Zoological Studies **60:**36 (2021)
doi:10.6620/ZS.2021.60-36

Zoological Studies

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Otolith Morphology: A Hidden Tool in the Taxonomic Study of Goatfishes (Teleostei: Perciformes: Mullidae)

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Received 13 December 2020 / Accepted 19 April 2021 / Published 7 July 2021
Communicated by Benny K.K. Chan

Goatfishes (Teleostei, Mullidae) are a group of mainly coastal fishes that form an important part of food chains, and also have commercial value. In the marine waters of Iran, they are found in the Persian Gulf and Oman Sea (Gulf of Oman). This study evaluated whether otolith morphology can be used to distinguish of species and genera, and also to interpret whether otoliths of the same species differ among two studied marine systems. To do this objective, the otolith morphology of ten species belonging to three genera were analyzed by SEM photography and shape analysis. Among the morphometric variables, relative rostrum length was found to be the most important otolith variable for discriminating *Mulloidichthys* (RRL = 32.29 ± 0.59) and *Parupeneus* (RRL = 37.39 ± 1.10), while rectangularity (REx) was the most important shape index for discriminating *Mulloidichthys* (REx = 0.99 ± 1.94) from *Parupeneus* (REx = 0.77 ± 1.21). By considering otolith morphology, *Upeneus* showed a better separation than the two other genera. The otoliths of *Upeneus* are diagnosed by short dorsal length (RDL = 69.35 ± 1.51), higher rostrum height (RRH = 53.63 ± 1.99), short rostrum (RRL = 31.12 ± 1.99), and antirostrum lengths (RanL = 12.38 ± 1.51). The most diverged phenotype within the genus *Parupeneus* was found for the otoliths of *P. rubescens*, and the most diverged phenotype within the genus *Upeneus* was found for the otoliths of *U. sundaicus*. The otoliths of the same species did not demonstrate large variation between the Persian Gulf and the Gulf of Oman. This study provides additional morphological evidence for the separation of goatfishes at the species and genus levels. We also underline that the slight observed differences between the otoliths of two marine systems are mainly caused by the ecological differences known between these two main systems.

Key words: Sagitta, Marine fish, Scanning Electron Microscope, Shape analysis, Phenotypic divergence.

BACKGROUND

Goatfishes (Teleostei, Mullidae) are predominantly benthivores that live in marine and brackish waters above sandy to muddy bottoms as well as coral reefs. They are distributed worldwide in tropical, subtropical, and temperate habitats between the upper littoral and the upper slope (Uiblein 2007). Goatfishes form an important part of food chains in coastal ecosystems, and have a commercial value in many regions around the world (Pavlov 2012). They are valuable fishes for ecosystem monitoring and management because they are sensitive to human-induced activities such as fisheries and habitat modification (Uiblein 2007). They

Citation: Echreshavi S, Esmaeili HR, Teimori A, Safaie M. 2021. Otolith morphology: a hidden tool in the taxonomic study of goatfishes (Teleostei: Perciformes: Mullidae). Zool Stud **60:**36. doi:10.6620/ZS.2021.60-36.

act as allochthonous ecosystem engineers through their vigorous foraging behavior with barbels and mouth, which leads to the stirring-up of sediments and associated detritus particles high into the water column (Uiblein 2007, table III, p. 279).

A total of six genera and 97 species are known worldwide, of which only three genera and 10 species are known from the Persian Gulf and the Gulf of Oman (Ketabi et al. 2017; Eagderi et al. 2019; Fricke et al. 2020). They are characterized by the deep body, forked caudal fin, widely separated dorsal fins, and a pair of well-developed long chemosensory barbels positioned on the chins of adult individuals (Kim et al. 2001). The chemosensory barbels help fish to detect food items on or slightly below the surface of the substrate (Lombarte and Aguirre 1997; Kim et al. 2001).

Goatfishes are usually small fishes, with the largest one (*i.e.*, the dash-and-dot goatfish; *Parupeneus barberinus*) reaching up to 550 mm in length (Rajan 2012) and used as valued food around the world. Goatfishes are also known for their high morphological variability (Golani and Galil 1990; Stepien et al. 1994). Various morphological traits (*e.g.*, body size, body coloration, head form, otolith shape, the scale ornamentation), and meristic characters (*e.g.*, the number of gill rakers, fin rays, and vertebrae) have been investigated and found to show interspecific variation (Thomas 1969; Aguirre 1997; Labropoulou et al. 1997; Platell et al. 1998; Uiblein et al. 1998; Kim and Nakaya 2002; Randall 2004; Teimori et al. 2020) or within-species variation (McCormick and Milicich 1993; McCormick and Molony 1995); Aguirre 1997; Uiblein et al. 1998; Mahé et al. 2005; Pothin et al. 2006; Sabatini et al. 2007).

Nevertheless, there is a considerable lack of basic systematic and taxonomic knowledge of these fishes (Uiblein 2007), and more information may still be hidden in morphological characters. New species are still being described and intraspecific morphological variation and genetic differentiation require more detailed studies. This study aimed to provide comprehensive data on otolith morphology as a possible means of discriminating between mullets from the Persian Gulf and the Gulf of Oman. It is proposed that some degrees of genetic isolation exist between the fishes of the Persian Gulf and the Gulf of Oman (Teimori et al. 2012b). Therefore, we compare qualitatively the otoliths between these marine systems to evaluate if the otoliths of the same species are different.

Otolith

Otoliths are hard tissues positioned in the membraneous labyrinth of the inner ear of all teleost fish and mainly serve as a balance organ and also contribute to hearing (Platt and Popper 1981; Gauldie and Nelson 1988; Campana and Thorrold 2001). They are composed mainly of calcium carbonate ($CaCO_3$), mostly in the form of aragonite, and are found in three pairs named sagittae (the largest pair), lapilli, and asterisci (Mendoza 2006).

The otolith structure is three-dimensional, but it does not necessarily grow at the same rate equally in all dimensions. Also, the size and shape vary considerably among species (Campana and Thorrold 2001; Panfili et al. 2002; Reichenbacher et al. 2007; Teimori et al. 2018; Ghanbarifardi et al. 2020; Sadeghi et al. 2020). The largest otolith (saccular) has a distinct general morphology and is known to be taxon-specific. In the following text, the term "otolith" refers to the saccular otoliths only.

For a long time, otoliths have been used traditionally to obtain information about paleodiversity (Nolf 1985), and were later applied to investigate various aspects of the fishes, including fish biology (Shen et al. 1998; Woydack and Morales-Nin 2001; Brazner et al. 2004), trophic ecology (de Carvalho et al. 2019), species separation and reconstruction of phylogenetic relationships and biogeographic distribution (Stransky and MacLellan 2005; Stransky et al. 2008; Teimori et al. 2012a 2020), the identification of fish stock (Campana and Casselman 1993; Volpedo and Echeverría 2000; DeVries et al. 2002) and the characterization of local populations (Mérigot et al. 2007).

Also, fish, seal, and seabird biologists, as well as taxonomists and archaeologists (Van Neer et al. 2002), often rely on the shape and size of preserved or undigested otoliths to measure fish biodiversity and to reconstruct the species and size compositions of the diet of fish predators (Lin et al. 2020) and determine specific developmental stages (Campana and Casselman 1993; Hare and Cowen 1994; Tuset et al. 2016; Sanjarani Vahed et al. 2018 2019; Motamedi and Teimori 2019; Motamedi et al. 2019a; Cerna et al. 2019; Souza et al. 2020; Teimori et al. 2020).

Available studies on the otoliths of goatfishes are limited to Pothin et al. (2006), Pavlov et al. (2012) and Osman et al. (2020). Pothin et al. (2006) used otolith morphometrics for discriminating between the yellow-striped goatfish *Mulloidichthys flavolineatus* juveniles from the Reunion Island and Mauritius Island (southwest Indian Ocean) to estimate the stocks of this species. A further study by Pavlov et al. (2012) demonstrated the characteristics of otoliths in the freckled goatfish *Upeneus tragula* and revealed an irregular rhomboid shape for the lapillus and a triangular shape with rounded edges for the asteriscus. In addition, three

goatfish species from the northern Red Sea were compared and discriminated by otolith morphometrics and ultrastructure (Osman et al. 2020).

