ORIGINAL PAPER

Cryoxcellia borchgrevinki gen. nov., sp. nov., a new parasitic X-cell species in an Antarctic nototheniid fish, the bald notothen *Trematomus borchgrevinki*

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Abstract

X-cells were first described as an unknown cell type in northern hemisphere flatfish in 1969. Almost a decade later they were described in an Antarctic fish, the bald notothen *Trematomus borchgrevinki*, thus demonstrating their global distribution. Since this time, X-cells from various northern hemisphere fish species and from three other Antarctic fishes, the emerald notothen *Trematomus bernacchii*, the crowned notothen *Trematomus scotti*, and the painted notothen *Nototheniops larseni* have been identified as perkinsozoan parasites of the Family Xcellidae. Currently there are seven X-cell species described within this family. Here we report the morphology of X-cells isolated from the gill filaments of the bald notothen and include details of some of its division forms. Using short-read high-throughput DNA sequencing technology we have sequenced, assembled, and verified a 5347-bp region of the X-cell rRNA repeat unit that includes the complete 18S gene. In all cases, phylogenetic analyses identified this sequence as a distinct taxon and placed it among the perkinsozoan alveolates alongside other previously identified species in the X-cell family. Using a combination of morphological and genetic evidence we now describe a new X-cell genus and species, *Cryoxcellia borchgrevinki* gen. nov., sp. nov., from McMurdo Sound, Antarctica.

Keywords Antarctica · McMurdo Sound · Perkinsozoa · X-cell disease · Xcellidae · 18S

Introduction

X-cells are known to have a global distribution and have been reported in several fish species that typically inhabit marine (sometimes brackish) bodies of water (Freeman et al. 2017). Different tissues and organs may be infected by the causal organisms (Miwa et al. 2004), subsequently identified as parasitic alveolates closely related to the genus *Perkinsus* (Freeman et al. 2017). All four X-cell species identified were placed in the Family Xcellidae within the Order Perkinsida (Eukaryota; Sar; Alveolata; Perkinsozoa; Perkinsea). Subsequent studies of X-cell disease have identified three new species in two new genera of X-cell organisms, which assist in establishing phylogenetic relationships within the family (Karlsbakk et al. 2021; Desvignes et al. 2022).

Members of the Family Xcellidae as defined by Freeman et al. (2017) are described as characteristically forming swellings or xenomas (tumor-like growths) in host tissues. In the classic xenoma, the causal parasites invade host cells within which they proliferate. This is not the case with X-cells, however, which although parasitic appear to remain independent entities (Freeman et al. 2017). In histological sections, X-cells are typically described as having a polygonal outline, but this can vary from being irregularly angular to more rounded in form (Freeman et al. 2017). The nucleus is weakly reactive in haematoxylin and eosin-stained histological sections, but more often than not it encloses one or sometimes two prominent, reactive nucleoli (Freeman et al. 2017). Although various morphological forms have been described, classic X-cells are relatively large (diameter may exceed 20 μm) and are often, but not always, surrounded by an envelope of host cells (Freeman et al. 2017). At certain stages of development, some X-cell species are enclosed within an extracellular capsule that is not always obvious microscopically (Watermann and Dethlefsen 1982), but overall their developmental stages are poorly described and their complete life cycle is unknown.

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Interestingly, X-cell disease has been identified in a number of trematomin fish species that inhabit high latitudinal waters of the Southern Ocean, including the bald notothen (*Trematomus borchgrevinki*), the striped rockcod (*Trematomus hansoni*), the emerald notothen *(Trematomus bernacchii)*, the sharp-spined notothen (*Trematomus pennellii*), and the spotted notothen (*Trematomus nicolai*) from McMurdo Sound, in which it is always found infecting the gills (Davison 1998; Evans and Tupmongkol 2014). It has also been reported in the grey rockcod (*Lepidonotothen squamifrons*) from South Georgia (Bucke and Everson 1992) and the icefish (*Chionodraco hamatus*) from McMurdo Sound (Montgomery and Wells 1993), but both of these are unconfirmed observations.

Generally, in fish gills, X-cells cram the basal region between adjacent gill lamellae of the host fish and may spread distally along the secondary lamellae between the surface epithelium and the lamellar capillary (Evans and Tupmongkol 2014). Recently, Desvignes et al. (2022) have reported further examples of X-cell disease in two other Antarctic fishes, the crowned notothen (*Trematomus scotti*), and the painted notothen (*Nototheniops larseni*), but in both cases the disease was found infecting the skin.

