



Population structure of the ornate goby, *Istigobius ornatus* (Teleostei: Gobiidae), in the Persian Gulf and Oman Sea as determined by otolith shape variation using ShapeR

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Abstract Otolith shape variation in the Ornate goby, *Istigobius ornatus* (Teleostei: Gobiidae), collected along the intertidal coasts of the Persian Gulf and Oman Sea, was analysed using Wavelet transform technique in the ShapeR package to determine population differentiation and structure. There were significant differences ($P < 0.001$, ANOVA) among geographically distant populations based on the variation in the anterior and posterior otolith rims. However, otolith shapes of neighbouring populations were more similar to each other ($P > 0.01$). Mantel test showed a positive correlation between the Euclidean distance of otolith shape and geographical distances among populations ($r = 0.93$, $P < 0.002$). This indicates that levels in otolith shape resemblance between populations are dependent on geographic distance. Different scenarios are discussed to explain the pattern of otolith shape variation and population structure. Among possible key mechanisms responsible for population differentiations are isolation by distance, Late Pleistocene sea level fluctuations, and

ecological and geographical differences between the studied locations. This study highlights otolith shape efficiency as an exceptionally convenient morphological marker to study intraspecific-level evolutionary and contemporary phenomena in marine fish.

Keywords Gobies · Otolith morphology · Discrete wavelet transform · Population discrimination · ShapeR

Introduction

Characterizing intraspecific variation is highly important to understand distributional range, migration behavior, ecology, systematics and genealogy of a species (Bohlen 2008; Libungan et al. 2015a). Many fish species show considerable intraspecific variation in their morphology, both among and within populations, which often is environmentally induced or behaviorally influenced (Turan 2006; Kocovsky et al. 2013).

Otoliths are hard structures, functionally associated with hearing and the sense of balance (Popper and Coombs 1982; Ramcharitar et al. 2006; Schulz-Mirbach et al. 2019), acoustic communication (Popper and Lu 2000; Cruz and Lombarte 2004), feeding strategy (Lombarte et al. 2010), swimming (Volpedo and Echeverria 2003; Volpedo et al. 2008) and spatial distribution (Gauldie and Crampton 2002; Lombarte and Cruz 2007; Sadighzadeh et al. 2014). Otolith analysis has made significant contributions to the understanding of evolution and phylogenetic relationships of different teleosts (e.g. Gaemers 1983; Nolf 1985; Reichenbacher

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et al. 2007; Reichenbacher et al. 2009a, b; Teimori et al. 2012a, b; Nolf 2013; Teimori et al. 2019), interpretation and explanation of historical fisheries (Van Neer et al. 2002; Limburg et al. 2008), ages and growth rates (e.g. Campana et al. 1985; Green et al. 2009), migration and duration of larval phases (e.g. Lord et al. 2010; Avigliano et al. 2014), as well as several other topics (see Vahed et al. 2018; Motamedi et al. 2019; Vahed et al. 2019). Otoliths can be found in plenty as fossils and are used to reconstruct ancient teleost fish diversity, zoogeography and evolution (e.g. Nolf 1995; Girone and Nolf 2009; Bratishko et al. 2015).

Sagittae, the largest otoliths in most teleosts, have been used extensively in taxonomic (e.g. L'Abée-Lund and Jensen 1993; Ponton 2006; Tuset et al. 2006) and phylogenetic studies in several groups of teleosts (e.g. Gaemers 1983; Nolf 1985; Monteiro et al. 2005; Lombarte et al. 2018; Teimori et al. 2019). Additionally, morphological variability of otoliths is a useful tool to measure fish population structure and to discriminate stocks (Burke et al. 2008; Lord et al. 2011; Reichenbacher and Reichard 2014; Libungan et al. 2015b; Libungan et al. 2016; Wujdi et al. 2017; Rashidabadi et al. 2019). The otolith shape is usually species specific, but intraspecific geographic variation may occur due to environmental factors (Mosegaard et al. 1988; Campana and Thorrold 2001; Reichenbacher et al. 2009a; Libungan et al. 2015a, b). While environmental factors essentially modify the otolith growth rate, which in turn alters the general otolith shape (mainly the length/height ratio), genetically induced changes may locally change otolith shape (Mosegaard et al. 1988; Cardinale et al. 2004; Vignon and Morat 2010; Berg et al. 2018). The extent to which these variations are genetically or environmentally stimulated usually remained unexplored (Cardinale et al. 2004; Annabi et al. 2013).

Three methods to study otolith shape have been applied traditionally: morphometry, landmarks and outline (Rohlf 1990). The outline method using the ShapeR package was first developed and applied for otolith analysis in the Atlantic herring *Clupea harengus* Linnaeus, 1758, but also has been recommended for other teleost fishes (Libungan et al. 2015a, b). Here, we used and tested this method on a gobioid species from the Persian Gulf and Oman Sea.

