

New Species of *Aphanius* (Teleostei, Cyprinodontidae) from Isfahan Province of Iran and a Reanalysis of Other Iranian Species

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A new killifish species, *Aphanius isfahanensis*, is described from the Isfahan basin of Iran. It is distinguished from the other Iranian species of *Aphanius* by adult color pattern, molecular character states of mitochondrial DNA sequence data, and in multivariate morphometric and meristic space. Based on the phylogenetic analysis of molecular sequence data, the new species is hypothesized to be sister taxon to *A. sophiae* plus *A. persicus*, which also occur in Iran.

THE extant and extinct species of the killifish genus *Aphanius* Nardo, 1827 (Cyprinodontiformes) are widely distributed along the late period Tethys Sea coastlines. The Tethys Sea closed at the Oligocene/Miocene boundary (Smith et al., 1995). The distribution includes coastal areas of the Mediterranean region and the Gir Peninsula of northwestern India to northeastern Somalia, including the Red Sea and the Persian Gulf. Inland distribution is restricted primarily to the Mediterranean and the Near East orogenic belt, including Turkey and Iran. Central Anatolia, Turkey, has been suggested to be the center of diversity of *Aphanius* (Wildekamp et al., 1999). However, high diversity appears to extend from Turkey to Iran and is observed in many faunal elements including cyprinodontoid killifishes (Hrbek et al., 2002), leuciscine cyprinids (Hrbek et al., 2004), true salamanders (Weisrock et al., 2001), and agamid lizards (Macey et al., 1998, 2000).

Distribution and taxonomy of *Aphanius* in Anatolia has been subject to several studies (e.g., Villwock, 1964; Scholl et al., 1978), and an overview of *Aphanius* in Turkish waters, including a discussion on the generic name and a review of the available literature, is given in Wildekamp et al. (1999). In contrast to Turkey, little work has been done on Iranian populations of *Aphanius*; however, basic data on reproduction, alimentation, and habitat of *A. vladkovi* exist (Keivany and Soofiani, 2004). Recent studies (Coad, 1996, 2000) show that, in addition to the three recognized species of central Iran, other populations may deserve species recognition. Specimens from these allopatrically distributed populations in isolated internal basins sometimes show color pattern, meristic, or morphometric differences from other populations of *Aphanius*.

The occurrence of marked genetic divergence among Iranian populations that may or may not be morphologically distinct resembles the pattern found in the central Anatolian *Aphanius*

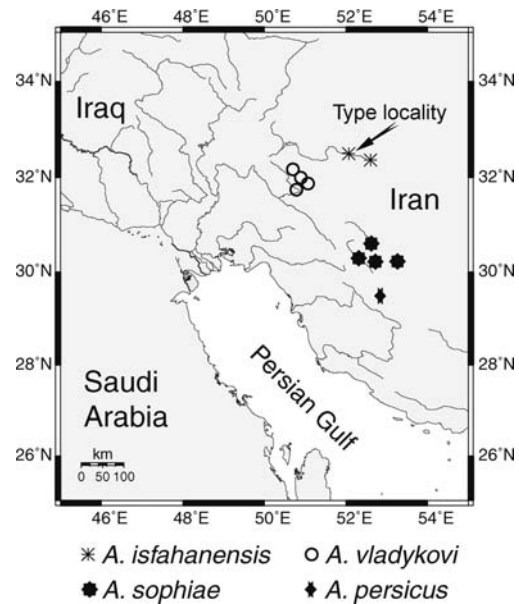


Fig. 1. Map of collections of Iranian *Aphanius* made for this study. Base map was created at Online Map Creations <http://www.aquarius.geomar.de/omc/>.

anatoliae species complex (Hrbek et al., 2002). We therefore suspect that Iran has a much greater ichthyofaunal diversity than currently recognized and that this diversity is organized in accordance with the geological units comprising Iran.

In 2002, we made collections of *Aphanius* from the Zagros Mountains of Iran, which harbor three known species: *A. vladkovi*, *A. sophiae*, and *A. persicus*. We also collected, from the Zayandeh River in the vicinity of Isfahan, Iran (Fig. 1), a suspected new species of *Aphanius* that differs in pigmentation pattern from all other Iranian species. We obtained morphometric, meristic, molecular, and distributional data, ultimately concluding that the Zayandeh River basin contains a species previously unknown to science.

MATERIALS AND METHODS

Measurements and counts follow Coad (1988, 1996). A set of 20 standardized measurements (Table 1) and eight counts (Table 2) was collected for all specimens. Measurements, including sub-units of head, are presented as percentages of standard length (SL) in Table 2. Cross-bar count (Table 2) was taken by counting the number of dark bars (males only) on the mid-lateral series of scales on the left side. To avoid potentially confounding effects of allometry (Atchley et al., 1976), the raw morphometric data were log-transformed and then transformed into residuals using the least-squares regression method (Sokal and Rohlf, 1994). Meristic data were analyzed untransformed. The morphometric residuals and meristic characters were analyzed using General Linear Model (GLM) as implemented in SYSTAT 10.2.05 (Systat Software, Inc.), where species and sex and their interaction effect were the independent variables. Multivariate data were further summarized and visualized using the principal component analysis (PCA) implemented in SYSTAT 10.2.05 (Systat Software, Inc.). The PCA of morphometric data were performed for each sex separately, but sexes were analyzed jointly in meristic PCA. Institutional abbreviations follow Leviton et al. (1985) with the addition of CMNFi, Canadian Museum of Nature, Ottawa, Canada.

Molecular analyses.—We obtained mitochondrial DNA sequence data from 18 individuals representing four species. *Aphanius asquamatus*, *A. fasciatus*, and the central Anatolian *Aphanius anatoliae* species complex were used as the outgroup data set. Choice of outgroup taxa was based on previous molecular studies of *Aphanius* (Hrbek et al., 2002; Hrbek and Meyer, 2003). DNA sequences included genes encoding a portion of 12S ribosomal RNA, complete sequences of NADH1 and NADH2, transfer RNAs coding for valine, leucine, isoleucine, glutamine, methionine, tryptophan, alanine, asparagine, cysteine, and tyrosine, and the light-strand replication origin. Amplification and sequencing primers and sequencing strategy are described by Hrbek and Meyer (2003).

In all instances, the two individuals sequenced per population were identical or nearly identical; therefore, we randomly selected one of the two individuals for all subsequent analyses. Maximum parsimony and maximum likelihood phylogenetic relationships were estimated using the program PAUP* v4.10b (D. L. Swofford, PAUP*: phylogenetic analysis using parsimony [*and other methods], Sinauer, Sunderland, MA,

2002). Bayesian likelihood phylogenetic relationships were estimated in the program MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003). Maximum likelihood estimates were done under the GTR model of evolution (Rodriguez et al., 1990) with rates for variable sites assumed to follow the gamma distribution and with a portion of sites assumed to be invariable; this model of molecular evolution was indicated by the program MODELTEST (Posada and Crandall, 1998) as best fitting the data. Sequence data for new Iranian populations have been deposited in GenBank under numbers AY593481–AY593498. Previously published sequence information is from Hrbek and Meyer (2003). Aligned sequences are available from the first author.

