Biological aspects of the life history of *Confluaria podicipina* (Cestoda, Hymenolepididae) from a hypersaline pampasic lagoon

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Abstract. The aim of the present study is to describe the larval stages and adults of *Confluaria podicipina*, parasitizing the brine shrimp *Artemia persimilis* and the silvery grebe *Podiceps occipitalis* from the Epecuén lagoon, to analyze its seasonal variation as well as to assess the damage in the gut of the definitive host. The Epecuén lagoon is an endorheic basin being the southernmost body water of the Lagunas Encadenadas del Oeste. There is a great concentration of silvery grebes attracted by the high population density of *A. pesimilis*, a basic food item. The present study reports a new host and geographical localization of *C. podicipina*. Also confirms that the life history of this hymenolepidid occurs between the brine shrimp *A. persimilis*, as the intermediate host and *P. occipitalis*, as its final host in the lagoon. *Confluaria podicipina* cysticercoids are present both in spring and autumn in these ecosystem and probably the transmission occurs all along the year. However both the prevalence and mean abundance are higher in spring, so this season could be the optimal moment for transmission. Tapeworms attach deeply in the crypts of Lieberkuhn by the rostellum and suckers, and cause traumatic lesions in the intestinal wall.

Key words: tapeworms, life cycle, hypersaline lagoon, *Confluaria podicina*, *Artemia*

Resumen: Aspectos biológicos de la historia de vida de *Confluaria podicipina* (Cestoda, Hymenolepididae) en una laguna pampeana hipersalina. En el presente trabajo se describen los estadios lavales y adultos de *Confluaria podicipina*, parasitando a *Artemia persimilis* y al macá plateado *Podiceps occipitalis* en el lago Epecuén, se analiza su variación estacional así como se evalúa el daño en el intestino del hospedador definitivo. El lago Epecuén constituye una cuenca endorreica siendo la laguna bonaerense más austral del sistema de las Encadenadas del Oeste y posee una gran concentración de macáes plateados atraídos por la abundancia de *A. persimilis*, su item trófico básico. El presente estudio comunica un nuevo registro de hospedador y de localidad geográfica para *C. podicipina*. Además confirma que el ciclo biológico de este cestode ocurre entre *A. persimilis* como hospedador intermediario y *P. occipitalis* como el definitivo en el lago. Los cisticercoides de *C. podicipina* estuvieron presentes tanto en primavera como en otoño en el hemocele del crustáceo anostraco y probablemente la transmisión ocurre a lo largo de todo el año. Sin embargo, tanto la prevalencia como la abundancia media de larvas fue mucho mayor en primavera, sugiriendo que esta época puede ser la óptima para la colonización del hospedador definitivo. Los helmintos se adhieren profundamente en las criptas de Lieberkühn por el rostelo y las ventosas, y causan lesiones traumáticas en la mucosa intestinal.

Palabras clave: cestodes, ciclo biológico, laguna hipersalina, *Confluaria podicipina*, *Artemia*.

