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Morphology of adhesive surfaces in the sisorid catfish, *Glyptothorax sinense sikkimensis*

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This paper deals with the surface morphology and structure of suctorial mouthparts, expanded paired fins and adhesive organ (AO) in a mountain stream catfish *Glyptothorax sinense sikkimensis* of India, as studied by scanning electron microscopy. It possesses type I taste buds on its barbels and lips. There are scattered unculiferous plaques on the lower lip and horny tubercles on outer fin rays, and knob like microstructures between fin rays of both paired fins. The thoracic region bears a prominent AO, which has 8-10 unculiferous ridges arranged parallel to the body axis, separated by many non-unculiferous grooves. The latter may act as the major attachment sites and function as a suction cup. A combined action of the adhesive devices with their unique surface specializations enables the fish for better anchorage to sandy-rocky substratum of glacier-fed, rapid mountain streams. The plaques on lower lip and horny tubercles and knob like substructures in fin rays perhaps help in additional anchorage support to the major AO located at the thoracic area.

Keywords: Adhesive organ, *Glyptothorax sinense sikkimensis*, Horny tubercles, Taste buds, Unculi

The integument of a fish, as in other vertebrates, is a natural biological surface that protects the animal from its environment, and establishes contacts with the outer world. It serves other functions, such as adhesion to the substratum of an aquatic system, by modifying some of its portions and forming peculiar structures called adhesive/attachment organs (AO), which are functional systems. The structural design of AO, which are even present in many invertebrate as well as vertebrate groups, varies rather strikingly[1]. Many teleost species that belong to the Sisoridae and Cyprinidae families of Himalayan streams have developed diverse adhesive surfaces in the form of major mouth associated or thoracic AO and also mouthparts, expanded paired fins and even most of the ventral body surface as additional supportive devices[2-8]. The morphology of adhesive devices depends on the species biology and particular role in which the adhesive device is involved[1].

The Sisorids are bottom-dwelling stream catfish found in southern and eastern Asia[9]. Several genera possess the widened outermost ray of the paired fins and a laminated AO on its ventral surface; others show a laminated thoracic AO[2,3,10]. *Glyptothorax* Blyth is a genus of Sisorid catfish, which are adapted to fast flowing streams and larger rivers. Some species reported from India are identified with a thoracic AO comprising of folded longitudinal pleats of skin, and unculiferous ridges extending anteriorly onto the gular region[11]. The Sisorid catfish *G. s. sikkimensis* are bottom-dwellers, predominantly nocturnal and carnivorous in nature. They prefer living in fast-flowing streams having rocky substratum, swims against the strong water current by their well-expanded pectoral and pelvic fins and adhere to the rock surface by the thoracic AO along with the modified lips of the suctorial mouth and two pairs of expanded fins. The adaptive modifications in different species of *Glyptothorax* were carried out earlier by several workers[2,12-15], but the same for *G. s. sikkimensis* are unknown. These studies focused on the morphology of the thoracic AO, primarily addressing the unculiferous ridges and unculi-free grooves. However, in several other sisorid taxa living in similar type of microhabitats, a thoracic AO is not found. These fishes possess similar folds of unculiferous skin along the leading edge of the snout, the ventral surface of the highly flattened barbels and the surface of the paired-fin pads.

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Interestingly, however, the species under study, *i.e.*, *G. s. sikkimensis* is unique in having both thoracic AO as well as additional attachment devices on the mouth parts and paired fins. Therefore, the present study was carried out to analyze the morpho-anatomical peculiarities present in the suctorial mouthparts, paired fins and thoracic AO in *G. s. sikkimensis* and how such adaptive modifications support the species to face its challenging habitat.

Materials and methods

Specimens and tissue fixation

Specimens of *G. s. sikkimensis* have elongated, and dorso-ventrally depressed body. They were identified following the description of Jayaram[16]. Specimens (n=12; standard lengths: 8-12 cm) were captured from the streams at Jorethang (altitude: 322 M above mean sea level), South Sikkim, India during the winter months. They were decapitated after mild anaesthesia with MS 222 dissolved in water and the entire head region and paired fins were fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 12 h for scanning electron microscopy (SEM). For analysis of Type I tastebuds on the mouthparts, barbels and lips of *G. s. sikkimensis* were dissected, fixed in 3% glutaraldehyde for 24 h and chemically dried.

