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GENERAL BIOLOGY

Nervous System and Musculature of the Parasitic Turbellarian *Notentera ivanovi* (Plathelminthes, Fecampiida)

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Abstract—The article reports the first description of the architecture of the musculature and cholinergic nervous system of the parasitic turbellarian *Notentera ivanovi* from the White Sea using histochemistry and confocal scanning laser microscopy. It has been demonstrated that the body wall is composed of layers of circular and longitudinal muscles between which there are diagonal muscles described here for the first time. The nervous system is of the regular closely spaced orthogon type with pronounced radiality. In order to make it clear whether the orthogon of this type is a phylogenetic characteristic of this group of worms or its shape is associated with the flat rounded body, the study of other representatives of the Fecampiida group is required.

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The nervous and muscular systems are considered to be the most evolutionarily conserved biological systems and, hence, are of potential interest for macroevolutionary studies. The transformations of the neuromuscular system in the course of evolution in the taxonomic groups closely related to the most basal parasitic flatworm taxons (Neodermata) draw special attention of zoologists and parasitologists. The commonly used molecular phylogeny approaches have already helped to conquer many issues of zoological taxonomy and phylogeny, but yet failed to uniquely define the turbellarian group which would be the most basic one among the parasitic flatworms in terms of evolution [1–3]. In view of this, we have chosen the object of research for the current study to be the turbellarian *Notentera ivanovi* Joffe, Selivanova, Kornakova, 1997, a parasitic organism living in the gut of the polychaete worm *Micronephthys minuta* from the White Sea. This worm is a representative of Fecampiida, a group of rare parasitic turbellarians remarkable for the fact that their spermiogenesis follows the same mechanism (Revertospermata) as in the Neodermata group of parasitic flatworms [4]. This observation underlies the interest taken in these turbellarians as the possible ancestors of Neodermata. However, the nervous and muscular systems of the representatives of this group have never been examined yet using the modern immunohistochemistry or histochemistry techniques in combination with confocal microscopy, whereas the descriptions of the biological structures made using the conventional histological techniques alone cannot be relied upon due to their poor resolution.

The current study presents the first description of the musculature and the nervous system of a fecampiid turbellarian.

About 30 *Notentera ivanovi* specimens were collected in the vicinity of the White Sea Biological Station of the Zoological Institute. The polychaete worms *Micronephthys minuta*, the hosts of *Notentera ivanovi,* were obtained by dredge sampling at the depths of about 10 m at Korov'ya Varaka. The material was fixed in Bouin's fixative modified by Stefanini–de Martino–Zamboni or in 4% neutral formalin. Histochemical staining with phalloidin [5] combined with confocal microscopy was used to study the muscular system. To study the nervous system, the histochemical Zherebtsov's method for the detection of cholinesterases [6] was used. The detection of cholinesterases should be regarded as an indirect demonstration of the presence of acetylcholine and acetylcholine-mediated signal transmission in the synapses. The incubation medium used in the experiments contains acetylcholine. Thus, if cholinesterase, the enzyme catalyzing the hydrolysis of acetylcholine and spatially associated with the nervous system, is present in the turbellarian tissues, histochemical reaction reveals the precise architecture of the major conduction pathways.

Here we present the results of our study.

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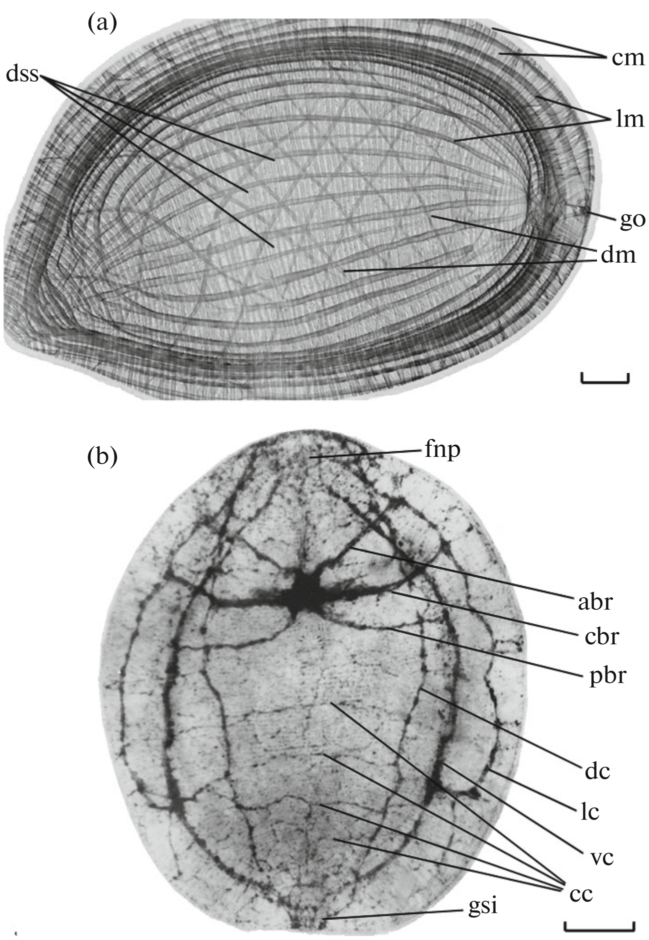

Fig. 1. Musculature of Notentera ivanovi (a), ventral view, phalloidin-TRITC staining and (b) its cholinergic nervous system, dorsal view, staining according to Zherebtsov. Abbreviations: abr, anterior brain root; cbr, central brain root; cc, circular commissure; cm, circular muscles; dc, dorsal nerve cord; dm, diagonal muscles; dss, diamond-shaped structures; fnp, frontal nerve plexus; go, genital opening; gsi, genital system innervation; lc, lateral nerve cord; lm, longitudinal muscles; pbr, posterior brain root; vc, ventral nerve cord. Scale bar, 100 μm.

