

Organization of Nervous System in *Lytocestus indicus* (Cestoda : Caryophyllidea)

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With 14 Figures

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Abstract

With the localization of nonspecific esterases and cholinesterase, the nervous system in toto could be visualized in *Lytocestus indicus* (Moghe, 1925) Woodland, 1926. The nervous system presents an orthogonal plan. A discrete cephalization in the form of cerebral ganglia is lacking, but slight thickenings of the main longitudinal nerves at the level of scolex-neck junction and their fine transverse connectives are suggestive of a brain. An intense enzyme activity indicates a dense array of nerve fibres and cells in the scolex region. Posteriad, 3 pairs of longitudinal nerves run through the most length of the worm, terminating at the excretory vesicle region. The thick outer longitudinals have nodal swellings at more or less regular gaps along their length. Numerous fine transverse connectives emerging from these thickenings join the various longitudinals and constitute fine subsurface plexuses. A conspicuous innervation is seen in association with the terminal genital ducts and the genital pore region. Small fine nerves, branching off mainly from the nodal thickenings of the outer longitudinals, extend towards the peripheral zone. A large number of nerve cell bodies are also associated with the longitudinal nerves. All the nerves are observed to be unmyelinated.

Introduction

Caryophyllids emerge as a unique group among cestodes with an unusual array of characteristic features such as monozoic body plan, vitelline cell nuclei serving as storage sites for glycogen and utilization of tubificid annelids as intermediate hosts, besides polyploidy and parthenogenesis exhibited among some species (MACKIEWICZ 1972, 1982). These non-strobilate cestodes are regarded as occupying a low phylogenetic position distinct from but closely related to Pseudophyllidea. The nervous system of caryophyllids seems interesting from an evolutionary point of view.

The nervous system in respect of several groups of cestodes has been studied in detail. While the histological studies provided a reconstructed view (SIDDIQI 1961, RAMSZ 1967), in toto organization of the nervous system was successfully studied in many polyzoic cestodes with histochemical demonstration of nonspecific esterases

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(NSE), cholinesterase (ChE) and butyrylcholinesterase (BChE) both in adult (KRALJ 1967, SHIELD 1969, WILSON and SCHILLER 1969, KOTIKOVA and KUPERMAN, 1977, 1978 a, b, RAMAKRISHNA et al. 1989, FAIRWEATHER et al. 1990 a) and larval forms (HART 1967, BOGITSH 1967, RYBICKA 1967, REES 1973, LEFLORE and SMITH 1976). Ultra-structural and immunocytochemical studies revealed further information regarding the nervous constitution and neuronal signal substances (MORSETH 1967, WEBB and DAVEY 1975, GUSTAFSSON and WIKGREN 1981 a, b, GUSTAFSSON 1984, GUSTAFSSON et al. 1985, PLUZHNIKOV et al. 1986, WIKGREN 1986, FAIRWEATHER et al. 1988, 1990 a, b, GUSTAFSSON and WIKGREN 1989). However, with regard to the nervous organization of monozoic cestodes while some information is available pertaining to gyrocotylids (ALLISON 1980, XYLANDER 1987), that for caryophyllids is fragmentary. Principal accounts of the caryophyllid nervous system are available only for *Caryophyllaeus mutabilis* (WILL 1893), *Archigetes appendiculatus* (MRÁZEK 1898) and *A. sieboldi* (WIŚNIEWSKI 1930), while minor descriptions are provided as part of the species description for some other species (COOPER 1920, HUNTER 1930). With the localization of NSE, RAMULU and KRISHNA (1982) described the nervous system of *Lytocestus indicus* in brief. The aim of the present investigation was to provide a complete picture of the nervous system in toto of this obligate parasite of siluroid catfishes and to determine the presence or absence of cerebral ganglia and various commissures.

