Notes on the Life History of *Pleurogonius malaclemys* Hunter, 1961 (Trematoda: Pronocephalidae) from Beaufort, North Carolina, with a Description of the Cercaria

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SUMMARY

A monostome cercaria developing in the marine snail, *Nassarius obsoleta* (Say), in the Beaufort, North Carolina area, is proved to be the larva of *Pleurogonius malaclemys* Hunter, 1961. This is the first marine cercaria definitely determined to be the larva of an adult of the Family Pronocephalidae Looss, 1902. Morphology of the metacercariae which commonly are encysted on and under the opercula of the snail host, as well as results of feeding experiments with the definitive host, *Malaclemys terrapin centrata* (Latreille), 1802, validate the above statement.

MATERIALS AND METHODS

Active, freshly emerged cercariae were killed in boiling seawater and measured under cover glass with the minimum amount of water necessary to prevent distortion from pressure; this method is that used by Cable (1956). Mature living cercariae could not be studied successfully under cover slip pressure due to their habit of rapid encystment when in contact with any suitable substrate. To facilitate observation of internal organs, cercariae were allowed to encyst on a slide and dissected out of their cysts within 2 or 3 min. This method allowed most of the cystogenous cells to empty, but did not alleviate entirely the difficulties of observation due to body pigments. Development of the excretory system was studied in living worms obtained by crushing the snail hosts; young, developing cercariae were dissected out of the rediae. Sections of mature as well as developing cercariae were made; hematoxylin and eosin staining was used.

Metacercariae of different ages were studied after removal from their cysts. These were photographed alive and then preserved in hot AFA solution under minimum cover glass pressure. Adult worms from experimentally fed turtles also were photographed before killing in hot AFA. Measurements of both the metacercariae and adults were made on stained and mounted specimens. Whole mounts were stained with either Semichon's or Ballard's. Photomicrographs of all living stages were made with a Polaroid camera; this method is particularly good for recording details of the excretory system which constantly vary in visibility.

DESCRIPTION OF CERCARIA

Measurements in mm from 10 heat-killed specimens:

	Range	*Average*
Body length	0.493–0.554	0.525
Body width (immediately posterior to collar)	0.208–0.281	0.227
Oral sucker		
Longitudinal diameter	0.040–0.054	0.049
Transverse diameter	0.038–0.054	0.045
Tail length	0.478–0.622	0.572
Tail width (near base)	0.053–0.068	0.059

Large, trioculate, monostome with smooth cuticle. Cephalic collar inconspicuous with ventral lobes approximately one-fifth to one-fourth of body length; lobes widely separated posteriorly, but joined medially behind oral sucker. Heavily pigmented body tends to be concave ventrally. Anterior end bluntly tapered with terminal, weak, subspherical oral sucker; body widens abruptly posterior to eyespots. Sides straight from posterior collar margins to rounded posterior end. Inconspicuous cup-shaped protrusible, apparently glandular, locomotor structures at posterolateral borders of body, extending beyond level of tail insertion. Cystogenous glands, filled with granular opaque material, immediately be-

