved by landmarks alone, and the question at hand. In the case of fish scales, a reasonable representation of form is achieved using very few landmarks (as we will show further on), and there are definite advantages to achieving a concise description of form by using a few variables because the resulting multivariate spaces are readily visualised and "noise" is minimised relative to "signal" in discriminant analysis.

According to Thomson (1981), the family Mugilidae contains 14 genera and 64 species that live in coastal waters and estuaries at all latitudes, except polar regions. The external morphology of this family is highly conservative, which contributes to uncertainty with regard to evolutionary relationships at both generic and specific levels (Crosetti et al. 1994). Mulletts are euryhaline species that can penetrate lagoons and estuaries, migrating back to the sea to spawn. They play an important part in small-scale coastal fisheries in several regions of the world. These fisheries constitute one of the 10 most important coastal fisheries in Mexico as a result of their catch volume, which exceeds 13 000 tonnes annually. Most of the catch takes place in the northern Gulf of Mexico in the Madre and Tamiahua lagoons. In Greek coastal lagoons, mulletts represent the 11th target of small-scale fisheries, accounting for 2.3% of total catches (Tzanatos et al. 2005).

The aim of this study was to evaluate whether the landmark-based, geometric, morphometric approach to describing fish scale morphology is useful in discriminating between species, populations, and geographic variants. This methodology was assessed using a case study based on fish species of the family Mugilidae collected from different localities from

the Gulf of Mexico and Aegean Sea. The null hypothesis being tested was that differences as described by landmarks in fish scale morphology between species and populations of mullet are insufficient to allow identification by genus, species, geographic variants, and local populations.

Materials and methods

Fish scale collection/age and size range of fishes

Scales of *Mugil cephalus* and *Mugil curema* were collected in Tamiahua Lagoon, Mexico, between April 1991 and March 1992. Further fish scales from these species were collected in December 2005 from the Mexico City central fish market: *M. cephalus* specimens came from Madre Lagoon and *M. curema* specimens came from Tecolutla River. All Mexican localities are near the Gulf of Mexico (Table 1; Figs. 1a, 1b).

Samples from the Aegean Sea were obtained from fish caught on the east coast of Greece in January 2006 (Figs. 2a, 2b): *M. cephalus* scales were acquired from Monolimni Lagoon on the River Evros and Agiasma Lagoon; *Chelon labrosus* (Risso 1827) scales were acquired from the Vassova, Eratino, and Agiasma lagoons (Nestos River); and *Liza saliens* (Risso 1810) were acquired from the Eratino Lagoon. Fish were measured to the nearest millimetre (mm) in situ, and scales were removed from the shoulder region in front of the 1st dorsal fin ray above the lateral line and stored dry in paper envelopes. In the laboratory, scales were cleaned with soft soap and tap water, dried, and examined using a microfiche projector. A digital image was taken from the flat screen of the projector. Only one scale per fish was used for the analysis (Table 1) as we are attempting to evaluate a rapid stock discrimination methodology that does not require repetitive sampling of individuals. All specimens were adults except for the sample of *M. cephalus* from the Tamiahua Lagoon, which contained 40% juveniles. In this paper, "area" refers to a large water body such as the Gulf of Mexico or Aegean Sea, and "local population" refers to specimens collected within a small geographic area such as a lagoon in the Gulf of Mexico or on the Greek coast.

Morphometrics

Preliminary visual assessment was used to identify potential landmarks on the scales. The landmarks chosen are located on key features of the scale that are common to all scales of the species or variants examined. This ensures that in subsequent interpretation of results, variations in particular landmarks can be related back to shared features of form (contrast with Fourier analysis where no such relationship exists except for the start point for the series; see Introduction). For mullet scales, the following landmarks were considered appropriate (Fig. 3): landmarks 1 and 3 are the ventro- and dorso-lateral tips of the anterior portion of the scale, respectively; landmark 2 is in the center of the anterior edge of the scale, landmarks 4 and 6 are at the boundary between the anterior area with circuli and the posterior area covered by cteni (spine-like ornamentations), respectively; landmark 5 is the focus of the scale; and landmark 7 is positioned at the tip of the posterior portion of the scale.

Table 1. Size of sample and site of collection of fish scales.

