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Micro-structure Consideration of the Adhesive Organ in Doctor Fish, *Garra rufa* (Teleostei; Cyprinidae) from the Persian Gulf Basin

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Abstract

This paper describes the morphology and microstructures of adhesive organ/disc (AO/AD) of the doctor fish, *Garra rufa* (Heckel, 1843) in the Persian Gulf basin. Ecologically, this species prefer to lives in the bottom of streams and rivers, where they adhere to submerged rocks and stones with its adhesive apparatus located beneath the mouth opening. Adhesive organ consists of a crescent callus part and a modified labial fold which bearing numerous tubercles. Furthermore, adhesive apparatus comprise four basic units (fringed anterior labial fold, posterior labial fold, callous portion of disc, and posterior free margin of disc). The mouth in *Garra rufa* is surrounded by the "fringed anterior labial fold" in anterior part, which bears a large number of posteriorly directed fringes. Posterior labial fold also include some semi-spherical tubercles similar to those on the "fringed anterior labial fold", but to some extend larger. However, it seems that there is a considerable relation between habitat characters (morphology and structure) and macro-micro shape and size of adhesive organs in fishes.

Keywords: Ecology, morphology, adhesive apparatus, Persian Gulf, SEM.

Basra Körfezi Havzasındaki Doktor Balık, *Garra rufa* (Cyprinidae Teleostei)'nın Yapışkan Organının Mikro Yapısının İncelenmesi

Özet

Bu çalışma, Basra Körfezi havzasındaki doktor balığının (*Garra rufa*) (Heckel, 1843) yapışkan organının/diskinin (AO/AD) morfolojik ve mikro yapısını tanımlamaktadır. Ekolojik olarak bu tür, akarsu ve nehirlerin dibinde, batık kaya ve taşlara ağız açıklığının altında bulunan yapışkan organıyla tutunarak yaşar. Yapışkan organı, sayısız yumru taşıyan hilal şeklinde kallus parça ve modifiye bir kıvrımdan oluşmaktadır. Ayrıca, yapışkan organ dört temel birimden (disk saçaklı ön kıvrım, arka kıvrım, hissiz kısım ve diskin arka serbest kısmı) oluşmaktadır. *Garra rufa*'nın ağzı, ön tarafta çok sayıda arkadan bağlı saçak taşıyan "saçaklı ön kıvrım" ile çevrilidir. Arka kıvrım, saçaklı ön kıvrıma benzer şekilde ancak daha büyük ölçüde bazı yarı küresel yumrular içerir. Ancak, balıkların yapışkan organlarının habitat karakteri (morfoloji ve yapısı) ile makro-mikro şekli ve boyutları arasında önemli bir ilişki olduğu görülmektedir.

Anahtar Kelimeler: Ekoloji, morfoloji, yapışkan aparatlar, Basra Körfezi, SEM.

Introduction

The *Garra rufa* as well as another cyprinid fish *Cyprinion macrostomum* have been identified as Doctor Fish because of their medical treatment (Undar *et al*., 1999) and also known as stone fish or mahi-e-sangi (in Farsi) because of its feeding activities. The distribution areas of this cyprinid fish were reported as Turkey and Iraq including Tigris-Euphrates basin, Ceyhan, Quwayq and Jordan river basins as well as inland waters of Iran such as Karoun and Persian Gulf drainages (Berg, 1949; Menon, 1964; Abdoli, 2000; Teimori, 2006; Coad, 2010) (Figure 1). It apparently feeds on skin scales and is used in the treatment of neurodermities (Arkhipchuk, 1999). Ecologically, this species can be found on the streams and rivers bed and also on under gravels and pebbles where they adhere to submerged rocks and stones with its adhesive apparatus located beneath the mouth opening (Figure 2a). Therefore, this species is a highly specialized fish inhibiting the bottom of medium water Temperature Rivers, springs and qanat habitats in these areas (Teimori, 2006; Esmaeili *et al*., 2006). Hence, this species is a bottom feeder and scraps its food such as diatoms and algae from the stones, pebbles or gravel surface using its adhesive

