INTRODUCTION

Protists are a highly diverse group of eukaryotic microorganisms that are distributed in almost all terrestrial and aquatic ecosystems. They play key ecological roles as important primary producers (autotrophic algae) and major predators/consumers of bacteria and other microorganisms (heterotrophic protozoa). Thus, they are crucial components of microbial communities linking lower and higher trophic levels (microbial loop), especially in aquatic habitats (Boenigk & Arndt, 2002; Caron, 2001; Grujcic et al., 2018; Laybourn-Parry & Parry, 2000; Meira et al., 2018; Okuda et al., 2014; Pomeroy et al., 2007).
Despite the formerly common assumption of ubiquitous dispersal of microorganisms ('Everything is everywhere, but the environment selects'; Baas-Becking, 1934; Beijerinck, 1913), some protist taxa were already shown to have dispersal limitations and, thus, show restricted distribution patterns (‘moderate endemicity model’; Foissner, 2006; Martiny et al., 2006; Bass & Boenigk, 2011) potentially reflecting biogeographical history. Such biogeographical distribution patterns can either be driven and influenced by evolutionary or ecological factors, as commonly described for plants and animals (Cox et al., 2016; Fine, 2015; Sanmartin, 2014; Schmitt, 2020; Wiens & Donoghue, 2004). Thus, apart from extant ecological conditions in specific habitats, biogeographical patterns can be strongly influenced by severe historical changes in environmental/climatic conditions, for example, during the Quaternary ice ages (Hewitt, 2000; Lister, 2004; Schmitt, 2007, 2020). The biogeographical patterns of higher organisms were commonly shown to comprise refugial areas and areas of expansion as a result of glaciation-dependent latitudinal/altitudinal shifts of their distribution ranges. Decreasing temperatures and increasing glacialiation in higher latitudes and altitudes forced organisms to shift their distribution ranges to lower latitudes and altitudes or even caused their extinction. Thus, warm-adapted/temperate taxa in the lowlands are supposed to have been forced to warmer areas in the south with post-Pleistocene migration from these lower latitude refugia where they survived glacial periods (Hewitt, 2004; Schmitt, 2007). Cold-adapted taxa are mainly assumed to have survived glacial phases in lower latitudes and altitudes and migrated to arctic and high-mountain refugia during interglacial and post-glacial periods (Hewitt, 2004; Schmitt, 2007). High-mountain ranges were repeatedly shown to play an important role in biogeography as island-like structures for alpine, cold-adapted species with putative lowland bridges between different mountain ranges in glacial periods (Albach et al., 2006; Schmitt & Haubrich, 2008; Schönswetter et al., 2005).

The patterns of alpine biogeography are well studied for higher organisms, especially in Europe, where large mountain ranges (i.e., Alps, Pyrenees, Carpathians) occur prominently across latitudes (Charrier et al., 2014; Ronikier, 2011; Theissinger et al., 2013). Alpine taxa were shown to have found glacial refugia either at lower altitudes in areas surrounding a mountain system (peripheral refugia) or on mountain peaks above the glacial ice shield (nunatak refugia), but there might also be more widespread lowland refugia (Holderegger & Thiel-Egenter, 2009). Since altitude (together with the related ecological factor temperature) is considered the crucial ecological factor limiting dispersal and acts as an important ecological filter, alpine regions are nowadays suggested to be island-like habitats for cold-adapted taxa with strongly restricted dispersal between different mountain ranges. Thus, the distribution of alpinespecific (cold-adapted) genetic lineages potentially provides prime examples to infer shared evolutionary history and/or post-glacial recolonization routes. Shared genetic lineages between different mountain ranges suggest rather a survival of taxa at lower altitudes between mountain ranges during glaciatication followed by retraction into both of them than a post-glacial dispersal between mountain ranges (Schmitt, 2017). In contrast, the exclusive occurrence of a genetic lineage within one single mountain system suggests its survival somewhere in the mountain range or its (post-glacial) evolution within the respective mountain system than a formerly widespread occurrence in the lowlands with its post-glacial retraction to one single area (Schmitt, 2017). However, direct dispersal between mountain ranges most probably by human impacts or appropriate vectors such as migrating birds is a possibility (Figueroa & Green, 2002; Foissner, 2006). Especially protist taxa that can form cysts and other robust dormant stages were predestined for such long-distance dispersal since active cells are often much more vulnerable to unfavorable conditions (Foissner, 2006).

Alpine protist communities on local scales as well as their lowland counterparts are highly diverse. They are supposed to be mainly structured by important environmental factors such as climate conditions, pH, nutrient levels, conductivity/salinity, and habitat size (Filker et al., 2016; Grossmann et al., 2016; Toletti et al., 2003; Triadó-Margarit & Casamayor, 2012; Wu et al., 2009). However, there are additional alpine-specific factors, mainly altitude and the related gradients of environmental conditions such as decreasing temperature and increasing UV radiation with altitude (Seppey et al., 2020; Sommaruga, 2001; Sonntag et al., 2011) facilitating alitudinal gradients of biodiversity. Especially high-mountain lakes are considered to be extremely challenging habitats due to low nutrient availability, low water temperature, and high ultraviolet radiation. These habitats require specific molecular and physiological adaptations of their inhabitants such as photo-protective pigmentation, cold-adapted enzymes, and dormancy stages (Morgan-Kiss et al., 2006; Sláveykova et al., 2016; Stamenković & Hanelt, 2017). The impacts of changing environmental conditions might strongly differ between taxonomic and functional groups and might promote or inhibit the occurrence and distribution of distinct groups: Chrysophyceae were shown to be predominant in lakes with oligotrophic conditions and lower pH values, whereas Cryptophyta were more abundant in lakes with high nutrient levels and higher pH values (Triadó-Margarit & Casamayor, 2012); apart from thermal conditions, Chrysophyceae were also shown to be more influenced by changing nitrate concentrations than Dinophyceae, which are rather influenced by alkalinity and altitude (Toletti et al., 2003); phytoplankton distribution was found to be mainly driven by catchment features and nitrate concentrations, whereas that of zooplankton is also influenced by trophic status and the prevailing phytoplankton structure (Toletti et al., 2006). Extreme conditions in terms of temperature, UV radiation, and nutrient availability in alpine regions might also facilitate diversification and create their specific communities. Geographical gradients and distances were, therefore, assumed to play a minor role in protist distribution (Castleley et al., 2010; Izaguirre et al., 2015). Nevertheless, their importance might increase with increasing isolation of a habitat type as supported by strong biogeographical patterns shown for alpine protist communities on three different continents (Filker et al., 2016).

Although recent studies demonstrated restricted distribution patterns of protist taxa in several habitat types (Azovsky & Mazei,
2013; Bates et al., 2013; Bik et al., 2012; Boenigk et al., 2018; Filker et al., 2016; Olefeld et al., 2020), there is still less known about the large-scale biogeographical patterns of protists and the evolutionary factors shaping and maintaining these communities. Thus, the investigation of protist distribution patterns on a European scale in the light of the well-studied post-glacial distribution patterns of macroorganisms offers a unique opportunity to identify general historical patterns and key protist players on a spatiotemporal scale ranging back to the last glaciation and possibly beyond. Recent studies of protist communities in European freshwater lakes based on sequence data identified biogeographical regions and supported the importance of mountain ranges and geographical distances for protist communities in Europe: High levels of biodiversity throughout European lakes with significant differences in richness, diversity, and taxon inventory between alpine and lowland lakes and a predominant occurrence of areas with high dissimilarity along alpine regions could be identified. This suggested the European mountain ranges as presumable biogeographical islands and dispersal barriers for protist freshwater communities (Boenigk et al., 2018). However, although geographical distances were shown to be relevant for protist dispersal, the mountain ranges as geographical barriers seemed to have only a low impact on structuring distribution patterns. Despite the high levels of endemicity in alpine communities they were supposed to have only a low effect on protist dispersal as derived from distribution patterns in lowland areas (Olefeld et al., 2020).

