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)

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BY

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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 alosoides, 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,