Aphanius isfahanensis, new species

کپوردندان اصفهان

Kapourdandan-e Isfahan (Farsi)

Figure 2A, B

Holotype.—CMNFi2004-0001, male, 25.0 mm SL, Iran, Isfahan Province, Zayandeh Rud (Zayandeh River) at Varzaneh Bridge, 32°25'32"N, 52°39'14"E, 1 July 2002, Y. Keivany and S. Asadollah.

Paratypes.—CMNFi2004-0002, 18 males, 20.8–30.9 mm SL, 18 females, 22.0–38.4 mm SL, of 49 total (13 not used in meristic and morphometric analyses), same locality as holotype; AMNH 233639, 1 male, 25.2 mm SL, 1 female, 21.6 mm SL, same locality as holotype; MRAC 2004-05-P-01-02, 1 male, 25.2 mm SL, 1 female, 21.6 mm SL, same locality as holotype; GenBank accession numbers AY593488, AY593489, AY593497, and AY593498.

Diagnosis.—*Aphanius isfahanensis* is clearly distinguished at the genetic level from all other species of *Aphanius*. It has 82 molecular apomorphies—19 transversions, two transversions/transitions (depending on comparison), and 61 transitions—that show fixed character state differences to homologous characters analyzed in *A. sophiae*, *A. persicus*, and *A. vladikovii* from Iran (Table 3). Thirty-seven of these character states are also apomorphies when compared to *A. anatoliae*, *A. danfordii*, *A. villwocki*, *A. asquamatus*, and *A. fasciatus* from Turkey. Alignment with apomorphies highlighted in yellow is available at www.cyprinodontiformes.org.

Males of *A. isfahanensis* (Fig. 2a) can be distinguished from those of all other Iranian species by having distinct black edge on the dorsal, anal, and pelvic fins. The dorsal fin is

TABLE 1. MORPHOMETRIC CHARACTERS OF THE HOLOTYPE AND ADDITIONAL SPECIES OF *Aphanius* OF THE EASTERN CLADE (HREBEK AND MEYER, 2003) FROM IRAN. n = number of specimens; each cell contains mean \pm standard deviation and range (minimum–maximum). m = male; f = female; * = significant pair-wise difference between *A. isfahanensis* and *A. sophiae*; \dagger = significant pair-wise difference between *A. isfahanensis* and *A. persicus*; \ddagger = significant pair-wise difference between *A. isfahanensis* and *A. vladychkovi*; cut-off $P = 0.0025$.

