



Scanning Electron Microscopy of Adhesive Apparatus of *Garra lissorhynchus* (McClelland) of Meghalaya, India

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Abstract

Garra lissorhynchus (McClelland) also known as the “Khasi Garra” is a species of ray finned fish in the genus *Garra*. The species is commonly found inhabiting the torrential rivers in the Khasi Hills Districts of the state of Meghalaya in India, and thus the name “Khasi Garra”. The adhesive organ of this species was studied with the help of Scanning Electron Microscopy (SEM). The study revealed that the mouth of the fish is guarded with upper fringed labial fold or lip and lower lip that has been modified into the main adhesive organ or disc. The adhesive disc consists of crescent callous pad, modified labial fold and posterior free margin. Many tubercles bearing curved spines were observed on the lips and labial folds whereas, numerous mucous openings were observed on the callous pad. The study suggested that spines and mucous together help the fish attach firmly to substratum under strong water currents.

Keywords: *Garra*, SEM, Suckers, Khasi, Tubercles, Spines.

Introduction

Garra lissorhynchus, McClelland is known as Khasi *Garra*¹ as it predominantly inhabits the streams and rivers of Khasi Hills Districts in Meghalaya, India. The fish is also found in other neighbouring North-Eastern states of India such as Manipur, Assam and Nagaland. In general, *G. lissorhynchus* inhabits the streams and rivers of the Himalayan foot hills.

River Rymben in Lapalang village is 75 km from Shillong, the capital of Meghalaya in India. It is a beautiful river with big rocks by the sides and underneath all through its length. At certain locations where the river makes its turn and the slope is gentle there is a collection of boulders, stones, pebbles and sand. River Rymben originates a few kilometers from Lapalang village and flows to Bangladesh. During the monsoon season when rainfall is plenty, the current of the water is very strong. In the winter months, water is less but flows freely with lesser force. The river is inhabited by a number of fish species such as golden mahseer (*Neolissochilus hexagonolepis*), catfishes (*Glyptothorax trilineatus*, *G. striatus*), Danio (*Devario aequipinnatus*), loach (*Balitora brucei*), *Badis* species, *Schistura multifasciata* and true suckers (*Garra gotyla gotyla* and *G. lissorhynchus*).

It is known that fishes inhabiting hill streams are able to adapt to the turbulent water current with an adhesive apparatus on their body parts to help them attach to substratum. The ventral surface of the fishes living in hill streams is often provided with an adhesive apparatus to facilitate adhesion to rocks and stones². However, different species developed different types of adhesive devices. In *Glyptothorax* species adhesive apparatus is seen in the form of ridges and grooves on the ventral side of the thorax. In other species such as *Crossocheilus*, irregular folds

and ridges of skin on the ventral side of the head act as anchorage device. Well developed adhesive apparatus is exhibited by fishes of the genus *Garra* in the form of true suckers.

Many authors in the early years described the adhesive apparatus of *Garra* species based on the observed morphology and light microscopy. Such studies on *G. annandalei*³, *G. lamta*⁴ and *G. mullya*⁵ were reported. In the recent years, scanning electron microscopy (SEM) due to its large depth of field and high resolving power has been successfully used by many authors in understanding the ultrastructural morphology of tissues and other structures. However, few authors studied the ultrastructure of adhesive apparatus of *Garra* species using SEM. The surface ultrastructure of the adhesive apparatus of *G. lamta*^{6, 7}, *G. gotyla gotyla*^{8, 9}, *G. rufa*¹⁰ were reported using SEM. However, there were no reports on the ultrastructure of the adhesive apparatus of *G. lissorhynchus*. Therefore, the present investigation has been undertaken with the aim to study and report the detail ultrastructure of the adhesive apparatus of *G. lissorhynchus* with the help of SEM.

