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Life cycle of *Ascocotyle* (*Phagicola*) *angeloi* (Digenea: Heterophyidae) in the Neotropical Region

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Abstract. Stages of the life cycle of *Ascocotyle* (*Phagicola*) *angeloi* Travassos, 1928 were experimentally obtained, from cercariae from naturally infected *Littoridina castellanosae* (Gaillard) collected in artificial ponds in the Zoological Garden of Buenos Aires. Metacercariae were found encysted mainly in muscles and ovary, but also in other parts of the body of naturally and experimentally infected fish *Jenynsia lineata* (Jenyns) (Atheriniformes: Jenynsidae). Adults were obtained experimentally from chicks and mice. *Ascocotyle* (*Phagicola*) *angeloi*, redescribed in the present paper, is distinguished from other species of the subgenus by the two rows of oral spines, each with 14 spines. The characteristics of the studied cercaria corresponds to those of the subgenus *Phagicola*.

Among the genera belonging to the family Heterophyidae Odhner, 1914, the species of the *Ascocotyle-Phagicola* complex are characterized by the presence of a crown of spines at the anterior end and a solid posterior prolongation of the oral sucker.

Within the subgenus *Phagicola* Faust, 1920 there are included 21 species which parasitize birds and mammals (Yamaguti 1972). The subgenus is characterized by the vitellaria confined laterally to the testicular-ovarian area, the uterus and intestinal caeca extending posterior to the acetabulum. Individual species differ in having one, two or no rows of oral spines and a single or double gonotyl. Some of the species are morphologically very similar difficult to distinguish from each other. Their possible synonymy has been discussed by different authors (Sogandares Bernal and Lumsden 1963, Font et al. 1984a, Ostrowski de Núñez 1993, Scholz et al. 1997).

Ascocotyle (*Phagicola*) *angeloi* was described by Travassos (1928) from *Ixobrychus exilis* in Brazil, and has not been found since. It is, together with *A.* (*P.*) *ampullacea* Miller et Harkema, 1962, the only species within the subgenus with two rows of oral spines and a simple gonotyl. In this paper a redescription is given of this species and developmental stages, redia, cercaria and metacercaria, are described.

MATERIALS AND METHODS

Snails, *Littoridina castellanosae* (Gaillard, 1974), were collected from artificial ponds at the Zoological Garden and two other ponds adjacent to the La Plata River, in Buenos Aires. Small fishes, *Jenynsia lineata* (Jenyns, 1842) (Jenynsidae), *Cnesterodon decemmaculatus* (Jenyns, 1842), *Gambusia affinis* (Baird et Girard, 1854) (Poeciliidae) and *Cichlasoma facetum* (Jenyns, 1842) (Cichlidae) were collected in the same ponds. Emerging cercariae were collected by exposing snails to light in 5 ml of tap water. When emergence of cercariae ceased, snails were dissected to study intramolluscan stages. Laboratory reared *C. decemmaculatus* and *J. lineata* were exposed during 24 hs to an undetermined number of cercariae and dissected on different days post infection (p.i.). One white mouse (*Mus musculus* L.) and six newly hatched, unfed chickens (*Gallus domesticus* L.) were force-fed mature metacercariae isolated from naturally infected *J. lineata* and autopsied 3 to 13 days p.i. All larval stages and adults were studied alive, with and without vital stains. Cysts were digested with trypsin at 37°C to free metacercariae. Adults and metacercariae were fixed, without pressure, in 70% ethanol, Raillet-Henry or AFA, stained with alcoholic hydrochloric carmine (Langeron 1949) and mounted in Canada balsam or cleared in lactophenol and mounted, unstained, in glycerine jelly. Measurements of heat killed and formalin-fixed cercariae, whole mounts of metacercariae and adults, and of living metacercarial cysts are in µm, if not otherwise stated (mean, followed by range within parentheses).

RESULTS

Littoridina castellanosae infected with cercariae of *Ascocotyle* (*Phagicola*) *angeloi* were found only in the ponds of the Zoological Garden. From all the fish collected, only *Jenynsia lineata* were found to be infected with metacercariae, mainly in the muscles and ovary, but also in the intestinal wall and near the gills, but not on the gill filaments. From experimentally exposed *Cnesterodon decemmaculatus* (15 specimens) and *J. lineata* (5), only the latter proved to be infected after 1, 2, 15, 75 and 105 days p.i.