Introduction

Tapeworms constitute the dominant group in the helminth communities from grebes (Podicipediformes) (Stock 1985, Storer 2000). In Argentina, digeneans (Sutton et al. 1982, Martorelli 1988, Etchegoin & Martorelli 1997, Núñez et al. 2017), nematodes (Escudero et al. 2007, Galeano & Tanzola 2012) and acanthocephalans (Vizcaíno 1989) were studied. Núñez et al. (op.cit.) reported an unidentified cestode in *P. gallardoi* Rumboll, 1974 from El Cervecero Lake, Santa Cruz province, Argentina. This finding represents the only record of a cestode in podicipediforms from Argentina. Also, only one record of tapeworms parasitizing the silver grebe *Podiceps occipitalis* Garnot 1826 from the central region of Chile, is known (González-Acuña et al. 2017). But parasitological data of this grebe are completely lacking in the rest of its dispersion area in the South of the Neotropical region. Vasileva et al. (2000) confirmed that *Confluaria podicipina* (Szymanski, 1905) Spasskaya, 1966 is a grebe specialist in the Palaearctic region, which parasites the black-necked grebe *P. nigricollis* Brehm, 1831 and the great crested grebe *P. cristatus* (Linnaeus 1758) in Europe. Redón et al. (2019) extend the geographical distribution of this hymenolepidid to Central Asia, North and South America. Gajardo et al. (2006) highlight the peculiar features of the inland hypersaline lakes due to its low biological diversity and the relatively simple food webs occurring in them. The Epecuén lagoon is located in the Lagunas Encadenadas del Oeste System, a great depression with an endorheic basin of interconnected lenthic water bodies. Wetmore (1926) remarked the enormous concentration of silver grebes in this wetland, with groups of more than a thousand individuals attracted by the high population density of *Artemia* spp., probably their main food item. The aim of the present study is to describe the larval stages and adults of *Confluaria podicipina*, parasitizing the brine shrimp *A. persimilis* and *P.occipitalis* from the Epecuén lagoon, to analyze its seasonal variation as well as to assess the damage in the gut of the definitive host.

Materials and methods

Study area, hosts sampling and dates: The Epecuén lagoon (37 ° 08′04 ″ S 62 ° 51′57 ″ W) with a surface area of 160 km^2 and mean depth of 7 m, is part of an endorheic basin being the southernmost body water of the Lagunas Encadenadas del Oeste System (Fig.1).

Figure 1. Geographic localization of Epecuén lagoon and sample sites. 1. Beach site. 2. Old municipal cemetery site.

Its waters are alkaline and hypersaline (320 g/l) establishing an oligotrophic environment with low biodiversity (Calcagno et al. 1995). The narrow and little flow Pigüé stream is the most important input of freshwater in the lagoon. Others minor tributaries are Pichi Pul stream and Arroyo Grande (Geraldi 2009). There is a total absence of hydrophytes and the phytoplankton is dominated by Bacillariophyceae and Chlorophyceae. The zooplankton presents low diversity and is dominated by three species of crustaceans resistent to high levels of salinity: *Moina eugenie* (cladoceran), *Boeckella poopoensis* (copepod) and *Artemia persimilis* (anostraca)(Calcagno op.cit). The wetland is colonized by aquatic birds both resident as *Phoenicopterus chilensis*, *Himantopus melanurus*, *Podiceps occipitalis*, *Anas* spp., *Coscoroba coscoroba* and seasonal visitors as several species of migratory charadriiforms from the Arctic (*Phalaropus tricolor*, *Calidris bairdi*, *C. fuscicollis*, *Tringa flavipes* and *T. melanoleuca*)(Fig. 2).

Nine hundred and twenty eight specimens of *A. persimilis* (506 females and 422 males) and one individual of *P. occipitalis* found dead in the shoreline, were examined. The crustaceans were sampled randomly, regardless the coloration of each individual, using a hand net (3x3 mm mesh) and a plankton net (250 µm mesh) from the shore at the northeast margin of the lagoon. An attempt was made to examine approximately the same number of males and females. They were kept alive and transported in aereated bottles until processed in the Laboratory of Parasitology (UNS). Brine shrimps were sexed based on the antenna morphology and the presence of ovisacs. Table 1 shows the seasonal collection of the hosts in two sampling sites.

Identification of parasites: The brine shrimps were fixed and preserved in 5% formalin. Semipermanent slides were made in glycerin, placing four specimens between porta and coverslips until complete trasparency (48-72 hs)(Georgiev et al. 2005, Redón et al. 2015). Helminths were identified under light microscopy inside the thorax and abdomen of the crustaceans. Taxonomical criteria follow that of Georgiev et al. (2005) and Redón et al. (2015), were based on the morphology, morphometry and number of the rostellar hooks, size and shape of cercomer. The specimen of *P. occipitalis* was dissected in the field and the visceral mass was extracted and preserved in formol 10%. This process may have

Figure 2. *Podiceps occipitalis* population in spring (Epecuén lagoon)

Table 1. Date and location of the samples in the Epecuén Lagoon (N= 928)

