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The nervous system of *Polystoma indicum* (Monogenea: Polystomatidae) as revealed by nonspecific esterase localization

M. Dutta and **V. Tandon***

Department of Zoology, North-Eastern Hill University, Shillong 793 014, India

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Abstract. Using the localization of nonspecific esterases, the nervous system of *Polystoma indicum* Diengdoh et Tandon, 1991 could be revealed *in toto*. The nervous system is bilaterally symmetrical. Cerebral ganglia, which are connected by a thick dorsal commissure, are present dorsal to the pharynx. From the cerebral ganglia arise five anterior pairs and four posterior pairs of nerve trunks. The anterior nerves and/or their fine branches join to form a conspicuous circumoral nerve ring complex. The posterior nerves unite in the opisthaptoral region to form two main haptoral nerves, the branches from which innervate the components of the opisthaptor. Presence of nerve cells is also revealed in association with the main nerves, more predominantly in the vicinity of the opisthaptoral suckers.

Information pertaining to the nervous organization of monogenean parasites is available only for a few species. Andre (1910) provided the first morphological account of the nervous system of *Polystoma integerrimum*. Using histochemical localization of nonspecific esterases (NSE), Halton and Jennings (1962) described the nervous system of *Diplozoon paradoxum*. Utilizing histochemical techniques, the nervous system *in toto* was also described for *Polystomoides* spp. and *Diclidophora merlangi* by Rohde (1968) and Halton and Morris (1962), respectively. Rahemo (1982) further worked out the morphology of the nervous system of about 30 species of Monogenea that included one polyopisthocotylean, using localization of cholinesterase *in toto* preparations. Rahemo and Gorgees (1987) also provided a detailed account of the nervous system of *P. integerrimum* using the same techniques. Advanced information is now available regarding the nature (serotoninergic, cholinergic and peptidergic) of the nervous components in *D. merlangi* (see Maule et al. 1990).

Polystoma indicum Diengdoh et Tandon, 1991 is a monogenean parasite of rhacophorid anurans in the north-eastern part of India. Its seemingly strict host-specificity and restricted geographical distribution (Diengdoh and Tandon 1991) invite an extended study on various aspects of the morphology and biology of this fluke. Therefore, the present communication aims at investigating the nervous organization *in toto* of *P. indicum*.

* To whom correspondence should be addressed

MATERIALS AND METHODS

Live specimens of *Polystoma indicum* were recovered from the urinary bladder of *Rhacophorus nigropalmatus* Boulenger, and collected in 0.7% saline. After fixation in 10% neutral buffered formalin at 4°C the specimens were processed for demonstration of nonspecific esterases (NSE) following the technique of Holt and Withers (1952). The specimens were washed in distilled water at 10°C and incubated at room temperature (10–18°C) for 12–14 h, using 5-bromoindoxyl acetate as the standard incubation medium (0-acetyl-5-bromoindoxyl –1.3 mg, dissolved in 0.1 ml ethanol and mixed with 2.0 ml of 0.1 M Tris, 1.0 ml of 0.05 M potassium ferricyanide, 1.0 ml of 0.05 M potassium ferrocyanide, 1.0 ml of 0.1 M calcium chloride, and final volume made to 10 ml with distilled water) at a pH between 5.9 and 7.4. After incubation they were washed in distilled water, dehydrated in ascending grades of ethylalcohol, cleared in methyl benzoate and mounted in Canada balsam. Various nervous elements were observed in whole mounts with the localization of NSE by their deep indigo blue colour. The terminology has been used according to Rahemo and Gorgees (1987).

RESULTS

The bilaterally symmetrical nervous system of *Polystoma indicum* could be revealed *in toto*. On the antero-lateral sides of the pharynx there are two prominent cerebral ganglia connected by a thick dorsal commissure (Figs. 1, 3–5). From the cerebral ganglia arise five anterior pairs of short nerves and four posterior pairs of distinct nerves (Figs. 1, 4–5).

