DESCRIPTION OF DIFFERENT DEVELOPMENTAL STAGES OF *CENTROCESTUS FORMOSANUS* (NISHIGORI, 1924) (DIGENEA: HETEROPHYIDAE)

DESCRIPCIÓN DE DIFERENTES ESTADIOS DE DESARROLLO DE *CENTROCESTUS FORMOSANUS* (NISHIGORI, 1924) (DIGENEA: HETEROPHYIDAE)

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ABSTRACT

Descriptions of the different immature stages (redia, cercaria, and metacercaria) and adult stage of *Centrocestus formosanus* (Nishigori) are presented. Between 32 and 36 circumoral spines are generally reported for the *C. formosanus*, and in this study 32 circumoral spines were found on the *C. formosanus* collected in Sucre state, Venezuela. Experimental infection on different types of hosts show a high susceptibility of infection for the *C. formosanus* metacercariae.

Key words: *Centrocestus formosanus, Bufo granulosus, Rivulus harti, Synbranchus marmoratus*, Venezuela.

RESUMEN

Se presentan las descripciones de los estadios inmaduros (redia, cercaria, y metacercaria) y el estadio adulto de *Centrocestus formosanus* (Nishigori). Entre 32 y 36 espinas circumorales son generalmente referidos para *C. formopsanus*, y en este estudio fueron encontradas 32 espinas circumorales para esta especie recolectada en el estado Sucre, Venezuela. Infecciones experimentales en varios hospederos mostraron una alta susceptibilidad a la infección de las metacercarias de *C. formosanus*.

Palabras clave: *Centrocestus formosanus, Bufo granulosus, Rivulus harti, Synbranchus marmoratus*, Venezuela.

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INTRODUCTION

The intestinal trematodes of the *Centrocestus* genus are (likewise many other worms) hermaphroditic worms equipped with a ventral sucker by which attach the wall of intestinal mucous. If eggs, passed in the faeces, reach fresh water, a miracidium is released and can enter and multiply in appropriate fresh water snails, *Melanoides tuberculata* (Muller, 1774). Here the parasite develop for several weeks into a cercarial form and these are discharged back into water and develop further as metacercariae in or on a second intermediate host, such as fishes and tadpoles. This complex cycle is completed when a susceptible animal (rats, monkey, pigeons, chicken, ducks) ingest the intermediate host, and the parasite develops into the adult form. Human infections with *C. formosanus* have been reported in Formosa, Japan and China [4,11].

The objective of this study is to elucidate all stages of the life cycle of *C. formosanus*, with the aid of light and scanning electron microscopy (SEM), and to try experimental infection on some susceptible animals.

MATERIALS AND METHODS

A total of 883 fresh water snail, *Melanoides tuberculata* (Mollusca: Prosobranchia: Thiaridae), 50 fishes belonging to *Rivulus harti* and one *Synbranchus marmoratus* were collected from a rivulet using small hand net in Aguasanta and Yaguaracual in Sucre state, Venezuela. In addition, 600 tadpoles of *Bufo granulosus* were collected by hand nets from different seasonal ponds in and around the campus of the Universidad de Oriente. All fish and tadpoles were brought to the laboratory and maintained in fresh water aquarium of size of 20 and 40 gallons. Two snails were kept in each finger bowls, with ¾ filled

with water. Each bowl was examined every 12-24 h with a binocular microscope to observe the emission of cercariae.

Infection free tadpole of *B. granulosus* were infected with naturally emerged cercariae of *C. formosanus* from naturally infected *M. tuberculata* and the cyst of the parasite were found in the gills and adipose tissue of the tadpole. Metacercariae of *C. formosanus* were found in naturally infected gill of fish, *R. harti* and *S. marmoratus*. Various mammals and birds were infected with metacercariae of *C. formosanus* collected from both naturally and experimentally infected hosts of 12 day old. Adult trematodes were found from the experimental hosts from the first day of infection (TABLE I).

All materials of *C. formosanus* were collected from the experimental and naturally infected sources and studied fresh in a normal saline solution of 0.75% and 0.85%. Most specimens were heat killed in hot saline, with or without coverslip pressure, and immediately fixed in FAA (Formalin ethyl alcohol and acetic acid), stained with Semichon's carmine for permanent mounts in Canada balsam. Camera lucida drawings of live and preserved trematodes were made. Definitive experimental hosts were supplied by the University bioterium. All measurements in the text are given in millimetres.

