A SURVEY OF THE CESTODE GENERA PROTEOCEPHALUS AND BOTHRIOCEPHALUS FROM THE FRESHWATER FISH OF WESTERN CANADA, INCLUDING A RE-DESCRIPTION OF PROTEOCEPHALUS luciopercae (WARDLE) AND PROTEOCEPHALUS stizostethi (HUNTER AND BANGHAM)

A Thesis Presented to the Faculty of Graduate Studies and Research, University of Manitoba, In Partial Fulfillment of the Requirements for the Degree of Master of Science

BY

Scott Wells Little B.Sc.

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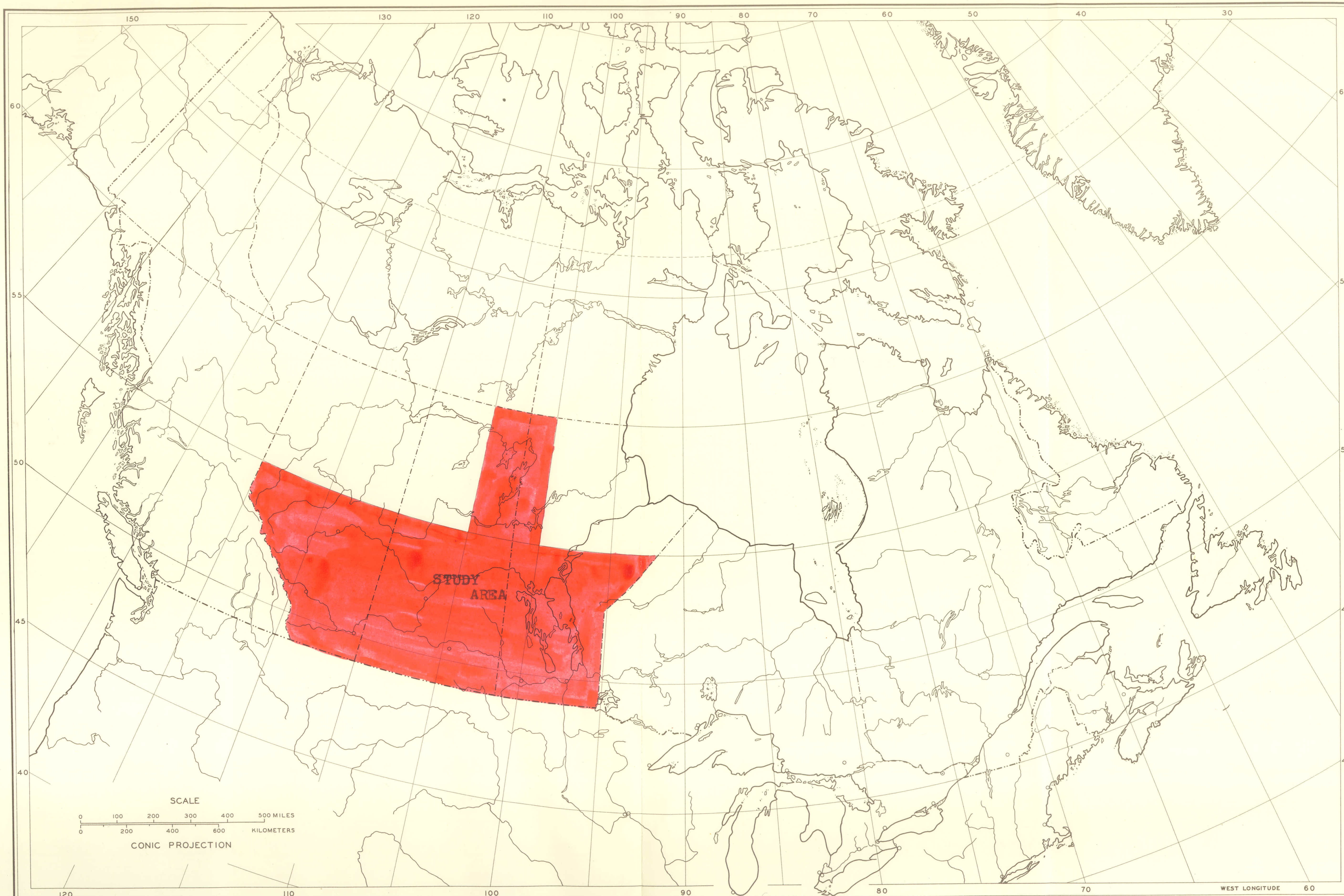
For Professor R.A.Wardle and Dr. J.A.McLeod for their guidance and patience I reserve a very special debt of gratitude.

CANADA AND NEWFOUNDLAND

No. III



GOODE'S SERIES OF BASE MAPS
HENRY M. LEPPARD, EDITOR

Prepared by Henry M. Leppard
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ABSTRACT

A systematic survey of the Cestode genera Proteocephalus and Bothriocephalus parasitizing the freshwater fish of Western Canada was carried out with a view to simplifying identification of species in these genera.

Laboratory examination of all material utilizing staining, sectioning, photomicrography, and camera lucida techniques was carried out. A new stain technique was developed to aid in this investigation. A combination of two separate species, Proteocephalus luciopercae (Wardle) and Proteocephalus stizostethi (Hunter and Bangham) under the single species of Proteocephalus luciopercae (Wardle) is suggested.

Findings show that among the more common freshwater fish of Western Canada the host-parasite interrelationship is remarkably specific. These findings materially aid in simplifying identification of tapeworm species in the area studied.

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INTRODUCTION

This work is intended to clarify and to some extent simplify the classification and identification of the principal proteocephalan and bothriocephalan tapeworms parasitizing the fish found in the lakes of Western Canada. The area from which material has been examined, roughly stretches from the Manitoba-Ontario boundary westward to the Great Divide in Alberta, and as far north as the 54th. parallel of latitude. The general area has been indicated on Map I.

The work includes a re-examination of all species found in the particular genera Proteocephalus and Bothriocephalus and from this the two species Proteocephalus luciopercae (Wardle) and Proteocephalus stizostethi (Hunter and Bangham) have been combined under the name Proteocephalus luciopercae (Wardle). From 297 fish samples obtained in Alberta, Saskatchewan and Manitoba, five consistantly occurring Proteocephalus species were recorded. Sixty-six other samples yielded Eubothrium, Triaenophoridae, Glaridacris and Spaethbothrid, forms which were of common occurrance. A single species of Bothriocephalus was recorded. This species was cuspidatus (Cooper).

The bulk of the material examined was accumulated by the Department of Zoology at the University of Manitoba from various fisheries and fisheries agencies during the period 1925 to 1953. Fresh fish were supplied by the Game and Fisheries Branch, Department of Mines and Natural Resources for Manitoba and the Central Fisheries Research Station, Winnipeg.

The basis for species identification follows the key and scheme suggested by Wardle and McLeod in their work, The Zoology of Tapeworms. This book combined with LaRue's monograph on a Revision of The Cestode Family Proteocephalidae has supplied the bulk of the reference material used. A special section on characteristic movements and holdfast variations recorded from live tapeworms by cinema photomicrography has been included. A specially developed haematoxylin staining technique which has yielded exceptional results is included in the section on methods of technique. A systematic investigation of staining properties of the common stains usually used in Cestode examination showed this particular stain to be superior in penetration and differentiating properties.

HISTORICAL

Ariola (1899) established the family Ichthyotaeniidae for the genus Ichthyotaenia which had been described by Riggenbach (1896) and founded by Loennberg in 1894. Rudolphi 1808-10, pladed forms recognized to day as distinctly proteocephalan like in the Linnaean genus Taenia (1758). In 1858 D.F.Weinland formed the genus Proteocephalus, based on the characteristics displayed in the species ambigua (Dujardin), filicollis (Rudolphi), and dispar (Goeze). Wardle and McLeod point out that:

> Presumably Weinland was unaware in 1858 that the term had been used previously by Blainville (1828) for a family of tapeworms that included the one genus Caryophyllaeus; or possibly he was aware of the fact, but believed that the grammatical difference between the terms "Proteocephala", and "Proteocephalus" sufficiently guarded the use of these terms from confusion. Dollfus (1932), however, pointed out the term "Proteocephalus" is really an orthographical error or "lapsus" for "Proteocephala", and since Article 19 of the International Code of Zoological Nomenclature specifically states that an orthographical error shall not be perpetuated, the correct term should be "Proteocephala". If we are to invoke the International Code, however it may be pointed out that according to Article 8 the generic name may be a Greek substantive for which the rules of Latin transcription should be followed. A generic name derivable from the Greek 'cephalo' would therefore be "Proteocephalus". Further, as Dollfus admitted, Blainville's term was used not for a genus but for a family. Here we shall use his term to cover the whole order.

In 1914 George LaRue published a Ph.D. thesis at the University of Illinois entitled A Revision of the Cestode Family Proteocephalidae. In this work, LaRue incorporated all the known forms of the tapeworm genus Proteocephalus as described by Weinland, while adding his own original material and that of several earlier and contemporary workers. He pointed out the apparent similarity between Loennberg's Ichthyotaenia (presumably based on filicollis (Rudolphi)) and Weinland's Proteocephalus (based on ambigua (Dujardin)) and suggested that on priority of nomenclature, Loennberg's Ichthyotaenia and Ariola's Ichthyotaeniidae were not valid. LaRue suggested the family name Proteocephalidae which is widely accepted. This divided proteocephalan tapeworms into two families as LaRue also indicated the homonymity of Monticelli's Tetracotylus with a group of larval Trematodes similarly named by Filippi (1854). Monticelli's Tetracotylus was renamed Monticellia by LaRue.

I Family: Proteocephalidae (LaRue)

Genera: Proteocephalus (Weinland, 1858)
Corallobothrium (Fritsch, 1886)
Acanthotaenia (von Linstow, 1903)
Crepidobothrium (Monticelli, 1899)
Choanoscolex (LaRue, 1911)
Ophiotaenia (LaRue, 1911)

II Family: Monticellidae (LaRue)

Genus: Monticellia (LaRue, 1911)

From 1914 to 1927 workers made little change or addition to LaRue's classification. Woodland between 1925 and 1937 attempted to improve LaRue's 1914 classification as he had available to him material collected in all parts of the world. If Woodland's view that holdfast characteristics are not valid recognition features between genera we may materially reduce our number of recognizable subgenera and species. In particular within the genus Proteocephalus alone it is found that where formerly distinct subgenera and species were described, the whole group became oversimplified and no distinct subgenera could be recognized. This weakness in Woodland's scheme was never overcome satisfactorily though he was able to attach the subgeneric term Teleostotaenia to forms from fresh water fishes whose members have an unusually small holdfast (breadth of 0.5mm. or less), complete lack of spines and hooks, testes distributed in a single continuous field (except P. longicollis) and have the vagina opening anterior to the cirrus pore. These forms include all those described by LaRue (1914) except a few from ganoids and siluroids.

Wardle and McLeod (1952) have accepted Woodland's eight subfamilies as described under LaRue's family of Proteocephalidae, order Proteocephala, but rejected his idea of not using holdfast characteristics to establish genera. They have accepted the generic list proposed by Fuhrman (1931) and have used the species-group concept suggested by Meggitt (1927) in the genus Proteocephalus.

The history of the order Proteocephala is not intended to cover the whole order in this paper, but, rather to follow the main highlights in the establishment of the family Proteocephalidae, subfamily Proteocephalinae, genus Proteocephalus as it is generally recognized at the present time.

