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THE LIFE CYCLE OF *DENDRITOBILHARZIA ANATINARUM* CHEATUM, 1941 (TREMATODA, SCHISTOSOMATIDAE)

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The life cycle of Dendritobilharzia anatinarum *was completed experimentally in the laboratory.* Cairina moschata domestica *(domestic duck) and* Biomphalaria straminea *served respectively as definitive and intermediate hosts.*

Eggs passed in duck faeces hatch miracidia in 10 minutes when placed in water. Eight days after the snail infection, the mother sporocyst contains daughter sporocysts ready to migrate. Cercariae are present within the daughter sporocysts 23 days after infection and emerge from the snail on the 25th day. They actively penetrate the skin of the duck and after a prepatent of 39 days, sexually mature trematodes are present in the blood vessels of the bird. The adult parasite is predominantly in the renal-portal system and to a lesser degree in the lungs and mesentery.

A detailed morphological description of the egg, miracidium, sporocyst and cercaria is presented.

Skrjabin and Zakharow (1920) described the genus *Dendritobilharzia* reporting the species *D. odhneri.* Later Skrjabin (1924) described *D. loossi* and changed the name *Bilharziella pulverulenta,* Braun, 1901 to *D. pulverulenta,* which became the type species of the genus. Mehra (1940) described *D. asiaticus* and Cheatum (1941) reported *D. anatinarum* as a synonym of *D. pulverulenta,* but Farley (1971), in a review of Schistosomatidae, mentions the species *D. pulverulenta* (Braun, 1901; Skrjabin, 1924), *D. loossi* (Skrjabin, 1924), *D. asiaticus* (Mehra, 1940) and *D. anatinarum* (Cheatum, 1941). Vande Vusse (1980) in a recent review of the genus *Dendritobilharzia* considered *D. anatinarum* as a synonym of *D. pulverulenta* (Macko, 1959).

In the present paper, the life cycle of *D. anatinarum* was completed experimentally under laboratory conditions.

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MATERIAL AND METHODS

Naturally infected ducks, *Cairina moschata domestica* (L), from Jequitibá County, Minas Gerais State, Brazil, were the source of *D. anatinarum* eggs for this study. Fresh faeces from ducks were placed in Petri dishes, diluted with tap water and examined for eggs. In each instance, 35 were measured in each of tap water, 0,85% saline, Bouin's and Gilson's solutions.

Miracidia were studied alive, both unstained and stained supravitally with alizarine, brilliant blue cresil, methyl red and methyl green. Methyl cellulose was used to immobilize living individuals. The number of miracidia measured after fixation at 65°C were: 35 in Bouin's, 35 in Gilson's and 25 stained with silver nitrate (Lynch, 1933).

Laboratory reared snails of the species *Aplexa (Stenophysa) rivalis* (Maton & Rachett, 1807), *Biomphalaria glabrata* (Say, 1818), *Biomphalaria straminea* (Dunker, 1848) and *Lymnaea (Pseudosuccinea) columella* (Say, 1817) were used to produce experimental infections. These snails when 15 days old were infected by the method of Leite, Costa & Costa (1979). At the same time, snails were collected from the place where the ducks were found naturally infected.

Sporocysts dissected from infected snails were studied alive unstained. Infected snails shedding cercariae were placed in Petri dishes with clean water at 28°C in artificial light for 2 to 3 hours. Cercariae were observed alive and 35 were measured after fixation in 10% formalin at 60° C. Measurements were also taken of heat-killed specimens.

Using a device placed against the skin, each of 5 laboratory-reared ducks were exposed to 80 cercariae, using the method of Leite, Costa & Costa (1979). Males and females of. *D. anatinarum* from experimentally infected ducks were fixed in Gilson's solution, stained by Gomori method and mounted in balsam.

RESULTS

Egg

The eggs of *D. anatinarum* (Fig. 1) are in various stages of development when passed in the faeces of infected ducks. They are subspherical and without a spine or operculum. The shell is thin, flexible and transparent. The egg measurements, in μm were: 111 (97 to 125) by 108 (94 to 124) in water: 101 (85 to 120) by 98 (84 to 120) in saline; 98 (82 to 121) by 94 (78 to 116) in Bouis's solution; and 97 (77 to 125) by 94 (74 to 122) in Gilson's solution.

Miracidium

Miracidia (Fig. 2) remain active for 8 hr in water at pH 5.5 to 6.0 and 20° to 21° C and require 4 min to penetrate the snail. The body is covered with cilia except for the terebratorium and subepithelial spaces. The body is uniform, except at the level of the first series of plates adjacent to the terebratorium where it is somewhat smaller. The measurements of fixed miracidia and those stained with silver nitrate are presented in Table I.

