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

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**ARTICLE INFO**

**Keywords:**
- Apicomplexan scallop
- *Aequipecten tehuelchus*
- Histopathology
- Phylogeny
- Ultrastructure

**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*, *Margoliessia islandica* and *Merocystis kathae* (Léger and Duboscq, 1917; Kristmundsson et al., 2011a; 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-Amercia (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...
(Karlsson, 1991) and *Pseudoklossia* sp. (Cawthorn et al., 1992) reported from Bay scallop *Argopecten irradians*.

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. 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), suggesting 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.
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 Argentinian 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 km²) connected by a narrow mouth to the much larger San Matías Gulf (18,000 km²) (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.
(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 mm², 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 (OsO₄) 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 hybridisation

Deparaffinized histological sections, 7 μm thick, were hydrated and permeabilized with 7 μm/mL protease 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 (H₂O₂) 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
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\'\text{ATCGAACCTGATTCTCACTCGGGAG} 3\' –biotin and Ags-1550-rev 5\'\text{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. 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).
Model selection was performed with an IT approach using Akaike information criterion (AIC) and Model averaging (Burnham et al., 2011). 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 to 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 exiting 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 appeared 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...
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 appearance, 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 of 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...
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 entraped 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 acquires 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)
Scallops from La Tapera were significantly smaller ($57.24 \pm 8.7$ mm) than those from Punta Conos ($74.06 \pm 5.6$ 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 ($\Delta$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).
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 1

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

| Site          | Summer | Autumn | Winter | Spring |
|---------------|--------|--------|--------|--------|
| Mean shell size (mm) | 53     | 54     | 57     | 65     |
| Condition index   | 100    | 108    | 85     | 126    |
| Gonad stage       | mature | spawning | proliferation | mature * |

East Domain

| Site          | Summer | Autumn | Winter | Spring |
|---------------|--------|--------|--------|--------|
| Mean shell size (mm) | 70     | 72     | 75     | 78     |
| Condition index   | 107    | 129    | 116    | 107    |
| Gonad stage       | mature | spent * | proliferation | mature |

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*<0.001 |
|                   |                | L             | 0.127   | 0.028          | 4.535   | 5.77*<0.001 |
|                   |                | Se            | -1.257  | 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* |
|                   |                | Se*Se_AU      | 2.653   | 0.907          | 2.822   | 0.0068 |
|                   |                | Se*Se_WI      | 1.875   | 0.857          | 2.188   | 0.028 |
|                   |                | Se*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.
In terms of bivalves in Patagonian waters, the bull crab 
Platxanthus 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 Cre-
mente, 2017). Possibly pectinid bivalves are susceptible intermediate 
hosts, similar to the apicomplexan parasite Merocystis kathae, which has 
itself acquired in a buccinid gastropod (Kristmundsson and Freeman, 
2018).

A positive relationship between the presence of the APXSc with the 
cold season and the size of the scallops was evidenced. Cold temper-
atures 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 phe-
nomenon 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 
infectet Tehuelche scallops was evidenced. Nevertheless, the severe 
host inflammation response evoked by the apicomplexan parasite would 
involve a metabolic energy cost for the host. Moreover, standing, 
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 standing 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 characteris-
tics of the two oceanographic domains, which present distinct hydro-
graphic 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 pro-
cess. 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 (Aggregati-
dae; 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. magellanicas, 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. magellanicas 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 
infectet 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 Lohmann, 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 McGladd-
dery 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 thraustochytriid/labyrinth-
inthuloid 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” (Lohmann, 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 
oragnisms. The Tehuelche scallop is the intermediate host for APXSc. It 
adquires infections via the gastrointestinal tract, while asexual repro-
duction (merogony) occurs in connective tissues. The absence of APXSc 
in other bivalve species in the San José gulf in previous studies (Vázquez 
and Cremente, 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.

Acknowledgements

We deeply acknowledge Christian Ituarte for providing valuable
TEM images and for joining us these years. This project was funded by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP 2021-2023 11220200102767CO). N.V. C.G. and F.C. are members of CONICET.

References

Akaie, H., 1973. Information theory as an extension of the maximum likelihood principle. In: Petrov, B.N., Csaki, F. (Eds.), Second International Symposium on Information Theory. Akademiai Kiado, Budapest, pp. 267–281.

Akaie, H., 1978. A Bayesian analysis of the minimum AIC procedure. Ann. Inst. Stat. Math. 30, 9–14.

Amoroso, R.O., Gagliardini, D.A., 2010. Inferring complex hydrographic processes using remote-sensed images: turbulent fluxes in the Patagonian Gulfs and implications for scallop metapopulation dynamics. J. Coast Res. 26, 320–332.