It is my own contention that Woodland is on a firm footing when he rejects the morphologic features of the holdfast for basic generic identification, however I do feel that the holdfast can be used to a degree in identification. Size may be unreliable, especially when measurements of suckers are used. Shape of relaxed scolices on the other hand appears to be quite uniform for any one species if the stage of maturity is uniform for the individuals being compared. This criterion of shape only holds good between individuals of approximately the same maturity. Appearance of the apical sucker or vestige of sucker also appears to yield an identification point but is not entirely a valid feature of recognition. It may even be that these latter holdfast features are indicative of a stage in the life cycle of the worm and are not generic differences. It is therefore left up to the investigator to use scolex characteristics as a guide to identification rather than a positive recognition feature. Positive identification must rely on internal anatomical features not unduly affected by fixing and on external proportion (not to be confused with actual measurement).

In Systema Naturae of Linnaeus (1758) a worm by the name of Taenia lata appeared. This was the "fish tapeworm", Dibothriocephalus latus and is probably the earliest description of a Pseudophyllidean tapeworm on record. In 1808 Rudolphi described a genera called Bothriocephalus. Blanchard (1849) established a family Bothriocephalidae covering most forms we now include in our present day order Pseudophyllidea. A family created by Carus in 1863 was called Pseudophyllidea and included four genera, Ligula, Triaenophorus, Schistocephalus, and Bothriocephalus. In 1899 Luhe established the family Bothriocephalidae to cover these pseudophyllidean forms. 1902 saw Luhe adopting Carus' term Pseudophyllidea as the name for the order. Cooper (1917) assigned the species name of cuspidatus to a tapeworm he found in the caeca of Stizostedion vitreum vitreum, Stizostedion canadense, Amphidon aloseides, Hidon tergisus, Esox lucius, Perca flavescens and rarely in Leucichthys. In 1932 Wardle (1932 a) described three subspecies of cuspidatus, subspecies hiodontos and subspecies luciopercae as well as a subspecies called cuspidatus. The majority of the cuspidatus species recorded in this investigation fit the original description of Cooper so for all practical purposes no subspecies will be dealt with though differences in adaptive and morphogenetic characters were observed in a minority of cases.

METHODS OF TECHNIQUE

The bulk of the material examined was already fixed and preserved in either 5% formalin or 70% ethyl alcohol. Of the two preservatives it was found that 5% formalin was noticeably superior. Formalin fixed material in particular, yielded better staining whole mounts and equally as good sections as alcohol fixed material. Fifty-two months appeared to be the limit of preservation afforded the tapeworms in either solution. After this period morphologic characteristics cannot be depended upon to yield consistently true or typical form.

With fresh fish material routine post mortem examination was carried out. Removal of tapeworm adults from the intestinal, caecal or stomach walls was accomplished by agitating the source material gently in a large beaker of lukewarm water. The material thus collected was separated by decantation. Muscular and internal organ examination was carried out under a low power lens, binocular microscope.

Fixing and killing of material was accomplished in hot 5% formalin. Tapes were stretched (using their own weight) on a wet 75x40mm. glass slide, then quickly dipped in a beaker containing the hot formalin solution. This tended to relax the material to an extent, but care was required to avoid a contraction of the material by leaving it in the hot solution too long. The killed and fixed material was then transferred to a Petri dish containing 5% formalin for at least 24 hours. This extra period in formalin insured a complete fixation.

Material which was to be mounted and stained whole was thoroughly washed under lukewarm tap water, then was transferred to a solution of physiological saline (7.0gm. sodium chloride to 1000 cc. water) made basic with two or three drops of 28% Ammonium hydroxide solution. The material was allowed to stand in this basic physiological saline solution for at least twenty-four hours for small specimens (breadth 2mm. or less) and seventy-two to one hundred and twenty-five hours for specimins exceeding this limit. This tended to relax the specimins still further and perfuse the internal tissues with a uniform medium that was compatible with the stain used.

For whole mount staining the following stains were tested but found to be lacking in one or more desirable properties; Lillie-Meyer acid haemalum, Harris' haematoxylin, Chatton's methyl blue-eosin, aceto carmine, picro carmine, borax carmine, Ehrlich's acid haematoxylin, Gower's aceto-carmine and alcoholic eosin. The general objection found in most whole mount stains is that they overstain the cuticle and parenchyma, obliterating all other structural features from the microscopist. One particular haematoxylin stain was found which rendered nearly all internal structures visible and at the same time did not overstain either the cuticle or the parenchyma. This same stain proved excellent for in toto staining before sectioning. In general it was found that strongly acidic stains fail to stain tapeworm material to any great extent. Weakly basic or acidic stains or near neutral stains in weak basic solutions yield good results.

The ingredients used to make up the haematoxylin stain and its method of preparation are recorded as follows;

<u>HARRIS HAEMATOXYLIN MODIFICATION</u> (based on a modification used by Dr. J.Isa, Manitoba Dept. Agriculture Vetrinary Laboratory, University of Manitoba, 1953)

I) Dissolve 1 gm. haematoxylin in 10cc. 95% ethyl alcohol if stain is to be used on sections, or 0.9gm. haematoxylin in 10cc. 95% ethyl alcohol if stain is to be used on whole mounts.
II) Dissolve 20gm. Potassium alum in 100cc. distilled water.
III) Mix I) and II) and bring to a rapid boil until solution turns purple.
IV) Add 0.5gm. Mercuric oxide and cool container <u>rapidly</u> in cold flowing water.
V) Add 100cc. glycerine.
VI) Add 4% by volume of glacial acetic acid.

This stain is moderately quick acting, the average worm being completely stained within 13 minutes or less. If a worm is left in the stain for longer than 13 minutes, destaining of the material in 70% acid alcohol may be required. Destaining rarely gives satisfactory results. Destained material may give excellent color rendition, but does not yield a maximum of definition or a minimum of distortion. Maximum sharpness is obtained when the stain retains a blue color, due to the basic influence of the internal basic physiological saline perfusing fluid. Use of green, blue and neutral optical filters on the microscope often improved definition in stained material.

Dehydration through a series of fresh ethyl alcohols (50%, 70%, 90%) to a fifty-fifty 95% alcohol / beechwood creosote solution is carried out. Clearing in beechwood creosote, followed by superclearing in fresh Methyl salicylate (artificial oil of Wintergreen) gives excellent results if permanent mounting iscarried out in toluene based Permount. Xylol based Clarite has been found to be less compatible with methyl salicylate supercleared material, but is nevertheless quite good.

Black and white line drawings were made using the camera lucida. Photomicrographs were made on 35mm still film and on 8mm motion picture Kodachrome.

RECOGNITION FEATURES

The forms of the genus Proteocephalus recorded from Western Canadian freshwater fish all fit the generalized description given by Wardle and Mcleod (1952) as follows:

> On the whole these worms are short slender forms, a few inches in length and distinctly segmented. The mature and gravid segments are longer than they are broad. The holdfast end is small, ranging in length from 0.1 to 1.0 mm. in material from fishes, and from 0.2 to 1.75mm. in material from amphibians and reptiles. It is dorso-ventrally flattened and rounded in surficial outline. There are four, simple cup-shaped suckers set flush with the body surface in most cases . Commonly they are so arranged as to form a cluster with their cavities facing forward.

Commonly proglottis shape is of either of the two following proportions. Arrangement of internal features shown is quite uniform.

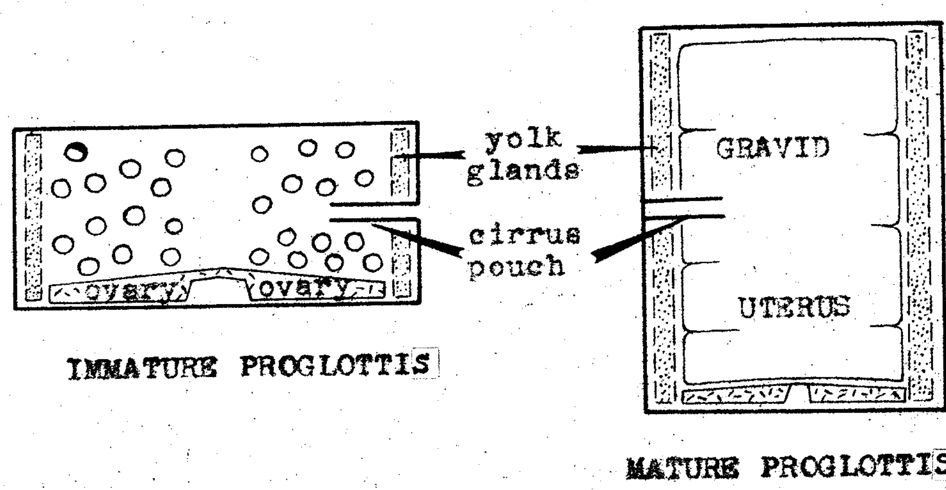


Figure 1.

In transverse section the appearance closely resembles the following generalized pattern (after Woodland 1925).

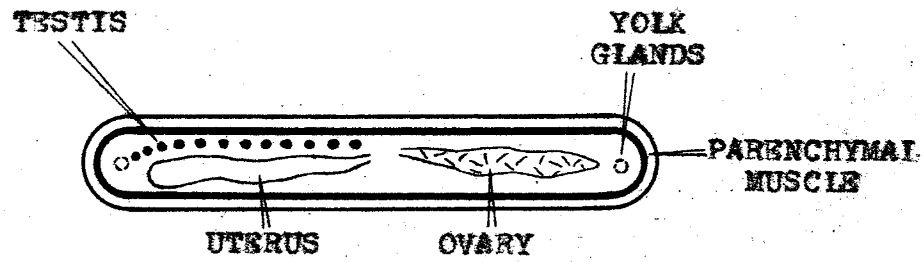


Figure 2.

Holdfast is of the following three general patterns.

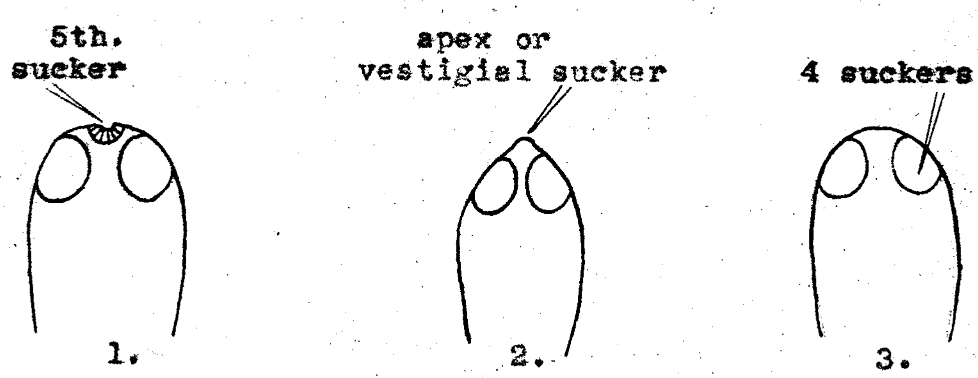


Figure 3.

The following set of pictures is taken from motion picture film and demonstrates a few of the contraction variations in the holdfast and neck region of *Proteocephalus pinguis*. The pictures are of living material in a physiological saline solution. The host was taken from Wellman Lake, Duck Mountains, Manitoba.

