

# The evolutionary history and taxonomy of *Aphanius* (Teleostei: Cyprinodontidae) species in Iran and the Persian Gulf region



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## Summary

The objective of this PhD project was to investigate evolutionary history and taxonomy of the *Aphanius* species in Iran, the Persian Gulf region and Gulf Oman. A comprehensive data set including morphological characters of fish body (i.e., morphometric and meristic characters), otolith morphology and morphometry, scale and teeth morphology, osteological characters as well as molecular data of mt-DNA sequences were analysed to study this objective. Considering the Iranian inland species, the results showed absence of strong interspecific differences in the external morphology, but differentiation of morphology in the multivariate space, and also differentiation of otolith morphology and mt-DNA sequences. In the case of *A. dispar* in the Persian Gulf region and Gulf of Oman, a clear intraspecific geographic differentiation is visible in external morphology, otolith morphology and mt-DNA sequences. Both the interspecific variation of inland *Aphanius* species and intraspecific differentiation of *A. dispar* are consistent with their biogeographic distribution and historical zoogeography.

Furthermore, my studies contributed to the recognition and description of three species (*A. pluristriatus* (Jenkins 1910); *A. arakensis* Teimori et al., 2012 and *A. furcatus* Teimori et al. subm.) and inferring their phylogenetic relationships and evolutionary history. Taking into account the newly discovered species in this study, the number of endemic *Aphanius* species in Iran increased from 6 to 9 (*A. vladykovi*, *A. isfahanensis*, *A. sophiae*, *A. farsicus*, *A. pluristriatus*, *A. arakensis*, *A. mesopotamicus*, *A. ginaonis* and *A. furcatus*). The Iranian plateau can be considered now as a center of speciation for *Aphanius* species and it is likely that additional species are present in remote areas, especially in the Zagros and Alborz Mountains.

The noticeable features of this diversification include clear genetic differentiation and also clear differences in otolith morphology, whereas only weak differences are present in the external morphology, morphometry and meristics. The only species that shows a unique morphology is the newly described *A. furcatus* from the Hormuzgan Basin in southern Iran.

In addition, the otolith morphology is recognized as a suitable tool to infer phylogenetic relationships between the studied *Aphanius* species. This may be related to the function of otoliths as parts of the inner ear as the otoliths provide a

mechanism for measuring motion and position of the head relative to gravity. This means that differences in otolith morphology can reflect changes in intraspecific communication and behaviors in fishes that may have acted as evolutionary pressures and consequently promote speciation. I have suggested the following evolutionary forces that probably have played an important role in diversification of Iranian *Aphanius* species:

**(i) Geological-based speciation.** The active geology of Iran, resulting from collisions of the Arabian with the Iranian plate during the Late Miocene (5–10 million years), Pliocene (1.8–5 million years) and Pleistocene (10,000 y.–1.8 million years) has led to rapid isolation of multiple areas owing to physical features such as migration barriers and changing hydrological networks. These events provided excellent conditions for the speciation of *Aphanius* species in Iran, particularly in the region of Zagros Mountains, where the number of *Aphanius* species is considerably high.

*Aphanius pluristriatus* is a good example of the geological-based allopatric speciation of inland *Aphanius* in Iran. At present it is restricted to the drainage system of the Mond River, but it originated from an ancient *A. sophiae* population in the Kor River Basin. During the Quaternary the Kor River and the Mond River were draining to the Persian Gulf, but in the Late Quaternary or Holocene, the Kor River was disconnected from the Persian Gulf as a result of tectonic uplift in the Zagros Mountains (the Kor River Basin is endorheic today). Thus, *A. pluristriatus* most likely is the relict of an ancient *Aphanius* population from the Quaternary Kor River drainage system.

*Aphanius dispar* is a further example for geological-based allopatric speciation. Based on my results this species displays clear intraspecific differentiation. I hypothesize that fragmentation of its habitats resulting from regional geological events during the Pleistocene, its environmental flexibility, vicariance events during the last glacial maximum (21,000-18,000 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 its diversification and also have led to the evolution of *A. ginaonis*.

**(ii) Sensory-driven speciation.** As it is usual in cyprinodontids, *Aphanius* species show clear sexual dimorphism with regard to body coloration. Based on the observed variation of flank bar numbers among males of inland *Aphanius* species, I hypothesize that flank bar patterns have played an important role in sexual selection. The specific male flank bar patterns in the individual *Aphanius* species may have evolved as a response to different light regimes in the respective habitats for increasing contrast and optimizing intraspecific communication such as mating preference. It can therefore be suggested that sensory-driven speciation might have played an additional role in *Aphanius* speciation.

**(iii) Regressive evolution.** In addition to the allopatric speciation that has played a major role in diversification of *Aphanius* species in Iran, I suggest that regressive evolution may also play a role in the evolution of the Iranian *Aphanius* species. *Aphanius furcatus* sp. nov. is a newly discovered species from the saline Shur River and hot sulphuric springs in the drainage of the Hormuzgan Basin in Southern Iran. It differs from all Iranian *Aphanius* species by the complete absence of scales, and from all known *Aphanius* species by its slightly forked caudal fin. In addition, *A. furcatus* demonstrates reduction in biomineralization of hard structures, particularly in the caudal skeleton and jaw teeth. This new species is thriving in extreme environments characterized by low oxygen, high water temperatures, high salt concentration and lack of predators. I have assumed that the reductive phenomena observed in *A. furcatus* have evolved as an evolutionary response to the extreme habitat conditions in order to save energy (because storage of  $\text{Ca}^{2+}$  is not necessary), and to transport oxygen efficiently. In addition, I confirm the assumption that reductive phenomena in *Aphanius* have occurred during multiple independent events.

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## 1. Introduction

### 1.1. Study system: genus *Aphanius* Nardo, 1827

The Old-World killifish genus *Aphanius* Nardo, 1827 (Teleostei: Cyprinodontidae) is the only genus of the Cyprinodontidae in Europe, Arabian Peninsula, and the drainages of the Persian Gulf (Villwock, 1999). Twenty five nominal species of *Aphanius* have been described until to date (Eschmeyer and Fong, 2013). As it is usual in cyprinodontids, all species show a clear sexual dimorphism, the males display vertical flank bars and the females show patterns of spot-like pigmentation.

Most species tolerate a wide range of temperature and salinity regimes, and their small size permits viable populations to persist in restricted habitats (e.g., Wildekamp, 1993). These abilities also allow the translocation of *Aphanius* via drainage shifting and stream capture and promote survival of relict populations (see Echelle and Echelle, 1992). A few *Aphanius* species are known to show some reductive phenomena. Examples are the various degrees of reduction or complete absence of scales in Anatolian *Aphanius* species (Grimm, 1980; Villwock, 1982), and the absence of pelvic fins in *A. apodus* from Algeria (Parenti, 1981; Sienknecht, 1999). *Aphanius* therefore represents a particularly interesting model for the study of evolutionary processes in vertebrates (e.g., Villwock, 1976; Tigano et al., 2006; Rocco et al., 2007).

Considering the habitat and distribution, *Aphanius* species typically thrive in coastal (brackish) and inland environments along the Mediterranean Sea, Red Sea, Persian Gulf and Arabian Sea that are not suitable as habitats for many other fishes, and thus often lack direct competitors and major predators (Clavero et al., 2007). The distribution areas represent former regions of the Tethys Sea (Hrbek and Meyer, 2003). Coastal distribution of *Aphanius* includes the regions of the Mediterranean Sea, Red Sea and Persian Gulf, the Gir peninsula of northwestern India up to northeastern Somalia (Kosswig, 1967; Villwock, 1999). Inland distribution is restricted to the Mediterranean and regions of the Near East including Turkey and Iran.

The greatest species diversity appears in the Near East, especially Turkey and Iran (Wildekamp et al., 1999; Coad, 2000; Hrbek and Meyer, 2003; Hrbek et al., 2006). Nine endemic and one widely distributed species (*A. dispar*) occur in drainages of Iran (e.g., Coad, 1988, 2000, 2009; Hrbek et al., 2006; this study).

Nevertheless, because of the small-scale spatial distribution in this group, additional not yet described species may occur in land-locked sites, high mountains and remote areas (Coad, 2000, 2009; Coad and Abdoli, 2000; Reichenbacher et al., 2009a).

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., Villwock, 1964; Maltagliati, 1999; Wildekamp et al., 1999; Tigano et al., 2006; Triantafyllidis et al., 2007; Hrbek et al., 2002). 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).

The morphology of the saccular otolith (or sagitta) is usually genus- and species-specific and therefore used for taxonomic studies (e.g., Nolf, 1985; 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 to detect genetic and environmental influences (Lombarte and Castellon, 1991; Lombarte and Leonart, 1993; Lombarte et al., 2010).

## **1.2. Iranian Inland *Aphanius***

The Iranian inland *Aphanius* species are currently distributed in the drainages of Central-, South-, West- and North-Iran. They are suggested to be relicts of the Tethys Sea (Kosswig, 1967; Bianco, 1995), having been trapped by the rising Iranian Plateau, i.e. the Zagros and Alborz mountain ranges. The most northerly and easterly distribution is the remote and endorheic Damghan drainage of the Alborz Mountains (Coad, 1996; Coad and Abdoli, 2000); the most southern distribution has been recorded for populations in the drainage of Hormuzgan drainage (observation of author in field works). Generally, it is thought that the current zoogeography has been shaped by the geological history of the Zagros and Alborz mountain systems (Coad and Abdoli, 2000; Hrbek et al., 2006).

