THE UNIVERSITY OF MANITOBA

THE TAXONOMY OF THE LUNG-WORM *Rhabdias* STILES AND HASSALL, 1905 (NEMATODA), PARASITIC IN *Bufo hemiophrys* COPE AND *Rana pipiens* SCHREBER, AND THE INTERSPECIFIC RELATIONSHIP OF HELMINTHS IN THE LUNGS OF THESE AMPHIBIANS

BY

MARTEY OSABU QUAYE, B. SC. (HONS.)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

WINNIPEG, MANITOBA

OCTOBER, 1972

ABSTRACT

The parasitic stage of Rhabdias in the lungs of Rana pipiens and Bufo hemiophrys is parthenogenic. Rhabdias from B. hemiophrys is larger than Rhabdias from R. pipiens. The life cycle of the lung-worms follow similar patterns. Both life cycles are heterogonic (alteration of generation). The heterogonic pattern reported for Rhabdias from R. pipiens is in contrast to the homogonic pattern (direct development) reported by Walton in 1929.

Experimentally second generation infective juvenile Rhabdias from R. pipiens infect B. hemiophrys by skin penetration. Infective juvenile Rhabdias from B. hemiophrys will not infect R. pipiens. For this reason, Rhabdias in R. pipiens is retained as Rh. ranae and that in B. hemiophrys is subjectively described as a variety of Rh. ranae; Rh. ranae var. hemiophrys.

Analysis of the nematode (Rhabdias) and trematode (Haematoloechus) in the lungs of the amphibians show no evidence of an inter-specific relationship between the two helminths.

ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. H. E. Welch, my supervisor and Head, Department of Zoology, University of Manitoba, for his guidance, encouragement and financial support during this study.

Gratitude is expressed to Drs. L. Graham and M. Lankester for assistance they extended in an advisory capacity while Dr. Welch was on sabbatical leave. Thanks are also due to Dr. J. D. McLaughlin for his advice and assistance. My thanks to the staff of the Animal Holding Facilities for looking after the animals in this study.

M. O. Quaye

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

INTRODUCTION

The parasitic adult female nematodes of the genus Rhabdias inhabit the lungs of amphibians and reptiles. Chu (1936a) pointed out the difficulties in the taxonomy of this group stressing that there are few morphological characteristics that can be used to separate species. Chabaud et al. (1961) stressed the state of flux of the systematics of this genus. Twenty-five Rhabdias species are described from amphibians, and of these only 5 species are from North America.

The Type Species, Rhabdias bufonis (Schrank, 1788) was first described from European toads. In 1929 Walton studied the lung nematodes of three North American frogs, Rana pipiens, R. palustris and Acris gryllus. He noted that in size and in the life cycle, the lung worms in the North American frogs differed from the original description of Rh.[1] bufonis. Hitherto, Rh. bufonis was reported in the North American frogs, R. clamitans, and R. palustris (cited from Walton 1929). On the basis of his observations, Walton named the species from the North American frogs as Rh. ranae. Some of his specimens of R. pipiens were from Winnipeg, Manitoba, and Walton also noted that the specimens from the frogs differed from toad specimens in the Winnipeg area.

[1] Rhabdias is abbreviated to Rh. to distinguish it from R. for Rana.

A general survey of helminths in R. pipiens and Bufo hemiophrys during the summers of 1968 and 1969 at the University Field Station, Delta by Hlynka (1970) revealed two types of Rhabdias spp. in the lungs of these two amphibians respectively. Rh. ranae was reported from R. pipiens and Rh. bufonis from B. hemiophrys. As these nematodes were only separated by size differences, and as Walton (1928, 1938) believed Rh. bufonis absent from North America though Fantham and Porter (1948) reported its occurrence in Quebec, further studies are needed to establish the identity of the nematodes.

The purpose of this study were threefold; namely,

1. To confirm the occurrence of Rh. ranae in R. pipiens and to establish the identity of Rhabdias found in B. hemiophrys.

2. To confirm the life cycle of Rh. ranae in R. pipiens and to work out that of Rhabdias species of B. hemiophrys.

3. To investigate the inter-specific relationships of helminths co-existing in the lungs of R. pipiens and B. hemiophrys.

LITERATURE REVIEW

LITERATURE REVIEW

THE GENUS RHABDIAS

A Historical Review of the Genus Rhabdias in North America:- Representatives of the genus Rhabdias frequently occur in the lungs of North American amphibians and reptiles. Although nematodes were reported from the lungs of amphibians in Europe as early as 1788 by Schrank, the first study of these worms in North America was in 1851 when Leidy reported Ascaris entomelas (Rhabdias entomelas) from Rana halecina in the U.S.A. In North America much has been done since Leidy's time, but work on lung nematodes has usually been part of faunal studies. Most research was prior to 1948, but since then little attention was given to the subject.

Stafford (1905) catalogued the nematode species of North America and included Rhabdias bufonis (Schrank 1788), Stiles and Hassell, 1905 as a North American species. Walton (1929) proposed that the European Rh. bufonis may not be present in North America, and that previous records may be due to mistaken identifications. Before Walton's work the only lung nematode of amphibians and reptiles that had been reported were Ascaris entomelas (Leidy 1851) from Rana halecina; Rhabdias serpenticola

(Linstow 1904), from Heterodon platyrhinus; Rhabdias eustreptos (McCallum 1920) Chitwood, 1934 from Lampropeltis getulus florida; Rhabdias vellardi Pereira, 1928, from various snakes and Rh. bufonis from R. clamitans and R. palustris. Walton re-examined fresh specimens of R. clamitans and R. palustris but failed to find Rh. bufonis in their lungs. He described a new species, Rhabdias ranae from the lungs of R. pipiens, R. palustris and Acris gryllus. Walton in a series of papers from 1929 to 1941 increased the number of known hosts for Rh. ranae. Tables (I) and (II) list the known species of Rhabdias, their hosts, and localities.

TABLE I

Rhabdias Species of World Amphibians Modified after Yamaguti 1961

Species	Hosts	Locality
1. *Rh. bufonis (Schrank 1788)	*Bufo* spp.	Europe
syn. *Ascaris nigrovenesa*		
Geoze 1800	*Rana* spp.	Sibera
Rhabdonema n. (G)	*Pelobates* sp.	China
Angiostomun n. (G)	*Bombinator* sp.	Canada
Leptodera n. (G)	*Angus fagilis*	U.S.A.
2. *Rh. bdellophis* Baylis 1929	*Bdellophis vittatus*	Africa
3. *Rh. bicornis* Lu 1934	*Bufo bufo asiaticus*	China
4. *Rh. brachylaimus* (Linstow 1903)	*Bufo melanosticus*	Siam
5. *Rh. elegans* Gutierrez, 1945	*Bufo arenarum*	Argentina
6. *Rh. entomelas* (Leidy 1851)	*Rana helecina*	U.S.A.
7. *Rh. escheri* Baer, 1930	*Uraeotyphlus oxyurus*	India
8. *Rh. fülleborni* Travassos 1926	*Bufo marinus*	Brazil
	Leptodactylus pentadactylus	Salvador
	Rana spp.	Guatemala
	Bufo horribilis	Guatemala

*North American Reports.

Table I cont'd,

Species	Hosts	Locality
9. Rh. globocephala Kung and Wu 1945	Microhyla ornata	China
10. Rh. halae Johnston & Simpson 1943	Hyla sp.	Austrailia
	Limnodynastes sp.	Australia
11. Rh. incerta Wilkie 1930	Bufo vulgaris japonicus	Japan
	Rana spp.	China
12. *Rh. joaquinensis Ingles 1936	Rana aurora	California
13. Rh. madascariensis Chabaud et al. 1961	Rana madascarensis	Madagascar
14. Rh. microotis Semenow 1929	Rana temporaria	USSR
	Bufo bufo	USSR
	Bufo viridis	USSR
15. Rh. montana Yamaguti 1954	Rana temporaria	Japan
16. Rh. multiproles Yuen 1965	Rana cancrivora oranativentris	Malaya
17. Rh. nipponica Yamaguti 1935	Rana rugosa	Japan
syn. Rh. bufonis Matuda (1939)	R. nigromaculata	Japan
	R. güntheri	China
	R. limnocharis	China
18. *Rh. plethodontis Chitwood 1933	Plethodon cinereus	Virgina
19. Rh. Polypedatis Yamaguti 1941	Polypedates buergeri	Japan
20. *Rh. ranae Walton 1929	Rana pipiens	N. America
	R. palustris	N. America
	R. catesbiana	N. America

Table 1 cont'd,

Species	Hosts	Locality
	R. halecina	N. America
	R. clamitans	N. America
	R. sylvatica	N. America
	R. sphenocephala	N. America
	Hyla squirella	" "
	Bufo terrestris	" "
	B. fowleri	" "
	Acris gryllus	" "
	Pseudacris brimleyi	" "
	Scaphiopus holdbrokii	" "
21. Rh. rhacophori Yamaguti 1941	Rhacophorus schlegeli var. arborea	Japan
22. Rh. rotundate (Linstow 1906	Bufo viridis	Europe
23. Rh. rubrovenosa (Schneider 1866)	Bufo cinereus	Europe
	Pelobates sp.	Europe
	Rana spp.	Europe
24. Rh. sphaerocephala Goodey 1924	Bufo vulgaris	Europe Costa Rica
	Bufo marinus	Vera Cruz
	B. horribillis	Chiapas
25. Rh. tokyoensis Wilkie 1930	Diemyctylus pyrrhogoster	Japan

TABLE II

Rhabdias Species of World Reptiles. Modified after Yamaguti 1961

Species	Hosts	Locality
1. Rh. annulosa Hsu 1933	Zoacys dhumnades	China
Relagated to subspecific rank of fuscovenosa	Naja sp.	China
	Holarchus spp.	China
2. *Rh. eustreptos (McCullum 1921) Chitwood 1934	Lampropeltis getulus floridana	U.S.A.
3. Rh. fuscovenosa (Railliet, 1899)	Tropidonotus natrix	Europe
	Agkistrodon halys brevicaudatus	China
	Naja nivea	S. Africa
	Sepedon haemachata	S. Africa
	Bitis arietans	S. Africa
4. *Rh. fuscovenosa var. catanensis (Rizzo 1902)	Coluber viridiflavus	Catania
	Tropidonotus natrix	Sicily
	Thamnophis sirtalis	China
	Natrix sipedon	China
	Thamnophis sp.	U.S.A.
	Storeria sp.	U.S.A.

Table II cont'd,

Species	Hosts	Locality
	Lampropeltis sp.	U.S.A.
	Liopeltis sp.	U.S.A.
	Natrix sp.	U.S.A.
	Elepha sp.	U.S.A.
	Coluber sp.	
5. Rh. fuscovenosa var. brevicauda Chu 1936 syn. R. fuscovenosa Hsu et Hoeppli 1931	Green snake	China
6. Rh. gemellipara Chabaud 1961 et al.	Chamaelea parsonii	Madagascar
7. Rh. horigutii Yamaguti 1943	Natrix tigrina	Japan
8. Rh. labiata Pereira 1927	Rhabinaea merrimi	Brazil
9. Rh. ophidia Goodey, 1924	Coluber leopardinus	London-Zoo
	Drymobius bifossatus	
	Natrix trigina	China
10. * Rh. sepentiocola (Linstow 1904)	Heterodon platyrhinus	N. America
11. * Rh. vellardi Pereira 1928	Philodryas schotti	Brazil
	Oxyrrhopus trigeminus	Brazil
	Agkistrodon bilineatus	Guatemala
	Heterodon sp.	Texas
	Storeria sp.	Texas
	Potomophis sp.	Texas
	Thamnophis sp.	Texas

TABLE III

Comparative Data on Nematodes of the Genus Rhabdias from Snakes

All measures are in mm. (Modified after Chu, 1936a)

Present	Rh. fuscovenosa (Railliet 1899) and Hassall 1905	Rh. (Strongylus) catanensis (Rizzo 1902) Chitwood 1933	Rh. ophidia Goodey 1927		Rh. annulosa Hsu 1933
Suggested Name	Rh. fuscovenosa fuscovenosa	Rh. fuscovenosa brevicauda	Rh. fuscovenosa catanensis	Rh. fuscovenosa ophidia	Rh. fuscovenosa ophidia	Rh. fuscovenos annulosa
Authority for data given	Railliet 1899 Goodey 1924	Hsu and Hoeppli 1931	Rizzo 1902	Goodey 1924	Hsu and Hoeppli 1931	Hsu 1933
Locality	England and France	China	Sicily	London (Z00)	China	China
Host	T. natrix	"Green snake"	T. natrix	Coluber leopardinus	Natrix tigrina	Zaocys dhumnade
Body length	3 - 6	5.27	3 - 4	6.0 - 6.5	4.65 - 4.98	5.65-6.30
Body width	0.15 - 0.19	0.27	0.18	0.2 - 0.25	0.18 - 0.186	0.25-0.31
Distance of nerve ring from anterior	Midway of oesophagus	0.145	---------	0.2	0.14 - 0.143	0.146-.16

Table III cont'd,

Distance of excretory pore from anterior end	.....	0.225	.....	.....	0.222-0.253	0.212-0.260
Oesophagus length	0.27	0.336	0.23	0.3 - 0.33	0.312-0.328	0.310-0.320
Distance of vulva from anterior end	Anterior half of body	2.66	.....	3.0 - 3.2	2.05-2.23	2.29-2.56
Tail length	0.11	0.092	0.23	0.27 - 0.30	0.34-0.35	0.153-0.179
Egg length	0.079 - 0.085	0.6 - 0.068	0.100	0.098 - 0.1	0.07-0.073	0.063-0.068
Egg width	0.045 - 0.048	0.033 - 0.04	0.060	0.04 - 0.041	0.037-0.048	0.03-0.037

Ingles (1936) described a new species, Rhabdias joaquinensis from the lungs of Rana aurora from California. He stressed that this new species could be distinguished from all other described species of the genus except Rhabdias ranae, Walton 1929, which it closely resembles in size, vulva position, oesophagus length and egg size. It differs from Rh. ranae in that it has only one pair of post-anal ventro-lateral papillae instead of the two pairs, and in the more anterior portion of the nerve ring. The same author reported finding another species of Rhabdias from the lungs of Rana boyli, Bufo boreas, and Triturus torosus. He did not describe this as a new species as he could not identify it with certainty.

In 1948 Fantham and Porter reported Rh. bufonis from the lungs and alimentary canal of Rana catesbieana, from Fourth Lake, Gaspé Country, Province of Quebec. In the summers of 1968 and 1969, Hlynka (1970) reported Rh. ranae from R. pipiens and Rh. bufonis from B. hemiophrys. He considered the possibility of these two nematode species being one and the same as their size ranges overlapped.