With the increasing use of genetic markers in systematics and the advent of next-generation sequencing technologies, the taxonomic status and phylogenetic relationships of this enigmatic group of organisms have been recently investigated (Freeman et al. 2017; Karlsbakk et al. 2021; Desvignes et al. 2022). All of the recent studies used the rRNA repeat unit or genes found within these repeat units (i.e., 18S) to support taxonomic description, to estimate phylogenetic trees, and to investigate the geographic distribution of identified species. For example, a distance-based phylogenetic tree using a partial 18S rDNA gene sequence from X-cells in the emerald notothen (*T. bernacchii*) has shown that they are closely related to those in the common dab (*Limanda limanda*), suggesting a global distribution of these organisms (Evans and Tupmongkol 2014).

We now provide descriptions of the gross morphology of X-cell disease in *T. borchgrevinki* and of various X-cell forms present in the diseased tissue. We also provide a verified rRNA repeat unit (which includes complete 18S and 5.8S sequences and a partial 28S sequence) of X-cells from this fish host that enables refinement of the X-cell phylogeny and a formal taxonomic description of a new X-cell genus and species, *Cryoxcellia borchgrevinki* gen. nov., sp. nov.

Materials and methods

Specimen collection

Specimens of the bald notothen (*T. borchgrevinki*) were collected by hook and line from various localities in McMurdo Sound, Antarctica. The fish bearing the type X-cell specimens was collected from an ice hole (77° 52.624′S, 166° 39.882′E) near the McMurdo Station sea-ice runway between 15 and 23 December 2011. X-cell lesions were identified in the gills by their characteristic white-tippy appearance (Fig. 1) and samples of infected tissue were fixed in either 2% paraformaldehyde, 95% ethanol, or 10% formalin for subsequent examination by light microscopy. Gills containing X-cells were also frozen in liquid nitrogen, transported to New Zealand in a dry shipper and stored at − 80 °C.
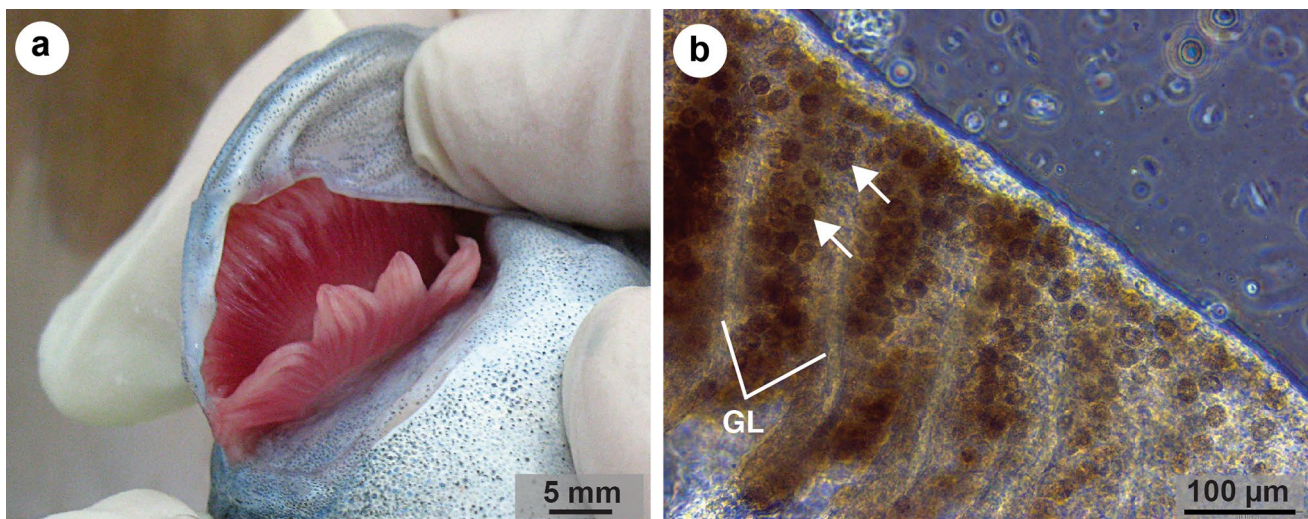
Fig. 1 *Cryoxcellia borchgrevinki* gen. nov., sp. nov. X-cell disease: gross and wet mount appearance. **a**, White-tipped gill filaments in a specimen of the bald notothen (*Trematomus borchgrevinki*). **b**, Dark spherical X-cells (arrows) pack the spaces between the secondary gill lamellae (GL) in the basal region (wet mount; 10% formalin)

Morphology and light microscopy

Fixed or fresh tissue samples, either as small pieces or teased apart to free the X-cells, were examined as wet preparations under transmitted light using differential interference contrast (DIC) or phase contrast optics.

