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A Description of three Acoela from the Gulf of Naples.

1. *Aphanostoma pulchella*, (Uljanin, non *A. pulchella* mihi Pereyaslawzewa).
2. *Monochoerus lineatus*, n. sp.
3. *Amphiscolops fuligineus*, n. sp.

by

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(With 12 text-figures and plate 10.)

Contents.

A. Introduction 292
B. Description 293
1. Aphanostoma pulchella 293
a) Historial review 293
b) External features and behaviour 294
c) Anatomy 295
2. Monochoerus lineatus 298
a) External appearance 298
b) Anatomy 299
c) Relation to other Acoela 302
3. Amphiscolops fuligineus 304
a) External appearance 304
b) Anatomy 305
c) Relation to other Acoela 307
C. Conclusion 308
D. Literature 310
E. Explanation of figures 311

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A. Introduction.

In a brief preliminary note (1913), I described a small acoelous flatworm found together with larger and less abundant forms, collected in the early spring, from the Mergellina, a sheltered harbor lying between Naples and Posilipo. Because of its close resemblance to the species described by Uljanin (1869) under the name *Nadina*[1] *pulchella*, I have given this worm the name *Aphanostoma pulchella*. Among the larger forms occurring in this region, two new species, *Amphiscolops fuligineus*, and *Monochoerus lineatus*, were discovered, but the material was not obtained in great abundance, and for this reason it was necessary to abandon temporarily the determination of the systematic position of the second form. In August of the same year a fresh supply of *Monochoerus* was collected, and serial sections have been made, in order to study the internal anatomy. Through the kindness of Professor Böhmig of Graz, I have had the opportunity of securing some specimens of *Monochoerus illardatus* from Dr. Micoletzky. After comparing this form from the Gulf of Trieste, with the species found at Naples, I am convinced that the latter belongs to the newly identified genus *Monochoerus*. In accordance with the nomenclature used by Löhner and Micoletzky (1911) I have given the Neapolitan form the specific name *lineatus*[2], to indicate the characteristic marking on the dorsal side.

The investigation of these worms was undertaken for the purpose of determining, by means of various experiments, the functions of the sense organs, and not for histological study. As the completion of the experiments is postponed until a comparison of the behaviour of European and American forms can be made, it seems wise at the present time to give a brief description of the external appearance and general structure of these interesting species.

Technique.

Four different fixing fluids were employed with varying success. Some worms were preserved in Hermann's fluid, others in Gilson's Mercuro-nitric, a third set in Zenker, and a fourth series in Corrosive-acetic. The latter method proved most satisfactory, although Hermann preserved the musculature better than the others. At best these worms are exceedingly difficult to preserve so that the finer structures are clear.

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[1] The generic name *Nadina* has been abandoned, as will be shown later.

[2] *lineatus* = striped, *illardatus* = flecked.

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Various stains were employed, among them APATHY's Haematein 1. A. Good results were obtained by combining DELAFIELD's Haematoxylin with either Orange G, or Eosin. The former combination stained the reproductive cells purple. the glands blue, and the parenchyma a light yellow. Glandular structures were also brought out well by using a triple stain consisting of equal parts of Methyl green, Acid fuchsin, and Orange G.

B. Description.

1. *Aphanostoma pulchella* (ULJANIN, non *A. pulchella* mihi, PEREYASLAWZEWA).

a) Historical review.

More than forty years ago ULJANIN (1869) included under the generic name *Nadina* several species of small worms found in the Bay of Sebastopol. According to his classification, the genus *Nadina* includes Aphanostomidae without a pharynx, with mouth opening on the ventral surface behind the otolith, two eyes, and a bursa with no hard parts. LUDWIG VON GRAFF (1886) first pointed out that the position of this genus was not definitely determined, for if there are two genital openings present *Nadina* belongs between *Aphanostoma*, and *Convoluta;* but if there is only one opening, it is intermediate between *Proporus* and *Convoluta.*

Nadina pulchella, which is, no doubt, the same species that occurs at Naples, is described by ULJANIN as pear-shaped, yellow in color, with many small rhabdites arranged in diagonal rows upon the surface of the body. The length according to his measurements, varies from 0,48 mm to 0,5 mm. In the region of the mouth and statocyst he observed a mass of dark "pigment" which he interpreted as "diffuse eyes". He also mentions the oil globules in the same region. The position of the ovaries is lateral, and in the center he observed a large round organ which he called a bursa seminalis and lying near it an egg-shaped bladder (Hode). The spermatozoa he represents with large heads and slender tails.

Some years later PEREYASLAWZEWA (1892) in her Monograph on the Turbellaria of the Black Sea, gave a detailed account of a worm which she called *Aphanostoma pulchella* mihi, and which she seemed to consider the same worm that ULJANIN named *Nadina pulchella.* PEREYASLAWZEWA describes this species as small, lively, and easy to

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distinguish on account of its size and apple-green color. The otolith is in the usual place, but no eyes are present; the body is covered with cilia. She recognizes that "pigment granules" first described by Uljanin, are not pigment at all, but are the granules of concrement, so often found in the Acoela. These granules appear black on account of their opacity. Much attention is given by this investigator to the pharynx whose structure she considers peculiar to this form. Two types of pharynx have been distinguished by von Graff, the "pharynx simplex", and the "pharynx compositus"; the latter type is divided into two kinds the "bulbosus" and the "plicatus". The pharynx of *Convoluta* belongs to the simplex type, but that of *Aphanostoma pulchella* mihi, according to Pereyaslawzewa, is bulbose. She describes it as formed of two superimposed tubes "qui sont liés par leurs ouvertures; il ne reste une space libre entre leurs parois formée de plusieurs couches de fibres musculaires tres fins. Le vide est traversé par de nombreux filaments musculaires très fins". These fibres are perpendicular to the surface. The outer layer of muscle cells is much more distinct than those composing the inner layer. These finer structures are however not clearly represented in her figures.

When von Graff examined the various species of Acoela from the Black Sea (1904) he found neither the species described by Uljanin, nor that of Pereyaslawzewa. He calls attention (p. 222) to the fact that nothing is said by either author about the number of genital openings, neither is there any accurate statement in regard to the presence of a chitinous mouth-piece on the bursa, and these two points must be clearly established before any definite conclusions are drawn.

The species that I found at Naples resembles that of Uljanin's description so closely that I feel justified in identifying it as *Aphanostoma pulchella*. The generic name *Aphanostoma* must be given because the bursa seminalis has no chitinous mouth-piece, and there are two genital openings (fig. 4). The only point of divergence from the description of Uljanin is in the shape of the mouth which he represents as slit-like instead of round, and he does not seem to have observed the pharynx. As we have only one "Habitusbild" by Uljanin, it is perfectly possible that the pharynx was overlooked by him.

b) External features, and behaviour.