The Ornate goby, *Istigobius ornatus* (Rüppell, 1830) is widely distributed throughout the tropical Indo-West Pacific including the Persian Gulf and Oman Sea

(Maugé 1984; Randall et al. 2003). It is a benthic spawner species that inhabits lower estuaries, mangroves, silty areas and crevices or interstices in rocky substrate with rubble or muddy substrate and, like other reef gobies, is detritivorous in addition to carnivorous (Cole 1990; Chiu et al. 2018). Due to cryptic behavior, its population structure, phylogeography and ecology along the coasts of the Persian Gulf and the Oman Sea largely remained unexplored. The Persian Gulf and Oman Sea, due to their known paleoclimatic history (Lambeck 1996) and high levels of both ecological and geographical heterogeneities (see Briggs 1974; Reynolds 1993; Carpenter 1997; Manilo and Bogorodsky 2003; Swift and Bower 2003; Béarez et al. 2008; Yao 2008; Thoppil and Hogan 2010a) offer a unique setting for studying the population structure of marine fish species.

The objectives of this work were to (i) evaluate otolith shape variation using an outline method based on a new function in the program R, named ShapeR, and (ii) to evaluate whether the spatial distribution of otolith shape showed a population discrimination meaning in the Ornate goby, *Istigobius ornatus*, in the Persian Gulf and Oman Sea.

Materials and methods

Study area and fish collection

In the intertidal zone, at the northern coasts of the Persian Gulf and the Oman Sea, 187 specimens of *Istigobius ornatus* (23.88–49.56 mm SL, mean: 37.89 mm) were collected at 10–20 cm water depth from November 2012 to September 2017 using hand net (1.30 mm mesh size) (Fig. 1, Table 1). After anesthesia with Quinaldine sulphate (Ross and Ross 2008), the specimens were documented photographically and fixed in 10% formaldehyde; subsequently stored in 70% ethanol for long-term storage. All the collected specimens are deposited in the Zoological Museum of Shiraz University, Collection of Biology Department, ZM-CBSU. Fishes were classified according to sex based on the shape of the genital papilla (Chiu et al. 2018). To reduce the possible effects of ontogenetic variations on otolith morphology data, only sexually mature specimens were used for analysis. Standard length (SL) (in mm) was measured from median anterior point of the upper lip to posterior end of the hypural plate for each

specimen using an Asimeto digital caliper (Miller 1988). Fishes were dissected under a stereomicroscope (Zeiss Stemi SV6) and the left sagittal otolith of each specimen was extracted and cleaned from organic remaining tissue of the macula and the vestibule using fine tweezers and incubation in 5% KOH solution for a few minutes. Otoliths were then washed in distilled water for 30 min and allowed to dry in room temperature before storage in plastic trays.

Otolith imaging and data analysis

The left sagittal otolith of each specimen was placed on a dark plate and digital images with high resolution at a same magnification of 4x were captured using a 14MP Industrial Microscope Camera 180x equipped with an S-EYE 1.2.4.128 image processing system (Fig. 2). The images were stored in JPEG format (*.jpg). Orientation of otolith images with their posterior rim to the left and manipulation on their contrast and brightness (Fig. 2) were implemented using ImageJ 1.50i (<https://imagej.nih.gov/ij/index.html>) and ACDSee Ultimate 9 (<https://acdid.acdsee.com/>). In addition, drawing of the otolith shape was prepared using the CorelDRAW Graphics Suite X8 (<https://www.coreldraw.com/>). For the terminology of otolith, Gierl et al. (2013) and Schwarzhans (2014) were followed (Fig. 2). Statistical analyses were done in the software R 3.6.3 (Team 2020) using the packages ShapeR 0.1–5 (Libungan and Pálsson 2015), Vegan 2.5–6 (Oksanen et al. 2013), Ipred 0.9–9 (Peters et al. 2019) and MASS 7.3–51.5 (Ripley et al. 2020).

Shape analysis

Here we focus on the outline analysis using the ShapeR package (Libungan and Pálsson 2015). To quantify otolith shape variation between populations we used several statistical functions provided in the program R (Team 2020). The otolith images were read into the software R. The ShapeR package analyzes otolith shape by extracting outlines from digital images of the otoliths and elimination of pixel noise (Fig. 2b) (see Libungan and Pálsson 2015).

Following the rotation of all otoliths horizontally along their longest axis, a matrix of x and y coordinates from all otolith outlines was estimated. Equally spaced radii were drawn from the centroid of the otolith to the otolith outline. The length of these radii acts as a

univariate shape descriptor. By using the Wavelet and Fourier transformation on the equally spaced radii, the Wavelet and Fourier coefficients, respectively, were extracted from the digital images using the Wavethresh 4.6.8 package (Libungan and Pálsson 2015; Nason 2016). By using a normalization technique based on regression to remove the allometric growth effect on otolith shape, the Wavelet and Fourier coefficients were scaled with SL and then those coefficients which showed significant interaction between populations and SL ($P < 0.05$), were omitted automatically from the analysis (Longmore et al. 2010; Libungan et al. 2015a). As an output of the ShapeR package, the remaining coefficients were directly imported into the statistical packages in the software R (see Libungan and Pálsson 2015). To get more detailed information of the shape differences between populations at angles of the otolith outline, Wavelet transform has proven to be more useful than Fourier transform (Libungan et al. 2015a).