Among the anterior nerves (Figs. 1, 3–5), there is a pair of most medial nerves, each of them arises from the

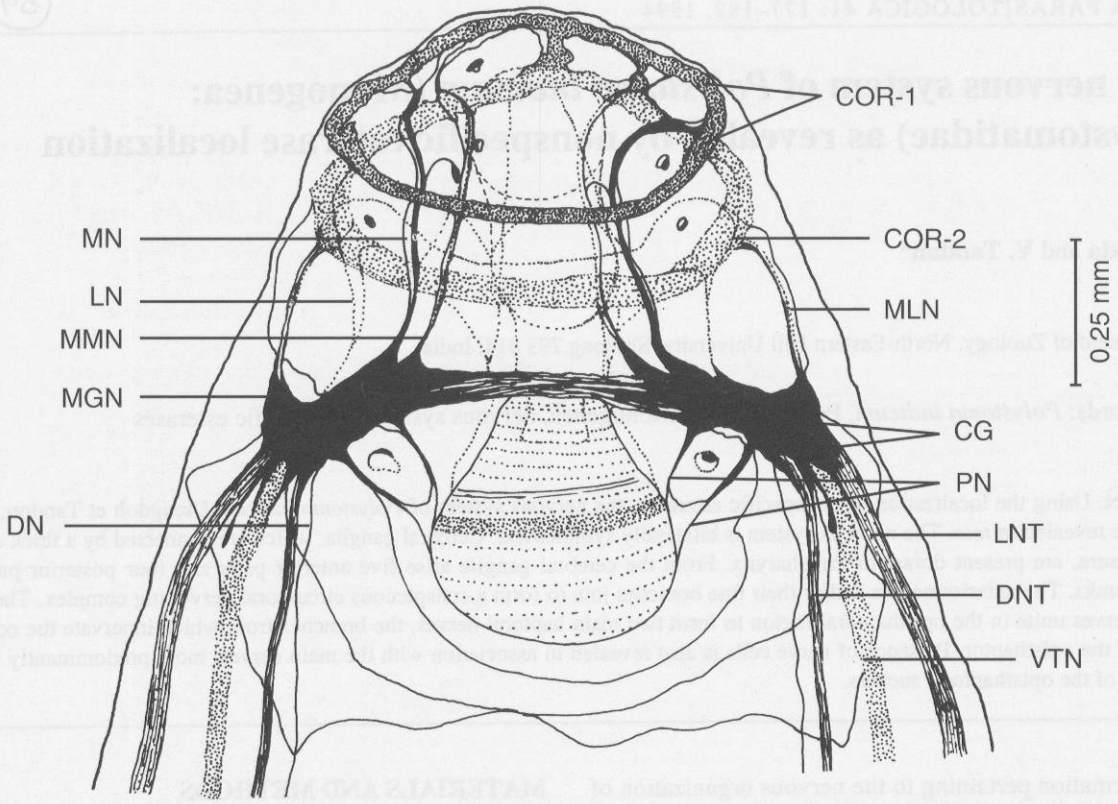


Fig. 1. Diagrammatic representation of the nervous system in *Polystoma indicum* Diengdoh et Tandon, 1991 (camera lucida diagrams) – anterior region. CG, cerebral ganglion; COR-1, anterior circumoral ring; COR-2, posterior circumoral ring; DN, dorsal nerve; DNT, dorsal nerve trunk; LN, lateral nerve; LNT, lateral nerve trunk; MGN, marginal nerve; MLN, most lateral nerve; MMN, most medial nerve; MN, medial nerve; PN, pharyngeal nerve; VNT, ventral nerve trunk.