DNA extraction, library construction, and short-read high-throughput sequencing

DNA was extracted from frozen-infected fish gill tissue of a single specimen. Details relating to the extraction process, library construction, and Illumina sequencing can be found in Patel et al. (2022). The Illumina HiSeq sequencing data were quality checked with FastQC (Andrews 2010) to ensure that there were no obvious problems with the sequencing process or the resulting data. All putative fish reads were identified and removed using the methods detailed in Patel et al. (2022). The resulting reduced dataset without potential fish nuclear or fish mitochondrial sequences was assembled using IDBA-UD (Peng et al. 2012) with the default parameters. The IDBA-UD assembler is designed for use in cases where a set of sequence reads are likely to contain several different genomes with different levels of coverage. All contigs that were longer than 1 kb in length were extracted and used for further analysis.

In an attempt to extract X-cell only DNA sequences, all contigs that had either no BLAST hit or a non-fish BLAST hit were extracted from the list of contigs produced by IDBA-UD. The X-cell rDNA was identified from this list.

Verification with PCR and Sanger sequencing

Primers were designed to produce overlapping fragments to the X-cell rDNA contig identified. Primer details can be found in Table 1.

PCRs were run in 25-μL volume reactions and included 10–30-ng template DNA, 2-mM $MgCl_2$, 0.4-μM forward and reverse primers, and 0.2-U Taq (Life Technologies). The thermal cycling conditions were as follows: 2 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 45 s at the annealing temperature (Ta; Table 1) and 60 s at 72 °C, and a final extension of 5 min at 72 °C.

All PCR products were purified, cycle sequenced using Big Dye 3.1 chemistry, and subsequently analyzed on an ABI Prism 3130xl genetic analyzer (Applied Biosystems). PCR products were sequenced in both directions. Sequences were edited manually using GENEIOUS (http://www.geneious.com/) and then aligned to the X-cell rDNA contig as well as to *Perkinsus marinus* rDNA (Accession Number AF497479.1) to establish boundaries of the 18S, 5.8S, and 28S sequences (Fig. 2).

Phylogenetic analyses

The initial analyses of X-cell sequences showed that the resulting phylogenetic trees are characterized by long branches, which can introduce uncertainty in the location of the root of the clade. In order to maximize the probability of correctly rooting the tree, our phylogenetic analysis included a series of outgroups with shorter branches. We included all X-cell sequences from Karlsbakk et al. (2021), as well as the newly identified 18S, 5.8S, and partial 28S sequences (this study) and all of their nonredundant close relatives (>85% identity) obtained

Table 1 PCR primers for amplification of the *Cryoxcellia borchgrevinki* gen. nov., sp. nov. rRNA repeat unit (complete 18S, 5.8S and partial 28S) from the bald notothen (*Trematomus borchgrevinki*)

Forward primer	Reverse primer	Amplicon size (base pairs)	Ta (°C)
GTTGTCTTGGAGCTTGAGAG	CGGAATCGAACCCTAATTCC	954	55
GCATATTTTGGTGAGGCGTA	CACTCCACCAACTAAGAACG	1092	55
TCGCAAGGCTGAAACTTAAA	ATTAGCATACCCACAATCGC	1156	55
GCGTAATCTTTACTTGCCCA	ACGCGATTTAGAATACAGGC	1005	55
GCGCGAACTAACTCTGTAAA	TACTTTCCCTTTCAGGAGCA	1143	55
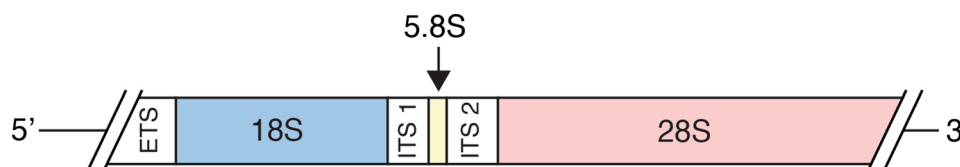
Fig. 2 Schematic representation of the genes of the rDNA repeat unit from *Cryoxcellia borchgrevinki* gen. nov., sp. nov. (ETS, external-transcribed spacer; ITS, internal-transcribed spacer)

by BLAST on GenBank. Beyond these very close relatives, BLAST detected many thousands of sequences with ~70–80% sequence identity, without clear evidence of which groups were the closest relatives, likely due to accelerated sequence evolution in the X-cell clade. To obtain outgroup sequences, we BLASTed GenBank starting with the shortest-branched close relative of our X-cell sequences, EF675616.1, a pathogenic perkinsozoan from Novel Alveolate Group 01 (NAG01) that infects tadpoles of the Southern leopard frog *Rana sphenocephala* (Chambouvet et al. 2015). Outgroup sequences were manually chosen from these BLAST results so as to ensure several sequences from each major outgroup clade were included, prioritizing sequences derived from type material. Outgroup sequences as distant as fungi were selected.

This resulted in 69 sequences that were aligned with Clustal Omega, available in EMBL-EBI (Madeira et al. 2019), using the maximum five iterations for guide tree and hidden Markov models. The resulting alignment was re-run again on the same settings, manually double checked in AliView (Larsson 2014), and edited to correct minor remaining alignment errors. On visual inspection the alignments were of high quality, with several highly conserved regions.

