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Nervous system in the monozoic cestode, *Djombangia penetrans* (Caryophyllidea) as revealed by nonspecific esterases and cholinesterase activity

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Abstract. The nervous system of *Djombangia penetrans* Bovien, 1926 was revealed in toto by staining for nonspecific esterases and cholinesterase. Five pairs of nerves radiated from a ring surrounding the sucker-like invagination at the scolex tip; of these, one pair was thicker than the others. Intense enzyme activity was evident at the points of origin of these nerves. Fine nerves ran transversely across the scolex, connecting various nerves and constituted a fine subsurface nerve net. At the scolex tip, these connective nerves had a concentric arrangement. Posteriad, one main thick nerve and three pairs of thin ones were present on either side of the median axis of the worm, terminating at the ovarian region; the 5th pair of nerves, terminated in the parenchyma near the scolex tip. Numerous connectives joined the various nerves, innervating the whole body of the worm. Throughout the length of the body fine nerves branched out from the outer longitudinals, extending towards the body surface and having a bulbous nerve cell at their tips. Like other cestodes and also the primitive platyhelminths, *D. penetrans* conforms to an orthogonal plan of nervous organization. The presence of a nerve ring in the scolex region appears to represent a step towards cephalization.

Introduction

Caryophyllidean cestodes, being single segmented with a monozoic body plan, are interesting organisms in respect of several aspects of their biology. The organization of their nervous system is of particular interest, especially when considered from an evolutionary perspective.

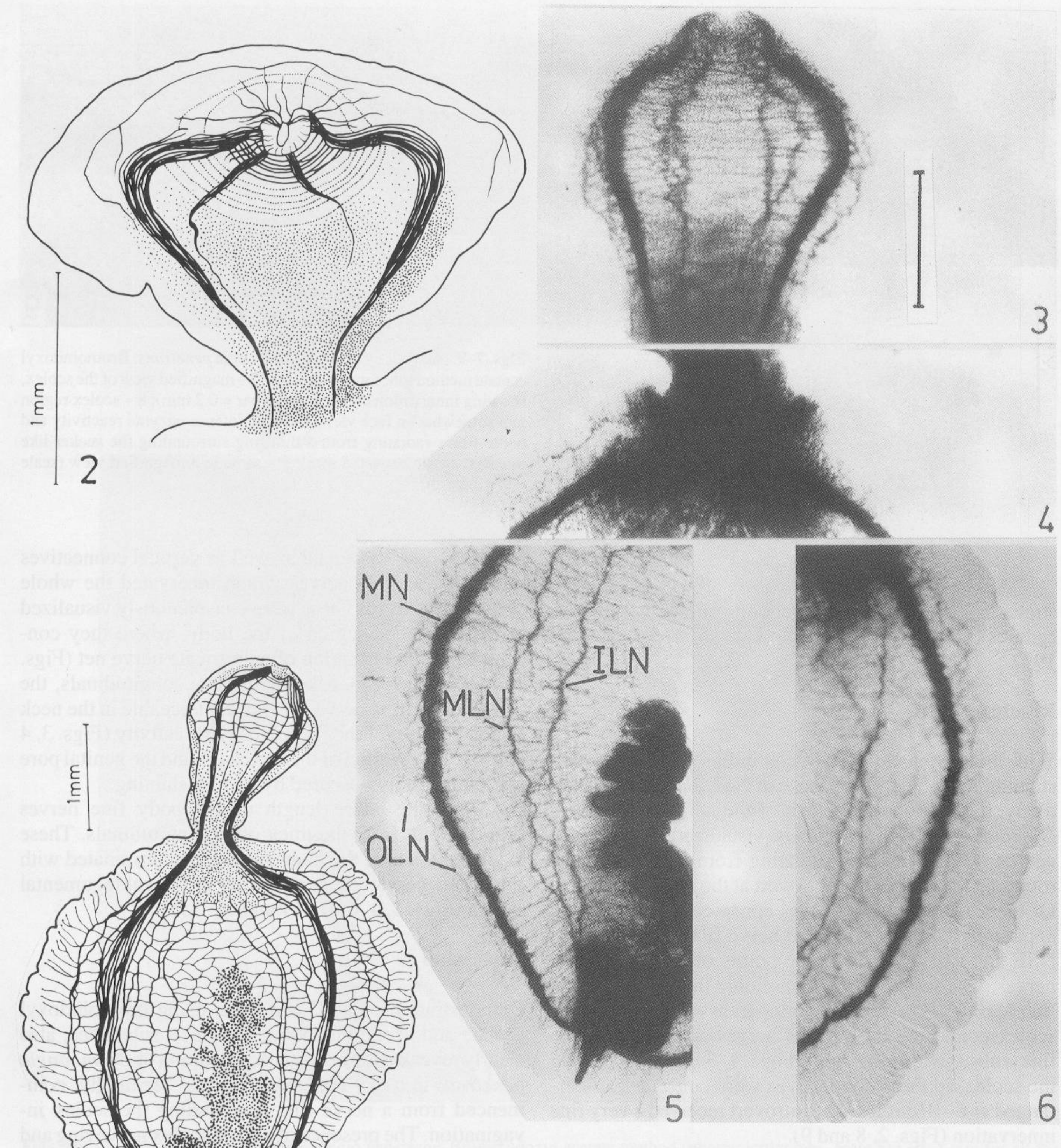
The details of the nervous organization have been worked out for several groups of cestodes. With the application of electron microscopy and immunocytochemical techniques, newer and interesting information has become available pertaining to the composition of the nervous tissues and the nature of their neuronal signals (Gustafsson 1990, 1991; Wikgren and Thorndyke 1990; Wikgren et al. 1990; Gustafsson and Eriksson 1991).

