

STUDIES ON FRESHWATER LARVAL TREMATODES.  
PART. VI. EXPERIMENTAL OBSERVATIONS ON VARIOUS  
STAGES IN THE LIFE CYCLE OF *ECHINOSTOMA DONO-*  
*SOI*. N. SP. (ECHINOSTOMATIDAE: TREMATODA) FROM  
VENEZUELA

P. Nasir  
Escuela de Biología,  
Universidad de Oriente,  
Cumaná, Venezuela.

INTRODUCTION

Since the inception of studies on freshwater larval trematodes from Cumaná, Venezuela, and the vicinity thereof, Nasir (1964a; 1964b; 1964c; 1964d; 1964e) has published a series of papers while another paper involving descriptions of "a new Virgulate Xiphidiocercaria, and another new Longifurcate, Pharyngeate Distome Furcocercaria" is in press. Still another paper (see Nasir and Acuña, 1964) about the description of a new Gymnocephalic cercaria has been presented in a scientific meeting. The paper advanced in this publication is the sixth and the very first in the current series dealing with experimental demonstration of different phases in the life cycle of a digenetic trematode.

On September 20, 1963 Dr. R. Donoso-Barros of the Escuela de Biología, Universidad de Oriente, brought 31 specimens of *Marisa cornuarietis* (L.) including 23 color-banded and 8 unicolor forms from Llanada de San Juan about five kilometers south of the University. One specimen of the unicolor forms was discharging echinostome cercariae. This very snail was placed in a small aquarium with five laboratory-bred seven months old color banded forms of *M. cornuarietis*, as these were the only clean laboratory-bred stock of snails available, in order to obtain cysts for infection experiments. After fourteen days all of the five individuals were dissected and a varying number of cysts was recovered exclusively from their pericardia. The maximum number of cysts obtained from the pericardium of a single snail was 81. These fifteen days

old cysts were divided in two group of 60 and 21 each and fed to two laboratory-bred pigeons of nine months age. Daily fecal examination was conducted and the echinostome eggs appeared on the thirteenth day of the introduction of cysts in the feces of a pigeon which had been infected with sixty cysts. This very pigeon was dissected the same day and four adult egg-discharging echinostomes were recovered from its small intestine.

#### MATERIALS AND METHODS

The laboratory-bred specimens of color banded form of *Marisa cornuarietis* (L). were fed exclusively on lettuce and from time to time sticks of black-board chalk were added to the aerated aquarium. The chalk was apparently a good source of calcium supply for the healthy condition of the shells of developing snails as the absence of chalk resulted in the formation of fragile and bitter shells.

The pigeons for experimental purpose were bred right from eggs in animal house and were given a dry diet of coarsely grounded maize and corn. Ordinary tap water was the only available supply to them. These pigeons were invariably restricted, except when necessary, to separate cages in the locked animal house. This was necessary in order to safeguard against curious intruders and inadvertent access to natural body of water. The only body of water, however, within reachable distance contains brackish water and there is no trace whatsoever of *Mrisa* or other fresh water genera like *Pomacea* which are frequent in distant localities.

Whenever a fresh supply of cercariae was needed the infected snail was confined periodically in a small standard finger bowl. Cercariae were studied alive and only freshly emerged specimens were employed. Addition of a weak solution of malachite green to water mounts was a great help for the determination of cuticular and collar spination. So were the other *intra vitam* stains like neutral red and methylene blue which greatly aided in making out various internal structures. Only measurements were taken on specimens killed by addition of an equal volume of 10% hot formalin.

Adult worms were killed by squirting into Gilson's fluid heated up to 60° C and stained with Aceto - Carmine.

*Redia (fig. 1-2)*

The rediae are so similar in color, shape, size, and general organization to those of the other species of the genus *Echinostoma* for which more or less complete life histories are known, namely *Echinostoma revolutum* as described by Beaver (1937), *E. lindoensis* as described by Sandground and Bonne (1940), *E. nudicaudatum* Nasir (1960; 1962), *E. pinnicaudatum* Nasir (1961) and *E. londonensis* Khan (1961) that it is almost impossible to separate the rediae of one species from the other.

The mature rediae enclosing fairly well differentiated cercariae may attain a length of 1.2 mm. and are orange yellow in color while the immature rediae are almost colorless. There is a typical pharynx, an undivided collar, and a pair of posterior locomotor appendages. Behind the collar, on one side of the body, slightly dorsal in position is a nipple-shaped birth pore considerably protruding in some specimens. In immature rediae birth pore is either lacking or poorly developed whereas in older rediae, in which the cercarial supply is exhausted, the birth pore apparently atrophies and is difficult to make out. Thus, the development of birth pore is seemingly related to the development of cercariae within redial lumen. In nine of the older rediae the collar and the locomotor appendages were also degenerate. The saccate gut normally extends to the level of the posterior locomotor appendages and contains flecks of yellow pigment. In some of the older rediae it is considerably reduced in extent, projecting only slightly posterior to collar region. At each side of the body two groups of flamecells were present, one at the level of the corresponding locomotor appendage and the other somewhat anterior to it. The exact number of flame cells in each group could not be determined with certainty mostly due to opaqueness of the body and a possible confusion with the flame-cells of abounding cercaria. However, in one suitable redia on one side of the body there were

29 flame cells in the anterior group and 34 in the posterior group.

There were all sorts of gradations from unicellular to variously differentiated germ balls. Three of the rediae contained not only developing cercariae but also rediae as well.

*Cercaria (Fig. 3-5)*

The body is furnished with transverse rows of minute spines both dorsally as well as ventrally. Behind the ventral sucker rows of spines are set further apart and spines are reduced in size. In addition to spines there are undetermined rows of "hair-like" processes. Similar processes have been observed in the cercaria of *Echinostoma nudicaudatum* Nasir, 1960 and *E. pinnicaudatum* Nasir, 1961.

Tail is aspinose but furnished with a dorsoventral fin fold running along its entire extent. The extreme posterior tip of the tail is invaginable at times. There are two pairs of longitudinal muscle bands, one dorsal and the other ventral, traversing almost the entire extent of caudal length.

