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# A survey of fresh-water oligochaeta and their commensal ciliates from the Richmond, Virginia area

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A SURVEY OF FRESH-WATER OLIGOCHAETA  
AND THEIR COMMENSAL CILIATES  
FROM THE RICHMOND, VIRGINIA AREA

BY  
ELSA QUEEN FALLS

A THESIS  
SUBMITTED TO THE GRADUATE FACULTY  
OF THE UNIVERSITY OF RICHMOND  
IN CANDIDACY  
FOR THE DEGREE OF  
MASTER OF ARTS IN BIOLOGY

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A SURVEY OF FRESH-WATER OLIGOCHAETA  
AND THEIR COMMENSAL CILIATES  
FROM THE RICHMOND, VIRGINIA AREA

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## INTRODUCTION

The distribution of North American aquatic oligochaetes and the ciliates inhabiting their guts have received very limited attention. For this reason, the present study was initiated to survey fresh-water oligochaetes of the families Aeolosomatidae, Naididae, Opistocystidae, and Lumbriculidae in four bodies of water of the Richmond, Virginia area and to determine the incidence of commensal ciliates within the gut.

### Oligochaetes

The fresh-water oligochaetes have been a notoriously troublesome group with which to work and appear not to have been a major point of interest to limnologists (Causey, 1953). Goodnight (1959) states that the present knowledge of aquatic oligochaetes is very limited, with many species remaining to be described.

The most recent comprehensive studies on North American oligochaetes were done by Brinkhurst. His surveys include the families Naididae and Opistocystidae (1964), Tubificidae (1965), and Lumbriculidae and Aeolosomatidae (1966). He is concerned primarily with the taxonomy and ecology of the Tubificidae and relies heavily on Sperber (1948) for much of his information on the Naididae and Opistocystidae, although he did examine several museum collections.

He states that the systematics of the family Aeolosomatidae need revision and that there are few references in American literature to the family, the named species of which often lack descriptions. He also states that the Lumbriculidae, although receiving more attention than most other aquatic Oligochaeta in the literature, are less well-known than the Naididae and Tubificidae.

Sperber's (1948) comparative morphological studies of the Naididae, in which she attempts a phylogenetical revision of the family, are based almost exclusively on specimens from Sweden; forms from other continents are studied solely from the literature.

Although the taxonomical descriptions and revisions of Brinkhurst (which include excellent keys to the Naididae, Opistocystidae, and Lumbriculidae) and of Sperber remain the most reliable guides to North American fresh-water oligochaetes, there are other helpful sources. Goodnight's (1959) revision of the fresh-water oligochaetes of North America, for example, includes a key to most species; Pennak (1953) is also useful, although it lacks recent taxonomic revisions and the key is incomplete. Authorities rely heavily on Stephenson's (1930) descriptions of British specimens, since many species are cosmopolitan.

Much of the early taxonomic work in the United States was done in the early 1900's by such workers as Smith (1900), Galloway (1911), and Hayden (1922).

Pennak (1953) states that less than 15 important papers have been

published since 1900 on fresh-water oligochaetes in this country. Several recent surveys have been undertaken in localized areas. (Causey, 1953, in Arkansas; Harman, 1966, in Mississippi; Wurtz and Roback, 1955, in some Gulf Coast rivers; and Harman and Platt, 1961, in Louisiana.)

Previous published surveys of the smaller oligochaetes (microdrili) of Virginia are non-existent, although Brinkhurst does mention some museum specimens which were collected in Virginia.

### Ciliates

Studies concerning the incidence of ciliates in oligochaetes have been carried out almost totally on European and Asian specimens. Rossolimo (1926) published a survey of ciliates found in oligochaetes from Lake Baikal. Heidenreich (1935b) compiled a review of ciliates occurring in annelids from ponds, ditches, and pools near Breslau, Germany. Research done by Raabe (1949) on the Hysteroecinetidae includes a complete history of the study of this family.

DePuytorac has contributed some of the most authoritative research on the Astomatida. Among the most comprehensive contributions is a detailed study, in 1954, of the cytology and taxonomy of the astomates found in fresh-water oligochaetes, as well as an extensive review of the literature. In 1959 and 1960, he published papers describing a number of astomates found in *Oligochaeta* from Ochrida Lake in Yugoslavia.



Meier (1954) made an extensive survey in Germany of oligochaetes and ciliates found within the gut; a similar study was undertaken by Frolova (1957) in Eastern Murman.

So far as could be determined, there are no published accounts of ciliates found within the gut of Naididae and Lumbriculidae in the U.S.A.; but Conklin (1930) and Williams (1942) both report finding ciliates in certain species of Lumbricidae, and Kozloff (1960) investigated, in Chapel Hill, North Carolina, two species of Hysteroconetidae, one from Eisenia longabergi (Lumbricidae), the other from a snail.

## MATERIALS AND METHODS

### Oligochaetes

Monthly collections, were made from February 1971 to February 1972, except as noted below, from:

1. Westhampton Lake on the University of Richmond campus.
2. Maymont Park Ponds, within the city limits, February 1971 to August 1971.

3. Swan Lake in Byrd Park, August 1971 to February 1972.  
(Since Maymont Ponds were drained in July, Swan Lake, which is on the same drainage shed as the latter, replaced it as a collecting site.)

4. Twin Lakes, on Twin Lakes Lane.

Bottom debris and mud from shallow portions of the lakes were collected in wide-mouthed gallon jars and examined in fingerbowls

under a stereoscopic binocular microscope within 24 hours. Worms were removed with a dropping pipette. This method of separation of worms from debris was utilized rather than a mesh screen, since screening results in fragmentation of the worms (Causey, 1953).

Tentative identifications were made on live organisms, as preservation renders the body opaque, making observations and measurements of chaetae difficult and the study of locomotion impossible.

Chaetal types were determined and measured under the microscope. The specimens were prepared for study by removing water from under a cover slip with paper toweling, causing them to burst, thus leaving the chaetae visible and in segmental position.

Specimens of all identified species were killed and fixed in hot Schaudinn's solution and preserved in 70% alcohol for future examination. Sperber's (1948) method was utilized for examination of chaetae in preserved material. The specimen was placed on a slide in a saturated solution of KOH for five to ten minutes, after which the KOH was replaced by distilled water. However, as this procedure caused chaetae to swell, their thickness could not be measured.

