1978

Migration and localization of Ornithodiplostomum ptychocheilus (Trematoda: Diplostomatidae) in the fish intermediate host

Gary Lee Hendrickson

Iowa State University

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MIGRATION AND LOCALIZATION OF
ORNITHODIPOLESTONUM PTYCHOCEILUS (TREMATODA:
DIPOLESTOMATIDAE) IN THE FISH INTERMEDIATE
HOST.

IOWA STATE UNIVERSITY, PH.D., 1978

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Migration and localization of Ornithodiplostomum ptychocheilus (Trematoda: Diplostomatidae) in the fish intermediate host

by

Gary Lee Hendrickson

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Zoology Major: Zoology (Parasitology)

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

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For the Graduate College

Iowa State University
Ames, Iowa

1978

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INTRODUCTION

That many parasites localize in specific habitats within their hosts is well-known. This phenomenon involves entry into the proper host and, once inside, localization in a suitable habitat for further development. Ulmer (1971) and others have referred to this process as "site-finding behavior".

Until recently, research into parasite localization has been limited to casual observations made during the course of routine life history studies (see reviews by Ulmer, 1971; Holmes, 1973; Crompton, 1976; and Cook, 1977). Only recently have experimental investigations into this problem begun to appear in the literature. This study was undertaken to determine the localization of larvae of Ornithodiplostomum ptychocheilus (Faust, 1917) Dubois, 1936, in the fish intermediate host. This trematode was selected as a model because of its unusual localization in and on the surface of the brain of certain cyprinid fish.

Although Hoffman (1958a) briefly described the life cycle, the brevity of his data particularly regarding the egg, miracidial, cercarial, and sporocyst stages, necessitated further life cycle study preceding any experimental work. Thus, the life cycle of O. ptychocheilus was reworked in detail.

The migration of cercariae of O. ptychocheilus to the brain of the fish intermediate host has never been examined, although occurrence of mature metacercariae on the brain has been known since
1953 (Hoffman, 1953). Research efforts were concentrated, therefore, in determining how the larvae "find their way" to the brain, and more specifically, the route taken. Mature metacercariae occur on the brain and in the mesenteries of the body cavity in some fish hosts, but only in the mesenteries in other hosts, adding additional aspects of interest to this problem.
Faust (1917, 1918) described the metacercaria of *Ornithodiplostomum ptychocheilus* as *Cercaria ptychocheilus* from specimens obtained from the mesenteries of *Ptychocheilus oregonensis* from Montana. He observed (1917, p. 110), "As a hemistome larva encysted in a vertebrate, it is technically a *Diplostomulum*," but he maintained the larval genus *Cercaria*. His description was inaccurate in that he mistook the tribocytic organ for the ventral sucker and the ventral sucker for the primitive genital pore.

Hughes and Piszczek (1928) redescribed the metacercaria, correcting Faust's errors on the basis of specimens found attached to the mesenteries of the body cavity and viscera and lying free in the ovaries of *Notropis deliciosus stramineus* collected at Douglas Lake, Michigan. They assigned the metacercaria to the larval genus *Neascus* Hughes, 1927.

Van Haitsma (1930) obtained adults by infecting young domestic ducks and also recovered them from naturally infected *Mergus americanus* from Douglas Lake, Michigan, and from *Mergus serrator*, *Lophodytes cucullatus*, and *Hafelda hyemalis* collected near Carthage, Illinois. Van Haitsma included the adult in the genus *Paradiplostomum* LaRue, 1926, erected for reptilian parasites, and based his generic diagnosis on the small body size, relatively short hindbody, crowded condition of the organs in the hindbody, unusual position of Mehlis' gland (posteroventral and to the left of the ovary), an ovary lying opposite
the anterior testis, and a large copulatory bursa. He observed no prostate gland in the genital cone of his specimens and stated that the generic diagnosis of *Paradiplostomum* may have been incorrect in this regard.

Dubois (1932) maintained this species in the genus *Paradiplostomum* on the basis of those characters mentioned by Van Haitsma (1930). However, Dubois (1936), in a revision of the higher classification of the Strigeida, erected the genus *Ornithodiplostomum* with *O. ptychocheilus* as the type and only species. This separation was based upon the presence of a prostate and an ovoid or spherical posterior testis in the family Proterodiplostomidae Dubois, 1936 a group composed of parasites of reptiles and containing the genus *Paradiplostomum*; and the absence of a prostate and a claviform, pyriform, cordiform, or bilobed posterior testis, characteristic of the family Diplostomidae Poirier, 1886 composed of parasites of birds and mammals and containing the genus *Ornithodiplostomum*. Dubois (1937) elaborated as follows his reasons for excluding Van Haitsma's species from the genus *Paradiplostomum*:

<table>
<thead>
<tr>
<th>Paradiplostomum</th>
<th>Ornithodiplostomum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Prostate present</td>
<td>1. Prostate absent</td>
</tr>
<tr>
<td>2. Posterior testis ovoid or spherical</td>
<td>2. Posterior testis bilobed or reniform</td>
</tr>
<tr>
<td>3. Ovary in the middle of the body and anterior to the anterior testis</td>
<td>3. Ovary opposite the anterior testis</td>
</tr>
<tr>
<td>4. Vitelline follicles confined to the anterior segment</td>
<td>4. Vitelline follicles in both segments of the body</td>
</tr>
</tbody>
</table>
5. Convergence of the uterus and ejaculatory canal emerging jointly at the middle of the beginning of the genital cone on its ventral side

6. Parasites of Alligatoridae (crocodilians)

Dubois (1938, 1953) presented detailed generic diagnoses of the genus *Ornithodiplostomum* and reaffirmed its monotypic status. He (1938) erected the larval genus *Ornithodiplostomulum* for metacercariae of this genus. Dubois (1944) discussed host specificity of *Ornithodiplostomum* and related forms and again noted host differences between this genus and *Paradiplostomum*.

Lyster (1940) described *Paraceonogonimus katsuradi* (Cyathocotylidae) from adult worms collected from the intestine of *Lophodytes cucullatus* from Quebec, Canada. Dubois (1946) examined Lyster's specimens and concluded that they were conspecific with *O. ptychocheilus*. Furthermore, the characters used by Lyster to differentiate his species from other members of *Paraceonogonimus* (*Paraceonogonimus* according to Dubois, 1946) were those same characters diagnostic of *Ornithodiplostomum*.

Yamaguti (1939) described *Ornithodiplostomum podicipitis* from the small intestine of *Podiceps ruficollis japonicus* from Lake Ogura, Japan, distinguishing this species from *O. ptychocheilus* on the basis of the fusiform shape of the body, its relatively larger body size, the larger size of the eggs, and other minor characters. Dubois (1944, 1953)
included *O. podicipitis* in the genus *Posthodiplostomum*, but Dubois (1970) transferred it back to *Ornithodiplostomum* on the basis of the fusiform shape of the body which was indistinctly divided into two regions.

Baer (1959) erected the genus *Prolobodiplostomum* to contain specimens collected from the intestine of the rodent *Dendromus pumilio lineatus* collected in the Belgian Congo. The type and only species was *P. garambense*. Characters diagnostic of this genus were recognized by Dubois (1961) as being those characteristic of *Ornithodiplostomum* (the presence of an evaginable copulatory bursa covering a genital cone and an ejaculatory pouch with its duct opening with the uterus at the axis of the cone). Both Baer (1959) and Dubois (1961) suggested that *Dendromus* may not be the normal host of *O. garambense*.

Odening (1963) described *Ornithodiplostomum ptychocheilus palaearcticum* from the gut of *Mergus m. merganser* from the Berlin zoo. This palearctic subspecies was differentiated from the nearctic form on the basis of a larger body size and different organ measurements.

Schulman (reported by Sudarikov and Kurochkin, 1968), in an unpublished dissertation, first observed metacercariae on the brain of rudd (*Scardinius erythrophthalmus*) from the West Dvina River, U.S.S.R. Schulman briefly described this species but there is some question as to whether or not he named it. Dubinin (1952) described and figured this species as *Neodiplostomulum scardinii* from the brain of rudd from the Volga Delta but gave full credit for the discovery to Schulman. Kozicka (1958) recovered similar metacercariae, referring
to them as Neascus scardinii, from the brains of rudd and tench (Rutilus rutilus) from Družno Lake, Poland. She examined a number of aspects of the biology of this parasite in the fish host, including the pathological effects on the brain. Sudarikov (1958) and Kozicka (1960) considered the larva in question to be the metacercaria of Neodiplostomum pseudattenuatum. Kozicka (1960) examined the development of the metacercariae in naturally infected fish hosts. Sudarikov and Kurochkin (1968) demonstrated experimentally that Neascus scardinii (=Neodiplostomulum scardinii) developed to adults of the genus Ornithodiplostomum. Additionally, they recovered adults from naturally infected Mergus albellus. Following rules of nomenclatural priority, they proposed Ornithodiplostomum scardinii Schulman for the adult stage of the parasite. They further believed O. ptychocheilus palaearcticum Odening, 1963 to be a junior synonym of O. scardinii and Odening (Akademie der Wissenschaften der DDR, personal communication) concurs with this view. Sudarikov and Kurochkin suggested that an earlier report of O. ptychocheilus from Mergus albellus from fish reservoirs in the U.S.S.R. (Shigin, 1954 from Bychovskaja-Pavlovskaja, 1962) was in error and felt certain that Shigin's specimens were O. scardinii. Skrjabin (1971) reviewed much of the work dealing with O. scardinii in the fish host. Dubois (1970), apparently unaware of the work of Sudarikov and Kurochkin, made no mention of O. scardinii in his comprehensive review of the Diplostomatidae. He, therefore, recognized three species in the genus Ornithodiplostomum: O. garambense, O. podicipitis, and O. ptychocheilus. Within the latter species he recognized two
subspecies: *O. ptychocheilus ptychocheilus* from North America and *O. p. palaearcticum* (=*O. scardinii*) from Europe.

At present it seems reasonable to recognize the following as valid species:

**Ornithodiplostomum ptychocheilus** (Faust, 1917) Dubois, 1936  
Synonyms: *Cercaria ptychocheilus* Faust, 1917  
Neascus *ptychocheilus* (Faust, 1917) Hughes and Piszczek, 1928  
*Paradiplostomum ptychocheilus* (Faust, 1917) Van Haitsma, 1930  
*Paraceoenogonimus katsuradi* Lyster, 1940  
*Paracoenogonimus katsuradi* (Lyster, 1940) Dubois, 1946

**Ornithodiplostomum podicipitis** Yamaguti, 1939  
Synonyms: *Posthodiplostomum podicipitis* (Yamaguti, 1939) Dubois, 1944

**Ornithodiplostomum garambense** (Baer, 1959) Dubois, 1961  
Synonyms: *Prolobodiplostomum garambense* Baer, 1959

**Ornithodiplostomum scardinii** (Dubinin, 1952) Sudarikov and Kurochkin, 1968  
Synonyms: *Neodiplostomulum scardinii* Dubinin, 1952  
Neascus *scardinii* Kozicka, 1958  
*Neodiplostomum psudattenuatum* Sudarikov, 1959 (see also Kozicka, 1960)  
*Ornithodiplostomum ptychocheilus palaearcticum* Odening, 1963

There seems little reason to retain *O. scardinii* Schulman. Apparently, Schulman gave a brief description of the metacercaria but some confusion
exists in the available literature as to whether or not he named it. Nevertheless, his manuscript was unpublished so priority rests with Dubinin (1952).

The relationship of *O. ptychocheilus* and *O. scardinii* warrants further investigation. Data regarding hosts, site specificity, and life cycles suggest that they are closely related if not specifically identical.

Parasites obtained in this study were identified as members of the genus *Ornithodiplostomum* based on the generic diagnosis of Dubois (1970). Specific identification was made by examining the descriptions of the adult by Van Haitsma (1930) and metacercaria by Faust (1917, 1918) and Hughes and Piszczek (1928). In addition, Van Haitsma's paratype specimen was obtained from the United States National Museum (USNM Number 7990).

**Life Cycle**

Van Haitsma (1930) first established the relationship between metacercarial and adult stages of *O. ptychocheilus*. Hoffman (1958a) completed the life cycle with brief descriptions of the miracidium, mother and daughter sporocysts, and cercaria. When Hoffman fed metacercariae recovered from the viscera or cranial cavities of *Notropis cornutus frontalis*, *N. d. dorsalis*, *Semotilus a. atromaculatus*, and *Pimephales p. promelas* to newly hatched unfed chicks, adult worms identical to those of Van Haitsma were recovered from the small intestines of experimental birds. Eggs were obtained either from
chick feces or by mechanically removing them from the adult worms. Miracidia hatched after a 9 day incubation period and penetrated the snail Physa anatina in which the mother and daughter sporocysts developed. Cercariae first emerged from the snails 45 days post-exposure. These cercariae were capable of infecting P. p. promelas, N. d. dorsalis, and S. a. atromaculatus and developing to metacercariae. Hoffman presented data on the development of the metacercaria and observed that in very heavy visceral infections, the abdomen of the fish host would burst, releasing parasites with subsequent healing of the wound.

A number of reports of natural infections of fish with metacercariae have been reported (Table 1). Although Paperna (1964) reported metacercariae of Ornithodiplostomum sp. from the musculature of Tilapia zilli (Cichlidae) in Israel, Hoffman (United States Fish and Wildlife Service, Fish Farming Experimental Station, personal communication) considered them to be Posthodiplostomum sp. Reports of natural infections in the definitive host are considerably fewer (Table 2). Hoffman (1958a) was able to infect the snail Physa anatina experimentally. However, the only report of a natural infection of O. ptychocheilus in the snail host is that of Sankurathri and Holmes (1976) who found naturally infected Physa gyrina in Alberta, Canada.

Aspects of the life cycle of Ornithodiplostomum scardinii were described by Sudarikov and Kurochkin (1968). These authors obtained metacercariae from the brains of naturally infected Scardinius
Table 1. Occurrence of metacercariae of *Ornithodiplostomum ptychocheilus* in fish second intermediate hosts

<table>
<thead>
<tr>
<th>Host Species</th>
<th>Location(s) in Host</th>
<th>Geographic Location</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catostomidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Catostomus clarki</em></td>
<td>Intestinal mesenteries</td>
<td>Arizona</td>
<td>Amin, 1968, 1969</td>
</tr>
<tr>
<td><em>Catostomus columbianus</em></td>
<td>On the liver</td>
<td>Washington</td>
<td>Griffith, 1953^a</td>
</tr>
<tr>
<td><em>Catostomus insignis</em></td>
<td>Intestinal mesenteries</td>
<td>Arizona</td>
<td>Amin, 1968, 1969</td>
</tr>
<tr>
<td><em>Catostomus macrocheilus</em></td>
<td>On the liver</td>
<td>Washington</td>
<td>Griffith, 1953^a</td>
</tr>
<tr>
<td>Cyprinidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hybognathus hankinsoni</em></td>
<td>Cranial cavity and</td>
<td>Ontario</td>
<td>Molnar et al., 1974</td>
</tr>
<tr>
<td></td>
<td>abdominal cavity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Choroid of eye</td>
<td>Wyoming</td>
<td>Hendrickson, 1978</td>
</tr>
<tr>
<td><em>Nocomis biguttatus</em></td>
<td>Cranial cavity and</td>
<td>Ontario</td>
<td>Molnar et al., 1974</td>
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<tr>
<td></td>
<td>abdominal cavity</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Notropis bifrenatus</em></td>
<td>Not specified</td>
<td>Connecticut</td>
<td>Hunter, 1942</td>
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<tr>
<td><em>Notropis cornutus</em></td>
<td>Fat bodies behind eyes,</td>
<td>North Dakota</td>
<td>Hoffman, 1953, 1954, 1958a^a</td>
</tr>
<tr>
<td></td>
<td>mesentery, cranial cavity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cranial cavity</td>
<td>North Dakota</td>
<td>Voth and Larson, 1968</td>
</tr>
</tbody>
</table>

^a Griffith reported these as *Posthodiplostomum minimum*, but Hoffman (1958b) examined the specimens and considered them to be *O. ptychocheilus*.