The body of *Aphanostoma pulchella* is pear-shaped, the broad anterior end measuring about 0,2 mm in diameter, the posterior end tapering to a blunt point (pl. 10, fig. 2). The entire worm never exceeds 0,5 mm

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in length. The color, a pale canary yellow, shading into white, is due to a diffuse pigment, and not to special pigment cells. There are no zoochlorella cells present. The "diffuse eyes" which Uljanin mentioned, are small masses of concrement in the region of the mouth. When the worm is swimming or suspended near the surface of the water this concrement appears as a dark mass in the anterior end of the body. Examination of the worm when slightly compressed under the cover-glass, proves that the black color is due to the opacity of the concrement. At times a great accumulation of oil globules in this region makes the animal appear almost translucent.

The behaviour of these small animals is characteristic. They swim rapidly from place to place, but when undisturbed in an aquarium, they gather in masses around the sides of the glass with the tail end down, and the blunt anterior end just below the surface of the water. This collecting of the individuals is of advantage to the investigator, for hundreds of worms may be drawn up together in a small pipette. They are exceedingly sensitive, and are therefore not at all well adapted for experimentation. One may succeed in keeping them alive in glass dishes for forty-eight hours if the water is changed at frequent intervals.

Aphanostoma pulchella is pelagic, and is obtained near the shore at a depth of one to two meters. I have never seen the worms crawling on seaweed.

c) Anatomy.

The surface of the body is ciliated, the cilia being arranged in longitudinal rows (pl. 10, fig. 1, *c*). So far as I can determine these rows are not oblique, but frequently appear so owing to the twisting and turning of the worm. The integument is very thick, and conspicuous, as seen in the transverse sections (figs. 1—4). It consists of tall epithelial cells with deeply staining nuclei. Scattered among these cells at more or less regular intervals, are large glands. The worm is not highly muscular, its movements are largely due to the lashing of the cilia. Definite muscle fibres were not visible in any of the preparations.

The large glandular mass (pl. 10, fig. 1, *fg*) to which the name frontal gland has been given, occupies the entire anterior end of the body. The occurrence of this gland among the acoelous flatworms is frequent. Löhner and Micoletzky distinguish two types of frontal glands those which open by separate ducts on the anterior surface as in *Polychoerus caudatus* Mark, and those in which the ducts converge into one common duct. This type is illustrated in *Convoluta roscoffensis*. The frontal gland of

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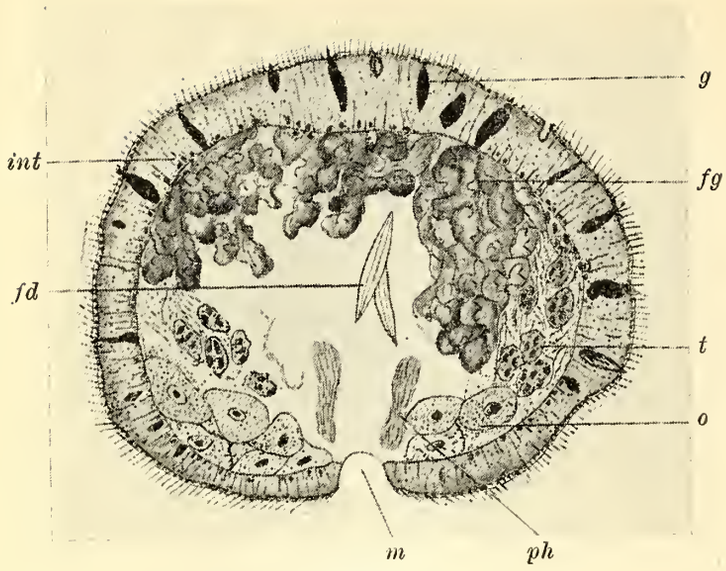

Fig. 1.

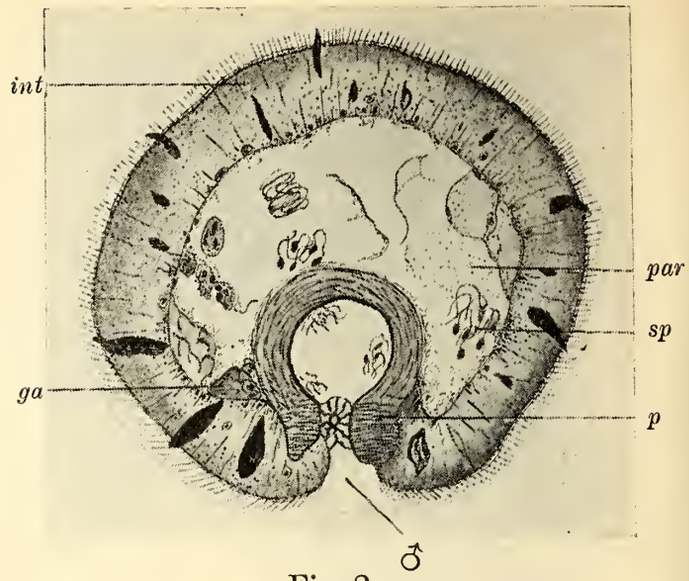

Fig. 2.

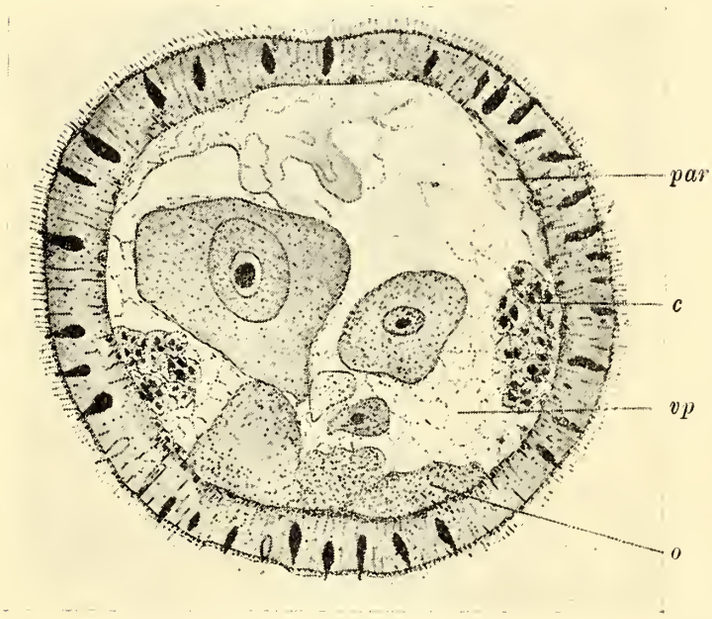

Fig. 3.

Figs. 1—4. *Aphanostoma pulchella.*

Fig. 1. Transverse section through the pharynx (*ph*); *fd*, ingested food; *fg*, frontal gland; *g*, slime gland; *int*, integument; *o*, ova; *t*, testes.

Fig. 2. Transverse section through the copulatory organ (*p*); *ge*, genital glands; *par*, parenchyma; *sp*, spermatozoa.

Fig. 3. Transverse section through the midale of the body: *p*, vacuolated parenchyma.

