



# Parasite–copepod interactions in Svalbard: diversity, host specificity, and seasonal patterns

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## Abstract

Copepods of the genera *Calanus* and *Pseudocalanus* are important components of Arctic marine ecosystems. Despite the key roles of these zooplankters, little is known about the organisms they interact with most intimately, their parasites and symbionts. We applied metabarcode sequencing to uncover eukaryotic parasites present within these two copepod genera from three areas around the high Arctic archipelago of Svalbard. Ten distinct parasite groups were observed: four different Apostome ciliates, four different dinoflagellates (*Chytriodinium* sp., *Ellobiopsis* sp., *Thalassomyces* sp., and *Hematodinium* sp.), a *Paradinium* sp., and a trematode. Apostome ciliates closely related to *Pseudocollinia* spp. were the most commonly observed parasite, with overall infection rates of 21.5% in *Calanus* and 12.5% in *Pseudocalanus*. Infection by these ciliates varied seasonally, with no infections observed in early winter, but infection rates exceeding 75% in spring. Host specificity varied between parasites, with significant differences in infection rate between the two host copepod genera for four parasites (two ciliates, *Chytriodinium*, and a trematode). The diverse assemblage of parasites observed in these copepods, and the frequency of infection, with over one in five copepod individuals infected, suggest parasites may be playing a greater role in Arctic plankton communities than generally acknowledged.

**Keywords** *Calanus glacialis* · *Pseudocalanus* spp. · Parasites · Metabarcoding · Arctic

## Introduction

Copepods are key components of most marine ecosystems. These small crustacean zooplankton serve as important prey for a variety of fish, seabird, and marine mammal species, and their population dynamics are important drivers in the production and recruitment of commercially important fish stocks (Runge 1988). Copepods also play essential roles in carbon cycling, contributing to the biological carbon pump by shuttling organic material to the deep sea through vertical migration and the production of rapidly sinking fecal pellets (Sanders et al. 2014; Jónasdóttir et al. 2015).

*Calanus* spp. copepods are typically the most abundant herbivore in Arctic marine waters, with *Calanus glacialis* dominating in Arctic shelf waters (Falk-Petersen et al. 2009). *C. glacialis* are relatively large and lipid-rich copepods, features which combine with their high abundance to make them essential components of Arctic food webs (Falk Petersen et al. 2009; Kwasniewski et al. 2010). *Pseudocalanus* spp. are among the most abundant of the smaller zooplankton in high latitude systems (Conover et al. 1988). This species complex of morphologically similar *Pseudocalanus*

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congeners, including *Pseudocalanus minutus*, *Pseudocalanus acuspes*, *Pseudocalanus newmani*, and *Pseudocalanus moultoni*, fill important niches in different trophic pathways in Arctic food webs, and are of particular importance in warmer years (Coyle et al. 2011).

Despite the depth of research which has been conducted on these two important high latitude copepod genera, little is known of the organisms with which they interact most intimately: their parasites and symbionts. Across all zooplankton groups, reports of parasites are sporadic, and they have typically been ignored in broadscale surveys and ecosystem models (Skovgaard 2014; Bass et al. 2021). Many parasites are cryptic and can be difficult or impossible to identify visually (Skovgaard and Daugbjerg 2008). This can be particularly challenging in early stages of infection, and when working with preserved samples (Skovgaard and Daugbjerg 2008). A variety of parasites have been identified in *Calanus* and *Pseudocalanus* spp. copepods. Both of these genera have been observed infected by Apicomplexa, ciliates, and dinoflagellates (Bass et al. 2021). Additionally, *Calanus* spp. have been observed infected by Acetosporea, diatoms, Mesomycetozoa, Ochrophyta, and Oomycote, and *Pseudocalanus* spp. have been observed infected by a Euglenozoan (Bass et al. 2021). However, much remains unknown about the abundance, diversity, distributions, and ecosystem roles of these parasites. In Svalbard waters, only two types of parasitic or symbiotic organisms have been documented interacting with copepods—*Ellobiopsis* sp. dinoflagellates and *Paracineta* sp. ciliates (Walkusz and Rolbiecki 2007). Parasite infections have been speculated to be behind observations of *Calanus* spp. carcasses observed in winter in Svalbard, but this remains uncertain (Daase et al. 2014).

There is increasing recognition that these oft ignored parasites and symbionts may be playing outsized roles in driving the structure and function of ecosystems. Theoretical estimates suggest there may be more parasitic species than free living species in the world's oceans, while empirical estimates suggest parasites can contribute as much to ecosystem biomass as top predators in estuarine systems (Kuris et al. 2008; Worden et al. 2015). At the level of an individual host, parasites can increase mortality, reduce respiration, growth and/or reproduction, and alter feeding and other behaviors (Kimmerer and McKinnon 1990; Kurtz 2007; Skovgaard 2014; Fields et al. 2015). Scaled up to the ecosystem level, parasites can influence carbon and nutrient cycling, shift balances between predators and prey or among competitors, and contribute to the maintenance of biodiversity across multiple trophic levels (Dobson and Hudson 1986; Vannatta and Minchella 2018).

A powerful emerging tool to investigate host–parasite interactions in planktonic communities is DNA analysis. DNA-based approaches are able to detect parasite infections in their earliest stages and samples can be preserved for

months or years prior to analysis. These approaches are also not reliant on taxonomic expertise, which is often the limiting factor in microscopy-based studies. By sequencing small “barcode” regions of DNA, and comparing these sequences to reference databases, it is possible to identify the parasites present within a sample. This metabarcoding approach has been applied to both individual zooplankters and size fractionated samples of bulk zooplankton. Results from bulk samples have indicated that parasitism is a very common interaction in planktonic communities, and plays important roles in structuring ecosystems (Lima-Mendez et al. 2015). Parasites identified from bulk samples have been diverse, and have exhibited seasonal and interannual variations (Lima-Mendez et al. 2015; Käse et al. 2021). Results from individual zooplankton have confirmed a diverse assemblage of parasites are present within zooplankton, and that parasite infection can be very common, for example, over 10% of Antarctic krill individuals contained DNA signatures of parasites (Cleary et al. 2019; Yeh et al. 2020).

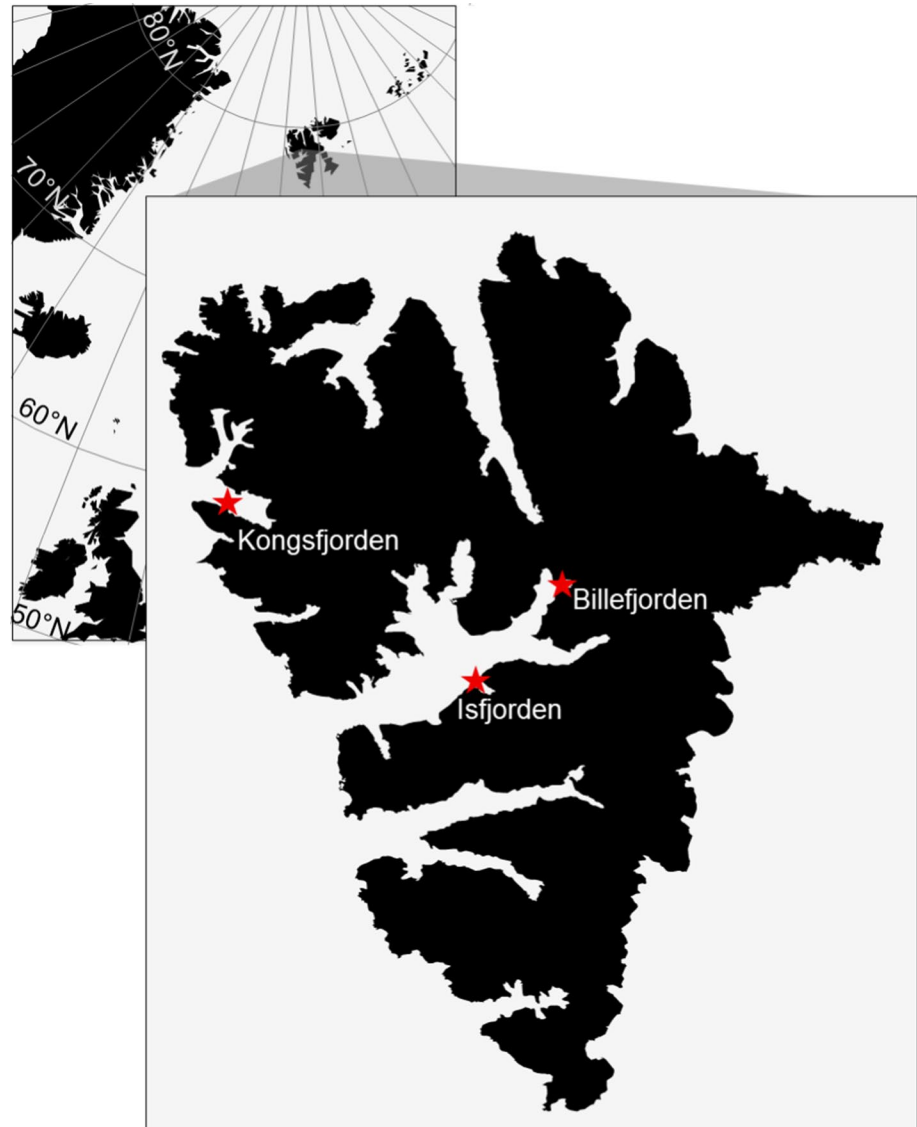
In this study, we applied metabarcoding to investigate all the eukaryotic parasites interacting with *Calanus glacialis* and *Pseudocalanus* spp. copepods in Svalbard waters. This provides a first survey of the diversity and prevalence of eukaryotic parasitic organisms interacting with these key zooplankters in the Arctic. We also explore questions of seasonality in parasite infection, and variations in host specificity.

