Ecological plasticity and divergence processes of the Iranian inland species of *Aphanius* (Teleostei, Cyprinodontidae), with focus on *A. sophiae* and *A. farsicus* in the Kor River and Maharlu Lake basins, Southwestern Iran

Dissertation zur Erlangung des naturwissenschaftlichen
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Tag der Disputation: 15.10.2014

Ehrenwörtliche Versicherung

Ich versichere hiermit ehrenwörtlich, dass die Dissertation von mir selbstständig, ohne Beihilfe angefertigt worden ist.

München, 15.07.2014

Erklärung

Hiermit erkläre ich, dass die Dissertation noch nicht in einem anderen Prüfungsverfahren vorgelegt und bewertet wurde.

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München, 15.07.2014

Summary

The main purpose of my PhD project was to investigate the ecological plasticity and inter- and intraspecific variation of the Iranian inland species of *Aphanius*, with focus on *A. sophiae* (Kor River Basin) and *A. farsicus* (Maharlu Lake Basin) in southwestern Iran, and to analyse their phylogenetic relationships, evolutionary history and possible links of speciation processes to geological events. A comprehensive dataset including meristics, morphometrics, scale surface microstructure and morphology, scale sizes (J-indices), otolith morphology and morphometry and also molecular data (*Cyt b*) was studied based on comparative morphology, uni- and multivariate statistics and genetic analyses using molecular programs (Geneious v. 6.4, SeaView v.4, jModelTest 2.1.1, MrBayes 3.2, FigTree v.1.4.0, MEGA5.2.2, DnaSP v.5.10.1, Tajima's D, TCS v.1.21).

The results revealed that scale surface microstructure and scale morphology cannot help in distinguishing between *A. sophiae*, *A. farsicus* and *A. pluristriatus*, but that some characters of the scale surface microstructures (lepidonts, tubercles, total radii and focus granules) can discriminate certain populations of *A. sophiae*. Relative scale sizes (J-indices) were found taxonomically useful to distinguish *A. sophiae*, *A. farsicus*, *A. pluristriatus* and also *A. arakensis*, *A. isfahanensis A. vladykovi*, *A. darabensis* and *A. kavirensis*. However, J-indices were also significantly different in three isolated populations of *A. sophiae* from the Kor, Helleh and Karun basins. This indicates that inter- and intraspecific differences of J-indices were evolving due to isolation of species or populations for at least several thousand years and that J-indices may not be taxonomically useful on the species level in every case as it was thought previously.

Furthermore, during my study on *Aphanius sophiae*, which is thought to be endemic in the Kor River Basin, I found out that a specific population from the upper reaches of the Kor River Basin (Paselari spring) is significantly different from *A. sophiae* and represents a new species, termed *A. shirini* sp. n. The new species was based on 17 fixed molecular apomorphies and the males showed the lowest number of white flank bars in comparison to all other Iranian inland *Aphanius* species. I additionally showed for the first time that there is a relation between the geological history and the present-day *Aphanius* species diversity in the tectonically still active Zagros Mountains of Iran. Accordingly, the Early to Middle Miocene phase of uplift in the northwestern regions of the High Zagros led to the split of *A. vladykovi* in the Karun Basin, while the Late Miocene uplift of the southeastern regions of the High Zagros resulted in the split of *A. shirini* sp. n. in the upper reaches of the Kor

River Basin. Both *A. vladykovi* and *A. shirini* represent the highest altitude records of *Aphanius*.

I have also investigated two previously not known isolated populations of *Aphanius* found in the Arjan Wetland (Helleh Basin) and the Semirom spring-stream (upper reaches of Karun Basin). The cytochrome b gene indicated that the individuals from the Arjan Wetland comprise two distinctive species. One of those clearly represents a population of *A. shirini*; the other represented a population of *A. sophiae* (as known from the Kor Basin). The population from the Semirom spring clearly represented another population of *A. sophiae* (as known from the Kor Basin). The presence of *A. shirini* in the Arjan Wetland is most likely the result of artificial introduction during 2000, after a severe drought that had affected the Arjan Wetland. The unexpected occurrence of isolated populations of *A. sophiae* outside the Kor Basin indicates that this species was widespread in the central Zagros Mountains until the Late Pleistocene or Early Holocene. Their present-day isolation can be linked to the onset of a new period of intensive tectonic activity during the Early and Middle Holocene in the Central Zagros that led to the interruption of formerly interconnected drainage systems.

In a further study, high phentotypic variation among four populations of *A. farsicus* was found based on meristics, morphometrics and otolith variables. However, no distinguishing molecular apomorphy was depicted among the populations and the phylogenetic tree failed to recover a supported genetic structure for an individual population, at least under the analysed fragment (*Cyt b*). Fu's Fs and R_2 statistical neutrality tests, pairwise Fst values, Tajima's D test and statistical parsimony analysis based on the haplotype frequencies clearly indicated high genetic connectivity among the studied populations of *A. farsicus*. Based on a literature survey and the new data it is concluded that population connectivity is maintained during times of droughts via large aquifers that formed during the Late Pliocene–Early Pleistocene, when the extant endorheic Maharlu Basin was created.

In the second part of my PhD project, I have collaborated to the description of three new species, *A. arakensis* Teimori et al., 2012, *A. darabensis* Esmaeili et al., 2014 and *A. kavirensis* Esmaeili et al., 2014. Furthermore, *A. pluristriatus* Esmaeili et al., 2012, from the Mond River Basin has been redescribed and was recognized as a valid species. Besides clear genetic divergence, the significant features of the described species are otolith morphology and morphometry, J-indices and number of white flank bars. Otolith morphology is generally known to represent an important taxonomic character for species discrimination. A possible explanation might be the function of the otoliths as parts of the

inner ear that provide a mechanism for hearing of fishes. Thus, differences in otolith morphology may reflect changes in intraspecific communication and behavior in closely related *Aphanius* species. An explanation for the taxonomically useful numbers of white flank bars in males of *Aphanius* species is that flank bar patterns may play an important role in sexual selection.

According to the outcome of my study and the collaborations it is clear that the Iranian plateau is a center of speciation for *Aphanius*. To date, 14 *Aphanius* species are known based on the cytochrome b gene, and their sites can be linked to distinctive tectonic units. It is likely that additional isolated populations can be found in the Zagros Mountains that may deserve species recognition. A combined analysis of molecular data together with comprehensive morphological datasets, as presented here, can disentangle the relationships of closely related Iranian inland *Aphanius* species and contribute to understand character evolution in the context of differing environments and/or allopatric divergence. Such studies are important for the identification of endangered local populations and species, and for conservation strategy and habitat management.

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Appendix 2: Gholami, Z., Esmaeili, H.R., Erpenbeck, D. & Reichenbacher, B. (2014). Phylogenetic analysis of *Aphanius* from the endorheic Kor River Basin in the Zagros Mountains, Southwestern Iran (Teleostei: Cyprinodontiformes: Cyprinodontidae). Journal of Zoological Systematics and Evolutionary Research, 52 (2), 130–141.

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Appendix 5: Teimori, A., Esmaeili, H.R., Gholami, Z., Zarei, N. & Reichenbacher, B. (2012). *Aphanius arakensis*, a new species of tooth-carp (Cyprinodontidae) from the endorheic Namak Lake Basin in Iran. ZooKeys, 215, 55–76.

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1. Introduction

1.1. Genus *Aphanius* Nardo, 1827

The genus *Aphanius* is the only native representative of the Cyprinodontidae (tooth-carps, killifish), and is widely distributed throughout the coastal habitats of the Mediterranean Sea, Red Sea and Persian Gulf, as well as in the brackish, freshwater and euryhaline inland water bodies in Iran, Pakistan and India (Wildekamp et al., 1999; Hrbek and Meyer, 2003; Reichenbacher et al., 2009a–b; Ferrito et al., 2013; App. 2). To date, 28 *Aphanius* species are recognized as valid, but the highest diversity appears in the endorheic basins of the Iranian plateau and central Anatolia (Hrbek et al., 2002, 2006; Esmaeili et al., 2010; Teimori et al., 2014; App. 2, 5–7).

A maximum length up to 8 cm appears in both sexes of *Aphanius* species and all species display clear sexual dimorphism. The males have generally vertical white flank bars and the females show often spot-like pigmentation or vertical pale dark flank bars. The males are predominantly aggressive and slightly smaller than females. Typical characters of *Aphanius* species include a rounded caudal fin, dorsal fin opposite to anal fin, tricuspid jaw teeth, cycloid scales, no lateral line, but lateral scales series dispersed on the head and flanks (Parenti, 1981). Few species show regressive characters e.g. reduction of scalation in the Anatolian *A. asquamatus* Sözer, 1942, lack of scales in the Iranian *A. furcatus* Teimori et al., 2014, absence of pelvic fins in *A. apodus* Gervais, 1853 from Algeria and in a Miocene fossil species of *Aphanius* (Villwock, 1982; Gaudant, 1993; Wildekamp et al., 1999; Nelson, 2006; Teimori et al., 2014).

Most species tolerate euryhaline and eurythermal habitats as well as relatively low levels of oxygen and wide range of pollution. In addition they are good candidates as biological control of malaria because they feed on mosquito larvae of *Anopheles* (Homski et al., 1994; Frenkel and Goren, 2000). Species of *Aphanius* inhabit environments such as small streams and calm regions of fresh and brackish waters that are not proper for other fishes and where competitors and major predators are absent (Bennett and Beittinger, 1997; Berra, 2007; Clavero et al., 2007). In addition, they have a high ability to survive in small and isolated populations and investigation of these populations is valuable to understand species diversification patterns (Hrbek and Meyer, 2003; Pappalardo et al., 2008; Ferrito et al., 2013; App. 2–3).

The diversity of *Aphanius* species in central Anatolia and the Mediterranean region has been largely studied to understand historical biogeography, vicariant speciation,

morphological and genetic differentiation, osteological variation and otoliths as species-specific criteria (e.g. Fernandez-Pedrosa et al., 1995; Reichenbacher et al., 2007; Hrbek and Meyer, 2002, 2003; Ferrito et al., 2007, 2013). In contrast, little work has been directed on the Iranian *Aphanius* species and populations until 2010. These species and populations are distributed in different drainage basins, and several of these populations have not yet been studied in detail. Taxonomic and genetic investigations of these populations are important to elucidate their phylogenetic relationships and evolutionary history (App. 1–7). Iran lies within a geologically active area with many geographically isolated regions, thus it is an ideal candidate for testing the geographic speciation hypothesis (Hrbek and Meyer, 2002).

1.2. Iranian *Aphanius* species

Based on previous studies (Coad, 1996, 2000, 2009; Hrbek et al., 2006; Teimori et al., 2011, 2014), my PhD work and the related collaborations (App. 2, 5–7), 14 extant *Aphanius* species have been now described in Iran. These species were reported from Northern (Alburz Mountains), Central and Southern (Zagros Mountains) Iran. Based on molecular data, it was indicated that these species are representatives of three large clades (App. 7):

(i) The *A. dispar* clade includes four species (*A. dispar*, *A. ginaonis*, *A. furcatus*, *A. sirhani*) that are distributed in the coastal habitats of the Persian Gulf, Gulf of Oman, Arabian and Red Sea, and locally in the Eastern Mediteranean (*A. dispar*), southern Iran (*A. ginaonis*, *A. furcatus*), and Jordan (*A. sirhani*).

(ii) The *A. mento* clade is a large clade distributed in the Eastern Mediterranean and especially in Anatolia (Wildekamp et al., 1999; Hrbek et al., 2002). *Aphanius mento* was also recorded from the Tigris Basin near the border of Iran to Iraq (Wildekamp, 1993), but recent studies have not confirmed its occurrence in this basin.

(iii) The Inland and Inland-related *Aphanius* species (IIRAS) clade comprises ten species (*A. vladykovi*, *A. darabensis*, *A. shirini*, *A. isfahanensis*, *A. farsicus*, *A. arakensis*, *A. sophiae*, *A. kavirensis*, *A. mesopotamicus* and *A. pluristriatus*) (Figs. 1–4). These species can be divided into three subclades (*A. vladykovi*/ *A. shirini*/ *A. sophiae* subclade) that are endemic to Iran (App. 7), except *A. mesopotamicus*, which was also found in Iraq (Coad, 2009) and perhaps occurs in Syria (Krupp, 1985). Some species of the three clades (i.e. *A. dispar*, *A. ginaonis*, *A. furcatus*, *A. mento*, *A. vladykovi*, *A. shirini*, *A. darabensis* and *A. isfahanensis*) show distinctive traits in color patterns, meristics or morphometrics, while

others (i.e. *A. farsicus*, *A. arakensis*, *A. kavirensis*, *A. mesopotamicus*, *A. sophiae* and *A. pluristriatus*) are superficially very similar and cannot be clearly distinguished by external characters. However, all species can be clearly separated by molecular characters (Hrbek and Meyer, 2003; Hrbek et al., 2006; App. 2–5, 7).

As *Aphanius* species or populations often show similar color patterns and external morphology, it is difficult to understand the taxonomy and phylogenetic relationships based on body morphology. An exception is *A. furcatus* from the saline Shur River and hot springs in the Hormuzgan Basin in southern Iran, which lacks scales and displays a slightly forked caudal fin (Teimori et al., 2014).

In my PhD project and the collaborations during the last four years (2010–2014), the Iranian inland and inland-related *Aphanius* species and populations have been studied using comprehensive datasets involving meristics, morphometrics, scale surface microstructures, scale morphology, scale sizes (J-indices), otolith morphology and morphometry, and mt-DNA (*cyt b*) gene sequences. This total evidence approach provided important information on multiple taxonomic levels and helped for a better understanding of the phylogenetic relationships and evolutionary history of closely related *Aphanius* species and populations (App. 1–7). The most important species for my PhD project were *A. sophiae* and *A. farsicus* (App. 1–4), which both are critically endangered because of land use, pollution and recent droughts.
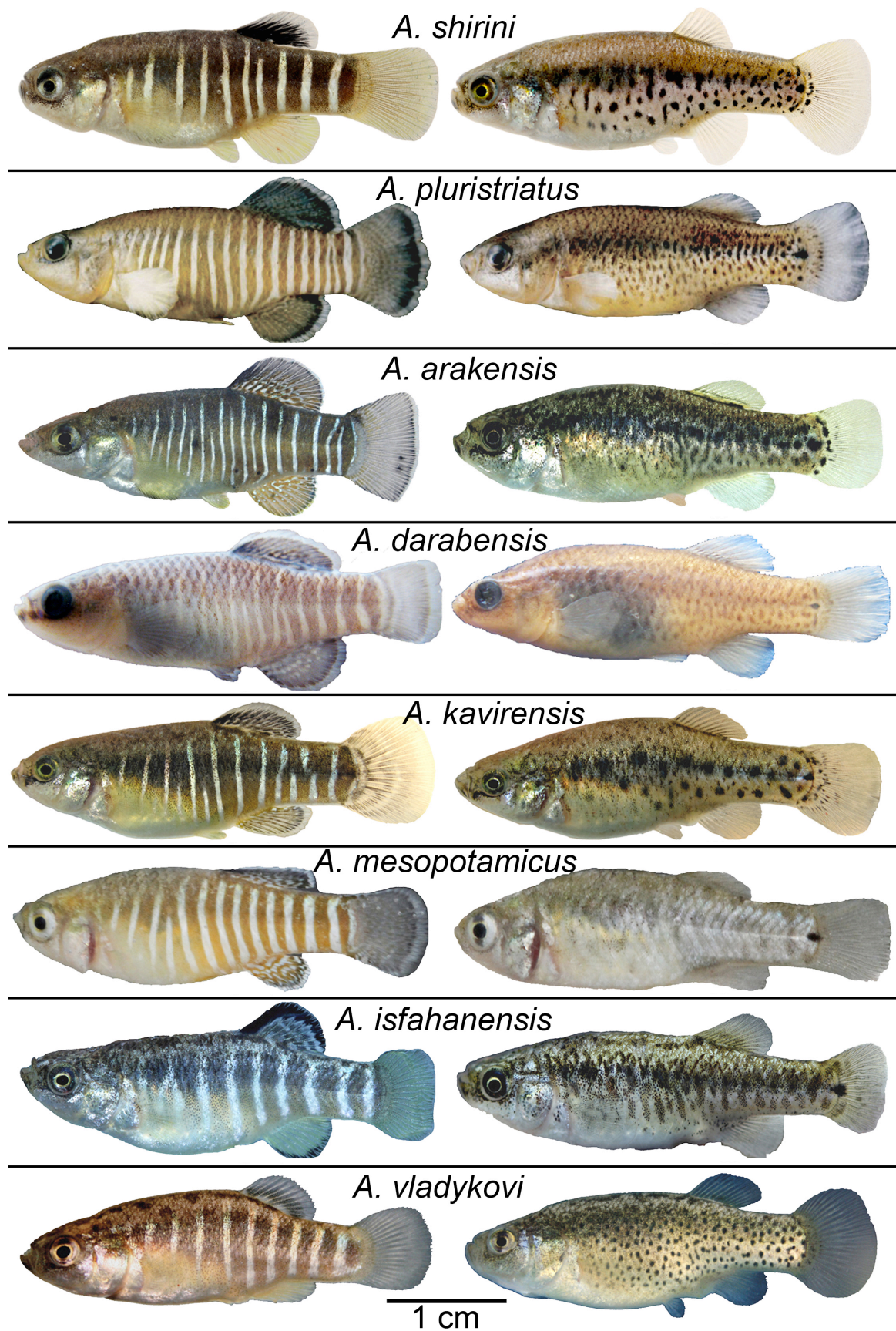
Figure 1. Color pattern and morphology of the Iranian inland and inland-related *Aphanius* species (IIRAS). Left images refer to male and right images to female specimens. Figure is compiled from App. 1–7.

1.3. *Aphanius sophiae*

Aphanius sophiae (Heckel, 1849) or soffia tooth-carp has been described as an endemic species from the endorheic Kor River Basin, southwest Iran (Coad, 1996; App. 1–3). The type locality of *A. sophiae* is in lukewarm salty springs near Persepolis (Heckel 1846–1849), and few populations of this species are known from several freshwater spring-streams in the mountainous areas of the Kor River Basin and around the hypersaline Tashk and Bakhtegan Lakes (Coad, 1996; App. 1–3) (Fig. 2A). The endorheic Kor River drainage occupies 26,440 km^2 north and east of Shiraz city (Coad, 1996) and includes two main subsystems, i) the freshwater subsystem of the Kor River, and ii) the subsystem of the Tashk and Bakhtegan salt lakes. *Aphanius* populations from these two subsystems are not commonly in connection, except between some sites during rainy years (e.g. between Kharameh and Gomban), thus it was not known whether gene flow is present (App. 1) (Fig. 2A).

