

SOME OBSERVATIONS ON THE STRUCTURE AND
LIFE-HISTORY OF *CERCARIA ANDHRAENSIS*
N.SP. (TREMATODA: ECHINOSTOMATIDAE)
FROM THE APPLE SNAIL *PILA GLOBOSA*
SWAINSON OF WALT AIR

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ABSTRACT

The morphology, anatomy and stages in life-history of *Cercaria andhraensis* n.sp. from the apple snail *Pila globosa* Swainson of Waltair have been described. *C. andhraensis* has 33 spines which are inconspicuous in the cercaria but are clearly seen in the metacercaria occurring in the snail host itself. The anatomy of the cercarial tail is given in detail. Differences in the tegument of the body and the tail are indicated. The tail tegument more clearly gives the impression that the so-called tegument of trematodes is a modified epidermis. It is suggested that the cercaria and the metacercaria may one day prove to be the stages of either *Echinostoma govindum* Moghe, 1932, or *E. crecci* Verma, 1936.

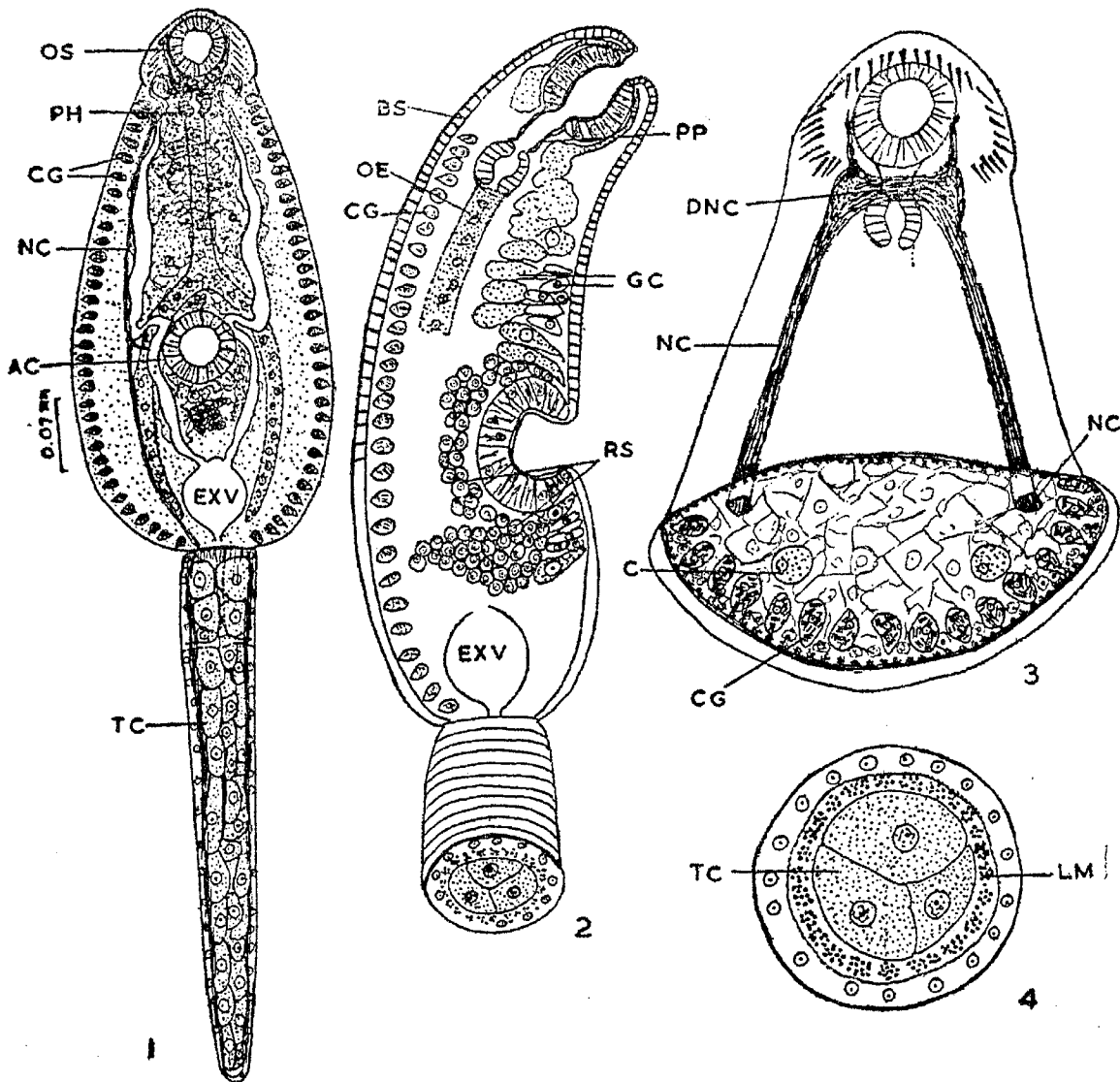
GANAPATI AND HANUMANTHA RAO (1968) have recently reported the occurrence and anomalous emission of larval stages of an echinostome cercaria with 33 spines in *Pila globosa* collected locally. Some further observations on the structure and life-history of this cercaria are given in the present communication.