## Materials and methods

### Field collections

Copepod samples were collected in three fjord areas of Svalbard: Billefjorden, Isfjorden, and Kongsfjorden (Fig. 1 and Table 1). These three areas are all influenced by a mixture of Atlantic and Arctic waters due to the dynamic current systems present along west coast of Spitzbergen (Dalpadado et al. 2016). Billefjorden is typically the most classically Arctic of these sampling locations, but both seasonal and interannual variability in the relative importance of different water masses is high in all three areas (Hop et al. 2006; Nilsen et al. 2008; Dalpadado et al. 2016). Billefjorden and Kongsfjorden sampling collected *Calanus glacialis*, while Isfjorden sampling collected the smaller *Pseudocalanus* spp. (*P. minutus*, *P. acuspes*, and *P. moultoni*). Billefjorden samples were collected across a seasonal cycle, with collections on October 5 2012, and January 10, March 13, April 26, June 17, and July 23 2013. The diet of these Billefjorden sampled copepods was reported in Cleary et al. (2017). Isfjorden samples were also collected over a seasonal cycle, with collections on January 27, May 30, September 6, September 19,

**Fig. 1** Map of sampling locations—*Calanus glacialis* were collected from Billefjorden and Kongsfjorden, *Pseudocalanus* spp. were collected from Isfjorden



**Table 1** Sampling—number of samples individuals (pools) analyzed from each season in each of the study regions

Area	Lat & Long	Species	Year(s)	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
Billefjorden	78° 40' N 16° 40' E	<i>Calanus glacialis</i>	2012–2013	22 (11)	26 (13)	18 (9)	10 (5)	6 (3)	–
Kongsfjorden	78° 57' N 11° 53' E	<i>Calanus glacialis</i>	2014	49 (19)	–	–	–	–	–
Isfjorden	78°15.6' N, 15°31.8' E	<i>Pseudocalanus moultoni</i> <i>Pseudocalanus acuspes</i> <i>Pseudocalanus mimus</i> <i>Pseudocalanus minutus</i>	2012	10 (10)	–	1(1)	–	18 (18)	3 (3)

November 29, and December 14, 2012. The taxonomic composition of these Isfjorden samples was reported in Stübner et al. (2016). Kongsfjorden samples were collected during the polar night, between January 16 and 19 2014. Combining samples across three different sampling campaigns allowed for increased sample sizes, which greatly

improves power to detect rarer parasites and the precision of detected infection rates.

Sampling was conducted from a variety of platforms, depending on sea ice conditions and vessel availability (snowmobiles, rigid inflatable boats, research, or coast guard vessels). Samples were collected in short vertical tows of

plankton nets (Billefjorden—standard WP2 net or multinet plankton sampler, Kongsfjorden—Method-Isaac-Kid net or multinet plankton sampler, Isfjorden—standard WP2 net or closing ring net with 0.25-m<sup>2</sup> mouth opening and 63- $\mu$ M mesh). Billefjorden sampling targeted the depth of peak copepod abundance (0–20 m, 100–150 m, or 120–180 m), Isfjorden sampling covered the complete water column in two short tows (0–25 m and 25–65 m), and Kongsfjorden sampling was restricted to the upper water column (0–30 m). All samples were preserved immediately after collection in 95% ethanol, with ethanol changed once after 24 h to maintain concentration.

### DNA extraction and sequencing

Samples were sorted under dissecting microscopes, and individuals of the target species, and most abundant stage, picked out for analysis. The most abundant stage varied across samples, as copepod cohorts progress through the seasonal cycle, and included copepodite four and five as well as adults. None of the sampled individuals had attached egg sacs. Species identification was confirmed genetically for all analyzed individuals, as per Gabrielsen et al. (2012). None of the sampled individuals showed visible signs of infection. To reduce the potential for external contamination, samples from Billefjorden and Isfjorden were rinsed thoroughly in deionized water. Samples from Kongsfjorden were additionally treated with a bath of 1% bleach for one minute prior to rinsing, and antenna and legs were removed. These processing differences reflect ongoing debate in the literature on best practices, but are unlikely to impact the detection of internal parasites (O’Rorke et al. 2012; Durbin and Casas 2014). All molecular processing was conducted in dedicated clean lab facilities, following standard protocols to minimize contamination risks.

DNA was extracted from all samples using the DNeasy Blood and tissue kit (Qiagen). Extracts from between one and three individuals from a single net tow and of the same stage and species were combined in each sample. The V7 region of the 18S rDNA gene was amplified from these extracts using a peptide nucleic acid-mediated polymerase chain reaction (PNA-PCR). This approach minimizes amplification of the host’s DNA (*Calanus/Pseudocalanus*), while allowing for amplification of DNA from all other eukaryotes, in order to detect all potential eukaryotic symbionts, without a priori assumptions. Each reaction contained 1  $\times$  DreamTaq buffer (Thermo-Fisher), 0.65 U  $\mu$ L<sup>-1</sup> DreamTaq polymerase, 0.25  $\mu$ M each dNTP, 0.5  $\mu$ M each of primers 960F and 1200R (Gast et al. 2004), approximately 0.5 ng  $\mu$ L<sup>-1</sup> extracted template DNA and either 5  $\mu$ M (Kongsfjorden) or 20  $\mu$ M (Billefjorden and Isfjorden) Cal-PNA5 (Durbin and Casas 2014). Thermocycling consisted of an initial denaturation at 95° for 30 s (Billefjorden and Isfjorden) or

2 min (Kongsfjorden), followed by 30 cycles (Billefjorden and Isfjorden) or 32 cycles (Kongsfjorden) of 94° for 30 s, 77° for 30 s, 58° for 30 s, and 60° for 45 s, with a final extension stage of 5 min at 60° (Billefjorden and Isfjorden only). Amplicons were purified with SPRI beads (Beckman-Coulter), following Rohland and Reich (2012).

Sequence identification tags of variable length (4–8 base pairs) were ligated onto amplicons with 16 U  $\mu$ L<sup>-1</sup> T4 DNA ligase (New England Biolabs) at 20° for 90 min. These tagged amplicons were then purified with SPRI beads as before. For Kongsfjorden samples, illumina adaptors were ligated onto the fragments, again using T4 DNA ligase. For Billefjorden and Isfjorden samples, illumina adaptors were added in a second round of PCR, which contained 1  $\times$  Q5 DNA polymerase (New England Biolabs), 1  $\times$  Q5 buffer, 0.5  $\mu$ M each forward and reverse primers (containing illumina adaptor sequences), 80  $\mu$ M each dNTP, and the tagged amplicons from the previous step. Thermocycling for this second PCR consisted of 98° for 30 s, followed by nine cycles of 98° for 10 s, 65° for 19 s, and 72° for 30 s, with a final extension at 72° for two minutes. This secondary PCR was followed by a third round of SPRI purification. All PCR runs included no template controls to confirm no contamination was present.

Amplicons were quantified for Billefjorden and Isfjorden samples based on comparison to standards on an agarose gel stained with GelRed (Biotium), and analyzed with GelAnalyzer2010. For Kongsfjorden samples, quantification was performed in a short qPCR (11 cycles) using Q5 polymerase. Equimolar ratios of samples were pooled prior to sequencing.

All samples were sequenced on Illumina MiSeq with V3 chemistry and 300 base pair paired-end reads. Billefjorden and Isfjorden samples were sequenced at a commercial sequencing center (ACGT, Illinois, USA). Kongsfjorden samples were sequenced at Biologie Intégrative des Systèmes at Larval University (Quebec, Canada).

### Bioinformatic analyses

All bioinformatic analyses were performed in Qiime2, in order to ensure traceability and reproducibility (Bolyen et al. 2019) (see electronic supplementary information for the data processing script). Sequences were first demultiplexed using the cut-adapt plug in, detecting barcodes present in either orientation, and trimming the barcodes off the sequence reads (Martin et al. 2011). Reads were then further trimmed to remove forward and reverse primers. As the read quality generally declines toward the end of reads, reverse primer matching was more lenient, allowing for up to 0.15% error, whereas forward primers were required to match exactly. Reads without forward primers were discarded, reads

without reverse primers were retained, to avoid introducing bias against longer amplicons.

