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## LIFE CYCLE OF *Culuwiya tilapiae* (Nasir y Gómez, 1976) (Trematoda: Haploporidae) IN VENEZUELA

### Ciclo de vida del *Culuwiya tilapiae* (Nasir y Gomez, 1976) (Trematoda: Haploporidae) en Venezuela

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#### ABSTRACT

The life cycle of *Culuwiya tilapiae* (Nasir y Gómez, 1976) Overstreet and Curran, 2005, a digenean belonging to the family Haploporidae Nicoll, 1914 is redescribed and illustrated. The parasite infects naturally the snail, *Pyrgophorus cf. spiralis* (Guppy 1864), and the definitive host, *Oreochromis mossambicus* (Peters, 1852). Both molluscs and fish were collected from the Parque Litoral Laguna de los Patos, Cumaná, Estado Sucre, Venezuela. The cercariae were developed in redia in the digestive gland of molluscs and encysted on the water surface and more frequently on the bottom and walls of dishes. The 2-4 days old cysts of *C. tilapiae* were fed to laboratory reared *O. mossambicus*, and the trematode reaches sexual maturity in 22-32 days after the experimental infection. All developmental stages of *C. tilapiae* are described and illustrated. This species was compared with other members of *Culuwiya* and with *Saccocoelioides tarpazensis* Díaz and Gonzalez, 1990, for being the only recorded haploporids species in Venezuela and its life cycle had been elucidated and for the similarity with *C. tilapiae*. This species is redescribed herein represents the first record of *Culuwiya* Overstreet and Curran, 2005 in Venezuela.

**Key words:** *Culuwiya*, trematode, life cycle, fish, Venezuela.

#### RESUMEN

El ciclo de vida de *Culuwiya tilapiae* (Nasir y Gómez, 1976) Overstreet y Curran, 2005, un digéneo perteneciente a la familia Haploporidae Nicoll, 1914 fue dilucidado. El parásito infecta naturalmente al caracol, *Pyrgophorus cf. spiralis* (Guppy,

1864), y al hospedador definitivo, *Oreochromis mossambicus* (Peters, 1852). Ambos, moluscos y peces fueron recolectados en el Parque Litoral Laguna de los Patos, Cumaná, estado Sucre, Venezuela. Las cercarias fueron desarrolladas en redias en la glándula digestiva del molusco, y enquistada en la superficie del agua y más frecuente en el fondo y paredes del recipiente. Los quistes de 2-4 días de edad de *C. tilapiae* fueron suministrados a *O. mossambicus* nacidos en el laboratorio, y los tremátodos alcanzan su madurez sexual en 22-32 días después de la infección. Todos los estadios de desarrollo de *C. tilapiae* fueron descritos e ilustrados. Esta especie fue comparada con los miembros del género *Culuwiya* y con *Saccocoelioides tarpazensis* Díaz y González, 1990, por ser la única especie de haploporido en Venezuela, cuyo ciclo de vida ha sido dilucidado y por su similitud con *C. tilapiae*. Esta especie redescribida aquí, representa la primera vez que se registra el género en Venezuela.

**Palabras clave:** *Culuwiya*, trematoda, ciclo de vida, peces, Venezuela.

#### INTRODUCTION

Nasir & Gómez [3] described *Carassotrema tilapiae* as new species from small intestine of *Tilapia mossambica* collected from the Parque Litoral Laguna de los Patos in Venezuela. Overstreet and Curran [5] revised the family Haploporidae Nicoll, 1914 and created a new sub-family, Chalcinotrematinae and the new genus *Culuwiya* to accommodate *C. tilapiae* which now recognized as *Culuwiya tilapiae* (Nasir & Gómez, 1976) Overstreet & Curran, 2005. During a routine study on cercariae of molluscs in Parque Litoral Laguna de los Patos, *Pyrgophorus c f. spiralis* were examined and found to emit distomads cercariae with ocular spots, which developed to

an adult of *Culuwiya tilapiae* (Nasir & Gómez, 1976) Overstreet & Curran, 2005, which is redescribed herein. The purpose of this study was to describe the life cycle and illustrate the different developmental stages of *C. tilapiae* in laboratory reared freshwater fish, *Oreochromis mossambicus* (Peters, 1852) which is also a natural host.