Exploratory phylogenetic analyses were performed with BIONJ and RAxML using the ETE3 pipelines at GenomeNet (Huerta-Cepas et al. 2016) under a variety of alignment filters, models, and run settings. These gave consistent topologies, so a full-partitioned model selection and bootstrapping analysis was conducted with IQ-TREE 2 (Minh et al. 2020), with a supplementary analysis in MrBayes (Ronquist and Huelsenbeck 2003) to estimate posterior probabilities. IQ-TREE 2 was run, testing all combinations of models on defaults for all three partitions (alignment positions: 18S: 1-1782; ITS1: 1783-1881; 5.8S: 1882-2033). The best model, as selected by BIC (Burnham & Anderson 2002), was a merged GTR+F+I+I+R4 model for 18S+ITS1 and TPM3+I+I+R3 for 5.8S. IQ-TREE 2 ran 1000 bootstrap replicates; the consensus tree with bootstrap branch supports was viewed in FigTree v1.4.3 (Rambaut 2016) and manually outgroup rooted to fungi. IQ-TREE 2 was also used to select from a simplified set of models available in MrBayes, selecting GTR+F+G4 for the merged 18S+ITS1 partition and HKY+F+G4 for 5.8S. Further details of the additional MrBayes analysis are available in the Supplementary Information.

Results

Phylogenetic analyses

The newly identified 18S, 5.8S, and partial 28S rDNA sequence (Fig. S1) groups as the deepest branch of the *Gadixcellia* / *Salmoxcellia* X-cell clade with 96% bootstrap support in the maximum likelihood IQTree analysis (Fig. 3; the full tree is shown in Fig. S2) and 100% posterior probability in the

MrBayes analysis (Fig. S3). The new sequence groups with all-known X-cell species, distinct from other perkinsozoans. As a basal branch in the *Gadixcellia* / *Salmoxcellia* X-cell clade, it is distinct from *Xcellia* and *Notoxcellia* species, which include *X. lamelliphila* responsible for X-cell disease in the Antarctic emerald notothen (*T. bernacchii*) and *N. coronata* and *N. picta* responsible for the disease in two Antarctic fishes, the crowned notothen (*T. scotti*) and the painted notothen (*N. larseni*). The genetic distance between sequences from the eight recognized X-cell species is shown in Table S1; these data support the results of the phylogenetic analyses. Collectively, the analyses support the identification of a new X-cell organism, formally named below as *Cryoxcellia borchgrevinki* gen. nov., sp. nov.

Taxonomic description

**Class Perkinsea** Levine, 1978
Order Perkinsida Levine, 1978
Family Xcellidae Freeman et al. (2017)

Parasitic alveolate cells found mostly in marine teleosts. Occur on the surface of the skin and fins, in the pseudobranchial region, and between the secondary gill lamellae. May be present on internal organs. The classic X-cell described from histological sections of fish tissues is a relatively large cell (sometimes exceeding 20 μm diameter) that is round to polygonal in shape and has a weakly-staining nucleus with one or more prominent nucleoli. X-cells may sometimes be associated with enveloping host cells. When released from host tissues, they may be found to have a capsule-like envelope. X-cells are globally distributed, infecting a broad range of fish. Their life cycle is unknown, developmental stages are uncertain, and dividing cells are reportedly rare.

Genus Cryoxcellia gen. nov. Evans, Patel, Matzke and Millar.

Diagnosis A type of X-cell forming lesions in the gills of the bald notothen (*Trematomus borchgrevinki*) endemic to the Southern Ocean. X-cells in this species expand in number to pack the primary gill filaments and extend between the secondary lamellae of their host fish. The cells are typically spherical and encapsulated and have a granular cytoplasm that varies in density. There is one or more centrally positioned nuclei, each with a distinct nucleolus. X-cells in this species also appear as rosettes or clusters of up to eight smaller cells, likely to be daughter cells derived from division of the parent. Not all rosettes appear to be encapsulated. To date, *Cryoxcellia* gen. nov. X-cells have not been identified in other fish species or in other tissues or organs from the host.