MATERIAL AND METHODS

Specimens of *P. globosa* were collected from ponds in and around Waltair and kept individually in finger bowls. The infected snails were spotted by the emergence of cercariae which took place in about 24 hours. The live cercariae were examined with or without neutral red. Bouin's fluid, Susa or Zenker's solutions were used as fixatives and the sections were stained with Iron haematoxylin-saffranin, Heidenhain's azan, PAS-light green or PAS-bromophenol blue. Measurements are given in mm.

OBSERVATIONS

The incidence of emergence was about one in fifty. The cercariae begin to emerge at dawn and continue to do so till late afternoon. The cercaria (Text-Fig. 1) swims briskly and occasionally the body is rolled into a ball. The body measures 0.53 in length when moderately extended with a breadth of 0.28. The oral sucker (OS) has a diameter of 0.06. There is a well-developed collar but the spines are inconspicuous. In sections processed according to the azan technique the spines appear more or less vividly in the fully developed cercariae. In the metacercaria (Text-Fig. 8) they could be seen and observed quite easily. There are in all 33 spines



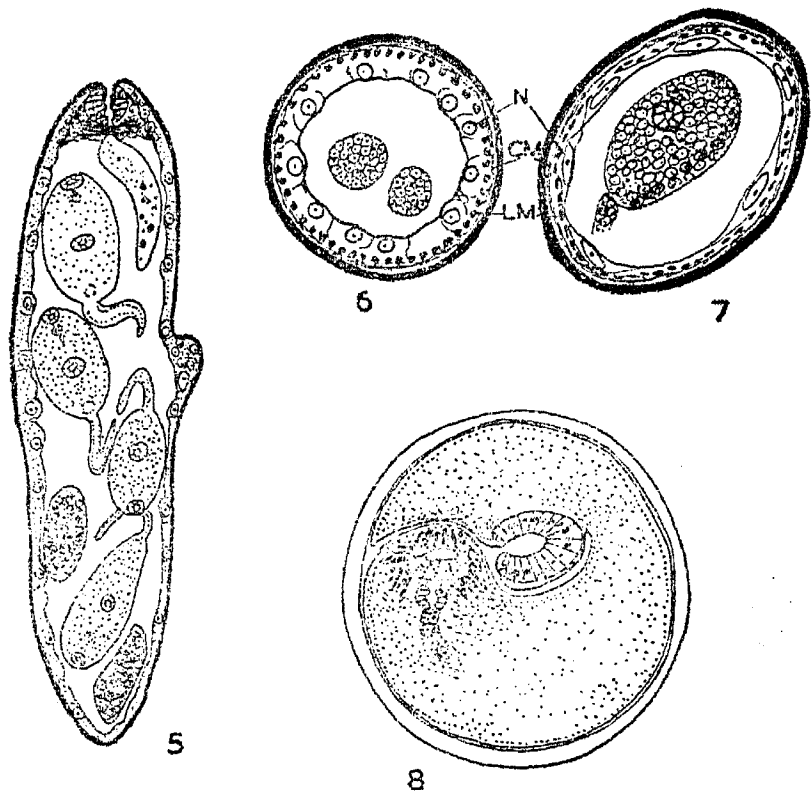
TEXT-FIGS. 1-4. *Cercaria andhraensis* n.sp. Fig. 1. Cercaria entire (ventral view). Fig. 2. Diagram of sagittal section of cercaria. Fig. 3. Diagram of anterior part of cercaria. Fig. 4. Diagram of T.S. of tail.

