


# A hole in the nematosphere: tardigrades and rotifers dominate the cryoconite hole environment, whereas nematodes are missing

K. Zawierucha<sup>1</sup> , D. L. Porazinska<sup>2</sup>, G. F. Ficetola<sup>3,4</sup>, R. Ambrosini<sup>3</sup>, G. Baccolo<sup>5</sup>, J. Buda<sup>1</sup>, J. L. Ceballos<sup>6</sup>, M. Devetter<sup>7,8</sup>, R. Dial<sup>9</sup>, A. Franzetti<sup>5</sup>, U. Fuglewicz<sup>10</sup>, L. Gielly<sup>4</sup>, E. Łokas<sup>11</sup>, K. Janko<sup>12,13</sup>, T. Novotna Jaromerska<sup>14</sup>, A. Kościński<sup>15</sup>, A. Kozłowska<sup>1</sup>, M. Ono<sup>16</sup>, I. Parnikoza<sup>17,18</sup>, F. Pittino<sup>5</sup>, E. Poniecka<sup>19</sup>, P. Sommers<sup>20</sup>, S. K. Schmidt<sup>20</sup>, D. Shain<sup>21</sup>, S. Sikorska<sup>1</sup>, J. Uetake<sup>22</sup> & N. Takeuchi<sup>23</sup>

<sup>1</sup>Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Poznań, Poland

<sup>2</sup>Department of Entomology and Nematology, University of Florida, Gainesville, FL, USA

<sup>3</sup>Department of Environmental Science and Policy, University of Milan, Milan, Italy

<sup>4</sup>Laboratoire d'Ecologie Alpine, University Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, LECA, Grenoble, France

<sup>5</sup>Earth and Environmental Sciences Department, University of Milano-Bicocca, Milan, Italy

<sup>6</sup>Institute of Hydrology, Meteorology and Environmental Studies, IDEAM, Bogota', Colombia

<sup>7</sup>Institute of soil Biology, Biology Centre CAS, České Budějovice, Czech Republic

<sup>8</sup>Centre for Polar Ecology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

<sup>9</sup>Institute of Culture and the Environment, Alaska Pacific University, Anchorage, AK, USA

<sup>10</sup>Private person, Wrocław, Poland

<sup>11</sup>Department of Mass Spectroscopy, Institute of Nuclear Physics Polish Academy of Sciences, Kraków, Poland

<sup>12</sup>Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechev, Czech Republic

<sup>13</sup>Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic

<sup>14</sup>Department of Ecology, Faculty of Science, Charles University, Praha, Czech Republic

<sup>15</sup>Private person, Wrocław, Poland

<sup>16</sup>Graduate School of Science and Engineering, Chiba University, Chiba, Japan

<sup>17</sup>State Institution National Antarctic Center of Ministry of Education and Science of Ukraine, Kyiv, Ukraine

<sup>18</sup>Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine

<sup>19</sup>School of Earth and Ocean Sciences, Cardiff University, Cardiff, UK

<sup>20</sup>Ecology and Evolutionary Biology Department, University of Colorado, Boulder, CO, USA

<sup>21</sup>Biology Department, Rutgers, The State University of New Jersey, Camden, NJ, USA

<sup>22</sup>The Arctic Environment Research Center, National Institute of Polar Research, Tachikawa, Japan

<sup>23</sup>Department of Earth Sciences, Graduate School of Science, Chiba University, Chiba, Japan

## Keywords

ecological selection; extremophiles; distribution; glaciers; Nematoda; psychrophiles; Rotifera; Tardigrada.

## Correspondence

Krzysztof Zawierucha, Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Poznań, Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland.  
Email: k.p.zawierucha@gmail.com

Editor: Gabriele Uhl

Received 25 December 2019; revised 27 July 2020; accepted 30 July 2020

doi:10.1111/jzo.12832

## Abstract

The worldwide distribution of microinvertebrates on glaciers, the coldest biome, is poorly known. Owing to their tolerance to hostile conditions, small size and dispersal abilities, nematodes, tardigrades and rotifers are considered cosmopolitan and together inhabit various ecosystems. In this study, we investigated their global distribution in cryoconite holes – a type of freshwater reservoir forming directly in the glacial ice that creates biodiversity hotspots on glaciers. We analysed cryoconite samples (using classical microscopic observations and environmental DNA metabarcoding) from 42 glaciers located around the world (the Arctic, Subarctic, Scandinavia, the Alps, the Caucasus, Siberia, Central Asia, Africa, South America and Antarctica), as well as using literature data. Samples from Antarctic, Karakoram and the Alps were analysed using next-generation sequencing (NGS) and classical observations under microscopes, while all other samples were analysed by microscope alone. Three general outcomes were found: (1) tardigrades and rotifers represented the most common invertebrates in cryoconite holes; (2) tardigrades and rotifers often coexisted together, with one or the other dominating, but the dominant taxon varied by region or by glacier; (3) nematodes – the most abundant, hyperdiverse and widespread metazoans on Earth, including in environments surrounding and seeding glacial surfaces – were consistently absent from cryoconite holes. Despite the general similarity of environmental conditions in cryoconite

holes, the distribution of tardigrades and rotifers differed among glaciers, but not in any predictable way, suggesting that their distribution mostly depended on the random dispersal, extreme changes of supraglacial zone or competition. Although nematodes have been found in supraglacial habitats, cryoconite hole environments seem not to provide the necessary conditions for their growth and reproduction. Lack of physiological adaptations to permanently low temperatures ( $\sim 0^{\circ}\text{C}$ ) and competition for different food resources in the cryoconite hole environment may explain the absence of nematodes in cryoconite holes.

## Introduction

The spatial distribution of life forms has been studied for centuries (Aristotle, 350 B.C.E.; Darwin, 1859; MacArthur & Willson, 1967). However, historically, these biogeographical surveys have been limited to macroorganisms. The emergence of new optical and molecular tools now allows the pursuit of similar questions for microorganisms, including single-cell microbes and multicellular micrometazoans (Nkem *et al.*, 2006a, 2006b; Guil, Sánchez-Moreno & Machordom, 2009; Velasco-Castrillón, Gibson & Stevens, 2014; Darcy *et al.*, 2018; Velasco-Castrillón, Hawes & Stevens, 2018; Zawierucha *et al.*, 2018b). Recent studies show that the distributions of micrometazoan taxa can be related to physiological and dispersal constraints (Nkem *et al.*, 2006a, 2006b; Dial *et al.*, 2012; Shain *et al.*, 2016; Zawierucha *et al.*, 2018b; Zawierucha *et al.*, 2019b), competition (Shaw *et al.*, 2018), geographical barriers (Jørgensen, Møbjerg & Kristensen, 2007; Czechowski *et al.*, 2016) and to ongoing shifts in environmental conditions (Andriuzzi *et al.*, 2018).

Since microinvertebrates (i.e. tardigrades, rotifers and nematodes) display similar size, dormancy and dispersal characteristics to unicellular organisms (Crowe & Madin, 1974; Sohlenius, 1977; Ramazzotti & Maucci, 1983), they may be expected to show worldwide distribution that is similar to microbes (Fontaneto, 2019). However, the long-standing expectation of cosmopolitan distributions for microbes – the ‘Everything small is everywhere’ hypothesis (Liu *et al.*, 2017; Segawa *et al.*, 2017, 2018; Fontaneto, 2019) – has been refuted for some of microinvertebrates by contemporary inventories of species diversity that reveal examples of endemism (Faurby *et al.*, 2012; Cesari *et al.*, 2016; Fontaneto, 2019). The evidence for endemism largely applies to higher precision taxonomic categories, like genus and species (Forró *et al.*, 2008), although not always, while cosmopolitan distributions apply to more coarse categories, like phylum and class. However, not all taxa at low taxonomic resolution are cosmopolitan. The phylum Onychophora (velvet worms), for example, inhabits the tropics, while the microinvertebrate phylum Loricifera (brush heads) await confirmation of their distribution (Monge-Najera, 1995; Kristensen, 2002).

A recent review (Fontaneto, 2019) of the distribution of limno-terrestrial rotifers, tardigrades and nematodes found a wide array of geographical ranges from worldwide, to biogeographically restricted, to completely endemic. Unfortunately,

typical studies of distribution patterns in microinvertebrates involve comparisons across very different habitats (e.g. soil, bryophytes or lichens, freshwater lakes, puddles) distributed across different biogeographical realms (Segers, 2008; Kaczmarek, Michalczyk, & McInnes, 2016; van den Hogen *et al.*, 2019). This lack of ecological focus can hamper biogeographical conclusions and generalizations. A worldwide analysis of microinvertebrates that focuses on a specific habitat characterized by a specific thermal regime and other environmental conditions is currently absent.

Terrestrial ecosystems constitute 30% of Earth’s surface. Although 10% of that is covered by glaciers and ice sheets, our knowledge about microinvertebrate diversity in these parts of the cryosphere is very incomplete. While polar terrestrial habitats have been the subject of many microinvertebrate diversity studies (Wu *et al.*, 2011; Coulson *et al.*, 2014; Iakovenko *et al.*, 2015), glacial habitats have not been well characterized (Zawierucha *et al.*, 2015; Zawierucha *et al.*, 2019a). During the melt season, warm temperatures and solar radiation transform glacial snow into liquid water, thereby providing a suitable environment for psychrophilic organisms (Hodson *et al.*, 2008; Cook *et al.*, 2016). Among habitats associated with glaciers, cryoconite holes – water-filled reservoirs ( $\sim 20\text{--}50$  cm deep) in the glacial ice (Wharton *et al.*, 1985; Hodson *et al.*, 2008; Stibal *et al.*, 2010; Cook *et al.*, 2016) – are known glacier biodiversity hotspots. The formation of these meltwater holes results from sediments (soil, rock fragments and mineral dust along with cryophilic organisms) that absorb solar radiation and so melt into the ice (Hodson *et al.*, 2008; Takeuchi, Nishiyama, & Li, 2010; Cook *et al.*, 2016; Uetake *et al.*, 2016). Cryoconite holes are inhabited principally by bacteria (both heterotrophic and photoautotrophic), algae and microinvertebrates (e.g. tardigrades and rotifers) (Porazinska *et al.*, 2004; Zawierucha *et al.*, 2015; Uetake *et al.*, 2016; Zawierucha *et al.*, 2018a, 2018b). The photosynthetic rates and organic matter content in cryoconite holes are comparable to other nutrient-poor freshwater ecosystems, such as stony-bottom oligotrophic lakes (Sävström *et al.*, 2002; Peters & Traunspurger, 2005).

Despite more than a century of research on cryoconite holes (Drygalski, 1897), the global biogeography of microinvertebrates – top consumers within cryoconite holes – remains poorly known; most studies generally focus on the worldwide distributions of bacteria and algae (Mueller *et al.*, 2001; Liu *et al.*, 2017; Segawa *et al.*, 2017, 2018; Darcy *et al.*, 2018).

Microinvertebrates of cryoconite holes feed on these producers and on dead organic matter as herbivores, microbivores and filter feeders (Takeuchi & Kohshima, 2004; Vonnahme *et al.*, 2016; Zawierucha *et al.*, 2016). Among microinvertebrates observed in cryoconite holes, studies indicate a ubiquitous presence of tardigrades and rotifers, yet an anecdotal absence of nematodes (Porazinska *et al.*, 2004; Zawierucha *et al.*, 2015). This absence of nematodes is very surprising, given their prevalence across all of Earth's aquatic and terrestrial habitats, including the most extreme (e.g. Borgonie *et al.*, 2011; Grzelak *et al.*, 2016; Andriuzzi *et al.*, 2018; van den Hoogen *et al.*, 2019).

Nematodes have been reported in supraglacial gravel in the Alps by Azzoni *et al.* (2015) and in moss balls (so-called 'glacier mice') in Iceland by Coulson & Midgley (2012). Additionally, Schwarz *et al.* (1993) found a high abundance of nematodes in mosses associated with a stream located near Taylor Glacier in the McMurdo Dry Valleys of Antarctica. Nematodes have also been detected within a cryoconite hole in the McMurdo Dry Valley using classical microscopy, but on a single occasion (Mueller *et al.*, 2001). Others (Christner, Kvitko, & Reeve, 2003; Sommers *et al.*, 2018) detected nematodes in Dry Valley glacier cryoconite holes using genetic barcoding, but not corroborated by microscopic inspection. Subsequent microscopic examination and high-throughput sequencing of cryoconite from holes on the same glaciers have failed to detect any further evidence of nematodes, neither living nor dead (Porazinska *et al.*, 2004; Sommers *et al.*, 2019). Taken together, these studies suggest that although cryoconite holes may be seeded from environments containing nematodes, they do not thrive there. Moreover, in Norway and the Alps, remnants of various arthropods in cryoconite holes were detected with microscopy, but nematodes were not (Dastych, 2019; Zawierucha *et al.*, 2019a).

As rotifers, tardigrades and nematodes exhibit similar life and feeding strategies (Sohlenius, 1979; Altiero & Rebecchi, 2001; Michiels & Traunspurger, 2005), occupy similar habitats (Sohlenius, 1977; Janiec, 1996; Sohlenius, Boström, & Jönsson, 2004) and could be passively dispersed by the same mechanisms into cryoconite holes, a reasonable null hypothesis would claim all three phyla should co-occur in cryoconite holes. Here, we synthesize original and previously published data to examine the distribution of microinvertebrates in cryoconite holes on glaciers around the world.

