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

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Abstract

Aphanius pluristriatus (Jenkins, 1910) (Cyprinodontidae) is a poorly known species from Fasa, located in the Mond River drainage system, east of Shiraz, southern Iran. It has not been investigated since its first description, its validity has been questioned and a synonymy with *A. sophiae* (Heckel, 1849) has been suggested. In this study, we describe a new collection of *Aphanius* specimens from the Zarjan spring system, which is probably the same spring system from where Jenkins (1910) collected the type specimens of *A. pluristriatus*. The morphological characters of our new series of specimens are consistent with those of *A. pluristriatus* as originally described by Jenkins (1910). We emend the original description of *A. pluristriatus* and add morphometric and meristic data. A comparison with the related taxa *A. sophiae*, *A. farsicus* (former *A. persicus*) and *A. isfahanensis* reveals that *A. pluristriatus* can be separated from them by a smaller caudal peduncle index, higher number of flank bars, lower number of gill rakers, and higher J scale index. Therefore *A. pluristriatus* represents a valid species, which is at present restricted to the drainage system of the Mond River. We suggest that *A. pluristriatus* originated from an ancient *A. sophiae* population in the Kor River Basin during the Quaternary. At that time, the Kor River was draining to the Persian Gulf by the "Paleo-Kor River" and the Mond River. During the Late Quaternary or Holocene, the connection between the Kor River and the Persian Gulf has been blocked as a result of tectonic uplift (the Kor River Basin is endorheic today). Thus, *A. pluristriatus* most likely is the relict of an ancient *Aphanius* population from the Quaternary "Paleo-Kor River" drainage system.

Key words: Cyprinodontidae, taxonomy, Paleo-Kor River, species validity, Mond River, conservation

Introduction

Aphanius is the only representative of the cyprinodontids in the Old World. Its distribution area includes coastal (brackish) and landlocked (freshwater to euryhaline) water bodies in the Mediterranean and Persian Gulf areas as far as Iran and Pakistan (Wildekamp 1993). The species diversity is highest in the endorheic basins of the mountainous regions of central Anatolia and the Iranian plateau (Coad 2000; Hrbek *et al*. 2002, 2006).

In Iran, *Aphanius* is represented by *A. dispar* (Rüppell, 1829), which is an euryhaline species also known as the Arabian (common) tooth-carp, and by six endemic species: *A. ginaonis* (Holly, 1929) or Genu tooth-carp from a hot spring near the Persian Gulf; *A. isfahanensis* Hrbek, Keivany & Coad, 2006 or Isfahan tooth-carp from the Zayandeh River Basin of central Iran; *A. farsicus* Teimori, Esmaeili & Reichenbacher, 2011 (former *A. persicus* (Jenkins, 1910)) or Farsi tooth-carp from streams and springs in the Maharlu Lake Basin; *A. sophiae* (Heckel, 1847) or Soffia tooth-carp from the endorheic Kor River Basin; *A. vladykovi* Coad, 1988 or Zagros tooth-carp from the central Zagros Mountains (Coad & Keivany 2000); and *A. mesopotamicus* Coad, 2009 or Mesopotamian tooth-carp from the Tigris-Euphrates Basin in Iran and Iraq. These endemic species occur in restricted areas, and most of them are threatened due to drought, land-use change and/or pollution around their native habitats, as well as due to the introduction of exotic fishes into their habitats.

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It is likely that additional endemic *Aphanius* species exist in Iran (Coad & Abdoli 2000). This is especially true for the mountainous regions of southern and central Iran with their complex geologic history that resulted in the creation of isolated basins and salt lakes, i.e. Maharlu, Tashk and Bakhtegan Lakes (Fig. 1). The geographic reorganization as a result of tectonic processes may have strongly affected speciation processes of *Aphanius* populations.

The aim of this study is to investigate *Aphanius pluristriatus* (Jenkins, 1910; as *Cyprinodon*), which has not been re-investigated since its original description. It has remained unclear to date whether it is valid species, a junior synonym of *A. sophiae* as suggested by Wildekamp (1993), or a junior synonym of another previously-described species. Jenkins (1910) described the type locality of *A. pluristriatus* as "East of Shiraz, stream running to Fussa, Southern Persia, 5,000 feet". The only spring system that exists near Fasa, which is the correct name for 'Fussa', is the Zarjan spring system (unpublished data of the authors), which flows to the Mond River (Fig. 1). Therefore it is clear that the types of *A. pluristriatus*, described by Jenkins (1910), came from that spring system. The here studied specimens were collected in the Zarjan spring system about 10 years ago, but further attempts (post-2003) to collect more specimens were unsuccessful because of the droughts of the last years.

