



General morphology of the muscle system in the female orthonectid, *Intoshia variabili* (Orthonectida)

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Abstract: The contractile system of the female *Intoshia variabili* (Orthonectida) was studied using rhodamine-phalloidin staining. The contractile fibres are true muscles. The muscle system consists of eight-twelve circular fibres, restricted to the anterior third of the body, and six-eight longitudinal fibres. The circular fibres are positioned beneath the longitudinal ones. The pattern of the musculature determines the bilateral body plan.

Résumé : Morphologie générale du système musculaire chez la femelle de *Intoshia variabili* (Orthonectida). Le système contractile des femelles de *Intoshia variabili* (Orthonectida) a été étudié par coloration avec la rhodamine-phalloïdine. Les fibres contractiles sont des muscles authentiques. Le système musculaire se compose de huit à douze fibres circulaires, limitées au tiers antérieur du corps, ainsi que de six à huit fibres longitudinales. Les fibres circulaires sont localisées au-dessous des fibres longitudinales. L'arrangement général de la musculature détermine le plan de symétrie bilatérale du corps.

Keywords: Orthonectida, muscle system, rhodamine-phalloidin staining.

Introduction

Among the vast diversity of invertebrate taxa, Orthonectida is one of the most enigmatic groups of lower Metazoa, whose phylogenetic relationships remain uncertain. Representatives of this group are parasites of a wide range of marine invertebrates. Their life cycle is rather simple and consists of a free-living sexual stage and a parasitic stage, commonly referred to as plasmodium. However, some authors consider the latter to be transformed host tissue, rather than a true plasmodial stage (Kozloff, 1994, 1997). The males and females develop from germinal cells within

the plasmodium and, after leaving the host, copulate. Ciliated larvae develop within the female and infect new hosts (Caullery & Lavallée, 1912; Caullery, 1961). The morphology of orthonectids is poorly known: only three species have been studied on the ultrastructural level (Kozloff, 1969, 1971; Slyusarev, 1994).

The main mode of locomotion of orthonectid males and females is ciliary swimming (Giard, 1877; Metschnikoff, 1879; Caullery & Mesnil, 1901), generally considered to be a primitive feature. In addition, females of *Intoshia variabili* Alexandrov and Slyusarev, 1992 were observed to bend the anterior body end (Slyusarev, 1994) which indirectly suggested the presence of a contractile system. Caullery and Mesnil (1901) reported supposedly contractile cells in orthonectids, and Kozloff (1969, 1971) described these cells

in *Rhopalura ophiocoma* Giard and *Ciliocincta sabellariae* Kozloff, 1965, both on the TEM level and in Protargol-impregnated preparations. Later, morphologically similar cells were described in the female of *I. variabilis* (Slyusarev, 1994). However, whether the contractile system of Orthonectida is composed of true muscle cells remained unknown.

The rhodamine-phalloidin staining of entire specimens is a very promising method which has recently been employed to study the three-dimensional muscle arrangements in small metazoans, including Platyhelminthes (Rieger et al., 1994; Reiter et al., 1996) and Acoela (Tyler & Hyra, 1998). We used this technique to reveal the F-actin component of the contractile fibres of *I. variabilis*, and in this paper we describe the general morphology of contractile cells in the female of this species.

Materials and methods

Intoshia variabilis is a parasite of the turbellarian *Macrorhynchus crocea* (O. Fabricius, 1826) (Platyhelminthes, Kalyptorhynchia). The hosts were collected from algae at the upper sublittoral level near the White Sea Biological Station of the Zoological Institute, Russian Academy of Sciences (Chupa Inlet, White Sea). The procedures for sampling and maintaining the turbellarians and obtaining the sex stages of *I. variabilis* have been described in detail by Slyusarev (1994). The orthonectid females were fixed in Stefanini's fixative (Stefanini et al., 1967), rinsed in PBS, stained in rhodamine phalloidin (Molecular Probes Inc., R-415) solution in PBS (1:20) for 40 min, mounted in glycerol-phenilendiamine medium, and viewed using a Zeiss Axioskop 50 epifluorescence microscope. A total of more than twenty specimens were examined and documented.

Results

The contractile system of *I. variabilis* females consists of longitudinal and circular fibres (Figs 1, 6). The longitudinal muscles run from the anterior tip of the animal to its posterior end. At anterior end, the longitudinal muscles converge, then spread, forming V-shaped forkings (Figs 1, 2, 6). Some of the longitudinal fibres bifurcate at or slightly behind the mid-body level, and the resulting branches may merge again closer to the hind body end (Fig. 6). In some specimens, a single longitudinal muscle appears to consist of two fibres running close together. The number of the longitudinal fibres varies from six (most frequent variant) to eight.