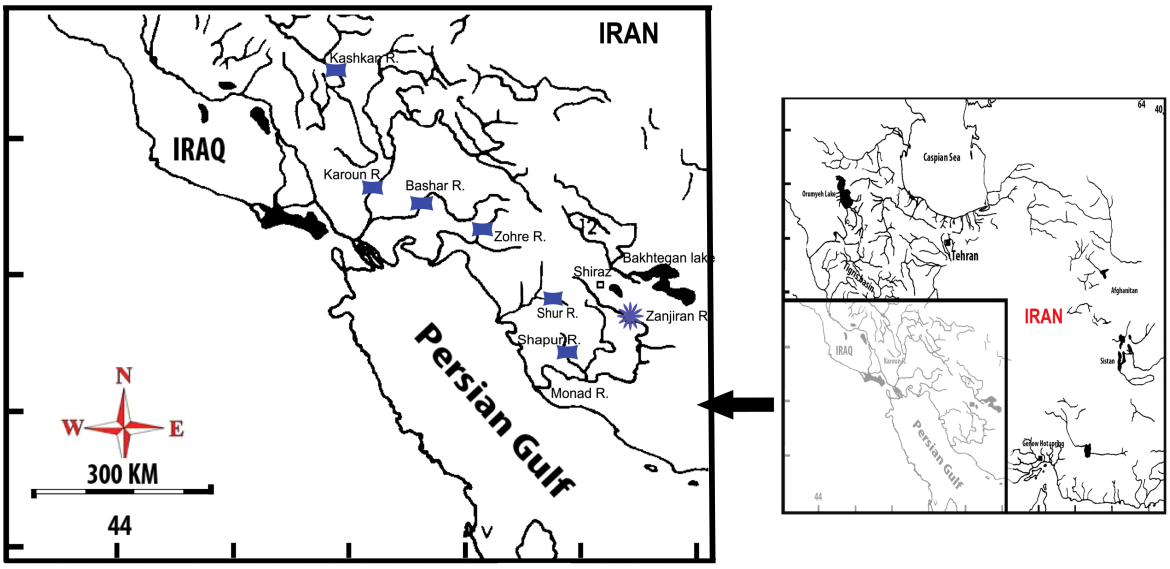

Figure. 1. Map of Iran as well as distribution and sampling site of stone lapper fish. (■) Distribution and (*) sampling site.

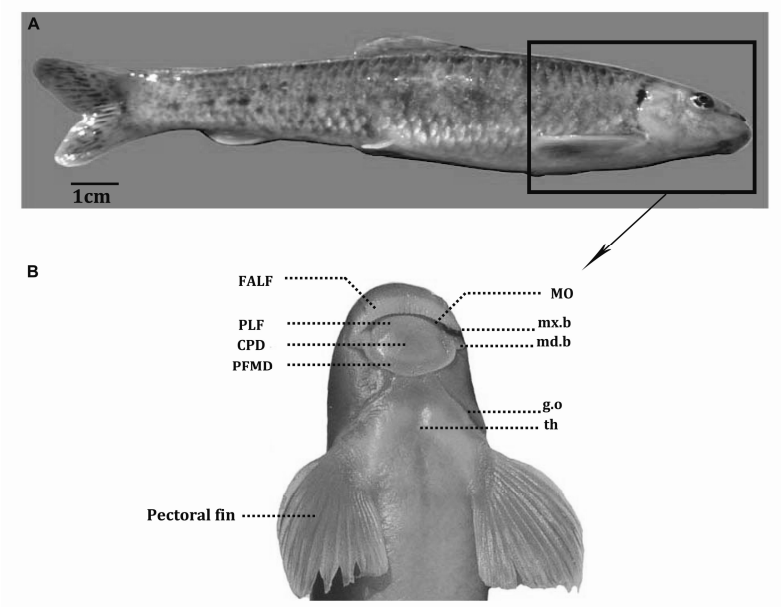

Figure 2. (A) photograph of *G. rufa* from Persian Gulf basin from south of Iran. (b) Ventral view of head and thorax parts, showing components of the adhesive apparatus. (MO) mouth opening; (FALF) fringed anterior labial fold; (PLF) posterior labial fold; (CPD) Callous portion of disc; (PFMD) posterior free margin of disc; (th) Thorax; (g.o) Gill opening; (mx.b) Maxillary barbel; (md.b) Mandibullary barbel.

