

ONYX AND DIPELTIS :

NEW NEMATODE GENERA, WITH A NOTE ON DORYLAIMUS.

By N. A. COBB.

I.

FIXATION AND PRESERVATION OF COMPRESSED OBJECTS.

Many sub-microscopic objects require to be compressed in order to give the best results at the final microscopical examination, and it is well known that compression cannot be accomplished conveniently (if at all) after hardening.

To illustrate by an example: the sub-cylindrical larvæ of dipterous insects if examined fresh are best seen in a compressorium, but much histological detail is thus seen with difficulty, or escapes observation altogether. If, however, it were possible to fix, stain and mount the larva while compressed, a distinct advantage would be gained. To describe a simple way of doing this is the object of these preliminary lines.

The object, say a dipterous larva or a rotifer or a tardigrade or nematode, is compressed between two small coverglasses of the same size. The amount of compression must be regulated by means of two hairs, or better by two pieces of spun glass, placed parallel to each other between the coverglasses. It will be found that hairs from the head, eyebrows, and backs of the hands are of different diameters, and a preliminary experiment will indicate which it is best to use. Having laid the animal, together with two hairs or bits of spun glass, on one of the covers in a drop of water which is too small to entirely fill the space between the covers

when they are finally placed together, lay the other cover on. The animal is compressed, and is unable to move. It will be found convenient to have laid the first cover on a minute drop of water on a glass object slide; by this means it will be held firmly in place on the slide, and the second cover can be laid squarely on; furthermore, after the second cover is adjusted the slide can be placed on the stage of a microscope and the animal then examined to see if its position is the correct one, and, if not, the fault can be rectified by sliding the upper cover slightly on the lower.

Supposing the object to be now correctly compressed and arranged, the next step is to fix the covers in place. This is done by moving the two covers to the edge of the slide by means of a needle and touching first one side of the pair and then the other side with the wick of a wax taper or candle which has been just now extinguished. The melted wax from the wick serves to cement the covers together, and they may be afterwards handled with considerable impunity. It will be remembered that directions were given to use less water than would fill the space between the covers; that was a precaution necessary to bringing both covers into close contact with the hairs that were placed between them, thus securing the requisite amount of compression, and also necessary to securing a firm cementing action of the wax. If there is space between the covers at the edge unfilled by the water, the wax enters it, and if melted wax is then also painted in small quantity on the adjacent outside edge of the covers, a firm union results.

Allow the covers thus united to lie until all or nearly all the water between them has evaporated. They will then present the appearance illustrated in the adjacent figure. Of course a small amount of water will sometimes remain immediately about the compressed animal, and this is often desirable.

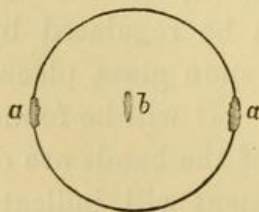


FIG. 1.—TWO ROUND COVER-GLASSES, cemented together with wax at *a a*, and having compressed between them an object *b*.

If now the animal could be fixed, stained and mounted without being allowed to change its attitude, a result often highly desirable

would be attained. To do this, proceed as follows:—Take an elongated piece of quill or other similar elastic non-metallic substance and make in it two cuts as shown at Fig 2 a,b. It will be

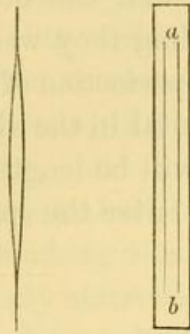


FIG. 2.—TWO VIEWS OF A PIECE OF QUILL, split and opened so as to form a compressorium.

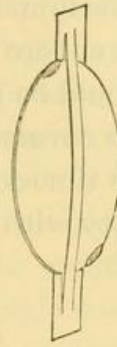


FIG. 3.—TWO ROUND COVER-GLASSES, cemented together and placed in a quill compressorium.

found that the piece of quill can then be opened and converted into a compressing machine. The covers are to be placed in this compressorium as shown in Fig. 3. Of course the compressorium of quill should be stiff enough to firmly hold the covers in place, *but should be no stiffer than will serve this purpose well.*

Our compressed animal is now ready for treatment, and may be handled like any other object. The quill will hold the covers firmly in place, even if the paraffin should become dissolved or melted. If no substance is to be used that will dissolve or melt the paraffin, then of course the compressorium of quill is unnecessary, as for instance when only cold solutions of glycerine are to be used and the object is to be mounted in glycerine. If, however, one wishes to fix in hot sublimate or to proceed at once to alcohols or other liquids that would have a loosening or solvent action on the paraffin, then of course the quill compressorium (or a different cement) is necessary.

