LYNXRUFUS WILSONI N.G., N.SP. (NEMATODA: METASTRONGYLIDAE) FROM THE LUNGS OF THE BOBCAT, LYNX RUFUS RUFUS (SHREBER)

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

INTRODUCTION

In February and March of 1952, many bobcats, Lynx rufus rufus (Schreber) collected for study (1) by The Virginia Cooperative Wildlife Research Unit were observed to be infected with a lungworm parasite. Of sixty-four bobcats examined, all collected in Virginia and North Carolina, twenty-five per cent were found to be infected with these parasites.

The infections were massive and in all specimens, only one type of lungworm was observed. Macroscopically, these nematodes were found in the bronchial tubes, the bronchioles and in the lung tissues. None were found in the pulmonary blood vessels.

1. This study was made by Donald R. Progulske, graduate fellow, and was reported (Progulske, 1952) in partial fulfillment of the requirements for the degree of Master of Science, Virginia Polytechnic Institute, June 1952.

REVIEW OF THE LITERATURE

There is perhaps no greater confusion, in the ever growing field of parasitology, than is encountered in the taxonomy. Seemingly, every taxonomic parasitologist employs some different criteria for the classification of nematodes. The roundworms, under which the parasite of this study falls, are in controversy even as to their position, in the Animal Kingdom. Most textbooks consider the roundworms to be representatives of the class or order Nematoda of the phylum Nemathelminthes while one very eminent worker would like to see them elevated to the status of a phylum (Chitwood 1950) and another would lower them to an aschelminth class. (Hyman 1951).

The nematodes of the family Metastrongylidae, the lungworms, have recently been reclassified into subfamilies from the host-parasite relationship, the morphology of the genera within the group and finally by the apparent evolutionary radiation. (Dougherty, 1943, 1945, 1946 a, 1946 b, 1949 a, 1949 b, 1951 a, 1951 b, 1952). His work is by far, the most extensive, conclusive, and detailed done with the metastrongylid worms.

The literature of the metastrongylid nematodes records nine species from the lungs or respiratory apparatus of the family Felidae. These species are as follows:(1) Aelurostrongylus abstrusus (Railliet, 1898) Cameron, 1927 from Felis catus; (2) Anafilaroides rostratus Gerichter, 1948, from Felis catus; (3) Bronchostrongylus subcrenatus (Railliet and Henry, 1913) Cameron, 1931, from Felis pardus and Felis tigris; (4) Gurltis paralysans Woffhugel, 1933, from Felis catus and

Felis g. guigna; Metathelazia californica Skinker, 1931, from Lynx rufus californicus; (6) Metathelazia felis (Vogel, 1928) Dougherty, 1943, from Felis pardalis; (7) Metathelazia massino (Davtian, 1933) Dougherty, 1943, from Felis catus; (8) Troglostrongylus brevior Gerichter, 1948, from Felis ocreatus; (9) Troglostrongylus troglostrongylus Vevers, 1922, from Felis bengalensis.

It is of importance to note that the literature does not record a lungworm parasite from the bobcat, Lynx rufus rufus. There is, however, a lungworm Metathelazia californica Skinker, 1931, from the lynx, Lynx rufus californicus. Skinker places the genus Metathelazia in the family Thelaziidae. Dougherty (1952) does not agree with this classification. He prefers, due to the morphology of the cephalic region and certain evolutionary deduction, to place the genus Metathelazia in the family Metastrongylidae.

The position of the genus Aelurostrongylus in the classification is greatly confused. Cameron 1927, who named the genus, considered it a member of the family Protostrongylidae. Skrjabin and Yershow (1933) placed Aelurostrongylus in the subfamily Synthecaulinae and Bohm and Gebauer (1934) regarded it as a member of the Metastrongylinae. Dougherty (1943, 1946) transferred it to the subfamily Filaroidinae. Gerichter (1948 a, 1948 b) has returned it to the family Protostrongylidae. A. abstrusus is the only species of this genus that has been described from the Felidae.

