

NUMBER 35.

JANUARY 27, 1917.

OCCASIONAL PAPERS OF THE MUSEUM OF
ZOOLOGY

UNIVERSITY OF MICHIGAN

ANN ARBOR, MICHIGAN.

PUBLISHED BY THE UNIVERSITY.

TWO NEW LARVAL TREMATODES FROM *THAM-*
NOPHIS MARCIANA AND *THAMNOPHIS*
EQUES.

BY GEORGE R. LA RUE.

The specimens forming the basis of this report were secured from specimens of *Thamnophis marciana* (Baird and Girard) and *T. eques* (Reuss) taken by Miss Crystal Thompson and Miss Myra Sampson, members of the University of Michigan-Newcomb expedition to the Davis Mountains in western Texas, during the summer of 1914. They captured four specimens of *T. marciana* which had distinctly swollen and shortened tails. Even at the time of capture these peculiarities together with the erect position of the ventral and lateral scutes of the tail were noted. At the time of injecting the preserving fluid into these snakes it was noted that some minute white granules or organisms visible to the naked eye burst forth from the lower margins of the swollen scutes and were collected in

the preserving fluid. These were soon seen to be parasites and were saved. A very brief preliminary study showed that they were larval trematodes or cercaria. The collectors further noted that all these snakes with the swollen tails came from a short part of Musquiz Creek, a small stream flowing through Musquiz canyon. At this time it was thought that none of the specimens of the two species of *Thamnophis* occurring in neighboring localities were infested with this parasite and thus a problem in local distribution of the parasite was presented.

Upon the return of the expedition the writer was given the opportunity to examine all of the snakes of these two species caught during the summer, and a superficial examination of thirteen other specimens of *T. marciana* and of two specimens of *T. eques* which had been collected along Limpia Creek and about artificial ponds at Fort Davis and the surrounding country revealed no external appearance of parasites. A careful internal examination showed, however, that two of the three specimens of *T. marciana* caught in the village of Fort Davis were infested with small numbers of this parasite and one of the two was parasitized with small numbers of another cercaria. Of the three specimens of *T. marciana* from Limpia canyon none were parasitized while one of the two *T. eques* from Limpia canyon was infested with a few of the second form of cercaria. There is then nothing remarkable about the local distribution of this parasite. Apparently the snakes about Musquiz Creek had more abundant opportunity for infestation than snakes from other creeks and ponds in the region of Fort Davis, or from Limpia canyon.

In appearance the tails of the heavily parasitized snakes are worthy of note. The tails are swollen; the scutes more or less erect thus giving the tails a roughened appearance. In three instances a large portion of the tail had been lost.

Whether the loss of a portion of the tail was due to the heavy parasitic infestation, as the collectors thought, is not determinable. Snakes sometimes lose a portion of the tail in encounters with other animals and it is not uncommon to find "bob-tailed" snakes.¹ In less heavily parasitized snakes the tails were slightly swollen and the scutes more nearly normal in position. In very lightly infested snakes the presence of parasites could only be determined by internal examination. A common feature of the preserved heavily infested snakes is the granular appearance of the ventral scutes of the tail region. These granules are minute, immature trematodes or cercariae readily visible to the naked eye and so abundant as to cause the scutes to appear swollen, their posterior margins to be elevated and somewhat projecting from the body. After being for several weeks in the preserving fluid many scales thus swollen by the parasites had become more or less detached and in some instances the tail was badly macerated although the remainder of the body was well preserved, and unparasitized snakes in the same museum jar were in good condition. The parasite present in such numbers proved to be an hitherto undescribed larval distome for which the name *Cercaria marciana* La Rue is proposed.

***Cercaria marciana*, new species.**

Cotypes: Museum of Zoology, University of Michigan, slide No. 150; paratypes on slides Nos. 149, 151, 152, 153, 154, also alcoholic material in the collection of the Museum, and slides and alcoholic material in the collection of the writer.

Description: The parasite is a typical tailless distome cer-

¹ One snake with a "bob-tail" but with no external appearance of parasites was caught in Fort Davis. Examination of internal organs showed that a few parasites were present but not in sufficient numbers to account for the loss of the tail.

caria presenting the usual features (Fig. 1). Its length is about 0.42 mm. and its breadth about 0.22 mm. It is much flattened dorsoventrally, oval in shape, usually narrow at the anterior end and broadly rounded at the posterior end. The surface of the body is covered with minute spines arranged in regular longitudinal rows. The spines on the anterior end of the body are a trifle longer than elsewhere. The oral sucker is terminal, protrusible, in some contraction states slightly sub-terminal, somewhat funnel-shaped, length exceeding the breadth. It measures about 0.081 mm. long by about 0.070 mm. broad. Well developed muscles encircle its lower hemisphere. The acetabulum is prominent, muscular, situated about the middle of the ventral surface, frequently posterior to the middle but in elongated specimens slightly anterior to the middle. It is almost circular and about 0.076 to 0.079 mm. in diameter.