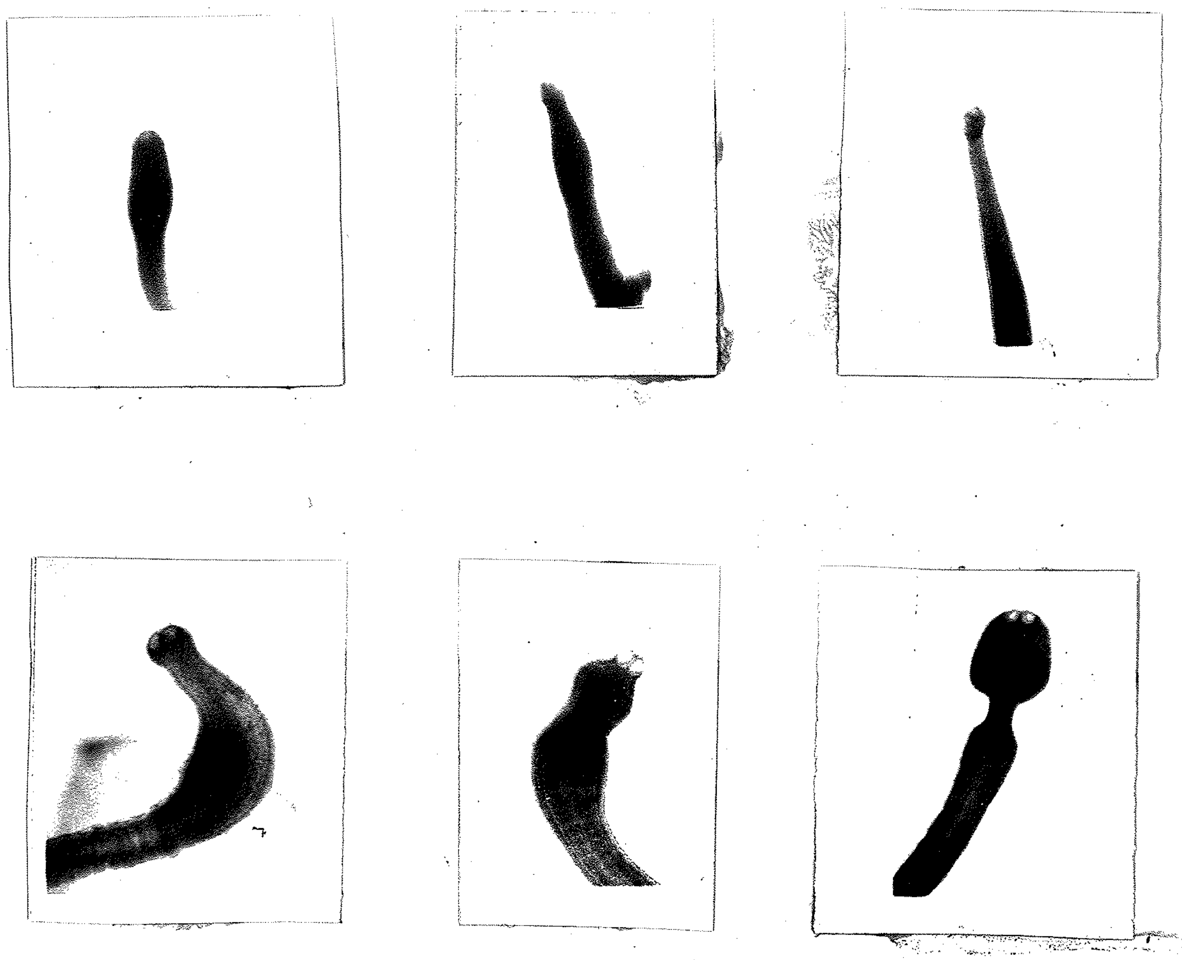


Figure 4. Variations in shape of holdfast of Proteocephalus *pinguis*.

The variations shown will appear in any fixed preparation if the animal is killed at that point. This of course would lead to a certain amount of confusion in identification if our holdfast shape was used to identify or classify the animal.

In living forms the longitudinal osmoregulatory canals appear quite distinctly , cortically to the yolk glands, but are not as distinct in fixed or stained material.

Life cycles of the various species found in Western Canada follow the general pattern outlined by Hunter (1929) for Proteocephalus pinguis. Connor (1953) in his studies on the seasonal cycle of Proteocephalus stizostethi further aids the worker in collecting his material at a stage of the life cycle most likely to yield best results. Connor found the peak of infestation by mature individuals to be late spring and early summer.

The proteocephalan egg appears to be eaten by a copepod Crustacean in which the onchosphere invades the haemocoel and develops rapidly into a procercoid larva. This seems a likely step, for in most fish examined copepods made up a fair proportion of the animal life found in the stomach and upper intestine. The procercoid tail-like cercomer is not always noticeable nor are the typical hooks. No second intermediate host is needed, the final vertebrate host being infected by swallowing the infected Crustacea. Plerocercoids have been removed from the liver, gut, muscle and spleen, indicating that procercoids have invaded these organs.

CLASSIFICATION

FAMILY: PROTEOCEPHALIDAE (LaRue, 1914)

SUBFAMILY: PROTEOCEPHALINAE (Mola, 1929)

GENUS : Proteocephalus (Weinland, 1858)

Ovary, testes, yolk glands and uterus in the medulla, yolk glands compactly arranged in two lateral bands. Holdfast with four suckers of normal type. A fifth or apical sucker, functional or vestigial may be present. No spines, hooks, or folds of tissue on the holdfast. Adults parasitic in freshwater fish, rarely in Amphibia. Genotype, filicollis (Rudolphi, 1810).

SPECIES OF PROTEOCEPHALUS ACCORDING TO OCCURRENCE IN THE COMMON FRESHWATER FISH OF WESTERN CANADA

HOST: Stizostedion vitreum vitreum (Mitchell)

Common name; Pickerel, dore, yellow, wall-eyed pike.

Parasitized by Proteocephalus luciopercae (Wardle, 1932)

Proteocephalus stizostethi (Hunter and Bangham 1933)

HOST: Esox lucius (Linnaeus)

Common name; Great northern pike, pickerel, jackfish.

Parasitized by Proteocephalus pinguis (LaRue, 1911)

HOST: *Leucichthys tullibee* (Richardson)

Common name; Tullibee.

Parasitized by *Proteocephalus pusillus* (Ward, 1910)

HOST: *Coregonus clupeaformis* (Mitchell)

Common name; Common whitefish.

Parasitized by *Proteocephalus singularis* (LaRue, 1911)

This foregoing list does not imply that no other tapeworm species are found associated in these particular hosts. Examination will show in most cases, two or more tapeworm species of different families or genera. Though specificity of occurrence of parasite in these particular hosts is marked, other workers have shown that this characteristic is not to be found in all cases. In general however, these are the main proteocephalan tapeworms likely to be encountered in these fish hosts in the area under study.

Proteocephalus lucioperceae (Wardle 1932)

1933: Proteocephalus stizostethi (Hunter and Bangham)

Pickerel intestines examined yielded proteocephalan forms that fitted both the description of Wardle's lucioperceae and Hunter and Bangham's stizostethi. As some of the original material used by Wardle in his description was available a re-examination of this material was undertaken in conjunction with the material which had been freshly mounted. It was apparent after this examination that variations that existed between stizostethi and lucioperceae could be traced to immaturity or individual variation caused principally by the killing and fixing technique.

Comparison of measurements in length of individual, dimensions of holdfast and proglottids in immature, mature and gravid states show that these measurements are close enough to be confusion factors in identification. On the basis of testis number an overlap occurs (lucioperceae 80-100 stizostethi 90-125). The fact also remains that in a single individual testis number does increase from early maturity to late maturity, and an increase in testis size can also be traced.

The genital pore on both species is located in the anterior half of the proglottis and has no genital papilla. While Hunter and Bangham state that the cirrus pouch ratio varies from 1/6 - 1/4 the width of the segment, Wardle gives a 1:3 ratio. The former ratio appears to be closer to the average than the 1:3 ratio which occurs more commonly in a gravid proglottis when the cirrus pouch diameter is reduced.

Transeverse sections show that the cirrus pouch is directed dorsally at its inner end, while the vagina dips ventrally in both types. Wardle notes that the testes occur in two layers, but this is not a constant feature. It is found that in late maturation as testes increase in size (and number) some are displaced towards the medulla of the proglottid giving the illusion of two layers. The number of coils in the ductus ejaculatorius appear to be the same in both species.

As has previously been noted the holdfst characteristics are variable, but two points may be made in this connection that are constant. One is that there are four distinct suckers,and secondly the apex,or rostellum described by Wardle is not as exaggerated nor distinct in all cases as he originally described.

A re-description of the two species under the priority of description rule assigning the name of luciopercae (Wardle) in preference over stizostethi (Hunter and Bangham) follows.

Proteocephalus luciopercae (Wardle, 1932)

Habitat:

Intestines and caecae of Stizostedion vitreum vitreum, Stizostedion canadense from Lakes Winnipeg, Dauphin, Hemming and Waskesiu, and from Stizostedion glaucum, Stizostedion canadense griseum, Stizostedion vitreum vitreum and Micropterus dolomieu Lake Erie and St.Lawrence River.

Gross Appearance:

Individuals measure up to 250 m.m. in length, 1.5-2.0 m.m. in average breadth, 0.5 - 0.6 mm. in thickness. Proglottization begins approximately 1/4 along the length away from the holdfast, with first segments at least 1/2 the maximum width of the worm. First proglottids are indistinct, being much wider than long. Segments in mature individuals are normally wider than long, gravid segments are nearly quadrate. Terminal segment is bluntly pointed. L-shaped yolk glands can be seen distinctly. Scolex posesses four cup shaped suckers, normally opening forward or laterally. Most often a rounded area, rostellar like, appears between the suckers, giving in some cases an apex like appearance. Overfixing in too strong a solution will produce an appearance in the scolex in which the suckers seem to be facing one another. The neck may in some cases be wider than the holdfast area in the flattened plane but is usually of equal breadth. The worm is acraspedote and anapolytic.

Internal Features:

Genital pore occurs in anterior half of segment, irregularly alternating from segment to segment. Vagina opens into the genital sinus anteriorly, but it is not unusual to find it entering posteriorly as well. The vas deferens is much coiled distal to the cirrus pouch. The cirrus pouch normally occurs in a ratio of 1:3 or 1:4 to the breadth of the proglottid, the latter ratio predominating in the majority of cases. The vagina dips ventrally after joining the genital sinus and passes nearly to the mid line of the proglottid before curving sharply posterior to the inter ovarial area. Slightly anterior to the ovary the vagina coils slightly and increases in diameter. It usually makes a complete loop before entering the isthmus of the ovary. Coiling of the oviduct and accessory female organs occurs ventral to the inter ovarial loop of the vagina.

The uterus in its early gravid shape has a basic four lobed pattern which increases in complexity to yield any number of branches up to twelve in number.

Figure 5. Basic four lobed pattern of the uterus.