Dougherty (1949a) places the genera Bronchostrongylus Cameron, 1931 and Troglostrongylus Vevers, 1923 in the subfamily Skrjabingylinae

(Skriabin, 1933). In a later paper (Dougherty, 1951) it is indicated that he wishes to combine these two genera into one. In his key (Dougherty, 1949 a) of the subfamily Skrjabingylinae, based chiefly on male characters, the genus Troglostrongylus and Bronchostrongylus differ only in the lateral rays of the pattern. The one species of Bronchostrongylus, B. subcrenatus has been found in the lungs of both Felis pardus and Felis tigris. Troglostrongylus troglostrongylus, from Felis bengalensis, and Troglostrongylus brevior, from Felis ocreatus, according to Gerichter, (1949) differ in the size of the worms, the length of the spicules and gubernaculum, and the pattern of the lateral bursal rays.

Two genera, of the subfamily Filariodinae, Gurlitia, Woffhugel, 1933, and Anafilaroides should cause no confusion in classification unless they gain more members. They are both represented by only one species each, they are Gurltia paralysans Woffhugel, 1933, and Anafilaroides rostratus Gerichter, 1949. Both of these genera parasitize the lungs of Felis catus while Gurlitia also parasitizes Felia g. guigna.

THE INVESTIGATION

Objective:

Since the literature does not record the existance of a lungworm parasite from the bobcat *Lynx rufus rufus* it is the purpose of this thesis to show that this is a previously unknown parasite. The description, naming and classification of this lungworm are to be considered in this objective.

Materials and Methods:

The bobcats were collected either by shooting or by traps. All specimens were placed in formalin until they could be studied conveniently. During subsequent examination of the preserved bobcats, all lungworms were removed and placed in seventy per cent alcohol. These nematodes were dehydrated in an alcohol series to one hundred percent and then transferred to beechwood cresote, to which had been added a small amount of fast green stain. After the parasites had cleared, they were mounted on slides in permount. These whole mounts were subjected to intensive microscopic study and all measurements were made by means of a calibrated ocular micrometer.

Stippled drawings were drawn to scale: of the anterior end of the worm, an *en face* view of the mouth, the posterior end of the female, and the posterior end of the male. These drawings were all photographed. In addition, photomicrographs were taken of slides showing the extended spicules of the male and the position of the vulva of the female. In one instance, a female worm was dissected at the caudal end and the reproductive organs allowed to float out in water. This was done in

an attempt to ascertain the position of these organs. A diagram of the terminal portion of this system was drawn and photographed.

After complete familiarity with this lungworm had been accomplished, a comparative investigation of the literature of related lungworms was undertaken.

A description of the parasite

The body of the parasite was found to be elongate with the outer body covering or teguminal sheath (Gerichter, 1948) thrown into folds at the anterior end (Fig. 1). On examining the cephalic end from an en face section (Fig. 2), there was observed to be a circular opening, displaying no teeth or papillae. A very thorough search was made under oil immersion for these structures but none could be seen. There were, however, four lip-like structures present. In whole mount slides, a shallow buccal capsule that joined the straight muscular esophagus could be distinguished. The esophagus was short and was observed to be larger at the anterior end than at the posterior. Measurements showed that this structure was 300 micra long by 50 micra wide. The nerve ring was situated 175 micra from the anterior end of the esophagus (Fig. 1). Both the male and female worms were identical at the anterior end. The esophagus was joined, in both male and female, to the simple straight intestine. This structure extended the length of the body and opened on the ventral surface, a short distance from the posterior extremity of the female and at the dorsal ray of the bursa in the male. In the female four unpaired pre-anal papillae were evident.

