






# Islands of ice: Glacier-dwelling metazoans form regionally distinct populations despite extensive periods of deglaciation

Karel Janko<sup>1,2</sup>  | Daniel H. Shain<sup>3</sup> | Diego Fontaneto<sup>1,4,5</sup> |  
 Marie Kaštánková Doležálková<sup>1</sup> | Jakub Buda<sup>6</sup> | Eva Štefková Kašparová<sup>1,7</sup> |  
 Marie Šabacká<sup>8</sup> | Jørgen Rosvold<sup>9</sup> | Jacek Stefaniak<sup>10</sup>  | Dag Olav Hessen<sup>11</sup> |  
 Miloslav Devetter<sup>12</sup>  | Marco Antonio Jimenez/Santos<sup>1,12</sup>  | Patrik Horna<sup>1</sup> |  
 Edita Janková Drdová<sup>13</sup> | Jacob Clement Yde<sup>14</sup> | Krzysztof Zawierucha<sup>6</sup> 

<sup>1</sup>Institute of Animal Physiology and Genetics AS CR, Liběchov, Czech Republic

<sup>2</sup>Department of Biology and Ecology, University of Ostrava, Ostrava, Czech Republic

<sup>3</sup>Biology Department, Rutgers the State University of New Jersey, Camden, New Jersey, USA

<sup>4</sup>National Research Council of Italy (CNR), Water Research Institute (IRSA), Molecular Ecology Group (MEG), Verbania, Italy

<sup>5</sup>National Biodiversity Future Center (NBFC), Palermo, Italy

<sup>6</sup>Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Poznań, Poland

<sup>7</sup>Department of Zoology and Fisheries, Faculty of Agrobiological Sciences, Czech University of Life Science Prague, Prague 6, Czech Republic

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

<sup>9</sup>Department of Terrestrial Biodiversity, Norwegian Institute for Nature Research (NINA), Trondheim, Norway

<sup>10</sup>Museum of Natural History, University of Wrocław, Wrocław, Poland

<sup>11</sup>Department Biosciences, University of Oslo, Oslo, Norway

<sup>12</sup>Institute of Soil Biology and Biogeochemistry, Biology Centre CAS, České Budějovice, Czech Republic

<sup>13</sup>Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague 6, Czech Republic

<sup>14</sup>Department of Civil Engineering and Environmental Sciences, Western Norway University of Applied Sciences, Sogndal, Norway

## Correspondence

Karel Janko, Laboratory of Non-Mendelian Evolution, Institute of Animal Physiology and Genetics AS CR, Rumburská 89, Liběchov 277 21, Czech Republic.  
 Email: [janko@iapg.cas.cz](mailto:janko@iapg.cas.cz)

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

## Funding information

National Science Center, Grant/Award Number: NCN 2013/11/N/NZ8/00597; Neuron Nadační Fond Na Podporu Vědy; Czech Science Foundation, Grant/Award Number: 22-28778S; Institutional

## Abstract

**Aim:** Glaciers cover considerable portion of land and host diverse life forms from single-celled organisms to invertebrates. However, the determinants of diversity and community composition of these organisms remain underexplored. This study addresses the biogeography, population connectivity and dispersal of these organisms, especially critical in understanding during the rapid recession of glaciers and increased extinction risk for isolated populations. By reconstructing the Quaternary biogeographic history of *Fontourion glacialis*, a widespread in Northern Hemisphere glacier obligate species of Tardigrada, we aim to understand how populations of glacier-dwelling metazoans receive immigrants, respond to disappearing glaciers and to what extent remaining glaciers can serve as refugia.

**Location:** Glaciers across Svalbard, Scandinavia, Greenland and Iceland.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Author(s). *Diversity and Distributions* published by John Wiley & Sons Ltd.

Research Concept, Grant/Award Number: RVO67985904; National Biodiversity Future Center of Italy, funded by the Italian Ministry of University and Research, PNRR, Missione 4 Componente 2, "Dalla ricerca all'impresa", Investimento 1.4; Secretaría de Educación, Ciencia, Tecnología e Innovación de la CDMX, Mexico; Norges Forskningsråd, Grant/Award Number: 302458 JOSTICE

Editor: Zhonghe Hou

**Methods:** We analysed mtDNA (COI gene) variability of 263 *F. glacialis* specimens collected across the distribution range. Phylogeographic and coalescent-based approaches were used to detect population differentiation patterns, investigate most likely models of gene flow and test the influences of geographical and climatic factors on the distribution of *F. glacialis* genetic variants.

**Results:** Our findings indicate that the distribution of *F. glacialis* genetic variants is primarily influenced by geographical rather than climatic factors. Populations exhibit a dispersal-limited distribution pattern, influenced by geographical distance and local barriers, even between neighbouring glaciers. Significantly, the genetic structure within Scandinavia suggests the existence of "southern" glacial or low-temperature refugia, where *F. glacialis* may have survived a period of extensive deglaciation during the Holocene climatic optimum (8–5 kyr ago).

**Main Conclusion:** The study uncovers complex metapopulation structures in *F. glacialis*, with impacts of local barriers, population bottlenecks as well as historical ice sheet fluctuations. It suggests that such populations can endure extended periods of deglaciation, highlighting the resilience of glacial refugia. The study highlights the necessity of understanding the diversity and population structure of ice-dwelling fauna in both spatial and temporal contexts.

#### KEYWORDS

cryoconite ecosystems, glacial ecology and biogeography, ice-fauna survival, impact of deglaciation, phylogeography, quaternary history, refugia identification, Tardigrada

## 1 | INTRODUCTION

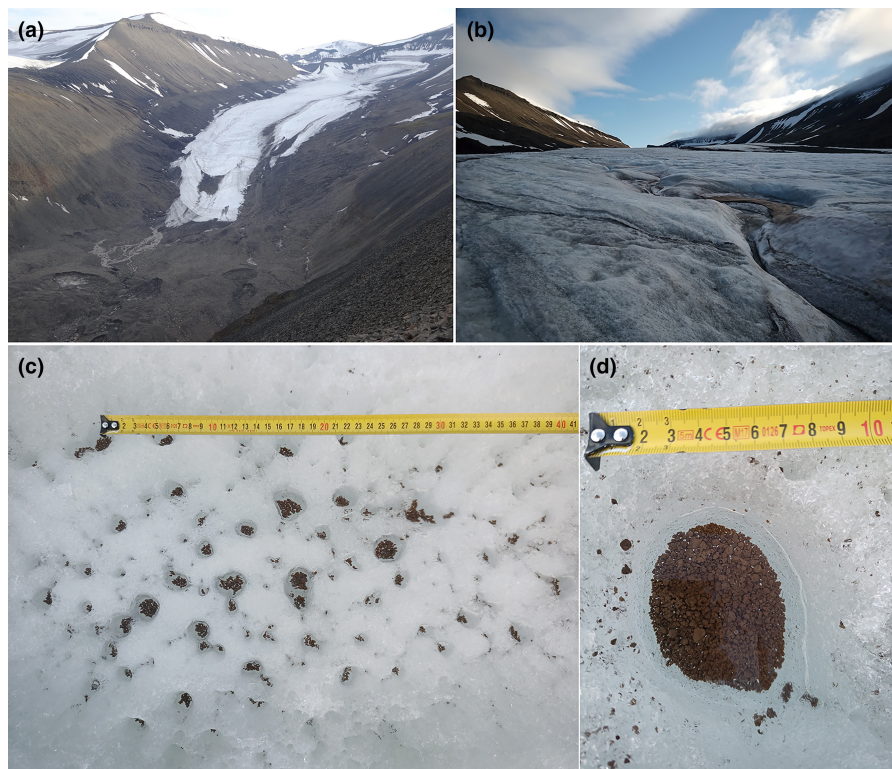
Spanning approximately 10% of the Earth's surface, glaciers significantly influence geomorphology, climate and productivity of ecosystems worldwide and have impacted biogeographical patterns and evolution by changing migration barriers and forming new habitats (Bosson et al., 2023; Ficetola et al., 2021; Hewitt, 2000; Weir & Schluter, 2004). However, glaciers themselves provide life-supporting services. They host diverse single-celled and microscopic multicellular organisms constituting flourishing, albeit relatively short, food webs (e.g., Anesio & Laybourn-Parry, 2012; Cook et al., 2016; Hodson et al., 2008; Zawierucha et al., 2015). Especially important is the dark sediment on glacier surfaces, known as cryoconite (Rozwalak et al., 2022), that reduces the albedo of ice and creates water-filled depressions called cryoconite holes (Figure 1). These constitute suitable habitats for primary producers, like algae and cyanobacteria, or chemolithotrophic bacteria (Poniecka et al., 2020), which sustain heterotrophic communities and consumers like tardigrades and rotifers (Cameron et al., 2012; Hodson et al., 2008; Lutz et al., 2015; Pittino et al., 2023; Zawierucha, Kašparová, et al., 2023).

Phylogenetic and ecophysiological evidence suggests that many glacial organisms are substantially diverged from relatives in other habitats, forming lineages adapted for harsh glacial conditions (Dastyk et al., 2003; Dial et al., 2016; Remias & Procházková, 2023; Shain et al., 2016; Singh et al., 2014; Zawierucha et al., 2022; Zawierucha, Buda, Azzoni, et al., 2019). Glacier surfaces (supraglacial

zone) are now recognized as a distinct ecosystem, albeit typically fragmented into patchy cryospheric islands surrounded by terrestrial habitats. Yet, glaciers may also be extensive, like Greenland, and have dramatically fluctuated in size over history. Understanding the biogeography of organisms dwelling on terrestrial ice masses thus presents a fascinating yet understudied research field with the utmost relevance for forecasting the future evolution of this ecosystem during ongoing climate change (Liu et al., 2017; Segawa et al., 2017; Zawierucha, Kašparová, et al., 2023).

High dispersal potential is common for many microorganisms (de Wit & Bouvier, 2006; Fenchel & Finlay, 2004; Foissner, 2005; Fontaneto, 2019). Thus, different populations on fragmented glacier patches may be highly connected by gene flow, but the current distribution of glacier-dwelling organisms may also follow an island biogeography model, with populations isolated on ice "islands" separated by land or oceans. In addition, during the Quaternary, the size, connectivity and even existence of these "glacier islands" varied from ice ages (glaciations) when much of the northern hemisphere was covered by large ice sheets, to warmer periods (interglacials) when ice sheets shrank in size and many contemporary glaciers presumably vanished for extended periods of thousands of years (Irvali et al., 2020; Nesje et al., 2008; Robinson et al., 2017). Cryophilic organisms may also "migrate in time" by becoming conserved in ice layers, potentially reviving decades, or even millennia later (Shatilovich et al., 2023; Shmakova et al., 2021; Sieger et al., 2022; Tsujimoto et al., 2016). Combination of all these processes may

**FIGURE 1** Glacial habitats. (a) View on the glacier Longyearbreen, Svalbard; (b) supraglacial zone of Longyearbreen, surface ice of weathering crust; (c) complex structure of cryoconite holes; (d) cryoconite hole with thin layer of cryoconite on the bottom.



create specific patterns in the distribution of genetic diversity of glacier-dwelling organisms, which remains poorly understood.

Available studies on bacteria and algae suggest that cryoconite holes on glaciers harbour regionally distinct communities (Darcy et al., 2018; Edwards et al., 2013; Liu et al., 2017; Millar et al., 2021; Pittino et al., 2023; Segawa et al., 2017, 2018). Their distribution is potentially affected by dispersal limitations (Liu et al., 2017; Segawa et al., 2017), contributing to biogeographical clustering within individual glaciers and sometimes even among individual cryoconite holes (Darcy et al., 2018). Biogeography of glacier-dwelling metazoans have received even less attention, although microscopic invertebrates are common on glaciers and were first observed in the 19th century (Novotná Jaroměřská et al., 2021; Valle et al., 2021; Zawierucha et al., 2015; Zawierucha, Buda, Azzoni, et al., 2019). Several studies revealed divergent biogeographic patterns ranging from allopatric fragmentation to long-distance dispersals or even inter-polar distribution (e.g., Dial et al., 2016; Hotaling et al., 2019; Shain et al., 2016; Zawierucha et al., 2022; Zawierucha, Kašparová, et al., 2023).

