A Comparative Study of the Helminths of the Anura from Five Given Localities

by

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Summary

A Comparative Study of the Helminths of the Anura from Five Given Localities

The frog is used probably more than any other animal for dissection in a zoological laboratory. The parasites found in the genus Rana have been extensively studied and described.

In this investigation an attempt is made to name and describe the different parasites found in frogs and toads from a few given localities and to compare their distribution. I was interested especially in finding out if the helminths harbored by the Anura secured from different localities would differ to any great extent as to species or even genera.

After searching the literature it was found that many new species named have varied so little from previously named parasites that much doubt has been raised as to whether they should be given a new specific name or not. The naming of the new genus Opheoraenia by La Rue in 1914 was especially objected to by Mybelin (1917) who considered it to be synonymous with Monticelli's genus Crepodobothrum . However the genus Ophiotaenia seems to stand.

The majority of parasites found in frogs were first described in Europe and when a similar one was found in America it was given the old world name. But upon further

study enough differences were often found to create a new species. For example, when one of the large intestinal flukes belonging to the genus Diplodiscus was described from Rana esculenta in Europe it was given the species name subclavatus. Later Stafford gave the same name to these flukes when found in American frogs. Upon more critical examination it was shown that in this country a new species name would have to be created.

Materials and Technique

The frogs studied were grouped according to species and locality from which secured. Altogether seven groups were studied, two of Rana pipiens from Illinois, one of the same species each from Wisconsin and Kansas, also a group of Rana areolatas from Kansas and Rana clamitans from Louisiana. A few Bufo americanus from Kansas were also examined.

The following method was employed in the examination of the frogs. The frogs were etherized and each organ was immediately removed from the body and examined in normal saline. The body cavity and the surface beneath the skin were thoroughly examined.

The trematodes and cestodes were fixed in Bouin's picro-formol fixing fluid. The time for fixation depended upon the size of the trematode. Ten hours proved to be sufficient for the large ones while two or three hours were sufficient for the smaller ones. Delafield's hemotoxylin

was used for staining. The modified method used by Ruth Shaw (1928) in staining cestodes from the dog was followed. Oil of Wintergreen was used as a clearer and proved to be much better than Xylol.

No attempt was made to stain the nematode parasites. They were fixed in acid-corrosive sublimate for two hours, washed in 50% alcohol to which enough iodine was added to color it slightly. Thereafter they were run up the series of alcohols to absolute, and cleared in glycerine and examined.

Discussion

In the frogs examined, parasites were found in the region beneath the skin, in the body cavity, lungs, bladder, and the digestive tract. No parasites were found in the liver or the kidneys. The blood was not examined in this study.

A. Parasites Beneath the Skin

There was only one kind of parasite found just under the skin of the frog namely, Clinostomum attenatum. Species of this genus were first discovered in fish and later in frogs. For a long time it was thought that these flukes were all of this same species, Clinostomum marginatum. In 1911 Osborne studied these trematodes in fish and frogs and found that the differences between the parasites of the two animals were so small that they should be assigned to the same specie, namely Clinostomum marginatum . Rudolph Cort, however, working along the same line at the same time, concluded that the differences in structure of the parasites

from the different hosts were pronounced enough to separate them and proposed the name Clinostomum attenatum for the type found in frogs.

I first noticed these encysted flukes in a specimen brought me from the Physiology Department at Kansas University. On examining the Rana pipiens from Chicago , twenyt-six cyst containing parasites were taken from the mesentaries and peritoneum, especially in the region of the neck and limbs. None, however, were found within the muscle tissue.

The average infection of the fourteen specimens was 7.5 flukes. The second group from Chicago was the only group in which Clinostomum attenatum was found.

On freeing these flukes from the cyst they became very active and had great power of expansion and contraction, sometimes reaching a length of over eleven millimeters. One mounted specimen measured ten and two-tenths millimeters, but the average length of the specimen was found to be six and seven-tenths millimeters in toto mount. The ratio of acetabulum to oral sucker was three to one. Two deeply lobed testes were located on the posterior third of the body and between them, to the right is located a small lobed ovary. An oviduct passes around the right end of the anterior testis, and connects with the uterus. The uterus at first is not distinct and appears as a branched stalk in toto mount. However several specimens were kept alive in saline solution for thirty-six hours and these specimen were

fixed and stained and showed the uterus as an elongate sac-like structure. The posterior part of the intestinal ceca are rough in appearance and when the specimen is alive has a yellow color. My measurements of the flukes are somewhat larger than those given by Cort, probably due to the differences in fixation.

B. Parasites of the Body Cavity

Only one kind of parasite was taken from the body cavity with the exception of Clinostomum attenatum, a nematode which we were unable to classify. This parasite was very abundant in numbers two and fifteen of Rana clamitans. Number two contained thirty-one of these nematodes, and number fifteen contained twenty-three (Chart 1). Both sexes of the nematode were present, the longest, a female measured fifty millimeters in length. The cuticle covering the body was transparent, allowing all the internal organs to be seen plainly. More work will be done on these parasites later.

C. Parasites of the Lungs

Three different parasites were found in the lungs of the specimen examined. The flukes belonged to the genus Pneumonoeces but were of two species namely: Medioplexus and parviplexus. The nematode was Rhabdonema nigrovenosum and belonged to the Ascarid group.

Pneumoneces representatives were found in the different groups with the exception of Rana areolata, the Rana pipiens from Wisconsin, and the Bufo americanus. They were

however, numerous in but one group, the Rana pipiens from Lawrence, Kansas. Within the intestines of one of these specimen twenty-six larval trematodes of Pneumonoeces medioplexus were found. They were migrating up the digestive tract to the mouth, from which point they enter the glottis, then to the lungs, the normal habitat of the adult. The life cycles of Pneumonoeces medioplexus and P. parviplexus have been thoroughly worked out by Krull, 1931.

Two species of Pneumonoeces were found in my collection. Those from the Rana pipiens were P. medioplexus, and those from Rana clamitans were P. parviplexus (Chart 2). The literature shows that there are seven species of Pneumonoeces including Irvin's P. parviplexus. The other six, as described by Cort, are Pneumonoeces simiplexus, P. complexus, P. medioplexus, P. longiplexus, P. breviplexus, and and P. coloradensis.

