

Sex dimorphism and evidence of sexually selected traits: A case study on the killifish *Aphaniops stoliczkanus* (Day, 1872)

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Abstract

Sex dimorphism is found in many organisms and is an important source of intraspecific variation. Among freshwater fishes, all members of the Aphaniidae (killifishes, Cyprinodontiformes) are known for their pronounced sex dimorphism, and it has been proposed that sexual selection has played a role in their diversification. However, few studies have assessed their sex dimorphism in detail. Here, we use *Aphaniops stoliczkanus* (Day, 1872) to analyse morphological variability within and between sexes and within the total sample using statistical analyses of body morphometry, meristic traits and otolith data. We found that sex dimorphism (i) is significant in many body morphometric variables, but (almost) absent in meristic traits and otolith morphometry, (ii) is most marked in anal- and dorsal-fin lengths (larger in males), and in anal- and dorsal-fin positions (more anteriorly positioned in males) and (iii) does not alter the overall variance of the population unless the character concerned is highly divergent. Our data also suggest that anal- and dorsal-fin lengths in males of *A. stoliczkanus* are sexually selected traits. Together with literature data, this reinforces the idea that sexual selection plays a role in the diversification of aphaniid species. Our results are also relevant to work on fossils, in which morphological variability is often difficult to interpret.

KEYWORDS

Aphaniidae, *Aphaniops*, morphological variability, sex dimorphism, sexually selected traits

1 | INTRODUCTION

Variability is a basic element of biology and is an essential component of evolutionary processes. Phenotypic variability within a species can be linked to ecological, environmental and ontogenetic factors, but also to sex-related differences (Martinez et al., 2019). The phenomenon of

sex-related variability often appears in the form of sex dimorphism, which is usually manifested in differences in the size, colour or shape of morphological structures (Kaliontzopoulou et al., 2015; Littleford-Colquhoun et al., 2019; Ruckstuhl & Clutton-Brock, 2005; Zajitschek et al., 2020). Generally, sex dimorphism is found in many organisms and is an important source of intraspecific

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variation (Aguirre et al., 2008; Hedrick & Temeles, 1989; Martinez et al., 2019). It can be the result of sexual selection for specific traits having to do with mate choice (e.g. male courtship), and of ecological selection for dimorphic niches due to reproductive constraints or resource competition (Herler et al., 2010; Sowersby et al., 2021; Walker & McCormick, 2009). Moreover, sexually dimorphic traits can play an important role in population divergence and adaptive radiations because such traits can reinforce interspecific differences in behaviour, feeding and habitat use, thus adding to species diversification (Bolnick & Doebeli, 2003; Martínez-Ruiz & Knell, 2017; Wasiljew et al., 2021). However, there is a clear gap in our knowledge about the range of variability of sexually dimorphic characters within and between the sexes, although such data are crucial to shed light on how sex dimorphism can contribute to speciation (Welsh & Fuller, 2015).

Killifishes (Cyprinodontiformes Berg, 1940) constitute a diverse order of freshwater fish and have been the subject of extensive research on multiple topics including (among others) species diversity, speciation events, phylogenetic relationships and biogeography (e.g. Bragança & Costa, 2019; Loureiro et al., 2018; Parenti, 1981; Teimori et al., 2018). They are distributed in tropical and temperate waters worldwide and are found in freshwater lakes, lagoons, brackish, hypersaline and ephemeral water bodies, salt marshes, sulphide-rich and alkaline habitats (Pohl et al., 2015; Wildekamp, 1993). The Old-World killifish family Aphanidiidae Hoedemann, 1949, is a survivor of the ichthyofauna of the Tethys Sea; its current distribution includes the Mediterranean, the Middle East and the western Indian Ocean (Esmaili et al., 2020; Hrbek & Meyer, 2003; Villwock, 2004). All species of the Aphanidiidae are known to exhibit sex dimorphism in the coloration of the flanks and the unpaired fins as well as in morphological traits (Esmaili et al., 2014; Freyhof & Yoğurtçuoğlu, 2020; Wildekamp, 1993). In addition, Aphanidiidae are known for rapid diversification when populations become isolated (Esmaili et al., 2014; Ferrito et al., 2013; Gonzalez et al., 2018; Tigano et al., 2006). Possible reasons for this potential include quick adaptation to different environments, limited gene flow and random genetic drift (e.g. Chiozzi et al., 2018; Reichenbacher, Feulner, et al., 2009; Teimori, Schulz-Mirbach, et al., 2012). Moreover, it has been suggested that sexual selection plays a role in the emergence of new species (Teimori, Esmaili, et al., 2012). The latter aspect, however, has not yet been closely investigated and quantitative data supporting, or refuting, the occurrence of sexual selection of traits in Aphanidiidae have not yet been presented.

An additional aspect of our study is that species delimitation of fossil killifishes greatly benefits from accurate data on variation within extant killifish species (Bidaye

et al., 2022). Fossils of Aphanidiidae and Valenciidae (=sister-group to Aphanidiidae, Bragança et al., 2018) are known from various locations in Europe and Asia, with the oldest species dating back to the lower Oligocene (34 Ma; Gaudant, 2012; Reichenbacher & Kowalke, 2009; Vasilyan et al., 2009). However, their ancient diversity is often obscured due to a high degree of intraspecific variability and the presence of few or only quite subtle differences between species, regardless whether fossil otoliths or fossil skeletons are concerned (Frey et al., 2016; Gaudant, 2009, 2011; Reichenbacher et al., 2007). Thorough analysis of morphological variability within an extant aphanidiid species, including sex dimorphism, will thus help to improve species concepts for their fossil counterparts.

The aim of this study is (i) to examine sex dimorphism in morphological traits within an aphanidiid population and (ii) to evaluate whether dimorphic traits are subject to sexual selection. Such data can contribute to a deeper understanding of the ability of Aphanidiidae to diverge into new species over short periods of time and are also important for correct taxonomic interpretation of their fossil ancestors.

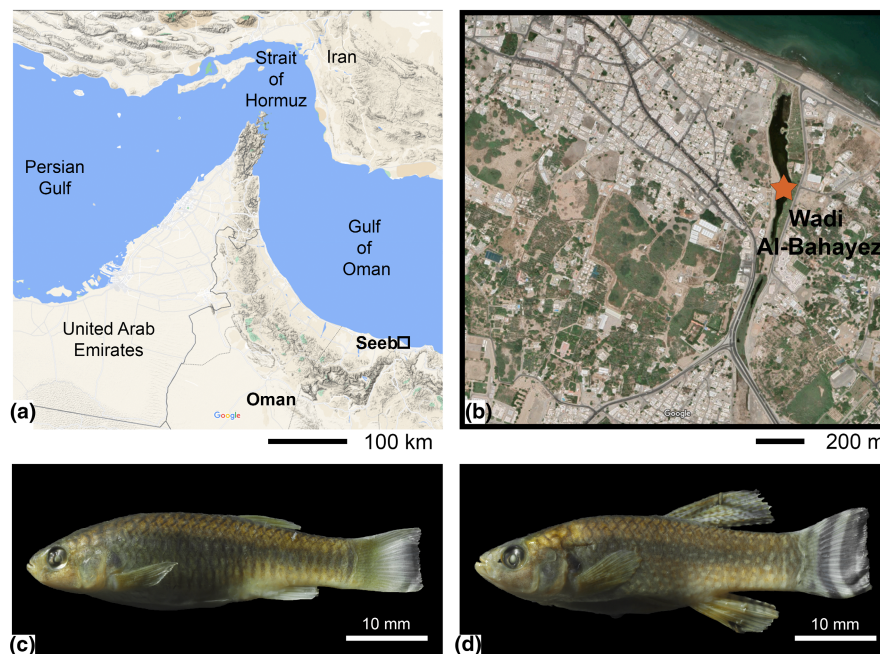
2 | MATERIALS AND METHODS

2.1 | Sample collection and preparation

The study was carried out on a sample of *Aphaniops stoliczkanus* collected over the course of a single day (23 July 2020) at Wadi Al Bahayez in northeastern Oman (Figure 1a). Wadi Al Bahayez is an inland water body near to the coast (Figure 1b), which drains into the Gulf of Oman during rainy seasons. The site lies within the natural range of the species (Freyhof et al., 2017). Species identification was based on the typical pigmentation of males and females (Figure 1c,d), and was additionally confirmed by molecular analysis (Bidaye et al., 2022). The fish were euthanized with an overdose of benzocaine (100 mg L⁻¹, see Barker et al., 2002) prior to fixation in 80% ethanol to ensure preservation of the otoliths. Sampling of the animals was carried out in accordance with legal policies, guidelines and protocols approved by the responsible governmental authorities of Oman (reference number CR/AGR/FISH/20/03).

The sample comprised 39 specimens: 23 males and 16 females. The standard lengths of all specimens ranged from 29.8 to 44.3 mm (mean 39.03 ± 3.34). Based on their sizes and typical pigmentation, no sorting into size classes was necessary, and all specimens were considered to be mature adults (Teimori, Motamedi, et al., 2021). Each specimen received a sample ID. All samples are stored in the Department of Earth and Environmental Sciences at Ludwig-Maximilian University (LMU) in Munich.

FIGURE 1 (a) Location of Wadi Al-Bahayez near Seeb in northern Oman; (b) close up of Wadi Al-Bahayez (black square in a); (c, d) female (c, specimen BAH5) and male (d, specimen BAH26) of *Aphaniops stoliczkanus* from Al-Bahayez, Oman. Source of map: Google (2021). [Colour figure can be viewed at wileyonlinelibrary.com]



To analyse meristic elements, all individuals were X-rayed with a Faxitron Bioptics machine at the Bavarian State Collection of Zoology (SNSB-ZSM). After X-raying, the saccular otoliths were extracted from 37 individuals through the opercle (Wakefield et al., 2016) and stored in Franke cells. The clean otoliths were coated with gold and imaged by scanning electron microscopy (SEM) on a HITACHI SU 5000 Schottky FE-SEM in the Department of Earth and Environmental Sciences at LMU.

