Adaptive Modifications in Four Fish Species of the Genus Garra (Teleostei; Cyprinidae) in Basistha River, Assam, India

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Adaptive Modifications in Four Fish Species of the Genus *Garra* (Teleostei; Cyprinidae) in Basistha River, Assam, India

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Abstract

This paper deals with the study of the surface morphology of barbels, upper lips, and adhesive discs in four hill stream fish species collected from Basistha River, a torrential river in Guwahati city, Assam, India. The four species belonging to the genus *Garra* namely, *Garra gotyla* (Gray, 1830), *Garra gravelyi* (Annandale, 1919), *Garra stenorhynchus* (Jerdon, 1849), and *Garra nasuta* (McClelland, 1838) were collected from the same torrential habitat. These fish revealed anatomical peculiarities in their barbels, lips, and adhesive discs which are believed to help them in adapting to such habitats. Organs exhibiting adaptive modifications (barbels, lips, and adhesive discs) were studied with the scanning electron microscope. The study primarily revealed the presence of type I and type II taste buds in the barbels, lips, and adhesive discs, and numerous unculi surrounded by microridges in the upper lips and adhesive discs. A cumulative, intercalated action of these organs enables these fish to adhere and adapt to rocky, torrential streams. Special ability to adapt to these habitats was reflected from the two types of taste buds (I and II) present in the barbels, and the clustering of excrescencies bearing unculi in the lips and adhesive discs of the fish.

Key words: *Garra*, hill stream, adaptive modifications, adhesive discs, unculi, taste buds

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Introduction

Most hill stream fish adapt differently and differentially to the environment they inhabit (Nagar et al., 2012). Development of a proper anchorage and mechanical stability to combat the force of swift water currents are some of the prerequisites for survival of hill stream fish (Nagar et al., 2012). The morphology of organs like the lips and adhesive discs in such fish has been reviewed by several workers. One of the pioneers in this field (Hora, 1921, 1922, 1930) studied the adaptive modifications, precisely on the adhesive organs (AO) in several fish; and gave a detailed note on the bionomics and evolution of torrential fauna giving special reference to the organs of attachment in these hill stream fish. The adaptive modifications of the hill stream fish *Glyptothorax telchitta* (Hamilton, 1822) along with the histological details of the structures of adaptation in this fish species were thoroughly studied (Bhatia, 1950). It was ascertained that adaptation of organisms to the environment and structural modifications are a part of evolutionary process and such adaptations are produced through changes of functions (Hora, 1952). It was further stated that changes in the environment are responsible for functional changes in organisms as a first step in evolution and such changes ultimately lead to structural modifications (Hora, 1952). Studies in related fields were carried forward in greater detail by various workers on *Garra mullya* (Sykes, 1839) (Saxena, 1959), *Glyptothorax pectinopterus* (McClelland, 1842) (Sinha et al., 1990), *Pseudecheneis sulcata* (McClelland, 1842) (Singh & Agarwal, 1991; Das & Nag, 2004, 2005), *Garra gotyla* (Das & Nag, 2006), and *Garra lamta* (Hamilton, 1822) (Nagar et al., 2012).

Fish belonging to the family Cyprinidae of the genus *Garra* (Hamilton, 1822) are elongate, small- to medium-sized, bottom-dwelling fish usually found in fast-flowing waters, where they adhere to the surface of the rocks using their highly modified lower lips which act as a sucker (Chen et al., 2009). A dorsoventrally flattened body, two pairs of dorsoventrally flattened fins and presence of an adhesive disc are the primary characteristics of fish belonging to the genus *Garra* (Talwar & Jhingran, 1991). Among the sub-Himalayan stream fish of India, *Garra* sp. are usually found inhabiting fast-flowing rivers and streams where they adhere to the substratum by means of a sucking disc present on the ventral side of the head, just behind the mouth (Lalronunga et al., 2013). The sucking disc is also known as developed AO (Das & Nag, 2006). Due to these special characteristics, these fish have become excellent models for studying adaptive modifications.

This tropical and Asian hill stream cyprinid genus consists of ~70 described species (Zhang & Chen, 2002), and constitutes of more than 85 described species (Chen et al., 2009) of which 68 species were reported to belong to Asia (Kullander & Fang, 2004).

Some researchers had reported on the ultrastructural details of the adhesive discs in some species belonging to this genus (Saxena, 1959; Singh et al., 1994; Das & Nag, 2006; Nagar et al., 2012). There are presently some reports on the adhesive apparatus of *G. gotyla* (Roberts, 1982; Singh et al., 1994; Das & Nag, 2006; Gaur et al., 2013); and few reports on the study of barbels in Siluroids (catfish) (Grover-Johnson & Farbman, 1976; Ovalle & Shinn, 1977; Abou-Zaid, 2014;

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Kaushik & Bordoloi, 2015, 2017); and in cyprinids (*Danio rerio*) (Hansen et al., 2002). A detailed study on adaptive modifications undergone by these hill stream dwellers however, remains incomplete.

The present paper discusses the surface topography of both pairs of barbels (rostral and maxillary), upper lips, and adhesive discs in four hill stream fish of the genus *Garra*, namely, *G. gotyla*, *Garra gravelyi*, *Garra stenorhynchus*, and *Garra nasuta* collected from Basistha hill stream, Guwahati, located at Assam, India. The aim of the study was to analyze the anatomical peculiarities present in these structures. The fish were selected on the basis of their availability in the hill stream, which is the only torrential river situated in the heart of Guwahati city, Assam, India.