To fix the object, take hold of the quill and place one edge of the covers in the fixing fluid; the fluid runs in by capillary attraction, and fixation takes place. The fixing fluid may be replaced by fresh fluid or can be washed out by the use of blotting paper in the ordinary way, *i.e.*, place one edge of the covers in the fluid it is desired to draw in and place fresh blotting paper in contact with the opposite edge of the covers.

An excellent way is to make the whole apparatus represented in Fig. 3 so small that it can be readily introduced into the object box of a differentiator. When the object returns from the differentiator the compressorium is carefully removed and the object will be found not to adhere to the covers, providing they were originally clean. It would be difficult to exceed the perfection of objects thus treated. The covers should not lie horizontal in the differentiator, otherwise the time occupied in treatment will be lengthened owing to the difficulty with which the fluids will enter the space between them.

II.

THE NEW GENUS ONYX.

In the worms constituting the genus *Onyx* the structure of the head and neck is very characteristic, but at the same time the kinship with the genus *Dorylaimus* is at once evident. It will be presently seen, however, that the two genera are very distinct from each other.

As one would expect from the foregoing remark the pharynx in *Onyx* is armed with a spear. As in *Dorylaimus*, so here, the spear is axial and attached to the dorsal side of the pharynx. The uncertainty with regard to the length of the spear is however lessened in *Onyx* by the presence of a *distinct pharyngeal swelling* or *bulb*, which is, as far as length is concerned, nearly co-extensive with the spear. This pharyngeal bulb is an elongated-ellipsoidal, muscular swelling, several times wider than the spear which it encloses; its function is by longitudinal contraction to protrude the spear. This latter organ is stout and tipped with a characteristic horny structure, from which the genus takes its name. As seen under the microscope, this tip presents itself as an inverted V-shaped, or more properly sagittate, body having an opalescent appearance. It is not quite symmetrical, for the ventral barb is uniformly slightly longer than the dorsal. I hardly need remark that this description refers to the view usually obtained, that is to say, the profile or lateral view, and that this body is in reality a hollow cone. The ring so constantly present in the throat of *Dorylaimus* is paralleled in *Onyx* by a three-fold growth from the walls of the anterior part of the pharynx, whose

function is the same as that of the ring in *Dorylaimus*, namely, to serve as a guide and support to the spear. Because of its affinity for carmine this threefold structure is usually conspicuous in specimens treated with that reagent. That portion of the oesophagus lying behind the pharyngeal bulb reminds one forcibly of the corresponding part in *Dorylaimus*, the narrow anterior half being surrounded near the middle of the neck by the nerve-ring, and joined to a stouter, nearly cylindrical, muscular posterior half, two-thirds as wide as the body.

Before describing the head it is necessary to premise that the cuticula is finely striated. The striæ in the single known species appear like plain transverse lines $\cdot 8\mu$ apart, so that the total number in the cuticula of an adult worm is calculated at about twenty-seven thousand. The nearly cylindrical neck terminates anteriorly in a rounded head, which bears, far forward, two large and conspicuous spiral lateral organs. These commonly lie opposite to, or a little in front of, the cap of the spear. The striations of the cuticula cease on the head to be transverse. One

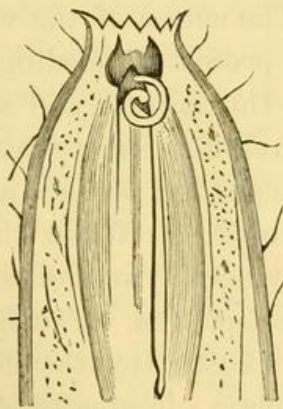


FIG. 4.—LATERAL VIEW OF THE HEAD OF ONYX PERFECTUS, with the mouth open and displaying lips. The pharyngeal bulb and its contained spear are clearly shown, as is one of the spiral lateral organs, and the spear-guide. The left hand side of the figure is dorsal. $\times 400$.