Materials and Techniques

Live specimens of *Lytocestus indicus* (MOGHE, 1925) WOODLAND 1926 (*Cestoda, Caryophyllidea*) were obtained in 0.7% saline from the intestine of freshly killed siluroid fish, *Clarias batrachus* (L.). The worms were fixed in 10% neutral buffered formalin at 4°C and were processed for histochemical localization of NSE and ChE. Staining for NSE was performed according to the method of HOLT and WITHERS (1952) using standard incubation medium containing bromoindoxyl acetate, and the method of GOMORI (1952) was followed for ChE using acetylthiocholine iodide as the substrate as described by RAHEMO and GORGEES (1987). The NSE and ChE were localized in the whole mounts of the methyl-benzoate cleared specimens by their deep indigo blue and brown staining, respectively.

Results

Judging by the NSE and ChE activity, a complete picture of the nervous system of *Lytocestus indicus* could be visualized. The nervous system presents an orthogonal plan. Brain in the form of two distinct cerebral ganglia joined by cerebral commissures was not discernible. However, in the basal region of the scolex just nearing the neck 2–3 very fine transverse connectives join the two main lateral nerves and these, with a slight thickening of the latter at this point of junction, suggest to be constituting the 'brain' (Figs. 1, 5). Anterior to these connectives, the scolex region, particularly its anterior and lateral extremities, show an intense enzyme activity indicating a very dense array of nerve fibres and cells (Figs. 4).

In the post-scolex region, running through the most length of the worm there are present 3 pairs of main longitudinal nerves which are medullary in position (Figs. 1–3, 5–7). Of these, the outermost are a pair of thick longitudinal nerves compris-