Date	Sampling site name	*A. persimilis*	*P. occipitalis*
17.10.2016	1. Beach (spring)	100 ♀♀, 99 ♂♂	0
03.12.2016	1. Beach (early summer)	120 ♀♀, 81 ♂♂	0
	2. Old municipal cemetery	85 ♀♀, 41 ♂♂	0
15.04.2017	1. Beach (autumn)	100 ♀♀, 101 ♂♂	0
	2. Old municipal cemetery	101 ♀♀, 100 ♂♂	0
06.10.2019	1. Beach (spring)	0	1

contracted *in situ* the intestinal helminths. Because of this, the taxonomical identification of adult worms was made by morphology and morphometry of the rostellar hooks, scolex and histological reconstruction of the internal anatomy of mature segments. The stomach, intestine and colic caeca were dissected and its contents observed under stereoscopic microscopy. The rostellar armature was studied in glycerine mounted scolices, and the hooks were counted and measured in µm. Drawings of the larvae were made using a camera lucida Olympus. All measurements are done in µm unless otherwise stated, and are shown as the mean (range, number of individuals).

Histopathological assessment: Small pieces (≈5mm lenght) of the intestinal wall were extracted for histopathological purposes. They were fixed in buffered formalin, dehydrated in an alcohol battery and embedded in Paraplast©. Transverse sections of gut between 5-7 µm thick stained with hematoxylin and eosin were performed. The protocol of necropsy and histopathological assessment follows Quse & Falzoni (2008). Photomicrographies were taken using a Motic BA 200 light microscopy.

Quantitative Parasitology: Prevalence, intensities and parasitic abundances were calculated following Bush et al. (1997). Parasitic prevalences between sexes and seasons were compared using the Z statistic for samples ≥100 hosts (Morales & Pino 1987). The parasitic abundances between sexes and seasons were tested using the U-Mann Whitney test (IBM SPSS Statistic 23).

Results

Description of the cysticercoid: The general aspect fit with that in Georgiev et al. (2005) description. Cyst oval, with a thin wall transversely striated, 40 (34-45, n=10) x 26.3 (23-31, n=10) in small cysts; 81.5 (75-87.5, n=10) x 41 (37.5-42.5, n=10) in fully developed. Young cysts without external envelope (Fig. 3). Fully developed cysticercoid with a very expanded, coiled and densely packed cercomer about 2.2 mm in length (25 times longer than cyst), which forms an external envelope surrounding the

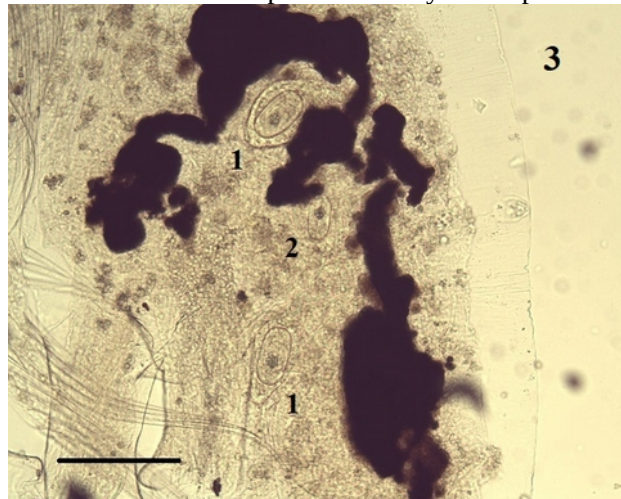scolex (12.5-20.00 in width) (Fig. 4). Scolex with several calcareous corpuscles mostly in the posterior

