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BY

J. O. HOBGOOD

BACHELOR OF ARTS OUACHITA COLLEGE ARKADELPHIA, ARKANSAS 1931

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APPROVED:

R. Chester Hughes — In Charge of Thesis

R. O. Whitenton — Head of the Department of Zoology

D. G. McIntosh — Dean of the Graduate School

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This paper presents an account of some penetration experiments with *Cercaria flexicorpa* Collins and a description of the metacercaria thus obtained.

In July, 1936, 27 of about 3000 specimens of *Helisoma trivolvis* (Say), collected from "Chandler Lake," a small old deep richly-faunal pond near Stillwater, Oklahoma, were found to harbor *C. flexicorpa*. The cercaria was studied in considerable detail, both alive and preserved; it agreed favorably in measurements and in anatomical minutiae (such as the flame-cell pattern) with the specimens (also taken in this community) from which the original description (Collins, 1935) was made. Particularly, the features cited by Collins to distinguish this form from its nearest relatives (*Cercaria hamata* Miller and *Cercaria bessiae* Cort and Brooks) were checked and confirmed. Collins expressed some doubt, however, concerning the distinctness of the three forms.

In July, 1936, about 100 small sunfish, *Apomotis cyanellus* (Rafinesque) were collected from a snail-free pond. Half of these were killed at once, carefully examined, and found to be apparently free from any trematode infections. The remaining fish were separated into two equal lots and kept in aquaria supplied with running water. One lot was used for experiments and the other kept for controls.

In order to have fresh cercariae in large numbers, the snails were changed to fresh water and placed in sunlight for about 3 hours before experiments. The fish were exposed to the cercariae daily from August 2 to 14 inclusive. For exposures the fish were removed to battery jars containing only water enough to cover them. The water was kept at about 25°C. and supplied with enormous numbers of cercariae. The periods of exposure ranged from 5 minutes to 4 hours. Spasmodic movements of the fish indicated severe irritation due supposedly to penetration by cercariae. Fish similarly placed in small quantities of warm water, but without cercariae showed much less agitation. None of the fish was killed by the exposures. McCoy (1928) working with *C. hamata* and Krull (1934) with "*C. bessiae*" both found that very small specimens of *Eupomotis gibbosus* (Linnaeus) often died within a few days after heavy exposures—the fishes in these cases, however, were smaller than my specimens. All efforts to actually observe a cercaria penetrate were in vain.

After August 14, the fish were left in the care of attendants for about

* Contribution from the Zoological Laboratory, Oklahoma Agricultural and Mechanical College, prepared under the direction of R. Chester Hughes.

8 weeks. On October 10 the experimental fish were all found to be very heavily infected with metacercariae in black-pigmented cysts. One fish harbored 1026 specimens. The control fish were all apparently entirely free.

Most of the cysts were readily visible through the skin of the living fish although they did not cause any external protrusions. The cysts were found everywhere in the skin, muscles, and connective tissue. They were most abundant at the bases of the fins and in the skin of the head. Some were found in the lining of the mouth and in the peritoneum and mesenteries.

The cyst consists of two parts, an outer ellipsoidal heavily-pigmented connective tissue cyst of host origin and an inner thin tough resilient transparent non-cellular capsule of parasite-origin. The latter (fig. 1) is ellipsoidal, slightly smaller at one end, and considerably flattened dorso-ventrally (with reference to the position of the closely invested worm). Measurements in microns: 7 outer cysts, length 598–904 (average 786), width 466–772 (average 572); 15 fresh inner cysts, free from pressure, length 345.5 to 418 (average 371), width, 172.8 to 252 (average 201). Three inner cysts, measured 120, 132, and 142 microns in thickness.

To free the parasite, intact and in good condition, from the inner cyst proved to be very difficult. In the most successful method the cyst is placed in a mere film of water on a glass slide. Then, under observation through a dissecting microscope, it is caught between the corrugated tips of a pair of forceps and gently squeezed. The limited amount of water reduces the tendency of the cyst to slip away from the instrument. About 20 per cent of the cysts thus opened yielded uninjured worms.

Within the inner cyst the parasite, usually exhibiting some movement, is folded, the fold running somewhat obliquely across the fore-body. The ends of the body are directed toward the smaller end of the cyst.