The presence of many isolated populations with uncertain taxonomic status, the difficulties to access the remote sites, the high variability between species (different

colour patterns, external morphology and genetic characters) makes it difficult to understand the evolutionary and taxonomic relationships. Examples are the populations from Namak Lake and Damghan basins (Seyfali et al., 2002; Coad and Abdoli, 2000) (see also Fig. 1). Since these variabilities, particularly different colour patterns are used in mate recognition; therefore the isolated populations could probably be considered as distinct taxonomic units such as species. *Aphanius ginaonis* (Holly, 1929) from the Genow hot sulphuric spring in southern Iran is also considered here as inland species.



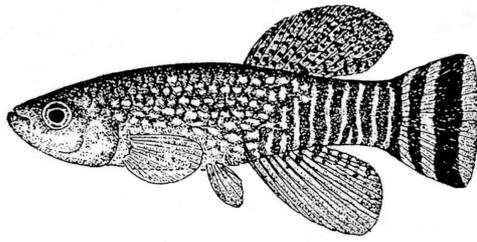
**Figure 1** Morphology and colour pattern of some Iranian inland *Aphanius* species. *Aphanius arakensis* from Namak Lake Basin (a); *A. farsicus* from Maharlu Lake Basin (b); *A. pluristriatus* from Mond River Basin (c); *A. sophiae* from Kor River Basin (d); *A. vladykovi* from Tigris Basin (e). Number 1 refers to female and number 2 refers to male. The photos of *A. arakensis*, *A. farsicus* and *A. pluristriatus* are from joint works of A. Teimori, H.R. Esmaeili, Z. Gholami and B. Reichenbacher. The photos of *A. sophiae* and *A. vladykovi* are from H.R. Esmaeili. Scale bar: 1 cm.

### 1.3. Iranian brackish water *Aphanius dispar*

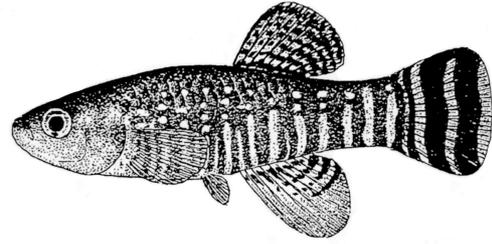
*Aphanius dispar* (Rüppell, 1829) is a brackish water *Aphanius* species, which is characterized by a moderately slender body with long dorsal and anal fins and a truncated to rounded caudal fin. It is the most widespread taxon among the species of *Aphanius* and is known to show considerable morphological variation (see Fig. 2). The principal habitats of *A. dispar* are coastal lagoons, but also inland waters such as endorheic drainage systems and hot sulphuric springs (Krupp, 1983; Wildekamp, 1993; Abdoli, 2000). The native distribution area ranges from the Red Sea and Persian Gulf to the Indian Ocean and southern coastline of Pakistan (Wildekamp, 1993). In addition, populations of *A. dispar* occur in the south-eastern portion of the Mediterranean basin, both in brackish water environments, as well as in Egyptian and Arabian fresh-water systems, and springs of the hypersaline Dead Sea Valley (Hrbek and Meyer, 2003; Reichenbacher et al., 2007). In Iran, *A. dispar* inhabits the coastal environments from the most south-western (border of Iran-Iraq) to the most south-eastern drainages (border of Iran-Pakistan).

The diversity within the Mediterranean and Anatolian species of *Aphanius* has been studied intensively (e.g., Villwock, 1964; Maltagliati, 1999; Wildekamp et al., 1999; Tigano et al., 2006; Triantafyllidis et al., 2007, Hrbek et al., 2002). However, *A. dispar* has received little scientific attention in previous studies although pronounced intraspecific differences were recorded for morphological characters and otolith traits (Wildekamp, 1993; Reichenbacher et al., 2009a)

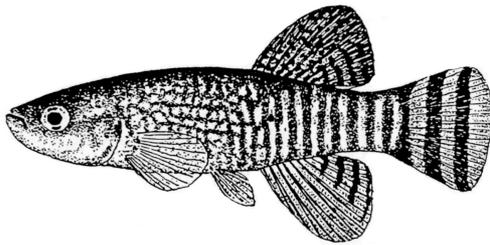
Hrbek and Meyer (2003) used mt-DNA data of five *A. dispar* populations to distinguish between two major *A. dispar* clades, that is, the Persian Gulf and the Red Sea clade. Moreover, the mt-DNA data strongly argue against monophyly of *A. dispar* because the Red Sea clade also includes *A. dispar richardsoni* (Boulenger, 1907), while the Persian Gulf clade also includes *A. ginaonis* (Holly, 1929). As a result, Hrbek and Meyer suggested that *A. dispar* does not constitute a species in terms of the phylogenetic species concept.



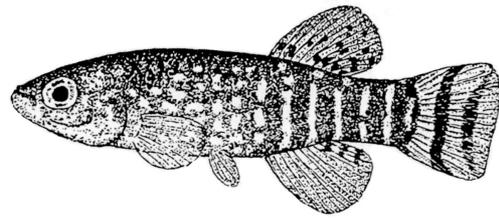
*Aphanius dispar*  
(Saudi Arabia)



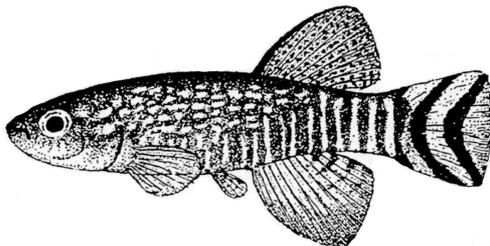
*Aphanius dispar*  
(Northwestern Egypt)



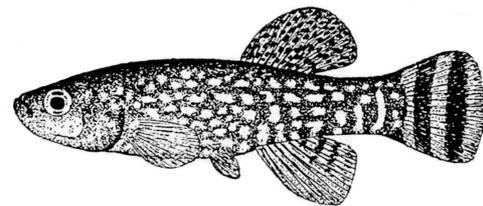
*Aphanius dispar*  
(Northern Somalia)



*Aphanius dispar*  
(Abaytou, Affars and Issas)



*Aphanius dispar*  
(Southern Iran)



*Aphanius dispar*  
(Dead sea drainage)

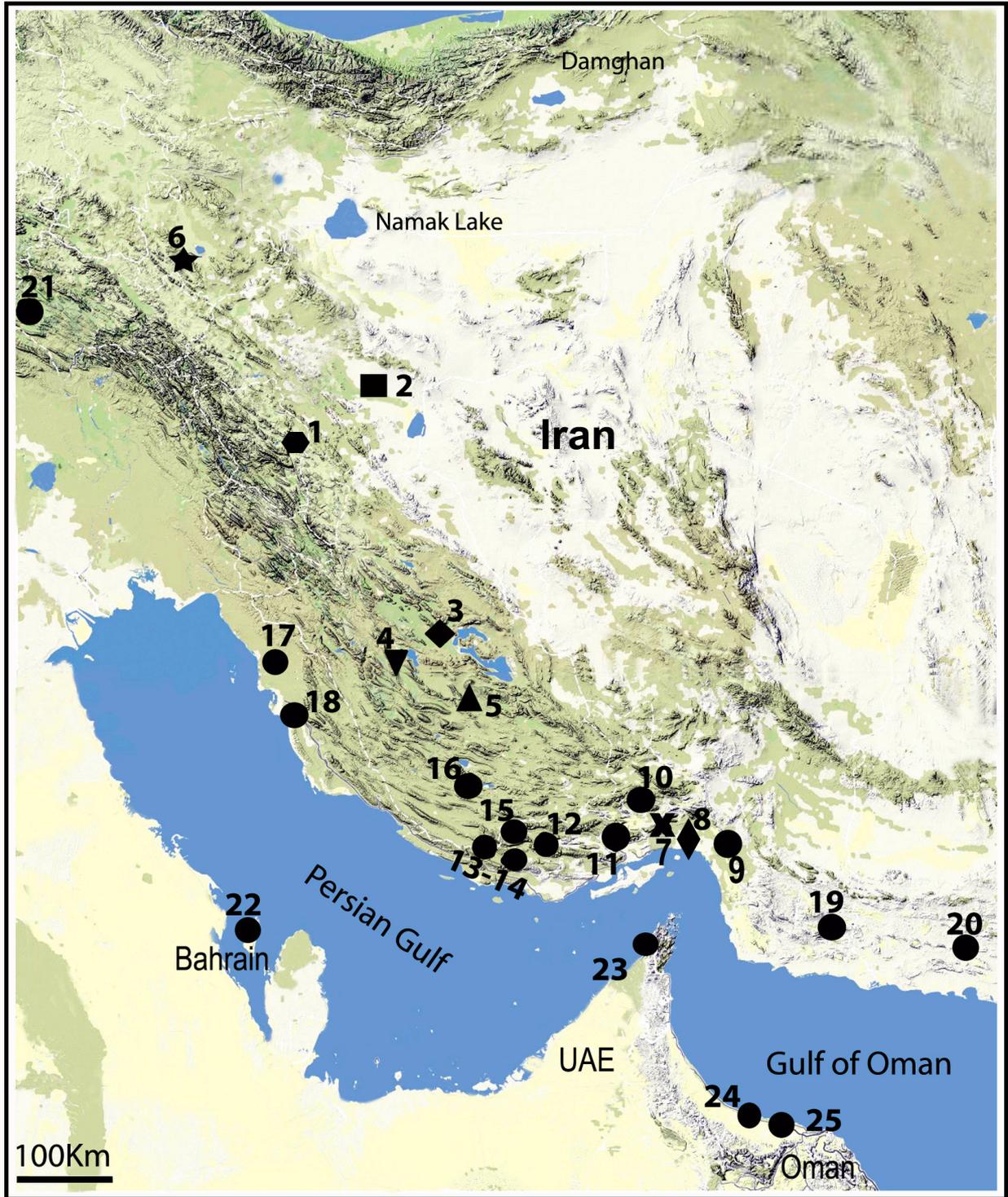
**Figure 2** Morphological variability among allopatric *Aphanius dispar* populations (from Wildekamp 1993).