Figure 3. *Confluaria podicipina*, cysticercoids. 1. Fully developed. 2. Juvenile. Scale bar= 0.1 mm
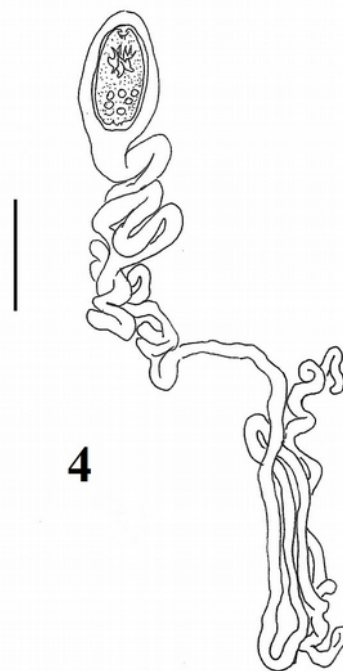

Figure 4. *C. podicipina*, fully developed cysticercoid (schematic). Scale bar= 0.1 mm.

part. Suckers were not visible in glycerine preparations. Ten refrigent aploparaksoid hooks in an invaginated rostellum, 17-21 (n=20) in total lenght (Fig. 5). Blade of hooks, 10-12 in lenght, always anteriorly directed. Handle small (5-6 in lenght). Cysticercoid mostly occupy the coelom of thorax. Table 2 summarizes comparative data of previous and present findings.

Adults (based on cleared individuals and histological sections): All of the strobilae were found in a mature but no gravide state. Detached gravid proglottides or mature eggs could not find in the intestinal tract. So, the following measures correspond to mature individuals. Scolex ovoidal 302.5 (270-330, n=4) in maximum width at level of middle of suckers having an apical protrusion (Fig. 6). Neck relatively short. Suckers round 112.5 (110-120), protruded with well-developed orbicular muscle. Rostellum dome in shape, powerfull with well developed musculature. Rostelar sheath to the level of the posterior margins of suckers. Single crown of 10 composite hooks, each of them with a refractile part (the larval hook conserved) and a strong epiphyseal thickening. Total length 38-40 (n= 10). Refractile part: 15-16 total length, 12.5 handle, 7.5 blade; epiphyseal thickening 32-35 x 5-8 (Fig. 7). Four pairs of gross longitudinal muscular bands 32 (22.5-40) in diameter, cover up the medullar reproductive organs (Fig. 8). Proglottides craspedote much wider than long (17-24 lenght; 350-380 maximum width). Genital pores unilateral. Testes 3, small (17-20 in maximum diameter), in the median field (Fig. 9). Genital ducts dorsal to excretory vessels. External seminal vesicle elongate. Cirrus sac elongated, cylindrical, with rounded proximal end, having an internal seminal vesicle which occupies 2/3 volume. Cirri could not be observed in evaginated condition to confirm the presence of a spiny cover. Ovary lobed dorsal to vitelary gland. Seminal receptacle in the midline of the proglottid. Uterus and mature eggs, not observed. Table 3 summarizes comparative data of previous and present findings.

Histopathology in the final host: The adults of *C. podicipina* were attached by their rostellum and suckers penetrating deeply the crypts of Lieberkuhn (Fig. 10). Attachment of the tapeworms caused traumatic lesions, erosion and atrophy of the mucosal epithelium. The helminths penetrated deeply into the crypts, caused an evident degeneration and complete destruction of intestinal villi. Microhemorrhagic foci were observed near the mucosa.
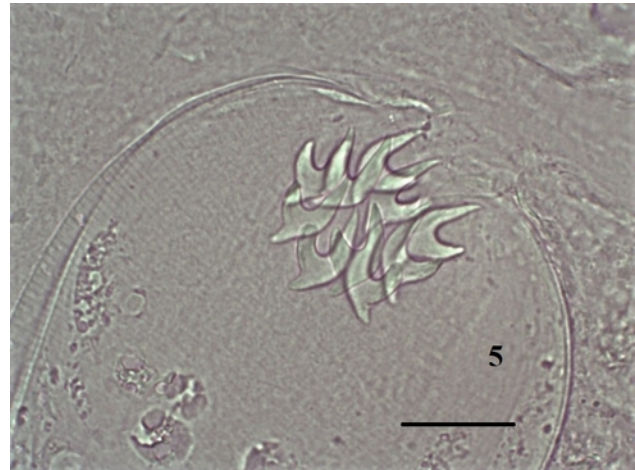

Figure 5. *C. podicipina* rostellar hooks (transverse strations are shown). Scale bar= 0.02 mm

Quantitative Parasitology of C. podicipina *in its intermediate hosts:* Table 4 shows the parasitological indexes from different sample sites and compare them with previous records of *C. podicipina* in different hosts and geographical regions.

