

Scanning Electron Microscopic study on mouth parts of hill stream fishes, *Psilorhynchus ngathanu*, Shangningam *et al.*, 2013 and *Garra abhoyai*, Hora, 1921 from Chindwin Basin, Manipur, India. (Teleostei: Cyprinidae)

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Abstract

Scanning electron microscopy (SEM) on the associated structures of mouth of *Psilorhynchus ngathanu* and *Garra abhoyai* has been carried out. SEM structures of mouth parts of *P. ngathanu* reveal the presence of elongate and pointed tubercles on the rostral cap. Papillae studded with uncini and taste buds are seen along with numerous mucus. In *G. abhoyai* hexagonal epithelial cells with elevated cell boundaries and scanty mucous gland opening are observed. Taste buds present at the apex of epithelial cells with micro-ridges pattern have also been observed. Histology of rostral cap and parts of Gular disc of *Garra abhoyai* are also carried out. Fringed and papillated rostral cap on upper lip are observed. Clavate cell, sensory cell, pigment cell of central callous are seen.

Keywords: *Garra abhoyai*, *Psilorhynchus ngathanu*, mouth, lips, adhesive organ, SEM, Histology.

Introduction

The *Psilorhynchus ngathanu*, Shangningam *et al.*, 2013 and *Garra abhoyai*, Hora, 1921 are true rheophilic fishes of the hill stream freshwater fishes. They are the cyprinid fish that include bottom dwelling fishes usually found in fast flowing streams where they cling to rocks using the highly modified mouth and its associated parts. The genus *Garra* (Hamilton, 1822) is widely distributed from southern China, across south east Asia, India and the middle east to northern and central Africa and the members of the genus *Psilorhynchus* McClelland, 1839 are small - size fishes with arched backs and flattened ventral surfaces that inhabits torrential rivers and streams of north-eastern and south-western India, Bangladesh, Myanmar, Nepal & and adjacent China .

The earlier study on histology of the adhesive apparatus of hill stream fishes has been described by Hora (1922) on *Garra annandalei* (Sunder Lal Hora). Worthy of mention is Hora's (1921) observations on the morpholaxis of the disc of *Garra rupeculus* (McClelland). More works on the adhesive organs of hill stream fishes are that of Saxena (1959, 1961). Hora, 1922 described *G. abhoyai* from the stream of Ukhrul district of Manipur, Chindwin basin. The species was characterised by having smoothly rounded snout tip, proboscis absent, pre-dorsal scales present but those towards head reduced. There are also very few structural reports of SEM and histological studies of the genus *Garra* (*G. annandalei*, Hora, 1921; *G. mullya*, Saxena and Chandu, 1959; *G. gotyla*, Das and Nag, 2006; *G. lamta*, Tripathi *et al.*, 2012). Recently, Linthoingambi *et al.*, 2013 has given an

account on the histological analysis of adhesive organ of *G. abhoyai* but there has not been analysis of these characters of *G. abhoyai* through SEM till now.

Genus *Psilorhynchus* has three groups of species such as *P. balitora* species group, *P. nudithoracicus* species group and *P. homaloptera* species group (Conway 2011, Conway *et al.* 2013). Shangningam *et al.*, 2013 described *P. ngathanu* from the stream of Dutah river, Chindwin basin, Manipur, India. The species belonged to *P. balitora* species group. As morphometric and meristic characters, the species of *P. ngathanu* was described as having head and eye large, mouth inferior, snout rounded, absence of scales from the midventral region between pectoral fins, the presence of two rows of spots on the dorsal-fin ray and two black bars on the caudal fin.

Thus, the aim of present study assigns with the scanning electron microscopic analysis of Mouth parts of *P. ngathanu* and SEM and histological analysis of adhesive organ of *Garra abhoyai*.

Materials and Methods

Live adult 11 specimens of *P. ngathanu* (Figure1.) and 15 specimens of *Garra abhoyai* (Figure2.) were collected from the Maklang river, Kamjong district, Manipur, Chindwin basin and from the Sekmai river, Awang Leikinthabi region, Manipur, Chindwin basin respectively.

For Scanning Electron Microscopic study:

Specimens were maintained in laboratory at $25 \pm 20^{\circ}\text{C}$. The fishes were cold anesthetized, tissues were cut from the mouth parts of *Psilorhynchus ngathanu* and *Garra abhoyai* as half of each. Halves of other sides were kept for later observation on. Then, it was rinsed in 70% ethanol and fixed in 3% Glutaraldehyde for 2 to 4 hours at 40°C . The tissues were washed in 2-3 changes of 15 minutes each at 40°C with 0.1M phosphate buffer and dehydrated in the graded series of ice-cold Acetone (30%, 50%, 70%, 90% and 100% approximate 20-30 min.) at 4°C . For drying, using TMS method, the tissues were immersed in Tetra Methyl Silane for 5 – 10 min. for two changes at 40°C . They were brought to room temperature ($25\text{-}26^{\circ}\text{C}$) to dry.

Tissues were mounted on Aluminium stubs using a double-sided adhesive tape and sputter coating was carried out by carbon coating. Samples were examined in a SEM at the Sophisticated Analytical Instrument Centre, IASST, Guwahati, India. The results were recorded using Zeiss

Sigma-Field emission scanning electron microscope.

For Histological study:

Tissues were fixed in Bouin's fluid for 24 hrs. After fixation, the tissues were dehydrated in a graded ethanol series as 30%, 50%, 70%, 90%, 100% and then in pure xylene for clarification and then it was embedded in paraffin wax. Paraffin block was cut into slices by using Microtome. 0.6 micro paraffin sections of the adhesive organs were subjected to routine histological staining procedures. Prepared histological slides were observed under Olympus Magnus MLX microscope (100X) and the images were captured with Olympus Pen system camera (E-PL1).