Mean otolith shape analysis

For visual assessment of otolith shape variation among populations, the average otolith shape of each population was plotted using the Wavelet and Fourier coefficients (Fig. 3). To evaluate which areas of the otolith shape indicated the most variation between populations, mean shape coefficients and their standard deviation of all combined otoliths were plotted against the angle of the outline using Fourier or Wavelet transform by the package gplots 3.0.3 (Warnes et al. 2020; Libungan and Pálsson 2015) (Fig. 4). Since the proportion of variation among groups (the intraclass correlation) provides more information about the partition of the variation along the outline of the otolith, to measure the differences between populations, intraclass correlation was calculated along the outline. Also using the Pearson correlation test, the correlation of the length of each radius that shows the most differences among populations was calculated. Since the plots based on Wavelet and Fourier were similar, solely the outcome of the Wavelet method was used for further analyses.

ANOVA-like permutation test

Using an ANOVA-like permutation test using the package Vegan 2.5–6 (Oksanen et al. 2013), the length of the radii as a univariate shape descriptor was used to

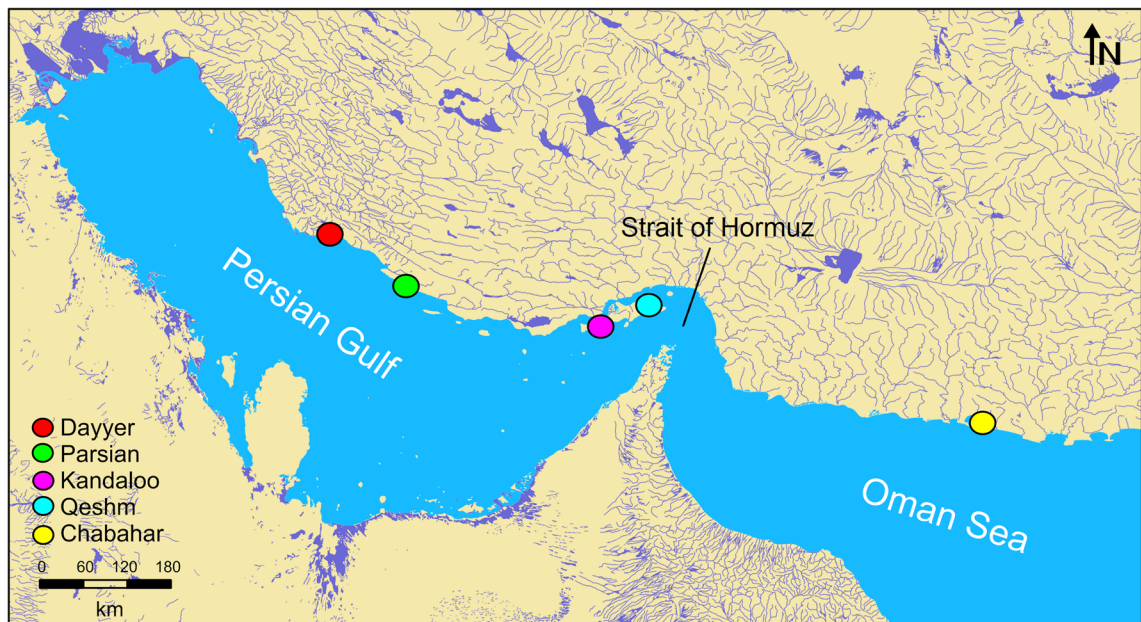


Fig. 1 *Istigobius ornatus* collection sites along the northern coasts of the Persian Gulf and Oman Sea

examine the significance of differences between populations based on 1000 permutations. To recognize if there is a significant difference between otolith shape of male and female and also if it is possible to combine the samples of two sexes, sexual stability in otolith shape was analyzed between sexes within each sampling locality (Table 2).

Canonical analysis of principal coordinates (CAP)

To evaluate the variation in otolith shape among the populations, the standardized Wavelet coefficients were transformed into principal coordinates and subjected to canonical analysis (CAP: Canonical Analysis of Principal coordinates) (Anderson and Willis 2003). The outcome was compared among populations using the

function capscale with the package Vegan 2.5–6 (Oksanen et al. 2013). In addition, Wavelet coefficients were used for visualizing the clustering of the CAP results in two discriminating axes CAP1 and CAP2. Also, a dendrogram based on the CAP outcome was derived by the Squared Euclidean Dissimilarity Distance performed in PAST 3.03 (Hammer et al. 2001).

