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LIFE HISTORY STUDIES OF HETEROPHYID TREMATODES IN THE NEOTROPICAL REGION: *ASCOCOTYLE (LEIGHIA) HADRA* SP. N.

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The life cycle of Ascocotyle (Leighia) hadra *n. sp. was experimentally reproduced, starting from cercariae from naturally infected* Littoridina parchappei, *collected from Los Ranchos stream, near Mercedes city, Buenos Aires Province, Argentina. Metacercariae were found encysted in the liver and mesentery of experimentally and naturally infected fishes* Cnesterodon decemmaculatus *and* Jenynsia lineata. *Adults were obtained experimentally in chicks and mice. The natural host is unknown. The new species is compared with* Ascocotyle (Leighia) mcintoshi *Price 1936 as described by Leigh, 1974, differing in behavior and morphology of cercarial, metacercarial and adult stages.*

Key words: *Ascocotyle (Leighia) hadra* n. sp. – Trematoda – Heterophyidae – life cycle

During studies of trematode larval fauna of Atheriniform fishes from La Plata River, a spherical cyst of an *Ascocotyle sp.* with two complete rows of 18-20 spines each was described (Ostrowski de Núñez, 1974). Recently a relatively big ophthalmogymnocephalous heterophyid cercaria was found to emerge from *Littoridina parchappei* and to develop into the same metacercaria found before. The experimentally obtained adult is related to *Ascocotyle (Leighia) mcintoshi* Price 1936, whose life cycle had been described by Leigh (1974), but differences in behavior and morphology of cercarial, metacercarial and adult stages lead to the proposal of a new species, *Ascocotyle (Leighia) hadra* sp. n.

MATERIALS AND METHODS

Naturally infected first intermediate host, *Littoridina parchappei,* were collected from Los Ranchos stream, near Mercedes city, Buenos Aires Province. Naturally infected second intermediate hosts, the Atheriniform fishes *Cnesterodon decemmaculatus* and *Jenynsia lineata,* were obtained in the same site. Laboratory reared *C. decemmaculatus* and *Gambusia affinis* were exposed individually and in mixed groups to naturally emerged cercariae. Newborn unfed chicks and laboratory mice were exposed to infection with metacercariae from natural and experimental infections.

Cercarial emergence was analyzed every two hours continuously during four days at room temperature (17-24 °C). The samples were collected by an automatic apparatus modified after Disko (1978) and the cercariae counted removing them with a Pasteur pipette.

All larval stages and adults were studied alive, with and without vital stains. Cysts were digested with trypsin at 37 °C to free the metacercariae. Adults were fixed in 70% alcohol, Bouin, 10% formalin or lactophenol, stained with carmine hydrochloride and mounted in Canada balsam, or mounted unstained in glycerine jelly. Measurements of heat-killed and formol-fixed cercariae, whole mounts of metacercariae and adults and living metacercarial cysts are in µm (minimum, maximum, followed by mean and standard deviation in parentheses).

RESULTS

Rediae (Fig. 2 – Measurements based on 24 Formol-fixed specimens). Body elongate, 512-1134 (872.5; 144.3) long by 92-143 (113.8; 12.8) wide. Pharynx 25-34 (30.3; 4.5) long by 25-34 (28.0; 4.3) wide, with an inconspicuous small gut attached to it. They contain 1-8 cercariae, in which pigment of eyespots already could be seen. Undifferentiated germ-

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balls fill the posterior region. Birth pore present near anterior end. Rediae infected hepatopancreas and gonads of snails.

Cercaria (Figs 1, 3 – Measurements based on 20 specimens). Body 132-145 (137.7; 3.3) long by 57-78 (66.8; 5.5) wide. Tegument of body thick, without spines, except anterior end; three pairs of sensory hairs on lateral margin of body, 2 pairs on anterior end. Scarcely distributed yellow-brown pigment granules present in body. Oral sucker 19-25 (21.9; 1.8) long by 21-27 (25.4; 1.5) wide, recessed within tegumental crypt, protrusible. Anterior border of oral sucker with 3 transverse rows of small spines. A mass of cells behind oral sucker belonged possibly to an undifferentiated pharynx. One pair of pigmented eyespots situated 34-44 (38.5; 3.1) from anterior end. Seven pairs of small granulated penetration glands arranged into two longitudinal rows of 3 and 4 cells on each side of body; their ducts run forward in two bundles passing between eyespots and open in four sets of 3-4-4-3 pores on anterior border. Ventral sucker not developed, at its site a cluster of small cells were observed. Excretory bladder epithelial, V-shaped, flame cell formula 2((2+2+2)+(2+2+2)) = 24. In immature cercariae, dissected from rediae, a short bifurcating excretory duct in the tail could be observed.

