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Study of cement apparatus, cement production and transportation in adult male *Neoechinorhynchus rutili* (Acanthocephala, *Eoacanthocephala*)

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**Abstract** Investigation with light and electron microscopy has been made of the cement apparatus of the mature male *Neoechinorhynchus rutili*, a parasite of *Gasterosteus aculeatus*. *N. rutili* possesses a single compact mass of glandular tissue (cement gland) with eight giant nuclei. The nucleus is lobe-shaped and has a conspicuous nucleolus with a granular appearance. The cytoplasm of the gland contains prominent rough endoplasmic reticulum, mitochondria and electron dense secretory granules. A short duct connects the gland with a single, separate cement reservoir. The duct appears to lack a lumen and is seen as an electron-dense, compact structure. Many microtubules of approximately 150 nm outer diameter are located throughout the cytoplasm of the duct. It is proposed that the microtubules in *N. rutili* are responsible for the transport of cement granules from the gland to the reservoir. The cement reservoir appears as a compact organ filled with granules measuring at least 0.5 μm in diameter.

**Introduction**

Cement glands and their products have considerable importance in the reproductive process; at the same time they are important elements in both the morphology and the taxonomy of acanthocephalans (Van Cleave 1949; Yamaguti 1963).

According to Meyer (1933), the cement glands of male acanthocephalans are of the exocrine type. Secretion from the cement glands seals the female gonopore with a cap after copulation and so prevents both the loss of sperm and subsequent insemination for some time (Abele and Gilchrist 1977; Dezfuli et al. 1999). Based on staining properties and the occurrence of well developed, rich, rough endoplasmic reticulum within the cytoplasm of the cement gland, protein is now recognised as a component of cement (Haley and Bullock 1952; Parshad and Crompton 1981; Dezfuli et al. 1998).

In a detailed description of the cement apparatus of *Neoechinorhynchus emydides* (Leidy, 1851; Eoacanthocephala), Van Cleave (1949) did not refer to the presence of a membrane between the gland and cement reservoir, nor did he explain how the glandular secretions moved from the gland to the reservoir. Recent work with other organisms has shown that microtubules are often involved in cytoplasmic streaming (Lye et al. 1987; Glikman and Salmon 1993; Theurkauf and Hazelringg 1998). It is not unreasonable to suppose that microtubular activity occurs in the cement apparatus of *N. rutili*. The major aims of this study were to describe for the first time: (1) the ultrastructure of the cement apparatus of *N. rutili* and (2) the mode of transportation of the cement from the cement gland to the reservoir through a duct lacking an obvious lumen.

**Materials and methods**

Specimens of *Neoechinorhynchus rutili* (Müller, 1780), were obtained from sticklebacks, *Gasterosteus aculeatus* (Linnaeus, 1758), ranging in length over 3.5–5.4 cm and sampled over several occasions by electrofishing in the Ceresina stream, north of Padua, Italy. For transmission electron microscopy, the entire male genital apparatus from each of ten *N. rutili* was removed and prepared as follows. Specimens were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide in the same buffer for 2 h, dehydrated in graded ethanol, transferred to propylene oxide and embedded in an Epon-Araldite mixture. Semi-thin sections were cut on a Reichert Om U2 ultramicrotome with glass knives and stained with azure-A methylene blue. Ultra-thin sections were stained in a 50% alcohol uranyl acetate solution and lead citrate. They were then observed with a Hitachi H-800 electron microscope.