The most outstanding features of separation are the size, shape, and position of the testes and ovary, and the corresponding length of the outside longitudinal folds of the uterus. The testes of P. medioplexus are almost spherical, while those of P. parviplexus are over twice as long as wide. The ovary in P. medioplexus is oval without lobes, while the ovary of the P. parviplexus is deeply lobed. there are no uterine folds outside the intestinal ceca in P. medioplexus, while such folds extend to the posterior testes in P. parviplexus. Figures eleven and twelve give a comparative picture of these species. The longest adult lung fluke

from Rana clamitans was ten and one-tenth millimeters. The ratio of acetabulum to oral sucker was two and six-tenths to one. The longest specimen from P. medioplexus measured eleven and four-tenths millimeters, the shortest, not including the larval forms taken from the intestines, was six millimeters long. The average length of the adults was seven and one-tenth millimeters. The ratio of acetabulum to oral sucker was three and one-tenth to one.

In examining the Rana clamitans, twenty-eight small flukes were taken from the upper part of the intestine, ranging in length from one and three-tenths millimeters to three and five-tenths millimeters. The description given by Cort in 1919 for a new Distome from Rana aurora which he named Margiana californiensis seems to fit exactly with that of the above named trematode with the exception that the largest of these specimens had its testes more elongate than spherical as Cort described them. Later in the Spring when Rana pipiens from Lawrence were collected several small flukes similar to those taken from R. clamitans were again found. This time all sizes were found measuring from one and eight-tenths millimeters, the longest of which was found in the lungs.

Cort describes Margiana californiensis as possessing a prepharnyx, short esophagus, intestinal ceca extending into the posterior fifth of the body, but not reaching the posterior end, excretory system of the two, six, three type, with club-shaped bladder, vitellaria extending from in

front of pharnyx to posterior linits of testes, cirrus sac-like, testes large and on the same plane, filling the width of the body almost entirely.

In my series of young flukes of Pneumonoeces medioplexus we found that there is present a prepharnyx in the smaller specimens, but as they increased in size the prepharnyx becomes shorter until in the adult trematode it disappears altogether. The esophagus was long in the smaller s specimens, but short in the larger ones. The intestinal ceca in the younger specimens were short. The vitellaria which Cort described as extending from in front of the pharnyx to the posterior limits of the testes without being arranged into groups was found also to be true in our smallest specimens, but they were located farther posteriorly in the larger specimens and were beginning to form into definite groups until the acini groups were full formed in the adults found in the lungs. The testes, as shown in Cort's figure of Margiana californiensis also was found in our small larval Pneumonoeces collection, but as the specimens became larger one testis arranged itself behind the other, as shown in figures nine and ten.

Our conclusions from the observations cited are that the small specimens taken from Rana clamitans were larval forms of Pneumonoeces parviplexus and that the difference in the shape of the testes was due to the species of Pneumonaeces. I believe that Margiana californiensis as described is the young form of the lung fluke of the genus Pneumonoeces medioplexus. Cort's naming of the in-

testinal fluke Margiana californiensis (1919) may be due to the fact that the life cycle of Pneumonoeces medioplexus as well as P. parviplexus was not fully worked out until 1931.

The third parasite of the lung of the specimens examined was Rhabdonema nigrovenosum. The parasite has long been known to inhabit the lungs of the majority of frogs and has a very wide range. The larval form of the nematode which is found in the rectum of the infested frog was for a long time thought to be another species, and was given the name Rhabditis nigrovenosa until the life cycle was fully worked out.

The larvae are early differentiated and in a relatively short time become sexually mature and mate within the excrement of the frog. The fertalized ova gives rise within the body of the female to young embryos that devour the mother's body and escape with the frog's excrement into the water where it lives a free life. Finally it forces its way into the body of a snail. When the snail is enjested by the frog the nematode escapes in the digestive tract and finds its way to the lungs where it reaches maturity. The mature nematode is a protandrous hermaphrodite which produces spermatozoa, and later ova which are fertilized by the former. The eggs pass out through the vagina and are pushed into the mouth cavity and injested, hatching within the rectum into sexually differentiated larvae. Of all the frogs examined, only two of the groups did not possess the parasites, namely Rana areolata, and Bufo americanus. Of the other five

groups the second group from Chicago was least infested. The longest specimen of Rhabdonema nigrovenosum found measured six and one-tenth millimeters. The shortest measured one and three-tenths millimeters. The average length was three and two-tenths millimeters.

D. Parasites of the Bladder

In 1800 Zeder gave the name Distomum cygnoides to the bladder flukes found in frogs. In 1851 Leidy in America was the first to notice them, but not until Bensley in 1897 described them did they receive an adequate description. He noted two varieties of the group and called them Distomum cygnoides A&B. The A type contained nine testes and the B type two. He suggested that the two forms should be separated generically, but made no attempt to do so.

Looss in 1899 thought the genus name Distomum inadequate for the group and designed the generic name Gorgodera, with Distomum cygnoides as the type species. When it was discovered that Gorgodera simplex had only two testes, Looss proposed the genus name Gorgoderina to those flukes with only two testes, and Gorgodera stood for those with nine testes. In the same year, 1902, this classification was accepted by Stafford.

In the frogs and toads which I examined both genera, Gorgodera and Gorgoderina were present. Gorgodera was represented by Gorgodera amplicava and Gorgodera minima. Gorgoderina was represented by Gorgoderina translucida. Both representatives of Gorgodera were found in the bladder of the

of the Rana clamitans from New Orleans and were not found in any of the other groups examined. Gorgoderina translucida were found in the bladders of Rana pipiens from Chicago. The specimens examined from Lawrence, Western Kansas, and Wisconsin did not harbor bladder flukes.

Of the eighteen Rana clamitans examined eight were infested with bladder flukes, one of which contained three specimen of Gorgodera minima and the remaining seven contained forty-two specimen of Gorgodera amplicava. The highest infection with G. amplicava in one frog was seventeen, but the average for the group was two and thirty-three hundredths flukes. The latter species varied from three to six and two-tenths millimeters. The relationship of acetabulum to oral sucker in G. amplicava was three to one, while in G. minima the ratio was two and one-tenth to one. The seminal vesicle in G. minima was more distinct than that in G. amplicava. Both species of Gorgodera possessed two groups of vitellaria on each side of the body directly below the acetabulum. The ovary is generally located on the right side of the body, but may occur on the left side as well. Five testes were located on the same side as the ovary and four on the opposite side.

These two species are very similar in structure, the only difference being in size, ratio of acetabulum to oral sucker and the shape of the ovary. In G. amplicava the ovary is generally a distinct three-lobed structure, while in G. minima the ovary was only slightly and irregularly lobed. No types were found with a distinct circular sheath as described by Guberlet and named G. circava.

Gorgoderina translucida was only found in Rana pipiens from Chicago. The Rana pipiens from Wisconsin and Lawrence, the Rana clamitans from New Orleans and Bufo americanus from Lawrence did not contain representatives of Gorgoderina.