measuring $11\ \mu$ each, arranged as follows: 4 (corner) + 6 + 6 + 1 (median) 6 + 6 + 4 (corner). Body spines (BS) are embedded in the cuticle dorsally and ventrally up to the level of the acetabulum. The acetabulum located at a distance of 0.8 from the anterior end is 0.07 in diameter.

The mouth leads into a prepharynx (PP) 0.03 in length followed by a pharynx (PH) 0.03 in diameter. The oesophagus and caeca are in the form of solid columns of cells. These cells are loaded with characteristic granules which stain blue-black with iron haematoxylin, red with azan, blue with bromophenol blue and green with light green. They are PAS-negative. It is thus possible to say that they represent protein granules. Cystogenous gland cells (CG) (Text-Figs. 2 and 3) and other gland cells (GC) could be noted clearly. The cystogenous gland cells (CG) are loaded with rod-shaped refringent bodies and are situated in the dorsal and dorsolateral regions beneath the tegument. Other gland cells are (Plate VIII, Fig. 1) located ventral to the digestive system. Some of the cells of this gland complex extend to the anterior end of the worm to open out by fine ducts. The reproductive system consists of a mass of cells situated posterior to the acetabulum and connected to a smaller preacetabular mass by a strand of cells. The excretory system is typical of the echinostome pattern and 21 pairs of flame cells could be counted. The nervous system consists of two conspicuous ventral lateral nerve cords. These nerve cords are connected by a transverse dorsal commissure at the level of oesophagus somewhat overlapping the anterior end of pharynx.

The matrix of the tegument or distal cytoplasm (Moreeth, 1967) of the tail (Text-Fig. 4) is relatively thin and the nucleated region of the cytoplasm lies immediately underneath it. These nuclei measure $3\ \mu$ in diameter. Striations which look like circular muscle fibres run around the tail (Plate VIII, Fig. 2). While reviewing the literature on this outer layer or tegument in helminths, Lee (1966) agreed with Hyman's (1950) theory that in trematodes the so-called cuticle represents an 'outer layer of an insunk epidermis the cells and nuclei of which have sunk beneath the subcuticular musculature'. It is clear that this outer layer should be considered as a modified region of epidermis which in fact is present. The epidermal nature is much more clear in the tail of the cercaria where there does not appear to be any so-called sinking of the nucleated region. It is of interest to mention here the remarks of Belton and Harris (1967) on the tail of the cercaria of *Acanthatrium oregonense* (Macy). From an electron microscope study these authors have revealed the contrasting characters of the body and tail tissues. The temporary nature of the tail which is destined to be cast off before

the cercaria gains entry into the second intermediate host is given out as the possible reason for this difference. Our observations are in complete agreement with these views. The epidermis is followed by a conspicuous layer of powerful longitudinal muscles. The interior of the tail is made up of large cells with glandular properties being PAS (saliva stable), alcian blue and aniline blue positive (Plate VIII, Figs. 1 and 3). The vesicular nuclei of these cells measure 6μ in diameter which is double the size of the nuclei of the epidermis. Lie and Basch (1967) have noted that in the tail of the cercaria of *Paryphostomum segregatum* Dietz, 1909 (echinostome) there is a central part consisting of dense tissue and a peripheral part of loose tissue with cross-striations. Obviously the dense tissue seems to represent the glandular cells and the peripheral layer the epidermis.



