

Late Pleistocene to Holocene diversification and historical zoogeography of the Arabian killifish (*Aphanius dispar*) inferred from otolith morphology

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SUMMARY: *Aphanius dispar* (Rüppell, 1829) is a common marine-euryhaline teleost fish in the Near East that has undergone considerable intraspecific differentiation. Otolith morphology is used to analyse the diversity within *A. dispar* in the Gulf of Oman (Sea of Oman) and the Persian Gulf. A total of 134 individuals from lagoons and inland habitats of Oman, the United Arab Emirates and southern Iran are analysed. The results revealed that otolith traits that are under genetic control are strikingly different from those that are under the influence of environmental factors. A clear spatial structure of the populations is detectable, suggesting that the environmental flexibility of *A. dispar*, vicariance events during the last glacial maximum (21000-18000 BP), dispersal in the course of the Early Holocene sea-level rise, and Holocene to present-day interruption of gene flow at the Strait of Hormuz have shaped the intraspecific differentiation of *A. dispar*. These factors may also be responsible for diversification within other marine-euryhaline fishes in the Near East and Mediterranean Sea, and thus the findings can contribute to successful conservation management.

Keywords: Cyprinodontidae, otolith, Arabian Sea, Persian Gulf, phylogeography, Pleistocene, biodiversity.

RESUMEN: DIVERSIFICACIÓN E HISTORIA ZOOGEGRÁFICA DEL PLEISTOCENO SUPERIOR AL HOLOCENO EN *APHANIUS DISPAR* DETERMINADA A PARTIR DE LA MORFOLOGÍA DEL OTOLITO. – *Aphanius dispar* (Rüppell, 1829) es un teleosteo eurihhalino común en aguas de Oriente Medio que presenta una considerable variación intraespecífica. El presente trabajo estudia la diversidad de *A. dispar* entre especímenes del Mar de Omán y del Golfo Pérsico a partir de la morfología de los otolitos. Se han analizado un total de 134 individuos procedentes de lagunas salobres y aguas interiores procedentes de Omán, Unión de Emiratos Árabes e Irán. Los caracteres morfológicos de los otolitos regulados genéticamente son distintos de los influenciados por factores ambientales. Se ha detectado una estructura espacial de las poblaciones, sugiriendo una plasticidad ambiental de *A. dispar*, un proceso de vicariancia durante la última glaciación (21000-18000 AP), una dispersión durante el aumento del nivel del mar a inicios del Holoceno y una interrupción del flujo genético en el estrecho de Ormuz desde el Holoceno a la actualidad. Los factores descritos podrían ser los causantes de la diversificación en otros peces eurihhalinos de Oriente Medio y del Mar Mediterráneo y determinarlos podría contribuir a su conservación.

Palabras clave: Cyprinodontidae, otolito, mar Árabe, golfo Pérsico, filogeografía, Pleistoceno, biodiversidad.

INTRODUCTION

The species of the Old-World killifish *Aphanius* (Teleostei: Cyprinodontidae) typically thrive in coastal and freshwater environments along the Mediterranean Sea,

Red Sea, Persian Gulf and Arabian Sea that are not suitable as habitats for other fishes, and thus often lack direct competitors and major predators (Clavero *et al.* 2007).

Aphanius species tolerate a wide range of temperature and salinity regimes, and their small size permits