Of the eighteen Rana pipiens examined from Chicago, eight were infested with Gorgoderina; the highest infection in one frog was five. The average number of parasites from each specimen of the group was two and five-tenths flukes. The largest fluke found measured ten and one-tenth millimeters in toto mount. The smallest was four and two-tenths millimeters. All these measurements were taken from fixed material.

The reproductive system especially the testes, were sometimes very faint if seen at all. The smallest flukes, however, showed the reproductive organs plainly. The vesicular seminalis is somewhat smaller in proportion to size than that in the body of Gorgodera amplicava. The two groups of vitellaria are farther removed from the acetabulum in Gorgoderina than they were in the genus, Gorgodera. The ovary appears as a slightly lobed structure beneath the right vitellarine follicle. The anterior testes is located on the opposite side, while the posterior one is found on the same side as the ovary. The intestinal ceca have no point of distinction. The ratio of acetabulum to oral sucker is two to one, which is much greater than that given by

Cont.

E. Parasites of the Digestive Tract

The digestive tract was infested with parasites more so than any other organ of the body as to numbers and kinds. Nine different species were found throughout the length of this organ, namely Oswaldocruzia leidyi, larval Pneumonoeces medioplexus, P. parviplexus, Ophiotaenia saphena, Diplodiscus temporanum, Opalina ranarum, Nyctotherus cordiformis, Tritrichomonas augusta, and Cylindrotaenia americanus.

Oswaldocruzia leidyi was found more anteriorly than any other parasites. It was found as far up as the stomach. The Rana pipiens from Wisconsin were the heaviest infected group. The average number of nematodes for each of the eleven specimen examined was eighteen and twenty-seven hundredths, as shown in Chart 2. Chart 1 shows that specimen number three contained the greatest number of these parasites. A total of seventy-nine nematodes was collected, eighteen of which were males, and the remaining sixty-one were females. The ratio of the females to males was nearly three and thirty-eight hundredths to one. The females were over twice as long as the males, the longest of which measured fifteen and three-tenths millimeters. The majority, however, measured thirteen and two-tenths millimeters. The posterior spine was the distinguishing feature which separated it from the other species of Oswaldocruzia. In A.C.Walton's studies of 1929 figures are given of the different shapes of spines of

the genus Oswaldocruzia. The three specimens of Rana areolata did not contain any of these parasites, but the first specimen of Bufo americanus was heavily infested with representatives of the genus. Rana clamitans were only slightly infested. As shown in Chart 2, only four of the eighteen specimen were infested.

At the anterior part of the intestine the larval forms of the genus Pneumonoeces were found. This was discussed in connection with the parasitic infection of the lungs and will not be taken up again here.

In this same region two genera of cestodes were found. One cestode was taken in each of two Rana clamitans and a second genus was found in two Rana pipiens from Lawrence. Those taken from the Rana clamitans were found to be representatives of the species Ophiotaenia saphena, a new species described by Osler in 1931. Those taken from the Rana pipiens of Lawrence were representatives of Cylindrotaenia americanus, a specie first described by Jewell in 1916.

In the revision of the cestode family of Proteocephalidae in 1911, La Rue created the new genus Ophiotaenia for those found in amphibians, thus separating them from those found in fish which had been given the name Cripodobothrum. La Rue bases his new genus on the fact that in Ophiotaenia the suckers are not notched, while they are in the Cripodobothrum, and the neck is longer in his new genus. Mybelin does not think this enough difference to create a new genus, and that Monticelli's Cripodobothrum should

stand if the law of priority be adhered to. However from the literature cited, there seems to be only one species of Cripodobothrum, namely C. gerrardii Baird. For some time new species have been assigned to the genus Ophiotaenia. Evidently this new genus has been generally accepted. Osler in 1931 assigned her new species to La Rue's genus and called it Ophiotaenia saphena.

In the eighteen specimens of Rana clamitans examined only two were infected. Each frog infested possessed one head and three or four chains of proglottids. The specimen as shown in figure six, seven, and eight fit exactly the description given by Osler.

The strobula of this cestode is continuous with its slender neck, and when placed into normal saline the scolex constantly changes, due to the action of the suckers. The width of the young proglottids when first distinguishable is six and seven-tenths times its length, but the ratio of the width to length of the largest proglottid found in one specimen was one and twenty-five hundredths to one. There are no hooks present on the scolex, but a small degenerate fifth sucker seems to be present in the center of the four oval shaped suckers. The genital pore out of which the cirrus sac projects in the gravid proglottid is marginal, alternating irregularly. In the mature proglottids such a protrusion was not seen. The testes are located on either side of the uterus whose branches in the gravid proglottids resemble a slightly lobed sac extending the entire length of

the proglottid anterior to the ovary. Beneath the ovary in the median plane is located Mehlis gland. The vitellaria are located on either side between the lateral nerve cord and the ventral excretory tube.

Cylindrotaenia americanus, the other cestode, was found only in the Rana pipiens from Lawrence. The first was found in a small Rana pipiens, (number three of Chart 1) which measured only two inches in length. The cestode was very small in comparison with those later found measuring only thirteen and five-tenths millimeters long. It was destroyed before a thorough examination could be made; however enough characteristics were noted to identify it as belonging to the same species as the two cestodes found later in Rana pipiens number thirteen which on examination was found to belong to the species Cylindrotaenia americanus. The two specimens measured twelve and thirteen and four tenths centimeters respectively. Its greatest breadth was forty-seven hundredths millimeters. Jewell describes the cestode as having an unarmed scolex, without rostellum, reproductive organs single in each proglottid, genital pores lateral and alternating irregular, vagina and cirrus sac dorsal to excretory canal and main nerve trunk, a single testis located dorsal, ovary and vitellaria ventral. The uterus breaks up into capsules surrounding the embryoes which ultimately passes into parauterine capsules.

The reproductive organs show better when the cestode is sectioned. No sections were made of the cestodes found,

but from whole mounts, our specimens coincided with Jewell's description of Cylindrotaenia americanus.

I observed that the mature proglottid had a greater breadth than the ripe proglottid. The gravid proglottids were very narrow and somewhat oval with a constriction in the median plane. The eggs could be plainly seen within the parauterine capsule which is located between the constriction. Many individual ripe proglottids were observed in the cloacae of the frog which had great power of contraction and expansion. On first observation the proglottids appeared to be trematodes.

In this study it was noted that cestode infection in frogs are rare. Only five cestodes were found from the seventy-seven specimens examined.

The only trematode found in the rectum of the frogs examined was Diplodiscus temperatus, Stafford. This trematode was first described in Europe by Goeze in 1872, and was named Amphistomum subclavatus of the superfamily Paramphistomidae with Diplodiscus subclavatus as the type species.

