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STOMODEAL CUTICULAR STRUCTURES IN THE DRAGONFLY *BRACHYTHEMIS CONTAMINATA* (FABRICIUS) (ANISOPTERA: LIBELLULIDAE)

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Light and scanning electron microscopic studies reveal various stomodeal cuticular structures. In the larvae and adults, microspines on the surface of the longitudinal folds of the pharynx, and dome-shaped, beaded structures on the inner surface of the oesophagous are evident. In the larvae, the folds of the crop bear long hairs laterally and parallel rows of microspines medially. In the larvae, the proventriculus is provided with 4 longitudinal plates; 2 large plates with teeth on each lateral side and 2 small plates each with 4 fine apical teeth, on either side. Scale-like acanthae are observed near the stomodeal valve. A whorl of long hairs is evident in the stomodeal valve. In the adult dragonfly, the acanthae and curved spines occupy the anterior and posterior regions of the proventricular dental plates, respectively. The functional significance of various stomodeal cuticular structures is discussed.

INTRODUCTION

The presence of a well-defined strong armature in the proventriculi of insects that feed on solid food material has been known for a long time (SNODGRASS, 1935; DAY & WATERHOUSE, 1953; WATERHOUSE, 1957; RICHARDS & DAVIES, 1984; CHAPMAN, 1985). SEM studies on the internal surface of proventriculi have revealed fine spines of variable size in orthopteroid insects (NOIROT & NOIROT--TIMOTHEE, 1969; MILLER & FISK, 1971) and Coleoptera (BALFOUR-BROWNE, 1944; ZHAVORONKAVA, 1969). Microspines throughout the foregut have been observed in *Blatta orientalis* (ELZINGA & HOPKINS, 1994), *Locusta migratoria* (HOCHULI et al., 1992) and some orthopteroid insects (BOUDREAUX, 1980; CHAPMAN, 1985).

In Odonata, strong armature in the proventriculus of the larva and a degenerated armature in the adult is reported (TILLYARD, 1917). There is, however, no

information on the cuticular processes of other regions of the foregut and hindgut, and no SEM studies have been carried out till now.

The present work was, therefore, undertaken to explore the various types of cuticular processes of the stomodaeum in the larva and adult of the dragonfly, *Brachythemis contaminata,* with the help of morphological, histological and scanning electron microscopic methods.

MATERIAL AND METHODS

The penultimate larvae were collected from ponds during the rainy season and brought to the laboratory. They were reared in specially designed tubs. Mosquito larvae were supplied twice a day and the water was renewed daily. Newly emerged adult dragonflies were transferred to cages covered with a mosquito net. The present study was carried out with the help of following morphological, scanning electron microscopic and histological staining methods.

MORPHOLOGICAL METHOD. – Last instar larvae, about a week old, were taken out from a tub and anaesthesized by CO_2 after keeping them in ice in a refrigerator for 15-20 minutes. The excess of water was soaked by wrapping clean blotting paper over the body. The proventriculus from the gut was removed, a long slit was made in it to expose the internal surface. The tracheae, fat bodies and muscles were removed and the proventriculus was washed thoroughly in distilled water. It was boiled for 15 minutes in 10% aqueous KOH solution and rinsed thoroughly in distilled water, dehydrated in ethanol and cleared in clove oil. The proventricular teeth and other cuticular structures were studied under the binocular microscope at various magnifications.

SCANNING ELECTRON MICROSCOPIC METHOD. – The alimentary canal was dissected out in Ringer's saline, removed from the body and the foregut was separated from the alimentary canal and opened with a longitudinal slit anteroposteriorly. The foregut was spread out and glued, with the internal surface exposed, to a piece of cardboard using a cynoacrylate glue, fixed in 10% formaldehyde for 24 hours, washed in distilled water and dehydrated in ethanol. Specimens were dried at room temperature, mounted on a stub, and coated with gold palladium alloy in a Poloron Automatic Unit. A Stereoscan 250 MK III Cambridge scanning electron microscope was used to examine the specimens at the Regional Sophisticated Instrumentation Centre (R.S.I.C.), Nagpur University Campus, Nagpur.

HISTOLOGICAL METHOD. – Alimentary canal of both, larvae and adults was dissected in Ringer's solution. The foregut was separated from the alimentary canal and fixed immediately in aqueous Bouin's fixative for 24 hours. The foregut was washed in distilled water, dehydrated in 30% to 100% ethanol, cleared in xylene and embedded in paraffin wax at 60°C. The 4-6 mm thick sections were cut, dehydrated and stained with (i) Ehrlich Haematoxylin-Eosin (HE), (ii) Heidenhain's Iron-Haematoxylin (FeH) and (iii) Mallory's Triple (MT).