Correct estimation of individuals to their sampling origin

The classification success into groups was evaluated using a leave-one-out cross-validation estimation. In this regard, the Linear Discriminant Analysis (LDA) on the standardized Wavelet coefficients was applied to show classification of individuals to original populations with

Table 1 Samples of *I. ornatus* from five localities along the coasts of the Persian Gulf and Oman Sea with measurements of standard length of fish specimens (SL, in mm)

Locality	Lat.	Long.	Male (N)	Female (N)	Sum (N)	SL Min.	SL Max.	SL Mean
Dayyer	27°50'	51°53'	18	19	37	35.05	43.85	38.90
Parsian	27°07'	53°01'	19	20	39	33.69	49.19	41.05
Kandaloo	26°40'	55°43'	14	25	39	32.15	46.70	38.10
Qeshm	26°55'	56°15'	19	29	48	23.88	49.56	33.01
Chabahar	25°21'	60°36'	13	11	24	32.62	48.84	38.43
Pooled data	–	–	83	104	187	23.88	49.56	37.89

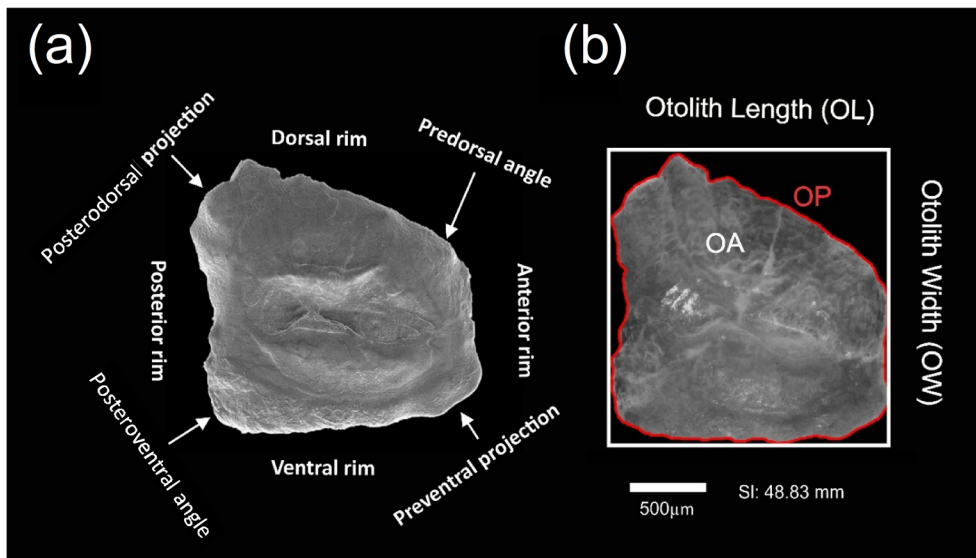


Fig. 2 Close-up view of the left sagittal otolith inner face of *I. ornatus*. a) SEM photo and terminology of characters; b) Otolith shape outline (red line) that marks the shape of the otolith. OA, otolith area, OP, otolith perimeter. Scale bar = 0.5 mm

cross-validation estimation using the functions errorest and lda in the ipred and MASS packages, respectively (Peters et al. 2019; Ripley et al. 2020)

Isolation by distance

The relationship between otolith shape variations and geographical distances among sampling sites was evaluated using the Mantel test (Mantel 1967) with a Pearson correlation test (1000 permutations) in the package Vegan. Therefore, the matrices of average Euclidean

distances based on otolith shape (CAP1 and CAP2) for each population, and the geographical distances between sampling sites (km as unit) were estimated. We used the software Geographic Distance Matrix Generator 1.2.3 (Ersts 2020) to create a geographic distance matrix.

Results

In total, otoliths of 187 individuals (23.88–49.56 mm SL, mean: 37.89 mm) were analyzed. The results of

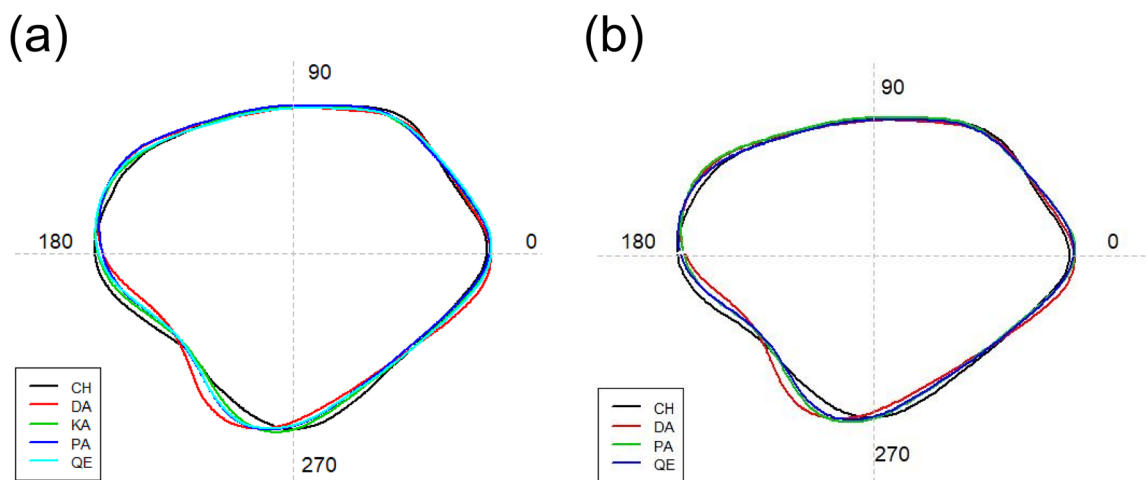


Fig. 3 Mean otolith shapes based on Wavelet reconstruction for the *I. ornatus* populations from five (a) and four (b) sampling sites (CH = Chabahar, DA = Bushehr (Dayyer), KA = Kandaloo, PA =