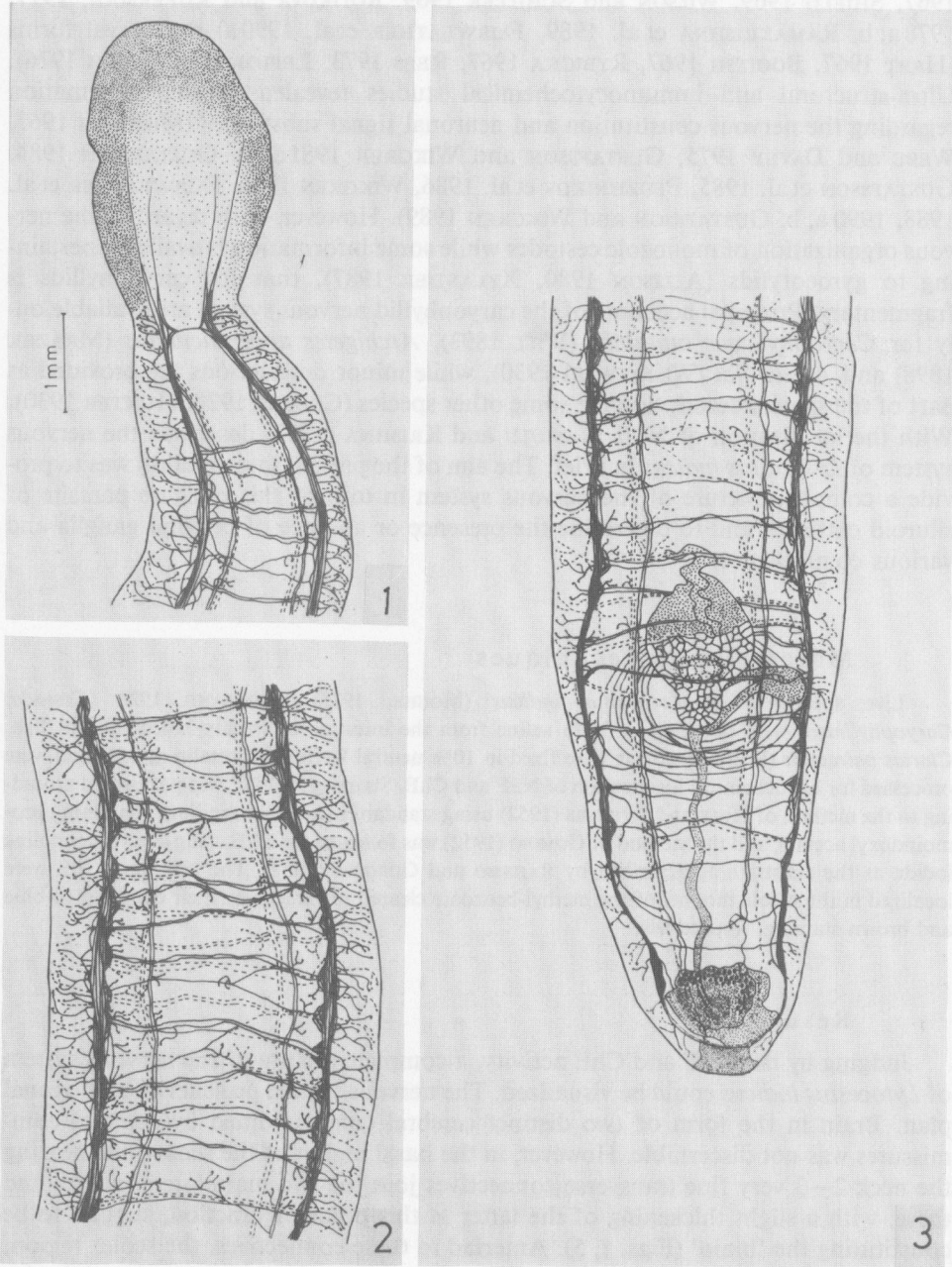


Fig. 1-3. Diagrammatic representation of nervous system in *Lytocestus indicus* (Moghe, 1925) Woodland, 1926 through camera lucida drawings.