## Materials and methods

### Original data

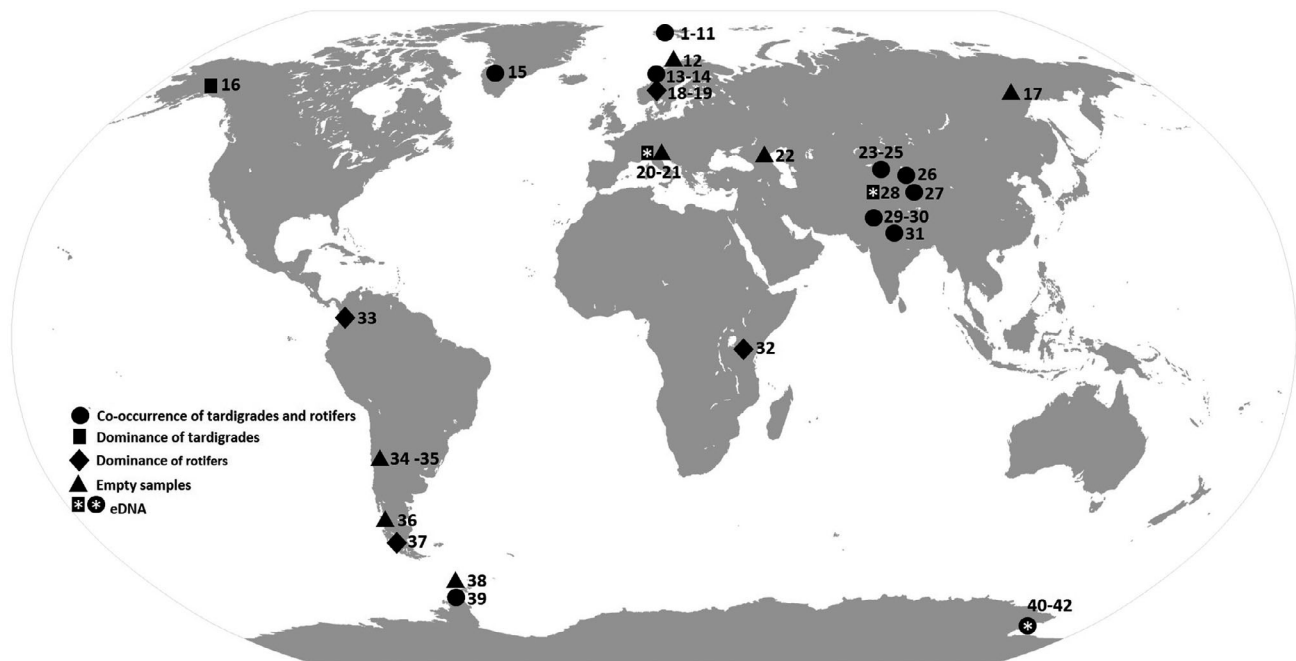
Cryoconite material was collected from cryoconite holes across a wide variety of glaciers differing in their (1) morphology (e.g. tidewater vs. valley), (2) thermal regime (i.e. polythermal vs. cold-base vs. temperate), (3) light and temperature [e.g. seasonal cycles (polar) vs. daily cycles (temperate)], and (4) elevation (i.e. terminating in the sea vs. high mountains up to 5200 m a.s.l.). A total of 42 glaciers (Fig. 1) were sampled in the Arctic [Greenland, Svalbard (Spitsbergen, Nordaustlandet)], the Subarctic (Alaska), Scandinavia (Norway, Sweden), the

Alps, the Caucasus, Siberia, Central Asia (the Karakoram, Pamir, Tien and Quilian Shan, Himalaya), Africa (Tanzania), South America (Colombia, Chile), Maritime Antarctica (Anvers Island, King George Island) and Continental Antarctica (McMurdo Dry Valleys). We sampled a wide range of cryoconite hole depths to reflect the variability in niche stability. The deepest holes were >60 cm deep with relatively stable water bodies; the shallowest were to <5 cm deep and subject to flooding and freeze/thaw cycles. Details of sampled cryoconite holes are provided in Supporting Information Table S1. Information on cryoconite holes from other sites was added by reviewing the published literature (Supporting Information Figure S1; Supporting Information Table S1). Data on the area and depth of cryoconite holes in particular regions and glaciers are available in Zawierucha *et al.* (2018a) for SW Greenland, Zawierucha *et al.* (2019c) and Łokas *et al.* (2016) for Svalbard, Zawierucha *et al.* (2019a) for the Alps, Takeuchi *et al.* (2000) for Himalaya, Buda *et al.* (2020) for Maritime Antarctica, and Porazinska *et al.* (2004) and Sommers *et al.* (2019) for Continental Antarctic.

The presence of microinvertebrates was evaluated using two complementary approaches; a classical approach based on microscopy (dissecting, inverted or both) and environmental DNA (eDNA) metabarcoding. Sampled cryoconite holes varied in shape and size across regions, across glaciers, and across the same glacier (Fig. 2). For microscopy, we collected sediment samples from the bottom of each hole by two methods determined by whether the holes were open or frozen. Sediments from open holes were sampled with independent sterile disposable Pasteur pipettes or scoops and placed directly in vials, zipp bags or jars. Sediments from frozen holes were retrieved from a 10-cm-diameter core and placed into sterile plastic bags. All sediment samples were either immediately frozen or preserved in 70% or 96% ethylic alcohol, then transported to home laboratories at the Adam Mickiewicz University in Poznań (Poland); Cardiff University (UK); Chiba University (Japan); University of Colorado Boulder (USA); or Charles University and Biology Centre AS CR (Czech Republic). At least 0.3–1.5 mL (in few cases for samples from Longyearbreen, Hansbreen and Marr) up to 10 mL of preserved cryoconite material was scanned from each sample for the presence of invertebrates on Petri dishes scored with perpendicular lines (for facilitating observations). Frozen cores collected from glaciers located in McMurdo Dry Valleys were melted and subset as described below in the Crary Lab in McMurdo, Antarctica, where microinvertebrates were extracted from 20 g of melted sediment subsamples using modified White Trays for 24 h (Porazinska *et al.*, 2018) and immediately counted.

In addition to microscopy counts, samples for eDNA metabarcoding analysis were collected from Forni Glacier in the Alps, Baltoro Glacier in the Pakistan Karakoram and McMurdo Dry Valleys glaciers in Antarctica (Taylor, Canada and Commonwealth glaciers). We performed metabarcoding analyses at two laboratories: University of Milan and University of Colorado.

For the University of Milan metabarcoding analysis, samples from Forni (18 samples on August 28th, and 19 samples on



**Figure 1** List of sampled glaciers (original data). Svalbard: Nordaustlandet: 1 – Area of Goosbukta, 2 – Dunerbreen, 3 – Nordre Franklinbreen, Spitsbergen: 4. Midre Lovénbreen, 5 – Svenbreen, 6 – Ebbabreen, 7 – Nordenskiöldbreen, 8 – Longyearbreen, 9 – Larsbreen, 10 – Werenskiöldbreen, 11 – Hansbreen, Scandinavia: 12 – Storglaciären (Sweden), 13 – Svartisen, 14 – Okstindbreen (Norway), Greenland: 15 – Dark zone of Greenland Ice Sheet, Alaska: 16 – Gulkana Glacier, Siberia: 17 – Suntarhyata no. 31, Scandinavia: 18 – Middalsbreen, 19 – Bläisen (Norway), Alps: 20 – Morteratsch, 21 – Forni, Caucasus: 22 – Gergeti, Pamir Mountains: 23 – Lenin Glacier, Tien Shan: 24 – Urumqi No.1, 25 – Grigoriev Ice Cap, 26 – Miaoergou, Quilian Shan: – 27 - Qiyi, Karakoram: 28 – Baltoro, Himalaya: 29 – Kang Yatse, 30 – Chamser Kangri, 31 – Yala, Kilimanjaro: 32 – Kersten, Andes: 33– Colombia (La Conojeras), 34 – Iver, 35 – El Morado, 36 – Exploradores, 37 – Tyndall, Maritime Antarctic: 38 – Marr Ice Piedmont, 39 – Blancmange, Continental Antarctic: 40 – Canada, 41 – Taylor, 42 – Commonwealth.

September 25th) and the Baltoro Glacier (19 samples from June 28th to July 9th) were collected in 2013. Cryoconite was deposited with a laboratory spoon (sterilized with alcohol) into 50 mL plastic tubes. Total DNA was extracted from 0.7 g of cryoconite using the FastDNA Spin for Soil kit (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. The extracted DNA was amplified in quadruplicates with the primers Euka02, which amplifies a ~123 bp fragment of 18S rDNA (Guardiola *et al.*, 2015; Taberlet, Bonin, Zinger, & Coissac, 2018). The primer pair amplifies all eukaryotes at the family/order level (Guardiola *et al.*, 2015; Taberlet *et al.*, 2018). The amplification success was checked using Capillary electrophoresis (QIAxcel System; Qiagen, Venlo, Netherlands). Extraction and PCR controls were also included (Parducci *et al.*, 2017). Sequencing was performed using Illumina MiSeq V2 platform (2 × 250 bp chemistry) at Fasteris, Geneva,

Switzerland. DNA sequences were filtered (i.e. removing sequences: with low quality score; missing both primers or exact tag sequences; containing ambiguous nucleotides, shorter than 7 bp; or occurring only once in the dataset) and chimera-checked using the OBITOOLS software (Boyer *et al.*, 2016). For the Forni + Baltoro metabarcoding experiment, 2.39 million reads were retained after the bioinformatic filtering steps and assigned to the samples (average number of reads per sample: 28 821). Potential contaminants – identified as those with highest occurrence in negative control samples – were removed. Sequences were retained in the final dataset only if their taxonomic assignment score was >80% and their occurrence was higher than 100 reads across the whole dataset. Pansu *et al.* (2015) provide a complete description of the filtering steps. Sequences were assigned taxonomy using the ecotag program and EMBL database (Boyer *et al.*, 2016).

**Figure 2** Cryoconite holes on glaciers around the world. (a) Svalbard, Longyearbreen, August 2016, (b) south-west Greenland, September 2015, (c) Norway, Bläisen, August 2018, photographs for all K. Zawierucha, (d) Alps, Forni, July 2017, photograph J. Buda, (e) Caucasus, Chaalati, July 2014, photograph K. Zawierucha (f) Caucasus, Gergeti, September 2019, photograph A. Kościński, (g) Himalaya, Kang Yatse, August 2017, photograph M. Devetter (h) Colombia, La Conejeras, 2015, photograph Jun Uetake, (i) Kilimanjaro, Kersten, September 2019, photograph U. Fuglewicz, (j) Andes, Iver, 2018, photograph R. Ambrosini, (k) Andes, Exploradores, 2018, photograph R. Ambrosini, (l) Maritime Antarctica, Ecology, January 2017, photograph T. Budzik, (m) Maritime Antarctica, Marr Ice Piedmont, February 2018, photograph I. Parnikoza, (n), o) Continental Antarctica, Canada, December 2017, photograph P. Sommers.



For the University of Colorado metabarcoding analysis, 90 samples were collected from frozen cryoconite holes on three glaciers in the McMurdo Dry Valleys, Antarctica using a SIPRE corer between 7 and 17 November 2016. Sampling details are described in Darcy *et al.* (2018) and Sommers *et al.* (2019). The cores were stored in sterile Whirl-Pak<sup>®</sup> bags (Nasco, Fort Atkinson, WI, USA) at  $-20^{\circ}\text{C}$  for up to 1 month. In the Crary Laboratory at McMurdo Station, the sediment portion was separated from the rest of the core and washed with deionized water to melt the outer layer and remove potential cross-contamination from the drill, then placed in separate acid-washed high-density polyethylene beakers covered with aluminium foil and melted at  $4^{\circ}\text{C}$  for 12–24 h. Final melting took place at room temperature when necessary immediately prior to homogenizing, subsampling and filtering. DNA was extracted from *c.* 0.3 g cryoconite using a PowerSoil DNA Isolation Kit (MoBio Inc., Carlsbad, CA, USA). Tubes were frozen at  $-20^{\circ}\text{C}$  until the DNA was extracted, up to 1 month later, following the manufacturer's protocol. Extracted genomic DNA was amplified in triplicate using 18S (1391f-EukBr primers, Amaral-Zettler, McCliment, Ducklow, & Huse, 2009; Caporaso *et al.*, 2012) SSU ribosomal gene markers. Amplified DNA was pooled and normalized to equimolar concentrations using SequalPrep Normalization Kit (Invitrogen Corp., Carlsbad, CA, USA) and sequenced using the Illumina MiSeq V2 ( $2 \times 250$  bp chemistry) at the BioFrontiers Sequencing Core Facility at the University of Colorado at Boulder. Sequences have been deposited in the NCBI SRA database under project PRJNA480849. At the Colorado laboratory, we used QIIME v1.9.1 (Caporaso *et al.*, 2010) to de-multiplex and quality filter the raw reads, and VSEARCH (Rognes *et al.*, 2016) to join paired-end reads. The reads were clustered into operational taxonomic units (OTUs) at 97% similarity using UCLUST (Edgar, 2010). Taxonomy was assigned using QIIME's `parallel_assign_taxonomy_blast.py` script with a hand-curated version of SILVA 128 Ref NR99 database (Quast *et al.*, 2013). Based on this classification, bacterial OTUs were removed from the eukaryotic OTU table. Singletons were discarded, as were any OTUs that made up at least 1% of the extraction blank sequences and were at least 1% of the samples, which were considered likely lab contaminants. A total of 2.36 million high-quality joined reads remained after this processing of reads from the 90 cryoconite hole samples, along with an additional 10 samples from stream microbial mats surrounding Canada Glacier in the Taylor Valley, Antarctica, included as a positive control for the presence of nematodes (see Supporting Information Figure S2).