On occasion worms were cultured for further study and to increase their numbers for preservation. For each culture a fingerbowl was filled with 200 ml spring water, and four boiled wheat grains were added.

## Ciliates

Since the ciliates died off when their hosts were cultured, destruction of the worms to search for ciliates was carried out within 24-48 hours after collection. After the body of the worm was squashed under a cover slip to release the ciliates, these were studied microscopically. On occasion, however, ciliates could be seen within the gut of living Dero digitata which had been flattened by the removal of water from beneath the cover slip with toweling.

A .65% saline solution was used for observation of ciliates, but, with all precautions, they lived only minutes outside the body of the host. In such preparations, methyl green was employed to stain the nucleus. Because of the delicate nature of the ciliates, permanent slides were made by squashing a number of worms on a slide and allowing the paste thus formed to almost dry. The fixation and staining procedure was as follows:

1. Cover slide with Schaudinn's fixative and allow to remain for 5 minutes.
2. Wash in distilled water.
3. Flood slide with 50% alcohol and allow to remain for 1 minute.
4. Stain with Delafield's hematoxylin for 5 minutes.
5. Rinse with 50% alcohol.
6. Flood with 70% alcohol for 1 minute.

7. Flood momentarily with 70% acid alcohol followed by 70% alkaline alcohol.
8. Flood with 95% alcohol for 5 minutes, followed by several changes of 100% alcohol for 5 minutes.
9. Clear in xylol for 10 minutes.
10. Drain excess xylol from slide and mount in balsam.

## RESULTS

### Oligochaetes

Collection data is summarized in Table I. A total of 19 species of fresh-water oligochaetes were collected and identified: 2 species belonging to Aeolosomatidae, 15 to Naididae, 1 to Opistocystidae, and 1 to Lumbriculidae. Sexual forms of one species, Dero digitata, were observed.

A total of 42 collections were made: 17 at Westhampton Lake with 11 species identified, 13 at Twin Lakes with 13 species, 6 at Swan Lake with 14 species, and 6 at Maymont with 5 species.

With the exception of Aeolosoma headleyi and Nais pseudobtusa, all species were successfully cultured.

The following section summarizes pertinent information concerning each species of oligochaete collected and identified. (Taxonomic data observed which does not deviate significantly from that described by other authors is included in the Addendum.)

Table I. Collection Data for Oligochaetes. Abbreviations: MM, Maymont Ponds; SL, Swan Lake; TL, Twin Lakes; WL, Westhampton Lake.

| Species                                | Collection Site | No. of Specimens Collected | No. of Collections Containing Specimens | Recorded in Va. Previously |
|--|-----------------|----------------------------|---|----------------------------|
| AEOLOSOMATIDAE                         |                 |                            |   |                            |
| <u>A. hemprichii</u>                   | SL, TL, WL      | 8                          | 6                                       | no                         |
| <u>A. headleyi</u>                     | SL              | 1                          | 1                                       | no                         |
| Naididae                               |                 |                            |   |                            |
| <u>C. langi</u>                        | SL, TL, WL      | 21                         | 6                                       | yes                        |
| <u>C. diaphanus</u>                    | MM, SL, TL, WL  | 105                        | 22                                      | no                         |
| <u>Paranais</u>                        | SL, WL          | 5                          | 4                                       | no                         |
| <u>N. communis</u>                     | SL, TL          | 21                         | 5                                       | yes                        |
| <u>N. variabilis</u>                   | SL, TL, WL      | 34                         | 11                                      | no                         |
| <u>N. pseudobtusa</u>                  | SL              | 3                          | 1                                       | no                         |
| <u>S. appendiculata</u>                | MM, SL, TL      | 27                         | 9                                       | no                         |
| <u>S. lacustris</u>                    | TL              | 1                          | 1                                       | no                         |
| <u>S. fossularis</u>                   | TL              | 4                          | 2                                       | no                         |
| <u>H. waldvogeli</u>                   | TL              | 6                          | 1                                       | no                         |
| <u>D. digitata</u>                     | MM, SL, TL, WL  | 400-600                    | 33                                      | yes                        |
| <u>D. obtusa</u>                       | SL, TL, WL      | 50                         | 12                                      | no                         |
| <u>A. furcatus</u>                     | SL, WL          | 1000-2000                  | 6                                       | no                         |
| <u>P. minuta</u>                       | SL              | 1000-2000                  | 1                                       | no                         |
| <u>P. longisetulae</u><br><u>leidy</u> | MM, SL, TL, WL  | 46                         | 16                                      | yes                        |
| OPISTOCYSTIDAE                         |                 |                            |   |                            |
| <u>O. flagellum</u>                    | WL              | 3                          | 1                                       | no                         |
| LUMBRICULIDAE                          |                 |                            |   |                            |
| <u>L. variegatus</u>                   | TL, WL          | 72                         | 15                                      | no                         |

### Family Aeolosomatidae

Aeolosoma hemprichii Ehrenberg, 1831: Small numbers of individuals were found on several occasions. Cultivation was successful, but preservation resulted in poor specimens for study. The color of the oil globules, important in identification, could not be determined.

A. hemprichii is a cosmopolitan species which has been recorded numerous times from various states, although never from Virginia.

Aeolosoma headleyi Beddard, 1880: Only one individual was collected, which died when culturing was attempted.

### Family Naididae

Chaetogaster langi Bretcher, 1896: This species is fairly common and was found at three of the four collecting sites. Chaetae of segment II measured as much as  $112\mu$  in length, which is somewhat longer than the maximum of  $100\mu$  recorded by Brinkhurst (1964).

Chaetogaster diaphanus Gruithuisen, 1828: This species was present in more collections than any other except Dero digitata. The longest chaetae of segment II varied from  $186-275\mu$  in length, which is less than the  $200-350\mu$  recorded by Brinkhurst (1964); the shortest measured  $130\mu$  in length, while Brinkhurst recorded  $145\mu$ .