Species	Area	Collection site	Group code	No. of fish	Fork length range (cm)	Sample collection period
<i>Mugil cephalus</i> (<i>Mce</i>)	GM	Ma	<i>Mce</i> -GMMa	33	27.0–31.0*	December 2005
		Ta	<i>Mce</i> -GMTa	29	22.5–35.6*	April 1991 to March 1992
	AS	Ag	<i>Mce</i> -ASAg	10	28.5–32.5	January 2006
		Mo	<i>Mce</i> -ASMo	40	24.8–47.2	January 2006
<i>Mugil curema</i> (<i>Mcu</i>)	GM	Ta	<i>Mcu</i> -GMTa	30	19.5–31.0*	April 1991 to March 1992
		Te	<i>Mcu</i> -GMTe	13	27.5–36.0*	December 2005
<i>Chelon labrosus</i> (<i>Cla</i>)	AS	Va	<i>Cla</i> -ASVa	10	23.3–29.6	January 2006
		Er	<i>Cla</i> -ASEr	27	20.0–28.6	January 2006
		Ag	<i>Cla</i> -ASAg	10	24.0–27.0	January 2006
<i>Liza saliens</i> (<i>Lsa</i>)	AS	Er	<i>Lsa</i> -ASEr	5	22.2–24.1*	January 2006

Note: Areas: AS, Aegean Sea; GM, Gulf of Mexico. Collection sites: Ag, Agiasma Lagoon; Er, Eratino Lagoon; Ma, Madre Lagoon; Mo, Monolimni Lagoon; Ta, Tamiagua Lagoon; Te, Tecolutla River; Va, Vassova Lagoon.

*Specimens were originally measured with total length, which was converted to fork length according to the equation given by Thompson et al. (1991).

Fig. 1. Sampling locations in the Gulf of Mexico: (a) Madre Lagoon; (b) Tamiagua Lagoon and Tecolutla River.

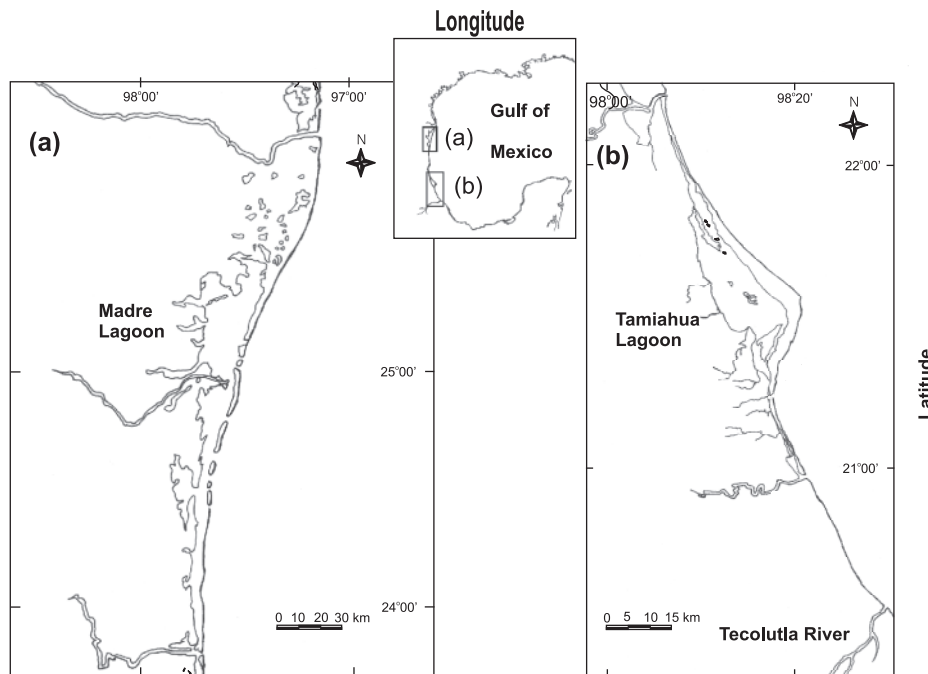


Fig. 2. Sampling locations in the Aegean Sea: (a) Agiasma, Eratino, and Vassova lagoons; (b) Monolimni Lagoon.