MATERIALS AND METHODS

Studied region and fish sampling

A total of 182 specimens belonging to 10 species were collected from six sites in the Persian Gulf and the Gulf of Oman using artisanal fishing gear (gill net and trammel net) from August 2017 to December 2019 (Fig. 1). All fish specimens were photographed immediately after sampling and were identified using the morphological-based diagnostic key described by Ben-Tuvin and Kissil (1998), Randall and Kulbicki (2006), Barman et al. (2007), and Uiblein and Heemstra (2010). The photographs of the studied fish species are shown in figure 2. The list of species, the details on sampling sites, and the number of examined individuals for each species are provided in table 1. The total length (TL) and standard length (SL) were measured to the nearest 0.05 millimeter following Smith (1949), Munro (1995), and Talwar and Kacker (1984).

Otolith preparation and imaging

The otolith extraction followed Reichenbacher et al. (2007). Otoliths were cleaned in distilled water, with the remaining tissue eliminated with 1% potassium hydroxide solution for 6 h, and eventually washed with distilled water. Scanning Electron Microscopy (SEM) was used to image the inner face of the left saccular otolith. To achieve this objective, the otoliths were air-dried and mounted on an aluminum stub using double-sided carbon tape. Thereafter, they were fixed on a specimen holder using sticker tape and coated with a 30-nm layer of gold. Electron micrographs were produced on a GAOL, GSMS 400 LV scanning electron microscope in back-scattering mode and on a Stereo Scan Cambridge Mark 2A (15 KV) at the Shiraz University Electron Microscope Center, Shiraz, Iran. Otolith terminology follows Tuset et al. (2008) (Fig. 3a).

In addition, a light microscope (Nikon Eclipse 80i Digital Imaging Head, New York, USA) integrated with a PC, was used to capture otolith images. For digital photography, the otoliths were oriented with the outer/lateral side down and ventral rim parallels to a horizontal line. The fish materials and their otoliths were deposited in the collection of the Zoological Museum of

Fig. 1. Map of six sampling locations from the Persian Gulf and the Oman Sea. The collected species from each location are indicated.

Shiraz University, Iran (ZM-CBSU).

Otolith morphometry

Digital images were used for morphometric analysis. Seven linear distances were measured for every otolith: relative dorsal length (RDL), relative medial length (RML), relative antirostrum height (RAH), relative rostrum height (RRH), relative antirostrum length (RAL), relative rostrum length (RRL), and Length-height-index (L.H index) (Fig. 3b) (Reichenbacher et al. 2007). These linear measurements were standardized as a function of length and height of otolith, respectively, and used for statistical analyses. In addition, 14 morphological characters of the sagittae were described and coded numerically. These data were used to prepare a data matrix, which was in turn used as input for morphological analysis (Tables 2–3).

Analysis of shape indices

Eight size parameters were calculated and used

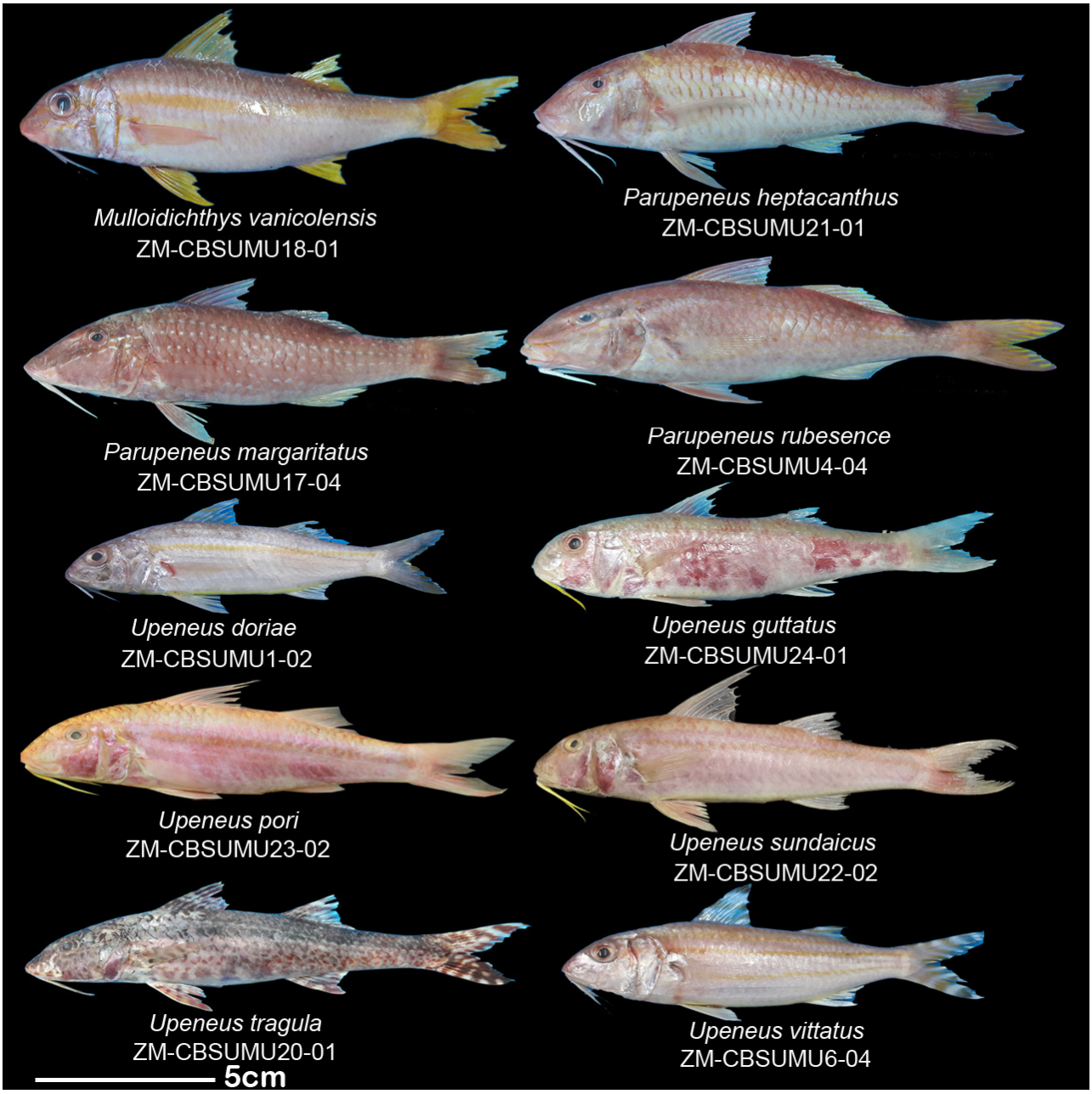

Fig. 2. Studied mullid species from the Persian Gulf and the Gulf of Oman.

to estimate the shape variation of otolith in each species as shown in figure 3c–d (Tuset et al. 2003). To estimate the shape index [sulcus area (SS)/otolith area (OS)] (Pothin et al. 2006), the otolith surface (OS) and sulcus surface (SS) were measured using the Image J software (Schneider et al. 2017) (Fig. 3c). Additionally, six size parameters related to the otolith dimension were measured: Feret length (FL), Feret width (FW), maximum radius (Rmax), minimum radius (Rmin), maximum Feret length (Fmax), and minimum Feret length (Fmin) (Tuset et al. 2003) (Fig. 3d). These parameters were used to calculate the following indices; Roundness [ROx] (4π), Rectangularity (REx), Ellipticity (ELx), Radius ratio (RAr), Feret ratio (FEr), and Aspect ratio (ASr). The larger values for Radius, Feret, and Aspect ratios show more elongation (Ponton 2006; Teimori et al. 2020). Also, the *E*-index was calculated to indicate the tendency of the otolith shape (*i.e.*,

Table 1. List of the studied species, sampling sites, and their coordinate points, and standard length (SL) ± s.d. of the fishes from each site. N refers to the number of examined individuals from each site

