

Identification of Lessepsian fish species using the sagittal otolith

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SUMMARY: Lessepsian species are marine organisms that enter the Mediterranean through the Suez Canal, a phenomenon that has dramatically increased in recent decades. The present study describes the morphological characteristics of the sagittal otoliths of 22 Lessepsian fish species collected at four locations in the Mediterranean Sea. These structures are commonly used in the identification of species found in the digestive tracts of predators, and their morphological description is mainly needed as a tool for trophic studies. Here we used the Automated Taxon Identification (ATI) system of the AFORO web database to determine the accuracy of classifying Lessepsian fish otoliths compared with otoliths of native Mediterranean species. The otolith contour analysis correctly classified 92.5% of the specimens, showing that these species have otoliths that can be clearly distinguished from native ones. Four different groups of otoliths were identified according to the morphological differences between the otoliths and the ATI analysis results: a) unusual shapes with no similarities to native species; b) unusual shapes with similarities to phylogenetically distant native species; c) common shapes with similarities to phylogenetically close native species; and d) common shapes with morphological characters related to other native congeners, although they are not classified with them.

Keywords: otolith, morphology, fish, Lessepsian species, Mediterranean Sea.

RESUMEN: IDENTIFICACIÓN DE ESPECIES DE PECES DE ORIGEN LESSEPSIANO MEDIANTE EL OTOLITO SAGITA. – Las especies lessepsianas son aquellos organismos marinos que invaden el Mediterráneo a través del Canal de Suez, fenómeno que se ha incrementado drásticamente en los últimos años. El presente trabajo describe las características morfológicas del otolito sagita de 22 especies de peces recolectadas en cuatro localidades del Mediterráneo. Estas estructuras son empleadas normalmente en la identificación de las especies presentes en los tubos digestivos de sus depredadores, y su descripción morfológica es necesaria en la mayoría de estudios tróficos. En este estudio, se empleó el sistema automatizado de reconocimiento específico (ATI) de la web AFORO con el objeto de determinar la precisión en la clasificación de estas especies respecto a otras especies nativas que cohabitan en el Mediterráneo. El porcentaje correcto de clasificación alcanzó el 92.5% de los casos, mostrando que estas especies tienen otolitos claramente diferenciables. Cuatro grupos de otolitos se identificaron teniendo en consideración las diferencias morfológicas encontradas y los resultados del análisis de ATI: a) formas peculiares no análogas a otras especies nativas, b) formas peculiares con analogías con especies nativas filogenéticamente muy distintas, c) formas comunes con analogías con especies nativas filogenéticamente cercanas, y d) formas comunes con caracteres morfológicos similares a otras especies nativas, si bien no fueron clasificadas con ellas.

Palabras clave: otolito, morfología, peces, especies lessepsianas, mar Mediterráneo.

INTRODUCTION

The invasion of Red Sea organisms through the Suez Canal, known as ‘Lessepsian migration’ (Por 1978) is today a major driver of change in the Mediterranean Sea. Due to the growing importance of this

phenomenon, in terms of the number of introductions, successful establishments and geographical expansions (Golani 2010, Zenetos *et al.* 2010), much attention is now being focussed on Lessepsian species. According to the most recent estimate (Zenetos *et al.* 2010), around one thousand immigrant species have

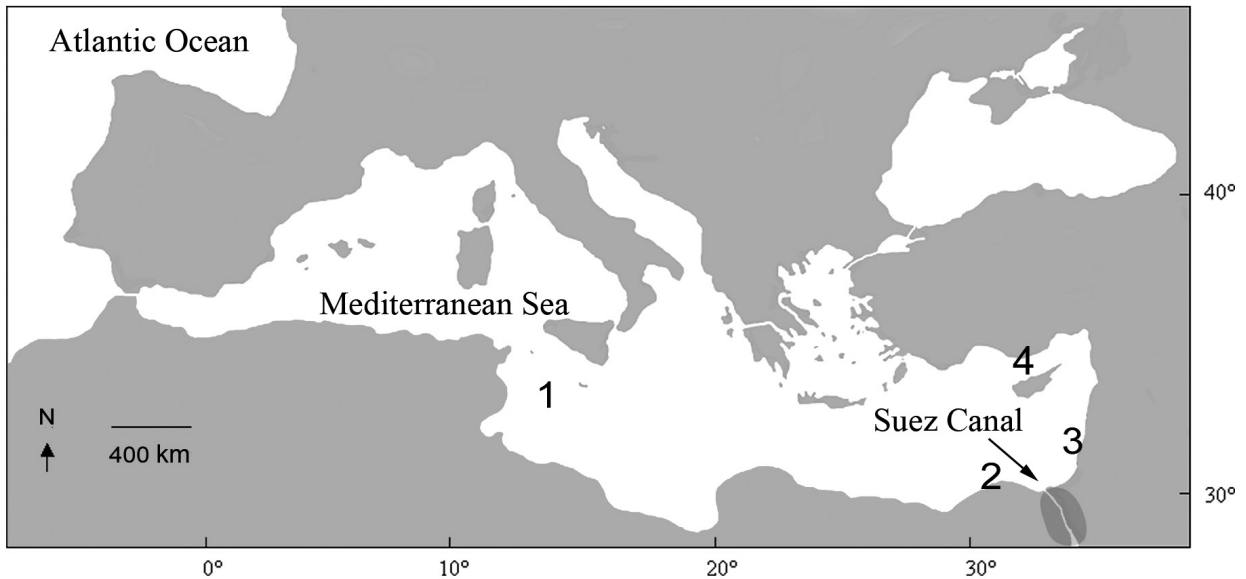


FIG. 1. – Sampling locations in the Mediterranean Sea: 1) Linosa Island, Italy; 2) Alexandria, Egypt; 3) Haifa, Israel; 4) Bozyayi, Turkey.

been recorded so far in the Mediterranean basin. Out of these, almost 60% come from the Suez Canal. The Mediterranean is therefore undergoing a tremendous increase in species richness due to the influx of Red Sea organisms. Hence, basic information about these new comers is urgently needed.

A total of 149 alien fish species have been recorded so far in the Mediterranean Sea and most of them are of Lessepsian origin (Zenetos *et al.* 2010). They are generally thermophilic species (Azzurro 2008), and it is very probable that the rising seawater temperature is giving them a distinct advantage over native temperate taxa (Galil 2007). The little information available on the biology and ecology of Lessepsian fish is mostly limited to certain abundant and commercially important families, such as Upeneidae and Siganidae (reviewed by Golani 2010). Some work has been dedicated to the trophic relationships of these species in their new environment (e.g. Golani 1993, 1994, Bariche 2006, Azzurro *et al.* 2007, Zouari-Ktari *et al.* 2008). Lessepsian fish can also be important prey (Aronov and Goren 2008) but research into their roles in the trophic network is extremely limited.