Results

Scanning Electron Microscopic (SEM) Observation:

Mouth parts of *Psilorhynchus ngathanu*:

Upper lip (UL) bears with numerous, horizontally arranged rows of heavily set, elongate and pointed Unculi (U). Rostral cap (RC) is fused with upper lip and separated by shallow groove. Numerous mucous pores (MP) are seen. Scattered tubercles(U) are present on the posterior margin of rostral cap. Lower Jaw (LJ) covered by thick squarish cushion that can be folded backwards. Blunt unculi arranged in the form of tiles are present over the lower lip surface. Lower jaw cushion is composed of two layers, one is anteriorly located in lower lips and another is posteriorly located in superficial layer. Unculiferous globular papillae present on surface of superficial portion are studded with multiple taste buds. Slightly papillated skinfold at posterolateral most corner of mouth connects the superficial layer with rostral cap. Taste buds are also present on the posterolateral skin folds (PLSF) (Figure3).

Adhesive Organ (AO) of *Garra abhoyai*:

SEM reveals the detailed surface features of the adhesive organ of *Garra abhoyai* as:

Stub Shaped Papillae,

At low magnification, the disc exhibits the presence of Stub shaped Papillae (SP) bearing spines. Large portion of the disc comprises the elevated pulvinus.

At higher magnification, the stub shaped papillae are seen covered with hexagonal epithelial cells with numerous mucous opening pores (figure-4, g). These cells have well-marked cell boundaries and are modified into spines.

Spines

The spines are broad at base and gently taper towards the tip. Some spines are short and have blunt ends whereas many spines are long and curved at the end. It is also noted that the spines tend to face the same direction, some spines are seen with no nucleus due to lack of development. This could be immature spines. Some papillae exhibit few hexagonal epithelial cells without spines. Some epithelial cells bear very short spines that may be in the process of development.

Lower lip (LP)

Papillae bearing pointed and longer spines are present. The pulvinus of adhesive disc is composed of irregular, rough epithelium that bears many mucous opening pores. At low magnification, it exhibits wide epithelial layers with small elevations throughout the entire area and at higher magnification, it shows the presence of concentrically irregular micro ridges with numerous mucous openings (MO and taste buds). This spread of mucus of pulvinus is facilitated by numerous canaliculi (microscopic canals) formed by epithermal micro ridges. Hence, this kind of cumulative action of spines and mucus enables the fish to make firm hold on the substratum. (Figure4)

Histological observation of *Garra abhoyai*:

Rostral cap in upper lip consists of numerous stub shaped papillae bearing spines and grooves between them. These papillae are formed by the rapid divisions of epithelial cells of stratum germinativum (basal cell layer producing new cells) that are covered with spines having nuclei. The surface of epithelium is keratinized. Spines are formed by the outermost layer of the epithelial cells, lying immediately below the stratum corneum. The nuclei of spines are somewhat oval in outline. Immature spines are present towards the snout portion of rostral cap. Some papillae which are devoid of spine are present. Two types of papillae are observed as large papilla formed by polygonal cells (PC) on upper region and lanceolate shape cells (LC) at base. They are observed on the peripheral region of skin fold and small papillae with 4 to 6 polygonal cells having large nuclei observed near the junction of pulvinus. These papillae are covered with spines (modification of stratum corneum layer).

Clavate Cells (CC)

Most of the central space of the epithelium is occupied by a number of CC which are distinguished at regular intervals and form a distinct layer of their own having distinct cell

wall and sometimes their nuclei may be as big as an ordinary epithelial cell and it also present more than one nucleus.

Sensory cells (SC) are present near the base of papillae and open in stratum corneum layer. Pigment cells are present in between the stratum compactum and stratum germinativum. (Figure5 a-d).

Discussion

Earlier SEM studies reported that fish taste buds fall into three categories based on their external surface morphology (Reutter *et al.*, 1974; Ezeasor, D.N. 1982). In addition to the three types of taste buds previously described from various teleost fish, a fourth type comprising very small buds, was found in some cardinal fish (Fishelson, 2004). The taste buds in the mouth cavity of *Rita rita* (Hamilton, 1822) are of three types which are elevated from the epithelium at different levels, which may be useful for ensuring full utilization of the gustatory ability of the fish, detection and analysing of taste substances, as well as for assessing the quality and palatability of food, during its retention in the mouth cavity (Yashpal *et al.*, 2006) In present study, only one type of taste bud is observed in *P. ngathanu*. Agrawal & Mittal (1992a) reported the presence of keratinized uncini on the ventral side of the upper lip and keratinized cone like structure with sharp cutting edge on the horny lower jaw sheath associated with the lower lip of an omnivorous bottom feeder, *Cirrhhina mrigala*. Agrawal & Mittal (1992b) observed keratinized uncini on the ventral side of the upper lip and on the dorsal side of the lower lip facing the mouth opening. In addition, they observed keratinized cone like structure on the horny upper jaw sheath and on the horny lower jaw sheath associated with the lips of the herbivorous column feeder, *Labeo rohita*. The present study shows that in *P. ngathanu*, the presence of uncini in both upper lip and lower lip are observed by SEM studies.

