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A comparative study of the parasite of certain salientia from Pocahontas State Park, Virginia

Ronald Arthur Campbell

A COMPARATIVE STUDY

OF THE PARASITES OF CERTAIN SALIENTIA

FROM POCAHONTAS STATE PARK, VIRGINIA

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ABSTRACT

Four species of salientia representing three selected habitats were collected from May 5th through October 4th, 1966 and examined for parasites. Of the 116 host specimens, 30 were Rana catesbeiana Shaw; 29 Rana clamitans Latreille; 29 Bufo fowleri Hinckley; 28 Hyla versicolor (Le Conte). A total of 34 species of parasites was recovered: Protozoa, 10; Trematoda, 9; Cestoda, 2; Nematoda, 11; Acanthocephala, 1; and Acarina, 1.

Although a relatively small number of hosts was examined, the results generally agree with those found in more extensive investigations of similar type. The aquatic hosts, R. catesbeiana and R. clamitans, harbored the greatest number of parasitic species. Infections of the terrestrial hosts, B. fowleri and H. versicolor, were fewer in number of species but significantly higher in number of specimens. Of the four representative hosts examined, the arboreal host, H. versicolor, showed fewer incidences of infection and harbored the least number of parasitic species.

INTRODUCTION

The variety of habitats in which salientians live provides excellent opportunities to study the relationship between parasites and environmental conditions. Although numerous important studies have dealt with amphibians and their helminth parasites, few comprehensive studies have been published concerning the ecology of salientian parasites in North America.

Comprehensive studies by Holl (1932), Brandt (1937), and Rankin (1945) have shown that salientians habitually associated with aquatic habitats were more often parasitized by a greater variety of species than terrestrial hosts. Attempts also have been made to correlate seasonal changes with the numbers of various parasites of salientia (Fortner, 1923; Holl, 1932; Brandt, 1937; Rankin, 1945)..Most studies agree that infections generally begin during the breeding season, increasing during the warmer summer months to a maximum prior to hibernation. Holl (1932), however, reports that "cricket frogs apparently become infected furing the breeding season and have no helminth parasites during the remainder of the year." Brandt's (1937) study comparing size, age, and habitats of aquatic salientia from North Carolina showed that they were infected with a variety of parasites. Larger and presumably older frogs harbored greater numbers of parasites than smaller individuals. Rankin (1945) found more parasitic species in aquatic than terrestrial salientia and concluded that the distribution of helminths depended upon three factors: habitat; life cycles of host and parasite; and host specificity.

Knowledge of the parasites of amphibians in Virginia is very limited. A review of the literature shows that Jordan and Reynolds (1932) made cytological studies on the trematode Diplodiscus temperatus (Stafford).

Walton (1933) reported Pharygodon armatus Walton from the intestine of Rana clamitans Latrielle and Rana pipiens Schreber collected near Charlottesville, Virginia. Walton (1931) reported another nematode, larval Physaloptera ranae Walton, encysted in the stomach of Rana catesbeiana Shaw. More recently, Miss C. M. Russell (1951) conducted a survey of the parasites of the newt Notophthalmus v. viridescens, also in the vicinity of Charlottesville.

It is surprising that, although frogs and toads have long been used as material for the study of parasites, and although comprehensive surveys of the parasites of these amphibia have been made in several other states, there has been no such study made in Virginia. The present paper attempts not only to survey the parasites of certain salientia in a selected area but to study the relations of these parasites to the habits and habitats of hosts.

COLLECTING AREA

The salientia used in the present study were collected in Pocahontas State Park, located in Chesterfield County in the east central part of Virginia. The park is a large natural undeveloped area consisting of 7, 604 acres of woodlands situated at the confluence of the gently rolling Piedmont hills and the Coastal Plains. The altitude within the park varies from 120-200 feet above sea level; drainage is poor and the soil is a sandy loam. The climate is hot and humid during the summer months and mild in the winter.

After several preliminary surveys of the area it was decided that several species of salientia representing different habitats would be studied. Aquatic specimens were collected from Beaver Lake as this area was relatively undisturbed and therefore in a more natural state than

the Swift Creek Lake which is provided with recreational facilities for public use. Beaver Lake is about 25 acres in area, surrounded by pine and hardwood forest and bordered by heavy underbrush. The dominant vegatation on the northeastern and northwestern banks is swamp azalea (Rhododendron viscosum Linnaeus), smooth alder (Alnus rugosa (DuRoi) Sprengel), and American hornbeam (Carpinus caroliniana Walter), while that of the southwestern and southern banks is a composite of hardwoods bordered by swamp fetter bush (Leucothoe racemosa Gray), and arrowhead (Saggitaria latifolia Willd). The dominant hardwood flora consists of beech (Fagus grandifolia Ehrhart), sweet gum (Liquidambar styraciflua Linnaeus), tulip tree (Liriodendron tulipfera Linnaeus), mocker nut hickory (Carya tomentosa Nuttall), red maple (Acer rubrum Linnaeus), and white oak (Quercus alba Linnaeus). A zone of river birch (Betula nigra Linnaeus) grows along all banks except the southern which is bordered by beech (F. grandifolia). Aquatic flora consists of an abundance of waterweed (Elodea canadensis (Michx.) Planchon), water pennywort (Hydrocotyle ranunculoides Linne), pondweed (Potamogeton strictifolius Benn), water shield (Brasenia schreberi Gmelin), and the common cat-tail (Typha latifolia Linnaeus). Approximately 75% of the lake is covered by the white water lilly (Nymphaea odorata Aiton).

MATERIALS AND METHODS

The three types of habitats selected for study were aquatic, terrestrial and arboreal. The aquatic species studied were the bullfrog (Rana catesbeiana), and the green frog (Rana clamitans). Fowler's toad (Bufo fowleri Hinckley) was selected as a representative of a terrestrial habitat. This toad is characteristically terrestrial but visits an aquatic environment during the breeding season from spring to mid-August. The gray tree

frog, Hyla versicolor (Le Conte), was selected as a representative of an arboreal habitat. It breeds in puddles and temporary surface water from April to mid-August.

Terrestrial specimens were collected by hand with the aid of a battery-powered spotlight. A row boat, spotlight, and a nylon net equipped with a long handle were used to capture aquatic specimens. All the animals were placed in damp cloth sacs.

The majority of examinations were made within 24 hours after the time of collection. No specimens were examined after 48 hours. Examinations were conducted externally and internally with the aid of a stereoscopic microscope. The alimentary tract was removed and the esophagus, stomach, small intestine, and colon were separated from each other. These organs plus the heart, liver, pancreas, kidneys, and bladder were placed in individual Syracuse watch-glasses containing 0.65% saline solution.

Helminth parasites were removed, counted, and either studied alive or prepared for permanent mounts. Identifiable remains from the stomach contents were recorded. Blood smears (to detect protozoa) were fixed in methyl alcohol and stained with Giemsa blood stain or Delafield's hematoxylin. Fecal smears for intestinal protozoa were fixed in Schaudinn's fixative and stained in Delafield's hematoxylin. Trematodes were washed in 0.65% saline solution, relaxed in tap water, fixed in acetic-formol-alcohol (A.F.A.:2-10-50), stained with alum cochineal or Grenacher's borax carmine, and mounted in balsam.

Cestodes were treated in the same manner as trematodes and stained with Harris' hematoxylin. Serial sections were studied both in transverse and frontal views.

Nematodes were killed and fixed in hot A. F. A., stored in 70%

glycerine alcohol, and mounted in glycerine jelly or Yetwin's mounting medium.

The single spiny-headed worm was washed in 0.65% saline solution, and relaxed in tap water until the proboscis was fully everted. The specimen was killed and fixed in A. F. A., stained with Harris' hematoxylin, cleared in benzene, and mounted in balsam.

Mites were fixed in A. F. A., stored in 70% glycerine alcohol, and mounted in glycerine jelly for examination.

All drawings accompanying this work were made with the aid of a camera lucida.

RESULTS

Thirty-four species of parasites from 116 hosts were identified from original literature, and classified according to the most recent literature. Five taxonomic groups are represented: Protozoa, 10 species; Trematoda, 9 species; Cestoda, 2 species; Nematoda, 11 species; Acanthocephala, 1 species; and Acarina, 1 species.