Parsian, QE = Qeshm). The numbers 0, 90, 180 and 270 indicate angle in degrees (°) on the outline (see Fig. 4)

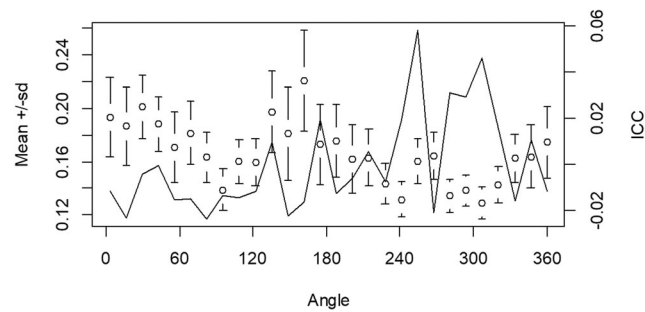


Fig. 4 Mean and standard deviation of the Wavelet coefficients for all combined otoliths and the proportion of variance between *I. ornatus* populations or the intraclass correlation (black solid

line). The horizontal axis shows angle in degrees ($^{\circ}$) as unit based on polar coordinates where the centroid of the otolith is the center point of the polar coordinates (see also Fig. 3)

ANOVA-like permutation test (Table 2) using the radii demonstrated no significant differences between sexes within each sampling site ($P > 0.05$). Likewise, the CAP analysis based on PC1 of the wavelet coefficients detected no differences between the male and female otoliths in each of the five populations (CAP, $P > 0.05$). Therefore, all the samples (male and female) from each sampling site were combined together for further analyses.

Univariate shape analysis between populations based on radii

According to the results of ANOVA-like permutation test, the lengths of the three major radii were statistically different between populations of the Oman Sea and the Persian Gulf ($P < 0.05$, Table 3). The variation in otolith shape between the Chabahar and Dayyer populations was large (F-value = 16.662; $P = 0.001$; see Table 3). On the other hand, the results of ANOVA-like permutation test indicated no significant difference in the otolith shape among Kandaloo and Qeshm from the Persian Gulf ($P = 0.43$), so the samples from these localities were combined together as Qeshm (QE).

Table 2 Variations in otolith shape between fish sex based on ANOVA-like permutation test based on 1000 permutations

Locality	df ^a	Var. ^b	F ^c	p ^d
Dayyer	1	0.36	0.42	0.87
Parsian	1	0.083	1.50	0.16
Kandaloo	1	0.48	0.78	0.48
Qeshm	1	1.23	1.69	0.15
Chabahar	1	1.11	1.02	0.43

^a degree of freedom; ^b variance; ^c F-value; ^d P value. $P < 0.05$ shows significant effect

Univariate analysis of otolith shape using the F and P value among populations demonstrated that the otolith shapes of populations correlated with geographical distances between sampling sites.

Differences of mean otolith shapes between populations based on wavelet

Examination of the mean shapes indicated minor differences between the shape of the otoliths from the geographically close populations Kandaloo and Qeshm (Fig. 3a). Therefore, we combined all specimens from these populations together as Qeshm, and the newly calculated mean shape was provided (Fig. 3b). Analysis of the remaining four populations showed that the mean shape of otoliths based on the Wavelet coefficients differed among the studied populations from the Oman Sea (Chabahar) and the Persian Gulf (Dayyer, Parsian

Table 3 Variations in otolith shape between populations of *I. ornatus* based on 1000 permutations

Comparison	df ^a	Var. ^b	F ^c	p ^d
Dayyer v. Parsian	1	2.272	3.8811	0.015
Dayyer v. Kandaloo	1	5.216	9.8736	0.001
Dayyer v. Qeshm	1	5.972	11.74	0.001
Dayyer v. Chabahar	1	8.3383	16.662	0.001
Parsian v. Kandaloo	1	2.919	5.0762	0.009
Parsian v. Qeshm	1	3.878	7.0463	0.002
Parsian v. Chabahar	1	6.097	10.86	0.001
Kandaloo v. Qeshm	1	0.410	0.8178	0.43
Kandaloo v. Chabahar	1	1.7856	3.6464	0.015
Qeshm v. Chabahar	1	2.072	4.3954	0.006

^a degree of freedom; ^b variance among populations; ^c F-value; ^d P value. $P < 0.05$ shows significant effect

and Qeshm), mainly at the posteroventral angle and the posterodorsal and preventral projections (Fig. 3b). This was confirmed by the partition of the variation along the outline of the otolith at 140–200°, 250–270° and 300–360°, respectively (Fig. 4). Most of the variation occurs at 240–270° angles, which confirms that the posteroventral angle was the most varied angle among the populations. Chabahar in the mean shape is in the outermost part of the posterodorsal projection, moving inwards towards the otolith centroid, Qeshm and Parsian are similar, and then in the innermost part is Dayyer. However, at the posteroventral angle, the pattern is reversed, Dayyer in the outermost part has a shape farthest from the centroid, and moving inwards Qeshm and Parsian have a similar shape at the edge, and then Chabahar in the innermost part has a shape closest to the centroid. This shape indicated that the Dayyer and Chabahar populations have the greatest distance from each other.

