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Scaning electron microscopy (SEM) study of caudal gills of *Ischnura senegalensis* (Rambur) and *Agriocnemis pygmaea* (Rambur) of Zygopteran Larvae (Odonata: Zygoptera)

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Abstract
Clearly there is need for extensive studies of the gills of such ecologically diverse species of damselfly larvae. In the present study an attempt has been made to study the Scanning Electron Microscopy (SEM) study of the caudal gills of damselfly larvae. 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.
Therefore, the objectives of the present study are to add information on the detailed fine ultrastructure of the caudal gills of these two species of Zygopteran larvae occupying different microhabitats of the inland waters.

Keywords: ischnura, agriocnemis, caudal gills, ultrascanning, dimensions, respiration, chloride cells

Introduction
The structure and function of the caudal gills of *the Ischnura senegalensis (Rambur) and Agriocnemis pygmaea (Rambur*) of Zygopteran Larvae 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) [13]
The fine structural organization of the caudal gills, chloride cells and histochemical demonstration has not been reported so far. In this insect taxa, the various chemoreceptors and proprioreceptors present on caudal gills which have also not been taken into account. Zwick (1973) [10] reported on the chloride cells of Plecopteran larvae, which were formerly interpreted as sensory or respiratory in function.
A detailed study of the surface specialization of the caudal gills *of Ischnura senegalensis* (Rambur) and *Agriocnemis pygmaea* (Rambur) of Zygopteran Larvae, 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) [1]. Considerable work on the structure of gills has been done in the past using light microscope by Goodrich (1930) [2]; Bevelader (1935); Kouch, H.J.A. (1938) [4]; Copeland, D.E. (1948) [5]; and Bijtel (1949) [6]. The notable morphological studies on the caudal gills of damselfly larvae which act as respiratory organs, are those by MacNeill, N. (1960) [7]; Zwick, P. (1973) [10]; Mill, P.J (1974) [11]; Diaz and Rodriguez (1977); Komnick, H. (1977) [12]; 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 and the caudal region of these two species, *Ischnura senegalensis* (Rambur) and *Agriocnemis pygmaea* (Rambur) of Zygopteran Larvae were removed and kept in tube separately. 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 ^{0}C for 2 hrs to 24 hrs. After 24 hours the gills were thoroughly washed in 0.1M phosphate buffer at 4 ^{0}C 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 ^{0}C 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 $Cacl_2$). 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

Ischnura senegalensis (Rambur) and *Agriocnemis pygmaea* (Rambur) 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 *Ischnura senegalensis* (Rambur) and *Agriocnemis pygmaea* (Rambur) of Zygopteran Larvae have been studied. The Comparative morphometric data on the cuticular structures and sensilla of the caudal gills of *Ischnura senegalensis* (Rambur) and *Agriocnemis pygmaea* (Rambur) of Zygopteran Larvae have been described below, Table 1

The SEM and fine structure of the caudal gills of *Ischnura senegalensis* (Rambur) and *Agriocnemis pygmaea* (Rambur) of Zygopteran Larvae, have been depicted by the microphotographs (Plate-I-VIII; Fig. 1A- 8A). Marked differences in the structure of epiproct and paraprocts have been found and shown by these microphotographs.

***Ischnura senegalensis* (Rambur):** The fine and ultrastructure of the epiproct and paraproct of *Ischnura senegalensis* (Rambur) has been depicted in Plate – 1; Fig. 1A. a, b, c and d and Plate – II – III; Fig. 2A. a,b, and c and Fig. 3A. d,e, and f respectively.

Epiproct: The ultastructural study of the epiproct of *Ischnura senegalensis* (Rambur) revealed that the surface is much corrugated with ridges and depressions and ramifications of the trachea-tracheoles at lower magnification (Fig. 1A. a and b). On higher magnification the surface epithelium is seen much corrugated with pores found in depressions. These pores are openings of the chloride cells. The chloride cells are caviform as seen under electron microscope.

Paraproct: The median longitudinal tracheal trunk is clearly visible at lower magnification with networking of the trachea-tracheoles on the epithelial surface. The sensilla are found on lateral and medion line on the paraprocts. The sensilla are socketed which are spherical in shape. The sensilla are paired structures with blunt tips. Several haemocoelomic structures are seen around the sensilla. The caviform chloride cells are present and around these cells networking of trachea-tracheoles are found. The chloride cells of epiproct and paraprocts of *Ischnura senegalensis* (Rambur) have similar shape and sizes.

