Morphology and Sexual Dimorphic Traits in the Scales and Fins of the Old-World Cyprinodontiform fish *Aphaniops sirhani* (Actinopterygii: Aphaniidae)

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Abstract: Sexual differences between males and females have been of special interest in the study of systematics/taxonomy, phenotypic evolution, and the farming of different invertebrate and vertebrate taxa including fishes. Sexual differentiation may be a result of natural selection, sexual selection, or a combination of the two. This study aims to examine the microscopic characteristics of scales and rays in the toothcarp *Aphaniops sirhani* (Cyprinodontiformes: Aphaniidae) that is endemic to Azraq wetland in northeastern Jordan. The study discusses the taxonomic and evolutionary significance of these structures and determines whether they can be used as secondary sexual dimorphism traits. The findings here indicate that the Azraq killifish males of the toothcarp have contact organs that exhibit sexual dimorphism in the form of spicule-like structures in the anal-fin rays and ctenus-like structures in the posterior margin of the scales. The contact organ variations in size, number, and location may offer a taxonomic and evolutionary signal for a deeper comprehension of the aphaniid species.

Keywords: Killifishes, toothcarps, sexual differences, ctenus-like structure, spicule-like structure, systematics

Introduction

Sexual differences between males and females have been of special interest in studying systematics/taxonomy, phenotypic evolution (Beltrán *et al*., 2022), and the farming of different invertebrate and vertebrate taxa including fishes. Sexual differentiation can arise from natural selection, sexual selection, or the combination of the two and can most likely be attributed to either the female mate choice or to the male-male competition (Moore *et al*., 1990; Abrahão *et al*., 2019; Beltrán *et al*., 2022). Sexual selection/secondary sexual dimorphism has triggered the evolution of remarkable morphological novelties among different groups of fishes: i) sexual size dimorphism (differences in the mean body size of adult male and female individuals). It includes female-biased sexual size dimorphism (females are larger than males e.g., *Gambusia holbrooki*, *Orestias gloriae*), and male-biased sexual size dimorphism (males are larger than females) e.g., *Iranocichla persa* and *I. hormuzensis*, ii) sexual colour dimorphism/dichromatism (differences in male and female coloration as observed in the genera *Aphanius*, *Aphaniops* and *Paraphanius*, iii) sexual shape dimorphism (comparative analysis of shape variation in males and females using geometric morphometrics or GM), e.g., *Caquetaia kraussi*, a cichlid fish with cryptic morphological behavior (Hernandez *et al*., 2022), iv) sexual structural dimorphism (differences in the presence or absence of a macrostructure: clasper, gonopodium, breeding tubercles, urogenital papilla in males and ovipositor in females (Esmaeili *et al*., 2017; Garcia and Zuanon, 2019; Esmaeili *et al*., 2020a); teeth variation as seen in the ray *Urotrygon microphthalmum* (de Sousa Rangel *et al*., 2016), and differences in the dentition on the fifth ceratobranchial of males and females of *O. gloriae* (Vila *et al*., 2011); gill glands in the mature males of Cheirodontinae; contact organs on the scales and fins of male profundulids (Velázquez-Velázquez *et al*., 2022; Esmaeili *et al*., 2023; Sungur *et al*. 2023); bony hooks along unbranched and

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anteriormost branched rays of the pelvic and anal fins of male *Tyttobrycon shibattai* (Abrahão *et al*., 2019), and differences in the length, width, and density of the dermal denticles on the pectoral fin, area posterior to the pectoral fin, caudal fin, and pelvic girdle of mature females and males of the lesser-spotted catshark, *Scyliorhinus canicula* (Crooks *et al*., 2013), v), sexual olfactory dimorphism (larger olfactory organs of mature males of characid fish *Tyttobrycon shibattai* (Abrahão *et al*., 2019), and vi), sexual glandular dimorphism (hypertrophied tissues, caudal fin gland, and caudal fin organ (see Malabarba and Weitzman, 1999; de Oliveira *et al*., 2012; Fukakusa, 2020).

In the order Cyprinodontiformes, secondary sexual dimorphism is associated with i) the occurrence of contact organs on scales and fins (Esmaeili *et al*., 2023; Sungur *et al*., 2023), ii) size differences and sexual dichromatism/colouration pattern (Esmaeili *et al*., 2020b; Velázquez-Velázquez *et al*., 2022), iii) specific structure e.g., gonopodium in males (Wiley and Collette 1970; Tripathi, 2018; Esmaeili *et al*., 2020b; Velázquez-Velázquez et al., 2022), and iv) otoliths of some aphaniid fishes (Motamedi *et al*., 2021; Teimori *et al*., 2021). However, comparatively, the contact organs have received less attention. These unique structures are found in some families currently classified in the order Cyprinodontiformes: Anablepidae, Cyprinodontidae, and Poeciliidae. Contact organs originate as bony dermal outgrowths of the scale margin or the ray (Wiley and Collette, 1970; Velázquez-Velázquez *et al*., 2022); they were first reported by Garman (1895) as “small spines appearing on the fins of males in several genera” of cyprinodontiform fishes during the breeding season (Wiley and Collette, 1970). The contact organs have been reported in some species of the family Aphaniidae classified in the genera *Aphanius*, *Aphaniops,* and *Paraphanius* based on Esmaeili *et al*., (2020b), or other genera (see Freyhof and Yoğurtçuoğlu, 2020) (Table 1).

Table 1. Taxonomic position of aphaniids mentioned in the present study based on Esmaeili et al. (2020), and Freyhof & Yoğurtcuoğlu (2020).

Esmaeili et al. (2020b)	**Freyhof & Yoğurtcuoğlu (2020)**
Aphanius anatoliae (Leidenfrost, 1912)	*Anatolichthys anatoliae* (Leidenfrost, 1912)
Aphanius chantrei (Gaillard, 1895)	*Anatolichthys chantrei* (Gaillard, 1895)
Aphanius darabensis Esmaeili, Teimori, Gholami & Reichenbacher, 2014	*Esmaeilius darabensis* (Esmaeili, Teimori, Gholami & Reichenbacher, 2014)
Aphanius iconii Akşiray, 1948	*Anatolichthys iconii* (Akşiray, 1948)
Aphanius marassantensis Pfleiderer, Geiger & Herder, 2014	*Anatolichthys marassantensis* (Pfleiderer, Geiger & Herder, 2014)
Aphanius meridionalis Akşiray, 1948	*Anatolichthys meridionalis* (Akşiray, 1948)
Aphanius shirini Gholami, Esmaeili, Erpenbeck & Reichenbacher, 2014	*Esmaeilius shirini* (Gholami, Esmaeili, Erpenbeck & Reichenbacher, 2014)
Aphanius sophiae (Heckel, 1847)	*Esmaeilius sophiae* (Heckel, 1847)
Aphanius villwocki Hrbek & Wildekamp, 2003	*Anatolichthys villwocki* (Hrbek & Wildekamp, 2003)
Aphaniops ginaonis (Holly, 1929)	*Aphaniops ginaonis* (Holly, 1929)
Aphaniops kruppi (Freyhof, Weissenbacher & Geiger, 2017)	*Aphaniops kruppi* (Freyhof, Weissenbacher & Geiger, 2017)
Aphaniops sirhani (Villwock, Scholl & Krupp, 1983)	*Aphaniops sirhani* (Villwock, Scholl & Krupp, 1983)
Aphaniops stoliczkanus (Day, 1872)	*Aphaniops stoliczkanus* (Day, 1872)
Paraphanius alexandri (Akşiray, 1948)	*Paraphanius alexandri* (Akşiray, 1948)
Paraphanius mento (Heckel, 1843)	*Paraphanius mento* (Heckel, 1843)
Paraphanius similis (Akşiray, 1948)	*Paraphanius similis* (Akşiray, 1948)

Materials and methods

Study Area

Azraq wetland is situated in northeastern Jordan (Figure 1), with an area of 74 km square featuring both seasonally flooded expanses and five permanently flooded and restored water bodies (Figure 2). It is the first Ramsar site in Jordan declared in 1977. It hosts the only endemic vertebrate in Jordan, namely the Azraq toothcarp *A. sirhani*. The whole area is protected and managed by the Royal Society for the Conservation of Nature as the Azraq Wetland Reserve where systematic monitoring and restoration programmes are carried out.
The climate in the Azraq region is harsh and arid, characterized by a hot and dry summer with temperatures reaching a height of 40 C°. Winters are somehow cool with temperatures reaching 0 C° sometimes. Water abstraction, habitat loss, and the existing of invasive species are the greatest threats facing the Azraq wetland and the ongoing restoration programmes.

