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NOTES ON AEOLOSOMA TENEBRARUM.

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Aeolosoma tenebrarum, a fresh-water oligochaete belonging to the Aphaneura, has been supposed to hold a unique position among annelids, having been described as having a brain but no ventral nerve cord.

Vejdovsky, Maggi, and Beddard have given the more detailed accounts of its structure. Vejdovsky[1] found a few cells supposed to represent a rudimentary nerve cord which was not connected with the bilobed brain: "Bei *A. tenebrarum* treten auf der Bauchseite zerstreute Elemente hervor, die auf eine nervöse Natur hinweisen." And again: "Man erkennt in der Centrallinie der Bauchseite, eine kurze Strecke hinter der Pharyngealregion, eigenthümliche, aber sehr undeutliche Zellen- und Fasserstränge, die jedoch mit dem Gehirnganglion nicht zusammenhängen." From the plates it is impossible to tell just what cells are referred to, but as they were found in the median line, they could not have been a part of the ventral cord.

Maggi[2] has described a brain, ventral cord, and lateral nerves for Aeolosoma: "Un cordone schiacciato che si estende lungo

[1] Vejdovsky, F. System und Morphologie der Oligochaeta. 1884.
[2] Maggi, L. Intorno al genere Aeolosoma. 1865.

tutto la linea mediana ventrale dell' animale mandando lateralamente degli esili fili nervosi, ed un ganglio cefalico constituiscono per quel che potei scogere, il sistema nervoso degli Aeolosoma." This description has not generally been credited. He gives no illustrations of the nervous system, and his drawing showing the mouth at the end of the prostomium, with the space inside the prostomium designated as the buccal cavity, leaves no place for a brain and is incorrect, as well as the statement that the nerve cord extends along the median ventral line.

Beddard[1] says of *A. tenebrarum*: "This species alone shows any traces of a ventral cord, which is very short and is not connected with the brain."

A further study of the subject was undertaken in the Zoölogical Laboratory of the University of Chicago, at the suggestion of Professor C. O. Whitman, to whom I am indebted for the supervision of my work.

Material for study was found among the water plants in the park ponds of Chicago, where it was especially abundant around the water hyacinth and *Victoria regia.*

The worm is not an active swimmer, but prefers to lie among the algae or to crawl between the meshes of a decaying leaf. It is white and semi-transparent, and the integument is studded with innumerable green oil drops contained in gland cells which have their large nuclei flattened against the cell wall, similar to the gland cells of the Turbellaria. A delicate chitinous covering may be seen after treatment with reagents. The worms feed upon algae or bits of decayed leaves and have a tendency to collect on the sunny side of the aquarium. They vary in length from 3 to 10 mm.

A number of worms will frequently get together, twist themselves into a ball, and remain so for a long time. It has been suggested that this was connected with conjugation, but that is improbable; they are presumably feeding upon each other, as one worm is usually found partly eaten, if the ball is pulled apart. They have great powers of regeneration; in one case, where the head had been eaten away to the first pair of setae, a new head was regenerated in about three days.

[1] Beddard, F. E. A Monograph of the Order Oligochaeta. 1895.

The head segment, which is broader than the following segments, is separated from them by a constriction, and seen from above, it appears to have a ciliated pit on each side like those of the Turbellaria. The mouth is on the ventral side and is overhung by the prostomium, which is ciliated on its lower surface, and serves as a tactile organ. There is no proboscis.

Intersegmental septa were not found, but the segmentation is defined by the nephridia and the setae. Each segment has one pair of nephridia and four bundles of setae, placed dorso- and ventro-laterally. There is also a segmental arrangement of single, nucleated muscle fibers which extend from the alimentary canal to the body wall between the setae sacs.

The alimentary canal comprises a circular mouth opening into a bell-shaped pharynx, followed by a narrow oesophagus, which extends through two segments and leads to a broad stomach with glandular walls, which extends through the sixth segment where it narrows into a straight intestine. As the worms are transparent the movements of the cilia lining the alimentary canal may be seen in the living specimen.

The muscular system is comparatively simple. There is one layer of longitudinal and one layer of circular muscle fibers just beneath the epidermal wall, and single nucleated fibers are connected with the setae and hold the various organs in place. These single nucleated fibers are especially numerous in the head and resemble the muscle plates of the Turbellaria.