SYSTEMATICS

Parthenogenic Adult Female (In the Lungs):- The parasitic generation of Rhabdias is much larger than the free-living generation. Six small lips surround the mouth, and may bear

lateral flanges which are broader anteriorly than posteriorly. Buccal capsule short and cup-shaped. The short cylindrical oesophagus ends in a club-shaped swelling posteriorly. Tail conical. Female reproductive tract consists of a vulva, a single and usually short vagina, a pair of uteri, oviducts and ovaries. Uteri are amphidelphic. Oviparous; eggs with a thin shell and containing fully developed juvenile or a morula at deposition.

Free-Living Generation:- Yamaguti (1961) gave the characteristics of the free-living generation as:- Sexes separate. Body fairly stout. Mouth without lips. A short buccal capsule present. Oesophagus with a fusiform prebulbar swelling and a pyriform posterior bulb.

Male:- Tail conical, with a short terminal spike and narrow lateral alae. Four pairs of preanal and three pairs of post anal papillae, all lateral in position. Spicules equal, short and stout. Gubernaculum apparently absent.

Female:- Tail conical, vulva somewhat behind middle of body. Uteri branches opposed. Eggs few, large. Embryo hatching *in utero*, retained until death of female.

According to Chabaud *et al.* (1961) the systematics of *Rhabdias*, is always changing. Twenty five species have been

described from amphibians and eleven from reptiles (Tables I and II). The three features most frequently used in the differentiation of species were the location of the nerve ring, length of oesophagus, and tail length. Chu (1936a) regarded the size of the worm as an unreliable diagnostic character because of its variability and noted that few distinct morphological characteristics exist between species. In a review of the Rhabdias parasitic in reptiles, Chu attributed the confusion in classification to (i) the absence of the free living male; (ii) the dearth of distinct morphological differences in the parasitic female, (iii) the inadequate descriptions of certain forms, and (iv) the incomplete information on the life history with the resulting lack of knowledge on the morphology of the free-living generation. The most commonly used characteristics to distinguish these species are the size of the worms, the position of the reproductive organs, the relative number of eggs in the uteri, the shape and length of the tail, the shapes of the oral opening and buccal cavity. Chu (1936a) showed that the host and the size of the worms are unsatisfactory bases for setting up species. He illustrated his point in a table which I have modified (Table III).

Chu concluded from the data of Table III that Rh. fuscovenosa (Railliet, 1899); Rh. catanensis (Rizzo, 1902) and Rh. annulosa Hsu 1938 are probably the same species and that the differences between them probably were only varietal or subspecific differences.

Chabaud et al. (1961) pointed out that species which were thought to be of restricted geographical distribution have been reported in other places and in different hosts. For example Williams (1960) and Bravo-Hollis and Caballero (1940) reported Rh. sphaerocephala in B. marinus from Bermuda and Vera Cruz, Mexico, respectively. Rh. sphaerocephala was originally described by Goodey (1924b) in the common British toad, B. vulgaris. Walton (1929) described Rh. ranae in R. pipiens, R. palustris and Acris gryllus. Since then many hosts were reported for Rh. ranae in North America. Gupta (1960) also reported the presence of Rh. ranae in Rana tigrina from East Pakistan (now Bangladesh).

Due to the emphasis on the characteristics mentioned previously in differentiating between species, much controversy exists in the literature as to what each species should be called. Rhabdias incerta Wilkie 1930 was reported also by Lu (1934) in some Nanking amphibians, R. japonica, R. nigromaculata, and R. plancyi. Lu (1934) noted that specimens from

these amphibians were much smaller and had longer tail lengths. Even in the various amphibians, he noted differences between species from host to host. Yamaguti (1941) pointed out the possibility of Rh. incerta of Lu (1934) as being synonymous with Rh. nipponica Yamaguti 1935. Rh. polypedatis in Polypedates burgeri was described as a separate species from Rh. nipponica by Yamaguti (1941) chiefly on the basis of egg size. He also separated Rh. rhacophori in Rhacophorus schlegeli var. arborea from a more closely related species, Rh. tokyoensis, by the much smaller buccal capsule and much shorter oesophagus.

Species found in different hosts were often described as new species because of the lack of distinct morphological differences. It is also possible that new species may have been due to mistaken identification. Thus Rh. entomelas; (Leidy 1851) nec. Duj., 1845 reported in R. halecina was synonymised with Rh. ranae Walton 1929.

LIFE HISTORY OF RHABDIAS

Adult parasitic Rhabdias females are found in the alveolar tissues of the lungs. The one exception is the report of Kurochkin and Guskov (1963) of mature females of Rh. martinoi in the eyes of Natrix natrix. Parasitic adults have eggs in their uteri, and lay these in the alvoelar spaces. At ovi-

position, the eggs are embryonated containing fully developed active juveniles. Some of these eggs have been reported to hatch in the lung tissues. Goodey (1924b) reported the recovery of large numbers of Rh. sphaerocephala juveniles when the lung cavities were hatched out with normal saline. Walton (1929) noted that the eggs of Rh. ranae usually were embryonated at the time of oviposition and hatch into rhabditoid juveniles in the lungs and upper end of the alimentary canal of the host. Chu (1936b), mentioned that eggs of Rh. fuscovenosa var. catanensis hatch readily when they are fully developed. He stressed that hatching does not seem to take place in the lungs in the case of Rh. fuscovenosa var. catanensis, because careful dissection of infected snakes revealed no first stage juveniles free in the lungs, trachea, or upper part of the alimentary canal. The eggs hatched immediately after contact with physiological salt solution or water, and according to Williams (1960) this might easily mislead "one into thinking larvae had been recovered". Williams (1960) working on Rh. sphaerocephala from Bufo marinus, repudiated Goodey's (1924b) idea and stated that eggs containing fully formed embryos hatched within six hours when placed in saline solution. Under natural conditions most eggs reach the mouth in an unknown manner, then are swallowed, and hatch only when they are evacuated from the gut. Other reports suggested that

the hatching of the eggs occurred in some part of the alimentary canal. Thus Chu (1936b) reported newly hatched juveniles and eggs in various states of development in the faeces of Thamnophis sirtalis and Natrix sipedon infected with Rh. fuscovenosa var. catanensis. Juveniles were often dead. Goodey (1924b) reported numerous rhabditiform juveniles in the intestinal contents of grass snake. Groups of eggs were found in food masses in various locations throughout the intestinal tract of Bufo marinus by Williams (1960). He reported some instances of hatching in the rectum, cloaca, and occasionally the lower ileum.

Prior to the breaking of the thin egg shell, the juveniles move vigorously inside the eggs. When the juvenile breaks the egg shell, its head emerges first followed by the rest of the body. Chu (1936b) reported that the newly hatched juveniles of Rh. fuscovenosa var. catanensis feed almost immediately. Walton (1929), Goodey (1924a), Chu (1936b), Williams (1960), Chabaud et al. (1961) and Yuen (1965) agree that first stage juveniles are rhabditiform.

As in Strongyloides, both homogonic and heterogonic types of development may occur in the free-living phase of the life cycle of Rhabdias. In most of the species one type or the other strongly predominates or may occur exclusively. Walton, (1929) reported the development of infective juveniles of

R. ranae from the eggs of the parasitic generation without the interposition of a free-living sexual generation in amphibians. Goodey (1924a) also reported this same type of development for Rh. fuscovenosa in a reptilian species. Such direct development was also reported for Rh. ophidia (Goodey 1924a), and Rh. sphaerocephala (Williams 1960). Chu (1936a) reported his own and unpublished observations of Chitwood that both types of development occur in several amphibian and reptilian species, (Rh. ranae; Rh. eustreptos, Rh. fullerborni, and Rh. fuscovenosa var. catanensis). Chu observed only homogonic development for Rh. fuscovenosa var. catanensis except when an especially favourable culture medium was used, and then a small percentage of free-living adults predominantly males, were usually found. He concluded that the direct type of development predominated and that a few free-living males and females failed to infect snakes. Recently, Chabaud et al. (1961) and Yuen (1965) showed that only the indirect type of development is present in Rh. madagascariensis, Rh. gemellipara and Rh. multiproles, Rh. brachylaimus respectively. Chandler (in Christie 1952) concluded that the type of development of Rhabdias is determined by factors similar to those operating in the case of Strongyloides.

The third stage juveniles of the homogonic cycle infect their hosts. Walton (1929) demonstrated the penetration of

Rh. ranae third stage infective juveniles to Rana pipiens and Rana clamitans. Williams (1960) also showed that the filariform juveniles of Rh. sphaerocephala infected Bufo marinus, and a tree frog, Eleuthrodactylis johnstonei. He showed that presence of numerous tufts of cast nematode cuticle were seen on the skin of the host animals after penetration by the infective stages. Chu (1936b) successfully infected snakes with the infective stage of Rh. fuscovenosa var. catanensis by both feeding and subcutaneous infections. He obtained negative results when he applied infective stages to the skin and beneath the scales of snakes. According to Goodey (1924a) the homogonic juveniles of Rh. fuscovenosa undergo two ecdyses outside the body of the host, the second shed cuticle being retained as a tight-fitting sheath for the infective juveniles. The sheath is shed upon entry to the host. Although the infective stages of Rh. bufonis were reported by Fulleborn (1920) (in Christie 1952) to penetrate the skin, and the same writer (1928) also described migration to the lungs via the circulatory system, Goodey (1924a) failed to get the infective juveniles of Rh. fuscovenosa to penetrate skin, though their behavior outside of the body was like that of skin-penetrating juveniles. Goodey suggested that the infective juveniles migrate to the lungs after penetrating the gut wall, by direct migration through the mesentery and not via the blood stream. Walton

(1929) also found that infective stages of Rh. ranae penetrate the body cavity of the host, and eventually move into the lungs via the mesenteries. Chu (1936b) confirmed Goodey's (1924a&b) observations, and suggested that the infective stages work their way to the lungs through the connective tissues and body cavities of snakes rather than by passive carriage in the blood stream. Williams (1960) suggested that the route to the lungs by Rh. sphaerocephala in some instances may be via the venous circulation through the right half of the heart. He also suggested that worms might reach the lungs by a direct migration without aid of blood for transportation.

Fulleborn (1928) stressed the fact that juveniles of Rh. bufonis could penetrate snails and other invertebrates, where they remain unchanged (paratenic hosts) for weeks, capable of infecting a frog when the snail is eaten. Similarly, juveniles may become encapsulated parenterally in frogs which eat them. Fulleborn suggested that as the skin of snakes is hard to penetrate, transport hosts may constitute the principal method of infection for these hosts. Chu (1936b) infected animals other than snakes with the first generation juveniles of Rh. fuscovenosa var. catanensis; tadpoles, snails and Cyclops fed actively on the juveniles. Infective juveniles were also fed to adult frogs, R. pipiens and R. clamitans, a toad Bufo americanus, and a painted turtle Chrysemys marginata.

Neither tissue penetration nor development occured in any of these hosts. Snails and tadpoles had live juveniles in the posterior end of their digestive tracts twenty-four hours after feeding. Chu concluded that snakes might possible acquire infection after they had ingested infective juveniles of Rh. fuscovenosa var. catanensis. This confirms Fülleborn's (1928) work.

MATERIALS AND METHODS

MATERIALS AND METHODS

COLLECTIONS

Rana pipiens and *Bufo hemiophrys* were collected at the University of Manitoba Field Station, Delta, Manitoba. These amphibians were usually collected in the afternoon along roadside ditches, meadows, and the beach of Lake Manitoba. The amphibians were collected with the aid of a large looped dip net of nylon mesh. The frogs were held in an aquarium containing sufficient water to cover the base; the toads were put in another container and both transferred to the laboratory. Collections were made twice a month between May and August, 1971.

An attempt was made to collect two sizes of each amphibian. These were arbitrarily selected as follows:-

For *R. pipiens*		and	*B. hemiophrys*	
	Male	Female	Male	Female
A.	Up to 70mm	Up to 70mm	Up to 45mm	Up to 45mm
B.	Over 70mm	Over 70mm	Over 45mm	Over 45mm

LABORATORY EXAMINATION OF AMPHIBIANS

In the laboratory the amphibians were measured, and then killed by pithing. Dissections were made of the following organs; right and left lung, the complete alimentary tract; and the peritoneal cavity. The organs were placed in tap water in separate petri-dishes. Lungs were dissected and nematodes and trematodes removed. The alimentary tract was examined for eggs and juvenile stages of Rhabdias. The peritoneal cavity was washed out with tap water and examined carefully for Rhabdias.

PROCESSING OF NEMATODES AND TREMATODES

Killing of Nematodes:- Nematodes recovered from the lungs and peritoneal cavities were placed in a drop of water on a plain microscope glass slide. The slide was held on a hot plate for about 5-6 seconds. Nematodes were watched carefully during heating and were immediately removed when they straightened out.

Fixing and Processing of Nematodes:- Nematodes were processed in two ways. Firstly, they were processed by a modification of the methods of Seinhorst (1959, 1962) and of Goodey (1963). Nematodes were fixed in formalin-acetic acid solution 10:4 for at least 12 hours. After fixation, the nematodes were transferred to a petri-dist (A) containing 3ml. of a mixture of 20 parts of 96% ethanol, 1 part glycerin, and 79 parts of distilled water. The container

with the nematodes was placed in a closed petri-dish (B) containing excess of 96% ethanol. This was kept at 35° - 40°C for at least 12 hours. After this, a solution containing 5 parts glycerin in 95% ethanol was added to the petri-dish, (A) containing the nematodes. The petri-dish (A) was then placed in a partly closed petri-dish (C) and kept at 40°C until all the ethanol evaporated. This took at least 3 hours. Nematodes were then mounted in anhydrous glycerine and ringed with glyceel. Secondly, the fixed nematodes were transferred to 10% of 70% alcohol glycerin mixture in a petri-dish which was partly covered. The nematodes were left until all the alcohol evaporated, leaving them in pure glycerine. Nematodes were mounted in anhydrous glycerine and ringed with glyceel.

Measurements and Drawing of Nematodes:- Outlines of nematodes were made with the aid of a camera lucida. The extent and positions of the various organs were marked. A piece of string was fitted to the outlined. This was measured against a meter rule.

Trematodes:- Trematodes from the lungs were flattened between two glass slides. They were counted and preserved in 5% formalin solution.

LIFE CYCLE STUDIES

Examination of lungs and alimentary tract:- The lungs of R. pipiens

and B. hemiophrys were carefully dissected, washed out with tap water and examined for eggs of Rhabdias. The same treatment was given the buccal cavity, oesophagus, stomach intestine, rectum and cloaca.

