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Description of Elements of the Life Cycle of a New Species of Leucochloridiomorpha (Trematode: Brachylaemidae) from Southwestern Michigan

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DESCRIPTION OF ELEMENTS OF THE LIFE CYCLE OF A NEW
SPECIES OF LEUCOCHLORIDIOMORPHA (TREMATODA:
BRACHYLAEMIDAE) FROM SOUTHWESTERN MICHIGAN

A Thesis
Presented to
The Faculty of the School of Graduate Studies
Western Michigan University

In partial fulfillment
of the requirement for
the degree of Master of Arts
in Biology

by
Jon Shoemaker
Kalamazoo, Michigan
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DESCRIPTION OF ELEMENTS OF THE LIFE CYCLE OF A NEW
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Introduction

During the period extending from September, 1959 to July, 1960, 553 snails, of the species Campeloma decisum, were collected from Portage Creek in the southeast corner of the SE 1/4, Sec. 34, T. 2 S., R. 11 W., Kalamazoo County, Michigan. Metacercariae, closely related to Leucochloridiomorpha constantiae of the monotypic genus, Leucochloridiomorpha, were found in the uteri of several snails. Subsequent studies showed the metacercariae to be an undescribed species. Life history studies showed that this form developed experimentally in the chicken and domestic duck. In this paper stages in the life cycle are described.

Allison (1943), after his preliminary report (1940), discussed in detail the life cycle of L. constantiae. He removed the family Brachylaemidae from the Prosostomata, placed it in the order Strigeatoidea, and erected the superfamily Brachylaemoidea. Allison (op. cit.) also emended the diagnoses of the family Brachylaemidae, subfamily Brachylaeminae, genus Leucochloridiomorpha, and

species constantiae.

Mueller (1935) found the metacercaria in the uterus of Campeloma decisum and described it as Cercariaeum constantiae.

Gower (1938) found two adults in the bursa cloaca of a black duck, Anas rubripes, and erected the genus, Leucochloridiomorpha, and species, macrocotyle. Allison (1943:134) noted, "The metacercaria, however, had been described by Mueller (1935) as Cercariaeum constantiae, which in the researches here reported, is proven to be conspecific with L. macrocotyle. Therefore, according to the law of priority, macrocotyle must be suppressed as a synonym of constantiae and the name for this species becomes L. constantiae (Mueller, 1935), new combination."

Methods

The snails were collected according to the methods reported by Allison (1942:1-4). Allison used "traps" consisting of small packets of chicken dung wrapped in cheesecloth. These were half-buried in mucky parts of the stream bed. Snails, of the genus Campeloma, being coprophagous, were attracted to the dung. I used small packets, four inches in diameter, of duck dung, wrapped in cotton cloth. The snails attracted to the traps were found not only on the packets of dung, but also in an area

two feet in diameter around them, and as much as six inches beneath the surface of the mud. Campeloma decisum was the only species of snail observed in the vicinity of the traps. Twenty-eight specimens were found three days after the first bait was placed in the stream. On subsequent occasions as many as forty Campeloma were collected.

In order to obtain metacercariae, the shell of each snail was crushed, and the viscera were removed and placed in a petri dish partially filled with water. Metacercariae, when present, were removed from the uterus with dissecting needles and forceps. They were not firmly attached to the uterine lining.

Adult flukes were obtained from the bursa cloacae of experimentally infested one-month-old chickens and domestic ducklings. Immature fowl were used because L. constantiae was found by Gower (1938:24) and Allison (1943:134) in the bursa cloacae, an organ not present in adults of either species (Hewitt 1952:32). The metacercariae were placed into the cloaca of each experimental host with a pipette, twenty-five metacercariae being inserted into each chick. Of the five experimentally infested chicks, one, autopsied twenty days following infestation, yielded nine adults. Examination of two chicks after one week, and one chick after ten days revealed no adult flukes. Two ducklings were in-

fested. Forty metacercariae were inserted into the cloaca of the first duckling, and 120 into the second. The bursa cloaca of the first duckling yielded two adult flukes ten days later, and that of the second duckling yielded twelve adult flukes five days after infestation.