Fig. 4. Median longitudinal section showing the relative positions of the genital openings: *bs*, bursa seminalis; *m*, mouth. All of these drawings were made with a camera lucida, *x* 250 diam

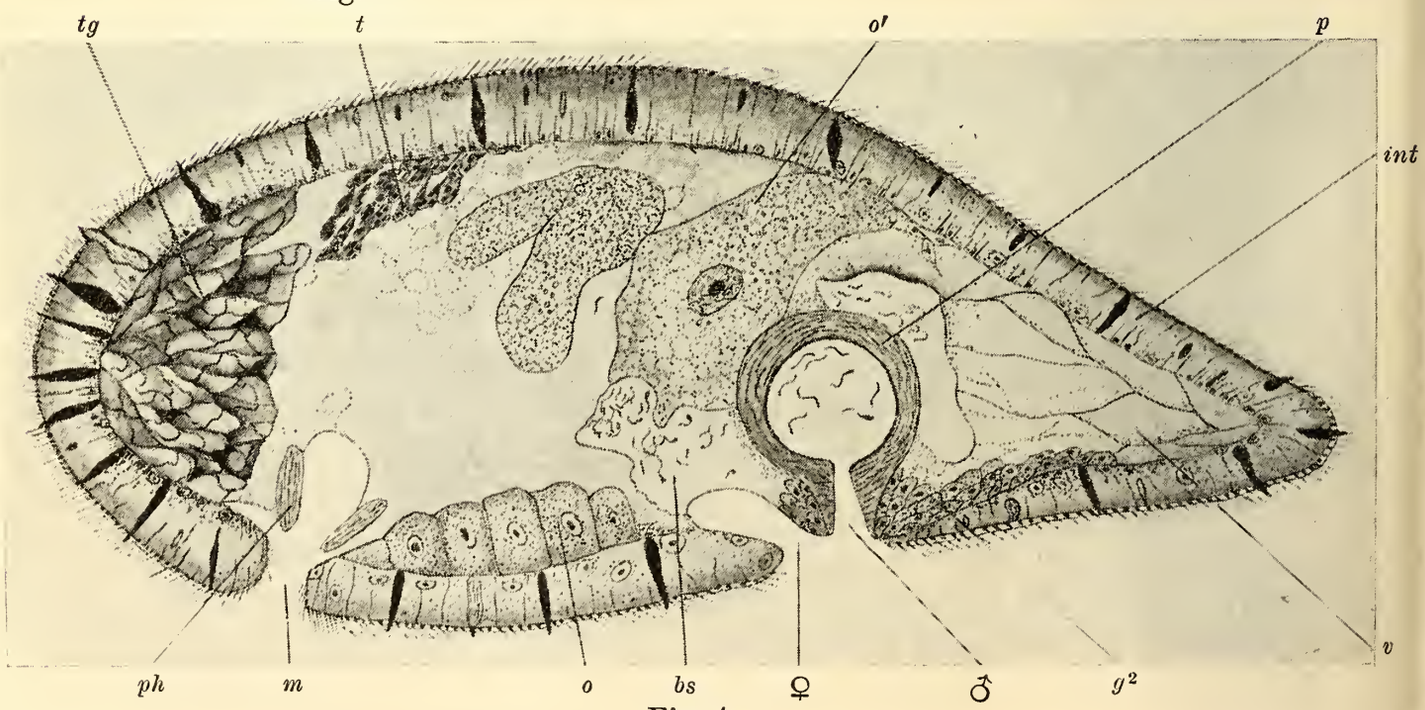

Fig. 4.

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Aphanostoma pulchella belongs to the later type. It is highly developed and typical in shape (pl. 10, fig. 1). The lobes extend posteriorly to the level of the mouth, and closely surround the brain and statocyst. This gland and the mucous glands of the integument as well as the glandular tissue of the reproductive organs, stain a brilliant blue in Delafield's Haematoyxlin.

The mouth, which is near the anterior end of the body (pl. 10, fig. 1 and text-fig. 4) opens on the ventral surface, posterior to the statocyst. The pharynx consists of a short circular tube with thick muscular walls. In my preparations I have not seen the "superimposed tubes" which Pereyaslawzewa described.

The nervous system is composed of a small mass of cells surrounding the statocyst. There are no eyes present. No nerve fibres have been observed.

The parenchyma is loosely aranged with large spaces between the cells giving it a vacuolated appearance (figs. 3 and 4). At the extreme posterior end the cells are large (pl. 10, fig. 1, and text-fig. 4), resembling those in the same region of *Aphanostoma diversicolor.*

The reproductive products ripen in *Aphanostoma pulchella* at different periods, the male organs maturing first, and the female later. For this reason when the eggs are mature the testes and vasa deferentia are small and inconspicuous. When fully developed the follicular testes extend forward on each side as far as the frontal gland (fig. 1, *t*). Their position is dorsal and lateral to the ovaries. The paired vasa deferentia are coiled in loose loops on each side of the copulatory organ. The ripe spermatozoa are frequently observed lying in bunches (fig. 2, *sp*) in the parenchyma. They have large rounded heads, and thread-like tails just as Uljanin described them in *Nadina pulchella.* The penis, or copulatory organ, is the largest and most conspicuous organ in the body (pl. 10, fig. 1, text-fig. 2 and 4). It opens to the exterior near the beginning of the posterior half. In the living worm, when slightly cempressed dorsoventrally, it resembles a muscular ball with a hole in the center (pl. 10, fig. 1, *p*). In transverse and sagittal sections (figs. 2 and 4, *p*) it is shaped like a stout flask, whose walls are composed of many layers of muscle. These muscles extend into the neck of the flask, and out to the surface. On each side of the orifice the muscular walls are surrounded by masses of glandular tissue. Whether or not the penis is evertible I have been unable to ascertain.

The antrum femininum lies immediately in front of the penis so close to the male genital opening that it might easily be overlooked. It leads

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into the thin-walled bursa seminalis (fig. 4, *bs*). The bursa usually contains a few spermatozoa. The ovaries lie on each side of the median line, near the ventral surface (fig. 3). The eggs when ripe grow to such a size that they distend the body. There is no chitinous mouth-piece on the bursa, and as two genital openings are present this worm falls undoubtedly, under the family Convolutidae. If only one genital opening were present it would belong among the Proporidae.

Until the two Black Sea species found by Uljanin and Pereyaslawzewa are re-investigated we must remain uncertain as to their systematic position. The yellow worm described by Uljanin, seems to correspond to *Aphanostoma pulchella* in every respect except the shape of the mouth, which is a minor point, but the color, shape, and much of the structure given by Pereyaslawzewa for *A. pulchella* mihi differ from the Neapolitan form.

1. *Monochoerus lineatus* n. sp.

a) External appearance.

This worm, which is fairly abundant at Naples, is easily detected when swimming in clear water on account of the two broad white stripes which lie parallel along the dorsal surface, extending from the anterior to the posterior end (pl. 10, fig. 4). When resting or crawling on the seaweed it is more difficult to discern, as the color and markings closely resemble the light and dark shades of the branches (pl. I0, fig. 3). The body when resting measures about 1,5 mm in length, and about 1,0 mm in breadth. When extended in swimming it reaches a length of nearly 2,5 mm, the breadth is at the same time decreased proportionately, as the edges turn in slightly toward the midventral line. The dorsal surface of the body is convex, so that in cross-section (figs. 5 and 7) it appears flattened ventrally, and rounded dorsaly. When swimming and crawling the posterior end tapers to a blunt tail-like point, the anterior end is rounded and slightly constricted at the sides giving the appearance of a head. The greatest diameter of the body is in the region of the bursa seminalis, at the beginning of the posterior one-half. This enlargement is undoubtedly due to the presence of great numbers of ripe eggs at this level (fig. 5). When contracted the worm assumes a polygonal shape (pl. 10, fig. 5) the sides lying parallel, and the anterior and posterior ends becoming rounded.