### Previously published distribution data

Published literature about cryoconite holes was identified using Scopus, Web of Science, Google Scholar and ResearchGate search engines with the following keywords: biota, cryoconite, cryoconite hole, ecosystems, glacier, glacial, animal, invertebrates, insects, Tardigrada, Rotifera, Nematoda, biodiversity and metazoans. All papers reporting microinvertebrate in cryoconite holes were included in the present study (Supporting Information Table S2), with an important exception. We

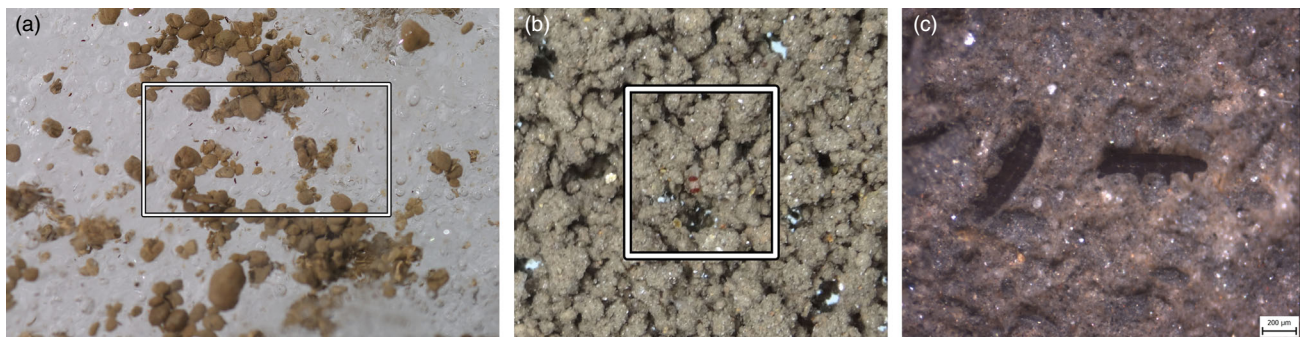
excluded from our literature search studies based solely on metagenomic DNA analysis that report the presence of sequences matching Nematoda alongside sequences of organisms such as members of Chordata, marine Echinodermata and tropical Onychophora (e.g. Edwards *et al.*, 2013). The reporting of these taxa that are certainly not part of the active cryoconite community makes it impossible without microscopic confirmation to know whether nematode sequences also originated from individuals living outside the cryoconite holes. Cases where microinvertebrates are known by the authors as present in samples but unreported in original papers are also listed in Supporting Information Table S2 (e.g. rotifers in Takeuchi & Kohshima, 2004 and Zawierucha *et al.*, 2016). As specific papers often focused a single taxon from cryoconite hole samples, omissions of other taxa from the paper were not taken as conclusive evidence of their absence from those samples. Although survey methods differed among studies (e.g. microscopy vs. molecular gene markers), we included publications from a variety of survey methods to better understand the biogeography of microinvertebrates in cryoconite holes.

## Results

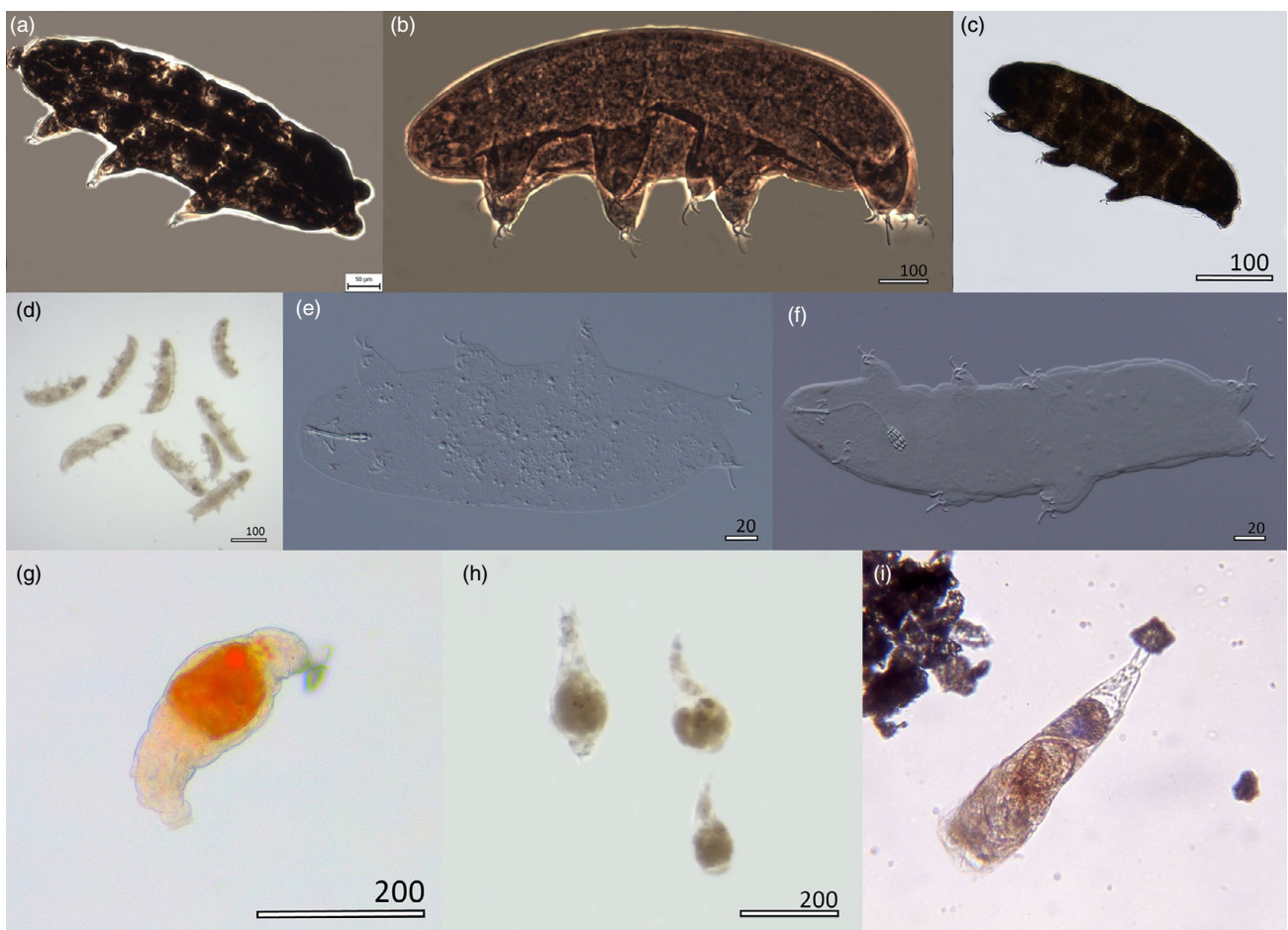
### Original data

We investigated material from 42 glaciers globally (Figs 1 and 7, Supporting Information Table S1). Using microscopy, we found that cryoconite holes were dominated mostly by tardigrades and rotifers (Figs 3, 4 and 7); unexpectedly, nematodes were never observed (Fig. 7). We found considerable differences among glaciers in taxonomic dominance. In the Arctic (Svalbard, Greenland) and Continental Antarctic, cryoconite holes were co-dominated by tardigrades and rotifers regardless of glacier thermal regimes or type (polythermal, cold base, valley, tidewater), or latitude or altitude in Svalbard and southwest Greenland. Cryoconite holes on Norway's Blåisen were dominated by rotifers, although a few tardigrades were also present in both 2018 and 2019. Cryoconite holes on Forni in the Alps during 2019 showed the opposite pattern with nearly exclusive presence of tardigrades and only a few rotifers. Dominance of tardigrades was similarly observed on Gulkana on Alaska sampled in 2015 and 2019. In contrast, cryoconite holes on the Alpine Morteratsch in 2017 and 2018 supported neither tardigrades nor rotifers. Similarly, cryoconite material from Gergeti in the Caucasus, Suntarhyata no. 31 in Siberia, Storglaciären in Sweden and Marr in the Maritime Antarctic, all appeared to be free of any animals in cryoconite holes. Out of five sampled glaciers in Colombia and Chile, rotifers were found on only two (La Conejeras and Tyndall), whereas no tardigrades have been reported in South America. No tardigrades, but many rotifers, were also detected from Kersten in Tanzania and Ecology on King George Island (South Shetland Islands).

Out of the 480 molecular operational taxonomic units (OTUs) recovered from eDNA analysis on Forni and Baltoro samples, the majority were assigned to protists (36%) and fungi (31%) (Fig. 5, Supporting Information Figure S3,



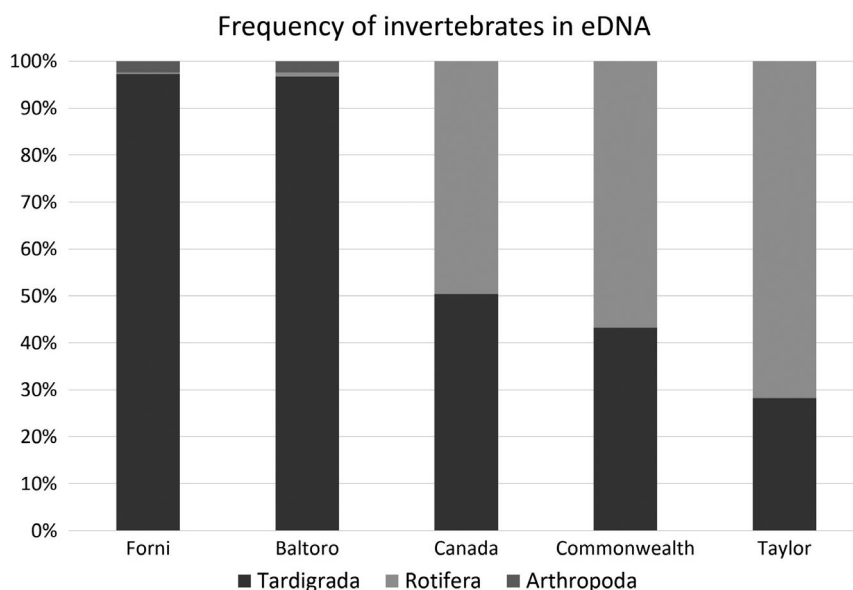
**Figure 3** Tardigrada. (a) *Cryoconicus kaczmareki* Zawierucha *et al.*, 2018 from Ürümqi Glacier (Central Asia) – dark small dots in the frame, and (b, c) *Cryobiotus klebelsbergi* (Mihelčič, 1959) from Forni Glacier (picture b – small brownish dots on cryoconite granules in the frame).



**Figure 4** Tardigrada (a–f) and Rotifera (g–i). (a) *Cryobiotus klebelsbergi* (PCM – phase contrast microscopy), (b) *Cryoconicus kaczmareki* (PCM), (c) eutardigrade from Gulkana glacier (DIC – differential interference contrast microscopy), (d) tardigrades from Svalbard (Longyearbreen) BFM – (bright-field microscopy), (e) *Hypsibius cf. dujardini* from Svalbard (DIC), (f) *Pilatobius* sp. from Greenland (DIC), (g) bdelloid rotifer from Svalbard, (h) bdelloid rotifers from Greenland (preserved in alcohol) and (i) bdelloid rotifer from Tyndall Glacier (BFM – g, h, i).

Supporting Information Table S3). Almost two per cent (1.8%) of MOTUs belonged to metazoan taxa from three phyla: Arthropoda, Rotifera and Tardigrada (Supporting Information

Table S3). None of the detected MOTUs were assigned to Nematoda. Comparison of sequences belonging to invertebrates from Forni, Baltoro and Continental Antarctic glaciers shows a



**Figure 5** Frequencies of invertebrate sequences detected in eDNA using fragment of 18S rDNA from cryoconite holes on Forni (Alps), Baltoro (Karakoram) and Antarctic glaciers (Canada, Commonwealth and Taylor).

clear dominance of tardigrades on Forni and Baltoro (with minority of arthropods and rotifers) and coexistence of rotifers and tardigrades (with dominance of the former) on Antarctic glaciers (Fig. 5).

### Previously published distribution data

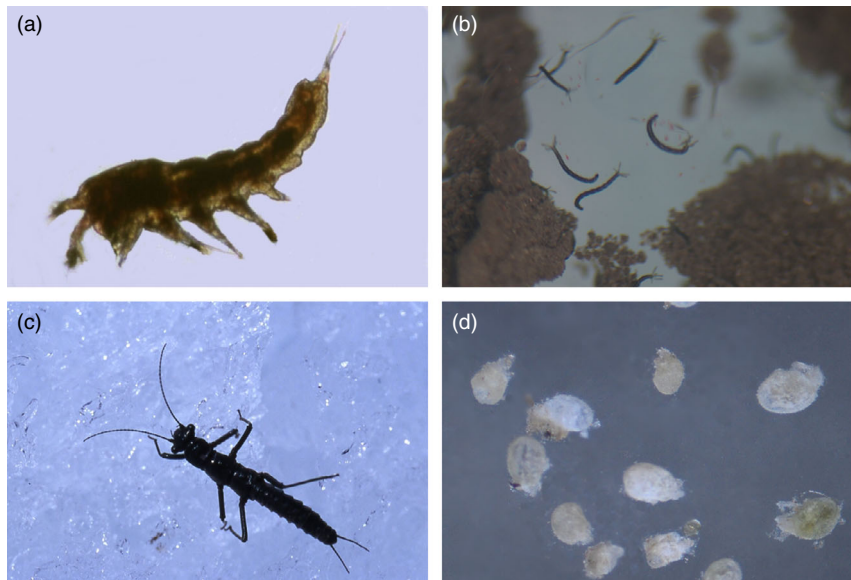
Our review of 46 publications in which 41 localities (Supporting Information Figure S1, Supporting Information Table S2) were investigated, confirmed that cryoconite holes around the world are mostly inhabited by either tardigrades, rotifers or both (Supporting Information Table S2). In some cases, cryoconite holes are inhabited by other animal groups such as Diptera in New Zealand (Odell, 1956; Boothroyd & Cranston, 1999) and the eastern Himalaya, Gangla Karchung La (R. Dial, pers. obs.), Plecoptera in the Andes, Copepoda in the Himalaya, or Acari in the Maritime Antarctica (Buda *et al.*, 2020) (Fig. 6). However, these animals have narrow geographical distributions and mostly represent endemic species (Kohshima, 1984, 1985). Some studies focused on the description or redescription of new microinvertebrate taxa (Mihelčič, 1959; Ramazzotti, 1968; Dastych, 1993, 2004, 2019; Kikuchi, 1994; Dastych, Kraus, & Thaler, 2003; Zawierucha *et al.*, 2018b). Others aimed to describe cryoconite communities, and authors discussed all invertebrates found in cryoconite holes (De Smet & Van Rompu, 1994; Gronggaard *et al.*, 1999; Porazinska *et al.*, 2004; Vonnahme *et al.*, 2016; Zawierucha *et al.*, 2018a; Lutz *et al.*, 2019; Zawierucha *et al.*, 2019a, 2019b, 2019c; Buda *et al.*, 2020). In some papers, animals were mentioned as an ecological variable; in others, the focus was on faunistic reports (Dastych, 1985; Dabert *et al.*, 2015; Łokas *et al.*, 2016).