In America, Leidy reported this species in 1856, and Stafford again in 1900, both referring it to the European Amphistomum. Diplodiscus subclavatus found in the rectum of Rana vereseins and Rana catesbiana. In 1905 Stafford redescribed the species from America and named it Diplodiscus temperatus.

In the specimens examined, the intestinal fluke Diplodiscus temperatus as shown in Chart 2 has a wider distribution than the bladder flukes. Diplodiscus temporatus was

taken from the rectum of Rana pipiens from Chicago and Lawrence, R. clamitans from New Orleans, and R. areolata from Kansas. The Rana pipiens from Wisconsin is the only group that did not harbor this parasite. A total of thirty-four of these parasites were taken from the frogs examined. The largest was taken from Rana areolata and measured ten and two-tenths millimeters. A larval form was also found in the small intestine of the host and when mounted measured one and five-tenths millimeters long.

The acetabulum opens slightly ventrad and is five times the size of the oral sucker. Immediately above it and located dorsally is the excretory bladder. The ovary is situated in a median plane in the posterior third of the body below which is located Mehlis gland. The vitellaria are situated on either side of the body beginning anteriorly near the posterior testes and extending posteriorly beyond the ovary as shown in Figure three. The testes are two in number, the anterior are situated slightly to the right of the median plane, and the posterior one slightly to the left. The testes are much larger than the ovary in the younger specimens..They appear to be not so large in the older flukes and in some cases the testes are smaller than the ovary. All specimens examined showed the testes distinctly separated and no attempt to coalesce was noted as Stile and Goldberg's key to the family Paramphistomidae states. The testes are connected at the vesicula seminalis by the vas deferens and pass into the penis sac which is located ven-

trad in the region where the intestinal ceca unite. In two specimens mounted on their side, rather than ventrad or dorsad, the genital pore is shown projecting from the ventral surface of the body, as shown in Figure five. The mouth opens into two pharyngeal pouches which are connected to a long esophagus possessing a terminal swelling, the pharnyx.

The protozoan parasites were also found in the rectum. No extensive study was made of them, however the presence or absence of Opalina ranarum and Nyctotherus cordiformis were noted when found merely for use of comparison between the frogs of the different localities. Tritrichomonas augusta was found frequently in the group of Rana pipiens from Lawrence but was not noted in any of the other groups examined. Of the protozoans found Opalina ranarum seemed to have the wider distribution. The frogs from the vicinity of Lawrence were more heavily infested with protozoan parasites than any of the other groups. In many cases

all three types were found in the cloaca of the same frog. In such cases it was estimated that Opalina ranarum was twenty times as numerous as Nyctotherus cordiformis, and when Tritrichomonas augusta was present they were more numerous than both the other two protozoans.

Results

As was stated in the introduction, one of the primary purposes of this study was to make a comparative study of the frogs taken from a few different localities.

Three charts have been prepared in connection with

this work. In Chart 1 each specimen for each group is listed and the name and number of parasites found in each are listed in the order in which they were examined. Chart 2 compares the infection of the groups as a whole, giving the per cent of infection of one group with that of another. Chart 3 gives the combined conclusions in this comparative study.

Because of the season of the year in which this problem was begun, it was necessary to secure frogs from different biological supply companies. The first group was received the first part of February. About forty-five days later a second group was secured from the same place and a difference was found in the parasites infesting the two groups.

In group 1 the specimens were heavily infested with Rhabdonema nigrovenosum the second specimen of harbering seventy-six of these parasites. All but one of the frogs had adult R. nigrovenosum in their lungs, and this specimen harbered four specimens of Pneumonoeces medioplexus in this organ.

Rhabdonema nigrovenosum infection was not as heavy in the second group from Chicago. In the first group the per cent of infection was seventy-five in contrast to fourteen and twenty-eight hundredths per cent infection in the second group. As shown in table 2 the average number of parasites for each specimen was twenty-nine, while the average in the second group was forty-two hundredths. The highest single

infestation in the second group was five in comparison to seventy-six in group 1.

The Rana pipiens from Wisconsin ranked first in percentage of infestation and second in average number of parasites for each specimen of the group. Bufo americanus and Rana areolata had the least percentage of infestation with Rhabdonema nigrovenosum.

Table 1 shows that Pneumonoecos medioplexus is specific for Rana pipiens, while P. parviplexus is specific for Rana clamitans. The highest percentage of infection with Pneumonoecos medioplexus as shown in Chart 2 was found among the Rana pipiens from Lawrence. P. parviplexus was found only in the group of Rana clamitans.

Among the bladder flukes based on Chart 1, the genus Gorgodera is specific for Rana clamitans. Two species were found in this group, namely Gorgodera amplicava and Gorgodera minima. Likewise based on the charts Gorgoderina translucida is specific for Rana pipiens. Only one frog of the group from Wisconsin was found to be infested with this form, and only one fluke was found in it.

Of all the trematodes found, Diplodiscus temporanum Stafford, seemed not to be specific to any one species of frogs. This parasite was found in both groups from Chicago, Rana areolata from Kansas, and Rana clamitans from New Orleans. The group possessing the highest percentage of infection was the second group from Chicago, However the

four groups in which they were found had a closely related percentage.

Cestode infestation among the frogs is rare as found in the specimens examined. Only five cestodes were found in the combined groups, and they were limited to Rana clamitans and Rana pipiens from Lawrence. From the results of our examinations, Ophiotaenia saphena is specific for Rana clamitans and Cylindrotaenia americanus is specific for Rana pipiens from Lawrence.

The unclassified nematode, as formerly stated, was found in Rana clamitans from New Orleans and Rana pipiens from Lawrence. Only one specimen from Lawrence contained the nematodes within the body cavity, and only three relatively small ones as compared with those found in Rana clamitans. Those from the latter averaged forty millimeters, i.e. the females, while the females in the Rana pipiens measured twenty-eight millimeters in length.

Clinostomum was found only in the second group from Chicago. All the groups except Rana clamitans contained protozoan parasites. The groups containing the heaviest infestation with protozoan parasites were the group of Rana pipiens and Bufo americanus from Lawrence. These groups were the only two groups in which Nyctotherus cordiformis was found.

Summary

1. Fifteen kinds of parasites were found, namely Rhabdonema nigrovenosum, Oswaldocruzia leidyi, Gorgodera

amplicava, Gorgodera minima, Gorgoderina translucida, Pneumonoeces medioplexus, P. parviplexus, Diplodiscus temporanum, Clinostomum attenatum, Ophiotaenia saphena, Cylindrotaenia americanus, Opalina ranarum, Nyctotherus cordiformis, and Tritrichomonas augusta. A short description was given of each as seen in toto mount.