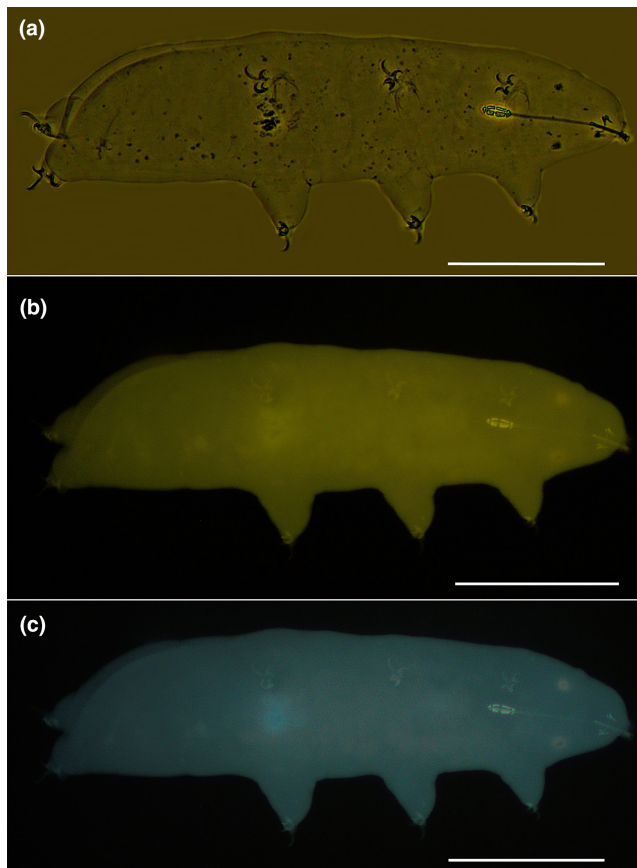
Tardigrades, microscopic invertebrates found across aquatic and terrestrial ecosystems worldwide, are among the few animal phyla that inhabit the supraglacial zone and are the apex consumers in cryoconite hole ecosystems (Nelson et al., 2015; Zawierucha et al., 2015, 2021). Tardigrade species diversity within cryoconite holes is relatively low; however, several new glacier-dwelling tardigrade species, belonging to the class Eutardigrada, have recently been described from diverse biogeographic regions, from the equator to high latitudes, presumably adapted to cryoconite hole environment (Zawierucha, Gąsiorek, et al. 2018; Zawierucha et al., 2020, 2022; Zawierucha, Stec, et al., 2018). Eutardigrades are gonochoric, parthenogenetic and rarely hermaphroditic (Bertolani, 2001; Poprawa & Janelt, 2019).

The biogeographic and demographic patterns of glacier-dwelling tardigrades can be intricate and may shed light on processes governing the glacial ecosystems in general. For instance, their local density can fluctuate dramatically from one day to another due to weather-mediated events (Zawierucha, Buda, & Nawrot, 2019), but on the other hand, *Cryoconicus kaczmareki*, an Asian-Arctic species, has established locally enduring population on one small Svalbard glacier (Ebbabreen) that persisted for millennia in genetic isolation from its Asian core range (Zawierucha, Kašparová, et al., 2023). Similarly, nearby New Zealand glaciers host phylogenetically separate lines, suggesting that glacier fragmentation during the Pleistocene might have triggered tardigrade speciation (Zawierucha et al., 2022).

*Fontourion glacialis* (class Eutardigrada, family Hypsiibidae, subfamily Pilatobiinae; Figure 2) has been recently delineated from its tundra and moss-dwelling congeners, *F. islandicus* and *F. recamieri*, as a species distinctively adapted to glacial environments that dominates on Arctic glaciers (Zawierucha et al., 2020; Zawierucha, Kašparová, et al., 2023). While the biology of *F. glacialis* remains largely unexplored, subfamily Pilatobiinae is a sister clade to the subfamily Diphasconinae with asexual reproduction. Occupying exclusive limnic niches within cryoconite holes, *F. glacialis* shares its habitat with other limnic tardigrades that are mostly parthenogenetic, irrespective of their phylogenetic lineage, such as *Dactylobiotus* and *Hypsibius* (Nelson et al., 2015; Poprawa et al., 2015). Considering its phylogenetic proximity to asexually reproducing taxa, specialized aquatic environment and the extreme glacial conditions it endures, *F. glacialis* is likely able of parthenogenesis.

Due to its common occurrence, *F. glacialis* represents a suitable model for understanding processes shaping the spatiotemporal distribution of apex consumers of glacier ecosystems. Reconstructing





**FIGURE 2** *Fontourion glacialis* (Zawierucha et al., 2020), ventro-lateral view; (a) Phase Contrast Microscopy; (b) blue light excitation; (c) UV light excitation. Scale bar corresponds to 100  $\mu$ m.

its phylogeography across four biogeographic provinces (Svalbard, Iceland, Greenland and Scandinavia), we test whether its dispersal connectivity is sufficient to genetically homogenize populations among currently isolated ice patches or whether significant population structure exists. We further test whether detected structure is primarily driven by geography or climatic parameters, and we reconstruct its demographic history to investigate how populations of this species responded to historical climatic oscillations. In particular, extensive sampling along its southern range, that is, glaciers across Norway, allows us to understand how such a glacial specialist coped with warmer phases leading to the temporary disappearance of therein glaciers.

## 2 | MATERIALS AND METHODS

### 2.1 | Tardigrade collection and genotyping using mitochondrial COI gene

We sampled 33 glaciers throughout the Arctic and sub-Arctic, covering central-western, central-northern and northern Norway, Iceland, southwestern Greenland and the Svalbard archipelago (Figure 3, Table 1). Cryoconite material from the ablation zone and the surface ice of the weathering crust from accumulation zone (i.e., above

the equilibrium line altitude – ELA) was collected from wide range of glaciers differing in their (i) morphology (e.g., tidewater, outlet or valley glaciers), (ii) thermal regime (i.e., temperate, polythermal or cold-based) and (iii) elevation (e.g., terminating in the sea or located in mountains).

Techniques for specimen collection, identification, DNA extraction and amplification of Cytochrome-c oxidase subunit I gene fragment (hereafter: COI) are provided in Data S1. Obtained sequences were visually inspected in the BioEdit (Hall & Hall, 1999) for accuracy of base calls and stop codons and final alignment was performed using the ClustalW program with the default settings to identify haplotypes.

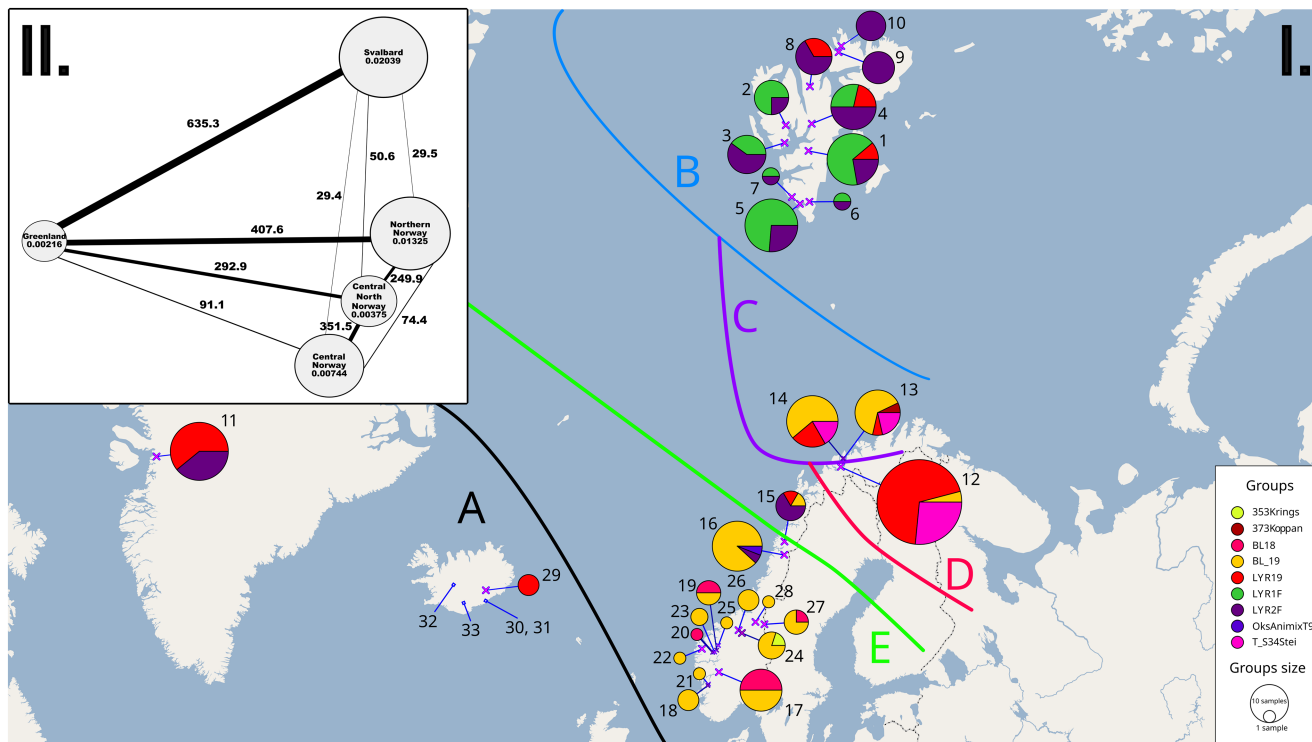
### 2.2 | Climatic variables

To obtain a quantitative description of the climatic differences between glaciers and to test their impact on observed distribution of genetic diversity, we obtained a broad set of climatic variables using the geographic coordinates of each glacier. We started from 19 unaltered bioclimatic variables in 30 arc seconds resolution, downloaded from WorldClim v2.1 (Fick & Hijmans, 2017) using QGIS software (version 3.30.1). Then, we calculated the full set of ENVIREM variables (Title & Bemmels, 2018) using R v4.1.3 (R Core Team, 2023), with packages envirem v2.3 (Title & Bemmels, 2018), provided in the Data S2.

To minimize redundancy, we first checked for correlation between all pairs of ENVIREM variables using the R package PerformanceAnalytics v2.0.4 (Peterson & Carl, 2020) and used only a set of non-correlated variables to explain haplotype distribution. Using a threshold of Pearson's  $r = .70$ , a subset of seven variables (namely the number of months with mean temperature greater than 10°C, PET (potential evapotranspiration) of the wettest quarter, topographic wetness index and yearly averages of temperature, precipitation, solar radiation and wind speed) was retained for the following analyses (Figure S1).

### 2.3 | Drivers of diversity

The sampling success at different regions may bias our inference as more animals may tend to be sequenced in denser populations, potentially affecting the estimates of spatial partitioning of genetic diversity. Therefore, we evaluated whether environmental variables or geographic position could affect the numbers of sequenced individuals, which indeed may be linked to differences in both the local animal abundances and in sampling effort. The tested model included the total number of sequenced individuals (response variable) as a function of the seven low-correlated ENVIREM variables. Then, we checked whether the genetic diversity of each glacier could be explained by environmental variables or geographic position. The model included the number of haplotypes (response variable as a proxy of genetic diversity) as a function of the seven uncorrelated



**FIGURE 3** (I) Sampling sites with indicated absolute frequencies of haplotypes of *F. glacialis* (haplotype labels correspond to Table 1). Five most important (A–E) barriers detected by the BARRIER software are indicated between populations with the run setting including virtual points. (II) An insert schematizing the migration connectivity and effective population sizes as estimated by MIGRATE-n for the five-population scenario.

ENVIREM (explanatory) variables, in addition to the confounding factor of sampling bias, expressed as abundance of sequenced individuals (see above).

We accounted for the existence of spatial effects by testing various correlation structures (spherical, linear, ratio, Gaussian and exponential) in spatially explicit generalized least square models in the R package nlme v 3.1-162 (Pinheiro & Bates, 2023). The model with the significantly lowest AIC (Akaike Information Criterion) was then selected to inspect the output. In cases when no spatial structure was needed in the model, we then used simpler linear models (LM) with the same structure of response and predictor variables. Model assumptions (i.e., normality of residuals, homogeneity of variance, influential observations, collinearity) were checked with the R package performance v0.10.2 (Lüdtke et al., 2021).

To assess the relationship between haplotype occurrence and the selected ENVIREM variables, accounting for the effect of geographical distances between glaciers, we used Mantel tests and partial Mantel tests with the R package vegan v2.6-4 (Oksanen et al., 2020). As a matrix of differences in haplotype composition between glaciers, we calculated an abundance-based Jaccard dissimilarity index in the R package BAT v2.9.2 (Cardoso et al., 2015); as a matrix of geographic distances, we calculated the geodesic distance in km between glaciers with the R package geodist v0.0.7 (Padgham & Sumner, 2021); as a matrix of ecological differences, we calculated the Gower distances using the seven selected ENVIREM environmental variables with the R package ecodist v2.0.9

(Goslee & Urban, 2007). To include phylogenetic relationships in the description of haplotype distribution, we also used a matrix of  $\Phi_{ST}$  distances between populations on each glacier in partial Mantel tests against geographical distances and ecological differences, calculating  $\Phi_{ST}$  only for a subset of 18 glaciers with at least four animals.