Both old (Drygalski, 1897; Dastych, 1985; De Smet & Van Rompu, 1994) and recent literature (Zawierucha *et al.*, 2018a;

Zawierucha *et al.*, 2019a, 2019b, 2019c) indicate that Arctic cryoconite holes are inhabited by tardigrades and rotifers without any visible general pattern at the global scale (Supporting Information Figure S1, Supporting Information Table S2). In the Alps, Zawierucha *et al.* (2019a) and Dastych *et al.* (2003) found cryoconite holes inhabited by tardigrades with only little or no rotifer presence, over multiple summers. However, tardigrades and rotifers were found earlier by Steinböck (1936) on other glaciers in the Alps (albeit without quantitative data). Analysis of material from Chalaati and Adishi glaciers in the Caucasus showed that neither tardigrades nor rotifers were present (Makowska *et al.*, 2016). The literature suggests that Central Asian glaciers are inhabited mostly by tardigrades; however, some of papers from this area were purely taxonomic (Supporting Information Table S2) and offered no descriptions of entire invertebrate assemblages.

In the Maritime Antarctica, cryoconite holes on the Ecology Glacier (King George Island, South Shetland Islands) were inhabited by rotifers, but no tardigrades nor nematodes were found (Zawierucha *et al.*, 2019b). Microscopy-based and eDNA surveys of cryoconite holes on Continental Antarctic glaciers showed the presence of both tardigrades and rotifers (Porazinska *et al.*, 2004; Sommers *et al.*, 2018; Lutz *et al.*, 2019). Porazinska *et al.* (2004) sampled five Dry Valley glaciers and found relatively equitable rotifer:tardigrade ratios in the wetter east but very high rotifer:tardigrade ratios in the drier west. Both groups were least abundant on the glaciers receiving the least snow. In cryoconite holes in Marie Byrd Land, Continental Antarctica, only rotifers were reported (Broady, 1989), whereas in cryoconite holes in Queen Maud Land both rotifer and tardigrade DNA were found together three times and separately five times (tardigrades once, rotifers four times) in ice covered cryoconite holes (Lutz *et al.*, 2019). Of the 5014 97% similarity OTUs recovered from the





**Figure 6** Arthropods found in cryoconite holes: (a) Copepoda in Himalaya (Kikuchi, 1994), (b) Chironomidae in Himalaya (Kohshima, 1984), (c) Plecoptera in Patagonia (Takeuchi & Kohshima, 2004) and (d) Acari in the Maritime Antarctica (Buda *et al.*, 2020).

cryoconite hole sediments in Continental Antarctica (Canada, Taylor, and Commonwealth glaciers), 14% were assigned to Rotifera and Tardigrada, and these OTUs accounted for 30–40% of the reads in the samples (Sommers *et al.*, 2019). Two studies from Antarctica detected nematodes in cryoconite holes. One used classical microscopy (Mueller, Vincent, Pollard, & Fristen, 2001), while the other used eDNA analysis (Christner *et al.*, 2003). Other studies from the same glaciers reported no nematodes in cryoconite holes (Porazinska *et al.*, 2004; Sommers *et al.*, 2019). While Sommers *et al.* (2018) reported the presence of nematode sequences using eDNA, these samples did not have microscopy associated with them and later, more extensive sampling of cryoconite holes from the same glaciers including microscopy (Sommers *et al.*, 2019) failed to find nematodes (Fig. 7). Also Murakami *et al.* (2018) detected nematodes in the faecal metagenomes of Plecoptera *Andiperla willinki* which also might be found in cryoconite holes. However, the presence of nematodes in faeces does not offer conclusive evidence of their presence in cryoconite holes because Plecoptera could find their preys directly on the glacier surface.

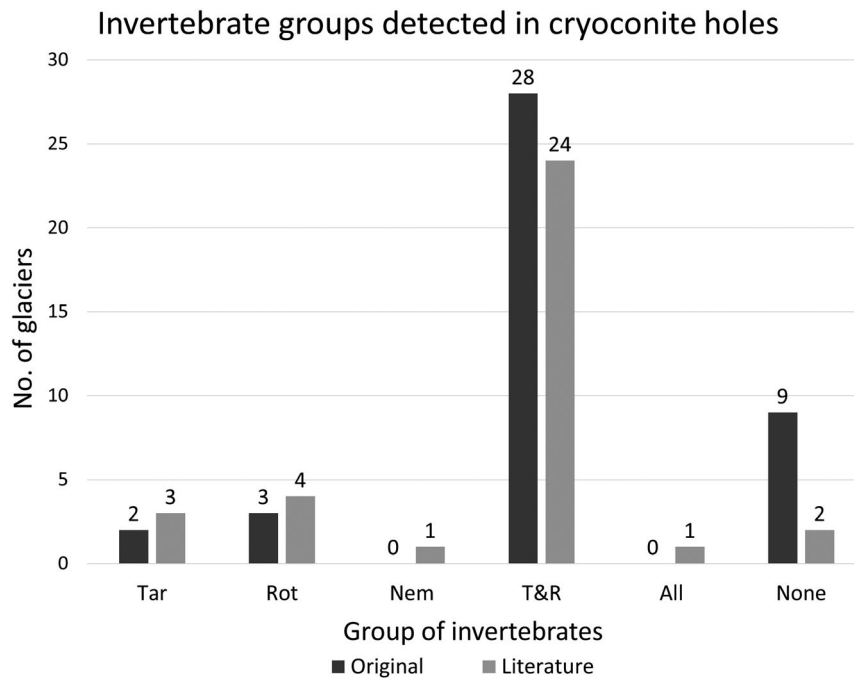
## Discussion

### Dominant taxa

In this study, we used both original data (Fig. 1, Supporting Information Table S1) and published records (Supporting Information Figure S1, Supporting Information Table S2) to show that the microinvertebrate communities in cryoconite holes from >70 locations consist of mostly rotifers, tardigrades or both, but, with the exception of two studies in Antarctica (one

microscopy-based and one genetic), never include nematodes. Although nematodes have been detected in glacial mosses (Coulson & Midgley, 2012), on the glacial ice (D. Shain pers. obs.), in sediments overlying glacial surface (Azzoni *et al.*, 2015; D. Porazinska pers. obs.), in sediments of glacial streams in Antarctica (P. Sommers & D. Porazinska pers. obs.) or even in glacial Plecoptera faeces (Murakami *et al.*, 2018), cryoconite hole environments seem not to provide the necessary conditions for growth and reproduction of nematodes.

Due to their physiological adaptations to survive low temperatures, freezing, high UV and osmotic stress (Altiero *et al.*, 2011; Guidetti *et al.*, 2012; Jönsson & Wojcik, 2017; Zawierucha *et al.*, 2018b; Zawierucha *et al.*, 2019a), dominance in cryoconite holes by tardigrades and rotifers is not surprising. Despite the similarity of conditions across cryoconite holes (e.g. low temperatures, periodic freezing), the composition of animal phyla varied within regions and even within glaciers. On Blåisen (Norway), Forni (the Alps), Ecology (Maritime Antarctic) and Kersten (Africa) glaciers, cryoconite holes were dominated by either rotifers or tardigrades. For example, in Norway (Blåisen, Midtdalsbreen), we observed the numerical dominance of rotifers over tardigrades. Regrettably, detailed records of the cryoconite hole community structure on the same glaciers in previous studies were missing; consequently, it was impossible to infer which group dominated in the past (Sømme, 1996). In the Alps, on Forni Glacier, there was almost exclusive dominance by tardigrades over multiple seasons (Zawierucha *et al.*, 2019a, present study). The same pattern was observed on Rotmoosferner in the Alps by Dastych *et al.* (2003), who reported few rotifers among thousands of tardigrades in cryoconite material. Arctic and Continental Antarctic cryoconite holes hosted both rotifers and tardigrades,



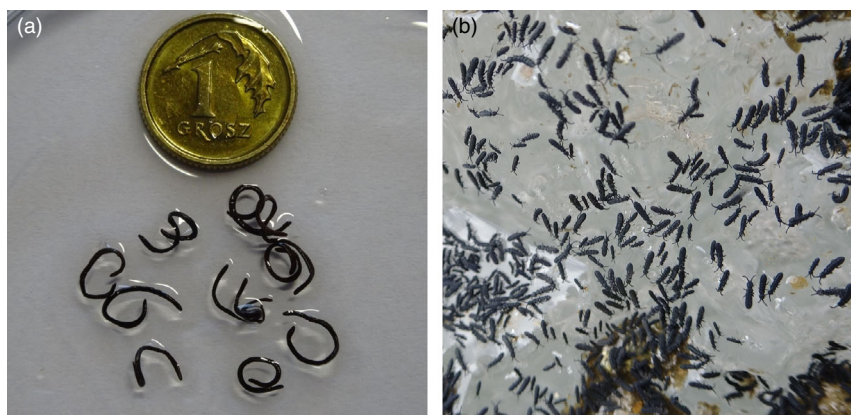
**Figure 7** Invertebrate groups (Tardigrada, Rotifera, Nematoda) detected in cryoconite holes on glaciers (original and literature data, Fig. 1, Supporting Information Figure S1, Supporting Information Tables S1 and S2). T&R: Tardigrada and Rotifera; Tar: Tardigrada; Rot: Rotifera; Nem: Nematoda; All: all three (Tardigrada, Rotifera and Nematoda); and None: absence of these groups from cryoconite holes of studied glaciers. In literature data, taxonomic papers focused only on particular groups were removed from this comparison. For selection criteria of literature records, see Supporting Information Table S1. In consulting original data, we considered dominance by a group when cryoconite was exclusively dominated by one group with few or no coexisting specimens of another.

while no invertebrates were detected in cryoconite holes on Morteratsch Glacier in the Alps (sampled during two seasons) or on Adishi, Chalaati and Gergeti glaciers in the Caucasus. Tardigrades were undetected in cryoconite holes in South America, whereas rotifers were found on one Colombian and one Chilean glacier. Taking into account the high dispersal abilities of both terrestrial and glacier invertebrates (Jørgensen, Møbjerg, & Kristensen, 2007; Ptatscheck, Gansfort, & Traunspurger, 2018; Zawierucha *et al.*, 2018b; Fontaneto, 2019; Zawierucha *et al.*, 2019a), we hypothesize that the nature of glaciers (thermal regime, type, altitude, latitude) is not the driving factor of microinvertebrates distribution patterns. Instead, local climatic or environmental factors and infrequent extreme weather events (e.g. strong rains (Zawierucha *et al.*, 2019c)) might be more important. More intense and standardized (using the same techniques and combining molecular and microscopy approaches) sampling, long-term monitoring and manipulative field experiments could resolve these questions.

### Other animals in cryoconite holes

Zawierucha *et al.* (2015) postulated that cryoconite holes should be inhabited by many groups of micrometazoans, including crustaceans and gastrotrichs, as found in other polar or high-alpine freshwater bodies (Janiec, 1996; Hinden *et al.*, 2005; Kolicka *et al.*, 2016). The habitat provides abundant organic matter as a food source and relatively low levels of

competition. However, our global survey did not corroborate this hypothesis. We found evidence of only a few other animals being found in some cryoconite holes. The Northern and Southern Patagonian Icefields were inhabited by arthropods such as Plecoptera *Andiperla willinki* (Aubert, 1956) and Collembola. In addition, some Himalayan cryoconite holes were inhabited by copepods (Copepoda) and chironomids (Insecta) (Kohshima, 1984, 1985; Takeuchi *et al.*, 2000; Dial pers. obs.). All of these examples are mostly endemic glacier-obligate invertebrates, which feed and reproduce in very narrow geographical areas (one mountain range, ice field or glacier). Recently, Zawierucha *et al.* (2019b) and Buda *et al.* (2020) reported presence of various instars of mite (*Nanorchestes nivalis*) in cryoconite holes on Ecology Glacier in the Maritime Antarctic during two seasons and showed that the mites on this glacier can be persistent residents of the cryoconite holes. Platyhelminthes have been reported by DNA in Continental Antarctica (Sommers *et al.*, 2018); however, confirmation of their affinity with cryoconite holes requires microscopic analysis. Glacier ice worms (*Mesenchytraeus solifugus*) – the only known glacier-obligate annelid, were reported in cryoconite holes in the Pacific Northwest of North America (Goodman, 1971); however, despite the habitat suitability formed by stable thermal structure of cryoconite microenvironments (e.g. Gulkana Glacier), they were absent from the material analysed in this study. Collembola have been found in the bottom of cryoconite holes in the Alps and the Caucasus



**Figure 8** Animals known from glaciers that are occasionally found in cryoconite holes, yet inhabit other glacier niches: (a) Annelida (glacier ice worms, *Mesenchytraeus solifugus*) from an Alaskan glacier and known to be abundant in the englacial zone of the maritime glaciers. Polish grosz as a scale. (b) Collembola from a glacier in the Alps and known to be widespread and abundant on ice and snow surfaces, particularly under stones and in weathering crust (Fjellberg, 2010; Zawierucha *et al.*, 2019a).

(Supporting Information Table S2), but benthic ecosystems are not their typical habitat and they have probably been passively dispersed to the holes by water, as observed in the Alps by Zawierucha *et al.*, 2019a (Fig. 8, Supporting Information Figure S4). Springtails have also been reported in weathering crust on glaciers but not in the holes bottom (Fjellberg, 2010).

Although arthropods sequences were detected in environmental DNA, their absence (complete or active specimens) from microscope observations suggests the detected DNA sequences were likely due to wind-blown material rather than active components of communities on Baltoro and Forni glaciers. In samples from Forni and Baltoro glaciers, remains of insects (wings, legs, heads), but not living individuals, suggest insects are primarily allochthonous (confirmed in this study and Zawierucha *et al.*, 2019a). Recently, Dastyh (2019) found along with tardigrades and rotifers, several small fragments of arthropod cuticles, a dead small aphid and a beetle of the genus *Bembidion* in cryoconite holes on the Gihccejiekna Glacier in northern Norway. All of those arthropods were not typical cryoconite hole dwelling invertebrates but accidental faunal elements. Although Edwards *et al.* (2013) reported DNA sequences from many animal taxa from cryoconite holes in the Alps including marine Echinodermata, and tropical Onychophora, without a confirmation with microscopy the actual presence of live individuals which belongs to these groups is seriously doubtful. The disparity in richness of animals between cryoconite holes and other freshwater environments remains poorly understood and is one of the most striking scientific questions in studies on the distribution of invertebrates in the cryosphere.