Culture Techniques:- Culture chambers were constructed after Chu (1936b), Fig. 1. Standard glass microscopic slides were covered with dental wax.[1] Chambers were cut out from the middle of the covered slides. The chamber had inner measurements of 3.0 x 1.0 x 0.5cm and other measurements of 7.5 x 2.5 x 0.5cm.

[1] Red wax square ropes; size 3/16 in. sqr. 11 inches long. Manufactures by Modern Materials Mfg. Co. St. Louis, Mo., U.S.A.

Fig. 1

Culture chamber

Explanation of abbreviations in Fig. 1

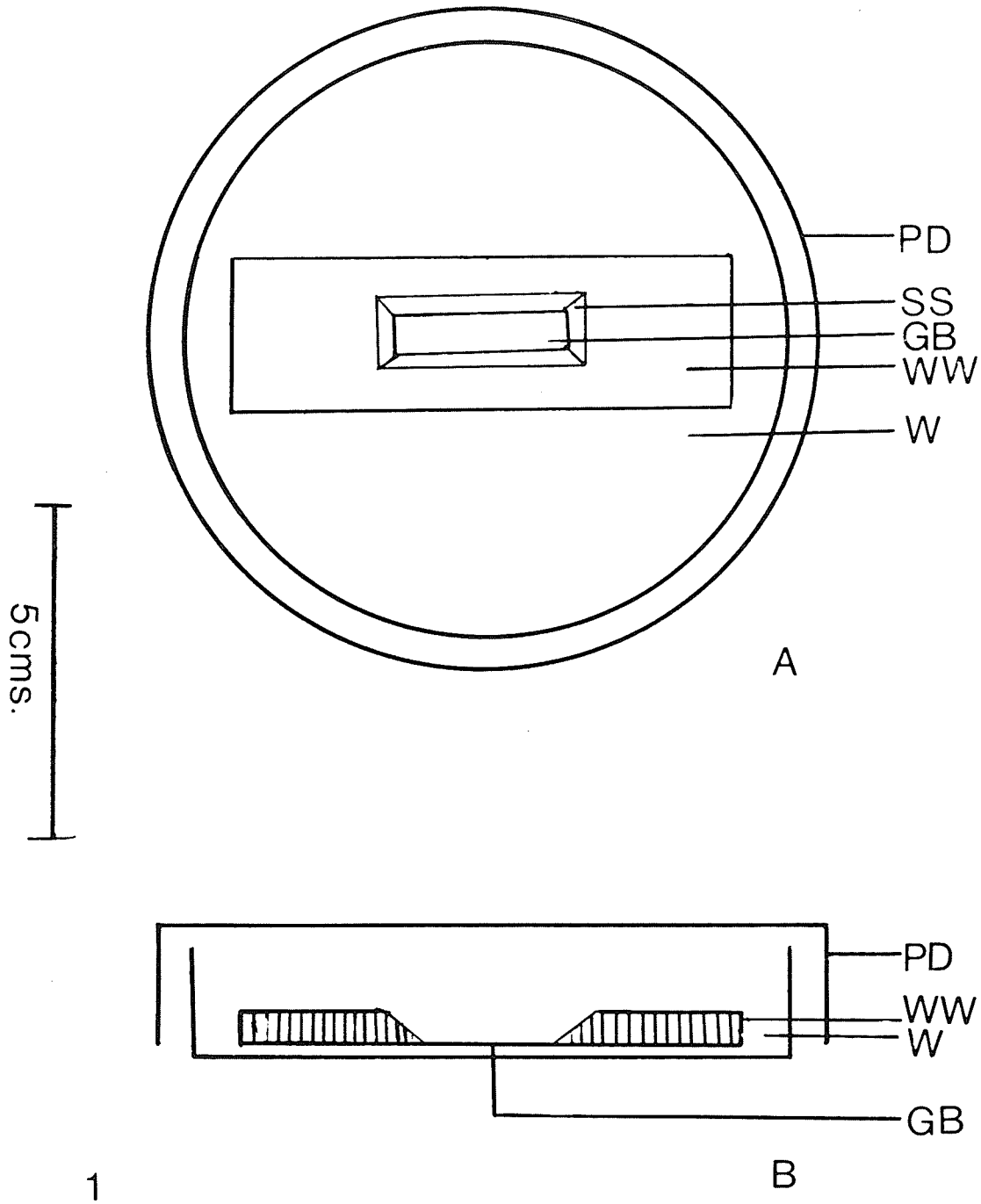
PD ..... Petri-dish

SS ..... Slanting side

GB ..... Glass bottom

WW ..... Wax wall

W ..... Water



1

CULTURE CHAMBER

Autoclaved frog and toad faecal material moistened with tap water was placed in the chambers and eggs from adult parasitic female Rhabdias were added to the faecal material. The chambers were then placed in petri-dishes. Water was added to the petri-dishes in such a manner that its level was half that of the chamber. The petri-dishes were covered. This assured that all surfaces of the chambers were moist. The cultures were left at room temperature (21.5 - 22.5°C) and examined under a binocular microscope for developing stages of the nematodes.

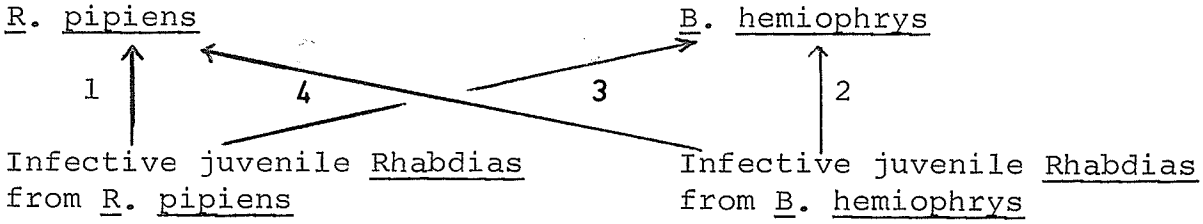
At various times samples were taken from the chambers for measurements and drawings after they had been killed by gentle heat. Samples were also studied alive in water. After 10 days, the infective stages were selected from the culture and these were used to establish infections in the amphibians.

Infection Experiments:- Experiments to determine the infectivity of filariform juveniles to R. pipiens and B. hemiophrys were conducted by the following tests:

1. Uninfected R. pipiens were exposed to third-stage infective juvenile Rhabdias from R. pipiens.
2. Uninfected B. hemiophrys were exposed to third-stage infective juvenile Rhabdias from B. hemiophrys.
3. Uninfected B. hemiophrys were exposed to third-stage infective juvenile Rhabdias from R. pipiens.

4. Uninfected R. pipiens were exposed to third-stage infective juvenile Rhabdias from B. hemiophrys.

The experiments can be represented thus:-



Experimental animals (frogs and toads) were placed in a bottle containing infective juveniles, sand and sufficient water. These animals were held in position by closing the opening of the glass bottle with cheese cloth. These were left up to a maximum of 2 days. At the end of the first day some of the experimental animals were sacrificed and the body cavity and lungs were examined for various stages of nematodes. Others were sacrificed at the end of 2 days. The rest were removed from the experimental glass-ware fed and the faeces passed (out by them) were examined from time to time.

All the experimental animals were known to be free from Rhabdias infection as determined by faecal examination for at least 10 weeks prior to the experiments.

RECORD KEEPING

All nematodes and trematodes encountered in the lungs were counted. The species, locality, date collected, sex, length,

class, helminths in the lungs, nematodes in the peritoneal cavities, and dimensions of the nematodes were tabulated in a set of data sheets (Appendix I and II).

RESULTS

RESULTS

THE DEVELOPMENT OF PARASITIC AND FREE-LIVING STAGES OF *RHABDIAS* FROM *R. pipiens* and *B. hemiophrys*

Early Developmental Stages in Naturally Infected Hosts:- Embryonated eggs were laid in the lungs by parasitic parthenogenetic females. No hatching was observed to occur in the lungs. The embryonated eggs were passed up the trachea to the buccal cavities of the amphibians where they were swallowed and ultimately passed to the rectum. Examination of alimentary canal showed that many of the embryonated eggs hatched while passing through the alimentary canal. A few eggs however did not hatch until evacuated from the rectum with the faeces. First stage juveniles were abundant and accumulated in the rectum. Development did not proceed beyond the third stage until they were passed out with the faeces.

Development of Free-Living Rhabdias in Laboratory Culture:- The development of *Rhabdias* from the lungs of both *R. pipiens* and *B. hemiophrys* was similar in laboratory faecal cultures. For this reason the description of the development of *Rhabdias* from both hosts are combined for convenience and the few minor differences indicated.

Embryonated eggs from the uteri of parasitic parthenogenetic

females hatched within a few minutes (10 - 30 mins.) in culture. First stage rhabditiform juveniles emerged from the eggs (Fig. 2). It was observed that moulting occurred approximately every six hours so that in about 24 hours free-living males and females were observed in the culture (Fig. 3, 4, 7 & 8).

Ratio of Free-Living Males to Females:- Samples were taken after 24 hours to determine the ratio of free-living females to males. Twenty samples were taken and in each there were approximately 20 worms. These were then killed by gentle heat and mounted under vaseline-ringed cover slips and the free-living males and females were counted using a Wild M-20 microscope. The ratio obtained were:

	Male	:	Female
Free-Living adult Rhabdias of R. pipiens	1	:	10
Free-Living adult Rhabdias of B. hemiophrys	4	:	10

Copulation of Free-Living Males and Free-Living Females:- Soon after free-living adults were found, males copulated with females. Males wrapped the posterior parts of their body around the vulva region of females. It was observed that sometimes males copulated with more than one female. Copulation normally lasted between one to two minutes. After copulation males became quiescent and died after 24 hours.

Figs. 2 - 6 Free-Living Rhabdias in R. pipiens

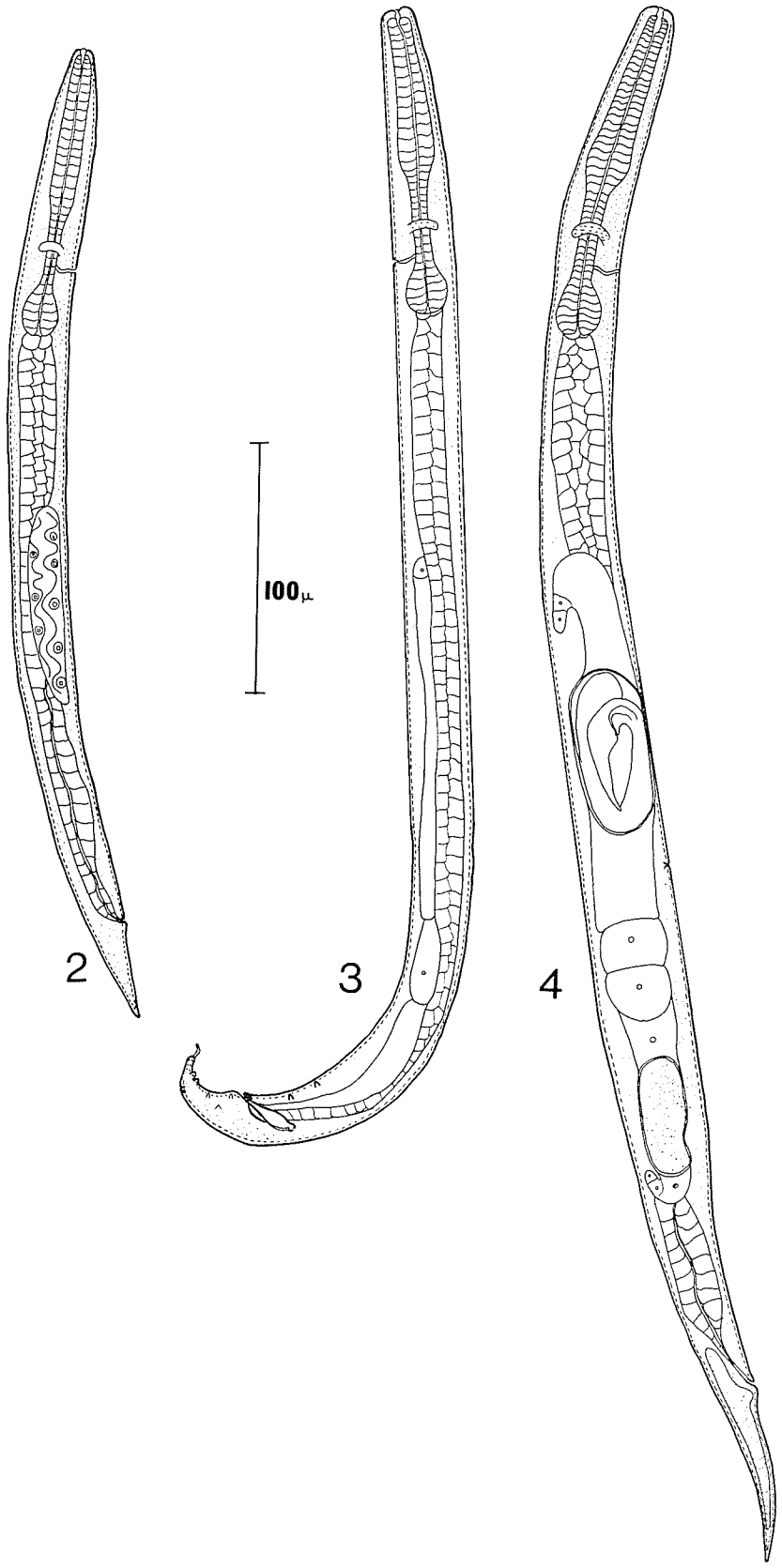
Fig. 2. First stage rhabdiform juvenile

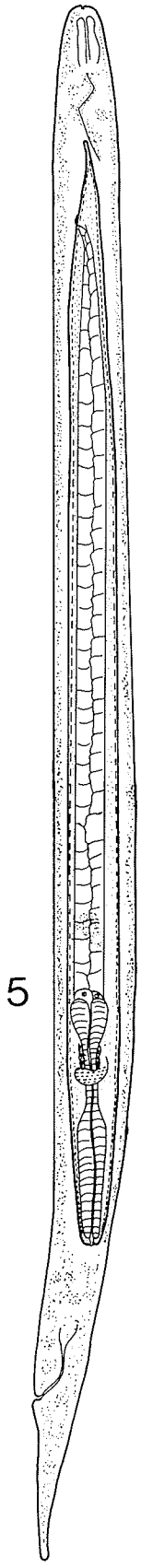
Fig. 3. Free-living male

Fig. 4. Free-living female with embryonated egg

Fig. 5. Free-living female combining 2nd. stage rhabditiform juvenile

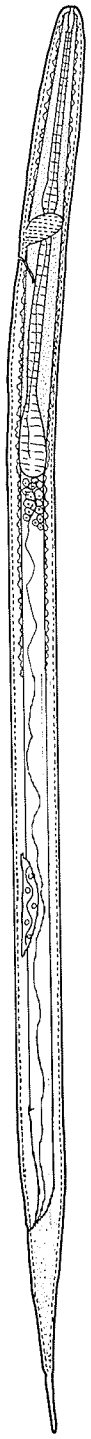
Fig. 6. Infective juvenile.