#### 1.4. Objective

The aim of this PhD project is to investigate evolutionary history and taxonomy of the *Aphanius* species in Iran, the Persian Gulf region and Gulf Oman. For this objective, a comprehensive data set including morphological characters of fish body (i.e., morphometric and meristic characters), otolith morphology and morphometry, scale and teeth morphology, osteological characters as well as molecular data of mt-DNA sequences was analysed.

## **2. Materials**

The specimens were collected by using hand net from selected drainage systems in Iran (Fig. 3, Table 1) (see also Teimori et al., 2011 (appendix 1); Esmaeili et al., 2012 (appendix 2); Teimori et al., 2012a-c (appendixes 3-5); Teimori et al. Submitted (appendix 6)). The studied material is deposited in the Collection of the Biology Department of the Shiraz University (ZM-CBSU), and in the collection of the Zoological Museum of Shahid Bahonar University of Kerman (ZM-SBUK), Iran.



**Figure 3** Distribution and sampling sites for Iranian inland *Aphanis* species and the *A. dispar* populations in Persian Gulf region and Gulf Oman. 1, *Aphanis vladkovi* (□); 2, *A. isfahanensis* (■); 3, *A. sophiae* (◆); 4, *A. farsicus* (▼); 5, *A. pluristriatus* (▲); 6, *A. arakensis* (★); 7, *A. ginaonis* (✕); 8,10,11,13,15, *A. furcatus* (◆); 9-25, *A. dispar* (●). Map Source: Google map 2013.

**Table 1** Overview of sampled sites, habitat types and corresponding drainage basins. N, number of collected specimens (number of females/males); SL, standard length (in mm); S.D., standard deviation. The *A. dispar* population from site 23 is from Reichenbacher et al. (2009a).

Sampled sites	Species	Habitat types	Drainage-Basin	GPS data	N (♀/♂)	Mean SL ± S.D.
<b>Inland <i>Aphanius</i></b>						
Chaghakhor (1)	<i>A. vladykovi</i>	Wetland	Tigris	E 50°, 56', 32.0" N 31°, 55', 30.7"	70 (35/35)	29.85 ± 3.25
Zayandeh River (2)	<i>A. isfahanensis</i>	River	Esfahan	E 52°, 39', 14.3" N 32°, 25', 26.3"	43 (25/18)	27.25 ± 2.85
Ghadamgah (3)	<i>A. sophiae</i>	Spring-stream	Kor River	E 52°, 25', 36.4" N 30°, 15', 23.0"	70 (35/35)	32.56 ± 3.80
Barm-e-shur (4)	<i>A. farsicus</i>	Spring-stream	Maharlu	E 52°, 42', 0.05" N 29°, 27', 9.51"	157 (78/79)	32.68 ± 2.95
Zarjan (5)	<i>A. pluristriatus</i>	Spring-stream	Mond River	E 53°, 38', 21.3" N 29°, 04', 37.3"	60 (28/32)	30.45 ± 3.40
Arak (6)	<i>A. arakensis</i>	Spring-stream	Namak Lake	E 49°, 50', 49.0" N 34°, 00', 34.2"	70 (35/35)	28.95 ± 3.65
Genow/ Iran (7)	<i>A. ginaonis</i>	Hot sulphuric spring	Hormuzgan	E 56°, 17', 97.0" N 27°, 26', 77.2"	10 (4/6)	30.5 ± 2.65
<b>Brackish water species (<i>Aphanius dispar</i>)</b>						
Shur/ Iran (8)	<i>A. dispar</i>	River	Hormuzgan	E 56°, 28', 10.2" N 27°, 19', 37.6"	10 (5/5)	30.6 ± 3.40
Rudan/Iran (9)	<i>A. dispar</i>	River	Makran	E 57°, 15', 14.5" N 27°, 28', 24.4"	42 (20/22)	28.5 ± 3.45
Khurgu/ Iran (10)	<i>A. dispar</i>	Hot sulphuric spring	Hormuzgan	E 56°, 28', 08.2" N 27°, 31', 34.1"	10 (5/5)	31.9 ± 3.80
Kol/ Iran (11)	<i>A. dispar</i>	River	Hormuzgan	E 55°, 45', 31.2" N 27°, 07', 40.3"	10 (5/5)	28.6 ± 3.70
Rasul/ Iran (12)	<i>A. dispar</i>	River	Hormuzgan	E 54°, 36', 30.5" N 27°, 16', 13.2"	10 (5/5)	27.7 ± 2.80
Mehran 1/ Iran (13)	<i>A. dispar</i>	River	Hormuzgan	E 54°, 15', 46.1" N 27°, 08', 39.8"	10 (5/5)	29.4 ± 3.35
Mehran 2/ Iran (14)	<i>A. dispar</i>	River	Hormuzgan	E 54°, 29', 13.1" N 27°, 04', 28.7"	10 (5/5)	33.5 ± 3.50
Faryab/ Iran (15)	<i>A. dispar</i>	Hot sulphuric spring	Hormuzgan	E 54°, 16', 28.0" N 27°, 25', 16.2"	10 (5/5)	29.6 ± 5.30
Howba/ Iran (16)	<i>A. dispar</i>	Hot sulphuric spring	Hormuzgan	E 53°, 53', 58.4" N 27°, 57', 30.5"	10 (5/5)	32.0 ± 4.00
Dalaki/ Iran (17)	<i>A. dispar</i>	Hot sulphuric spring	Helleh	E 51°, 16', 35.4" N 29°, 24', 07.9"	10 (5/5)	33.0 ± 2.45
Mirahmad/ Iran (18)	<i>A. dispar</i>	Hot sulphuric spring	Helleh	E 51°, 16', 50.9" N 28°, 47', 56.4"	31 (16/15)	28.9 ± 4.40

**Table 1** Continued

Sampled sites	Species	Habitat types	Drainage-Basin	GPS data	N (♀/♂)	Mean SL ± S.D.
<b>Brackish water species (<i>Aphanius dispar</i>)</b>						
Irandedgan/ Iran (19)	<i>A. dispar</i>	Qanat	Mashkid	E 62°, 24', 50.9" N 27°, 18', 13.4"	6 (female)	29.3 ± 2.95
Saravan/ Iran (20)	<i>A. dispar</i>	River	Makran	E 62°, 24', 50.9" N 27°, 34', 39.3"	Tissue	Tissue
Sar Tang/ Iran (21)	<i>A. dispar</i>	Spring-stream	Tigris	E 45°, 51', 48.5" N 33°, 41', 24.3"	12 (6/6)	34.6 ± 3.65
Al-Manameh/ Bahrain (22)	<i>A. dispar</i>	aquarium	Persian Gulf	E 50°, 29', 59.7" N 26°, 10', 05.4"	4 (2/2)	34.4 ± 2.95
Khor Hulaylah/ UAE (23)	<i>A. dispar</i>	Coastal habitat	Persian Gulf	E 56°, 03', 23.9" N 25°, 53', 55.4"	5 (3/2)	33.6 ± 2.70
Sib city 1/ Oman (24)	<i>A. dispar</i>	Coastal marsh	Gulf of Oman	E 58°, 12', 21.3" N 23°, 40', 19.6"	26 (14/12)	32.5± 2.80
Sib city 2/ Oman (25)	<i>A. dispar</i>	Coastal marsh	Gulf of Oman	E 58°, 11', 33.3" N 23°, 40', 28.7"	16 (9/7)	32.8± 2.35
<b>Brackish water species (<i>Aphanius furcatus</i> sp. nov.)</b>						
Shur/ Iran (9)	<i>A. furcatus</i>	River	Hormuzgan	E 56°, 28', 10.2" N 27°, 19', 37.6"	14 (11/3)	24.6 ± 3.40
Khurgu/ Iran (10)	<i>A. furcatus</i>	Hot sulphuric spring	Hormuzgan	E 56°, 28', 08.2" N 27°, 31', 34.1"	6 (female)	24.9 ± 2.80
Kol/ Iran (11)	<i>A. furcatus</i>	River	Hormuzgan	E 55°, 45', 31.2" N 27°, 07', 40.3"	9 (7/2)	23.5 ± 3.70
Mehran 1/ Iran (13)	<i>A. furcatus</i>	River	Hormuzgan	E 54°, 15', 46.1" N 27°, 08', 39.8"	3 (male)	19.9 ± 1.30
Faryab/ Iran (15)	<i>A. furcatus</i>	Hot sulphuric spring	Hormuzgan	E 54°, 16', 28.0" N 27°, 25', 16.2"	5 (2/3)	24.8 ± 2.20