The Kor River subsystem involves two large tributaries, the Sivand (Pulvar) and Main Rivers that originate from the north and northwest of the Zagros Mountains, join the Kor River and drain into the northwest corner of the Bakhtegan Lake; a continuous water flow appears throughout the year (Nadji, 1997) (Fig. 2A). Thus a permanent interconnection of fish populations along these two rivers probably appears. However, the upper reaches of the Kor River in the northwest of the Zagros Mountains (high up to 4400 m) geologically underwent different tectonic events, which might have led to vicariant events and population differentiation (see App. 2).

The subsystem of the Tashk and Bakhtegan salt lakes involves the hypersaline and fishless waterbodies of the Tashk Lake and the southward bordering Bakhtegan Lake (Houtum-Schindler, 1981) in the lowest area of the Kor River Basin (1525 m). Further waterbodies are several isolated freshwater springs that drain into the salt lakes, but are not regularly connected to each other (see App. 1).

In recent years, the habitats of many spring-streams in the Kor River Basin were severely affected due to drought, water pollution, heavy pumping, habitat loss and degradation (App. 1). However, there was no information with regard to the taxonomic state and intraspecific variation of *A. sophiae* before this PhD project. The males of *A. sophiae* display narrow white flank bars, whereas the females have a small dark spot at the base of the caudal fin and irregular spots on the flanks that may be oval, round or elongate (Fig. 2B). During my PhD project I have examined the scale surface microstructure, scale morphology, and J-indices between the populations of *A. sophiae* (App. 1).
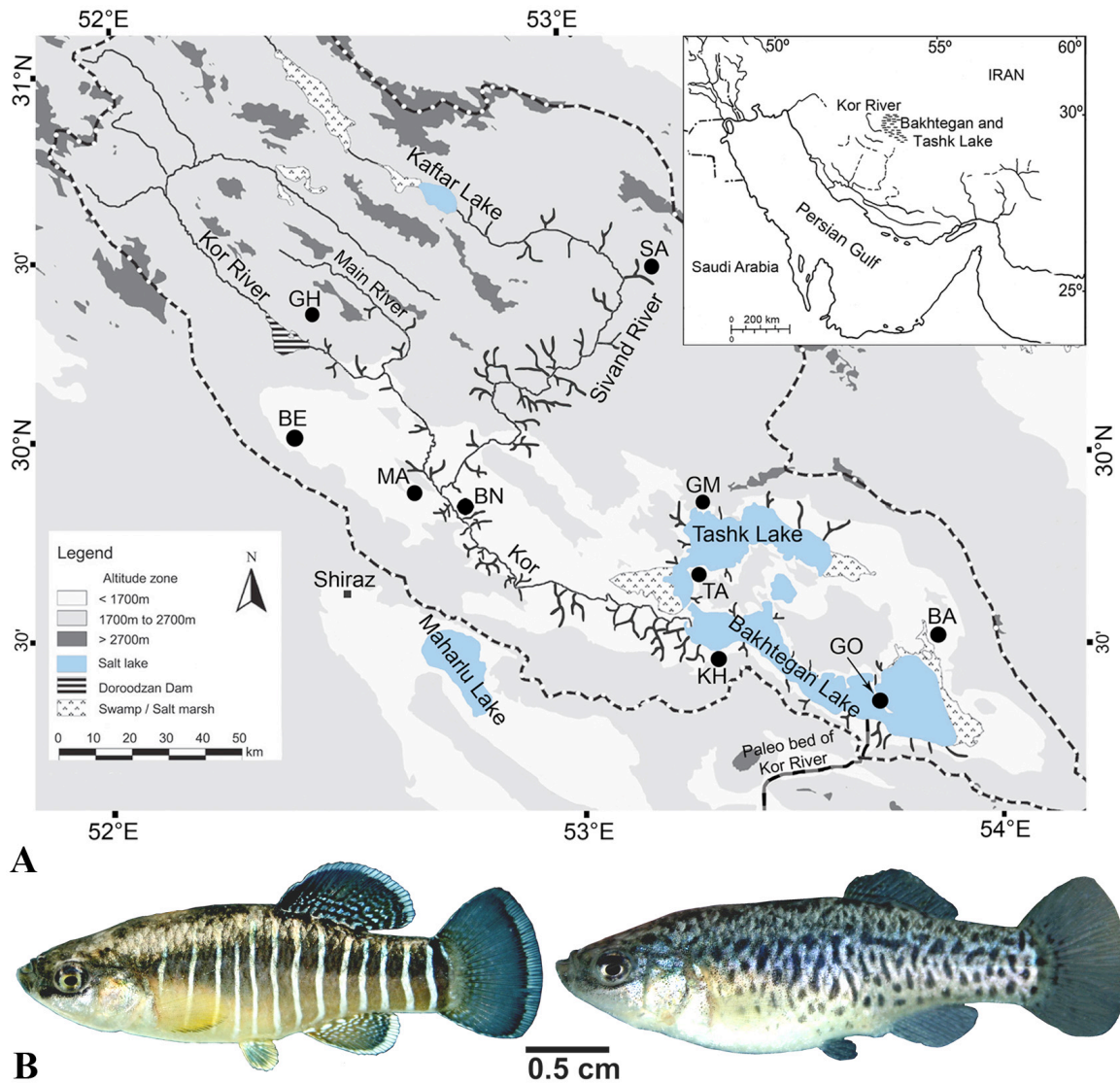
Figure 2. A) Geographic overview and studied sites of the Kor River Basin (SA; Safashahr, GH: Ghadamgah, BE: Beyza, MA: Maloosjan, BN: Bandeamir, KH: Kharameh; GM: Gomban, TA: Tashk, GO: Gol and BA: Bakhtegan. B) Male (left) and female (right) of *A. sophiae* from the Maloosjan spring-stream. Figure modified from App. 1–2; source of map from Kehl et al., (2009).

1.4. *Aphanius farsicus*

The Farsi tooth-carp, *Aphanius farsicus* Teimori, Esmaeili and Reichenbacher, 2011 is distributed in the endorheic Maharlu (Shiraz) Basin, southeast of the city Shiraz (SW Iran, Fars Province) (Fig. 3A). Jenkins (1910) described this species as *Cyprinodon persicus* from a spring on the edge of "Shiraz Lake"; the type locality is not exactly clear. Later, Coad, (1996) recognized this species as *Aphanius* and he named it *A. persicus* (Jenkins, 1910) based on meristic and morphometric characters. In 2011, it became clear that the fossil tooth-carp *Brachylebias persicus* Priem, 1908 from the Upper Miocene of

Northwestern Iran is a species of *Aphanius* (see Gaudant, 2011; Reichenbacher et al., 2011). Thus, the extant *A. persicus* (Jenkins, 1910) became a homonym of the fossil *A. persicus* (Priem, 1908), and *A. farsicus* was introduced as a replacement name (see Teimori et al., 2011). The habitats of *A. farsicus* are freshwater streams and springs around the hypersaline fishless Maharlu Lake (salt concentration >200 ppt, Esmaeili et al., 2007; see here Fig. 3B).

The Maharlu Basin covers nearly 4,200 km^2 (see Agard, 2005; Zare et al., 2005; Faghih et al., 2012). The northern part of this basin is mountainous with elevations of 1,455–2,990 m and the southern part is mainly plain (Khaksar et al., 2004). Main sources of freshwater in the Maharlu Basin are seasonal rivers that drain from the Zagros Mountains (Shiraz (Khoshk) and Babahaji rivers), and several karstic spring-streams (Fayazi et al., 2007; Faghih et al., 2012) (Fig. 3B). The spring-streams around the hypersaline fishless Maharlu Lake may be connected via the rivers during rainy winter seasons. In summer, when the rivers are dry, several spring-streams still hold freshwater and represent essential habitats for the populations of *A. farsicus*.

In recent years, the habitats of many spring-streams were severely affected because of drought, heavy pumping, water pollution, habitat degradation and loss and by the introduction of exotic species (App. 1, 4). Before my PhD project, only the study of Coad (1996) was available; he found no clear separation between nine *A. farsicus* populations based on meristics and morphometrics. The males of *A. farsicus* show comparatively thick white flank bars, whereas the females display irregular dark thin flank bars and a dark, large spot at the base of the caudal fin that may be oval, elongate or lozenege-shape (Fig. 3C). In the course of my PhD project I have examined the morphological (meristics, morphometrics, otoliths, scale surface microstructure, scale morphology, J-indices) and genetic variation (*Cyt b*) between the populations of *A. farsicus* (App. 1, 4).
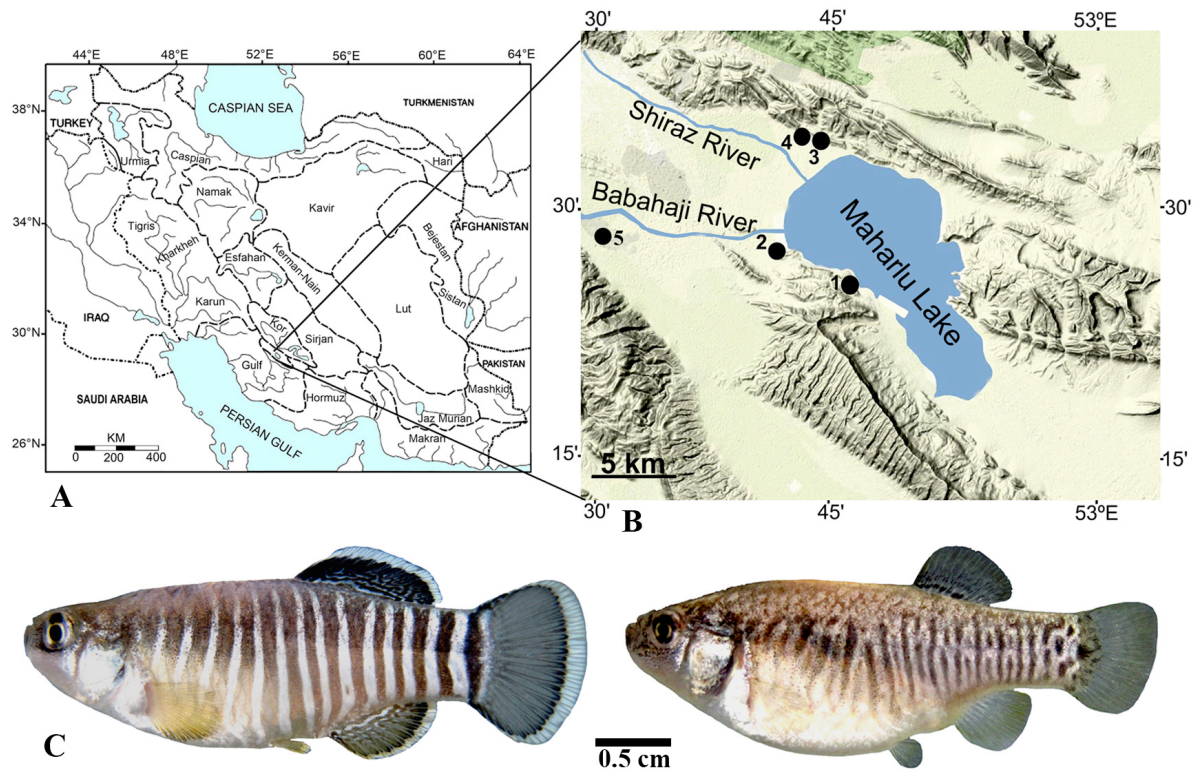
Figure 3. A) Geographic overview and B) sites of the studied populations of *Aphanius farsicus* (1-Dobaneh, 2- Barmeshoor, 3- Babunak, 4- Nearbabunak, 5- Pirbanoo). C) Male (left) and female (right) of *A. farsicus* from the Barmeshoor spring-stream. Figure from App. 4; source of maps Coad (2014) for A and Google Map for B.

1.5. Objective

The main purpose of this PhD project was; i) to investigate the phenotypic variation, phylogenetic relationships and evolutionary history of the populations of *A. sophiae* from the Kor River and *A. farsicus* from the Maharlu Lake basins; and ii) to understand connectivity and gene flow between the populations of each species, which is a major subject for conservation strategy and habitat management. Moreover, I have investigated the taxonomic status and phylogenetic relationships of two previously not known populations from the Helleh and upper reaches of the Karun basins (Fig. 4). Furthermore, I have collaborated to the studies on several other Iranian inland *Aphanius* species, of which the taxonomic status, phylogenetic relationships and evolutionary history were unknown (populations from the Mond River, Namak Lake, Kavir and Kol River basins).

2. Materials

Specimens were collected using dip net from selected basins and sites in Iran (Fig. 4, Table 1), transferred to 5% ethanol for 10 min to avoid shrinkage and then preserved in 96% ethanol (we did not use formalin solution because it corrodes otoliths, and inhibits molecular studies). In total, 26 different populations belonging to 10 inland basins were studied in the course of my PhD project (see App. 1–7). All specimens are deposited in the Zoological Museum of the Shiraz University, Collection of Biology Department, Iran (ZM-CBSU).

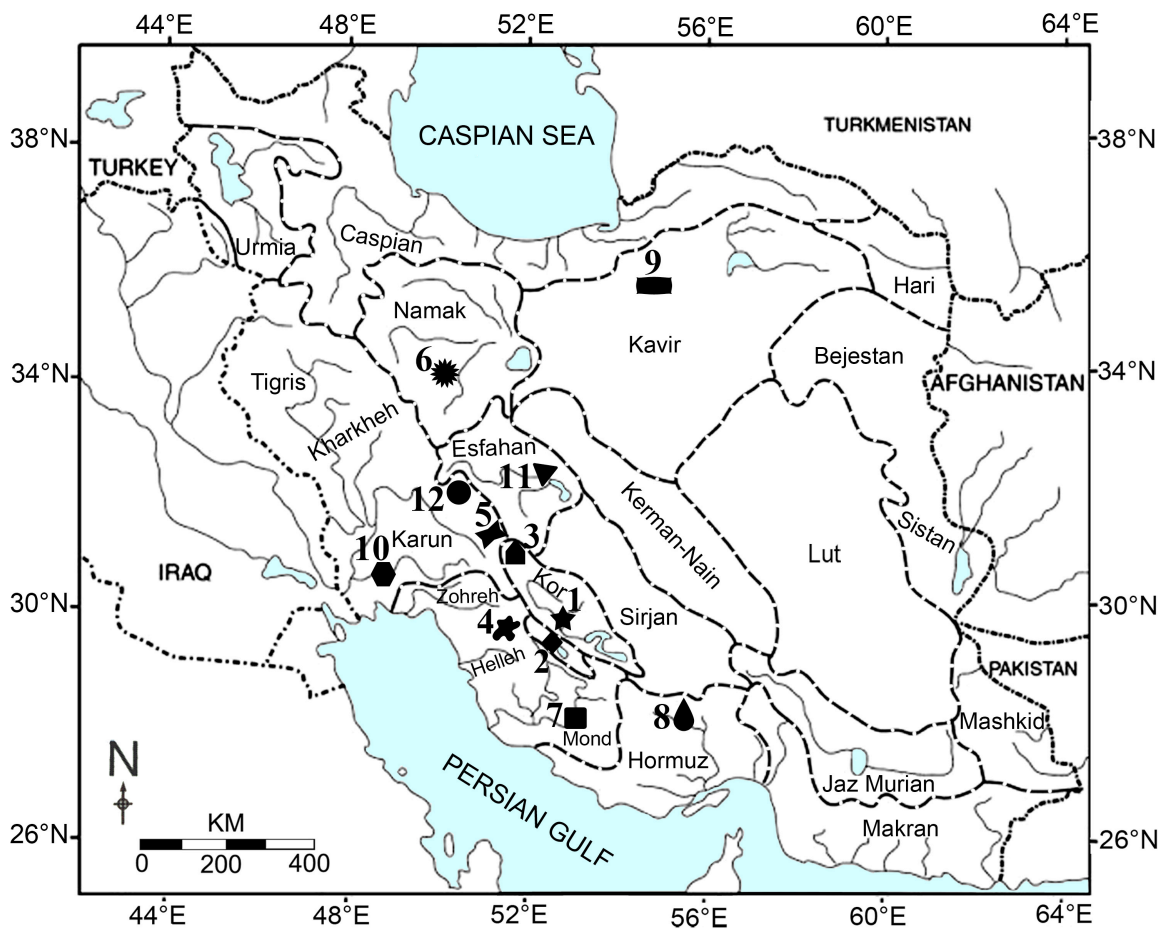Figure 4. Distribution of the Iranian inland and inland-related *Aphanius* species. 1) *Aphanius sophiae* (Kor River Basin), 2) *A. farsicus* (Maharlu Lake Basin), 3) *A. shirini*, 4) *A. sophiae* (Arjan Wetland), 5) *A. sophiae* (Semirom spring), 6) *A. arakensis*, 7) *A. pluristriatus*, 8) *A. darabensis*, 9) *A. kavirensis*, 10) *A. mesopotamicus*, 11) *A. isfahanensis*, 12) *A. vladykovi*. Source of map: Coad (2014), modified.

Table 1. Details of the samples studied. SD = standard deviation, SL = standard length in mm, N= number of specimens.