When the experimentally-raised parasites were removed from the cysts in October (at about 2 months of age) they showed considerable activity. This was not true of specimens collected from Chandler Lake in June, 1937. The latter were probably several months old. When freshly removed from cysts they generally showed little if any movement. Their internal structures were less clearly recognizable. They were more easily damaged by manipulation and cover-glass pressure. They seemed to be moribund. Indeed many of the outer cysts examined at this time contained only dead and disintegrating matter. Krull (1934: 69) working with a similar (perhaps identical) form, *Neascus ambloplitis* Hughes, in *Salvelinus fontinalis* (Mitchill) at Ann Arbor, Michigan, found that most of the specimens collected in June were degenerating.

After their removal from the inner cyst studies were made of both living and preserved specimens. The latter were fixed in hot sublimate-acetic and stained with borax carmine.

The Metacercaria

Description. Encysted strigeid larva of the neascus type with typical fore- and hind-bodies (fig. 2), separated by deep constriction. Axis of body, flexed dorsally at level of constriction. Fore-body thin, foliaceous, spoon-shaped, concave ventrally, widest at about two-thirds of length from anterior end. Hind-body ellipsoidal with maximum diameter posterior to middle. Entire animal apparently aspinous except on hold-fast organ. Subcuticular circular and longitudinal muscle fibers, typical.

Measurements in microns. Thirty living specimens at rest and not under pressure: fore-body 486 (338–627) long by 272 (230–446) wide; hind-body 421 (292–584) long by 212 (130–306) wide; oral sucker 95 (79–107) wide; pharynx 36 (21–49) wide; acetabulum 46 (29–53) wide; hold-fast organ 84 (61–100) wide. Ten preserved specimens mounted *in toto;* fore-body 311 (288–381) long by 210 (194–266) wide; hind-body 270 (247–321) long by 129 (116–161) wide; oral sucker 72 (61–87) wide; pharynx 18 (16–26) wide; acetabulum 39 (31–44) wide; hold-fast organ 49 (41–62) wide.

Oral sucker terminal, mouth ventro-terminal, wall of sucker conspicuously thin (about 21 microns thick in fixed specimens). No lateral suckers or lappets. Acetabulum small, weak, posterior to middle of fore-body. Hold-fast organ prominent, immediately posterior to acetabulum, its cuticle densely armed with minute spines. Adhesive gland small, transversely elongate, postero-dorsal to hold-fast organ.

Prepharynx practically wanting; pharynx prominent; esophagus somewhat elongate; caeca, narrow anteriorly, gradually wider toward hind-body, widest in hind-body, very gradually divergent, lie close to acetabulum and hold-fast organ, extend almost to posterior end of hind-body.

Explanation of Plate

Parts of "reserve bladder": *ar*, ring around the base of acetabulum; *dl*, dorso-lateral vessel; *el*, extra-lateral vessels; *hr*, ring about the base of hold-fast organ; *hv*, median vessel in hold-fast organ; *il*, intra-lateral vessel; *lc*, lateral collecting vessel; *md*, median dorsal vessel; *mv*, median ventral vessel; *mve*, marginal vessel; *pl*, primary lateral vessel; *sc*, semicircular commissural vessels; *tc*, transverse commissural vessels.

