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Neurons containing catecholamine in juveniles of eight species of free–living marine nematodes

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Summary. The occurrence, number and distribution of neurons containing catecholamine (CC) was determined using histofluorescent techniques with first stage juveniles of eight species belonging to the subclass Adenophorea. This feature was demonstrated to be species-specific. In all species, two pairs of cephalic CC neurons innervating four cephalic sensillae were observed. The number and localization of the remaining CC neurons (somatic neurons) vary between different species and correspond to the somatic sensillae. The pattern of CC neurons in the juveniles of marine Adenophorea is similar to that in juveniles and adults of Secernentea but not to that of adult Adenophorea.

Key words: catecholamines, neurons, first stage juveniles, *Enoplus brevis, E. communis, Pontonema vulgare, Odoncholaimus lepidus, Chromodoropsis vivipara, Paracanthonhus macrodon, Theristus setosus, Sphaerolaimus balticus.*

Neurons containing catecholamines as neurotransmitters have been described for various nematode species. All the representatives of the subclass Secernentea and some Adenophorea (orders Dorylaimida and Mermithida) have a small, constant number of identifiable neurons containing catecholamines (CC) (Plotnikova et al., 1969; Sulston et al., 1975; Goh & Davey, 1976; Wright & Awan, 1978; Zhuchkova & Shishov, 1979; Sharpe & Atkinson, 1980; Lee & Ko, 1991). However, adults of various species of free-living marine nematodes from the subclass Adenophorea have numerous unidentifiable CC cells present in their nervous system (Malakhov & Nezlin, 1982; Malakhov et al., 1983). First stage juveniles belonging to one of these species, *Enoplus brevis*, possess a constant number of identifiable CC neurons, and some of these correspond to the CC neurons of Secernentea (Nezlin et al., 1990). It had not been determined if the existence of these CC neurons was a unique event in *E. brevis* or if juveniles of other marine nematodes also contained a similar, simple set of CC neurons. Therefore, we examined the presence and diversity in the number and localization of CC neurons in the nervous system of juvenile nematodes in several species of free-living marine Adenophorea.

MATERIALS AND METHODS

The species of free-living marine nematodes examined for the presence and diversity of CC neurons are listed in Table 1.

Female nematodes were collected from the sandy littoral at Kandalaksha Bay, the White Sea (Biological Station of the Zoological Institute, Russian Academy of Sciences). Female *Ch. vivipara* and *P. macrodon* were cultured in Petri dishes with filtered sea water until juveniles appeared. With the other species, fertilized eggs were extracted and kept in Petri dishes with filtered sea water until they hatched and the juveniles were then collected.

The presence and localization of catecholamines was determined using whole-mount preparations with a glyoxylic acid-induced fluorescent technique

Table 1. Nematode species examined for the presence and diversity of CC neurons.

Order Enoplida	Order Chromadorida
Family Enoplidae	Family Cyatholaimidae
Enoplus brevis Bastian, 1865	*Paracanthonhus macrodon* (Ditlevsen, 1910)
Enoplus communis Bastian, 1865	Order Monhysterida
Family Oncholaimidae	Family Monhysteridae
Pontonema vulgare (Bastian, 1865)	*Theristus setosus* (Butschli, 1874)
Odoncholaimus lepidus De Man, 1889	Family Sphaerolaimidae
Order Desmodorida	*Sphaerolaimus balticus* Schneider, 1906.
Family Spiriniidae	
Chromodoropsis vivipara (De Man, 1907)	

(Lindvall & Bjorklund, 1974; de la Torre & Surgeon, 1976), and a formaldehyde technique (Sakharova & Sakharov, 1971), both slightly modified as described by Nezlin et al., (1990). Specimens were embedded in paraffin oil and examined with a UV epifluorescent microscope LUMAM I-3 (LOMO, Russia) with the fluorescence combined with bright-field microscopy. A minimum of 30-40 juveniles of each species were examined.

