

PARTIAL LIFE CYCLE OF *Pygidiopsis australis* Ostrowski de Nuñez, 1996 (DIGENEA: HETEROPHYIDAE) IN VENEZUELA

Ciclo vital parcial de *Pygidiopsis australis* Ostrowski de Nuñez, 1996 (Digenea: Heterophyidae) en Venezuela

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ABSTRACT

Partial life cycle of *Pygidiopsis australis* Ostrowski de Nuñez was elucidated from encysted metacercariae which was found in the cranial cavity, musculature and abdominal cavity of the naturally infected freshwater fish, *Anablepsoides hartii* from El Naranjal, Montes Municipality, Sucre State, Venezuela. Metacercariae were fed to rats and chickens experimentally, and adult trematodes were recovered from the small intestines of the experimental hosts, 5-18 days after infection. Descriptions of metacercariae and adult from both naturally and experimentally infected hosts are provided. These adult parasites were compared with the closely related species of *Pygidiopsis* Looss, 1907, such as: *P. ardea*, *P. australis*, *P. genata*, *P. marivillae*, *P. anterouteria* and *P. himantopae*, and it was concluded that the species is undoubtedly *P. australis*, which was also reported from chickens and mice in Argentina. However, the metacercariae are showing slight differences in the structure of the cyst wall, the site of infection and the shape of excretory bladder, while variation in number of digitiform pockets in the gonotyl of adults, could be due to geographical and intraspecific variations.

Key words: Digenea, *Anablepsoides hartii*, fresh water fish parasites, experimental infection, Argentina, Venezuela.

RESUMEN

El ciclo vital parcial de *Pygidiopsis australis* Ostrowski de Nuñez, 1996 fue dilucidado desde metacercarias encontradas naturalmente enquistadas en la cavidad craneal, musculatura y cavidad abdominal del pez de agua dulce, *Anablepsoides har-*

tii, capturados en El Naranjal, municipio Montes, estado Sucre, Venezuela. Las metacercarias fueron suministradas a ratas y polluelos, los trematodos adultos fueron recobrados del intestino delgado de estos hospedadores experimentales, después de 8-15 días de la infección. Se redescubren las metacercarias y adultos de hospedadores infectados natural y experimentalmente. Los parásitos adultos fueron comparados con las especies más cercanamente relacionadas del género *Pygidiopsis* Looss, 1907, tales como: *P. ardea*, *P. australis*, *P. genata*, *P. marivillae*, *P. anterouteria* y *P. himantopae*, concluyéndose que se trata indudablemente de *P. australis*, la cual fue obtenida experimentalmente en polluelos y ratones en Argentina. Las ligeras diferencias observadas en la estructura de la pared del quiste, el sitio de infección y la forma de la vejiga excretora de la metacercaria, así como el número de bolsas digitiformes en el gonotilo de los adultos, podrían ser debido a las variaciones geográficas e interespecíficas.

Palabras clave: Digenea, *Anablepsoides hartii*, parásitos de peces de agua dulce, infección experimental, Argentina, Venezuela.

INTRODUCTION

Pygidiopsis was erected by Looss [4] to include *P. genata* from *Pelecanus onocrotalus* in Egypt, as type species. The representatives of the genus *Pygidiopsis* are small digenetic trematodes belonging to the family Heterophyidae, parasites of piscivorous birds and mammals, including humans when consume raw or undercooked infected fish. During a survey of freshwater molluscs in El Naranjal, Montes Municipality, Sucre State, Venezuela in search of the snail *Aroapyrgus vivens* (Baker, 1930) (Gastropoda: Cochliopidae), first intermediate host of *Paragonimus* spp, several specimens of *Anablep-*