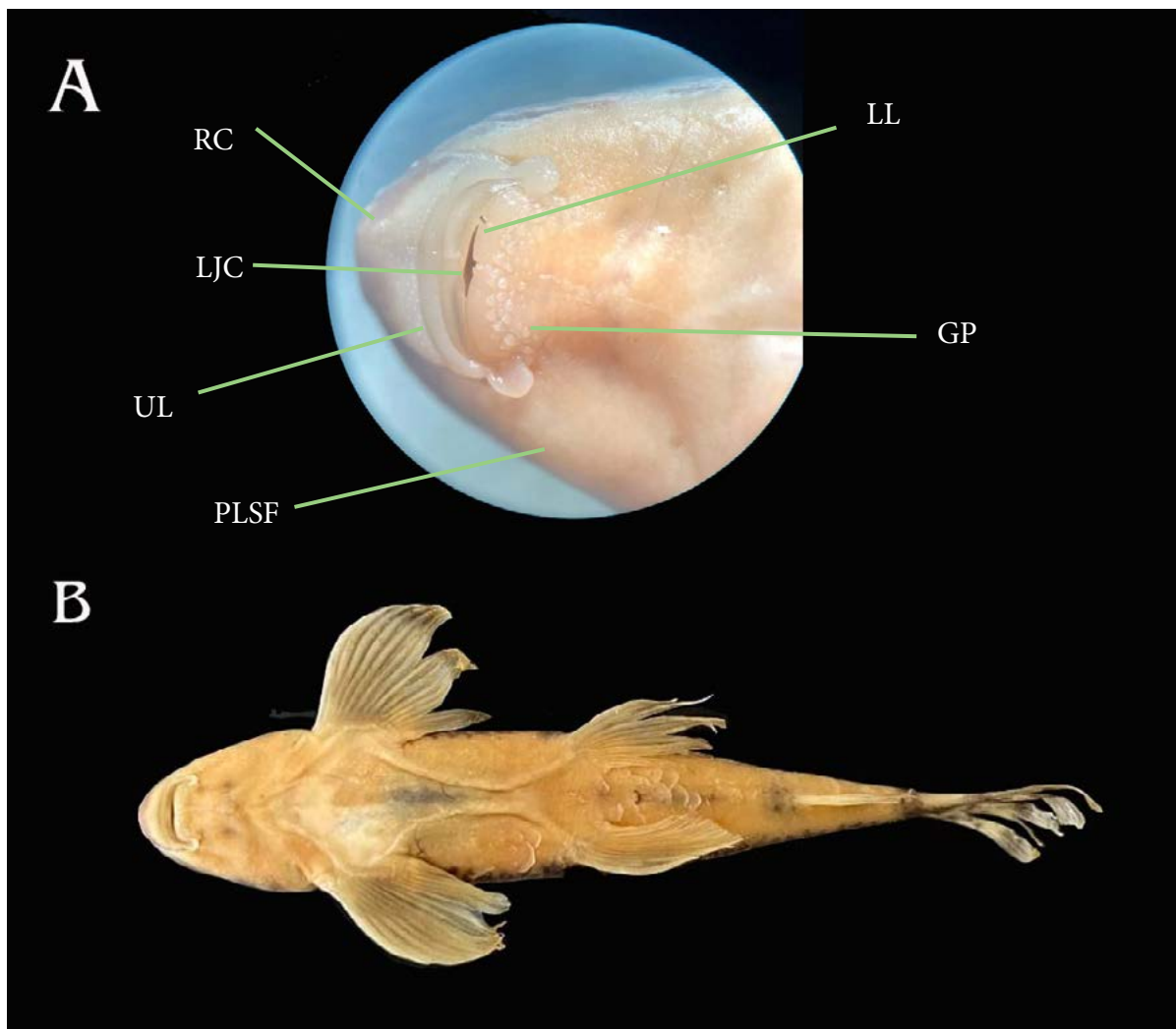
Garra abhoyai:

The study on lips and associated structures began about 200 years ago, as described by Anson, 1929. He made an attempt to define lips and on Danforth's interpretation of homology, homologous lips were found at certain stages of development in some representatives of all classes of vertebrates. Lips were varied in structure to accord with their physiological functions, whether sensory, prehensile, or adhesive (Anson, 1929). Fishelson, (1984) correlated the variations in micro-ridge pattern with the locomotory activity of the fish. He

suggested that in faster swimming fishes, micro-ridges were most developed and served to trap mucus on the epithelial surface. *G. abhoyai* has also well-developed micro-ridges. Secretions elaborated by the epithelial cells and the mucous cells in the mucogenic epithelia could be regarded as an adaptation to lubricate and protect the epithelia from abrasion (Pinky *et al.*, 2002). The role of mucus was likewise postulated previously to inhibit the invasion and proliferation of pathogenic micro-organisms and to prevent their colonisation in fish epidermis (Nigrelli, 1937; Nigrelli *et al.*, 1955; Hildemann, 1962; Liguori *et al.*, 1963; Lewis, 1970). For such kind functions numerous mucous opening pores in *Garra abhoyai* are also shown in squamous epithelial cells. A very prominent taste bud in large numbers is observed in *Garra lamta* (Tripathi, A., 2012) but only one type of taste

bud is also observed in *G. abhoyai* on lower lip.

G. abhoyai and *P. ngathanu* occupy peculiar trophic niche and they scrape epilithic or epiphytic algae and other food items from submerged substrates. This specialized feeding type is possible due to the remarkably formed, ventrally placed sucker mouth (adhesive disc) just behind the arched lower lip for *Garra* species and for *Psilorhynchus* species lower jaw cushion in lower lip is present. In spite of this highly specialized feeding apparatus, diversity in both thickness of the different regions of lips and in shape of lips exists, and these fishes actually feed on a broad range of food. As such, Cyprinidae are the most specialized and successful fish family within the order Cypriniformes (Mittal, A.K, *et al.*, 2012).



