UiT The Arctic University of Norway

Faculty of Biosciences, Fisheries and Economics

Seasonal dynamics of four cryptic species of *Crepidostomum* spp. in a subarctic lake in Northern Norway

Kristine Drage

Master thesis in Akvamedisin BIO-3955, May 2023

Acknowledgements

First, I would like to thank my supervisors Rune Knudsen, Isabel Blasco-Costa, and Christian Selbach. Rune, thank you for the opportunity to work on this project, for always encouraging me with your kind words, and for all the great feedback on my writing. You are a great supervisor and a true inspiration. Isa, thank you for letting me stay with you and for your guidance with my lab work in Geneva. Thank you Chris for all the writing tips, your helpful feedback on my paper, and for always being so positive. Also, a special thanks to Sigurd Slåteng. Thank you for the help in the lab and your patience with all my (sometimes stupid) questions. Your help throughout this year has been invaluable.

I would also like to thank the Freshwater Ecology Group. You have all been so welcoming and have made my master so much fun with all the social activities of the group. Thanks to Hedda Auestad Nilsen for the talks and parasite solidarity, to my office companion Ingrid Udø, and to everyone who has helped me with my thesis and in the lab. Thanks to my friends and classmates, especially Samaneh, Marte, Martine and Ingrid, for five great years. Lastly, I would like to thank my parents and my sister for encouraging and supporting me in whatever I do. You are the best.

Kristine Drage

May 2023

Abstract

The function of parasites in ecosystems is often overlooked, and there is a general lack of knowledge about parasite biodiversity down to species levels, especially for cryptic species. This study looks at the seasonal dynamics of four cryptic species of the trematode *Crepidostomum* spp. in Lake Takvatn, Northern Norway. Samples were collected between June 2017 and May 2018, and a total of 560 specimens of *Crepidostomum* spp. were sequenced in this study. The species were identified by phylograms based on novel cytochrome c oxidase subunit 1 (CO1) sequences. The species from Takvatn grouped into four distinct species-level lineages: *C. farionis*, *C. pseudofarionis*, *C. metoecus* and *C. brinkmanni*. The dominant species were *C. farionis* and *C. brinkmanni*, while *C. pseudofarionis* and *C. brinkmanni* were much rarer. The species showed clear dissimilarity in host distribution. Brown trout had a more diverse infracommunity than Arctic charr, and comprised all four species, while Arctic charr was mainly parasitised by the two dominant species. The species showed some variance in seasonality. The dominant species had a peak in frequency during autumn and winter, while the rarer species displayed a peak in frequency during autumn. In general, juveniles were mostly found in winter. The seasonal patterns of the *Crepidostomum* species could be linked to the seasonality of their intermediate hosts and the seasonal dietary shifts of their final hosts. Infection of *C. farionis* and *C. metoecus* could be connected to the fish preying upon the second intermediate host *Gammarus lacustris* during autumn and winter, especially for Arctic charr. Infection with *C. pseudofarionis* and *C. brinkmanni* for brown trout is likely due to the trout preying upon the second intermediate hosts mayflies and stoneflies over the summer months. The two rarer species might be more prevalent in Takvatn than indicated in this study due to the lack of samples of brown trout during spring and summer.

Contents

1 Introduction

Parasites have a far greater and more important function in ecosystems than just their role as pathogens. By definition, parasites have negative effects on their hosts directly by causing disease, or indirectly by stealing nutrients (Araújo et al., 2003). In addition to regulating/altering host populations through pathogenic effects (Holmstad et al., 2005), parasites are also important in aquatic food webs as consumers and prey (Johnson et al., 2010; Thieltges et al., 2013). Their importance in food webs is evident in many ecosystem studies were the links in the webs increase significantly when including parasites (Amundsen et al., 2009; Huxham et al., 1995; Thompson et al., 2005). Parasites can also be used to measure the health of an ecosystem as the abundance and diversity of some parasites are highly linked to the abundance and diversity of their hosts (Hudson et al., 2006). The presence of parasites, especially those with complex life cycles who require several hosts, are therefore good bioindicators of a diverse and healthy ecosystem (Dzikowski et al., 2003). However, for most ecosystems, parasites are not included in food web analyses (Lafferty et al., 2008) and in general there is a lack of details on parasite communities down to a species level (Pappalardo et al., 2020; Selbach et al., 2020). Parasites are estimated to account for a significant portion of the biodiversity on earth, ranging to over half of all existing species, most of which have not yet been discovered (Poulin, 2014). This means that in systems that are otherwise well known, much of the parasite biodiversity is still hidden.

Seasonality is an important factor in the context of understanding parasites and their place in ecosystems (Studer & Poulin, 2012). For parasites with complex life cycles the timing of their life cycle depends both on host availability and specific environmental factors, like temperature, during their transmission stages (Selbach & Poulin, 2020; Studer & Poulin, 2012). The subarctic lakes of Northern Norway experience strong seasonal variations in climate (Amundsen & Knudsen, 2009). Organisms inhabiting these systems are subject to profound changes in both biotic and abiotic factors throughout the year which leads to strong seasonal patterns (Varpe, 2017), and this is also the case for parasites (Prati et al., 2020b). The subarctic lakes and their ecosystems are especially vulnerable to the changing climate (Rouse et al., 1997). In the Arctic, climate change develops more rapidly and is more prevalent than at lower latitudes, shown by the air temperature increase at 2.4 times the rate of the Northern Hemisphere average (Box et al., 2019). Environmental changes will affect the parasites, especially when it comes to the environment they face between host during transmission (Selbach & Poulin,

2020). Changes in the parasite's seasonality will also affect their host populations and other connected species in the ecosystem (Studer & Poulin, 2012). Studies have shown that some trematodes (parasitic flatworms) react positively to higher temperatures with regards to cercarial productivity and output in the first intermediate host, but negatively when it comes to cercarial survival, activity and infectivity between hosts (Selbach & Poulin, 2020). In general, it is thought that trematode infections will increase with the warming climate (Goedknegt et al., 2015), though this will depend on the specific ecosystems and how their hosts react to these changes. Arctic charr (*Salvelinus alpinus*) is a cold-water adapted fish (Klemetsen et al., 2003) and is predicted to experience great habitat loss with the warming climate (Hein et al., 2012; Jeppesen et al., 2012). As they are an important host species for a variety of trophically transmitted parasites (Kuhn et al., 2016), a reduction in their population will have adverse effects on parasite populations and community structure. More knowledge about the seasonality of parasites may therefore help with better understanding the impacts of future changes.

Crepidostomum (Braun, 1900) is a genus of trematodes that are geographically widespread and parasitise a variety of fish (Faltýnková et al., 2020). For their first intermediate host, they utilize freshwater bivalves (Atopkin & Shedko, 2014). Therein, they undergo asexual reproduction which produces great numbers of free-living cercariae (Goater et al., 2014). After being shed from the bivalves they infect their second intermediate host, freshwater arthropods (Atopkin & Shedko, 2014), where they encyst into a resting, metacercariae stage (Goater et al., 2014). As final hosts they use a wide variety of fish species, and can often be found in freshwater salmonids (Faltýnková et al., 2020). In the fish they mature into adults, and the worms reside in the fish's intestine and pyloric caeca. Here they undergo sexual reproduction which produces eggs that hatch into free-living miracidia when released into the water (Goater et al., 2014) (fig. 1). Most of the free-living stages in the life cycle will not successfully infect their hosts, and some may be directly eaten by predators or utilized in detrital food webs after they die (Johnson et al., 2010; Preston et al., 2013).

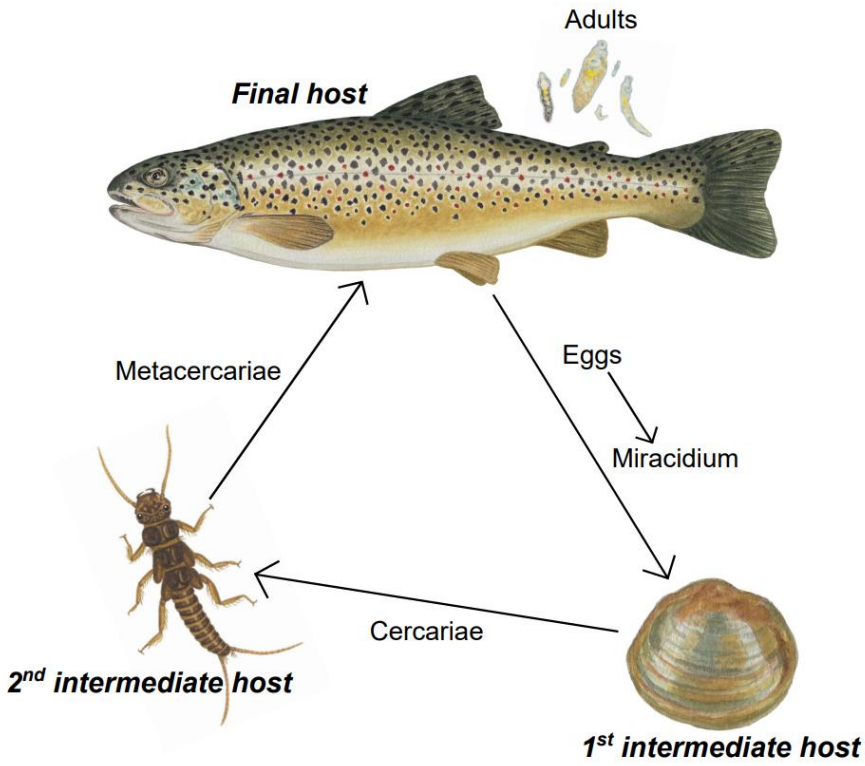

Fig. 1: *Crepidostomum* spp. life cycle: freshwater bivalves as 1st intermediate host (e.g., *Euglesa casertana*) → freshwater arthropods as 2nd intermediate host (e.g., *Diura bicaudata*) → freshwater fish as final host (e.g., *Salmo trutta*). Illustration by: Kristine Drage

The morphological traits and characteristics of different species of *Crepidostomum* have a high plasticity (Faltýnková et al., 2020), and the species are considered cryptic. Cryptic species can be defined as morphologically indistinguishable populations that are reproductively isolated, and which are often incorrectly classified under one species name (Struck et al., 2018). *Crepidostomum* species are often confused with one another (Moravec, 2002; Petkevičiūtė et al., 2018), and there is a high possibility of inaccurate identification in studies where molecular methods have not been utilized. The prevalence of certain species may be overestimated as new information about the genus and its cryptic diversity is continuously uncovered (Soldánová et al., 2017). Because of this, the genus has a complex taxonomy, but as molecular methods are becoming more available and increasingly used on parasites (Selbach et al., 2019; Soldánová et al., 2017), a more accurate estimation of the diversity of this genus will become possible to achieve. Still, a lot of work remains to be done on their phylogenetic relationships. One study suggested that the genus may be paraphyletic, and not monophyletic as previously believed (Vainutis et al., 2021), which further complicates the status. Recent studies have also been able

to describe new species of *Crepidostomum* using molecular methods (Faltýnková et al., 2020). As the genus has such a wide geographical and host range (Faltýnková et al., 2020), one can assume that more species are yet to be discovered.

In Lake Takvatn, Northern Norway (hereafter Takvatn), four species of *Crepidostomum* have been confirmed to reside in the lake. These species are: *C. farionis, C. pseudofarionis, C. metoecus and C. brinkmanni* (Faltýnková et al., 2020; Soldánová et al., 2017). *Crepidostomum farionis* and *C. metoecus* are recorded as the most common species in European salmonids (Moravec, 2002). *Crepidostomum* infections in Takvatn have a high prevalence in both brown trout (*Salmo trutta*) and Arctic charr (Prati et al., 2020b), have been shown to be stable over time (Kuhn et al., 2016), and have low pathogenicity for the fish (Henriksen et al., 2019). Takvatn has been extensively researched when it comes to the fish's feeding behaviour, as well as their parasite communities (Amundsen et al., 2015). Therefore, it is a suitable system for studying the seasonality of *Crepidostomum*, as possible seasonal patterns of the parasites can be linked to factors already know about the system. For example, the different fish species, and fish within the same species, inhabit various trophic niches and have seasonal shifts in their diet (Amundsen et al., 2008; Prati et al., 2020a). This will likely influence which *Crepidostomum* species can be found in which fish at different times throughout the year. Previously, seasonal patterns suggest the highest infection in autumn/winter (Knudsen et al., 2008; Prati et al., 2020b). Closely related species, like these *Crepidostomum* species, may therefore react differently to changing environmental factors, and more knowledge about their seasonality is a step towards uncovering more about their cryptic diversity.

No studies have previously looked at the seasonality of these cryptic species of *Crepidostomum*. The object of this study is to look at the seasonal dynamics and host specificity of the four species of *Crepidostomum* in Takvatn in their final hosts: brown trout and Arctic charr. My first hypothesis is that the four species will utilize brown trout and Arctic charr as a final host to varying degrees, and that *C. farionis* and *C. metoecus* will be the most common species. My second hypothesis is that for all *Crepidostomum* species there will be seasonal patterns, with the highest infection levels in autumn/winter, and with differences in the distribution of juveniles and adults within the same species over the seasons. My third hypothesis it that the seasonal distributions of each *Crepidostomum* species will be linked to the fish hosts feeding behaviour of potential intermediate hosts.

