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SOME HELMINTHS OF NATRIX RHOMBIFERA RHOMBIFERA AND NATRIX ERYTHROGASTER
TRANSVERSA IN PAYNE COUNTY, OKLAHOMA

By

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CHAPTER I

INTRODUCTION

The helminthic fauna of a region must be known before extensive parasitological research can be undertaken. Likewise, pure science must be considered before applied scientific investigations can be planned with accuracy. The writer undertook an investigation involving pure science and selected to determine what helminths occurred in water snakes. They were preferred because (1) no report of a similar survey was found in the literature; (2) the snakes live in a moist habitat and should be abundantly parasitized; (3) they are quite plentiful so that the removal of a few would not endanger the continuation of the species; (4) the snakes are of little or no tangible economic importance; and (5), as anyone knows, the general public approves of their removal.

The writer began to collect snakes for the study in April, 1949 and finished in May, 1950, but no snakes were taken during the hibernation period. They were taken from as many different water impoundments as possible. By making a collection of parasites over such a period of time, it was hoped not only to obtain the various helminths and classify them, but to obtain data on the variety, incidence, abundance and relative abundance in these snakes during the several seasons.

Matrix rhombifera rhombifera and Matrix erythrogaster transversa were first described by Hallowell (1852) and are commonly called brown water snakes. The parasite data for this thesis are based on these hosts. A third species, Matrix grahami (Baird and Girard) exists in the area, but it is exceedingly rare and was observed only on two occasions. Therefore, the writer chose not to include this snake in the

investigation but to leave it for future biological collecting groups. The type locality for both species considered in this thesis is the Arkansas River and its tributaries near the northern boundary of the Creek Nation.

The review of literature for the parasites of each species is incorporated in the body of the thesis following the respective parasite description.

CHAPTER II

METHODS AND MATERIALS

Natricine snakes were collected by hunting the waters edge of lakes and farm impoundments. When encountered, usually in a hiding place provided by tall semi-aquatic weeds, an ordinary garden rake was used to pin the snake to the ground until it could be picked up and placed in a bag. If alders or willows rendered the rake useless, a 22 caliber revolver loaded with "dust shot" was employed; however, nothing but a direct head hit would stop the reptile. When females were gravid and distended with numerous eggs, they were usually found in groups beneath a brush pile close to the waters edge. This is the only time that snakes were found congregated.

Snakes are known to writhe and twist for some time after death. Xylene, placed on a cotton wad in a closed jar containing the snake, was used as a killing agent for the smaller snakes. Death usually took place in ten minutes and the body was in a relaxed condition. Larger snakes were drowned in lukewarm water, and this method relaxed them. Killing by decapitation was not desirable because relaxation was not accomplished.

In preparation for examination, the dead snake was stretched out ventral side up, the body length measured, sex determined, and data recorded. When this had been accomplished, two longitudinal incisions were made through the body wall, extending from the base of the head to the anus, and at the level of the lateral margins of the ventral scales. This ventral section was removed, revealing the entire peritoneal cavity and viscera. The under surface of the removed section was examined for

filarid nematodes and other encysted helminths before it was discarded. The organs were freed by severing mesenteric attachments, and removing them from the body cavity. Organs were carefully separated and placed in marked finger bowls containing tap water. Before the remaining portion of the carcass was discarded, all the skin was removed and examined for filarids and cysts.

Body organs having a lumen were slit open and turned inside out; solid organs were teased apart with needles. All tissues were removed from the finger bowls and examined with the unaided eye for possible attached helminths. When parasites were thus observed, the tissue was returned to the bowl and the organism worked loose into the water. When it was certain the tissue was free of parasites, it was placed in a jar containing water for further examination if such was deemed necessary.

The finger bowls, now ready for decantation, were filled with water, allowed to stand for two minutes and decanted. The process was repeated until the water was clear so that the bottom of the container could be examined for parasites.

The writer always made a casual examination of all bowls for possible nematodes. Such specimens were removed to physiological saline solution until fixed. Then, a more intense examination was made of each bowl to recover trematodes or cestodes large enough to be visible to the unaided eye. When found, they were placed in separate, labeled watch glasses or petri dishes containing clear water. Finally, a thorough examination of the sediment in each bowl was made with the binocular dissecting microscope. If any parasites were observed, which frequently was the case, they were placed in their respective watch glasses with other parasites from the same organ.

The fixation of all helminths was accomplished as soon after discovery as possible, except for some trematodes which were relaxed in water to bring about the ejection of eggs from the uterus. Nematodes were fixed in hot 70% alcohol containing 4% glycerine. The solution was left standing to allow the alcohol to evaporate and thus leave the cleared nematodes in glycerine.

Cestodes were washed in water and then individually held up on an applicator stick while either hot 70% alcohol or 10% formalin was poured over them. This fixed the specimen in an extended condition which is desirable in order to reveal the internal structures to the best advantage in stained specimens. Trematodes were separated into groups and put into individual watch glasses or petri dishes containing water. The excess water was removed and the fixative dropped on each with a pipette. Alcohol-Formalin-Acetic Acid, either hot or cold, was found very satisfactory for this purpose. The AFA fixative was removed from the worms after penetration had taken place (twelve hours minimum) and replaced with 70% alcohol to prevent hardening.

The flukes and tapes were preserved in individual vials containing either 10% formalin if fixed in same, or 70% alcohol if fixed in AFA or 70% alcohol, labeled, numbered and cataloged. All small vials were placed in closed quart jars of similar solutions to prevent evaporation.

Trematodes and cestodes were stained either in Cochineal, Carmine, or Ehrlich's Acid Hematoxylin stain, dehydrated in alcohol, cleared in methyl salicylate and mounted in balsam. The slides were labeled as to host number and organ. The parasites were identified and twenty slides of each species were selected at random for the descriptions of species.

CHAPTER III

DATA CONCERNING PARASITES

General: Water snakes for this study were collected from water impoundments in the vicinity of Stillwater, Payne County, Oklahoma. The largest number of snakes collected at Lake Carl Blackwell was taken from Arms 6 and 13. Collections were made from numerous other impoundments and the snakes taken in Stillwater were captured from Theta Pond and the Park impoundment. The snakes are represented by red dots at the approximate locality of capture on the following scaled map of the county (Figure 1).

