CHAPTER VI

MORPHOLOGY AND LIFE HISTORY OF

*ECHINOOSTON LUNOJII N.SP.*
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ECHINOSTOMA LUTEOLE N.SP.

On 4th November, 1969 *Lymnaea luteola f. australis* (Lamarck) were collected from a particular area of Polsapara tank in Durg (M.P.). The snails were individually isolated and tested for cercarial infection, and were found to be infected with a 37 collar spined echinostome cercaria. From an average of 30 snails isolated at a time, 2-5 were found to be infected with this cercaria.

Two lots of about 100 *Lymnaea luteola f. australis* were brought to the laboratory on 25th and 31st March, 1970 from Mohan Nagar tank of Durg. These snails when individually isolated were found to be infected with the same cercaria, which was found to be emerging from *Lymnaea luteola* collected from Polsapara tank.

MATERIALS AND METHODS

Cercariae and rediae from natural infection were studied in fresh and living conditions stained with neutral red and methylene blue. Unstained cercariae were used for the study of number and arrangement of collar spines and also the excretory system. Redia, cercariae and metacercariae
were fixed in 10% hot formalin and 70% alcohol for measurements. The adults were fixed in warm Bouin's fluid and stained with Mayer's paracarmine and eosin. Naturally shed cercariae encysted in clean laboratory raised *Lymnaea luteola f. australis* (Lamark) and *Indoplanorbis exustus* (Deshayes). In the absence of second intermediate snails, (snails of the above mentioned species in this case) cercariae encyst in the same snail from which they have been shed. These experimentally obtained metacercariae were fed to two 10-12 days old clean duckling for the adult worms. Though hatching experiments were set up, miracidia could not be obtained. Drawings were made with the aid of camera lucida. All measurements are in millimeters.

**RESULTS**

**DESCRIPTION OF STAGES IN THE LIFE CYCLE**

**Rediae** (Plate XIII Fig. 2; Plate XIV Fig. 2)

The cercariae develop in rediae which are found in large number in the liver of the first intermediate host. Mature rediae 2.00 to 2.03 long by 0.367 to 0.390 wide contain developed and undeveloped cercariae and germ balls. They are provided with conspicuous procusculae and collar, the pharynx being 0.101 in diameter. The gut is comparatively small. The birth pore is situated 0.219 from the anterior extremity.

**Cercaria** (Plate XIII Fig. 1, 3, 4 and 5 Plate XIV fig. 1)
In the laboratory the cercariae emerge in large numbers from the snail host throughout the day. Measurements are based upon 25 living and 10 fixed (in 10% hot formalin) specimens. Body spinose, 0.389 to 0.392 long by 0.120 to 0.167 wide. Collar distinct, (Plate XIII, Fig. 5) 0.120 to 0.127 broad with 37 collar spines of equal size, 0.012 long and arranged in double rows uninterrupted dorsally. Oral sucker subterminal, spherical 0.049 - 0.050 in diameter. Prepharynx 0.015 to 0.018 long. Pharynx nearly spherical, 0.044 to 0.051 long by 0.046 to 0.049 wide. Oesophagus solid made up of 6 to 7 cells, and 0.119 to 0.122 long. Intestinal caeca thick, containing granular material, extending up to the posterior end of body. Three pairs of penetration glands lie in lateral oesophageal field. Besides these glands there are 5 pairs of other glands, present in the anterior region of the body of the cercaria. Such glands (Plate XIII, Fig. 1) have been referred to as para oesophageal glands by Kian Joe Lie (1966). These glands and their ducts stain deeply with neutral red. The ducts dilate and constrict at intervals throughout their course, have granular contents and open in the collar region laterally at the base of oral sucker. Acetabulum strongly muscular, post equatorial, slightly larger than the oral sucker, 0.025 to 0.027 in diameter. Genital primordia two masses of cells, one located at the anterior margin of the acetabulum
0.013 by 0.024, the other between acetabulum and the excretory bladder 0.036 by 0.039, both connected by a string of cells passing dorsal to the acetabulum. Cystogenous cells stain very lightly with neutral red, have granular and rod-like contents and extend laterally from level of pharynx to the posterior end of body.