Sequence reads were denoised and separated into Amplicon Sequence Variants (ASVs) using dada2 as implemented in qiime2 (Callahan et al. 2016). Sequences were first further trimmed (forward reads—200 bp, reverse reads—150 bp). These trimming lengths were determined by testing a range of values on a subset of the data, in order to maximize the proportion of reads which successfully merge forward and reverse. Reads were quality filtered, with truncation after the first base with a quality of two or lower, and an estimated maximum errors per read of four (forward and reverse). Forward and reverse reads were merged using default parameters (minimum 12 base overlap, 100% identity across the overlap region). The resulting merged reads were checked for chimeras with a minimum parent:chimera fold difference of two. The reads discarded in the dada2 process were randomly spot-checked to ensure filtering was not introducing any systematic biases. These checks showed only very short fragments and poor quality reads most similar to the host copepods.

Taxonomy was assigned to each ASV using SK-learn with a pre-trained classifier built on the Silva-138 99% identity database (Pedregosa et al. 2011). Each ASV was then classified as host (sequences identified as *Calanus* or *Pseudocalanus*, or only identified to a higher level grouping which includes these, such as Maxillopoda or Metazoan), potential parasite or symbiont (any organism known to form symbiotic relationships with any crustacean, based on Bass et al. 2021, literature searches, and BLAST searches against the NCBI nr database), plausible prey (any organism not in the previous two categories and known to be present in marine habitats and of a size to be consumed by copepods), or potential contaminant (a handful of sequence reads from humans and agriculture crops). In the few cases where the taxonomic resolution in this gene fragment was not sufficient to distinguish between symbiotic and potential prey organisms (mainly fungal sequences), we took the conservative assumption that they were prey. In order to focus on those sequences abundant enough to represent ecologically meaningful interactions, ASVs with less than 100 reads were at this stage excluded from further analysis. Similarly, samples which contained less than 100 reads after all filtering steps were not included in further analyses. All of the identified symbiotic organisms have been referred to previously in the literature as parasitic, so we will refer to this group as parasites, while recognizing that the exact nature of their interactions with copepods is in some cases poorly understood.

Parasite ASVs were placed into higher groups to minimize impacts of any sequencing errors which were not removed by the denoising process, and of variation between the many 18S copies present in each individual. For most parasites, this was based on the taxonomy described above, which was confirmed

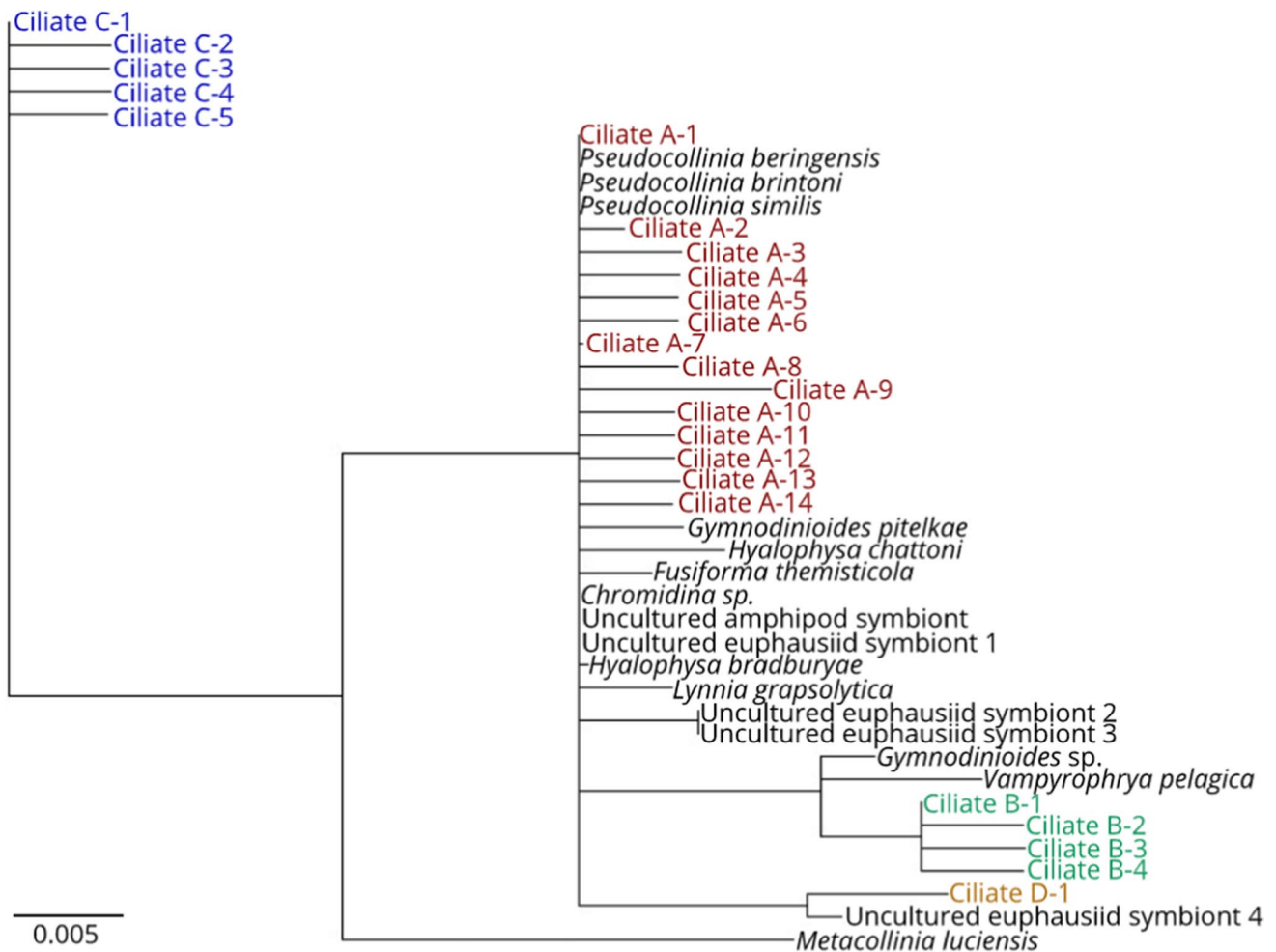
with BLAST searches against the nr database (Altschul et al. 1990). Ciliates were not well resolved by either the taxonomy assignment process or manual BLAST searches, so a phylogenetic tree was constructed to group these ASVs and attempt to refine their taxonomy. All ciliate ASV sequences, their closest matches from BLAST, and a variety of other reference sequences were aligned in Geneious v. 6.1.6 with standard parameters (gap open penalty 12, gap extension penalty 11, cost matrix 93% similarity (5.0/-9.026168)), and a neighbor-joining consensus tree was constructed using Tamura-Nei distances, 2000 bootstraps, and a branch support threshold of 50%. This tree is not meant to provide definitive information on evolutionary relationships, but rather simply to place the ciliate ASVs into meaningful groups (Fig. 2).

### Infection rate analysis

Samples were considered infected by a parasite if they contained 50 or more reads from a single ASV of that parasite group. Because many of the samples analyzed contain more than one individual, we applied a bias corrected maximum likelihood estimation approach to calculate the population-level infection rate, using PooledInfRate (Biggerstaff 2009). This approach takes into account that a pooled sample which contains a parasite may include a single infected host or multiple infected hosts, and estimates how many of the hosts in each parasite-containing pool are infected based on the proportion of all pooled samples which contain that parasite. The power to accurately detect parasite or pathogen infection rate is a function of the number of sample pools, the number of individuals within each pool, and the infection rate itself. At small sample sizes and/or low infection rates, the stochastic effects of capturing (or not) a single infected individual is relatively more important than it is at larger sample sizes and/or higher infection rates (Gu et al. 2004). We therefore present infection rates for all parasites for each copepod host genera, to allow for the maximum meaningful sample size (Fig. 3 and Table S3). For the most abundant parasite, calculations are additionally presented for each season separately (Fig. 4). Infection rates were calculated in single sample tests to determine the bias corrected maximum likelihood estimate and 95% confidence intervals. Infection rates were additionally calculated for the two host genera together in a paired test to determine the significance of the differences between them; rates are reported as significantly different if the 95% confidence intervals around the difference between the two rates do not include zero.