For SEM, living specimens of rediae, cercariae, encysted and excysted metacercariae, and adult worms were fixed in 2.5% Glutaraldehyde in a 0.1 M sodium Cacodylate buffer, pH 7.2 for a period of 4-6 h and later postfixed in 2% Osmium tretraoxide for 1h in the same buffer. Dehydration was in a graded series of ethanol with 20 min in each. Specimens were dried in a critical point drying apparatus for 30 min, coated with gold palladium for 3 min and examined in a S-800 Hitachi SEM.

RESULTS

Description of different stages of ***C. formosanus***

REDIA (FIG. 1: a-d): Direct observation on the body of redia show that they exhibit some movement and contraction, measure 0.217-0.333 X.080-0.101. The SEM slide show that there are short and long ciliae on either side of anterior end (FIG. 3b) and body folds (FIG. 3a). The neck is well developed. Pharynx is large and muscular, measuring 0.02-.03 X.025-0.030 and joins with short intestinal caecum. Birth pore opens laterally at the level of intestinal caecum which possess six digitiform prolongation when inverted (FIG. 1b). Body of redia is full of germinal mass (FIG.1c) and different stages of cercaria. A rudimentary acetabulum is the most prominent organ of cercaria when is inside the redia. Two short locomotory appendages was observed in young redia (FIG.1d) but never seen in the adult redia. Elongated gland cells (FIG. 1a), transverse and longitudinal muscles were observed in the posterior part of body when it was not occupied with germinal mass.

CERCARIA (FIG. 2:a-g): The newly emerged cercaria is very active and swim by beating tail synchronized with the shaking of body in abundant water. Immediately after activity the cercaria becomes quiet and start to sink down slowly. The majority rest on the bottom with body and tail become almost horizontal line and they live up to 3 days.

Body is oval and sometimes heart shaped when rest, measuring 0.045 -0.078 x 0.023- 0.072. On SEM, the body is spinose with short scale like spine arranged in transverse rows (FIG. 3 c-d). Tegument is thick, provided with 7 uniciliated pairs of papillae, 4 of which with long cilias and other 3 with short cilias. The tail subterminal without spines, possessing transverse and longitudinal muscles all along the tail (FIG.2 a), 0.09-0.137 x 0.01-0.018 long. It contains 7 uniciliated papillae, 3 on right side and 4 on left. The tail possess an undulated membrane dorsoventrally, well developed originating from the anterior –third of the tail continuing up to the tip (FIG. 2 e,f). There are two ocular spots with lens containing dark brown pigment (FIG. 2 g), localized on either side of the body just below the pharynx. The left spot is double and the right one single. Oral sucker subterminal, well developed, measuring 0.021-0.024 x 0.018-0.024. The external border of the mouth is surrounded with papillae while internal border with minute spines (FIG. 2b,c). Guarding the mouth are nine small hooklets in two rows, 4 anteriorly and 5 posteriorly. Prepharynx short and pharynx muscular followed by a thin long esophagus. Pharynx measures 0.008-0.010. Intestinal caecum is absent. Acetabulam rudimentary, inconspicuous, located above the ru-

TABLE I

EXPERIMENTAL INFECTIONS IN DIFFERENT HOSTS USING ENCYSTED METACERCARIAE OF *CENTROCESTUS FORMOSANUS* RECOVERED FROM TADPOLES OF *BUFO GRANULOSUS* DURING 1995-96 (ALL PARASITES RECOVERED FROM SMALL INTESTINE OF INFECTED HOSTS)

Type of animals	No.of hosts infected artificially	Age of metacercariae	Duration of infection	No. of worms recovered & mean intensity	No. Infected & Prevalence (%)
Chicken	7	15-30	5-7	21 / 7	3/ 43
Rats	7	23	1-21	5038/ 719.7	7/ 100
Ducks	3	26	5-6	2/ 2	1/ 33
Pigeon	2	26	3	6/ 3	2/ 100
Hamster	1	34	3	46/ 46	1/ 100