Materials and Methods

Collection of Fish

Live specimens of *G. gotyla*, *G. stenorhynchus*, *G. gravelyi*, and *G. nasuta* ($n = 5$ for each species), all adults but not in the breeding phase, were collected during the winter months of 2013–2014 from the Basistha River, Guwahati. Collections were done in the intermediate zone of the river (between 26°05′31.2″ N and 91°46′50.3″ E and 26°05′39.4″ N and 91°47′01.4″ E). Morphometric measurements were done following Talwar & Jhingran (1991), Jayaram (1999), and Vishwanath et al. (2007). Total length (TL) of the fish was measured with a dial calliper (Mitutoyo) to the nearest 0.01 cm. Body weight (BW) was measured with a digital balance (Sartorius, BS-223 S, Goettingen, Germany) to the nearest 0.01 g. Adult fish were in the range of 7–11 cm TL and 3.6–23 g BW. The fish were cold anaesthetized in the laboratory. Barbels (rostral and maxillary), upper lips, and adhesive discs were dissected carefully using a sterilized surgical blade. Subsequently, the excised tissues were rinsed in physiological saline.

Sample Preparation

The parts dissected out were superficially cleaned with 70% alcohol using a fine brush, to remove any attached debris. Samples were initially fixed in 3% glutaraldehyde solution for 24 h. Buffer treatment consisted of washing the samples three times with 0.1 M sodium cacodylate buffer at an interval of 15 min to remove traces of glutaraldehyde, and finally storage in the same buffer until processed further. Samples were then gradually dehydrated in acetone grades (30–100%) and were finally treated with tetramethylsilane following the method of Dey et al. (1989). Gold sputtering (6 min) was done on clean and dried samples before viewing in the scanning electron microscopes (SEM) [JSM-6360 JEOL (Peabody, MA, USA) at North East Hill University (NEHU)] and ZEISS SIGMA VP (Carl Zeiss AG, Oberkochen, Germany), SL. NO. 01-94 at the Institute of Advanced Study in Science and Technology) at 20 kV.

Statistical Analysis

ImageJ software (National Institute of Health, USA) was used to calculate the total length of the taste buds. Sizes of the taste buds were statistically analyzed by one-way analysis of variance (ANOVA) in Excel for Windows. GIMP software was used to change the resolution of the images.

Results

Light microscopic images of the dorsal and ventral surfaces of a *Garra* species are shown in Figures 1a and 1b, respectively. Upon viewing under the light microscope, an adhesive disc was observed located ventrally. The adhesive disc is continuous with the lower lip of the species. *Garra* sp. possesses a pair of maxillary barbels and a pair of rostral barbels. The dorsal surface revealed the presence of the proboscis with tubercles.

Observations Under the SEM

The summary of observations of rostral and maxillary barbels, upper lips, and adhesive discs is presented in Table 1, with average sizes of the taste buds at Table 2.

Rostral Barbels

Surface topography of the rostral barbels revealed that taste buds of type I are primarily found in these species. Figures 2a to 2h represents the ultra-structures observed in the rostral barbels of the four species.

In *G. gotyla*, type I taste buds were observed towards the tip of the barbels (Fig. 2a). Each taste bud revealed an elevation from the general surface epithelium when observed with a higher on screen magnification of ~9000 × (Fig. 2b). The average height of these taste buds was $9.4 \pm 0.2\,\mu$m. Sensory cells bearing microvilli were observed at the apical regions of these taste buds.

In *G. gravelyi*, type I taste buds, also concentrating towards the barbels' apical regions (Fig. 2c), were observed with epithelial elevations with depressions in the surrounding areas as the primary observation. The surfaces of the taste buds were covered with microridges. The average height of the taste buds was $9.73 \pm 3.5\,\mu$m. When viewed with a higher on screen magnification of ~5300 × (Fig. 2d), a single taste bud was observed to be an elevated papilla with numerous microridges enclosing the bud and microvilli at its apical region.

In *G. stenorhynchus* (Fig. 2e), approximate height of the taste buds was $9.99 \pm 0.9\,\mu$m. It was observed that the taste buds were enclosed by microridges and the apical regions of the taste buds were covered with sensory microvilli (Fig. 2f).

In case of *G. nasuta*, mucous pores were observed in the adjoining areas of the taste buds (Fig. 2g). Mucosal openings were located adjacent to the taste buds, and higher magnification of ~2500 × revealed numerous microvilli in the sensory area of a single taste bud (Fig. 2h). The average height of the taste buds was $23.8 \pm 3.4\,\mu$m.

Hence, a similar orientation of taste buds was observed in the rostral barbels of *G. stenorhynchus* and *G. nasuta.*

Maxillary Barbels

Figures 3a to 3f represents the ultra-structures observed in the maxillary barbels of the four species. The maxillary barbels of these fish revealed that type II taste buds were abundant in *G. gotyla*; with an average height of $10.9 \pm 1.5\,\mu$m. These taste

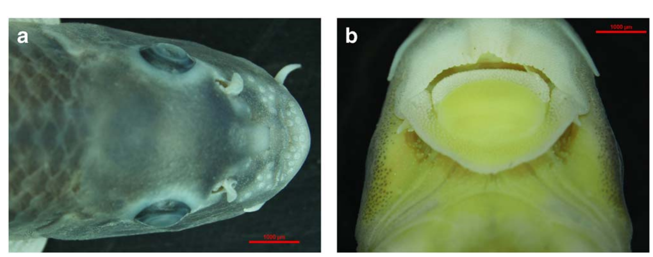

Figure 1. Light microscopic images of the dorsal side (**a**) and ventral side (**b**) of a *Garra* species.

Table 1. Summary of observations in rostral and maxillary barbels, upper lips and adhesive discs in the four species of *Garra*.

	Characteristics	*Garra gotyla*	*Garra gravelyi*	*Garra stenorhynchus*	*Garra nasuta*
Rostral barbels	Type I taste buds	+	+	+	+
	Type II taste buds	–	–	–	–
	Microvilli	+	+	+	+
	Microridges	–	+	+	–
	Mucous pores	–	–	–	+
Maxillary barbels	Type I taste buds	–	–	+	–
	Type II taste buds	+	+	–	+
	Microvilli	+	+	+	+
	Microridges	+	+	–	–
	Mucous pores	–	–	+	+
Upper lips	Type I taste buds	–	+	–	+
	Type II taste buds	–	+	–	–
	Mucous pores	–	–	–	+
	Excrescencies loaded with unculi	+	+	+	+
Adhesive discs	Type I taste buds	–	–	–	–
	Type II taste buds	–	–	–	–
	Mucous pores	+	+	+	+
	Excrescencies loaded with unculi	+	+	+	+

Table 2. Types and Sizes of Taste Buds in the Rostral and Maxillary Barbels in the Four Species of *Garra*.