^b Larson reported metacercariae of *O. ptychocheilus*, "on the brain, peritoneum," but did not indicate their specific location in individual host species.
<table>
<thead>
<tr>
<th>Host Species</th>
<th>Location(s) in Host</th>
<th>Geographic Location</th>
<th>Reference(s)</th>
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<tr>
<td>Notropis oornutis</td>
<td>Cranial cavity</td>
<td>Ontario</td>
<td>Molnar et al., 1974</td>
</tr>
<tr>
<td>(Continued)</td>
<td>and abdominal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cavity</td>
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<tr>
<td>Notropis stramineus</td>
<td>Peritoneum,</td>
<td>Michigan</td>
<td>Hughes and Piszczek, 1928</td>
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<tr>
<td></td>
<td>mesentery, free</td>
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<tr>
<td></td>
<td>in ovaries</td>
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<tr>
<td>Notropis dorsalis</td>
<td>Mesentery</td>
<td>North Dakota</td>
<td>Hoffman, 1953, 1954, 1958a</td>
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<td>Notropis heterolepis</td>
<td>Cranial cavity</td>
<td>Ontario</td>
<td>Molnar et al., 1974</td>
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<td>Notropis volucellus</td>
<td>On the brain,</td>
<td>Minnesota</td>
<td>Larson, 1966</td>
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<tr>
<td></td>
<td>peritoneum</td>
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<tr>
<td>Ptychocheilus oregonensis</td>
<td>Mesentery</td>
<td>Montana</td>
<td>Faust, 1917, 1918</td>
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<tr>
<td>Semotilus atromaculatus</td>
<td>Mesentery</td>
<td>North Dakota</td>
<td>Hoffman, 1953, 1954, 1958a</td>
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<tr>
<td></td>
<td>Cranial cavity</td>
<td>Ontario</td>
<td>Molnar et al., 1974</td>
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<td>and abdominal</td>
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<td>Intestinal</td>
<td>Wisconsin</td>
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<td>Retina, sclera,</td>
<td>Wyoming</td>
<td>Hendrickson, 1978</td>
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<td>choroid of eyes;</td>
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</tr>
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<td><strong>Cyprinodontidae</strong></td>
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<td>Viscera</td>
<td>Nova Scotia</td>
<td>Wiles, 1975</td>
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<td><strong>Percidae</strong></td>
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<tr>
<td><em>Etheostoma exile</em></td>
<td>On the brain, peritoneum&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Minnesota</td>
<td>Larson, 1966</td>
</tr>
<tr>
<td><em>Etheostoma nigrum</em></td>
<td>Mesentery</td>
<td>North Dakota</td>
<td>Hoffman, 1958a</td>
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<td><em>Perca flavescens</em></td>
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<td>Minnesota</td>
<td>Larson, 1966</td>
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<td><strong>Poeciliidae</strong></td>
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<tr>
<td><em>Gambusia affinis</em></td>
<td>Ovary, eyes</td>
<td>Texas</td>
<td>Davis, 1975</td>
</tr>
</tbody>
</table>
Table 2. Occurrence of adult *Ornithodiplostomum ptychocheilus* in definitive hosts

<table>
<thead>
<tr>
<th>Host species</th>
<th>Geographic Location</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anatidae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Harelda hyemalis</em></td>
<td>Illinois</td>
<td>Van Haitsma, 1930</td>
</tr>
<tr>
<td><em>Lophodytes cucullatus</em></td>
<td>Illinois</td>
<td>Van Haitsma, 1930</td>
</tr>
<tr>
<td></td>
<td>Quebec</td>
<td>Lyster, 1940 (as <em>Paraceonogonimus katsuradi</em>)</td>
</tr>
<tr>
<td><em>Mergus americanus</em></td>
<td>Michigan</td>
<td>Van Haitsma, 1930</td>
</tr>
<tr>
<td><em>Mergus merganser</em></td>
<td>Germany</td>
<td>Odening, 1963 (as <em>O. ptychocheilus palaearcticum</em>)</td>
</tr>
<tr>
<td><em>Mergus serrator</em></td>
<td>Illinois</td>
<td>Van Haitsma, 1930</td>
</tr>
<tr>
<td><em>Ardeidae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Botaurus lentiginosus</em></td>
<td>Unknown</td>
<td>Hoffman, 1960</td>
</tr>
<tr>
<td><em>Experimental Hosts</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domestic chicks</td>
<td>-</td>
<td>Hoffman, 1958a</td>
</tr>
<tr>
<td>Domestic ducks</td>
<td>-</td>
<td>Van Haitsma, 1930</td>
</tr>
</tbody>
</table>

aAlthough reported as *O. ptychocheilus*, Shigin's specimens were considered to be *O. scardinii* by Sudarikov and Kurochkin (1968).

bSudarikov and Kurochkin (1968) considered this species to be synonymous with *O. scardinii*. 
erythrophthalmus from the Volga Delta. Whole brains were fed to 16 avian species including most of the fish-eating birds in the area. A single adult was recovered from a grey heron and numerous adults were recovered from pigeons and young ducks. Natural infections, at very low levels, were found in the smew (merganser), Mergus albellus. The authors described the adult parasite in detail but did not examine development of the eggs, miracidium, mother or daughter sporocysts. Snails (Physa fontinalis) naturally infected with this species were collected from nature. The authors noted the striking similarity between cercariae of O. scardinii and those of Posthodiplostomum cuticola but apparently did not follow the development of the metacercariae on the brain. Kozicka (1960) studied development of metacercariae, but had available only naturally infected fish. Sudarikov and Kurochkin found it intriguing that fish were so heavily infected (up to 324 metacercariae per fish), although the natural definitive host (Mergus albellus) was only a part-time visitor to the area as a spring and fall migrant. Furthermore, the merganser was present only at a time when snail hosts had burrowed into the mud for winter; trematode eggs apparently overwinter and hatch in spring when miracidia are able to contact snails. These authors also noted that infection levels had increased in fish from the Volga Delta, but could offer no explanation.

Skrjabin (1971), reviewing much of the biology of the metacercariae of O. scardinii, reported them from the ventricular cavities of the brain and more rarely in the tissues of various regions of the
cerebrum in the following species of fish: rudd (Scardinius erythrophthalmus), tench (Tinca tinca), white bream (Blicca bjoerkna), Caspian roach (Rutilus rutilus caspicus), and ruffe (Acerina cernua). Metacercariae have been reported principally from the European U.S.S.R. (including West Dvina, Kursh Gulf of Baltic Sea, Braslav Lakes, Northern Donets, Krasnoskol Reservoir, delta of Dnieper, lower Danube, delta of Volga, Priazor Estuary, reservoirs of Daghestan U.S.S.R.) and from Poland (Mazurian Lakes, e.g. Lake Drużno).

Migration of Strigeoid Trematodes

That strigeoid metacercariae localize in particular regions within their hosts has been established since von Nordmann's (1832) pioneering work on Diplostomum volvens. Steenstrup (1845) appears to have been the first to consider how such larvae had arrived at their specific location. In reference to D. volvens he noted (p. 97), "... I sometimes found these pupae on the internal surface of the cornea, and at the same time noticed a finely granular, unorganized streak, which passing through the cornea to the pupa clearly indicated the way by which the animal had penetrated previous to becoming a pupa. That these animals had entered from without, was shown also by some pupae which were situated in the skin around the eye, or a little beneath it, or even on the muscles of the globe. ..." Blochmann (1910) investigated the death of aquarium fish and believed that the migration of Cercaria fissicauda within the fish had killed them, for he recovered cercariae from the
heart and brain and also observed numerous small hemorrhages in the regions of the branchial arteries, orbit and lens, and pericardium.

Szidat (1924) working with Cercaria C (= cercaria of Diplostomum spathaceum) examined fish which died following cercarial penetration. He recovered cercariae from under the skin, the brain, gills (where large blood vessels were full of cercariae), pharynx, and the orbit of the eye. Szidat suggested that the circulatory and nervous systems formed easy routes which cercariae could follow to the lens of the eye.

Dubois (1929) studied the migration of Cercaria letifera in small trout by means of serial sections and observed that cercariae penetrated very rapidly and that the host's cranial region was most frequently penetrated. In fatal infections, cercariae were recovered from all the major blood vessels, heart, skin, connective tissue, mucous membrane of the mouth, body musculature, and brain. Wesenberg-Lund (1934), in reference to Cercaria C (= cercaria of Diplostomum spathaceum), stated (pp. 153-154), "... the Cercariae do not dart at the fish; it is not covered with a coating of creeping cercariae; I have never seen a Cercaria fasten itself to the fish; there is no doubt that most of them are drawn in through the mouth or especially enter the fish by piercing the skin." Wesenberg-Lund found cercariae virtually everywhere in Carassius vulgaris killed by cercarial penetration: creeping on the gills, below the scales, in the stomach, in the heart, and creeping on the lens of the eye. In examining young Leuciscus sp. for newly penetrated cercariae he noted (p. 154), "... I was especially struck by the great numbers in the veins of the gills and in those in the heart..."
There is of course no doubt that these larvae are carried with the blood into the body, and that their main organ is the lens of the eye. . . ."

An experimental approach to the problem of strigeoid migration was finally made by Davis (1936) who studied the penetration of Diplostomum spathaceum cercariae into frog tadpoles. Examination of tadpoles following cercarial penetration revealed cercariae in the brain, eye and eye lens, lumen of the heart, liver, gills, kidney, and intestinal tract. Additionally, petechial hemorrhages were evident on the external body surface. Davis exposed two frog tadpoles by immersing only their tails in a cercarial suspension. Tadpoles were examined 3 hrs later and metacercariae were recovered from the eye of one and from the brain of the other. Davis concluded that this rapid rate of migration was not possible by movement through tissues and suggested that metacercariae were passively carried to the head in the bloodstream.

Ferguson (1943a) studied the problem of migration of Diplostomum spathaceum using the normal second intermediate (fish) host. By performing a series of complex surgical operations, he was able to show that cercariae did not localize in the orbits from which the eye had been removed. Furthermore, cercariae were unable to reach the lens when the optic blood vessels had been cut, yet they could reach the lens when the optic nerve had been cut. Cercariae were not attracted to lenses implanted in various regions of the body. Ferguson concluded that the presence of at least one eye was necessary for large numbers of cercariae to reach the head although the lens itself was not essential. The eye tissues, according to Ferguson, provide a cercaria-
attracting stimulus. Finally he stated (p. 397),

The passage of the organisms through the body toward the head, largely by the blood stream, may to some extent represent a chance migration. However, once the anterior part of the body is reached apparently some factors must be present there which exert a considerable pull. It does not appear to be consistent with the facts available to assume at this time that the cercariae, once they are within the body, are not subject to certain influences which result in an active response on their part.

Erasmus (1959) followed the migration of Cercaria X Baylis to the eye lens of Gasterosteus aculeatus by serially sectioning whole fish at various intervals post-exposure. He concluded (p. 188) that,

The majority of migrating cercariae are present in connective tissue and muscle. Very few occur in the blood system and even fewer in the other organs of the body. The numbers in the blood system show a local anterior concentration in the blood vessels anterior to the heart and in the region of the gills. It seems probable that the blood system does not form the major route of migration.

Erasmus also suggested that the rapid rates of migration recorded by Davis (1936) and by Ferguson (1943a) might be explained by cercariae creeping over the external surface of the host's body and entering in the immediate vicinity of the eye. These findings were corroborated by Ratanarat-Brockelman (1974) who followed the migration of cercariae of Diplostomum spathaceum to the eye lens of Phoxinus phoxinus by serially sectioning fish killed at various intervals post-exposure. He noted (p. 123),

Migration occurred mostly in subcutaneous connective tissue and muscles of the trunk. Neither peritoneal cavity nor circulatory system served as a major migratory route.

Johnson (1971), in examining the migration of Cotylurus erraticus cercariae in Salmo gairdneri via serial sections, stated (p. 244),
The circulatory system and loose connective tissue served as migratory routes to the pericardium. The evidence suggests that the former was the more common route.

Johnson's data, however, seem to suggest that the connective tissue was the more common route. He stated (p. 247), "The migration to the pericardium was essentially complete by 8.0 hr." Up to and including this time, only small numbers of observed cercariae were in the blood vessels (82 of 3920 cercariae), whereas rather large numbers were in the connective tissue (1334 of 3920 cercariae).

Johnson (1971) suggested that increases in numbers of cercariae in the area of the pericardium with increased time post-infection, indicated that migration to the pericardium was progressive and nonrandom. In support of this hypothesis he cited the works of Ferguson (1943a) and Erasmus (1959). Johnson, however, seems to have confused concepts of migration and habitat selection. High percentages of cercariae in the pericardium may indicate that cercariae "select" this site nonrandomly but do not explain how they arrived at this site. Furthermore, Erasmus (1959) never claimed to have shown that migration of Cercaria X was directional and nonrandom and he clearly indicates this (p. 187), "Whether or not the migration is a directive one still remains to be proven. . . ."

Previous studies have, therefore, failed to show conclusively that migration of strigeoid trematodes is directed, oriented, nonrandom movement. They have suggested that migration routes are poorly defined and variable. Studies postulating a well-defined route have suggested the circulatory system as a means for such movement.
Only Szidat (1969) has addressed the question as to how parasites are able to localize in specific regions within their hosts. He theorized that strigeoid metacercariae are somehow able to obtain a plan (engram) of the total properties of their environment and noted (p. 784),

In those metacercariae which are successful in reaching their goal, the "engram" is retained and through thousands of generations is increasingly strongly fixed, until the metacercariae proceed quite "instinctively" and without hesitation to the part of the body which offers the best chance of protection against the destructive influences of the environment. . . . Those larvae which follow different paths build up, of course, a false "engram" which dies with them and cannot be perpetuated.
MATERIALS AND METHODS

Life Cycle

Metacercariae of *Ornithodiplostomum ptychocheilus* were obtained from naturally infected fathead minnows, *Pimephales promelas*, collected by seining or trapping from Frontage Road Pond, Eau Claire, Eau Claire County, Wisconsin (R-9W, T-27N, Section 35). Live fish were transported to the laboratory in styrofoam coolers with forced aeration. In the laboratory, fish were maintained in 50 gallon fiberglass tanks of artificial spring water (Ulmer, 1970) and fed a diet of artificial fish food (Tetramin) and frozen brine shrimp.

Uninfected fathead minnows were obtained from the Neosho National Fish Hatchery, Neosho, Missouri. Uninfected fish of other species were collected by seining from Squaw Creek, Ames, Story County, Iowa (R-24W, T-84N, Section 33). Uninfected fish were maintained in the same manner as infected fish.

All fish were treated for external parasites with a solution of formalin (1:6000) prior to their release in the laboratory. Similar prophylactic treatments were made at irregular intervals throughout the study.

Newly hatched chicks were obtained from the Veterinary Medical Research Institute, Ames, Iowa. Chicks were force fed whole brains removed from infected fathead minnows and were maintained in the laboratory without food for up to 7 days post-exposure.

Fecal material from infected chicks was collected by placing white
enamel pans containing artificial spring water beneath the chick cages. This material was then decanted 2-3 times in a 2000 ml graduated cylinder, macerated in a Waring blender, strained through a layer of cheesecloth and 3 graded sieves (Fisher 50, 60, 100 mesh), and decanted 2-3 more times in a 250 ml graduated cylinder. Often it was necessary to concentrate eggs even more by placing the sediment in a 5 or 9 cm petri dish and gently swirling the dish on a desk top.

When eggs were incubated in a darkened incubator at 30°C for 9-10 days, many would hatch when exposed to light. This technique greatly facilitated experimental snail exposures. When very large numbers of eggs were available, some were stored at 10°C for as long as 3 months to retard development. Such eggs maintained their hatchability and infectivity of miracidia was not impaired.

Hatched miracidia were drawn into a mouth pipette and placed into cylindrical plastic vials (2.5 cm by 1.2 cm). One snail was added to each vial and remained there with miracidia and enough water to cover the bottom for 2-3 hours. If the snail crawled above water level, it was carefully pushed back down. After this 2-3 hr period, vials were filled with water (about 1.2 ml), corked so as to allow a small air space, and left overnight. Snails were exposed to 2, 5, or 10 miracidia each. The latter proved most successful as more snails became infected and there was little, if any, post-penetration mortality.

Laboratory reared snail cultures of a variety of species were established by collecting snails from nature, maintaining them in the laboratory, and harvesting their eggs, which were the source of the
laboratory reared stocks. Snails were maintained in 10 gallon aquaria containing artificial spring water and fed a diet of leaf lettuce (ad libidum) and artificial fish food (Tetramin) once weekly. Crushed oyster shell served as a source of calcium carbonate.

Snails exposed to miracidia were reared in lots in 1 gallon aquaria for approximately 2 weeks and were then isolated in 5 cm stender dishes to permit observation for first emergence of cercariae so as to determine the prepatent period of the sporocysts. If cercariae were not observed within 2 months post-exposure, experimental snails were crushed and examined for sporocysts.

Whole mounts of all stages were prepared by washing in the appropriate saline solution and fixing in either hot (60°C) 10% formalin or hot (60°C) A.F.A. Adult worms were usually subjected to light coverslip pressure during fixation. Specimens were stained with Ehrlich's acid hematoxylin with either Mayer's paracarmine or Semichon's carmine counterstained with fast green. Following staining, specimens were dehydrated in ethanol, cleared, and mounted in Permount.

Specimens for sectioning were fixed in 10% formalin, A.F.A., Bouin's fixative, or alcoholic Bouin's fixative; they were then dehydrated with a tertiary-butyl alcohol series, and embedded in Paraplast or Tissuemat at 56-58°C. Serial sections were cut at 7-15 μm on an AO Spencer microtome, affixed to slides with Haupt's gelatin adhesive, and stained with either Mallory's triple connective tissue stain or Harris' hematoxylin with an eosin counterstain.

