Microscopic Examination of Scale Morphology in the Doctor Fish *Garra ghorensis* (Cyprinidae: Labeoninae): Structural Features as Taxonomic Tools

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Abstract

This study presents a detailed analysis of scale morphology and morphometric traits in *Garra ghorensis*, a cyprinid species adapted to fast-flowing waters of the Southeastern Dead Sea basin. Optical and scanning electron microscopy were employed to investigate structural characteristics of scales and to evaluate their potential taxonomic value. The scales of *G. ghorensis* are primarily cycloid, exhibiting distinctive radial patterns and unique topological features that contribute to both hydrodynamic efficiency and protection. Morphological traits—including scale type, size, shape, lateral surface, focal position, circuli, radii types, lepidonts, and margin forms—were examined across six body regions. Findings highlight strong attachment, overlapping arrangement, and a thin yet robust structure that enhance defense, flexibility, and transparency, while reducing drag and body weight. Variations in striae (radii) types (primary, secondary, tertiary) across anterior, posterior, and lateral fields produced a characteristic four-cut pattern typical of the genus *Garra*. Scale margin morphology (smooth or wavy) and lepidont variability (smooth, rounded, fine, short) further support their utility for species identification, classification, and phylogenetic interpretation among freshwater fishes.

Keywords: Cypriniformes, Freshwater fish, Doctor fish, Scale morphology, Ultrastructural features, Scanning electron microscopy (SEM), Cycloid scales

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Introduction

Scales are rigid structures on the fish's skin, playing a protective role against physical damage and contamination (Lagler, 1947). Made of collagen, calcium, and phosphorus, they have historically been used for fish classification, as first introduced by Agassiz in 1833. Different scale types help fish adapt to their environments (Baudelot, 1873).

Extensive research has been conducted on fish scale morphology, especially in *Garra* species. These studies have provided valuable insights into species identification, phylogenetic relationships, and environmental changes. For example, morphological features like scale shape and size serve as indicators for distinguishing species and assessing environmental impacts. Studies on fish scales show their use as environmental and phylogenetic markers (Roberts and Aspy, 1993; Esmaeili and Gholami, 2011; Viertler *et al*., 2021). In studying scales, it is especially important to focus on the scale morphology of species found in unique habitats, such as desert streams, as in the case of the cyprinid fish *Garra ghorensis*. Understanding these traits can aid biological adaptation and conservation efforts and improve ecosystem management (Komarova *et al*., 2022).

Members of the genus *Garra* (Hamilton, 1822) display a small to medium size, an elongated and almost cylindrical body, a round snout with a lower and crescent-shaped mouth, a horned lower jaw, usually an edged upper lip continuous with the snout, and a needle disk with a free posterior margin. These fish have sucking mouth feeding on algae, plankton, and small invertebrates (omnivores), and are usually kept in aquariums (Esmaeili *et al*., 2016). Several species in the genus *Garra* commonly refer to Doctor fish/doctorfish that are known for their keratinous "doctoring" behavior: they nibble on dead skin and minor abrasions of other fish or, in some contexts, on human skin. The behavior is sometimes marketed as therapeutic foot or body scrubbing in spa settings, though the ecological and ethical contexts vary (Faal *et al*., 2024).

Garra ghorensis (Krupp, 1982) a rare freshwater cyprinid fish (Cypriniformes: Cyprinidae: Labeoninae) inhabits springs and small rivers in the Ghor Valley, Jordan. Due to habitat specificity and adaptability to slow currents and fluctuating temperatures, it plays a critical ecological role. However, threats like habitat destruction and water resource mismanagement have placed it at high risk of extinction. Conservation is vital to preserving the biodiversity of these sensitive ecosystems (Kottela, 2020).

Comprehensive morphological studies on this species' scales are still lacking. Investigating scale characteristics can provide insights into its biological adaptations and aid conservation strategies. Since scales help identify species and analyze evolutionary relationships, examining *G. ghorensis* scales can offer valuable

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information on its conservation status and habitat management (Krupp *et al*., 1989). This study represents the first attempt to describe the ultrastructural features of *G. ghorensis* scales using digital light microscopy (LM) and scanning electron microscope (SEM) imaging. The research aims to (i) reveal hidden morphological traits, (ii) examine scale plasticity across different body regions, and (iii) identify topographical and structural variations in scales that may serve as taxonomic tools.

