NEW ASPIDOGASTRID TREMATODE, *COTYLASPIS REELFOOTENSIS*, FROM SOME TENNESSEE MUSSELS*

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It is generally accepted that the subclass Aspidogastrea Faust and Tang, 1936 represents a group of worms intermediate in morphology and life history between monogenetic and digenetic trematodes. This paper describes a new aspidogastrid species, *Cotylaspis reelfootensis*, n. sp., from some mussels (listed below) collected in the Bayou du Chien region of Reelfoot Lake, Tennessee. Its anatomical features are compared with other species previously described in the genus *Cotylaspis* Leidy 1857: *C. insignis* Leidy 1857, *C. lenoiri* Poirier 1886, *C. cokeri* Barker and Parsons 1914, *C. stankardi* Rumbold 1927, and *C. sinensis* Faust and Tang 1936.

MATERIALS AND METHODS

The pelecypods were collected by hand and the soft parts exposed by cutting the adductor muscle with a scalpel. The mantle, mantle cavity, gills, foot, visceral mass, and kidney-heart region were then inspected under a dissecting microscope for the presence and location of worms. Worms were removed either by a wide-mouth medicine dropper or by an L-shaped dissecting needle, and then placed in tap water or saline. Morphological observations were made mostly on mounted living specimens with a minimum of cover glass pressure. Worms were also fixed in hot A.F.A. or 10 percent formalin and later stained with Mayer's HCL carmine or Delafield's hematoxylin. Material for sectioning was fixed and stained in a similar manner. All average and range measurements given are in millimeters and are based on 25 specimens fixed in hot 10 percent formalin and mounted in toto.

Cotylaspis reelfootensis, n. sp.

Diagnosis: Aspidogastrid trematode with characters of genus; body elongate, 0.7 to 2.2 long (average 1.7); ventral sucker large, 0.5 to 1.2 long (average 1.1) and 0.5 to 0.9 (average 0.8) wide; ventral sucker divided into 29 alveoli (20 peripheral and 9 medial); 40 marginal organs, 20 on dorsal and 20 in medial borders of peripheral rim of alveoli; cuticula aspinose; body wall with three layers of muscle, circular, longitudinal and oblique; oral sucker lacking; mouth at tip of eversible oral cone (diameter 0.3) capable of assuming many shapes; pharynx large, protrusible, diameter 0.15; paired eye spots 0.016 by 0.007 at anterior end of pharynx; prepharynx and esophagus lacking; intestine single, saeculate (average length 1.4, average width 0.15), extending almost to posterior end of body, intestinal epithelium very glandular; excretory pore single, dorsal, posterior; excretory bladder slightly bilobed (0.17 by 0.08) just posterior to intestine; excretory system stenostomate, main excretory collecting ducts branching into anterior and posterior secondary collecting ducts near mid-length of body; anterior secondary collecting vessel arising near oral cone in three capillaries, each ending in a single flame cell; posterior secondary collecting duct arising in several collecting tubules with complex branching; several capillaries and flame cells observed, always in groups of three; ciliated tufts in both arms of main collecting ducts mostly in second fourth of body; testis single, large, spherical, 0.25 in diameter, anterior and to right of end of intestine; vas efferens single, narrow, at right of intestine, extending anteriorly and widening into sinuous seminal vesicle (usually filled with spermatozoa), latter crossing over to left side at one-third level of body in entering cirrus sac; cirrus sac muscular, 0.165 long and 0.10 wide, at left of intestine; cirrus and prostate gland cells absent; cirrus sac entering genital atrium, latter located slightly to left of mid-line, some distance posterior to pharynx; genital atrium muscular, opening ventrally by single genital pore; vitelline follicles large, 0.45 to 0.6 in diameter, in posterior two-thirds of body, located mostly laterally; ovary small (0.066 in diameter) on left side about mid-level of body; oviduct short; ootype and Mehlis' gland present; seminal receptacle absent; large vitelline reservoir on left side just posterior to ootype; common vitelline duct entering oviduct just before and to left of ootype; uterus thin-walled, very elastic, capable of narrowing to extremely small diameter; ascending loop of uterus on left side; uterus then bending posteriad and running ventral to posterior end of intestine, crossing over to right side of body and extending anteriorly on right side of body, entering genital atrium from right just posterior to pharynx; eggs large, 0.165 to 0.180 long (average 0.168) by 0.92 to 0.115 wide (average 0.98), kidney-shaped and operculate at narrower end; average of 6 eggs per

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mature worm; eggs in uterus undeveloped or at most in two-cell stage.

Hosts: *Anadonta grandis* Say, *Ligumia subrostrata* Say, and *Uniomerus tetralasmus* Say.

