

ANATOMICAL AND HISTOLOGICAL OBSERVATIONS ON THE TRACHEAL GILL OF PALINGENIA LONGICAUDA OLIV. (EPHEMEROPTERA)

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The respiratory organ of the Ephemeroptera larvae, which is suitable for the respiratory metabolism of the aquatic animal, consists of tracheal trunks branching richly in the body, and of branchial lamellae arranged on both sides of the abdomen.

The anatomical observations previously reported on the respiratory apparatus of the Ephemeroptera extended to the study of both sections (LANG, 1913; SCHOENEMUND, 1929; LANDA, 1948). In addition to the macroscopic descriptions there are also important papers seeking phylogenetic correlations between the individual groups of the Ephemeroptera on the basis of the development and state of the respiratory organ and other systems (nervous system, Malpighian tubules) (LANDA, 1948; LANDA, 1969).

The paired branchial lamellae, which are situated on the 1st–6th abdominal segments, are characteristic generic features.

The present paper deals with the structures of the tracheal gill of a single species, *Palingenia longicauda* OLIV., with particular regard to the electron-microscopic structure.

Materials and Methods

May-fly larvae were collected from the Szeged reaches of the rivers Tisza and Maros. After collection the material was fixed appropriately for light and electron-microscope studies.

After Bouin, Carnoy and formalin fixation, sections either stained with haematein-eosin or impregnated with silver were prepared for light microscope histological observations. For the electron-microscope studies, excised tracheal branchial lamellae or their filaments were treated with 2% glutaraldehyde adjusted to pH 7.6 with 0.15 M phosphate buffer, and then for 2 hours with Palade fixing solution (1% OsO₄ in veronal acetate buffer (Pease, 1964), and embedded in Durcupan ACM (Araldite) resin after dehydration in an ascending alcohol series. The material was checked by examination of semi-thin sections, and the positions of the fine sections determined. The thin sections were contrasted with a lead citrate solution prepared according to REYNOLDS (1963). Photographs were prepared on Tesla BS 242 D and JEM 100 B electron-microscopes.

Abbreviations

F	= filament	M	= mitochondrion
Ct	= connective tissue	Go	= Golgi apparatus
cf	= interstitial bundles	N	= nucleus
tr	= trachea	GER	= granulated endoplasmatic reticulum
K	= chitin	r	= ribosome

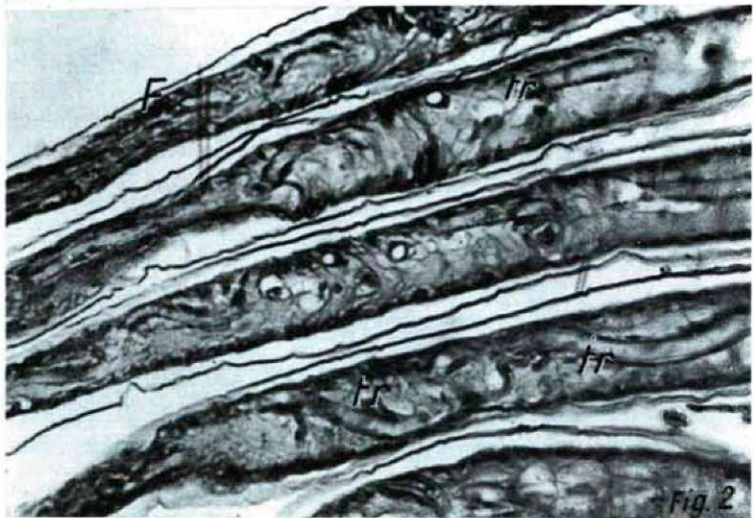


Fig. 1. Longitudinal section of the large tracheal gill.

In the lamella, interstitial cells, interstitial fibre-bundles and small tracheal branches are visible, which enter the filaments. The surface of the filaments is covered with homogeneous material (arrows). x 380

Fig. 2. Longitudinal section of filaments. Cross- and longitudinal sections of the tracheae can be seen inside the filaments. x 340

Results and discussion

Anatomy of tracheal branchia

The members of the paired branchial lamellae of the mayfly larvae are of different shapes and sizes (LANG, 1913; SCHOENEMUND, 1929; ÚJHELYI, 1959). The smaller are more rounded and nearer to the dorsal side of the abdomen than the longer, larger ones. Ventrally from them is a further densely piliated chitinous lamella, but this does not participate in the gaseous exchange.

The tracheal gill of *Palingenia longicauda* OLIV. are structured, but this structuring does not result in a symmetric arrangement on the two sides, for the filaments in the structuring are thinner on the side farther from the surface of the body and follow each other densely in a double line, in contrast with the opposite side, where the filaments are larger and arranged in a single row.

Along the entire length of the centre of every branchial lamella runs a thick tracheal branch, which originates from the visceral tracheal trunk in the body of the larva.