Sampling site/altitude			SL range and Mean (±SD)	
Kor River Basin, *Aphanius sophiae*	**GPS data**	**N ♂/♀**	**Male**	**Female**
Safashahr spring-stream/2557 m/	E 53º31'51.73″ N 30º34'50.78″	31/32	25.2–38.5 (29.8±4.4)	26.4–40.8 (30.2±3.2)
Ghadamgah spring-stream/1660 m/	E 52º25'36.42″ N 30º15'23″	30/30	23.0–33.3 (26.1±2.2)	25.3–38.8 (26.7±3.3)
Beyza spring-stream/1631 m/	E 52°21'11.1″ N 29°57'41.8″	18/19	16.9–29.1 (22.5±4.2)	21.3–37.1 (22.5±3.6)
Maloosjan spring-stream/1656 m/	E 52º29'48.4″ N 29º52'19.7″	6/8	24.7–35.6 (30.1±4.5)	23.7–42.8 (25.1±3.7)
Bandeamir spring-stream/1861 m/	E 52°50'23.56″ N 29°45'49.03″	15/15	15.9–26.2 (20.3±2.9)	13.6–27.5 (19.2±3.9)
Kharameh spring-stream/1576 m/	E 53°31'08.4″ N 29°27'33.6″	20/20	23.1–32.2 (26.4±2.5)	27.2–37.3 (32.8±2.9)
Gomban spring-stream/1558 m/	E 53°28'57.87″ N 29°47' 48.42″	26/24	20.7–27.2 (23.3±1.7)	19.4–35.3 (26.1±4.3)
Bakhtegan spring/1582 m/	E 54°6'35.74″ N 29°26'23.60″	20/19	14.8–28.1 (20.0±3.4)	20.1–30.6 (24.1±3.0)
Gol spring/1632 m/	E 53°55'58.38″ N 29°21'12.65″	11/11	14.3–18.6 (15.6±1.4)	14.1–19.5 (16.0±1.8)
Tashk spring/1644 m/	E 53°30'9.11″ N 29°39'35.08″	20/20	15.3–24.4 (18.7±1.8)	14.7–20.1 (17.3±1.7)
Maharlu Lake Basin, *A. farsicus*				
Dobaneh spring-stream/1453 m/	E 52º46'24.7″ N 29º25'48.1″	20/23	20.6–33.3 (23.3±1.6)	21.3–38.2 (27.5±3.8)
Barmeshoor spring-stream/1465 m/	E 2º42'0.051″ N 29º27'9.51″	35/32	17.1–26.8 (19.7±1.9)	19.6–35.2 (24.1±3.1)
Babunak spring-stream/1480 m/	E 52º44'23.2″ N 29º33'58.68″	20/28	16.20–25.4 (18.7±2.8)	16.6–35.6 (18.8±2.7)
Nearbabunak spring-stream/1479 m/	E 52º43'58.2″ N 29º33'12.3″	11/11	19.0–25.4 (22.0±2.2)	20.6–34.6 (27.7±3.8)
Pirbanoo spring-stream/1501 m/	E 52°30'58.68″ N 29°28'12.72″	20/20	16.0–24.5 (19.8±2.8)	17.8–22.8 (19.9±1.4)
Upper reaches of the Kor River Basin, *A. shirini*				
Khosroshirin spring-stream/2327 m/	E 52º00'36.8″ N 30º53'29.5″	4/5	21.2–29.6 (24.3±3.1)	33.9–35.5 (34.6±0.7)
Helleh Basin, *A. sophiae*				
Arjan Wetland/2004 m/	E 51º59'14.39″ N 29º39'20.99″	16/14	20.6–29.4 (25.0±2.5)	21.1–36.5 (28.6±5.0)
Upper reaches of the Karun Basin, *A. sophiae* & *A. vladykovi*				
Semirom spring-stream/1855 m/	E 51º26'59.2″ N 31º11'2.3″	9/13	22.0–27.5 (24.3±2.1)	17.5–34.6 (26.6±5.4)
Chaghakhor spring-stream/2278 m/	E 50°56'30.5″ N 31°55'30.7″	13/11	21.2–29.2 (25.0±2.4)	22.4–41.4 (27.8±5.3)
Namak Basin, *A. arakensis*				
Arak spring-stream/1786 m/	E 49°50'50.8″ N 34º00'35.1″	12/11	22.6–32.7 (27.3±3.2)	22.5–34.0 (27.9±3.8)
Mond Basin, *A. pluristriatus*				
Zarjan spring-stream/1455 m/	E 53°36'59.23″ N 29°3'39.44″	20/20	15.3–22.7 (18.2±2.2)	16.3–23.3 (18.7±2.0)
Khonj spring-stream/576 m/	E 53º12'59″ N 28º06'32″	4/4	21.6–28.8 (24.2±3.2)	22.7–24.7 (24.1±0.9)
Kol River Basin, *A. darabensis*				
Korsiah Banaki spring-stream/1027 m/	E 54°23'35.48'' N 28°46'24.96''	35/35	29.3±1.3 (25.9–31.9)	28.2±1.1 (26.0–31.8)
Kavir Basin, *A. kavirensis*				
Cheshmeh Ali spring/1569 m/	E 54°05'01.6'' N 36°16'45.6''	32/42	29.05±1.1 (26.6–32.1)	27.61±1.54 (24.8–31.6)
Tigris Basin, *A. mesopotamicus*				
Shadegan Wetland/7 m/	E 48°32'22.7″ N 30º41'01.4″	4/4	16.6–23.4 (19.3±3.2)	16.1–29.1 (22.4±5.3)
Isfahan Basin, *A. isfahanensis*				
Varzaneh spring-stream/1479 m/	E 52°39'14.3″ N 32º25'26.28″	10/11	20.1–23.8 (21.8±1.3)	20.2–34.0 (24.9±3.8)

3. Methods

3.1. Meristic and morphometric characters

Nine meristic characters were counted under a stereomicroscope. They included the numbers of the branched and unbranched dorsal, anal, pectoral and pelvic fin rays, the numbers of gill rakers of the first branchial arch, and the numbers of lateral scale series, caudal peduncle scales, predorsal scales and white flank bars (Holcik, 1989; App. 2–7). Based on standard morphometry (Holcik, 1989), 15 morphometric parameters of each fish were measured using a vernier caliper adjusted to the nearest 0.5 mm (Fig. 5). Measurements were standardized in relation to the standard length (SL) in order to eliminate size effects (Lahnsteiner and Jagsch, 2005). In total, 14 morphometric variables were calculated and served as input for the statistical analyses (Table 2, App. 2–7).
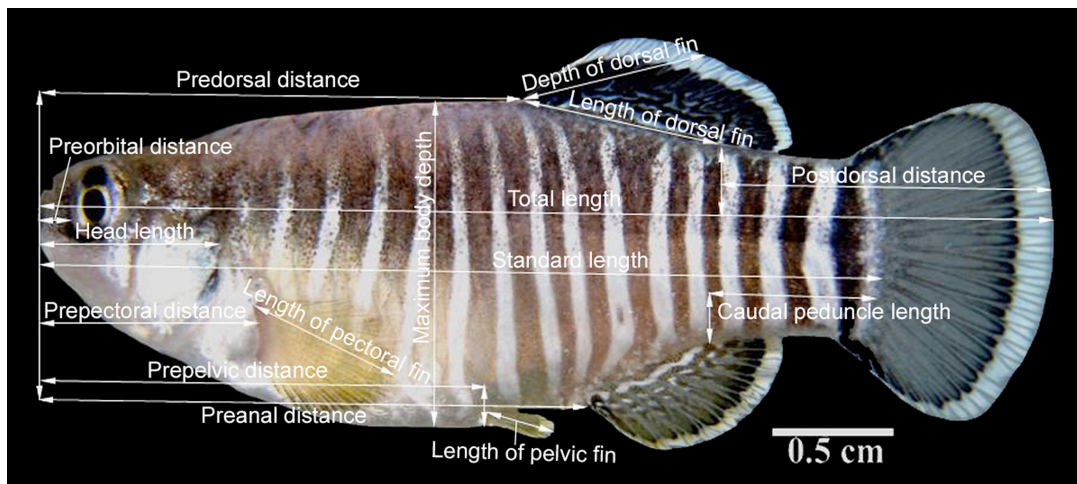
Figure 5. *Aphanius farsicus* (♂) from the Barmeshoor spring-stream (Maharlu Lake, SW Iran) and morphometric characters used in this PhD project.

Table 2. Meristic and morphometric characters of the studied *Aphanius* individuals.

Meristic characters	Morphometric characters	
Dorsal fin rays	Total length	Dorsal fin Depth
Anal fin rays	Standard length	Dorsal fin length
Pectoral fin rays	Head lengt	Pelvic fin length
Pelvic fin rays	Predorsal distanc	Pectoral fin length
Gill rakers	Postdorsal distanc	Prepelvic distance
Caudal peduncle scales	Preanal distance	Prepectoral distance
Lateral scale series	Preorbital distance	
Predorsal scales	Maximum body depth	
White flank bars	Caudal peduncle length	

3.2. Analysis of the scale surface microstructure, scale morphology and scale sizes (J-indices)

For scale morphology, from each fish of the studied populations or species, five scales from the 3rd or 4th row below the dorsal fin, refered as key scale, were removed from the left side of two males and two females, respectively (Lindroth, 1963; Esmaeili, 2001; Johal et al., 2006; App. 1) (Fig. 6A). Scales were rinsed in triple distilled water, cleaned mechanically using a fine brush to remove extranous matter or soft tissue from the surface and transfered into 1% KOH solution for 30 to 60 min. Following the procedure outlined in Lippitsch (1990), the cleaned scales were dehydrated through an ascending ethanol series (30%, 50%, 70% and 90%) at 30 min intervals, then dried on Whatman filter paper (No. 10), kept for several hours between two glass slides to avoid curling of the scale margins, mounted dorsal-up on Scanning Electron Microscopy (SEM) stubs with double self-adhesive carbon stickers, and coated with an 100 A° thick gold layer using a Polaron E 5100. Five to twelve images per scale were imaged with a LEO 1430VP at 15kV.

For measurment of scale sizes, four scales from the 3rd or 4th row below the dorsal fin from the left side of each fish were removed, and then mounted between two microscope slides. The length and width of the scales were measured to the nearest 0.1 mm using an oculometer attached to a stereomicroscope (Fig. 6B). For each individual, the measurements of scale length and scale width were averaged to obtain a single length and a single width value and relative length and width of scales in relation to SL were calculated (J-indices= length or width of scale/fish standard length ×100) (Esmaeili, 2001; App. 1, 3, 5–7).
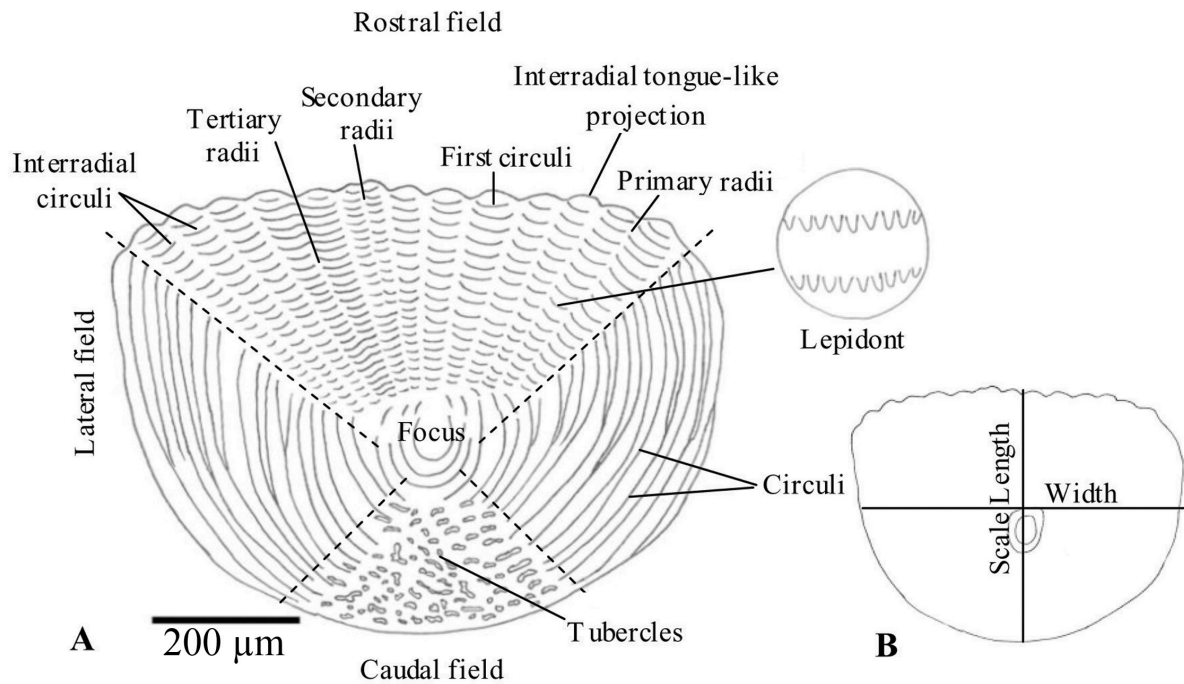
Figure 6. A) Schematic drawing of *Aphanius* scale, including the terms used in this study. Terminology follows Lippitsch (1990), Kuusipalo (1998), Jawad (2005a) and Jawad and Al-Jufaili (2007). Dashed lines delimit the different fields. B) Measurements of scale length and width after Esmaeili (2001).

3.3. Analysis of otoliths

For otolith analysis, fish skulls were opened dorsally, and left and right otoliths were removed. Otoliths were cleaned from organic remains with 1% KOH solution for 3–4 hours, rinsed in distilled water for 4–5 hours, and then washed several times with distilled water (Reichenbacher et al., 2007, 2009a–b; App. 2–5, 7). Otolith morphology was studied using a stereomicroscope and scanning electron microscopy (SEM) (LEO 1430 VP) (Fig. 7A). For otolith morphometrics, digital images from left otoliths were captured using Leica DFC 295 camera and three angles and eight linear distances of each left otolith were measured using the Leica Image Access Software (IMAGIC 1000, Imagic Bildverarbeitung AG, Glattbrugg, Switzerland). Ten otolith variables were calculated and served as as input for statistical analyses (see Reichenbacher et al., 2007) (Fig. 7B).
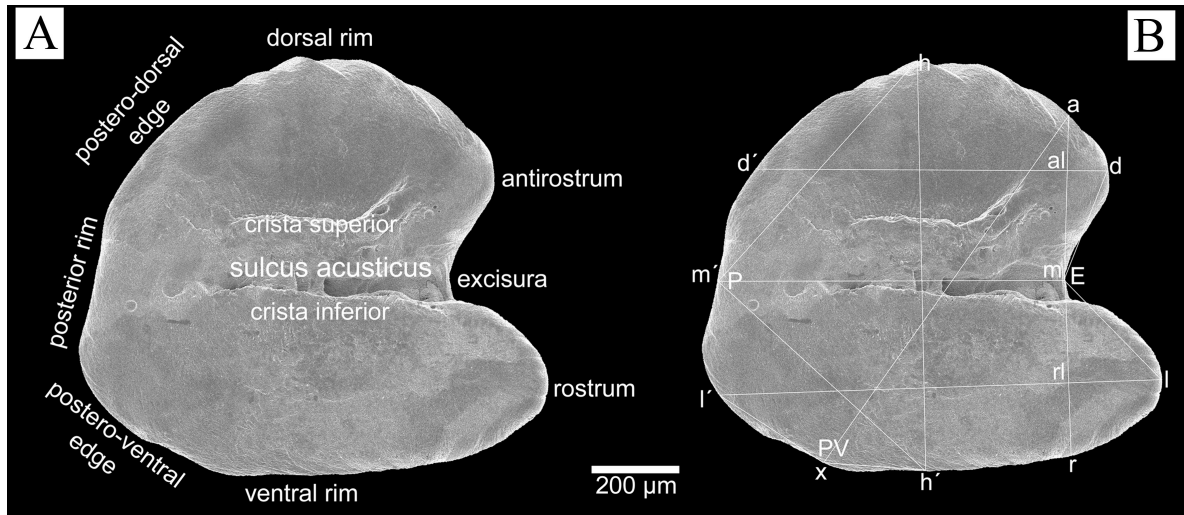
Figure 7. A) SEM micrograph of left otolith of *Aphanius farsicus* (male) from the Barmeshoor spring-stream showing terminology of otolith characters (according to Nolf 1985), B) linear measurements and angles according to Reichenbacher et al. (2007), E= excisura angle, P= posterior angle, PV= posteroventral angle, l–l´= maximum length, h–h´= max. height, m–m´= length of medial part, rl–l= length of rostrum, r–m= height of rostrum, al–d= length of antirostrum, m–a= height of antirostrum, d–d´= length of dorsal part.

3.4. Statistical analysis

Statistical analyses were carried out using PASW v.19, v.20, v.21 (SPSS Inc, 2011, 2012, 2013). Univariate analysis of variance (ANOVA, with Duncan's post hoc test, $p < 0.05$) was used to test the significance of phenotypic differences among populations and also between sexes. The t-test ($p < 0.05$) was applied to test whether variables show significant differences between two groups. The Canonical discriminant analysis (CDA) was used for multivariate analyses in order to show the classification success between the groups. To determine the discriminatory importance of each variable (i.e. the value of each variable that contributed most to the separation of the groups) across all discriminant functions, the mean discriminant coefficient was calculated using the following equation (Backhaus et al., 2006): Mean discriminant coefficient $b_j = \Sigma \_|b_{jk}|*EAK$ (k=1, k=….) where b_{jk} is the standardized discriminant function coefficient for variable j with respect to the discriminant function k and where EA_k is the proportion of the eigenvalue of the discriminant function k relating to the sum of all eigenvalues. Moreover, 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 distinct populations.

3.5. Molecular analysis

3.5.1. DNA extraction, PCR amplification, nucleotide sequencing and phylogenetic analyses

A small piece of dorsal muscle of each alcohol preserved fish was removed in sterile condition and placed in 96-well Eppendorf PCR plates until further processing. Total genomic DNA was purified by extraction of tissues with high concentrations of Guanidinium Thiocyanate and passage of the extracts through fiberglass membranes (AcroPrep 1 µM glass fiber; PALL 5051) (Vargas et al., 2012). The entire cytochrome b gene was amplified via PCR using the primers (forward: L14724 (5'-GTGACTTGAAAAACCACCGTTG-3'; reverse: H15915 (5'-CAACGATCTCCGGTTTAGAAGAC-3') (Schmidt and Gold, 1993; Perdices et al., 2001). Amplification was performed under the following temperature regime: initial 92ºC for 3 min, 34 cycles at 92ºC for 1 min, 53ºC for 90 sec, 72ºC for 3 min, followed by a final extension at 72ºC for 4 min. PCR products were purified with the PEG (Polyethylene glycol) method (after Rosenthal et al., 1993). Cleaned DNA was sequenced in a cycle sequencing reaction using BigDye 3.1 chemistry (Applied Biosystems, Munich, Germany) according to the protocol provided by the manufacturer and the same forward and reverse primers as used for PCR and follows: initial 96ºC for 1 min, 30 cycles at 96ºC for 15s, 52ºC for 10 min, 60ºC for 2 and 30 min. Sequencing was performed on an ABI 3730 automated sequencer in the Genomic Sequencing Unit, Department of Biology, LMU, Munich. Sequences were assembled, edited and aligned with MUSCLE (Edgar, 2004) under default setting as incorporated in Geneious version 6.4 (Drummond et al., 2012). Additional *Aphanius* and outgroup species sequences were obtained from the NCBI GenBank (http://www.ncbi.nlm.nih.gov) and included in the analyses to assess the phylogenetic position of the new taxa. Maximum likelihood reconstructions with PhyML were assessed using SeaView version 4 (Guindon et al., 2010; Gouy et al., 2010). The best-fitting model of nucleotide substitution was estimated using the program jModelTest 2.1.1 under the Akaike Information Criterion (Darriba et al., 2012).

