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# LIFE-CYCLE STAGES OF A *POSTHODIPLOSTOMUM* SPECIES (DIGENEA: DIPLOSTOMIDAE) FROM PATAGONIA, ARGENTINA

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ABSTRACT: In Patagonia, populations of the galaxiid fish *Galaxias maculatus* are parasitized by metacercariae of a species of ?1 *Posthodiplostomum* (Digenea: Diplostomidae). The aim of this work was to describe larval and adult stages of this species in experimental and natural hosts from an Andean Patagonian lake. Specimens of *G. maculatus* and the pulmonate snail, *Anisancylus obliquus,* were collected in Patagua Lake. The snails were isolated in individual containers to observe emergence of cercariae, dissected, and examined under a stereoscopic microscope to record sporocysts and cercariae. Fish were examined to obtain metacercariae, and uninfected fish from Gutiérrez Lake were exposed to cercariae from *A. obliquus* to obtain experimental metacercariae. Chicks and mice were infected with metacercariae from naturally infected *G. maculatus* to obtain experimental adults. Specimens recovered belong to *Posthodiplostomum* sp. on the basis of morphological features. This is the first description of sporocysts, cercariae, metacercariae, and adults stages of a *Posthodiplostomum* species in Patagonia, including data about its natural intermediate hosts.

Species of the genus *Posthodiplostomum* are distributed around the world (Niewiadomska, 2002). Although many species have been described, Palmieri (1976) suggests the need for a revision of the species since there is a wide variation of morphological structures associated with the type of definitive host. In the American continent, 11 species were described from piscivorous birds, which are the definitive hosts. In North America, the following species were described: *Posthodiplostomum boydae* Dubois, 1969 and *Posthodiplostomum opisthosicya* Dubois, 1969 in Canada; *Posthodiplostomum minimum* (MacCallum, 1921) Dubois, 1936 and *Posthodiplostomum prossostomum* Dubois & Rausch, 1948 (Dubois, 1952 comb. emend.) in the United States (Dubois, 1970). *Posthodiplostomum minimum* was also registered in Cuba (Dubois, 1970). In South America, *Posthodiplostomum grande* (Diesing, 1850) Dubois, 1936, *Posthodiplostomum macrocotyle* Dubois, 1937, and *Posthodiplostomum microsicya* Dubois, 1936 were described in Brazil (Travassos et al., 1969); *Posthodiplostomum giganteum* Dubois, 1988 in Paraguay (Dubois, 1988); *Posthodiplostomum obesum* (Lutz, 1928) Dubois, 1977 comb. nov. and *Posthodiplostomum mignum* Boero, Led, & Brandetti, 1972 in Argentina (Dubois, 1977; Lunaschi et al., 2007). *Posthodiplostomum nanum* Dubois, 1937 was recorded in Argentina and Brazil (Travassos et al., 1969; Lunaschi et al., 2007). Larval stages of only 2 species, i.e., *P. minimum* and *P. nanum*, were described in United States and in Argentina, respectively (Miller, 1954; Ostrowski de Núñez, 1973).

During a survey of parasites of the galaxiid fish *Galaxias maculatus* (puyens) (Jenyns) in Patagonia, metacercariae of *Posthodiplostomum* sp. were found parasitizing the abdominal cavity (Viozzi et al., 2009). The aim of the present study was to describe life stages of a *Posthodiplostomum* species obtained naturally from snails and fish, and experimentally from chicks and mice, although they could not be assigned to any species.

## MATERIALS AND METHODS

### Sampling of intermediate hosts

In summer 2011, samples of the pulmonate snail *Anysancilus obliquus* (Broderip and Sowerby) (Ancylidae) and the fish *G. maculatus* were collected from Patagua Lake (40°47′S, 71°37′W), located in Arrayanes National Park, Argentina. Naturally infected snails were collected by hand from the reed *Schoenoplectus californicus,* and transported alive to the laboratory. Each snail was placed in a container with 5 ml of tap water to observe emerging cercariae. After 48 hr, snails were measured, dissected under stereoscopic microscope, and examined to obtain the sporocysts and record their infection site.

Specimens of *G. maculatus* were collected using baited traps, transported alive to the laboratory, and necropsied to examine the abdominal cavity with the aid of a stereoscopic microscope to detect and count the metacercariae.

