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First report of cestode infection in the crustacean *Artemia persimilis* from Southern Chilean Patagonia and its relation with the Neotropical aquatic birds

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The brine shrimp *Artemia* Leach (Crustacea, Branchiopoda), a keystone species in extremely saline wetlands all over the world, offers an excellent model to study species interactions (parasitism) and to elucidate “hidden fauna” (avian endoparasites). The present study is the first report on the parasite infection of the South American species *Artemia persimilis* from the Southern Chilean Patagonia (50°S-53°S). Samples were collected in Los Cisnes and Amarga lagoons, the two most austral populations of this crustacean described to date, during two seasons (spring and autumn). A total of 98 larvae of cestodes of the family Hymenolepididae (Cestoda, Cyclophyllidea) were found and identified as belonging to the following taxa: *Confluarina podicipina* (adult parasitic in grebes), *Flamingolepis* sp. (a cestode parasite of flamingos), *Fimbriarioides* (?) sp. (adults of the species of this genus infect waterfowl and shorebirds) and *Wardium* sp. (definitive host unknown, most probably charadriiform birds). This is a new geographical record of *C. podicipina* and the undescribed species *Fimbriarioides* (?) sp. for the Neotropical Region, the latter being the most widely distributed species at both localities and seasons surveyed, and the only species recorded in autumn (April). Cestode community composition in the Los Cisnes population was characterised by dominance of *Flamingolepis* sp., representing more than 65% of the total cestode species recorded, whereas in the Amarga population the most abundant parasite (> 83%) was *Fimbriarioides* (?) sp. Significant seasonal variations were detected in Cisnes lagoon for *Flamingolepis* sp. and *C. podicipina*, with exclusive presence of them in spring (November). Besides providing novel information on cestodes infection in *A. persimilis*, this study provides new data on the life-cycle of cestodes of Neotropical aquatic birds such as South American flamingos and grebes. Our finding expands the knowledge on the biodiversity and population dynamics of extreme and unique environments from high latitudes (Patagonia), but makes evident the need of further taxonomical and ecological studies to better understanding the life cycle of

avian helminth parasites in the Neotropics, and to elucidate the role of aquatic invertebrates in the parasite life cycle in hypersaline wetlands from the Americas.

Title

First report of cestode infection in the crustacean *Artemia persimilis* from Southern Chilean Patagonia and its relation with the Neotropical aquatic birds

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21 Introduction

22 Conservation of biodiversity is one of the greatest challenges in this century given the
 23 accelerated rate of species loss due to global threats such as habitat degradation, biological
 24 invasions, overexploitation, and co-extinctions. Parasites are important components of
 25 biodiversity and they are exposed to the same threats as their hosts (Gómez & Nichols, 2013).
 26 However, maintenance of the parasitic biodiversity has not been traditionally considered as a
 27 priority strategy (Dunn et al., 2009) despite their fundamental role as ecological and evolutionary
 28 drivers, biomarkers of the ecology of their hosts and of ecosystems health, and the benefits they
 29 can provide for host populations (reviewed by Gómez & Nichols, 2013). Understanding the host-
 30 parasite relationships in a given ecosystem, either in terms of the host specificity, virulence,
 31 transmission pathways, or life-cycles, is essential to know and preserve global biodiversity.
 32 Parasites with complex life cycles, i.e. those that involve more than one host, which are usually
 33 part of a common food web, may be used as good indicators of the aquatic biodiversity
 34 (Hechinger & Lafferty, 2005), and can reflect the specific diet of the final host and their
 35 population dynamics.

36 Hypersaline wetlands (i.e. salinity > 50g/L; *sensu* Hammer, 1986) are natural laboratories
 37 for biodiversity key-studies due to their relatively simple trophic webs (Gajardo, Sorgeloos &
 38 Beardmore, 2006). The crustaceans of the genus *Artemia* Leach (Branchiopoda: Anostraca) have
 39 a main ecological role both as valuable food source for aquatic birds communities (Sánchez,
 40 Green & Castellanos, 2006; Varo et al., 2011) and as intermediate hosts of helminth parasites of
 41 aquatic birds such as flamingos, grebes, shorebirds, and ducks (Georgiev et al., 2005; Vasileva et
 42 al., 2009; Redón et al., 2015a). Previous studies on helminths of *Artemia* spp. from the Western
 43 Mediterranean and USA have demonstrated the participation of brine shrimps in the life cycles

of 15 cestode species of the order Cyclophyllidea van Beneden in Braun, 1900, and a nematode species of the family Acuariidae (Georgiev et al., 2005; Vasileva et al., 2009; Redón et al., 2015a).

Two species of *Artemia* have been described as native for the American continent: *A. franciscana* Kellogg widely distributed in the continent, and *A. persimilis* Piccinelli & Prosdocimi restricted to Argentina and Southern Chile. Information about their parasites is rather limited; there is just one study demonstrating the participation of *A. franciscana* in the circulation of helminth parasites in the Great Salt Lake (Utah, Western USA) (Redón et al., 2015a) while for South American *Artemia* populations parasites are almost unexplored. The aim of the present study is to fill this gap of knowledge by presenting the first parasitological study of *A. persimilis* from Southern Chilean Patagonia. The sampling sites include two of the most austral populations of *A. persimilis* described to date in Chile (Gajardo et al., 2002; De los Ríos-Escalante, 2013). The main goal of our work is to provide a first glance to the biodiversity of cestodes parasitizing the species, including seasonal effects on the dynamics of infection, and to propose a hypothesis about the potential relationships within the system “cestodes – brine shrimps – aquatic birds” of these unique salty lakes in Southern Chile, located in protected areas due to their bird biodiversity.

Material and methods

Study area and Artemia sampling

The study area comprises two saline aquatic ecosystems from the Southern Chilean Patagonia (50°-53°S), the region of Magallanes and Chilean Antarctica (Fig. 1). The Los Cisnes lagoon (53°15'S, 70°22'W) is located in Tierra del Fuego Island close to the main town Porvenir. It has

an extension of 25.3 hectares and represents an important habitat for flamingos, swans, grebes, and shorebirds. In 1982 it was declared Natural Monument to protect its high diversity of aquatic birds (around 50 species reported; CONAF, 2009; CONAF, 2014). The Amarga lagoon (50°58'S, 72°43'W) is a lagoon of 2.5 km length, 1.1 km breadth, and 2.6 m of depth (Campos et al., 1996). It is located in the province of Última Esperanza just at the entrance of Torres del Paine National Park, one of the 102 wild protected areas of Chile. The park was created in 1959 by the Ministry of Agriculture and declared Reserve of Biosphere in 1978 by UNESCO (CONAF, 2007). It harbours a great diversity and abundance of birds with around 118 species using the area as permanent residents or migratory species, including flamingos, ducks, grebes, swans, and geese (Matus & Barría, 1999; CONAF, 2007).

The local populations of *Artemia* were identified as *A. persimilis*, a species previously considered endemic to Argentina (Triantaphyllidis, Abatzopoulos & Sorgeloos, 1998) but later were discovered in the Southern Chile, first in Amarga lagoon (Gajardo et al., 1998) and few years later in Los Cisnes (De los Ríos-Escalante, 2010). Sampling was carried out on spring (November 2017) and autumn (April 2018) as a part of an expedition dedicated to a comprehensive field study along Chile. Salinity of the water were measured *in situ* with a portable refractometer ATAGO®. *Artemia* individuals were collected with a planktonic hand net (180 µm mesh size), washed with fresh water to remove salt and fixed with 70% ethanol. *Artemia* samples were transported to the laboratory and preserved at 4°C for future parasitological examination. Additional living specimens were transferred into plastic bottles of 5 l filled with lake brine and transported to the laboratory of the Genetics, Aquaculture & Biodiversity for further examination. Field work was conducted under an authorization provided

by the National Forest Corporation of Chile (CONAF), Department of Conservation of Biological Diversity, for research activities in protected wild areas (Permission N° 025/2017).

Processing brine shrimp samples and helminth identification

A total of 800 brine shrimps (200 individuals per site and season) were mounted in glycerol and examined under a compound light microscope Olympus BX50 for the presence of cestodes.

After screening, some cysticercoids (i.e. the larval stage of cestodes developing in the intermediate host) were isolated and mounted in Berlese's medium for a thorough examination of the morphology of parasite larvae. In addition to the qualitative samples, some extra *Artemia* individuals were examined in order to obtain more than one cysticercoids per species for the preparation of accurate morphological descriptions. Several *Artemia* individuals infected with one parasite species (single infection) were kept at –20°C for subsequent molecular studies.

Identification of cysticercoids was based on a comparison with the previous descriptions of larvae of cestodes using branchiopods as their intermediate hosts (Gvozdev & Maksimova, 1978; Maksimova, 1973, 1976, 1981, 1989; Georgiev et al., 2005; Vasileva et al., 2009; Redón et al., 2015a). Terminology of the cysticercoids follows Lenta Chervy (2002). The morphological observations of parasites (including the measurements and the preparations of drawings and photographs) were done using the facilities of the Division of Biodiversity and Ecology of Parasites at IBER-BAS, Sofia, Bulgaria. Metrical data are given as a range, followed by the mean and number of observations (n) in parentheses. The measurements are in micrometres unless otherwise stated. The photographs were prepared with Zeiss Axio Imager 2 light microscope equipped with differential interference contrast (DIC) and Jenoptik ProgRes® microscope camera incorporated. Pencil drawings were made with Olympus BX51 microscope

equipped with a drawing tube. The opensource graphic software, GIMP and Inkscape were used for the image processing of the illustrative material.

Representative slides with voucher specimens have been deposited at the Parasitological collection of the Natural History Museum in Geneva.