The process and lateral papillae are located between the first and second tiers of epidermal plates. The 22 epidermal plates are arranged in 4 transverse rows of 6, 9, 4, and 3 cells respectively from anterior to posterior end (Fig. 3).

The terminal portion of the sensitive papillae and the pores of the apical and penetrating glands are located anteriorly in the cone-shaped terebratorium (Fig. 4).

TABLE I

Measurements (μm) of *D. anatinarum* miracidia

	Bouin	*Gilson*	*Silver nitrate*
Length	147±10	134±12	126±9
Width			
anterior 1/3	58±10	64±10	70±80
posterior 1/3	46± 9	44± 9	60± 7
Terebratorium			
length	5±1	4±1	4±1
width at base	10±1	10±1	10±1

The apical gland is usually pyriform, has granular contents and contains 4 nuclei in its posterior end. This gland stains strongly with neutral red and brilliant blue cresil.

The tube-shaped penetration glands are located lateral to the apical gland; an eccentric nucleus in visible in their bulb-like posterior end. They stain strongly with neutral red. The neural mass is ovoid and composed of small round cells each with its eccentric nucleus, which become very distinct when stained by neutral red. An elastic membrane contains 30 to 40 germ-cells connected in a compact mass that is readily observed when stained with neutral red or methyl green. An individual cell is ovoid, has a transparent cytoplasm and an eccentric nucleus which, in turn, contains a nucleolus.

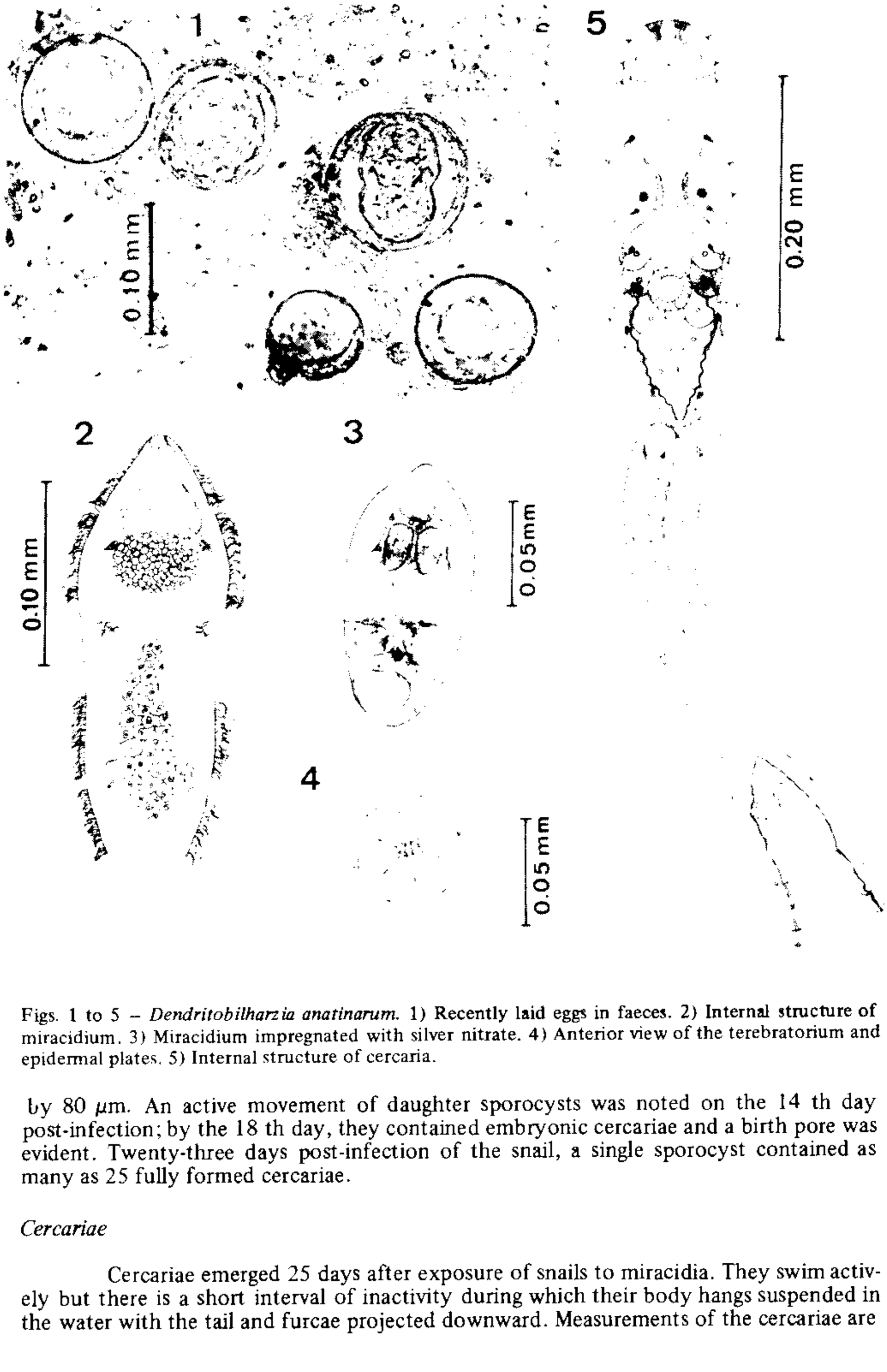
The excretory system consists of two pairs of flame cells and their connecting tubules. The anterior pair is located adjacent to the bulblike portion of the penetrating glands and the other pair is located at the origin of the fourth row of the epidermal plates. Two sinuous tubes arising from the flame cells connect above the posterior flame cell. The excretory pore opens laterally between the third and fourth, or occasionally, near the posterior margin of the third row of epidermal cells.