| Character | Holotype ♂ | <i>A. isfahanensis</i> ♂ ($n = 18$) | <i>A. isfahanensis</i> ♀ ($n = 19$) | <i>A. sophiae</i> ♂ ($n = 40$) | <i>A. sophiae</i> ♀ ($n = 34$) | <i>A. persicus</i> ♂ ($n = 177$) | <i>A. persicus</i> ♀ ($n = 228$) | <i>A. vladychkovi</i> ♂ ($n = 36$) | <i>A. vladychkovi</i> ♀ ($n = 16$) |
|---|------------|--|--|-------------------------------------|-------------------------------------|---------------------------------------|---------------------------------------|---|---|
| Standard length | 25 | 25.8 \pm 3.29 (20.8–30.9) | 29.2 \pm 5.36 (22.0–38.4) | 26.7 \pm 4.49 (18.1–39.3) | 29.2 \pm 4.84 (20.0–42.0) | 22.8 \pm 3.88 (15.9–33.1) | 26.0 \pm 5.79 (15.7–45.9) | 27.0 \pm 4.53 (21.6–36.6) | 29.1 \pm 5.13 (23.5–40.2) |
| Body depth | 31.60 | 32.05 \pm 1.91 (28.82–36.49) | 29.98 \pm 2.81 (25.41–35.94) | 31.25 \pm 2.36 (25.97–36.66) | 29.71 \pm 2.37 (25.95–36.48) | 32.06 \pm 2.06 (27.32–37.92) | 30.88 \pm 1.75 (26.18–37.00) | 33.18 \pm 1.70 (29.30–36.16) | 31.94 \pm 1.18 (30.12–33.83) |
| Head depth ^{ms,mv,fp} | 27.20 | 27.03 \pm 1.40 (23.97–30.07) | 25.04 \pm 1.06 (23.43–27.37) | 24.70 \pm 1.27 (22.55–27.44) | 23.84 \pm 1.63 (21.20–28.34) | 26.54 \pm 1.52 (23.39–30.64) | 25.91 \pm 1.44 (22.34–30.25) | 25.70 \pm 1.04 (23.48–28.62) | 25.18 \pm 0.94 (23.94–27.11) |
| Head length ^{ms,fp,lv} | 30.40 | 31.20 \pm 1.19 (29.45–34.31) | 29.24 \pm 0.81 (27.94–31.08) | 29.08 \pm 1.00 (27.74–31.28) | 28.16 \pm 1.47 (25.26–31.00) | 30.90 \pm 1.24 (27.43–34.34) | 30.70 \pm 1.49 (27.05–34.56) | 31.13 \pm 0.94 (29.00–32.88) | 30.48 \pm 1.16 (28.96–33.08) |
| Head width ^{ms} | 20.40 | 20.90 \pm 1.03 (19.42–22.55) | 21.14 \pm 0.60 (20.06–22.26) | 18.87 \pm 0.88 (17.12–21.20) | 19.07 \pm 1.30 (15.74–21.50) | 20.37 \pm 1.07 (17.94–24.26) | 20.77 \pm 1.47 (17.87–25.81) | 20.36 \pm 0.73 (18.83–21.88) | 20.84 \pm 0.85 (19.69–23.13) |
| Snout length ^{ms,mv} | 8.00 | 8.59 \pm 0.61 (7.92–10.46) | 8.12 \pm 0.66 (7.19–9.60) | 7.47 \pm 0.43 (6.63–8.40) | 7.44 \pm 0.63 (6.44–9.15) | 8.28 \pm 0.81 (6.36–10.44) | 8.34 \pm 0.94 (6.12–12.04) | 8.12 \pm 0.55 (7.33–9.32) | 7.95 \pm 0.72 (7.23–9.95) |
| Mouth width ^{ms,mv,fs} | 10.40 | 10.81 \pm 0.87 (9.59–12.68) | 10.59 \pm 0.78 (8.92–11.72) | 9.32 \pm 0.71 (8.36–11.45) | 9.07 \pm 0.92 (7.48–11.49) | 11.05 \pm 1.07 (8.44–14.87) | 11.27 \pm 1.47 (8.33–17.65) | 9.86 \pm 0.68 (8.54–11.56) | 9.89 \pm 0.82 (8.75–11.44) |
| Orbit diameter ^{ms,fp,lv} | 9.20 | 9.89 \pm 0.64 (8.74–11.21) | 8.68 \pm 0.58 (7.69–9.70) | 9.18 \pm 0.56 (8.33–10.13) | 8.82 \pm 0.48 (7.87–9.77) | 10.05 \pm 0.57 (8.55–11.60) | 9.40 \pm 0.59 (7.91–11.88) | 9.91 \pm 0.62 (8.67–11.34) | 9.52 \pm 0.58 (8.46–10.42) |
| Interorbital width ^{mp,fp} | 11.60 | 12.20 \pm 0.68 (11.11–13.70) | 11.90 \pm 0.87 (10.16–13.54) | 11.81 \pm 0.57 (11.00–13.22) | 11.20 \pm 0.75 (9.80–13.03) | 12.71 \pm 0.70 (10.78–15.15) | 12.49 \pm 0.83 (10.19–15.16) | 12.40 \pm 0.73 (10.99–13.74) | 12.18 \pm 0.77 (10.94–13.62) |
| Postorbital length ^{mp,mv,fp,lv} | 13.60 | 13.46 \pm 0.57 (11.87–14.53) | 13.65 \pm 0.71 (12.50–15.41) | 13.31 \pm 0.76 (11.68–14.66) | 13.08 \pm 0.91 (11.84–14.92) | 13.75 \pm 1.00 (11.54–16.36) | 14.32 \pm 1.24 (11.64–18.01) | 14.81 \pm 0.88 (13.33–16.47) | 14.79 \pm 1.18 (12.92–17.91) |
| Predorsal length ^{mp,mv,fp,lv} | 59.60 | 58.35 \pm 1.71 (54.71–60.75) | 61.38 \pm 2.27 (57.14–64.60) | 59.07 \pm 1.81 (55.74–62.82) | 60.10 \pm 1.52 (57.74–63.19) | 60.96 \pm 1.39 (56.71–64.60) | 62.14 \pm 1.63 (57.38–66.43) | 64.99 \pm 1.47 (62.17–67.83) | 65.68 \pm 1.51 (62.81–69.15) |
| Dorsal length ^{mv} | 27.60 | 28.03 \pm 1.70 (23.61–31.05) | 22.53 \pm 0.99 (20.62–24.59) | 29.15 \pm 2.63 (23.20–33.92) | 23.22 \pm 1.78 (20.75–27.06) | 28.75 \pm 2.46 (22.16–34.41) | 23.32 \pm 1.48 (19.62–26.54) | 25.34 \pm 1.58 (21.90–29.90) | 21.57 \pm 1.08 (20.00–23.40) |
| Prepelvic length ^{ls,fp} | 53.60 | 54.15 \pm 3.06 (50.00–59.87) | 57.10 \pm 2.65 (51.36–62.16) | 52.56 \pm 1.91 (49.03–57.14) | 53.37 \pm 2.52 (48.93–59.52) | 54.03 \pm 1.84 (48.40–59.51) | 55.00 \pm 2.05 (49.31–63.99) | 54.50 \pm 1.38 (51.84–57.34) | 54.97 \pm 1.35 (52.77–58.46) |
| Prealanal length ^{ls} | 64.40 | 65.72 \pm 2.33 (62.04–69.93) | 69.85 \pm 1.99 (65.77–73.02) | 65.90 \pm 1.41 (63.18–68.55) | 67.29 \pm 1.87 (64.56–70.57) | 66.68 \pm 1.83 (61.75–72.54) | 68.25 \pm 2.02 (63.59–78.78) | 66.26 \pm 4.05 (43.33–69.23) | 68.47 \pm 0.99 (66.38–70.00) |
| Anal length ^{mv} | 22.40 | 22.65 \pm 1.26 (20.49–24.84) | 19.16 \pm 1.11 (16.80–20.83) | 23.07 \pm 1.60 (19.35–26.67) | 19.59 \pm 1.30 (16.82–22.44) | 22.51 \pm 1.61 (18.05–26.98) | 23.32 \pm 1.48 (19.62–26.54) | 21.21 \pm 1.08 (18.18–24.44) | 19.38 \pm 1.05 (17.89–22.13) |
| Caudal peduncle length ^{mp,mv} | 25.20 | 25.33 \pm 0.91 (23.65–27.54) | 23.59 \pm 1.46 (20.36–25.77) | 24.74 \pm 1.32 (22.34–27.48) | 24.27 \pm 0.93 (22.41–25.87) | 23.68 \pm 1.02 (20.65–26.41) | 23.08 \pm 1.22 (18.37–26.70) | 23.23 \pm 1.03 (21.54–25.48) | 22.65 \pm 0.63 (21.57–24.21) |

TABLE 1. CONTINUED.

| Character | Holotype ♂ | <i>A. isfahanensis</i> ♂ (n = 18) | <i>A. isfahanensis</i> ♀ (n = 19) | <i>A. sophiae</i> ♂ (n = 40) | <i>A. sophiae</i> ♀ (n = 34) | <i>A. persicus</i> ♂ (n = 177) | <i>A. persicus</i> ♀ (n = 228) | <i>A. vladychkovi</i> ♂ (n = 36) | <i>A. vladychkovi</i> ♀ (n = 16) |
|--|------------|--------------------------------------|--------------------------------------|---------------------------------|---------------------------------|-----------------------------------|-----------------------------------|-------------------------------------|-------------------------------------|
| Caudal peduncle depth | 17.20 | 17.75 ± 0.94 (16.10–19.59) | 15.30 ± 0.85 (13.84–16.73) | 17.39 ± 1.29 (15.08–19.92) | 15.93 ± 0.85 (14.21–17.62) | 16.97 ± 1.23 (13.36–20.08) | 15.43 ± 1.00 (13.11–18.44) | 17.31 ± 0.82 (15.51–19.21) | 15.78 ± 0.48 (15.09–16.92) |
| Pectoral fin length ^{ms,fp} | 17.20 | 17.23 ± 1.21 (14.84–18.96) | 15.79 ± 1.12 (13.06–17.84) | 18.23 ± 1.19 (16.02–21.59) | 16.87 ± 1.41 (14.64–20.13) | 19.26 ± 1.39 (15.49–23.48) | 17.23 ± 1.37 (13.58–21.76) | 17.80 ± 0.85 (15.84–19.91) | 16.23 ± 1.13 (13.11–18.16) |
| Pelvic fin length ^{ms,mp,fs,fp} | 6.40 | 6.89 ± 0.82 (5.71–8.45) | 6.80 ± 0.61 (5.65–8.09) | 9.78 ± 1.05 (6.63–12.00) | 8.82 ± 0.87 (7.33–10.56) | 7.88 ± 1.13 (2.35–11.93) | 7.59 ± 0.94 (4.63–10.45) | 7.48 ± 0.77 (5.63–9.04) | 7.21 ± 1.14 (5.42–9.51) |
| Pectoral–pelvic fin distance ^{ms,mp,ms,fp,lv} | 25.20 | 24.73 ± 1.86 (21.49–28.62) | 28.18 ± 2.39 (23.64–31.20) | 22.80 ± 1.47 (18.29–25.19) | 25.25 ± 2.96 (20.17–34.18) | 22.63 ± 1.49 (18.77–28.57) | 24.29 ± 2.02 (19.43–31.22) | 23.33 ± 1.30 (20.44–26.01) | 25.10 ± 1.22 (22.55–27.22) |
| Pelvic–anal fin distance ^{ms,mp} | 10.80 | 11.26 ± 1.33 (8.17–14.02) | 13.33 ± 0.79 (12.31–14.77) | 13.35 ± 1.25 (10.50–15.97) | 13.80 ± 1.55 (9.48–17.17) | 12.26 ± 1.14 (9.27–16.92) | 12.94 ± 1.18 (10.27–16.48) | 12.44 ± 1.05 (10.17–15.11) | 13.95 ± 1.01 (12.45–15.94) |