Phylogenetic relationship based on Bayesian inference (BI) was estimated using MrBayes 3.2 (Ronquist et al., 2012) with two runs of four Markov Chain Monte Carlo (MCMC) chains over a total of 2,000,000 generations under the most generalized model (GTR+G+I), because overparametrization obviously does not negatively affect Bayesian analyses (Huelsenbeck and Ranala, 2004). Chains were terminated when the standard deviation of split frequencies fell below 0.01, and likelihood values of sampled trees from

both runs reached a stationary distribution. The first 25% of trees before were disregarded as "burnin".

To distinguish models of population growth from the null hypothesis of constant population size, Fu's Fs (Fu, 1997) statistic was calculated and the R_2 neutrality tests (Ramos-Onsins and Rozas, 2002) were perfomed with DnaSP (version 5.10.1; Librado and Rozas, 2009). Neutral evolution was also tested by calculating Tajima's D (Tajima, 1989) in DnaSP. The probability of neutrality tests was calculated based on 1000 replicates. In addition, pairwise Fst values were calculated to assess gene flow (Nmf) and estimate the degree of genetic connectivity. In order to assess the relationships between haplotypes, a haplotype network based on haplotype frequency was constructed using TCS v. 1.21 (Clement et al., 2000). This method estimates an unrooted tree and provides a 95% plausible set of parsimonious relationships between the haplotypes.

4. Results

4.1. Results on *Aphanius sophiae* and *A. farsicus*

According to the accomplished data in my PhD project (App. 1–4, see below), one new species and several populations have been described from the Kor River and Maharlu Lake basins and their phenotypic variation, phylogenetic relationships and evolutionary history have been analysed. A link between geological events and species divergence in the active tectonic areas of the Iranian plateau has been elaborated for the first time.

The main findings of my PhD projects are as follows:

4.1.1. Scale surface microstructure and scale size in the tooth-carp genus *Aphanius* (Teleostei, Cyprinodontidae) from endorheic basins in Southwest Iran

The study was based on four populations of *Aphanius farsicus* from the Maharlu Basin (128 specimens), nine populations of *A. sophiae* from the Kor River Basin (317 specimens) and a single population of *A. pluristriatus* from the Mond River Basin (40 specimens) (App. 1).

Scale morphology. – The results showed that the scales of both species are cycloid, that no differences of the scales between males and females are present and that the overall shape and surface morphology is highly variable between and within species. In contrast, several scale surface microstructures (Fig. 6A), i.e. focus position, granules, total number of radii,

first circuli, tubercles, and lepidonts exhibited clear intra- and interspecific differences. Uni- and multivariate analyses showed that the characters related to the lepidonts (size, shape, number and distance), tubercles (shape, number and position), total radii and focus granules can contribute to discriminate certain populations of *A. sophiae* (those from Safashahr, Kharameh, Tashk, Gol), whereas *A. farsicus* did not reveal such clear within-species differentiation (App. 1).

Scale sizes. – Relative scale sizes (J-indices) represented a valuable tool for species separation, and highlighted the important role of J-indices in *Aphanius* taxonomy, but as expected, J-indices were not significant for a given population. All males and females of the three studied species had large scales relative to the standard length of fish body, and males had often relatively larger scale sizes than females and the relative scale width (Jsw.sl) was higher than the relative scale length (Jsl.sl). Also, *A. farsicus* showed higher J-indices than *A. sophiae* (App. 1).

Appendix 1. Gholami, Z., Teimori, A., Esmaeili, H.R., Schulz-Mirbach, T. & Reichenbacher, B. (2013). Scale surface microstructure and scale size in the tooth-carp genus *Aphanius* (Teleostei, Cyprinodontidae) from endorheic basins in Southwest Iran. Zootaxa, 3619 (4), 467–490.

4.1.2. Phylogenetic analysis of *Aphanius* from the endorheic Kor River Basin in the Zagros Mountains, Southwestern Iran (Teleostei: Cyprinodontiformes: Cyprinodontidae)

The collected specimens for the study included nine *Aphanius* individuals from the Khosroshirin River (upper reaches of the Kor River Basin), 14 specimens of *A. sophiae* from the Maloosjan spring (Kor River Basin), eight specimens of *A.* cf. *pluristriatus* from the Mond Basin, 21 individuals of *A. isfahanensis* from the Isfahan Basin, 23 specimens of *A. arakensis* from the Namak Basin, 21 individuals of *A. farsicus* from the Maharlu Basin and 24 specimens of *A. vladykovi* from the Karun Basin (Table 1) (App. 2).

Only few populations of *A. sophiae* were previously described from the Kor River Basin based on external morphology and no genetic data was available. During my study of the Kor River Basin, several new populations were discovered. Based on meristics, morphometrics, otolith characters and molecular analysis I found out that a specific population from the upper reaches of the Kor River Basin (Khosroshirin River) is significantly different from *A. sophiae* from the site Maloosjan, which is near to the type locality Persepolis. Therefore, this population has been recognized as a new species, *A. shirini* sp. n., which is diagnosed by 17 fixed molecular apomorphies. In comparison to the

known Iranian inland *Aphanius* species, this new species showed the lowest number of white flank bars, a higher predorsal, postdorsal and caudal peduncle length, the smallest dorsal fin depth, and a straight ventral rim of the otoliths in combination with a short and truncated rostrum and long dorsal part. Moreover, I found that there is a relation between the geological history and the present-day *Aphanius* species diversity in the tectonically still active Zagros Mountains of Iran. The molecular data indicated that the divergence of *A. shirini* sp. n. is much older than the divergence of *A. sophiae* from the Kor River Basin. It is suggested that the divergence of *A. shirini* sp. n. can be linked to the Miocene-Pliocene period of defomation in the High Zagros Mountains, whereas *A. sophiae* appears to be a very young species that may have diverged during the Early to Middle Holocene (11,700 to 4000 y. ago) (App. 2).

Appendix 2. Gholami, Z., Esmaeili, H.R., Erpenbeck, D. & Reichenbacher, B. (2014). Phylogenetic analysis of *Aphanius* from the endorheic Kor River Basin in the Zagros Mountains, Southwestern Iran (Teleostei: Cyprinodontiformes: Cyprinodontidae). Journal of Zoological Systematics and Evolutionary Research, 52 (2), 130–141.

4.1.3. Evidence for wide presence of *Aphanius sophiae* (Teleostei: Cyprinodontidae) during the Early and Middle Holocene in the Central Zagros

This study was based on two previously not known isolated populations of *Aphanius*, i.e. 30 specimens from the Arjan Wetland (Helleh Basin) and 22 individuals from the Semirom spring-stream (upper reaches of Karun Basin) (Fig. 4). For comparison, 14 individuals of *A. sophiae* from the Maloosjan spring (near to the type locality of this species in the Kor Basin) and nine specimens of *A. shirini* from the Paselari spring (upper reaches of the Kor Basin, the type locality) were used (App. 3).

Molecular data (*Cyt b* gene) indicated that the individuals from the Arjan Wetland represent two distinctive species, but phenotypic differences were not observed between them. One of those formed a monophyletic unit with *A. shirini* (as known from the type locality) and clearly represents a population of *A. shirini*; it is termed *A. shirini* (Arjan). The other represented a population of *A. sophiae* (as known from the Kor Basin) and is termed *A. sophiae* (Arjan). *Aphanius sophiae* (Arjan) is sister to *A. sophiae* (Maloosjan, Kor Basin) + the population from the Semirom spring.

The population from the Semirom spring comprised a single monophyletic group, which is sister to *A. sophiae* (as known from the Kor Basin), and together with this population, is

sister to *A. sophiae* (Arjan). This lineage clearly represents another population of *A. sophiae*, which is termed *A. sophiae* (Semirom).

However, significant phenotypic variation was observed among *A. sophiae* (Arjan), *A. sophiae* (Semirom) and *A. sophiae* (Maloosjan) based on meristics, morphometrics, J-indices, otolith morphology and morphometry. As *Aphanius* species have a high potential of phenotypic plasticity, the observed morphological differences may result from the special habitats. The occurrence of isolated populations of *A. sophiae* in the Arjan Wetland and the Semirom spring can be linked to the intensive tectonics of the Main Zagros Reverse Fault and the Kazerun Fault System in the Central Zagros. The onset of the tectonic activity has been estimated within the time span of the Late Pliocene to Early Holocene (Authemayou et al., 2009), and might have caused isolation of populations because former drainage systems were interrupted (App. 4).

Appendix 3. Gholami, Z., Esmaeili, H.R. & Reichenbacher, B. (submitted). Evidence for wide presence of *Aphanius sophiae* (Teleostei: Cyprinodontidae) during the Early and Middle Holocene in the Central Zagros. Limnologica.

4.1.4. Genetic connectivity and phenotypic plasticity in the cyprinodont *Aphanius farsicus* from the Maharlu Basin, Southwest Iran

A total of 92 specimens from four populations of *Aphanius farsicus* were collected for this study. The result showed high variation in meristics, morphometrics and otolith variables among these populations. The variation was related to the caudal peduncle scales, lateral scale series, postdorsal distance, caudal peduncle length, pectoral fin length, dorsal fin length and depth, and otolith variables (relative rostrum height, medial and dorsal relative otolith length). However, no distinguishing molecular apomorphy was found among the populations and the phylogenetic tree failed to recover a supported genetic structure for an individual population, at least under the analysed fragment (*Cyt b*). Fu's Fs and R_2 statistical neutrality tests, pairwise Fst values, Tajima's D test and statistical parsimony analysis based on the haplotype frequencies clearly indicated high genetic connectivity among the studied populations (App. 4).

Appendix 4. Gholami, Z., Esmaeili, H.R., Erpenbeck, D. & Reichenbacher, B. (2014). Genetic connectivity and phenotypic plasticity in the cyprinodont *Aphanius farsicus* from the Maharlu Basin, Southwest Iran. Journal of Fish Biology, in review.

4.2. Results on further inland and inland-related *Aphanius* species from collaborations

As a result from collaborations (Apps. 5–7, see below), three new species have been described and one species has been redescribed and revalidated. All four species are endemic and threatened species. Moreover, the phylogenetic relationships and evolutionary history between all ten currently known Iranian inland and inland-related *Aphanius* species (IIRAS) have been investigated and a link between geological events and divergence of *Aphanius* species in the active tectonic areas of the Iranian plateau has been elaborated. The main findings are as follows:

4.2.1. *Aphanius arakensis*, a new species of tooth-carp (Cyprinodontidae) from the endorheic Namak Lake Basin in Iran

Based on a compiled dataset, 70 individuals from the Namak Lake Basin and as comparative materials, 323 specimens from five Iranian inland and inland-related *Aphanius* species (*A. sophiae*, *A. farsicus*, *A. isfahanensis*, *A. pluristriatus* and *A. vladykovi*) were investigated. The result showed that the population from the Namak Basin was morphologically and genetically different in comparison to the other studied *Aphanius* species. Therefore, this population was described as a new species, *A. arakensis* sp. n. In comparison to the studied Iranian inland *Aphanius* species, the new species showed the highest number of white flank bars, the highest value of interorbital distance, the lowest value of eye diameter, otoliths with a short, high antirostrum, a wide excisura angle, and a small drop-like process of the ventral rim, as well as 19 molecular apomorphy (App. 5).

Appendix 5. Teimori, A., Esmaeili, H.R., 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.

4.2.2. Re-validation and re-description of an endemic and threatened species, *Aphanius pluristriatus* (Jenkins, 1910) (Teleostei, Cyprinodontidae), from southern Iran

A total of 339 specimens of four Iranian inland *Aphanius* species (*A. pluristriatus*, *A. sophiae*, *A. isfahanensis* and *A. farsicus*) were studied. Before this study, *A. pluristriatus* (Jenkins, 1910) was a poorly known species from Fassa (Fussa) in the Mond River Basin, Southern Iran. Moreover, the validity of *A. pluristriatus* was questionable and a synonymy with *A. sophiae* (Heckel, 1949) was suggested by Wildekamp (1993). Our study documented that *A. pluristriatus* is a valid species. In comparison to *A. sophiae*, *A.*

isfahanensis and *A. farsicus* it displayed the lowest value of gill rakers and caudal peduncle length and the largest scale sizes (J-indices). This species is an endemic and threatened species due to restricted habitat numbers, recent drought and introduction of exotic fishes (App. 6).

Appendix 6. Esmaeili, H.R., 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.

4.2.3. Two new species of the tooth-carp *Aphanius* (Teleostei: Cyprinodontidae) and the evolutionary history of the Iranian inland and inland-related *Aphanius* species

In this study, 70 specimens of *Aphanius* from the Kol River (Hormuzgan Basin) and 74 individuals of *Aphanius* from the Cheshmeh Ali spring (Kavir Basin) were examined. As comparative materials, 393 specimens from six Iranian inland and inland-related *Aphanius* species (*A. farsicus*, *A. arakensis*, *A. isfahanensis*, *A. pluristriatus*, *A. sophiae* and *A. vladykovi*) were considered. Based on genetic data (*Cyt b*) and morphological differentiation, these two populations were described as *A. darabensis* sp. n. and *A. kavirensis* sp. n. In contrast to the other studied Iranian *Aphanius* species, *A. darabensis* displayed longer anal and pelvic fins, larger scale width, and symmetrically triangular to trapezoid otoliths with a rostrum that is longer than the antirostrum. *Aphanius kavirensis* showed comparatively short pectoral fins and asymmetrically triangular to trapezoid otoliths with a pronounced predorsal portion and a rostrum that is of equal size or slightly longer than the antirostrum (App. 7).

Based on the molecular phylogeny, two evolutionary groups within the IIRAS clade were recognized (Fig. 8). The "old" group comprises species that may have diverged in the Late Miocene and Early Pliocene, the *A. vladykovi* and *A. shirini* subclades as well as the *A. isfahanensis* lineage belong to this group. The "young" group is represented by the *A. sophiae* subclade, but without *A. isfahanensis*. The young group apparently diverged in the course of the Late Pleistocene to Middle Holocene. Within this group, the splits of *A. farsicus*, *A. sophiae, A. mesopotamicus* and *A. pluristriatus* are clearly related to vicariance events caused by the closure of the formerly exorheic Kor Basin in the Late Pleistocene to Early Holocene. However, the splits of *A. kavirensis* n. sp. and *A. arakensis* are difficult to explain by vicariance events owing to the geological history.
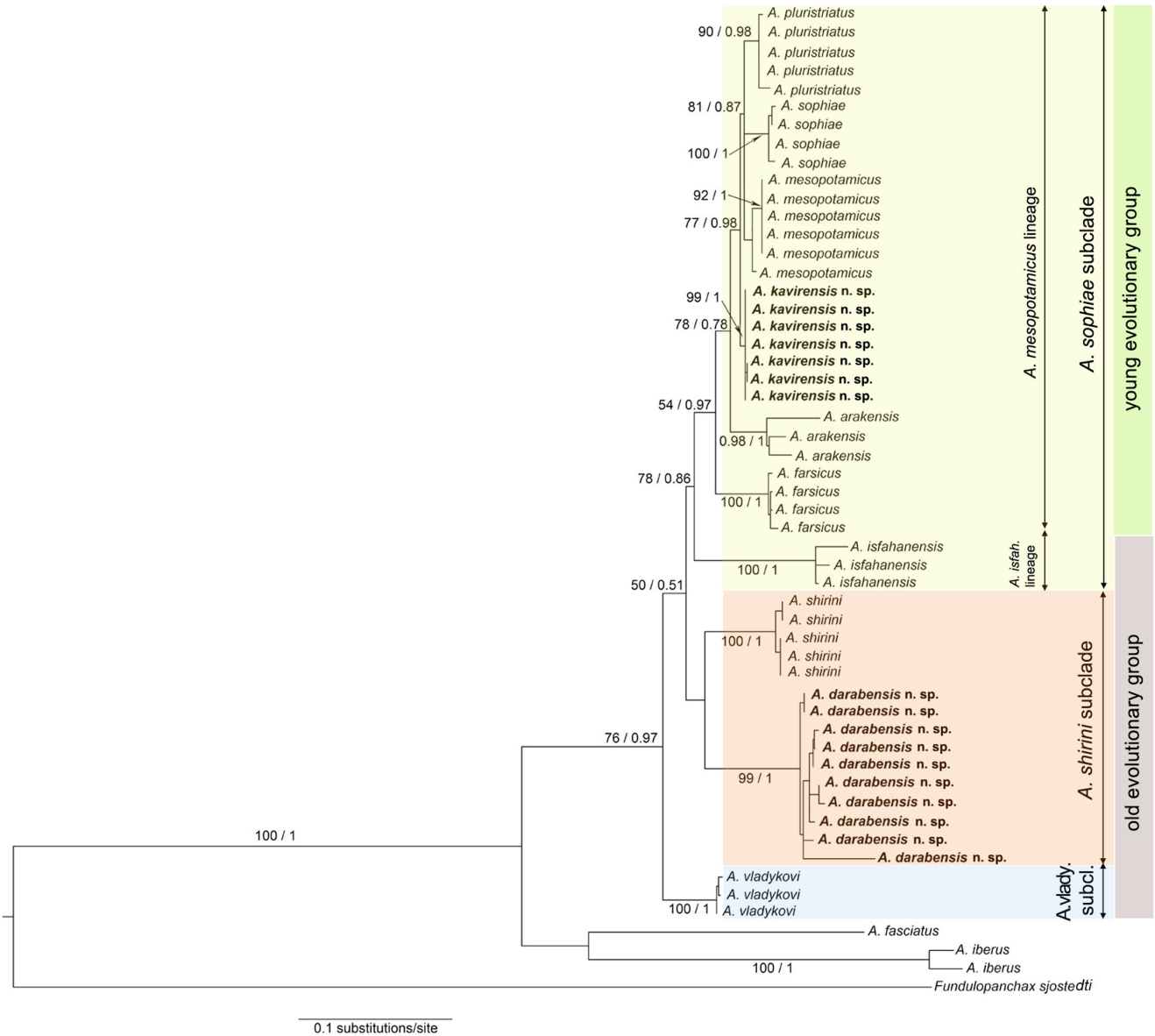
Figure 8. Maximum likelihood estimate (based on cytochrome b gene sequences) of phylogenetic relationships of the Iranian inland and inland-related *Aphanius* species (IIRAS). Numbers above nodes represent maximum likelihood bootstrap values based on 2,000 replicates followed by Bayesian likelihood values. Scale bar indicates substitutions per site. Figure is from App. 7.