Etymology The genus name is derived from the Greek *kryos* reflecting the icy habitat of the host fish and from the common name of the parasite (X-cell) given by Brooks et al. (1969).
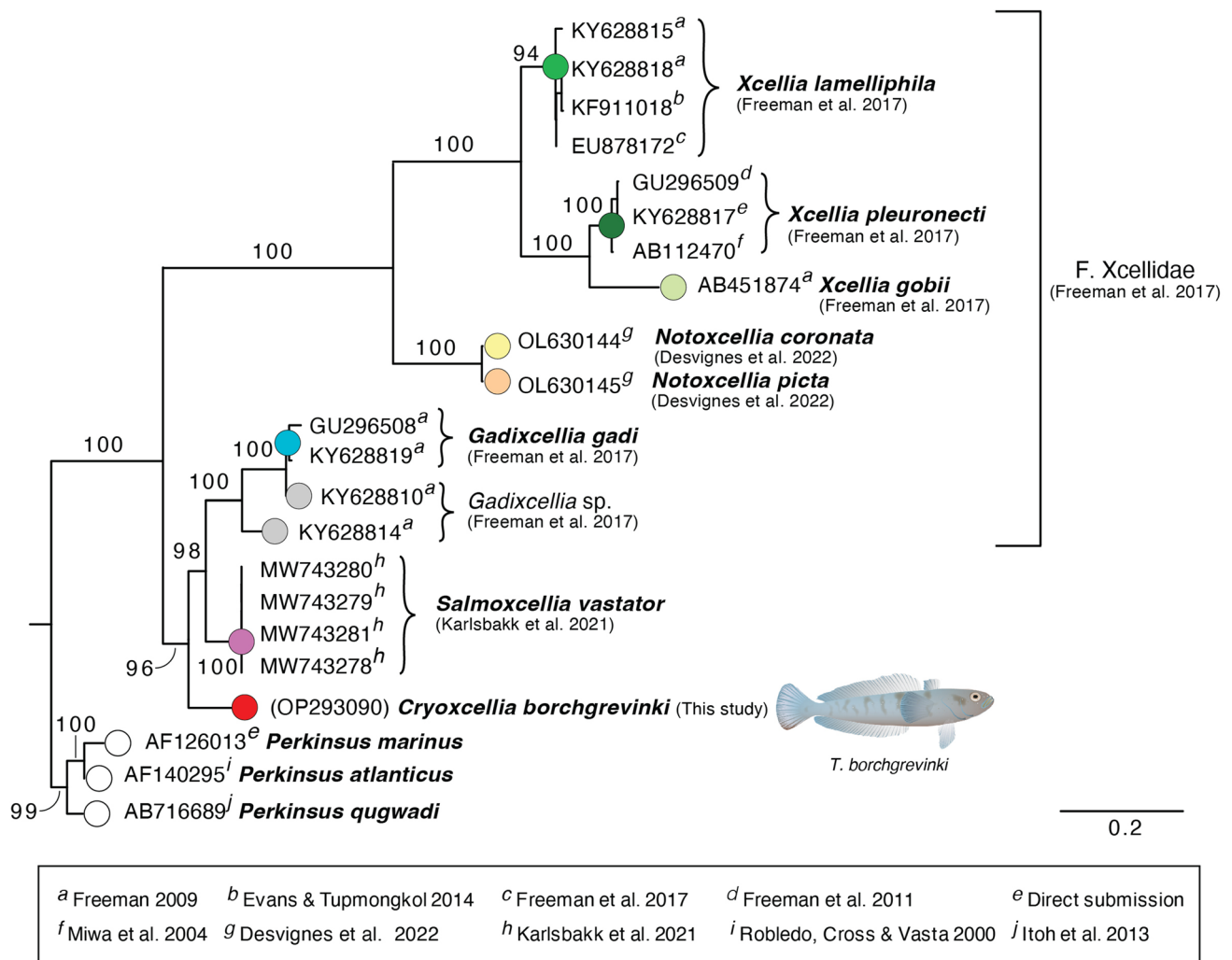
Fig. 3 rDNA maximum likelihood phylogenetic tree (IQTree run, partitioned, with bootstrapping) showing the position of *Cryoxcellia borchgrevinki* gen. nov., sp. nov. in relation to all other currently published X-cell lineages. As an outgroup, sequences of three *Perkinsus* species are shown. Maximum likelihood bootstrap values are indicated at major nodes. GenBank Accession numbers are given for each sequence used in the analysis. The currently recognized species from the Class Perkinsea are highlighted in bold text and the associated reference for each sequence is provided (boxed)

ZooBank registration The LSID for the new genus is urn:lsid:zoobank.org:act:AECDF4AE-B466-4A66-B9F6-1493EFAE696B.

Cryoxcellia borchgrevinki gen. nov., sp. nov. Evans, Patel, Matzke and Millar.

Type host The bald notothen, *Trematomus borchgrevinki*.

Type location McMurdo Sound, Antarctica.

Type material Fixed gill tissue (70% ethanol) from the bald notothen containing X-cells has been deposited in the Museum of New Zealand Te Papa Tongarewa, located in Wellington, New Zealand (registration number: holotype MONZ PR.001000). The *C. borchgrevinki* gen. nov., sp. nov. verified rDNA sequence from the holotype sample (containing multiple X-cells) has been submitted to GenBank, Accession number OP293090.