Tail aspinose, longer than body, 0.704 to 2.451 long by 0.043 to 0.047 wide at base and 0.015 wide at tail tip. Two dorsolateral and one ventrolateral fin folds present, at posterior end of tail. (Plate XIV Fig. 4).

Excretory bladder bipartite, 0.078 to 0.081 wide. Excretory pore situated at junction of body and tail. The upper smaller chamber communicates with the main lateral excretory ducts of both sides, which pass anteriorly in a sinuous course and contain numerous excretory granules of different shapes and size, 0.010 to 0.013 long by 0.006 to 0.009 wide in the distended portion between the pharyngeal region and the acetabulum. Anteriorly at the level of prepharynx the main ducts of each side make a triangular loop, then extend posteriorly to the distal end of the body and turn again anteriorly. As far as could be ascertained, there are 42 flame cells in the body. They are arranged as shown in figure 1 of Plate XV; Flame cell formula:

\[
2 \left(\begin{array}{c} 3 + 3 + 3 + 3 + 3 + 3 + 3 \end{array}\right) = 42
\]
Caudal excretory ducts run into anterior one-sixth of tail, bifurcating into two lateral branches. There are no flame cells in the tail.

Related species:

The above described cercaria resembles the cercaria of *Echinostoma revolutum* (Froelich) Beaver, 1937; *Cercaria helvetica* XXIV Dubois, 1929; 'unnamed cercaria' Johnston and Muirhead, 1949; *C. cuneata* Fain, 1953; *Cercaria lindoensis* Khan, 1960 and cercariae of *Echinostoma lindoense* (Sandground and Bonne, 1940) Lie, 1968; *E. radicatum* Nasir, 1960; *E. binnicaudatum* Nasir, 1961; *E. audyi* Lie and Umathey, 1965; *E. barbosi* Lie and Basch, 1966; *E. paraesot* Lie and Basch, 1967 and *E. rodriguezi* Hsu, Lie and Dasch, 1968 in the possession of 37 collar spines and fin fold on the tail. It, however, differs from the above mentioned species in some remarkable characters, which differentiate it from other known echinostome cercariae.

The present form differs from the cercaria of *Echinostoma revolutum* in the nature of the excretory system and the number of flame cells. There are 43 flame cells in the latter species and 42 in the present form. *C. helvetica* XXIV has been shown by Beaver to be identical with cercaria of *E. revolutum*.

In the present form the fin folds are confined to the
distal region of the tail while in the 'unnamed' cercaria of Johnston and Hairhead. There are fin folds at the base of the tail as well.

There are 5 corner spines in the present from, while there are 4 corner spines in _C. cuneata_ which also differs in the extension of the tail fin fold.

The present species of cercaria differs from _Cercaria londonensis_, cercariae of _E. lindoense_, _E. audyi_; _E. barbosi_, _E. paraensei_, and _E. rodriguesi_ in the nature of tail fin folds, the number of penetration glands, distribution of paraccesophageal glands (if present) and the number and distribution of flame cells.

In _C. londonensis_ the dorsoventral tail fin continues from the base of the tail to its distal end, while in the present form there are 2 dorsolateral and 1 ventrolateral small fin folds at the distal end of the tail. The former species has long hair-like structures on the body and tail, while such structures are absent in the present species.

In cercaria of _E. lindoense_ there are 6 pairs of penetration glands and 42 flame cells, the fin folds are
seen on the dorsal and ventral parts of the tail. The cystogogenous cells have granular contents. Cercaria of *E. audyi* has a pattern of duct outlets completely different from that of the present species. It has a total number of 54 flame cells and a tail identical with that of cercaria of *E. lindoense*.

Cercaria of *E. barbosi* has 4 gland duct openings on the dorsal lip of oral sucker and 48 flame cells in total.

Cercaria of *E. paraensei* has 42 flame cells in the body and 6 to 8 gland duct openings, while there are 3 pairs of penetration glands, and paraoesophageal glands are absent in cercaria of *E. paraensei* while these glands are present in the present form.

There are 4 pairs of penetration glands in cercaria of *E. pinnicaudatum* and 48 flame cells and there is no mention of paraoesophageal glands in this form.