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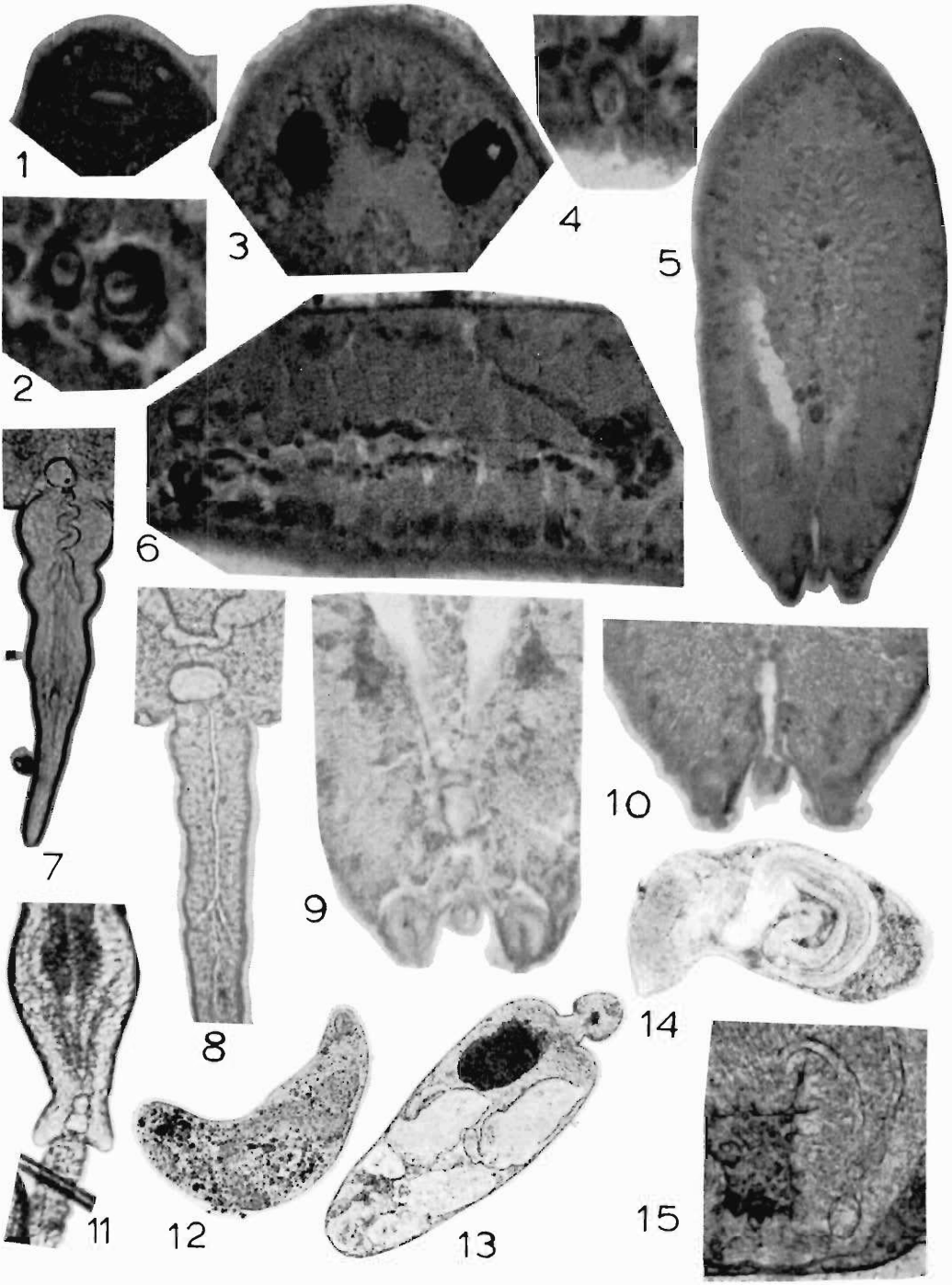

neath entire body surface except for extreme anterior end. Six cephalic glands surround oral sucker and their ducts open at extreme anterior end (Fig. 1, 3, 29). Median eyespot smaller, more irregular, less organized, usually lacks lens, and lies slightly anterior to other two, close to oral sucker. The three eyespots closely associated with bilobed ganglionic mass of nervous system (Fig. 3, 18, 29).

Pharynx lacking, esophagus thin, long, bifurcating posterior to collar in second one-fourth of body length. Cecae long, with irregular thick-walled diverticula throughout their length; diverticula more prominent and regular in anterior parts of cecae (Fig. 5, 17, 26).

Primordia of all genital organs present; two small extratesticular masses widely separated from each other in posterior part of body (Fig. 9, 26, 27); primordia of vitellaria anterior and lateral to testes (Fig. 26, 27). A more conspicuous median line of cells (Fig. 5, 6, 17, 18, 26) extends anteriorly from position of future adult ovary–ootype complex (Fig. 5, 6, 26, 27); this begins in front of excretory bladder and runs into anterior half of body where it turns to left and ends in post-bifurcal region of future genital pores. Rudiments of male and female ducts (Fig. 2), including one interpreted as Laurer's canal (Fig. 6, 26, 27) (not observed in adult, Hunter, 1961), as well as location of future genital pores, can easily be traced in sections (Fig. 4, 26, 27).