Materials and methods

Sampling site and studied taxon

To investigate the morphological characteristics and ultrastructural patterns of scales in *G. ghorensis*, a total of 10 fish specimens (SL=43.64±0.08 mm) were collected from the Ibn Hammad region (N31° 18′, E35° 38′) in Northwestern Jordan (Fig. 1), where Wadi Ibn Hammad is characterized as a fast-flowing and shallow habitat (Fig. 2). The fish were caught using electrofishing in February 2024. Initially, the specimens were fixed in a 4% formalin solution and later transported to the laboratory, where they were preserved in 70% ethanol for further analysis.

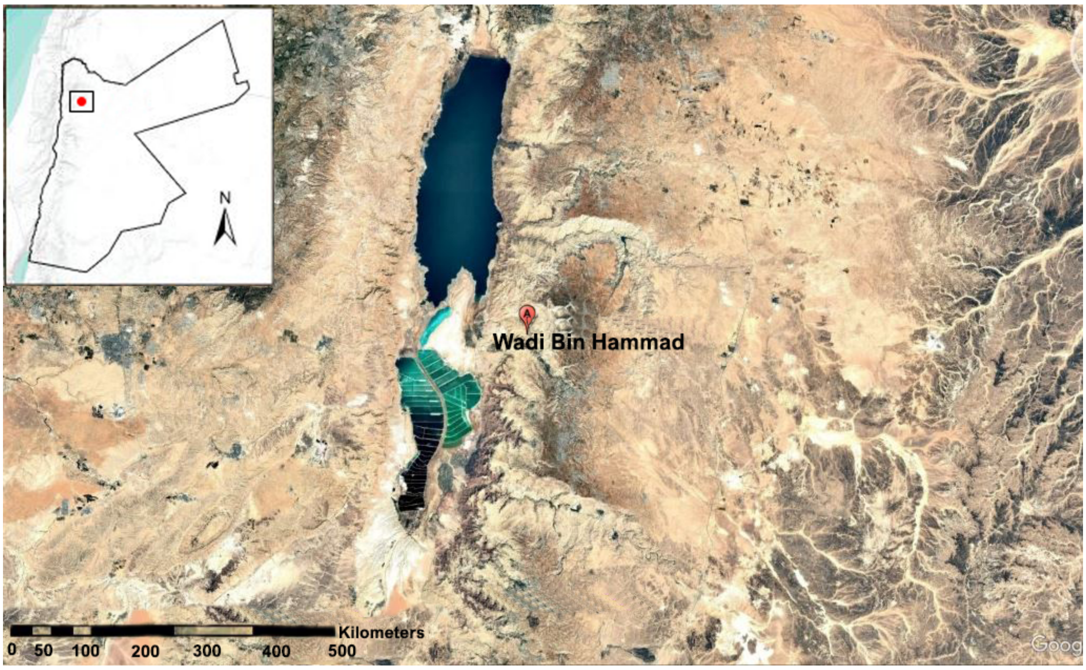

Figure 1: Location of the Ibn Hammad in North Jordan.

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Figure 2: Fast-flowing and shallow habitat of Wadi Ibn Hammad.

Scale preparation and digital and scanning electron microscopy imaging

Initially, before removing the scales, the total length (TL) and standard length (SL) of the fish were measured with calipers to the nearest 0.05 mm (Echreshavi *et al*., 2021). All fish specimens were deposited in the Zoological Museum and the Department of Biology, Shiraz University, Iran (ZM-CBSU), with corresponding voucher numbers.

For scale preparation, the methods of Gholami *et al*. (2013) and Esmaeili *et al*. (2019) were followed. Scales were removed from six specific regions on the left side of the fish (Fig. 3a). These regions included: Region A (head area), Region B (third to fourth row between the origin of the dorsal fin and the lateral line), Region C (lateral line), Region D (caudal peduncle area), Region E (area in front of the anal fin), Region F (area behind the pectoral fin; Fig. 3b). For each fish sample, five scales were removed from selected areas (i.e., 30 scales per sample: 6 areas × 5 scales per area). Thus, a total of 300 scales were examined in this study. The scales were carefully removed from the left side with fine tweezers to avoid damage. They were immediately rinsed in distilled water, mechanically cleaned with a fine brush to remove any debris, and then transferred to a 4% KOH solution for approximately 20 minutes to remove soft tissues from the surface. The cleaned scales were sequentially dehydrated in 30%, 50%, 70%, and 90% ethanol for 20 minutes each and then dried on filter paper (Lippitsch, 1990). To prevent the margins of the scales from curling, they were kept between two microscope slides for 2 to 3 days.