We also applied Moran's Eigenvector Mapping to assess the unique and combined effect of spatial structure and environmental variables in shaping the distribution of haplotypes. To do so, we used the R package adespatial v0.3-21 (Dray et al., 2023) on a subset of 18 glaciers with at least four animals.

To test the detailed effects of environmental variables on the occurrence of the different haplotypes on the glaciers, we used a model-based approach to the analysis of multivariate abundance data (Wang et al., 2012). The model included the multivariate occurrence data of the haplotypes (response variable) as a function of the seven selected ENVIREM variables, in addition to latitude (as a proxy for spatial patterns) and abundance of individuals (as a proxy for sampling bias) in the R package mvabund v4.2.1 (Wang et al., 2012). The R script for all the analyses is provided as Data S3, together with the datasets Data S2 and S4.

## 2.4 | Phylogeographic analyses

Summary statistics of genetic diversities were calculated in DNAsp v. 5 (Librado & Rozas, 2009), phylogenetic relationships among

**TABLE 1** Sampling sites and distribution of genetic diversity among sites (Locality IDs and Haplotype labels correspond to Figure 3) and calculated Tajima's D and Fu's  $F_s$  indices. Bottom half of the table summarizes the results of SAMOVA, indicating the sequential grouping of populations into k clusters ( $k = 2-18$ ). Significance of  $F_{sc}$ ,  $F_{st}$  and  $F_{ct}$  indices is denoted by \* ( $<.05$ ) and \*\*\* ( $<.001$ ). Note that SAMOVA used only localities with >4 sampled individuals (the exception is represented by an Icelandic site with three samples).

Region	Locality name	Locality ID	Haplotype label	Svalbard										Greenland										Northern Scandinavia										Middle Scandinavia										Icelandic Scandinavia										Iceland																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220	1221	1222	1223	1224	1225	1226	1227	1228	1229	1230	1231	1232	1233	1234	1235	1236	1237	1238	1239	1240	1241	1242	1243	1244	1245	1246	1247	1248	1249	1250	1251	1252	1253	1254	1255	1256	1257	1258	1259	1260	1261	1262	1263	1264	1265	1266	1267	1268	1269	1270	1271	1272	1273	1274	1275	1276	1277	1278	1279	1280	1281	1282	1283	1284	1285	1286	1287	1288	1289	1290	1291	1292	1293	1294	1295	1296	1297	1298	1299	1300	1301	1302	1303	1304	1305	1306	1307	1308	1309	1310	1311	1312	1313	1314	1315	1316	1317	1318	1319	1320	1321	1322	1323	1324	1325	1326	1327	1328	1329	1330	1331	1332	1333	1334	1335	1336	1337	1338	1339	1340	1341	1342	1343	1344	1345	1346	1347	1348	1349	1350	1351	1352	1353	1354	1355	1356	1357	1358	1359	1360	1361	1362	1363	1364	1365	1366	1367	1368	1369	1370	1371	1372	1373	1374	1375	1376	1377	1378	1379	1380	1381	1382	1383	1384	1385	1386	1387	1388	1389	1390	1391	1392	1393	1394	1395	1396	1397	1398	1399	1400	1401	1402	1403	1404	1405	1406	1407	1408	1409	1410	1411	1412	1413	1414	1415	1416	1417	1418	1419	1420	1421	1422	1423	1424	1425	1426	1427	1428	1429	1430	1431	1432	1433	1434	1435	1436	1437	1438	1439

**TABLE 2** Coalescent models compared in MIGRATE; results of 5-population and 7-population datasets are presented with model probabilities.

	Model	Ln(prob(D model))	Model probability
5-population dataset	a	-1341.205498	1.8558E-37
	b	-1396.775899	1.36337E-61
	c	-1257.231421	0.547028328
	d	-1278.710825	2.56817E-10
	e	-1315.127521	3.92693E-26
	f	-1324.64155	2.89843E-30
	g	-1257.420092	0.452971672
7-population dataset	a	-1338.541072	7.39066E-36
	b	-1435.759272	4.43972E-78
	c	-1261.642427	0.018422142
	d	-1281.678092	3.66405E-11
	e	-1317.516657	9.98781E-27
	f	-1328.250384	2.17707E-31
	g	-1257.666819	0.981577858

Model	Parameters	Description
a	Model assumes one panmictic population	Scenario assumes that all sampled populations belong to a single Arctic-wide panmictic population
b	Model assumes recent split of populations from single ancestor without subsequent migration connectivity	Scenario assumes recent (e.g. post-LGM) split of all current population clusters from a single large ancestral population with no gene flow after the split
c	Model assumes variable (symmetric) migration rates among long time separated populations	Migration-drift equilibrium scenario assuming old population structure with ongoing gene flow (i.e. that all population clusters split from each other effectively long time ago and have been interconnected by migrations ever since); migration rates are allowed to vary between population pairs
d	Source-sink model assuming that Scandinavian populations receive migrants from High Arctic but do not send migrants outside Scandinavia	Scenario assumes a source-sink model when the split of all population clusters occurred effectively long time ago, but was followed by unidirectional migration from high Arctic sites (acting as a source) to Norwegian sites (acting as a sink)
e	Model assumes that Scandinavian populations were recently colonized from Svalbard and received migrants subsequently	Scenario assumes that Scandinavian sites were recently (e.g., during post-LGM or Holocene times) established by colonists from Svalbard with possible immigration after the establishment
f	Model assumes that Scandinavian populations were recently colonized from Greenland cluster and received migrants subsequently	Scenario assumes that Scandinavian sites were recently (e.g., during post-LGM or Holocene times) established by colonists from Greenland with possible immigration after the establishment
g	Model assumes the same (symmetric) migration rates among long time separated populations	Migration-drift equilibrium scenario assuming old population structure with ongoing gene flow (i.e. that all population clusters split from each other effectively long time ago and have been interconnected by migrations ever since); migration rates are not allowed to vary between population pairs. The model thus tests whether there was a statistical support for claiming that some population pairs exchange migrants more intensively than other pairs

from High Arctic to Scandinavia (d) and recent colonization of Scandinavia from High Arctic (d, e). Details and descriptions are provided in [Table 2](#).

All models were run in three replicates of 10,000,000 generations, sampling every 100th step and applying a burn-in value of 1000 first sampled genealogies. The best models for each grouping were the ones with the highest value of likelihood, and we calculated model probabilities and ranked the models relative to the best-selected one ([Table 2](#)). When bi-directional migrations were assumed in any pair of populations (such as in models c and g),

symmetric migrations were assumed to avoid over parameterization of the coalescent computation.

Demographic histories of *F. glacialis* population clusters were estimated using Tajima's D in DnaSP version 5.0 (Librado & Rozas, 2009) and with the Extended Bayesian skyline plot analysis (EMSP) implemented in the BEAST v.1.8.0 (Drummond et al., 2012), with subsequent analysis in Tracer v 1.5 (Rambaut et al., 2009). The clock rate was set to strict, and the mutation model was set to Tamura-Nei with invariant sites site heterogeneity (as suggested by jModelTest) and base frequencies set to empirical values.

To estimate the divergence time of major haplotype groups, we first run the BEAST 1.8.0 on the same dataset and substitution model as above, constructing the trees under a coalescence model of constant size, strict molecular clock assumption and running three independent runs of  $1 \times 10^8$  iterations, sampling every 10,000th generation. Results were inspected in Tracer v 1.5 (Rambaut et al., 2009), combined in the LogCombiner using 10% burn-in and harvested in TreeAnnotator. As a second approach, we used the method of Saillard et al. (2000) to infer the average distance from the most recent common ancestor (MRCA) of the clade in the number of mutation steps ( $\rho = \{n_1 l_1 + \dots + n_k l_k\} / n$ , where  $n$  is the number of specimens and  $l$  is the length of  $k^{\text{th}}$  branch expressed in mutation steps, and the variance of the estimate  $\sigma_H^2 = \{n_1^2 l_1 + \dots + n_k^2 l_k\} / n^2$ ). Since the tardigrade mtDNA mutation rate is unknown, we calibrated the trees with the estimates of COI gene substitution rates of northern polar arthropods calibrated by the divergence of the Beringian species, suggesting the rate of  $2.55\text{E-}08$  per site per year (Loeza-Quintana et al., 2019).

### 3 | RESULTS

#### 3.1 | *Fontouion glacialis* distribution and sequence variability

568bp alignment of COI gene was retrieved from 263 sequenced *F. glacialis* (Figure 1) individuals sampled on 29 out of 33

investigated glaciers (Table 1, Figure 3), altogether yielding unique 9 haplotypes characterized by 12 polymorphic sites (haplotype diversity = 0.798, SD = 0.008) and 3.22753 average nucleotide differences ( $\pi = 0.00568$ , SD = 0.00025) (Figure 4). Newly obtained haplotypes were deposited into Gene Bank under accession numbers OR898234 – OR898241, OR958747. Sampling success notably differed among glaciers, with *F. glacialis* being quite common in High Arctic sites, and less abundant in Iceland, where only three individuals were recovered in one out of 5 sampled glaciers.

#### 3.2 | Haplotype diversity

The effect of climatic variables on *F. glacialis* haplotype richness was not supported: the model on richness of haplotypes as a proxy for local genetic diversity was not affected by spatial autocorrelation (delta AIC ratio vs non-spatial model = 1.86, degrees of freedom = 12 and 10), and none of the selected ENVIREM variables had any significant effect. As expected, the haplotype richness on each glacier increased with the abundance of individuals sampled (LM:  $t = 2.4$ ,  $p = .0253$ ; Table S1). However, the model evaluating the sampling bias using numbers of sampled individuals as a proxy was not affected by spatial autocorrelation (delta AIC spherical vs. non-spatial model = 1.26, degrees of freedom = 11 and 9), and none of the selected ENVIREM variables had any significant effect (Table S2).

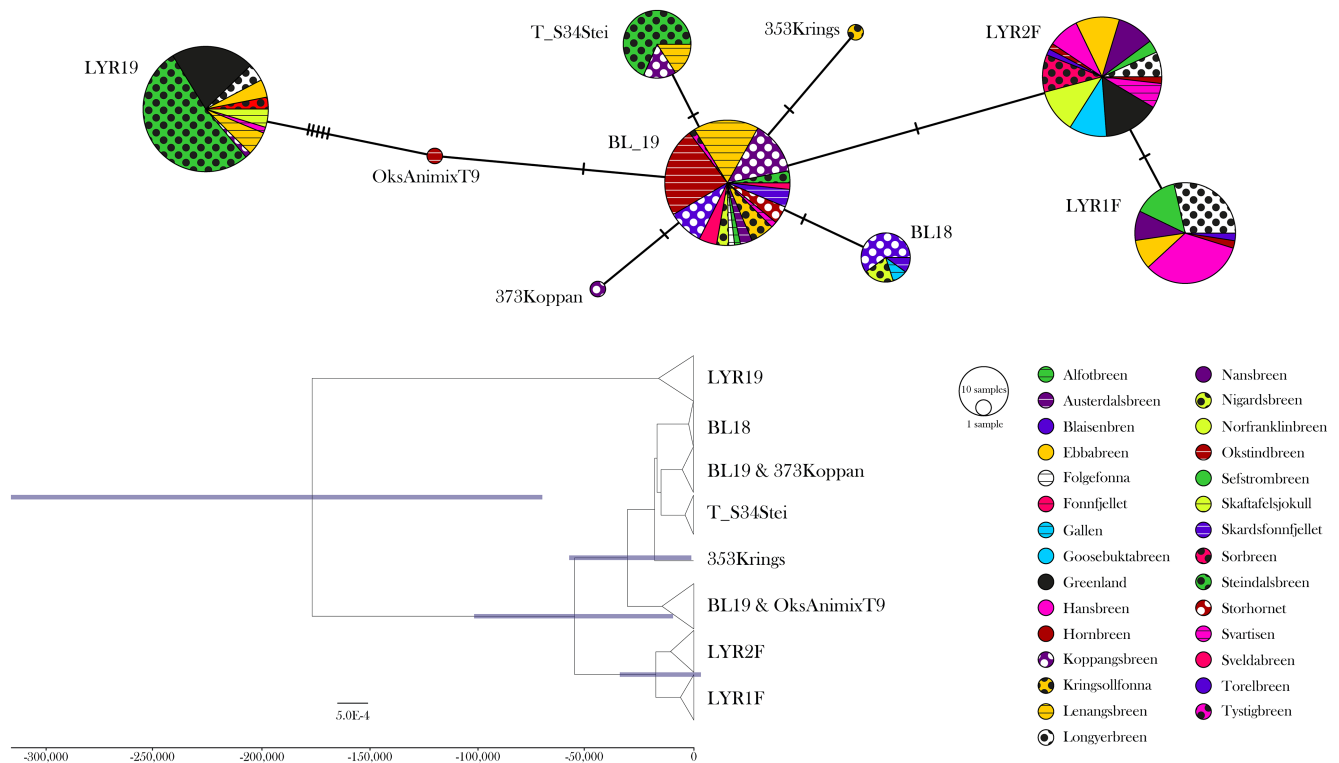


FIGURE 4 Upper panel: Haplotype network reconstructed using the TCS. Lower left panel: Time-calibrated phylogenetic tree with the revealed haplotypes. Large-scale bar indicates the time in years before present, while the small-scale bar indicates the mutation-time units.