During the months of February and March, when the investigations were in progress, the ground color of the worms was a light brick-red

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which contrasted sharply with the white stripes. These stripes are joined anteriorly by a transverse band, and posteriorly they converge forming a point (pl. 10, fig. 4). In August the color is much lighter, and therefore the stripes are less conspicuous altough still present. The red color is due to pigment cells which are distributed over the surface of the body (pl. 10, fig. 6, *pi*) not in rows as they are in *Anaperus*, and *Monochoerus illardatus*, but irregularly. The red pigment granules are in the eyes, and also in the eggs (pl. 10, figs. 6 and 7).

The movements of *Monochoerus lineatus* are slow; the worm often lying motionless for hours. It swims and crawls, but the characteristic "feeling about" with the anterior end has not been observed. The food consists of small marine animals, usually worms, which are engulfed regardless of size. The mouth is capable of great distension, and often worms of large proportions are found within the parenchyma.

b) Anatomy.

The body is covered everywhere with a thick coat of cilia of uniform length, but the cilia are not arranged in definite rows as they are in *Aphanostoma pulchella*, and other closely related forms. The thin epithelium consists of a layer of cells whose outline is not easily determined. The large round nuclei are scattered at intervals throughout the tissue (figs. 5, 7 and 8), but distinct cell boundaries were not brought out by the methods of fixing and staining which were employed.

The integument is richly supplied with mucous or slime glands (*g*). Although found all over the surface of the body, these glands are especially abundant at the anterior and posterior ends. These masses replace the frontal and tail glands of other species. The glands in *Monochoerus lineatus* are pear-shaped, opening to the exterior at the narrow end, while the broad end extends into the parenchyma below the epithelium (fig. 5, *g*). In specimens stained with Haematoxylin the glands are plainly visible for they stain a brilliant blue. It is possible to see them in the living worm. As in *Monochoerus illardatus*, there are no rhabdites present.

The musculature is strongly developed. Longitudinal and circular fibres form a layer under the epithelium, and strong dorso-ventral fibres are present in the parenchyma (fig. 8, *cm* and *dvm*). The body shows great power of contraction when stimulated.

The mouth lies in the mid-ventral line about as far from the anterior end as the genital opening is from the posterior end (Fig. 8). There is no well defined pharynx, but the mouth opening is surrounded by muscle cells (*ms*). The space into which the mouth leads is usually filled with

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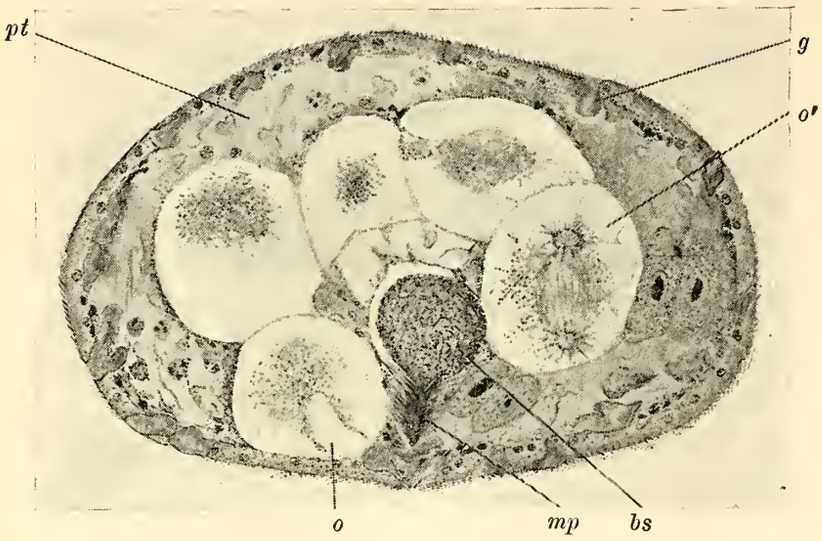

Fig. 5.

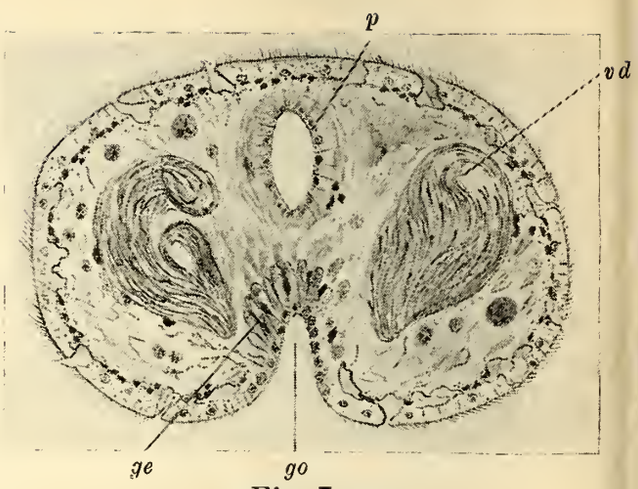

Fig. 7.

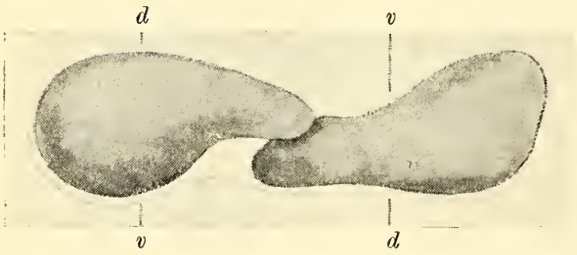

Fig. 6.

Figs. 5—8. *Monochoerus lineatus.*

Fig. 5. Transverse section through the middle of the body: *bs*, bursa seminalis; *g*, slime glands; *mp*, chitinous mouth-piece; *o*, *o'*, ova; *pt*, parenchyma. 150 diam.

Fig. 6. Two copulating individuals united by their ventral surfaces: *v*, ventral; *d*, dorsal surface. 25 diam.

Fig. 8.

Fig. 7. Transverse section through the region of the male reproductive organs: *ge*, genital glands; *go*, genital orifice; *p*, penis; *vd*, vasa deferentia. 150 diam.

Fig. 8. Median longitudinal section through the entire worm; *aa* and *ap*, anterior and posterior adenodactylus; *br*, brain: *cm*, circular muscles of the body wall; *dvm*, dorso-ventral muslce strands; *fd*, food; *gd*, genital duct; *ms*, muscles surrounding the mouth. 150 diam.

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food (*fd*). The parenchyma of *Monochoerus illardatus* contains zoochlorella cells, but that of *Monochoerus lineatus* does not. In other respects the two tissue agree in structure and arrangement.

