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## Light and ultrastructure modifying the endocrine character of *Moniezia expansa* (Rud., 1805, Cestoda Cyclophyllidea) interproglottidal gland

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### Abstract

*Moniezia expansa* could be considered as the most important cestode parasites infecting sheep causing monieziasis which constitutes a problem in sheep breeding. The aim of the present study is to investigate on the anatomical cellular and tissue structure and development of the interproglottidal gland and tegument correspondig to the mechanism of its secretion. The detailed structures of the tegument and microtriches could not be seen by light microscopy examination and also the interproglottidal glands appear as interrupted non differential cellular chain in between worm proglottides or segments. The ultrastructure study done by transmission electron microscopy (TEM) revealed that the glands develop separately from the tegument and compromising an endocrine character. It was observed that the gland consists of subglobular follicles or vesicles, which are embedded in parenchyma and pushing the tegument at the posterior border of the proglottid, formulated crypt-like structures with central hilum or sinus. The gland secretions were diffused to the tegument and parenchyma of the proglottid through cellular channels (protoplasmic channels) upward to the anucleate syncytial layer of tegument; apical cytoplasm through cytoplasmic channels.

**Keywords:** *Moniezia expansa*, Interproglottidal gland, Endocrine character, Light and Ultrastructure

### 1. Introduction

Significant advances have been made towards understanding anatomical and cellular structures in cestodes. Many studies on the morphological and structural features of cestodes, having a particular relevance to taxonomy, phylogeny, physiology and development of the worm have been accumulated <sup>[1,2]</sup>. By light microscopy the glands arrange as a round small separate pits in a one row at the posterior border of each proglottid <sup>[3]</sup>. The genus *Moniezia* is also unique in possessing interproglottidal glands in the parenchyma along the posterior edge of each proglottid. The interproglottidal glands were examined by the light and electron microscopy and considered as a specialized portion of the tegument and developed separately and have an alkaline phosphatase activity in the cytoplasm of the gland especially in their protoplasmic connection with the distal tegument <sup>[4,5]</sup>.

The glands are first developed by infolding of the surface tegument and their subjacent cell bodies into the parenchyma along the posterior margin of each immature proglottid. As development proceeds, new crypt-like infoldings of tegument are formed and the older ones separate from the tegument to become rosette-like glandular structure<sup>[6]</sup>. Under TEM, different kinds of secretory granules can be seen in the cytoplasm of the glandular cell bodies and in connecting glandular tegument. Each glandular complex is syncytial, glandular cell bodies surrounds and open into an elliptical spherical or irregular-shaped tegumental cavity<sup>[7]</sup>. The syncytial tegument consists of the uninucleate fusiform tegumentary cells on the epithelium of *M. expansa* and is connected to the apical cytoplasm through cytoplasmic processes<sup>[8, 9]</sup>. The tegumental microvilli or microtriches developed from the tegument, cover the whole external surface of the worm and consider, as a complex apical complex cytoskeleton structure modified for attachment of worms and receive tegumental and interproglottidal gland secretions<sup>[10, 11, 12]</sup>. TEM of *M. expansa* interproglottidal glands consist of multiple spherical or subspherical bodies of different sizes in parenchyma and having secretory vesicles<sup>[13]</sup>. The objective of the present study is to describe the cellular and tissues of the interproglottidal gland and tegument by light and electron microscopy signifying the endocrine pathway of its secretion.

## 2. Materials and methods

Mature specimens of *M. expansa* were collected from the small intestine of freshly slaughtered lambs at Cairo abattoir, Egypt. Worms were placed in warm 0.9% physiological saline and maintained at 35-37 °C in a vacuum flask for transport to the laboratory. In the laboratory, worms were subjected to whole mount and histological preparation of *M. expansa* mature proglottides or segments for light microscopy and also ultrastructure studies by the transmission electron microscopy.

### 2.1 Whole mount and stained *M. expansa* mature proglottides

The mature specimens were washed with distilled water and compressed between two slides and fixed in 10 % formaldehyde for 24 h. and then through washing with distilled water for removing excess formalin. The specimen was stained with acetic acid alum carmine and then mounted on glass slide with canada balsam for examination by light microscopy<sup>[14]</sup>.

### 2.2 Histological preparation of *M. expansa* mature proglottides:

The adult worms were sliced broadly into strips of 5 mm wide at mature segment locations before being fixed for three hours in 10% neutral formalin, alcohol-dehydrated and embedded in parafin wax. Serial sections (5µm thick) were cut using a Rotatory microtome. All sections were dewaxed in xylene, hydrated in serially-diluted alcohol and finally washed with distilled water. For light microscopy, hydrated sections were stained with haematoxylin for four min and eosin for two min. Stained sections were dehydrated in gradual alcohol series, cleared in xylene and mounted in canada balsam for comparative study by light microscopy.