Metacercariae were flushed into the stomachs of a guinea pig and a white rat by means of a pipette. Autopsies a week later failed to reveal either metacercariae or adults.

The metacercariae and adult flukes which were preserved were fixed in acetic sublimate, stained in Mayer's paracarmin, and mounted in damar. I made the drawings from both living and mounted specimens. A five per cent aqueous solution of methylene blue aided greatly in revealing gland cells of living specimens. Eggs removed from a twenty-day-old adult were placed in a watch glass filled with tap water and observed carefully over a period of one month.

Description of Leucochloridiomorpha papillata, sp. nov.

Adult. An illustration of the adult may be seen on page 17, fig. 1. Measurements in millimeters of four living specimens:

Table I

Measurements	Maximum	Minimum	Average
length	2.36	1.42	1.70
width	1.17	0.81	0.91
oral sucker	0.31	0.18	0.23
acetabulum	0.85	0.59	0.67
pharynx	0.11	0.08	0.09

Fixed and stained specimens shrunk to approximately one-half their natural size. Averages of six fixed and mounted five-day-old adults were:

Table II

Measurements	Maximum	Minimum	Average
length	0.95	0.64	0.85
width	0.58	0.48	0.51
oral sucker	0.19	0.13	0.15
acetabulum	0.49	0.36	0.38
pharynx	0.06	0.05	0.06
right testis	0.16x0.09	0.11x0.06	0.14x0.08
left testis	0.13x0.09	0.09x0.06	0.12x0.07
ovary	0.11	0.06	0.09

The body is flat and broadly oval, the width at the acetabulum being one-half the length. It is rounded anteriorly and broadly pointed posteriorly. The acetabulum is 2.72 times the diameter of the oral sucker, and lies in the middle of the body. The body is straw-colored, the acetabulum red. The cuticle is covered with conspicuous, irregularly spaced papillae, and is rugose bearing many small transverse and longitudinal folds.

The excretory system consists of a small, thin-walled, triangular shaped bladder and a pair of slightly coiled ducts which pass dorso-laterad to the cecae, and extend anteriorad to the level of the oral sucker. At this point each turns and passes posteriorad to the level of the posterior margin of the acetabulum where it divides into posterior and anterior branches. The anterior branch extends anteriorad and terminates lateral to the oral sucker. The posterior branch passes posteriorad ending at the level of the bladder. Ampulla-like enlargements are present on each side of the bladder at the bladder-excretory duct junctions. Tufts of cilia (flames) are present in the main duct in the region preceding its divisions into anterior and posterior branches. Their number was not ascertained as most of them were obscured by vitellaria and eggs in the specimens I examined. The bladder opens directly at the posterior tip of the body.

The digestive system consists of a mouth in the center of the oral sucker, a short prepharynx, a small, oval pharynx, a very small esophagus, not always apparent, and two smooth, unbranched cecae extending posteriad and laterad past the acetabulum to the level of the ovary in the posterior end. The cecae are usually filled with small, granular materials which are occasionally regurgitated.

The two oblong testes are located slightly anteriad to the bladder and on the right and left sides. The single, spherical ovary lies dorso-anteriad to the right testis. The elongate, protrusile cirrus bears many small spines covering its entire length. The cirrus pouch is twice as long as it is wide, and contains only the cirrus. The muscular metraterm joins the cirrus pouch near the genital pore, which opens ventrad, and lies anteriad to the bladder, and between the testes. The uterus, enlarged with numerous eggs, loops once laterad to and at the middle of the acetabulum on either side of the body, and descends on the left side to the metraterm. Vitellaria are present on the right and left sides of the body, anteriad to the acetabulum, posteriad to the cecal bifurcation, and laterad to the uterus. The vitelline ducts join to form a reservoir between the testes. Laurer's canal begins at the reservoir and opens dors-

ally at mid-bladder level.

