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A phylogenetically unresolved apicomplexan (APXSc) causing swirl lesions in the Tehuelche scallop, *Aequipecten tehuelchus*, from the Southwest Atlantic coast

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ABSTRACT

The study reports a previously unknown apicomplexan (APXSc) parasite infecting wild scallops *Aequipecten tehuelchus* (d'Orbigny, 1842) from two separate areas (La Tapera and Punta Conos) of the San José gulf, in Patagonia Argentina. Histology, transmission electron microscope, molecular analyses and *in situ* hybridization were performed to describe the morphology of APXSc, and confirm its phylogenetic status. The prevalence of APXSc infection was 24% and 72% in scallops from La Tapera and Punta Conos, respectively. Seasonal variation was observed for scallops from La Tapera, recording highest prevalence in summer. A positive relationship between the presence of the APXSc and the size of the scallops was observed. A SSU rDNA consensus sequence of 1758 base pairs was generated which has a 94.8% identity to sequences obtained from a pathogenic apicomplexan parasite infecting *Ostrea chilensis* in New Zealand, but not closely related to other apicomplexans. The asexual reproduction, i.e. merogony, occurs in the Tehuelche scallop whilst the gamogonic and sporogonic stages were absent, suggesting a yet unknown definitive host. Severe host inflammation response involving fibroblast-like hemocytes surrounding the APXSc in the form of granuloma-like "swirls" is characteristic for this apicomplexan infection. Further studies are needed to reveal the life cycle, and presumable pathogenicity of APXSc.

1. Introduction

The phylum Apicomplexa forms a large and diverse group of parasitic protists that comprises around 6000 described species of either facultative or obligate intracellular parasites (Kwong et al., 2021; Rueckert et al., 2010). Many lineages of apicomplexans are closely associated with marine invertebrates, including a number from commercially significant bivalve species from around the world (Morado et al., 1984; Whyte et al., 1994; Aranda et al., 2011; Kristmundsson et al., 2015; Vázquez and Cremonte, 2017). Data on apicomplexan parasites infecting scallops are scarce with only three nominal species reported, i.e. *Pseudoklossia pectinis*, *Margolisiella islandica* and *Merocystis kathae* (Léger and Duboscq, 1917; Kristmundsson et al., 2011a, b; Kristmundsson and Freeman, 2018), all of which within a recently formed Order Marosporida (Mathur et al., 2019). The former two species have only been observed in one host each in the NE Atlantic, i.e. *Pecten maximus* and *Chlamys islandica*, respectively. *Merocystis kathae* has however, been observed in five different scallop species, i.e. *C. islandica, P. maximus, Aequipecten opercularis*, in European waters (Léger and Duboscq, 1917; Kristmundsson et al., 2011a; Kristmundsson and Freeman, 2018), *Placopecten magellanicus* off the East coast of N-America (Inglis et al., 2016) and *Patinopecten caurinus* in Alaskan waters in the NE Pacific (Ferguson et al., 2021). While *M. islandica* is monoxenous, with all life stages present in the scallop host, *M. kathae* is heteroxenous, with a buccinid gastropod as definitive host and pectinid bivalves as intermediate hosts (Kristmundsson et al., 2011a; Kristmundsson and Freeman, 2018). The life cycle of *P. pectinis* is presently unknown, but initially suggested to be heteroxenous (Léger and Duboscq, 1917). To date, that is considered questionable, along with other *Pseudoklossia* species (Duszynski et al., 1999; Kristmundsson and Freeman, 2018). Some further reports of anonymous apicomplexan parasites infecting scallops exist in the literature, e.g., *Pseudoklossia pectinis*-like coccidian

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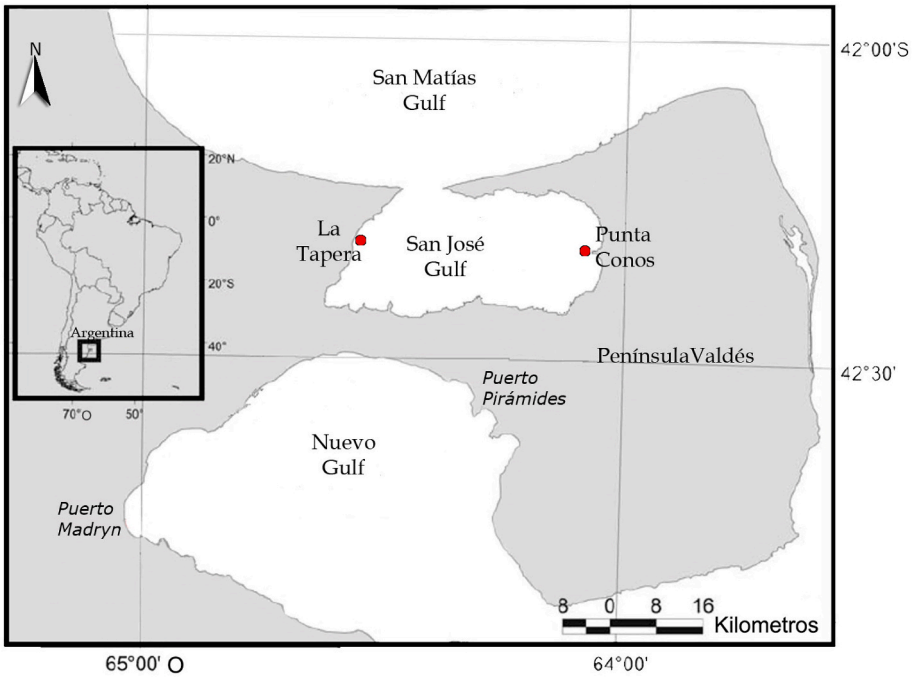

Fig. 1. Sites where Tehuelche scallops were collected in this study. Sampling collection sites within San José Gulf (Chubut province, Argentina): in the West domain, La Tapera, and in the East domain, Punta Conos.

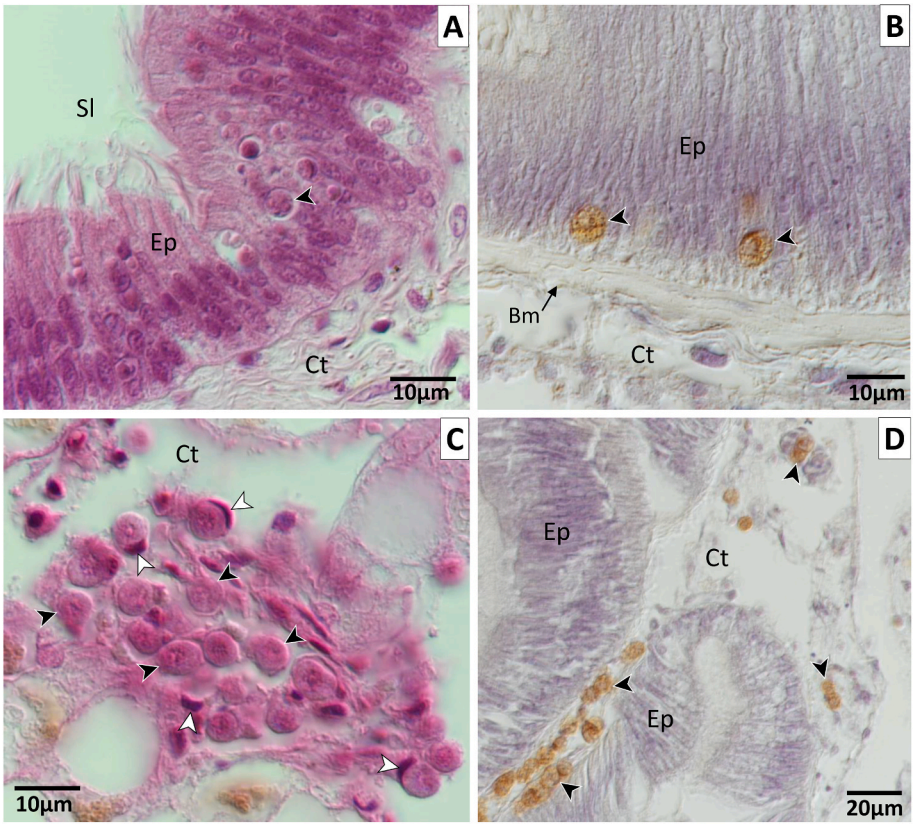

Fig. 2. Intra- and extracellular forms of APXSc within the Tehuelche scallop. (A–B) H&E stained section (A) and ISH (B) showing APXSc forms within the stomach epithelium (Ep), suggestion that infections are via the gastrointestinal tract. (C–D) H&E stained section (C) and ISH (D) showing APXSc in the connective tissue adjacent to the gastrointestinal tract. Many of the parasite forms are found inside hemocytes with the host nuclei marginalized (white arrowheads) whilst other forms are still free (black arrowheads) in the connective tissue. Sl = Stomach lumen; Ct = connective tissue; Bm = basement membrane.

