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Review Article

Review of *Aphanius farsicus*: a critically endangered species (Teleostei: Cyprinodontidae) in Iran

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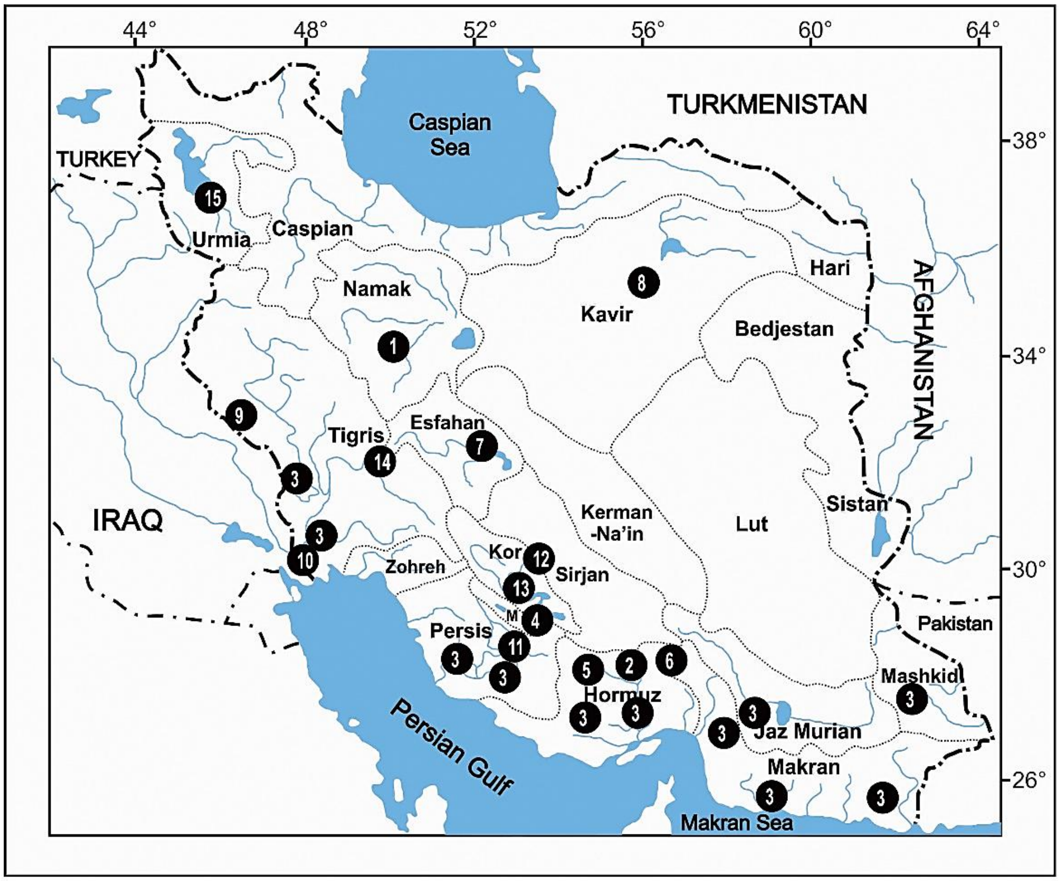
Abstract: The systematics, morphology, phylogeny, distribution, biology, economic importance and conservation of the Fars tooth-carp, *Aphanius farsicus* of Iran are described, the species is illustrated, and a bibliography on this endemic, critically endangered species is provided. Iran and central Anatolia show the highest diversity of *Aphanius*, and 14 extant (including *Aphanius farsicus*, an endemic species in the endorheic Lake Maharlu basin, South-western Iran) and one fossil species are known to occur in Iran based on data derived from fish morphometry and meristics, otoliths, scales and mtDNA sequences. Human-induced disturbance or anthropogenic activities, including hydrological alteration, introduction of exotic species, intensive aquaculture industry, water pollution, rapid sedimentation, natural disturbance (climate change and drought) and also limited distribution, have forced *Aphanius farsicus* populations to the edge of extinction. *Aphanius farsicus* can benefit from a combination of reintroduction, assisted colonization and capture release which potentially increase success rate of a conservation management plan. Encouraging the local communities, NGOs and media to involve voluntarily in long-term conservation programs is highly recommended.

Keywords: Fish diversity, Threatened fishes, Endemic, Maharlu Lake basin, Iran.

Introduction

Being part of Irano-Anatolian hot spot, Iran harbors high biodiversity especially in freshwater fish diversity (e.g., Esmaeili et al. 2010, 2014a, 2014b) exceeding more than 263 fish species distributed in different endorheic and exorheic basins. This high biodiversity is now under severe threats due to human-induced disturbance or anthropogenic activities (hydrological alteration, introduction of exotic species, over-fishing, unusual methods of fishing, rapid sedimentation and land erosion) and natural disturbance (climate change and drought) (see Teimori et al. 2016). Due to these threats, some species have been considered as critically endangered and it is already clear that some others might be added to the list; hence the area is of great conservation concern. This issue is particularly important for those groups that contain most endemicity such as cyprinodontids, which show strong endemic diversity within Iranian basins. Cyprinodontids of the genus *Aphanius* Nardo, 1827 inhabit a wide range of coastal and landlocked habitats in the Mediterranean, the Red Sea and the Persian Gulf, and 32 species of *Aphanius* are currently recognized (Wildekamp 1993; Eschmeyer & Fricke 2016). Iran and central Anatolia show the highest diversity of *Aphanius*, and 14 extant and one fossil species are known to occur in Iran (Fig. 1)



1 Aphanius arakensis
2 Aphanius darabensis
3 Aphanius dispar
4 Aphanius farsicus
5 Aphanius furcatus
6 Aphanius ginaonis
7 Aphanius isfahanensis
8 Aphanius kavirensis
9 Aphanius mento
10 Aphanius mesopotamicus
11 Aphanius pluristriatus
12 Aphanius shirini
13 Aphanius sophiae
14 Aphanius vladykovi
*15 Aphanius persicus**

Fig.1. Geographic distribution map of *Aphanius* species in Iran. * Fossil record. M, Maharlu Lake basin.