In this study, we focused on protist communities in 43 alpine lakes (based in parts on the same dataset used by Boenigk et al., 2018 and Olefeld et al., 2020) located in the Alps, Carpathians, Pyrenees, and the Sierra Nevada to answer the following questions: How do alpine protist communities differ from non-alpine ones? Are there differences in biodiversity of protists between alpine specialists and generalists? Are there differences in biodiversity of protists between the mountain ranges (separated genetic lineages in geographically separated mountain ranges)? Is there diversification in alpine lakes?

2 | MATERIALS AND METHODS

2.1 | Sampling and sample processing

Eukaryotic amplicon sequences of samples collected in 244 natural freshwater lakes and ponds across Europe in August 2012 were used in this study from the NCBI BioProject PRJNA414052 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA414052). Sampling, DNA isolation, and sequencing were previously done and described in detail by Boenigk et al. (2018) for two technical replicates per sample. Forward primer (5’-GTA CAC ACC GCC CGT C-3’) and a combination of two reverse primers with different wobble positions (5’-GCT GCG CCC TTC ATC GKT G-3’ (ITS2_Dino; 10%) and 5’-GCT GCG TTC TTC ATC GWT R-3’ (ITS2_broad; 90%) were used to amplify the V9-ITS1 region of the 18S SSU and ITS region of the rDNA. All samples were commercially sequenced using paired-end Illumina HiSeq 2500 sequencing in ‘rapid run’ mode applying 2 × 300 bp reads with subsequent adapter trimming, quality trimming, and demultiplexing (FASTERIS; Geneva, Switzerland).

An additional 12 lakes were sampled from the Balkans region in August 2018. As described by Boenigk et al. (2018) water samples were filtered onto 0.22 µm Isopore Membrane Filters (47 mm diameter, Merck Millipore, Darmstadt, Germany) until the filters clogged (50–500 ml water per filter). Subsequently, filters were air-dried and then immediately frozen in liquid nitrogen (Cryoshippers). The filters were stored at ~80°C in the laboratory until further processing. DNA isolation was conducted in two technical replicates per sample using my-Budget DNA Mini Kit (Bio-Budget Technologies GmbH) following the protocol of the supplier with the following modifications: Filters were homogenized in 800 µl Lysis Buffer TLS within lysing Matrix E tubes (MP Biomedicals) using the FastPrep instrument (MP Biomedicals). Homogenization was run three times for 45 seconds each at a speed setting of 6 m/s and then incubated for 15 min at 55°C. The next steps followed the standard protocol supplied by Bio-Budget Technologies GmbH. The V9 region of the 18S SSU of the rDNA was amplified using forward (5’-GTA CAC ACC GCC CGT C-3’) (Lane, 1991; Stoeck et al., 2010) and reverse (5’-TGA TCC TTC YGC AGG TTC ACC TAC-3’) (Zhang et al., 2015) primers. Samples were commercially sequenced using paired-end Illumina HiSeq 3000/4000 sequencing in ‘Version1’ mode applying 2 × 150 + 8 bp reads with subsequent adapter trimming, quality trimming, and demultiplexing (FASTERIS; Geneva, Switzerland).

2.2 | Sequence analyses

The bioinformatic procession of raw data sequences was performed using the open-source bioinformatics pipeline Natrix (https://github.com/MW55/Natrix, accessed 11/2019, Welzel et al., 2020). After quality filtering and assembly of reads using the Natrix-pipeline, mothur v.1.39.1 (Schloss et al., 2009) was used to check all sequences for orientation (pcr.seqs, reverse.seqs, rdiffs=2 (fwd)) and to cut all sequences to V9 region (pcr.seqs, rdiffs=3 (rev)) including removal of the reverse primer sequence (5’-TGA TCC TTC YGC AGG TTC ACC TAC-3’).

Sequencing results then underwent dereplication based on 100% identity including length variability (CD-HIT-EST algorithm; Fu et al., 2012), chimera removal via VSEARCH uchime3_denovo algorithm (Edgar, 2016; Rognes et al., 2016), and filtering using the AmpliconDuo pipeline (Lange et al., 2015) as implemented in Natrix. Finally, reads were clustered into operational taxonomic units (OTUs) using the SWARM algorithm (Mahé et al., 2015). Representative sequences (SSU fragment V9) of all OTUs were taxonomically assigned by searching the SILVA database r132 (Quast et al., 2013; Yilmaz et al., 2014) as implemented in Natrix. Obtained taxonomic affiliations (pident >90%) were manually revised, partially corrected/harmonized, and questionable levels (uncultured, unidentified, etc.) were removed. All reads assigned to Embryophyta, Dikarya, and Metazoa as well as unassigned reads were excluded from further
analyses. Presence-absence or abundance (sum of sequence numbers of each two split samples) data of V9-SWARMs (OTUs) were used for all subsequent analyses.

2.3 | Diversity analyses

Biodiversity analyses were performed using the R package ‘vegan’ version 2.5-5 (Oksanen et al., 2019) in R version 3.6.3 (R Core Team, 2020; RStudio Team, 2015). OTU-based alpha diversity (richness (specnumber(x)) and Shannon diversity (diversity(x, method = “shannon”))) were calculated per sample. For phylogenetic studies, the representative sequences per V9-SWARM were aligned using the multiple alignment program MAFFT version 7.453 with the progressive FFT-NS-2 method (Katoh & Standley, 2013). A maximum likelihood (ML) tree with rapid bootstraps (100 replicates, GTRGAMMA) was constructed in RAxML Version 8.2.12 (Stamatakis, 2014). Based on this ML tree (phy) and the abundance community matrix (comm) phylogenetic diversity was analyzed in R version 3.6.3 (R Core Team, 2020; RStudio Team, 2015) using the R package ‘picante’ version 1.8 (Kembel et al., 2010). Faith’s phylogenetic diversity (PD = total of the unique branch length in the tree (pd(comm, phy))) (Faith, 1992), mean pairwise distance (MPD, (phy, dist <- cophenetic.phylo(phy)), mpd(comm, phy, dist, abundance.weighted = FALSE)), mean nearest taxon distance (MNTD (phy, dist <- cophenetic.phylo(phy)), mntd(comm, phy, dist, abundance.weighted = FALSE))) (Webb et al., 2002) were calculated according to the developer’s instructions (http://picante.r-forge.r-project.org/picante-intro.pdf, 2010).

2.4 | Environmental parameters

Three environmental parameters were measured for all samples (256 lakes) directly on the sampling site (water temperature, pH, conductivity (EC/TDS)) using a portable ‘Combo tester HI 98129 (Hanna Instruments Deutschland GmbH, Vöhringen). Bioclimatic variables were calculated based on GPS data of the sampling sites using the R package ‘raster’ version 3.0-7 (Hijmans, 2019) in R version 3.6.3 (R Core Team, 2020; RStudio Team, 2015) and the current ‘worldclim’ dataset with a spatial resolution of 2.5 minutes (https://biogeo.ucdavis.edu/data/worldclim/v2.1/base/wc2.1_2.5m_bio.zip, accessed 07/20, based on averaged values for the years 1970–2000 (Fick & Hijmans, 2017)).

2.5 | Biogeographical analyses

For biogeographical analyses, the investigated European lakes were clustered into groups designated as ‘alpine’ and ‘non-alpine (lowland)’ based on their geographical location within mountain ranges (the Alps, Carpathians, Pyrenees, and the Sierra Nevada), their altitude above sea level (m a.s.l.) and additionally an important extreme or limiting environmental factor in high altitudes, the minimum temperature of the coldest month (bio6 variable, https://worldclim.org/data/bioclim.html). After sorting the investigated lakes according to their bio6 temperature the dataset comprised one obvious gap between −8.4°C and −7.9°C. This gap of 0.5°C in the otherwise more or less continuous distribution of temperatures among the sampled lakes coincided largely with an altitude of 1500m a.s.l. and divided most of the high-altitude (>1500m a.s.l.) lakes of the European mountain regions together with some low-temperature Scandinavian lakes (arctic) from the low-altitude (<1500 m a.s.l.) non-arctic ones (Figure A1 in Appendix 1, Table A1 in Appendix 2). Thus, lakes with less or equal −8.4°C (bio6) were classified as ‘alpine’ (bio6 temperature range of −11.6°C to −8.4°C) except the lakes of Scandinavia that were classified as ‘non-alpine’ (arctic lowland) despite low temperatures (bio6 temperature range of −13.2°C to −8.5°C). Lakes with bio6 temperatures equal to or greater −7.9°C were classified as ‘non-alpine’ (non-arctic lowland, bio6 temperature range of −7.9°C to 5.1°C). This classification assigned some low-altitude lakes (<1500 m a.s.l.) to the ‘alpine’ cluster (altitudinal range of 527 m a.s.l. to 3120 m a.s.l., 1656 m a.s.l. on average) due to low bio6 temperatures especially in the Alps and Carpathians, whereas some high-altitude lakes (>1500 m a.s.l.) were assigned to the ‘non-alpine’ cluster (range −3 m a.s.l. to 2378 m a.s.l., 445 m a.s.l. on average) due to higher bio6 temperatures especially in the Pyrenees and Sierra Nevada (Figure A1 in Appendix 1, Table A1 in Appendix 2). Thus, especially the less abundant alpine specialists of the Pyrenees and the Sierra Nevada are likely to be underestimated within our dataset, although there are presumably fewer lakes in the Pyrenees and the Sierra Nevada at all than in the Alps and Carpathians solely based on their total area, meaning that these Sierra Nevada and Pyrenees taxa are possibly globally rare.