***Agriocnemis pygmaea* (Rambur):** The fine and ultrastructural studies of ther epiproct ad paraprocts of *Agriocnemis pygmaea* (Rambur) has been depicted in Plate – VI – V; Fig. 4A. a,b and c; 5A. 12 d,e and f; and Plate – VII – VIII; Fig. 6A. a,b and c; Fig. 7A. d,e and f and fig. 8A. g, h, and i respectively.

Epiproct: The surface epithelium at lower magnification indicate the presence of socketed sensilla on the median longitudinal tracheal trunk and ramification of trachea-tracheoles in the epiproct. On higher magnification ridges and depressions are present and abundance of caviform chloride cells on the dorsal surface of the epiproct present (Fig. 5A. f). On further higher magnification the attachment of epiproct with abdominal segment showing cuticular spiral structures prominently (Fig. 6A. g and h). The sensilla has been lodged in spherical socket with slightly bent spine surrounded by differentiated epithelial cells.

Paraproct: The alternately ridges and depressions are observed in the paraproct with pores. The ramifications of trachea-tracheoles are seen under low magnification. The significant cuticular spiral structures are present on the longitudinal tracheal trunks as seen under higher magnification. The sensilla are unsocketed present on the lateral side of the paraproct. The sensilla present on the general surface of the paraproct are very long, spinous and hair-like (Fig. 7A. e). On the outer surface of the paraproct an ectoparasite is found attached (Fig. 7A. f).

The morphometric measurement of the chloride cells and various cuticular structures with sensilla have been depicted in Table 1. The diameter of chloride cells was found more in *Ischnura senegalensis* (Rambur) (6.60mm.) of epiproct and paraproct (7.40mm) in Zygopteran species studied. However, minimum diameter was found in *Agriocnemis pygmaea* (Rambur) as 2.0 mm. The sensilla length was also found more in the epiproct of *Ischnura senegalensis* (Rambur) (6.80mm) and paraproct of *Agriocnemis pygmaea* (Rambur). The shape and sizes of sensilla varied considerably in different species of damselfly larvae.

Table 1: Table showing the comparative morphometry data of length and width (diameter - mm) of Zygopteran larvae

Species name	Tissue	Cells name	Length (mm)	Width (mm)			Diameter (mm)
				Tip	Middle	Base	
Ischnura senegalensis (Rambur)	Epiproct	Sensilla	7.80 mm	0.40 mm	0.60 mm	0.60 mm	
		Chloride cell					6.60 mm
	Paraproct	Sensilla	3.40 mm	0.40 mm	1.0 mm	0.70 mm	
		Chloride cell					7.40 mm
Agriocnemis pygmaea (Rambur)	Epiproct	Sensilla	4.40 mm	0.30 mm	0.65 mm	0.60 mm	
		Chloride cell					2.0 mm
	Paraproct	Sensilla	6.10 mm	0.08 mm	0.09 mm	1.0 mm	
		Chloride cell					

PLATE-I

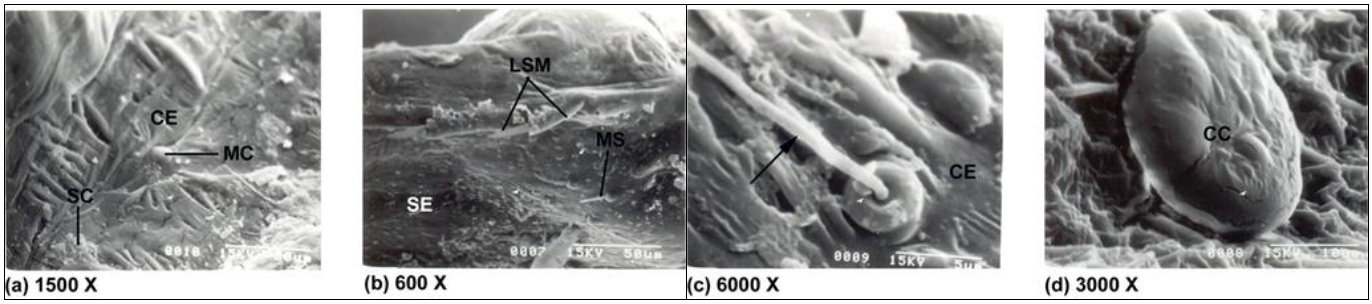

Fig 1A: SEM-microphotograph showing Epiproct of *Ischnura senegalensis* (Rambut)