Differences in haplotype composition correlated more strongly with geographical distance (Mantel test:  $r = .559$ ,  $p = .001$ ) than with climatic differences among glaciers ( $r = .179$ ,  $p = .011$ ). Correlation between haplotype composition was still significant with geographical distance when controlling for ecological differences (partial Mantel test:  $r = .556$ ,  $p = .001$ ) but not with ecological differences when controlling for geographical distance ( $r = .040$ ,  $p = .215$ ). The same pattern was confirmed when using the subset of 18 glaciers with at least four animals: MEMs revealed that spatial structure alone explained 11% of the variability in haplotype composition, with additional 23% shared with environmental variables, but environment alone did not add anything (Figure S2).  $\Phi_{ST}$  distances between populations were

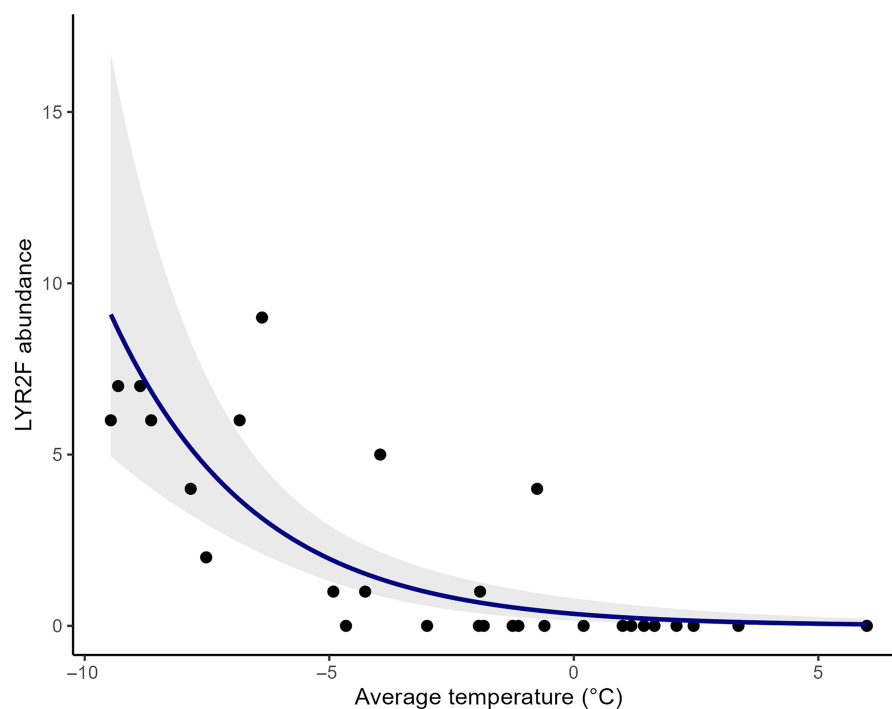
again better explained by geographical distance, when controlling for ecological differences (partial Mantel test:  $r = .409$ ,  $p < .001$ ), than by ecological differences, when controlling for geographical distance ( $r = .140$ ,  $p = .066$ ).

Multivariate abundance analysis revealed relatively small but significant effects of temperature and precipitation on haplotype distribution on 29 glaciers with *F. glacialis*, in addition to latitude and sample size (Table 3). Specifically, the univariate models for occurrence of each haplotype suggested that haplotypes LYR2F and T\_S34stei were negatively affected by temperature ( $p = .001$ ) and by precipitation ( $p = .007$ ), respectively (Figure 5; Table S3). To avoid geographic confounding effect, not included in the multivariate

**TABLE 3** Output of the model-based approach to the analysis of multivariate abundance data (mvabund) to explain the occurrence of haplotypes. Residual and differential degrees of freedom, deviance and  $p$  values are reported. Bold  $p$  values highlight significant predictors, below the .05 threshold.

Predictor	Residual d.f. <sup>a</sup>	Differential d.f. <sup>a</sup>	Deviance	$p$
(Intercept)	28			
Average temperature yearly average	27	1	42.91	<b>.0010</b>
Precipitation yearly average	26	1	41.34	<b>.0020</b>
Solar radiation yearly average	25	1	19.36	.0670
Wind speed yearly average	24	1	12.52	.3510
Month count by temp 10	23	1	8.52	.3550
PET wettest quarter	22	1	5.74	.7730
Topographic wetness index	21	1	16.51	.1640
Latitude	20	1	26.60	<b>.0045</b>
Total	19	1	34.12	<b>.0090</b>

<sup>a</sup>Degrees of freedom.



**FIGURE 5** The effect of yearly average temperature on the abundance of haplotype LYR2F.

analysis, we ran models independently for both haplotypes using spatially explicit GLS and confirmed the negative effect of temperature on LYR2F even in a zero-inflated model, while the effect of precipitation on T\_S34SteI was not supported (Data S5). To particularly assess the effect of temperature on the abundance of LYR2F, we used negative binomial generalized linear models with log-link function implemented using glmmTMB, comparing it with the null model using the Chi-sq test ( $\text{Chi-sq} = 22.718$ ,  $\text{df} = 1$ ,  $p < .001$ ; Figure 5).

### 3.3 | Geographic differentiation of *Fontourion glacialis*

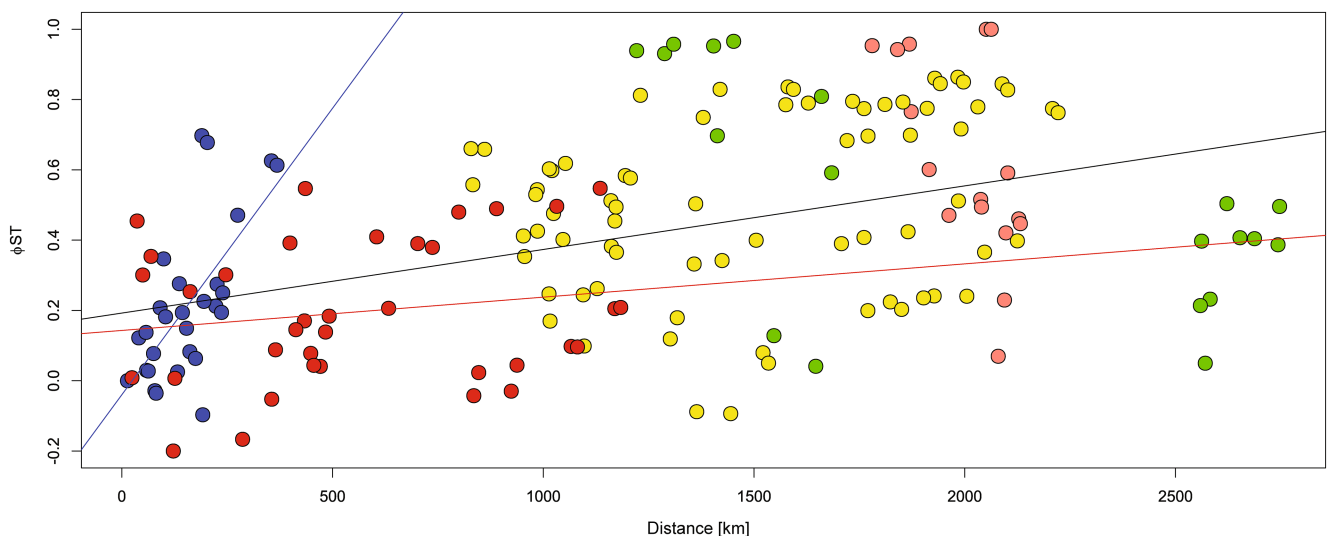
Despite significant correlation between genetic ( $\Phi_{ST}$ ) and geographic distances among sampling sites (see above), we noticed some outliers when nearby population pairs received high  $\Phi_{ST}$  values (Lenangsbreen & Koppangsbreen vs. Steindalsbreen or Svartisen vs. Okstindbreen), while some geographically distant pairs possess low  $\Phi_{ST}$  values (Greenland vs Steindalsbreen or Svalbard glaciers vs. Svartisen; Figure 6). At regional scales, the correlation was significant within Svalbard archipelago (Mantel test:  $r = .6650$ ,  $p = .0035$ ), but not within Scandinavia ( $r = .1694$ ,  $p = .124$ ; Figure 6).

The SAMOVA reached highest  $F_{CT}$  values at 5–7 population clusters ( $k$ ), with ~48% of variation attributable to among-group differentiation, after which the  $F_{CT}$  values dropped and rose again at higher  $k$ , where individual glaciers formed separate groups, providing uninformative clustering (Table 1). Several patterns emerged at  $k = 5$ –7. Population clusters generally reflected geographical proximity but Iceland and SW Greenland populations clustered together with the distant population from Northern Norwegian Steindalsbreen. While Svalbard sites formed a geographically distinct cluster also comprising the central-north Norwegian Svartisen

glacier, possible differentiation was indicated between central-south Svalbard and north-eastern Svalbard (Sorbreen and Nordauslandet with Norfranklinbreen and Goose Buktabreen). Finally, central-west Norwegian glaciers formed a cluster grouped with more northerly Okstindbreen and even Lenangsbreen and Koppangsbreen (depending on  $k$ ; Table 1).

The BARRIER results were consistent with SAMOVA when most prominent barriers were not only located among geographically distant regions but also among some nearby population pairs. The two highest-ranking barriers separated the Scandinavian sites from Greenland–Iceland cluster and Svalbard. However, prominent barriers were also detected between Steindalsbreen and the Lenangsbreen–Koppangsbreen population cluster in northern Norway and between neighbouring Svartisen and Okstindbreen populations in central-northern Norway (Figure 3). The BARRIER run without virtual points provided similar results (Figure S3) with the most prominent barrier again separating Scandinavia from Greenland–Iceland cluster. North-Eastern Svalbard was again separated from Central and Southern Svalbard, this time appearing on the same side of the barrier with Greenland. Apart from that, other barriers between Svalbard and Scandinavia as well as those within Scandinavia appeared consistent with the previous run.

The coalescent analysis of migration connectivity in MIGRATE-n suggested that both the 5- and 7-population cluster datasets are best explained by migration–drift equilibrium models (models c and g) assuming ongoing migration among long-term diverged populations. Other model designs assuming panmixia (a), recent split without migration (b), source–sink scenarios, (d) or recent colonization of Norway from High Arctic (e and f; Table 2) were significantly outperformed. Model (c) indicated higher connectivity of Svalbard with the Greenland cluster than with Scandinavian sites (Figure 3, Table S4) and, in the 7-population design, it also suggested high connectivity



**FIGURE 6** Scatter plot of pairwise geographic and genetic  $\Phi_{ST}$  distances among sampling sites. Colours are used to specifically indicate the pairwise distances among sites within Svalbard (blue), within Scandinavia (red) between Svalbard and Scandinavia (yellow) and between Scandinavia and Greenland and Iceland clusters (green). Linear model fits are presented in black (total dataset), blue (within Svalbard) and red (within Scandinavia) pairwise distances.

of Svalbard with the Svartisen population (Figure S3, Table S4). The remote Greenland and Iceland population cluster appeared intensively interconnected with northern Norway and the central-west Norwegian population cluster exchanged migrants at much higher rate with Okstindbreen and potentially with northern Norwegian sites than with any High Arctic population (Table S4). However, the model (c) allowing variable migration rates among population pairs did not significantly outperform the model (g) assuming equal migration rates, suggesting that our data do not contain enough information to detect significant differences in migration connectivity among population pairs. Therefore, the differences in population pairwise connectivity indicated by the models (c) must be considered with care (Table 2).

Only one value of Tajima's  $D$  and Fu's  $F_s$  indices was significantly negative and, in general, both indices tended to positive values, indicating signals of population admixture, rather than growth (Table 1). EBSP was applied to geographically defined population clusters used in the MIGRATE-n and sufficient mixing and Effective Sample Sizes were obtained apart from combined Greenland-Iceland population clusters and the Steindalsbreen populations where no convergence was achieved despite extended run times and heating schemes. Obtained EBSP plots generally indicated stable populations (Figure 7) with the possible exception in the central-western Norwegian population cluster, where a possible population increase was indicated, and its recent onset ( $\sim 10^{-4}$  mutational time units) may be translated to ca. 4 kya using the northern arthropod COI calibration (Loeza-Quintana et al., 2019).