Specimens studied for each species are as follows:

Confluaria podicipina: Los Cisnes lagoon, 29 November 2017, 5 cysticercoids, isolated and mounted in Berlese's medium; MHNG-PLAT XXX XXX, a cysticercoid isolated and mounted in Berlese's medium; Amarga lagoon, 26 November 2017, 2 cysticercoids, one of them isolated and mounted in Berlese's medium.

Fimbriarioides (?) sp.: Los Cisnes lagoon, 29 November 2017, 6 cysticercoids, 17 April 2018, 7 cysticercoids; all cysticercoids mounted and measured in glycerol, subsequently 7 cysticercoids isolated and mounted in Berlese's medium; MHNG-PLAT XXX XXX, a cysticercoid isolated and mounted in Berlese's medium; Amarga lagoon, 26 November 2017, 3 cysticercoids, 27 November 2017, 2 cysticercoids, 18 April 2018, 1 cysticercoid; all cysticercoids mounted and measured in glycerol, subsequently 2 cysticercoids isolated and mounted in Berlese's medium.

Flamingolepis sp.: Los Cisnes lagoon, 29 November 2017, 14 cysticercoids, mounted and measured in glycerol; subsequently 12 cysticercoids isolated and mounted in Berlese's medium; MHNG-PLAT XXX XXX, a cysticercoid isolated and mounted in Berlese's medium.

Wardium sp.: Los Cisnes lagoon, 29 November 2017, 2 cysticercoids mounted and measured in glycerol; subsequently both cysticercoids isolated and mounted in Berlese's medium.

Parameters of infection and statistical analysis

Infection descriptors prevalence (P%), mean intensity (MI) and mean abundance (MA) for each cestode species and for the overall infection were calculated according to Bush et al., (1997). For

cestode community composition, relative abundance (RA%) of each cestode species was determined for each site and season. To analyse differences in the prevalence of infection between seasons within each location we used Z tests. Mann-Whitney U test was applied for testing temporal variations on the abundance of parasites and the intensity of infection. The same procedure was applied to analyse variations of infection parameters between the localities within the same season. Significance was assumed at $p \leq 0.05$. Statistical analyses were performed using SPSS 15.0 for Windows (SPSS Inc. Chicago, IL, USA).

Results

Systematic survey of cestode larvae

Family Hymenolepididae Perrier, 1897

***Confluaria podicipina* (Szymanski, 1905) (Figs. 2, 6A)**

Remarks

We do not provide a full redescription of the cysticercoids from *A. persimilis* in Chile because they possess similar morphological characters as previously described cysticercoids of *Confluaria podicipina* (Maksimova, 1981; Georgiev et al., 2005; Redón et al., 2015a), i.e. the presence of an external envelope formed by the anterior part of cercomer (Figs. 2A, B); invaginable rostellum armed with 10 aploparaksoïd hooks (Figs. 2 C, D; 6A); a very long and coiled cercomer, densely packed in thin membranous envelope (Fig. 2A). The measurements taken from isolated cysticercoids mounted in Berlese's medium also correspond well to the previous data from *A. parthenogenetica* in Spain (Georgiev et al., 2005) and *A. franciscana* in USA (Redón et al., 2015a) (see Table 1). Instead, the external capsule of the cysticercoids, which are smaller in the specimens from Chile. Our results revealed less variations in the total size of

the external capsule in the samples of *C. podicipina* from *A. persimilis* in comparison to the samples from USA and Spain, where we found cysticercoids of various sizes, including some specimens with intermediate measurements (Redón et al., 2015a). Maybe this difference is due to the smaller populations of the parasite and its definitive hosts at the two sampling sites in Southern Chile comparing to the populations of *C. podicipina* and *P. nigricollis* Brehm at the Great Salt Lake and the Odiel marshes. Despite the differences in the external capsule, the morphology of the cysticercoids, the shape of their rostellar hooks and the remaining metrical data are similar. On this basis, we identify these cysticercoids from *A. persimilis* as *C. podicipina*.

The species of the genus *Confluaria* Ablasov in Spasskaya, 1966 are specific parasites of grebes (Podicipediformes) recorded mostly from the Holarctic region (Vasileva, Georgiev & Tenov, 1999a; Vasileva, Georgiev & Tenov, 1999b; Vasileva, Georgiev & Genov 2000; Vasileva, Korniyushin & Genov, 2001; Vasileva, Skirnisson & Georgiev, 2008; Sitko & Heneberg, 2015). In South America, there are only two records of *Confluaria* spp., e.g. *C. capillaris* (Rudolphi, 1810) in *Tachybaptus dominicus* (L.) in Brazil (Fuhrmann, 1906; Vasileva, Georgiev & Genov, 1999a) and “*Confluaria* sp.” reported from the Southern silvery grebe *Podiceps occipitalis* Garnot in the central Chile (González-Acuña et al. 2017). Until now, *Confluaria podicipina* has only been recorded in the Holarctic Region (Vasileva, Georgiev & Genov, 2000; Stock & Holmes, 1987). In North America, as definitive hosts of *C. podicipina* have been reported *Podiceps auritus* (L.) and *P. nigricollis* from Alberta (Canada) (Stock & Holmes, 1987) and its confirmed naturally infected intermediate host is *A. franciscana* (Redón et al. 2015a).

The present study is the first report of this species in the Neotropic Region and might be

associated to grebes which distribution comprises the Southern Patagonia. These are the Silvery grebe *Podiceps occipitalis*, the Hooded Grebe *Podiceps gallardoi* Rumboll, the White-tufted grebe *Rollandia rolland* (Quoy & Gaimard), the Pied-billed grebe *Podylimbus podiceps* (L.), and the Great grebe *Podiceps major* (Boddaert) (Sylvester, 1995; Storer, 2000; CONAF, 2014).

***Fimbriarioides* Fuhrmann, 1932**

***Fimbriarioides* (?) sp. (Figs. 3, 6B, E)**

Description of cysticercoids (metrical data based on specimens mounted in Berlese's medium):

Diplocysticercoïd. Outer envelope thin-walled, formed by packed cercomer, usually encircling internal cyst, often with irregular shape (Figs. 3B, 6E). Internal cyst lemon-shaped, thick-walled. Scolex almost oval, $195\text{--}210 \times 163\text{--}195$ (203×170 , $n = 5$), with short apical protrusion. Suckers oval, muscular, unarmed; diameter $45\text{--}65$ (56 , $n = 11$). Rhynchus thick-walled, with well-visible radial musculature (Fig. 6E). Rostellar sheath sac-like, $95\text{--}100 \times 53$ (98×53 , $n = 2$); thin-walled (Fig. 6B). Rostellum invaginable, cup-like, muscular, with apical invagination. Rostellar hooks diorchoid-like, 10 in number. Each hook with long straight, long handle, with flattened, spatulate posterior end (Figs. 3C, D, 6B); blade sickle-shaped, slightly shorter than handle; guard short, with slightly bifurcated thickening (Figs. 3C, 6B). Measurements of rostellar hooks: total length $32\text{--}35$ (33 , $n = 10$), length of blade $13\text{--}15$ (14 , $n = 10$), length of handle $16\text{--}20$ (17 , $n = 10$), length of guard $3\text{--}4$ (4 , $n = 10$). Calcareous corpuscles concentrated anterior to scolex. Cercomer thick, long, usually forming compact additional thin-walled envelope surrounding cyst; unpacked cercomer thin-walled (Fig. 3A).

Additional measurements based on specimens in temporary glycerol mounts: diplocyst with outer envelope $200\text{--}315 \times 155\text{--}265$ (254×209 , $n = 6$); internal cyst $175\text{--}215 \times 130\text{--}155$ (192×151 , $n = 13$); scolex $118\text{--}130 \times 98\text{--}125$ (124×112 , $n = 9$); diameter of suckers $38\text{--}53$ (46 , $n =$

204 21); rostellar sheath $75-108 \times 45-60$ (99×52 , $n = 7$), rostellum $45-58 \times 33-48$ (49×41 , $n = 8$);
 205 longest fragment of cercomer 470×88 .

206

207 *Remarks*

208 The overview of the valid avian genera of Hymenolepididae revealed 15 genera with 10
 209 diorchoid rostellar hooks (see Czaplinski in Czaplinski & Vaucher, 1994; Mariaux et al., 2017).
 210 Among them three genera possess invaginable rostellum (for the generic morphology see
 211 Czaplinski & Vaucher, 1994), i.e. *Fimbriaria* Fröhlich, 1802, *Fimbriarioides* Fuhrmann, 1932
 212 and *Fimbriasacculus* Alexander & McLaughlin, 1996. They belong to the subfamily
 213 Fimbriariinae Wolffhügel, 1898 and include parasites of aquatic birds (mainly Anseriformes and
 214 Charadriiformes), accidentally Galliformes (Spasskaya, 1966; Czaplinski in Czaplinski &
 215 Vaucher, 1994).

216 The hooks of the cysticercoids from *A. persimilis* in Chile characterise with sickle-shaped
 217 blade, slightly shorter than the handle, a spatulate posterior end of handle and a slightly
 218 bifurcated thickening of the guard. This shape differs from the hooks of *Fimbriaria* spp. which
 219 have blades much shorter than handle and guard is not bifid; a spatulate handle has been
 220 illustrated only for *F. czaplinski* Grytner-Ziecina, 1994 (see fig. 4 of Cielecka et al., 2011). In
 221 addition, the data about the life-cycle of *Fimbriaria* spp. indicated that these species have
 222 freshwater life-cycles which involved various groups of crustaceans as intermediate hosts, i.e.
 223 mainly Copepoda, Ostracoda and Amphipoda (Jarecka, 1958; Spasskaya, 1966).