### Cryoconite holes as suitable habitats for cryophilic invertebrates

In some respects, cryoconite holes are not as extreme as often described (Maccario *et al.*, 2015). Liquid water makes them oases of life in a frozen landscape, supporting abundant and diverse microbial communities and potentially providing

sufficient food resources (Stibal *et al.*, 2010; Cook *et al.*, 2016; Zawierucha *et al.*, 2018a; Buda *et al.*, 2020). Even though cryoconite holes are harsh environments in many respects, they provide an adequate food resources for various invertebrates. Cryoconite holes are considered biodiversity hotspots for cryophilic species of algae, cyanobacteria, heterotrophic bacteria, amoebas, fungi and ciliates (Takeuchi, 2002; Hodson *et al.*, 2008; Cook *et al.*, 2016; Pittino *et al.*, 2018; Sommers *et al.*, 2018, 2019; Zawierucha *et al.*, 2018a; Buda *et al.*, 2020; Stibal *et al.*, 2020). The biodiversity of photoautotrophs includes many opportunistic and glacier specific taxa which may attain the biomass of  $0.998 \mu\text{g dm}^{-3}$  and the photosynthetic rates from  $0.63 \text{ C L}^{-1} \text{ h}^{-1}$  to  $156.99 \mu\text{g C L}^{-1} \text{ h}^{-1}$  (S awstr om *et al.*, 2002; Buda *et al.*, 2020). Metagenomic studies showed that copy number of 16S rRNA gene (a proxy for bacterial concentration) ranges between regions, but is generally very high ( $10^5$ – $10^7$  copies  $\text{g}^{-1}$  on Adishi in the Caucasus,  $10^8$ – $10^9$  copies  $\text{g}^{-1}$  on the Greenland ice sheet (Makowska *et al.*, 2016, Stibal *et al.*, 2015)). Cryoconite holes generate and store organic matter on glaciers (Stibal *et al.*, 2010), for example up to 18% in Greenland (Gerdel & Drouet, 1960), 16% in the Caucasus ( okas *et al.*, 2018), 14% in Svalbard ( okas *et al.*, 2016), 13% in Central Asia (Takeuchi, 2002) and 7% in Maritime Antarctic (Buda *et al.*, 2020). Moreover, apart from autochthonous production, cryoconite holes food webs on a small glacier receive allochthonous wind-blown organic input from fragments of mosses, leaves, insects and others windborne materials (Stibal & Tranter, 2007; Dastyh, 2019; Zawierucha *et al.*, 2019a). Primary production in holes is comparable to other oligotrophic water pools in Arctic regions (S awstr om *et al.*, 2002; Michelutti *et al.*, 2005), and the bacterial number is similar to other freshwater ecosystems (Bomberg *et al.*, 2019). Values of organic matter in cryoconite holes surpass the organic matter content in oligotrophic lakes (*c.* 10% in stony-bottom lakes) that support diverse meiofaunal groups (Peters & Traunspurger, 2005). Tardigrades in cryoconite holes are mostly herbivores and microbivores, while rotifers are filter feeders. Thus,

microbivorous and omnivorous (even carnivorous) nematodes should find adequate resources for growth and reproduction. However, it is possible that specific food sources required by invertebrates such as nematodes are missing from these environments. As cryoconite holes are the most productive and species-rich habitats on glacial surfaces, more factors than only trophic resources must prevent nematodes from occupying this aquatic habitat. More holistic and thorough approaches to studying cryoconite communities are therefore necessary to describe their ecological networks and limiting factors.

### Limiting factors

This study spanned a worldwide collection of glaciers from a broad variety of environmental conditions, from cold-polar, to wet-temperate, to dry, high-altitude glaciers. We therefore suggest that factors other than biogeographical region, climate, latitude and light cycles explain the presence of tardigrades and rotifers and the absence of nematodes in cryoconite holes.

The harsh physical conditions of glacial environments may reduce the faunal diversity in cryoconite holes. Surrounded by ice instead of soil and rock, cryoconite holes remain near freezing even during summer days and freeze solid over winter. For instance, the temperature in cryoconite holes on Arctic glaciers in summer averages 0.08°C with a maximum of 0.22°C, (Zawierucha *et al.*, 2019c). In comparison, shallow polar ponds or lakes may reach 10°C (Sheath, 1986; Schneider *et al.*, 2016) and consequently can be inhabited, not only by tardigrades and rotifers, but by a diversity of freshwater fauna (Coulson *et al.*, 2014; Kolicka *et al.*, 2016). The permanently low temperatures in cryoconite holes may suppress basic life activities, including reproduction, hatching and foraging.

In addition to low temperatures, cryoconite holes are dynamic environments and characterized by periodic freezing, removal of sediments from the bottom, ablation and/or collapse during extreme weather events (e.g. rain and föhn winds) (Mueller *et al.*, 2001; Fountain *et al.*, 2004; Zawierucha *et al.*, 2019c). Consequently, animals can be exposed to intense UV radiation directly on the surface or simply be flushed out into the proglacial field. Most importantly, not even all tardigrades are able to enter anhydrobiosis (Bertolani *et al.*, 2004; Nelson, Bartels, & Guil, 2018), which might be a crucial mechanism to survival on the ice surface during the drying of removed sediment (Zawierucha *et al.*, 2019a). Even though cryoconite tardigrades are able to survive desiccation, their recovery success is lower in comparison with other limno-terrestrial species (e.g. Bertolani *et al.*, 2004; Schill & Hengherr, 2019). Nevertheless, their anhydrobiotic state may favour dispersal (Zawierucha *et al.*, 2019a). Indeed, desiccation enables their passive dispersal by wind (Nkem *et al.*, 2006b) and successful colonization of new habitats on other glaciers. In contrast, limited anhydrobiosis may lead to endemism. For example, Himalayan copepods and chironomids, and New Zealand chironomids, Patagonian Plecoptera (Odell, 1956; Takeuchi *et al.*, 2000; Takeuchi & Kohshima, 2004; Murakami *et al.*, 2018) are restricted to mountain glaciers in relatively small areas. Larger body size and a lack of anhydrobiosis abilities probably limit other microinvertebrates in terms of passive aerial dispersal and

successful establishment on glaciers, specifically in cryoconite holes.

The low concentration of available phosphorous or nitrogen in water to support primary producer populations could furthermore limit the diversity of invertebrates in cryoconite holes. Such a hypothesis related to freshwater ecosystems was previously suggested by Hart & Robinson (1990) and demonstrated in experimental freshwater mesocosms (Urabe, Clasen, & Sterner, 1997; Michiels & Traunspurger, 2005). The negative influence of phosphorous limitation on zooplankton and benthic grazers is well known in freshwater systems (Hart & Robinson, 1990; Urabe *et al.*, 1997). Glaciers are very likely ecosystems where organisms are limited by dissolved phosphorous availability (Holland *et al.*, 2019), which is a crucial biogenic element. This, in turn, may limit the presence of other taxa in cryoconite holes, but to our knowledge nematodes are not more sensitive to the resulting effects on the food web of phosphorous limitation than are other microinvertebrates.

Anthropogenic contaminants might constitute another factor potentially reducing animal diversity, albeit perhaps a minor role (Lorimore *et al.*, 1998; Bezchlebová *et al.*, 2007; Hussain *et al.*, 2009). Worldwide, contamination of supraglacial ecosystems by pollutants includes black carbon, POPs (persistent organic pollutants), radionuclides and heavy metals (Hodson, 2014; Baccolo *et al.*, 2017; Ferrario *et al.*, 2017; Łokas *et al.*, 2018). For example, on Adishi Glacier in the Caucasus, unexpected high concentrations of radionuclides have been detected, and a concomitant absence of animals (except for few collembolans) has been observed (Łokas *et al.*, 2018). A similar observation was made on Morteratsch in the Alps (Baccolo *et al.*, 2017). However, radionuclide contamination has only been investigated for one glacier in the Caucasus and a few in the Alps, suggesting that this topic deserves further investigation. Moreover, in Continental Antarctica, contamination of the cryosphere is low (Khan *et al.*, 2017), but nematodes are absent from cryoconite holes there as well. Thus, contaminants alone cannot explain nematode absence.

### Absence of nematodes

Generally, tardigrades, rotifers and nematodes all enjoy large geographical ranges across a multitude of habitats. The three phyla often coexist in both terrestrial and aquatic (freshwater and marine) ecosystems (Schwarz *et al.*, 1993; Janiec, 1996; Albuquerque *et al.*, 2007). Nematodes are considered one of the most hyperdiverse taxa on Earth and the most abundant multicellular organisms (Hodda, 2007; van den Hoogen *et al.*, 2019). Besides taxonomic diversity, they also present a variety of feeding habits, including algal, bacterial and fungal feeders, omnivores, predators and parasites. Although many species are well adapted to survive desiccation, they are aquatic organisms needing at least a film of water for movement, feeding, growth and reproduction to occur (Wharton, 1986). Consequently, cryoconite holes, due to the presence of liquid water and potential food sources, appear to be suitable habitats for nematodes.

Though nematodes are one of the most resilient invertebrate groups, able to survive under many unfavourable conditions

(Treonis, 2005; Nkem *et al.*, 2006a, 2006b; Hodda, 2007; Wharton & Raymond, 2015), it seems odd that the conditions of cryoconite holes appear unsuitable for their growth. Since nematodes were absent from all the cryoconite holes we investigated, we suspect that evidence of nematodes in cryoconite by Mueller *et al.* (2001), Christner *et al.* (2003) and Sommers *et al.* (2018) may be allochthonous. In support of this interpretation, Nkem *et al.* (2006b) reported mostly dead nematodes in air traps on Antarctic glaciers. Similarly, sediments on the surface of Antarctic glaciers often support abundant populations of bacterial-feeding nematode *Plectus* sp. (D. Porazinska, pers. obs.), but never inside cryoconite holes. Christner *et al.* (2003) used a small subset of samples from Porazinska *et al.* (2004), where a large amount of sediments were processed for counts without detecting a single nematode. Similarly, nematode DNA presence reported by Sommers *et al.* (2018) was not supported by microscope counts from subsequently collected samples from the same glaciers.

Even though nematodes are well adapted to tolerate cold in terrestrial habitats, permanently low temperatures in cryoconite holes may limit their reproduction or limit their ability to compete for food with rotifers and tardigrades. For instance, nematodes survive freezing and desiccation in Antarctic soils through dormancy but reproduce when water and temperature conditions are favourable (Wharton, 2003). The presence of nematodes overlying glaciers in supraglacial debris (Azzoni *et al.*, 2015) and in glacier mice (Coulson & Midgley, 2012) indicates the presence of these favourable conditions in these habitats. Overhoff, Freckman & Virginia (1993) found that temperatures of 10°C were required for growth of *Scottinema lindsayae*, the most dominant Antarctic nematodes adapted to cold soils – a temperature unlikely achieved in ice-bound cryoconite holes. This hypothesis is corroborated by previous observations of Zawierucha *et al.* (2019a) who did not find nematodes in cryoconite holes on Forni Glacier, but did find them in sediments from a proglacial lake. Similarly high Arctic tundra tardigrades (different than Arctic cryoconite hole species (K. Zawierucha *et al.*, under review)) reproduce faster in higher temperatures (D. Stec pers. comm.). It could be that – as with the North American ice worm, an obligate glacier-dwelling organism that can not survive below about –7°C (Dial *et al.*, 2016) – the flexible cuticle of a vermiform morphology cannot survive the cold damage of being immersed in nearly pure frozen water, something that tardigrades and rotifers in cryoconite holes can withstand. This hypothesis would seem amenable to experimentation.

As nematodes are among the most numerous benthic animals in oligotrophic lakes (Ristau & Traunspurger, 2011; Majdi & Traunspurger, 2015), low levels of nutrients do not appear to determine their presence/absence. To explain the absence of nematodes from the otherwise apparently suitable habitat offered by cryoconite holes, we hypothesize the existence of some other biological or ecological barriers. Cyanobacteria are reported to be inhibitory towards some nematode species: for example, in permanent cyanobacteria mats or blooms in freshwater ecosystems (Muñoz *et al.*, 2006; Nascimento *et al.*, 2009). However, this hypothesis fails for some environments, as demonstrated by Jungblut, Vincent &

Lovejoy (2012) who showed that nematodes are found in Arctic and Antarctic cyanobacterial mats (see also Supporting Information Figure S2). Another barrier may be rapid changes in oxygen. While cryoconite holes are considered ultraoligotrophic environments, rapid development of anoxic zones in the sediment has been observed by Poniecka *et al.* (2018). Quick shifts from oxic to anoxic conditions may be a potential barrier for survival and reproduction, enabling the existence of only some taxa that can respond equally rapidly. Finally, microbial assemblages in cryoconite holes are distinct from those in soil or benthic environments (e.g. Franzetti *et al.*, 2017), such that crucial components of the nematode diet are missing. Thus, competition and absence of necessary resources may prevent nematode occupancy of cryoconite holes. Undoubtedly, further studies will be necessary to confirm the absence of nematodes from cryoconite holes and other englacial and subglacial habitats characterized by temperatures that are almost constantly near-freezing. Subglacial ecosystems, considered for years by the scientific community to be sterile, have now gained scientific attention by biologists and bio-geochemists, with many studies currently addressing their diversity and productivity (Yde *et al.*, 2011; Achberger *et al.*, 2017; Dubnick *et al.*, 2017; Zdanowski *et al.*, 2017). We cannot exclude the hypothesis that some specialized nematodes inhabit both subglacial ecosystems and englacial channels thermally similar to cryoconite holes. For example, Northern American glaciers host oligochaetes inhabiting the englacial zone (Dial *et al.*, 2012; Dial *et al.*, 2016). Similarly, deeper layers of weathering crust are inhabited by rotifers on Icelandic glaciers (Shain *et al.*, 2016) and also nematodes on New Zealand (D. Shain pers. obs.).

## Conclusion

The microinvertebrate communities of cryoconite holes worldwide appear to be dominated by Tardigrada and Rotifera. In some locations, tardigrades dominated (e.g. Gulkana on Alaska, Forni in the Alps); in others, rotifers (e.g. Ecology in maritime Antarctic, Blåisen in Norway). We found, however, that tardigrades and rotifers mostly co-dominated in communities. In contrast, nematodes were absent in material originated from cryoconite holes from 42 glaciers. This absence may be related to a combination of factors including consistently low temperatures, periodic freezing, UV irradiation, nutrient limitation, low solute content, high levels of contamination in cryoconite worldwide (often higher than in other benthic ecosystems) and competition for food. The most likely explanations include the effects of permanently low temperatures in cryoconite holes, which may limit nematode reproduction, or their ability to compete for specific food with glacial rotifers and tardigrades. This observation lays the groundwork for comparisons that can shed light on the limits to multicellular life in the cryosphere.

