

## THE LIFE CYCLE OF CENTROCESTUS UNEQUIORCHALIS N. SP. (HETEROPHYIDAE: CENTROCESTIINAE)

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*SUMMARY: The life cycle of Centrocestus unequiorchalis n. sp. was described from parapleurolophocercous cercariae emerging from Melania tuberculata snails, encysted metacercaria in Oreochromys niloticus fishes and adult stage in albino rats and ducklings. Detailed description of different stages included:*

*- Description of cercariae for the first time and the differences between it and cercariae of Haplorchis pumilio.*

*- Description of metacercaria and adult stage.*

*- Comparison between the present parasite and the previously described Egyptian forms*

*Key Words: Centrocestus unequiorchalis, melania tuberculata, trematoda, albino rats.*

### INTRODUCTION

Looss (5) created the subfamily Centrocestiinae as well as the genus *Centrocestus* (5) Looss, 1899 from *Milvus parasiticus* in Egypt.

According to Yamaguti (12), Martin (7) obtained the adult stage of *C. cuspidatus* experimentally from metacercaria found in naturally infected *Gambusia sp.* Kurokawa (4) studied the life cycle of *C. nycticoracis* Kobayasi (3). Nishigori (8), Chen (1), and Kobayasi (3) studied the life cycle of *C. formosanus* (8) Price, 1932.

During a survey on the larval trematodes of *Melania tuberculata* snails in Aswan province; parapleurolophocercous cercariae emerged. The completion of its life cycle has been investigated to identify its exact taxonomic position.

### MATERIALS AND METHODS

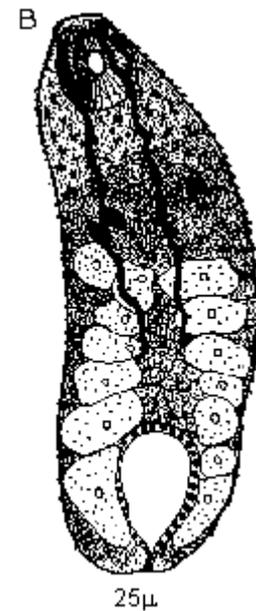
*Melania tuberculata* snails were collected from different irrigation canals from Aswan Governorate. Out of 2876 snails collected, two snails (0,0069%) were found shedding parapleurolophocercous cercariae. Cercariae were described in details from living specimens stained with supravital stains (Nile blue sulphate and neutral red). Some specimens were permanently mounted after staining in aceto-carmine. Measurements of the cercariae were taken from 20 moist-heat killed specimens. Very young clean *Oreochromys niloticus*, *Gambusia affinis* and *Ctenopharyngodon idellus* fishes were experimentally infected with these cercariae by keeping them together with infected snails for 48 hours. Fishes were dissected after fifteen days post infection to examine encysted metacercaria. Five albino rats and five ducklings were left to feed for fifteen days on infected fishes. The experimental animals were dissected after ten days post infection. Mature worms were collected, fixed in 70% alcohol, stained with aceto-carmine stain and mounted. All measurements are in

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Figure 1a: Camera lucida drawing for the whole cercariae.



Figure 1b: Camera lucida drawing for the body of cercariae showing the arrangement of penetration glands.



microns unless otherwise mentioned. Camera lucida drawings were done with different magnifications as indicated.

## RESULTS

### Cercariae

Out of 2876 snails collected, two snails (0.0069%) were found shedding parapleurolophocercous cercariae. The cercariae were usually found in the lower half of the water in the container. Cercariae emerge from infected snails during day and night but commonly in the morning and before noon. They are very sensitive to light. They are not good swimmers, with frequent resting phases. During rest, the body was curved attaining the characteristic pipe-shape of parapleurolophocercous cercariae. Diving movements are characteristic. In the absence of second intermediate host, cercariae live between 36-48 hours.

It is a biocelate parapleurolophocercous cercariae (Figure 1a). The body measures 150-170 $\mu$  x 55-65 $\mu$  and its cuticle is covered with minute spines (Figure 1b). Oral sucker is oval in shape and measures 30-33 $\mu$

x 27.5-29 $\mu$  in size. It is provided by a muscular protrusible organ (the penetrating apparatus) armed with three rows of spines arranged as 6:9:11 spines in each row (Figure 1c). No trace of alimentary canal could be seen except for a small muscular pharynx that measures 9-11 $\mu$  in diameter and located in between eye spots. There are two triangular eye spots at the posterior part of the anterior third of the body. The distance between them varied from 18-23 $\mu$ . The body contains seven pairs of small penetration glands arranged as seven glands in longitudinal series at the right side, while at the left side six glands in longitudinal series and one gland is located to the left of the first gland (Figure 1b). They extend from a little distance below the eye spots to the posterior part of the body surrounding the excretory vesicle and they are separated by genital primordium. The penetration glands occupied 90-100 $\mu$  from the whole length of the body. These glands lead to two bundles of ducts which open anteriorly by 14 large openings arranged as 3:4:4:3 on the terminal edge of the oral sucker. The anterior part of

the body is filled with dark brownish pigments, while the rest of the body contains cystogenous glands. Excretory vesicle is oval in shape. It leads to excretory duct which is inverted Y-shaped and opens at the lateral margins of the tail by two excretory openings located at 40-50 $\mu$  from the anterior edge of the tail. Excretory ducts and flame cells could not be traced in the body due to the heavy pigmentation and excess cystogenous glands. The tail measures 300-320 $\mu$  x 15-20 $\mu$  in size. It is provided with dorso-ventral finfold.

