

Axonal sheaths in two reportedly myelinated polychaete nervous systems: *Asychis elongata* and *Capitella sp. I*.

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Myelin has evolved independently in three invertebrate groups: copepods (Crustacea), malacostracans (Crustacea) and oligochaetes (Annelida)^{2,4}. Described over 100 years ago in malacostracans and oligochaetes based on staining and other properties of the sheath, it has since been confirmed in all three groups by electron microscopy. In 1889, Friedländer also described myelin in a capitellid polychaete¹. In a 1948 review, Nicol identified myelinated giant axons in three polychaete families: Maldanidae (“bamboo worms”), Capitellidae and Spionidae³. To verify these light-microscopic reports, we examined the large axons of the ventral nerve cord (VNC) of specimens from the Maldanidae and Capitellidae using modern transmission electron microscopy (TEM).

Capitella sp. I (Capitellidae), were obtained from a culture maintained by Dr. Elaine Seaver of the Kewalo Marine Laboratory, PBRC, University of Hawaii. Pieces of body wall containing the VNC were fixed overnight at 4°C in 4% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4, with 0.24M sucrose and 2mM CaCl₂. The tissues were then rinsed in 0.1M sodium cacodylate buffer with 0.31M sucrose, postfixed in buffered 1% OsO₄ for 1 hour, dehydrated in a graded ethanol series and propylene oxide, and then embedded in LX-112 epoxy resin. After resin polymerization, ultrathin (75-90 nm) sections were taken, double-stained with uranyl acetate and lead citrate, and photographed in a LEO912 EF transmission electron microscope at 100 kV. *Asychis elongata* (Maldanidae) were collected from mud flats at Mt. Desert Narrows, Frenchman Bay, Hancock Co., ME. Tissues were fixed using the same protocol; however, after fixation in glutaraldehyde and prior to postfixation in OsO₄, the tissues were transferred to 0.2M phosphate buffer (Sorensen’s) and shipped cold to Hawaii.

Figure 1A1 is a light micrograph of a section from the anterior end of the *Asychis* VNC. In this region, a single dorsal medial giant axon (gax) is present. It is surrounded by a thick sheath consisting of multiple layers of spindle-shaped overlapping cellular profiles. Many sheath cells contained elongate fibrous bodies (possible sources of birefringence) and dark granules ranging upward in size from 6 nm (Fig. 1A2, 3). Adjacent cells were separated by spaces of 13 nm, measured between midpoints of the membranes. An example of partially-overlapping margins of sheath cells, with close apposition between, is shown in Figure 1A3. No extracellular material was noted in the sheath. Adjacent small axons were not ensheathed (Fig. 1A2). While the spacing between sheath cells was narrow and might contribute to insulating properties, there was no evidence of the membrane condensation or attachment structures typical of oligochaete, crustacean or vertebrate myelins.

An optimal fixation protocol for the *Capitella* VNC was never resolved. Although we adjusted the osmolarity of the fixative, membranes appeared more disordered than those of *Asychis* (Fig. 1B). Nevertheless, even among the larger axons (ax), we observed no evidence of multilamellar membranous ensheathments resembling the myelin of myelinate groups (Fig. 1B2).

We failed to verify with TEM the occurrence of myelin in axonal sheaths of two genera (*Asychis* and *Capitella*) from the same families reported to possess myelin, based on light microscopy (genera *Clymenella* and *Mastobranchus*, respectively^{1,3}). While this calls into question the light microscopic conclusions, possibilities still exist for inter-generic differences in myelination patterns or myelin presence in other regions within the nervous systems of these organisms.