The circular fibres lie under the longitudinal ones and are restricted to the anterior third of the body. In all specimens

examined, they are arranged in two loose rings, each consisting of four-five circular fibres (Figs 1, 2, 6). The two rings lie in non-parallel planes, in such a way that they are positioned very close together on one side of the body but separated by a considerable gap on the opposite side (Figs 1, 3–5). Such an arrangement violates the otherwise radial symmetry of the orthonectid and determines a specific plane of bilateral symmetry, which may be tentatively referred to as dorso-ventral. The side on which the two rings of circular fibres converge is termed the dorsal side, and the side with the gap, the ventral one. The bilateral symmetry is observed in the longitudinal fibres as well: even though their arrangement is not strictly symmetrical, the fibres are confined to two opposed lateral groups and always absent dorsally and ventrally.

Besides the longitudinal and circular fibres, a less intensely stained superficial orthogonal network is observed in some preparations; this network corresponds to the borders of ciliated and non-ciliated epidermal cells (partly observable in Fig. 1). These cells have at least two different systems of filaments, one of which forms the subapical belt (Slyusarev, 1994). It is difficult to state whether this weak fluorescent signal reveals epidermal actin filaments or represents a processing artefact. The superficial epidermal network was, however, easily distinguished from the subepidermal contractile fibres.

Discussion

The results of rhodamine-phalloidin staining are in good agreement with the previously reported TEM data on the number and position of contractile cells (Slyusarev, 1994). Thus, the contractile system of the female of *I. variabilis* is composed of true muscles.

The muscle pattern is generally considered one of the main aspects of the body plan, characterizing taxa of high taxonomic rank. For over a hundred years since their description by Giard (1877), Orthonectida have been assumed to be related to a number of invertebrate taxa, including Cnidaria (Hatschek, 1888; Caullery & Mesnil, 1901; Hartmann, 1925), Platyhelminthes (Stunkard, 1954; Ivanov, 1983), Rotifera (Giard, 1879), and Archiannelida (Metschnikoff, 1881). Although the available information is insufficient for a detailed comparison, it is evident that the arrangement of muscles revealed in *I. variabilis* shows no close resemblance to any of these groups.

The general pattern of the muscle system in *I. variabilis* females is characterized by a combination of three main features: position of longitudinal muscles outside of circular ones; presence of circular muscles only in the anterior body part; and the general bilateral symmetry of the musculature. Although such a combination of characters appears unique,