Paranais Czerniavsky, 1880: Inclusion in this genus is questionable as dorsal chaetae begin in segment II rather than V, as indicated by Sperber (1948) and Brinkhurst (1964), although all other characters fit generic descriptions.

Nais communis Piguet, 1906: N. communis, which is cosmopolitan, did not always appear readily distinguishable from Nais variabilis,

as far as chaetal characteristics were concerned. Much reliance, therefore, was put upon whether or not the individual worms swam, which Sperber (1948) states is a reliable characteristic. N. communis does not swim, whereas most other species of Nais do. One individual identified as this species did not have eyes; Brinkhurst (1964) states that eyes are sometimes absent.

Nais variabilis Piguet, 1906: This species, which swims with spiral movements, was common at three collecting sites and was often collected with N. communis. The distal tooth of the ventral chaetae of segment II was longer than the proximal, whereas in N. communis the teeth generally appear sub-equal. The hair chaetae were usually longer than those of N. communis.

Nais pseudobtusa Piguet, 1906: Specimens were found only once, in January in Swan Lake; the same collection also contained N. communis and N. variabilis. N. pseudobtusa is distinguishable from the other two species by its small size and particularly by its single-pointed needles. This species has been recorded previously from Michigan (Brinkhurst, 1964). The nodulus of ventral chaetae posterior to segment VI appeared slightly proximal, rather than distal as Brinkhurst (1964) states.

Slavina appendiculata d'Udcken, 1855: This oligochaete, found at three sites, is easily distinguishable by a thick crust of foreign material surrounding the body and the long hairs of segment VI, up to 700  $\mu$ . in length.

Stylaria lacustris Linnaeus, 1767: One individual was encountered, at Twin Lakes, with what appeared to be a broken-off proboscis projecting from between two lobes. After culture, the proboscis regenerated, and several worms subsequently were produced by budding. There were 2-4 needles present per bundle. Brinkhurst (1964) states needles vary in number from 3-4. Pennak (1953), however, contains an illustration of S. proboscidea, which is synonymous with S. lacustris, showing two needles per bundle. The needles were from 52-65 $\mu$  long, whereas Brinkhurst (1964) lists 75-120 $\mu$  as length variations; he also states that two hairs are present per bundle, whereas I found only one hair per bundle in some segments. Ventral chaetae varied from 114-125 $\mu$  in length, but Brinkhurst (1964) states they vary from 159-222 $\mu$ .

Stylaria fossularis Leidy, 1852: This oligochaete was found in two collections at Twin Lakes. Brinkhurst (1964) reports that S. lacustris often has been merged with S. fossularis and that fresh material would be required to be certain that characters used in separation do not vary with fixation. From the present study of fresh material of both species, the point of attachment of the proboscis appears to be definitely different. S. fossularis has the proboscis projecting from the tip of the pointed prostomium, whereas it projects from between two lobes in S. lacustris. Also, S. fossularis has no serrations on the hairs, as does S. lacustris; and, in agreement with Sperber (1948), the ventral chaetae of S. lacustris are distinctive,



having two sharp proximal bends.

Haemonais waldvogeli Bretscher, 1900: Six individuals were identified from one Twin Lakes collection. The species has only recently been established definitely as a part of the North American fauna, Brinkhurst (1966) reporting its collection in Boone County, Missouri. Hairs measured 140-171 $\mu$  long, whereas Brinkhurst (1964) gives 132-160 $\mu$  as length. For needles, he gives 115 $\mu$  as the upper limit of length; 126 $\mu$  was measured as the upper limit in the present study. Some anterior ventral chaetae were as long as 127 $\mu$ ; the upper limit, according to Brinkhurst (1964), is 117 $\mu$ .

Dero (Dero) digitata (Müller), 1773: Specimens were found in 33 of 42 collections, making the species the most common encountered. On one occasion (December collection at Westhampton Lake) the specimens were so numerous it was impossible to separate and classify all to species.

D. digitata was the only oligochaete for which sexual forms were observed. Sexual individuals were present in collections from Westhampton Lake in April and May and from Maymont in April; these specimens were maintained in culture, as were non-sexual worms from the same collections. Many non-sexual forms developed into sexual individuals while under culture. Initially, in sexual individuals, the clitellum, which extended over segments V-VII, appeared white and opaque, with marked swelling in the genital region. The testes are

found in segment V, the ovaries in segment VI (Stephenson, 1930).

The ovisacs, which extended posteriorly for several segments beyond the genital region, continued to enlarge and darken for several weeks until they occupied nearly the entire diameter of the body. No sexual individuals were observed to reproduce by fission. By June 10, all cultured sexual worms were dead, with no discharge of ova having been observed.

Dero (Dero) obtusa d'Udekem, 1855: Individuals occurred in three of the four sites, often in the same collections as D. digitata, making it necessary to examine each specimen microscopically to determine species. D. obtusa ordinarily was not as large as D. digitata, gills were not foliate, and hair chaetae and needles measured less in length.

Dero (Aulophorus) furcatus (Müller), 1773: Members of this species were discovered most often floating on the surface of a collection in portable tubes of cemented woody debris and green plant material. At the time of the August collections at Westhampton Lake, the water surface along the shore for approximately 30 square feet was covered with small bits of woody debris in which thousands of worms were found.

Dorsal needles of A. furcatus measured as short as  $41 \mu$ ; Brinkhurst (1964) lists  $45 \mu$  as the lower limit.

Pristina minuta (Stephenson), 1914: Brinkhurst (1964) states that P. minuta has not been established definitely in the North American fauna. Specimens fitting Brinkhurst's description (chaetal length

measurement not given) numbered in the thousands in the October collection at Swan Lake. Dorsal hairs of specimens measured 65-68  $\mu$  long; needles were 25-26  $\mu$  long.

Pristina longiseta leidy Smith, 1896: Individuals commonly were collected at all four sites. The teeth of serration on the dorsal hair chaetae, which are characteristic for the species, were difficult to see on some specimens; they were never visible except under optimum light conditions with the oil immersion lens. Brinkhurst (1964) and Sperber (1948) give no length measurements for chaetae; in the present study hair chaetae measured 172-228  $\mu$  long, except in segment III where they were 601-688  $\mu$  long, and needles were 33-52  $\mu$  long.