Additional records of another cestode larvae: Additional records in the present study indicate the presence of *Wardium* sp. and *Flamingolepis* sp. in the ecosystem but with low presence (only 2 and 3 cysticercoids found, respectively, in 928 hosts).

Discussion

Maximova (1981) described the cysticercoid of *C. podicipina* in *Artemia salina* from Tengiz Lake, a saline lake from the central region of Kazakhstan. Ryzhikov et al. (1985 in Storer 2000) recorded two species of cladocerans as intermediate hosts in freshwater environment from the Paleartic region. Redón et al. (2019) reported by the first time the presence of *C. confluaria* as cysticercoid in the South American brine shrimp *Artemia persimilis* Piccinelli & Prosdocimi 1968 (Crustacea, Anostraca) from two hypersaline lagoons in South Chile. These authors, based on anatomical traits, trophic behavior, diet and abundance suggest that *P.occipitalis* is the most probable definitive host of *C. podicipina* in that ecosystems. In the present study we confirm that suggestion. Because of only one bird was analyzed, here we present only quantitative data from the intermediate host of the cestode in the Epecuén lagoon. In the sample studied from Argentina, the prevalence was higher than South Chile population as were the mean intensity and mean abundance (Table 4). This fact positioned *C. podicipina* as the dominant parasites in the helminth fauna of *A. persimilis.* González-Acuña et al. (2017) recorded

Table 2. Comparative data of *Confluaria podicipina* cysticercoids from several intermediate hosts and geographical records.

Host		*Artemia parthenogenetica*			*A. franciscana*			*A. persimilis*			*A. persimilis*	
Locality		Spain			USA			Chile			Argentina	
Refs		(1)			(2)			(3)			(Present study)	
		Range	Mean	n	Range	Mean	n	Range	Mean	n	Range	n
External	L	195-255	215	9	144-313	212	15	113-185	135	5	82.5-127.5	5
capsule	W	135-204	152	9	72-192	118	15	73-110	87	5	52-75	7
Cyst	L	93-147	121	12	72-143	106	17	80-110	90	7		
	W	47-87	73	12	36-89	61	17	43-55	49	7		
Scolex	L	72-104	92	12	38-52	48	15	50-63	57	6	34-45	10
	W	38-72	59	12	25-47	37	15	33-40	38	6	23-31	10
Suckers	L	26-32	28	10	21-26	22	6	16-23	19	10	w/d[a]	---
Rostellum	W	42-47	46	7	23-31	27	10	25	25	3	w/d[a]	---
	L	---	---	---	---	---	---	18-23	20	3	w/d[a]	---
Rostellar hooks	Total length	21-24	22	12	19-21	20	11	18-21	19	10	17-21	10
	Blade	---	---	---	11	---	11	10-12	11	10	10-12	10
	handle	---	---	---	3-5	4	11	3-4	4	10	5-6	10
	guard	---	---	---	5-6	6	11	5-6	6	10	w/d[a]	---

References: (1) Georgiev et al. (2005); (2) Redón et al. (2015); (3) Redón et al. (2019). w/da= without data

Figure 6. *C. podicipina* adult, anterior end. Scale bar= 0.1 mm. Lower box= rostellar detail. Scale bar= 0.02 mm.