Most prominent part of excretory system consists of two lateral collecting tubes, filled with refractile bodies. In collar region, these ducts unite by a very small tubule to complete cycloid system characteristic of Family (Fig. 26, 28). Posteriorly large ducts meet and connect with bladder by thin-walled duct which regularly expands to form an anterior bladder, or vestibule; this alternates in pulsations with thicker-walled posterior bladder. (See Fig. 7, 8, 11, 14, 15, 16.) Therefore, bladder variable in shape, definitely of two parts, and when empty disappears from view and arms of large collecting ducts may then be easily mistaken for parts of a Y-shaped bladder. In a mature cercaria, the heavy-lipped excretory pore is in the dorsal wall of the bladder, some distance from the posterior margin of the body (Fig. 26); it is nonfunctional until the tail is lost. Small caudal atrium present; its two posterolateral openings function as excretory pores until tail is cast off at encystment (Fig. 10, 11, 26). Caudal excretory tubes prominent in developing forms (Fig. 7, 8), but at emergence, no evidence of a caudal duct or pores. Branches of main collecting ducts as described for adult (Hunter, 1961) visible in living forms. Flame cell pattern expressed as $2(3 + 3 + 3) + (3 + 3 + 3)$ (Fig. 28).

Tail straight, slightly longer than body, tapering gradually to tip. Few, irregularly scattered, small cells throughout length. Few, long, and somewhat spiral strands run lengthwise, independent of scattered cells. Cuticle with fine striations.

Development occurs in simple rediae (Fig. 12, 13, 14) which contain much yellow–orange and brown pigment, and which have a relatively long, wide gut. Young rediae often show marked constriction near anterior end. Birth pore inconspicuous, relatively close to pharynx. Cercariae leave rediae at early stage and mature in tissue spaces of host. Usually but two or three (often only one) cercariae were found developing in each redia; several small germ balls present in rediae with advanced larvae. No second-generation rediae found.