Material and Methods

A few samples of *G. lissorhynchus* (McClelland.) were collected from river Rymben of Meghalaya, India in the month of April, 2014. A local fisherman was engaged for this purpose since this species inhabits specific locations in the river making it difficult for a common man to catch it. Fishing was done at night with the help of small fishing nets. The fish were anesthetized and preserved by exposing them to a freshly prepared solution of 5 % formaldehyde. The fish were taken to Zoological Survey of India, NER region, Shillong for identification.

Scanning Electron Microscopy (SEM): The mouth part of the fish was dissected and washed in distilled water to remove waste materials and fixed in 2.5 - 3 % glutaraldehyde prepared in 0.1M sodium cacodylate buffer (pH 7.2) for 4 hours at 4 °C. The tissue was washed in 0.1 M sodium cacodylate buffer for 15 - 30 minutes, dehydrated in ascending grades of acetone (30 %, 50 %, 70 %, 80 %, 90 %, 95 %, 100 % and dry acetone) with two changes of 15 minutes each. (Dried acetone was prepared by adding excess amount of copper sulphate to absolute acetone followed by filtration). Dehydrated samples were dried in Tetramethylsilane (TMS) drying technique¹¹ through replacement of acetone by TMS at 4°C for 10 minutes, followed by drying the samples in TMS at 26°C. Dried tissue samples were secured to brass stub (10 mm diameter × 30 mm height) with double coated adhesive tape connected via a patch of silver paint to ensure charge conduction. A conductive coating of gold was applied to the samples using JFC-1100 (JEOL) Ion-Sputter Coater by establishing a low vacuum (10^{-3} Torr) in the sputtering chamber. The coated samples were examined in JSM-6360 (JEOL) SEM.

Results and Discussion

G. lissorhynchus or the Khasi Garra belongs to the family Cyprinidae, order Cypriniformes and class Actinopterygii. The local name for this species from where it was collected is “*sher*”. *G. lissorhynchus* is a small hill stream fish varying in length from 10 to 12 cm (figure-1a). The fish is well adapted to fast flowing hill streams and rivers. This adaptation is seen in the form of well developed adhesive apparatus situated at the ventral part of the head. The adhesive apparatus of *G. lissorhynchus* consists of fringed upper lip and lower lip that has been modified in the form of a specialized suction disc. The disc is oval in shape, shorter than wide measuring about 1cm in breadth and 0.6 - 0.7 cm in length. The middle and major part of the disc consists of fleshy, swollen callous pad guarded by concentric anterior labial fold at the anterior end, posterior lateral free margin on both sides and posterior free labial fold at the posterior end (figure-1b).

