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Probolitrema tomalis sp. nov. (Trematoda) from *Dasyatis dipterura* and *Brachylaemus virginianus* (Dickerson) (Trematoda) from *Didelphys virginiana*

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PROBOLITREMA TOMALIS SP. NOV. (TREMATODA)

FROM DASYATIS DIPTERURA

AND

BRACHYLAEMUS VIRGINIANUS (Dickerson) (TREMATODA)

FROM DIDELPHYS VIRGINIANA

By .

Elsie Mae Graves

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PROBOLITREMA TOMALIS SP. NOV.
(GORGODERIDAE)

HISTORICAL

The genus Probolitrema was established by Looss (1901) in recognition of the location of vitellaria and multiple testes entirely outside the extra caecal field in Anaporrhutum richiardii Lopez (1888) which differentiated it from A.albidum Ofenheim (1900) with the vitellaria within and the testes partly within and partly without the intra-caecal field. Ofenheim had created the genus Anaporrhutum for the above two species because of their multiple testes. A.albidum was a new species described by him; his A.richiardii Lopez specimens he considered to be the same species as Distomum richiardii Lopez, described in detail by Monticelli in 1893. Looss pointed out that they were not identical; Ofenheim's specimens possessed suckers of nearly equal size while Monticelli's specimens (which were more probably D.richiardii Lopez) possessed a very large and powerful ventral sucker. Looss therefore retained P.richiardii Lopez as the type species of the genus and created a new species, P.canense, for A.richiardii Ofenheim.

OCCURRENCE

About sixty individuals of Probolitrema tomalis were obtained from the coelom of a sting ray, Dasvatis dipterura, on July 2, 1933, from Tomales Bay, California. No observations concerning the living animal were recorded.

METHODS

Specimens were fixed in ten percent formalin. Whole mounts were stained with either borax or alum carmine. Series of frontal and a broken series of cross sections were made (by the paraffin method) and stained with Mallory's.

MORPHOLOGY

External features. The body form is rounded, the animal being slightly longer than wide. That portion bearing the oral sucker is sometimes slightly protruded, giving a gentle tapering appearance to the anterior end. The rounded posterior end is more or less deeply indented. The body is widest in the region just posterior to the ventral sucker. The average body length is 13.7 (10.8-18.5)mm. and the average maximum width is 12.5 (10.5-15)mm. The thickness of the body in flattened specimens is about 0.7mm., slightly less at the margins. The body is semi-transparent and weakly muscular. It is covered with a fairly thick cuticle, which, in flattened specimens is irregularly ridged. The cuticle is thicker at the anterior than at the posterior end and is thinnest over the sucker surfaces.

The oral sucker is well developed, ovoid, somewhat broader than long, and measures 0.8181 X 1.149 (0.643-1.018 X 0.959-1.352)mm. The circular mouth opening is sub-terminal.

The acetabulum is larger and more powerful than the oral sucker and is located from one quarter to one third the length of the body distant from the anterior end. Its average

measurements are 1.417 X 1.617(1.2-1.657 X 1.308-2.326)mm. The longitudinal diameter is the shorter. Conspicuous radial muscle bands extend obliquely from the rim of the sucker to the dorsal wall of the body. The thickness of the sucker is about .5mm., the cavity is very shallow, however, and its circular opening measures about 0.5 X 0.5 mm. The size ratio of the two suckers does not appear to be constant.

Muscles, nerves. Just beneath the cuticle a narrow band of longitudinal muscles occurs; and, beneath these, diagonal muscles extend obliquely inward and anteriorly. These muscles form a criss-cross pattern over the entire body, the individual bands being relatively far apart and so forming a loose network. The parenchyma is very loose and is penetrated by the numerous diagonal muscle bands. A few small cells with prominent nuclei are scattered through it, most of them concentrated near the body wall. Large nerve cells are prominent. The connective tissue of the parenchyma is very loose and barely serves to hold the organs in place; in the flattening process it is often broken.