#### Family Opistocystidae

Opistocysta flagellum (Leidy), 1880: Sperber (1948) states that Pristina flagellum Leidy was transferred to a family of its own, Opistocystidae, by Cernosvitov (1936) because the sexual organs are found in segments XXI-XXIII, whereas the organs in the Naididae are located more anteriorly. The species has been recorded previously in Pennsylvania and South America (Brinkhurst, 1964). The fine lateral hairs on the dorsal hairs described by Brinkhurst were not visible on the present specimens. The dorsal needles measured 57-65  $\mu$  in length compared to Brinkhurst's measurements of 90-105  $\mu$ . Ventral chaetae of segments II-V were 73-82  $\mu$  long compared with his measurements of 100-120  $\mu$ , and in remaining segments ventral chaetae measured 82-93  $\mu$  long compared with his length of 128-130  $\mu$ .

## Family Lumbriculidae

Lumbriculus variegatus (Muller), 1774: Specimens in culture underwent reproduction by fragmentation, a phenomenon common within the family.

Brinkhurst (1966) proposes that Lumbriculus be made a monotypic genus, retaining the other taxa as subspecies so that the various atrial positions can be recorded. According to this proposal, there are two subspecies: L. variegatus typica Brinkhurst, 1966 (atria usually located in segment VIII) and L. variegatus inconstans Smith, 1895 (atria usually located in segment X). Both subspecies apparently are cosmopolitan.

Although whole mounts were fixed, cleared, and stained and cross-sections were made, the atria could not be located in any collected specimens, and, therefore, the subspecies could not be determined.

## Ciliates

Of the 19 species of oligochaetes studied and identified, only D. digitata and L. variegatus had ciliates within the gut. D. digitata harbored Radiophrya deronis; L. variegatus harbored Ptychostomum lumbriculi and Mesnilella clavata; some specimens contained both ciliate species, some only one.

When host worms of both species were cultured longer than 2-3 days, the number of ciliates found within them was fewer than in freshly collected worms. In worms cultured for one-three months, the percentage of infection decreased to zero.

Collection data, as it applies to ciliates, is presented in Table II.

The following section summarizes pertinent information concerning each ciliate species identified. (Taxonomic data observed which does not deviate significantly from that of other workers is included in the Addendum.)

Order Thigmotrichida, Family Hysteroecinetidae

Ptychostomum lumbriculi Heidenreich, 1935 (Figure 1): This ciliate species, which was found in the midgut and hindgut of L. variegatus, varied in length from 48-103 $\mu$ , which is somewhat greater than the 60-95 $\mu$  range reported by Heidenreich (1935a) for specimens examined from Breslau, Germany. The number of ciliates occurring in one worm varied from none to approximately 80, with the majority having 10-20.

Order Astomatida, Family Intoshellinidae

Radiophrya deronis Heidenreich, 1935 (Figure 2): The number of ciliates present in D. digitata varied from none to 17 per host, with 4-6 being found most frequently. Although Kudo (1966) states that an attaching organelle is present in the genus, ciliates observed within the gut of living D. digitata appeared to be unattached and moving from segment to segment. The ciliates (without satellites) varied from 138-315 $\mu$  in length and from 28-48 $\mu$  in width; Heidenreich (1935b) reports 190-280 $\mu$  for length and 36-50 $\mu$  for width, whereas Meier (1954) states the length varies from 200-450 $\mu$  and the width from 45-75 $\mu$ . Two rows of contractile vacuoles were present, as reported also by Heidenreich (1935b); Kudo (1966) states that one row

Table II. Collection Data for Ciliates. Abbreviations: MM, Maymont Ponds; SL, Swan Lake; TL, Twin Lakes; WL, Westhampton Lake.

| Species              | Host                 | Collecting Site | Hosts Harboring Ciliates |
|----------------------|----------------------|-----------------|--------------------------|
| <u>P. lumbriculi</u> | <u>L. variegatus</u> | TL              | 4 of 11 (36.4%)          |
|                      |                      | WL              | 5 of 26 (19.2%)          |
| <u>M. clavata</u>    | <u>L. variegatus</u> | TL              | 5 of 11 (45.5%)          |
|                      |                      | WL              | 19 of 26 (73.1%)         |
| <u>R. deronis</u>    | <u>D. digitata</u>   | MM              | 6 of 35 (17.1%)          |
|                      |                      | SL              | 2 of 24 ( 8.3%)          |
|                      |                      | TL              | 20 of 36 (55.6%)         |
|                      |                      | WL              | 54 of 86 (62.8%)         |

of vacuoles is present in the genus.

Mesnillella clavata (Leidy), 1855 (Figure 3): The species, which occurs in the greatest numbers in the foregut of L. variegatus, measured 69-241  $\mu$  in length and 21-34  $\mu$  in width. Heidenreich (1935b) reports great variation in length, up to 240  $\mu$ ; Meier (1954) reports lengths of 75-135  $\mu$ . The ciliates varied in number from none to several hundred per host. Six of the worms harboring M. clavata contained over 100 ciliates; the remaining 18 contained 1-30.

#### DISCUSSION

##### Oligochaetes

This study is the first survey of the microdrili for Virginia. Of

the 19 species collected and identified, C. langi, N. communis, D. digitata, and P. longiseta have been recorded previously in Virginia (Brinkhurst, 1964).

The specimens which are placed provisionally under the genus Paranais possess dorsal chaetae beginning on segment II rather than segment V, as indicated by Sperber (1948) and Brinkhurst (1964). Cultured worms multiplied by fission, placing them in the family Naididae. Except for the segment in which dorsal chaetae begin, they fit descriptions for Paranais. The individuals collected could not be placed in a species even provisionally. Pennak (1953) states only that there are several poorly recognized species; Brinkhurst (1964) lists only one salt or brackish water species for the U. S. A., although Goodnight (1959) states that this species, P. litoralis (Müller), probably also occurs in adjacent fresh waters.