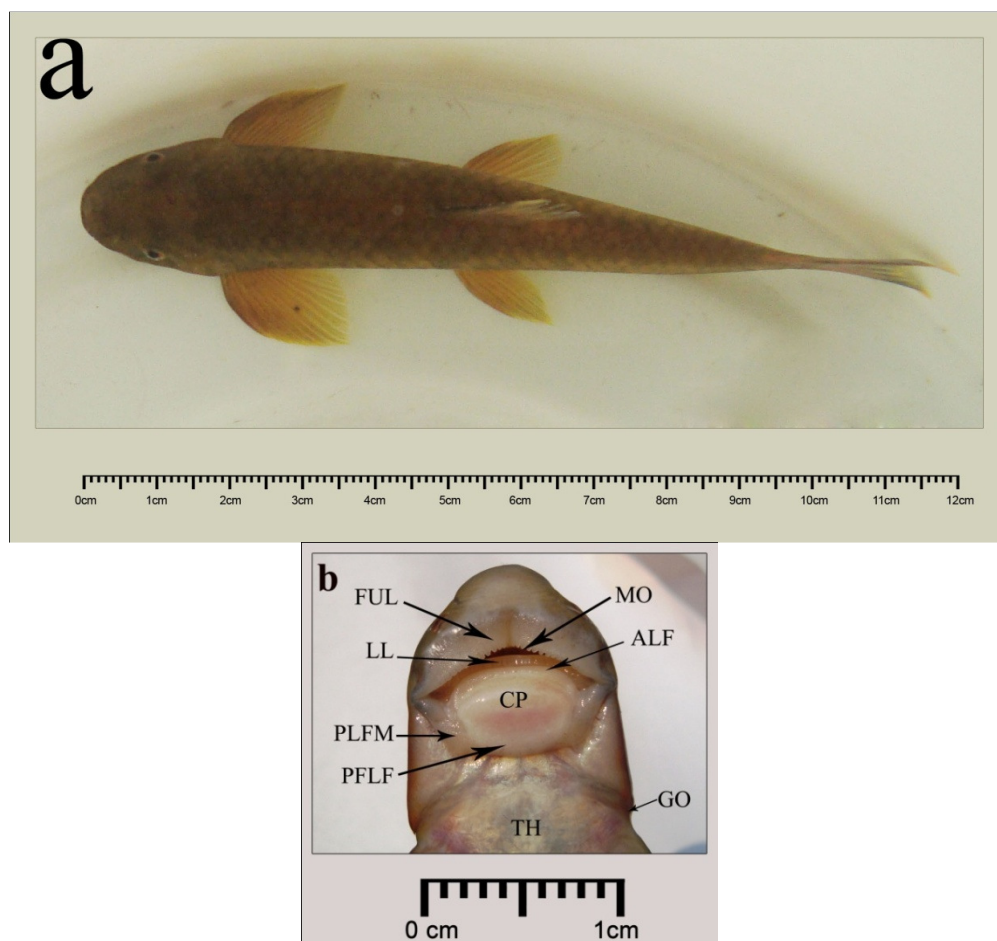


Figure-1

Photographs of *G. lissorhynchus*. (a) Whole fish, dorsal side view. (b) Ventral part of the head showing the adhesive apparatus. Fringed upper lip (FUL); mouth opening (MO); lower lip (LL); anterior labial fold of the disc (ALF); callous pad (CP); posterior lateral free margin of the disc (PMLF); posterior free labial fold (PFLF); thorax (TH) and gill opening (GO)

SEM reveals the detailed surface feature of the adhesive apparatus of *G. lissorhynchus*. At low magnification, the disc exhibits the presence of stub shaped tubercles on the lips and on the labial folds. Large portion of the disc comprises the elevated callous pad (figure-2a). At higher magnification, the stub shaped tubercles are covered by squamous epithelium (figure-3b). It is also evident that the tubercles exhibit many hexagonal epithelial cells that are modified into spines (figure-2d, e, f; figure-3a, b, c, d). Moreover, hexagonal epithelial cells show the presence of

well marked cell boundaries (figure-3c, d, e, f). The spines are broad at the base and gently taper towards the end (figure-2f, figure-3a,b,c,d). Some spines are short and have blunt ends (figure-3c) whereas many spines are long and curved at the end (Figure-3d). It is also noted that the spines tend to face the same direction (Figure-3a,b,d). Moreover, some tubercles exhibit few hexagonal epithelial cells without spines and also epithelial cells with very short spines that may be in the process of development (figure-3b,c,d).