The ‘alpine’ cluster included 43 lakes of four mountain ranges (Figure 1): Alps (AL, 29 lakes), Carpathians (CP, 10 lakes), Pyrenees (PY, 1 lake), and Sierra Nevada (SN, 3 lakes), the ‘non-alpine’ cluster comprised the remaining 213 lakes across Europe. Alpine OTUs were classified as ‘specialists’ if they were only detected within one or more lakes in alpine regions and as ‘generalists’ if they occurred additionally within at least one lake in a non-alpine region. Kruskal-Wallis tests (kruskal.test) and linear regression analyses (lm(y~x)) were conducted using R version 3.6.3 and package ‘stats’ v3.6.2 (R Core Team, 2020; RStudio Team, 2015) to detect significant differences (p < 0.05) of environmental parameters and diversity estimates between groups and along altitudinal gradients, respectively.

Binary-State Speciation and Extinction (BiSSE) models (Maddison et al., 2007) were used to compare the evolutionary characteristics (speciation (κ), extinction (μ) and state-transition rates (q)) of different groups of observed taxa (OTUs) (e.g., specialists vs. generalists). BiSSE models were calculated as implemented in the R package ‘diversitree’ v0.9.13 (FitzJohn, 2012) using R version 3.6.3 (R Core Team, 2020; RStudio Team, 2015). First, the ML trees (phy) were forced to be ultrametric by extending their branches (force.ultimetric(phy, method="extend"), R package ‘phytools’ v0.7-20 (Revell, 2012)) as well as to be bifurcated (mult2dil(phy), R package ‘ape’ v5.3 (Paradis & Schliep, 2019)). Based on these trees together with an
appropriate set of binary character states (e.g., generalists/specialists) of each tip the initial full models were constructed (lik <- make.bisse(phy, states)) and ML searches were performed (find.mle(lik, p)) after determining an appropriate starting point (p <- starting.point.bisse(phy)). Full models were compared to constrained ones (e.g., equal speciation rates ($\lambda_0$~$\lambda_1$), Birth/death ($\lambda_0$~$\lambda_1$, $\mu_0$~$\mu_1$, $q_{01}$~0.01, $q_{10}$~0.02)). The best model was chosen based on ANOVA analyses. To assess the stability of the final estimate 1000- step Markov Chain Monte Carlo (MCMC) simulations were performed using the ‘mcmc’ function (R package ‘dissertree’ (FitzJohn, 2012)) with an exponential prior value (prior <- make.prior.exponential(1/(2*($\lambda$-$\mu$))) and the step size ‘w’ obtained as widths range of high-probability regions for observed samples of a short pre-chain (100 steps).

### RESULTS

#### 3.1 | Ecological characterization of sampling sites

The 43 investigated alpine lakes were located in the Alps (29), Carpathians (10), Pyrenees (1), and Sierra Nevada (3) (Figure 1, Table 1). They differ significantly in their environmental conditions from 213 non-alpine lakes distributed predominantly in the lowlands from Scandinavia to Spain, Italy, and the Balkans (Kruskal-Wallis p-values < 0.001, higher altitudes and lower temperatures, conductivity and pH values in alpine compared to non-alpine lakes, Table A2 in Appendix 2). Alpine lakes differ significantly between the four mountain ranges in altitude and maximum temperature of

### TABLE 1 Description of sampled regions with lake numbers and OTU richness; percentages of (non-)specific OTUs are related to total OTU numbers per region

| Region     | ID   | Description | # of lakes | # of OTUs | # of (non-) alpine-specific OTUs (specialists) | # of non-specific alpine OTUs (generalists) | # of region-specific OTUs | # of lake-specific OTUs |
|------------|------|-------------|------------|-----------|-----------------------------------------------|---------------------------------------------|----------------------------|------------------------|
| Alps       | AL   | Alpine      | 29         | 3207      | 730 (23%)                                     | 2477 (77%)                                  | 690 (22%)                  | 654 (20%)              |
| Carpathians| CP   | Alpine      | 10         | 2248      | 419 (19%)                                     | 1829 (81%)                                  | 379 (17%)                  | 375 (17%)              |
| Pyrenees   | PY   | Alpine      | 1          | 303       | 21 (7%)                                       | 282 (93%)                                   | 17 (6%)                    | 17 (6%)                |
| Sierra Nevada | SN | Alpine   | 3          | 557       | 174 (31%)                                     | 383 (69%)                                   | 160 (29%)                  | 155 (28%)               |
| Alpine (total) |     | Alpine  | 43         | 4754      | 1293 (27%)                                    | 3461 (73%)                                  | 1246 (26%)                 | 1201 (25%)             |
| Non-alpine |      | Non-alpine  | 213        | 20,008    | 16,547 (83%)                                  | 16,547 (83%)                                | 16,547 (83%)               | 10,783 (54%)           |
the warmest month (Kruskal-Wallis p-values < 0.001, Table A2 in Appendix 2): All sampled lakes in the Pyrenees and Sierra Nevada that matched our definition of alpine lakes (minimum temperature of the coldest month < -8°C) were located above 2800 m a.s.l., while the sampled lakes of the Alps and Carpathians were predominantly below 2000 m a.s.l.; the maximum temperatures of the warmest month in alpine regions of the Alps, Carpathians and Pyrenees are predominantly below 20°C, but that at Sierra Nevada lakes reached more than 24°C (Table A1 in Appendix 2).

3.2 | Biodiversity and distribution of protist communities

The final dataset of all 256 lakes (Figure 1) contained 118,907,804 sequences clustering into 21,301 eukaryotic OTUs (V9-SWARMs) classified as protists. Taxonomic affiliations of representative sequences per OTU revealed Alveolata (mainly Dinoflagellata, Ciliophora), Stramenopiles (mainly Chrysophyceae, Diatomae), Oïpsthokonta (mainly Chytromycycota), and Archaeplastida (mainly Chlorophyta) as the most abundant and diverse taxa within all investigated lakes (in terms of sequence and OTU abundance, respectively. Minor parts of the communities were classified as Cryptophyceae, Rhizaria (mainly Cercozoa), Excavata, Amoebozoa, Uncertae sedes (mainly Telonema), Centrohelida, Haptophyta, and Piczoa (Figure A2 in Appendix 1, Table A3a, b in Appendix 2).