based on data derived from fish morphometry and meristics, otoliths, scales and mtDNA sequences (Hrbek et al. 2002, 2006; Hrbek & Meyer 2003; Coad 2009; Esmaeili et al. 2012, 2014a, b; Gholami et al. 2014, 2015a, b; Teimori et al. 2012a, b, 2014). Twelve out of 14 Iranian *Aphanius* species are endemic to this country. Most of the endemic species are distributed in the closed endorheic basins in the mountainous regions of the Zagros and therefore, they are characterized by small population size. These include the widely distributed *A. dispar* (Rüppell, 1829), *A. sophiae* (Heckel, 1849) from the endorheic Kor River basin, *A. pluristriatus* from the Mond River drainage (Esmaeili et al. 2012), *A. ginaonis* (Holly, 1929) from the Genow hot spring, *A. mento* (Heckel, 1843) from Tigris basin in border of Iran and Iraq, *A. vladykovi* Coad, 1988 from the upper reaches of the Karoun drainage (Coad, 1988), *A. mesopotamicus* Coad, 2009 from the Tigris and Karoun drainage (Coad 2009), *A. farsicus* Teimori, Esmaeili & Reichenbacher, 2011 from the endorheic Lake Maharlu basin (Teimori et al. 2011), as well as *A. arakensis* from the Lake Namak basin (Teimori et al. 2012b), *A. isfahanensis* from the endorheic Esfahan basin (Hrbek et al. 2006), *A. shirini* from the upper reaches of the Kor River drainage (Gholami et al. 2014), *Aphanius darabensis* from upper reaches of Kol river (Hormuz basin) (Esmaeili et al. 2014c), *A. kavirensis* from Kavir basin (Esmaeili et al. 2014c) and

A. furcatus from the Shur River, Hormuzgan Basin (Teimori et al. 2014). There is also a record of the fossil *A. persicus* (Priem, 1908) from Urmia basin (Gaudant 2011).

Among the Iranian cyprinodotids, *A. farsicus* (originally described as *A. persicus*), is an endemic cyprinodontid species in the endorheic Lake Maharlu Basin, South-western Iran, which may be extinct in the wild since 2013. Therefore, the aim of this study is to review the past and current status of *A. farsicus* from the Lake Maharlu Basin.

This work has been compiled from extensive field expeditions till 2016 in the Maharlu Lake Basin, the works listed in the references and also by examination of the material in the Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz (ZM-CBSU); and those available in Coad, 2016 (available in www.briancoad.com).

Systematic ichthyology of the genus *Aphanius*: We follow the classification of the Teleostei in Arratia (1999), which has been confirmed by recent molecular analyses (e.g., Near et al. 2012; Broughton et al. 2013; Betancur et al. 2013).

Subdivision Teleostei sensu Arratia, 1999
Order Cyprinodontiformes Berg, 1940
Suborder Cyprinodontoidei Parenti, 1981
Family Cyprinodontidae Gill, 1865
Subfamily Cyprinodontinae
Genus *Aphanius* Nardo, 1827 (type species *A. fasciatus* Valenciennes, in Humboldt & Valenciennes, 1821)

Order Cyprinodontiformes (Killifishes): Monophyly of this order is recognized on the basis of several derived characters: e.g., caudal fin truncate or rounded; caudal fin skeleton symmetrical, with one epural; first pleural rib on second vertebra rather than third; pectoral fin insertion ventrolateral (primitively, low-set pectoral girdle); scale-like first postcleithrum; an alveolar arm of the premaxillae; extended developmental period (Parenti 1981; Rosen and Parenti 1981; Costa 1998a, b; Nelson 2006). In addition, they possess the following characters: lateral line canal and pores chiefly on head, lateral line represented on body only by pitted scales; narial opening paired; branchiostegal rays 3-7; pelvic fins and girdle present or absent; upper jaw bordered by premaxilla only, protrusible; vomer usually present and supracleithrum always present; metapterygoid usually absent and ectopterygoid always absent; parietals present or absent; vertebrae 24-54. Marked sexual dimorphism with the males often brightly colored. Members of this order are popular aquarium and experimental fishes.

Family Cyprinodontidae (Pupfishe); Greek, kyprinos = goldfish + Greek, odous = tooth, teeth. The term pupfish was coined by Hubbs & Miller (1948) because of their behavioral pattern of rapid movements punctuated by period of inactivity that resembled puppies at play. The killifishes represent a large group of secondary freshwater fishes (i.e., fishes normally occurring in inland aquatic systems, but being tolerant of brackish waters and capable of occasionally crossing narrow sea barriers). They have a long evolutionary history of dispersal and vicariance that provides insights as to their success in desert habitats. Geological records, fossil evidence, and phylogenetic comparisons of cyprinodontids suggest their origins in the shallow seas and estuarine environments of the west Tethys Ocean (Parker & Kornfield 1995). With the breakup of the North American and Eurasian land masses some 80 million years ago (mya), one cyprinodontid lineage remained in what was to become the Mediterranean/Middle Eastern region, while another moved with the North American land mass.

Nelson (2006) gives the following characters for members of this family: Dorsal processes of maxillaries expanded medially, nearly meeting in the midline; lateral arm of maxilla expanded. Origin of dorsal fin (10-18 soft rays) anterior to origin of anal fin (8-13 soft rays). Fertilization external. Maximum length 8cm SL.

They found in freshwater, brackish water and coastal marine of United States, Middle America, West Indies, parts of northern South America, North Africa, and Mediterranean Anatolian region (Nelson

2006).

Genus Aphanius Nardo [G. D.] 1827:34, 39-40 (Nardo, 1827). Masc. *Aphanius nanus* Nardo 1827. Type by subsequent designation. Also in Isis, v. 20:482. Type designated by Jordan (1917:121). Proposal submitted to the ICZN to conserve this name (Kottelat & Wheeler 2001:110; conserved in Opinion 2057. Name was placed on the Official List of Specific Names in Zoology (Opinion 2057). •Synonym of *Lebias* Goldfuss 1820 -- (Lazara 1995:502, Lazara 2001:179). •Valid as *Aphanius* Nardo 1827. Sethi (1960) advocated placing *Aphanius* in a separate family, Aphaniidae, but this did not find general acceptance. The genus *Aphanius* Nardo, 1827 has been used for these tooth-carps for many years. However, Lazara (1995) designated *Lebias fasciata* Valenciennes, 1821 as the type species for *Lebias* Goldfuss, 1820, making *Lebias* a subjective senior synonym of *Aphanius*. On this basis *Lebias* must be used rather than *Aphanius* but Lazara's type species designation is invalid (Kottelat & Wheeler 2001). Since Lazara (1995) could have chosen another species as type species, the change involves a large number of species and these species are threatened and listed in various legislation, a petition is before the International Commission on Zoological Nomenclature to suppress Lazara's designation (Kottelat 1997; Kottelat & Wheeler 2001; Wildekamp 2001; Villwock et al. 2002). Wildekamp et al. (1999) present evidence that *Lebias* is a synonym of *Cyprinodon*.