Sl. No.	Name of the Fish	Rostral/Maxillary Barbel	Type of Taste Buds Observed	Size of the Taste Buds (µm) (Mean ± SD)	*p*-Value
1.	*Garra gotyla*	Rostral	Type I	9.4 ± 0.2	0.3
		Maxillary	Type II	10.9 ± 1.5	
2.	*Garra gravelyi*	Rostral	Type I	9.73 ± 3.5	0.3
		Maxillary	Type II	9.7 ± 2.4	
3.	*Garra stenorhynchus*	Rostral	Type I	9.99 ± 0.9	0.3
		Maxillary	Type I	19.63 ± 4.3	
4.	*Garra nasuta*	Rostral	Type I	23.8 ± 3.4	0.3
		Maxillary	Type II	23.41 ± 0.4	

The statistical analysis (one-way analysis of variance) reveals no statistically significant differences in the taste buds among the species ($p > 0.05$).

buds were enclosed by microridges and the apical regions revealed the presence of both shorter and longer microvilli (Fig. 3a). Similar taste buds of type II were observed towards the anterior tips of the maxillary barbels in *G. gravelyi*. The average height of the taste buds was $9.7 \pm 2.4\,\mu$m and each bud was enclosed by microridges (Fig. 3b). However, in *G. stenorhynchus*, taste buds of type I bearing microvilli were observed towards the anterior tip of the barbels (Fig. 3c). The average height of the taste buds was $19.63 \pm 4.3\,\mu$m. The areas adjacent to these taste buds were composed of irregular polygonal cells bearing mucous pores (Fig. 3d). Type II taste buds were also prominent in *G. nasuta* with an average height of $23.41 \pm 0.4\,\mu$m (Fig. 3e). Mucous pores were distinct near the areas where such taste buds were highly concentrated (Fig. 3f).

Hence, in case of the maxillary barbels, type II taste buds were observed in *G. gotyla*, *G. gravelyi* and *G. nasuta* and type I taste buds in *G. stenorhynchus*.

Upper Lips

The upper lips in *Garra* species are continuous with the lower lips, which are modified to form the adhesive discs. Figures 4a to

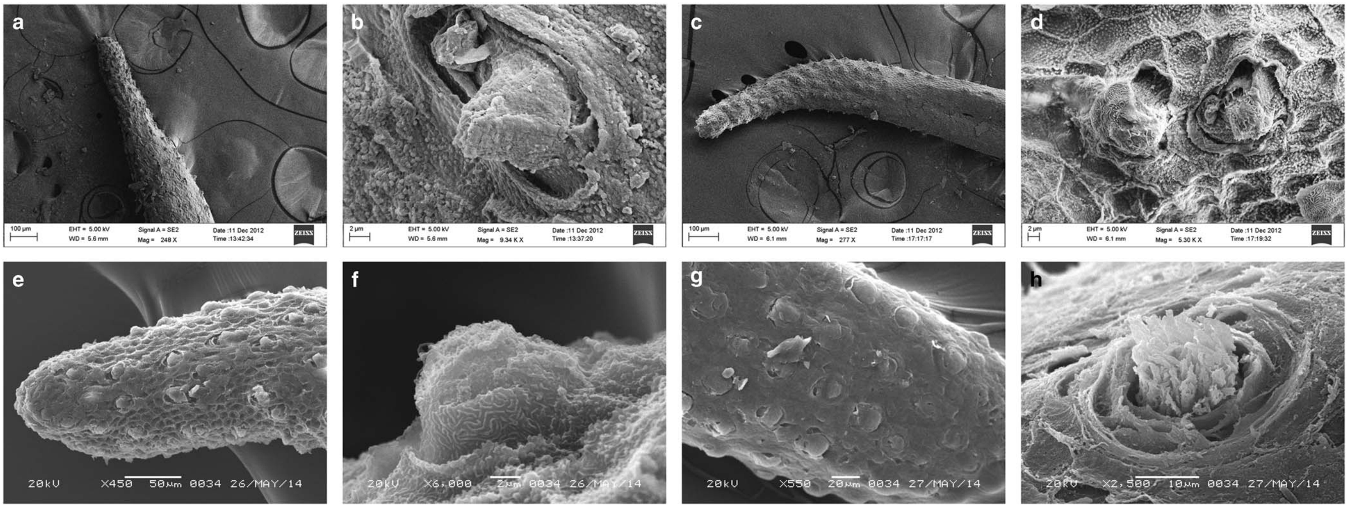

Figure 2. Scanning electron microscope images: (**a**) rostral barbel (RB) of *Garra gotyla* with taste buds (TB) at the apex, (**b**) higher magnification of **a** showing type I TB; (**c**) RB of *Garra gravelyi* with type I TB, (**d**) type I TB in *G. gravelyi* with microridge (Mr) and microvilli (Mv); (**e**) apical region of RB of *Garra stenorhynchus* with type I TB, (**f**) type I TB in *G. stenorhynchus* with Mv and Mr (6000×); (**g**) RB of *Garra nasuta* with TB and mucous pore, (**h**) type I TB in the RB of *G. nasuta* with Mv (2500×).