The sensory organs of *P. vulgare* juveniles were studied using a modification of Ranvier's silver staining method (Retzius, 1906; Filipjev, 1924; Voronov et al., 1989). Juveniles were incubated for 10-20 sec. in a 0.1% solution of silver nitrate in distilled water, transferred for 30 seconds to photographic developer Kodak D-76, fixed for 1 h in 4% formaldehyde and embedded in glycerol using an evaporation method.

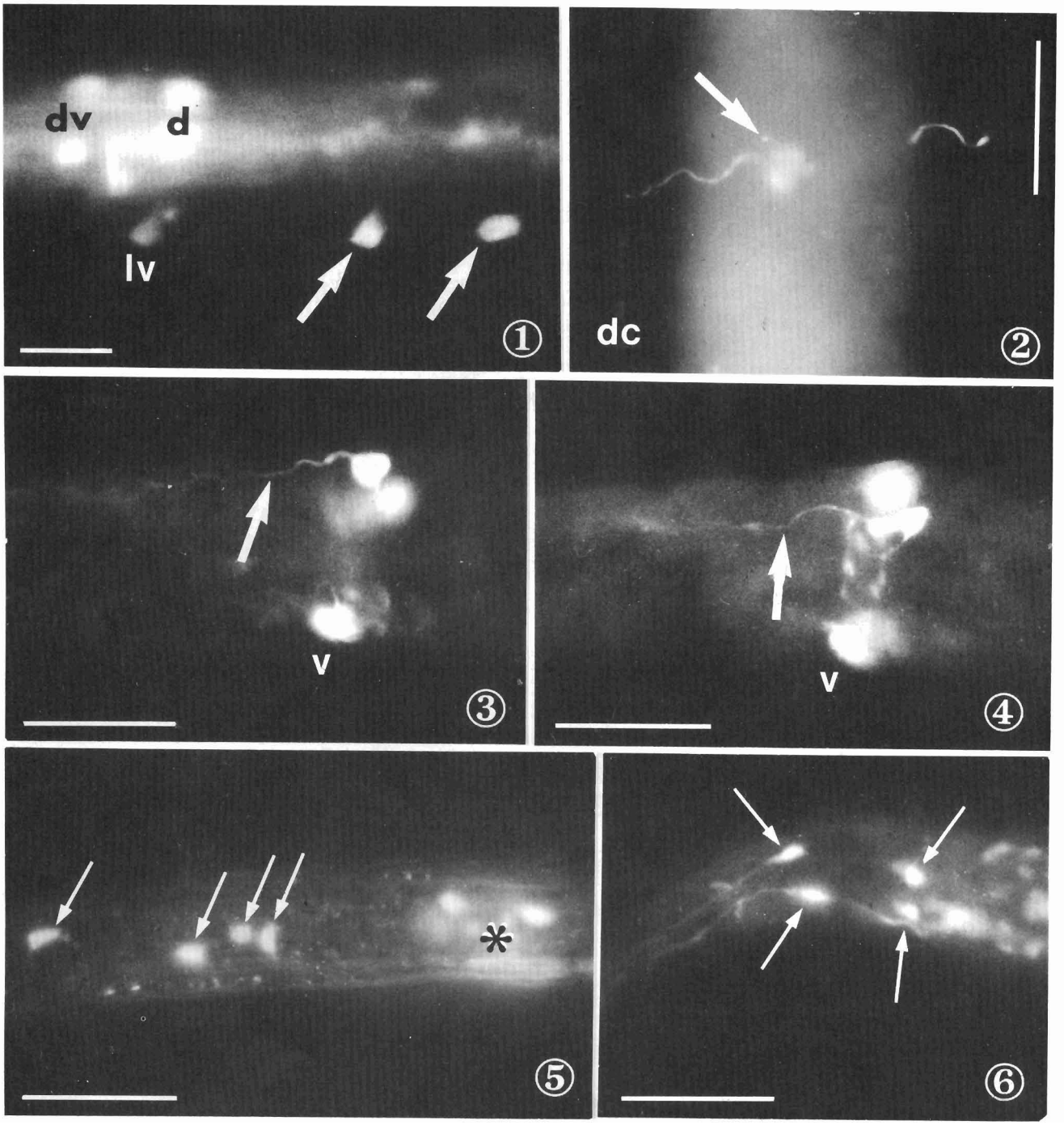
RESULTS

The widely accepted terminology is followed for describing the sensory structures and to distinguish the three circles of anterior head sensillae (six inner labial, six outer labial, and four cephalic papillae or bristles). All other papillae or bristles are considered being somatic.