TABLE 2. MERISTIC CHARACTERS OF THE HOLOTYPE AND ADDITIONAL SPECIES OF *Aphanius* OF THE EASTERN CLADE (HRBEK AND MEYER, 2003) FROM IRAN. n = number of specimens; each cell contains mean ± standard deviation and range (minimum–maximum). ^s = significant pair-wise difference between *A. isfahanensis* and *A. sophiae*; ^p = significant pair-wise difference between *A. isfahanensis* and *A. persicus*; ^v = significant pair-wise difference between *A. isfahanensis* and *A. vladychkovi*; cut-off P = 0.0063.

| Character | Holotype ♂ | <i>A. isfahanensis</i> ♂ (n = 18) | <i>A. isfahanensis</i> ♀ (n = 19) | <i>A. sophiae</i> ♂ (n = 40) | <i>A. sophiae</i> ♀ (n = 34) | <i>A. persicus</i> ♂ (n = 177) | <i>A. persicus</i> ♀ (n = 228) | <i>A. vladychkovi</i> ♂ (n = 36) | <i>A. vladychkovi</i> ♀ (n = 16) |
|-----------------------------------|------------|--------------------------------------|--------------------------------------|---------------------------------|---------------------------------|-----------------------------------|-----------------------------------|-------------------------------------|-------------------------------------|
| Gill rakers ^{sp,v} | 13 | 12.2 ± 0.62 (11–13) | 12.3 ± 0.89 (10–13) | 11.4 ± 0.70 (10–13) | 11.3 ± 0.72 (10–12) | 11.2 ± 0.73 (9–14) | 11.2 ± 0.86 (9–14) | 10.9 ± 0.52 (10–12) | 11.0 ± 0.37 (10–12) |
| Lateral-line scales ^{sv} | 28 | 26.8 ± 1.10 (25–28) | 27.4 ± 0.77 (26–29) | 28.3 ± 1.26 (27–31) | 28.6 ± 1.07 (25–31) | 26.9 ± 1.01 (24–29) | 27.1 ± 1.06 (24–30) | 39.4 ± 2.35 (36–47) | 39.0 ± 1.86 (36–43) |
| Precaudal vertebrae ^v | 11 | 11.4 ± 0.61 (10–12) | 12.0 ± 0.58 (11–13) | 12.0 ± 0.66 (11–13) | 12.0 ± 0.58 (11–13) | — | — | 12.0 ± 0.69 (10–13) | 12.2 ± 0.40 (12–13) |
| Caudal vertebrae | 17 | 16.2 ± 0.71 (15–17) | 15.9 ± 0.88 (15–18) | 16.3 ± 0.63 (15–17) | 16.3 ± 0.63 (15–17) | — | — | 16.5 ± 0.56 (15–17) | 16.3 ± 0.45 (16–17) |
| Pectoral-fin rays ^s | 16 | 15.4 ± 0.62 (14–16) | 15.1 ± 0.91 (13–16) | 16.0 ± 0.82 (14–18) | 16.1 ± 1.04 (15–19) | 15.0 ± 0.79 (13–17) | 15.0 ± 0.85 (11–18) | 15.7 ± 0.78 (14–17) | 15.4 ± 0.81 (14–17) |
| Pelvic-fin rays ^{sv} | 6 | 5.3 ± 0.58 (4–6) | 5.5 ± 0.51 (5–6) | 5.8 ± 0.41 (5–6) | 5.6 ± 0.49 (5–6) | 5.4 ± 0.59 (3–6) | 5.5 ± 0.54 (4–6) | 5.9 ± 0.52 (5–8) | 5.9 ± 0.50 (5–7) |
| Dorsal-fin rays ^s | 13 | 12.2 ± 0.71 (11–14) | 12.1 ± 0.88 (11–14) | 13.1 ± 0.85 (12–15) | 13.0 ± 0.76 (11–15) | 11.9 ± 0.86 (8–14) | 12.0 ± 0.77 (10–14) | 12.5 ± 0.61 (11–14) | 12.4 ± 0.81 (11–13) |
| Anal-fin rays ^{sp,v} | 12 | 11.2 ± 0.51 (10–12) | 11.3 ± 0.67 (10–13) | 11.8 ± 0.79 (10–13) | 11.8 ± 0.64 (11–13) | 10.9 ± 0.71 (9–13) | 10.9 ± 0.69 (9–13) | 12.1 ± 0.72 (11–13) | 11.9 ± 0.77 (11–13) |
| Male flank bars ^{sv} | 11 | 10.2 ± 0.99 (8–12) | — | 14.4 ± 2.52 (10–21) | — | — | — | 11.5 ± 1.48 (9–14) | — |

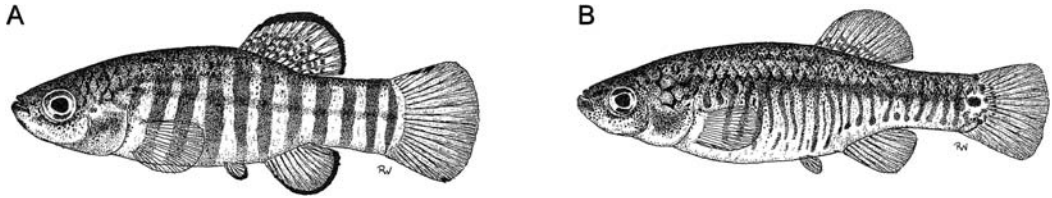


Fig. 2. (A) *Aphanius isfahanensis*, male holotype, 25.0 mm SL. (B) *Aphanius isfahanensis*, female paratype, 38.3 mm SL from the type locality.

covered with a high density of black blotches. However, this color pattern is also observed in males of *Aphanius anatoliae* from Turkey.

Females of *A. isfahanensis* (Fig. 2b) can be distinguished from females of *A. sophiae* and *A. vladykovi* by having flank-bars rather than spots. It can also be distinguished from *A. persicus* by less well-defined bars terminating at a mid-flank stripe and a relatively light gray stripe at the caudal-fin base rather than a black spot or blotch. Flank-bars are also characteristic of females of *Aphanius dispar* and *Aphanius fasciatus*, however.