(Karlsson, 1991) and *Pseudoklossia* sp. (Cawthorn et al., 1992) reported from Bay scallop *Argopecten irridians*.

The pathogenicity of apicomplexans varies significantly between species and/or their hosts, some species being highly pathogenic, e.g. human pathogens like *Babesia* spp., *Plasmodium* spp., *Toxoplasma gondii* (e.g. Seed, 1996), whilst others seem to have minor impact on their host. Apicomplexan infections are very common in fish, in some cases causing severe coccidiosis (Lom and Dyková, 1992; Kristmundsson et al., 2018). However, being obligate intracellular parasites (with some exceptions), they cause some degree of pathology in all cases. The general knowledge

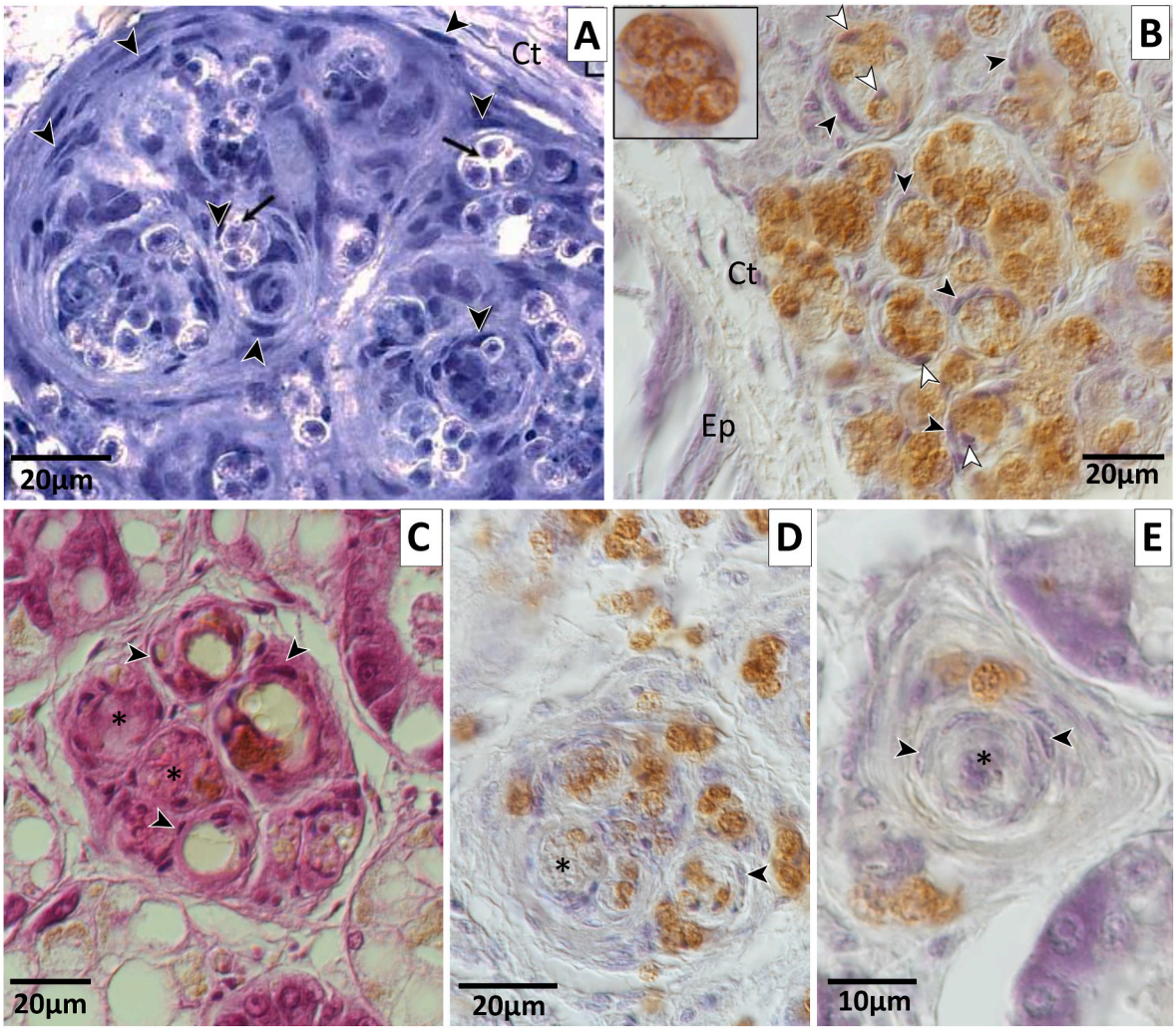

Fig. 3. Host reaction and pathology related to APXSc. (A–E) Granulomatous swirl lesions within the Tehuelche scallop, a common host reaction to APXSc infection. (A) Semithin section stained with toluidine blue, showing typical swirl-like lesion, where fibroblast-like hemocytes (black arrowheads) surround groups of parasites, commonly 4–8 (black arrow). A larger granuloma is then formed around a cluster of these groups, forming a kind of double granuloma. (B) ISH sections of a similar swirl lesion as in (A), note the formation of numerous groups of parasites, each group apparently within one hemocyte (host nuclei: white arrowheads) and surrounded by fibroblast like hemocytes (black arrowheads). The insert shows a higher magnification of a cluster of the parasites. (C–E) Granulomatous swirl lesions with apparently degenerating parasites (*), surrounded by fibroblast like hemocytes (arrowheads). (C) = HE) = ISH. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

on the effect of apicomplexan parasites on bivalve molluscs is limited. However, a number of apicomplexans have been associated with mortalities in cultured and wild molluscs, including scallops (Leibovitz et al., 1984; Whyte et al., 1994; Friedman et al., 1995; Winstead et al., 2004; Cheng, 2012; Kristmundsson et al., 2015; Muehl et al., 2021) and oysters (Hine, 2002).

The Tehuelche scallop *Aequipecten tehuelchus* is a simultaneous hermaphrodite (Christiansen and Olivier, 1971), and is iteroparous species. It is distributed in the Southwest Atlantic, from Río de Janeiro (23°S, Brazil) to the north of San Jorge Gulf (45°S, on the Argentinean Patagonian coast), inhabiting sandy bottoms at depths shallower than 130 m. This scallop is the target of small inshore fisheries that operate within the northern Patagonian Gulfs, involving commercial diving. In spite of the small volumes landed at present, these fisheries are of considerable significance for the local economies (Soria et al., 2014). To date, only a survey reporting low infestation levels of parasites or low pathological effects has been described from the Tehuelche scallop (Cremonte et al., 2005). A critical risk concern for bivalve aquaculture and fishery operations is the management of pathogens and/or diseases that could affect the production, causing severe economic losses (Bondad-Reantaso and Arthur, 2008).

In the present study, we report for the first time an apicomplexan parasite infecting *A. tehuelchus* using light and transmission electron microscopy, *in situ* hybridization and molecular analyses. In addition, we investigate by using Generalized Linear Model analysis, how the presence of the apicomplexan parasite is influenced by the environmental and biological variables (site of collection, water temperature, shell length, gonad developmental stages, and condition index of the scallop).