A fundamental pre-requisite for trophic studies is to be able to identify fish in the digestive tract of other fish based on the morphology of the sagittal otolith (e.g. Pierce and Boyle 1991, Granadeiro and Silva 2000, Moreno-López *et al.* 2002, García-Rodríguez and De La Cruz-Aguero 2011). The otolith shape has a high degree of inter-specific variation (Nolf 1985), and in most species there is low intra-specific variability. Otolith collections and atlases (Sanz-Echeverría 1926, Nolf 1985, Härkönen 1986, Smale *et al.* 1995, Rivaton and Bourret 1999, Volpedo and Echeverría 2000, Assis 2003, 2004, Campana 2004, Reichenbacher *et al.* 2007, Tuset *et al.* 2008) can be used

to identify fish, but more recently the web AFORO (<http://www.cmima.csic.es/aforo/>) allows automated taxon identification (ATI) based on a database of 950 species and 3300 images of sagittal otoliths. ATI is based on iterative classification using the Euclidean distances of the compared wavelet multiscale descriptors of otolith outlines (Parisi-Baradad *et al.* 2010). Therefore, each otolith is tested against all the other otoliths in the database. However, the description of the otolith is still relevant because morphological characteristics other than the outline can also help to identify the species, such as the type of sulcus, ostium and cauda (Tuset *et al.* 2008). Therefore, the main goals of this study were (i) to provide a morphological description of the otoliths of Lessepsian fish, (ii) to compare the otolith morphology of Lessepsian and native species, and (iii) to use the otolith contour to determine the accuracy of the AFORO web as a tool for species identification.

MATERIALS AND METHODS

Sampling and data collection

A total of 22 Lessepsian species (18 families and 8 orders) inclusive of the most successful taxa were collected at four different locations of the central and eastern Mediterranean (Table 1; Fig. 1): 1) Linosa island, Italy (35°51'N 12°51'E); 2) Alexandria, Egypt (31°19'N 30°03'E); 3) Haifa, Israel (32°47'N 34°56'E); and 4) Bozyayi, Turkey (36°5'N 32°59'E). Fish were obtained with the voluntary collaboration of local fishermen, who mainly use trammel nets and trawling. The classification system used was based on Nelson (2006) and the species were named following the criteria of the online fish catalogue of the California Academy of Science (Eschmeyer and Fricke 2011).

TABLE 1. – Taxonomical identification, area and total length range of Lessepsian species studied in the Mediterranean Sea.

Order	Family	Species	Number of specimens	Length range (cm)
Clupeiformes	Clupeidae	<i>Etrumeus teres</i> (DeKay, 1842)	4	12.0-12.5
Siluriformes	Plotosidae	<i>Plotosus lineatus</i> (Thunberg, 1787)	2	7.7-17.0
Aulopiformes	Synodontidae	<i>Saurida undosquamis</i> (Richardson, 1848)	3	15.0
Atheriniformes	Atherinidae	<i>Atherinomorus forskalii</i> (Rüppell, 1838)	1	-
Beryciformes	Holocentridae	<i>Sargocentron rubrum</i> (Forsskål, 1775)	1	19.8
Gasterosteiformes	Fistulariidae	<i>Fistularia commersonii</i> Ruppell, 1838	3	10.3-49.0
Perciformes	Apogonidae	<i>Apogon smithi</i> (Kotthaus, 1970)	3	10.0
	Sillaginidae	<i>Sillago sihama</i> (Forsskål, 1775)	1	-
	Leiognathidae	<i>Equulites kluzingeri</i> (Steindachner, 1898)	4	5.3-8.6
	Haemulidae	<i>Pomadasys stridens</i> (Forsskål, 1775)	3	12.0-12.1
	Teraponidae	<i>Terapon puta</i> Cuvier, 1829	2	11.4-11.7
	Mullidae	<i>Upeneus moluccensis</i> (Bleeker, 1855)	1	17.8
		<i>Upeneus pori</i> Ben-Tuvia and Golani, 1989	4	10.5-11.5
	Pempheridae	<i>Pempheris vanicolensis</i> Cuvier 1831	2	15.0-16.0
	Labridae	<i>Pteragogus pelycus</i> Randall, 1981	1	10.1
	Siganidae	<i>Siganus luridus</i> (Rüppell, 1829)	1	7.6
		<i>Siganus rivulatus</i> (Forsskål, 1775)	5	-
	Sphyrnidae	<i>Sphyrna chrysotaenia</i> Klunzinger, 1884	1	-
	Scombridae	<i>Scomberomorus commerson</i> (Lacepède, 1800)	1	43.0
Tetraodontiformes	Tetraodontidae	<i>Stephanolepis diaspros</i> Fraser-Brunner, 1940	2	13.0-17.5
		<i>Lagocephalus sceleratus</i> (Gmelin, 1789)	2	42.0
		<i>Lagocephalus suezensis</i> Clark & Goher, 1953	5	-

Otolith morphology

Images of the left otolith were taken with a digital camera under a binocular microscope. The image was taken of the internal side (medial or proximal) of the otolith because this side has the sulcus acusticus (a groove along the surface of the sagitta). To obtain a clear sagittal contour, the image must be well contrasted with a homogeneous black background. The otoliths were always represented with the respective dorsal margin at the top of the image and the anterior (rostral) region to the right. The terms outlined in Tuset *et al.* (2008) were used for the morphological description. The following anatomical features were considered: otolith shape, type of sulcus acusticus, shape and size of ostium and cauda, and the characteristics of the anterior and posterior regions (Fig. 2).

Automated identification

Automated taxon identification (ATI) uses wavelet descriptors to extract specific characters of the orthogonal projection of a standard oriented left sagitta according to the methodology given by Parisi-Baradad *et al.* (2005) and Parisi-Baradad *et al.* (2010). A multi-scale signal representation is obtained from the wavelets. Outline singularities can be located more accurately with wavelets than with Fourier analyses (Mallat 1989). The recognition system searches the AFORO database iteratively, from the coarsest to the finest wavelet scale, to find the otoliths that most resemble the tested otolith. In each iteration, the approximation signal of the tested otolith is compared with each otolith of the database using a Euclidean distance (ED) (Parisi-Baradad *et al.* 2005, 2010). The otoliths were ordered according to their decreasing similarity to the specimen test, measured as ED.