5. Discussion

5.1. Phenotypic variation of *Aphanius sophiae* (Kor River Basin/Maloosjan spring, Helleh subbasin/Arjan Wetland and Karun Basin/Semirom spring) and *A. farsicus* (Maharlu Lake Basin)

The results of my PhD project showed that the scale shape and scale surface microstructure could not be used for the identification of *A. farsicus* and *A. sophiae* (populations from the Kor Basin). This outcome supported the results of previous studies on the scales of cichlids and cyprinids (De Pontual and Prouzet, 1987; Coburn and Gaglione, 1992; Kuusipalo, 1998; Jawad, 2005a–b; Jawad and Al-Jufaili, 2007), indicating that the scale shape varies with age and size of the fish (App. 1).

In contrast, the relative scale sizes (J-indices) that had received little attention in the field of fish taxonomy, appeared as a reliable character to distinguish *A. sophiae* and *A. farsicus* (App. 1), and also *A. isfahanensis*, *A. pluristriatus*, *A. vladykovi*, *A. arakensis*, *A. darabensis* and *A. kavirensis* (App. 5–7). However, J-indices were found significantly different in three isolated populations of *A. sophiae* from the Kor, Helleh and Karun basins (App. 3). It can therefore be concluded that differences of J-indices were evolving due to isolation of populations for at least several thousand years (e.g. since the Early or Middle Holocene), and that J-indices may not be taxonomically useful on the species level in every case.

Furthermore, the high phenotypic differentiation found among the studied three *A. sophiae* populations from the Kor, Helleh and Karun basins might be related to the isolation of these populations due to the tectonically induced habitat fragmentation (see 5.3.). In addition, the high phenotypic plasticity of *Aphanius* individuals when exposed to different habitats might also has played a role. For example, a long postdorsal distance and caudal peduncle can enhance efficient swimming (see studies on *Salvelinus fontinalis* and *S. alpinus* by Dynes et al., 1999 and Peres-Neto and Magnan, 2004). Thus, the long postdorsal distance in *A. sophiae* (Semirom) may be linked to improve swimming capability because of the relatively large size of the Semirom spring. Moreover, the small caudal peduncle and postdorsal distance in *A. sophiae* (Maloosjan) may indicate no demand for proficient swimming due to the rather small size of the Maloosjan spring.

Moreover, high phenotypic variation among four *A. farsicus* populations was found, while analysis of genetic data (*Cyt b*) showed high genetic connectivity among populations (App. 4; see Fig. 9). The observed phenotypic variation may be related to the environmental parameters, swimming demands, different sizes of the spring-streams and the occurrence

of competing or predating fish (see App. 4 for more details). Furthermore, intraspecific variation in the otolith variables of *A. farsicus* was observed. Previous studies have shown that intraspecific otolith variation of the relative length of the medial and dorsal part may depend on otolith growth rates, and also on water temperature, habitat productivity and diet availability (Lombarte and Lleonart, 1993; Mérigot et al., 2007; Reichenbacher et al., 2009b; Lombarte et al., 2010; App. 4). On the other hand, otolith variables such as the relative height of the rostrum and antirostrum and the relative rostrum length were usually constant within a species in previous studies. It is possible that the here observed variation of these otolith characters is an artefact of small sample sizes of *A. farsicus* populations.

We conclude that the phenotypic plasticity of *A. farsicus* promotes morphological divergence even between interconnected populations as a result of adaptation to different environmental conditions and swimming demands. In the case that a population becomes isolated, a given trend of evolution may continue to develop and the character may become taxonomically useful on the species level.
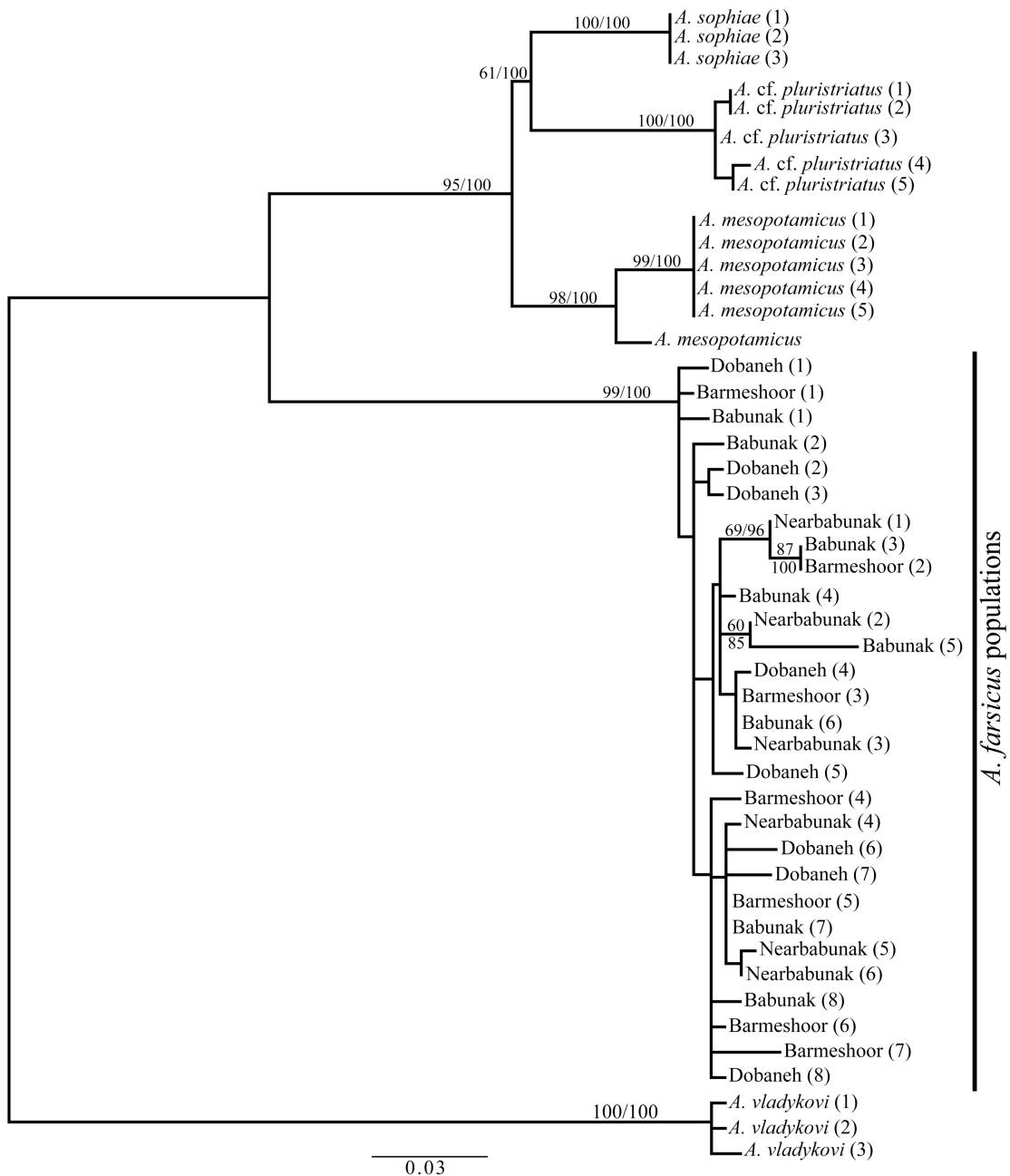
Figure 9. Maximum likelihood phylogenetic reconstruction (based on cytochrome b sequences) of the sample sets of *Aphanius farsicus* and comparative material. Numbers above nodes represent maximum likelihood bootstrap values based on 2000 replicates followed by Bayesian posterior probabilities. 1n(L)= –2674.8280, pinvar = 0.7730. Scale bar indicates substitutions per site. Figure is from App. 4.

5.2. Zoogeography of *Aphanius sophiae* in the Kor River Basin and divergence of *A. shirini*

The Zagros Mountains trends from northwest to southeast Iran and is made up of five main structural units of which the Zagros Folded Belt and the High Zagros include a zone of collision that is active until today (Agard et al., 2005; Gavillot et al., 2010; Mouthereau, 2011) (Fig. 10). The Kor River Basin belongs to the High Zagros and is located within the collision zone; altitudes are between 1,557 to 3,978 m above sea level (Kehl et al., 2005; App. 2) (Fig. 10). Previous work has reported a prominent phase of tectonic deformation in the Late Miocene and Early Pliocene (ca. 10–5 Ma) that has led to the rise of the Iranian plateau and new configurations of mountain ranges and drainage patterns (Homke et al. 2004; Agard et al. 2005; Mouthereau et al. 2007). As the Kor River Basin is located just within the collision zone, its initial configuration is probably linked to this Late Miocene to Early Pliocene period of geological activity (App. 2). Notably, this initial Kor River Basin (Paleo-Kor River) was exorheic and drained to the Persian Gulf through the Mond River (Nadji, 1997; Kehl et al., 2009; Teimori et al., 2012b).

It was shown that the speciation of *A. shirini* can be linked to vicariance events due to the tectonic activity and uplift in the southwestern part of the High Zagros during the Late Miocene (App. 2). Furthermore, it has been pointed out that the present-day endorheic Kor Basin has formed in the Late Pleistocene or Early Holocene, and that the "young" age of *A. sophiae* is clearly related to this history (App. 2). The latter assumption has been supported by the recent study of Esmaeili et al. (2014/App. 7).
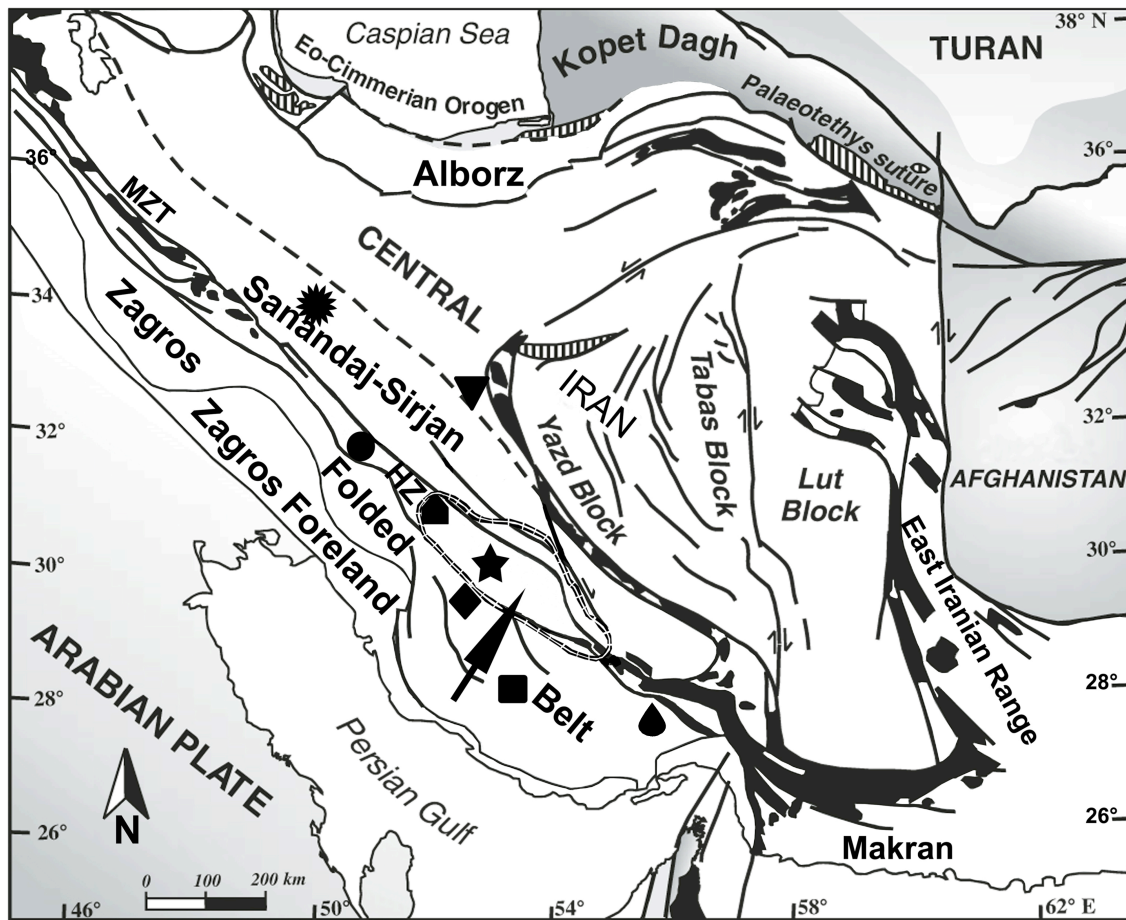
Figure 10. Geological overview of the Iranian plateau and position of the study sites from the endemic inland *Aphanius* species. ◆ *A. farsicus*, ▲ *A. shirini*, ★ *A. sophiae*, ■ *A.* cf. *pluristriatus*, ▼ *A. isfahanensis*, ✸ *A. arakensis*, ● *A. vladykovi*, ⬤ *A. darabensis*. HZ, High Zagros; MZT, Main Zagros Thrust. Arrow shows the Kor River Basin. Figure is newly compile; source of map from Angiolini et al. (2007).

5.3. Zoogeography of *Aphanius sophiae* outside the Kor River Basin

Based on the genetic data (*Cyt b*) and otolith characters, the newly discovered *Aphanius* populations from the Arjan Wetland (Helleh subbasin) and the Semirom spring (upper reaches of the Karun Basin) have been identified as populations of *A. sophiae* (Kor Basin). This outcome showed, for the first time, that *A. sophiae* populations are distributed in the Central Zagros in isolated drainage basins (Helleh and upper reaches of Karun), and that this species is not restricted to the Kor Basin as previous authors had stated (e.g. Coad 1996; Esmaeili et al., 2010).

Hatzfeld et al. (2010) have shown that the still active tectonics in the Central Zagros is mainly due to the activities of the Main Zagros Reverse Fault (MZRF) and the Main

Recent Fault (MRF) that prograded to the Southwest (Fig. 11); the onset of the geological activity probably was Late Miocene. The lateral component (right lateral strike-slip motion) of the MRF had a severe impact on the Kazerun Fault System (KFS), which is an important tectonic structure in the Central Zagros, and drove the development of its individual faults (Hatzfeld et al., 2010) (Figs. 11–12). The KFS includes several individual faults, i.e. the Kazerun fault consisting of three segments (Dena, Kazerun, Borazjan), and the Kareh-Bas, Sabz-Pushan and Sarvestan faults (Fig. 12).

The Arjan Wetland is located near to the Kareh-Bas fault (Fig. 12), which represents the most active fault of the KFS (see Hatzfeld et al., 2010) and has started its activity ca. 2.8–0.8 Ma (Late Pliocene–Early Holocene) (Authemayou et al., 2009). The Semirom spring is near to the Dena segment of the Kazerun fault (Fig. 12), which is active since ca. 3 Ma (Late Pliocene) (Authemayou et al., 2009; Hatzfeld et al., 2010). It is therefore possible that the isolation of the *A. sophiae* populations in the Arjan Wetland and the Semirom spring can be linked to the motion of these faults, which caused interruption of formerly interconnected drainage systems and isolation of populations (see App. 3). However, the increased aridity in the course of the Holocene (Kehl, 2009) might have played an additional role in population isolation and divergence.
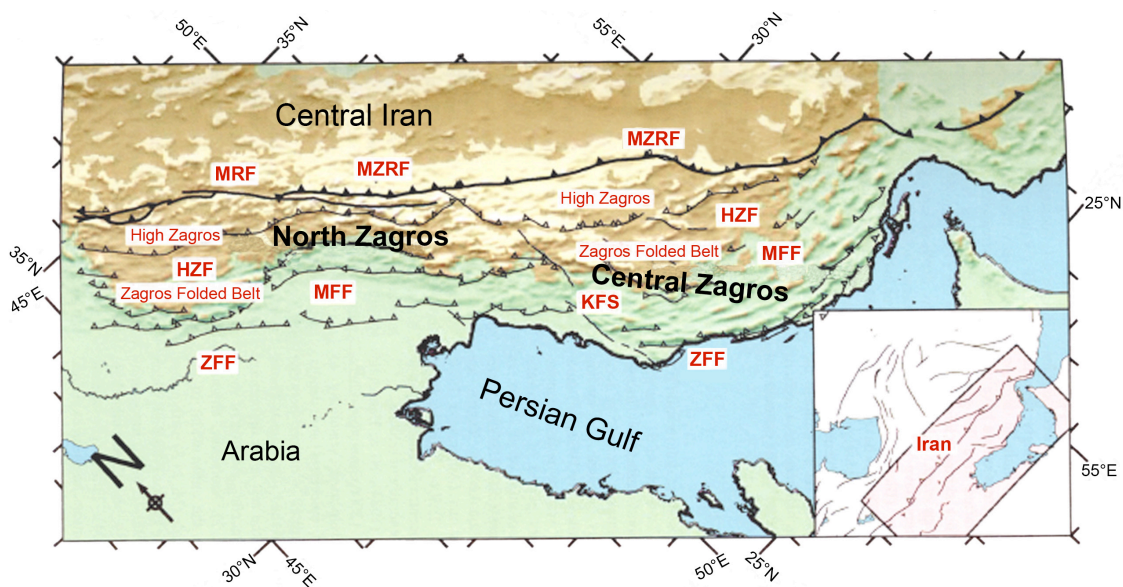