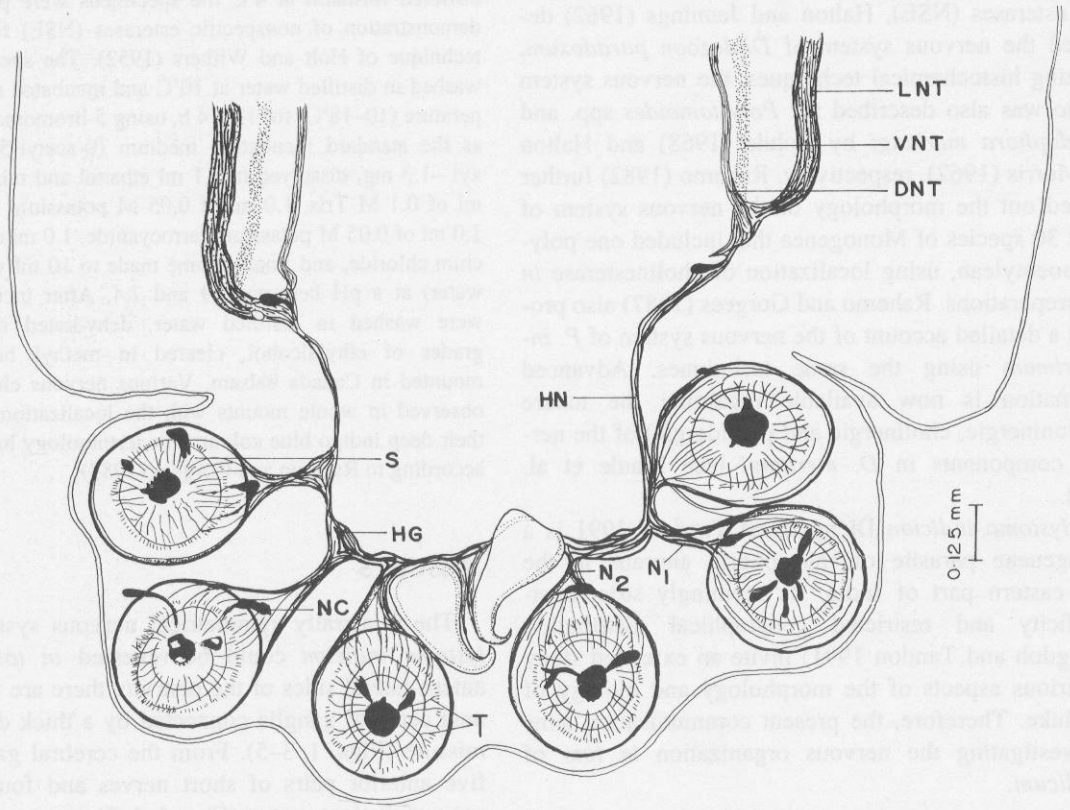


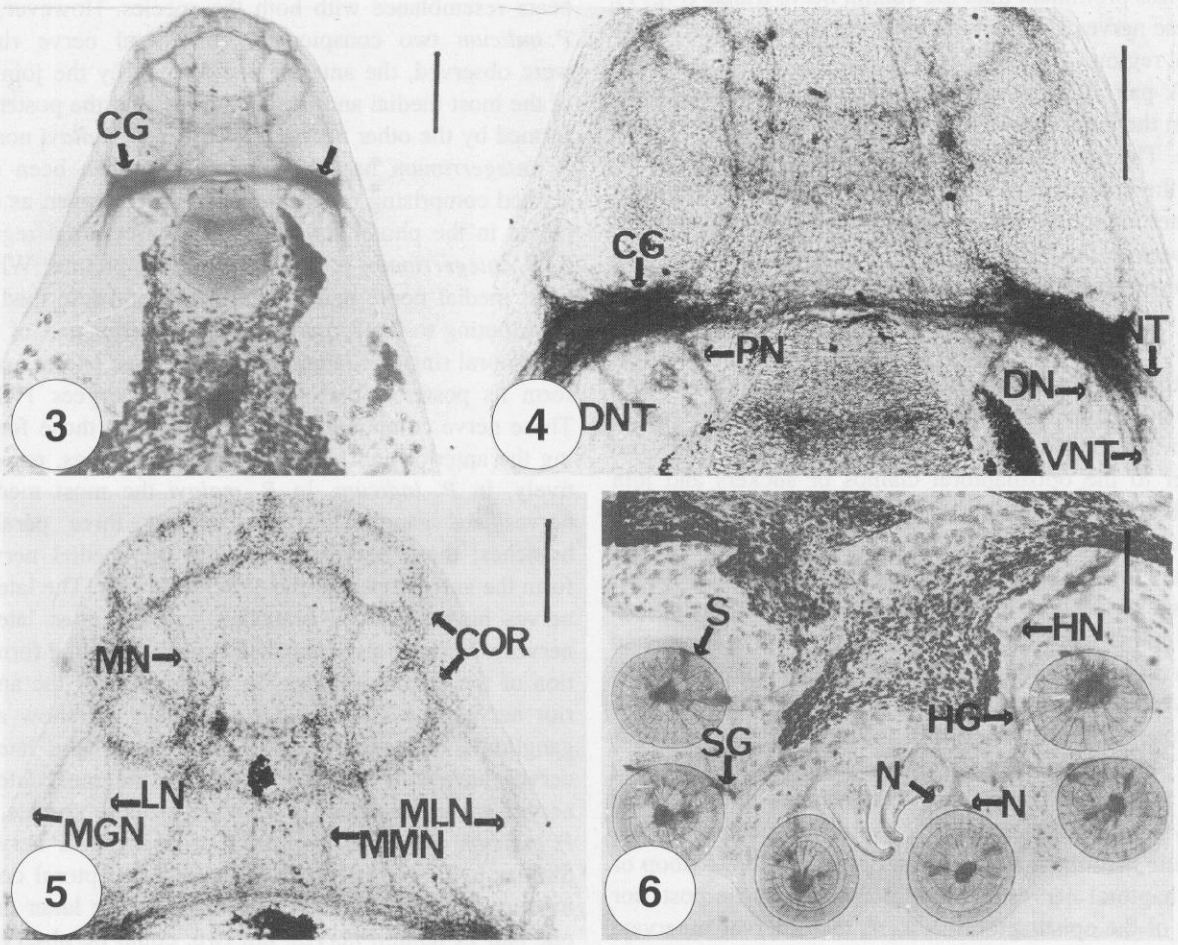
Fig. 2. Diagrammatic representation of the nervous system of *Polystoma indicum* – posterior and opisthaptor region (camera lucida diagrams). DNT, dorsal nerve trunk; HG, haptoral ganglion; HN, haptoral nerve; LNT, lateral nerve trunk; N_1 and N_2, two nerves supplying the suckers; NC, nerve cell; S, sucker; SG, sucker ganglion; T, tendons; VNT, ventral nerve trunk.

anterior facet of the cerebral ganglion of either side and innervates the oral sucker. These short and slender nerves occupy the most medial region. The other anterior nerves, in accordance with their dorsal position, include a pair of thick and stout medial nerves, slightly less distinct lateral nerves that arise ventrally one on either side from the middle part of the cerebral ganglion, branching into two nerves distally; thick most lateral nerves, arising from the most lateral side of each cerebral ganglion and stout like the most medial nerves; marginal nerves are very slender and least conspicuous of all the nerves. All these nerves and their fine branches join to form circumoral complex comprising two conspicuous circumoral rings. While the most medial nerves, the medial nerves and their tributaries participate to form the anterior circumoral ring which lies in close approximation of the oral opening, the most lateral nerves, the lateral nerves and the marginal nerves contribute to the formation of the second circumoral ring, just a little posterior to the anterior one.

Among the posterior nerves (Figs. 1–2, 4), there are two dorsal nerve trunks, each arising from the cerebral ganglion. These nerves run posteriad somewhat parallel to each other until they combine with the other posterior nerve trunks in the region just in front of the opisthaptor. An additional pair of dorsal nerves are the innermost of all the posterior long nerves, which are very slender and run parallel to the dorsal nerve trunks.