1. Scolex and neck region. 2. Mid-body region. 3. Posterior region (scale bar, 1 mm for all figures)

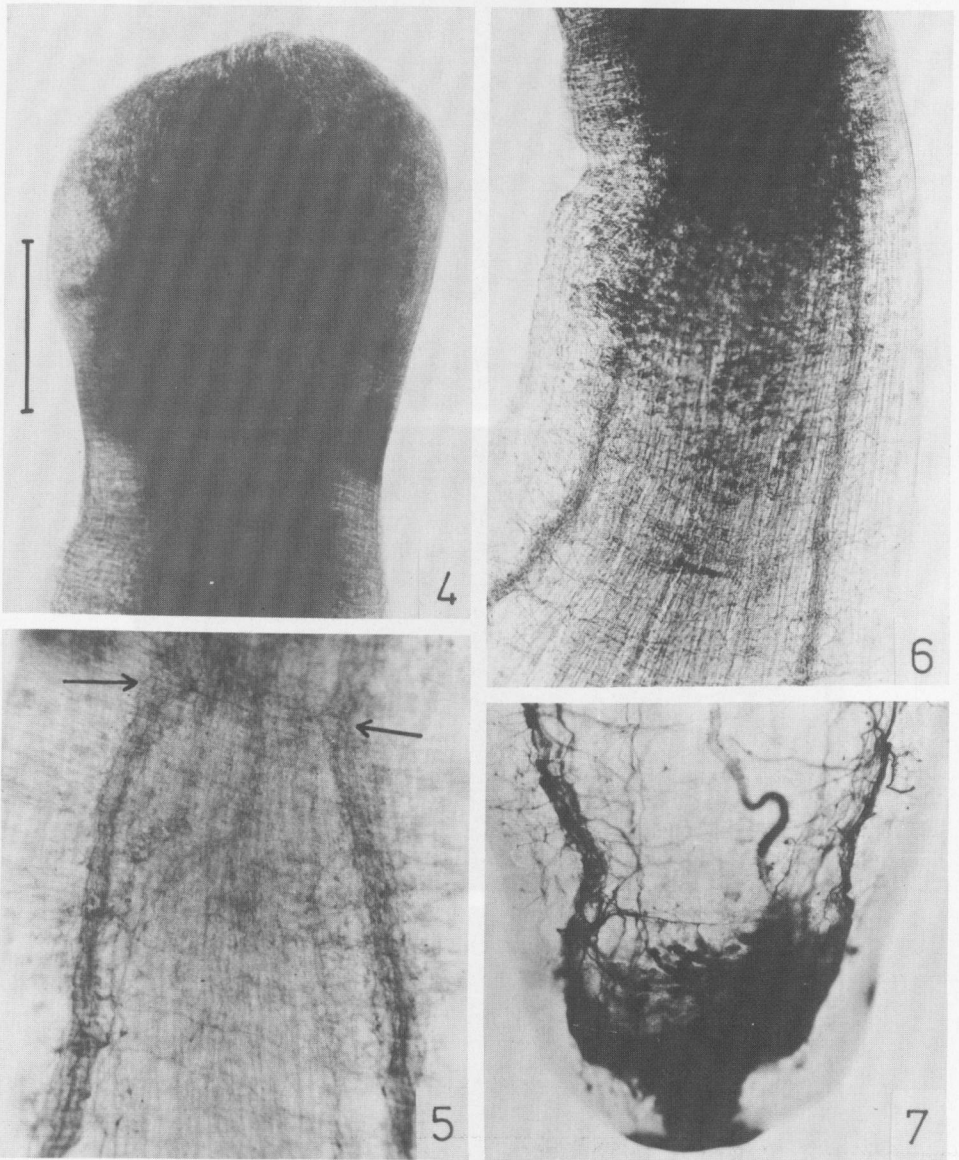
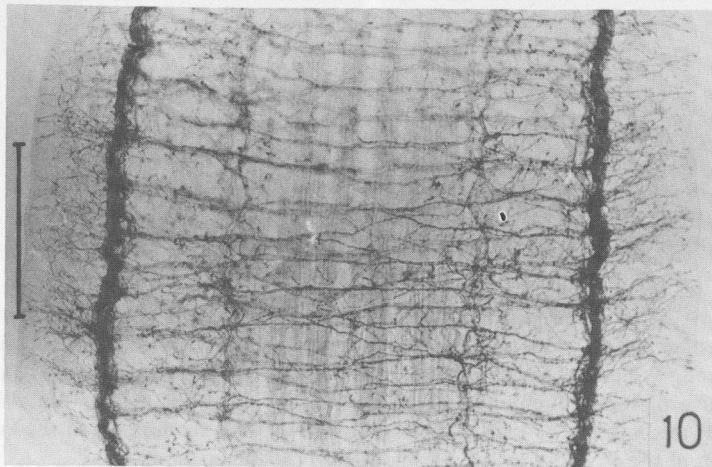
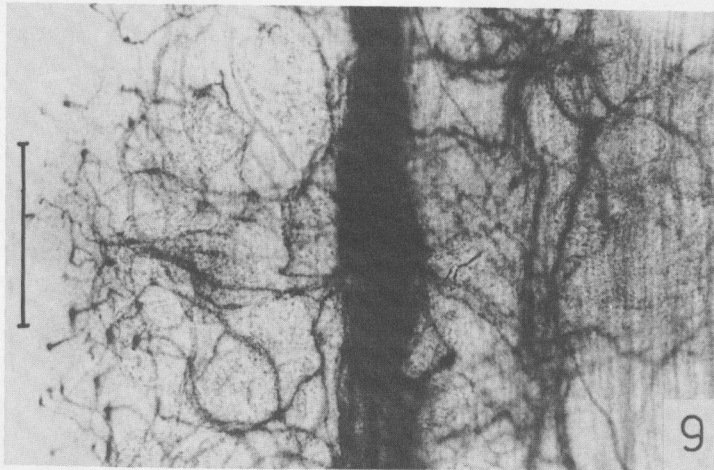
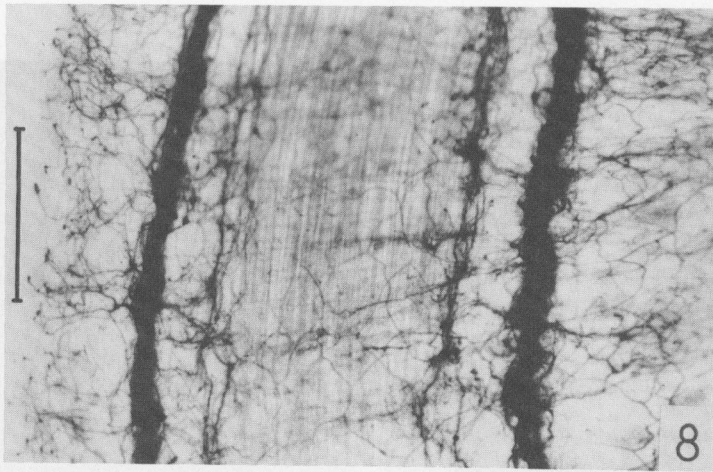