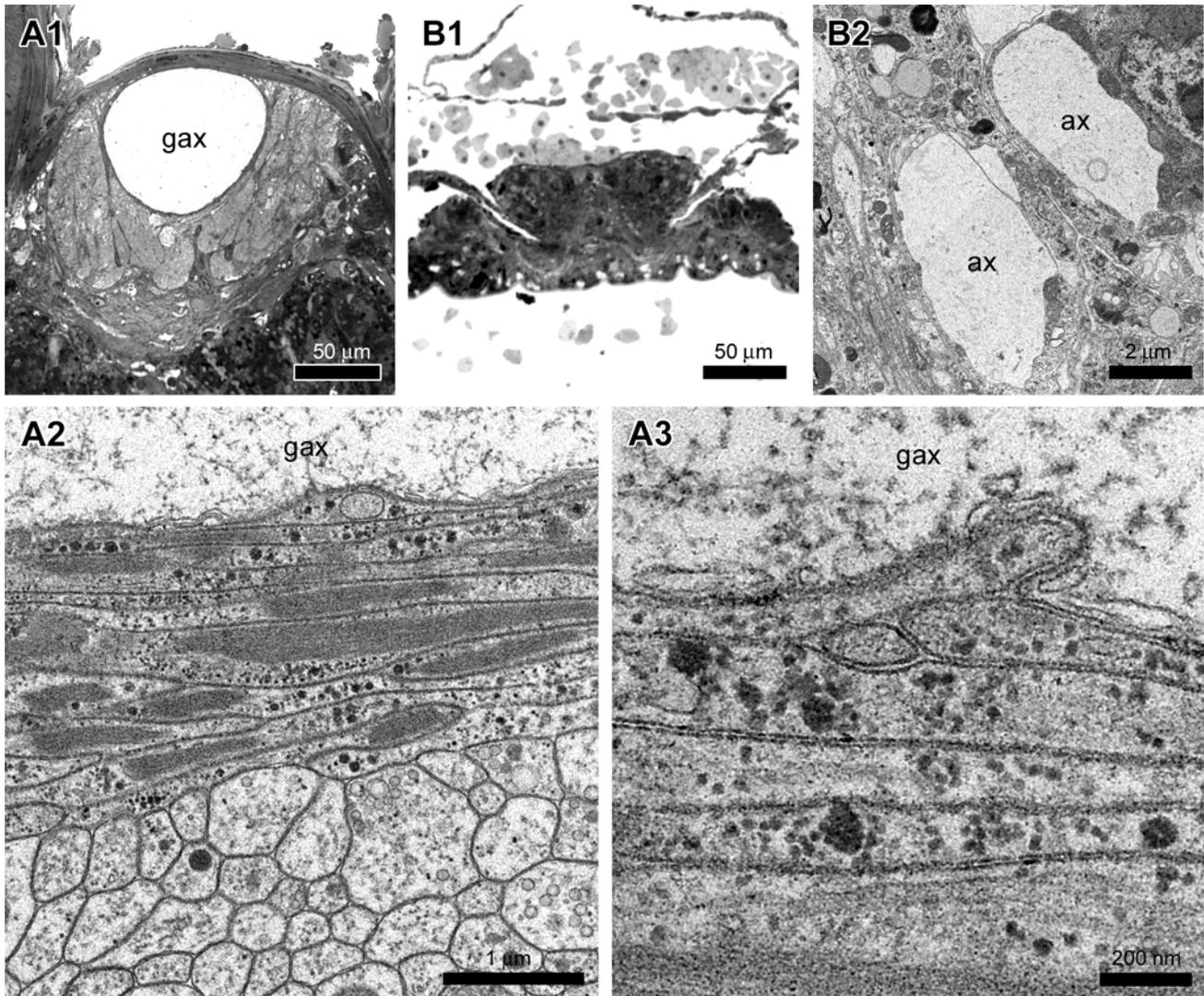


Fig. 1. Micrographs from the ventral nerve cord (VNC) of (A) *Asychis elongata* and (B) *Capitella sp. I.* A1, B1. Light micrographs (LM) of cross sections from the anterior VNC. A2, A3 two different TEM magnifications of the portion of sheath bordering the giant axon of A1. B2. Oblique TEM cross-section of two VNC axons in *Capitella*.

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