Bright green fluorescence was revealed in cell bodies and processes after treatment with glyoxylic acid and with formaldehyde. The former was best for visualizing thin fibers whereas the latter was best for detecting perikaryons. The reasons for these differences is not known, but both methods were combined to obtain optimal results.

The length of first stage *E. brevis* juveniles is about 1 mm. The pattern of fluorescence was similar in all specimens examined. The results are illustrated in Figs. 1 and 2 and schematically summarized in Figs. 7 and 8. Fluorescence was observed in four CC cell bodies near the nerve ring (Fig. 1), and in ten cell bodies situated posterior to the nerve ring, along the body (Fig. 1 and 2). The neurons of the nerve ring (cephalic neurons) were arranged in two bilaterally symmetrical pairs: dorsal and ventral. In addition, six large varicosities were revealed around the nerve ring (two dorsal, two ventral and two lateral). The varicosities fluoresced in a similar way as the cell bodies, but could be distinguished from them by the absence of nuclei. Some processes were observed in the nerve ring and four processes were observed passing from the cephalic neurons and innervate the four cephalic bristles.

Ten CC cell bodies (somatic neurons) were observed in the lateral hypoderm ridges close to the cuticle surface, five each on the left and right sides, and each innervates a somatic bristle. Two pairs of CC bodies are situated in the posterior part of oesophageal region, one pair on the tail. Between these paired CC bodies four unpaired ones positioned: anteriormost on the right side adjacent to ventricular portion of the intestine, next CC body is on the left side, then again



Figs. 1-6. Glyoxylic acid induced histofluorescence of catecholamines in the juveniles of free-living marine nematodes. Fig. 1. Anterior portion of *Enoplus brevis*, latero-dorsal view, anterior at the left. d - a pair of dorsal cephalic neurons; dv - a pair of dorsal varicosities; lv - left lateral varicosity; arrows indicate the first and second left somatic neurons. Fig. 2. Third (unpaired) right somatic neuron in *E. brevis*, lateral view, anterior at the top. dc - dorsal nerve cord; arrow indicates sensory ending at the base of the somatic bristle. Figs. 3, 4. Cephalic neurons of *P. vulgare*, lateral view, anterior at the left; arrows indicate the processes passing from the right dorsal (Fig. 3) and left dorsal (Fig. 4) neurons and innervating cephalic bristles; v - a pair of ventral neurons. In this example the large varicosities near the nerve ring were not detected. Fig. 5. Anterior portion of *O. lepidus*, laterial view, anterior at the right; asterisk indicates the nerve ring region and arrows indicate four paired right somatic neurons. Fig. 6. The tail region of *P. macrodon*, latero-ventral view, anterior at the right; arrows indicate two pair of tail neurons. Scale bar - 20μm.