SEM

The aldehyde fixed tissue samples were osmicated for 1 h and dehydrated in graded acetone at 4°C. The samples were critical-point dried, mounted onto aluminium stubs and sputter-coated with colloidal gold. They were viewed under a Zeiss EVO18 scanning electron microscope at an operating voltage of 15 kV, available at the Sophisticated Analytical Instrumentation Facility, AIIMS, New Delhi, India. Images were acquired using SmartSEM software and morphometric parameters of features (size and length) were determined on the digitized images during SEM observation.

Results

General Morphology

The stream catfish *G. s. sikkimensis* is dorso-ventrally flattened and dark brown in coloration. Ventrally, a prominent adhesive disc is present at the thoracic region having no concavity at the centre; it is not continuous with the mouthparts. The mouth opening is surrounded by upper and lower lip. A pair each of maxillary and rostral barbels is also evident at the corners of mouth cavity.

SEM observations

Suctorial Mouthparts

Figure 1A represents the surface ultrastructure of the suctorial mouth parts in *G. s. sikkimensis*. Both upper and lower jaw sheaths were studded with villiform teeth of variable size. The teeth were arranged in several parallel rows, the smaller ones were located interior to the oral cavity (Fig. 1B). Surface topography of the upper lip revealed randomly arranged elevations/tubercles that were devoid of superficial epithelial projections/unculi. No mucous pores (outlets of mucous glands) were noticed on the entire upper lip surface. A distinct sign of scratch mark on the surface of upper lip indicated its ability to aid in anchorage towards sandy-rocky substratum (Fig. 1A). Randomly arranged adhesive plaques were observed towards the posterior margin of the lower lip. Each adhesive plaque was studded with randomly arranged unculi. Non-unculiferous plaques of small sizes at the anterior region of the lower lip were observed. Several type I taste buds were also observed on the slightly elevated surface of the plaques, being identified by their formation of a depression from the neighbouring epithelial cells,

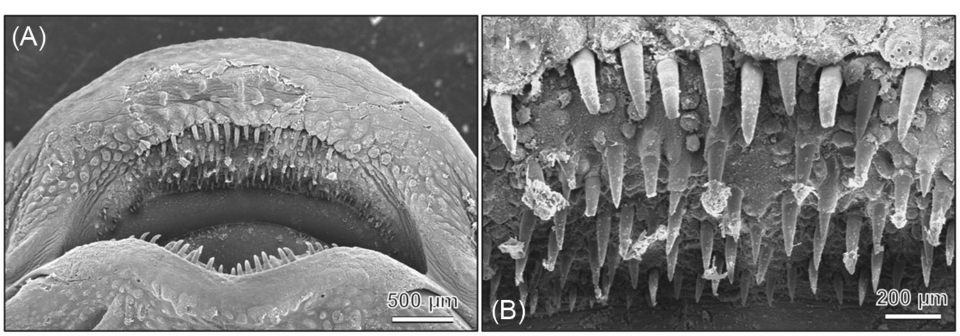

Fig. 1 — SEM micrographs (A) showing upper lip, lower lip and mouth opening with teeth; and (B) which are shown in magnified view. The teeth are arranged in several parallel rows, the smallest ones are located interior to the oral cavity. Magnifications: (A) 51x; and (B) 182x

forming a demarcation (Fig. 2A-C). No mucous pores were observed on the surface of lower lip (Fig. 2A).

Barbels

Surface topography of the barbels revealed that taste buds of type I are primarily found in this species. Figure 3A & C represent the ultrastructure of type I taste buds located towards the entire ventro-lateral sides of the barbels (Fig. 3A-D). At higher magnification, each taste bud revealed as an elevated structure developed from a circular depression from the general surface epithelium and form unique demarcated structures between neighbouring epithelial cells (Figs. 2C & 3C-D). Sensory cells bearing microvilli were observed at the apical regions of these taste buds. No distinct mucous pores were found on the surface epithelium of the barbels where taste buds were highly concentrated.