Many of the oligochaetes identified are cosmopolitan, although P. longiseta leidyi is restricted to North America, N. pseudobtusa is best known from Europe, S. lacustris appears to have an holarctic distribution, and S. fossularis is restricted to North America and Asia (Brinkhurst, 1964). While H. waldvogeli seems to have an holarctic distribution, this is only the second recorded occurrence for North America. P. minuta, as a result of this present study, definitely appears to be part of the North American fauna; it occurs also in South America and Asia (Brinkhurst, 1964).

Causey (1953), in his report on the microdrili in artificial lakes

in Northwest Arkansas, was able to identify four species of Naididae, three of Tubificidae, and one of Lumbriculidae. Three species-- D. obtusa and S. lacustris (Naididae) and L. variegatus (Lumbriculidae)--were also identified in the present study. He reports difficulties with fragmentation and chaetal loss, resulting in an inability to identify many species. His method involved obtaining bottom material from a .25 ft<sup>2</sup> area with an Ekman dredge, screening through 40 mesh screen, killing in 5% formalin, and preserving in 70% alcohol. The method utilized in the present study, which involved examination of debris with a stereoscopic binocular microscope and separation of live worms for examination, was more tedious but yielded more intact specimens. Examination of live material is advocated by Sperber (1948), Pennak (1953), and Brinkhurst (1964).

Cole (1955) includes the Oligochaeta in his investigation of the microbenthic fauna of two Minnesota Lakes but gives no descriptions of his material. His list of identified Oligochaeta (all Naididae and Aeolosomatidae) includes only five classified to genus and three to species; two of the species, A. hemprichii and S. lacustris, were also identified in the present study.

Of eight species of oligochaetes identified and described by Harman (1966) from Mississippi, four -- A. hemprichii, D. digitata, A. furcatus, and P. longiseta leidy -- were identified in the present survey.

Only D. digitata was observed to become sexual, this occurring



in April and May. Such worms were recognized easily, as in the microdrili, the clitellum is a temporary development found only in sexual individuals. Although sexual forms are relatively uncommon in the Aeolosomatidae and Naididae and indeed unknown in some genera, they have been reported in D. digitata in May, as well as from Aeolosoma, Nais, Chaetogaster, Pristina, and Stylaria (Stephenson, 1930). L. variegatus, according to Stephenson (1930), is rarely sexual and reproduces ordinarily by fragmentation, as was observed in this investigation.

#### Ciliates

The present survey, in which 2 of 19 species harbored ciliates, supports evidence presented by other workers that fewer of the Aeolosomatidae and the Naididae are hosts to ciliates than larger worms, such as the Enchytraeidae, Tubificidae, Lumbriculidae, Branchiobdellidae, and Lumbricidae. Many workers concerned with ciliates in oligochaetes have not included the smaller oligochaetes in their surveys (Frolova 1957, Kozloff 1960, Conklin 1930, and Rossolimo 1926).

Ciliates were absent from the Aeolosomatidae (in A. hemprichii and A. headleyi); Meier (1954), likewise, found none in A. hemprichii, the only member of the family she investigated. Naidu (1961) reports that several species of Radiophryoides (Family Astomatida) have been found in India in the Aeolosomatidae, including in A. hemprichii. Heidenreich (1935b) reports a dubious species, Radiophrya (?) aeolosomatis Anderson from A. chlorosticum Wood-Mason.

As far as could be determined, there is no record of O. flagellum (Family Opistocystidae) containing ciliates; likewise, no ciliates were encountered in the present study.

Of the 15 species of Naididae identified, only D. digitata harbored a protozoan (R. deronis). Similarly, of the 32 species of ciliates reported by Heidenreich (1935b), D. limosa (digitata) was the only naid with ciliates; he reports R. deronis as well as Metaradiophrya stammeri sp. nov. DePuytorac (1954) found 41 astomate species in Oligochaeta, 2 of which occurred in Naididae (both in Nais obtusa Gervais), the remainder being present in larger oligochaetes (macrodrili).

Of the 17 species of Naididae identified by Meier (1954), 2 harbored ciliates: N. variabilis, in which no ciliates were found in the present study, contained Radiophrya naidos Mackinnon and Adam, and D. digitata harbored R. deronis, as in this survey. In agreement with Meier, the following Naididae were found free of ciliates in this study: C. langi, C. diaphanus, N. pseudobtusa, S. appendiculata, S. lacustris, D. obtusa, and A. furcatus.

The ciliates P. lumbriculi and M. clavata identified in the present study have been reported in L. variegatus by other workers, too: P. lumbriculi by Heidenreich (1935a) and M. clavata by Heidenreich (1935b), DePuytorac (1954), and Meier (1954). Workers have reported in L. variegatus other species not found in this survey: Mesnilella trispiculata Kejensky by DePuytorac (1954), Heidenreich (1935b) and

Meier (1954); Hoplitophrya secans Stein by DePuytorac (1954); and Ptychostomum chattoni Rossolimo by Heidenreich (1935b) and Meier (1954).

As noted in the results, attempts to maintain ciliates within the oligochaetes in the laboratory were unsuccessful. As far as could be determined, there have been no previous comparable experiments with oligochaetes. Jones and Rodrigues (1971), however, working with a holotrichous ciliate occurring in the intestine of a polychaete, Hermodice carunculata, determined that 91% of 121 freshly collected worms harbored the ciliate whereas only 35% of 44 worms carried the protozoan when maintained in the laboratory longer than 3 days.

#### SUMMARY

1. Nineteen species of fresh-water oligochaetes were identified from four lakes of the Richmond, Virginia area over a twelve-month period. Two species belonged to the family Aeolosomatidae, 15 to Naididae, 1 to the Opistocystidae, and 1 to the Lumbriculidae.
2. Fifteen of the species have not been recorded previously from Virginia.
3. P. minuta is established definitely as part of the North American fauna.
4. Two of the 19 species harbored ciliates within the gut. R. deronis was found in D. digitata; M. clavata and P. lumbriculi in L. variegatus.
5. All of the ciliates are reported for the first time in microdrili of

North America.