Larval stages were examined alive under both light and phase contrast
microscopy. Intra vitam dyes further aided the examination of internal structures of miracidia and cercariae. Aqueous solutions of neutral red proved useful in detecting gland cells and Nile blue sulfate was useful in examining the excretory system.

Miracidia were also fixed in hot (70°C) 0.5% silver nitrate, rinsed in distilled water, exposed to light, and mounted in glycerine to determine the boundaries of the ciliated epidermal plates.

Developing eggs were examined on glass slides whose coverslips were ringed with vaseline petroleum jelly. This technique enabled the observation of a single slide throughout the 9 day incubation period.

Photomicrographs were made using Panatomic X, Kodachrome II type A, or High Speed Ektachrome roll film in a Nikon M-35 S single lens reflex camera mounted on an AO Spencer Microstar Microscope with an AO Spencer orthoilluminator. Drawings were made with the aid of a Leitz micro-projector or from 35 mm color slides which were projected and drawn.

Route of Migration

To determine the route of migration, uninfected fathead minnows (2.3-2.8 cm in total length) were allowed to swim for 30 minutes in 5 cm stender dishes containing 20-25 ml water with 200 newly emerged cercariae of O. ptychocheilus. Using this technique, about 100 cercariae penetrated each experimental fish. This number was sufficiently large to provide significant results, yet small enough so as not to disrupt the "normal" pattern of migration. Following exposure, fish were removed and maintained individually. Fish were fixed in alcoholic Bouin's fixative (room
temperature) at each of the following intervals post-exposure: 0, 1, 2, 3, 4, 8, 12, 24, 48, and 72 hours. Time 0 was considered to be the time at which the 30 minute exposure period ended. Following a 3-4 day period of fixation, fish were washed, dehydrated, and embedded as discussed previously.

The basic assumption in this method was that host tissues or regions consistently containing the greatest number of migrating cercariae would represent those most frequently having been entered and traversed. Furthermore, migration of cercariae from one tissue to another would be indicated by appropriate changes in numbers of cercariae from the respective tissues.

Serial sections of experimentally exposed fish were cut sagittally at 15 μm. This facilitated visibility of the cercariae as they tended to align themselves with the longitudinal axis of the host.

Sagittal serial sections were systematically examined under light microscopy at 200 X. Once observed, the approximate position of a cercaria in the body of the fish and the tissue type in which it was found were noted. Tissue types were diagnosed with the aid of Anderson and Mitchum (1974) and Grizzle and Rogers (1976).

Rate of Migration

To determine the rate of migration, uninfected fathead minnows (2.2 to 3.0 cm in length) were exposed in an apparatus permitting cercarial penetration through the caudal fin (Figure 1). With the entire apparatus
filled with water and inverted, a fish was placed into the tube head first such that its snout contacted the pipette tip. Nonabsorbant cotton was packed around the caudal peduncle. The apparatus was then turned right side up (as figured) and the caudal fin (up to the posterior extremity of the caudal peduncle) was immersed in a cylindrical plastic vial containing 1 ml water and large numbers of cercariae. The apparatus employed is very similar to that described by Ratanarat-Brockelman (1974). However, the parafilm sheath which he employed to seal the fish in the tube was not water tight when very small fish, like those used in this study, were employed. Cook (1977) had used a similar apparatus on frog tadpoles, substituting a small piece of a rubber glove with a small hole cut in it for the parafilm sheath. This technique, when used on fish in the present study, apparently inhibited blood circulation to the tail, destroying the tissues; cercariae, therefore, never left the caudal fin and use of this technique was abandoned.

Fish were exposed for 30 minutes in the tail immersion apparatus described above and were then removed. Their caudal fins were rinsed thoroughly with artificial spring water from a squeeze bottle and brushed lightly with a camel hair brush to prevent cercariae which had not yet penetrated from creeping along the body surface to the head. Experimental fish were maintained individually in the fashion previously described.

It was assumed in this technique that all cercariae penetrated the middle of the longitudinal axis of the caudal fin (excluding fulcra), it is likely that equal numbers of cercariae penetrated anterior to and posterior to this plane; the error in either direction would be offset
by an equal error in the other direction.

In practice, the following procedures were followed: Serial sections were cut sagittally at 15 μm and systematically examined under light microscopy at 100 or 200 X. When a cercaria was observed, the distance from the middle of the caudal fin to the cercaria was determined. This distance, when divided by the time from the end of the exposure interval until the fish was fixed, gave an estimate of the average rate of speed for the cercaria in question.

Additionally, the tail immersion method of exposure indicated whether or not cercariae were capable of traversing the entire length of the fish's body and, if so, provided an estimate of the length of time required for such movement.
LIFE CYCLE

General Statement

Adults of *Ornithodiplostomum ptychocheilus* are intestinal parasites of piscivorous ducks, particularly mergansers. In the laboratory, unfed chicks, domestic ducklings, nestling English sparrows, or domestic mice may serve as definitive hosts. Eggs, passed with the feces of the definitive host, hatch in water after 8-9 days at 30°C. Free-swimming miracidia emerge and penetrate the snail *Physa gyrina* where they continue their life cycle. Within the snail (first intermediate) host, two generations of asexually reproducing sporocysts are produced, ultimately giving rise to a generation of free-swimming cercariae. The latter are capable of penetrating fathead minnows in which they migrate to the brain. Metacercariae remain within the brain tissues for approximately 1 week, then emerge from its dorsal surface, undergo a 2-3 week period of growth and development, and eventually encyst as neascus-type metacercariae at 4-5 weeks post-infection. The definitive host is infected via the ingestion of fish harboring infective metacercariae.

Adult

Van Haitsma (1930) presented an excellent description of adult *Ornithodiplostomum ptychocheilus*. His description and paratype specimen (United States National Museum Number 7990) agree so closely in morphology with specimens examined during the present study that it is unnecessary to redescribe the adult.
Van Haitsma (1930) did not note that the reproductive organs of
*O. ptychocheilus* are amphitypical. Of 118 specimens examined for this
character, the ovary in 66 (55.9%) was situated in the right anterior
region of the forebody and the anterior testis on the left (Figure 2).
In 52 (44.1%) the ovary lay in the left anterior region of the forebody
and the anterior testis on the right. Other reproductive structures of the
hindbody were correspondingly reversed. Van Haitsma's description was
based on specimens in which the ovary was on the left, a condition which
was observed in less than half of the specimens in my studies. Amphitypy
is relatively common among strigeoid trematodes (e.g. Van Haitsma, 1931;
Olivier, 1940; and others).

In the present study, adult worms were obtained from experimentally
infected domestic chicks (*Gallus gallus*), domestic ducks (Rowen strain),
estling English sparrows (*Passer domesticus*), and domestic mice (*Mus
musculus*). The specificity exhibited in nature by adults of this
species is, apparently, largely ecological. In chicks, adults were re­
covered from the duodenum (posterior third) and ileum (anterior half).
Only rarely were they observed attached in other locations.

**Egg**

Although numbers of intrauterine eggs are few (usually 1-2) in gravid
adults, they are produced rapidly. With concentration techniques, enough
eggs were obtained in only 4-5 days from 20-30 adults to completely cover
the bottom of a 90 cm petri dish.

Eggs were obtained from lab reared adults developed from metacercariae
originating from two localities: Frontage Road Pond, Eau Claire, Wisconsin, and Garlock Slough, Dickinson County, Iowa (R-37W, T-99N, S-35, 36). Thirty freshly passed eggs from Wisconsin measured 78-94 (88.2) µm long by 62-74 (66.6) µm wide. In 17 of these, the operculum was 16-24 (20.6) µm in diameter. Twelve eggs from Iowa measured 77-100 (87.5) long by 56-68 (63.0) wide. Van Haitsma (1930) reported that intrauterine eggs of O. ptychocheilus measured 70-75 (71) µm long by 60-65 (61) µm wide, whereas Sudarikov and Kurochkin (1968) observed that eggs of O. scardinii, presumably intrauterine, measured 80-90 by 61-62. Valid comparisons with results presented by these authors cannot be made as they did not state whether measurements of eggs had been based on specimens in living worms or on specimens measured in stained and mounted preparations of adults. Assuming the latter, smaller sizes of such eggs could be accounted for by their contraction and wrinkling during dehydration and clearing.

At 10°C, development was arrested or proceeded so slowly that it could not be detected. However, eggs held at this temperature for as long as 3 months maintained their hatchability, and infectivity of miracidia was not noticeably impaired. Eggs developed rapidly at 30°C and all observations on development were made on specimens incubated at this temperature. Measurements are in micrometers and are based only on 1-4 specimens.

Day 0 (Figure 3): Freshly passed eggs are operculate, asymmetrical, and are slightly attenuated at the opercular end. They are golden brown, transparent, and contain a nearly spherical zygote measuring 19-21 by 18-21. The zygote contains two large, oval pronuclei each measuring 8-12 by 5-9, with prominent nucleoli about 2-3 in diameter. Granular vitelline
cell nuclei are dispersed throughout the remainder of the egg but boundaries of individual vitelline cells are not apparent in freshly passed eggs. Although Pearson (1961), Dönges (1964), and others have observed these boundaries in freshly passed eggs of strigeoid trematodes, Hugghins (1954) could not observe them in freshly passed eggs of Hystero-morpho triloba. The granular material of the vitelline cell nuclei exhibits considerable brownian movement throughout the incubation period.

Shortly after incubation is initiated, the first division takes place. Pronuclei presumably fuse and the zygote divides unequally and generally at right angles to the longitudinal axis of the egg. The larger daughter cell, presumably the somatic cell of Hyman (1951) and others, measures 16-22 in diameter. Ishii (1934) and Rees (1940) have termed this cell the ectodermal cell; Pearson (1961) called it the E cell. Erasmus (1972) indicated that there is no formation of germ layers in digenetic trematode development, hence the term "somatic" is preferable to "ectodermal" to describe one of the products of unequal cleavage. The smaller daughter cell, the propagatory cell of Ishii (1934) and Rees (1940), measures 12-15 in diameter. Pearson (1961) called this cell the P cell, and observed marked size differences and differences in the granulation of the nucleoplasm of these two cell types in Neodiplostomum intermedium. In O. ptychocheilus, size differences are much more subtle and differences in granulation of the nucleoplasm could not be detected. Thus, it is often very difficult to differentiate the somatic cell line from the propagatory cell line. Between divisions, the nucleus reappears in each daughter cell.
The second division occurs only a short time (a few hours at most) after the first division. It apparently involves the somatic cell, this cell dividing into two similar daughter cells. Daughter cells are somewhat smaller, measuring 11-17 in diameter. The axis of this division is at right angles to the first division such that the propagatory cell is not in the same plane as the daughter somatic cells.

The third division appears to be an unequal division of the propagatory cell, producing a P cell like the parent about 13 in diameter and a smaller cell called a P₁ cell by Pearson (1961). Pearson observed that the nucleoplasm of P₁ cells was more granular than that of P cells in N. intermedium but this difference was not observed in O. ptychocheilus. The axis of the third division seems to be at right angles to the axes of the first and second divisions. The resultant 4-celled embryo consists of 2 large somatic cells, a medium sized P (propagatory cell), and a smaller P₁ cell.

The fourth and fifth divisions occur in rapid sequence and involve the two somatic cells. The result is a flat group of 4 somatic cells with the P and P₁ cells either on the top or bottom of the quartet of somatic cells. Pearson (1961) suggested that these divisions were simultaneous in N. intermedium, but in O. ptychocheilus it was sometimes possible to recognize a 5-celled embryo.

Because only small differences in size differentiate cell types, further divisions are difficult to follow. Development to this point proceeds in a fashion nearly identical to that reported by Pearson (1961) and it is likely that further divisions take place in the manner
described by him.

Day 1 (Figure 4): The embryo, consisting of about 12-20 small cells at the opercular end of the egg, is not much larger than the 6-celled stage measuring only 20-25 by 24-30. The boundaries of individual vitelline cells are readily apparent. Vitelline cells are spherical to hexagonal and measure 21-22 by 15-21 and have prominent nuclei measuring approximately 8-9 in diameter. It seems likely that vitelline cell membranes are present in freshly passed eggs but are not visible by the techniques employed. Hugghins (1954) observed vitelline cell boundaries during the second day of incubation of *H. triloba* at 37°C and when they were apparent he termed the egg "embryonated". In my material, it was sometimes possible to detect an apparent connection between the embryo and the inside of the shell at the opercular end. Rees (1940) and Pearson (1961) described formation of the vitelline membrane in *Parorchis acanthus* and *N. intermedium*, respectively. Apparently, a cell (or cells) separates from the embryo, flattens against the inside of the shell at the opercular end forming the vitelline membrane. Pearson suggested that a single cell of the P₁-type gave rise to the vitelline membrane in *N. intermedium*. Apparently, this "connection" in *O. ptychocheilus* suggests that the vitelline membrane is either forming or has been formed.

Day 2 (Figure 5): The embryo is almost three-fourths as long as the egg, and measures about 58 by 26. Growth apparently proceeds from the original cellular mass near the operculum toward the anopercular end of the egg. By this time, the embryo is situated near the middle of the egg.
The vitelline cell membranes are apparent only near the surface of the egg.

Day 3 (Figure 6): The embryo is very similar to that seen at Day 2. The membranes of vitelline cells, however, are well pronounced in the peripheral regions.

Day 4 (Figures 7,8): The multicellular embryo is quite large, measuring about 50 by 37. Although still situated near the middle of the egg, it begins to take on the appearance of a miniature miracidium with rather rudimentary eye spots. Occasionally, the beating of flame cells may be observed. Vitelline cells appear reduced in number and those remaining are somewhat smaller, measuring 16-17 by 10-13, and are more oval due to the pressure of the contained embryo.

Day 5 (Figure 9): The embryo, now 57 by 37, is nearly as long as the egg shell. Eyespots are well-developed measuring about 7-8 in diameter and the apical papilla is clearly evident. Vitelline cells, reduced in number (10-15), are concentrated in small areas not occupied by the embryo.

Day 6 (Figures 10,11): The embryo is about 1.5 times as long as the entire egg, making accurate measurement difficult. The apical papilla lies near the operculum and the embryo is folded upon itself near the anopercular end of the egg. Clearly evident are miracidial morphological features including conspicuous eyespots and apical papilla, four flame cells, and cilia. The miracidium is very active within the egg and cilia occasionally beat irregularly.
Day 7 (Figure 12): The developing miracidium is nearly twice as long as the egg. Both anterior and posterior ends of miracidium are near the opercular end and the larva appears U-shaped. The few vitelline cells (less than 10) remaining are concentrated laterally near the opercular end.

Day 8 (Figures 13,14): Prior to miracidial hatching, vitelline cells are almost entirely gone; the miracidium fills almost the entire area within the egg shell (Figure 13). The operculum opens, although remaining attached to the shell, the miracidium emerges, leaving a small amount of residual material near the opercular end. This residual material (Figure 14) appears to be cellular, at least in part, and may represent remnants of the vitelline membrane and/or vitelline cells. As hatching itself was not observed, no further conclusions can be made.

When eggs were incubated at 30°C in the dark and then were brought into the light on day 8, large numbers of miracidia hatched. However, day 9 appeared to be the peak day of miracidial hatching; eggs for experimental purposes were consequently incubated for 9 days prior to bringing them into the light. Most eggs hatched when exposed to light, although some required as many as 5 additional days of incubation.

Published accounts of very few strigeoid life history studies have included details of embryological development of the miracidium. Pearson (1961) presented an excellent account of the early divisions in N. intermedium and the pattern in O. ptychocheilus, as far as could be determined, is similar. Further growth occurs in a manner very similar to that described for Hysteromorpha triloba by Huggins (1954). Patterns
of embryological development of strigeoid trematodes do not appear to be unique and numerous similarities in this regard exist between members of this group and other groups of digeneans (Ishii, 1934; Bennett, 1936; Chen, 1937; Rees, 1940; and others).

Miracidium

Miracidia of the genus Ornithodiplostomum are poorly known and warrant description. The miracidium of O. ptychocheilus was briefly described by Hoffman (1958a) on the basis of 3 specimens, however he did not illustrate this larval stage. Sudarikov and Kurochkin (1968), in examining the life cycle of O. scardinii did not include data on the miracidium. Measurements recorded below are in micrometers and based on specimens fixed in hot 10% formalin unless otherwise specified.

The free-swimming miracidium is elongate, fusiform, rounded at both ends, and widest in the anterior half (Figure 15). Ten fixed specimens measured 95-127 (109.4) in length by 31-44 (37.2) in width at the level of the second tier of epidermal cells. The body is very flexible, and capable of nearly doubling back on itself when the living miracidium changes direction.