Sensory papillae are located in the base of the first row of epidermal plates. Eighteen additional sensory papillae are located between the first and second row of plates.

Experimental infection of snails — *A. rivalis, B. glabrata, B. straminea* and *L. columella* were tested for susceptibility to *D. anatinarum* infection. Only *B. straminea* proved to be susceptible and their infection rates was more than 50%. This species of snail was also found naturally infected in the same place as infected ducks.

Sporocysts

Forty-eight hr after exposure, mother (or micaridium-sporocysts) were found in the cephalic region of the snails. They measured 250 by 90 μm, were smooth and contained germinal elements. At 6 days post-exposure, mother sporocysts measured 130 by 950 μm and contained elongated germ balls. They were mostly in the perirenal area but also in the digestive gland, and showed slow, weak movements. Eight days post-exposure mother sporocysts containing daughter sporocysts, measuring 370 by 60 μm and showing germinal elements, were observed in the perirenal region, digestive gland and ovotestis. The daughter sporocysts were smooth except for spines anteriorly. By the 10th day post-infection, they were free in the digestive gland and ovotestis and measured 510



Figs. 1 to 5 – *Dendritobilharzia anatinarum.* 1) Recently laid eggs in faeces. 2) Internal structure of miracidium. 3) Miracidium impregnated with silver nitrate. 4) Anterior view of the terebratorium and epidermal plates. 5) Internal structure of cercaria.

by 80 μm. An active movement of daughter sporocysts was noted on the 14 th day post-infection; by the 18 th day, they contained embryonic cercariae and a birth pore was evident. Twenty-three days post-infection of the snail, a single sporocyst contained as many as 25 fully formed cercariae.

Cercariae

Cercariae emerged 25 days after exposure of snails to miracidia. They swim actively but there is a short interval of inactivity during which their body hangs suspended in the water with the tail and furcae projected downward. Measurements of the cercariae are

TABLE II

Measurements (μm) of the epidermal cells of *D. anatinarum* miracidia stained with silver nitrate

Row of Cells	*Measurements*	*Average*	*Range*
1st	Length	22	19-27
	Width	17	13-21
	Distance between 1st and 2nd row	4	3-5
2nd	Length	23	19-27
	Width	15	12-20
	Distance between plates	3	1-15
	Distance between 2nd and 3rd row	28	20-34
3rd	Length	17	13-21
	Width	31	25-36
	Distance between plates	3	3-5
	Distance between 3rd and 4th row	7	4-9
4th	Length	17	13-25
	Width	28	19-34
	Distance between plates	2	1-4

presented in Table III. Cercariae are ovoid and brevifurcous with a surface having minute spines except on the fund-folds and tail papillae. Thin and proeminent spines are evident on the body surface.

TABLE III

Measurements (μm) of cercariae of *D. anatinarum* fixed in 10% formalin or heat-killed specimens

Measurement	*Total*	*Body*	*Oral sucker*	*Ventral sucker*	*Eyespot*	*Tail stem*	*Furcae*	*Papillae of the furcae*
Fixed in formalin								
Length	446(412-495)	182(169-207)	68(58-80)	22(19-27)	7(5-9)	198(180-219)	66(53-74)	10(8-13)
Width		67(58-74)	41(37-49)	25(21-30)	6(4-7)	29(9-16)	12(9-16)	5(4-7)
Anterior end				121(104-144)	83(70-100)			
Posterior end				39(33-48)				
Between eyespot					22(19-27)			
Heat-Killed								
Length	541(504-602)	232(210-257)	68(62-73)	30(27-33)	7(5-8)	214(183-253)	86(84-97)	9(7-11)
Width		77(64-90)	48(34-56)	29(27-33)	6(5-7)	35(29-44)	17(13-20)	6(4-7)
Anterior end				136(116-160)	100(85-106)			
Posterior end				69(66-82)				
Between eyespot					27(21-32)			

The oral sucker occupies the anterior fourth of the body and its anterior end is provided with numerous spines. The esophagus is bifurcated into two caeca at the level of the eyespots. The highly muscular ventral sucker is situated in the anterior third of the body between the second and third pair of penetrating glands. There are 5 pairs of these glands. Two, which have granular contents, are anterior to the ventral sucker and have homogenous contents.