Literature on these Mediterranean and Southwest Asian fishes may appear under either of these names or under *Cyprinodon* Lacepède, 1803, the latter now restricted to American species. Bănărescu (1995) disagrees with Parenti's (1981) relationship of *Aphanius* to South American *Orestias* (see also Parker & Kornfield 1995; Stevenson 1997; Costa 1997 for conflicting views).

This genus is characterised by a thick oval body, large to moderate cycloid scales, the head flattened on top, a small, superior mouth with tricuspid teeth, the upper jaw bordered by the premaxillaries only, lateral line system present only on the head, dorsal fin positioned somewhat posteriorly with 1-2 unbranched rays and 7-13 branched rays, anal fin rays 1-2 unbranched and 7-14 branched, dorsal and anal fins larger in males than in females, dorsal fin inserted opposite the anal fin origin (in contrast to *Gambusia*), and colouration of males and females distinct. All species show a clear sexual dimorphism.

Aphanius is the only genus in the family currently recognised in Iran. However Parenti (1981) distinguishes derived members of the genus *Aphanius* as `*Aphanius*' without formally describing a new genus. One of the distinguishing features of `*Aphanius*' is the reduction of cephalic sensory pores to neuromasts, a character found in *A. mento*, *A. sophiae* and *A. vladykovi* in Iran.

These fishes are known generally as gour-e khar in Farsi (= literally "striped donkey" which means zebra here although usually in Iran this term refers to the wild ass or onager), Gormahi (literally "striped fish), kapurdandandar or kopurdandandar (= tooth-carp), or even آفانیوس (= *Aphanius*).

To date, 14 species of *Aphanius* have been described from the Iranian endorheic and exorheic basins (e.g., Hrbek et al. 2006; Coad 2009; Esmaeili et al. 2012; 2014d; Gholami et al. 2014; Teimori et al. 2012a; 2014). Based on the mtDNA phylogenies, it has been suggested that they are representatives of three large clades i.e., the *A. dispar* clade, the *A. mento* clade, and the Inland and Inland-related *Aphanius* species (IIRAS clade) (see Esmaeili et al. 2014c).

Aphanius farsicus Teimori, Esmaeili and Reichenbacher, 2011
(Fig. 2)

Common name: Kapour-e-dandandare Farsi, gour-e-khar mahye Fars (in Farsi); Fars tooth-carp (in English)

Synonyms: *Cyprinodon blanfordii* Jenkins [J. T.] 1910:124, Pl. 6 (fig. 3) [Records of the Indian Museum (Calcutta) v. 5 (art. 12)] East of Shiraz, southern Iran.

Cyprinodon persicus Jenkins [J. T.] 1910:125,

Fig.2. Male and female *Aphanius farsicus* from Maharlu Lake basin, Iran.

Pl. 6 (fig. 4) [Records of the Indian Museum (Calcutta) v. 5 (art. 12)] spring on the edge of Shiraz Lake, southern Iran.

Lebias punctatus Heckel [J. J.] 1847:268, Pl. 22 (fig. 3) [Reisen in Europa, Asien und Africa v. 2 (pt 3)] Nemek-Deria or Salt Lake, flowing into the Saadi below Schiraz, Iran.

Jenkins (1910) described from the area near Shiraz besides *Cyprinodon persicus* two further species, i.e. *C. blanfordii* ("East of Shiraz") and *C. pluristriatus* ("East of Shiraz, near Fassa"). Wildekamp (1993) has indicated that all three species may be synonymous with *Aphanius sophiae* (Heckel in Russegger, 1846). Coad (2016) agrees with this interpretation only with regard to *A. blanfordii*, of which he found three syntypes to be identical with female *A. sophiae* based on the pigment pattern (i.e. spotted on the flank with a lozenge-shaped spot at the caudalfin base). It has not up to date been ascertained whether *A. pluristriatus* too, is a synonym of *A. sophiae* or another described species. As a result, we refrain from using the epithet *blanfordii* (because it represents a synonym of *A. sophiae*), and we also refrained from using the name *pluristriatus* (because future studies may reveal that it represents a valid species). Consequently, *A. farsicus* is used here as a replacement name for *A. persicus* (Jenkins, 1910).

Type locality: Spring on the edge of Shiraz Lake (= Maharlu Lake), Fars Province, southern Iran (Fig. 3).

Etymology: *farsicus* is a noun (not declinable), which refers to the Fars Province, in which the type locality is located.

Key characters: Females of this species are barred while; all the other *Aphanius* species in this area of southern Iran have spotted females (Coad 2016).

Morphology: Although populations are isolated in springs and streams around Lake Maharlu, Coad (1996) found them to be relatively homogenous. Lateral line scales 24(3), 25(34), 26(69), 27(176), 28(100) or 29(22). Scales above the lateral line 4-6, scales between lateral line and the anal fin 4-8, scales between the lateral line and the pelvic fin 5-9, and scales around the caudal peduncle 12-17. Esmaeili et al. (2007) determined a chromosome number of 2n=48 with 11 pairs of submetacentric and 13 pairs of subtelocentric chromosomes and an arm number of NF=70. Karyotype 16Sm + 32St and arm number 32 (Esmaeili et al., 2008). Total dorsal fin rays 8(1), 10(8), 11(97), 12(204), 13(89), or 14(7); total anal fin rays 9(3), 10(111), 11(225), 12(64) or 13(3); total pectoral fin rays 13(9), 14(82), 15(211), 16(94), 17(9) or 18(1); total pelvic fin rays 4(11), 5(181) or 6(213); total gill rakers 9(5), 10(51), 11(220), 12(108), 13(19) or 14(3); abdominal vertebrae 9(1), 10(1), 11(27), 12(355), or 13(22); and caudal vertebrae 12(1), 14(16), 15(285) or 16(104) (Coad 2016).

