

über die Biologie der meisten Gymnotiden noch sehr spärlich, und es wäre sehr wünschenswert, durch weitere Beobachtungen unsere Kenntnis über diese äußerst interessante Familie zu erweitern.

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The morphology and taxonomy of *Macrostomum virginianum* n. sp.

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(With 6 Figures.)

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Macrostomum virginianum is a member of the family Macrostomidae, of the sub-order *Opisthandropora*, and of the order *Rhabdocoelida*¹.

The material was collected from a small stream on the campus of the University of Virginia. A suitable culture of the animals, taken from the collected mud and water, was furnished by covering a sprig of *Elodea* with spring water in a Petri dish. Finely cut bits of the annelid *DERO* were used for food. Specimens were held slightly compressed under coverglasses supported by a wall of vaseline, the height of which could be modified by pressure. In this manner their morphological details could be examined under the various objectives, including the oil immersion. HEIDENHAIN'S hemalum stain was employed for general histological information. BELLING'S iron-aceto-carmine method gave good results in the study of chromosomes.

The dorso-ventrally compressed body (Fig. 1) has two pairs of shallow lateral indentations. The first is at the anterior level of the mouth and marks off the cephalic region; while the second is found at the anterior level of the oviducts. Posterior to these shallow indentations there is a third pair of more pronounced lateral depressions, beyond which the spatulate posterior end of the body extends. The animal is 1 mm. lang.

The epidermis is composed of a flattened layer of pentagonal cells which are studded with rhabdites and bear cilia, lateral sensory hairs and terminal spines. The rod-shaped rhabdites (Fig. 1, *rh*) are distributed over the entire epidermis, but are more

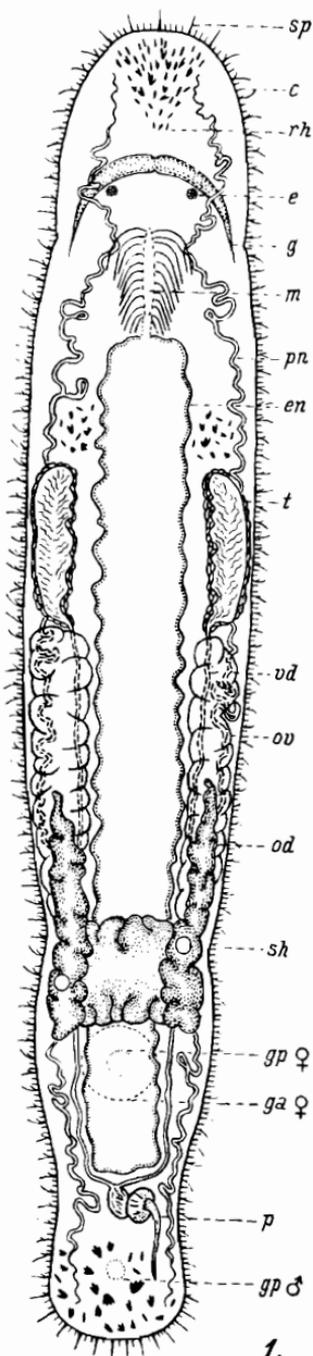
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numerous at the anterior end. They appear in groups of from two to six and are about 8 micra by 1 micra in dimension. Many single ones appear in the anterior region. A dense coat of cilia (Fig. 1, *c*), which are approximately 6 micra long, covers the entire body surface. Lateral sensory hairs (Fig. 1, *sh*) about twice as long as the cilia are interspersed among the latter at irregular intervals. A short spine projects anterolaterally from the base of each sensory hair. Terminal semi-rigid spines (Fig. 1, *sp*) of approximately the same length as the sensory hairs are found at the extremities of the body. This triple variation of epidermal processes is peculiar to this species.

The crescent-shaped "brain" (Fig. 1, *g*) is composed of two ganglia connected by a commissure which is so short that the cephalic neural mass appears to be but slightly indented along its mid-line. It is located equidistant from the dorsal and ventral sides of the body. Paired eyes lie immediately behind and dorsal to the "brain" (Fig. 1, *e*).