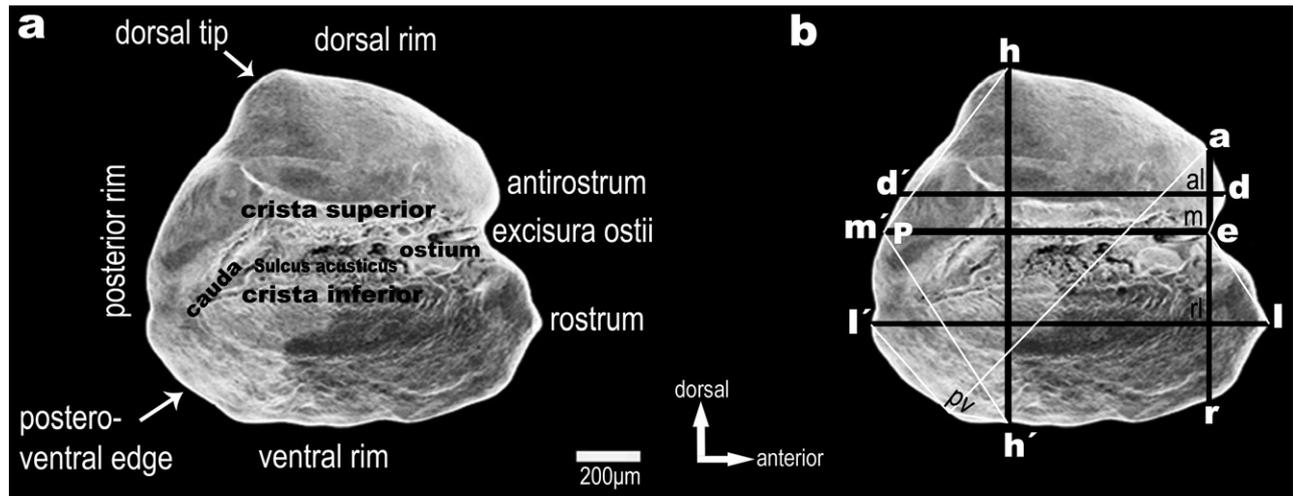


FIG. 1. – Otolith morphology (a) and morphometry (b) of *Aphanius dispar* from a coastal lagoon near Sib, Oman (site 2); otolith is shown in medial view. Terminology of otolith characters follows Nolf (1985); linear measurements and angles after Reichenbacher *et al.* (2007). l-l' length, h-h' height, m-a antirostrum height, m-r rostrum height, rl-l rostrum length, al-d antirostrum length, m-m' medial length, d-d' dorsal length, e excisura angle, p posterior angle, pv posteroventral angle.

viable populations to persist in restricted habitats (e.g. Wildekamp 1993). These abilities also allow the translocation of individuals via drainage shifting and stream capture and promote survival of isolated and relict populations (Echelle and Echelle 1992). This group of fishes therefore represent particularly useful organisms for the study of microevolutionary processes in vertebrates (e.g. Tigano *et al.* 2006; Rocco *et al.* 2007).

The diversity within the Mediterranean and Anatolian species of *Aphanius* has been studied intensively for successful conservation management, reconstruction of phylogeography, and identification of the factors that affect genetic differentiation (e.g. Maltagliati 1999, Tigano *et al.* 2006, Triantafyllidis *et al.* 2007). Recent methods used include analyses of molecular, meristic, morphometric and osteological characters (e.g. Doadrio *et al.* 2002, Hrbek and Meyer 2003, Maltagliati *et al.* 2003), as well as the study of coloration (Wildekamp 1993) and otoliths (Reichenbacher and Sienknecht 2001, Schulz-Mirbach *et al.* 2006, Reichenbacher *et al.* 2007).

However, the Arabian killifish (*Aphanius dispar*), which is quite common in the Near East, has received little scientific attention to date, although molecular data suggest that it does not represent a monophylum (Hrbek and Meyer 2003). The principal habitats of *A. dispar* are coastal lagoons, but also inland waters such as endorheic drainage systems and hot springs (Krupp 1983, Wildekamp 1993, Abdoli 2000). Pronounced intraspecific differences are known to exist in morphological characters of the individuals and otolith traits (Wildekamp 1993, Reichenbacher *et al.* 2009a, Teimori *et al.* 2012a). These differences suggest that some of the landlocked populations have been isolated since the last humid phase of the Holocene, approximately 5500 years ago (Preusser 2009), and perhaps represent species *in statu nascendi*.

Otoliths

Otoliths are aragonitic mineralizations located in the membranous labyrinth of the inner ear of bony fishes. They play an important role in the senses of hearing and balance (see Popper *et al.* 2005 for a recent overview). The saccular otolith (or sagitta) is the largest otolith in most teleost fishes and the main type that has been studied (Fig. 1a). The morphology of this structure is usually genus- and species-specific and therefore used for taxonomic studies (e.g. Nolf 1985, Smale *et al.* 1995, Volpedo and Echeverría 2000, Lombarte *et al.* 2006, Jawad *et al.* 2008, Tuset *et al.* 2008, 2012) and also to examine phylogeny (Knudsen *et al.* 2007, Nolf and Tyler 2006). In addition, variations in otolith size and contour are used in the discrimination of individual populations or stocks (e.g. Campana and Casselman 1993, Torres *et al.* 2000, Stransky *et al.* 2008), and also to detect genetic and environmental influences (Lombarte and Castellon 1991, Lombarte and Lleonart 1993, Lombarte *et al.* 2010).

In the case of *Aphanius*, particular otolith characters related to the length-height-index, rostrum and antirostrum remain stable within a given species also in the case of ecologically different habitats (Reichenbacher *et al.* 2007, Teimori *et al.* 2012a). They can be used to discriminate confidently not only the various coastal Mediterranean *Aphanius* species, but also closely related inland species such as those from Anatolia (Schulz-Mirbach *et al.* 2006) and Iran (Teimori *et al.* 2012b). The consistency of these otolith characters indicates that their formation is under genetic control and is scarcely influenced by environmental factors. Therefore, they can provide valuable information with regard to the diversification and phylogeography of *Aphanius*, and probably also those of other teleost fishes that possess otoliths