Figure 11. Main geographical and tectonic descriptions of the Zagros Mountain. MZRF, Main Zagros Reverse Fault; MRF, Main Recent Fault; HZF, High Zagros Fault; MFF, Main Frontal Fault; ZFF, Zagros Frontal Fault; KFS, Kazerun Fault System. Figure is from App. 3; source of map from Hatzfeld et al., (2010).
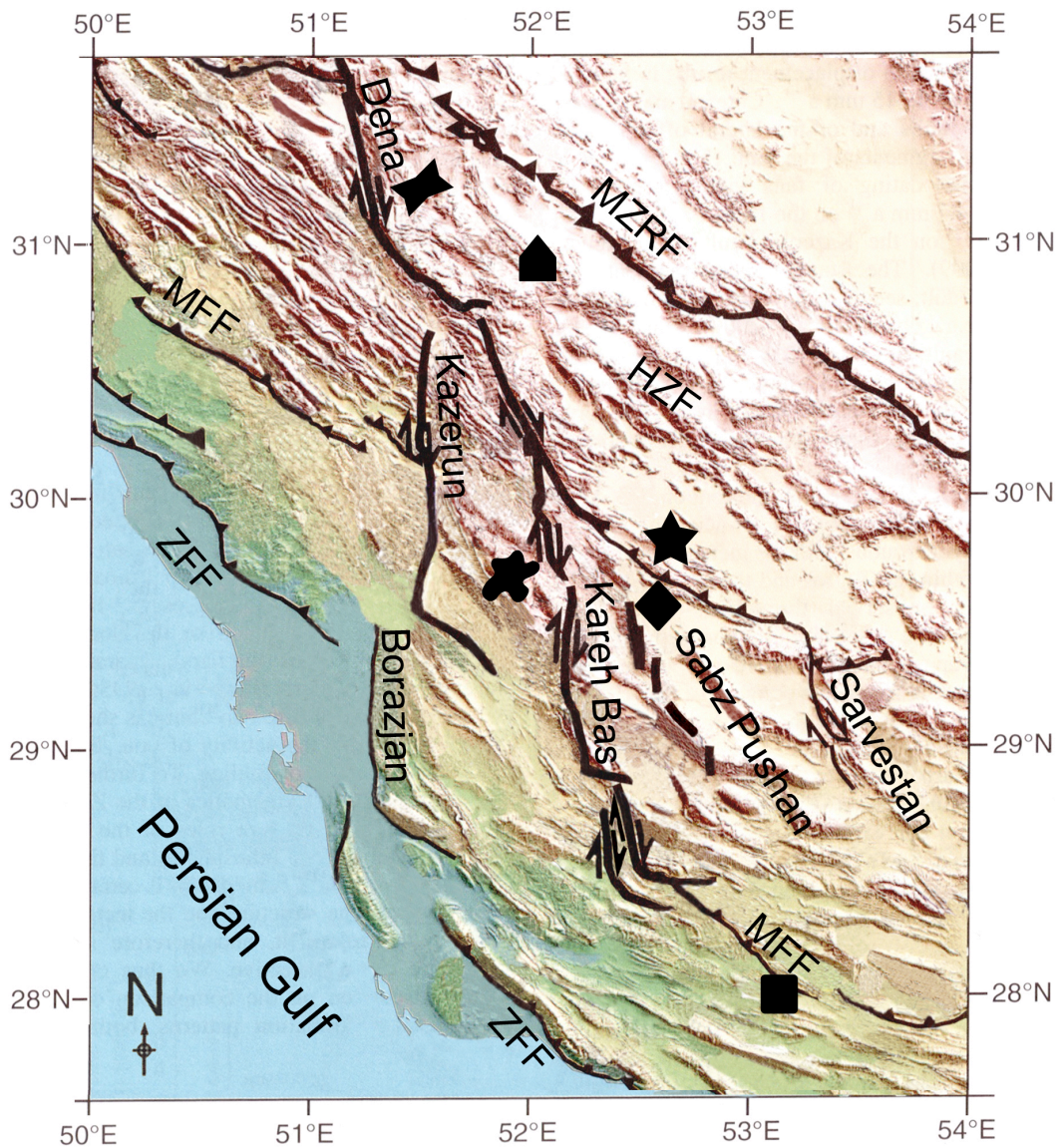
Figure 12. Geological overview of the study area and position of the Kazerun Fault System; Kazerun, Kareh Bas, Sabz Pushan, Sarvestan. HZF: High Zagros Fault, MZRF: Main Zagros Reverse Fault, MFF: Main Frontal Fault. Modified from Hatzfeld et al. (2010). ★ *A. sophiae* (Kor Basin), ⬖ *A. sophiae* (Arjan), ▬ *A. sophiae* (Semirom), ◆ *A. farsicus*, ■ *A.* cf. *pluristriatus*, ⬟ *A. shirini*. Figure is from App. 3; source of map from Hatzfeld et al., (2010).

5.4. Biogeography of the Maharlu Lake Basin and divergence of *Aphanius farsicus*

The Maharlu Basin is an endorheic basin within the Zagros Folded Belt of the Zagros Mountains (Fig. 10). Tectonically, it is delimited by the Sabz-Pushan Fault in the Southwest and the Sarvestan Fault in the Northeast; both faults belong to the Kazerun Fault System (KFS), which is one of the most active fault systems in southern Iran (Agard, 2005; see also 5.3.) (Fig. 12). The formation of the Maharlu Basin is probably related to the tectonic activity that characterized the geological history of the Zagros Mountains during the Pliocene (5.0–1.8 Ma) and Pleistocene (1.8 Ma–11.700 y. ago) periods (Khormali et al., 2003; Hatzfeld et al., 2010; Faghih et al., 2012) and the present endorheic Maharlu Basin probably was created in the Late Pliocene to Early Pleistocene (Khormali et al., 2003; Hatzfeld et al., 2010; Faghih et al., 2012). This young geological age of the Maharlu Basin is additionally supported by its elongate shape, which is typical of basins located in tectonically active mountain ranges (Keller and Pinter, 2002; Faghih et al., 2012).

Based on the geological data and the phylogenetic tree derived from the *cyt b* data, *A. farsicus* belongs to the "young" evolutionary group of the IIRAS clade, which developed in the Late Pleistocene (100.000–11.700 y. ago) and Early to Middle Holocene (11.700–4.000 y. ago) (App. 4, 7) (Fig. 8). Furthermore, it was shown for the first time, that connectivity of populations of *A. farsicus* is present in the Maharlu Basin, which probably results from the establishment of river networks during rainy seasons, but also from migration via underground passages through large alluvial aquifers during both wet and dry seasons (App. 4). The presence of such aquifers that probably formed during the Late Pliocene–Early Pleistocene in the Maharlu Basin had also been demonstrated by the hydrological study of Zare et al. (2005).

5.5. Differentiation patterns of the Iranian inland and inland-related *Aphanius* species

Since 2006, several *Aphanius* species have been described based on molecular data (Hrbek et al., 2006; App. 2–7). Besides molecular data, significant differences in otolith morphology and morphometry and numbers of white flank bars of males were shown to be useful for species discrimination, whereas variation in meristics and morphometrics was relatively low (App. 1–7).

It is well known that otoliths are distinctive for a species and that this is also true for the

species of *Aphanius* (e.g. Reichenbacher et al., 2007, 2009a–b; App. 2–3, 5, 7) (Fig. 13). Otoliths are parts of the inner ear and functionally important for the balance and orientation; this biological function may help fishes to distinguish between different sounds and to respond to the most relevant sounds (Manley et al., 2004; Popper et al., 2005). Therefore, variation in otolith morphology between species can reflect changes in intraspecific communication and behavior in fishes that may have acted as evolutionary pressures.

Moreover, the number and patterns of white flank bars are known to play an important role in sexual selection (Boughman, 2001; Kraaijeveld et al., 2010). The species specific numbers of white flank bars in males in some of the studied *Aphanius* species (see Fig. 1) may have evolved as a response to different light regimes prevalent in the respective habitats for optimizing intraspecific communication. Sensory-driven speciation can therefore be suggested as a prominent additional factor for the divergence of *Aphanius* species (App. 5). This suggestion is additionally supported by the study of Hrbek et al. (2002), who showed that the number and thickness of white flank bars are taxonomically useful to discriminate *Aphanius* species from central Anatolia.
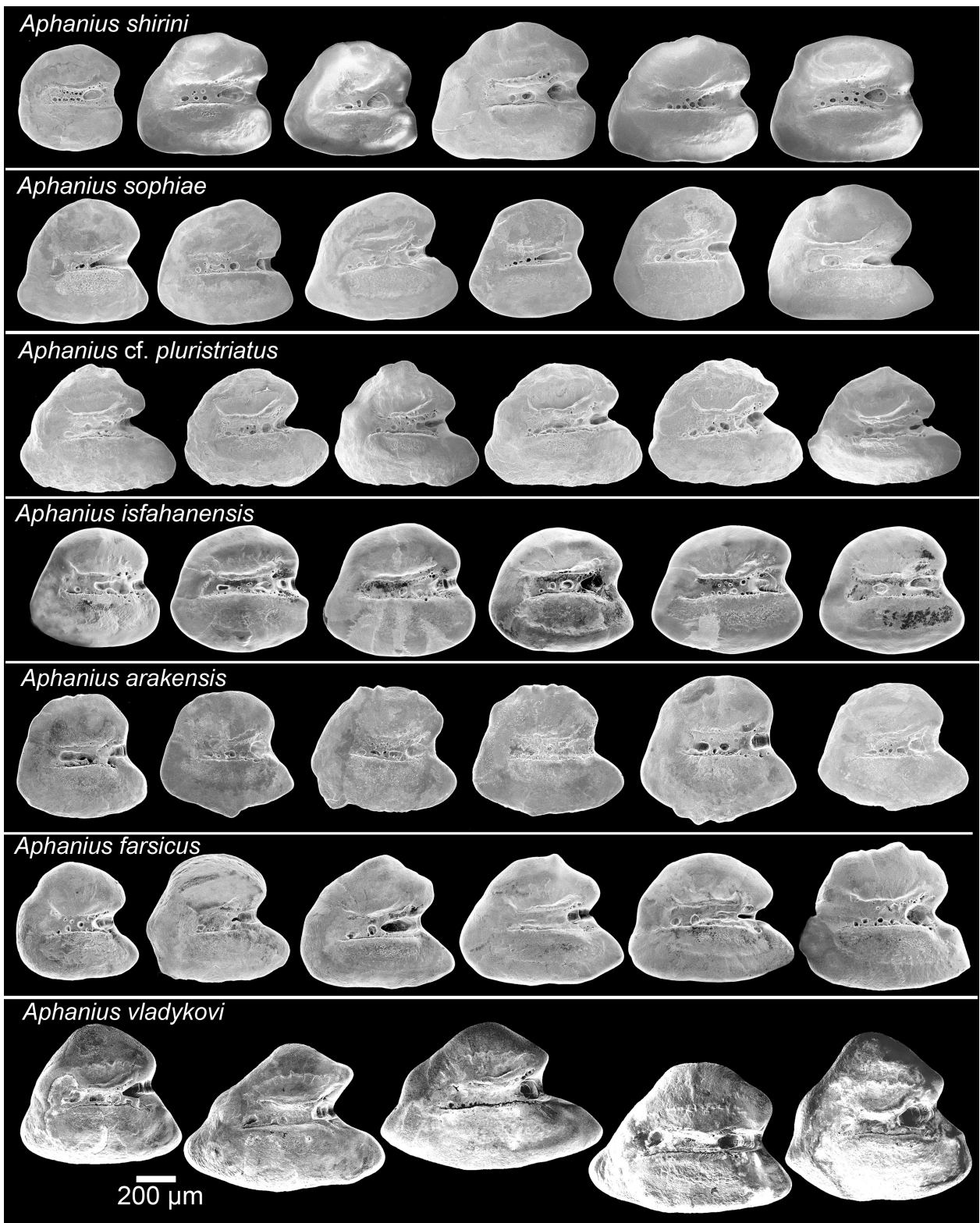
Figure 13. SEM micrographs of left otoliths (in medial view) from the Iranian inland and inland-related *Aphanius* species (IIRAS). Otoliths are sorted in three males and three females (two females in the case of A. vladykovi) in each species, successively. Figure is from App. 2.

5.6. Historical zoogeography of the Iranian inland and inland-related *Aphanius* species

Not much information was available about the biogeographic history of the Iranian *Aphanius* species until 2010. This PhD project and related collaborations tried to find out the relationship between the evolutionary history of the Iranian *Aphanius* species and the geological events that had affected the Iranian Plateau in the past.

The Iranian plateau faced different periods of geological activity during the Late Miocene (5–10 Ma), Pliocene (1.8–5 Ma), Pleistocene (1.8 Ma–100.000 y. ago) and Late Pleistocene to Holocene (20,000–4,000 y. ago) (Löffler, 1959; Dercourt et al., 1986; Nadji, 1997; Hatzfeld et al., 2010). These caused new configurations of mountain ranges, rapid isolation of multiple areas and changes of hydrological networks and drainage patterns (Dercourt et al., 1986; Hrbek and Meyer, 2003; Hrbek et al., 2006). In the course of my PhD project and collaborations, we could show that the phylogenetic relationships of the studied Iranian *Aphanius* species (Fig. 8) are largely consistent with the association of their sites to the various tectonic units. Thus it can be assumed that their speciation is closely linked to vicariance events due to the tectonic activity and uplift, especially for the species in the Zagros Mountains (App. 2–7). In detail, the following scenario has been hypothesized based on the Gholami et al. (2014) (App. 2), Esmaeili et al. (2012) (App. 6) and Esmaeili et al. (2014) (App. 7):

Aphanius vladykovi is the first species that has split, followed by *A. shirini* + *A. darabensis* and *A. isfahanensis*, and their splits can be linked with different Late Miocene and Early Pliocene phases of tectonic activity in the High Zagros. An age of ca. 5 Ma can be assumed for the split of *A. shirini* based on the estimated splits of *A. vladykovi* (*c.* 5.5 Ma) and *A. isfahanensis* (ca. 4.8 Ma) by Hrbek et al. (2006) (see App. 2). Moreover, a divergence age of 5 Ma can also be suggested for *A. darabensis* because it is sister to *A. shirini* (Fig. 8) (see App. 7).

The close phylogenetic relationship between *A. shirini* and *A. vladykovi* indicates that the pre-Pliocene drainage in the northwestern part of the Kor River Basin was directed to the northwest (to the Karun Basin), and not to the southeast as in the present-day Kor Basin (see App. 2). Furthermore, based on the distribution of the closely related *A. shirini* and *A. darabensis* in isolated drainage basin, i.e. in the upper reaches of the Kor Basin (*A. shirini*) and in the Hormuzgan Basin (*A. darabensis*), it can be assumed that the Early Pliocene episode of geological activity separated the Hormuzgan Basin from the other basins of the Zagros Mountains (see Fig. 10 and App. 7).

The strong uplift of the Zagros Mountains during the Late Pleistocene to Holocene apparently created the endorheic Kor River Basin, which led to the isolation of the formerly interconnected *Aphanius* populations in the Kor River and Mond River basins (see App. 2, 6). This resulted in the divergence of *A. pluristriatus* in the Mond Basin, which can be considered as a relict of the ancient *Aphanius* population of the Paleo-Kor River drainage (Fig 3A; App. 6). Further species that appear to have evolved in the course of the Late Pleistocene or Early to Middle Holocene comprise *A. farsicus, A. sophiae, A. mesopotamicus, A. arakensis* and *A. kavirensis* (see App. 2, 7). However, the splits of *A. kavirensis* and *A. arakensis* are difficult to explain by vicariance events alone and factors such as sensory-driven speciation and the increased aridity in the course of the Holocene (Kehl, 2009) might have played an additional role for their divergence (see App. 5, 7).

6. Conclusion

I.   Inter- and intraspecific phenotypic variations appear as a result of multiple factors such as environmental parameters, physiological adaptation, swimming demands, absence or presence of connectivity, and allopatric divergence.

II. The phenotypic plasticity of certain characters promotes morphological divergence as a result of adaptation to different environmental conditions. In the case that a population becomes isolated, a given trend of evolution may continue to develop and the character may become taxonomically useful on the species level.

III. Inter- and intraspecific differences of J-indices were evolving due to isolation of species or populations for at least several thousand years, and J-indices are not taxonomically useful on the species level in every case as previously thought.

IV. The still active tectonics of the Iranian plateau is largely responsible for the high endemicity of the Iranian inland *Aphanius* species because divergence of *Aphanius* species is clearly linked to periods of intense geological activity.

V.   A combined analysis of molecular data and comprehensive morphological datasets can disentangle the relationships of closely related Iranian inland *Aphanius* species and contribute to understand character evolution in the context of differing environments and/or allopatric divergence. Such studies are important for the identification of endangered local populations and species, and are important for their conservation strategy and habitat management. The unexpexted record of *A. shirini* in the Arjan Wetland (where the native species is *A. sophiae*) has shown that careful taxonomic studies are necessary before fishes are introduced.

7. Acknowledgements

8. References

Agard, P., Omrani, J., Jolivet, L. & Mouthereau, F. (2005). Convergence history across Zagros, Iran; constraints from collisional and earlier deformation. Iranian Journal of Earth Sciences, 94, 401–419.

Angiolini, L., Gaetani, M., Muttoni, G., Stephenson, M.H. & Zanchi, A. (2007). Tethyan oceanic currents and climate gradients 300 m.y. ago. Geology, 35, 1071–1074.

Authenmayou, C., Bellier, O., Chardon, D., Benedetti, L., Malekzadeh, Z., Claude, C., Angeletti, B., Shabanian, E. & Abbassi, M.R. (2009). Quaterny slip-rates of the Kazerun and the Main Recent Faults: active strike-slip partitioning in the Zagros fold-and-thrust belt. Geophysical Journal International, 178, 524–540.

Backhaus, K., Erichson, B., Plinke, W. & Weiber, R. (2006). Multivariate Analysemethoden. Eine anwendungsorientierte Einführung. Springer, Berlin, 831 P.

Bennett, W.A. & Beittinger, T.L. (1997). Temperature tolerance of the sheepshead minnow, *Cyprinodon variegatus*. Copeia, 1997, 77–87.