Studied taxa

The scales and anal fin were removed from the alcohol fixed (70%) specimens collected from the Azraq Oasis, Jordan, 31°49’59.0”N, 36°49’19.1”E.

Scale preparation

Light microscopy

The scales were removed from six specific regions along the longitudinal axis of both

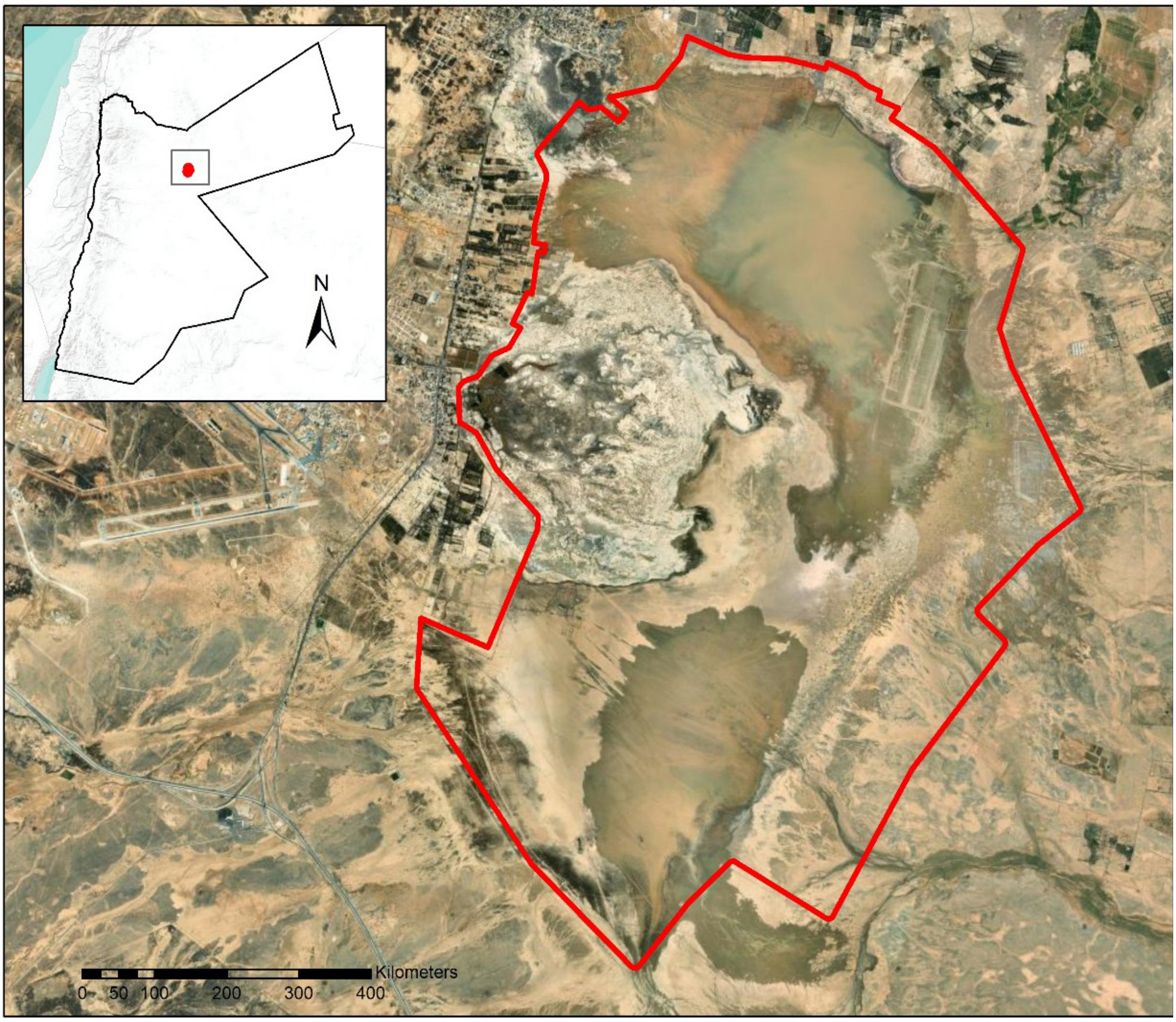

Figure 1. Location of the Azraq wetland in northeastern Jordan.

Figure 2. The permanent flooded type of habitats in the Azraq wetland.

male and female specimens. This extraction process was performed meticulously using tiny forceps, as seen in Figure (3). To prepare the scales used in this study, the researchers followed Lippitsch (1990), Esmaeili (2001), Gholami *et al*. (2013), Echreshavi *et al*. (2021), and Esmaeili *et al*. (2023). After removal, the scales were promptly rinsed with distilled water, subjected to a cleansing process, and were then immersed in a 1% potassium hydroxide (KOH) solution for forty minutes. This procedure aimed to eliminate the presence of soft and mucous tissues adhering to the scales' surface, using a suitable brush for this purpose. Subsequently, the scales underwent dehydration in a sequential series of ethanol solutions with concentrations of 30%, 50%, 70%, and 90% (with each step lasting thirty minutes), followed by thorough drying using filter paper. Finally, to mitigate the potential torsion of the scale edges, the scales were placed between two glass slides (Lippitsch, 1990; Figure. 3).

Scanning electron microscopy

As for the scanning electron microscopy, the scales were removed with fine forceps from the left side of the body, without damaging the scale (Lippitsch, 1990, 1995). Immediately after their removal under a dissecting microscope, the scales were rinsed in distilled water, and the adhering and irrelevant tissues were detached mechanically using a fine brush and were transferred into a 1% KOH solution for forty minutes to remove the soft tissues from the surface (Sadeghi *et al*., 2020; Echreshavi *et al*., 2021; Esmaeili *et al*., 2023, Sungur *et al*., 2023). After dehydration in 30, 50, 70, and 90% ethanol at thirty-minute intervals, the cleaned scales were dried on the Whatman filter papers, and to avoid curling the margins of the scales, they were immediately mounted on aluminium stubs using a double adhesive tape with the dorsal surface being upward. The stubs were coated with gold to a thickness of 100 Å in a gold coating unit.