The collar spines are 31 in all including a group of 4 angle spines at each lateral side of the pharynx and arranged in a continuous dorsolateral series, broken only ventrally in the pharyngeal region. The general pattern of collar spination agrees with that of the adult.

The ventral sucker is heart-shaped and situated behind middle of the body. A tubular prepharynx is present. The pharynx is anteroposteriorly elongate. The esophagus divides immediately anterior to the ventral sucker and the intestinal ceca extend almost to the posterior end of body. Both esophagus and intestinal ceca are lined with columns of transversely elongate well demarcated cells containing granular material and each with a vesicular nucleus.

The cystogenous gland cells contain granular and not "rod-shaped" cyst material.

About half-way along esophagus is a group of gland cells apparently different in shape and size from the surrounding cystogenous gland cells. These esophageal gland cells readily stand out when treated with neutral red.

Bordering the extreme anterior tip of the body there are

eight openings leading into eighth duct-like extensions which may extend slightly to the posterior border of oral sucker. No glandular associations could be connected with these duct-like extensions.

The cerebral mass lies dorsally over the prepharynx.

The genital rudiments are represented by three groups of globular cellular masses, one anterior to the ventral sucker and two posterior to it.

The excretory vesicle is a bipartite structure, consisting of a more or less rectangular large posterior division and a smaller somewhat V-shaped anterior division. The anterior V-shaped division is more or less rhythmically disappearing while the posterior division exhibits rather slow rhythmic contractions. The main excretory tubes, only in the preacetabular region enclose refractile excretory granules of a double nature.

There is a characteristic anterior as well as a posterior echinostome loop at each side of the body. The secondary excretory tubules are ciliated throughout their entire extent. The caudal excretory duct divides into two laterally slanting branches in proximal region of the tail. There are 42 flame cells on each side of body. The arrangements of the flame cells, primary, secondary, and main tubes are shown in Fig. 5.

Cercarial emergence takes place during daytime, especially before noon. Cercariae are not particularly attracted by light.

Measurements (in mm.) of ten cercariae: Body, 0.528 - 0.721 by 0.286 - 0.403. Tail, 0.650 - 0.858 by 0.091 - 0.117. Oral sucker, 0.057 - 0.075. Ventral sucker, 0.099 - 0.123 by 0.087 - 0.108. Prepharynx, 0.010 - 0.017 long. Pharynx, 0.039 - 0.045 by 0.027 - 0.039.

#### *Cyst (Fig. 6)*

The cysts are almost spherical and measure 0.31 - 0.33 mm. (live material) in diameter. The cyst wall consists of an outer gelatinous layer and an inner tougher opaque layer. Both layers are approximately equal in thickness, measuring 0.006 -

0.012 mm. in thickness. Thickness of layers is often variable, so that inner layer may have a maximum thickness in contrast to minimum thickness of outer layer and vice-versa. Fibrous material, apparently of host origin, is often attached to the outer gelatinous covering. After four weeks the enclosed metacercaria is still active within the cyst wall.

The naturally infected snail continued to discharge cercariae for 21 days until its cercarial supply was exhausted. On dissection 497 cysts were found in its pericardium whereas only 13 cysts were encountered in its hepato-pancreas. None of the rediae contained any echinostome cyst, however, some of the rediae harbored a varying number of unidentified precystic tetracotyloform larvae. It was interesting to note that in most of the rediae not only the cercarial formation had ceased, but also there was hardly any visible trace of germ balls. At the same time, there was a large number of immature cercariae in intercecal spaces of hepato-pancreas.

These immature cercariae were very inactive, exhibiting a very weak swimming tendency, with a poorly developed fin fold and the cuticular as well as the collar spines were barely recognizable. Moreover, the body was very transparent, in contrast to naturally emerged cercariae, apparently due to the fact that the cystogenous gland cells were not yet filled with fully developed cyst material. These observations suggest that a period of extra-redial development similar to the one described for the cercariae of *Echinostoma nudicaudatum* Nasir 1960, is essential before the cercariae can make their natural escape in to the surrounding water.

*Adult (Fig. 7-9)*

(Measurements in mm.)

Genus *Echinostoma* Rudolphi, 1809 *sensu* Dietz (1909; 1910) emend. Mendheim (1943). Shape of the body is typical of other species of the genus. Length of the body varies from 3.627 - 5.370 and width from 0.702 - 1.631. The greatest width is invariably in the testicular region. Posterior end of the body may be produced into a conical protuberance. The cuticular spination is unequal in distribution. Ventrally spines may ex-

tend only slightly posterior to posterior border of posterior testis, but dorsally they are limited anterior to the anterior border of ventral sucker. The collar is well developed, measuring 0.325 - 0.379 in transverse diameter and beset with a row of 31 collar spines. The collar spines are arranged in a continuous series uninterrupted dorsally but interrupted ventrally in the pharyngeal region. Of these 31 spines there is a group of 4 angle spines, located at each lateral side of the pharynx. Two spines of each angle group are oral and 2 aboral in position. The median spine of dorsal series is aborally placed and measures 0.036 - 0.039 in length. The aboral lateral angle spine varies 0.045 - 0.053 in length. The rest of the spines measure 0.048 - 0.057 in length. The oral sucker is rather weak measuring 0.117-0.127 by 0.104-0.115. The protrusible ventral sucker is heart-shaped and measures 0.611-1.479 in diameter. It is located at a distance of 0.429 - 0.503 from extreme anterior end of the body while its location from the posterior end varies 2.847 - 3.630. The prepharynx measures 0.015 - 0.031 in length. The muscular pharynx is antero-posteriorly elongate, measuring 0.111 - 0.185 by 0.087 - 0.169. The esophagus divides immediately anterior to the ventral sucker and the ceca extend almost to posterior end of the body. The ovary is spheroidal, 0.169 - 0.182 in diameter, not lobed, median, and located at distance of 0.598 - 1.479 from posterior border of the ventral sucker. The true receptaculum seminis, Laurer's canal, ootype and receptaculum seminis uterinum are present. The uterus is intricately coiled and the muscular metraterm after traversing right side of the cirrus sac opens through a female genital pore into a common genital aperture. The eggs are yellowish, operculate, and the extra-uterine eggs measure 0.105 - 0.126 by 0.057 - 0.072. They are not embryonated when discharged. Mehlis' gland is diffuse, 0.130 - 0.186 by 0.182 - 0.293, and located at a distance of 0.026 - 0.037 from the anterior testis. The vitelline follicles of two sides are almost entirely non-encroaching upon intercecal space and stop short of the posterior border of the ventral sucker, rarely extending as far as slightly anterior to posterior border of the ventral sucker. In the post-testicular region a few follicles may extend into intercecal space but follicles of the two sides are never confluent.