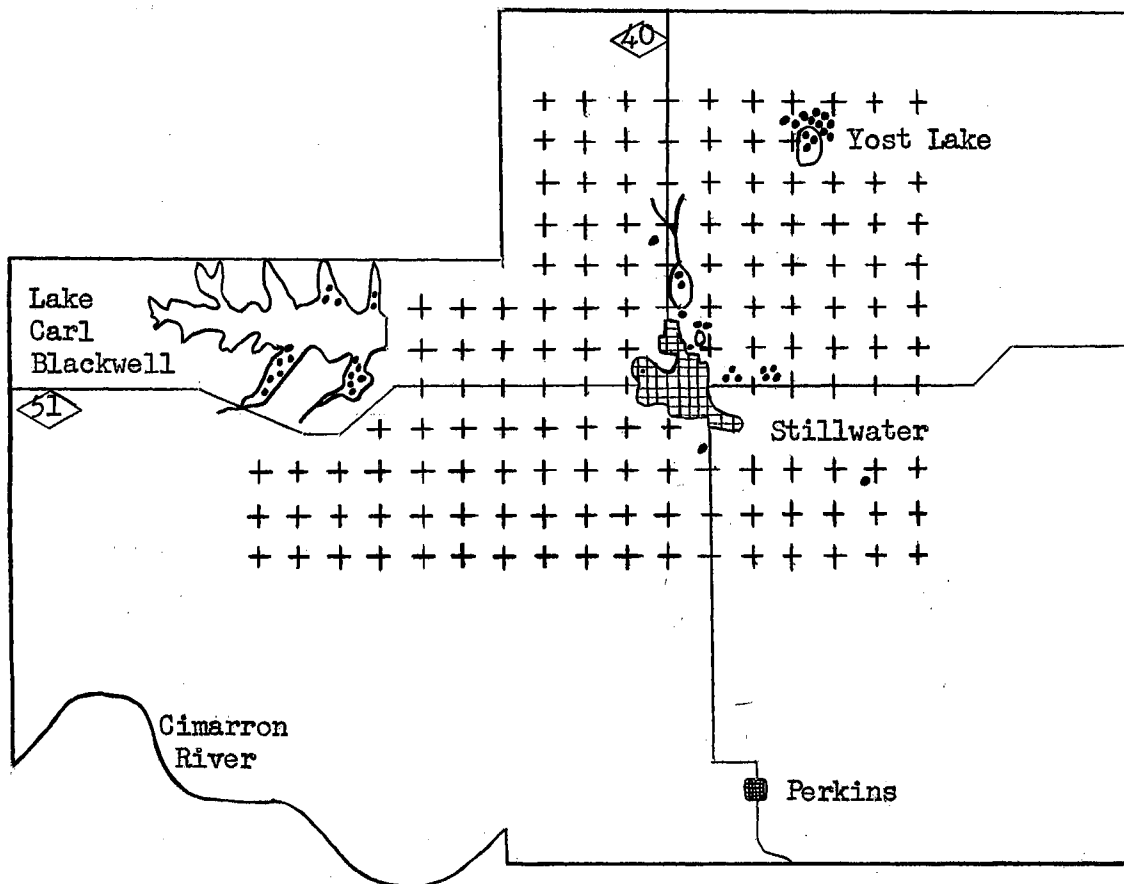


FIGURE 1

MAP OF COUNTY SHOWING WHERE SNAKES WERE CAPTURED

The writer examined a total of forty-seven hosts for helminth parasites and these snakes were taken from eleven separate localities. Each host was found to be parasitized by either mature or immature helminths or by representatives of both groups. The abundance of mature helminths in a host varied from one to two hundred forty-eight individuals while the immature helminths ranged from one to approximately twenty-five hundred. Data concerning the parasites recovered from Natrix rhombifera rhombifera and Natrix erythrogaster transversa are presented in Tables I and II, respectively.

Table I shows that forty-one specimens of Natrix r. rhombifera, with their respective dates of collection, were examined, of which twenty-three were females, thirteen were males and five were undetermined. The data presented indicate that mature trematodes, those showing eggs in the uterus, were not found in any of the organs except the mouth, esophagus and lung, and that cestodes were found only in the intestine. Mature mouth trematodes were found in thirty-nine percent of the hosts with an average abundance of ten, mature esophageal trematodes in fifty-one percent with an average abundance of nine, mature lung trematodes in fifty-one percent with an average abundance of four and mature cestodes in eighty-two percent with an average abundance of eighteen per host. Therefore, the incidence ranges from thirty-nine percent of the hosts infected with mouth trematodes to eighty-two percent infected with cestodes while the comparative average abundance ranges from four for lung trematodes to eighteen for cestodes. Thus, cestodes were encountered in more individuals and in larger numbers than any other helminth.

TABLE I

PARASITES RECOVERED FROM MATRIX RHOMBIFERA RHOMBIFERA

Hosts		Date Collected	Mature Trematodes			Mature Cestodes
No.	Sex		Mouth	Esophagus	Lung	Intestine
1	*	4-22-49		3		5
2	*	6-11-49	1	3		19
4	*	6-11-49			3	
5	*	6-11-49			2	1
6	*	6-18-49				1
8	F.	6-23-49	1			4
9	F.	7-5-49			1	247
10	F.	7-6-49	5		8	6
11	F.	7-6-49	14	8	2	12
13	M.	7-7-49	27	1	1	47
14	F.	7-12-49				14
15	F.	7-15-49				12
16	F.	7-15-49				21
17	F.	7-15-49		4	1	8
18	F.	7-15-49		1	1	26
19	M.	7-15-49	3			7
20	F.	7-15-49				28
22	F.	7-19-49		1		12
23	F.	7-19-49	1	29		18
24	F.	9-21-49			1	
26	M.	9-24-49	1			13
27	F.	9-25-49				5
28	F.	9-30-49	1	9	1	20
29	M.	9-30-49			1	
30	M.	10-3-49		1	15	
31	F.	10-18-49				9
32	F.	4-29-50	71	26	2	7
33	M.	4-29-50		9		29
34	M.	4-29-50		34		6
35	F.	4-29-50	2	8	2	6
36	F.	4-29-50	8	10		
37	M.	4-29-50				8
38	M.	4-29-50	3	3		2
39	M.	4-29-50	9	1	2	3
41	F.	4-29-50		9	10	13
42	F.	4-29-50			10	
43	M.	4-29-50		1	15	7
44	F.	5-13-50	12		1	8
45	M.	5-13-50	3	5		
46	F.	5-13-50		31	2	7
47	M.	5-13-50			5	2
Total			162	197	86	633

* Undetermined

TABLE II

PARASITES RECOVERED FROM NATRIX ERYTHROGASTER TRANSVERSA

Hosts		Date Collected	Mature Trematodes			Mature Cestodes
No.	Sex		Mouth	Esophagus	Lung	Intestine
3	*	6-11-49	127	21		
7	M.	6-23-49	4	17	2	
12	F.	7-7-49				
21	F.	7-19-49		13		
25	F.	9-21-49				
40	F.	4-29-50			2	
Total			131	51	4	

* Undetermined

Table II shows that fewer Natrix erythrogaster transversa than N. r. rhombifera were collected for the study. This is a direct result of natural distribution because the former snake is not as plentiful as the latter and the writer did not show any partiality when collecting specimens. The data indicate collection dates and show that four females, one male and one specimen for which the sex was undetermined were examined. Mature trematodes were only recovered from the mouth, esophagus and lung, but no cestodes were encountered. Mature mouth trematodes were found in thirty-three percent of the hosts with an average abundance of twenty-two, mature esophageal trematodes in fifty percent with an average abundance of eight and mature lung trematodes in thirty-three percent with an average abundance of two specimens. However, this species was collected in too few numbers to permit any further conclusions concerning the parasites.