Species	Sampling site and coordinate points	SL ± s.d. (mm)	N
Mulloidichthys vanicolensis	Gulf of Oman (Chabahar) N: 25°21'14.1" E: 60°36'04.5"	210.33 ± 23.35	20
Parupeneus heptacanthus	Gulf of Oman (Chabahar) N: 25°21'14.1" E: 60°36'04.5"	251.64 ± 31.43	20
Parupeneus margaritatus	Gulf of Oman (Chabahar) N: 25°21'14.1" E: 60°36'04.5"	198.55 ± 12.60	10
	Gulf of Oman (Pozm) N: 25°16'12.29" E:60°28'36.60"	243.66 ± 14.31	10
Parupeneus rubescens	Gulf of Oman (Chabahar) N: 25°21'14.1" E: 60°36'04.5"	324.25 ± 39.16	20
Upeneus doriae	Gulf of Oman (Chabahar) N: 25°21'14.1" E: 60°36'04.5"	148.45 ± 12.36	10
	Persian Gulf (Bandarabbas) N: 27°10'18.78" E: 56°16'00.29"	121.78 ± 13.01	10
Upeneus guttatus	Persian Gulf (Bandarabbas) N: 27°10'18.78" E: 56°16'00.29"	157.87 ± 1.16	2
Upeneus pori	Persian Gulf (Dayyer) N: 27°49'43.85" E: 51°56'39.29"	163.73 ± 10.98	20
Upeneus sundaicus	Persian Gulf (Dayyer) N: 27°49'43.85" E: 51°56'39.29"	177.72 ± 24.93	20
Upeneus tragula	Gulf of Oman (Chabahar) N: 25°21'14.1" E: 60°36'04.5"	228.33 ± 34.44	9
	Persian Gulf (Bushehr) N: 28°54'20.61" E: 50°46'45.43"	175.63 ± 10.16	11
Upeneus vittatus	Gulf of Oman (Chabahar) N: 25°21'14.1" E: 60°36'04.5"	189.53 ± 14.27	10
	Persian Gulf (Jask) N:25°41.1'6.8" E:57°53'26.46"	175.11 ± 10.93	10

circular or elongate). The *E*-index = gh/ef, where gh is the maximum width of otolith and ef is the maximum length of otolith (Volpedo and Echeverría 2000).

Statistical analysis

The univariate analysis of variance (ANOVA, with Duncan's post hoc test, $P < 0.05$) was applied to test the differences in morphometric variables and otoliths shape indices among the studied taxa. The Canonical discriminant analysis (CDA) was used for multivariate analyses to show the classification success of the groups. Otolith variables were analysed using the IBM SPSS Statistics software v.25 and PAST, Palaeontological Statistics, version 2.7 (Hammer et al. 2001).

RESULTS

General otolith characteristics in 10 Mullidae species

Generally, the otoliths of ten Mullidae species were lanceolated, elliptic to oval, fusiform, triangular, and trapezoid in shape (Figs. 4–5). The sculpture of otolith margin varied from dentate to crenate to lobed. Sulcus acusticus: heterosulcoid, ostial, median (rarely supramedian). Ostium: rectangular or funnel-like, shorter than the cauda. Cauda: elliptic, curved, slightly or markedly flexed from the middle region, ending close to the posterior margin. Anterior region: peaked and blunt; rostrum thick, broad, pointed, blunt or irregular; antirostrum short, broad, pointed, sometimes absent or fused with the rostrum; excisura wide with a deep and acute notch, sometimes excisura not clearly

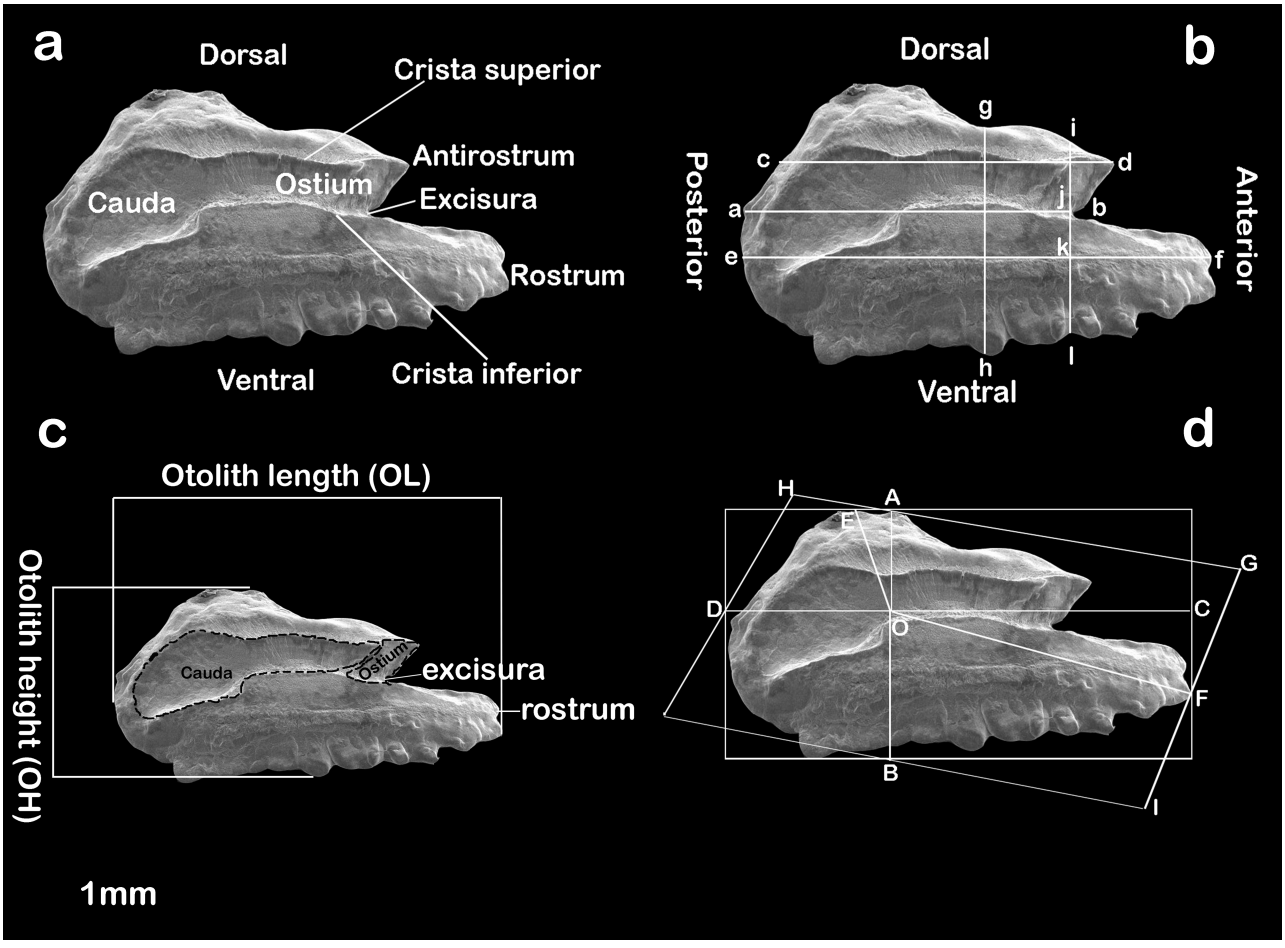

Fig. 3. (a) Left sagitta otolith showing the terminology of otolith characters according to Tuset et al. (2008), (b) linear measurements according to Reichenbacher et al. (2007), a–b = medial length, c–d = dorsal length, e–f = length, g–h = height, i–b = antirostrum height, j–d = antirostrum length, k–f = rostrum length, b–l = rostrum height, (c) measurements related otolith surface area, OS = otolith surface, SS = sulcus surface (D) measurements related to the otolith dimension for calculating of shape indices, O = core, AB = Feret width, CD = Feret length, OE = minimum radius, OF = maximum radius, GH = longest Feret length, GI = smallest Feret length.