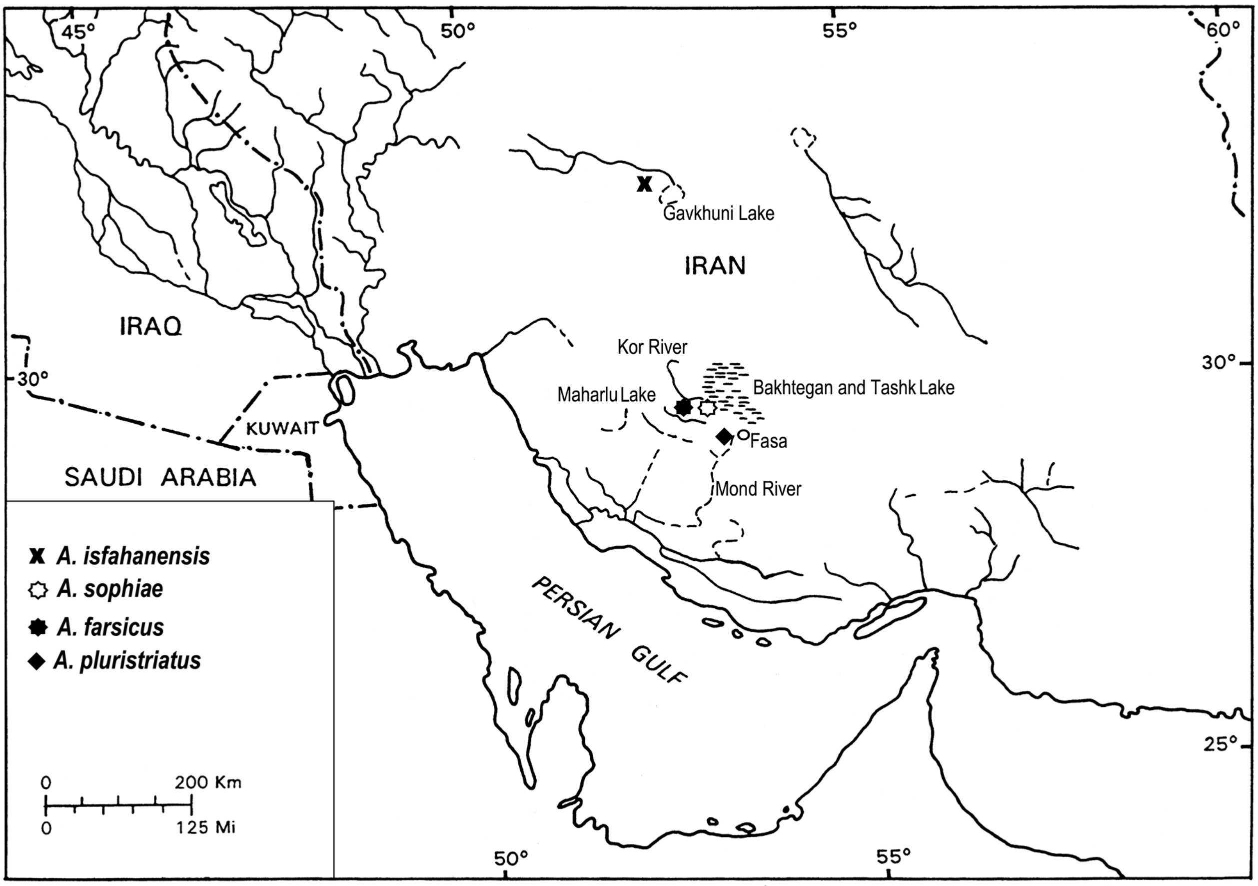

FIGURE 1. Drainage systems and type localities of *Aphanius* species in south and central Iran.

Material and methods

Localities, sampling. A total of 340 specimens of *Aphanius sophiae*, *A. pluristriatus*, *A. farsicus*, and *A. isfahanensis* were collected using hand nets from their type localities (with the exception of *A. sophiae*) in south-western and central Iran (Fig. 1). The material includes 35 males/35 females of *A. sophiae* (Kor River Basin, Ghadamgah spring), 32 males/38 females of *A. pluristriatus* (Mond River drainage system, Zarjan spring); 79 males/78 females of *A. farsicus* (Maharlu Lake Basin, Barm-e-shur spring), and 18 males/25 females of *A. isfahanensis* (Esfahan Basin, Varzaneh River). All studied specimens are deposited in the Collection of the Biology Department of the Shiraz University (ZM-CBSU), Iran.

TABLE 1. List of the 58 morphometric variables and abbreviations used in text.

Abbreviation	Morphometric characters	Abbreviation	Morphometric characters
TL/SL	Total length/Standard length	Daf/SL	Depth of anal fin/ Standard length
TL/Prad	Total length/Preanal distance	Daf/Prad	Depth of anal fin/ Preanal distance
HL/SL	Head length/Standard length	Prad/SL	Preanal distance/ Standard length
HL/Prad	Head length/ Preanal distance	Lpcf/SL	Length of pectoral fin/ Standard length
HD/SL	Head depth/ Standard length	Lpcf/Prad	Length of pectoral fin/ Preanal distance
HD/Prad	Head depth/ Preanal distance	Lplf/SL	Length of pelvic fin/ Standard length
HW/SL	Head width/ Standard length	Lplf/Prad	Length of pelvic fin/ Preanal distance
HW/Prad	Head width/ Preanal distance	Prplf/SL	Prepelvic fin/ Standard length
Prod/SL	Preorbital distance/ Standard length	Prplf /Prad	Prepelvic fin/ Preanal distance
Prod/Prad	Preorbital distance/ Preanal distance	Maxbd/SL	Maximum body depth/ Standard length
Pood/SL	Postorbital distance/ Standard length	Maxbd/Prad	Maximum body depth/ Preanal distance
Pood/Prad	Postorbital distance/ Preanal distance	Minbd/SL	Minimum body depth/ Standard length
Inod/SL	Interorbital distance/ Standard length	Minbd/Prad	Minimum body depth/ Preanal distance
Inod/Prad	Interorbital distance/ Preanal distance	Dbpcaf/SL	Distance between pectoral and anal fins/ Standard length
Inod/HW	Interorbital distance/Head width	Dbpcaf /Prad	Distance between pectoral and anal fins/ Preanal distance
ED/SL	Eye diameter/ Standard length	Dbpcplf/SL	Distance between pectoral and pelvic fins/ Standard length
ED/Prad	Eye diameter/ Preanal distance	Dbpcplf /Prad	Distance between pectoral and pelvic fins/ Preanal distance
ED/HL	Eye diameter/Head length	Dbplaf/SL	Distance between pelvic and anal fins/ Standard length
Prdd/Prad	Predorsal distance/ Preanal distance	Dbplaf /Prad	Distance between pelvic and anal fins/ Preanal distance
Prdd/SL	Predorsal distance/ Standard length	Lcauf/SL	Length of caudal fin/ Standard length
Podd/SL	Postdorsal distance/ Standard length	Lcauf/Prad	Length of caudal fin/ Preanal distance
Podd/Prad	Postdorsal distance/ Preanal distance	Lcaup/SL	Length of caudal peduncle/ Standard length
Prod/ED	Prorbital distance/Eye diameter	Lcaup/Prad	Length of caudal peduncle/ Preanal distance
Ldf/SL	Length of dorsal fin/ Standard length	Lcaup/Minbd	Length of caudal peduncle/Minimum body depth
Ldf/Prad	Length of dorsal fin/ Preanal distance	MW/SL	Mouth width/ Standard length
Ddf/SL	Depth of dorsal fin/ Standard length	MW/Prad	Mouth width/ Preanal distance
Ddf/Prad	Depth of dorsal fin/ Preanal distance	BW/SL	Body width/ Standard length
Laf/Prad	Length of anal fin/ Preanal distance	BW/Prad	Body width/ Preanal distance
Laf/SL	Length of anal fin/ Standard length	SL/Prad	Standard length/Preanal distance