The ventral nerve trunks are also a pair of stout nerves that arise from the postero-ventral sides of the cerebral ganglia (Fig. 1). These long prominent nerves run parallel to the dorsal nerve trunks. In the posterior haptoral region they form two main haptoral nerves as a result of their combination with the other trunks.

The lateral trunks, two in number, also arise from the lateral sides of the cerebral ganglia, being marginal in



Figs. 3–6. Nervous system of *Polystoma indicum* (photomicrographs). **Fig. 3.** Anterior region, showing the CG, cerebral ganglia, with commissure and anterior nerves (scale bar = 0.2 mm). **Fig. 4.** Magnified view of anterior region, showing the CG, cerebral ganglion; PN, pharyngeal nerve, and 4 pairs of nerves posteriorly; DNT, dorsal nerve trunk; LNT, lateral nerve trunk; VNT, ventral nerve trunk; DN, dorsal nerve (scale bar = 0.05 mm). **Fig. 5.** Anterior region, showing COR, circumoral rings and 5 pairs of anterior nerves: MN, medial nerve; LN, lateral nerve; MMN, most medial nerve; MLN, most lateral nerve; MGN, marginal nerve (scale bar = 0.125 mm). **Fig. 6.** Opisthaptor region showing the six suckers; S, the sucker; HN, haptoral nerve; HG, haptoral ganglion; SG, sucker ganglion; and N_1 and N_2, two nerves supplying the suckers (scale bar = 0.2 mm).

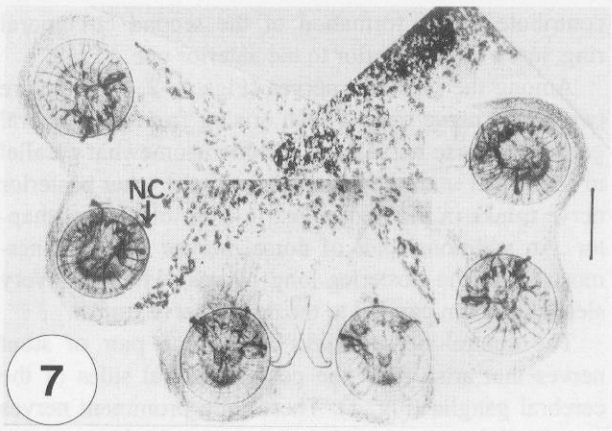


Fig. 7. Opisthaptor magnified and reconstructed from three micrographs, showing innervation to suckers and the nerve cells (NC-arrows) (scale bar = 0.125 mm).

position and much stouter than the dorsal nerve trunks but less prominent than the ventral nerve trunks (Fig. 1). These nerves also join the other nerve trunks in the haptoral region.