The mouth (Fig. 1, *m*) is located on the ventral surface about 170 micra from the anterior end. It is bounded by two ciliated lips and opens into the pharyngeal cavity which leads into the enteron. Glands are directed laterally and then posteriorly from the mouth.

The slender, sac-like, slightly lobed enteron (Fig. 1, *en*) extends



1.

Fig. 1. *M. virginianum*. *sp* = spines; *c* = cilia; *rh* = rhabdites; *e* = eye; *g* = "brain"; *m* = mouth; *pn* = protonephridium; *en* = enteron; *t* = testis; *vd* = vas deferens; *ov* = ovary; *sh* = sensory hairs; *gp ♀* = female gonopore; *ga ♀* = female genital atrium; *p* = penis; *gp ♂* = male gonopore. Mag. 150.

to the posterior lateral constriction of the body. Its epithelium is ciliated.

The excretory system is composed of two latero-ventral tubes of about 2.5 micra in caliber (Fig. 1, *pn*). The tubules extend from one extremity of the body to the other, and are believed to empty separately into the lumen of the pharyngeal cavity.

The male reproductive system is composed of testes, vasa deferentia, vesicula seminalis, vesicula granulorum, penis-stilette, and male gonopore. The elongated, semi-oval testes (Fig. 1, *t*) are located latero-ventrally in the posterior part of the first half of the body. The mesial surface of a testis is irregular, while its lateral surface conforms more or less to the contour of the body. Interlacing muscular bands are present within the walls of the testes. From the posterior tip of each testis, a vas deferens (Fig. 1, *vd*) extends caudally until it reaches the posterior end of the enteron where it bends mesially to join with its counterpart of the opposite side in the mid-line of the body. A false vesicula seminalis may be formed at this point during the passage of sperm cells. The entrance of the common vas deferens into the vesicula seminalis is ciliated. The empty vas deferens is a tubule of approximately the same caliber as that of the protonephridium, but may be distended to accommodate a group of two to three sperm cells lying side by side. In the vesicula seminalis the bolus of sperm cells is always rotated in an apparent clock-wise fashion when observed through a compound microscope. Three interlacing layers of muscles are found within the walls of the vesicula seminalis. The entrance into the vesicula granulorum is guarded by a sphincter. Ciliated, vertically disposed septa divide the anterior region of the vesicula granulorum into two to four chambers (Fig. 2, *c*). Granular material is always found in the vesicula granulorum near its exit at the base of the stilette (Fig. 2, *g*).

The wall of the vesicula granulorum is supplied with diagonal muscles which are continuous with the muscles surrounding the penis-stilette (Fig. 2, *m*). The penis lies to the right of the mid-line of the body. Its muscular tunic houses a stilette, the widened base of which, leads from the vesicula granulorum. The stilette (Fig. 2, *p*) is curved and obliquely truncated on its convex side. This conical tube is bent at a right angle near its proximal end. It measures 62 micra in length and 18.5 micra at the base. The middle portion of the stilette lies in a mid-dorso-ventral plane. The penis communicates with the male gonopore which lies

approximately 50 micra from the posterior end. This gonopore is guarded by a circular, ciliated, epithelial margin.

The stilettes of forty mature specimens were measured according to the scheme of figure three. The following figures show the range of variation in the stilette of this species: (a) varies from 5 micra to 16 micra; (b) varies from 3 micra to 7 micra; (c) varies from 15 micra to 55 micra. In none of these has the

