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SCANNING ELECTRON MICROSCOPE (SEM) STUDIES OF RADULA OF THREE GANGETIC RIVER MOLLUSCA FROM INDIA

BASUDEV TRIPATHY[1*], SUBHANKAR PAL[2], AMIT MUKHOPADHYAY[3], PARTHA BISWAS[4]

[1,3] *Zoological Survey of India, Kolkata - 700 053*

[2]*Department of Zoology, Netaji Subash Open University, Kolkata*

[4]*Ramakrishna Mission, Vivekananda Centenary College, Rahara, Kolkata*

Corresponding author – E.mail: tripathyb@yahoo.co.uk

INTRODUCTION

The most important characteristic feature of the buccal cavity in Mollusca (except Bivalves) is the presence of radula, one of the hallmarks of the phylum (Purchon, 1977). It is a chitinous ribbon bearing many transverse rows of firmly fixed teeth. Each row of teeth on the radula normally repeats precisely the number and shape of the teeth in the rows in front of it and behind. The radular teeth are usually in odd numbers because the row consists of a single tooth centrally placed, which is a central or rachidian teeth, with a series of others on either side broadly known as lateral teeth. The shapes of radular teeth are important for determining the effectiveness of the radula as a tool for feeding (Padilla, 1989).

Radula has been frequently investigated because of its importance as a tool in supra-specific systematic to diagnose the species. Radula has also been recognized as an important morphological criterion for the taxonomic allocation of species. It shows general similarities at family and generic levels with consistent differences at the species level. The respective similarities and differences have been utilized to an increasing extent in the classification of many gastropods as the shape of the radula teeth is directly related to the kind of food, which the gastropods eat, and the way in which it is manipulated. Radular teeth of gastropods are adapted for a variety of feeding methods in different species (Padilla, 1989).

Traditionally, studies have focused on the morphology of hard structures associated with the radula, especially radular teeth. Hard structures usually do not require chemical fixation, and therefore maintain their shape when removed from the animal. Even most descriptions of the shape and morphology of hard structures however, are qualitative rather than quantitative. In addition to the focus on hard structures, soft structures should be studied, especially the morphology of structures auxiliary to the radula that are essential for function. Soft body morphology, including the muscles, muscle attachments, the chitinous ribbon, and other structures such as the odontophore must be integrated into our understanding of the morphology of this whole structure. In addition, an understanding of their development will aid in our interpretations of evolutionary changes in form and function (Guralnick & Smith, 1999). The scanning Electron microscope (SEM) is one of the powerful tool in the studies of radula. The Scanning Electron Microscope (SEM) permits the examination of radula with higher magnification without elaborate preliminary preparation and without squashing or fragmentation.

The radulae of marine gastropods have been described by Freeman and Silva (1973) using SEM technique. The most obvious use of SEM in the study of gastropod radulae is to investigate the cusp structures and patterns of tooth wear (Carriker, 1961), normal tooth functioning position and chemical composition of the teeth (Runham, 1969) and radular growth (Isarankura & Runham, 1968). The recent study on freshwater molluscs from Western Ghat by Reid *et al.* 2013 given indepth

information on six species of *Cremnoconchus* using SEM. Nevertheless, studies on riverine gastropods *viz. Neritina* and *Assiminea* are limited and the present study on these two genus is the first work on the feeding ecology of freshwater mollusca.

MATERIALS AND METHODS

Specimens of *Neritina (Dostia) violacea* (Gmelin, 1791) of three (3) individuals, *Vittina smithi* (Wood, 1828) of four (4) individuals and *Assiminea francessi* (Wood, 1828) of ten (10) individuals were collected from the river Ganges at Raskhola Ghat, Khardah, North 24 Paraganas (22°43'14"N, 88°21'48"E) in the state of West Bengal, India (Fig. 1).

Fig. 1. Map of collection location

Fig. 2. Dorsal view of *Neritina (Dostia) violacea*

At each sampling site, live specimens of the above species were collected and stored in 7.5 % Magnesium chloride for relaxing or anaesthetizing the animal as per methodology suggested by Reid (2000). This helps facilitate easy removal of operculum and radula. Subsequently, these were transferred to prelabeled zip-lock bags and temporarily stored in ice. The specimens were subsequently identified up to species level using taxonomic methods based on shell morphology, colour, texture, sculpture and meristic counts of shells those aided by taxonomic literature (Subba Rao, 1989). The shells were subsequently stored in pre-labelled plastic containers.