6. Attempts to maintain ciliates within cultured worms were unsuccessful.

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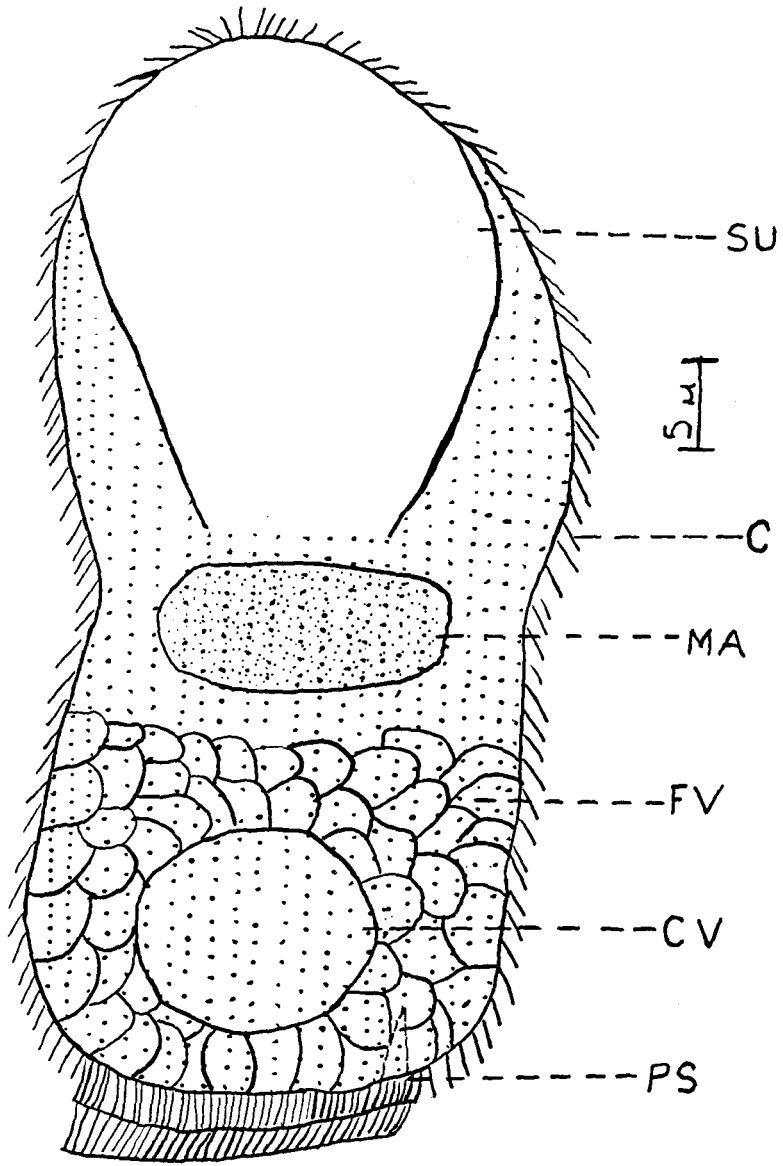


## FIGURE 1

Ptychostomum lumbriculi Rossolimo, 1925

Composite drawing based on living and stained ciliates. Stained vitally with methyl green and with Delafield's hematoxylin after fixation with Schaudinn's.

Abbreviations: SU, sucker; C, cilia; MA, macronucleus; FV, food vacuole; CV, contractile vacuole; PS, peristome.

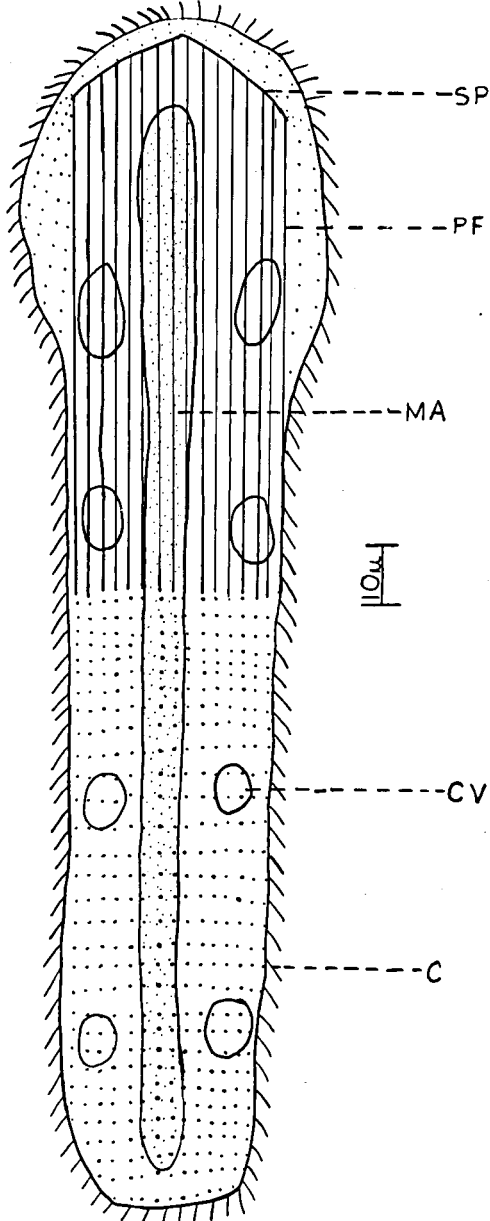


## FIGURE 2

Radiophrya deronis Heidenreich, 1935

Composite drawing based on living and stained ciliates. Stained vitally with methyl green and with Delafield's hematoxylin after fixation with Schaudinn's.

Abbreviations: SP, spicule; PF, plasmic fibril; MA, macronucleus; CV, contractile vacuole; C, cilia.