## MATERIALES AND METHODS

Specimens of *Pyrgophorus cf. spiralis* were collected between February and May of 2001, from the Parque Litoral Laguna de los Patos, Cumaná, Sucre State, Venezuela. The snails were maintained alive in aerated aquariums in the laboratory at 24°C. Once acclimatized, the snails were isolated in groups of five in small glass bowl with water, and examined under a stereoscopic microscope (Marca Bausch & Lomb, Modelo FSB, New York, USA) to observe the emission of cercariae. The rediae, cercariae, and metacercariae were examined live from the natural hosts. The collected larvae and adults were fixed in formol alcohol acetic acid (FAA) and subsequently stained in Semichon's acetocarmine and mounted in Canada balsam. All measurements are in micrometers ( $\mu\text{m}$ ) and drawings were done with the help of a camera lucida, with some details incorporated by hand. This trematode was identified taxonomically according to Overstreet and Curran [5]. The experimental definitive hosts, *Oreochromis mossambicus* were raised in the laboratory, and the same species was found as a natural host of *Culuwiya tilapiae* in the Parque Litoral Laguna de los Patos.

## RESULTS AND DISCUSSIONS

### Redia

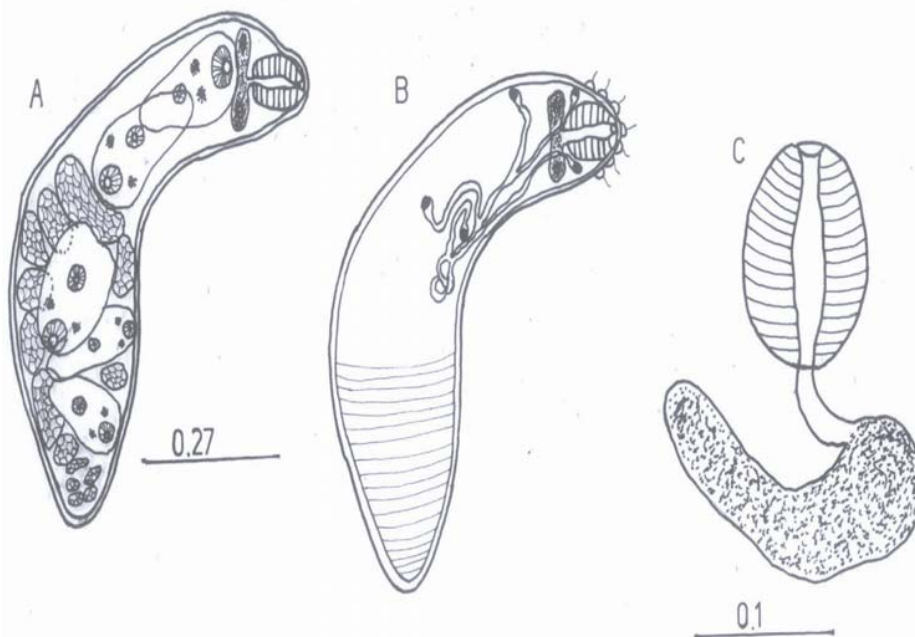
Description and measurements based on 15 live and mounted rediae (FIGS. 1, A-C).

The rediae develop in the digestive gland of *Pyrgophorus cf. spiralis* in large numbers with little activities. Body elongate, 353-505 X 106-136, thick tegument, with longitudinal and traverse muscles. Anterior part of the body contains papillae with hair. Mouth is terminal. Pharynx muscular and well developed, 35-55 X 35-50, communicates with intestine, with a dark brown granular content (FIG. 1C). No birth pore was observed. Well developed rediae contains 4-7 cercariae of several stages (FIG. 1A). In some cases, immature cercariae moves to leave quickly, probably to complete the growth in the tissues of snails. Germinal balls are generally located in the posterior part of body of the redia. The protonephridial system (FIG. 1B) is constituted by a small excretory bladder, located in the equatorial part of the body. Primary excretory ducts are sinuous, reaching on postlateral sides of pharynx, later descending at the level of the excretory vesicle. Flame cell 5 (FIG. 1B) and no excretory pore observed.