TEXT-FIGS. 5-8. Fig. 5. Diagram of redia. Figs. 6 and 7. T.S. of redia. Fig. 8. Metacercarial cyst. AC, acetabulum; BS, body spines; C, caecum; CG, cystogenous glands; CM, circular muscles; DNC, dorsal nerve connective; EXV, excretory viscle; GC, gland cells; LM, longitudinal muscles; N, nuclei; NC, nerve cord; OE, oesophagus; OS, oral sucker; PH, pharynx; PP, prepharynx; RS, reproductive system; TC, tail cell.

The cercariae develop in elongate rediae (Text-Fig. 5) measuring upto 2.5 in length. A fully developed redia may contain 5 to 6 cercariae and 2 to 3 germinal masses. The wall of the redia (Text-Figs. 6, 7) is provided with circular and powerful longitudinal muscles. Internal to the muscle layer there

is a wall of cells containing vesicular nuclei. The outermost layer is a tegument. In younger rediae the cells of the inner layer appear cuboidal. In older and longer rediae these cells become flattened, there being considerable stretching during the movements of rediae.

The cercariae swim vigorously and are capable of encystment in a short time in the snail host itself. The problem of intraredial encystment of the cercaria and anomalous emission of larval stages has already been reported by us (Ganapati and Hanumantha Rao, 1968). The spherical cyst measures 0.152 in diameter. The metacercaria as in other echinostomes lies folded in a characteristic way. All traces of gland cells in the body disappear. The spines become conspicuous. Attempts to obtain the adult stages by feeding experiments with the Indian gerbil *Tatera indica* have been partly successful. The gerbils were examined for previous infections by daily examination of faeces. After ensuring that they were negative for trematode infections they were starved for two days and fed with pieces of infected snails containing metacercariae. Seven days later immature specimens with well-developed collar and partly developed reproductive system were recovered from the intestines of the experimental hosts.

DISCUSSION

Some of the earlier reports on echinostome cercariae from India are those of Sewell (1922), Faruqui (1930), Chatterji (1932) and Peter (1955). It would appear that the trematode infestation in the apple snail *P. globosa* has not hitherto received adequate attention in India. Among the echinostome cercariae so far known from this country the present cercaria resembles *C. indicae* XII Sewell, 1922 and *C. indicae* XXIII Sewell, 1922 in the inconspicuous nature of collar spines. But the number of spines in the form reported here is 33 while both *C. indicae* XII and *C. indicae* XXIII possess 43 spines.

It may be of some interest to make a few comments on the structure of the tail. Cardell Jr. and Philpott (1960) have studied the ultra-structure of the tail of the cercaria of the echinostome *Himasthla quissetensis* (Miller and Northup, 1926). Their electron micrographs revealed the presence of an outermost cuticle charged with mitochondria and vacuoles though there is no indication of nuclei in this layer. In the present echinostome small nuclei with distinct nucleoli have been seen in the outermost layer which appears to be syncytial. Since the tail is used only for locomotion and is cast off when the cercaria enters the intermediate host it may be

