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Neascus nolfi n. sp. (Trematoda: Strigeida) from Cyprinid Minnows with Notes on the Artificial Digest Recovery of Helminths

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Neascus nolfi n. sp. (Trematoda: Strigeida) from Cyprinid Minnows with Notes on the Artificial Digest Recovery of Helminths

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During an examination of fish from Turtle River, Arvilla, North Dakota, a new *Neascus* (larval strigeid) was recovered from the musculature and integument of the northern creek chub, *Semotilus a. atromaculatus* (Mitchell) and the northern common shiner, *Notropis cornutus frontalis* (Agassiz). This new species is named in honor of Dr. L. O. Nolf, University of Iowa.

Neascus nolfi n. sp.

The following description is based mostly on living material except for the measurements, which were all done on metacercariae freed from their cysts, fixed in hot AFA, and stained with celestin blue B.

The outer host cyst is heavily pigmented, is nearly spherical, and ranges from 472 to 576μ in diameter. The inner thin cyst, which is probably of parasitic origin, is also nearly spherical and measures between 202 and 324μ in diameter. It cannot be separated easily from the host cyst as is the case with *Uvulifer* (*Neascus*) *ambloplitis*. However, the metacercaria itself of *N. nolfi* is more easily freed from the cyst with the use of small forceps and needle.

The metacercaria, which is clearly divided into fore and hind bodies, measures 290-382μ (340 average of 4) in length and 137-153μ (147 average of 4) in greatest width. The forebody, which is thin, flat and leaf-shaped with posterior edges forming a shallow cup like most Diplostomatidae metacercariae, averages 229μ in length by 147μ in greatest width. In living worms, the great width of the forebody is very striking even while still in the cyst. The conical, rounded hindbody measures 61-107μ in length by 52-61μ in greatest width. The oral sucker is 44μ wide x 41μ long and faces anteroventrally. No prepharynx could be seen. The pharynx measures 13 x 12μ and the esophagus is 24 to 27μ long. The intestinal crura can be seen extending into the hindbody in stained specimens but the terminations could not be ascertained; they could not be seen at all in unstained worms. The ventral sucker, which is located about mid-way in the forebody, is 17μ in diameter. The round holdfast organ is approximately 55μ in diameter.

In no instances could a reserve bladder of the excretory system be discerned; this is unusual in a *Neascus*. There is little likelihood that these are "young" forms of other *Neascus* species in which the reserve bladder system has not yet developed, because the host cyst is already pigmented. In the only black-spot *Neascus* which has been studied experimentally, the metacercaria is fully developed by the time the cyst becomes melanated (Hunter and Hamilton, 1941).

No evidence of reproductive fundament or holdfast gland could be discerned.

Neascus nolfi differs from: (1) *Uvulifer* (*Neascus*) *ambloplitis* (Hughes, 1927) in the more spherical shape and larger size of the parasite cyst, smaller size of the metacercaria, lack of reserve excretory system and intolerance of pepsin solution; (2) *Crassiphiala* (*Neascus*) *bulboglossa* (Van Haitsma, 1925) in the round shape and smaller size of the parasite cyst, smaller size of the metacercaria, lack of reserve excretory system, and in possession of a ventral sucker; (3) *N. pyriformis* Chandler (1951) in the round shape of the parasite cyst, smaller size of the metacercaria, and lack of reserve excretory system; (4) *N. ellipticus* Chandler (1951) in the round shape and smaller size of the parasite cyst, smaller size of the metacercaria, lack of reserve excretory system, and in possession of

host cyst pigmentation; (5) *N. rhinichthysi* Hunter (1933) in the absence of a discernible reserve excretory system and the smaller size of the metacercaria.

The type specimen has been deposited in the United States National Museum Collection as No. 47898.