According to the BEAST estimate using the same clock calibration, the divergence of "Norwegian" haplotype group (Bl19, Bl18, T\_34Ste1, OksAnimixT9, 373Koppa, 353Kring) from the MRCA occurred  $\sim 31$  kya (8–67 kya), while its divergence from the "Svalbard" (Lyr1F1, Lyr2F1) haplotypes occurred  $\sim 55$  kya (C.I.: 16–109 kya); Figure 4. The method of Saillard et al. (2000) estimated the divergence of "Norwegian" group from its MRCA to  $\sim 23$  kya  $\pm 3.4$  kya.

## 4 | DISCUSSION

### 4.1 | Spatial distribution of *F. glacialis* and new data on its distribution

Previously, *Fontourion glacialis* was documented in Norway's Blåisenbreen, south-eastern Greenland and several glaciers in Svalbard, where it typically cohabits with six to seven other tardigrade species and some rotifers (Novotná Jaroměřská et al., 2021; Zawierucha et al., 2020; Zawierucha, Buda, et al., 2018; Zawierucha, Buda, Azzoni, et al., 2019; Zawierucha, Buda, & Nawrot, 2019). Our research has significantly expanded its known range, finding it throughout the entirety of Svalbard, including Nordaustlandet, and, though infrequently, in Iceland. It is also present on all Norwegian glaciers we studied, as the sole tardigrade species in the cryoconite holes there. An interesting parallel is observed with temperate glaciers in the Alps, wherein cryoconite holes are also inhabited by a

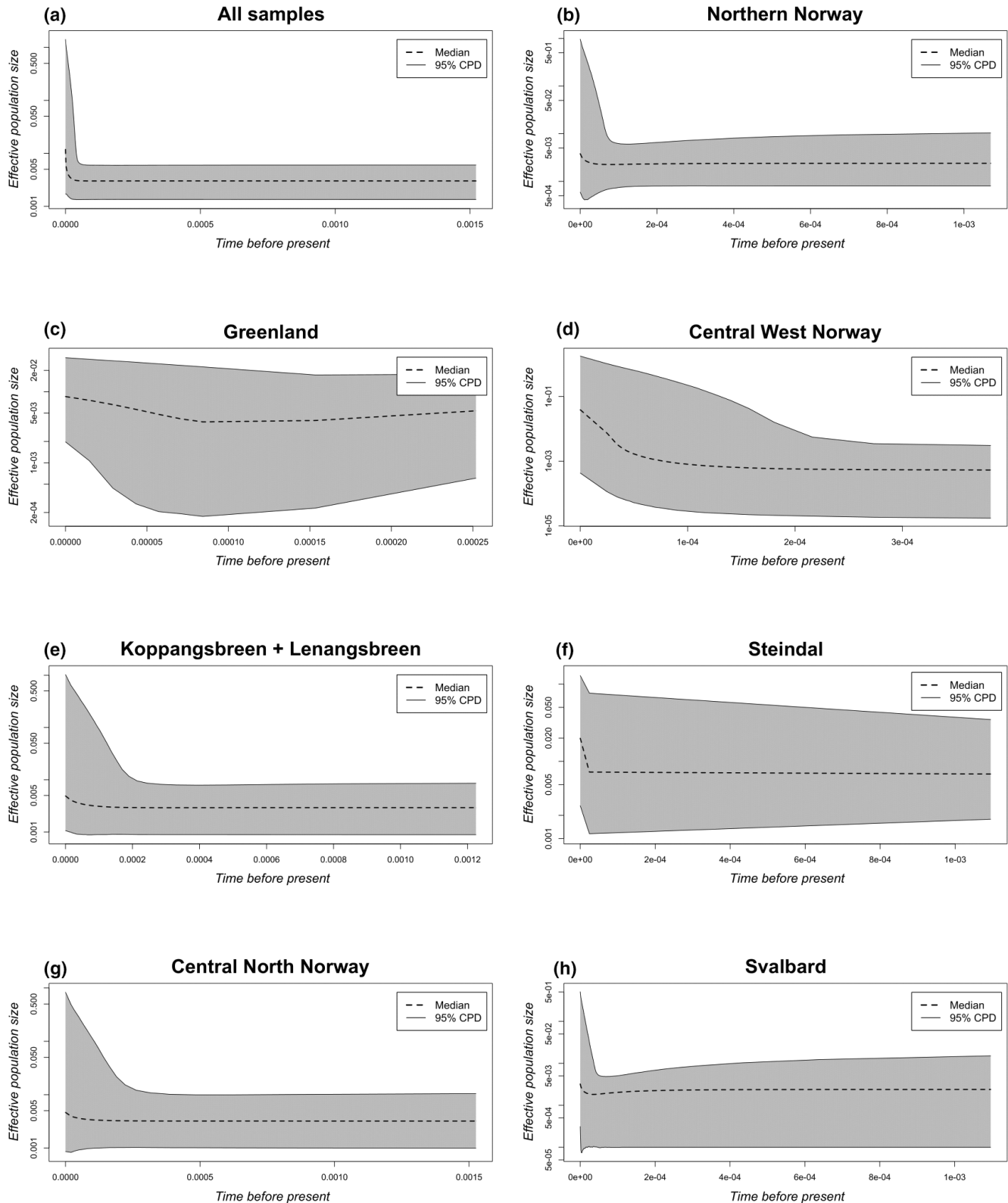
single tardigrade species, *Cryobiotus klebelsbergi* (Zawierucha, Buda, Azzoni, et al., 2019). This pattern of species exclusivity contrasts with the higher species richness typically found in the cryoconite holes of High Arctic glaciers.

### 4.2 | Population differentiation, dispersal and barriers to migration connectivity

Geographical partitioning explained the largest part of variability in the distribution of *F. glacialis* haplotypes. However, beyond spatial structure alone, additional variability was explained by ecological and climatic differences between glaciers and abundance of Lyr2F1 variant significantly negatively correlated with the tested parameter of average temperature. Since habitats of cryoconite holes constantly face similar near-zero temperatures even during summer, the air temperature and other variables evaluated by the ENVIREM dataset likely do not affect therein tardigrades directly. More likely, these variables may affect the supraglacial environment which, together with stochastic events like weather-driven changes in population density (Zawierucha, Buda, & Nawrot, 2019), may drive the changes in local genetic diversity of *F. glacialis* populations and their response to local conditions.

In any case, our results imply that geography rather than climatic variables is the primary factor driving the non-random distribution of *F. glacialis* haplotypes, similar to the pattern of regional clustering also found in other invertebrates inhabiting Arctic and sub-Arctic aquatic habitats, like lakes and ponds (Alfsnes et al., 2016; Hessen et al., 2004). This suggests that distribution of allelic variants may be dispersal-limited even in such microscopic invertebrates, otherwise expected to have high dispersal potential, similar to the patterns explained by the monopolization hypothesis as observed for example, in aquatic microscopic invertebrates (e.g., De Meester et al., 2002). The coalescent analysis supports this view by rejecting the models assuming panmixia as well as recent fragmentation and isolation from historically large populations (MIGRATE-n models a and b) and providing the best data fit by models assuming an interplay between ongoing migrations among regional populations and local drift causing their differentiation (coalescent models c and g).

Such results suggest that dispersal among sampled populations is not potent enough to distribute haplotypes in space uniformly, but still sufficient to prevent the isolation of individual glaciers in Holocene after their split from the large glacial sheets. This aligns with a correlation between genetic and geographic distances, observed on both a total (whole dataset) as well as regional (within Svalbard) scales (Figure 6), which conforms to Isolation by Distance (IBD) scenario (Slatkin, 1993) and is consistent with regionally limited distribution of terminal, that is, more recent, haplotypes, like Lyr1, TSte134 and Bl18 (Figure 3). However, analyses in SAMOVA (Table 1) and particularly BARRIER (Figure 3, Figure S3) also indicated sharp barriers to migration among some nearby sites. In northern Norway, Lenangsbreen and Koppangsbreen, characterized by high frequencies of the common Scandinavian haplotypes Bl19 and TSte134,



**FIGURE 7** Demographic reconstruction within the major geographical population clusters as suggested by the ESBP method. X-axis represents time from the present scaled in mutation-time units, Y-axis the median value and 95% central posterior density (CPD) of population size estimate. A – total dataset; B – Northern Norwegian population cluster; C – Greenland; D – central-west Norwegian population cluster; E – Koppangsbreen and Lenangsbreen population cluster; F – Steindalsbreen population; G – central-north Norwegian population cluster; H – Svalbard population cluster.



notably differed from nearby Steindalsbreen with a high frequency of the common High Arctic allele Lyr19. Neighbouring glaciers in central-northern Norway, Okstindbreen and Svartisen, also notably differed in proportions of “Scandinavian” vs “High Arctic” haplotypes (Figures 3 and 4). Monmonier’s algorithm is robust against false detection of barriers when real data are generated by pure IBD mechanism (Blair et al., 2012), suggesting that such differences among nearby sites reflect biological reality.

These results jointly imply that gene flow among populations of glacial inhabitants like *F. glacialis* may not only be limited by geographical distance and obvious barriers such as open oceans and large ice-free regions, but also by obstacles between closely located glaciers.

### 4.3 | Scandinavian population cluster and the survival through long periods of potential deglaciation

The Scandinavian region hosts unique *F. glacialis* genotypes but differentiation exists between glaciers of the Central West, Central North and Northern Norway (Figure 3, Table 1). While each cluster contains regionally endemic haplotypes (Figure 3), last two regions also hosted “High Arctic haplotypes,” suggesting their links to populations further north (Figure 3, Table 1, Table S4). The full migration model (model c in MIGRATE-n) highlights limited connectivity between central west Norway and other regions, but indicated stronger migration between more northern Norwegian and High Arctic areas, albeit these results should be interpreted cautiously due to our data’s limitations in testing variable migration intensities.

That said, a similar split between populations inhabiting southern/central Scandinavia and more northerly regions with possible admixture has been observed in various other boreal organisms, such as mammals (Bray et al., 2013; Haanes et al., 2011; Horn et al., 2014; Jaarola et al., 1999; Marková et al., 2020), birds (Hansson et al., 2000) or aquatic crustaceans (Hessen et al., 2004) and has been explained by alternative postglacial recolonization routes from distinct glacial refugia. However, inhabitants of glaciers are likely subject to inverse time frame of population expansion/fragmentation since their populations putatively flourished during LGM when terrestrial organisms were restricted to fragmented refugia, while their ranges shrank and fragmented when postglacial expansion of terrestrial species begun. Notwithstanding the substantial differences in dispersal capabilities between these organisms, similarities in their population structure may suggest that the south-central Scandinavian region is not prone to massive immigration events from more northerly regions and generally harbours autochthonous populations of organisms inhabiting various types of habitat, including glaciers.

This finding is intriguing, given temporary disappearance of many sub-Arctic glaciers during the Marine Isotope Stage 11c (~425–395 ka BP) (Irvali et al., 2020; Robinson et al., 2017) and potentially complete deglaciation of Scandinavia during the Holocene climatic optimum (ca. 8–5,000 years ago) (Linge et al., 2020; Nesje et al., 2008). Yet, we observed a distinctive population structure

within Scandinavia and coalescent models rejected the scenario of unidirectional source–sink immigration. Molecular clock analysis suggested that divergence between “Scandinavian” and “High Arctic” alleles and even the divergence of the “Scandinavian” clade itself predates Holocene. This jointly indicates that central-west Norway was not recently colonized from a single northern source, but therein *F. glacialis* population likely survived in situ during the Holocene deglaciation.

How *F. glacialis* survived when all or most debris-free glaciers melted remains unclear. The species might have taken refuge in other habitats like debris-covered or rock glaciers, snowfields or high mountain aquatic bodies in shady places that keep low temperatures during summer, from which it might have expanded as glaciers began to reform. A similar survival strategy is seen in snow and glacier algae, which use cysts to repopulate icy surfaces in subsequent seasons (Hoham & Remias, 2020; Ono et al., 2021). However, existing surveys of periglacial habitats (Zawierucha, Buda, Azzoni, et al., 2019; Zawierucha, Kašparová, et al., 2023) do not indicate that glacier-dwelling tardigrades can persist long-term outside of glaciers.

Alternatively, some Scandinavian glaciers and ice patches might have persisted during the Holocene climatic optimum (Bakke et al., 2010). Considering that populations of glacial tardigrades can flourish in extremely fragmented habitats for extended periods (Zawierucha, Kašparová, et al., 2023), even small ice patches might have acted as refuges from which *F. glacialis* could have expanded to larger, reforming glaciers. Melting and re-establishment of Scandinavian glaciers might have also been asynchronous across regions, allowing *F. glacialis* to survive via “wandering refugial populations,” a concept proposed for the persistence of Antarctic terrestrial and shallow water marine biota (Janko et al., 2007; Kašparová et al., 2015; Thatje et al., 2005). Such a range restriction–extinction–recolonization dynamics at southern regions is in line with demographic analyses showing rather stable population sizes in northern *F. glacialis* populations, and possible population growth of the Central West Norwegian population during the Holocene (Figure 7).