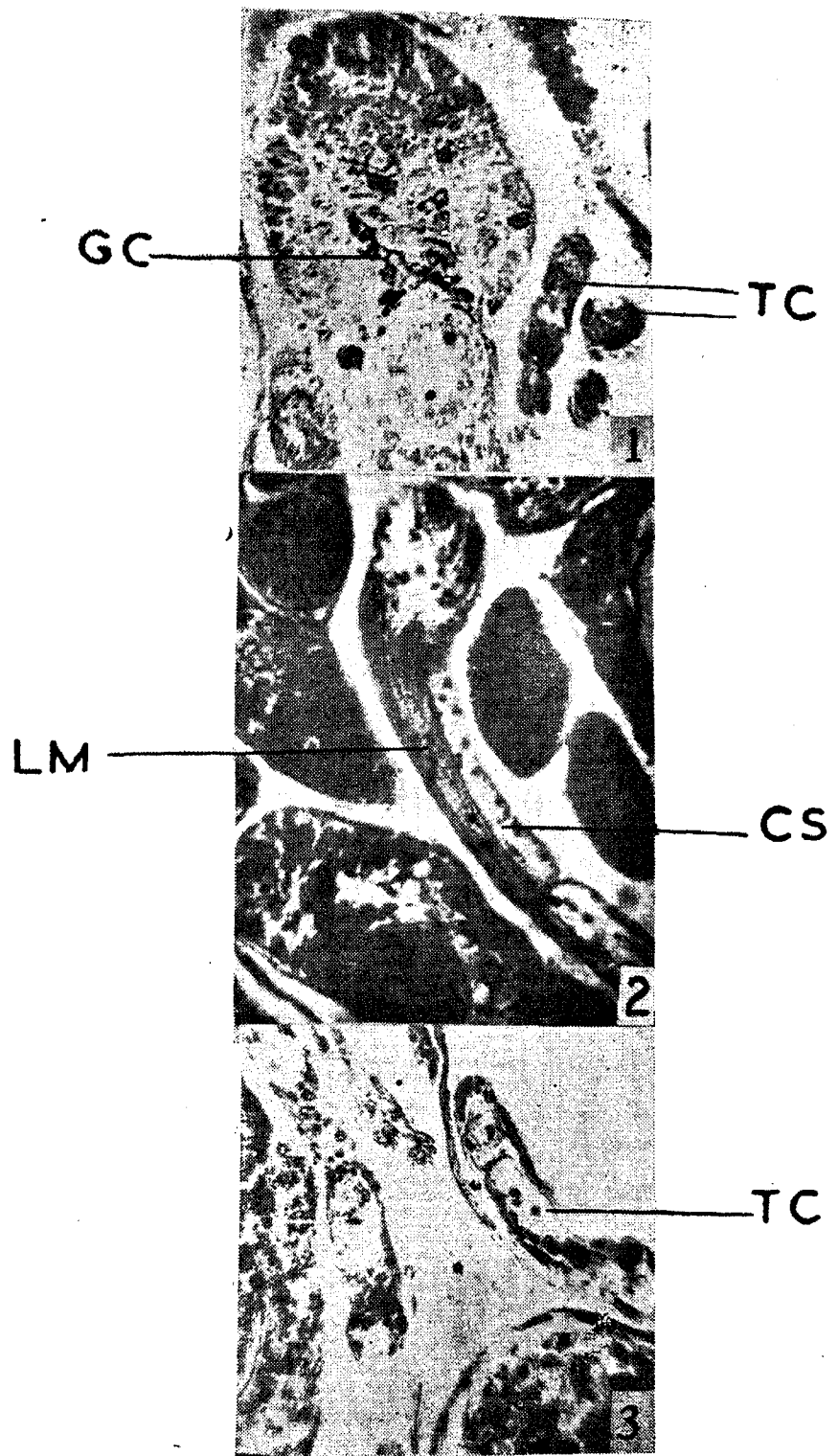
expected that the organization of the so-called cuticle of this region is somewhat different in comparison to the tegument of the body. It is tempting to call the outermost layer of the tail simply as epidermis rather than considering it a cuticle or tegument of the main body.

Powerful longitudinal muscles are present. The interior of the tail is packed with big cells containing larger nuclei almost double the size of the nuclei found in the outermost layer. The tail cells generally regarded as 'parenchymal cells' are histochemically different from the typical cells of parenchyma in being alcian blue, PAS and aniline blue positive. Similar observations have been made by Rees (1967) in the case of the tail cells of the cercaria of *Parorchis acanthus* Nicoll. Thus they conform more nearly to the gland cells comparable to those in the ventral region of the body of the cercaria.

As far as the adults are concerned it is reported that *Echinostoma govindum* Moghe, 1932 and *E. crecci* Verma, 1936 possess 32 spines. At present it is not known how far this number of spines can be taken as correct. Because it is usual for echinostomes to display an odd number of spines. It is possible that the present cercaria may one day prove to be close to either *E. govindum* or *E. crecci*. The name *Cercaria andhraensis* is suggested for the present form.

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FIGS. 1-3