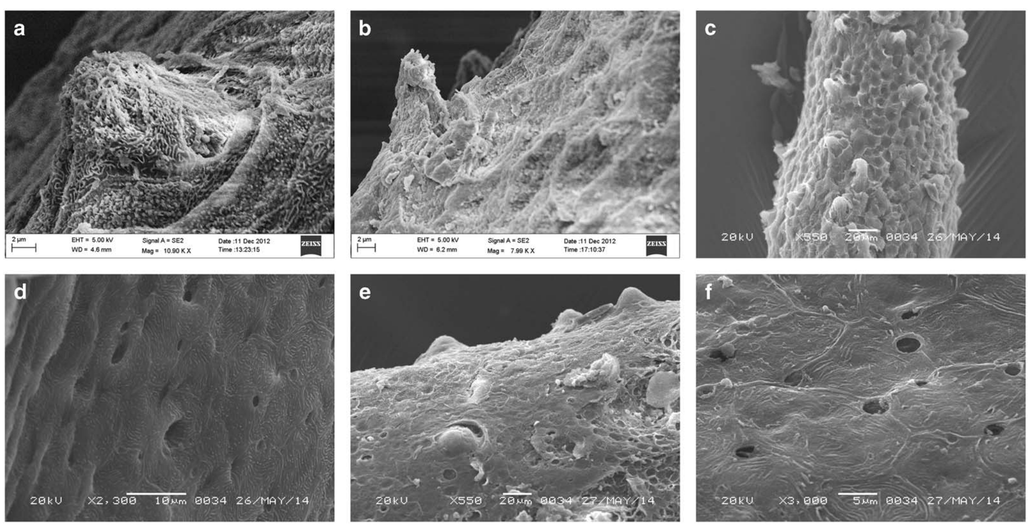

Figure 3. Scanning electron microscope images: (**a**) type II taste buds (TB) in the maxillary barbel (MB) of *Garra gotyla* with the presence of microvilli and microridge; (**b**) apical tip of a MB in *Garra gravelyi* displaying a type II TB; (**c**) type I TB in the MB of *Garra stenorhynchus*; (**d**) irregular polygonal cells (IPC) bearing mucous pore (MP) in the MB of *G. stenorhynchus*; (**e**) type II TB in the MB of *Garra nasuta*; (**f**) MP in the MB of *G. nasuta* when viewed with higher magnification (3000×).

4f represents the ultra-structures observed in the upper lips of the four species. Surface topography studies on the upper lips in the four species revealed that in *G. gotyla*, the upper lip possessed dense aggregations of excrescencies loaded with superficial epithelial projections called unculi (Fig. 4a). The anterior regions of the lips were mucogenic in nature, while the posterior regions were strictly keratinized, possessing such horny epidermal outgrowths which are otherwise known as unculi. Unculiferous structures were observed in all the species. In case of *G. gravelyi*, type I taste buds were observed in the anterior region comprising of both light and dark cells bearing microvilli (Fig. 4b), while the posterior region was loaded with similar clusters of excrescencies bearing unculi. Towards the extreme posterior region of the lips, type II taste buds were observed adjacent to the clusters of excrescencies (Fig. 4c). Unculi were located as depressions burrowed within the surface epithelium. Prominent curved unculi stacked together and loaded in excrescencies were observed in case of *G. stenorhynchus* (Fig. 4d). In *G. nasuta*, in addition to clusters of excrescencies, mucous pores and type I taste buds were observed towards the anterior regions of the lips (Figs. 4e and 4f).

Hence, clusters of excrescencies with densely packed unculi were observed in all four species with the presence of type I taste buds and mucous pores in *G. nasuta*; and type I and II taste buds in *G. gravelyi*.

Adhesive Discs/Suctorial Discs

Under the electron microscope, the adhesive disc or the suctorial disc in *Garra* species was observed to comprise of two distinct regions: the central callous pad, mucogenic in nature, bearing numerous mucous pores and the peripheral keratinized region, bearing epithelial projections bearing outgrowths called unculi. Figures 5a to 5d represents the ultra-structures observed in the adhesive discs of the four species.

In *G. gotyla*, dense aggregations of unculi on clusters of excrescencies were observed in the keratinized areas on the

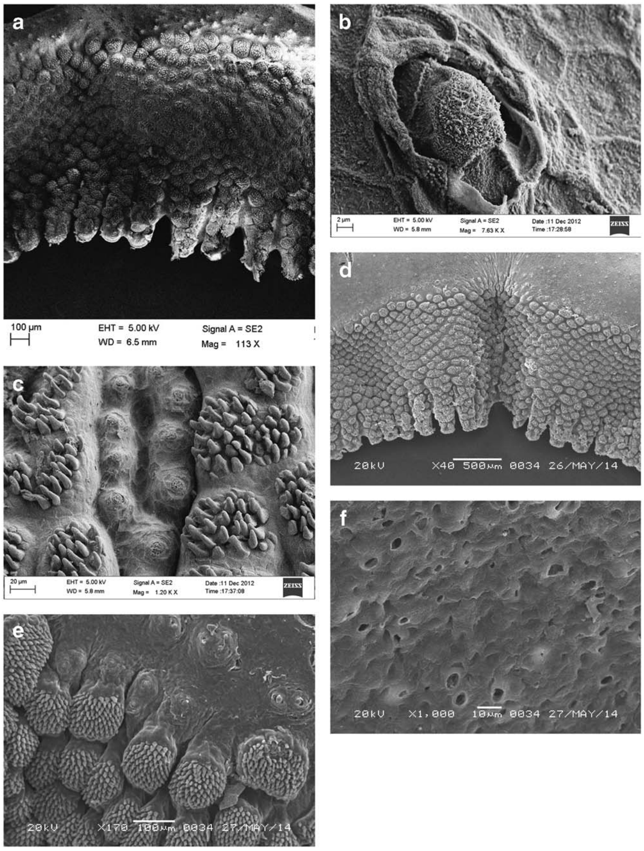

Figure 4. Scanning electron microscope images: (**a**) an overview of the upper lip (UL) in *Garra gotyla* showing epithelial projections; (**b**) type I taste buds (TB) in the anterior region (AR) of the UL in *Garra gravelyi* possessing microvilli; (**c**) type II TB in the posterior region of the UL of *G. gravelyi* located adjacent to clusters of excrescency (EX); (**d**) clusters of EX in the UL of *Garra stenorhynchus* loaded with unculi; (**e**) clusters of EX in the posterior region and type I TB and mucous pore in the AR of UL in *Garra nasuta*; (**f**) higher magnification (1000×) of the AR of UL of *G. nasuta* revealing MP.