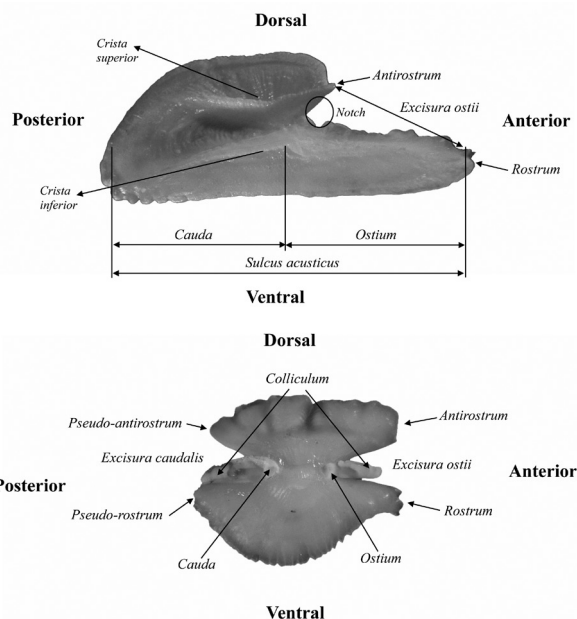


FIG. 2. – Anatomical features of otoliths used for the morphological descriptions (from Tuset *et al.* 2008).

RESULTS

Anatomical descriptions

Etrumeus teres. *Shape*: elliptic, ventral margin dentated. *Sulcus acusticus*: heterosulcoid, ostial, median. *Ostium*: funnel-like, longer than the cauda. *Cauda*: tubular, straight, ending far from the posterior margin. *Anterior region*: peaked; rostrum broad, long, pointed; antirostrum short, broad, rounded or absent; excisura wide with an acute notch or absent. *Posterior region*: round to angled with suprmedian apex in the larger otoliths (Fig. 3A).

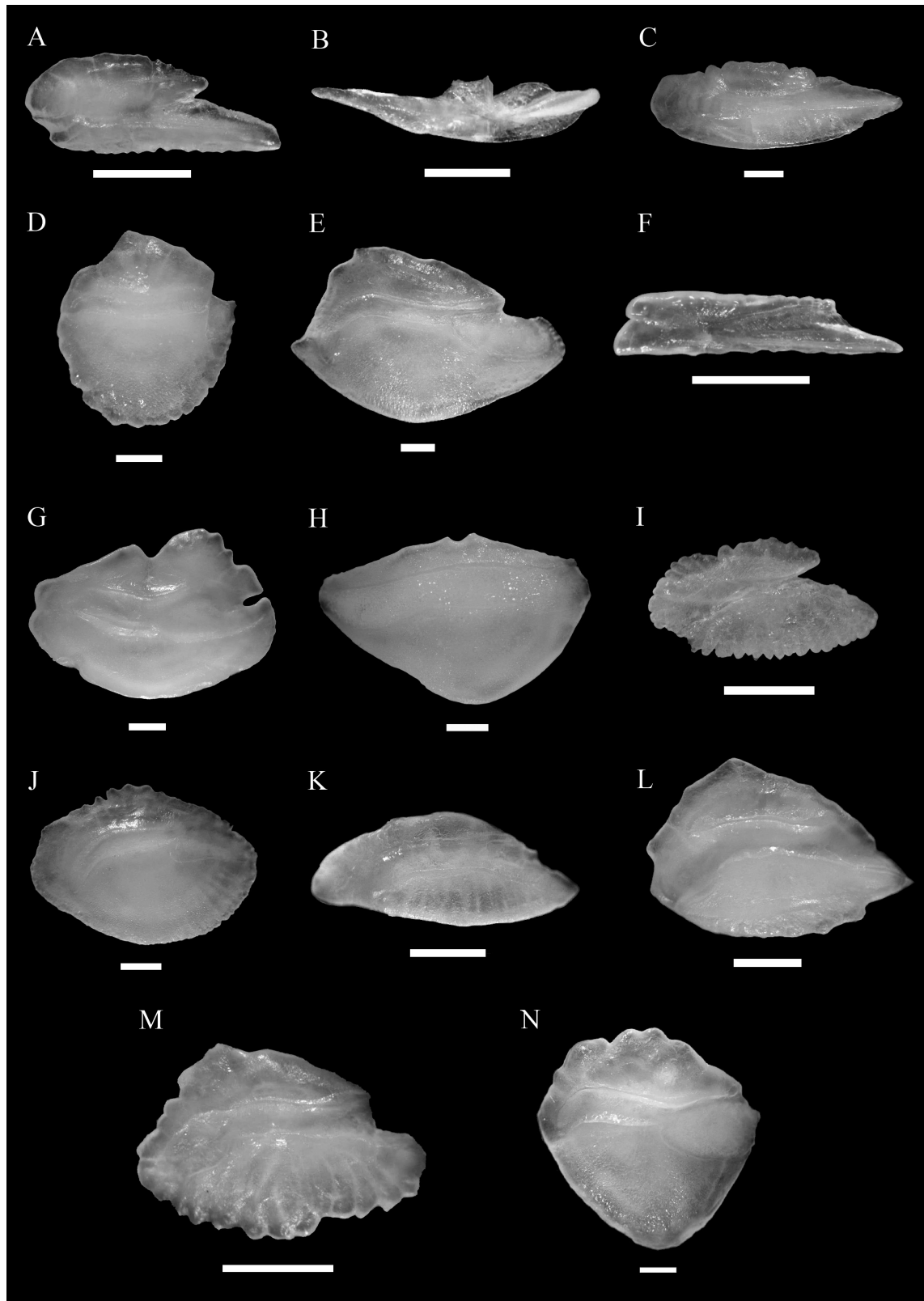


FIG. 3. – Sagittal otoliths of Lessepsian species from the Mediterranean Sea. A, *Etrumeus teres* (12.5 cm total length [TL]); B, *Plotosus lineatus* (17.0 cm TL); C, *Saurida undosquamis* (12.0 cm TL); D, *Atherinomorus forskalii* (no fish length); E, *Sargocentron rubrum* (19.8 cm TL); F, *Fistularia commersonii* (49.0 cm TL); G, *Apogon smithi* (10.0 cm TL); H, *Sillago sihama* (14.6 cm TL); I, *Equulites klunzingeri* (8.6 cm TL); J, *Pomadasystris stridens* (12.1 cm TL); K, *Terapon puta* (11.7 cm TL); L, *Upeneus moluccensis* (no fish length); M, *Upeneus pori* (13 cm TL); N, *Pempheris vanicolensis* (16 cm TL). Scale bars = 1 mm.