(a) SEM-microphotograph showing corrugated epithelium (CE), Mucous cells (MC), secretory cell (SC) and other haemocoelmoic structures at higher magnification. (1500X)
(b) SEM-microphotograph showing surface epothlium (SE), lateral peg-like sensilla microtrichia (LSM), socketed median sensilla (MS) and other haemocoelomic structure. (600X)
(c) SEM-microphotograph showing socketed very long sensilla (Arrow) and corrugated epithelium (CE) at higher magnifiation. (6000X)
(d) SEM-microphotograph showing caviform chloride cell (CC) with pores at higher mangification. (3000X)

PLATE-II

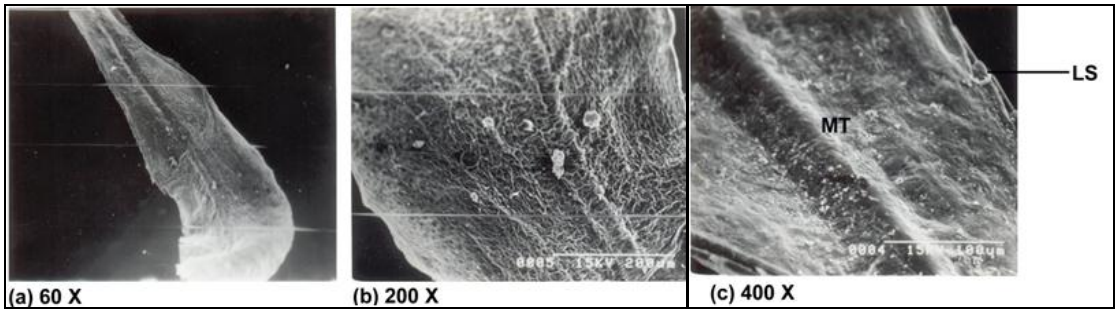

Fig 2A: SEM-microphotograph showing Paraproct of *Ischnura senegalensis* (Rambur)

(a) SEM-microphotograph showing whole structure of paraproct at lower magni-fication. (60X)
(b) SEM-microphotograph showing surface epithelium, mucous cells, median trunk networking of trachea-tracheoles ND finer ramifications at lower magnification. (200X)
(c) SEM-microphotograph showing surface epithelium, median trunk (MT) and socketed lateral sensilla (LS) pointed peg-like and other haemocoelomic structures at lower magnification. (400X)

PLATE-III

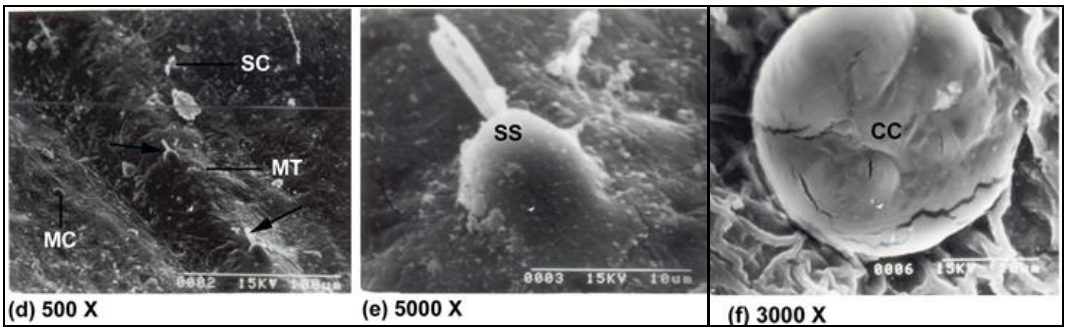

Fig 3A: SEM-microphotograph showing Paraproct of *Ischnura senegalensis* (Rambur)

(d) SEM-micrphotograph showing many small scketed sensilla (Arrow), median trunk (MT), mucous cells (MC), secretory cells (SC) and other haemocoelomic structuress at lower magnification. (500X)

(e) SEM-microphotograph showing presence of socketed sensilla (SS) on the surface of the caudal gill at higher mangnification. (5000X)

(f) SEM-microphotograph showing caviform chloride cell (CC) on epithelial surface (ES) at higher mangification. (3000X)

PLATE-IV

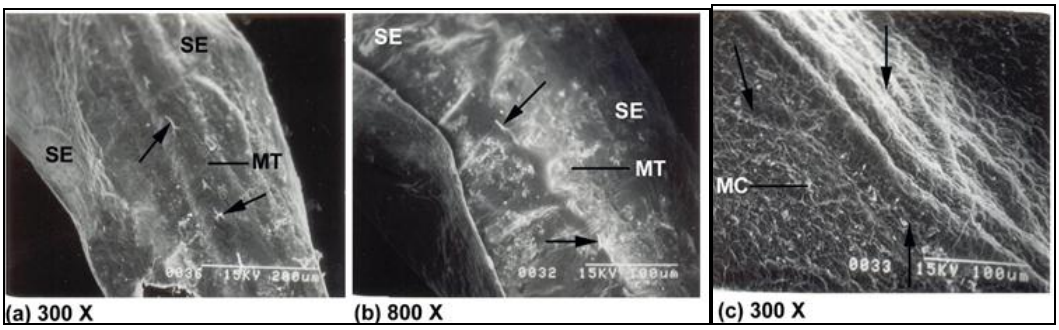

Fig 4A: SEM-microphotograph showing Epiproct of Agriocnemis pygmaea (Rambur)

