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ULTRASTRUCTURAL ORGANIZATION OF THE LARVAL SPIRACLES IN *LIBELLULA DEPRESSA* L. (ANISOPTERA: LIBELLULIDAE)

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In the last larval instar (F-0) of *L. depressa*, 2 paired spiracles, in the form of elongated eye-shaped structures, are located in the anterior region of the mesothorax segment. A fine structural analysis of these spiracles under the scanning and electron microscopes reveals that each spiracle consists of a well-developed cuticular peritreme with a dorsal-anterior lip bearing a thin laminar coat and a ventral-posterior lip bearing a filter apparatus. The filter apparatus derives from a series of folds forming discrete groups adhering to one another to delimit empty spaces and producing a honeycomb-like structure. This structure is coherent with the need to avoid entry of water when the larva is submerged. The function of these spiracles during the insect development is discussed, noting that in anisopteran larvae the rectal epithelium, forming the so called branchial basket, is the main respiratory organ.

INTRODUCTION

In pterygote insects, spiracles are important structures involved in the balance between gas exchange and transpiratory water loss. The gross morphology of the spiracles has been studied in representatives of a number of lepidopteran families (SCHMITZ & WASSERTHAL, 1999); using electron microscopy they showed important details of the organization of the spiracle apparatus in both larvae and adults. In the Diptera, studies on the spiracle apparatus of water-living larvae showed that the morphology of this system differs markedly from group to group, not only in organization but also in location and in relation to the developmental stadium considered (FAUSTO et al., 1999). In addition, in immature

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stadia of Diptera, it has been demonstrated that the organisation of the spiracle apparatus varies according to environmental conditions (KHOLE, 1979; NIKAM & KHOLE, 1989; FAUSTO et al., 1999). Spiracle structure is also a useful tool for taxonomic purposes (MAROLI et al., 1992; FAUSTO et al., 1998; MILL, 1998a).

Anatomical studies of the adult and larval spiracles of Odonata have been carried out (TILLYARD, 1917; WOLF, 1935). Differences in the morphology of thoracic and abdominal spiracles have been described in the adults of *Pantala flavescens* (TONAPI, 1960). In certain adult African dragonflies, MILLER (1962) carried out an exhaustive analysis of spiracle control and of the factors altering its activity, such as changes in water balance (MILLER, 1964a), degree of hypoxia and changes in temperature (MILLER, 1964b).

In anisopteran larvae the rectal epithelium, forming the so called branchial basket, is the main respiratory organ and the function of the two mesothoracic spiracles is unclear.

The goal of the present study is the fine structural analysis of the spiracles of the last larval instar of *Libellula depressa*. This species is a pond-dwelling dragonfly, common and widespread in Italy (UTZERI, 1995) and throughout the rest of Europe (CARCHINI, 1983).

MATERIAL AND METHODS

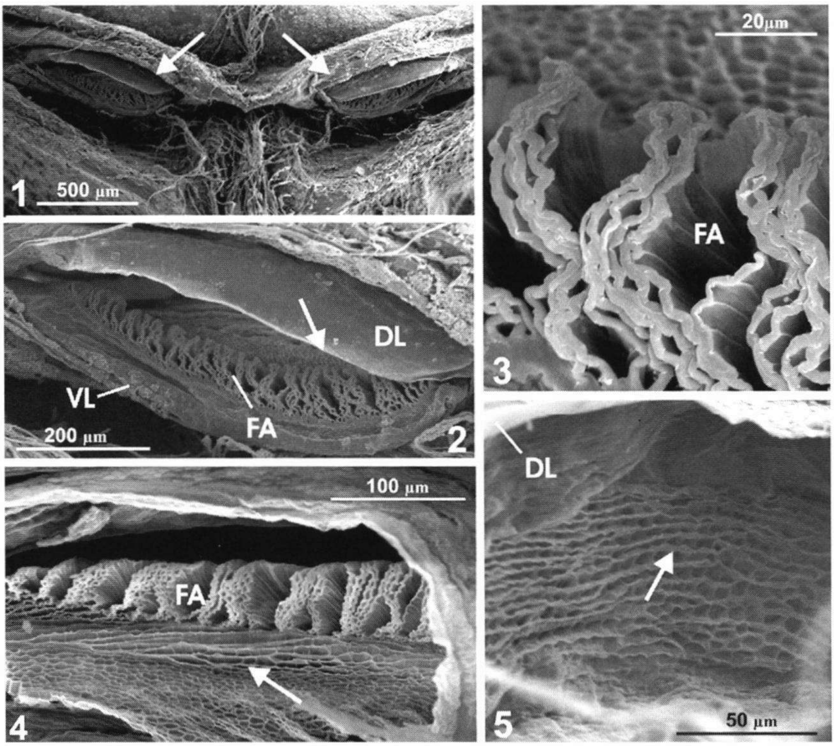
Larvae of *Libellula depressa*, attributed to the ultimate stage (F-0) on the base of their head width and body length (DI GIOVANNI et al., 2000), were collected in ponds in central Italy (Perugia, Umbria). In the laboratory, the specimens were kept in plastic containers (60×40×40 cm) with water, detritus, flora and fauna from the collecting site. The larvae were fed ad libitum with plankton (*Daphnia* sp. and *Cyclops* sp.).