A prominent feature of the stained specimen is the group of four large cephalic glands which lie in a row across the body ventral to the intestinal bifurcations. These are ovoidal or irregular organs composed each of a single cell provided with a nucleus measuring about 0.01 mm. in diameter. A large nucleolus or net-knot is a prominent feature of each nucleus. The glands are arranged in two groups of two each, right and left, and the ducts leading from the members of a group appear to unite to form a single duct which passes forward penetrating the tissue of the oral sucker and discharging near the external opening of the latter. This apparent union of the ducts is to be found in toto preparations of specimens unstained or stained with carmine, eosin, orange G, or haematoxylin. Even sections stained in haematoxylin alone or in contrast with eosin present this appearance. If, however, the specimens be stained in toto or after sectioning with an aqueous

solution of toluidin blue or thionin with or without a contrast stain of erythrosin it may be readily determined that there is no union of these ducts, but that they lie parallel to each other and very close together. The toluidin blue or the thionin stains only the contents of the glands and their ducts while the membrane remains unstained. Between the deeply stained masses of contents of the ducts may be perceived the thin line marking the membranes of the ducts. The other stains used color the membranes as well as the contents of the ducts, hence the two ducts appear as one. It seems that Nicoll (1912) in describing the cephalic glands of *Cercaria ordinata* may have erred in considering that the ducts of each pair have united to form a single duct.

The nature of the secretion produced by the cephalic glands has not, as far as the writer is aware, been determined. Cort (1915) called these glands cephalic or stylet glands. However, since they are present in cercaria in which a stylet is not present it seems doubtful that the cephalic glands have anything to do with the formation of the stylet. It is here suggested that these glands produce mucus. In support of this view may be presented the evidence that their secretion is in the condition of fine globules which stain blue or purplish blue with toluidin blue or thionin. These are specific mucin dyes yielding a metachromatic stain. Haematoxylin also stains mucin deeply but it cannot be depended upon as a test for mucin.²

² The writer is here constrained to make an appeal for the use of better and more varied staining methods in the study of parasitic worms. Haematoxylin and carmine dyes because of the ease with which they may be used are employed to the almost total exclusion of other dyes. It is admitted that eosin and erythrosin are used with haematoxylin as contrast stains. These stains, however, reveal but little. The result of the employment of this limited technique is that the products of many glands, their function, and the finer histological structure of numerous parasitic worms is little understood or the wrong ideas have come to prevail. The well trained pathologist, histologist, or bacteriologist makes use of a large number of stains and micro-chemical tests in securing his data. The time is ripe for the helminthologist, as well, to employ a varied technique.

The pharynx is small, almost globular, and in contact with the oral sucker. It measures about 0.021 mm. in diameter. No oesophagus is present. Immediately behind the pharynx is the bifurcation of the intestine. The coeca are simple wide tubes which extend to a point about midway between the acetabulum and the posterior end of the body. Their non-granular contents stain readily with eosin, erythrosin, orange G, and very slightly green with thionin. The excretory system is simple. An excretory pore is terminally situated and leading into this is a short vesicle with two short branches which extend forward. It was impossible to find any such branches as Nicoll (1912) has shown in *Cercaria ordinata* and it is doubtful if they exist in this species.

In the region just anterior to the excretory vesicle may be seen a small group of large cells and granules the significance of which is not understood. They may be the anlagen of sexual organs.

Measurements of *Cercaria marcianae* in millimeters.

Structure	Average dimension	Number measured	Maximum	Minimum
Body	length	10	0.459	0.354
	breadth	10	0.286	0.184
Oral sucker	length	9	0.095	0.068
	breadth	9	0.086	0.057
Acetabulum	length	8	0.09	0.068
	breadth	8	0.096	0.068
Pharynx	diameter	9	0.266	0.02
Cephalic glands	length	12	0.110	0.046
	breadth	12	0.066	0.030

A study of the distribution of these cercariae in the tissues of the host shows that they occur chiefly (1) in the mesenteric and peritoneal tissues, fat bodies, and in the sub-peritoneal lymph spaces, (2) in the large lymph spaces beneath the skin of the tail, and immediately beneath many of the scutes of the

tail, (3) rarely in the muscles of the tail. A careful examination has failed to reveal their presence in the connective tissues or lymph spaces about the oral cavity, or in the muscles or connective tissues of the body anterior to the tail except as above noted.