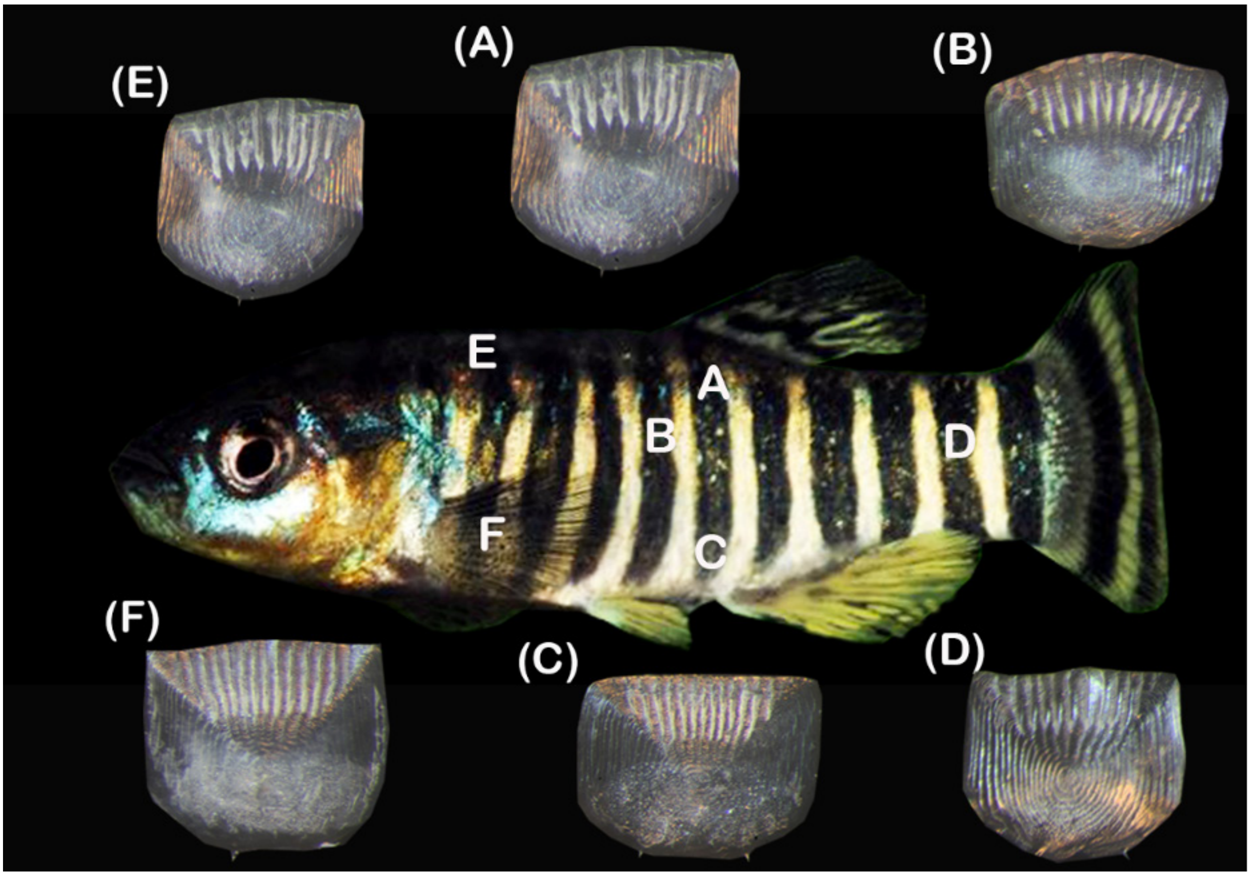

Figure 3: Image of male *Aphaniops sirhani* showing six different studied body regions where scales were removed from the left side of the fish. (A) Key scale below the dorsal fin; (B) middle of the body; (C) dorsal area of the pelvic fin; (D) caudal peduncle; (E) head region; (F) beneath the pectoral fin.

Digital imaging

The cleaned scales were then subjected to digital imaging using a Canon EOS 7D Camera connected to a computer for light microscopy. The gold coated scales were subjected to digital imaging using a TESCAN vega3 SEM instrument (Shiraz University, Iran) at 15 or 20 kV., and several images per scale were captured. The digital images were then used to do the morphological descriptions.

Fin preparation and digital imaging

The fins were removed, cleaned, and stained (bone alizarin and cartilage counter-stained with Alcian blue), following the technique recommended by Taylor and van Dyke (1985). The cleaned and stained fins were then subjected to digital imaging using a Canon EOS 7D Camera connected to the computer for light microscopy (Esmaeili *et al*., 2023).

Terminology of scales and fin rays

The terms used to describe the characteristics of scales and fins (Esmaeili *et al*., 2023) are as follows:

Contact organs: Dermal protrusions that are made of bone and are located on the posterior margin of the scales (ctenus-like structure on the posterior margin of the scales in males) or fin ray (spicule-like structure in the rays of the anal fin of males) (Figure. 4F).

Fields: The parts of the scale surface in the anterior, posterior, and two lateral parts (Figure. 4A).

Focus: The first area of the scale that appears. The geometrical position of the focus varies in different forms of scales and may be in the posterior, anterior, or posterior-central and central areas of the scale (Figure. 4A).

Circulus/circuli: Continuous concentric lines that approximately follow the outline of the scale and are commonly interrupted

by radii in the anterior part of the scale (Figure. 4C).*Radius/radii*: Groove/grooves that usually radiate from the focus to the edges (Figure. 4A).
Primary radii: Radii that extend from the focus to the edge of the scale (Figure. 4A).
Secondary radii: The radii that are formed with the distance from the center and toward the outer edge of the scale (Figure. 4A).
Tertiary radii: Radii that are positioned between the scale margin and the focus and are the shortest radii (Figure. 4A).
Ctenus/cteni: Tooth-like structure(s) that become ossified. The cteni appear in one or more rows on the margin of the posterior field (Figure. 4E).
Granules/tubercles: Protrusions of different shapes, sizes, and numbers are located on the posterior part of the scale (Figure. 4D).
Lepidonts: Small tooth-like structures that are located on the crown of circuli and have different shapes (Figure. 4B).
Sectioned scales: Scales with well-developed radii (Figure. 4).

Results

Light microscopy

The overall shapes of the scales in six body regions in *A. sirhani* are given in Figure (5). Scales were relatively large, of the cycloid type, with numerous radii (sectioned scale). These cycloid scales were further categorized into several subtypes being quadrilateral/square in all body regions of males, and quadrilateral/square in the A, B, and D regions, circular/true circular in the C region, and Intermediate/calyx in the E and F regions of the female specimens. The scales showed the general characteristics of the aphaniid scales. Each scale displayed a rostral field, two lateral fields, and a caudal field, with numerous circuli. The rostral field is embedded in the dermis, and only the caudal field is visible on the surface. The scales from all six regions demonstrated ctenus-like structures in males (Figure. 5), but these structures were not present in the scales of female individuals.

Scanning electron microscopy

The overall shapes of the scales in six body regions in *A. sirhani* are given in Figure 6. The scales were large, of the cycloid type, with numerous radii (sectioned scale), and presented general characteristics of aphaniid scales. Each scale displays a rostral field, two lateral fields, and a caudal field, with numerous circuli. Circuli in the rostral field were closely spaced and interrupted vertically by primary, secondary, and sometimes by tertiary radii, while they were widely spaced and continuous on the lateral and caudal fields. The change in the curvature of the circuli, the absence of radii and a lower number of circuli on the lateral fields determined the boundary between the rostral and lateral fields. Fusion of some of the circuli were mainly on the lateral fields. The caudal field contained tubercles which give colour to the fish body. The caudal field was recognizable due to the remains of the thin, soft tissue of the skin on its borders, the widening of the intercircular spaces and fewer circuli. The caudal field beard tubercles