The transverse vitelline duct occupies posterior half of the Mehlis'gland. The common vitelline duct opens on the left side of ootype. Posteriorly, the vitelline follicles terminate with posterior terminations of intestine but never extending to extreme posterior end of the body. Post-vitelline space varies 0.169 - 0.257 in length. The testes are entire, median, tandem and the anterior testis is always smaller than the posterior testis measuring 0.351 - 0.46 by 0.195 - 0.271, lying at a distance of 0.143 - 0.270 from posterior border of the ovary. The posterior testis measuring 0.390 - 0.574 by 0.195 - 0.307 lies at a distance of 1.118 - 1.126 from posterior end of the body. The intertesticular space varies from nil - 0.135. The cirrus sac is muscular, somewhat pyriform in shape, measuring 0.210-0.330 by 0.105 - 0.165, and bulk of it lies dorsally about middle of the ventral sucker, never extending more than one-eighth of its length from anterior border of the ventral sucker. Posteriorly, the cirrus sac may extend about halfway in post-equatorial region of ventral sucker. The seminal vesicle contains a swollen basal region and a coiled narrow anterior region. Several prostatic glands open in a spindle shaped *pars prostatica*. A coiled ejaculatory duct emerges from anterior end of *pars prostatica* and opens through a male genital aperture into the common genital pore. The vasa efferentia enter independently at posterior end of cirrus sac. The cirrus is unspined and may attain a length of 0.386. The common genital pore is ventral, bifurcal and measures 0.015 - 0.021 by 0.021 - 0.03. The excretory vesicle is tubular and has three branches on each lateral side of it. It encloses numerous fine granulations.

#### *Miracidium* (Fig. 10 - 11)

Description: The apparently mature miracidium escapes from its egg in 17-21 days in ordinary tap water at a temperature of 21-23° C. The naturally escaped miracidia, narcotized with a weak solution of neutral red, measure 0.081 - 0.133 by 0.042 - 0.056 mm. At anterior end of the body is a typical apical papilla bordered with seven hair-like processes similar to those described for the miracidia of *Echinostoma nudicau-*

*datum* Nasir, 1960. Rest of the body is covered with vibratile cilia borne on epidermal plates arranged in four tiers separated by intervening spaces with the resultant interruption in distribution of cilia. There are 18 epidermal plates and the arrangement of these in corresponding tiers, from anterior to posterior, is as follows: in the anteriormost tier, two of the plates are dorsal, two ventral and two lateral; in the second tier three are dorsal and three ventral; in the third tier two of the plates are dorsolateral and two ventrolateral; finally, in the posteriormost tier one of the plates is dorsal and other ventral in position. This number and arrangement that is 6,6, 4,2, (=18) of epidermal plates is identical with those of the miracidia of *E. nudicaudatus* and *E. londonensis* Khan, 1961. Anterior to the pre-equatorial level of body is a pair of dark brown pigmented eye-spots, usually separated but in some of the specimens almost overlapping each other. Immediately posterior to the eye-spots lies the cerebral mass, surrounded by nucleated cells. The very position of these cells indicates that they are probably nerve cells. In the interspace, on each side of the body, between first and second tiers of epidermal plates is a pair of lateral processes. Each of these processes consists of a narrow duct-like basal portion and a swollen distal portion. In other words, each of the lateral processes simulates an inverted flask placed horizontally. Anterior to and in immediate contact with each lateral process is an hair-like prolongation of uniform thickness. In the region of anteriormost tier and sometimes extending posterior to it is a pair of the so-called "cephalic glands" each one of which contains granular contents and a spherical nucleus in its basal region. Dorsal to the cephalic glands, in direct communication with extreme anterior tip of body, lies the "apical gland", containing coarsely granular material, in contrast with finely granular material of cephalic glands. In its distal region are four spherical nuclei. There are two flame cells, one anterior and ventral the other posterior and dorsal. The anterior flame cell may occupy a position at the level of interspace between second and third tier of epidermal plates. Both of these flame cells lead into corresponding capillaries opening, slightly dorsally, between the third and fourth tiers of epidermal

plates. There are many uninucleated germ cells, limited almost exclusively to posterior half of the body.

#### DISCUSSION

*Echinostoma anseris* Yamaguti, 1939, found in the intestine of *Anser fabalis sibiricus*, Japan, and *E. sudanense* Odhner, 1911, found in the intestine of *Ephippiorhynchus senegalensis* and *Scoups umbretta*, Africa, are the only two other species belonging to the genus *Echinostoma* Rudolphi, 1809 which like *E. donosoi* possess a definite number of 31 collar spines and thus are markedly in close specific relationship. However, there are important diagnostic characters which set these species as distinct entities. *E. anseris* differs from *E. donosoi* in that, it has 3 corner spines, larger size of these spines, oral sucker three times as large, pharynx two times as large, ovary four times as large, post-equatorial position of ovary (being equatorial or preequatorial in *E. donosoi*) anterior limits of vitelline follicles more posteriorly located, vitelline follicles confluent behind testes, smaller size of eggs, testes "scoop-shaped" or shoulder-blade shaped, cirrus sac three times as large, and finally the latter lying between intestinal bifurcation and ventral sucker.