2 Materials and methods

2.1 Study site

The samples in this study were collected from Takvatn (69°07'N, 19°05'E) between June 2017 and May 2018 (Prati et al., 2020b). The lake is part of the Målselv watercourse in Troms and Finnmark county, Northern Norway, located at 214 MSL. It has an area of 15.2 km^2 and two basins with a depth of maximum 80 m. The lake is subarctic, oligotrophic and dimictic, and is usually covered by ice from November to May/June (Amundsen et al., 2009). During the winter of 2017/2018, the lake was covered in ice from the end of November to the end of May (Prati et al., 2020b). The area experiences midnight sun from late May to late July, and polar night from late November to late January. In summer the average air temperature is around 13°C, while in the coldest winter months the average air temperature lies around - 10°C (Amundsen et al., 2009). The main water inlet and outlet is Takelva which enters the lake in the north-western part and exits in the south-eastern part. The area around the lake consists of mountains and woodland dominated by birch trees (*Betula pubescens*) as well as pines (*Pinus sylvestris*), in addition to some farmland (Amundsen et al., 2009).

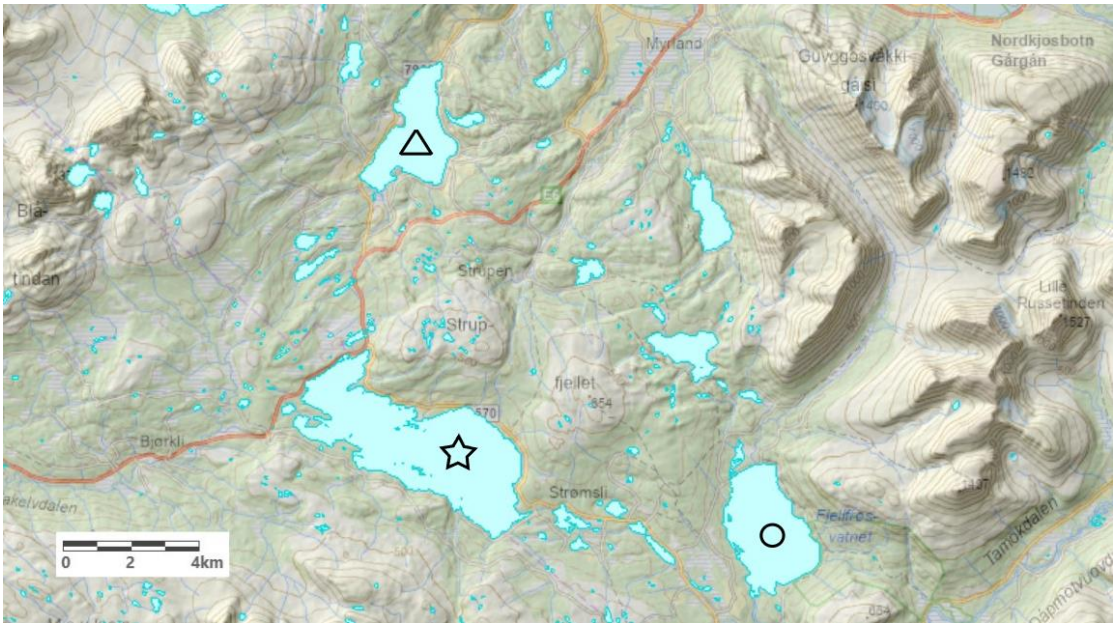

Fig. 2: Map of the study lake, Takvatn (star), and the nearby lakes Sagelvvatn (triangle) and Fjellfrøsvatn (circle) from which the fish were introduced (see text). Source: https://atlas.nve.no/html5Viewer/?viewer=nveatlas

The fish community in Takvatn consists of resident brown trout (hereafter trout), Arctic charr (hereafter charr) and three-spined stickleback (*Gasterosteus aculeatus*). Trout is the only fish species native to the lake, while charr was introduced from the nearby Lake Fjellfrøsvatn (69°05'N, 19°20'E) in the 1930s, and three-spined stickleback from another nearby lake, Lake Sagelvvatn, in the 1950s (69°11'N, 19°06'E) (Klemetsen et al., 1989) (fig. 2). Due to overpopulation and stunted growth of charr, an intensive fishing program was carried out between 1984 to 1991 to reduce the fish density in the lake. During this period over 690,000 charr were removed from the lake. This reduced the charr density and helped increase their growth rates. It also increased the trout population which had been in decline since the introduction of the two other species (Amundsen et al., 2019). Takvatn has been extensively researched, and has been sampled annually since 1979 (Amundsen et al., 2009; Amundsen et al., 2015). A large focus of the research has been placed on the parasite community of the fish (Amundsen et al., 2009). The presence of *Crepidostomum* spp. in Takvatn has been known since the 1980s, but molecular analysis carried out in 2012/2013 by Soldánová et. al (2017) confirmed the presence of four species *C. farionis, C. metoecus* and two unnamed species-lineages later confirmed to be *C. pseudofarionis* and *C. brinkmanni* (Faltýnková et al., 2020).

2.2 Sampling

2.2.1 Fish sampling

Between June 2017 and May 2018, 203 trout and 354 charr were sampled from the littoral zone of Takvatn using multi-meshed gillnets. The sampling occurred almost each month during the ice-free season and every second month during the ice-covered season (June, August, September, October, November, January, March, May). The nets were left overnight for approximately 12 h in the ice-free season, and 16 h in the ice-covered season. The weight, fork length, sex, gonad maturation and stomach fullness degree of the caught fish were recorded in the laboratory. Stomach content were preserved in a 96% ethanol solution and the intestine plus pyloric caeca were frozen for later parasite sampling (Prati et al., 2020b).

2.2.2 Parasite sampling

The intestines and pyloric caeca were cut open and sieved in a 120-micron mesh size nylon net under running water. The remaining content and parasites were placed in a physiological saltwater solution for parasite taxa identification. *Crepidostomum* spp. were identified as one of the taxa present, but no further molecular analysis was performed in 2017/2018 (Prati et al., 2020b). A subsample of the parasites present in the intestine and pyloric caeca were placed in a 96% ethanol solution for further analysis.

In 2022 the parasite samples from 2017/2018 were processed for this study. All samples had experienced varying degrees of ethanol evaporation. Samples with high ethanol levels were prioritised, but samples with lower levels were used when no other alternatives were available. *Crepidostomum* spp. specimens were sorted from the rest of the intestinal parasites, and the number of juvenile and adult stages from each fish was recorded. Classification of specimens into two stages; adults (gravid and non-gravid) and juveniles, was done under a stereomicroscope. If a specimen had visible eyespots, no eggs, no/undefined internal organs and were smaller in size compared to the other specimens, it was classified as a juvenile. A specimen without eggs might also be defined as an adult depending on the presence of other features mentioned above, these non-gravid adults were classified as adults. *Crepidostomum* spp. samples from each fish were stored separately as juveniles and adults in a 96% ethanol solution until further molecular analysis could be performed.

2.3 DNA extraction

For the DNA extraction a 5% solution was made mixing 2.5 g of BT Chelex ® 100 Resin Biotechnology grade, 100-200 mesh, sodium form (BIO-RAD) with 50 ml MQwater. For each tube containing one *Crepidostomum* specimen, 200 - 300 µl of the Chelex solution was added, followed by 2 - 3 µl of proteinase K. The tubes were incubated at 56°C overnight (or for a minimum of 5 h) in an Eppendorf Thermomixer®C (Eppendorf Smartblock™). After incubation, the tubes were vortexed at low shake for homogenization and boiled at 90°C for 8 min. They were then vortexed again at low shake before being centrifuged at max speed (~ 16000 RCF) for 10 min. The tubes were stored refrigerated until PCR was conducted.

2.4 PCR protocol and clean-up

Combinations of different cytochrome c oxidase subunit 1(CO1) primers were run on gradient PCRs to test which combination worked best. An overview of these primers, reagent volumes (master mix) and gradient PCR temperatures can be found in the appendix (table 3), while the description below is for the primer combination that worked best. CO1 DNA sequences of the *Crepidostomum* samples were amplified for the first time using the forward primer JB3 (5'- TTTTTTGGGCATCC TGAGGTTTAT- 3') (Bowles et al., 1993) and the reverse primer trem.cox1.rrnl (5'- AATCATGATGCA AAAGGTA -3') (Králová-Hromadová et al., 2008). PCR was carried out using 4.5 µl DNA extraction in 1.8 µl MQwater, 7.5 µl MyFiMix2x and 0.6 µl of both primers at 10 pmol/µl. The thermocycling profile used was initial DNA denaturation for 3 min at 95°C, 39 cycles of amplification: denaturation for 50 s at 94°C, annealing for 40 s at 55°C, extension for 60 s at 72°C, and final extension for 4 min at 72°C and cooling for 5 min at 5 - 15°C.

To confirm the presence of DNA, the samples were run through gel electrophoresis. To prepare the gel, 0.6 g of agar was mixed with 40 ml of TBE and then heated until it formed a clear, even solution. 1 µl of ethidium bromide (Et-Br) was added to the mix before the gel was poured in and solidified for a minimum of 40 min. 1 µl of blue dye was mixed with 2 µl of PCR-DNA for each specimen and added to separate wells. 2 µl of GeneRuler 100 bp Plus ladder (peqlab) was added to the separate wells. Electrophoresis was carried out at around 94 hz.

If the presence of DNA was confirmed, 1.1 µl of Exo1-Fast AP (0.1 µl Exonuclease 1 + 1 µl Fast AP Thermosensitive Alkaline Phosphatase enzyme) was added to the non-purified PCR tubes. The tubes were spun to mix the solution and remove any bubbles. Afterwards they were placed in the PCR machine on a thermocycling profile of 40 min incubation at 37°C, and deactivation for 15 min at 94°C. The tubes were then stored in the freezer at - 20°C. The purified DNA was diluted by mixing the PCR products with 10 µl of MQwater to account for any evaporation during PCR. In each well of a Macrogen EZ-seq-plate, 5 µl of forward or reverse primer was added at 5 pmol/µl (JB3 and trem.cox.1.rrnl), followed by 5 µl of purified DNA. The plate was sent for sequencing at Macrogen Europe BV.

2.5 Sequence alignment and phylogenetic analyses

Geneious Prime (*Geneious Prime® 2023.0.4*, 2023) was used for sequence alignment. Sequences were assembled using De Novo Assemble with the standard settings and scanned for any errors. Outgroup and reference sequences were downloaded from NCBI GenBank (Benson et al., 2013). All sequences were aligned using MAFFT Alignment with standard settings and trimmed to the same number of nucleotides.

The alignment was uploaded to the CIPRES Science Gateway V. 3.3 (Miller et al., 2010) for phylogenetic analyses. The alignment was run on two programs: RAxML-HPC2 on XSEDE for maximum likelihood (ML) and MrBayes on XSEDE v3.2.7a for Bayesian inference (BI). For ML, GTRCAT was used, and the number of bootstrap iterations was set to 1000. BI analysis was carried out using two independent Markov chain Monte Carlo runs with 4 chains for 10^7 generations. The sample tree frequency was set to every 1000^{th} generation, with a burn-in frac of 0.25. The consensus tree were uploaded to FigTree Version 1.4.4. (Rambaut, 2018) to visualise the phylograms. The phylogram presented in the results below was edited in Inkscape Version 1.2 (*Inkscape*, 2022).

Species assignments of each specimen represented in the phylogram were transferred to Microsoft Excel for further analyses, including host distribution, infracommunity analyses, seasonal distribution, and adult and juvenile distribution. To account for low/no occurrence of some species in certain months, the sampling months were grouped together into four seasons for looking at seasonal distributions: summer (June and August), autumn (September and October), winter (November and January) and spring (March and May). As the specimens in this dataset is a subsample of the original data, there was not done any statistics for the distributions. Frequency is therefore used to describe seasonal patterns instead of statistical terminology like abundance and prevalence. For infracommunity analyses of the fish hosts, only fish with four or more *Crepidostomum* specimens were selected. The infracommunity analyses does not include the complete infracommunity of any fish, as only a subsample was kept from the original study, in addition to many samples not being successfully amplified during PCR due to DNA degradation.

3 Results

In total, 1701 *Crepidostomum* spp. specimens were collected from 158 fish (1587 adults and 114 juveniles). DNA extractions were carried out for 812 of these, and 744 were run through PCR. Of these, 560 were successfully amplified for CO1 and used further for genetic analyses. These specimens were collected from 106 fish: 364 specimens from 67 charr, and 196 specimens from 39 trout. For both fish species, around 19% of the fish from the original study were used in this dataset. Of the sequenced parasites, 486 were classified as adults and 74 as juveniles. Four species of *Crepidostomum* from Takvatn were molecularly detected in this study: *C. farionis*, *C. pseudofarionis*, *C. metoecus* and *C. brinkmanni* (table 1).

Table 1: Details of sequences used in phylogenetic analyses based on CO1 gene fragments with information about total number of sequences (n), host species, locality and geographic region, origin of sequences (reference) and GenBank ID. Data from this study are marked in bold. All sequences are CO1. No CO1 sequences for *C. pseudofarionis* and *C. brinkmanni* existed in GenBank.