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Subsequently, the scales were imaged using a Nikon Eclipse 80i digital imaging system connected to a computer. The digital images were then used for morphological descriptions and measurements (Echreshavi *et al*., 2021). For scanning electron microscopy, to prevent curling of the scale margins, the scales were immediately mounted on aluminum stubs using double-sided tape with the adhesive side facing down. The upper surfaces of the scales were coated with a 100 Å layer of gold in a gold sputter coater, and various images of the scales were obtained using a TESCAN Vega3 scanning electron microscope (Shiraz University) at a voltage of 20 kV.

Terminology for Scale characteristics and features

Various terms are used to define the characteristics of scales; therefore, the sections of the scale are described in Figure 3c. The terminology for defining different parts of the scale follows the descriptions introduced by Lagler (1947) and Lippitsch (1990).

Scale measurements

Since scale size varies between different regions of a fish's body, scales from various sections were measured (Echreshavi *et al*., 2021). Five linear parameters of scale shape/size were measured using ImageJ ver.1.8.0 software (Schneider *et al*., 2012) as follows: (i) the anteroposterior length or maximum longitudinal diameter of the scale (SCL=scale length), (ii) the dorsoventral height or maximum transverse diameter of the scale (SCW = scale width), (iii) the anterior focus length (AFL), (iv) the distance from the upper left corner to the lower right corner of the left cross (LI=d–d′), and (v) the distance from the upper right corner to the lower left corner of the right cross (RI=e–e′; Fig. 3c; Echreshavi *et al*., 2021). Based on these parameters, five indices were calculated, including two relative scale size indices (J indices), shape index (Si), scale extension index (SE), and focus index (Fi). The two J indices, JSL and JSW, were calculated to provide information about scale size (Esmaeili *et al*., 2014; Table 1). The J indices were calculated as follows: JSL (or JSW)=(Scale length or width / Standard length of the fish) * 100 (Esmaeili, 2001; Esmaeili *et al*., 2014). The mean of these two indices was considered as SSI (Scale Size Index). The shape index (Si) was calculated as: Si=SCL/SCW or Si=a–a′/b–b′ (Figure 3c), and the focus index (Fi), which indicates the position of the focus on the scale, was calculated as: Fi = [(a-c) / (a-a′)] * 100. The focal index (Fi) was calculated by dividing the distance from the outermost edge of the anterior field to the focus by the distance from the outermost edge of the anterior field to the posterior edge (Teimori *et al*., 2017). The position of the focus was classified based on the focal index as follows: <0.20, anterior; 0.21-0.40, anterior-central; 0.41–0.60, central; 0.61-0.80, posterior-central; >0.81, posterior (Sabbah *et al*., 2020; Echreshavi *et al*., 2021).

