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EVIDENCE OF TUBULIN IN THE SCOLEX GLAND DUCTS OF *GYMNORHYNCHUS GIGAS* PLEROCERCOID (CESTODA: TRYPANORHYNCHA)

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The presence of the microtubules in the ducts of scolex gland cells of cestodes has been reported by numerous researchers (e.g. Öhman-James C. 1973: Z. Parasitenkd. 42: 77-86; Richards K.S., Arme C. 1981: Parasitology 83: 477-487; Kuperman B.I., Davidov V.G. 1982: Int. J. Parasitol. 12: 285-293; Richards K.S., Arme C. 1983: Parasitology 86: 83-88; McCullough J.S., Fairweather I. 1989: Parasitol. Res. 75: 575-582; Žd'árská Z., Nebesářová J. 1997: Folia Parasitol. 44: 139-146; Žd'árská Z., Nebesářová J. 1999: Parasite 6: 49-56).

The existence of peripheral system of microtubules in the ducts is a general feature of many gland cell types and it is thought to be involved in the transport of secretory granules towards the distal cytoplasm (Richards and Arme 1981, op. cit.; McCullough and Fairweather 1989, op. cit.). However, up to this date, the presence of microtubules has been detected by ultrastructural observations only, and the lack of immunocytochemical studies does not allow to ascertain whether they are microtubules or contractile microfilaments. In the present study we investigated the presence of tubulin (which is the basic structural protein of microtubules), in the duct wall of secretory glands in the scolex of *Gymnorhynchus gigas* (Cuvier, 1817). The mentioned task was carried out by relying on immunocytochemical analysis with monoclonal anti-α and anti-β tubulin antibodies as tubulin's markers.

Plerocercoids of *G. gigas* were removed from muscles of *Brama raii* (Bloch, 1791) captured in the Strait of Gibraltar close to the Tarifa coast, Spain. The blastocysts containing the scolices were carefully extracted from surrounding host tissue and washed in a physiological solution before fixation. In order to study the samples under transmission electron microscope (TEM), the original scolices were first processed according to the technique elaborated by Perez-Serrano J., Casado N., Denegri G., Rodriguez-Caabeiro F. 1994: Int. J. Parasitol. 24: 219-224, and an M10 TEM at 60 kV was used. Prior to the immunocytochemical study, the scolices were fixed in a mixture of glutaraldehyde (0.3%) and paraformaldehyde (4%) in cacodylate buffer for 6 h at 4ºC, then washed twice (15 min each) at 4ºC in a cacodylate buffer containing sucrose (5%), dehydrated in a graded ethanol series, then infiltrated in an intermediate solution of LR White resin and 100% ethanol (1 : 1) for 1 h, and embedded in fresh LR White resin overnight at 4ºC. After a few minute's contact with fresh pure resin at room temperature, a final change was made, and then the resin was polymerised in gelatin capsules at 50ºC for 24 h. Ultrathin sections were incubated in TBG (30 mM Tris, 150 mM NaCl, pH 8.2 plus 0.1% BSA and 1% gelatin) containing 5% goat serum, in order to block non-specific binding, and then they were transferred to a drop of the mouse monoclonal anti-tubulin at optimal dilution (1 : 200 anti-α tubulin plus 1 : 100 anti-β tubulin, both from Sigma Immuno Chemicals, in TBG) for 120 min. After final wash, the sections were incubated with 10-nm gold-labelled anti-mouse IgG from Electron Microscopy Sciences (dilution 1 : 50 in TBG). Similar immunostaining protocol was observed when using other primary antibody (anti-actin; dilution 1 : 100 in TBG) in order to detect existing contractile microfilaments.

The ultrastructural study showed that the secretory cells gave rise to ducts that penetrated the distal cytoplasm and contained granules, which were often accumulated in reservoirs (Fig. 1). These ducts seemed to be strengthened by a system of longitudinally oriented microtubules or microfilaments and opened through the tegument (neodermis) without any projections. They discharged their secretion into the environment by an eccrine secretion mechanism (Fig. 2), similar to that suggested for the plerocercoid and adult of *Diphyllobothrium* spp. (Kuperman and Davidov 1982, op. cit.), the scolex glands of *Caryophyllaeus laticeps* (Richards and Arme 1981, op. cit.), the frontal glands of the metacestode and juvenile forms of *Tentacularia coryphaenae* (Farooqi H.U. 1986: Z. Parasitenkd. 72: 653-659), as well as the glandular cells of *Trilocularia acanthiaevulgaris* (McCullough and Fairweather 1989, op. cit.). The discharge did not involve destruction of the duct, since some microtubules or microfilaments from the duct wall were positively identified after consecutive discharge (Fig. 3).