2.2 | Body morphometry and meristic counts

Eighteen measurements, combining different characters assessed in previous research (Freyhof et al., 2017; Holčík, 1989; Scott et al., 2020; Teimori, Schulz-Mirbach, et al., 2012), were taken under a stereomicroscope (Leica EZA) with a digital calliper (Kraftixx) and recorded to 0.1 mm precision (Figure 2a). Standard length (SL) was measured from the tip of the upper jaw to the end of the hypural complex. Head depth and length were measured from the farthest end of the opercle vertically (HD), respectively horizontally to the tip of the upper jaw (HL) (Figure 2a). Fin lengths were taken following Miller (1948). Measurements were standardised as a percentage of SL ($\text{Measurement/SL} * 100$) or of the head length ($\text{Measurement/HL} * 100$), resulting in 16 morphometric variables (Table 1).

Fifteen meristic characters (Figure 2b, Table 1) were obtained from the X-ray images, and two additional characters (counts of pectoral and pelvic-fin rays) were examined under the stereomicroscope, since their visibility on the X-ray images was low. Counts of vertebrae were divided

into total, abdominal and caudal vertebrae; the last caudal vertebra includes the terminal centrum (TC; Teimori & Esmaeili, 2020). The ordinal numbers of the vertebrae opposite to the point of insertion of the dorsal and anal fin, respectively, were added as new meristic characters (indicated as DF-V and AF-V in Figure 2b); they were taken from the most anterior point of the fin following a straight line to the vertebra. In cases where the position of the first ray lay between two vertebrae, the ordinal number refers to the posterior vertebra. Rays of the anal and dorsal fins were each counted in total, and, where possible, we distinguished between branched and unbranched rays (Freyhof et al., 2017; Teimori, Schulz-Mirbach, et al., 2012). The pterygiophore formula of the dorsal fin follows Birdsong et al. (1988); that is the first number refers to the ordinal number of the vertebra behind which the first pterygiophore inserts; this is followed by a hyphen, and then by the number of pterygiophores in each interneural space (e.g. 10–122311). For the anal fin, the number of pterygiophores anterior to the first haemal spine was noted (Charpila et al., 2020). Caudal-fin ray counts were done separately for ventral and dorsal unbranched rays and principal branched rays (Arratia, 2008; Charpila et al., 2020).

2.3 | Otolith terminology and morphometry

Otolith terminology is shown in Figure 2c. Eight linear distances and three angles were measured based on 35 left otoliths using ImageJ (v. 1.53c; Figure 2d), and 10 variables were derived from the linear measurements, following the methods described by Reichenbacher et al. (2007) and Teimori, Iranmanesh, et al. (2021).

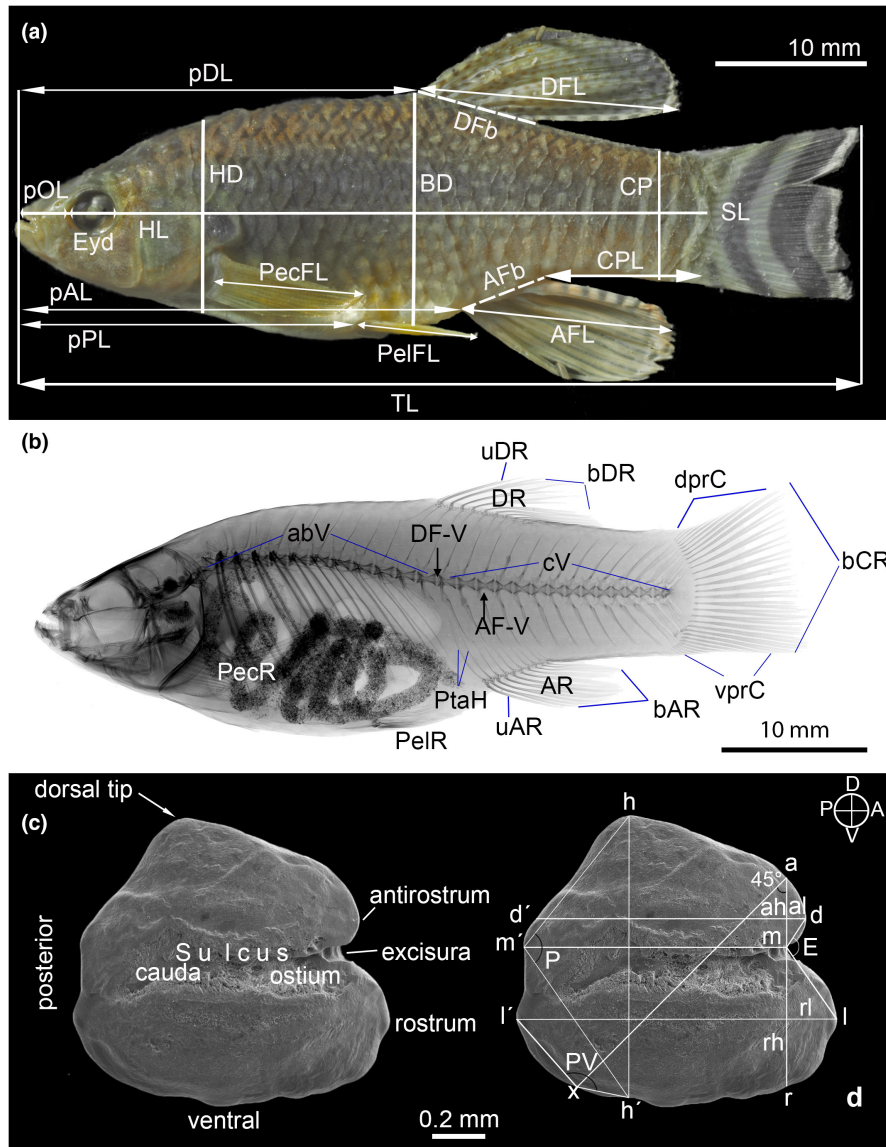


FIGURE 2 (a) Definition of the morphometric measurements analysed. AFL, anal-fin length; AFb, anal-fin base length; BD, body depth; DFb, dorsal-fin base length; DFL, dorsal-fin length; Eyd, eye diameter; HD, head depth; HL, head length; CP, minimum peduncle depth; pAL, preanal length; pDL, predorsal length; PecFL, pectoral-fin length; PelFL, pelvic-fin length; CPL, peduncle length; pOL, pre-orbital length; pPL, prepelvic length; SL, standard length; TL, total length. (b) X-ray image showing the meristic characters analysed. AF-V, ordinal number of vertebra opposite to the point of anal-fin insertion; AR, anal-fin rays; abV, abdominal vertebrae; bAR, branched anal-fin rays; bCR, branched caudal-fin rays; bDR, branched dorsal-fin rays; cV, caudal vertebrae (including terminal centrum); dprC, dorsal procurrent caudal-fin rays; DF-V, ordinal number of vertebra opposite to the point of dorsal-fin insertion; DR, dorsal-fin rays; PecR, pectoral-fin rays; PelR, pelvic-fin rays; PtaH, anal-fin pterygiophores anterior to first haemal spine; uDR, unbranched dorsal-fin rays; uAR, unbranched anal-fin rays; vprC, ventral procurrent caudal-fin rays. (c, d) general morphology and nomenclature of *Aphaniops stoliczkanus* otoliths from Al-Bahayez, Oman, with definition of otolith measurements according to Reichenbacher et al. (2007). L-l', ventral length; m-m', medial length; d-d', dorsal length; h-h', height; m-a, antirostrum height; m-r, rostrum height; al-d, antirostrum length; rl-l, rostrum length; P, posterior angle; PV, posteroventral angle (lines a-x and a-r define an angle of 45°); E, excisura angle. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/azo.12356)]

2.4 | Variance of characters

We used the standard deviation to quantify the degree of variability with respect to the mean value of each character studied. We did not use the coefficient of variation, a common parameter used for comparison of variances

between different traits and variable scales, because previous reports have shown that the value obtained is influenced both by the quantitative relationship between the standard deviation and the mean, and the nature of the data concerned (Pélabon et al., 2020; Van Valen, 2005; Zajitschek et al., 2020).