Tail without fin fold, 235-277 (259.6; 9.5) long by 32-42 (36.5; 2.9) wide at base, with yellow brownish pigment granules in the posterior half and conspicuous annulations in the tegument of whole length, which give a serrated appearance to the borders; the posterior fifth is smooth.

Remarks – Cercariae escaped from snail during day and night, but the majority emerged during the morning h and early afternoon (Fig. 10). The peak-hour varied during the four days analyzed (2.00 PM, 10.00 AM, 6.00 AM, 12.00 AM), perhaps related with the simultaneous slight increase in room temperature. A second, lower peak was observed before or at midnight (10.00 PM, 12.00 PM). They were positively phototactic and during approximately 24 h they alternate active swimming periods, with the oral sucker recessed and describing an eight with body and tail, and resting periods, in which the body is spherical and the tail curved to the side. After 48 h they lie jerking on the bottom of container, surviving for nearly 24 h more. The daily production of cercariae was 3-202 (79 cercariae/day/snail, mean from six snails during four days at 17-24 °C).

L. parchappei is a small snail, measuring up to 9 mm; snails over 3 mm were found to be infected with *A. hadra*, the 5.1 – 6 mm class was the most infected.

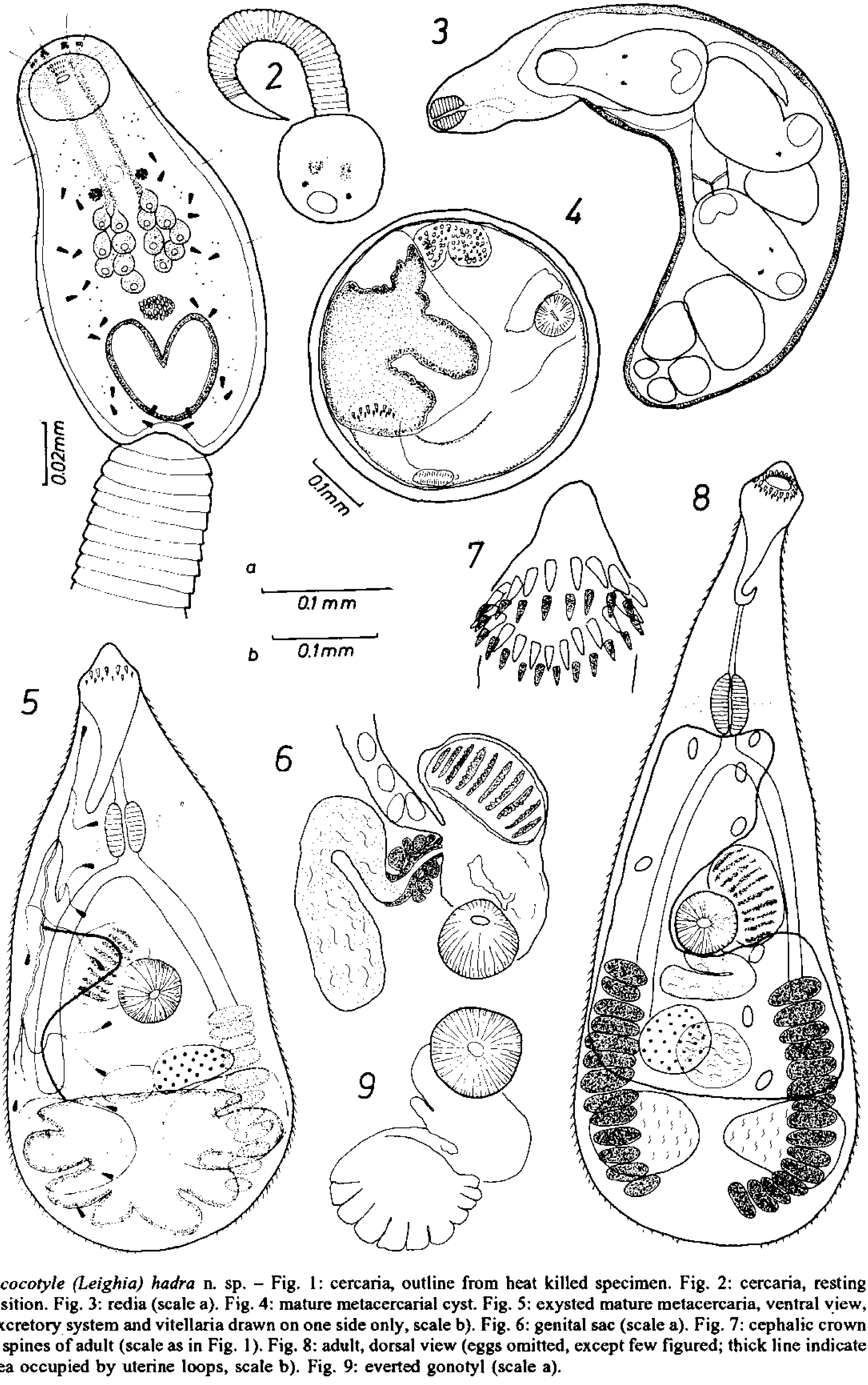
Ascocotyle hadra cercariae were present in Los Ranchos stream during the investigated period (Jan 88 – Feb 89) with a prevalence ranging from 0.4-4.1, representing 2.6-14.5% of all 18 different species of larval trematodes found in *L. parchappei*. It was also present in adjacent ponds on the coast of the La Plata River in Buenos Aires city.