224 Most closely the hooks of the cysticercoids from *A. persimilis* resemble those of the
 225 species of *Fimbriarioides* (see Webster, 1943; Maksimova, 1976, 1989) and *Fimbriasacculus*
 226 (see Alexander & McLaughlin, 1996). Webster (1943) reported the presence of “long, bifid

guard” in *Fimbriarioides haematopodis* Webster, 1943 and *F. lintoni* Webster, 1943. Maksimova (1989) also mentioned a peculiar structure of the rostellar hooks of *Fimbriarioides spasskyi* Maksimova, 1989 in which the blade and the guard form a “clamp-like” anterior part of hook. A spatulate posterior end of the handle has been illustrated for two species of *Fimbriarioides*, i.e. *F. tadornae* Maksimova, 1976 (see Vasileva et al., 2009) and *F. spasskyi*, and for *Fimbriasacculus africanensis* Alexander & McLaughlin, 1996 described from three species of Anatinae in South Africa (see fig. 5 of Alexander & McLaughlin, 1996). Unfortunately, the generic differentiation between *Fimbriarioides* and *Fimbriasacculus* is based mainly on the strobilar morphology which is not applicable to our study. There are no published records of species belonging to these genera from waterfowl in South America. By this reason, the definitive generic identification of our larvae could not be proposed. We prefer to keep the identification of cysticercoids from *A. persimilis* as “*Fimbriarioides* (?) sp.” by two reasons: i) the hooks of *Fimbriasacculus* have no bifurcation of the guard; ii) the previous data on the life-cycles of *Fimbriarioides* spp. confirmed the role of branchiopods as their intermediate hosts (Maksimova, 1976; Vasileva et al., 2009). Another reason could be the fact that a similar type of cysticercoïd, i.e. “diplocysticercoïd” (Lenta Chervy, 2002) has been recorded for *Fimbriarioides intermedia*. Belopolskaya (1953) described the cysticercoïd of this species isolated from naturally infected *Semibalanus balanoides* (L.) (Cirripedia) in Russia. The author mentioned that the cysticercoïd has a very long cercomer (1.75-2.1 mm length) which is usually surrounding the cysticercoïd forming an oval outer cyst. The diplocysticercoïds have been also described for other hymenolepidid genera parasitising in aquatic bird, i.e. *Aploparaksis* Clerc, 1903 and *Wardium* Mayhew, 1925 (see Bondarenko & Kontrimavichus, 2006).

The present finding is the first geographical record of a species of *Fimbriarioides* in

Chile and in South America. The list of possible definitive hosts of *Fimbriarioides* (?) sp. in Los Cisnes and the Amarga lagoons includes the representatives of Anseriformes and Charadriiformes, i.e. the shelducks (Tadorninae) *Chloephaga rubidiceps* Sclater and *C. picta* (Gmelin); dabbling ducks (Anatinae) *Specularias specularis* (King), *Anas georgica* Gmelin, *Lophonetta specularioides* (King), *Tachyeres patachonicus* (King), *Mareca sibilatrix* Poeppig; as well as some charadriiform birds as *Haematopus leucopodus* Garnot, recorded at Los Cisnes and the Torres del Paine National Park (Garay, Johnson & Franklin, 1991; Matus & Barria, 1999).

***Flamingolepis* sp. (Figs. 4, 6C, F)**

Description of cysticeroids (metrical data based on specimens mounted in Berlese's medium, for some measurements see Table 2): Cercocysticeroid. Cyst elongate oval, with very thick wall, consisting of several layers; outer layer thick, with fine radial striations (Figs. 4A, 6F); numerous calcareous corpuscles situated in anterior part of cysticeroid. Scolex with conical apical protrusion and maximum width at level of suckers. Suckers unarmed, elliptical, with well-developed musculature. Rhynchus unarmed, thin-walled. Rostellar sheath thick-walled, 125-133 \times 33-38 (130 \times 36, n = 3), passing far beyond posterior margins of suckers; glandular cells presented in its cavity (Fig. 6F). Rostellum retractile, highly elongated, apical part with conical protrusion, posterior end usually coiled near bottom of rostellar sheath; walls thick, consisting of strong radial musculature (Figs. 4B, D, 6F); cavity with glandular cells. Rostellar hooks 8, skrjabinoid; each hook with long handle and very short guard, blade as long as handle or slightly longer, with smooth curvature; length of handle 21-24 (24, n = 15) (Figs. 4E, 6C). Cercomer considerably thick, coiled; cysticeroid with complete cercomer not available

Additional measurements based on specimens in temporary glycerol mounts: Measurements of

274 cyst 158-170 × 95-123 (164 × 111, n = 5); thickness of wall 7-12 (10, n = 5); scolex 118-125 ×
275 73-96 (123 × 88, n = 5); diameter of suckers 43-50 (47, n = 16); rostellum 123-125 × 25-28 (125
276 × 26, n = 5); rostellar sheath 118-130 × 30-38 (123 × 35, n = 5); longest fragment of cercomer
277 40-75 wide 600 long.

278

279 *Remarks*

280 According to the latest taxonomic revision of the family Hymenolepididae only two genera
281 possessing eight skrjabinoid rostellar hooks have been considered valid, i.e. *Cladogynia* Baer,
282 1938 and *Sobolevicanthus* Spasskii & Spasskaya, 1954 (Czaplinski & Vaucher, 1994). The
283 concept of Czaplinski & Vaucher (1994) postulated numerous new generic synonymies in these
284 two genera, based on very few morphological criteria (for example number and shape of hooks,
285 presence of stylet) and ignored numerous distinguishing morphological and life-cycle
286 characteristics, which have been used to distinguish a number of well-defined avian
287 hymenolepidid genera. Due to these reasons most of the proposed synonymies are not widely
288 accepted and the genera as *Pararetinometra* Stock & Holmes, 1982, *Retinometra* Spasskii, 1955
289 and *Flamingolepis* Spasskii & Spasskaya, 1954 are still considered valid (see Vasileva, Georgiev
290 & Genov, 1996; Mariaux et al., 2017).

291 Judging on the presence of 8 skrjabinoid hooks, the extremely long, retractile muscular
292 rostellum, the cysticercoids from *A. persimilis* in Chile most closely resemble species of the
293 genus *Flamingolepis*. Some other hymenolepidid genera parasitising aquatic birds have also
294 similar characters, i.e. *Cladogynia*, *Retinometra*, *Sobolevicanthus* and *Calixolepis* Macko &
295 Hanzelova, 1997. However, there are differences in the shape of hooks of the species from this
296 group despite that all of them have been assigned to “skrjabinoid type”. Comparing to the hooks

of cysticercoids from Chile, the type species of *Cladogynia*, *C. phoeniconaiadis* (Hudson, 1934), has much bigger hooks with very long and thick base and much shorter blade (see Baer, 1938). The majority of the species of the remaining three genera have hooks with a different proportion of their parts, i.e. the blades are much longer than the handles (see Spasskaya, 1966; Maksimova, 1989). Moreover, the data about the life-cycles of species of *Retionometra* and *Sobolevicanthus* indicated the role of freshwater crustaceans from Copepoda, Ostracoda and Amphipoda as their intermediate hosts (Jarecka, 1960; Maksimova, 1989). *Flamingolepis* is the only hymenolepidid genus with 8 skrjabinoid hooks which have been proven to use Branchiopoda as intermediate hosts in the life-cycles of its species. The definitive hosts are various species of Phoenicopteriformes. On this basis we consider the cysticercoids from *A. persimilis* as belonging to *Flamingolepis*.

Cysticercoids of five species of *Flamingolepis* have been recorded so far from naturally infected *Artemia* spp. (see Table 2). Judging on the length of hooks (45-50 μm) the cysticercoids from the present material are most similar to the cysticercoids of *F. flamingo* (Skrjabin, 1914) and *F. tengizi* Gvozdev & Maksimova, 1968, both species are parasites of flamingos in Eurasia (Maksimova, 1989). *F. flamingo* has been recorded from *A. salina* and *A. parthenogenetica* in France (Robert and Gabrion, 1991; Sánchez et al. 2012) and Spain (Georgiev et al., 2005; Georgiev et al., 2007; Redón et al., 2011; Redón et al., 2015b; Sánchez et al., 2013). The cysticercoids from *A. persimilis* differ from *F. flamingo* by the bigger cysticercoid and the bigger scolex (see Table 2). The scolex of the cysticercoid is highly elongated and much thinner in *F. flamingo*, i.e. 8–12 μm wide (Georgiev et al., 2005) versus 40–75 μm in *Flamingolepis* sp. from Chile. The cysticercoids of *F. tengizi* were described from two different intermediate hosts in Kazakhstan, i.e. *A. salina* (Maksimova, 1973) (for metrical data see Table 2) and from a species

of Ostracoda *Eucypris inflata* (Sars) (Gvozdev & Maksimova, 1978). The cysticercoïd of *F. tengizi* described from *A. salina* differs from *Flamingolepis* sp. from Chile by the bigger cyst with thicker envelopes, the slightly bigger hooks with their blades comprising more than 55% of the total length of the hook. The cysticercoïds of the remaining three species of *Flamingolepis* differ significantly from the present material by the length of the hooks.

In addition to the above mentioned species which use brine shrimps in their life cycles, *Flamingolepis* includes two more species. *Flamingolepis megalorchis* (Lühe, 1898) uses Ostracoda as its intermediate hosts and has been characterised with bigger hooks compared with the present material (68–85 µm long, see Gvozdev & Maksimova, 1978). Another species is *F. chileno* Babero, Cattán & Jensen, 1981, described from *Phoenicoparrus andinus* (Philippi) in Chile, the only record of *Flamingolepis* from South America. The species characterises with much bigger hooks (163–189 µm) of a very different shape compared with cysticercoïds from *A. persimilis* and obviously the present material does not belong to this species.