## Results

Following dada2 denoising, the complete data set included 7,824,330 sequence reads. As part of QC, 69,714 reads from ASVs of low overall abundance (< 100 total reads



**Fig. 2** Phylogenetic tree of parasitic ciliate ASVs, and reference sequences from GenBank. Ciliate ASVs are cluster into four distinct groups, indicated by both letter (A, B, C, D) and color, but it is not possible to assign them to specific taxonomy. Note that within each lettered group, ASV's are numbered based on total read abundance, with "1" the most abundant. The scale bar indicates substitutions per base position across the complete amplicon. Reference sequences: *Pseudocollinia beringensis* (HQ591486), *Pseudocollinia brintoni* (HQ591468), *Pseudocollinia similis* (HQ591478) (Lynn et al. 2014), *Gymnodinioides pitelkae* (EU503534), *Hyalophysa chat-*

*toni* (EU503537), *Gymnodinioides* sp. (EU503535), *Vampyrophrya pelagica* (EU503539) (Clamp et al. 2008), *Fusiforma themisticola* (KF516511) (Chantangsi et al. 2013), *Chromidina* sp. (LT546662) (Souidenne et al. 2016), Uncultured amphipod symbiont (KF609459) (Olsen et al. 2014), Uncultured euphausiid symbionts 1 (MH259897), 2 (MH259894), 4 (MH259903) (Cleary et al. 2019), Uncultured euphausiid symbiont 3 (DQ201519) (Martin et al. 2006), *Lynnina grapsolytica* (MT906161) (Metz & Hechinger 2021), *Metacollinia luciensis* (MH200622) (Lynn and Strüder-Kypke 2019)

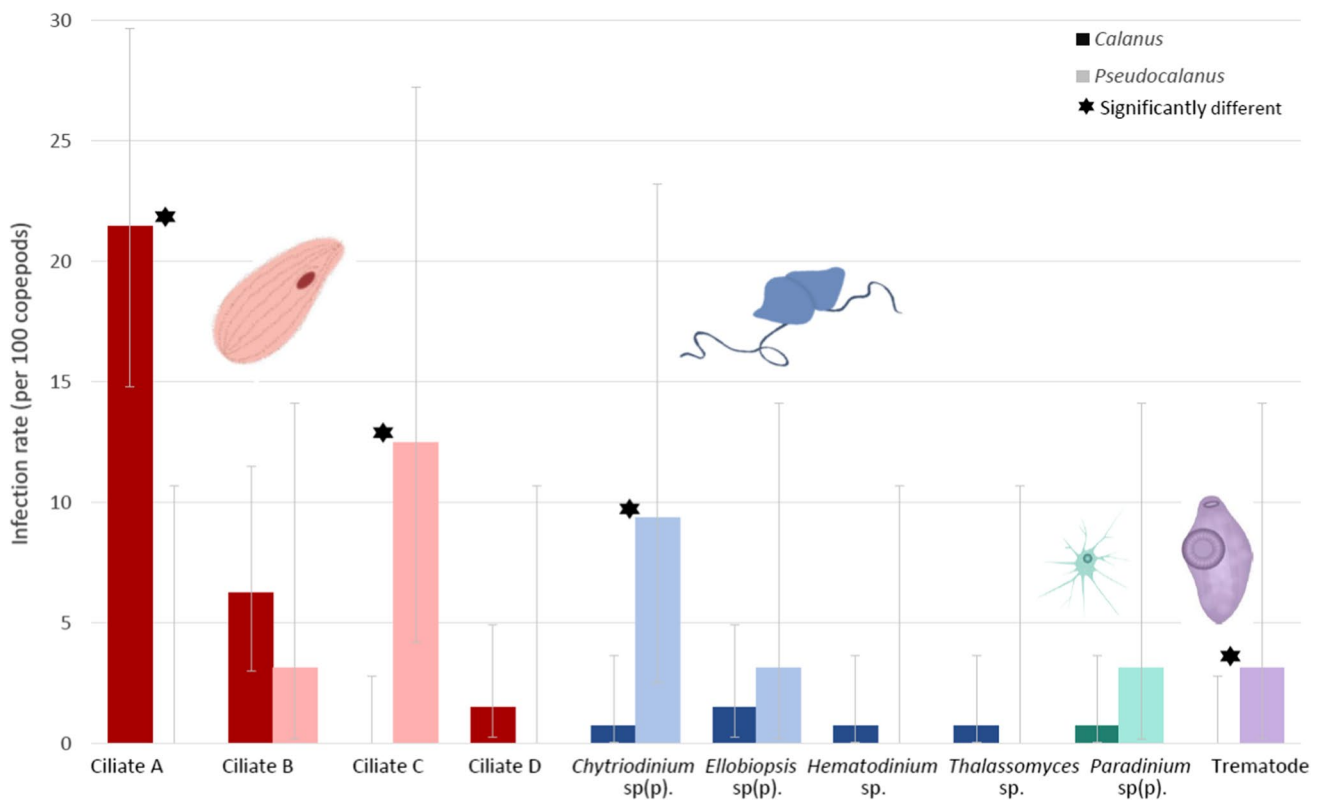
across all samples) were removed, along with 9,152 reads which were attributed to organisms which could potentially represent contamination as they are expected to be rare in the polar marine environment (terrestrial grasses). The remaining sequence reads were divided between 6,416,665 sequence reads attributable to the host copepods, 807,558 sequence reads attributable to prey in gut contents, and 521,241 sequence reads attributable to parasites (Figure S1).

Parasites identified fell into ten groups: four ciliate groups containing a total of 24 ASVs, four dinoflagellate groups containing a total of nine ASVs, a *Paradinium* sp(p). group containing four ASVs, and a trematode group containing five

ASVs (Tables 2 and S1). We have used the notation sp(p). to denote that a group may contain one or more species.

## Ciliates

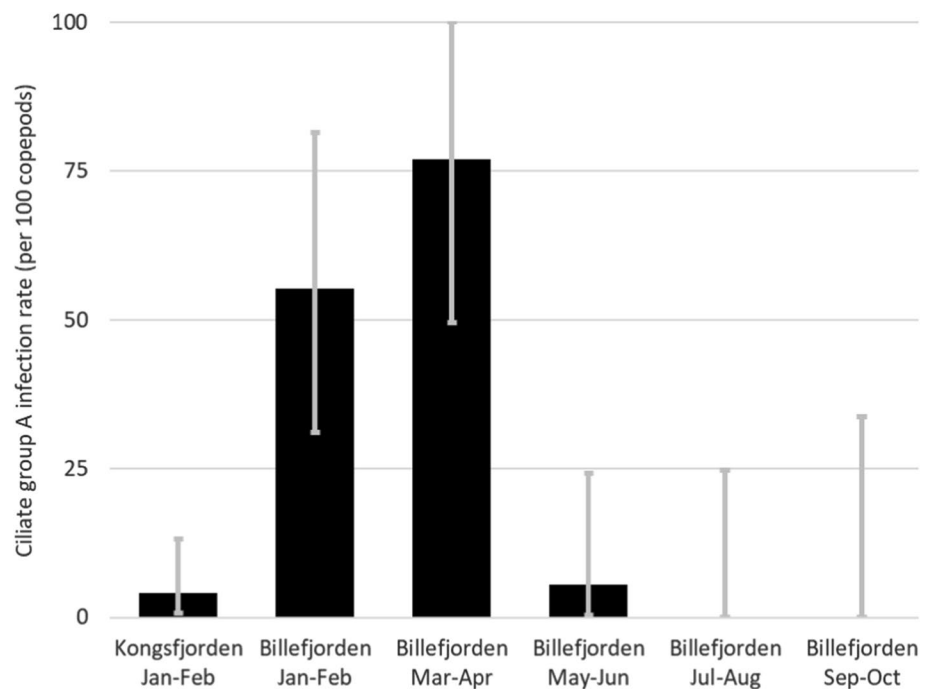
All ciliates were classified as Apostomatia, with all closest BLAST matches parasitic. Further refinement of taxonomy was not possible, but a phylogenetic tree showed four distinct groups, identified here as A, B, C, and D (Fig. 2). Group A ciliate sequences mainly showed highest similarity to reference sequences of *Pseudocollinia* spp. isolated from euphausiids, with sequence identities of 99.11% to 100%, with greatest similarity in the most abundant sequences



**Fig. 3** Infection rates for each parasite group for both host genera, including samples from all seasons. Parasites are colored by group (ciliates—red, dinoflagellates—blue, *Paradinium*—green, trematode—purple), with darker bars on the left showing *C. glacialis* and

lighter bars on the right showing *Pseudocalanus* spp.. Bar height indicates the maximum likelihood estimate, error bars indicate 95% confidence intervals. Stars indicate parasite groups with significantly different infection rates between the two host genera

**Fig. 4** Seasonal infection rate by ciliate group A. Error bars indicate 95% confidence intervals. Note that the infection rate for March–April is a lower estimate—all pools in this season were infected with Ciliate A, but as the maximum likelihood estimator is not defined in such a case, an artificial pool of one non-infected individual was included in the calculation



**Table 2** Parasite ASVs, the number of unique ASVs, and the total number of reads in each, as well as the number of samples in which at least 50 reads from that parasite were found (infected)

Parasite type	Number unique ASVs	Total reads	Number of samples infected
<i>Chytriodinium</i> sp(p).	4	105,559	4
Ciliate Group A	14	331,427	25
Ciliate Group B	4	19,388	9
Ciliate Group C	5	1991	4
Ciliate Group D	1	1815	2
<i>Ellobiopsis</i> sp(p)	3	24,285	3
<i>Hematodinium</i> sp.	1	1316	1
<i>Paradinium</i> sp(p).	4	2713	2
<i>Thalassomyces</i> sp.	1	367	1
Trematode	5	32,380	1

(Lynn et al. 2014). The closest reference sequences for the remaining three groups B, C, and D were, respectively, a parasitic *Gymnodinioides* sp. ciliate isolated from a gammarid amphipod at 98.67–99.11% sequence identity (Clamp et al. 2008), a *Lynnina grapsolytica* isolated from a striped shore crab (*Pachygrapsus crassipes*) at 96.89–97.33% identity (Metz and Hechinger 2021), and a *Hyalophysa Iwoffi* isolated from a glass shrimp (*Palaemonetes paludosus*) at 98.67% identity (Clamp et al 2008). These last mentioned reference sequences did not however consistently cluster with the respective ciliate groups in phylogenetic trees (Fig. 2).