Thirty-three adults were obtained after 3 days in the

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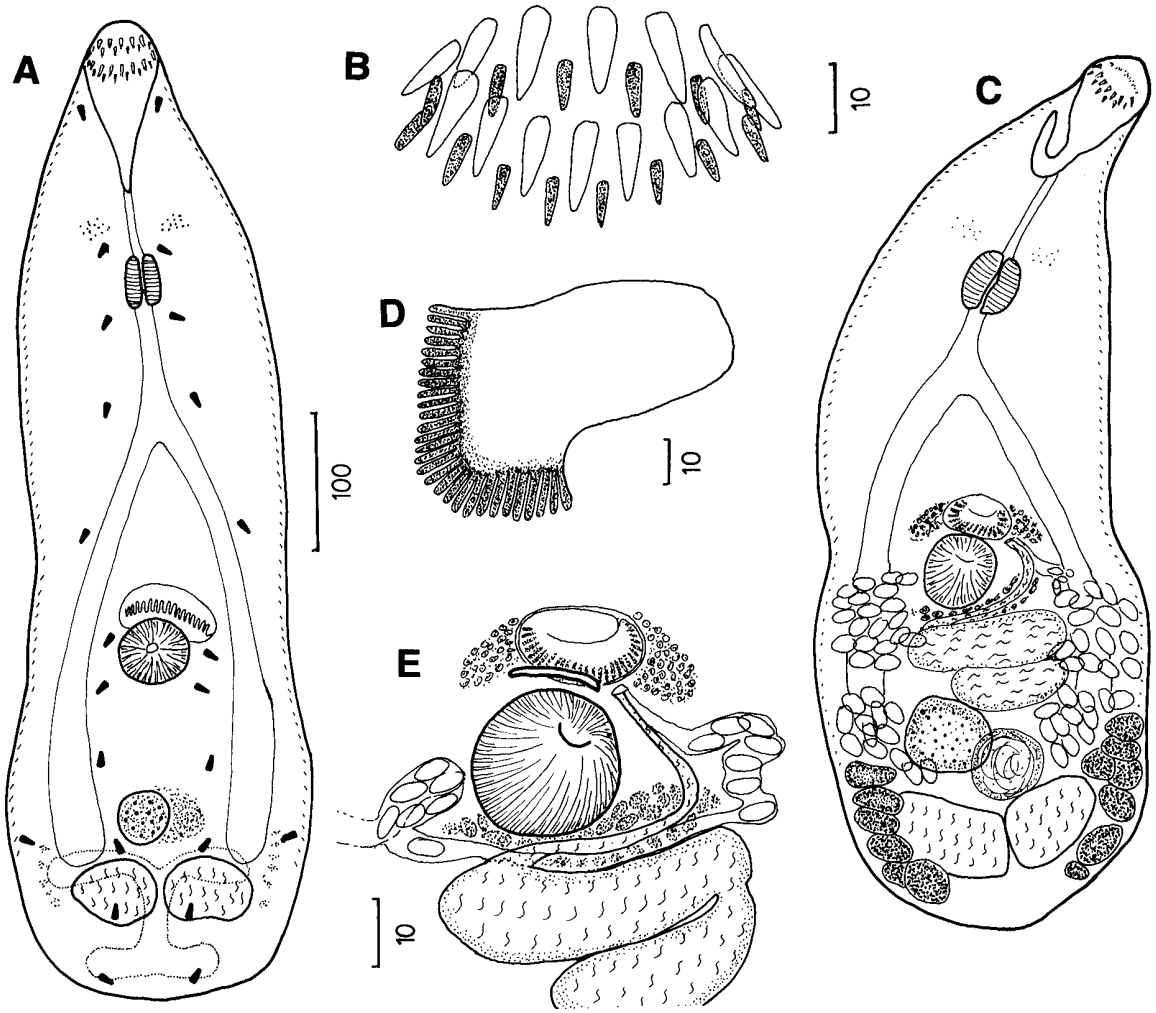

Fig. 1. *Ascocotyle* (*Phagicola*) *angeloi.* **A** - excysted metacercaria from muscles of naturally infected *Jenynsia lineata* (ventral view); **B** - crown of spine of the same; **C** - adult from experimentally infected chick, 5 days p.i. (ventral view); **D** - everted gonotyl of living adult specimen; **E** - detail of terminal genitalia of adult of Fig. 1C, completed with observations *in vivo*. Scale bars in μm.

mouse, and 7 and 15 after 3 and 5 days, respectively, in the chicks, in the first third of intestine. Three chicks, autopsied at 7, 8 and 13 days p.i. were negative. Adults with eggs were found only in specimens 5 days p.i.