Plotosus lineatus. *Shape*: sagitiform, slightly concave. *Sulcus acusticus*: heterosulcoid, ostial, median, ventral ridge well developed. *Ostium*: funnel-like, shorter than the cauda. *Cauda*: tubular, straight, ending close to the posterior margin. *Anterior region*: peaked; rostrum very short, broad, colliculum lanceolate, pointed, extensively protruding; antirostrum absent. *Posterior region*: lanceolated (Fig. 3B).

Saurida undosquamis. *Shape*: slightly lanceolated, with dorsal margin irregular. *Sulcus acusticus*: heterosulcoid, ostial, median. *Ostium*: funnel-like, longer than the cauda. *Cauda*: tubular, straight, ending far from the posterior margin. *Anterior region*: peaked; rostrum narrow, long, pointed; antirostrum absent; excisura wide without a notch. *Posterior region*: round (Fig. 3C).

Atherinomorus forskalii. *Shape*: discoidal, dorsal-ventral axis more developed. *Sulcus acusticus*: heterosulcoid, ostial, suprmedian. *Ostium*: lateral, markedly asymmetric, noticeably shorter than the cauda. *Cauda*: tubular, curved, markedly flexed posteriorly, ending close to the posterior margin. *Anterior region*: double-peaked; rostrum short, broad, flattened-irregular; antirostrum short, broad, round; excisura wide with a deep notch. *Posterior region*: round (Fig. 3D).

Sargocentron rubrum. *Shape*: elliptic. *Sulcus acusticus*: heterosulcoid, ostial, suprmedian. *Ostium*: discoidal, shorter than the cauda. *Cauda*: tubular, curved, markedly flexed from the middle region, ending close to the posterior-ventral margin. *Anterior region*: peaked; rostrum short, broad, round; antirostrum short, small, peaked; excisura wide with a shallow notch. *Posterior region*: angled-pointed (Fig. 3E).

Fistularia commersonii. *Shape*: trapezoidal-elongated, the ventral margin longer. *Sulcus acusticus*: heterosulcoid, ostial, median. *Ostium*: funnel-like, longer than the cauda, separated from the cauda by a conspicuous collum. *Cauda*: elliptic, ending close to the posterior margin. *Anterior region*: lanceolated; rostrum long, narrow, pointed; antirostrum absent; excisura very wide without a notch. *Posterior region*: oblique (Fig. 3F).

Apogon smithi. *Shape*: elliptic, dorsal margin irregular. *Sulcus acusticus*: heterosulcoid, ostial, slightly concave, median. *Ostium*: discoidal, longer than the cauda. *Cauda*: tubular, straight, ending far from the posterior-dorsal margin. *Anterior region*: round; rostrum short, broad, round; antirostrum short, broad, peaked; excisura narrow with a deep notch. *Posterior region*: round to blunt (Fig. 3G).

Sillago sihama. *Shape*: rhomboidal. *Sulcus acusticus*: archaesulcoid, ostial, suprmedian. *Ostium* and *cauda*: undifferentiated, cauda ending close to the

posterior margin. *Anterior region*: round; rostrum and antirostrum very small, short, broad and round or absent; excisura wide without notch. *Posterior region*: peaked (Fig. 3H).

Equulites klunzingeri. *Shape*: pentagonal to elliptic, anterior-ventral margin crenate to irregular. *Sulcus acusticus*: heterosulcoid, ostial, median. *Ostium*: funnel-like, longer than the cauda. *Cauda*: tubular, slightly curved, ending far from the posterior margin. *Anterior region*: angled to peaked; rostrum short to long, broad, pointed; antirostrum short, broad, pointed; excisura wide with an acute and shallow notch. *Posterior region*: angled to oblique (Fig. 3I).

Pomadasys stridens. *Shape*: oval, ventral area very developed. *Sulcus acusticus*: heterosulcoid, ostial, suprmedian. *Ostium*: rectangular, shorter than the cauda. *Cauda*: tubular, curved, strongly flexed posteriorly, ending in the posterior-ventral region. *Anterior region*: round to oblique; rostrum short, broad, oblique; antirostrum absent; excisura very wide, without a notch. *Posterior region*: round to oblique (Fig. 3J).

Terapon puta. *Shape*: oblong. *Sulcus acusticus*: heterosulcoid, ostial, median. *Ostium*: funnel-like, shorter than the cauda. *Cauda*: tubular, curved, strongly flexed ending far from the posterior margin. *Anterior region*: peaked; rostrum short, broad, pointed; antirostrum absent or short, broad, round; excisura wide, without or with a shallow notch. *Posterior region*: oblique (Fig. 3K).

Upeneus moluccensis. *Shape*: elliptic, dorsal margin angled. *Sulcus acusticus*: heterosulcoid, ostial, median. *Ostium*: funnel-like, shorter than the cauda. *Cauda*: tubular, curved, strongly flexed from the middle region, ending close to the posterior margin. *Anterior region*: peaked; rostrum short, broad, pointed; antirostrum short, broad, round; excisura wide, without notch. *Posterior region*: blunt (Fig. 3L).

Upeneus pori. *Shape*: elliptic, ventral margin crenate. *Sulcus acusticus*: heterosulcoid, ostial, median. *Ostium*: funnel-like, shorter than the cauda. *Cauda*: tubular, curved, strongly flexed from the middle region, ending close to the posterior margin. *Anterior region*: peaked; rostrum short, broad, round; antirostrum short, broad, pointed; excisura wide, with a shallow notch. *Posterior region*: oblique (Fig. 3M).

Pempheris vanicolensis. *Shape*: pentagonal, ventral area more developed, margin dorsal irregular. *Sulcus acusticus*: heterosulcoid, ostial, suprmedian. *Ostium*: bent-concave, similar in size to the cauda. *Cauda*: tubular, curved, markedly flexed, ending close to the posterior margin. *Anterior region*: round; rostrum short, very broad, flattened; antirostrum very short, broad, slightly peaked; excisura very wide without a notch. *Posterior region*: angled (Fig. 3N).