Originally, this study was chosen because a vast number and variety of helminths were expected to be found parasitizing water snakes, and no complete surveys had been made. The literature, however, showed that trematodes could be expected in the mouth, esophagus, lung, intestine, gall bladder and uterus of N. r. rhombifera and that at least one nematode had been reported from the stomach of this species of snake taken in Texas. Acanthocephalid worms had been reported from some water snakes. Therefore, the writer was surprised when so relatively few species were recovered from the hosts in this study.

It is theoretically possible that certain helminths, not native to this locality but existing in the same species of snakes in other regions, have never been introduced or that, if introduced, their life cycle may have demanded a species of intermediate host that did not

inhabit this area. This idea is substantiated by the fact that only two species of snails inhabit the local water impoundments in large numbers, and since a molluscan intermediate host is always necessary for a trematode life cycle, this then may be one reason why certain trematodes did not appear in the study. The dearth of nematode species in animals with such habits and living in such an environment is surprising and not readily explainable.

Along with the above perplexing problem, the writer came across another unusual situation. This time it was a matter of immature trematodes. They were encountered in fifty-four percent of N. f. rhombifera and in one hundred percent of N. e. transversa examined. Due to their small size, their large numbers, their general distribution through visceral organs and the scarcity of definite characters for diagnosis, they prevented the writer from making a survey of the helminths of water snakes as originally planned. This plan was abandoned in favor of the study as it is entitled, and to include a discussion of the peculiar phenomenon.

These immature trematodes were found in such organs as mouth, esophagus, stomach, intestine, gall bladder and liver. They even were recovered from the container in which the snakes were dissected, which indicates that young forms also may be free in the body cavity or in the blood stream. Adult trematodes, however, were limited to only a few of the above organs and, comparatively, their numbers were never as great as the immature ones. Thus, the writer concludes that probably trematode species of other reptilian or amphibian hosts are present.

This may be explained by citing an example. Garter snakes, for instance, have been found to be parasitized by large numbers of trematode species. They very frequently inhabit the boundaries of water impoundments. They were common in the area studied and even were observed by the writer swimming in the same pond with water snakes. Likewise, they are known to eat some of the same organisms as do the water snakes and, without question, both get most of their metacercariae from such organisms. When this happens, it is likely that the metacercariae of garter snake parasites linger for a variable period of time in the organs of the water snake, without showing any development. This may account for the large number of larvae in the water snakes.

However, it is theoretically possible that these large numbers of immature ones are of the species found, but that the species are poorly adapted to the host, and as a consequence, the mortality during development is high. These findings uncover an interesting situation and present a problem that can be solved experimentally, and by which much may be learned concerning specificity and behavior of immature forms in abnormal hosts.

Pneumatophilus variabilis (Leidy, 1856) Odhner, 1910: Reniferidae
Baer, 1924.

Synonyms: Distomum variabile Leidy, 1856 and Renifer variabilis
Pratt, 1903.

Description: Body pear-shaped in outline, dorso-ventrally flattened, moderately muscular and whitish-cream colored. Cuticula beset with moderately heavy conical spines, largest and most numerous in neck and central regions, smaller and less numerous at body extremities. Body longer than wide, attenuated anteriorly and broadly rounded posteriorly, averaging 3.85 mm. long (3.0 to 4.48) by 2.0 mm. wide (1.54 to 2.49) at level of testes. Oral sucker nearly circular, 0.55 mm. long (0.39 to 0.72) by 0.57 mm. wide (0.42 to 0.72). Acetabulum larger than oral sucker, 0.75 mm. long (0.48 to 1.04) by 0.73 mm. wide (0.51 to 0.90). Distance from anterior end of worm to anterior margin of acetabulum averaging 1.19 mm. Prepharynx short. Pharynx muscular, nearly circular, 0.21 mm. long (0.15 to 0.30) by 0.20 mm. wide (0.15 to 0.41). Esophagus 0.18 mm. long (0.11 to 0.30), bifurcates to form ceca which pass posteriorly to region of testes. Their extent is variable, not necessarily symmetrical and frequently they have lateral outpocketings distally. Excretory pore usually prominent at median posterior margin. Paired, lateral excretory tubules proceed posteriorly from region of pharynx across ceca and laterally to acetabulum, uniting behind öotype to form median common duct which proceeds directly to excretory pore. There is a small excretory bladder terminally. Testes in same transverse plane, irregular to lobed in outline, located short distance behind acetabulum, left testis 0.57 mm. long (0.39 to 0.83) by 0.52 mm.

wide (0.29 to 0.77), right testis 0.54 mm. long (0.41 to 0.83) by 0.47 mm. wide (0.26 to 0.71). Vasa efferentia proceed to posterior end of cirrus sac. Cirrus sac large, extending from mid-dorsal region of acetabulum to genital pore and containing deeply stained, voluminous, convoluted vesicula seminalis. Ovary spherical, ovate or lobed in outline, situated left of mid-ventral line with greatest area dorsal to acetabulum, 0.36 mm. long (0.27 to 0.47) by 0.33 wide (0.23 to 0.44). Oviduct short. Öotype and Mehlis' gland to right and adjacent to ovary. Laurer's canal present. Vitellaria consist of oval to elongated follicles, lateral and extra-cecal in position (forty percent of specimens show one or more follicles occupying intra-cecal region), anterior limits variable between level of genital pore and acetabulum, and posterior limits reaching to anterior region of testes. Longitudinal vitelline collecting tubules on either side unite in region just behind ovary and the common duct from either side proceeds medially to region of öotype, the two uniting to form common vitelline duct. Uterus voluminous, occupying entire post-testicular region, ascending and descending loops numerous, terminal portion passing over right side of acetabulum to genital pore. Metraterm well developed. Genital pore immediately posterior to origin of ceca, located medially or slightly to right of median line. Ova numerous, filling entire uterus, intra-uterine eggs averaging 37 microns by 19 microns.

Discussion: The above description of Pneumatophilus variabilis (Leidy, 1856) Odhner, 1910 is based on microscopic examinations and measurements of twenty mature flukes. These worms were removed from the lungs of nine water snakes, eight Natrix rhombifera rhombifera and one Natrix

erythrogaster transversa. This fluke has previously been taken from the former host, but this is the first time it has been reported from N. erythrogaster transversa. The measurements were made from permanent whole mounts of worms that were not pressed in fixation.