Eight miracidia fixed in hot 0.5% silver nitrate measured 86-124 (105.2) in length by 30-49 (40.5) in width and were of approximately the same size as those fixed in hot 10% formalin. The body is covered by 22 ciliated epidermal (tegumental) plates arranged in four tiers (Figure 16). Plates are separated from one another by a narrow space which, according to Erasmus (1972), is an extension of the underlying cytoplasmic layer.
The first (anteromost) tier consists of 6 triangular plates: 2 dorsal, 2 ventral, and 2 lateral. Nineteen of these plates measured 20-27 (22.4) in length by 12-17 (14.1) in greatest width. Tips of the plates of the anterior tier form a ring surrounding the base of the apical papilla and their bases are at a level just posterior to the eyespots. Cilia are short (2-3) at the anterior region of the plates of the first tier but lengthen progressively until a length of 8-12 is attained at the posterior margin of this tier. The second tier consists of nine rectangular plates, more widely separated than those of the anterior tier. Thirty-nine of these plates measured 26-40 (33.9) in length by 6-13 (9.7) in width and were arranged such that 2 plates were dorsal, 2 dorsolateral, 2 lateral, 2 ventrolateral, and 1 ventral. Cilia of the second tier are uniform in length, measuring from 7-10. The third tier consists of 4 wide, well-separated plates, 12 of which measured 18-43 (30.5) long by 12-17 (14.2) wide. Two of these plates are dorsolateral; 2 are ventrolateral. The anterior edges of these plates are nearly perpendicular to the sides; however, the posterior edge is typically convex. Cilia of the third tier are uniform in length, measuring from 6-13. The fourth tier consists of 3 well-separated, nearly diamond shaped plates, 9 of which measured 16-33 (24.7) long by 10-17 (13.0) wide. One plate is situated dorsally; 2 are situated ventrolaterally. The cilia of the fourth tier increase in length from 7-10 anteriorly to about 10-13 posteriorly.

Epidermal plates were counted in 17 specimens, 15 of which had the 6:9:4:3 formula described above. A single specimen had the formula 6:8:4:3. In this specimen, 2 plates of the second tier were dorsal,
2 dorsolateral, 2 ventrolateral, and 2 ventral. Other tiers were arranged in the "normal" fashion.

The apical papilla (termed anterior papilla, rostrum, rostellum, terebratorium, tactile organ by various authors) is a low, retractable mound bounded by the apices of the first tier of epidermal plates, and in 10 specimens measured 2-6 (4.1) long by 6-10 (7.9) wide. It lacks cilia, but bears numerous short hair-like processes. In miracidia fixed in silver nitrate, a number of pore-like "openings" were observed. Their exact number, arrangement, or function could not be determined but some may represent openings of the cephalic glands and the multiple openings of the apical gland.

In miracidia fixed in silver nitrate, a number of structures were observed in the spaces between the ciliated plates. Most prominent of these is a pair of large, lateral papillae near the midlateral line between plates of the first and second tiers; such papillae measured approximately 2 in length. Six apparently similar structures, each located in an indentation in the middle of the posterior margin of each plate of the first tier, were also observed. These structures appear as blackened areas. The functions of these structures are not clearly understood, for some authors (Cort, 1919; Faust and Meleney, 1924; Stunkard, 1923) have stated that substances were extruded from the lateral papillae, suggesting a glandular function. However, Price (1931), Rees (1940), and others have suggested that they are sensory with direct connections to the neural mass and presumably function in response to tactile or chemical stimuli facilitating host detection. Excretory pores appeared as black
circular areas located at the margins of the dorsolateral and ventrolateral plates of the third tier on each side. Pores were not always at the junction of the third and fourth tiers as has been reported for some strigeoid miracidia (Pearson, 1956, 1961; Dönges, 1965), but often were located about 2/3 of the distance between anterior and posterior margins of plates of the third tier. A similar situation has been observed in miracidia of Posthodiplostomum cuticola (Dönges, 1964).

A sac-like apical gland (Figure 15) nearly fills that portion of the miracidium anterior to the eyespots. Extending anteriorly from it are at least 4 minute ducts apparently opening to the exterior via the apical papilla. Posteriorly, the gland narrows just anterior to the eyespots, then widens again, ending in a second sac-like expansion near the middle of the body. The apical gland is apparently unicellular, although 4-5 nuclei are typically clustered in the posterior sac-like expansion. Nucleoli were not apparent. The nuclei seem to float freely in the cytoplasm as their location is quite variable depending upon the movements of the miracidium. The cytoplasm of the apical gland is fluid or semi-fluid and coarsely granular and takes up both neutral red and Nile blue sulfate stain.

Lying adjacent to the lateral portion of the anterior swelling of the apical gland are paired cephalic glands (termed salivary or pharyngeal glands by early authors). These are unicellular, flask-shaped, and contain dense nuclei and finely granular cytoplasm. Each cephalic gland opens to the exterior at the base of the apical papilla. Cephalic glands
were evident only under phase contrast microscopy and also stain readily with Nile blue sulfate.

At the posterior end of the body is a coarsely granular, sac-like body which has been called the posterior glandular body (Pearson, 1956, 1961) or caudal sac (Dönges, 1964, 1965). It appeared to be unicellular but contained up to 4 nuclei lacking prominent nucleoli. In some specimens, this structure appeared attached by a thin strand to the posterior body margin. No opening to the exterior was observed.

Paired eyespots made up of densely packed pigment granules occur near the dorsal surface near the junction of the first and second tiers of epidermal plates. Their position is quite variable and in 10 specimens the distance from the anterior tip of the miracidium to the anterior tip of the eyespots measured 13-33 (21.9). Eyespots are nearly spherical except for their anterolateral surfaces which are concave and in 20 specimens (from 10 miracidia), each measured 7-9 (7.9) in length by 5-8 (6.5) in width. No lenses were observed, although according to Pearson (1956, 1961) and Erasmus (1972), they are usually present and are occasionally doubled (Van Haitsma, 1931). Eyespots are present in all known strigeoid miracidia except Cercariae Indicae XV Sewell, 1922 (see Yamaguti, 1940; Pearson, 1956, 1961; Dönges, 1964, 1965).

Just posterior to the eyespots is a relatively complex aggregate of fibers and nuclei which is presumably the neural mass (termed "cerebral mass" or "brain" by various authors). Pearson (1956, 1961) and Dönges (1964, 1965) described a number of commissures extending from the
neural mass to various structures of miracidia of *Alaria* and *Posthodiplostomum*, respectively. Such commissures extend to the eyespots, lateral papillae, apical papilla, and posterolateral papillae. Although commissures were not observed in my study, it seems likely that they occur in a pattern similar to that described by these authors.

Germinal cells number approximately 6, possess large nuclei with prominent nucleoli, and are grouped in two clusters immediately posterior to the posterior swelling of the apical gland. Pearson (1961) and Dönges (1964, 1965) described "hyaline cells" or "parenchymal cells" enclosing the germinal cells of miracidia of *Alaria* and *Posthodiplostomum*, respectively. Such cells were not observed during the present study.

The flame cell formula $2[(1+1)]$ is characteristic of strigeatoid miracidia as indicated by LaRue (1957). The location of the excretory pores appears fixed and, as discussed previously, pores lie between the dorsolateral and ventrolateral plates on either side of the third tier of cells. Each primary excretory tubule bears a slight swelling just medial to the excretory pore. Pearson (1961) has considered this to be a "small bladder". The primary tubule extends posteroventrally, forms a loop near the posterior end of the body, then meanders anterodorsally, occasionally crossing over upon itself or crossing over the tubules from the other side, to a level just posterior to the eyespots. It then describes an anterior loop, runs posteriad, and finally bifurcates at a level near the middle of the anterior cluster of germinal cells. The anterior tubule extends forward to the anterior flame cell which is just posterolateral to the eyespots and is typically directed anteriorly. The posterior
tubule extends posteriad to a level near the posterior glandular body. Each flame cell of the posterior pair usually lies in a direction different from its counterpart.

The miracidium of *Ornithodiplostomum ptychocheilus* is similar in general morphology to previously described strigeoid miracidia (see Pearson, 1961 and Peters, 1966 for reviews). As far as could be determined, it is nearly identical to the miracidia of *Posthodiplostomum brevicaudatum* and *P. cuticola* as described by Dönges (1965, 1964, respectively). This is not surprising when the close relationship between these two genera is considered.

The similarity between strigeoid miracidia and miracidia of the families Clinostomatidae, Schistosomatidae, and Spirorchiiidae has been discussed by a number of authors including LaRue (1957), Pearson (1961), and Peters (1966). LaRue characterized the order Strigeatoidea, which included these families, in part as possessing a large miracidium with 2 pairs of flame cells.

Perhaps the most controversial matter regarding morphology of strigeoid miracidia is the pattern of distribution of the ciliated epidermal plates (Table 3). All authors are agreed that plates are arranged in 4 tiers. However, a variety of epidermal cell patterns has been proposed, from 6:6:6:3 in *Uvulifer ambloplitis*, to 6:8:4:3 in a number of species, to 6:9:4:3 as observed in this study and in a variety of other species. Pearson (1961) has suggested that 6:9:4:3 = 22 is the correct formula and will be found throughout the strigeate trematodes and pointed out that many past errors were based on the assumption
Table 3. Ciliated epidermal cell formulas reported for some strigeoid miracidia (based on Peters, 1966)

<table>
<thead>
<tr>
<th>Species</th>
<th>Formula</th>
<th>Reference(s)</th>
</tr>
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<tbody>
<tr>
<td><strong>Family Strigeidae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apatemon cobitidus cobitidis</td>
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<td>Vojtek, 1964</td>
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<td>Cotylurus cornutus (=Strigea tarda)</td>
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<td>Mathias, 1925</td>
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<td>Cotylurus erraticus</td>
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<td>Cotylurus lutzi</td>
<td>6:9:4:3</td>
<td>Basch, 1969</td>
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<td><strong>Family Diplostomatidae</strong></td>
<td></td>
<td></td>
</tr>
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<td>Alaria arisaemoides</td>
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<td>Pearson, 1956</td>
</tr>
<tr>
<td>Alaria intermedia</td>
<td>6:8:4:3</td>
<td>Odlaug, 1940</td>
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<tr>
<td>Didelphodiplostomum variable</td>
<td>6:9:4:3</td>
<td>Harris et al., 1967</td>
</tr>
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<td>Diplostomum flexicaudum</td>
<td>6:8:4:3</td>
<td>Van Haitsma, 1931; Cort et al., 1951</td>
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<td>Diplostomum phoxini</td>
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<td>Dönges, 1969</td>
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<td>Fibricola cratera</td>
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<td>Turner, 1957</td>
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<td>Fibricola texensis</td>
<td>6:9:4:3</td>
<td>Chandler, 1942</td>
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<tr>
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<td>6:9:4:3</td>
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<tr>
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<td>6:9:4:3</td>
<td>Park, 1936</td>
</tr>
<tr>
<td>Ornithodiplostomum ptychocheilus</td>
<td>6:9:4:3</td>
<td>This study</td>
</tr>
<tr>
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<td>Beckerdite et al., 1971</td>
</tr>
<tr>
<td>Posthodiplostomum brevicaudatum</td>
<td>6:9:4:3</td>
<td>Dönges, 1965</td>
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<td>Dönges, 1964</td>
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<td>Harris et al., 1970</td>
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<td>Cyathocotyle bushiensis</td>
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<td>6:8:4:3</td>
<td>Vojtková, 1970</td>
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<tr>
<td>Procyotrema industrius</td>
<td>6:8:4:3</td>
<td>Tang, 1941</td>
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that plates of the second tier were arranged symmetrically, when in fact there is a mid-ventral, unpaired plate. However, Pearson (1956) determined epidermal cell patterns in 12 miracidia of *Alaria arisaemoides* and observed 8 specimens with the pattern 6:9:4:3, 3 with the pattern 6:8:4:3, and 1 with the pattern 6:10:4:2. In the present study, only a single miracidium was observed with the pattern 6:8:4:3. Furthermore, Lynch (1934) has demonstrated considerable variation in epidermal plate counts of miracidia of *Heronimus chelydrae* and noted the dubious value of their number and arrangement in assigning family relationships. It is difficult to imagine why the epidermal plate formula should be constant in an order or family whose species are known to be highly variable.

As noted by Pearson (1961), the so-called cephalic glands were once thought to be present in schistosomatid miracidia but absent in strigeoid miracidia, with the exception of *Uvulifer ambloplitis*. Pearson's study, published data by Dönges (1964, 1965), and this study suggest that they are present throughout the Strigeoidea and, as Pearson has suggested, may occur in all miracidia. Erasmus (1972) has observed that such glands are small in some miracidia (e.g. *Fasciola hepatica*, *Diplodiscus temperatus*, *Cotylophoron cotylophorum*) and may easily be overlooked.

Structures similar to the posterior granular body of *O. ptychocheilus* miracidia have been described in a variety of other strigeoid miracidia including *Alaria arisaemoides* (by Pearson, 1956), *Diplostomum flexicaudum* (by Van Haitsma, 1931), *Hysteromorpha triloba* (by Huggins, 1954), *Neodiplostomum intermedium* (by Pearson, 1961), *N. lucidum* (by Park, 1936), *Posthodiplostomum brevicaudatum* (by Dönges, 1965), and *P. cuticola* (by
Dönges, 1964). Van Haitsma, Hugghins, and Park considered the posterior granular body to be a posterior group of germinal cells, but studies by Cort et al. (1951) and Pearson (1956) have shown that in *D. spathaceum* and *A. arisaemoides*, respectively, this body does not contribute to the germinal material of the mother sporocyst. Pearson (1961) noted its glandular appearance but neither he nor Erasmus (1972) suggested a possible function.

Although most authors refer to the presence of a "neural mass" in miracidia, Chu and Cutress (1954) considered it to be neither nervous nor glandular in the miracidium of *Australobilharzia variglandis*. Erasmus (1972), however, in ultrastructural studies of the "neural mass" of miracidia of *Fasciola hepatica* demonstrated an organization similar to that of the nervous system of cercariae of that species.

**Sporocysts**

Experimental infection of snails with miracidia of *O. ptychocheilus* was successful only in *Physa gyrina*, although experimental exposures with the following snails were also conducted: 2 *Helisoma trivolvis*, 6 *Lymnaea catascopium*, 17 *L. palustris*, and 65 *Physa integra*. Hoffman (1958a) was able to experimentally infect only *Physa anatina*, even though he exposed *Stagnicola (=Lymnaea) palustris* and *Helisoma anceps* as well. Sankurathri and Holmes (1976) found *P. gyrina* naturally infected with *O. ptychocheilus* in Alberta, Canada.

Sporocyst development appears to follow the general pattern observed in other strigeoid trematodes (Cort and Olivier, 1941; Cort et al.,
1951; Van der Woude et al., 1953; Cort et al., 1954; Pearson, 1956, 1961; Dünges, 1964, 1965; and others) with modifications associated with differing rates of development. For this reason, sporocyst development was not followed in detail.

Mother sporocysts typically developed in tissues surrounding the lobes of the hepatopancreas (Figure 17), although a single specimen was recovered from the mesenteries behind the cerebral ganglion in one host and from the tissues of the mantle in another (Figure 18). Mother sporocysts were in the tissues of the hepatopancreas within 2 days post-infection. By 2-3 days they were relatively thick-walled, up to 100 μm in length and were filled with small, densely packed cells, presumably germinal cells. By 6-9 days post-infection, mother sporocysts had grown to 375 μm in length and embryos of daughter sporocysts could be observed within them.

Between 9 and 12 days post-infection, daughter sporocysts were released from mothers and by 12 days post-infection, daughter sporocysts containing early cercarial embryos were interspersed throughout the tissues of the hepatopancreas. By 15 days post-infection, daughter sporocysts contained nearly fully formed cercarial embryos (Figures 19, 20). Shedding of cercariae commenced as early as 22 days and as late as 32 days post-infection, even when snails had been incubated at a constant temperature (22°C). Usually, however, shedding was first observed on or near the 24th day after infection.

Daughter sporocysts obtained from the hepatopancreas of experimentally
exposed *P. gyrina* are relatively thick-walled, and up to 5.1 mm in length. They are beaded in appearance and are as wide as 120 μm where large embryo(s) occur, or as narrow as 45 μm. Daughter sporocysts are relatively muscular, hollow sacs, rounded posteriorly, and slightly attenuated and solid anteriorly. Near the anterior end is a subterminal birth pore. Each daughter sporocyst contains a variable number of cercarial embryos in different stages of development.

**Cercaria**

The cercaria of *O. ptychocheilus* (Figures 21, 22) was briefly described by Hoffman (1958a). He did not, however, illustrate the cercaria, and discrepancies between his material and that used in the present study suggest the need for a redescription. All measurements are in micrometers and based on ten specimens fixed in 10% hot formalin unless otherwise stated. Ranges are given followed by means in parentheses.

The cercaria of *O. ptychocheilus* is a freshwater, bioculate, monostomate longifurococercaria. The digestive system is rudimentary and represented only by two clusters of cells immediately posterior to the penetration organ. The first cluster is presumably the rudimentary pharynx (about 17 long by 12 wide in living specimens) and the second is presumably the rudiment of the intestinal ceca. This rudimentary digestive system seems to be characteristic of cercariae of the genera *Posthodiplostomum* and *Ornithodiplostomum* (Wiśniewski, 1958; Dönges, 1964, 1965; Hoffman, 1958a).