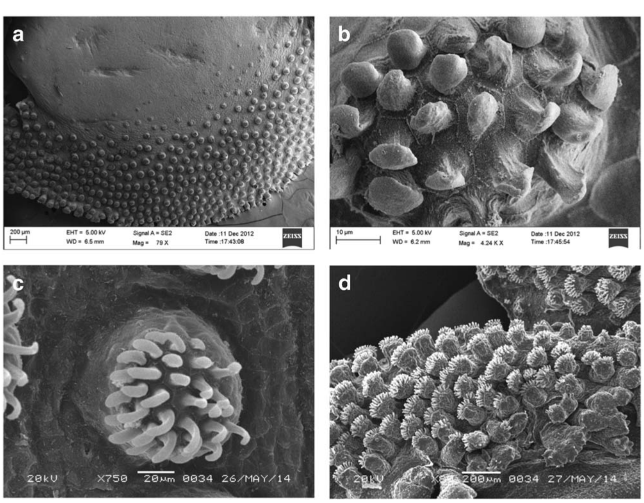

Figure 5. Scanning electron microscope images: (**a**) an overview of the adhesive disc (AD) in *Garra gravelyi* with the callous pad and the peripheral keratinized area; (**b**) higher magnification of **a** (4000×) displaying uncluli loaded in a single excrescency (EX); (**c**) a cluster of curved UN borne on a single EX in the AD of *Garra stenorhynchus*; (**d**) dense clusters of UN loaded EX observed in the peripheral regions of the AD in *Garra nasuta*.

peripheral regions, while the central regions of the adhesive discs were seen to possess mucosal openings as well as taste buds. A similar organization of the adhesive discs was observed in *G. gravelyi*, *G. stenorhynchus*, and *G. nasuta*. In *G. gravelyi*, a continuous stretch of epidermal outgrowths was observed in the keratinized regions with numerous excrescencies bearing unculi (Fig. 5a). Upon higher magnification, numerous curved unculi loaded on a single excrescency were observed (Fig. 5b). Such epidermal outgrowths were surrounded by hexagonal epithelial cells. The central regions of the adhesive discs were strictly mucogenic in nature with the presence of mucosal openings. In *G. stenorhynchus*, both pentagonal and hexagonal cell types bearing microridges were observed adjacent to the growing excrescencies and unculi were borne on hexagonal cell types (Fig. 5c). In *G. nasuta*, similar clusters of excrescencies bearing unculi were observed in the peripheral keratinized regions of the adhesive discs (Fig. 5d). Unculi observed loaded on each excrescency were in clusters, a single unculus being a curved structure with a smooth surface.

Discussion

Morphological variations visible under the SEM in barbels, lips, and adhesive discs of the four species belonging to the genus *Garra* revealed specialized structures and structural peculiarities that are responsible for assisting them in adapting differentially to a torrential environment. These topographical peculiarities are also said to be related to their feeding habits, feeding biology, and various sensory mechanisms (Singh et al., 1994).

Taste Buds

For their peculiarities in adaptations, certain olfactory organs in certain hill stream fish have been studied earlier. Such organs of olfaction were responsible for mechano-sensory functions which were well related with ecological-feeding habits and feeding times of the hill stream fish (Singh et al., 1994). Apart from olfaction, taste buds of various types are also observed in fish and are adaptations. The Cyprinids and the Siluroids are the two major groups among the fish, possessing taste buds throughout their body (Hara & Barbara, 2007). These epithelial projections represent vital sensory organs that are common to all vertebrates and they have been used as simple and convenient models for studying sensory interactions (Ovalle & Shinn, 1977). Taste buds have been grouped into four different types (Murray, 1971, 1973); of which three types were found to occur in fish (Reutter, 1971). These indicate vital details on the species characteristics depending upon their differences and their presence or absence on the body. Type I taste buds form a depression from the neighboring epithelial cells, forming a demarcation; while type II taste buds are not surrounded by a rim into which the base is sunk, forming no demarcations as such (Reutter et al., 1974). It was stated that the terminals of both types of buds are usually similar; and they bear sensory cells called the longer and shorter microvilli (Reutter et al., 1974).

In the present study, the external morphology of the taste buds in the rostral and maxillary barbels of the four species of *Garra* studied under the SEM revealed that taste buds of type I and II predominantly occurred in the barbels of these fish. Type I taste buds were observed in the apical regions of the rostral barbels in all the four species and only in the maxillary barbels of *G. stenorhynchus*. Type II taste buds were observed in the maxillary barbels of *G. gotyla*, *G. gravelyi*, and *G. nasuta*. Hence, both type I and II taste buds were found in both barbels only in case of *G. stenorhynchus*. Type I taste buds function as mechanoreceptors and also as chemoreceptors whereas type II taste buds are chemoreceptive only (Reutter et al., 1974). Hence, the rostral barbels in these four fish species function both as mechanoreceptors as well as chemoreceptors while the maxillary barbels are chemoreceptive only, with an exception in *G. stenorhynchus* which shows an enhanced function of adaptation being in possession of both types of taste buds in its barbels. The four fish studied have high chemoreceptor and mechanoreceptor density in the barbels providing sensitivity to chemical stimulus (Gomahr et al., 1992).

The measurements of type I and type II taste buds that originated in these four fish were statistically analyzed by one-way ANOVA which revealed no statistically significant differences ($p > 0.05$) in the taste buds of these fish (Table 2). Hence, it could be ascertained that the presence or absence of different types of taste buds in the barbels result in the indication of functional characteristics of the species.

Barbels primarily help in the fishes' gustation and chemoreception. So far taste buds in barbels of fish were studied and reported from some catfish (Grover-Johnson & Farbman, 1976; Reutter & Witt, 1999; Kaushik & Bordoloi, 2015, 2017) and in *Devario* species (*D. rerio*) (Hansen et al., 2002); while the present investigation revealed the presence of similar taste buds in the barbels of the described four species of *Garra* species, which has not been reported earlier.