Paired fins

The pectoral- and pelvic fins of *G. s. sikkimensis* are expanded and fan-shaped in appearance. These fins, instead of being situated on the under surface of the body, are pushed outward and placed horizontally on the sides of the body. The outer rays of pectoral fins have a cushion like pad over the surface but no striations including prominent transverse ridges and grooves in an alternated fashion was not seen (Fig. 4A). In higher magnification of the image, numerous knob-like microstructures are quite evident and suggest that they may aid in additional support to other anchorage devices (Fig. 4B & C). The inner rays of pectoral fins do not show any such cushiony pad over their surface but are instead significantly loaded with multicellular horny tubercles/pearl organs showing evidence in favour of providing extra surfaces helping further as attachment devices of this species (Fig. 4D & E). No mucous pores are found in the epidermal cells of pectoral fin surface. In higher magnification of the outer ray of the pectoral fin cushion like pad with longitudinal striations are also visible (Fig. 5A). Many multicellular horny tubercles arranged on the margin of the outer ray are also evident (Fig. 5B).

The surface structure of the outer rays of pelvic fins has lesser number of specializations of the integument (Fig. 6A). No cushion-like pads over the surface were

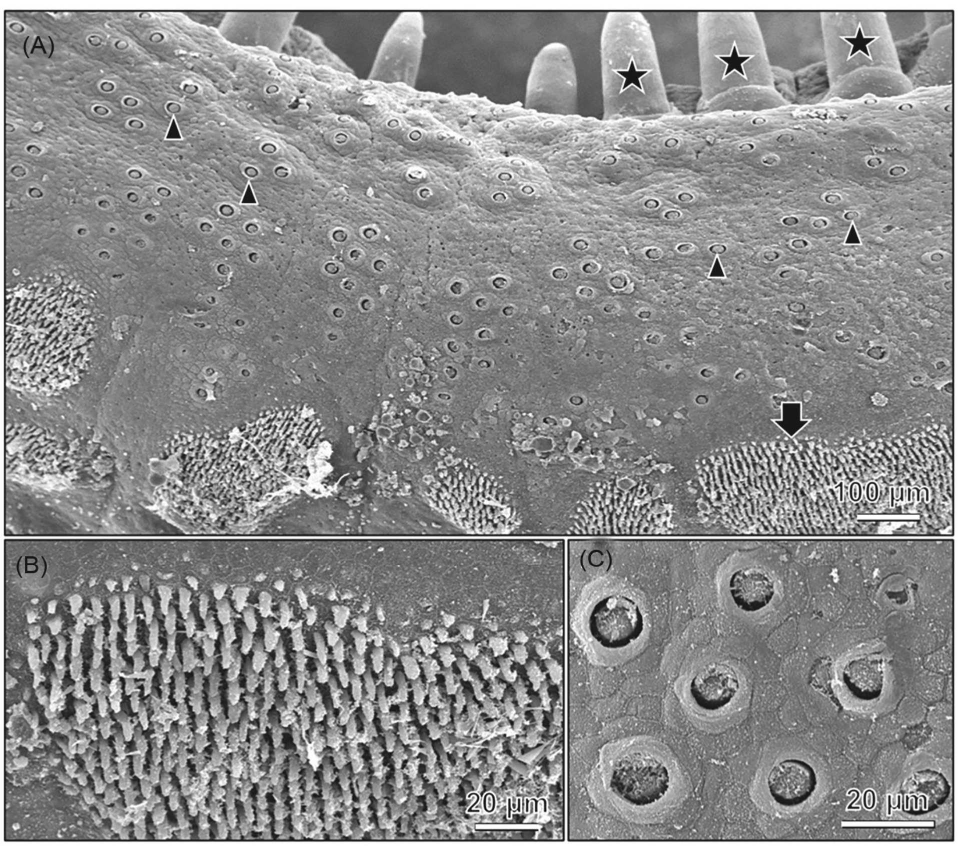

Fig. 2 — (A) SEM micrographs showing lower lip of mouth with teeth (stars), unculiferous plaques (arrow) and type I taste buds (arrowheads) on the outer covering surface; and (B & C) The latter two structures are shown in magnified, respectively. Magnifications: (A) 302x; (B) 1000x; and (C) 1960x

