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Scanning Electron Microscopy (SEM) Study of Caudal Gills of *Ceriagrion coromandelianum* (Fabricius) of Zygopteran Larvae (Odonata: Zygoptera)

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Abstract:
In the present study an attempt has been made to study the Scanning Electron Microscopy (SEM) study of the caudal gills of damselfly larvae *Ceriagrion coromandelianum* (Fabricius) Family Coenagronidae. Scanning electron microscopy greatly clarifies the orientation, structures and arrangement of trachea, its ramification, tracheoles, and chloride cells apart with the arrangement of complex cuticular components because of its depth of field and high resolving power.

Keywords: *Ceriagrion*, Caudal gills, Dimensions, Scanning, Chloride Cells, Respiration.

INTRODUCTION

The structure and function of the caudal gills of the damselfly larvae is directly indicative of the habitat conditions. The shape, size, dimensions, histology, histochemistry and bioenergetics of these larvae are some detrimental parameters governing the functioning of these larvae in their habitat. The organization of caudal gills enables them to adapt in their microhabitats which are generally diverse types of inland waters ranging from O_2 rich as well as deficient water bodies. Organizational characteristics are important factors in determining the uptake of oxygen from the habitat by these larvae, which are respiratory strategy, body size, respiratory surface area and morphological thickness of the respiratory barrier between water and tracheoles in case of these larvae. Several aquatic respiratory strategies have been emerged from the basic open tracheal system, including epithelial gas exchange systems utilizing body walls and gills (Eriksen et. al., 1996).

A detailed study of the surface specialization of the caudal gills of *Ceriagrion coromandelianum* (Fabricius), Zygopteran larvae and accessory respiratory organs using some recent techniques and tools are needed. The electron microscopy helps to study the fine structures like cell organelles and chemical aggregates of the caudal gills (Anonymous, 2006). Considerable work on the structure of gills has been done in the past using light microscope by Goodrich (1930); Bevelader (1935); Kouch, H.J.A. (1938); Copeland, D.E. (1948) and Bijtel (1949). The notable morphological studies on the caudal gills of damselfly larvae which act as respiratory organs are those by Mac Neill, N. (1960); Zwick, P. (1973); Mill, P.J (1974); Diaz and Rodriguez (1977); Komnick, H. (1977); Gupta, S and Gupta, A. (2004). Like most fishes, the caudal gills of the damselfly larvae particularly play the dual role of gas exchange and osmoregulation and thus, have to design for doing these two essential functions.

MATERIALS AND METHODS:

Live specimens of the Zygopteran larvae (Odonata) has been collected from local fish ponds. The collected specimens were sorted out and kept in glass aquarium in the entomological research laboratory of the department with pond water. Aquatic weeds like Hydrilla were supplied to help the insects in clinging to the plant. The gills were dissected the caudal region of the species. The dissected gills were taken on slide and wash with distilled water. The gills were fixed by immersion with 2.5% gluteraldehyde in 0.1M phosphate buffer at pH 7.4 at 4 0C for 2 hrs to 24 hrs. After 24 hours the gills were thoroughly washed in 0.1M phosphate buffer at 4 0C for 24 hours with two changes (first for 1½ hour and second for hour or more).

The materials were dehydrated in ascending concentration of ethanol (1½ hour each at 4 0C temperature, 30% to 70%) and from 90% to absolute alcohal dehydrated at room temperature. After that the material were dehydrated in mixed solution of absolute alcohol and acetone in different concentration and fixed in pure acetone and finally fixed in anhydrous acetone (Acetone + Fused Cacl2). The fixed gills in anhydrous acetone were taken to USIC, Burdwan University, Burdwan, where they were subjected to critical point dry (CPD) in liquid carbon dioxide. These dried tissues were taken for gold coating and then were studied under scanning electron microscope (SEM) at different magnification and photographed with Kodak Technical Pan Film TP-120.