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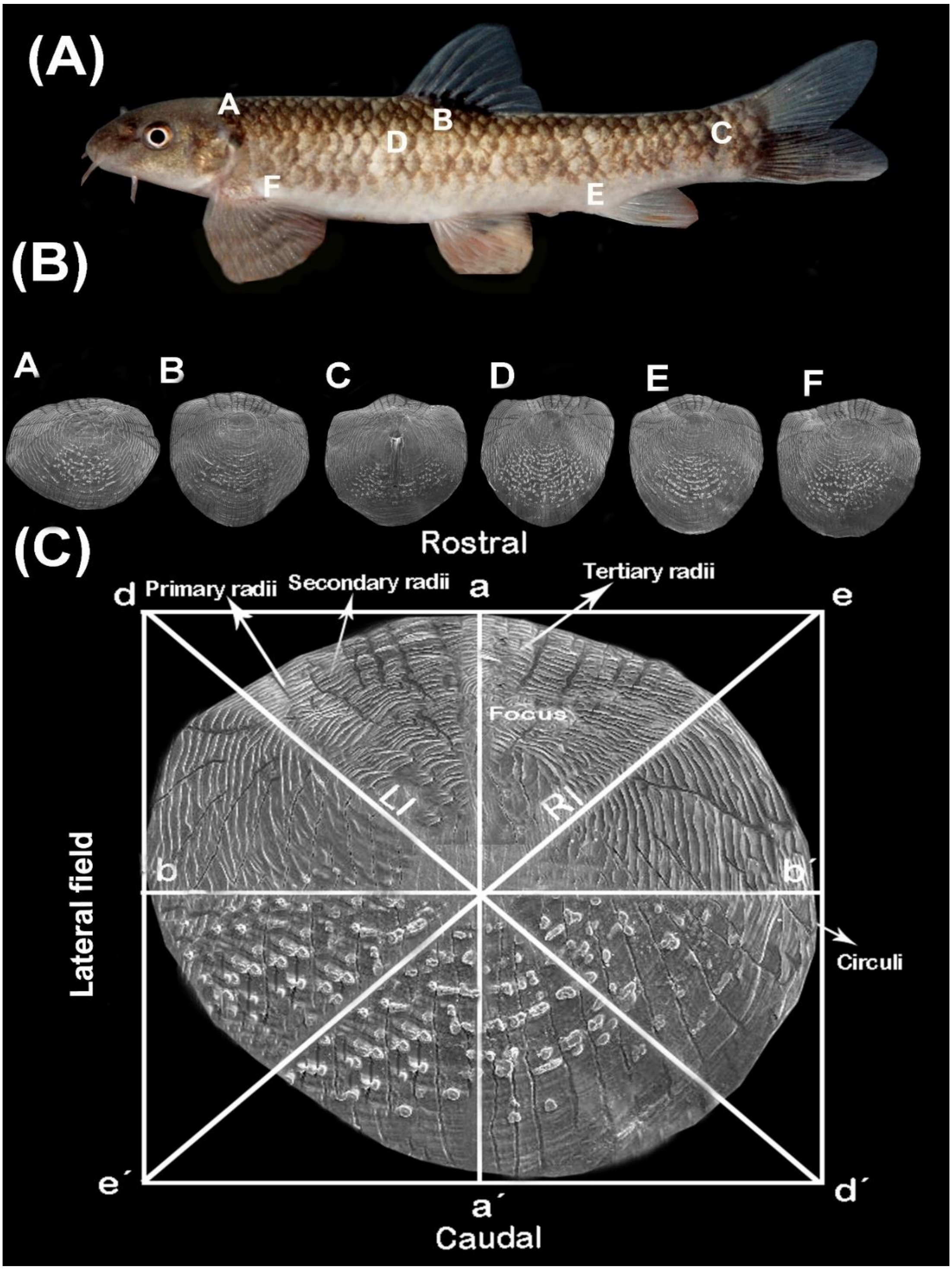

Figure 3: (A) Image of *Garra ghorensis* showing six different studied body parts where scales were removed from the left side of the fish, (B) scales of six body regions (A, B, C, D, E, and F), (c) morphological terminology of the scale. The linear measurements used for the morphometric analyses and for the estimation of the focal index (Fi) as (a c/ a a´) 100. "a c" is the distance from the outermost part of the anterior field to the focus, "a a´" is the distance from the outermost part of the anterior field to the outermost part of the posterior field, and "b b´" is dorsal–ventral height (Bräger et al., 2017; Echreshavi et al., 2021; Sabbah et al., 2020).

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Table 1: Descriptive analysis of J-indices (JSL.SL and JSW.SL), SSI (average value of JSL.SL and JSW.SL), S index (SCL.SCW), F index (AFL.SCL), and SE index (RI/LI) for the *Garra ghorensis* species.