Ciliates were the most abundant group in terms of total sequence reads, and the most frequently observed infecting samples. The ciliate groups also showed host-genus specific patterns in infection rates (Fig. 3). Ciliate group A was only found in *C. glacialis*, where it was the most common parasite group, with an infection rate of 21.47% (95% CI 14.82–29.66). This was significantly higher than in *Pseudocalanus* (0%, 0–10.72). By contrast, ciliate group C was only found in *Pseudocalanus* spp., with an infection rate of 12.50% (4.20–27.25), which was significantly different from *Calanus* (0%, 0–2.80). Ciliate group D was only found in *Calanus*, but due to its relative rarity (1.53%, 0.28–4.91), this difference was not significant (*Pseudocalanus* 0%, 0–10.72). Ciliate B was present in both *Calanus* (6.27%, 2.99–11.53), and *Pseudocalanus* (3.13%, 0.18–14.13), with no significant difference in infection rate. Multiple ciliate types were occasionally observed within the same sample, with four samples each containing two ciliate types, and one sample containing three of the ciliate types.

Ciliate A showed a seasonal pattern to infection rates in *C. glacialis*. Infections were highest in early spring, when every single sample contained this parasite group. Infections were present at a lower level in Jan–Feb and May–June, and

absent in the late summer and fall samples (Fig. 4). The statistical significance of this trend was not assessed.

## Dinoflagellates

*Chytriodinium* sp(p). was observed infecting one *C. glacialis* sample (infection rate 0.76%, 0.04–3.63) and three *Pseudocalanus* spp. samples (9.38%, 2.53–23.21), all from January. *Chytriodinium* sp(p). sequences all showed highest sequence similarity to sequences isolated from copepod eggs (unknown species), at 96.9–97.4% identity (Gómez and Skovgaard 2015).

*Ellobiopsis* sp(p). was observed infecting two samples of *C. glacialis* (1.53%, 0.28–4.91), and 1 sample of *P. minutus* (3.13%, 0.18–14.13), with no seasonal pattern. *Ellobiopsis* sp(p). sequences were 97.4 to 98.7% identical to reference sequences of *Ellobiopsis chattoni* isolated from a mixed copepod assemblage in the Mediterranean sea (Gómez et al. 2009b).

*Hematodinium* sp. was observed as a single ASV, infecting a single sample (*C. glacialis* in Billefjorden in October) (infection rates *Calanus* 0.76%, 0.04–3.63, *Pseudocalanus* 0%, 0–10.72). This sequence was 96.97% identical to a reference sequence for *Hematodinium* sp. isolated from Norway Lobster (*Nephrops norvegicus*) (Jensen et al. 2010).

*Thalassomyces* sp. was also observed as a single ASV infecting a single sample, a *C. glacialis* in Billefjorden in January (infection rates *Calanus* 0.76%, 0.04–3.63, *Pseudocalanus* 0%, 0–10.72). This sequence was 100% identical to a reference sequence of *Thalassomyces* sp. isolated from a hyperiid amphipod (Silberman et al. 2004).

## Paradinium

Four ASVs of *Paradinium* sp(p). were observed, infecting two samples, *C. glacialis* in Billefjorden in January and *P. minutus* in Isfjorden in January, (infection rates *Calanus* 0.76%, 0.04–3.63, *Pseudocalanus* 3.13%, 0.18–14.13). *Paradinium* sp(p). ASVs all were most similar to a single reference sequence of *Paradinium pouchetti* isolated from copepods, with similarities between 90.46% and 95.71% (Skovgaard and Daugbjerg 2008). Both of the samples infected with *Paradinium* sp(p). also contained another parasite (Ciliate A in *Calanus* and trematode in *Pseudocalanus*).

## Trematode

Five trematode ASVs were observed, all in a single sample, *P. minutus* in Isfjorden in January (infection rates *Calanus* 0%, 0–2.80, *Pseudocalanus* 3.13%, 0.18–14.13). Trematode sequences were all most similar to a reference sequence of *Lecithaster gibbosus* isolated from the whiting



fish (*Merlangius merlangus*), with similarities of 98.98% to 99.66% (Olson et al. 2003).

## Discussion

### Methodological considerations

The metabarcoding approach applied here identified a wide range of parasites, across samples of copepods which did not show obvious outward signs of infection. This suggests metabarcoding may be an effective approach for the future, allowing high sensitivity and high throughput parasite detection. However, this approach is not without its limitations, many of which may be overcome by future methodological improvements.

Using freshly captured copepods, there is a possibility that some of the parasite sequences may represent prey. Parasite spores may be ingested, and occasionally digested without necessarily leading to a successful infection (Poulin and Keeney 2007). Parasites may also potentially be present within ingested prey. We minimized the impact of these two possibilities by setting a lower threshold of 50 reads for a sample to be considered infected. Prey DNA is rapidly digested, whereas parasites are not being digested, so any parasites-as-prey DNA is likely to be in lower abundance. Alternatively, there may be organisms which are indistinguishable from prey, that are missed as parasites. In this study, ctenophore sequences were fairly common, and since they were most similar to free living ctenophore sequences were classified as non-parasitic. However, there is some uncertainty, as parasitic ctenophores are known, and there are likely additional groups which have yet to be described and sequenced (Haddock 2007). Future studies aimed at parasite–zooplankton interactions can avoid both of these potential issues by incubating zooplankton in filtered seawater prior to preservation, so that gut contents are evacuated. In a similar issue, the methods employed here may not accurately sample all epibiotic parasites, as they may have been affected by the steps taken to decontaminate the exterior of the copepods prior to analysis. The impact of this appears to be small, as there were no clear differences in the detection of external parasites across samples which were treated with different decontamination procedures, but future work will need to consider the optimal balance between decontamination and potential loss of epibiotic parasites.

Lastly, while this approach efficiently captures most eukaryotic symbionts, our data do not accurately capture fungal assemblages. Fungi are not generally well detected or resolved by 18S sequencing, so future work would benefit from parallel analyses using gene regions which are more appropriate for this group, such as ITS. Similarly, these data are not informative about bacterial or viral symbiont, groups

which would again require the use of differently targeted markers.

### Parasite diversity

A diverse assemblage of parasites was observed across the sampled copepods, including Apistome ciliates (4 types), dinoflagellates (4 types), *Paradinium*, and trematodes. Apistome ciliates, dinoflagellates, and trematodes are all among the most species rich of known copepod parasite groups in the marine environment (Bass et al. 2021). *Hematodinium* spp. dinoflagellates are common among other groups of decapod crustaceans, including various commercially important stocks of crabs, shrimps, and lobster, but have not previously been reported from copepods (Stentiford and Shields 2005; Bass et al. 2021).

Within these groups, there is some uncertainty how many distinct parasite species these sequences represent, hence our use of sp(p). There is both intraspecific and interspecific variation in DNA sequences, and for multicopy genes, such as the 18S used here, even intraindividual variation (Gómez et al. 2009b; Coissac et al. 2012). It is often unclear where the boundaries between these different types of variation lie, particularly with poorly studied organisms such as parasites. Additionally, even with best practices in bioinformatics and quality control, there may be some level of PCR and sequencing error in the data, resulting in sequences which do not represent true biological variation. One notable example in our data is the group of trematode sequences; five unique trematode sequences were found with over 100 reads each, but all five were present only in the same one sample, and all are most similar to the same sequence in GenBank. Thus, the most parsimonious explanation would be that these sequences all arise from a single trematode, as a result of either differences between multiple 18S copies in the genome or errors occurring early in the PCR. On the other hand, sequence variation may in some cases be lower than true parasite species variation. For example, the four described species of parasitic *Pseudocollinia* spp. ciliates are all identical over this section of the 18S gene (Cleary et al. 2019). For these reasons, we report the number of unique sequences, but focus our discussion on the broader groups. In the future, these challenges may be improved by the use of multiple gene markers, and by increasing depth of reference databases.

Some of the parasites observed here likely represent species for which there are no existing sequence data. While it is difficult to determine an exact similarity cut-off for the reasons described above, sequences showing much less than 97% similarity to anything currently in genbank, such as the *Paradinium* sp(p). parasites identified here, likely reflect novel, species-level diversity.

## Parasite prevalence

Rates of infection by parasite vary widely across studies of copepods. While comparisons between studies using different methods should be made cautiously, as molecular methods are likely to detect earlier stage infections compared to microscopy-based studies, for several of the parasites observed here, infection rates are similar to published rates from other studies.

Apostome ciliates were the parasites with the highest infection rate observed here, with overall rates of infection for the most common group A in *Calanus* of 21.47%, with higher rates seasonally, including in spring where every sample was infected. These high rates fit with previous observations, which have found up to 100% of copepods in a sample infected (Ohtsuka et al. 2004).