Figure 7. *C. podicipina* adult, scolex in frontal view. Lower box= isolated rostellar hook. Scale bar= 0.05 mm.
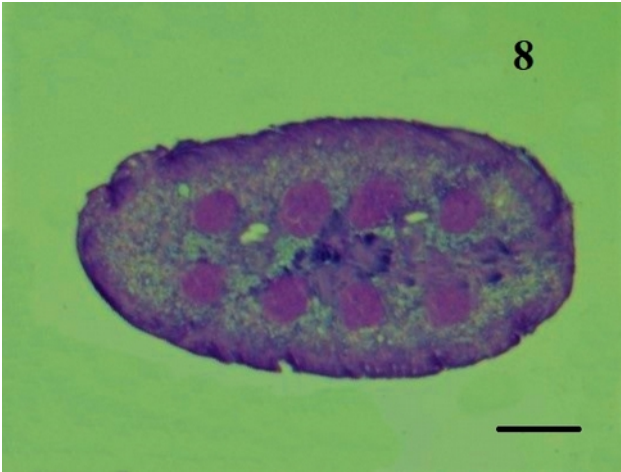
Figure 8. *C. podicipina* adult, histological transverse section of the mature strobila, showing four pairs of gross longitudinal muscular bands and the reproductive organs in the middle field of proglottid. Scale bar= 0.05 mm
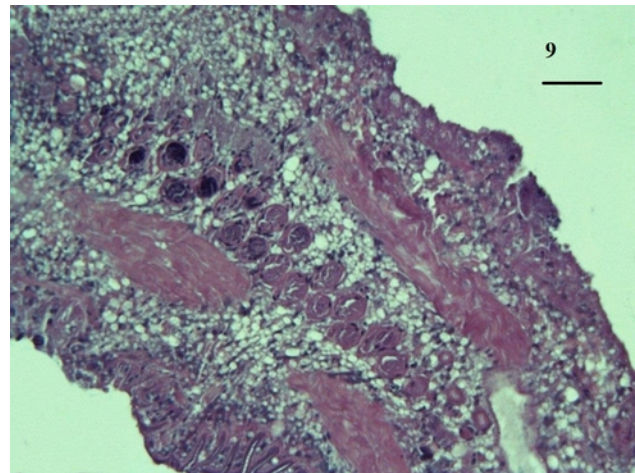

Figure 9. *C. podicipina* adult, histological sagittal section of the mature strobila, showing the testes in the middle field of the proglottids. Scale bar= 0.02 mm.

Table 3. Comparative measurements of *C. podicipina* adults from various hosts and geographic localities.

| Hosts	*Podiceps auritus*	*P.nigricollis P.cristatus*	*P.nigricollis*	*P.nigricollis*	*P. nigricollis*			*P. occipitalis*	
Locality Refs.	Poland Szymanski (1905)	Slovak Republic Macko (1959)	Poland Sulgostowska & Korpaczewska (1972)	Kazakhstan Maksimova (1989)	Bulgaria Vasileva et al. (2000)			Argentina Present study	
	Range	Range	Range	Range	Range	Mean	n	Range	n
Strobila length (mm)	140	269	250-310	170-290	37-71	---	2	45-55	3
Strobila width (mm)	0.4-0.7	1.063	1.2	0.9-1.0	0.34-0.45	---	2	0.35-0.38	4
Scolex length (µm)	372	336-459	670-740	420	252-378	318	6		4
Scolex width (µm)	296	---	450	340	164-353	269	6	270-330	4
Rostellar hooks length	43-46	42-47	55	50-58	45-60	54	24	38-40	10
Testes diameter	---	17	20-25	17-25	18-28	22	40	17-20	10
Cirrus sac length	---	107	150-200	170	98-132	113	33		
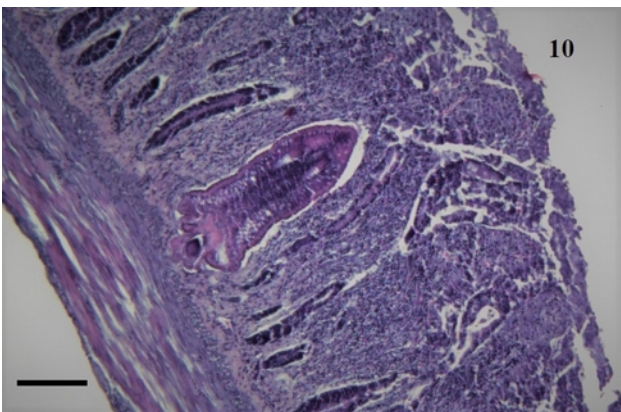
Figure 10 (opposite column). Histological transverse section of the intestinal wall of *P. occipitalis* showing the scolex deeply attached in a crypt of Lieberkhun. Scale bar= 0.1 mm