Sexual dimorphism: Most apparent in colour and pigmentation detailed below. Females are longer and heavier than males (Esmaeili & Shiva 2006).

Colour: The flank in females bears numerous alternating light and dark bars, the light bars varying in width from about one half to twice that of dark bars. The bars gradually merge with background pigmentation anteriorly on the flank and while clearly defined on the rear flank are difficult to distinguish anteriorly.

The caudal fin spot in females can be oval, tear-drop shaped or elongate but is usually in the form of a lozenge. Occasionally, single, smaller, dark, subsidiary spots may be found antero-dorsally and antero-ventrally to the basal spot or scattered spots may be found irregularly before and behind the basal spot.

Males have a pigmentation very similar to that of *A. sophiae* and the description here is identical. Some minor observed variation is attributed to variation in size and maturity of the fish compared. Males have light flank bars half the width or much narrower than alternating dark bars. The margins of the dorsal, anal and caudal fins are clear while the rest of these fins is dark. Some fish have up to 3, but usually 2, thin, light bars on the basal half of the caudal fin; these are generally larger fish. The margin of the lower half of the pectoral fin has concentrations of pigment on the membranes such that this area is darker than the rest of the fin. The anal fin is darkest posteriorly where pigment is concentrated on membranes. The distal third of the fin is pigmented to form a dark band, becoming lighter proximally. The dorsal fin is the darkest fin (except for the clear margin) and the anterior base is the darkest part of the fin. Bands are not always evident but pigment spots are large proximally. Some fish have 2, sometimes 3, thin light bars at the base separated by thin dark bars and paralleling the body profile while others have none. The dorsal fin base may have instead a series of lighter spots, sometimes irregular and not paralleling the body profile.

Reproduction: Esmaeili & Shiva (2006) found the gonado-somatic index in females to increase from November to June, decreasing slowly from late June to November. The reproductive period lasted six months. Males had two peaks, in April and August. Egg diameters reached 1.71mm, absolute fecundity ranged from 45 to 250 eggs (average 115.7 eggs) and relative fecundity was 21.6 to 244.1 eggs with a mean of 90.01 eggs per gram body weight. Even small fish had hydrated eggs and, with the extended reproductive period, this shows adaptation to an unstable habitat. Monsefi et al. (2009) found that this species is a batch spawner in the Barm-e Shur spring with a spawning period from April to November. This species lives in unstable environments such as temporary lagoons or very small pools and batch spawning of relatively large eggs gives a greater chance of survival.

Feeding ecology: Fars tooth-carp diet is based on detritus, algae (particularly diatoms, green algae, and cyanobacteria), and small invertebrates. Seasonal variation in diet is more important than variation due to fish size and the Fars tooth-carp consume more green algae in spring and early summer and more diatoms and insects the rest of the year. Herbivory was considerable, similar to a few other tooth-carps, and increased with fish size, particularly because of higher consumption of green algae (Alcaraz et al. 2015). As with species composition in diet, season was more important than size in the variation of number, biovolume, mean size, and diversity of prey captured, with higher number, richness and size of prey captured in summer. The ontogenetic diet shift was less marked in this cyprinodont than in many other *Aphanius* species, probably due to its reduced size and the resource availability of its habitat, but was also shown by size-dependent feeding selectivity for a few invertebrates (Alcaraz et al. 2015).

Age and growth: In the studies of Esmaeili & Shiva (2006), the sex ratio was 1.67:1 for females: males, highly significant, the age groups were 0^+ to 3^+ years, and positive allometric growth was determined (*b* value significantly greater than 3). The condition factor for females was highest in February and March and lowest in September while in males it was lowest in December and increased until February. Fishes smaller than 25mm had an equal sex ratio, suggesting selective predation on males, or better survival or longevity of females. Esmaeili & Ebrahimi (2006) give a significant length-weight relationship based on 62 fish measuring 1.86-4.27cm standard length. The α-value was 0.0222 and the *b*-value 3.395 (a *b*-value <3 indicating a fish that becomes less rotund as length increases and a *b*-value >3 indicating a fish that becomes more rotund as length increases). Alavi-Yeganeh et al. (2011) give length-weight data on this species from Kaftarak spring with a (intercept) being 0.0097and *b* (slope) being 3.303 for sexes combined.

Habitat: This species is found in fresh streams and springs and in springs of varying saline content or saline influence from hypersaline chloride Lake Maharlu. The lake at 124% is an impassable barrier to dispersal but is very shallow and dries out periodically, e.g. in 1967 (Cornwallis 1968). The larger springs and their streams can then meet and transfer fishes on the dried-out lake bed. Some springs are 27km apart along the lake margin and have probably had no contact or fish exchange over many generations. This is especially the case for the smaller springs which emerge from the ground with a diameter of only 1m and restricted flow. When lake water rises, lower springs are inundated with hypersaline water and the fishes are killed. The spring is presumably recolonised from a higher spring when lake level falls.

Fish placed in pure lake water die within minutes. Springs discharging directly into the lake contain tooth-carps but the fish do not venture into the lake. Some fish, if disturbed or deliberately harassed, will swim out for a short distance into the hypersaline lake water but they blanch and rapidly retreat to fresher water (Coad 1996).

Esmaeili and Shiva (2006) found the bottom of these water bodies to be muddy but the water was clear and slow-running. Conditions in October at three different sites were 16.9-19.0ºC, pH 6.70-6.74, dissolved oxygen 3.96-6.11 mg/l, nitrate 0.9-1.6 mg/l, nitrite 0.029-0.062 mg/l, phosphate 0.35-0.65 mg/l and ammonium 1.55-2.60 mg/l.

Karyotype: Esmaeili et al. (2007) determined a chromosome number of 2n=48 with 11 pairs of submetacentric and 13 pairs of subtelocentric chromosomes and an arm number of NF=70. Karyotype 16Sm + 32St and arm number 32 (Esmaeili et al. 2008).