organ (disc). The ventral view of the mouth of *G. rufa* is given in the Figure 2b. This species has dramatically adapted to live in the undersides of rivers and streams because of it inferior mouth with fleshy lips and developed "adhesive disc" (AD). It is one of the principle morpho-eco-phisiological adaptations required for the survival in these habitats. The lower lips of G. *rufa*, is modified to a developed organ as well as many papillas-like structure and also many tubercles. There is an obvious relationship between morpho-ecological understanding and mechanical-function aspects of the adhesion process which is a manifestation of the adhesive apparatus (Singh *et al*., 1994). The surface morphology of the adhesive organ of *G. rufa* under scanning electron microscope (SEM) has not been studied so far. For that reason, along with its medical treatment character in some parts of Iran and Turkey, we studied morphology and various constituents of the adhesive organ of this species using scanning electron microscope in order to understanding the micro structure of mouth apparatuses.

Materials and Methods

In order to study the morphology and ultrastructure of adhesive organ of stone lapper fish, the fish specimens were collected from Ghara-aghaj River (29°9', 10.16" N, 52°50', 11.94" E), Persian Gulf basin (see Figure. 1) using an electro fishing device. The samples were fixed in alcohol 96% and transferred to the laboratory. We selected a normal sample (because some fishes such as *G. rufa* show some abnormalities in different pollutioned habitats, so they can be used as an appropriate pollution indicator). A delicate layer of its adhesive organ (thickness 0.5 mm) was cut and then, cleaned with 70% ethanol to remove rubbish. After cleaning, the tissue was dehydrated in a graded ethanol series 30, 50, 70, and 90% ethanol and dried on filter paper, placed on blotting paper to avoid folding and fixed in 3% gluteraldehyde at 4^{oC} (for Celsius) for 24 hours. The selected pieces were mounted on metallic stubbs by double adhesive tape with the dorsal surface upward and the ventral surface sticking to the tape and coated with a thick layer of gold in a gold coating unit (SC7640 SPUTTER COATER, Model: FISONS).

Results

The adhesive organ (disc) of *G. rufa* consists of a central circular, an involutedly smooth callous portion with a postero-latral tuberculated border. The adhesive organ in this species is one of the most complex combinations of integumentary modifications. The involuted surface of the border is devoid of tubercles. Morphologically, it comprises the modified anterior and posterior labial folds and integument between the pharyngeal openings. Anteriorly, mouth is surrounded by the "fringed anterior labial fold" (FALF) which bears a large number of posteriorly directed fringes. The fringes mostly cover the mouth, and probably provide filter contrivance during food gathering besides being a part of the adhesive apparatus. The exposed surface of FALF has numerous small spherical and cylindrical tubercles. Some of these tubercles are elongated (Figure 3a). Just behind the mouth, there is a hard scrapping plate being partially covered by "posterior labial fold" (PLF) (Figure 3a). Posterior labial fold also has a number of semi-spherical tubercles similar to those on the FALF, but to some extend larger. The most appear part of the adhesive organ in *G. rufa* is smooth-surfaced oval area called "Callous portion of disc" (CPD) (Figure 3b). The posterior labial fold is followed by Callous portion of disc. There are no any scales in this portion, but there are some small spherical tubercles. All sides of the periphery of CPD except the anterior margin have the "posterior free margin of disc" (PFMD) being firmly attached to CPD but outer rims of PFMD are free (Figure 3c, 3d). There are many round and spherical tubercles in PFMD but in posterior margin parts of the PFMD, the shape of tubercles are elongated. Between these tubercles, there are some opening pores secrete adhesive substance (Figure 3e). The "mouth opening" (MO) is located between FALF and PLF (Figure. 3a). The fringed anterior labial fold (FALF) has many small and large tubercles with different shapes such as spherical, cylinders and elongated. Each tubercle bears numerous glandular secretive device and possibly keratinized spines (Figure 3f, 3g, 3h, 3i). There are several opening pores in the anterior part of FALF which secrete mucous in order to gather food particle and sticking to bottom (Figure 3j).