The body length and width of twenty unmounted mature worms of each sex were measured. The average length of the male worms was 10.9 mm with

with a range of from 10.2 to 12.0 mm. The average male width, excluding the bursal portion, was .22 mm ranging from .19 to .24 mm. The female worms were somewhat larger with an average length of 21.6 mm and ranging from 19.6 to 23.4 mm. The average female width was .36 mm, with a range of from .35 to .40 mm.

The body width of the male diminished in size to an average of 100.8 micra just anterior to the bursa. The bursa of the male, measuring 137.5 micra long by 175.0 micra wide, was small and fan-shaped. From a ventral view the rays of the bursa were paired and symmetrical. The fused ventroventral and lateroventral rays of the bursa were short, adhered closely to the body and were bifurcated at the extremity. Upon measurement with an ocular micrometer, these fused rays were determined to be 30 micra long and the bifurcation began 6.2 micra from the base. Measurement of the fused externolateral and mediolateral rays showed them to be 40.7 micra and 34.5 micra respectively in length, while the bifurcation occured 10.1 micra from the base. The fused posterolateral and externodorsal rays were 42.5 micra and 37.5 micra long and the bifurcation was found to be 12.3 micra from the base. The dorsal ray was single, broad at its origin and tapering caudally to enlarge at its end. It was without bifurcation, and measured 50.3 mm from base to tip. The bursal pattern may be seen in Figure 4. The paired spicules of the male worm were extremely long, delicate, equal in length and provided with striated laminae. They measured from 1.70 to 1.85 mm in length and were 5.6 micra wide. Each spicule was provided with an accessory appendage at the terminal end. These appendages were joined to the spicules 100 micra from the posterior extremity of the spicules, and measured 150 micra in length (Fig. 5). There was a paired gubernaculum present that measured laterally 171 micra in

length and 6.2 in width.

In considering the female reproductive system of the lungworm parasite, whole mount slides and a dissected female were studied. The posterior end of the worm when dissected and floated out in water showed that there was no ovejectoral apparatus and the organs themselves were arranged in a prodelphic didelphic pattern (Fig. 7). That is, the vulva was found situated at the posterior end of the worm just anterior to the anus (Fig. 3 & 6). The vagina ran toward the anterior and ended in paired uteri that coiled about themselves. The uteri extended to the paired oviducts and ovaries. The vulva was .097 mm from the anus.

The uteri of the mature females were filled with embryonated eggs. These ova measured 50.1 to 53.4 micra in length by 25.3 to 27.6 micra in width. The layers of the egg shell were undeterminable but the entire shell measure 3.75 micra thick (Fig. 3 & 6).

These descriptive measurements have been arranged in tabular form in Tables I, II, and III.

Taxonomic Diagnosis:

As a result of the previous description a new genus, Lynx rufus, and a new species, Lynxrufus wilsoni are assigned to the family Metastrongylidae, subfamily Skrjabingylinae.

Lynxrufus, n.g.

Generic diagnosis-Skrjabingylinae; body elongate, cuticle thrown into folds at anterior end. Mouth circular, displaying no

teeth or papillae but four lip-like structures present when viewed from en face view (Fig. 2). Shallow buccal capsule present joining a straight muscular esophagus short and larger at anterior end than at posterior. Bursa of male small and fan shaped; ventroventral and laterolateral rays of bursa short and fused on each side, adhering closely to body and bifurcating at extremity; externolateral and mediolateral rays largely fused on each side, but bifurcate at extremity; fused postero-lateral and externodorsal rays, bifurcation at extremity; dorsal ray single, broad at origin then tapering only to enlarge again at unbifurcating extremity (Fig. 4). Spicules very long, delicate and equal; each provided with accessory appendage at posterior end; spicules provided with striated laminae. Gubernaculum present. Female with slightly pointed tail; vulva located at posterior end of body close to anus (Fig. 3 & 4); no ovejectoral apparatus; didelphic, prodelphic (Fig. 7). Type species: Lynxrufus wilsoni.

Lynxrufus wilsoni, n. sp.