The following section includes the classification of parasites from the four species of salientia, and pertinent observations pertaining thereto:

Phylum PROTOZOA Goldfuss, 1818, emend. von Siebold, 1845

Subphylum SARCOMASTIGOPHORA Honigberg and Balamuth, 1963

Superclass MASTIGOPHORA Diesing, 1866

Class ZOOMASTIGOPHORA Calkins, 1909

Order KINETOPLASTIDA Honigberg, 1963

Family TRYPANOSOMATIDAE Doflein, 1901

Trypanosoma rotatorium (Mayer, 1843)

Reference: Kudo, R. R., (1966), and Wenyon, C. M., (1924).

Hosts: R. catesbeiana, R. clamitans, H. versicolor, and B. fowleri.

Habitat: Blood.

Remarks: Seventy-five % of the specimens of R. catesbeiana and 56% of the R. clamitans harbored this haemoflagellate. Low incidences of infection, 6.9 and 7.1%, were noted for B. fowleri and H. versicolor respectively.

Study of the morphological characters of this protozoan reveals a very close similarity to T. rotatorium Kudo (1966). However, two forms, large and small, of this species were found in the same host. Mean body measurements of the large forms were somewhat greater than those described by Wenyon (1924). The large forms lacked a free flagellum and measured 49.8u in length by 6-12u in width. The small forms possessed a long trailing flagellum and measured 32.7u in body length by 3.3u in width.

Order OXYMONADIDA Grasse, 1952

Family HEXAMITIDAE Kent, 1880

Octomitus intestinalis Prowazek, 1904

Reference: Kudo, R. R., 1966.

Hosts: R. catesbeiana, R. clamitans, H. versicolor, and B. fowleri.

Habitat: Colon.

Remarks: This protozoan was found in more than 50% of the specimens of H. versicolor and B. fowleri. Infections of R. clamitans and R. catesbeiana with the protozoan were more numerous, however, being 82.7 and 90% respectively.

Order TRICHOMONADIDA Kirby, 1947

Family TRICHOMONADIDAE Wenyon, 1926

Tritrichomonas augusta Alexieff, 1911

Reference: Kudo, R. R., 1966.

Hosts: R. catesbeiana, R. clamitans, H. versicolor, and B. fowleri.

Habitat: Colon.

Remarks: Tritrichomonas augusta was the most common of all the protozoans found in this study. The percentage of infections was found to be higher in R. catesbeiana and R. clamitans than in H. versicolor and B. fowleri (Table 1).

Superclass OPALINATA Corliss and Balamuth, 1963

Order OPALINIDA Poche, 1913

Family OPALINIDAE Stein, 1860

Opalina hylaxena Metcalf, 1923

Reference: Metcalf, M. M., 1923.

Host: H. versicolor.

Habitat: Colon.

Remarks: Fifty % of the specimens of H. versicolor harbored this opalinid. Measurements of the general body dimensions were somewhat smaller than those described by Metcalf (1923), however, diameters of the nuclei and endospherules, and the cilia line intervals, agree with his measurements.

Opalina triangulata Metcalf, 1923

Reference: Metcalf, M. M., 1923.

Hosts: H. versicolor and B. fowleri.

Habitat: Colon.

Remarks: Heavy infections of O. triangulata were found in approximately 21% of the specimens of H. versicolor and 7% of those of B. fowleri.

Opalina virguloidea Metcalf, 1923

Reference: Metcalf, M. M., 1923.

Host: R. catesbeiana.

Habitat: Colon.

Remarks: A single specimen of R. catesbeiana was found to be infected with this opalinid.

Superclass SARCODINA Hertwig and Lesser, 1874

Class RHIZOPODEA von Siebold, 1845

Order AMOEBIDA Kent, 1880

Family ENDAMOEBIDAE Calkins, 1902

Entamoeba ranarum Grassi, 1879

Reference: Wenyon, C. M., 1924.

Hosts: R. catesbeiana and R. clamitans.

Habitat: Colon.

Remarks: Three specimens each of R. clamitans and R. catesbeiana were found to harbor this amoeba.

Subphylum SPOROZOA Leuckart, 1879

Class TELOSPOREA Schaudinn, 1900

Order EUCOCCIDIA Leger and Duboscq, 1910

Family HAEMOGREGARINIDAE Neveu-Lemaire, 1901

Haemogregarina sp. Danilewsky, 1885

Reference: Kudo, R. R., 1966.

Hosts: R. catesbeiana, R. clamitans, and B. fowleri.

Habitat: Blood.

Remarks: Merozoites of a haemogregarine were found in approximately 24% of the specimens of R. catesbeiana and R. clamitans studied. It was impossible to determine the species because of lack of other stages in the life cycle.

Subphylum CILIOPHORA Doflein, 1901

Class CILITEA Perty, 1852

Order TRICHOSTOMATIDA Butschlii, 1889

Family BALANTIDIIDAE Doflein and Reichenov, 1879

Balantidium sp. Claparede and Lachmann, 1858

Reference: Kudo, R. R., 1966.

Host: H. versicolor.

Habitat: Colon.

Remarks: It was impossible to determine the species due to distortion resulting from fixation.

Order SPIROTRICHA Butschlii, 1889

Family PLAGIOTOMIDAE Butschlii, 1889

Nyctotherus cordiformis Ehrenberg, 1838

Reference: Kudo, R. R., 1966.

Hosts: R. catesbeiana, R. clamitans, H. versicolor, and B. fowleri.

Habitat: Colon.

Remarks: This ciliate was found to be a common parasite in terrestrial hosts (present in 24.1% of specimens of B. fowleri and 50% of H. versicolor) but was rarely encountered in aquatic specimens (present in 3.3% of R. catesbeiana and 3.4% of R. clamitans).

Phylum PLATYHELMINTHES (Minot, 1876)

Class TREMATODA Rudolphi, 1808

Order MONOGENEA Carus, 1863

Family POLYSTOMATIDAE Gamble, 1896

Subfamily POLYSTOMATINAE Gamble, 1896

Polystoma nearcticum Paul, 1938 (Figure 1)

Reference: Paul, A. A., 1938.

Host: H. versicolor.

Habitat: Colon.

Remarks: A single bladder form of this trematode was obtained from H. versicolor. Few records of this trematode have been reported from the eastern United States.

Order DIGENEA V. Beneden, 1858

Family PARAMPHISTOMIDAE Fischoeder, 1901

Megalodiscus temperatus (Stafford, 1905) (Figure 2)

Reference: Stafford, J., 1905.

Hosts: *R. catesbeiana*, *R. clamitans*, and *B. fowleri*.

Habitat: Colon.

Remarks: All 60 specimens of this trematode consistently agreed with Stafford's description. The worms varied from 2.4 to 5.8 mm in length and in all cases the acetabulum was wider than the body. Two testes and a small sucker in the center of the acetabulum were present in all specimens, but some authors (Harwood, 1932; Manter, 1938) have noted the absence of these structures in small specimens.

One of the 28 specimens of *B. fowleri* harbored a single worm, probably acquired during the breeding season.

Family BRACHYCOELIDAE S. J. Johnston, 1913

Glypthelmins subtropica Harwood, 1933

Reference: Harwood, P. D., 1933.

Hosts: *R. catesbeiana* and *R. clamitans*.

Habitat: Small intestine.

Family GORGODERIDAE Looss, 1901

Gorgodera amplicava Looss, 1899

Reference: Stafford, J., 1902a.

Host: *R. catesbeiana*.

Habitat: Colon and urinary bladder.

Remarks: One specimen of *G. amplicava* was found in each of five specimens of *R. catesbeiana*.

Gorgoderina bilobata Rankin, 1937

Reference: Rankin, J. S., Jr., 1937.

Host: *R. catesbeiana*.

Habitat: Urinary bladder.

Remarks: This trematode occurred only in a single instance of infection.

Family PLAGIORCHIDAE Luhe, 1901

Haematoloechus breviplexus Stafford, 1902

Reference: Stafford, J., 1902.