Region	N	J index	J index				Si index			
			Min	Max	Mean	SD	Min	Max	Mean	SD
Head	1	Jsl	2.11	2.62	2.28	0.1	0.96	1.19	1.07	0.06
	0	Jsw	2.09	2.70	2.45	0.1				
Dorsal	1	Jsl	3.20	3.35	3.27	0.07	0.96	1.02	0.99	0.03
	0	Jsw	3.08	3.43	3.25	0.1				
Lateral line	1	Jsl	3.49	4.03	3.78	0.2	1.06	1.08	1.07	0.01
	0	Jsw	3.59	4.00	3.79	0.2				
Caudal peduncle	1	Jsl	3.49	3.72	3.60	0.1	1.05	1.08	1.06	0.01
	0	Jsw	3.46	3.5	3.52	0.06				
Pre-Anal	1	Jsl	3.10	3.67	3.40	0.1	1.01	1.05	1.03	0.01
	0	Jsw	3.28	3.59	3.45	0.09				
Pre-Pectoral	1	Jsl	3.25	3.77	3.46	0.1	1.06	10.8	1.07	0.00
	0	Jsw	2.83	3.59	3.09	0.2				
			Fi index				**SE index**			
Head	1 0	Jsl Jsw	0.69	1.11	0.87	0.1	0.78	1.02	0.94	0.08
Dorsal	1 0	Jsl Jsw	1.0	1.36	1.18	0.1	0.98	1.04	1.01	0.03
Lateral line	1 0	Jsl Jsw	-	-	-	-	0.97	1.01	0.99	0.02
Caudal peduncle	1 0	Jsl Jsw	1.21	1.32	1.26	0.05	0.97	1.08	1.02	0.05
Pre-Anal	1 0	Jsl Jsw	1.42	1.82	1.62	0.1	0.89	1.12	0.99	0.06
Pre-Pectoral	1 0	Jsl Jsw	0.86	1.4	1.1	0.1	1.05	1.19	1.12	0.04

The scale extension index (SE) was calculated as SE=RI/LI, where "RI" is the right cross and "LI" is the left cross. The scale extension index indicates the tendency of the scale dimension towards the right or left. An SE>1 indicates a rightward tendency, SE<1 indicates a leftward tendency, and SE=1 indicates a central dimension. Descriptive morphological analysis was conducted using IBM SPSS v.25 statistical software.

Results

Optical light and scanning electron microscopy

The overall morphology of the scales is illustrated in Figure 4. These scales, similar to other bony fish scales, consist of four parts: rostral, caudal, ventral, and dorsal fields. The morphology reveals distinct surface patterns and arrangements, comprising circuli, grooves, and a focus (Figs. 4 and 5).

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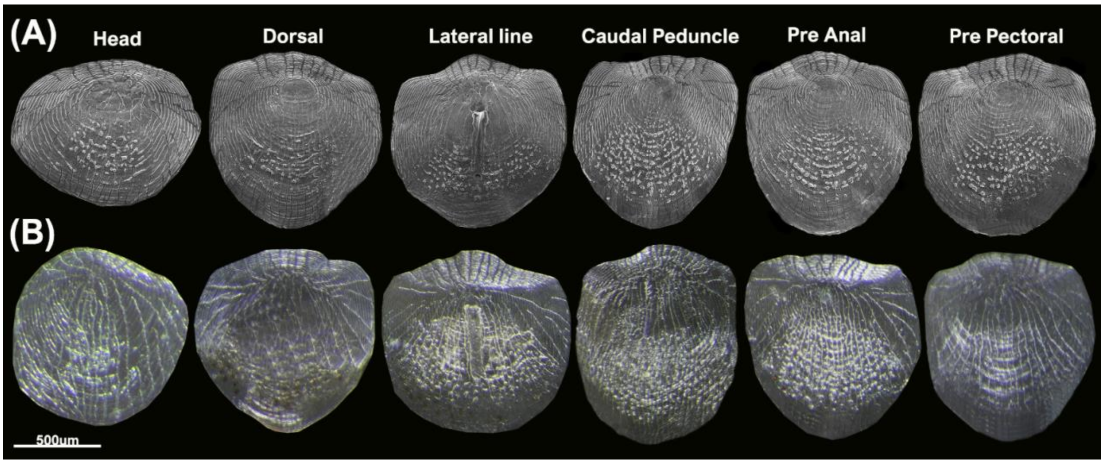

Figure 4: (A) Scanning electron microscopy, and (B) Light microscopic photography of scales for *Garra ghorensis* from the Wadi Ibn Hammad of Jordan.