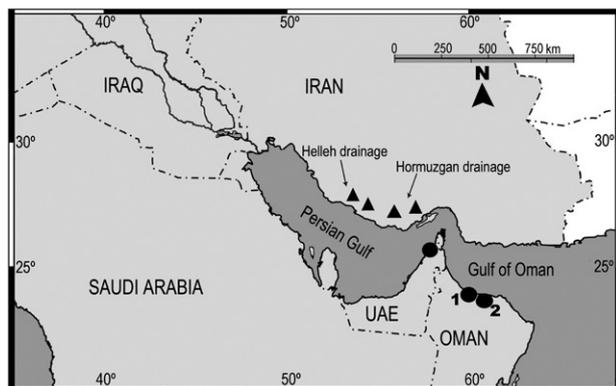


FIG. 2. – Map of sampling locations. Circles indicate coastal sites, triangles landlocked sites. 1 site 1 near Sib, Oman; 2 site 2 near Sib, Oman.

with a clear rostrum and antirostrum (see also Vignon and Morat 2010).

The objective of this study is to evaluate the divergence of coastal and landlocked *A. dispar* populations in the Gulf of Oman (Sea of Oman) and Persian Gulf based on qualitative and quantitative otolith data. Those otolith traits that can be assumed to be under genetic controls were used to analyse the phylogeography of *A. dispar* in the study area. The outcomes significantly enhance our understanding of the intraspecific diversity in *A. dispar*, and are probably also valid for other marine-euryhaline species in the Near East and the Mediterranean Sea.

MATERIALS AND METHODS

Samples collection

A total of 43 specimens of *Aphanius dispar* were collected from two coastal habitats near Sib city, Oman (site 1, 12 males, 14 females, 23°40'19"N, 58°12'21"E; site 2, 7 males, 9 females, 23°40'28"N, 58°11'33"E) (Fig. 2). All specimens were adults with a standard length greater than 20 mm (total length 24.60–39.20 mm). Fishes were preserved in 99.9% ethanol until dissection.

Samples for comparison

A total of 32 specimens (8 males, 24 females, total length 25.0–41.4 mm) were available from a coastal habitat in the United Arab Emirates (25°53'55"N, 56°03'23"E) (Fig. 2; see Reichenbacher *et al.* 2009a). In addition, a total of 60 specimens from southern Iran were studied, i.e. 10 males and 10 females (total length 24.40–39.35 mm) from hot springs within the Helleh Basin (29°24'08"N, 51°16'35"E; 28°47'56"N, 51°16'51"E), and 23 males and 17 females (total length 24.40–42.35 mm) from the Mehran and Shur river drainages within the Hormuzgan Basin (27°08'40"N, 54°15'46"E; 27°04'29"N, 54°29'13"E; 27°31'34"N, 56°28'08"E) (Fig. 2, see Teimori *et al.* 2012a). All

specimens were adults with a standard length greater than 20 mm. Fishes were kept in 99.9% ethanol until dissection.

According to the habitat descriptions for *A. dispar* from the United Arab Emirates and southern Iran (Reichenbacher *et al.* 2009a, Teimori *et al.* 2012a) and own observations at the sites in Oman, the general environmental conditions of the sites are comparable, i.e. all are shallow pools, the water energy is low, temperature and pH are generally within the range of 25–28°C and pH 8.0–9.5, and salinity is about 0.05–3%. However, it is possible that food availability, predator pressure, spawning success or other factors that we could not evaluate during our observations are differing among the sites.

Otolith preparation and analysis

Skulls were opened ventrally and right and left otoliths were removed. Otoliths were cleaned from tissue remains in 1% potassium hydroxide (KOH) solution for 6 h and then rinsed in distilled water for 12 h. Otolith morphology was studied with a stereomicroscope. In addition, morphological comparisons were conducted using SEM images (LEO 1430 VP) of five representative otoliths from each population. For morphometric analyses, only left otoliths were used. They were positioned on plasticine with the lateral face down, and digital images were captured using a Leica DFC 295 camera and the IMAGIC 1000 software. Following the methodology introduced in Reichenbacher *et al.* (2007), eight linear distances and three angles were measured for each of the otoliths (Fig. 1b). Linear measurements were standardized as a function of length and height of otolith, respectively, and, together with the angle values, used as otolith variables in the statistical analyses. Moreover, the groupwise registration approach (Zöllei *et al.* 2005, Balci *et al.* 2007) was used for a comprehensive illustration of the otolith contour of a given population. This method provides a representative image based on the so-called average image. The representative image is close to all images in the data set but is not necessarily one of them.