Hosts: R. catesbeiana and R. clamitans.

Habitat: Lungs and body cavity.

Remarks: Four specimens of R. catesbeiana and 7 specimens of R. clamitans harbored trematodes of this species. One specimen of R. clamitans harbored 44 adults, 34 of which were found in the body cavity. It was not ascertained as to how these trematodes gained access to the body cavity as there were no signs of active penetration. Subsequent examinations failed to reveal a similar infection in this situation.

Haematoloechus varioplexus Stafford, 1902 (Figure 3)

Reference: Stafford, J., 1902.

Hosts: R. catesbeiana and R. clamitans.

Habitat: Lungs.

Remarks: A low frequency of infection of H. varioplexus was found in R. catesbeiana and R. clamitans. Some specimens of this fluke showed a slight lobing of the testes, a deviation from the entire margin described by Stafford (1902).

Haematoloechus sp. Looss, 1899

Reference:

Host: B. fowleri.

Habitat: Lungs.

Remarks: A single specimen was recovered. Measurements of the general body structures are somewhat larger than those given for H. similiplexus Stafford (1902). It also differs from H. similiplexus in that the lateral folds of the uterus posterior to the testes extend extracecally thus

completely transversing the posterior body field. Stafford (1902) states that the lateral uterine folds of H. similiplexus are always median and posterior to the testes never extending extracecally. As no additional specimens were recovered subsequently, a mean could not be established.

Family LECITHODENDRIIDAE Odhner, 1910

Loxogenoides bicolor (Krull, 1933) (Figure 4)

Reference: Krull, W. H., 1933a.

Host: R. clamitans.

Habitat: Bile duct and stomach.

Remarks: This pigmented fluke was found in the bile duct of 31% of the specimens of R. clamitans. On two occasions several of these worms were also found in the stomach; Krull (1933) made the same observation. The testes and egg size of these trematodes were slightly larger and the body was more attenuated posteriorally than described by Krull (1933).

Class CESTODA Rudolphi, 1819

Order CYCLOPHYLLIDEA Braun, 1900

Family NEMATOTAENIIDAE Luhe, 1910

Cylindrotaenia americana Jewell, 1916

Reference: Jewell, M. E., 1916.

Hosts: R. catesbeiana and B. fowleri.

Habitat: Small intestine.

Remarks: Approximately 7% of the R. catesbeiana and 11% of the B. fowleri harbored this tapeworm.

Distoichometra bufonis Dickey, 1921

Reference: Dickey, L. D., 1921.

Host: B. fowleri.

Habitat: Small intestine.

Remarks: Sixteen % of the specimens of B. fowleri were parasitized by this worm.

Phylum ASCHELMINTHES Grobben, 1908

Class NEMATODA Grobben, 1910

Family RHABDIASIDAE Railliet, 1915

Rhabdias ranae Walton, 1929

Reference: Walton, A. C., 1929.

Hosts: *R. catesbeiana* and *R. clamitans*.

Habitat: Lungs.

Remarks: In this study *R. ranae* was found only in aquatic species. The frequency of infection (24.1%) was about four times that found in *R. catesbeiana*.

Rhabdias bufonis (Schrank, 1788)

Reference: Walton, A. C., 1935.

Host: *B. fowleri*.

Habitat: Lungs.

Remarks: Approximately 71% of the specimens of *B. fowleri* were heavily parasitized by this nematode.

Family TRICHOSTRONGYLIDAE Leiper, 1912

Oswaldocruzia pipiens Walton, 1929

Reference: Walton, A. C., 1929.

Hosts: *R. catesbeiana* and *B. fowleri*.

Habitat: Stomach and small intestine.

Remarks: The frequencies of infection of *B. fowleri* and *R. catesbeiana* with this nematode were 32.1 and 10% respectively.

Oswaldocruzia waltoni Ingles, 1936

Reference: Ingles, L. G., 1936.

Host: *R. clamitans*.

Habitat: Small intestine.

Remarks: This nematode infected 17% of the *R. clamitans* examined.

Family OXYURIDAE Cobbold, 1864

Cosmocerocoides dukae Holl, 1928

Reference: Holl, F. J., 1928.

Hosts: R. catesbeiana, R. clamitans, H. versicolor, and B. fowleri.

Habitat: Colon.

Remarks: The incidence of infection of this nematode was lower than that noted by other investigators (Holl, 1932; Brandt, 1937; Rankin, 1945) (10% or less in all hosts). This round worm differed in the number of caudal plectanes from those described by Holl (1928). Holl described twelve pairs of caudal plectanes in males; one pair post-anal and eleven pairs pre-anal. In the present material the pre-anal plectanes vary in number from 9-11 pairs while the number of post-anal plectanes remain constant.

Cosmoceralla haberi Steiner, 1924

Reference: Steiner, G., 1924.

Host: H. versicolor.

Habitat: Colon.

Remarks: Three of the specimens of H. versicolor showed heavy infections (50 or more worms per specimen).

Oxysomatium variabilis Harwood, 1930

Reference: Harwood, P. D., 1930.

Hosts: R. catesbeiana, R. clamitans, H. versicolor, and B. fowleri.

Habitat: Colon.

Remarks: The highest incidence of infection with this nematode was observed in the terrestrial B. fowleri (78.5%), while the lowest incidence of infection was in the arboreal H. versicolor (10.7%).

Family FILARIIDAE (Cobbold, 1864) Claus, 1885

Foleyella americana Walton, 1927

Reference: Walton, A. C., 1929.

Hosts: R. catesbeiana, R. clamitans, and B. fowleri.

Habitat: Abdominal mesenteries and bladder.

Remarks: The highest incidence of infection with this nematode was observed in R. catesbeiana. These worms are, on an average, smaller than described by Walton (1929). All infections were light.

On two occasions adult worms were removed from the lumen of the urinary bladder. These nematodes were identical in character with those recovered from the abdominal mesenteries. Reiber (1941) found a similar condition in Rana pipiens and B. fowleri from Reelfoot Lake, Tennessee.

Family SPIRURIDAE Orley, 1885

Physaloptera ranae Walton, 1931

Reference: Walton, A. C., 1931.

Hosts: R. catesbeiana, R. clamitans, H. versicolor, and B. fowleri.

Habitat: Stomach.

Remarks: Twenty-eight % of the specimens of B. fowleri were parasitized by this helminth. One heavy infection yielded 25 specimens from the stomach of H. versicolor.

Collective Names

Agamonema sp. Diesing, 1851

Reference: Bayliss, H. A. and R. D. Aubney. 1930.

Hosts: R. catesbeiana, R. clamitans, H. versicolor, and B. fowleri.

Habitat: Viscera.

Remarks: The terrestrial host, B. fowleri, showed the largest number of infections (38.5%) of larval nematodes.

Family FILARIIDAE Claus, 1885

Microfilaria sp. Cobbold, 1880

Reference: Bayliss, H. A. and R. D. Aubney. 1930.

Host: R. catesbeiana.

Habitat: Blood.

Phylum ACANTHOCEPHALA Rudolphi, 1809

Class METACANTHOCEPHALA Van Cleave, 1948

Family POLYMORPHIDAE Meyer, 1931

Centrorhyncus wardae Holloway, 1956 (Figure 5 and 6a, b, c, d)

Reference: Holloway, H. L., 1958.

Host: R. clamitans.

Habitat: Small intestine.

Remarks: A single immature female acanthocephalan closely conforming to the characters and measurements described by Holloway (1956) for Centrorhyncus wardae was found in the intestine of a specimen of R. clamitans. The known species of this worm normally infect birds but also are known to occur in a variety of mammals. Read (1950) has shown that members of the genus Centrorhyncus have a very low host specificity. Adult acanthocephalans from frogs are rare and this is the first report of an adult of this genus from a frog.