The body of the cercaria is 112-169 (146.7) long by 25-44 (32.2) at
its widest point. The tailstem is approximately 1.1 times as long as the body and measures 153-199 (167.8) long by 24-38 (29.5) wide. Furcae are as long or longer than the body, and measure 147-204 (165.8) along their greatest curvature by 11-20 (15.9) wide at their base. Pigmented eyespots are located about two-thirds of the distance from the anterior to the posterior of the body. In living specimens, eyespots are prominent and are 3-5 in diameter. Eyespots were not evident in fixed specimens. Hoffman (1958a) observed one pair of laterally projecting "flagellets" emerging from the body between the posterior pair of penetration glands and the posterior end of the body. "Flagellets" were observed only in a few specimens in this study.

The penetration organ is large, occupying nearly half of the body length. It is oval, slightly attenuated anteriorly and measures 56-86 (72.0) long by 17-35 (24.7) at its widest point. The thick-walled but nonmuscular "oral sucker" bears little morphological resemblance to a "true" sucker. For this reason, Sewell (1922) suggested the term "anterior protrusible penetrating organ" which H. M. Miller (1926) shortened to anterior organ. The anterior organ is eversible and in living cercariae is constantly everted and inverted when in contact with a substrate. It is armed with scattered penetration spines covering its anterior third. Posterior to these penetration spines are about 8 rows of smaller spines extending almost one-third of the length of the body.

Three pairs of unicellular penetration glands are located posteromedial to the eyespots. Gland cells are of a single kind and possess
large refractile nuclei. A penetration gland duct leads from each
gland, those ducts from each side running parallel as a group. Each
gland duct runs anteriorly into the penetration organ, where it then
enlarges to almost 9 in diameter. Prior to reaching their terminations,
ducts are reduced in diameter to about 1-4. They terminate in two groups
of three at each lateral margin of the anterior tip of the penetration
organ. Fluid secretions are released from their terminations in living
cercariae under coverslip pressure.

The excretory bladder is bipartite and situated at the posterior of
the body. The small "island of Cort" noted by Hoffman (1958a) was not
observed; however, the small size of the cercaria made accurate observa-
tions difficult. The primary collecting tubules arise from the antero-
lateral margins of the bladder, proceed anterolaterally to a level be-
tween the second and third pairs of penetration glands, where a
transverse commissure joins the two primary tubules. They continue
anteriorly for a short distance, then coil and presumably branch at the
level of the second pair of penetration glands. Primary ducts, in the
region of coiling, bear 2, perhaps 3 ciliated patches. Because the
precise point of branching of the primary tubules into secondary tubules
could not be discerned, the flame cell formula remains uncertain.
There are, however, 8 pairs of flame cells in the body: 2 pairs lateral
to the penetration organ, 2 pairs lateral to the eyespots, 2 pairs lateral
to the penetration glands, and 2 pairs near the posterior end of the body.
Two pairs of flame cells are located in the tailstem: one near the
anterior margin, the other about halfway along its length. The flame
cell formula is probably $2[(2+2)+(2+2+2s)] = 20$, as has been described for *Posthodiplostomum cuticola* and *P. brevicaudatum* by Dönges (1964, 1965). The excretory duct leads from the posteromedial portion of the bladder, through the center of the tailstem, then bifurcates at the origin of the furcae, each branch leading to an excretory pore on the anterior margin of each furca.

The genital primordium is located immediately anterior to the bladder and consists of a number of rather large, tightly packed cells. A similar cluster of cells occurs between the second and third pairs of penetration glands and probably represents the primordium of the acetabulum. The genital primordium and primordium of the acetabulum could not be seen in all specimens.

The tailstem is aspinose but 2 pairs of patches of hair-like processes (called flagellets by Hoffman, 1958a) are found on its lateral surfaces. One pair is located near the anterior extremity, the other near the posterior extremity. The tailstem is almost as wide as the body except at its posterior end where it widens to form the furcae. In newly emerged cercariae, the tailstem contains 5-6 pairs of oval caudal bodies. In older cercariae (e.g. 12-24 hrs after emergence) caudal bodies become stellate and then either disappear or become obscured.

Furcae are equipped with small, lateral fin folds evident only under phase contrast microscopy and only when the cercaria is somewhat flattened. Fin folds extend from almost midway along both lateral edges of each furca to and around their tips. Furcal fin folds are equipped with 2-4 pairs of very short, supporting, spine-like processes.
A structure similar to the cerebral ganglion described by Dönges (1964) in the cercaria of _P. cuticola_ was observed in a single cercaria during this study. He illustrated it in the shape of a flattened H located just posterior to the penetration organ, but whether or not the corresponding structure in my specimen was the cerebral ganglion could not be determined.

The activity of the cercaria after its escape from the snail host consists of alternating periods of hanging motionless in the water and periods of rapid upward swimming. When large numbers of cercariae are observed at a given time, most are motionless in the water. Generally they do not hang perpendicular to the surface; most seem to align themselves at various angles to the surface. In this resting position, furcae are held so that lines running down their centers form angles of about 80° each with the tailstem. These angles appear never to be less than 45° and are usually greater than 60°. In this position, the cercariae sink quite slowly and upon nearing the bottom, swim rapidly upward. The ascent, however, is not perpendicular to the bottom but upwards toward the walls of the container. Resting periods are quite long (up to 30-45 seconds) if the cercaria is undisturbed. Swimming periods may be as long as 2 seconds, although usually they are less than 1 second and vertical ascent is usually only a few millimeters. Cercariae emerge from the snail host in large numbers when the container is placed under light. Large numbers also emerge in the morning hours when snail hosts are maintained in the laboratory under a normal photoperiod. Cercariae are positively phototactic and gather on the brightest side of a stender dish.
held under a dissecting microscope.

Except for the number of flame cells, the morphology of cercariae as determined in this study is consistent with that reported by Hoffman (1958a) (Tables 4, 5). However, Hoffman observed 6 pairs of flame cells in the body and 2 pairs in the tailstem; I observed 8 pairs in the body and 2 pairs in the tailstem. The small size of the cercaria of this species, the occurrence of ciliated patches in the primary excretory tubules, and the dense nature of the parenchyma make exact observation of flame cells difficult. The findings of Hoffman (1958a) are supported by Sudarikov and Kurochkin (1968) who found a similar pattern in _O. scardinii_. I have found that refrigeration of cercariae overnight causes the parenchyma to clear considerably, greatly enhancing the observation of flame cells. Ciliated patches do not become evident until just before the living cercaria begins to disintegrate under a coverslip, at a time when the flame cells beat very slowly. Ciliated patches, however, beat very rapidly and their cilia are nearly twice as long as those of flame cells.

As noted by Hoffman (1958a), the cercaria of _O. ptychocheilus_ bears a remarkable resemblance to cercariae of the genus _Posthodiplostomum_, especially _P. minimum_ J. H. Miller and _P. brevicaudatum_ (Tables 4, 5). Assuming my data on the flame cell number are correct, cercariae of both genera have 20 flame cells in approximately the same location, with primary excretory tubules that are equipped with ciliated patches and possess a transverse commissure. It is difficult to understand how
Table 4. Biological and morphological characters of the Rhadboecaeca group of furcocercous cercariae

<table>
<thead>
<tr>
<th>Host</th>
<th>Body at rest</th>
<th>Eyespots</th>
<th>Caudal bodies</th>
<th>Lateral filaments on tailstem</th>
<th>Folds</th>
<th>Digestive system</th>
<th>Pigmented cyst</th>
<th>Flame cell number</th>
<th>Transverse commissure in excretory system</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cercaria bessiae</em> Cort and Brooks, 1928</td>
<td>Pl</td>
<td>B</td>
<td>U</td>
<td>-</td>
<td>+</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. flexiorpa</em> Collins, 1935</td>
<td>Pl</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>+,-</td>
<td>-</td>
<td>R</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td><em>C. furcolineata</em> E. L. Miller, 1936</td>
<td>Pl</td>
<td>B</td>
<td>-</td>
<td>4,6</td>
<td>-</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. hamata</em> H. M. Miller, 1923, 1926</td>
<td>Pl</td>
<td>B</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>R</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td><em>C. isomi</em> Goodman, 1951</td>
<td>Ph</td>
<td>S</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. louisiana</em> E. L. Miller, 1935, 1936</td>
<td>Ph</td>
<td>B</td>
<td>+</td>
<td>-</td>
<td>(-)</td>
<td>+</td>
<td>R</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><em>C. metadena</em> Johnston and Angel, 1942</td>
<td>Pl</td>
<td>B</td>
<td>U</td>
<td>-</td>
<td>+,-</td>
<td>-</td>
<td>R</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td><em>C. multicellulata</em> H. M. Miller, 1923, 1926</td>
<td>Ph</td>
<td>S</td>
<td>+</td>
<td>10-15</td>
<td>+</td>
<td>O</td>
<td>-</td>
<td>20 (--)</td>
<td></td>
</tr>
<tr>
<td><em>C. paramulticellulata</em> Goodman, 1951</td>
<td>Ph</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>R</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td><em>C. physae</em> Cort and Brooks, 1928</td>
<td>Ph</td>
<td>S</td>
<td>+</td>
<td>10</td>
<td>+</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. rhabdobaeca</em> Faust, 1919</td>
<td>Pl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td><em>Crassiphiala bulboglossa</em> Hoffman, 1956</td>
<td>Pl</td>
<td>B</td>
<td>-</td>
<td>(-)</td>
<td>+</td>
<td>R</td>
<td></td>
<td></td>
<td>+ 18</td>
</tr>
</tbody>
</table>

Abbreviations: + character present; - character absent; B body bent or hooked when at rest; 0 digestive system not present or observed; Ph physid snail; Pl planorbid snail; R digestive system rudimentary; S body straight when at rest; U eyespots present but unpigmented. Authors are those upon whose data the table is based and are not necessarily the original describers.
<table>
<thead>
<tr>
<th>Name</th>
<th>Host</th>
<th>Body attitude at rest</th>
<th>Eyespots</th>
<th>Caudal bodies</th>
<th>Lateral filaments on tailstem</th>
<th>Fimbriae</th>
<th>Digestive system</th>
<th>Pigmented cyst</th>
<th>Flame cell number</th>
<th>Transverse commissure in excretory system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ornithodiplostomum ptychocheilus</td>
<td>Ph</td>
<td>+</td>
<td>10,12</td>
<td>+</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td><em>O. ptychocheilus</em> This study</td>
<td>Ph</td>
<td>S</td>
<td>+</td>
<td>10,12</td>
<td>+</td>
<td>+</td>
<td>R</td>
<td>-</td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td><em>O. scardinii</em> Sudarikov and Kurochkin, 1968</td>
<td>Ph</td>
<td>S</td>
<td>+</td>
<td>10</td>
<td>+</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Posthodiplostomum brevicaudatum Donges, 1965</td>
<td>Pl</td>
<td>S</td>
<td>+</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>R</td>
<td>-</td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td>P. cuticola Donges, 1964</td>
<td>Pl</td>
<td>B</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td>P. minimum Bedinger and Meade, 1967</td>
<td>Ph</td>
<td>S</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>-</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>P. minimum J. H. Miller, 1954</td>
<td>Ph</td>
<td>+</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>R</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Uvulifer ambloplitis</td>
<td>Pl</td>
<td>-</td>
<td>(-)</td>
<td>+</td>
<td>(R)</td>
<td>+</td>
<td>(16)</td>
<td>(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Hoffman and Putz, 1965)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Mensural characters of the Rhabdocaeca group of furocercous cercariae (length - width) (ranges are given followed by means, all measurements are in micrometers and are based on same authors as in Table 4, unless specified otherwise)

<table>
<thead>
<tr>
<th></th>
<th>Body</th>
<th>Tailstem</th>
<th>Furcae</th>
<th>Penetration organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cercaria bessiae</td>
<td>176</td>
<td>255</td>
<td>178</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. flexicorpa</td>
<td>107-182(159)</td>
<td>156-227(188)</td>
<td>136-198(169)</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>19-32(26)</td>
<td>13-35(22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. furculinaeata</td>
<td>168</td>
<td>280</td>
<td>252</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>39</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>C. hamata</td>
<td>207</td>
<td>276</td>
<td>276</td>
<td>29-39^c</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>39^c</td>
<td>28^c</td>
<td></td>
</tr>
<tr>
<td>C. isomi</td>
<td>180</td>
<td>265</td>
<td>230</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. louisiana</td>
<td>168</td>
<td>224</td>
<td>224</td>
<td>59^c</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>33</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>C. metadene</td>
<td>175-225</td>
<td>167-250</td>
<td>184-250</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>33-50</td>
<td>29-38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. multicellulata</td>
<td>165</td>
<td>198</td>
<td>147</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. paramulticellulata</td>
<td>170</td>
<td>182</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>C. physae</td>
<td>143</td>
<td>222</td>
<td>194</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^aBased on fixed specimens.
^bBased on living specimens.
^cBased on E. L. Miller, 1935, 1936.
<table>
<thead>
<tr>
<th>Species</th>
<th>Body</th>
<th>Tailstem</th>
<th>Furcae</th>
<th>Penetration Organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. rhabdocaeca</td>
<td>140</td>
<td>Nearly two times body</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crassiphiala bulboglossa</td>
<td>106-230 (152)</td>
<td>190-360 (250)</td>
<td>23-30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28-54 (41)</td>
<td>24-42 (31)</td>
<td>18-28</td>
<td></td>
</tr>
<tr>
<td>Ornithodiplostomum ptychocheilus Hoffman</td>
<td>144-196 (169)</td>
<td>162-220 (196)</td>
<td>159-196 (177)</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>26-39 (32)</td>
<td>24-34 (27)</td>
<td>12-14 (13)</td>
<td>19</td>
</tr>
<tr>
<td>Ornithodiplostomum ptychocheilus This study</td>
<td>112-169 (147)</td>
<td>153-199 (168)</td>
<td>147-204 (166)</td>
<td>56-86 (72)</td>
</tr>
<tr>
<td>Ornithodiplostomum scardinii</td>
<td>203-249</td>
<td>238-241</td>
<td>206-217</td>
<td>66-78</td>
</tr>
<tr>
<td></td>
<td>26-34</td>
<td>20-31</td>
<td>14</td>
<td>2-29</td>
</tr>
<tr>
<td>Posthodiplostomum brevicaudatum</td>
<td>200-274</td>
<td>166-216</td>
<td>175-235</td>
<td>43-63</td>
</tr>
<tr>
<td></td>
<td>27-44</td>
<td>26-35</td>
<td>12-16</td>
<td>14-25</td>
</tr>
<tr>
<td>P. cuticola</td>
<td>188-302</td>
<td>220-314</td>
<td>196-298</td>
<td>66-98</td>
</tr>
<tr>
<td></td>
<td>35-70</td>
<td>33-55</td>
<td>15-29</td>
<td>20-39</td>
</tr>
<tr>
<td>P. minimum Bedinger and Meade</td>
<td>143-158</td>
<td>172-199</td>
<td>126-176</td>
<td>32-61</td>
</tr>
<tr>
<td></td>
<td>23-25</td>
<td>21-25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. minimum J. H. Miller</td>
<td>112-226 (192)</td>
<td>171-254 (247)</td>
<td>175-226 (219)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20-46 (32)</td>
<td>19-38 (28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uvulifer ambloplitis</td>
<td>168-208 (193)</td>
<td>276-288 (282)</td>
<td>228-247 (237)</td>
<td>21-43 (36)</td>
</tr>
<tr>
<td></td>
<td>34-36 (35)</td>
<td>31-46 (34)</td>
<td>17-21 (19)</td>
<td>17-21 (19)</td>
</tr>
</tbody>
</table>

^d Condition of cercariae measured not specified or unknown.
two nearly identical cercariae can represent two supposedly distinct genera.

The cercaria of *O. ptychocheilus* belongs to the Rhabdobaeca group established by H. M. Miller (1926). Cercariae of this group are characterized as longifurcate monostomes lacking a ventral sucker, having 3 pairs of penetration glands, and a rudimentary digestive system which may or may not be detectable (H. M. Miller, 1926; Dönges, 1964). Some biological, morphological, and mensural characters of the described Rhabdobaeca cercariae are presented in Tables 4 and 5.

Krull (1934), using cercariae which he identified as *Cercaria bessiae*, experimentally infected fish and recovered metacercariae of *Uvulifer ambloplitis*. However, Hoffman and Putz (1965), in working the life cycle of *U. ambloplitis*, observed that their cercariae were not identical to *C. bessiae* in that they lacked unpigmented eyespots and had furcae 25% longer than those of *C. bessiae*. Further investigations into this problem are needed. Hoffman (1960, 1967) has suggested that the metacercaria of *C. flexicorpa* is similar to, if not identical with, the metacercaria of *U. ambloplitis*. The differing number of flame cells in these two cercariae, however suggests the need for further investigation.