Berra, T.M. (2007). Freshwater fish distribution. The University of Chicago Press, Ltd., London, pp 321–350.

Boughman, J.W. (2001). Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature, 411, 944–948.

Clavero, M., Blanco-Garrido, F. & Prenda, J. (2007). Population and microhabitat effects of interspecific interactions on the endangered Andalusian toothcarp (*Aphanius baeticus*). Environmenatl Biology of Fishes, 78, 173–182.

Clement, M., Posada, D. & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. Molecular Ecology, 9, 1657–1660.

Coad, B.W. (1996). Systematics of the tooth-carp genus *Aphanius* Nardo, 1827 (Actinopterygii: Cyprinodontidae) in Fars Province, southern Iran. Biologica Bratislava, 51, 163–172.

Coad, B.W. (2000). Distribution of *Aphanius* species in Iran. Journal of American Killifish Association, 33, 183–191.

Coad, B.W. (2009). A new species of tooth-carp, *Aphanius mesopotamicus*, from Iran and Iraq (Actinopterygii, Cyprinodontidae). ZooKeys, 31, 149–163.

Coad, B.W. (2014). Freshwater fishes of Iran. Available from http://www.briancoad.com (accessed February 2014).

Coburn, M. M. & Gaglione, J.I. (1992). A comparative study of percid scales (Teleostei: Perciformes). Copeia, 4, 986–1001.

Darriba, D., Taboada, G., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. Nature Methods, 9, 772–772.

Dercourt, J., Zonenshain, L.P., Ricou, L. E., Le Pichon, X., Knipper, A.L., Grandjaquet, C., Sbortshikov, I.M., Geussant, J., Lepvrier, C., Pechersku, D.H., Boulin, J., Bazhenov, M.L., Lauer, J.P. & Biju-Duval, B. (1986). Geological evolution of the Tethys belt from the Atlantic to the Pamirs since the Lias. Tectonophysics, 123, 241–315.

De Pontual, H. & Prouzet, P. (1987). Atlantic salmon, *Salmo salar* L., stock discrimination by scale-shape analysis. Aquaculture and Fish Manage, 18, 277–289.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled. J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T. & Wilson, A. (2012). Geneious v6.4, Available from http://www.geneious.com.

Dynes, J., Magnan, P., Bernatchez, L. & Rodriguez, M.A. (1999). Genetic and morphological variation between two forms of lacustrine brook char. Journal of Fish Biology, 54, 955–972.

Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32, 1792–1797.

Esmaeili, H.R. (2001). Some aspects of biology of an exotic fish *Hypophthalmicthys molitrix* (Val., 1844) from Gobindsagar reservoir Himachal paradesh, India. PhD Thesis, Department of Zoology, Panjab University, Chandigarh, 287p.

Esmaeili, H.R., Teimori, A., Dehghani, N., Mashgholzekr, E., Ebrahimi, M. & Nikookar, K. (2007). Monitoring of three salty lakes in Fars Province (Maharlu, Tashk and Bakhtegan), Southern Iran (Phase I). Shiraz University.

Esmaeili, H.R., Coad, B.W., Gholamifard, A., Nazari, N. & Teimori, A. (2010). Annotated checklist of the freshwater fishes of Iran. Zoosystematica Rossica, 19, 361–386.

Esmaeili, H.R., Teimori, A., Gholami, Z., Zarei, N. & Reichenbacher, B. (2012). Re-validation and redescription of an endemic and endangered species, *Aphanius pluristriatus* (Jenkins, 1910) (Teleostei, Cyprinodontidae), from southern Iran. Zootaxa, 3208, 58–67.

Esmaeili, H.R., Teimori, A., Gholami, Z. & Reichenbacher, B. (2014). Two new species of the tooth-carps *Aphanius* (Teleostei: Cyprinodontidae) and the evolutionary history of the Iranian inland and inland-related *Aphanius* species. Zootaxa, 3786, 246–268.

Faghih, A., Samani, B., Kusky, T., Khabazi, A. & Roshanak, R. (2012). Geomorphologic assessment of relative tectonic activity in the maharlu Lake Basin, Zagros Mountains of Iran. Geology Journal, 47, 30–40.

Fayazi, F., Lak, R. & Nakhaei, M. (2007). Hydrogeochemistry and brine evolution of Maharlu Saline Lake, southwest of Iran. Carbonate and Evaporites, 22, 34–42.

Ferrito, V., Mannino, M.C., Pappalardo, A.M., & Tigano, C. (2007). Morphological variation among populations of *Aphanius fasciatus* Nardo, 1827 (Teleostei, Cyprinodontidae) from the Mediterranean. Journal of Fish Biology, 70, 1–20.

Ferrito, V., Pappalardo, A.M., Canapa, A., Barucca, M., Doadrio, I., Olmo, E. & Tigano, C. (2013). Mitochondrial phylogeography of the killifish *Aphanius fasciatus* (Teleostei, Cyprinodontidae) reveals highly divergent Mediterranean populations. Marine Biology, 160, 3193–3208.

Fernabdez-Pedrosa, V., Gonzalez, A., Planelles, M., Moya, A. & Latorre, A. (1995). Mitochondrial and variability in three Mediterranean populations of *Aphanius iberus*. Biological Conservation, 251–256.

Frenkel, V. & Goren, M. (2000). Factors affecting growth of killifish, *Aphanius dispar*, a potential biological control of mosquitoes. Aquaculture, 184, 255–265.

Fu, Y.X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics, 147, 915–925.

Gavillot, Y., Axen, G.J, Stockli, D.F, Horton, B.K. & Fakhari, M.D. (2010). Timing of thrust activity in the High Zagros fold-thrust belt, Iran, from (U-Th)/He thermochronometry. Tectonics, 29, TC4025.

Gaudant, J. (1993). Un exemple de "régression évolutive" chez des poisons cyprinodontidae du miocène supérieur d'Espagne: *Aphanius illunensis* nov. sp. Geobios, 26 (4), 449–454.

Gaudant, J. (2011). *Aphanius persicus* (Priem, 1908) (Pisces, Teleostei, Cyprinodontidae): une nouvelle combinaison pour *Brachylebias persicus* Priem, 1908, du Miocène supérieur des environs de Tabriz (Iran). Geodiversitas, 33, 347–356.

Gholami, Z., Teimori, A., Esmaeili, H.R., Schulz-Mirbach, T. & Reichenbacher, B. (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.

Gholami, Z., Esmaeili, H. R., Erpenbeck. & Reichenbacher, B. (2014). Phylogenetic analysis of *Aphanius* from the endorheic Kor River Basin in the Zagros Mountains, South-western Iran (Teleostei: Cyprinodontiformes: Cyprinodontidae). Journal of Zoological Systematics and Evolutionary Research, 52 (2), 130–141.

Gholami, Z., Esmaeili, H. R., Erpenbeck, D. & Reichenbacher, B. (2014). Genetic connectivity and phenotypic plasticity in the cyprinodont *Aphanius farsicus* from the Maharlu Basin, South-western Iran. Journal of Fish Biology, in review.

Gholami, Z., Esmaeili, H.R. & Reichenbacher, B. (submitted). Evidence for wide presence of *Aphanius sophiae* (Teleostei: Cyprinodontidae) during the Early and Middle Holocene in the Central Zagros. Limnologica.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010). New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology, 59, 307–21.

Gouy, M., Guindon, S. & Gascuel, O. (2010). SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution, 27, 221–224.

Hatzfeld, D., Authemayou, C., Vanderbeek, P., Bellier, O., Lave¢, J., Oveisi, B., Tatar, M., Tavakoli, F., Walpersdorf, A. & Yamini-fard, F. (2010). The kinematics of the Zagros Mountains (Iran). Geological Society of London, 330, 19–42.

Holcik, J. (1989). The Freshwater Fishes of Europe. General Introduction to Fishes: Acipenceriformes. AULA-Verlag, Wiesbaden, 469 p.

Homke, S., Verges, J., Garces, M., Emami, H. & Karpuz, R. (2004). Magnetostratigraphy of Miocene Pliocene Zagros foreland deposits in the front of the Push- e Kush Arc (Lurestan Province, Iran). Earth and Planetary Science Letters, 225, 397–410.

Homski, D., Goren, M., Gasith, A. (1994). Comparative evaluation of the larvivorous fish *Gabmusia affinis* and *Aphanius dispar* as mosquito control agents. Hydrobiologia, 283, 137–146.

Houtum-Schindler, A. (1981). Note on the Kor River in Fars, its sources and dams and the districts it irrigates. Proceedings of Royal Geographical Society, 13, 287–291.

Huelsenbeck, J. P. & Ranala, B. (2004). Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Systematic Biology, 53, 904–913.

Hrbek, T., Küçük, F., Frickey, T. N., Stölting, K., Wildekamp. R.H. & Meyer, A. (2002) Molecular phylogeny and historical biogeography of the *Aphanius* (Pisces, Cyprinodontiformes) species complex of central Anatolia, Turkey. Molecular Phylogeny and Evolution, 25, 125–137.

Hrbek, T. & Meyer, A. (2003). Closing of the Tethys Sea and the phylogeny of Eurasian killifishes (Cyprinodontiformes: Cyprinodontidae). Journal of Evolutionary Biology, 16, 17–36.

Hrbek, T., Keivany, Y. & Coad, B.W. (2006). New species of *Aphanius* (Teleostei, Cyprinodontidae) from Isfahan Province of Iran and a reanalysis of other Iranian species. Copeia, 2, 244–255.

Jawad, L.A., (2005a). Comparative morphology of scales of four teleost fishes from Sudan and Yemen. Journal of Natural History, 39, 2643–2660.

Jawad, L.A. (2005b). Comparative scale morphology and squamation patterns in triplefins (Pisces: Teleostei: Perciformes: Tripterigyiidae). Tuhinga. 16, 137–168.

Jawad, L.A., Al-Jufaili, S.M. (2007). Scale morphology of greater lizardfish *Saurida tumbil* (Bloch, 1795) (Pisces: Synodontidae). Journal of Fish Biology, 70, 1185–1212.

Jenkins, J. T. (1910). Notes on fish from India and Persia, with descriptions of new species. 1. On a collection of fishes made by W. T. Blainford in 1872 in Persia and Baluchistan. Record of Indiana Museum, 5, 123–128.

Johal, M.S., Esmaeili, H.R. & Sharma, M.L. (2006). Scale structure of a cobitid fish, *Cobitis linea* (Heckel, 1849) using different modes of SEM. Current Science, 91, 1464–1466.

Kehl, M., Frechen, M. & Skowronek, A. (2005). Paleosols derived from loess and loess-like sediments in the Basin of Persepolis, Southern Iran. Quaternary International, 140/141, 135–149.

Kehl, M. (2009). Quaternary climate change in Iran, the state of knowledge. Erdkunde, 63, 1–17.

Kehl, M., Frechen, M. & Skowronek, A. (2009). Nature and age of Late Quaternary basin fill deposits in the Basin of Persepolis/Southern Iran. Quaternary International, 196, 57–70.

Keller, E. A. & Pinter, N. (2002). Active Tectonics: Earthquakes, Uplift, and landscape (2nd Ed). Prentice Hall, NewJersey.

Khaksar, K., Goodarzi, M., Gharibreza, M. R. & Rahmati, M. (2004). A study on the sensitivity of geological formations to erosion in the Maharlu Basin. Geology Sciences, Number 62, Volume 16, Issue Winter.

Khormali, F., Abtahi, A., Mahmoodi, S. & Stoops, G. (2003). Argilic horizon development in calcareous soils of arid and semiarid regions of southern Iran. Catena, 53, 273–301.

Kraaijeveld, K., Kraaijeveld-Smit F, & Emaan, M. (2010). Sexual selection and speciation: the comparative evidence revisited. Biological Reviews, 86, 367–377.

Krupp, F. (1985). A new species of *Chondrostoma* from the Orontes river drainage basin of Turkey and Syria (Pisces: Osteichthyes: Cyprinidae). Senckenbergiana Biologica, 66(1/3), 27–33.

Kuusipalo, L. (1998). Scale morphology in Malawian cichlids. Journal of Fish Biology, 52, 771–781.

Lahnsteiner, F. & Jagsch, A. (2005). Change in phenotype and genotype of Austrain *Salmo trutta* populations during the last century. Environmental Biology of Fishes, 74, 51–65.

Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25, 1451–1452.

Lindroth, A. (1963). The body/scale relationship in Atlantic salmon (*Salmo salar* L.), A preliminary report. Journal du Conseil International pour Exploration de la Mer, 1, 37–52.

Lippitsch, E. (1990). Scale morphology and squamation patterns in cichlids (Teleostei, Perciformes): A comparative study. Journal of Fish Biology, 37, 265–291.

Löffler, H. (1959). Beiträge zur Kenntnis der iranischen Binnengewässer I, der Niriz-See und sein Einzugsgebiet. International Review of Hydrobiology, 44, 227–276.

Lombarte, A. & L1eonart, J. (1993). Otolith size changes related with body growth, habitat depth and temperature. Environmental Biology of Fishes, 37, 297–306.

Lombarte, A., Palmer, M., Matallanas, J., Gómez-Zurita, J. & Morales-Nin, B. (2010). Ecomorphological trends and phylogenetic inertia of otolith sagitae in Ototheniidae. Environmental Biology of Fishes, 89, 607–618.

Manley, G.A. & Clack, J.A. (2004). An outline of the evolution of vertebrate hearing organs. In: Manley GA, Popper AN, Fay RR (eds), Evolution of the Vertebrate Auditory System. Springer, New York, pp 1–26.

Mérigot, B., Letourneur, Y. & Lecomte-Finiger, R. (2007). Characterization of local populations of the common sole *Solea solea* (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis. Marine Biology, 151, 997–1008.

Mouthereau, F., Tensi, J., Bellahsen, N., Lacombe, O., De Boisgrollier, T. & Kargar, S. (2007). Tertiary sequence of deformation in a thin-skinned/ thick-skinned collision belt: the Zagros Folded Belt (Fars, Iran). Tectonics, 26: TC5006.

Mouthereau, F. (2011). Timing of uplift in the Zagros belt/Iranian plateau and accommodation of late Cenozoic Arabia-Eurasia convergence. Geological Magazine, 148, 726–738.

Nadji, M. (1997). Rerouting of the Kor River from the Zagros Region into the Persian Gulf–a proposed solution to the problem of salinization in the Persepolis Basin, Iran. Zeitschrift für Angewandte Geologie, 43, 171–178.

Nelson, J. (2006). Fishes of the World. Fourth Edition, Published by John Wiley & Sons, Inc., Hoboken, New Jersy. Fourth Edition. xix + 601 pp.

Nolf, D. (1985). Otolithi piscium. Handbook of paleoichthyology, vol. 10. Gustav Fischer, Stuttgart.

Pappalardo, A.M., Ferrito, V., Messina, A., Guarino, F., Patarnello, T., De Pinto, V. & Tigano, C. (2008). Genetic structure of the killifish *Aphanius fasciatus*, Nardo 1827 (Teleostei, Cyprinodontidae) results of mitochondrial DNA analysis. Journal of Fish Biology, 72, 1–20

Parenti, L.R. (1981). A phylogenetic and biogeographic analysis of the cyprinodontiform fishes (Teleostei, Atherinomorpha). The Bulletin of the American Museum of Natural History, 168, 335–557.

Perdices, A., Carmona, J.A., Fernandez-Delgado, C. & Doadrio, I. (2001). Nuclear and mitochondrial data reveal high genetic divergence among Atlantic and Mediterranean populations of the Iberian killifish *Aphanius iberus* (Teleostei, Cyprinodotidae). Heredity, 87, 314–324.

Popper, A.N., Ramcharitar, J. & Campana, S.E. (2005). Why otolith? Insights from inner ear physiology and fisheries biology. Marine and freshwater Research, 56, 497–504.

Peres-Neto, P.R. & Magnan, P. (2004). The influence of swimming demand on phenotypic plasticity and morphological integration: a comparison of two polymorphic charr species. Oecologia, 140, 36-45.

Ramos-Onsins, S. E. & Rozas, J. (2002). Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution, 19, 2092–2100.

Reichenbacher, B., Sienknecht, U., Küchenoff, H. & Fenske, N. (2007). Combined otolith morphology and morphometry for assessing taxonomy and diversity in fossil and extant killifish (*Aphanius*, †*Prolebias*). Journal of Morphology, 268, 898–915.

Reichenbacher, B., Feulner, G.R. & Schulz-Mirbach, T. (2009a). Geographic Variation in Otolith Morphology Among Freshwater Populations of *Aphanius dispar* (Teleostei, Cyprinodontiformes) from the Southeastern Arabian Peninsula. Journal of Morphology, 270, 469–484.

Reichenbacher, B., Kamrani, E., Esmaeili, H. R. & Teimori, A. (2009b). The endangered cyprinodont *Aphanius ginaonis* (Holly, 1929) from southern Iran is a valid species: evidence from otolith morphology. Environmental Biology of Fishes, 86, 507–521.

Reichenbacher, B., Alimohammadian, H., Sabouri, J., Haghfarshi, E., Faridi, M., Abbasi, S., Matzke-Karasz, R., Fellin, M.G., Carnevale, G., Schiller, W., Vasilyan D. & Scharrer, S. (2011). Late Miocene stratigraphy, palaeoecology and palaeogeography of the Tabriz Basin (NW Iran, Eastern Paratethys). Palaeogeography, Palaeoclimatology, Palaeoecology, 311, 1–18.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. Systematic Biology, doi: 10.1093/sysbio/sys029.

Rosenthal, A., Coutelle, O. & Craxton, M. (1993). Large-scale production of DNA sequencing templates by microtitre format PCR. Nucleic Acids Research, 21, 173–174.

Schmidt, T.R. & Gold, J.R. (1993). Complete sequence oft he mitochondrial cytochrome b gene in the cherryfin shiner, *Lythrurus roseipinnis* (Teleostei, Cyprinidae). Copeia, 3, 880–883.

SPSS Inc. (2011). PASW 19.0 Base. Chicago, SPSS Inc, 845 p.

SPSS Inc. (2012). IBM SPSS Statistics for Mac. Version 20.0. Armonk, NY: IBM Corp.

SPSS Inc. (2013). IBM SPSS Statistics for Mac. Version 21.0. Armonk, NY: IBM Corp.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123, 585–595.