*Echinostoma sudanensis* was first described by Odhner, later on Shigin (1955) gave a redescription of it from U.S.S.R. and added a new host, *Ardea cinerea*, record to it. This worm possesses a group of four angle spines, and is therefore more closely allied to *E. donosoi* but it is characterized with the following details which are in contradistinction to their counterparts met within *E. donosoi*: the oral sucker and the cirrus sac are twice as large, larger collar spines and smaller eggs. According to Odhner (1911) anterior testis is transversely elongate and posterior testis triangular but Shigin describes the anterior testis as having a shape from rounded to transversely elongated while posterior testis rounded to triangular. In *E. donosoi* both of the testes are invariably anteroposteriorly elongated and thus this characteristic divergence alone affords a ground sufficiently strong enough for the separation of *E. donosoi* as a definitely distinct species from *E. sudanense*.

Lutz (1924) described *Echinostoma parcespinosum* from *Gallinula galeata* and *Aramides cayennensis* in Brazil. The same author (1928) made another record of it from various species of *Aramides* in Venezuela. From the Brazilian species he reported 27-31 collar spines and from the Venezuelan representatives, 29-33. It is interesting to note that *E. parcespinosum* has a group of four angle spines, two of which oral and two aboral, and a series of dorsal spires arranged in a single row. This arrangement is in a perfect agreement with that of the collar spines of *E. donosoi*. Consequently, the two species are inseparable insofar as the arrangement of the collar spines is concerned but *E. donosoi* has an unerring number of 31 collar spines. From the inadequate description of *E. parcespinosum* a reasonable comparison, insofar as the other structural details are concerned, is not possible. However, from the fragmentary account of the same it is noticed that its oral sucker is not very conspicuous and the ventral sucker is very large. The latter peculiarity, as shown in diagram (Fig. 16, pl. 8), alone constitutes a remarkable feature of *E. parcespinosum* in contrast with a condition found in *E. donosoi*. Moreover, *E. parcespinosum* has smaller eggs (only 0.1 mm. long.), rounded testes and last but not the least is the limitation of the vitellaria only to the posttesticular region.

Experimental demonstration of various stages, right from miracidium to adult, in the life cycle of *Echinostoma donosoi* by using clean laboratory-bred stocks of hosts is the first account of the life cycle of a digenetic trematode ever accomplished in Venezuela. Lutz (1928) alludes to the presence of pre-cercarial stages of *E. parcespinosum* in Ampullarid snails and cysts in various species of aquatic mollusks, as well as makes a mention about the attainment of a certain degree of development of the adult echinostome in domestic pigeons but the experimental proof, unfortunately, is lacking. The same author (1924) gives a fragmentary account of the life cycle of the sister specimens of *E. parcespinosum* in Brazil but here again the details of various stages are missing so as to render a comparative developmental study quite impossible. There are three other species of the genus *Echi-*

*nostomsa*, namely, *Echinostoma revolutum* as described by Beaver 1937), *E. nudicaudatum* Nasir (1960; 1962), *E. londonensis* Khan (1960; 1961), and *E. pinnicaudatum* Nasir (1961), in which a reasonably good account of different stages from miracidia (with the exception of *E. pinnicaudatum*) to adults is available and thus a comparative study with a great deal of certainty can be made out. At the same time a relative taxonomic study with that of *E. donosoi* is out of question, as all the above mentioned four species of echinostomes possess a definite number of 37 collar spines in relation to 31 collar spines of *E. donosoi*. However, a useful taxonomic clue, for the separation of genera *Echinostoma* and *Echinoparyphium*, is inferred from the arrangement of the epidermal plates of the miracidia of *E. revolutum*, *E. nudicaudatum*, *E. londonensis* and *E. donosoi* on one hand and that of *Echinoparyphium recurvatum* as described by Rasin (1933) and *E. flexum* as described by Najarian (1954) on the other hand. In the miracidia of *E. revolutum*, *E. nudicaudatum*, *E. londonensis* and *E. donosoi* the plates of the posteriormost tier lie one above the other whereas in the miracidia of *Echinoparyphium recurvatum* and *Echinoparyphium flexum* the plates of the posteriormost tier are lateral. As already pointed out by Nasir (1962) "the exact arrangement of epidermal plates in a particular tier" is of taxonomic importance. Consequently, the dorsoventral position of epidermal plates, in the last tier of the miracidia of the species of the genus *Echinostoma* for which life cycles are known, and the lateral position of epidermal plates in the last tier of the miracidia of the species of genus *Echinoparyphium* for which life cycles are known, might well be taken for the separation of these two allied genera. And this view is further strengthened by studies on the subject of present investigation that is the miracidium of *E. donosoi*.

It must be mentioned here that whereas the miracidium of *E. donosoi* is very much like the miracidia of *E. nudicaudatum* and *E. londonensis*, it differs from these miracidia in lacking a transverse collar which is characteristically present between the first and second tiers of epidermal plates in the miracidia of *E. nudicaudatum* and *E. londonensis*. This

absence of transverse fold in the miracidium of *E. donosoi* might be taken into consideration for specific determination. Presently, there is no other life history study of 31-spined species of genus *Echinostoma* on record so as to make a comparison of structural details of miracidia for specific identity where the adults have an identical number of collar spines.

The author has been unable within his reach of literature to find any other echinostome cercaria with 31 collar spines, fin-fold on tail, cystogenous glands with granular contents, and a total number of 42 flame cells on each side of the body with which the cercaria of *E. donosoi* may be compared.

The natural host of *Echinostoma donosoi* is unknown and it is presumed that some aquatic birds serve as the definitive host for egg-discharging adults of this echinostome species. Efforts are in progress to make a parasite-survey of aquatic birds abounding in the vicinity of the molluscan intermediate host.

#### SUMMARY

The cercaria of *Echinostoma donosoi* develops in *Marisa cornuarietis* (L), unicolor form, Llanada de San Juan about five kilometers south of the university, and possesses the following characters: 31 collar spines including a group of four angle spines, tail with a dorsoventral fin-fold, cystogenous glands with granular contents, short prepharynx, muscular pharynx, esophagus extending to ventral sucker, ceca reaching almost posterior end of the body, main excretory tubes enclosing refractile excretory granules in preacetabular region only, characteristic anterior as well as a posterior echinostome loop on each side of the body, secondary excretory tubules ciliated throughout their entire extent, caudal excretory duct bifurcating in proximal region of the tail and 42 flame cells on each side of the body. Encysting in pericardia of *M. cornuarietis*. Rediae with a pharynx, an undivided collar, a saccate gut and posterior locomotor appendages.