Table 2. The numerical coding of otolith morphological features

Character	State	Code
Shape	Oval	0
	Elliptic to Oval	1
	Elliptic to triangular	2
	Round to slightly flattened	3
	Wedge shape/ Elliptic	4
Anterior region	Ovate	0
	Ovate to elliptic/ Irregular	1
	Triangular to elliptic	2
	Peaked	3
	Peaked to blunt	4
	Pointed to round	5
Posterior region	Round to angled	0
	Round to angle/ Oblique irregular	1
	Round to angle/ Oblique/ more or less straight	2
	oblique	3
	Round/ Irregular	4
Sulcus acusticus	Heterosulcoid, ostial, median	0
	Angle to oblique/ Heterosulcoid, ostial, medium	1
	Heterosulcoid, ostial, median/ longitudinal	2
	C-Heterosulcoid	3
Crista superior	Distinct and ridge-like/Well development	0
	Distinct and ridge-like	1
	Distinct and ridge-like/ well development but broken	2
Crista inferior	Distinct and ridge-like/Well development	0
	Distinct and ridge-like	1
Ostium	Funnel-like to Elliptic	0
	Funnel-like	1
Cauda	Curved to Oval, descending/Tubular, strongly curved	0
	Curved, descending	1
	Curved to elliptic, descending	2
	Curved to elliptic/ Tubular, slightly curved	3
	Curved to Oval/ descending	4
	Curved to Oval or elliptic, descending	5
Dorsal margin	Wavy forming or less conspicuous round/ slightly irregular	0
	Slightly irregular, Slightly emarginated/ Enteric	1
	Composed of conspicuous and irregularly spaced protuberances	2
	Enteric/ well development/ slightly emarginated	3
	Enteric	4
	Ridge like /slightly irregular, slightly emarginated	5
Ventral margin	Wavy forming or less conspicuous round/ slightly irregular	0
	Slightly irregular, slightly emarginated/ Enteric	1
	Composed of conspicuous and irregularly spaced protuberances	2
	Enteric/ well development/ slightly emarginated	3
	Enteric	4
	Ridge like /slightly irregular, slightly emarginated	5
Rostrum	Short, very broad, blunt	0
	Broadly pointed	1
	Broad, short pointed to round	2
	Pointed to round	3
	Medium, wide, irregular	4
	Pointed	5
Rostrum size	Large	0
	Medium	1
Rostrum shape	Pointed	0
	Long Pointed	1
	Pointed to round	2
	Broadly pointed	3
Excisura	U-Shape	0
	V-Shape	1

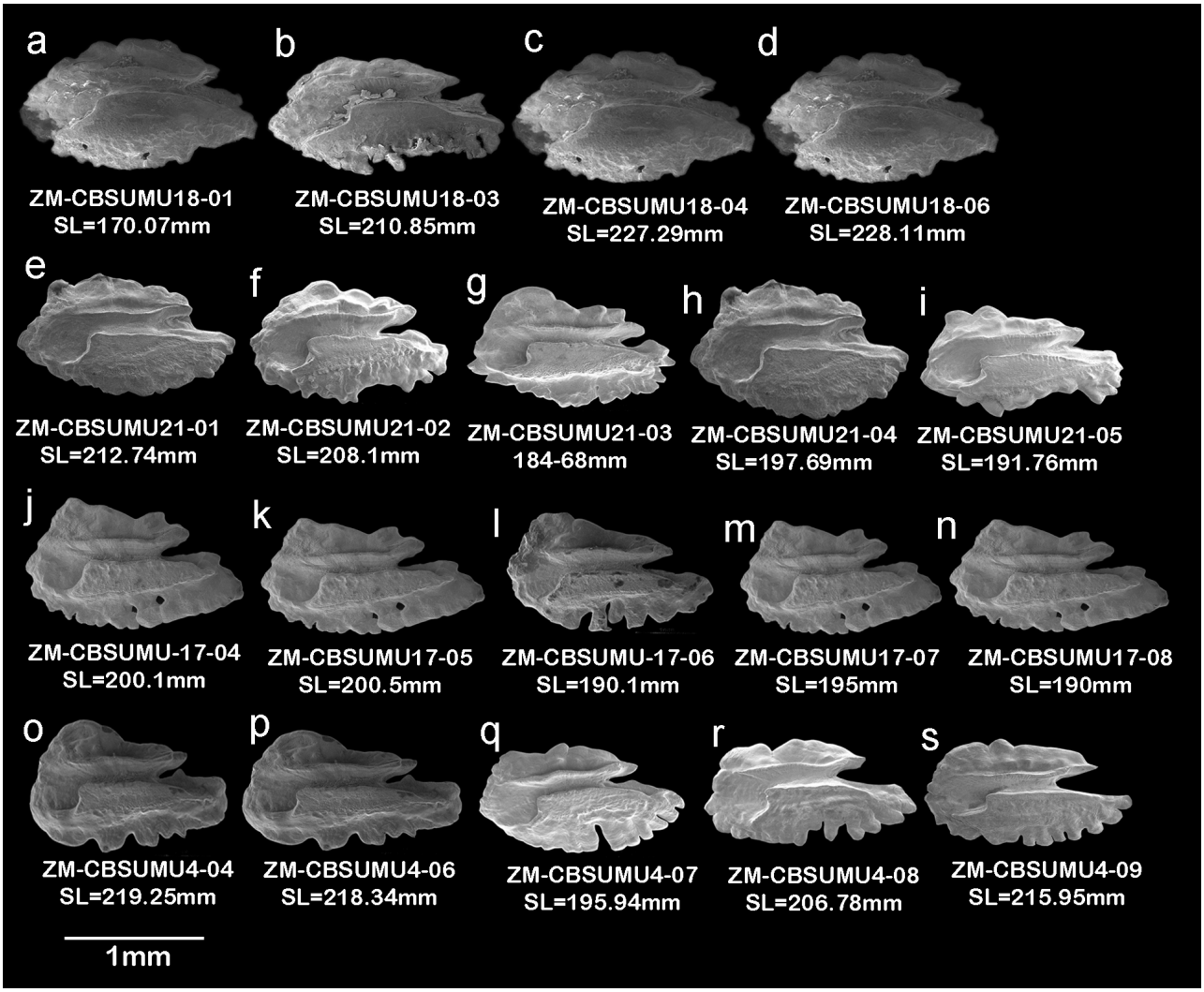

Fig. 4. Otoliths of four mullid species from the Gulf of Oman. *Mulloidichthys vanicolensis* (a–d), *Parupeneus heptacanthus* (e–i), *Parupeneus margaritatus* (j–n), and *Parupeneus rubescens* (o–s). All images are SEM micrographs, showing left otoliths in medial view.

Table 3. Data matrix of the otolith morphological features

Characters \ Species	*M. vanicolensis*	*P. heptacanthus*	*P. margaritatus*	*P. rubescens*	*U. doriae*	*U. guttatus*	*U. pori*	*U. sundaicus*	*U. tragula*	*U. vittatus*
Shape	5	6	7	1	0	5	4	3	2	1
Anterior region	1	1	1	3	0	4	1	2	1	0
Posterior region	5	5	4	5	0	3	4	3	2	1
Sulcus acusticus	2	2	3	0	0	0	0	0	1	0
Crista superior	2	2	0	0	0	0	1	1	0	0
Crista inferior	0	0	0	1	0	0	1	1	0	0
Ostium	1	1	0	2	0	0	0	0	0	0
Cauda	0	0	5	3	0	4	4	3	2	1
Dorsal margin	0	0	5	6	0	0	4	3	2	1
Ventral margin	4	4	5	6	0	0	3	2	1	1
Rostrum	4	4	5	4	0	1	3	2	1	1
Rostrum size	0	0	0	1	0	0	0	0	0	0
Rostrum shape	2	2	3	4	0	0	1	2	0	1
Excisura	1	1	1	0	0	0	0	0	0	0

distinguishable. Posterior region: round to angled-irregular (Figs. 4–5).

The otoliths of a single species of *Mulloidichthys* (*M. vanicolensis*) were elliptic (Fig. 4a–d), and otoliths of one species of *Parupeneus* (*i.e.*, *P. heptacanthus*) were elliptic to oval (Fig. 4e–i), while the otoliths of *P. margaritatus* (Fig. 4j–n) and *P. rubescens* (Fig. 4o–s) were elliptic to lanceolate in shape.