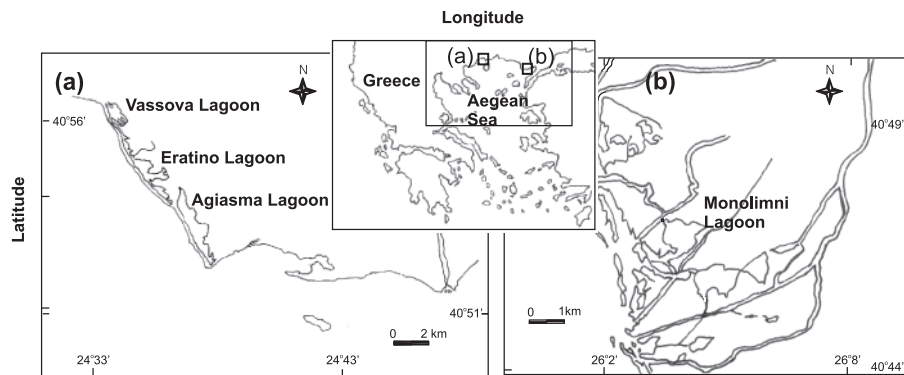
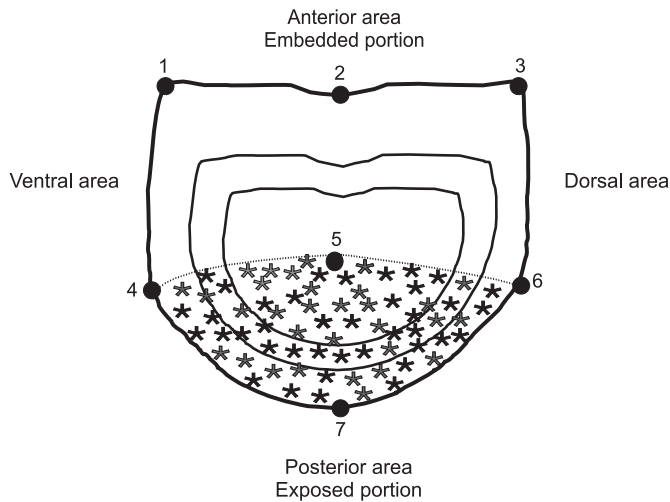


Fig. 3. Landmark definitions used in the fish scales.



The configurations of landmark coordinates were scaled, translated, and rotated using generalised procrustes analysis (GPA). They were then submitted to tangent projection (Dryden and Mardia 1993) and subsequently to principal components analysis (PCA) (Dryden and Mardia 1993; Kent 1994). The aim was to examine the potential for differences in shape to allow classification of unknown specimens, and so the scores of specimens on all non-zero principal components (PCs) were submitted to discriminant analysis (SPSS ver. 13.0) to compute generalised Mahalanobis distances and discriminant functions and to assess the efficacy of the latter in classification. This was carried out using cross-validation in which multiple repeated analyses were carried out leaving out one individual in the construction of the discriminant function before classifying this individual according to the function. This avoids overestimating the efficacy of discriminant functions by using them to classify specimens employed in their construction. Percentage correct classification rates were recorded. The aspects of shape variability represented by the discriminant functions were estimated by multivariate regression of PC scores on discriminant function scores. The PC scores predicted by the extremes of each discriminant function were then used to reconstruct the expected shape of landmark configurations with those particular scores by adding to the mean tangent coordinates the products of these PC scores and the eigenvectors for those PCs before projecting back from the tangent to the configuration space (O'Higgins et al. 2001). Finally, the differences in shape between the mean and the shapes represented by the extremes of discriminant functions of interest were visualised using transformation grids computed using thin-plate splines (TPS) (Bookstein 1989; Marcus et al. 1996; Dryden and Mardia 1998).

Sequential analyses were carried out to assess the extent to which scale shape differences, as described by landmarks, allow identification by genus, species, geographic variant, and local population.

To assess the quality of discrimination by genus relative to that by species, GPA of the whole sample (207 fish scales) was carried out, and the PC scores were submitted to two discriminant analyses: one in which the scales were labeled

by genus and the other, by species. Differences in fish scale shape between geographic variants were assessed by GPA/PCA and subsequent discriminant analysis of the *M. cephalus* specimens (112 fish scales), 62 from the Gulf of Mexico and 50 from the Aegean Sea. Differences in fish scale shape between species and geographic variants were assessed by GPA/PCA and subsequent discriminant analysis of the PC scores of specimens of the genus *Mugil* (155 fish scales). Classifications of specimens were carried out by species (*M. cephalus* and *M. curema*) and geographic variant, Gulf of Mexico and Aegean Sea. Finally, the differences between local populations were assessed using the same methods applied to *M. cephalus* from the Gulf of Mexico (62 fish scales: 33 for Madre Lagoon and 29 for Tamiahua Lagoon) and Greece (50 specimens: 10 for Agiasma and 40 for Monolimni Lagoon), *M. curema* from the Gulf of Mexico (43 specimens: 30 for Tamiahua Lagoon and 13 for Tecolutla River), and *C. labrosus* from the three Greek lagoons (47 specimens: 10 fish scales for Vassova, 27 for Eratino, and 10 for Agiasma Lagoon).

Results

The first canonical discriminant function for the 207 individuals classified by genus explained 90.7% of the total between-genus variance, whereas the second accounted for 9.3% (Wilks' lambda = 0.560, $p < 0.001$). The discriminant analysis correctly classified 74.4% of the fish scales to genus, whereas the cross-validated analysis correctly classified 71.0% of the fish scales (Table 2). The best classification rate, 73.5%, was for *Mugil* compared with 63.8% and 60.0%, respectively, for *Chelon* and *Liza*; most misclassifications occurred between the latter two genera (Table 2).