Location in Hosts: Cavities bordering gills, heart, kidney, and foot.

Locality: Reelfoot Lake, Tennessee.

Type Specimens: Holotype and paratypes are deposited in the Helminthological Collection, U. S. National Museum; No. 39069.

DISCUSSION

The genus *Cotylaspis* was erected by Leidy (1857) with *C. insignis* as type species from mussels (Unionidae) collected in Pennsylvania. *C. reelfootensis* is the second species described from mussels, and the genus *Anodonta* is most frequently infected with this trematode (Najarian, 1955). There have been four other species described in the genus *Cotylaspis*, all from the small intestine of fresh-water turtles: *C. lenoiri* Poirier, 1886, from Africa, *C. sinensis* Faust and Tang, 1936, from China and *C. cokeri* Barker and Parsons, 1914, and *C. stunkardi* Rumbond, 1927, from the United States.

The most noticeable character which distinguishes *C. reelfootensis* from the other described species in the genus is the number and arrangement of the so-called marginal organs. There are 20 in *C. insignis*, none in *C. lenoiri*, 22 in *C. cokeri*, 34 in *C. stunkardi*, 18 in *C. sinensis*, and 40 in *C. reelfootensis*. When described, each marginal organ has been located at the distal border of each peripheral alveolus of the ventral sucker, whereas in *C. reelfootensis* in addition to 20 organs being located peripherally, 20 are also present on the medial side of each peripheral alveolus bordering on the medial alveoli. The exact nature of these organs has not been demonstrated. Dujardin (1845) in *Aspidogaster*, Looss (1902), and Osborn (1903) in *Cotylaspis* considered them as glandular, whereas Voeltzkow (1888) in *Aspidogaster*, Monticelli (1892) and Nickerson (1902) in *Cotylogaster*, and Stunkard (1917) in *Cotylaspis* regarded them as sensory structures. The marginal organs in *C. reelfootensis* are eversible, and contract in a manner similar to the excretory bladder. The bulbous portion of the organs never protrudes. Glandular concretions were observed both in the bulb and leaving the narrow channel to the exterior, indicating possible secretory function.

In general appearance *C. reelfootensis* most closely resembles *C. insignis* but, in addition to the number and arrangement of the marginal organs, differs from the latter in the absence of both prepharynx and esophagus, a slightly bilobed excretory bladder instead of in two parts, and the absence of a cirrus. Mehlis' gland and the ootype have not been described for *C. insignis* but are present in *C. reelfootensis*. In *C. insignis* ciliated tufts are present only in the main posterior collecting duct next to the pharynx, whereas in *C. reelfootensis* ciliated tufts are present in both ascending and descending limbs of the main collecting duct at the same level of the body. Osborn (1903) stated that the excretory capillaries ending in flame cells are never branched tricotvonously in *C. insignis*. In *C. reelfootensis* they always end in groups of three. In *C. insignis* the ovary is on the right side whereas in *C. reelfootensis* it is on the left. The testis of *C. insignis* is located more posteriorly than in *C. reelfootensis*. The concretionary bodies in the vas efferens of *C. insignis* where it expands into the seminal vesicle are absent from *C. reelfootensis*. There is disagreement as to whether or not *C. insignis* has a genital atrium. Stunkard (1917) stated the genital ducts open to the exterior separately, whereas Osborn (1903) observed a single genital atrium and pore into which the two ducts lead, the latter condition being present in *C. reelfootensis*. The single recurrent loop of the uterus in *C. reelfootensis* is distinctly different from the winding nature of the uterus of *C. insignis*.

C. cokeri differs from *C. reelfootensis* in the nature of the host, the number of alveoli on the ventral sucker, the number and arrangement of the marginal organs, in the presence of an esophagus, cirrus, prostate gland, and double genital pore, the absence of a genital atrium, and the position of ovary and testis. Stunkard (1917) described an ootype in *C. cokeri* but did not designate it as such.

C. stunkardi differs from *C. reelfootensis* in the nature of the host, number of alveoli, number and arrangement of marginal organs, presence of esophagus, size, shape and position of ovary and testis, extent of vitellaria, convoluted nature of the uterus, and the greater average number of eggs per worm.

C. sinensis differs from *C. reelfootensis* in the nature of the host, number of alveoli, number and arrangement of marginal organs, size and position of ovary and testis, absence of ootype, presence of prostate gland and seminal

receptacle, and a small vitelline reservoir.

C. lenoiri differs from *C. reelfootensis* in the nature of the host, number of alveoli, absence of marginal organs, size and position of ovary and testis, the extent of the vitellaria, and the large size of the vitelline reservoir.