Analysis of fish specimens. Based on the morphometry introduced in Holcik *et al.* (1989), 30 morphometric parameters were measured using a Vernier calliper and recorded to the nearest 0.5 mm. They describe the following characters: Total length (TL), standard length (SL), predorsal distance (Prdd), postdorsal distance (Podd), preanal distance (Prad), preorbital distance (Prod), postorbital distance (Pood), interorbital distance (Inod), eye diameter (ED), length of caudal peduncle (Lcaup), maximum body depth (Maxbd), minimum body depth (Minbd), head length (HL), head depth (HD), head width (HW), length of dorsal fin (Ldf), depth of dorsal fin (Ddf), length of anal fin (Laf), length of pectoral fin (Lpcf), length of pelvic fin (Lplf), prepectoral distance (Prpcd), prepelvic distance (Prpld), distance between pectoral and anal fins (Dbpcaf), distance between pectoral and pelvic fins (Dbpcplf), distance between pelvic and anal fins (Dbplaf), length of caudal fin (Lcauf), body width (BW), mouth width (MW), scale length (SL) and scale width (SW).

According to Lahnsteiner & Jagsch (2005), the values of selected measured morphometric parameters (= Mmp) were standardized in order to eliminate size effects. Standardization was achieved by using the standard length (Mmp/SL*100), preanal distance (Mmp/Prad*100), head length (Mmp/HL*100), head width (Mmp/HW*100), eye diameter (Mmp/ED *100) and minimum body depth (Minbd) (Mmp/ Minbd *100). We also calculated the caudal peduncle index (= length of caudal peduncle/minimum body depth = Lcaup/Minbd; see Doadrio *et al*. 2002). In total, 58 variables were calculated from the measurements (Table 1).

In addition, four normal scales from the left side of each fish from the 3rd or 4th row below the dorsal fin were removed. Scales were kept between two glass micro slides and length and width of scales were measured using a scale reader (Xerox 320) to the nearest 0.1 mm. For each fish, length and width measurements were averaged to obtain one length and width value per individual. To study potential size differences of scales between species, we calculated four J scale indices (after Esmaeili 2001): JSL/TL = scale length/total length*100; JSW/TL = scale width/total length*100; JSL/SL = scale length/standard length*100; JSW/SL = scale width/standard length*100.

The meristic characters were counted under a stereomicroscope and consist of the numbers of (i) gill rakers, (ii) lateral-line series scales, (iii) caudal-peduncle scales, (iv) flank bars of males, (v) pectoral, (vi) pelvic, (vii) dorsal and (viii) anal fin rays.

Statistical analyses. Univariate analysis of variance (ANOVA, with Duncan's post hoc test, $p< 0.05$) was used to test the significance of phenotypic differences among species and also between sexes (see below). The Canonical discriminant analysis (CDA) was used for multivariate analyses in order to show the classification success of the groups. The statistical analyses were carried out using PASW 18.00 (SPSS Inc, 2010) and PAST (Hammer *et al.* 2001: PAlaeontological STatistics, version 1.81).

Results

Variation between species. The four *Aphanius* species included in this study, *A. sophiae*, *A. pluristriatus*, *A. farsicus* and *A. isfahanensis*, exhibit a strong sex dimorphism, as is normal for *Aphanius* species. As a result, differences between females and males have to be studied in order to understand the variation between the species.

Results of morphometric data. Two variables are significantly different between the females of the four studied species (Duncan post hoc test, $p< 0.05$), i.e. the predorsal distance/standard length (Prdd/SL) and the mean value of the caudal peduncle index (Table 2A). In males, only the eye diameter/standard length (ED/SL) is different between all species. A clear separation of the four species, based either on females or males, is indicated by the CDA, which reveals a classification success of 98.6% (based on females) and 98.9% (based on males) (Fig. 2, Table 3).