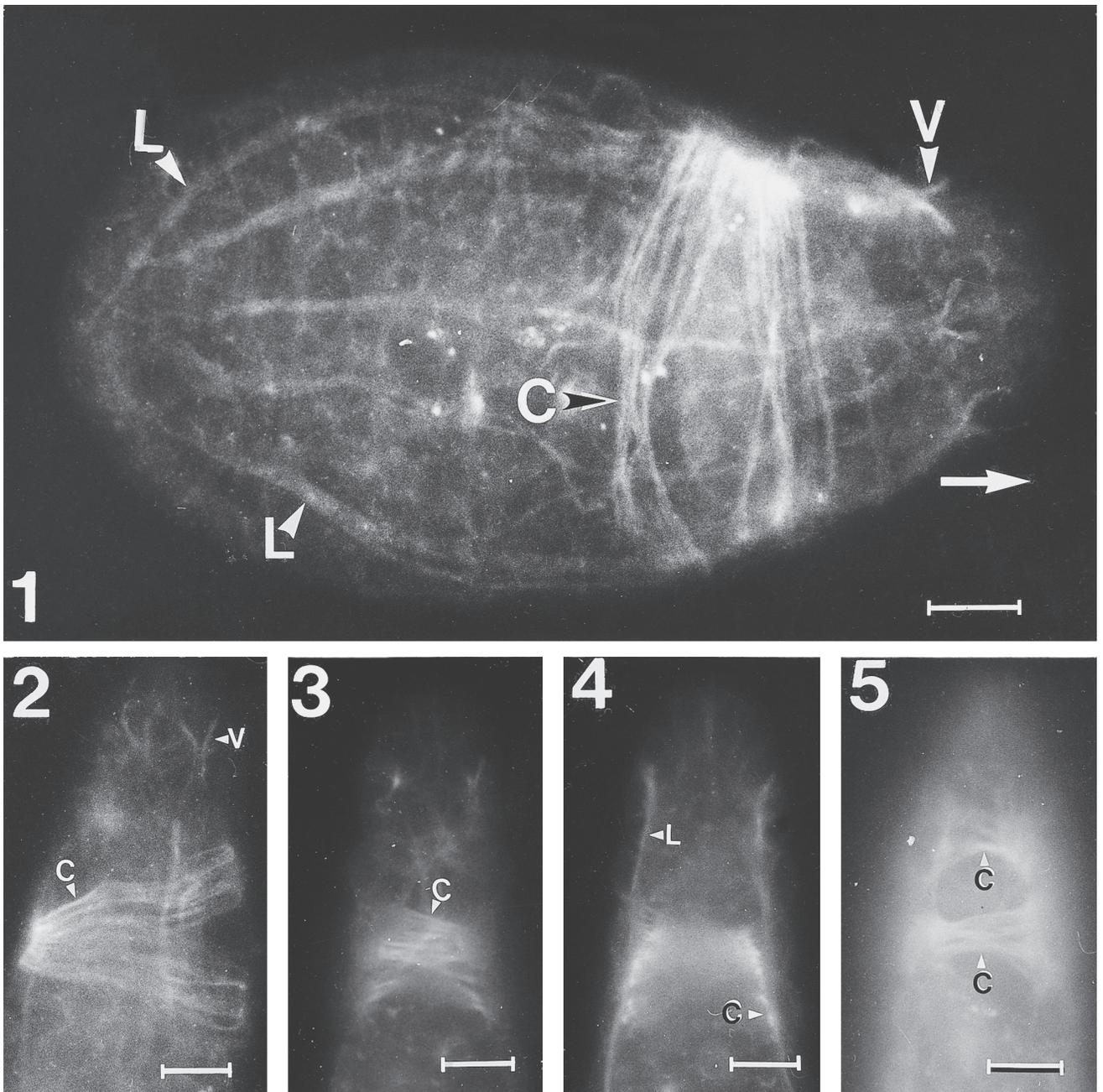


Figure 1. Ventrolateral view of female *Intoshia variabili*. (C) circular muscles; (L) longitudinal muscles; (V) v-shaped forking at anterior end. (Arrow) shows the direction of ciliary swimming. Scale bar: 10 μ m.

Figure 1. Vue ventrolatérale de la femelle de *Intoshia variabili*. (C) muscles circulaires ; (L) muscles longitudinaux ; (V) bifurcation en forme de v à l'extrémité antérieure. La flèche indique la direction du mouvement de l'Orthonectide. Echelle : 10 μ m.

Figure 2. Lateral view of anterior part of female *Intoshia variabili*. (C) circular muscles; (L) longitudinal muscles. Scale bar: 5 μ m.

Figure 2. Vue latérale de la partie antérieure de la femelle de *Intoshia variabili*. (C) muscles circulaires ; (L) muscles longitudinaux. Echelle : 5 μ m.

Figures 3–5. Frontal optical sections at different planes of the same specimen of female *Intoshia variabili* showing the dorsal (3), median (4), and ventral (5) planes. (C) circular muscles; (L) longitudinal muscles. Scale bars: 5 μ m.

Figures 3–5. Sections frontales optiques différemment focalisées du même spécimen femelle de *Intoshia variabili*, faisant voir les plans présumés dorsal (3), médian (4) et ventral (5). (C) muscles circulaires ; (L) muscles longitudinaux. Echelle : 5 μ m.

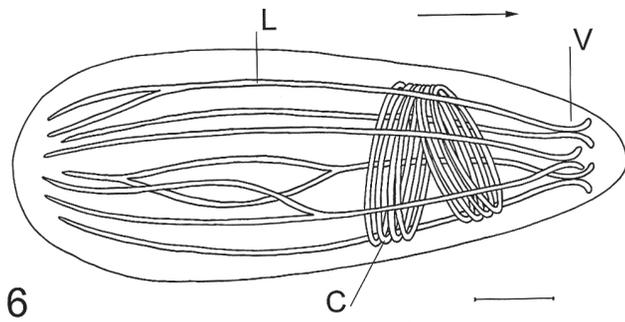


Figure 6. Diagrammatic representation of muscle arrangement in female *Intoshia variabili*, as revealed by rhodamine-phalloidin staining. (C) circular muscles; (L) longitudinal muscles; (V), v-shaped forking at anterior end. Scale bar: 20 μ m.