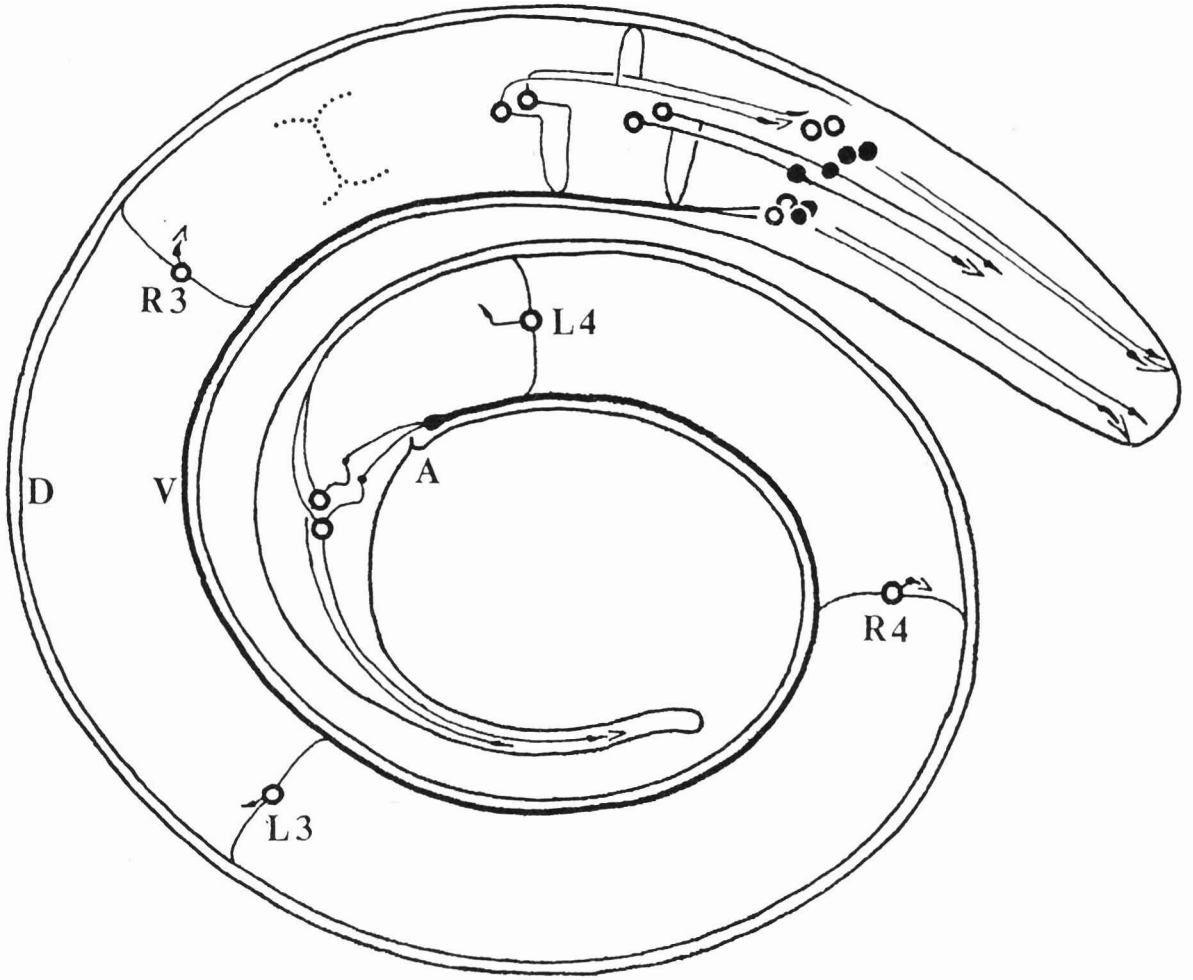


Fig. 7. Schematic presentation of nerve elements containing catecholamine in first stage juveniles of *Enoplus brevis*, right view; open circles, cell bodies of neurons containing catecholamine (CC); solid circles, large varicosities of CC neurons near the nerve ring; dotted line, pharyngeal-intestinal junction; A - anal opening; D - dorsal nerve cord; V - dorsal nerve cord; L3 and L4 - third and fourth left somatic unpaired neurons; R3 and R4 - third and fourth right somatic unpaired neurons. Only the right cephalic and somatic bristles are depicted, the thin processes in the nerve ring are not presented.

Table 2. Distribution of neurons containing catecholamine (CC) and somatic setae in first stage juveniles of *P. vulgare* and *O. lepidus*.

№ of juveniles		1	2	3	4	5	6	7	8	9	10
P. vulgare											
somatic CC neurons	left	11	11	10	10	11	11	10	10	11	10
	right	11	11	10	11	11	10	11	11	10	11
	total	22	22	20	21	22	21	21	21	21	21
somatic setae:	left	11	11	11	11	10	10	10	10	10	11
	right	11	11	10	10	11	10	11	11	10	11
	total	22	22	21	21	21	20	21	21	20	22
O. lepidus											
somatic CC neurons	left	10	9	11	10	9	10	10	9	10	9
	right	9	10	10	9	9	11	10	9	10	10
	total	19	19	21	19	18	21	20	18	20	19

one on the right side and posteriormost unpaired CC body on the left side.

