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ORIGINAL PAPER

A potential zoonotic parasite, the digenean *Gymnophalloides nacellae*, on the Magellanic coast in the Southwestern Atlantic Ocean: its life cycle and geographical distribution

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Abstract
This is an integrative study of a potential zoonotic digenean from the Magellanic Southwestern Atlantic coast. The life cycle of the gymnophallid *Gymnophalloides nacellae* Cremonte, Pina, Gilardoni, Rodrigues, Chai and Ituarte, 2013 (Digenea) at the type locality, Puerto Deseado (47° 45′ S, 65° 51′ W), Santa Cruz province, was elucidated. This digenean uses the upper subtidal clam *Gaimardia trapesina* (Lamarck) (Gaimardiidae), which lives on the fronds of the giant kelp, as first intermediate host. A very short-stem furcocercous cercaria emerges and enters the limpets, *Nacella magellanica* (Gmelin) and *N. deaurata* (Gmelin) (Nacellidae), which live in the lower rocky intertidal zone. The unencysted metacercariae inhabit the extrapallial space of the limpet at high prevalences and intensities of infection. When the black oystercatcher *Haematopus ater* Vieillot and Oudart (Charadriidae) preys upon it, it becomes infected, acting as the definitive host. This parasite seems to exhibit a high specificity for their first and second intermediate hosts. Its geographical distribution is from 47 to 55° S in Patagonia, and it is restricted to those sites where the giant kelp reaches to lower intertidal and upper subtidal zones where the limpets are present. *Gymnophalloides seoi* Lee, Chai and Hong, 1993, a parasite of the Pacific oyster *Magallana gigas* (Thunberg) (Ostreidae), causes a zoonotic disease in Korea; thus, *G. nacellae* represent a risk of being a zoonotic parasite if infected limpets are consumed.

Keywords Parasite · Gymnophallidae · Host–parasite relationship · Life cycle · Black oystercatcher · Southwestern Atlantic coast

Introduction

The family Gymnophallidae was recently reviewed using morphological and molecular data and currently approximately 50 species from seven genera are considered valid. Most members infect in shorebirds as definitive hosts and marine bivalves as first and second intermediate hosts (Cremonte et al. 2015).

The genus *Gymnophalloides* was erected for *Gymnophalloides tokiensis* by Fujita (1925) from metacercariae found in the Pacific oyster in Japan. This species is probably synonymous with *G. seoi*, which was described from adults found in a Korean woman by Lee et al. (1993). *Gymnophalloides seoi*, whose metacercarial and adult stages are well known, infects the Eurasian oystercatcher *Haematopus ostralegus* Linnaeus (Charadriidae) in Korea. The Pacific oyster is highly infected by the metacercariae in the intertidal zone of Korea (Chai et al. 2001). Because it is consumed raw, this species causes an endemic zoonotic disease that is mainly characterized by gastroenteritis (Chai et al. 2000; Guk et al. 2006; Lee and Chai 2001). This species has been extensively studied regarding its several pathologic effects in mammals (e.g., Seo et al. 2006; Lee et al. 2010; Song et al. 2018). The

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mollusk that acts as the first intermediate host of this parasite is still unknown (Lee and Chai 2001).

In Patagonia on the Southwestern Atlantic coast, *Gymnophalloides nacellae* has been described from its metacercarial stage, which parasitizes the limpets *Nacella magellanica* and *N. deaurata* (Cremonte et al. 2013). Later, Bagnato et al. (2015) reported sporocysts of this species parasitizing the gonad and digestive gland of the clam *Gaimardia trapesina* at the type locality; these authors did not describe the cercaria but established its correspondence with the metacercaria of *G. nacellae* by molecular data.

Nacella magellanica is the most abundant limpet in the Magellanic biogeographic Province of Argentina and Chile (Aranzamendi et al. 2011). It is widely distributed along the Atlantic coast from Río Negro (41° S, Argentina) southwards, and the Pacific coast from Valdivia (39° S, Chile) southwards, including the Staten and Malvinas Islands (Castellanos and Landoni 1988; Valdovinos and Rüth 2005). As this mollusk is usually collected and eaten, both by pre-Columbian people and currently by artisanal fishermen (Elías et al. 2011) on the Patagonian coast and other species of *Gymnophalloides* (i.e., *G. seoi*) cause of zoonotic diseases, it is important to know if there are other second intermediate hosts besides the limpet, their prevalence, and infection intensity and its distribution range.

The aim of the present work is to elucidate the life cycle of *Gymnophalloides nacellae* at the type locality (Puerto Deseado) on the Magellanic Patagonian coast by discovering the entire host spectrum. The sporocyst, cercaria and adult forms are described for the first time. Additionally, the geographical distribution based on records of metacercariae in the limpet acting as the second intermediate host is provided.

Materials and methods

Specimens of the clam *Gaimardia trapesina* ($N = 1299$), which lives adhered on the fronds of the giant kelp, *Macrocystis pyrifera* (Linnaeus) on the upper subtidal, were collected bimonthly during the lowest tides between December 2012 and March 2014 by hand from a boat, near the mouth of the estuary of the Deseado river (47° 45′ S, 65° 55′ W), at Puerto Deseado, Santa Cruz Province, Argentina, Southwestern Atlantic Ocean. The clams were transported to the laboratory and some specimens ($N = 202$) were placed on small flasks filled with seawater at room temperature (20–22 °C) where they were inspected twice daily under a stereomicroscope looking for emerging of cercariae. The maximum length of each clam was measured with a caliper with a precision of 0.1 mm. After 48 h, all clams were necropsied to detect prepatent infections and to study sporocysts and cercariae. To find other possible hosts acting in this life cycle, the most abundant mollusks (gastropods and bivalves) were dissected at the type locality (see Bagnato et al. 2015). Scientific names are used according to WoRMS (WoRMS Editorial Board 2019).