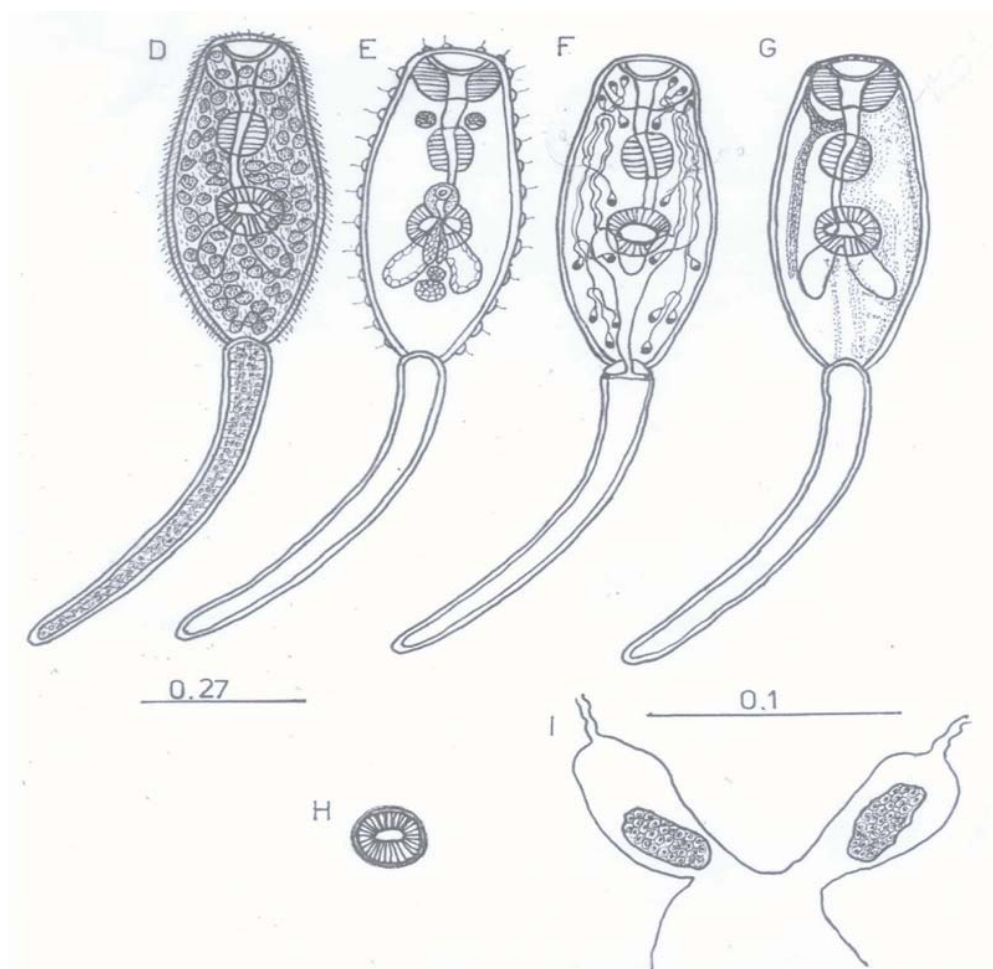
### Cercaria

Description and measurements based on 18 live and mounted cercariae (FIGS. 2 D-I).

Cercaria of *Culuwiya tilapiae* (Nasir & Gómez, 1976) was found in snails, *Pyrgophorus cf. spiralis*. Of 578 snails, 25



FIGURES 1 (A-C). *Culuwiya tilapiae*. A. REDIA, SHOWING CERCARIAE IN SEVERAL DEVELOPMENT STAGES AND GERMINAL BALLS/ REDIA MOSTRANDO CERCARIAS EN VARIOS ESTADOS DE DESARROLLO y BOLAS GERMINALES; B. REDIA, SHOWING PROTONEPHRIDIAL SYSTEM / REDIA, MOSTRANDO SISTEMA PROTONEFRIDIAL, C. REDIA, SHOWING PHARYNX AND INTESTINAL CAECUM/ REDIA MOSTRANDO FARINGE Y CIEGO INTESTINAL.