Species	n	Host	Locality	Geographic region	Reference	GenBank ID
						CO1
Crepidostomum farionis	**135**	***Salvelinus alpinus***	**Lake Takvatn**	**Troms and Finnmark,**	**This study**	**-**
	95	***Salmo trutta***		**Norway**		**-**
Crepidostomum farionis	1	*Onchorhyncus masou*	Kedrovaya River	Primorsky Region, Russia	Vainutis et al., 2021	MW729434
	1	*Salvelinus leucomaenis*	Belaya River	Sakhalin Island, Russia	Vainutis et al., 2021	MK818867
Crepidostomum pseudofarionis	**1**	***S. alpinus***	**Lake Takvatn**	**Troms and Finnmark,**	**This study**	**-**
	31	***S. trutta***		**Norway**		
Crepidostomum metoecus	**224**	***S. alpinus***	**Lake Takvatn**	**Troms and Finnmark,**	**This study**	**-**
	39	***S. trutta***		**Norway**		
Crepidostomum metoecus	1	*O. masou*	Mamachi River	Hokkaido Island, Japan	Vainutis et al., 2021	MK818837
	2	*Barbatula toni*	Artyomovka River	Primorye, Russia	Vainutis et al., 2021	MT214991 MW729427
Crepidostomum brinkmanni	**35**	***S. trutta***	**Lake Takvatn**	**Troms and Finnmark,**	**This study**	**-**
				Norway		
Crepidostomum nemachilus	1	*B. toni*	Belaya River	Sakhalin Island, Russia	Vainutis et al., 2021	MK818851
Bunodera mediovitellata	1	*Gasterosteus aculeatus*	Azabachya River	Kamchatsky Krai, Russia	Vainutis et al., 2021	MK818872
Bunodera luciopercae	1	*Thymallus thymallus*	Kamennaya River	Karelia, Russia	Vainutis et al., 2021	MK818873
Bunodera acerinae	1	*Gymnocephalus cernua*	Kamennaya River	Karelia, Russia	Vainutis et al., 2021	MK818874

3.1 Phylogram

Based on 560 CO1 sequences obtained from Takvatn, as well as nine reference sequences, a phylogram was built using a maximum likelihood analysis (ML) (fig. 3). The sequences formed two main clades: *Crepidostomum* spp. and *Bunodera* spp.. Within the *Crepidostomum* clade, the sequences from Takvatn grouped into four distinct lineages; *C. farionis*, *C. pseudofarionis*, *C. metoecus* and *C. brinkmanni*, while the reference sequence for *C. nemachilus* formed its own separate lineage. The support for these five lineages was strong. Within this clade, *C. farionis* and *C. pseudofarionis* were more related to each other than the other three species. *Crepidostomum metoecus* and *C. brinkmanni* were more related to each other and to *C. nemachilus* than to the other two.

For *C. pseudofarionis* all sequences clustered together. There were no available CO1 reference sequences for this lineage, and it was identified by its sister relationship to *C. farionis* (see Faltýnková et al., 2020; Soldánová et al., 2017). The specimens of *C. farionis* from Takvatn and the reference sequences from GenBank grouped together. Within the *C. metoecus* linage there was separation between the Takvatn specimens and the reference sequences. The three reference sequences for *C. metoecus* were slightly divergent from sequences of the Takvatn specimens but were still more related to the *C. metoecus* Takvatn specimens than any of the other species. *Crepidostomum brinkmanni* formed its own lineage and all the specimens clustered together. There were no available CO1 reference sequences for *C. brinkmanni* either, and the species was also identified by its relation to the other species. The three outgroup sequences of *Bunodera* spp. (*B. mediovitellata*, *B. luciopercae*, *B. acerinae*) formed a separate lineage from all the *Crepidostomum* sequences with strong support.

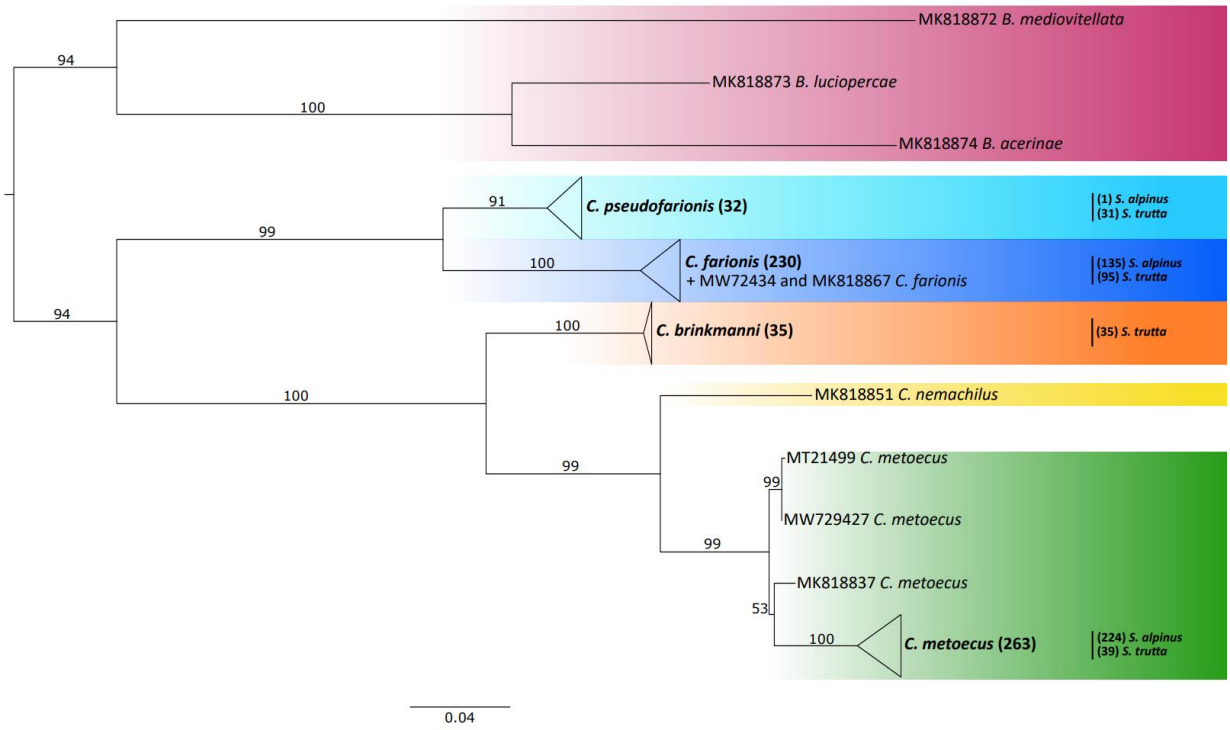

Fig. 3: Phylogram built from maximum likelihood (ML) analysis based on CO1 gene fragments (n = 888), showing *Crepidostomum* species sampled from Takvatn in addition to *Crepidostomum* spp. reference sequences and outgroup sequences (*Bunodera* spp.) from GenBank. Support for the lineages is shown as bootstrap values on the nodes. Lineages are highlighted with different colours and sequences from this study are in bold. Behind the species names, the number of sequences from this study is written in brackets. On the right in brackets is the number of sequences collected from each of the fish host species in this study: *S. alpinus* and *S. trutta*.

3.2 Species and host distribution

The dominant species of *Crepidostomum* from trout and charr in Takvatn were *C. farionis* and *C. metoecus*, accounting for 88% of the specimens. *Crepidostomum farionis* made up 41% (n = 230), while *C. metoecus* made up 47% (n = 263). Both *C. pseudofarionis* and *C. brinkmanni* were rarer and had an occurrence of 6%, with 32 and 35 specimens belonging to each respectively (fig. 4). For *C. metoecus*, 224 specimens came from charr and 39 came from trout. For *C. farionis*, 135 came from charr and 95 from trout. Almost all *C. pseudofarionis* specimens were found in trout (n = 31), while only one came from charr. All 35 *C. brinkmanni* specimens came from trout (table 1).

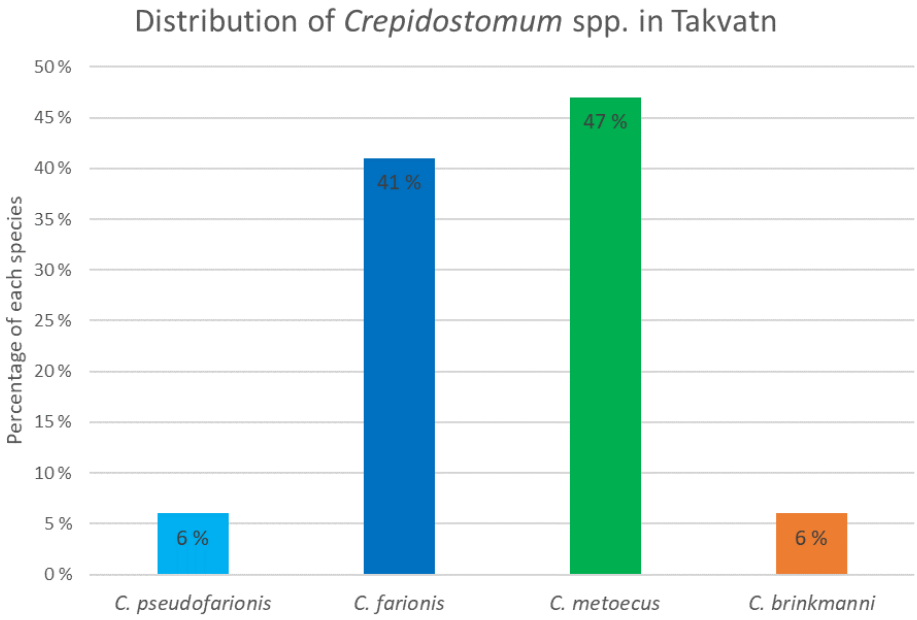

Fig. 4: Distribution of *Crepidostomum* spp. in Takvatn, showing the percentage each species accounts for, sampled from trout (n = 39) and charr (n = 67).

To account for the uneven number of trout and charr in the sequenced dataset, the number of *Crepidostomum* specimens in the trout were multiplied with a factor of 1.72 to even out the difference. *Crepidostomum farionis* was more frequent in trout (55%) than charr (45%), while *C. pseudofarionis* was almost exclusively found in trout (98%) with only 2% coming from charr. *Crepidostomum metoecus* mostly utilized charr as a final host (77%) but could also be found in trout (23%). *Crepidostomum brinkmanni* was exclusively found in trout (fig. 5).

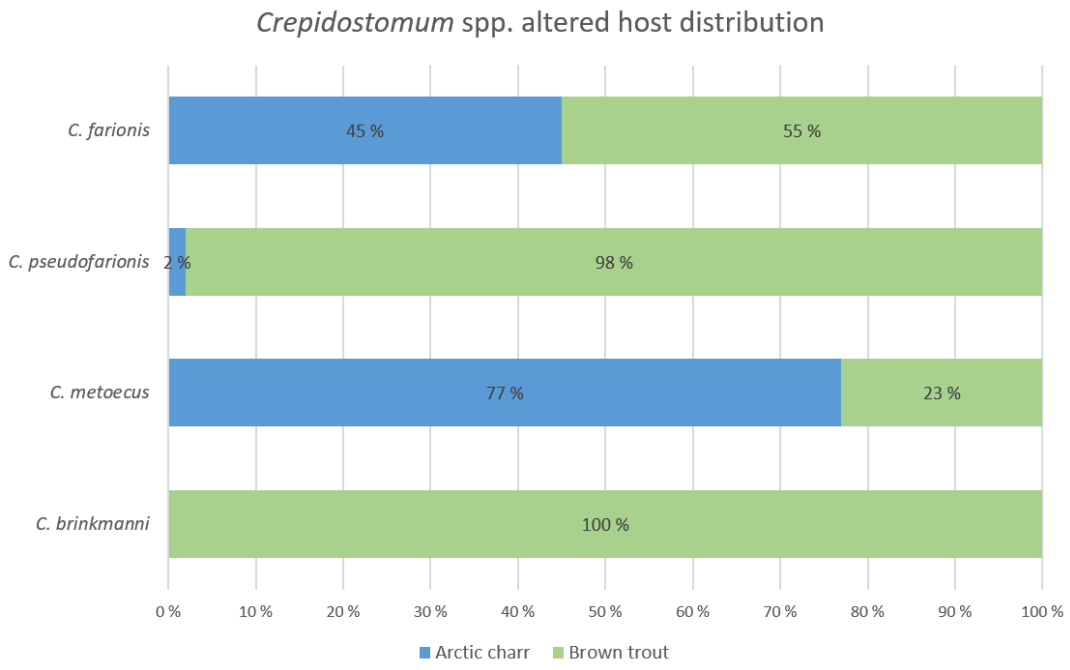

Fig. 5: Distribution of *Crepidostomum* spp. in trout and charr in Takvatn, showing the expected distribution in the two hosts after accounting for the uneven number of charr (n = 67) and trout (n = 39) in the sequenced dataset.

3.2.1 Infracommunity in the final hosts

The average number of *Crepidostomum* species per host species was 1.4 for charr and 2.2 for trout. The two most common *Crepidostomum* spp. infracommunity structures of was one (n = 24, 42.9%) or two species (n = 25, 44.6%) in a host. The most frequent combination of two species was *C. farionis and C. metoecus*, as these were the most abundant species in general. Most charr was infected by only one *Crepidostomum* spp. at a time (57.6%) and had no fish infected with more than two species. Most trout were infected with more than one *Crepidostomum* spp. (78.3%) and had five trout individuals infected with a combination of three species (8.9%), and two trout infected with a combination of four species (3.6%) (fig. 6).