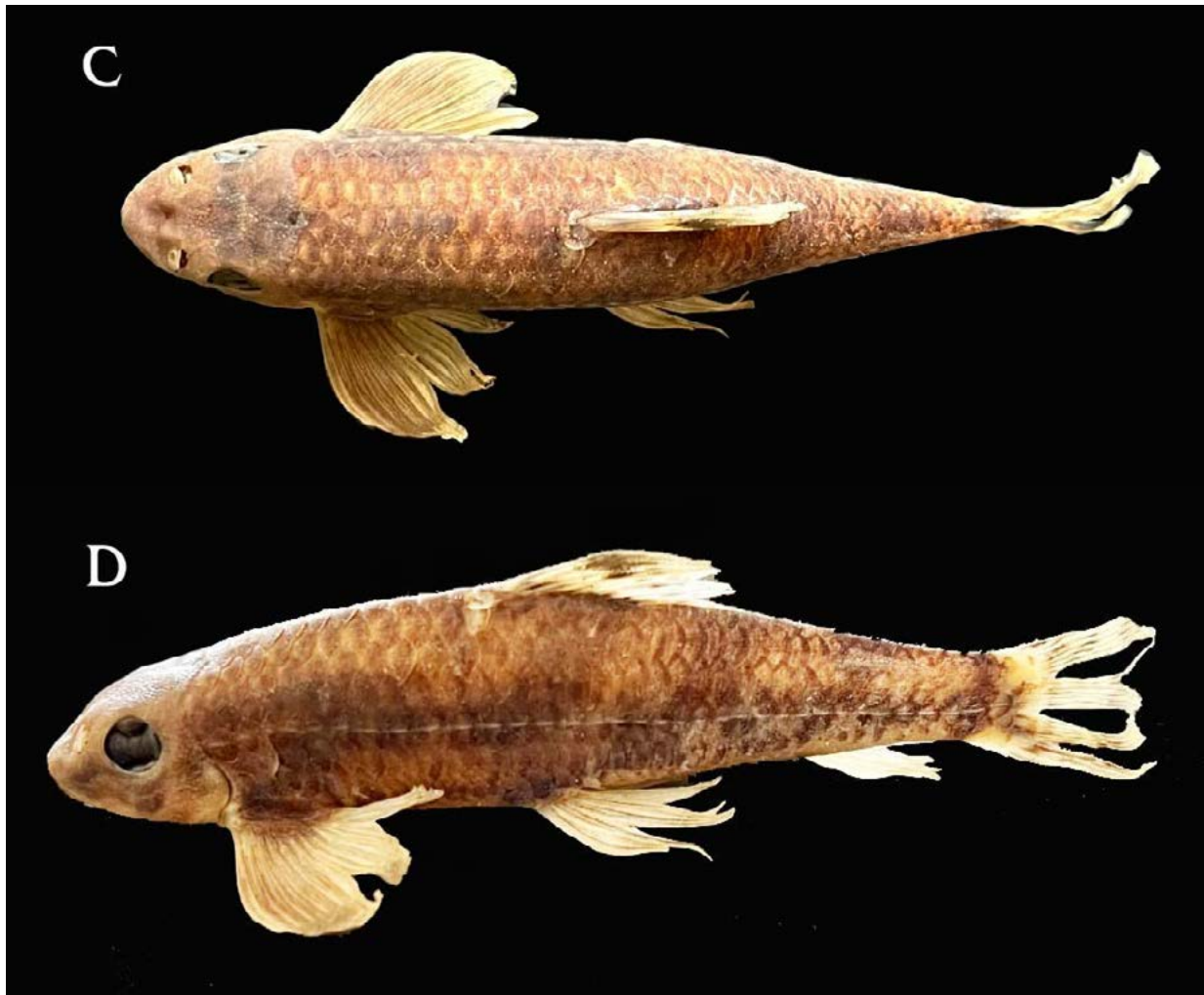


Fig 1. A – Mouth of *Psilorhynchus ngathanu* showing Rostral Cap (RC), Posterolateral skin folds (PLSF), Lower Jaw Cushion (LJC), Globular papillae (GP), Upper Lip (UL), Lower Lip (LL) using Light Electron Microscope.