On the basis of the above mentioned discussion, we consider that the cysticercoïds from *A. persimilis* do not fit the morphological and metrical characters of no one of the described *Flamingolepis* spp. Most probably it belongs to an undescribed species parasitizing flamingos in South America. Its identification needs an examination of adult specimens from their most probable definitive host, i.e. Chilean flamingo *Phoenicopterus chilensis* Molina which is most abundant in saline lagoons from Central Argentina and southern Chilean Patagonia, mainly concentrated between latitudes 51°– 53°S (Battauz et al., 2013; Gibbson, Vilina & Cárcamo, 2007), corresponding to the area of our study. The remaining two species of flamingos, the Andean flamingo *Phoenicoparrus andinus* and the James' flamingo *Phoenicoparrus jamesi* (Sclater) live in the high altitudes of Andean plateaus of Peru, Chile, Bolivia, and Argentina.

343

344 ***Wardium* sp.** (Fig. 5, 6D)

345 *Description of cysticercoids* (metrical data based on specimens mounted in Berlese's medium):

346 Cercocysticercoid. Cyst lemon-shaped, thick-walled, $300\text{--}315 \times 215\text{--}225$ ($n = 2$). Scolex oval,
347 $150\text{--}195 \times 118$ ($n = 2$), with maximum width at level of suckers (Fig. 5A). Suckers oval,
348 unarmed, muscular, with diameter $43\text{--}65$ (51 , $n = 6$). Rhynchus short. Rostellar sheath deep, thin-
349 walled, passing beyond level of posterior margins of suckers; 125×45 ($n = 2$). Rostellum
350 invaginable, thick-walled, with apical enlargement and conically-tapering posterior part, $55\text{--}75 \times$
351 $45\text{--}50$ ($n = 2$) (Fig. 5A). Rostellar hooks aploparaksoid-like, 10 in number (Figs. 5C, 6D); blade
352 sickle-shaped, slightly longer than guard; handle distinct, but very short, guard thick; base of
353 hook comprised by well-developed epiphyseal thickening; total length of hooks $25\text{--}26$ (25 , $n =$
354 4), length of blade $12\text{--}13$ (12 , $n = 4$), length of base $18\text{--}19$ (18 , $n = 4$), distance between handle-
355 tip and guard-tip $9\text{--}10$ (10 , $n = 4$). Calcareous corpuscles mostly concentrated in anterior part of
356 the cyst. Cercomer 125 thick, length of longest fragment 1.4 mm. Cysticercoid with complete
357 cercomer not available.

358 *Additional measurements based on specimens mounted in temporary glycerol mounts:*

359 Measurements of cyst $300\text{--}312 \times 202\text{--}205$ ($n = 2$); scolex 150×120 ($n = 1$); suckers $45\text{--}64$ (51 , n
360 $= 6$); rostellar sheath 113×48 ($n = 1$); rostellum 50×43 ($n = 1$).

361

362 *Remarks*

363 The present material corresponds mostly to the cysticercoids of the genus *Wardium* described
364 previously in *A. franciscana* in Great Salt Lake, USA (Redón et al. 2015a). The shape and the
365 measurements of the cyst, the suckers and rostellum are very similar. The rostellar hooks possess
366 the same shape and epiphyseal thickening. The lengths of the hooks are also similar, i.e. $25\text{--}26$

μm in cysticercoids from *A. persimilis* vs. 24-26 μm in *Wardium* sp. from *A. franciscana*. Based on these similarities we identify the cysticercoids from the present material as *Wardium* sp. and consider it as conspecific to the specimens from *A. franciscana* in USA. The exact identification of the species is difficult because of the small number of cysticercoids, e.g. two larvae per each of the localities (USA and Chile). The small sample makes difficult to obtain a representative range of the measurements and the intraspecific variability. Nevertheless, the detailed comparison of the rostellar hooks of the cysticercoids of *Wardium* sp. with the known species of *Wardium* Mayhew, 1925 and *Branchiopodataenia* Bondarenko & Kontrimavichus, 2004 revealed that none of the species of these two genera can be matched with the hooks of the cysticercoids of *Wardium* sp. (see Redón et al. 2015a).

Several species of *Wardium* and *Branchiopodataenia* have been recorded so far in South America. However, no one of them has rostellar hooks of the shape and the length similar to these of the cysticercoids from Chile. These species are: *Branchiopodataenia arctowskii* (Jarecka & Ostas, 1984) from *L. dominicanus* from the King George Island (Antarctica) (the hooks are 14-20 μm long with elongate handle, but without epiphyseal thickening, see Bondarenko & Kontrimavichus, 2004); *W. neotropica* Deblock & Vaucher, 1997 from *Himantopus melanurus* in Paraguay (hooks are 9 μm long) (see Bondarenko & Kontrimavichus, 2006); *W. paucispinosum* Labriola & Suriano, 2000 described from *L. maculipennis* in Argentina (hooks are 13-18 μm long, see Labriola & Suriano, 2000); and *W. tinamoui* (Olsen, 1970) from *Nothoprocta perdicaria* in Chile (16.6 μm long, see Bondarenko & Kontrimavichus, 2006). *Wardium semiductilis* (Szidat, 1964) recorded from *L. maculipennis* and *L. dominicanus* from Argentina (Szidat, 1964) has hooks of completely different shape (assuming diorchoid, see Szidat, 1964) and a different shape of scolex, possessing a very long rhynchus.

The definitive hosts of the species of *Wardium* and *Branchiopoddataenia* include aquatic birds mainly of the order Charadriiformes (Spasskaya, 1966; Bondarenko & Kontrimavichus, 2006). The list of potential hosts at Southern Chile includes many species of this order, as *Pluvianellus socialis* Gray, *Charadrius falklandicus* Latham, *C. modestus* Lichtenstein, *Vanellus chilensis* (Molina), *Haematopus leucopodus*, *Larus dominicanus* Lichtenstein, etc. (Garay, Johnson & Franklin, 1991; Matus & Barría, 1999). In addition, some of the migration charadriiform birds have areas including both North America and South America as *Limosa haemastica* (L.) (Lepage, 2019).

Spatial and temporal variations of cestode infections

Among 800 brine shrimp individuals examined 92 were infected by cestodes ($P = 11.5\%$). A total of 98 cestode larvae were identified in *A. persimilis* from the two localities in the Southern Chile with the majority of them ($> 87\%$) found at Los Cisnes lagoon (see Table 3). The cysticeroids were identified as belonging to four species from four genera of the family Hymenolepididae. Two of the four species, *Fimbriarioides* (?) sp. and *C. podicipina*, were recorded in both localities. The other two, *Flamingolepis* sp. and *Wardium* sp., were found in Los Cisnes lagoon only. The total intensity ranged from 1 to 3 cysticeroids; 96.3% of the infected individuals harboured just one cysticeroid (single infections). Multiple infections has been also detected in 4 individuals, all of them infected with *Flamingolepis* sp.

The cestode community in Los Cisnes lagoon was dominated by the parasite of flamingos, *Flamingolepis* sp. (RA = 65.1%), followed by *Fimbriarioides* (?) sp. (Table 3). The later species was the most abundant in the cestode community at Amarga lagoon, (RA = 83.3%). The overall cestode infection was much higher at Los Cisnes lagoon.

Variations of the cestode community were also detected between seasons at both localities (Table 4). Higher values of the overall cestode infection parameters were detected in spring (November) than in autumn (April) in Los Cisnes lagoon ($P=34.5\%$ versus 6% ; $p=0.000$; $MA=0.37 \pm 0.04$ versus 0.06 ± 0.02 , $p=0.000$). Seasonal effect on the infection dynamics of *C. podicipina* and *Flamingolepis* sp. was also found, registering an exclusive presence of these two parasites in spring (Table 4). On the contrary, for *Fimbriarioides* (?) sp., the most widely distributed parasite recorded in samples from both sites and both seasons, the highest parameters of infection were registered in autumn, although no significant differences between seasons were detected for the prevalence ($p=0.252$) either the abundance of this species ($p=0.347$). No seasonal differences were detected for the intensity of the overall infection ($p>0.05$), either for intensity of infection of any cestode species in Los Cisnes lagoon or Amarga lagoon.

Discussion

Species composition of cestode larvae in relation to the avian communities

The present study provides the first evidence that *A. persimilis* participates in the life cycles of four cestode species from the family Hymenolepididae. These include a specific parasite of grebes *C. podicipina*, an unidentified cestode species of flamingos *Flamingolepis* sp., and two unidentified species that could be parasites of ducks or charadriiform birds (gulls or shorebirds) (i.e. *Fimbriarioides* (?) sp. and *Wardium* sp.).

Previous studies in the Western Mediterranean and in North America have revealed that the prevalence of avian cestodes in brine shrimps may depend on the seasonal presence of definitive hosts at certain hypersaline wetlands (Georgiev et al. 2005, Georgiev et al., 2014; Sánchez et al. 2013; Redón et al., 2015a). The differences between the parasite community of

brine shrimp populations from Los Cisnes and Amarga indicate that specific characteristics of the habitat and the differences in the aquatic birds community may affect the parasite community in the crustacean intermediate host *A. persimilis*. Unfortunately, the information on the population dynamics of aquatic birds at both sampling sites in the Southern Chile is very limited.