This main branch divides into thinner and thinner branches, and thus the entire branchial lamellar area is covered by a network of tracheae of various diameters resulting from the gradual ramifications. The main tracheal trunk can readily be seen with the naked eye to be accompanied by a pigment layer, the thickness of which is not uniform in the region of the two lamellae. The pigment layer is thicker and denser for the larger lamella than for the smaller one. The amount of the pigment layer progressively decreases towards the tracheal gills tips, and finally disappears completely. Besides this, varying numbers of sensillae can be observed on the tracheal gills, and as described by EASTHAM (1936) for *Caenis macrura* and *Caenis boraria* they also differ in form.

Histological structure of the tracheal gill

The branchial lamellae and the filaments are covered from outside by epithelial cells arranged closely beside each other (Figs. 1-2), one of the wide bases of which becomes progressively narrower towards the inside, while at the same time the neighbouring cells appear as triangles of reversed position, since they proceed with a narrow base (Fig. 3). Such an arrangement can be particularly well seen on the epithelial cells in the region of the filaments (WICHARD et al., 1971). In addition to this, the very intense interdigitation formed with the neighbouring cells is also characteristic. The membranes of the interdigitating surfaces are frequently uniformly thickened on both sides (Fig. 5).

On the basis of their own data and also literature results on invertebrates, SATIR et al. (1970) consider the septate junction or the septate desmosome and the zonula adherens to be the characteristic forms of the epithelial junction structures. They note that there are differences, and that the tight junction too occurs for instance. This was described by LOCKE (1965) in the epidermis of insects, and by SMITH et al. (1969) in epithelial cells of the insect midgut epithel and in a certain form of the campaniform sensillae. It is striking that similar, but shorter, thickened membrane sections are found on the surface of

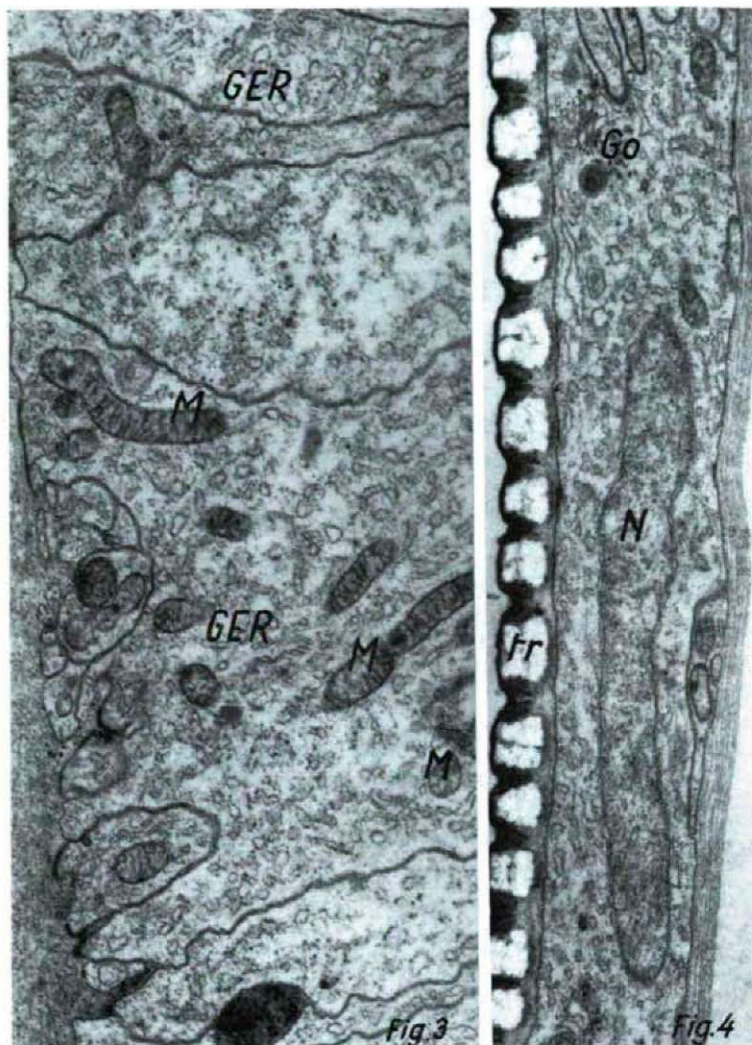


Fig. 3. Electron-microscope photograph of epithelial cells. x 19 000

Fig. 4. Longitudinal section of thin filament. Basal membrane with lamellar structure can be seen on the outside surface of the epithelial cells. Sections of the chitin rings are visible on the left side of the picture. x 12 000

the epithelial cells adjacent to the basal membrane which will be discussed below (Fig. 7). These may correspond structurally to the hemidesmosomes described by BLOOM and FAWCETT (1970). The basal membrane does not take part itself in the thickening. Desmosomes can be observed only rarely (FAWCETT, 1966) (Fig. 6).