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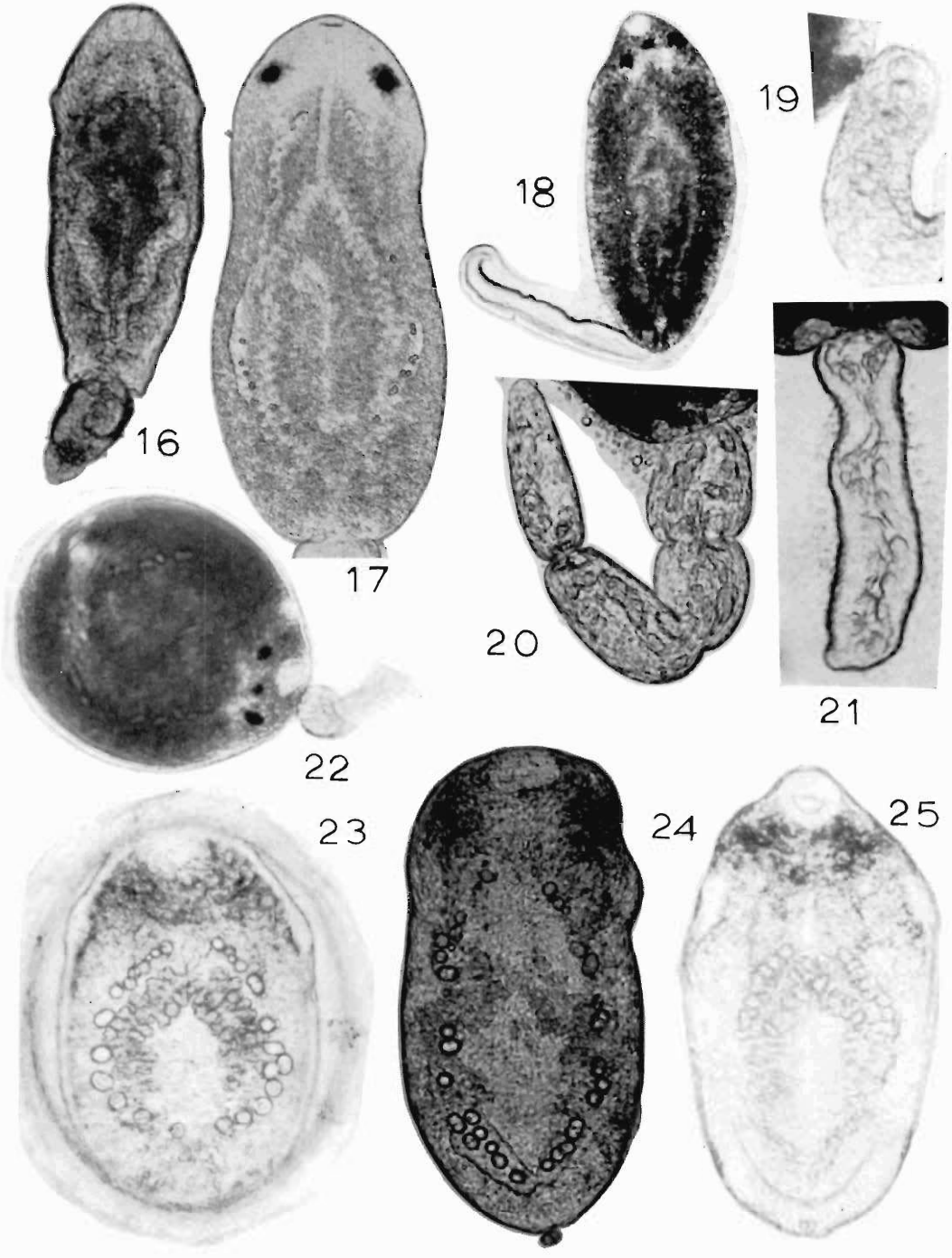
FIGS. 1–15. 1. (Frontal sec.) Ducts of cephalic glands. (200×). 2. (Cross sec.) Male and female ducts immediately posterior to developing cirrus pouch. (970×). 3. (Frontal sec.) Cephalic glands, ganglia, eyespots. (430×). 4. (Sagittal sec.) Cirrus pouch and genital pore area. (970×). 5. (Frontal sec.) Gut, genitalia, bladder. (200×). 6. (Sagittal sec.) Genital ducts, ootype region, Laurer's canal. (430×). 7. Live, developing cercaria ex snail. Thick-walled part of excretory bladder and caudal ducts. (150×). 8. Live, developing cercaria. Bladder and caudal ducts. Note contracted anterior bladder. (200×). 9. (Frontal sec.) Bladder, ducts, testes, locomotor organs. (430×). 10. (Frontal sec.) Bladder, atrium, and atrial pores. (430×). 11. Live cercaria ex redia. Two parts of bladder and atrial pores. (100×). 12. Live redia. (48×). 13. Live redia with young cercariae. Note constriction. (48×). 14. Live redia with older developing cercariae. Note double excretory bladder. (48×). 15. Live cercaria within redia. Note thicker wall of posterior part of bladder. (150×).



DISCUSSION OF CERCARIA

Infected snails with emerging cercariae are to be found throughout the year, being somewhat more prevalent during the late fall and early months. The percentage of infected snails with emerging cercariae when isolated in the laboratory is usually less than 1%, although two collections (in November and December of different years) from isolated pens where infected turtles had been confined showed unusually high 3.3 and 4% infections. In any area where infected snails are found, cysts are conspicuous on and under opercula of both infected and noninfected snails.

In the laboratory, midday is the optimum time for emergence of cercariae; relatively few emerge daily as would be expected from the observations of the numbers developing within each redia. The cercariae are comparatively slow, often steady swimmers, moving in circles by means of violent tail-lashings. When they stop swimming, they soon encyst on snails as well as on the bottom of the glass culture dishes in which the hosts have been isolated. In the laboratory, many of the cercariae fail to encyst and die within a few hours. Failure of encystment of many cercariae suggests the premature emergence of some of the larvae due to laboratory conditions. Bioculate and trioculate cercariae are found at the same time and from the same snail host, the trioculate being the more numerous. During development, the poorly organized third eyespot appears after the more posterior two are well formed and when the cercariae are free in the host's tissue spaces. Emergence of the bioculate forms also probably is due to unnatural laboratory conditions.