Parasites and predators: Mokhayer (1989) reported metacercariae of the eye fluke, *Diplostomum spathaceum* from *Aphanius sophiae*, probably this species, in Iran. The fluke can cause complete blindness and death in commercially important species of fish. This fish was also infested with yellow grub, *Clinostomum complanatum*. González-Solís et al. (1997) report *Contracaecum* sp. larvae from this species in the Lake Maharlu drainage, Fars.

Amin et al. (2013) described *Acanthogyrus* (*Acanthosentis*) *barmeshoori* (Acanthocephala: Quadrigyridae) from the Fars tooth-carp, *A. farsicus* in the Barm-e-Shoor Spring, the Maharlu Lake basin, southern Iran based on the collected fish specimens during July 2006 to June 2007. According to them, the parasite was observed all year, with the highest abundance and intensity in May while the prevalence was highest in February. The prevalence of acanthocephalans decreased with increasing fish size. While most worms were recovered in fish within the length range of 18-29.9mm, one of the longest parasites (1.68mm long) was found in fish within the range of 30-33.9mm long (see Amin et al. 2013). Some fishes and birds, and even snakes, feed on *A. farsicus*.

Distribution: In 1976, this species had a patchy distribution and was common in small springs and streams, pools and qanats around the hypersaline lake and was found in at least 15 sites (Fig. 3). While the situation was more or less stable in the period of 1976-2000, the species rapidly disappeared from all but one site in the 21st century. A Recent investigation in 2015 failed to collect it from the last locality too. *Aphanius farsicus* has not been listed in the IUCN Red List of Threatened Species as critically endangered (CR) species, even though it should be considered.

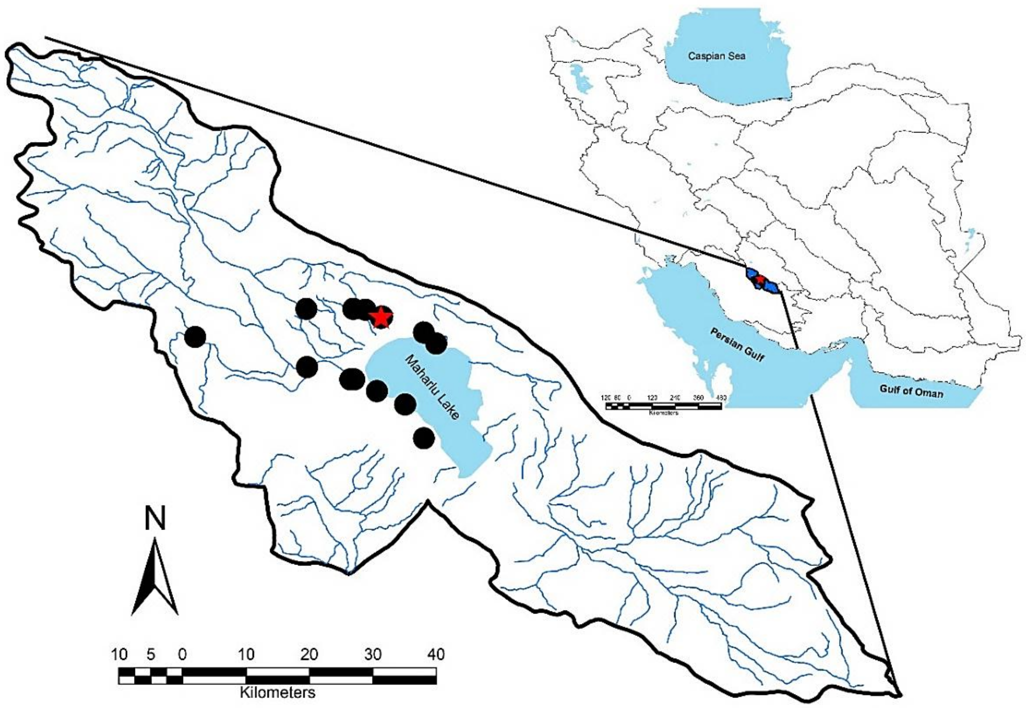


Fig.3. Male and female *Aphanius farsicus* from Maharlu Lake basin, Iran.

Genetic connectivity: The *Cytb* data derived from the phylogenetic tree and the molecular tests provided by Gholami et al. (2015b) give clear evidence that population connectivity was present in 4 studied *A. farsicus* populations. This was consistent with the assumptions of previous studies based exclusively on morphological characteristics (Coad 1996; Gholami et al. 2013). Gholami et al. (2015b) suggested that population connectivity of *A. farsicus* resulted not only 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. The presence of such aquifers in the Maharlu Basin has been demonstrated by the hydrological study of Zare et al. (2005). These aquifers probably formed during the late Pliocene to early Pleistocene, when the present endorheic Maharlu Basin was created (Khormali et al. 2003; Hatzfeld et al. 2010; Faghih et al. 2012). A report on the natural recolonization of the Maharlu springs after the drought event of 1967 (Coad 1996) provides additional support for the existence of these underground passages.

Based on the high haplotype diversity and low nucleotide diversity detected in all 4 studied *A. farsicus* populations Gholami et al. (2015b) concluded the rapid population expansion of this species after a period in which effective population size was low (Grant & Bowen 1998; Yang et al. 2012). It is possible that the above-mentioned drought in 1967 may have resulted in a genetic bottleneck, which was followed by population expansion.

Phylogenetic position and biogeography: The phylogenetic relations of *Aphanius* based on the cytochrome *b* data are shown in Fig. 4. The Maximum Likelihood and Bayesian Likelihood trees clearly indicate that *A. farsicus* is monophyletic and distinct from the other species of the IIRAS clade. *Aphanius farsicus* is a member of the *A. mesopotamicus* lineage, in the *A. sophiae* clade of a young evolutionary group. It is sister to a group of seven species comprising *A. arakensis* + *A. kavirensis*, *A. mesopotamicus*, *A. sophiae* and