A large pair of ganglia occurs opposite and close to the posterior border of the pharynx with a thick commissure joining them dorsally (fig.3). A pair of outer lateral nerve cords extends forward obliquely laterally almost to the anterior limits of the body and then bends posteriorly, near the margin of the body, to within about 1.5 mm. of the posterior end. No trace of the union of these cords posteriorly, as mentioned by Monticelli (1893) for D.richiardii, were found. Another pair of inner lateral cords extends

posteriorly from the main ganglia in the extra-caecal spaces to the length of the outer cords. These inner cords lie about 2 mm. distance from the intestinal caeca and about 1.59 mm. distance from the margins of the body. A double ring of nerve cells occurs in the dorsal portion of the ventral sucker; the inner ring lying around the rim of the sucker opening and the outer ring around the rim of the sucker. Similar double rings of nerve cells were plainly seen in the dorsal portions of the oral sucker and of the pharynx.

Digestive system. The cavity of the oral sucker opens directly into the rounded, muscular pharynx which lies slightly dorsal to and just posterior to the oral sucker, sometimes slightly overlapping its posterior part. Average measurements of the pharynx are 0.442 X 0.558 (0.378-0.538 X 0.480 -0.654)mm., the longitudinal diameter being the shorter. Longitudinal muscle bands extend from its anterior border to the dorsal wall. Bands of circular muscles lie beneath the longitudinal bands and encircle the pharynx. No muscle bands connect the pharynx with the oral sucker.

The pharyngeal cavity opens posteriorly and medianally into a relatively long esophagus which, in turn, bends slightly ventrally and gradually broadens as it nears the intestinal bifurcation. The average length of the esophagus in specimens not very extended is about 0.5 mm., while in individuals with more protruded anterior ends it is about 0.9 mm.; and its width at the juncture with the caeca is about 0.364 mm. The wall of the esophagus is thrown into deep folds along its entire length.

The intestinal caeca extend to within 2 mm. distance from the posterior border of the body. They do not lie along the outer margins of the body but leave extra-caecal spaces each of which is approximately equal to one third the width of the body or slightly less. The blind ends of the caeca are usually rounded, sometimes tapering, and are of unequal length. The caeca are narrowest in their anterior constricted portions near the esophagus where they average 0.305-0.363 mm. in width. They quickly widen and range from 0.465-1.163 mm. along their remaining lengths. They are thrown into bulging folds along their entire length.

Cells which appear to be secretory are grouped at the sides of the pharynx and extend along the lateral borders of the esophagus to the intestinal bifurcation.

Female reproductive system. The ovary, seminal receptacle, and shell gland lie in a group just posterior to the ventral sucker; usually to the left of the mid-body longitudinal axis, sometimes almost directly on it, and sometimes to the right. The seminal receptacle is the largest and the most anterior member of the group and is usually the most laterally placed. It lies dorsally, is oval in outline, and in all specimens examined it is closely packed with spermatozoa. It measures 0.881 X 1.189 (0.713-2.0 X 0.974-2.40)mm., the longitudinal diameter being the shorter. There is a great variation in its size; in a few specimens the seminal receptacle approximated the size of the ventral sucker, and in one case it was slightly larger.

The ovary, usually lobed (3-4 lobes), sometimes rounded,

lies just posterior, slightly ventral, and to the right of the receptacle. Average measurements for the ovary are 0.666×0.907 ($0.499-0.945 \times 0.654-1.192$)mm., the longitudinal diameter being the shorter.

Mehlis' gland usually lies wedged between the ovary and the receptacle, sometimes freely to the right of both. It lies lateral and ventral to the receptacle and extends still further to the right of it than does the ovary. The average measurements for the extent of the gland are 0.296×0.549 ($0.203-0.392 \times 0.479-0.727$)mm., the longitudinal extent usually being the shorter.