Statistical analyses

Otolith variables were analysed using SPSS 19.00 (SPSS Inc. 2011) and PAST (PALaeontological STATistics, version 1.81 (Hammer *et al.* 2001)). The *t* test was applied to determine whether otolith variables within a given population exhibit sex dimorphism. One-way ANOVA with Duncan Post-hoc test ($P < 0.05$) was used to test the significance of individual otolith variables. For multivariate analysis of otolith variables, canonical discriminant analysis (CDA) was performed (covariance homogeneity for all groups, all variables analysed together); classification success was tested with jackknifed cross-validation.

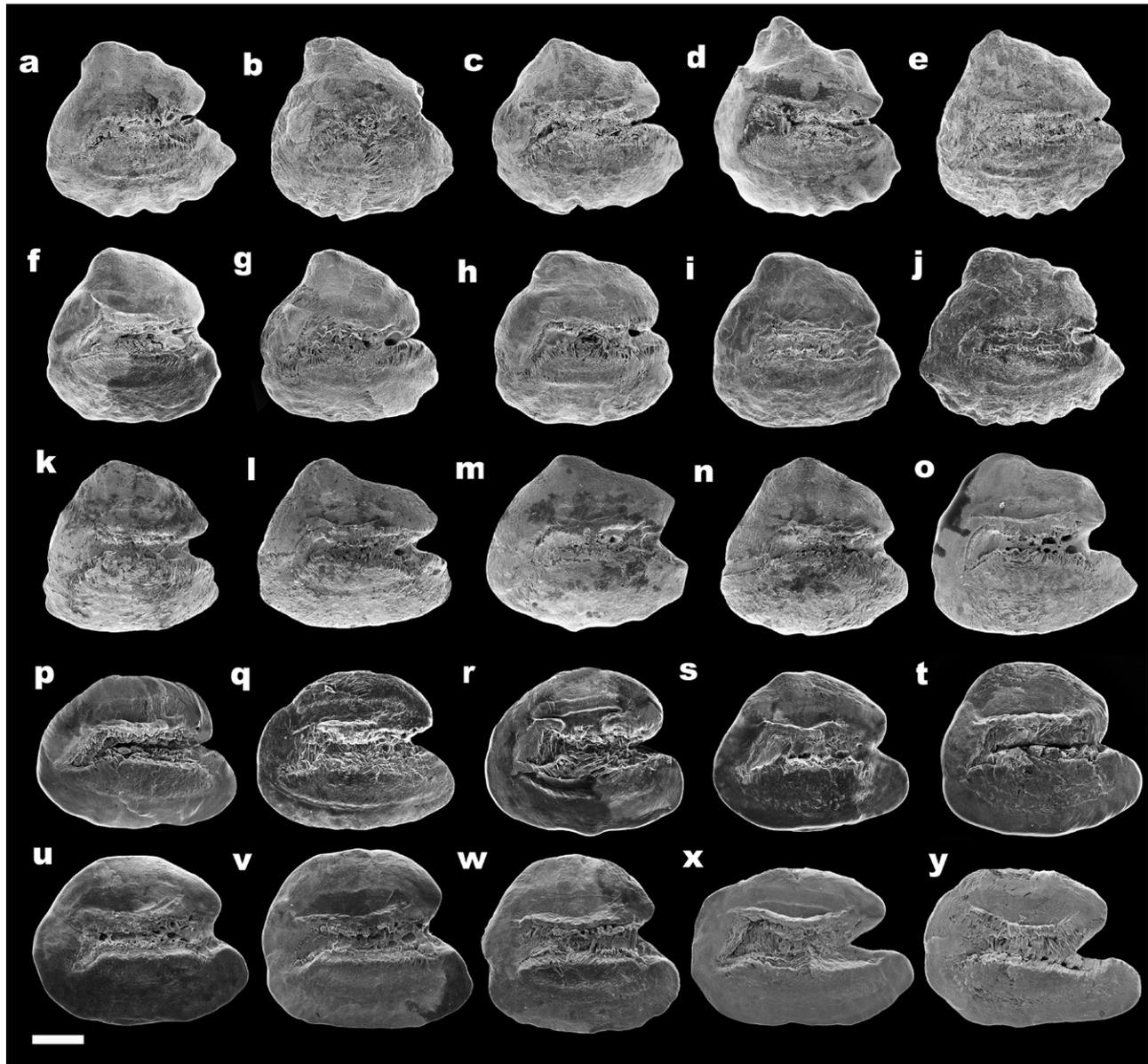


FIG. 3. – Otoliths of *Aphanius dispar* from the studied sites. a-e, site 1 near Sib, Oman; f-j, site 2 near Sib, Oman; k-o, Khor Hulaylah, United Arab Emirates; p-t, Hormuzgan Basin, Iran; u-y, Helleh Basin, Iran. Note presence and absence of a dorsal tip, distinctive shapes and different proportions of rostrum and antirostrum. All images are SEM micrographs, showing left otoliths in medial view. Scale bar 200 μm .