The worm (Figure 5) is 3.24 mm in length with a maximum diameter of 0.59 - 0.65 mm slightly posterior to mid-body. The proboscis (Figure 6a) is 0.97 mm in overall length with a width of 0.32 mm at the anterior end increasing posteriorally to a width of 0.35 mm at the insertion of the proboscis receptacle. The proboscis is 0.61 mm long from the apex to the insertion of the proboscis receptacle and 0.36 mm in length from that point to the basal row of posterior hooks. The proximal portion of the proboscis varies from 0.29 - 0.31 mm in width. The proboscis is armed with from 34 - 36 longitudinal rows of 18 - 19 hooks each. The first five hooks in each row are strongly recurved, stout, and possess well developed root plates. These hooks measure 0.041 - 0.048 mm from the tip of the

thorn to the base (Figure 6b). Hooks from rows 6 through 12 are slightly curved and are 0.036 - 0.042 mm in length (Figure 6c). The root plates of these hooks are poorly developed and are absent on the basal hooks of rows 13 through 19. The basal hooks exhibit a slighter curvature than the preceding hooks and measure 0.032 - 0.034 mm in length (Figure 6d). Lemnisci are paired, long, and measure 0.81 mm in length by 0.14 in width. The proboscis receptacle is a two-layered sac measuring 1.2 mm in length by 0.19 mm in width at the point of insertion. The proboscis receptacle tapers posteriorally to a point from which two proboscis retractors emerge. Each of the retractors originates at the apex of the proboscis, passes through the proboscis receptacle, and terminates on the body wall (Figure 5). The reproductive organs, though well stained, are not developed well enough to permit accurate description.

Two differences were noted between this specimen and those described by Holloway. The posterior portion of the trunk of Holloway's specimens is attenuated, whereas in the present specimen it is rounded. Furthermore, the length of the lemnisci is slightly greater (0.989 - 1.060 mm) compared to the single specimen recovered from R. clamitans (0.81).

According to Holloway (1958), "C. wardae can be clearly differentiated from all other species of this genus upon the basis of the number of longitudinal rows of hooks, the number of hooks per row and the size of the hooks". As the present specimen so closely agrees with the measurements given for the general characters this worm is tentatively identified as C. wardae until further knowledge concerning the members of this genus is obtained.

It is interesting to note that larval forms of this genus were obtained only from aquatic hosts.

Phylum ARTHROPODA von Siebold, 1845

Class ARACHNIDA Latreille, 1809

Order ACARINA Latreille, 1809

Family TROMBICULINAE Ewing, 1944

Hannemania penetrans Ewing, 1931

Reference: Ewing, H. E., 1931.

Hosts: R. catesbeiana, R. clamitans, and B. fowleri.

Habitat: Skin.

Collection data are summarized in Tables 1 through 5. In all the tables B. fowleri and H. versicolor, representing terrestrial and arboreal habitats respectively, are grouped alongside the aquatic representatives, R. catesbeiana and R. clamitans, to facilitate comparison. Tables 1 through 3 summarize the species of protozoan, trematode, cestode, and nematode parasites recovered and the percentages of hosts infected with each. These tables indicate that there is little variation in the percentage of infection with a particular parasite between salientian species from the same type of environment. However, noticeable differences may be seen in the variety of parasites in hosts from an aquatic environment as compared with hosts from a terrestrial environment (Table 2). Furthermore, marked differences in the extent of representation of parasites exist between hosts from aquatic and terrestrial environments, for example Nyctotherus cordiformis and Trypanosoma rotatorium (Table 1).

The data in Table 1 show that the salientia examined were commonly parasitized by a variety of protozoans, especially those that live in the intestine. Intestinal ciliates (Opalina spp., Nyctotherus cordiformis, and Balantidium sp.) were more often associated with hosts from a terrestrial environment whereas blood protozoa (Haemogregarina and Trypano-

soma) and the intestinal amoeba, Entamoeba ranarum, were found in aquatic hosts. Species of Opalina were consistently absent from R. clamitans. The intestinal flagellates Octomitus and Tritrichomonas were common to both terrestrial and aquatic hosts.

Table 1. Protozoan parasites of salientia examined. Figures in the heading indicate the number of each species of host examined. Figures in the table indicate the percentage of hosts infected.

	Terrestrial	Arboreal	Aquatic	Aquatic
	29	28	30	29
PROTOZOA	B. fowleri	H. versicolor	R. catesbeiana	R. clamitans
Entamoeba ranarum	-	-	10.0	10.3
Octomitus intestinalis	58.6	64.2	90.0	82.7
Haemogregarina sp.	-	-	23.3	24.1
Opalina virguloidea	-	-	3.3	-
O. triangulata	6.9	21.4	-	-
O. hylaxena	-	53.5	-	-
Nyctotherus cordiformis	24.1	50.0	3.3	3.4
Trypanosoma rotatorium	6.9	7.1	56.6	31.3
Tritrichomonas augusta	89.6	71.4	96.6	93.1
Balantidium sp.	-	14.2	-	-
% hosts infected by one or more sp. Protozoa.	89.6	78.5	96.6	93.1

Table 2 shows that trematode parasites were largely limited to aquatic hosts as would be expected. Cestode infection does not show this limitation.

Data concerning nematode parasites recovered is presented in Table 3. The number of species of nematodes from B. fowleri and H. versicolor represent the extremes of infection with 100% and 25% of the hosts parasitized respectively. Bufo fowleri averaged 52 worms per host while H. versicolor averaged 7.1 worms per host.

Parasites are arranged in subgroups in Table 4 according to the body system infected. This table summarizes the parasites, according to phylum, harbored by terrestrial and aquatic hosts. It is apparent that aquatic hosts were infected by a greater variety of parasites than terrestrial hosts. In general, Table 4 shows that infections of the aquatic salientia were heavier than the terrestrial species, with the exception of the terrestrial B. fowleri, which was predominantly infected by nematodes. H. versicolor was parasitized by the smallest number of species.

Multiple infections (Table 5) were common and some idea of the extent of these is indicated by the greatest number of parasitic species collected from a single individual.

Table 2. Trematode and Cestode parasites of salientia examined. Figures in the heading indicate the number of each species of host examined. Figures in the table: upper figures indicate the percentage of infection; lower figures indicate the average number of parasites per host.

	Terrestrial	Arboreal	Aquatic	Aquatic
	29	28	30	29
TREMATODA	B. fowleri	H. versicolor	R. catesbeiana	R. clamitans
Megalodiscus temperatus	3.4 0.04	-	36.6 1.23	48.2 0.75
Gorgodera amplicava	-	-	13.3 0.16	-
Gorgoderina bilobata	-	-	3.3 0.03	-
Glypthelmins subtropica	-	-	6.6 2.00	13.7 0.13
Haematoloechus breviplexus	-	-	13.3 0.43	24.1 2.31
Haematoloechus varioplexus	-	-	13.3 0.63	6.8 0.24
Haematoloechus sp.	3.4 0.04	-	-	-
Loxogenoides bicolor	-	-	-	31.0 1.06
Polystoma nearcticum	-	3.5 0.04	-	-
% hosts infected by one or more sp. Trematoda.	6.9 0.68	3.5 0.04	56.6 2.76	68.9 4.51
CESTODA				
Distoichometra bufonis	13.8 0.79	-	-	-
Cylindrotaenia americana	10.3 0.18	-	6.6 0.23	-
% hosts infected by one or more sp. Cestoda.	24.1 0.92	-	6.6 0.23	-

Table 3. Nematode parasites of salientia examined. Figures in the heading indicate the number of each species of host examined. Figures in the table: upper figures indicate the percentage of hosts infected; lower figures, when present, indicate the average number of parasites per host.

	Terrestrial	Arboreal	Aquatic	Aquatic
	29	28	30	29
NEMATODA	B. fowleri	H. versicolor	R. catesbeiana	R. clamitans
Agamonema sp.	38.5 39.20	7.1 3.60	13.3 27.23	10.3 10.3
Cosmoceralla haberi	-	10.7 40.50	-	-
Cosmocercoides dukae	10.7 1.32	3.5 0.50	3.3 0.04	6.9 0.14
Foleyella americana	10.6 0.14	7.1 0.14	19.9 0.50	10.3 0.14
Microfilaria sp.	-	-	6.6	-
Oswaldocruzia pipiens	32.1 0.61	-	10.0 0.10	-
Oswaldocruzia waltoni	-	-	-	17.2 0.45
Oxysomatium variabilis	78.5 3.00	10.7 1.96	36.6 0.13	27.5 0.83
Physaloptera ranae	28.5 1.39	10.7 1.00	6.6 0.30	17.2 0.21
Rhabdias bufonis	71.4 5.35	-	-	-
Rhabdias ranae	-	-	6.6 0.17	24.1 1.48
% hosts infected by one or more sp. Nematoda.	100 52.06	25.0 7.14	46.6 28.21	62.1 13.45

Table 4. Comparison, according to system infected, of parasites recovered from hosts representing different habitats. Figures in the heading indicate the number of each species of host examined. Figures in the table: upper figures indicate the percentage of hosts infected; lower figures, when present, indicate the average number of parasites per host.