Pneumatophilus variabilis was named and described in 1856, by Joseph Leidy, the "Grandfather of Parasitology" in the United States. Leidy named the worm Distomum variabile, but divided his specimens into two groups, variety "a" and variety "b" on the basis of a longer body and a distinct neck in variety "a". It is interesting to note that his specimens, number not recorded, were removed from the lung of a water snake, Tripidonotus sipedon, which was obtained in December when snakes were in hibernation. The description of the parasite is lacking in completeness and is of little taxonomic value. The name of the snake is now Natrix sipedon.

In 1900, Lühe obtained specimens from the Museum für Naturkunde in Berlin, and described briefly and without figures variety "b" of Distomum variabile as a distinct species. He proposed to confine the specific name to variety "b" without proposing a name for variety "a". He was the last writer to mention either variety "a" or variety "b".

Before considering the species further, it will be necessary to consider a discussion of the Family, since the further history of the genus and species is intimately associated with its development. The family Plagiorchiidae apparently arose through great confusion. A question concerning the status of the family name Plagiorchiidae arose from the fact that two genera, Lepoderma Looss 1899 and Plagiorchis Lühe 1899, were based on the same species. Stiles (1901) reviewed the question of synonymy in these two genera, and submitted his findings to

the International Zoological Congress. He concluded that, since the journal containing Looss' description of the genus Lepoderma was not distributed until one day after the journal containing Lühe's description of Plagiorchis, the name Plagiorchis had priority and the family name should therefore be Plagiorchiidae. The Congress apparently concurred in the conclusions of one of its members, and established Plagiorchis as valid. The family Plagiorchiidae has become one of the largest trematode families with approximately forty-four genera included in it.

Pratt (1903) subdivided the family Plagiorchiidae, and created the subfamilies Reniferinae and Plagiorchinae; he also created the genus Renifer and designated Renifer ellipticus Pratt, 1903, a trematode inhabiting the mouth and air passages of certain North American water snakes, as the type. Pratt also recovered three worms from the lung of Natrix sipedon which he considered to be Distomum variable Leidy, 1856, even though they exhibited some variations not mentioned in Lühe's description. To make known these variations, he redescribed the species, and included it in the new genus Renifer. This description of the species is extensive and complete with measurements and plates, and the genus has remained valid.

Odhner (1910) created a new genus, Pneumatophilus, and designated Renifer variabilis (Leidy, 1856) as the type species in the genus. Pneumatophilus differs from Renifer in that the genital pore is posterior instead of anterior to the fork of the intestine. This genus has remained valid.

Talbot (1934) reviewed the systematics of the subfamily Reniferinae. He accepted the generic description of Pneumatophilus, but modified it

to include configurations of the uterus that were peculiar to the species in the genus. In this configuration, the coils of the uterus are nearly all arranged vertically and both ascending and descending loops are equally coiled. In the same paper he described P. foliaformis, basing his description on twenty-five specimens taken from the lung and trachea of Natrix sipedon. This species differs from the type species, P. variabilis, by having a longer esophagus and longer cirrus sac, while arms of the excretory collecting tubules are shorter.

McMullen (1937) discussed the taxonomy of the family Plagiorchiidae and related trematodes. He concluded that classification of nearly all the major groups of trematodes is based upon adult characters. This is often misleading, and the use of one adult character by one author, and a different character by another, often places the same species in two different families. McMullen proposed to use larval characters as a foundation on which to base the superfamily Plagiorchioidea Dollfus, 1930, and proposed that it should include all trematodes that develop from Xiphidiocercariae. McMullen compared thirty-five species that develop from Xiphidiocercariae. They represent three families: Plagiorchiidae, Lecithodendriidae and Lissorchiidae. Many of the adults show diverse characters but they all have certain larval characteristics in common. The morphology of the excretory system is important in separating the larger group into natural subgroups, despite the fact that the flame cell pattern in cercariae and in adults is quite different. Not enough is known about the miracidia of the trematodes to make a statement on their use in the taxonomic scheme.

With the proposal to amend the definition of the superfamily Plagiorchioidea, McMullen suggested that the family Plagiorchiidae be

redefined and be restricted to those forms that at present are included in the subfamily Plagiorchiinae. The cercariae of all the species in this subfamily, except one, belong to a distinct group of Xiphidiocercariae characterized by a well developed Y-shaped excretory bladder and a flame cell pattern of $2 (3+3+3) + (3+3+3)$.

The writer recognizes the superfamily Plagiorchioidea Dollfus, 1930, amended by McMullen to include all trematodes which develop from Xiphidiocercariae. It will be necessary for final arrangement to await further information on life histories and all contributions will be of help to clarify this taxonomic problem.

Byrd and Denton (1937) described Pneumatophilus leidyi. The description was based on nine mature specimens removed from the lung and trachea of the water snake Natrix sipedon fasciata in Georgia. They pointed out that P. leidyi differs from P. variabilis by its larger body, constant length of ceca, differences in shape and size of ovary, larger ova and smaller testes.

Pneumatophilus leidyi is the closest relative of P. variabilis and the writer has compared the two worms in every detail. Results show that the differences between P. variabilis and P. leidyi, as indicated by Byrd and Denton, are not of sufficient magnitude nor constant enough to separate the species. Dr. Byrd graciously loaned the writer five slides of P. leidyi for study and comparison. These specimens, labeled P. leidyi, showed organ variations that had been omitted or overlooked in the description and which were peculiar to P. variabilis, as originally described. The writer, therefore, considers P. leidyi to be a synonym of P. variabilis, and presents a detailed discussion in support of this action. However, the writer in the discussion, for convenience

and clarity, refers to P. leidy as a species even though he considers it a synonym of P. variabilis.

As already indicated, Pratt based his description of P. variabilis on three flukes from Natrix rhombifera rhombifera, while Byrd based the description of P. leidy on nine worms from Natrix sipedon fasciata. McMullen mistakenly reported the garter snake, Thamnophis, as the definitive host of P. variabilis (Leidy, 1856) Odhner, 1910. The writer had seventy-two mature specimens to study from N. rhombifera rhombifera. Therefore, anatomical variations were made more obvious in the latter and largest group.

Pratt reported that the entire body of P. variabilis is thickly beset with minute spines which are more numerous in the anterior than the posterior portion. Byrd states that the cuticula of P. leidy is thickly beset with rather large, triangular shaped spines from the level of equatorial plane of oral sucker to level slightly behind the testes. Examination of the mounted specimens supplied by Byrd disclosed spines in patches in the posterior portion suggesting that some of the cuticula had been sloughed before fixation. The writer checked this condition by placing some freshly recovered P. variabilis flukes in water and studying them at intervals under the binocular microscope. It was found that the cuticula blistered in less than one hour after the worms were placed in water and that sloughing did occur. Therefore, description of spines cannot be relied on to distinguish species.