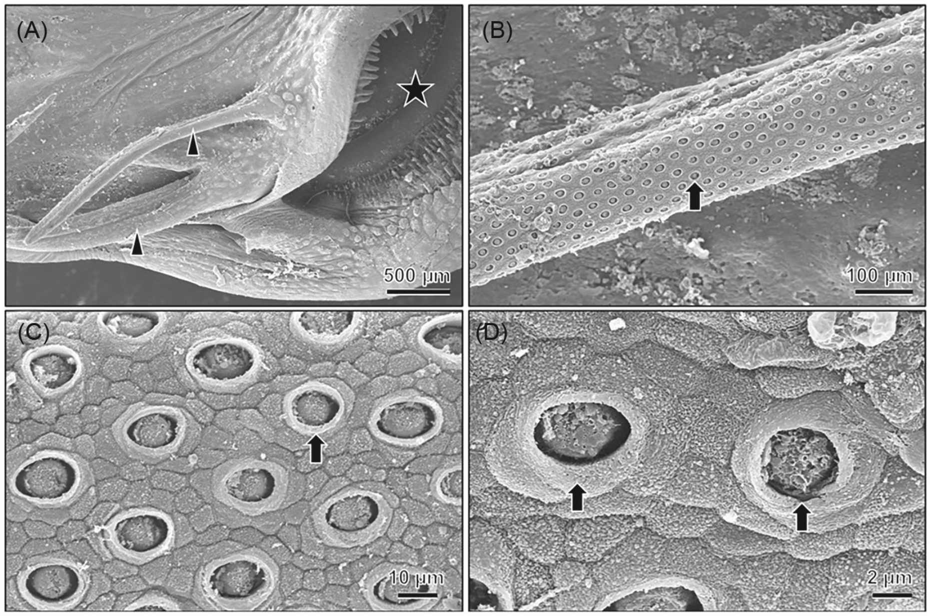

Fig. 3 — SEM micrographs of (A) barbels (arrowheads) and corner of mouth cavity of *G. s. sikkimensis*; and (B-D, arrows) showing presence of type I taste buds. Magnifications: (A) 51x; (B) 391x; (C) 2340x; and (D) 4950x. Star in (A) denotes mouth cavity