	Terrestrial	Arboreal	Aquatic	Aquatic
	29	28	30	29
PARASITE	B. fowleri	H. versicolor	R. catesbeiana	R. clamitans
PROTOZOA				
Blood	6.9	7.1	76.6	55.1
Intestine	89.6	64.2	96.6	93.1
TREMATODA				
Lungs	3.4 0.03	-	26.6 1.07	31.0 2.55
Intestine	3.4 0.03	3.5 0.04	46.6 1.48	65.5 1.97
Bladder	-	-	3.3 0.03	-
CESTODA				
Intestine	24.1 0.92	-	6.6 0.23	-
NEMATODA				
Blood	-	-	6.6	-
Lungs	71.4 5.35	-	6.6 0.17	24.1 1.48
Intestine	89.6 3.89	28.5 7.14	36.6 0.83	55.1 8.55
Encysted	37.9 46.10	7.1 0.14	26.6 27.72	10.3 0.14
ACANTHOCEPHALA				
Intestine	-	-	-	3.4 0.03
Encysted	-	-	33.3 5.20	24.1 6.41
ACARINA				
Skin	3.4 0.03	-	10.0 1.37	3.4 0.31

Table 5. Multiple infections of salientia examined. Figures indicate the greatest number of parasitic species (by taxonomic groups) in a single host.

	Terrestrial	Arboreal	Aquatic	Aquatic
PARASITIC TAXA	B. fowleri	H. versicolor	R. catesbeiana	R. clamitans
Protozoa	5	6	6	5
Trematoda	1	1	3	3
Cestoda	1	0	1	0
Nematoda	5	3	4	4
Acanthocephala	0	0	1	1
Acarina	1	0	1	1
Parasites of all the above groups.	9	7	9	11

DISCUSSION

The present study is the first of its kind in the State of Virginia and the occurrence of all the parasites except M. temperatus and Physaloptera ranae in the salientia studied constitute new distribution records. The four species of salientia are of special interest here in that they represent three different habitats thus permitting a correlation of the habitat of the host and its parasites.

Although the data presented in Tables 1 through 5 are based on a relatively small number of specimens examined, they are in agreement with similar findings of previous investigators (Holl, 1932; Brandt, 1937; Rankin, 1945) whose data were based on a larger number of host specimens.

Table 1 shows that the salientia studied are commonly parasitized by protozoans, especially those inhabiting the intestinal tract. Intestinal ciliates were more prevalent in the terrestrial hosts, being unusually abundant in the arboreal H. versicolor. These observations are in agreement with those of Brandt (1937) who made a seasonal study of the parasites of salientia from different habitats in North Carolina. The fact that blood protozoans were found more often in aquatic than in terrestrial hosts agrees with the findings of Brandt (1937) and Hegner (1929) who studied the protozoans in the aquatic and terrestrial phases of the newt Notophthalmus viridescens. This observation supports the opinion of these authors that an aquatic blood sucking vector is involved as an intermediate host in the transfer of the parasites. Hazard (1941) has shown that R. clamitans seems to have an immunity to infections of opalinas. Of the three species of opalinas recovered none was found in R. clamitans. The absence of Entamoeba ranarum from B. fowleri and H. versicolor suggests an aquatic adaptation by this parasite. This observation is in agreement with those of Brandt (1937).

Rana catesbeiana was infected by a greater variety of metazoan parasites than any other species studied, although the number of individuals infected was less than in the case of R. clamitans (Tables 2-3). The metazoan parasites found in B. fowleri and H. versicolor were largely nematodes. All of the specimens of B. fowleri were infected but only 25% of those of H. versicolor were infected. These data suggest that the arboreal habitat is less conducive to metazoan parasitism and is in agreement with the work of Brandt (1937) and Rankin (1945). Considering all the species of metazoan parasites recovered, there is a high correlation between the aquatic environment and the variety of metazoan parasites.

Trematode parasites were largely limited to aquatic hosts (Table 2). This observation is in agreement with those of Brandt (1937), Rankin (1945), and Bouchard (1952). In the light of the work done by Krull (1930, 1932, 1933) on life-histories of trematodes, these data are understandable as all of these trematodes, with the exception of Polystoma nearcticum, require aquatic intermediate hosts in addition to snails. The infections in the case of B. fowleri and H. versicolor were very light both in numbers of species and specimens. Due to the terrestrial nature of B. fowleri it is believed that the two infections observed were obtained accidentally during the return of this host to the aquatic habitat to breed. However, the monogenetic trematode, P. nearcticum, recovered from H. versicolor seems to be specific for H. versicolor and Hyla cinerea (Schneider) having never been reported from a member of the genus Rana (Paul, 1938).

Brandt (1937) reported that Glypthelmins subtropica and Haematoloechus breviplexus were restricted to a single host and that from May to December he found 36 to 50% of the specimens of R. catesbeiana infected with Loxogenes bicolor. In the present study G. subtropica and H. breviplexus were found in both R. clamitans and R. catesbeiana. Although a

relatively small number of specimens of R. catesbeiana were examined (30 over a period of 5 months compared to the 71 examined by Brandt over a period of 12 months), it seems unusual that L. bicolor was never found in R. catesbeiana whereas 31% of the specimens of R. clamitans were infected. Krull (1933) also noted that L. bicolor was apparently absent from the specimens of R. catesbeiana collected in Maryland.

Although a comparison of cestode infections is presented in Table 2, the number of specimens obtained was not sufficient to show the correlation with habitat that was so clearly indicated for trematodes.

The data in Table 3 concerning nematode infections shows little, if any, correlation of the habitats of hosts and their parasites. A lower percentage of infection (25%) appears in the arboreal host H. versicolor than in the more terrestrial B. fowleri (100%) or the aquatic hosts R. catesbeiana (46%) and R. clamitans (62%). This is especially noticeable in the absence of nematodes from the lungs and the low frequency of intestinal worms (Table 4). These observations and the lower percentage of infection of the arboreal hosts support those of Brandt (1937), Pearse (1932), and Rankin (1945). The evidence presented in Table 3 also supports the findings of Brandt (1937) and Reiber (1941) that Rhabdias ranae is not prevalent in terrestrial hosts. However, adaptation by Rhabdias bufonis to a terrestrial host is clearly indicated by the high percentage of infection of specimens of B. fowleri (71.4%). This observation suggests that the free living stages of R. bufonis may be adapted to optimum development in the terrestrial habitat.

A careful search of the literature and Walton's lists did not show the following nematodes as parasites of the salientia that were used in this study. Oxysomatium variabilis was found in all four hosts studied, and Cosmoceralla haberi was recovered from H. versicolor. Their occurrence,

therefore, constitutes a new host record.

The infection of aquatic hosts by the larval acanthocephalan, Centrorhyncus sp., supports Brandt's (1937) observation correlating the residence of this parasite with intermediate hosts from an aquatic habitat (Table 4). Adult acanthocephala are rarely mentioned in the literature dealing with salientian parasites. However, larval forms of the genus Centrorhyncus have been reported by several authors (Millzner, 1924; Subramanian, 1928; Brandt, 1937; Das, 1952). Sandground (1926) has reported Moniliformis moniliformis from Bufo marinus in Honduras. However, an adult Centrorhyncus appears never to have been reported from any salientian host. Therefore the recovery of the adult acanthocephalan, Centrorhyncus wardae, from R. clamitans constitutes a new host record, and so far as can be determined, is the first record of an adult of this genus in any salientian from North America.