Results of J scale indices. In comparison to *Aphanius sophiae*, *A. farsicus* and *A. isfahanensis*, JSL/TL and JSL/SL are significantly higher in both sexes of *A. pluristriatus*, while JSW/TL and JSW/SL are significantly higher only in males of *A. pluristriatus* (Duncan post-hoc test, $p< 0.05$; Table 4). These differences indicate the larger scale size of *A. pluristriatus*.

Results of meristic data. Numbers of gill rakers, lateral-line series scales, caudal-peduncle scales, and pectoral-fin rays are important characters for species discrimination based on females, while the number of gill rakers, flank bars and anal fin rays differs significantly between species if the data set from the males is used (ANOVA, Duncan post-hoc test, $p< 0.05$, Table 2). The separation power of the meristic characters is supported by the CDA, which reveals a classification success of 89.6% for females and 86.5% for males (data not shown).

The above-described analyses clearly indicate that *Aphanius pluristriatus* from the Zarjan spring system represents a distinct, valid species.

Re-validation and re-description of *Aphanius pluristriatus*. Jenkins (1910) listed as typical characters of *Aphanius pluristriatus*: "Height of body 3.5 times in length of head and 4 in total length inclusive of caudal. Body elevated and compressed, snout obtuse. Diameter of eye nearly equal to length of snout. Interorbital space about I2/3 diameter of eye. Origin of dorsal much nearer to root of caudal than to eye."

This description fits well with our observations. The interorbital distance (Inod, 2.55 mm) is clearly greater than the eye diameter (ED, 1.79 mm). Moreover, the original description can be complemented based on the caudal-peduncle index and gill-raker numbers. Small caudal-peduncle index values appear more frequent in *Aphanius pluristriatus* specimens than in *A. sophiae*, *A. farsicus* and *A. isfahanensis* (Table 2A). The gill-raker number is lower in *A. pluristriatus* than in the other species, ranging from 8–11 in both females and males (Table 2B). Furthermore, the percentage of specimens possessing low numbers of gill rakers is significantly higher in *A. pluristriatus* than in the other species (Table 2B, Duncan post-hoc test, $p < 0.05$).

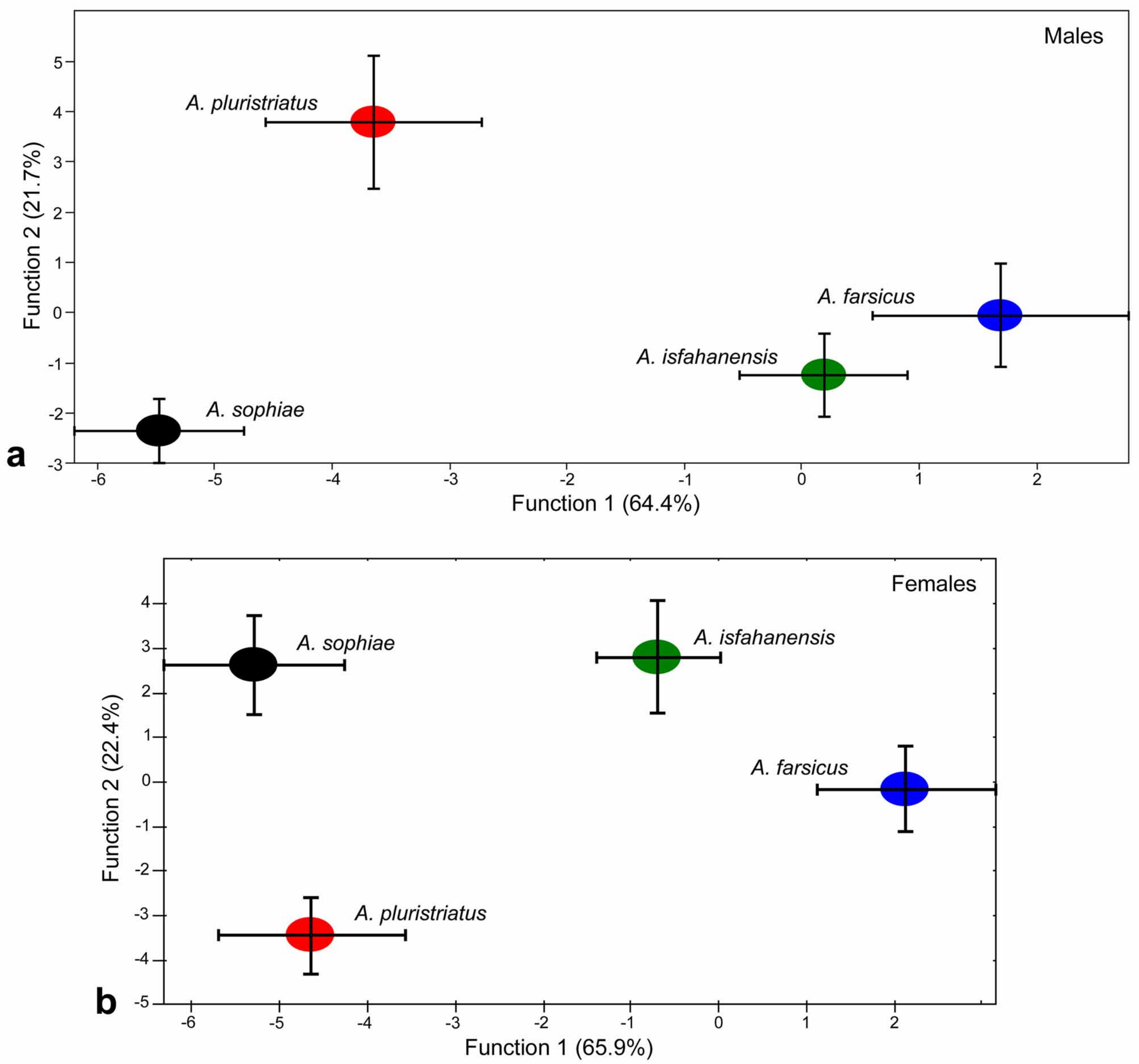

FIGURE 2. Group centroids (± S.D.) of discriminant function analyses (DFAs) for separation of the four studied *Aphanius* species based on all morphometric and meristic characters of males (a) and females (b). Numbers of flank bars are excluded from this analysis.