Amoroso, R.O., Parma, A.M., Orenzana, J.M., (Lobo), Gagliardini, D.A., 2011. Zooming the microscope: medium-resolution remote sensing as a framework for the assessment of a small-scale fishery. ICES J. Mar. Sci. 68, 696–706.

Aranda, D.A., Frenkeli, L., Bruné, T., Montero, J., Cárdenas, E.B., 2011. Occurrence of Apicomplexa-like structures in the digestive gland of Stroumbus gigas throughout the Caribbean. J. Invertebr. Pathol. 106, 174–178.

Bondad-Reantaso, M.G., Arthur, J.R., 2008. Pathogen risk analysis for aquaculture production. In: Bondad-Reantaso, M.G., Arthur, J.R., Subasinghe, R.P. (Eds.), Understanding and applying risk analysis in aquaculture, 519. FAO Fisheries and Aquaculture Technical Paper, Rome, pp. 27–46.

Brand, A.R., 2006. Scallop ecology: distribution and behaviour. In: Shumway, S.E., Bondad-Reantaso, M.G., Arthur, J.R., Cawthorn, R.J., Upton, S., (Eds.), Scallops: Biology Ecology and Aquaculture. Elsevier Press, Amsterdam, p. 131–157.

Cremonte, F., Figueras, A.M., Burreson, E.M., 2005. A histopathological survey of some lesions in bay scallops (Argopecten purpuratus) from Chile. A histopathological survey. Dis. Aquat. Org. 51, 14–20.

Diyova, L., 1992. Protozoan Parasites of Fishes. Elsevier Science Publishers B.V., Amsterdam, p. 315.

Ho, A., Rasouli, A., Kasler, B.K., 2012. Genetic diversity and population structure of the Pacific oyster, Crassostrea gigas, through CR and mitochondrial DNA markers. Mar. Genet. Genomics 4, 351–359.

Hughey, R., 2005. FastPhyML: rapid maximum likelihood phylogenetic estimation. Bioinformatics 21, 434–435.

Jerez, I., 2020. How effective are vaccination strategies against Adenovirus in the marine bivalve mollusks and crustaceans. NOAA Tech. Memo. NOS NGS 5, 218.

Kristmundsson, A., Hansen, H., Alarón, M., Freeman, M.A., 2018. An eimerian apicomplexan causing pathology in wild and farmed lampfish, Cyclopterus lumpus. Bull. Mar. Sci. 89, 213–222.

Kwong, W.K., Irwin, N.A., Mathur, V., Na, I., Okamoto, N., Vermeij, M.J., Keeling, P.J., 2021. Taxonomy of the apicomplexan symbionts of coral, including Corallolidia ord. Nov., reassignment of the Genus Gemmocystis, and description of new species Corallolidia aquarius gen. Nov. sp. nov. And Anthozaophyllum gratus gen. Nov. sp. nov. J. Eukaryot. Microbiol. 68, e125852.

Leder, M., Duboscq, O., 1917. Pseudoklossia pectinis n. sp. et lorigine de ses adéochres. Arch. Zool. Éppl. Exp. Gen. (Suppl Notes Rev.) 68, 88–94.

Leibovitz, L., Schoot, E.F., Korney, R.C., 1984. Diseases of wild captive and cultured scallops. J. World Maricult. Soc. 14, 69–83.

Lucas, A., Benninger, P.G., 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture 44, 187–200.

Maurin, V., Koloso, M., Hebert, P.D.N., Irwin, N.A.T., Leander, B.S., Kristmundsson, A., Freeman, M.A., Keeling, P.J., 2019. Multiple independent origins of apicomplexan-like parasites. Curr. Biol. 29, 2936–2941 e5.

McCladghery, S.E., Cawthorn, R.J., Bradford, B.C., 1991. Perkinus kauensis n. sp. in Scallop (Pectinidae) in New Zealand. Dis. Aquat. Org. 10, 127–137.

Morado, J.F., Sparks, A.K., Reed, S.K., 1984. A coccidian infection of the kidney of the native littleneck clam, Protothaca staminea. J. Invertebr. Pathol. 43, 207–217.

Moran, E.M., 2008. Impact on biodiversity of scallop dredging in san Matias gulf, northern Patagonia (Argentina). Hydrob. (Sofia) 619, 167–180.

Muehl, M., Pales Espinosa, E., Tettelbach, S., Geraci-Yee, S., Farhat, S., Kristmundsson, A., Allam, B., 2021. Is an apicomplexan parasite responsible for the collapse of the bay scallop (Argopecten irradians irradians) population in New York? In: National Shellfisheries Association Meeting, Virtual Meeting, March 2021.