To find the adult stage of *G. nacellae*, several shorebirds (4 *Larus dominicanus* Lichtenstein*,* 2 *Leucophaeus scoresbii* Traill (Laridae), and 2 *Haematopus ater* (Charadriidae)) were captured with an airsoft gun under permits provided by Wildlife Secretary of Santa Cruz province (Resolution Number 861/08) in May 2016 at the type locality, Puerto Deseado. Birds were euthanized with carbon dioxide, necropsied and inspected for endoparasites under a stereomicroscope. The gastrointestinal tract was separated into esophagus, stomach, and intestine; this last organ was divided into three equal sections. The body cavity, liver, pancreas, biliary vesicle, gall bladder, gonads, lungs, heart, bursa of Fabricius, cloaca, and kidneys were also examined for parasites. Emerged cercariae and adults were studied alive, stained with neutral red or Nile blue under a light microscope before being fixed, then killed with hot saline solution, immediately fixed with 10% formalin (Cribb and Bray 2010), stained with Semichon′s acetocarmine or Gomori′s trichrome, dehydrated through ascendant ethanol series, cleared with methylsalicylate and mounted on glass-slides with Canada balsam. Drawings and measurements were obtained for 10 unflattened stained and mounted specimens (cercariae and adults) with the aid of a light microscope with drawing device. Measurements are presented in micrometers as mean values followed by the range in parentheses. Forebody length was measured as the distance from the anterior end to the anterior edge of ventral sucker. The sucker ratio was calculated as oral sucker length/ventral sucker length. For scanning electron microscopy (SEM), some specimens were fixed in a 2.5% glutaraldehyde solution buffered with 0.1 M sodium cacodylate, dehydrated through an ascendant ethanol series and dried by rinsing for few minutes in hexamethyldisilazane. Photomicrographs were obtained with a Jeol JSM-6460LV SEM operating at 15 kV. Mounted specimens of the different stages (cercariae, metacercariae and adults) were deposited at the Parasitological Collection (CNP-Par) of the Instituto de Biología de Organismos Marinos (CCT CONICET -CENPAT), Puerto Madryn, Argentina. Metacercariae from the type locality were previously deposited at the Parasitology Collection of the Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina and U.S. National Parasite Collection, Beltsville, Maryland, USA (Cremonte et al. 2013).

Overall prevalences of sporocysts on *G. trapesina*, and overall prevalences and mean intensity of infection of metacercariae in *N. magellanica* and adults in *H. ater* were calculated following Bush et al. (1997). To determine the range of geographical distribution of *G. nacellae*, specimens of *N. magellanica*, which is known to act as second intermediate host (Cremonte et al. 2013), were sampled between February and April 2017 at different localities along the Patagonian coast encompassing most of the distribution area in the Southwestern Atlantic Ocean, from the northern Patagonia (42° 05′ S, 63° 54′ W) to the tip of South America (54° 80′ S, 68° 29′W) (Fig. 1). Limpet specimens from each locality were collected with a spatula during the lowest tides, dissected under a stereomicroscope and the metacercariae were counted to calculate prevalence and infection intensity.

Molecular data on the sporocyst and cercariae were taken from Bagnato et al. (2015), and the adults found for first time in this study were treated by applying the method described