Pratt described P. variabilis as a broad, flat worm ranging between 3 and 4 mm. in length and 1.5 and 2 mm. in width. Byrd reported P. leidy to average 4.3 mm. long by 2.6 mm. wide. The specimens selected at random for this study ranged from 3.0 to 4.9 mm. in length

to 1.5 to 2.5 mm. in width, thus covering the range of published data.

Pratt stated that the oral sucker of P. variabilis is subterminal and measures 0.5 mm. in diameter while the acetabulum has a diameter of 0.6 mm. Byrd listed the oral sucker of P. leidyi as being subterminal and averaging 0.54 mm. long by 0.66 mm. wide and the acetabulum as averaging 0.82 in diameter. The writer's specimens displayed the following measurements: oral sucker averaged 0.55 mm. (0.39 to 0.72) long by 0.57 mm. (0.42 to 0.72) wide and the acetabulum averaged 0.75 mm. (0.48 to 1.0) long by 0.73 mm. (0.5 to 0.9) wide. The range given for each of the two suckers fully includes the range of measurements listed for P. variabilis and P. leidyi.

Pratt only recorded the length of the pharynx, 0.22 mm., of P. variabilis, while Byrd described the pharynx of P. leidyi as being nearly round, averaging 0.23 mm. long by 0.24 mm. wide. The specimens used in this study showed the pharynx to average 0.21 mm. (0.15 to 0.3) long by 0.20 mm. (0.15 to 0.4) wide. Again, the range for the pharynx of the latter specimens includes the range for both species. With the more complete series one can readily see that the variation in the size of these organs is considerable, which would not be obvious in lesser series.

Byrd specified that P. leidyi has ceca which are constant in their length and reach to the testes. An examination of Byrd's specimens showed that in one specimen one cecum terminated after overlapping the testis three-fourths of its length, and in others the ceca terminated at any place from the anterior margin of the testis to the halfway mark. Pratt stated that the ceca in young flukes, P. variabilis, terminate at the anterior end of the testes, while in old worms they terminate at the

posterior end, but Pratt gives no evidence for his statement. The ceca of the worms in this study exhibited a tendency to terminate at any level between the ends of the testes. Several specimens, approximately fifteen percent, showed one cecum, sometimes both, turning medially and passing for a short distance posteriorly between the testes before terminating.

Pratt recorded the ovary of P. variabilis as irregularly ovoid and measuring 0.33 mm. in length. Byrd stated that the ovary of P. leidy is slightly irregular in outline, averaging 0.34 mm. long by 0.27 mm. wide. Yet, of his five mounts examined by the writer, one was found to be spherical, another ovate, a third lobed and two were as he described. Similar variations were observed in my specimens of P. variabilis. Furthermore, the ovary averaged 0.36 mm. (0.27 to 0.47) long by 0.33 mm. (0.23 to 0.44) wide and the measurements cover the range of both species.

Pratt recorded testicular measurements of 0.8 mm. in P. variabilis. Comparisons made with specimens used in this study showed that 0.8 mm. was very large and seldom existed. However, it is entirely probable, that Pratt measured the longest diameter whether it was transverse, oblique or longitudinal. Byrd specified smaller testes in P. leidy and recorded measurements of right testis averaging 0.61 mm. long by 0.52 mm. wide and left testis averaging 0.61 mm. long by 0.59 mm. wide. He stated also that the testes were deeply notched and elongated obliquely. The five mounted specimens of P. leidy from Byrd and all the writer's specimens of P. variabilis showed the testes to be deeply notched or irregular in outline, but only the type specimen, not the paratypes, of P. leidy proved to have testes that were elongated obliquely. Furthermore, the specimens in this study showed the right testis to range from

0.41 to 0.83 mm. in length by 0.26 to 0.71 mm. in width and the left testis to range from 0.39 to 0.83 mm. in length by 0.29 to 0.77 mm. in width. These figures cover the range of measurements made by both Pratt and Byrd.

Byrd states that among the characteristics separating P. leidy from P. variabilis, the position of the genital pore is of value. Pratt stated that the genital pore is halfway between the two suckers and very nearly on the median line, while Byrd stated that it is located at the bifurcation of the ceca and slightly to the right of the median line. The writer, using the five specimens of P. leidy from Byrd and numerous specimens of P. variabilis, was unable to distinguish the slightest difference in the position of the genital pore on the two worms, and found it to be variable and within the ranges already specified.

Egg measurements of P. leidy were recorded by Byrd as 38 to 42 microns long by 26 microns wide. The writer found P. variabilis yielded intra-uterine ova averaging 37 microns long by 19 microns wide. Since Byrd did not specify whether his measurements were made from fixed or viable material, the writer made separate measurements of approximately a dozen fixed eggs of P. leidy and found them to average 38 microns long by 21 microns wide. These compare very closely to the eggs of my specimens of P. variabilis. Furthermore, the writer searched for the largest eggs and made frequent measurements of their width, but was unable to find a single egg that was 26 microns wide. It is possible that Byrd made measurements of fresh eggs, but since he failed to state how they were made, the likeness of the intra-uterine eggs of both species is sufficient from which to draw conclusions.

Byrd failed to include the course of the vitelline ducts in the drawing of P. leidyi. Therefore, the writer, using the five mounted specimens received from Byrd, compared P. leidyi and P. variabilis on this basis. The species were similar, and the small tubules from groups of follicles on each side unite just posterior to the ovary and a single duct from each side proceeds medially to the region of the oötype where the two unite to form the common vitelline duct. Variations were evident as to the sizes of the small collecting tubules in both species.

Therefore, it is shown that there are variations in both forms, but that there are no discontinuous variations in any of the organs discussed that demonstrate distinctive characters for separating the species.

Dasymetra conferta Nicoll, 1911: Reniferidae Baer, 1924.