### 3. Methods

#### 3.1. Morphometry of fish specimens

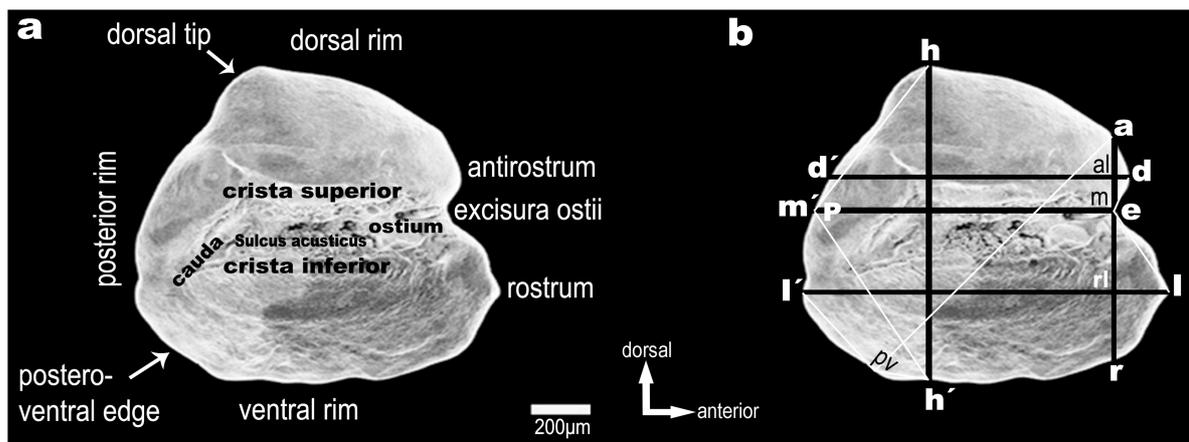
Based on the morphometry introduced in Holcik (1989), 14 morphometric parameters of fish body were measured using a vernier caliper adjusted to the nearest 0.5 mm. In addition, seven meristic characters were counted under a stereomicroscope. In total, 27 variables were calculated from the measurements and served as input for the statistical analyses. Table 2 lists the 27 variables + seven meristic characters:

**Table 2** Morphometric variables and abbreviations used in the text.

Morphometric characters	Morphometric characters
Total length/Standard length	Head length/Preanal distance
Predorsal distance/Standard length	Head length/Length of pectoral fin
Predorsal distance/Head length	Length of dorsal fin/Standard length
Predorsal distance/Preanal distance	Length of dorsal fin/Head length
Postdorsal distance/Standard length	Length of dorsal fin/Length of pectoral fin
Postdorsal distance/Preanal distance	Length of pectoral fin/Standard length
Preanal distance/Standard length	Length of pectoral fin/Head length
Preorbital distance/Standard length	Length of pectoral fin/Preanal distance
Preorbital distance/Head length	Length of pelvic fin/Standard length
Preorbital distance/Length of pectoral fin	Length of pelvic fin/Head length
Length of caudal peduncle/Standard length	Length of pelvic fin/Preanal distance
Length of caudal peduncle/Preanal distance	Length of pelvic fin/Length of pectoral fin
Maximum body depth/Standard length	Depth of dorsal fin/Standard length
Head length/Standard length	
Meristic characters	
Gill rakers	
Lateral line series scales	
Caudal peduncle scales	
Pectoral fin rays	
Pelvic fin rays	
Dorsal fin rays	
Anal fin rays	

### 3.2. Analysis of otoliths

For preparation of otoliths, I used the standard method provided in Reichenbacher et al. (2007). Otolith morphology was studied with a stereo-microscope. In addition, SEM images were captured with a LEO 1430 VP at the Zoological State Collection Munich (ZSM) for some representative otoliths. Terminology of otolith characters follows Nolf (1985) and linear measurements and angles follow Reichenbacher et al. (2007) (see also Fig. 4a,b).



**Figure 4** Otolith morphology (a) and morphometry (b) of *Aphanius dispar* otolith is shown in medial view. l-l', length; h-h', height; m-a, antirostrum height; m-rl, 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.

### 3.3. Osteology and Jaw teeth morphology

The specimens were stained mainly following the protocol of Potthoff (1984). Terminology for osteological structures follows Arratia and Schultze (1992). Jaw teeth were removed from both the upper and lower jaw, and representative teeth were examined by a scanning electron microscope (SEM) with a LEO 1430 VP at ZSM.

### 3.4. Statistical analyses

The statistical analyses were carried out using PASW v.18.00, v.19.00, v.20.00 (SPSS Inc, 2010, 2011, 2012) and PAST (Palaeontological Statistics, version 1.81 (Hammer et al., 2001). Univariate analysis of variance (ANOVA, with Duncan post hoc test,  $p < 0.05$ ) was used to test the significance of phenotypic differences among populations and also between the sexes within populations. The Canonical discriminant analysis (CDA) was used for multivariate analyses to show the classification success of the groups. To find the discriminatory importance of each variable (i.e., the values of each variable that contributed most to the separation of the groups) across all discriminant functions, the mean discriminant coefficients were calculated by using the following equation (Backhaus et al., 2006):

Mean discriminant coefficient  $b_j = \sum |b_{jk}| * EA_k$  ( $k = 1, k = \dots$ )  $b_{jk}$ , standardized discriminant function coefficients for variable  $j$  with respect to discriminant function  $k$ ,  $EA_k$ , proportion of eigenvalue of discriminant function  $k$  of the discriminant. Furthermore, a dendrogram was constructed based on the Euclidean distance as a measure of dissimilarity. The between groups linkage method was used as the clustering algorithm to show the phenotypic relationships between isolated populations.

### 3.5. Molecular phylogenetic analyses

#### 3.5.1. DNA extraction, nucleotide sequencing and phylogenetic analyses

Total DNA was extracted from few mm<sup>3</sup> muscle tissue of the right caudal peduncle of the *Aphanius* specimens using a commercial DNA extraction kit (DNeasy Tissue Kit, Qiagen) following the manufacturer's protocol, or either using the phenol/chloroform DNA extraction procedures (Sambrook et al., 1989).

Two mitochondrial genes i.e., complete cytochrome *b* and partial 12S rDNA were amplified by polymerase chain reaction (PCR). Table 3a,b lists information of the primers and the amplification processes.

The resulting products of PCR were visualized on a 1% agarose gel, and then purified with PEG solution (PolyEthylene Glycol, modified from Rosenthal et al., 1993), Forward and reverse strand were sequenced with the PCR primers and BigDye 3.1 chemistry (Applied Biosystems) following the manufacturers protocol on an ABI3730 automated sequencer of the Genomic Sequencing Unit LMU Munich.

Sequences were trimmed and assembled in Geneious R6 (Biomatters). Sequences were subsequently aligned using the Muscle 3.6 (Edgar, 2004), as incorporated in Geneious, with additional *Aphanius* sequences published in Genbank in order to gain a representative data set for the assessment of the phylogenetic position of the new taxa.

Bayesian analyses of nucleotide sequences were run with the parallel version of MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) on a Linux cluster with one processor assigned to each Markov chain under the most generalizing model (GTR+G+I) because overparametrization apparently does not negatively affect Bayesian analyses (Huelsenbeck and Ranala, 2004). Each Bayesian analysis comprised two simultaneous runs of four Metropolis-coupled Markov-chains at the default temperature (0.2). Analyses were terminated after the chains converged significantly, as indicated by the average standard deviation of split frequencies < 0.01. Maximum likelihood reconstructions were performed using RAxML 7.2.5 (Stamatakis, 2006) under the GTR model of nucleotide substitution, with CAT approximation of rate heterogeneity and fast bootstrap (2000 bootstrap replicates). See also nucleotide sequencing and the phylogenetic analyses in appendix 2.

**Table 3** Primers used in this study for amplification of 12S rDNA and cytochrome *b* (a), as well as details of the amplification processes (b). AT: Annealing temperature

Gene	AT	Primer	Sequence (5'→3')	Reference
12S rDNA	58	H1782	TTTCATCTTTCCCTTGCGGTAC	Hrbek and Larson, 1999
		L1090	AAACTGGGATTAGATACCCCACTA	Hrbek and Larson, 1999
Cytochrome <i>b</i>	54	Glu-F	AACCACCGTTGTATTCAACTACAA	Machordom and Doadrio, 2001
		Thr-R	ACCTCCGATCTTCGGATTACAAGACCG	Machordom and Doadrio, 2001

Cycle number	Phase	Temperature	Time
1	First denaturation	95°C	4 min
30	Denaturation	95°C	30 sec
	Annealing	Specific annealing temperature (AT) in Table 3	1min
	Elongation	72°C	1min
1	Final elongation	72°C	7min

## 4. Results

### 4.1. Diversification and taxonomy of Iranian Inland *Aphanius* species

With regard to Iranian *Aphanius*, primary data was available for local distribution, reproductive biology, and habitat characteristics of some species such as *A. vladkovi*, *A. sophiae*, and *A. farsicus* (as *A. persicus*). In contrast, little was known concerning the evolutionary history of the known species and on the taxonomic status of many other *Aphanius* populations. The main aim of my study was to use sufficient data sets including morphometric, meristic and osteological characters of the fish, jaw teeth morphology, otolith morphology and morphometry, and also molecular data of mt-DNA sequences to investigate the evolutionary history and taxonomic status of the *Aphanius* species and populations in Iran.