Specific diagnosis-Lynxrufus: Male: 10.2-12.0 mm long. 0.19-0.24 mm wide. Esophagus 300 micra long by 50 micra wide; nerve ring 175 micra from anterior end of esophagus. Bursa 137.5 micra long by 175 micra wide. Bursal rays, with exception of dorsal, in fused groups, each tapering toward extremity and bifurcating near extremity; dorsal ray narrowing from base toward extremity enlarging at extremity; without bifurcation. Fused ventroventral and latero-ventral rays 30 micra long bifurcating 6.2 micra from base; fused

externolateral and mediolateral rays 40.7 and 34.5 micra long, bifurcate 10.0 micra from base; fused posterolateral and externodorsal rays 42.5 micra and 37.5 micra long, bifurcation 12.3 micra from base; dorsal ray 50.3 micra long (Fig. 4). Spicules 1.70-1.85 mm long by 5.7 micra wide measured dorsally; accessory appendages joined to spicules 100 micra from end; accessory appendage 150 micra long (Fig. 5). Gubernaculum, lateral measurement, 171 micra long by 6.2 micra wide. Body proper diminishing in size to 100 micra just previous to bursa.

Female: 19.6-23.4 mm in length by 0.35-0.40 mm in width. Anterior end identical with male. Vulva .097 mm from anus. Four small paired preanal papillae situated longitudinally just anterior to anus on ventral surface (Fig. 3). Internal reproductive organs didelphic prodelphic. Embryonated eggs 50.1-53.4 micra long by 25.3-27.6 micra wide; layers of shell undeterminable but entire shell 3.75 micra thick.

Host: Lynx rufus rufus (Shreber)

Location: Lungs

Geographic distribution: Virginia and North Carolina

TABLE 1. Comparable measurements of the male and female of Lynxrufus wilsoni

	Male	Female
Body length	10.2-12.0 mm Av. 19.9mm	19.6-23.4 mm Av. 21.6 mm
Body width	0.19-0.24mm Av. 0.22mm	0.35-0.40 mm Av. 0.36 mm
Length of esophagus	300 micra	300 micra
Width of esophagus	50 micra	50 micra
Situation of nerve ring from anterior end of esophagus	175 micra	175 micra

TABLE 2 . A tabulation of the measurements of the male reproductive parts of Lynxrufus wilsoni

Reproductive Parts Male	Measurements
Bursa length and width	137.5 X 175.0 micra
Length of fused ventroventral & lateroventral rays bifurcation	30 micra 30 micra 6.2 micra
Length of fused externolateral & mediolateral rays bifurcation	40.7 micra 34.5 micra 10.1 micra
Length of fused posterolateral & externodorsal rays bifurcation	42.5 micra 37.5 micra 12.3 micra
Length of dorsal ray	50.3 micra
Length and width of spicules	1.70-1.85 mm X 5.6 micra
Length of accessory appendage	150 micra
Gubernaculum	171 X 6.2 micra

TABLE 3 . A tabulation of some of the measured female parts of *Lynxrufus wilsoni*

Female Parts	Measurements
Distance from vulva to anus	.097 mm
Length of ova	50.1-53.4 micra
Width of ova	25.3-27.6 micra
Thickness of shell	3.75 micra