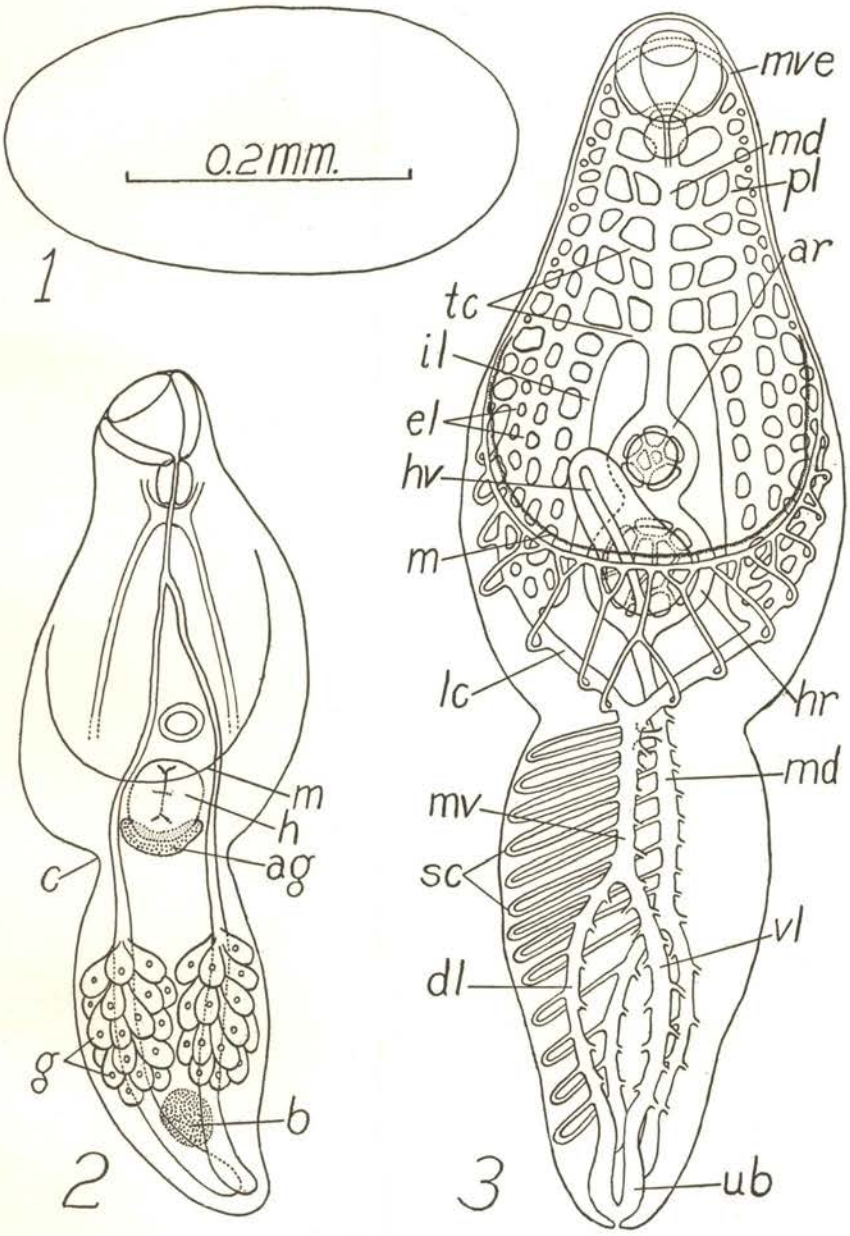
Other structures: *ag*, adhesive gland; *b*, supposed fundament of bursa copulatrix; *c*, constriction between fore- and hind-bodies; *g*, glandular cells of undetermined function and relationship; *h*, hold-fast organ; *m*, postero-ventral margin of fore-body.

Figures 1 and 2 concern the metacercaria of *Cercaria flexicorpa* Collins. They were drawn to the same magnification with the aid of a camera lucida.

Fig. 1. Outline sketch of an inner cyst which contained a live worm.

Fig. 2. Ventral view of preserved neascus from *in toto* mount.

Fig. 3. Diagram, in ventral view, of the "reserve bladder" of *Neascus ambloplitis*, redrawn somewhat simplified from Hughes (1927). The reserve bladder of the neascus of *C. flexicorpa* is essentially the same, but was not studied in so much detail because sufficient time and suitable specimens were not concomitantly available.



PLATE

No reproductive fundaments observed. Bursa copulatrix not distinctly developed but apparently represented by vaguely outlined fundament in posterior part of hind-body. Suprapharyngeal commissure and bases of a pair of anterior and a pair of posterior nerves, observed.

The middle third of the hind-body is filled with large, conspicuous spheroidal, apparently glandular, cells (fig. 2) having small nuclei and coarsely granular cytoplasm. These cells appeared to be grouped about the intestinal caeca like bananas on the stalk—actual connections, however, to the caeca were not positively identified. The cells were readily visible in both living and preserved material.

Urinary bladder with four anterior lobes (two dorsal and two ventral) continuous with principal tubules of "reserve bladder," discharges to exterior through median posterodorsal pore. Details of the primary excretory apparatus (the flame-cell system) were not observed. The secondary apparatus or "reserve bladder" is very similar to that of *Neascus ambloplitis* described in detail by Hughes (1927); to avoid repetition of the lengthy description a copy of Hughes's fully-labelled illustration is included (fig. 3).