2. Materials and methods

2.1. Study area

The San José Gulf (42°20′S 64°20′W) is located north of Península Valdés, northern Patagonia, Argentina. It is a small semi-enclosed gulf (817 km^2) connected by a narrow mouth to the much larger San Matías Gulf (18,000 km^2) (Fig. 1). The stocks of Tehuelche scallop inhabiting the San José Gulf are structured as a metapopulation whose component sub-populations (‘grounds’) are interconnected by larval dispersal

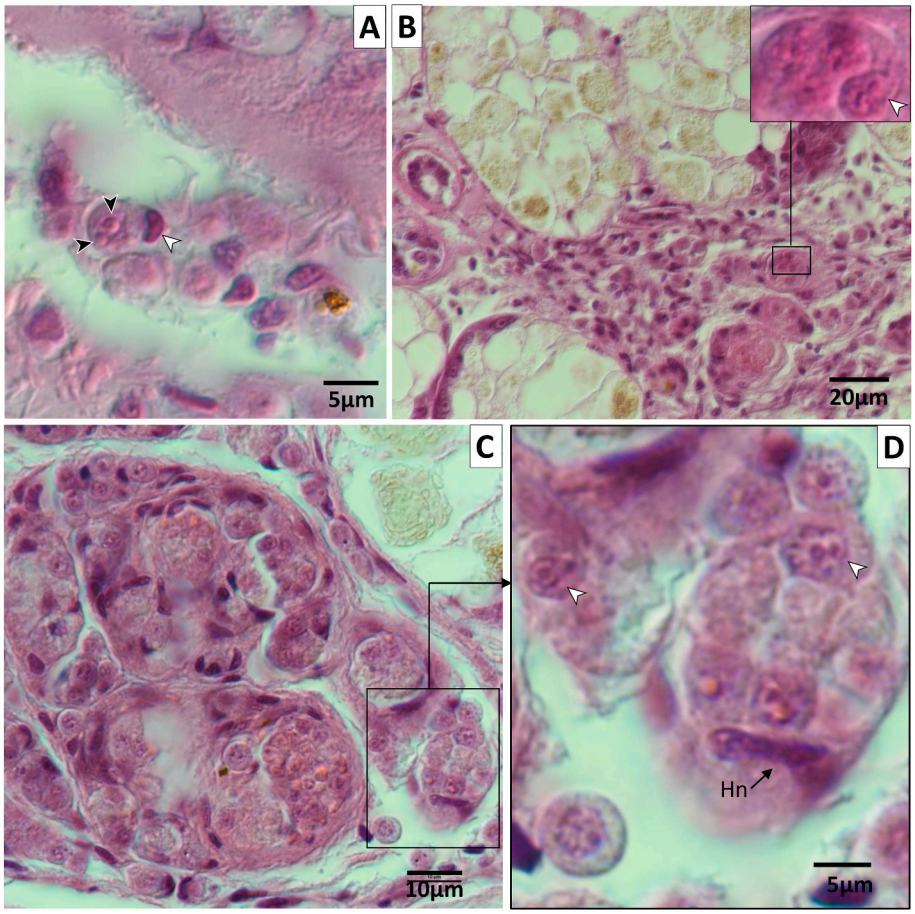

Fig. 4. HE stained section of merogony/schizogony of APXSc within the host. (A) APXSc trophozoite within a free hemocyte (white arrowhead: hemocyte nucleus) in the digestive gland connective tissue, showing signs of cleavage (black arrowheads). (B) A cluster of APXSc parasites in the digestive gland. The insert shows signs of early schizogony/ectomerogony (arrowhead). (C–D) Granulomatous swirl lesion with more advanced schizogony/ectomerogony (D) (arrowhead). Within a cyst of 6–8 parasites in one hemocyte nucleus (Hn).

(Amoroso et al., 2011). A persistent frontal system running in the north-south direction, dividing the gulf into two hydrographic domains (termed West- and East domains). These domains present clear differences in water circulation and vertical stability, causing higher larval retention in the East domain, favouring the spatial persistence and resilience of high scallop abundance east of the front, where the most important fishing grounds have historically been located (Crespi-Abril et al., 2014).

2.2. Scallop sampling and processing

A total of 250 scallops of commercial size (65.9 ± 10.8 mm) were seasonally collected by scuba diving at La Tapera (42°33′S, 64°55′W, West domain) and Punta Conos (42°19′S, 64°02′W, East domain) in the San José Gulf (Fig. 1) during 2009, approximately 30 specimens in each season from each sampling site. Live scallops were transported to the laboratory and maintained in aquaria with filtered and aerated seawater for 24 h until processing. Shell length (size) of each specimen was measured, shell and flesh were weighed separately to calculate the condition index, as the ratio of the wet flesh weight to shell weight × 100 (Lucas and Benninger 1985). The gonad stages were determined according to Lasta and Calvo (1978) (1: proliferation, 2: partially mature, 3: mature, 4: spawning; 5: spent. For histological examination, soft parts of each scallop, including mantle, gills, gonad, digestive gland, intestine and kidney, were fixed in Davidson's fixative (Howard et al., 2004) for 24 h and subsequently embedded in paraffin, sectioned at 5 µm thickness, and stained with haematoxylin and eosin (H & E). Histological sections were examined under a light microscope (Leica DM 2500) for the presence of pathological conditions and parasites as well as for the gonad developmental stage. Specimens positive for "swirl" lesions from histopathological observations were processed for TEM and *in situ* hybridization (ISH). For molecular analysis, small bits of digestive gland were fixed in 95% ethanol, until further processing.

2.3. Transmission electron microscopy and semithin sections

Small pieces of tissues from paraffin blocks containing "swirl" lesions from microscopic observations were cut at 1 mm^2, deparaffinized and extracted by dewaxing in xylene overnight, hydrating through two changes of 100, 90, and 70% ethanol. Subsequently, the tissue pieces were fixed for 2 h in 2.5% glutaraldehyde and stored in 0.05M phosphate buffered saline (PBS), pH 7.4 at 4 °C, followed by a post-fixation in 1% osmium tetroxide (OsO_4) buffered with 0.1 M PBS, pH 7.2, for 1.0–1.5 h at 4 °C, an rinsing in PBS, dehydratation in an ethanol series (30, 50, 70, 80, 95% and absolute for 30 min each), and lastly embedding in a mixture of Epon-812 resin. For TEM, ultrathin sections were double stained with 2% uranyl acetate and 1% lead citrate and observed in a JEM 1200EX II, JEOL transmission electron microscope. For light microscopy, semithin sections were made and stained with toluidine blue.

2.4. In situ hybridization

Deparaffinized histological sections, 7 µm thick, were hydrated and permeabilized with 7 µm/mL proteinase K in Tris-buffered saline (TBS) pH 8 for 12 min at 37 °C followed by a 2 × 5 min washing in PBS. Samples were then post-fixed in 0.4% paraformaldehyde in PBS for 15 min and subsequently washed for 2 × 5 min in distilled water. In order to prevent non-specific binding, sections were exposed to 10% hydrogen peroxide (H_2O_2) in methanol for 10 min and then washed in distilled water for 2 × 5 min. Subsequently, the samples were enclosed with Frame-Seal™ chambers (Bio-Rad, Sundbyberg, Sweden) and