Fig. 4-7. Nervous system in *Lytocestus indicus* (photomicrographs, scale bar, 0.5 mm).
 4. Scolex region, showing an intense enzyme reactivity. Acetylthiocholine iodide method. 5. Scolex - neck region, showing fine transverse connectives (arrows) indicative of brain. Bromoindoxyl acetate method. 6. Neck region; Acetylthiocholine iodide method. 7. Posterior end of the worm, showing termination of the longitudinal nerves at the excretory vesicle. Bromoindoxyl acetate method



ing a mass of 7–12 unmyelinated nerve fibres each. These extend up to the posterior end of the body where they are seen to terminate near the excretory vesicle. Inner to these nerves there are 2 pairs of fine nerves, one on either side of the median axis of the body. These nerves, termed as inner and median longitudinals, are very slender in comparison to the outer longitudinals and each is made up of two strands. Slight swellings conspicuously visualized at more or less regular gaps along the length of the outer longitudinals are suggestive of a metameric ganglionated arrangement (Figs. 2, 3, 8, 9). Numerous fine transverse connectives, clearly observable posterior to the 'brain' are seen to be given out from these ganglionic thickenings all through the body. These connectives join the various longitudinal nerves with one another and constitute the fine subsurface plexuses both on dorsal and ventral aspects of the worm (Figs. 10, 13, 14). An intricate nerve net is seen associated with the wall of the cirrus sac and also the seminal vesicle (Fig. 11). A prominent complex of concentrically arranged fine nerves innervates the region of the genital pore (Fig. 12). Like the outer longitudinals, the inner and median longitudinal nerves also terminate near the excretory vesicle where also an intense staining reaction hinders a clear view of the course taken by the nerves.

Small fine nerves, branching off in bunches mainly from the 'ganglionic' thickenings of the main nerves, but occasionally from the other longitudinal nerves as well, extend towards the peripheral zones and supply the musculature and the tegument. A large number of nerve cell bodies are associated with the lateral nerve cords and also with the plexus and other nerves, too. These cell bodies extend into the tegument (Figs. 8, 9, 13).

Discussion

Using indoxyl acetate and acetylthiocholine iodide as substrates, the nervous system of *Lytocestus indicus* is revealed to have an orthogonal organization characterized by transverse connectives. The orthogon with varying number of longitudinal nerve trunks is known to occur in primitive platyhelminth group of Turbellaria (KOTIKOVA 1986); among cestodes, an orthogon with 3 pairs of longitudinal nerves represents the initial state of the nervous system among Pseudophyllidea (KOTIKOVA and KUPERMAN (1977, 1978 a).