These results are confirmed by the multivariate analyses. The first two discriminating axes of the CAP analysis based on the Wavelet coefficients explained 91.5% of the variation between the four populations (CAP 1: 78.5%, CAP 2: 12.8%) and demonstrated clear differences between Chabahar (Oman Sea) and Dayyer (Persian Gulf), and Chabahar and Parsian (Persian Gulf) (Fig. 5). A Box's M test in our data showed that the covariance matrices are homogeneous ($P = 0.128$), which showed that LDA can be used. The overall classification success (Table 4) with a leave-one-out cross-validation estimation based on all samples from the four populations was 55.9%, the highest classification success was achieved for Chabahar (87%).

A hierarchical cluster analysis based on the values of the CAP1 and CAP2 analysis and using the Euclidean distance as a measure of dissimilarity revealed a dendrogram with two main clusters, the Persian Gulf populations, i.e. Dayyer, Parsian, Kandaloo, Qeshm (Fig. 6a) or Dayyer, Parsian, Qeshm (Fig. 6b) in one cluster, and the Oman Sea population (i.e. Chabahar) in the second cluster.

Isolation by distance

Geographically, the populations found in habitats near to each other were more similar in their otolith shape than populations further apart. The Mantel test based on 1000 permutations also confirmed that there was a significant positive correlation between the otolith shape

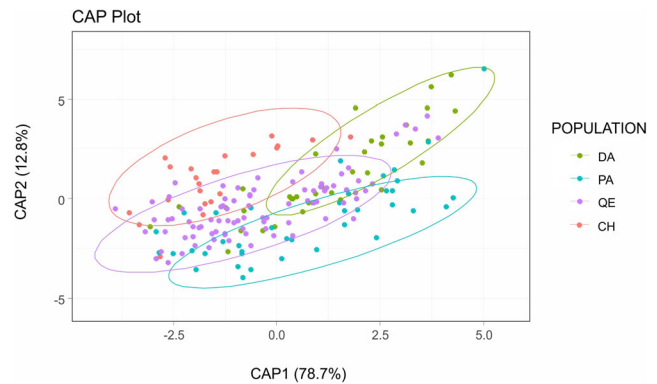
and the geographical distance matrices ($r = 0.93$, $P < 0.002$).

Discussion

The *Istigobius ornatus* populations are distributed in heterogeneous habitats over a large geographical range along the Iranian intertidal coasts of the Persian Gulf and Oman Sea, and hence, this species is a suitable candidate for population studies. Our results based on radii length as well as based on CAP analysis using the Wavelet coefficients demonstrated no significant sex differences regarding otolith shapes, which is consistent with the results of Yu et al. (2014) on five gobiid species from the northern Chinese coastal seawaters. Generally, significant differences in otolith shape between sexes can occur, as has been shown for the trichiurid *Aphanopus carbo* from the Portuguese coast based on elliptic Fourier descriptor analysis (Farias et al. 2009), but for gobiids it has not yet been proven.

All analyses revealed significant differences among the studied *I. ornatus* populations. CAP analyses using the Wavelet coefficients further demonstrated differences among the *I. ornatus* populations that are correlated with their geographical distances, i.e. the otolith shapes of the geographically close populations of *I. ornatus* from the Persian Gulf are more similar to each other than to the more distant Chabahar population in the Oman Sea. These results reinforce the outcome of Sadeghi et al. (in review), who detected high gene flow between four populations of *I. ornatus* along the coast of the Persian Gulf, but a clear separation between these populations and the Oman Sea population using the D-loop marker of mitochondrial DNA. Based on two mitochondrial markers Ghanbarifardi et al. (2018) demonstrated that the populations of *Periophthalmus waltoni* (Teleostei: Gobiidae) along the northern coasts of the Persian Gulf and Oman Sea are divided into two well-separated clades, the Persian Gulf clade and the eastern clade (i.e. the Strait of Hormuz and Oman Sea clade). Likewise, the studies of Ghanbarifardi et al. (2014) and Ghanbarifardi et al. (2020), who analyzed morphometric data of the Walton's mudskipper *Periophthalmus waltoni* Koumans, 1941 and the Indian Ocean slender mudskipper *Scartelaos tenuis* (Day, 1876), respectively, revealed significant differences between the corresponding Persian Gulf and Gulf of Oman populations. Several further studies have indicated

Fig. 5 Canonical scores on discriminating axes 1 and 2 for each *I. ornatus* population. CH = Chabahar, DA = Bushehr (Dayyer), PA = Parsian, QE = Qeshm



population differentiation between these two regions. Sadighzadeh et al. (2014) showed that the otolith shape in John's snapper, *Lutjanus johnii* (Bloch, 1792), exhibits a geographically structured dissimilarity between the two gulfs, which allowed them to consider these populations as separate stocks. According to these authors, the observed dissimilarity in the otolith shape is likely associated with genetic heterogeneity and environmental factors (Sadighzadeh et al. 2014). Similar results have been documented for the *Aphanius dispar* species group (Teimori et al. 2018) (now *Aphaniops*, see Esmacili et al. 2020). Moreover, studies investigating the whole morphology for some other inhabitants of the Persian Gulf and Oman Sea have demonstrated the same geographic structure. An example is the study of Afkhami et al. (2016), who, based on significant morphometric differences among populations of the crab *Leptodius exaratus* (Brachyura, Xanthidae) from the Persian Gulf and Oman Sea, suggested that the Strait of Hormuz represents a significant biogeographic barrier.