PROTEOCEPHALUS __luciopercae__

HOLDFAST

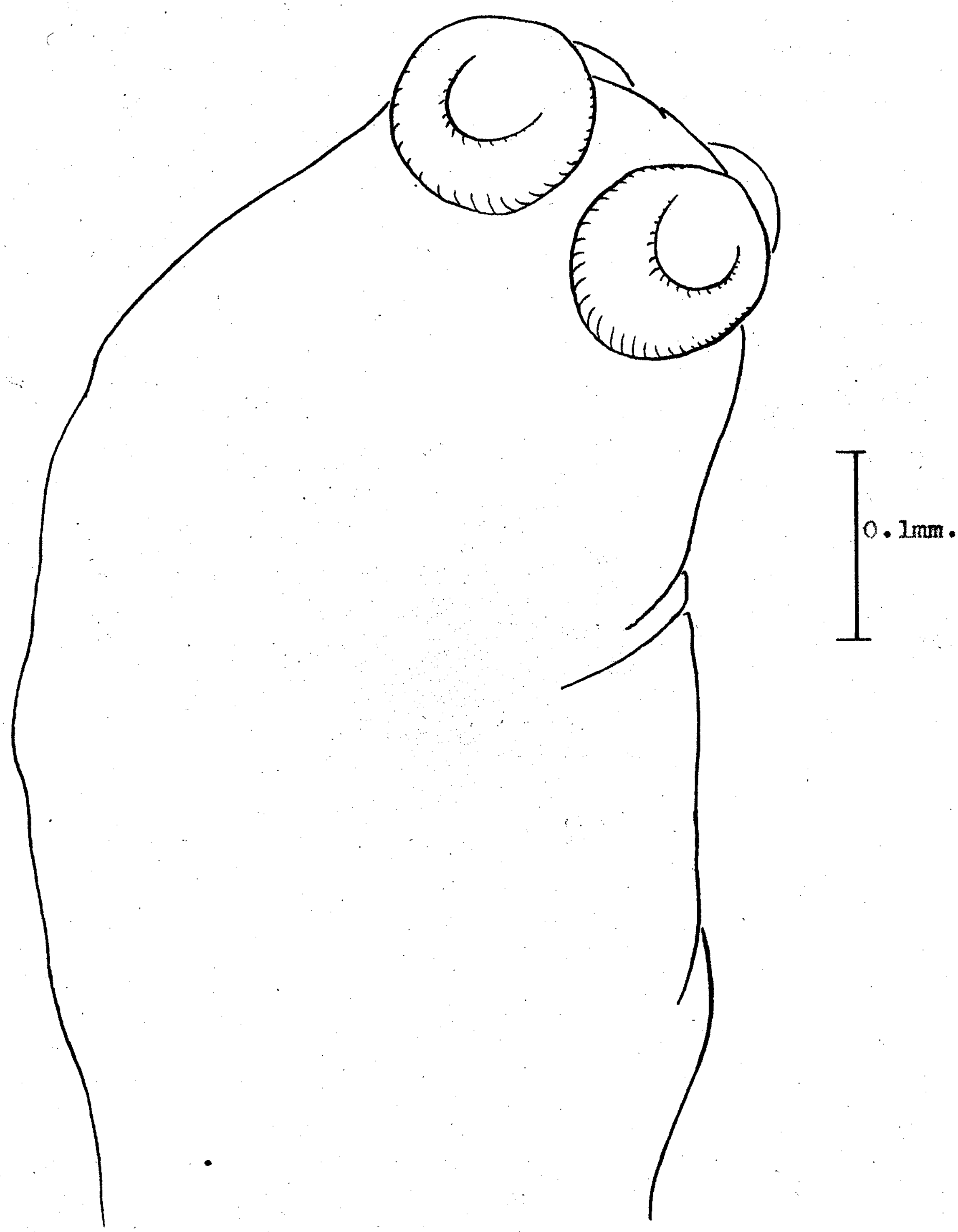


Figure 6.

PROTEOCEPHALUS luciopercae

HOLDFAST VARIATION 'B'

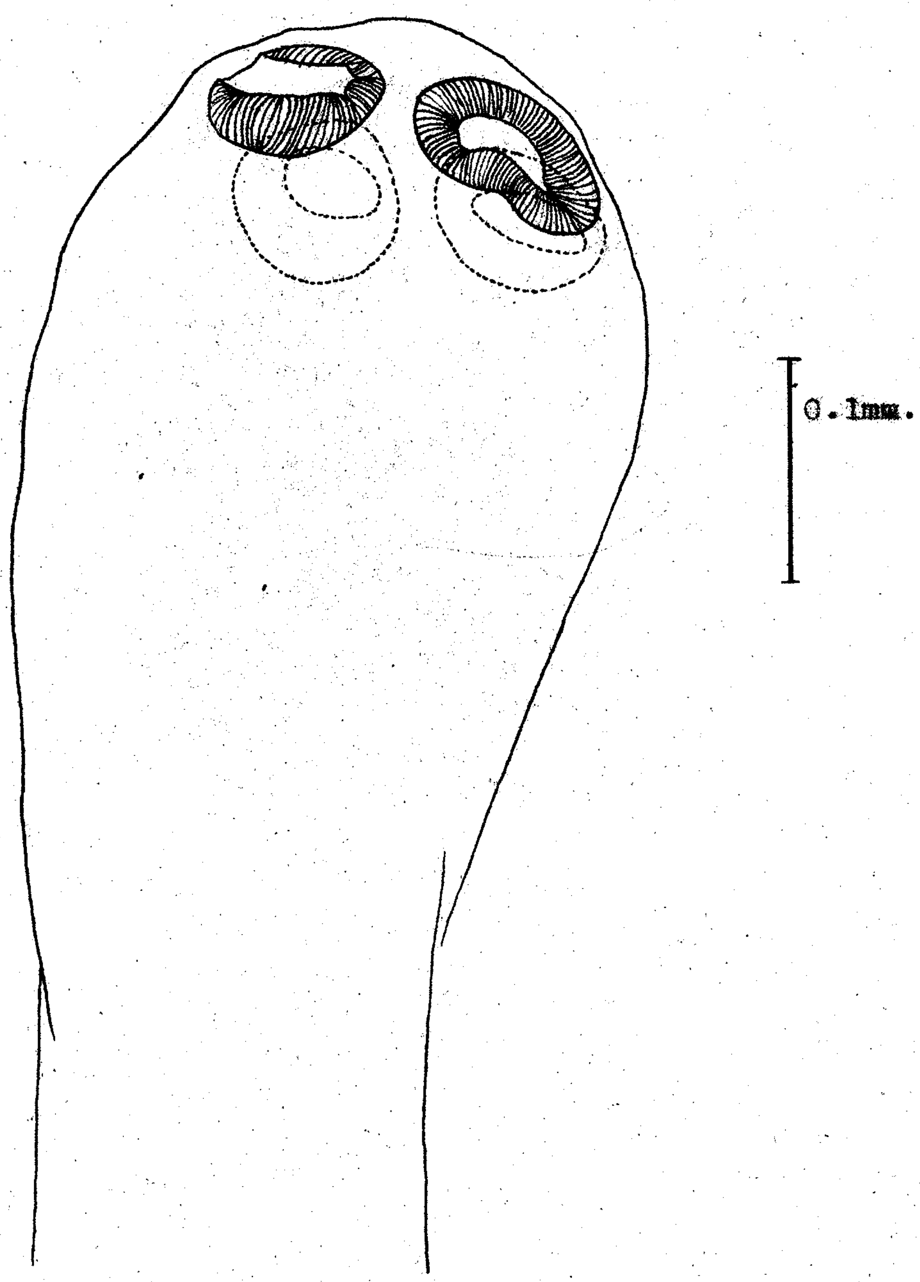
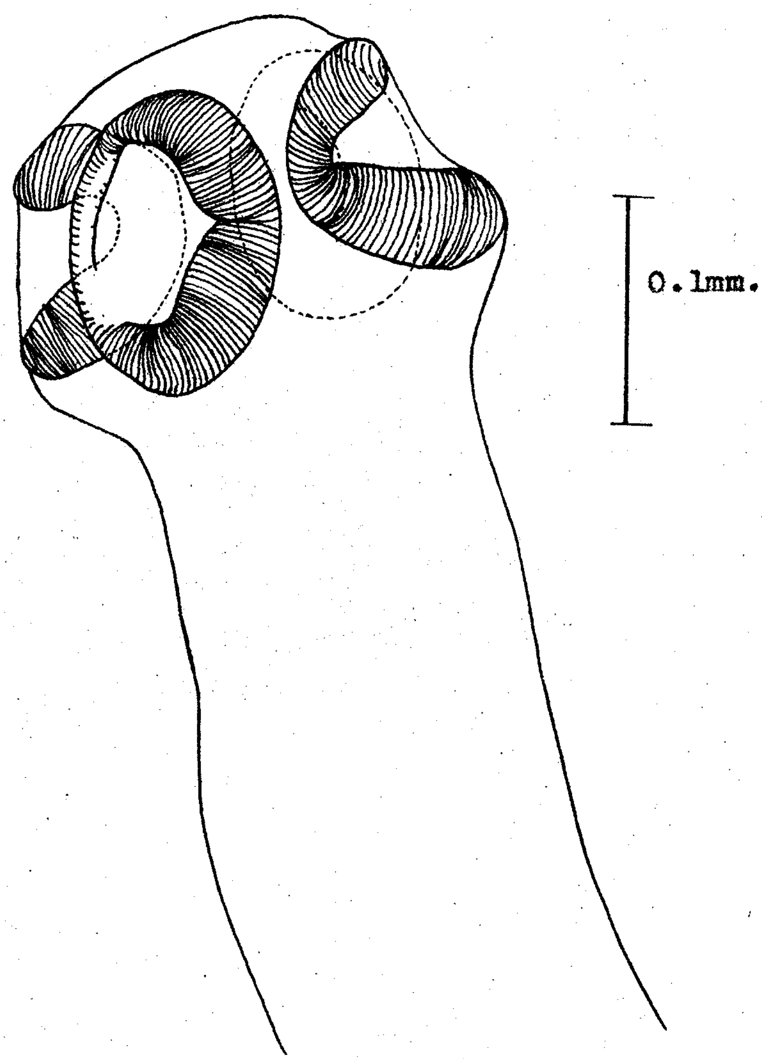


Figure 7.

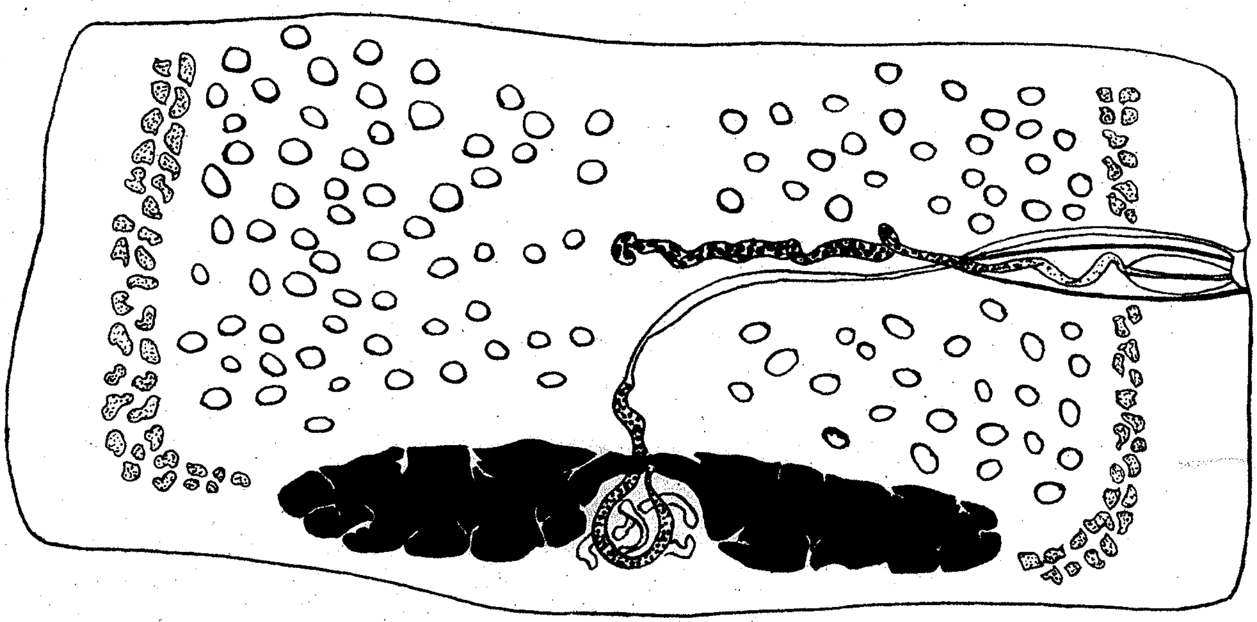
PROTEOCEPHALUS <u>luciopercae</u>



HOLDFAST VARIATION 'C'

Figure 8.