The worms were under observation from October until July, and during that time they were constantly reproducing by fission, with sometimes as many as three zoöids developing at once. Back of the seventh setigerous segment there is a fission zone in which all the tissues of the epidermal wall are greatly thickened, especially on the ventral side, where they nearly obliterate the body cavity. The new brain arises as a dorsal thickening of the epidermis. No sexual reproduction was observed.

Methods. — At the slightest irritation the worms will coil in a circle, throw off the contents of the gland cells, and contract so violently that the tissues are injured for study. To

secure good specimens for sectioning they were mounted on a slide and held in place by a cover-glass that pressed on them slightly. They were then placed on ice for a few moments until chilled and unable to contract any portion of the body, when they were treated with the fixing fluid. The cold also prevented abnormal activity of the glands, so that very perfect preparations were obtained, although the worms will go to pieces if left on the ice too long.

The fixing fluid after the second formula of vom Rath (picric + acetic + osmic + platinum-chloride) was found most effective in demonstrating the nervous system. Specimens were left in this for fifteen minutes, washed in alcohol, and placed in a 20 per cent solution of tannin in acetic acid for periods of time varying from twenty-four hours to four days, or else they were stained in section with safranin or iron-haematoxylin after vom Rath.

Paraffin was used for imbedding, and sections were cut from 3–20 μ thick. Sections from 10–15 μ thick were found most favorable for study.

Nervous System. — The brain lies in front of the mouth in close contact with, and partly imbedded in, the epidermal wall (Pl. XXI, Figs. 1, 6–8), the lower part projecting more or less into the cavity of the prostomium. Its ventral and lateral surface, so far as free from the epidermis, is covered by a delicate nucleated membrane which may be seen in section. It has a slightly bilobed appearance, as seen from above, each half having a rounded anterior margin and a large posterior lobe, the latter composed entirely of nerve cells (Pl. XXI, Figs. 2, 3). Closely packed nerve cells with large granular nuclei cover the whole dorsal surface, and are from three to four cells deep in the anterior and posterior lobes, but only one layer deep in the middle region where the anterior lobes meet (Pl. XXI, Figs. 8, 10).

A pair of nerves composed of fibers partly from the brain and partly from the oesophageal commissure, runs forward from the brain into the prostomium, and another pair runs back from the angle between the posterior lobes and the commissures (Pl. XXI, Figs. 1, 2, 5, 6, 9).

Commissure. — Immediately after leaving the brain the oesophageal commissure passes into an accessory ganglion, from which a nerve runs forward into the prostomium (Pl. XXI, Fig. 1). It then passes downward and backward, in close connection with the epidermal wall, to the ventral side, where it expands into a second ganglion before passing into the ventral cord (Pl. XXI, Fig. 1). The fibers of the commissure form a broad band which is clearly distinguishable, but it is often difficult to determine whether the cells along its course belong to it or to the epidermis.

Ventral Cord. — The two parts of the ventral cord are separated by about one-fifth of the diameter of the body and communicate with each other by fibrous commissures, forming the ladder type of nervous system (Pl. XXI, Figs. 1, 12).

There is one pair of ganglia in each segment, and each ganglion is deeply bilobed, the anterior lobe being somewhat smaller, while the posterior lobe extends out farther in the body wall. The fibrous portion forms the greater part of the ganglion, and is covered by cells one layer deep (Pl. XXI, Fig. 11). In the posterior segments the ganglia are crowded together more closely than in the anterior segments (Pl. XXI, Fig. 1).

Lateral Nerves. — Four distinct lateral nerves are given off from each ganglion, two from the anterior and two from the posterior half (Pl. XXI, Figs. 1, 11).

This whole system of brain, ventral nerve cord, commissures, and nerves is connected throughout with the epidermal wall, no portion of it being entirely free in the body cavity. The cells of the ventral ganglia, as well as those of the brain, are often so closely connected with the epidermis that it is hard to find the boundary line between them. The nuclei of the ganglion cells are of about the same size as those of the epidermis, but stain a little more deeply.

Ciliated Pits. — Vejdovsky[1] states that in Aeolosoma we find the only instance of an oligochaete possessing a pair of lateral ciliated pits, and he compares them with the ciliated pits of the Turbellaria. From the dorsal side the appearance is very similar to these organs in the Turbellaria, but frontal

[1] Vejdovsky, F. Thierische Organismen der Brunnenwässer von Prag. 1882.

sections of the ventral side show that they are not pits at all, but the terminations of deep ciliated grooves which curve forward and outward from the mouth to the edge of the prostomium (Pl. XXI, Fig. 13).