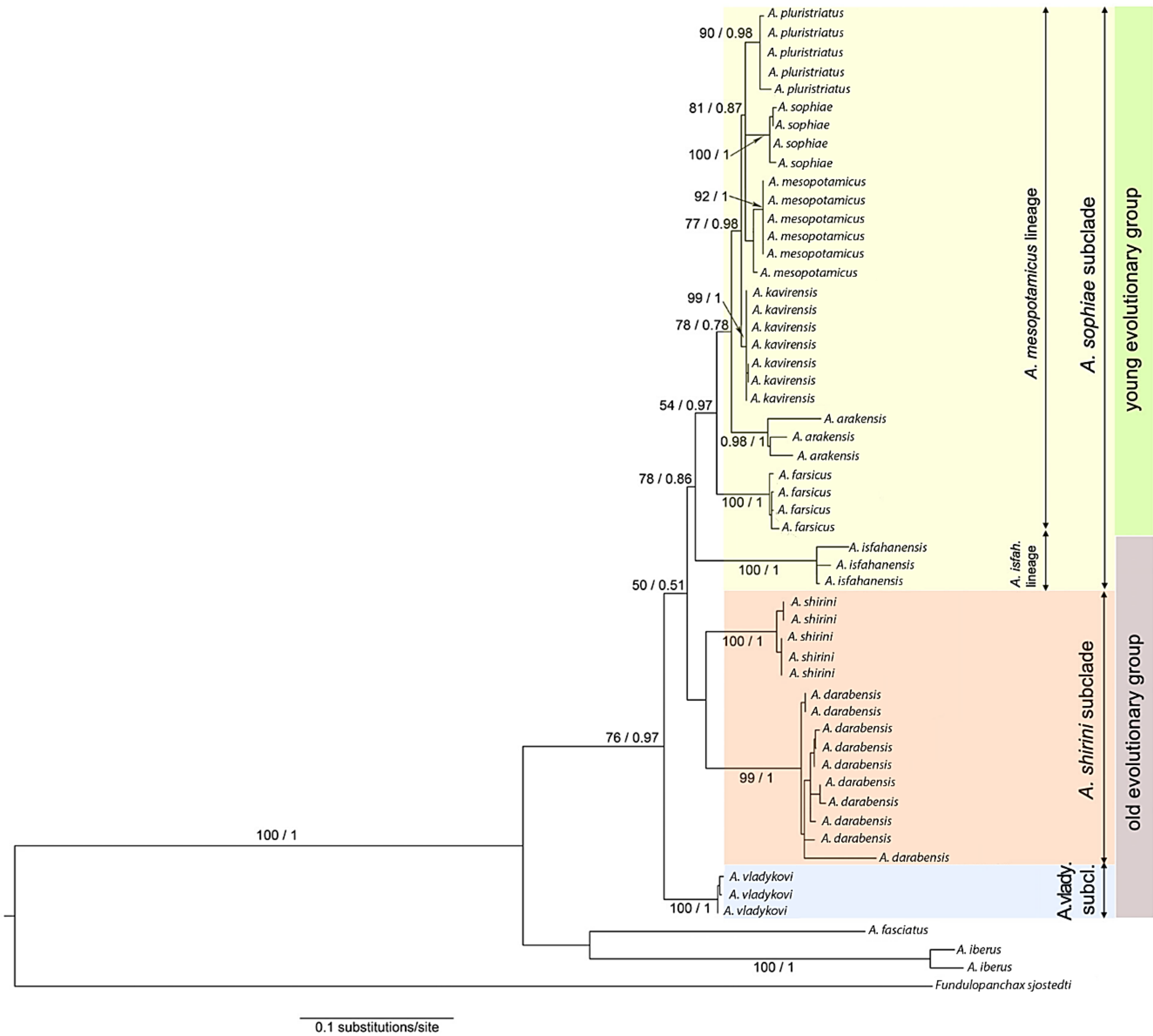


Fig.4. Maximum likelihood estimate (based on cytochrome b sequences) of phylogenetic position of *Aphanius farsicus* and its relations to other relatives Iranian inland and inland-related *Aphanius* species (IIRAS clade). Numbers represent maximum likelihood bootstrap values based on 2000 replicates followed by Bayesian likelihood values (see Esmaeili et al. 2014c).

A. pluristriatus. Sister to all species mentioned above is *A. isfahanensis*. All of these species are sister to *A. shirini*, *A. darabensis* + *A. vladykovi* (Fig. 4).

Based on the previous molecular phylogenetics (Hrbek & Meyer 2003; Hrbek et al. 2006, Teimori et al. 2012a, Gholami et al. 2014, Esmaeili et al. 2014c), the clade of the Iranian inland and inland-related *Aphanius* species (IIRAS clade) can be divided into three subclades comprising (i) the *A. vladykovi* subclade, (ii) the *A. shirini* subclade, and (iii) the *A. sophiae* subclade, which may also termed the *A. sophiae* species group. All species of the three subclades are endemic to Iran, with the exception of *A. mesopotamicus*, which was also found in Iraq (Coad 2009). The *A. vladykovi* subclade includes *A. vladykovi* itself as the most distinct species of the

IIRAS clade (Hrbek & Meyer 2003; Teimori et al. 2012a; Esmaeili et al. 2014c). *The A. shirini* subclade comprises *A. shirini* and *A. darabensis*. The *A. sophiae* subclade includes the remaining species of the IIRAS clade. It is the most diverse subclade and is subdivided here in three lineages according to the temporal diversification. The lineage of *A. isfahanensis* contains only this species, which diverged much earlier (4.8. m.y.a. according to Hrbek et al. 2006) than all other species of the *A. sophiae* subclade. *A. farsicus* + *A. arakensis* probably diverged in the Late Pleistocene (100,000–11,700 y. ago), whereas the lineage of *A. mesopotamicus* comprises a group of very closely related species (*A. sophiae, A. mesopotamicus, A. pluristriatus,* and *A. kavirensis*) that may have diversified 11,700 to 4000 y. ago (Early to Middle Holocene) (see also Gholami et al. 2014). Notably, external characters do not unambiguously distinguish between the species of the *A. sophiae* subclade, with the exception of *A. isfahanensis*. However, differences in cytochrome *b* and also between the otoliths clearly show that the species of the *A. sophiae* subclade are distinct. In addition, they inhabit widely separated distribution areas without any hydrological networks or connectivity (Fig. 1). These are the reasons that we consider the species of the *A. sophiae* subclade as distinct species rather than as populations of a single species. The diversification of the species of the IIRAS clade has been shaped mainly by vicariance events during the Miocene to Holocene geological processes in the Zagros and Alborz mountains and associated changes in drainage systems (Hrbek & Meyer 2003; Hrbek et al. 2006; Esmaeili et al. 2012; Teimori et al. 2012a; Gholami et al. 2014). Examples of such geological-based speciation include *A. vladykovi* from the High Zagros (Coad 1988), *A. isfahanensis* from the endorheic Esfahan Basin (Hrbek et al. 2006), *A. pluristriatus* from the Mond River Basin (Esmaeili et al. 2012, 2014c), and *A. shirini* from the High Zagros (Gholami et al. 2014).