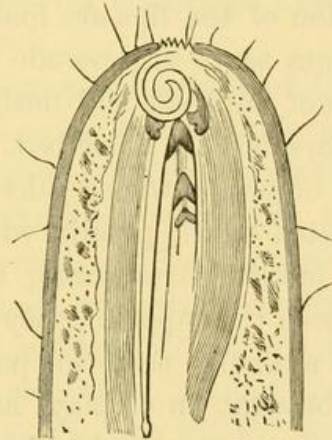


FIG. 5.—LATERAL VIEW OF THE HEAD OF ONYX PERFECTUS, with the mouth closed. The head of the spear is shown just behind the spiral lateral organ. Under the cap of the spear are two developing caps for future use, showing neatly the manner of dentition. The guides for the spear are partially hidden by the spiral organ. The right hand side of the figure is dorsal. $\times 400$.

may observe them passing circularly around the lateral organs and on some parts of the head they are arranged *longitudinally*. This

latter fact harmonizes with the manner in which the cuticula at the head expands to allow of the protrusion of the lips, which are ordinarily so far withdrawn that only the tips of their papillæ are visible. The peculiar action of the cuticula on the head may be compared to the opening and shutting of an inverted umbrella. When the lips and other mouth-parts are withdrawn, the cuticula is drawn together and disposes itself in longitudinal folds. When the mouth parts are thrust forth, and they can be thrust forth to a remarkable extent (see Figs. 4 and 5), the cuticula unfolds to permit the action, and the striæ become less visible.

I am not altogether clear about the muscles by means of which the foregoing movements are accomplished. It is possible that the mouth may be closed by an orbicular muscle or even by the elasticity of the cuticle. Threads, doubtless muscular, pass obliquely backward from the pharyngeal bulb and attach themselves to the body wall. These elements, if muscular, are of course retractile in function. The pharyngeal bulb is also supplied with internal threads, also contractile, which if followed in the direction of the lips are found to pass obliquely outward. These filaments serve to protrude the spear. The action of the various organs of the head and neck during the process of feeding may, therefore, be thus described. The lips are thrust forth and applied to the organism whose juices are to be sucked. This operation is facilitated by the papillæ which act as feelers and perhaps also by other mouth parts acting as clutching organs. When the lips have been thus applied, they are made to adhere by suction exerted in the muscular posterior portion of the œsophagus. The spear is next brought into play, an operation effected by the muscles inside the pharynx which act against the close adhesion of the lips as a resistance. Thus the spear is made to glide forward through its guide and to pierce the surface held by the lips. When the surface has been pierced, the liquid food beneath it is made accessible and is sucked in and swallowed by means of the large muscular posterior œsophageal swelling.

This method of using the spear is somewhat unique. In *Dorylaimus*, with which *Onyx* will naturally be compared as a

related genus, the manner of using the spear is quite different. The differences will be most clearly apprehended if their consideration be preceded by a short discussion of the mechanics of the Nematode spear taken in a general sense. The office of the spear is to puncture membranes which enclose the food-materials of its possessor—in most cases the walls of cells. For this operation it is necessary to have an opposing pull or inertia greater than the force which moves the spear forward. The inertia of the animal is not a sufficient reaction because of its small size and consequent lightness; therefore we find, for the production of a pull, in all cases where a spear is present, well-developed lips and a powerful sucking apparatus in the shape of a highly muscular portion of the œsophagus specially adapted to producing a partial vacuum. The lips are applied, suction is then exerted, and the mouth is thus made to firmly adhere to the membrane to be pierced. This force of suction is the mechanical "base of operations" for the action of the spear, and the pull of the suction must be greater than the force required to thrust the spear forward, otherwise the lips will let go their hold before the spear can accomplish its work.

In all the genera possessed of a spear, the action of the lips in obtaining a purchase is much the same, and in this respect, therefore, *Onyx* cannot be said to present marked peculiarities. When we come, however, to the manner in which the spear is thrust forward, we find marked differences, and *Onyx* presents one of the most marked types. The most emphatic morphological expression of the difference existing between *Onyx* and its congeners is the possession by the former of a distinct muscular pharyngeal bulb. There is no such bulb in any known species of *Tylenchus*, *Aphelenchus*, *Dorylaimus*, or other spear-carrying genus. In *Tylenchus* the spear is believed to be moved backward and forward by means of muscles attached to the three chitinous bulbs which constitute its posterior extremity. I believe, however, that no such muscles have been observed in *Dorylaimus*; in fact the spear in this genus appears to me often to be moved forward, not so much by muscles attached to itself as by muscles attached solely

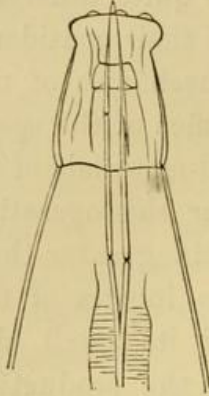


FIG. 6.—EXTENDED CONDITION OF THE HEAD OF DORYLAIMUS LATUS. That portion beyond the line marking a transverse constriction can be retracted within the skin of the posterior part. The spear is slightly protruded, and the ring through which it slides is clearly shown. $\times 450$.