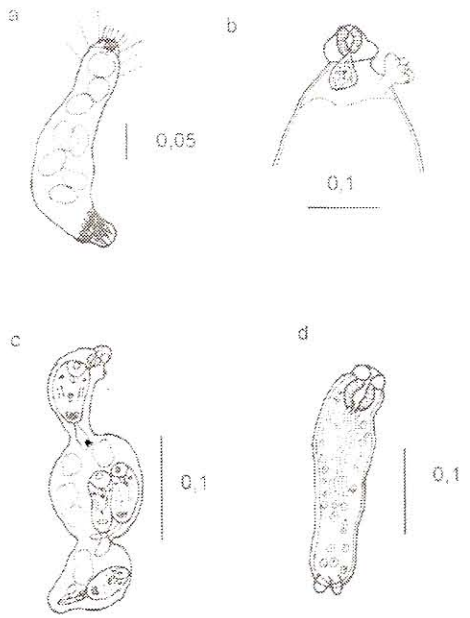

FIGURE 1. DIFFERENTS STAGES OF REDIA OF *Cetrocestus formosanus*. A) YOUNG REDIA WITH CILIAS AT ANTERIOR END AND GLAND CELLS AT THE POSTERIOR END. B) ANTERIOR END OF ADULT REDIA SHOWING EVERTED BIRTH PORE AND SHORT INTESTINE. C) ADULT REDIA WITH CERCARIAE AND GERMINAL MASS. D) POSTERIOR LOCOMOTOR APPENDAGES IN YOUNG REDIA.

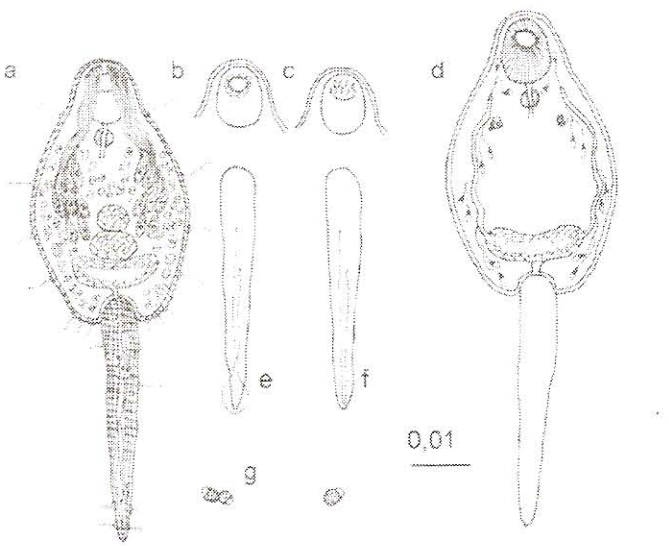

FIGURE 2. CERCARIA OF *Centrocestus formosanus*. A) CERCARIA WITH PENETRATION GLAND CELLS, CYSTOGENIC GLAND CELLS AND RUDIMENTARY ACETABULUM. B) MOUTH SURROUNDED WITH PAPILLAE EXTERNALLY AND MINUTE SPINES INTERNALLY.

dimentary genital primordial. It is constituited with a mass of undifferentiated cells between the acetabulum and excretory bladder. The cephalic glands consist of 14 granular cells, each provided with a nucleus and grouped in two of 7 cells each and located on either side of body between esophagus and excretory bladder. Each cell is connected at its anterior end with a finely granular duct. These ducts go upward anteriorly and opening up at the anterior part of the body in four groups of 3- 4- 4- 3. The excretory bladder is transversally enlarged and its wall consist of unnucleated cells with granular cytoplasm. The primary duct is originated on the margin of antero-lateral side of bladder extending up to the level of pharynx, receiving the anterior and posterior collecting tubules of flame cells (FIG. 2 d). The formula of flame cells: 2(2+ 2) + (2+ 2) = 16.