Previous work in Svalbard found infections of *Ellobiopsis* sp. on *C. glacialis* and *C. finmarchicus*, with rates of 0.09% and 0.06%, respectively (Walkusz and Rolbiecki 2007). Similarly, low infection rates ranging from 0.05% to 2.27% were found for *Ellobiopsis* on *C. finmarchicus* by Timofeev in waters northeast of Svalbard (Timofeev 1997). These are both similar to the infection rate observed here for *C. glacialis* of 1.53% (95% confidence interval 0.28 – 4.91). Our sampling may have underestimated *Ellobiopsis* sp(p). in Kongsfjorden due to the enhanced decontamination protocols applied to those samples, as this parasite is mainly external to the copepod body. But as *Ellobiopsis* sp(p). was also rare in Billefjorden samples, this is unlikely to have made a significant impact on the overall rates. In contrast, Timofeev (1997) found much higher infection rates for *Ellobiopsis* sp. in *C. finmarchicus* in more southern sampling areas with a mean infection rate near Iceland of 19.7% (range 8.3–45.4), and near the Shetlands of 9.3% (range 0–20), and similar rates (8.9%) have been observed in *C. helgolandicus* populations in oceanic waters of the Bay of Biscay (Albaina and Irigoien 2006). It has been suggested these differences indicate temperature limitation of this parasite (Walkusz and Rolbiecki 2007). Our data seem to fit the observed pattern of relatively low infection rates in the Svalbard copepod assemblage, but more data are needed to conclude the drivers of this patterns.

Trematodes were relatively rare in our data, with an infection rate of 3.1% in *Pseudocalanus* and not present at all in *Calanus*. Previous observations of the closely related trematode *Lecithaster gibbosus* found infection rates of 4% in samples of *Pseudocalanus minutus* and *Centropages abdominalis* in coastal British Columbia, Canada (Boyce 1969).

## Host specificity

Across ecosystems generally, the vast majority of parasites exhibit high host specificity, infecting only one, or less often

two to four, host species; broad generalist parasites certainly exist, but are uncommon (Poulin and Keeney 2007). Little is known about host specificity in zooplankton parasites. Patterns in specificity are often correlated with infection pathway, with trophically transmitted parasites less specific than parasites transmitted by near contact, but these pathways are also uncertain for most zooplankton parasites (Poulin and Keeney 2007).

Ciliate parasites showed different patterns of infection across the two host copepod genera investigated, with two of the ciliate groups (A and C) showing a significant preference for one of the host genera (*Calanus* and *Pseudocalanus*, respectively). Apostome ciliates as a group vary in their specificity, with some preferring certain host species, or selecting calanoid copepods over cyclopoids, while others infect a wide range of hosts, such as *Hyalophysa chattoni* which parasitizes 18 different decapod species (Ohtsuka et al. 2015; Savage 2020).

*Chytriodinium* sp(p). also showed a significant host preference, being found in three *Pseudocalanus* spp. samples and only a single *Calanus* sp. sample. *Chytriodinium* sp. have previously been observed on copepod eggs and nauplii of unknown species (Chatton 1912; Chatton 1920; Cachon and Cahon 1968; Gómez et al. 2009a; Strassert et al. 2019; Bass et al. 2021).

Trematode sequences were only present in a *Pseudocalanus* spp. sample. While it is difficult to draw conclusions from such small sample sizes, it is interesting to note that previous studies of this trematode found infections in samples of *P. minutus* and *Centropages abdominalis*, but not in samples of *Calanus pacificus* and *Calanus plumchrus* collected at nearby locations (Boyce 1969).

## Seasonal patterns

There is remaining uncertainty about seasonal patterns in zooplankton parasites generally. Both biotic and abiotic factors in the environment can contribute to seasonal variation in parasite–host interactions. Here, we saw highest infection rates by ciliate A in spring samples, with lowest rates in late summer and autumn. This is similar to previous observations, with highest infection rates by Apostome ciliates on *Calanus pacificus* near Vancouver observed in spring, with much lower rates in summer fall and winter (Savage 2020). Potential drivers of this observed seasonality could include reduced immune defences in the period before the spring bloom when both available food and lipid reserves are low, or seasonal changes in the vertical distribution of copepods bringing them into greater contact with parasites. Ohtsuka et al. (2004) also found highest infection rates from late winter into early summer and lowest rates in fall and winter for Apostome ciliates infecting *Calanus sinicus*, but they found inverse patterns in other copepod host spp., with

higher infection rates in late summer and fall. Thus, complete understanding of the seasonality in ciliate infections will require further investigation, and may necessitate considering other host zooplankton.

## Broader implications

What are the potential impacts of parasite infections of the types identified here on the populations of these keystone copepods? *Pseudocollinia* spp. ciliates, with DNA sequences identical to the most common parasite observed here, have been shown to lead directly to euphausiid mortality, and mass mortality events have been attributed to outbreaks of these ciliates (Gómez-Gutiérrez et al. 2003). *Ellobiopsis* spp., though not lethal, leads to reduced egg production in infected female hosts (Albaina and Irigoien 2006). Too little is known about most other copepod parasites to confidently assess their potential impacts at either the individual or population level.

Looking to a future with ongoing changes in environmental conditions, the role of parasites already common in Svalbard copepod populations could change, in ways that remain difficult to predict. The responses of parasite–host systems to environmental change are complex and multifaceted; various environmental factors such as changes in temperature, salinity, acidification, and circulation may influence different parasites and hosts in multiple ways, with combined responses which are nonlinear and difficult to predict (Harvell et al. 2002; Hamilton et al. 2009; Lafferty 2009; Bates et al. 2010).

In addition to changes in the dynamics of present-day host–parasite interactions, climate change may also lead to the introduction of new parasites. While most parasites show high host specificity, others are more generalist, and even parasites which are typically highly specific may rapidly evolve to new hosts when an advantageous opportunity presents itself (Poulin and Keeney 2007). In that context it is interesting to compare the parasite assemblage observed here in Arctic *C. glacialis*, with the parasites previously observed in *C. finmarchicus*, a species closely related to *C. glacialis* which is abundant in temperate North Atlantic waters. *C. finmarchicus* has been increasingly observed in Svalbard waters in recent years, reflecting a general trend of Atlantification in the archipelago (Wassmann et al. 2006). While not entirely comparable in methodology, there is an extensive review of the parasites found in *C. finmarchicus* in coastal waters of Scotland (Jepps 1937). Jepps identified three of the same groups of parasites identified here, *Ellobiopsis* spp., *Paradinium* spp., and Apostome ciliates, as well as four groups not observed here, *Blastodinium* spp., *Syndinium* spp., gregarines, and *Ichthyosporidium* spp. (Jepps 1937). Absent in Jepps's observations are three of the parasite types observed here, *Chytriodinium* spp., *Hematodinium*

spp., and *Thalassomyces* spp.. This raises concern that increasing overlap between *C. finmarchicus* and *C. glacialis* may create the conditions to allow transfer of parasites in either or both directions.

This study provides a first baseline measure of the breadth and prevalence of eukaryotic parasites in these keystone zooplankton species in Arctic ecosystems. While there is still much to be done to understand spatial and temporal trends, and impacts on physiology, populations, and ecosystems, the frequency with which parasites were detected in this study suggests they merit further attention. Understanding the roles that parasites play today, and incorporating them into our understanding and modeling of ecosystems structure and function will also be essential to robust predictions of how ecosystems will respond to a future warmer Arctic.

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**Author contributions** TMG conceived of the idea, conducted field work, and supervised laboratory analyses. TAC conducted Kongsfjorden field work, laboratory analyses, and initial data analyses. JB contributed to Kongsfjorden initial analyses. ACC conducted final data analyses, and drafted the manuscript. All authors contributed to the final manuscript text and agree to its publication.

**Data availability** Sequence data are freely available from NCBI under Bioproject PRJNA799346, including both raw demultiplexed reads (accessions SAMN25159405 – SAMN25159602) and assembled ASV sequences (accessions OM337878 – OM337919). Infection status for each parasite type in each sample is available in the electronic supplementary information (Table S2).

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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## References