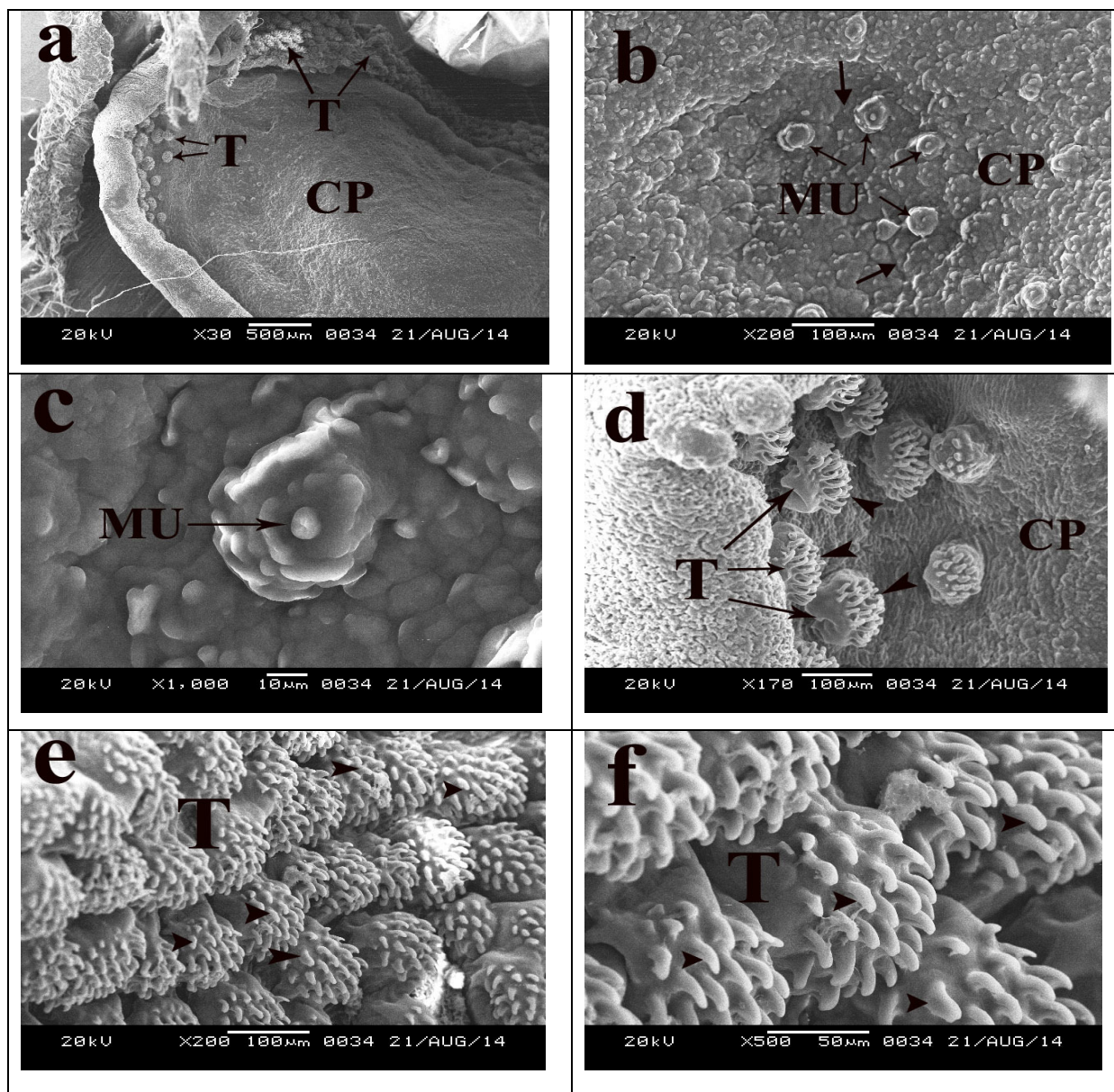


Figure-2

Scanning electron micrographs of adhesive disc of *G. lissorhynchus*. (a) Part of the adhesive disc at low magnification showing tubercles (T); callous pad (CP) (Scale bar = 500 μm). (b) Portion of the callous pad (CP) showing mucous openings (MU) and micro ridges (arrow) (Scale bar = 100 μm). (c) Enlarged view of Fig. 2a showing mucous opening (MU) (Scale bar = 10 μm). (d) Enlarged view of Fig. 2a showing tubercles (T) with spines (arrow head); callous pad (CP) (Scale bar = 100 μm). (e) Higher magnification of part of the adhesive disc showing tubercles (T) with spines (arrow head) (Scale bar = 100 μm). (f) Enlarged view of Fig. 2e showing tubercles (T) with spines (arrow head) (Scale bar = 50 μm)