Site of infection Gills, bilaterally distributed, with affected regions appearing white and often swollen. In severe cases, the affected gills may extend from under the cover of the operculum on each side.

ZooBank registration To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological nomenclature (ICZN), details of the new species have been submitted to ZooBank. The LSID for the publication is urn:lsid:zoobank.org:pub:DEC43534-E4D5-4358-B5A1-A40505A2F417.

The LSID for the new species *Cryoxcellia borchgrevinki* is urn:lsid:zoobank.org:act:CACCDC22-442C-4D1C-A16E-3795F89AFA44.

Etymology The species name is derived from the host fish, the bald notothen *Trematomus borchgrevinki*, which honors Carsten Borchgrevink, the leader of the British Antarctic Expedition (1898–1900).

Description

Morphology

X-cell disease in the bald notothen (*T. borchgrevinki*) presents as swollen (fluffy) gill filaments with a characteristic whiteness extending proximally to variable proportions along affected filaments (Fig. 1a). The disease affects the gills on both sides of the body and the swollen filaments can extend beyond the margins of the operculum. The disposition of X-cells in the basal region of the gill lamellae and extending distally between them is apparent in wet mounts of gill fragments (Fig. 1b). There are no other overt signs indicating the presence of the disease.

Microscopic appearance

The predominant form of X-cells in the gills of the bald notothen (*T. borchgrevinki*) presents as a more-or-less spherical cell within a limiting capsule. As is well documented by others (Franklin and Davison 1988; Davison 1998; Powell 2007), apparently encapsulated X-cells in the bald notothen have a prominent single nucleolus and appear rounded in tissue sections, packing the spaces between adjacent secondary gill lamellae. This distribution is apparent in wet mounts of whole infected gill tissue (Fig. 1b).

In samples of infected gill tissue teased apart and examined as wet preparations (in 10% formalin or in seawater) under phase contrast or DIC optics, a distinctly spherical, granular X-cell form (diameter 22.40 ± 1.09 μm; n = 10) circumscribed by an external capsule is obvious. As revealed by phase microscopy, some X-cells have a pale nucleus with a single dark nucleolus (Fig. 4a), equivalent to the classic X-cell form previously described in stained histological sections of infected bald notothen gills (Franklin and Davison 1988; Davison 1998; Powell 2007), while others appear to have multiple nuclei, each with a single, dark nucleolus (Fig. 4b, arrows). Granule density varies among the X-cells (Fig. 4c).

We also report here an X-cell form, termed rosettes (diameter 17.96 ± 2.06 μm; *n* = 10), which consist of clusters of smaller, separate daughter cells (up to at least eight) that may not always lie within a limiting capsule (Fig. 4d). The component cells are typically triangular or wedge-shaped (cuneiform), with one tip of each daughter cell directed toward the center of the rosette. Their average size measured across their greatest width is 9.37 ± 1.52 μm (*n* = 10).

Remarks *C. borchgrevinki* gen. nov., sp. nov. infection manifests as white, often swollen gill filaments similar to X-cell disease of the gills reported in other fish species, including Antarctic fishes (Evans and Tupmongkol 2014). The similar gross morphological appearance of X-cell disease in the gills of Antarctic fishes led initially to them being considered together as a single disease-causing entity, with different cellular forms being attributed to different developmental stages, but we now show conclusively that X-cell disease in Antarctic fishes is caused by more than one X-cell species. Microscopically, *C. borchgrevinki* gen. nov., sp. nov. is distinct from known xcellids in the abundance of spherical, encapsulated cells, which have one or many nuclei. Phylogenetic analyses groups it deep within the *Gadixcellia / Salmoxcellia* clade and distinct from all-known *Xcellia* species, which include *Xcellia pleuronecti*, *Xcellia gobii*, and *Xcellia lamelliphila* as well as the recently described *Notoxcellia coronata* and *Notoxcellia picta*. *Xcellia lamelliphila* is a globally distributed species that infects a variety of fishes including the common European dab (*Limanda limanda*), the eelpout (*Lycodes* spp.), and the Antarctic emerald notothen (*T. bernacchii*). Notably, the Antarctic host of *C. borchgrevinki* gen. nov., sp. nov. is different from that infected by *X. lamelliphila*. *X. gobii* forms mostly epidermal lesions in the Japanese goby (*Acanthogobius flavimanus*), while *X. pleuronecti* forms similar lesions in flatfish (Freeman et al. 2017), thus helping distinguish these *Xcellia* species from *Cryoxcellia* gen. nov. The two *Notoxcellia* species described by Desvignes et al. (2022) are found in different Antarctic host fishes and occur typically in the dermis while being absent from the gills, thus helping delineate them from *C. borchgrevinki* gen. nov., sp. nov. Additionally, unlike *C. borchgrevinki* gen. nov., sp. nov., the tumor-like lesions in the *Notoxcellia* species maybe unilateral, and the component X-cells are compartmentalized by host cells.