The spermatic duct emerges from the posterior right border of the seminal receptacle and, after extending about 0.058 mm. gives off a short canal, which appears to be homologous with Laurer's canal; it proceeds dorsally and laterally to the right for 0.087 mm. where it enlarges into an oval sac, measuring 0.072×0.063 mm., the longitudinal diameter being the shorter. (fig. 4).

The oviduct proceeds almost directly anterior from the anterior right margin of the ovary and joins the spermatic duct just dorsal to the right vitelline duct. It then extends directly laterally and to the right into the midst of Mehlis' gland where it is joined ventrally by the short common vitelline duct and runs into the ootype with which it is continuous.

The uterus extends directly laterally of its point of emergence from Mehlis' gland and to the right; immediately upon leaving the gland, it makes an anterior loop, then

proceeds in loose coils along the left portion of the uterine mass to the level of the caecal tips of slightly more posterior to them. It then coils back upon and ventral to its descending branch in tighter and wider coils, more or less filling the intra-caecal space up to the region of the ovary. Still wider coils then proceed to the right of the ovary and ventral sucker. Anterior to the sucker, the uterus curves inward to the mid-body line where it makes a loop to the right and then continues as a median metraterm, whose thickened wall is thrown into deep folds. It terminated in a genital pore at the posterior border of the intestinal bifurcation. Just before reaching the genital pore, the metraterm constricts into a narrow tube. (fig.2). The size of the female genital pore is approximately 0.086×0.063 mm., the longitudinal diameter being slightly shorter. The average width of the ascending branch of the uterus is 0.216 ($0.174-0.290$)mm. Upon emerging from Mehlis' gland it is only about 0.043 mm. wide. The eggs are oval, operculated, thin shelled, yellow, and measure 0.064×0.042 ($0.056-0.069 \times 0.033-0.048$)mm.

The vitellaria, which occur in two irregularly branched compact groups, lie in the extra-caecal fields either on a level with the ventral sucker or posterior to it. (fig. I.). The space occupied by each group of vitellaria varies greatly but on the average it is about 1.4×1.5 mm., the length usually being greater than the breadth. The vitelline duct, averaging about 0.049 mm. in width, proceeds from the inner border of each group, passes ventral to the caecum, and

curves slightly posterior to fuse ventrally to the mid-region of Mehlis' gland into a triangular shaped reservoir and the short common vitelline duct. (fig. 4). The right vitelline duct passes beneath the receptacle and proceeds between it and the ovary to the point of fusion.

Male reproductive system. The multiple testes lie in the extra-caecal spaces, approximately midway between the caeca and the lateral margins of the body. Their anterior limit is usually just posterior to a line level with Mehlis' gland and their posterior limit is usually at least 2 mm. distance anterior to the tips of the caeca. The testes (fig. 1) vary in number and distribution, ranging from 18 to 29 on the right side and from 21 to 29 on the left side of specimens examined. In only one individual was an equal number of testes, 27, found on each side.

An individual testis is irregularly lobed (sometimes almost rounded) and has an average size of 0.480 X 0.596(0.348 -0.654 X 0.436-1.01)mm., the longitudinal diameter usually being the greater. The testes usually occur in clusters of three or four, though are often single, and sometimes grouped closely together in great numbers. A duct leads from the inner dorsal border of each testis and converges with other ducts to form the wider vas efferens (averaging from 0.02 -0.03 mm. in width) which crosses the caecum dorsally at a point some distance posterior to the level of the ovary and then curves anterior, extending just within the caecal borders and always outside the uterine coils. Anterior to the

ventral sucker, the vas efferens from each side curves gradually inward to the mid-body line where they fuse into the short, swollen vas deferens. The vasa efferentia, particularly in the extra-caecal spaces, are surrounded with longitudinal muscles. They are conspicuous throughout their entire lengths because of the presence of great numbers of spermatozoa.