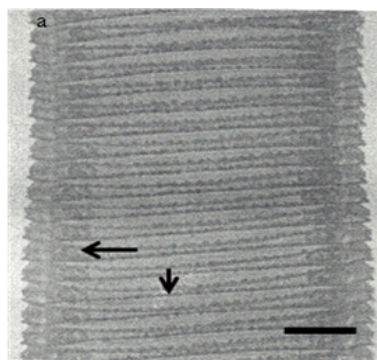
### 2.3 TEM of *M. expansa*:

The mature specimens of *M. expansa* segment were quickly cut into small (approximately 0.4 cm) pieces, fixed immediately in ice-cold 4% gluteraldehyde in 0.1 M cacodylate-HCl buffer containing 3% (WV) sucrose, pH 7.4, for 4-5 hrs, followed by two 0.5 hrs washes in cacodylate buffer and stored for 24 hrs in cacodylate buffer prior to post-fixation in 1% aqueous osmium tetroxide for 2 hrs. Specimens were then dehydrated through an ethanol series and orientated appropriately prior to embedment in Epon 812 resin. Ultrathin sections were cut and collected on uncoated 200-mesh copper grid, stained with alcoholic uranyl acetate (15mins) and aqueous lead citrate (8mins), and finally examined using a JEOL 100-cx electron microscope operated at 100 k.v<sup>[15]</sup>.

## 3. Results

### 3.1 Light microscopy observations of *M. expansa* tegument and interproglottidal glands

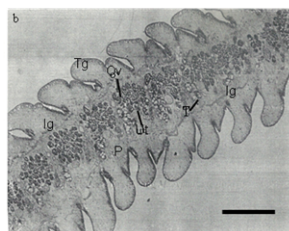
The morphological characteristics of *M. expansa* acetic acid alum carmine stained mature segment, broader than longer and shows two sets of genital organs; the ovaries and vitelline glands formed a ring on either side median to the longitudinal excretory canals. Each ovary is oval and located more laterally and the testes concentrate towards the sides. The interproglottidal glands arrange as small pits at the posterior border of each proglottide in a straight row (fig1).



**Fig 1:** Light microscopy of *M.expansa* mature proglottides; representing the morphological characteristics of each segment, appears broader than longer with two sets of genital organs; the ovaries and vitelline glands (long arrow).The interproglottidal glands arrange as small separate pits at the posterior border of each proglottide in a straight row(short arrow). Scale bar: 100 mm.

### 3.2 Histological structures of *M. expansa* mature proglottides

The *M. expansa* body wall shows a thick tegument (Tg) which is metabolically an active cytoplasm layer. Parenchyma (P) is of peculiar branching cells fill the whole space around the internal organs; ovoid testes (T) and semicircular lobulated ovary (Ov). Uterus (Ut) and longitudinal excretory canal (LEC) can be distinguished. The detailed structures of tegument with microtriches (Mi) could not be seen. The interproglottidal glands (Ig) appear as interrupted non differential cellular chain in between worm segments (fig2&3).



**Fig.2**

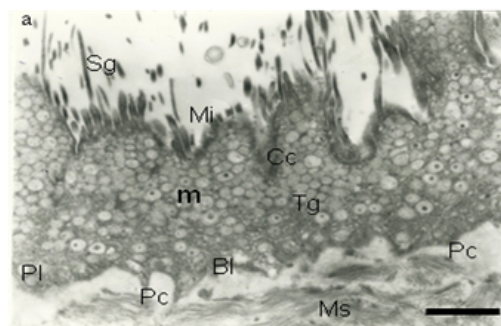


**Fig.3**

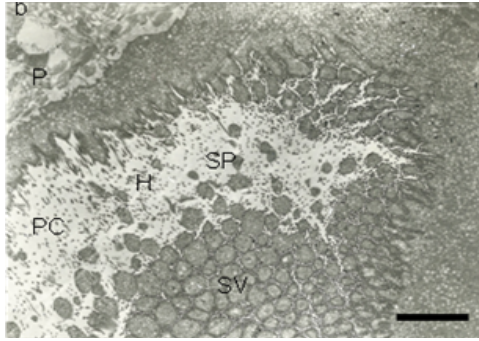
**Fig 2&3:** Light microscopy of *M.expansa* mature proglottides shows the histological structures of *M. expansa* mature proglottides with the following structures; thick tegument (Tg), Parenchyma (P), ovoid testes (T), lobulated ovary (Ov), Uterus (Ut) and longitudinal excretory canal (LEC). The interproglottidal glands (Ig) appear as interrupted non differential cellular chain in between worm segments. Scale bars : 150µm.