The relatively big cercariae possibly attracted the attention of fish intermediated hosts, which swallowed them.

Metacercaria (Figs 4, 6 – Measurements based upon 20 mounted metacercariae and 21 living metacercarial cysts). Metacercarial cysts were found in the liver and mesentery of naturally infected native *Cnesterodon decemmaculatus* and *Jenynsia lineata*. All *C. decemmaculatus* experimentally exposed to emergent cercariae became infected, but never the introduced species, *Gambusia affinis*.

In summer months (Dec-Mar, 21-32 °C) experimental cysts 7-10 days p.i. measured 185/168, the most conspicuous feature was the excretory vesicle with its thick epithelial layer. Cysts 22-25 days p.i. were 300-320 in diameter with a cyst wall of 16-25, the oral spines were already developed. Cysts from natural infections were 328-386 (364.9; 15.5) long by 336-378 (361.7; 14.2) wide, with a cyst-wall of 25-34. Cover glass pressure distorted the measurements, appearing the cysts 40% bigger than without it. The excysted metacercaria were 311-605 (493.1; 87.4) long by 160-302 (228.9; 35.1), without body pigment, with some remnant granules of eyepigment near pharynx and similar in development to adult, except presence of eggs. The vitelline follicles were clearly visible and the opaque excretory vesicle showed 4 pairs of branches.

Adult (Figs 7-9 – Measurements based on 20 mounted specimens obtained experimentally from chicks). One to 68 adults were obtained from each of five of seven exposed chicks, 2-6 days p.i. Twenty specimens were recovered from one of two exposed white mice four days p.i. Adults became sexually mature with numerous eggs within 48 h.



Ascocotyle (Leighia) hadra n. sp. – Fig. 1: cercaria, outline from heat killed specimen. Fig. 2: cercaria, resting position. Fig. 3: redia (scale a). Fig. 4: mature metacercarial cyst. Fig. 5: exysted mature metacercaria, ventral view, (excretory system and vitellaria drawn on one side only, scale b). Fig. 6: genital sac (scale a). Fig. 7: cephalic crown of spines of adult (scale as in Fig. 1). Fig. 8: adult, dorsal view (eggs omitted, except few figured; thick line indicate area occupied by uterine loops, scale b). Fig. 9: everted gonotyl (scale a).