The thick, relatively short vas deferens extends anterior, curves more or less sharply to the right, then bends back to the left and is sharply constricted before joining the seminal vesicle. The vas deferens is surrounded with longitudinal muscles and thick connective tissue and is greatly distended. It averages 0.125 (0.102-0.145) mm. in width.

The seminal vesicle consists of two parts, a larger oval posterior portion and a smaller, round anterior portion, with thicker wall. (fig. 2). The average measurements for the posterior portion are 0.389 X 0.671 (0.290-0.538 X 0.567-0.872) mm., the longitudinal diameter being the longer. The average measurements for the anterior portion of the vesicle are 0.230 X 0.262 (0.218-0.240 X 0.218-0.290) mm., the longitudinal diameter sometimes being slightly greater.

Small prostate cells lie free in the parenchyma, surrounding the anterior portion of the vesicle, which tapers into a thick walled ejaculatory duct. (fig. 2). The prostate cells are more numerous along the later and ventral borders of the vesicle. The ejaculatory duct proceeds anteriorly and ventrally and bends slightly inward to enter the male

genital pore lying beneath the posterior border of the intestine on the mid-body line and right beside the female pore. There is no trace of a cirrus or a genital atrium.

The size of the male genital pore is about 0.069 X 0.86 mm., the longitudinal diameter being slightly greater.

Excretory system. The excretory pore is median, ventral, and at the posterior border at the deepest part of the characteristic indentation there. A narrow excretory tube proceeds anterior in the mid-body region and broadens into a small elongate bladder just posterior to the ovary. (fig. 5). The bladder forks from its anterior lateral borders into two narrow tubes which curve anterior-laterally, cross the caeca dorsally in the general region opposite the ventral sucker, extend outward over the posterior portion of the vitellaria to just beyond their outer limits where it forks again into anterior and posterior branches. The anterior branch is the narrower and curves inward, passing close to the caecal border to within a short distance of the esophagus. The wider posterior branch extends along the outer limits of the testes and, in their anterior portion, gives off a branch which curves inward and posterior toward the caecum. The general pattern of the excretory vesicle is that of a shallow Y.

COMPARISONS

Probolitrema tomalis differs most strikingly from the two species of the genus in the pattern of its excretory vesicle, which is that of a shallow Y, almost like the T form of Anaporrhutum (Ofenheim 1900) rather than like the

deep Y pattern listed as characteristic of the genus Probolitrema. Also, the excretory vesicle of P.tomalis is much narrower than those of the other two species. P.tomalis is like P.richiardii in possessing a powerful acetabulum which is much larger than the oral sucker; it differs in this respect from P.capense which possesses suckers of almost equal size. P.tomalis differs from both species in the following features: (1) a much less powerful body; (2) a rather small ovoid pharynx rather than a large cup shaped one embracing the oral sucker; (3) fewer testes, which extend only about one third rather than one half the length of each side of the body; (4) narrower uterus.

BRACHYLAEMUS VIRGINIANUS (Dickerson 1930)
(BRACHYLAEMIDAE)

OCCURRENCE

About fifteen specimens that have been referred to the species, Brachylaemus virginianus were obtained from the small intestine of a female opossum, Didelohys virginiana, caught near Stockton, California, December 10, 1934.

METHODS

Specimens were fixed in ten percent formalin. Whole mounts were stained either with alum or borax carmine. A series of cross-sections was stained with Ehrlich's and another series with Mallory's haematoxylin. A frontal section series was also stained with Mallory's haematoxylin.

HISTORICAL

Two forms of the species from the North American opossum have been previously described; specimens from Virginia were described by Dickerson (1930) and placed by him in a new variety, virginiana, of H.opisthotrias (Lutz 1895). More recently Chandler (1932) studies a form from Texas which he found to be intermediate in many respects between the Virginia form and the Brazilian form, H.opisthotrias, from the South American opossum, Didelohys aurita; he questioned the validity of Dickerson's new variety for the Virginia form. Sinitsin (1931) in his revision of the Har-mostomidae (syn. Brachylaemidae) placed the North and South

American forms in separate groups. In 1934 Joyeaux revived the genus name Brachylaemus for Harmostomum; he had previously, in 1930, revived the older name, Brachylaemidae (Dujardin 1843) in place of Harmostomidae (Odhner 1913). Dollfus (1935) regrouped the species of Brachylaemus and, evidently because of geographical distribution and a different species of host, created a new species, B. virginianus, for the North American forms and retained the name B. opisthotrias for the South American forms.