As the climate cooled down again, newly formed glaciers might have been populated by both nearby refugial populations and long-distance migrants, including those from the High Arctic. Indeed, some pairs of neighbouring glaciers in Scandinavia show stark genetic differences with high incidences of High Arctic haplotypes on one side of identified barriers and their rarity or absence on the other side (Figure 3, Table 1). Especially Okstindbreen, one of the few Scandinavian glaciers that may have potentially survived the Holocene climatic optimum (Bakke et al., 2010), hosts local “typically” Scandinavian alleles, while the neighbouring Svartisen glacier appears colonized by the High Arctic phylogroup. Abrupt genetic differentiation among some neighbouring sites might result from just such stochastic extinction–recolonization events rather than being driven by specific environmental conditions, since we have not identified any environmental variables explaining this pattern and no major differences in microbial community compositions, albeit on high taxonomical resolution, have been documented among Scandinavian and Svalbard cryoconite (Millar et al., 2021).

#### 4.4 | Past and future of psychrophilic metazoans on glaciers in changing world climate

Connectivity and extent of glacial ecosystems have dramatically varied over the Pleistocene, posing special challenges for their inhabitants, especially given their currently precarious state in the face of global warming. Similar to other terrestrial tardigrades and rotifers (Fontaneto et al., 2008; Morek et al., 2021), we revealed that populations of glacier-dwelling *F. glacialis* are far from panmixia. Instead, they exhibit complex metapopulation dynamics involving an interplay of dispersal-limited connectivity, historical extinction–recolonization dynamics and occasional long-distance migrations, which, particularly at range margins in lower latitudes, can lead to the swift establishment of locally abundant populations under favourable conditions (De Meester et al., 2002; Hessen et al., 2004). This can promote increased genetic divergence among neighbouring sites, especially when recolonizing migrants reproduce asexually (Fontaneto, 2019; Kokko & López-Sepulcre, 2006). Remarkably, the distribution of tardigrade species is shaped by a multitude of factors beyond just their reproductive mode. For example, while the widespread presence of *Paramacrotus fairbanksi* might imply a connection to parthenogenesis, the asexual species *Acutuncus antarcticus* is exclusively found in Antarctica, underscoring that dispersal capacity, habitat and thermal preferences, and cryptobiotic abilities are also fundamental in dictating tardigrade distribution patterns.

The complexity of patterns revealed by our study underscores the need for multidisciplinary approaches to understand mechanisms creating them. Key areas of focus should include: (1) determining the extent to which communities living on glaciers are strictly glacier-dependent or can thrive in non-glacial habitats; this would benefit from greater integration of metabarcoding approaches across studies, since some markers have insufficient power to differentiate between locally adapted sister species, while others have taxon-specific amplification efficiencies. (2) Better understanding the dispersal mechanisms; indeed, both wind (e.g., Brendonck & Riddoch, 1999; Cáceres & Soluk, 2002; Dabert et al., 2015; Fontaneto, 2019; Jørgensen et al., 2007; Lopes et al., 2016; Ptatscheck et al., 2018) and animals, like birds (e.g., Green et al., 2023; Hessen et al., 2019), are likely dispersal vectors for meiofauna. However, although many birds and mammals actively visit glaciers especially in Scandinavia and other lower latitudes (Rosvold, 2016), their role in dynamics of glacier-dwelling species is worth examining (Dial et al., 2016; Hotaling et al., 2019). Finally, (3) understanding the adaptability and fate of glacial biota should combine phylogeographic analyses with examinations of physiological parameters, like their response to increasing freeze–thaw cycles (Zawierucha, Vecchi, et al., 2023), and continuous monitoring of local glacial populations across seasons and years (Pittino et al., 2018; Zawierucha, Buda, & Nawrot, 2019; Zawierucha, Kašparová, et al., 2023), which unfortunately remain largely unexplored research fields. With many of these organisms newly discovered and their genomes largely unexplored, there is a pressing need

to prioritize genomic studies. These could not only uncover genes key to habitat-specific adaptations but also facilitate the development of new, more variable markers for a nuanced understanding of their microevolutionary processes. The challenge lies in the scarcity of universal primers that extend beyond a few standard markers, often targeting repetitive loci, which presents a unique set of challenges for their application in a phylogenetic context (e.g., Alvarez & Wendel, 2003).

In summary, our results suggest that glacial inhabitants establish regionally distinct population structure that may have locally endured through extended periods of considerable habitat loss, such as during the deglaciation in Scandinavia. This is in line with recent findings that populations of glacier-dwelling tardigrades have the resilience to endure locally in extremely fragmented habitats (Zawierucha, Kašparová, et al., 2023). Hence, just as there is a long-lasting debate about “northern” ice-free refugia hosting terrestrial organisms during the last glaciation (e.g., Kotlík et al., 2006), so our data contribute to the possible existence of “southern” glacial refugia for glacier-dwelling organisms during the Holocene or other warm periods. Understanding these aspects may thus provide crucial insight into the resilience of life in extreme environments and aid in predicting biological responses to future climatic changes.

#### ACKNOWLEDGEMENTS

Authors are immensely grateful to Johanka Anna Janková for her help with sampling and for her patience, when parents left her on side of glaciers while performing other sampling procedures. We are also grateful to Šárka Pelikánová and Petra Šejnohová for kind help in laboratory. The study was supported by the Neuron Endowment Fund to K.J. and M.Š., by Czech Science Foundation no. 22-28778S and Institutional Research Concept RVO67985904 to K.J., M.K, M.D., M.Š., P.H., E.Š.K., National Biodiversity Future Center of Italy, funded by the Italian Ministry of University and Research, PNRR, Missione 4 Componente 2, “Dalla ricerca all’impresa”, Investimento 1.4, Project CN00000033 to DF, Secretaría de Educación, Ciencia, Tecnología e Innovación de la CDMX to JSMA, Norwegian Research Council (project no. 302458 JOSTICE) to J.C.Y., National Science Center grant no. NCN 2013/11/N/NZ8/00597 to K.Z. Samples in Svalbard were collected under RIS no. 10574 and RIS no. 11997.

#### CONFLICT OF INTEREST STATEMENT

Authors declare no conflicts of interest.

#### PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ddi.13859>.

#### DATA AVAILABILITY STATEMENT

Newly obtained haplotypes were deposited into Gene Bank under accession numbers OR898234 – OR898241, OR958747.

## ORCID

Karel Janko  <https://orcid.org/0000-0002-7866-4937>

Jacek Stefaniak  <https://orcid.org/0000-0003-3299-5025>

Miloslav Devetter  <https://orcid.org/0000-0003-1767-4266>

Marco Antonio Jimenez/Santos  <https://orcid.org/0000-0002-1486-9351>

Krzysztof Zawierucha  <https://orcid.org/0000-0002-0754-1411>

## REFERENCES

- Alfsnes, K., Hobæk, A., Weider, L. J., & Hessen, D. O. (2016). Birds, nutrients, and climate change: mtDNA haplotype diversity of Arctic daphnia on Svalbard revisited. *Polar Biology*, 39, 1425–1437. <https://doi.org/10.1007/s00300-015-1868-8>
- Alvarez, I., & Wendel, J. F. (2003). Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution*, 29, 417–434. [https://doi.org/10.1016/s1055-7903\(03\)00208-2](https://doi.org/10.1016/s1055-7903(03)00208-2)
- Anesio, A. M., & Laybourn-Parry, J. (2012). Glaciers and ice sheets as a biome. *Trends in Ecology & Evolution*, 27, 219–225. <https://doi.org/10.1016/j.tree.2011.09.012>
- Bakke, J., Dahl, S. O., Paasche, Ø., Riis Simonsen, J., Kvisvik, B., Bakke, K., & Nesje, A. (2010). A complete record of Holocene glacier variability at Austre Okstindbreen, northern Norway: An integrated approach. *Quaternary Science Reviews*, 29, 1246–1262. <https://doi.org/10.1016/j.quascirev.2010.02.012>
- Beerli, P., Mashayekhi, S., Sadeghi, M., Khodaei, M., & Shaw, K. (2019). Population genetic inference with MIGRATE. *Current Protocols in Bioinformatics*, 68, e87. <https://doi.org/10.1002/cpbi.87>
- Bertolani, R. (2001). Evolution of the reproductive mechanisms in tardigrades — A review. *Zoologischer Anzeiger - A Journal of Comparative Zoology*, 240, 247–252. <https://doi.org/10.1078/0044-5231-00032>
- Blair, C., Weigel, D. E., Balazik, M., Keeley, A. T., Walker, F. M., Landguth, E., Cushman, S., Murphy, M., Waits, L., & Balkenhol, N. (2012). A simulation-based evaluation of methods for inferring linear barriers to gene flow. *Molecular Ecology Resources*, 12, 822–833. <https://doi.org/10.1111/j.1755-0998.2012.03151.x>
- Bosson, J. B., Huss, M., Cauvy-Fraunié, S., Clément, J. C., Costes, G., Fischer, M., Poulenard, J., & Arthaud, F. (2023). Future emergence of new ecosystems caused by glacial retreat. *Nature*, 620, 562–569. <https://doi.org/10.1038/s41586-023-06302-2>
- Bray, S. C. E., Austin, J. J., Metcalf, J. L., Østbye, K., Østbye, E., Lauritzen, S. E., Aaris-Sørensen, K., Valdiosera, C., Adler, C. J., & Cooper, A. (2013). Ancient DNA identifies post-glacial recolonisation, not recent bottlenecks, as the primary driver of contemporary mtDNA phylogeography and diversity in Scandinavian brown bears. *Diversity and Distributions*, 19, 245–256. <https://doi.org/10.1111/j.1472-4642.2012.00923.x>
- Brendonck, L., & Riddoch, B. J. (1999). Wind-borne short-range egg dispersal in Anostracans (Crustacea: Branchiopoda). *Biological Journal of the Linnean Society*, 67, 87–95. <https://doi.org/10.1111/j.1095-8312.1999.tb01931.x>
- Cáceres, C. E., & Soluk, D. A. (2002). Blowing in the wind: A field test of overland dispersal and colonization by aquatic invertebrates. *Oecologia*, 131, 402–408. <https://doi.org/10.1007/s00442-002-0897-5>
- Cameron, K. A., Hodson, A. J., & Osborn, A. M. (2012). Structure and diversity of bacterial, eukaryotic and archaeal communities in glacial cryoconite holes from the Arctic and the Antarctic. *FEMS Microbiology Ecology*, 82, 254–267. <https://doi.org/10.1111/j.1574-6941.2011.01277.x>
- Cardoso, P., Rigal, F., & Carvalho, J. C. (2015). BAT – Biodiversity assessment tools, an R package for the measurement and estimation of alpha and beta taxon, phylogenetic and functional diversity. *Methods in Ecology and Evolution*, 6, 232–236. <https://doi.org/10.1111/2041-210X.12310>
- Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9, 1657–1659. <https://doi.org/10.1046/j.1365-294x.2000.01020.x>
- Cook, J., Edwards, A., Takeuchi, N., & Irvine-Fynn, T. (2016). Cryoconite: The dark biological secret of the cryosphere. *Progress in Physical Geography: Earth and Environment*, 40, 66–111. <https://doi.org/10.1177/0309133315616574>
- Dabert, M., Dastych, H., & Dabert, J. (2015). Molecular data support the dispersal ability of the glacier tardigrade *Hypsibius klebelsbergi* Mihelčič, 1959 across the environmental barrier (Tardigrada). *Entomologische Mitteilungen aus dem Zoologischen Museum Hamburg*, 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. *Frontiers in Ecology and Evolution*, 6, 180. <https://doi.org/10.3389/fevo.2018.00180>
- 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. *Mitteilungen aus dem Zoologischen Museum in Berlin*, 100, 73–100.
- De Meester, L., Gómez, A., Okamura, B., & Schwenk, K. (2002). The monopolization hypothesis and the dispersal–gene flow paradox in aquatic organisms. *Acta Oecologica*, 23, 121–135. [https://doi.org/10.1016/S1146-609X\(02\)01145-1](https://doi.org/10.1016/S1146-609X(02)01145-1)
- de Wit, R., & Bouvier, T. (2006). “Everything is everywhere, but, the environment selects”; what did baas Becking and Beijerinck really say? *Environmental Microbiology*, 8, 755–758. <https://doi.org/10.1111/j.1462-2920.2006.01017.x>
- 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). *Arctic, Antarctic, and Alpine Research*, 48, 199–211. <https://doi.org/10.1657/AAAR0015-042>
- Dray, S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guénard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N., & Wagner, H. H. (2023). adespacial: Multivariate Multiscale Spatial Analysis. R package version 0.3–23. <https://CRAN.R-project.org/package=adespacial>
- Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29, 1969–1973. <https://doi.org/10.1093/molbev/mss075>
- Dupanloup, I., Schneider, S., & Excoffier, L. (2002). A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, 11, 2571–2581. <https://doi.org/10.1046/j.1365-294x.2002.01650.x>
- 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. *Environmental Research Letters*, 8, 035003. <https://doi.org/10.1088/1748-9326/8/3/035003>
- Fenchel, T., & Finlay, B. J. (2004). The ubiquity of small species: Patterns of local and global diversity. *Bioscience*, 54, 777–784. [https://doi.org/10.1641/0006-3568\(2004\)054\[0777:TUOSSP\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2004)054[0777:TUOSSP]2.0.CO;2)
- Ficetola, G. F., Marta, S., Guerrieri, A., Gobbi, M., Ambrosini, R., Fontaneto, D., Zerboni, A., Poulenard, J., Caccianiga, M., & Thuiller, W. (2021). Dynamics of ecological communities following current retreat of glaciers. *Annual Review of Ecology, Evolution, and Systematics*, 52, 405–426. <https://doi.org/10.1146/annurev-ecolsys-010521-040017>
- Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37, 4302–4315. <https://doi.org/10.1002/joc.5086>
- Foissner, W. (2005). Biogeography and dispersal of microorganisms: A review emphasizing protists. *Acta Protozoologica*, 45, 111–136.