46.39% prevalence, 14.7 mean intensity and 10.05 mean abundance of adults *Confluaria* sp. from 97 *P. occipitalis* died by an oil spill in Central Chile. Assuming that these tapeworms belong to *C. podicipina* may be that this tapeworm inhabits commonly the silvery grebe in Chile. Consequently the differences between parasitological indexes registered in our sample of brine shrimps and the previous Neotropical record by Redón et al. (2019)

Table 4. Parasitological indexes of *C. podicipina* cysticercoids from different hosts and geographical localities

Host Locality References	*Artemia parthenogenetica* Spain (Odiel Marshes) Georgiev et al. (2005)	*A franciscana* USA (Great Salt Lake) Redón et al. (2015)	*A. persimilis* Chile (Los Cisnes lagoon and Amarga lagoon) Redón et al. (2019)	*A. persimilis* Argentina (Epecuén lagoon) Present study
N° hosts examined	3300	2487	800	928
N°cysticercoids (total)	Not stated	1471	12 (10 Los Cisnes lagoon) (2 Amarga lagoon)	334
Prevalence(%)	6.5	34.9	2.5 (Los Cisnes lagoon) 0.5 (Amarga lagoon)	21.39
Mean Intensity (range)	1.42 (1-7)	1.69 (1-11)	1 (Los Cisnes lagoon) 1 (Amarga lagoon)	1.69 (1-11)
Mean Abundance (±SD)	0.09 (±0.40)	0.592 (±0.02)	0.025 (Los Cisnes lagoon) 0.005 (Amarga lagoon)	0.36 (±0.02)

could be explained for the relative importance of *A. persimilis* in the diet of the silvery grebe populations. Whereas in South Chile *C. podicipina* was found only in spring samples, in Epecuén lagoon was present in spring as in autumn with highly significative differences in prevalences (38.8% vs 23.8%, Z= 15.09, p<0.001). Silvery grebes are present throughout the year in Epecuén and form dense agglomerations of mating individuals during spring (Fig. 2). Prevalences were not different between sexes (Z= 1.06, p>0.05) as well as neither the mean abundances (U Mann-Whitney= 104023.5, p>0.05). These data show that both sexes are exposed equally to the infective stage of the parasite.

Silvery grebes potentially may be colonized both in spring and autumn by *C. podicipina*, keeping their parasite populations at a relatively constant level. However, the mean abundances were statistically different between spring and autumn (U Man-Whitney= 16362 p<0.001) and it could be that the optimal instance for parasite transmission is the spring. All of crustaceans parasitized were adults. They were not recorded neither metanauplii nor juvenile brine shrimps parasitized. This feature confirm previous findings, that possibly by the size of the parasite eggs, *C. podicipina* only colonized adults of *Artemia sp.* (Redón et al. 2011). The sample of adult tapeworms collected in spring had no gravid proglottids. It could be that the infection in the grebe was recent.

Conclusion

The present study records new host and geographical localization of *C. podicipina*. Also

confirms that the life history of this hymenolepidid occurs between the brine shrimp *A. persimilis*, as the intermediate host and *P. occipitalis*, as its final host in the hypersaline Epecuén lagoon, from the Southwest of Buenos Aires province. *Confluaria podicipina* cysticercoids are present both in spring and autumn in this ecosystem and probably the transmission occurs all along the year. However both the prevalence and mean abundances are higher in spring, so this season could be the optimal moment for transmission. Tapeworms attach deeply in the crypts of Lieberkühn by the rostellum and suckers, and cause traumatic lesions in the intestinal mucosa.

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