B – Ventral view of *P. ngathanu*

C – Dorsal view of *P. ngathanu*

D – Lateral view of *P. ngathanu*

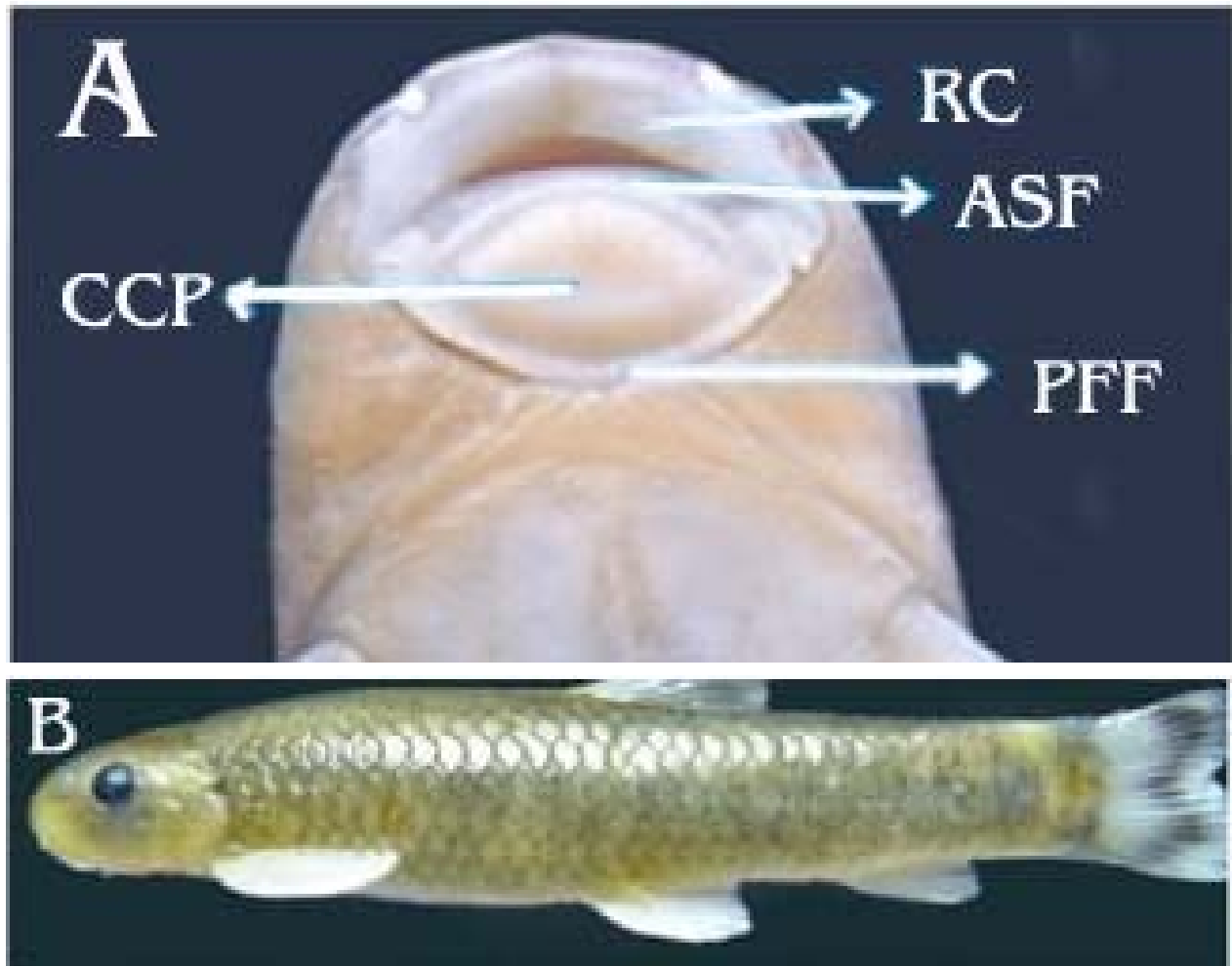
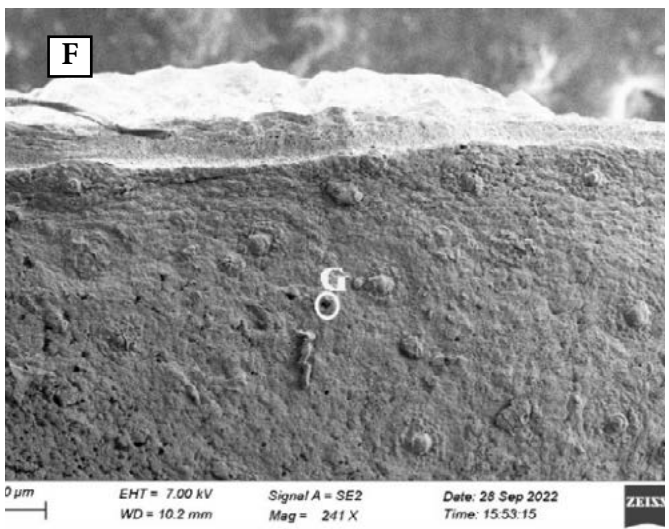
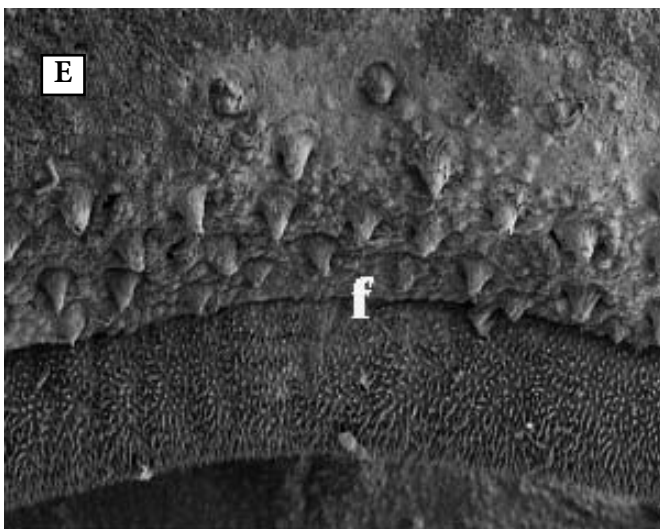
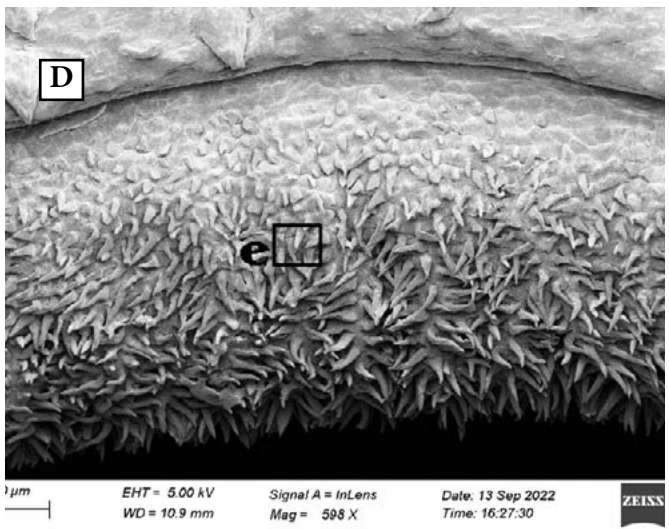
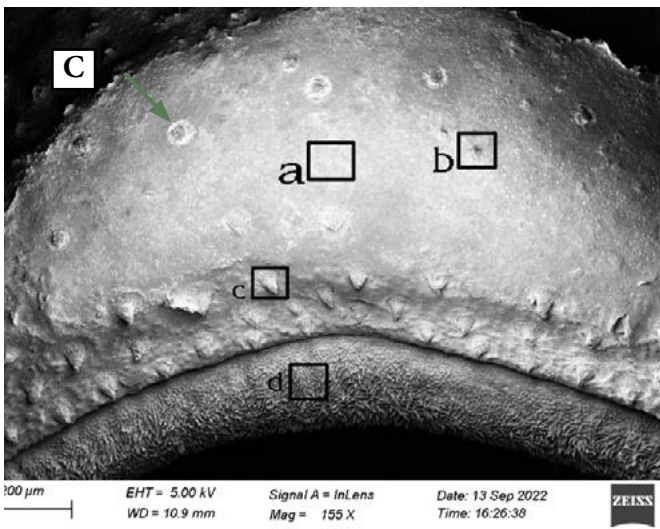
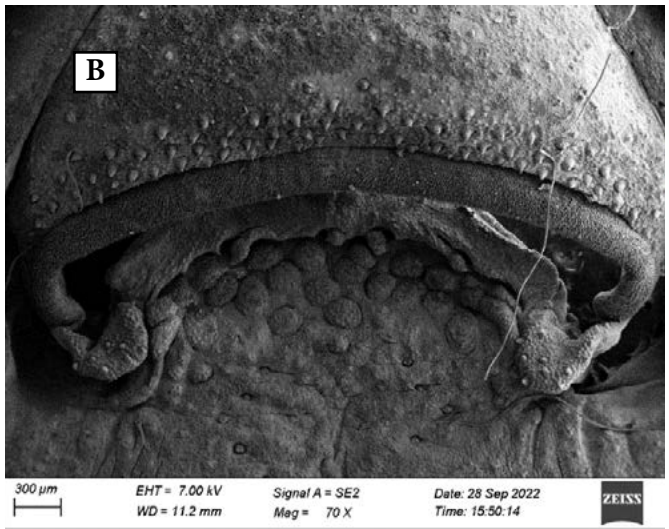
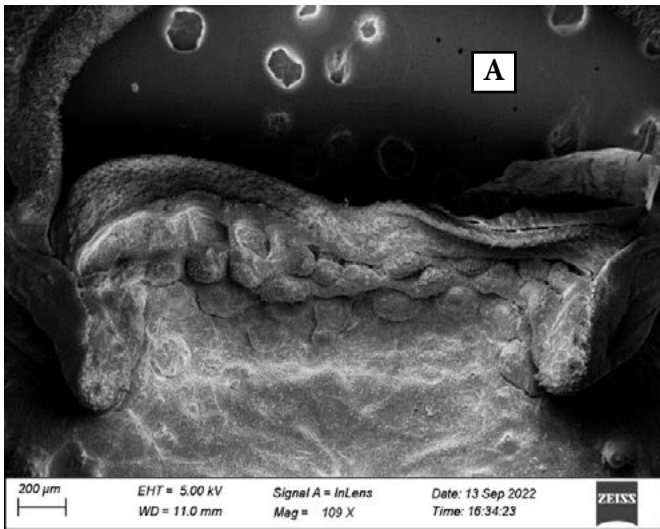