### 3.3 TEM observation of *M. expansa* tegument and interproglottidal glands

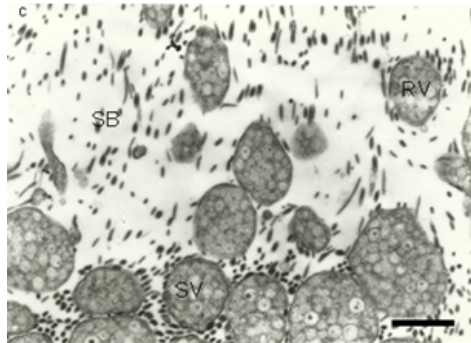
The apical syncytial cytoplasm of *M. expansa* tegument (Tg) is rich in mitochondria (m) and is covered with minute microvilli or microtriches (Mi) mixed with secretory granules (Sg). The plasma membrane is continuous with the apical syncytial cytoplasm; plasmalemma (Pl) and the anucleate syncytial layer of tegument is resting on the basal lamina (Bl) which in turn connected with perikarya and the parenchyma (P) through the protoplasmic channels or connections (pc) (Fig4). The TEM ultrastructure of the interproglottidal glands of *M. expansa* shows clusters embedded in the parenchyma along the posterior margin of each proglottid. These clusters make various semi-rounded follicles formulated crypt-like structures. The gland follicles or vesicles are globular, mostly filled with immature secretory cells and the mature ripened ones are fewer and passed towards the center of the glandular follicles; hilum (H) (Fig5). With the progress of maturation, the gland vesicles increase in size and take a sub-globular or ovoid shape and the outlined flat cells are stretched and separated to extrude crystals or secretions outside the vesicles. Each gland vesicle varies in morphology according to the stages of development; ripened vesicles (RV), secretory vesicles (SV) and secretory products (SP) at the center of the vesicles. Each follicle is provided with hilum or sinus (H) at the center of follicle for collection of gland secretions transferring it through the protoplasmic connections (pc) into the adjacent tegument and parenchyma (P) (Fig6). The gland secretions reach to the apical cytoplasm through the cytoplasmic channels (Cc) at the syncytial surface of the tegument.



**Fig 4:** TEM observation of *M. expansa* tegument; tegument (Tg) is rich in mitochondria (m) and is covered with minute microvilli or microtriches (Mi) mixed with secretory granules (Sg). plasmalemma (Pl) and the basal lamina (Bl) which in turn connected with perikarya and the parenchyma (P) through the protoplasmic channels or connections (pc). Scale bar: 500 µm.



**Fig 5:** TEM observation of the interproglottidal glands of *M. expansa* with low magnification; consists of multiple follicles embedded in parenchyma with secretory vesicles (SV), secretory products (SP) and hilum (H). The gland secretions were drained through the protoplasmic connections (pc) into adjacent tegument and parenchyma. Scale bar: 200  $\mu$ m.



**Fig 6:** TEM observation of the interproglottidal glands of *M. expansa* with higher magnification; shows ripened vesicles (RV) and secretory products (SP) liberated from ruptured ones. Scale bar: 500  $\mu$ m.

#### 4. Discussion

*Moniezia* is a unique member among cyclophyllidea in possessing interproglottidal glands along the posterior edge of each proglottid. These glands are distributed in follicles embedded in parenchyma. The present study revealed that the interproglottidal glands develop separately as one of the parenchymatous organs producing its specific secretion. Some authors reported that interproglottidal glands are specialized regions of tegumentary epithelium and they do not differentiate between the mechanism of tegument and gland secretions [15, 5]. At the same time, the present investigation concluded that secretions of both glands and tegument pass distally up to the syncytial tegument and finally to the surface microtriches. The present study described the interproglottidal glands as cells grouped in follicles and secretory vesicles embedded in parenchyma and neighboring tegument at the posterior margin of the mature proglottid. The secretions is

distributed from gland in parenchyma to tegument through the cellular arranged pathway or channels (protoplasmic connections) reach to the apical cytoplasm through the cytoplasmic channels at the syncytial surface of the tegument to microtriches in an endocrine character. Some researches deal with the ultrastructure of *M. expansa*, interproglottidal glands but did not add scientific data on the development and mechanism of the gland secretion pathway [13]. The present study described how the gland secretion pass to the parenchyma, tegument and microtriches through the protoplasmic connections of cells in an endocrine manner. In conclusion, the interproglottidal glands secretions and its distribution to the parenchymal, tegumental and microtriches structures in an endocrine character will help in detection of metabolic enzymes of worm and surface antigens for immunological studies.

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