## Acknowledgements

The authors would like to thank two anonymous reviewers for their valuable comments and corrections. Studies on Arctic glaciers were supported by the National Science Centre under

Grant No. NCN 2013/11/N/NZ8/00597 awarded to K.Z. Samples from the Blåisen and Midtdalsbreen glaciers were collected within the Grant [ETANGO] awarded by INTERACT Transnational Access to K.Z. GFF was funded by the European Research Council under the European Community's Horizon 2020 Programme, Grant Agreement no. 772284 (IceCommunities). NT was supported by the funds from JSPS KAKENHI (19H01143 and 20K21840), and from the Arctic Challenge for Sustainability II (ArCS II), Program Grant Number JPMXD1420318865. Work on glaciers from the McMurdo Dry Valleys of Antarctica was supported by the National Science Foundation Award #1443578 to S.K.S. Fieldwork on Marr Ice Piedmont was realized according to kindly assistance of National scientific foundation (USA) and Palmer station crew. All of the samples were properly collected under the permission of local organizations or governments. The samples from the Ecology Glacier used in the paper were collected and transported based on the logistic support of Henryk Arctowski Polish Antarctic Station. The sampling on Ecology Glacier were initiated by the seminar, 'The role of cryoconite in the functioning of glacial systems' supported by the Centre for Polar Studies (the Leading National Research Centre in Earth Sciences for 2014–2018 No. 03/KNOW2/2014). K.Z. is grateful to Ela Wiejaczka for her support in gaining permission and access to samples from Africa, Joseph Cook for collection of samples in the dark zone of Greenland Ice Sheet, E.L. thanks Łukasz Pawłowski for collection of samples from Werenskiöld Glacier, and T.J. thanks Matěj Pokorný (Department of Ecology, Charles University) for collection of samples from Blancmange Glacier. Swedish samples were kindly provided to E.P. by Alexandre Anesio.

### Data availability statement

The data that support the findings of this study are openly available in Supplementary material and under NCBI SRA database under project PRJNA480849.

### References

- Achberger, A.M., Michaud, A.B., Vick-Majors, T.J., Christner, B.C., Skidmore, M.L., Priscu, J.C. & Tranter, M. (2017). Microbiology of subglacial environments. In *Psychrophiles: from biodiversity to biotechnology*: 83–110. Margesin, R. (Ed.). New York: Springer.
- Albuquerque, E.F., Pinto, A.P.B., Perez, A.Q. & Veloso, V.G. (2007). Spatial and temporal changes in interstitial meiofauna on a sandy ocean beach of South America. *Braz. J. Oceanogr.* **55**, 121–131.
- Altiero, T. & Rebecchi, L. (2001). Rearing tardigrades: results and problems. *Zool. Anz.* **240**, 217–221.
- Altiero, T., Guidetti, R., Caselli, V., Cesari, M. & Rebecchi, L. (2011). Ultraviolet radiation tolerance in hydrated and desiccated eutardigrades. *J. Zool. Syst. Evol. Res.* **49**, 104–110.
- Amaral-Zettler, L.A., McCliment, E.A., Ducklow, H.W. & Huse, S.M. (2009). A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLoS One* **4**, e6372.
- Andriuzzi, W.S., Adams, B.J., Barrett, J.E., Virginia, R.A. & Wall, D.H. (2018). Observed trends of soil fauna in the Antarctic Dry Valleys: early signs of shifts predicted under climate change. *Ecology* **99**, 312–321.
- Aristotle. *350 B.C.E. Historia Animālium. Book VIII, part 2.* Athens. Translated by D'Arcy Wentworth Thompson.
- Aubert, J. (1956). *Andiperla willinki* n. sp. plécoptère nouveau des Andes de Patagonie. *Bull. Soc. Entomol. Suisse* **29**, 229–232.
- Azzoni, R.S., Franzetti, A., Fontaneto, D., Zullini, A. & Ambrosini, R. (2015). Nematodes and rotifers on two Alpine debris-covered glaciers. *Ital. J. Zool.* **82**, 616–623.
- Baccolo, G., Di Mauro, B., Massabò, D., Clemenza, M., Nastasi, M. & Delmonte, B., Prata, M., Prati, P., Previtali, E. & Maggi, V. (2017). Cryoconite as a temporary sink for anthropogenic species stored in glaciers. *Sci. Rep.* **7**, 9623.
- Bertolani, R., Guidetti, R., Jönsson, I.K., Altiero, T., Boschini, D. & Rebecchi, L. (2004). Experiences with dormancy in tardigrades. *J. Lim.* **63**, 16.
- Bezchlebová, J., Černohlávková, J., Lána, J., Sochová, I., Kobetičová, K. & Hofman, J. (2007). Effects of toxaphene on soil organisms. *Ecotoxicol. Environ. Saf.* **68**, 326–334.
- Bomberg, M., Claesson Liljedahl, L., Lamminmäki, T. & Kontula, A. (2019). Highly diverse aquatic microbial communities separated by permafrost in Greenland show distinct features according to environmental niches. *Front. Microbiol.* **10**, 1583. <https://doi.org/10.3389/fmicb.2019.01583>
- Boothroyd, I. & Cranston, P. (1999). The 'Ice Worm' - the immature stages, phylogeny and biology of the glacier midge *Zelandochlus* (Diptera: Chironomidae). *Aquat. Insects* **21**, 303–316.
- Borgonie, G., García-Moyano, A., Litthauer, D., Bert, W., Bester, A., van Heerden, E., Möller, C., Erasmus, M. & Onstott, T.C. (2011). Nematoda from the terrestrial deep subsurface of South Africa. *Nature* **474**, 79–82.
- Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P. & Coissac, E. (2016). Obitools: a unix-inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.* **16**, 176–182.
- Broadly, P.A. (1989). Survey of algae and other terrestrial biota at Edward VII Peninsula, Marie Byrd Land. *Antarct. Sci.* **1**, 215–224.
- Buda, J., Łokas, Ł., Pietryka, M., Richter, D., Magowski, W., Iakovenko, N.S., Porazinska, D.L., Budzik, T., Grabiec, M., Grzesiak, J., Klimaszuk, P., Gaca, P. & Zawierucha, K. (2020). Biotope and biocenosis of cryoconite hole ecosystems on Ecology Glacier in the maritime Antarctic. *Sci. Total Environ.* **724**, 138112.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tu, P.J. & Knight, R. (2010). QIIME allows

- analysis of high-throughput community sequencing data. *Nat. Methods* **7**, 335–336.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Bauer, M. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* **6**, 1621–1624.
- Cesari, M., McInnes, S.J., Bertolani, R., Rebecchi, L. & Guidetti, R. (2016). Genetic diversity and biogeography of the south polar water bear *Acutuncus antarcticus* (Eutardigrada: Hypsibiidae) – evidence that it is a truly pan-Antarctic species. *Invertebr. Syst.* **30**, 635–649.
- Christner, B.C., Kvitko, B.H. & Reeve, J.N. (2003). Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. *Extremophiles* **7**, 177–183.
- Cook, J., Edwards, A., Takeuchi, N. & Irvine-Fynn, T. (2016). Cryoconite: The dark biological secret of the cryosphere. *Prog. Phys. Geog.* **40**, 66–111.
- Coulson, S.J. & Midgley, N.G. (2012). The role of glacier mice in the invertebrate colonisation of glacial surfaces: the moss balls of the Falljökull, Iceland. *Pol. Biol.* **35**, 1651–1658.
- Coulson, S.J., Convey, P., Aakra, K., Aarvik, L., Avila-Jimenez, M.L., Babenko, A., Biersma, E.M., Boström, S., Brittain, J.E., Carlsson, A.M., Christoffersen, K., De Smet, W.H., Ekrem, T., Fjellberg, A., Füreder, L., Gustafsson, D., Gwiazdowicz, D.J., Hansen, L.O., Holmstrup, M., Hullé, M., Kaczmarek, Ł., Kolicka, M., Kuklin, V., Lakka, H.-K., Lebedeva, N., Makarova, O., Maraldo, K., Melekhina, E., Ødegaard, F., Pilskog, H.E., Simon, J.C., Sohlenius, B., Solhøy, T., Sjøli, G., Stur, E., Tanasevitch, A., Taskaeva, A., Velle, G., Zawierucha, K. & Zmudczyńska-Skarbek, K. (2014). The terrestrial and freshwater invertebrate biodiversity of the archipelagoes of the Barents Sea; Svalbard, Franz Josef Land and Novaya Zemlya. *Soil Biol. Biochem.* **68**, 440–470.
- Crowe, J.I. & Madin, K.A. (1974). Anhydrobiosis in tardigrades and nematodes. *Trans. Am. Microsc. Soc.* **93**, 513–524.
- Czechowski, P., White, D., Clarke, L., McKay, A., Cooper, A. & Stevens, M.I. (2016). Age-related environmental gradients influence invertebrate distribution in the Prince Charles Mountains, East Antarctica. *R. Soc. Open Sci.* **3**, 160296.
- Dabert, M., Dastych, H. & Dabert, J. (2015). Molecular data support the dispersal ability of the glacier tardigrade *Hypsibius klebelsbergi* Mihelcic, 1959 across the environmental barrier (Tardigrada). *Mitt. Hamb. Zool. Mus. Inst.* **17**, 233–240.
- Darcy, J.L., Gendron, E.M.S., Sommers, P., Porazinska, D.L. & Schmidt, S.K. (2018). Island biogeography of cryoconite hole bacteria in Antarctica's Taylor valley and around the world. *Front. Ecol. Evol.* **6**, 180. <https://doi.org/10.3389/fevo.2018.00180>
- Darwin, C.R. (1859). *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. London: John Murray.
- Dastych, H. (1985). West Spitsbergen Tardigrada. *Acta Zool. Crac.* **28**, 169–214.
- Dastych, H. (1993). Redescription of the cryoconital tardigrade *Hypsibius klebelsbergi* Mihelcic, 1959, with notes on the microslide collection of the late Dr. F. Michelcic (Tardigrada). *Veröff. Mus. Ferdinandeum* **73**, 5–12.
- Dastych, H. (2004). *Hypsibius thaleri* sp. nov., a new species of a glacier-dwelling tardigrade from the Himalayas, Nepal (Tardigrada). *Mitt. Hamb. Zool. Mus. Inst.* **101**, 169–183.
- Dastych, H. (2019). *Cryobiotus roswithae* gen. n., sp. n., a new genus and species of glacier-dwelling tardigrades from Northern Norway (Tardigrada, Panarthropoda). *Entomol. Heute* **31**, 95–111.
- Dastych, H., Kraus, H.J. & Thaler, K. (2003). Redescription and notes on the biology of the glacier tardigrade *Hypsibius klebelsbergi* Mihelcic, 1959 (Tardigrada), based on material from Ötztal Alps. *Austria. Mitt. Hamb. Zool. Mus. Inst.* **100**, 73–100.
- De Smet, W.H. & Van Rompu, E.A. (1994). Rotifera and Tardigrada from some cryoconite holes on a Spitsbergen (Svalbard) glacier. *Belg. J. Zool.* **124**, 27–37.
- Dial, C.R., Dial, R.J., Saunders, R., Lang, S.A., Lee, B., Wimberger, P., Dinapoli, M.S., Egiazarov, A.S., Gipple, S.L., Maghirang, M.R., Swartley-McArdle, D.J., Yudkovitz, S.R. & Shain, D.H. (2012). Historical biogeography of the North American glacier ice worm, *Mesenchytraeus solifugus* (Annelida: Oligochaeta: Enchytraeidae). *Mol. Phylo. Evol.* **63**, 577–584.
- Dial, R.J., Becker, M., Hope, A.G., Dial, C.R., Thomas, J., Slobodenko, K.A., Golden, T.S., Shain, D.H. (2016). The role of temperature in the distribution of the glacier ice worm, *Mesenchytraeus solifugus* (Annelida: Oligochaeta: Enchytraeidae). *Arct. Antarct. Alp. Res.* **48**, 199–211.
- Drygalski, E.V. (1897). Die Kryoconitlcher. In *Grönland-Expedition der Gesellschaft für Erdkunde zu Berlin 1891–1893*: 93–103. Kuhl, W.H. (Ed.). Berlin: Heerausgegeben von dek.
- Dubnick, A., Kazemi, S., Sharp, M., Wadham, J., Hawkings, J., Beaton, A. & Lanoil, B. (2017). Hydrological controls on glacially exported microbial assemblages: subglacial hydrology and microbiology. *J. Geophys. Res. Biogeosci.* **122**, 1049–1061.
- Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460–2461.
- Edwards, A., Pachebat, J.A., Swain, M., Hegarty, M., Hodson, A.J., Irvine-Fynn, T.D.L., Rassner, S.M.E. & Sattler, B. (2013). A metagenomic snapshot of taxonomic and functional diversity in an alpine glacier cryoconite ecosystem. *Environ. Res. Lett.* **8**, 1–11.
- Faurby, S., Jørgensen, A., Kristensen, R.M. & Funch, P. (2012). Distribution and speciation in marine intertidal tardigrades. *J. Biogeo.* **39**, 1596–1607.
- Ferrario, C., Pittino, F., Tagliaferri, I., Gandolfi, I., Bestetti, G., Azzoni, R.S., Diolaiuti, G., Franzetti, A., Ambrosini, R. & Villa, S. (2017). Bacteria contribute to pesticide degradation in cryoconite holes in an Alpine glacier. *Environ. Pollut.* **230**, 919–926.
- Fjellberg, A. (2010). Cryophilic Isotomidae (Collembola) of the Northwestern Rocky Mountains, U.S.A. *Zootaxa* **2513**, 27–49.
- Fontaneto, D. (2019). Long-distance passive dispersal in microscopic aquatic animals. *Mov. Ecol.* **7**, 10. <https://doi.org/10.1186/s40462-019-0155-7>