Three groupings were defined in the plot of the first two discriminant functions from the analysis of the 207 individuals. The centroids and specimens of *M. cephalus* and *M. curema* were separated principally on the first discriminant function, whereas those of *C. labrosus* and *L. saliens* occupied a central position with considerable overlap of specimens and lower scores than *M. cephalus* and *M. curema* on the second function (Fig. 4). The first discriminant function explained 79.2% of the total variance, whereas the second accounted for 17.6% (Wilks' lambda = 0.252, $p < 0.001$). The discriminant analysis correctly classified 78.7% of the original grouped cases, whereas cross-validation correctly classified 73.9% (Table 3). *Mugil cephalus* has the best classification rate, with 83.9% correctly classified after cross-validation. Misclassifications were common between *M. curema* and *C. labrosus* and between *L. saliens*, *M. cephalus*, and *C. labrosus* (Table 3).

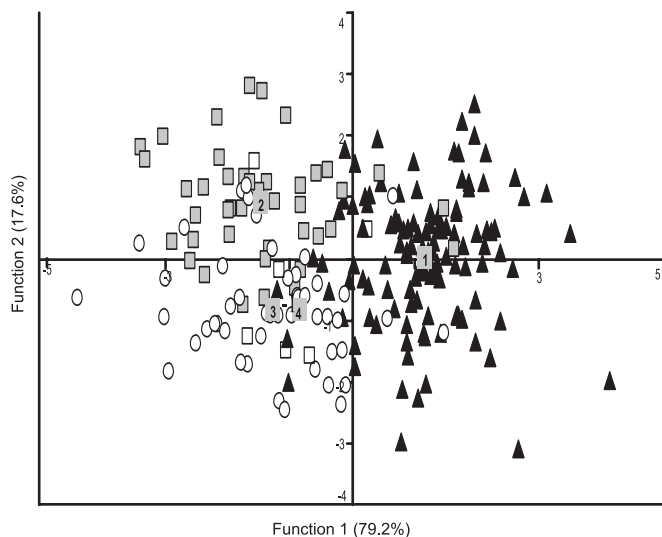
The shape variation represented by the first discriminant function of Fig. 4 is shown (Fig. 5), computed by regressing shape on that function. The leftmost grid (Fig. 5a) represents the mean shape warped to a score of -0.4, the central grid (Fig. 5b) represents the mean with a score of 0, and the rightmost grid (Fig. 5c) represents a score of 4.0. Scales from *C. labrosus*, *L. saliens*, and *M. curema* (which lie to the negative pole of the first discriminant function and so have shapes that differ from the mean as represented in Fig. 5a) were characterized by a relatively shorter distance

Table 2. Classification results^a for the cross-validation testing procedure for the three genera of the Mugilidae family.

Genus	Predicted group membership			Total
	<i>Mugil</i>	<i>Chelon</i>	<i>Liza</i>	
Count				
<i>Mugil</i>	114	27	14	155
<i>Chelon</i>	5	30	12	47
<i>Liza</i>	1	1	3	5
Percent				
<i>Mugil</i>	73.5	17.4	9.0	100.0
<i>Chelon</i>	10.6	63.8	25.5	100.0
<i>Liza</i>	20.0	20.0	60.0	100.0

^aOf cross-validated grouped cases, 71.0% were correctly classified.

Fig. 4. Scatter plot of the discriminant function scores from the analysis of species: solid triangles, *Mugil cephalus*; shaded squares, *M. curema*; open circles, *Chelon labrosus*; open squares, *Liza saliens*. Numbers over the shaded squares are the number of centroids.



between the focus and landmark 7, with a convex anterior edge, whereas this distance for *M. cephalus* is relatively larger, with the focus more central and the anterior edge concave (Fig. 5c). Likewise the shape variation represented by the second discriminant function visualised with a transformation grid is illustrated (Fig. 6). From left to right, each grid portrays the shape for the sample with the following discriminant function scores (DS): -3.0 (Fig. 6a), 0.0 (Fig. 6b), and 3.0 (Fig. 6c). The degree of shape variation appears less than in Fig. 5, in part because the transformation grids span a smaller range on the discriminant function. The key difference is in the relative location of the landmark, which becomes more posterior with respect to the remaining landmarks. The grid is distorted principally between this landmark and the focus. Thus, the first discriminant function of Fig. 4 principally relates to contrasts between groups in the relative locations of the focus and landmark 2, whereas the second relates to contrasts in

Table 3. Classification results^a for the cross-validation testing procedure for the four mullet species.