(a) SEM-microphotograph of a caudal gill showing surface epithelium (SE), median trunk (MT), with many small socketed sensilla (Arrow) and other heamocoelomic structures at lower magnification. (300X)

(b) SEM-microphotograph of caudal gill showing surface epithelium (SE), median trunk (MT) with many small socketed sensilla (Arrow) and other heamocoelomic structures at higher magnification. (800X)

(c) SEM-microphotograph of caudal gill showing mode of tracheation, mucous cells (MS) and sectretory materials (Arrow) at lower magnification. (300X)

PLATE-V

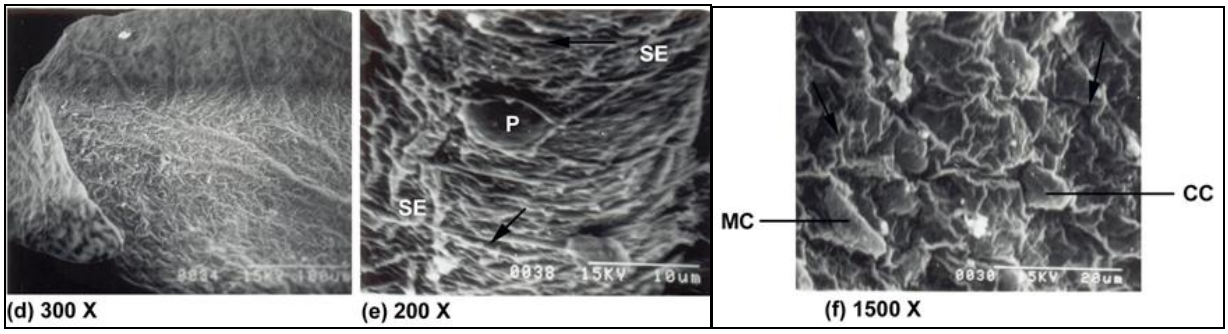

Fig 5A: SEM-microphotograph showing Epiproct of *Agriocnemis pygmaea* (Rambur)

(d) SEM-microphotograph of a caudal gill showing mode of tracheation and finer branching of trachea inctrasing surface area and reducing morphological thickness of the barrier at lower magnification. (300X)

(e) SEM-microphotograph of surface epithelium showing ridges (Arrow) alternately with pores (P) at lower magnification. (300X)

(f) SEM-microphotograph showing at higher magnification of surface epithelium (Arrow), mucous cells (MC) and chloride cells (CC) at giher mangification (1500X)

PLATE-VI

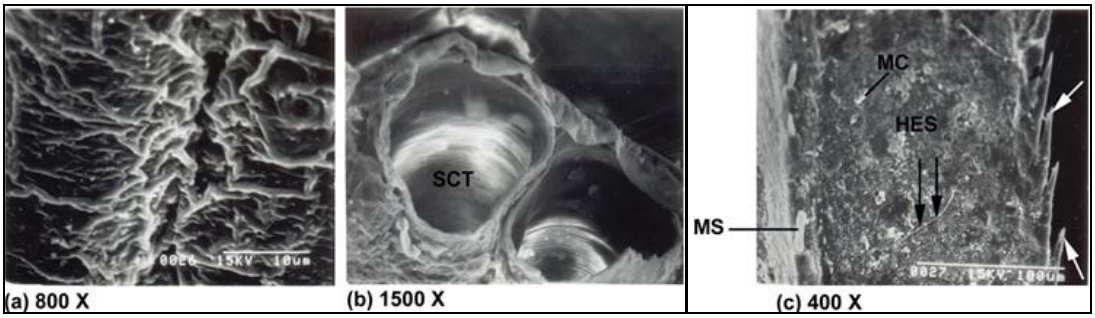

Fig 6A: SEM-microphotograph showing Epiproct of Agriocnemis pygmaea (Rambur)