Large vesicular bodies, first described by Faust, 1917, are very numerous and prominent in the tail of developing forms (Fig. 19, 20, 21). These become smaller and less prominent in the tail of older developing and actively swimming forms. Kruidenier and Mehra (1957) described the distribution and character of the mucoid glands in the freshwater pronocephalid *Macrovestibulum eversum* Hsü. They found 10 pairs of irregular glands in the tail. Kruidenier, 1953, postulates the function of the body and tail gland secretions to be involved with the cuticle. No histochemical studies have as yet been carried out by me on *Cercaria P. malaclemys*. However, Fig. 21 does suggest that there is some secretion from cells within the tail. No constant number of these caudal bodies is to be found; in young developing forms the tails are packed with them (Fig. 19). I suggest that certain of these bodies may be homologous to the glycogen-containing caudal vesicles of certain cercaria including *Notocotylus* which were described by Genetzinskaja and Dobrovolski, 1962. They, therefore, logically would disappear as the actively swimming cercariae age.

Posterolateral locomotor organs are more easily seen in developing cercariae than in the emerged forms. This is probably due to the development of pigmentation in the older forms; they are extremely important in any crawling movements.

Study of the development of the excretory system indicate that it is in general agreement with that described for the Family (Kuntz, 1951). However, Figures 8, 11, 14, 15, and 16 show that there are two parts to the functional bladder. The double caudal duct, leading from the atrium as seen in developing forms dissected from rediae or lymph spaces of the snail host, cannot be observed by the