### Experimental obtaining of metacercariae and adults

To obtain adults worms, metacercariae were removed from the abdominal cavity of naturally infected *G. maculatus* from Patagua Lake, and 18 newborn chicks *Gallus gallus domesticus* and 12 adult mice *Mus musculus* were force-fed with 50 metacercariae each and necropsied at 14 days postinfection (dpi).

To establish the correspondence between the cercariae obtained from *A. obliquus* and the metacercariae of *Posthodiplostomum* sp. obtained from *G. maculatus* in Patagua Lake, uninfected *G. maculatus* from Gutiérrez Lake (41°10′S, 71°24′W, Viozzi et al., 2009) were exposed to cercariae released from naturally parasitized snails. One infected snail was placed with 8 fish for 5 days; then the snails were removed and fish were examined 20 days postexposure (dpe).

### Morphological study of larval stages and adults of Posthodiplostomum sp. obtained from natural and experimental infections

All stages were observed alive. Sporocysts and cercariae from naturally infected *A. obliquus* were fixed in 4% hot formalin and measured. Metacercariae and experimentally obtained adults were fixed in 4% hot formalin, stained with hydrochloric carmine or Gomori's trichrome, cleared in creosote, mounted in Canada balsam, and measured. All mesurements are given in micrometers. Measurements of eggs, sporocysts, cercariae, and metacercariae are given with the range, followed by the mean between parentheses. Measurements of experimentally obtained adults of 14 dpi from *G. gallus domesticus* are given, with range followed by the mean in parentheses, and those of mice are given in brackets. Forebody is the measurement between the anterior end of body and the anterior border of ventral sucker. Drawings of eggs, sporocysts, metecercariae, and adults were made using a camera lucida, and cercariae were drawn freehand.

Specimens were deposited in the Colección Nacional de Parasitología, Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina (MACN-Pa) and Colección Parasitológica de la Universidad Nacional del Comahue, Bariloche, Argentina (UNCo-Pa). Specimens studied in the present work were compared with the following museum specimens: *P. nanum* from naturally infected *Butorides striatus* MACN-Pa No. 236/6, and 236/8 (11 vouchers), experimental adults from chicks MACN-Pa No. 228/7–8 (7 vouchers).

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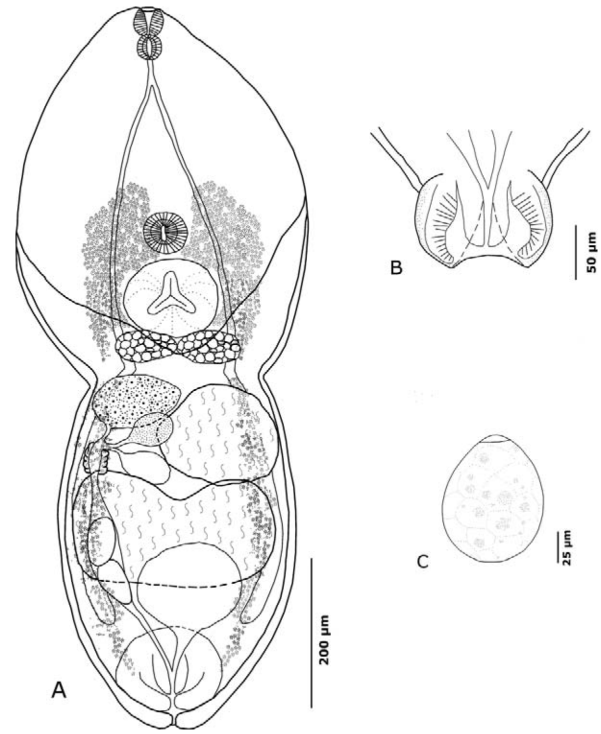


FIGURE 1. *Posthodiplostomum* sp. (**A**) Experimental adult of 14 days postinfection obtained from chicks (composite, ventral view). (**B**) Detail of evaginated copulatory bursa. (**C**) Operculated egg.