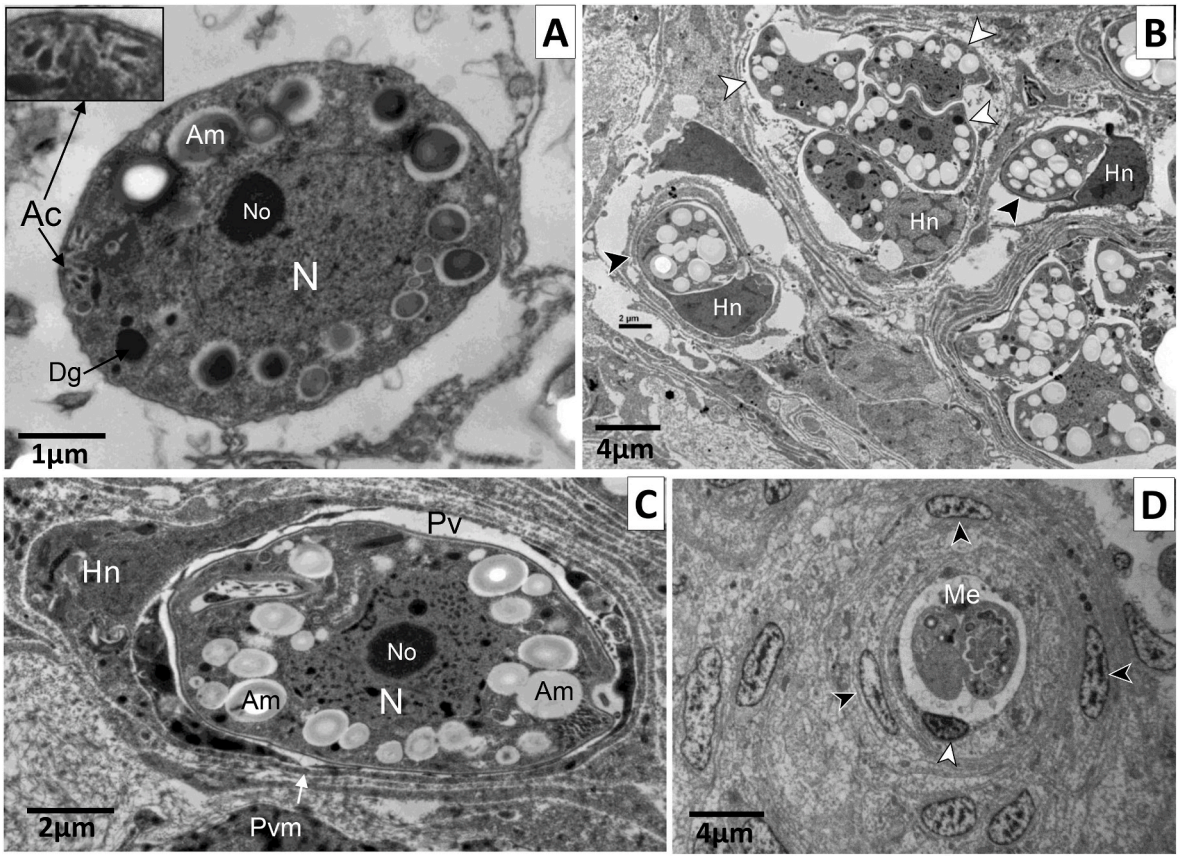

Fig. 5. Ultrastructure of common forms of APXSc. (A) An early trophozoite with a prominent nucleus (N) and nucleolus (No), amylopectin like structures (Am) and dense granules (Dg). The apical complex (Ac) is visible, presumably remains of the initial infective sporozoite. (B) A cluster of dividing pleomorphic/amoeboid forms (white arrowheads) and early trophozoites (black arrowhead) within hemocytes (Hn = hemocyte nucleus). (C) A developing trophozoite within a parasitophorous vacuole (Pv) surrounded by a parasitophorous vacuolar membrane (Pvm). (D) Two merozoites (Me) within a granuloma. Note the host cell nucleus (white arrowhead) and the surrounding fibroblast like hemocytes (black arrowheads).

equilibrated in "ready to use" hybridization buffer (Roche, Mannheim, Germany, REF: 11717472001) with added 1.5 ng ml^{-1} of each of two biotin labelled oligonucleotide probes, i.e. Ags-660-rev 5′ATCGAACCTGATTCTCACTCGGGAG 3' –biotin and Ags-1550-rev 5′GTGAGTCGAGAACGTTGAAAGTTC 3′-biotin, specially designed for this parasite.

The sections were sealed, denatured at 95 °C for 4 min followed by 18 h hybridization at 45 °C. Hybridization was followed by non-stringent and stringent washes with 2 × SSC and SSC with 0.1% Tween 20 at 42 °C, respectively. Signal detection was achieved using incubation with horseradish peroxidase-labelled streptavidin (Dako, Agilent Technologies, Glostrup, Denmark) for 20 min at room temperature, followed by 3 × 5 min washing in PBS (pH 7.4) and visualized with a DAB Peroxidase Substrate (Vector Laboratories, Burlingame, USA). Haematoxylin was applied as a counterstain, after which sections were rapidly dehydrated in series of ethanol, transferred to xylol and mounted in resin based medium.

2.5. DNA amplification and phylogenetic analyses

The genomic DNA from small pieces (approximately 20 mg) of ethanol fixed infected tissues, from five individual scallops, was extracted using a GeneMATRIX kit (EURx Poland) following the tissue protocol. Apicomplexan small subunit ribosomal DNA (SSU rDNA) was amplified using the primer pairs SFC-340f/SFC-1260r, SFC-1120f/18gM and 18eAP/AP-1010r as previously described (Kristmundsson et al., 2011a, 2015, 2018).

Amplified DNA of the expected size were recovered from the PCR products using a GeneMATRIX PCR extraction kit (EURx Poland). All PCR reactions were performed in triplicate. Sequencing reactions were performed using BigDyeTM Terminator Cycle Sequencing chemistry utilising the same oligonucleotide primers that were used for the original PCRs. DNA sequencing was performed in both forward and reverse directions for all PCR products and nucleotide BLAST searches performed for each sequence to confirm an apicomplexan origin. The contiguous sequence was obtained manually using CLUSTAL_X and BioEdit (Hall, 1999).

SSU rDNA sequences highlighted during BLAST searches with others chosen to represent all major apicomplexan lineages, plus other related basal taxa, for use as an outgroup, were selected from NCBI databases and aligned with our sequence using CLUSTAL-X. Alignment files containing 49 taxa and 2061 characters were used in phylogenetic analyses performed on PhyML 3.3.2 (Guindon et al., 2010) and Bayesian inference (BI) using MrBayes v3.2.1 (Ronquist et al., 2012). PhyML used the maximum likelihood methodology, selecting the general time-reversible substitution model (GTR) via the Smart Model Selection (Lefort et al., 2017) and used 1000 bootstrap repeats. For the BI analysis, models of nucleotide substitution were first evaluated using MrModeltest v2.3 (Nylander, 2004). The most parameter-rich evolutionary model based on the AIC was the general time-reversible, GTR + I + G. Settings used were nst = 6, with the gamma-distributed rate proportion to invariable sites (rates = invgamma). Default setting used for priors on state frequency (Prset statefreqpr = dirichlet (1,1,1,1)), and *Noctiluca scintillans* (AF022200) set as the outgroup. Posterior probability distributions were generated using the Markov Chain Monte Carlo (MCMC) method with four chains being run simultaneously for 2,000,000 generations.

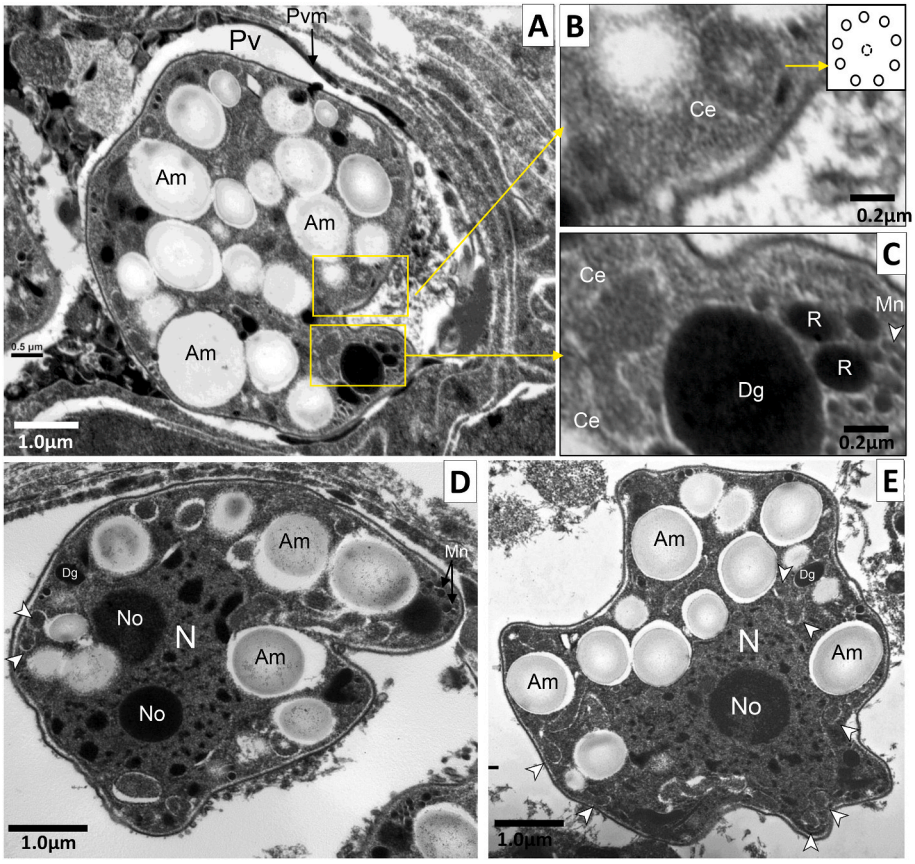

Fig. 6. TEM microphotographs showing ectomerogony of APXSc. (A–E) Pleomorphic maturing meronts, dividing by ectomerogony. (A) A developing meront in schizogony, within a parasitophorous vacuole (Pv). (B–C) Higher magnification of visible centriole (Ce) from (A), indicative of mitosis. (B) A centriole characteristic for apicomplexans, i.e. with 9 singlet microtubules arranged in a circular fashion, usually there is also one in the middle, i.e. 9 + 1, but this looks like 9 + 0, which is also known. (C) Dividing centriole (Ce), mother- (upper Ce) and daughter (lower Ce) centrioles. (D) Pleomorphic, dividing meront. Note the duplication of the nucleolus (No) within the nucleus (N) and the formation of two adjacent centrioles by division (white arrowheads). (E) A meront during schizogony, with pairs of centrioles (white arrowheads) visible at various sites in the cell. Abbreviations: N = nucleus; No = nucleolus; Ce = centriole; Dg = dense granules; Am = amylopectin; R = rhoptries; Mn = micronemes; Pv = parasitophorous vacuole; Pvm = parasitophorous vacuolar membrane.