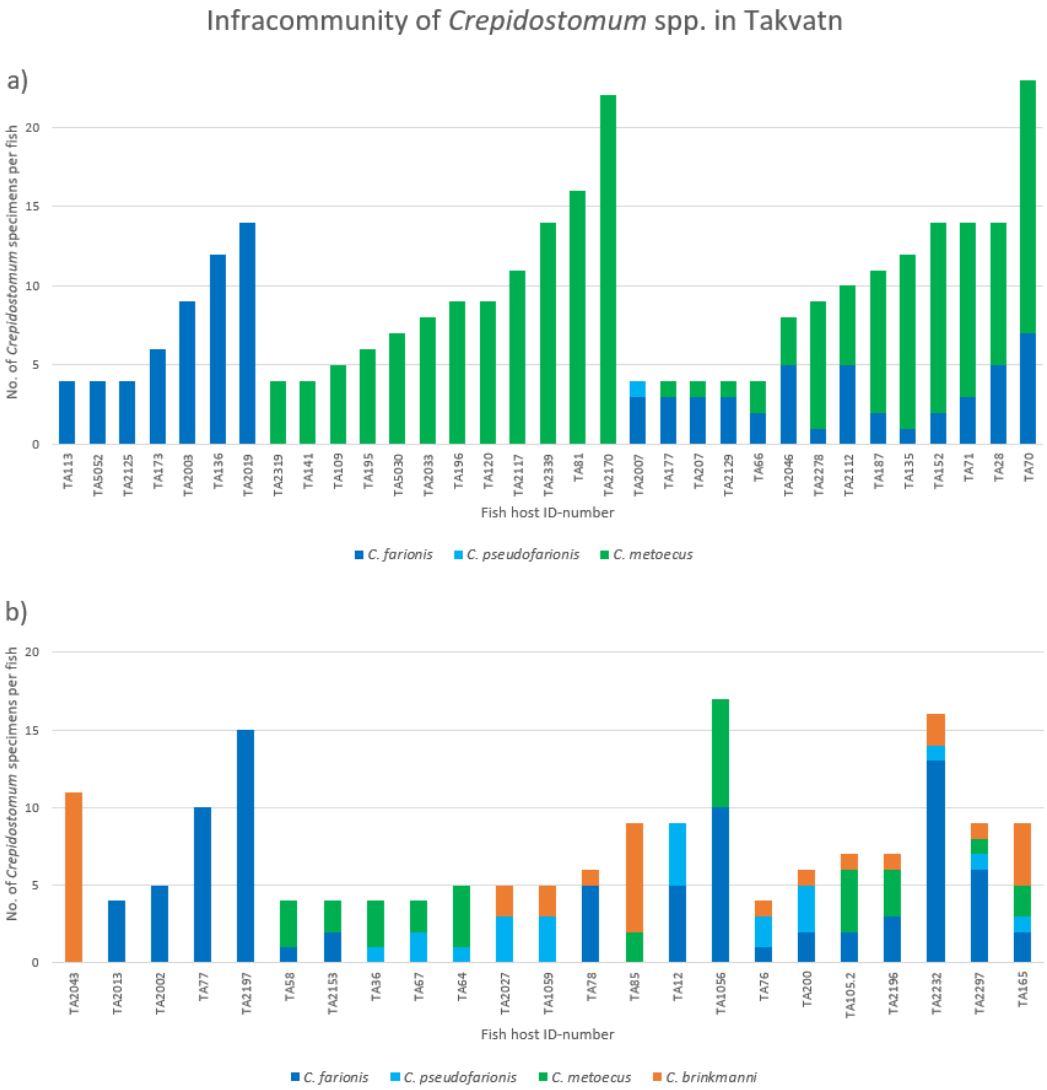

Fig. 6: *Crepidostomum* spp. infracommunity (based on sequenced specimens) in Takvatn for a) charr and b) trout. Each column represents one fish host and has the fish ID-number below it.

3.3 Seasonal distribution

There was a large sample size for each season with 92 specimens from the summer months (June and August), 156 from autumn (September and October), 197 from winter (November and January) and 115 from spring (March and May) (table 2). For most of the months, there was a lower number of trout sampled compared to charr.

Table 2: Total number of fish hosts and specimens of *Crepidostomum* spp. in the study. Below the fish number for each month is the number of *Crepidostomum* specimens sampled from them (in brackets). *Crepidostomum* specimens are divided into adults (AD) and juveniles (JV). The total number of *Crepidostomum* specimens for each month is shown in the far-right column. The last row shows the total number of fish hosts, and the total number of each *Crepidostomum* species.

Sampling month	Arctic charr	Brown trout	*C. farionis*	*C. pseudofarionis*	*C. metoecus*	*C. brinkmanni*	Total
June, 2017	11	0	15 AD	0 AD	8 AD	0 AD	26
	[26]	[0]	2 JV	0 JV	1 JV	0 JV	
August, 2017	7	4	20 AD	7 AD	29 AD	3 AD	66
	[35]	[31]	5 JV	0 JV	2 JV	0 JV	
September, 2017	8	9	45 AD	9 AD	11 AD	14 AD	82
	[48]	[34]	3 JV	0 JV	0 JV	0 JV	
October, 2017	5	7	31 AD	5 AD	25 AD	7 AD	74
	[32]	[42]	2 JV	0 JV	2 JV	2 JV	
November, 2017	10	8	32 AD	2 AD	40 AD	4 AD	112
	[60]	[52]	11 JV	1 JV	22 JV	0 JV	
January, 2018	9	7	28 AD	4 AD	37 AD	0 AD	85
	[62]	[23]	2 JV	2 JV	12 JV	0 JV	
March, 2018	6	2	19 AD	0 AD	43 AD	1 AD	64
	[56]	[8]	0 JV	0 JV	1 JV	0 JV	
May, 2018	11	2	14 AD	1 AD	28 AD	4 AD	51
	[41]	[10]	1 JV	1 JV	2 JV	0 JV	
Total	**67**	**39**	**230**	**32**	**263**	**35**	**560**

Crepidostomum farionis had a peak in frequency during autumn and winter, with the highest frequency in autumn. For autumn in general, *C. farionis* was the most numerous species making up 51.9% of the total amount that season (table 4, appendix). *C. farionis* was least frequent in spring and summer, though it was still the most frequent of all the *Crepidostomum* species in summer at 47.7%. *Crepidostomum pseudofarionis* had a slight peak in frequency during autumn, but it only accounted for 9% of the total amount that season. For summer and winter there was a low and almost equal frequency of *C. pseudofarionis*, while the lowest frequency was in spring with only two individuals.

Crepidostomum metoecus had a peak in frequency in winter, while there was also a high frequency in spring. It was the most numerous species both in winter and spring making up respectively 56.3% and 64.3% of the total amount for those seasons. The lowest frequency for *C. metoecus* was in summer and autumn, which was almost equal. Tough, in summer this accounted for 42.5% of the total amount, but only 24.4% in autumn. *Crepidostomum brinkmanni* had a low and similar frequency for spring, summer, and winter. In autumn, there is a peak in frequency which makes up 14.7% of the total amount for that season (fig. 6).

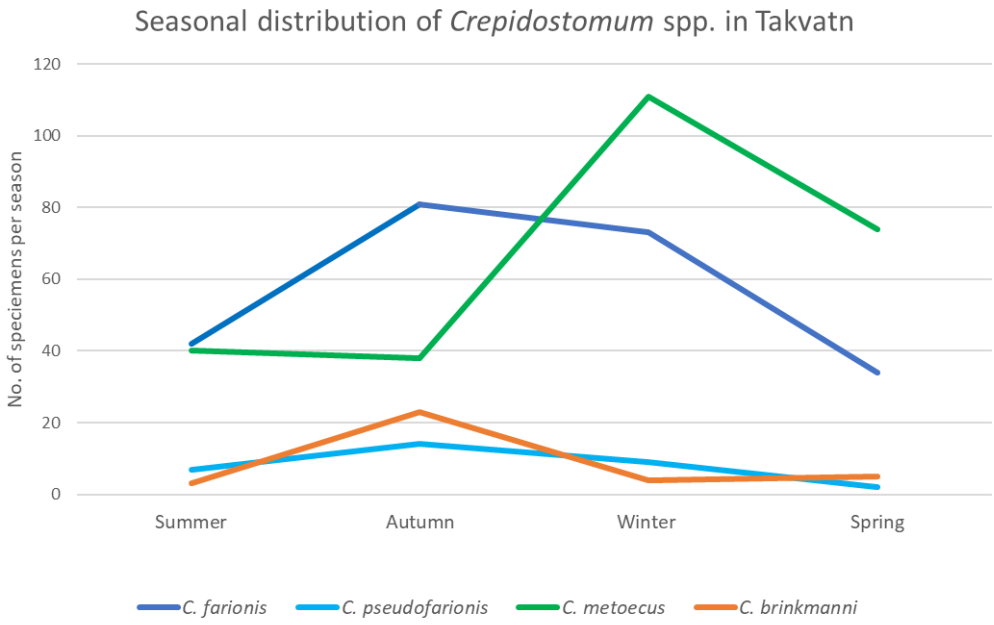

Fig. 6: Seasonal distribution of *Crepidostomum* spp. from trout and charr combined in Takvatn. Summer = June and August, autumn = September and October, winter = November and January, spring = March and May. See figure 8 for differences between the seasonal distribution of *C. farionis* and *C. metoecus* split between charr and trout.

3.4 Adult and juvenile distribution

In general, for all species and sampling seasons, mostly adult specimens were found. In the pre-sequenced dataset, 114 were classified as juveniles (7.2%) and 1587 as adults (92.8%). In the pre-sequenced data, the largest number of juveniles were found in winter (n = 61). Of the 560 sequenced specimens from the present study, 74 were juveniles (15.2%) and 486 adults (84.8%). The largest number of juveniles for the sequenced specimens were also from winter (n = 50).

Few juveniles were collected for both *C. pseudofarionis* (n = 4) and *C. brinkmanni* (n = 2), and there is not enough data to see any trends in the distribution of the developmental stages for these two species. For *C. farionis* and *C. metoecus* there is a trend in the seasonal distribution of their developmental stages with a peak of juveniles in winter, though the trend is less clear for *C. farionis* (fig. 7). The largest amount of *C. farionis* juveniles were found in winter (n =13) and makes up 5.7% of the total amount of *C. farionis* for all seasons. For the other seasons there is a low percentage of juveniles. For *C. metoecus* there is a significant peak in the number of juveniles in winter with 34 individuals. This accounts for 12.9% of the total *C. metoecus* specimens for all season. For the other season there is a low and equal number of juveniles (fig. 7).

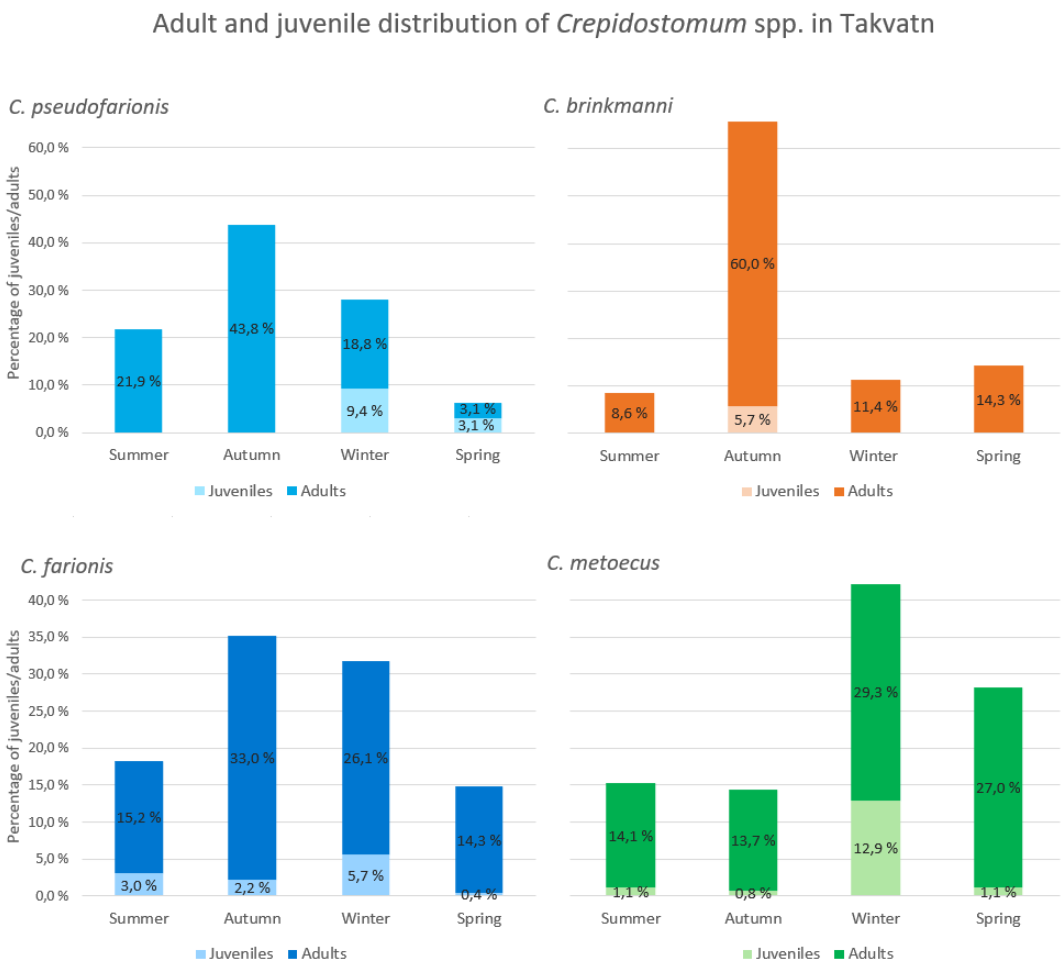

Fig. 7: Distribution of adults and juveniles for each *Crepidostomum* species from trout and charr in Takvatn. The amount each stage for each season accounts for in total is shown in percentage (e.g., out of all *C. metoecus* specimens, juveniles in winter accounts for 12.9%).