TABLE 1 Descriptive statistics of considered variables for females and males of *Aphaniops stoliczkanus* from Al-Bahayez, Oman

Variables	Total population	Females (n = 16)		Males (n = 23)	
	Mean ± SD	Mean ± SD	Range	Mean ± SD	Range
TL (mm)	47.2 ± 3.9	48.0 ± 2.4	43.1–52.4	46.6 ± 4.7	36.0–55.1
SL (mm)	39.0 ± 3.3	40.2 ± 1.9	36.2–43.5	38.2 ± 3.9	29.8–44.3
Morphometry in % of SL					
Preanal length	69.3 ± 3.1	71.9 ± 2.2	66.6–74.8	67.6 ± 2.3	64.8–72.1
Prepelvic length	50.2 ± 2.2	51.5 ± 1.9	47.0–55.0	49.2 ± 1.9	46.4–53.2
Predorsal length	60.1 ± 3.0	62.3 ± 1.7	59.6–65.9	58.6 ± 2.7	53.3–63.5
Peduncle length	22.0 ± 1.8	20.6 ± 1.5	17.7–23.3	22.9 ± 1.2	19.9–25.1
Head length	25.3 ± 1.2	24.6 ± 1.1	21.8–25.7	25.7 ± 1.1	24.0–27.6
Pelvic-fin length	12.8 ± 2.4	11.0 ± 1.2	8.9–13.3	14.1 ± 2.1	10.4–18.4
Pectoral-fin length	21.9 ± 2.4	19.9 ± 1.4	17.8–22.7	23.2 ± 2.0	19.5–27.6
Anal-fin length	23.9 ± 6.0	18.0 ± 1.5	15.0–20.8	27.9 ± 4.2	20.8–33.4
Dorsal-fin length	28.4 ± 7.9	21.0 ± 0.8	19.5–22.7	33.5 ± 6.2	22.8–47.1
Anal-fin base length	9.7 ± 1.9	8.2 ± 1.3	6.3–10.7	10.7 ± 1.5	8.2–13.4
Dorsal-fin base length	12.9 ± 1.9	11.4 ± 0.9	9.5–13.0	14.0 ± 1.7	10.5–16.9
Head depth	24.9 ± 1.6	24.8 ± 1.4	22.3–27.4	25.0 ± 1.7	21.4–28.6
Body depth	30.3 ± 2.2	29.8 ± 2.0	26.6–33.4	30.6 ± 2.3	26.3–34.8
Minimum peduncle depth	17.6 ± 1.4	16.4 ± 0.8	14.5–17.8	18.4 ± 1.0	16.4–20.9
Morphometry in % of HL					
Preorbital length	17.1 ± 3.4	16.3 ± 3.0	10.2–21.2	17.6 ± 3.7	12.0–25.2
Eye diameter	30.3 ± 2.6	30.3 ± 2.1	26.5–33.4	30.3 ± 3.0	25.0–35.8
Meristic counts					
Dorsal-fin rays	9 ± 0.5	8.9 ± 0.2	8–9	9.0 ± 0.6	8–11
Unbranched dorsal-fin rays	1.0 ± 0.0	1.0 ± 0.0	1	1.0 ± 0.0	1
Branched dorsal-fin rays	8.0 ± 0.5	7.9 ± 0.3	7–8	8.1 ± 0.6	7–10
Anal-fin rays	10.1 ± 0.4	10.2 ± 0.4	10–11	10.0 ± 0.4	9–11
Unbranched anal-fin rays	1.0 ± 0.1	1.0 ± 0.0	1–1	1.0 ± 0.2	0–1
Branched anal-fin rays	9.1 ± 0.4	9.3 ± 0.5	9–10	9.1 ± 0.4	8–10
Anal-fin pteryg. anterior to first haemal spine	1.9 ± 0.6	2.1 ± 0.7	1–3	1.8 ± 0.5	1–3
Pelvic-fin rays	7.2 ± 0.4	7.1 ± 0.3	7–8	7.3 ± 0.5	7–8
Pectoral-fin rays	15.3 ± 1.0	15.1 ± 0.9	13–16	15.5 ± 1.0	14–17
Total vertebrae	27.0 ± 0.5	26.9 ± 0.2	26–27	27.1 ± 0.6	26–29
Abdominal vertebrae	12.1 ± 0.4	12.2 ± 0.4	12–13	12.0 ± 0.4	11–13
Caudal vertebrae	15.0 ± 0.5	14.7 ± 0.4	14–15	15.1 ± 0.5	14–16
Ord. numb. of vert. opp. dorsal-fin insert.	13.1 ± 0.6	13.4 ± 0.5	13–14	12.8 ± 0.6	12–14
Ord. numb. of vert. opp. anal-fin insert.	14.7 ± 0.7	14.9 ± 0.2	14–15	14.5 ± 0.8	13–17
Branched caudal-fin rays	15.9 ± 0.6	15.7 ± 0.7	14–17	16.0 ± 0.6	15–18
Dorsal procurrent caudal-fin rays	6.1 ± 0.5	6.1 ± 0.5	5–7	6.1 ± 0.6	5–7
Ventral procurrent caudal-fin rays	6.5 ± 0.6	6.5 ± 0.5	6–7	6.4 ± 0.7	5–8
Otolith morphometry (in % of OL or OH), otolith angles and otolith ratios					
Dorsal length	84.0 ± 4.7	83.2 ± 5.3	74.3–93.3	84.7 ± 4.1	77.0–90.2
Medial length	80.0 ± 3.0	80.4 ± 3.5	74.5–85.9	79.8 ± 2.7	75.5–85.0
Antirostrum length	8.4 ± 3.2	7.8 ± 3.6	1.8–13.3	8.8 ± 3.0	4.0–15.0
Rostrum length	18.3 ± 2.8	18.4 ± 3.0	13.6–25.4	18.3 ± 2.6	13.4–24.4
Antirostrum height	27.1 ± 5.7	27.5 ± 6.4	16.3–36.5	26.9 ± 5.3	18.1–37.1
Rostrum height	48.3 ± 2.6	48.2 ± 2.5	42.4–53.0	48.3 ± 2.8	41.9–53.2
Posterior angle	105.8 ± 6.7	104.4 ± 6.6	93.5–119.1	106.8 ± 6.8	92.3–121.8

(Continues)

TABLE 1 (Continued)

Variables	Total population	Females (n = 16)		Males (n = 23)	
	Mean ± SD	Mean ± SD	Range	Mean ± SD	Range
Posteroventral angle	144.9 ± 9.7	143.4 ± 9.8	129.0–162.8	146.0 ± 9.7	134.6–167.6
Excisura angle	101.0 ± 14.3	102.9 ± 17.8	72.1–142.8	99.5 ± 11.2	79.9–117.3
Length-height index	1.1 ± 0.1	1.1 ± 0.1	1.0–1.2	1.1 ± 0.1	1.0–1.2
OL:SL	2.8 ± 0.2	2.8 ± 0.2	2.6–3.2	2.8 ± 0.2	2.4–3.3
OL:HL	11.2 ± 0.9	11.5 ± 0.5	10.6–12.5	11.1 ± 1.1	9.2–13.1
OL:EyeD	37.1 ± 2.8	37.7 ± 2.9	34.0–43.6	36.7 ± 2.8	32.0–42.4

Note: Variables in **bold** and/or *italic* refer to **characters** and/or *variances* that were significantly different between the sexes ($p < .01$, see Methods for details).

2.5 | Statistical analyses

Statistical analysis was performed with R v. 4.0.3. (R Core Team, 2021) using basic functions along with the “DescTools” package for statistical tests (Signorell et al., 2021). Descriptive statistics were based on data for the total population and for each sex, and data for males and females were compared. Tests for normality (Shapiro–Wilk) and homogeneity of variance (Levene test) were done to choose between parametric and nonparametric tests with corrections (Welch’s *T*-test) in cases where the variance of the data for each sex differed. A 99% confidence level was chosen ($p < .01$) to obtain more robust results. Sex dimorphism of body morphometric variables was analysed using the *T*-test ($p < .01$) for normally distributed morphometric variables (all variables, except two). A nonparametric test (Mann–Whitney, $p < .01$) was conducted for the two not normally distributed morphometric variables (preanal length in males and head length in females) and for meristic counts.

3 | RESULTS

3.1 | Variation in body morphometric measurements and meristic counts

As explained in the Methods, we use the values of the standard deviation (SD) to evaluate the variability of a given character within the population (all sampled specimens) and within each sex.

3.1.1 | Total population

With respect to morphometric variables, the most variable parameters ($SD > 5$) were dorsal-fin length and anal-fin length (Figure 3a, Table 1). Standard deviations for preorbital, preanal and predorsal lengths, together with eye diameter, were also high (> 2.5 , Figure 3a). On the contrary, there is considerably less variability in the parameters

head length, head depth and minimum peduncle depth (Figure 3a, Table 1). Meristic characters show low levels of variation overall (Figure 3b), and most of the counts vary by one (Table 1). The only markedly variable character is the pterygiophore formula of the dorsal fin, with a total of 25 different formulas, the most frequent being 10–112222 (present in seven specimens; Figure S1).

3.1.2 | Females, males

Females exhibit lower variability than males in all morphometric body variables, except for the caudal peduncle length (Figure 3a). In particular, very low variability is observed for dorsal-fin length in females, for which the standard deviation is lower than that for any other body morphometric variable (Figures 3a and 4d). In males, both dorsal- and anal-fin lengths show the greatest variability (Figures 3a and 4c,d). With regard to meristic traits, females show again slightly lower variance than males (Figure 3b), except in the numbers of branched rays in the anal and caudal fins, the total number of anal-fin rays and the number of anal-fin pterygiophores anterior to the first haemal spine. Generally, females and males each show relatively little variation in meristic traits, as observed in the total population (Table 1).

3.2 | Sex dimorphism in body morphometric measurements and meristic counts

We used parametric and nonparametric statistics ($p < .01$) to test whether the mean values of the studied traits differ significantly between females and males. In addition, we performed Levene tests ($p < .01$) to determine whether variances also differ significantly between females and males (see Methods).