Redescription

Ascocotyle* (*Phagicola*) *angeloi Travassos, 1928

A d u l t Fig. 1C,D

Measurements based on 9 egg-bearing specimens from chickens, 5 days p.i., mounted in toto in Canada balsam. Body elongated to pyriform, 697 (592-992) long by 206 (192-240) wide at ovarian level. Tegument spinose, with conspicuous spines 6-8 long at pharyngeal level, diminishing in size, reaching back posteriorly to posterior level of testes. Remnant of cercarial eye-spot pigment seen close to pharynx. Oral sucker subterminal, 44 (38-47) wide with solid prolongation of variable length, in living specimens reaching pharynx, in fixed and mounted specimens reaching half length of prepharynx, 130 (73-221). Small pre-oral dorsal lip present. Anterior extremity bearing crown of spines with anterior row of 14 spines, 12-17 long and posterior row of 14 smaller spines, 6-12 long (Fig. 1B). Pharynx muscular; 45 (38-50) long by 34 (25-38) wide; oesophagus relatively long, 67 (28-142), dividing into two blind caeca reaching up to testicular level. Ventral sucker in posterior half of body, 55 (50-63) long by 53 (41-60) wide. Genital sac preacetabular, sinistral, 32 (28-35) long by 56 (50-69) wide, with conspicuous gonotyl containing approximately 29 narrow, digitiform pockets (Fig. 1D). Mouth of genital sac slit-shaped, anterior to ventral sucker. Seminal vesicle folded in two chambers separated by a constriction, situated between ventral sucker and ovary. Prostatic gland cells surrounded terminal part of duct. Genital pore on the left side of the genital sac. Ovary oval, 48 (44-60) long