COMPARISONS

The California specimens closely resemble the Virginia form; however, in two respects, they agree with Chandler's description of the Texas form; and, in addition, show smaller sized suckers and pharynx than those of either of the two above forms.

The body is tongue shaped with rounded ends, slightly tapering posteriorally. Like the Virginia form, it is weakly spinose in the anterior region, especially on the lateral and ventral sides. These blunt spines, barely projecting through the cuticula, appear to be arranged in groups of two.

California specimens range in length from 1.352-1.905 mm. with an average of 1.654 mm. The range for the Virginia form is given as 1.535-2.541 mm. with an average of 1.945 mm. Thus, although length range for the two forms overlaps to some extent, the maximum for the Virginia form is slightly less than the average for the California form and also has a shorter minimum length than the latter. Greatly contracted

forms seemed well extended; all specimens were mature. The average width through the region of the ventral sucker (.368mm.) appears to be the same for both forms. The range in size of the California form (0.305-0.465 mm.) is greater than that of the Virginia form (0.314-0.450 mm.).

The oral sucker is noticeably smaller; the average for the California specimens is 0.164 X 0.154 (0.145-0.190 X 0.145-0.170)mm. Dickerson gives an average size of 0.259 X 0.285 mm. with a minimum of not less than 0.2 mm. and a maximum of 0.3 mm. The maximum diameter of the California form is thus less than the minimum of the Virginia form. The ventral sucker is also smaller in the California specimens, averaging 0.137 X 0.141 (0.123-0.145 X 0.131-0.150)mm. The Virginia form averages 0.167 X 0.198 mm. Thus the two suckers are more closely approximate in size in the California form. There is a difference of 0.1-0.12 mm. between the oral suckers of the two forms and only 0.03-0.05 mm. between the ventral suckers.

The size of the pharynx, averaging 0.083 X 0.092 (0.0727-0.099 X 0.092-0.102)mm. also falls below that of the Virginia form, which averages 0.109 X 0.127 mm. There is no trace of a definite pre-pharynx noted by Chandler in the Texas forms. There is present a very short gullet, approximately 16 microns long, which was also noted by Dickerson.

The shorter diameter of the ovary averages 0.104 mm. (0.073-0.138) mm. as compared to 0.091 (0.082-0.102) mm. for the Virginia form. The shorter diameter of the anterior testis averages 0.198 (0.196-0.2) mm. as compared to an

average of 0.186 (0.177-0.205) mm. in the Virginia specimens.¹ The posterior testis averages 0.134 (0.102-0.204) mm. in its short diameter while the testis of the Virginia form averages 0.177 (0.123-0.204) mm. The California form agrees with the description of the Texas form in the lack of any close relationship (noted by Dickerson) between the variations in the dimensions of the ventral sucker and the anterior testis, the latter always being the larger in the California form.

The California form is also like the Texas form in showing more variation in the extent of the vitelline glands than occurs in the Virginia form. The anterior limit of the vitellaria is sometimes at the level of the middle of the ventral sucker; more often just posterior to the sucker.

The eggs are operculated, oval, thin shelled, pale yellow, and somewhat smaller (29 microns in length) than the measurements given for the Virginia form (31 microns in length). This average length of egg (31 microns) in the Virginia form is the maximum length for the California form, which also possess narrower eggs (average 14.8 microns) than the former (Dickerson gives a minimum width of 15 microns for the eggs of his specimens). Eggs as narrow as 13.2 microns and as wide as 16 microns were measured in the California form.