RESULTS

The scanning electron microscopic studies and fine structure of the caudal gills of *Ceriagrion coromandelianum* (Fabricius), of Zygopteran larvae (Odonata: Zygoptera) belonging to the family Coenagrionidae have been studied. The shape, size, dimension and structure of the caudal gills of the species have been investigated and marked differences in the morphometric parameters have been known. The arrangement of sensilla, general plan of tracheation, surface epithelium, orientation and other microstrutures of the caudal gills of *Ceriagrion coromandelianum* (Fabricius), have been studied. The Comparative morphometric data on the cuticular structures and sensilla of the caudal gills of *Ceriagrion coromandelianum* (Fabricius), have been described below, Table 1.0

The SEM and fine structure of the caudal gills of *Ceriagrion coromandelianum* (Fabricius), have been depicted by the microphotographs (Plate-I-III; Fig. 1, 2, 3). Marked differences in the structure of epiproct and paraprocts have been found and shown by these microphotographs.

***Ceriagrion coromandelianum* (Fabricius)**

The fine and surface ultrastructure using electron microscopic studies of the caudal gills of *Ceriagrion coromandalianum* (Fabricius) has been shown in (Plate – I; Fig 1. a, b, c and d) for epiproct and (Plate – II - III; Fig. 2-3. a, b, c, d and e) for paraproct.

Epiproct

This is median caudal gill and remain covered with two lateral paraprocts. The unsocketed sensilla are arranged on the margin (Plate – I; Fig. I. a). Each sensillum is situated in the form of projections on the epiproct facing outside towards the environment. The peg-like projections are arranged in a fashion showing teeth-like arrangement. The outer surface of the epiproct is subjected to come in direct contact with the environment, thus, various modifications occurred in its structures. Copepod ecto-parasite is found attached to the outer surface of the epiproct (Fig. 1 d).

Paraproct

These are laterally arranged and are larger structure of caudal gills covering epiproct from two sides. The attachment of paraprocts with last abdominal segment indicates the position of median tracheal trunk passing into these two lateral caudal gills. The median tracheal trunk (MTT) after entering into paraprocts repeatedly divide into primary, secondary, tertiary and quarternary tracheoles which has been seen clearly in the apical region (Fig. 2 a, b and c). The surface epithelium is corrugated with ridges and depressions favourably suitable for gaseous exchange. Between ridges pores are situated

which are openings of the mucous and chloride cells (Fig. 3 d). The fine and ultrastructure of the tracheal trunk revealed clearly circularly arranged spiral cuticular structures (Fig. 2 c) at the proximal region of the paraproct. The spiral structures protect the collapse of the tracheal trunk. The sensilla are arranged in the definite rows. These cuticular structures are unsocketed. The lateral sensilla are much longer than the median which are paired spinous structures.

Table 1: Table showing the comparative morphometry data of length and width (diameter - mm) of *Ceriagrion coromandelianum* (Fabricius) of Zygopteran larvae protective

Species name	Tissue	Cells name	Length (mm)	Width (mm)			Diameter (mm)
				Tip	Middle	Base	
Ceriagrion coromandelianum (Fabricius)	Epiproct	Sensilla	2.0 mm	0.30 mm	0.90 mm	0.60 mm	
		Chloride cell					
	Paraproct	Sensilla	1.0 mm	0.10 mm	0.25 mm	0.35 mm	
		Chloride cell					