The mouth is circular, bordered posteriorly and laterally with a thick swollen lip, which may be greatly extended, and which is continued as the posterior wall of the ciliated furrows. The cilia of the grooves, and those around the mouth, are exceptionally long. Sense organs are as numerous along either side of the furrows as on the prostomium.

Vejdovsky describes a nerve connecting the lateral pits with the brain. I find muscle fibers here, but no nerve, and from the nature of the structure should not expect to find one.

Sense Organs. — There are many large pear-shaped cells, that have the appearance of sensory cells, lying in all parts of the prostomium and disposed through the body segments (Pl. XXI, Fig. 14). The cytoplasm of these cells is finely granular and deep-staining, the nucleus is of medium size, coarsely granular, and usually eccentric, taking its position at the base of the cell. Between the nucleus and the opposite end of the cell there is a large, sharply outlined, clear space containing a refractive body with peculiar granulations at its periphery (Fig. 14) which may represent an otolith. These cells are sometimes isolated, but are often collected into small groups (Fig. 15), as seen to best advantage in the prostomium. They suggest sense organs of some kind. They have no pigment.

At the anterior end of the prostomium there is a group of about fifteen of these large compound organs, crowded together so closely that their sides are somewhat flattened against each other (Fig. 16). Back of these there are smaller compound sense organs, some distance apart, arranged in rows across the ventral surface of the prostomium, and there are large sense organs along both sides of the ciliated furrows leading to the mouth (Fig. 16). The smaller compound sense organs are also found on the ventral side of the segments back of the mouth. All of these sense organs lie immediately under the epidermis, so that they project slightly into the body cavity.

Aeolosoma undoubtedly possesses the essential annelidan

characteristics, although Vejdovsky favored classifying it with the Turbellaria on account of the similarities which he found between the ciliated pits, muscle plates, and gland cells of these forms, together with the structure of the brain and the supposed lack of a ventral cord.

The course of the large nerves running back from the brain has not yet been traced for an annelid; they present an anomalous feature which is most interesting from its suggesting a possible transitional form of nervous system between unsegmented and segmented worms. The position of the brain in the first segment, the continuity of the entire nervous system with the epidermis, and the wide separation of the halves of the ventral cord are primitive characteristics which would be consistent with such a form.

REFERENCE LETTERS.

ag. accessory ganglion.
b brain.
c. connecting commissures of the ventral nerve cord.
cl. cluster of large sense organs in the end of the prostomium.
d. second ganglion of the oesophageal commissure.
ep. epidermal cells.
f. lateral ciliated furrows leading to the mouth.
g. gland cells.
l. lip bordering the mouth and the ciliated furrows.
ln. lateral nerves.
m. mouth.
mb. membrane lining the body cavity and covering the free, lower surface of the brain.
mf. muscle fibers attached to the brain and connecting it with the epidermis of the ventral side.
n. nucleus.
$n_{.1-3}$ first, second, and third pairs of cephalic nerves.
nm. nuclei of the lining membrane.
o. refractive body.
oc. oesophageal commissure.
P. posterior ganglionic lobes.
v. vesicle containing a refractive body.
vc. ventral nerve cord.

EXPLANATION OF PLATE XXI.

FIG. 1. Frontal view of the central nervous system, reconstructed from sections. (× 210.)

FIG. 2. Ventral view of the brain. (× 1200.)

FIG. 3. Dorsal view of the brain, showing its bilobed form. (× 1200.)

FIG. 4. Frontal section near the middle of the brain, showing the first pair of nerves. (× 800.)

FIG. 5. Frontal section next above Fig. 4. (× 800.)

FIG. 6. Sagittal section of the brain in the plane of the anterior nerve and the posterior lobe. (× 800.)

FIG. 7. Sagittal section of the brain a little nearer the middle than Fig. 6. (× 800.)

FIG. 8. Sagittal section near the middle of the brain, showing a single layer of cells on the dorsal side. (× 800.)

FIG. 9. Oblique section of the brain, showing the posterior lobe and the roots of the first and second cephalic nerves. (× 800.)

FIG. 10. Cross-section of the brain in the plane of the commissure. (× 800.)

FIG. 11. Frontal section through a segment of the ventral nerve cord, showing the roots of the lateral nerves and of the connecting commissures. (× 1200.)