The structure of the nervous system has not been studied. In the anterior end a large statocyst is surrounded by nerve cells and on each side of the statocyst at the same level with it are the red eyes (pl. 10, fig. 6, *e*).

Monochoerus linaeatus, like other Acoela, is hermaphrodite, the male and female sexual organs occurring in the same individual, the former ripening before the latter. Paired testes lie on each side of the body, and extend far forward, almost reaching the level of the statocyst. In the testes which are follicular in structure, the developing spermatozoa can be seen.

The large penis is enclosed in a sheath (fig. 8, *ps*) surrounded by glandular tissue. On each side of the penis are two rudimentary canals (*aa* and *ap*) which no doubt correspond to the "Adenodactylus anterior" and "posterior" of *Monochoerus illardatus*. The penis is evertible, and at the time of copulation it is extruded from the genital opening. Two copulating individuals were found and were killed in order to study from sections, the relation of the copulating organs. Unfortunately during the process of imbedding, the animals were broken apart, and the structures were so much injured that it was impossible to determine the relation of the parts. When the worms copulate they unite by their ventral surfaces (fig. 6), so that the posterior ends overlap, while the anterior ends extend in opposite directions.

Careful investigation of serial sections, both transverse and sagittal, has failed to reveal the presence of a female genital orifice in front of the male opening. There seems to be no direct communication between the bursa seminalis and the exterior. Löhner and Micoletzky (1912) have found in *Monochoerus illardatus*, that a ciliated tube, the vagina, leads from the antrum femininum, and enters the posterior side of the bursa seminalis. No such canal has been found in *Monochoerus lineatus*. Here there is a canal leading from the male genital opening, first in a dorsal direction then toward the anterior, and finally entering a cavity which may be a "Penisblase" such as has been demonstrated in *Monochoerus illardatus*, but which appears to lead directly into the bursa (fig. 8, *gd*). As no other communication between the bursa and the exterior has been found I am inclined to think that the spermatozoa from another worm enter the bursa in this way. If this be true then the opening to the exterior (*go*) should be called the atrium genitale commune.

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In what manner the eggs are extruded from the body is still unsettled. It is possible that they are forced out through the mouth, or they may pass between the parenchyma cells through a temporary opening which closes immediately after the eggs are laid. At the time when these worms were collected the eggs were mature, but none were observed in the act of extruding the eggs. It is evident that they are fertilized within the body, for various stages of maturation, fertilization, and the first segmentation spindle (fig. 5, *o'*) have been found in cross-sections of the worms.

The ovaries of *Monochoerus lineatus* consist of two thin-walled sacs, extending along the body from the region of the bursa to the brain. These sacs unite in the middle of the body to form one large sac which when distended, with eggs, occupies the entire region between the mouth and the bursa.

The testes which are follicular, also extend anteriorly to the level of the brain. The vasa deferentia broaden out into large coils in the region of the penis (fig. 7, *vd*).

According to Löhner and Micoletzky, the genus *Monochoerus* is distinguished by the fact that the chitinous mouth-piece opens into the parenchyma, and not to the exterior as it does in *Convoluta*. As shown in the sagittal section in fig. 8, the mouth-piece of *Monochoerus lineatus* opens into the parenchyma at some distance from the ventral surface of the body. In transverse section (fig. 5), it also shows no connection with the exterior.

c) Relation to other Acoela.

It is evident that *Monochoerus lineatus* and *Monochoerus illardatus* are closely related species. In order to compare them the following tabulation of their chief characteristics has been made.

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	Monochoerus lineatus	*Monochoerus illardatus*
Habitat.	Pelagic, among seaweeds near shore.	Plankton.
Length	From 1,5—2,5 mm.	1,2 mm.
Breadth.	From 0,8—1,00 mm.	0,6 mm.
Form.	Cylindrical, anterior end rounded and separated from the body by a slight constriction. Posterior end blunt. When swimming three times as long as broad. Sides turn under slightly.	Cylindrical, rounded at the ends. Sides turn under slightly.
Color.	Light brick-red, with two broad bands of white concrement on the dorsal surface. Pigment cells througout the integument.	Dark olive to dull green. Dark brown pigment in the integument.
Glands.	Slime glands large, most numerous at anterior and posterior ends. No definite frontal gland. No rhabdites.	Diffuse frontal gland. No rhabdites.
Mouth.	Slightly anterior to the center.	Same.
Pharynx.	Reduced to few muscles at the mouth opening.	Long and muscular, with glands.
Eyes.	Two reddish eyes, one on each side of the statocyst.	No eycs.
Statocyst.	Spherical.	*Convoluta* type.
Genital openings.	One.	Two, female in front of male.
Penis.	Large, with sheath. Two short canals.	Large, with two canals.
Bursa.	Chitinous rod straight. Mouth-piece opening into parenchyma.	Chitinous rod straight. Mouth-piece opening into parenchyma.

In form, color, and general structure *Monochoerus lineatus* follows the *Convoluta* type, but the fact that the chitinous mouth-piece opens into the surrounding parenchyma, instead of leading to the exterior, prevents it from belonging to that genus. If we adopt the classification of the Acoela, made by von Graff (1912) which be bases upon the number of genital openings *Monochoerus lineatus* would fall under the Family Proporidae. The other characteristics make this impossible. Luther (1912) on the other hand, bases his division upon the presence or absence of a bursa seminalis, grouping under the Proporidae those in which

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there is no bursa, and under the Convolutidae those possessing this organ. The latter family he subdivides into those with one genital opening (*Otocelis*), and those with one or two genital openings, the female in front of the male. *Monochoerus illardatus* and *Monochoerus lineatus* will then be contained in this last division. I shall therefore identify *Monochoerus lineatus* as an Acoela with a bursa seminalis, one genital opening, the mouth-piece lamellate, and opening into the parenchyma. It is distinguised from *Monochoerus illardatus* not only by its external form and markings, but by the absence of the female genital orifice.

3. *Amphiscolops fuligineus*, n. sp.

a) External appearance.

As *Amphiscolops fuligineus* so closely resembles the green form *Amphiscolops cinereus*, described by von Graff (1891), I shall limit myself to a brief account of the more important anatomical features, and omit the detailed description of the finer structure. *Amphiscolops fuligineus* is a larger worm than either of those described in the preceeding pages, its size exceeds that of the Neapolitan form of *cinereus*. When the body is extended in swimming the length is more than twice the breadth, but when in a contracted state the worm is almost square in outline. When fully extended the largest worms measured from 2,00 to 3,00 mm in length, and from 0,8 to 1,2 mm in breadth. At rest they assume the characteristic shape indicated in pl. 3, fig. 16. The deep depression between the ear-like projections of *Amphiscolops cinereus* is here replaced by a rounded anterior end, and instead of a tail filament like that found in *Polychoerus caudatus*, there is a knoblike projection on the posterior end. This slight enlargement is due to the terminal position of the large penis. The animal is capable of great variation of form; when swimming it is cylindrical, but when partially contracted the dorsal surface is convex, and the ventral flat. The edges do not turn under ventrally.