The fifteen days old cysts recovered from the pericardia of laboratory-bred and experimentally infected specimens of *M. cornuarietis* were fed to two laboratory-bred pigeons. Daily fecal examination was conducted. The echinostome eggs first appeared in the feces of a pigeon on the thirteenth day after the experimental introduction of cysts. This very pigeon was dissected on the same day and four egg-discharging adults were recovered from its small intestine. A detailed comparison has been made with three other species of the genus *Echinostoma* having 31 collar spines. The other species of the genus are: *Echinostoma anseris* Yamagutti, 1939, *E. sudanense* Odhner (1911) Shigin (1955) and *E. parcespinosum* described by Lutz (1924; 1928) from Brazil and Venezuela.

The mature miracidium escapes from the egg in 17-21 days and is marked with the under mentioned characters: 18 epidermal plates arranged in 4 tiers, plates of posteriormost tier lying one above the other, vibratile cilia, a pair of pigmented eye-spots, an apical gland a pair of cephalic glands, 2 flame cells and scattered germ cells.

The natural definitive host of *Echinostoma donosoi* is not yet determined but efforts are in progress.

#### ACKNOWLEDGEMENTS

The author expresses his appreciation to Dr. J. P. E. Morrison of Smithsonian Institute, Washington D. C., U. S. A. for the identification of the snail intermediate hosts and thanks are due also to Dr. F. Balda, Instituto Oceanográfico, Universidad de Oriente for going through this manuscript.

#### RESUMEN

La cercaria de *Echinostoma donosoi* se desarrolla en *Marisa cornuarietis* (L.), forma unicolor, de la Llanada de San Juan, alrededor de 5 Kms. al oeste de la Universidad. Posee los siguientes caracteres: 31 collares de espinas que incluyen un grupo de 4 espinas angulares, cola con un pliegue en aleta dorsoventral, glándulas cistógeas con contenido granular, prefaringe corta, faringe muscular, esófago se extiende hasta la ventosa ventral, según alcanza el término posterior

del cuerpo. Los tubos excretorios encierran granulas refractantes en la región preacetabular; los caracteres anteriores a los posteriores de las espiras de cada lado del cuerpo son semejantes. Túbulos secretores secundarios, ciliados en toda su extensión. Tubo caudal excretorio es bifurcado en la región proximal de la cola y se encuentran a cada lado del cuerpo 42 células flamíferas. Se enquista en el pericardio de *M. cornuarietis*. Redia con faringe y collar no dividido, intestino saculiforme, apéndices locomotores posteriores; los quistes de 15 días de edad recogidos del pericardio de *M. cornuarietis* infestados experimentalmente fueron administrados a dos (2) pichones no infestados, cuyo control previo se hizo por exámenes fecales diarios. Cuatro (4) *Echinostomas* adultos fueron encontrados en el intestino de uno de los pichones 13 días después de la introducción experimental de los quistes. Comparaciones detalladas con *Echinostomas* con 31 collares de espinas, como *Echinostoma anseris* Yamagutti, 1931, *E. sudanense* Odhner, 1911 y *E. parcespinosus* Lutz, 1924 fueron realizadas resultando *E. donosoi* una entidad distinta.

Los miracidios maduros abandonan el huevo entre 17 a 21 días y se caracterizan por lo siguiente: 18 placas epidérmicas dispuestas en cuatro hileras, cilios vibrátiles, una serie de manchas oculares pigmentadas, glándulas apicales, glándulas cefálicas, 2 células flamíferas y células germinales dispersas.

El huésped definitivo natural de *Echinostoma donosoi* no ha sido identificado aún.

#### REFERENCES

BEAVER, P. C.

1937. Experimental studies on *Echinostoma revolutum* (Froel.), fluke from birds and mammals. III. Biol. Monogr. vol. 15, p. 1-96.

DIETZ, E.

1909. Die Echinostomiden der Vögel. Zool. Anz. 34:180-192.  
1910. Die Echinostomiden der Vögel. Zool. Jahrb. Supp. 12, p. 265-512.

KHAN, D.

1961. The life history of *Echinostoma Londonensis* N. Sp., the adult of *Cercaria londonensis* Khan, 1960. *J. Helminth.* vol. 35, p. 119 - 132.

LUTZ, A.

1924. Untersuchung über die entwicklungsgeschichte brazilianscher Trematoden. Spezieller. Teil 1. Echinostomidae. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, vol. 17, p. 55 - 93.

1928. *Estudios de zoología y parasitología venezolana.* 133 pp. Río de Janeiro.

MENDHEIM, H.

1943. Beiträge zur systematik und biologie der familie "echinostomatidae", *Arch. Natur. Leipzig* (N. F.) vol. 12, p. 175 - 302.

MORRISON, J. P. E.

1964. Personal communication.

NAJARIAN, H. H.

1954. Developmental stages in the life cycle of *Echinoparyphium flexum* (Linton, 1892) Dietz, 1910, (Trematoda: Echinostomatidae). *J. Morph.* vol. 94, p. 165 - 197.

NASIR, P.

1960. Studies on the life of *Echinostoma nudicaudatum* N. Sp. (Echinostomatidae: Trematoda) *J. Parasitol.* vol. 46, p. 833 - 847.
1961. Observations on the life cycle of *Echinostoma pinnicaudatum* (Echinostomatidae: Trematoda). *Helminth. Soc. Wash.* vol. 28, p. 207 - 212.
1962. Further observations on the life cycle of *Echinostoma nudicaudatum* Nasir, 1960. (Echinostomatidae: Trematoda). *Proc. Helm. Soc. Wsh.* vol. 29, p. 115 - 127.