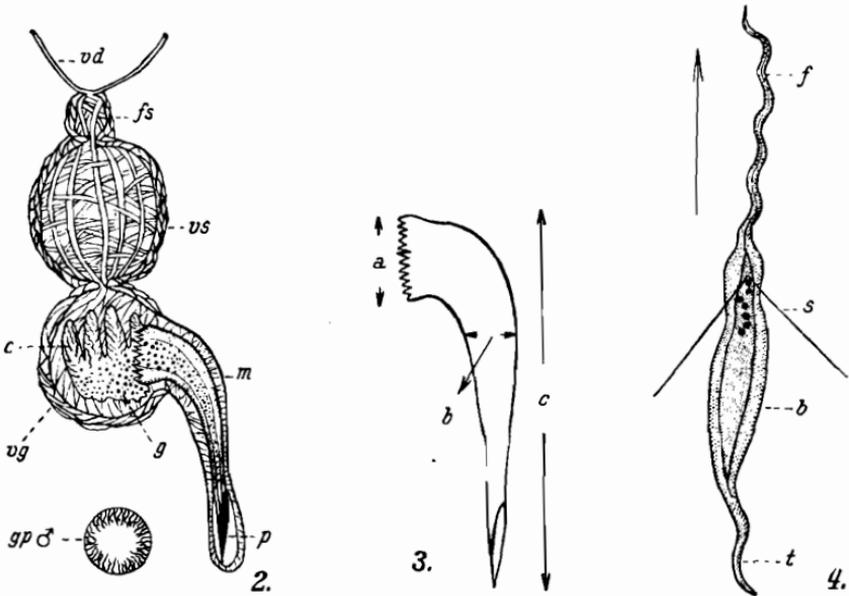


Fig. 2. Male Sex Apparatus of *M. virginianum*. *vd* = vas deferens; *fs* = false vesicula seminalis; *vs* = vesicula seminalis; *c* = ciliated pits; *g* = granular material; *vg* = vesicula granularum; *gp* = male gonopore; *m* = muscles of penis sheath; *p* = penis stilette. Mag. 400.

Fig. 3. Scheme for Measuring Stilette of *M. virginianum*. Mag. 835.

Fig. 4. Mature Sperm Cell of *M. virginianum*. *f* = feeler; *s* = setae "Nebengeisseln"; *b* = body of cell; *t* = tail. Mag. 2250.

flexed region shifted from the proximal end. A few specimens with large stilettes had neither vesicula seminalis nor vesicula granularum. In such cases, it appears that the active parts of the system had disappeared leaving only the inert cuticular stilette.

The mature sperm cell (Fig. 4), possessing a row of chromatin granules, measures 35 micra by 2 micra, and is composed of feeler (*f*), body (*b*), and tail (*t*). Two setae "Nebengeisseln" (*s*) extend latero-posteriorly from the body of the cell. Observations show that a sperm cell can arrive in the vesicula seminalis from the testes in about five minutes. I have observed, on frequent occasions, copulation in this genus which involved the stilettes and female gonopores of the two individuals. This, however, may

not be the only means whereby sperm cells are exchanged, for they have been observed, in this species, within the pseudocoel near the eyes. This unusual position for sperm cells suggests that the stylette may be used as a weapon, during which use, sperm cells are discharged from one animal into another. LUTHER (1905, p. 36) comments upon the penetration of the wall of the female genital atrium by the sharpened stylette of *M. hystrix*, by which act, masses of sperm cells are lodged in the mesenchymal tissues surrounding the atrium.

The female reproductive system consists of two ovaries, oviducts, common oviduct, genital atrium, female gonopore, and accessory glands. The paired ovaries (Fig. 1, *ov*) are embedded in the pseudocoel in a lateral and dorsal position, lying anterior to the middle pair of lateral constrictions of the body. From the mesial margin of the posterior third of each ovary, an oviduct runs mesio-dorsally to unite with the one from the opposite side (Fig. 1, *od*). The common oviduct extends, from this point, postero-ventrally to enter the female genital atrium. A muscular sphincter controls the entryway. The spheroidal genital atrium is at times distended with a spherical egg which is brown to reddish-brown in color. The female gonopore