Color. Jenkins (1910) writes: "The head and body are of a dark brown colour [in spirit]. Along the sides of the body there is a number of vertical white stripes, running from the ventral surface to just below the dorsal. The number is greater than in *Cyprinodon persicus* [= *Aphanius farsicus*], being from 14 to 16. The operculum has a number of small brownish spots irregularly arranged. The fins are yellowish brown and, except the pectoral and pelvic, are white-edged. The lower edge of the pectoral is tinged with black."

The color description can be emended as follows: Males show vertical white flank stripes and bars that extend from the ventral side to just below the dorsal region. Numbers of flank bars are higher in *Aphanius pluristriatus* than in *A. sophiae*, *A. farsicus* and *A. isfahanensis* (Table 2C, Duncan post-hoc test, $p < 0.05$). Females do not possess flank bars, but may have thin or thick, dark, wavy and irregular vertical patches of pigments (Fig. 3a–b). In addition, females display an oval to lozenge-shaped spot at the central base of their caudal fin, which is often broken up into small spots and which has a high concentration of melanophores. Both sexes reveal an operculum with small and irregularly arranged spots (Fig. 3a–b).

TABLE 2. (A) Mean values and frequencies of caudal peduncle indices in the four studied *Aphanius* species. In *A. pluristriatus*, the caudal peduncle indices 1.2-1.4 and 1.4-1.6 are dominant (in bold). **(B)** Mean values and frequencies of gill raker numbers in the four studied *Aphanius* species. In *A. pluristriatus*, specimens with 10 gill rakers are most abundant (in bold). **(C)** Mean values and frequencies of flank bar numbers in the four studied *Aphanius* species. In *A. pluristriatus*, specimens with 12-16 flank bars are most abundant (in bold). N=total number of individuals.

(A) Caudal peduncle index				**1-1.2**	**1.2-1.4**	**1.4-1.6**	**1.6-1.8**	**1.8-2.0**	**2.0-2.2**				
	Species	N	Mean (±S.D.)	**Number of specimens, percentages in brackets**									
female	*A. sophiae*	35	**1.59** (±0.15)	0	3 (8.6)	**17 (48.6)**	**11 (31.4)**	4 (11.4)	0				
	A. pluristriatus	38	**1.43** (±0.12)	0	**16 (42.1)**	**20 (52.6)**	2 (5.3)	0	0				
	A. farsicus	78	**1.56** (±0.17)	2 (2.5)	8 (10.2)	**42 (53.8)**	**22 (28.2)**	3 (3.8)	1 (1.3)				
	A. isfahanensis	25	**1.62** (±0.13)	0	1 (4)	**10 (40)**	**13 (52)**	1 (4)	0				
male	*A. sophiae*	35	**1.38** (±0.15)	5 (14.3)	**15 (43)**	**12 (43)**	3 (8.6)	0	0				
	A. pluristriatus	32	**1.35** (±0.12)	3 (2.4)	**18 (56)**	**10 (31)**	1 (3.1)	0	0				
	A. farsicus	79	**1.52** (±0.18)	2 (2.5)	17 (21.5)	**35 (44.3)**	**19 (24)**	5 (6.3)	1 (1.26)				
	A. isfahanensis	18	**1.61** (±0.11)	0	0	**9 (50)**	**8 (44.4)**	1 (5.6)	0				
(B) Number of gill rakers				**8**	**9**	**10**	**11**	**12**	**13**				
female	*A.sophiae*	35	**10.71** (±0.78)	0	1 (2.9)	**14 (40)**	**14 (40)**	6 (17.1)	0				
	A. pluristriatus	38	**9.58** (±0.68)	2 (5.3)	**14 (36.8)**	**20 (52.6)**	2 (5.3)	0	0				
	A. farsicus	78	**10.43** (±0.80)	1 (1.28)	12 (15.4)	**19 (24.3)**	**41 (52.5)**	4 (5.1)	1 (1.3)				
	A. isfahanensis	25	**11.16** (±0.74)	0	0	4 (16)	**14 (56)**	**6 (24)**	1 (4)				
male	*A.sophiae*	35	**10.66** (±0.72)	0	1 (2.9)	**14 (40)**	**16 (45.7)**	4 (11.4)	0				
	A. pluristriatus	32	**9.84** (±0.67)	1 (3.1)	**7 (22)**	**20 (62.5)**	4 (12.5)	0	0				
	A. farsicus	79	**10.47** (±0.95)	2 (2.5)	13 (16.5)	**20 (25.3)**	**35 (44.3)**	7 (8.9)	2 (2.5)				
	A. isfahanensis	18	**10.78** (±0.54)	0	0	**5 (27.8)**	**12 (66.7)**	1 (5.6)	0				
(C) Number of flank bars				**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
male	*A. sophiae*	35	**11.89** (±1.53)	1 (2.9)	1 (2.9)	4 (11.4)	**7 (20)**	**10 (28.6)**	**7 (20)**	4 (11)	1 (3)	0	0
	A. pluristriatus	32	**13.84** (±1.71)	0	0	0	3 (9.4)	**5 (15)**	**6 (18.8)**	**6 (19)**	**5 (15)**	**6 (19)**	1 (3)
	A. farsicus	79	**11.56** (±1.32)	0	3 (3.8)	13 (16.5)	**20 (25.3)**	**29 (36.7)**	7 (8.9)	5 (6)	1 (1.2)	1 (1.2)	0
	A. isfahanensis	18	**10.67** (±0.91)	0	1 (5.6)	**7 (38.9)**	**8 (44.4)**	1 (5.6)	1 (5.6)	0	0	0	0