The Natural Monument Los Cisnes lagoon is an area with a great variety of habitats. Originally this wetland comprised seven islets which were used by aquatic birds for nesting. Because of the decrease of the water level, actually the system is formed by the lagoon with a peninsula connected with the land, and several temporally shallow water bodies and muds areas used mainly as feeding places for charadriiform birds such as White-rumped sandpiper *Calidris fuscicollis*, Baird's sandpiper *C. bairdii*, Two-banded plover *Charadrius falklandicus*, Rufous-chested plover *C. modestus*, and the Magellanic Plover *Pluvianellus socialis*. It is frequent to observe *Podiceps gallardoi* and *Phoenicopterus chilensis* in the lagoon, particularly in winter and spring time (CONAF, 2014; Roesler, 2015). The Amarga lagoon is a shallow saline lake situated close to the eastern border of the Torres del Paine National Park. Among the most abundant birds year-round are waterfowl like the Black-neck swan *Cygnus melancoryphus*, Coscoroba swan *Coscoroba coscoroba* and Upland goose *Chloephaga picta*, several species of dabbling ducks, the grebes *Rollandia roland* and *Podiceps occipitalis* (Garay, Johnson & Franklin, 1991). Among the most abundant birds at Amarga lagoon is also *Phoenicopterus chilensis* especially in spring (Campos et al. 1996; Matus & Barría, 1999). These data correspond to the species composition of cestode parasites in *A. persimilis*. The most abundant species at Los Cisnes lagoon are *Flamingolepis* sp., the parasite of flamingos, and *Fimbriarioides* (?) sp., which potential definitive hosts could be a species of shelducks (Tadorninae) or dabbling ducks (Anatinae). Dietary studies of flamingos in South America confirmed that most probably the

459 definitive host of *Flamingolepis* sp. is the Chilean flamingo, which diet includes mainly brine
 460 shrimps (Hulbert, López & Keith, 1984); in contrast, the Andean flamingo *Phoenicoparrus*
 461 *andinus* is primarily herbivorous feeding with microalgae such as *Surirella* (Tobar et al., 2012).
 462 The specific parasite of grebes *C. podicipina* was presented in both sampling sites. However,
 463 comparing with the previous studies and the infection descriptors of *C. podicipina* from *A.*
 464 *parthenogenetica* in Spain (Georgiev et al., 2005) and from *A. franciscana* in USA (Redón et al.,
 465 2015a), the prevalence, the mean abundance and the mean intensity of *C. podicipina* from *A.*
 466 *persimilis* are lower. As for example, the prevalence in *A. persimilis* from Los Cisnes is 2.5%
 467 versus 28.7% in a population from *A. parthenogenetica* from Spain (Redón et al., 2011) and
 468 varying from 27.4% to 40.6% in *A. franciscana* from the Great Salt Lake (Redón et al., 2015a).
 469 Most probably, the difference is due to the host-specificity of the parasite and the different host
 470 species, i.e. *P. nigricollis* in the Holarctic and most probably *P. occipitalis* or *P. gallardoi* in the
 471 Neotropic Region. Another reason could be the great differences in the abundance of the grebes
 472 populations at the sampling sites which reflected the prevalence of the cestode larvae (Sánchez et
 473 al., 2013). The second largest staging population of the eared grebe *Podiceps nigricollis* in North
 474 America (over 1 million grebes) is nesting at the Great Salt Lake (Redón et al., 2015a). The
 475 studies on the comparative anatomy, the diet and the ecology of Podicipedidae in the latest
 476 century confirmed that the grebes possess evolutionary established adaptations to avoid the
 477 interspecific competition and the food overlap, especially when the species live sympatrically
 478 (Fjeldså, 1983; Storer, 2000). The structure of the bill is a very important character related to the
 479 diet of grebes and the possibility to ingest the potential intermediate hosts of cestodes.
 480 Concerning this character, the Neotropic grebe species are highly specialised (Storer, 2000), i.e.
 481 *Podylimbus podiceps* is adapted for feeding on crabs, crayfish and other hard-bodied animals;

Rollandia rolland is considered as a generalist, feeding on oligochaets, crustaceans, fish, and molluscs; *Podiceps major* is a fish-eating species. The Hooded grebe *Podiceps gallardoi* is feeding on rather big amphipods, leaches and especially snails; its bill is not much longer than that of the Silvery grebe, but considerably thicker (Fjelds , 1983). Despite the similarities between the Hooded and the Silvery grebe *P. occipitalis*, the later species has a different diet, feeding on small invertebrates, freshwater and saltwater crustaceans, including *Artemia* (Wetmore, 1926; Storer, 2000). *P. occipitalis* has a unique mechanism to swallow the prey. After the prey is captured, the bird is pressing the large fleshy tongue against the roof of the mouth, squeezing water from around the prey before it is swallowed (Fjelds , 1981). It is interesting that the same mechanism of feeding, which is necessary to minimise the salt intake in the hypersaline environments, has been confirmed for the Holarctic relative of the Silvery grebe, *P. nigricollis* (Mahony & Jehl, 1985), one of the typical definitive hosts of *C. podicipina*. On the basis of these similarities and the diet specialisation, it could be expected that the most probable definitive host of *C. podicipina* in the Southern Patagonia is the Silvery grebe *P. occipitalis*. The most reliable confirmation of this hypothesis could be done after some further examination of adult cestodes obtained from the same locality.

The low prevalence of the parasite of charadriiform birds (*Wardium* sp.) and the lack of cysticercoids from the family Dilepididae were unexpected. With the diversity of shores and shallow water bodies at both sampling sites and with the variety of shorebirds inhabiting them (Garay, Johnson & Franklin, 1991; Matus & Bar a, 1999) it can be expected that *A. persimilis* could play a role of an intermediate host for more cestode species, especially dilepidids, parasitic in shorebirds and gulls (Georgiev et al., 2005).

Spatial and temporal variations of cestode communities and bird communities

The present study revealed both spatial and temporal variations in prevalence and mean abundance of two species: *Flamingolepis* sp. and *C. podicipina* (Table 4), which influenced temporal and spatial variations of the overall cestode infection in *A. persimilis* at Southern Chile. The seasonal abundance of *Flamingolepis* sp. in spring samples from Los Cisnes lagoon may be explained with the abundance of flamingos, which are one of the main *Artemia* predators in Patagonian hypersaline wetlands (Campos et al., 1996). It is also considered as a common summer resident at Torres del Paine National Park (Garay, Johnson & Franklin, 1991). *C. podicipina* was recorded only in spring at both localities, indicating a seasonal influence on the infection dynamics of this species and suggesting the presence of grebes in a determined period. The Hooded grebe *P. gallardoi* has been reported in Los Cisnes lagoon (CONAF, 2014) and Torres del Paine National Park especially in winter and spring (Donoso et al., 2015); although it has been considered as occasionally appearing species in low numbers and perhaps only during migration to the Atlantic Coast (Roesler, 2015). The White-tufted grebe *Rollandia rolland* and the Great grebe *Podiceps major* were considered to be year-round residents at the sampling sites (Garay, Johnson & Franklin, 1991). However, they cannot be considered as most possible definitive hosts of *C. podicipina* due to their more generalist diet including a variety of freshwater invertebrates, molluscs and fish (Storer, 2000). The most probable definitive host the Silvery grebe *Podiceps occipitalis*, is also resident at the two localities but it migrates to the Atlantic Coast and to Paraguay in non-breeding season (Matus & Barría, 1999), which might explain the lack of records of cysticeroids of *C. podicipina* in the autumn samples.

Cysticeroids of *Fimbriarioides* (?) sp. were recorded in both localities and seasons and its infection parameters did not demonstrate apparent seasonal trends (Table 4). This could be explained by the presence of waterfowl during the entire annual cycle at both sampling sites,

especially the shelducks *Cloephaga picta* and *C. poliocephala*, the dabbling ducks and some species of shorebirds (for example *Haematopus leucopodus*) which are present almost all year at Los Cisnes and the Torres del Paine National Park (Garay, Johnson & Franklin, 1991; Matus & Barria, 1999).

The limited amount of information on population and seasonal dynamics of aquatic birds at both sites demands further investigations for a more comprehensive knowledge of host-parasite interactions in the “cestode-*Artemia*-aquatic birds” system in the hypersaline habitats at the austral part of the Chile.

Contribution to the cestode fauna of the Neotropic aquatic birds

Previous surveys on helminth parasites from aquatic birds in Chile are scarce with a few studies from the last decades including parasites of gulls (*Larus dominicanus* and *L. maculipennis*), cormorants (*Phalacrocorax olivaceus*), grebes (*Podiceps occipitalis*, *P. major* and *P. gallardoi*), and swans (*Cygnus melanocoryphus*) (see Torres et al., 1991, 1993; González-Acuña et al., 2009, 2010, 2017; Núñez et al. 2017b). The available data on the parasitofauna associated to Chilean flamingo is very limited; there are two reports of a nematode species found in two captive animals of the San Francisco Zoological Garden (Fox et al., 1974) and two more nematodes from Central Argentina (Núñez et al., 2017a). The data about cestodes from the aquatic birds in Chile are highly limited mainly to a few cestode taxa recorded in several grebe species (see Storer, 2000 for a survey). The Neotropic Region has been considered as not sufficiently studied area in terms of the diversity of the cyclophyllidean cestodes of birds (Mariaux et al., 2017). In this sense, our results are an important contribution to the biodiversity of cestodes of aquatic birds in the region, providing the first geographical records of *C.*

podicipina and a species of *Fimbriarioides* for the Neotropics. Our observations on *Flamingolepis* sp. from *A. persimilis* also assumed that it belongs to a new undescribed species of this genus, most probably a specific parasite of the Chilean flamingo. In addition, the present work shows that hypersaline wetlands and their most conspicuous inhabitant (*Artemia*) are an excellent model system for parasitological studies and a useful tool to evaluate and monitor aquatic biodiversity (including hosts and parasites). The Magallanean and Chilean Antarctica region is a pristine area whose salty wetlands provide habitat for a great diversity of aquatic birds, thus we could expect further to increase the knowledge on parasites using various groups of the crustaceans as their intermediate hosts. Long-term monitoring of *Artemia* will provide more data on cestode diversity of austral aquatic birds and probably new species to science.