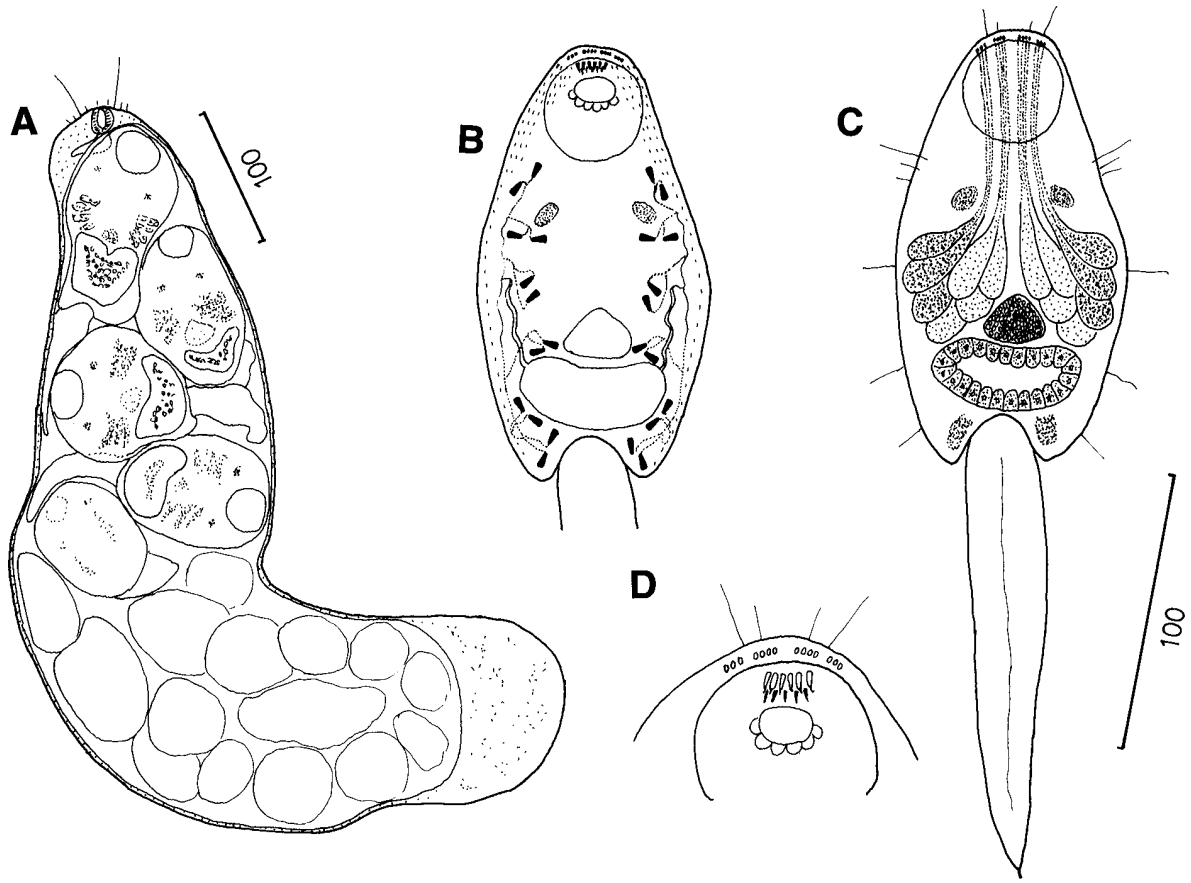

Fig. 2. *Ascocotyle* (*Phagicola*) *angeloi.* **A** - redia; **B** - cercaria from naturally infected *Littoridina castellanosae*, ventral view; **C** - same, dorsal view; **D** - free hand sketch of ventral anterior end of cercaria. Scale bars in µm.

by 66 (54-85) wide, in front of right testis, seminal receptacle on its left side. Mehlis' gland and Laurer's canal present. Two oval testes, 55 (41-69) long by 75 (57-91) wide. Vitelline glands arranged in 5-8 well separated, oval follicles, lateral to testes on each side of body. Uterus extending in transverse loops between testes and ventral sucker, containing numerous eggs, 18 (15.5-21) long by 9.5 (8-11) wide.

Remarks: The specimens 3 days p.i. from chicks had more developed vitelline glands than those found in the mouse. Accurate counts of the oral spines at the anterior extreme could not be performed in all mounted specimens. In 13 adults obtained from chicks and the mouse, 7 mounted metacercariae, several metacercariae and adults that were observed *in vivo*, the number of oral spines in each of the two rows was always 14.

M e t a c e r c a r i a Figs. 1A,B, 2D

Living metacercarial cysts were: 310 (270-333) long by 218 (201-232) (16 days p.i., n = 8); 362.5 (352-383) long by 264 (257-276) wide (105 days p.i., n = 10). Cyst wall delicate and thin.

Cysts were found in the muscles, body cavity, on internal organs, and in the gill chamber, but not on gill filaments of naturally and experimentally infected *Jenynsia lineata*. *Cnesterodon decemmaculatus* and *Gambusia affinis* occurring in the same pond were not infected. Also *C. decemmaculatus* could not be infected experimentally.

Excysted metacercariae were similar to adults, except for the development of vitelline follicles, which were seen as little coloured areas, and the absence of eggs. Living specimens contained round platelets of 19-23 µm in diameter in the intestinal caeca. The excretory bladder had two larger anterior and two smaller posterior branches, which extend anterior and posterior to the testes, respectively, filled with opaque lipid droplets. The flame cell formula was 2 [(2 + 2 + 2) + (2 + 2 + 2)] = 24, as in the cercaria.

C e r c a r i a Figs. 2B,C,E

(Measurements of 16 heat-killed, formalin-fixed cercariae.) Body 136 (122-152) long by 63 (50-81) wide; tail 159 (142-191) long by 21 (13-27) wide; oral sucker 35 (30-41) long by 29 (24-40) wide; distance from anterior extremity to eye spots 48 (40-55).

Body pyriform or triangular in resting position, oval in formol-fixed specimens, covered with minute spines.