In addition to taste buds, mucous pores were observed in case of *G. nasuta* (both barbels) and *G. stenorhynchus* (maxillary barbels). Mucous pores help in mucous secretion and could be regarded as an adaptation to lubricate and protect the barbel epithelia from abrasion (Singh & Agarwal, 1991; Pinky et al., 2002). Presence of mucous pores in barbels may be an adaptation to peculiar bottom-scooping habit of *G. nasuta* and *G. stenorhynchus* disturbing bottom mud, since mucous has a remarkable power to precipitate mud held in suspension (Hora, 1934).

Sensory Cells

Two types of sensory cells are usually found in taste buds, the dark cells comprising of short microvilli and light cells comprising of longer microvilli and associated with specialized functions (Ovalle & Shinn, 1977). Although the light cells are believed to be associated with gustatory chemoreception, the dark cells are thought to mainly involve in providing greatly increased surface area for secretion of material into the taste bud pore (Ovalle & Shinn, 1977). The distribution pattern in taste buds reflects fishes' feeding habits, strategies, and habitats (Kiyohara et al., 1980; Gomahr et al., 1992; Fishelson & Delarea, 2004) and also is indicative of possible mechanisms of foraging and food recognition (Fishelson et al., 2004). In the present study, we visualized the presence of both shorter and longer microvilli comprising of the dark and light cells, respectively, in the rostral and maxillary barbels of the four fish. Microvilli are believed to contribute to taste buds' receptor area (Boudriot & Reutter, 2001), with both types (short and long microvilli) possessing chemosensitive membranes (Reutter et al., 1974). Hence, the presence of dark and light cells comprising of shorter and longer microvilli, respectively, describes the mechano- and chemo-receptive ability of the barbels in these torrential fish.

Microridges

Microridges are unique nonsensory cell types. These are modifications of the general surface epithelium and are believed to serve in facilitating the metabolic gas exchanges of the skin and in respiration (if found in fish gills); and help in holding mucous secretions to the skin surface (Reutter et al., 1974). In the present study, networks of microridges were observed in areas adjacent to the taste buds in both barbels of *G. gravelyi*, rostral barbels of *G. stenorhynchus* and in the maxillary barbels of *G. gotyla*. Microridges may also provide some mechanical defense to trauma (Hawkes, 1974) in stabilizing the surface of epidermal cells. Microridges observed in both barbels of *G. gravelyi* indicate the increased facilitation towards mechanical defense in this species.

Microridges were also observed in the keratinized regions of the lips and adhesive discs of the four species. Presence of such nonsensory cell types in these areas enables these fish to withstand the eddies of strong water currents (Hora, 1930), involving in adhesive mechanism to the substratum (Saxena & Chandy, 1966) and maintaining a secretory activity of the skin (Das & Nag, 2006).

Upper Lips

Unique morphological modifications in lips and their associated regions reveal the diverse food and feeding habits of the fish as well as the ecological niches inhabited by them (Tripathi & Mittal, 2012). In the present study, the upper lips in the four fish were found continuous and associated with the rostral caps. The upper lips bearing a broad and flat surface was associated with horny jaw sheaths or excrescencies loaded with unculi that helped these fish in scraping food, like algal matter from the surface of the rocks. The areas surrounding these horny jaw sheaths were found interwoven with microridges. Tripathi & Mittal (2012) stated the presence of mucogenic layer of irregular polygonal epithelial cells of varied dimensions in the lips of *Glossogobius giuris* (Hamilton, 1822), and had further characterized the presence of microridges in them. Such structures were observed in hill stream fish like *Botia almorhae* (Gray, 1831), *Homaloptera brucei* (Gray, 1830) (now named as *Balitora brucei*), and *Schizothorax richardsonii* (Gray, 1832) (Singh & Bisht, 2014). In the four fish studied here, clusters of excrescencies were observed towards the posterior regions of the lips. Both types of taste buds (I and II) were observed in the lip regions of *G. gravelyi* and were specifically located adjacent to the clusters of excrescencies loaded with unculi. Type I taste buds were also observed in the anterior mucogenic region of the lips in *G. nasuta.* The presence of taste buds in the upper lip regions of *G. gravelyi* and *G. nasuta* describes the mechanoreceptive and chemoreceptive behavior in these two fish. Mucous pores observed in the mucogenic regions of the upper lips in *G. nasuta* allow lubrication provided in the lips during attachment to rocks thereby protecting the lips from physical damage and abrasion (Das & Nag, 2006).

Unculi

Unculi or horny projections arising from single cells were described by Roberts (1982), as epidermal outgrowths that arise from single cells and are observed in a wide array within the fish kingdom. The presence of unculiferous structures (unculi) has been an important tool for studying the adaption of hill stream fish. Different types of unculi are observed in different species of fish, namely, truncate polygonal unculi, and elongate polygonal unculi (Roberts, 1982). In the present study, hook-shaped unculi were observed with a higher magnification under the SEM in the lip regions and adhesive discs of the four fish. These structures (unculi) could be well related to the role of adaptations in such hill stream fish (Hora, 1922). Possible functions of unculi include mechanical protection of the skin, rasping, adhesion and hydrodynamic effects (Roberts, 1982). The rostral caps associated with lip regions of *Garra* often exhibit deep scratches resulting from their stone-sucking habits.