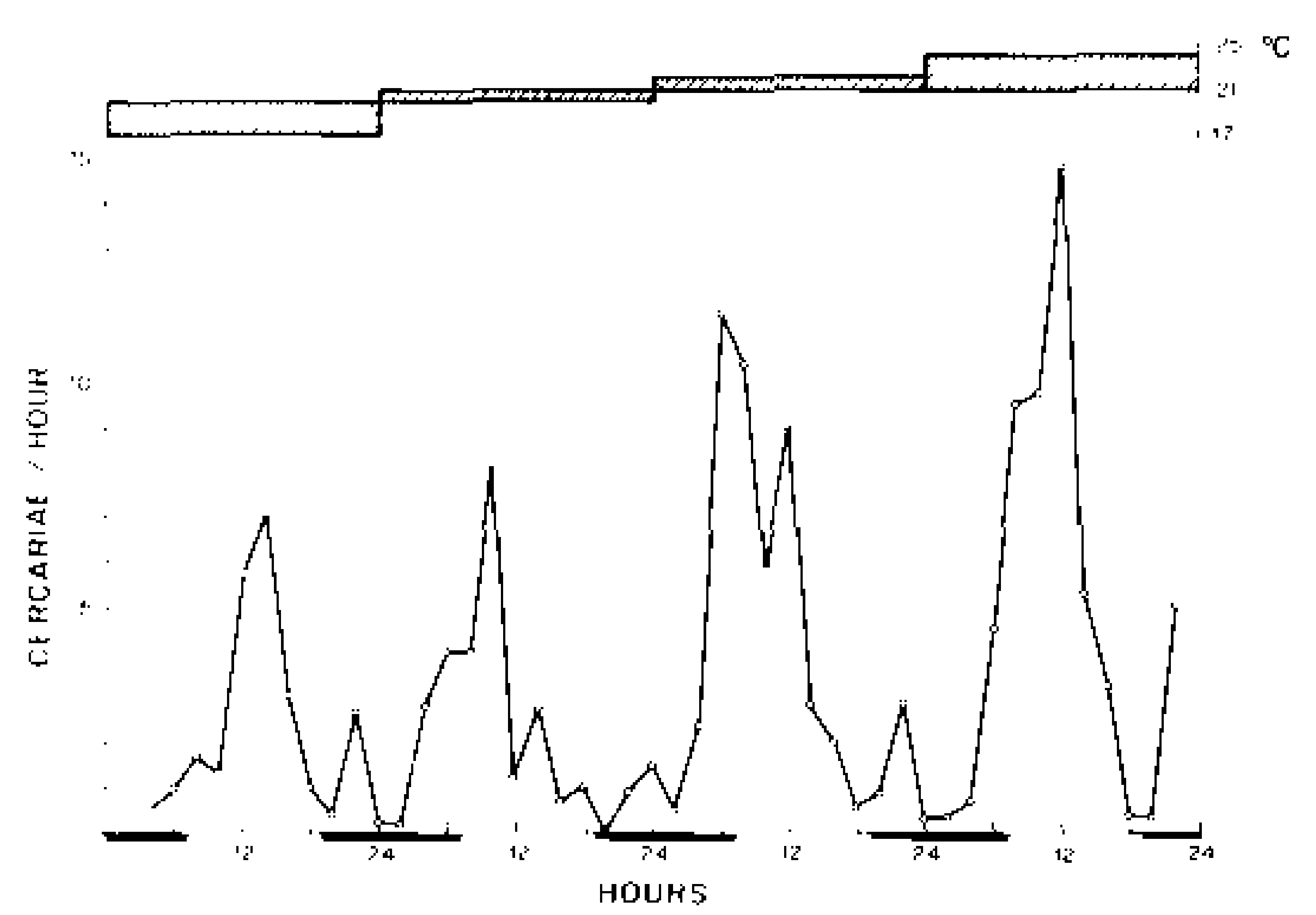


Fig. 10: emergence of cercariae, natural photoperiod, mean from six snails. Temperature bars represent minimum and maximum °C/day.

Body pyriform, 496-798 (614.9; 88.3) long, with maximum width 260-353 (289.2; 26.6) at ovarian level and a conspicuous cavity when observed *in vivo*. Tegument spinose with spines 6.4 long up to end of body. Oral sucker terminal, preoral lip present. Solid prolongation of oral sucker variable in length, but never exceeding pharynx; including oral sucker it was 84-162 (119.7; 20.8) long by 46-67 (56.6; 8.7) wide. Anterior end bearing two rows of spines, the anterior row with 18-21 spines 11-13 long, the posterior with 18-21 spines 6.3-8.4 long. Prepharynx 84-195 (143.1; 31.2). Pharynx muscular, 36-63 (50.9; 6.8) long by 32-53 (40.1; 5.0) wide; oesophagus short, dividing into two blind caeca ending just anterior to testes. Ventral sucker postequatorial, 42-69 (54.0; 6.9) long by 46-74 (57.1; 7.2) wide, situated 235-428 (316.7; 60.3) from anterior end. Genital sac in front of left border of ventral sucker, with a conspicuous gonotyl, with 10-11 folds or projections, containing refracting material *in vivo*. Seminal vesicle immediately posterior to ventral sucker. Ovary spherical, 53-84 (62.9; 9.5) long by 53-95 (74.9; 15.6) wide, dextral, near right testis. Seminal receptacle approximately the same size, on left side of ovary. Testes two, oval to spherical, side by side, posterior to ovary, at end of body. Right testis 63-88 (71.4; 8.8) long by 84-147 (105.3; 16.8) wide, left testis 53-95 (72.6; 12.8) long by 74-137 (108.8; 18.3) wide. Vitellaria composed of transversely elongate follicles, tight together, approximately 11-16, lateral to testes on each side of body, extending behind ventral sucker and exceeding posterior border of testis. Uterus extending in transverse, ascending and descending loops between testes and pharynx. Eggs numerous, 19-23 (21.2; 0.8) long by 12.6-16.8 (14.1; 1.1) wide. Excretory vesicle branched, excretory pore terminal.