2. The conclusion was reached that Cort's new genus Margeana californiensis, which was described in 1919, is very similar in structure to the young specimens of Pneumonoeces medioplexus.

3. The various species from different localities showed a difference in kind and number of parasites which they contained. Likewise it was found that the same species from different localities differed in kinds of parasites found. The difference in the two groups from Chicago was thought to be due to a difference in the locality in which they might have been collected. Systematic charts bringing out the comparisons in number, kinds, and distribution accompany the study.

To Miss Mary E. Larson, under whose direction this work has been carried on, I wish to express my appreciation for her interest and helpful suggestions.

I also wish to thank Dr. H.H.Lane for his assistance in furnishing material on which to work and Dr. E. H. Taylor for verifying the classification of the frogs used,as well as for several of the specimens from the Kansas fauna.

Abbreviations
Used in Plates Made

a- acetabulum

ap-apical portion of parauterine organ

bp-basal portion of parauterine organ

cir-cirrus sac

dg-degenerate sucker

exb-excretory bladder

gp-genital pore

i-intestine

m-mouth

n-neck

o-ovary

os-oral sucker

p-parauterine capsule

ph-pharnyx

ph p-pharnygeal pouch

pph-prepharnyx

s-sucker

sg-shell gland

t-testes

u-uterus

us-uterine sac

v-vagina

vd-vas deferens

vit-vitellaria

Explanation of Plates

All figures are drawn with the aid of the Projectoscope.

Plate 1

Figure 1--Ventral view of *Gorgodera amplicava*. x 15

Figure 2--Ventral view of *Gorgodera minima*. x 25

Figure 3--*Gorgoderina Translucida* drawn from the side x25

Figure 4--Ventral view of *Clinostomum attenatum*. x 14

Figure 5--Side view of *Diplodiscus temporanum* showing genital pore projected. x 14

Plate 2

Figure 6--Head of *Ophiotaenia saphena*. x 20

Figure 7--*Ophiotaenia saphena* mature proglottid. x 20

Figure 8--Gravid proglottid of *Ophiotaenis saphena*. x24

Figure 9--Head of *Cylindrotaenia americanus*. x 55

Figure 10--Mature proglottid of *C. americanus*. x 86

Figure 11--Ripe proglottid of *C. americanus* x 86

Plate 3

Figure 12--Young *Pneumonoeces medioplexus* x 17

Figure 13--Young *Pneumonoeces parviplexus* x 13

Figure 14--Adult *Pneumonoeces parviplexus* x12

Figure 15--Adult *Pneumonoeces medioplexus* x 9

-Note-

These magnification numbers were attained with the aid of a micrometer, compass, Spencer microscope with a 10x eye piece and a millimeter rule. The specimens under five millimeters in length were measured under the microscope and those over five millimeters were measured on the rule with the aid of the compass

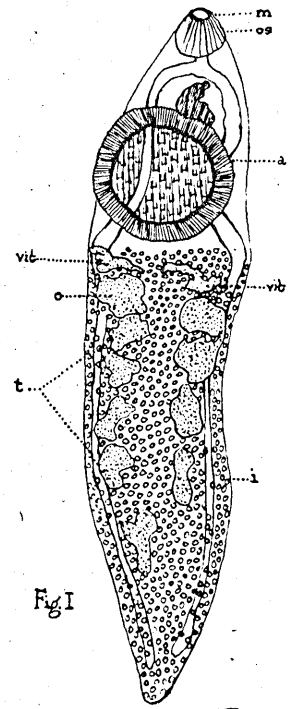

Fig I

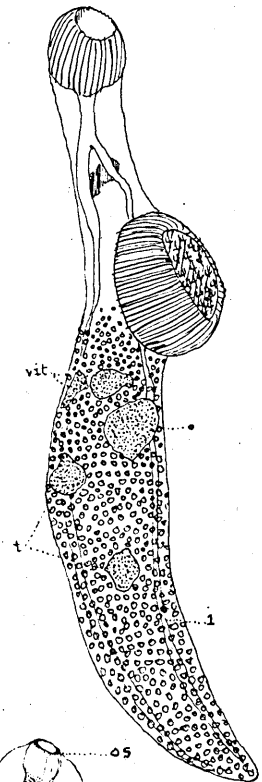

Fig. III

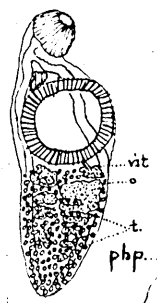

Fig II

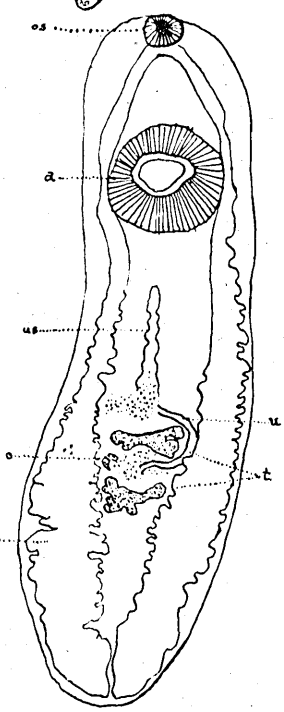

Fig IV

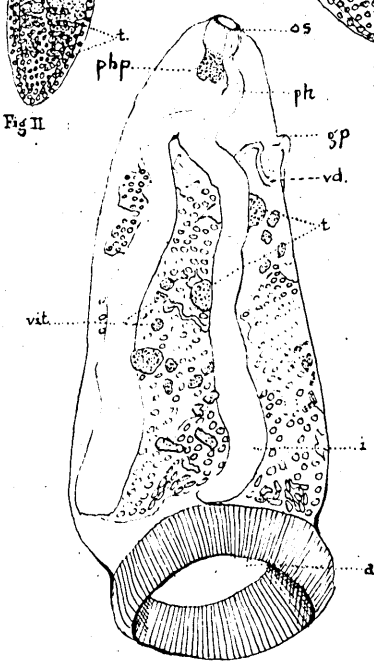

Fig. V

PLATE II

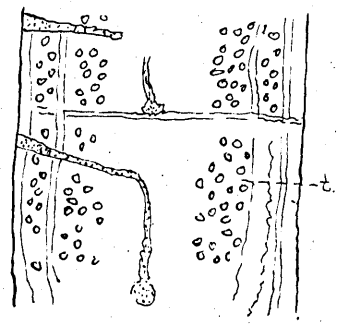

Fig XII.