to the walls of the body, the facts being as follows. The species of *Dorylaimus*, as they ordinarily come under observation, present a rather low lip region, offering anteriorly no very remarkable peculiarities. An examination of the figures given by various authors of various species of *Dorylaimus* soon discovers a peculiar loop-like appearance apparently inside the head just behind the base of the lips. I say *apparently*, for these loops, which are visible *in whatever position the animal be viewed*, are in reality the optical expression of an *infolding* of the skin,—exactly such an infolding as occurs in the skin of a turtle's neck when the head is drawn partly within the carapace. The extended condition of the head of *Dorylaimus latus*,* an unpublished Australian species, are

* *Dorylaimus latus*, n. sp. $\frac{4}{1} \frac{8.5}{3.5} \frac{25}{4.5} \frac{52.30}{4.6} \frac{98}{2.5}$ 1.75 to 2.5 mm. The transparent skin of this interesting species is destitute of hairs and is possessed of a distinct, finely striated sub-cuticula in which are to be seen the structures denominated "pores" by Bastian. The pores did not seem to me to perforate the outer cuticula. The neck is conoid to somewhat behind the expanded lip-region, where it becomes convex-conoid. Each of the six lips is, as usual, supplied with two papillae. I could observe neither eyes nor lateral organs, unless, indeed, the latter be the external openings of the glands which I believed to be discernible in the anterior part of the neck when the head was protruded. Under those circumstances these organs, each longer than the head, lay as far behind the fold in the cuticula as the latter was behind the lips. Each appeared like a unicellular gland with a short neck, indistinct ampulla and short chitinous lateral (?) outlet. The pharynx and spear are normal. The cesophagus expands suddenly near the middle, the anterior part being only one-fourth as wide as the neck, while the posterior part is twice that width. The brownish-green intestine is two-thirds as wide as the body, and is set off from the cesophagus by a distinct constriction; the intestine is composed of large cells filled with small granules. The pre-rectal portion of the intestine is twice as long as the adjacent body diameter, its anterior end being less distinctly marked

represented in the accompanying cuts. The manner in which this peculiar arrangement is made of service to the animal may be thus reasoned out. The head having been thrust out and the lips having obtained a purchase, the spear is moved forward by contracting the length of the body by means of muscles attached to the body wall inside the head. This contraction results in an infolding of the skin of the head. This reasoning is exactly in harmony with the usual position of the spear in *Dorylaimus*, for it is well known to be situated well forward, being in fact often normally a little exerted. Attention might also be called to the sinuous condition of the narrow anterior portion of the œsophagus as being also in harmony with the above view. The apparent disproportion between the length of the neck and that of the œsophagus might be thus explained.

We return now to *Onyx*. Passing from the œsophagus the food enters the intestine through a narrow cardia. The connection between the œsophagus and the intestine is unusually small, the diameter at the cardiac collum being not more than a sixth as great as that of the base of the neck. The thick wall of the intestine is built of a single layer of large cells, which are of such a size that half-a-dozen side by side make up a circumference. The width of the intestine where it is the sole occupant of the internal cavity is not far from four-fifths as great as the width of

off than its posterior. The distinct lateral fields are of a lively brown colour and appear to terminate posteriorly in pores near the rounded terminus of the tail. Anteriorly they become narrower and apparently cease altogether in the neighbourhood of the nerve-ring. This latter is oblique and as wide as the œsophagus at the point encircled. The short tail is conical to the blunt terminus and is traversed transversely by distinct anal muscles. To the indistinct vulva succeeds a vagina supplied with a chitinous lining and the usual glands. The reflexed portions of the ovaries are narrow and filled with double rows of developing ova, and extend as far back as the vulva. The eggs are one-half as wide as the body and two to three times as long as wide, and are deposited before segmentation begins. The male is unknown.

Hab. Roots and stems of grass, Sydney, Australia, at all seasons.