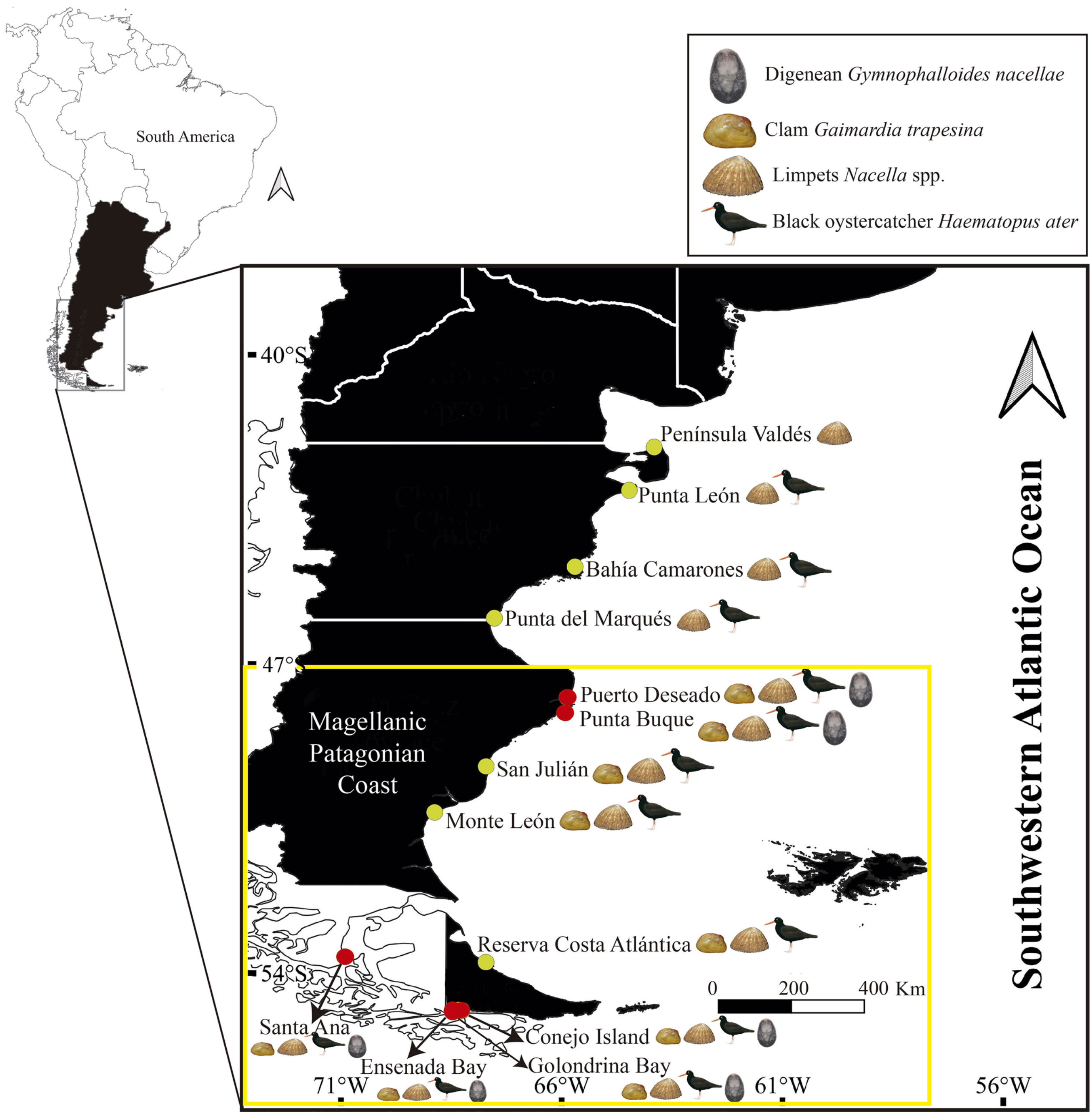

Fig. 1 Map showing the geographical distribution of the parasite *Gymnophalloides nacellae* (Gymnophallidae) along the Patagonian coast, Southwestern Atlantic Ocean (between 47° S to 55° S); where the distribution of the first intermediate host, the clam *Gaimardia trapesina*, the second intermediate host, the limpet *Nacella magellanica* and the definitive host, the black oystercatcher *Haematopus ater* overlap in the lower intertidal and upper subtidal zones. Data of Puerto Deseado (type locality) and Conejo Island were extracted from Martorelli and Morriconi (1998) and Cremonte et al. (2013), respectively. Data regarding host geographic distributions were extracted from Rios (1994), Aranzamendi et al. (2011) and Woods (2014)

by Cremonte et al. (2013, 2015) to obtain the 18S region of ribosomal DNA which has been deposited in GenBank. The adult sequence was alignment and compared with 18S sequences of metacercaria using Multalin software (available at https://bioinfo.genotoul.fr/multalin/multalin.html).

Results

Description of intramolluscan stages

Sporocysts

Furcocercous cercariae develop in colorless, immobile, and thin-walled daughter sporocysts (not measured because they were broken inside the clam).

Cercariae

(measurements based on 10 naturally emerged cercariae) (Figs. 2a, 3a): Cercaria furcocercous, very short stem, with two sucker-like structures with setae at tip of each furca. Body small, elongated, 84 (60–102) long by 17 (12–20) in maximum width. Forebody 50 (33–66) long. Spines on body surface not seen. Oral sucker sub-terminal, oval, 20 (13–23) long by 15 (13–18) wide. Ventral sucker post-equatorial, oval, 14 (11–17) long by 16 (13–18) wide. Sucker ratio 1:1.22 (1:1.06–1:1.23). Pharynx rounded, 7 (4–10) long by 5 (4–6) wide. Esophagus 12 (8–19) long. Ceca short, extend to level of anterior edge of ventral sucker or first third of ventral sucker, 17 (11–22) long. Four pairs of penetration glands open dorsally at anterior edge of body; anterior pair stain with neutral red; cytons located at ceca level. Excretory vesicle small, oval with thick wall, 13 (9–16) long by 8 (5–10) wide. Flame cell formula: 2 [(2 + 2) + (2 + 2)] = 16. Tail stem very short 7 (5–9) long by 6 (5–8) wide at base; furcae 45 (38–53) long by 5 (3–6) wide.

Behavior and life span

One from 202 clams emerged cercariae (0.49%). Emerged cercariae do not swim in the water column as typical gymnophallid cercariae; instead they descend to the bottom and slowly move by contracting and stretching the body and the tail. Some rest the dorsal part of body on the bottom and rotate the furcae; others attach to the bottom by the sucker-like structures and rotate the body. Their life span was about 24 h at room temperature (20-22°C).