3.2.1 | Alpine vs. non-alpine lakes

A total number of 4754 OTUs (14,543,467 sequences) was observed within 43 alpine lakes, while the 213 non-alpine lakes comprise a total number of 20,008 OTUs (Table 1). Thus, 3461 OTUs were detected within alpine and non-alpine lakes (generalists), whereas 1293 and 16,547 OTUs were exclusively detected in alpine and non-alpine lakes (specialists), respectively (Table 1, Figure 2a). The proportions of OTUs classified as specialists per lake differed between alpine (10% on average) and non-alpine lakes (30% on average). The OTU-based richness and diversity of alpine protist communities per lake were significantly lower than those of non-alpine communities (Kruskal-Wallis p-values < 0.001, Figure A3a in Appendix 1). Phylogenetic diversity estimates also revealed significant differences between alpine and non-alpine lakes (Kruskal-Wallis p-values < 0.001) in terms of Faith's Phylogenetic Diversity (PD is lower in alpine communities) and Mean Nearest Taxon Distance (MNTD is higher in alpine communities), but not for Mean Pairwise Distance (MPD) (Figure A3b in Appendix 1). Based on linear regression analyses these differences in richness and diversity estimates (except MPD) also revealed significant altitudinal gradients (p-values < 0.001, Figure A4 in Appendix 1). Although the richness per taxon was significantly lower in alpine than non-alpine lakes for all major taxonomic groups (Kruskal-Wallis p-values < 0.05), we found in total comparable proportions of these major taxa in lakes of alpine and non-alpine regions (Figure A2 in Appendix 1, Table A3a, b in Appendix 2). Nevertheless, relative OTU abundances per lake revealed significantly lower proportions for OTUs classified as Amoebozoa, Archaeplastida, and Diatomae and higher proportions for OTUs classified as Incertae Sedis, Ciliophora, and Cryptophyceae (Kruskal-Wallis p-values < 0.05) in alpine compared to non-alpine lakes. Except for the phyllum Piczoa that was only detected in a few non-alpine lakes, alpine- and non-alpine-specific taxa (specialists) could only be identified at higher taxonomic resolution (e.g., Koliella sempervirens, Colpidium sp. aAcq1, Paramecium woodruffi or Hemiamphisiella terricola that were only detected in alpine lakes).

3.2.2 | Mountain ranges

Community composition differed between lakes of different mountain ranges. We detected considerable amounts of region-specific OTUs (6–29% of all OTUs per mountain range (Table 1). A total number of 3595 alpine OTUs (76% of all alpine OTUs, 1,110,055 alpine sequences) was not shared between the investigated alpine regions (Figure 2b, groups AL, CP, PY, SN), although many of them were also detected in non-alpine lakes (2349 OTUs). The proportions of these non-shared OTUs per lake ranged from 5% (Gigerwaldsee, Switzerland) to 75% (Štrbské Pleso, Slovakia). They amount on average to more than 30% in lakes of the Alps, Carpathians, and the Sierra Nevada, but only 13% in the lake of the Pyrenees (Figure A5b in Appendix 1). Summarized proportions of non-shared OTUs per mountain range revealed even higher values of 66% (Alps), 54% (Carpathians), and 45% (Sierra Nevada), but 13% for the single Pyrenees lake (Figure 3, Table A3c in Appendix 2), since most of them were exclusively detected within one single lake (Table 1). Nevertheless, 1159 alpine OTUs (24%) were shared between mountain ranges in different combinations, but only 82 of them (2%) were detected in lakes of all four mountain ranges (Figure 2b). Only one OTU was detected within all investigated alpine lakes (classified as Kathablepharidae). The highest number of shared alpine OTUs was detected between lakes of the Alps and Carpathians (994 OTUs), much less between both of them and lakes of the Pyrenees plus Sierra Nevada (207–268 OTUs) and least between lakes of the Pyrenees and Sierra Nevada (93 OTUs, Figure 2c).

Lakes of all mountain ranges comprise the major taxonomic groups in similar proportions per mountain range (Table A3a, b in Appendix 2) and lake (Figure A5c in Appendix 1). Significant differences of OTU proportions for these major taxonomic groups per lake between the mountain ranges (Kruskal-Wallis p-values < 0.05) could be observed for Archaeplastida and Cryptophyceae: Sierra Nevada lakes comprise a higher proportion of OTUs classified as Archaeplastida but a lower proportion of OTUs classified as Cryptophyceae than lakes of the other mountain ranges. No significant differences in OTU-based richness and diversity, as well as phylogenetic diversity estimates (PD, MPD, MNTD), could be observed between mountain ranges (Figure A3a in Appendix 1).
FIGURE 2  Venn diagrams showing shared OTUs of alpine and non-alpine lakes (a) and lakes within the four alpine regions (b) (AL, Alps; CP, Carpathians; PY, Pyrenees; SN, Sierra Nevada) and non-alpine regions; (underlined) bold, italic and standard numbers describe OTU numbers of (region-specific) alpine specialists, alpine generalists and lowland specialists, respectively; (c) network graph showing shared OTUs between mountain ranges, numbers within brackets are OTUs classified as specialists and generalists, respectively, node size reflects total OTU numbers per mountain range and edge width number of shared OTUs; bold, italic and standard numbers describe OTU numbers of alpine specialists, alpine generalists and total alpine OTUs per region and shared group, respectively
3.3 | Biogeography of alpine specialists and generalists

Protist communities of alpine lakes comprised OTUs classified as alpine specialists and generalists differing significantly in OTU richness and diversity estimates: Compared to alpine generalists the alpine specialists within all mountain ranges comprised significant lower richness and Shannon diversity estimates per lake as well as significantly lower phylogenetic diversity (PD), but higher mean nearest taxon distances (MNTD) per lake (Kruskal-Wallis p-values < 0.0001); no significant differences could be observed in mean pairwise distances (MPD) (Figure A3 in Appendix 1). Proportions of alpine OTUs classified as specialists and generalists per mountain range differed from each other with 23%, 19%, 7%, and 31% alpine-specific OTUs (specialists) in lakes of the Alps, Carpathians, Pyrenees, and Sierra Nevada (Table 1, Figure 3). However, the OTU numbers and proportions of alpine specialists and generalists per lake revealed no significant differences between the mountain ranges (Kruskal-Wallis p-values > .2, Figure A5 in Appendix 1).

3.3.1 | Alpine specialists

About one-fourth of the alpine OTUs (1293 OTUs/27%) were exclusively detected in alpine lakes and therefore classified as alpine specialists. However, these 1293 alpine-specific OTUs include only 0.7% of all alpine sequences and thus, represent predominantly rare taxa (in terms of sequence abundance with an average of 80 sequences and a maximum number of 7000 sequences per OTU). About 10% of all alpine OTUs per lake (2% of all alpine sequences per lake) were on average classified as alpine specialists (Figure A5a in Appendix 1). In total the lakes of the Sierra Nevada and the Pyrenees revealed the highest (31%) and lowest (7%) proportions of OTUs classified as alpine specialists (alpine-specific OTUs per mountain range), respectively (Table 1). No alpine specialists could be detected within two lakes of the Alps (Eibsee (986m) and Großer Arbersee (935 m), Germany) and one lake of the Carpathians (Lacul Bâlea (2004 m), Romania, Figure A5 in Appendix 1). Most of the alpine specialists were exclusively detected within one single mountain range (1246 region-specific OTUs, 96% in total) or even within one single lake (1201 lake-specific OTUs, 93% in total; Table 1). In summary, more than 90% (Alps, Carpathians, and the Sierra Nevada) and 80% (Pyrenees) of the OTUs classified as alpine specialists per mountain range were region-specific, whereas only minor parts were shared between mountain ranges (47 OTUs in total, 5–20% of all alpine-specific OTUs per mountain range) (Figures 2b,c and 3).

Protist communities of the Alps and Carpathians shared 33 OTUs classified as alpine specialists (Figure 2c), whereas 29 of them were exclusively detected within these two mountain ranges (AL-CP). Two more alpine-specific OTUs were shared each with lakes in the Pyrenees (AL-CP-PY) and Sierra Nevada (AL-CP-SN). Lakes of the Sierra Nevada shared an additional seven and five alpine-specific OTUs with lakes in the Carpathians (CP-SN) and Alps (AL-SN), respectively, but none with the investigated Pyrenean lake. Two more alpine-specific OTUs were shared between the Pyrenean lake and lakes in the Alps (AL-PY) (Figure 2b). No alpine-specific OTUs could be detected that were shared by lakes of all four mountain ranges (AL-CP-PY-SN).