Teimori, A., Esmaeili, H. R. & Reichenbacher, B. (2011). *Aphanius farsicus*, a replacement name for *A. persicus* (Jenkins, 1910) (Teleostei, Cyprinodontidae). Zootaxa, 3096, 53–58.

Teimori, A., Esmaeili, H.R., Gholami, Z., Zarei, N. & Reichenbacher, B. (2012a). *Aphanius arakensis*, a new species of tooth-carp (Actinopterygii, Cyprinodontidae) from the endorheic Namak Lake basin in Iran. ZooKeys, 215, 55–76.

Teimori, A., Schulz-Mirbach, T., Esmaeili, H.R. & Reichenbacher, B. (2012b). Geographical differentiation of *Aphanius dispar* (Teleostei: Cyprinodontidae) from Southern Iran. Journal of Zoological Systematic and Evolutionary Research, 50, 289–304.

Teimori, A., Esmaeili, H. R. Erpenbeck, D. & Reichenbacher, B. (2014). A new and unique species of the genus *Aphanius* (Teleostei: Cyprinodontidae) from Southern Iran: A case of regressive evolution. Zoologischer Anzeiger, 253 (4), 327–337.

Vargas, S., Schuster, A., Sacher, K., Büttner, G., Schätzle, S., Läuchli, B., Hall, K., Hooper, J.N.A., Erpenbeck, D. & Wörheide, G. (2012). Barcoding Sponges: An Overview Based on Comprehensive Sampling. PLoS ONE, 7, e39345.

Villwock, W. (1982). *Aphanius* (Nardo, 1827) and Cyprinodon (Lacépède, 1803) (Pisces: Cyprinodontidae), an attempt for a genetic interpretation of speciation. Zoological Systematics and Evolutionary Research, 20, 187–197.

Wildekamp, R. H. (1993). A world of Killies. Atlas of the Oviparous Cyprinodontiform Fishes of the World. The Genera *Adamas*, *Adinia*, *Aphanius*, *Aphyoplatys* and *Aphyosemion*. Indiana, American Killifish Association, 311 p.

Wildekamp, R.H., Kücük, F., Ünlüsayin, M. & Neer, W.V. (1999). Species and subspecies of the genus *Aphanius* Nardo 1827 (Pisces: Cyprinodontidae) in Turkey. Turkish Journal of Zoology, 23, 23–44.

Zare, M., Nematollahi, A. R. & Rahnemaei, M. (2005). Effect of monitoring time interval on the results of stochastic analysis of karst water resources. Iranian Journal of Sciences & Technology, 29, 387–399.

Curriculum Vitae

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Education

- 1996–1999: High school in experimental Sciences, Babol, Iran

- Oct 1999–Feb 2003: Bachlor degree in Animal Biology, Department of Biology, Agriculture Sciences and Natural Resources of Gorgan University, Gorgan, Iran.

- Oct 2005–Sep 2008: Master degree in Animal Biosystematics (Ichthyology), Biology Department, Shiraz University, Shiraz, Iran. Thesis: Taxonomy and Distribution of Genus *Aphanius* Nardo, 1827 (Actinoptrygii: Cyprinodontidae) in Iran.

- Oct 2010–Sep 2014: Promotion at Department of Earth and Environmental Sciences, Palaeontology & Geobiology & GeoBio-Center LMU, Ludwig-Maximilians-University, Munich, Germany. Dissertation: Ecological plasticity and divergence processes of the Iranian inland species of *Aphanius* (Teleostei, Cyprinodontidae), with focus on *A. sophiae* and *A. farsicus* in the Kor River and Maharlu Lake basins, Southwestern Iran.

Awards

- Award for a German course at the Goethe-Institute, Freiburg, Germany, funded by the German Academic Exchange Service (DAAD), Jun 2010–Sep 2010.

- Scholarship for a PhD program at the LMU, Munich, Germany funded by the German Academic Exchange Service (DAAD), Oct 2010–Oct 2014.

- A member of LMU Mentoring Program since Jan 2014–Mar 2015.

Lectures

- Fish osteology course for Master students at Department of Earth and Environmental Sciences, Palaeontology & Geobiology & GeoBio-Center, Ludwig-Maximilians-University, Munich, Germany. May–July 2011.

Workshops attended

- LMU Mentoring Program."Werden Sie Partnerinnen der Medien" (Become partners of Media). 17.July.2014.

- LMU Mentoring Program."Frauen in Führungspositionen-Souverän und Klug agieren (Women in management positions-Act supreme and intelligent)". 23.May.2014.

- LMU Mentoring Program."Am Seminar Drittmittel–Wie wird mein Antrag zum Erfolg? (How proceed my application to success)" 07.February.2014.

- Advanced Molecular Palaeobiology. LMU, Munich, Geo- and Palaeobiology Lab, Department of Earth and Environmental Sciences. February 2013.

- Preliminary Molecular Paleobiology. LMU, Munich, Molecular Geo- and Palaeobiology Lab, Department of Earth and Environmental Sciences. 2011.

- German language (Jun 2010–Sep 2010) in the Goethe Institute, Freiburg, Germany (certificate for level B1/B2, Grundstufe) and B2.1 and B2.2 (Mittelstufe) in the Volkshochschule Munich, Germany.

- Karyotype of vertebrates (fish, reptiles and amphibians). Shiraz University, Iran. 2007.

- Farming of Algae. Shiraz University, Shiraz, Iran. 2007.

- Farming of Artemia. Shiraz University, Shiraz, Iran. 2006.

- Rainbout trout farming. Shiraz University, Shiraz, Iran. 2005.

- Farming of Daphnia. Shiraz University, Shiraz, Iran. 2005.

Projects

- Cooperator in investigation of annual food variation in *Alburnoides bipunctatus* in the rivers and springs of Caspian Sea Basin. Alzahra University, Tehran, Iran. 2009.

- Executive responsible in farming of Farsi tooth-carp (*Aphanius farsicus*). Shiraz University, Shiraz, Iran. Oct 2007–Sep 2008.

- Cooperator in farming of Hormuz cichlid (*Iranocichla hormuzensis*). Shiraz University, Shiraz, Iran. Oct 2006–Sep 2007.

In review and submitted manuscripts

Alcaraz, C., Gholami, Z., Esmaeili, H.R. & Garcia, E. (in review). First data on the food of a highly endemic cyprinodont (*Aphanius farsicus*): annual variation and ontogeny. Environmental Biology of Fishes.

Esmaeili, H. R., Masoudi, M., Sayyadzadeh, G., Mehraban, H.R., Gholami, Z., Teimori, A. (in review). Length–weight relationships for four *Aphanius* species of Iran (Teleostei: Cyprinodontidae). Journal of Applied Ichthyology.

Gholami, Z., Esmaeili, H.R. & Reichenbacher, B. (submitted). Evidence for wide presence of *Aphanius sophiae* (Teleostei: Cyprinodontidae) during the Early and Middle Holocene in the Central Zagros. Limnologica.

Gholami, Z., Esmaeili, H.R., Erpenbeck, D. & Reichenbacher, B. (2014). Genetic connectivity and phenotypic plasticity in the cyprinodont *Aphanius farsicus* from the Maharlu Basin, Southwest Iran. Journal of Fish Biology, in review.

Publications (peer-reviewed)

2014

Gholami, Z., Esmaeili, H.R., Erpenbeck, D. & Reichenbacher, B. (2014). Phylogenetic analysis of *Aphanius* from the endorheic Kor River Basin in the Zagros Mountains, Southwestern Iran (Teleostei: Cyprinodontiformes: Cyprinodontidae). Journal of Zoological Systematics and Evolutionary Research, 52 (2), 130–141.

Esmaeili, H.R., Teimori, A., Gholami, Z. & Reichenbacher, B. (2014). Two new species of tooth-carps *Aphanius* (Teleostei: Cyprinodontidae) and the evolutionary history of the Iranian inland and inland-related *Aphanius* species. Zootaxa, 3786 (3), 246–268.

Gholami, Z., Rahimi, M.T., Kia, E.B., Esmaeili, H.R. & Mobedi, I. (2014). *Capoeta damascina* (Cyprinidae), a new host of *Contracaecum* sp. and *Capillaria* sp. (Nematoda) from Kor River Basin, Southern Iran. Asian Pacific Journal of Tropical Biomedicine, 4 (Supp 1), S139–S142.

Gholami, Z., Rahimi, M.T., Zarei, R., Kia, E.B., Mobedi, I. & Vatandoost, S. (2014). First report of *Ichthyophthirius multifiliis* (Ciliophora: Oligohymenophorea) from *Aphanius dispar* (Cyprinodontidae) in Iran. Journal of Coastal Life Medicine, 2 (6), 490–495.

2013

Gholami, Z., Teimori, A., Esmaeili, H.R., Schulz-Mirbach, T. & Reichenbacher, B. (2013). Scale surface microstructure and scale size in the tooth-carp genus *Aphanius* (Teleostei, Cyprinodontidae) from endorheic basins in Southwest Iran. Zootaxa, 3619 (4), 467–490.

Amin, O.M., Gholami, Z., Akhlaghi, M. & Heckmann, R.A. (2013). The description and host-parasite relationships of a new species of *Acanthosentis* (Acanthocephala: Quadrigyridae) from the Persian toothcarp, *Aphanius farsicus* (Actinopterygii: Cyprinodontiade) in Iran. Journal of Parasitology, 99 (2), 257-263.

Rahimi, M.T., Gholami, Z., Esmaeili, H.R. & Mobedi, I. (2013). Survey on ectoparasite of *Aphanius sophiae* (Cyprinodontidae) from Southern Iran. Journal of Coastal Life Medicine, 1 (2), 89-91.

2012

Esmaeili, H.R., 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.

Teimori, A., Esmaeili, H.R., Gholami, Z., Zarei, N. & Reichenbacher, B. (2012). *Aphanius arakensis*, a new species of tooth-carp (Actinopterygii, Cyprinodontidae) from the endorheic Namak Lake basin in. Zookeys, 215, 55–76.

2011

Esmaeili, H.R. & Gholami, Z. (2011). Scale structure of a cyprinid fish, *Rutilus frisii* (Kamenskii, 1901) (Actinopterygii: Cyprinidae) using scanning electron microscope (SEM). Iranian Journal of Fisheries Sciences, 10 (1), 155–166.

Gholami, Z., Akhlaghi, M., Mobedi, I. & Esmaeili, H.R. (2011). First Report of *Aphanius sophiae* (Heckel, 1849) (Actinoptrygii: Cyprinodontidae) with *Tetracotyle* sp. in Cheshmeh-Ali Damghan, Semnan Province, North of Iran. Veterinary Research Forum, 2 (1), 605–608.

Gholami, Z., Akhlaghi, M. & Esmaeili, H.R. (2011). Infection of *Aphanius dispar* (Rüppell, 1829) with *Ligula intestinalis* plerocercoides in Mehran River, south of Iran. Iranian Journal of Fisheries Sciences, 10 (2), 346–351.

Gholami, Z., Kia, B.E., Mobedi, I. & Esmaeili, H.R. (2011). Occurrence of *Clinostomum complanatum* in *Aphanius dispar* (Actinoptrygii: Cyprinodontidae) collected from Mehran River, Hormuzgan Province, South of Iran. Asian Pacific Journal of Tropical Biomedicine, 189–192.

2010

Monsefi, M., Gholami, Z. & Esmaeili, H.R. (2010). Histological and Morphological Studies of digestive tube and liver of the Persian tooth-carp, *Aphanius persicus* (Actinopterygii: Cyprinodontidae). Istanbul University Faculty of Science Journal of Biology, 69 (1), 57–64.

Esmaeili, H. R., Gholami, Z., Hojat Ansari, T. & Baghbani. S. (2010). Morphology of otoliths in some fishes of Caspian Sea Basin. Journal of Science, 22, 18–27.

Esmaeili, H.R., Zarei, N., Teimori, A & Gholami, Z. (2010). An introduction to *Aphanius persicus* in Maharlu basin, south of Iran. Fars Province Counseling, 15, 1–3.

Esmaeili, H.R., Zareian, H., Gholamhosseini, A., Ebrahimi, M., Gholami, Z., Teimori, A. & Hojat Ansari, T. (2010). Karyotype analysis of the king nase fish, *Chondrostoma regium* (Heckel, 1843) (Actinopterygii: Cyprinidae) from Iran. Turkish Journal of Fisheries and Aquatic Science, 10, 477–481.

2009

Esmaeili, H.R. & Gholami, Z. (2009). Scale structure of a cyprinid fish, *Alburnoides bipunctatus* (Blotch, 1782) Using scanning electron microscope (SEM). Ferdowsi University International Journal of Biological Sciences, 1 (1), 19–27.

Esmaeili, H. R., Gholami, Z., Teimori, A. & Baghbani, S. (2009). Morphology of urohyal bone and its importance in taxonomy of some fishes of Caspian Sea basin. Iranian Journal of Biology, 23 (3), 448–459.

Esmaeili, H.R., Gholami, Z., Nazari, N., Gholamifard, A., Sharyari, F., Baghbani, S. & Ebrahimi, M. (2009). First karyotype analysis of an endemic sucker catfish, *Glyptothorax silviae* Coad, 1981 (Actinopterygii: Siluriformes) from Iran. Turkish Journal of Zoology, 33, 409–412.

Gholami, Z., Akhlaghi, M. & Esmaeili, H.R. (2009). Report of the infection of tooth-carp Isfahan *Aphanius isfahanensis* Hrbek, Keivany and Coad, 2006 with *Diplostomum spathaceum*. Journal of Veterinary Research, 3 (2), 129–131.

2008

Esmaeili, H.R., Teimori, A., Coad, W.B. & Gholami, Z. (2008). Threatened fishes of the world: *Cobitis linea* (Cyprinoforme: Cobitidae). Environmental Biology of Fishes, 83 (4), 407–408.

Esmaeili, H.R., Teimori, A., Coad, W.B. & Gholami, Z. (2008). Threatened fishes of the world: *Seminemacheilus tongiorgii* (Cyprinoforme: Balitoridae). Environmental Biology of Fishes, 84 (4), 375.

2007

Esmaeili, H.R. & Gholami, Z. (2007). Investigations on the surface ultrastructure of scale of Genow tooth-carp, *Aphanius ginaonis* (Holly, 1929) (Actinopterygii: Cyprinodontidae) using scanning electron microscope. Iranian Journal of Biology, 20 (2), 307–314.

2006

Esmaeili, H.R., Teimori, A., Gholami, Z. & Hosseini, F. (2006). Range extension report for *Barbus sublimus* Coad and Najafpoor, 1997 (Actinopterygii: Cyprinidae) along with its sympatric species in southwest Iran. Iranian Journal of Animal Biosystematics, 2 (1), 19–24.

Abstracts in Conferences

2014

Gholami, Z., Reichenbacher, B., Esmaeili, H. R. & Teimroi, A. (2014). Morphology of the sacular otoliths in species identification, phylogeny and systematics of the Iranian *Aphanius* (Cyprinodontidae: Cyprinodontidae) species. 5th International Otolith Symposium (IO2014). Mallorca, Balearic Islands, Spain, 20–24 October 2014.

2012

Gholami, Z., Teimori, A., Eemaeili, H. R., Schulz-Mirbach, T. & Reichenbacher, B. (2012). Do scale size indices and scale surface morphology allows identification of endemic *Aphanius* species from southern Iran? 14th European Congress of Ichthyology, Liege, Belgium, 3–8 July 2012. Poster presentation.

Amin, O.M., Gholami, Z., Akhlaghi, M. & Heckmann, R.A. (2012). The description and host-parasite relationships of a new species of *Acanthosentis* (Acanthocephala: Quadrigyridae) from the Farsi toothcarp, *Aphanius farsicus* (Actinopterygii: Cyprinodontiade) in Iran. XIth European Multicolloquium of Parasitology (EMOP XI) in Cluj-Napoca, Romania, 24–29 July 2012. Oral presentation.

2011

Gholami, Z., Zarei, R., Mobedi, I. & Kia, E. (2011). Characterization of a new species, *Ichthyophthirius mehrani* (Ciliophora: Oligohymenophorea) isolated from *Aphanius dispar* (Rüppell, 1829) (Cyprinodontidae) collected from Mehran River, South of Iran. Biosystematic Congress in Berlin, 21–27 February 2011. Poster presentation.

Esmaeili, H.R., Zarei, N., Gholami, Z. & Teimori, A. (2011). Biodiversity and distribution of Cyprinodontiform fish species (Actinoptrygii: Cyprinodontiformes) of Iran. Biosystematic Congress in Berlin, 21–27 February 2011. Oral presentation.

2009

Gholami, Z., Esmaeili, H.R., Akhlaghi, M. & Peyghan, R. (2009). First report of *Ligula intestinalis* infection of *Aphanius persicus* (Jenkins, 1910) in Barme-e-Shoor Spring-Stream System, Maharlu Basin, Shiraz, Iran. 1st International Congress on Aquatic Animal Health Management and Diseases. 27–28 January 2009. Oral presentation.

2008

Esmaeili, H.R., Gholami, Z., Teimori, A., Ebrahimi, M. & Hojat Ansary, T. (2008). Distribution, morphology and karyology of the genus *Aphanius* Nardo, 1826 (Actinopterygii: Cyprinodontidae) in Iran. International Symposium, Documenting, Analysing and Managing Biodiversity in the Middle East, 19–21 October 2008-Amman, Jordan. Oral presentation.

2006

Esmaeili, H.R. & Gholami, Z. (2006). Investigation on the surface ultrastructure of scale of Geno tooth-carp, *Aphanius ginaonis* (Holly, 1929) (Actinopterygii: cyprinodontidae) using scanning electron microscope. 14th National and 2th International Conference of Biology. Tehran, Iran. Poster presentation.

2003

Kami, H.G. & Gholami, Z. (2003). Introduction on Morphology, Identification and Biology Sunspider in Golestan Province. Second Conference of Biology and Biotechnology of biology students of Iran's Universities. Tonekabon, Iran. Oral presentation.

Kami, H.G. & Gholami, Z. (2003). Introduction on biology and Morphometric and Meristic characters *Typhlops vermicularis* in Gorgan city, Golestan Province. Third Conference of Biology and Biotechnology of Biology students of Iran's Universities. Kermanshah, Iran. Oral presentation.