Of the 16 morphometric variables examined, 12 differ significantly between females and males; the four variables that show no sex dimorphism are preorbital length, eye

diameter, body depth and head depth (Figure 3a, Table 1). Dimorphic variables are related to anal- and dorsal-fin positions (more posterior in females, Figure 4a,b), fin lengths (longer in males, Figure 4c-f), and head and

peduncle lengths (Table 1). However, although these variables differ significantly between the sexes, their mean values are relatively close in most cases (Table 1). Only the anal- and dorsal-fin lengths, for which mean values differ

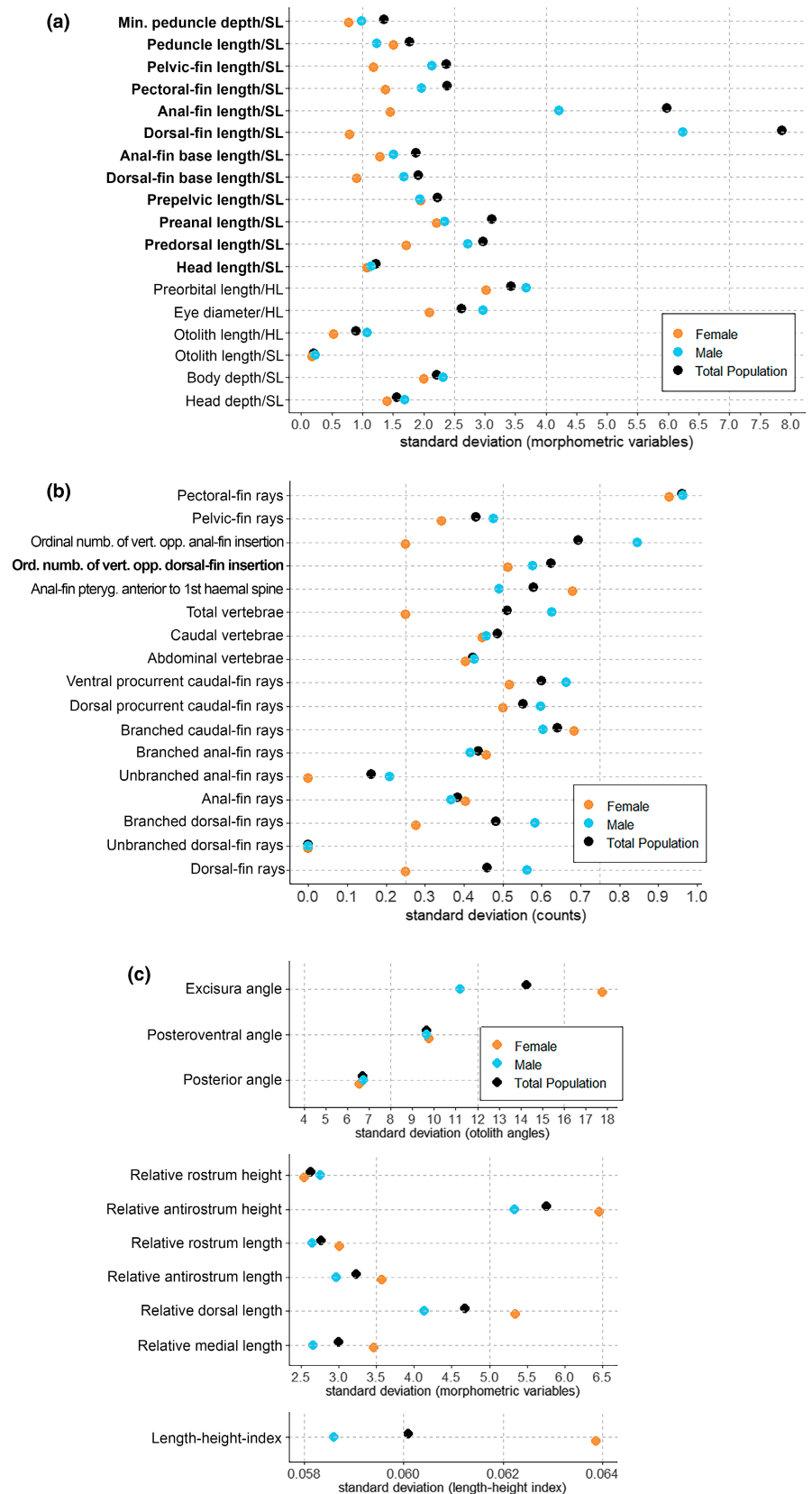


FIGURE 3 Scatter plots of standard deviations of the studied variables for the total population, the females and the males of *Aphanicops stoliczkanus* from Al-Bahayez, Oman. (a) Variables related to body morphometry and maximum otolith length; (b) meristic counts; (c) variables related to otolith morphometry. Variables indicated in bold differ significantly between the sexes (T -test, $p < .01$ for morphometric, Mann–Whitney test, $p < .01$ for meristic variables). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

markedly between the sexes (anal-fin length in females vs. males 18.0 vs. 28.0% SL; dorsal-fin length in females vs. males 21.0 vs. 33.5% SL), are striking exceptions (Table 1). Notably, these two variables are the only ones that also display significantly different variances between the sexes (Figure 4c,d, Table 1).

Among the meristic variables, only the ordinal number of the vertebra opposite to the dorsal-fin insertion differs significantly between males and females (Figure 3b, Table 1, Figure S2). Significant dimorphism in terms of the variance is solely detected in the ordinal number of the vertebra opposite to the anal-fin insertion (Table 1).

3.3 | Variation in otolith morphology

The general otolith morphology of the here studied specimens of *A. stoliczkanus* from Wadi Al Bahayez is consistent with the description provided by Bidaye et al. (2022) on the otoliths from the same site, but from a different sampling campaign. Most of the otoliths have a rounded-triangular or rounded-trapezoid shape (Figure 5). Usually, the rostrum is slightly longer than the antirostrum, and the excisura is deep and narrow. The sulcus is long and terminally inclined towards the ventral rim. The dorsal margin shows a prominent tip in most specimens. The ventral margin is weakly to moderately crenulated (Figure 5).

3.3.1 | Total population

There is considerable variation in the general shape, the lengths of rostrum and antirostrum, and the projection of the dorsal tip or the excisura (Figure 5). Some otoliths reveal very obvious deviations, for example an unusually long rostrum (BAH18), an almost straight dorsal margin (BAH5), a very shallow excisura or an excisura with extrusions (BAH25 and BAH37), and also in the overall outline (BAH16 and BAH3; Figure 5). The observed otolith variability is at least partially reflected in the relatively high standard deviation of the otolith morphometric variables related to the antirostrum height and dorsal length (Figure 3c, Table 1).

3.3.2 | Females, males

Contrary to body morphometric and meristic traits, most otolith variables display greater variability in females than in males, with equal variance between females and males restricted to the posteroventral and posterior angle and the rostrum height (Figure 3c).

3.4 | Sex dimorphism in otolith morphology

Sex dimorphism in otolith morphology was not detectable based on the visual inspection of the SEM images of all specimens (see also Figure 5). Likewise, none of the otolith variables reveal significantly different means or variances between females and males (Table 1).

3.5 | Effect of sex dimorphism on total population variability (all traits)

For each sexually dimorphic morphometric variable, as well as for the sole dimorphic meristic trait, the standard deviation for the total population is greater than the variance observed for each sex (Figure 3a,b). In contrast, not sexually dimorphic morphometric and meristic traits and also the otolith variables reveal standard deviations for the total population that generally lie between the values for the sexes (Figure 3a-c).

4 | DISCUSSION

Analysis of intrapopulation variability tells us which traits vary significantly, and one can then ask why this is so. Based on the results of this study, our specimens of *A. stoliczkanus* from the coastal site Al-Bahayez show clear signs of sex dimorphism in most external morphometric characters, the most striking of which are the lengths and positions of the anal and dorsal fins (i.e. anal- and dorsal-fin lengths, preanal and predorsal lengths). Meristic characters show little overall variability; the only sexually dimorphic meristic character (i.e. the ordinal number of the vertebra opposite the dorsal-fin insertion) is related to a sexually dimorphic morphometric trait (i.e. predorsal length). The absence of sex dimorphism in most meristic traits is consistent with the results of previous studies of Aphaniidae (e.g. Esmaeili et al., 2014; Gholami et al., 2014, 2015; Teimori et al., 2014, 2018; Teimori, Iranmanesh, et al., 2021). Otoliths are highly variable, but no sex dimorphism is detectable.

4.1 | Evidence for sex dimorphism in morphometric body characters in previous publications

Morphometric data derived from each of the sexes are available in the literature for several aphaniid species (e.g. Esmaeili et al., 2014; Gholami et al., 2014,