- Forró, L., Korovchinsky, N.M., Kotov, A.A. & Petrusek, A. (2008). Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. *Hydrobiologia* **595**, 177–184.
- Fountain, A.G., Tranter, M., Nysten, T.H., Lewis, K.J. & Mueller, D.R. (2004). Evolution of cryoconite holes and their contribution to meltwater runoff from glaciers in the McMurdo Dry Valleys, Antarctica. *J. Glaciol.* **50**, 35–45.
- Franzetti, A., Navarra, F., Tagliaferri, I., Gandolfi, I., Bestetti, G., Minora, U., Azzoni, R.S., Diolaiuti, G., Smiraglia, C. & Ambrosini, R. (2017). Potential sources of bacteria colonizing the cryoconite of an Alpine glacier. *PLoS One* **12**, e0174786.
- Gerdel, R.W. & Drouet, F. (1960). The cryoconite of the Thule area, Greenland. *Trans. Am. Microsc. Soc.* **79**, 256–272.
- Goodman, D. (1971). *Ecological investigations of ice worms on Casement Glacier, Southeastern Alaska (No. 39)*. Columbus: Ohio State University Research Foundation.
- Grøngaard, A., Pugh, P.J. & McInnes, S.J. (1999). Tardigrades, and other cryoconite biota, on the Greenland ice sheet. *Zool. Anz.* **238**, 211–214.
- Grzelak, K., Gluchowska, M., Gregorczyk, K., Winogradow, A. & Weslawski, J.M. (2016). Nematode biomass and morphometric attributes as biological indicators of local environmental conditions in Arctic fjords. *Ecol. Ind.* **69**, 368–380.
- Guardiola, M., Uriz, M.J., Taberlet, P., Coissac, E., Wangenstein, O.S. & Turon, X. (2015). Deep-Sea, deep-sequencing: metabarcoding extracellular DNA from sediments of marine canyons. *PLoS One* **10**, e0139633.
- Guidetti, R., Rizzo, A.M., Altiero, T. & Rebecchi, L. (2012). What can we learn from the toughest animals of the Earth? Water bears (tardigrades) as multicellular model organisms in order to perform scientific preparations for lunar exploration. *Planet. Space Sci.* **74**, 97–102.
- Guil, N., Sánchez-Moreno, S. & Machordom, A. (2009). Local biodiversity patterns in micrometazoans: Are tardigrades everywhere? *Syst. Biodivers.* **7**, 259–268.
- Hart, D.D. & Robinson, C.T. (1990). Resource limitation in a stream community: phosphorus enrichment effects on periphyton and grazers. *Ecology* **71**, 1494–1502.
- Hinden, H., Oertli, B., Menetrey, N., Sager, L. & Lachavanne, J.B. (2005). Alpine pond biodiversity: what are the related environmental variables? *Aquat. Conserv.* **15**, 613–624.
- Hodda, M. (2007). Phylum Nematoda. *Zootaxa* **1668**, 265–293. <https://doi.org/10.11646/zootaxa.1668.1.14>
- Hodson, A.J. (2014). Understanding the dynamics of black carbon and associated contaminants in glacial systems. *Wiley Interdiscip. Rev.* **1**, 141–149.
- Hodson, A.J., Anesio, A.M., Tranter, M., Fountain, A., Osborn, M., Prisco, J., Laybourn-Parry, J. & Sattler, B. (2008). Glacial ecosystems. *Ecol. Monogr.* **78**, 41–67.
- Holland, A.T., Williamson, C.J., Sgouridis, F., Tedstone, A.J., McCutcheon, J., Cook, J.M., Poniecka, E., Yallop, M.L., Tranter, M., Anesio, A.M.; The Black & Bloom Group (2019). Nutrient cycling in supraglacial environments of the Dark Zone of the Greenland Ice Sheet. *Biogeoscience* **16**, 3283–3296.
- van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D.A., de Goede, R.G.M., Adams, B.J., Ahmad, W., Andriuzzi, W.S., Bardgett, R.D., Bonkowski, M., Campos-Herrera, R., Cares, J.E., Caruso, T., de Brito Caixeta, L., Chen, X., Costa, S.R., Creamer, R., Mauro da Cunha Castro, J., Dam, M., Djigal, D., Escuer, M., Griffiths, B.S., Gutiérrez, C., Hohberg, K., Kalinkina, D., Kardol, P., Kergunteuil, A., Korthals, G., Krashevskaya, V., Kudrin, A.A., Li, Q., Liang, W., Magilton, M., Marais, M., Martín, J.A.R., Matveeva, E., Mayad, E.H., Mulder, C., Mullin, P., Neilson, R., Nguyen, T.A.D., Nielsen, U.N., Okada, H., Rius, J.E.P., Pan, K., Peneva, V., Pellissier, L., Carlos Pereira da Silva, J., Pitteloud, C., Powers, T.O., Powers, K., Quist, C.W., Rasmann, S., Moreno, S.S., Scheu, S., Setälä, H., Sushchuk, A., Tiunov, A.V., Trap, J., van der Putten, W., Vestergård, M., Villenave, C., Waeyenberge, L., Wall, D.H., Wilschut, R., Wright, D.G., Yang, J.-I. & Crowther, T.W. (2019). Soil nematode abundance and functional group composition at a global scale. *Nature* **572**, 194–198.
- Hussain, S., Boland, S., Baeza-Squiban, A., Hamel, R., Thomassen, L.C., Martens, J.A., Billon-Galland, M.A., Fleury-Feith, J., Moisan, F., Pairon, J.-C. & Marano, F. (2009). Oxidative stress and proinflammatory effects of carbon black and titanium dioxide nanoparticles: role of particle surface area and internalized amount. *Toxicology* **260**, 142–149.
- Iakovenko, N.S., Smykla, J., Convey, P., Kašparová, E., Kozereetska, I.A., Trokhymets, V., Dykyy, I., Plewka, M., Devetter, M., Duriš, Z. & Janko, K. (2015). Antarctic bdelloid rotifers: diversity, endemism and evolution. *Hydrobiologia* **761**, 5–43.
- Janiec, K. (1996). The comparison of freshwater invertebrates of Spitsbergen (Arctic) and King George Island (Antarctic). *Pol. Polar Res.* **17**, 173–202.
- Jönsson, K.I. & Wojcik, A. (2017). Tolerance to X-rays and Heavy Ions (Fe, He) in the Tardigrade *Richtersius coronifer* and the Bdelloid Rotifer *Mniobia russeola*. *Astrobiology* **17**, 163–167.
- Jørgensen, A., Møbjerg, N. & Kristensen, R.M. (2007). A molecular study of the tardigrade *Echiniscus testudo* (Echiniscidae) reveals low DNA sequence diversity over a large geographical area. *J. Limnol.* **66**, 77.
- Jungblut, A.D., Vincent, W.F. & Lovejoy, C. (2012). Eukaryotes in Arctic and Antarctic cyanobacterial mats. *FEMS Microbiol. Ecol.* **82**, 416–428.
- Kaczmarek, L., Michalczyk, L. & McInnes, S.J. (2016). Annotated zoogeography of non-marine Tardigrada. Part III: North America and Greenland. *Zootaxa* **4203**, 1–249.
- Khan, A.L., Wagner, S., Jaffe, R., Xian, P., Williams, M., Armstrong, R. & McKnight, D. (2017). Dissolved black carbon in the global cryosphere: Concentrations and chemical signatures. *Geophys. Res. Lett.* **44**, 6226–6234.
- Kikuchi, Y. (1994). *Glaciella*, a new genus of freshwater Canthocamptidae (Copepoda, Harpacticoida) from a glacier in Nepal, Himalayas. *Hydrobiologia* **292**, 59–66.



- Kohshima, S. (1984). A novel cold tolerant insect found in a Himalayan glacier. *Nature* **310**, 225–227.
- Kohshima, S. (1985). Patagonian glaciers as insect habitats. In *Glaciological studies in Patagonia Northern Icefield*: 94–99. Nokajima, C. (Ed.). Kyoto: Data Center for Glacier Research Japanese Society of Snow and Ice.
- Kolicka, M., Dabert, M., Dabert, J., Kånneby, T. & Kisielewski, J. (2016). *Bifidochaetus*, a new Arctic genus of freshwater Chaetodontida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebr. Syst.* **30**, 398–419.
- Kristensen, R.M. (2002). An Introduction to Loricifera, Cycliophora, and Micrognathozoa. *Int. Compar. Biol.* **42**, 641–651.
- Liu, Y., Vick-Majors, T.J., Priscu, J.C., Yao, T., Kang, S., Liu, K., Cong, Z., Xiong, J. & Li, Y. (2017). Biogeography of cryoconite bacterial communities on glaciers of the Tibetan Plateau. *FEMS Microbiol. Ecol.* **93**, fix072.
- Lokas, E., Zaborska, A., Kolicka, M., Różycki, M. & Zawierucha, K. (2016). Accumulation of atmospheric radionuclides and heavy metals in cryoconite holes on an Arctic glacier. *Chemosphere* **160**, 162–172.
- Lokas, E., Zawierucha, K., Cwanek, A., Szufa, K., Gaca, P., Mietelski, J.W. & Tomankiewicz, E. (2018). The sources of high airborne radioactivity in cryoconite holes from the Caucasus (Georgia). *Sci. Rep.* **8**, 10802.
- Lorimore, S.A., Kadhim, M.A., Pocock, D.A., Papworth, D., Stevens, D.L., Goodhead, D.T. & Wright, E.G. (1998). Chromosomal instability in the descendants of unirradiated surviving cells after  $\alpha$ -particle irradiation. *Proc. Natl. Acad. Sci. USA* **95**, 5730–5733.
- Lutz, S., Ziolkowski, L.A. & Benning, L.G. (2019). The biodiversity and geochemistry of cryoconite holes in Queen Maud Land, East Antarctica. *Microorganisms* **7**, 160.
- MacArthur, R.H. & Wilson, E.O. (1967). *The theory of island biogeography*: 224. Princeton: Princeton University Press.
- Maccario, L., Sanguino, L., Vogel, T.M. & Larose, C. (2015). Snow and ice ecosystems: not so extreme. *Res. Microbiol.* **166**, 782–795.
- Majdi, N. & Traunspurger, W. (2015). Free-living nematodes in the freshwater food web: a review. *J. Nemat.* **47**, 28–44.
- Makowska, N., Zawierucha, K., Mokracka, J. & Koczura, R. (2016). First report of microorganisms of Caucasus glaciers (Georgia). *Biologia* **71**, 620–625.
- Michelutti, N., Wolfe, A.P., Vinebrooke, R.D., Rivard, B. & Briner, J.P. (2005). Recent primary production increases in arctic lakes. *Geophys. Res. Lett.* **32**, L19715. <https://doi.org/10.1029/2005GL023693>
- Michiels, I.C. & Traunspurger, W. (2005). Impact of resource availability on species composition and diversity in freshwater nematodes. *Oecologia* **142**, 98–103.
- Mihelčič, F. (1959). Zwei neue Tardigraden aus der Gattung *Hypsibius* Thulin aus Osttirol (Österreich). Systematisches zur Gattung *Hypsibius* Thulin. *Zool. Anz.* **163**, 254–261.
- Monge-Najera, J. (1995). Phylogeny, biogeography and reproductive trends in the Onychophora. *Zool. J. Linn. Soc.* **114**, 21–60.
- Mueller, D.R., Vincent, W.F., Pollard, W.H. & Fristen, C.H. (2001). Glacial cryoconite ecosystems: a bipolar comparison of algal communities and habitats. *Nov. Hedw. Beih.* **123**, 173–197.
- Muñoz, I., Gaudes, A., Sabater, S. & Vilalta, E. (2006). The nematode community in cyanobacterial biofilms in the river Llobregat, Spain. *Nematology* **8**, 909–919.
- Murakami, T., Segawa, T., Takeuchi, N., Barcaza Sepúlveda, G., Labarca, P., Kohshima, S. & Hongoh, Y. (2018). Metagenomic analyses highlight the symbiotic association between the glacier stonefly *Andiperla willinki* and its bacterial gut community. *Environ. Microbiol.* **20**, 4170–4183.
- Nascimento, F.J.A., Karlson, A.M.L., Näslund, J. & Gorokhova, E. (2009). Settling cyanobacterial blooms do not improve growth conditions for soft bottom meiofauna. *J. Exp. Mar. Biol. Ecol.* **368**, 138–146.
- Nelson, D.R., Bartels, P.J. & Guil, N. (2018). Tardigrade ecology. In *Water bears: the biology of tardigrades*, Vol. 2: 163–210. Schill, R.O. (Ed.). Berlin: Zoological Monographs Springer.
- Nkem, J.N., Virginia, R.A., Barrett, J.E., Wall, D.H. & Li, G. (2006a). Salt tolerance and survival thresholds for two species of Antarctic soil nematodes. *Pol. Biol.* **29**, 643–651.
- Nkem, J.N., Wall, D.H., Virginia, R.A., Barrett, J.E., Broos, E.J., Porazinska, D.L. & Adams, B.J. (2006b). Wind dispersal of soil invertebrates in the McMurdo Dry Valleys, Antarctica. *Pol. Biol.* **29**, 346–352.
- Odell, N. (1956). So-called ‘Glacier-Worms’ in New Zealand. *Nature* **177**, 534.
- Overhoff, A., Freckman, D.W. & Virginia, R.A. (1993). Life cycle of the microbivorous Antarctic Dry Valley *Scottmema lindsayae* (Timm 1971). *Pol. Biol.* **13**, 151–156.
- Pansu, J., Giguet-Covex, C., Ficetola, G.F., Gielly, L., Boyer, F., Zinger, L., Arnaud, F., Poulenard, J., Taberlet, P. & Choler, P. (2015). Reconstructing long-term human impacts on plant communities: an ecological approach based on lake sediment DNA. *Mol. Ecol.* **24**, 1485–1498.
- Parducci, L., Bennet, K.D., Ficetola, G.F., Alsos, I.G., Suyama, Y., Wood, J.R. & Pedersen, M.W. (2017). Ancient plant DNA in lake sediments. *New Phytol.* **214**, 924–942.
- Peters, L. & Traunspurger, W. (2005). Species distribution of free-living nematodes and other meiofauna in littoral periphyton communities of lakes. *Nematology* **7**, 267–280.
- Pittino, F., Maglio, M., Gandolfi, I., Azzoni, R.S., Diolaiuti, G., Ambrosini, R. & Franzetti, A. (2018). Bacterial communities of cryoconite holes of a temperate alpine glacier show both seasonal trends and year-to-year variability. *Ann. Glaciol.* **59**, 1–9.
- Poniecka, E.A., Bagshaw, E.A., Tranter, M., Sass, H., Williamson, C.J., Anesio, A.M.; Black and Bloom Team (2018). Rapid development of anoxic niches in supraglacial ecosystems. *Arct. Antarct. Alp. Res.* **50**, S100015.
- Porazinska, D.L., Fountain, A.G., Nysten, T.H., Tranter, M., Virginia, R.A. & Wall, D.H. (2004). The biodiversity and biogeochemistry of cryoconite holes from McMurdo Dry Valley glaciers, Antarctica. *Arct. Antarct. Alp. Res.* **36**, 84–91.
- Porazinska, D.L., Farrer, E.C., Spasojevic, M.J., Bueno de Mesquita, C.P., Sartwell, S.A., Smith, J.G., Schmidt, S.K.