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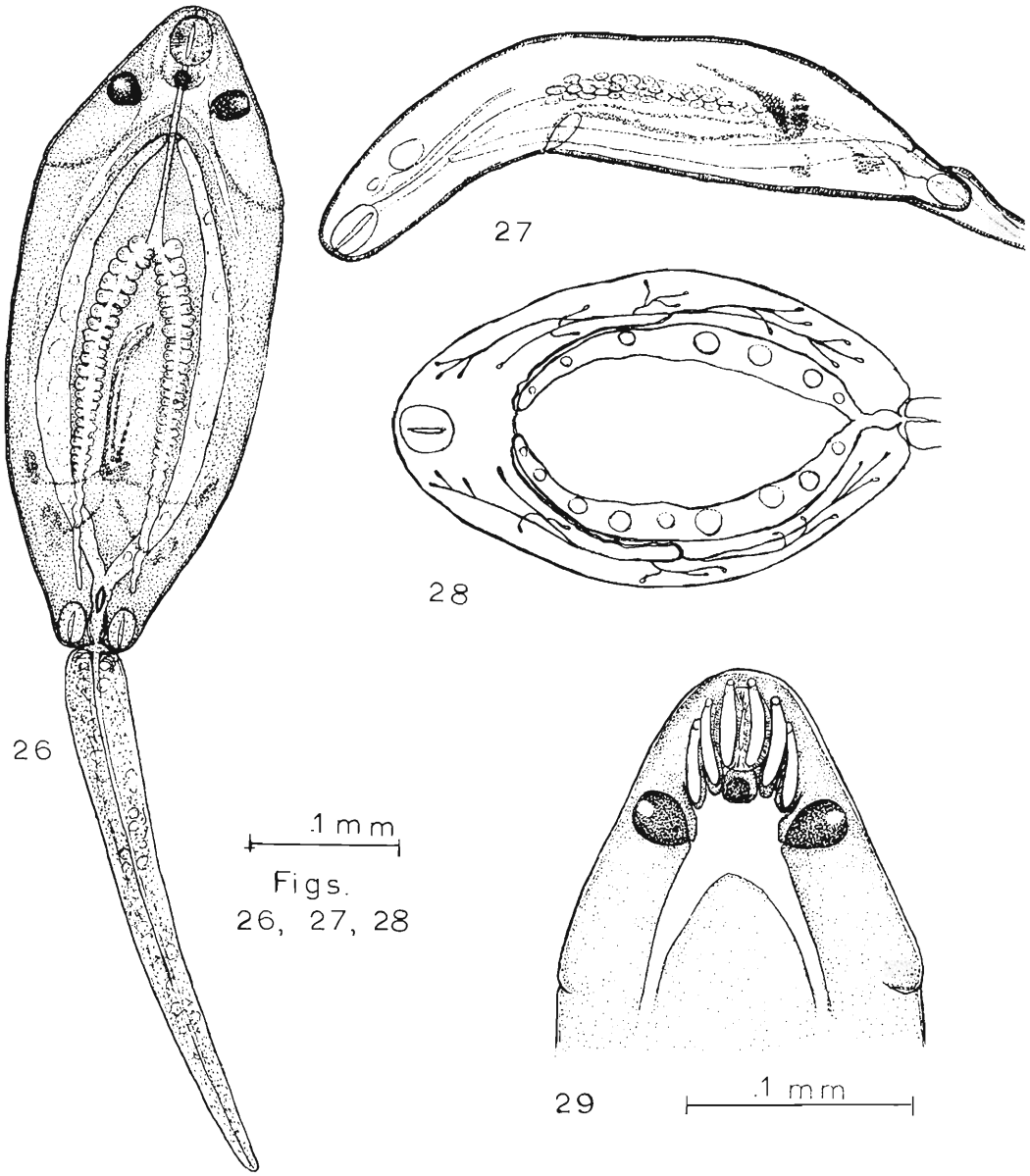
FIGS. 16–26. 16. Live, developing cercaria from snail. Collar, rudiments of reproductive organs, excretory system. (48×). 17. Live, emerged bioculate cercaria. Digestive, excretory, and reproductive systems. (100×). 18. Live, emerged trioculate cercaria. Collar and all systems evident. (48×). 19. Live, tail of young developing cercaria ex snail. Numerous vesicular bodies. (150×). 20. Live tail of older developing cercaria ex snail. Fewer vesicular bodies present. (150×). 21. Live tail of developing cercaria. Arching caudal ducts, apparent mucoid secretions around tail. (150×). 22. Live cercaria beginning to encyst. First, thin irregular layer visible. (100×). 23. Live. Metacercaria approximately 50 days old. (150×). 24. Live metacercaria dissected ex cyst. Approximately 50 days old. (150×). 25. Live adult recovered from experimentally fed and laboratory-raised *Chelonia mydas*. Ten days old. (150×). 26. Composite drawing of "mature" cercaria. Ventral view. Note primordia of reproductive system as observed in sections.



FIGS. 27–29. 27. Composite sagittal section to show relationship of digestive, excretory, and primordia of reproductive systems. 28. Schematic representation of flame-cell pattern in "mature" cercaria. 29. Detailed relationship of cephalic glands, nervous system, and three eyespots in "mature" cercaria.

time the cercariae emerge. The tubes in a developing cercaria end blindly in the mid-region of the tail (Fig. 7, 8). Aforementioned cystogenous cells and pigmentation make it very difficult to see the smaller ducts and complete flame-cell pattern. The anterior part of the cycloid duct (Fig. 26, 28) is inconspicuous indeed in this larva and only careful observations confirm its presence. Repeated observations and plotting the location of flame cells have definitely established the pattern to be that ascribed to the Family by Kuntz, 1951.

CYST FORMATION AND METACERCARIA

Cyst formation occurs very rapidly. The cercaria settles on a suitable substrate, contracts strongly, and secretes cystogenous material from the entire body surface. While the secretion is taking place, the violently lashing tail detaches from the body; often the posterior body region contracts to form a small rounded knob between the locomotor pockets, aiding in the tail detachment. The body of the cercaria revolves actively, is contracted so as to be almost circular in outline, molding the cyst wall during its movements (Fig. 22). The cyst wall is formed in layers, the first secreted being irregular and spread out on the substrate. The completed cyst is round, dome-shaped, and firmly attached. Although a thin wall is formed within 2 to 3 min, cyst wall formation is not completed for approximately 8 to 12 min. A well-formed cyst wall averages 0.026 mm in thickness, and with age often appears yellow–brown in color. The cyst diameters exclusive of the attachment layer range from 0.315 to 0.341, 10 averaging 0.330. The diameter of the space within the cyst averages 0.294 (Fig. 23).