Aphanius isfahanensis can be distinguished unambiguously only from *A. vladykovi* by a lower lateral-line scale count. It cannot be distinguished unambiguously by any morphometric or meristic character from *A. sophiae* or *A. persicus*.

Description.—Morphometric and meristic characters are summarized in Tables 1 and 2 and Figure 3. Snout rounded, 26% head length. Lower jaw directed upward at 30° angle, posterior tip of maxilla halfway between snout and edge of orbit. Head depth and head length is on average relatively larger, but head width relatively smaller than in other Iranian species. Eye 30% of head length, and in males the eye relatively smaller than in other Iranian species. Body depth approximately 32% body length. Head profile straight and dorsal profile rounded. Anal fin with superior border curved, pectoral fin rounded and inserted at below midline of body, ventral fins short and inserted just anterior to anal fin, caudal fin rounded. Total vertebrae 28. Males bulky and high-bodied, reaching 31 mm SL. Greatest body depth just anterior of pelvic fins. Females larger than males, reaching over 38 mm SL. Body and fin morphology identical to that of males, but females have relatively short pelvic fins and relatively long belly relative to other Iranian species. Anus and oviduct separated.

Pigmentation.—Counts of male flank bars are given in Table 2; there are significantly fewer flank bars than in *A. sophiae*. The bars are broad with interspaces about equal or slightly narrower

(Fig. 2a). The bars extend from behind the head to the tail. Anterior bars fade on the belly, whereas, posteriorly on the caudal peduncle, they encircle the body. Dorsally, the head is dark gray and the body is lighter but still heavily pigmented with melanophores; the belly lacks pigmentation. The sides of the head are densely speckled with melanophores, more thinly on the ventral side; in most specimens the chin is darker than the rest of the ventral head surface. The eye is bounded ventrally and postero-ventrally by a thin line of black pigment. The defining male coloration is the black margins of the pelvic, anal, and dorsal fins. The dorsal and anal fins may present a halo effect, the margins being so dark in relation to the rest of these fins. The tips and outer margin of the pelvic fin are blackish. The anal fin has a broad, blackish margin with the rest of the fin light cream-colored. The dorsal fin has the blackest margin. The rest of the dorsal fin is variably blotched, the blotches being much lighter than the fin margin. Most specimens have a contrasting pigmentless area just below the fin margin. The pectoral fin has sparse pigmentation along rays and ventrally on the interradiial membranes but lacks the concentrated black pigmentation seen on the pelvic fins. The caudal-fin rays and membranes are sparsely pigmented, and the whole margin may be blackish but in most fish pigment is restricted to the upper and lower margins, the lower margin only, or is absent.

Large females have a grayish dorsal surface on the head, a lighter back and upper flank covered with scattered melanophores, and a mid-flank

→

Fig. 3. Principal component analysis of 20 standardized morphometric measurements in males (A) and females (B) and of six meristic counts analyzed jointly for both sexes (C). Open circles (○) represent specimens of *A. isfahanensis*, vertical crosses (+) represent *A. sophiae*, diagonal crosses (×) represent *A. persicus*, and triangles (▲) represent *A. vladykovi*. Ellipses denote the 68.27% density limits of each sample.

stripe terminating on the caudal peduncle in a blackish, short stretch covering up to three scales (Fig. 2b). This short stretch of pigment is present in all females, faint in some, rarely forming a blotch and in some small fish tapering anteriorly. The flank stripe may be broken into a series of blotches in some smaller females, or it may be continuous as in large fish. Starting anterior to the belly there is a ventrolateral series of thin bars (up to ten) separated by cream-colored interspaces 1–3 bars wide. These are absent in some smaller females, which may only have blotches at various levels presenting this region. Even some large females have faint flank pigmentation so that bars and the stripe are weakly expressed. At about the origin of the anal fin, the flank bars may continue onto the caudal peduncle in regular form or become irregular, breaking up into blotches. Anteriorly the bars terminate ventrally at about the level of the lower edge of the pectoral fin. The belly and lower head have sparse pigment although the chin and sides of head are speckled with melanophores. The eye is bounded ventrally and posteroventrally by a thin line of black pigment. Fins lack any distinctive pigment pattern. Fin rays are outlined with melanophores, and interradial membranes of the caudal and anal fins have melanophores at varying degrees of density. The dorsal fin has the most interradial pigmentation, particularly near, but clear of the fin base.

Distribution and habitat.—The type locality (Fig. 1) is near the town of Varzaneh on the lower reaches of the Zayanadeh River, about 30 km upriver from the terminal sump, the Gav Khuni marsh. Water temperature was 27 C, pH 6.7, water was brackish, conductivity 10.9 mS, dissolved solids 5450 ppm, dissolved oxygen 12.3 mg/L, river width was 50 m, and capture depth 0.5 m. Current was slow, and there was no cover. *Gambusia holbrooki* was captured at the same locality. *Aphanius isfahanensis* appears to be restricted to the internal Zayanadeh River basin.

Phylogenetic relationships.—The genus *Aphanius* is divided into two major clades, the western and the eastern clade (Hrbek and Meyer, 2003). The western clade contains the main radiation found in the orogenic belt from central Anatolia, Turkey to southern Iran. This radiation also contains the Mediterranean brackish-water species *A. fasciatus*. The Iranian group is monophyletic and forms the sister group to *A. fasciatus* + *A. asquamatus* + *A. anatoliae* species complex of central Anatolia. A total of 3303 characters were included in the analyses; 1012 were variable and 697 were parsimony informative; data showed no