Species	Predicted group membership				Total
	<i>Mce</i>	<i>Mcu</i>	<i>Cl</i>	<i>Lsa</i>	
Count					
<i>Mce</i>	94	4	7	7	112
<i>Mcu</i>	3	30	5	5	43
<i>Cl</i>	3	5	26	13	47
<i>Lsa</i>	1	0	1	3	5
Percent					
<i>Mce</i>	83.9	3.6	6.3	6.3	100.0
<i>Mcu</i>	7.0	69.8	11.6	11.6	100.0
<i>Cl</i>	6.4	10.6	55.3	27.7	100.0
<i>Lsa</i>	20.0	0.0	20.0	60.0	100.0

Note: *Mce*, *M. cephalus*; *Mcu*, *M. curema*; *Cl*, *Chelon labrosus*; *Lsa*, *Liza saliens*.

^aOf cross-validated grouped cases, 73.9% were correctly classified.

Fig. 5. Shape variation along the first discriminant function of Fig. 4, visualized using a transformation grid. From left to right, each grid shows the predicted shape for DS: (a) -3.0, (b) 0.0, and (c) 3.0.

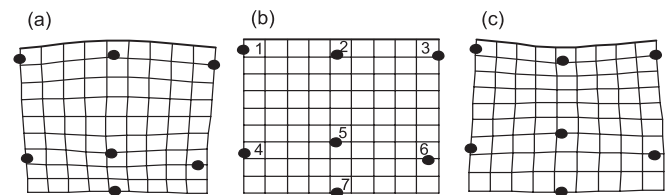
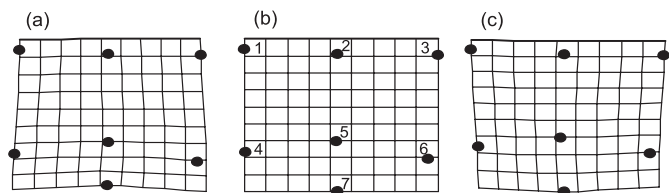


Fig. 6. Shape variation along the second discriminant function of Fig. 4, visualized using a transformation grid. From left to right, each grid shows the predicted shape for DS: (a) -3.0, (b) 0.0, and (c) 3.0.



landmark 7. All of these landmarks are arranged along the axis of the scale, and so it is variation in the longitudinal axis that accounts for the discrimination between groups.

The first discriminant function from the analysis of the 112 fish scales of *M. cephalus* specimens explained 100% of the total variance and was highly significant (Wilks' lambda = 0.320, $p < 0.001$). The discriminant analysis correctly classified 92.0% of the original grouped cases; whereas cross-validation resulted in 91.1% correct classification (Table 4).

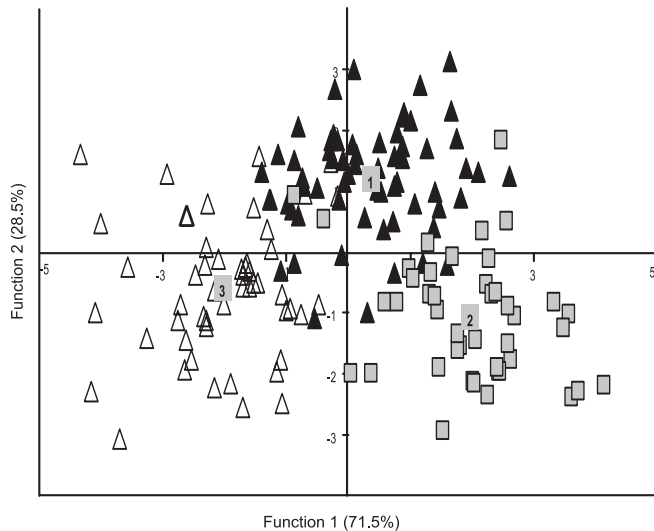
Three groups were distinguished by the discriminant analysis of the *Mugil* species (155 individuals) by area (Wilks' lambda = 0.138, $p < 0.001$). The first function (71.5% of the total variance; Fig. 7) separated *M. cephalus* from the Gulf of Mexico and the Aegean Sea and both species of *Mugil*;

Table 4. Classification results^a for the cross-validation testing procedure for *Mugil cephalus* in the Gulf of Mexico (GM) and Aegean Sea (AS).

Areas	Predicted group membership		Total
	GM	AS	
Count			
GM	56	6	62
AS	4	46	50
Percent			
GM	90.3	9.7	100.0
AS	8.0	92.0	100.0

^aOf cross-validated grouped cases, 91.1% were correctly classified.