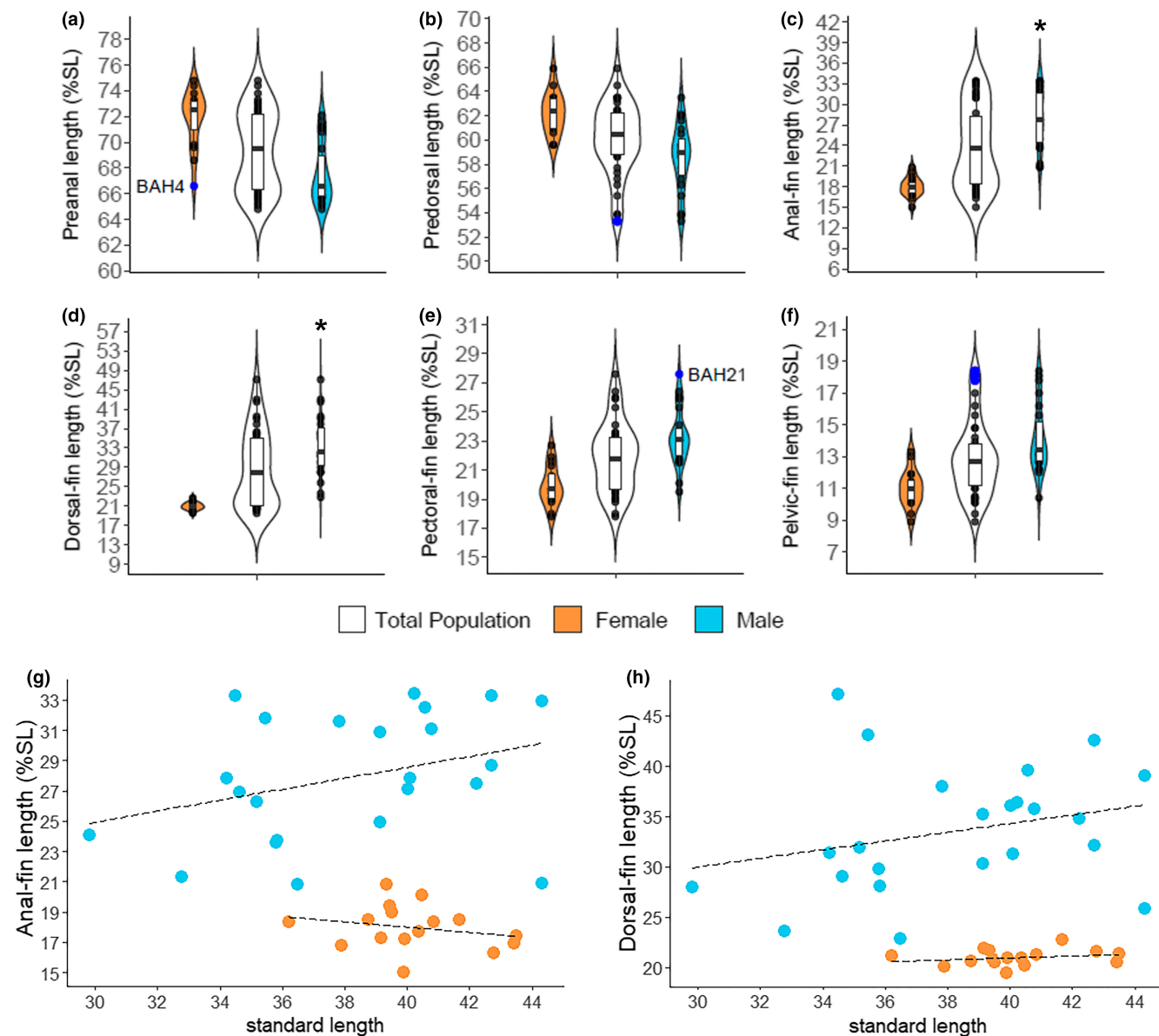


FIGURE 4 (a–f) Violin plots of sexually dimorphic variables (T -test, $p < .01$) of *Aphaniops stoliczkanus* that revealed clearly distant mean values between females and males. Note the significantly different variances of the standard deviation (indicated with *) between sexes in anal-fin length (c) and dorsal-fin length (d) (Levene test, $p < .01$). Blue dots represent outliers. (g, h) scatter plots of anal- and dorsal-fin lengths (% SL) of females and male specimens. The trend lines are based on a general linear model (glm) algorithm. [Colour figure can be viewed at wileyonlinelibrary.com]

2015; Teimori, Esmaili, et al., 2012; Teimori, Schulz-Mirbach, et al., 2012; Teimori et al., 2014, 2018; Teimori, Iranmanesh, et al., 2021). However, only the study by Teimori, Iranmanesh, et al. (2021) focused on sex dimorphism in body morphometric traits. In the other studies, sex dimorphism was recorded in the context of descriptions of species and populations but statistical significance of differences was not tested and in no case were all four of the traits highlighted here (anal- and dorsal-fin lengths, preanal and predorsal lengths) included. We used the mean values reported in these studies for females and/or

males to assess whether results similar to those obtained in our work can be inferred.

4.1.1 | Data on preanal and predorsal lengths

Teimori, Iranmanesh, et al. (2021), who addressed the issue of sex dimorphism in morphological traits in *Aphaniops hormuzensis*, recorded significant dimorphism in the preanal and predorsal lengths for specimens from a hot spring habitat (both traits) and from an urban canal

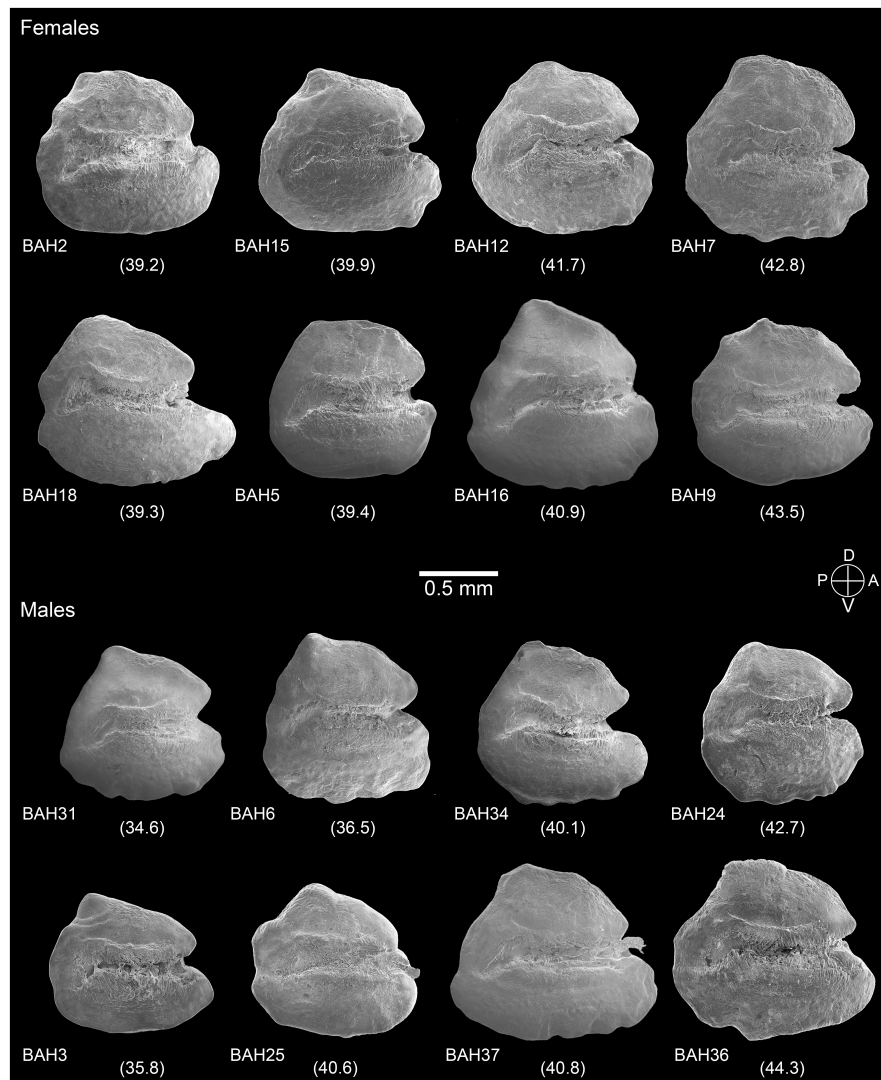


FIGURE 5 General morphology (1st and 3rd rows) and variants (2nd and 4th rows) of otoliths from females and males of *Aphaniops stoliczkanus* from Al-Bahayez, Oman. Number in brackets represents the standard length of the corresponding fish individual in mm.

(preanal length only), while no such differences were found in a sample from a saline river. As in our study, sex dimorphism—when present—was expressed in a more posterior position of anal and dorsal fins in females. Data for two other species of *Aphaniops*, that is *A. furcatus* and *A. ginaonis*, also reveal slightly more posterior positions of the anal and dorsal fins in females than in males (Teimori et al., 2014: table 1). The same trend is found in most of the inland species of *Aphaniops* (*Esmaeilius* according to Freyhof & Yoğurtçuoğlu, 2020) in Iran (e.g. Esmaeili et al., 2014; Gholami et al., 2014; Teimori, Esmaeili, et al., 2012). Notably, in three of the inland *Aphaniops* species, a relatively clear difference in preanal length has been reported, namely in *A. shirini* (70.5 vs. 66.9% SL in females vs. males), *A. isfahanensis* (71.5 vs. 66.8% SL) and *A. farsicus* (71.0 vs. 68.6% SL), while predorsal lengths in these species did not show such clear differences (Gholami et al., 2014: table 2). However, exceptions to this general trend can also occur as predorsal lengths in *A. pluristriatus*

males exceed those seen in females (64.8 vs. 61.0% SL in males vs. females; Esmaeili et al., 2014: table 2).

4.1.2 | Data on anal- and dorsal-fin lengths

No difference between the sexes in the anal-fin length is detectable in three species of *Aphaniops* from southern Iran (*A. stoliczkanus*, *A. hormuzensis*, *A. ginaonis*; Teimori et al., 2018: table S2). Solely in *A. furcatus*, mean values of anal-fin lengths are slightly higher for males than for females (9.7 vs. 8.7% SL; Teimori et al., 2014: table 1). On the contrary, in the Iranian inland species of *Aphaniops*, males have slightly longer anal and dorsal fins than females do (as in our study), but only two species show a relatively clear sex-related difference in one of those traits (anal-fin length in *A. darabensis*, 15.5 vs. 12.1% SL in males vs. females; dorsal-fin length in *A. cf. pluristriatus*, 17.7 vs. 14.9% SL in males vs. females; see Esmaeili et al., 2014:

table 1; Gholami et al., 2014: table 2). However, in none of these species were the differences in anal- or dorsal-fin lengths between the sexes as marked as those observed in *A. stoliczkanus* in our study.