ENCYSTED METACERCARIAE (FIG. 4): The cysts are found normally on the branquial filaments of fishes and on adipose tissues of tadpole. Cysts are small oval or spherical. The cyst is composed of two walls, the outer one thick and the inner one thin. Body is oval shaped, measuring 0.115-0.202 x 0.136- 0.191 and the tegument is completely covered with spines. Two small ocular spots appear at a level of pharynx. Oral sucker terminal, measuring.040-.0446 X.030-.050 and armed with two rows of 32 spines. Acetabulum well developed, measuring.016-.037 X.031-.035.Prepharynx short, measures 0.004-0015 x0.004-0.015 and pharynx oval shaped, measuring 0.030- 0.037 X 0.021-.030. Oesophagus is shorter than pharynx. Caecum wider and reaches up to the level of excretory bladder, filled with discoidal bodies of brilliant green color. Testes symmetrical reaching close to the extreme end and left one measure 0.016-0.022 X 0.032- 0.018 and right one measure 0.015-0.022 X 0.026-0.045. Ovary long, pre-testicular, measures 0.012-0.015 X 0.0228- 0.0335. Excretory bladder X-shaped and main collecting ducts reaches anterior to acetabulum. Flame cell formula: 2 (2+ 2) +(2+ 2) = 16.

EXCYSTED METACERCARIAE (FIG. 5 a-b): Body somewhat elongate (FIG. 5a), narrower in extreme anterior end, with cuticuler spines upto the extreme posterior end, measures 0,244-0.322 X.083-.086. The oral sucker is 0.026-0.056 x.032-0.051, armed with 32 circumoral spines (FIG. 5b). The prepharynx, measures 0.008- 0.024 X.008-.010 and oesophagus, measures 0.013-.034. Pharynx large and muscular, measuring 0.021- 0.043 X.013-.026. Intestinal caeca long, reaching at a level of ovary, filled with crystal mass. Acetabulum post equatorial, spherical, measure 0.029-.040 X 0.034-.043. SEM photograph of oral sucker shows two rows of 16 circumoral spines (FIG. 6 b) and uniciliated sensorial papillae. The acetabulum possess 6 non ciliated papillae on border (FIG. 6a). Testes oval, lightly lobulated in posterior border, symmetrical. and displaced towards lateral side due to the expansion of excretory bladder, measuring 0.021-.034 X.032-.043 (left) and 0.024-.034 X.026-.037 (right).Genital atrium located anterior to acetabulum and oval genital pore located immediately at the anterior margin of acetabulum. Ovary

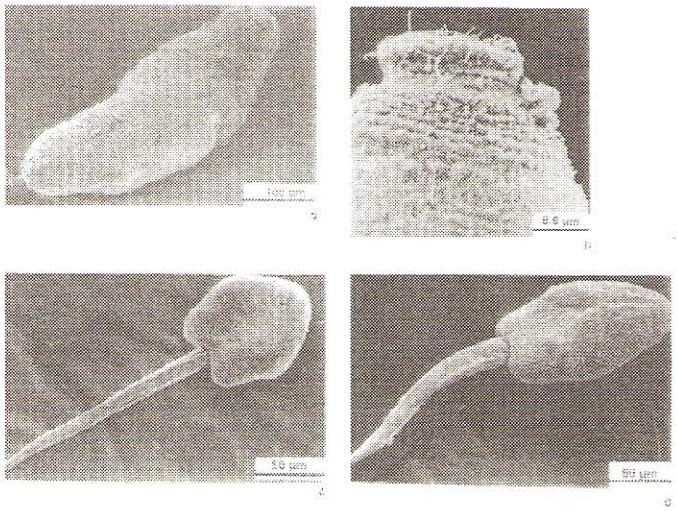

FIGURE 3. THE STRUCTURE OF REDIA AND CERCARIA OF *Centrocestus formosanus.* A)REDIA SHOWING CIRCUNFERNTIAL FOLDS. B) UNICILIATED PAPILLAE AND MICROVILLI AT ANTERIOR OF REDIA. C,D) CERCARIA IN VENTRAL VIEW AND DORSAL VIEW BODY COVERED WITH SPINES.