TABLE 3. Canonical discriminant analysis for females and males of the four studied *Aphanius* species based on 58 variables derived from morphometric data, with numbers and percentages of correctly classified cases. Overall classification success is 98.6% for females, and 98.9% for males. The correlation is 0.92 for function 1, and 0.84 for function 2 in females, and 0.92 for function 1, and 0.82 for function 2 in males, Lambda is 0.018 and 0.118 (in females) 0.021 and 0.133 (in males).

Species		Predicted Group Membership				
		A. sophiae	*A. pluristriatus*	*A. farsicus*	*A. isfahanensis*	Total
female	*A. sophiae*	35 **(100.0)**	0	0	0	35
	A. pluristriatus	0	38 **(100.0)**	0	0	38
	A. farsicus	0	0	75 (**98.3)**	3 (1.7)	78
	A. isfahanensis	0	0	1 (4.0)	24 **(96.0)**	25
males	*A. sophiae*	35 (**100.0)**	0	0	0	35
	A. pluristriatus	1 (3.2)	30 **(96.8)**	0	0	31
	A. farsicus	1 (0.6)	0	77 **(98.9)**	1 (0.6)	79
	A. isfahanensis	0	0	0	18 **(100.0)**	18

TABLE 4. Maximum and minimum values (in brackets mean value ± S.D.) of the J indices of the four studied *Aphanius* species. JSL.TL = scale length/total length*100; JSW.TL = scale width/total length*100; JSL.SL = scale length/standard length*100; JSW.SL = scale width/standard length*100. N = total number of individuals.

Species		N	JSL.TL	JSW.TL	JSL.SL	JSW.SL
females	*A. sophiae*	35	2.18–3.12 (2.65 ± 0.25)	2.21–3.37 (2.82 ± 0.30)	2.59–3.77 (3.14 ± 0.32)	2.62–4.07 (3.35 ± 0.37)
	A. pluristriatus	38	2.94–3.64 (3.31 ± 0.19)	2.49–4.51 (3.37 ± 0.32)	3.39–4.50 (3.93 ± 0.25)	2.83–5.45 (4.01 ± 0.41)
	A. farsicus	78	1.59–3.69 (2.75 ± 0.33)	2.00–4.18 (3.08 ± 0.42)	1.97–4.61 (3.34 ± 0.40)	2.45–4.97 (3.74 ± 0.51)
	A. isfahanensis	25	2.26–3.32 (2.70 ± 0.26)	2.59–4.15 (3.21 ± 0.36)	2.66–4.00 (3.24 ± 0.34)	3.14–4.96 (3.85 ± 0.45)
males	*A. sophiae*	35	2.23–3.15 (2.66 ± 0.28)	2.43–3.54 (2.89 ± 0.31)	2.68–3.74 (3.17 ± 0.33)	2.84–4.18 (3.44 ± 0.37)
	A. pluristriatus	32	3.05–3.78 (3.37 ± 0.16)	3.00–3.94 (3.47 ± 0.20)	3.77–4.53 (4.09 ± 0.19)	3.72–4.79 (4.22 ± 0.23)
	A. farsicus	79	1.84–3.64 (2.79 ± 0.34)	1.86–4.39 (3.18 ± 0.46)	2.31–4.51 (3.43 ± 0.40)	2.34–5.45 (3.91 ± 0.55)
	A. isfahanensis	18	2.25–2.92 (2.52 ± 0.19)	2.65–3.58 (3.07 ± 0.25)	2.70–3.52 (3.06 ± 0.23)	3.31–4.34 (3.37 ± 0.31)

Etymology. Jenkins (1910) proposed the name *pluristriatus* for this species because of the many stripes of the males. Here we propose as common and Farsi names: multi-striped (Mond) tooth-carp and Kapour-e-dandandar-e-Mond.

Distribution and conservation. At present, *A. pluristriatus* appears not to be present in the Zarjan spring, but it may still be found in a qanat system near Jahrom city, which also belongs to the Mond River drainage system (unpublished data of the authors). However, drought and introduction of exotic fishes (e.g. *Xiphophorus hellerii*, Esmaeili *et al.* 2010) are major threats for the survival of this species.

Discussion

Taxonomic significance of characters. *Aphanius* species in Iran and adjacent countries show a distinctive pigmentation that can be used to identify and discriminate between species. Otolith morphology and morphometry represent other useful characters for the identification of *Aphanius* species (Reichenbacher *et. al.* 2007, 2009a,b), but are poorly known for the endemic species from Iran. In contrast, morphometric and meristic characters generally overlap, and it has been suggested in previous studies that these characters are only useful in multivariate space for species separation (Coad 2006, 2009, 2011; Hrbek *et al.* 2006).