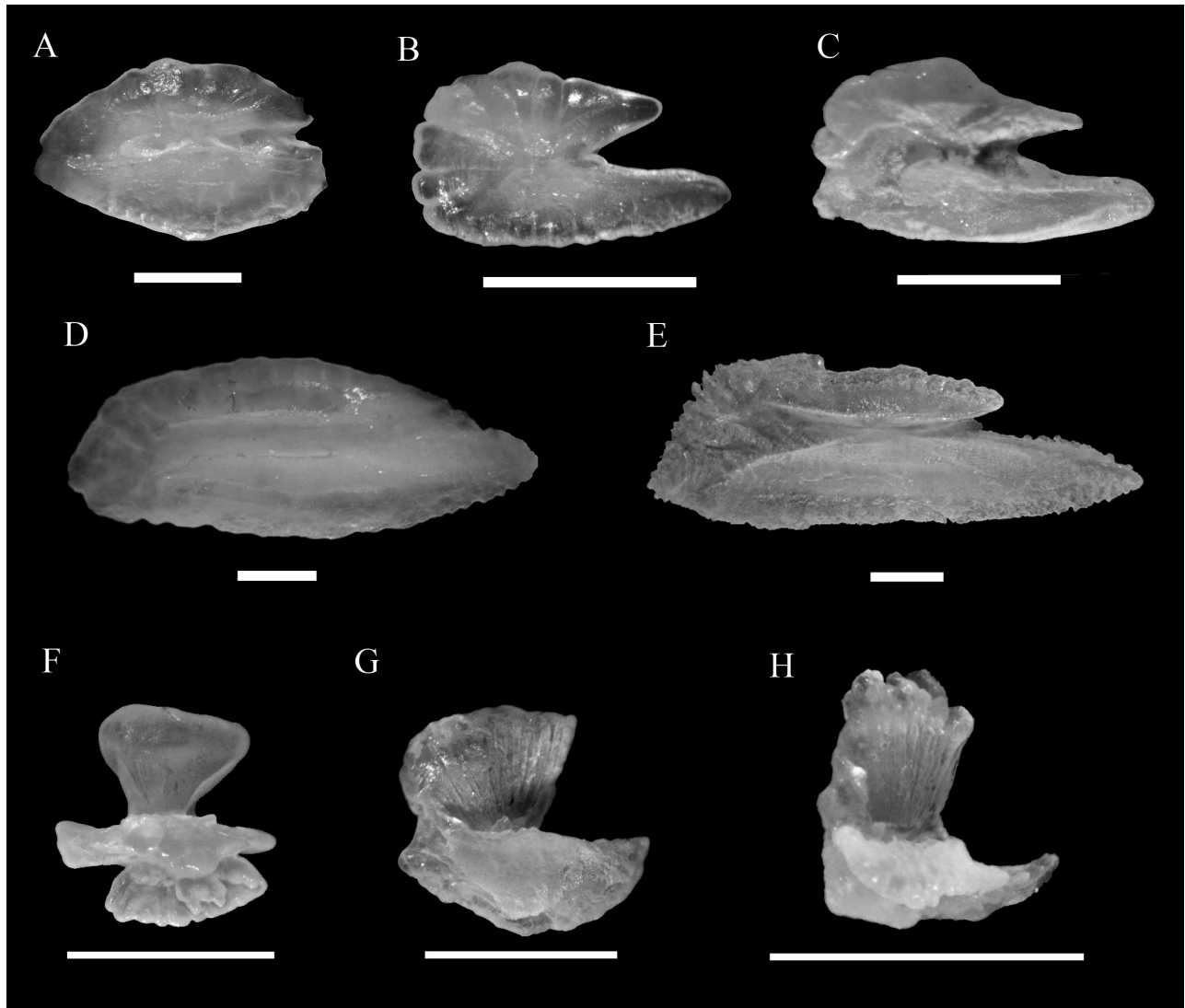


FIG. 4. – Sagittal otoliths of Lessepsian species from the Mediterranean Sea. A, *Pteragogus pelycus* (10.1 cm TL); B, *Siganus luridus* (7.6 cm TL); C, *Siganus rivulatus* (no fish length); D, *Sphyaena chrysotaenia* (no fish length); E, *Scomberomorus commerson* (43.0 cm TL); F, *Stephanolepis diaspros* (17.5 cm TL); G, *Lagocephalus sceleratus* (42.0 cm TL); H, *Lagocephalus suezensis* (no fish length). Scale bars: 1 mm.

***Pteragogus pelycus*.** *Shape:* oval-rhomboidal, margins entire. *Sulcus acusticus:* heterosulcoid, ostial, median. *Ostium:* funnel-like, shorter than the cauda. *Cauda:* elliptic, straight, ending close to the posterior margin. *Anterior region:* peaked; rostrum short, broad, blunt; antirostrum short, narrow, pointed; excisura narrow, with a shallow notch. *Posterior region:* oblique (Fig. 4A).

***Siganus luridus*.** *Shape:* elliptic, posterior margin lobed. *Sulcus acusticus:* heterosulcoid, ostial, median. *Ostium:* funnel-like, longer than the cauda. *Cauda:* elliptic, curved, ending close to the posterior margin. *Anterior region:* double-peaked; rostrum long, narrow, pointed; antirostrum long, narrow, pointed; excisura wide, with an acute and deep notch. *Posterior region:* round (Fig. 4B).

***Siganus rivulatus*.** *Shape:* elliptic, posterior margin irregular. *Sulcus acusticus:* heterosulcoid, ostial, median. *Ostium:* funnel-like, longer than the cauda. *Cauda:* elliptic, curved, ending close to the posterior margin. *Anterior region:* double-peaked; rostrum long, narrow, pointed; antirostrum long, very narrow, lanceolated; excisura wide, with an acute and deep notch. *Posterior region:* round-irregular (Fig. 4C).

***Sphyaena chrysotaenia*.** *Shape:* fusiform. *Sulcus acusticus:* heterosulcoid, ostial, median, ventral margin sinuated. *Ostium:* funnel-like, shorter than the cauda. *Cauda:* tubular, curved, strongly flexed posteriorly, ending close to the ventral margin. *Anterior region:* peaked; rostrum short, broad, pointed; antirostrum poorly defined; excisura wide without a notch. *Posterior region:* oblique (Fig. 4D).

***Scomberomorus commerson*.** *Shape:* lanceolated, serrate margins, especially the margin. *Sulcus acusticus:* heterosulcoid, ostial, median. *Ostium:* funnel-like, longer than the cauda. *Cauda:* elliptic, ending close to the posterior margin. *Anterior region:* peaked; rostrum long, narrow, pointed; antirostrum long, broad, pointed; excisura wide, with an acute notch. *Posterior region:* oblique (Fig. 4E).