Nylander, J.A.A., 2004. MrModeltest v.2. Program distributed by the author. Bioinformatics 24, 581–583.

Orenzana, J.M., 1986. Size, environment, and density: the regulation of a scallops stock and its management implications. Can. Spec. Publ. Fish. Aquat. Sci. 92.

Orenzana, J.M., Parma, A.M., Irbarine, O., 1991. Population dynamics and management of natural stocks. In: Shumway, S.E. (Ed.), Scallop Biology, Ecology and Aquaculture. Developments in Aquaculture and Fisheries. Elsevier, Amsterdam, pp. 625–714.

R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, ISBN 3-900051-07-0. Available at: http://www.R-project.org.

Ronquist, F., Teslenko, M., van der Mark, F., Ayres, D.L., Darling, A., Hobma, S., Larteg, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 593–594. http://dx.doi.org/10.1093/sysbio/yys029.

Rueckert, S., Chantangis, C., Leander, B.S., 2010. Molecular systematics of marine gregarines (Apicomplexa) from North-eastern Pacific polychaetes and nemertean species, with descriptions of three novel species: Lecudia phyllochaetopteri sp. nov., Diffillicula tubulina sp. nov. and Diffillicula paramerites sp. nov. Int. J. Syst. Evol. Microbiol. 60, 2681–2690.

Seed, J.R., 1996. Protozoa: pathogeneses and defenses. In: Baron, S. (Ed.), Medical Microbiology, fourth ed. Galveston, Texas, pp. 184–199.

Sokal, R.R., Rohlf, F.J., 1979. Biometry. Principles and statistical methods in biology research. (Biometría. Principios y métodos estadísticos en la investigación biológica). Blume (Bulmej), Madrid, p. 832.

Soria, G., Orenzana, J.M., (Lobo), Morsan, E.M., Parma, A.M., Amoroso, R., 2014. Scallop biology, fisheries and management in Argentina. Dev. Aquacult. Fish. Sci. 40, 1019–1046.

Suong, N.T., Webb, S., Banks, J., Wakeman, K.C., Lane, H., Jeffs, A., Bronnah, C., Jones, B., Fidler, A., 2017. Partial 18S rRNA sequences of apicomplexan parasite ‘X’ (APX), associated with flat oysters Ostrea chilensis in New Zealand. Dis. Aquat. Org. 127, 1–9.

Suong, N.T., Banks, J.C., Fidler, A., Jeffs, A., Wakeman, K.C., Webb, S., 2019. PCR and histology identify new bivalve hosts of Apicomplexan-X (APX), a common parasite of New Zealand flat oyster Ostrea chilensis. Dis. Aquat. Organ 132, 181–189.

Toms and Omni Online Visualization and Analysis System using Giovanni. GES-DISC, NASA URL: http://giovanni.gsfc.nasa.gov/.

Kristmundsson, A., Helgason, S., Bambir, S.H., Eyðali, M., Freeman, M.A., 2011b. Previously unknown apicomplexan species infecting Iceland scallop, Chlamys islandica (Müller, 1776) (Apicomplexa: Eimerididae) infecting Iceland scallop Chlamys islandica (Müller, 1776) in Icelandic waters. J. Invertebr. Pathol. 108, 139–146.

Margelostia islandica sp. nov. (Apicomplexa: Eimerididae) infecting Iceland scallop Chlamys islandica (Müller, 1776) in Icelandic waters. J. Invertebr. Pathol. 108, 139–146.

Kristmundsson, A., Helgason, S., Bambir, S.H., Eyðali, M., Freeman, M.A., 2011a. Margelostia islandica sp. nov. (Apicomplexa: Eimerididae) infecting Iceland scallop Chlamys islandica (Müller, 1776) in Icelandic waters. J. Invertebr. Pathol. 108, 139–146.
Vázquez, N., Cremonte, F., 2017. Review of parasites and pathologies of the main bivalve species of commercial interest of Argentina and Uruguay, Southwestern Atlantic coast. Arch Parasitol. 1, 2.

Whyte, S.K., Cawthorn, R.J., McGladdery, S.E., 1994. Co-infection of bay scallops Argopecten irradians with Perkinsus kalmii (Apicomplexa, Perkinsea) and an unidentified coccidian parasite. Dis. Aquat. Org. 18, 53–62.

Winstead, J.T., Volety, A.K., Tolley, S.G., 2004. Parasitic and symbiotic fauna in oysters (Crassostrea virginica) collected from the Caloosahatchee River and Estuary in Florida. J. Shellfish Res. 23, 831–840.