Regarding the otolith shape differences between the *I. ornatus* populations along the intertidal coasts of the

Persian Gulf and Oman Sea, four different scenarios can be put forward to explain the observed population structure. It is likely that some of these factors may have acted together to promote otolith shape differentiation among the Persian Gulf and Oman Sea populations.

(i) According to the result from different studies (e.g. L'Abée-Lund and Jensen 1993; Cardinale et al. 2004; Pothin et al. 2006; Vignon 2012; Teacher et al. 2013; Yu et al. 2014; Libungan et al. 2015a), the formation of the otolith shape may be under the control of genetic processes. This was further investigated for *I. ornatus* populations from the Persian Gulf and Oman Sea by Sadeghi et al. (in review/preparation a, b) using the mitochondrial D-loop marker and morphological characteristics. The plasticity in the larval stage duration between geographical populations of a species might correspond to the expression of selected strategies, which are defined as genetically determined life histories or behaviors (Robinet et al. 2007; Reveillac et al. 2008). Some authors have pointed out that the shape of otolith is associated to the traits of early life history during ontogeny, which may reflect the genetic factors (Volpedo and Echeverría 2003; Lord et al. 2011).

(ii) Ghanbarifardi et al. (2018) speculated that the phylogeographic break between the Persian Gulf and Gulf of Oman could be related to the geo-hydrological history of the area during the Late Pleistocene, when inconsistencies of habitat areas due to sea-level fall and rise considerably affected fish populations (Fairbanks 1989). From approximately 63,000 until 18,000 years ago the western basin of the Persian Gulf has been free of water and from the Last Glacial Maximum (LGM) at about 18,000 until 14,000 years ago the Persian Gulf has been free of marine influence out to the Biaban Shelf edge (Randall 1995; Lambeck 1996). The dryness of the Strait of Hormuz during these times probably

Table 4 Classification success (cross-validated) based on Linear Discriminant Analysis of the standardized Wavelet coefficients of the studied *I. ornatus* populations. The numbers in rows are percentages that denote the classification into the population given in columns (correctly classified populations are bold-faced)

Population	Dayyer	Parsian	Qeshm	Chabahar
Dayyer	48.6	21.6	21.6	8.1
Parsian	12.8	66.7	17.9	2.6
Qeshm	20.7	19.5	46.0	13.8
Chabahar	4.3	0	8.7	87.0

Overall classification success (cross-validated): 55.9%

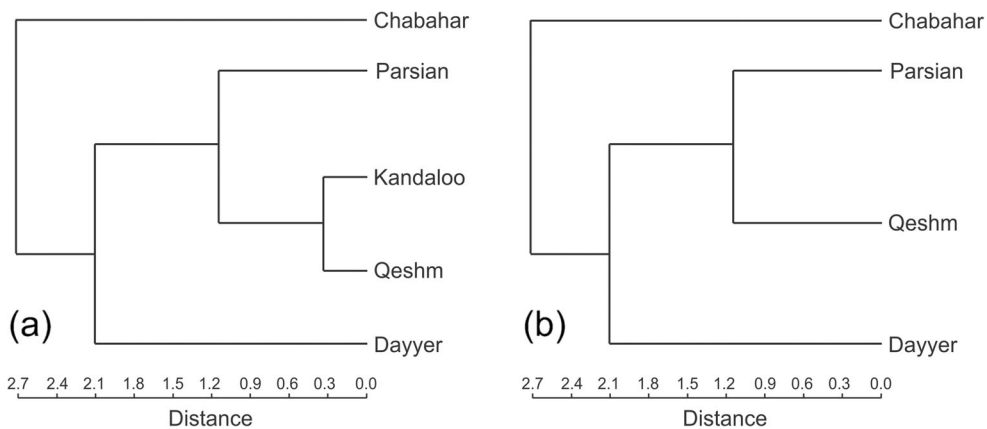


Fig. 6 Relationships between the *I. ornatus* populations from five (a) and four localities (b) using hierarchical Cluster Analysis based on otolith shape data

contributed to the isolation between the Persian Gulf and Gulf of Oman populations of several species, as has been demonstrated for the gobiids *Periophthalmus waltoni* (Ghanbarifardi et al. 2018), *P. argentilineatus* (Polgar et al. 2014), *I. ornatus* (Sadeghi et al. in review and this study), and the blenniid *Istiblennius pox* (Mehraban et al. 2020).