One possible reason why the expression and degree of sex dimorphism in these characters differ among aphaniiid species and populations may be found in variations in their habitats. Our specimens, which display distinctive sex dimorphism, originate from a coastal marsh environment, which can be considered to be an optimal environment for *Aphaniops* (Wildekamp, 1993). Optimal habitats favour fitness of specimens, while more stressful environments will have negative effects on the physical condition of the fish, which can eventually suppress sex dimorphism in morphometric body traits due to metabolic constraints (see Bonduriansky, 2007). However, also predation pressures, reproductive cycles and life history may impact fitness in a killifish, with low predation pressure favouring increase of sexual dimorphism in fin sizes (Sowersby et al., 2021). Based on our field observations, a cichlid, the sailfin molly (*Poecilia latipinna*) and the Arabian goby (*Cryptocentroides arabicus*) co-occur with *A. stoliczkanus* in Al Bahayez, but if any of these species is preying on *A. stoliczkanus* is not known. Likewise, information on predation pressure is rarely available for the ephemeral and hot sulfuric streams of the Iranian killifish habitats. Thus, future research should be done on other aphaniiid populations from different habitats to fully understand the interrelation between sexual and natural selection, and how sexual dimorphism is affecting morphological variation.

4.2 | Are anal- and dorsal-fin lengths sexually selected in *Aphaniops stoliczkanus*?

Our data reveal the presence of clear-cut sex dimorphism in the anal- and dorsal-fin lengths in the sample of *A. stoliczkanus* from Wadi Al-Bahayez. Not only do the mean values differ clearly between the sexes (Table 1), so too does the variance of these traits (Figure 3a), which has not been reported before for any aphaniiid species. Theory predicts that sexually selected characters usually exhibit greater variability in the sex in which this character is subject to selection (Zajitschek et al., 2020). In the case of our study, males of *A. stoliczkanus* present significantly greater variance in the anal- and dorsal-fin lengths than the females (Figure 4c,d). This suggests that the fins of the males are under sexual selection; males with longer fins may be more successful during courtship and/or may be at an advantage in antagonistic interactions with competitors. Observations on *A. stoliczkanus* in their natural habitats in the Hajar Mountains (United Arab Emirates)

are in agreement with this idea, as mating males cruise with highly extended dorsal fin in wide circles around the females (observation by BR).

Moreover, it is known that the variability of sexually selected traits can be controlled by variations in the physical condition of the fish, a form of developmental plasticity, with smaller individuals usually showing less sex dimorphism than larger ones (Bonduriansky, 2007). This is also seen in the male specimens of our data set, although some outliers can be observed (Figure 4g,h). Outliers include some large males with relatively short fin lengths (in % SL) similar to those of females, as well as some small males that had the relatively longest fins (in % SL) of all (Figure 4g,h). These exceptions may be related to differences in environmental conditions during growth or in the intensity of male–male competition, which can have an impact on the allocation of resources to body growth or sexual ornaments (dorsal- and anal-fin size), the latter being a metabolically cheaper way to increase perceived size than increasing total body size (Rosenthal & Evans, 1998; Sowersby et al., 2021). Furthermore, a relatively slow growth rate of the anal fin resulting in negative allometry between body size and anal-fin length has been noted in females of the killifish *Nothobranchius orthonotus* (suborder Aplocheiloidei; Vrťilek & Reichard, 2016). Similar allometry appears to be present in females of *A. stoliczkanus* (Figure 4g), but statistical analysis was not robust enough to confirm its significance. Future research with a higher sample size in both sexes (for static allometry) and different size classes (for ontogenetic allometry) should be considered.

4.3 | Otolith variability

Previous work on the otolith morphometry of *A. hormuzensis* has shown that sex dimorphism occurs in the otolith length/head length ratio, otolith height/head depth ratio and in antirostrum height and length, and that the highest variability is present in antirostrum height, dorsal-, medial- and rostrum lengths (Teimori, Motamedi, et al., 2021). Another study of otolith morphology in *A. furcatus* found sex dimorphism in the antirostrum- and rostrum lengths in adult specimens (Motamedi et al., 2021). The variation observed in these populations has been explained as the result of possible social and spatial segregation due to possible differences in lifestyles and habitat occupation of each sex (see Ruckstuhl, 2007). In our analysis, the otoliths revealed no differences between sexes, as was also found for *A. ginaonis* (Reichenbacher, Kamrani, et al., 2009), but we found high levels of variability in overall otolith shape, antirostrum height and length, and in the excisura angle

(Figures 3c and 5). Since all studied specimens were of similar size, the variability in these characters cannot be attributed to ontogenetic variation (Reichenbacher, Kamrani, et al., 2009; Teimori, Iranmanesh, et al., 2021). It may be linked to the ecological diversity of their habitat and/or genetic connectivity with other populations from ecologically diverse habitats in the nearby mountains, as has previously been suggested by Bidaye et al. (2022).

4.4 | Effect of sex dimorphism on overall variability

Aside from the anal- and dorsal-fin lengths and positions, other body morphometric traits also differed between the sexes, albeit to a lesser extent, with the means and variances not differing greatly between the sexes (Figure 3a, Table 1). Additionally, the variances of these traits (within the sexes and overall) were similar to those of traits that did not differ significantly between the sexes (Figure 3a). Notably, variances for some of the latter (i.e. preorbital length and eye diameter) were greater than those measured for some dimorphic traits (Figure 3a). It thus appears that sex dimorphism does not affect the overall variance of the population unless it is particularly pronounced. This has been previously noted by Mallon (2017) in a study of nonavian dinosaurs, where he concluded that, unless a significant dimorphism is present, it will not be possible to discriminate between the sexes due to overlap in morphospace between them.

4.5 | Implications for fossil killifish

Understanding morphological variability in extant populations is also relevant for studies of fossil species. In our study, the dorsal- and anal-fin bases of *A. stoliczkanus* were found to be sexually dimorphic and revealed a pattern of variance between the sexes similar to that observed in the dorsal- and anal-fin length, although not as pronounced (Figure 3a). Previous work on two extinct killifishes (†*Prolebias* Sauvage, 1874, †*Paralebias* Gaudant, 2013) reported that the dorsal- and anal-fin bases were among the most variable traits (Frey et al., 2016), but sex dimorphism was not considered as possible reason. We assume that the fin bases could be a good substitute for evaluating possible sex dimorphism in the corresponding fins of a fossil species, where fins are often distorted or missing. Detection of sex dimorphism in fossil assemblages avoids over estimation of species numbers and thus helps to better understand past diversity and biogeographic and evolutionary patterns of a lineage.

5 | CONCLUSIONS

Intrapopulation variability is usually the outcome of genetic diversity, ecological flexibility, patterns of ontogeny (life history) and sex dimorphism. Here, we present detailed information regarding sex dimorphism in morphological traits in a population of *Aphaniops stoliczkanus*. Sex dimorphism was most marked in four morphometric body traits related to the anal and dorsal fins. Among these, anal- and dorsal-fin lengths showed particularly pronounced dimorphism, with males having significantly longer fins than females. Moreover, these two traits were the only variables for which also the variances showed sex dimorphism, with high variance in males and markedly low variance in females. Based on these results, it can be assumed that anal- and dorsal-fin sizes of *A. stoliczkanus* males are subject to sexual selection; males with longer fins may be more competitive during courtship behaviour. Our data provide the first evidence of sexually selected traits in a species of the Aphaniidae and support the hypothesis of Teimori, Esmaeili, et al. (2012) that sexual selection plays a role in the diversification of aphaniid species. Our results will be relevant for future research regarding the role of sexual selection in aphaniid speciations, as well as for interpretation of killifish palaeodiversity and evolution.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