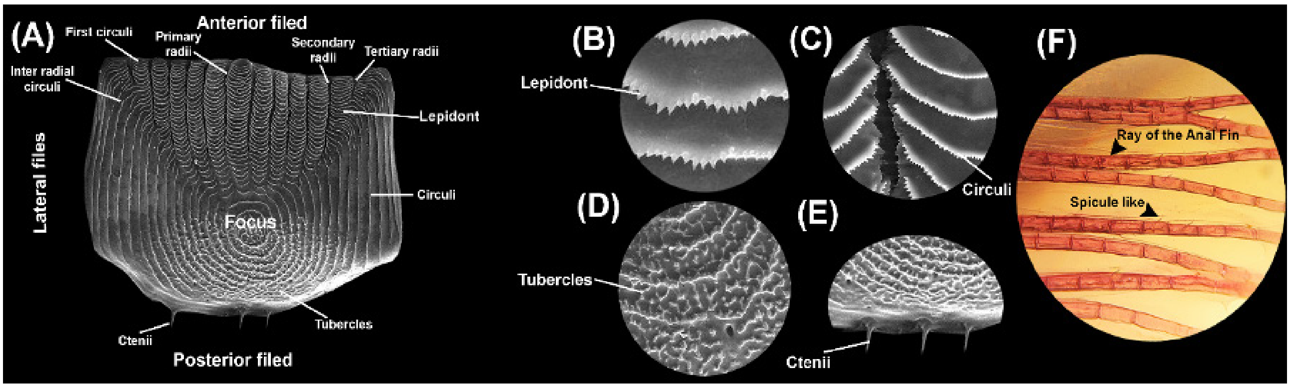

Figure 4: Morphological terminology of an aphaniid fish scale and ray of the anal fin. (A), general morphology; (B-E), microstructures on the scale. (F), Digital illustration of the anal fin and its rays.

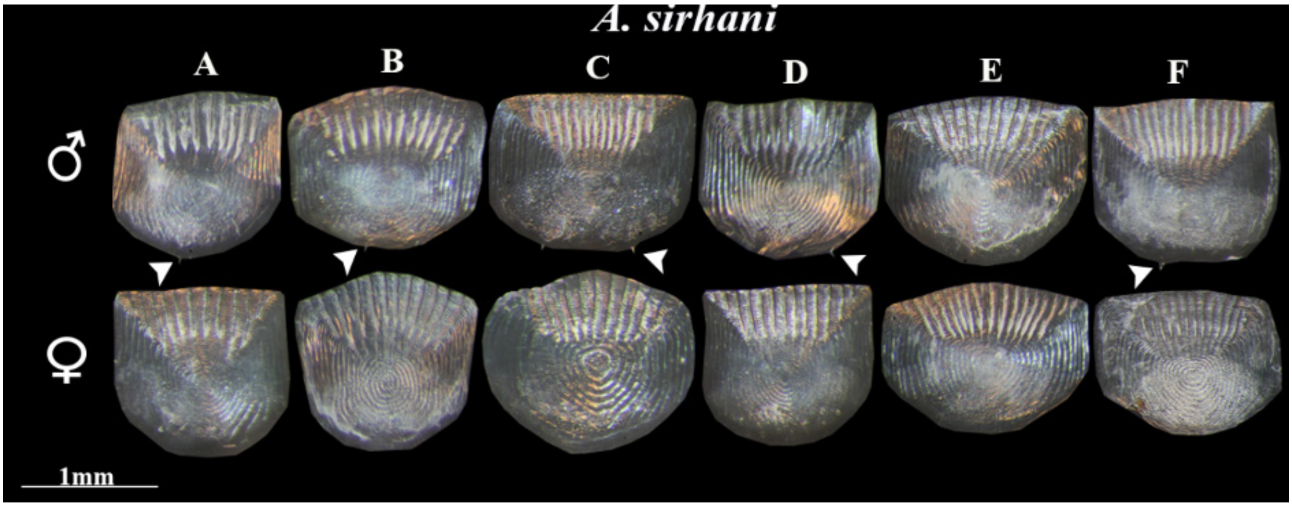

Figure 5: Light microscopic photographs of scales from different regions (A–F) in male and female individuals of *Aphaniops sirhani*. Arrows show ctenus-like structures (contact organs).

or was without tubercles. Focus was distinct and clear, with different sizes, almost with tubercles located in the central position or slightly toward the posterior.

Based on these SEM figures, none of the examined females had contact organs in the form of ctenus-like structures in the posterior part of their scales, while the male specimens displayed one to three ctenus-like structures in the posterior part of the scales (Figure. 6), revealing remarkable structural sexual dimorphism.

Fin ray

The examined males of *A. sirhani* presented contact organs in the form of spicule-like structures on the distal end of the anal-fin rays. These organs were in rows along the inner surface of the fin rays (Figure. 7), and spicules were numerous, thin, and small (almost no spicules on the proximal and middle parts). No spicule-like structures were seen on the caudal-fin rays. Female specimens had no contact organs on the anal-fin rays (Figure. 7).

Discussion

The study provides details of the macro and microstructure of scales, and the existence of novel characteristics related to sexual dimorphism in the scales and anal-fin rays of *Aphaniops sirhani*, and aphaniid species restricted to the Azraq Oasis in Jordan.

Scale morphology

The scales of *A. sirhani* show the general main characteristics of aphaniid scales, and this finding is consistent with the findings in previous studies on other aphaniids

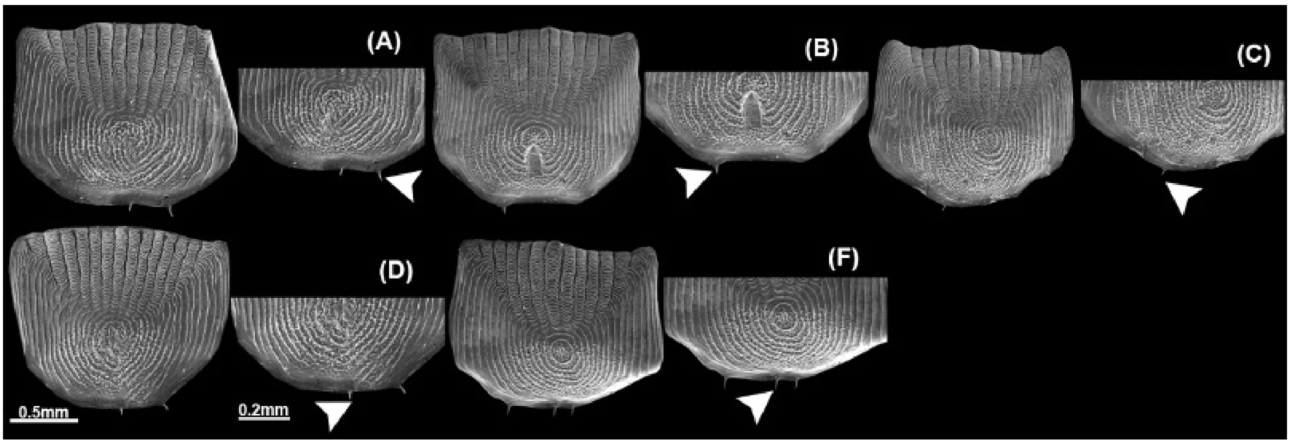

Figure 6: Scanning microphotographs of scales from several regions in male individuals of *Aphaniops sirhani*. Arrows show ctenus-like structures (contact organs).