Fig 2. A. Adhesive Organ of Lower Lip of *Garra abhoyai* showing Rostral Cap (RC) and remove (ASF-Anterior Skin Fold (Torus), CCP-Central Callous Part (Pulvinus), PFF-Papillated Fee Fold(Labrum)

B. Lateral view of *G. abhoyai*



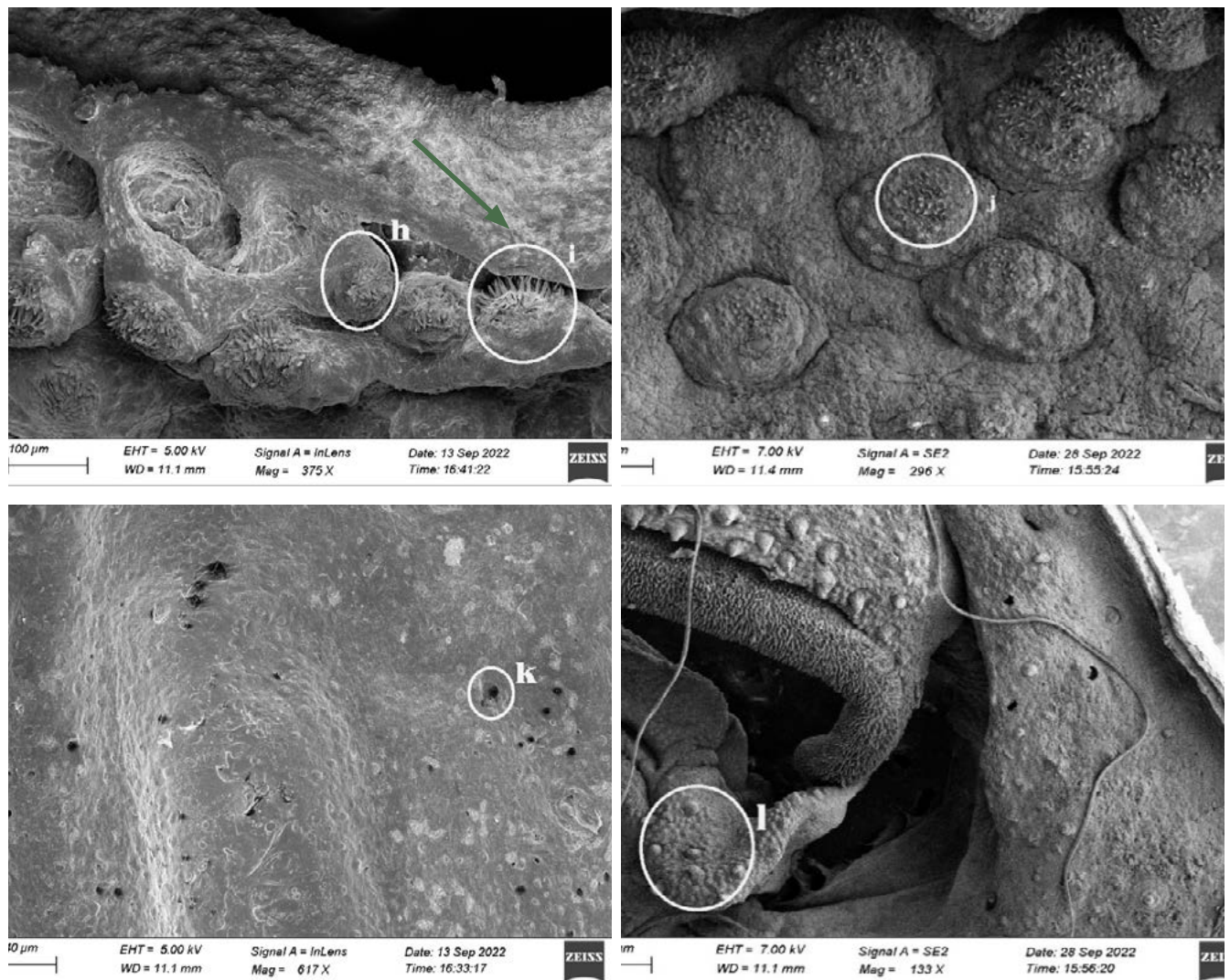
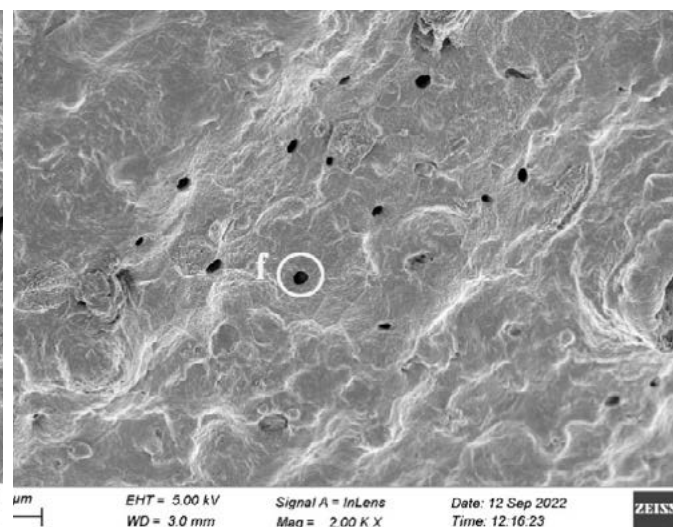
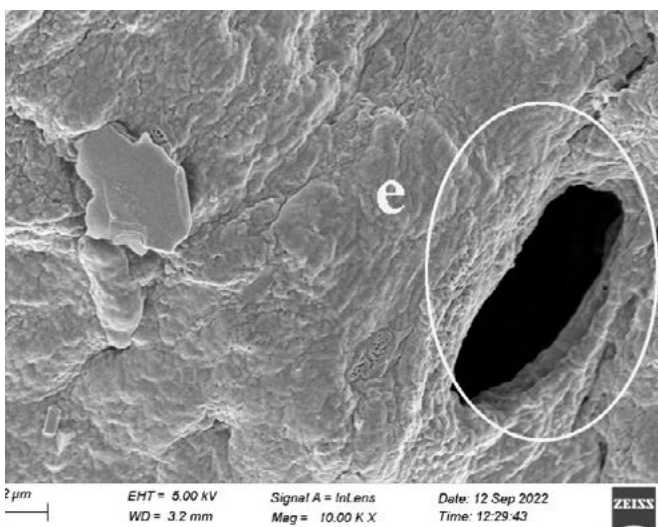
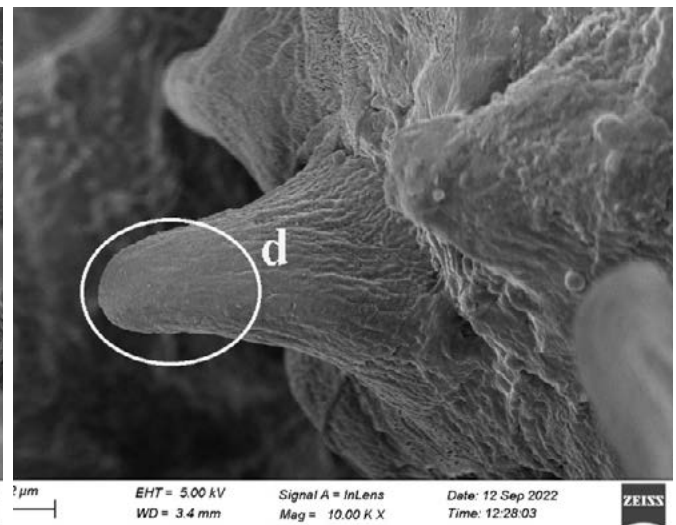
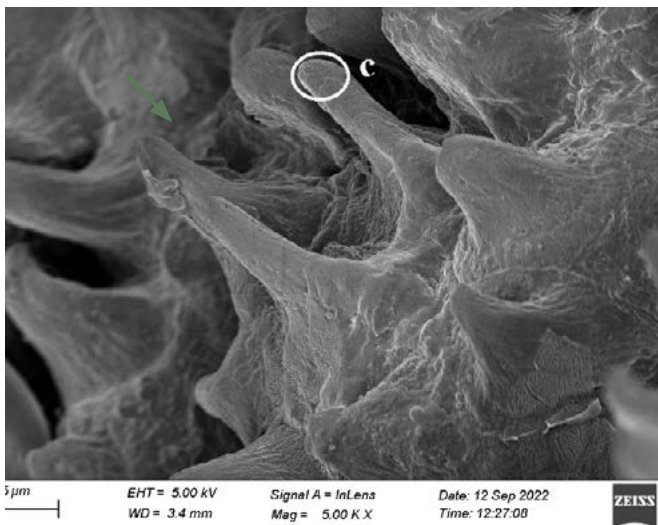
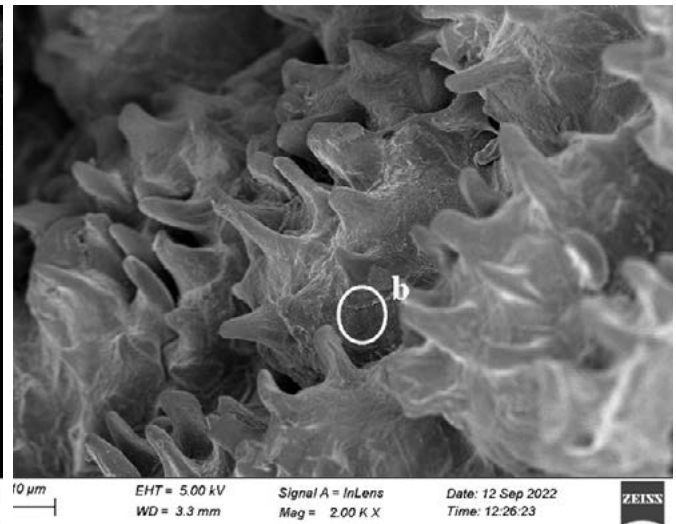


Figure 3. Scanning Electron Microscopic pictures of Oromandibular structures of *Psilorhynchus ngathanu*, 57.86 mm SL.

- A. Showing blunt unculi on lower jaw
- B. Showing whole parts of the mouth of *Psilorhynchus ngathanu* i-rostral cap, ii-upper lip, iii- lower lip, iv- superficial layer densely papillated, v-skinfolded
- C. a- Rostral Cap (RC) on the mouth, b. showing taste buds on RC with eroded tubercles, c. Pointed Tubercles (PT) on Rostral Cap, d. Upper Lip (UL) with heavily set elongated and pointed unculi, arrow shows eroded tubercles
- D. e. magnifying image of elongated and Pointed Unculi (U) on UL,
- F. Shallow groove (SG) between the RC & UL,
- G. MO-Mucous opening pore on upper lip,
- H & I- Unculiferous globular papillae on superficial layer of lower jaw cushion (LJC) on LL, and arrow shows the taste buds on lower lip
- J. Superficial layer having densely unculiferous globular papillae covered with taste buds,
- K. Mucus opening pore on lower lip,
- L. Posterolateral skin folding (PLSF) with taste buds on corner of the mouth.