In confirmity with the observations of RAMULU and KRISHNA (1982) in the present study of *L. indicus* also, the 'brain' was found to be weakly pronounced and represented by 2–3 transverse connectives in the neck region, there being no distinct cerebral ganglia. However, in the present study the occurrence of ganglionic thickenings along the outer longitudinals and nerve cell bodies have been detected. In *Caryophyllaeus mutabilis* WILL (1893) described a nerve ring in the neck region,

- ◀ Fig. 8–10. Nervous system in *Lytocestus indicus* (photomicrographs).
8. Mid-body region; longitudinal nerves and nerve cell bodies are clearly seen. Bromoindoxyl acetate method (scale bar, 0.5 mm). 9. Same in a magnified view (scale bar, 0.2 mm). 10. Mid-body region, showing longitudinal nerves, transverse connectives, ganglionic thickenings and nerve cell bodies, Acetylthiocholine iodide method (scale bar, 0.5 mm)

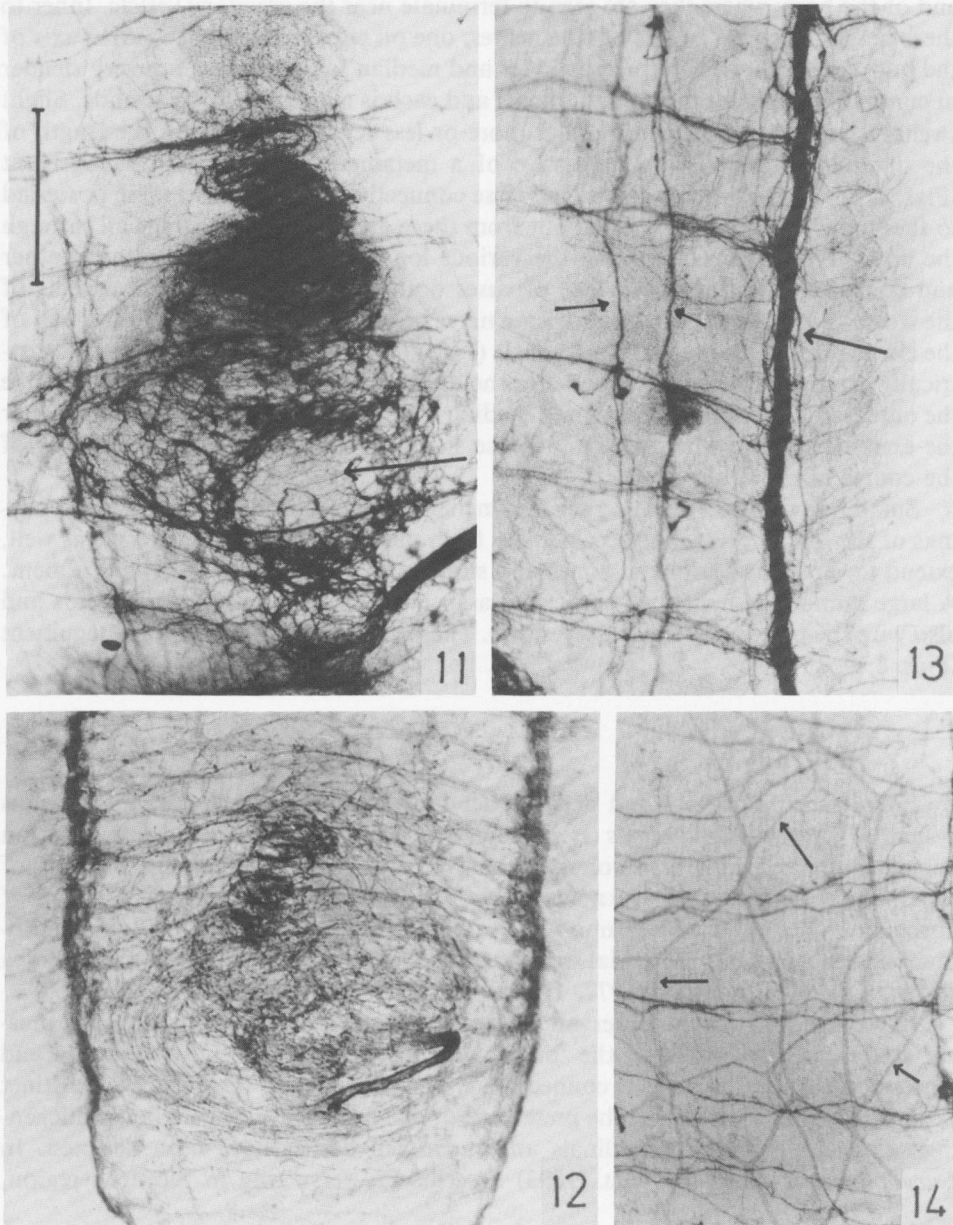


Fig. 11–14. Nervous system in *Lytocestus indicus* (photomicrographs, scale bar, 0.5 mm).

11. Innervation of the male terminal genitalia and the genital pore (arrow), forming an intricate nerve net in the region. Bromoindoxyl acetate method. 12. Concentric arrangement of fine nerves in the genital pore region as revealed by Acetylthiocholine iodide method. 13. Course of outer longitudinal (large arrow) and inner longitudinal nerves (small arrows) and their transverse connectives in a closer view. Bromoindoxyl acetate method. 14. A portion of the mid-body region, revealing the excretory network (arrows) along with the transverse nerves. Bromoindoxyl acetate method

anteriad from which 12 nerve cords emerge and join a second nerve ring in the scolex; 10 longitudinal nerve cords joined by about 20 transverse strands run in the post-neck region. MRÁZEK (1898) reported a single large scolex nerve ring and two pairs of longitudinal laterals with 16 nodal thickenings in *Archigetes appendiculatus*. In *Glaridacris catostomi*, two scolex ganglia and two lateral cords are described (COOPER 1920), while in several other species, namely *Monobothrium