When splitting charr and trout, the distribution of life stages for *C. farionis* shows that most of the juveniles came from trout (17 out of 26), with the largest amount in winter with 12 specimens. For charr the largest amount was found in autumn with five specimens. For *C. metoecus* almost all juveniles came from charr (n = 41), with most of them being found in winter. Only one juvenile came from trout, and it was found in winter (fig. 8).

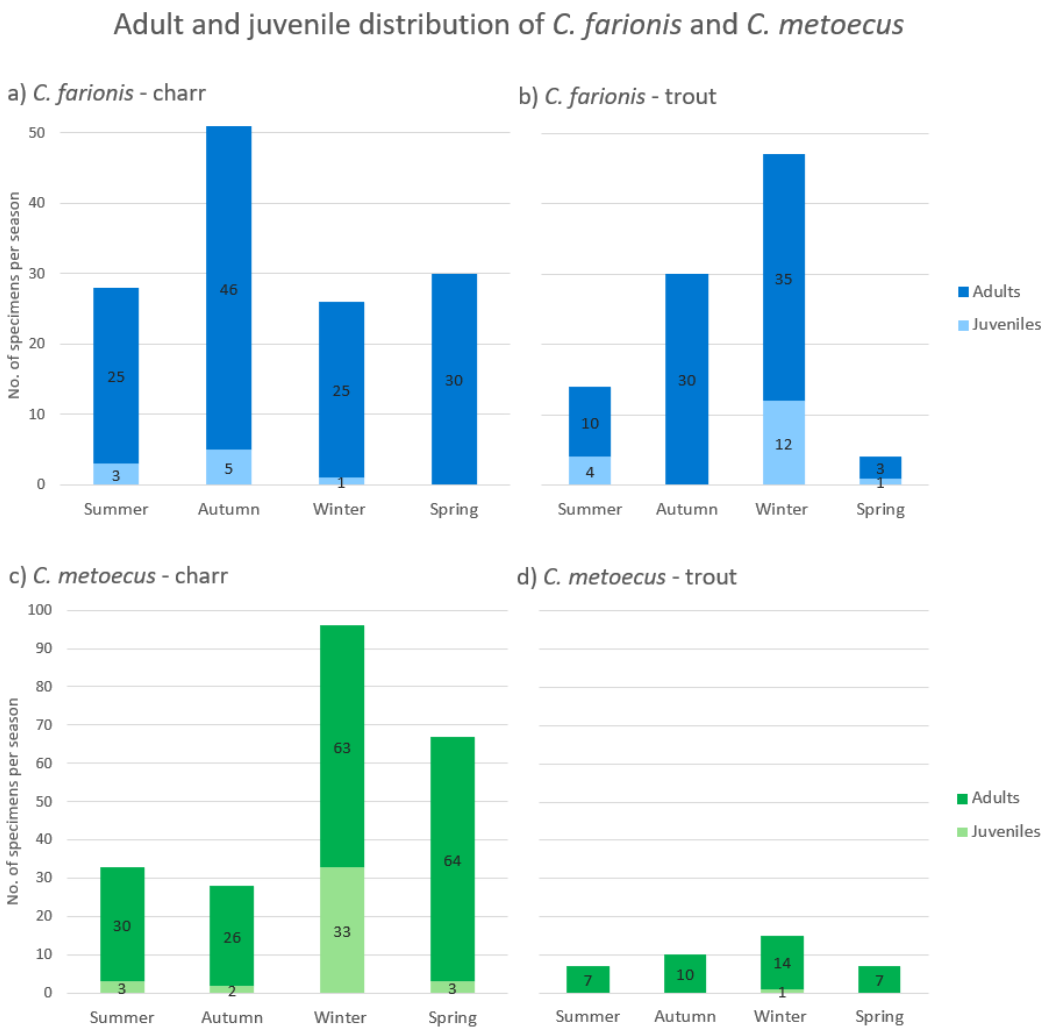

Fig. 8: Distribution of adults and juveniles for *C. farionis* and *C. metoecus* in Takvatn, split between charr and trout.

4 Discussion

This study has been able to uncover seasonal patterns and host distributions of four cryptic *Crepidostomum* species in Takvatn. Few studies have looked at the seasonality of *Crepidostomum* spp. (e.g., (Mariaux, 1986) and this is the first time CO1 has been used on *Crepidostomum* spp. to this extent. *Crepidostomum* species showed distinct patterns both when it came to their seasonal distribution and host distribution as I originally hypothesised. As expected, four species of *Crepidostomum* were detected in the two final hosts, trout and charr, from Takvatn. The infracommunity of trout was more diverse than for charr and comprised all four species. In contrast, charr was mainly parasitised by the two dominant species: *C. farionis* and *C. metoecus*. *Crepidostomum farionis* and *C. metoecus* showed a peak in frequency during autumn and winter, while *C. pseudofarionis* and *C. brinkmanni* showed a peak in frequency during autumn. Patterns of adult and juvenile distribution throughout the season were harder to uncover as the sample size was too small. Juvenile specimens were mostly found in winter, though this is very likely to differ from one *Crepidostomum* species to another.

The most numerous species were as expected *C. farionis* and *C. metoecus* which made up 88% of the sequenced *Crepidostomum* samples from Takvatn, while *C. pseudofarionis* and *C. brinkmanni* made up a much smaller proportion at 12%. *Crepidostomum farionis* and *C. metoecus* have previously been recorded as the most common species in European salmonids (Moravec, 2002). However, their populations in their final hosts are likely overestimated as new species like *C. pseudofarionis* and *C. brinkmanni* are discovered (Faltýnková et al., 2020; Soldánová et al., 2017). The low frequency of *C. pseudofarionis* and *C. brinkmanni* in Takvatn and the morphological similarities within this group explain why it has taken so long to detect and describe these species. They were observed as possible separate species from *C. farionis* and *C. metoecus* in Takvatn already in 1992 (Rune Knudsen personal communication) but were first officially detected molecularly in their first intermediate host in 2013 (Soldánová et al., 2017) and described morphologically in 2020 (Faltýnková et al., 2020). Moreover, Kuhn et al. 2016 hypothesised that the most common species in Takvatn was *C. farionis* based on the placement of the parasites in the intestine. *Crepidostomum farionis* is known to infect the lower intestine, while *C. metoecus* infects the upper intestine and pyloric caeca (Kuhn et al., 2016; Mariaux, 1986). Most of the *Crepidostomum* specimens from Takvatn in previous studies were found in the lower intestine, and it was therefore concluded that *C. farionis* represented the most common species (Kuhn et al., 2016). In contrast, *C. metoecus* was shown to be the most

common species in Takvatn in the present study. This highlights the importance of molecular methods to study cryptic parasite diversity and how it can help to re-assess the conclusions of previous host-parasite studies from this ecosystem and subarctic lakes in general.

Trout and charr showed differences in *Crepidostomum* community composition where trout was infected with all four *Crepidostomum* species, while charr was mainly parasitised by the two dominant species. This is the first time such differences are discovered using a large dataset. Such patterns could be caused by different immune systems between the two host species as even closely related fish species can have different immune reaction to parasites (Wegner et al., 2006), but a more likely cause may be the differences in the fish species feeding behaviour. When living in sympatry, charr has a broader diet than trout and thus a richer community of tropically transmitted intestinal parasites (Knudsen et al., 2008). In the ice-free season their diets are more segregated than under ice over (Eloranta et al., 2013; Prati, 2019). In addition to insect larvae, zooplankton is also an important prey item for charr during the ice-free season. Trout has insect larvae and surface insects as a main prey item during summer, while both fish species additionally prey upon amphipods (Prati, 2019). Naturally, one would assume that charr have a more diverse infracommunity of *Crepidostomum* than trout due to their broader diet, though this was not shown to be the case. The host distribution and infracommunity analyses clearly shows the difference between the two fish host species for the first time. Trout had a much more diverse community at the individual level with many different species combinations, while charr was mainly infected with only one species. Even though the present study only analysed a subsample of the *Crepidostomum* populations in the two fish hosts, the infracommunity analyses indicate a clear pattern and should reflect the total infracommunity of the fish.

Crepidostomum farionis was well represented in both fish hosts, while *C. metoecus* preferred charr. Both *C. pseudofarionis* and *C. brinkmanni* almost exclusively utilized trout as their final host in the current study. Historically, trout was almost eradicated in Takvatn due to the overcrowded charr population (Amundsen et al., 2015). Thus, these two rarer parasite populations may have been larger historically before the charr was introduced. Although rare species can be overlooked in studies if the sample size is low, the 560 sequenced samples in the present study likely adequately captures the composition and distribution of the *Crepidostomum* community in Takvatn and allows comparisons to other systems. Similar to this study, a study from Iceland found *C. brinkmanni* exclusively in trout, while *C. pseudofarionis* were only

found in charr (Faltýnková et al., 2020) in contrast to this study. The Icelandic study was on a smaller scale with 22 *Crepidostomum* samples collected from four lakes. A study from Scotland found *C. pseudofarionis* in charr (Rochat et al., 2022), while another from Switzerland found *C. brinkmanni* in trout (Rochat et al., 2021). In a previous study from Takvatn, *Crepidostomum* specimens were collected from the intermediate hosts, as well as some of their final hosts (Soldánová et al., 2017). The samples of *C. farionis* came from their first intermediate clam hosts *Sphaerium* sp. and *Euglesa casertana* (previously *Pisidium casertaum*). *Crepidostomum pseudofarionis* were collected from their first intermediate host *Sphaerium* sp. and second intermediate Ephemeropteran (mayfly) host *Siphlonurus lacustris*. *Crepidostomum metoecus* were collected from their first intermediate host *Euglesa casertana* and their second intermediate amphipod host *Gammarus lacustris*. Lastly, *Crepidostomum brinkmanni* were collected from their second intermediate hosts *S. lacustris* and Plecopteran (stonefly) host *Diura bicaudata*. (Soldánová et al., 2017) (table 5, appendix). Another study from Takvatn and Lake Skogsfjordvatn (Troms and Finnmark) also found trout to be parasitised by all four *Crepidostomum* species, while only *C. farionis* and *C. metoecus* were found in charr (Slåteng, 2022). Life cycle data of *C. pseudofarionis* and *C. metoecus* in Takvatn are therefore partly available. With the knowledge of their seasonal distribution in their fish hosts, as well as complete life cycle data for some species, we can connect this to the fish's diet in Takvatn.

During autumn and early winter, *G. lacustris* constitute a large portion of the charrs prey items (Prati et al., 2020b). One can therefore expect to find an increased abundance of *C. metoecus* in charr in autumn and early winter. This was the case in the present study, as the highest frequency of *C. metoecus* were found in charr in winter. *Gammarus lacustris* may be the most important or only intermediate host for this species, as they have not yet been found in other arthropods. Fewer specimens of *C. metoecus* were found in trout, which could be explained by amphipods being a less important prey item for trout compared to charr (Prati, 2019). *Crepidostomum farionis* had its highest peak in autumn but remained frequent in winter. Their second intermediate host has not yet been confirmed molecularly, but they have been described morphologically from *Gammarus pulex* (Awachie, 1968). From their seasonal distribution in Takvatn and the fish's diets, we can infer that *C. farionis* likely utilize *G. lacustris* as a host. As *C. farionis* were well represented in both fish species, but mainly found in trout, it could suggest that this *Crepidostomum* species also uses another second intermediate host, such as insect larvae, which are an important prey item for trout throughout the year (Prati, 2019). Another study from Switzerland also found similar seasonal patterns to the present study with

a predominance of *C. metoecus* in winter, a predominance of *C. farionis* in autumn, and with a lower prevalence of both species over spring and summer (Mariaux, 1986).