(a) SEM-microphotograph showing networking of trachea-tracheoles and finer ramifications. (800X)
(b) SEM-microphotograph showing spiral cuticular structures of tracea (SCT) at the middle region of caudal gill at higher magnification. (1500X)
(c) SEM-microphotograph of middle region of a caudal gill showing lateral rows of peg-like sensilla (Arrow), many small median socketed sensilla (MS), fine long hair-like sensilla (Double Arrow), mucous cells (MS) and other haemo-coelomic structures (HES) at lower mangification. (400X)

PLATE-VII

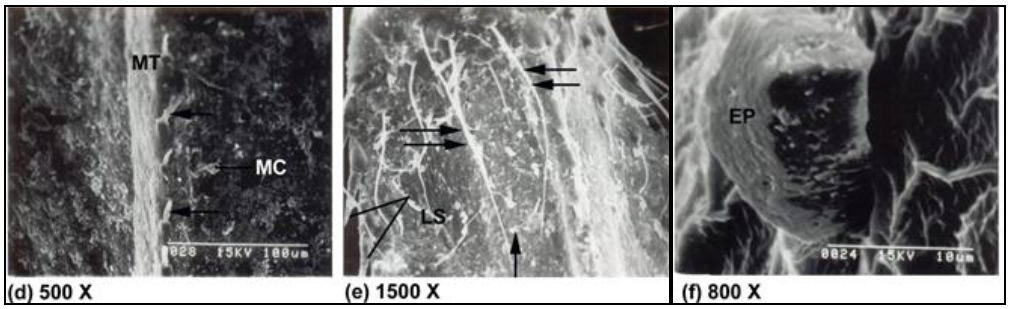

Fig 7A: SEM-microphotograph showing Paraproct of *Agriocnemis pygmaea* (Rambur)

(d) SEM-microphotograph showing many small median socketed sensilla (Arrow), mucous cells (MS), median trunk (MT) and other haemocoelomis structures at lower magnification. (500X)
(e) SEM-microphotograph showing many fine very long hair like socketed sensilla (Double Arrow), many peg-like lateral sensilla (LS) and secertory materials (SM) at higher magnification. (1500X)
(f) SEM-microphotograph of outer surface of caudal gill showing external parasite (EP) attachment - a good example of eco-friendly fauna at higher mangification. (800X)

PLATE-VIII

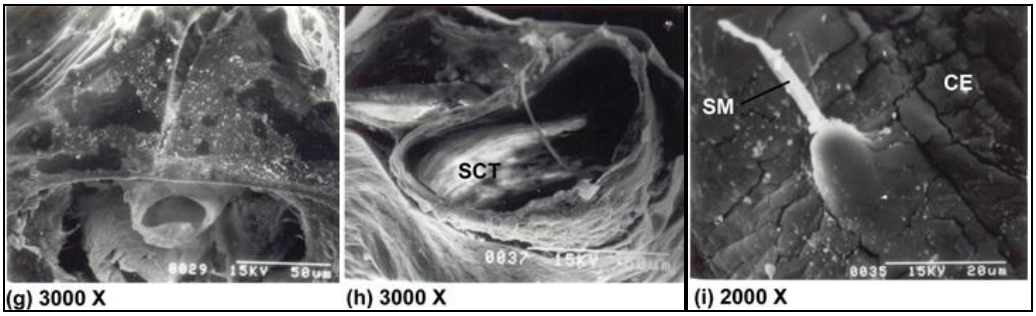

Fig 8A: SEM-microphotograph showing Epiproct of Agriocnemis pygmaea (Rambur)

(g) SEM-microphotograph of caudal gill showing attachement site of epiproct with abdominal segment at higher magnification. (3000X)
(h) SEM-microphotograph showing spiral cuticular structure of trachea (SCT) at the proxmal region of caudal gill at higher mangification. (3000X)
(i) SEM-microphotograph showing scketed sensilla microtrirhis (SM), long peg-like and corrugated epithelium (CE) at higher magnification. (2000X)

Discussion

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 area 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, 1971, 1974) [9]. 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.

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 two different species.

The structure of epiproct and paraprocts which has been modified as one median and two lateral caudal gills, differs significantly in in these two species of Zygoptera: Coenagrionidae with regard to the arrangement of sensilla and the cells and other haemocoelomic structures. The role of sensilla is to function as mechanoreceptors has been emphasized by Gewecke, 1970; Gupta, *et al*. 1999, 2000; Kapoor and Zachariah 1984. The unsocketed sensilla have been shown to the non-innervated (Schmidt and Smith, 1985). However, the basal areas of peg-like structures are suggestive of a possible secretory role.

It has been observed that the nature 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 these two 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.

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