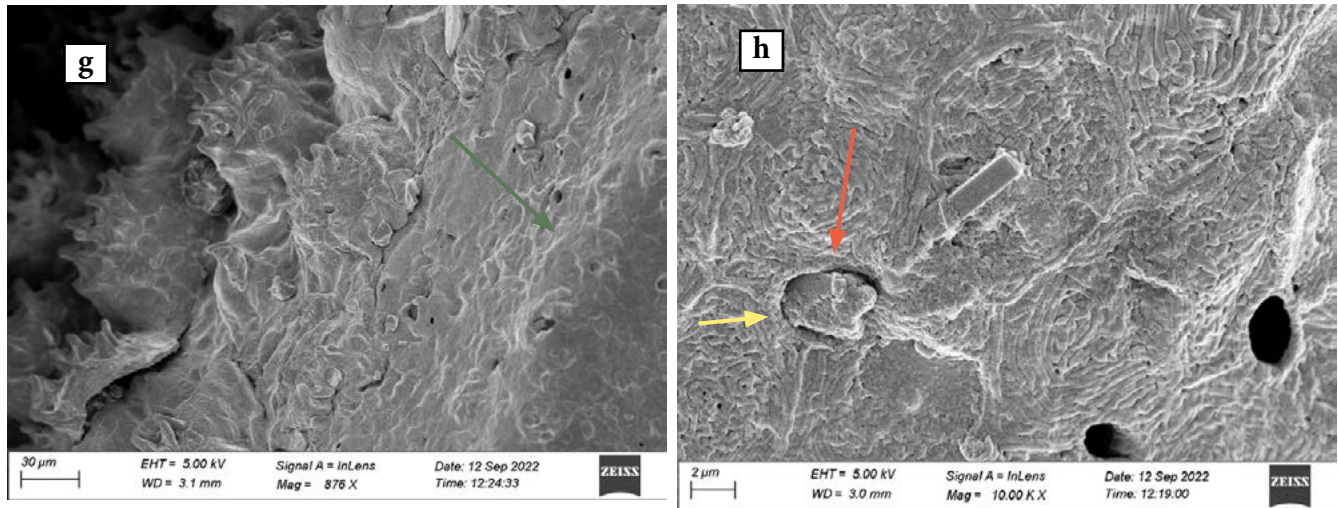


Figure 4: S.E.M. micrograph of Adhesive organ of mouth parts of *Garra abhoyai*: a. at low magnification, showing Short Stub shaped Papillae (SP) bearing spines on lower lip (LL), b. groove between the papillae, c. at higher magnification, SP showing bearing elongated spines on lower lip (LL), d. blunt spines e. magnifying mucous opening pore on pulvinus, f. mucous opening pores on corner side of lower lip, g. arrow- showing squamous epithelium on the substratum, h- micro-ridges (arrow) with mucous opening pores on squamous epithelium.

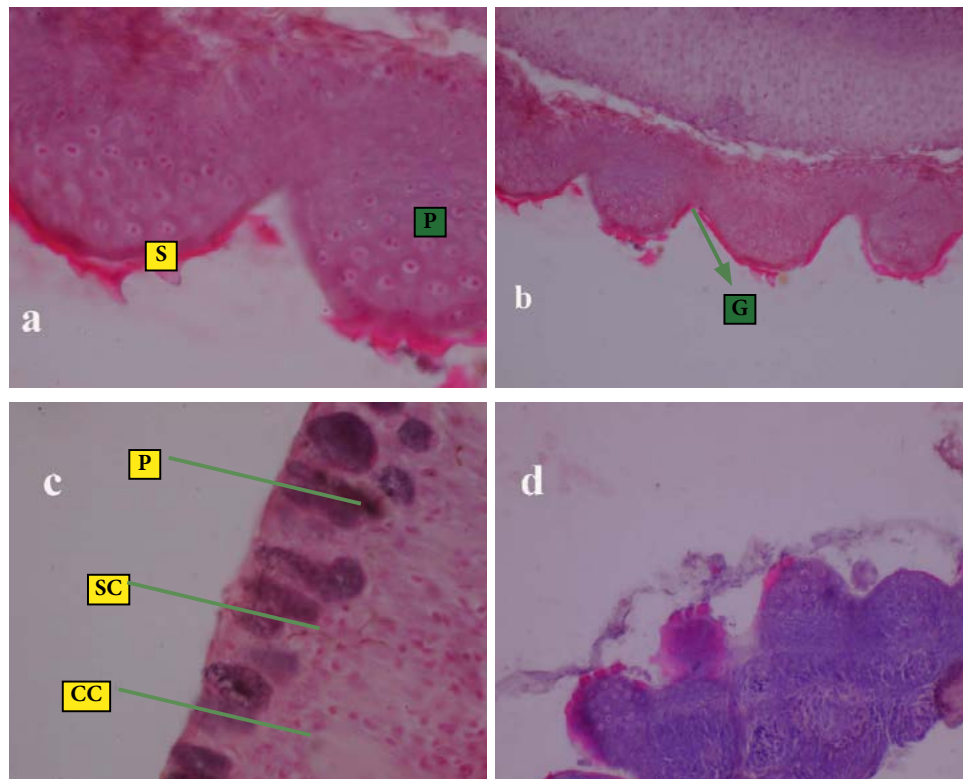


Figure 5. Histological images of adhesive organ on mouth paths of *Garra Abhoyai* a. Papillae(P) with spine(S), b. groove (G) between papillae, c. PC - Pigment cells of pulvinus, SC- Sensory cells of pulvinus, CC- Cavate cell of pulvinus part, d. Papillae without spine present in papillated fold area (SC-Sensory cells).

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