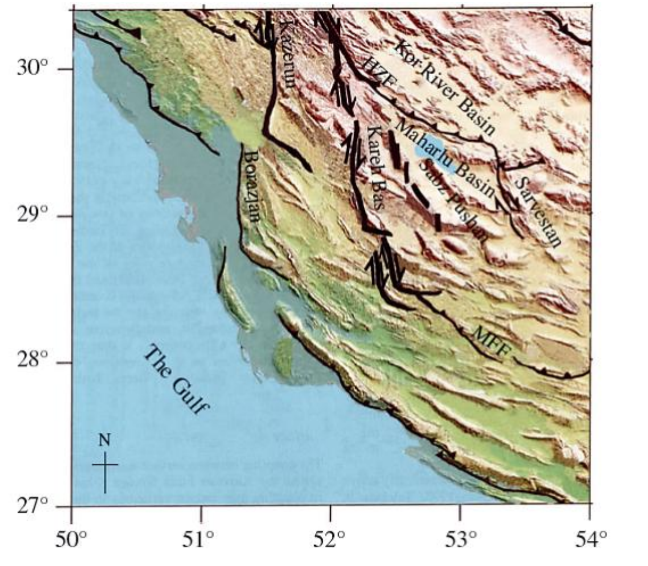


Fig.5. The geological overview of south-western Iran illustrating the tectonic structures that delimit the Maharlu and Kor River basins (HZF, High Zagros Fault; MFF, Main Frontal Fault) (after Hatzfeld et al. 2010 and Gholami et al. 2015b).

The Maharlu Basin (Fig. 5) is a tectonically constrained endorheic basin within the Simple Folded Belt of the Zagros Mountains (Agard et al. 2005; Gholami et al. 2014). It lies close to the Sabz–Pushan Fault and forms part of the Kazerun Fault System, which is one of the most active fault systems in southern Iran and includes the Kazerun, Kareh-Bas, Sabz-Pushan and Sarvestan faults. 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 (5·0-1·8M b.p.) and Pleistocene (1·8M b.p.–10 000 b.p.) (Khormali et al. 2003; Hatzfeld et al. 2010; Faghih et al. 2012). These events led to rapid isolation of multiple areas, created new barriers to migration and altered hydrological networks (Hatzfeld et al. 2010). The young geological age of the Maharlu Basin is additionally supported by its elongated shape, which is typical of basins located in tectonically active mountain ranges (Keller & Pinter 2002; Faghih et al. 2012).

Based on the available published information it appears that tooth-carps in inland waters may have risen with the post-Pliocene uplift of the Zagros Mountains rather than being the result of relatively recent inter-basin dispersal.

Fig.6. Road construction has affected natural habitat of *A. farsicus* in Maharlu basin.

Threats: Changes in fish diversity and community structure are mainly due to two reasons. The first is human-induced disturbance: anthropogenic activities include hydrological alteration, introduction of exotic species (e.g., *Mauremys caspica*), over-fishing, unusual methods of fishing (i.e. toxins and dynamite), rapid sedimentation, and land erosion. Among them, hydrological alteration, over-fishing, and unusual methods of fishing probably exert the largest effects on the endemic fish biodiversity in Iran. The second reason is natural disturbance such as drought, which may exert the greatest effect on fish diversity in Iran including *A. farsicus* (see Teimori et al. 2016).

There are several major threats affecting *A. farsicus* population (Figs. 6-10):

(I) Human-induced disturbance:

Introduction of exotic species: The introduction of exotic fish species to the Iranian hydrological

Fig.7. Water pollution in natural habitat of *A. farsicus* (11 March 2010).

Fig.8. Water pumping from natural habitat of *A. farsicus* (14 May 2010).

systems dates back to the 1920s, when mosquito fish *Gambusia* was introduced as an antimalarial agent (Coad 1996). Since then, 29 fish species have been introduced to Iranian inland waters and many more fishes have also been translocated. To date 8 fish species in three families have been introduced to the Maharlu basin all in sympatry with *A. farsicus*: *Carassius gibelio*, *Cyprinus carpio*, *Hypophthalmichthys molitrix*, *H. nobilis*, *Ctenopharyngodon idella*, *Pseudorasbora parva* (Cyprinidae), *Oncorhynchus mykiss* (Salmonidae) and *Gambusia holbrooki* (Poeciliidae). Aquaculture, mosquito control and accidental introduction have been the main reasons for the introduction of these

Fig.9. Drought of natural habitat of *A. farsicus* in Maharlu Lake basin (15 July 2010).

Fig.10. Carnivorous Caspian turtle or striped-neck terrapin (*Mauremys caspica*) in semi-dried and polluted pool in Maharlu Lake basin, Iran (January 2016).

exotic fishes to the Maharlu basin. The processes involved in species invasions are almost similar in every biological invasion (see Esmaeili et al. 2014a). An alien species must surmount geographic barriers to be introduced into a new area, then barriers to survival and reproduction, to become established within the expanded range, and finally barriers to dispersal to become invasive (Esmaeili et al. 2014a).

Water pollution: Development of the human community has increased demand for consumption on one hand and has increased ecosystem pollution on the other hand (Teimori et al. 2016). Agricultural and industrial activities in the Maharlu basin are currently the main sources of water pollution in this small basin. Domestic households, industrial and agricultural practices produce wastewater that can cause pollution and inject wastes and toxins directly into the natural water bodies of the Maharlu Lake basin. They have also added a high nutrient load.

Hydrological alteration: Dam and road constructions and pumping water from the aquifer which feeds the systems of the Maharlu Lake basin, are the main hydrological alterations which have affected *A. farsicus* populations, especially during the last decade. These factors have led to the habitat fragmentation of Fars tooth-carp.