**FIGURES 2 (D-I). *Culuwiya tilapiae*. D. CERCARIA SHOWING BODY SPINES, DIGESTIVE SYSTEM AND CYSTOGENIC GLAND CELLS/ CERCARIA MOSTRANDO ESPINAS DEL CUERPO, SISTEMA DIGESTIVO Y CÉLULAS GLANDULARES CISTOGENICAS, E. CERCARIA, SHOWING PAPPILLAE WITH SENSITIVE HAIR, OCULAR SPOTS AND GENITAL RUDIMENTS/ CERCARIA MOSTRANDO PAPPILLAS CON PELOS SENSORIALES, MANCHAS OCULARES Y RUDIMENTOS GENITALES; F. CERCARIA, SHOWING PROTONEPHRIDIAL SYSTEM/ CERCARIA MOSTRANDO SISTEMA PROTONEFRIDIAL; G. CERCARIA, SHOWING THE NERVOUS SYSTEM (LEFT SIDE) AND PIGMENTS OF BODY (RIGHT SIDE)/ CERCARIA MOSTRANDO SISTEMA NERVIOSO (LADO IZQUIERDO) Y PIGMENTOS DEL CUERPO LADO DERECHO; H. ACETABULUM OF CERCARIA SURROUNDED BY CIRCULAR MUSCLES/ ACETABULO DE LA CERCARIA RODEADO POR MÚSCULOS CIRCULARES; I. BRANCHES OF THE EXCRETORY BLADDER WITH REFRACTILES GRANULES/ RAMAS DE LA VEJIGA EXCRETA CON GRANULOS REFRACTILES.**

(4.3%) emitted gymnocephalid cercariae with ocular spots, swimming actively. Newly emitted cercaria encysted on the surface of water, and on bottom and sidewalls of the glass recipient. Cercariae emerged from snails in the first hours of the day, between 7-9 am. Some cercariae remained more than 12 hours without encysting, while other encysted in hours and some in minutes. Body elongated, measuring 202-333 X 111-146, of brown colour due to pigments that are distributed in the whole body (FIG. 2G). Tegument spinose, surrounded by papillae with sensitive hair. Four small papillae located anterior to oral sucker (FIGS. 2 D-E). Two prominent ocular spots with glass located on both sides of the pharynx anterolaterally, and constituted by pigments of dark brown colour (FIG. 2E). Tail, 353 - 494 X 30-35, without spines with numerous longitudinal and circular muscles; containing abundant small cells with

granules, located in the central part and in their borders (FIG. 2D). Oral sucker terminal 48-59 X 61-80, spinose, larger than acetabulum, 10 rows of small spines on the oral opening. Prepharynx short, 10-32 X 5-13, which communicates with an oval shaped muscular pharynx, measuring 32-48 X 34-45. Oesophagus long and sinous, 53-69 X 8-24, which bifurcates at equatorial level of acetabulum in two short intestinal caeca. Intestinal caeca, 61-94 X 18-29, reaching below the branches of the excretory bladder. Some openings and its ducts are observed at the anterior part of oral sucker, containing thick granules arranged in a group of 3-3-3-3-3. Acetabulum, 51-64 X 51-61, located slightly in the posterior part of the body, protusible covered by a thick muscular membrane with rows of spines (FIG. 2H). Preacetabular distance, 141-161 and postacetabular distance, 101 X 126. Cystogenic gland cells are distributed

throughout the body with granules (FIG. 2D). The nervous system is constituted by a transverse commissure, located above the pharynx and connected to two cerebral ganglions. Two short anterior and two long posterior nerves originate in each ganglion (FIG. 2G). Hermaphroditic sac not well developed, overlap the acetabulum. Genital pore preacetabular. Testis, ovary, external and internal vesicle difficult to observe (FIG. 2E). Protonephridial system (FIG. 2F) consists of a Y-shaped tubular excretory bladder; the main branches extend to the equatorial margin of the acetabulum; each branch containing a group of refractile granules (FIG. 2I). The primary excretory duct terminal or subterminal, moves anteriorly and bends at the level of the oral sucker, later grows posteriorly, dividing at the level of the pharynx. Excretory caudal channel emerges at the base of the excretory bladder, originating into two lateral branches close to the tail which opens to their respective excretory pores. Flame cells formula is:  $2[(3+2)+(3+2)] = 20$ .