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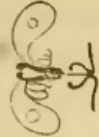


FIG. 7.—PROFILE VIEW OF THE VULVA OF ONYX PERFECTUS. The chitinous vagina is shown dark, and two unicellular glands are shown light. $\times 225$.

supplied with a chitinous lining and the usual vaginal glands. The male sexual apparatus is double and commonly directed forward throughout its extent, but sometimes having the ends of the testicles reflexed. The ductus ejaculatorius extends along that portion of the belly occupied by the row of accessory organs, and appears to be composed of a double row of cells much flattened in the direction of the axis of the body. The free extremity of each testicle is filled with from fifty to one hundred elongated structures arranged radially, but directed obliquely towards the axis of the organ. These bodies are granular and stain in carmine. They increase rapidly in size posteriorly and become the mother-cells of the spermatozoa, which they appear to do by a condensation of the granular matter contained in them into a distinct nucleus. The flattened mother-cells are packed in two or three rows after the manner of a string of dried figs, but begin so soon to break up that it is often impossible to count more than twenty of them. The spermatozoa resulting from the breaking up of the mother-cells are distinctly nucleated, spheroidal, granular bodies whose diameter is one-fifth to one-fourth as great as that of the testicle. There are two spicula, and they are supplied with accessory pieces. On the ventral line a single row of preanal accessory organs is found, coextensive with the ductus ejaculatorius. Caudal glands are found in both sexes. The posterior part of the tail, or terminus, is larger than usual, conical and destitute of striæ.

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* Jenaische Zeitschrift für Naturwissenschaft. xxiii. Bd.

the remark that other species if carefully examined would perhaps prove to be also striated. Since making those observations I have confirmed the impression under which they were written, by the discovery of striæ in a number of other species of *Dorylaimus*. These striæ are generally most clearly visible near the posterior extremity of the animal. The occurrence of spiral markings on the head of *Onyx*, and of fine transverse striæ in its cuticula, coupled with the general resemblance to *Dorylaimus*, when taken in conjunction with the observation of fine striæ on many species of the latter genus and obscure spiral markings on two species, obviously give a new character to the group of Nematodes of which *Onyx* and *Dorylaimus* are representatives, and suggest new phylogenetic probabilities.

The worms belonging to the genus *Onyx* are readily recognised by the cylindrical neck and peculiar head. The single species now first described is called on account of the perfection of its development

ONYX PERFECTUS, n.sp. $\frac{4.1}{2.7}$ $\frac{7.5}{2.9}$ $\frac{15.5}{3.1}$ $\frac{51.30}{3.6}$ $\frac{91.5}{2.2}$ 1.94 mm. The cuticula is traversed by twenty-seven thousand transverse striæ and bears throughout the length of the body very slender and rather long hairs. These latter are, as usual, longer and more numerous near the head, where their length is about half as great as that of the diameter of the body. When the mouth is closed the anterior extremity is hemispherically rounded. The conspicuous lateral markings are so curved that the right hand one appears as a left-handed spiral passing through about 450° of angular space, and the left hand one as a corresponding right-handed spiral. When the worm is placed in profile the spirals appear to be one-half as wide as the pharyngeal bulb, the latter being itself one-half as wide as the head. There are no eyes. When the mouth is closed the summits of the twelve pointed papillæ with which the lips are armed may be seen crowded together at the small orifice. When the mouth-parts are thrust forward, the points of the papillæ become separated from each other and then sometimes have the appearance commonly presented by the lips and papillæ of

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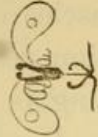


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Chromadora. The pharyngeal bulb is about one-fourth, the posterior or cardiac bulb about two-fifths, and the intermediate



FIG. 8.—I, THE MALE OF ONYX PERFECTUS ($\times 40$); II, III, IV and V, the tail end, head, accessory organs and anal region, respectively, of the same worm, more highly magnified (II, $\times 750$; III, $\times 225$; IV, $\times 900$; V, $\times 175$). In the anterior part of I the oesophagus is shown, surrounded by the nerve-ring (white); following the oesophagus is the intestine, the view of which is interrupted near the middle of the body by the testicles (drawn lighter).

canal about one-third as long as the neck. While the cylindroid cardiac swelling is three-fourths as wide as the neck, the pharyngeal swelling is only one-half and the intermediate canal only one-third as wide as the neck. The slightly oblique nerve-ring has about the same width as the oesophageal canal it surrounds, and is accompanied by the usual groups of nerve-cells. The tail is slightly convex-conoid to the large conical terminus, which begins with a slight expansion. The widest portion of the terminus is one-third as wide as the base of the tail. The caudal glands are situated in the anterior part of the tail, and are three in number. The reflexed portion of the ovaries reach one-third the distance to the vulva.

