

## The neuromuscular junction in the amphioxus myotomes revealed by an ultrastructural and immunohistochemical study

I. Bočina<sup>1</sup>, M. Saraga-Babić<sup>2</sup>

1. Department of Biology, Faculty of Science, Teslina 12, 21 000 Split, Croatia.

2. Department of Anatomy, Embriology and Histology, School of Medicine, Šoltanska 2, 21 000 Split, Croatia

bocina@pmfst.hr

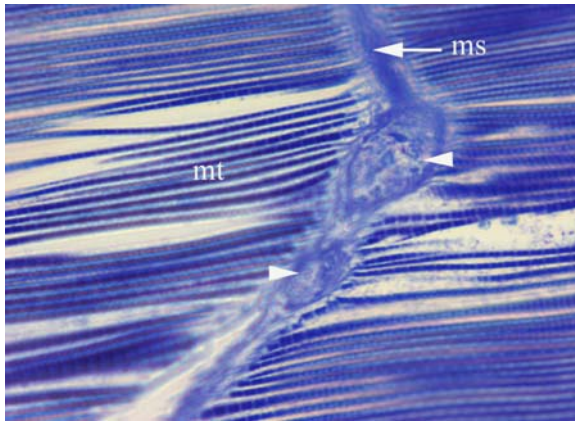
Keywords: amphioxus, locomotion, myosepta, neuromuscular spindle

The amphioxus muscles constitute the bulk of its body consisting of about sixty V-shaped segments (myotomes) separated by connective tissue septa called myosepta. In amphioxus, muscle fibers never attach directly to axial structure of the notochord, so the important role in transmission of muscular forces to the notochord is taken by the myosepta. In order to define this specific role of the myosepta in amphioxus during locomotion, 10 adult specimens were analyzed ultrastructurally and immunohistochemically.

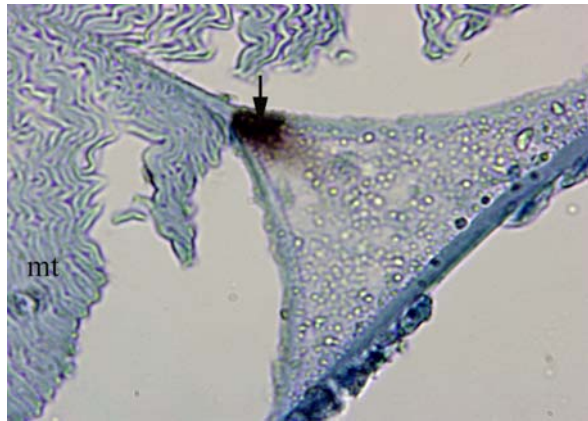
In the semi-thin sections, each myosepta contains two round-shaped structures resembling the peripheral nerves surrounded by the connective tissue (Fig. 1). In the ultra-thin sections, the smaller oval structure resembled very much the neuromuscular spindle containing nerve and muscle fibers encircled by collagen fibers (Fig 3). The nearby structure within the septa resembled the peripheral nerve made of neural fibers encircled by perineurium (Fig. 4). Applied antibodies to neurofilament and  $\beta$ -tubulin showed positive reaction in the described area, as well (Fig. 2). Although the myoseptal innervations in amphioxus have already been described earlier, the neuromuscular spindle-like structure is described in this study for the first time. We suppose that the neuromuscular spindle controls the position and movement of the amphioxus body in the space, thus helping the notochord to maintain the body stiffness during undulatory locomotion.

1. I. Bočina, M. Saraga-Babić, *Int. J. Bio. Sci.* **2** (2006) p73.
2. I. Bočina, M. Saraga-Babić, *Coll Antropol.* **30** (2006) p361.
3. P. R. Flood, *Symposia of the Zoological Society of London.* **36** (1975) p81.
4. S. Gemballa, F. Vogel, *Compar Biochem Phys A.* **133** (2002) p1013.
5. S. Gemballa et al., *Zoomorphology* **122** (2003) p169.
6. A. Urano et al., *Evol Dev.* **5** (2003) p447.
7. T. Nakao, *J. Comp. Neur.* **165** (2004) p1.

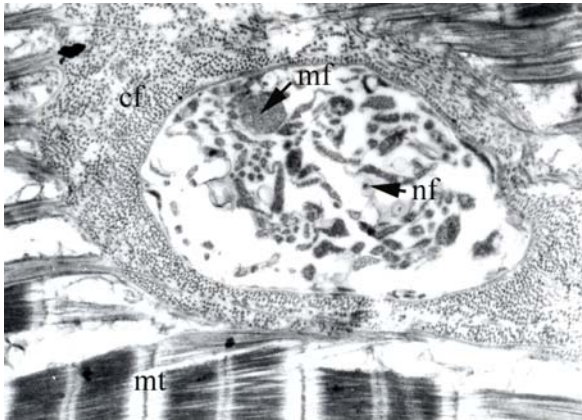
This research was supported by Croatian Ministry of Science and Education; grant number: 216-2160528-0507. The aid of Dr. Nikola Ljubešić and Laboratory for Electron microscopy of “Ruder Bošković” Institute is gratefully acknowledged. We are also grateful to Mrs. Asja Miletić for her skilful technical assistance.



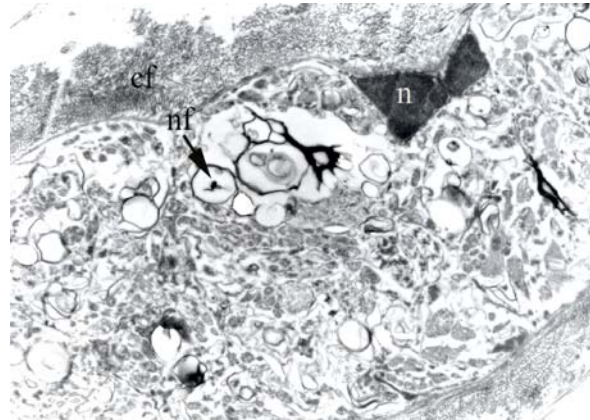
**Figure 1.** Semi-thin section through the amphioxus myotomes: myotome (mt), myosepta (ms), myoseptal innervation (arrowheads). Methylene-blue, 100x.



**Figure 2.** Positive reaction to neurofilament antibody in the area of myoseptal innervation (↑): myotome (mt). DAB, 100x.



**Figure 3.** Ultra-thin section through myosepta: myotome (mt), collagen fibers in myosepta (cf), muscle fiber (mf), nerve fiber (nf). TEM, 25 000x.



**Figure 4.** Peripheral nerve in myosepta: nerve fiber (nf), nucleus of the epithelial cell in perineurium (n), collagen fibers (cf). TEM, 12 500x.