Muscular system architecture. The musculature of the body wall of *Notentera ivanovi* includes circular, longitudinal, and diagonal muscles. It is in this precise order that these types of muscles are located under the basal lamina of the epidermis, forming a muscular sac typical of turbellarians. Outer circular muscles 6.5–7 μm thick form a closely-packed layer at the proximal and the distal ends of the body with the distance of no more than 3.5 μm between them. The distance between the muscles increases to 4–7 μm towards the center of the body. Under the circular muscles, there are diagonal muscles with the muscle fibers thickness of approximately 10 μm. Diagonal musculature all along the turbellarian body is represented by the inter-crossing of two layers of muscles running diagonally toward each other; one layer being formed by muscles running from the proximal end down and another layer by the muscles running from the distal end up with overlap. Diamond-shaped structures can be observed where the two layers of diagonal muscles cross over; they are more regular in the middle part of the body. Their size directly depends on the body length, with the largest being invariably observed within the anterior third of the body (Fig. 1a). The deepest layer of the body wall is composed of strong longitudinal muscle fibers 13 μm thick, which lay at intervals of 10–17 μm, with the largest intervals in the middle part of the body. In the lateral regions, up to six marginal longitudinal muscles 13 μm thick come closer to one another and partly fuse to form massive muscular bands (Fig. 1a). The presence of these muscular structures disrupts the geometry of diagonal muscles. At the proximal end of the body, the right and left muscular bands are interconnected by their thinner parts, which are 5–7 μm thick. Other longitudinal muscles turn back, in some cases not reaching the anterior end of the animal, and continue running backwards along the opposite side of the body (Fig. 1a). All the above measurements were made using a 1.3-mm-long specimen.

N. ivanovi lacks the digestive system and mouth opening, and, consequently, has no specific organization of the muscular sac on the ventral side of the body. The reproductive system is primitive. It is located at the distal end of the body and ends with a posterior terminal opening surrounded by a muscular sphincter, a derivative of the longitudinal musculature (Fig. 1a). The genital opening is located at the junction of the two longitudinal muscles with the approaching neighbor longitudinal muscle fibers and a number of thin radial muscle strands.

Architecture of the nervous system. The brain in *N. ivanovi* is located at the edge of the proximal third of the body dorsally and looks like a star with six rays from which three pairs of long brain roots of uniform thickness extend (Fig. 1b). With the body length of the worm of 740 μm, the length of the brain neuropile along the middle axis is 50 μm. Brain roots run to the paired ventral, dorsal, and lateral longitudinal cords, which are shifted laterally. The shortest posterior brain roots extend to the strong ventral cords. Central roots fork approximately at the mid-length, with short branches running to the dorsal nerve cords and longer branches running to the ventral cords and further to the lateral cords. Anterior roots extend straight to the marginal lateral cords. Longitudinal cords are interconnected by seven circular commissures (Fig. 1b). The first three commissures join the brain roots, and the remaining four commissures are evenly distributed along the postcebral part of the body. The lateral cords are connected at the anterior end, while both the ventral and dorsal cords reach them. All cords divide into branches which innervate the anterior body end forming a frontal nerve plexus (Fig. 1b). The ventral cords are connected with the lateral cords at the posterior end with the dorsal cords reaching them. The posterior parts of the nerve cords divide into branches innervating the posterior terminal genital opening and the primitive genital system (Fig. 1b). The thin and diffuse submuscular plexus can be seen along the whole length of the body.

Notentera ivanovi, the new parasitic turbellarian living in the gut of polychaete worms, was first described by Joffe et al. [7]. In their study, the authors reported the description of the musculature of the worm. However, they detected only the layers of circular and longitudinal muscles, but did not show the presence of the diagonal muscles. It seems that it was challenging to differentiate between the more widely spaced diagonal muscles and the circular muscles, which are almost of the same thickness, on the histological cross sections. The authors also did not describe the massive longitudinal lateral muscular bands, the contraction of which, apparently, allows the worm to adhere to the gut wall of the polychaete host by its ventral side.

With regard to the nervous system, the orthogon of *Notentera* should be defined as regular closely spaced orthogon with pronounced elements of radiality according to Kotikova's classification [8]. The causes underlying the presence of the orthogon of such type in *Notentera* are not yet clear. The rounded and flattened body of this turbellarian may in some way account for the transition from regular orthogons to radial ones. A similar observation was made, e.g., for monogeneans with the broad leaf-shaped body [8]. On the other hand, the radial-type orthogons may be a characteristic philogenetic trait of all fecampiids. However, this assumption may be confirmed or rejected only after studying other representatives of this group, which has never been investigated earlier in respect of their nervous system. Thus, the architecture of the nervous system may appear to be an important phylogenetic trait when the issue of the Neodermata sister group is addressed.

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