Before starting this study, six species have been described from Iranian inland water systems, i.e., *Aphanius sophiae* (Heckel, 1847), *A. vladkovi* (1988), *A. persicus* (Jenkins, 1910), *A. ginaonis* (Holly, 1929), *A. isfahanensis* (Hrbek, Keivany and Coad, 2006), and *A. mesopotamicus* (Coad, 2009). My studies contributed to the recognition and description of two species (*A. pluristriatus* (Jenkins, 1910) and *A. arakensis* Teimori et al., 2012) and reconstructed phylogenetic relationships between

them. In addition, I showed that the name *A. persicus* (Jenkins 1910) was preoccupied and I replaced it by *A. farsicus* (For details see Teimori et al., 2011, Appendix 1). I also used genetic and morphological data along with available geological data in order to infer the historical zoogeography of the Inland *Aphanius* species. Additionally, I showed that otolith differentiation among the inland endemic species is high and can support their phylogenetic relations (For details see Teimori et al., 2012a/Appendix 2).

The outcome includes the following articles:

**Appendix (1).** Teimori A, Esmaeili HR, Reichenbacher B (2011) *Aphanius farsicus*, a replacement name for *A. persicus* (Jenkins, 1910) (Teleostei, Cyprinodontidae). *Zootaxa* 3096: 53–58.

**Appendix (2).** Teimori A, Esmaeili HR, Gholami Z, Zarei N, Reichenbacher B (2012a) *Aphanius arakensis*, a new species of tooth-carp (Cyprinodontidae) from the endorheic Namak Lake Basin in Iran. *ZooKeys* 215: 55–76.

**Appendix (3).** Esmaeili HR, Teimori A, Gholami Z, Zarei N, Reichenbacher B (2012). Re-validation and re-description of an endemic and threatened species, *Aphanius pluristriatus* (Jenkins, 1910) (Teleostei, Cyprinodontidae), from southern Iran. *Zootaxa* 3208: 58–67.

#### **4.2. Diversification and taxonomy of *Aphanius dispar***

It was obvious from the previous studies that a reliable phylogenetic species concept is not available for *Aphanius dispar*. The hypothesis of my research was that the numerous *A. dispar* populations may reflect high geographical differentiation and that some of the landlocked populations have been isolated since several thousand years, and could represent separate taxonomic units. To test this hypothesis, I used a large data set of phenotypic and molecular characters from non-isolated as well as from isolated *A. dispar* populations from three drainage basins in southern Iran (Helleh, Hormuzgan and Makran), and other sites in the regions of the Persian Gulf and Gulf of Oman (see Table 1).

The univariate analysis of the populations from the Helleh, Hormuzgan and Makran basins revealed that among the morphometric and meristic characters, only the characters related to the head are significant for the discrimination of the populations from each basin (i.e., predorsal distance/head length, preorbital distance/standard length, and head length/standard length). Moreover, the otoliths of populations of these basins show clear morphological differentiation. The most differentiated otoliths were detected from the Helleh drainage, and the relative medial length and relative rostrum length were the most important otolith characters for the separation of the populations. The obtained results are not only suitable for the discrimination of intra-specific variation in *Aphanius dispar*, but also useful for distinctness of *A. dispar* versus its geographically nearest species, *A. ginaonis* (For details see Teimori et al., 2012b/Appendix 4).

In a further study, I focused on the otoliths of *Aphanius dispar* from two coastal populations from Oman and one coastal population from the United Arab Emirates (UAE) and compared them with the otoliths of *A. dispar* from southern Iran (Helleh and Hormuzgan Basins). The otolith characters show clear intraspecific differentiation among the populations that is consistent with zoogeographic distribution of *A. dispar* populations in the Persian Gulf and Gulf of Oman drainages. This is particularly supported by the significant differences of relative antirostrum height, relative rostrum height, and relative antirostrum length among the populations (For details see Teimori et al., 2012c/Appendix 5). The phylogenetic analyses based on the mitochondrial DNA sequences support the intraspecific diversification of *A. dispar* in southern Iran and the Persian Gulf region in addition.

Moreover, my study on the populations of *A. dispar* in southern Iran has also led to the discovery of another new *Aphanius* species (i.e. *Aphanius furcatus* sp. Nov.) from the salty Shur River and hot sulphuric springs in the Hormuzgan Basin based on a unique set of morphometric data, osteology, otolith, jaw teeth, and mt-DNA sequences. The new species is sympatric to *A. dispar* and differs from the Iranian *Aphanius* species by a complete absence of scale, color pattern of male and female, and from all known *Aphanius* species by a slightly forked caudal fin. Based on the phylogenetic analysis of molecular sequence data, the new species forms a sister taxon to *A. dispar* plus *A. ginaonis*, the latter is occurring in a geographically near location. In addition to the complete absence of scale, the new species demonstrates

reduction in the biomineralization of hard structures, particularly in the caudal skeleton and jaw teeth (For details see Teimori et al. subm/Appendix 6).

The outcome includes mostly the following articles:

**Appendix (4).** Teimori A, Schulz-Mirbach T, Esmaeili HR, Reichenbacher B (2012). Geographic differentiation of *Aphanius dispar* (Teleostei: Cyprinodontidae) from Southern Iran. *Zoological Systematics and Evolutionary Research* 50: 289–304.

**Appendix (5).** Teimori A, Jawad LAJ, Al-Kkarusi LH, Al-Mamry JN, Reichenbacher B (2012). Late Pleistocene to Holocene diversification and zoogeography of the Arabian killifish *Aphanius dispar* inferred from otolith morphology. *Scientia Marina* 76: 637–645. [Featured Article]

**Appendix (6).** Teimori A, Esmaeili HR, Erpenbeck D, Reichenbacher B (under review). A new and unique species of the genus *Aphanius* (Teleostei, Cyprinodontidae) from Southern Iran; A case of regressive evolution. *Journal of Comparative Zoology*.

## 5. Discussion

### 5.1. Patterns of differentiation in inland *Aphanius* species

The noticeable features of the present-day diversity of the endemic inland *Aphanius* species in Iran include high genetic divergence and clear differences in otolith morphology, but only weak differences in general external morphology, morphometry and meristics. Examples are *A. isfahanensis* from central Iran, *A. sophiae* and *A. farsicus* from southern Iran (Hrbek et al., 2006, this study); another example from the Mediterranean area is *A. baeticus* from Spain (Doadrio et al., 2002). *Aphanius arakensis* Teimori et al., 2012a (Appendix 2), from the Namak Lake Basin represents another example for a species that is difficult to distinguish from its relatives based on external morphology. These patterns are probably caused by different rates of evolution in the mentioned characters that may be linked to the similarity of the individual environments, intraspecies communication, high ability of adaptation, and vicariance events.

The outcome of my PhD study clearly showed that otolith morphology is different between Iranian inland *Aphanius* species despite their similarities with regard to other morphological characters. This differentiation in otolith morphology corresponds well with the phylogenetic analyses. A good example is *A. vladykovi* because it is phylogenetically the most distinctive species (Hrbek et al., 2003; this study) in comparison to the other studied inland species, and its otoliths are highly differentiated (see Fig. 3 in Appendix 2). Consequently I hypothesize that *Aphanius* has a higher rate of divergence in otolith morphology than in overall morphology, and that this difference in divergence rate may be related to the function of the otoliths as parts of the inner ear. Functionally, otoliths provide a mechanism for measuring motion and position of the head relative to gravity (see Manley and Clack, 2004). However, it is quite important for a fish to know from where a sound is coming, and to be able to distinguish between different sounds and pick out the biologically most relevant sounds (Popper et al., 2005). In addition, differences in otolith morphology are related to the balance and orientation of a fish (Popper et al., 2005). This means that differences in otolith morphology can reflect changes in intraspecific communication and behaviour in fishes that may have acted as evolutionary pressures and consequently diversification.

In addition, the outcome of my PhD study suggests that flank bar numbers are significant characters for the identification of the male inland *Aphanius* species. However, the mechanisms that control male flank bar variation have not been studied. I hypothesize that flank bar numbers can contribute in mating selection and thus promote species divergence (see Kraaijeveld et al., 2010 for a review, and Teimori et al., 2012b for details/Appendix 2).