With regard to the neurobiology of the monozoic cestodes, the gyrocotylids and amphilinids have received some attention (Allison 1980, Rohde and Garlick 1985, Rohde et al. 1986, Xylander 1987). Among the caryophyllids a detailed description of the nervous system of *Lytocestus indicus* was reported by Lyngdoh and Tandon 1992.

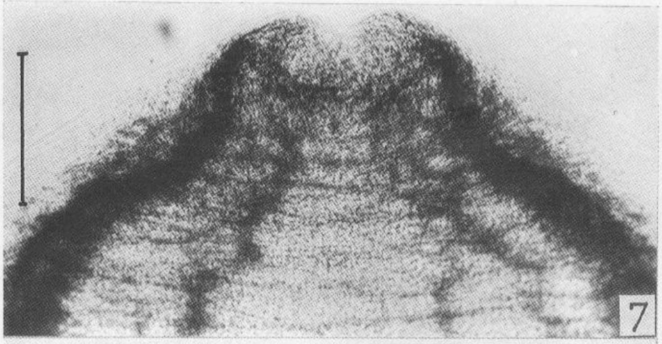
Djombangia penetrans (Lytocestidae), a common caryophyllid cestode parasitizing the duodenum of the catfish, *Clarias batrachus* in the oriental region, is different from *Lytocestus* (subfamily Lytocestinae) in having a distinct scolex, well differentiated from the rest of the body and with a sucker-like introvert at its tip. The present study aimed to provide a detailed account of the neuronal organization in this species.