1964. Studies on freshwater larval trematodes. Part. V. A new Polyadenous Xiphidiocercaria, *C. baldai*, from Venezuela. *Proc. Helminth. Soc. Wash.* vol. 31, p. 28 - 30.
1964. b Studies on freshwater larval trematodes. Part. III. A new species of a strigeid cercaria, *C. manzanarensis*, from Venezuela. *Proc. Helminth. Soc. Wash.* vol. 31, p. 31 - 33.
1964. c. Estudios on freshwater larval trematodes. Part. II. A new echinostome cercaria, *C. penalveri*, from Venezuela. *Proc. Helminth. Soc. Wash.* vol. 31, p. 33 - 36.
1964. d. Estudios de larvas de Trematodes de aguas dulces. Parte VI. Observaciones experimentales de varios estados en el ciclo de vida de *Echinostoma donosoí*. N. Sp. (Echinostomatidae: Trematoda) de Venezuela. *Asociación Venezolana para el Avance de la Ciencia. Resúmenes de los trabajos presentados en la XIV convención anual.* Pág. 92 - 93 (Abstract). 3 al 9 de Mayo de 1964. Caracas. Venezuela.
1964. e. Estudios de larvas de Trematodes de agua dulce. Parte I. Una nueva Microcotylous Xiphidiocercaria, *C. cumanensis* de Venezuela. *Ibd.* Pág. 93 - 94. (Abstract).
1964. Studies on freshwater larval trematodes. Part. IV. A new Virgulate Xiphidiocercaria, an another new longifurcate, Pharyngeate Distome Furcocercaria, from Venezuela. In Press.
- NASIR, P. y ACUÑA, C. A.
1964. Estudios sobre larvas de Trematodes de agua dulce. Parte VII. Observaciones sobre una nueva cercaria gimnocephalic encontrada en Venezuela. *Ibd.* Pág. 87. (Abstract).

ODHNER, T.

1911. Nordostafrikanische Trematoden grösstenteils von Weissen Nil (Von der Schwedischen Zoologischen Expedition gesammelt). *Res. Swed. Zool. Exp. Egypt. and White Nile IV*, 170 pp.

RASIN, K.

1933. *Echinoparyphium recurvatum* (Linstow, 1873) und seine Entwicklung. *Biol. Spis. Vys. Sk. Zverolek.* vol. 12, p. 1 - 104.

RUDOLPHI, C. A.

1803. Beobachtungen über die Eingewidewürmer. *Arch. Zool. U. Zool.* Braunschweig, Bd. 3, H. 2, SS. 61 - 32.

SANDGROUND, J. H. and BONNE, C.

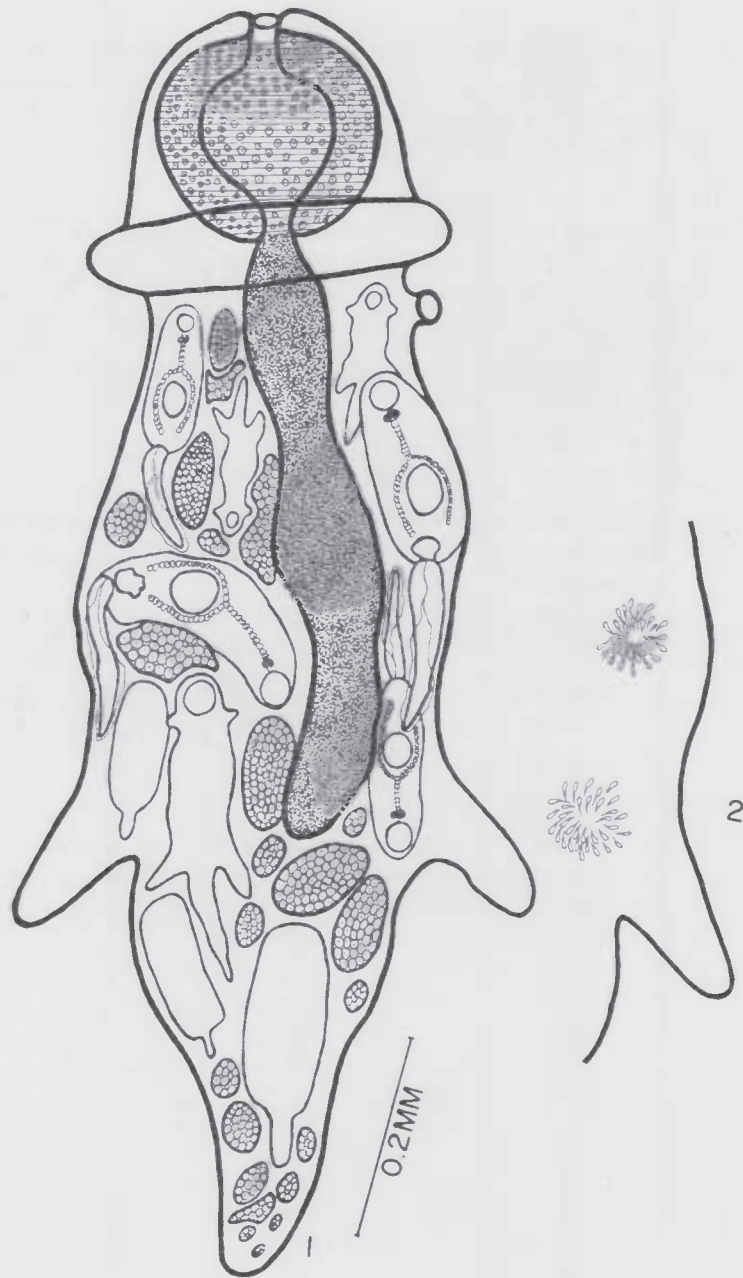
1940. *Echinostoma lindoensis*, a new parasite of man in the Celebes with an account of its life history and epidemiology. *Amer. J. Trop. Med.* vol. 20, p. 511 - 535.