100μ
2
3
4



100μ

5



6

Further Development in Free-Living Females:- Inseminated females developed embryonated eggs within their uteri (Fig. 4 & 8). There were marked differences between the number of embryonated eggs produced by free-living female Rhabdias of R. pipiens and that of B. hemiophrys. Free-living female Rhabdias of R. pipiens developed one embryonated egg while B. hemiophrys developed three or four embryonated eggs:- occasionally one or two developed (Fig. 8). Normally the embryonated egg in the free-living female Rhabdias of R. pipiens hatched, while two or three embryonated eggs hatched in the case of Rhabdias of B. hemiophrys.

The hatched juveniles which were apparent after 48 hours were rhabditiform. They were similar to the first stage rhabditiform juveniles of the parasitic parthenogenetic females except in length, development of genital primordium and for the fact that these were "imprisioned" within their "mother". These juveniles devoured the contents of their mother (endotokia matricida) and within 48 hours changed the shape of their oesophagus from rhabditiform to filariform. The filariform juveniles escaped from their mother at about 92 hours and migrated from the culture chamber into the surrounding water medium. At the end of the 5th day most of the filariform juveniles had moved out from the culture chamber into the surrounding water in the petri-dish.

Development did not proceed further after this stage. It was observed that the filariform stages moulted but retained their

moulted cuticle as a sheath (Fig. 10). The filariform juveniles lived as long as there was sufficient water in the petri-dish.

EXPERIMENTAL CROSS-INFECTION OF *R. pipiens* AND *B. hemiophrys* WITH RHABDIAS FROM THE TWO HOSTS

Filariform juveniles were exposed to uninfected *R. pipiens* and *B. hemiophrys*. After a day, a number of cast cuticles were observed in the infection containers. The filariform juveniles were observed in the body cavities of the amphibians where they increased greatly in size (Table IV), to fourth stages. The fourth stages molted to fifth and final parasite states while still in the body cavities. These fifth stages were actually pre-adults as the vulva had just formed and eggs had started developing in their uteri.

Some fifth stages were observed in the lungs. Once in the lungs they increased in length and width and started producing embryonated eggs in their uteri. It must be pointed out that the pre-adult could stay in the body cavities of the amphibians for a long time, as in two instances of *B. hemiophrys* infected with *Rhadbias* of *R. pipiens*, these stages were seen in the cavities when the toad was sacrificed after 25 days.

Infection of R. pipiens with Rhabdias originating from B. hemiophrys:- All attempts to infect *R. pipiens* with infective juvenile *Rhabdias* of *B. hemiophrys* failed.

Infection of B. hemiophrys with Rhabdias originating from R. pipiens:- Ten Bufo hemiophrys were exposed to infective juvenile Rhabdias originating from R. pipiens. Five of these toads were exposed to 30 infective juveniles each. The other five were exposed to about 200 infective juveniles. Three of the five given 30 infective juveniles were autopsied 24 hours later. Only one had two pre-adults in its body cavity. The other two autopsied in 48 hours. Again only one had three pre-adults in its body cavity and three pre-adults in its lungs. Examination of the alimentary tract revealed neither eggs nor juveniles stages. Of the 5 toads infected with 200 infective juveniles three were autopsied 6 days later. None of them had any nematodes in their body cavities or lungs. The other two were autopsied 25 days later. One had 21 pre-adults and the other 17 pre-adults in the body cavities. None were found in the lungs.

MORPHOMETRICS OF SELECTED DEVELOPMENTAL STAGES OF RHABDIAS FROM R. pipiens AND B. hemiophrys

Parasitic Females originating from R. pipiens and B. hemiophrys:- Lung nematodes. Parasitic parthenogenetic females. Mouth surrounded by six small lips. Cuticle inflated. Oesophagus club-shaped. Posterior extremity tapers rapidly behind the anus and ends in a finely conical point, (Fig. 11). Vulva near middle of body. Uteri divergent.

TABLE IV

Rhabdias in Body Cavities of R. pipiens and B. hemiophrys

	R. pipiens	B. hemiophrys
Number	20	20
Length	3.65 - 4.6(4.27±0.29)mm* 0.065+	3 - 5.2(4.2±0.70)mm** 0.154
Width	90 - 132(116.3±14.2)u 3.13	80 - 147u(125.6±21.26)u 4.72
B. C.	12 - 20(14.2±2.49)u 0.56	12 - 19u(15.5±2.54)u 0.57
Oesophagus	0.45 - .51(0.49±0.02)mm .0045	0.46 - 0.55(0.56±0.03)mm .0067
Vulva	2.35 - 3.1(2.79±0.21)mm .047	1.7 - 3.05(2.21±0.3)mm 0.067
Tail	180 - 226(208.3±14.66)u 3.21	172 - 250(223±23.7)u 5.2
N. R.	160 - 225(193.1±19.8)u 4.43	180 - 220(199.2±13.42)u 2.96

* All measurements unless otherwise stated are in mm.

** Range followed by mean in brackets, ± standard deviation. These terms are defined in Appendix III. All tables follow similar patterns.

\+ Standard error: As a simple rule it can be stated that two samples are probably different if the difference between the means ($M_1 - M_2$) is more than twice the sum of the standard errors ($S.E.M_1 + S.E.M_2$) and almost certainly different if it is more than three times the sum of the standard errors.

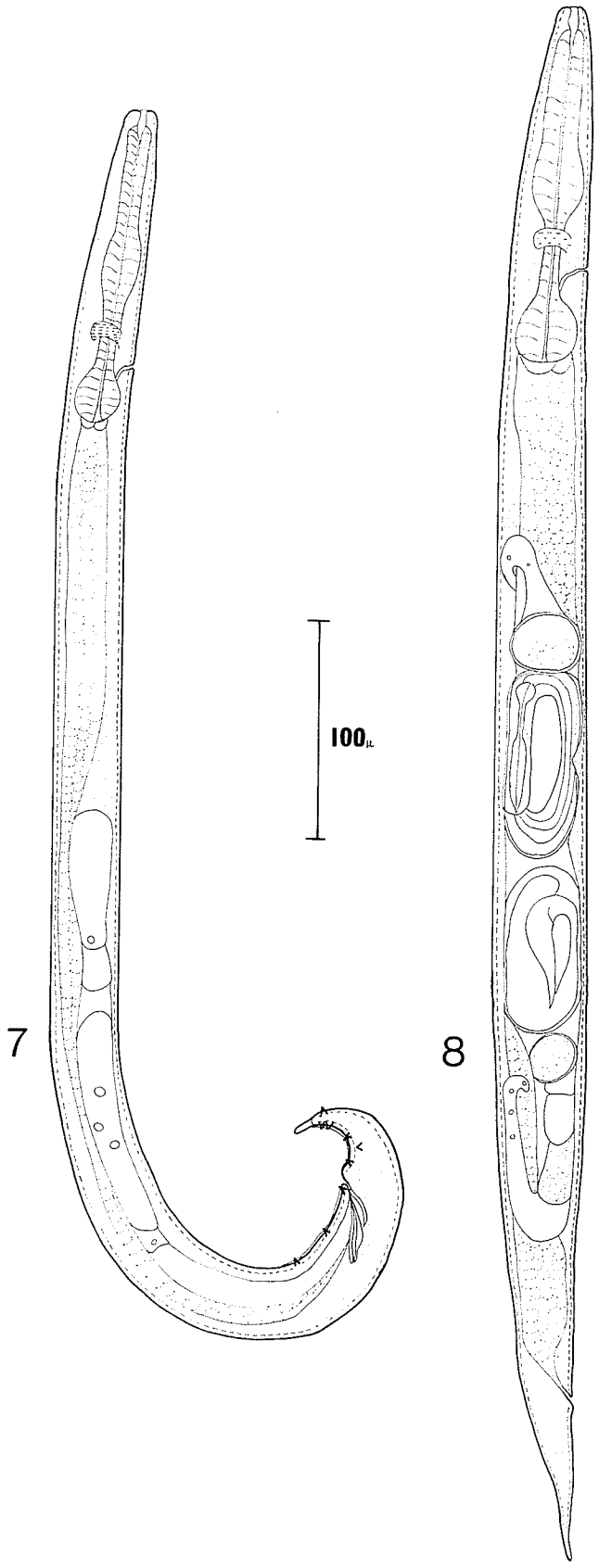
Figs. 7 - 10 Free-Living *Rhabdias* of *B. hemiophrys*

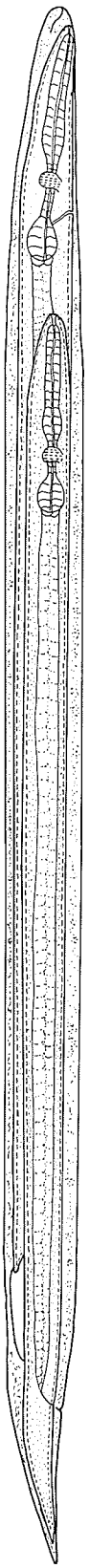
Fig. 7. Free-living male

Fig. 8. Free-living female with embryonated eggs

Fig. 9. Free-living female with 2nd. generation rhabditiform juveniles

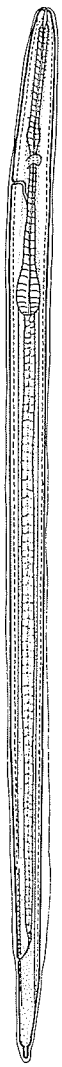
Fig. 10. Infective juvenile.


100μ
7
8



100µ

9



10

Rhabdias originating from R. pipiens * (Table V):- Length 6.9 - 9 (8.1)mm. Width 0.22 - 0.31 (0.27)mm, near the region of the vulva.** Bucal cavity 0.02 - 0.015mm deep. Oesophagus club shaped and is 0.49 - 0.75 (0.64)mm in length. Nerve ring 0.17 - 0.29 (0.25)mm from anterior extremity. Excretory pore slightly below nerve ring; difficult to see in dead specimen. A pair of excretory glands run along side the oesophagus terminating slightly behind the oesophagus. The intestine is dark coloured and opens by way of cuticularised rectum to the anus. Vulva 3.9 - 5.2 (4.6)mm from anterior extremity; near middle of body. Tail 0.2 - 0.34 (0.26)mm.

Phasmids situated laterally in the middle of the tail (Fig. 12). Eggs of various developmental stages present in the reproductive tract.

* 20 specimens, range followed by mean in brackets. ** Width does not include inflated cuticle.

Rhabdias originating from B. hemiophrys (Table V):- Length 7.4 - 16 (12.2)mm. Maximum width 0.16 - 0.38 (0.27)mm. Buccal cavity deep. Oesophagus 0.63 - (0.75)mm; club shaped. Intestine dark coloured and opens by way of cuticularised rectum at posterior end of body. Nerve ring 0.21 - 0.27 (0.24)mm from anterior extremity. Excretory pore and glands similar to Rhabdias in R. pipiens. (Fig. 13 & 14). Vulva 3.9 - 8.3 (6.4)mm from head and; near middle of body. Tail 0.29 - 0.57 (0.45)mm. (Fig 15). Phasmids situated laterally in mid-tail region. (Fig. 16). Embryonated eggs 92 - 125 (110) x 50 - 68 (60.5)μ.

FIRST STAGE RHABDITIFORM JUVENILES FROM EGGS IN THE UTERI OF PARASITIC FEMALES (TABLE VI)

First Stage Juveniles from R. pipiens:- Length 370 - 483 (434)μ. Maximum width 22 - 27 (23.6)μ. Buccal cavity 9 -14 (11.25)μ deep. Oesophagus 108 - 139 (122.15)μ; rhabditiform in shape.

Corpus, Isthmus and bulb of oesophagus clearly distinguished. They measure 58 - 75 (64.8), 30 -42 (36.4)μ and 19 - 23 (20.95)μ respectively. Nerve ring 68 - 88 (76.7)μ; from anterior extremity. Excretory pore difficult to see. Tail 50 - 69 (57.1)μ. Genital primordium 60 - 90 (74.25)μ; near mid-region of body.

First Stage Juveniles from B. hemiophrys (Table VI):- Length 380 - 470 (415.3)μ. Maximum width 22 - 25 (22.95)μ. Buccal cavity 9 -

TABLE V

Dimensions of Parasitic Female Rhabdias from the Lungs of Rana pipiens and Bufo hemiophrys

	R. pipiens	B. hemiophrys
Number	20	20
Length	6.9 - 9(8.1±0.53)*	7.4 - 16(12.2±2.5)
Width	0.22 - 0.31(0.27±0.02)	0.16 - 0.38(0.27±0.06)
Oesophagus	0.49 - 074(0.64±0.06)	0.63 - 0.84(0.75±0.05)
% of body length	6 - 11(7.9±1.0)	4 - 9(6±1.0)
Nerve ring (from anterior)	0.17 - 0.29(0.25±0.03)	0.21 - 0.27(0.24±0.02)
% of body length	2 - 4(3±0.3)	2 - 3(2±0.4)
Vulva (from anterior)	3.9 - 5.2(4.6±0.35)	3.9 - 8.3(6.4±1.24)
% of body length	52 - 61(57±2)	50 - 57(53±1.7)
Tail	0.2 - 0.34(0.26±0.04)	0.29 - 0.57(0.45±0.09)
% of body length	2 - 4(3±0.3)	3 - 4(3.6±3)
Embryonated eggs	94 - 107(101±3.5)μ	98 - 125(110±5.8)μ
	43 - 56(48.7±3.7)	58 - 68(60.5±4.7)u

* All measures unless otherwise stated are in mm.

TABLE VI

Dimensions of First Stage Rhabditiform Juvenile Rhabdias originating from R. pipiens and B. hemiophrys from Laboratory Culture

	R. pipiens	B. hemiophrys
Number	20	20
Length	270 - 483(434.24±46.75)*	380 - 470(415.3±23.6)
Width	22 - 27(23.6±1.39)	22 - 25(22.95±0.99)
Buccal cavity	9 - 14(11.25±1.29)	9 - 14(11.65±1.30)
Oesophagus	108 - 139(122.15±8.7)	101 - 129(118.45±6.7)
% of body length	23.8 - 47.03(28.5±0.2)	25.9 - 32.1(28.6±1.4)
Corpus	58 - 75(64.8±5.55)	57 - 73(64.5±5.08)
Isthmus	30 - 42(36.4±3.47)	28 - 40(34.05±3.57)
Bulb	19 - 23(20.95±1.39)	18 - 26(20.4±1.88)
Nerve ring (from anterior)	68 - 88(76.7±5.88)	60 - 86(73.65±6.55)
% of body length	15.8 - 30.7(17.9±0.1)	15.8 - 19.9(17.7±0.9)
Genital primordium (length)	60 - 90(74.25±6.83)	52 - 89(65.65±9.87)
% of body length	14.08 - 27.8(17.3±2.9)	12.4 - 22.1(15.9±0.1)
Tail	50 - 69(57.1±4.2)	36 - 53(48.05±4.41)
% of body length	11.8 - 21.4(13.3±2.06)	8.5 - 12.8(11.6±1.02)

All measurements unless otherwise stated are in μ.