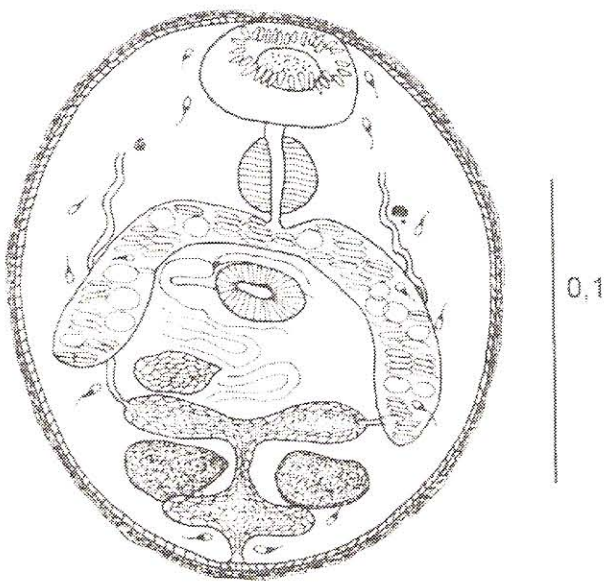

FIGURE 4. ENCYSTED METACERCARIA OF *Centrocestus formosanus*

oval in shape, located in front of right branch of excretory bladder. Excretory vesicle is X shaped. Excretory pore subterminal. Flame cell formula: 2 (2+ 2) + (2+ 2) =16.

EXPERIMENTAL INFECTIONS OF DEFINITIVE HOSTS

The results of the experimental infections are summarized in TABLE I and II.

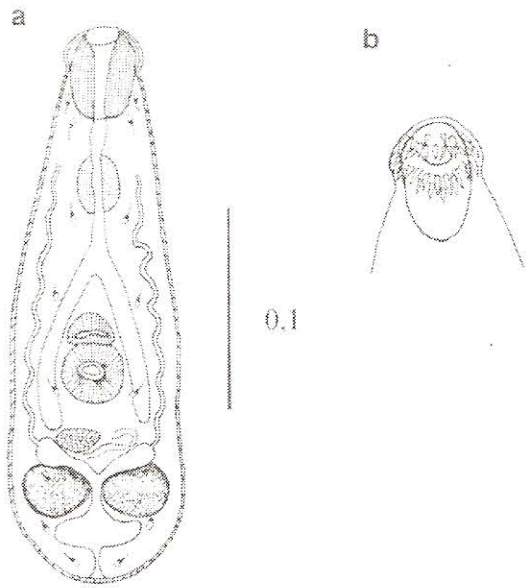

FIGURE 5. METACERCARIA OF *Centrocestus formosanus.* A) VENTRAL VIEW OF EXCYSTED METACERCARIA. B) EXTREME ANTERIOR END OF EXCYSTED METECERCARIA SHOWING 32 CICUMORAL SPINES.

DESCRIPCION OF ADULT (FIG. 7 a- d):Body small, truncated and spinose (FIG. 7a), measured 0.232-0.469 X 0.106- 0.237. Oral sucker terminal, 0.029-.067 x 0.032-.061 long. It is armed with two circles of 32 spines (FIG. 7b). SEM photograph of oral sucker showing two rows of spines and uniciliated papillae (FIG. 7c), and 6 large papillae on the border of acetabulum (FIG. 7d) Prepharynx short. Pharynx muscular measuring 0.026-.040 X.021-.037. and followed by short oesophagus, 0.005-.0032 X.005-.008. Caeca large and diverge towards acetabulum and extend to level of ovary. Acetabulum at middle of body, 0.026-.053 x 0.032-.061 long. The nervous system consists of a pair of ganglia located on each side of the pharynx and connected by a transverse commissiure (FIG. 8a). Each of two cerebral ganglia give rise to two antero lateral nerve fibers reaching the oral sucker and two postero lateral nerve fibers reaching the level of the testes.