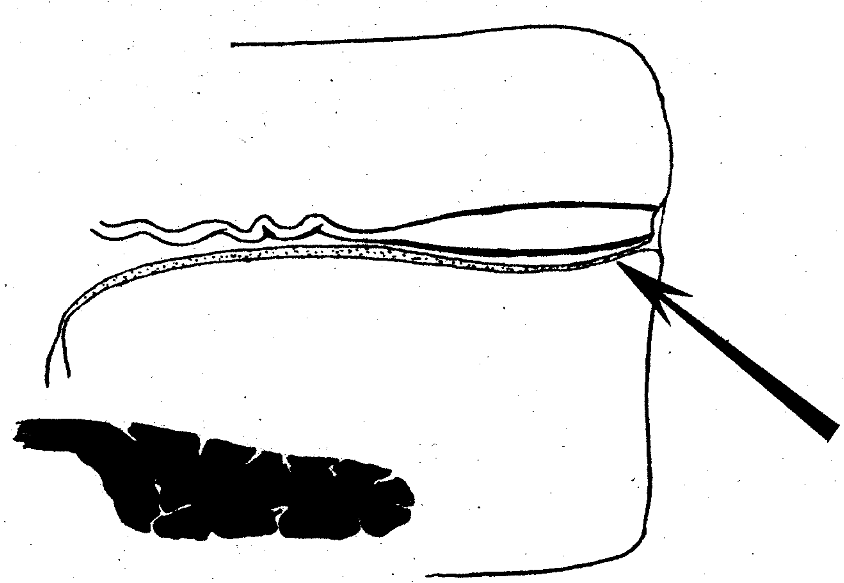
PROTEOCEPHALUS luciopercae



NORMAL MATURE PROGLOTTIS

1.0mm.

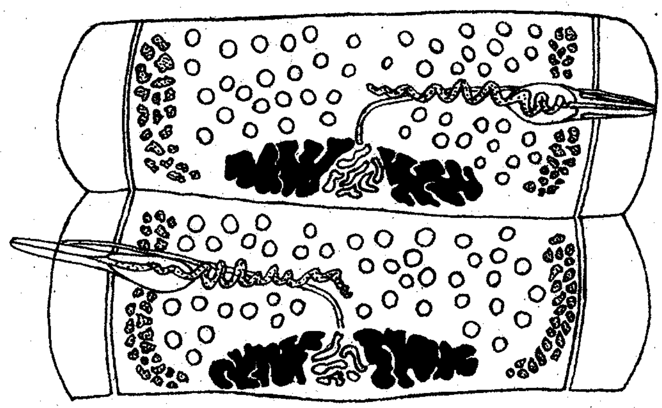
Figure 9.



COMMON VARIATION OF VAGINA OCCURRING
POSTERIOR TO CIRRUS POUCH

Figure 10.

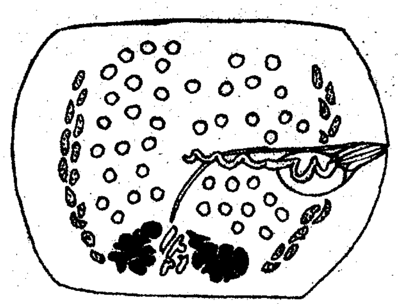
PROTEOCEPHALUS luciopercae



MATURE PROGLOTTIS
(EARLY)

1.0mm.

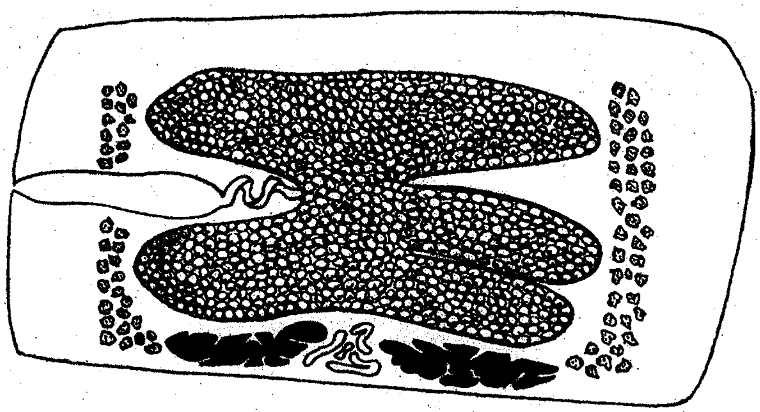
Figure 11.



QUADRATE PROGLOTTIS
VARIATION

Figure 12.

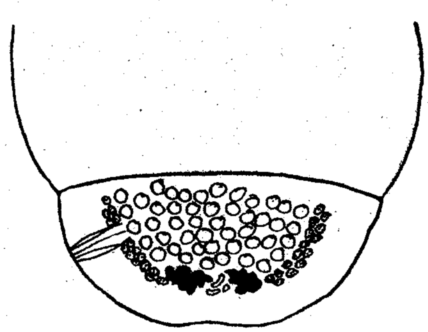
PROTEOCEPHALUS lucioperCae



EARLY GRAVID PROGLOTTIS

Figure 13.

1.0mm.



TERMINAL PROGLOTTIS

Figure 14.

PROTEOCEPHALUS <u>luciopercae</u>

TRANSVERSE SECTION OF GRAVID SEGMENT

SHOWING DORSAL BREAKTHROUGH OF UTERINE

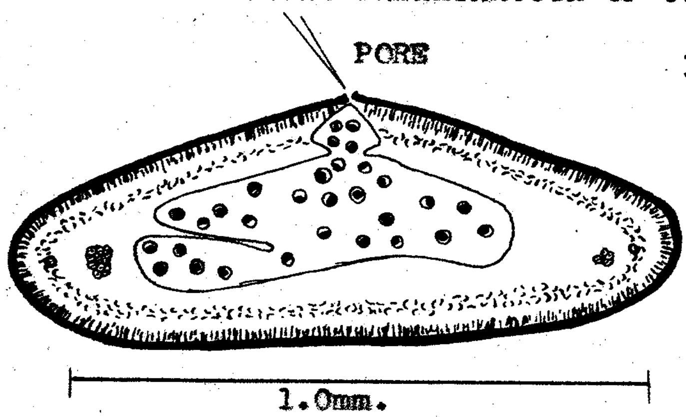


Figure 15.

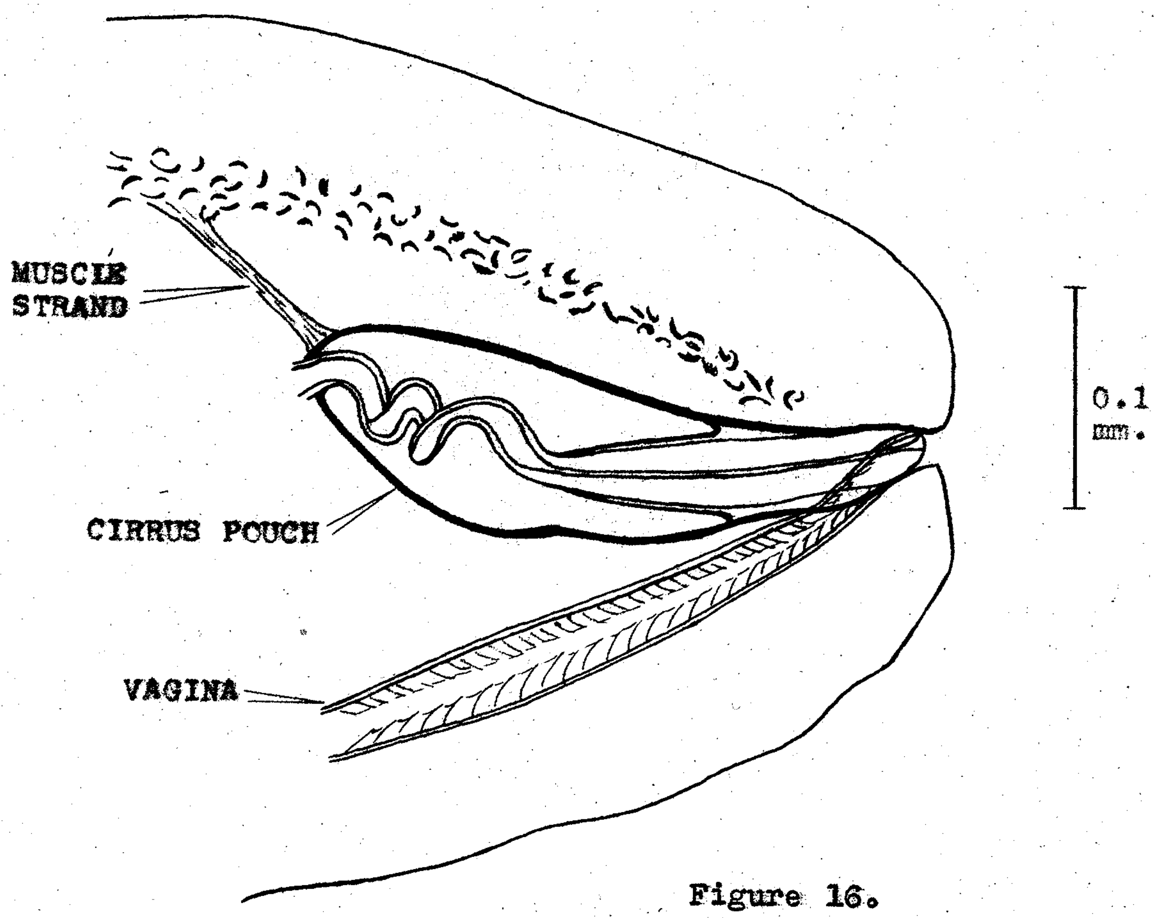
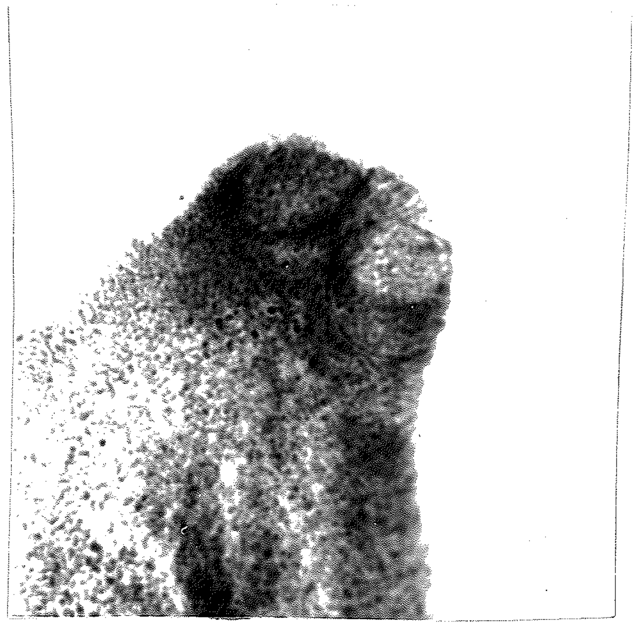


Figure 16.