The excretory system consists of 6 pairs of flame cells; 5 in the body and one in the anterior end of the tail.

The flame cell formula is $2\,[\,(3)+(2)+(1)\,]=12$. From each flame cell there arises a primary collecting tubule which drains into a secondary collecting tubule. These

form a main collecting tube at the level of the ventral sucker. This tube extends to the excretory vesicle in the posterior end of the body. Arising from the excretory vesicle there are two short tubules that converge into a common collecting tube which runs through the center of the tail stem and bifurcates, extending to the tips of the furcae.

Infection of Ducks

Eggs of *D. anatinarum* were first found in the faeces of the 5 experimentally infected ducks 39 days after exposure and were still present in the faeces of one bird at the end of one year.

Sexually mature trematodes (34 females and 17 males) were observed within the blood vessels of a single infected duck at necropsy. Forty-five trematodes were located in the renalportal system and, 3 each, in the lungs and mesentery.

DISCUSSION

The eggs of recently laid *D. anatinarum* described in this paper are morphologically similar to those described by Cheatum (1941) and by Freitas & Costa (1972). The mature egg is also morphologically similar to that of *D. pulverulenta* (Vande Vusse, 1968).

The miracidium is morphologically similar to that of *Trichobilharzia cameroni* (Wu, 1953).

Vande Vusse (1968) found 20 sensory papillae between the first and second row of plates in the miracidium of *D. pulverulenta.* For *D. anatinarum* we found 22 epidermal plates (6+9+4+3, respectively in rows 1, 2, 3 and 4), and 18 sensory papillae.

The number, distribution and shape of the epidermal plates is the same as found by Basch (1966) for *Trichobilharzia brevis.* The sensory papillae, situated in the middle of the base of each epidermal plate of the first row (radial papillae of Donges, 1964), are similar to those observed by Morgono (1968) for *T. brevis.*

The 4 pores in each penetration gland observed by Otollina (1957) in miracidia *S. mansoni,* were also seen in the terebratorium of *D. anatinarum.* Probably they are the end portion of the sensory papillae, similar to those found by Donges (1964) in *Posthodiplostomum cuticola.*

The 4 pairs of small pores at the end of the sensory papillae are comparable to those found by Price (1931) in *S. douthitti.*

The two small pores on the midline of the terebratorium, probably are the secretory pores of the apical gland as found by Otollina (1957) and Lengy (1962) in miracidia of *S. mansoni* and *S. bovis,* respectively.

The development of sporocysts into cercariae is similar to that described by Cort, Ameel & Woude (1955) for *T. physella.*

The cercaria behaviour has no difference from that of *Bilharziella polonica* (Khalifa, 1972) and *Dendritobilharzia anatinarum* (Khalifa, 1976). The general morphology resembles that of *Schistosomatium douthitti, Heterobilharzia americana, Bilharziella polonica* and *Gigantobilharzia mazuriana* described respectively by Price (1913), Lee (1962), Khalifa (1972) and Khalifa (1974). In morphological aspect, it is more similar to cercariae reported by Khalifa (1976), except in the following points:

1. The arrangement of the prominent spines of the body.
2. The penetration glands are postacetabular.

3. The flame cells formula is 2 [(3+2+1)] = 12.

4. There are striking difference in measurements. The tail stem and furcae are half the size.

Among the snails tested in the present study, only *B. straminea* proved to be susceptible to experimental infection. The finding of naturally infected *B. straminea* suggests that this snail is the intermediate host of *D. anatinarum.*

RESUMO

O ciclo biológico de *Dendritobilharzia anatinarum* foi desenvolvido experimentalmente em laboratório. Patos domésticos *Cairina (moschata) domestica,* e moluscos *Biomphalaria straminea,* foram os hospedeiros definitivo e intermedário, respectivamente. Os miracídios começaram a eclodir 10 minutos após a permanência dos ovos em água. Esporocistos primários continham esporocistos secundários aptos a migrar, após 8 dias da infecção dos moluscos. Decorridos 23 dias da infecção, esporocistos secundários já continham cercárias, as quais começaram a migrar dos moluscos no 25º dia. Após a penetração das cercárias nas aves, o período de pré-patência foi de 39 dias. Os parasitas adultos foram localizados nos vasos sanguíneos dos pulmões, mesentério e, principalmente, do sistema porta-renal. Detalhes morfológicos do ovo, miracídio, esporocisto e cercária são apresentados.

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