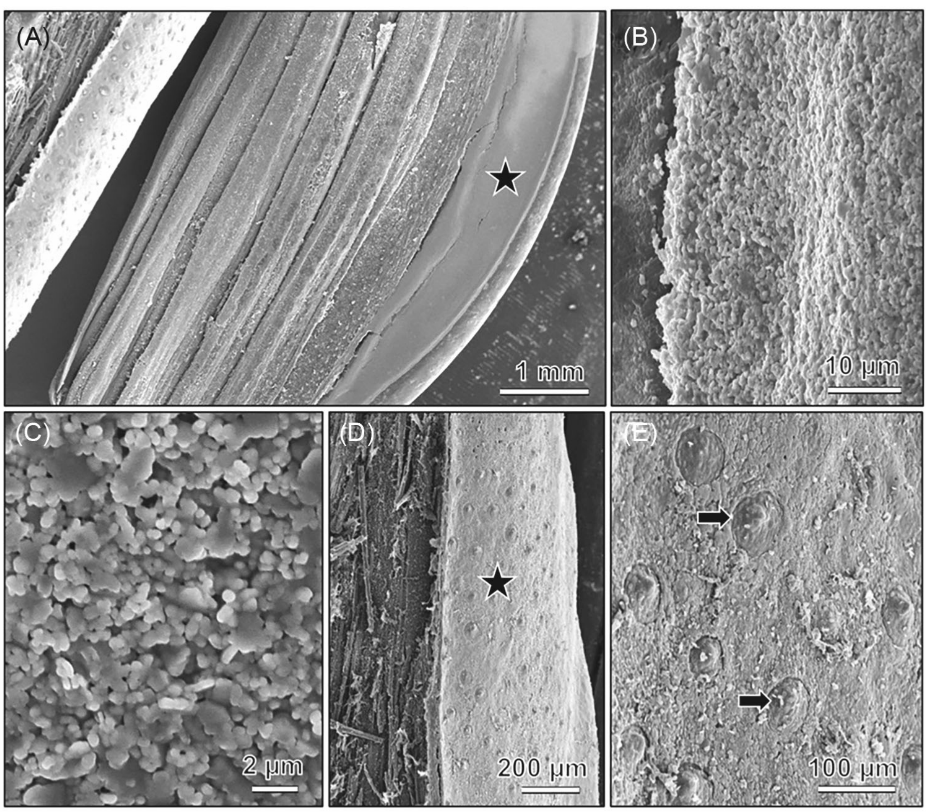

Fig. 4 — SEM micrographs showing details of substructures present in pectoral fin and associated tissue of *G. s. sikkimensis*; (A) Low magnification view of pectoral fin; (B) the extreme right fin (A, star) of which is showing substructures; (C) magnified view of part (B), showing knob-like projections from the surface that may aid in anchorage; and (D & E) The round part of pectoral fin (star, in D), which contains elevated structures as multicellular horny tubercles (arrows, E). Magnifications: (A) 43x; (B) 3000x; (C) 8000x; (D) 8x; and (E) 287x

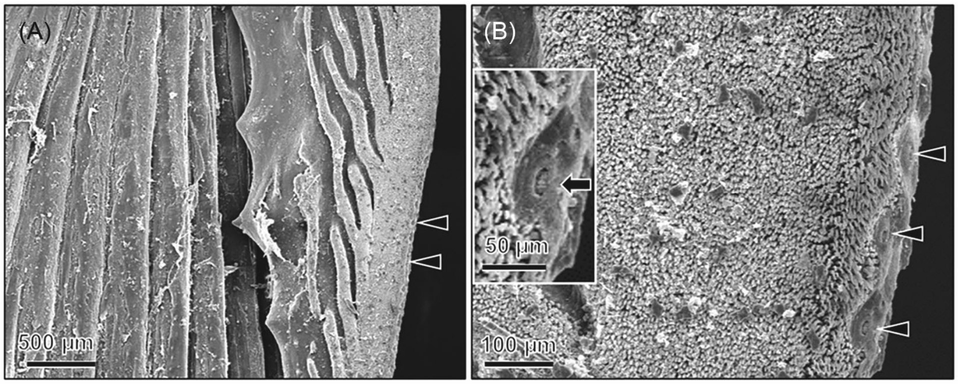

Fig. 5 — SEM micrographs showing tubercles (arrowheads) near the margin of pectoral fin. Inset shows the magnified view of a multicellular horny tubercle (arrow). Magnifications: (A) 54x, (B) 237x; and 467x (inset in B)