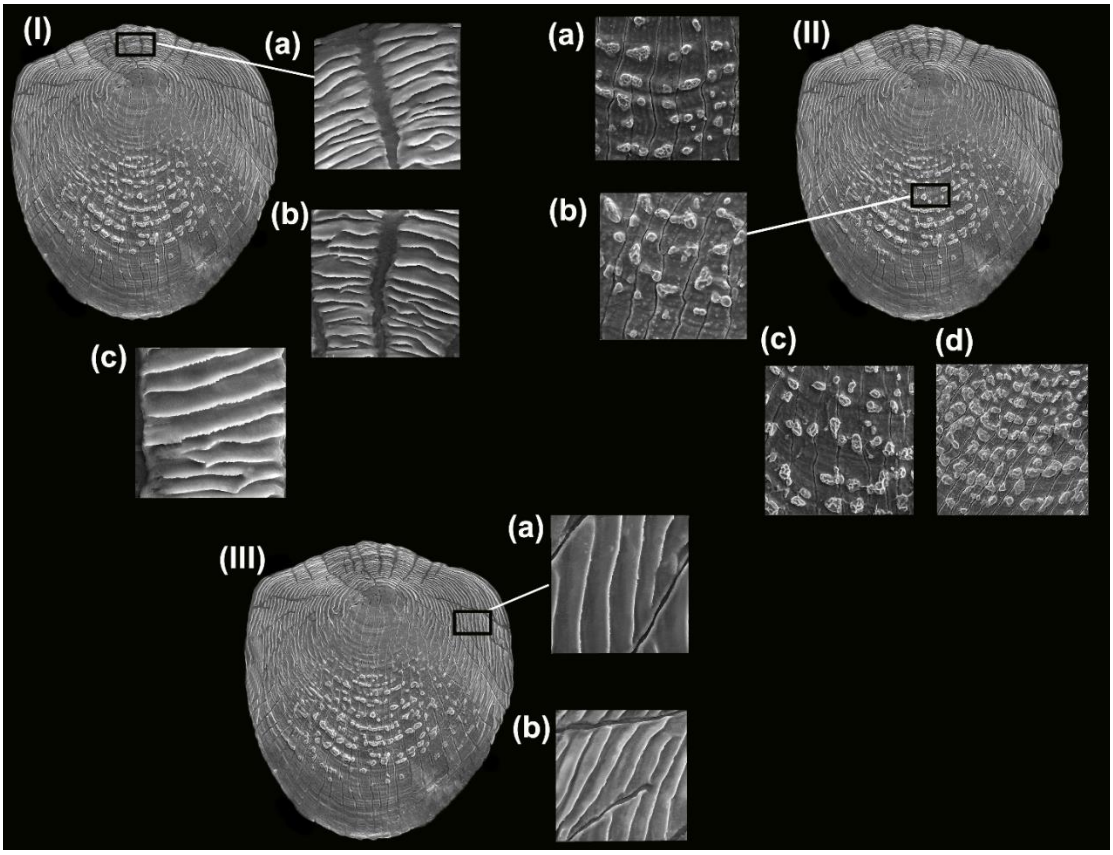

Figure 5: SEM microphotograph of *Garra ghorensis* scale: (I) shape of the first circuli in the anterior field and lepidonts, (II) shape, size, and number of tubercles on the posterior part of the scale, (III) lepidonts in the circuli of the lateral field.

Scale type

The general shape of the scales in *G. ghorensis* was cycloid, and no cteni were observed in the posterior region of the scales. Different types of radii were present. The overall shape of the scale in all the sampled regions was cordate except for the head area which was true circular (Fig. 4).

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Radius/radii

In the scales of all body regions, branched radii were observed in the four fields (anterior, posterior, and lateral). The anterior radii were deeper and broader, extending from the central area to the margins. The posterior-directed radii were longer than others. Overall, three types of radii (primary, secondary, tertiary) were present, with parallel orientations, well-aligned in the posterior field (Fig. 4). Primary radii outnumbered secondary and tertiary radii.

Rostral margin

Usually, the rostral margin of the scales is waved, but in head region was smooth (Fig. 4).

Lateral filed

The right and left regions of scales were convex in all studied regions (Figure 4).

Posterior filed

The posterior area of the studied scales was tapered and round end (almost all regions) or rounded (head region), and without ctenii (Fig. 4).

Focus

The focus, formed early during ontogenesis, appeared circular in all *G. ghorensis* samples. It was located anteriorly. Focus size varied, classified as medium (head, caudal peduncle, and pre-pectoral areas) or small (dorsal and pre-anal areas) (Fig. 4).