Description: Body slightly ovoid in outline, dorsally convex, ventrally flattened and moderately muscular, averaging 3.37 mm. long (2.33 to 4.59) by 1.13 mm. wide (0.8 to 1.5). Cuticula thickly beset with small spines throughout. Oral sucker conspicuously sub-terminal, nearly circular averaging 0.5 mm. long (0.38 to 0.6) by 0.51 mm. wide (0.24 to 0.62). Acetabulum circular located at end of first half of body, averaging 0.48 mm. in diameter (0.32 to 0.56). Distance from anterior end of worm to anterior margin of acetabulum averaging 1.19 mm. Prepharynx short. Pharynx muscular, nearly spherical, averaging 0.22 mm. long (0.17 to 0.29) by 0.21 mm. wide (0.18 to 0.33). Esophagus short, bifurcates to form ceca which are long and voluminous extending to posterior body region behind testes. Excretory pore usually prominent at median

posterior margin. Entire excretory system not observed but small lateral collecting tubules filled with dark pigment granules are conspicuous in some mounted specimens. Testes oblique, left testis always anterior to right testis, irregular to lobate in outline. Left testis averaging 0.4 mm. long (0.28 to 0.59) by 0.36 mm. wide (0.18 to 0.57), right testis 0.48 mm. long (0.29 to 0.65) by 0.35 mm. wide (0.26 to 0.53). Cirrus sac short and broad, containing the slightly coiled vesicula seminalis and extends from posterior or mid-acetabular region to genital pore. Genital pore located on the median line ventral and at the level of the bifurcation of the ceca or immediately behind. Cirrus long, heavy and slightly bent when extended, measuring approximately 0.405 mm. long and 0.135 mm. diameter at distal end. Ovary spherical or elongated in outline, located to right of median line and, in ninety percent of the specimens, partly overlapping acetabulum. Oviduct short. Mehlis' gland and öotype located medially and slightly posterior to ovary. Vitellaria loosely dendritic, small and inconspicuous, lying lateral, dorsal and ventral to ceca in most specimens, but in some there were intercecal follicles, and in some of these a few follicles even met at median line on a level with ovary. Extent of vitellaria from or little behind level of genital pore to approximately level of posterior margin of right testis. Uterus proceeds posteriorly from öotype to posterior tip where it coils several times and then passes directly to genital pore becoming very voluminous in region between right testis and ovary. Metraterm very well developed. Intra-uterine eggs average 34 microns by 17 microns.

Discussion: The previous description of Dasymetra conferta Nicoll, 1911 is based on microscopic examinations and measurements of twenty mature flukes. These worms were removed from the esophagus of seven water snakes, four Natrix rhombifera rhombifera and three Natrix erythrogaster transversa. This is the first time Dasymetra conferta Nicoll, 1911 has been reported from Natrix erythrogaster transversa.

Nicoll recovered the parasites on which his description is based from an American water snake, Natrix rhombifera rhombifera. The number of specimens was not recorded. McCoy (1928) reported D. conferta from another water snake, Natrix sipedon.

The specimens used in this study closely resemble Dasymetra conferta as described by Nicoll (1911). However, the writer found three characters that varied somewhat and should be listed here. Nicoll states that the genital pore is located at the bifurcation of the ceca. This writer found such to be the case in many of the specimens used in this study, but some, perhaps due to contraction at the time of fixation, showed the genital pore a little posterior to the bifurcation of the ceca. Nicoll states that the vitellaria are peripheral and overlap the ceca occasionally. The specimens used in this study showed the vitellaria to vary considerably in position from lateral and dorsal to the ceca to even a few follicles from each side meeting at the mid-line on a level with the ovary. The third characteristic is the dark pigment granules in the small excretory tubules making them conspicuous. Nicoll believed that they might be the result of post-mortem changes. The writer, however, found that the tubules, containing chalky white granules, could be observed in all the living flukes but that the darkened ducts showed up in only a few specimens after staining and mounting. The writer

proposes that the specific diagnosis of Dasymetra conferta Nicoll, 1911 be emended to include the previous observations as recorded in the writer's description.

Other species in the genus have been described. Byrd (1935), working in Louisiana, described Dasymetra villicaeca from the upper esophagus and mouth cavity of Natrix sipedon fasciata, N. sipedon erythrogaster, N. rhombifera rhombifera, and N. cyclopion, while Hall and Allison (1935), working in New York, described Dasymetra nicolli from the stomach of Natrix sipedon.

Dasymetra conferta Nicoll, 1911 shows close affinities to D. villicaeca Byrd, 1935 and D. nicolli Hall and Allison, 1935. It may be separated from D. villicaeca by its larger testes and smaller pharynx, while D. nicolli has lobate testes which are wider than long and vitellaria that extend to the posterior limits of the ceca.

Renifer acetabularis Crow, 1913: Reniferidae Baer, 1924.

Synonym: Neorenifer acetabularis Byrd and Denton, 1938.

Description: Body elliptical in outline, dorsally convex and ventrally flattened, moderately muscular, averaging 1.59 mm. long (0.96 to 2.43) by 0.49 mm. wide (0.3 to 0.68). Cuticula beset with small spines throughout. Oral sucker 0.24 mm. long (0.18 to 0.3) by 0.21 mm. wide (0.16 to 0.26). Acetabulum located mid-ventrally, 0.31 mm. long (0.2 to 0.42) by 0.3 mm. wide (0.2 to 0.45). Distance from anterior end of worm to anterior margin of acetabulum averaging 0.50 mm. Prepharynx short. Pharynx spherical, averaging 0.09 mm. in diameter (0.07 to 0.10). Esophagus short, not apparent in some specimens, bifurcates to form ceca which pass posteriorly to level of anterior margin of testes. Excretory

pore median at posterior margin. Paired, lateral excretory tubules proceed posteriorly from region of origin of ceca and lateral to acetabulum, uniting posterior to this organ and forming a single voluminous vesicle with numerous outpocketings which occupies a large portion of the body posterior to the testes. Testes oval to slightly irregular in outline, located immediately posterior to acetabulum in same transverse plane, left testis 0.15 mm. long (0.1 to 0.25) by 0.12 mm. wide (0.09 to 0.16), right testis 0.17 mm. long (0.1 to 0.3) by 0.12 mm. wide (0.07 to 0.17). Cirrus sac long and narrow, often bent, containing the vesicula seminalis and extends from the anterior median margin of acetabulum to genital pore. Genital pore located in left lateral region opposite oral sucker. Ovary spherical in outline, located medially or slightly to right of median line with greatest area dorsal to acetabulum and averaging 0.12 mm. in diameter (0.08 to 0.16). Oviduct short. Mehlis' gland, surrounding ootype, located immediately to left of ovary. Vitellaria consist of oval to elongated follicles located in four groups, two on each side of body, lying both ventral and lateral to ceca and testes. The anterior pair of groups extend approximately from level of bifurcation of ceca to a level slightly behind anterior margin of acetabulum, and the posterior pair extend from posterior margin of acetabulum to level of mid-testicular region. Longitudinal vitelline collecting tubules on either side unite at level of ovary and proceed toward the ootype where the two from each side unite to form common vitelline duct. Uterus proceeds posteriorly from ootype to region behind testes where it coils several times and then passes anteriorly across acetabulum to genital pore. Metraterm not developed.

Intra-uterine eggs average 37 microns by 18 microns.

Discussion: The previous description of Renifer acetabularis Crow, 1913 is based on microscopic examinations and measurements of twenty mature flukes. These worms were removed from the mouth cavity of eight water snakes, six Natrix rhombifera rhombifera and two Natrix erythrogaster transversa. All measurements were made from permanent whole mounts.