Morphological analysis : Shell dimensions were measured i.e. shell length (SL) and shell width (SW) with precision calipers to ascertain differentiate between adult and juvenile specimens.

Preparation of Radula for SEM study: The radulae from the specimens were dissected out from their buccal mass with the help of scissors, watchmaker forceps, fine needles and magnifying glass. Dissected radulae were then taken on a drop of water on slides for further clearing with needle and forceps. The cleaned radulae were kept in increasing concentration (15% > 30% > 50% > 70% > 90% > 100%) of alcohol for dehydration. The dehydrated radulae were then stored in absolute alcohol for Scanning Electron Microscopic (SEM) study at University Science Instrumentation Centre (JUSIC), Jadavpur University, Kolkata. SEM photographs of the radulae were taken at the JUSIC using a JEOL JSM 5200 Scanning Electron Microscope (SEM) at 15 to 20 KV accelerating voltage. Initially, the radulae mounted on brass stubs with a double-sided tape were coated with gold and placed into the specimen chamber of the SEM (Roberts, 2000). Subsequently, photographs were taken at various magnifications depending on the size of the radula. Radula length and rachidian width were measured from the photographs. The terminology used in the description of radula follows Kool (1987).

RESULTS

Phylum MOLLUSCA

Class GASTROPODA

Order CYCLONERITIMORPHA

Family NERITIDAE

Neritina (Dostia) violacea (Gmelin, 1791)

Morphometry: Shell length ~7 mm (range 6-9 mm),

width ~ 5 mm (4-6 mm); shell thick, ovate, crepidula shaped, whorl 2, spire minute, anteriorly twisted inwards, protruding out as a beak; aperture broad, columellar plate porcellaneous, operculum semiluner (Fig.2).

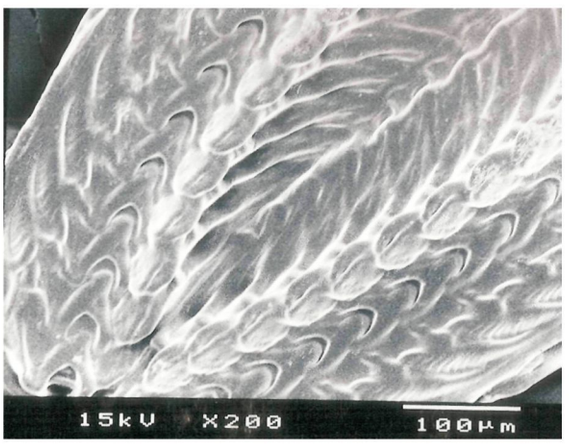

Fig.-2. SEM view of *Neritina (Dostia) violacea*'s radula ribbon; scale bar = 100 µm

Habitat : It is common in creeks and rivers having tidal influence and generally found adhering to the roots and branches of trees within the high water limit and getting exposed during low tide. Sometimes, it may also be found creeping on the mudflats (Subba Rao, 1989).

Description of Radula : The buccal cavity consists of ribbon like compact radula rhipidoglossate with the formula á-5-1-5-á. There are five laterals and teeth are serrated minutely. The first lateral has a concave inner and outer side (Fig. 5,a). The cusps of the R-central are a little broader, without any projection, and the anterior end is strikingly thin, so that the anterior half-ellipse is more transparent than usual. The reflection of the E-central is somewhat more extensive. The cusps are not conspicuous or (Fig. 5,b). There are numerous marginals present.

Order NERITIMORPHA

Family NERITIDAE

Neritina (Vittina) smithi (Wood, 1828)

Morphometry : Shell length ~ 11 mm (range 10-13 mm), width ~ 9 mm (range 8-11 mm); shell solid, oval, higher than board; whorls 5, rapidly increasing in diameter; spire sub-conical, apex blunt; shell dark yellowish brown in colour, smooth and glistering with strong black longitudinal undulating lines and bands forming a zigzag pattern; aperture broad, semilunar; columelar callus laterals, small and smooth; outer lip thickened operculum semilunar, black or light flesh colour (Fig. 3).

Fig.3. Dorsal view of *Neritina (Vittina) smithi*

Habitat : It is reported only from the estuaries of Ganges in India and Irrawady in Burma. It is primarily a brackish water species occasionally extending into freshwater systems. This species is mainly attached with hard substratum viz. rocks, banks of the river, bathing Ghats etc. This species also often seen climbing into bricks, stones and cement stones close to water bodies.