In tap water the adults move about slowly on the bottom of a dish by frequent contractions and elongations of the body. They will remain alive in tap water at room temperature for one day.

Metacercaria. An illustration of the metacercaria may be seen on page 17, fig. 2. Measurements in millimeters of thirty living specimens under slight cover glass pressure:

Table III

Measurements	Maximum	Minimum	Average
length	2.06	1.35	1.66
width	0.87	0.57	0.71
oral sucker	0.26	0.19	0.20
acetabulum	0.47	0.33	0.37
pharynx	0.11	0.08	0.09

Preserved specimens shrunk to about one-half their natural size. Measurements of ten fixed and mounted metacercaria in millimeters were:

Table IV

Measurements	Maximum	Minimum	Average
length	0.99	0.66	0.86
width	0.44	0.29	0.36
oral sucker	0.16	0.11	0.13
acetabulum	0.31	0.19	0.24
pharynx	0.06	0.06	0.06
right testis	0.06x0.04	0.05x0.02	0.06x0.03
left testis	0.06x0.03	0.03x0.02	0.05x0.03
ovary (at widest point)	0.09	0.05	0.06

The metacercariae are longer and thinner than five day old adults, but similar to them in shape. The acetabulum is 1.85 times the diameter of the oral sucker, and lies in the posterior half of the body. The body color varies from light pink to yellow, the acetabulum being a darker pigmented pink-orange. Under low power (100x) the whole worm is straw-colored. The color is apparently influenced by the seasons and the food of the host snail; straw color predominates in the fall and winter, and pink becomes more frequent in the late spring and early summer.

Mueller (1935:99) described "a number of wart-like cuticular bosses in head region, especially abundant around

lip of oral sucker." Structures fitting Mueller's description are present on L. papillata; moreover, the cuticle is covered with conspicuous, irregularly spaced papillae, which are more abundant than those in the adults. Papillae are more numerous in the anterior region, especially so on the anterior tip of the body. The cuticle is rugose, consisting of many small transverse and longitudinal folds.

The excretory system is essentially the same as in the adult. The slightly coiled excretory ducts extend anteriorly to the posterior edge of the oral sucker. The flames in the "ascending duct," which starts at mid-acetabular level, end before the cecal bifurcation. Eight to twelve flames were counted per side from thirty individuals.

Flame cells are present at the ends of short ducts leading to the larger ducts. Only a few flame cells were observed, consequently a definite flame cell pattern could not be ascertained. Those seen were located near the posterior edge of the oral sucker, anterior and lateral to the acetabulum, and posteriad to the level of the ovary.

The digestive system consists of a sub-terminal mouth located in the center of the oral sucker, a short prepharynx, a small, round pharynx, a short esophagus,

and two smooth cecae. The cecae continue posteriad and laterad past the acetabulum to the level of the ovary in the posterior end.

The genital primordia are located posteriad to the acetabulum. Two elongate testes are located on the right and left sides, and slightly anterior to the bladder. A single, pear-shaped ovary lies dorso-anteriad to, and between the testes.

The uterus ascends on the right side, crosses at a tangent to the acetabulum and descends on the left side to the metraterm. The metraterm joins with the cirrus pouch to form a genital pore which opens on the ventral side of the body, anterior to the bladder and between the testes. The cirrus pouch contains an elongate, spineless cirrus.

Vitellaria are not present in the metacercaria. Yolk ducts descend laterally and turn medially to form a reservoir near the genital pore. Laurer's canal begins at the reservoir, extends posteriorly a short distance, and opens dorsally near the bladder.

The metacercariae are unencysted and active. In tap water they move about slowly by frequent contractions and elongations. They will live in tap water at room temperature for forty-eight hours or longer.