PROTEOCEPHALUS <u>luciopercae</u>

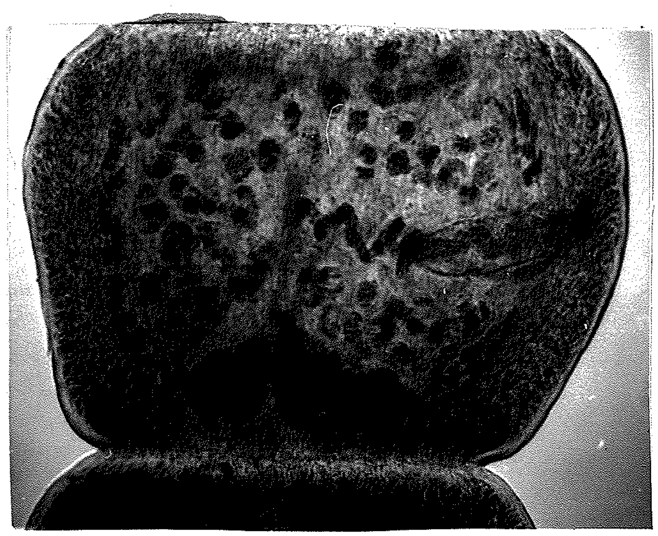


HOLDFAST
Figure 17.

HOLDFAST TYPE 'B'
Figure 18.

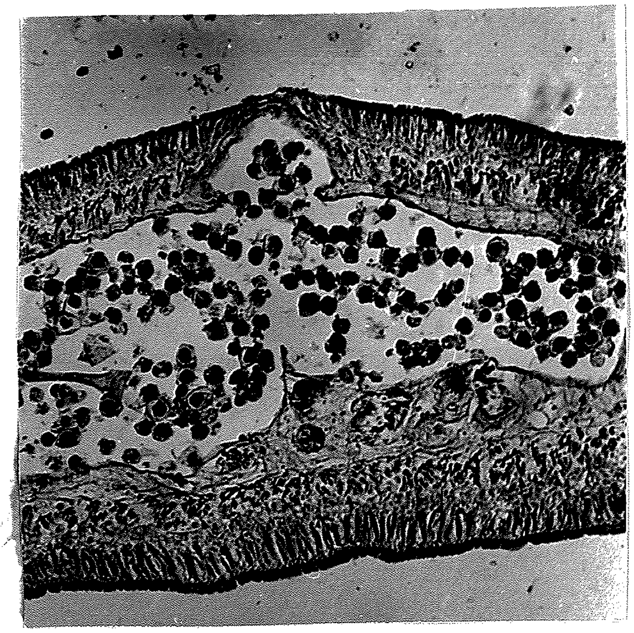
PROTEOCEPHALUS <u>luciopercae</u>

HOLDFAST TYPE 'C'
Figure 19



QUADRATE PROGLOTTIS VARIATION
Figure 20

PROTEOCEPHALUS <u>luciopercae</u>



UTERUS BEGINNING BREAKTHROUGH
OF DORSAL BODY WALL
Figure 21.

PLEROCERCOID
Figure 22.

Proteocephalus pinguis (LaRue, 1911)

Habitat:

In intestines of Esox lucius from Lakes Dauphin, Snow, Hemming, Athapapuskow, Burton, Bell, Steeprock and Wellman in Manitoba and Wapus River, Reindeer Lake, Lake Waskesiu, Kingsmere Lake and Deep Lake in Saskatchewan. In general this parasite is found in Esox in all parts of North America.

Gross Appearance:

Individuals measure up to 90mm in length, with a maximum breadth of 1.3mm. The strobila is moderately short but slender. The 'head' although roughly conical is flattened dorso-ventrally and shows a great variation in shape. A peculiarity noted in many individuals is the cobra-like swelling of the holdfast region which seems to aid the animal in locomotion. This peculiarity was noted in all live specimins seen, and contrary to LaRue(1914) I believe that this shape is the normal, rather than a contraction phase. The genital pore occurs mid way, laterally along proglottis, irregularly alternating from side to side from segment to segment. Mature and ripe proglottids are nearly quadrate with the terminal segment bluntly rounded.

Internal Features:

Testes number between 54-70 in a single layer between the yolk glands and anterior to the ovary. Ductus ejaculatorius is fairly straight, although in some cases a slight torsion is noted. Cirrus pouch length ratio to proglottis width is 1:3 or less commonly 1:4. Vagina crosses the inner end of the cirrus sheath. Two or three ventral uterine pores may be seen in ripe proglottids. Ten to fourteen uterine branches may be seen on each side in a ripe proglottis. The animal is acraspedote and anapolytic.

PROTEOCEPHALUS *pinguis*

HOLDFAST

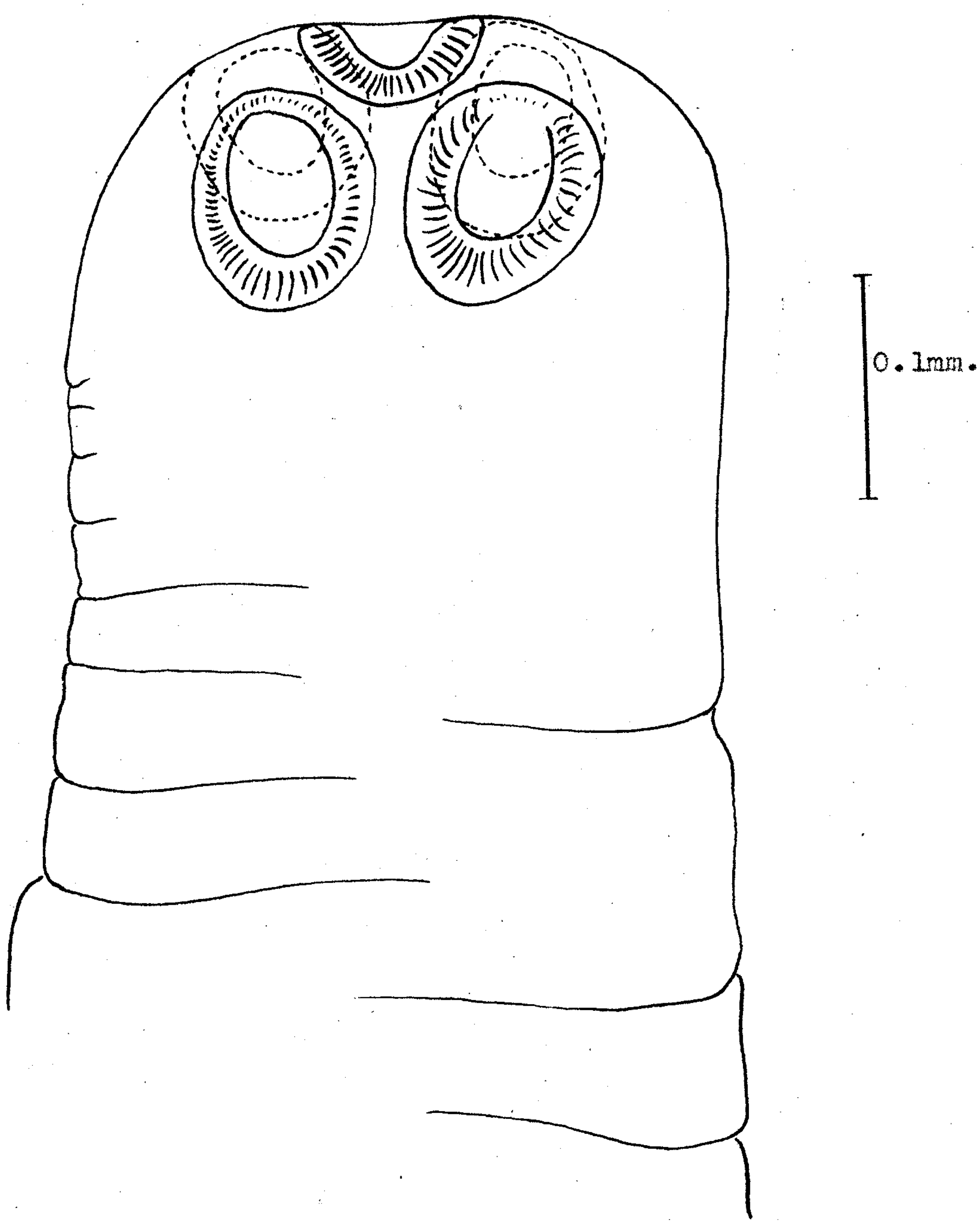
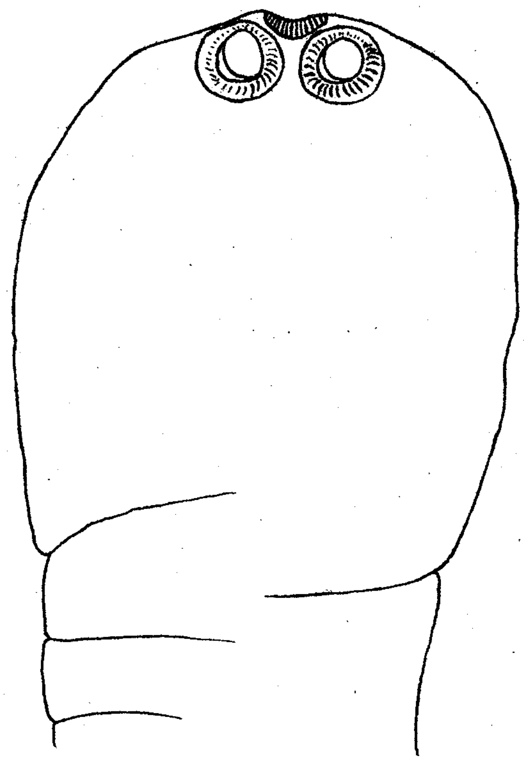


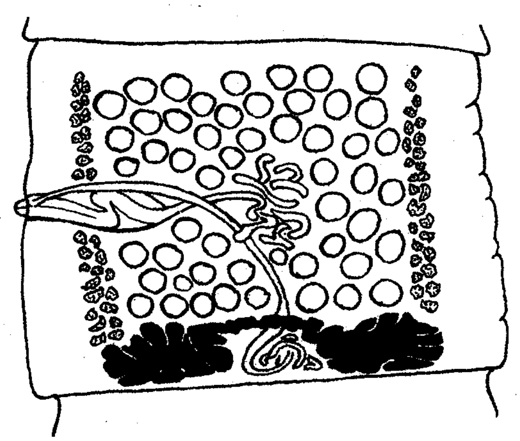
Figure 23

PROTEOCEPHALUS __pinguis__



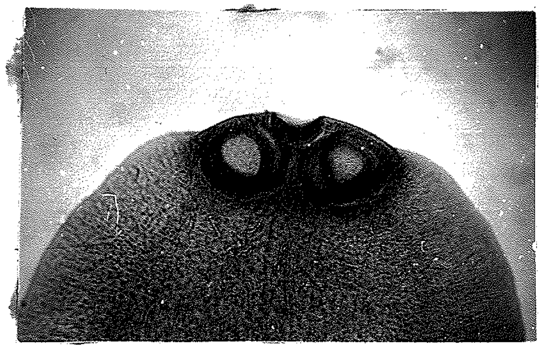
HOLDFAST VARIATION
Figure 24

1.0mm.



DORSAL VIEW OF MATURE
PROGLOTTIS SHOWING VAGINA
DORSAL TO CIRRUS POUCH
Figure 25.