Some attempt was made to determine the relative numbers of the parasite in the various parts of a heavily infested snake. In the serosa about the extreme upper oesophagus they were not numerous while in the thickened mesenteries of the anterior part of the body they were present in considerable numbers. From this point to the posterior limit of the body cavity the parasites occurred very numerous, the greatest number occurring in the posterior third of the body. In the fat bodies they were abundant. A small piece of mesentery taken from this region and mounted on a slide contained after mounting more than 300 cercaria while more than 60 were shaken free in the course of manipulations incident to mounting. A small group of cercaria as they occur under the mesentery is shown in Fig. 2. A rough but very conservative estimate of the number of parasites in the mesentery, fat-bodies and peritoneum is about 500,000 to 700,000. In the muscles and lymph spaces immediately back of the anus there were but few cercaria but a little farther back they occurred in enormous numbers. In arriving at an estimate of the number of parasites in the tail region the following points were taken into consideration: the number of parasites in a single section of the tail, the length of the tail, the swollen condition of the tail, and the extremely large number of cercaria shaken out from the loosened scutes and found in the bottom of the container. Judging from these criteria there must have been at least 250,000 to 350,000 parasites in the tail region, a grand total for this snake of 750,000 to 1,050,000 parasites.

Nicoll found *Cercaria ordinata* in thin cysts. This species occurs in extremely delicate cysts in the fat bodies but no cysts are produced in other parts of the host. Where cysts occur they appear to be made up of about one layer of flattened connective tissue cells differing from the surrounding fat cells only through the loss of fat globules. Even in the fat bodies the formation of definite cysts is rare.

The method of entrance of this cercaria into the host is unknown but since *Thamnophis marciana* feeds largely on tadpoles when they are available it seems likely that the cercaria are taken with the tadpoles which may be a still earlier host. Work upon the preserved hosts has not revealed the method by which the cercaria get into the lymph spaces. Dr. W. W. Cort in a letter to the writer dated January 10, 1915, states, "Trematodes seem to be able to worm their way through tissue. I think there is no doubt that larval forms in muscles usually come by way of the alimentary canal. I have seen holostome larvae pushing their way through the muscles of the tadpole." Since these cercaria are provided with a close armature of fine spines they should find no difficulty in penetrating the tissues of their host. Why they congregate in the lymph spaces of the tail rather than in the body cavity is not known. Nor is it known why they collect in great numbers in the spaces so near to the scutes of the tail.

***Cercaria vegrandis*, new species.**

The second species of larval trematode, *Cercaria vegrandis* n. sp., was found occurring in small numbers in cysts in the fat bodies only of one specimen of *Thamnophis marciana* from a tank or pond in Fort Davis and of one specimen of *T. eques* from Limpia canyon. In the first mentioned host it occurred

in association with still smaller numbers of *Cercaria marciana*. The cysts enclosing individuals of *C. vegrandis* differed in the two host species. In *T. eques* the cyst membrane was tough, moderately thick and the enclosed cercaria was released with difficulty while in *T. marciana* the cysts were scarcely discernible, the cercaria were released with ease, almost as readily as *C. marciana* were released. The cysts in *T. marciana* always ruptured without being torn from the fat body. This difference in the response of closely allied host species to the presence of the same parasitic species is rather remarkable.

Type: Museum of Zoology, University of Michigan, slide No. 155; paratypes alcoholic material in collection of the Museum, slides and alcoholic material in the collection of the writer.

Description: The study of this species is based entirely on preserved specimens removed from fat bodies. Such specimens were frequently so rolled or twisted that they could not be studied while others were ovate in form, being sometimes very broadly ovate, the greatest breadth occurring near the middle or near the posterior end. More rarely the specimens are somewhat slender (Fig. 3). No specimens have either one or both ends acute. Rarely an elliptical form was assumed. They were in all cases very flat and tailless. The parenchyma differs greatly from that found in *C. marciana*, being filled with small round or oval cavities measuring about 0.010×0.010 or 0.010×0.012 mm. These are best seen in unstained but cleared specimens. Nuclei are numerous. In all specimens studied, about twenty, the parenchyma which was opaque masked the details of various structures to such an extent as to make their study difficult and in some cases impossible.

The usual structures of tailless cercaria were found except the cephalic glands which if they had ever been present were now absent. With some difficulty in unstained specimens or even in those stained with thionin, haemalum, or erythrosin one can make out a rather weakly muscled oral sucker, slender or broadly oval in outline, followed by a weakly muscled pharynx, usually slender and difficult to see. A short oesophagus gives rise to the intestinal bifurcations which as slender indistinct tubes, sometimes containing a few small non-staining ellipsoidal bodies, extend well into the hinder end of the body.