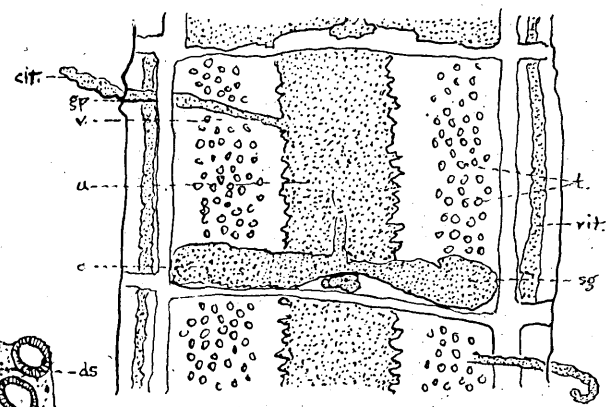

Fig XIII.

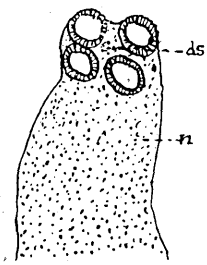

Fig XI

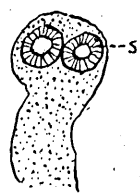

Fig IX.

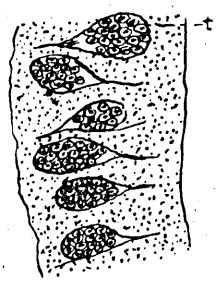

Fig X

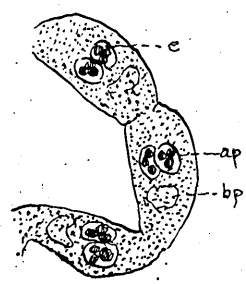

Fig XI

PLATE II

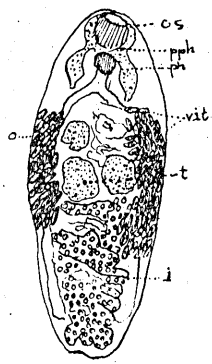

Fig XII

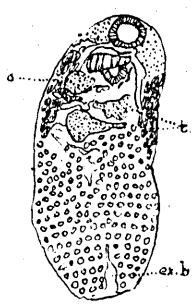

Fig XIII

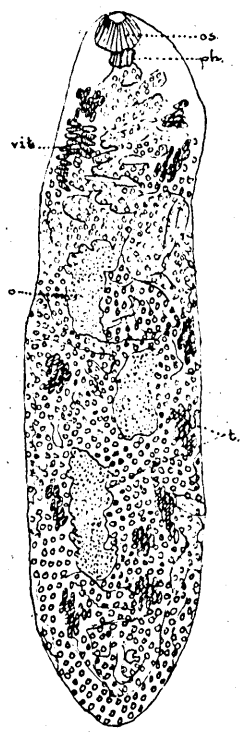

Fig XIV

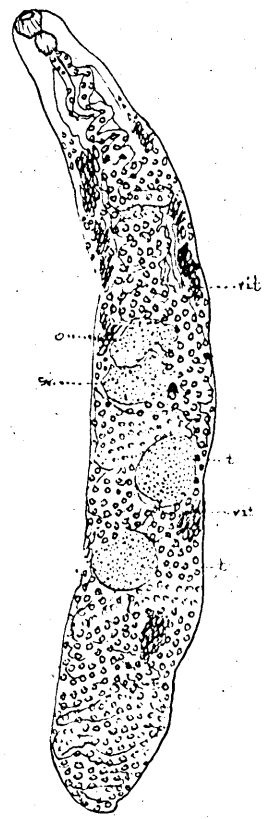

Fig XV

PLATE III

Bibliography

Bensley, R.R.
1897-Two forms of Distomum cygnoides. Centra. f. Bakt., Infedt., 21:326-331

Chandler, A.C.
1928-Three new trematodes from Amphiuma means. Proc. U.S. Nat. Mus., 63. Art 3

Chapin- E.A.
1926-Report of the Helminthological Society of Washington. Jour. Parasit., 12:180

Cort, W.W.
1912- North American frog bladder flukes. Trans. Amer. Mic. Soc. 31:,151-166
1915-North American frog bladder flukes Trans. Amer. Mic. Soc. 34:203-240
1919-A New Distome from Rana aurora. Univ. Calif. Publ. Zool., 19:283-298
1926-Report of Helminthological Society of Washington. Journ. Parasit. 12:190

Dickey, L.B.
1921-A New Amphibian Cestode. Jour. Parasit. 7:129-136

Guberlet, John E.
1920- A new bladder fluke from the frog. Trans. Am. Mic. Soc. 39:142-148

Hunter, G.W.
1930-Diplodiscus intermedius- sp. nov. from Rana Catesbiana Shaw. Jour. of Parasit. 17:74-79

Irwin, Marion S.
1929-A new lung fluke from Rana Clamitans Latreille. Trans. Amer. Mic. Soc. 48:74-79

Jewell, Minna E.
1916-Cylindrotaenia americana nov. spec. from the Cricket Frog Jour. Parasit. 2: 181-192

Krull, W.
1930-The life history of two North American Frog Lung Flukes 16: 207-212

Leidyi, J.
Researches in Helminthology and Parasitology. Smithsonian Miscellaneous collection 46: Art 3
1851-Contributions to Helminthology. Proc. Acad. Nat. Sci. Phila. 5:205-209;239-244

Mac Callum, W.G.
1899-On the species Clinostomum heterostomum. Journ. Morph., 15:697-710

Osborn, H.L.
1911-On the distribution and mode of occurrence in the

United States and Canada of Clinostomum margenatum, a trematode parasitic in fish, frogs, and birds. Biol. Bull., 20:350-366

Osborne, H.L.
1912-On the structure of Clinostomum margenatum, a trematode parasitic of the frog, bass, and heron. Journ. Morph., 23:189-222

Osler, C.P.
1931-A new cestode from Rana clamitans. Jour. Parasit., 17:183-186

Shaw, Ruth
1928-A Critical Study of the Taeneoid Cestodes of the Dogs (canis familiares) Master's Thesis, Univ. Kan.

Stafford, J.
1902-The American Representative of Distomum cygnoides. Zool. Jahrb. Syst., 16:411-424
1905-Trematodes from Canadian vertebrates. Zool. Anz., 28:681-694

Stile and Goldberger
1910- A Study of the anatomy of Watsonius (N.G.) Watsoni of man and of nineteen allied species of mamalian trematode worms of the superfamily Paramphistomidea Hygienic Laboratory Bulletin, 60:1-264 205 figs.