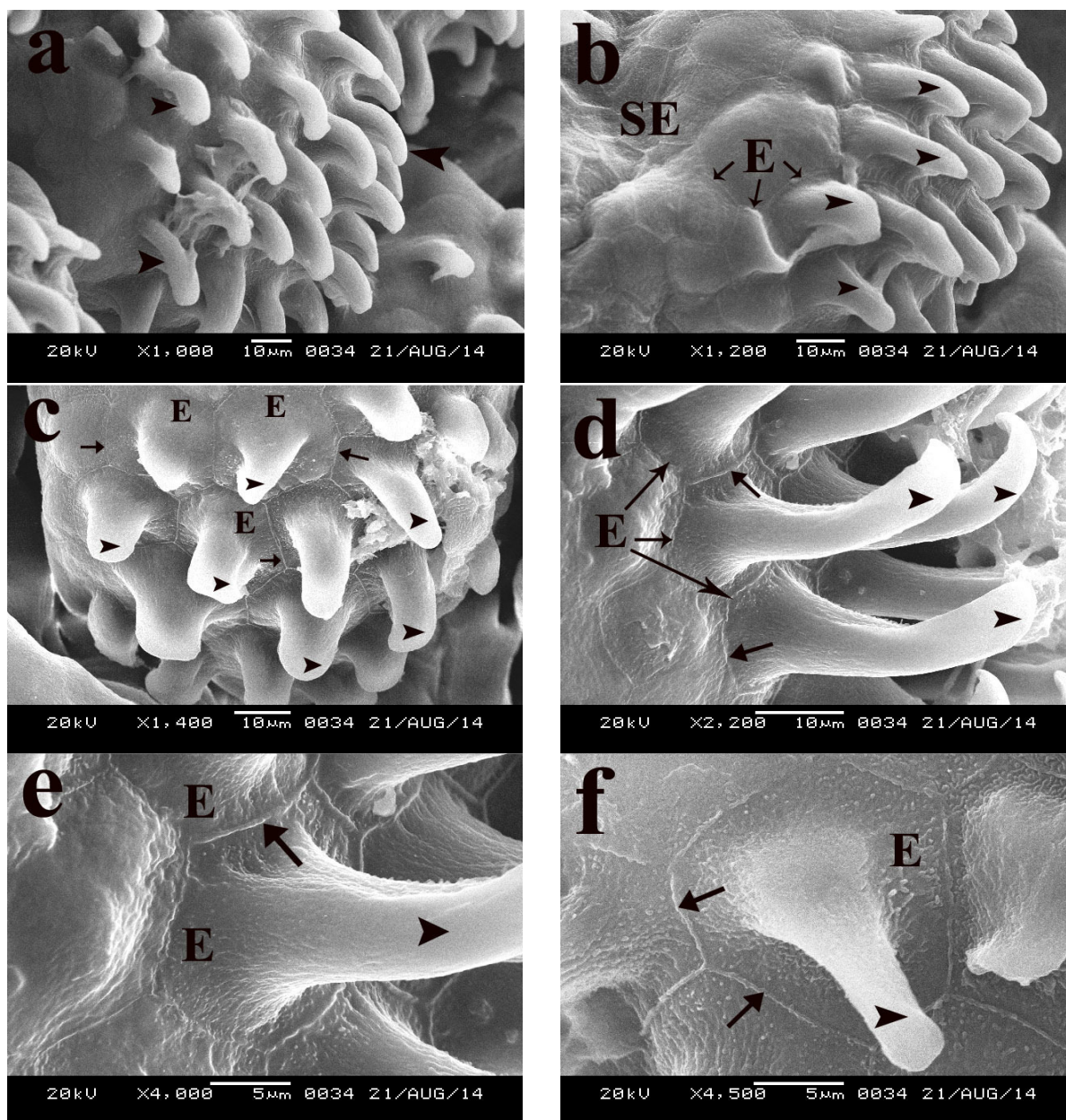


Figure-3

Scanning electron micrographs of adhesive disc of *G. lissorhynchus*. (a) Tubercles at higher magnification showing curved spines (arrow head) that tend to face the same direction (Scale bar = 10 μ m). (b) Part of the disc showing squamous epithelium (SE) in between the tubercles; squamous epithelial cells (E) and spines on tubercles (arrow head) that face the same direction (Scale bar = 10 μ m). (c) Short stub tubercle showing squamous epithelial cells (E) with short spines (arrow head) and cell boundary (arrow) (Scale bar = 10 μ m). (d) High magnification of tubercle showing hexagonal epithelial cells with long hooked spines that face the same direction (arrow head) and cell boundary (arrow) (Scale bar = 10 μ m). (e) Enlarged view of Fig. 3d showing hexagonal epithelial cell (E) with spine that is broad at the base (arrow head) and cell boundary (arrow) (Scale bar = 5 μ m). (f) High magnification of hexagonal epithelial cell (E) with spine (arrow head) and cell boundary (arrow) (Scale bar = 5 μ m)

The callous pad of the adhesive disc composes of irregular, rough epithelium that gives rise to many mucous openings. At low magnification, the callous pad exhibits wide epithelial layers with small elevations throughout the entire area (figure-

2a). At higher magnification, the callous pad shows the presence of irregular micro ridges with numerous mucous openings (figure-2b).

Gross morphology and SEM of the adhesive apparatus of *G. lissorhynchus* resemble other *Garra* species as reported by many authors^{7,9,10,12}. The presence of numerous tubercles with many spines on the lips and the labial folds observed in the present study are of great importance as far as adhesion to the substratum is concerned. The spines are used for anchorage to the substratum⁹. The curved tips of spines observed in the tubercles in the present study act like hooks enabling the fish to cling on to rough and irregular surfaces of stones and rocks. Moreover, unidirectional spines observed in the tubercles suggest their synchronized action to hold on to the substratum against strong current of water.

The presence of some epithelial cells without spines in the present study suggested that the fish are subjected to turbulent habitat that causes loss or damage of the spine due to constant abrasion with rough surfaces. At the same time very short and under developed spines exhibited by the tubercles in the present study, suggest continuous growth of the epithelial cells.