The acetabulum is nearly round, shallow, and situated always posterior to the middle of the body. While of fair size it is not a prominent organ. Posterior to the acetabulum but near it is situated a group of deeply staining cells, making a round or somewhat rectangular mass measuring about 0.03×0.026 mm. or even 0.036×0.043 mm. A slit like aperture on the ventral surface marks the middle of the mass. The opening is probably the genital pore and the mass of cells a portion of the developing sexual organs. The cells are large massively club-shaped, radiating irregularly from the genital orifice. The nuclei of these cells do not stain well but the cytoplasm stains densely with haemalum and diffusely with thionin. Close behind the first mass of cells in some more fully developed individuals (Fig. 4) lies a second irregularly triangular mass of cells. These cells are indistinct. The mass when well developed measures about 0.032×0.035 mm. It lies dorsal to the excretory vessels although in the drawing it appears to lie within the excretory vessels.

The excretory system is not well marked and only a small portion of it can be traced. In the hinder part of the body can sometimes be found a small terminal excretory pore by which through a narrow duct the excretory vessels discharge

to the exterior. These vessels sometimes take on the appearance of a broad **Y** with a short slender stem and broad thick arms. From the anterior ends of the arms of the **Y** broad, somewhat coiled tubes may be traced for a short distance anteriorly where their outlines are lost in the parenchyma.

Portions of the nervous system can be seen in some specimens extending from the circumoesophageal commissure toward the posterior part of the body, in a few cases as far as the developing sexual organs. The cuticula is about 0.005 mm. thick and is unarmed with spines. Measurements of the various structures are given in the accompanying table.

Measurements of *Cercaria vegrandis* in millimeters.

Organ	Type	Average dimension of paratypes	Number measured	Maximum	Minimum
Body	length	0.273	10	0.316	0.243
	breadth	0.193	10	0.273	0.176
Oral sucker	length	0.033	9	0.046	0.033
	breadth	0.030	9	0.032	0.022
Acetabulum	length	0.023	7	0.046	0.023
	breadth	0.025	7	0.046	0.025
Pharynx	length	0.022	6	0.04	0.02
	breadth	0.008	6	0.02	0.008

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University of Michigan

PLATE I.

(All drawings made with the aid of the camera lucida.)

Figure 1. *Cercaria marciana*, ventral view, drawn from whole specimen on slide No. 150.

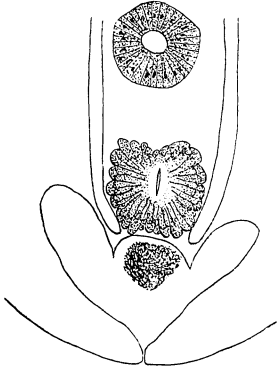
Figure 2. A group of *Cercaria marciana* as they occur under the mesentery of heavily infested snakes. Magnified.

Figure 3. *Cercaria vegrandia*, ventral view, drawn from whole specimen on slide No. 155. All structures are shown much more distinctly than in the preparation.

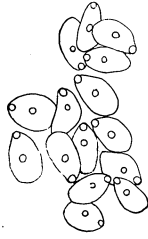
Figure 4. Detail of posterior end of *Cercaria vegrandia*. This specimen is somewhat more mature than the one shown in figure 3. Note especially the second mass of cells. Drawn to the same scale as figure 3.

CERCARIA MARCIANAE
AND
CERCARIA VEGRANDIS

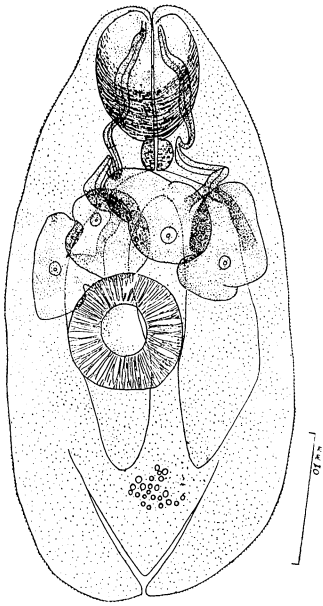
PLATE I



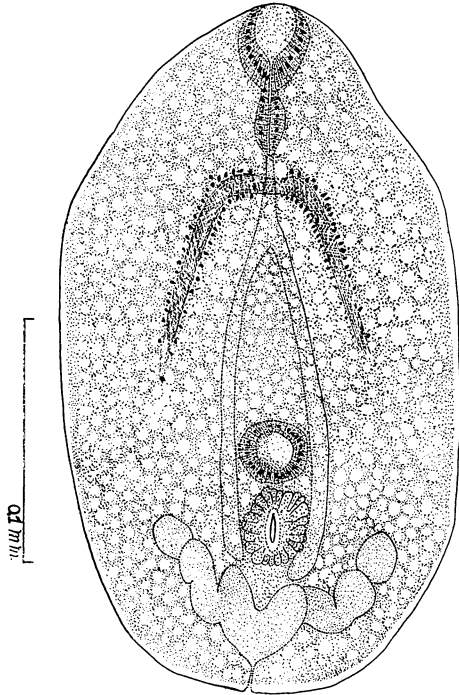
4



2



1



3