***Stephanolepis diaspros*.** *Shape:* hour-glass, asymmetric. *Sulcus acusticus:* homosulcoid, ostio-caudal, median, with a cristal ventral well developed, high, round, protruding in both excisurae. *Ostium:* funnel-like, as long as the cauda. *Cauda:* straight. *Anterior region:* notched; rostrum and antirostrum very short, very broad, flattened; 1 ventral. *Posterior region:* notched, excisura cauda very wide with a shallow notch hidden by the crista (Fig. 4F).

***Lagocephalus sceleratus*.** *Shape:* hour-glass, asymmetric, ventral area more developed, fan-shaped. *Sulcus acusticus:* homosulcoid, ostio-caudal, median. *Ostium:* funnel-like, as long as the cauda. *Cauda:* straight. *Anterior region:* notched; rostrum long, angled, with triangular shape; antirostrum absent; excisura very

wide without notch. *Posterior region:* flattened to irregular (Fig. 4G).

***Lagocephalus suezensis*.** *Shape:* hour-glass, asymmetric, ventral area more developed, fan shaped. *Sulcus acusticus:* homosulcoid, ostio-caudal, median. *Ostium:* funnel-like, as long as the cauda. *Cauda:* straight. *Anterior region:* notched; rostrum long, angled, with triangular shape; antirostrum absent; excisura very wide without notch. *Posterior region:* flattened to irregular (Fig. 4H).

Image analysis

The otolith contour analysis correctly classified 92.5% of the specimens. Only the otolith of *Pomadasys stridens* was confused with *Pomadasys incisus*, and the two specimens of *Stephanolepis diaspros* were incorrectly assigned to *Sphoeroides marmoratus* and *Lagocephalus lagocephalus* (Fig. 5). A few species showed morphological analogies with other species, reflecting their taxonomic proximity. This was the case of *Upeneus pori* and *Mullus surmuletus* (Mullidae), *Pomadasys stridens* and *Pomadasys incisus* (Haemulidae), *Etrumeus teres* and *Sardina pilchardus* (Cupleidae),

Order of classification

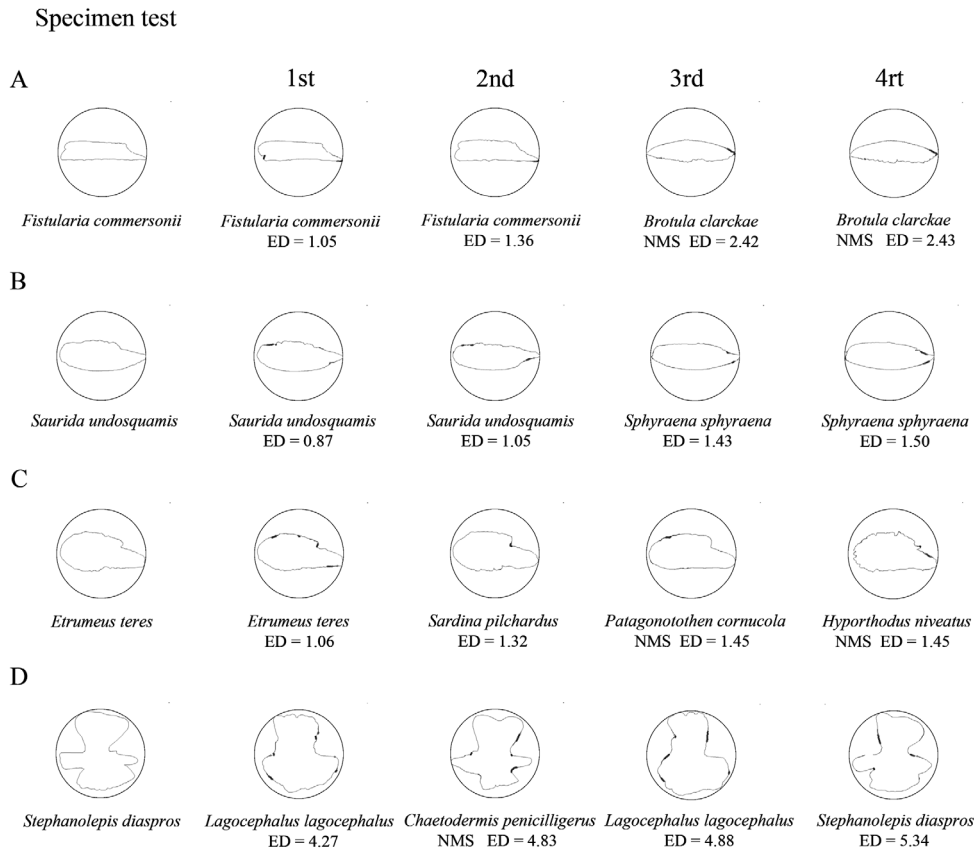


FIG. 5. – Classification example of four Lessepsian species using the automated taxon identification system (ATI) from the AFORO web. A, *Fistularia commersonii*; B, *Saurida undosquamis*; C, *Etrumeus teres*; D, *Stephanolepis diaspros*. ED, Euclidean distance; NMS, non-Mediterranean species.

TABLE 2. – Comparison of the otolith contour of Lessepsian species with other species from the Mediterranean Sea using the automatic system analysis of AFORO web. ED, Euclidean Distance.