The brown color of this worm is its chief distinguishing feature, therefore the specific name *fuligineus*, was selected. This peculiar shade of brown is found in Saccardo's (1894) list of colors. The vivid contrast produced by the brown and white markings is striking, and this sturdy little robber is easily detected when swimming in clear water, but like *Monochoerus lineatus*, it is protected by its resemblance to the seaweeds upon which it is so often found. The anterior end of the body is entirely white, and for this reason the eyes and statocyst are plainly visible. The

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white area (pl. 10, fig. 8) extends back for nearly one-fourth of the body length. The brown pigment begins abruptly, forms a transverse band reaching beyond the middle of the body, and gradually fades off into white again. The dorsal surface is distinctly mottled in appearance (pl. 10, fig. 8), but the ventral surface is light with the exception of two large brown patches on each side of a median white area (pl. 10, fig. 9). The margin is flecked with a more or less symmetrical row of brown spots. The brown color is due to the presence of granules immediately under the epithelium while the white areas are caused by the accumulation of the concrement which is so common in the Acoela.

b) Anatomy.

The body is covered everywhere with a thick coat of fine cilia which are uniform in length and distribution. There is no smooth ventral disc such as that of *Haplodiscus* (1895). The cilia also line the orifices of the body. Small mucus glands open at intervals along the surface (figs. 9 and 12). The muscular system consists of a thick layer of circular and longitudinal fibres under the epithelium. Strong dorso-ventral strands traverse the parenchyma.

The frontal gland fills the anterior end of the body (pl. 10, fig. 10). This organ in *Amphiscolops* resembles the type found in *Proporus venosus*. The various lobes of the gland unite at the anterior end and open on the surface where they form a distinct frontal organ. There are many glandular cells surrounding the reproductive organs. A collection of these accessory glands are shown in fig. 12 where they enclose the female genital opening, and in fig. 9, in the bursa seminalis.

Between the muscle strands the bulk of the body of *Amphiscolops fuligineus* is filled with vacuolated cells with large open spaces between them (figs. 9 and 12). The cells toward the outer wall are more closely packed together. Their nuclei stain deeply and are conspicuous in sections.

The nervous system resembles that of *Amphiscolops cinereus*, and therefore a description of its structure is omitted. The large spherical statocyst (fig. 11) lies near the anterior end of the body, and at the same level are situated the prominent red eyes. The eyes are not mere aggregates of pigment as in *Otocelis rubropunctata*, instead they possess a distinct lens such as occurs in *Proporus venosus*.

The reproductive organs of *Amphiscolops fuligineus* resemble those of *Amphiscolops cinereus* so closely that one description might easily apply to both species. The penis is situated at the extreme posterior end of

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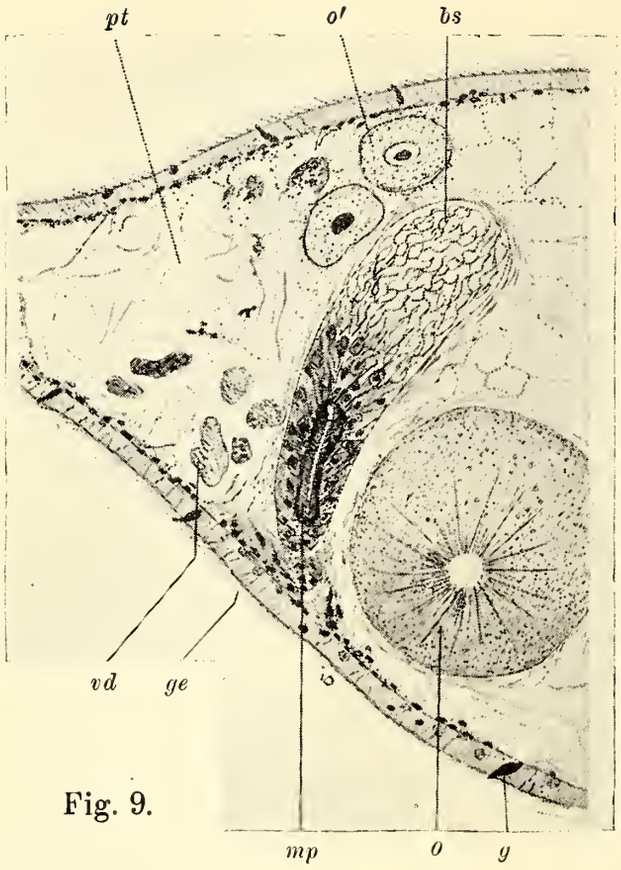

Fig. 9.

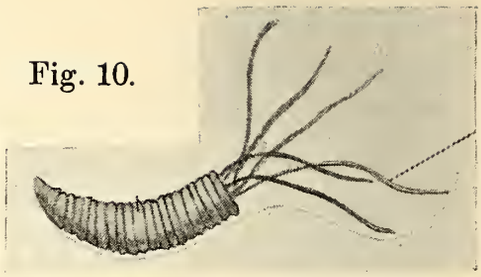

Fig. 10.

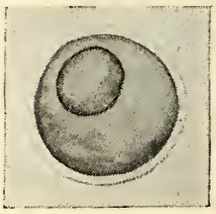

Fig. 11.

Figs. 9—12. *Amphiscolops fuligineus.*

Fig. 9. Longitudinal section on one side of the median line: *bs*, bursa seminalis; *g*, slime glands; *ge*, genital glands; *mp*, mouth-piece; *o*, *o'*, ova; *pt*, parenchyma; *vd*, vasa deferentia; 250 diam.

Fig. 10. Chitinous mouth-piece with spermatozoa protruding from the basal end; 400 diam.

Fig. 10.