The otoliths of six species of *Upeneus* demonstrated three morphotypes. The elliptic shape was found in *U. doriae*, *U. guttatus*, *U. tragula*, and *U. vittatus*, the fusiform to trapezoid were found in *U. pori*, and the elliptic to fusiform shape was detected in *U. sundaicus*. The morphological characteristics of otoliths are summarised in appendix 1. The following features were described for the otoliths of each species; sulcus acusticus, ostium, cauda, anterior region, rostrum and antirostrum, excisura, posterior region, dorsal and ventral rimes.

Genus *Mulloidichthys* Whitley, 1929
Mulloidichthys vanicolensis (Valenciennes, 1831): Yellowfin goatfish

Shape: elliptic. *Anterior region*: double peaked; rostrum: round and irregular; antirostrum: short and pointed; excisura: U-shape and deep. *Posterior region*: angled. *Margin*: ventral dentate to lobed. *Sulcus acusticus*: heterosulcoid with a median ostial: ostium rectangular; cauda: tubular, slightly curved ending close to the posterior margin (Fig. 4a–d). Roundness (ROx) = 0.31 ± 2.22, Rectangularity (REx) = 0.99 ± 1.94, Ellipticity (ELx) = 0.16 ± 1.70.

Genus *Parupeneus* Bleeker, 1863
Parupeneus heptacanthus (Lacepède, 1802): Cinnabar goatfish

Shape: elliptic to oval. *Anterior region*: peaked to blunt; rostrum blunt; antirostrum slightly pointed and very short (Fig. 4e and h), or elongated (Fig. 4f–g and i); excisura U-shape (Fig. 4e and h) or V-shape and deep (Fig. 4f–g and i). *Posterior region*: round to angle. *Margin*: ventral dentate; dorsal lobed and irregular. *Sulcus acusticus*: median and ostial; ostium funnel-like; cauda elliptic, curved, markedly flexed from the middle region, ending close to the posterior margin (Fig. 4e–i). Roundness (ROx) = 0.75 ± 1.96, Rectangularity (REx) = 0.85 ± 1.67, Ellipticity (ELx) = 0.18 ± 1.34.

Parupeneus margaritatus Randall & Guézé, 1984: Pearly goatfish

Shape: elliptic to lanceolated. *Anterior region*: peaked to blunt; rostrum thick and blunt; antirostrum elongated, peaked, smaller than rostrum; excisura V-shape and deep. *Posterior region*: round. *Margin*: ventral dentate to lobed; dorsal irregular, sometimes with dorsal tip. *Sulcus acusticus*: heterosulcoid, ostial, median, straight; ostium funnel-like; cauda elliptic, curved, markedly flexed from the middle region, ending close to the posterior margin (Fig. 4j–n). Roundness (ROx) = 0.93 ± 1.65, Rectangularity (REx) = 1.04 ± 1.52, Ellipticity (ELx) = 0.17 ± 1.46.

Parupeneus rubescens (Lacepède, 1801): Rosy goatfish

Shape: elliptic to lanceolated. *Anterior region*: peaked to blunt; rostrum thick, blunt or pointed; antirostrum elongated, peaked, smaller than rostrum; excisura V-shape and deep. *Posterior region*: round. *Margin*: ventral dentate; dorsal irregular. *Sulcus acusticus*: heterosulcoid, ostial, median, straight; ostium funnel-like; cauda elliptic, curved, markedly flexed from the middle region, ending close to the posterior margin (Fig. 4o–s). Roundness (ROx) = 0.36 ± 2.07, Rectangularity (REx) = 0.42 ± 1.92, Ellipticity (ELx) = 0.19 ± 2.08.

Genus *Upeneus* Cuvier, 1829
Upeneus doriae (Günther, 1869): Gilded goatfish

Shape: elliptic. *Anterior region*: peaked; rostrum short, very broad, round; antirostrum absent; excisura wide, without a notch. *Posterior region*: oblique. *Margin*: ventral crenate, sometimes smooth; dorsal entire. *Sulcus acusticus*: heterosulcoid, ostial, supramedian; ostium funnel-like, shorter than the cauda; cauda: tubular, curved, markedly flexed posteriorly, ending in the posterior-ventral region. (Fig. 5a–e). Roundness (ROx) = 0.43 ± 2.52, Rectangularity (REx) = 0.32 ± 2.52, Ellipticity (ELx) = 0.28 ± 2.54.

Upeneus guttatus (Day, 1868): Two-tone goatfish

Shape: elliptic. *Anterior region*: blunt, a well-developed tip that forms acute angle; rostrum blunt to round; anti-rostrum short and pointed; excisura U-shape, sometimes V-shape. *Posterior region*: oblique and angled, regularly curved. *Margin*: ventral crenate; dorsal tip present in the dorsal margin. *Sulcus acusticus*: heterosulcoid, ostial, median; ostium funnel-like to elliptic, shorter than the cauda; caudal curved to oval, ending close to the posterior margin (Fig. 5f–g). Roundness (ROx) = 0.57 ± 0.86, Rectangularity (REx) = 0.72 ± 0.98, Ellipticity (ELx) = 12.7 ± 1.69.

Upeneus pori Ben-Tuvia & Golani, 1989: Por's goatfish

Shape: fusiform to a trapezoid. *Anterior region*: blunt, a well-developed tip, sometimes pointed; rostrum blunt to pointed; anti-rostrum short and pointed, smaller than rostrum; excisura V-shape, deep. *Posterior region*: rounded, oblique to angled. *Margin*: ventral dentate; dorsal entire, sometimes irregular. *Sulcus acusticus*: heterosulcoid, ostial, median; ostium funnel-like to elliptic, shorter than the cauda; caudal curved to oval, ending close to the posterior margin (Fig. 5h–i).