Intensive aquaculture industry: During the last few years, several fish farms were developed to culture exotic Chinese carps (*C. carpio*, *H. molitrix*, *H. nobilis* and *C. idella*). Moreover, *C. gibelio* and *P. parva* were also introduced accidently along with the Chinese carps.

These Aquatic Invasive carp Species (AIS) and other bio-invasions (e.g., *O. mykiss* and previously introduced *G. holbrooki*) had major effects on *A. farsicus* populations through competition, habitat changes and introduction of parasites. Moreover, due to changes in water regimes of springs and pools caused by these fish farms, severe habitat modifications have occurred, resulting in the disappearance of *A. farsicus* from the majority of its natural habitats.

(II) Natural disturbance: In general, the climate of Iran can be classified as arid to semiarid, with more than 80% of the country including the Maharlu basin characterized by less than 250mm of annual rainfall (Teimori et al. 2016). Therefore, the presence of fresh water in this country is always a considerable issue. Drought can be considered as the most important natural disturbance of freshwater fishes of Iran, and it has recently become a critical problem for the freshwater ecosystems, particularly in the small endorheic basin of Maharlu Lake. Due to severe drought, many small springs and pools located in this basin dried out periodically and led to the loss of *A. farsicus* populations.

Fig.11. Captive breeding of *Aphanius farsicus* in aquarium (Shiraz University Aquatic Animal Breeding Center).

Fig.12. Captive breeding of *Aphanius farsicus* in different tanks (Shiraz University Aquatic Animal Breeding Center).

Conservation Action: Our investigation in spring 2013 showed a metapopulation existed in one locality in the Maharlu Lake basin (Fig. 3). A rescue mission started immediately, and part of the wild population (founder population) was collected for special

Fig.13. Male and female *Aphanius farsicus* in Shiraz University Aquatic Animal Breeding Center.

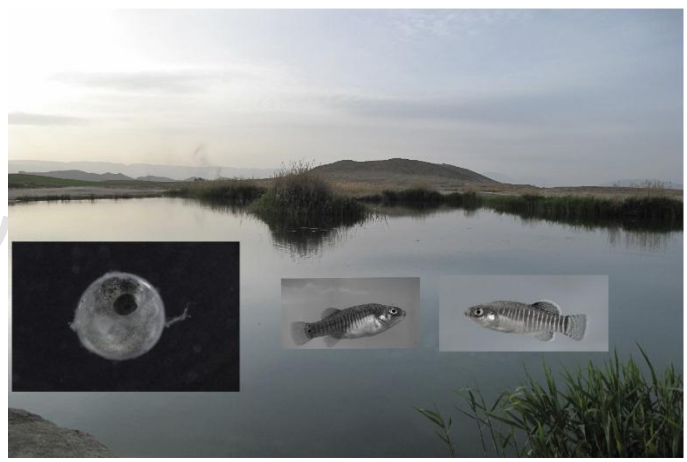


Fig.14. Can we have *Aphanius farsicus* back to its natural habitat in Maharlu Lake basin, Iran?.

stocking and propagating programs. A few months later, in 2013, the first breeding took place successfully in small glass aquariums and large plastic tanks in Shiraz University Aquatic Animal Breeding Center (ABCSU) (Figs. 11-14), so far we have 4 populations in (ABCSU). While it is no major problem to breed this species in numbers, the possibilities to reintroduce it into nature are still unexplored due to drought and instability of natural water bodies in the Maharlu basin. As all, but one, of the natural water bodies have dried out, alternative habitats need to be found in the area.

Management and conservation implication: There are several conservation strategies available for the species, such as reintroduction, assisted colonization and capture release. *Aphanius farsicus* can benefit from a combination of these strategies which potentially increase success rate of its conservation management plan (Fig. 14). To achieve a long term conservation plan, we can encourage the local communities to volunteer in our programs. This is particularly true when endemic species and their habitats are often known as significant ecotourism attractions. In this case it can also boost the local economy.

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مقاله مروری

مروری بر گورماهی فارسی *Aphanius farsicus*، گونه در معرض انقراض ایران (ماهیان استخوانی عالی: کپوردندان ماهیان)

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چکیده: در این مقاله مروری، سیستماتیک، ریخت‌شناسی، تبارشناسی، پراکنش، زیست‌شناسی، اهمیت اقتصادی و حفاظت گورماهی فارسی *Aphanius farsicus* شرح داده شده، تصاویری از آن ارائه گردیده و فهرستی از منابع موجود درباره این گونه بومی در معرض خطر انقراض لیست شده است. ایران و آناتولی مرکزی بالاترین تنوع گورماهی جنس *Aphanius* را نشان می‌دهند. بر اساس داده‌های ریخت‌سنجی و شمارشی، سنگ ریزه شنوایی، فلس، توالی DNA میتوکندریایی و شواهد دیرینه‌شناختی مشخص گردیده است که ۱۴ گونه موجود امروزی (از جمله گور ماهی فارسی، *Aphanius farsicus* ، گونه بومی در حوضه بسته دریاچه مهارلو، جنوب غربی ایران) و یک گونه فسیلی (*Aphanius persicus*) در ایران وجود دارد. دخالت‌ها و یا فعالیت‌های انسانی از جمله تغییرات هیدرولوژیکی، معرفی گونه‌های بیگانه، صنعت آبزی‌پروری، آلودگی آب، رسوب سریع، تغییرات طبیعی (تغییر آب و هوا و خشکسالی) و پراکنش محدود باعث شده که جمعیت‌های گورماهی فارسی، *Aphanius farsicus* به مرز انقراض کشانده شود. به‌کارگیری استراتژی‌های ترکیبی شامل، معرفی دوباره آن به محیط طبیعی، ایجاد کلونی‌هایی تکثیر یافته در اسارت و آزادسازی آن‌ها که بطور بالقوه می‌تواند باعث افزایش میزان موفقیت طرح مدیریت حفاظت شوند، ممکن است سبب بهبود وضعیت جمعیت گورماهی فارسی در حوضه مهارلو گردد. تشویق جوامع محلی، سازمان‌های غیر دولتی و رسانه‌ها جهت شرکت داوطلبانه در برنامه‌های بلند مدت حفاظتی بسیار توصیه می‌شود.

کلمات کلیدی: تنوع ماهی، ماهی‌های در معرض تهدید، بومزاد، حوضه دریاچه مهارلو، ایران.