PLATE I

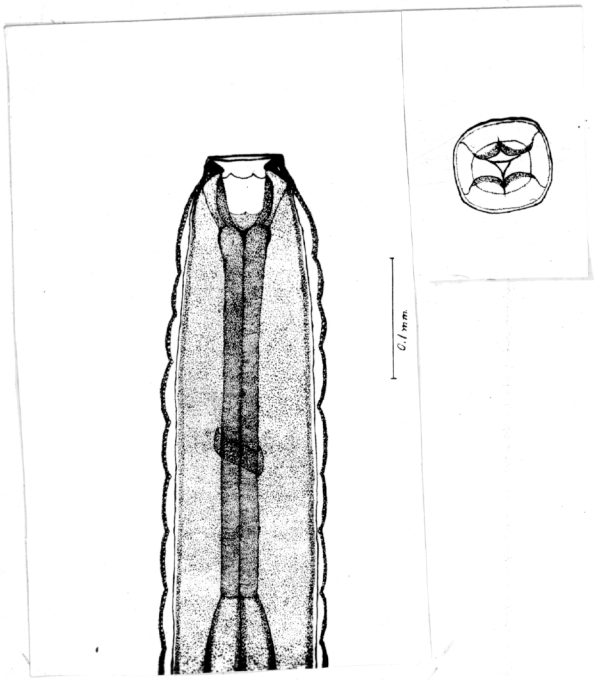

Fig. 2

Fig. 1

Figure 1. Anterior end, showing oral region, esophagus, nerve ring and the beginning of the intestine of Lynxrufus wilsoni.

Figure 2. Lynxrufus wilsoni, en face view of anterior end.

PLATE II

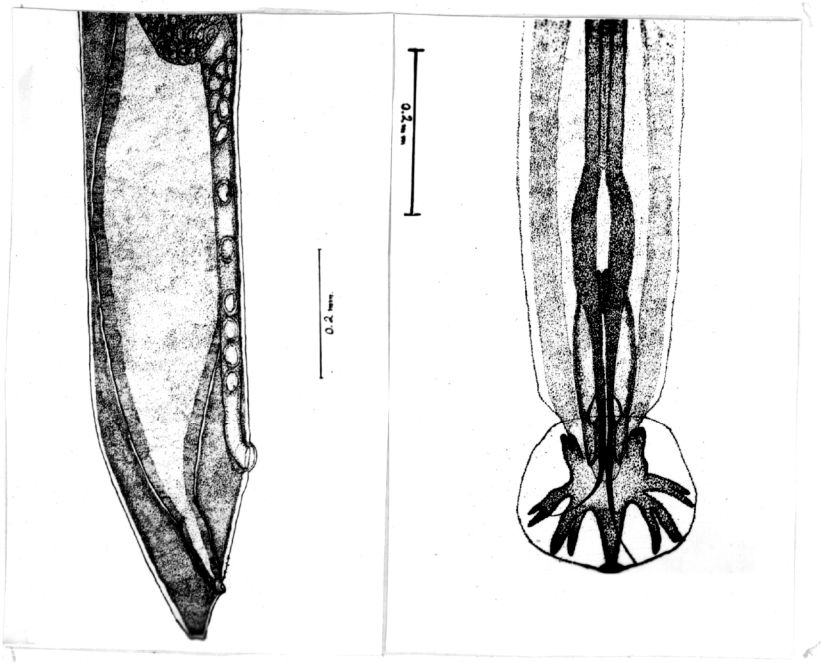

Fig. 3 Fig. 4

Figure 3. Posterior end of female of Lynxrufus wilsoni, ventral view, showing small amount of the egg filled uterus, the vagina, the vulva and the anus with preanal papillae.

Figure 4. Posterior end of male, ventral view, showing pattern of the bursal rays and contracted spicules of Lynxrufus wilsoni.