Both mayflies and stoneflies emerge from the substrate in large numbers over the warmer months (Britannica, 2010; Kjær et al., 2021; Tikkanen et al., 1997). At that time, they are an abundant host for parasites as well as important prey items for fish (Britannica, 2010). Stoneflies hatch early in the spring usually around the time the ice breaks, while mayflies hatch over the summer months (Kirkemo, 1990). The fish can then feed on nymphs emerging from the substrate, adult insects and eggs on the surface, as well as dead insects in the water column (Kirkemo, 1990). In this study, *C. pseudofarionis* and *C. brinkmanni* specimens were mainly found as adults during autumn in trout. Both stoneflies and mayflies are important prey for trout (Kirkemo, 1990), but less important for charr (Prati, 2019), which could explain their extremely low presence in charr. Few trout were sampled in May, and none in June/July. August was the only summer month during which a substantial number of trout were sampled (Prati et al., 2020b). Based on the life cycles of their intermediate hosts, it is likely that May, June and July are the most important months for transmission of these rarer *Crepidostomum* species from the insect host to the fish host. Finding the highest number of established infections of these species in August could therefore be expected. Sampling during the other summer months might also contribute to a larger number of juveniles. In general, more sampling across the summer months might reveal a higher frequency of these species in Takvatn than presented in this study. In other systems, grayling (*Thymallus thymallus*) may be an important final host for *C. pseudofarionis* and *C. brinkmanni* as they also prey upon mayflies and stoneflies (Kirkemo, 1990). This could mean that in systems with more diverse fish communities, these two *Crepidostomum* species may be more prevalent.

When classifying *Crepidostomum* samples into life stages, some specimens did not fit into the characteristics set for each stage because the transition in the morphological development of the organs is a continuum. Consequently, the number of juveniles might have been over- or underrepresented in this study. The seasonal distribution with a peak of juveniles in winter is therefore only a preliminary pattern and need to be interpreted carefully. In the pre-sequenced dataset, most of the juveniles came from the winter months, and based on the seasonal patterns of the sequenced specimens they should mainly be *C. metoecus*. A preliminary pattern for differential life stage distributions of *C. farionis* and *C. metoecus* was also observed at a smaller scale study in Takvatn and Lake Skogsfjordvatn (Slåteng, 2022). Juvenile *C. farionis* were more

frequent in August (from both charr and trout) (Slåteng, 2022), in contrast to the present study where most juveniles were found in winter in trout. For *C. metoecus*, mostly juveniles were found in November in charr (Slåteng, 2022) similar to the present study. Future studies with a distinct focus on life stages would be helpful to get more insight into the transmission periods and intra-host development of these parasites. Some trematode species have also been confirmed to be progenetic, meaning that the trematode matures into adults while still encysted in their second intermediate host (Villa & Lagrue, 2019). This was the case for many of the *C. metoecus* specimens examined from *G. lacustris* in the study by Slåteng (2022). Thus, looking at the distribution of life stages in the final host may not accurately reflect the time of infection and the time they develop into adults, as they may already have evolved into adults in their second intermediate hosts. However, data from both studies indicate that for *C. farionis* and *C. metoecus*, as expected, autumn/winter is an important recruitment period in the fish.

Parasites can serve as environmental indicators, and host-parasite associations and community composition can reveal valuable insights into ecological processes, such as biological invasions. Parasites can hitchhike with introduced fish hosts, which has likely occurred in Takvatn (Amundsen et al., 2013). In Sagelvvatn, the fish community consists of trout, charr and three-spined stickleback (Kuhn et al., 2015). The salmonids in the lake have been confirmed to be infected with *Crepidostomum* spp., but not the sticklebacks (Kuhn et al., 2015). In a study by Kuhn et al. 2015, no *Crepidostomum* specimens were found in the stickleback from Sagelvvatn, while very low numbers were found in the sticklebacks from Takvatn (Kuhn et al., 2015). It is therefore unlikely that any *Crepidostomum* species were introduced with the sticklebacks from Sagelvvatn. However, the sampling in the aforementioned study was conducted during the summer, which is one of the seasons with the lowest intensity of *Crepidostomum* infection (Prati et al., 2020b). This means that sampling in another season might have revealed a higher intensity of infection. In Fjellfrøsvatn, only charr and trout are native to the lake (Klemetsen et al., 1997). *Crepidostomum* infection have also been confirmed here (Knudsen et al., 1997), but not molecularly identified down to a species level. As *C. pseudofarionis* and *C. brinkmanni* were almost exclusively found in trout, the original fish species in Takvatn, it is likely that these two species are also native to Takvatn. *Crepidostomum farionis* was commonly found in both charr and trout and could therefore be either introduced or native. *Crepidostomum metoecus* was mainly found in charr and is more likely to have been introduced to the lake with the charr from Fjellfrøsvatn. Alternatively, all *Crepidostomum* species may be native to Takvatn, and the differences in distribution in the final hosts could be

just connected to the fishes' diets. The variable seasonal patterns of *Crepidostomum* spp. could be a result of competition-avoidance when the parasites occur in sympatry, though only a small proportion of the host had high levels of infection (Prati et al., 2020b). It could also be because of differences in biotic and abiotic factors in the three lakes, if *Crepidostomum* spp. were introduced with their final hosts. In both Sagelvvatn and Fjellfrøsvatn, no molecular analyses have been carried out on *Crepidostomum*. Conducting these analyses could help to uncover which species are native to which lake, and could possibly uncover even more cryptic diversity.

A thorough understanding of the diversity and distribution of *Crepidostomum* spp. will allow us to better assess how they will respond to climate and/or environmental changes. Warmer temperatures will have effects on both the lake environment and on the host species involved in their life-cycle (Jeppesen et al., 2012; Magee & Wu, 2017; Magnuson et al., 2000). A warmer climate means longer summers and shorter winters, with a decrease in the ice-covered season of the lakes (Magnuson et al., 2000). *Crepidostomum* species that are more frequent in summer and use insects as intermediate hosts will get a longer window for transmission, which could increase their populations. On the other hand, *Crepidostomum* species that have their recruitment period in winter might decrease if they are dependent on colder temperatures, either directly or indirectly through their different hosts. For the final hosts of *Crepidostomum* spp. in Takvatn, charr is most likely negatively affected by increasing temperatures. Charr is a cold-water adapted species, and their populations are expected to, and have already started to decline as an effect of the warming climate (Hein et al., 2012; Jeppesen et al., 2012). Trout will likely handle these effects better as they are more adapted to warmer temperatures than charr, but they are still vulnerable to the warming climate (Mitro et al., 2019). Fewer charr in Takvatn would also reduce competition in the lake and could positively affect the trout population as it did during the mass removal in the 1980s (Klemetsen et al., 2002). I would therefore hypothesise that *C. pseudofarionis* and *C. brinkmanni* populations will increase with the warming climate as they were mainly found in trout during the summer months. In contrast, *C. farionis*, and especially *C. metoecus*, might decrease as they were most frequent in charr during winter. The present study therefore provides a valuable insight into the diversity and distribution of several *Crepidostomum* species, and future studies should investigate the effects of temperature on the intermediate hosts, as well as on survival rates for the parasite free-living stages. This would help to further predict the impacts of a warmer climate on parasites in general and specifically on *Crepidostomum* spp.

5 Conclusion

In this study the seasonal dynamics and host distribution of *Crepidostomum* spp. in Takvatn was uncovered. The dominant species were *C. farionis* and *C. metoecus*, with *C. pseudofarionis* and *C. brinkmanni* being much rarer. Trout was parasitised by all four *Crepidostomum* species, while charr was mainly parasitised by the two dominant species. There was some variance in the seasonality of *Crepidostomum* spp.; the dominant species were more frequent in autumn and winter, while the rarer species were more frequent autumn. Based on the distribution of adults and juveniles, autumn and winter is an important recruitment period in the fish for *C. farionis* and *C. metoecus*. Infection of *C. farionis* and *C. metoecus* could be connected to the fish, especially charr, preying upon the second intermediate host *Gammarus lacustris* during autumn and winter. Trout preys upon the second intermediate hosts mayflies and stoneflies over the summer months, which can be connected to the trout being infected with *C. pseudofarionis* and *C. brinkmanni*. The two rarer *Crepidostomum* species might be more prevalent in Takvatn than indicated in this study, due to the lack of trout sampled during the summer months when the second intermediate hosts of these species are more abundant. There is a possibility that *C. farionis* and *C. metoecus* hitchhiked with the introduced charr from Fjellfrøsvatn. *Crepidostomum farionis* and *C. metoecus* are vulnerable to climate change due to their use of charr as a final host, as the charr population is expected to decline with the warming climate.

Fig. 9: Artistic illustration of *Crepidostomum* spp. from Takvatn. Illustration: Kristine Drage

6 References

Amundsen, P.-A., & Knudsen, R. (2009). Winter ecology of Arctic charr (Salvelinus alpinus) and brown trout (Salmo trutta) in a subarctic lake, Norway. *Aquatic Ecology*, *43*(3), 765-775.

Amundsen, P.-A., Knudsen, R., & Klemetsen, A. (2008). Seasonal and ontogenetic variations in resource use by two sympatric Arctic charr morphs. *Environmental Biology of Fishes*, *83*(1), 45-55.

Amundsen, P.-A., Lafferty, K. D., Knudsen, R., Primicerio, R., Klemetsen, A., & Kuris, A. M. (2009). Food web topology and parasites in the pelagic zone of a subarctic lake. *Journal of Animal Ecology*, 563-572.

Amundsen, P.-A., Lafferty, K. D., Knudsen, R., Primicerio, R., Kristoffersen, R., Klemetsen, A., & Kuris, A. M. (2013). New parasites and predators follow the introduction of two fish species to a subarctic lake: implications for food-web structure and functioning. *Oecologia*, *171*, 993-1002.

Amundsen, P.-A., Smålås, A., Kristoffersen, R., Knudsen, R., Siwertsson, A., & Klemetsen, A. (2015). Takvatnprosjektet-Forskning og kultivering av en overbefolka røyebestand. *Septentrio Reports*(5).

Amundsen, P. A., Primicerio, R., Smalås, A., Henriksen, E. H., Knudsen, R., Kristoffersen, R., & Klemetsen, A. (2019). Long‐term ecological studies in northern lakes−challenges, experiences, and accomplishments. *Limnology and Oceanography*, *64*(S1), S11-S21.

Araújo, A., Jansen, A. M., Bouchet, F., Reinhard, K., & Ferreira, L. F. (2003). Parasitism, the diversity of life, and paleoparasitology. *Memórias do Instituto Oswaldo Cruz*, *98*, 5-11.

Atopkin, D. M., & Shedko, M. B. (2014). Genetic characterization of far eastern species of the genus Crepidostomum (Trematoda: Allocreadiidae) by means of 28S ribosomal DNA sequences. *Advances in Bioscience and Biotechnology*, *2014*.

Awachie, J. (1968). On the bionomics of Crepidostomum metoecus (Braun, 1900) and Crepidostomum farionis (Müller, 1784)(Trematoda: Allocreadiidae). *Parasitology*, *58*(2), 307-324.

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2013). GenBank. *Nucleic Acids Res*, *41*(Database issue), D36-42. https://doi.org/10.1093/nar/gks1195

Bowles, J., Hope, M., Tiu, W. U., Liu, X., & McManus, D. P. (1993). Nuclear and mitochondrial genetic markers highly conserved between Chinese and Philippine Schistosoma japonicum. *Acta tropica*, *55*(4), 217-229.

Box, J. E., Colgan, W. T., Christensen, T. R., Schmidt, N. M., Lund, M., Parmentier, F.-J. W., Brown, R., Bhatt, U. S., Euskirchen, E. S., & Romanovsky, V. E. (2019). Key indicators of Arctic climate change: 1971–2017. *Environmental Research Letters*, *14*(4), 045010.

Britannica, I. E. (2010). *Encyclopaedia*. Nabu Press.

Dzikowski, R., Paperna, I., & Diamant, A. (2003). Use of fish parasite species richness indices in analyzing anthropogenically impacted coastal marine ecosystems. *Helgoland Marine Research*, *57*(3), 220-227.

Eloranta, A. P., Knudsen, R., & AMUNDSEN, P. A. (2013). Niche segregation of coexisting Arctic charr (Salvelinus alpinus) and brown trout (Salmo trutta) constrains food web coupling in subarctic lakes. *Freshwater Biology*, *58*(1), 207-221.

Faltýnková, A., Pantoja, C., Skírnisson, K., & Kudlai, O. (2020). Unexpected diversity in northern Europe: trematodes from salmonid fishes in Iceland with two new species of Crepidostomum Braun, 1900. *Parasitology Research*, *119*(8), 2439-2462.

Geneious Prime® 2023.0.4. In. (2023). www.geneious.com

Goater, T. M., Goater, C. P., & Esch, G. W. (2014). *Parasitism: the diversity and ecology of animal parasites*. Cambridge University Press.

Goedknegt, M. A., Welsh, J. E., Drent, J., & Thieltges, D. W. (2015). Climate change and parasite transmission: how temperature affects parasite infectivity via predation on infective stages. *Ecosphere*, *6*(6), 1-9.

Hein, C. L., Öhlund, G., & Englund, G. (2012). Future distribution of Arctic char Salvelinus alpinus in Sweden under climate change: effects of temperature, lake size and species interactions. *Ambio*, *41*(3), 303-312.

Henriksen, E. H., Smalås, A., Strøm, J. F., & Knudsen, R. (2019). The association between parasite infection and growth rates in Arctic charr: do fast growing fish have more parasites? *Hydrobiologia*, *840*(1), 261-270.

Holmstad, P., Hudson, P., & Skorping, A. (2005). The influence of a parasite community on the dynamics of a host population: a longitudinal study on willow ptarmigan and their parasites. *Oikos*, *111*(2), 377-391.