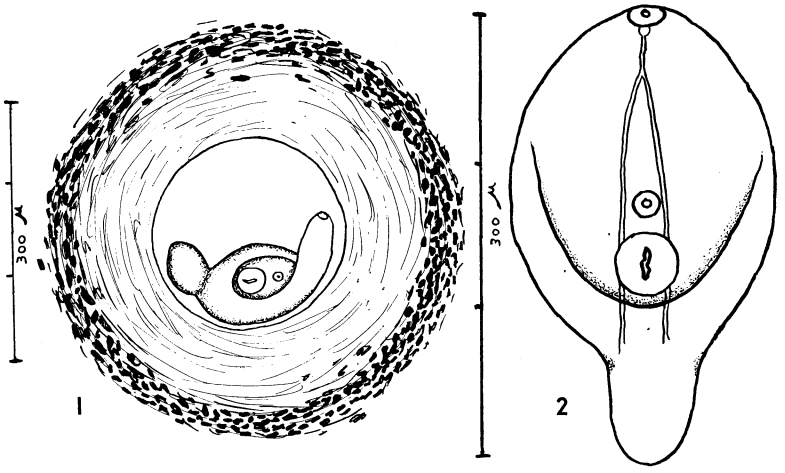
PEPSIN DIGEST OF FISH

Because *Neascus nolfi* and gasterostome metacercariae had been digested and overlooked during routine digest of fish tissue it was decided to compare three methods of isolating trematode larvae: (1) Routine pepsin digest followed by saline washings; (2) Waring blendor maceration of tissue followed by a very short pepsin digest and saline washings; (3) Waring blendor maceration followed only by saline washings.

The pepsin solution digest method of freeing encysted nematode and trematode larvae has been used by many workers and has been particularly useful in *Trichinella spiralis* studies. Its usefulness is great but since not all larvae can tolerate pepsin solution for very long, and some cannot tolerate it at all, it must be used with caution. Hemenway (1948), Hughes (1928c), and Wallace (1935, 1939) have used trypsin to assist metacercariae in excysting, and Lyster (1940) has used the plant proteolytic enzyme, papain, for liberating *Trichinella spiralis* larvae.

A water bath shaker was used which consists of a metal basket that fits inside the bath and is powered by a geared-down Kahn shaker. The unit makes 72 excursions per minute, and the digest bottles are held to the basket by natural rubber strips from discarded automobile inner tubes. A temperature of 39°C was chosen instead of 37°C because most of the worms recovered were those which develop to maturity in birds, and it was hoped that the 2°C increase would hasten digestion.

The digest solution consisted of 0.5% pepsin in 0.25% HCl made up in 0.65% 3-salt Ringer's (Parker, 1938). One percent HCl has been used by most workers, particularly for *Trichinella* digest, but I hoped to minimize the possibility of acid damage to the worms. I had previously found pH2 and



Figs. 1, 2.—1. Free hand drawing of living *Neascus nolfi* within the cyst.
2. Composite drawing of living, and fixed and stained *Neascus nolfi*.

lower to be inimical to the survival of the metacercariae of *Posthodiplostomum minimum* (Hoffman, 1950). Washings were made with 0.65% 3-salt Ringer's The digest solution was used in the approximate proportion of 20 cc of solution to 1 gram of tissue.

Routine digestion consisted of cutting the fish into small pieces and digesting until the material appeared all digested. Rapid digestion consisted of macerating the tissue for 1 minute in the Waring blendor and then digesting for 10 to 15 minutes; this was used for muscle and integument. For viscera, the Waring blendor was omitted. The parasites were allowed to settle and the solution was decanted, followed by three or four saline washings.

Neascus nolfi was first observed after the rapid digestion technique. Although metacercariae of the common black-spot *Neascus, Uvulifer ambloplitis,* were alive after this treatment, none of the new metacercariae were. The cysts remained intact, however, and the gross morphology of the larvae did not appear distorted. One to 8 minutes in the Waring blendor followed by saline washing with no pepsin digest yielded a few living metacercariae which appeared normal in all respects. It was extremely difficult to macerate the tissue just enough in the Waring blendor to free large numbers of the cysts but not to damage them; consequently, only a few could be recovered alive from each infected fish. It was impossible to determine accurately the number of larvae present or the number of fish infected except that they were far less numerous than *U. ambloplitis* in the final samples. During routine pepsin digestion of fish no *Neascus nolfi,* gasterostome metacercariae, or cestode larvae were recovered although it was known that all were present. Only a few *Posthodiplostomum minimum* metacercariae survived the routine method, although the fish were heavily infected. Nematode larvae remained alive and active. *Uvulifer ambloplitis* metacercariae survived this treatment, and many were active and appeared uninjured, but they failed to survive when transplanted into the muscle or visceral cavity of the same species of fish. When recovered from Waring blendor washings only, both *U. ambloplitis* and *N. nolfi* were alive 5 weeks after transplantation into fish of the same species.