Description of adult

Figures 2b, c, 3b, c, 4: Body oval, anterior end rounded, posterior end slightly pointed, 401 (360–454) long by 210 (198–228) wide at ventral sucker level; maximum wide 217 (200–230). Digitiform spines arranged transversely, covering entire dorsal body surface, bigger and dense in anterior part of body, smaller and sparse in posterior part of body (Fig. 4c). Spines covering entire ventral body surface,

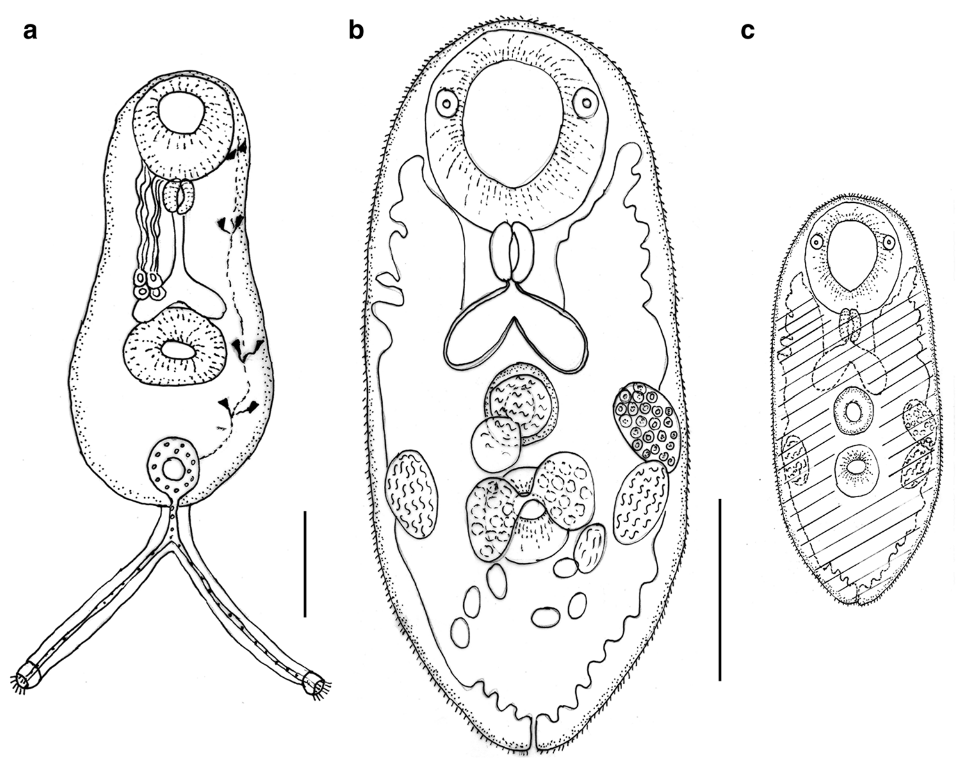

Fig. 2 Line drawings of *Gymnophalloides nacellae* (Gymnophallidae) from Patagonian coast, Southwestern Atlantic Ocean. **a** Emerged cercaria from the clam *Gaimardia trapesina*, ventral view, excretory cells on the right side and penetration glands on the left side omitted. **b** Adult from the black oystercatcher, *Haematopus ater*, dorsal view. **c** Adult, detail of the uterine extension (field indicated with oblique lines). Scales bar: **a** 20 µm, **b**, **c** 100 µm

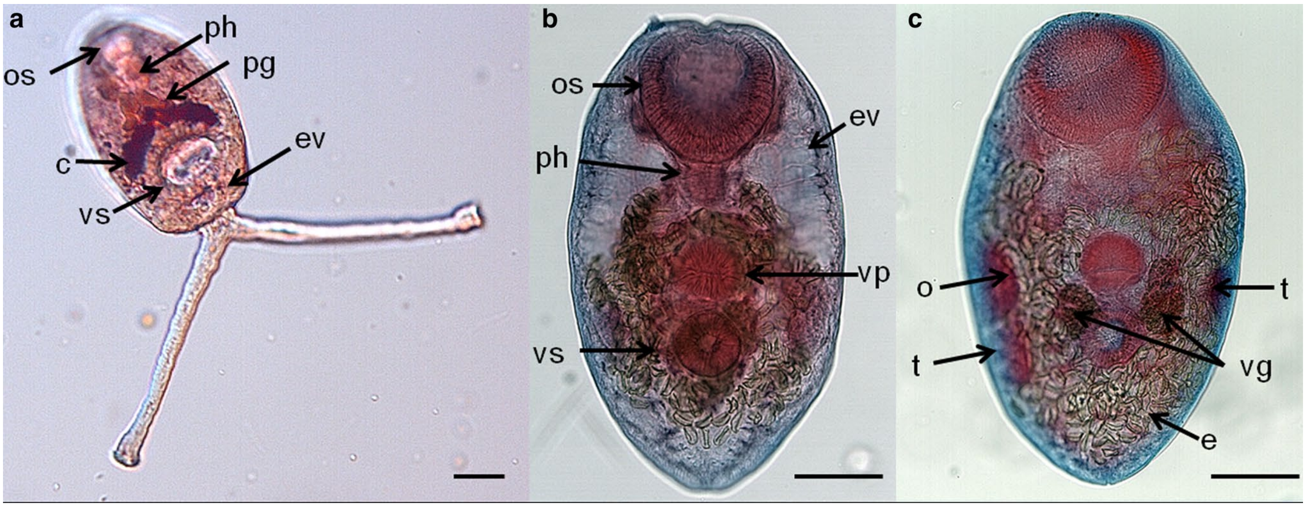

Fig. 3 Microphotographs of *Gymnophalloides nacellae* (Gymnophallidae) from Patagonian coast, Southwestern Atlantic Ocean. **a** Emerged cercaria in vivo stained with Neutral Red, ventral view. **b** Adult *in toto* stained with Gomori trichrome, ventral view. **c** Adult *in toto* stained with Gomori trichrome, dorsal view. *c* ceca, *e* eggs, *ev* excretory vesicle, *o* ovary, *os* oral sucker, *pg* penetration glands, *ph* pharynx, *t* testis, *vg* vitelline gland, *vp* ventral pit, *vs* ventral sucker. Scale bars: **a** 10 µm, **b**, **c** = 100 µm