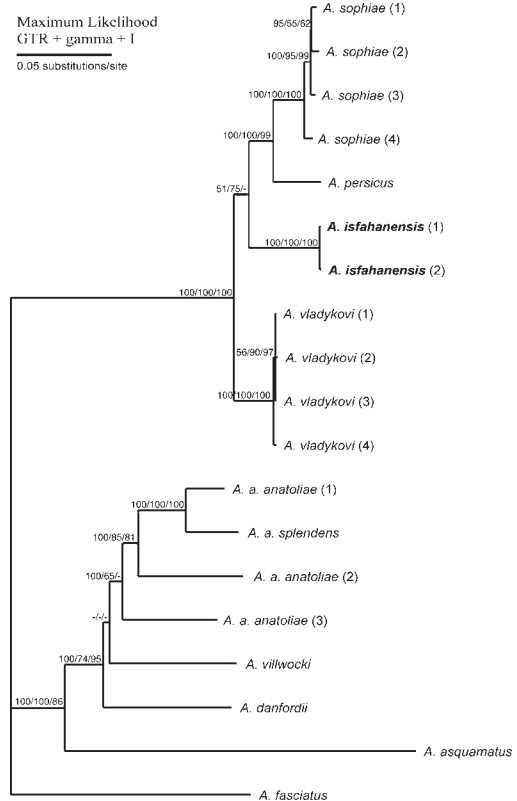


Fig. 4. Maximum likelihood estimate of phylogenetic relationships of *Aphanius isfahanensis* to other species of *Aphanius* of the western clade of species of *Aphanius* (Hrbek and Meyer, 2003). $-\ln L = 14164.01742$, $\alpha = 0.950747$, $\text{pinvar} = 0.538377$. Numbers above nodes represent Bayesian likelihood values followed by maximum likelihood and maximum parsimony bootstrap values. Species and geographic localities correspond to those listed in Material Examined and those from Hrbek and Meyer (2003). Localities of *Aphanius sophiae* are: (1) Dolarabad, Iran; (2) Malaskuh, Iran; (3) Abdolmahdi, Iran; and (4) Kor River basin near Shiraz, Iran. *Aphanius persicus*: Nasrabad Spring near Imamzadeh Ibrahim, Iran. *Aphanius vladkovi*: (1) Boldaji, Iran; (2) Nadar-Bokhtri, Iran; (3) Ebrahimbabad, Iran; and (4) Taqanak, Iran. *Aphanius anatoliae anatoliae*: (1) Lake Egirdir at Karaot, Turkey; (2) Lake Isikli near Beydilli, Turkey; and (3) Thaliye Canal by Sulamasi, Turkey. *Aphanius anatoliae splendens*: Lake Salda, Turkey. *Aphanius danfordii*: Soysalii springs in Sultan Swamps, Turkey. *Aphanius villwocki*: Pinarbaşı in upper Sakarya River, Turkey. *Aphanius fasciatus*: Lake Tuzla near Tarsus, Turkey. *Aphanius asquamatus*: Lake Hazer, Turkey. Both individuals of *Aphanius isfahanensis* are from Zayandeh River near Varzaneh, Iran.

visible effect of saturation. The parsimony phylogeny has a CI of 0.650 and RI of 0.747. The maximum-likelihood phylogeny (Fig. 4) is

identical to the maximum parsimony phylogeny; parameters are $-\ln L = 14201.78889$, $\alpha = 0.800049$, $\text{invar} = 0.504877$. Phylogenetic analyses support the hypothesis that *A. isfahanensis* is sister to *A. sophiae* from the Kor basin + *A. persicus* from the Maharlou basin (Fig. 4); sister to these three taxa is *A. vladykovi* from the highland (>2000 m) plains of the Kukalar mountains in the upper reaches of the Karun River drainage system (Fig. 1). All species are monophyletic and clearly divergent. Hrbek and Meyer (2003) estimated that *A. sophiae* and *A. vladykovi* diverged from each other approximately five million years ago. These estimates were based on several geological (Bender, 1968; Krijgsman et al., 1999) and fossil (Sauvage, 1874; Gaudant, 1978; Reichenbacher and Weidmann, 1992) calibration points. Using these same calibrations points, *A. isfahanensis* is estimated to have diverged approximately 4.8 million years ago from a lineage leading to *A. sophiae* and *A. persicus*.

Etymology.—Named after the province and its capital city, Isfahan, central Iran.

DISCUSSION

The geological history of Iran is similar to Anatolia in that it has been shaped by geological events associated with the closing of the Tethys Sea. Detailed sampling and analysis of central Anatolian populations of *Aphanius* revealed at least seven deeply divergent (Hrbek et al., 2002), and in cases where investigated, reproductively isolated (Villwock, 1964) lineages, but only three scientifically recognized species (Wildekamp et al., 1999; Hrbek and Wildekamp, 2003). All lineages investigated are allopatrically distributed in separate hydrological basins.

Similar to Anatolia, rapid isolation of multiple areas in Iran was caused by northward movement of the Arabian plate into the Iranian plate starting ten MYA (Dercourt et al., 1986). The initial phase caused the uplift of the Zagros Mountains at the southern edge of the Iranian plate. Continued north-eastern movement of the Arabian plate and a northerly movement of India resulted in additional mountain building by five MYA (Dercourt et al., 1986) along the northern edge of the Iranian plateau as well as along the sutures of the Iranian, Lut, Helmand, and Farah plates which comprise present-day Iran. If geological events had a similar effect on the fauna of Iran as did geological events on Anatolian taxa (Weisrock et al., 2001; Hrbek et al., 2002, 2004), we would expect the various populations of *Aphanius* occurring in isolated basins of the Zagros, Little Caucasus, and the Elburz moun-

tains and intervening Iranian plateau to form monophyletic groups. Due to the rapid and nearly simultaneous isolation of these areas from one another, we also would expect relationships among these populations to approximate a hard polytomy.

Until this study, relatively little attention has been paid to the role of geological events in generating the biological diversity of Iran. To our knowledge, only in the case of agamid lizards (Macey et al., 1998, 2000; Rastegar-Pouyani and Nilson, 2002) have geological events been implicated and tested as the causal mechanism in faunal diversification. However, geological events associated with the accretion of present-day Iran also seem to play a role in generating biodiversity of *Aphanius*. Based on estimates of five MYA divergence of *A. sophiae* and *A. vladykovi* (Hrbek and Meyer, 2003) derived from several independent geological (Bender, 1968; Krijgsman et al., 1999) and fossil (Sauvage, 1874; Gaudant, 1978; Reichenbacher and Weidmann, 1992) calibration points, we estimate a 4.8 MYA divergence of *A. isfahanensis* and *A. sophiae* + *A. persicus*. This divergence time is in accord with our hypothesis of a near-simultaneous diversification of ~5 MYA of organisms occupying different geological units of central Iran.

If this pattern is corroborated, and if it reflects the pattern observed in Anatolia, then Iran probably harbors numerous other species in addition to those currently recognized. Four species, including *Aphanius isfahanensis*, are presently recognized from central Iran, but *Aphanius* lives in at least two additional geologically and hydrologically isolated internal basins (Coad, 2000). Additional studies probably will result in recognition of greater Iranian fish diversity than currently appreciated, as well as in the biodiversity of other organismal groups.

A noticeable feature of the ichthyofauna of the Near East is the relative high degree of conservatism of external morphology in spite of substantial genetic, and when studied, reproductive (Villwock, 1964) divergence. In our analysis of the Iranian members of the eastern clade of *Aphanius* there are no fixed external morphological characters that would distinguish *A. isfahanensis* from its closest relatives *A. sophiae* and *A. persicus*. *Aphanius isfahanensis* can be distinguished by non-overlapping lateral-line scale number from *A. vladykovi*, the fourth Iranian member of this clade. *Aphanius isfahanensis*, *A. sophiae*, and *A. persicus* are not distinguishable qualitatively and difficult to distinguish even quantitatively by multivariate analyses of morphological data (Table 4, 5; Fig. 3a, b, c) but are clearly distinguishable by molecular data

TABLE 4. SUMMARY OF MORPHOMETRIC CHARACTER LOADINGS FOR PCA SEPARATED BY SEX OF *A. isfahanensis*, *A. sophiae*, *A. persicus*, AND *A. vladykovi* AND PERCENT VARIANCE EXPLAINED BY EACH EIGENVECTOR. First three eigenvectors explain over 50% of total variance. Effects of size were removed by converting log-transformed morphometric data into residuals using the least-squares regression method. Heavy loadings (>75%) are indicated in **bold**.