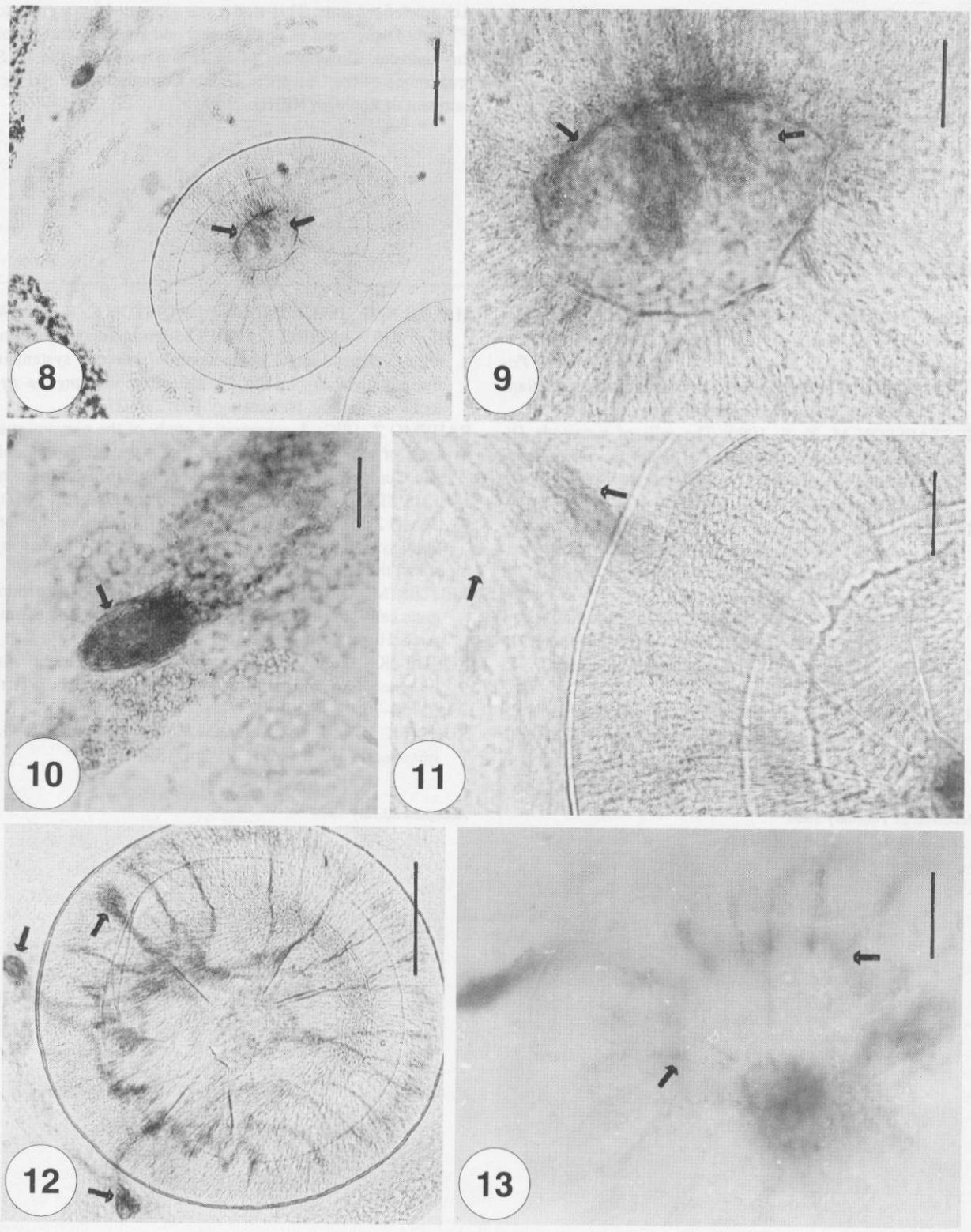
A pair of thin and short pharyngeal nerves arises from the inner aspects of the cerebral ganglion on either side. The four pharyngeal nerves innervate the pharynx. All the nerve trunks are connected along their length by numerous transverse commissures. While commissures present in the vicinity of the pharynx and in the post pharyngeal region (Fig. 1) are quite prominent, the others cannot be traced exactly as they are very fine and thin. The haptoral nerves (Figs. 2, 6–8, 11–13) formed by the merger of the dorsal, ventral and lateral nerve trunks of either side just prior to the opisthaptoral region and extend further into the opisthaptor. They run inner to the opisthaptoral clamps or suckers and join medially, thus forming a U-shaped commissure. Along the course of this "U" of the haptoral commissure, there occurs a thickening confronting each of the opisthaptoral suckers. These thickenings or "haptoral ganglia" are the points from each of which arise two or more fine nerves leading to the corresponding sucker. These nerves run divergently from each other but along the periphery of the sucker, thus forming a ring by eventually joining each other. These nerves innervate the suckers from their dorsal and ventral facets and give out very fine tributaries in the tissue of the suckers. A pair of fine hamuli nerves is given out from the "U" loop of the haptoral nerves in the region between the posterior pair of the opisthaptoral suckers; these nerves innervate the hamuli from their basal part.