except in region from pharynx to ventral pit (Fig. 4a, d). Forebody 246 (228–280) long. Oral sucker 114 (100–136) long by 122 (105–140) wide, 18–27 papillae sparse and surrounding mouth; lateral lips present, 2 in number, eversible (Fig. 4b). Ventral pit 46 (39–53) long by 52 (43–63) wide. Large papillae present between oral sucker and ventral pit, about 41–46 in number, arranged in two groups, anterior group with 31–35 well-developed papillae and posterior group close to ventral pit with 10–14 less developed papillae (Fig. 4d). Ventral sucker 47 (30–62) long by 47 (42–52) wide, with outer circle of 6 papillae (Fig. 4e). Sucker ratio 1:0.41 (0.26–0.52). Pharynx ovoid, 35 (30–45) long by 30 (23–35) wide. Esophagus 8 (2–38) long. Ceca sacciform, 63 (47–90) long by 29 (16–46) wide, reach to mid-ventral pit region, rarely reach anterior edge of ventral sucker. Testes ovoid, located at level of ventral sucker or between ventral pit and ventral sucker. Left testis 50 (32–63) long by 36 (30–43) wide; either right testis 52 (35–65) long by 37 (32–44) wide. Seminal vesicle bipartite, anterior part usually overlapping ventral pit and fill with spermatozoa, 48 (42–58) in diameter, posterior part usually empty, 42 (32–52) diameter. Pars prostatica absent; prostatic cells not seen. Genital pore small, inconspicuous, located close to anterior edge of ventral sucker. Ovary rounded, 51 (32–70) long by 34 (30–48) wide, located anterior to right or left testis. Seminal receptacle sinister to ventral sucker, 28 (23–30) in diameter. Uterus filling entire body, from pharynx level to posterior end, except in ventral pit and ventral sucker regions (Figs. 2c, 3c). Vitelline gland paired, oval, compact close to ventral sucker and united by common vitelline duct, left mass 41 (30–50) long by 36 (25–43) wide and right mass 39 (32–45) long by 32 (20–40) wide; each follicle measuring 9 (7–11). Eggs 17 (15–18) long by 10 (8–11) wide. Excretory vesicle Y-shaped with very short stem, excretory granules present only in young adults.

Taxonomic summary

First intermediate host *Gaimardia trapesina* Lamarck (Bivalvia: Gaimardiidae).

Second intermediate hosts *Nacella magellanica* (Gmelin) and *Nacella deaurata* (Gmelin) (Gastropoda: Nacellidae).

Definitive host *Haematopus ater* Vieillot & Oudart (Aves: Charadriidae).

Infection sites Gonad and digestive gland in the clam first intermediate host, extrapallial space (between mantle and shell) in the limpet second intermediate host and intestinal ceca (~ 100 specimens), cloaca (39) and intestine (~ 200) in the oystercatcher definitive host.

Type locality Puerto Deseado (47° 45′ S, 65° 51′ W), province of Santa Cruz, Argentina.

Other localities Punta Buque (48° 06′ S, 65° 55′ W), province of Santa Cruz, Ensenada Bay (54° 80′ S, 68° 29′ W), Ushuaia, province of Tierra del Fuego, Argentina.

Prevalences and intensities of infection Sporocysts 2% overall prevalence (22 from 1299) in the clam first intermediate host at the type locality. Metacercariae 46.4% overall prevalence (77 from 166) and 43 overall mean intensity (range intensity 1–500) in the limpet second intermediate host. One black oystercatcher infected (339 worms) of 2 examined at the type locality.

Material deposited Cercariae CNP-Par 48, metacercariae CNP-Par 174 (Punta Buque), 175 (Ensenada Bay)