Lessepsian specimens tested	Correctly identified	ED	Nearest species	ED
<i>Etrumeus teres</i>	Yes	1.49	<i>Scomber scombrus</i>	1.64
<i>Etrumeus teres</i>	Yes	1.06	<i>Caranx rhonchus</i>	1.45
<i>Etrumeus teres</i>	Yes	1.06	<i>Sardina pilchardus</i>	1.32
<i>Etrumeus teres</i>	Yes	1.27	<i>Trachinotus ovatus</i>	1.56
<i>Plotosus lineatus</i>	Yes	1.88	no similar specimens	-
<i>Plotosus lineatus</i>	Yes	1.88	not similar specimens	-
<i>Saurida undosquamis</i>	Yes	1.05	<i>Sphyraena sphyraena</i>	1.48
<i>Saurida undosquamis</i>	Yes	1.05	<i>Sphyraena sphyraena</i>	1.43
<i>Saurida undosquamis</i>	Yes	0.87	<i>Sphyraena sphyraena</i>	1.27
<i>Atherinomorus forskalii</i>	only 1 specimen	-	no similar specimens	-
<i>Sargocentron rubrum</i>	only 1 specimen	-	<i>Pagellus bogaraveo</i>	1.31
<i>Fistularia commersonii</i>	Yes	1.24	no similar specimens	-
<i>Fistularia commersonii</i>	Yes	1.05	no similar specimens	-
<i>Fistularia commersonii</i>	Yes	1.05	no similar specimens	-
<i>Apogon smithi</i>	Yes	1.12	<i>Coelorinchus caelorhincus</i>	1.59
<i>Apogon smithi</i>	Yes	1.12	<i>Coelorinchus caelorhincus</i>	1.21
<i>Apogon smithi</i>	Yes	1.4	<i>Coelorinchus caelorhincus</i>	1.87
<i>Sillago sihama</i>	Yes	1.03	<i>Gnathophis mystax</i>	1.67
<i>Sillago sihama</i>	Yes	0.99	<i>Gnathophis mystax</i>	1.42
<i>Equulites klunzingeri</i>	Yes	1.3	<i>Mullus barbatus</i>	1.57
<i>Equulites klunzingeri</i>	Yes	1.3	<i>Atherina hepsetus</i>	1.41
<i>Equulites klunzingeri</i>	Yes	1.54	no similar specimens	-
<i>Pomadasys stridens</i>	Yes	0.77	<i>Spondyliosoma cantharus</i>	0.9
<i>Pomadasys stridens</i>	No	1.25	<i>Pomadasys incisus</i>	1.16
<i>Pomadasys stridens</i>	Yes	0.77	<i>Spicara maena</i>	1.01
<i>Terapon puta</i>	Yes	0.95	<i>Scorpaena porcus</i>	1.11
<i>Terapon puta</i>	Yes	1.11	<i>Scorpaena elongata</i>	1.12
<i>Upeneus moluccensis</i>	only 1 specimen	-	no similar specimens	-
<i>Upeneus pori</i>	Yes	1.12	<i>Mullus surmuletus</i>	1.5
<i>Upeneus pori</i>	Yes	1.12	<i>Mullus surmuletus</i>	1.56
<i>Upeneus pori</i>	Yes	1.31	<i>Mullus surmuletus</i>	1.56
<i>Pempheris vanicolensis</i>	Yes	0.8	<i>Epigonus constanciae</i>	1.21
<i>Pempheris vanicolensis</i>	Yes	0.8	<i>Epigonus constanciae</i>	1.34
<i>Pteragogus pelycus</i>	only 1 specimen	-	<i>Apogon imberbis</i>	1.25
<i>Siganus luridus</i>	only 1 specimen	-	no similar specimens	-
<i>Siganus rivulatus</i>	Yes	1.23	<i>Labrus mixtus</i>	1.87
<i>Siganus rivulatus</i>	Yes	2.72	<i>Synodus saurus</i>	3.12
<i>Siganus rivulatus</i>	Yes	1.6	<i>Lichia amia</i>	1.93
<i>Siganus rivulatus</i>	Yes	1.78	<i>Sardinella aurita</i>	1.89
<i>Siganus rivulatus</i>	Yes	1.23	<i>Sardina pilchardus</i>	1.99
<i>Sphyraena chrysotaenia</i>	only 1 specimen	-	<i>Serranus cabrilla</i>	0.97
<i>Scomberomorus commerson</i>	only 1 specimen	-	<i>Seriola dumerili</i>	1.46
<i>Stephanolepis diaspros</i>	No	5.34	<i>Sphoeroides marmoratus</i>	5.17
<i>Stephanolepis diaspros</i>	No	5.34	<i>Lagocephalus lagocephalus</i>	4.27
<i>Lagocephalus scleratus</i>	only 1 specimen	-	no similar specimens	-
<i>Lagocephalus suezensis</i>	Yes	3.28	<i>Lagocephalus scleratus</i>	7.42
<i>Lagocephalus suezensis</i>	Yes	3.28	no similar specimens	-
<i>Lagocephalus suezensis</i>	Yes	4.05	no similar specimens	-
Mean ED		1.62		1.83
Standard deviation ED		1.12		1.28
Percentage correctly identified	92.5%			

Stephanolepis diaspros and *Sphoeroides marmoratus* (Tetraodontidae), and *Lagocephalus suezensis* and *L. scleratus* (Tetraodontidae). Nevertheless, in most of the species the morphological affinity among otoliths did not have any taxonomic meaning, for example, otoliths of *Saurida undosquamis* (Synodontidae) where classified as *Sphyraena sphyraena* (Sphyraenidae) (Table 2).

DISCUSSION

The Lessepsian phenomenon, once limited to the eastern sectors of the Mediterranean (Por 1978), is today a matter of concern for the entire basin. In the last two

decades it has increased dramatically, as is evidenced by the increase in the number of invaders and their fast geographical expansion (Golani 2010, Zenetos *et al.* 2010). Species such as *Siganus luridus* and *Fistularia commersonii* have expanded rapidly in the central and western Mediterranean. The latter is now considered well established throughout the entire Mediterranean Sea, as it has been recorded in western areas (Sánchez-Tocino *et al.* 2007) on the opposite side to the Suez Canal where it originally entered. However, these species have the most impact in the eastern basin. Golani (2010) counted a total of 73 species, which is 14.9% of all the fish known for this sector. Therefore, considering the

number of new families that have entered the Mediterranean, it can be hypothesized that the morphological and taxonomic diversity of Mediterranean fish communities has increased. Our range of species includes the more abundant and widespread Lessepsian fish in the Mediterranean Sea, such as *Siganus luridus*, *S. rivulatus*, *Fistularia commersonii*, *Upeneus moluccensis*, *U. pori*, *Scomberomorus commerson*, and *Stephanolepis diaspros*. Some recent colonists, such as *Apogon smithi* (Golani *et al.* 2008) and *Plotosus lineatus* (Golani *et al.* 2002), were also considered together with occasional species such as *Pteragogus pelycus* Randal, 1981 (Golani and Sonin 1992).

Our morphological description of Lessepsian fish otoliths provides new and valuable information for these species. It is also a robust tool for species classification, and agrees in most cases with the ATI system of AFORO. The percentage of correct classifications (92.5%) obtained in this study is greater than that (72%) obtained by Parisi-Baradad *et al.* (2010) in a study that used the same methodology but with native species. This difference of more than 20% can be attributed to the high morphological specificity of Lessepsian sagittae in comparison with those of Mediterranean native species. In fact, many of these invaders belong to families or genera that are not represented in the native fish fauna of the Mediterranean Sea. Nevertheless, ecomorphological characters of otoliths are generally related to the ecological niche that the species occupies (Lombarte 1992, Arellano *et al.* 1995, Aguirre and Lombarte 1999, Lombarte and Popper 2004, Ramcharitar *et al.* 2004, Schulz-Mirbach *et al.* 2008, Colmenero *et al.* 2010, Tuset *et al.* 2010). Thus, the highly differentiated otolith morphology of Lessepsian species could reflect specific adaptations (Schoener 1974, Motta *et al.* 1995), and hence novel opportunities to thrive in the newly colonized habitat. This hypothesis recalls the controversial concept of ‘niche opportunity’ that has often been used to explain the success of these invaders (Por 1978, Lundberg and Golani 1995, Oliverio and Taviani 2003). This concept is not within the scope of the present research, and further ecological studies are necessary to evaluate it.