The systematics of aspidogastrid trematodes has been reviewed by Nickerson (1902), Stunkard (1917), Dawes (1941), and Dollfus (1956, 1958). The most definitive account was given by Dollfus (1958) who listed three characters which distinguish the genus *Cotylaspis* from other genera in the subfamily Aspidogastrinae: (1) three longitudinal rows of alveoli, (2) a single testis, and (3) a cirrus sac. Since the alveoli of the ventral sucker are not actually in three longitudinal rows, this character might better be re-stated as a peripheral rim of alveoli surrounding a single longitudinal row of alveoli.

SUMMARY

Cotylaspis reelfootensis, n. sp. is described from the mussels *Anadonta grandis* Say, *Lipsites subrostrata* Say, and *Uniomerus tetralasmus* Say collected from Reelfoot Lake, Tennessee. This is the sixth species described in the genus *Cotylaspis* Leidy and the second species of *Cotylaspis* reported from pelecypods. Its anatomical characters are compared with previously described species in the genus.

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EXPLANATION OF PLATES

PLATE I

FIGURES 1–3. Sketches of *C. reelfootensis* from ventral, dorsal, and lateral aspects.
FIGURE 4. Single alveolus of ventral sucker with four marginal organs.
FIGURE 5. Optical section of marginal organ, showing protrusibility, and communication with exterior.
FIGURE 6. Three views of an eyespot.
FIGURE 7. *C. reelfootensis*, type specimen, dorsal view.
FIGURE 8. Optical section of pharynx and anterior portion of intestine.
FIGURE 9. Alveoli of posterior end of worm; dotted lines indicate positions of borders before contraction.
FIGURE 10. Ventral sucker showing number and arrangement of alveoli and marginal organs.

PLATE II

FIGURES 11–14. Sketches of anterior end of worm showing various positions of pharynx in relation to oral cone.
FIGURES 15–17. Sketches of various shapes of oral cone.
FIGURE 18. Vitelline cells of single vitelline follicle.

FIGURE 19. Portion of uterus with single egg, indicating elasticity of uterus.
FIGURE 20. Optical section of uterus showing fibrous condition.
FIGURE 21. Incomplete sketch of excretory system showing main collecting ducts and their branches, ciliated tufts, and tripartite nature of capillaries and flame cells.
FIGURE 22. Optical section of body wall showing cuticle, parenchyma, and musculature.
FIGURE 23. Egg of *C. reelfootensis* after incubation at room temperature for 3 days; morula stage present and vitelline cells broken down.
FIGURE 24. Views of eggs from uterus showing germinal cells, vitelline cells, and opercula.
FIGURE 25. Sketches showing successive stages in operation of excretory bladder.

RESEARCH NOTE

ON THE ECOLOGY OF *LEIDYNEMA APPENDICULATA* (LEIDY, 1850), (NEMATODA: OXYUROIDEA).

During attempts to raise parasite-free *Blatta orientalis* for experimental purposes, nymphs hatching from isolated oothecae obtained from the Quartermaster Research and Engineering Center, Natick, Massachusetts, were found to be protozoa-free, but had very large numbers (up to 85 adults) of *Leidynema appendiculata*. This high infection occurred in subsequent experiments, even though the oothecae were rinsed in 50 percent alcohol before incubation. However, when the egg cases were cleaned with detergent (Tide) solution, the nymphs which hatched were *Leidynema*-free. The ability to obtain nematode-free *Blatta* only from thoroughly cleaned oothecae indicates that *Leidynema* eggs are transferred by adhering to the ootheca, as well as directly from adult to nymph. There is also some evidence that *Leidynema* eggs may occasionally be carried within roach oothecae.

It was further noted that average populations of *Leidynema* in protozoon-free *Blatta* were usually greater than those of protozoon infected hosts. One experiment was undertaken in which 12 protozoon-free *Blatta* were experimentally infected with *Nyctotherus ovalis*, *Lophomonas striata*, and *L. blattarum*. Twelve roaches from the same source were isolated as controls. After 42 days of incubation the hosts of both groups were killed and the populations of *Leidynema* tabulated. Data are presented in table I.

TABLE I. *Relation of protozoon infection to populations of* Leidynema appendiculata *in* Blatta orientalis.