In an attempt to free readily the intestinal nematode *Rhabdochona cascadilla,* and the metacercariae of *Posthodiplostomum minimum, Ornithodiplostomum ptychocheilus,* and other helminths present in the viscera, it was found that a 10 to 15-minute pepsin digest in a 39°C water-bath shaker was satisfactory. The intestine and stomach were slit open previous to digestion. Adult *Rhabdochona cascadilla, Phyllodistomum* spp., and *Allocreadium lobatum* were recovered intact and alive and freed of all mucus. The visceral metacercariae were alive and nearly all of them were freed from their cysts. Many other workers have used a pepsin digest technique for recovering larval helminths but, to my knowledge, there is no record of others using it to free adult helminths from the mucus and mucosa. This method proved more satisfactory for recovering intestinal helminths from mucus than the 0.7% $NaHCO_3$ method of Bangham (1951).

The following larval trematodes have been found to survive pepsin digest methods: *Amphimerus elongatus* (Wallace, 1939); *Apophallus venustus* (Cameron, 1945); *Caecincola parvulus,* 2 hrs. (Lundahl, 1941); *Clinostomum marginatum* (Hemenway, 1948; Nolf, 1952); *Diplostomulum corti* (Hoffman, 1953a); *Fibricola cratera, ibid; Metorchis conjunctus* (Cameron, 1945);

Ornithodiplostomum ptychocheilus (this paper); *Posthodiplostomum minimum* (Ferguson, 1940; Hoffman, 1950, 1953b; this paper); *Sellacotyle mustelae* (Wallace, 1935); *Uvulifer ambloplitis* (Nolf, 1952; this paper); An unidentified distome metacercariae in musculature of the brown bullhead (Hoffman, 1953a).

The following have been found not to survive pepsin digest methods: Gasterostome metacercariae, can be recovered dead after 10 min. digest (Hoffman, 1953a); *Linstowiella szidati,* can be recovered dead (Anderson and Cable, 1950); *Neascus nolfi,* can be recovered dead (this paper); an unidentified distome metacercaria in the mucosa of the creek chub can be recovered dead after 10 min. digest (Hoffman, 1953a).

KEY TO THE KNOWN LARVAL STRIGEIDS OF NORTH AMERICAN FISH*

1. Typical strigeid constriction lacking but holdfast present; no lateral pseudo-suckers or cotylae; no ventral sucker ..... Cyathocotylidae 2

   Typical strigeid constriction present although sometimes reduced; if no evidence of constriction there are lateral pseudo-suckers or cotylae 3

2. In flesh of cyprinids; cyst nearly round, about 180μ; adult exp. in unfed chicks (Anderson and Cable, 1950) ..... *Linstowiella szidati*

   In flesh of centrarchids; possibly adult in snakes; much like *L. szidati* above (also a *Prohemistomulum* in Europe; Ciurea, 1930) (Vernberg, 1952) ..... *Prohemistomum chandleri*

3. Encysted forms 4

   Not encysted; two lateral pseudo-suckers; hind body usually not apparent; usually in eyes, one sp. in musculature, one sp. in brain ..... *Diplostomulum* sp. and *Diplostomum* sp.[1]

   a. In lens of many spp. fish; adult in gulls (Hughes and Berkhout, 1929) ..... *Diplostomum flexicaudum*

   b. In musculature of Siluridae; large (1 mm); adults in cormorants and herons. (Ciurea, 1930; Hugghins, 1953) ..... (*Diplostomulum corti*) *Hysteromorpha triloba*

   c. In vitreous humor; over 3 times as long as broad (Hughes, 1929b) ..... *Diplostomulum scheuringi*

   d. In optic tecti and in cyst-like structure at posterior of optic tecti of *Eucalia inconstans* (Hoffman, 1953; to be described later) ..... *Diplostomulum* sp.

   e. In vitreous humor; less than 3 times as long as broad; adult in gulls ..... *Diplostomum huronense* and *Diplostomulum* spp.[2]

4. Two lateral cotylae (suckers); in pericardial cavity, visceral cavity, and eye muscle; cyst nearly round; in many spp. fish ..... *Cotylurus* (*Tetracotyle*) sp.

   Adult demonstrated in one instance in the intestine of gulls; known as *Cotylurus communis* (*Tetracotyle communis* Hughes, 1928). Hughes (1928a) described 3 other sp. of *Tetracotyle* but because of the difficulty in identifying spp. of this genus the metacercariae are known as *Tetracotyle* sp. unless shown by experimentation to be metacercariae of known spp. Further life history is needed on this group.

   No lateral cotylae 5

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* The references in parentheses are not necessarily those of the original author.

1 *Diplostomulum* denotes larval genus where adult is unknown; perhaps is the metacercaria of *Diplostomum* sp.