The alpine-specific community within all mountain ranges comprised the major taxonomic groups that were also detected within the entire alpine community (except Haptophyta) in comparable proportions (Table A3a,b in Appendix 2). However, the proportions strongly differed between lakes within each mountain range (Figure A5c in Appendix 1). The 47 alpine-specific OTUs that were exclusively detected between mountain ranges were affiliated to Alveolata (13, mainly Ciliophora (8) and Dinoflagellata (3)), Archaeplastida (12, Chlorophyta (9) and Charophyta (3)), Opisthokonta (12, mainly Chytridiomycota (7) and Holozoa (4)), Stramenopiles (9, mainly Chrysophyceae (5)) and Rhizaria (1, Cercozoa), whereas the region-specific cluster (non-shared) comprise all major taxonomic groups of the entire alpine dataset.

No significant differences in richness and diversity, as well as phylogenetic diversity estimates (PD, MPD, and MNTD) of alpine specialists, could be detected between mountain ranges (Kruskal-Wallis p-values >.5).

3.3.2 | Alpine generalists

Approximately three fourth of the alpine OTUs (3461 OTUs/73%) were detected in alpine and non-alpine lakes and therefore classified as alpine generalists (Figure 2a). These OTUs include 99.3% of all alpine sequences and, thus, represent more abundant taxa than alpine specialists (in terms of sequence abundance with 4200 (compared to 80) sequences per OTU on average and a maximum number of 650,000 sequences (compared to 7000)). Thus, protist communities of alpine lakes were dominated by generalists in terms of OTU and sequence abundance (on average 90% of all OTUs and 98% of all sequences per lake (Figure A5 in Appendix 1)).

Although the OTUs classified as generalists were detected in alpine and non-alpine regions, they might be specific for one distinct alpine region (2349 OTUs, Figure 2b). The percentages of such alpine region-specific OTUs amount to 67% in total of all alpine generalists (57% (Alps), 45% (Carpathians), 8% (Pyrenees), 24% (Sierra Nevada), Figure 3) and reached on average about 25% per lake (Figure A5 in Appendix 1). Only about one-third of the alpine OTUs classified as generalists were shared between mountain ranges. Nevertheless, with 1112 shared OTUs generalists made the major part of the overall 1159 alpine OTUs shared between mountain ranges. They also included all 82 OTUs that were shared by all four investigated mountain ranges (Figure 2b, type AL-CP-PY-SN). These 82 OTUs included 45% of all alpine sequences (80,000 alpine sequences on average ranging from 1 to 84% of the sequences per lake) and comprised the most abundant alpine OTUs (in terms of total sequence abundance with >494,000 sequences) classified as Ciliophora (Strombidium), Kathablepharidae and Dinophyceae (Woloszynskia). One OTU was
detected within all alpine and 212 non-alpine lakes (classified as K. Kathablepharidae), 19 OTUs occurred in more than 200 lakes and only seven in less than 20 lakes, whereas 17 OTUs occurred in more than 30 alpine lakes and only three in less than five alpine lakes.

Similar to the observations made for alpine specialists, the highest number of shared generalist OTUs was detected between lakes of the Alps and Carpathians (961 generalist OTUs including 653 OTUs exclusively shared between these two mountain ranges) and only about one fourth each between both of them and lakes of the Pyrenees and Sierra Nevada (205–261 OTUs), but even 93 generalist OTUs were shared between lakes of the Pyrenees and Sierra Nevada (Figure 2c).

Lakes of the Alps and Carpathians were dominated by OTUs classified as generalists occurring in one or two mountain ranges (on average 56% and 61% per lake, 87% and 83% in total, predominantly group AL-CP), whereas lakes of the Pyrenees and the Sierra Nevada comprise higher proportions of generalist OTUs occurring in more than two mountain ranges (on average 71% and 64% per lake, 71% and 54% in total, Figure 3, Figure A5b in Appendix 1).

There were no significant differences in OTU-based richness and diversity as well as phylogenetic diversity (PD) and mean nearest taxon distances (MNTD) between mountain ranges. However, mean pairwise distances (MPD) of alpine generalists were slightly higher in lakes of the Pyrenees and the Sierra Nevada than in those of the Alps and Carpathians (Kruskal-Wallis p-value < 0.05) (Figure A3b in Appendix 1).

3.4 Evolutionary characteristics of alpine protist communities

The results of Binary-State Speciation and Extinction (BiSSE) models revealed diversification in generalists and widely distributed taxa and almost no diversification in specialists and geographically restricted (in terms of altitude, latitude, and longitude) taxa (Figure 4). Averaged transition rates from generalists toward specialists were about 3- to 7-fold higher than in the opposite direction. Similar patterns were also found for the major taxonomic groups (Archaeplastida, Opisthokonta, Ciliophora, Dinoflagellata, Chrysophyceae, and Diatomeae), but the rate values differed between the taxonomic groups (Figure A6 in Appendix 1).

4 DISCUSSION

Alpine lakes of the four European mountain ranges Alps, Carpathians, Pyrenees, and the Sierra Nevada were shown to comprise a high protist diversity (Figure 2, Figure A3 in Appendix 1) comprising all major taxonomic groups that were also detected in non-alpine lakes (Figure A2 in Appendix 1). In line with previous studies in similar regions (Bock et al., 2018; Filker et al., 2016; Grossmann et al., 2016; Kammerlander et al., 2015; Ortiz-Álvarez et al., 2018; Triadó-Margarit & Casamayor, 2012) the investigated alpine communities were dominated by OTUs (and sequences) classified as...
Alveolata (mainly Ciliophora and Dinoflagellata (Dinophyceae)) and Stramenopiles (mainly Chrysophyceae) (Figure A5c, Figure A2 in Appendix 1). Due to their small cell sizes, motility (flagellates), and physiological properties members of these taxa (especially Chrysophyceae and Dinophyceae) are assumed to be well-adapted to live under low-temperature and low-nutrient conditions that are commonly found in (high) alpine lakes (Kammerlander et al., 2015; Ortiz-Álvarez et al., 2018; Tolotti et al., 2003). The high sequence abundances of these taxa observed here in almost all alpine lakes compared to other taxa and a significantly higher relative sequence abundance of Chrysophyceae in alpine lakes (11% on average) compared to non-alpine lakes (7.5% on average). Kruskal-Wallis p-value < 0.05, data not shown) supported these assumptions and indicated their success in such extreme environments. Especially their common ability to live mixotrophically and to form resting cells such as cysts in unfavorable conditions were previously shown to be advantageous in oligotrophic high-mountain lakes (Kammerlander et al., 2015; Waibel et al., 2019). Consequently, taxon inventory and community composition on OTU level differed between alpine and non-alpine regions, mountain ranges, and lakes within each mountain range (Figure 2, Figure A5c in Appendix 1) indicating restricted distribution patterns of most of the detected taxa.

Although there were large differences in OTU inventory between the mountain ranges (Figure 2b), but also between lakes within one region (Figure A3a in Appendix 1), no significant differences in richness and diversity per lake could be observed between the four different mountain ranges suggesting basic comparability of the investigated alpine habitats in terms of general living conditions and harshness of the environment.

### 4.1 Altitudinal diversity gradients of protist freshwater communities

Our results revealed significantly lower richness and diversity of protist freshwater communities in alpine than non-alpine lakes (Figure A3a in Appendix 1). The resulting altitudinal diversity gradient of protist freshwater communities across Europe (Figure A4 in Appendix 1) verified the findings of previous studies (Boenigk et al., 2018; Macingo et al., 2019; Olefeld et al., 2020). This matched the classical patterns of macroorganisms with an overall decreasing richness along altitudinal gradients of environmental conditions (Amori et al., 2019; Peters et al., 2016; Rahbek, 1995). However, the patterns of single taxa might strongly differ from each other since the general trend only shows interference of all taxa (Peters et al., 2016). Bryant et al. (2008) could show, that bacterial taxa rather show a monotonal decrease of richness with increasing altitude, while plants and animals often tend to follow a unimodal pattern with the highest richness in mid-altitudes (Bryant et al., 2008; Peters et al., 2016). The patterns observed here for major taxonomic groups of protists rather suggests a monotonical decrease of protist richness and diversity (linear regression p-values < 0.05, data not shown) similar to those shown for bacteria, but they still might differ on lower taxonomic levels.