Host	*Blatta orientalis*					
	Stock culture (75 per jar) protozoon-free 30 examined		Control group (12 per jar) protozoon-free 11 examined		Experimentally protozoon infected (12 per jar) 11 examined	
	Numbers of adult female *Leidynema* present					
Sex	Range	Ave.	Range	Ave.	Range	Ave.
Males	4–19	15	5–15	9.25	4–11	6
Females	2–85	24.7	6–31	15.3	2–19	11.2
Nymphs	1–18	8	5–18	8.5	1–5	2.5
Totals	1–85	18.2	5–31	10.6	1–19	5.7

The smaller average number of *Leidynema* in both experimental and control groups of hosts compared with the protozoon-free stock culture is probably due to the decrease in contact between roaches under less crowded conditions. The further decrease of *Leidynema* in experimentally protozoon-infected hosts is apparently directly related to the presence of protozoa. Further investigations are required to determine the extent and basis of this limitation of *Leidynema* population by associated protozoa.

In a brief series of in vitro experiments on *Leidynema* it was found that addition of albumin and glucose to Ringer's solution extended the viability of the nematode by about 2 days. Microscopic examination of numerous living specimens failed to reveal whether the worms ingested either fluid or particulate material of their medium. In order to visualize particulate ingestion, more than 15 *Leidynema* were kept for periods of 24 hours in suspensions of Congo Red stained yeast or India ink. Since no ingestion of particulate matter was observed under these conditions, it is suggested that the worms gain nutrition by absorption.—C. M. FAY, *Department of Biological Sciences, University of Cincinnati.*

PLATE I

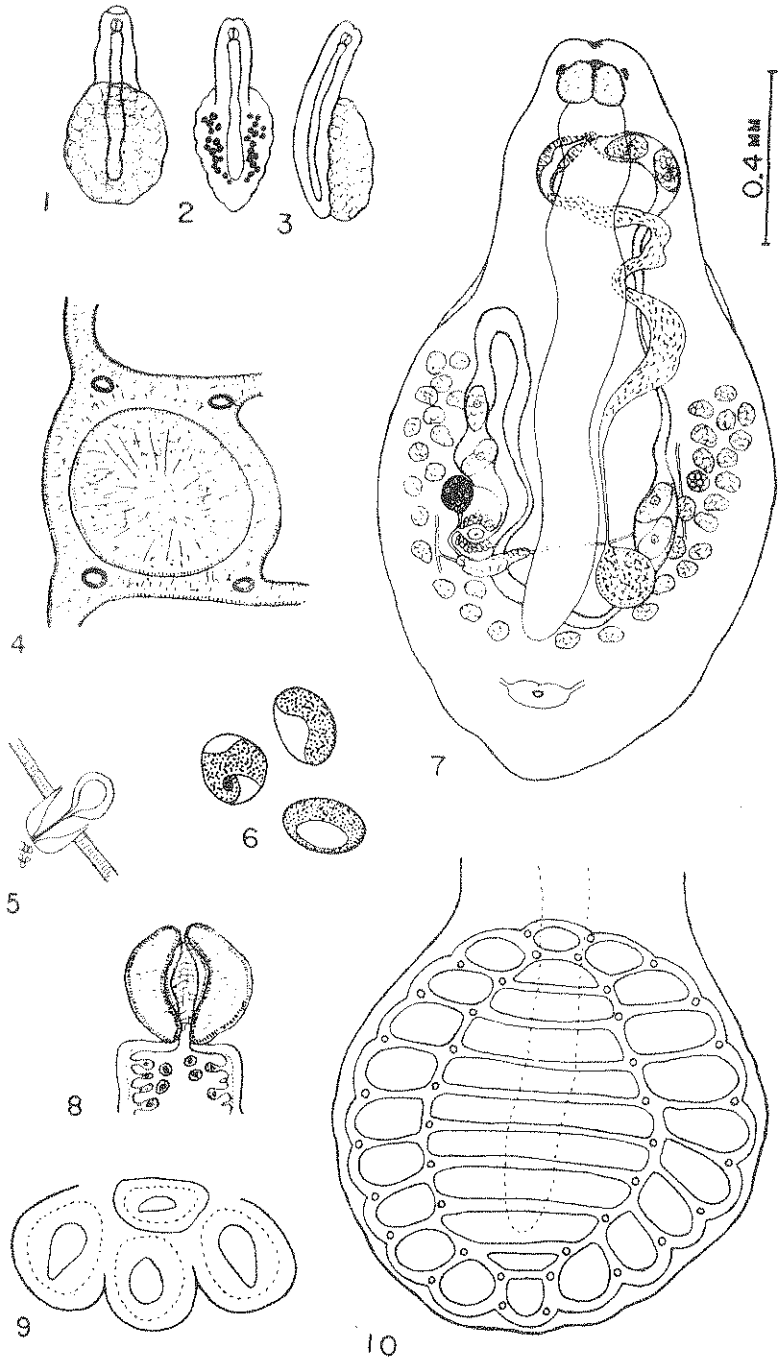

PLATE II