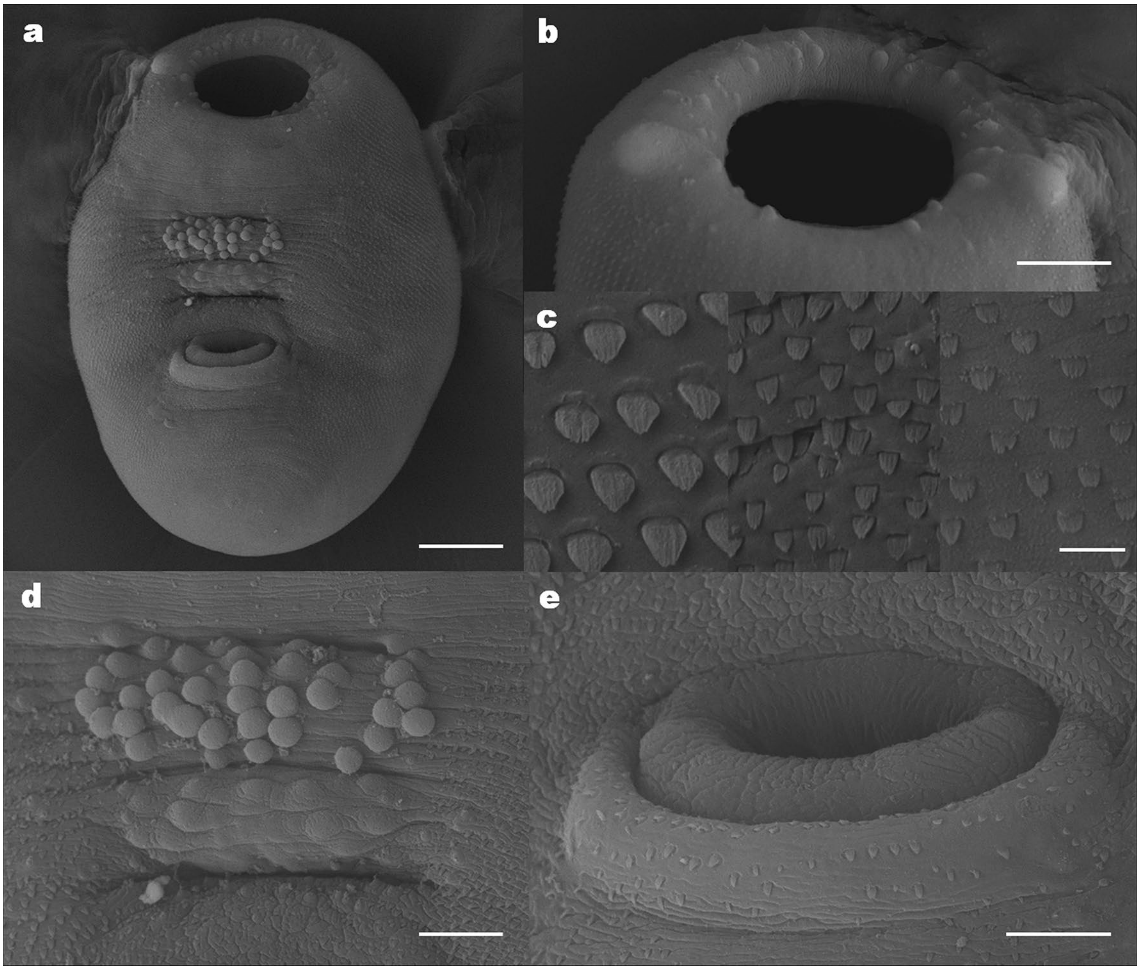

Fig. 4 Scanning electron microscope (SEM) photographs of the adult stage of *Gymnophalloides nacellae* (Gymnophallidae) from Patagonian coast, Southwestern Atlantic Ocean. **a** Entire body, ventral view. **b** Detail of anterior sucker with lateral lips and papillae around the mouth. **c** Spines on dorsal surface of body at anterior, middle and posterior zone (from left to right). **d** Detail of papillae and body surface without spines on the ventral pit zone. **e** Detail of ventral sucker. Scale bars: **a**, **b** 25 µm, **c** 2.5 µm, **d**, **e** 10 µm

and adults CNP-Par 171. Records of metacercariae from Cremonte et al. (2013) CNP-Par 50, 51, MACN-Pa 532/1, 532/2 and USNPC 105642.

GenBank accession numbers KF575168 (sporocyst, 18S-ITS1-5.8S-ITS2-28S), JN381025 (metacercaria18S-ITS1-5.8S-ITS2-28S) from Bagnato et al. (2015) and MH887406 (adult, 18S) from present study.

Taxonomic remarks

Just two species of genus *Gymnophalloides* have been described in addition to *G. nacellae* and the cercaria has been recorded for neither. The peculiarity of the cercaria described here is the presence of sucker-like structures at the tips of the furcae; this characteristic has only been previously recorded in the cercaria of *Parvatrema margaritense* (Ching, 1982) (Gymnophallidae) which parasitizes the bivalve *Turtonia minuta* (Fabricius) (Veneridae) (Galaktionov et al. 2006). These authors studied the life cycle of *P. margaritense* and described the same behavior of the cercaria here described, where it attaches to the substrate with their furcae and rotates their body. Cercaria of *P. margaritense* is larger than the cercaria here described (148 [130– 185] long × 73 [60–80] wide vs. 84 [60–102] long × 17 [12–20] wide) and they have excretory vesicle with a different shape (V-shaped vs. oval). Both morphology and behavior of cercaria of *G. nacellae* and *P. margaritense* are different to all gymnophallid cercariae described at present.

Adults of *G. nacellae* share some features with *Gymnophalloides seoi*, in the presence of lateral lips in the oral sucker, a bipartite seminal vesicle and vitelline glands paired close to ventral sucker. However, these species differ in the position of the seminal vesicle (overlapping the ventral pit in *G. nacellae* and distinctly anteriorly to the ventral pit in *G. seoi*), the position of the vitelline glands (overlapping the ventral sucker in *G. nacellae* and at both sides of the ventral sucker in *G. seoi*) and the extension of the uterine field (filling the entire body, from level with the pharynx to the posterior end in *G. nacellae* and occupying the middle third of the body in *G. seoi*). *Gymnophalloides heardi* Ching 1995 was described from the rodent *Oryzomys palustris* (Harlan) (Ching 1995). This species differs from *G. nacellae* in the smaller body size, the presence of a single lobed vitellarium and an elongated and undivided seminal vesicle (Ching 1995).