Adhesive Discs

Adhesive discs/suctorial discs form important organs of attachment for fish living in torrential habitats. The adhesive discs in these fish possessed two distinct regions, namely the mucogenic and the keratinized regions. The mucogenic regions possessed mucous pores and mucosal gland openings, and formed the central area of the adhesive disc. The keratinized regions contained numerous clusters of excrescencies, also known as adhesive islets and were observed bearing unculiferous structures (unculi). Many workers described the presence of these structures as a means to mainly associate with firm adhesion to the substratum (Nagar et al., 2012). Excrescencies bearing unculi in the adhesive discs were earlier termed as stub shaped tubercles, bearing spines in a related species called *G. lamta* (Nagar et al., 2012). The clusters of excrescencies, otherwise known as adhesive islets, were also observed in the adhesive disc of *G. telchitta* (Subba & Pandey, 2014). It was mentioned that such structures were observed in the upper lip, lower lip, and the labial fold of *G. lamta*. Pinky et al. (2002) reported the functions of such structures in the upper and the lower lips in *G. lamta*; and explained that the mucogenic region is secretory in behavior, which is evident by the presence of mucous cells, and such substances secreted from the surface prevent the fish from abrasion and physical damage. While for the keratinized region, bearing numerous excrescencies loaded with unculi, they serve to adhere firmly to the rocks and stones in fast-flowing waters. Roberts (1982) observed that previously described literature on unculiferous structures observed in the lips of two species of genus *Garra*; *G. lamta* and *G. mullya*. The presence of unculi in the general body epidermis, snout epidermis, lip epidermis, adhesive apparatus epidermis, and paired fin epidermis of *P. sulcata* was also reported (Joshi et al., 2012), revealing the functional significance of these structures.

The adhesive discs in the four fish of genus *Garra* in the present study were found loaded with numerous unculi, which were found interspersed throughout the peripheral areas. A common observation was made with respect to adhesive discs in all the four fish of *Garra*. The callous pad present in the center of the adhesive disc has a distinctive functional significance. A large number of mucosal openings, in addition to the clusters of excrescencies bearing unculi towards the periphery, facilitates a firm hold on the substratum (Nagar et al., 2012). Similar structures were named spines (Bhatia, 1950) when the anterior and posterior lips of the hill stream catfish *G. telchitta* were studied.

Conclusion

Unique morphological adaptations observed in these four fish provide examples of excellent adaptive modifications. Dwelling in the same habitat, these fish are probably separated by a microhabitat that might have influenced the morphological specialization in their structures. While the barbels of *G. stenorhynchus* show an enhanced mechano and chemoreceptive behavior in foraging food and sensing the surrounding water, the lip regions of *G. nasuta* and *G. gravelyi* show increased adaptive characteristics. The combined and cumulative functions of these organs and their associated structures are the unique adaptive modifications observed in these fish.

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References

Abou-Zaid DFA (2014) A comparative study of the distribution and morphology of the external taste buds in the siluroid fish, *Malupterus electricus* and *Clarias lazera*. *Int J Adv Res* **2**(5), 1083–1095.

Bhatia B (1950) Adaptive modifications in a hill stream catfish *Glyptothorax telchitta* (Hamilton). *Proc Natl Inst Sci India* **XVI**(4), 271–285.

Boudriot F and Reutter K (2001) Ultrastructure of the taste buds in the blind cave fish *Astyanax jordani* ("Anoptichthys") and the sighted river fish *Astyanax mexicanus* (Teleostei, Characidae). *J Comp Neurol* **434**, 428–444.

Chen Z, **Zhao S and Yang J** (2009) A new species of the genus *Garra* from Nujiang River Basin, Yunnan, China (Teleostei: Cyprinidae). *Zool Res* **30** (4), 438–444.

Das D and Nag TC (2004) Adhesion by paired pectoral and pelvic fins in a mountain-stream catfish, *Pseudocheneis sulcatus* (Sisoridae). *Environ Biol Fishes* **71**, 1–5.

Das D and Nag TC (2005) Structure of adhesive organ of the mountain-stream catfish, *Pseudocheneis sulcatus* (Teleostei: Sisoridae). *Acta Zool* **86** (4), 231–237.

Das D and Nag TC (2006) Fine structure of the organ of attachment of the teleost, *Garra gotyla gotyla* (Ham). *Zoology* **109**(4), 300–309.

Dey S, **Baul TSB**, **Roy B and Dey D** (1989) A new rapid method of air-drying for scanning electron microscopy using tetramethylsilane. *J Microsc* **156**(2), 259–261.

Fishelson L and Delarea Y (2004) Taste buds on the lips and mouth of some blenniid and gobiid fishes: Comparative distribution and morphology. *J Fish Biol* **65**(3), 651–665.

Fishelson L, **Delarea Y and Zverdling A** (2004) Taste bud form and distribution on lips and in the oropharyngeal cavity of cardinal fish species (Apogonidae, Teleostei), with remarks on their dentition. *J Morphol* **259**(3), 316–327.

Gaur KS, **Sharma V**, **Sharma MS and Verma BK** (2013) Food and feeding habits of the hill stream fish *Garra gotyla gotyla* (Teleostei: Cyprinidae) in the streams of South-Eastern Rajasthan. *Ecol Environ Conserv* **19**(4), 1025–1030.

Gomahr A, **Palzenberger M and Kotrschal K** (1992) Density and distribution of external taste buds in cyprinids. *Environ Biol Fishes* **33**(1-2), 125–134.

Grover-Johnson N and Farbman AI (1976) Fine structure of taste buds in the barbel of the catfish, *Ictalurus punctatus*. *Cell Tissue Res* **169**(3), 395–403.

Hara TJ and Barbara SZ (2007) *Fish Physiology: Sensory Systems and Neuroscience*. San Diego, CA: Elsevier Publishing House.

Hansen A, **Reutter K and Zeiske E** (2002) Taste bud development in the zebrafish, *Danio rerio*. *Dev Dyn* **223**(4), 483–496.

Hawkes JW (1974) The structure of fish skin. I. General organization. *Cell Tissue Res* **149**, 147–158.

Hora SL (1921) Indian Cyprinoid fishes belonging to the genus *Garra*, with notes on related species from other countries. *RecIndian Museum* **22**, 633–687.

Hora SL (1922) Structural modifications in the fish of mountain torrents. *Rec Indian Museum* **24**(1), 31–61.

Hora SL (1930) Ecology, bionomics and evolution of the torrential fauna, with special reference to the organs of attachment. *Phil Trans Roy Soc Lond B* **218**, 171–282.

Hora SL (1934) A note on the biology of the precipitating action of the mucus of Boro fish, *Pisodonophis boro* (Ham.-Buch). *J Proc Asiat Soc Beng* **29**(4), 271–274 1pl.