RESULTS

The otoliths of *Aphanius dispar* individuals from the two coastal sites in Oman (near Sib city) were quite similar in morphology (Fig. 3a-j); they are therefore considered here as belonging to one population. Their characters include a trapezoid-triangular shape, a prominent dorsal tip, slightly to strongly crenulated ventral and dorsal rims, a short rostrum and antirostrum, and a very narrow excisura. The otoliths of *A. dispar* from the Helleh and Hormuzgan Basins (southern Iran) (Fig. 3p-y) differed clearly from the Oman population because of their narrow and rounded-triangular shape, absence of a dorsal tip, mostly non-crenulated rims,

and the presence of a long rostrum and prominent antirostrum. The otoliths from the United Arab Emirates (Fig. 3k-o) are somewhat intermediate in morphology because they resemble the Oman specimens with regard to the trapezoid-triangular shape and dorsal tip, whereas their pronounced rostrum and antirostrum and widely opened excisura make them different from the Oman specimens but similar to the southern Iranian otoliths. This intermediate morphology is supported by the multivariate analysis, which plots the otoliths from the United Arab Emirates between the southern Iranian and Oman otoliths (Fig. 4).

In the statistical analyses, males and females were used together as only one otolith character (posterior

TABLE 1. – Values of otolith variables (means and standard deviations) of the studied *Aphanius dispar* populations (one-way ANOVA with Duncan Post-hoc test, $P<0.05$); significantly different values of variables are highlighted in bold; N, number of individuals; m, males; f, females; UAE, United Arab Emirates.

Location	Oman	UAE	Hormuzgan/Iran	Helleh/Iran
N (m/f)	42 (19/23)	32 (8/24)	40 (23/17)	20 (15/13)
Length-height-index ^a	1 (± 0.09)	1.1 (± 0.05)	1.1 (± 0.09)	1.2 (± 0.1)
Relative antirostrum height ^a	22.4 (± 6.2)	29.3 (± 4.1)	29.4 (± 5.2)	32.2 (± 6.2)
Relative rostrum height ^a	41.9 (± 5.6)	44.1 (± 3.2)	44.8 (± 5.1)	49.9 (± 3.1)
Relative rostrum length ^a	14.2 (± 4.3)	17.1 (± 3.4)	15.5 (± 4.4)	22.5 (± 5.8)
Relative antirostrum length ^b	6.4 (± 3.2)	10.7 (± 2.7)	8.3 (± 3.1)	11.4 (± 5.1)
Relative medial length ^b	86.3 (± 4.8)	81.8 (± 4.4)	83.5 (± 5.2)	78.7 (± 5.3)
Excisura angle ^b	120.6 (± 11.1)	105.3 (± 12.6)	115.3 (± 14.2)	104.8 (± 22.3)
Posterior angle ^b	138.8 (± 9.5)	97.6 (± 7.3)	89.4 (± 8.9)	87.6 (± 7.4)
Relative dorsal length	85.6 (± 5.5)	85.9 (± 5.6)	85.9 (± 7.5)	82.3 (± 3.3)
Postero-ventral angle	146.9 (± 8.3)	149.4 (± 7.4)	144.8 (± 8.3)	143.4 (± 7.9)

^a variables that are usually consistent within a species (regardless of the ecological situation) and thus under genetic constrains

^b variables that are known to display variation due to different ecological conditions

angle) showed sexual dimorphism (t test, $P<0.05$). Six otolith variables were significantly different for *A. dispar* from Oman and the Helleh Basin, respectively, whereas a single and no otolith variable was significant for the United Arab Emirates and Hormuzgan Basin populations, respectively (one-way ANOVA with Duncan post-hoc test, $P<0.05$; Table 1). The otolith variables that were significantly different for the Oman and Helleh Basin populations include the length-height index, relative antirostrum height, relative rostrum height, relative antirostrum length and relative medial length. In addition, the posterior angle separated the Oman population from the others, whereas the relative rostrum length was significant for *A. dispar* from the Helleh Basin (Table 1). The high value of the posterior angle in the Oman individuals was related to the position of the dorsal rim slightly behind or in the middle of the dorsal rim (Fig. 3a-j). Additionally, the populations studied were separated with high overall classification success (88.6%) based on the multivariate analysis (CDA) of all otolith variables (Table 2, Fig. 4). The classification was even better for the Oman individuals (92.5%).

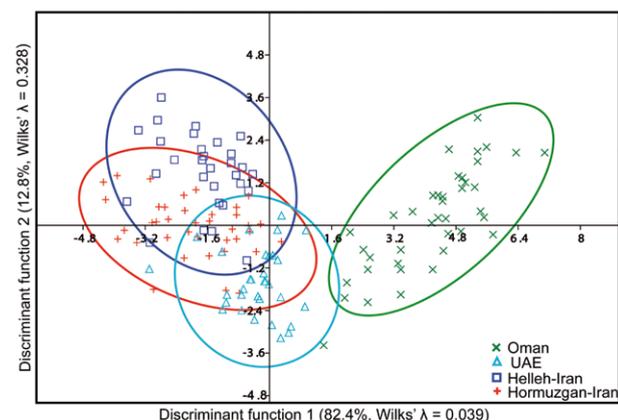


FIG. 4. – Discriminant function scores with 95% ellipses for all otolith variables of *Aphanius dispar* populations.