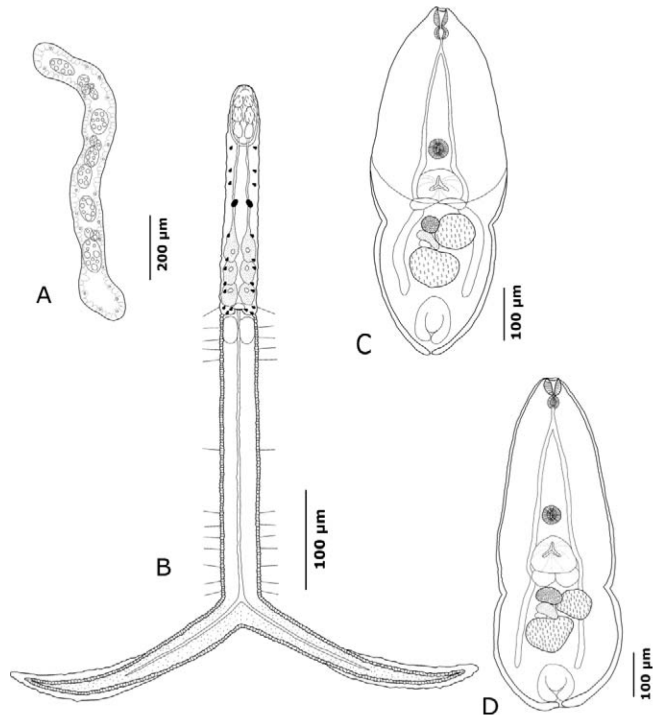


FIGURE 2. Larval stages of *Posthodiplostomum* sp. (**A**) Sporocyst obtained from *Anisancylus obliquus*. (**B**) Cercariae emerged from *A. obliquus*. (**C**) Natural metacercariae obtained from abdominal cavity of *Galaxias maculatus*. (**D**) Experimental metacercariae obtained from *Galaxias maculatus*.

On the basis of the information about the intermediate host and the life-cycle stages, we consider that the *Posthodiplostomum* sp. described herein appears to be a new species. But considering the high morphological variation observed in species of *Posthodiplostomum* depending on the experimental definitive host (Palmieri, 1976, 1977a, 1977b, 1977c; Pérez-Ponce de León, 1995), and the lack of information of the life cycle of the other species described, we are hesitant to name it as a new species. Our findings represent a further step in clarifying the taxonomic status of this genus and also contribute to enhancing the knowledge of the parasitic fauna of *G. maculatus* and *A. obliquus* in Argentina.

## DESCRIPTION

### *Posthodiplostomum* sp.

(Figs. 1, 2)

*Diagnosis (experimentally obtained ovigerous adults at 14 dpi; measurements on the basis of 8 specimens from chicks and 16 specimens from mice):* Body 852–1,193 (1,022.4) long by 398–540 (475.7) wide [767-1136 (885.7) long by 284–426 (326.6) wide] with body differentiated into anterior and posterior segments. Anterior segment 499–624 (590.3) long [470–624 (529.1) long], concave, oval, elongate. Posterior segment 384–595 (466.8) long [250–499 (370.8) long], subcylindrical. Tegument covered with spines on anterior segment of body, from oral sucker to posterior border of holdfast organ. Pseudosuckers absent. Oral sucker 48–67 (56.4) long by 48–48 (48) wide [(29–48 (42.6) long by 29–48 (40.2) wide], subterminal, slightly oval. Pharynx 29–48 (40.8) long by 29–38 (37.2) wide [38–48 (40.2) long by 29–48 (38.4) wide]. Ventral sucker 41–67 (57) long by 53–96 (70.8) wide [38–58 (53.9) long by 38–67 (56.3) wide], circular. Holdfast organ 77–154 (110) long by 134–221 (164.4) wide [144–192 (135) long by 144–202 (161.4) wide], round, trirradiate slit, with adhesive glands on each side. Testes different in size and shape; anterior testis 77–211 (149.5) long by 67–221 (171.1) wide [96–144 (124) long by 106–264 (161.2) wide], asymmentrical, sinistrolateral. Posterior testis 86–202 (146.7) long by 307–403 (353.8) wide [86–163 (132.5) long by 173–346 (243.2) wide], symmetrical with an anterior slit. Ovary 67–96 long (82.1) by 96–125 (110.9) wide [38–86 (60.8) long by 67–96 (73.6) wide] pretesticular, lateral, tangential to anterior testis, dextral, ellipsoid. Vitellarium extends from almost the middle of anterior segment to copulatory bursa. Vitelline follicles distributed densely around ventral sucker and holdfast organ, forming 2 ribbons on posterior segment. Oviduct connected with vitelline reservoir, Mehlis' gland, and ootype. Evaginable copulatory bursa with terminal opening. Genital cone surrounded by a prepuse, enclosing hermaphroditic duct, which is formed by union of uterus and ejaculatory duct, from the seminal vesicle. When genital cone is everted, an inverted V-shaped notch is observed in dorsal view. Maximum number of eggs observed in uterus 7 (3).