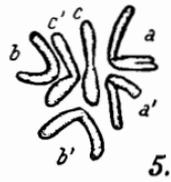
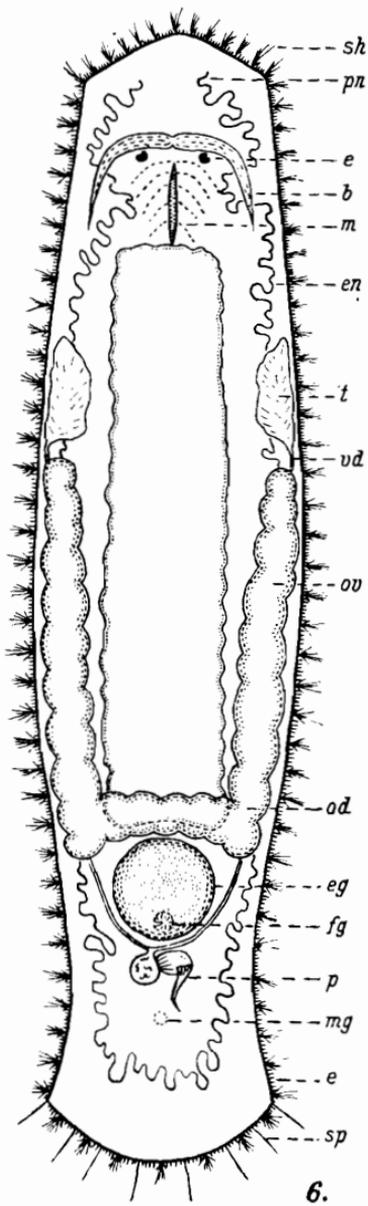


Fig. 5. Metaphase Plate of Spermatogonial Mitosis in *M. virginianum*. Mag. 2400.
 Fig. 6. *M. appendiculatum* (O. Fabr.). *sh* = sensory hairs; *pn* = protonephridium; *e* = eye; *b* = "brain"; *m* = mouth; *t* = testis; *vd* = vas deferens; *ov* = ovary; *od* = oviduct; *eg* = egg; *fg* = female gonopore; *p* = penis; *mg* = male gonopore; *c* = cilia; *sp* = spines. Mag. 112.

is located mid-ventrally, mid-way between the second and third lateral constrictions of the body. The opening is guarded by ciliated lips and a system of radiating accessory glands.

The haploid chromosome number in this species is three and the diploid number is six (Fig. 5). Spermatogonial tissue yields the best results in the study of chromosomes. A suggested pairing is indicated in the metaphase plate. PHILLIPS (1936) has reported the chromosome number of *M. tuba* GRAFF as N equals 3, $2N$ equals 6. In a spermatogonial mitosis, PHILLIPS shows three pairs, including a large pair, a medium pair, and a small pair, all possessing median attachment points. *M. virginianum* has one pair (Fig. 5, *a a'*) with sub-median attachment points and two other pairs (Fig. 5, *bb', cc'*) with median attachment points.

Taxonomically, *M. virginianum* must be compared with the members of the group whose stilettes possess oblique distal openings upon their convex sides. In previous descriptions of species, this feature has been used as a diagnostic character. This list includes *M. hystrix* OERSTED, *M. erinaceum* GIRARD and *M. ventriflavum* PEREYASLAWCEWA which have formerly been shown to be synonymous with *M. appendiculatum* O. FABRICIUS, by GRAFF (1913, p. 49). *M. appendiculatum* O. FABRICIUS, *M. viride* BENEDEN, *M. obtusum* VEDJOVSKY, *M. lineare* ULJANIN, and *M. sensitivum* SILLIMAN remain to be considered.

GRAFF (1913, p. 50, Fig. 52) depicts the stilette of *M. appendiculatum* O. FABRICIUS as being bent to the right in a sharp point, varying in thickness of the termination as well as in the width of the base. The opening is sub-terminal and is in the convexity of the curve. BRONN (1908, Taf. XVII, Fig. 13, 14) presents the stilette as possessing a small sub-terminal opening located about mid-way in the convexity of the curve. The sperm cells are shown to be slender, bent, threads lacking differential regions and setae "Nebengeißeln", BRONN (1908, Taf. XXV, Fig. 3). Since *M. virginianum* more closely resembles *M. appendiculatum* O. FABRICIUS (Fig. 6) than it does the others with which it is compared, a short description of the latter species, which also occurs in this community, is given. The average length of ten sexually mature individuals as determined by me is 1.25 mm. The description of the animal corresponds to that given by GRAFF (1913, p. 49) for this species, with the exception that a three fold epidermal ciliary differentiation (Fig. 6, *c, sh, sp*), including cilia, lateral tufts of sensory hairs and posterior, semi-rigid spines, may be noted. Meseasurements taken on the stilette (Fig. 7) show these average figures; length 59.8 micra, width of base 19.5 micra, length of somewhat elliptical opening 8 micra. The shape of the penis-stilette (Fig. 7) agrees with the illustrations of BRONN (1908, Taf. XVII, Fig. 13, 14), in that the funnel-shaped stilette gradually narrows from a widened, crenated base and terminates in a distal right angle bend. The opening (Fig. 7, *op*) is located in the convexity of the curve, subterminal to the pointed tip of the stilette. The sperm cells (Fig. 8) are spindle-shaped. They measure 50 micra in length