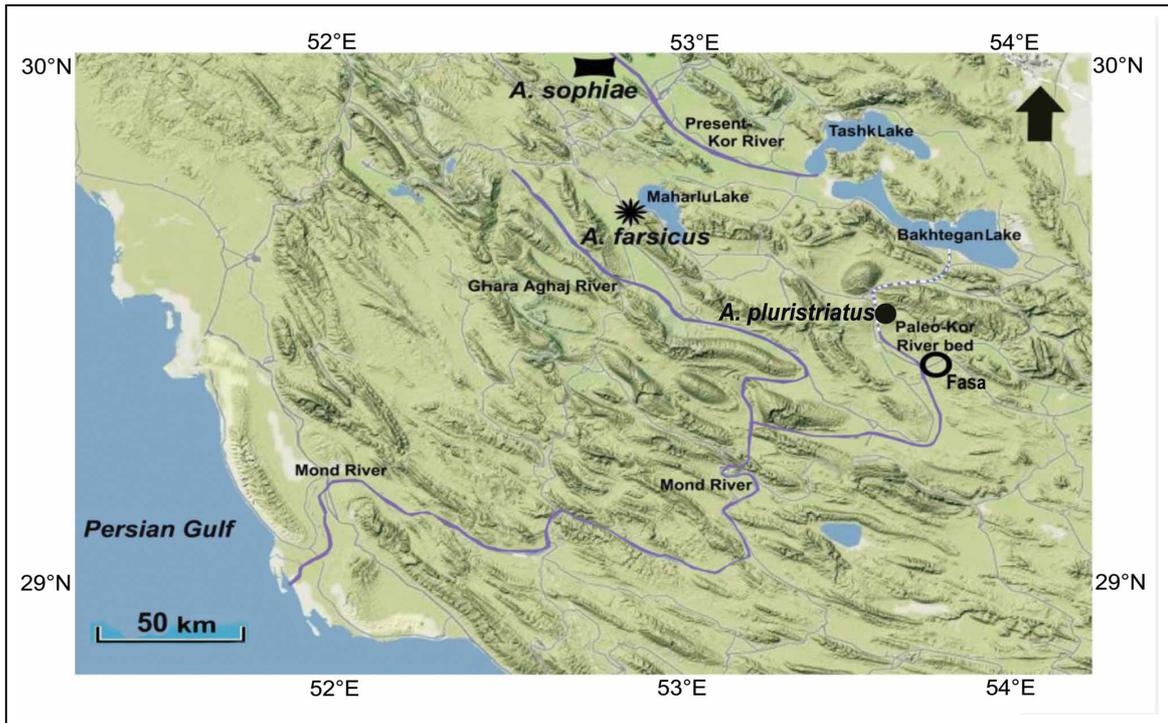
## **5.2. Historical zoogeography of inland *Aphanius* species**

The zoogeographic relationships of the Iranian inland *Aphanius* species have not been studied sufficiently. In their recent study, Hrbek et al. (2006) have suggested *Aphanius isfahanensis* from Esfahan drainage to be sister to *A. farsicus* (Maharlu Basin) + *A. sophiae* (Kor River Basin), and *A. isfahanensis* diverged from the *A. sophiae* + *A. farsicus* lineage at approximately 4.8 million years ago (early Pliocene). It remains unknown, however, when *A. sophiae*, *A. farsicus*, *A. pluristriatus* and *A. arakensis* have been diverged from each other. Phylogenetic analyses in this study

suggested that *Aphanius pluristriatus* from Mond River drainage in southern Iran is sister to *A. sophiae* from Kor River drainage and these together are sister to *A. farsicus* from their geographically closed drainage, Maharlu Lake.

*Aphanius pluristriatus* is a good example of inland species to explain the historical zoogeography of *Aphanius* species in Iran. I suggest that the present day *A. pluristriatus* is a relict of the ancient *Aphanius* population of the Kor River drainage. It has diverged from an *Aphanius* population in the ancient Kor River due to a prominent re-organisation of the drainage systems (Kehl et al., 2009). During the Quaternary, a Paleo-Kor River (crossing the type locality of *A. pluristriatus*), connected the present-day Kor River with the Mond River and drained the Kor River to the Persian Gulf (Fig. 5). In the Late Pleistocene or later, this drainage of the Kor River was closed due to the strong tectonic uplift of the Zagros Mountains (Nadji 1997), and the present-day endorheic Kor-River Basin with the Bakhtegan and Tashk Lakes formed (Kehl et al., 2009).

The timing of the Paleo-Kor River closure is probably Late Pleistocene (20,000–10,000 years ago; Nadji, 1997) or Holocene (6,000–2,000 years ago; Löffler, 1959). Since that time the connection between the *Aphanius* populations in the present-day Kor River Basin (as *A. sophiae*) and those in the Mond River drainage system (as *A. pluristriatus*) has been blocked and the populations began to diverge (see Fig. 5 and appendix 3 for details).



**Figure 5** Geographic distributions of the inland *Aphanius* species in southern Iran. The ancient Kor River (dotted line) and the present Kor River were connected until the end of the Quaternary. Then the connection was blocked and the endorheic Kor River Basin with the Bakhtegan and Tashk Lakes developed (see text for details and references). Source of map: Google Earth; modified.

It is likely that at the same time *A. farsicus* diverged from both *A. sophiae* and *A. pluristriatus* because of the above mentioned geological histories of the endorheic (Kor River and Maharlu Lake) and exorheic (Mond River) basins in the Fars area (Zagros mountains), as detailed by Ramsey et al. (2008) and Kehl et al. (2009). These events have probably played a major role in speciation of *Aphanius* species in this region (see Appendix 3 for details).

### 5.3. Patterns of differentiation in *Aphanius dispar*

The results of this PhD project revealed clear differentiation among *Aphanius dispar* populations in the drainages of southern Iran. This is obviously supported by significant differences of several characters among *A. dispar* populations from three basins i.e., (1) predorsal distance (predorsal distance/head length), (2) preorbital distance (preorbital distance/ standard length) and (3) head length (head length/ standard length). This intraspecific differentiation is also supported by the taxonomic meaning of the character “head length/standard length” for the separation between

*A. ginaonis* and *A. dispar* (this study) and for the discrimination of four closely related *Aphanius* species from central Iran (Hrbek et al., 2006).

The intraspecific differentiation of *Aphanius dispar* is particularly supported by the significant differences in otolith characters that are consistent with the zoogeographic distribution of *A. dispar*. The otolith characters that clearly showed these patterns are the relative antirostrum height, relative rostrum height, and relative antirostrum length (see Fig. 6). These characters have already suggested being significantly different in the otoliths of closely related *Aphanius* species (see Reichenbacher et al., 2007, 2009b). By comparing observed differentiation with the available geological information of the region (see below), I suggested a vicariance-based diversification for *A. dispar* in the studied regions, and also hypothesised that each individual basin contains its distinct taxonomic units (see Appendix 4 for details).

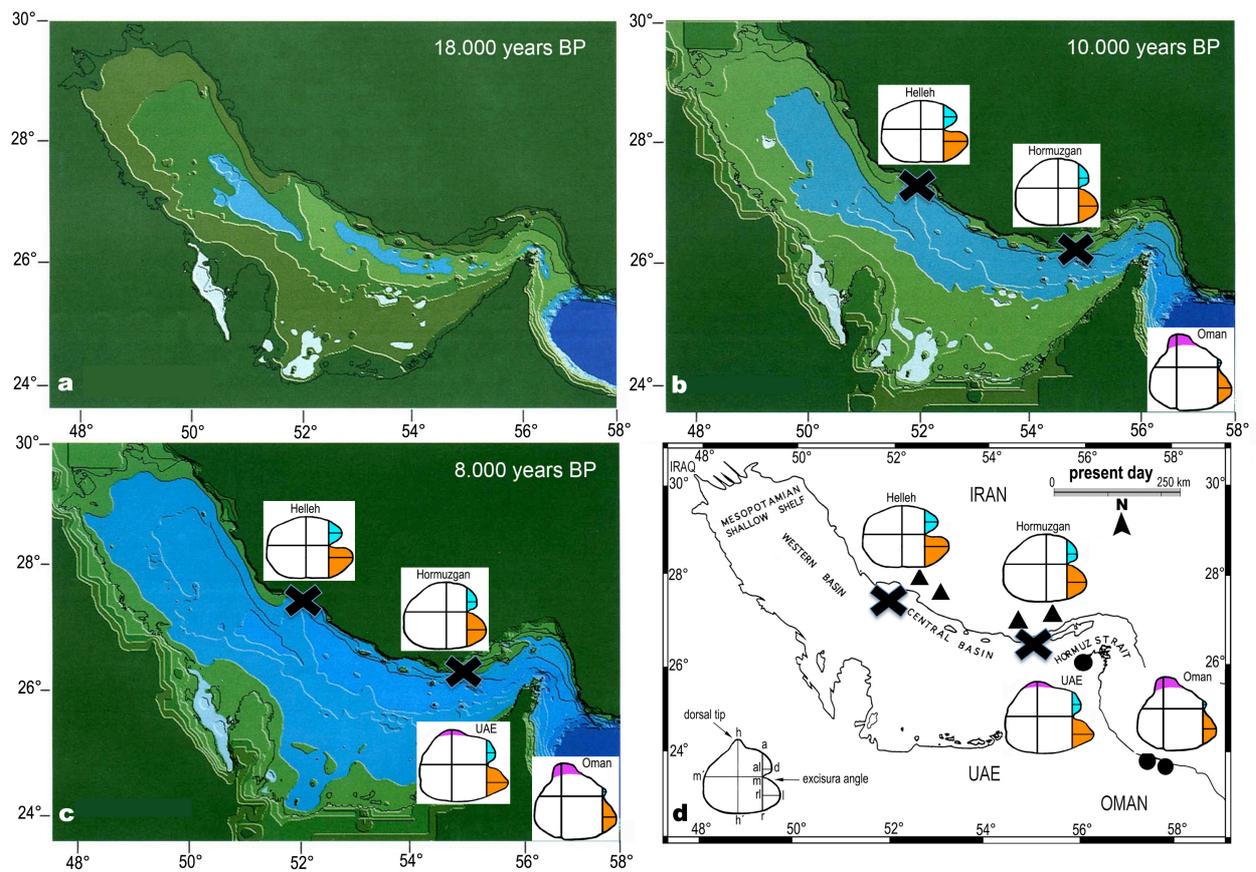
#### **5.4. Historical zoogeography of *Aphanius dispar***