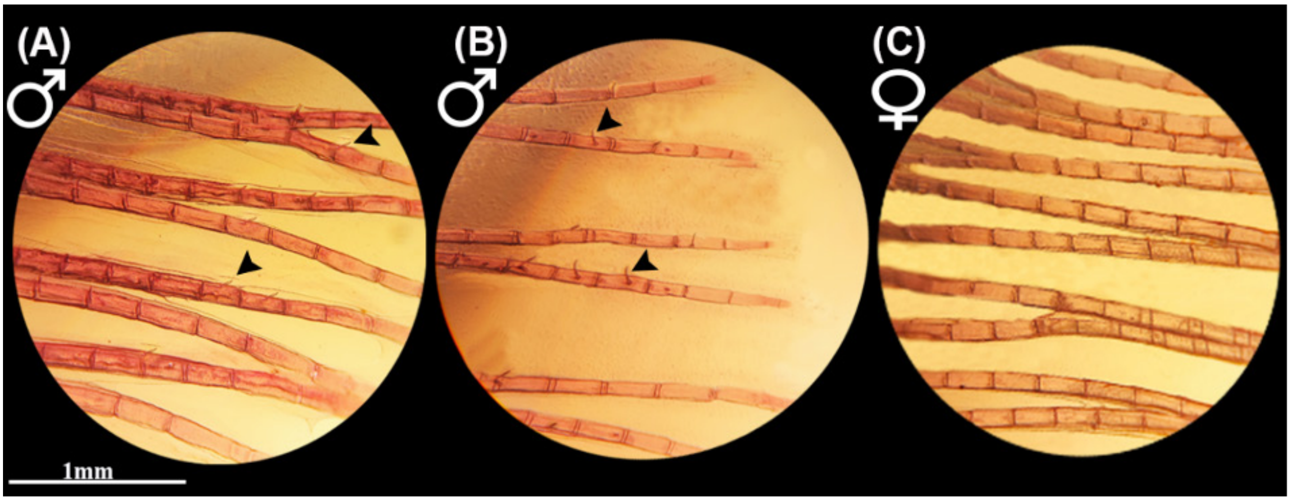

Figure 7: Light microscopic photographs of anal fin rays in male (A, B), and female (C) individuals of *Aphaniops sirhani*. Arrows show spicule-like structures (contact organs).

(e.g., Gholami *et al*., 2013; Teimori *et al*., 2017 a,b; Esmaeili *et al*., 2019, 2023; Esmaeili *et al*., 2023; Sunger *et al*., 2024). The scales exhibited a cycloid morphology, distinguishing them from the placoid, ganoid, or ctenoid scales of other bony fishes. These scales displayed typical features such as anterior, posterior, and lateral fields, as well as fin architectural structures including focus, radii, circuli, inter-circular space, and lepidonts. The cycloid scales were in the forms/subtypes of quadrilateral/square, circular/true, and intermediate/calyx depending on the sex and their locations on the fish body. Based on Sunger *et al*. (2024), the scales of the genera *Anatolichthys* and *Paraphanius* exhibit a notable level of diversity, including circular (true circular) 48%, circular (cordate) 24%, quadrilateral (square) 10%, intermediate (calyx) 8.2%, polygonal (pentagonal) 4.5%, oval (reversed ovoid) 2.6%, oval (ovoid) 1.8% and oval (oblong) 0.9%. Based on Esmaeili *et al*. (2023), the spinoid cycloid type scales are found in *Aphanius arakensis, Ap. darabensis, Ap. kavirensis, Ap. mesopotamicus, Ap. pluristriatus, Ap. shirini, Ap. sophiae*, and *Ap. vladykovi* species (placed in the genus *Esmaeilius* by Freyhof and Yoğurtçuoğlu, 2020) (Table 1). In *A. farsicus, Paraphanius mento, Aphaniops ginaonis, A. hormuzensis, A. kruppi,* and *A. stoliczkanus*, the overall shape is polygonal (pentagonal). *Aphanius baeticus* presents quadrilateral (square) scales, while *Ap. isfahanensis* demonstrates polygonal (pentagonal) or circular (discoidal) scales (Esmaeili et al., 2023). However, it should be noted that these subtypes exhibited no sexual dependency, a characteristic shared with other members of the Aphaniidae family, as shown by Esmaeili *et al*. (2023), and Sunger *et al.* (2024). Generally, the morphological variation observed in nature may be the result of phenotypic plasticity, ecological character displacement, local adaptation, genetic divergence, or the interaction of any of these main factors (Nicieza, 1995) in the species, subspecies, populations and even different body parts of the same individual. At the species level, morphological differences among the species are often considered genetic divergent as consequences of competition and ecological preferences so that different species exploit various resources (e.g. Ehlinger and Wilson, 1988; Dynes *et al*., 1999). However, the among-population differences are often considered to be the result of adaptation to local environmental conditions (e.g. Mittelbach *et al*., 1992). To sum up, variation in morphology has resulted either from environmental effects on phenotypic characters or by counteracting genetic differences between populations (Marcil *et al*., 2006).

The scales of many fish taxa show variable shapes and possess a high degree of morphological plasticity within species, specimens, and body parts, often making a clear identification at the species level difficult for some taxa (Ganias, 2014; Braeger *et al*., 2017). Similarly, in other fish body parts, the scale phenotype is also affected by genetic, environmental and their covariate effects, during the lifespan of the fish (Garduno-Paz *et al*., 2010; Ibanez *et al*., 2012; Staszny *et al*., 2013, 2019). Scale morphology varies considerably at the level of species, population (e.g., age, size, sexual maturity and sex) and different body regions (e.g., Gholami *et al*., 2013; Teimori *et al*., 2017 a,b; Esmaeili *et al*., 2019, 2023; Motamedi *et al*., 2020; Sabbah *et al*., 2021; Al Jufaili *et al*., 2021; Sunger *et al*., 2024). Within the same population, the plasticity in scale morphology is likely to be in connection with the ontogenetic development of the fish body shape, which was described in detail in several studies (see Zelditch and Fink, 1995; Reis *et al*., 1998; Braeger *et al*., 2017; Staszny *et al*., 2019).

Some scale morphological characteristics can be used as diagnostic features at the genus level for aphaniids. Males of the genus *Anatolichthys* can be distinguished from the genus *Paraphanius* by having a higher number of ctenus-like structures and a wider distribution range (vs. a low number of ctenus-like structures and a narrow distribution range). Esmaeili *et al*. (2023) revealed variations in the position, quantity, and dimensions of contact organs across many typical species of the Aphaniidae family, such as *Aphanius* (=*Esmaeilius*), *Aphaniops,* and *Paraphanius*.

Sexual dimorphism

Sexual dimorphism refers to differences between males and females of a species in secondary sex-related features, including body size, colour pattern, morphological details of specific body parts, and behaviour (Esmaeili *et al*., 2023). Sexual colour dimorphism/dichromatism (SCD) is the main and primary sexual dimorphism recorded for the aphaniid species (see Esmaeili *et al*., 2020). As in all members of the family Aphaniidae, sexual colour dimorphism is pronounced in *Aphaniops sirhani* (Figure. 8). Males of *A. sirhani* exhibit a series of dark vertical bars on the flanks with usually two bars in the caudal fin. The fins are yellowish with some dark markings, especially in the dorsal and caudal fins. Females are larger and much plainer possessing only a series of irregular dark spots on the body and completely hyaline finnage. Male individuals of *Paraphanius* display a colour pattern consisting of various shades of grey, blue, or nearly black bodies, often accompanied by irregularly shaped and positioned iridescent blue-white to silvery spots (Freyhof and Yoğurtcuoğlu, 2020; Esmaeili *et al*., 2020, 2023; Sunger *et al.,* 2024). These spots may form narrow vertical rows along the flanks, particularly in juvenile specimens. Furthermore, males possess a caudal fin that features very narrow rows of blue–white or silvery spots, or small blotches, arranged in bands against a black or blue background (Freyhof and Yoğurtcuoğlu, 2020). Male *Anatolichthys* present black or dark-brown bars in the caudal fin, a series of black or brown patterns in the flanks, and black dorsal and anal-fin margins. Female *Anatolichthys* present a bold, black spot at the center of the caudal-fin base (Freyhof and Yoğurtcuoğlu, 2020).