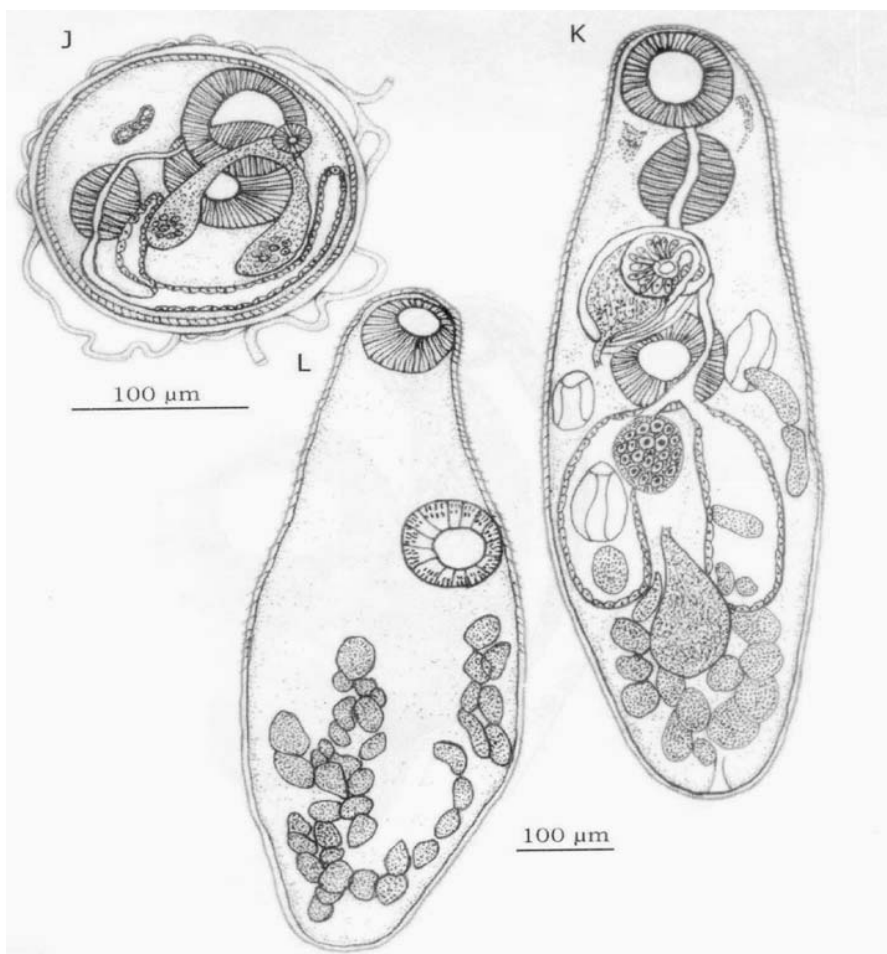
### Metacercaria

Descriptions and measurements based on 12 live cysts (FIG. 3 J).

The cysts are oval, almost spherical, 153-182 X 147-166, covered with fibrous materials and filaments of different sizes, formed by cystogenic materials. Body of metacercaria occupies whole space of the cyst and reflects all the characteristics of cercaria, except the tail and contents of cystogenic gland cells. Oral sucker measures 40 - 53 X 59- 75, Pharynx 40-53 X 29-43, Acetabulum 56-67 X 61-80. External wall 5-8 thick.

### Experimental infection of natural hosts

Six of 25 naturally infected snails *Pyrgophorus cf. spiralis*, collected from the Laguna de los Patos, were placed in a recipient (finger bowls) with water. Cercariae emitted around 7-9 am, encysted in water. The cysts of 2-4 days old were