The trends of decreasing richness and diversity with increasing altitude could also be observed within the alpine regions (altitudinal range 500 to 3100 m a.s.l.), where they were mainly driven by alpine generalists with significantly decreasing richness and diversity with increasing altitude (linear regression p-value < 0.01, data not shown). Contrary to our results, Grossmann et al. (2016) found no decrease in protist richness along an alpine elevation gradient in the Alps (429 to 2072 m a.s.l., 29 lakes). This might be probably caused by a different and presumably less resolving sequencing technology (454 compared to Illumina HiSeq) and differences in the setup of sampling (smaller sampling area and fewer lakes in their study). Within single mountain ranges investigated here significant diversity gradients could only be detected for OTU richness and Shannon diversity within the Alps (532–2785 m a.s.l., linear regression p-values < 0.05), whereas not more than slight trends could be found within the Carpathians (527–2030 m a.s.l.).

As expected, Faith’s Phylogenetic Diversity (PD), representing the sum of branch lengths connecting all OTUs within a phylogenetic tree (phylogenetic distances), was strongly correlated to OTU-based richness (linear regression p-value: < 0.001, R-squared: 0.91) and thus, revealed a comparably decreasing altitudinal gradient. Although the Mean Pairwise Distances (MPD) per lake did not show any significant trend across altitudes, there was a significant increase of Mean Nearest Taxon Distances (MNTD) with altitude. We interpret this as an effect of the decreased richness in alpine regions, which still comprise the full range of major taxonomic groups also found in non-alpine lakes (resulting in comparable MPD values), but fewer closely related species per taxonomic group due to a reduction of potential niches to be occupied in alpine regions (resulting in higher MNTD values). Such a reduction of potential niches could be caused by the widening of niche breadths with higher altitude (Rasmann et al., 2014) and, consequently, higher competition would reduce the possible number of species coexisting in alpine lakes. Testing this altitudinal niche breadth hypothesis in protists seems a worthwhile field of study, although the definition of niche is crucial since the temperature niches of alpine specialists seem to be smaller (see below).

### 4.2 Ecological patterns

Altitudinal gradients of environmental conditions such as temperature and UV radiation are known as important ecological factors structuring community composition across altitudes (Sommaruga, 2001; Sonntag et al., 2011). Especially the significantly lower temperatures in alpine than non-alpine lakes (Table A2 in Appendix 2) are here suggested to facilitate the observed shifts in community composition between alpine and non-alpine lakes. Although temperature within a mountain range usually decreases with altitude, there might also be microclimatic changes independent of altitude, but influenced by other local conditions (e.g., slope and shading). Thus, the classification of ‘alpine’ conditions solely according to altitude seems not sufficient here. Since temperature is commonly supposed
as the most important factor of altitudinal diversity gradients (Peters et al., 2016), we decided to define and classify alpine lakes according to the minimum temperature of the coldest month. Lakes with less extreme conditions due to higher minimum temperatures (>−8°C) were excluded from the alpine dataset, even if they are located in higher altitudes (>1500 m a.s.l.) and lakes at lower altitudes (<1500 m a.s.l.) were included if they experience low minimum temperatures (<−8°C) during a year (survival under ice and snow). Members

**FIGURE 4** Estimation of evolutionary characteristics for generalists (0) and specialists (1) as well as widely distributed (0) and more restricted (1) taxa (altitude, latitude, and longitude) using Binary-state speciation and extinction (BISSE) models providing distinct speciation (λ), extinction (μ), and state-transition (q) rates per state; diversification rates (div) were calculated as a difference of speciation and extinction rates; posterior probability density was calculated by 1000-step Markov Chain Monte Carlo (MCMC) simulations.
of alpine communities are expected to be either cold-adapted or at least cold-tolerant. Usually, the cold-adapted specialists are supposed to have narrow niche widths and thus, fewer dispersal capabilities along temperature gradients than cold-tolerant generalists with much wider niche widths (Kassen, 2002). This could be verified in our study by high proportions of the region- and lake-specific alpine specialists (cold-adapted) and high numbers of alpine generalists (cold-tolerant) with wide distribution in alpine and non-alpine lakes (Figure 3, Figure A5 in Appendix 1).

### 4.2.1 Alpine generalists

Wide distribution ranges of alpine generalists in alpine and non-alpine lakes indicate high dispersal capabilities as a result of wide tolerance ranges toward diverse environmental conditions allowing survival even in increasingly extreme environments like the alpine one. Thus, alpine generalists are highly likely to be able to move easily between mountain ranges and lowlands as supported by high proportions of shared OTUs within alpine generalists (Figure 2c, Figure 3) and their wide distribution patterns in lowland lakes. However, since about two-thirds of alpine generalists were only detected in one of the investigated mountain ranges (Figure A5 in Appendix 1), direct movement between mountains is unlikely. On the other hand, the high relative sequence abundances of OTUs shared by all (on average 44% of all sequences per lake) or at least three mountain ranges (on average 25% of all sequences per lake) suggested an overall dominance of widely distributed generalists in alpine freshwater communities and a more or less free dispersal of these protists via lowland lakes connecting different mountain ranges.

### 4.2.2 Alpine specialists

Alpine specialists were predominantly shown to be low in abundance (based on OTU and sequence abundances), but they make the crucial part of the alpine communities distinguishing them from that of non-alpine lakes. On average alpine lakes comprise lower proportions of specific OTU (about 10% alpine-specific OTUs per lake) and sequence proportions (about 2% per lake) than non-alpine lakes with about 30% lowland-specific OTUs per lake (about 6% of all sequences per lake). It is questionable if this could probably be an effect of lower numbers of investigated lakes within the alpine (43) than the non-alpine regions (213) in our dataset. However, apart from naturally given differences in the area of both regions, subsampling of non-alpine lakes to reach equal numbers of lakes would not only decrease the total numbers of non-alpine specialists per subsample without any effect on numbers per lake but would also create false-positive alpine specialists still occurring evidently in other non-alpine lakes that are not part of the respective subsample. Thus, the classification of real alpine and non-alpine specialists was proposed to get more accurate the more lakes are included. Although non-alpine regions comprised a much larger area than alpine regions, the overall density of investigated alpine lakes seemed to be equal to that of non-alpine lakes (Figure 1). Minimum distances between alpine lakes were even significantly lower than between non-alpine lakes (Kruskal-Wallis p-value = 0.001).

Mountain ranges are often considered as biogeographical islands for alpine specialists (Schmitt, 2017) as supported by high levels of endemism within alpine specialists (Table 1, Figure 3) and a restricted distribution for the great majority of detected alpine specialists (Figure 2b, Figure 3). High proportions of the lake- and region-specific OTUs (Table 1, Figure 3) indicate a separation of mountain ranges and suggested that the lowlands in between are putative dispersal barriers for cold-adapted alpine specialists. Thus, the question is whether alpine specialists are dead-end or whether they actively speciate.

### 4.3 Evolutionary patterns

Alpine specialists are considered to have either evolved continuously from lowland progenitors or radiate and disperse within and between mountain ranges. If they are restricted to one distinct, formerly glaciated, alpine region, they can either (re-)colonize them post-glacially from glacial refugia in lower areas (peripheral or lowland refugia) or glacial refugia within the mountain systems (nunatak refugia) (Holderegger & Thiel-Egenter, 2009; Schmitt, 2020). These hypotheses have been studied extensively for plants and animals but rarely for protists and provided the basis for our understanding of frequent and prominent alpine radiations for larger organisms (Hughes & Atchison, 2015). Our analysis of protists across European mountain systems paints a different picture for protist taxa, which lack alpine specialist radiations, at least in Europe. High amounts of region-specific alpine specialists (96%) and low levels of shared alpine specialists between mountain ranges (4%) as shown here (Figure 3) rather indicate colonization of each mountain region from separate glacial refugia than parallel colonization of mountain regions from a common pool of specialists surviving in lowland glacial refugia. Whether this colonization occurred from periglacial lakes comparable to plant refugia (Schönswetter et al., 2005) or whether they survived within the respective mountain systems as shown for several plant species (Holderegger & Thiel-Egenter, 2009; Schönswetter et al., 2005; Stehlik et al., 2002) could not be concluded for protist taxa based on our results, although suitable habitats would be more difficult to imagine and survival as dormant stages in lakes under ice would be a more probable scenario. Survival of glacial periods in peripheral and lowland refugia was commonly shown to result in shared genetic lineages between different mountain systems since they are highly likely to serve as lowland bridges for cold-adapted species during glacial periods followed by a post-glacial retreat into different mountain refugia. Such overlaps in community composition (shared OTUs) were here mainly observed in alpine generalists and especially between lakes located in the Alps and
Carpathians (Figure 2c), but only 4% of the alpine specialists were detected within two or more mountain ranges. Thus, protists classified as alpine generalists matched the patterns commonly found in alpine macroorganisms with identical genetic lineages found in different mountain ranges that were retrieved from shared glacial lowland refugia (Paun et al., 2008; Schmitt, 2017; Triponez et al., 2011). This suggests that cold tolerance is widespread among protists, possibly by being dormant in cold phases, and alpine specialists are rather characterized by lack of heat stress tolerance excluding them from lowlands, which suggests considerable conservation concern with the warming climate.