- Aguirre, W. E., Ellis, K. E., Kusenda, M., & Bell, M. A. (2008). Phenotypic variation and sex dimorphism in anadromous threespine stickleback: Implications for postglacial adaptive radiation. *Biological Journal of the Linnean Society*, 95(3), 465–478. <https://doi.org/10.1111/j.1095-8312.2008.01075.x>
- Arratia, G. (2008). Actinopterygian postcranial skeleton with special reference to the diversity of fin ray elements, and the problem of identifying homologies. In G. Arratia, H.-P. Schultze, & M. V. H. Wilson (Eds.), *Mesozoic fishes – Systematics, homology and nomenclature* (Vol. 4, pp. 49–101). Verlag Dr. Friedrich Pfeil.
- Barker, D., Allan, G., Rowland, S., & Pickles, J. (2002). *A guide to acceptable procedures and practices for aquaculture and fisheries research. State of New South Wales through Department of Industry and Investment (Industry & Investment NSW)*. Port Stephens.
- Bidaye, R. G., Al-Jufaili, S. M., Charmpila, E. A., Jawad, K., Vukić, J., & Reichenbacher, B. (2022). Possible links between phenotypic variability, habitats and connectivity in the killifish *Aphaniops stoliczkanus* in Northeast Oman. *Acta Zoologica*. <https://doi.org/10.1111/azo.12428>
- Birdsong, R. S., Murdy, E. O., & Pezold, F. L. (1988). A study of the vertebral column and median fin osteology in gobioid old fishes with comments on gobioid relationships. *Bulletin of Marine Science*, 42(2), 174–214.
- Bolnick, D. I., & Doebeli, M. (2003). Sex dimorphism and adaptive speciation: Two sides of the same ecological coin. *Evolution*, 57(11), 2433–2449. <https://doi.org/10.1111/j.0014-3820.2003.tb01489.x>
- Bonduriansky, R. (2007). The evolution of condition-dependent sexual dimorphism. *The American Naturalist*, 169(1), 9–19. <https://doi.org/10.1086/510214>
- Bragança, P. H. N., & Costa, W. J. E. M. (2019). Multigene fossil-calibrated analysis of the African lampeyes (Cyprinodontidae: Procatopodidae) reveals an early Oligocene origin and Neogene diversification driven by palaeogeographic and palaeoclimatic events. *Organisms, Diversity & Evolution*, 19, 303–320. <https://doi.org/10.1007/s13127-019-00396-1>
- Bragança, P. H. N., Amorim, P. F., & Costa, W. J. E. M. (2018). Pantanodontidae (Teleostei, Cyprinodontiformes), the sister group to all other cyprinodontoid killifishes as inferred by molecular data. *Zoosystematics and Evolution*, 94, 137–145. <https://doi.org/10.3897/zse.94.22173>
- Charmpila, E. A., Teimori, A., Freyhof, J., Weissenbacher, A., & Reichenbacher, B. (2020). New osteological and morphological data of four species of *Aphaniops* (Teleostei; Aphaniidae). *Journal of Applied Ichthyology*, 36(5), 724–736. <https://doi.org/10.1111/jai.14074>
- Chiozzi, G., Stiassny, M. L. J., Alter, S. E., De Marchi, G., Mebrahtu, Y., Tessema, M., Asmamaw, B., Fasola, M., & Bellati, A. (2018). Fishes in the desert: Mitochondrial variation and phylogeography of *Danakilia* (Actinopterygii: Cichlidae) and *Aphaniops* (Actinopterygii: Cyprinodontidae) in the Danakil depression of northeastern Africa. *Mitochondrial DNA Part A*, 29(7), 1025–1040. <https://doi.org/10.1080/24701394.2017.1404043>
- Esmaeili, H. R., Teimori, A., Gholami, Z., & Reichenbacher, B. (2014). Two new species of the tooth-carp *Aphaniops* (Teleostei: Cyprinodontidae) and the evolutionary history of the Iranian inland and inland-related *Aphaniops* species. *Zootaxa*, 3786, 246–268. <https://doi.org/10.11646/zootaxa.3786.3.2>
- Esmaeili, H. R., Teimori, A., Zarei, F., & Sayyadzadeh, G. (2020). DNA barcoding and species delimitation of the Old World tooth-carps, family Aphaniidae Hoedeman, 1949 (Teleostei: Cyprinodontiformes). *PLoS One*, 15(4), e0231717. <https://doi.org/10.1371/journal.pone.0231717>
- Ferrito, V., Pappalardo, A. M., Canapa, A., Barucca, M., Doadrio, I., Olmo, E., & Tigano, C. (2013). Mitochondrial phylogeography of the killifish *Aphaniops fasciatus* (Teleostei, Cyprinodontidae) reveals highly divergent Mediterranean populations. *Marine Biology*, 160(12), 3193–3208. <https://doi.org/10.1007/s00227-013-2307-4>
- Frey, L., Maxwell, E. E., & Sánchez-Villagra, M. R. (2016). Intraspecific variation in fossil vertebrate populations: Fossil killifishes (Actinopterygii: Cyprinodontiformes) from the Oligocene of Central Europe. *Palaeontologia Electronica*, 19(2), 14A. <https://doi.org/10.26879/628>
- Freyhof, J., Weissenbacher, A., & Geiger, M. (2017). *Aphaniops kruppi*, a new killifish from Oman with comments on the *A. dispar* species group (Cyprinodontiformes: Aphaniidae). *Zootaxa*, 4338(3), 557–573. <https://doi.org/10.11646/zootaxa.4338.3.10>
- Freyhof, J., & Yoğurtcuoğlu, B. (2020). A proposal for a new generic structure of the killifish family Aphaniidae, with the description of *Aphaniops teimorii* (Teleostei: Cyprinodontiformes). *Zootaxa*, 4810(3), 421–451. <https://doi.org/10.11646/zootaxa.4810.3.2>
- Gaudant, J. (2009). Occurrence of the genus *Aphaniops* Nardo (cyprinodontid fishes) in the lower Miocene of the Cheb Basin (Czech Republic), with additional notes on *Prolebias egeranus* Laube. *Journal of the National Museum (Prague), Natural History Series*, 177(8), 83–90.
- Gaudant, J. (2011). *Aphaniops persicus* (Priem, 1908) (Pisces, Teleostei, Cyprinodontidae): une nouvelle combinaison pour *Brachylebias persicus* Priem, 1908, du Miocène supérieur des environs de Tabriz (Iran). *Geodiversitas*, 33(2), 347–356. <https://doi.org/10.5252/g2011n2a9>
- Gaudant, J. (2012). Révision de *Prolebias stenoura* Sauvage, 1874 du Stampien (=Rupélien) de Limagne (centre de la France), espèce type du genre *Prolebias* (poisson téléostéen, Cyprinodontiformes). *Geodiversitas*, 34, 409–423. <https://doi.org/10.5252/g2012n2a9>
- Gholami, Z., Esmaeili, H. R., Erpenbeck, D., & Reichenbacher, B. (2014). Phylogenetic analysis of *Aphaniops* from the endorheic Kor River basin in the Zagros Mountains, South-Western Iran (Teleostei: Cyprinodontiformes: Cyprinodontidae). *Journal of Zoological Systematics and Evolutionary Research*, 52(2), 130–141. <https://doi.org/10.1111/jzs.12052>
- Gholami, Z., Esmaeili, H. R., Erpenbeck, D., & Reichenbacher, B. (2015). Genetic connectivity and phenotypic plasticity in the cyprinodont *Aphaniops farsicus* from the Maharlu Basin, South-Western Iran. *Journal of Fish Biology*, 86(3), 882–906. <https://doi.org/10.1111/jfb.12599>
- Gonzalez, E. G., Cunha, C., Ghanavi, H. R., Oliva-Paterna, F. J., Torralva, M., & Doadrio, I. (2018). Phylogeography and

- population genetic analyses in the Iberian Toothcarp (*Aphanius iberus* Valenciennes, 1846) at different time scales. *Journal of Heredity*, 109(3), 253–263. <https://doi.org/10.1093/jhered/esx076>
- Hedrick, A. V., & Temeles, E. J. (1989). The evolution of sex dimorphism in animals: Hypotheses and tests. *TREE*, 4(5), 136–138.
- Herler, J., Kerschbaumer, M., Mitteroecker, P., Postl, L., & Sturmbauer, C. (2010). Sex dimorphism and population divergence in the Lake Tanganyika cichlid fish genus *Tropheus*. *Frontiers in Zoology*, 7, 4. <https://doi.org/10.1186/1742-9994-7-4>
- Holčík, J. (1989). Part II. General introduction to fishes Acipenseriformes. In *The freshwater fishes of Europe* Vol. 1 (1st ed.). AULA-Verlag.
- Hrbek, T., & Meyer, A. (2003). Closing of the Tethys Sea and the phylogeny of Eurasian killifishes (Cyprinodontiformes - Cyprinodontidae). *Journal of Evolutionary Biology*, 16, 17–36.
- Kalioztopoulou, A., Carretero, M. A., & Adams, D. C. (2015). Ecomorphological variation in male and female wall lizards and the macroevolution of sex dimorphism in relation to habitat use. *Journal of Evolutionary Biology*, 28(1), 80–94. <https://doi.org/10.1111/jeb.12540>
- Littleford-Colquhoun, B. L., Clemente, C., Thompson, G., Cristescu, R. H., Peterson, N., Strickland, K., Stuart-Fox, D., & Frere, C. H. (2019). How sexual and natural selection shape sexual size dimorphism: Evidence from multiple evolutionary scales. *Functional Ecology*, 33(8), 1446–1458. <https://doi.org/10.1111/1365-2435.13337>
- Loureiro, M., Sá, R. d., Serra, S. W., Alonso, F., Lanés, L. E. K., Volcan, M. V., Calviño, P., Nielsen, D., Duarte, A., & Garcia, G. (2018). Review of the family Rivulidae (Cyprinodontiformes, Aplocheiloidei) and a molecular and morphological phylogeny of the annual fish genus *Austrolebias* Costa 1998. *Neotropical Ichthyology*, 16(3), e180007. <https://doi.org/10.1590/1982-0224-20180007>
- Mallon, J. C. (2017). Recognizing sex dimorphism in the fossil record: Lessons from nonavian dinosaurs. *Paleobiology*, 43(3), 495–507. <https://doi.org/10.1017/pab.2016.51>
- Martínez-Ruiz, C., & Knell, R. J. (2017). Sexual selection can both increase and decrease extinction probability: Reconciling demographic and evolutionary factors. *Journal of Animal Ecology*, 86(1), 117–127. <https://doi.org/10.1111/1365-2656.12601>
- Martínez, C. M., Kao, B. H., Sparks, J. S., & Wainwright, P. C. (2019). Pectoral dimorphism is a pervasive feature of skate diversity and offers insight into their evolution. *Integrative Organismal Biology*, 1–14. <https://doi.org/10.1093/iob/obz012>
- Miller, R. R. (1948). The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada. *Miscellaneous publications Museum of Zoology*, 68, 7–196.
- Motamedi, M., Teimori, A., & Iranmanesh, A. (2021). Ontogenetic pattern, morphological sexual and side dimorphism in the saccular otolith of a scaleless killifish *Aphanius furcatus* (Teleostei: Aphaniidae). *Acta Zoologica*, 102(1), 38–50. <https://doi.org/10.1111/azo.12313>
- Parenti, L. R. (1981). A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). *Bulletin of the AMNH*, 168(4), 335–557.
- Pélabon, C., Hilde, C. H., Einum, S., & Gamelon, M. (2020). On the use of the coefficient of variation to quantify and compare trait variation. *Evolution Letters*, 4(3), 180–188. <https://doi.org/10.1002/evl3.171>
- Pohl, M., Milvertz, F., Meyer, A., & Vences, M. (2015). Multigene phylogeny of cyprinodontiform fishes suggests continental radiations and a rogue taxon position of *Pantanodon*. *Vertebrate Zoology*, 65(1), 37–44.
- R Core Team. (2021). *A language and environment for statistical computing*. 4.0.3 ed. R Foundation for Statistical Computing.
- Reichenbacher, B., & Kowalke, T. (2009). Neogene and present-day zoogeography of killifishes (*Aphanius* and *Aphanolebias*) in the Mediterranean and Paratethys areas. *Palaeogeography Palaeoclimatology Palaeoecology*, 281(1–2), 43–56. <https://doi.org/10.1016/j.palaeo.2009.07.008>
- Reichenbacher, B., Sienknecht, U., Kuchenhoff, H., & Fenske, N. (2007). Combined otolith morphology and morphometry for assessing taxonomy and diversity in fossil and extant killifish (*Aphanius*, *Prolebias*). *Journal of Morphology*, 268(10), 898–915. <https://doi.org/10.1002/jmor.10561>
- Reichenbacher, B., Feulner, G. R., & Schulz-Mirbach, T. (2009). Geographic variation in otolith morphology among freshwater populations of *Aphanius dispar* (Teleostei, Cyprinodontiformes) from the southeastern Arabian peninsula. *Journal of Morphology*, 270(4), 469–484. <https://doi.org/10.1002/jmor.10702>
- Reichenbacher, B., Kamrani, E., Esmaeili, H. R., & Teimori, A. (2009). The endangered cyprinodont *Aphanius ginaonis* (Holly, 1929) from southern Iran is a valid species: Evidence from otolith morphology. *Environmental Biology of Fishes*, 86, 507–521. <https://doi.org/10.1007/s10641-009-9549-5>
- Rosenthal, G. G., & Evans, C. S. (1998). Female preference for swords in *Xiphophorus helleri* reflects a bias for large apparent size. *Proceedings of the National Academy of Sciences, United States of America*, 95(8), 4431–4436. <https://doi.org/10.1073/pnas.95.8.4431>
- Ruckstuhl, K. E. (2007). Sexual segregation in vertebrates: Proximate and ultimate causes. *Integrative and Comparative Biology*, 47(2), 245–257. <https://doi.org/10.1093/icb/icm030>
- Ruckstuhl, K. E., & Clutton-Brock, T. H. (2005). Sexual segregation and the ecology of the two sexes. In K. E. Ruckstuhl & P. Neuhaus (Eds.), *Sexual segregation in vertebrates: Ecology of the two sexes* (pp. 3–6). Cambridge University Press. <https://doi.org/10.1017/cbo9780511525629>
- Scott, S., Rojas, P., & Vila, I. (2020). Meristic and morphological differentiation of *Orestias* species (Teleostei; Cyprinodontiformes) from the southern Altiplano. *Environmental Biology of Fishes*, 103(8), 939–951. <https://doi.org/10.1007/s10641-020-00995-4>
- Signorell, A., Aho, K., Alfons, A., Anderegg, N., Aragon, T., Arachchige, C., Arppe, A., Baddeley, A., Barton, K., Bolker, B., Borchers, H. W., Caeiro, F., Champely, S., Chessel, D., Chhay, L., Cooper, N., Cummins, C., Dewey, M., Doran, H. C., ... Zeileis, A. (2021). *DescTools: Tools for descriptive statistics*. R package version 0.99.42, <https://cran.r-project.org/package=DescTools>
- Sowersby, W., Eckerstrom-Liedholm, S., Rowinski, P. K., Balogh, J., Eiler, S., Upstone, J. D., Gonzalez-Voyer, A., & Rogell, B. (2021). The relative effects of pace of life-history and habitat characteristics on the evolution of sexual ornaments: A comparative assessment. *Evolution*, 76, 114–127. <https://doi.org/10.1111/evo.14358>
- Teimori, A., & Esmaeili, H. R. (2020). Axial skeleton morphology of the Western palearctic aphaniid fishes (Teleostei: Cyprinodontiformes; family: Aphaniidae). *Acta Zoologica*, 103(3), 282–296. <https://doi.org/10.1111/azo.12370>