Each cell of the anterior somatic pair had a process running anteriorly and dividing into two branches. One branch passed to the ventral nerve cord, and the other passed the nerve ring, connecting with the large lateral varicosity and innervating the first left and right somatic bristles. All other CC somatic neurons were connected with ventral and dorsal nerve cords by a pair of comissures (Fig. 2). Neurons of the second pair innervated the second pair of somatic bristles. The unpaired neurons innervated the unpaired somatic bristles. The tail neurons innervated a pair of somatic bristles on the tail.

The number of CC neurons and other features of catecholamine distribution in juvenile *E. communis* were identical to those in *E. brevis*.

Juvenile specimens of the other species examined contained catecholamines only in the ventral nerve cord with none observed in the dorsal cord. Therefore, somatic CC neurons in these nematodes produced only ventral but not dorsal comissures. Also, these species, as with *Enoplus* juveniles, had four cephalic CC neurons which innervated four cephalic sensillae (Fig. 3 and 4). The bodies of these neurons were situated as ventral and dorsal pairs near the nerve ring. Six large varicosities could be observed in the vicinity of the nerve ring, but their detection was not always reliable. These varicosities were arranged as ventral, lateral, and dorsal pairs and were similar to analogous structures in *Enoplus* juveniles. The processes of the first pair of somatic neurons were in contact with lateral varicosities as occurs in *Enoplus*. All juveniles had several somatic CC neurons and, as in *Enoplus* juveniles, these neurons were situated in the lateral hypodermic ridges.

The length of the first stage juvenile of *P. vulgare* is about 1.6 mm and *O. lepidus* about 1 mm. The number of CC somatic neurons in the juveniles of these species was variable (Table 2). Both species had four pairs of cell bodies at the level of the pharynx behind the nerve ring (Fig. 5). Immediately posterior were unpaired CC neurons and, as in *Enoplus* juveniles, the left and right neurons alternated. Their number varied in different specimens. In contrast to *Enoplus*, the first unpaired neuron was observed either on the right or left side, depending on the specimen being examined. Two CC neurons were present in the tail, innervated caudal bristles and produced processes to the ventral nerve cord. The number and localization of somatic CC neurons in *P. vulgare* appeared to correspond to that of the bristles (Table 2).

First stage juveniles of *P. macrodon* are about 0.3 mm long and have eight somatic CC neurons: one pair between the nerve ring and the mid-gut, one pair near the mid-gut anterior end, and two pairs in the tail (Fig. 6). First stage juveniles of *Ch. vivipara*, *S. balticus*, and *Th. setosus* are 0.4-0.5 mm long, and only have four somatic CC neurons: one pair at the level of the mid-gut anterior end and one pair in the tail.