There is currently little information on the morphology of Lessepsian fish otoliths. Tuset *et al.* (2008) published an otolith atlas that included an exhaustive description of the sagittae of the most common species inhabiting the Mediterranean Sea, but no Lessepsian species were included. Lessepsian species can be classified into four different groups according to morphological differences between otoliths, ATI analysis and the otolith descriptions of species from the Mediterranean Sea (Tuset *et al.* 2008):

a) Unusual otolith shapes with no similarities to other native species. This is the case of *Plotosus lineatus*, which is a Siluriform and like other otophysian teleosts has a sagitta that is very different from that of non-otophysian fish (Popper and Platt 1981, Nolf 1985); *Fistularia commersonii*, which is a syngnathi-

form with a very unusual body shape and size compared with the Syngnathiformes native to the Mediterranean; *Atherinomorus forksalii* is an atheriniform but does not show any similarities to otoliths from Mediterranean Atherinidae.

b) Unusual otolith shapes with similarities to phylogenetically distant native species. Otoliths of *Saurida undosquamis* showed similarities to the Sphyraenidae, although this group is characterized by a well-differentiated ostium and cauda; *Apogon smithi* matched with *Coelorinchus caelorhincus* (Risso 1810) (Macrouridae) and with the Epigonidae, but the sulcus acusticus and shape were different; *Sillago sihama* was similar to *Gnathophis mystax* (Delaroche 1809) (Congridae) and *Phycis* spp. (Phycidae), although *G. mystax* has a heterosulcoid sulcus and *Phycis* spp. has a more lanceolate or spindle-shaped otolith; *Sargocentron rubrum* had a similar shape to some sparids, especially to *Pagellus bogaraveo* (Brünnich 1768), although the otolith was easily identified due to its thickness, as occurs in other Holocentridae; *Equulites klunzingeri* joined with *Atherina* spp. (Atheriniidae) and *Mullus barbatus* Linnaeus 1758 (Mullidae); *Atherina* spp. showed an excisura ostii with or without a shorter notch, while *Mullus* had a curved sulcus; *Terapon puta* was classified as *Scorpaena* spp., although the otolith was characterized by a more acute rostrum, a larger ostium area and a less curved cauda; the otoliths of *Pempheris vanicolensis* resembled those of *Epigonus constanciae* (Giglioli 1880) (Epigonidae), which have a curved cauda, a pointed rostrum and an entire dorsal margin without sculpture; the otoliths of *Pteragogus pelycus* were similar to those of *Apogon imberbis* (Linnaeus 1758) (Apogonidae) and to the otoliths of Labridae. Nevertheless, they can be easily identified due to the unusual cauda form and the V-shaped ventral margin; *Siganus rivulatus* was close to species with a double-peaked anterior region, e.g. *Sardinella aurita* Valenciennes 1847 (Clupeidae), *Labrus mixtus* Linnaeus 1758 (Labridae) and adult *Lichia amia* (Linnaeus 1758) (Carangidae). In Clupeidae and Labridae the end cauda is not curved, while *L. amia* has more irregular margins and a tubular shaped cauda. Finally, *Sphyraena chrysotaenia* otoliths have a similar shape to those of *Serranus cabrilla* (Linnaeus 1758) (Serranidae), which can have a shallow notch in the excisura ostii.

c) Common otolith shapes with similarities to phylogenetically close native species. The otolith morphology of *Etrumeus teres* is comparable to that of *Sardinella* spp. and *Sardina* spp., and also resembles otoliths of other pelagic species, such as *Scomber scombrus* Linnaeus 1758 (Scombridae), *Trachinotus ovatus* Linnaeus 1758 and *Caranx rhonchus* Geoffroy Saint-Hilaire 1817 (Carangidae). Nevertheless, the antirostrum was less developed, short and rounded, with a shallower notch in the excisura ostii in comparison with other clupeids. It also differed in the shape of the sulcus acusticus and the rostrum and postrostrum regions, which are more pointed in carangids and

scombrids. *Pomadasys stridens* was similar to *Pomadasys incisus* (Bowdich 1825) and to sparids such as *Spondyliosoma cantharus* (Linnaeus 1758). The most identifiable feature of *P. incisus* was an indentation in the anterior-dorsal margin, whilst *S. cantharus* shows thinner otoliths with a median sulcus acusticus and slightly curved cauda; *Upeneus pori* resembled *Mullus* spp., although there were no deep or strong depressions at the end of the cauda; *Scomberomorus commerson* had a shape characteristic of pelagic species, such as young *Seriola dumerili* (Risso 1810) (Carangidae), and Scombridae, such as *Sarda sarda* (Bloch 1793); the otoliths of young *S. dumerili* have a more peaked rostrum and antirostrum as well as lobulated dorsal margins, and the otoliths of *S. sarda* have a triangular shape and a more elongated rostrum. Finally, the otoliths of *Stephanolepis diaspros* and *Lagocephalus suezensis* were easy to identify and comparable with otoliths of other Tetraodontiformes, including *Sphoeroides* spp.

d) Common otolith shapes with morphological characters related to other native congeners, although they are not classified with them. *Upeneus moluccensis* is similar to *Mullus* spp., *Siganus luridus* resembles Cupleidae and Labridae because of a double-peaked anterior region, and *Lagocephalus scleratus* has an otolith shape that is very representative of Tetraodontidae.

To conclude, the unusual otolith shapes of Lessepsian fish species led to a very high classification success. In addition, the analysis of Lessepsian otoliths provided new morphological information that can be used in species identification with the ATI system, and applied in other scientific fields, such as establishing marine trophic networks (Moreno-López *et al.* 2002, García-Rodríguez and De La Cruz-Aguero 2010). Future studies are needed to complete the morphological catalogue of Lessepsian otoliths. This information can be used not only as a descriptive tool, but also to test theories in the ecomorphological field of invasion biology.

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