14 (11.65)μ deep. Oesophagus 101 - 129 (118.45)μ from head end. Corpus, Isthmus and bulb 57 - 73 (64.5)μ, respectively. Excretory pore difficult to see. Nerve ring 60 - 86 (73.65)μ from anterior extremity. Tail 36 - 53 (48.05)μ long. Genital primordium in middle of body; 52 - 89 (65.65)μ.

FREE-LIVING MALES (TABLE VII)

Oesophagus rhabditiform. Tail conical with narrow lateral alae. Three pairs of pre-anal papillae and 6 pairs of anal papillae. Spicules equal, slightly rounded at distal portion and pointed at proximal portion with middle portion slightly inflated. Gubernaculum triangular in shape.

Free-Living Male Rhabdias originating from R. pipiens:- Length 645 - 850 (730.25)μ. Width 26 - 40 (31.7)μ. Buccal cavity 10 - 16 (13)μ. Oesophagus divided distinctly into corpus, Isthmus and bulb. These measure 68 - 83 (73.8)μ, 36 - 50 (43.6)μ and 18 - 25 (22)μ respectively. Nerve ring and excretory pore are 90 - 112 (98.55)μ and 112 - 130 (121.45)μ respectively from anterior extremity. Tail 41 - 61 (54.35)μ long. Spicules 29 - 37 (31.2)μ; equal in length. Gubernaculum half the size of spicule; 14 - 21 (17.95)μ.

TABLE VII

Free-Living Male Rhabdias Originating from R. pipiens and B. hemiophrys

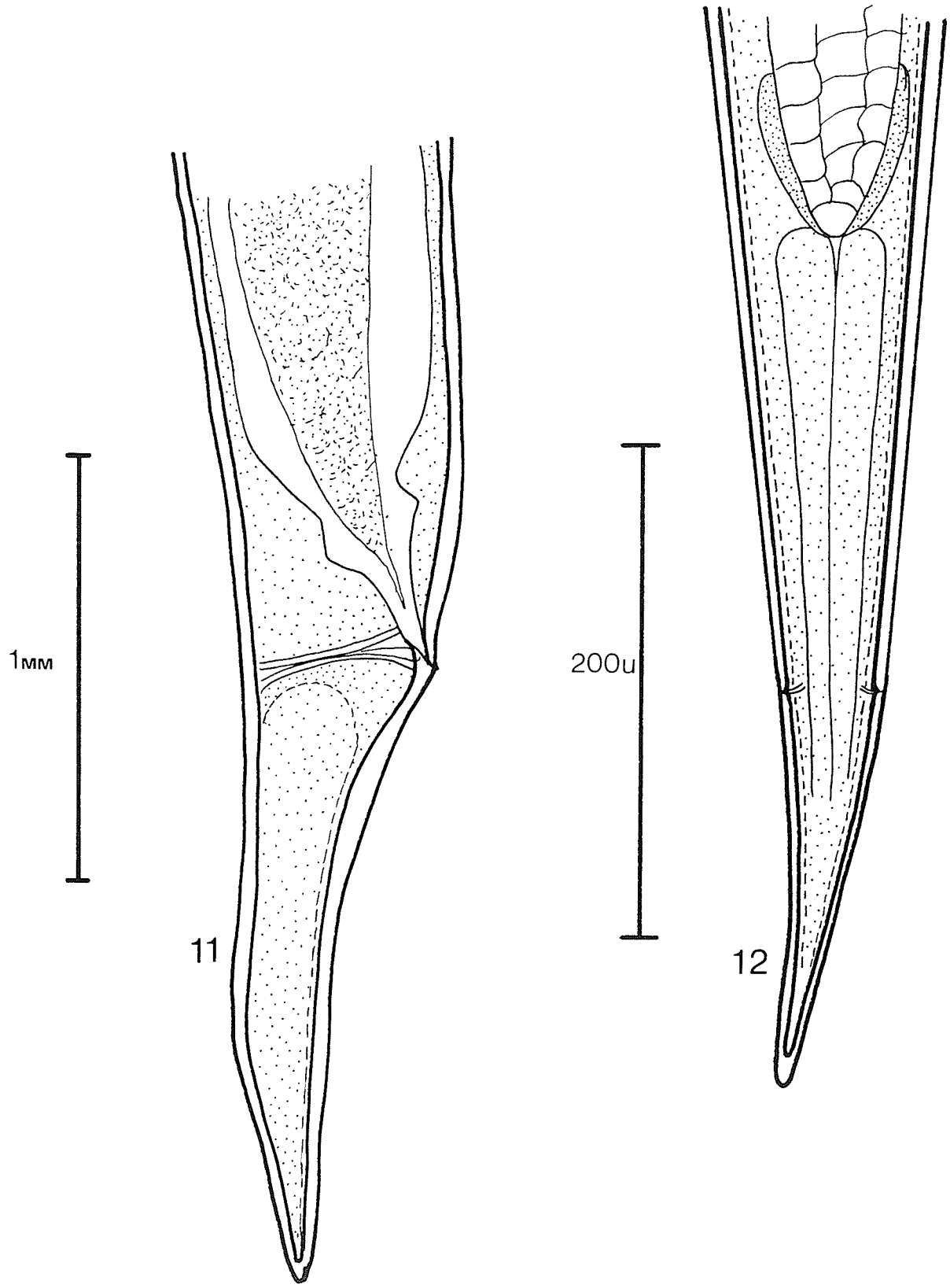
	R. pipiens	B. hemiophrys
Number	20	20
Length	645 - 850(730.25±44.7)*	545 - 770(670.25±53.15)
Width	26 - 40(31.7±3.36)	27 - 32(28.9±1.78)
Buccal cavity	10 -16(13±1.86)	11 - 15(12.65±1.49)
Oesophagus (from anterior)	126 - 149(139.4±5.77)	138 - 167(146.05±6.8)
% of body length	17.5 - 20.6(19.1±0.7)	19.2 - 26.4(219±1.9)
Corpus	68 - 83(73.8±3.14)	73.94(81.55±4.9)
Isthmus	36 - 50(43.6±4.12)	36.49(42.05±3.48)
Bulb	18 - 25(22±1.86)	20 - 26(22.45±1.73)
Nerve ring (from anterior)	90 - 112(98.55±5.99)	94 - 115(102.4±4.84)
% of body length	11.7 - 15.6(13.5±0.9)	13.2 - 17.8(15.4±1.34)
Excretory pore (from anterior)	113 - 130(121.45±4.85)	112 - 143(123.6±6.55)
% of body length	15.2 - 18.2(16.6±0.6)	16.4 - 22.4(18.5±1.7)
Tail	41 - 61(54.35±5.29)	50 - 70(56.45±5.7)
% of body length	6.1 - 8.4(7.4±0.7)	7.04 - 10.58(5±1.1)
Spicule	29 - 37(31.2±2.04)	27 - 36(31.05±2.2)
Gubernaculum	14 - 21(17.95±1.82)	14 - 21(16.3±1.89)

All measurements unless otherwise stated are in μ.

Figs. 11 - 12 Parasitic adults Rhabdias of R. pipiens

Fig. 11. Tail end, lateral view

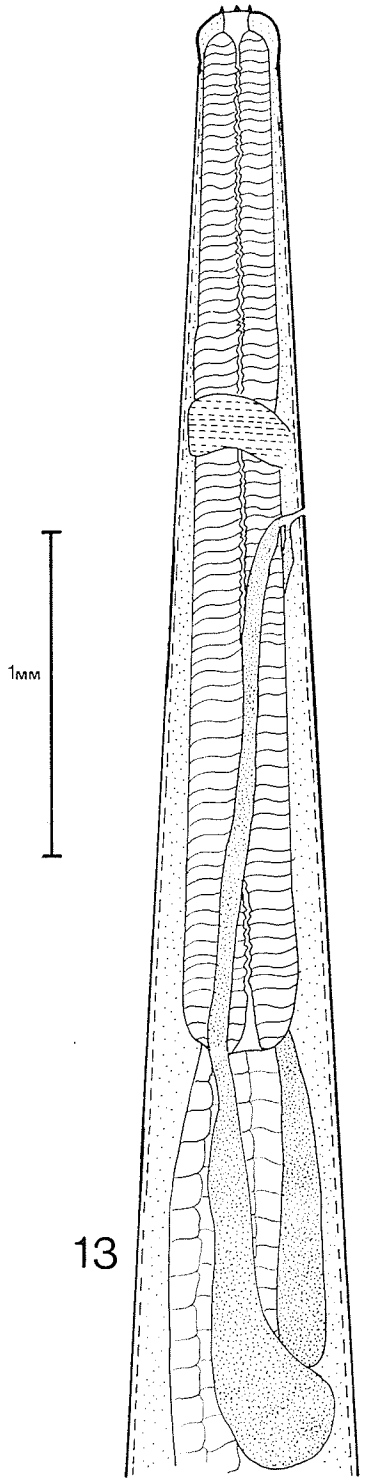
Fig. 12. Tail end, ventral view.


1мм
11
200u
12

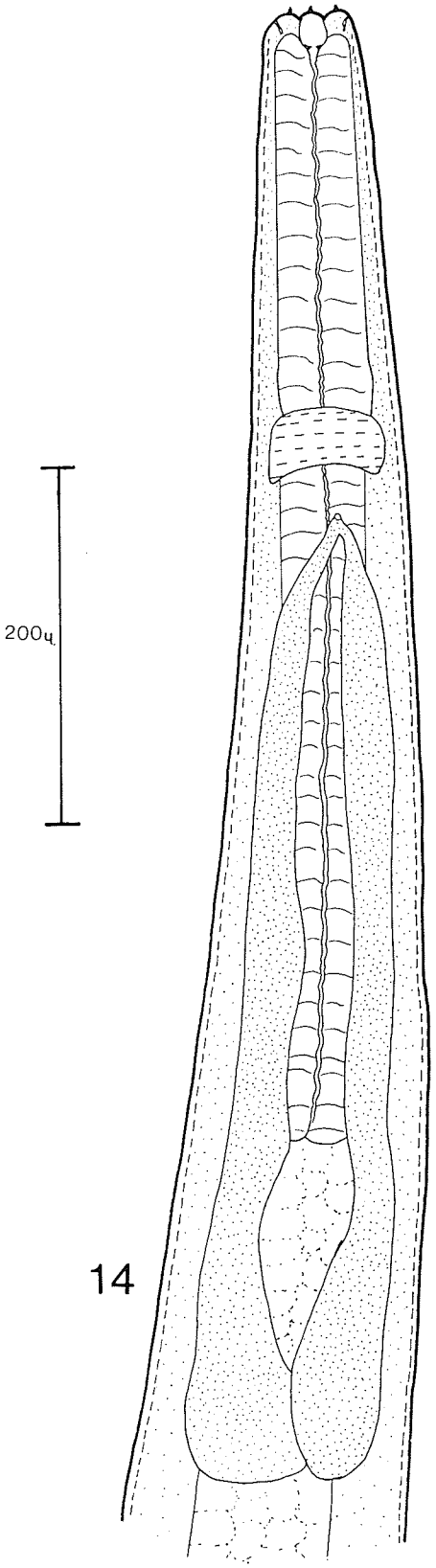
Figs. 13 - 14 Parasitic stages Rhabdias of B. hemiophrys

Fig. 13. Head end, lateral view

Fig. 14. Head end, ventral view.