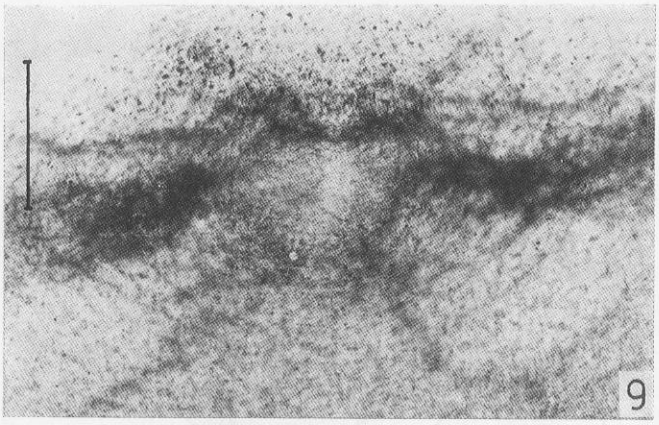
Material and methods

Specimens of *Djombangia penetrans* Bovien, 1926 were recovered from the duodenum of freshly killed *Clarias batrachus* (L.). The worms, while still active were washed in 0.7 saline and fixed in cold 10% neutral buffered formalin. Nonspecific esterases (NSE) and cholinesterase (ChE) were localized using bromoindoxyl acetate and acetylthiocholine iodine, respectively, as the sub-



Figs. 1 and 2. Diagrammatic representation of nervous system in *Djombangia penetrans* Bovien, 1926 through camera lucida drawings. **1** – whole representation of the worm; **2** – magnified view of the scolex, sucker is clearly shown at a different orientation. **Figs. 3–6.** Nervous system in *D. penetrans.* Bromoindoxyl acetate method (photomicrographs, scale bar = 0.5 mm for all the figures). **3** – whole view of the scolex region, showing thick and thin pairs of nerves with transverse connectives; **4** – neck region showing intense enzyme reactivity; **5** – half of the worm body on one side of its median axis; the main thick nerve (MN), outer, median and inner longitudinal nerves (OLN, MLN and ILN respectively) and different connectives are seen; **6** – nerves in other half of the body





Figs. 7–9. Nervous system in *Djombangia penetrans*. Bromoindoxyl acetate method (photomicrographs). **7** – magnified view of the scolex, showing innervation at its tip (scale bar = 0.2 mm); **8** – scolex region in a somewhat en face view, showing intense enzyme reactivity and nerve fibres radiating from a thin ring surrounding the sucker-like introvert (scale bar = 0.5 mm); **9** – same in a magnified view (scale bar = 0.2 mm)

strates in the incubation medium following the procedures published earlier by Lyngdoh and Tandon 1992.

Results

With the demonstration of deep indigo blue and brown staining revealing the presence of NSE and ChE, respectively, the entire arrangement of the nervous system in *Djombangia penetrans* could be visualized. Five pairs of nerves were discernible radiating from a thin ring surrounding the sucker-like introvert at the tip of the scolex. Of these nerves, one pair was conspicuously thick and apparently comprised several nerve fibres. The intense NSE and ChE activity at the points of origin of these nerves, was suggestive of ganglionic thickening on the "nerve ring". Fine nerves running transversely across the scolex connected the various nerves and constituted a fine subsurface nerve net (Figs. 1–3 and 7–9). Near the scolex tip these connectives were concentrically arranged as 8–10 circles. The introvert received a very fine innervation (Figs. 2, 8 and 9).