2 If a *Diplostomulum* is not readily identified as one of the first 4 spp. it is designated as *Diplostomulum* sp. Further life history work is needed to straighten out this group.

5. In cranial cavity of *Notropis cornutus frontalis* and *Pimephales p. promelas;* small oval cyst; adult in chick exp.; (Hoffman, 1954)....*Ornithodiplostomum ptychocheilus*

In viscera, mesenteries, peritoneum 6

In musculature and integument 7

6. Relatively short hindbody; constriction slight; relatively small (cyst 750μ); in mesenteries of Cyprinidae; adult in mergansers, squaw duck, and exp. in domestic duck and unfed chick (Hughes and Piszcek, 1928; Van Haitsma, 1930; Hoffman, 1954 .....*Ornithodiplostomum ptychocheilus*

Relatively large hindbody; constriction pronounced; much larger (metacercaria over 1 mm); adults in herons, exp. in unfed chicks .....*Posthodiplostomum* sp.

a. In liver, kidneys and on heart of centrachids (Ferguson. 1943: Hoffman, 1950; Hughes, 1928b; Van Cleave and Mueller, 1934:249) .....*P. minimum centrarchi*

b. In mesenteries of cyprinids (Hughes, 1928b; Hoffman, 1950) .....*P. m. minimum*

c. In mesenteries of *Umbra limi;* forbody very large; ventral sucker in center of forebody; adult unknown (Mueller and Van Cleave, 1932:93) .....*Neascus* (*Posthodiplostomum?*) *grandis*

7. Black pigment surrounding cyst 8

No black pigment surrounding cyst 13

8. Metacercaria nearly fills the parasite cyst 9

Metacercaria does not fill the parasite cyst 12

9. Parasite cyst pyriform 10

Parasite cyst oval or round 11

10. Parasite cyst about 330 x 200μ; in many fish; adult in kingfisher (Hughes, 1927) .....*Uvulifer* (*Neascus*) *ambloplitis*

Parasite cyst smaller (about 270 x 160μ), abruptly narrowed at one end; in perch; adult not demonstrated, possibly is *Uvulifer semicircumcisus* in kingfisher. (Chandler, 1951) .....*Neascus pyriformis*

11. No ventral sucker; reserve excretory system similar to that of *N. ambloplitis* but forebody greatly cup-shaped (Hughes, 1928c) ....*Crassiphiala* (*Neascus*) *bulboglossa*

Ventral sucker present; reserve excretory system similar to *N. ambloplitis* although simpler; in dace; adult unknown (Hunter, Wanda, 1933) .....*Neascus rhinichthysi*

12. Smaller (parasite 200 to 330μ); reserve excretory system indistinct; in *Semotilus a. atromaculatus, Notropis cornutus frontalis,* possibly others; adult unknown (this paper) .....*Neascus nolfi*

Quite large (parasite cyst 450μ long, metacercaria 870μ long); metacercaria with a finger-like anterior papilla; in perch; adult unknown (Chandler, 1951) .....*Neascus longicollis*

13. Oral sucker smaller than ventral sucker; parasite cyst 570μ long; metacercaria 760μ long; resembles *P. minimum* somewhat; in perch; adult unknown (Chandler, 1951) .....*Neascus ellipticus*

Oral sucker larger than ventral sucker; parasite cyst about 1 mm long; metacercaria about 1½ mm long; in perch, pike, cyprinids IN EUROPE, adult in pelicans, Europe and U.S. (Ciurea, 1930; Dubois, 1938:158) ....*Bolbophorus confusus*

I wish to thank Dr. Asa C. Chandler, Rice Institute, Houston, Texas, for reading the manuscript and for suggestions concerning the preparation of the above key.

SUMMARY

A new strigeid metacercaria, *Neascus nolfi,* from the creek chub, *Semotilus a. atromaculatus,* and shiner, *Notropis cornutus frontalis,* is described.

Neascus nolfi, gasterostome metacercariae, and a distome metacercaria in the stomach mucosa of the chub do not survive pepsin digest whereas *Uvulifer (Neascus) ambloplitis, Posthodiplostomum minimum, Ornithodiplostomum ptychocheilus, Diplostomulum corti, Fibricola cratera,* and an unidentified distome metacercaria in the musculature of the bullhead do survive for varying lengths of time. The adult helminths, *Phyllodistomum* sp., *Allocreadium lobatum,* and *Rhabdochona cascadilla* can be freed from mucus and mucosa by 10 to 15-minute exposure to pepsin solutions.

A key to the strigeid metacercariae of North American fish is presented.

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