The present form differs from the cercaria of *E. pinnicaudatum* as the latter species is devoid of any fin fold in the tail.

**Metacercaria** (Plate XIV, Fig. 3)

Metacercarial cysts spherical, 0.258 to 0.261
in diameter. Cyst wall 0.012 to 0.015 thick double layered, the outer layer is hyalinous 0.009 in thickness and the inner layer is opaque measuring about 0.005 in thickness. Hundreds of metacercarial cysts may be found in the mantle, pericardial sac and foot of the snail host. Cysts become viable after 48 hours encystment and remain so till a long period of 30 to 35 days.

Altogether 63 infected snails lodging 2 to 14 days old metacercariae were fed to 2 ducklings 10 to 12 days old. On autopsy 20 days post-infection, fifty five 37 collar-spined echinostome adults belonging to the genus Echinostoma were recovered from the rectum of the experimental hosts.

Echinostoma luteoli n.sp.

Adult (Plate XVI Fig.1)

All measurements are based on 20 specimens fixed in warm Bouin's fluid.

Diagnosis: Echinostomatidae; Echinostoma Rudolphi 1809 sensu Dietz, 1909, emend, Kendhjem, 1943. Body 7.520 to 7.880 long
by 0.601 to 0.608 wide at level of pharynx; 1.106 to 1.131 wide at level of acetabulum and 0.858 to 0.962 wide at level of genitalia. Minute cuticular spines 0.024 long by 0.006 wide present on the body surface. They extend up to anterior one third of body. Collar well developed (Plate XVI, Fig. 2; Plate XVIII) 0.273 to 0.299 long by 0.468 to 0.715 wide, with conspicuous collar spines arranged in double dorsally uninterrupted rows measuring 0.105 to 0.115 long by 0.025 wide. Five corner spines 0.110 to 0.114 long by 0.027 wide in each ventral lappet, 3 orals and two aborals; 5 lateral spines on each side, arranged in a single row; 17 dorsal 0.130 to 0.132 by 0.020 to 0.021 wide arranged in an uninterrupted double row, 8 oral and 9 aboral.

Oral sucker slightly subterminal, 0.247 to 0.273 by 0.160 to 0.208. Prepharynx comparatively small 0.065 to 0.156 long by 0.052 to 0.073 wide. Pharynx subglobular, 0.182 to 0.195 long by 0.169 to 0.182 wide. Oesophagus with an irregular margin, 0.390 to 0.421 long by 0.065 to 0.073 wide. Acetabulum strongly developed in anterior one-fourth of body, 0.753 to 0.794 by 0.375 to 0.900. Genital pore situated immediately in front of acetabulum. Cirrus sac (Plate XVI, Fig. 3; Plate XVII, Fig. 1) anterodorsal to acetabulum slightly lateral, 0.390 long by 0.246 wide,
containing coiled vesicula seminalis, pars prostatica, and cirrus. Testes tandem post equatorial and ovoid, anterior testis 0.457 to 0.463 long by 0.325 to 0.468 wide; posterior testis 0.461 to 0.470 long by 0.221 to 0.234 wide. Ovary (Plate XVII, Fig. 2) spherical post equatorial, median in position measuring 0.143 to 0.156 in diameter. Mehlis' gland complex 0.167 long by 0.260 broad. Uterus intercaecal extends in the region from posterior margin of acetabulum and anterior margin of ovary, consisting of several coils lodging numerous, operculate eggs. Receptaculum seminis is absent, A receptaculum seminis uterinum is present. Laurer's canal opens dorsally, observed in living specimens. Vitellaria lateral extending from the level of the first one-fifth of the uterine coils, upto the level of the posterior half of excretory bladder. In testicular region some follicles may cover the intestinal caeca entirely or even enter the intercaecal zone. Excretory bladder bifurcates immediately posterior to posterior testis. The stem of the excretory bladder, 1.56 by 0.130 and opens posteriorly through the excretory pore.