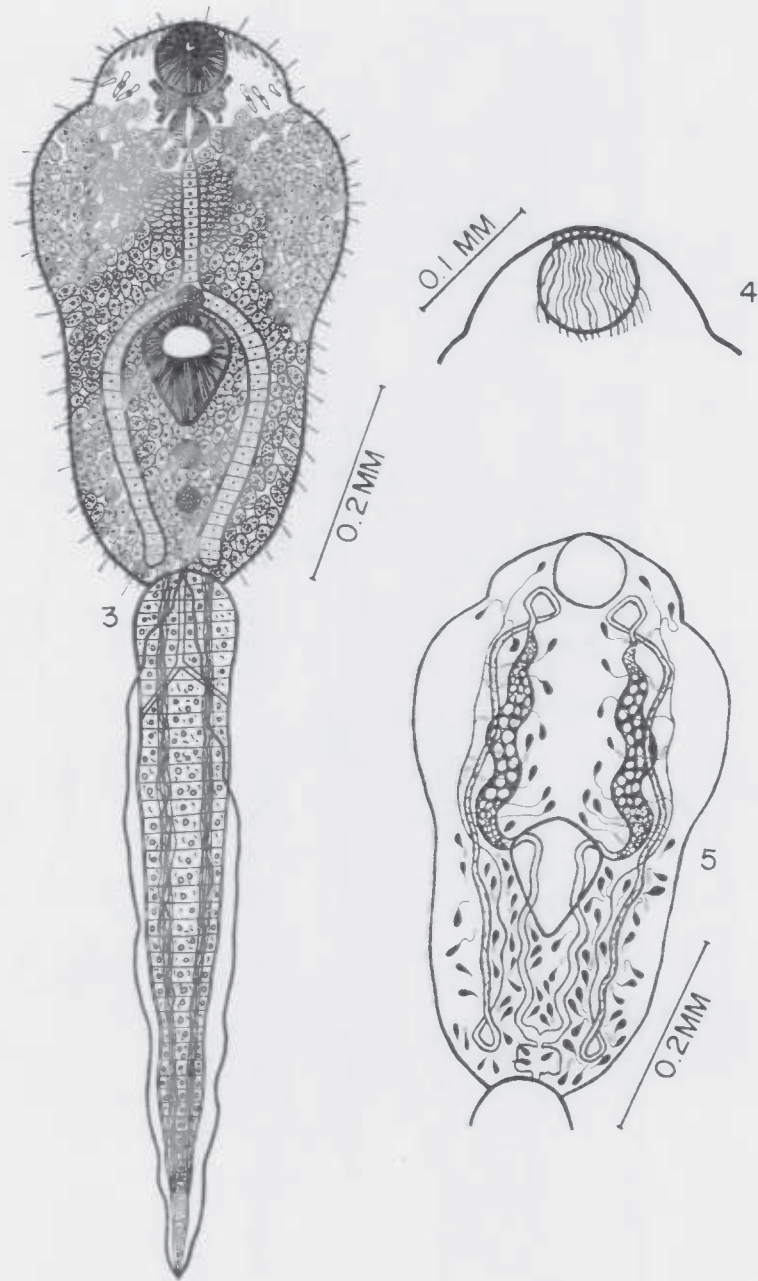
SHIGIN, A. A.

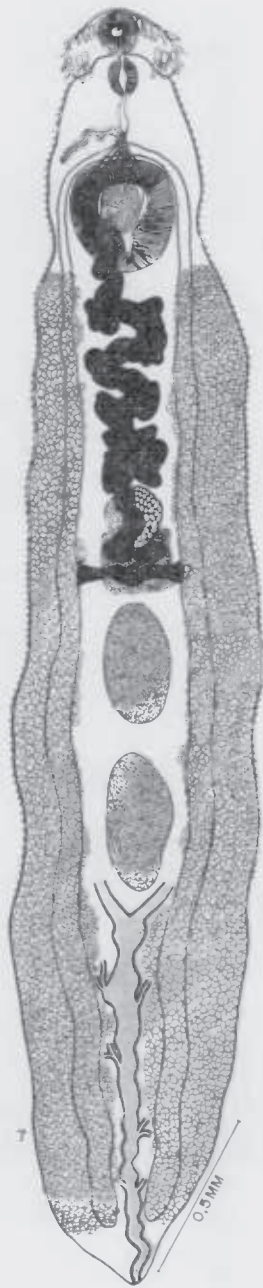
1955. In Skrajabin, K. I. 1956, Trematody Zhivotryj cheloveka. Osmoy trematology. XII: 1 - 932. *Iskd. Askd. Nauk SSSR.* Moskava-Lynengrad.

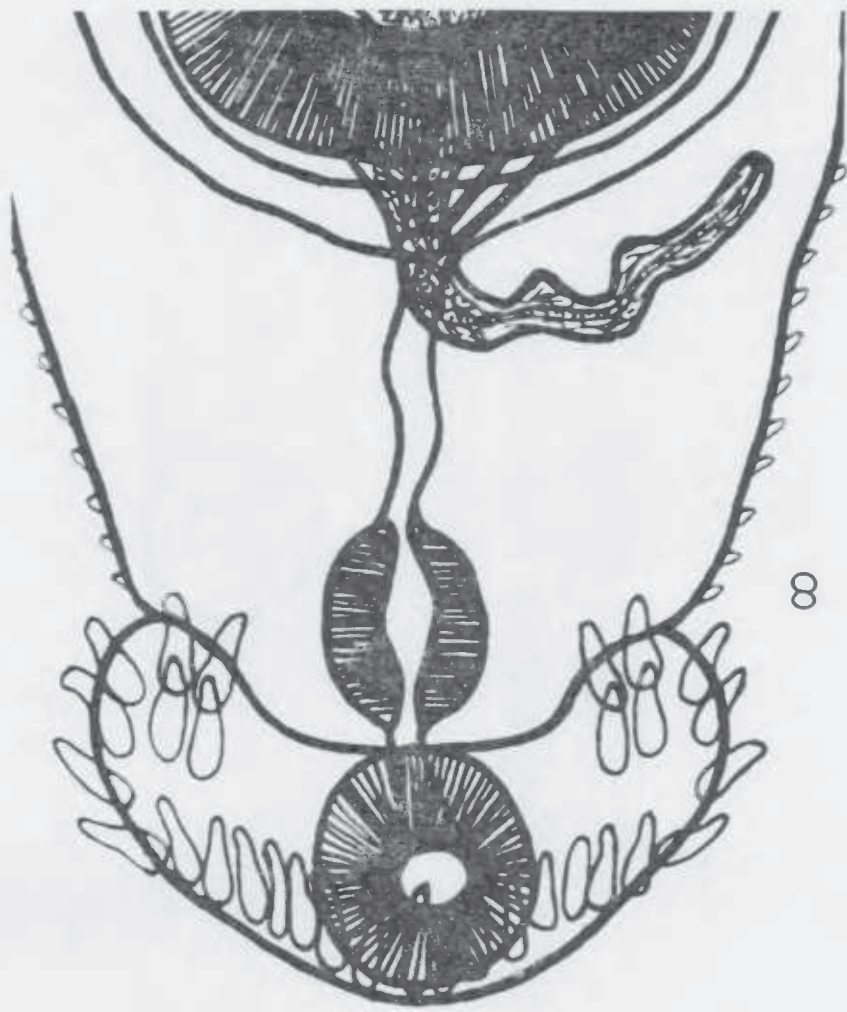
YAMAGUTI, S.