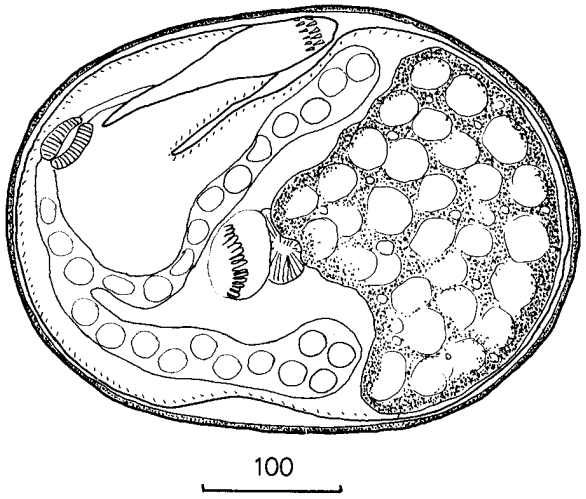

Fig. 3. Metacercarial cyst from naturally infected *Jenynsia lineata.* Scale bars in μm.

Body pigment absent. Six pairs of sensory hairs on lateral margins of body: one pair of longer hairs and two shorter at level of eye spots, one at level of penetration glands, one at level of excretory bladder, and one near posterior end of body. Two sensory hair pairs directed forward at anterior end. Oral sucker spherical, protrusible from a tegumental crypt; its anterior border with anterior row of 6 larger and posterior row of 5 smaller acicular spines. Posterior border of oral opening with 6 small papillae (Fig. 2D). Anterior end of body covered more densely with spines, arranged in several more or less regular rows; first two rows consisting of conspicuous spines, remaining diminishing gradually in size. One pair of pigmented eye spots posterior to oral sucker. Seven pairs of penetration glands arranged in 2 groups on each side of body, differently stainable with neutral red: 3 glands coarsely and 4 glands finely grained; their ducts running forward in four groups passing between eyespots, dorsal to oral sucker, and opening by sets of 3-4-4-3 pores at anterior extremity. A triangular mass of cells visible posterior to penetration glands, corresponding to anlagen of genitals and ventral sucker. No independent sucker primordium could be distinguished in this mass, either in ventral or in lateral view.

Excretory bladder epithelial, transversely elongate. Excretory canals arising from antero-lateral margins of bladder, passing forward to mid-level of body and bifurcating into anterior and posterior canals, each associated with 3 pairs of flame cells; flame cell formula probably 2 [(2 + 2 + 2) + (2 + 2 + 2)] = 24. Small glandular region staining darkly with neutral red posterior to excretory bladder and around tail base.

Tail longer than body, fitting into groove postero-ventrally. Finfolds absent; small tegumental crests located dorsally and ventrally at midline of tail; small spine present at caudal extremity.

Remarks: Cercariae emerged at irregular intervals during day-time and were positively phototactic, accumulating on the illuminated side of the container. They were intermittent swimmers that alternated periods of active swimming and resting. While resting, cercariae slowly sink to the bottom of the container, remaining motionless with their triangular bodies and tails maintained straight, at a variable angle between 90° and 120°. The maximum life-span recorded was 48 hours at 17-20°C.

R e d i a Fig. 2A

(Based on observations of several living specimens and measurements of 10 of them.) Mature rediae with one to seven cercarial embryos with visible eye spots; rediae 342 (283-427) long and 130 (113-176) wide. Pharynx 23 (14-28) long and 20 (13-25) wide; intestinal caecum small and inconspicuous. Several sensory hairs present on anterior end. Newly emerged rediae measuring 170-255 × 41-44, with pharynx 60 long and 22-25 wide and 1-2 small gland cells, with ducts opening into the pharynx.

Rediae were found in the hepatopancreas and gonad of snails. The sporocyst was not observed.

DISCUSSION

The present specimens agree with the description and drawings of *Ascocotyle* (*Phagicola*) *angeloi* made by Travassos (1930). The prepharynx in the specimens of Travassos is slightly longer (150-230 vs 130), but the other measurements are within the variation of the present material. This species is easily distinguished from all the other members of the genus by the presence of two rows of oral spines, each with 14 spines.

The systematics of the species of the *Ascocotyle*-complex has been discussed by different authors. Sogandares-Bernal and Lumsden (1963) accepted one valid genus *Ascocotyle* Looss, 1899 with 3 subgenera, *Ascocotyle* Looss, 1899, *Phagicola* Faust, 1920 and *Leighia* Sogandares-Bernal et Lumsden, 1963. Yamaguti (1971) accepted 3 genera, *Ascocotyle* (with subgenera *Ascocotyle* and *Leighia*), *Phagicola* (with subgenera *Phagicola* and *Metascocotyle* Ciurea, 1933) and *Pseudascocotyle* Sogandares-Bernal et Bridgman, 1960.