soides hartii (Boulenger, 1890) (Pisces) were collected from the local stream, and found infected with metacercariae of heterophyids. These metacercariae were fed to rats (*Rattus norvegicus* Berkenhout, 1769) and chickens (*Gallus gallus f. domestica* Linnaeus, 1758), which developed to adults of *Pygidiopsis australis* Ostrowski de Núñez (1996). The life cycle of this digenean was first completed by Ostrowski de Núñez [6] in Argentina working with Pleurolophocercous cercaria released from the naturally infected *Litoridina castellanosa* (= *Heleobia castellanosa* (Gaillard, 1974) (Gastropoda: Hydrobiidae). Ostrowski de Núñez [6] exposed specimens of the laboratory-reared *Cnesterodon decemmaculatus* (Jenyns, 1842) Garman, 1895 (Pisces: Poeciliidae) to this cercaria and the metacercaria were recovered several days post infection and experimentally infected mice and chicken with these metacercariae and obtained adults trematodes which were identified as *P. australis*. The present study reports the partial life cycle of *P. australis*, and redescribes the metacercariae and adults collected both from naturally and experimentally infected hosts and compared them with that of *P. australis* as described by Ostrowski de Núñez [6]. However, the first intermediate host, cercaria and definitive host still unknown which need to be studied in Venezuela.

MATERIALS AND METHODS

Thirty-eight specimens of *Anablepsoides hartii* (Boulenger, 1890) (Teleostei: Cyprinodontiformes: Rivulidae) were collected from a stream in El Naranjal, Montes Municipality, Sucre State, Venezuela (10° 29' N and 64° 12' W), between May and October, 2009. They were brought to the laboratory of Parasitology, IIBCA-UDO, Venezuela and kept alive in aquariums for acclimatization. After examination, 20 out of 38 fish were infected with heterophyid metacercariae encysted in the cranial cavity, muscles and the abdominal cavity. These encysted metacercariae were fed experimentally to chickens and whole fish to white rats, and adult trematodes were recovered from small intestine. The encysted metacercariae and some adults were studied *in vivo* in 0.85% saline. Other specimens were heat fixed between gently pressured glass slide in AFA (alcohol-formalin-acetic acid), and stained with Semichon's acetocarmine, dehydrated in a series of ethanol, cleared in clove oil and mounted in Canada balsam. Figures were drawn with the help of a camera-lucida attachment on a Zeiss Axioskop Microscope, Switzerland and all measurements (length x wide) were in millimeters (mm). The taxonomic identification up to genus was done using the keys of Bray et al. [1], Pearson [7] and Yamaguti [9].

RESULTS AND DISCUSSION

Twenty of the 38 fish, *Anablepsoides hartii*, collected from El Naranjal were found infected with metacercariae of *Pygidiopsis australis*. These encysted metacercariae were recovered from cranial cavity, muscles and abdominal cavity of the fish hosts. The collected metacercariae were fed experimen-

tally to chickens and some infected fish were fed to white rats. A total of 358 adults digeneans were recovered from small intestines of experimental hosts, 5-18 days after infection, of which 24 adults from chickens and 334 from white rats.

Metacercaria

Description of encysted metacercariae: Based on 12 flattened specimens obtained from *Anablepsoides hartii* (FIG. 1).

Cyst oval, 0.242-0.333 X 0.232-0.292, surrounded by three walls, a fibrous outer wall, fragile, produced by host tissue, 0.005-0.025 thick. Very frequently, it forms a dark lump on both ends of the cyst; some small granules present Brownian motion, the content of these granules differ from the excretory bladder; a middle transparent layer, 0.01-0.025 thick, an internal very resistant layer, 0.005-0.010 thick. Body covered with tegumental spines reaching almost back to the testes. Penetration glands, almost completely empty on both sides of the pharynx. Oral sucker, 0.050-0.075 x 0.030- 0.070, armed with one complete row of 16-17 spines and 4-5 additional spines in dorsal side. Prepharynx short, 0.045-0.050 long, pharynx oval, strongly muscular, 0.030-0.085 x 0.030; relatively short esophagus. Intestinal caeca short, surrounding the ventral sucker and ending posterior to it, filled with platelets. Ventral sucker spherical, well developed, 0.026-0.032 X 0.024-0.059. Testes symmetrical close to end of the body. Ventrogenital sac sinistral to ventral sucker. Genital pore oval; gonotyl with 20-23 digitiform pockets. Excretory bladder X-shaped, with short and wide lateral branches. Excretory pore subterminal. Flame cells formula: 2 [(2 +2) + (2 +2)] = 16.

Second intermediate host: *Anablepsoides hartii* (Boulenger, 1890).