PLATE III

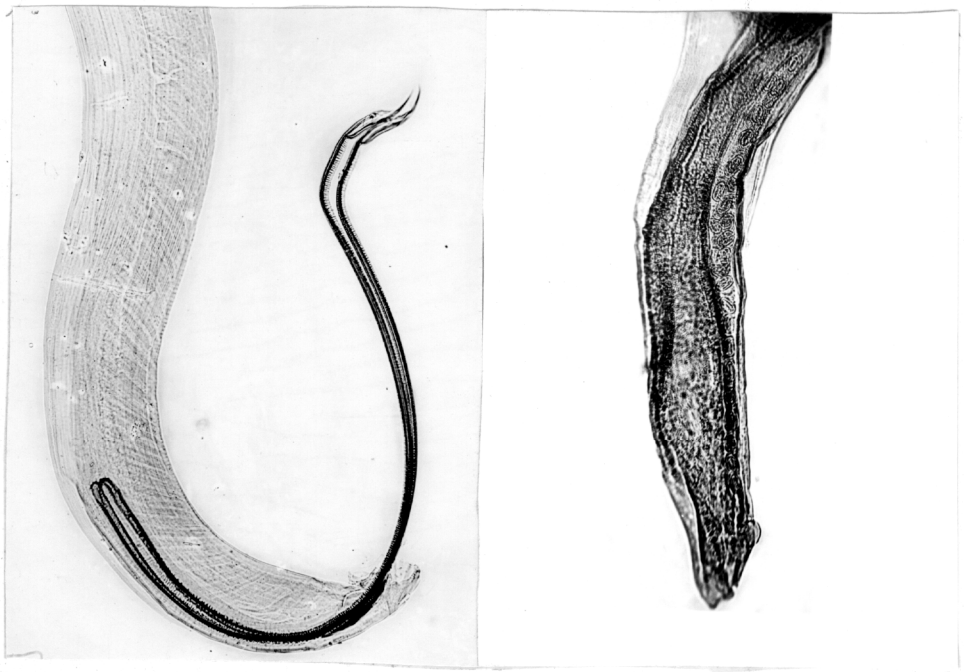

Fig. 5 Fig. 6

Figure 5. Photomicrograph of the posterior end of the male Lynxrufus wilsoni, lateral view, showing spicules, accessory piece and gubernaculum. 100 x.

Figure 6. Photomicrograph of posterior end of female, Lynxrufus wilsoni, showing position of vulva.

PLATE IV

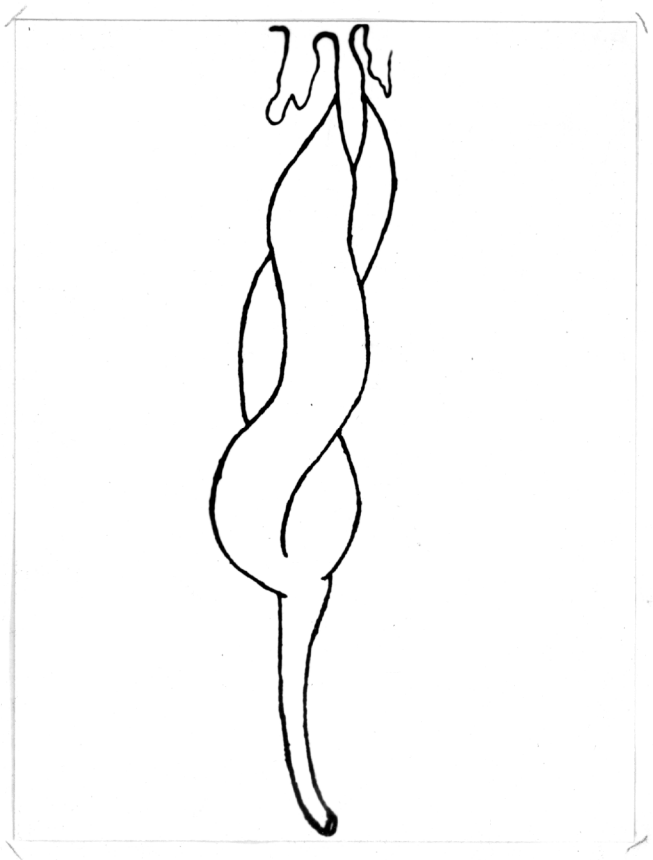

Fig. 7

Figure 7. Diagram of the female reproductive system of Lynxrufus wilsoni.