OBSERVATIONS

Although the alimentary canal is secondarily modified in the larva and adult in relation to the aquatic and terrestrial mode of life respectively, the foregut is commonly differentiated into the pharynx, oesophagus, crop and proventriculus (Fig.1). The foregut is typically composed of outer circular muscle, middle longitudinal muscle and inner epithelial layers. The epithelium is composed of tall columnar cells. It is folded and internally lined with the cuticular intima equipped with various cuticular structures.

The pharynx consists of the enormously folded epithelium and thick chitinous intima, due to which the lumen is greately reduced in both the larva and adult. The cuticular intima is differentiated into outer stained and inner unstained areas. The rows of fine spines and the folds are clear in the histological preparations of the pharynx of both, the larva and adult (Figs 2-5).

The oesophagus resembles the pharynx in structural organisation but the internal folds occupy most of the lumen. The cuticular intima is differentiated into outer stained and inner unstained regions similar to that in the pharynx. The SEM reveals that the entire inner surface of the cuticular intima is rough due to the presence of spherical, dome-shaped projections giving a beaded appearance to the entire surface of the oesophagus (Figs 6, 7).

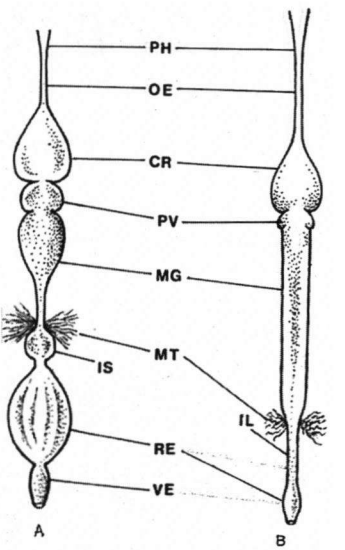


Fig. 1. Morphology of the alimentary canal of *Brachythemis contaminata* (diagrammatic): (A) the last instar larva; – (B) the adult. – [CR: crop, – IL: ileum, – IS: iliac sac, – MG: midgut, – MT: malpighian tubules, – OE: oesophagus, – PH: pharynx, – PV: proventriculus, – RE: rectum, – VE: vestibule].

The crop is greately enlarged, sac-like structure in both the larva and the adult, enclosing a large lumen. In the larva, the cuticular intima is thin measuring 16.66±2.35 μm in thickness and with long hairs laterally and some parallel rows of microspines medially along the folds (Figs 8-10). The lumen is extensive. In the adult, the cuticular intima is invaginated with a large number of folds. Four large diverticula-like folds of ephithelium lined with thin cuticular intima are developed in the lumen.

The proventriculus is a prominent structure in the larva but is ill--defined in the adult dragonfly. The wall of the proventriculus is commonly composed of an outer muscle layer and an inner, thin, indistinct epithelium. The cuticular intima is thick and consists of dental armature in the lumen.

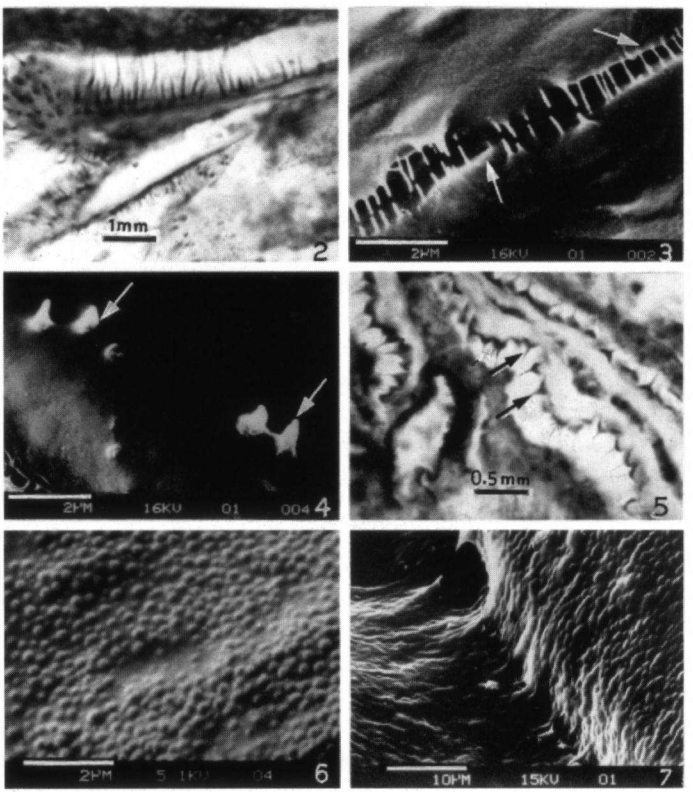
In the larva, the cuticular intima is differentiated into four dental plates, two large and two small (Fig. 11). The large plates bear two large teeth on each lateral side, while the small plates bear four fine teeth apically, of decreasing size, on either side. In addition, the dental plates are provided with scale-like microspines (acanthae) in the posterior region (Figs 12-14). They are arranged in a large number of parallel rows all over the surface. The majority of them are uniramous but some are multiramous structures.