- Teimori, A., Esmaili, H. R., Gholami, Z., Zarei, N., & Reichenbacher, B. (2012). *Aphanius arakensis*, a new species of tooth-carp (Actinopterygii, Cyprinodontidae) from the endorheic Namak Lake basin in Iran. *Zookeys*, 215, 55–76. <https://doi.org/10.3897/zookeys.215.1731>
- Teimori, A., Schulz-Mirbach, T., Esmaili, H. R., & Reichenbacher, B. (2012). Geographical differentiation of *Aphanius dispar* (Teleostei: Cyprinodontidae) from southern Iran. *Journal of Zoological Systematics and Evolutionary Research*, 50(4), 289–304. <https://doi.org/10.1111/j.1439-0469.2012.00667.x>
- Teimori, A., Esmaili, H. R., Erpenbeck, D., & Reichenbacher, B. (2014). A new and unique species of the genus *Aphanius* Nardo, 1827 (Teleostei: Cyprinodontidae) from southern Iran: A case of regressive evolution. *Zoologischer Anzeiger - A Journal of Comparative Zoology*, 253(4), 327–337. <https://doi.org/10.1016/j.jcz.2013.12.001>
- Teimori, A., Esmaili, H. R., Hamidan, N., & Reichenbacher, B. (2018). Systematics and historical biogeography of the *Aphanius dispar* species group (Teleostei: Aphaniidae) and description of a new species from southern Iran. *Journal of Zoological Systematics and Evolutionary Research*, 56(4), 579–598. <https://doi.org/10.1111/jzs.12228>
- Teimori, A., Iranmanesh, N., Hesni, M. A., & Motamedi, M. (2021). Within-and among-population differentiation of *Aphaniops hormuzensis* from ecologically diverse environments (Cyprinodontiformes; Aphaniidae). *Acta Zoologica*, 102(4), 420–436. <https://doi.org/10.1111/azo.12350>
- Teimori, A., Motamedi, M., & Zeinali, F. (2021). Intrapopulation variation of otolith associated with ontogeny and morphological dimorphism in Hormuz tooth-carp *Aphanius hormuzensis* (Teleostei: Aphaniidae). *Acta Zoologica*, 102(3), 250–264. <https://doi.org/10.1111/azo.12332>
- Tigano, C., Canapa, A., Ferrito, V., Barucca, M., Arcidiacono, I., Deidun, A., Schembri, P. J., & Olmo, E. (2006). A study of osteological and molecular differences in populations of *Aphanius fasciatus* Nardo 1827, from the Central Mediterranean (Teleostei, Cyprinodontidae). *Marine Biology*, 149(6), 1539–1550. <https://doi.org/10.1007/s00227-006-0300-x>
- Van Valen, L. (2005). The statistics of variation. In B. Hallgrímsson & B. K. Hall (Eds.), *Variation* (p. 592). Elsevier Academic Press Publications. <https://doi.org/10.1016/B978-0-12-088777-4.X5000-5>
- Vasilyan, D., Reichenbacher, B., & Carnevale, G. (2009). A new fossil *Aphanius* species from the upper Miocene of Armenia (eastern Paratethys). *Palaeontologische Zeitschrift*, 83(4), 511–519. <https://doi.org/10.1007/s12542-009-0034-4>
- Villwock, W. (2004). Synopsis of classic and molecular investigations of Old World cyprinodontids of the genus *Aphanius* NARDO, 1827 (Teleostei: Cyprinodontidae), with special concern of the Anatolian species, their speciation phenomena and their probable historic development. *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut*, 101, 35–46.
- Vrtílek, M., & Reichard, M. (2016). Patterns of morphological variation among populations of the widespread annual killifish *Nothobranchius orthonotus* are independent of genetic divergence and biogeography. *Journal of Zoological Systematics and Evolutionary Research*, 54(4), 289–298. <https://doi.org/10.1111/jzs.12134>
- Wakefield, C. B., Boddington, D. K., & Newman, S. J. (2016). Rapid lateral extraction of otoliths that maintains the integrity of fish product to improve access to catches and reduce potential sampling biases. *The Open Fish Science Journal*, 9(1), 26–28. <https://doi.org/10.2174/1874401x01609010026>
- Walker, S. P., & McCormick, M. I. (2009). Sexual selection explains sex-specific growth plasticity and positive allometry for sexual size dimorphism in a reef fish. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 276(1671), 3335–3343. <https://doi.org/10.1098/rspb.2009.0767>
- Wasiljew, B. D., Pfaender, J., Wipfler, B., Gabelaia, M., Utama, I. V., Wantania, L. L., & Herder, F. (2021). Sex dimorphism in an adaptive radiation: Does intersexual niche differentiation result in ecological character displacement? *Ecology and Evolution*, 11(21), 14615–14629. <https://doi.org/10.1002/ece3.8137>
- Welsh, D., & Fuller, R. (2015). Influence of sex and habitat on the size and shape of anal and dorsal fins of the blackstripe topminnow *Fundulus notatus*. *Journal of Fish Biology*, 86(1), 217–227. <https://doi.org/10.1111/jfb.12564>
- Wildekamp, R. H. (1993). Atlas of the oviparous Cyprinodontiform fishes of the world. In B. R. Watters (Ed.), *A world of Killies: The genera Adamas, Adinia, Aphanius, Aphyoplatys and Aphyosemion* Vol. 1 (p. 67). The American killifish Association, Inc.
- Zajitschek, S. R., Zajitschek, F., Bonduriansky, R., Brooks, R. C., Cornwell, W., Falster, D. S., Lagisz, M., Mason, J., Senior, A. M., Noble, D. W., & Nakagawa, S. (2020). Sex dimorphism in trait variability and its eco-evolutionary and statistical implications. *eLife*, 9, e63170. <https://doi.org/10.7554/eLife.63170>

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