DISCUSSION OF RESULTS

It seems pertinent at this point to discuss Lynxrufus wilsoni and its position in the family Metastrongylidae. Dr. Ellsworth C. Dougherty, Department of Zoology, University of California, Berkley, California, was kind enough to look over and criticize the primary research on Lynxrufus wilsoni. At that time a female worm had not been dissected to ascertain the anatomy, position, et cetera of the reproductive organs. It was thought that the female was monodelphic. Dr. Dougherty, in a note on this point wrote:

" I think Lynxrufus wilsoni would make a perfect member of the genus Troglostrongylus but for one thing-the remarkable position of the vulva and monodelphy." Further he wrote:

"I should also point out that in the posterior position of the vulva of Lynxrufus is transitional between the rest of the subfamily Skrjabingylinae (Wherein I should place it) and members of the other subfamilies of the Metastrongylidae (except Dictyocaulus). However, the fact of monodelphy of which you apparently are unsure, makes this less significant. This last is such a crucial point that I strongly recommend your dissecting out the terminal female reproductive tract and settling this point once and for all. If Lynxrufus wilsoni is in fact didelphic, it represents a very important transitional form in the Metastrongylidae as I conceive them."

After receiving the foregoing suggestion of Dr. Dougherty's, the posterior portion of a female worm was dissected and proved to be didelphic, as had been indicated in the previous section. Also, there are the facts that the female reproductive organs of Lynxrufus wilsoni run anteriorly from a posteriorly located vulva and there is no ovejectoral apparatus. The genera of the subfamily Skrjabingylinae (Skriabin, 1933) as recognized by Dougherty (1948, 1949) consist of Dictyocaulus, Skrjabingylus, Crenosoma, Otostrongylus, Troglostrongylus,

and Bronchostrongylus. The females of these genera have three things in common; namely; an ovejectoral apparatus, a vulva situated toward the middle of the body and amphidelphy. Dougherty has since modified the Skrjabingylidae by placing Dictyocaulus into a subfamily all its own.

Dougherty (1945) felt in his earlier studies on the evolution of the metastrongylid family that the Skrjabingylinae, because of the position of the vulva and the structure of the ovejectoral apparatus, were a link between the Trichostrongylidae and the family Metastrongylidae (i. e. they had a common ancestor, Strongylina) and he placed the skrjabins under Trichostrongylidae. He has since removed the subfamily Skrjabingylinae to the family Metastrongylidae (Dougherty 1951 b). This change of view was brought about by the work of Gerichter (1948 and 1949) which clearly showed that the morphology of the larval forms of Troglostrongylus brevior were closer to the metastrongylids than to the trichostrongylids. Gerichter further suggested to Dougherty that the position of the vulva near the mid-portion of the body in the skrjabins might be a secondary evolutionary shift from the preanal position found in most of the metastrongylids. Since the male of Lynxrufus rather closely resembles two genera of the subfamily Skrjabingylinae, it is understandable that Dougherty in the previously mentioned communication should feel that the posterior position of the vulva and the didelphy of the female of Lynxrufus wilsoni causes it to become "a very important transitional form in the Metastrongylidae".

The male of Lynxrufus wilsoni resembles two genera of the

subfamily Skrjabingylinae, Bronchostrongylus Cameron, 1931, and Troglostrongylus Vevers, 1923. The males of these three genera have in common (Vevers, 1922, Cameron 1931, Dougherty 1947) a dorsal ray that is a single stalk, a small, though normal, bursa with a full complement of rays, a teguminal sheath that is not thrown into striated annulations and thin delicate spicules with striated laminae. The bursal pattern of the three genera differs mostly in the arrangement and fusion of the paired lateral bursal rays. The externolateral and mediolateral rays are fused in the proximal portion as are the posterolateral and externodorsal in genus Lynxrufus. The externolateral and mediolateral rays are fused in Troglostrongylus but the posterolaterals arise independently. In Bronchostrongylus, the externolateral rays arise independently and the mediolaterals and posterolaterals are partly fused.