13

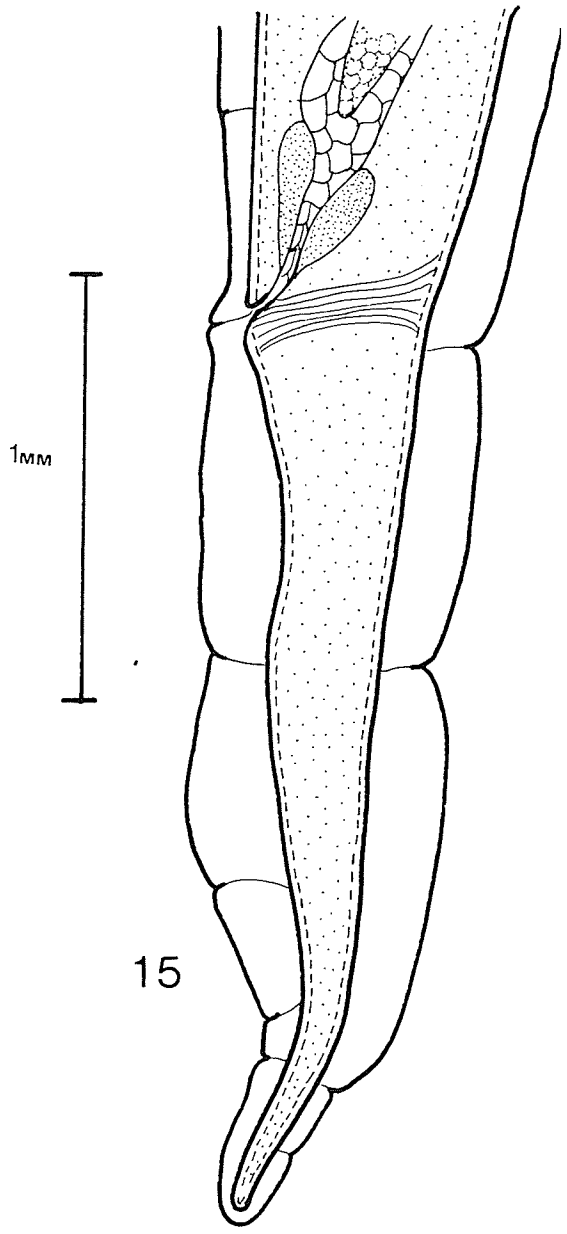


14

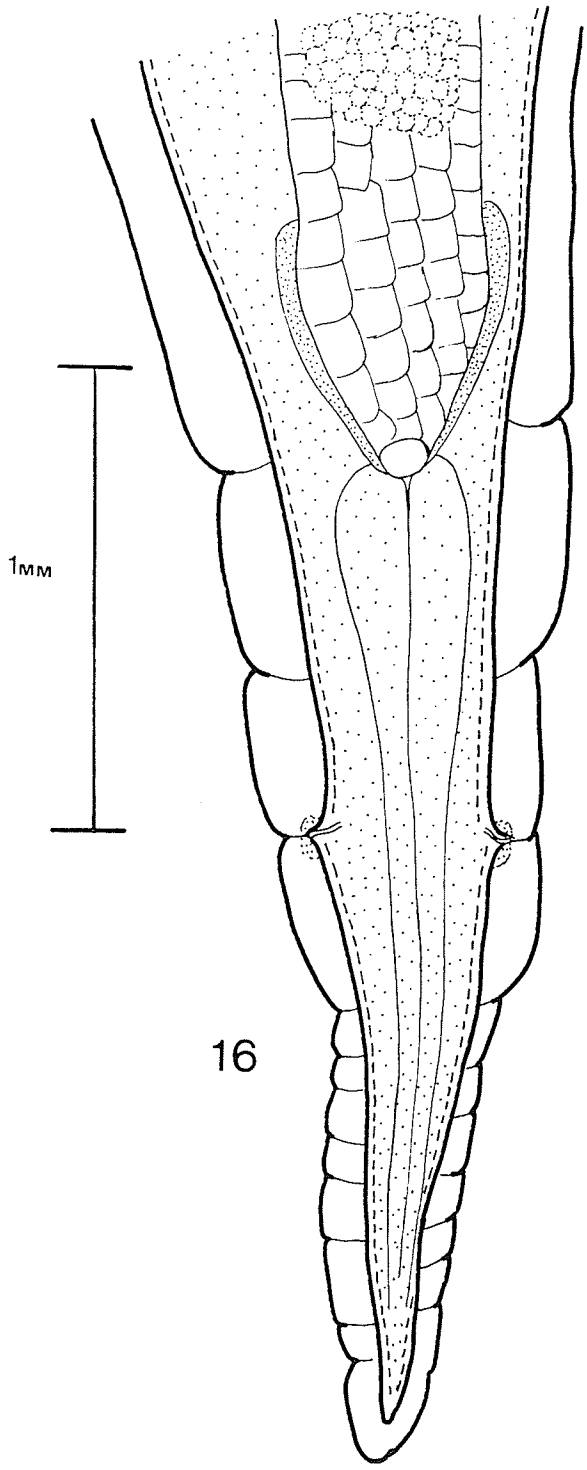
Figs. 15 - 16 Parasitic adult Rhabdias of B. hemiophrys

Fig. 15. Tail end, lateral view

Fig. 16. Tail end, ventral view.



15



16

Free-Living Male Rhabdias Originating from B. hemiophrys:- Length 545 - 770 (670.25)μ. Width 27 - 32 (28.9)μ. Buccal cavity 11 - 15 (12.65)μ deep. Oesophagus rhabditiform; 138 - 167 (146.05)μ. Corpus, Isthmus and bulb 73 - 94 (81.55)μ, 36 - 49 (42.05)μ and 20 - 26 (22.45)μ respectively. Nerve ring and excretory pore 94 - 115 (102.4)μ and 112 - 143 (123.6)μ respectively. Tail 50 - 70 (56.45)μ. Spicules equal; 27 - 36 (81.55)μ. Gubernaculum 14 - 21 (16.3)μ.

TABLE VIII

Free-Living Female Rhabdias Originating from R. pipiens and B. hemiophrys

	R. pipiens	B. hemiophrys
Number	20	20
Length	750 - 990(88.5±68.24)*	625 - 872(738.3±75.36)
Width	40 - 63(47.15±5.89)	37 - 49(43.55±2.92)
Buccal cavity	13 - 19(14.85±1.53)	10 - 20(13.8±3.3)
Oesophagus	156 - 176(164.5±5.41)	139 - 188(160.25±11.5)
% of body length	16.8 - 21.2(18.6±1.16)	18.3 - 24.4(21.8±2.1)
Corpus	83 - 98(89.7±3.96)	80 - 112(92.35±2.1)
Isthmus	40 - 54(48.6±4.25)	39 - 52(45.35±4.9)
Bulb	25 - 32(26.7±1.66)	20 - 26(22.55±2.04)
Nerve ring	102 - 121(112±5.63)	96 - 130(114.05±8.3)
% of body length	11.3 - 14.9(12.7±0.8)	13.7 - 16.7(15.5±0.8)
Excretory pore	129 - 149(137.75±5.31)	119 - 162(138.2±10.72)
% of body length	14.04 - 17.9(15.6±1.0)	15.9 - 21.7(18.8±1.9)
Tail	61 - 80(72.1±5.09)	55 - 73(64.15±5.5)
% of body length	7.3 - 9.9(8.1±0.5)	7.4 - 10(8.7±0.6)
Embryonated eggs	85 - 90(82.03±4.1)X	80 - 91(84.65±3.51)X
	34 - 40(35.36±2.7)	33 - 40(36.35±2.3)

* All measurements unless otherwise stated are in μ.

FREE-LIVING FEMALES (TABLE VIII)

Usually larger than free-living males. Oesophagus rhabditiform. Vulva situated in the mid-region of the body; difficult to see. Uteri divergent; continue as oviducts which turn backward extending towards middle of body.

Free-Living Females Rhabdias originating from R. pipiens:- Length 750 - 990 (885.5)μ. Width 40 - 63 (47.15)μ. Buccal cavity 13 - 19 (14.85)μ. Oesophagus 156 - 176 (164.5)μ from anterior extremity. Corpus, Isthmus and bulb 83 - 98 (89.7)μ, 40 - 54 (48.6)μ and 25 - 32 (26.7)μ respectively. Nerve ring and excretory pore 102 - 121 (112)μ and 128 - 149 (137.75)μ respectively from anterior end. Tail 61 - 80 (72.1)μ. Embryonated eggs in uteri of free-living females 71 - 92 (815) x 30 - 40 (36)μ.

Free-Living Female Rhabdias originating from B. hemiophrys:- Length 625 - 872 (738.3)μ. Width 37 - 49 (43.55)μ. Buccal cavity 10 - 20 (13.8)μ. Oesophagus 139 - 188 (160.25)μ and 20 - 26 (22.55)μ respectively. Nerve ring and excretory pore 96 - 130 (114.05)μ and 119 - 162 (138.2)μ respectively from anterior end. Tail 55 - 73 (64.15)μ. Embryonated eggs.

RHABDITIFORM JUVENILES OF THE FREE-LIVING FEMALE RHABDIAS

These juveniles are similar to the first stage rhabditiform juveniles of the parasitic females except that they are "imprisoned" in the free-living females. Genital primodium is less developed.

SECOND GENERATION RHABDITIFORM JUVENILES OF THE FREE-LIVING FEMALES (TABLE IX)

The dimensions of the second generation rhabditiform juveniles of the free-living Rhabdias of R. pipiens are: Length 418 - 674 (553.55)μ. Width 10 - 16 (12.95)μ. Oesophagus is 114 - 145 (129.5)μ from the anterior end. Those of the free-living females Rhabdias of B. hemiophrys are 551 - 712 (592.65)μ in length and 20 - 28 (23.55)μ in width. Oesophagus is 131 - 151 (139.2)μ from anterior extremity.

INFECTIVE JUVENILES (TABLE X)

Body slender with numerous longitudinal striations. Oesophagus club-shaped (long tubular pharynx lacking bulb). Genital primordium rudimentary; near middle of body. Body ensheathed (Fig. 10).

Infective Juvenile Rhabdias originating from R. pipiens:- Length 520 - 670 (576.75)μ. Width 18 - 22 (19.65)μ. Oesophagus 165 - 207 (187.5)μ from anterior extremity; somewhat filariform in shape. Nerve ring and excretory pore 81 - 98 (87.5)μ and

97 - 124 (107)μ from anterior end respectively. Tail length 72 - 95 (84.6)μ.

Infective Juveniles Rhabdias originating from B. hemiophrys:- Length 630 - 805 (722.25)μ. Maximum width 15 - 22 (19.8)μ. Oesophagus 172 - 225 (201.05)μ from anterior end; somewhat filariform in shape. Nerve ring and excretory pore 87 - 120 (102.85)μ and 117 - 136 (125.9)μ from head end. Tail length 62 - 87 (74.1)μ.

TABLE IX

Second Generation First Stage Rhabditiform Juveniles

	R. pipiens	B. hemiophrys
Number	20	20
Length	418 - 674(553.55±62.53)*	551 - 712(592.65±36.03)
Width	10 - 16(12.95±2.14)	20 - 28(23.55±1.93)
Oesophagus	114 - 145(129.5±94)	131 - 151(139.2±5.19)

*All measurements unless otherwise staged are in μ.

TABLE X

Infective Juveniles

	R. pipiens	B. hemiophrys
Number	20	20
Length	520 - 670(576.75±39.4)	630-805(722.25±38.95)
Width	18 - 22(19.65±1.30)	15 - 22(19.8±1.64)
Oesophagus	165 - 207(187.5±10.95)	172 - 225(201.05±12.24)
% of body length	29.8 - 35.4(32.59±1.4)	25.3 - 30.1(27.8±1.1)
Nerve ring	81 - 98(87.5±5.1)	87 - 120(102.85±8.39)
% of body length	17.2 - 23(21.1±1.5)	11.6 - 15.4(14.24±0.9)
Excretory pore	97.124(107±5.98)	117 - 136(125.9±5.98)
% of body length	15.9 - 20(18.6±1.0)	15.0-19(17.45±0.7)
Tail length	72 - 95(84.6±5.97)	62.87(74.1±5.83)
% of body length	11.5 - 15.4(14.4±0.9)	8.5 - 11.2(10.26±0.7)

* All measurements unless otherwise staged are in μ.

DISCUSSION

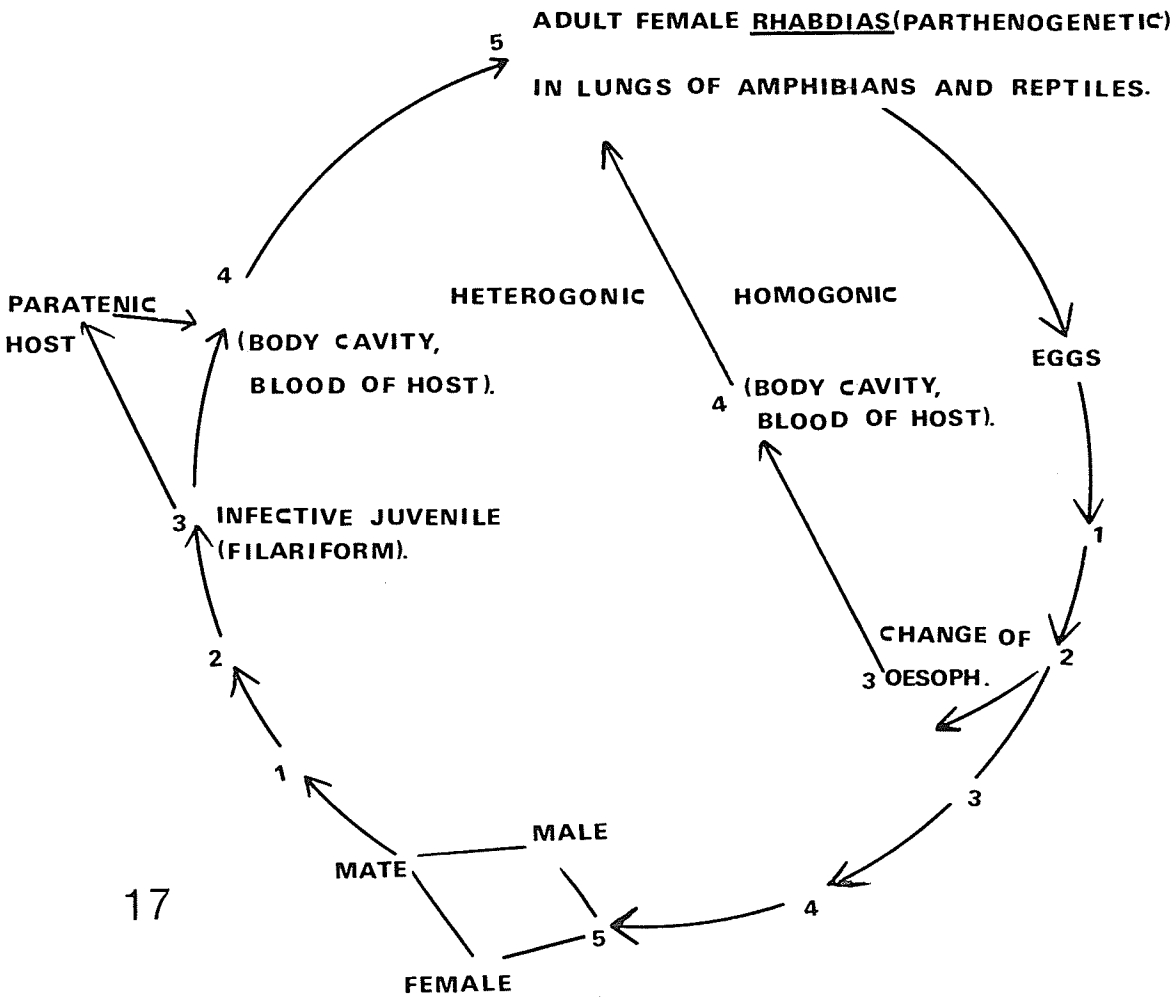
DISCUSSION

Travassos (1926) recognized two Rhabdias life cycle types: a) Heterogonic (Indirect), in which there is parasitic and free-living male and female generation and b) Homogonic (Direct) in which there is only the parasitic phase (Fig. 17).

He stated that the heterogonic type of life cycle was reported for Rh. bufonis, Rh. entomelas, Rh. fulleborni and Rh. dujardini. The number of eggs produced by the free-living females differed. Rh. bufonis, Rh. entomelas and Rh. fulleborni had their free-living females producing one to two eggs which hatched in the females while Rh. dujardini produced two to eight eggs, normally three to five eggs which were shed by the females before hatching.

In the present study Rhabdias from both amphibian hosts exhibited a heterogonic development in laboratory faecal cultures. Free-living male and female developed in 24 hours, copulated and development to the infective filariform juveniles proceeding within the body of the free-living females. A heterogonic life cycle of this type has also been described for Rh. ranae by Chitwood (unpublished, cited from Chu 1936), Rh. spaerocephala Williams (1960), Rh. brachylaimus and Rh. multiproles Yuen (1965). Walton (1929) who first studied the life cycle of Rh. ranae concluded Rh. ranae did not follow a heterogonic development

Fig. 17 Life cycle pattern of Rhabdias


ADULT FEMALE RHABDIAS(PARTHENOGENETIC)
IN LUNGS OF AMPHIBIANS AND REPTILES.
5
4
PARATENIC
HOST
(BODY CAVITY,
BLOOD OF HOST).
HETEROGONIC
HOMOGONIC
EGGS
4
(BODY CAVITY,
BLOOD OF HOST).
1
3
INFECTIVE JUVENILE
(FILARIFORM).
CHANGE OF
3 OESOPH.
2
2
1
3
MALE
MATE
4
5
FEMALE

but developed directly from faeces of frogs to the filariform juveniles omittine free-living adults. However, Chu (1936b) demonstrated that Rhabdias identified as Rh. ranae from R. pipiens could undergo a heterogonic development under certain circumstances.