and have two long flagella "Nebengeißeln" (Fig. 8, *fl*) extending from the anterior part of the cell.

M. virginianum may not be confused with *M. viride* BENEDEN. Three authors, OKUGAWA (1930, p. 78), GRAFF (1913, S. 51, Fig. 54), and LUTHER (1905, p. 28—37, Taf. III, Fig. 10, 11) have commented upon the S-shaped spiralling nature of the penis-stilette of *M. viride* BENEDEN. Its sperm cell has a long spindled head and possesses lateral ciliation at the insertion of the tail filament.

M. obtusum VEDJOVSKY has several characteristics which mark it as separate and distinct from *M. virginianum*. GRAFF (1913, p. 55, Fig. 62)

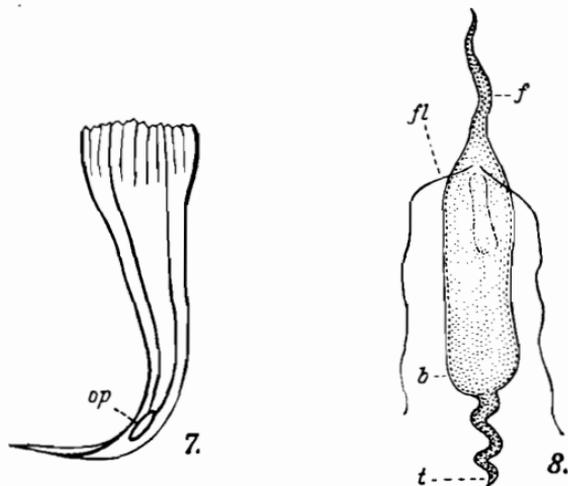


Fig. 7. Penis-stilette of *M. appendiculatum* (O. Fabr.). *op* = penis opening. Mag. 1150.
Fig. 8. Mature Sperm cell of *M. appendiculatum* (O. Fabr.). *fl* = flagellum "Nebengeißeln".
Mag. 2250.

remarked upon and gave a figure of the bent and sharply pointed penis of *M. obtusum* VEDJOVSKY. VEDJOVSKY (1895) described this species as having ciliated pits, a single definite opening to the excretory system, and a peculiar sperm cell lacking "Nebengeißeln".

The description and figure upon *M. lineare* ULJANIN in GRAFF (1913, p. 54, Fig. 61) show that this species is markedly different from *M. virginianum*. The fully spiralling stilette of *M. lineare* ULJANIN bears no resemblance to that of *M. virginianum*.

M. virginianum and *M. sensitivum* SILLIMAN are shown to be dissimilar according to the descriptions and figures of GRAFF (1913, p. 51, 52, Fig. 55), GRAFF (1911, Taf. II, Fig. 28, 29, 30), LUTHER (1905, p. 28—37), SILLIMAN (1885, S. 50, Fig. 18). The penis-stilette of *M. sensitivum* SILLIMAN is described as almost straight and bent to the right only at the point.

In recapitulation *M. virginianum* is described as having these distinctive characteristics:

- (1) The body has a three fold lateral indentation.
- (2) There are three types of epidermal ciliary processes.

- (3) The sperm cell is differentiated into feeler, body, and tail, and bear setae "Nebengeisseln".
- (4) The stilette has a right angle bend near its base plus an oval distal opening that lies on the convexity of the curve and is distinctly terminal.
- (5) The chromosome count is N equals 3, $2N$ equals 6. The chromosome morphology is peculiar to this species.

Based upon the criteria employed by previous workers in this field, *M. virginianum* is therefore a clearly defined species.

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