Hora SL (1952) Presidential address delivered at the anniversary meeting of the National Institute of Sciences of India. *Adapt Evol* **18**, 161–170.

Jayaram KC (1999) *The Freshwater Fishes of the Indian Region*. Delhi: Narendra Publishing House.

Joshi SC, **Bisht I and Agarwal SK** (2012) Study of the unculi of *Pseudocheneis sulcatus* (McClelland) (Sisoridae) fish of Kumaun Himalaya. *J Am Sci* **8**(2), 413–418.

Kaushik G and Bordoloi S (2015) Adaptive modifications in lip and barbel of an endangered catfish *Amblyceps arunchalensis* Nath & Dey, 1989. *Curr Sci* **109**(9), 1554–1556.

Kaushik G and Bordoloi S (2017) Ultrasurface structure of oromandibular area in a hill stream teleost *Glyptothorax trilineatus* Blyth, 1860. *Acta Zool* **98**(4), 362–369.

Kiyohara S, **Yamashita S and Kitoh J** (1980) Distribution of taste buds on the lips and inside the mouth in the minnow, *Pseudorasbora parva*. *Physiol Behav* **24**(6), 1143–1147.

Kullander SO and Fang F (2004) Seven new species of *Garra* (Cyprinidae: Cyprininae) from the Rakhine Yoma, Southern Myanmar. *Ichthyol Explor Freshwaters* **15**(3), 257–278.

Lalronunga S, **Lalnuntluanga and Lalramliana** (2013) *Garra dampaensis*, a new ray-finned fish species (Cypriniformes: Cyprinidae) from Mizoram, North Eastern India. *J Threatened Taxa* **5**(9), 4368–4377.

Murray RG (1971) Ultrastructure of taste receptors. In: *Handbook of Sensory Physiology IV Chemical Senses Part 2 Taste*, Beidler LM (Ed.), pp. 31–50. Berlin, Heidelberg, New York: Springer.

Murray RG (1973) The structure of taste buds. In: *The Ultrastructure of Sensory Organs 1*, Friedman I (Ed.), pp. 1–81. New York: Elsevier.

Nagar KC, **Sharma MS**, **Tripathi AK and Sansi RK** (2012) Electron microscopic study of adhesive organ of *Garra lamta* (Ham.). *Int Res J Biol Sci* **1**(6), 43–48.

Ovalle WK and Shinn SL (1977) Surface morphology of taste buds in catfish barbels. *Cell Tissue Res* **178**, 375–384.

Pinky MS, **Ojha J and Mittal AK** (2002) Scanning electron microscopic study of the structures associated with lips of an Indian hill stream fish *Garra lamta* (Cyprinidae, Cypriniformes). *Eur J Morphol* **40**(3), 161–169.

Reutter K (1971) The taste-buds of *Amiurus nebulosus* (Lesueur). Morphological and histochemical investigations. *Z Zellforsch Mikrosk Anat* **120**(2), 280–308.

Reutter K, **Breipohl W and Bijvank GH** (1974) Taste bud types in fish. *Cell Tissue Res* **153**, 151–165.

Reutter K and Witt M (1999) Comparative aspects of fish taste bud ultrastructure. In *Advances in Chemical Signals in Vertebrates*, Johnston, E, Muller-Schwarze, D and Sorensen, PW (Eds.), pp. 573–581. Boston, MA: Springer.

Roberts TR (1982) Unculi (horny projections arising from single cells), an adaptive feature of the epidermis of ostariophysan fish. *Zool Scr* **11**(1), 55–76.

Saxena SC (1959) Adhesive apparatus of a hill stream cyprinid fish *Garra mullya* (Sykes). *Proc Natl Inst Sci India* **B25**(4), 205–214.

Saxena SC and Chandy M (1966) Adhesive apparatus in certain Indian hillstream fish. *J Zool London* **148**, 315–340.

Sinha AK, **Singh I and Singh BR** (1990) The morphology of the adhesive organ of the sisorid fish, *Glyptothorax pectinopterus*. *Ichthyol Res* **36**(4), 427–431.

Singh N and Agarwal NK (1991) The SEM surface structure of the adhesive organ of *P. sulcatus* McClelland (Family Sisoridae), from Garhwal Himalayan hill streams. *Acta Ichthyol Piscatoria* **XXI**(2), 29–35.

Singh N, **Agarwal NK and Singh HR** (1994) SEM investigation on the adhesive apparatus of *Garra gotyla gotyla* (Family - Cyprinidae) from Garhwal Himalaya. In: *Advances in Fish Biology and Fisheries*, Singh HR (Ed.), vol. 1, pp. 281–291. Delhi: Hindustan Publication Corporation.

Singh H and Bisht I (2014) Comparative study of lips in three hill stream fish (*Botia almorhae, Homaloptera brucei and Schizothorax richardsonii*) of Kumaun Region: A SEM investigation. *Int J Sci Res* **3**(5), 715–721.

Subba BR and Pandey MR (2014) Morphology of adhesive organ of a hill-stream fish *Glyptothorax telchitta* (Ham.) (Teleostei: Sisoridae) from Saptakoshi River, Barahkshetra, Nepal. *Lasbela Un J Sci Technol* **3**, 1–4.

Talwar PK and Jhingran AG (1991) *Inland Fish of India and Adjacent Countries*. New Delhi: Oxford and IBH Publishing Co.

Tripathi P and Mittal AK (2012) Diversity of Lips and Associated Structures in Fish by SEM. In: *Scanning Electron Microscopy*, pp. 147–178. London, UK: IntechOpen.

Vishwanath W, **Lakra WS and Sarkar UK** (2007) *Fish of North-East India*. Lucknow: NBFGR.

Zhang E and Chen YY (2002) *Garra tengchongensis*, a new cyprinid species from the upper Irrawaddy river basin in Yunnan, China (Pisces: Teleostei). *Raffles Bull Zool* **50**(2), 459–464.