*Cercaria louisiana, C. multicellulata, C. P. minimum* Bedinger and Meade, and *C. P. minimum* J. H. Miller have been shown experimentally to develop into *Posthodiplostomum minimum* by Hoffman (1958b), Hunter (1936), Ferguson (1936, 1937, 1938, 1943b), Bedinger and Meade (1967), and by J. H. Miller (1954). Although different subspecies of *P. minimum* are known,
it seems very doubtful that flame cell number varies between subspecies. Bedinger and Meade (1967) may have observed ciliated patches and considered them to be flame cells. Until concurrent experimental work is done on both subspecies, this taxonomic problem cannot be resolved. Furthermore it is likely that *C. paramulticellulata* and *C. physae* are involved in the *P. minimum* complex as well.

Cort and Brooks (1928) considered *C. hamata* to be a synonym of *C. rhabdocaeca*. Although Faust's original description of *C. rhabdocaeca* was lacking in detail, the fact that both species were obtained from the same host species and from similar localities (the same locality, according to Cort and Brooks) suggests that they are specifically identical.

The remaining *Rhabdocaeca* cercariae, where life history studies have been completed, represent the genera *Crassiphiala* and *Uvulifer* of the tribe *Crassiphialini* Dubois, 1936, and *Posthodiplostomum* and *Ornithodiplostomum* of the tribe *Diplostomatini* Dubois, 1936 *ex* Poirier, 1886. These cercariae penetrate fish and develop to neascus-type metacercariae. *Cercaria metadena*, however, penetrates fish and develops to a diplostomulum-type metacercaria, and Johnston and Angel (1942) suggested that these larvae might be those of the genus *Bolbophorus* (tribe *Diplostomatini*).

*Rhabdocaeca* cercariae occur sporadically in the tribe *Diplostomatini* and in all those members of the tribe *Crassiphialini* whose life cycles are known, suggesting that either Dubois' (1970) scheme of classification requires modifications, that the *Rhabdocaeca*-type cercaria has evolved independently at least twice in the subfamily *Diplostomatinae* Monticelli, 1888 *ex* Poirier, 1886, or that the *Crassiphialini* is an evolutionary
branch of the Rhabdocoaecan line of the Diplostomatini. Until further life history studies are completed, such hypotheses must remain speculative.

Metacercaria (Neascus)

The metacercaria of *O. ptychocheilus* belongs to the larval genus *Neascus* Hughes, 1927. Hoffman (1960, 1967) has characterized this larval genus as possessing: (1) a foliaceous forebody, concave ventrally; (2) a hindbody more extensively developed than the small conical prominence on the posterodorsal portion of the forebody of *Diplostomulum*; (3) a reserve bladder more extensively developed than in *Diplostomulum*, with calcareous concretions not confined to the termini of small branches which do not end blindly but constitute anastomoses; (4) no lateral pseudosuckers; (5) a cyst partially of parasite origin. Synopses of the larval genus *Neascus* have been presented by Hughes (1928b) and by Hoffman (1960). Hoffman (1967) reviewed species known to occur in North American freshwater fish.

Although the metacercaria of *O. ptychocheilus* has been described by Faust (1917, 1918) and by Hughes and Piszczek (1928), a redescription including additional data is presented here. Measurements are in micrometers and based on 12 specimens fixed in hot 10% formalin unless otherwise stated. Ranges are given followed by means in parentheses.

The cyst is oval and much larger than the enclosed parasite. Ten cysts containing metacercariae measured 504-735 (638.9) in length by 299-426 (325.0) in width. Living cysts vary in appearance from colorless
and relatively clear to a more opaque yellowish color, depending upon the nature of the surrounding host tissue. The thin cyst wall is composed of two layers, the outer presumably of host origin and the inner presumably of parasite origin. In the living condition, the two layers are closely applied to one another and appear as a single membrane. However, during fixation and/or staining they often become separated. In sections, the parasite cyst appears acellular and stains a light blue with Mallory's triple stain; the host cyst contains a number of flattened nuclei and stains light red. Apparently, a number of parasite cysts may be enclosed in a single host cyst resulting in the "syncyst" of Sudarikov and Kurochkin (1968). Cysts are rather fragile and are easily broken, but without injury to the parasite. They contain a rather thick, clear liquid surrounding the parasite. Often excretory (?) concretions are suspended in this liquid. Living metacercariae were recovered from cysts taken from fathead minnows which had died 48 hrs previously. Although the ability of metacercariae to remain viable after death of the host was not evaluated experimentally, it seems likely that the cyst offers some protection to the enclosed metacercaria.

The body of the parasite is divided by a shallow constriction into a thin foliaceous forebody and a short, bulbous, almost spheroidal hindbody. This division into fore- and hindbodies is distinct only in living specimens. In preserved and/or stained specimens, the hindbody is much reduced and the parasite appears as a flat oval, resembling a diplostomulum. The forebody is concave ventrally and about twice as long as the hindbody, measuring 261-364 (308.3) long by 136-200 (165.3) wide.
In living specimens, the shape of the forebody is changeable in accordance with movements of the parasite. The hindbody is relatively short and measures 110-153 (130.1) long by 125-173 (145.0) wide. The shape of the hindbody seems to vary only with the amount of excretory substance contained within it and is relatively immobile.

Movements of living metacercariae consist of alternate expansion and contraction of the body, particularly the forebody. This is true when metacercariae are enclosed within or freed from their cysts. In specimens freed from cysts, no actual locomotion was accomplished. The leech-like movement observed in *Neascus ambloplitis* by Hughes (1927) was never observed.

The tribocytic organ ("holdfast") is located medially near the posterior margin of the forebody. In fixed specimens it is nearly rectangular and in 11 specimens measured 52-93 (66.6) long by 62-105 (81.1) wide. In living specimens, it is nearly round. Its surface is marked by a median triangular opening. The tribocytic organ is very contractile. In relaxed specimens, it protrudes from the body and in lateral aspect appears mushroom-shaped.

The oral sucker is terminal, circular and measures 20-30 (25.5) long by 17-30 (23.1) wide. The acetabulum is located just anterior to the tribocytic organ and is circular, measuring 22-30 (25.0) long by 22-30 (27.9) wide. In living specimens, neither sucker shows any signs of contraction.

The oral sucker is followed by a short prepharynx which in 7 specimens measured 5 or less. In living specimens, the prepharynx is
considerably longer. The pharynx is bulbous, muscular, and quite pronounced, measuring 23-30 (28.0) long by 15-19 (16.7) wide. The esophagus is short and slender. The ceca diverge at a small angle, parallel each other posteriorly to a level between the ventral sucker and tribocytic organ, and then diverge more widely. At the level of the constriction between fore- and hindbodies, they bend medially, then laterally, and finally terminate blindly lateral to the copulatory bursa. At the bifurcation of the esophagus, ceca are quite narrow and gradually widen posteriorly until a maximum diameter is attained at about the middle of the hindbody.

Because of the presence of large numbers of excretory concretions in the hindbodies of living specimens, the rudimentary reproductive system was visible only in stained and mounted specimens. Gonadal rudiments are composed of 3 well-defined, compact, darkly staining, cellular masses located in the anterior portion of the septa between the two large excretory spaces in the hindbody. Two of these are situated anteriorly, are oval and may represent rudiments of the ovary and anterior testis. Immediately posterior to these is the third which is somewhat transversely elongate and presumably represents the primordium of the posterior testis. When metacercariae are fixed under any type of pressure, septa and/or excretory spaces of the hindbody often rupture, the gonadal primordia spread out and resemble the situation seen in the adult worm. The copulatory bursa is located posteriorly and in morphology closely resembles that seen in the adult parasite. However, in metacercariae it is rarely everted and in 5 specimens measured 30-43 (35.5)
long by 42-50 (46.0) wide. The bursa appears to be associated with the posterior portion of the septa dividing the hindbody into the two large excretory spaces.

As has been observed by Faust (1922), Hughes (1927, 1928a,b), Hoffman (1960, 1967) and others, the excretory system of strigeoid metacercariae is composed of two parts; the primary excretory system consisting of the flame cells and their associated tubules, and the reserve excretory system. As in most neascus-type larvae, the primary excretory system of *O. ptychocheilus* is obscured by the thickness of the worm and by the development of the reserve system. During this study, a few scattered flame cells and tubules were observed but not in sufficient number or detail to permit one to determine the arrangement of the primary excretory system.

The reserve excretory system (Figure 23) consists of a network of large and small vessels; the smaller are somewhat variable, the larger show some degree of regularity. In the forebody, these vessels contain a clear fluid in which are suspended spheroidal calcareous corpuscles. This fluid and contained corpuscles flows from one body region to another in response to muscular movements.

The principal vessels of the reserve excretory system are essentially the same as those described by Hughes (1928a) in *N. van-cleavei* and Hughes and Piszczek (1928) in *N. ptychocheilus*. The terminology employed for the vessels of the reserve excretory system is that of Hughes (1928a). The principal longitudinal vessels of the forebody include: (1) a single median dorsal vessel extending from a region immediately posterior to
the pharynx to the posterior margin of the forebody, where it appears to end in a bulbous expansion; (2) paired intra-lateral vessels located lateral to the median dorsal vessel and paralleling it; and (3) paired primary lateral vessels located laterally to each intra-lateral vessel. Just posterior to the oral sucker, the median dorsal vessel is connected with each intra-lateral vessel by 3 anterior transverse commissural vessels. Hughes (1928a) suggested that 4 pairs were the normal condition in N. van-cleavei; however, Hughes and Piszczek (1928) indicated 3 pairs in the case of N. ptychocheilus. My observations agree with the latter authors, although the middle pair of transverse commissural vessels appears, at times, to be incomplete. The median dorsal vessel is also connected to each intra-lateral vessel by a single posterior transverse commissural vessel at a level just posterior to the acetabulum.

The intra-lateral vessels are small in comparison to the primary lateral vessels. Lateral to the primary vessels are irregular networks of spaces which have been referred to as extra-lateral networks (Hughes, 1928a). The primary lateral vessels are connected with their respective intra-lateral vessels and extra-lateral networks by numerous irregular anastomoses.

Posteriorly, intra-lateral vessels and the primary lateral vessels are collected into a large dorsal lateral collecting vessel and a small marginal vessel. The latter courses along the posteroventral margin of the forebody. A short, broad median ventral vessel is formed by the union of the lateral collecting vessels and is located between the tribocytic organ and the posterior margin of the forebody. The median
dorsal vessel does not divide to form a ring around the ventral sucker and tribocytic organ as has been reported in *N. ambloplitis* (Hughes, 1927), but sends off two fine branches appearing to anastomose with the lateral collecting vessels or the median ventral vessel as has been reported in *N. van-cleavei* (by Hughes, 1928a) and *N. ptychocheilus* (by Hughes and Piszczek, 1928).

In the hindbody, the excretory vessels are very large and appear to consist of two large, lateral excretory "spaces" divided by a median septum and filled with spheroidal concretions much larger than the calcareous corpuscles. These larger concretions are, apparently, made up of a heavy gelatinous fluid similar to that reported by Hughes (1928a). Under coverslip pressure, some of these concretions were released from the excretory pores of living specimens. Freed in this manner, they remain intact for only a short time and then disintegrate.

Hughes (1928a) suggested that in *N. van-cleavei*, the median ventral vessel divided upon passing into the hindbody, giving rise to distinct vessels in the latter so that larger concretions were in definite rows. Neither the division of the median ventral vessel, nor the definite channels in the hindbody could be observed in the present study. The metacercariae of *O. ptychocheilus* examined here appear to have concretions of the hindbody arranged irregularly in the two excretory "spaces" mentioned previously.

The metacercaria of *O. ptychocheilus* has been described previously by Faust (1917, 1918) and Hughes and Piszczek (1928). The only other metacercaria from this genus which has been described is that of
O. scardinii described by Kozicka (1960) and Sudarikov and Kurochkin (1968). Mensural characters observed by these authors and those obtained in the present study are shown in Table 6.

Morphological, biological, and mensural characteristics leave little doubt that metacercariae examined in the present study and those of Hughes and Piszczeck (1928) are identical. Differences observed in measurements may be explained by differences in the condition of the worms when they were measured (alive, fixed, or stained and mounted).

Metacercariae of O. ptychocheilus have been reported from a variety of fish hosts and from primarily two locations within these hosts; in the cranial cavity and on the brain or in the mesenteries of the abdominal cavity (Table 1). Hoffman (1958a) has supported this lack of host and site specificity with life history studies. However, experimental studies conducted during the course of this investigation indicate otherwise (Table 7). Of 6 species of fish exposed to cercariae, 5 of which have been previously reported as suitable hosts, only Pimephales promelas and P. notatus became infected. Additionally, all metacercariae were recovered from the brain, cranial cavity, and rarely the eyes. It is interesting that 10 Semotilus atromaculatus, a species heavily infected with the abdominal form (according to Hoffman, 1958a), did not become infected under experimental conditions. It seems likely that there are several species, strains, or races, in the O. ptychocheilus complex which may vary in fish host(s), "preferred" site within the fish host, and/or host and site specificity. The evidence indicates that the strain used in the present study is both host and site specific. The
Table 6. Comparative measurements (micrometers) of metacercariae of the genus Ornithodiplostomum (ranges are given followed by means in parentheses)

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<tr>
<th></th>
<th>O. ptychocheilus</th>
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<th>O. scardinii</th>
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<tr>
<td></td>
<td>Hughes and Piszczek, 1928</td>
<td>Living Mounted</td>
<td>Sudarikov and Kurochkin, 1968</td>
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<td>Forebody length</td>
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<td>261-364(308.3)</td>
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<td>Hindbody length</td>
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<tr>
<td>width</td>
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<td>Acetabulum length</td>
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<td>width</td>
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<td>width</td>
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<td>15-19(16.7)</td>
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<td>Copulatory bursa</td>
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<tr>
<td>width</td>
<td>28-35(34)</td>
<td>42-50(46.0)</td>
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Table 7. Experimental exposures of fish to cercariae of *Ornithodiplostomum ptychocheilus*

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<th>Species</th>
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<td><em>Catostomus commersoni</em></td>
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<td><em>Notropis cornutus</em></td>
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<tr>
<td><em>Notropis dorsalis</em></td>
<td>0/6</td>
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<tr>
<td><em>Pimephales promelas</em></td>
<td>86/86</td>
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<tr>
<td><em>Pimephales notatus</em></td>
<td>5/5</td>
</tr>
<tr>
<td><em>Semotilus atromaculatus</em></td>
<td>0/10</td>
</tr>
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</table>

The fact that the strains are morphologically indistinguishable is supported by the variety of hosts and sites given in the literature. I have observed metacercariae on the brain as well as in the abdominal cavity of Wyoming cyprinids; they appear morphologically indistinguishable (Hendrickson, 1978). Hoffman (1958a) apparently had a mixed infection or a nonspecific strain at the time he elucidated the life cycle.

Dubois (1938) suggested the larval genus *Ornithodiplostomulum* for metacercariae of the genus *Ornithodiplostomum* so as to differentiate them from metacercariae of the genus *Posthodiplostomum*, for which he proposed *Posthodiplostomulum*. On the basis of similar, if not identical, morphology and life histories such a division seems undesirable. The reduced size of the hindbody in "*Ornithodiplostomulum*" may be a valid diagnostic character, but is pronounced only in fixed specimens. The occurrence of neascus larvae on the brain cannot be relied upon as a
valid character until the taxonomic tangle of the O. ptychocheilus complex is unraveled. Thus, it is suggested that the larval genus Neascus Hughes, 1927 sensu latu be maintained.
MIGRATION

Longitudinal Migration

The longitudinal distribution of tailless cercariae within the fish host (*Pimephales promelas*) at various times after infection is shown in Table 8. The head of the fish includes regions 1, 2, and 3 (in part). Region 1 usually involves the olfactory bulb and lobe; region 2 contains the remainder of the brain. The percentages indicated in Table 8 are shown in Graph 1. Each histogram represents results obtained from a single fish host. All time periods given in the text refer to the time elapsed from the end of the 0.5 hr exposure until the fish was fixed.

Cercariae penetrated along the entire length of the fish, although a large percentage (57.8% at time 0) penetrated the head region (regions 1 to 3). Penetration generally occurred between the scales, at the bases of the fins and opercula, and other sites where the surface of the fish was irregular. Gills were not commonly penetrated. At 0 and 1 hr, most cercariae were near the external body surface regardless of the area penetrated.