Etymology: the specific Greek adjectival epithet meaning "thick, big" refers to the relatively big and thick specimens in comparison with the other members of the genus in the area.

DISCUSSION

Ascocotyle (Leighia) hadra n. sp. is closely related to *A. (Leighia) mcintoshi* Price 1936, of North America, but differs in morphologic and behavioral features of the life cycle, as described by Leigh (1974). In the redia of *A. mcintoshi* no gut was observed to be attached to pharynx, while a gut is present in *A. hadra*. The most important differences are seen in the cercariae. In *A. mcintoshi* the penetration glands are big, occupying 37% of body size (estimated from Fig. 1), whose ducts open in a single bundle of 7 pores on each side of body; the dorso-lateral extension of excretory bladder is filled with fine glandular concretions; pigment granules are reported only for the tail and the cercariae emerge at night and early morning hours. In *A. hadra* the penetration glands are small, occupying only 23-26% of body size, the ducts open in sets of 3-4-4-3 pores; no concretions were observed in the excretory bladder, pigment granules are present in body and tail and the peak of emergence varies between 6.00 AM and 2.00 PM.

The fully developed metacercarial cyst of *A. mcintoshi* is reported to be marked by a brownish color of the enclosed larva, while the present species is not pigmented. Differences in measurements may be due to techniques. The two species show different specificity for the intermediate host: *A. mcintoshi* infected *Gambusia affinis*, *A. hadra* only the native poecilid fishes, *Cnesterodon decemmaculatus* and *Jenynsia lineata*.

The adults differ in body size (680-1200 in *A. mcintoshi*) and in configuration of gonotyl. The latter was not described by Leigh (1974), but from his Fig. 7 it is evident that it has 13 narrow projections or folds, while *A. hadra* has 10-11 folds of different appearance and contain refringent material, as cited also for *A. gemina* by Font et al. (1984).

Leigh (1974) figured 7-8 vitelline follicles, *A. hadra* has 11-16, but a similar number is figured by Price (1936) for *A. mcintoshi*.

Font et al. (1984) erected *A. gemina* as a new species differing from its sibling species *A. sexidigita* Martin and Steele in minor morphologic features (7-10 pockets vs 6 projection in the gonotyl, egg measurements 19/9 vs 16/8 respectively, and possibly uterine configuration), in different specificity for the second intermediate host (*Cyprinodon variegatus vs Fundulus parvipinnis*) and in different geographic distribution in North America (Louisiana *vs* California). They explain the existence of these two species as possible relicts of an ancestral species that had specificity for two or more cyprinodontid fishes and was distributed over a wide geographic area. Geographical separation and subsequent speciation may have occurred after splitting of the ancestral range.

It may be similarly argued for *A. hadra* n. sp and *A. mcintoshi*. The morphologic differences in adults and cercariae are few. There is a different spcificity for the second intermediate host: *A. hadra* could not infect the North American *Gambusia affinis*, and the poecilid fishes infected had only Neotropic distribution. *Littoridina* (Hydrobiidae) is a genus mainly distributed in the neotropics, existing in a small geographic area at the Atlantic coast from New Yoirk to Florida and in Texas, Gulf of Mexico (Gaillard & Castellanos, 1976). *L. parchappei* is only cited for the central area of Argentina, from the provinces Entre Rios to Rio Negro.

A. hadra may not be very specific for the definitive host, as concluded from the fact that chicks and mice could be experimentally infected. Piscivorous birds, as egrets, ibis, herons; etc., frequent in the area, are suspected as the natural hosts.

The described differences in the stages of life cycle, the different endemic intermediate hosts and the separated geographic areas were considered to support the description of *A. hadra* as a new species.

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