Nevertheless, there were at least 47 OTUs classified as alpine specialists, which were shared between lakes of different mountain systems (Figure 2c), conforming to the pattern potentially caused by shared glacial refugia in lower altitudes and a retraction into different mountain ranges as post-glacial refugia (Schmitt, 2020; Stewart et al., 2010). Nevertheless, a putative post-glacial dispersal of alpine specialists across mountain ranges could not be excluded, since many of the detected taxa (especially ciliates and flagellates) can form cysts facilitating the long-distance dispersal capabilities (Foissner, 2006). The Alps are considered Europe's most important high-mountain system with biogeographical links to all other European mountain systems in the surrounding sharing identical genetic lineages (e.g., Paun et al., 2008; Schmitt, 2017; Triponez et al., 2011). The strongest connection between mountain ranges in terms of alpine specialists within protist freshwater communities could be observed here between lakes of the Alps and Carpathians (33 OTUs) and less between the other mountain ranges (Figure 2c).

Our model of source-sink dynamics between alpine generalists and alpine specialists was supported by the estimates of the BiSSE models: Protist diversification (specification) in alpine lakes was shown to be mainly driven by generalists with wide distribution ranges (along altitudes, latitudes, and longitudes) and putatively wide tolerance ranges toward environmental conditions. In contrast, there was hardly any diversification in specialists and the transition rates from generalists toward specialists were significantly higher than vice versa (Figure 4). These patterns could be verified for all major taxonomic groups (Figure A6 in Appendix 1). Whereas this initially seems to contradict the patterns revealed by plants, protists resemble alpine specialists in plants and insects with poor dispersal. These have been shown to speciate faster than lowland plants, but this relationship is erased by a higher extinction rate (Smyčka et al., 2017). Unfortunately, little is known about dispersal and extinction patterns in protists to confirm the relationship of poor dispersal and high extinction risk found in plants and insects (Marta et al., 2019; Smyčka et al., 2017).

With limited abilities to diversify, low dispersibility, and high extinction risk, alpine specialist protists form a group of interesting taxa to study ecological adaptation in protists. In general, such adaptations can be diverse from temporal differentiation (earlier emergence after dormancy), reproductive advantages or higher motility at lower temperatures, or life history changes. Unfortunately, little is known about the biology of these common alpine specialist protist taxa that we detected in our sampling since an exact taxonomic classification on species level is challenging based on the V9 region of the 18S SSU of the rDNA. Comparison of our sequences with sequences at GenBank often led to ambiguous best hits, for example, alpine-specific OTUs (3075 sequences, 4 OTUs, 1–2 lakes) were classified as Koliellopsis inundata, Koliella sempervirens, Koliella longiseta, or Raphidonema nivale (98–100% sequence identity). Additionally, there is still the chance that our alpine specialists were not found in the lowlands and arctic regions by chance. For example, Koliella sempervirens (98–100% sequence identity, 3075 sequences, 4 OTUs, 1–2 lakes), Colpidium sp. aAcq1 (100% sequence identity, 2498 sequences, 2 OTUs, 1–4 lakes), and Hemiarmaphisiella terricola (> 98% sequence identity, 1461 sequences, 1 OTU, 2 lakes) were found by us only in alpine lakes but they were also described from glaciers in Iceland (Lutz et al., 2015) and Svalbard (Stibl & Elster, 2005), Tuscan freshwater biotopes (Rossi et al., 2016), and the Austrian lowlands (Foissner et al., 2005), respectively. Finally, the problem of species identification also resulted in OTUs that were inferred to be alpine specialists here but blast hits at GenBank suggested it to be for example Paramecium woodruffi (>96% sequence identity, 2242 sequences, 3 OTUs, 1–4 lakes), which is considered a lowland species occurring in marine or brackish water (Wenrich, 1928). Unfortunately, the origin of the sequence with high similarity to our sequence is not known. Nevertheless, there appears to be little chance to diversify for alpine specialists, although we could not exclude the occurrence of additional alpine specialists in other parts of the lake than the sampled one. Spatial restriction, smaller niche breadth, putatively young age, and an increased threat to extinction are highly likely to reduce the chances of specialists to diversify. Opposed to that, higher abundances, wider tolerance ranges toward changing environmental conditions, and an increased ability to disperse in and adapt to new environments facilitate the opportunities of generalists to diversify, also in alpine habitats.

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CONFLICT OF INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

Janina C. Vogt: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Project administration (supporting); Resources (lead); Validation (equal); Visualization (lead); Writing-original draft (lead); Writing-review &
and shallow water sediments. Molecular Ecology, 21, 1048–1059. https://doi.org/10.1111/j.1365-294X.2011.05297.x

Bock, C., Salcher, M. M., Jensen, M., Pandey, R. V., & Boenigk, J. (2018). Synchrony of Eukaryotic and Prokaryotic Planktonic Communities in three seasonally sampled Austrian Lakes. Frontiers in Microbiology, 9, 1290. https://doi.org/10.3389/fmicb.2018.01290

Boenigk, J., & Arndt, H. (2002). Bacteriivory by heterotrophic flagellates: Community structure and feeding strategies. Antonie Van Leeuwenhoek, Int J Gen Mol Microbiol, 81, 465–480.

Boenigk, J., Wodniok, S., Bock, C., Beisser, D., Hempel, C., & Grossmann, L. (2018). Geographic distance and mountain ranges structure freshwater protist communities on a European scale. Metabarcoding and Metagenomics, 2, e21519.

Bryant, J. A., Lamanna, C., Morlon, H., Kerkhoff, A. J., Enquist, B. J., & Green, J. L. (2008). Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. Proceedings of the National Academy of Sciences, 105, 11505–11511. https://doi.org/10.1073/pnas.0801920105

Caron, D. A. (2001). Prostikan herbivory and bacteriovory. In J. H. Paul (Ed.), Marine Microbiology. Methods in Microbiology (pp. 289–315). Academic Press.

Casteleyn, G., Leliaert, F., Backeljau, T., Debeer, A., Kotaki, Y., Rhodes, L., Lundholm, N., Sabbe, K., & Vyverman, W. (2010). Limits to gene flow in a cosmopolitan marine planktonic diatom. Proceedings of the National Academy of Sciences, 107, 12952–12957. https://doi.org/10.1073/pnas.1001380107

Chiarri, O., Dupont, P., Pornon, A., & Escaravage, N. (2014). Microsatellite marker analysis reveals the complex phylogeographic history of Rhododendron furfuraceum (Ericaceae) in the pyrenees. PLoS One, 9, e92976. https://doi.org/10.1371/journal.pone.0092976

Cox, C. B., Moore, P. D., & Ladle, R. J. (2016). Biogeography: an ecological and evolutionary approach (9th edn). In C. B. Cox, P. D. Moore, & R. J. Ladle (Eds), John Wiley & Sons, Ltd.