*Encysted metacercaria* (based on ten mounted specimens): Cercariae encysted in gills of *Ore-*

*ochromys niloticus* but failed to encyst in *Gambusia affinis* and *Ctenopharyngodon idellus* fishes. Fifteen-days old metacercaria measure 180-200 $\mu$  x 200-210 $\mu$  in size. Cyst wall measures 5 $\mu$  in thickness. Traces of circumoral spines could be seen. Experimental infection was successful in all five albino rats and five ducklings. Adult stage was obtained after ten days post infection (Figure 2).

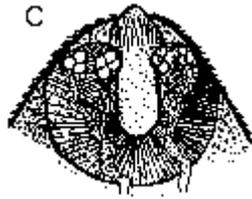
#### Adult stage

The body is small (Figure 3), pyriform and measures 265-300 $\mu$  in length and 70-125 $\mu$  in maximum

Table 1: Comparison between previously described species from Egypt and *C. unequiorchalis* n. sp.

The authors The parasites	<i>Centrocestus cuspidatus</i> (Loss, 1896) Loss 1899 described by Sakla and Monib (1982)	<i>Centrocestus</i> sp. Sakla and Monib (1982)	<i>C. unequiorchalis</i> n. sp. (present work)
Body size	490-530 x 230-250 $\mu$	570-600x180-195 $\mu$	265-300 x 70-125 $\mu$
Oral sucker	70 $\mu$ in diam.	59 $\mu$ in diam	40-55 x 37.5-50 $\mu$
No. of circumoral spines	32 spines	28 spines	31-33 spines
Size of spines	17 $\mu$ x 14 $\mu$	16 x 4 $\mu$	10 x 5 $\mu$ ant. row 7.5 x 5 $\mu$ post. row
Prepharynx	53-55 $\mu$ in length	59 $\mu$ in length	10-15 $\mu$ in length
Pharynx	53-55 x 40-43 $\mu$	40-43 x 35-39 $\mu$	25-30 in diam.
Oesophagus	78 $\mu$ in length	113 $\mu$ in length	40-50 $\mu$ in length
Ventral sucker	51-55 in diam	39-47 in diam.	35-40 in diam.
Right testis	51-55 $\mu$ in diam	74-78 $\mu$ in diam	30-40 x 35-45 $\mu$
Left testis	symmetrical	symmetrical	45-60 x 45-55 $\mu$ asymmetrical
Ovary	40-43 x 50-55 $\mu$	59-60 x 75-78 $\mu$	45-65 $\mu$ in diam
Egg	37-39x19-21 $\mu$	39-41 x 20-22 $\mu$	21-23 x 27-30 $\mu$
No. of uterine eggs	numerous	numerous	4-6 eggs
Host	<i>Ardeola ibis</i>	<i>Hoplopterus spinosus</i>	Exp. in Ducklings and albino rats
Locality	Assiut province	Assiut province	Aswan province

Figure 1c: Free hand drawing for the anterior part of the body of cercariae showing the penetrating apparatus and the arrangement of penetration gland openings.



breadth. Cuticle is covered with small scale-like spines. The oral sucker is sub-terminal, oval,  $45\text{-}50\mu \times 37.5\text{-}50\mu$  in size. Oral sucker is surrounded by 31-33 circum-oral spines arranged in two alternated rows. Spines of the anterior row measure  $10 \times 5\mu$  while spines of the posterior row measure  $7.5 \times 5\mu$ . Oral sucker is followed by a short pre-pharynx which measures  $10\text{-}15\mu$  in length. Pharynx is well developed measuring  $25\text{-}30\mu$  in diameter. Oesophagus is short,  $40\text{-}50\mu$  in length. Intestinal caeca are slender, short and could not be traced beyond the level of the posterior margin of acetabulum. The acetabulum is rounded, post-equatorially measures  $35\text{-}40\mu$  in diameter and located about  $100\text{-}155\mu$  from the anterior end. Testes are large, rounded and opposite in position. The right testis is usually smaller ( $30\text{-}40\mu \times 35\text{-}45\mu$ ) than the left one ( $45\text{-}60\mu \times 45\text{-}55\mu$ ). They are situated at a short distance from the posterior end of the body and narrowly separated by X-shaped excretory bladder. The ovary is large, globular and measures  $45\text{-}65\mu$  in diameter and is situated at the left side of acetabulum above the left testis. Vitellaria extend along the lateral margin starting from the posterior level of the pharynx to the posterior end of the body and confluent at the fore body. Uterus is short and contains from 4-6 eggs. The egg is oval and measures  $27\text{-}29\mu \times 21\text{-}23\mu$  in size.