Besides the sexual colour dimorphism, in a recent study conducted by Esmaeili *et al.* (2023) on sixteen aphaniid species (classified under the three genera *Aphanius*, *Aphaniops,* and *Paraphanius*), the presence of a new morphological characteristic (contact organs/ctenus-like structures) on the scales of male individuals were observed and documented. In another work by Sunger *et al*. (2024) on nine species and two genera of aphaniids including *Anatolichthys anatoliae*, *An. chantrei*, *An. iconii*, *An. marassantensis*, *An*. cf. *meridionalis*, *Anatolichthys* sp., *An. villwocki*, *Paraphanius alexandri*, and *P. similis*, the researchers documented the presence of contact organs in the forms of ctenus-like structures in the posterior margin of the scales, and spicule-like structures in the anal-fin rays of males in all examined species. The present study revealed that these contact organs are present in the

Figure 8: Sexual colour dimorphism in *Aphaniops sirhani*. male (upper) and female (lower).

posterior margin of the scales and anal-fin rays of males in another aphaniid species (*A. sirhani*). The contact organs are not present in the scales of female specimens of *A. sirhani* (present study) and all other studied aphaniids except *Aphaniops ginaonis* though females had fewer contact organs (Esmaeili *et al*., 2023; Sunger *et al*., 2024).

Aphaniops sirhani presents ctenus-like structures in the scales of all the six examined regions. This also applies to some other congeneric species including *A. kruppi* and *A. stoliczkanus* (Esmaeili *et al*., 2023).

The variation is observed in the scales of other male aphaniids being in one or more areas of the fish body in the male specimens: being in one region (*An. Anatoliae*, *Aphanius darabensis*), two regions (*An. marassantensis*), three regions (*An. iconii* and *An*. cf. *meridionalis*), four regions (*Aphaniops ginaonis*), five regions (*An. chantrei*, *An. villwocki*, *Paraphanius alexandri*, and *P. similis*, *Aphanius vladykovi*), and six regions (*Anatolichthys* sp.), (Esmaeili *et al*., 2023; Sunger *et al*., 2024).

In addition, the location, number, and extent of contact organ development varied intra- and interspecifically in the aphaniid species.

The number of contact organs on the scales of the genus *Aphaniops* are more than those in the genera *Aphanius* and *Paraphanius*, and they were short, pointed, and wide covering almost the entire posterior area of the scales (Esmaeili *et al*., 2023). Most of the species of the genus *Anatolichthys* are also characterized by a higher number of ctenus-like structures and a wider distribution range and were variously short, pointed and wide in shape (Sunger *et al*., 2024). However, the species of the genus *Paraphanius* are characterized by a low number of long and pointed ctenus-like structures limited to a region in the posterior part of the scales (Sunger *et al*., 2024). Hence, these characteristics might provide a taxonomic and evolutionary signal. Variations are also found in the position of contact organs in the fin rays of aphaniids as presented in Figure (9) (Esmaeili *et al*., 2023; Sunger *et al*., 2024, and the present study).

Contact organs have long been known in fish-related publications under a confusing variety of terms (Kang *et al*., 2013; Tripathi, 2018; Velázquez-Velázquez *et al*., 2022). Esmaeili *et al*. (2023) have shown that contact organs in male aphaniid fishes mostly manifest in mature individuals and during periods of active reproduction. During the spawning season, several species of aphaniids, including *Aphanius fasciatus*, show courtship displays. In this habit, both male and female individuals exhibit a strong affinity for one another, maintaining close physical proximity by adhering to each other's posterior regions. Notably, the male fish envelops the whole of the female fish body with its own. The conduct serves as evidence for the presence and importance of contact organs in reproductive processes (Grech and Schembri, 1993; Cavraro *et al*., 2013).

Conclusion

The findings of this study demonstrate that *Aphaniops sirhani* (i) exhibit sexual colour dimorphism, (ii) their cycloid scales display phenotypic flexibility across several body regions, (iii) sexual dimorphism in their contact organs is seen in the forms of ctenus-like structure (in the posterior margin of the scales), and in the form of spicule-like structures on the distal end of the anal-fin rays in male individuals; such structures are

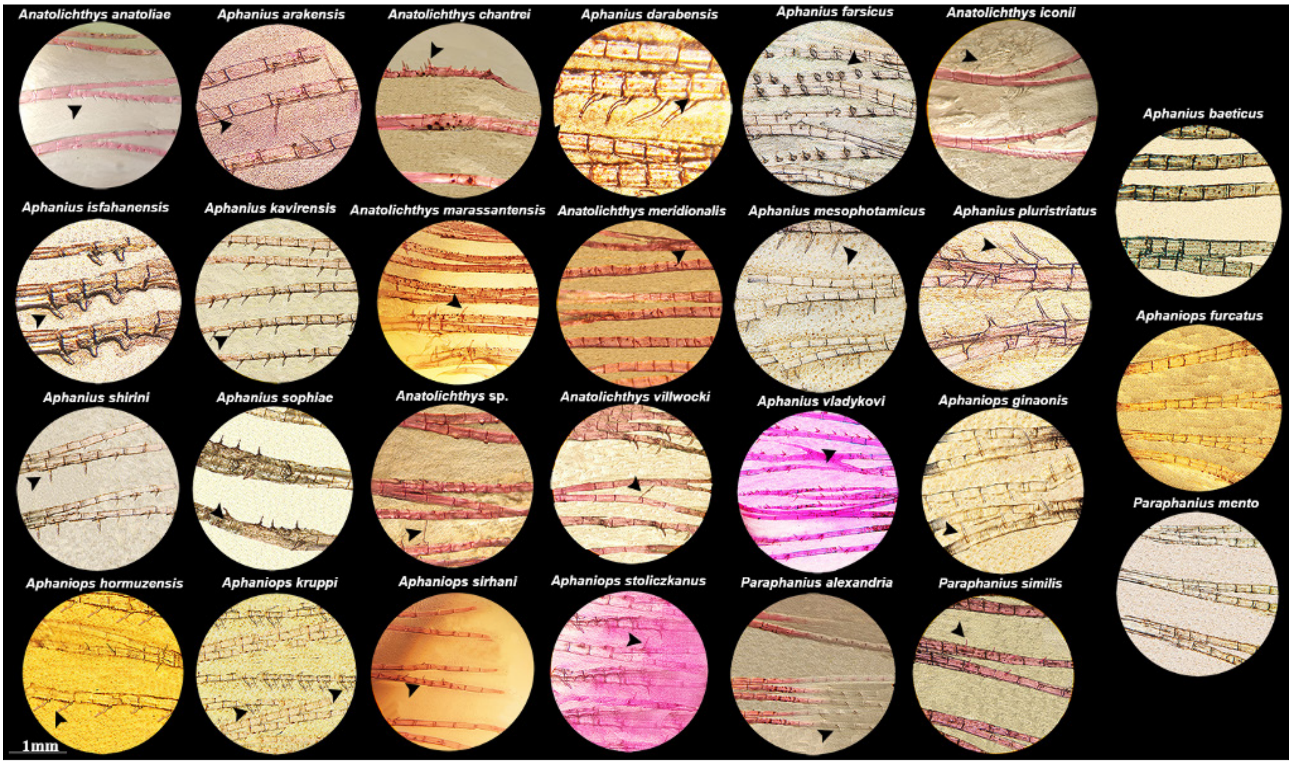

Figure 9: Light microscopic photographs of anal fin rays in male individuals of 27 aphaniid species. Arrows show spicule-like structures (contact organs), based on Esmaeili et al. (2023), Sunger et al. (2024), and the present study.

mostly functional and facilitate the physical contact between male and female individuals during the active phase of reproduction, (iv) females do not exhibit contact organs, and (v) variations in size, number, and position of contact organs in aphaniids might provide a taxonomic and evolutionary signal.