*Eggs; measurements on the basis of 7 specimens from chicks and 13 specimens from mice:* Smooth, operculated, oval, yellowish, 77–96 (85.1) long by 48–58 (52.1) wide [58–96 (77) long by 38–57 (43.8) wide].

*Sporocyst; measurements on the basis of 7 specimens:* Found in hepatopancreas of *A. obliquus*, they constitute long, narrow sacs with a central cavity containing free-moving cercariae and germ balls; cylindrical with rounded ends, 576–1,136 (877.3) long by 67–105 (89.6) wide.

*Cercariae; measurements on the basis of 20 specimens:* Oculate furcocercariae. In resting position, cercariae remain in water with the body slightly curved downward, forming the tail with furcae an angle of 90°, approximately. Body 192–240 (219.8) long by 38–58 (44.6) wide, tail stem 221–365 (307.7) long by 19–48 (35.5) wide. Ratio tail stem to body length 1:1.2–1.5. Furcae with fin folds 182–317 (244.8) long by 10–29 (21.6) wide. Tail stem without spines, small spines on furcae. Sensorial hairs paired and lateral, 1 pair at level of third pair of penetration glands of body, 4 pairs at the proximal end of tail stem, 2 in the middle, and 7 pairs near the furcae bifurcation. There were no sensorial hairs in the furcae. Penetration organ 48–62 (52.6) long by 19–36 (23.0) wide, covered with 11 rows of interspersed spines. Pigmented eyespot located in the second third of body at 96 µm from the anterior end. Ventral sucker not developed. Three pairs of penetration glands in posterior half of body. The 3 ducts of each side of these glands run together into the penetration organ and flow individually. Mouth located at the anterior end of body, goes through penetration organ reaching posterior end of it; ceca not developed. The excretory system consists of 11 pairs of flame cells whose protonephridial formula is $(1 + 1 + 1 + [1 + 2] + 2 + [1 + 2]) \times 2 = 22$. Excretory duct of both sides end in a small excretory vesicle, from where the excretory canal runs along the midline of the tail stem and bifurcates to penetrate the furcae, ending approximately at its midpoint.

*Natural metacercariae; measurements on the basis of 15 specimens:* Metacercariae in different development stages, even some without cyst. Metacercariae neascus, surrounded by a transparent cyst, thin walled, and oval, in which metacercariae extended or folded on themselves. Excysted metacercaria 566–777 (672) long by 134–278 (194.8) wide, has a clear division between the foliaceous anterior segment and the posterior one. Tegument covered with spines visible only on border of the anterior segment of body. Oral sucker 29–43 (37.3) long by 19–34 (26.0) wide, terminal. Pharynx 14–31 (25.4) long by 10–29 (20.2) wide. Forebody 240–346 (291.8) long, ventral sucker 34–48 (40.4) long by 22–41 (30.8) wide, rounded. Holdfast organ 62–96 (77.8) long by 60–125 (87.5) wide, rounded, with 2 posterior adhesive oval glands. Undeveloped reproductive system at the posterior end of posterior segment, formed by two morphologically different testes and 1 ovary. Anterior testis 38–74 (53.3) long by 38–79 (58.6) wide, posterior testis 43–74 (62.4) long by 48–113 (73.7) wide, and ovary 19–34 (24.9) long by 19–53 (29.4) wide.

Experimental metacercariae were recovered from the experimentally infected *G. maculatus* from Gutiérrez Lake. Only 1 of 8 fish was infected and 6 metacercariae were recovered at 20 dpe and measured, 738–824 (766.8) long by 114–142 (123.1) wide.

*Taxonomic summary: First intermediate host Anisancylus obliquus* (Broderip and Sowerby).

*Site:* Hepatopancreas.

*Second intermediate host: Galaxias maculatus* (Jenyns).

*Site:* Mesenteries of abdominal cavity.

*Type locality:* Patagua Lake (40°47′S, 71°37′W), Patagonia, Argentina.

*Prevalence in first intermediate host (type locality)*: 3.5% (summer).

*Prevalence in second intermediate host (type locality)*: 100% (summer).

*Mean intensity in second intermediate host (type locality)*: 763 (summer).

*Experimental definitive host*: *Gallus gallus domesticus* and *Mus musculus*.