The historical zoogeography of *Aphanius dispar* in southern Iran has mainly been linked to the active geological history of Zagros Mountains in southern Iran. It is likely that drainages of Makran, Hormuzgan and Helleh in southern Iran have been separated since the Pliocene and Pleistocene because of geological events in this region (Regard et al., 2004; Hatzfeld et al., 2010). During the late Pliocene, the NW-SE extending of Kazeroun Fault System (KFS) separated the Helleh Basins from Hormuzgan basin (Kompani-Zare and Moore, 2001; Hatzfeld et al., 2010). Therefore, it is likely that this fault has caused the isolation of the *Aphanius dispar* populations east and west of it, that is, the isolation of the Helleh (west of KFS) and Hormuzgan populations (east of KFS). Accordingly, the distinct differences in otolith morphology between Helleh populations and the populations from Hormuzgan basin could be explained by the geographical isolation and allopatric divergence since at least 1-2 million years. Moreover, separation of the Makran Basin from the Central Zagros Mountains and Hormuzgan Basin happened by the Zendan Fault (ZF) system, which was active during the late Pliocene and lower Pleistocene and probably caused separation of the populations between Makran and Hormuzgan drainages (see Appendix 4 for details).

Moreover, it is likely that the local geologic events within the Hormuzgan Basin are responsible for the presence of many unusual and isolated habitats (e.g., hot

sulphuric springs). This habitat fragmentation probably increased the species diversity among fishes (see Esmaeili et al., 2010 and unpublished Fig. 7) and also among the populations of *A. dispar* in Hormuzgan Basin owing to genetic drift, founder effects and local adaptation to specific environmental conditions (see review in Barton and Charlesworth, 1984; Plath et al., 2010). The examples for the *Aphanius* species that probably evolved locally in the Hormuzgan Basin because of the above-mentioned factors are *A. ginaonis* Holly, 1929, which is represented by a single isolated population in the Genow hot sulphuric spring (Abdoli, 2000; Hrbek and Meyer, 2003; Teimori, 2006; Reichenbacher et al., 2009b) and *A. furcatus* within the *A. dispar* distribution region (see Appendix 6 for details).

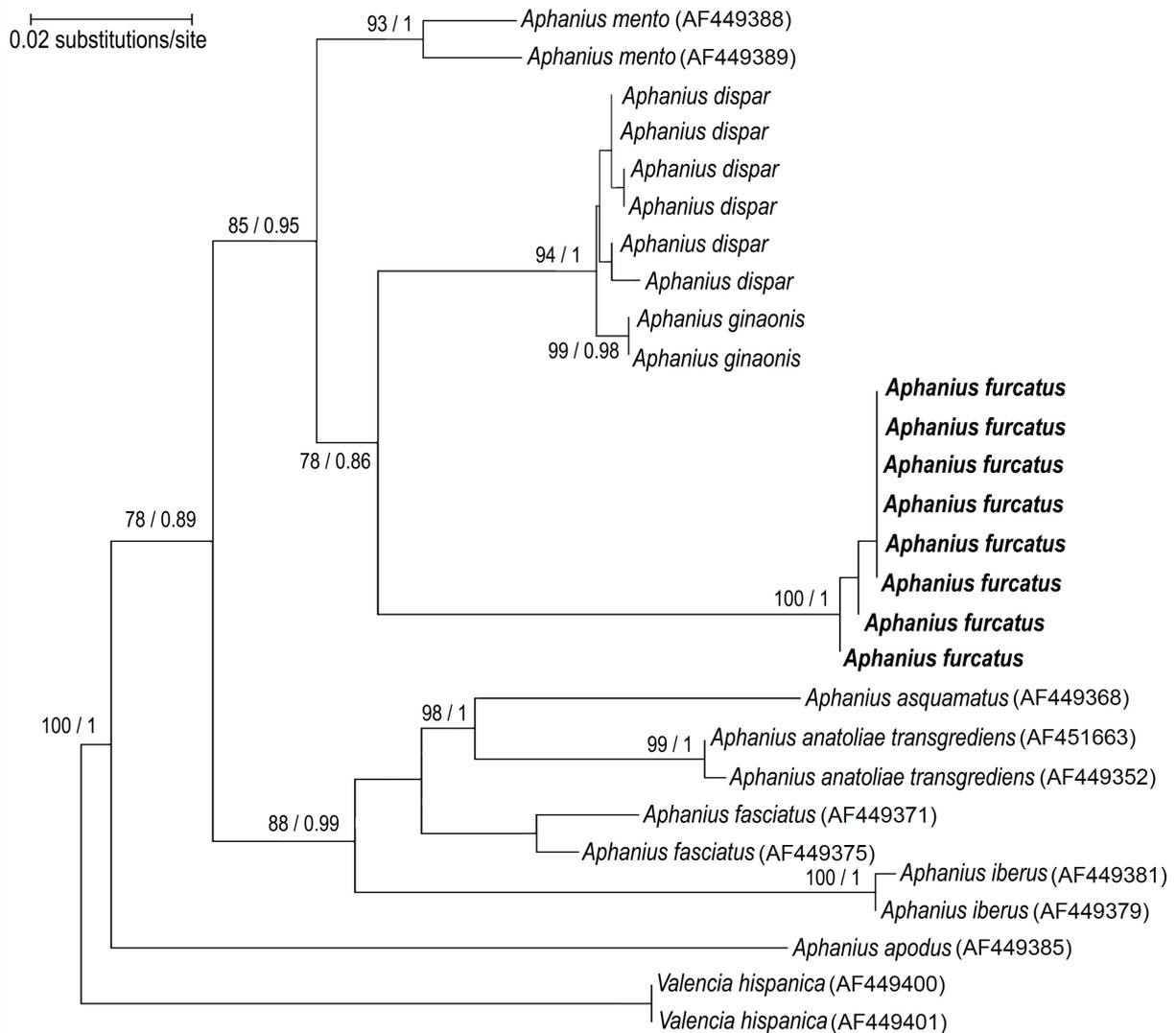
In addition, the historical diversification of *Aphanius dispar* in the region of the Persian Gulf and Gulf of Oman has been inferred by a clear spatial distribution pattern of the otolith characters antirostrum, rostrum and dorsal tip (Fig. 6, see Appendix 5 for details). These otolith-based results can now be supported by my new molecular results (unpublished, Fig. 7). According to the known geological history of the Persian Gulf, the Persian Gulf sea floor was subaerially exposed during the Late Pleistocene (18,000 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). Based on both otolith and molecular data it can be suggested that the environmental flexibility of *A. dispar* enabled several populations of this species to survive that period of time in the freshwater environments on the exposed sea floor and also in the rivers coming from Mesopotamia and southern Iran. On the other hand, several other populations of *A. dispar* continued to thrive in the Oman Sea, but there was no gene flow between these populations and those within the freshwater habitats of the Persian Gulf. However, the Oman Sea populations had a good connection to the drainages in Southeastern Iran, which is clearly shown by the molecular data (Fig. 7). Eventually, dispersal of marine animals during the Early Holocene sea-level rise (11,000-8,000 BP, Lambeck, 1996; Teller et al., 2000; Smith et al., 2011), and Holocene to present-day ecological barriers to gene flow at the Hormuz Strait have promoted intraspecific diversification in *A. dispar* (see Appendix 5 for details).



**Figure 6** 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. Source of maps: Purser and Seibold (1973), Lambeck (1996), Teller et al. (2000).



geological data suggest an age of about 16 m.y. for this event (Reuter et al., 2009). Thus, an age of about 16 m.y. can be assumed now for the divergence between *A. dispar* and *A. mento*. I suggest that the divergence between the lineages of *A. furcatus* and *A. dispar* happened in a comparatively short time after the split of *A. mento*. Taking into account the newly estimated age of 16 m.y. for the *A. mento*–*A. dispar* split, I estimate that the divergence between *A. furcatus* and *A. dispar* is about 12–14 m.y. old.



**Figure 8** Maximum likelihood and Bayesian likelihood estimations of phylogenetic relationships of *Aphanius furcatus* sp. nov. to other *Aphanius* species. Numbers at the branches are (from left to right): Maximum Likelihood bootstrap support values on 2000 replicates / Bayesian Posterior Probabilities.

## 5.6. Regressive evolution in *Aphanius*

Regressive characters such as scale reduction and reductions of pelvic (ventral) fins have been described for several teleost taxa and have been linked with adaptations and speciation processes (e.g., Villwock, 1982; Harris, 2012). Notably, these phenomena appear in different lineages of teleosts (Bell et al., 1993; Nelson, 1971; Harris, 2012). Also within the genus *Aphanius*, regressive evolution has been reported for several species (see below) that clearly belong to separate lineages (Hrbek and Meyer, 2003; Hrbek et al., 2002; this study).