- Fontaneto, D. (2019). Long-distance passive dispersal in microscopic aquatic animals. *Movement Ecology*, 7, 10. <https://doi.org/10.1186/s40462-019-0155-7>
- Fontaneto, D., Barraclough, T. G., Chen, K., Ricci, C., & Herniou, E. A. (2008). Molecular evidence for broad-scale distributions in bdelloid rotifers: Everything is not everywhere but most things are very widespread. *Molecular Ecology*, 17, 3136–3146. <https://doi.org/10.1111/j.1365-294X.2008.03806.x>
- Goslee, S., & Urban, D. (2007). The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, 22, 1–19. <https://doi.org/10.18637/jss.v022.i07>
- Green, A. J., Lovas-Kiss, Á., Reynolds, C., Sebastián-González, E., Silva, G. G., van Leeuwen, C. H. A., & Wilkinson, D. M. (2023). Dispersal of aquatic and terrestrial organisms by waterbirds: A review of current knowledge and future priorities. *Freshwater Biology*, 68, 173–190. <https://doi.org/10.1111/fwb.14038>
- Haanes, H., Røed, K. H., Solberg, E. J., Herfindal, I., & Sæther, B. E. (2011). Genetic discontinuities in a continuously distributed and highly mobile ungulate, the Norwegian moose. *Conservation Genetics*, 12, 1131–1143. <https://doi.org/10.1007/s10592-011-0214-0>
- Hall, T. A., & Hall, T. A. (1999). Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. [10.14601/Phytopathol\\_Mediterr-14998u1.29](https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29)
- Hansson, M. C., Bensch, S., & Brännström, O. (2000). Range expansion and the possibility of an emerging contact zone between two subspecies of chiffchaff *Phylloscopus collybita* ssp. *Journal of Avian Biology*, 31, 548–558. <https://doi.org/10.1034/j.1600-048X.2000.1310414.x>
- Hessen, D. O., Jensen, T. C., & Walseng, B. (2019). Zooplankton diversity and dispersal by birds; insights from different geographical scales. *Frontiers in Ecology and Evolution*, 7, 1–11.
- Hessen, D. O., Rueness, E. K., & Stabell, M. (2004). Circumpolar analysis of morphological and genetic diversity in the Notostracan *Lepidurus arcticus*. *Hydrobiologia*, 519, 73–84. <https://doi.org/10.1023/B:HYDR.0000026486.16615.06>
- Hewitt, G. (2000). The genetic legacy of the quaternary ice ages. *Nature*, 405, 907–913. <https://doi.org/10.1038/35016000>
- Hodson, A., Anesio, A. M., Tranter, M., Fountain, A., Osborn, M., Priscu, J., Laybourn-Parry, J., & Sattler, B. (2008). Glacial ecosystems. *Ecological Monographs*, 78, 41–67.
- Hoham, R. W., & Remias, D. (2020). Snow and glacial algae: A review. *Journal of Phycology*, 56, 264–282. <https://doi.org/10.1111/jpy.12952>
- Horn, S., Prost, S., Stiller, M., Makowiecki, D., Kuznetsova, T., Benecke, N., Pucher, E., Hufthammer, A. K., Schouwenburg, C., Shapiro, B., & Hofreiter, M. (2014). Ancient mitochondrial DNA and the genetic history of Eurasian beaver (*Castor fiber*) in Europe. *Molecular Ecology*, 23, 1717–1729. <https://doi.org/10.1111/mec.12691>
- Hotaling, S., Shain, D. H., Lang, S. A., Bagley, R. K., Tronstad, L. M., Weisrock, D. W., & Kelley, J. L. (2019). Long-distance dispersal, ice sheet dynamics and mountaintop isolation underlie the genetic structure of glacier ice worms. *Proceedings of the Royal Society B: Biological Sciences*, 286, 20190983. <https://doi.org/10.1098/rspb.2019.0983>
- Irvali, N., Galaasen, E. V., Ninnemann, U. S., Rosenthal, Y., Born, A., & Kleiven, H. K. F. (2020). A low climate threshold for south Greenland ice sheet demise during the late Pleistocene. *Proceedings of the National Academy of Sciences of the United States of America*, 117, 190–195. <https://doi.org/10.1073/pnas.1911902116>
- Jaarola, M., Tegelström, H., & Fredga, K. (1999). Colonization history in Fennoscandian rodents. *Biological Journal of the Linnean Society*, 68, 113–127. <https://doi.org/10.1006/bjil.1999.0333>
- Janko, K., Lecoindre, G., DeVries, A., Couloux, A., Cruaud, C., & Marshall, C. (2007). Did glacial advances during the Pleistocene influence differently the demographic histories of benthic and pelagic Antarctic shelf fishes? – Inferences from intraspecific mitochondrial and nuclear DNA sequence diversity. *BMC Evolutionary Biology*, 7, 220.
- 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. *Journal of Limnology*, 66, 77–83. <https://doi.org/10.4081/jlimnol.2007.s1.77>
- Kašparová, E., de Putte, A. P. V., Marshall, C., & Janko, K. (2015). Lifestyle and ice: The relationship between ecological specialization and response to Pleistocene climate change. *PLoS One*, 10, e0138766. <https://doi.org/10.1371/journal.pone.0138766>
- Kokko, H., & López-Sepulcre, A. (2006). From individual dispersal to species ranges: Perspectives for a changing world. *Science*, 313, 789–791. <https://doi.org/10.1126/science.1128566>
- Kotlík, P., Deffontaine, V., Mascheretti, S., Zima, J., Michaux, J. R., & Searle, J. B. (2006). A northern glacial refugium for bank voles (*Clethrionomys glareolus*). *Proceedings of the National Academy of Sciences of the United States of America*, 103, 14860–14864. <https://doi.org/10.1073/pnas.0603237103>
- Leigh, J., & Bryant, D. (2015). PopART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6, 1110–1116. <https://doi.org/10.1111/2041-210X.12410>
- Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>
- Linge, H., Nesje, A., Matthews, J. A., Fabel, D., & Xu, S. (2020). Evidence for rapid paraglacial formation of rock glaciers in southern Norway from <sup>10</sup>Be surface-exposure dating. *Quaternary Research*, 97, 55–70. <https://doi.org/10.1017/qua.2020.10>
- 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 Microbiology Ecology*, 93, fix072. <https://doi.org/10.1093/femsec/fix072>
- Loeza-Quintana, T., Carr, C. M., Khan, T., Bhatt, Y. A., Lyon, S. P., Hebert, P. D. N., & Adamowicz, S. J. (2019). Recalibrating the molecular clock for Arctic marine invertebrates based on DNA barcodes 1. *Genome*, 62, 200–216. <https://doi.org/10.1139/gen-2018-0107>
- Lopes, P. M., Bozelli, R., Bini, L. M., Santangelo, J. M., & Declerck, S. A. J. (2016). Contributions of airborne dispersal and dormant propagule recruitment to the assembly of rotifer and crustacean zooplankton communities in temporary ponds. *Freshwater Biology*, 61, 658–669. <https://doi.org/10.1111/fwb.12735>
- Lüdecke, D., Ben-Shachar, M. S., Patil, I., et al. (2021). Performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, 6, 3139. <https://doi.org/10.21105/joss.03139>
- Lutz, S., Anesio, A. M., Edwards, A., & Benning, L. G. (2015). Microbial diversity on Icelandic glaciers and ice caps. *Frontiers in Microbiology*, 6, 307.
- Manni, F., Guérard, E., & Heyer, E. (2004). Geographic patterns of (genetic, morphologic, linguistic) variation: How barriers can be detected by using Monmonier's algorithm. *Human Biology*, 76, 173–190. <https://doi.org/10.1353/hub.2004.0034>
- Marková, S., Horníková, M., Lanier, H. C., Henttonen, H., Searle, J. B., Weider, L. J., & Kotlík, P. (2020). High genomic diversity in the bank vole at the northern apex of a range expansion: The role of multiple colonizations and end-glacial refugia. *Molecular Ecology*, 29, 1730–1744. <https://doi.org/10.1111/mec.15427>
- Millar, J. L., Bagshaw, E. A., Edwards, A., Poniecka, E. A., & Jungblut, A. D. (2021). Polar cryoconite associated microbiota is dominated by hemispheric specialist genera. *Frontiers in Microbiology*, 12, 738451.
- Monmonier, M. (1973). Maximum-difference barriers: An alternative numerical regionalization method. *Geographical Analysis*, 3, 245–261.
- Morek, W., Surmacz, B., López-López, A., & Michalczyk, Ł. (2021). “Everything is not everywhere”: Time-calibrated phylogeography of the genus *Milnesium* (Tardigrada). *Molecular Ecology*, 30, 3590–3609. <https://doi.org/10.1111/mec.15951>
- Nelson, D. R., Guidetti, R., & Rebecchi, L. (2015). Chapter 17 – Phylum Tardigrada. In J. H. Thorp & D. C. Rogers (Eds.), *Thorp and Covich's freshwater invertebrates* (4th ed., pp. 347–380). Academic Press.