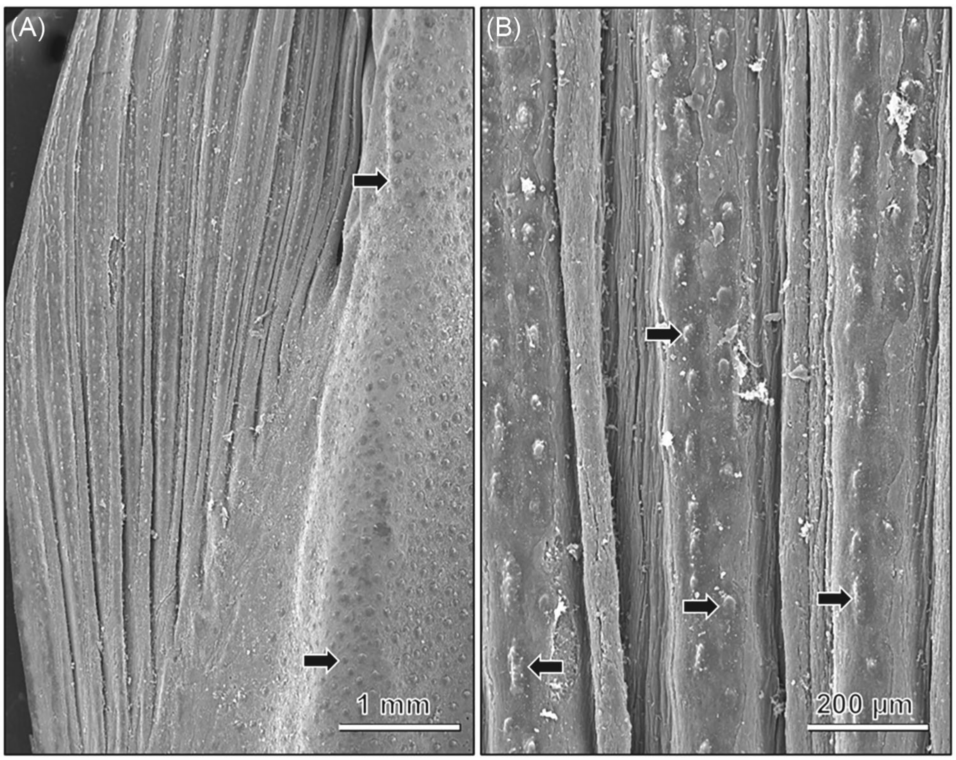

Fig. 6 — SEM micrographs showing details of substructures present in pelvic fin of *G. s. sikkimensis*, (A) Low magnification view of pelvic fin. Note surface knobs/horny tubercles (arrows) on the skin surfaces of fin rays and nearby pelvic skin; and (B) Magnified view showing substructures from the surface that may aid in anchorage. Magnifications: (A) 43x; and (B) 209x

observed, instead multicellular horny tubercles/pearl organs were observed over the all fin rays, respectively, (Fig. 6B).

Thoracic AO

The AO in *G. s. sikkimensis* is a chevron-shaped structure. The surface ultrastructure possesses numerous alternate ridges and grooves. Ridges and grooves are 18-24 in number depending on the size AO and body length of the specimens (standard length 8-14 cm, n=12). The integument at the anterior portion of the AO is thrown into alternate ridges (180-200 µm) and grooves (80-100 µm), that appear to be arranged parallel to the body axis and converge towards the posterior end at the longitudinal furrow (Fig. 7A). Occasionally, the ridges may branch either at anterior or at the posterior end of AO (Fig. 7A). All ridges present in AO also possess numerous conical spines/unculi of similar appearance (Fig. 7B-D). No mucous pores (outlets of mucous