Fig. 7. Scatter plot of the discriminant scores from the analysis of the *Mugil* species: solid triangles, *M. cephalus* from the Gulf of Mexico; open triangles, *M. cephalus* from the Aegean Sea; shaded squares, *M. curema* from the Gulf of Mexico. Numbers over the shaded squares represent the number of centroids.



the second function (28.5% of the total variance) separated *M. cephalus* from the Gulf of Mexico from *M. curema* and *M. cephalus* from the Aegean Sea. The discriminant analysis correctly classified 90.3% of the original grouped cases and 89.7% when cross-validated (Table 5). *Mugil cephalus* from the Aegean Sea was classified with 92% accuracy in the original and cross-validated analyses, whereas *M. cephalus* and *M. curema* from the Gulf of Mexico were correctly classified in 87.1% and 90.7% of cases, respectively (Table 5).

The shape variation represented by the first discriminant function of Fig. 7 is visualized using transformation grids, showing a DS of -4.0 (Fig. 8a), the mean (Fig. 8b), and the score 4.35 (Fig. 8c). As DS I increases, the exposed portion of the scale becomes relatively narrower (between landmarks 4 and 6) and the embedded portion becomes relatively broader (between landmarks 1 and 3). Likewise, the shape variation represented by the second discriminant function is illustrated (Fig. 9). From left to right, each grid shows DS -3.0 , 0, and 3.0 (Figs. 9a, 9b, 9c, respectively). The deformations of panels in Figs. 9a and 9c indicate that with increasing DS II, the

Table 5. Classification results^a for the cross-validation testing procedure for the *Mugil* species (*M. cephalus*, *M. curema*) in the Gulf of Mexico (GM) and Aegean Sea (AS).

Species	Area	Predicted group membership			Total
		<i>M. cephalus</i>		<i>M. curema</i>	
		GM	AS	GM	
Count					
<i>M. cephalus</i>	GM	54	4	4	62
	AS	4	46	0	50
<i>M. curema</i>	GM	4	0	39	43
Percent					
<i>M. cephalus</i>	GM	87.1	6.5	6.5	100.0
	AS	8.0	92.0	0.0	100.0
<i>M. curema</i>	GM	9.3	0.0	90.7	100.0

^aOf cross-validated grouped cases, 89.7% were correctly classified.

Fig. 8. Shape variation along the first discriminant function of Fig. 7, visualized using a transformation grid. From left to right, each grid shows the predicted shape for DS: (a) -4.0 , (b) 0.0, and (c) 4.0.

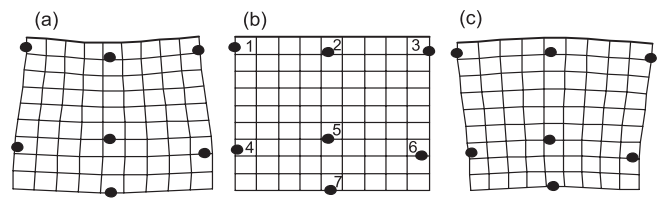
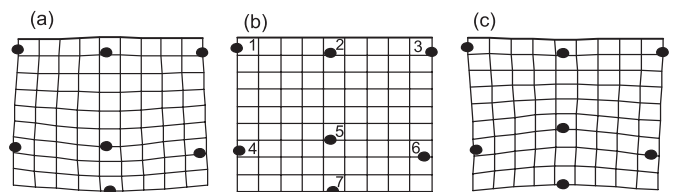


Fig. 9. Shape variation along the second discriminant function of Fig. 7, visualized using a transformation grid. From left to right, each grid shows the predicted shape for DS: (a) -3.0 , (b) 0.0, and (c) 3.0.



focus becomes more central with a little narrowing between landmarks 4 and 6.

The cross-validated classification results from the analysis between local populations for the *Mugil* species (Table 6) identified significant discrimination in all cases ($p < 0.001$). The cross-validated results for *M. cephalus* from the Gulf of Mexico showed a 74.2% correct overall classification: 72.7% for Madre Lagoon and 75.9% for Tamiahua Lagoon (Wilks' lambda = 0.536, $p < 0.001$). The cross-validated results for the Greek lagoons correctly classified 82.0% of specimens overall: with 70.0% and 85.0% for Monolimni and Agiasma lagoons, respectively (Wilks' lambda = 0.565, $p < 0.001$). *Mugil curema* from the Gulf of Mexico was correctly classified 74.4% overall: 83.3% for Tamiahua Lagoon and 53.8% for Tecolutla River (Wilks' lambda = 0.463, $p < 0.001$).

Lower cross-validated correct classification rates were obtained for *C. labrosus* with respect to the three Greek coastal

Table 6. Classification results for the cross-validation testing procedure for pair local populations of *Mugil* (*M. cephalus* (*Mce*), *M. curema* (*Mcu*)).