There is little evidence of host-specificity in the 34 species of parasites recovered. The following 12 species of parasites were found to be restricted to a single host: Opalina hylaxena, Balantidium sp., Gorgodera amplicava, Gorgoderina bilobata, Haematoloechus sp., Loxogenes bicolor, Polystoma nearcticum, Distoichometra bufonis, Cosmoceralla haberi, Microfilaria sp., Oswaldocruzia waltoni, Rhabdias bufonis, and Centrorhyncus wardae. However, all of these parasites with the exception of C. wardae, Balantidium sp., and the probably undescribed Haematoloechus sp. have been reported from other hosts by numerous authors.

Multiple infection was common among all the hosts studied, the condition appearing to be the rule rather than the exception (Table 5). The aquatic hosts were heavily parasitized by the greatest number of species. Of the four representative hosts studied, R. clamitans harbored the greatest number of species in any single individual.

SUMMARY

1. One hundred sixteen salientia, comprised of 30 specimens of R. catesbeiana, 29 of R. clamitans, 29 of B. fowleri, and 28 of H. versicolor, were examined from May to October, 1966.

2. Thirty-four species of parasites from five phyla were recovered. The total numbers of species of parasites found in each of the hosts studied were as follows: R. catesbeiana, 24; R. clamitans, 21; B. fowleri, 17; and H. versicolor, 14.

3. Trematodes, acanthocephalans, and blood protozoa were more often associated with aquatic hosts whereas intestinal ciliates were found more often in terrestrial hosts.

4. The arboreal H. versicolor yielded far fewer metazoan parasites than the terrestrial and aquatic salientian hosts.

5. Multiple infections were common. As many as 11 species of parasites were recovered from a single specimen of R. clamitans.

6. All of the parasites reported herein, with the exception of M. temperatus and P. ranae, constitute new distributional records for the state of Virginia.

7. An adult acanthocephalan of the genus Centrorhyncus, C. wardae, is reported from a salientian for the first time.

8. Five other new host-parasite records are also reported.

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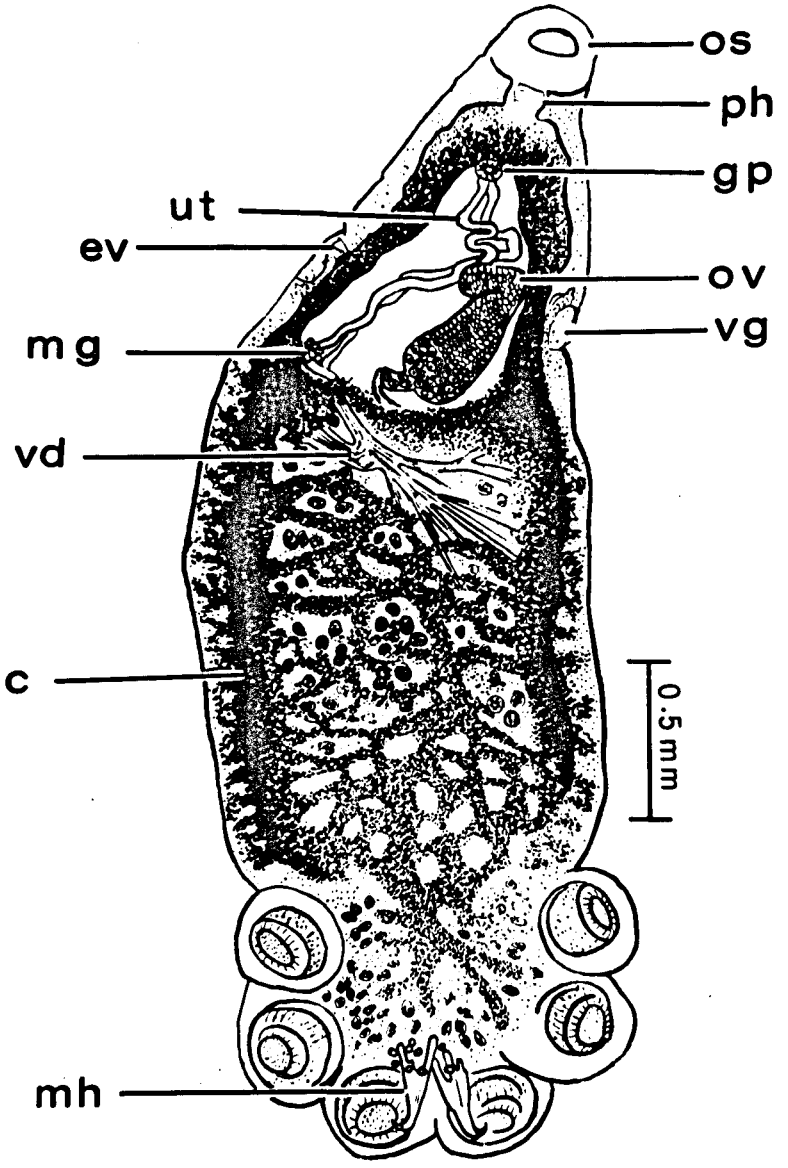
FIGURE 1

Polystoma nearcticum Paul, 1938

Abbreviations: os, oral sucker; ph, pharynx; gp, genital pore; ov, ovary; mh, marginal hooks; c, caecum; vd, vas deferens; mg, Mehlis' gland; ev, excretory vesicle; ut, uterus.


os
ph
gp
ut
ev
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vg
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c
0.5 mm
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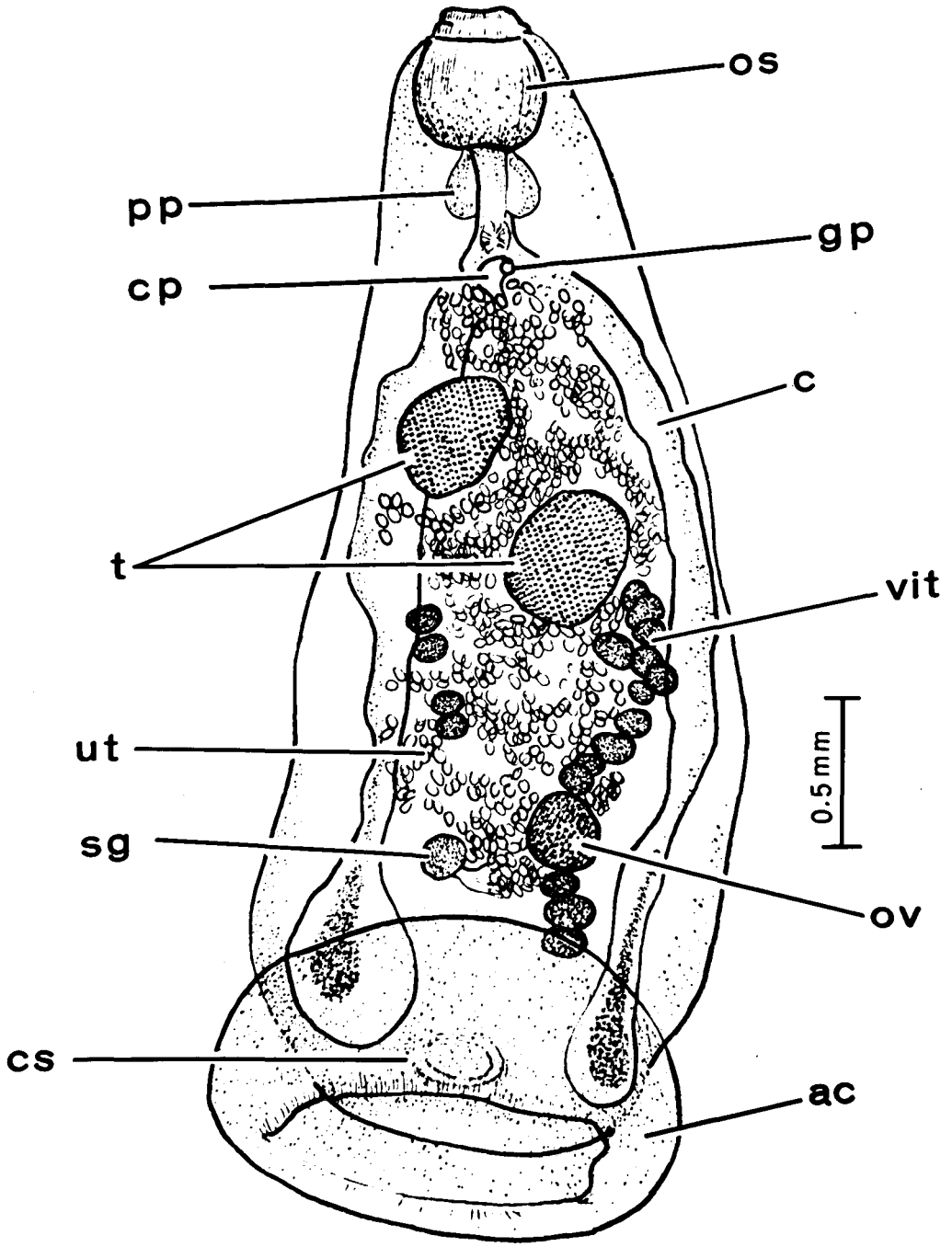
FIGURE 2