Discussion

In all the species of *Garra*, pre and post mouthy opening components of the adhesive apparatus comprise four basic units (the fringed anterior labial fold, the posterior labial fold, the callous portion of disc, and the posterior free margin of disc) of integumentary specializations which improve on such modifications described so far in outer hill stream fishes (*Glyptothorax* and *Schizothorax*) which have only one unit (described as adhesive pad or adhesive apparatus) (Singh *et al*., 1994). The other fish with similar pre and post mouth opening component is *Crossocheilus latius* but it also lags behind with regard to structural details of post mouth opening components (Singh *et al*., 1994). The habitats condition of studied species in the Persian Gulf basin is comparatively the same as *Glyptothorax silviae* and *Crossocheilus latius* habitat's but the ecologically, are not so comparable. As an ecologically perspective, *Glyptothorax silviae* typically have been adapted for living in rapid running water with large gravels and pebbles and normally, this species moved against the water flow. In this condition, adhesive organ help fishes to attack on gravels and pebbles for doing feeding function. In contrast to this species, the *G. rufa* has been adapted to live in the rivers and springs with a relative lower water current. Therefore, the adhesive apparatus of *Glyptothorax silviae* has been more developed than of *G. rufa*. Habitat observations substantiate that there is a considerable relation between habitat characters (morphology and structure) and adhesive organ (macro and micro shape and size) of fishes. The snout of *G. rufa* conforms more or less in shape to that of less adapted hill-stream fishes such as *Crossocheilus* and *Schizothorax*. The abundance of mucous cells in the epidermis of the adhesive organ in Doctor Fish is remarkable, which renders the fish with an oily appearance. Thus, the dorso-ventrally compressed form of the snout with mucous coating, offers minimum resistance to water current. There are particular mode of feeding to the adhesive organ in the hill-stream fishes belonging to the genera *Garra* and *Discognathus* (Annandale, 1919). Hora (1920), especially in case of the genus *Garra*, confirmed that the adhesive organ of *Garra*