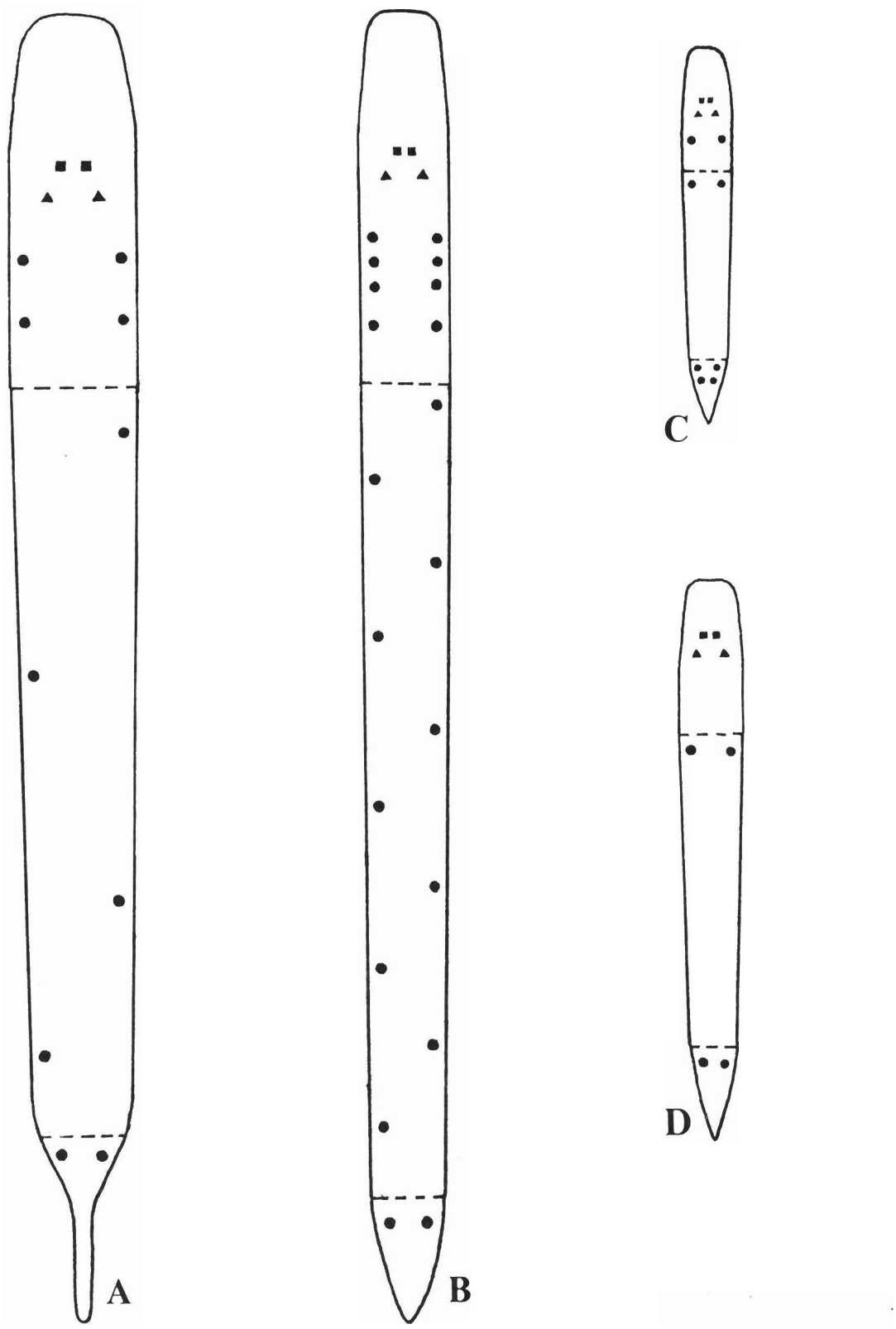


Fig. 8. Schematic presentation of the number and distribution of neurons containing catecholamine in juveniles of different species of free-living marine nematodes, dorsal view. A: *Enoplus brevis* and *E. communis*; B: *Pontonema vulgare* and *Odoncholaimus lepidus*; C: *Paracanthonhus macrodon*; D: *Chromodoropsis vivipara*, *Theristus setosus* and *Sphaerolaimus balticus*. The dashed lines indicate the level of the pharyngeal-intestinal junction and the level of the anal opening; squares, ventral cephalic neurons; triangles, dorsal cephalic neurons; circles, somatic neurons.

DISCUSSION

We have identified that juveniles of different marine nematodes possess constant numbers of cephalic CC neurons. As occurs with somatic neurons their number is constant for the majority of species, and each somatic CC neuron innervates a single sensilla. The distribution of the CC neurons in the species examined is summarized in Fig. 8.

The determination of cell bodies using the fluorescence technique with whole mount preparations is not entirely reliable as the neurons can not always clearly be distinguished from large varicosities. Previously, this resulted in a mistake when counting the cephalic neurons in *E. brevis* (Nezlin et al., 1990). In the present study both fluorescent and bright field microscopy were used as described by Sulston at al. (1975) to distinguish between large varicosities and nucleus-containing cell bodies.