For electron microscopy, the larvae were dissected under a stereomicroscope, after having cut off the head to reduce stress, and placed into 2.5% glutaraldehyde in cacodilate buffer (pH, 7.2) for five hours. Afterwards, the material was rinsed in the same buffer and post-fixed in 1% cacodilate buffered osmium tetroxide for 30 minutes. For scanning electron microscopy (SEM), the material was dehydrated in a crescent ethanol series up to 100%, critical-point dried in a CPD 030 Bal-Tec. Specimens were then mounted on stubs with silver conducting paint, sputter-coated with gold-palladium in an Emitech K550X sputter, and observed with a Philips EM 515 SEM. For transmission electron microscopy (TEM), dehydrated specimens were infiltrated with an Epon-Araldite mixture. Ultrathin sections were cut with a diamond knife on a Leica EM UC6 ultramicrotome, collected on formvar-coated copper grids, stained with uranyl-acetate and lead citrate, and observed in a Philips EM 208 TEM.

RESULTS

In the last stage (F-0) of *Libellula depressa* larvae, two paired spiracles, in the form of elongated eye-shaped structures, are located in the anterior region of the mesothorax segment (Fig. 1). Each spiracle measures about 800 μm in length. The view from the exterior shows that each spiracle consists of a well developed cuticular peritreme with a dorsal-anterior lip and a ventral-posterior lip (Figs 1-2). Along its external border, the ventral lip bears a filter apparatus while the dorsal lip bears a thin laminar coat (Fig. 2). The filter apparatus consists of a series of long folds, emerging from the cuticular peritreme. The folds form discrete groups where several folds are in contact. The overall effect is a honeycomb-like surface (Figs 3-4). Moving inwards towards the atrium, its walls appear completely covered with short folds that show an irregular honeycomb-like pattern both on the ventral (Fig. 4) and on the dorsal surface (Fig. 5).

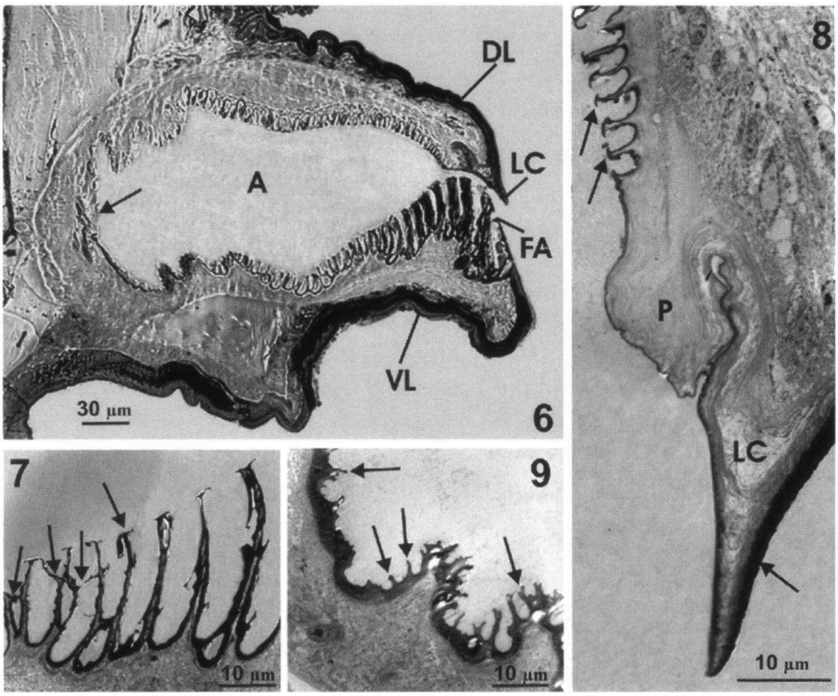
The atrium measures about 100 µm in high and about 250 µm in depth (Fig. 6).



Figs 1-5. Scanning electron micrographs of the mesothoracic spiracles of the last larval instar (F-0) of *Libellula depressa*: (1) two mesothoracic spiracles (arrows) in the form of elongated eye-shaped structures; – (2) detail of the left spiracle showing the dorsal-anterior lip (DL), bearing a thin laminar coat (arrow), and the ventral-posterior lip (VL) with the filter apparatus (FA); – (3) detail of the filter apparatus (FA) deriving from a series of folds forming discrete groups (where each fold in the group adheres to the adjacent ones) to delimit empty spaces producing a honeycomb-like surface; – (4) view of a spiracle from the inside looking outwards (the bottom of the spiracle has been removed). Note the sequence of folds forming the filter apparatus (FA). The ventral wall of the atrium shows an irregular honeycomb-like structure (arrow); – (5) detail of the upper wall of the atrium consisting of short folds with a similar, irregular, honeycomb-like pattern (arrow); DL, dorsal lip.