(iii) Marine environments are often regarded as open habitats in which isolation by distance is the main mechanism that may promote differentiation among populations (Palumbi 1994), and patterns of isolation by distance (Wright 1943; Slatkin 1993) has been well-documented in some marine fish species (e.g. Gold et al. 1994; Pogson et al. 2001; Ashe et al. 2015; Barnes et al. 2016; Gonzalez et al. 2016; Drinan et al. 2018). Since our study populations are distributed along a west-east axis at the northern coasts of the Persian Gulf and Oman Sea, we expected geographic isolation to contribute to otolith shape affinities, which was confirmed (Mantel test, see Results). It thus appears that isolation by distance is one of the main mechanism that promoted otolith shape differentiation in *I. ornatus*. Possible reasons are that the fertilized eggs of *I. ornatus* are adhesive and demersal (Patzner et al. 2012; Chiu et al. 2018), and that members of *I. ornatus* seem to avoid the open water, i.e. they often accumulate during the low tide at the margin of water, and in the high tide they hide in the submerged burrows to avoid being attacked by predatory fish (Murdy and Hoese 1985). For these reasons, adults of *I. ornatus* probably do not move between the Persian Gulf and Oman Sea. On the other hand, their larvae have a planktonic life stage (Patzner et al. 2012; Chiu et al. 2018); therefore, passive transport of larvae

via currents within the Persian Gulf and/or the Oman Sea might also have contributed to the dispersion of *I. ornatus*, keeping the geographically close populations similar in their general morphological and genetic features. Similarly, Ghanbarifardi et al. (2014) concluded that the main factor to connect *P. waltoni* populations from different sites within the Persian Gulf or within the Oman Sea and keep them similar, morphologically and probably genetically, is likely to be dispersal of larvae.

(iv) In general, fishes demonstrate greater variances in morphological traits both within and between populations than any other vertebrates (see Hossain et al. 2010; Brraich and Akhter 2015), which could be (amongst others) a result of physical and ecological habitat characteristics (e.g. water temperature, salinity, depth, substrate, diet and niches) (McElroy and Douglas 1995). Therefore, geographically or ecologically separated populations of a species that occupy different habitats may display unique otolith shape throughout their lifetime (Yu et al. 2014). Accordingly, differences in the ecological settings within the Persian Gulf as well as between the Persian Gulf and Oman Sea may explain the here observed population differentiations in *I. ornatus*. Due to its unique oceanographic conditions, the Persian Gulf should be regarded as a distinct ecosystem (Carpenter 1997; Manilo and Bogorodsky 2003) and the Oman Sea should be considered as a zoogeographic boundary between the Western Indian Ocean and the Indo-Polynesian provinces (Briggs 1974; Béarez et al. 2008). This distinctness affects aquatic organisms. The waters of the Persian Gulf and the Oman Sea have markedly different physical and chemical characteristics. The Persian Gulf is a relatively closed

and very shallow sea (average 35 m) connected to the Oman Sea through the narrow Strait of Hormuz (Swift and Bower 2003; Thoppil and Hogan 2010b). In comparison, the Oman Sea is deeper than the Persian Gulf (in most cases deeper than 1000 m), has more stable physical and chemical conditions and an open direct connection to the Indian Ocean (Reynolds 1993). In addition, the Persian Gulf experiences wide seasonal temperature fluctuation of its surface water, low rainfall and high evaporation rates and has a higher level of salinity than the Oman Sea (Yao 2008; Thoppil and Hogan 2010a). Moreover, environmental factors such as temperature and salinity are different in the western part of the Persian Gulf and its other parts, most probably caused by the discharge of major rivers (i.e. Heleh, Zohrehand Arvand) into the western part (Ghanbarifardi et al. 2014). The dendrogram of fig. 6 showed two sub-clusters within the Persian Gulf cluster, the western sub-cluster (Dayyer) and the eastern sub-cluster (Parsian, Kandaloo and Qeshm). Therefore, we might conclude that the observed otolith shape differences between the western and eastern population of *I. ornatus* along the coast of the Persian Gulf may be due to ecological differences.

Conclusion

This study demonstrated the high efficiency of otolith shape analysis (in comparison to otolith morphometry and landmarks analysis) using the Wavelet transform technique to discriminate populations of *I. ornatus* along the northern coasts of the Persian Gulf and Oman Sea. Our results indicated significant variations in otolith shape among the *I. ornatus* populations. Possible reasons for these differences include genetic factors, paleoclimatic history of the study area, isolation by distance, and different ecological settings. It is likely that some of these factors have contributed together to promote otolith shape differences between the Persian Gulf and Oman Sea populations. However, further investigations including habitat and phylogeographic analyses are required to complement these results and evaluate the role of each of these factors. This study could provide a basis for further extensive researches to be done on the otolith shape variation and implementing this highly efficient morphological marker to investigate population structure of gobiid species.

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Data availability Specimens used in the present study are deposited in the Zoological Museum of Shiraz University, Collection of Biology Department, ZM-CBSU.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethics approval The research work was approved by Ethics Committee of Biology Department, Shiraz University (SU-9330207).

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