'Burn-in' was set at 2500 and trees were sampled every 100 generations. Output files were viewed using FigTree v1.4.4 and edited in Adobe Illustrator.

2.6. Statistical analyses

Shell length comparison between collection sites was performed using analysis of variance (ANOVA), followed by Turkey's HSD test (Sokal and Rohlf, 1979). Prevalence (P) of apicomplexan infection was calculated as the ratio of infected scallops by the total scallops examined. Data on superficial seawater temperature corresponding to each season was taken monthly from the Internet web page by Toms and Omi (NASA).To evaluate the factors affecting the presence of the apicomplexan, generalized linear models (GLMs) were applied. Presence-absence (binary response) of the parasite was evaluated by GLMs with binomial distribution and a logit link function with regard the main effects as explanatory variables (Site of collection: La Tapera-Punta Conos; Season (water temperature): 1 winter, 2 autumn, 3 spring, 4 summer; Size (shell length); Gonadal development stages: 1 to 5; and Condition index). The Akaike information criterion (AIC) was used to determine the best model for the analyzed data set (Burnham and Anderson, 2002). Descriptive plots were performed to determine observed patterns and propose a full model. The full model included the independent effects of site, season, size, gonadal development stages and condition index, and one interaction effect between the site and season. Model selection was performed with an IT approach using Akaike's information criterion (AIC) and Model averaging (; Grueber et al., 2011). The AIC values (Akaike, 1973) were calculated for each model. From the AICc differences (Δi), where Δi = AICc(i) – AICc(min), Akaike weights (wi) (Akaike, 1978) were obtained for all candidate models. For each data set, the models were ranked by their wi values; the model with the highest wi was considered the one with the best supporting data (Burnham and Anderson, 2002). For the parameters of the best model, p-values below 0.05 were considered significant in all analyses. The predicted prevalences for the best model were calculated and plotted. All statistical analyses were performed in R (R Development Core Team, 2011).

3. Results

3.1. Histological examination

Apicomplexan forms, APXSc, were exclusively observed in connective tissues, most commonly adjacent to the gastrointestinal tract and between digestive gland tubules, but also, on rare occasions in mantle and gonads. Furthermore, forms were seen inside the gastrointestinal epithelium and between the epithelial cells and the basement membrane (Fig. 2A and B). The great majority of the forms observed looked very much alike in histological sections, i.e. ellipsoid and sometimes almost circular, with a prominent nucleus and nucleolus. Although the parasites were found free in the connective tissue, they were more commonly observed in a parasitophorous vacuole, within the host's hemocytes, either one or several parasites in each (Fig. 2C and D). The APXSc parasites were exclusively histozoic and not found at sites that would suggest that they were exititing the host, e.g. kidney or the lower intestine.

A severe inflammatory response was typically observed in association with the presence of APXSc, characterized by the formation of dense granulomatous aggregations forming "swirls" lesions containing necrotic hemocytes, parasites and ceroid bodies (brown cells) enclosed by fibroblast-like hemocytes (Fig. 3). The fibroblast-like hemocytes surrounded groups of parasites, commonly 4–8, many of which apparently still within a hemocyte as its marginated nucleus was seen in many cases. Most of the swirl lesions were a kind of a double granuloma, with the smaller ones formed around a group of APXSc forms, and the larger

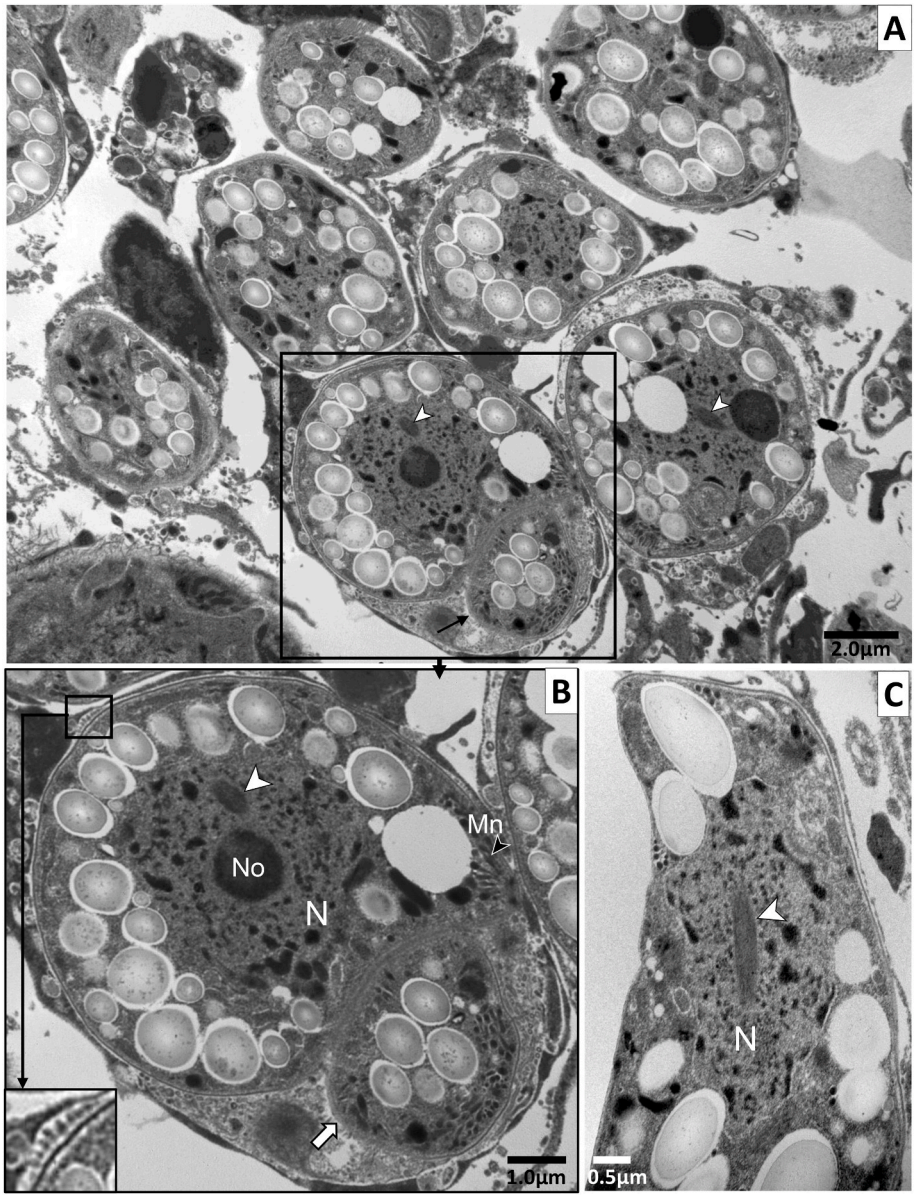

Fig. 7. TEM microphotographs showing signs of endomerogony of APXSc. (A) A group of meronts, some of which with signs of spindle microtubules within the nucleus (white arrowhead) and one showing signs of asynchronous endomerogony (within the rectangle), i.e. where the merozoites develop inside the mother cell. (B) Higher magnification of presumable endomerogony, showing two forms inside a mother cell. Remains of the conoid of the mother cell are still visible (insert) but at the other end an apical complex a "progeny" seems to be forming (micronemes = Mn), while another merozoite has completely detached from the mother cell (arrow). (C) A dividing meront with visible spindle microtubules (arrow). N = nucleus; No = nucleolus; Mn = micronemes.

one formed around a group of the smaller granulomas (Fig. 3A and B). Some of the APXSc within these granulomas are apparently undergoing degeneration (Fig. 3C–E).

Although the APXSc forms, were at first sight quite similar in appearence, they differ somewhat, both in size and morphology. In terms of size, they ranged from 3.7 to 7.2 µm (median 5.6 µm) in width. Many of the trophozoites/meronts showed obvious signs meront/schizont production by schizogony/ectomerogony, e.g. from division of the nucleoli (Fig. 4A and B), or by infolding of the membrane into the meront/schizont, apparently giving rise to 6–8 merozoites (Fig. 4C and D).