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References

Abrahão, V. P., Pastana, M. and Marinho, M. (2019). On a remarkable sexual dimorphic trait in the Characiformes related to the olfactory organ and description of a new miniature species of *Tyttobrycon* Ge´ry (Characiformes: Characidae). *PLoS ONE,* **14**(12): e0226130. https://doi.org/10.1371/journal.pone.0226130

Al Jufaili, S. M., Masoumi, A. H., Esmaeili, H. R., Jawad, L. and Teimori, A. 2021. Morphological and microstructural characteristics of scales in longnose goby *Awaous jayakari* (Teleostei: Gobiidae): Light and scanning electron microscopy approaches. *Microscopy Research and Technique*, **84**(12): 3128-3149.

Andersson, M. 1994. **Sexual Selection**. Princeton University Press: Princeton, NJ, USA.

Beltrán, D. F., Araya-Salas, M., Parra, J. L., Stiles, F. G. and Rico-Guevara, A. 2022. The evolution of sexually dimorphic traits in ecological gradients: an interplay between natural and sexual selection in hummingbirds. *Proceedings of the Royal Society B*, **289**(1989): 20221783. https://doi.org/10.1098/rspb.2022.1783

Braeger, Z., Staszny, A., Mertzen, M., Moritz, T. and Horvath, G. 2017. Fish scale identification: from individual to species-specific shape variability. *Acta Ichthyologica et Piscatoria*, **47**(4): 331-338.

Cashner, R. C., Schaefer, J., Warren Jr, M. L., Echelle, A. A., Galvez, F. and Ghedotti, M. J. (2020). Fundulidae: **Topminnows. *Freshwater Fishes of North America***. Edited by ML Warren, Jr. and BM Burr, with AA Echelle, BR Kuhajda, and ST Ross. Johns Hopkins Univ. Press, Baltimore, Md*,* pp.549-608.

Cavraro, F., Torricelli, P. and Malavasi, S. 2013. Quantitative ethogram of male reproductive behavior in the South European toothcarp *Aphanius fasciatus*. *The Biological Bulletin*, **225**(2): 71-78. https://doi:10.1086/BBLv225n2p71.

Costa, C., Loy, A., Cataudella, S., Davis, D. and Scardi, M. 2006. Extracting fish size using dual underwater cameras. *Aquacultural Engineering,* **35**(3): 218-227. https://doi.org/10.1016/j.aquaeng.2006.02.003

Crooks, N., Babey, L., Haddon, W. J., Love, A. C. and Waring, C. P. 2013. Sexual dimorphisms in the dermal denticles of the lesser-spotted catshark, *Scyliorhinus canicula* (Linnaeus, 1758). *PLoS ONE,* **8**(10): e76887. doi: 10.1371/journal.pone.0076887

de Oliveira, C. L., Malabarba, L. R. and Burns, J. R. 2012. Comparative morphology of gill glands in externally fertilizing and inseminating species of cheirodontine fishes, with implications on the phylogeny of the family Characidae (Actinopterygii: Characiformes). *Neotropical Ichthyology*, **10**: 349-360.

de Sousa Rangel, B., Santander-Neto, J., Rici, R. E. G. and Lessa, R. 2016. Dental sexual dimorphism and morphology of *Urotrygon microphthalmum*. *Zoomorphology,* **135**: 367-374. https://doi.org/10.1007/s00435-016-0312-0

Dynes, J., Magnan, P., Bernatchez, L. and Rodriguez, M. A. 1999. Genetic and morphological variation between two forms of lacustrine brook charr. *Journal of fish biology*, **54**(5): 955-972.

Echreshavi, S., Esmaeili, H. R., Teimori, A., Safaie, M. and Owfi, F. 2021. Hidden morphological and structural characteristics in scales of mullid species (Teleostei: Mullidae) using light and scanning electron digital imaging. *Microscopy Research and Technique,* **84**: 2749–2773. https://doi.org/10.1002/jemt.23837

Ehlinger, T. J. and Wilson, D. S. 1988. Complex foraging polymorphism in bluegill sunfish. *Proceedings of the National Academy of Sciences*, **85**(6): 1878-1882.

Esmaeili, H. R. (2001). **Biology of an exotic fish, silver carp *Hypophthalmichthys molitrix* Val., 1844) from Gobindsagar Reservoir, Himachal Pradesh, India.** Ph.D: Panjab University, India.

Esmaeili, H. R., Echreshavi, S., Masoumi, A. H. and Nejad, A. M. 2023. On a remarkable sexual dimorphic trait on scales and fins of the old world Cyprinodontiformes (Actinopterygii: Aphaniidae). *Acta Zoologica*. https://doi.org/10.1111/azo.12469

Esmaeili, H. R., Sayyadzadeh, G., Japoshvili, B., Eagderi, S., Abbasi, K. and Mousavi-Sabet, H. (2020a). *Rhodeus caspius*, a new bitterling from Iran (Teleostei: Cypriniformes: Acheilognathidae). Zootaxa, 4851(2): 319-337.

Esmaeili, H. R., Teimori, A., Zarei, F. and Sayyadzadeh, G. 2020b. 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

Esmaeili, H.R., Sayyadzadeh, G. and Amini Chermahini, M. 2017. Sexual dimorphism in two catfish species, *Mystus pelusius* (Solander, 1794) and *Glyptothorax silviae* Coad, 1981 (Teleostei: Siluriformes). *Turkish Journal of Zoology*, **41**: 144-149. https://doi.org/10.3906/zoo-1509-22

Freyhof, J. Ö. R. G., and YoĞurtÇuoĞlu, 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): zootaxa-4810. https://doi.org/10.11646/zootaxa.4810.3.2

Fukakusa, C. K. 2020. Behavioral evidence of chemical communication by male caudal fin organs of a glandulocaudine fish (Teleostei: Characidae). *Ichthyological Exploration of Freshwaters*, **IEF-1127**: 1-11. https://doi.org/10.23788/IEF-1127

Ganias, K. (Ed.). 2014. **Biology and ecology of sardines and anchovies**. CRC Press.

Garcia, E. Q. and Zuanon, J. 2019. Sexual dimorphism in the electric knifefish, *Gymnorhamphichthys rondoni* (Rhamphichthyidae: Gymnotiformes). *Acta Amazonica*, **49**: 213-220. https://doi.org/10.1590/1809-4392201804392

Garduno-Paz, M. V., Couderc, S., and Adams, C. E. 2010. Habitat complexity modulates phenotype expression through developmental plasticity in the threespine stickleback. *Biological Journal of the Linnean Society*, **100**(2): 407-413.

Garita-Alvarado, C. A., Naranjo-Elizondo, B. and Barrantes, G. 2018. Mating and aggressive behaviour of *Brachyrhaphis olomina* (Cyprinodontiformes: Poeciliidae). *Journal of Ethology*, **36**: 1-13. https://doi.org/10.1007/s10164-017-0523-y

Garman, S. 1895. The cyprinodonts. *Memoirs of the Museum of Comparative Zoology,* **19**: 1–179, pls. 1–12.