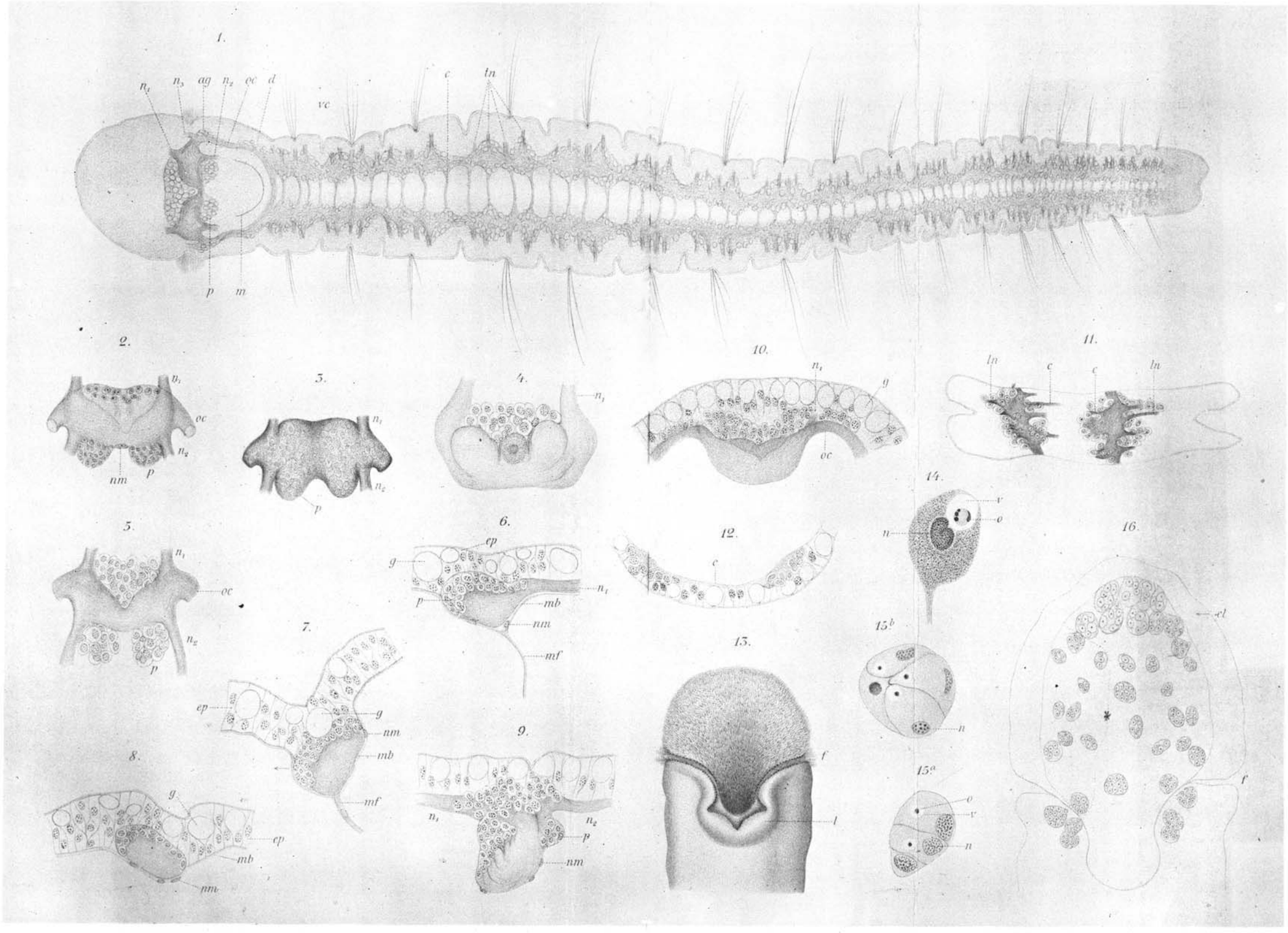
FIG. 12. Cross-section of the ventral nerve cord in the thoracic region, showing the ganglia connected by a commissure. (× 800.)

FIG. 13. Drawing showing the mouth with the furrows leading to it, and the prostomium, ciliated on the ventral side. (× 1200.)

FIG. 14. Pear-shaped sensory cell, with vesicle containing a refractive body. (× 1200.)

FIG. 15. Sense organs composed of several cells similar to those of Fig. 14. (× 1200.)

FIG. 16. Partial diagram showing the position of the compound sense organs on the ventral surface of the prostomium. (× 1200.)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