The organs innervated by fine nerves arising from the commissures and also from the main nerve trunks are ovary, ootype, uterus, cirrus sac and testes. The pharynx and the digestive tract receive nerves from the pharyngeal nerves and the various transverse commissures.

Many dark staining nerve cells were observed in association with the cerebral ganglia, anterior nerves and the main nerve trunks and also with the fine branches more predominantly of the nerves supplying to the opisthaptoral suckers. The ones associated with the latter are mostly unipolar small club-shaped cells, with their cytoplasm showing intense staining reactivity (Figs. 9–10, 13).

DISCUSSION

The present study on the nervous system of *Polystoma indicum* exhibits a similar basic pattern as that described for other species of Polystomatidae, namely *Polystomoides malayi* by Rohde (1972) and *Polystoma integerrimum* by Rahemo and Gorgees (1987). Presence of five anterior pairs of nerves in *P. indicum*, which participate in the formation of the circumoral complex bears resemblance with both the species. However, in *P. indicum* two conspicuous circumoral nerve rings were observed, the anterior one formed by the joining of the most medial and medial nerves, and the posterior formed by the other nerves. Neither in *P. malayi* nor in *P. integerrimum* has a circumoral complex been described comprising of two nerve rings. However, as depicted in the photomicrographs, the circumoral region in *P. integerrimum* presents a complex picture. While most medial nerve and medial nerve are described as contributing to the formation of the anterior part of the circumoral ring, the lateral nerve and most lateral nerve form its posterior part (Rahemo and Gorgees 1987). These nerve components do correspond to those forming the anterior and posterior circumoral rings, respectively, in *P. indicum*. In *P. malayi* the most medial nerves are ganglionated and having three parallel branches; these nerves along with the medial nerves form the anterior part of the circumoral ring. The lateral nerves having several branches, and the most lateral nerves which are also branched contribute to the formation of the circumoral ring. In *P. indicum*, of the anterior nerves, the most medial nerves do not show any ganglionic thickenings along their course, the lateral nerves have two branches each and the most lateral nerves are unbranched. In contrast to these species, in *P. indicum* there are two pairs of pharyngeal nerves. Similar to *P. malayi*, in *P. indicum* the haptoral commissure is quite prominent; however, in the latter only one pair of hamuli nerves, not two, could be observed. In *P. integerrimum* also, there are only two hamuli nerves. In *P. indicum* many nerve cells were observed associated with the ganglia and the main nerves, more abundantly with the haptoral suckers. Rahemo and Gorgees (1987) also observed the occurrence of neurons in *P. malayi*, but found only a few unipolar cells in the



Figs. 8–13. Nervous system of *Polystoma indicum* (photomicrographs). **Fig. 8.** Opisthaptoral sucker showing enzymatic reactivity, arrows (scale bar = 0.125 mm). **Fig. 9.** A magnified view of the same, showing intense enzymatic reactivity, arrows (scale bar = 0.025 mm). **Fig. 10.** A nerve cell associated with an opisthaptoral sucker, showing intense nonspecific esterase activity, arrow (scale bar = 0.025 mm). **Fig. 11.** A closer view of the nerve innervating the sucker, arrows (scale bar = 0.025 mm). **Fig. 12.** Another opisthaptoral sucker in a magnified view; several nerve cells are evident, arrows (scale bar = 0.05 mm). **Fig. 13.** Innervation of the ventral region of the sucker in Fig. 12, arrows (scale bar = 0.025 mm).

vicinity of the haptoral suckers. These authors suggested these cells to be neurosecretory in nature. Reports and information regarding the occurrence of neurosecretory components in platyhelminth parasites have accumulated in the past few years (Reuter and Gustafsson 1989, Gustafsson 1990). Further investigation seems desirable to ascertain the nature of nerve cells in *P. indicum*.

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