Gholami, Z., Teimori, A., Esmaeili, H. R., Schulz-Mirbach, T. and Reichenbacher, B. 2013. Scale surface microstructure and scale size in the tooth-carp genus *Aphanius* (Teleostei, Cyprinodontidae) from endorheic basins in Southwest Iran. *Zootaxa,* **3619**(4): 467–490. https://doi.org/10.11646/zootaxa.3619.4.5

Grech, M. and Schembri, P. J. 1993. Observations on courtship and mating behaviour in Maltese populations of the killifish *Apnonius fasciatus*

(Pisces: Cyprinodontidae). *The Central Mediterranean Naturalist,* **2**(2): 28-34.

Herler, J., Kerschbaumer, M., Mitteroecker, P., Postl, L. and Sturmbauer, C. 2010**.** Sexual dimorphism and population divergence in the Lake Tanganyika cichlid fish genus *Tropheus*. *Frontiers in Zoology,* **7**: 1–10.

Hernandez, J., Villalobos-Leiva, A., Bermúdez, A., Ahumada, C. D., Suazo, M. J. and Benítez, H. A. 2022**.** An Overview of Interlocation Sexual Shape Dimorphism in *Caquetaia kraussi* (Perciformes: Cichlidae), a geometric morphometric approach. *Fishes,* **7**: 146. https://doi.org/10.3390/fishes7040146

Ibáñez, A. L., Pacheco-Almanzar, E. and Cowx, I. G. 2012. Does compensatory growth modify fish scale shape? *Environmental Biology of Fishes*, **94**: 477-482.

Kang, J. H., Schartl, M., Walter, R. B. and Meyer, A. 2013. Comprehensive phylogenetic analysis of all species of swordtails and platies (Pisces: Genus *Xiphophorus*) uncovers a hybrid origin of a swordtail fish, *Xiphophorus monticolus*, and demonstrates that the sexually selected sword originated in the ancestral lineage of the genus, but was lost again secondarily. *BMC Evolutionary Biology*, **13**: 1-19.

Lima-Filho, P. A., Bidau, C. J., Alencar, C. E. R. D. and Molina, W. F. 2017. Latitudinal influence on the sexual dimorphism of the marine fish *Bathygobius soporator* (Gobiidae: Teleostei). *Evolutionary Biology,* **44**: 374–385. https://doi.org/10.1007/s11692-017-9416-9

Lippitsch, E. 1990. Scale morphology and squamation patterns in cichlids (Teleostei, Perciformes): A comparative study. *Journal of Fish Biology,* **37**(2): 265–291. https://doi.org/10.1111/j.1095-8649.1990.tb05858.x

Lippitsch, E. 1995. Scale and squamation character polarity and phyletic assessment in the family Cichlidae. *Journal of Fish Biology,* **47**(1): 91-106.

Malabarba, L. R. and Weitzman, S. H. 1999. A new genus and species of South American fishes (teleostei: characidae: cheirodontinae) with a derived caudal fin, including comments about inseminating cheirodontines. *Proceedings of the Biological Society of Washington*, **112**(2): 410-432.

Marcil, J., Swain, D. P. and Hutchings, J. A. 2006. Genetic and environmental components of phenotypic variation in body shape among populations of Atlantic cod (*Gadus morhua* L.). *Biological Journal of the Linnean Society*, **88**(3): 351-365.

Mittelbach, G. G., Osenberg, C. W. and Wainwright, P. C. 1992. Variation in resource abundance affects diet and feeding morphology in the pumpkinseed sunfish (*Lepomis gibbosus*). *Oecologia*, **90**: 8-13.

Moore, A. J. 1990. The evolution of sexual dimorphism by sexual selection: the separate effects of intrasexual selection and intersexual selection. *Evolution,* **44**: 315–331. https://doi.org/10.1111/j.1558-5646. 1990. tb05201.x PMID: 28564385

Motamedi, M., Teimori, A., Amiri, V. and Hesni, M. A. 2020. Characterization of age-dependent variability in the flank scales of two scorpaeniformes fishes by applying light and scanning electron microscopy imaging. *Micron*, **128**: 102778.

Motamedi, M., Teimori, A. and 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

Nicieza, A. G. 1995. Morphological variation between geographically disjunct populations of Atlantic salmon: the effects of ontogeny and habitat shift. *Functional Ecology*, **1**: 448-456.

Reis, R. E. and Schaefer, S. A. 1998. New cascudinhos from southern Brazil: systematics, endemism, and relationships (Siluriformes, Loricariidae, Hypoptopomatinae). *American Museum novitates*; no. **3254**.

Rodríguez, J., Rojas, G. C. and Castro, K. R. 2005 Selección de pareja y comportamiento sexual de *los guppys* (*Poecilia reticulata*). *Orinoquia,* **9**: 38–44.

Sabbah, N., Teimori, A. and Hesni, M. A. 2021. Digital light microscopy to characterize the scales of two goatfishes (Perciformes; Mullidae). *Microscopy Research and Technique*, **84**(2): 180-191.

Sadeghi, R., Esmaeili, H. R., Teimori, A., Ebrahimi, M. and Gholamhosseini, A. 2021. Comparative ultrastructure and ornamentation characteristics of scales in gobiid species (Teleostei: Gobiidae) using the scanning electron microscope. *Microscopy Research and Technique,* **84**(6): 1243–1256. https://doi.org/10.1002/jemt.23683

Staszny, Á., Havas, E., Kovács, R., Urbányi, B., Paulovits, G., Bencsik, D. and Csenki, Z. 2013. Impact of environmental and genetic factors on the scale shape of zebrafish, *Danio rerio* (Hamilton 1822): a geometric morphometric study. Acta Biologica Hungarica, **64**(4): 462-475.

Staszny, Á., Paulovits, G., Takács, P., Juhász, V., Prigl, S., Sziráki, B. and Ferincz, Á. 2019. The Role of Ontogenetic Development in Fish Scale Shape Change. *Applied Ecology and Environmental Research*, **17**(2): 3535-3544.

Sungur, S., Esmaeili, H. R., Echreshavi, S. and Çiçek, E. 2024. Light and scanning electron imaging confirm sexual dimorphism in scales and anal-fin rays of the genera *Anatolichthys* and *Paraphanius* (Teleostei: Cyprinodontiformes: Aphaniidae). *Acta Zoologica*. https://doi.org/10.1111/azo.12493

Taylor, W. R. and van Dyke, G. C. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium,* **9**(2): 107–119.

Teimori, A., Motamedi, M. and 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

Tripathi, P. 2018. Mystery of unicellular and multicellular keratinous tubercles of the epidermal or dermal origin in fish. *International Journal of Cell Science and Molecular Biology,* **4**(2): 35–40. https://doi.org/10.19080/IJCSMB.2018.04.555633

Velázquez-Velázquez, E., Domínguez-Cisneros, S. E., Anzueto-Calvo, M. J. and Maza-Cruz, M. F. 2022. Occurrence of contact organs and sexual dimorphism in fishes of the family Profundulidae (Actinopterygii: Cyprinodontiformes). *Acta Ichthyologica et Piscatoria,* **52**(4): 261–265. https://doi.org/10.3897/aiep.51.93669

Vila, I., Scott, S., Mendez, M. A., Valenzuela, F., Iturra, P. and Poulin, E. 2011. *Orestias gloriae*, a new species of cyprinodontid fish from saltpan spring of the southern high Andes (Teleostei: Cyprinodontidae). *Ichthyological Exploration of Freshwaters*, **22**(4): 345.

Wiley, M.L. and Collette, B.B. 1970. Breeding tubercles and contact organs in fishes: Their occurrence, structure, and significance. *Bulletin of the American Museum of Natural History,* **143**: 143–216.

Zelditch, M. L., Fink, W. L. and Swiderski, D. L. 1995. Morphometrics, homology, and phylogenetics: quantified characters as synapomorphies. *Systematic Biology*, **44**(2): 179-189.