**FIGURES 3 (J-L). *Culuwiya tilapiae*. J. METACERCARIA. BODY COVERS WITH FILAMENTS PRODUCED BY CYSTOGENIC MATERIALS / METACERCARIA- LOS FILAMENTOS FORMADOS POR EL MATERIAL CISTOGENICO RODEADO LA PARED DEL CUERPO. K. ADULT OF *C. tilapiae*. SIZE OF PHARYNX IN RELATION TO SUCKERS AND INTESTINAL CAECA/ ADULTO DE *C. Tilapiae*: EL TAMAÑO DE LA FARINGE EN RELACIÓN CON LAS VENTOSAS, Y CIEGO INTESTINAL; L. ADULT OF *C. tilapiae*, SHOWING THE DISTRIBUTION OF VITELLINE GLAND CELLS IN THE POST ACETABULAR PART OF THE BODY / ADULTO DE *C. tilapiae*, MOSTRANDO LA DISTRIBUCIÓN DE LAS CÉLULAS GLANDULAE VITELOGÉNICAS EN LA REGIÓN POST ACETABULAR DEL CUERPO.**

used to infect 5 laboratory raised *Oreochromis mossambicus*. A total of 11 digeneans (8 adults with eggs and 3 adults without eggs) were recovered 22-32 days after infection from intestines of 4 experimental hosts (TABLE I). A similar experiment was carried out, using metacercariae of 4 days old with three *O. mossambicus*, and a total of 5 digeneans (4 adults with eggs and 1 adult without eggs) were recovered from 2 of the 3 infected fish, 24 to 32 days after infection (TABLE I).

**TABLE I**  
**EXPERIMENTAL INFECTION OF LABORATORY RAISED DEFINITIVE HOSTS, *O. mossambicus* WITH CYSTS OF *Culuwiya tilapiae*** INFECCIÓN EXPERIMENTAL DE HOSPEDADOR DEFINITIVO, *O. mossambicus* MANTENIDO EN LABORATORIO CON QUISTES DE *Culuwiya tilapiae*

No. of host	Age of cysts (days)	Duration(days)	No. recovered
Fish no. 1	2	22	3a+2b
Fish no. 2	2	22	1a+1b
Fish no. 3	2	27	2a
Fish no. 4	2	27	2a
Fish no. 5	2	32	0
Fish no. 6	4	24	2a+1b
Fish no. 7	4	28	0
Fish no. 8	4	32	2a

a = adult with eggs. b = adult without eggs.

**Adult.** Description based on 10 mounted specimens recovered from experimental hosts (FIGS. 3 K-L and FIGS. 4 M-N).

Body small, elongated, measuring 629-923 X 172-284, covered with short spines reaching to the testicular region (FIG. 3L). Pigmented granules of cercaria and metacercaria are spread in both sides anterior to the pharynx. Oral sucker terminal or subterminal, with spines slightly larger than the acetabulum, 67-110 X 75-121. Prepharynx short 21-40 X 13-24. Pharynx strongly muscular 56-83 X 64-112. Oesophagus long and sinous 81-107 X 11-13. Intestinal caeca wide reaching the region of testes. Acetabulum pre-equatorial, with spines 75-101 X 81-99. Preacetabular distance 203-314 and postacetabular distance 304-507. Hermaphroditic sac well developed 129-228 X 61-83, located anterior or anterolateral to the acetabulum; consisting of internal seminal vesicle, pars prostatic, prostatic gland cells, eyaculatory duct, metraterm and a hermaphroditic duct (FIG. 4M). Testis large, elongated, measuring 112-191 X 67-137 and located in the posterior fourth of the body. Post testicular distance 50-18. Efferent duct originate from the anterior part of testis and ascends and penetrates into posterior part of external seminal vesicle. External seminal vesicle 64-107 X 40-86 (FIG. 4M). Internal seminal vesicle may be larger or smaller than the external seminal vesicle, which leads to pars prostatic and continues to form short ejaculatory duct. Prostatic gland cells scarce. Ovary pre-testicular, ovoid, non lobuled 43-81 X 29-81. Laurel's canal not observed. Uterus

short, principally in acetabulum-testicular region, containing large non embryonated eggs. Eggs measure 81-91 X 40-48. Posterior end of uterus enters into the hermaphroditic sac and transforms into short muscular metraterm uniting to ejaculatory duct to form a muscular hermaphroditic duct. Genital pore median or submedian, located between pharynx and acetabulum. Vitelline follicles of different sizes, distributes in two marginal rows from the border of posterior acetabulum to posterior part of the body where it converges (FIG. 3L). Excretory bladder Y shaped and reaching to acetabulum. Excretory pore terminal or sub-terminal.