Another important characteristic of the fish observed in the callous pad of the adhesive disc is the presence of mucous openings. Mucous is a thick fluid secretion of mucous glands and mucous membranes that line the body cavities that open to the exterior¹³. In the present study, numerous mucous openings observed in the callous pad suggest the presence of mucous glands which secrete mucous, thus acting as a lubricant protecting the adhesive apparatus from injury. Moreover, mucous enables the fish to make firm hold to substratum⁷. It has been reported that the sudden spread of mucous in the callous pad is facilitated by canaliculi formed by epidermal micro ridges⁷ as observed in the present study.

Light microscopy of adhesive apparatus of *G. mullya*⁵ revealed that adhesion to substratum is performed suctionally by the callous portion of the disc whereas, the lips and tuberculated border of the disc act as frictional device to prevent skidding. However, recent studies performed on different *Garra* species revealed that both the spine bearing lips and the callous pad are important devices for adhesion. SEM analysis of adhesive apparatus of *G. gotyla gotyla*⁸ revealed that protrusions bearing spines present on both lips and disc and mucous pores on callous pad function based on the suction principle. It has been reported that the anchorage apparatus of *Garra* is in the form of a ventrally placed, cup-shaped adhesive disc (0.031 cm²) just behind the arched lower lip and separated from it by a crescent-shaped groove¹⁴. The adhesive disc is capable of generating formidable sticking force if applied against the substratum and pressed carefully to create a vacuum by draining the underlying water¹⁴. Moreover, mucous displaced water and air and thus help the fish to obtain near absolute or absolute vacuum so that fish adheres formidably with the substratum⁹. Thus, the present study supports the idea that both spines and mucous are necessary to attach the fish to the substratum.

Conclusion

SEM analysis of the adhesive apparatus of *G. lissorhynchus* reveals that there are many spines in one tubercle and these spines tend to face the same direction and are curved. The finding suggests that the curved spines hook the fish to the substratum, and at the same time, the mucous that spread out from the numerous mucous openings display the water and air to create a vacuum. The action of these two factors, spines and mucous collectively help the fish to fight the strong current of water and attach firmly to the substratum.

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