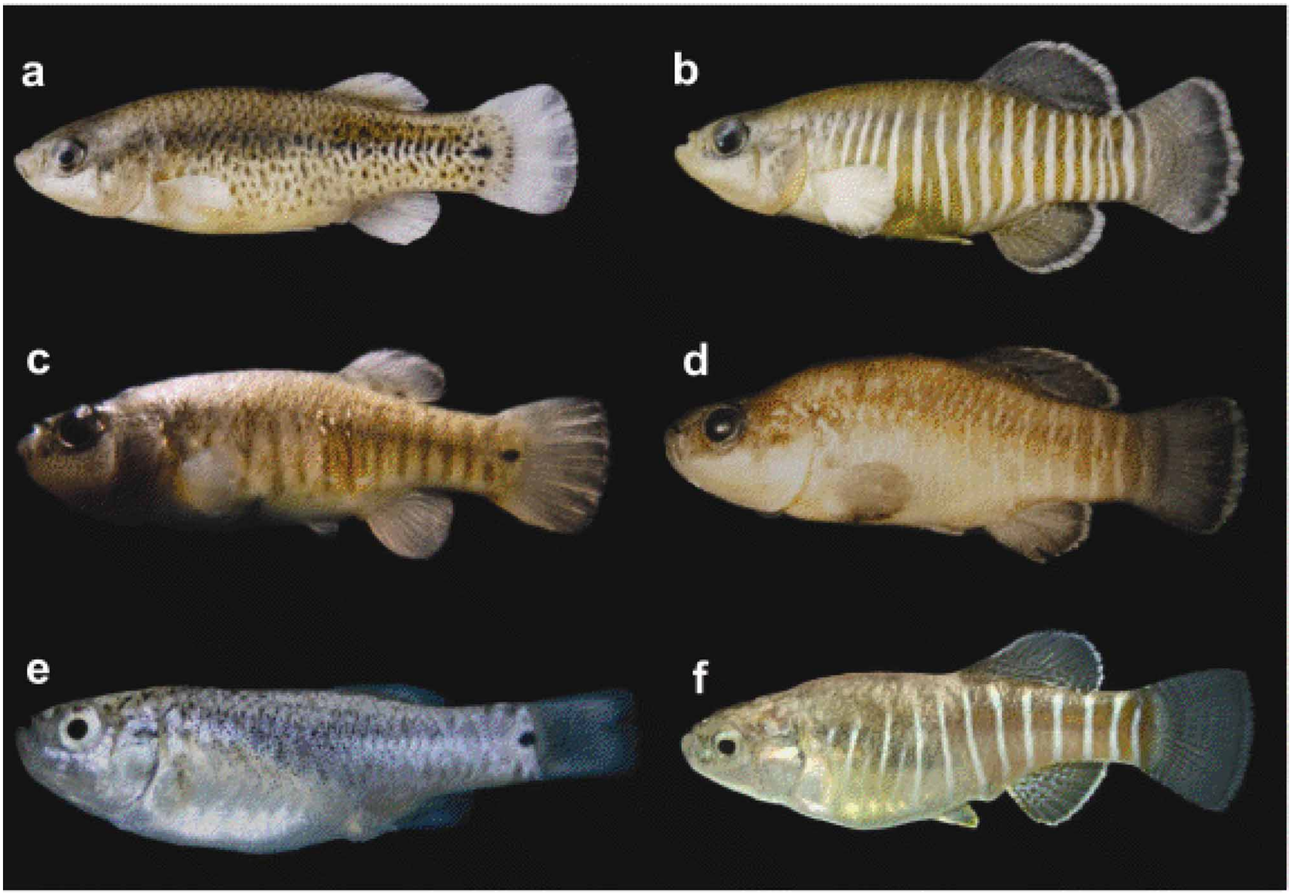

FIGURE 3. Photos of the studied *Aphanius* species from southern Iran: a-b, *A. pluristriatus* (a, female; b, male); c-d, *A. farsicus* (c, female, d, male); e-f, *A. sophiae* (e, female, f, male).

Our data indicate that in the case of *Aphanius sophiae*, *A. pluristriatus*, *A. farsicus*, and *A. isfahanensis* specific phenotypic characters, i.e. the caudal-peduncle index, numbers of gill rakers and flank bars and also J indices, are important diagnostic characters. Some of these phenotypic characters have already been demonstrated to be useful for the identification of other *Aphanius* species such as *A. baeticus* (see Doadrio *et al*. 2002), *A. saourensis* (see Blanco *et al.* 2006), *A. isfahanensis* (see Hrbek *et al*. 2006), and *A. mesopotamicus* (see Coad 2009).

Zoogeography. According to Hrbek *et al*. (2006), *Aphanius isfahanensis* is sister to *A. farsicus* and *A. sophiae* (*A. pluristriatus* was not included in that study) and *A. isfahanensis* diverged from the *A. sophiae* + *A. farsicus* lineage at approximately 4.8 million years ago (early Pliocene). It remains unknown, however, when *A. sophiae* and *A. farsicus* diverged from each other.