TABLE 2. – Classification matrix of the CDA (jack-knifed) based on all otolith variables of the studied *Aphanius dispar* populations.

Location	Predicted classification				N
	Oman	UAE	Hormuzgan	Helleh	
Oman	92.5 (38)	5.0 (3)	2.5 (1)	0	42
UAE	0	62.5 (20)	25 (8)	12.5 (4)	32
Hormuzgan/Iran	2.5 (1)	15 (6)	80 (32)	2.5 (1)	40
Helleh/Iran	0	0	20.2 (4)	77.8 (16)	20

The percentages in rows represent the classification into the populations given in columns (correct classifications are highlighted in bold); corresponding numbers of individuals are given in brackets. Overall classification success is 88.6% (Wilks' $\lambda=0.039$ for function 1 and 0.328 for function 2). N, number of individuals; UAE, United Arab Emirates.

DISCUSSION

Environmentally vs. genetically influenced otolith characters

In fish species, some otolith characters may display intraspecific variation resulting from habitat-specific physical or ecological parameters such as water depth, water temperature, habitat productivity and diet availability (e.g. Lombarte and Leonart 1993, Torres *et al.* 2000, Lombarte *et al.* 2010). In the case of *Aphanius*, these environmentally influenced otolith characters comprise the relative antirostrum length, relative medial length, excisura angle, and posterior angle (Reichenbacher *et al.* 2007, 2009b, Teimori *et al.* 2012a). Thus, their variation between *A. dispar* individuals from the studied localities (see Table 1) may indicate some environmental differences between the habitats, e.g. differences in food availability, predator pressure or spawning success.

However, in addition to the otolith variation caused by environmental factors, the individuals of *A. dispar* from Oman and the Helleh Basin are characterized by otolith variables that can be assessed as genetically controlled on the basis of previous studies on *Aphanius* (Reichenbacher *et al.* 2007, 2009a,b, Teimori *et al.* 2012a). These otolith variables include the length-height-index, relative antirostrum height and relative rostrum height