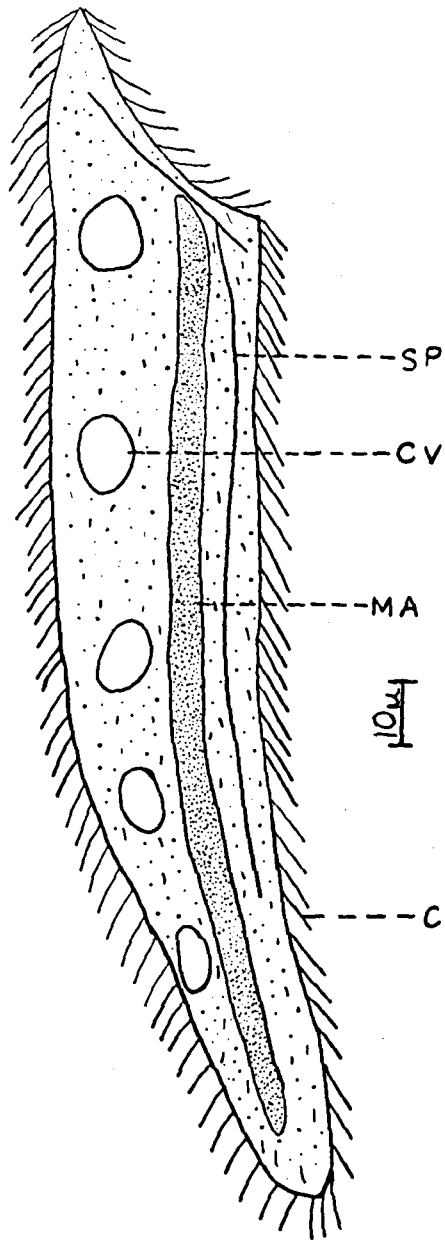


## FIGURE 3

Mesnilella clavata (Leidy), 1855

Composite drawing based on living and stained ciliates. Stained vitally with methyl green and with Delafield's hematoxylin after fixation with Schaudinn's.

Abbreviations: SP, spicule; CV, contractile vacuole; MA, macro-nucleus; C, cilia.



## ADDENDUM.

Oligochaetes

## Family Aeolosomatidae

Aeolosoma hemprichii: Prostomium wider than subsequent segments. Cilia on ventral surface of prostomium. Orange epidermal pigment globules. Dorsal and ventral bundles of chaetae. Length = 1-2 mm.

Aeolosoma headleyi: Prostomium wider than subsequent segments. Cilia on ventral surface of prostomium. Green epidermal pigment globules. Dorsal and ventral bundles of chaetae. Length = 1 mm.

Chaetogaster langi: Anterior end obtuse. No dorsal chaetae; ventral chaetae usually 5 per bundle, 60-112  $\mu$  in length in segment II, in other segments averaging 52  $\mu$  long. Length = 1 mm.

Chaetogaster diaphanus: No dorsal chaetae; ventral chaetae longer than in any other species; chaetae of segment II 8-11 per bundle, 3.4-4.9  $\mu$  thick, the longest chaetae measuring 186-275  $\mu$ , the shortest down to 130  $\mu$ ; in other segments chaetae 102-138  $\mu$  long and approximately 3.3  $\mu$  thick. Length = 4-5 mm.

Paranais: No eyes. Chaetae all bifid crochets, from 41-52  $\mu$  long; ventral chaetae, 2-3 per bundle, with distal nodulus; dorsal chaetae, 2-3 per bundle, beginning in segment II. Swimming response absent. Length = 5-10 mm.

Nais communis: Eye spots usually present. Often brownish-yellow pigment in anterior segments. Dorsal chaetae consisting of

needles and hairs; needle chaetae 1-2 per bundle, 57-70  $\mu$  long, with inconspicuous nodulus and short teeth; hairs, one per bundle, 84-163  $\mu$  long; ventral chaetae, 2-4 per bundle, those segment II with median nodulus and sub-equal teeth, 70-98  $\mu$  long; in segment V, 65  $\mu$  long; in segments behind V, chaetae up to 68  $\mu$  long, with distal nodulus. Swimming response absent. Length = 3-6 mm.

Nais variabilis: Eye spots always present. Brownish-yellow pigment present in anterior segments. Dorsal chaetae consisting of hairs and needles; needles, 1 per bundle, 41-46  $\mu$  in length, with short teeth; hairs, 1 per bundle, 138-555  $\mu$  long; ventral chaetae, 2-5 per bundle, those of segment II having distal tooth longer than proximal, being 76-103  $\mu$  in length. Swims with spiral movements. Length = 3-6 mm.

Nais pseudobtusa: Eye spots present. Brown pigment anteriorly. Dorsal chaetae consisting of hairs and needles; needle chaetae, 1 per bundle, 59-62  $\mu$  long, with pointed tip and distal nodulus; hairs 1-2 per bundle, 196-326  $\mu$  long; ventral chaetae with distal tooth longer than proximal and proximal nodulus; those of segment II being slender and 82-90  $\mu$  long; those of segment VI, 65-68  $\mu$  long, being thicker and more curved than segment II with proximal tooth thicker than distal one. Swims with spiral movements. Length = 2 mm.

Slavina appendiculata: Covered with thick crust of foreign material. Eye spots present. Dorsal chaetae (hairs and needles) beginning in segment VI; hairs, 1 per bundle, being very coarse, from 525-700  $\mu$  long



in segment VI, up to 400  $\mu$  long in other segments; needles, 1-2 per bundle, 52-60  $\mu$  in length; ventral chaetae 2-5 per bundle. Length = 5-10 mm.

Stylaria lacustris: Probosis projecting from between two lateral lobes. Eye spots present. Dorsal chaetae (hairs and needles) beginning in VI; hairs, 1-2 per bundle and serrated, 490-560  $\mu$  long; needles, 2-4 per bundle, 52-65  $\mu$  long; ventral chaetae, 4-6 per bundle and 114-125  $\mu$  long, displaying two sharp proximal bends with a long curved distal tooth and a weak proximal one. Swims. Length = 6-8 mm.

Stylaria fossularis: Probosis projecting from tip of prostomium. Eye spots present. Dorsal chaetae (hairs and needles) beginning in VI; hairs, one per bundle; needles, one per bundle; ventral chaetae, 5-7 per bundle. Swims. Length = 8-10 mm.

Haemonais waldvogeli: Dorsal chaetae (hairs and needles) beginning in segment XVIII; hairs, 1 per bundle, 140-171  $\mu$  long; needles curved and bifid, 99-126  $\mu$  long; ventral chaetae, 2-4 per bundle, in anterior segments up to 127  $\mu$  in length; in posterior segments ventral chaetae thicker and more curved, from 98-106  $\mu$  long. Swimming response absent. Length = 10-15 mm.