R. acetabularis is reported here for the first time from N. e. transversa.

Renifer acetabularis was named and described in 1913 by Crow. The parasites were recovered from the mouth cavity of a water snake, Natrix rhombifera rhombifera, collected in Kansas.

Byrd and Denton (1938) discussed the systematics of the subfamily Reniferinae Pratt, 1902. They subdivided the genus Renifer and created the new genus Neorenifer. The genus Renifer was to retain those species that had a weakly developed cirrus sac and a genital pore lateral in position and to one side of the bifurcation of the ceca; while the genus Neorenifer was proposed to include those species with a well developed cirrus sac and a genital pore located to one side of the mid-line and on a level with the oral sucker and pharynx. As a result, Renifer acetabularis was made a synonym of Neorenifer acetabularis.

Kagan (1947) described a new species of Renifer and emended the definition of the genus as a result of his investigations. He found that the genital pore of R. septicus MacCallum, 1921 is located midway between the bifurcation of the ceca and pharynx and is, therefore, between the two designated areas as defined for Renifer and Neorenifer by Byrd and Denton (1938). Kagan also found evidence of variability in the position of the genital pore within species. He, therefore,

redefined the genus Renifer to include all the species whose genital pores are either lateral, extracecal, or lying between the bifurcation of the ceca and oral sucker. He also proposed that the genus be emended to include the species of the genus Neorenifer.

As a result of this study, the writer agrees with Kagan in that the position of the genital pore and the development of the cirrus sac are not constant enough to be used to separate two genera.

The specimens used in this study closely resembled R. acetabularis as described by Crow (1913) except for two characteristics. Crow stated that the intestinal ceca branch off directly from the posterior end of the pharynx. This writer found that a short esophagus is present in most specimens but not apparent in a few and that, when it is present, the length is variable. Crow described the testes as two partially lobate organs, but the plate accompanying the description shows them as being slightly irregular in outline. In this study, the testes were never found to be lobate but were oval or slightly irregular in outline. Therefore, the writer proposes that the specific diagnosis of R. acetabularis be emended to include these observations as recorded in the writer's description.

Renifer acetabularis shows close affinities to R. wardi Byrd, 1936, R. texanus Harwood, 1932, R. aniarum Leidy, 1890 and R. natricis MacCallum, 1921. It may be separated from R. wardi by its larger body, larger ovary and more posteriorly extending ceca. Renifer texanus, aniarum and natricis are all larger than R. acetabularis and, also, R. natricis has ceca which do not extend beyond the posterior margin of the acetabulum.

Proteocephalus (Ophiotaenia) perspicua (LaRue, 1911) Harwood, 1933:

Proteocephalidae LaRue, 1911.

Synonyms: Taenia lactea Leidy, 1855; Ophiotaenia lactea (Leidy, 1855) LaRue, 1911; Ophiotaenia perspicua LaRue, 1911.

Description: Longest strobila measured 64 cm., others only 25 to 35 cm., maximum breadth 2.2 mm., average 1.5 mm. Scolex triangular with apex pointed to broadly oval, averaging 0.225 mm. long (0.17 to 0.32) by 0.295 mm. wide (0.17 to 0.44). Suckers circular or oval, averaging 0.121 mm. in transverse diameter (0.08 to 0.18), being separated from each other by pronounced grooves. Neck averages 20 mm. long (13 to 30) by 0.288 mm. broad (0.165 to 0.75) behind scolex. First proglottids wider than long and very inconspicuous. Mature proglottids usually quadrate but soon becoming longer than broad, averaging 1.83 mm. long (1.2 to 2.55) by 1.343 mm. broad (1.12 to 1.86). Gravid proglottids conspicuously longer than wide, averaging 3.24 mm. long (2.1 to 6.0) by 1.48 mm. broad (0.98 to 2.33). Proglottids usually smooth and rectangular shaped, some ripe ones near end of strobila may be slightly ovoid with a single median furrow, are connected by their full widths, pseudosegmentation indistinct. Some may appear translucent after fixation but most are opaque. Genital pore marginal, irregularly alternating in strobila, situated at end or middle of first third of proglottid, and always marked by a slight rise in elevation of the margin. Vagina slender, located anterior or posterior to cirrus sac, proceeding medially with a gradual bending posteriorly until it reaches the median line which it then follows unerringly to near ovary. Testes 150-215, spherical to oval, situated in two lateral zones

separated by the uterus, follicles average 0.076 mm. in diameter (0.05 to 0.11). Vas deferens pronounced in gravid proglottids as a heavy mass of coils reaching from median line to cirrus sac. Cirrus sac prominent, averaging 0.336 mm. long (0.23 to 0.38) by 0.143 mm. wide (0.08 to 0.36). Ratio of length of cirrus sac to width of proglottid 1:3 to 1:6. Cirrus long, averaging 0.48 mm., slender and with small inflated region at proximal end when fully protruded. Ovary irregular in outline, lobes long, situated conspicuously in posterior region of the mature proglottid. Vitellaria follicular and consist of two rows, one in each lateral field, running the length of the proglottid. Paired vitelline collecting tubules proceed medially at the posterior end crossing the ovarian lobes. Uterus, when gravid, with 20 to 30 large lateral pouches with numerous interspersed smaller pouches on each side. Uterine pore not evident. Eggs oval or polygonal in outline, averaging 35.06 microns in diameter (31.05 to 41.40). Embryos averaging 14.5 microns (10.35 to 24.15).

Discussion: The above description of Proteocephalus (O.) perspicua (LaRue, 1911) Harwood, 1933 is based on microscopic examinations and measurements of six entire strobila and miscellaneous segments from at least fifteen other worms. The worms were removed from the intestine of ten water snakes, Natrix rhombifera rhombifera. All strobila measurements were made after fixation.

LaRue (1911) named and described briefly and without figures Ophiotaenia perspicua from N. rhombifera rhombifera, and created the new genus for the species. At that time, LaRue placed Taenia lactea Leidy, 1855, from N. sipedon, a species inquirenda, in his newly established genus Ophiotaenia.

LaRue (1914) published a revision of the cestode family Proteocephalidae in which he redescribed, complete with diagrams, and discussed Ophiotaenia perspicua. Some specimens were obtained from the water snake Natrix rhombifera rhombifera in Illinois and others from the same host in Oklahoma. This description, as well as the entire monograph, is complete in all details and is of great taxonomic value.

LaRue's (1914) monograph of the Proteocephalidae first placed that family in some degree of order and it has provided a starting place for subsequent writers discussing the classification of the Family. Since 1914, many writers, however, have departed from LaRue's system and at the present time the classification of the family is in a very confused state. One important reason for this confusion is that they are very similar morphologically and only a small percentage of the known species have had their life histories worked out. The natural classification cannot be attained until more is known concerning the larval stages and their characteristics, and these then compared with the general anatomy of the adults. However, such a study does not fall within the scope of this paper and the writer is mainly interested in placing the cestode in its correct taxonomic position.