In the northern hemisphere, the two known hosts of *Salmoxcellia* are both salmonids, namely the rainbow trout (*Oncorhynchus mykiss*) and the Atlantic salmon (*Salmo salar*) (Karlsbakk et al. 2021). Apart from these host differences and geographic separation, *Cryoxcellia* gen. nov. does not cause systemic infection, as in *Salmoxcellia*, being confined to the gills of the host fish, the bald notothen (*T. borchgrevinki*), which is restricted to Antarctic waters. *Gadixcellia* is found in Atlantic cod (*Gadus morhua*) from the northern Atlantic (Freeman et al. 2017). Unlike *Cryoxcellia* gen. nov., *Gadixcellia* is found mostly on the pseudobranch where it can form X-cell masses that are compartmentalized by fibrous septa and are often associated with an active host response.
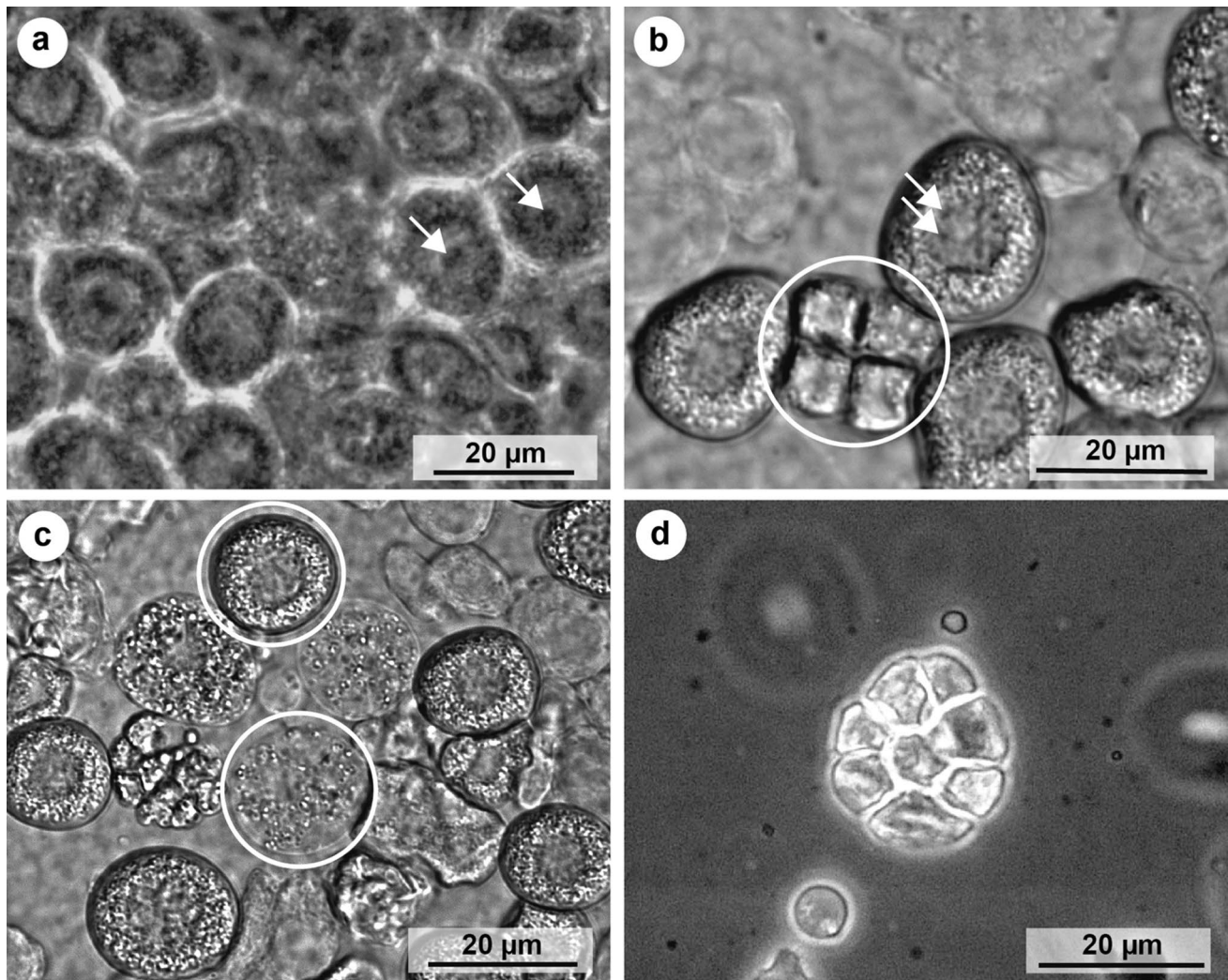
Fig. 4 Microscopic appearance (phase optics) of different *Cryoxcellia borchgrevinki* gen. nov., sp. nov. X-cell forms. **a**, Some X-cells have a prominent single nucleolus (arrows). **b**, Two dark nucleoli (arrows) in the nuclei of each multinucleate X-cell and a X-cell rosette (4-cell stage, circled). **c**, X-cells have different granule densities (ringed cells) **d**, X-cell rosette (8-cell stage)