Positive staining of tubulin with immunogold method was observed in peripheral structures of the ducts (Fig. 4), which confirmed the presence of tubulin and therefore helped identifying microtubules. The absence of contractile microfilaments was verified by the negative actin immunostaining observed in the ducts. However, in other sections incubated with anti-actin, positive immunostaining was detected in tegumental and parenchymal muscles. No gold labelling was detected in sections where mouse serum was used or in sections where the primary antiserum was omitted from the labelling protocol. The specificity of the immunostaining was confirmed by the positive tubulin staining of microtubules in the bundle of cilia of the flame cells (Fig. 5) that were surrounded by the negatively immunostaining membranous cytoplasmic extensions. The contractile microfilaments in the tegumental and parenchymal muscles did not show traces of immunostaining with anti-tubulin, nor did other parenchymal structures of the scolex. The present study is the first confirmation of the presence of tubulin, and therefore microtubules, in the ducts that transport secretory granules from glandular cells in a cestode, namely, plerocercoid of *G. gigas*, by means of an immunocytochemical technique, never used before in such type of studies.

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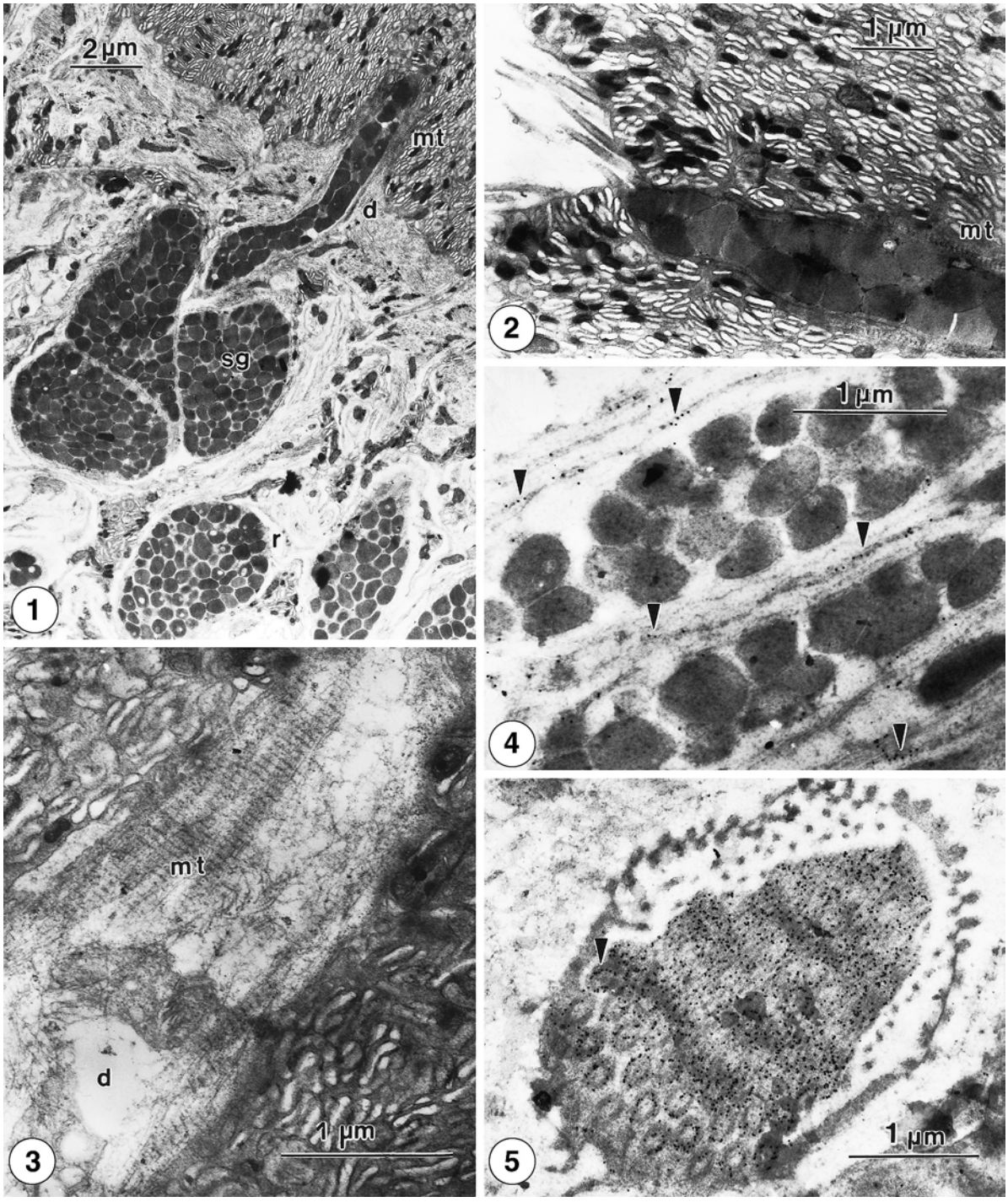

Figs. 1-3. Transmission electron micrographs of scolex glands of *Gymnorhynchus gigas* plerocercoid. **Fig. 1.** Duct penetrating the tegument and several reservoirs containing secretory granules. **Fig. 2.** Discharge of the secretory material from the distal cytoplasm by eccrine secretion. **Fig. 3.** Microtubules in the wall of an empty duct. **Figs. 4, 5.** Immunocytochemical localisation of tubulin in scolex of *Gymnorhynchus gigas*. **Fig. 4.** Gold-labelled (▼) tubulin in gland duct walls. **Fig. 5.** Gold-labelled (▼) tubulin in flame cell cilia (positive control). d – duct; mt – microtubules; r – reservoir; sg – secretory granules.

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