3.2. Ultrastructure of common forms of APXSc

Various different developmental forms of APXSc were identified in TEM. The youngest stages, the trophozoites, were ovoid and usually in a parasitophorous vacuole inside hemocytes. They had a relatively large nucleus and a prominent nucleolus, a number of amylopectin-like structures and dense granules. The apical complex was usually visible, presumably remaining from the infective sporozoites. Other forms were pleomorphic or amoeboid, seemingly in a schizogonic process (Fig. 5A, B,C). As seen in the H & E stained sections, many of the forms were inside a granulomatous "swirl" lesion, enclosed by fibroblast-like hemocytes (Fig. 5D). In terms of schizogony, seemingly two types were identified, i.e. ectomerogony (typical schizogony) (Fig. 6) and, less common, endomerogony (Fig. 7). When examining the pleomorphic meronts at higher magnification, clear signs of schizogony were observed (Fig. 6). Centrioles were commonly seen composed of 9 singlet microtubules arranged in a circular fashion, i.e. 9 + 0, as the central microtubule was not observed (Fig. 6A and B). Furthermore, some were dividing showing a mother and daughter centrioles (Fig. 6C and D). Two nucleoli were also seen within the same nucleus (Fig. 6E). At closer look at the whole schizonts, pairs of centrioles were seen, suggesting development of several merozoites (Fig. 6D). On rare occasions, a different kind of schizogony was observed, apparently endomerogony where the merozoites develop within the original trophozoite, i.e. the mother cell (Fig. 7A and B). Signs of mitotic spindle were also commonly seen inside the nuclei (Fig. 7A–C). Mature merozoites had all the organelles characteristic of apicomplexan zoites, i.e. an apical complex with a conoid, micronemes and four rhoptries, three layered pellicle, mitochondria and a microphore (Fig. 8A–D). However, we did not detect apicoplast with

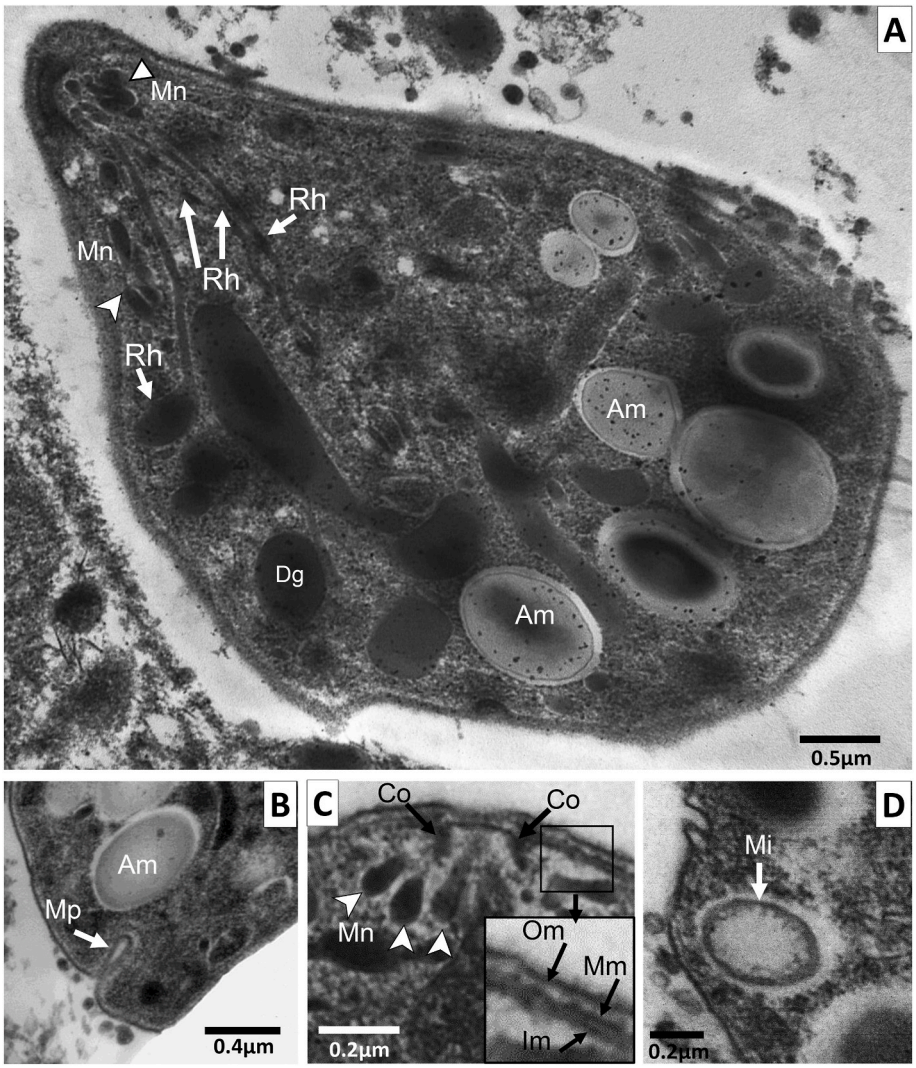

Fig. 8. Ultrastructure of mature merozoites of APXSc. (A–D) The ultrastructure of a mature merozoites showing four rhoptries (Rh), micronemes (Mn), amylopectin (Am), conoid (Co), dense granules (Dg) (A, B, C). Furthermore, a micropore (Mp) (B), a typical pellicle with an outer- middle- and inner membrane (Om, Mm, Im) (C) and a mitochondrion (Mi) (D).

any certainly. Possibly it is greatly reduced or even absent.

3.3. Hypothetical development inside the scallop host

The transmission of APXSc seems to be via the gastrointestinal tract, by active filtering of the scallop host. The infective forms invade the connective tissues by penetrating the gastrointestinal epithelium. When in connective tissues, the infect hemocytes, either passively by hemocyte phagocytosis or by active invasion. Subsequently, the young trophozoite initiates asexual reproduction by ectomerogony, producing several merozoites (presumably 4–8). It seems that a second generation of merozoites are formed by endomerogony. The infective merozoites are entrapped in host tissue, i.e. do not exit the host via urine or feces. To pass on the APXSc infection, the most likely scenario is that the scallop needs to be eaten by an unknown definitive host, either by active predation or scavenging on dead scallops remains. Gamogony and sporogony most likely occur in the unknown definitive host, from which the infective sporozoites are released to the outside environment. The scallop intermediate host then aquires infections by its filter feeding apparatus.

3.4. Phylogenetic analyses of APXSc

A SSU rDNA consensus sequence of 1758 base pairs was generated and has been deposited in GenBank under the accession number MF12345. This sequence has a 94.8% identity to sequences obtained from an apicomplexan parasite infecting the oyster, *Ostrea chilensis*, in New Zealand. However, it is not closely related to other apicomplexans using nucleotide BLAST searches, with the next most similar sequences belonging to colpodellids (<90% similarity) with respect to the SSU rDNA sequence. These findings are supported by the phylogenetic analyses, where the sequence generated in the present study, forms a fully supported clade with the oyster parasite from New Zealand, but this grouping is not associated with any of the known apicomplexan clades and is placed at the base of our tree next to the Chrompodellids (Fig. 9). Bayesian inference also showed the novel sequence grouping with oyster parasite from New Zealand, where it formed an unresolved polytomy at the base of the apicomplexan tree next to the Chrompodellids and the dinoflagellate outgroup (data not shown).