In the adult, the cuticular intima is differentiated into four elongated dental plates. The plates posses small, very fine teeth all over the surface. The anterior region is

occupied by a large number of fine, uniramous acanthae measuring 2.85±0.15 μm in length while the posterior region is covered by a large number of large, curved, apically pointed and basally broad spines measuring 3.48±0.16 μm in length (Figs 15-17).

DISCUSSION

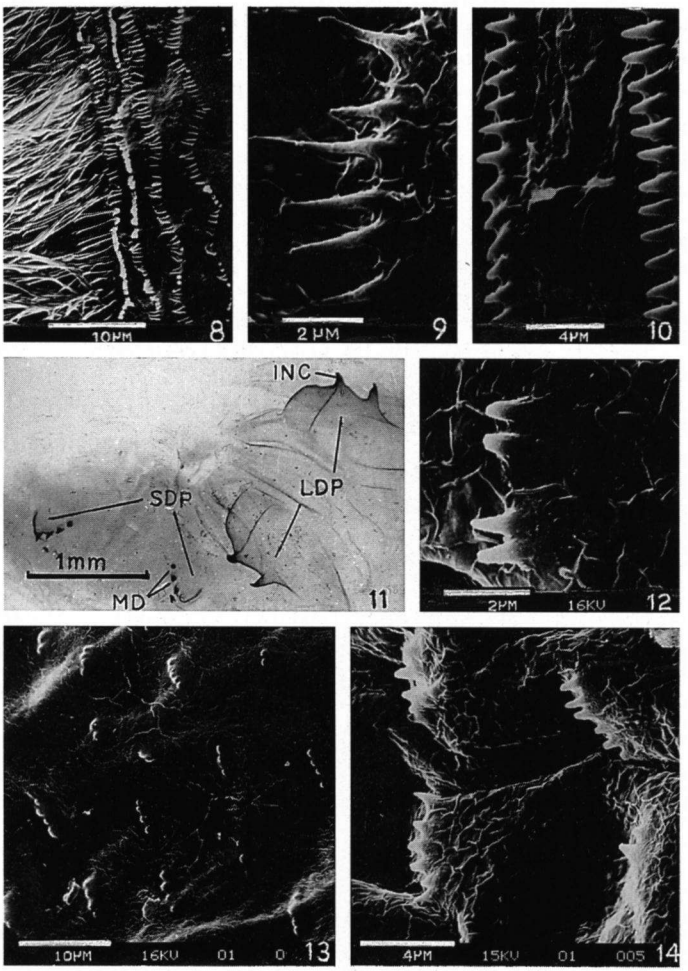
The morphological and structural organisation of the alimentary canal of the larva and adult of *Brachythemis contaminata* represents basically the orthopteroid type of gut, although it is extensively modified in the larva and the adult in accordance with the aquatic and terrestrial mode of life respectively (MARSHALL, 1914;



Figs 2-7. Cuticular structures of pharynx and oesophagus showing a row of spines along an epithelial fold: (2) cross section of pharynx in the larva, FeH; – (3) SEM of cuticular surface of the pharynx in the larva showing typical cuticular armature (→); – (4) SEM view of pharyngeal spines in the larva (→); – (5) cross section of pharynx of adult showing rows of spines along epithelilal folds (→), FeH; – (6) SEM of cuticular surface of oesophagus in the larva showing spherical, dome-shaped projections; – (7) SEM of inner surface of oesophagus in the adult showing spherical dome-shaped projections.

TILLYARD, 1917; SNODGRASS, 1954; RICHARDS & DAVIES, 1984).