Stiles, C.W. and Hassall, A.
1912-Index catalog of Medical and Veterinary Zoology: Cestodes and Cestodaria. Bull. Hyg. Lab.85

Walton, A.C.
1929-Studies on some nematodes of North American frogs Jour. Parasit., 15:227-249
1931-Note on some larval nematodes found in frogs. Jour. Parasit., 17:228-230

Ward, H.B.

1917-On the structure and classification of North American parasitic worms. Jour. Parasit., 4:1-11

Ward, H.B. and Whipple, G.C.
1918-Fresh-water biology. John Wiley and Sons, New York. 1111 pp.

Number and Kind of Parasite per Frog

Chart I

Species of Frog and Locality	Number	Cylindrotaenia americanus	Ophiotaenia saphena	Rhabdonema Nigrovenosum	Oswaldocruzia leidyi	Unclassified nematode	Gorgodera minima	Gorgoderina translucida	Gorgodera amplicava	Pneumonoeces Paruiplexus	Pneumonoeces medioplexus	Diplodiscus temporanum	Clinostomum attenatum	Opalina ranarum	Nyctotherus cordiformis
Rana pipiens Chicago Group 1	1	-	-	9	2	-	-	-	-	-	-	-	-	++	-
	2	-	-	76	5	-	-	4	-	-	1	-	-	-	-
	3	-	-	-	-	-	-	2	-	-	4	2	-	-	-
	4	-	-	13	2	-	-	-	-	-	-	-	-	-	-
Rana pipiens Chicago Group 2.	1	-	-	-	-	-	-	1	-	-	-	-	26	-	-
	2	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	3	-	-	-	16	-	-	5	-	-	-	3	1	-	-
	4	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	51	++	-
	6	-	-	-	11	-	-	-	-	-	-	-	-	-	-
	7	-	-	1	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	9	-	-	5	1	-	-	-	-	-	-	-	12	-	-
	10	-	-	-	-	-	-	-	-	-	-	2	-	-	-
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	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	13	-	-	-	-	-	-	-	-	-	-	3	16	-	-
	14	-	-	-	-	-	-	5	-	-	-	-	-	-	-
Rana pipiens Wisconsin	1	-	-	2	-	-	-	-	-	-	1	-	-	-	-
	2	-	-	5	1	-	-	-	-	-	-	-	-	-	-
	3	-	-	11	79	-	-	-	-	-	-	-	-	-	-
	4	-	-	27	48	-	-	-	-	-	-	-	-	-	-
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	8	-	-	4	16	-	-	-	-	-	-	-	-	-	-
	9	-	-	24	8	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	21	-	-	-	-	-	-	-	-	-	-
	11	-	-	2	10	-	-	-	-	-	-	-	-	-	-
Rana clamitans New Orleans	1	-	1	3	3	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	1	-	-	31	1	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	3	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	4	1	2	-	-	-	-	-	1	-	-	-
	6	-	-	-	-	-	-	-	-	12	-	-	-	-	-
	7	-	-	2	-	-	-	-	-	4	-	-	-	-	-
	8	-	-	2	-	-	-	-	1	11	-	-	-	-	-
	9	-	-	3	-	2	-	-	16	2	-	2	-	-	-
	10	-	-	2	-	-	-	-	17	2	-	1	-	-	-
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	12	-	-	4	-	-	-	-	1	2	-	1	-	-	-
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	18	-	-	7	-	1	-	-	-	-	-	-	-	-	-
5 Rana areolata Kansas	1	-	-	-	-	-	-	-	-	-	-	3	-	++	-
	2	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6 Rana pipiens Lawrence	1	-	-	1	-	-	-	-	3	-	9	-	-	++++	++
	2	-	-	-	-	-	-	-	-	-	17	-	-	++++	++
	3	1	-	-	-	-	-	-	-	-	-	-	-	++++	-
	4	-	-	3	4	-	-	-	-	-	-	-	-	++++	++
	5	-	-	1	1	-	-	-	-	-	1	-	-	++++	-
	6	-	-	10	-	-	-	-	-	-	1	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	26	-	-	++++	++
	8	-	-	-	-	-	-	-	-	-	6	-	-	+++	++
	9	-	-	-	-	-	-	-	-	-	2	-	-	++	+
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	13	2	-	5	-	-	-	-	-	-	2	4	-	++++	++
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	16	-	-	-	-	-	-	-	-	-	-	-	-	+++	++
	17	-	-	3	-	-	-	-	-	-	8	-	-	+++	++
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	23	-	-	-	-	-	-	-	-	-	3	-	-	+++	++
7 Bufo americanus Lawrence	1	-	-	-	26	-	-	-	-	-	-	-	-	++++	++
	2	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note:
No attempt was made to count the protozoans. Their presence is noted by the plus sign(+), and their absence by the minus sign (-). The greater number of plus signs used the greater was the infestation.

A Comparative Chart Giving Percentage of Infection with Each Parasite within the Different Groups