Megalodiscus temperatus (Stafford, 1905)

Abbreviations: os, oral sucker; gp, genital pore; c, caecum; vit, vitellaria; ov, ovary; ac, acetabulum; cs, central sucker; sg, shell gland; ut, uterus; t, testis; cp, cirrus pouch; pp, pharyngeal pouches.


os
pp
gp
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t
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0.5 mm
sg
ov
cs
ac

FIGURE 3

Haematoloechus varioplexus Stafford, 1902

Abbreviations: os, oral sucker; gp, genital pore; ac, acetabulum; t, testis; ph, pharynx; c, caecum; vit, vitellaria; ut, uterus; ov, ovary.

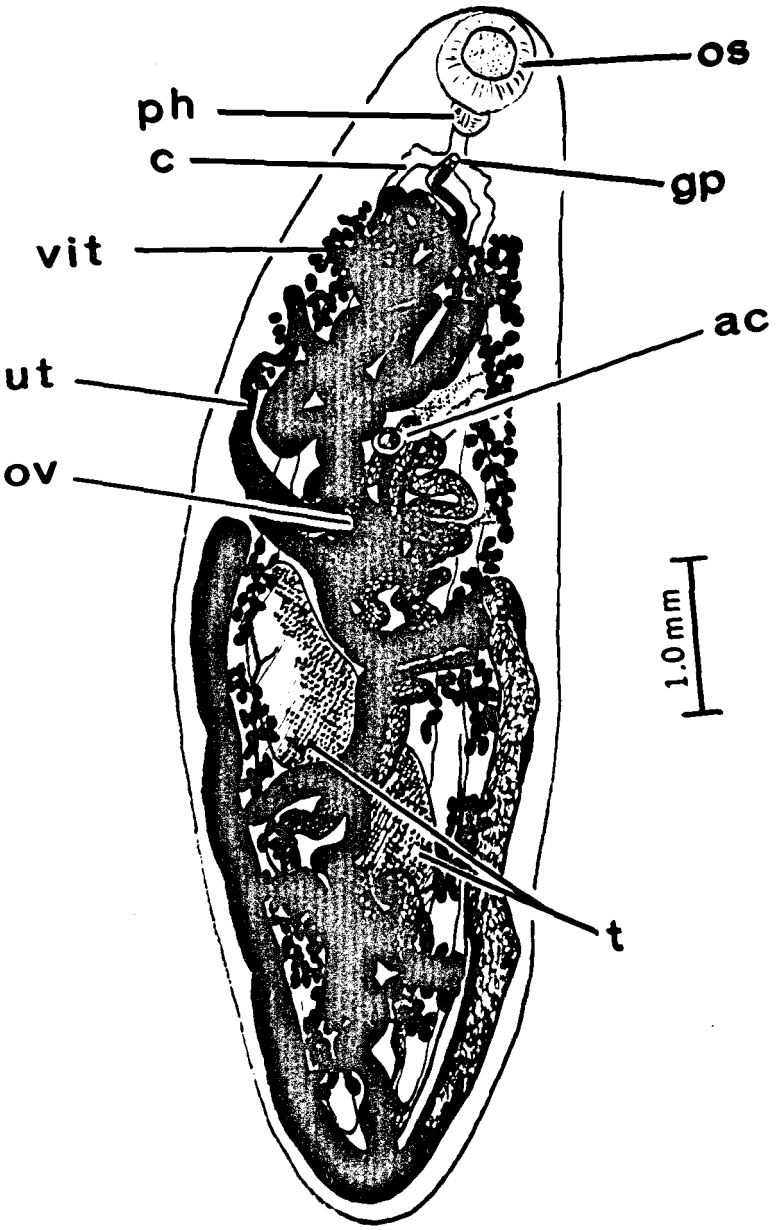
os
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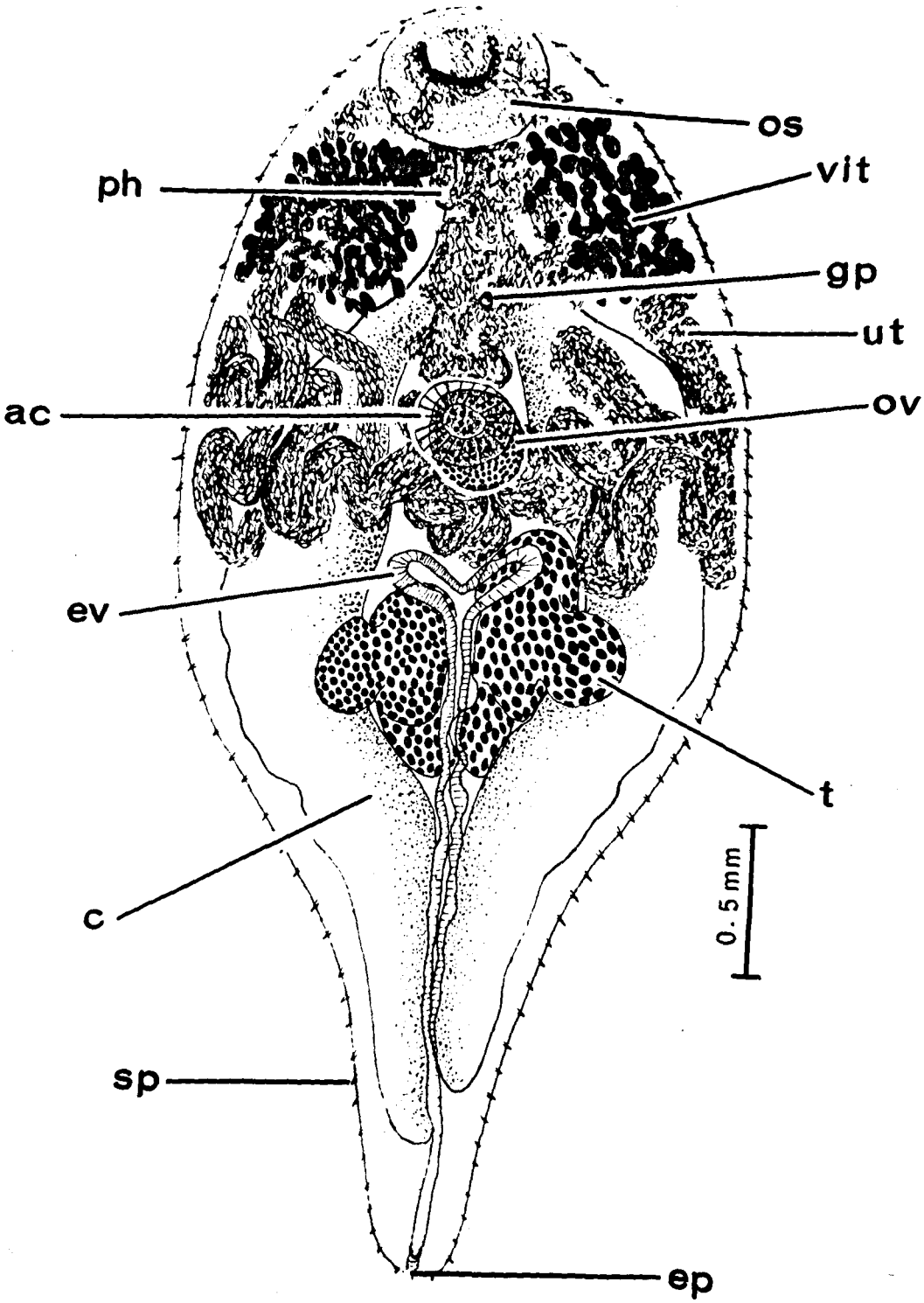
FIGURE 4

Loxogenoides bicolor (Krull, 1933)

Abbreviations: os, oral sucker; vit, vitellaria; gp, genital pore; ut, uterus; ov, ovary; t, testis; ep, excretory pore; sp, spine; c, caecum; ev, excretory vesicle; ac, acetabulum; ph, pharynx.


os
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sp
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FIGURE 5

Centrorhyncus wardae Holloway, 1956

Abbreviations: ipr, insertion proboscis receptacle; pr, proboscis receptacle; rm, retractor muscle; ga, genital apparatus; ls, ligament sac.