#### DISCUSSION

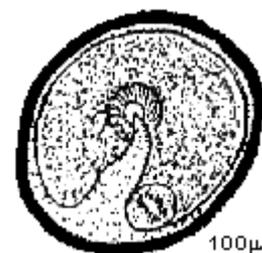
According to Yamaguti (10) the parasite under discussion belongs to the genus *Centrocestus* Looss,

(5,6) described *Centrocestus cuspidatus* for the first time from the small intestine of *Milvus parasiticus* in Egypt. Gohar (2) listed *C. cuspidatus* from the intestine of *Milvus Mgranus* in Egypt. Martin (7) obtained the adult of *C. cuspidatus* experimentally by feeding metacercaria to chicks for the first time. Sakla and Monib (9) redescribed this species from *Ardeola ibis* in Assiut province as a new host record for this parasite. They also described unnamed species from the Spur-winged plover, *Hoplopterus spinosus*. By comparing the present parasite with the common Egyptian species *C. cuspidatus* it was found that they are completely different (Table 1) where, the present parasite is smaller in size, testes are asymmetrical in size, the circumoral spines are in two different sizes, the number of uterine eggs are few, oesophagus is short, pharynx is small and the size of egg is small. Moreover, *Centrocestus* species Sakla and Monib (9) differs from the present material in having equal-sized 28 circumoral spines (Table 1) parasite.

Martin (7) found the metacercaria of *C. cuspidatus* in the gills of *Gambusia* sp. but during the present study, the encystment occurred only in *Oreochromys niloticus*. This might add an evidence that the present material is not *C. cuspidatus*.

*C. formosanus* (8) Price, 1932 which synonymized by Yamaguti (11) as *C. yokogawai Kobayasi*, 1942 has 32 circumoral spines alternating in two rows, where spines of the anterior row ( $11.2\text{-}15.8\mu$ ) are larger than spines of the posterior one ( $11.2\text{-}13\mu$ ). On the basis of the larval stages (cercariae and metacercaria) and

Figure 2: Camera lucida drawing for fifteen-days old metacercaria.



adult stages, the present parasite and *C. formosanus* are completely different, where in the latter parasite:

\* Cercarial has seven pairs of penetration glands anterolateral to the acetabulum.

\* Cercarial tail with weakly developed dorso-ventral finfold which extends approximately posterior three-fourth of tail.

\* *Gambusia affinis* and *Ctenopharyngodon idellus* fishes act as second intermediate hosts.

\* The adult stage is large in size, has spherical, or lobate ovary, and symmetrical testes which transversely elongated with marginal incisions.

The present cercariae emerged from *Melania tuberculata* snails in Aswan and described here for the first time is apparently similar to that of *Haplorchis pumilio* (5) Looss, in having the same penetrating apparatus, but different in the size and arrangement of penetration glands; the size of penetration gland openings; and in the behavior and position of encystment in the intermediate hosts.

The present work included a hitherto unknown species of *Centrocestus*, to which the name of *Centrocestus unequiorchalis* is suggested due to the characteristic unequal testes of the adult parasite. Moreover, complete life cycle was successfully done experimentally giving the opportunity to describe the detailed morphology of the cercariae and metacercaria.

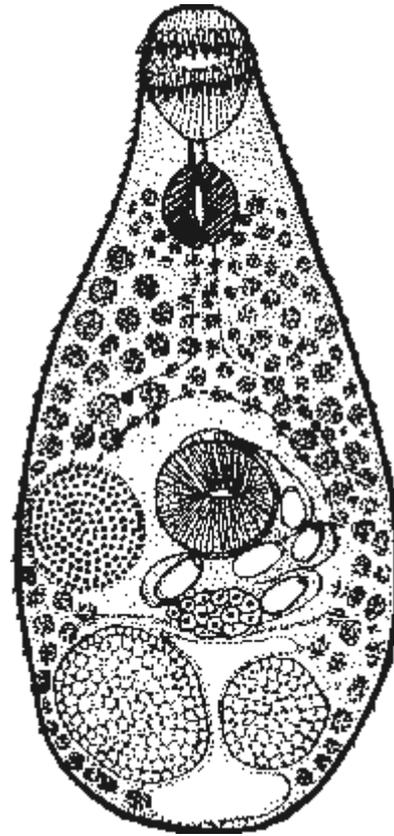
#### ACKNOWLEDGEMENT

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Figure 3: Camera lucida drawing for ten-days old mature stage.



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