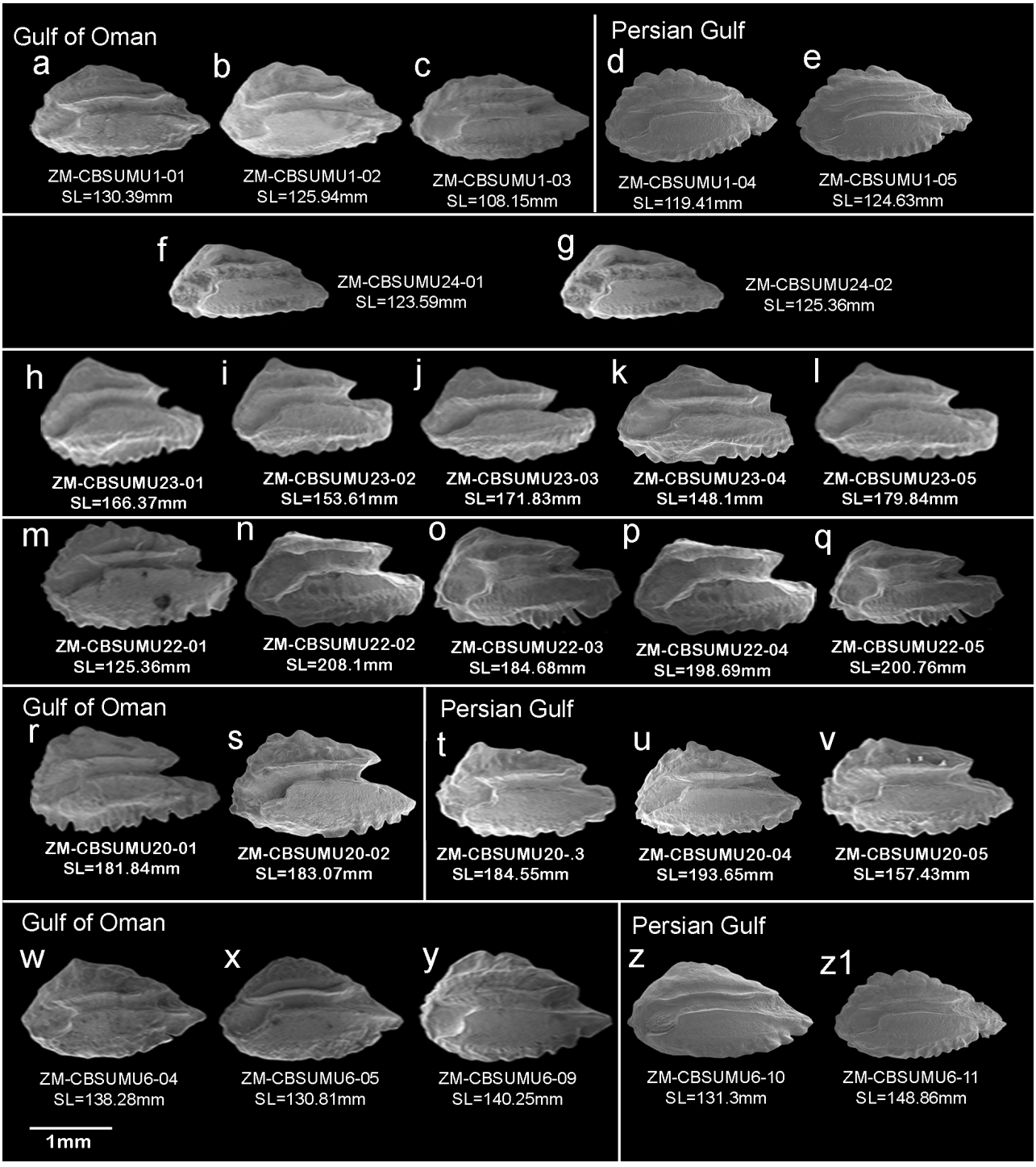

Fig. 5. Otoliths of six species of the genus *Upeneus* from the Gulf of Oman and the Persian Gulf. *Upeneus doriae* (a–e), *U. guttatus* (f–g), *U. pori* (h–i), *U. sundaicus* (m–q), *U. tragula* (r–v), and *U. vittatus* (w–z1). All images are SEM micrographs, showing left otoliths in medial view.

Roundness (ROx) = 0.11 ± 1.1, Rectangularity (REx) = 1.96 ± 1.14, Ellipticity (ELx) = 0.24 ± 1.69.

Upeneus sundaicus Bleeker, 1855: Ochrebanded goatfish

Shape: elliptic to fusiform. *Anterior region*: wide and thick; rostrum pointed, sometimes rounded; antirostrum short and pointed; excisura U-shape, sometimes V-shape. *Posterior region*: oblique and angled, rounded. *Margin*: ventral lobed; dorsal lobed to irregular. *Sulcus acusticus*: heterosulcoid, ostial, median; ostium funnel-like to elliptic, shorter than the cauda; caudal curved to oval, ending close to the posterior margin (Fig. 5m–q). Roundness (ROx) = 0.31 ± 0.3, Rectangularity (REx) = 0.34 ± 0.32, Ellipticity (ELx) = 0.17 ± 0.34.

Upeneus tragula Richardson, 1846: Freckled goatfish

Shape: elliptic. *Anterior region*: peaked; rostrum short, very broad, pointed, sometimes blunt; antirostrum short and pointed; excisura V-shape and deep or U-shape, without a notch. *Posterior region*: oblique. *Margin*: ventral crenate; dorsal lobed, sometimes irregular. *Sulcus acusticus*: heterosulcoid, ostial, supramedian; ostium funnel-like, shorter than the cauda; cauda: tubular, curved, markedly flexed posteriorly, ending in the posterior-ventral region (Fig. 5r–v). Roundness (ROx) = 0.59 ± 2.54, Rectangularity (REx) = 0.79 ± 2.22, Ellipticity (ELx) = 0.26 ± 2.13.

Upeneus vittatus (Forsskål, 1775): Yellowstriped goatfish

Shape: elliptic. *Anterior region*: peaked; rostrum short, very broad, pointed; antirostrum absent; excisura wide, without a notch. *Posterior region*: oblique. *Margin*: ventral crenate; dorsal lobed, sometimes irregular. *Sulcus acusticus*: heterosulcoid, ostial, supramedian; ostium funnel-like, shorter than the cauda; cauda: tubular, curved, markedly flexed posteriorly, ending in the posterior-ventral region (Fig. 5w–z1). Roundness (ROx) = 0.43 ± 2.41, Rectangularity (REx) = 0.49 ± 2.23, Ellipticity (ELx) = 0.18 ± 2.25.

Analysis of otolith morphology and shape indices

The details of descriptive analysis of the otolith morphometric variables and the otolith shape indices were provided in tables 4 and 5, respectively. The univariate analysis showed that three studied genera differ significantly in both morphometric and shape indices. *Mulloidichthys* and *Parupeneus* differed significantly in two shape indices, including REx and RRL (ANOVA with post-hoc test, Duncan, $P < 0.05$). The genus *Upeneus* significantly differed from two other genera in four morphometric variables (RDL, RRH, RRL, and RanL) and two shape indices *i.e.*, FEr, and ELx (ANOVA with post-hoc test, Duncan, $P < 0.05$) (Table 6).

We also used ANOVA with post-hoc test to compare differences within *Parupeneus* and *Upeneus*. Since only a single species was studied for *Mulloidichthys*, we only examined differences among the species of the two other genera. Whitin *Parupeneus*, *P. heptacanthus* was different in a single morphometric variable (RML) and a single shape index (FF), while *P. margaritatus* was differed from other two species only in a single shape index (FF), and *P. rubescens* was differed in a single morphometric variable (FF) and

Table 4. The otolith morphometric variables (means ± standard deviations) for the studied fish species. RDL (Relative dorsal length), RML (Relative medial length), RanH (Relative antirostrum height), RRH (Relative rostrum height), RanL (Relative antirostrum length), RRL (Relative rostrum length). N refers to the number of examined individuals from each site

Morphometric variable	RDL	RML	RanH	RRH	RanL	RRL	LH	N
Mulloidichthys vanicolensis	75.34 ± 0.68	82.56 ± 0.79	11.02 ± 0.32	46.98 ± 0.59	16.00 ± 0.76	32.29 ± 0.59	1.36 ± 0.59	20
Parupeneus heptacanthus	74.10 ± 0.82	71.06 ± 0.71	12.74 ± 0.48	39.53 ± 0.56	14.83 ± 0.65	37.80 ± 0.78	1.36 ± 0.78	20
Parupeneus margaritatus	73.63 ± 0.72	79.59 ± 0.88	10.92 ± 0.54	37.51 ± 0.50	13.47 ± 0.58	37.51 ± 0.82	1.31 ± 0.82	20
Parupeneus rubescens	76.45 ± 0.64	82.34 ± 0.74	11.79 ± 0.34	46.92 ± 0.4	17.40 ± 0.43	36.88 ± 0.56	1.32 ± 0.56	20
Upeneus doriae	69.43 ± 1.29	84.66 ± 1.44	8.06 ± 0.66	31.30 ± 0.86	10.69 ± 0.93	39.20 ± 1.33	1.44 ± 1.31	20
Upeneus guttatus	66.08 ± 0.04	85.32 ± 0.03	8.61 ± 0.12	38.87 ± 0.03	14.91 ± 0.06	29.78 ± 0.09	1.53 ± 0.09	2
Upeneus pori	64.82 ± 0.24	71.95 ± 0.24	8.76 ± 0.09	53.82 ± 0.16	8.19 ± 0.21	29.19 ± 0.19	1.74 ± 0.19	20
Upeneus sundaicus	69.29 ± 0.25	77.06 ± 0.34	13.13 ± 0.5	116.44 ± 0.33	18.53 ± 0.26	30.22 ± 0.60	3.56 ± 0.60	20
Upeneus tragula	70.50 ± 0.84	77.20 ± 0.9	9.77 ± 0.43	44.40 ± 0.58	10.68 ± 0.66	29.76 ± 0.77	1.67 ± 0.77	20
Upeneus vittatus	76.03 ± 0.86	84.71 ± 0.88	8.99 ± 0.37	36.99 ± 0.56	11.33 ± 0.64	28.62 ± 0.81	1.46 ± 0.81	20

five shape indices (FEr, ELx, REx, RRL, and RanL) (ANOVA with post-hoc test, Duncan, $P < 0.05$) (Table 6).