*Site:* Anterior region of intestine.

*Specimens deposited:* Two voucher specimens from chick No. 558/11–12 (MACN–Pa), 3 from mice No. 558/ 8–10 (MACN–Pa), 3 natural metacercariae No. 558/5–7 (MACN–Pa), 3 experimental metacercariae No. 558/2–4 (MACN–Pa), 1 sporocyst No. 558/1 (MACN–Pa); 6 vouchers from chick No. 239/1–6 (UNCo-Pa), 13 from mice No. 240/1–13 (UNCo-Pa), 12 natural metacercariae No. 241/1–12 (UNCo-Pa), 3 experimental metacercariae No. 242/1–3 (UNCo-Pa), 4 sporocyst No. 243/1–3 (UNCo-Pa).

*Remarks:*The adult worms experimentally obtained in chicks and mice were identified as members of the genus *Posthodiplostomum* (sensu Niewiadomska, 2002) on the basis of morphological characteristics, i.e., body bipartite; pseudosuckers absent; oral and ventral sucker developed; pharynx small; testes in tandem with differences in size and shape, the anterior one being asymmetrical and lateral and the posterior testis larger; ovary pretesticular, lateral to anterior testis; copulatory bursa evaginable, with terminal opening and genital cone surrounded by prepuse. Besides, metacercariae are neascus, parasitizing fish, and cercariae emerge from pulmonate snails, with a rudimentary alimentary system, ventral sucker absent, pigmented eyespots, and 3 pairs of penetration glands.

It has been demonstrated, at least for some species of *Posthodiplostomum* such as *P. minimum* and *P. nanum*, that they have a high intraspecific morphological variability. Studies on *P. minimum* described aspects of the host-induced variation using various classes of tetrapod vertebrates as experimental definitive hosts, concluding that egg production and the level of maturity reached depend on the host parasite species. Some morphological characteristics as shape and size of body, oral sucker, and ventral sucker showed also a great variation (Palmieri, 1976, 1977a, 1977b, 1977c; Pérez-Ponce de León, 1995). Considering the high intraspecific variation registered between specimens obtained from chicks and mice, the use of morphological characters as the only source of information to describe a new species would not be appropriate. Palmieri (1976) suggested the need for an extensive and complete revision of the genus *Posthodiplostomum*, as well as the necessity for experimental determination of relationships between parasite species and their definitive host, which was not done yet.

The specimens described herein differ from other species of the genus mainly in the distribution of vitellarium, the shape and body size, as well as the morphometry of some organs. *Posthodiplostomum boydae, P. opisthosicya, P. mignum,* and *P. minimum* have the vitellarium in anterior segment reaching the anterior border of the ventral sucker, whereas in this species it reaches the half of this segment. Furthermore, those species have smaller suckers and pharynx (*P. boydae*: oral sucker 34–35, ventral sucker 32–38, pharynx 24–26; *P. opisthosicya*: 23–34, 37–44, 21–26, and *P. mignum* 40, 40–41, 28–30) (Dubois, 1970) than the Patagonian specimens of *Posthodiplostomum* (oral sucker 48–67, ventral sucker 41–67, pharynx 29–48). Besides, *P. minimum* differs from these specimens described herein by having a longer body size (1,700 vs. 852–1,193) (Dubois, 1970). In addition, *P. prossostomum*, *P. grande, P. microsicya,* and *P. giganteum* differ from our *Posthodiplostomum* specimens since their vitellarium extend beyond the middle of the anterior segment, reaching the pharynx or the cecal bifurcation. Also all these species have a bigger body size (*P. prossostomum* 2,500, *P. grande* 2,250*, P. microsicya* 1,950, and *P. giganteum* 4,060–4,610) than the Patagonian specimens (Dubois, 1970, 1988; Boero et al., 1972). *Posthodiplostomum macrocotyle, P. obesum, and P. nanum* have a similar vitellarium distribution than our specimens, but *P. macrocotyle* differs by having a bigger body size (1,200) and a smaller oral sucker (24–34); *P. obesum* differs by having bigger eggs (96–104) than our species (77–96) (Ostrowski de Núñez, 1970). *Posthodiplostomum nanum* sensu Dubois (1970) has smaller ventral sucker (24–46), holdfast organ (43–76), and eggs (62–76) than our species (41–67, 77–154, 77–96, respectively) (Dubois, 1970). The morphology and the meristic features of the present species is very similar to that obtained experimentally in chicks of *P. nanum* sensu Ostrowski de Núñez (1973), but they differ from the voucher specimens of *P. nanum* obtained from *B. striatus* (Linnaeus) in the form of the anterior segment of body (oval in these specimens and discoidal in *P. nanum*).