The metacercaria of *G. nacellae* was described by Cremonte et al. (2013) and the seminal vesicle was erroneously reported as unipartite; this organ is undeveloped in the metacercariae and thus, it was not possible to observe the two parts of seminal vesicle. *Gymnophalloides* metacercariae are poorly developed on the second intermediate host and use a non-migratory bird as definitive host. In contrast, *Bartolius pierrei* Cremonte, 2001 that uses the red knot, *Calidris canutus rufa* Linnaeus (Scolopacidae), a Neotropical migratory bird as definitive host presents a well-developed metacercaria on the clam *Darina solenoides*, the second intermediate host (Cremonte 2004).

Life cycle

Gymnophalloides nacellae utilizes the clam *G. trapesina* as first intermediate host, the limpets *N. magellanica* and *N. deaurata* as second intermediate hosts and the black oystercatcher *H. ater* as definitive host (Fig. 5). The 18S sequence of the adult from *H. ater* has 905 bp with 100% similarity with 18S sequences of metacercariae from *N. magellanica* (JN381025). The other mollusks and birds dissected where found not to be infected with *G. nacellae*.

Geographical distribution

From the nine sampled sites in this study (Fig. 1 and Table 1), *G. nacellae* in *N. magellanica* (second intermediate host) was present on two sites, (1) Punta Buque, a beach about 100 km from Puerto Deseado, with a prevalence of 92%, and (2) Ensenada Bay, Ushuaia, Tierra del Fuego province, a beach located in the Beagle Channel, the most southern site, with a prevalence of 16%. Previous and current records of metacercariae *G. nacellae* on limpets are showed in Table 1. The presence of *G. nacellae* is possible in those sites as all three hosts involved in the life cycle are present, and moreover, the giant kelp is close to the shoreline, when the clams *G. trapesina* (first intermediate hosts) live adhered to its fronds.

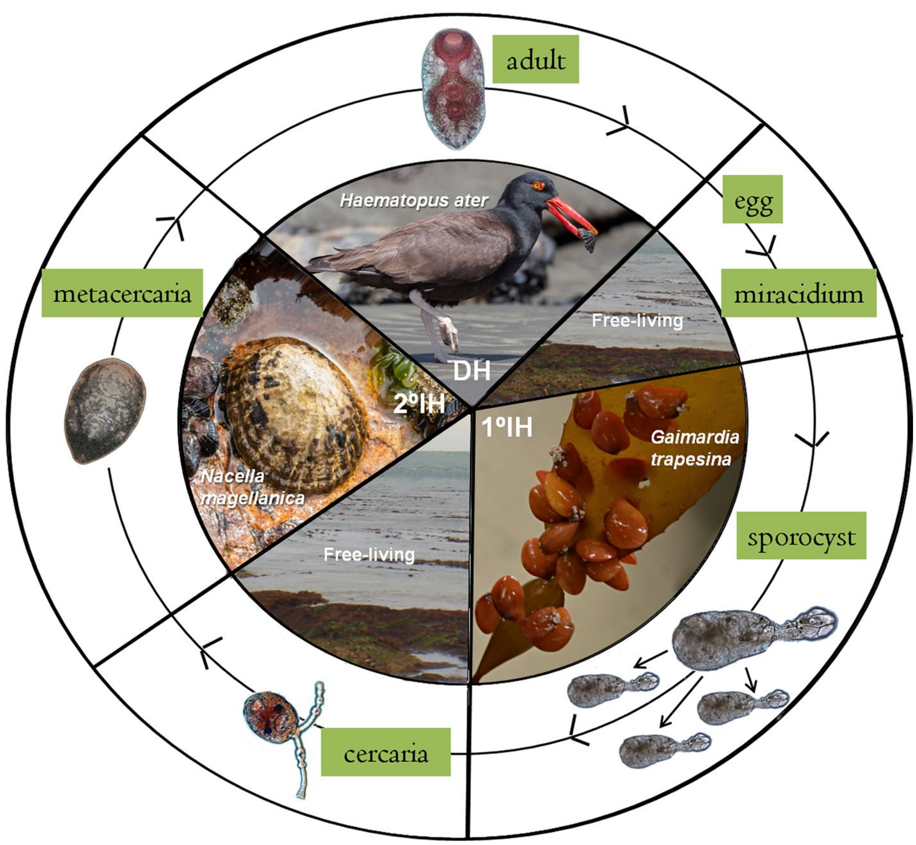

Fig. 5 Life cycle of *Gymnophalloides nacellae* (Gymnophallidae) from the Patagonian coast, Southwestern Atlantic Ocean. *DH* definitive host, *1°HI* first intermediate host, *2°IH* second intermediate host

Table 1 Records of metacercariae of the gymnophallid digenean *Gymnophalloides nacellae* parasitizing Nacella *magellanica* (*Nm*) and *N. deaurata* (*Nd*) along the Magellanic Patagonian coast, Southwestern Atlantic Ocean