The reproductive system consists of well developed organs which mature in a day or so in the definitive host. The male system (FIG. 8b) consists of two oval shaped testes, smooth or slightly lobular, somewhat transversely opposite to each other at the posterior end of the body and localized between the branch of excretory bladder (FIG. 7a). The left testes measured 0.040-.094 X.029-.067 and the right testis measured 0.051-.096 X.026-.059. The efferent duct extends anteriorly from the mid border of each testis and unite to form a short deferent duct before entering the posterior part of seminal vesicle. The seminal vesicle is bifurcated, distal part of it is shorter than the proximal one. The seminal vesicle narrows considerably towards anterior part forming a prostatic complex which is

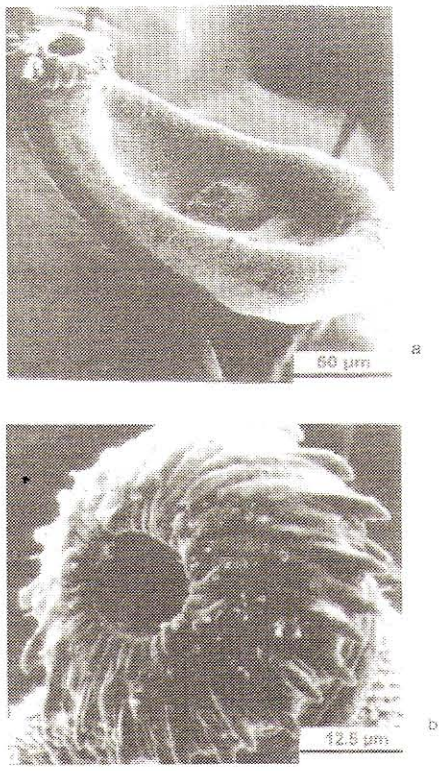

FIGURE 6. THE STRUCTURE OF METECERCARIA OF *Centrucestus formosanus*. A) ENTIRE VENTRAL VIEW OF EXCICED METACERCARIA SHOWING BODY SPINES CONCAVITY. B) ORAL SUCKER METACERCARIA SHOWING DOUBLE ROWS OF SPINES AND UNICILIATED PAPILLAE.

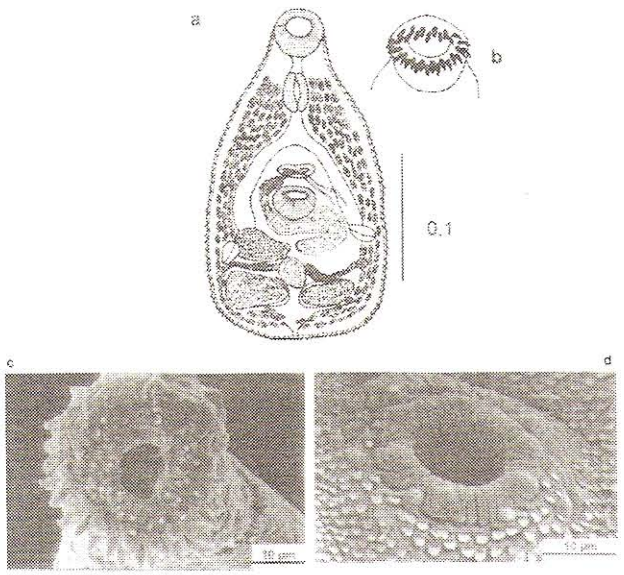

FIGURE 7. THE STRUCTURE OF ADULT OF *Centrocestus formosanus*. A) ADULT IN DORSAL VIEW. B) EXTREME ANTERIOR END SHOWING 32 CIRCUMORAL SPINES. C,D) MICROPHOTOGRAPH OF ORAL SUCKER WITH TWO ROWS OF SPINES AND UNICILIATED PAPILLAE AND PAPILLAE ON THE BORDER OF ACETABULUM.