Conclusions

The present study provides the first evidence that *A. persimilis* play an important role in the life cycles of at least four cestode species from the family Hymenolepididae, i.e. a specific parasite of grebes and flamingos, and two unidentified species most probably parasitising ducks or charadriiform birds (gulls or shorebirds). Despite the lack of data on the adult cestodes, our finding represents the first data of the presence of a specific cestode parasite of flamingo (*Flamingolepis* sp.) transmitted via brine shrimps in hypersaline ecosystems of the Southern Chilean Patagonia. A more comprehensive research of wildlife parasite biodiversity of aquatic birds in South America is needed for a better knowledge of the parasite life-cycles and the factors affecting the host-parasite interactions. Understanding the parasitofauna associated to *Artemia* is relevant in order to elucidate environmental factors affecting aquatic bird populations, and particularly essential for species with conservation problems such as the Chilean flamingo and the Hooded grebe (CR, critically endangered; IUCN Red List of Threatened Species). The

role of anostracans as prey for birds evidenced its importance as bottom-up determinants of the waterbird communities in salty wetlands (e.g. Verkuil et al., 2003; Sánchez, Green & Castellanos, 2006; Horváth et al., 2013). In what extent and in which manner variations of food item (in terms of composition and availability) can be structuring parasites communities, particularly those trophically transmitted as cestodes, and their potential relation to the distribution patterns of the avian populations are interesting questions to be further explored in order to shed light on the species interactions (parasitism) and trophic ecology of hypersaline wetlands.

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Table 1 (on next page)

Metrical data of cysticercoids of *Confluaria podicipina* (Szymanski, 1905) recorded in *Artemia* spp. from various localities.

All measurements in μm .

Host		<i>A. parthenogenetica</i>			<i>A. franciscana</i>			<i>A. persimilis</i>		
Locality		Odiel marshes (Spain)			Great Salt Lake (USA)			Los Cisnes lagoon and Amarga lagoon (Chile)		
References		Georgiev et al. (2005)			Redón et al. (2015a)			Present study		
		Range	Mean	n	Range	Mean	n	Range	Mean	n
External	length	195-255	215	9	144-313	212	15	113-185	135	5
capsule	width	135-204	152	9	72-192	118	15	73-110	87	5
Cyst	length	93-147	121	12	72-143	106	17	80-100	90	7
	width	47-87	73	12	36-89	61	17	43-55	49	7
Scolex	length	72-104	92	12	38-52	48	15	50-63	57	6
	width	38-72	59	12	25-47	37	15	33-40	38	6
Suckers	diameter	26-32	28	10	21-26	22	6	16-23	19	10
Rostellum	length	42-47	46	7	23-31	27	10	25	25	3
	width				-	-	-	18-23	20	3
Rostellar	total length	21-24	22	12	19-21	20	11	18-21	19	10
hooks	blade	-	-	-	11	-	11	10-12	11	10
	handle	-	-	-	3-5	4	11	3-4	4	10
	guard	-	-	-	5-6	6	11	5-6	6	10

Table 2 (on next page)

Metrical data of cysticercoids of *Flamingolepis* spp. recorded in *Artemia* spp.

Type host and locality are also indicated. All measurements are in μm except where otherwise stated. * maximum length of longest fragment of cercomer.

1

Source		Robert & Gabrion (1991)		Georgiev et al. (2005)					Maksimova (1973)		Present study		
Intermediate hosts		<i>A. salina</i>		<i>A. parthenogenetica</i>					<i>A. salina</i>		<i>A. persimilis</i>		
Locality		France		Spain					Kazakhstan		Chile		
<i>Flamingolepis</i> spp.		<i>F. caroli</i>	<i>F. liguloides</i>		<i>F. flamingo</i>			<i>F. dolguschini</i>	<i>F. tengizi</i>	<i>Flamingolepis</i> sp.			
		Range	Range	Mean	n	Range	Mean	n	Range	Range	Range	Mean	n
Cyst	length	300	560–810	671	14	168–270	231	14	418	180	150–163	156	8
	width	200	372–597	479	14	126–207	177	14	287	130	110–145	121	8
Scolex	length	-	358–771	614	14	141–225	182	12	254	96	113–128	122	7
	width	-	339–490	423	14	108–183	145	12	164	84	90–100	94	7
Suckers	diameter	-	181–288	233	18	45–75	66	16	121	40–42	45–53	49	14
Rostellum	length	-	446–485	465	14	108–180	119	5	-	-	100–125	114	8
	width	-	116–149	125	14	33–48	43	5	-	-	23–28	25	8
Rostellar	total length (TL)	110–130	186–201	189	15	55–61	57	15	184	53–54	45–50	49	15
hooks	length of blade (Lb)	-	105–117	110	15	28–30	29	15	102	30	24–27	25	15
	Ratio Lb/TL	-	0.56–0.60	0.58	15	0.47–0.53	0.5	15	app. 0.55	app. 0.56	0.50–0.54	0.52	15
Cercomer	length (mm)	0.40–0.50	0.62–0.81	0.71	8	6.5–7.4	-	3	0.610	-	0.60*	-	1

2

Table 3(on next page)

Descriptive parameters of cestodes in brine shrimps *Artemia persimilis* from two hypersaline ecosystems in Southern Chile.

SE, standard error.

1

Los Cisnes lagoon						
Cestode species	Number of cysticercoids	Prevalence (%)	Intensity		Abundance	
			Range	Mean \pm SE	Relative (%)	Mean \pm SE
<i>Confluaria podicipina</i>	10	2.5	1	1.00 \pm 0.00	11.6	0.025 \pm 0.01
<i>Fimbriarioides</i> (?) sp.	19	4.8	1	1.00 \pm 0.00	22.1	0.048 \pm 0.01
<i>Flamingolepis</i> sp.	56	13.8	1-2	1.02 \pm 0.02	65.1	0.14 \pm 0.02
<i>Wardium</i> sp.	1	0.3	1	1.00	1.2	0.003 \pm 0.00
Overall infection	86	34.5	1-3	1.06 \pm 0.03	-	0.215 \pm 0.02
Amarga lagoon						
<i>Confluaria podicipina</i>	2	0.5	1	1.00 \pm 0.00	16.7	0.01 \pm 0.00
<i>Fimbriarioides</i> (?) sp.	10	2.5	1	1.00 \pm 0.00	83.3	0.03 \pm 0.01
Overall infection	12	2.5	1-2	1.09 \pm 0.02	-	0.03 \pm 0.01

2

3

4

Table 4(on next page)

Temporal variations of cestode infection in *Artemia persimilis* in two localities from Southern Chile.

P (%), prevalence; MI, mean intensity; MA, mean abundance; RA (%), relative abundance; SE, standard error; S, salinity measured *in situ*. Spatiotemporal variations at *p* level 0.05 are shown.* indicates significant differences between seasons, † indicates significant differences between sites in the same month.

1

		Los Cisnes lagoon		Amarga lagoon	
Cestode species		Spring 2017 (S = 55 g/L)	Autumn 2018 (S = 51g/L)	Spring 2017 (S = 86 g/L)	Autumn 2018 (S = 81g/L)
<i>Confluaria podicipina</i>	P (%)	5.0*†	0.0	1.0	0.0
	MI ± SE	1.00 ±0.00	0.00	1.00 ±0.00	0.00
	MA ± SE	0.050 ±0.02*†	0.00	0.01 ±0.01	0.00
	RA (%)	13.5		33.3	
<i>Fimbriarioides (?) sp.</i>	P%	3.5	6.0	2.0	3.0
	MI ± SE	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00
	MA ± SE	0.035 ±0.01	0.06 ±0.02	0.02 ±0.01	0.03 ±0.01
	RA (%)	9.5	100	66.7	100
<i>Flamingolepis sp.</i>	P%	27.5*†	0.0	0.0	0.0
	MI ± SE	1.02 ±0.02	0.00	0.00	0.00
	MA ± SE	0.28 ±0.03*†	0.00	0.00	0.00
	RA (%)	75.7			
<i>Wardium sp.</i>	P%	0.5	0.0	0.0	0.0
	MI ± SE	1.00	0.00	0.00	0.0
	MA ± SE	0.005±0.01	0.00	0.0	0.0
	RA (%)	1.4			
Overall infection	P%	34.5*†	6.0	2.5	3.0
	MI ± SE	1.07 ±0.04	1.00 ±0.00	1.2 ±0.03	1.00 ±0.00
	MA ± SE	0.37 ±0.04*†	0.06 ±0.02	0.025 ±0.01	0.03 ±0.01
No. of cestode specimens		74	12	6	6
No. of <i>Artemia</i> individuals examined		200	200	200	200

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Figure 1

Geographical distribution of the study sites in the Magallanes Region.