Embryonated eggs which were dissected out of the parasitic females or recovered from the rectum hatched into rhabditiform juveniles a few minutes after setting up the culture. As mentioned previously, hatching did not occur in the lungs. This is in contrast to the life cycle of Rh. ranae reported by Walton (1929) that embryonated eggs hatched in the lungs of the amphibians. Chu (1936b), Williams (1960) and Yuen (1965) did not observe hatching in the lungs of various amphibians.

The first stage rhabditiform juveniles moulted approximately every six hours so that between 24 - 30 hours both adult free-living males and females were evident in the culture. Yuen (1965) working on the life cycle of Rh.brachylaimus and R. multiproles showed that free-living males and females were formed within 24 hours. In the rectum of the amphibians the oldest Rhabdias juveniles recovered was in the 1st. stage. No further development apparently occurred until these stages were passed out with the faeces. Chu (1936b) showed that embryonated eggs of Rh. fuscovenosa var. catanensis hatching in the rectum of the grass snake showed no evidence of development until after

being passed out with the faeces and they usually die if retained for a long time. Walton's (1929) observation that infective juveniles of Rh. ranae accumulated in the rectum was not confirmed by me, as the only stages of Rhabdias found in the rectum of amphibians were first stage rhabditiform juveniles.

According to Travassos, the homogonic cycle was represented by Rh. ophidia and Rh. fuscovenosa both of which are reptilian species. Other reptilian studies of Rhabdias life cycle confirm this with slight differences. Chu (1936b) demonstrated the direct type of development for Rh. fuscovenosa var. catanensis a reptilian parasite, even though he recovered a few free-living forms, most of which were males, when he used a "special culture chamber". Williams (1960) studied the life cycle of Rh. sphaerocephala an amphibian species and found that the homogonic cycle prevailed with a small but negligible proportion of the worms developing into free-living stages. It is probable that most amphibian Rhabdias follow the heterogonic pattern while reptilian Rhabdias follow the heterogonic type, with a few amphibians and reptiles exhibiting both patterns.

In this study, the free-living male and female Rhabdias mate and the females produced second generation rhabditiform juveniles. This procedure was also reported by Chu (1936b) for Rh. fuscovenosa var. catanensis, by Chabaud et al. (1961) for Rh. madagascariensia and Rh. gemillipara and by Yuen (1965) for

Rh. brachylaimus and Rh. multiproles. The rhabditiform juveniles eventually changed the shape of the oesophagus from rhabditiform to strongyloid type.

These juveniles then broke loose from the mother cuticle and moved out into the surrounding water from the growth chamber. Chu (1936b) demonstrated this fact earlier for Rh. fuscovenosa var. catanensis.

The filariform juveniles in the surrounding water of the growth chambers were able to live in this medium as long as the inside of the chamber was moist. Goodey (1924a) noted that infective juveniles of Rh. fuscovenosa were resistant to desiccation on a glass surface for about a day, as when a drop of water containing ensheathed juveniles of Rh. fuscovenosa were allowed to evaporate overnight, they all revived and resumed active mobility on being remoistened. He concluded that air drying on a glass surface was a much severer form of desiccation that the infective stages would be likely to encounter under natural conditions. Chu (1936b) found that most infective juveniles of Rh. fuscovenosa var. catanensis lived three weeks or longer in water but died as the "water became stale". He noted, however, that if the juveniles were washed in sterile water and then kept in clean fresh oxygenated water in a petri-dish they lived in one case as long as 82 days. He therefore suggested that while infective, the juveniles did not feed, just as is true for similar stages of

other nematode infective juveniles.

Stages recovered in the body cavities in both naturally and experimentally infected animals fell within the same range of measurements.

In R. pipiens the data agree closely to that given by Walton (1929) for stages he recovered in the body cavity (Table XI). No stages were recovered in the blood. As pointed out earlier, Williams (1960) showed that infective juveniles of Rh. sphaerocephala reached the lungs either by the mesenteries or through the blood stream. Chu (1936b) confirmed Goodey's (1924) report that an active migration of infective juveniles passed through connective tissues and body cavity of snakes rather than a passive carriage in the blood stream as in Rh. bufonis.

Lung nematodes in B. hemiophrys were described as Rh. bufonis by Hlynka (1970). Those in R. pipiens were described by Walton (1929) as Rh. ranae on the basis of morphological and biological difference between this form and Rh. bufonis (Schrank 1788). Walton also noted that Rh. ranae differed materially from specimens obtained from toads of the same region (Temperate North America).

My description of the parasite in R. pipiens differed substantially from the one by Walton (Table XI). The excretory pore is a short distance behind the nerve ring. From Walton's picture, the nerve ring is situated near the end of the club shaped oesophagus. Also there were no lateral post-anal papillae present

TABLE XI

Measurements of Rh. ranae by different authors.

	Walton (1929)	Gupta (1960)	Quaye (1972)
Length	3.5-40mm	5.54-7.6mm	6.9-9(8.1)mm
Width	0.3-0.4mm	0.3-0.38mm	0.22-0.31(0.27)mm
Oesophagus	0.45-55mm	0.34-0.37mm	0.49-0.75(0.64)mm
N.R.	0.2-0.3mm	0.17-0.19mm	0.17-0.29(0.25)mm
Tail	0.15-0.25mm	0.21-0.39mm	0.2-0.34(0.26)mm
Eggs	40 x 75u	100 x 550-650u	94-107(101)X 43-56(48.7)u

as suggested by Walton. Gupta (1960) did not find any post-anal papillae in Rh. ranae from East Pakistan in R. tigrina.

I found a pair of phasmids situated laterally on the adult Rhabdias specimen in both R. pipiens and B. hemiophrys. On the basis of the two pairs of post-anal ventro-lateral papillae of Walton (1929), Ingles (1936) described a new species, Rh. joaquinensis from the lungs of R. aurora. This new species is closely related to Rh. ranae. It seems to me that what Ingles described as a pair of ventro-lateral papillae are phasmids.

If the papillae of Ingles are phasmids and as Gupta (1960) and I found that there are no ventro-lateral papillae in Rh. ranae then, Ingles basis for erecting a new species is consequently not valid. If these are true then, Rh. joaquinensis becomes a synonym of Rh. ranae.

The free-living males Rhabdias of both R. pipiens and B. hemiophrys have nine pairs of papillae in the anal region. These agree with the number of papillae found on the free-living males of Rh. brachylaimus, Rh. multiproles and Rh. gemellipara. The suggestion by Chu (1936b) that careful study of the free-living males might help in species identification, might not carry much weight after all.

The cross infection experiments suggested that infective juveniles Rhabdias from R. pipiens infect B. hemiophrys. Even though only a small proportion of the juveniles eventually reached

the lungs of B. hemiophrys, it is conceivable that with time a lot of the juveniles might have reached the lungs. Chabaud et al. (1961) found that Rh. gemellipara in the lungs of Chamaeleo parsonic measured on the average 8.41mm. When C. lateralis was infected orally with material from C. parsonii the Rhabdias which were recovered in the lungs were morphologically similar to Rh. gemellipara except they were smaller and measured on the average 4.65mm.

Material of Rh. sphaerocephala in B. marinus that Williams (1960) sent to Mrs. B. Chitwood were smaller than those reported by Goodey (1924) or by Bravo-Hollis and Caballero (1940) though Williams claimed that many specimens that he retained fell within the range given by those authors. In an experiment to determine the infectivity of Rh. sphaerocephala to other amphibians, Williams (1960) found that the tree frog Eleuthrodactylis johnstonei which were not known for this nematode became infected.

INTERSPECIFIC RELATIONSHIP

THE GENUS HAEMATOLOECHUS

REVIEW OF LITERATURE

North American Reports of Haematoloechus:- The genus Haematoloechus (synonyms Pneumonoeces, Pneumobites and Ostilium) is a large genus of lung flukes of amphibians. Leidy (1851) was the first to report flukes from the lungs of North American amphibians. He described Distomum variegatum from the lungs of Rana pipiens. Stafford (1905) in a systematic study of the flukes and other helminths of vertebrates of North America, described five new species of Haematoloechus from Canada and later designated them as Pneumonoeces longiphexus; P. breviplexus; P. varioplexus; P. similiplexus and P. medioplexus.

Cort's (1915) studies on the North American lung flukes were the first extensive ones since Stafford. Further studies of this type were carried out by Harwood (1932). Ingles (1936) reported Haematoloechus from California amphibians; Brandt (1936) reported long flukes from certain North American Salientia; Manter (1938) recovered Haematoloechus species from Florida amphibians; Kuntz and Self (1944) reported some lung flukes of the Salientia of Comanche Country, Oklahoma and Rankin (1945) reported lung flukes from the amphibians of Western Massachusetts and vicinity.

SYSTEMATICS

Bouchard (1951) recognized Looss (1899) as the authority for Haematoloechus. Earlier Stal (1875), (in Bouchard 1951) had employed Haematoloecha for a Hemipteran genus. Looss (1902), (in Bouchard 1951) on the assumption that the name Haematoloechus was a homonym from Haematoloecha, proposed Pneumonoeces to replace his earlier taxa. Leidy (1851) upon re-examination of his Distomum variegatum material, found that it included three distinct species. The largest of these he designated the type of the genus H. variegatus and the others he named H. similis and H. asper.

Stafford, Cort and others accepted the taxa Pneumonoeces. Cort (1912) accepted the name Pneumonoeces because that seemed to him "a logical application of the rule of priority". Harwood (1932) and Ingles (1932) independently concluded that the Law of Priority as interpreted by Looss and Cort did not apply in this case and they re-established the genus Haematoloechus. In the opinion of Bouchard (1951) and on the basis of the decision of the Commission of Zoological Nomenclature relative to Leucochila vs. Leuchochilus (Opinion 115, 131, Smithsonia Miss. Coll. 73 (:1-5), Pneumonoeces should assume priority. In this manuscript, I use the term Haematoloechus because I agree with Harwood (1932) and Ingles (1932). Also according to Art. 35 of the International Rules as amended at Paris in 1948, the use of a and us should not be considered homonyms. Subsequent workers were divided in

usuage of these two names. The genus contains many species of which about 17 are reported from North America, as parasites of amphibians of the genera Rana and Bufo (Table XII).

TABLE XII

Tabulation of Haematoloechus. From Manther (1938)

Species	Hosts	Locality
1. H. coloradensis (Cort) Ingles, 1932	R. pipiens	U.S.A.*
	R. montezumae	Mexica
2. H. complexus (Seely) Krull, 1933	R. pipiens	N. Carolina
	R. clamitans	
3. H. medioplexus Stafford, 1902	R. clamitans	Canada*
	R. pipiens	U.S.A.
4. H. oxyorchis, Ingles, 1932 Synonym: H. confusus Ingles, 1932	R. aurora draytoni	California
5. H. breviplexus Stafford, 1902	Bufo americanus	Canada
	R. catesbeina	U.S.A.
	R. clamitans	U.S.A.
6. H. buttensis Ingles, 1936	R. boyli	California
7. H. elongatus Caballero & Sokoloff, 1934	R. montezumae	Mexica
8. H. floedae Harwood, 1932	R. catesbeiana	Texas, Florida
	R. clamitans	
9. H. kernensis Ingles, 1923	R. aurora draytoni	California
10. H. longiplexus Stafford, 1902	R. catesbeina	Canada
	R. pipiens	United States
11. H. parviplexus (Irvin) Ingles, 1932	R. clamitans	Michigan

Table XII cont'd,

Species	Hosts	Locality
.2. H. tumidus Ingles, 1932	R. aurora draytoni	California
.3. H. uniplexus Harwood, 1932	R. sphenocephala	U.S.A.
.4. H. varioplexus Stafford, 1902	Bufo lentiginosus	Canada
synonym: H. similiplexus, Stafford, 1902	R. clamitans	U.S.A.
	R. pipiens	

* The locality "United States" and "Canada" in the above list indicate a probable wide distribution of the Haematoloechus species.

GENERIC DIAGNOSIS

Yamaguti (1958) gave the generic diagonis of Haematoloechus as:- Body elongate, more or less attenuated anteriorly, spinulate or not. Acetabulum small, in anterior or middle third of body. Oral sucker well developed. Oesophagus short. Caeca simple terminating out near posterior extremity. Testis diagonal or nearly symmetrical, usually in posterior half of body; cirrus pouch cylindrical or claviform, may be very long containing seminal vesicle, prostatic complex and eversible ducts ejaculatorius. Genital pore ventral to pharynx or oesophagus. Ovary lobed or not, near acetabulum. Receptaculum seminis large. Laurer's canal absent. Vitellaria forming bunches of follicles extending along caeca for their greater part of entire length. Uterus occupying all available space of hindbody as well as intercaecal field of forebody, describing longitudinal extracaecal loops which may extend forward to specifically different levels; eggs exceedingly numerous, brown, embryonated. Excretory vesicle Y-shaped. Parasitic in lungs of amphibians.

LIFE CYCLE

The life cycles of some lung flukes of amphibians were worked out by Krull. In a series of papers from 1930 to 1934, Krull found that frogs acquired certain lung flukes by ingesting Odonata infected with metacercariae and that frogs annually lost and renewed

their infections. The parasites produced eggs in great numbers which pass from the lungs through the glottis into the mouth cavity. They are swallowed, pass through the digestive tract, and are voided with the faeces. Eggs containing mature miracidia are deposited in this way in the shallow water along the margins of ponds and lakes where snails are usually abundant. The miracidia hatch only when snail feed on the eggs.

The embryonated eggs of H. parviplexus are ingested by the planorbid snail, Gyraulus parvus, in which miracidia hatch, penetrate the intestinal wall and metamorphose to the sporocyst stage. Xiphidiocercariae of the ornatae group develop in sporocysts and after leaving the snail, are drawn into the bronchial basket of dragonfly nymphs where they encyst. The infected insects are eaten by R. clamitans. Mature flukes develop in the lungs.

The life cycle of H. medioplexus is similar to H. parviplexus except that the host snail is Planorbula armigera, and the definitive host R. pipiens.