Host: Natural host unknown,
Experimental host: Ducklings
Location: Rectum
Locality: Durg (M.P.) India.
DISCUSSION

There are several 37 collar-spined species in the genus *Echinostoma*, as enumerated by Lie (1964) in his paper on the life history of *E. lindoense* Sandground and Bonne, 1940. Deaver (1937) reduced eight species of *Echinostoma* with 37 collar spines to synonymy with *E. revolutum*. He considered the other 3 species as doubtful and transferred the remaining two 37 collar spined forms, *E. aconiatum* Dietz, 1909 and *E. contignum* Barker and Bastron, 1915 to the genus *Echnonarychium*.

Deaver (1937) did not include in his critical discussion, the two forms described by Yamaguti, and 13 additional new species have been erected since, therefore, sixteen 37 collar spined forms have been recognised in the genus *Echinostoma* Rudolphi, 1809.

Lie (1964) enlisted 12 species as having 37 collar spines. To his list four new species described later on, could be added as below:

*Echinostoma revolutum* (Froelich)
*E. ralli* Yamaguti, 1934
*E. robustum* Yamaguti, 1935
*E. splendens* Verna, 1936
*E. lindoense* Sandground and Bonne, 1940
*E. revolutum* tenuicollis Baschkirova, 1941
E. stromi Bradski rova, 1946
E. sp. Sedovskaja, 1952
E. goldi, Ochmarin, 1956
E. rudicaudatum Nasir, 1960
E. vinnicaudatum Nasir, 1961
E. londonensis Khan, 1961
E. audyi Lie and Umathevy, 1965
E. barboesi Lie and Basch, 1966
E. paraensei Lie and Basch, 1967 and
E. noelgei Hsu, Lie and Basch 1968.

The present form resembles the above mentioned forms in the possession of 37 collar spines. It, however, differs from them in other remarkable features such as the arrangement of the collar spines, a factor that has been emphasized to be the most important feature in determining the species of echinostomes (Mathias, 1925; Beaver, 1937; Bonne, Bras and Lie, 1953).

E. ralli has end groups of 4 collar spines, E. goldi had end groups of 6 collar spines on each side. Echinostoma sp. has 3 end group spines on one side and a single one on the other, all the remaining spines are arranged in a row. E. splendens has 5 corner spines on each side and all the remaining spines are arranged in double rows. E. rudicaudatum
has 5 corner spines on each side and 7 laterals in a single row.

The collar spine arrangement is not known for *E. revolutum* tenuicollis and *E. robustum*, therefore, a careful comparison is not possible. *E. lindoense* has 5 corner spines, and 6 laterals on each side, 15 dorsals are arranged in double dorsally uninterrupted rows. This arrangement is similar to that of *E. revolutum*.

The present form resembles *E. pinnicaudatum* in the arrangement of the collar spines. It, however, differs from this species in size and nature of cirrus sac, the disposition of the female genital organs, the nature of vitellaria and the cercarial characters. *E. pinnicaudatum* has 5 corner spines and 5 laterals on each side. There are 17 dorsal spines, 8 orals and 9 aborals. This arrangement is exactly similar to that of the present form except that the dorsal collar spines of the oral row in *E. pinnicaudatum* are only slightly shorter than the aborals. In the present form the dorsals are of the same size. Nasir (1961) further states that the adults of *E. pinnicaudatum* and *E. nudicaudatum* are similar but the arrangements of the collar spines in both the forms is different.

The present form further differs from *E. pinnicaudatum* in the relative position of ovary, which is postequatorial in
the present form, and equatorial in *E. pinnicaudatum*.

The present form resembles *E. audyi*, *E. barbosi*, *E. paracensai* and *E. rodriguezii* in the number of collar spines but differs in their arrangement, in the general morphology of the adult and in the cercarial characters.

The adult of the present form resembles *E. audyi* in a general way but differs in the measurement of the body organs, the extend of the body spination, it extends until the middle of the level of excretory bladder which is simple and tubular in *E. audyi*. The smaller cirrus sac in *E. audyi* is situated anterior to the acetabulum in a dorsomedian position.

Following the traditional method of establishing a new form, the distinctive morphology of the adult and larval stages has been used as a criteria to identify and establish a trematode at the species level, and this new form has been named *Echinostoma luteoli* n.sp.