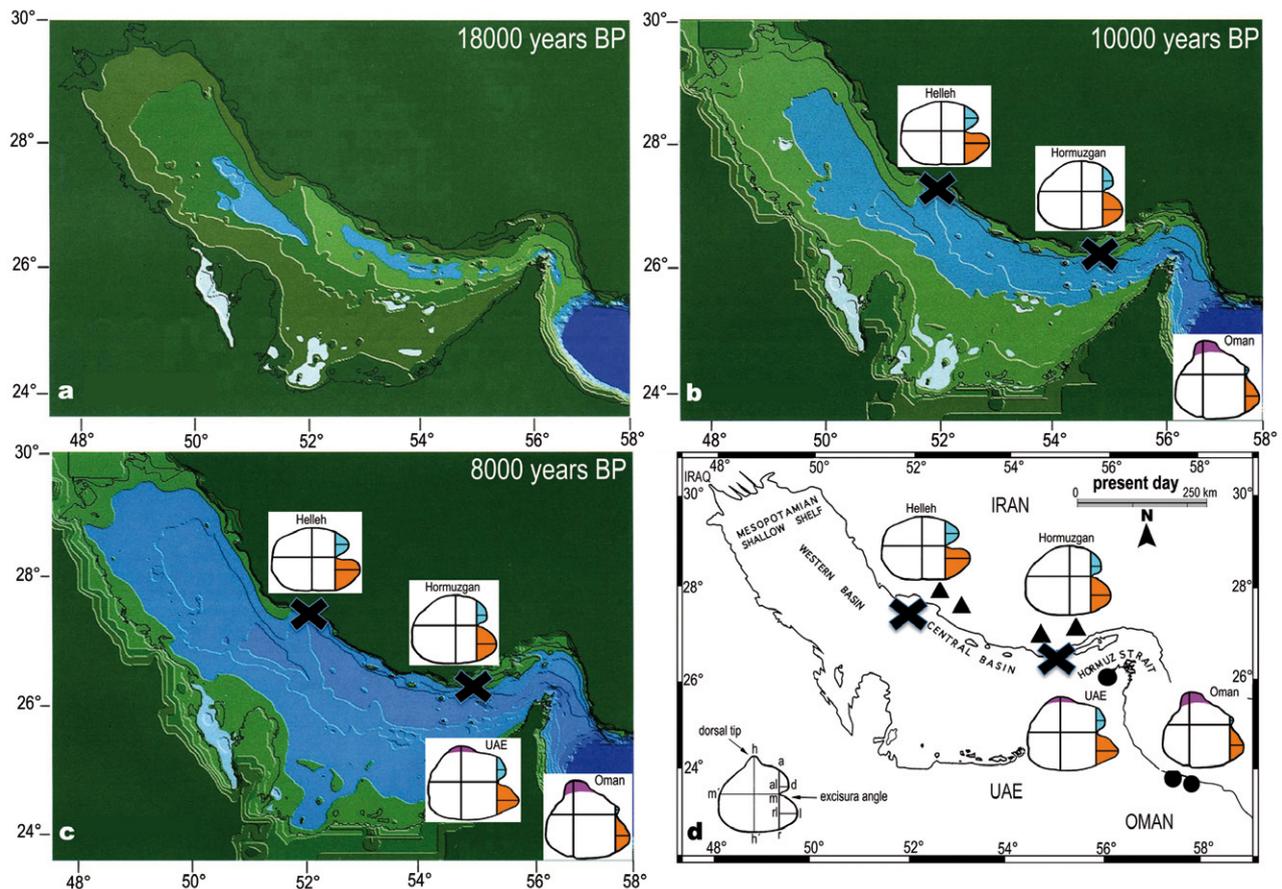


FIG. 5. – Historical zoogeography of *Aphanius dispar* as indicated by genetically constrained otolith characters. **a**, subaerially exposed Persian Gulf sea floor during the last glacial maximum of the Late Pleistocene; **b-c**, Early Holocene re-flooding of the Persian Gulf, black crosses indicating absence of gene flow between *A. dispar* inland populations and immigrants from the Gulf of Oman; **d**, present-day situation of the Persian Gulf, circles showing coastal sites, triangles landlocked sites, black crosses absence of gene flow between *A. dispar* inland populations and coastal *A. dispar* individuals. Otolith images are representative images of the groupwise registration method; linear distances in otolith images represent selected morphometric distances (see inset in d and Figure 1). Source of maps: Purser and Seibold (1973), Lambeck (1996), Teller *et al.* (2000).

(Table 1) and their variation indicates considerable genetic diversification within *A. dispar*. A further line of evidence to support this assumption comes from a study on the coral reef snapper *Lutjanus kasmira* (Vignon and Morat 2010), which found that intra-specific genetic divergence due to long-time separation affects otolith shape locally and re-shapes mainly the antirostrum and rostrum. This finding reinforces the significance of the antirostrum and rostrum variation for a tentative evaluation of intraspecific diversity in *A. dispar*, and probably also in many other teleost species that possess otoliths with an antirostrum and rostrum.

Glacial-postglacial Persian Gulf history and historical zoogeography of *Aphanius dispar*

Our data show a clear spatial distribution pattern of the otolith characters antirostrum and rostrum and additionally of the dorsal tip. A weakly developed antirostrum and rostrum co-occur with a long dorsal tip in the individuals from Oman; a prominent antirostrum

and rostrum, but no dorsal tip are present in the individuals from the Helleh and Hormuzgan Basins; and a moderately developed antirostrum co-occurs with a comparatively short dorsal tip in the individuals from the United Arab Emirates and the Hormuzgan Basin (Figs 3, 5).

We now consider the factors that may have contributed to the development of this spatial pattern within *A. dispar*.

The average depth of the Persian Gulf today is about 35 m, while the maximum depth, restricted to the central basin off the southern coast of Iran, is 100 m (Purser and Seibold 1973). The runoff into the Persian Gulf derives from the Tigris and Euphrates rivers at the northwestern margin, and from rivers draining the Zagros Mountains at the northern central margin (the present-day Helleh and Hormuzgan Basin, southern Iran); almost no runoff comes from the arid Arabian Peninsula (Teller *et al.* 2000).

During the last glacial maximum of the Late Pleistocene (21000-18000 BP), global sea-level was at

–120 m and the Persian Gulf sea floor was subaerially exposed (Sarnthein 1972, Lambeck 1996, Teller *et al.* 2000; Fig. 5a). During that time and until the Holocene sea-level rise in 11000 BP, the Tigris and Euphrates rivers, as well as the rivers from the Zagros Mountains, probably fed a large ancient stream that flowed across the exposed Persian Gulf sea floor to the Hormuz Strait (Lambeck 1996, Teller *et al.* 2000).

It is clear that all stenohaline-marine species must have disappeared from the sea in the Persian Gulf between 21000 and 11000 BP. However, *A. dispar* is a marine-euryhaline species, and today also occurs in rivers and hot springs (see Introduction). It can therefore be hypothesized that the *A. dispar* individuals, which existed in the Persian Gulf sea before the last glacial maximum, could adapt to the changing conditions and were able to thrive in the freshwater environments on the exposed sea floor and also in the rivers coming from Mesopotamia and southern Iran.