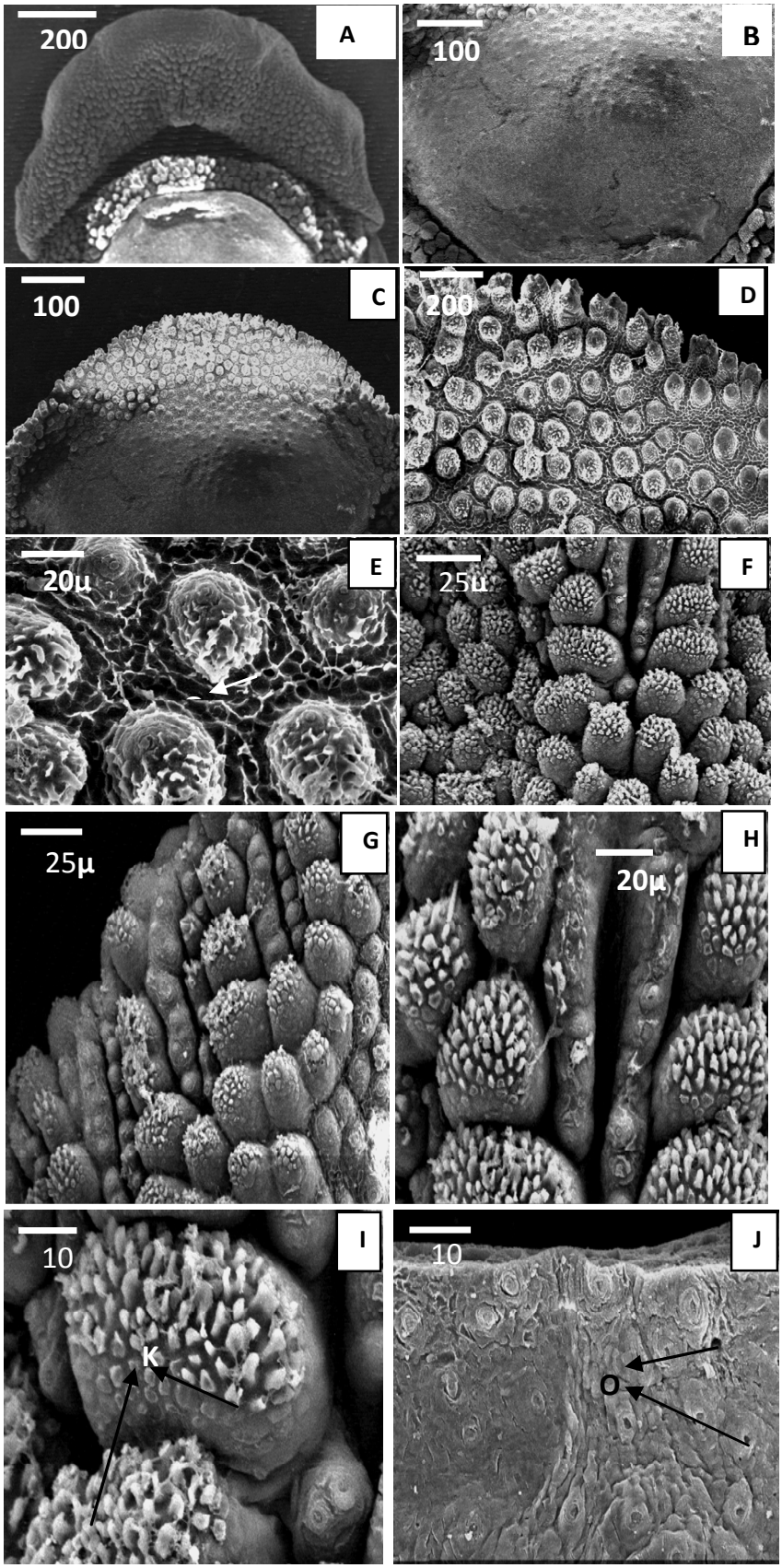

Figure 3. Scanning electron microphotographs of adhesive organ in *G. rufa*. A) Fringed anterior labial fold (FALF) and posterior labial fold (PLF); B) Callous portion of disc; D and C) posterior free margin of disc (PFMD); E) tubercles in PFMD and opening pores; G, H, I) glandular tubercles (secretive device) and keratinized spines (KS); J) opening pores (OP) in the anterior part of FALF.

has not mainly evolved for the strange mode of feeding which is practically similar in all the genera of hill-stream fishes but for securing adhesion to rocks in rapid running water. The disc with its round shape (CPD), a smooth shallow depression in the center, is an effective and admirable device for adhesive. It is the callous portion of the adhesive organ which forms an effective sucker when fish rests on the substratum and bottom. In addition, the numerous glandular secretive devices and keratinized spines on fringed anterior labial fold (FALF) help fish to scratch food from surface of pebbles and gravels. This species apparently feeds on skin scales (see introduction) and is used in the treatment of some parasitism of skin (Undar *et al*., 1999) in several countries such as Iran and Turkey. Consequently, micro study of adhesive organ of the doctor fish is necessary in order to study of its feeding behavior, ecology and develops its treatment function as well. Furthermore, study of the physiological and ecophysiological adaptations for its medical treatments is suggested.

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