Discussion

The estimated prevalence of X-cell disease in *Trematomus borchgrevinki* collected from McMurdo Sound can vary, with one study reporting that overall, 22% of the fish caught showed signs of X-cell disease, although the prevalence in individual fishing sessions ranged from 9.2 to 43.9% (Davison 1998). Little is known of the life cycle of the parasites responsible for the disease. Taken collectively, reported microscopic observations suggest X-cells exhibit different forms and thus there is no single X-cell archetype. The predominant X-cell form in *C. borchgrevinki* gen. nov., sp. nov. is spherical, granulated, encapsulated, and free within the gill tissue of its host. It does not elicit an obvious host cell inflammatory response, but infected hosts have a lower condition factor when compared to uninfected fish (Davison

1998), they are physiologically compromised with a disrupted lamellar blood supply (Franklin et al. 1993) and have a reduced oxygen exchange capacity (Davison et al. 1990).

C. borchgrevinki gen. nov., sp. nov. X-cells have one or more nuclei, each with a nucleolus. The closely related X-cell, *S. vastator*, exists as multinucleate plasmodia, which divide by plasmotomy to form cellular aggregates in the host tissues (Karlsbakk et al. 2021). The similarity with *C. borchgrevinki* gen. nov., sp. nov. is unclear; however, since there is no evidence in the latter species that the parent X-cell divides by fission to yield two multinucleate daughter cells, as is typical of plasmotomy. The fate of the multinucleate X-cells in *C. borchgrevinki* gen. nov., sp. nov. is unknown (Fig. 4b, c). Significantly, we also describe small groups (rosettes) of up to eight cells in *C. borchgrevinki* gen. nov., sp. nov., which likely derive from division of the

predominant X-cell form within its capsule. At some point these daughter cells break free of their limiting capsule, presumably increasing the X-cell population. There is no direct evidence at present to suggest that the multinucleate X-cell forms described above transition into rosettes, but this remains a possibility.

Franklin and Davison (1988) describe a group of multinucleate X-cells in infected bald notothens (*T. borchgrevinki*), which possibly represent the forms described here. These authors also suggest the presence of two cell types, dark and light cells, the former with dense granules and dark cytoplasm and the latter with relatively light cytoplasm but still with electron-dense granules. We report similar cell differences here as judged by light microscopy. Their significance is unknown, although Franklin and Davison (1988) assumed that the two forms represent different X-cell developmental stages. We note the similarity of the more and less granular forms in Fig. 4c with the early and late sporocyte stages of *Parvilucifera corolla* as described by Reñé et al. (2017, Fig. 1 E and G, respectively). The presence of electron-dense cytoplasmic granules helps morphologically to differentiate *C. borchgrevinki* gen. nov., sp. nov. from *S. vastator*, which lacks such structures (Karlsbakk et al. 2021).

Masses of *X. lamelliphila* X-cells form between the secondary gill lamellae leading to lamellar fusion and loss of gill function. This pattern of infection and its physiological consequences have close parallels with the situation in the bald notothen (*T. borchgrevinki*) infected with *C. borchgrevinki* gen. nov., sp. nov. but microscopic observation (Fig. 4) in combination with phylogenetic analyses indicates significant differences. Indeed, phylogenetic analyses group *Xcellia* species distinct from the *Gadixcellia/Salmoxcellia/Cryoxcellia* clade.

It is not known how X-cell organisms spread the disease. There is no evidence of any dispersal form, such as the biflagellate zoospore seen in *Perkinsus* species (Petty 2010). Dispersal is arguably direct, from fish to fish without any intermediate stage, although other strategies such as dispersal through the substrate are possible (Freeman et al. 2011).

In conclusion, based on morphological and phylogenetic analyses, we have confidence in naming a distinct new X-cell genus and species, *Cryoxcellia borchgrevinki* gen. nov., sp. nov. Finally, we note that Karlsbakk et al. (2021) suggested the Family Xcellidae be emended to exclude the genera *Gadixcellia* and *Salmoxcellia* and by extension *Cryoxcellia* gen. nov., but we suggest this interpretation await further analysis. For the purpose of further discussion, however, we broaden the family as originally described by Freeman et al. (2017) to include *Salmoxcellia* and *Cryoxcellia* gen. nov., while retaining *Gadixcellia*.

Supplementary Information The online version contains supplementary information available at https://doi.org/10.1007/s00300-023-03132-w.

Declarations

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