When cyst formation is complete, the larva relaxes, may lie in a bent or folded position; or as it ages, may shorten and lie straight across the cavity. Older metacercariae are less active than the younger, and actually show little development beyond that of the cercariae. The eyespots tend to lose their organization; remnants of them are carried over into the adult worms as dark blotches of pigment. As would be expected, the digestive system is much more visible than in the cercaria. Most of the internal organs are obscured by the natural opacity of the animal which was first described for the adult. The number of flame cells is increasing over that of the cercaria, this is evident by the spotting of an additional pair in the mid-lateral region. Pigmentation of the animals prevents following this development through to include the adult. Worms have remained viable within cysts kept in the laboratory for a period of 5 to 6 months (Fig. 24).

The larvae decrease in size with age; the average size of those dissected from month-old cysts measured 0.369 long and 0.166 wide. Measurements of the testes in a 42-day-old larva were 0.0208 by 0.0204 and 0.0194 by 0.0218; overall size of this metacercaria was not determined because of damage during dissection.

In unpublished data, Dr. John J. McDermott, Jr., found similar cercariae in *Nassarius obsoleta* from tidal ponds and marshes of the southwestern coast of New Jersey. He noted that mature cercariae are often much larger than the parent redia; this is true of my observations. The cercariae are also larger than the metacercariae which tend to decrease in size with the duration of encystment. McDermott also noted the primordia of the gonads (testes and ovary), but did not see the entire cecae nor excretory system. In spite of the failure to see the complete details, such as collar, diverticula on gut, etc., I believe that he worked with the same larva; his measurements, comparisons of the general morphology, behavior, method of encystment, the metacercaria, and the fact that many northern diamond-back terrapins were present in his collecting area permit me to name the New Jersey coast as a second locality for *Pleurogonius malaclemys*.

Detailed comparisons with other marine monostomate cercariae, e.g., *C. ephemera* Lebour, 1911, *C. lebouri* Stunkard, 1932, and *C. caribbea I* Cable, 1956, are not being given. The forms can be compared only as to the overall size and other general characteristics due to the difficulties of observing details which resulted in incomplete descriptions. *C. caribbea I* is the only one definitely described as a marine pronocephalid. Size, host differences, as well as Cable's observations on the ecological relationships and probable adult

affinities, allow me to take the liberty of assuming it to be a different species.

FEEDING EXPERIMENTS

Although morphology of both the cercaria and metacercaria strongly indicate that the above described forms are larvae of *Pleurogonius malaclemys* Hunter, 1961, feeding experiments were undertaken to corroborate the identification. Snail opercula heavily covered with cysts were fed to the experimental hosts used. Cysts on the opercula were checked for viable metacercariae before feeding them.

Three small and immature adults were obtained from the posterior region of the small intestine of an adult female *Malaclemys t. centrata* 10 days after feeding. Forty-three mature adult worms were obtained from the same host, indicating that the experimental infection was superimposed on a natural one even though the turtle had been kept in the laboratory for 3 months prior to the infection. All turtles kept in the laboratory were fed only frozen shrimp.

Through the courtesy of Dr. Peter Klopfer of the Zoology Department of Duke University, eight 11-month-old *Chelonia mydas* which had been laboratory-raised and shrimp-fed were made available to me. These turtles change their feeding habits from carnivorous to herbivorous during their second year and using them as experimental hosts was considered questionable. However, in one of these hosts, eight immature worms were recovered 10 days after cyst-feeding. Turtles which were kept 16 to 24 days after feeding yielded no *Pleurogonius*. Besides using an unnatural host, it is very probable that the changing physiological conditions naturally occurring in them played a significant role in the results. So far, it has been impossible to obtain laboratory-raised *Malaclemys* for the experiments. However, the two successful feedings do confirm the accurate identification (Fig. 25).

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