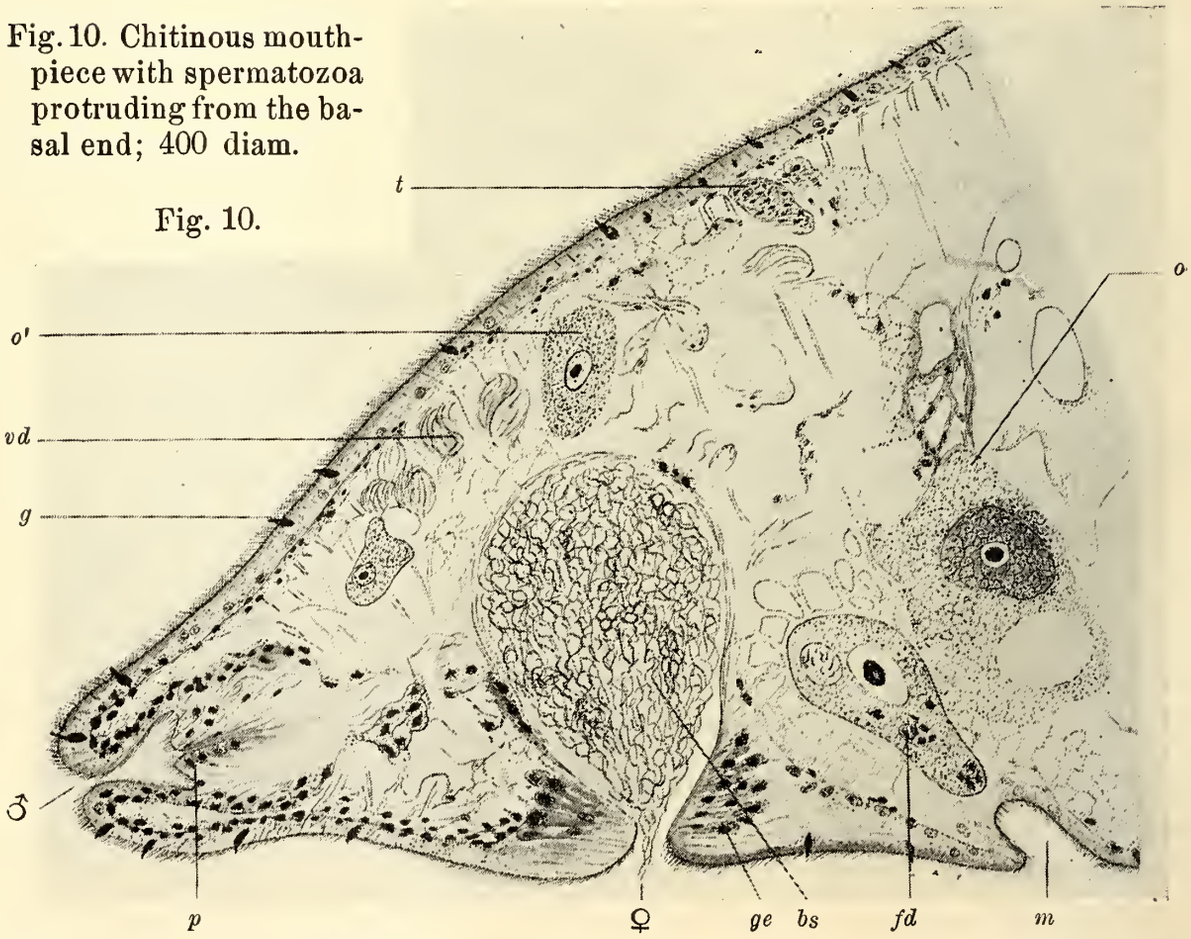

Fig. 11. Statocyst, 400 diam.

Fig. 12. Median longitudinal section through the posterior end of the worm: *fd*, food; *m*, mouth; *p*, penis; *t*, testes; 250 diam.

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the body. It is surrounded by much accessory glandular tissue and opens not directly to the exterior but into a depression formed by the extension of the body wall to form a sheath (fig. 12, *p*). The follicular testes lie in the usual position, dorsal to the ovaries. The vasa deferentia form heavy coils on each side, and finally lead through a bladder-like cavity, into the penis (pl. 10, fig. 10). Unfortunately the process of copulation was not observed in these worms, and therefore it is impossible to determine exactly how the spermatozoa enter the bursa seminalis. There is certainly no duct leading from the region of the penis.

The large bursa is situated immediately behind the mouth (pl 10, fig. 10). It possesses two curved chitinous mouth-pieces. These mouth-pieces are lamellate in structure (figs. 9 and 10). They lie close to the sides of the bursa, with their openings directed toward the ventral side. In his description of *Amphiscolops cinereus* von Graff (1891) represents the three openings of the bursa lying close together, the central one for the reception of the spermatozoa, the two lateral ones for the mouth-pieces. I have not been able to see the actual opening, but I conclude it is present in *Amphiscolops fuligineus*, because the spermatozoa were frequently seen protruding from the bursa in that region. There is a definite antrum femininum surrounded by accessory glands. In fig. 9 the glandular structure of the bursa itself can be seen. The main cavity is usually filled with tangled balls of spermatozoa.

c) Relation to other Acoela.

The systematic position of *Amphiscolops fuligineus* is easily determined, for it possesses a bursa seminalis and there are two genital openings present. It therefore belongs to the Family Convolutidae, and to the genus *Amphiscolops* because of the presence of two chitinous mouth-pieces, and the absence of a tail filament. Löhner has recently (1913) published a brief statement in regard to the position of the genus *Amphiscolops*. He includes in this genus Convolutidae with two or more chitinous mouth-pieces opening into the antrum femininum. Under the genus *Polychoerus* he puts those with two or more mouth-pieces opening into the body parenchyma; thus he finds the same essential point of difference between these two genera as exist between *Convoluta* and *Monochoerus*.

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C. Conclusion.

Within the last ten years several new genera have been discovered among the Acoela and now at least twelve have been identified and described two of these, *Childia* and *Anaperus*, were found by von Graff (1912) on the eastern coast of the United States, *Rimicola* was discovered by Böhmig (1908) on one of the Belgian expeditions, and *Monochoerus* by Löhner and Micoletzky (1911) in the Adriatic. The following classification by von Graff (1912) is given here for the sake of comparison with one made by Luther (1912) later in the same year.

The classification of the Acoela, by von Graff (1912).

I. Acoela with a single genital opening.

Family 1. Proporidae.

A. Proporidae without bursa seminalis.

aa) Definite chitinous mouth-pieces wanting.

a) Penis simple.

1. Pharynx a long tube, body elongated.

Genus 1. *Proporus.*

2. Pharynx wanting, or short, body shield-shaped.

Genus 2. *Haplodiscus.*

b) Penis paired.

Genus 3. *Childia.*

bb) Several chitinous mouth-pieces present.

Genus 4. *Anaperus.*

B. Proporidae with bursa seminalis, and a mouth-piece.

Genus 5. *Otocelis.*

I. Acoela with two genital openings, and a bursa seminalis.

Family 2. Convolutidae.

A. Chitinous mouth-piece wanting

Genus 6. *Aphanostoma.*

B. Chitinous mouth-piece present.

a) With one mouth-piece.

1. Mouth-piece opening into antrum femininum.

Genus 7. *Convoluta.*

2. Mouth-piece opening into the parenchyma.

Genus 8. *Monochoerus.*

b) With two or more mouth-pieces.

1. Two mouth-pieces, no tail filament.

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Genus 9. *Amphiscolops.*

With many (—50) mouth-pieces, tail filament present.

Genus 10. *Polychoerus.*

The classification of the Acoela by Luther (1912).

I. Acoela without bursa seminalis.

Family I. Proporidae.

A. Pharynx a long tube.

Genus 1. *Proporus.*

B. Pharynx wanting, or short.

a) Penis paired.

Genus 2. *Childia.*

b) Penis single.

aa. Body shield-shaped.

Genus 3. *Haplodiscus.*

bb. Body cylindral.

Genus 4. *Rimicola.*

II. Acoela with bursa seminalis.

Family II. Convolutidae.

A. With one genital opening, duct of female posterior to male.

Genus 5. *Otocelis.*

B. With one or two genital openings, female in front of male.

a) Mouth-piece a soft tube or wanting.

Genus 6. *Aphanostoma.*

b) Mouth-piece lamellate.

aa) With one mouth-piece.