- Albaina A, Irigoien X (2006) Fecundity limitation of *Calanus helgolandicus*, by the parasite *Ellobiopsis* sp. J Plankton Res 28:413–418. <https://doi.org/10.1093/plankt/fbi129>
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Bass D, Cleary AC, Huys R, Reuckert S, Taylor JD, Ward GM, Stern R (2021) Parasites, pathogens, and other symbionts of copepods. Frontiers in Parasitology 37:875–889. <https://doi.org/10.1016/j.pt.2021.05.006>
- Bates AE, Stickle WB, Harley CD (2010) Impact of temperature on an emerging parasitic association between a sperm-feeding scuticociliate and Northeast Pacific sea stars. J Exp Mar Biol Ecol 384:44–50. <https://doi.org/10.1016/j.jembe.2009.12.001>
- Biggerstaff BJ (2009) PooledInfRate, Version 4.0: a Microsoft® Office Excel® Add-In to compute prevalence estimates from pooled samples. Centers for Disease Control and Prevention, Fort Collins, CO, U.S.A.
- Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. <https://doi.org/10.1038/s41587-019-0209-9>
- Boyce NP (1969) Parasite fauna of pink salmon (*Oncorhynchus gorbuscha*) of the Bella Coola River, central British Columbia, during their early sea life. Journal of the Fisheries Board of Canada 26:813–820
- Cachon J, Cachon M (1968) Cytologie et cycle évolutif des *Chytridinium* (Chatton). Protistologica 4:249–262
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. <https://doi.org/10.1038/nmeth.3869>
- Chantangsi C, Lynn DH, Rueckert S, Prokopowicz AJ, Panha S, Leander BS (2013) *Fusifforma themisticola* n. gen., n. sp., a new genus and species of apostome ciliate infecting the hyperiid amphipod *Themisto libellula* in the Canadian Beaufort Sea (Arctic Ocean), and establishment of the Pseudocolliniidae (Ciliophora, Apostomatia). Protist 164:793–810. <https://doi.org/10.1016/j.protis.2013.09.001>
- Chatton É (1912) Diagnoses préliminaires de Péridiniens parasites nouveaux. Bull Soc Zool Fr 37:85–93
- Chatton É (1920) Les Péridiniens parasites. Morphologie, reproduction, éthologie. Archives De Zoologie Expérimentale Et Générale 59:1–475
- Clamp JC, Bradbury PC, Strüder-Kypke M, Lynn DH (2008) Phylogenetic position of the apostome ciliates (Phylum Ciliophora, Subclass Apostomatia) tested using small subunit rRNA gene sequences. Denisia 23:395–402. [https://doi.org/10.1111/j.1550-7408.2005.05202003\\_1\\_54.x](https://doi.org/10.1111/j.1550-7408.2005.05202003_1_54.x)
- Cleary AC, Søreide JE, Freese D, Niehoff B, Gabrielsen TM (2017) Feeding by *Calanus glacialis* in a high arctic fjord: potential seasonal importance of alternative prey. ICES J Mar Sci 74:1937–1946. <https://doi.org/10.1093/icesjms/fsx106>
- Cleary AC, Casas MC, Durbin EG, Gómez-Gutiérrez J (2019) Parasites in Antarctic krill guts inferred from DNA sequences. Antarct Sci 31:16–22. <https://doi.org/10.1017/S0954102018000469>
- Coissac E, Riaz T, Puillandre N (2012) Bioinformatic challenges for DNA metabarcoding of plants and animals. Mol Ecol 21:1834–1847. <https://doi.org/10.1111/j.1365-294X.2012.05550.x>
- Conover RJ, Bedo AW, Herman AW, Head EJ, Harris LR, Horne EP (1988) Never trust a copepod—some observations on their behavior in the Canadian Arctic. Bull Mar Sci 43:650–662
- Coyle KO, Eisner LB, Mueter FJ, Pinchuk AI, Janout MA, Cieciel KD, Farley EV, Andrews AG (2011) Climate change in the southeastern Bering Sea: impacts on pollock stocks and implications for the oscillating control hypothesis. Fish Oceanogr 20:139–156. <https://doi.org/10.1111/j.1365-2419.2011.00574.x>
- Daase M, Varpe Ø, Falk-Petersen S (2014) Non-consumptive mortality in copepods: occurrence of *Calanus* spp. carcasses in the Arctic Ocean during winter. J Plankton Res 36:129–144. <https://doi.org/10.1093/plankt/ftb079>
- Dalpadado P, Hop H, Rønning J, Pavlov V, Sperfeld E, Buchholz F, Rey A, Wold A (2016) Distribution and abundance of euphausiids and pelagic amphipods in Kongsfjorden, Isfjorden and Rijpfjorden (Svalbard) and changes in their relative importance as key prey in a warming marine ecosystem. Polar Biol 39:1765–1784. <https://doi.org/10.1007/s00300-015-1874-x>
- Dobson AP, Hudson PJ (1986) Parasites, disease and the structure of ecological communities. Trends Ecol Evol 1:11–15. [https://doi.org/10.1016/0169-5347\(86\)90060-1](https://doi.org/10.1016/0169-5347(86)90060-1)
- Durbin EG, Casas MC (2014) Early reproduction by *Calanus glacialis* in the Northern Bering Sea: the role of ice algae as revealed by molecular analysis. J Plankton Res 36:523–541. <https://doi.org/10.1093/plankt/ftb121>
- Falk-Petersen S, Mayzaud P, Kattner G, Sargent JR (2009) Lipids and life strategy of Arctic *Calanus*. Mar Biol Res 5:18–39. <https://doi.org/10.1080/17451000802512267>
- Fields DM, Runge JA, Thompson C, Shema SD, Bjelland RM, Durif CMF, Skiftesvik AB, Browman HI (2015) Infection of the planktonic copepod *Calanus finmarchicus* by the parasitic dinoflagellate, *Blastodinium* spp: effects on grazing, respiration, fecundity and fecal pellet production. J Plankton Res 37:211–220. <https://doi.org/10.1093/plankt/fbu084>
- Gabrielsen TM, Merkel B, Søreide JE, Johansson-Karlsson E, Bailey A, Vogedes D, Nygård H, Varpe Ø, Berge J (2012) Potential misidentifications of two climate indicator species of the marine arctic ecosystem: *Calanus glacialis* and *C. finmarchicus*. Polar Biol 35:1621–1628. <https://doi.org/10.1007/s00300-012-1202-7>
- Gast RJ, Dennett MR, Caron DA (2004) Characterization of protistan assemblages in the Ross Sea, Antarctica, by denaturing gradient gel electrophoresis. Appl Environ Microbiol 70:2028–2037. <https://doi.org/10.1128/AEM.70.4.2028-2037.2004>
- Gómez F, Moreira D, López-García P (2009a) Life cycle and molecular phylogeny of the dinoflagellates *Chytridinium* and *Dissodinium*, ectoparasites of copepod eggs. Eur J Protistol 45:260–270. <https://doi.org/10.1016/j.ejop.2009.05.004>
- Gómez F, López-García P, Nowaczyk A, Moreira D (2009b) The crustacean parasites *Ellobiopsis Caullery*, 1910 and *Thalassomyces Niezabitowski*, 1913 form a monophyletic divergent clade within the Alveolata. Syst Parasitol 74:65–74. <https://doi.org/10.1007/s11230-009-9199-1>
- Gómez F, Skovgaard A (2015) Molecular phylogeny of the parasitic dinoflagellate *Chytridinium* within the Gymnodinium clade (Gymnodinales, Dinophyceae). J Eukaryot Microbiol 62:422–425. <https://doi.org/10.1111/jeu.12180>
- Gómez-Gutiérrez J, Peterson WT, De Robertis A, Brodeur RD (2003) Mass mortality of krill caused by parasitoid ciliates. Science 301:339–339. <https://doi.org/10.1126/science.1085164>