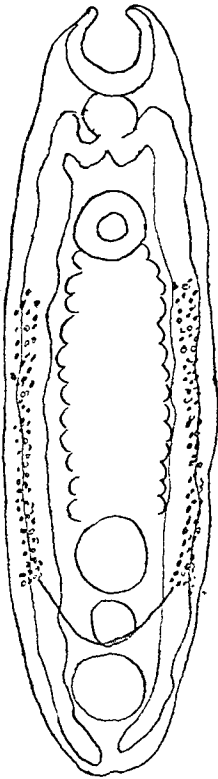
¹ It is assumed that the short diameter of the testis was the one given by Dickerson (1930, p. 37-46) in his measurements of the Virginia form.

DISCUSSION

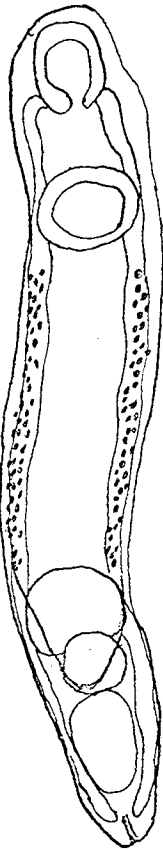
It is the opinion of the writer that the Texas form is intermediate (as previously suggested by Chandler, 1932) between the Brazilian and Virginia forms. The differences seem to be chiefly in regard to size; and study of the California forms has further emphasized this size variation, especially in regard to the suckers, as compared to the Virginia form which it so closely resembles. Dollfus in examining different specimens of B.opisthotrias from Brazil found variations in the extent of the vitellaria; similar variations (table 1 & chart 1) have been noted among the North American forms. Dollfus is doubtful whether or not to place the B.opisthotrias specimens of Braun (1899) in the same species with the B.opisthotrias specimens of Lutz (1895). There seems to be no less difference as to size between these two and between B.opisthotrias Lutz and Chandler's specimens. If the South American forms are retained in one species it seems valid to place the North American forms in the same species, or at most in different varieties, with them. California specimens have been given the species name, B.virginianus, recently created by Dollfus but it seems probable that intermediate forms will eventually be found that will necessitate the inclusion of both North and South American forms in the one species, B.opisthotrias.

	Brazil (Lutz)	Virginia (Dickerson)	Texas (Chandler)	California (Graves)
length	4 mm.	1.945 mm. (1.535-2.541)	1.78-5.33 mm.	1.654 mm. (1.352-1.905)
width	0.9-1.1 mm.	0.368 mm. (.314-.450)	0.314-.450 mm.	0.368 mm. (0.305-0.465)
oral sucker	0.3-0.4 mm.	0.259 X 0.285 mm.		0.164 X 0.154 mm.
ventral sucker		0.167 X 0.198 mm.		0.137 X 0.141 mm.
prepharynx		absent	present	absent
pharynx		0.109 X 0.127 mm.		0.83 X 0.092 mm.
spines	present	rudimentary	absent	rudimentary
ant. testis		0.186 mm.		0.198 mm.
post. testis		0.177 mm.		0.133 mm.
ovary		0.091 mm.		0.104 mm.
size relation of ant. testis to vent. sucker		same size	anterior testis larger	anterior testis larger
beginning of vitellaria	back of ventral sucker	back of ventral sucker	level of middle of ventral sucker	sometimes at level of middle of ventral sucker
eggs		0.016 X 0.031 mm.		0.0148 X 0.029mm.

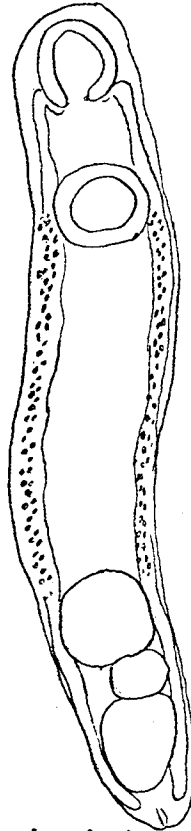
TABLE 1. A comparison of the californian form of Brachylaemus virginianus with two other North American forms of the species and with the closely related South American species, Brachylaemus opisthotrias.