The Early Holocene re-flooding of the Persian Gulf around 11000 BP advanced through the Hormuz Strait, but was initially restricted to the deepest part of the central basin off the southern Iranian coast (Lambeck 1996, Teller *et al.* 2000, Smith *et al.* 2011; Fig. 5b). In the course of this flooding new marine species from the Gulf of Oman and the Arabian Sea migrated into the sea of the Persian Gulf (see Hoolihan *et al.* 2004, Kennett and Kennett 2006) and, together with these species, also individuals of *A. dispar*. However, since the last glacial maximum (Late Pleistocene), i.e. for about 10000 years, these immigrants had been isolated from those *A. dispar* individuals that had survived the Late Pleistocene non-marine phase within the Persian Gulf. It is therefore likely that the immigrants did not interbreed with the “native” individuals inhabiting freshwater environments, because of either genetic divergence or environmental constraints. This assumption offers an explanation as to why the individuals in the Helleh Basin, which today live in endorheic systems and hot springs, show the highest divergence of their otolith characters in comparison with the marine individuals from Oman: the former are descendants of the “native” non-marine Late Pleistocene population, while the latter are descendants from the Early Holocene marine immigrants. Notably, the Helleh Basin individuals display not only high divergence of otolith traits, but also high divergence of the morphometric and meristic characters of the fish body (Teimori *et al.* 2012a). The *A. dispar* population in the rivers of the Hormuzgan Basin (which drain to the Persian Gulf) are somewhat transitional between the populations from the Helleh Basin and Oman due to their elongate to rounded shape and medium-sized antirostrum (Figs 3, 5). A possible explanation would be that they are “hybrids” of some ancient non-marine Late Pleistocene individuals and Early Holocene marine immigrants.

By 8000 BP, the southern and westernmost areas of the Persian Gulf had also become marine (Lambeck 1996, Teller *et al.* 2000; Fig. 5c). The fauna of the

now drowned ancient waterway on the Persian Gulf sea floor (including the non-marine populations of *A. dispar*) probably retreated to the lower reaches of the present-day Tigris and Euphrates rivers in Mesopotamia, and the marine immigrants from the Gulf of Oman and Arabian Sea became widespread in the entire region of the Persian Gulf (Hoolihan *et al.* 2004, Kennett and Kennett 2006).

Following this scenario, the existence of genetic differences between present-day *A. dispar* individuals from coastal habitats inside and outside the Persian Gulf is unlikely, as the populations can be assumed to be in contact with one another. However, the results of this study indicate that genetic divergence exists between the coastal individuals from Oman (outside the Persian Gulf) and those from the United Arab Emirates (inside the Persian Gulf) (Fig. 5d). Hoolihan *et al.* (2004) presented similar results for marine sailfish populations (*Istiophorus platypterus*) based on mt-DNA analyses. These authors suggested as the most plausible explanations not only the restricted physical size of the Hormuz Strait, but also the presence of ecological barriers to gene flow such as higher salinity level and more extreme water temperature shifts in the Persian Gulf than in the Gulf of Oman and Arabian Sea. This hypothesis is very appropriate for explaining also the genetic divergence between the coastal *A. dispar* individuals from Oman and the United Arab Emirates reported herein.

CONCLUSIONS

The intraspecific variation of *A. dispar* from the Gulf of Oman and the Persian Gulf, as inferred from otolith differences, can be linked to (i) vicariance events due to the sea-level drop of the last glacial maximum (Late Pleistocene; 21000-18000 BP), (ii) environmental flexibility of *A. dispar*, (iii) dispersal during the Early Holocene sea-level rise (11000-8000 BP), and (iv) Holocene to present-day ecological barriers to gene flow at the Hormuz Strait. The most distant *A. dispar* populations are those from the Gulf of Oman and the Helleh Basin in southern Iran; these populations have probably been isolated since the last glacial maximum, i.e. for about 20000 years. The Helleh populations may be closely related to the present-day *A. dispar* populations of the Tigris and Euphrates river systems in Iraq, with which they may have shared the freshwater habitats on the exposed Persian Gulf sea floor between the Late Pleistocene last glacial maximum and Early Holocene sea-level rise. Present-day divergence between coastal populations in and outside the Persian Gulf most likely results from ecological barriers to gene flow at the Hormuz Strait, as suggested in a previous study on a marine sailfish species. The same factors that have shaped the pattern of diversity within *A. dispar* have probably also formed within-species diversity of other marine-euryhaline fish species in the Persian Gulf, the Near East and the Mediterranean Sea. Study

of their otolith differences should be a suitable tool for contributing to a successful conservation management of these species.

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