Egg and Miracidium. An illustration of the egg without the embryo may be seen on page 17, fig. 3. Measurements

in millimeters of ten eggs removed from a twenty-day-old adult were:

Table V

Measurements	Maximum	Minimum	Average
length	0.036	0.032	0.033
width	0.019	0.014	0.016
projection	0.003x0.003	0.003x0.003	0.003x0.003

Eggs of five to twenty-day-old adults are golden brown in color. The eggs of five-day-old adults are soft; some are embryonated; the operculum is not distinct, and no projection is present. The eggs of twenty-day-old adults possess a small projection opposite a distinct operculum, and all contain embryos within a firm shell.

Living miracidia did not hatch in tap water. The eggs probably hatch after being eaten by an intermediate host. The embryos observed did not possess stylets or cilia, and no definite movements were seen. Partially emerged miracidia were observed, but all were dead, the structures were not well defined, and none possessed stylets or cilia.

Specific Diagnosis: Leucochloridiomorpha (Gower, 1938). Body straw-colored, acetabulum red. Body flat and twice as long as it is wide. Margin crinkled, not

smooth. Acetabulum 2.72 times larger than oral sucker. Cuticle covered with small irregularly spaced papillae. Esophagus present or absent. Coiled excretory ducts extend anteriorly to posterior edge of oral sucker. Small, oblong testes; left smaller than right.

Metacercaria flat and more than twice as long as wide. Widest point just anteriorly to acetabulum. Excretory ducts extend to posterior edge of oral sucker. Ascending duct ciliated, eight to twelve tufts of cilia per side. Cilia begin at start of ascending duct, end before cecal bifurcation. Ascending duct starts at mid-acetabular level. Acetabulum in posterior half of body, diameter 1.85 times diameter of oral sucker. Cuticle papillate. Ovary median, dorso-anteriorly to testes. A cirrus pouch present, contains an elongate, spineless cirrus. Genital pore median-ventral, between testes. Laurer's canal from vitelline reservoir, opens dorsally near bladder.

Egg 0.033 mm. x 0.016 mm.; projection opposite operculum, 0.003 mm. high x 0.003 mm. at the base.

Miracidium without cilia and stylet.

Host: Chicken and domestic duck (experimental)

Habitat: Bursa cloaca

Locality: SE 1/4, SE 1/4, Sec. 34, T. 2 S., R. 11 W., Kalamazoo County, Michigan.

Cotypes: Adult cotypes, U.S.N.M. Helm. Coll. No. 39453
Metacercaria, U.S.N.M. Helm. Coll. No. 39454

Discussion

The ratio of infested to uninfested snails was one to four during most of the year. The ratio decreased to about one to ten in March and April at which times many empty shells were observed. The ratio increased during May and June. The number of metacercariae per snail varied from one to 75 with an average of 33.

Two kinds of cercariae were taken from the snails. Cercaria trigonura, described by Cort (1915:44-49), which develops from mother and daughter rediae, and an unidentified form developing from within a saccular sporocyst. Both were found in the uterus, and C. trigonura was also present in the liver. C. trigonura was found in several snails, and the unidentified form only once, in June, 1960. The unidentified form has a stylet and a tail as long as the body. The size and position of the suckers, and the presence of only two testes in the posterior end, indicate a possible relationship to L. papillata. Cercariae of the Brachylaemidae develop from sporocysts; however, the presence of a stylet and long tail is unusual.

The natural hosts are possibly young mallard ducks. Many were seen on Portage Creek and in the area around it. None were examined as shooting is prohibited in the city limits.

If, after further experimentation, eggs are successfully fed to snails, and the unidentified cercaria is shown to be a stage of L. papillata, and the definite morphology of the miracidium ascertained, the life history will then be complete.

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Explanation of Drawings

Abbreviations

- C - Cecum
- CED - Ciliated excretory duct
- CP - Cirrus pouch
- GP - Genital pore
- LC - Laurer's canal
- P - Papillae
- U - Uterus
- V - Vitellaria
- VD - Vitelline duct

Figures

- 1 - Adult of *L. papillata*. Ventral view.
- 2 - Metacercaria. Ventral view.
- 3 - Egg showing operculum and projection.