Natural hosts. Apomotis cyanellus (Rafinesque) and *Helioperca machrochira* (Rafinesque).

Experimental host. A. cyanellus

Habitat. Skin, muscles, connective tissue, peritoneum, etc.

Locality. Stillwater, Oklahoma.

Museum material. Several slides of specimens mounted *in toto* in the parasite Collection, Museum of Zoology, University of Michigan.

Discussion

Close relatives of the present species include those members of the larval group *Neascus* that occur in black-pigmented cysts in the skin and muscles of fish. Other American forms are (1) *N. ambloplitis* Hughes, (2) the metacercaria of *Cercaria hamata* Miller, (3) *Neascus wardi* Hunter, (4) *Neascus bulboglosa* (Van Haitsma), and (5) *Neascus rhinichthysi* Hunter.

The neascus of *C. flexicorpa* seemingly differs from *N. ambloplitis* [found originally in *Ambloplites rupestris* (Rafinesque) and *Micropterus dolomieu* Lacépède] as described by Hughes (1927) in the following respects: (1) somewhat larger in size in all features measured; (2) thin-wall of oral sucker; (3) longer esophagus and more narrowly divergent caeca; (4) apparent absence of spines on ventral surface of fore-body; (5) undeveloped condition of bursa copulatrix; (6) the absence of distinct fundaments of gonads; (7) hind-body more nearly cylindrical with widest region farther back; (8) inner cyst more nearly ellipsoidal and dorso-ventrally flat; (9) found naturally in different hosts; (10) the description of *N. ambloplitis* does not mention peculiar glandular cells in the hind-body. To what ex-

tent these differences may be only apparent, due to differences in interterpretation on the part of investigators, or real but due only to the effects of various external factors because of different geographic distributions or different host species, are apparently questions to be answered only by further work on life histories. Krull (1932, 1934), working with a cercaria collected near Cushing (about 25 miles from Stillwater) which he identified as *C. bessiae* Cort and Brooks, obtained the metacercaria (which he identified as *N. ambloplitis*) experimentally in *E. gibbosus* and *A. cyanellus*. The writer fully realizes that he is in no position to question the accuracy of Krull's work. Since, however, several students of cercariae in this laboratory all have failed to find *C. bessiae* (if it may be assumed that *C. flexicorpa* is a distinct form) and since one of his experimental hosts was the same as the writer's, the possibility suggests itself that Krull may have been working with *C. flexicorpa* instead of *C. bessiae*. Hunter and Hunter (1930, 1931) fed *Neascus ambloplitis* from *M. dolomieu* to fledgling kingfishers, *Streptoceryle alcyon* (Linnaeus), and obtained the new *Crassiphiala ambloplitis* (Hughes) described by G. W. Hunter (1933), the life history of which was described in more detail by Hunter and Hunter (1934, 1935).

McCoy (1928) raised the metacercaria of *C. hamata* experimentally in *E. gibbosus*. Early developmental stages were described at different ages but the fully developed agamodistome was very briefly discussed, giving too little information for detailed comparisons. An illustration, however, shows clearly that it is a neascus similar to the present form. Incidentally, Collins (1935) regarded *C. hamata* as the closest relative of *C. flexicorpa*.

Neascus wardi (found in the same host "*Lepomis cyanellus*" that I used for experiments) was described (Hunter, 1928) as having reproductive fundaments in the fore-body. The name *N. wardi* was listed as a synonym of *N. ambloplitis* by Van Cleave and Mueller (1934).

In *Neascus bulboglossa*, described by Hughes (1928) as the larva of *Crassiphiala bulboglossa* Van Haitsma, the hind-body is very slender, the hold-fast organ enormous, and the acetabulum entirely wanting.

Neascus rhinichthysi, described by W. S. Hunter (1933) from *Rhinichthys atronasus* (Mitchill), differs clearly from the present species in having (1) a smaller, easily-opened, inner cyst, (2) smaller size with relatively smaller oral sucker and larger hold-fast organ, (3) a short plump form, and (4) simpler reserve bladder.

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