Circulus/circuli

The circuli on the scales were clear and discontinuous across all regions. Grooves between the circuli, termed "circular grooves," were present. The first circulus in the anterior region was straight (Figs. 4 and 5 I). Circuli were either with or without lepidonts (Fig. 5 I and III).

Lepidonts

Small denticles on the circuli's crowns in the anterior and lateral regions were termed lepidonts. Microscope analysis revealed varying sizes, with some lepidonts being very small and others prominent. Their shapes ranged from round to short, and their spacing varied from close to distant. Typically, circuli had lepidonts or were without them. The anterior circuli contained numerous large lepidonts (Fig. 5 I), while lateral regions had smaller or no lepidonts (Fig. 5 III). The size, shape, number, and distribution of lepidonts varied between the anterior and lateral regions (Fig. 5 I and III).

Granules / Tubercles

The posterior area of the scales was covered by large granules of varying shapes and sizes, referred to as tubercles (Figs. 4 and 5 II). These tubercles were observed in scales from different body regions, with significant variations in size, located in the inter-radial spaces, covering a large portion of the caudal field. The number of tubercles varied from high (Fig. 5 IId), medium (Figures 5 II b,c), to low (Fig. 5 IIa). The external surface of the scale was not smooth but exhibited numerous warts and ridges.

Expansion

Most of the studied scales expanded along the elongated anterior-posterior axis, while in the head region, they extended laterally (Fig. 4).

Scale indices

By analyzing the J indices (JSL and JSW) for the scales of *G. ghorensis*, which provide the mean relative scale length and width, the values of 2.28±0.1 and 2.45±0.1 respectively were the lowest. The value of JSL and JSW is 3.78 ± 0.2 and 3.79 ± 0.2 respectively, the highest values of JSL and JSW were obtained. In addition, the focus index (Fi) is less than 0.2, which indicates focus located in the front. In addition, the SE index (SE>1) shows the trend of the scale dimensions to the right in the dorsal, caudal peduncle and pre pectoral regions, and the rest of the scales sampled from the fish body showed the trend of the scale dimensions to the left. Finally, the Si index showed that the length of the scales, especially in the scales of the head, lateral line, caudal peduncle and pre-pectoral regions, is greater than their width, and the scales of the dorsal region have a scale width greater than its length (Table 1).

Discussion

The structural materials found in animals, including fish, can inspire the creation of new armor designs with enhanced resistance to penetration, flexibility, lightness, and unique traits like transparency and breathability. These characteristics are seen in the scales of *G. ghorensis*, a cyprinid fish native to the Middle East (Southern basin of the Dead Sea Valley). This study, for the first time, uses scanning electron microscopy and light microscopy to describe the general morphology, surface topography, and microstructures of *G. ghorensis* scales, linking their mechanical properties to fish classification. Six different body regions were analyzed to explore potential variations in scale structure across various body parts.

Scales vary greatly in size, shape, and structure across different fish species, ranging from tough armor plates like those in shrimpfishes and boxfishes to microscopic scales in eels and anglerfishes (Nelson *et al*., 2016; Wainwright *et al.*, 2019). In general, four main types of scales exist: placoid, ganoid, cosmoid, and elasmoid. Cycloid scales are common in various fish orders, including *G. ghorensis*, which exhibits cycloid scales across all six studied regions, with no ctenii present on the posterior (Nelson *et al*., 2016; Esmaeili *et al*., 2019; Wainwright *et al.*, 2019). The overlapping arrangement of these scales allows for greater flexibility compared to cosmoid and ganoid scales. Distinct concentric lines and radial grooves characterize cycloid scales, aiding in reducing body friction with the surrounding water (Schultze, 2016). *Garra ghorensis* shows discontinuous circuli in all fields, a feature commonly seen in other species with similar scale types. Cycloid scales in species like

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Capoeta damascina, *Catla catla*, and *Tor putitora* display comparable traits, enhancing their adaptability and function within aquatic environments (Esmaeili *et al*., 2007, 2012; Esmaeili and Gholami, 2011; Raffealla and Nath, 2020).