| Character | Eigenvector | | | | | |
|------------------------------|--------------|--------------|--------|--------------|--------|--------|
| | Female | | | Male | | |
| | 1 | 2 | 3 | 1 | 2 | 3 |
| Body depth | 0.713 | 0.038 | -0.112 | 0.558 | -0.207 | 0.008 |
| Head depth | 0.843 | 0.060 | 0.077 | 0.872 | -0.097 | 0.011 |
| Head length | 0.847 | -0.101 | -0.077 | 0.899 | 0.035 | -0.120 |
| Head width | 0.739 | -0.300 | 0.137 | 0.794 | -0.218 | -0.053 |
| Snout length | 0.704 | 0.040 | 0.160 | 0.732 | 0.050 | -0.229 |
| Mouth width | 0.587 | -0.033 | -0.070 | 0.559 | 0.157 | 0.025 |
| Orbit diameter | 0.693 | 0.071 | -0.062 | 0.814 | 0.168 | -0.165 |
| Interorbital width | 0.683 | -0.188 | -0.366 | 0.838 | 0.053 | -0.125 |
| Postorbital length | 0.319 | -0.469 | -0.679 | 0.379 | -0.272 | -0.030 |
| Predorsal length | -0.238 | 0.384 | 0.538 | -0.098 | 0.429 | -0.436 |
| Dorsal length | 0.573 | 0.448 | 0.109 | 0.573 | 0.265 | -0.098 |
| Prepelvic length | 0.468 | 0.482 | -0.200 | 0.431 | 0.521 | 0.323 |
| Preal length | -0.090 | 0.574 | -0.283 | 0.135 | 0.533 | 0.447 |
| Anal length | 0.215 | -0.447 | 0.536 | -0.163 | -0.734 | 0.340 |
| Caudal peduncle length | -0.242 | 0.021 | -0.387 | -0.112 | 0.112 | 0.003 |
| Caudal peduncle depth | 0.623 | -0.354 | 0.210 | 0.420 | -0.712 | 0.277 |
| Pectoral fin length | 0.373 | -0.242 | 0.126 | 0.332 | -0.682 | 0.293 |
| Pelvic fin length | 0.381 | 0.793 | 0.051 | 0.251 | 0.371 | 0.633 |
| Pectoral-pelvic fin distance | 0.314 | 0.770 | -0.048 | 0.249 | 0.355 | 0.679 |
| Pelvic-anal fin distance | 0.729 | -0.029 | 0.217 | 0.792 | -0.039 | -0.167 |
| Percent variance explained | 32.433 | 14.159 | 8.928 | 31.952 | 14.421 | 8.215 |

TABLE 5. SUMMARY OF MERISTIC CHARACTER LOADINGS FOR PCA OF *A. isfahanensis*, *A. sophiae*, *A. persicus*, AND *A. vladykovi*, AND PERCENT VARIANCE EXPLAINED BY EACH EIGENVECTOR. Total variance explained by the first three eigenvectors is 68.94%. Heavy loadings (>75%) are indicated in **bold**.

| Character | Eigenvector | | |
|----------------------------|--------------|--------|--------|
| | 1 | 2 | 3 |
| Gillraker number | 0.018 | 0.843 | 0.361 |
| Lateral-line scales | 0.638 | -0.238 | -0.412 |
| Pectoral-fin rays | 0.511 | 0.348 | -0.380 |
| Pelvic-fin rays | 0.512 | 0.427 | -0.326 |
| Dorsal-fin rays | 0.723 | -0.138 | 0.504 |
| Anal-fin rays | 0.807 | -0.198 | 0.314 |
| Percent variance explained | 35.054 | 18.820 | 15.066 |

(Table 3; Fig. 4). Species of *Aphanius* also often differ in male color pattern, differences potentially attributable to sexual selection, which is major evolutionary force in killifishes (e.g., Fuller, 2002). However, these color patterns often appear to be convergent among species of *Aphanius*, a phenomenon clearly observed in other taxa (West-Eberhard, 2003; Coyne, 2004). Although taxonomically not satisfactory, knowledge of collecting locality or drainage basin is the most practical information for rapid species identification, followed by color pattern, if

approximate collection data are known. However, only molecular analyses can unambiguously distinguish among different species of *Aphanius*.

This and our previous studies (Hrbek et al., 2002, 2004) suggest that, in active orogenic areas with opportunities for allopatric speciation, much of the extant biodiversity remains and will remain unrecognized under many species concepts. Allopatrically distributed species are often very similar in external morphological and meristic characters and, thus, difficult to distinguish, as should be evident from this study. This

similarity is probably the result of stabilizing selection on morphological characters driven by a common set of ecological/environmental variables. However, the species in these different areas are not genetically interchangeable (Villwock, 1964) and are genetically distinguishable (Hrbek et al., 2002) in spite of extensive morphological similarity. The species occupying these disjunct areas are natural in that they embody an evolutionary process, but do not necessarily demonstrate the pattern in their external morphology. It is important to remember that real evolutionary groups need not be morphologically distinct, whereas morphological categories are created as a direct function of perceived distinction (Hey, 2001), and the two are not the same.

Multivariate analyses of 20 morphometric characters collected in males indicate that *A. isfahanensis* differs significantly from *A. sophiae* (Wilks' Lambda $F = 20.711$, d.f. = 40, 60, $P < 0.001$), from *A. persicus* (Wilks' Lambda $F = 18.670$, d.f. = 40, 350, $P < 0.001$) and from *A. vladykovi* (Wilks' Lambda $F = 26.209$, d.f. = 40, 68, $P < 0.001$). Same results are obtained for morphometric comparisons of females: *A. isfahanensis* differs significantly from *A. sophiae* (Wilks' Lambda $F = 10.772$, d.f. = 40, 46, $P < 0.001$), from *A. persicus* (Wilks' Lambda $F = 24.570$, d.f. = 40, 448, $P < 0.001$), and from *A. vladykovi* (Wilks' Lambda $F = 12.184$, d.f. = 40, 28, $P < 0.001$). Multivariate analyses of six meristic characters analyzed jointly for both sexes indicate that *A. isfahanensis* differs significantly from *A. sophiae* (Wilks' Lambda $F = 777.686$, d.f. = 12, 176, $P < 0.001$), from *A. persicus* (Wilks' Lambda $F = 2617.349$, d.f. = 12, 860, $P < 0.001$), and from *A. vladykovi* (Wilks' Lambda $F = 1914.332$, d.f. = 12, 166, $P < 0.001$). For pair-wise differences see Tables 1 and 2.