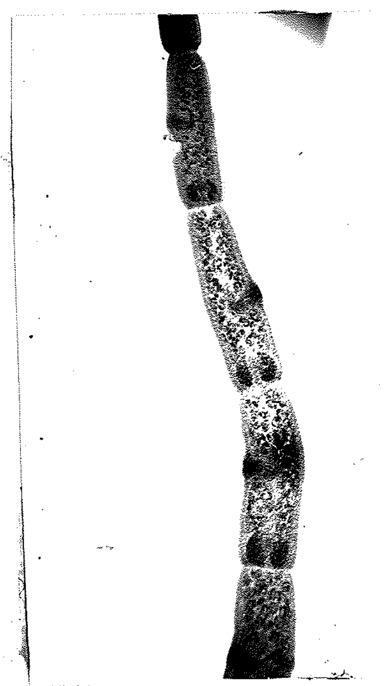
Proteocephalus pinguis



ARRANGEMENT OF THE FIVE SUCKERS
ON HOLDFAST OF P. pinguis

Figure 26

Proteocephalus pusillus



DORSAL VIEW OF SEVERAL PROGLOTTIDS
FROM P. pusillus

Figure 27

Proteocephalus pusillus (Ward, 1910)

Habitat:

In the oesophagus , intestine, and intestinal caecae of *Leucichthys tullibee* from Lake Athapapuskow Manitoba. Also found in *Cristivomer namaycush* , Lake Timagami Ontario and *Salmo sebago*, Sebago Lake, Maine (type locality).

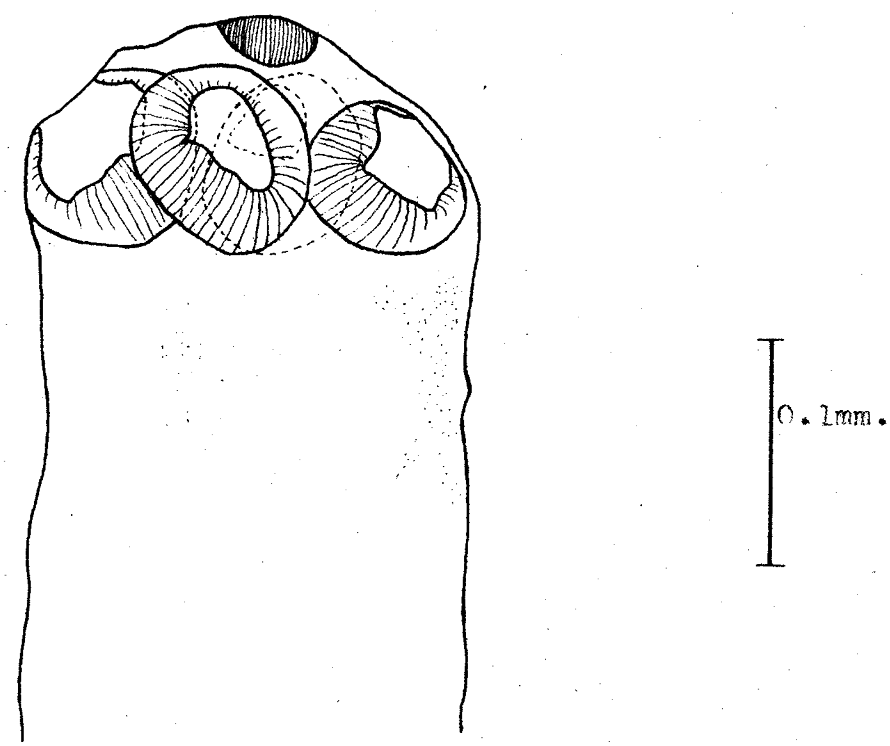
Gross Appearance:

Individuals measure up to 50.0 mm in length, maximum breadth of 0.35mm with few proglottids. First proglottids usually broader than long but mature segments are longer than broad. The end segment is fertile and of nearly equal length to the other segments. Genital pore is marginal, situated in the posterior 1/3-2/5 of the proglottis but appearing to be closer to mid segment line. The pore irregularly alternates from side to side in successive segments. Head is spheroidal, occasionally much contracted. Suckers located on the broadest part of the head include a muscular fifth apical sucker. Segmentation is quite distinct.

Internal Features:

Testes number fourty-four to seventy and are located in two layers between yolk glands and anterior to the ovaries. Ovaries are short, thick with free ends frequently pressed posteriad. The coiled vas deferens appears quite centrally anterior to the cirrus pouch. The ductus ejaculatorius coils once or twice within the cirrus pouch. The vagina is usually anterior and dorsal to the cirrus pouch. Uterine branches in ripe segments number ten to sixteen on either side. The animal is acraspedote and anapolytic.

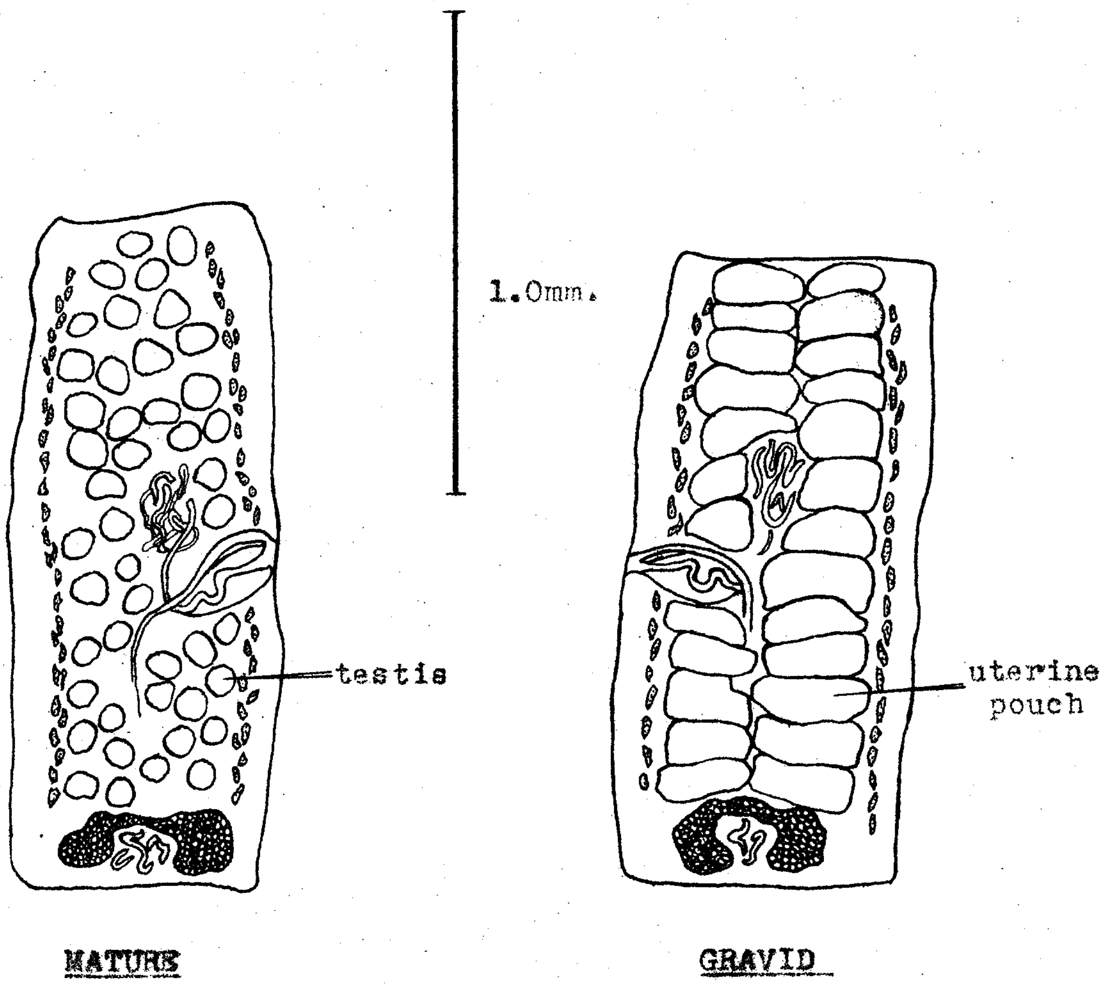
PROTEOCEPHALUS *pusillus*



HOLDFAST

Figure 28

PROTEOCEPHALUS pusillus



PROGLOTTIS
APPEARANCE

Figure 29

Proteocephalus singularis (LaRue, 1911)

Habitat:

In intestines of *Coregonus clupeaformis* from South Indian Lake, Lake Wellman and Lake Manitoba in Manitoba and Lakes Bow, Waterton and Jackfish in Alberta. Also found in *Lepisosteus platystomus* from the Illinois River at Havana Illinois (type locality).

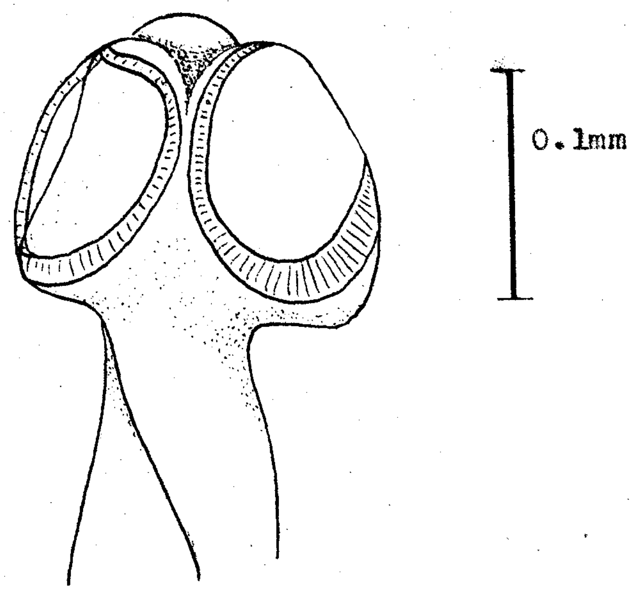
Gross Appearance:

Individuals measure up to 250mm in length, with maximum breadth of 1.0mm. Strobila is considered long and slender. First proglottids are broader than long. Normal mature proglottids usually approach a quadrate form. Older spent proglottids were observed slightly longer than broad. The genital pore usually occurs laterally about 1/2 the distance along the segment. Occasionally the pore may appear to be slightly in the posterior half. There is no genital papilla. Four large suckers are mounted at the maximum breadth of the head. The apical region of the head is frequently prolonged into an unarmed rostellum like organ. Deep grooves appear between the suckers. The neck is slender compared to the holdfast region. Terminal segment which is bluntly rounded is as long as segments immediately anterior to it.

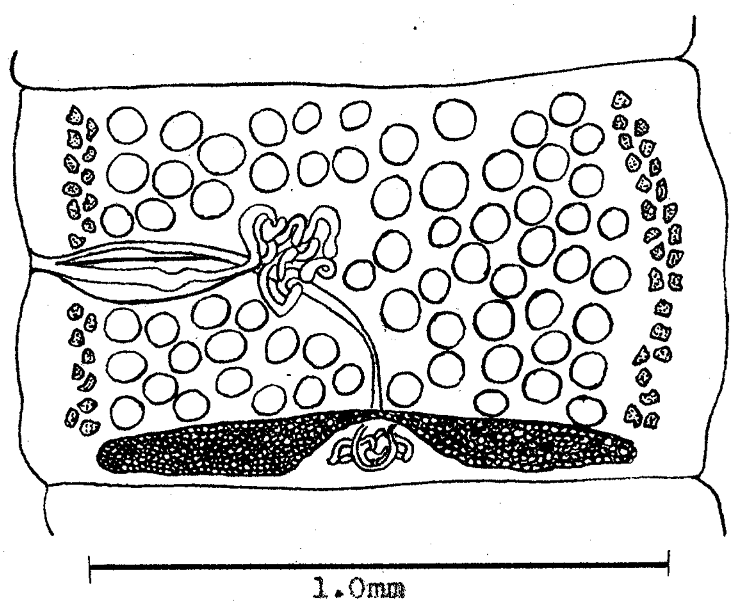
Internal Features:

Testes number seventy-five to ninety in a single field between the yolk glands and anterior to the ovaries. The vas deferens is a large coiled mass about mid-field in the proglottis. Ductus ejaculatorius with few or usually no coils. Cirrus pouch is slender, straight and muscular. Cirrus pouch length compared to proglottis width is 1:3. The vagina never crosses the cirrus pouch and always remains anterior to it. Care must be taken to view the whole mount from both dorsal and ventral surfaces to determine these latter features which are quite distinctive. Ripe proglottids have up to twenty-five lateral uterine pouches on either side. The animal is anapolytic and acraspedote.