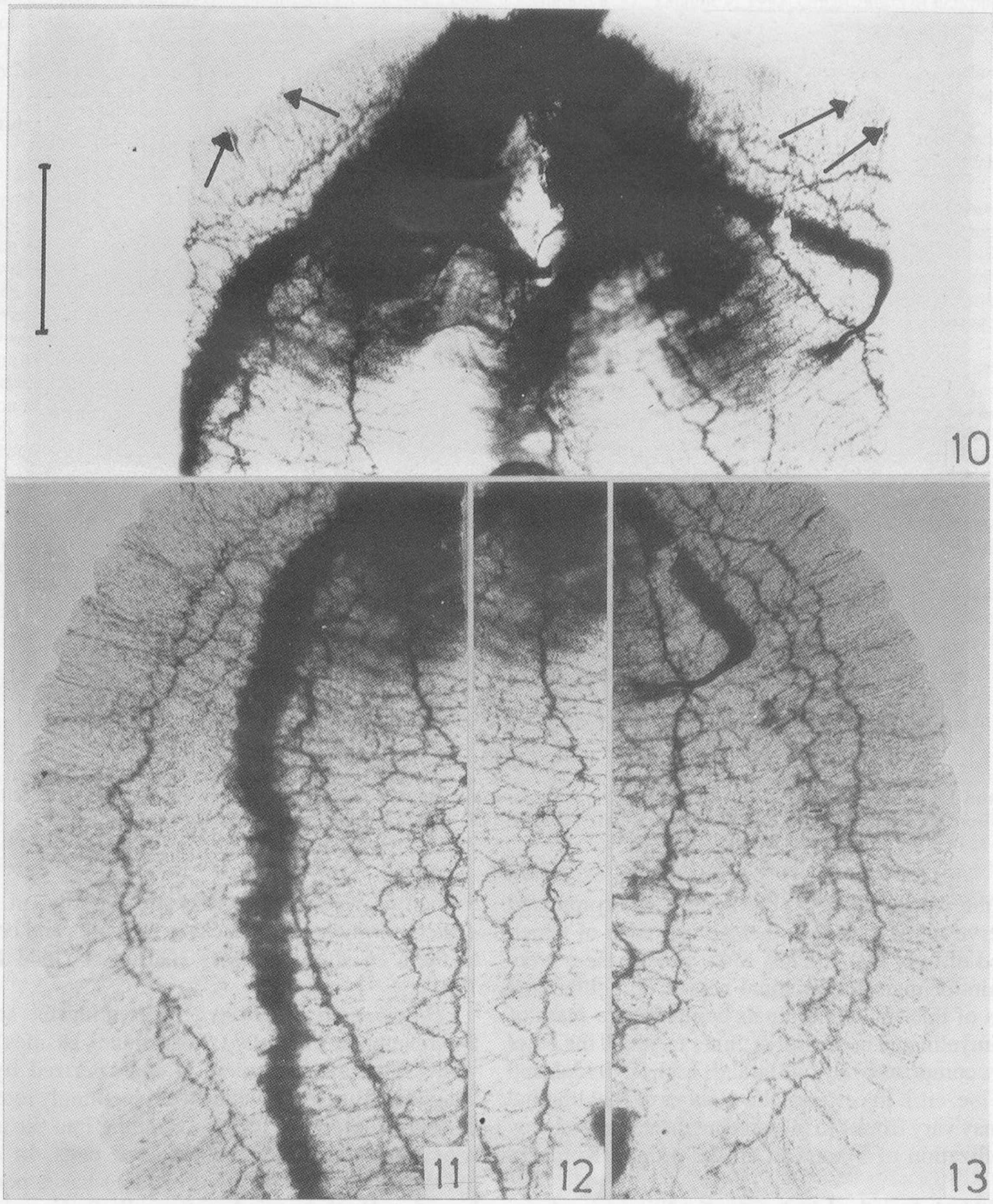
The main thick nerve and three thinner nerves (each of the latter comprising a pair of nerve fibres) proceeded posteriad from the nerve ring on either side of the median body axis, ran towards the posterior region and terminated at the ovarian region (Figs. 1 and 3–6). The 5th pair of nerves contributing to the nerve ring seemed to end in the parenchyma near the scolex tip (Figs. 1–3 and 8). Intense enzyme activity in the neck region hindered the localization of thinner nerves throughout this region (Figs. 3, 4 and 10).

Numerous horizontal as well as vertical connectives joined the various nerves which innervated the whole body of the worm. These were conspicuously visualized in the post-neck region of the body, where they contributed to the formation of an intricate nerve net (Figs. 1, 5, 6 and 10–13). Like the thinner longitudinals, the course of the fine nerve net was not traceable in the neck region because of the intense enzyme activity (Figs. 3, 4 and 10). Innervation of the region around the genital pore was comparably obscured by intense staining.