ingens*, *Hypocaryophyllaeus paratarius*, *Glaridacris confusus* and *Biacetabulum infrequens* ganglia are reported to be present (HUNTER 1930). The presence of nodes along the longitudinal trunks, as observed in the present study, appears similar to the observations by MRÁZEK (loc. cit.). Segmental bilateral ganglionic thickenings are known to occur in the developing proglottids of strobilate cestodes (SIDDIQI 1961, FAIRWEATHER et al. 1990a) and a somewhat nodal appearance of the main longitudinal trunks in the monozoic *L. indicus* may be taken as representing a primitive or initial state of metameric organization of the nervous elements. Transverse connectives joining the longitudinal nerves prominently occur in many cestodes, both larval and adult cyclophyllideans (HART 1967, SHIELD 1969) and pseudophyllideans (KOTIKOVA and KUPERMAN 1978 a, b).

The nerve fibres in *L. indicus* were found to be unmyelinated. Among the platyhelminth parasites a nerve sheath has been described in *Multicotyle purvisi*, an aspidogastreaean (ROHDE 1971) and a *Temnocephala* sp. from cray fish (ROHDE and WEBB 1986). However, in many cestodes such as *Echinococcus granulosus*, *Hymenolepis microstoma*, *H. nana* and *Gyrocotyle urna* the nerves are reported to be of unmyelinated nature (MORSETH 1967, WEBB and DAVEY 1975, FAIRWEATHER and THREADGOLD 1983, XYLANDER 1987).

The gross neuroanatomy of the post-scolex region in *L. indicus* indicates a closeness with that of tetraphyllideans (FAIRWEATHER et al. 1990a). As in *Trilocularia acanthiaevulgaris*, one pair of main laterals is predominantly conspicuous and a substantial population of nerve cells is associated with the nerve trunks in this caryophyllidean cestode.

The histochemical methods used in the present study provide a more detailed information on the cholinergic nervous components in *L. indicus*, and extend the current knowledge on the neuroanatomy of caryophyllidean cestodes. As evident in *L. indicus* the nervous organization of caryophyllids may be considered a prelude to that in strobilate polyzoic cestodes.

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