Sogandares-Bernal and Lumsden (1963) attempted to attribute a certain cercarial type to each of the above mentioned subgenera and Ostrowski de Núñez (1993) summarized the characteristics of the cercariae that develop into adults of the subgenera *Ascocotyle* ("*Ascocotyle*-form") and *Phagicola* ("*Phagicola*-form"). The general characteristics of the cercaria of *A. angeloi* corresponds to the *Phagicola*-form, but differed from it by having two rows of acicular spines, 24 proto-nephridia, and tail devoided of finfolds (one row of 5 spines, 16 protonephridia and presence of small dorsal and ventral fin folds in the other members).

Only two cercariae (which could be named "*Leighia*-form") are known to develop into adults of the subgenus *Leighia* (Leigh 1974, Ostrowski de Núñez 1992). These cercariae have subspherical bodies, protrusible oral sucker recessed within tegumental crypt, 7 pairs of penetration glands, 24 pairs of protonephridia, large excretory bladder V-shaped or transversally elongated and tail with annulations but lacking fin folds. Typical acicular spines are absent, although rows of fine spines may be present, and a terminal spine at the tail is present or absent. The cercaria of *A*.(*P.*) *angeloi* only shares with this form the number of penetration glands and protonephridia.

The relationship of each subgenus to a certain cercarial type is not very strict, as the "*Phagicola*-form" and "*Leighia*-form" are also known for the genus *Pygidiopsis* (Køie 1990, Ostrowski de Núñez 1994, 1995), which differs from *Ascocotyle* in lacking the solid prolongation of the oral sucker. Adults of *A. sexidigita* Martin et Steele, 1970, *A. gemina* Font, Heard et Overstreet, 1984 and *A. nunezae* Scholz, Vargas-Vázquez, Vidal-Martínez et Aguirre-Macedo, 1997 do not fit well in any of the three subgenera: they are similar to the subgenus *Leighia* in the extension of the uterus and the two rows of oral spines (except *A. nunezae*, which has one row and additional spines), but differ in the pre-acetabular position of the vitelline follicles, and *A. gemina* has a cercaria of the *Ascocotyle*-form.

Ascocotyle (*Phagicola*) *angeloi* shows intermediate characters between the subgenera *Ascocotyle* and *Phagicola*. It has a double row of oral spines, like the former one, but the arrangement of ovary, testes, and vitellaria correspondes to the latter one.

The cercaria is similar to that of *A.* (*A.*) *tenuicollis*, *Pygidiopsis pindoramensis* (see Ostrowski de Núñez, 1976), *A.* (*P.*) *diminuta* (see Ostrowski de Núñez 1993), *Pygidiopsis ardeae* Køie, 1990, *A.* (*P.*) *nana*, *A.* (*Ascocotyle*) sp. of Ditrich et al. (1997) and pleurolophocercaria sp. III Martorelli et Etchegoin, 1996 in the number of penetration glands, but differs from them in the number of protonephridia (8 pairs versus 12 in *A.* (*P.*) *angeloi*).

The cercaria of *A.* (*P.*) *angeloi* is similar in protonephridial formula to *A.* (*L.*) *hadra* Ostrowski de Núñez, 1992 and *Pygidiopsis crassus* Ostrowski de Núñez, 1995, but differs in body dimensions, morphology of tail, swimming behaviour and emergence pattern.

The metacercaria of *A.* (*P.*) *angeloi* seems to be very host-specific, as only *Jenynsia lineata* was found to be infected, and *Cnesterodon decemmaculatus* and *Gambusia affinis* never contained cysts in their muscles, despite their coexistence in the same pond. The experimental infections were positive also only in *Jenynsia lineata*.

Contrary to other species of the *Ascocotyle*-complex, which mature after 2-3 days in the definitive (experimental) host, the adults of *A.* (*P.*) *angeloi* were found with eggs on the fifth day after infection. The final host in Brazil, *Ixobrychus exilis*, is present in the northeast of Argentina (Misiones and Corrientes Province) but not in Buenos Aires; other ardeid birds may act as final hosts here.

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