Ingles (1933) investigated the life cycle of H. oxyorchis from R. aurora and found that Gyraulus parvus and G. vermicularis serve as first intermediate hosts. Ingles observed a 50-day-old daughter sporocyst and found that the cercariae encyst in dragonfly naiads of genus Sympetrum. Schell (1965) described the life cycle of H. breviplexus. Mother and daughter sporocysts developed and later produce xiphidiocercariae of the ornatae

group. The host snail is Gyraulus similaris. Dragonfly nymphs of Aeschna multicolor serve as second intermediate host and definitive host is Rana pretiosa.

INTERSPECIFIC RELATIONSHIPS

Little work has been done on the interspecific relationships of helminth parasites of the lungs of amphibians. Mazurmovich (1957) observed that the lung trematode Haematoloechus, and the lung nematode, Rhabdias bufonis never occurred together. On the other hand Markow (1955) showed that in the lungs of Rana temporaria the trematode, Haplometra cylinderacea, and Rhabdias bufonis occurred together fairly often (20-60%) and that they therefore are not antagonistic. A drop in the intensity of trematode infestation was observed when a large number of nematodes were in the lungs.

RESULTS

Of the lungs of 117 R. pipiens and 77 B. hemiophrys examined, most were infected with either Rhabdias spp., Haematoloechus spp., or both. Of the R. pipiens examined 33 had both nematode and trematode infections, 55 nematodes alone, 17 trematodes alone and 12 no helminths in their lungs. Of the B. hemiophrys examined 26 had both helminth infections, 22 nematodes alone, 18 trematodes alone, and 11 no helminths in their lungs. Table XIII shows the percentage incidence of parasites in the two sexes of the amphibian host. It is evident from the table that in general amphibians in Class B were more parasitized by both nematodes and trematodes than in Class A. The incidence of parasites (mean number per lung) in left and right lungs is about equal in both amphibians.

Analysis of the occurrence of Rhabdias and Haematoloechus was carried out using the Chi square method. In this analysis the hypothesis that the occurrence of one form of helminth precludes the occurrence of the other was tested. A two by two contingency test indicated that occurrence of one form of helminth did not preclude the occurrence of the other form: (Tables XIV and XV).

TABLE XIII

Incidence of helminths in the lungs of Male and Female amphibians

ss Parasite	Amphibian	Males parasitized %	Mean no. of parasite per male	Mean no. of parasites in left lung of male	Mean no. of parasites in right lung of male
Rhabdias sp. (13)*	R. pipiens	61.5	10.1	5.1	5.0
Haematoloechus (13) ssp.	R. pipiens	23	2.1	1.1	0.9
Rhabdias sp. (13)	B. hemiophrys	37.4	0.4	0	0.4
Haematoloechus (13) ssp.	B. hemiophrys	46.2	3.3	1.8	1.4
Rhabdias sp. (26)	R. pipiens	76.9	4.6	2.5	2.1
Haematoloechus (26) ssp.	R. pipiens	69.2	4.4	2.2	2.2
Rhabdias sp. (30)	B. hemiophrys	70	3.9	1.2	2.5
Haematoloechus (30) ssp.	B. hemiophrys	63.3	3.9	1.9	2.1

Number in brackets refer to number of individuals examined.

Table XIII cont'd,

ss Parasite	Amphibian	Females parasitized %	Mean no. of parasites per female	Mean no. of parasites in left lung of female	Mean no. of parasites in right lung of female
Rhabdias sp. (28)	R. pipiens	92.9	7.3	3.1	4.1
Haematoloechus (28) ssp.	R. pipiens	10.7	0.4	0.3	0.2
Rhabdias sp. (12)	B. hemiophrys	83.3	2.8	1.2	1.6
Haematoloechus (12) ssp.	B. hemiophrys	33.3	1.6	0.8	0.6
Rhabdias sp. (50)	R. pipiens	70	7.9	3.9	3.9
Haematoloechus (50) ssp.	R. pipiens	50	3.4	1.7	1.6
Rhabdias sp. (22)	B. hemiophrys	54.5	6.2	2.4	3.7
Haematoloechus (22) ssp.	B. hemiophrys	68	6.8	3.3	3.5

TABLE XIV

Lungs of R. pipiens. Nematodes vs. Trematodes

(Figures represent the number of frogs examined)

		Trematodes		
		present	absent	total
Nematodes	present	33	55	88
	absent	17	12	29
	total	50	67	117

$$X^2 = \frac{(/396-935/ - 58.5)^2 \times (117)}{8,549,200}$$

$$= 3.16$$

TABLE XV

Lungs of B. hemiophrys. Nematodes vs. Trematodes

(Figures represent the number of toads examined)

		Trematodes		
		present	absent	total
Nematodes	present	26	22	48
	absent	18	11	29
	total	44	33	77

$X^2 = 1.25$

CONCLUSIONS

Rhabdias in R. pipiens closely agrees with the description given by Gupta, (1960) and differs in the description given by Walton (1929) in the following:- a/ the size of nematodes, are larger, b/ heterogonic pattern of development, c/ smaller infective juveniles, d/ position of excretory pore in the parasitic forms. The species in R. pipiens is retained as Rhabdias ranae and the species of Rh. joaquinensis is subjectively synonymized with Rh. ranae.

The species in B. hemiophrys is closely related to Rh. bufonis and Rh. bicornis. It is closely related to Rh. bicornis in length, but differs in the shape of the mouth. Since filariform stages of Rhabdias from R. pipiens could infect B. hemiophrys; the Rhabdias in B. hemiophrys is designated as a variety of R. ranae; Rh. ranae var. hemiophrys.

The life cycle of the parasitic lung nematodes originating from R. pipiens and B. hemiophrys follow the heterogonic pattern of development.

Hlynka (1970) showed that competition did not exist between parasitic adult Rhabdias and Haematoloechus in both R. pipiens and B. hemiophrys of the University Field Station, Delta Marsh. My findings agree with his as I found no degree of interspecific relationsbip between the two helminths. The reason for this lack

of competition might be due to the way in which these parasites occur in the lungs. Even though both feed on blood extracted from the capillaries of the amphibians lungs, Haematoloechus spp. are known to be sanguivorous while Rhabdias spp. lie free in the lumen of the lung or occur coiled up in the alveoli.

REFERENCES

REFERENCES

Bouchard, J. L. 1951. The Platyhelminthes parasitising some Northern Maine Amphibia. Trans. Am. Micro. Soc. 70: 245-250.

Brandt, B. G. 1936. Parasites of certain North Carolina Salientia. Ecol. Monorg. 6: 491-532.

Bravo-Hollis, M. and y.C.E. Caballero, 1940. "Nematodes parasitos de los batracios de Mexico, IV". An. Inst. Biol. Uni. Mex, 11: 239-247.

Chabaud, A. G. E. R. Nrygoo et A. J. Petter. 1961. Description et caracters biologiques de deux nouveaux Rhabdias Malgaches. Ann. de Parasit. Humaine et Comp. 38: 752-763.

Chandler, A. C. Life history. Parasites of Verterbrates. (In Nematology Sec. 11). Edited by Christie. pp. 269-272.

Chu, T. 1936a. A review of the status of the reptilian nematodes of the genus Rhabdias with a redescription of Rh. fuscovenosa var. catanensis (Rizzo 1902) new rank. Jour. Parasit. 22: 130-139.

_____.1936b. Studies on the life history of Rhabdias fuscovenosa var. catanensis (Rizzo, 1902) Ibid.22: 140-160.

Cort, W. W. 1915. North American frog lung flukes. Trans. Amer. Micro. Soc. 34: 203-243.

Fantham, H. B. and A. Porter, 1948. The parasitic fauna of vetebrates in certain Canadian fresh waters,with some remarks on their ecology, structure and importance. Proc. Zool. Soc. Lond. 117: 609-649.

Fulleborn, 1920. Life history. Parasites of vertebrates. (In Nematology, Section 11). Edited by Christie. pp. 269-272.

_____. 1928. Ueber der Infektionsweg bei Rhabdias bufonis (Rhabdonema nigrovenosum) des Frosches nebst Versuchen uber die Pymphzirkulation des letzteren. Centralblatt fur Bakt., Parasit., und Infektionskrankheitn 109: 444-462.

Goodey, T. 1924a. The anatomy and life history of the nematode Rhabdias fuscovenosa (Railliet) from the grass snake Tropidonotus natrix. Jour. Helm. 2: 51-64.

Goodey, T. 124b. Two new species of the nematode genus Rhabdias. Ibid. 2: 203-208.

Goodey, J. B. 1963. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture, Fisheries and Food. Technical Bulletin No. 2. London.

Gupta, S. P. 1960. Nematode parasites of Vertebrates of East Pakistan. Can. Jour. Zool. 38: 745-750.

Harwood, P. D. 1932. The helminths parasitic in the Amphibian and Reptilia of Houston, Texas and vicinity. Proc. U.S.A. Nat. Mus. 81: 1-71.

Hlynka, L. J. 1970. Helminths in Rana pipiens and Bufo hemiophrys. from the Delta Marshes, Manitoba. University of Manitoba M.Sc. thesis. Unpublished.

Ingles, L. G. 1932. Four new species of Haematoloechus from Rana aurora Draytoni from California Univ. Cal. Publ. Zool. 37: 189-201.

_____. 1933. Studies on the structure and life history of Ostolium oxyorchis (Ingles) from the California red-legged frog, Rana aurora draytoni. Univ. Calif. Publ. Zool. 39: 135-161.

_____. 1936. Worm parasites of California Amphibia. Trans. Am. Micro. Soc. 55: 73-92.

Krull, W. H. 1930. The life history of two North American frog lung flukes. Jour. Parasit. 16: 207-212.

_____. 1931. Life history studies on two lung flukes. Pneumonoeces medioplexus and Pneumobites parviplexus. Trans. Am. Micro. Soc. 50: 215-277.

_____. 1934. Some additional notes on the life history of a frog lung fluke, Haematoloechus complexus (Seely 1906) Krull. Ibid. 53: 196-199.

Kuntz, R. E. and J. T. Self. 1944. An ecological study of the metazoan parasites of the Salientia of Comanche Country, Oklahoma. Proc. Ikla. Acad. Sci. 25: 35-38.

Kurochkin, Y. V. and E. P. Guskov. 1963. (A new nematode species from the eye of Natrix natrix). Izdatelskvo Akad. Nauk. SSSR. pp. 183-185.

Leidy, J. 1851. Contributions to helminthology. Proc. Acad. Nat. Sc. Phila. 5: (12) 349-351.

Looss, A. 1899. Weiter Beitrage sur Kenntniss der Trematoden Fauna Aegyptens. Zool. Jahr. 12: 521-784. (Not examined but cited in Bouchard 1951).

Lu, S. C. 1934. On Rhabdias, a genus parasitic nematode of Nankin. Sinensi 5: 164-172.

Manter, H. W. 1938. A collection of Trematodes from Florida Amphibia. Trans. Amer. Micro. Soc. 57: 26-38.

Markov, G. S. 1955. (Annual differences in the parasitic fauna of grass frogs Rana temporaria). Russian text. Zool. Zh. 34: 1203-1209.

Mazurmovich, B. N. 1957. (On the interrelationship between the parasitic helminths of amphibia). Trud. Leningr. Obshch. Estestvoisp. 73: 204-207.

Rankin, J. S. 1945. An ecological study of the helminth parasites of amphibians and reptiles of western Massachusetts and vicinity. Journ. Parasit. 31: 142-156.

Schell, S. C. 1965. The life history of Haematoloechus breviplexus. Stafford 1902, (Trematoda: Haplometridae McMullen, 1937), with emphasis on the development of the sporocysts. Jour. Parasit. 51: 587-593.

Seinhorst, J. W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematogica 4: 67-69.

_____. 1962. On the killing, fixation and transferring to glycerin of nematodes. Nematologica 8: 29-32.

Stafford, J. 1905. Trematodes from Canadian vertebrates. Zool. Anz. 28: 681-694.

Stal, C. 1875. Enumeratio Hemipterorum. Bidragtill en Forteckning Ofver aller Hittils Kanda Hemiptera jempte Systematiska Meddelanden Pt. 4. K. Svenska Vetenskapsakademien Handlingar n.s. 12(1): 3-186. (Not examined but cited in Bouchard 1851).

Stilles, C. C. and H. Hassall 1905. The determination of generic types, and a list of roundworm genera, with their original and type species. U.S. Dept. Agri; Bur. Anim. Ind. Bul 79: 150 pp.

Travassos L. 1926. Entwicklung des Rhabdias fulleborni n. sp. Arch. Schiffs-u Tropenhyg 30: (9), 594-602.

Walton, A. C. 1929. Studies on some nematodes of North American frogs. Jour. Parasit. 15: 227-240.

_____. 1938. The nematoda as parasites of amphibia. Tr. Am. Micro. Soc. 57: (1) 38-53.

_____. 1941. Amphibian nematodes from the Gaspe Peninsula and vicinity. Jour. Parasit. 27: 59-61.

Williams, R. W. 1960. Observations on the life history of Rhabdias sphaerocephala Goodey, 1924 from Bufo marinus L. in the Bermuda Islands. Jour. Helm. 34: 93-98.

Yamaguti, S. 1941. Studies on the helminth fauna of Japan. Part 34. Amphibian Nematodes, 11. Jap. J. Zool. 9: (3) 397-399.

_____. 1958. Systema Helminthum. Vol. 1. The Digenetic Trematodes of Vertebrates. New York Interscience.

_____. 1961. Systema Helminthum. Vol. 111. The nematodes of vertebrates Part 1. New York Interscience.

Yuen, P. H. 1965. Some studies on the taxonomy and development of some Rhabdisoid and Cosmocercoid nematodes from Malayan Amphibians. Zool. Anz. 174: 275-285.

APPENDIX

APPENDIX I

Collection and Dissection Data

Species Locality

Date collected: Species no.:

Sex: Species length: Class

Date dissected:

HELMINTHS TREMATODES NEMATODES

Lungs Right: Left Right: Left

Peritonial cavity NEMATODES

Additional Information.

APPENDIX II

Lung Nematode Data

Species

Nematode no:

Location:

Date processed:

1. Length:

2. Length of oesophagus:

3. Position of vulva from anterior end.

4. Tail length:

5. Nerve ring from cephalic extremity:

6. Excretory pore from cephalic extremity:

APPENDIX III

Mean: The mean of a set of n measurements is equal to the sum of the measurements divided by n.

Range: The smallest and the largest specimens of a sample.

Standard deviation: The square root of the sum ($\leqslant$) of the squared deviations (d) from the mean, divided by N.

$$S.D. = \sqrt{\frac{\leqslant d^2}{N}}$$

Standard error: The standard deviation divided by the square root of the sample size (N).

$$S.E. = \frac{S.D.}{\sqrt{N}}$$