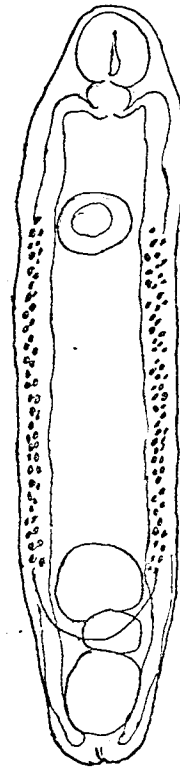
B. opisthotrias
Lutz 1895
Brazil



B. virginianus
Dickerson 1930
Virginia



B. virginianus
Chandler 1932
Texas



B. virginianus
Graves 1935
California

CHART 1. Sketches B. opisthotrias and B. virginianus showing variation in extent of the vitellaria and in size of suckers.

SUMMARY

Fixed specimens of Probolitrema tomalis sp. nov. are described from the coelom of the sting ray, Dasvatis diptera. The animal is 13.7(10.8-18.5)mm. in length by 12.5 (10.5-15)mm. in width, having a greater body width than the other two species of the genus and having a less powerful body. The pattern of its excretory vesicle differs from those of the other two species but it has testes and vitellaria entirely outside the intra-caecal space and, as this the chief basis of differentiation of the genus Probolitrema from the other genera of the sub-family Anaporrhutinae it is placed in the genus Probolitrema.

Fixed specimens of Brachylaemus virginianus (Dickerson 1930) obtained from the small intestine of the California opossum, Didelohys virginiana, are compared to other forms of the species from the opossum in North America. Study of the California form further emphasizes the great variation among members of this species and the closely related South American species, B. opisthotrias, especially as to size. The validity of the creation of separate species for the North and South American forms is questioned.

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EXPLANATION OF PLATE

The following abbreviations are used:

cvd.....common vitelline duct	ov.....oviduct
cm.....circular muscles	os.....oral sucker
ep.....excretory pore	pr.....prostate cells
es.....esophagus	ph.....pharynx
ev.....excretory vesicle	rvd.....rt.vitell.duct
g.....ganglion	sd.....spermatic duct
gp.....genital pore	sr.....seminal receptacle
i.....intestine	sv.....seminal vesicle
lc.....Laurer's canal	t.....testis
lm.....longitudinal muscles	u.....uterus
mg.....Mehlis'glands	vd.....vas deferens
mt.....metraterm	ve.....vas efferens
nc.....nerve cord	vs.....ventral sucker
o.....ovary	vt.....vitellaria
ot.....ootype	

Fig.1.Probolitrema tomalis x 4.6.Ventral view.Drawn with aid of the projection lantern.

Fig.2.Terminal part of reproductive system.X Drawn with the aid of a camera lucida.

Fig.3.Ganglia and beginnings of nerve cords.Dorsal view. Sketch. x 12

Fig.4.Female reproductive organs.Ventral view. X Camera lucida drawing.