Hudson, P. J., Dobson, A. P., & Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in ecology & evolution*, *21*(7), 381-385.

Huxham, M., Raffaelli, D., & Pike, A. (1995). Parasites and food web patterns. *Journal of Animal Ecology*, 168-176.

Inkscape. In. (2022). (Version 1.2) Danilo Šegan et. al. www.inkscape.org

Jeppesen, E., Mehner, T., Winfield, I. J., Kangur, K., Sarvala, J., Gerdeaux, D., Rask, M., Malmquist, H. J., Holmgren, K., & Volta, P. (2012). Impacts of climate warming on the long-term dynamics of key fish species in 24 European lakes. *Hydrobiologia*, *694*, 1-39.

Johnson, P. T., Dobson, A., Lafferty, K. D., Marcogliese, D. J., Memmott, J., Orlofske, S. A., Poulin, R., & Thieltges, D. W. (2010). When parasites become prey: ecological and epidemiological significance of eating parasites. *Trends in ecology & evolution*, *25*(6), 362-371.

Kirkemo, O., Ulnitz, S., Oglesby, A., Öste, B., Kreh, L. (1990). *Teknologisk forlags store bok om fluefiske*. AB Nordbok, Göteborg.

Kjær, C., Sørensen, P. B., Wiberg‐Larsen, P., Bak, J., Bruus, M., Strandberg, B., Larsen, S. E., Rasmussen, J. J., & Strandberg, M. (2021). Vulnerability of aquatic insect species to insecticides, depending on their flight period and adult life span. *Environmental Toxicology and Chemistry*, *40*(6), 1778-1787.

Klemetsen, A., Amundsen, P.-A., Grotnes, P. E., Knudsen, R., Kristoffersen, R., & Svenning, M.-A. (2002). Takvatn through 20 years: long-term effects of an experimental mass removal of Arctic charr, Salvelinus alpinus, from a subarctic lake. *Ecology, behaviour and conservation of the charrs, genus Salvelinus*, 39-47.

Klemetsen, A., Amundsen, P.-A., Muladal, H., Rubach, S., & Solbakken, J. I. (1989). Habitat shifts in a dense, resident Arctic charr Salvelinus alpinus population. *Physiology and Ecology Japan*, *1*(spl), 187-200.

Klemetsen, A., Amundsen, P., Knudsen, R., & Hermansen, B. (1997). A profundal, winter-spawning morph of Arctic charr Salvelinus alpinus (L.) in lake Fjellfrøsvatn, northern Norway. *Nordic Journal of Freshwater Research*, *73*, 13-23.

Klemetsen, A., Amundsen, P. A., Dempson, J., Jonsson, B., Jonsson, N., O'connell, M., & Mortensen, E. (2003). Atlantic salmon Salmo salar L., brown trout Salmo trutta L. and

Arctic charr Salvelinus alpinus (L.): a review of aspects of their life histories. *Ecology of freshwater fish*, *12*(1), 1-59.
Knudsen, R., Amundsen, P.-A., Nilsen, R., Kristoffersen, R., & Klemetsen, A. (2008). Food borne parasites as indicators of trophic segregation between Arctic charr and brown trout. *Environmental Biology of Fishes*, *83*, 107-116.
Knudsen, R., Kristoffersen, R., & Amundsen, P.-A. (1997). Parasite communities in two sympatric morphs of Arctic charr, Salvelinus alpinus (L.), in northern Norway. *Canadian Journal of Zoology*, *75*(12), 2003-2009.
Králová-Hromadová, I., Špakulová, M., Horáčková, E., Turčeková, L., Novobilský, A., Beck, R., Koudela, B., Marinculić, A., Rajský, D., & Pybus, M. (2008). Sequence analysis of ribosomal and mitochondrial genes of the giant liver fluke Fascioloides magna (Trematoda: Fasciolidae): intraspecific variation and differentiation from Fasciola hepatica. *Journal of Parasitology*, *94*(1), 58-67.
Kuhn, J. A., Knudsen, R., Kristoffersen, R., Primicerio, R., & Amundsen, P.-A. (2016). Temporal changes and between-host variation in the intestinal parasite community of Arctic charr in a subarctic lake. *Hydrobiologia*, *783*, 79-91.
Kuhn, J. A., Kristoffersen, R., Knudsen, R., Jakobsen, J., Marcogliese, D. J., Locke, S. A., Primicerio, R., & Amundsen, P.-A. (2015). Parasite communities of two three-spined stickleback populations in subarctic Norway—effects of a small spatial-scale host introduction. *Parasitology Research*, *114*, 1327-1339.
Lafferty, K. D., Allesina, S., Arim, M., Briggs, C. J., De Leo, G., Dobson, A. P., Dunne, J. A., Johnson, P. T., Kuris, A. M., & Marcogliese, D. J. (2008). Parasites in food webs: the ultimate missing links. *Ecology letters*, *11*(6), 533-546.
Magee, M. R., & Wu, C. H. (2017). Effects of changing climate on ice cover in three morphometrically different lakes. *Hydrological processes*, *31*(2), 308-323.
Magnuson, J. J., Robertson, D. M., Benson, B. J., Wynne, R. H., Livingstone, D. M., Arai, T., Assel, R. A., Barry, R. G., Card, V., & Kuusisto, E. (2000). Historical trends in lake and river ice cover in the Northern Hemisphere. *Science*, *289*(5485), 1743-1746.
Mariaux, J. (1986). Helminthes des poissons de l'Areuse. *Bulletin de la Société Neuchâteloise des Sciences Naturelles*, *109*, 57-64.
Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 2010 gateway computing environments workshop (GCE),
Mitro, M. G., Lyons, J. D., Stewart, J. S., Cunningham, P. K., & Griffin, J. D. (2019). Projected changes in Brook Trout and Brown Trout distribution in Wisconsin streams in the mid-twenty-first century in response to climate change. *Hydrobiologia*, *840*, 215-226.
Moravec, F. (2002). External morphological differences between Crepidostomum farionis and Crepidostomum metoecus (Trematoda: Allocreadiidae), parasites of salmonids, as revealed by SEM. *Folia Parasitologica*, *49*(3), 211-216.
Pappalardo, P., Morales‐Castilla, I., Park, A. W., Huang, S., Schmidt, J. P., & Stephens, P. R. (2020). Comparing methods for mapping global parasite diversity. *Global Ecology and Biogeography*, *29*(1), 182-193.
Petkevičiūtė, R., Stunžėnas, V., Zhokhov, A. E., Poddubnaya, L. G., & Stanevičiūtė, G. (2018). Diversity and phylogenetic relationships of European species of Crepidostomum Braun, 1900 (Trematoda: Allocreadiidae) based on rDNA, with special reference to Crepidostomum oschmarini Zhokhov & Pugacheva, 1998. *Parasites & Vectors*, *11*(1), 1-17.
Poulin, R. (2014). Parasite biodiversity revisited: frontiers and constraints. *International Journal for Parasitology*, *44*(9), 581-589.

Prati, S. (2019). *Seasonal and ontogenetic variation in the infection of intestinal parasites in Arctic charr (Salvelinus alpinus) and brown trout (Salmo trutta) in a subarctic lake* UiT Norges arktiske universitet].

Prati, S., Henriksen, E. H., Knudsen, R., & Amundsen, P.-A. (2020a). Impacts of ontogenetic dietary shifts on the food-transmitted intestinal parasite communities of two lake salmonids. *International Journal for Parasitology: Parasites and Wildlife, 12*, 155-164.

Prati, S., Henriksen, E. H., Knudsen, R., & Amundsen, P. A. (2020b). Seasonal dietary shifts enhance parasite transmission to lake salmonids during ice cover. *Ecology and Evolution*, *10*(9), 4031-4043.

Preston, D. L., Orlofske, S. A., Lambden, J. P., & Johnson, P. T. (2013). Biomass and productivity of trematode parasites in pond ecosystems. *Journal of Animal Ecology*, *82*(3), 509-517.

Rambaut, A. (2018). *FigTree*. In (Version 1.4.4) Institute of Evolutionary Biology, University of Edinburgh. http://tree.bio.ed.ac.uk/

Rochat, E. C., Brodersen, J., & Blasco-Costa, I. (2021). Conspecific migration and environmental setting determine parasite infracommunities of non-migratory individual fish. *Parasitology*, *148*(9), 1057-1066.

Rochat, E. C., Paterson, R. A., Blasco‐Costa, I., Power, M., Adams, C. E., Greer, R., & Knudsen, R. (2022). Temporal stability of polymorphic Arctic charr parasite communities reflects sustained divergent trophic niches. *Ecology and Evolution*, *12*(11), e9460.

Rouse, W. R., Douglas, M. S., Hecky, R. E., Hershey, A. E., Kling, G. W., Lesack, L., Marsh, P., McDonald, M., Nicholson, B. J., & Roulet, N. T. (1997). Effects of climate change on the freshwaters of arctic and subarctic North America. *Hydrological processes*, *11*(8), 873-902.

Selbach, C., Jorge, F., Dowle, E., Bennett, J., Chai, X., Doherty, J.-F., Eriksson, A., Filion, A., Hay, E., & Herbison, R. (2019). Parasitological research in the molecular age. *Parasitology*, *146*(11), 1361-1370.

Selbach, C., & Poulin, R. (2020). Some like it hotter: trematode transmission under changing temperature conditions. *Oecologia*, *194*(4), 745-755.

Selbach, C., Soldánová, M., Feld, C. K., Kostadinova, A., & Sures, B. (2020). Hidden parasite diversity in a European freshwater system. *Scientific Reports*, *10*(1), 1-14.

Slåteng, S. (2022). *Molecular study of digenean diversity in aquatic organisms in northern Norway, with a focus on the seasonality of Crepidostomum [Braun, 1900]* UiT Norges arktiske universitet].

Soldánová, M., Georgieva, S., Roháčová, J., Knudsen, R., Kuhn, J. A., Henriksen, E. H., Siwertsson, A., Shaw, J. C., Kuris, A. M., & Amundsen, P.-A. (2017). Molecular analyses reveal high species diversity of trematodes in a sub-Arctic lake. *International Journal for Parasitology*, *47*(6), 327-345.

Struck, T. H., Feder, J. L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V. I., Kistenich, S., Larsson, K.-H., Liow, L. H., & Nowak, M. D. (2018). Finding evolutionary processes hidden in cryptic species. *Trends in ecology & evolution*, *33*(3), 153-163.

Studer, A., & Poulin, R. (2012). Seasonal dynamics in an intertidal mudflat: the case of a complex trematode life cycle. *Marine Ecology Progress Series*, *455*, 79-93.

Thieltges, D. W., Amundsen, P. A., Hechinger, R. F., Johnson, P. T., Lafferty, K. D., Mouritsen, K. N., Preston, D. L., Reise, K., Zander, C. D., & Poulin, R. (2013). Parasites as prey in aquatic food webs: implications for predator infection and parasite transmission. *Oikos*, *122*(10), 1473-1482.

Thompson, R. M., Mouritsen, K. N., & Poulin, R. (2005). Importance of parasites and their life cycle characteristics in determining the structure of a large marine food web. *Journal of Animal Ecology*, *74*(1), 77-85.

Tikkanen, P., Muotka, T., Huhta, A., & Juntunen, A. (1997). The roles of active predator choice and prey vulnerability in determining the diet of predatory stonefly (Plecoptera) nymphs. *Journal of Animal Ecology*, 36-48.

Vainutis, K. S., Voronova, A. N., & Urabe, M. (2021). Systematics of Crepidostomum species from the Russian Far East and northern Japan, with description of a new species and validation of the genus Stephanophiala. *Parasitology International*, *84*, 102412.

Varpe, Ø. (2017). Life History Adaptations to Seasonality. *Integrative and Comparative Biology*, *57*(5), 943-960. https://doi.org/10.1093/icb/icx123

Villa, M., & Lagrue, C. (2019). Progenesis and facultative life cycle abbreviation in trematode parasites: Are there more constraints than costs? *International Journal for Parasitology*, *49*(5), 347-354.

Wegner, K., Kalbe, M., Rauch, G., Kurtz, J., Schaschl, H., & Reusch, T. (2006). Genetic variation in MHC class II expression and interactions with MHC sequence polymorphism in three‐spined sticklebacks. *Molecular ecology*, *15*(4), 1153-1164.