- (2018). Plant diversity and density predict belowground diversity and function in an early successional alpine ecosystem. *Ecology* **99**, 1942–1952.
- Patscheck, C., Gansfort, B. & Traunspurger, W. (2018). The extent of wind-mediated dispersal of small metazoans, focusing nematodes. *Sci. Rep.* **8**, 6814.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucl. Acids. Res.* **41**, D590–D596.
- Ramazotti, G. (1968). *Tardigradi dei pozzetti glaciali di fusione (Kryokonitlöcher) dell'Himalaya*, Vol. 3: 1–3. Khumbu Himal: Innsbrück, München: Universitätsverlag, Wagner.
- Ramazotti, G. & Maucci, W. (1983). II Philum Tardigrada (III. edizione riveduta e aggiornata). *Mem. Ist. Ital. Idrobiol.* **41**, 1–1016.
- Ristau, K. & Traunspurger, W. (2011). Relation between nematode communities and trophic state in southern Swedish lakes. *Hydrobiologia* **663**, 121–133.
- Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**, e2584.
- Sävström, C., Mumford, P., Marshall, W., Hodson, A. & Laybourn-Parry, J. (2002). The microbial communities and primary productivity of cryoconite holes in an Arctic glacier (Svalbard 79° N). *Polar Biol.* **25**, 591–596.
- Schill, R.O. & Hengherr, S. (2019). Environmental adaptations: desiccation tolerance. In *Water bears: the biology of tardigrades*: 273–293. Schill, R.O. (Ed.). Berlin: Springer.
- Schneider, A., Wetterich, S., Schirmeister, L., Herzsuh, U., Meyer, H. & Pstryakova, L.A. (2016). Freshwater ostracods (Crustacea) and environmental variability of polygon ponds in the tundra of the Indigirka Lowland, north-east Siberia. *Pol. Res.* **35**, 25225.
- Schwarz, A.M.J., Green, J.D., Green, T.G.A. & Seppelt, R.D. (1993). Invertebrates associated with moss communities at Canada Glacier, southern Victoria Land, Antarctica. *Pol. Biol.* **13**, 157–162.
- Segawa, T., Yonezawa, T., Edwards, A., Akiyoshi, A., Tanaka, S., Uetake, J., Irvine-Fynn, T., Fukui, K., Li, Z. & Takeuchi, N. (2017). Biogeography of cryoconite forming cyanobacteria on polar and Asian glaciers. *J. Biogeogr.* **44**, 2849–2861.
- Segawa, T., Matsuzaki, R., Takeuchi, N., Akiyoshi, A., Navarro, F., Sugiyama, S., Yonezawa, T. & Mori, H. (2018). Bipolar dispersal of red-snow algae. *Nat. Comm.* **9**, 3094. <https://doi.org/10.1038/s41467-018-05521-w>
- Segers, H. (2008). Global diversity of rotifers in freshwater. *Hydrobiologia* **595**, 49–59.
- Shain, D.H., Halldórsdóttir, K., Pálsson, F., Aðalgeirsdóttir, G., Gunnarsson, A., Jónsson, P., Lang, S.A., Pálsson, H.S., Steinórsson, S. & Arnason, E. (2016). Colonization of maritime glacier ice by bdelloid Rotifera. *Mol. Phylo. Evol.* **98**, 280–287.
- Shaw, E.A., Adams, B.J., Barrett, J.E., Lyons, W.B., Virginia, R.A. & Wall, D.H. (2018). Stable isotopes reveal soil food web structure and the nematode, *Eudorylaimus antarcticus*, as an omnivore-predator in Taylor Valley, Antarctica. *Pol. Biol.* **41**, 1013–1018.
- Sheath, R.G. (1986). Seasonality of phytoplankton in northern tundra ponds. *Hydrobiologia* **138**, 75–83.
- Sohlenius, B. (1977). Numbers, biomass and respiration of Nematoda, Rotatoria and Tardigrada in a 120-year-old Scots pine forest at Ivantjärnsheden, Central Sweden. Technical Report - Swedish Coniferous Forest Project (Sweden) no. 9, ISSN : 0346–7708.
- Sohlenius, B. (1979). A carbon budget for nematodes, rotifers and tardigrades in a Swedish coniferous forest soil. *Holarct. Ecol.* **2**, 30–40.
- Sohlenius, B., Boström, S. & Jönsson, I. (2004). Occurrence of nematodes, tardigrades and rotifers on ice-free areas in East Antarctica. *Pedobiologia* **48**, 395–408.
- Sømme, L. (1996). Anhydrobiosis and cold tolerance in tardigrades. *Eur. J. Entomol.* **93**, 349–357.
- Sommers, P., Darcy, J.L., Gendron, E.M.S., Stanish, L.F., Bagshaw, E.A., Porazinska, D.L. & Schmidt, S.K. (2018). Diversity patterns of microbial eukaryotes mirror those of bacteria in Antarctic cryoconite holes. *FEMS Microbiol. Ecol.* **94**(1). <https://doi.org/10.1093/femsec/fix167>
- Sommers, P., Darcy, J.L., Porazinska, D.L., Gendron, E.M.S., Fountain, A.G., Zamora, F., Vincent, K., Cawley, K.M., Solon, A.J., Vimercati, L., Ryder, J. & Schmidt, S.K. (2019). Comparison of microbial communities in the sediments and water columns of frozen cryoconite holes in the McMurdo Dry Valleys, Antarctica. *Front. Microbiol.* **10**, 65. <https://doi.org/10.3389/fmicb.2019.00065>
- Steinböck, O. (1936). Über Kryokonitlöcher unter ihre biologische Bedeutung. *Z. Gletsch.* **24**, 1–21.
- Stibal, M. & Tranter, M. (2007). Laboratory investigation of inorganic carbon uptake by cryoconite debris from Werenskiöldbreen, Svalbard. *J. Geophys. Res.* **112**, G04S33.
- Stibal, M., Lawson, E.C., Lis, G.P., Mak, K.M., Wadham, J.L. & Anesio, A.M. (2010). Organic matter content and quality in supraglacial debris across the ablation zone of the Greenland ice sheet. *Ann. Glaciol.* **51**, 1–8.
- Stibal, M., Schostag, M., Cameron, K.A., Hansen, L.H., Chandler, D.M., Wadham, J.L. & Jacobsen, C.S. (2015). Different bulk and active bacterial communities in cryoconite from the margin and interior of the Greenland ice sheet. *Environ. Microbiol. Rep.* **7**, 293–300.
- Stibal, M., Bradley, J.A., Edwards, A., Hotaling, S., Zawierucha, K., Rosvold, J., Lutz, S., Cameron, K.A., Mikucki, J.A., Kohler, T.J., Šabacká, M. & Anesio, A.M. (2020). Glacial ecosystems are essential to understanding biodiversity responses to glacier retreat. *Nat. Ecol. Evol.* **4**, 686–687.
- Taberlet, P., Bonin, A., Zinger, L. & Coissac, E. (2018). *Environmental DNA: For biodiversity research and monitoring*. Oxford: Oxford University Press.
- Takeuchi, N. (2002). Optical characteristics of cryoconite (surface dust) on glaciers: the relationship between light

- absorbency and the property of organic matter contained in the cryoconite. *Ann. Glaciol.* **34**, 409–414.
- Takeuchi, N. & Kohshima, S. (2004). A snow algal community on Tyndall glacier in the Southern Patagonia Icefield, Chile. *Arct. Antarct. Alp. Res.* **36**, 92–99.
- Takeuchi, N., Kohshima, S., Yoshimura, Y., Sekto, K. & Fujita, K. (2000). Characteristics of cryoconite holes on a Himalayan glacier, Yala Glacier Central Nepal. *Bull. Glaciol. Res.* **17**, 51–59.
- Takeuchi, N., Nishiyama, H. & Li, Z. (2010). Structure and formation process of cryoconite granules on Ürümqi glacier No. 1, Tien Shan, China. *Ann. Glaciol.* **51**, 9–14.
- Treonis, A.M. (2005). Soil nematodes and desiccation survival in the extreme arid environment of the antarctic dry valleys. *Int. Comp. Biol.* **45**, 741–750.
- Uetake, J., Tanaka, S., Segawa, T., Takeuchi, T., Nagatsuka, N., Motoyama, H. & Aoki, T. (2016). Microbial community variation in cryoconite granules on Qaanaaq Glacier, NW Greenland. *FEMS Microbiol. Ecol.* **92**, fiw127.
- Urabe, J., Clasen, J. & Sterner, R.W. (1997). Phosphorus limitation of *Daphnia* growth: is it real? *Limnol. Oceanogr.* **42**, 1436–1443.
- Velasco-Castrillón, A., Gibson, J.A.E. & Stevens, M.I. (2014). A review of current Antarctic limno-terrestrial microfauna. *Pol. Biol.* **37**, 1517–1531.
- Velasco-Castrillón, A., Hawes, I. & Stevens, M.I. (2018). 100 years on: a re-evaluation of the first discovery of microfauna from Ross Island, Antarctica. *Antarct. Sci.* **30**, 209–219.
- Vonnahme, T.R., Devetter, M., Zárský, J.D., Sabacká, M. & Elster, J. (2016). Controls on microalgal community structures in cryoconite holes upon high Arctic glaciers, Svalbard. *Biogeoscience* **13**, 659–674.
- Wharton, D.A. (1986). *A functional biology of nematodes*: 192. London: Croom Helm.
- Wharton, D.A. (2003). The environmental physiology of Antarctic terrestrial nematodes: a review. *J. Comp. Physiol. B* **173**, 621–628.
- Wharton, D.A. & Raymond, M.R. (2015). Cold tolerance of the Antarctic nematodes *Plectus murrayi* and *Scottinema lindsayae*. *J. Comp. Physiol. B* **185**, 281–289.
- Wharton, D.A., McKay, C.P., Simmons, G.M. & Parker, P.C. (1985). Cryoconite holes on glaciers. *Biogeoscience* **35**, 499–503.
- Wu, T., Ayres, E., Bardgett, R.D., Wall, D.H. & Garey, J.R. (2011). Molecular study of worldwide distribution and diversity of soil animals. *Proc. Natl. Acad. Sci. USA* **108**, 17720–17725.
- Yde, J.C., Bárcena, T.G. & Finster, K.W. (2011). Subglacial and proglacial ecosystem responses to climate change. *Clim. Chang. Geophysical Foundations and Ecological Effects*, Juan Blanco and Houshang Kheradmand, IntechOpen. <https://doi.org/10.5772/24236>
- Zawierucha, K., Kolicka, M., Takeuchi, N. & Kaczmarek, Ł. (2015). What animals can live in cryoconite holes? A faunal review: cryoconite holes fauna. *J. Zool.* **295**, 159–169.
- Zawierucha, K., Ostrowska, M., Vonnahme, T.R., Devetter, M., Nawrot, A.P., Lehmann, S. & Kolicka, M. (2016). Diversity and distribution of Tardigrada in Arctic cryoconite holes. *J. Limnol.* **75**, 545–559.
- Zawierucha, K., Buda, J., Pietryka, M., Richter, D., Łokas, E., Lehmann-Konera, S., Makowska, N. & Bogdziewicz, M. (2018a). Snapshot of micro-animals and associated biotic and abiotic environmental variables on the edge of the south-west Greenland ice sheet. *Limnology* **19**, 141–150.
- Zawierucha, K., Stec, D., Lachowska-Cierlik, D., Takeuchi, N., Li, Z. & Michalczyk, Ł. (2018b). High mitochondrial diversity in a new water bear species (Tardigrada: Eutardigrada) from mountain glaciers in Central Asia, with the erection of a new genus *Cryoconicus*. *Ann. Zool.* **68**, 179–201.
- Zawierucha, K., Buda, J., Azzoni, R.S., Niškiewicz, M., Franzetti, A. & Ambrosini, R. (2019a). Water bears dominated cryoconite hole ecosystems: densities, habitat preferences and physiological adaptations of Tardigrada on an alpine glacier. *Aquat. Ecol.* **53**, 543–556.
- Zawierucha, K., Buda, J., Fontaneto, D., Ambrosini, R., Franzetti, A., Wierzoń, M. & Bogdziewicz, M. (2019b). Fine-scale spatial heterogeneity of invertebrates within cryoconite holes. *Aquat. Ecol.* **53**, 179–190.
- Zawierucha, K., Buda, J. & Nawrot, A. (2019c). Extreme weather event results in the removal of invertebrates from cryoconite holes on an Arctic valley glacier (Longyearbreen, Svalbard). *Ecol. Res.* **34**, 370–379.
- Zdanowski, M.K., Bogdanowicz, A., Gawor, J., Gromadka, R., Wolicka, D. & Grzesiak, J. (2017). Enrichment of Cryoconite Hole Anaerobes: Implications for the Subglacial Microbiome. *Microbial. Ecol.* **73**, 532–538.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Previously studied glaciers in terms of invertebrates in cryoconite holes.

**Figure S2.** Additional information regarding the high-throughput sequencing data from continental Antarctica.

**Figure S3.** The most dominant eukaryotic taxa (constituting more than 1% of all sequences) detected in eDNA using 18S rDNA fragment from Baltoro in Karakoram and Forni in the Alps.

**Figure S4.** (a) Supraglacial zone on Forni Glacier in the Alps covered by stones, (b) and (c) black springtails (*Collembola*) found under stones on Forni.

**Table S1.** List of samples analysed in this study.

**Table S2.** Literature records of invertebrates in cryoconite holes.

**Table S3.** OTUs (operational taxonomic units) recovered from eDNA analysis on Forni and Baltoro samples.