Common local name: raja talón.

Site of infection: Cranial cavity, muscles and abdominal cavity.

Distribution: El Naranjal, Montes Municipality, Sucre State, Venezuela.

Prevalence: 52.63



FIGURE 1. ENCYSTED METACERCARIA FROM NATURALLY INFECTED ANABLEPSOIDES HARTII.

Adults

Description of adults: (FIGURES, 2, 3, 4, 5, 6, 7, 8).

A total of 358 adult trematodes were recovered from small intestine, 5-18 days after infections, of which 334 from white rats and 24 from chickens. No difference was observed at random samples between adults recovered from chicken and rats: descriptions based on 28 adult specimens from experimentally infected rats and chickens.

Body small, 0.328-0.570 X 0.156-0.297, elongated pear-shaped, sometimes oval-shaped, or almost spherical. Tegument spined, posttesticular area free of spines, Oral sucker, 0.045-0.064 X 0.040-0.053, terminal or slightly subterminal, armed with one complete row of 16-17 spines and 4-5 additional spines in dorsal side. Prepharynx relatively short, 0.010-0.056 X 0.008-0.016. Pharynx oval, strongly muscular, 0.040-0.053 X 0.032-0.048. Oesophagus short, 0.024-0.061 X 0.008-0.013. Intestinal caeca short, ending posterior to ventral sucker. Ventral sucker, 0.032-0.069 X 0.053-0.075, equatorial, transversely oval or round. Testes symmetrical, near posterior extremity of body, right testis, 0.040-0.069 X 0.053-0.131, left testis, 0.037-0.075 X 0.053-0.134. Seminal vesicle bipartite, 0.026-0.040 x 0.068-0.121, posterior to ventral sucker. Ventronephros surrounded by glandular cells, with a conspicuous gonotyl with at least 23 digitiform pockets, genital pore large, oval shaped, immediately preceding the genital sac. Ovary, 0.040-0.075 X 0.037-0.091, oval, anterior to right testis. Seminal receptacle large, 0.029-0.053 X 0.037-0.067, pretesticular, lateral to the ovary. Vitelline glands formed by 6-8 follicles, ventro-lateral to testes. Uterus tubular, sinuous, extending between the ventral sucker region and anterior to testes. Opercu-

lated eggs, 0.010-0.016 X 0.018-0.024. Excretory pore subterminal. The number and position of the flame cells are similar to metacercariae.

Experimental host: *Rattus norvegicus* and *Gallus gallus f. domestica*.

Site of infection: Small intestine.

The genus *Pygidiopsis* Looss [4] belong to the subfamily Pygidiopsinae Yamaguti, 1958 of the family Heterophyidae Leiper. Of the 13 species of this genus described to date: *P. genata* Looss [4]; *P. marivillai* Refuerzo and Garcia [8]; *P. ardea* Køie [3]; *P. australis* Ostrowski de Núñez [6]; *P. anterouteri* (Nasir and Díaz) [4] and *P. himantopae* (Dronen *et al.*) [2] are morphologically very similar to the digeneans of this study. However, it was found that both the metacercariae and adults collected from experimentally infected definitive and intermediate hosts, correspond morphometrically to *P. australis* Ostowski de Nunez [6]. The metacercaria of the Argentinian species was found naturally in the poeciliid fish species *Cnesterodon decemmaculatus*, *Gambusia affinis* and *Phalloceros caudimaculatus*. There are slight differences in structure of the cyst wall, the site of infection and the shape of the excretory bladder of the specimens examined in the present study in comparison to the Argentinian specimens. The metacercariae of *P. australis* in Argentina are covered by a thin, transparent wall, while the Venezuelan specimens are covered by three walls with the outer wall bearing one or two protrusions on its tip, showing black contents. The ovary of *C. decemmaculatus* was found heavily infected, while the cranial cavity of *A. hartii* was the preferred site for metacercarial infection. The metacercariae and adults of the Argentinian *P. australis* showed



FIGURES 2, 3 AND 4. ADULT FROM EXPERIMENTAL INFECTED *RATTUS NORVEGICUS* AND *GALLUS GALLUS F. DOMESTICA*.