7 Appendix

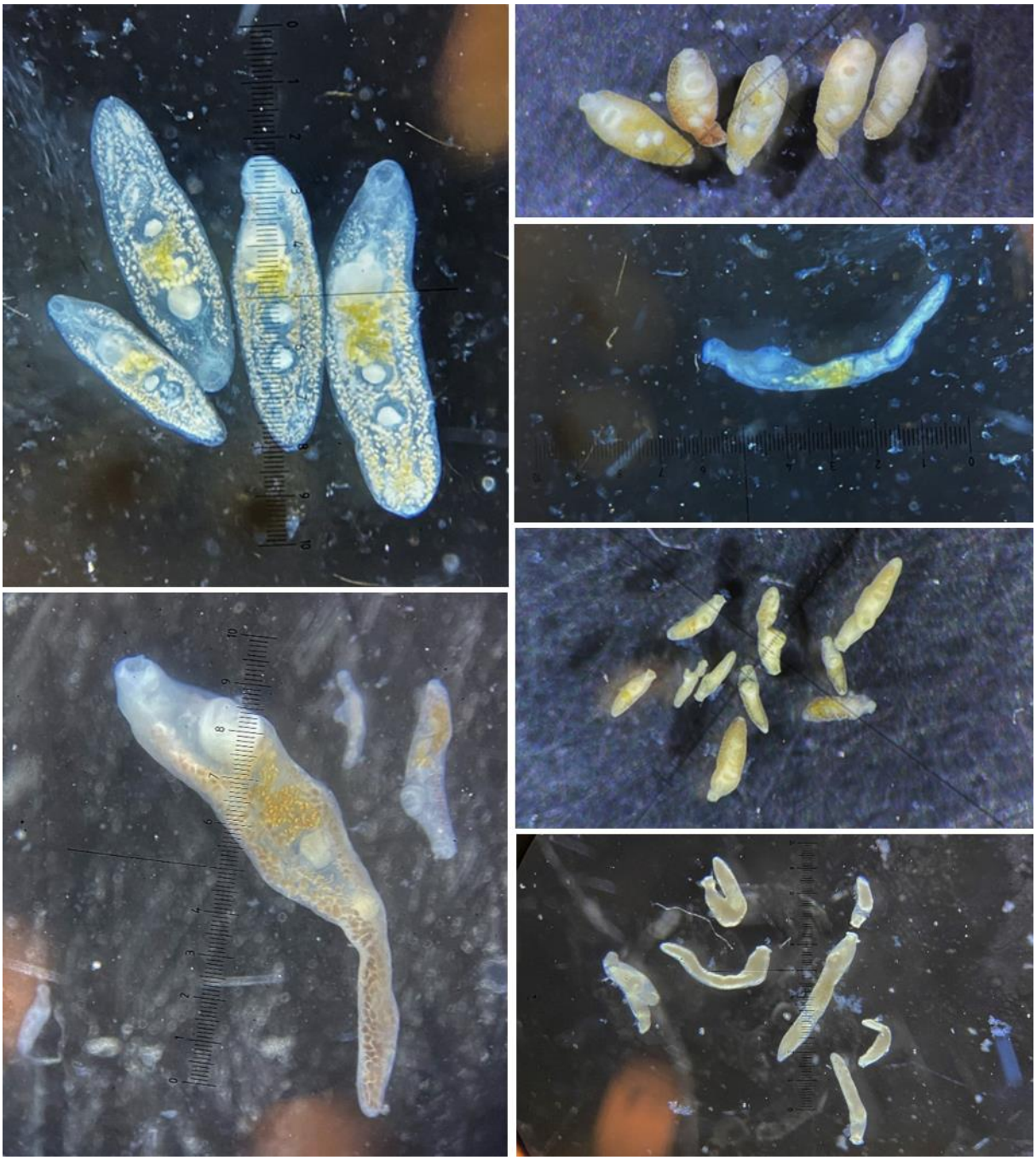

Fig. 10: Stereomicroscope pictures of *Crepidostomum* spp. from Takvatn showing the morphological diversity of the cryptic species. The scale in the pictures measures 10 mm.

Table 3: Overview of the different primer combinations tested for CO1 for *Crepidostomum* spp. with gradient PCR, also showing the master mix with the volume of reagents used, and the gradient PCR temperatures.

Primer F	Primer R
PlagDipCO1hF	CO1R-trema
PlagDipCO1hF	trem.cox1.rrnl
PlagDipCO1gF	CO1R-trema
PlagDipCO1gF	trem.cox1.rrnl
JB3	trem.cox1.rrnl

Master Mix	10 µl	Gradient PCR temperature (°C)
MQwater	1,2	48,4
Primer F	0,4	51,3
Primer R	0,4	55,0
MyFiMix2x	5,0	57,9
CO1 DNA	3,0	59,4

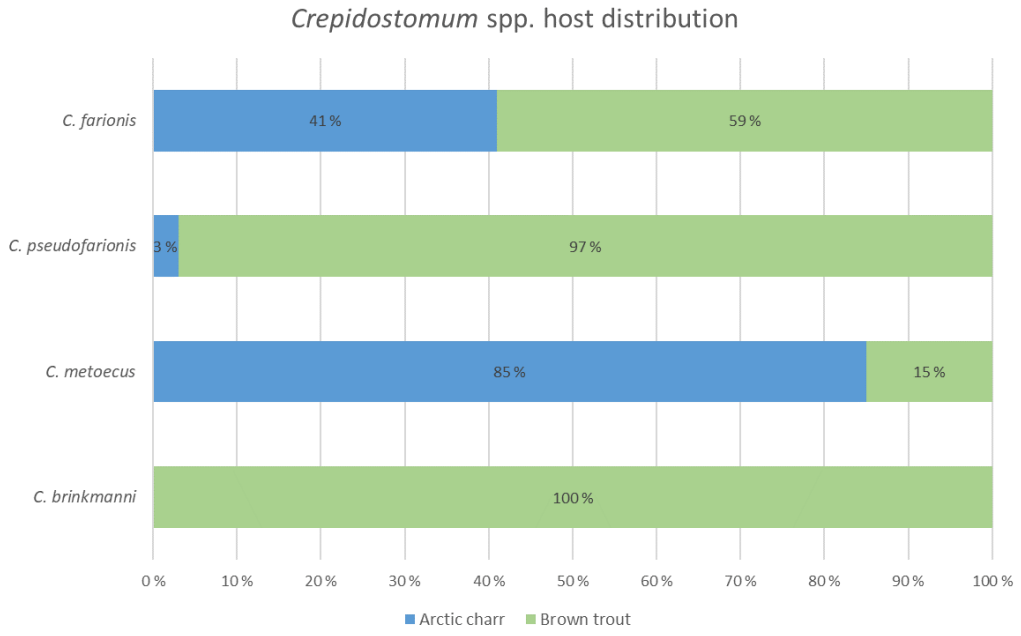

Fig. 11: Distribution of *Crepidostomum* spp. in trout (n = 39) and charr (n = 67) combined in Takvatn. Showing the percentage of each *Crepidostomum* species found in each fish host.

Table 4: The seasonal distribution of *Crepidostomum* spp. in trout (n = 39) and charr (n = 67) in Takvatn. Showing the number of specimens found for each parasite species for each season. Also showing the percentage this makes up for that season in total.

	C. farionis		*C. pseudofarionis*		*C. metoecus*		*C. brinkmanni*		**Total**	
Summer	42	45.7%	7	7.6%	40	43.5%	3	3.3%	92	100 %
Autumn	81	51.9%	14	9.0%	38	24.4%	23	14.7%	156	100 %
Winter	73	37.1%	9	4.6%	111	56.3%	4	2.0%	197	100 %
Spring	34	29.6%	2	1.7%	74	64.3%	5	4.3%	115	100 %

Table 5: Identified *Crepidostomum* spp. hosts in Takvatn based on this study and the study by Soldánová et al. 2017, showing molecularly confirmed first intermediate hosts, second intermediate host, and final hosts.

Identified *Crepidostomum* spp. hosts in Takvatn

Species	First intermediate host	Second intermediate host	Final host
C. farionis	*Sphaerium* sp. *Euglesa casertana*	-	*Salmo trutta* *Salvelinus alpinus*
C. pseudofarionis	*Sphaerium* sp.	*Siphlonurus lacustris*	*S. trutta* *S. alpinus*
C. metoecus	*E. casertana*	*Gammarus lacustris*	*S. trutta* *S. alpinus*
C. brinkmanni	-	*S. lacustris* *Diura bicaudata*	*S. trutta*

Table 6: Complete data of the number of sequenced *Crepidostomum* specimens of each species from Takvatn divided into sampling months, showing which fish species they were sampled from, with accompanying fish host ID numbers (Arctic charr = AC, brown trout = BT).

Sampling month	Year	Fish species	Fish host ID	*C. farionis*	*C. pseudofarionis*	*C. metoecus*	*C. brinkmanni*
June	2017	AC	TA5010	1	0	0	0
June	2017	AC	TA5025	3	0	0	0
June	2017	AC	TA5029	1	0	0	0
June	2017	AC	TA5030	0	0	7	0
June	2017	AC	TA5034	1	0	0	0
June	2017	AC	TA5038	1	0	1	0
June	2017	AC	TA5040	1	0	1	0
June	2017	AC	TA5043	1	0	0	0
June	2017	AC	TA5044	2	0	0	0
June	2017	AC	TA5046	2	0	0	0
June	2017	AC	TA5052	4	0	0	0
August	2017	BT	TA1010	2	1	0	0
August	2017	BT	TA1056	10	0	7	0
August	2017	BT	TA1059	0	3	0	2
August	2017	AC	TA109	0	0	5	0
August	2017	AC	TA141	0	0	4	0
August	2017	AC	TA152	2	0	12	0
August	2017	AC	TA153	3	0	0	0
August	2017	AC	TA173	6	0	0	0
August	2017	AC	TA179	0	0	1	0
August	2017	AC	TA189	0	0	2	0
August	2017	BT	TA200	2	3	0	1
September	2017	AC	TA2007	3	1	0	0
September	2017	BT	TA2047	0	2	0	0
September	2017	BT	TA2002	5	0	0	0
September	2017	AC	TA2003	9	0	0	0
September	2017	BT	TA2008	1	0	0	0
September	2017	BT	TA2013	4	0	0	0
September	2017	BT	TA2014	1	1	0	0
September	2017	AC	TA2017	1	0	0	0
September	2017	BT	TA2018	0	1	0	1
September	2017	AC	TA2019	14	0	0	0
September	2017	BT	TA2022	1	1	0	0
September	2017	BT	TA2027	0	3	0	2
September	2017	AC	TA2032	3	0	0	0
September	2017	AC	TA2033	0	0	8	0
September	2017	AC	TA2035	1	0	0	0
September	2017	BT	TA2043	0	0	0	11
September	2017	AC	TA2046	5	0	3	0
October	2017	AC	TA2111	3	0	0	0
October	2017	AC	TA2112	5	0	5	0
October	2017	AC	TA2117	0	0	11	0
October	2017	AC	TA2125	4	0	0	0
October	2017	AC	TA2129	3	0	1	0
October	2017	BT	TA2153	2	0	2	0
October	2017	BT	TA64	0	1	4	0
October	2017	BT	TA67	0	2	2	0

October	2017	BT	TA76	1	2	0	1
October	2017	BT	TA77	10	0	0	0
October	2017	BT	TA78	5	0	0	1
October	2017	BT	TA85	0	0	2	7
November	2017	BT	TA2163	0	1	0	0
November	2017	AC	TA2170	0	0	22	0
November	2017	BT	TA2173	0	0	1	0
November	2017	BT	TA2196	3	0	3	1
November	2017	BT	TA2197	15	0	0	0
November	2017	AC	TA2202	0	0	1	0
November	2017	AC	TA2219	2	0	1	0
November	2017	BT	TA2232	13	1	0	2
November	2017	AC	TA2234	1	0	0	0
November	2017	AC	TA2278	1	0	8	0
November	2017	BT	TA2284	1	0	0	0
November	2017	BT	TA2297	6	1	1	1
November	2017	BT	TA2298	1	0	1	0
November	2017	AC	TA2319	0	0	4	0
November	2017	AC	TA2326	0	0	1	0
November	2017	AC	TA2339	0	0	14	0
November	2017	AC	TA2341	0	0	3	0
November	2017	AC	TA2344	0	0	2	0
January	2018	BT	TA12	5	4	0	0
January	2018	BT	TA15	1	0	0	0
January	2018	BT	TA17	0	1	1	0
January	2018	AC	TA28	5	0	9	0
January	2018	AC	TA29	1	0	0	0
January	2018	AC	TA35	0	0	1	0
January	2018	BT	TA36	0	1	3	0
January	2018	BT	TA38	0	0	2	0
January	2018	AC	TA45	0	0	1	0
January	2018	AC	TA5	2	0	0	0
January	2018	BT	TA58	1	0	3	0
January	2018	AC	TA61	2	0	0	0
January	2018	AC	TA66	2	0	2	0
January	2018	AC	TA70	7	0	16	0
January	2018	AC	TA71	3	0	11	0
January	2018	BT	TA74	1	0	0	0
March	2018	BT	TA105.2	2	0	4	1
March	2018	AC	TA106	0	0	3	0
March	2018	AC	TA113	4	0	0	0
March	2018	AC	TA120	0	0	9	0
March	2018	BT	TA126	0	0	1	0
March	2018	AC	TA135	1	0	11	0
March	2018	AC	TA136	12	0	0	0
March	2018	AC	TA81	0	0	16	0
May	2018	AC	TA105	1	0	0	0
May	2018	AC	TA150	2	0	0	0
May	2018	AC	TA151	0	0	1	0

May	2018	AC	TA155	1	0	0	0
May	2018	BT	TA165	2	1	2	4
May	2018	AC	TA169	1	0	0	0
May	2018	BT	TA176	0	1	0	0
May	2018	AC	TA177	3	0	1	0
May	2018	AC	TA181	0	0	1	0
May	2018	AC	TA187	2	0	9	0
May	2018	AC	TA195	0	0	6	0
May	2018	AC	TA196	0	0	9	0
May	2018	AC	TA207	3	0	1	0
			106	**230**	**32**	**263**	**35**