Plate–I

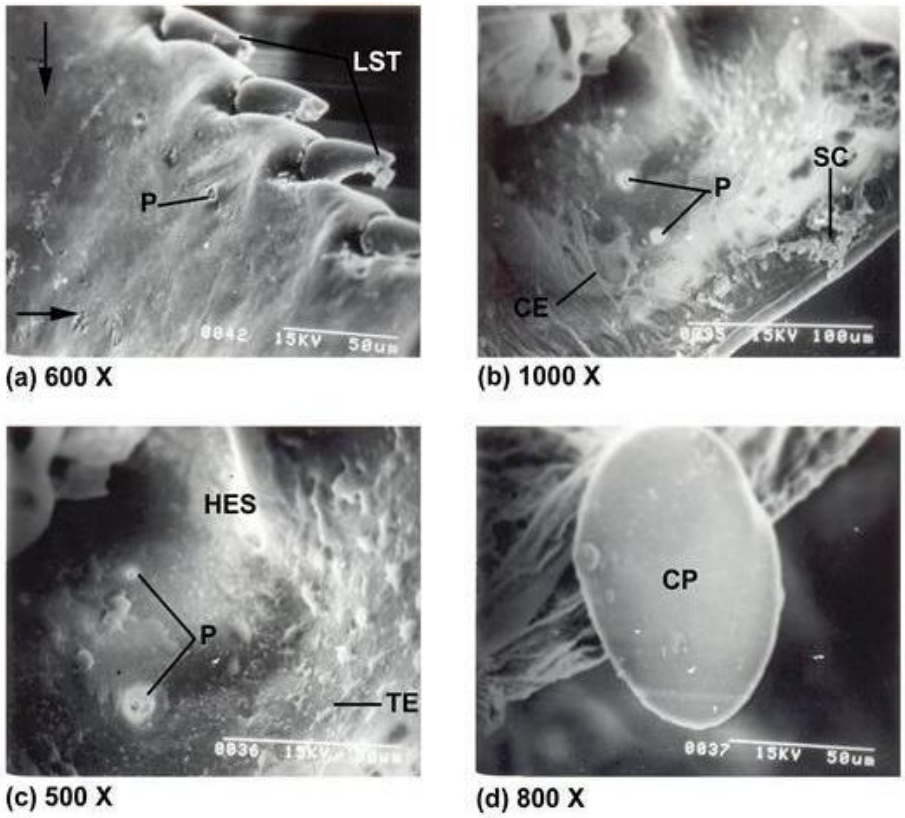

Figure 1: SEM-microphotograph showing Epiproct of *Ceriagrion coromandalianum* (Fabricius) bar showing the magnification.

(a) SEM-Microphotogaph showing unsocketed lateral sensilla trichidea-blunt peg like (LST), surface epithelium (Arrow), other haemocoelomic structures and pore (P) at higher magnification (600X).

(b) SEM-microphotograph surface structure showing ridges alternately with pores (P), secretory cells (SE) and corrugated epithelium (CE) at higher magnification (1000X).

(c) SEM-microphotograph surface structure showing fine tracheoles (TE) and haemocoelomic structures (HES). (5000X)

(d) SEM-microphotograph of outer surface of caudal gill showing a Copepod parasite (CP) attachment-a good example of eco-friendly fauna. (800X).