Previously we demonstrated that maximum fluorescence emission, 480 nm, in nerve-like cells of *E. brevis* occurred after treatment with glyoxylic acid was 480 nm, as expected for catecholamines (Corrodi & Jonsson, 1967; Lindvall & Bjorklund, 1974).

The data obtained from juvenile specimens revealed that the number and localization of CC neurons in these nematodes differed from that known for adults of the same species (Malakhov & Nezlin, 1982; Malakhov et al., 1983). The adults had numerous (up to several hundred) CC neurons and probably their quantity is not constant. The perikaryons were situated mostly laterally, rare ventrally and dorsally, especially in the nerve ring region. The distribution of CC neurons corresponds to the distribution of somatic sensillae and probably, all the described CC neurons are somatic.

All adult Secernentea are known to have a constant set of four cephalic and four somatic CC neurons (Plotnikova et al., 1969; Sulston et al., 1975; Goh & Davey, 1976; Wright & Awan, 1978; Zhuchkova & Shishov, 1979; Sharpe & Atkinson, 1980). Additionally, they have several genital CC neurons, with each set being characteristic for the different species. The most detailed data have been obtained for CC neurons in *Caenorhabditis elegans* for which there is a complete electron microscopic reconstruction available (White et al., 1986) and the CC neurons of *C. elegans* are known to contain dopamine (Sulston et al., 1975). All CC neurons are sensory with each being connected with a particular sensilla. Four cephalic papillae in *C. elegans* are innervated by four cephalic neurons which are arranged in ventral and dorsal pairs near the nerve ring. Two deirids and two postdeirids are innervated also by CC neurons.

In the vicinity of the nerve ring the cephalic and deirid neurons of *C. elegans* form three pairs of large synaptic endings which are situated similarly as large varicosities in juveniles of marine nematodes. Therefore, the cephalic and anterior somatic CC neurons of Secernentea and of juveniles of marine nematode species seem to correspond. All nematodes investigated previously have this minimal set of CC neurons and, in particular, *C. elegans* juveniles have only these neurons present (Sulston et al., 1975). A single exception is *Trichinella spiralis*, which had eight cephalic neurons present in each development stage studied (Lee & Ko, 1991). However, as in *T. spiralis*, the pattern of catecholamine distribution near the nerve ring is similar to that in *C. elegans*, and it can be concluded that four of the eight CC neurons reported in *T. spiralis* actually are large varicosities.

The remaining somatic CC neurons of juveniles marine nematodes are similar to the postdeirid neurons present in Secernentea, but the number and distribution of the somatic neurons in the former vary between different species or between different specimens, as in representatives of the Oncholaimidae. Therefore, marine Adenophorea differ from Secernentea by the presence of a pair of CC neurons in the tail region where they innervate caudal sensillae. However, one Secernentea species, *Panagrellus redivivus*, has a pair of CC neurons in the tail where they innervate lateral papillae situated in the region of intestinal-rectal junction (Sharpe & Atkinson, 1980).

In conclusion, the number and distribution of CC neurons in the juveniles of marine Adenophorea is similar to that in juveniles and adults of Secernentea but not to that of adult Adenophorea. The simple set of CC neurons is characteristic for Secernentea and

juveniles of Adenophorea and is probably fundamental for Nematoda.

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Воронов Д.А., Незлин Л.П. Катехоламинсодержащие нейроны у личинок восьми видов свободноживущих морских нематод.

Резюме. Число и локализация катехоламинсодержащих (КС) нейронов, изученных водным формальдегидным и глиоксилатным методами у личинок первой стадии восьми видов свободноживущих морских нематод из подкласса Adenophorea видоспецифичны. У всех видов обнаружены две пары цефалических КС нейронов, иннервирующих четыре цефалические щетинки. Расположение и число остальных КС нейронов (соматических) различно у разных видов и в целом соответствует соматическим сенсиллам. По распределению КС нейронов личинки морских нематод ближе к нематодам подкласса Secernentea, чем ко взрослым особям своего вида.