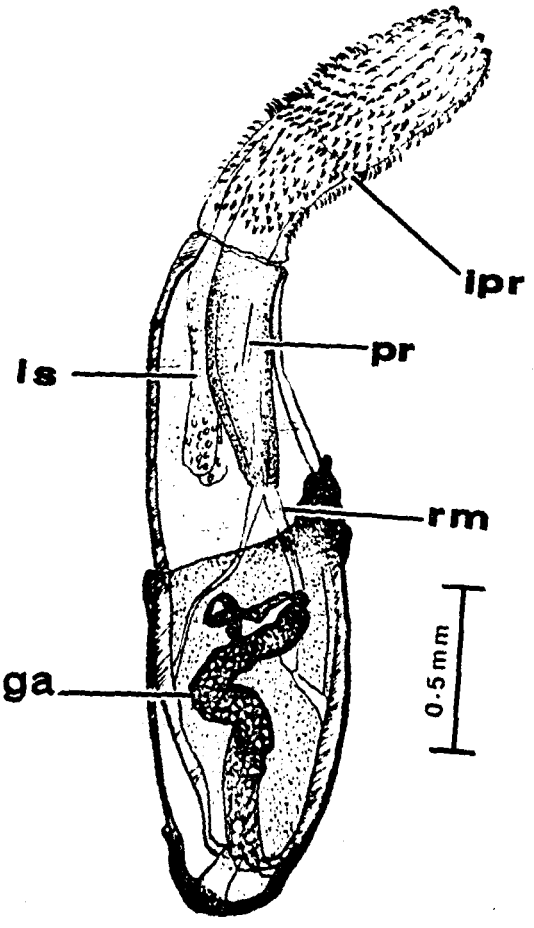
lpr
pr
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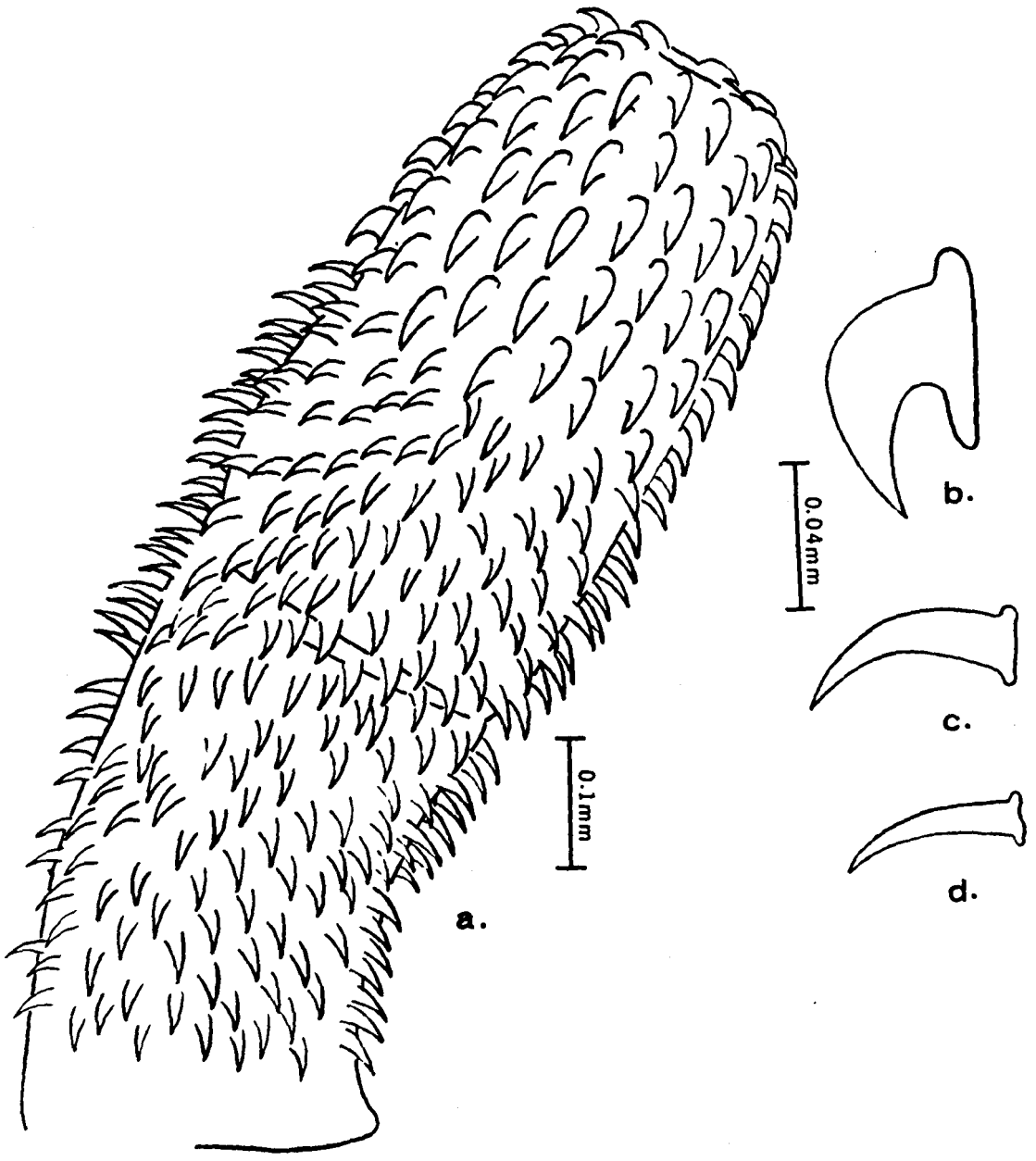
FIGURE 6

Centrorhyncus wardae Holloway, 1956

a. Proboscis; b. Anterior hook; c. Medial hook; d. Basal hook.


0.1mm
a.
0.04mm
b.
c.
d.

VITA

Ronald Arthur Campbell was born in Norfolk, Virginia on March 24, 1943. He attended elementary and secondary schools in Roanoke, Virginia and was graduated from Cave Spring High School in June, 1961.

He entered Roanoke College in Salem, Virginia in September, 1961 and received the Bachelor of Science degree in biology on June 5, 1965. While attending the college he was elected a provisional member of Beta Beta Beta Biological Honor Society and was a member of the Men's Independent Club and the Men's Athletic Association.

He began graduate work at the University of Richmond in September, 1965 and completed the requirements for the Master of Arts degree in biology in May, 1967. During this time he assisted in the general biology laboratories and was honored with a William's Fellowship at the end of his first year of study. He was promoted to full membership in the Beta Theta Chapter of Beta Beta Beta and was elected to the office of vice-president in May, 1966. He served as president of that chapter from January to June, 1967. He was elected to membership in the Virginia Academy of Science and the American Society of Zoologists in the fall of 1966.

He was appointed in March, 1967 to a graduate assistantship in the Department of Zoology and Entomology at Iowa State University to continue graduate work toward the degree of Doctor of Philosophy in parasitology.