Dero (Dero) digitata: No eye spots. Dorsal chaetae (hairs and needles) beginning with segment VI; 1 hair, 240-269  $\mu$  long; 1 needle 86-90  $\mu$  long with distal tooth longer than proximal; ventral chaetae 3-5 per bundle, those of segment V, 83-106  $\mu$  long. Branchial fossa with 4 pairs of gills, 1 small dorsal and 3 ventral foliate. Lives in

fixed mucous tube. Swims with spiral movements. Length = 6-10 mm.

Dero (Dero) obtusa: No eye spots. Dorsal chaetae (hairs and needles) beginning in segment VI; 1 hair, 106-109  $\mu$  long; 1 bifid needle, 57-62  $\mu$  long, with equal teeth; ventral chaetae, 2-4 per bundle, those of segment II 96-110  $\mu$  in length; chaetae behind segment V 62-71  $\mu$  long. Branchial fossa with 3 pairs of gills, 2 ventral digitate and 1 pair dorsal ciliated swellings. Lives in fixed mucous tube. Swims. Length = 4-5 mm.

Dero (Aulophorus) furcatus: No eye spots. Dorsal chaetae beginning in segment V, consisting of 1 hair, 130-138  $\mu$  long, and 1 needle, 41-57  $\mu$  long, with distal tooth thinner and shorter than proximal; ventral chaetae 2-4 per bundle, those of segment II-IV 62-65  $\mu$  long, with distal tooth longer, from V on, 54-63  $\mu$  long, with equal teeth. Branchial fossa with pair of long palps and 3 pairs of digitate gills. Usually in portable tubes composed of mucous and debris. Swims. Length = 4-10 mm.

Pristina minuta: No eye spots. No probosis. Dorsal chaetae beginning segment II, consisting of 1 non-serrated hair 65-68  $\mu$  long, and 1 needle, 25-26  $\mu$  long, with equal, divergent teeth and distal nodulus; ventral chaetae from 3-5 per bundle anteriorly, 2-3 posteriorly; in segment II, distal tooth longer than proximal and nodulus proximal; behind II nodulus distal and teeth becoming equally long. Length = 2.0-2.4 mm.

Pristina longiseta leidyi: No eye spots. Prostomium forming a

proboscis. Dorsal chaetae, consisting of hairs and needles, beginning segment II; hair chaetae, 2 per bundle, those of segment III elongate, 601-688  $\mu$  long, others 172-228  $\mu$  long; needles, single-pointed, 33-52  $\mu$  long; ventral chaetae 5-9 per bundle, with distal prong 3 times as long as proximal. Length = 3-6 mm.

#### Family Opistocystidae

Opistocysta flagellum: Long prostomium. No eye spots. Chaetae beginning in segment II; dorsal chaetae consisting of long hair chaetae, 2-3 per segment, 293-326  $\mu$  long and 2 dorsal hair-like needles, 57-65  $\mu$  long; ventral chaetae, 3-5 per segment, teeth about equally long; in segments II-V, chaetae 73-82  $\mu$  long; in remaining segments, 82-93  $\mu$  long. Posterior end with one median and two lateral projections. Length = 4-5 mm.

#### Family Lumbriculidae

Lumbriculus variegatus: Prostomium conical. Lateral caecae of posterior vascular system well-developed. Four bundles of chaetae per segment, typically with 2 chaetae per bundle, chaetae bifid with upper tooth reduced. Reproduction by fragmentation. Length = 25-30 mm.

#### Ciliates

##### Order Thigmotrichida, Family Hysteroconinetidae

Ptychostomum lumbriculi: 48-103  $\mu$  long, 27-34  $\mu$  wide. In the midgut and hindgut of Lumbriculus variegatus. Body flattened laterally; cytostome at posterior end; cup-shaped sucker, surrounded by

horseshoe-shaped ridged area, occupying entire anterior surface on one side. Ciliation uniform. Ovoid macronucleus located medially below sucker with long axis vertical to that of body. One contractile vacuole located posteriorly in that portion of body filled with food vacuoles. Multiplication by transverse fission.

Order Astomatida, Family Intoshellinidae

Radiophrya deronis: 138-315  $\mu$  long, 28-48 wide. In the midgut of Dero digitata. Elongate with anterior end appearing club-shaped and posterior end being narrower. V-shaped spicule present in anterior region of body and 16-20 plasmic fibrils extending longitudinally over approximately half of body. Cilia arranged in rows. Band-form macronucleus, wider in anterior region (10.0  $\mu$ ) and narrower in posterior (7.0  $\mu$ ). Three to four contractile vacuoles present in two longitudinal rows. One to two satellites (each 48-70  $\mu$  long) attached frequently, forming chains.

Mesnilella clavata: 69-241  $\mu$  long, 21-34  $\mu$  wide. Mainly in the foregut of Lumbriculus variegatus. Body elongate and slightly curved, flattened with long cilia covering body. Band-form macronucleus. Anterior hatchet-shaped end occupied by 2 spicules, the longer extending backward and paralleling the macronucleus for approximately half the body length. Three to five contractile vacuoles in one row.

## VITA

Elsa Carolyn Queen was born in Charlottesville, Virginia on January 25, 1942. She attended elementary and secondary schools in Culpeper County, Virginia and was graduated second in her class from Culpeper County High School in June 1960.

She entered Westhampton College, University of Richmond, Virginia in September 1960 and received the Bachelor of Arts degree in biology in June 1964. At Westhampton she served as treasurer of College Government and the Senior Class, received Intermediate Honors, and was elected to Phi Beta Kappa, Beta Beta Beta, and Kappa Delta Pi.

From September 1964-June 1965 she taught biology at Douglas S. Freeman High School in Henrico County, Virginia. The following year she was a laboratory instructor in biology and chemistry at University of Virginia Extension in Roanoke, Virginia.

In September 1967 she began graduate work at the University of Richmond and completed requirements for the Master of Arts degree in biology in May 1972. She was elected to membership in the American Society of Zoologists in the spring of 1971.

She is married to Donald Parker Falls and is the mother of two children, Melissa Arnold, born August 1966, and Mark Parker, born August 1968.

She is a member of River Road Presbyterian Church, where she teaches Sunday school. Outside interests include the Westhampton

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