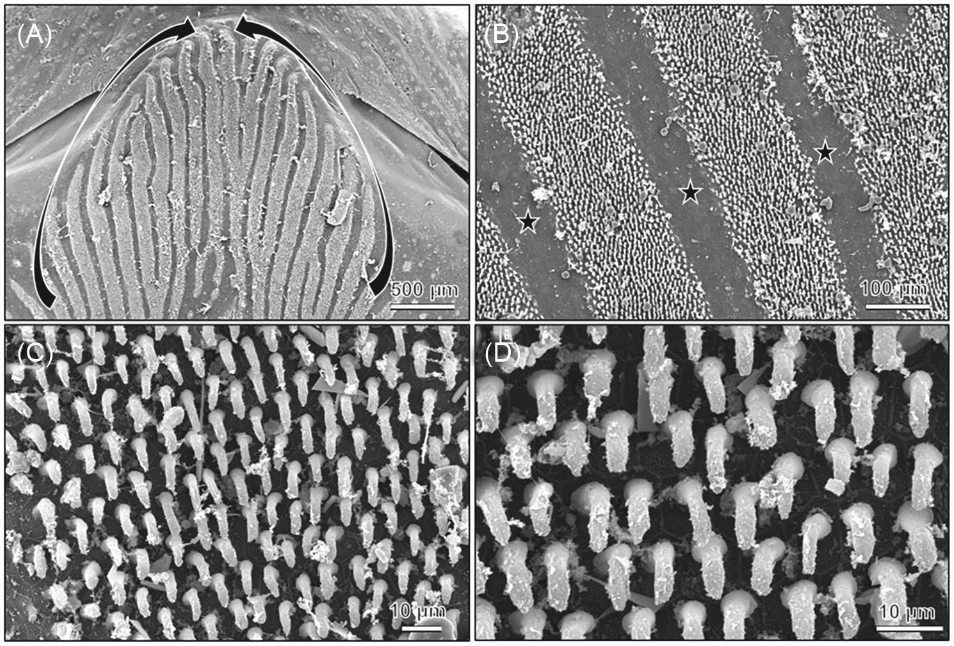

Fig. 7 — SEM micrographs showing (A) the adhesive organ of *G. s. sikkimensis* (bordered with arrows). The structure is magnified in (B) and further in (C) to show the spines/unculi. The spines/unculi are arranged in distinct rows, separated by depressed non-unculiferous grooves (80-100 µm in diameter; stars, B); and (D) They appear as curved hooks. Magnifications: (A) 49x;(B) 361x;(C) 2000x; and (D) 3730x

glands) were observed between spinous/unculiferous ridges and in non-unculiferous groove (Fig. 7B).

Discussion

Fishes belonging to family Sisoridae and some species of the Cyprinidae family are essentially bottom-dwellers in mountain streams of the Eastern Himalayas. In order to thrive successfully in the mountain streams, fishes have developed adhesive or attachment devices in the form of major AO on the thorax or mouth associated area, which are organized to act as an adaptive functional surface that primarily interact with the diverse underwater surfaces including submerged rocks and stones of their unique habitat[2-8,17,18]. Due to their special anatomic peculiarities, cyprinids and sisorids belonging to stream habitats have therefore become an excellent model for studying adaptive modifications from the perspective of microhabitat specific challenges encountered in glacier fed sub-Himalayan mountain streams of India[2,3].

The present study was aimed to describe the structural peculiarities in barbels, suctorial mouthparts, paired fins and AO, in the Sisorid *G. s. sikkimensis* and correlated those features how they take part in adapting successfully in the natural habitat of the species for survival and dispersal into diverse niches of the mountain torrents. AO forms the primary organ of attachment for fish living in torrential habitats. In *G. s. sikkimensis*, the integument at the anterior portion of the AO is thrown into alternate ridges and grooves that appear to be arranged parallel to the body axis and converge towards the posterior end at the longitudinal furrow. Apart from their major AO located at the thoracic region, several other morphological specializations are observed in expanded paired fins, suctorial mouth parts and mouth associated regions including barbels which provide opportunities to manage diverse foraging behaviours required to explore special ecological niches inhabited by them. To our knowledge, the assemblage of such diverse types of adhesive devices on a stream fish, as in *G. s. sikkimensis*, has not been reported earlier.

In majority of the teleosts, barbels are skin appendages having specific sensitivity towards taste and mechano-sensation. The number and form of barbells vary extensively from species to species and they are located on varied areas of the jaws, lips and head[19,20]. The taste buds present in the barbels guide the fish towards source of food. The Cyprinids and the Silurids are the two major groups among the ostariophysian fishes, possessing taste buds throughout their body[21]. These epithelial projections represent vital sensory organs that are common to all vertebrates and they have been used as convenient models for understanding sensory interactions[22]. Taste buds have been grouped into four different types[23,24], of which three types are found to occur in fishes[25]. Type I taste buds form a depression from the neighbouring epithelial cells, forming a demarcation, while type II taste buds are not surrounded by a rim into which the base is sunk[26-29]. Under SEM, the surface topography of both pairs of barbels (rostral and maxillary) revealed that taste buds of type I are primarily found in this species. Type II taste buds were not encountered anywhere of the body, including lips and barbels. In yellow catfish, *Pelteobagrus fulvidraco*, three types of taste buds were reported in the barbels[30], whereas, no taste buds were found in the barbels of the catfish *Mystus vittatus*[31]. The keratinised epidermis of an Indian catfish, *Bagarius bagarius* was reported to lack taste buds, which were, however, present in the non-keratinised portions on epidermal projections[32]. Abou-ZAID[33] examined two siluroid catfishes and found that the species inhabiting the turbid water (*Malupterus electricus*) possesses larger taste buds than the species dwelling shallow water.