- Gu W, Lampman R, Novak RJ (2004) Assessment of arbovirus vector infection rates using variable size pooling. *Med Vet Entomol* 18:200–204. <https://doi.org/10.1111/j.0269-283X.2004.00482.x>
- Haddock SH (2007) Comparative feeding behavior of planktonic ctenophores. *Integr Comp Biol* 47:847–853. <https://doi.org/10.1093/icb/icm088>
- Hamilton KM, Shaw PW, Morrill D (2009) Prevalence and seasonality of *Hematodinium* (Alveolata: Syndinea) in a Scottish crustacean community. *ICES J Mar Sci* 66:1837–1845. <https://doi.org/10.1093/icesjms/fsp152>
- Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. *Science* 296:2158–2162. <https://doi.org/10.1126/science.1063699>
- Hop H, Falk-Petersen S, Svendsen H, Kwasniewski S, Pavlov V, Pavlova O, Søreide JE (2006) Physical and biological characteristics of the pelagic system across Fram Strait to Kongsfjorden. *Prog Oceanogr* 71:182–231. <https://doi.org/10.1016/j.pocean.2006.09.007>
- Jensen PC, Califf K, Lowe V, Hauser L, Morado JF (2010) Molecular detection of *Hematodinium* sp. in Northeast Pacific *Chionoecetes* spp. and evidence of two species in the Northern Hemisphere. *Dis Aquat Org* 89:155–166. <https://doi.org/10.3354/dao02193>
- Jepps MW (1937) Memoirs: on the protozoan parasites of *Calanus finmarchicus* in the Clyde sea area. *J Cell Sci* 2:589–658
- Jónasdóttir SH, Visser AW, Richardson K, Heath MR (2015) Seasonal copepod lipid pump promotes carbon sequestration in the deep North Atlantic. *Proc Natl Acad Sci* 112:12122–12126. <https://doi.org/10.1073/pnas.1512110112>
- Käse L, Metfies K, Neuhaus S, Boersma M, Wiltshire KH, Kraberg AC (2021) Host-parasitoid associations in marine planktonic time series: Can metabarcoding help reveal them? *PLoS ONE* 16:e0244817. <https://doi.org/10.1371/journal.pone.0244817>
- Kimmerer WJ, McKinnon AD (1990) High mortality in a copepod population caused by a parasitic dinoflagellate. *Mar Biol* 107:449–452. <https://doi.org/10.1007/BF01313428>
- Kuris AM, Hechinger RF, Shaw JC, Whitney KL, Aguirre-Macedo L, Boch CA, Dobson AP, Dunham EJ, Fredensborg BL, Huspeni TC, Lorda J (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* 454:515–518. <https://doi.org/10.1038/nature06970>
- Kurtz J (2007) Evolutionary ecology of immune defense in copepods. *J Plankton Res* 29:27–38. <https://doi.org/10.1093/plankt/fbl063>
- Kwasniewski S, Gluchowska M, Jakubas D, Wojczulanis-Jakubas K, Walkusz W, Karnovsky N, Blachowiak-Samolyk K, Cisek M, Stempniewicz L (2010) The impact of different hydrographic conditions and zooplankton communities on provisioning Little Auks along the West coast of Spitsbergen. *Prog Oceanogr* 87:72–82. <https://doi.org/10.1016/j.pocean.2010.06.004>
- Lafferty KD (2009) Calling for an ecological approach to studying climate change and infectious diseases. *Ecology* 90:932–933. <https://doi.org/10.1890/08-1767.1>
- Lima-Mendez G, Faust K, Henry N, Decelle J, Colin S, Carcillo F, Chaffron S, Ignacio-Espinosa JC, Roux S, Vincent F, Bittner L (2015) Determinants of community structure in the global plankton interactome. *Science* 348:1262073. <https://doi.org/10.1126/science.1262073>
- Lynn DH, Gomez-Gutierrez J, Strüder-Kypke MC, Shaw CT (2014) Ciliate species diversity and host-parasitoid co-diversification in *Pseudocollinia* infecting krill, with description of *Pseudocollinia similis* sp. nov. *Dis Aquat Org* 112:89–102. <https://doi.org/10.3354/dao02796>
- Lynn DH, Strüder-Kypke MC (2019) The sanguicolous apostome *Metacollinia luciensis* Jankowski 1980 (colliniidae, apostomatia, ciliophora) is not closely related to other sanguicolous apostomes. *J Eukaryot Microbiol* 66:140–146. <https://doi.org/10.1111/jeu.12638>
- Martin DL, Ross RM, Quetin LB, Murray AE (2006) Molecular approach (PCR-DGGE) to diet analysis in young Antarctic krill *Euphausia superba*. *Mar Ecol Prog Ser* 319:155–165. <https://doi.org/10.3354/meps319155>
- Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *Embnet J* 17:10–12. <https://doi.org/10.14806/ej.17.1.200>
- Metz DC, Hechinger RF (2021) *Lynnina grapsolytica* n. gen, n. sp. (Ciliophora: Apostomatida: Colliniidae), a deadly blood parasite of crabs with a novel pseudocyclopharynx. *J Eukaryot Microbiol* 68:e12847. <https://doi.org/10.1111/jeu.12847>
- Nilsen F, Cottier F, Skogseth R, Mattsson S (2008) Fjord–shelf exchanges controlled by ice and brine production: the interannual variation of Atlantic Water in Isfjorden. *Svalbard Continental Shelf Research* 28:1838–1853. <https://doi.org/10.1016/j.csr.2008.04.015>
- Ohtsuka S, Hora M, Suzaki T, Arikawa M, Omura G, Yamada K (2004) Morphology and host-specificity of the apostome ciliate *Vampyrophrya pelagica* infecting pelagic copepods in the Seto Inland Sea, Japan. *Mar Ecol Prog Ser* 282:129–142. <https://doi.org/10.3354/meps282129>
- Ohtsuka S, Suzaki T, Kanazawa A, Ando M (2015) Biology of symbiotic Apostome Ciliates: their diversity and importance in the aquatic ecosystems. In *Marine Protists* Springer, Tokyo. [https://doi.org/10.1007/978-4-431-55130-0\\_18](https://doi.org/10.1007/978-4-431-55130-0_18)
- Olsen BR, Troedsson C, Hadziavdic K, Pedersen RB, Rapp HT (2014) A molecular gut content study of *Themisto abyssorum* (Amphipoda) from Arctic hydrothermal vent and cold seep systems. *Mol Ecol* 23:3877–3889. <https://doi.org/10.1111/mec.12511>
- Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DT (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *Int J Parasitol* 33:733–755. [https://doi.org/10.1016/S0020-7519\(03\)00049-3](https://doi.org/10.1016/S0020-7519(03)00049-3)
- O’Rourke R, Lavery S, Chow S, Takeyama H, Tsai P, Beckley LE, Thompson PA, Waite AM, Jeffs AG (2012) Determining the diet of larvae of western rock lobster (*Panulirus cygnus*) using high-throughput DNA sequencing techniques. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0042757>
- Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M, Prettenhofer P, Weiss R, Dubourg V, Vanderplas J (2011) Scikit-learn: machine learning in Python. *J Mach Learn Res* 12:2825–2830
- Poulin R, Keeney DB (2007) Host specificity under molecular and experimental scrutiny. *Trends Parasitol* 24:24–28. <https://doi.org/10.1016/j.pt.2007.10.002>
- Rohland N, Reich D (2012) Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Res* 22:939–946. <https://doi.org/10.1101/gr.128124.111>
- Runge JA (1988) Should we expect a relationship between primary production and fisheries? The role of copepod dynamics as a filter of trophic variability. In *Biology of Copepods* Springer, Dordrecht, pp 61–71
- Sanders R, Henson SA, Koski M, Christina LD, Painter SC, Poulton AJ, Riley J, Salihoglu B, Visser A, Yool A, Bellerby R (2014) The biological carbon pump in the North Atlantic. *Prog Oceanogr* 129:200–218. <https://doi.org/10.1016/j.pocean.2014.05.005>
- Savage, Rose-Lynne (2020) Characterizing the diversity of the eukaryotic microbiome in marine crustacean zooplankton. Electronic Thesis and Dissertation Repository 7442. <https://ir.lib.uwo.ca/etd/7442>
- Silberman JD, Collins AG, Gershwin LA, Johnson PJ, Roger AJ (2004) Ellobiopsids of the genus *Thalassomyces* are alveolates. *J Eukaryot Microbiol* 51:246–252. <https://doi.org/10.1111/j.1550-7408.2004.tb00555.x>

- Skovgaard A, Daugbjerg N (2008) Identity and systematic position of *Paradinium poucheti* and other *Paradinium*-like parasites of marine copepods based on morphology and nuclear-encoded SSU rDNA. *Protist* 159:401–413. <https://doi.org/10.1016/j.protis.2008.02.003>
- Skovgaard A (2014) Dirty tricks in the plankton: diversity and role of marine parasitic protists. *Acta Protozoologica* 53:51–62
- Souidenne D, Florent I, Dellinger M, Justine JL, Romdhane MS, Furuya H, Grellier P (2016) Diversity of apostome ciliates, *Chromidina* spp. (Oligohymenophorea, Opalinopsidae), parasites of cephalopods of the Mediterranean Sea. *Parasite* 23:33. <https://doi.org/10.1051/parasite/2016033>
- Stentiford GD, Shields JD (2005) A review of the parasitic dinoflagellates *Hematodinium* species and *Hematodinium*-like infections in marine crustaceans. *Dis Aquat Org* 66:47–70. <https://doi.org/10.3354/dao066047>
- Strassert JF, Hehenberger E, Del Campo J, Okamoto N, Kolisko M, Richards TA, Worden AZ, Santoro AE, Keeling PJ (2019) Phylogeny, evidence for a cryptic plastid, and distribution of *Chytriodinium* parasites (Dinophyceae) infecting copepods. *J Eukaryot Microbiol* 66:574–581. <https://doi.org/10.1111/jeu.12701>
- Stübner EI, Søreide JE, Reigstad M, Marquardt M, Blachowiak-Samolyk K (2016) Year-round meroplankton dynamics in high-Arctic Svalbard. *J Plankton Res* 38:522–536. <https://doi.org/10.1093/plankt/fbv124>
- Timofeev SF (1997) An occurrence of the parasitic dinoflagellate *Ellobiopsis chattoni* (Protozoa: Mastigophora) on the copepod *Calanus finmarchicus* (Crustacea: Copepoda) and a possibility to use the parasite as a biological tag of local populations. *Parazitologiya* 31:334–340
- Vannatta JT, Minchella DJ (2018) Parasites and their impact on ecosystem nutrient cycling. *Trends Parasitol* 34:452–455. <https://doi.org/10.1016/j.pt.2018.02.007>
- Walkusz W, Rolbiecki L (2007) Epibionts (*Paracineta*) and parasites (*Ellobiopsis*) on copepods from Spitsbergen (Kongsfjorden area). *Oceanologia* 49:369–380
- Wassmann P, Reigstad M, Haug T, Rudels B, Carroll ML, Hop H, Gabrielsen GW, Falk-Petersen S, Denisenko SG, Arashkevich E, Slagstad D (2006) Food webs and carbon flux in the Barents Sea. *Prog Oceanogr* 71:232–287. <https://doi.org/10.1016/j.pocean.2006.10.003>
- Worden AZ, Follows MJ, Giovannoni SJ, Wilken S, Zimmerman AE, Keeling PJ (2015) Rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes. *Science* 347:1257594. <https://doi.org/10.1126/science.1257594>
- Yeh HD, Questel JM, Maas KR, Bucklin A (2020) Metabarcoding analysis of regional variation in gut contents of the copepod *Calanus finmarchicus* in the North Atlantic Ocean. *Deep Sea Res Part II* 180:104738. <https://doi.org/10.1016/j.dsr2.2020.104738>

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