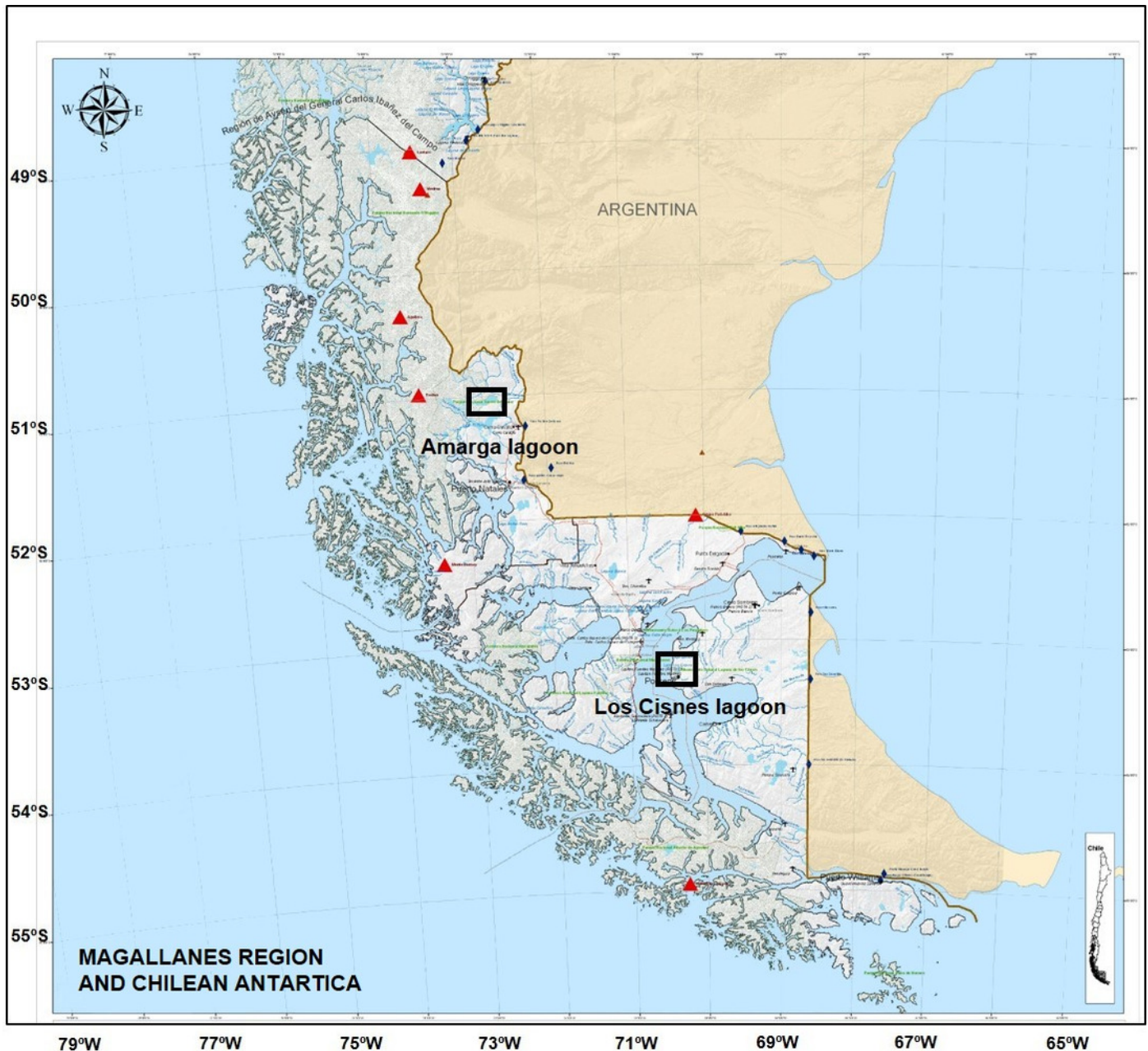


Figure 2

Confluaria podicipina (Szymanski, 1905) from *Artemia persimilis*, Los Cisnes lagoon (A) and Amarga lagoon (B-D), Chile (mounts in Berlese's medium).

(A) Isolated cysticeroid with unpacked cercomer. (B) Fully developed cysticeroid with a part of unpacked cercomer. (C) Internal cysts. (D) Rostellar hooks.

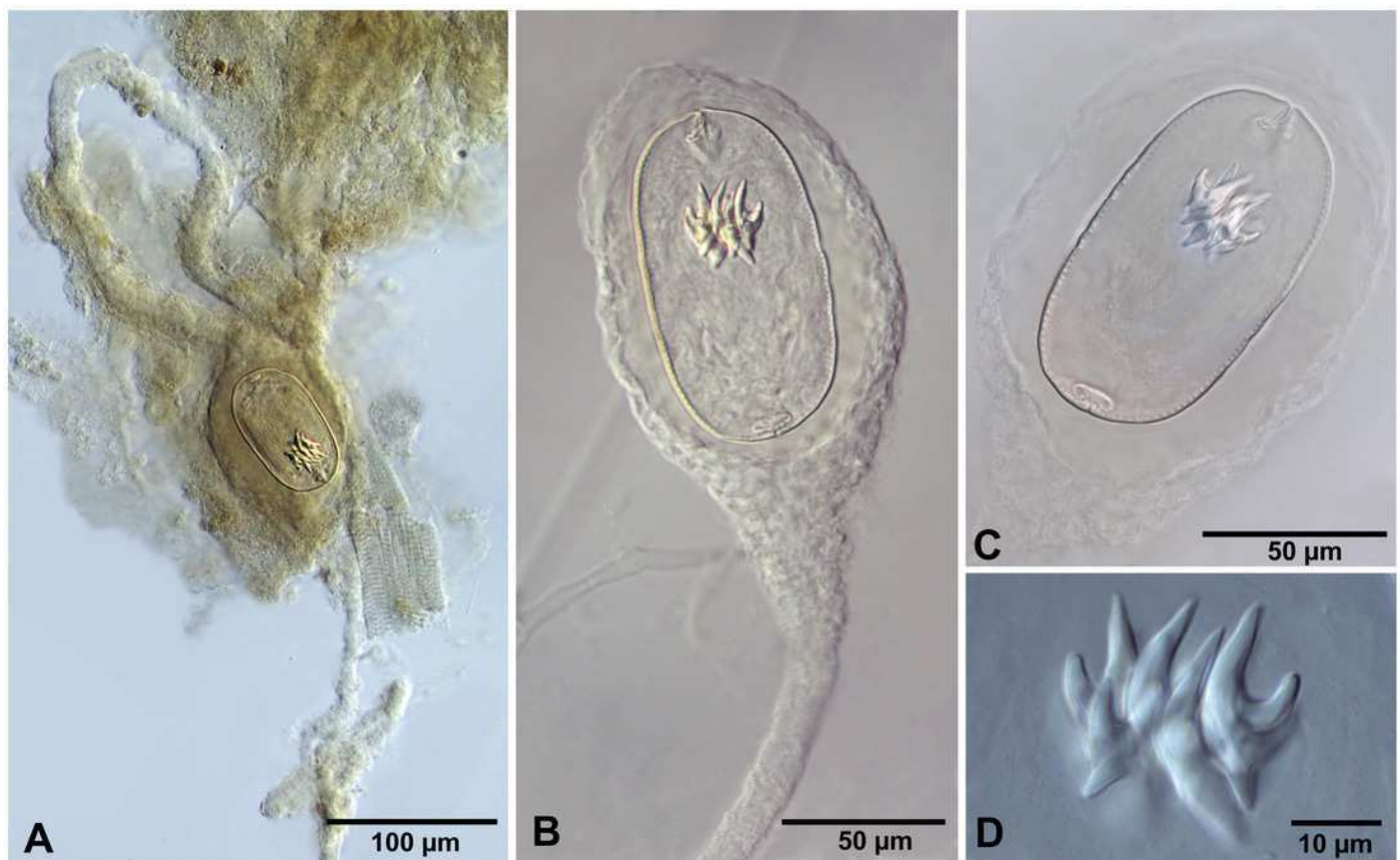


Figure 3

Fimbriarioides (?) sp. from *Artemia persimilis*, Los Cisnes lagoon (A, D) and Amarga lagoon (B, C), Chile.

(A) Cysticercoid with smashed outer cyst and unpacked cercomer (temporary glycerol mount). (B) Acaudate diplocyst with fully developed cysticercoid (temporary glycerol mount). (C, D) Rostellar hooks of isolated cysticercoids (mounts in Berlese's medium).

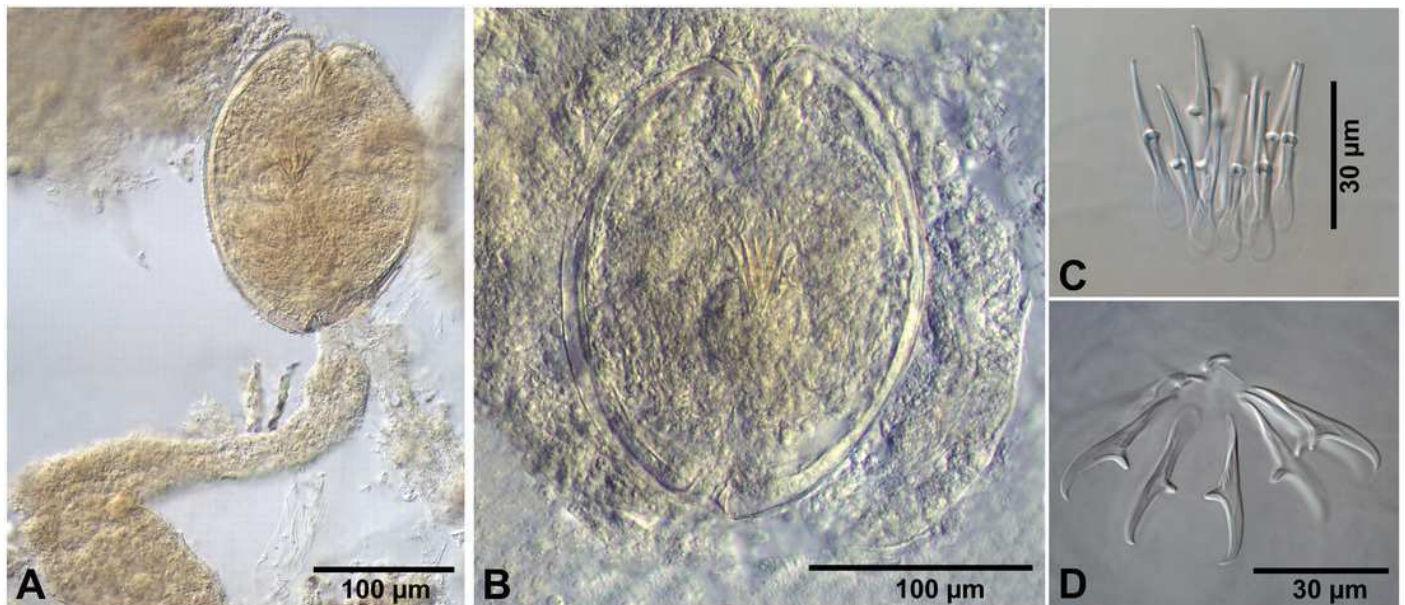


Figure 4

Flamingolepis sp. from *Artemia persimilis*, Los Cisnes lagoon.