On its ventral surface the folds delimiting the walls become progressively shorter from the filter apparatus to the inner end of the atrium, where they are hardly visible. The laminar coat fits over the edge of the filter apparatus (Fig. 6).

Thin sections, observed under the TEM, reveal that all the atrium is bordered by a thin electron-dense cuticle (Figs 7-9). The folds of the filter apparatus are 30-50 µm high. Their inner core consists of a fibrous component covered by thin cuticle which expands at the apex to produce contact points linking the folds to one another (Fig. 7). The dorsal lip bears a thin laminar coat bordered by cuticle, which is thicker on the external surface, and includes a fibrous region interposed



Figs 6-9. A semithin section under the light microscope (6) and transmission electron micrographs (Figs 7-9) of the mesothoracic spiracles of the last larval instar (F-0) of *Libellula depressa*: (6) section of a spiracle showing the atrium (A), the ventral surface of which has folds that become progressively shorter from the filter apparatus (FA) to its inner limit (arrow), where they are hardly visible. Note that the laminar coat (LC) on the dorsal lip (DL) fits over the edge of the filter apparatus (FA) on the ventral lip (VL); – (7) detail of the folds of the filter apparatus with their inner core consisting of a fibrous material covered by thin cuticle which expands to produce contact points linking the folds to one another (arrows); – (8) detail of the thin laminar coat (LC) of the dorsal lip bordered by cuticle which is thicker on the external surface (arrow). The fibrous material interposed between the two cuticular layers also fills a protrusion (P) at the base of the laminar coat. Double arrows point out short folds on the upper wall of the atrium; – (9) details of the ventral surface of the atrium showing that the walls of its inner limit are decorated by very short cuticular folds (arrows).

between the two cuticular layers (Fig. 8). A fibrous region also fills a protrusion (P) at the base of the laminar coat, between the latter and the first folds (3-5 µm high) of the atrium (Fig. 8). The ventral surface of the atrium, in its inner limit, shows walls decorated by very short cuticular folds, irregularly expanded at their apices (Fig. 9).

DISCUSSION

The two mesothoracic spiracles of Anisoptera larvae seem to be open only in the final stadium, even if they are also present in earlier developmental stages (CORBET, 1999). According to CORBET (1999), larvae of many species of Anisoptera, in their final stadium, leave the water partly or wholly and intermittently or irrevocably, days or even weeks before emergence. This behaviour may reflect the need to expose the thoracic spiracles to the air. The larvae of pond-dwelling Anisoptera commonly congregate at the pond margin with their spiracles open as metamorphosis proceeds (CORBET, 1999). In particular, the larvae of *Libellula depressa*, close to the final moult, tend to spend time in such locations, sometimes returning into the water (personal observation).

The fine structure of the spiracles of the final larval stadium of *L. depressa* is coherent with the need to avoid water entering when the larva is submerged. Indeed, each spiracle has a dorsal laminar lip fitting over the filter apparatus with a fibrous protrusion on its base on the internal side. This protrusion could help to seal the lip to the edge of the filter. The honeycomb-like surface of the filter and of the atrium walls may function as a hydrofuge structure, able to resist water entry into the tracheal system. A honeycomb-like structure with this function has been described in the spiracles of *Periplaneta americana* (MILLER, 1981; MILL, 1998a).

In Anisoptera larvae the main respiratory organ is represented by the rectal epithelium (MILL,1998b). In fact, by comparing various structural models for gas exchange in the internal gills of the anisopteran *Aeshna cyanea*, KOHNERT et al. (2004) concluded that rectal gills alone can satisfy the metabolic demand of this active carnivorous insect. In addition, CORBET (1999) reports that larval Anisoptera, with their thick integument, probably rely almost entirely on their rectal gills, except perhaps in very early stadia, when the integument is relatively unsclerotized and transpiration *via* the cuticle is possible.

Previous investigations on the larvae of *L. depressa*, belonging to the final stadium, gave evidence that these insects are able to survive for several days out of water, if kept in air with high relative humidity values (PIERSANTI et al., 2007; REBORA et al., 2007). In such conditions we can hypothesize that the larvae could take oxygen from the air through their rectal epitelium. Indeed, as reported by CORBET (1999), terrestrial or semiterrestrial larvae commonly employ rectal respiration and larvae of aquatic species, in response to low oxygen concentration, climb backward to the water surface and place their anal pyramid in the air (see review in CORBET, 1999).

Even though during the experiments with *L. depressa* larvae it was not detected if the thoracic spiracles were involved in oxygen uptake, we cannot exclude a possible role of these structures in gas exchange. An involvement of the spiracles in dragonfly larval breathing has never been shown, nevertheless it has been hypothesized in some primitive semiterrestrial species, like *Uropetala carovei* (Petaluridae) (GREEN, 1977). In addition, the final larval stadium of the living fossil anisozygopteran *Epiophlebia superstes* leaves the water four or five months before moulting and, during this period, gas exchange probably occurs through the mesothoracic tracheal spiracles (see review in WICHARD et al., 2002).

The present work represents a first approach to the fine morphology of the spiracles in larval anisopteran dragonflies and gives an essential base for physiological studies of the role that these structures may perform during development.

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