Also, the CDA was conducted based on the data matrix of the otolith morphological features, all seven morphometric variables, and eight shape indices. The *Parupeneus* species revealed a high classification success (80.0%) (Wilk's lambda k = 0.197, Table 7 and Fig. 6A).

Three morphometric variables (*i.e.*, RRH, RanH, and LH) and seven otolith shape indices were different among the six examined species of *Upeneus* (Table 6). Among them, two indices (*i.e.*, PA and FF) were significantly different among all the six examined species (Table 6). The CDA analysis for all *Upeneus* species indicated 91.2%, classification success (Wilk's Lambda'k = 0.009, Table 7 and Fig. 6B).

Table 5. The otolith shape indices (means ± standard deviations) for the studied fish species. ROx (Roundness), REx (Rectangularity), ELx (Ellipticity), RAr (Radius ratio), FEr (Feret ratio), ASr (Aspect ratio), PA (Squared perimeter to area ratio), and FF (Form factor). N refers to the number of examined individuals from each site

Morphometric variable	ROx	REx	ELx	RAr	FEr	ASr	PA	FF	N
Mulloidichthys vanicolensis	0.31 ± 2.22	0.99 ± 1.94	0.16 ± 1.70	3.28 ± 0.72	2.86 ± 1.21	1.51 ± 1.68	15.41 ± 3.24	0.81 ± 3.21	20
Parupeneus heptacanthus	0.75 ± 1.96	0.85 ± 1.67	0.18 ± 1.34	3.66 ± 0.84	2.59 ± 1.67	1.44 ± 1.31	12.93 ± 3.41	0.97 ± 3.38	20
Parupeneus margaritatus	0.93 ± 1.65	1.04 ± 1.52	0.17 ± 1.46	3.56 ± 0.75	2.53 ± 1.05	1.42 ± 1.42	38.37 ± 3.20	0.32 ± 3.17	20
Parupeneus rubescens	0.36 ± 2.07	0.42 ± 1.92	0.19 ± 2.08	3.63 ± 0.86	2.95 ± 1.49	1.48 ± 2.02	8.86 ± 2.65	1.41 ± 2.61	20
Upeneus doriae	0.43 ± 2.52	0.32 ± 2.52	0.28 ± 2.54	2.73 ± 0.99	2.58 ± 1.81	0.95 ± 2.50	12.20 ± 4.32	1.02 ± 4.30	20
Upeneus guttatus	0.57 ± 0.86	0.72 ± 0.98	0.26 ± 1.69	4.22 ± 1.01	3.01 ± 2.01	1.61 ± 1.36	10.91 ± 0.02	1.80 ± 0.01	2
Upeneus pori	0.11 ± 1.1	1.96 ± 1.14	0.24 ± 1.69	3.03 ± 1.01	3.00 ± 2.01	1.64 ± 1.36	3.46 ± 0.02	3.62 ± 0.01	20
Upeneus sundaicus	0.31 ± 0.3	0.34 ± 0.32	0.17 ± 0.34	3.05 ± 0.19	4.08 ± 0.23	1.42 ± 0.30	3.54 ± 0.41	3.53 ± 0.40	20
Upeneus tragula	0.59 ± 2.54	0.79 ± 2.22	0.26 ± 2.13	3.79 ± 0.81	2.82 ± 1.44	1.71 ± 2.01	23.43 ± 4.58	0.53 ± 4.52	20
Upeneus vittatus	0.43 ± 2.41	0.49 ± 2.23	0.18 ± 2.25	3.20 ± 0.54	3.07 ± 0.98	1.46 ± 2.21	20.78 ± 3.46	0.60 ± 3.42	20

Table 6. Significant differences in otolith morphometric variables and shape indices among the three studied genera of Mullidae, and also among the species within each genus. Characters with a significant difference are indicated

Genera *Mulloidichthys*, *Parupeneus*, *Upeneus*									
	RDL	RRH	RanL	RRL	REx	ELx	FEr		
Mulloidichthys				✓	✓				
Parupeneus				✓	✓				
Upeneus	✓	✓	✓	✓	✓	✓	✓		
Genus *Parupeneus*									
	RML	RRH	RanL	REx	ELx	FEr	FF		
P. heptacanthus	✓						✓		
P. margaritatus							✓		
P. rubescens		✓	✓	✓	✓	✓	✓		
Genus *Upeneus*									
	RanH	RRH	RanL	ROx	REx	FEr	ASr	PA	FF
U. doriae							✓	✓	✓
U. guttatus			✓		✓			✓	✓
U. pori			✓	✓		✓		✓	✓
U. sundaicus	✓	✓	✓		✓			✓	✓
U. tragula				✓	✓			✓	✓
U. vittatus				✓				✓✓	✓

DISCUSSION

Taxonomical significance of otolith morphology in Mullidae

Typically, the mullid fishes are difficult to identify using the available morphological literature. A particular problem in the taxonomy of these fishes is the lack of diagnostic morphological and meristic characters (Kim and Nakaya 2002). Some important meristic characters are the number of dorsal-fin spines, the number of pectoral-fin rays which may differ among species by only one ray, and the number of gill rakers. However, all these characters require careful examination to detect the minute, recumbent first spine in the eight-spine species group that distinguishes it from the seven-spine group (Lachner 1954; Kim and Nakaya 2002). In this context, the examination of hard structures such

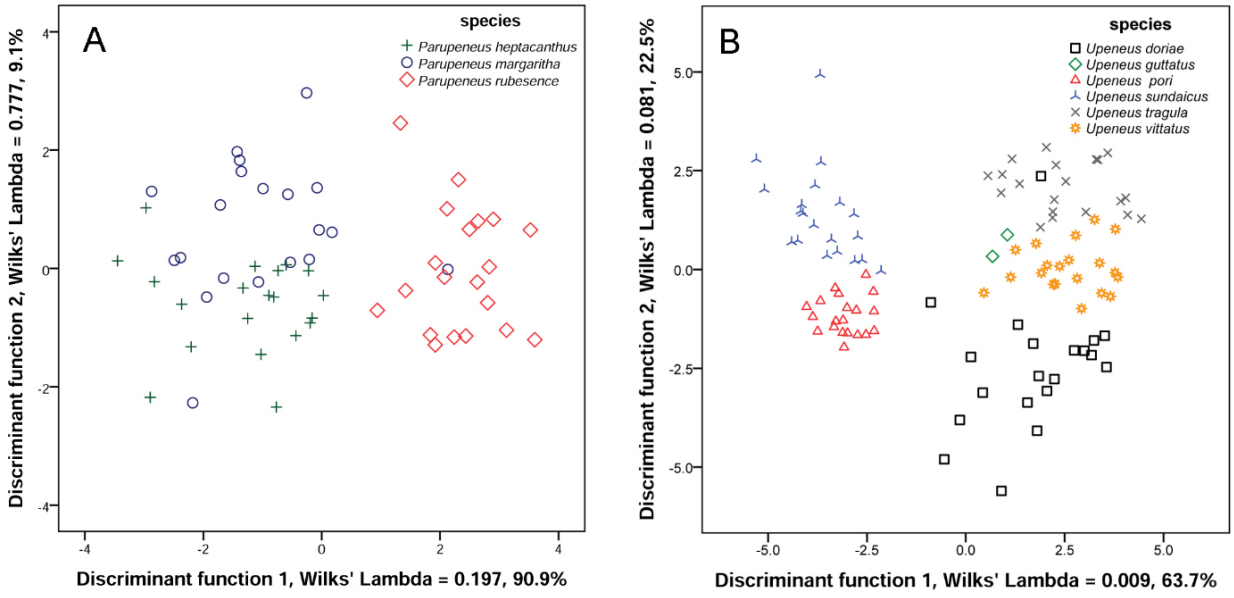

Fig. 6. Discriminant function scores (95% ellipses) based on all seven morphometric variables and eight shape indices of the otolith of the studied species of the genus *Parupeneus* (A) and the genus *Upeneus* (B).