Description of Radula : The radula is rhipidoglossate type with the formula á-5-1-5-á (Fig.6,a). The central tooth (rachis) is with a small middle depression bounded by two small rounded raised heads. Wider than its length [length: width=1:3]. Anterior end forms slop for articulation. The laterals are razor like; 1st laterals are wider than higher, with a flat broad cutting surface. The outer end is broader than their inner (toward rachis) end. The posterior corner of the outer end is recurved toward anterior side and the anterior corner is slightly raised. The 2nd and 3rd laterals are cup shaped. 3rd is longer than 2nd and is with dagger like pointed tip. 2nd and 3rd laterals are arranged concentrically with their concave surface toward the central rachis (Fig. 6,b). The 4th laterals are wider than their length. Their inner end forms a conical trapezoidal 'tusk' like projected tip, which ends in a notch. The outer margin following the notch is serrated with triangular sharp tips. The

gradually tapering outer end curves toward the anterior end. The marginals are long, slender, hook like with the raised pointed tips bent towards anterior side (Fig.6,c). They are numerous in number and arranged in basket like series. The razor/blade like 1st lateral with its wide cutting surface certainly helps to scrape off loose moss or algae from the surfaces of fixed substrates like boulders etc. This can be supported by the fact that these snails were collected from the moist surface of the brick steps along the river. The pointed 2nd and 3rd laterals may aid in tearing the food materials from the adjacent stuff. The 4th laterals and the hook like marginals perhaps used for munching and mincing of food stuff respectively. The 5th lateral is slender and hook shaped bend towards the marginals and embedded partially under the 4th lateral. From the overall diagnostic features of radula structure, the feeding habit of *Neritina (Vittina) smithi* seems to be of scraping/rasping type.

Order RISSOOIDEA

Family ASSIMINEIDAE

Assiminea francessi (Wood, 1828)

Morphometry : Shell length ~ 7 mm (range 6-9 mm), width ~ 6 mm (range 5-8 mm); approximately ~ 7 mm (range 6-9 mm), ; shell elongated, conical; yellowish brown to brown in colour with brown rings surrounding the body whorls, whorls are 7 in number and gradually increasing in width; aperture oblique, ovate; operculum very thin, brownish and translucent (Fig.4).

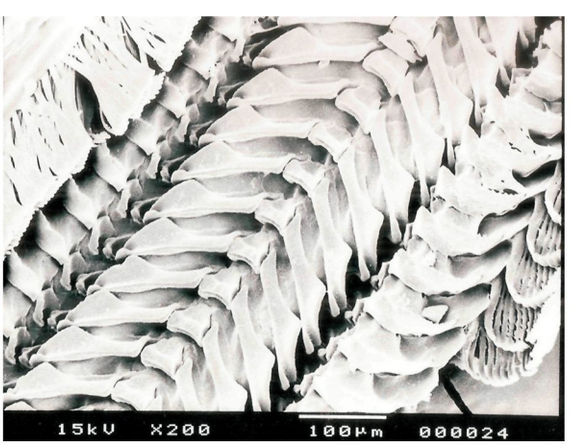

Fig.4. - SEM view of *Neritina (Vittina) smithi*'s radula ribbon; scale bar = 100 µm

Habitat : It is the only species occurs in fresh water ponds, canals having link with Hugli river (Ganges) and extends further north. This species is mainly attached with hard substratum viz. rocks, banks of the river, bathing Ghats etc.

Fig.5. Dorsal view of *Assiminea francessi*

Fig.6. - SEM view of *Neritina (Vittina) smithi*'s radula ribbon; scale bar = 50 µm.

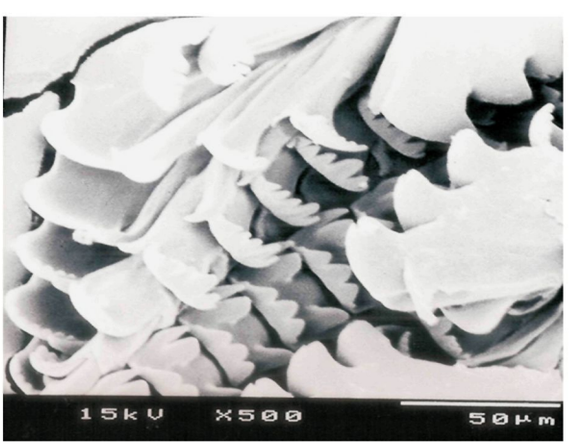

Fig.7. SEM view of radula of *Assiminea francessi*'s radula ribbon; scale bar = 50 µm.