- Nesje, A., Bakke, J., Dahl, S. O., Lie, Ø., & Matthews, J. A. (2008). Norwegian mountain glaciers in the past, present and future. *Global and Planetary Change*, 60, 10–27.
- Novotná Jaroměřská, T., Trubač, J., Zawierucha, K., Vondrovicová, L., Devetter, M., & Žárský, J. D. (2021). Stable isotopic composition of top consumers in Arctic cryoconite holes: Revealing divergent roles in a supraglacial trophic network. *Biogeosciences*, 18, 1543–1557. <https://doi.org/10.5194/bg-18-1543-2021>
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2020). *Vegan: Community ecology package*. <https://CRAN.R-project.org/package=vegan>
- Ono, M., Takeuchi, N., & Zawierucha, K. (2021). Snow algae blooms are beneficial for microinvertebrates assemblages (Tardigrada and Rotifera) on seasonal snow patches in Japan. *Scientific Reports*, 11, 5973. <https://doi.org/10.1038/s41598-021-85462-5>
- Padgham, M., & Sumner, M. D. (2021). *geodist:Fast, Dependency-Free Geodesic Distance Calculations. R package geodist version 0.0.7*. <https://CRAN.R-project.org/package=geodist>
- Peterson, B., & Carl, P. (2020). *PerformanceAnalytics: Econometric Tools for Performance and Risk Analysis; R package version 2.0.4*. <https://CRAN.R-project.org/package=PerformanceAnalytics>
- Pinheiro, J., & Bates, D. (2023). *nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-162*. <https://CRAN.R-project.org/package=nlme>
- 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. *Annals of Glaciology*, 59, 1–9. <https://doi.org/10.1017/aog.2018.16>
- Pittino, F., Zawierucha, K., Poniecka, E., Buda, J., Rosatelli, A., Zordan, S., Azzoni, R. S., Diolaiuti, G., Ambrosini, R., & Franzetti, A. (2023). Functional and taxonomic diversity of anaerobes in supraglacial microbial communities. *Microbiology Spectrum*, 11, e0100422. <https://doi.org/10.1128/spectrum.01004-22>
- Poniecka, E. A., Bagshaw, E. A., Sass, H., Segar, A., Webster, G., Williamson, C., Anesio, A. M., & Tranter, M. (2020). Physiological capabilities of cryoconite hole microorganisms. *Frontiers in Microbiology*, 11, 1783.
- Poprawa, I., Hyra, M., & Rost-Roszkowska, M. M. (2015). Germ cell cluster organization and oogenesis in the tardigrade *Dactylobiotus parthenogeneticus* Bertolani, 1982 (Eutardigrada, Murrayidae). *Protoplasma*, 252, 1019–1029. <https://doi.org/10.1007/s00709-014-0737-6>
- Poprawa, I., & Janelt, K. (2019). Reproduction, gonad structure, and oogenesis in tardigrades. *Results and Problems in Cell Differentiation*, 68, 495–513. [https://doi.org/10.1007/978-3-030-23459-1\\_20](https://doi.org/10.1007/978-3-030-23459-1_20)
- Ptatscheck, C., Gansfort, B., & Traunspurger, W. (2018). The extent of wind-mediated dispersal of small metazoans, focusing nematodes. *Scientific Reports*, 8, 6814. <https://doi.org/10.1038/s41598-018-24747-8>
- R Core Team. (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Rambaut, A., Ho, S. Y. W., Drummond, A. J., & Shapiro, B. (2009). Accommodating the effect of ancient DNA damage on inferences of demographic histories. *Molecular Biology and Evolution*, 26, 245–248. <https://doi.org/10.1093/molbev/msn256>
- Remias, D., & Procházková, L. (2023). The first cultivation of the glacier ice alga *Ancylonema alaskanum* (Zygnematophyceae, Streptophyta): Differences in morphology and photophysiology of field vs laboratory strain cells. *Journal of Glaciology*, 69, 1080–1084. <https://doi.org/10.1017/jog.2023.22>
- Robinson, A., Alvarez-Solas, J., Calov, R., Ganopolski, A., & Montoya, M. (2017). MIS-11 duration key to disappearance of the Greenland ice sheet. *Nature Communications*, 8, 16008. <https://doi.org/10.1038/ncomms16008>
- Rosvold, J. (2016). Perennial ice and snow-covered land as important ecosystems for birds and mammals. *Journal of Biogeography*, 43, 3–12. <https://doi.org/10.1111/jbi.12609>
- Rozwalak, P., Podkowa, P., Buda, J., Niedzielski, P., Kawecki, S., Ambrosini, R., Azzoni, R. S., Baccolo, G., Ceballos, J. L., Cook, J., di Mauro, B., Ficetola, G. F., Franzetti, A., Ignatiuk, D., Klimaszuk, P., Łokas, E., Ono, M., Parnikoza, I., Pietryka, M., ... Zawierucha, K. (2022). Cryoconite – From minerals and organic matter to bioengineered sediments on glacier's surfaces. *Science of the Total Environment*, 807, 150874. <https://doi.org/10.1016/j.scitotenv.2021.150874>
- Saillard, J., Forster, P., Lynnerup, N., Bandelt, H. J., & Nørby, S. (2000). mtDNA variation among Greenland Eskimos: The edge of the Beringian expansion. *American Journal of Human Genetics*, 67, 718–726.
- Segawa, T., Matsuzaki, R., Takeuchi, N., Akiyoshi, A., Navarro, F., Sugiyama, S., Yonezawa, T., & Mori, H. (2018). Bipolar dispersal of red-snow algae. *Nature Communications*, 9, 3094. <https://doi.org/10.1038/s41467-018-05521-w>
- 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. *Journal of Biogeography*, 44, 2849–2861. <https://doi.org/10.1111/jbi.13089>
- Shain, D. H., Halldórsdóttir, K., Pálsson, F., Aðalgeirsdóttir, G., Gunnarsson, A., Jónsson, Þ., Lang, S. A., Pálsson, H. S., Steinþórsson, S., & Arnason, E. (2016). Colonization of maritime glacier ice by bdelloid Rotifera. *Molecular Phylogenetics and Evolution*, 98, 280–287. <https://doi.org/10.1016/j.ympev.2016.02.020>
- Shatilovich, A., Gade, V. R., Pippel, M., Hoffmeyer, T. T., Tchesunov, A. V., Stevens, L., Winkler, S., Hughes, G. M., Traikov, S., Hiller, M., Rivkina, E., Schiffer, P. H., Myers, E. W., & Kurzhalia, T. V. (2023). A novel nematode species from the Siberian permafrost shares adaptive mechanisms for cryptobiotic survival with *C. elegans* dauer larva. *PLoS Genetics*, 19, e1010798. <https://doi.org/10.1371/journal.pgen.1010798>
- Shmakova, L., Malavin, S., Iakovenko, N., Vishnivetskaya, T., Shain, D., Plewka, M., & Rivkina, E. (2021). A living bdelloid rotifer from 24,000-year-old Arctic permafrost. *Current Biology*, 31, R712–R713. <https://doi.org/10.1016/j.cub.2021.04.077>
- Sieger, J., Brümmer, F., Ahn, H., Lee, G., Kim, S., & Schill, R. O. (2022). Reduced ageing in the frozen state in the tardigrade *Milnesium inceptum* (Eutardigrada: Apochela). *Journal of Zoology*, 318, 253–259. <https://doi.org/10.1111/jzo.13018>
- Singh, P., Hanada, Y., Singh, S. M., & Tsuda, S. (2014). Antifreeze protein activity in Arctic cryoconite bacteria. *FEMS Microbiology Letters*, 351, 14–22. <https://doi.org/10.1111/1574-6968.12345>
- Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution: International Journal of Organic Evolution*, 47, 264–279. <https://doi.org/10.1111/j.1558-5646.1993.tb01215.x>
- Thatje, S., Hillenbrand, C.-D., & Larter, R. (2005). On the origin of Antarctic marine benthic community structure. *Trends in Ecology & Evolution*, 20, 534–540. <https://doi.org/10.1016/j.tree.2005.07.010>
- Title, P. O., & Bemmels, J. B. (2018). ENVIREM: An expanded set of bioclimatic and topographic variables increases flexibility and improves performance of ecological niche modeling. *Ecography*, 41, 291–307. <https://doi.org/10.1111/ecog.02880>
- Tsujimoto, M., Imura, S., & Kanda, H. (2016). Recovery and reproduction of an Antarctic tardigrade retrieved from a moss sample frozen for over 30 years. *Cryobiology*, 72, 78–81. <https://doi.org/10.1016/j.cryobiol.2015.12.003>
- Valle, B., Cucini, C., Nardi, F., et al. (2021). *Desoria calderonis* sp. nov., a new species of alpine cryophilic springtail (Collembola: Isotomidae) from the Apennines (Italy), with phylogenetic and ecological considerations. *European Journal of Taxonomy*, 787, 32–52. <https://doi.org/10.5852/ejt.2021.787.1599>

- Wang, Y., Naumann, U., Wright, S. T., & Warton, D. I. (2012). Mvabund—An R package for model-based analysis of multivariate abundance data. *Methods in Ecology and Evolution*, 3, 471–474. <https://doi.org/10.1111/j.2041-210X.2012.00190.x>
- Weir, J. T., & Schluter, D. (2004). Ice sheets promote speciation in boreal birds. *Proceedings of the Royal Society B: Biological Sciences*, 271, 1881–1887. <https://doi.org/10.1098/rspb.2004.2803>
- Zawierucha, K., Buda, J., Azzoni, R. S., Niškiewicz, M., Franzetti, A., & Ambrosini, R. (2019). Water bears dominated cryoconite hole ecosystems: Densities, habitat preferences and physiological adaptations of Tardigrada on an alpine glacier. *Aquatic Ecology*, 53, 543–556. <https://doi.org/10.1007/s10452-019-09707-2>
- Zawierucha, K., Buda, J., Jaromerska, T. N., Janko, K., & Gąsiorek, P. (2020). Integrative approach reveals new species of water bears (*Pilatobius*, *Grevenius*, and *Acutuncus*) from Arctic cryoconite holes, with the discovery of hidden lineages of *Hypsibius*. *Zoologischer Anzeiger*, 289, 141–165. <https://doi.org/10.1016/j.jcz.2020.09.004>
- Zawierucha, K., Buda, J., & Nawrot, A. (2019). Extreme weather event results in the removal of invertebrates from cryoconite holes on an Arctic valley glacier (Longyearbreen, Svalbard). *Ecological Research*, 34, 370–379. <https://doi.org/10.1111/1440-1703.1276>
- Zawierucha, K., Buda, J., Pietryka, M., Richter, D., Łokas, E., Lehmann-Konera, S., Makowska, N., & Bogdziewicz, M. (2018). 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. <https://doi.org/10.1007/s10201-017-0528-9>
- Zawierucha, K., Gąsiorek, P., Buda, J., Uetake, J., Janko, K., & Fontaneto, D. (2018). Tardigrada and Rotifera from moss microhabitats on a disappearing Ugandan glacier, with the description of a new species of water bear. *Zootaxa*, 4392, 311–328. <https://doi.org/10.11646/zootaxa.4392.2.5>
- Zawierucha, K., Kašparová, E. Š., McInnes, S., Buda, J., Ambrosini, R., Devetter, M., Ficetola, G. F., Franzetti, A., Takeuchi, N., Horna, P., Jaroměřská, T. N., Ono, M., Šabacká, M., & Janko, K. (2023). Cryophilic Tardigrada have disjunct and bipolar distribution and establish long-term stable, low-density demes. *Polar Biology*, 46, 1011–1027. <https://doi.org/10.1007/s00300-023-03170-4>
- Zawierucha, K., Kolicka, M., Takeuchi, N., & Kaczmarek, Ł. (2015). What animals can live in cryoconite holes? A faunal review. *Journal of Zoology*, 295, 159–169.
- Zawierucha, K., Porazinska, D. L., Ficetola, G. F., Ambrosini, R., Baccolo, G., Buda, J., Ceballos, J. L., Devetter, M., Dial, R., Franzetti, A., Fuglewicz, U., Gielly, L., Łokas, E., Janko, K., Novotna Jaromerska, T., Kościński, A., Kozłowska, A., Ono, M., Parnikoza, I., ... Takeuchi, N. (2021). A hole in the nematosphere: Tardigrades and rotifers dominate the cryoconite hole environment, whereas nematodes are missing. *Journal of Zoology*, 313, 18–36. <https://doi.org/10.1111/jzo.12832>
- Zawierucha, K., Stec, D., Dearden, P. K., & Shain, D. H. (2022). Two new tardigrade genera from New Zealand's southern alp glaciers display morphological stasis and parallel evolution. *Molecular Phylogenetics and Evolution*, 178, 107634. <https://doi.org/10.1016/j.ympev.2022.107634>
- Zawierucha, K., Stec, D., Lachowska-Cierlik, D., Takeuchi, N., Li, Z., & Michalczuk, Ł. (2018). 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*. *Annales Zoologici*, 68, 179–201. <https://doi.org/10.3161/00034541ANZ2018.68.1.007>
- Zawierucha, K., Vecchi, M., Takeuchi, N., Ono, M., & Calhim, S. (2023). Negative impact of freeze–thaw cycles on the survival of tardigrades. *Ecological Indicators*, 154, 110460. <https://doi.org/10.1016/j.ecolind.2023.110460>

## BIOSKETCH

Dr. Karel Janko's research is centred on the genomic consequences of reproductive mode variations and the ecology and evolution of animals in extreme environments, with a particular focus on polar regions. He is affiliated with the Institute of Animal Physiology and Genetics at the Czech Academy of Sciences and with University of Ostrava. His work aims to understand how various reproductive strategies influence genetic diversity and organismal adaptation. Dr. Janko actively participates in field expeditions to the Arctic and Antarctica, enhancing our knowledge about biodiversity and the survival strategies of life in extreme climatic conditions.

Author contributions: Project conceptualization (KJ, DHS, MŠ, KZ), Manuscript drafting (KJ), Sampling (KJ, DHS, MK, EKŠ, MŠ, JR, MD, MAJS, PH, EJD, JB, JCY, KZ), Sample identification (MD, MAJS, JB, KZ), Molecular analysis (DHS, MK, EKŠ, JB, KZ), Phylogenetic and phylogeographic analysis (KJ, MK, PH), Ecological analysis (DF, JS, MD), and Data interpretation (KJ, DHS, DF, MŠ, JR, JS, DOH, MD, JB, JCY, KZ). All authors contributed to manuscript writing and editing.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Janko, K., Shain, D. H., Fontaneto, D., Kaštánková Doležalková, M., Buda, J., Štefková Kašparová, E., Šabacká, M., Rosvold, J., Stefaniak, J., Hessen, D. O., Devetter, M., Jimenez/Santos, M. A., Horna, P., Janková Drdová, E., Yde, J. C., & Zawierucha, K. (2024). Islands of ice: Glacier-dwelling metazoans form regionally distinct populations despite extensive periods of deglaciation. *Diversity and Distributions*, 00, e13859. <https://doi.org/10.1111/ddi.13859>