The "preference" for the head region was not nearly so marked in *O. ptychocheilus* as has been observed for *Cercaria X*, *Cotylurus erraticus*, and *Diplostomum spathaceum* (Erasmus, 1959; Johnson, 1971; Ratanarat-Brockelman, 1974). This "preference" in *O. ptychocheilus* may be artificial and due to greater surface area and more surface irregularities (fins, opercula, eyes, etc.) in this area of the body. The marked "preference" of *Cercaria X*, *C. erraticus*, and *D. spathaceum* for penetration in the head region may be due to the fact that large numbers
Table 8. Distribution of developing metacercariae of O. ptychocheilus in longitudinal body divisions of Pimephales promelas at various intervals post-infection

<table>
<thead>
<tr>
<th>Time post-exposure (hours)</th>
<th>Percentage (number) of developing metacercariae in longitudinal body divisions of Pimephales promelas (Region 1 - anterior; Region 10 - posterior)</th>
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<td>72</td>
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Graph 1. Percentage of developing metacercariae of *O. ptychocheilus* in longitudinal body divisions of *Pimephales promelas* at various intervals post-infection.
of cercariae of these species penetrate the gills (Erasmus, 1959; Johnson, 1971; Ratanarat-Brockelman, 1974). Donges (1964) demonstrated that cercariae of *Posthodiplostomum cuticola* were stimulated to swim actively when water was turbulent, particularly near the gills and bases of the fins. Such turbulence allowed swimming larvae to contact the host more readily. Erasmus (1959) suggested that many *Cercaria X* were drawn in with respiratory currents and subsequently penetrated the pharynx.

The percentage of cercariae in different regions changed markedly with increased time (Graph 1). Data obtained 1 hr post-infection suggest a short orientation period following penetration involving posterior movement to some extent. However, from 2 to 72 hrs the percentages in region 2 increased with corresponding decreases in other regions. These rapid changes can be explained only on the basis of relatively well-oriented movement, primarily directed toward the anterior end of the fish. By 48 hrs, longitudinal migration was essentially complete.

Similar patterns of longitudinal migration by strigeoid cercariae in fish have been observed by Erasmus (1959), Johnson (1971), and Ratanarat-Brockelman (1974). These investigators indicated that longitudinal migration was essentially complete within 24 hrs for *Cercaria X*, 8 hrs for *C. erraticus*, and 4 hrs for *D. spathaceum*. Longitudinal migration in *O. ptychocheilus* apparently occurs at a slower rate than in some other strigeoid cercariae, possibly because of the rather diffuse pattern of penetration throughout the length of the
fish. Because gills are not penetrated by cercariae of *O. ptychocheilus*, larvae of this species penetrate regions distant from the head and migration time is consequently increased.

Changes in Cercarial Distribution in Tissues and Organs

The distribution of cercariae in various tissues and organs varied markedly with time (Table 9) in experimental infections. Some of these data are shown in Graph 2. Most cercariae observed 0 or 1 hr post-infection were in the integument (epidermis (Figure 24), dermis, and subdermal connective tissue - 48.5%) or fins (16.0%). Cercariae observed in the fins were generally in the epidermis or in the connective tissue between the rays. Gills were only rarely penetrated (2.2% at 0 hrs - Figure 26).

By 1 to 2 hrs, cercariae had migrated into the deeper tissues, primarily body musculature (Figure 25) and connective tissues (41.7%) (Figure 27). Only 11% remained in the integument after 2 hrs. This suggests that *O. ptychocheilus* cercariae do not migrate on the external body surface. Erasmus (1959) criticized Ferguson (1943a) for failing to take an external migration of *D. spathaceum* into account.

At 2 to 8 hrs, a large percentage of cercariae (41.8%) were within the cranial nerves and ganglia (Figure 28), spinal nerves and ganglia (Figures 29, 30), and in the neural canal and spinal cord (Figures 33-35). Cercariae observed in nerves or ganglia were almost always in close proximity to the central nervous system, the one exception being a
Table 9. Distribution of developing metacercariae of *O. ptychocheilus* in various tissues or regions of *Pimephales promelas* at various intervals post-infection (abbreviations: Int. - integument (includes subdermal connective tissue); B.M. - body musculature; F.M. - fin musculature; G.C. - general connective tissue; F.C. - fin connective tissue; Br. - brain; S.C. - neural canal and spinal cord; N.&G. - nerves and ganglia; S.O. - sense organs; Oth. - other locations)

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Graph 2. Percentage of developing metacercariae of *O. ptychocheilus* in various tissues or regions of *Pimephales promelas* at various intervals post-infection.
Graph 2. Percentage of developing metacercariae of *O. ptychocheilus* in various tissues or regions of *Pimephales promelas* at various intervals post-infection.
Integument

Connective Tissue and Musculature

Neural Canal and Spinal Cord

Brain

Time Post-Exposure (Hours)
considerable number of cercariae observed at various times along the entire length of the vagus nerve (Figure 31). Cercariae were usually within nerve tissue, but in a few cases appeared to be following the nerves along their surfaces. Most cercariae within the vertebral column were in the neural canal between neural arches and spinal cord (Figure 35). However, some cercariae were within the tissues of the cord (Figures 33, 34). The fact that some of these appeared to be partially in the canal and partially within the cord made precise localization difficult.

Percentages of cercariae in the neural canal and spinal cord peaked at 46.0% at 3 hrs, but remained at high levels until 24 hrs post-infection. These high values suggest that most cercariae arrive at the brain via the neural canal and spinal cord. Much variation regarding the time required to reach and/or traverse the neural canal and spinal cord is indicated by the fact that cercariae were observed in these regions from 1 to 72 hrs post-infection.

Cercariae were first observed in the brain at 1 hr. Generally, percentages increased with time and the migration was essentially complete by 48 hrs (Figures 36, 37). These data further support the hypothesis that migration of *O. ptychocheilus* is relatively slow in comparison to that of some other strigeoids.

Only a single cercaria was observed in the abdominal cavity. It was in the lumen of the stomach with tail intact, and undoubtedly had been swallowed. Of the cercariae recovered from the sense organs, one was in the membranous labyrinth of the ear; the remainder were in the various regions of the eye (Figure 32).
The increases in percentages of cercariae in the neural canal and spinal cord and, finally, brain were accompanied by corresponding decreases in other tissues or organs. The decline in percentage in the integument was very rapid and penetration of this layer was essentially complete by 2 hrs. Declines in percentages in the body musculature and connective tissues occurred somewhat later, but were equally apparent.

Variation is evidenced by the fact that cercariae were observed in the integument and body musculature after 12 hrs, in the body connective tissue after 24 hrs, and in the neural canal and spinal cord after 72 hrs. This may have resulted from variation in rates of movement of individual cercariae, from different tissue types encountered, or to other factors.

Although all brain regions have been shown to be involved in migration and/or localization to some extent, there is some evidence to suggest that there is a selective localization within the brain with the majority of metacercariae occurring in the optic lobes and cerebellum (Table 10). Estimates made 1 week after infection further support this hypothesis: olfactory bulb - 0.6%, olfactory lobe - 3.3%, optic tecta - 53.8%, cerebellum - 20.6%, hypothalmus - 0.3%, medulla oblongata - 5.3%, brainstem - 15.9%, neural canal and spinal cord - 0.3%. Because cercariae were so large by 1 week, estimates were made through examination of every fifth section and by counting the cercariae in the various brain regions. Some cercariae were undoubtedly counted more than once, but this technique should indicate an approximate percentage of cercariae
Table 10.  Percentage of developing metacercariae of *O. ptychocheilus* in regions of the nervous system of *Pimephales promelas* at various intervals post-infection (brainstem includes all regions of the brain which could not be morphologically distinguished from the brainstem proper such as tegmenta of the mesencephalon and thalamus)

<table>
<thead>
<tr>
<th>Time post-exposure (hours)</th>
<th>Olfactory bulb</th>
<th>Olfactory lobe</th>
<th>Optic tecta</th>
<th>Cerebellum</th>
<th>Hypothalamus</th>
<th>Medulla oblongata</th>
<th>Brainstem</th>
<th>Neural canal and spinal cord</th>
<th>Cranial nerves and ganglia</th>
<th>Spinal nerves and ganglia</th>
<th>Other nervous system</th>
<th>Number of cercariae in nervous system/total number observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>1.0/1/90</td>
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<td>1</td>
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<td></td>
<td></td>
<td></td>
<td>0.9/11/116</td>
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<td>9.2/66/87</td>
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<td>28.3/86/106</td>
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<td>12</td>
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<td>65.2/103/107</td>
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<td>24</td>
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<td>14.8/76/81</td>
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<td>48</td>
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<td></td>
<td></td>
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<td></td>
<td>21.4/72/92</td>
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<tr>
<td>72</td>
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<td></td>
<td></td>
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<td>30.8/117/117</td>
</tr>
</tbody>
</table>
in each brain region. This "preference" for the optic lobes and cerebellum may be artificial and merely a result of the fact that these two areas and the brainstem are the largest regions of the fathead minnow brain.

It remains a possibility that developing metacercariae do not remain in a given region but continue to wander through the brain. The "preference" for the optic lobes and cerebellum, if not artificial, may be due to more arrivals than departures.

Rate of Migration

Results obtained from caudal fin immersion exposures at 4 and 24 hrs post-infection are shown in Table 11. Cercariae are capable of migrating almost the entire length of the fish, from caudal fin to brain, within 24 hrs. Although no fish were examined between 4 and 24 hrs, the fact that after 4 hrs a number of cercariae were already in cranial ganglia in close proximity to the brain suggests that the time required to reach the brain is approximately 4 hrs. Ratanarat-Brockelman (1974) has shown that in a similar situation, 40% of cercariae of *D. spathaceum* traveled from caudal fin to eyes in 4 hrs after infection.

It appears that some cercariae penetrating the caudal fin can move to the brain more rapidly than some of those penetrating much closer to the brain (it should be recalled that in total immersion experiments, only 66.7% of the cercariae were in the brain after 24 hrs). This might be explained as follows: Once the neural canal has been reached, migration proceeds quite rapidly. Cercariae penetrating the caudal fin
Table 11. Rate of travel of cercariae observed in fish fixed 4 (FHM-86) and 24 (FHM-84) hours after infection

<table>
<thead>
<tr>
<th>Cercaria Number</th>
<th>Distance Traveled (mm)</th>
<th>Approximate Rate of Travel (mm/hr)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHM-86-1</td>
<td>16.5</td>
<td>4.1</td>
<td>Vagus nerve</td>
</tr>
<tr>
<td>FHM-86-2</td>
<td>12.0</td>
<td>3.0</td>
<td>Vagus nerve</td>
</tr>
<tr>
<td>FHM-86-3</td>
<td>7.8</td>
<td>2.0</td>
<td>Vagus nerve</td>
</tr>
<tr>
<td>FHM-86-4</td>
<td>22.0</td>
<td>5.5</td>
<td>Cranial ganglion</td>
</tr>
<tr>
<td>FHM-86-5</td>
<td>22.5</td>
<td>5.6</td>
<td>Cranial nerve</td>
</tr>
<tr>
<td>FHM-86-6</td>
<td>2.5</td>
<td>0.6</td>
<td>Spinal nerve</td>
</tr>
<tr>
<td>FHM-86-7</td>
<td>5.0</td>
<td>1.3</td>
<td>Spinal nerve</td>
</tr>
<tr>
<td>FHM-86-8</td>
<td>5.9</td>
<td>1.5</td>
<td>Neural canal</td>
</tr>
<tr>
<td>FHM-86-9</td>
<td>3.8</td>
<td>1.0</td>
<td>Neural canal</td>
</tr>
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<td>FHM-86-10</td>
<td>6.0</td>
<td>1.5</td>
<td>Neural canal</td>
</tr>
<tr>
<td>FHM-86-11</td>
<td>2.3</td>
<td>0.6</td>
<td>Caudal fin</td>
</tr>
<tr>
<td>FHM-86-12</td>
<td>6.8</td>
<td>1.7</td>
<td>Neural canal</td>
</tr>
<tr>
<td>FHM-86-13</td>
<td>6.0</td>
<td>1.5</td>
<td>Spinal cord</td>
</tr>
<tr>
<td>FHM-86-14</td>
<td>5.0</td>
<td>1.3</td>
<td>Vagus nerve</td>
</tr>
<tr>
<td>FHM-86-15</td>
<td>21.8</td>
<td>5.5</td>
<td>Cranial nerve</td>
</tr>
<tr>
<td>FHM-86-16</td>
<td>11.0</td>
<td>2.8</td>
<td>Vagus nerve</td>
</tr>
<tr>
<td>FHM-86-17</td>
<td>12.0</td>
<td>3.0</td>
<td>Vagus nerve</td>
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<tr>
<td>FHM-86-18</td>
<td>10.5</td>
<td>2.6</td>
<td>Vagus nerve</td>
</tr>
<tr>
<td>FHM-84-1^</td>
<td></td>
<td></td>
<td>Optic lobe</td>
</tr>
<tr>
<td>FHM-84-2^</td>
<td></td>
<td></td>
<td>Cerebellum</td>
</tr>
<tr>
<td>FHM-84-3^</td>
<td></td>
<td></td>
<td>Cerebellum</td>
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<tr>
<td>FHM-84-4^</td>
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<td></td>
<td>Optic lobe</td>
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<tr>
<td>FHM-84-5^</td>
<td></td>
<td></td>
<td>Optic lobe</td>
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</tbody>
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^These cercariae were already in the brain and had, presumably, been there for some time. Thus, the rate of travel could not be accurately determined.
reach the canal almost immediately. Cercariae penetrating further anteriad have a shorter distance to travel to the brain, but require a longer period of time to reach the neural canal.

The actual rate of migration was determined to be from approximately 1 to 6 mm per hour. There are indications that rate of travel, however, is highly dependent upon the tissue type(s) traversed. Erasmus (1959) observed migration of Cercaria X in the connective tissue between the rays of isolated caudal fins and observed that they traversed a distance of 0.4 mm in about 8 minutes (3 mm per hr). Ratanarat-Brockelman (1974) indicated that the maximum rate of migration of cercariae of Diplostomum spathaceum was at least 5.1 mm per hr.

The maximum distance traveled by a single cercaria (FHM 86-5) was between 20 and 25 mm. If cercariae can migrate at an average rate of 3.5 mm per hr and can travel at least 22 mm, they must have sufficient energy reserves to sustain migratory activity for at least 6 hrs. The data (Table 8, Graph 1, Table 9) suggest that migratory activity may be sustained much longer, perhaps up to 48 hrs post-infection.

Success of Migration

Data shown in Table 9 suggest that the success rate of migration is very high. Forty-eight hrs after experimental infection, all observed cercariae were in the brain, and after 72 hrs, 98.3% had reached this site. It seems likely that in nature where small numbers of cercariae probably penetrate at any given time throughout the life of the fish host, they are equally efficient. The inhibition of metacercarial
development by the simultaneous exposure of fish to many cercariae has been observed by many authors. Hoffman (1958a) termed this the "crowding effect".

Ratanarat-Brockelman (1974) attempted to correlate the success rate of migration with the number of cercariae exposed to an individual fish at a given time. Although his table (Table 1, p. 125) is somewhat unclear, it appears that with increasing numbers of cercariae, an increasing percentage successfully penetrated the fish. When fish were exposed to 30-100 cercariae, the percentage recovered from the lenses 24 hrs post-infection remained relatively constant (96.7-100%).

Route of Migration

Various authors (Wesenberg-Lund, 1934; Davis, 1936; Ferguson, 1943a; Miller, 1954; Hoffman and Hundley, 1957; and others) have suggested the bloodstream as the main route of migration of strigeoid trematodes. This hypothesis has been based largely on the rapid rates of migration observed in these trematodes. Most authors indicated that such rates could be explained only by cercariae being passively carried in the bloodstream. Some work has supported this view (Ferguson, 1943a; Hoffman and Hoyme, 1958; Johnson, 1971), but Erasmus (1959) and Ratanarat-Brockelman (1974) have shown that Cercaria X and cercariae of D. spathaceum respectively, migrate principally via the subcutaneous tissues (connective tissues and muscles).

The evidence suggests that migration of cercariae of O. ptychocheilus to the brain of Pimephales promelas takes place in a well-ordered manner
by means of a clearly definable route, with cercariae entering the brain via either the neural canal or spinal cord, or the cranial nerves and their foramina. The route is sufficiently well-defined that it can be concluded that migration in this species is by directed, nonrandom movement. The high success rate of migration further supports this hypothesis.

Penetration of the epidermis occurs rapidly and is essentially complete by 2 hrs. The occurrence of cercariae in the integument up to 12 hrs post-infection (Table 9) suggests that cercariae can remain and probably migrate in this layer for extended periods.

Migration to the nervous system occurs almost exclusively via the general body musculature and connective tissues, although a few cercariae gain direct access to the nervous system via the eyes. A few remain in the eyes, eventually encysting as Hendrickson (1978) and others have reported.

The low percentages of cercariae recovered from the peripheral nerves may suggest that cercariae become associated with them only where the latter are in close proximity to the central nervous system. Cercariae remain associated with nerves only long enough to follow them through the foramina of the vertebral column or cranium. Hence, they are rarely observed within or upon the nerves. It seems unlikely that cercariae enter the neural canal or brain by any route other than the nerve foramina. It is unlikely that they are capable of penetrating bone or dense cartilage. Additionally, a single cercaria was observed in section passing through the foramen of a spinal nerve. Its anterior end, in the neural canal, was directed toward the brain; its posterior end trailed in the
tissue of the spinal nerve (Figure 30).