Type hosts: Natural definitive host: *Oreochromis mossambicus* (Peters, 1852)

Experimental host: *Oreochromis mossambicus* (Peters, 1852)

Type locality: Parque Litoral Laguna de los Patos, Cumaná, Estado Sucre, Venezuela.

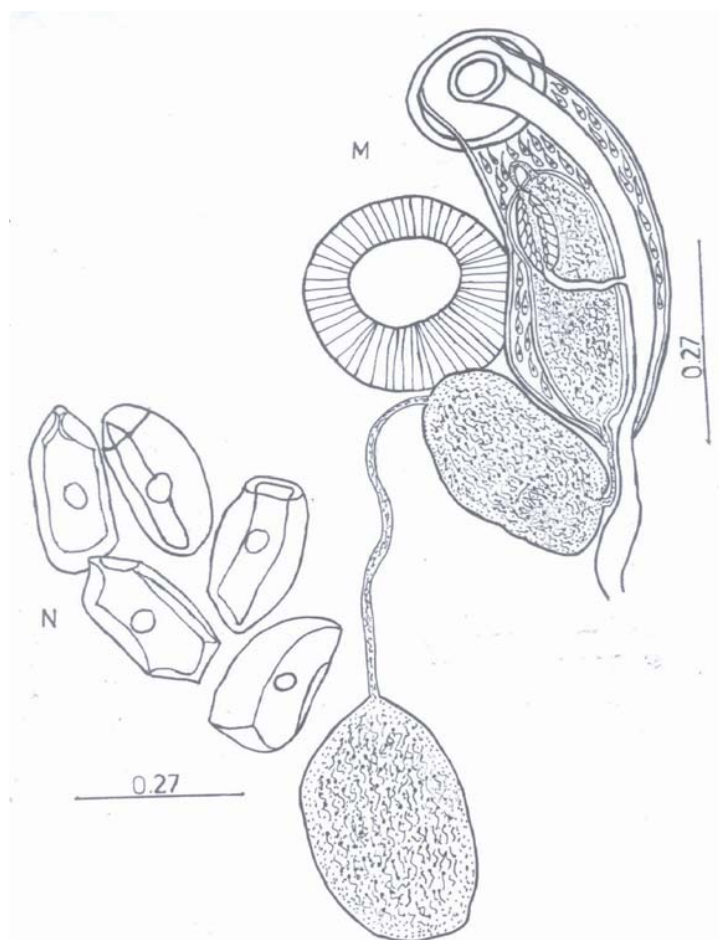
Site of infection: Adults in small intestine and metacercariae in water.

Holotype: Adult deposited in the Parasitological collections of the Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas, Universidad de Oriente, Cumaná, Venezuela, IBCA No. 1226.

Paratype: IBCA No. 1227, in above mentioned collections.

Nasir & Gómez [3] described *Carassotrema tilapiae* as new species, belonging to the family Haploporidae from the small intestine of *Tilapia mossambicus* (= *Oreochromis mossambicus*) collected from the Laguna de los Patos, Cumaná, Sucre, Venezuela. Kohn et al. [2] transferred this species to the genus *Chalcinotrema* Freitas, 1947, which was considered as synonym of *Crassotrema* Park, 1938 by Nasir & Gomez [3], accepted by Wang & Pan [7] but rejected by Overstreet [4], Rekharani & Madhavi [6], Kohn et al. [2], Overstreet & Curran [5]. Overstreet & Curran [5] revised the family Haploporidae Nicoll, 1914 and create a new sub family Chalcinotrematinae to accommodate the genus *Chalcinotrema* Freitas, 1947 as type genus and include the genera *Paralecithobotrys* Szidat, 1954, *Saccocoelioides* Szidat, 1954, *Megacoelium* Szidat, 1954 and *Unicoelium* Thatcher & Dossman, 1975. The authors [5] transferred the genus *Saccocoelioides* Szidat, 1954 from the sub family Haploporinae Nicoll, 1914 to the newly created sub family Chalcinotrematinae; and changed *Saccocoelioides platensis* Lunaschi, 1984 to the genus *Chalcitrema* as *C. platense* (Lunaschi, 1984) Overstreet y Curran, 2005.