Plate–II

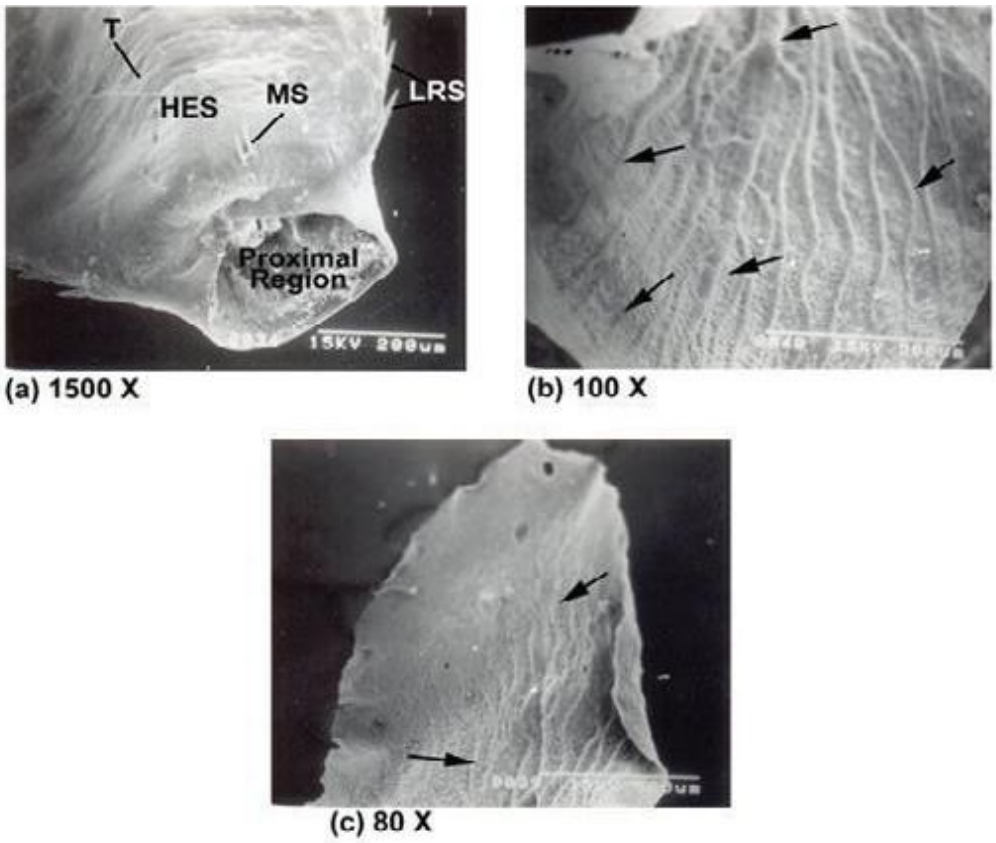

Figure 2: SEM-microphotograph showing Paraproct of *Ceriagrion coromandalianum* (Fabricius).
(a) SEM-microphotogaph of a caudal gill showing attachment site with abdominal segment and definite lateral rows of peg-like sensilla (LRS). Median sensilla (MS), fine trachea (T) and other haemocoelomic structures (HES) at higher magnification. (1500X).
(b) SEM-microphotograph of caudal gill showing mode of tracheation and finer branching of trachea increasing surface area, reducing morphological thickiness of the barrier (Arrow) at lower magnification. (100X).
(c) SEM-microphotograph of caudal gill showing very fine branching of tracheoles in the apical portion (Arrow) at lower magnification (80X).