If cercariae become associated with a cranial nerve, they continue to the brain. Cercariae associated with spinal nerves, however, must journey up the neural canal and/or spinal cord to the brain. The evidence suggests that the great majority of cercariae arrives at the brain via the neural canal and spinal cord. At 3 hrs, 40 (46.0%) of 87 cercariae observed in one fish were in the canal or cord. In addition, 14 (16.1%) were already in the brain and it seems reasonable to assume that half of these arrived via the same route. Furthermore, it seems likely that at least half of the remaining 33 cercariae will use this route as well. This gives a theoretical total of 64 (73.6%) cercariae employing this route. Studies on the rate of migration refute the possible argument that movement along the neural canal is very slow, with this accounting for the high percentages of cercariae observed in this region from 3 to 24 hrs.

Within the brain, the majority of developing metacercariae occurs in the optic lobes and cerebellum. This holds true from 48 hrs to 1 week post-infection. Whether this is "selective localization" or merely due to the larger size of these brain regions could not be determined.

Discussion

Migration of O. ptychocheilus cercariae to the brain of the fish intermediate host represents directional, nonrandom movement. The fact that the migratory route of this species differs markedly from those of some other members of the Diplostomatidae (Cercaria X, Diplostomum
spathaceum) suggests that patterns of migration may be species specific. This does not seem unreasonable when one considers the wide range of hosts and habitats within these hosts occupied by metacercarial stages of species assigned to this family. Data on theories regarding the migrations of cestodes or nematodes, therefore, cannot necessarily be applied to trematodes and vice versa.

This well-ordered migration of *O. ptychocheilus* suggests a long period of coevolution between host and parasite. The host, no doubt, provides the stimuli and the parasite responds to them. Thus, both host and parasite are, in effect, involved in the migration. Patterns of migration can, therefore, be expected to vary when either the host (stimuli) or parasite (responses) are changed.

The unusual theory of Szidat (1969) may explain how cercariae of *O. ptychocheilus* "find their way" to the brain. It may be, however, that natural selection has favored those larvae of this species traveling via the nervous system to the exclusion of other routes. It would seem that the ability to invade the nervous system and reach the brain is, by this time, part of the genetic makeup of this parasite.

A further question to be answered is why does *O. ptychocheilus* invade the nervous system? Other trematode larvae invade sites favoring continuation of their life cycles (e.g. **Diplostomum spathaceum** in the eye lens) and this factor may be of importance for *O. ptychocheilus* as well. However, mergansers, natural definitive hosts of this parasite, generally ingest whole fish and no behavioral changes in infected fish have, as yet, been demonstrated. Further studies, both behavioral and
physiological, on this intriguing problem are called for.

Most authors have referred to movements of parasites within their hosts as migrations. Crompton (1976), apparently influenced by Kennedy (1975), used the term "emigration" for journeys from a place of entry to a site in a host or when a change of site occurs within a host. He reserved "migration" for movements involving phases of coming and going between or within sites. Odum (1971) has defined population dispersal as (p. 200),

... the movement of individuals or their disseminules or propagules (seeds, spores, larvae, etc.) into or out of the population or population area. It takes three forms: 
- emigration - one-way outward movement; 
- immigration - one-way inward movement; and 
- migration - periodic departure and return.

The fact that release of trematode cercariae from a molluscan host and their subsequent penetration into a second intermediate host is a means of dispersal in space seems clear (Kennedy, 1975). What remains unclear is the fact that after penetration, aggregation of cercariae within a specific region(s) of the host also represents population dispersal. The movements involved in this aggregation are one-way directional movements into a population area and should, therefore, be termed immigrations.

Ulmer (1971), Holmes (1973), Crompton (1973, 1976), and others have used the term "site" to denote the "place" in the host in which a parasite is found. Holmes likened sites to microhabitats. Croll (1976) criticized the use of "site" and discussed the merits of "habitat" instead. Croll's arguments were based on the fact that adult nematodes, *Nippostrongylus brasiliensis*, dispersed in the rat intestine with respect to the presence of food in the lumen and the condition of the mucosa. If this is the
case, he argued (p. 446),

... the 'site' is not measureable in linear units alone, neither is it a 'position', or 'niche'... It is however, a 'habitat'; habitats are the result of dynamic environmental interactions.

Croll further suggested,

The habitat of an earthworm is not described in centimeters below the soil, nor a barnacle's in centimeters up a cliff-side -- both are understood in units of environmental variables: pH, redox potential, water availability, nutrient, osmotic pressures, temperature and so on.

It appears that Croll has likened "habitat" to the multidimensional hypervolume of Hutchinson (1958) and has then suggested that the habitat of a parasite can be measured in centimeters along the length of the intestinal tract!

Odum (1971) has defined "habitat" as the place where an organism lives or the place where one would go to find it. Andrewartha and Birch (1954) considered a "habitat" to be an area which seems to possess a certain uniformity with respect to some quality which the investigator decides is important in his study. In a parasitological sense, "habitat" has been equated with the organ of the host in which the parasite lives such as the small intestine (Noble and Noble, 1976; and others). On the basis of Andrewartha and Birch, Crompton (1976) suggested that each organ system of the body might be assigned the status of habitat with the physiological function of an organ system of the host providing the necessary uniformity.

Croll's criticism of the term "site" is warranted as this term, parasitologically speaking, is undefined and may be used with reference
to the host (e.g. mouse), organ system within a host (e.g. mouse digestive system), organ in a host (e.g. mouse small intestine), portion of an organ in a host (e.g. mouse duodenum), or location within a portion of an organ in a host (e.g. first centimeter of mouse duodenum). Thus, "site" tells little if not defined by individual authors. The absence of any ecological ties to this term may be its most desirable characteristic. Croll's arguments in favor of "habitat" also seem valid, but his concept of habitat appears far too narrow to be of practical use.
SUMMARY AND CONCLUSIONS

1. The life cycle of Ornithodiplostomum ptychocheilus has been examined experimentally.

2. Experimental adults were reared in unfed chicks, domestic ducks, nestling English sparrows, and laboratory mice. In chicks, the parasites are most often encountered in the posterior duodenum and anterior ileum of the small intestine.

3. Eggs, passed with chick feces, hatch in water after 8-9 days incubation at 30°C. Miracidia emerge and penetrate the snail Physa gyrina. Morphologically, O. ptychocheilus miracidia are "typical" for members of the family Diplostomatidae.

4. Two generations of sporocysts occur in the hepatopancreas of the snail host. Mother sporocysts release daughter sporocyst embryos at 9-12 days post-infection and daughter sporocysts begin to shed cercariae at 22-32 days post-infection at 22°C.

5. Cercariae belong to the Rhabdocaeca group of furocercariae and are morphologically similar to other members of this group. Cercariae penetrate fathead minnows and migrate to the brain.

6. Developing larvae remain within the brain tissues for a short time but eventually encyst on the brain's surface as neascus-type metacercariae. Experimentally reared metacercariae develop into gravid adults when fed to chicks.
7. The migration of cercariae to the brain of the fish intermediate host was examined experimentally. Cercariae penetrate between the scales, at the bases of the fins and opercula, and other areas where the surface of the fish is irregular. Cercariae only rarely penetrate the gills.

8. Cercariae penetrate along the entire length of the fish host, although a large percentage penetrates in the region of the head. With increased time following infection, the percentage in the head increased with corresponding decreases in other regions of the body. Migration to the head was essentially complete by 48 hrs post-infection.

9. The distribution of larvae in various tissues and organs also varied markedly with time. Most larvae observed 0 or 1 hr post-infection were in the integument or fins. By 1 to 2 hrs post-infection, they had migrated into deeper tissues, primarily body musculature and connective tissues.

10. Data suggest that cercariae gain access to the central nervous system via the peripheral nerves and their associated foramina, although they appear to remain in the peripheral nerves for only a short time.

11. If cercariae become associated with cranial nerves, they continue to the brain. Those becoming associated with spinal nerves, however, must journey up the neural canal and/or spinal cord to the brain. The high percentages of larvae observed in the neural
canal and spinal cord can only suggest that most arrive at the brain via this route.

12. Larvae were first observed in the brain at 1 hr post-infection. Generally, percentages in the brain increased with time post-infection, and migration was essentially complete by 48 hrs post-infection. All brain regions are, to some extent, involved in the migration and localization of *O. ptychocheilus*.

13. When cercariae penetrated only in the caudal fin, they were still able to complete migration to the brain within 24 hrs. Data suggest that the actual time required to travel from caudal fin to brain is just over 4 hrs. The actual rate of migration was from approximately 1 to 6 mm per hr.

14. The success rate of migration is extremely high. Under experimental conditions, nearly all penetrating cercariae are able to complete the migration to the brain.

15. Migration of *O. ptychocheilus* to the brain of the fish intermediate host is directional, nonrandom movement. It proceeds in a well-ordered and predictable fashion. Because the migratory route of this species differs markedly from related species, patterns of migration may be species specific.


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ACKNOWLEDGMENTS

I express my sincere gratitude to Dr. Martin J. Ulmer for his guidance and encouragement during this study, but it is his personal warmth and friendship that will be longest remembered.

Thanks are also due to Dr. Glenn L. Hoffman and Normal R. Hines of the United States Fish and Wildlife Service. The former aided in a number of aspects of this study and allowed me to share his knowledge and enthusiasm for the study of fish parasites during a two week visit to his laboratory and home. The latter provided uninfected fathead minnows used throughout the course of this study and kindled my early interest in fish culture and its problems.

Appreciation is expressed to my fellow graduate students whose enthusiasm for parasitology was always contagious and whose willingness to share their ideas never diminished. I am especially indebted to Darwin Wittrock and Sam Loker for training in photography and histological techniques.

Chicks were graciously supplied by Hy-Line Hatchery, Spencer, Iowa, and by Bonnie Shearer, Veterinary Medical Research Institute, Ames.

I am especially grateful to the people of the state of Iowa for their continued, albeit unknowing, financial support via the Graduate College, Iowa State University.

I gratefully acknowledge the constant support and encouragement of my parents who have given much but asked little in return.
My wife Susan has made my graduate education all worthwhile. Her love and enthusiasm for my work have made the completion of this dissertation a relatively easy task.
PLATES
Abbreviations

A - acetabulum
AG - apical gland
AO - anterior organ
AP - apical papilla
AR - acetabulum rudiment
AT - anterior testis
ATC - anterior transverse commissural vessel
B - body
BC - bursa copulatrix
BS - brainstem
C - caudal sac (posterior glandular body)
CB - caudal body
CBL - cerebellum
CG - cerebral ganglion
CGL - cephalic gland
CN - cranial nerve
CS - cercarial suspension
D - disposable pipette (cut)
E - eyespot
EC - epidermal cell
ELN - extra-lateral networks
F - furca
GC - germinal cell
GP - genital primordium
GT - glass tube
H - hypothalamus
ILV - intra-lateral vessel
LP - lateral papilla
M - medulla oblongata
MDV - median dorsal vessel
MVV - median ventral vessel
N - nonabsorbant cotton
NC - neural canal
O - ovary
OL - optic lobe
OLF - olfactory lobe
PG - penetration gland
PLV - primary lateral vessel
PT - posterior testis
PTC - posterior transverse commissural vessel
R - retina
RS - ringstand clamp
SC - spinal cord
SN - spinal nerve
SV - seminal vesicle
T - tribocytic organ
TS - tailstem
V - vagus nerve
VA - ventral aorta
VR - vitelline reservoir
Plate I

Fig. 1. Apparatus for exposing only the caudal fin of *Pimephales promelas* to cercarial penetration (X 0.80)
Plate II

Fig. 2. Diagram of experimentally developed adult *Ornithodiplostomum ptychocheilus* from unfed chick (*Gallus gallus*)
Plate III

Figs. 3-8. Egg development of *O. ptychocheilus* at 30°C (X 535)

Fig. 3. Freshly passed egg. Note operculum and absence of vitelline cell membranes

Fig. 4. After 1 day of incubation. Note well-defined vitelline cell membranes

Fig. 5. After 2 days of incubation. Note embryo near opercular end of the egg

Fig. 6. After 3 days of incubation. Note spherical vitelline cells

Fig. 7. After 4 days of incubation. Note large embryo situated near middle of egg

Fig. 8. After 4 days of incubation. Note presence of miracidial eyespots
Plate IV

Figs. 9-14. Egg development of *O. ptychocheilus* at 30°C (X 535)

Fig. 9. After 5 days of incubation. Note well-developed eyespots and vitelline cells. The latter are pushed to the periphery by growth of the embryo.

Fig. 10. After 6 days of incubation. Note well-developed miracidial features.

Fig. 11. After 6 days of incubation. Note reduced numbers of vitelline cells.

Fig. 12. After 7 days of incubation. Note reduced numbers of vitelline cells and the U-shape of the embryo.

Fig. 13. After 8 days of incubation and just prior to hatching. Note large size of miracidium, well-developed eyespots, and vitelline cells at periphery.

Fig. 14. After 8 days of incubation and just following hatching. Note operculum and residual material. The later appears cellular in part.
Plate V

Fig. 15. Diagram of the miracidium of *O. ptychocheilus*
Fig. 16. Diagram of the miracidium of *O. ptychocheilus* treated with silver nitrate to demonstrate arrangement of ciliated epidermal plates. Note that plates are arranged in 4 tiers in a 6:9:4:3 pattern.
Plate VII

Figs. 17-20. Sporocysts of *O. ptychocheilus* from experimentally infected *Physa gyrina*

Fig. 17. Mother sporocyst (arrow) in hepatopancreas 9 days post-infection (X 200)

Fig. 18. Mother sporocyst in mantle 21 days post-infection. Note presence of daughter sporocyst embryos (arrow) (X 200)

Fig. 19. Distribution of daughter sporocysts in hepatopancreas 10 months post-infection (X 50)

Fig. 20. Daughter sporocysts in hepatopancreas 10 months post-infection. Note presence of cercarial embryos (arrow) with penetration glands (X 200)
Plate VIII

Fig. 21. Cercaria of *O. ptychocheilus* showing body, tailstem, and furcae (X 210)
Plate IX

Fig. 22. Drawing of body and anterior tailstem of the cercaria of *O. ptychocheilus*
Plate X

Fig. 23. Drawing of the metacercaria (neascus) of *O. ptychocheilus* depicting the major vessels of the reserve excretory system. Metacercarial cyst not shown.
Plate XI

Figs. 24-27. Migrating cercariae of *O. ptychocheilus* in experimentally infected *Pimephales promelas* (X 190)

Fig. 24. Recently penetrated cercaria (arrow) in the integument (between epidermis and dermis). Note melanin containing cells apparently attacking cercaria (0 hrs post-infection)

Fig. 25. Cercaria (arrow) in dorsal musculature of caudal peduncle (1 hr post-infection)

Fig. 26. Cercaria (arrow) within blood vessel of gill arch. This was the only cercaria observed within the circulatory system (4 hrs post-infection)

Fig. 27. Cercaria (arrow) in loose connective tissue adjacent to ventral aorta. Note expansion of aorta to right of figure where it emerges from the heart (4 hrs post-infection)
Plate XII

Figs. 28-32. Migrating cercariae of O. ptychocheilus in experimentally infected Pimephales promelas (X 190)

Fig. 28. Cercaria (arrow) within or on the surface of a cranial nerve. Note close proximity to brain (2 hrs post-infection)

Fig. 29. Two cercariae (arrows) within or on the surface of a spinal nerve. Note close proximity to spinal cord (1 hr post-infection)

Fig. 30. Cercaria (arrow) passing through foramen of a spinal nerve (3 hrs post-infection)

Fig. 31. Cercaria (arrow) in or on the surface of the vagus nerve (8 hrs post-infection)

Fig. 32. Cercaria (arrow) partially within humor and partially within retina of the eye (1 hr post-infection)
Plate XIII

Figs. 33-35. Migrating cercariae of *O. ptychocheilus* in neural canal and/or spinal cord of experimentally infected *Pimephales promelas*

Fig. 33. Cercariae (arrows) 3 hrs post-infection. Note one cercaria within nervous tissue of the spinal cord (X 290)

Fig. 34. Cercariae (arrows) 8 hrs post-infection. Note one cercaria in neural canal; two, at least partially, within spinal cord (X 190)

Fig. 35. Cercariae (arrows) 16 hrs post-infection. Note that cercaria on right is directed posteriad (brain is to the left) (X 190)
Plate XIV

Figs. 36-37. Developing metacercariae of *O. ptychochailus* in brain of experimentally infected *Pimephales promelas* (X 66)

Fig. 36. Forty-eight hrs post-infection. Note cercariae (arrows) in optic lobe, cerebellum, medulla oblongata, and brainstem

Fig. 37. Seventy-two hrs post-infection. Note cercariae (arrows) in optic lobe, cerebellum, medulla oblongata, brainstem, and hypothalamus