The surface of the cells is surrounded by a homogeneous layer which can also be studied by light microscope (Fig. 1), and on this interlacing (particularly in the region of the filaments) (Figs. 1-2) or breaks (at the sensillae)

can be observed in places. The electron-microscope reveals that this homogeneous substance is composed of lamellae 80–100 Å thick, and depending on the thickness of the filaments the number of lamellae layered one on the other ranges from 2–3 to 20–25 (Fig. 4). This basal membrane also surrounds nerve bundles running on the periphery (on the outer edge of the epithelial cells) (GUPTA et al., 1969).

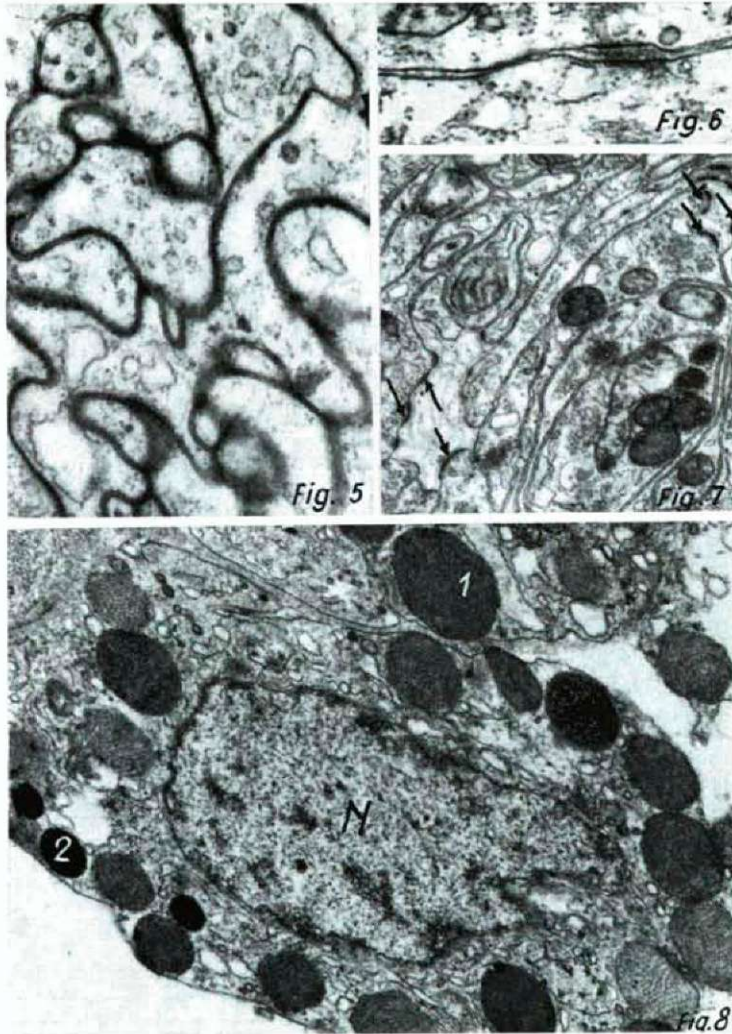


Fig. 5. Closely connected section of epithelial cells. x 31 000

Fig. 6. Desmosome between two epithelial cells. x 52 000

Fig. 7. Hemidesmosomes between the epithelial cells and the basal membrane (arrows). x 192 000

Fig. 8. Haemocytes with granules of type 1 and 2. x 16 000

The nucleus of the epithelial cells is large, and is situated approximately at the centre. A few organelles can be observed in the cytoplasm bordering the nucleus. In the entire region of the cells are found the short channels of the granulated endoplasmatic reticulum, with more rarely a parallel-running network of channels and a smooth-surfaced endoplasmic reticulum. The Golgi apparatus is slight in extent (Fig. 4), and in general dense material can be seen in the vesicles; the mitochondria are cristate type. Besides these organelles in the cytoplasm a large amount of free ribosome can also be observed. On the sections prepared from the thin filaments only epithelial cells can be seen, and between them tracheal cross- and longitudinal-sections. This structure exhibits a large degree of agreement with the structure of the branchia of *Glypbataelius pellucidus* RETZ. (Insecta, Trichoptera) (WICHARD et al., 1971).

If the histological structure of the tracheal gill itself is examined, then connective tissue can be observed below the epithelial cells covering the lower and upper surfaces; this is characteristic of an extremely loose structure, containing few cellular elements and relatively more interstitial fibres. The fibres are arranged in small bundles, some of which frequently run from the larger tracheal branches to the base of the filaments (Fig. 1).

Between the interstitial cells and fibres haemocytes are frequently to be found; these are recognizable by their typical organelles. It is characteristic of these cells that their nuclei are elliptical, while in the cytoplasm of the cell bodies of variable density can be observed, the structures of some of which can be clearly recognized whereas others appear homogeneous (Fig. 8). These special organelles can be classified according to their structure in types 1 and 2 of the haemocyte granules as systematized by SCHARRER (1972).

Summary

Anatomical and histological observations on the tracheal gill of *Palingenia longicauda* OLIV. are reported. The studies deal with the structures of the branchial lamella and the filaments. The connective structures of the epithelial cells and their electron-microscope structures are reported.

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