Description of Radula : The radula is taenioglossate type with formula 2-1-1-1-2. The central tooth (rachis) is hollow, half cylindrical in shape (Fig.7,a). The anterior raised margin is with two forked tips (the outer of which is pointed) separated with a low flat region which articulates with the anterior rachis. The posterior raised margin is broad with three triangular blunt projections. The middle of these projections is little longer than the other two. The lateral teeth are spoon shaped with the raised posterior margin curved towards anterior side. The posterior margin is with a 'thumb' like projection towards outer side which is not raised anteriorly. The 'thumb' is followed by 5 anteriorly raised conical projections, of which the outer 4 are gradually increasing in length. The 4^{th} is the longest and with rounded head. The 5^{th} (innermost) projection is smaller than the 4^{th} but taller than the outer three (Fig.7,b). The 'handle' like flat, slender anterior part is inserted below the lateral anterior to it. The marginals are plate like, raised posterior margin is serrated. Middle portion of the plate is broader. The triangular cusps of the central rachis seem to help in piercing (clipping) the vegetation. The multi level projections of the laterals can be well suited for chewing. While the serrated margin plate like marginals indicates ability of through mastication. So the forms and the overall stability of the structure observed in its radula structure may suggest that the feeding habit of *Assiminea francessi* is of grazing type.

DISCUSSION

Of the 409 recognized families of recent gastropods, 26 are almost or entirely restricted to freshwater and only four span freshwater, brackish and marine environments (Strong *et al.*, 2008). Some of the freshwater members of the families raise interesting, but still largely unanswered, questions about their phylogenetic relationships, antiquity, geographical origins, physiological adaptations and the selection regimes under which they evolved (Vermeij & Wesselingh, 2002). The distribution of aquatic gastropods was found to be restricted to a particular zonation of the aquatic habitat indicating that prevailing environmental factors such as degree of exposure to wave impact, duration of wetting by immersion and by splashes from waves, the presence or absence of competitors, predators, food source, air and sea temperatures anomaly and the duration of exposure (Purchon, 1968). Evidently, the most primitive type of gastropod feeding involved browsing and grazing of algae from rocks (Purchon, 1977). Amongst the most primitive gastropods, the very survival of *Neritina* and *Assiminea* was probably because of their ability to live sympatrically. The species belonging to genus *Neritina* and *Assiminea* are common gastropods in the river systems of Ganges in the Gangetic delta region, although they are brackish water species (Subba Rao, 1989). They are found attached to the substratum, more often seen attached to rocks in the bathing Ghats. They graze on microscopic algae from the substratum by means of radula. SEM observations on the radula of two species of *Neritina* and one species of *Assiminea* elucidated interesting features. The comparison of the radular morphology with the feeding habitats and food preferences of both the genus reflects the fundamental role of radula. The SEM study reveals a significant difference in the structure of centrals and laterals in the radulae of all the three species which indicate the feeding behavior to be different among them, although live sympatrically. Whereas, the *Neritina (Dostia) violacea* is predominantly scrapping and rasping feeder, the *Neritina (Vittina) smithi* is advance than the former with modified laterals in the radula. This also shows the evolutionary significance of the genus *Neritina*. In the case of *Assiminea francessi* the feeding is grazing type due to modification of the central and lateral teeth. In terms of their evolution, from the SEM study of radula, *Assiminea francessi* is seems to be youngest among the three, being developed with advanced structures in the radulae. The SEM study on the radula structure in mollusca is definitely an useful tool for determine the phylogenetics, antiquity, geographical origins and physiological adaptations. Nevertheless, this is a preliminary observation of radula through application of SEM and radula study using SEM is quite recent in India and future malacological research of terrestrial and aquatic gastropods will reveals many important information through such advance techniques.

SUMMARY

The similarities and differences in the feeding organs utilized to an increasing extent in the classification of many gastropods as the shape of the radula teeth is directly related to the kind of

food which they feed. Radular teeth of gastropods are adapted for a variety of feeding methods. Traditionally studies have focused on the morphology of hard structures associated with the radula, especially radular teeth. The Scanning Electron Microscope (SEM) permits the examination of radula with higher magnification without elaborate preliminary preparation and without squashing or fragmentation. This paper discuss about the radula structure and feeding biology of three freshwater gastropods using SEM.

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