(A, C) Fully developed cysticeroid with different stage of aggregation of the cercomer (temporary glycerol mounts). (B) Fully developed cysticeroid with focus on the morphology of the scolex, rostellum and the rostellar hooks (temporary glycerol mount). (D) Isolated cysticeroid (mount in Berlese's medium). (E) Rostellar hooks of an isolated cysticeroid (mount in Berlese's medium).

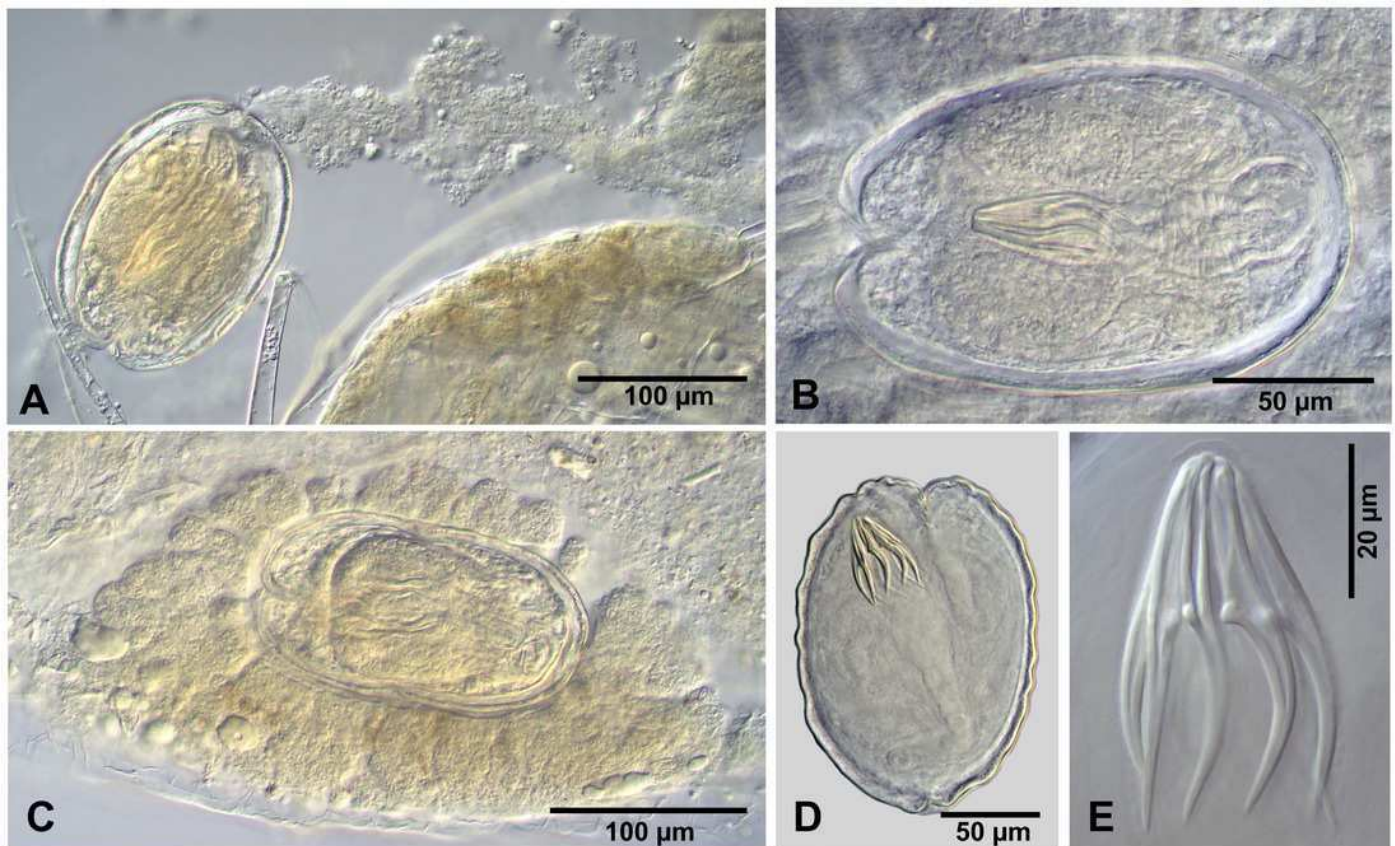


Figure 5

Wardium sp. from *Artemia persimilis*, Los Cisnes lagoon.

(A) Fully developed cysticeroid (temporary glycerol mount). (B) Isolated cysticeroid (mount in Berlese's medium). (C) Rostellar hooks of an isolated cysticeroid (mount in Berlese's medium).

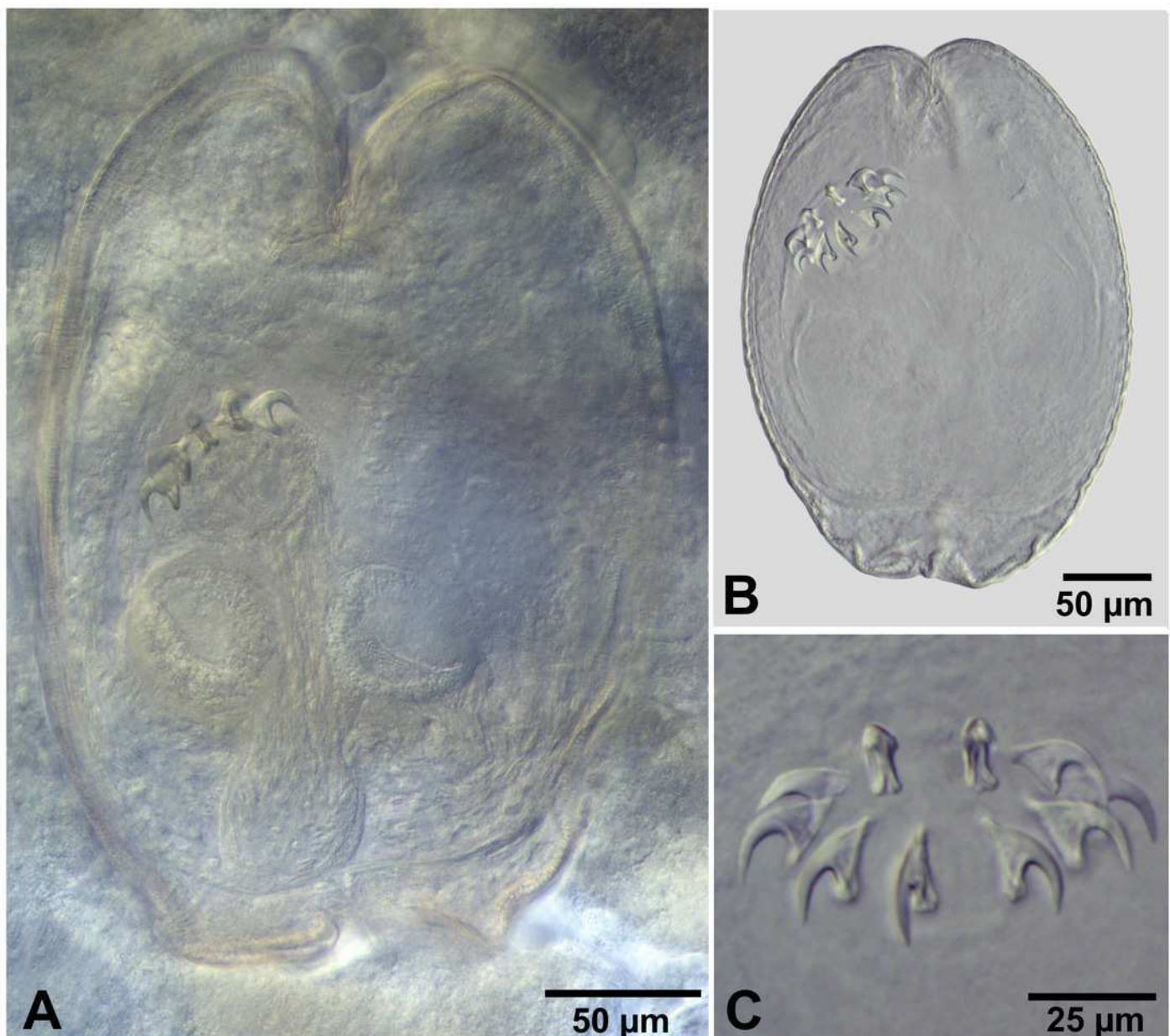


Figure 6 (on next page)

Cestode larvae from *Artemia persimilis*, Amarga lagoon (A, E) and Los Cisnes lagoon (B – F), Chile.

(A) Hook of *Confluaria podicipina* (Szymanski, 1905). (B, E) Hooks and cysticeroid of *Fimbriarioides* (?) sp. (C, F) Hooks and cysticeroid of *Flamingolepis* sp. 1. (D) Hook of *Wardium* sp.