Along the entire length of the body fine nerves branched out from the thick outer longitudinals. These extended towards the body surface and terminated with a bulbous nerve cell at their tips in the subtegumental region (Figs. 1, 4–6, 10, 11 and 13–15).

Discussion

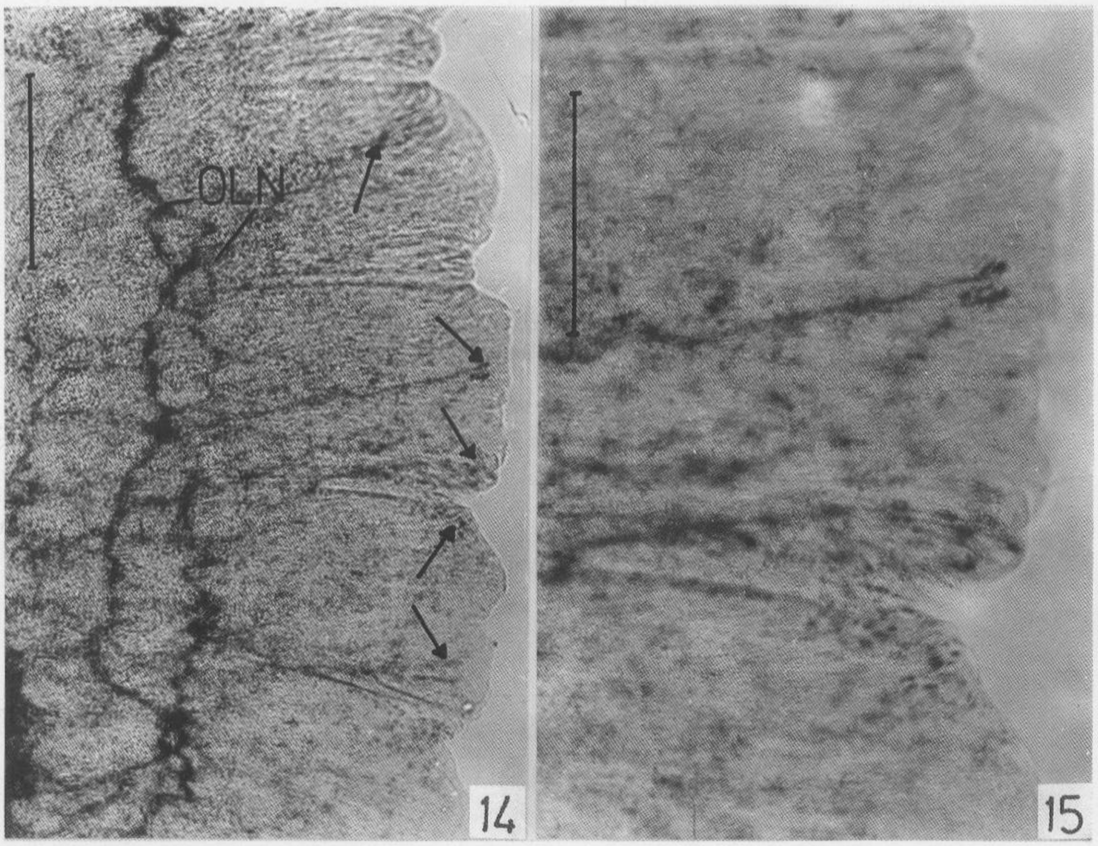
Demonstration of NSE and ChE, using bromoindoxyl acetate and acetylthiocholine iodide as substrates, also clearly revealed the nervous organization of *Djombangia penetrans* in toto. The main nerves seemingly commenced from a nerve ring surrounding the scolex invagination. The presence of a large scolex nerve ring and a pair each of short and longer laterals with 16 nodes has been reported in *Archigetes appendiculatus* (Mrázek 1898). In *Caryophyllaeus mutabilis* also reportedly 12 nerve cords branch off from a neck nerve ring and join in the centre of the scolex at a second nerve ring (Will 1893). In *Lytocestus indicus* only 2–3 transverse connectives join the main laterals at the junction between the scolex and the neck region and there is no nerve ring (Lyngdoh and Tandon 1992). However, like *L. indicus*, *D. penetrans* also has a well pronounced brain (Ramulu