In addition, the same authors transferred seven other species of *Saccocoelioides* to the sub family Waretrematinae Srivastava, 1937, leaving about 15 other species to the genus *Saccocoelioides* which were all distributed in the western hemisphere, majority of them were from the south America. Three of the seven species of *Saccocoelioides*: *S. martini* Madhavi,



**FIGURES 4 (M-N). *Culuwiya tilapiae*. M. ADULT: MALE REPRODUCTIVE SYSTEM, SHOWING THE UNION EJACULATORY DUCTS AND METRATERM, FORMING HERMAPHRODITIC DUCT / ADULTO: SISTEMA REPRODUCTIVO MASCULINO, MOSTRANDO LA UNIÓN DEL CONDUCTO EJACULADOR Y EL METRATERMO, FORMANDO EL CONDUCTO HERMAFRODITA. N. EGGS: NON EMBRYONATED OF *C. tilapiae*, OBTAINED EXPERIMENTALLY/ HUEVOS- NO EMBRIONADO DE *C.tilapae* OBTENIDO EEXPERIMENTALMENTE.**

1980, *S. pearsoni* Martin, 1973 and *S. buidogensis* Lu, 1993, were transferred to the genus *Pseudohapladena*, Yamaguti, 1952, and proposed a new genus *Culuwiya* to accommodate the remaining four species of *Saccocoelioides* and a species of *Carassotrema*, which are different from *Saccocoelioides* due to the following characteristics: body fusiform to pyriform, small pre-pharynx, long esophagus, ovary lightly separated from testes, vitelline follicles large and eggs large but few. The species are: *Culuwiya beauforti* (Hunter y Thomas, 1961) Overstreet and Curran, 2005, *C. overstreeti* (Bargiela, 1988) Overstreet and Curran, 2005, *C. papernai* (Bargiela, 1988) Overstreet and Curran, 2005, *C. agonostomum* (Dyer, Bunckley-Williams and Williams, 1999) Overstreet and Curran, 2005 and *C. tilapiae* (Nasir y Gómez, 1976) Overstreet and Curran, 2005.

*S. tarpazensis* Diaz & Gonzalez 1990, only haploporid that infects cichlid fishes of Venezuela, complete life cycle of which were studied using a mollusc, *Pyrgophorus cf. spiralis* as the first intermediate host and a fish, *Poecilia reticulata* as defini-

tive host [1]. The cercariae of both *S. tarpazensis* y *C. tilapiae* share the same intermediate host, and very similar morphometrically but differ in excretory system. *C. tilapae* do not bear accessory excretory bladder in the tail, and the division of the excretory duct is located at the level of pharynx, which is located at the level of acetabulum in *S. tarpazensis*. The number of flame cells in *C. tilapiae* is 20 while 30 in *S. tarpazensis*. In adult, body measurements, hermaphroditic sac, testes, pre & post acetabular distance, are larger in *C. tilapiae* than *S. tarpazensis*, while the size of ovary and eggs are smaller. Eggs do not bear miracidium in *C. tilapiae* and lack spines in hermaphroditic duct.

Body measurements of *C. tilapiae* in this study coincides with that of the original description of *C. tilapiae* [3], but differs in the presence of tegumentary spines upto the level of anterior testis, small spines on both suckers, absence of internal seminal receptacle, larger oral sucker than ventral sucker, well developed Pars prostatic and internal seminal vesicle covering almost all the area of hermaphroditic sac.

## CONCLUSIONS

The *Culuwiya tilapiae* (Nasir y Gómez, 1976) Overstreet and Curran, 2005 was redescribed reporting new morphometric characteristics. The life cycle of this species was studied, using the snail, *Pyrgophorus cf. spiralis* as intermediate host and fresh water fish, *Oreochromis mossambicus* (Peters, 1852) as definitive host from the Parque Litoral Laguna de los Patos, Cumaná, Venezuela.

The life cycle was studied from cercariae to adult both under natural and experimental conditions. The trematode reaches sexual maturity in 22-32 days after the experimental infection. All developmental stages of *C. tilapiae* were described and illustrated.

This species was compared with other members of *Culuwiya* and with *Saccocoelioides tarpazensis* Díaz and González, 1990, for being the only recorded haploporids species in Venezuela. This species represents the first record of *Culuwiya* Overstreet and Curran, 2005 in Venezuela.

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