Here we suggest that *Aphanius pluristriatus* diverged from an *Aphanius* population in the ancient Kor River (see Figs. 1, 4) due to a prominent re-organisation of the drainage systems (Kehl *et al.* 2009). During the Quaternary, a "Paleo-Kor River" (crossing the type locality of *A. pluristriatus*), connected the Kor River with the Mond River and drained the Kor River to the Persian Gulf (Fig. 4). In the Late Quaternary or later, this drainage of the Kor River was closed due to the strong tectonic uplift of the Zagros Mountains (Nadji 1997), and the present-day endorheic Kor-River Basin with the Bakhtegan and Tashk Lakes formed (Kehl *et al*. 2009). The timing of the Paleo-Kor River closure can be Late Quaternary (20,000–10,000 years ago, Nadji 1997) or Holocene (6,000–2,000 years ago, Löffler 1959). Since that time the connection between the *Aphanius* populations in the Kor River Basin and those in the Mond River drainage system has been blocked and the populations began to diverge. The present-day *A. pluristriatus* is thus a relict of the ancient *Aphanius* population of the Paleo-Kor River drainage (see Fig. 4).

The re-validation of *Aphanius pluristriatus* increases the number of endemic *Aphanius* species reported from Iran to seven. A similarly high species diversity is only found in Anatolia, where it is related to a comparable complex geological history as in southern and central Iran (Hrbek *et al*. 2002). The geological events caused rapid isolation of mountainous areas and subsequent fragmentation of populations, which gave rise to the evolution of new species. Speciation processes similar to that observed for *Aphanius* have also been described for *Pseudophoxinus* (Pisces, Cypriniformes) and salamandrids (Amphibia) from Anatolia (Hrbek *et al.* 2004; Weisrock *et al.* 2001). Moreover, the individual geological histories of the endorheic (e.g. Kor, Maharlu) and exorheic basins (Mond, Persian Gulf) of the Fars area (Zagros mountains), as detailed by Ramsey *et al.* (2008) and Kehl *et al*. (2009), may have affected speciation and thus contributed to the unusually high *Aphanius* species diversity in this region.

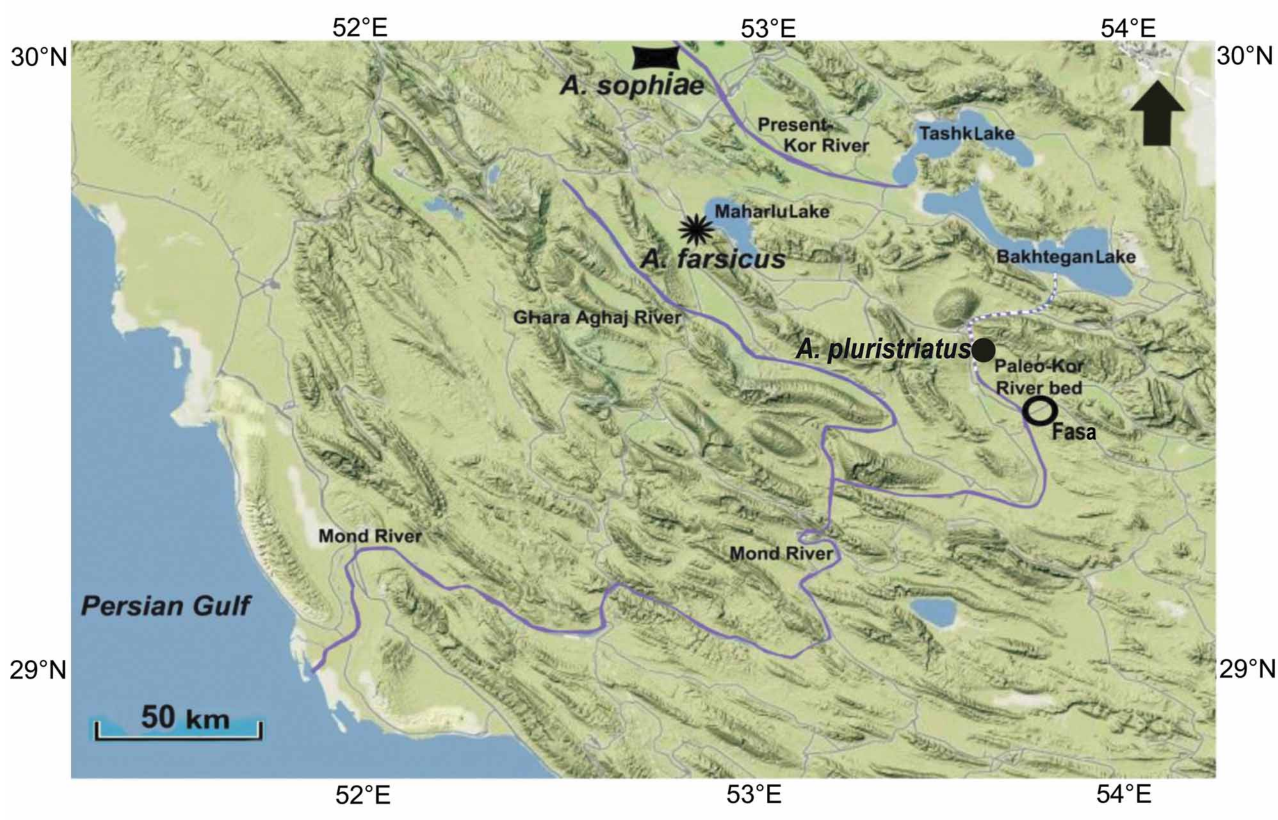

FIGURE 4. Geographic distribution of the studied *Aphanius* species in southern Iran and location of Fasa, which is near the type locality of *A. pluristriatus*. The ancient Paleo-Kor River (dotted line) and the present Kor River were connected until the end of the Quaternary. Then the connection was blocked and the endorheic Kor Basin with the Bakhtegan and Tashk Lakes developed (see text for details and references). Source of map: Google Earth; modified.

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