**Results**

The typical cement gland found in the Acanthocephala is the simple, undivided syncytial gland which is char-
acteristic of all species of the Eoacanthocephala (Figs. 1, 2). In *Neoechinorhynchus rutili*, the boundary of the cement gland is a membrane formed by a material of fibrous nature and appears as an extension of the posterior region of the ligament sac (Figs. 1, 3B). The gland has its own envelope, which is about 0.2 μm thick (Fig. 3B). Beneath this envelope is a single compacted mass of glandular material, provided with a relatively small number of giant nuclei (Figs. 2, 3A). No membranes have been observed to separate the mass into follicles. Eight is the characteristic number of nuclei, each having a diameter of 40–75 μm. Each nucleus is lobe-shaped with an irregular outline and a conspicuous nucleolus of granular appearance (Figs. 2, 3A). In the peripheral portion of the gland, the occurrence of translucent vesicles is commonly observed (Fig. 3B). Within the gland, mitochondria with lamellar cristae can be observed (Fig. 3B, C). One feature of the *N. rutili* gland is that the cytoplasm is filled with a high number of free ribosomes as well as a prominent rough endoplasmic reticulum which is frequently in close relationship with secretory granules (Fig. 3C).

At higher magnification, a secretory granule appears as an electron-dense granule and presents a narrow outer granular area enclosing a dense, amorphous component (Fig. 3B). Granule diameters range over 0.5–2 μm and are observed within the cytoplasm of the gland together with microtubules (Fig. 2, near open arrows).

![Fig. 1 Semi-thin section in longitudinal view of posterior end of a male *Neoechinorhynchus rutili*. The undivided syncytial gland (asterisk) is enclosed in the ligament sac (arrow). A short duct (open arrow) connects the gland to the cement reservoir (cr)](image)

A cement duct arises from one edge of the basal part of the gland (Figs. 1, 2, 3D) and leads to the cement reservoir. In the connection between the gland and the duct (Fig. 3D), cement granules appear to be much more numerous, in comparison with the rest of gland (Fig. 3A). The duct is found to be compact and electron-dense in appearance with its apical part being about 26 μm and its basal portion about 10 μm (Fig. 2). The basal portion is in direct contact with the reservoir. Within the duct, longitudinal rows or chains of cement granules are observed (Figs. 2, 3D), as are groups consisting of a few granules (Figs. 2, 4A, B), together with single granules. The cement duct includes many microtubules which are generally oriented parallel to the long axis of the duct (Fig. 4A, B). Each microtubule has a total diameter of about 150 nm, with an inside diameter of approximately 100 nm and two membranous walls, each 25 nm thick (Fig. 4B). It seems that these microtubules may determine the pathway for the transport of cement granules along the axis of the duct (Fig. 4A, B). In fact, in many instances at a point along the duct, microtubules have been noticed around granules and so appear to facilitate the movement of secretory granules towards the reservoir (Fig. 4B). Within the duct, the presence of narrow empty channels is observed; these spaces are assumed to represent sites previously occupied by granules (Figs. 2, 3D).

The next most distinctive feature of the cement apparatus in *N. rutili* is the presence of a single, separate cement reservoir, external to the gland, for the storage of the secretory products (Figs. 1, 2). The cement reservoir is similar in structure to both the gland and the duct, but it has a thicker wall of about 2 μm (Fig. 4C), which appears to be formed from muscle fibres. The apical part of the reservoir lies within a slight concavity of the gland (Figs. 1, 2), ensuring that the reservoir’s envelope and the basal part of the gland are often in intimate contact (Fig. 4C). Within the cement reservoir, many electron-dense granules, with diameters ranging over 0.5–2 μm, are visible (Fig. 4D, E). In the central part of the reservoir, some empty spaces (Figs. 2, 4D), degenerated mitochondria (Fig. 4E), rarely intact ones and a few microtubules are seen among the secretory granules.

**Discussion**

The cement glands of representatives of the Acanthocephala vary in number, shape and structural features; and so they have become important elements in the systematics of genera and higher taxons (Meyer 1933; Van Cleave 1949). According to Amin and Redlin (1980), the cement gland pattern remains of crucial significance in the diagnostic characteristics of some genera, e.g. *Echinorhynchus* (Müller, 1776). According to Van Cleave (1949), the cement apparatus of the Eoacanthocephala is the most primitive and, by recognising its progressive modification, the glands of the Archiacanthocephala and the Palaeeacanthocephala respectively are derivable.