It is of interest to note that with one exception, all the species of the previously mentioned genera parasitize the respiratory system of the family Felidae. Troglostrongylus delicatus Travassos, 1946 is that one exception; it is a parasite of the opossum, Didelphis.

The cephalic region of Lynxrufus should certainly be discussed. Dougherty insisted, in the personal communication about Lynxrufus, that papillae could be seen if a slide were properly prepared and viewed under oil immersion. The author has tried many times to see papillae but even with the clearest of en face preparations studied under oil immersion, papillae were not discerned. In a new classification of the suborder Strongylina (Dougherty 1951), the family

Metastrongylidae is described in part as having unfused submedian papillae of the external circle.

CONCLUSIONS

It is concluded that the new genus Lynxrufus should be placed tentatively in the subfamily Skrjabingylinae because of the similarity of the male reproductive organs to those of the genera Troglostrongylus Vevers, 1923, and Bronchostrongylus Cameron, 1931. Further, it is concluded that the genus represents a transitional form between the subfamily Skrjabingylinae and the other subfamilies of the family Metastrongylidae because of the posterior position of the vulva, the lack of ovejectoral apparatus and the paired condition of the female reproductive organs.

Since actually, it has not been ascertained, the author concludes that the cephalic region of Lynxrufus wilsoni may have cephalic papillae but with the technique and ocular equipment at hand, papillae could not be seen.

SUMMARY

Of sixty-four bobcats, Lynx rufus rufus, collected in Virginia and North Carolina, twenty-five percent were found to be infected with an unidentified lungworm.

As the literature showed no record of lungworm parasites from Lynx rufus rufus, a study was made of the lungworms of the cat family, Felidae.

A new metastrongylid genus and species is here described, named and classified. The name is designated as Lynxrufus wilsoni. Lynx-rufus wilsoni is placed tentatively in the subfamily Skrjabingylinae due to the similarity of its male reproductive organs to the male reproductive organs of the other members of that subfamily.

ACKNOWLEDGEMENTS

As one thinks over the work put into a thesis, it becomes very evident that ones' teachers, friends and associates have given more than can be expressed in a page of typewritten words. Therefore, with a great feeling of inadequacy, I should like to pay tribute to some of the many who have made this work possible.

First, I would like to express appreciation to the entire Biology Department. At some time or another every member has helped me. Special appreciation is felt for my graduate committee, whose suggestions, guidance, and judgement have proven sound. Particularly, I would like to thank Drs. Threlkeld, Wilson, and Grayson. I am deeply indebted to Dr. W. L. Threlkeld. Without his confidence and faith in the abilities of his fellow man, I am sure that this work would never have been begun. Of his intelligence, understanding, and energy, he has given unsparingly. To Dr. James M. Grayson, I owe a debt of gratitude. Dr. Grayson has given me, by his ability to simplify the complicated and clarify the confused, a clearer insight into research and study. I am grateful to Dr. I. D. Wilson. He has given encouragement, advice and kindness freely and has been a mainstay in time of need. It is for him that this nematode is named. To these three men, whom I hold in highest respect, I give my most sincere thanks.

The opinions, assistance, and friendship of my fellow graduate students in parasitology, Lucia Sarmiento, Helen Jordan, P. Riggin, Richard Crosby, and Barney Jennings have added immeasurably. To Donald R. Progulske, recently of the Wildlife Unit, I express thanks.

Had he not been curious about the nematodes he found in the lungs of the bobcats and called our attention to them, the research of this thesis would never have been conceived. Also, I wish to express sincerest gratitude to William Spencer Davis for enriching this task by his interest.

I would like to thank Mr. Frank Burleson for his presentation of a paper at a meeting of the Association of Southeastern Biologists. I was unable to attend and Mr. Burleson very kindly agreed to present a short paper of this work at the 1953 meeting of the organization.

Finally, I would like to thank Virginia Polytechnic Institute for giving me the opportunity to study and work.

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