For fish species in the genus *Garra*, particularly those inhabiting fast-flowing streams, cycloid scales can be seen as an evolutionary advantage. In this study, *G. ghorensis* was found to have circular (true circular and cordate) scales in various regions. Prior research has explored inter- and intra-species scale shape variations in several fish, including polygonal, hexagonal, and oval forms (Gholami *et al*., 2013; Teimori *et al*., 2017; Echreshavi *et al*., 2021; Sadeghi *et al*., 2021). The general shape of the scales might aid in reducing friction, a hypothesis that requires further validation (Echreshavi *et al*., 2021; Sadeghi *et al*., 2021). Additionally, the scale focus, which forms during early development, displayed distinct positioning and shape in *G. ghorensis*, with variations noted across species (Fig. 4). The reliability of scale morphology as a fish identification marker is confirmed in *G. ghorensis*, whose tightly attached cycloid scales offer effective protection. Due to their durability, these scales could serve as pollution indicators, unlike species like Clupeiformes, which tend to lose scales more easily (Patterson *et al*., 2002; Drago *et al*., 2009; Bräger *et al*., 2017). Furthermore, *G. ghorensis* scales are thin, lightweight, and flexible, contributing to reduced drag and enhanced protection. These characteristics, also observed in other Cyprinids, could be valuable in studying tissue regeneration, given their resemblance to mammalian bone structures (Nara *et al*., 2018).

The scales of *G. ghorensis* exhibit a unique tetra radial pattern, with radii present in all four fields (anterior, posterior, and lateral) (Fig. 4). This distinctive feature has also been reported in species like *G. rossica*, *G. shamal*, and *G. sharq* (Esmaeili *et al*., 2012; Al Jufaili *et al*., 2023; Echreshavi *et al*., 2023). Unlike other cyprinids, such as *Rutilus frisii* and *Capoeta damascina*, this architectural design is specific to the genus *Garra* (Johal *et al*., 1999; Esmaeili *et al*., 2007; Esmaeili and Gholami, 2011). The number of radii in *G. ghorensis* scales ranges from 11 to 12, which can be used as a key characteristic in fish identification and classification (Esmaeili *et al*., 2012). Several detailed studies have focused on lateral line scales to highlight their unique morphology and potential use in fish classification (Kaur and Dua, 2004). In studies by Mekkawy (1980) and Matondo *et al*. (2010), the presence of lateral line canals on scales was identified as a distinctive feature. In *G. ghorensis*, these lateral line scales lack a focus but possess a canal running along the anterior-posterior axis, with a wider anterior opening. The granules in the posterior field increase with fish age, consistent with findings on *G. rossica* and *G. sharq* (Esmaeili *et al*., 2012; Echreshavi *et al*., 2023). Microscopic structures that can be used for fish

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classification through electron microscopy images include circuli, lepidonts, tubercles, and the size and position of the focus (Esmaeili *et al*., 2012). In *G. ghorensis*, the first circulus is straight, similar to what has been observed in *G. shamal* and *G. sharq* (Al Jufaili *et al*., 2023; Echreshavi *et al*., 2023). Lepidonts vary in size and shape, appearing in different parts of the scales (Fig. 5 I, III). Tubercles also show diverse forms like oval or spherical, and their number increases with the fish's size, which mirrors findings in other species of *Garra* (Esmaeili *et al*., 2012) (Fig. 5 II). The scale index study on *G. ghorensis* involved both morphological and morphometric analyses to determine relative size, focus position, and scale spread, aiding in species identification and classification. The J index was used to calculate scale length and width, with dorsal and lateral line scales showing larger dimensions compared to other fish species, J index for the scales of the dorsal area (JSL=3.27±0.07, JSW=3.25±0.1) and for the scales of the lateral line (JSL=3.78±0.2, JSW=3.79±0.2). Additionally, the Fi index indicated that the focus is located in the anterior section of the scales, and the Si index showed that scale length exceeded width, while the SE index suggested rightward extension of the scales (Table 1).

Conclusion

In conclusion, the detailed morphological and morphometric analysis of *G. ghorensis* scales reveals significant structural adaptations that contribute to their survival in fast-flowing streams. The unique arrangement of cycloid scales, their robust attachment to the body, and the presence of distinct radial patterns across all four scale fields provide essential protective features and reduce hydrodynamic drag. These characteristics, combined with the morphometric indices and architectural features, support the classification of *G. ghorensis* within the genus *Garra*, highlighting the ecological and evolutionary significance of scale morphology in fish taxonomy.

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