1. Bursa opening into antrum femininum.

Genus 7. *Convoluta.*

2. Bursa opening into parenchyma.

Genus 8. *Monochoerus.*

bb) With two or more mouth-pieces.

1. With one genital opening.

a) Bursa single.

Genus 9. *Palmenia.*

b) Bursa composed of many sacs.

Genus 10. *Anaperus.*

2. With two genital openings.

a) Mouth-piece 2—14 parts, no tail filament.

Genus 11. *Amphiscolops.*

b) Mouth-piece many (—50) parts, with filament.

Genus 12. *Polychoerus.*

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It can be readily seen by comparing these two tables that *Convoluta* and *Monochoerus* are very closely related, the distinction resting upon the position of the opening of the chitinous mouth-piece. Further study of the species now included under the genus *Convoluta* may result in changing their position.

As our knowledge of the Acoela increases it seems probable that the classification based upon the presence or absence of a bursa seminalis will prove more satisfactory than one based upon the number of genital openings.

In conclusion, I wish to express my grateful appreciation of the kindness shown me by the members of the staff at the Zoological Station in Naples. My thanks are especially due to Dr. Cerruti, for his untiring efforts to secure the material that I desired. I am glad also to have this opportunity to thank the Naples Association for again allowing me to use the American Woman's Table; and Prof. F. Doflein for the permission to continue my investigations in his laboratory, while spending the summer in Freiburg. Lastly it is fitting to mention the Boston Branch of the Association of Collegiate Alumnae whose fellowship I held while engaged in this and other investigations.

Bryn Mawr, Pa., June 10th, 1914.

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Explanation of Figures.

Plate 10.

Figures 1—2. *Aphanostoma pulchella.*

Fig. 1. Living worm, slightly compressed. *bs*, bursa seminalis; *c*, cilia; *f*, oil globules; *fg*, frontal gland; *m*, mouth; *o*, ovum; *p*, copulatory organ; *s*, statocyst; *v*, vacuolated tail cells; *vd*, vasa deferentia. 120 diam.

Fig. 2. Sketch of living worm, showing form when swimming. 30 diam.

Figures 3—7. *Monochoerus lineatus.*

Fig. 3. Branch of seaweed upon which three worms are resting. About twice the natural size.

Fig. 4. Dorsal view of worm showing form when swimming. 25 diam.

Fig. 5. Dorsal view of animal when contracted. Same magnification.

Fig. 6. Living worm, slightly compressed under cover-glass. *bs*, bursa seminalis; *e*, eye; *m*, mouth; *mp*, chitinous mouth-piece; *o*, ovum; *p*, penis; *pi*, pigment; *s*, statocyst; *vd*, vasa deferentia. 35 diam.

Fig. 7. Mature egg showing pigment granules. 150 diam.

Figures 8—10. *Amphiscolops fuligineus.*

Fig. 8. Sketch of dorsal surface. Animal partially contracted. 15 diam.

Fig. 9. Ventral view of same worm.

Fig. 10. Sketch of living worm, compressed under the cover-glass. *bs*, bursa seminalis; *c*, cilia; *e*, eye; *fd*, food; *fg*, frontal gland; *m*, mouth; *mp*, mouth-piece; *o*, ovum *p*, penis; *s*, statocyst; *vd*, vasa deferentia. 50 diam.

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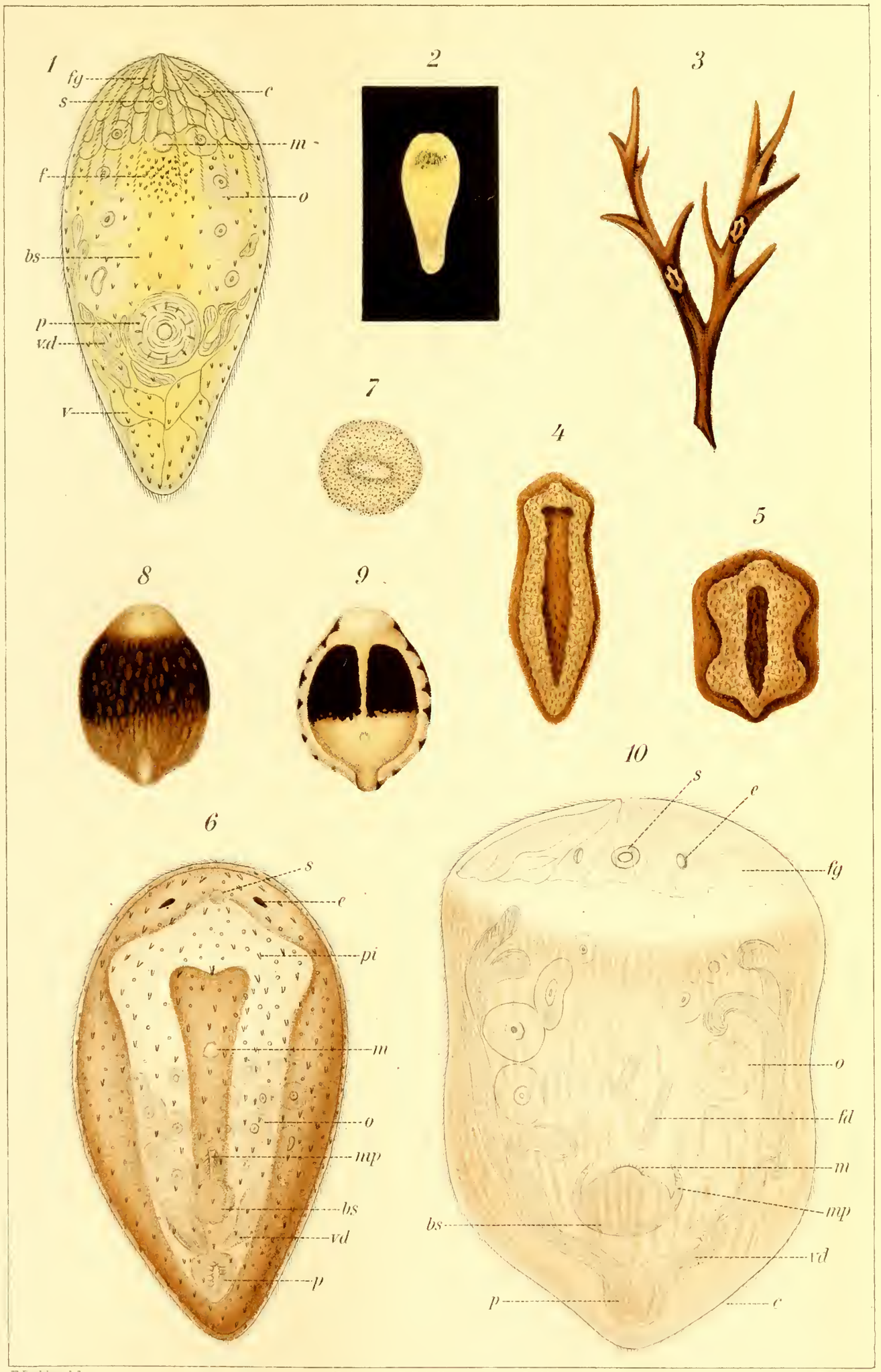

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