Although *A. isfahanensis* is statistically distinguishable in multivariate space as well as in certain pair-wise comparisons from *A. sophiae*, *A. persicus*, and/or *A. vladykovi*, with the exception of lateral-line scale number in *A. vladykovi*, there is no univariate or combination of univariate characters that will unambiguously distinguish *A. isfahanensis* from these other three Iranian species. All morphometric and meristic characters overlap among species, and PCA analyses show extensive overlap among species for both meristic and morphometric characters (Fig. 3A, B, C). The defining male color pattern of black margins of the pelvic, anal, and dorsal fins is also observed in *Aphanius anatoliae* from Turkey (Wildekamp et al., 1999; Hrbek et al., 2002); thus, this pattern is not exclusive to *A. isfahanensis*. The female flank-bar pattern, which is similar

to that of *A. persicus*, is also found in females of *A. dispar* and *A. fasciatus*. The only characters that unambiguously distinguish *A. isfahanensis* from all other species of *Aphanius* are 83 molecular apomorphies in the 3303 mitochondrial DNA characters analyzed in this study. Therefore unambiguous species identification can only be obtained through molecular analysis which in principle is molecular bar-coding (Hebert et al., 2003).

MATERIAL EXAMINED

Aphanius sophiae: NMC 79-0025, female, 31.6 mm SL, Iran, Fars, Kor River at Marv Dasht, 29°51'N, 52°46'30"E; NMC 79-0059, 19 males, 18.1–39.3 mm SL, and 11 females, 24.5–31.9 mm SL, Iran, Fars, Pulvar River, 8 km south of Sivand, 30°01'30"N, 52°57'E, 18 April 1976, B. W. Coad and S. Coad; NMC 79-0061, 3 females, 23.2–32.1 mm SL, Iran, Fars, stream tributary to Pulvar River, 14 km south of Sa'adatabad, 30°04'N, 53°01'E, 18 April 1976, B. W. Coad and S. Coad; NMC 79-0062, 2 males, 24.0–26.6 mm SL, and 4 females, 26.5–42.0 mm SL, Iran, Fars, spring, 17 km south of Sa'adatabad, 30°05'N, 53°00'E, 18 April 1976, B. W. Coad and S. Coad; NMC 79-0067, male, 25.7 mm SL, Iran, Fars, qanat at Zarqan, 29°46'N, 52°43'E, 27 April 1976, B. W. Coad and S. Coad; NMC 79-0292, 4 males, 26.5–31.1 mm SL, and 2 females, 29.7–34.4 mm SL, Iran, Fars, Lapu'i spring near Zarqan, 29°48'N, 52°39'E, 30 June 1974, H. Assadi; NMC 79-0342, female, 39.4 mm SL, Iran, Fars, Kor River at Band-e Amir, 29°49'N, 52°51'E, 22 Nov. 1977, B. W. Coad; NMC 79-0498, 4 males, 19.7–25.2 mm SL, and 2 females, 17.4–20.0 mm SL, Iran, Fars, spring in Kor River basin, 30°05'N, 52°27'E, 4 Oct. 1978, B. W. Coad; CMNFi2004-0003 (GenBank AY593483, AY593492), 7 of 47, 20.7–28.8 mm SL, Fars Province, Abolmahdi Spring, 17 km south of Sa'adat-shahr (Saadatabad), 30°06'12"N, 52°58'38"E, 14 April 2002, Y. Keivany and S. Asadollah; CMNFi2004-0004 (GenBank AY593482, AY593492), 8 of 38, 19.5–34.0 mm SL, Fars Province, Malasskuh Spring, 29°52'04"N, 52°29'20"E, 15 April 2002, Y. Keivany and S. Asadollah; uncatalogued (GenBank AY593481, AY593490), 6, 25.7–37.0 mm SL, Fars Province, Dolatabad Spring, 29°43'05"N, 52°50'11"E, 14 April 2002, Y. Keivany and S. Asadollah. *Aphanius persicus*: CMNFi2004-0006 (GenBank AY593484, AY593493), 15, 22.2–41.8 mm SL, Fars Province, Nasrabad Spring near Imamzadeh Ibrahim, 29°35'05"N, 52°39'08"E, 15 April 2002, Y. Keivany and S. Asadollah. *Aphanius vladykovi*: NMC 79-0247, male, 36.6 mm SL, Iran, Shahrestan-e Bakhtiari va Chahar Mahall, large pool, 3 km

west of Boldaji, 31°57'N, 51°01'E, 9 June 1977, B. W. Coad and S. Mansoorabadi; NMC 79-0247A, 35 males, 21.6–36.4 mm SL, and 16 females, 23.5–40.2 mm SL, same locality as NMC 79-0247; NMC 79-0248, male, 30.3 mm SL, Iran, Shahrestan-e Bakhtiari va Chahar Mahall, stream, 3 km east of Boldaji, 31°55'N, 51°05'E, 9 June 1977, B. W. Coad and S. Mansoorabadi; uncatalogued (GenBank AY593486, AY593495), 4, 25.6–37.0 mm SL, Chahar Mahall va Bakhtiari Province, Ebrahimabad, 31°52'30"N, 51°10'10"E, 13 July 2002, S. Asadollah; uncatalogued (GenBank AY593487, AY593496), 2, Chahar Mahall va Bakhtiari Province, Taqanak, 32°12'35"N, 50°49'29"E, 26 March 2002, Y. Keivany and S. Asadollah; uncatalogued (GenBank AY593485, AY593494), 5, Chahar Mahall va Bakhtiari Province, Madar-Dokhtar, 31°52'12"N, 51°08'29"E, 13 July 2002, Y. Keivany and S. Asadollah.

Additional molecular material included: *Aphanius anatoliae splendens*: MRAC 99-072-P-0040-0041 (GenBank AF449287, AF449349), Lake Salda, Turkey. *Aphanius anatoliae anatoliae*: MRAC 96-053-P-0508-0517 (GenBank AF449292, AF449354), Lake Eğirdir at Karaot, Turkey. *Aphanius anatoliae anatoliae*: MRAC 97-033-P-0172 (GenBank AF449293, AF449355), Lake Isıklı near Beydilli, Turkey. *Aphanius anatoliae anatoliae*: MRAC 92-136-P-0035-0043 (GenBank AF449297, AF449359), Thaliye Canal by Sulamasi, Turkey. *Aphanius danfordii*: MRAC A3-031-P-0004-0009 (GenBank AF449299, AF449361), Soy-salii springs in Sultan Swamps, Turkey. *Aphanius villuociki*: MRAC A1-030-P-0001-0003 (GenBank AF449303, AF449365), Pinarbaşı in upper Sakarya River, Turkey. *Aphanius asquamatus*: uncatalogued (GenBank AF449306, AF449368), Lake Hazer, Turkey. *Aphanius fasciatus*: MRAC 92-136-P-0078-0097 (GenBank AF449310, AF449372), Lake Tuzla near Tarsus, Turkey. *Aphanius sophiae*: uncatalogued (GenBank AF449314, AF449376), Kor River basin near Shiraz, Iran. *Aphanius vladkovi*: uncatalogued (GenBank AF449315, AF449377), Boldaji basin, Iran.

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