Figs. 10–13. Nervous system in *Djombangia penetrans*. Bromoindoxyl acetate method (photomicrographs, scale bar = 0.5 mm for all the figures). **10** – post-neck region; longitudinal nerves, nerve connectives as well as nerve cells (arrows) are seen; **11** and **13** – longitudinal nerves running at the sides of the worm body, vertical and horizontal connectives as well as nerve cells terminating in lateral fields are clearly seen; **12** – inner longitudinal nerves passing through the mid longitudinal region of the body

and Krishna 1982, Lyngdoh and Tandon loc. cit.). In the cyclophyllidean, *Hydatigera taeniaeformis*, large ganglia and commissures are present in the scolex, from which nerves emerge and extend into the rostellum and the suckers (Lee et al. 1963). The bilobed brain with ganglionic commissures has been reported in the pseudophyllidean cestode *Diphyllobothrium dendriticum* by Gustafsson and Eriksson 1991.

Unlike *L. indicus*, the longitudinal nerves of *D. penetrans* did not show any ganglionic thickening along their course. In *D. penetrans* there were three thin longitudinal nerves and one thick nerve on either side of the median



Figs. 14 and 15. Nervous system in *Djombangia penetrans*. Bromoindoxyl acetate method (photomicrographs). **14** – nerve cells (arrows) given out from outer longitudinal nerves (OLN) (scale bar = 0.2 mm); **15** – same in a closer view (scale bar = 0.1 mm)

axis of the worm; of these, the thick nerve comprised more nerve fibres than the other nerves, each of which consisted of only two strands. In *L. indicus* there were three pairs of main longitudinal nerves, the outermost pair was of thicker longitudinals comprising a mass of 7–12 unmyelinated nerve fibres, inner to which the other two pairs comprised only two strands each (Lyngdoh and Tandon loc. cit.). In cestodes the number of longitudinal trunks may vary from 1 to 60 pairs but there is a tendency for stabilization of 5 pairs in all the orders (Kotikova 1979).

Will 1893 described 10 longitudinal nerve cords interconnected by about 20 transverse strands in *C. mutabilis*. In *L. indicus*, numerous fine transverse connectives emanating from the ganglionic thickening of the main longitudinals join the various longitudinal nerves (Lyngdoh and Tandon loc. cit.). In *D. penetrans*, no ganglionic thickening were observable along the length of the main nerves.

In *D. penetrans* the subtegumental nerve cells, which are connected to the outer longitudinals, were to some extent similar to those found associated with the lateral nerve cords and also with the plexus and other nerves in *L. indicus* (Lyngdoh and Tandon loc. cit.). Numerous nerve cell bodies are reported in *D. dendriticum*, which are adjacent to the cords and connected to them by thin processes (Gustafsson and Eriksson 1991).

Djombangia penetrans (representing the subfamily Djombanginae of family Lytocestidae), besides having a well differentiated scolex, is characterized by highly fringed body margins which were seemingly reminiscent of strobilization, the uterus extending into the testicular zone, and containing embryonated eggs. In contrast, *Lytocestus* (subfamily Lytocestinae) has a poorly differentiated bothriate scolex, uterus limited in extent to the post-testicular zone, and unembryonated eggs. Organizational differences between the two caryophyllids are also evident at the level of elaboration of the nervous system. With an apical introvert at the scolex, *D. penetrans* exhibited the presence of a "nerve ring", which is suggestive of a step towards cephalization, characteristic of polyzoic cestodes. However, the concentric nerve complex occurring around the genital pore in *L. indicus*, was not evident in *D. penetrans*.

Like other cestodes and also primitive platyhelminths, *D. penetrans* conforms to an orthogonal plan of nervous organization (Kotikova 1979).

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