Chart 2

Species of Parasite	Species of Frog	Locality	Number Examined	Number Infected	Percentage of Infection	No. of Parasites Found	Maximum Infection	Minimum Infection	Av. No. of Parasites for Each
1. Rhabdonema nigrovenosum	Rana pipiens	Chicago Gr. 1	4	3	75%	98	76	13	29
	" "	" "2	14	2	14.28%	6	5	1	.42
	" "	Wisconsin	11	9	81.81%	126	32	2	11.45
	" "	Lawrence	23	8	34.78%	30	10	1	1.26
	Rana clamitans	New Orleans	18	13	72%	51	7	2	2.83
	Rana areolata	Kansas	3	-	0.00%	0	-	-	-
	Bufo americanus	Lawrence	4	-	0.00%	-	-	-	-
2. Oswaldocruzia leidyi	Rana pipiens	Chicago Gr. 1	4	3	75%	9	5	2	2.25
	" "	" "2	14	4	27.14%	32	16	1	2.28
	" "	Wisconsin	11	10	90%	201	79	1	18.27
	" "	Lawrence	23	2	8.69%	5	4	1	.21
	Rana clamitans	New Orleans	18	2	11.11%	4	3	1	.22
	Rana areolata	Kansas	3	-	0.00%	0	-	-	-
	Bufo americanus	Lawrence	4	1	25%	26	26	26	6.5
3. Unclassified nematode	Rana pipiens	Chicago Gr. 1	4	-	0.00%	-	-	-	-
	" "	" " 2	14	-	0.00%	-	-	-	-
	" "	Wisconsin	11	-	0.00%	-	-	-	-
	" "	Lawrence	23	1	4.35%	3	3	3	.13
	Rana clamitans	New Orleans	18	7	38.88%	67	31	1	3.72
	Rana areolata	Kansas	3	-	0.00%	-	-	-	-
	Bufo americanus	Lawrence	4	-	0.00%	-	-	-	-
4. Pneumonoeces parviplexus	Rana pipiens	Chicago Gr. 1	4	-	0.00%	0	-	-	-
	" "	" " 2	14	-	0.00%	0	-	-	-
	" "	Wisconsin	11	-	0.00%	0	-	-	-
	" "	Lawrence	23	-	0.00%	0	-	-	-
	Rana clamitans	New Orleans	18	7	38.88%	29	12	1	1.61
	Rana areolata	Kansas	3	-	0.00%	0	-	-	-
	Bufo americanus	Lawrence	4	-	0.00%	0	-	-	-
5. Gorgodera amplicava	Rana pipiens	Chicago Gr. 1	4	-	0.00%	0	-	-	-
	" "	" " 2	14	-	0.00%	0	-	-	-
	" "	Wisconsin	11	-	0.00%	0	-	-	-
	" "	Lawrence	23	-	0.00%	0	-	-	-
	Rana clamitans	New Orleans	18	7	38.88%	42	17	1	2.33
	Rana areolata	Kansas	3	-	0.00%	0	-	-	-
	Bufo americanus	Lawrence	4	-	0.00%	0	-	-	-
6. Gorgodera minima	Rana pipiens	Chicago Gr. 1	4	-	0.00%	0	-	-	-
	" "	" " 2	14	-	0.00%	0	-	-	-
	" "	Wisconsin	11	-	0.00%	0	-	-	-
	" "	Lawrence	23	-	0.00%	0	-	-	-
	Rana clamitans	New Orleans	18	1	5.55%	3	3	3	.16
	Rana areolata	Kansas	3	-	0.00%	0	-	-	-
	Bufo americanus	Lawrence	4	-	0.00%	0	-	-	-
7. Gorgoderina translucida	Rana pipiens	Chicago Gr. 1	4	2	50.00%	6	4	2	1.5
	" "	" " 2	14	6	42.86%	15	5	1	1.07
	" "	Wisconsin	11	-	0.00%	0	-	-	-
	" "	Lawrence	23	-	0.00%	0	-	-	-
	Rana clamitans	New Orleans	18	1	5.55%	3	3	3	.16
	Rana areolata	Kansas	3	-	0.00%	0	-	-	-
	Bufo americanus	Lawrence	4	-	0.00%	0	-	-	-
8. Diplodiscus temporanum	Rana pipiens	Chicago Gr. 1	4	1	25%	2	2	2	.5
	" "	" " 2	14	5	35.71%	10	3	1	.71
	" "	Wisconsin	11	-	0.00%	0	-	-	-
	" "	Lawrence	23	-	17.39%	11	4	2	.47
	Rana clamitans	New Orleans	18	5	27.07%	7	2	1	.38
	Rana areolata	Kansas	3	2	33.33%	4	3	1	1.33
	Bufo americanus	Lawrence	4	1	0.00%	0	-	-	-
9. Clinostomum attenatum	Rana pipiens	Chicago Gr. 1	4	1	25%	0.	-	-	-
	" "	" "2	14	5	35.71%	106	51	1	7.57
	" "	Wisconsin	11	-	0.00%	0	-	-	-
	" "	Lawrence	23	-	0.00%	0	-	-	-
	Rana clamitans	New Orleans	18	-	0.00%	0	-	-	-
	Rana areolata	Kansas	3	-	0.00%	0	-	-	-
	Bufo americanus	Lawrence	4	-	0.00%	0	-	-	-
10. Cylindrotaenia americanus	Rana pipiens	Chicago Gr. 1	4	-	-	-	-	-	-
	" "	" " 2	14	-	-	-	-	-	-
	" "	Wisconsin	11	-	-	-	-	-	-
	" "	Lawrence	23	2	8.69%	3	2	1	.13
	Rana clamitans	New Orleans	18	-	-	-	-	-	-
	Rana areolata	Kansas	3	-	-	-	-	-	-
	Bufo americanus	Lawrence	4	-	-	-	-	-	-
11. Pneumonoeces medioplexus	Rana pipiens	Chicago Gr, 1	4	2	50%	5	4	1	1.25
	" "	" "2	14	-	0.00%	0	-	-	-
	" "	Wisconsin	11	1	9.09%	1	1	1	.09
	" "	Lawrence	23	13	56.52%	98	26	1	4
	Rana clamitans	New Orleans	18	-	0.00%	0	-	-	-
	Rana areolata	Kansas	3	-	0.00%	0	-	-	-
	Bufo americanus	Lawrence	4	-	0.00%	0	-	-	-
12. Opalina ranarum	Rana pipiens	Chicago Gr. 1	4	1	25%	++	++	++	++
	" "	" " 2	14	-	0.00%	0	-	-	-
	" "	Wisconsin	11	2	18.18%	+	+	+	+
	" "	Lawrence	7	6	85.71%	++++	++++	++++	+
	Rana clamitans	New Orleans	18	-	0.00%	0	-	-	-
	Rana areolata	Kansas	3	1	33.33%	++	++	++	++
	Bufo americanus	Lawrence	1	1	100.00%	++++	++++	++++	++
13. Nyctotherus cordiforms	Rana pipiens	Chicago Gr. 1	4	-	0.00%	0	-	-	-
	" "	" " 2	14	-	0.00%	0	-	-	-
	" "	Wisconsin	11	-	0.00%	0	-	-	-
	" "	Lawrence	7	4	57.14%	++	++	++	++
	Rana clamitans	New Orleans	18	-	0.00%	0	-	-	-
	Rana areolata	Kansas	3	-	0.00%	0	-	-	-
	Bufo americanus	Lawrence	1	1	100%	++	++	-	-

Chart 3

A Comparative Chart Giving the Percentage of Total Infection Per Group

Name of Species and Locality	No. of Specimens Examined	No. of Parasites Found	Av. No. of Parasites per Specimen	No. of Kinds of Parasites per Gr.	Percentage of total no. of Parasites Found per Gr.
1. Rana pipiens Chicago Gr. 1	4	120	30	6	11.38%
2. Rana pipiens Chicago Gr. 2	14	169	12	6	16.03%
3. Rana pipiens Wisconsin	11	328	29.82	4	31.12%
4. Rana pipiens Lawrence	23	139	6.04	8	13.04%
5. Rana clamitans New Orleans	18	268	14.88	2	25.42%
6. Rana areolata Kansas	3	4	1.33	8	.37%
7. Bufo americanus	4	26	6.5	3	2.57%
TOTAL	77	1054	13.68	15	100%