FIGURE 5. ADULT FROM *RATTUS NORVEGICUS*, TERMINAL GENITALIA.

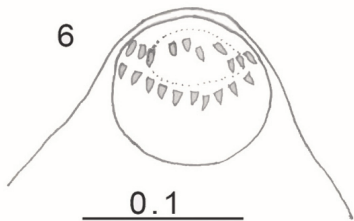


FIGURE 6. ADULT FROM *RATTUS NORVEGICUS*, SPINATION PATTERN OF CIRCUMORAL SPINES (16+4).

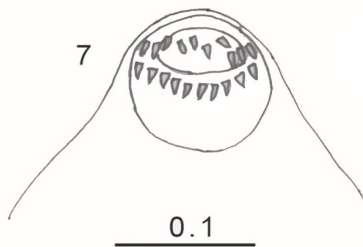


FIGURE 7. ADULT FROM *RATTUS NORVEGICUS*, SPINATION PATTERN OF CIRCUMORAL SPINES (15+5).

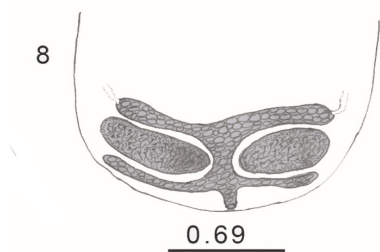


FIGURE 8. ADULT FROM *GALLUS GALLUS F. DOMESTICA*, X-SHAPED EXCRETORY BLADDER.

(16+4) and (16-17+4) collar spines, respectively [6] while in the present study, the majority of 28 adults examined appear to possess 16+4 collar spines, but in some specimens 15 +5 were found. It was difficult to establish the exact number of spines in live specimens. The excretory bladder of the Argentinian is T-shaped, while it is X shaped in both metacercariae and adults of the specimens in the present study. However, it can be inferred from FIGS, 7 and 8 of Ostrowski de Núñez [6] that the excretory bladder of the metacercariae is X-shaped, but with short posterior branches depending on their stage of development. The author did not mention the excretory bladder in the adult. The structure and morphology of the ventro-genital sac of adult worms of this study seem to be comparable to that of *P. australis* of Argentina [6]. However, there are slight differences in the number of digitiform pockets in the gonotyl, 20-23 in the present study and at least 19 in Argentina. There are also differences in the number of vitelline follicles, 5-8 in the Argentinian specimens and 6-8 in the Venezuelan. Of these species only *P. anterouteria* (Nasir and Diaz) Pearson [7] was known from Venezuela, and was recovered 8 days after infecting cats (*Felis catus*) with metacercariae from the intestine of the fish *Poecilia reticulata*, a naturally infected host collected from San Juan de Macarapana, Sucre State, Venezuela. *Pygidiopsis himantopae* (Dronen et al.) Pearson [7], collected from the gut of *H. mexicanus* at Galveston Bay, Texas, USA, which shares morphological features with *P. anterouteria*, such as: absence of spines on oral sucker, vitelline follicles extending anterior to the level of acetabulum, and posterior to the testicular region, as well as the uterus reaches pre-acetabular region. These characters easily separate *P. australis* from *P. himantopae* and *P. anterouteria*. This is the first report of the occurrence of *P. australis* in Venezuela and the cyprinodontid fish *Anablepsoides hartii* represents a new second intermediate host for this digenean, and the first intermediate host of this species is not known from Venezuela.

CONCLUSIONS

The studies with both natural and experimental hosts in laboratory facilitated to elucidate the life cycle of *Pygidiopsis australis*, using *Anablepsoides hartii* as second intermediate host, while chicks and rats as experimental definitive hosts. Morphological characteristics obtained from both natural and experimental hosts of metacercariae and adults correspond to *Pygidiopsis australis*, reported in Argentina. The differences in the structure of the cyst wall, the site of infection, the form of excretory bladder in metacercariae, the numbers of digitiform pockets in the gonotyle and number of vitelline follicles in adults may be due to geographic and interspecific variations. This is the first record of *Pygidiopsis australis* in Venezuela and *Anablepsoides hartii* represents a new intermediate host for this species. The first intermediate host of this species needs to be further clarified.

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