PROTEOCEPHALUS singularis



HOLDFAST
Figure 30



DORSAL VIEW OF MATURE PROGLOTTIS
Figure 31

CLASSIFICATION

ORDER: PSEUDOPHYLLIDEA (Carus, 1863)

FAMILY : BOTHRIOCEPHALIDAE (Blanchard, 1849)

GENUS: Bothriocephalus (Rudolphi, 1808, emended Luhe, 1899)

Characteristics of Genus: Elongated holdfast, depressed, bilobed apical disc with indented bothrial edges connected by a groove. Holdfast margin surfaces truncatedly oval and slightly concave. Surficial surfaces rectangular, each with an elongated bothrium wider anteriorly than posteriorly, narrow and deep when relaxed, wide and shallow when constricted. Complete segmentation of body, craspedote and anapolytic. Testes medullary in lateral zones. Yolk glands are cortical and continuous from segment to segment. No seminal receptacle.

As has been pointed out by several parasitologists the occurrance of a Bothriocephalus species in freshwater fish is considered unusual. The genus is distinctly a marine type. Other Bothriocephalus species come from marine Teleosts, anadromous teleosts or catadromous teleosts such as Anguilla rostrata (common eel). This suggests that Bothriocephalus species can live successfully in the bodies of either marine or freshwater fish. How these forms entered lakes in Western Canada which are isolated from the sea is a matter for speculation. It is suggested that upon the retreat of Lake Agassiz from 5,000 to 15,000 years ago some marine fish were isolated in the pockets of water that were left. It is possible that these bodies of water slowly lost their salinity and became freshwater lakes. It is also possible that these lakes continued to harbour marine animals that were adaptable to freshwater habitats. If this is the case it is understandable that this distinctly marine form appears in our Western Canadian lakes.

Bothriocephalus cuspidatus (Cooper, 1917)

Habitat:

In intestinal caecae and intestines of *Stizostedion vitreum vitreum*, *Esox lucius*, *Cristivomer namaycush* and Dolly Varden trout from Lakes Wellman, Snow, Dauphin, Glad, Whitefish, Child, Pickerel, Athapapuskow and Mossy River in Manitoba and Lakes Waskesiu and Deep in Saskatchewan and Lakes Waterton, Bow and Bow River in Alberta. Also found in *Stizostedion canadense*, *Amphidon alosoides*, *Hiodon tergisus*, *Perca flavescens* and rarely in *Leucichthys* in Northern United States and across Canada.

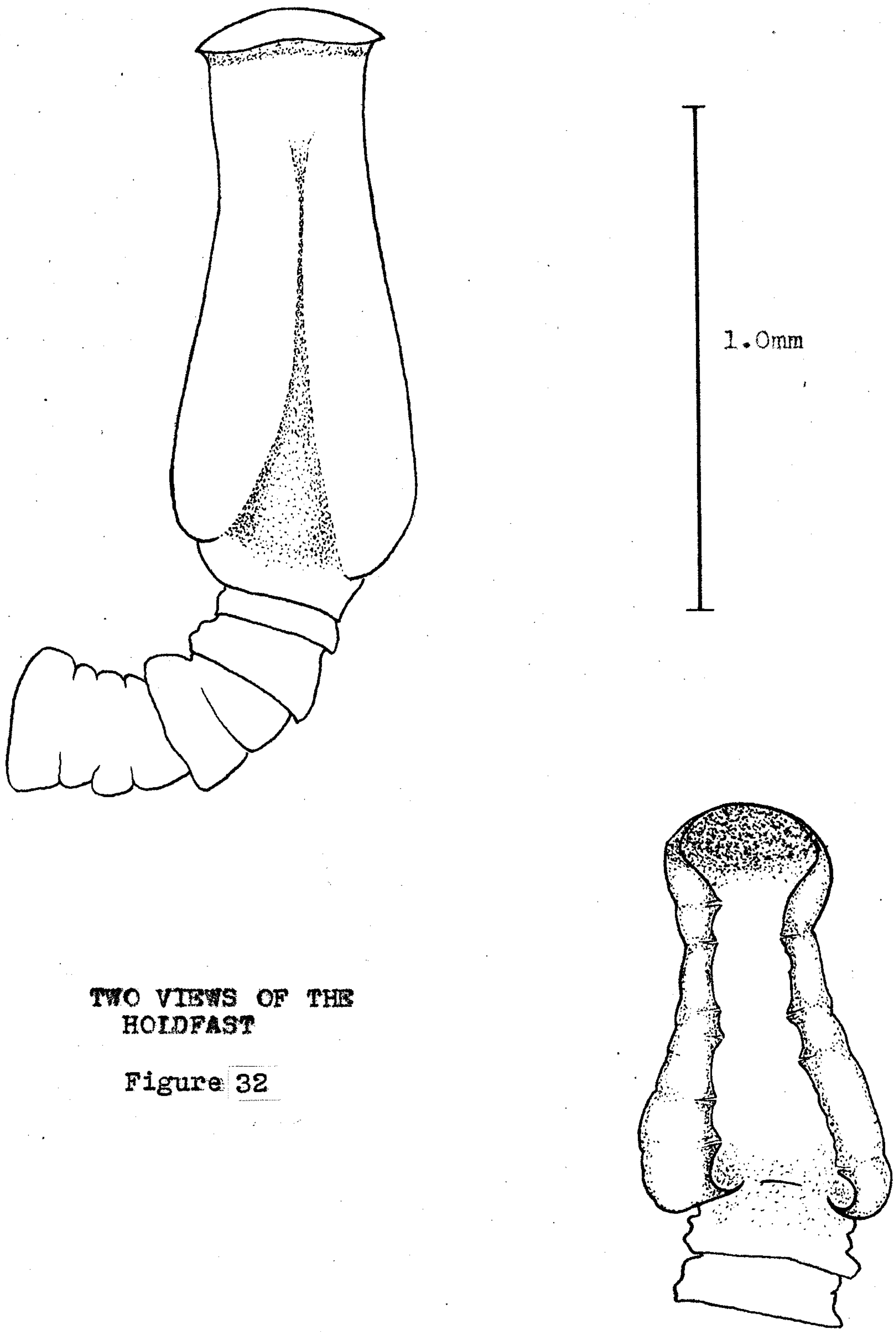
Gross Appearance:

Individuals measure up to 150mm in length with maximum width averaging 1.3mm. Strobila is long and slender. Genital apertures in the first third of the segment surface. Segments usually wider than long. Holdfast is roughly rectangular, apical disc is bilobed, with bluntly rounded apex.

Internal Features:

Testes number up to 50 in a single field medullary, lateral and anterior to the ovaries. Yolk glands rather large and ovid and cortical in position with mid dorsal and mid ventral gaps of one third to one fifth the segment width, but continuous ventrally. Ovaries appear as thin strips in the posterior portion of the segment, often obliterated by yolk glands and testes. It is suggested that small specimens in which the proglottids are not fully mature are best for whole mount study. The cirrus pouch is about one half the dorso-ventral thickness of the segment, Cirrus is often seen protruding. Uterus occupies the anterior half of the segment and opens externally through a distinct genital aperture. The animal is craspedote and anapolytic.

BOTHRIOCEPHALUS **cuspidatus**



TWO VIEWS OF THE HOLDFAST

Figure 32

BOTHRIOCKPHALUS cuspidatus

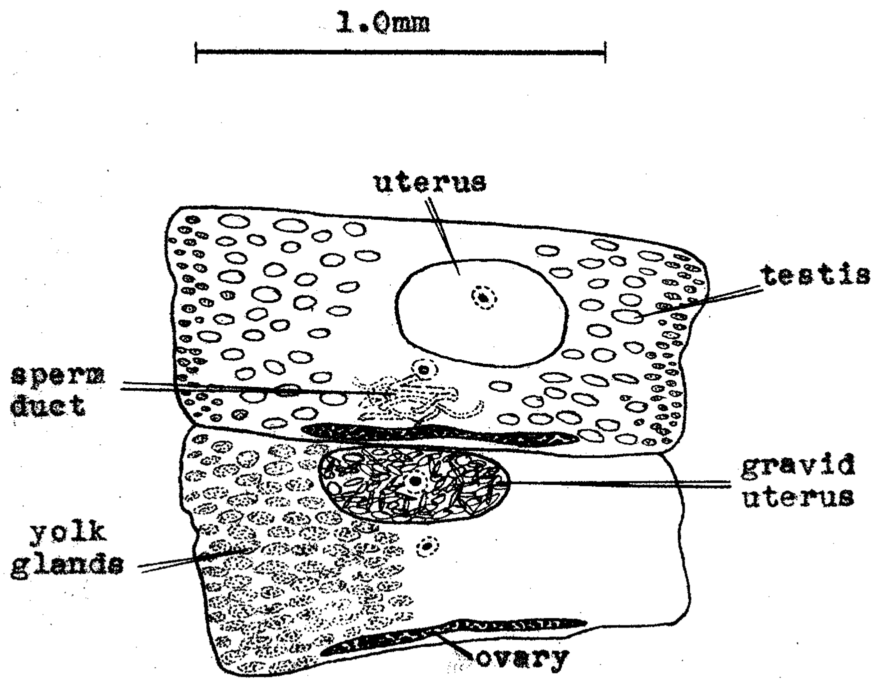
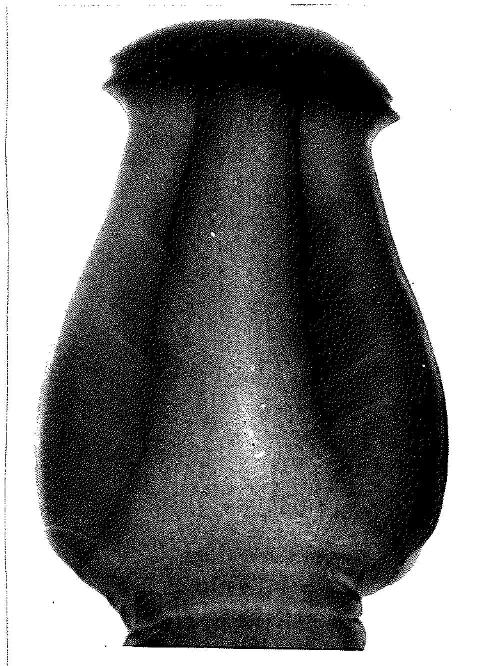


Figure 33

Bothriocephalus cuspidatus



HOLDFAST CONTRACTED
Figure 34

HOLDFAST
Figure 35

Bothriocephalus cuspidatus

IMMATURE INDIVIDUAL
Figure 36

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