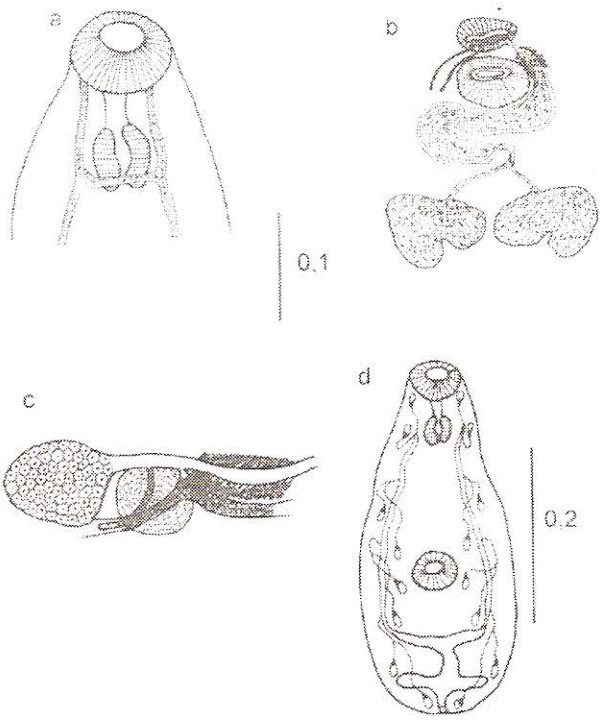

FIGURE 8. CAMERA LUCIDA DRAWING OF ADULT *Centrocestus formosanus*. A) NERVOUS SYSTEM. B) MALE REPRODUCTIVE SYSTEM WITH A PART OF METRATERM. C) A PART OF FEMALE REPRODUCTIVE SYSTEM. D) ADULT WITH PROTONEPHRIDIAL SYSTEM.

surrounded by glandular prostatic cells. The terminal part of the ejeculatory duct entering common genital atrium, which opens to the genital pore, usually located at the anterior border of acetabulum.

The female reproductive system (FIG. 8c) consists of an oval ovary, smooth or slightly lobulated, measured 0.034-.110 X.032 -.080, located in the region immediately anterior to right testes. Oviduct begins from the left lateral side of ovary, passing transversely and receives a short duct from rounded seminal receptacle which measures 0.026-0.053 x 0.21- 0.088. Oviduct next receives a short duct from the Laurer's canal and the vitelline reservoir and then passes on as ootype surrounded by Mehlis gland. Uterus bends several times between anterior border of testes and genital atrium. Common vitelline duct is just in front of testes and reservoir is triangular in shape. Vitellaria form in small group of follicles located outside of caeca and extending from the level of pharynx to region behind the testes at posterior end of body.Eggs elongated, operculated and not embryonated, measuring 0.021-.040 X.014-.018. The protonephridial system (FIG. 8d) is characterized by X- shaped excretory bladder which opens to a terminal excretory pore. From each side of bladder a principal excretory duct extends upward to the level of pharynx where it receives anterior and posterior collecting ducts of flame cells. The formula of flame cells: 2 (2+2) + (2+ 2) = 16.

TABLE II

EXPERIMENTAL INFECTION OF RATS USING ENCYSTED METACERCARIAE OF *C.FORMOSANUS* RECOVERED FROM NATURALLY INFECTED FISHES, *RIVULUS HARTI* AND *SYNBRANCHUS MARMORATUS* DURING 1996-1997 (ALL PARASITES RECOVERED FROM SMALL INTESTINE OF INFECTED HOSTS)

No. rat infected	Duration of infection(in days)	No. of worms recovered	Prevalence(%)
1	5	2	100
1	7	300	100
1	11	150	100
1	53	230	100
1	74	100	100

DISCUSSION

The differentiation of species of *Centrocestus* has been mainly based on the number of circumoral spines, form of the margin of the testes and ovary, maturity stage of egg, and the shape and form of excretory bladder. The number of circumeral spines appears to be definite in the three species according to the various authors. Looss [12] mentioned 36 spines *in C. cuspidatus*, Tanabe [24] gave 44 spines in *C. armatus* and Nishigori [15] counted 32 spines in *C. formosanus*. In the present study, it has been observed 32 spines in two rows which is within the range of 32- 36, mentioned by Chen [4], Kobayasi [8] and Premvati and Pande [20]. Srisawangwong et al. [23] observed also 32 circumoral spines, 16 spines in each row which agree with our observations. With reference to the form of the margin of the testes and ovary in *C. formosanus* were found to be smooth or lobulated with agree with the earlier studies. The excretory bladder in the present study was found to be "X" shaped which was found earlier by Chen [4], Pande and Shukla [17] and Premvati and Pande [20]. The eggs in the present study lacked developed larva in the egg which is in contrast to Faust and Nishigori [6], Chen [5] and Srisawangwong et al. [23]. Nishigori [15] in his original description of cercaria of *C. formosanus* did not report about penetration glandular cells and flame cells. He described complete digestive system. Presence of 7 pairs of penetration glandular cells, 8 pairs of flame cells and incomplete digestive system are described in this studies. These results coincide with Martin [13] and Arizmendi [3]. Chen [5] describes the cercaria but reports only 4 flame cells in the crecaria but 16 in the metacercaria. The external border of the mouth is surrounded with a row of papillae and minute spines on the inner side (FIG. 2b)which was not reported earlier in this species. Similarly, in some rediae, posterior part of the body is composed of well developed transverse and longitudinal muscles, elongated gland cells, and two small caudal projections. The birth pore shows 6 short digitiform prolongations.