Species	Area	Predicted group membership						Total
		<i>Mce</i>				<i>Mcu</i>		
		GMMa	GMTa	ASAg	ASMo	GMTa	GMTe	
Count								
<i>Mce</i>	GMMa	24	9					33
	GMTa	7	22					29
	ASAg			7	3			10
	ASMo			6	34			40
<i>Mcu</i>	GMTa					25	5	30
	GMTe					6	7	13
Percent								
<i>Mce</i>	GMMa	72.7	27.3					100.0*
	GMTa	24.1	75.9					100.0*
	ASAg			70.0	30.0			100.0 [†]
	ASMo			15.0	85.0			100.0 [†]
<i>Mcu</i>	GMTa					83.3	16.7	100.0 [‡]
	GMTe					46.2	53.8	100.0 [‡]

Note: GMMa, Gulf of Mexico, Madre Lagoon; GMTa, Gulf of Mexico, Tamiahua Lagoon; ASAg, Aegean Sea, Agiasma Lagoon; ASMo, Aegean Sea, Monolimni Lagoon; GMTe, Gulf of Mexico, Tecolutla River.

*Of cross-validated grouped cases, 74.2% were correctly classified

[†]Of cross-validated grouped cases, 82.0% were correctly classified.

[‡]Of cross-validated grouped cases, 74.4% were correctly classified.

lagoons. The first canonical discriminant function explained 69.3% of the total variance, whereas the second function accounted for 30.7%, but overall discrimination was significant (Wilks' lambda = 0.434, $p < 0.001$). The discriminant analysis correctly classified 70.2% of the original grouped cases, whereas the cross-validated procedure correctly classified 48.9% (Table 7). *Chelon labrosus* from Eratino Lagoon gave the best classification rate, 55.6%, whereas 40% of specimens from Vassova and Agiasma lagoons were correctly classified.

Discussion

The analysis provides sufficient evidence to reject the null hypothesis; scale morphology is appropriate to allow discrimination by genera, species, geographic variants, and local populations.

Although the discrimination between genera and species in all cases was over 60%, there are similarities between the clusters comprising *C. labrosus* and *L. saliens*. Despite the limited sample of *L. saliens*, results were consistent with the original and cross-validated classifications in the genus and species discrimination analyses. The close resemblance of these two species has been reported based on chromosome complements and mitochondrial DNA sequence analysis (Cataudella et al. 1974; Papisotiropoulos et al. 2001, 2002). Turan et al. (2005) also suggested that the separation of *Chelon* and *Liza* into separate genera might be inappropriate, based on allozyme electrophoresis. Similarly, Guinea and Fernandez (1992) showed broad similarities between the gill rake morphology of three species of *Liza* and *C. labrosus*. However, despite *M. curema* and *C. labrosus* sharing similarities in the shape of the scales, it is possible to discriminate between them in a large proportion of cases, even with seven landmarks. This might be further improved by increas-

Table 7. Classification results^a for the cross-validation testing procedure for local populations of *Chelon labrosus* in Nestos River lagoons from the Aegean Sea.

Areas	Predicted group membership			Total
	ASVa	ASEr	ASAg	
Count				
ASVa	4	3	3	10
ASEr	7	15	5	27
ASAg	3	3	4	10
Percent				
ASVa	40.0	30.0	30.0	100.0
ASEr	25.9	55.6	18.5	100.0
ASAg	30.0	30.0	40.0	100.0

Note: ASVa, Aegean Sea, Vassova Lagoon; ASEr, Aegean Sea, Eratino Lagoon; ASAg, Aegean Sea, Agiasma Lagoon.

^aOf cross-validated grouped cases, 48.9% were correctly classified.

ing the number of landmarks. Thus, discrete differences between species are prevalent, supporting their separate species nomenclature.

The genus *Mugil* was even better segregated, with *M. cephalus* well separated from other species. *Mugil curema* more closely resembled *C. labrosus* and *L. saliens*. These results agree with genetic studies of the Mugilidae that indicate remarkable genetic divergence in *M. cephalus* and *M. curema* compared with other members of the family (Caldara et al. 1996).

The correct classification rates based on fish scale shape differences between populations of *M. cephalus* agree with genetic analyses, which indicates extensive genetic diversity

between populations from around the world (Crosetti et al. 1994; Rossi et al. 1998; Rocha-Olivares et al. 2000). Rocha-Olivares et al. (2000) identified the existence of a single population within the northern Gulf of Mexico. In the present study, specimens from different lagoons in the Gulf were well separated, pointing to the prevalence of several stocks. Similarly, *M. cephalus* in Greek waters could be reasonably well identified, but *C. labrosus* stocks were poorly discriminated. Not all differences should be attributed solely to genetic differences; environmental influences might also influence fish morphology (West-Eberhard 1989; Shepherd 1991; Kinsey et al. 1994). Such environmental influences on morphology have led to the definition of phenotypic stocks characterized by phenotypic differences that may be exclusively environmentally induced (Swain and Foote 1999). Temporal differences in sampling might also be a source of variability. The 13- to 14-year time lag between the collection periods of the *Mugil* species from the Gulf of Mexico might have confounded geographic variation to some extent, but further, more-intensive sampling will address this issue. Allometry presents a further confounding source of shape variability that we were unable to take into account in the present study. Similar effects are seen in otoliths that undergo morphology changes with age and environmental conditions (Felix-Uraga et al. 2005; Monteiro et al. 2005). Nevertheless, the aim of this study was to evaluate a rapid assessment tool for stock discrimination, and it is expected that accounting for ontogenic variability will only improve the output.