Harwood (1933) discussed the systematics of the family Proteocephalidae. He found that the genus Ophiotaenia LaRue, 1911 contained a large number of cestodes. Many of the Ophiotaenia cestodes, described since LaRue created the genus, demonstrated testes in two fields that were distinctly separated while in others the testes were not so clearly separated. Therefore, Harwood recognized two subgenera on the basis of this character, designating Proteocephalus as the subgenus for those species in which testes were in one field and Ophiotaenia for those species in which the testes were divided into two fields.

Anderson (1935) recovered several cestodes from Natrix sipedon which he identified as Ophiotaenia perspicua and reported an incidence of thirty-one percent infection. He reviewed the question of O. lactea as a species inquirenda and suggested that the name Ophiotaenia perspicua LaRue, 1911 be retained for the species and that Ophiotaenia lactea (Leidy, 1855) LaRue, 1911 be considered a synonym, in recognition of complete, clear description rather than of priority. Anderson emended certain characters in the description of O. perspicua.

Herde (1938) reported the incidence of Proteocephalus (Ophiotaenia) perspicua as about sixty percent in Natrix r. rhombifera at Stillwater, Oklahoma.

The specimens used in this study closely resembled Proteocephalus (Ophiotaenia) perspicua as described by LaRue (1914) under the name of Ophiotaenia perspicua, and as emended by Anderson. The writer found that Proteocephalus (O.) perspicua reaches a total of 64 cm. often enough to be acknowledged. Also, the length of the neck although exceedingly variable averaged 20 mm., while LaRue described it as 5 to 7 mm. long and Anderson emended this character to read ". . . 4 to 10 mm. long" LaRue reported the ratio of the length of cirrus sac to width of proglottid as 1:3 to 1:4 while Anderson described it as 1:3 to 1:6. The specimens in this study demonstrated this character to vary from 1:3 to 1:6. Due to foregoing data, the writer believes the specific diagnosis of Proteocephalus (O.) perspicua (LaRue, 1911) Harwood, 1933 should be emended to read, ". . . strobila length up to 64 cm., neck averages 20 mm. long (13 to 30)"

Proteocephalus (O.) perspicua is the only cestode in this genus reported from Natrix rhombifera rhombifera. Oochoristica natrixis

Harwood, 1932 was reported from N. rhombifera rhombifera in Texas and is easily distinguished from P. (O.) perspicua by its centrally located ovary and vitellaria. Other species of cestodes that show close affinities to Proteocephalus (O.) perspicua are: Proteocephalus (Ophiotaenia) agkistrodontis Harwood, 1933; P. (O.) nattereri (LaRue, 1911) Harwood, 1933; P. (O.) grandis (LaRue, 1911) Harwood, 1933 and P. (O.) marenzelleri (LaRue, 1911) Harwood, 1933. Proteocephalus (O.) perspicua differs from both P. (O.) agkistrodontis and P. (O.) nattereri by having fewer testes. P. (O.) marenzelleri has more testes and P. (O.) grandis has more lateral uterine pouches than P. (O.) perspicua.

Spiruroidea larva: The only nematodes encountered during the study were four larva recovered from three Natrix rhombifera rhombifera taken at Lake Carl Blackwell. Three were found encysted on the outer wall of the esophagus while the fourth was encysted on the outer wall of the stomach. All four larva were alive when removed from the cysts and the largest measured 3.5 cm. long. Specific determination was not possible due to their immaturity.

The writer did not hesitate to place these larva in the Order Spiruroidea because morphologically they possessed spirurid characters and it has been previously and repeatedly demonstrated that many of the Spiruroid larva will reencyst in an accidental host instead of being passed on through as are many of the larva of other Orders. Likewise, it was believed that the hosts were accidentally infected because so few snakes contained the larva and in no instance were adult nematodes encountered. Spirurids usually inhabit the alimentary canal, respiratory system or orbital, nasal or oral cavities of vertebrates.

The life cycle of a Spiruroid nematode is indirect and it usually involves an arthropod intermediate host.

A review of the literature indicates no report of encysted larvae on the esophagus of any Natrix.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Summary:

1. The helminths of Natrix rhombifera rhombifera and Natrix erythrogaster transversa were investigated in the vicinity of Stillwater, Payne County, Oklahoma and forty-seven hosts were examined.
2. No species of helminths new to science were found, but all those that were encountered were identified, and are listed and described.
3. Pneumatophilus variabilis (Leidy, 1856) Odhner, 1910, a lung fluke, is reported with an incidence of fifty-one percent in Natrix rhombifera rhombifera and thirty-three percent in Natrix erythrogaster transversa.
4. Dasymetra conferta Nicoll, 1911, an esophageal trematode, is reported with an incidence of fifty-one percent in Natrix rhombifera rhombifera and fifty percent in Natrix erythrogaster transversa.
5. Renifer acetabularis Crow, 1913, a mouth fluke, is reported with an incidence of thirty-nine percent in Natrix rhombifera rhombifera and thirty-three percent in Natrix erythrogaster transversa.
6. Proteocephalus (Ophiotaenia) perspicua (LaRue, 1911) Harwood, 1933, a cestode, is reported with an incidence of eighty-one percent in Natrix rhombifera rhombifera but was not recovered from Natrix erythrogaster transversa.
7. Natrix erythrogaster transversa is reported for the first time as a host for Pneumatophilus variabilis, Dasymetra conferta and Renifer acetabularis.

8. Pneumatophilus leidy Byrd and Denton, 1937 was studied and determined by the writer to be a synonym of Pneumatophilus variabilis (Leidy, 1856) Odhner, 1910.
9. No nematodes, except larvae, or acanthocephalids were found in the snakes examined.
10. Immature trematodes were encountered in fifty-four percent of the Natrix rhombifera rhombifera and in one hundred percent of the Natrix erythrogaster transversa hosts examined.

Conclusions: This study revealed that the number of species of trematodes in these hosts is limited and that several forms described from the same host in other regions do not occur here.

It is indicated by the results of host examinations and perusal of literature that nematodes, cestodes and acanthocephalids are exceedingly scarce in these species of snakes.

In the opinion of the writer, the dearth of helminth parasites in these hosts is unusual and interesting for animals with such habits and ecological relations. Also, it is surprising that the one species of cestode recovered with such a high incidence from Natrix r. rhombifera was not found in its close relative Natrix e. transversa.

The unusual phenomenon of a general distribution of larval trematode forms throughout the visceral organs is not fully explained and constitutes material that could be used in the elucidation of physiological parasite problems.

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Typist

Helen Dunham