The only sporocysts described for *Posthodiplostomum* species in the American continent are those of *P. nanum* obtained from *Uncancylus concentricus* (d' Orbigny), which have 1 end sharp and the other rounded, in contrast to those of the sprorocyst of the Patagonian species, which are both rounded. There were also differences in sporocyst width, being narrower (42–63) in those of *P. nanum* than those described herein (67–106) (Ostrowski de Núñez, 1973). The only 2 cercariae of *Posthodiplostomum* described correspond to those of *P. minimum*, which were released from the physiid snail *Physa halei* Lea in United States (Bedinger and Meade, 1967), and those of *P. nanum* from the ancylid snail *U. concentricus* in Argentina (Ostrowski de Núñez, 1973). The cercariae described herein have a larger body (192–240) and penetration organ (48–62) than those of *P. nanum* (148–190, 31–42) and *P. minimum* (143–158, 32–61). Other differences between the cercariae is the number of flame cells, which is larger in our species (22) than in *P. nanum* (18), and lower than in *P. minimum* (26) (Bedinger and Meade, 1967; Ostrowski de Núñez, 1992). Particularly the Patagonian specimen differs from *P. nanum* in number and distribution of sensory hairs; *P. nanum* has 2 pairs in the body, 1 at level of the first pair of penetration glands and the other at level of excretory bladder, 3 pairs on the anterior part of tail stem, and on the furcae (Ostrowski de Núñez, 1973), whereas our specimen has only 1 on the body at level of excretory bladder, 4 pairs on the anterior part of tail stem, and has no hairs on furcae. The rest position in *P. nanum* and the cercariae described in this work are similar; no further behavioral differences have been observed.

The metacercariae of *P. minimum* were obtained from centrarchid and cyprinid fishes in the United States, and those of *P. nanum* from the poeciliid fish *Cnesterodon decemmaculatus* (Jenyns) and *Phalloceros caudimaculatus* (Hensel) in Argentina (Miller, 1954; Ostrowski de Núñez, 1973), whereas metacercariae of the Patagonian specimens were only recovered from *G. maculatus* (Ortubay et al., 1994), and it has never been recorded in other Patagonian galaxiid fishes like *Aplochiton zebra* Jenyns and *Galaxias platei* Steindachner (Ortubay et al., 1994; Fernández et al., 2012). They also differ in the site of infection, since metacercariae of *P. minimum* are encysted in the parenchyma of the liver and the kidney (Miller, 1954; Palmieri, 1976), and those of Patagonian specimens are located in the mesenteries of theabdominal cavity. The metacercariae of the species described herein are smaller (566–778/134–278) than those of *P. minimum* (1,230–1,360/640–680) (Miller, 1954), but have a similar size than those of *P. nanum* (620–806; 217–310) (Ostrowski de Núñez, 1973).

## DISCUSSION

In this paper we described life-cycle stages of a species of *Posthodiplostomum* on the basis of natural infections of intermediate hosts, adult worms obtained experimentally from chicks and mice fed with metacercariae from naturally infected *G. maculatus*, and metacercariae obtained experimentally from fish infected with cercariae. The life cycle of this species includes the snail *A. obliquus* as first intermediate host, being the first parasitological record for this pulmonate snail. The second intermediate host is the fish *G. maculatus*, which is parasitized by highly host-specific metacercariae, widely distributed in Andean Patagonian lakes (Ortubay et al., 1994; Viozzi et al., 2009). The natural definitive host is still unknown; because the Andean Patagonian lakes are included in Argentinean national parks, avian hosts cannot be hunted. It is probable that the definitive hosts are piscivorous birds, since the experimental adult worm size and egg production were higher in chicks than in mice. Other species of *Posthodiplostomum* parasitize mainly birds of Ciconiiformes and to a lesser extent, Pelecaniformes and Charadriiformes (Dubois, 1970). All these avian hosts live in Patagonia, and it is known that *Phalacrocorax atriceps* King and *Phalacrocorax brasilianus* (Gmelin) prey on *G. maculatus* in this region (Rasmussen et al., 1993; Alarcón et al., 2012).

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**Queries for para-99-05-24**

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