Fig.5 Excretory vesicâ. Sketch. x 2.6

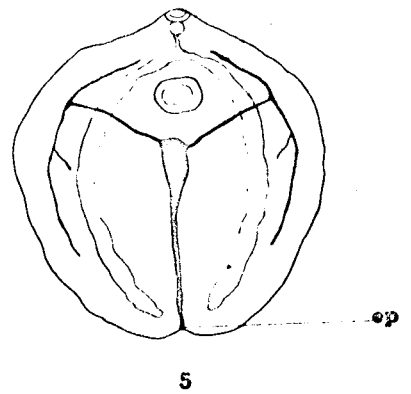
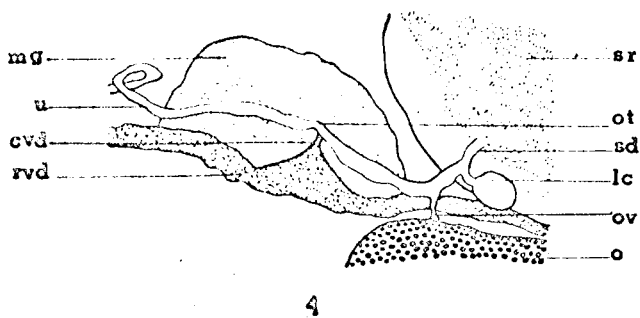
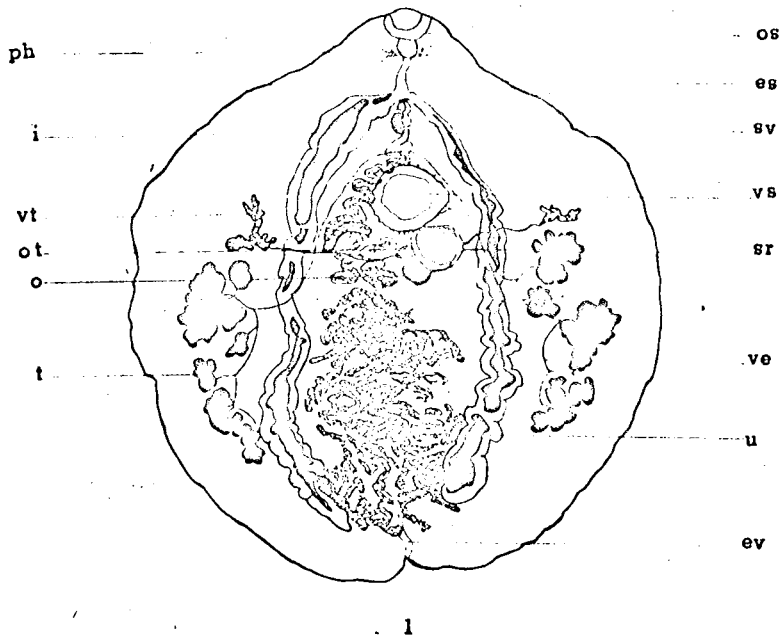
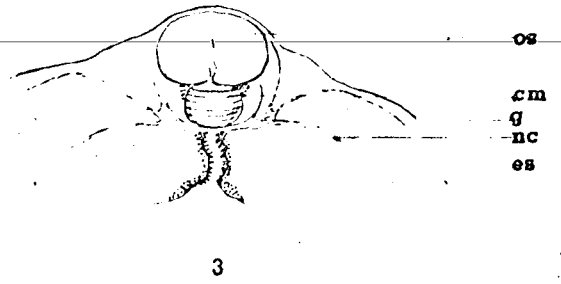
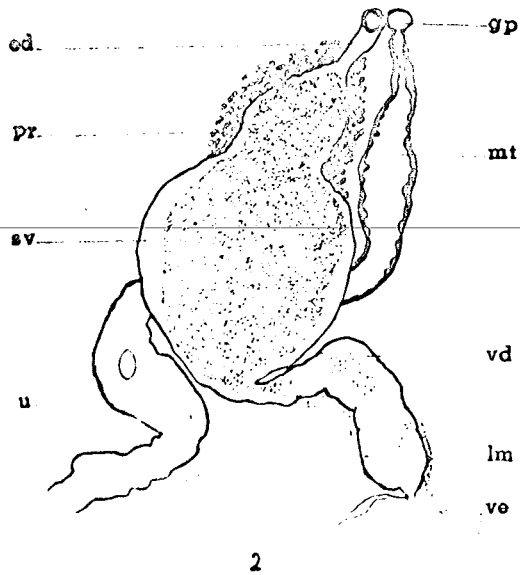


Plate 1