The lips surrounding the mouth cavity in *G. s. sikkimensis* are highly modified to assist in additional anchorage for the species. Both upper and lower jaw sheaths are studded with villiform teeth of variable size. Such arrangements of teeth on jaw sheaths are comparable to heterodont dentition and exhibit carnivorous habits of some other Sisorid catfishes[11].

Barbels loaded with type I taste buds and replacement of villiform teeth on jaw sheaths in bottom-dwelling Sisorid species, *G. s. sikkimensis* represent the indications of its evolutionary success achieved as a result of new innovations; these new innovations acted as major keys to their adaptation to torrential habitat and made them capable of occupying available niches and diversifying their feeding habits[10].

The paired pectoral and pelvic fins in teleosts are used for various functions. In the Sisorid catfishes, the pectoral and pelvic fins are effectively used for adhesion, at rest, besides their common use during swimming[3-5]. The integument covering the outer rays under surfaces of paired pectoral and pelvic fins of the stream-catfishes *Pseudocheneis sulcatus*, *Glyptothorax pectinopterus*[4,5,8] and *Bhavania annandalei* and *Glyptostenum labiatum* possess adhesive devices[2].

Various types of epidermal outgrowths and derivatives are reported to occur in the teleosts. Unculi or horny, spinous, cornified projections of different types arising from single cells were described by Roberts[10] as epidermal outgrowth and are observed in ostariophysian fishes, a key adaptive feature. Possible functions of unculi include mechanical protection of the skin, rasping, adhesion and hydrodynamic effects[10]. Unculi are especially prominent features of portions of the epidermis in Cyprinids and Silurids; they also occur in Characoidei (characins) and Chanoidei (gonorynchs), but have not been found in any non-ostariophysian fishes. The presence of such spinous structures has been an important tool for studying the adaption of teleosts that belongs to mountain streams of the Himalayas. Unculi are prominent on the lips and other mouth parts (especially in groups lacking jaw teeth), on the ventral surface of paired fins, the thoracic AO of sisorid catfishes, and the mental adhesive disc of the cyprinid genus *Garra*, which are strictly bottom-dwellers. Appearance of unculi at upper and lower lips could be considered as a secondary function of the unculi assisting the fish to scrap the food material from the substratum[17]. Therefore, unculi seem to have been especially important in the diversification of cyprinoid feeding habits and in the adaptation of bottom-dwelling cyprinids and silurids to swift-water habitats[10].

Based upon the structural organization of the AO, it is likely that this device in the Sisorid catfish functions by way of suction for adhesion. A well-articulated and orchestrated action of the AO and additional adhesive devices such as mouthparts, expanded paired fins observed in this species can manage better anchorage to sandy-rocky substratum of stream habitat. The presence of numerous type I taste buds on the lips and barbel surface provides additional mechano-sensory guidance prior to manoeuvre over the stream substratum. The anatomic specializations present on barbels, suctorial mouthparts, paired fins and AO are permanent keratinized epithelial derivatives, irrespective of sexes and maturity of fishes. Such specialized features are developed as a result of adaptive evolution and suggest the functional phenotypic plasticity of fish integument towards meeting the challenges prevailed in the microhabitats of the mountain torrents.

Conclusion

The present study of adhesive devices in a bottom dwelling Sisorid catfish (*Glyptothorax sinense sikkimensis*) revealed the thoracic adhesive organ to act as the major attachment sites and function *via* suction during attachment to the substratum. Additional adhesive structures, which are present surrounding the mouth, include specialized structures such as unculiferous plaques on lower lips, horny teeth on jaws, and type I taste buds on lower lip and paired barbels. The paired fins also possess additional adhesive devices as horny tubercles on outer fin rays, and knob- like substructures between fin rays of both paired fins. A combined action of the specialized adhesive devices guided by sensory inputs by taste buds on mouthparts enables the fish for selection of sites for better anchorage to sandy-rocky substratum of its habitat.

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Conflict of interest

All authors declare no conflict of interest.

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