The variable discrimination between regions and species might be explained by the life history of mullets (Ibañez and Gallardo-Cabello 2004; Ibañez and Gutiérrez-Benítez 2004). *Mugil cephalus* in the Gulf of Mexico waters spawn in depths of 40–1650 m and beyond the continental shelf 50–100 km off the coast (Arnold and Thompson 1958). They do not migrate extensively (Idyll and Sutton 1951; Funicelli et al. 1989) and return to their original coastal area after spawning (Mahmoudi 1991). The larvae remain in the coastal waters for 2 to 3 months (Ditty and Shaw 1996) where they are confined to surface currents that transport them to estuarine and coastal regions that could not be the same as those of their parents. Similar reproductive behaviour is shown by *M. cephalus*, *C. labrosus*, and *L. saliens* from the Mediterranean Sea (Koutrakis et al. 1994; Koutrakis 2004), where floating eggs and larvae of mullet are transported by sea currents (Rossi 1986). The distances between the mouths of the Madre and Tamiahua lagoons and between the Tamiahua Lagoon and the Tecolutla River are ~370 km and ~96 km, respectively; these distances seem sufficient to maintain stock integrity. The extent to which they can be identified by lagoon depends on the balance between larval migration and adult stasis. Similarly for the Aegean systems, Monolimni Lagoon belongs to the River Evros delta, which is some 100 km from Agiasma Lagoon, a part of the River Nestos delta.

By contrast, the samples of *C. labrosus* were taken from three coastal water bodies, the Vassova, Eratino, and Agiasma lagoons, which form part of the delta of the River Nestos; the mouths of the Vassova and Eratino are only 3.7 km apart, whereas the Eratino and Agiasma are separated by 5.3 km, and there is a possible narrow connection

between Vassova and Eratino (Sotiris Orfanidis, National Agricultural Research Foundation Fisheries Research Institute, Kavala, Greece, personal communication, 2006). These geographical conditions go a long way to explaining the poor discrimination rates as they are likely predisposed to considerable gene flow with similar environmental conditions.

Although landmarks varied between the groups of fish in the present study, the central axes formed by landmarks 2, 5, and 7 appear most significant in the analysis of all 207 scales. The distinctiveness of the *M. cephalus* fish scales with respect to all other species is mainly due to a more centrally placed focus. Scales of the genus *Mugil* are generally relatively longer and narrower than those of other taxa within the Mugilidae. Such scale shape differences may well reflect adaptations to different functions and swimming characteristics, but there is a paucity of information with relation to these species in the literature. The ornamentations of the scales have been used to discriminate between congeneric *Mugil* species (Ibañez and Gallardo-Cabello 2005); likewise, the roughness of scale surfaces has been studied from the viewpoint of hydrodynamics (Sudo et al. 2002). Thus, scale shape variations possibly arise from adaptations to varying hydrodynamic conditions, and further analyses such as the present one combined with biomechanical analyses offer promise in approaching the study of form–function relationships.

Although the scales used in this study were sampled from the same anatomical region of the fish (the shoulder region), some variation exists within this region in each fish. Further, there is considerable variation over the whole body (A. Ibañez, unpublished data). The localised variations doubtless add to error by artificially inflating intragroup variability. This could be reduced by being more specific with regard to line and row of scales in future studies. The variations over the whole body might, in turn, be applied to improve fish identification rates because these larger scale variations might also be informative.

Finally, the analyses presented involved shape rather than size and shape. The centroid size of each scale was not included in the discriminant analyses. This should be factored into the analysis to test whether it improves or worsens discrimination. Considering the efficacy of discriminating stocks using scale shape as assessed by landmarks, it is somewhat surprising that the methodology has not been developed more strongly in fisheries science. This approach seems potentially useful in discriminating between closely related species that are otherwise difficult to distinguish, between fish of hybrid origin, and between conspecifics in polyploid populations (e.g., Martins et al. 1998; Ribeiro et al. 2003). The method is nondestructive, thus allowing rare and endangered species to be returned to the water. It is also quick and less costly than genetic analysis, thus allowing many individuals from a population or community to be screened quickly.

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