$\frac{4}{2.8} \frac{7}{2.9} \frac{14.8}{3.3} = \frac{M}{3.3} \frac{92.8}{3} 2.1$ mm. The tail of the male closely resembles that of the female, the only difference being an arcuate form and the presence of an inconspicuous, low, broad, ventral,

bristle-bearing papilla near the middle. The ventral series of twenty to twenty-eight equi-distant accessory organs lies immediately in front of the anus and occupies a distance a little more than twice as great as the length of the tail. The linear spicula, two-thirds as long as the tail, are cimetar-shaped, being of quite uniform diameter. They are rather strongly and uniformly arcuate in the distal four-fifths. The proximæ are cephaloid by unusually great expansion. The thin accessory pieces are one-third as long as the spicula.

This species is common in the Bay of Naples, living in sand in situations occupied by *Amphioxus lanceolatus*. The absence of large marine algæ in its habitat leads me to surmise that it is a carnivorous species.

III.

THE NEW GENUS *DIPELTIS*.

Nearly thirty years ago Eberth described in his "Untersuchungen über Nematoden," under the name of *Enoplus cirrhatus*, a peculiar marine Nematode whose like has not since been observed. I am interested, therefore, to find in my Ceylon collection a similar worm which enables me to confirm Bastian's statement that Eberth's species mentioned above was not an *Enoplus*. The observations I have made on the Ceylon species, coupled with observations on a new species taken in the Mediterranean, lead to the establishment of the new genus *Dipeltis*. The characteristics of this new genus are not numerous, but they are well marked. The head was described by Eberth as bearing on either side a peculiar oval plate. These "plates" are in reality an hitherto unknown form of the lateral organs. Each is an ellipsoidal structure nearly as wide as the head and having a thickened margin. Being rather more pointed anteriorly than posteriorly and extending to the very base of the lips, they give to the head of the worm when seen in profile a peculiar eel-like or fish-like appearance. In other particulars *Dipeltis* is in nowise very remarkable.

The cuticula, which may or may not bear conspicuous hairs, is very finely striated. The mouth was said by Eberth to be furnished with three papillæ. It appears to me, however, that these "papillæ" are rather to be denominated lips. One of them seems to be more pointed than the others—to be, in fact, spear-like. The œsophagus is simply conoid. The ventrally arcuate tail is supplied with caudal glands. Ocelli are present in some species.

1. DIPELTIS MINOR, n.sp. Female unknown.

$\frac{1'(?)}{1'}$ $\frac{8'2}{2'}$ $\frac{19'}{2'3}$ $\frac{M}{2'5}$ $\frac{92'}{1'9}$ 1.26 mm. The cuticula bears no conspicuous hairs. The neck is conoid to near the slightly oblique nerve-ring, becoming thence more and more decidedly convex-conoid until it at last becomes rather suddenly almost acute at the mouth. The length of the ellipsoidal lateral organs is one-fifth as great as the distance between the mouth and the nerve-ring, and they are about one-half as wide as long. Their thickened margins present a double contour. Posteriorly the œsophagus becomes three-fifths as wide as the neck. The portion of the alimentary canal immediately behind the distinct cardiac collum is usually pressed to one side by the large ventral gland, which is two-thirds as wide as the body and twice as long as wide. The position of the porus is unknown to me. The simple, arcuate, linear spicula are of nearly uniform size throughout and are about as long as the anal diameter. An accessory piece less than half as long as the spicula is seen to curve inward and backward from the anus. The tail is conoid to the convex conical terminus, which is one-third as wide as the base of the tail and is supplied with an outlet for the caudal glands similar to that commonly seen in species of *Chromadora*.

Hab. The single male specimen seen was taken from sand on the coast of Ceylon in the month of March.

2. DIPELTIS CIRRHATUS, Eb. $\frac{?}{?}$ $\frac{9'7}{?}$ $\frac{16'}{?}$ $\frac{7?}{3'}$ $\frac{91'(?)}{2'5(?)}$ 4 mm. The cuticula is said to be smooth. Submedian rows of conspicuous hairs occur near the head, extending from the anterior extremity as far back as the two eye spots. These latter are situated half way between the nerve-ring and the mouth. The neck is conoid to near the head, where it becomes convex-conoid. The mouth is said to be surrounded by three papillæ. The conoid œsophagus is on the average one-third as wide as the neck. The cardiac collum, shallow but distinct, marks off the beginning of an intestine which is two-fifths as wide as the body. The rectum would seem to be longer than the anal diameter. The conoid tail is ventrally arcuate and ends in a distinct outlet for the caudal glands.