3.5. Epidemiology of the APXSc infection

A summary of the main characteristics (size, condition index, gonad development stage) of *Aequipecten tehuelchus* from West (La Tapera site)

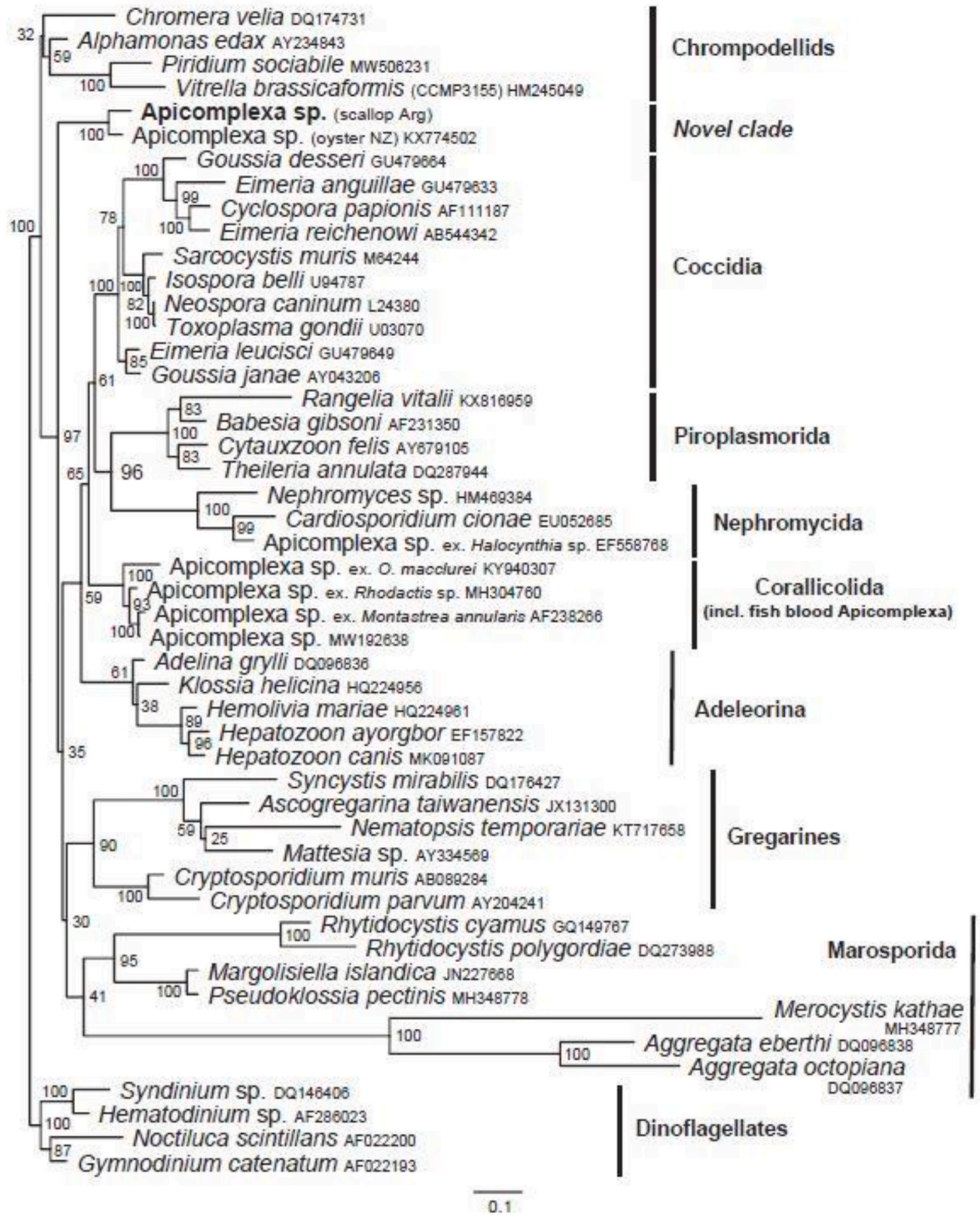

Fig. 9. Maximum likelihood topology from phylogenetic analysis of the SSU rDNA of the main clades of apicomplexans and the basal Chrompodellids, using the dinoflagellates as an outgroup. Numbers at the nodes denote bootstrap support values (1000 replications) with the scale shown at the bottom of the tree. The sequence generated in this study is shown in bold and groups together with a parasite known to infect oysters in New Zealand. This novel clade does not show any phylogenetic affinity to the known clades of apicomplexans infecting marine and terrestrial animals and is placed at the base of the tree next to the Chrompodellids.

and East (Punta Conos site) domains in the San José Gulf are presented in the Table 1.

Scallops from La Tapera were significantly smaller (57.24 ± 8.7 mm) than those from Punta Conos (74.06 ± 5.6 mm) (Tukey HSD test, $p < 0.01$). The highest prevalence of APXSc was recorded in scallops from Punta Conos (72.0%) versus La Tapera (24.3%). The best model (<AICc) included the independent effect of size and the interaction effect of site and season (Table 2). A positive relationship was found between the predicted prevalence of APXSc and the size of the scallop, indicating that bigger (older) scallops were more frequently parasitized than the smaller ones (Fig. 10). Regarding the site and season, the prevalence was higher and relatively similar (60–90%) in all seasons in Punta Conos. In contrast, the predicted prevalence was significantly lower on La Tapera reaching a prevalence of 40% in summer (Fig. 11).

Table 1
Main characteristics of *Aequipecten tehuelchus* (size, condition index and gonad development stage) from the West and East domains in San José Gulf.

Site	Summer	Autumn	Winter	Spring
West Domain				
Mean shell size (mm)	53	54	57	65
Condition index	100	108	85	126
Gonad stage	mature	spawning	proliferation *	mature *
East Domain				
Mean shell size (mm)	70	72	75	78
Condition index	107	129	116	107
Gonad stage	mature	spent *	proliferation	mature

4. Discussion

This study reports APXSc, a novel apicomplexan and an unusual one, particularly in terms of host reaction towards infection, i.e. the formation of swirl-lesions and its unique phylogenetic status.

4.1. APXSc phylogeny

The DNA data obtained from the novel apicomplexan in this study was most related to the parasite described infecting oysters and other bivalves in New Zealand. Whilst phylogenetic analyses consistently and robustly grouped these two parasites together, there was no relationship with any of the known clades within the Apicomplexa. Apicomplexans have been generally considered to be a single group of obligate animal parasites, but recent single-cell genomic data has revealed that at least three separate lineages of apicomplexans have independently evolved to become parasitic (i. modern apicomplexans (largest group), ii. *Piridium*, iii. *Platyproteum*) (Mathur et al., 2019). Our phylogenetic analyses place the novel parasite from this study at the base of the main apicomplexan lineage (i.e. not related to *Piridium* or *Platyproteum*), which suggests that it may be the ancestral form for some or all clades of modern apicomplexans, however, additional phylogenomic data is going to be required to demonstrate this reliably.

APXSc life stages and life cycle A parasite, morphologically similar to APXSc, has not previously been observed in molluscs of the region nor the host inflammation response (Vázquez and Cremonte, 2017). With only merogonic stages observed, and an apparent absence of gamogony and sporogony, strongly suggests that it is a heteroxenous apicomplexan, with the Tehuelche scallop as an intermediate host, whilst the definitive host is unknown at present. However, histopathological examination did not imply that the parasite was exiting the host as they were exclusively histozoic and not found in the kidneys or the intestinal lumen. That might indicate that the Tehuelche scallop must be eaten by the parasite's definitive host to complete APXSc's full reproductive life cycle, making animals predating or scavenging on the scallops likely candidates as definitive hosts. In general, scallops have various predators, the most significant ones being different species of decapod crustaceans, gastropods, octopuses as well as many benthic finfish species

Table 2
Selected model presented the lowest AIC value from a set models according to the step function of R program for the apicomplexan APXSc infection to Aequipecten tehuelchus. Models included as factors: site (West and East) and season (summer, autumn, winter and spring) and covariables included (scallop length). In each model, the intercept is represented by the level West site and Summer season. Significant probabilities (<0.05) are highlighted with an asterisk. Abbreviations: AU (autumn) CI (condition index), E (east) L (scallop length), Se (season) Si (site), SP (spring), WI (winter).