Chen [5] reported that metacercaria was infective after 30 days while it was 12 days in the present study. Arizmendi [3] described the life history of *C. formosanus* but did not describe intestinal caecum in the redia and complete protonephidial system. All those are reported in this study. Nasir and Diaz [14] described *Cercaria pleuroloparapleuriformis* for the first time in Venezuela, a cercaria belonging to the group of Pleurolophocerca and the 2nd cercaria, cercaria of *C. formosanus* of the same group is being reported here, which is easily differentiated in number of flame cells and penetration glandular cells.Ciliated papillae and microvilla of the body surface of redia are observed on Scanning photograph which are important diagnostic characters. No such are normally seen in light microscopy and are not reported earlier in *C. formosanus*. Page et al. [16] reported that the ciliated papillae in the region of anterior region of redia of *Ribeiroia marini* may function as chemoreceptors, presumibly in the orientation during the migration in tissues of the snail. Koie [9] reported that short cilias around the mouth of redia of *Neophasis lageniformes* may be involved in feeding and orientation during the migration in snail, while the long cilias on the body surface may be used for removing and circulating the liquid around the surface of redia. Abdul Salam and Sreelatha [1] sustained that the microvilla are essential in the body surface for absorbtion of nutrients in the redia, as the digestive system in this stage does not function properly.

Some changes in the surface morphology of metacercarae during development to adults have reported in *Clonorchis sinensis* and *Ophistorchis viverrini* (Fujini et al. [7] and Apinhasmit et al. [2]. In contrast, the tegument appearance of cercariae to adult worms of *C. formosanus* are closely similar. The spines on the surface probably work as grater to break the surface of the host as indicated by Rees [22]. Surface spines may serve to have a good grip during the penetration process on hosts. Similarly, surface spines in coordination with pre oral spines and cephalic glandular secretion may help in adhering and penetrating the host tegument as indicated by Abdul Salam and Sreelatha [1]. Ciliated papillae of cercariae may be used in localizing the intermediate host and appropriate site for encysment. Rees [22] and Koie [10] reported similar structures in cercariae of *Cryptocotyle lingua*. Ciliated papillae on the dorsal and ventral body surface and tail may function as mechanoreceptor. These papillae may work as tango receptors by stopping the movement of the tail as soon as it is in contact with the hosts. Some ciliated papillae around the oral opening may function as tango receptors in localizing the specific site

for encystment. Page et al. [16] described similar function of ciliated papillae in *R. marini*.

The tegumental appearance of metacercariae and adults are closely similar, perhaps because the metacercariae take a very short time to reach to adult stage. The rapid maturation of the worms may be due to the precocious development of the genitalia in the metacercaria, which coincides with the results of Srisawangwong et al. [23]. The papillae of oral sucker assist in feeding of the worms and may perhaps use in detection of surface tissue; while the papillae of ventral sucker may serve in the control of adhesion of worms. Progressive degeneration of certain organs reported by Srisawangwong et al. [23] have not been observed in this study.

CONCLUSIONS

This report of *Centrocestus formosanus* in Venezuela is a new geographical record. Cercariae of *C. formosanus* were found in naturally infected snails, *Melanoides tuberculata*. Metacercariae were found in gill of naturally infected fish, *Rivulus harti* and *Synbranchus marmoratus* and experimentally infected tadpole of *Bufo granulosus*. These are new secondary intermediate hosts for *C. formosanus*. Rats are found to be the most appropriate experimental definitive hosts of adult *C. formosanus* for harboring high intensity of infection. The differentiation of species of *Centrocestus* has been mainly based on the number of circumoral spines and reported to vary between 32-36 spines for *C. formosanus* and 32 circumoral spines are found in this study.

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