Plate–III

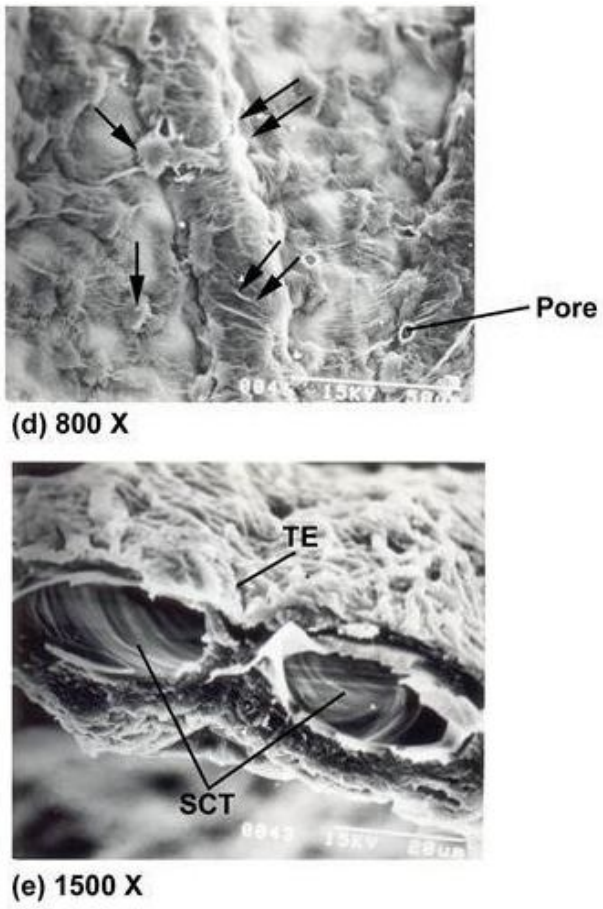

Figure 3: SEM-microphotograph showing Paraproct of *Ceriagrion coromandalianum* (Fabricius).
(d) SEM-microphotogaph showing corrugated epithelium (Double Arrow) and specialized cells (Arrow) to lower magnification. (800X).
(e) SEM-microphotograph showing spiral cuticular structures of trachea (SCT) at the proximal region of the caudal gill and finer branching of tracheoles (TE) at higher magnification (1500X)

DISCUSSION

The scanning electron microscopy was used to study the distribution of chloride cells, sensilla as well as other structures of the caudal gills of these larvae. The main longitudinal tracheal trunk divide repeatedly into primary, secondary, tertiary and quarternary fine structures called tracheoles. These branching of tracheoles increase the surface area for the gaseous exchange and reduced the morphological thickness of the respiratory barrier, allowing maximum gaseous exchange. Interestingly a great modification of sensillum has been observed in the epiproct and paraprocts of these four different species.

It has been observed that the natures of cuticular magnifications found in the different species of the Zygopteran larvae are entirely different, although they inhabit the same ecosysterm. It may be due to the fact that the four species occupying different niches of the same ecosystem and thus, seem to be modified their cuticular structures for monitoring and responding the changes in the environment. Hence, the position and orientation of different types of sensilla and other cuticular structures provide a basis for understanding of the relationship between their structures and functions.

In discussing the problems associated with the fine ultrastructure and surface scanning electron microscopic study of caudal gills of the Zygopteran larvae, a distinction has to be made between the structure and function of fish gills, invertebrate gills and caudal gills of insects. Gills are generally outpushings of the body surface which increase the surface area for gas exchange. Such surface areas become reduced in thicknes and achieve the function of gaseous exchange. This type of gills present in majority of invertebrates.

The structure and organization of caudal gills of Zygopteren larvae is completely different from that of fish gills and invertebrate gills. The caudal gills are tracheated evaginations of the bodywall. They developed an outpocketings of the specialized regions of the bodywall, more or less lamellate structures that are well supplied with tracheae and tracheoles. The tracheoles are arranged regularly at optimal distances apart and very close to the cuticle (Wichard, 1973; Wichard and Komnick, 1972, 1973). These tracheal gills borne on the last abdominal segment where the two paraprocts from the paired lateral and one epiproct form the median caudal gills. The characteristic features of caudal gills are uniformly thin cuticle overlying a richly tracheated bodywall, a concentration of tracheoles and cuticular permeability into delineated regions of the bodywall.

However, the study on the structures of epiproct and paraprocts which after their modification form one median and two lateral caudal gills, some conclusions may be drawn as follows

- Epiproct is median and two paraprocts are lateral.
- Epiproct is almost smaller than paraproct.
- The two lateral paraprocts almost cover the epiproct.
- The sensilla are shorter peg-like in epiproct and long socketed in paraproct.
- Trachea is more in paraproct than the epiproct.
- Cellular structures are more in paraprocts than the epiproct.
- Structurally the paraprocts are more complex than epiproct.
- It seems that paraprocts has more role in gaseous exhange than epiproct.

CONCLUSION

Structurally, the paraprocts are more complex with diversity of trachea-tracheoles, socketed long sensilla and presence of coniform chloride cells a part with abundance of ridges and depressions in it, which are responsible for gaseous exchange in aquatic medium. It has been concluded that the paraprocts have major role in dissolved O2 uptake in these larvae.

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