Parasite	Selected model	Parameters	Estimate	Standard Error	Z value	Pr (>|z|)
APXSc apicomplexan	P = Si + L + Se + Si*Se	Intercept	−7.181	1.579	−4.546	5.47^{e-06}*
		L	0.127	0.028	4.535	5.77^{e-06}*
		Si_E	−1.227	0.736	−1.665	0.095
		Se_{Au}	−1.388	0.702	−1.976	0.048*
		Se_{Wi}	−1.703	0.679	−2.508	0.012*
		Se_{Sp}	−2.761	0.762	−3.621	0.0002*
		Si_E*Se_{Au}	1.653	0.907	1.822	0.068
		Si_E*Se_{Wi}	1.875	0.857	2.188	0.028
		Si_E*Se_{Sp}	2.385	0.907	2.628	0.0085*

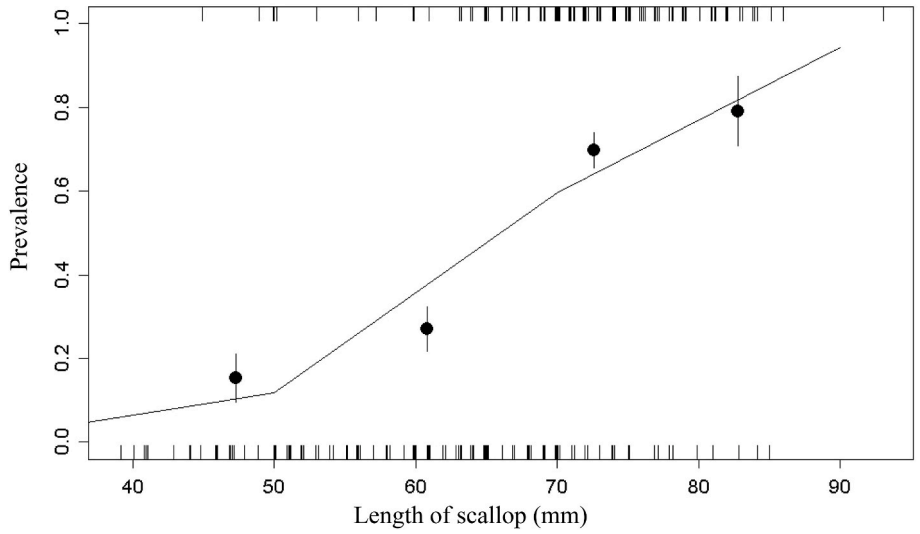

Fig. 10. Logistic regression of the presence or absence of apicomplexan (APXSc) parasite as a function of size (shell length) in the wild Tehuelche scallop *Aequipecten tehuelchus*. The tick marks along the bottom and top lines show the locations of the data points along the x-axis. The black dots indicate the mean and SE of the predicted proportions.

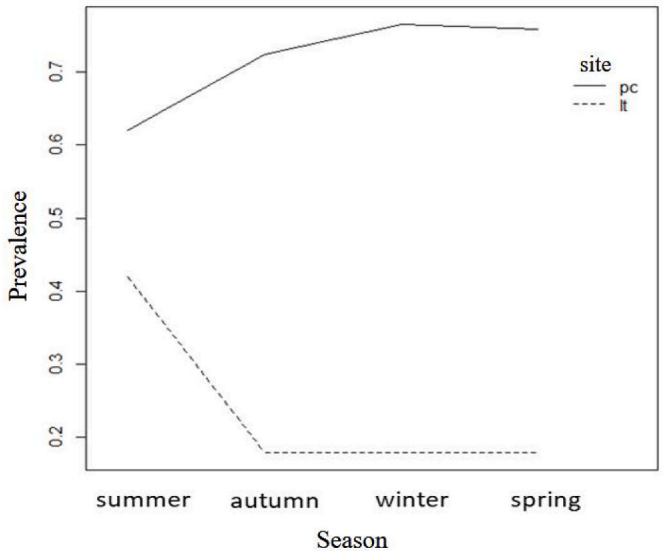

Fig. 11. Interaction plot of the predicted prevalences of aplicomplexan (APXSc) as function of season in the wild Tehuelche scallop *Aequipecten tehuelchus*.

(Brand, 2006). In terms of bivalves in Patogonian waters, the bull crab *Platyxanthus patagonicus*, is a well known predator and scavenger (Morsan, 2008).

Considering previous research on bivalves from the same geographic area (*Ostrea puelchana, Pododesmus rudis, Mytilus edulis, Aulacomya atra, Panopea abbreviata, Ensis macha, Leukoma antiqua*) it seems that APXSc is specific, at least to some extent, to the Tehuelche scallop, as none of the other bivalve species examined were found infected (Vázquez and Cremonte, 2017). Possibly pectinid bivalves are susceptible intermediate hosts, similar to the apicomplexan parasite *Merocystic kathae*, which has its definitive host in a buccinid gastropod (Kristmundsson and Freeman, 2018).

A positive relationship between the presence of the APXSc with the colder season and the size of the scallops was evidenced. Cold temperatures would favor the transmission of the parasite, when the bivalve seemed to be more susceptible due to minima of food availability (Soria et al., 2014). Likewise, older scallops were more infected. This phenomenon is usually attributed to the fact that larger hosts are older and had longer exposure time for infection. In the case of *A. tehuelchus*, where older scallops are sedentary filter-feeders, it is assumed that larger specimens have higher filtering rates facilitating the entry of the parasite with water currents.

4.2. Bivalve mortality and apicomplexans

No mass mortalities or detrimental effect on the condition index of the infected Teheulche scallops was evidenced. Nevertheless, the severe host inflammation response evoked by the apicomplexan parasite would involve a metabolic energy cost for the host. Moreover, stranding, induced by storms and strong winds, was widely reported to be the main source of mortality along the north coast of San José gulf (Orensanz, 1986; Orensanz et al., 1991). These stranding mortalities seemed to increase with scallop size; suggesting that infected specimens are more likely to be removed from the bottom. APXSc parasitized mostly scallops from the East. These differences could be explained by the characteristics of the two oceanographic domains, which present distinct hydrographic regimes. The highest prevalence of the apicomplexan parasites may be due to the calm and more homogeneous domain of the San José gulf (SJG), where the nutrients from the continental shelf are 'trapped in' and larvae are retained (Amoroso and Gagliardini, 2010; Amoroso et al., 2011). These protozoans were found most prevalent in the East domain, where the oceanographic characteristics would favor the retention of the apicomplexan life stages involved in the infection process. Moreover, Amoroso et al. (2011) reported that scallops from the East domain exhibit higher growth rates as that nutrient-rich water flows eastward of the SJG, finding the biggest specimens in this domain.

Mass mortality in bivalves related to apicomplexans are known from the literature. The apicomplexan parasite *Merocystis kathae* (Aggregatidae; Marosporida) has been identified in five different scallop species from different geographic areas, the Iceland scallop, *Chlamys islandica* from West Iceland, *Pecten maximus* from UK waters, *A. opercularis*, from UK and Faroese waters, *P. magellanicus*, off the East coast of USA and *Patinopecten caurinus* from Alaskan waters. It caused mass mortalities in Iceland scallop in the 2000s and has furthermore been a suspected cause of m in *P. magellanicus* in the NW Atlantic and *P. caurinus* in Alaskan waters (Kristmundsson et al., 2011b, 2015; Inglis et al., 2016; Ferguson et al., 2021). It is interesting to note that the closest known relative of APXSc, i.e. the apicomplexan observed in New Zealand waters (termed APX), is considered to contribute to a disease process in the oyster *Ostrea chilensis* which is caused by the oyster pathogen *Bonamia exitiosa* (see Hine 2002). A species genetically very similar to APX has furthermore been identified in three other bivalve species (Suong et al., 2017, 2019).

4.3. Swirl lesions in scallops

Unusual lesions of "swirls", similar to those observed in APXSc infected Tehuelche scallop, have been observed in scallops in previous studies, i.e. in Bay scallop *Argopecten irradians* in the Northwest Atlantic (McGladdery et al., 1991; Whyte et al., 1994) and Chilean scallops *A. purpuratus* (see Lohrmann, 2009). In case of the Bay scallop, these swirl lesions, which were observed in a range of host tissues, were originally thought to be a response to *Perkinsus karlssoni* (see McGladdery et al., 1991; Whyte et al., 1994). However, at present *P. karlssoni* is not considered a perkinsids at all and therefore not a valid species. It did not give positive reaction in *Perkinsus* targeted *in situ* hybridization (Goggin et al., 1996; Getchell et al., 2016). Its etiology is still unknown, but has been suggested to be related to the thraustochytrid/labyrinthuloid complex, based on ultrastructural features (Goggin et al., 1996). In the Chilean scallop, swirl lesions were observed in the visceral connective tissues underneath the epithelium of stomach or secondary ducts. The authors were not able to identify the parasite and described it as "Type 1 granuloma" (Lohrmann, 2009).

5. Conclusions

Further research are needed to obtain a better understanding of APXSc. Its phylogenetic status is unresolved as it does not fit in any of the known apicomplexan clades, due to lack of molecular data on similar organisms. The Tehuelche scallop is the intermediate host for APXSc. It aquires infections via the gastrointestinal tract, while asexual reproduction (merogony) occurs in connective tissues. The absence of APXSc in other bivalve species in the San José gulf in previous studies (Vázquez and Cremonte, 2017), suggests that it is, at least somewhat, host specific in terms of its intermediate host. The pathological conditions caused by the apicomplexan, APXSc, warrants regular monitoring of the stocks. The definitive host of this apicomplexan is unknown at present. Species feeding on the Tehuelche scallop seem the most likely candidates as definitive hosts.

Declaration of interests

The authors declare no competing interests.

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