Table 7. Classification matrix of the canonical discriminant analysis based on seven morphometric variables and eight otolith shape indices in the *Parupeneus* and *Upeneus*. The percentages in rows represent the classification into the species of each genus given in columns (correct classifications are bold-typed). The corresponding numbers of individuals are given in brackets

Genus *Parupeneus* (80.0% of original grouped cases correctly classified)				
Species	*P. heptacanthus*	*P. margaritatus*	*P. rubescens*	N
P. heptacanthus	**75%(15)**	5(25%)	0	20
P. margaritatus	6(30%)	**65%(13)**	1(5%)	20
P. rubescens	0	0	**100%(20)**	20

Genus *Upeneus* (91.2% of original grouped cases correctly classified)							
Species	*U. doriae*	*U. guttatus*	*U. pori*	*U. sundaicus*	*U. tragula*	*U. vittatus*	N
U. doriae	**95%(19)**	0	0	0	5%(1)	0	20
U. guttatus	0	**100%(2)**	0	0	0	0	2
U. pori	0	0	**100%(20)**	0	0	0	20
U. sundaicus	0	0	20%(4)	**80%(16)**	0	0	20
U. tragula	0	15%(3)	0	0	**85%(17)**	0	20
U. vittatus	0	0	0	0	1(5%)	**95%(19)**	20

as otolith may help to discriminate different genera and species. The present study examined the otolith morphology of 10 mullid species belonging to three genera (*Mulloidichthys*, *Parupeneus*, and *Upeneus*) for the first time from the Iranian marine waters of the Persian Gulf and the Gulf of Oman. The most important key morphological characteristics for distinguishing these genera are the teeth on the vomer or palatines (Uiblein and Heemstra 2010). *Mulloidichthys* and *Parupeneus* do not have teeth on the vomer or palatines, while in the genus *Upeneus*, teeth are present on vomer and palatines (Uiblein and Heemstra 2010). In addition to the meristic characters, Ramteke et al. (2016) have recently examined morphological characters and hard structures such as osteological elements to separate several species of goatfishes. Here we added additional morphological evidence to distinguish species and genera of goatfishes.

The relative rostrum length was the most important character for discriminating *Mulloidichthys* (RRL = 32.29 ± 0.59) and *Parupeneus* (RRL = 37.39 ± 1.10), while rectangularity (REx) was the most important shape index for discriminating two genera (0.99 ± 1.94 in *Mulloidichthys* vs. 0.77 ± 1.21 in *Parupeneus*). It means that *Mulloidichthys* has a more rectangular otolith than the genus *Parupeneus*, while its otolith rostrum is significantly shorter. However, otolith morphology has more power to distinguish the genus *Upeneus* than the two others, because four morphometric variables and two shape indices participated in separating it. The otoliths of *Upeneus* are diagnosed by short dorsal length (RDL = 69.35 ± 1.51), higher rostrum height (RRH = 53.63 ± 1.99), short rostrum (RRL = 31.12 ± 1.99), and antirostrum (RanL = 12.38 ± 1.51) lengths in comparison to the two other genera. Also, based on our analysis, *Upeneus* has more elliptical otoliths than the other genera.

Otolith morphology seems to be an appropriate tool for discrimination between species of goatfishes. Within *Parupeneus*, *P. rubescens* showed the most diverged otoliths. It showed significant differences in seven otolith characters consisting of RRH and RanL (morphometric variables) and REx, ELx, FEr, and FF (shape indices) (see also Table 6). Within *Upeneus*, *U. sundaicus* showed the most diverged otolith. It showed significant differences in six otolith characters consisting of RanH, RanL, and RRH (morphometric variables), REx, PA, and FF (shape indices) (Table 6).

The taxonomic significance of otoliths has already been examined in *Upeneus* from Indian marine waters by Ramteke et al. (2016). They found wedge shape otoliths in *U. guttatus*, elliptic to truncate in *U. vittatus* and *U. sundaicus*. They also distinguished the otoliths of *U. tragula* by its short rostrum and poorly developed antirostrum, which is in agreement with our data (see also Fig. 5r–v).

Moreover, based on a combination of the number of dorsal-fin spines, gill rakers, the number of pectoral-fin rays, and bars on the caudal fin, four major species complexes were distinguished within the genus *Upeneus* (*e.g.*, Thomas 1969; Sainsbury et al. 1985; Golani and Galil 1990; Kim and Nakaya 2002; Randall 2004). Of these, we examined otoliths of two complexes *i.e.*, "tragula" and "vittatus" groups. Based on our examination, the otoliths of *U. tragula* and *U. vittatus* were largely similar in general shape: in which both were elliptic in shape with a short rostrum and had no clear antirostrum (see also Fig. 5r–v and w–z1). They did, however, show a difference in shape indices, particularly in the Roundness (ROx) and Rectangularity (REx). Therefore, we concluded that otolith shape dimensions have more power to separate these two complexes than morphometry.

Moreover, the otoliths of three *Upeneus* species (*i.e.*, *Upeneus doriae*, *U. tragula*, and *U. vittatus*) were compared from between the Persian Gulf and the Gulf of Oman (see also Fig. 1 and Table 1) to see if the otolith of the same species is different between the two main water systems. In the case of *U. doriae*, otoliths from the Gulf of Oman (Chabahar, Fig. 5a–c) were similar in general shape (elliptic) to those from the Persian Gulf (Bandar Abbas, Fig. 5d–e); however, they do not display crenate margins in comparison to the otoliths from the Persian Gulf. *Upeneus doriae* was described for the first time from the coastal waters of Bandar Abbas and therefore the otoliths with almost smooth margins in the Gulf of Oman could be considered as morphological variation in comparison to its type morphology. The comparison of *U. doriae* from the Persian Gulf and the Gulf of Oman by Kim and Nakaya (2002) has also revealed geographic differences in orbit size and caudal-fin length but they considered the allometric changes during ontogeny for the observed variation. In the present study, the ontogenetic changes could be excluded because we used almost the same fish size for the otolith comparison (see also Table 1).

In the case of *U. tragula*, otoliths from the Gulf of Oman (Chabahar, Fig. 5r–s) were similar in general shape (elliptic) to those from the Persian Gulf (Bushehr, Fig. 5t–v). However, they displayed almost a deep and V-shape excisura as well as a more developed antirostrum (see Fig. 5r–s), in comparison to the U-shape and wide excisura in the otoliths from the Persian Gulf (Fig. 5t–v). Based on the previous studies, changes in the excisura angle and shape are mostly influenced by environmental factors (Reichenbacher et al. 2009; Vignon and Morat 2010; Teimori et al. 2012a). The otoliths *U. vittatus* from both the Persian Gulf and the

Gulf of Oman displayed almost the same morphotype.

CONCLUSIONS

It is concluded that otolith shape dimensions have more power to separate the species and genera of the studied Mullidae fishes. Also, the otoliths of the same Mullidae species did not show large variation between the two main studied marine systems, and slight observed differences in the otoliths of the two marine systems are mainly caused by environmental factors, as highlighted in previous studies.

Acknowledgment: We would like to thank Dr. F. Owfi for helping with fish collection and H. Hashemi for providing fish survey facilities. We thank Dr. B.W. Coad (Canadian Museum of Nature) for editing the manuscript and respected reviewers for their constructive and valuable comments.

Authors' contributions: SE collected and analyzed the data, prepared the manuscript, and revised the drafts. HRE contributed to the study design, analyzed the data, prepared the manuscript, and revised the drafts. AT contributed to data analysis, preparing the manuscript, and revising the drafts. MS performed sample collection. All the authors approved the final manuscript. This study was funded by Shiraz University (grant number SU-9531462).

Competing interests: The authors declare that they have no conflicts of interest.

Availability of data and materials: The data generated and analyzed during the present study are available from the corresponding author. The fish specimens are available in ZM-CBSU.

Consent for publication: Not applicable.

Ethics approval consent for participate: The research work was approved by the Ethics Committee of Biology Department (SU-9531462).

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Supplementary Materials

Appendix 1. Morphological characters and states of each character in species used in this study. (download)