In the new species *Aphanius furcatus* (Fig. 9), several phenotypic characters show reduction; most remarkable is the complete lack of scales. In a recent study, Harris (2012) suggested that scale reductions or loss may be linked with oxygen poor environments because the loss of scales enables the fish to transport additional oxygen via the skin. In such environments, phenotypes with reduced scalation or lack of scales would have a higher chance of survival and would be selected.

*Aphanius furcatus* is thriving in extreme environments characterized by low oxygen, high water temperatures, and high salt concentration (see habitat description in Appendix 6). I conclude that the reductive phenomena observed in *A. furcatus* have evolved as an evolutionary response to the extreme habitat conditions in order to save energy (because storage of  $\text{Ca}^{2+}$  is not necessary), and to transport oxygen efficiently, and that reductive phenomena in *Aphanius* have occurred during multiple independent events (see Appendix 6 for details).



**Figure 9** Male (left) and female (right) of *Aphanius furcatus* sp. nov., from Hormuzgan Basin, Southern Iran.

## 6. Conclusion

The evolutionary history and taxonomy of *Aphanius* species in Iran and the regions of Persian Gulf as well as the Gulf of Oman were studied in my PhD project. The outcome of this study demonstrated that;

1. The endemism of Iranian inland *Aphanius* species is high. The noticeable features of this diversity, however, include high genetic differentiation and clear differences in otolith morphology, but only weak differences in general external morphology, morphometry and meristics. These patterns of differentiation are probably caused by different rates of evolution in the mentioned characters that can be linked to the similarity of the environments, intraspecific communication, and vicariance events.
2. *Aphanius dispar* populations in three drainage basins of Southern Iran and those from the regions of the Persian Gulf and Gulf of Oman showed clear geographical differentiation with regard to all examined data sets. Thus, they represent different taxonomic units. Major driving forces of the differentiation probably include geographical isolation and habitat fragmentation resulting from the geologic history of Zagros Mountains in southern Iran, local adaptation to unusual habitats (hot sulphuric springs), vicariance events due to the sea-level drop of the last glacial maximum (Late Pleistocene; 21,000-18,000 BP), dispersal during the Early Holocene sea-level rise (11,000-8,000 BP), and Holocene to present-day ecological barriers to gene flow at the Hormuz Strait.
3. In addition to vicariance-based speciation that plays a major role in diversification of Iranian *Aphanius* and the *Aphanius dispar*, sensory-driven speciation and regressive evolution also suggested playing important role.

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## 8. References

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# Curriculum vitae

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## Education

1994-1997: High school in experimental Sciences, Kazeroun, Iran.

2000-2004: Bachelor degree at Department of Biology, Shahid Bahonar University of Kerman (SBUK), Kerman, Iran. Major of biology

2004-2008: Master of Sciences at Department of Biology, Shiraz University, Iran. Major of Animal Biosystematics (Ichthyology). Thesis: Biodiversity of freshwater fishes in drainages of Southern Iran (Fars Province).

Nov. 2009-present: Promotion. Department of Earth and Environmental Sciences, Geobiology and Palaeontology, Ludwig-Maximilians-University (LMU), München, Germany.

Supervisor: Prof. Dr. Bettina Reichenbacher

## Honors and Awards

- Awarded with a Complementary Grant by the Graduate Center LMU, March–Jun. 2013, Munich.
- **Featured article:** Teimori et al., 2012: Late Pleistocene to Holocene diversification and historical zoogeography of the Arabian killifish (*Aphanius dispar*) inferred from otolith morphology. *Scientia Marina* 76: 637–645.
- Travel Grant Award by German Academic Exchange Service (DAAD) for Attending the XIV European Congress of Ichthyology, July 3–8, 2012 Belgium.
- Awarded with a grant from the German Academic Exchange Service (DAAD) in 2011-doctoral program for a teaching-research assistantship at Ludwig-Maximilians-University (LMU), München, Germany.
- ICSEB Student Award. International conference of Biosystematics. Freie Universität Berlin 2011 (Oral presentation).
- Awarded by the Ministry of Science, Research and Technology of Iran to do Ph.D. research in 2009–2013.
- Best researcher student at Shiraz University. Shiraz, Iran. 2007–2008.

## Lectures

- Course for Master Students on fish osteology at Ludwig-Maximilians-University (LMU), München. May–July 2011.
- Biological diversity of Iranian inland fishes and its conservation (2006). In *The biodiversity conservation imperative: biological, ethical, and economic approaches in the Middle East and Europe*. Tehran University, Iran.
- An introduction to the Iranian international wetlands-focus on international wetlands in Southern Iran (2005). Shiraz University, Shiraz, Iran.

## Research projects

### Current

Hamid Reza Esmaeili and **Azad Teimori** (2010–2013). An Introduction to the Biological, Ecological and Conservation management of *Aphanius farsicus* in Mahalru Basin, Southern Iran.

**Azad Teimori** and collaborators (Nov. 2009–July 2013). The Evolutionary history and taxonomy of *Aphanius* species in Iran and the Persian Gulf (Cyprinodontiformes: Cyprinodontidae). PhD project.

### Completed (2004–2009)

Hamid Reza Esmaeili and **Azad Teimori** et al. (2004–2006). Monitoring of three salty lakes (Maharlu, Bakhtegan and Tashk) in Southern Iran, Fars province. Shiraz University, Iran.

Hamid Reza Esmaeili and **Azad Teimori** et al. (2005–2007). Investigation of the habitat structure and distribution of *Ursus arctos* (Linnaeus, 1758) (Carnivora: Ursidae) in Zagros Mountains, Southern Iran. Shiraz University, Iran.

Hamid Reza Esmaeili and **Azad Teimori** et al. (2007–2009). Study of the habitats and taxonomic evaluation of the vertebrate fauna in Gorm protected area in Southern Iran using GIS. Shiraz University, Iran.

Hamid Reza Esmaeili and **Azad Teimori** et al. (2007–2009). Land-use planning of Fars, Kohgeloye and Boirahmad Provinces in Southern Iran. Shiraz University, Iran

### **List of publications**

#### **2013**

**Azad Teimori**, Hamid Reza Esmaeili, Dirk Erpenbeck, Bettina Reichenbacher (under review). A new and unique species of the genus *Aphanius* (Teleostei: Cyprinodontidae) from Southern Iran: A case of regressive evolution.

Zeinab Gholami, **Azad Teimori**, Hamid Reza Esmaeili, Tanja Schulz-Mirbach, Bettina Reichenbacher (2013). Scale surface microstructure and scale size in the tooth-carp genus *Aphanius* (Teleostei: Cyprinodontidae) from endorheic basins in Southwest Iran. *Zootaxa* 3619: 467–490.

#### **2012**

**Azad Teimori**, Laith Abd Jalil Jawad, Lubna Hamoud Al-Kharusi, Juma Mohamed Al-Mamry, Bettina Reichenbacher (2012) Late Pleistocene to Holocene diversification and historical zoogeography of The Arabian killifish (*Aphanius dispar*) inferred from otolith morphology. *Scientia Marina* 76: 637–645. **Featured Article.**

**Azad Teimori**, Hamid Reza Esmaeili, Zeinab Gholami, Neda Zarei, Bettina Reichenbacher (2012) *Aphanius arakensis*, a new species of tooth-carp (Cyprinodontidae) from the endorheic Namak Lake Basin in Iran. *Journal of ZooKeys* 215: 55–76.

**Azad Teimori**, Tanja Schulz-Mirbach, Hamid Reza Esmaeili, Bettina Reichenbacher (2012) Geographical differentiation of *Aphanius dispar* (Teleostei: Cyprinodontidae) from Southern Iran. *Zoological Systematics and Evolutionary Research* 50: 289–304.

Hamid Reza Esmaeili, **Azad Teimori**, Zeinab Gholami, Neda Zarei, Bettina Reichenbacher (2012) Re-validation and re-description of an endemic and endangered species, *Aphanius pluristriatus* (Jenkins, 1910) (Teleostei, killifishes) from southern Iran. *Zootaxa* 3208: 58–67.

Abouzar Eslami, **Azad Teimori**, Mehmet Yigitsoy, Nassir Navab (2012) A new framework for morphological and morphometric study of fish species based on groupwise registration of otolith images. *Biomedical Imaging (ISBI)* 1679–1682.

## 2011

**Azad Teimori**, Hamid Reza Esmaeili, Bettina Reichenbacher (2011) *Aphanius farsicus*, a replacement name for *A. persicus* (Jenkins, 1910) (Teleostei: Cyprinodontidae). *Zootaxa* 3096: 53–58.

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## 2010

**Azad Teimori**, Hamid Reza Esmaeili (2010) The Ichthyofauna of Kor and Helleh River basins in Iran with reference to taxonomic and zoogeographic features of native fishes. *Iranian Journal of animal Biocystematics (IJAB)* 6: 1–8.

Ali Gholmahosseini, Hamid Reza Esmaeili, Haj Gholi Kami, Mehregan Ebrahimi, **Azad Teimori** (2010) Study of vertebrate fauna coexists in Brown bear habitats in Southern Iran. *Journal of life sciences* 5: 17–28.

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