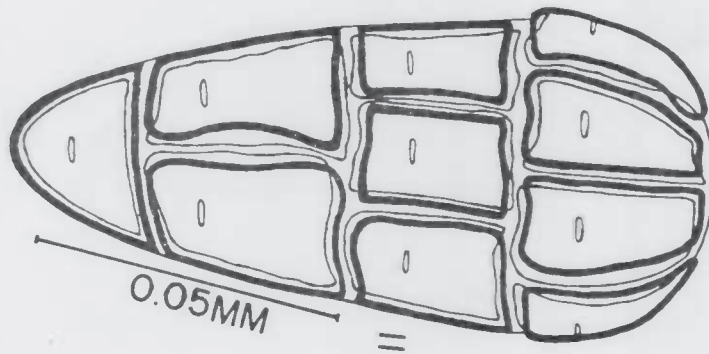
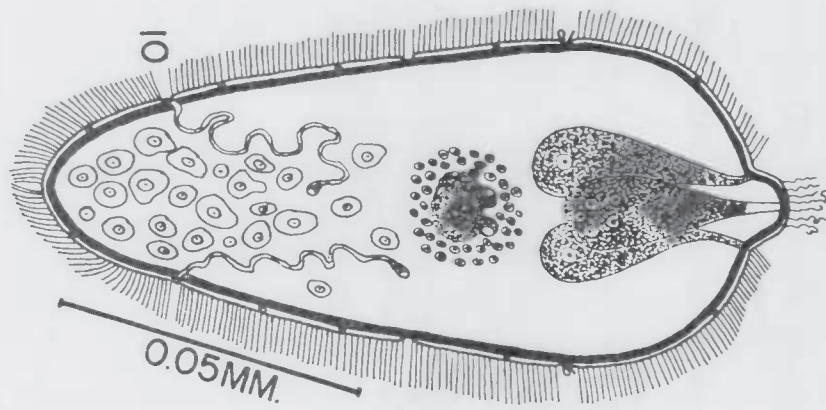
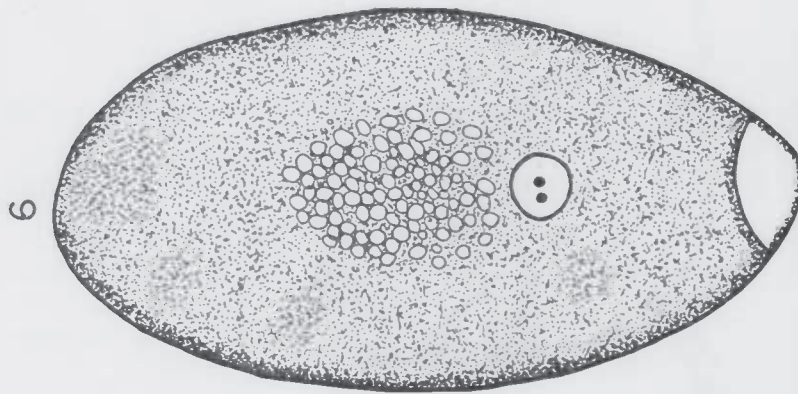
1939. Studies on the helminth fauna of Japan. Part. 25. Trematodes of birds. IV. *Jap. J. Zool.* vol. 8, p. 130 - 210.











### EXPLANATION OF FIGURES

- Fig. 1. The mature redia of *Echinostoma donosoi* N. Sp. containing rediae as well as cercariae inside.
- Fig. 2. Flame - cell system of the redia drawn on one side only.
- Fig. 3. Cercaria of *E. donosoi* showing "hair-like" processes, collar spines, cuticular spines, suckers, digestive system, genital rudiments, cystogenous glands, cerebral mass, and fin-fold on tail.
- Fig. 4. Anterior region of cercaria showing eight terminal apertures leading into eight duct-like extensions.
- Fig. 5. Cercaria showing flame-cell system.
- Fig. 6. Cyst of Cercaria *E. donosoi*.
- Fig. 7. Adult *Echinostoma donosoi*.
- Fig. 8. Anterior region of *E. donosoi* greatly modified to show the arrangement of collar spines and prostatic equipment.
- Fig. 9. Naturally discharged egg of *E. donosoi*.
- Fig. 10. Free-swimming miracidium of *E. donosoi*.
- Fig. 11. Epidermal plates of miracidium showing transver-

\* EL PRESENTE BOLETIN SE  
TERMINO DE IMPRIMIR EN LAS  
PRENSAS DE LA EDITORIAL  
UNIVERSITARIA DE ORIENTE /  
CUMANA, ESTADO SUCRE, VE-  
NEZUELA / EL DIA 16 DE DI-  
CIEMBRE DE MIL NOVECIE-  
TOS SESENTA Y CUATRO \*