Figure 6. Représentation schématique de la disposition des fibres musculaires chez la femelle de *Intoshia variabili* révélée par la coloration avec la rhodamine-phalloïdine. (C) muscles circulaires ; (L) muscles longitudinaux ; (V), bifurcation en forme de v à l'extrémité antérieure. Echelle : 20 μ m.

we consider it premature to assign any decisive phylogenetic meaning to these features. It should be noted that the general muscle pattern characterizing the entire taxon Orthonectida may be different from that described in *I. variabili*. No data on the male musculature of this species is available at present. Furthermore, the female of *I. variabili* is the smallest of all known orthonectids, and it is quite possible that its muscle system is reduced to some extent. The variability of the musculature observed in this species indirectly confirms this reasoning.

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References

Caullery M. 1961. Classe des Orthonectides (Orthonectida Giard 1877). In: *Traité de Zoologie* (P.P. Grassé, ed.), IV, pp. 695–706. Paris.

Caullery M. & Mesnil F. 1901. Recherches sur les orthonectides. *Archives d'Anatomie Microscopique*, **4**: 381–470.

Caullery M. & Lavallée A. 1912. Recherches sur le cycle évolutif des orthonectides. Les phases initiales dans l'infection expérimentale de l'ophiure, *Amphiura squamata*, par *Rhopalura ophiocomae* Giard. *Bulletin Scientifique de la France et de la Belgique*, **46**:139–171.

Giard A. 1877. Sur les Orthonectida, classe nouvelle d'animaux parasites des Echinodermes et des Turbellariés. *Comptes Rendus des Séances de l'Académie des Sciences*, **85** (5): 812–814.

Giard A. 1879. On the organization and classification of the Orthonectida. *Annals and Magazine of Natural History*, Series 5, **4** (5): 471–473.

Hartmann D.W. 1925. Mesozoa. In: *Handbuch der Zoologie*, Bd 1, pp. 996–1014.

Hatschek B. 1888. *Lehrbuch der Zoologie*. Jena. 144 pp.

Ivanov A.V. 1983. [On the taxonomic position of Mesozoa] *Trudy Zoologicheskogo Instituta Akademii Nauk SSSR*, **109**: 76–89 [in Russian with English summary].

Kozloff E.N. 1969. Morphology of the orthonectid *Rhopalura ophiocomae*. *Journal of Parasitology*, **55**: 171–195.

Kozloff E.N. 1971. Morphology of the orthonectid *Ciliocincta sabellariae*. *Journal of Parasitology*, **57**: 377–406.

Kozloff E.N. 1994. The structure and origin of the plasmodium of *Rhopalura ophiocomae* (Phylum Orthonectida). *Acta Zoologica (Stockholm)*, **75**:191–199.

Kozloff E.N. 1997. Studies on the so-called plasmodium of *Ciliocincta sabellariae* (Phylum Orthonectida), with notes on an associated microsporan parasite. *Cahiers de Biologie Marine*, **38**:151–159.

Metschnikoff E. 1879. Zur Naturgeschichte der Orthonectiden. *Zoologischer Anzeiger*, **2**: 547–549.

Metschnikoff E. 1881. Untersuchungen über Orthonectiden. *Zeitschrift für wissenschaftliche Zoologie*, **35**: 282–303.

Reiter D., Boyer B., Ladurner P., Mair G., Salvenmoser W. & Rieger R.M. 1996. Differentiation of the body wall musculature in *Macrostomum hystricinum marinum* and *Hoploplana inquilina* (Plathelminthes), as models for muscle development in lower Spiralia. *Roux's Archives of Developmental Biology*, **205** (7/8): 410–423.

Rieger R.M., Salvenmoser W., Legniti A. & Tyler S. 1994. Phalloidin-rhodamine preparations of *Macrostomum* (Platyhelminthes): functional morphology and postembryonic development of the musculature. *Zoomorphology*, **114**: 133–148.

Slyusarev G.S. 1994. The fine structure of the female *Intoshia variabili* (Alexandrov & Sljusarev) (Mesozoa: Orthonectida). *Acta Zoologica (Stockholm)*, **75**: 311–321.

Stefanini M., Martino C. & Zamboni L. 1967. Fixation of ejaculated spermatozoa for electron microscopy. *Nature*, **216**: 173–174.

Stunkard H.W. 1954. The life-history and systematic relations of the Mesozoa. *Quarterly Review of Biology*, **29**: 230–244.

Tyler S. & Hyra G.S. 1998. Patterns of musculature as taxonomic characters for the Turbellaria Acoela. *Hydrobiologia*, **383**: 51–59.