Locality	Prevalence (*n*)	References
Península Valdés 42° 45′ S, 63° 54′ W	0 (75) *Nm*	Present study
Punta León 43° 04′ S, 64° 29′ W	0 (37) *Nm*	Present study
Bahía Camarones 44° 49′ S, 65° 43′ W	0 (100) *Nm*	Present study
Punta del Marqués 45°57′S, 67°32′W	0 (99) *Nm*	Present study
Puerto Deseado 47° 45′ S, 65° 51′ W	**96** (186) *Nm*	Cremonte et al. (2013)
Punta Buque 48° 06′ S, 65° 54′ W	**92** (66) *Nm*	Present study
Puerto San Julián 49° 17′ S, 67° 42′ W	0 (67) *Nm*	Present study
Monte León 50° 13′ S, 68° 55′ W	0 (100) *Nm*	Present study
Reserva Costa Atlántica 53° 20′ S, 68° 30′ W	0 (97) *Nm*	Present study
Conejo Island 54° 49′ S, 68° 13′ W	**91** (637) *Nm* **97** (548) *Nd* **100** (15) *Nm* **100** (2) *Nd*	Martorelli and Morriconi (1998) Unpublished data
Golondrina Bay 54° 48′ S, 68° 17′ W	**50** (2) *Nm* **75** (4) *Nd*	Unpublished data
Ensenada Bay 55° 12′ S, 67° 26′ W	**16** (100) *Nm*	Present study
Santa Ana 53° 37′ S, 70° 54′ W	**12** (214) *Nd*	Flores et al. (2019)

Bold indicate the presence of the parasite

Data of prevalences (%), number of examined hosts (*n*) and references are provided

Discussion

This work provides the first descriptions of the cercaria and adult forms of *Gymnophalloides nacellae*, its life cycle elucidated by molecular tools, and an expanded geographical distribution. One of the most fundamental characteristics of a parasite is the spectrum of host species used at each stage in its life cycle. However, this information is not available for many parasite species where only one host is reported for each life stage (Skirnisson and Galaktionov 2002). Taking into account the high degree of specificity exhibited by digeneans for its first intermediate host (Skirnisson and Galaktionov 2002), that low prevalences are common among trematodes using clams as first intermediate host (Lauckner 1983), and considering that in the type locality, the most abundant gastropods and bivalves were examined with negative results (Bagnato et al. 2015); it is most likely that *Gymnophalloides nacellae* uses only the clam *Gaimardia trapesina* as a first intermediate host. This clam occurs in the upper subtidal, and only limpets of the family Nacellidae, which occur in the lower intertidal zone as a second intermediate host. Although the adult was only found parasitizing the black oystercatcher, *G. nacellae*, it probably utilizes other oystercatcher species and some gulls (as for example, *Larus dominicanus*) as definitive host, since these shorebirds also prey on the limpets (Yorio and Bertellotti 2002).

Loos-Frank (1969) categorized five types of gymnophallid life cycles. According to this classification, the life cycle of *G. nacellae* belongs to type Ia where swimming cercariae enter a second intermediate host different to the first one (for example, *Parvatrema borinquenae* Cable 1953). However, in most of the elucidated life cycles of the family, the bivalve acting as second intermediate host is usually the same species as that which acts as first intermediate host [for example, *Bartolius pierrei*, *Gymnophallus gibberosus* Loos-Frank, 1971 and *Parvatrema duboisi* (Dollfus, 1923)] (Pekkarinen 1987; Cremonte 2004; Yanagida et al. 2009).

Gymnophalloides seoi, another species identified from the genus, was recorded in Korea and also uses an oystercatcher, *Haematopus ostralegus,* as definitive host (Lee and Chai 2001). The first intermediate host for this zoonotic parasite is unknown and the second intermediate host is the Pacific oyster *Magallana gigas* (Lee and Chai 2001). In general, gymnophallids are not specific to their definitive host and may use birds or mammals (Lee and Chai 2001; Cremonte et al. 2015). *Gymnophalloides seoi* was firstly recorded in a woman diagnosed with acute pancreatitis and later, at fecal examination, trematode eggs were found and after a single dose of an anthelmintic was given, a thousand small trematodes were recovered and the worms were described (Lee et al. 1993). Other wading birds including the Kentish plover *Charadrius alexandrinus* Linnaeus (Charadriidae) as well as gerbils, hamsters, cats and several strains of mice were highly susceptible to experimental infections (Lee and Chai 2001).

Metacercariae of *G. seoi* are transmitted to humans by eating raw oysters; the larvae mature to the adult form, and cause an endemic disease on western and southern coastal islands in the Republic of Korea (Chai et al. 2001; Lee and Chai 2001). Infected people suffer variable degrees of gastrointestinal troubles because the adult worms inhabit the small intestine, pinching and sucking the root of villi with their oral suckers (Lee and Chai 2001). Even though limpets are not the principal shellfish harvested on the Patagonian coast, they are currently collected and eaten by artisanal fishermen (Elías et al. 2011) and this parasite constitutes a potential zoonotic disease on the Patagonian coast of the Southwestern Atlantic Ocean.

This parasite species was found to be geographically distributed from Puerto Deseado (47° S) to Ushuaia (55° S) along the Southwestern Atlantic coast (Fig. 1). This result indicates that the parasite's distribution is restricted to the distribution of the clam first intermediate host and where the other two hosts involved in the life cycle are present (i.e., limpets and oystercatcher). Taking into account that the three hosts (clam, limpet and oystercatcher) are also present on the Pacific coast of the Magellanic Biogeographic Province (Rios 1994; Aranzamendi et al. 2011; Woods 2014), it seems very probably that *G. nacellae* is also present in Chile. Because of the worldwide popularization of Pacific and Peruvian cuisine, the traditional seafood dishes like sushi, sashimi and ceviche cause fishborne parasitic zoonoses (Nawa et al. 2005; Praveen et al. 2015). Then, special measures should be taken with the consumption of undercooked mollusk from intertidal environments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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