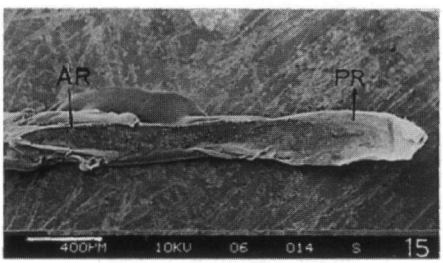
The well developed larval proventriculus contrasts with that in the adult and the difference might be due to varied nature of food material they consume i.e. the larvae feed upon crustaceans and other hard-bodied aquatic organisms, whereas the adults feed selectively on soft-bodied prey, such as mosquitoes, midges, small butterflies and other insects. Adaptations in the alimentary canal of insects in relation

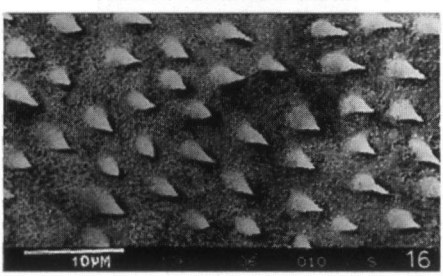


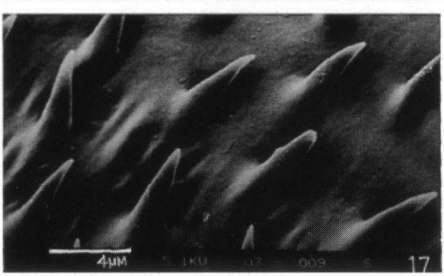
Figs 8-14. Cuticular structures of crop and proventriculus: (8-10) SEM of inner surface of wall of crop in the larva showing rows of hairs and spines; – (11) in-situ preparation of proventriculus in the larva showing paired large and small dental plates with teeth [INC: incisor, – MD: minute denticles, – LDP: large dental plate, – SDP: small dental plate]; – (12-14) SEM of large proventricular dental plates showing spines on surface in the larva.

to the nature of their food has been noticed widely (WATERHOUSE, 1957; DADD, 1970; CHAPMAN, 1985). The thick cuticular surface in the oesophagus and the complex dental apparatus in the proventriculus of *B. contaminata* are presumably associated with the structural modifications of the foregut in relation to a carnivorous feeding habit in both, the larva and adult (SNODGRASS, 1954; POPHAM & BEVANS, 1979). Similar modifications have been noticed in the foregut of other carnivorous insects (CHAPMAN, 1985).

The microspines in the oesophagus, along with the bundles of hairs, has also been reported in *Blatta orientalis* (MIALL & DENNY, 1886; ELZINGA & HOPKINS, 1994), *B. germanica* (ELZINGA & HOPKINS, 1994), *Periplaneta americana* (MURTHY, 1975; BRACKE et al., 1979) and other blattids (Mc KITTRICK, 1964). The microspines or acanthae are found commonly in the foregut of some orthopteroid insects (MURALIRANGAN & ANANTHAKRISHNAN, 1974; BOUDREAUX, 1980; HOCHULI et al., 1992; BENTOS-PEREIRA & LORIER, 1992). The adaptive function of microspines in the pharyngeal region seems to be related to cutting and driving the food material from the buccal cavity to the oesophagus (ELZINGA & HOPKINS, 1994). The pharyngeal spines of *B. contaminata* are tanned and stiffened like those of *P. americana,* and they may act as a natural valve, in colaboration with the epithelial folds, to prevent regurgitation and loss of ingested food (ELZINGA & HOPKINS, 1994). In *B. contaminata* the pharyngeal microspines and the oesophageal cuticular beaded surface may play a vital role in chewing, cutting and grinding the food material in relation to the carnivorous habit (CHAPMAN, 1985).







Figs 15-17. SEM of proventricular dental plates in the adult: (15) dental plate, showing anterior region (AR) and posterior region (PR) with fine spines; – (16) spines in anterior region; – (17) spines in posterior region.

BOUDREAUX (1980) and CHAPMAN (1985) discussed the proventricular microspines or acanthae in different insect orders, and suggested their possible utilization in

systematics. The microspines or acanthae are reported in various orthopteroid and other insects, adapted mostly to the phytophagous or carnivorous feeding habit (MURALIRANGAN & ANANTHAKRISHNAN, 1974; BOUDREAUX, 1980; NATION, 1983; HOCHULI et. al., 1992; CHAPMAN, 1985). They are also reported in adephagous Coleoptera (ZHAVORONKOVA, 1969), Mecoptera and Siphonaptera (RICHARDS & RICHARDS, 1969), along with the hairs. In blattids, there are, moreover, scale-like microspines at the tips of blade-like, proventricular teeth in addition to the multi- and unispinose microspines (ELZINGA & HOPKINS, 1994). The present study demonstrates clearly proventricular modifications in the larva and adult independently, and rules out the general statement made by earlier workers that, "in adult dragonflies the armature is weak or absent" (CHAPMAN, 1985). Similar to other insects, the microspines and acanthae in the proventriculus may perform the function of grinding the food material, while the whorl of hairs of the stomodeal valve might be to prevent backflow of enzymes from the midgut in the larva and adult of *Brachythemis contaminata.*

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