Edgar, R. C. (2016). UCHIME2: improved chimera prediction for amplicon sequencing. bioRxiv, 74252. https://www.biorxiv.org/content/10.1101/074252v1

Faith, D. P. (1992). Conservation evaluation and phylogenetic diversity. Biological Conservation, 61, 1–10. https://doi.org/10.1016/0006-3207(92)91201-3

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

Fiquerola, J., & Green, A. J. (2002). Dispersal of aquatic organisms by waterbirds: A review of past research and priorities for future studies. Freshwater Biology, 47, 483–494. https://doi.org/10.1046/j.1365-2427.2002.00829.x

Fikser, S., Sommaruga, R., Vila, I., & Stoeck, T. (2016). Microbial eukaryote plankton communities of high-mountain lakes from three continents exhibit strong biogeographic patterns. Molecular Ecology, 25, 2286–2303. https://doi.org/10.1111/mec.13633

Fine, P. V. A. (2015). Ecological and evolutionary drivers of geographic variation in species diversity. Annual Review of Ecology Evolution and Systematics, 46, 369–392. https://doi.org/10.1146/annurev-ecolsys-112414-054102

FitzJohn, R. G. (2012). Diversitree: Comparative phylogenetic analyses of diversification in R. Methods in Ecology and Evolution, 3, 1084–1092.

Foißner, W. (2004). Biogeography of microorganisms: a brief review emphasizing protists. Acta Protozool, 45, 111–136.

Foißner, W., Berger, H., Xu, K., & Zechmeister-Boltenstern, S. (2005). A huge, undescribed soil ciliate (Protozoa: Ciliophora) diversity in natural forest stands of Central Europe. Biodiversity and Conservation, 14, 617–701. https://doi.org/10.1007/s10531-004-3923-6

Fu, L., Niu, B., Zhu, Z., Wu, S., & Li, W. (2012). CD-HIT: Accelerated for clustering the next-generation sequencing data. Bioinformatics, 28, 3150–3152. https://doi.org/10.1093/bioinformatics/bts565
workflow for processing, clustering, and taxonomically assigning amplicon sequencing reads. BMC Bioinformatics, 21(1), 1–14.

Wenrich, D. H. (1928). Paramecium woodruiffi, n. sp. (Protozoa, Ciliata). Transactions of the American Microscopical Society, 47, 256–261. https://doi.org/10.2307/3222177

Wiens, J. J., & Donoghue, M. J. (2004). Historical biogeography, ecology and species richness. Trends in Ecology & Evolution, 19, 639–644. https://doi.org/10.1016/j.tree.2004.09.011

Wu, Q. L., Chatzinotas, A., Wang, J., & Boenigk, J. (2009). Genetic diversity of eukaryotic plankton assemblages in eastern Tibetan lakes differing by their salinity and altitude. Microbial Ecology, 58, 569–581. https://doi.org/10.1007/s00248-009-9526-8

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., & Glöckner, F. O. (2014). The SILVA and "all-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Research, 42, 643–648. https://doi.org/10.1093/nar/gkt1209

Zhang, J., Zhao, S., Zhang, Y., Sun, P., Bu, D., & Wang, J. (2015). New Primers Targeting Full-Length Ciliate 18S rRNA Genes and Evaluation of Dietary Effect on Rumen Ciliate Diversity in Dairy Cows. Current Microbiology, 71, 650–657. https://doi.org/10.1007/s00284-015-0898-3

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APPENDIX 1

Figure A1  Classification of alpine and non-alpine (arctic and non-arctic) lakes according to their minimum temperatures of the coldest month (bio6) in relation to altitude.

Figure A2  Taxonomic affiliations and relative abundances of OTUs detected in alpine and non-alpine (lowland) lakes; Chl, Chlorophyta; Chy, Chytridiomycota; Cil, Ciliophora; Din, Dinoflagellata; Chr, Chrysophyceae; Dia, Diatomeae.

Figure A3  (a) OTU-based alpha diversity estimates (richness, Shannon diversity) for all eukaryotic OTUs (left), alpine/non-alpine specialists (mid), and alpine/non-alpine generalists (right) per region (alpine regions, non-alpine); (b) Phylogenetic alpha diversity estimates based on a maximum likelihood tree of the representative sequences per OTU (Faith's Phylogenetic Diversity (PD), Mean Pairwise Distance (MPD), and Mean Nearest Taxon Distance (MNTD)) for all eukaryotic OTUs (left), alpine/non-alpine specialists (mid) and alpine/non-alpine generalists (right) per mountain range; p-values of Kruskal-Wallis tests (alpine (AL + CP + PY + SN) vs. non-alpine lakes and between mountain ranges AL, CP, PY, SN); AL, Alps; CP, Carpathians; PY, Pyrenees; SN, Sierra Nevada.
Figure A4  Linear regression analyses of OTU- and phylogeny-based alpha diversity estimates per lake in correlation to altitudes
Figure A5  (Relative) OTU abundances per lake: (a) Alpine OTUs classified as specialists and generalists; (b) Affiliations of alpine OTUs (top), alpine specialists (mid), and alpine generalists (bottom) per lake to distinct groups of (non-) sharing regional groups (mountain ID); (c) Affiliations of alpine OTUs (top), alpine specialists (mid) and alpine generalists (bottom) per lake to main taxonomic groups; AL, Alps; CP, Carpathians; PY, Pyrenees; SN, Sierra Nevada
Figure A6 (Continued)
Figure A6  Estimation of evolutionary characteristics of major taxonomic groups using Binary-state speciation and extinction (BiSSE) models providing distinct speciation (λ), extinction (μ), and state-transition (q) rates per state; diversification rates (div) were calculated as a difference of speciation and extinction rates; posterior probability density was calculated by 1000-step Markov Chain Monte Carlo (MCMC) simulations: (a) for generalists (0) and specialists (1), (b) for altitudinally widely distributed (0) and more restricted (1) taxa, (c) for latitudinally widely distributed (0) and more restricted (1) taxa, and (d) for longitudinally widely distributed (0) and more restricted (1) taxa.
### TABLE A1 Description, geographical location, environmental parameters and classification of sampling sites with OTU and sequence numbers; Bioclimatic variables ('worldclim' dataset, https://biogeography.ucdavis.edu/data/worldclim/v2.1/base/wc2.1_2.5m_bio.zip, accessed 07/20, averaged values for the years 1970–2000 (Fick & Hijmans, 2017)): bio1 = annual mean (air) temperature, bio5 = max (air) temperature of warmest month, bio6 = min (air) temperature of coldest month; WTemp = water temperature at sampling time; Conductivity and pH at sampling time; OTUs = number of OTUs (V9-SWARMs) classified as protists; seqs = number of sequences classified as protists

| Name       | Lake          | Country   | Latitude [°N] | Longitude [°E] | Altitude [m a.s.l.] | Wtemp [°C] |
|------------|---------------|-----------|---------------|-----------------|--------------------|------------|
| Alps       | Z201LI        | Switzerland | 46.5631       | 8.4301          | 2646               | 15.6       |
|            | A251SC        | Austria    | 46.9654       | 10.9448         | 2785               | 13.7       |
|            | A191GI        | Italy      | 46.4919       | 10.7176         | 1839               | 17.8       |
|            | A122HU        | Austria    | 47.3543       | 13.8096         | 1505               | 15.5       |
|            | A123OB        | Austria    | 47.3515       | 13.8177         | 1628               | 13.3       |
|            | A152WI        | Austria    | 46.8864       | 13.8026         | 1889               | 15.4       |
|            | Z132AG        | Italy      | 45.4696       | 7.1402          | 2295               | 15.8       |
|            | Z121PI        | Switzerland | 46.5566       | 8.5674          | 2089               | 16.9       |
|            | Z331Gxx       | Switzerland | 46.4169       | 9.6753          | 2485               | 15.3       |
|            | A041BE        | Austria    | 47.7248       | 14.9239         | 1580               | 16.5       |
|            | A093GO        | Austria    | 47.5250       | 13.4921         | 927                | 19.8       |
|            | A201SE        | Italy      | 46.8714       | 11.6546         | 2366               | 17.1       |
|            | A261SI        | Austria    | 46.9176       | 10.0915         | 2033               | 12.5       |
|            | A051OB        | Austria    | 47.8060       | 15.0785         | 1113               | 21.5       |
|            | A211BR        | Austria    | 47.0169       | 11.5022         | 1259               | 20.2       |
|            | A033DU        | Austria    | 47.6048       | 15.2827         | 908                | 12.2       |
|            | A091WO        | Austria    | 47.7429       | 13.3728         | 532                | 21.5       |
|            | Z122OU        | France     | 45.4298       | 6.9951          | 2523               | 18.1       |
|            | Z141GM        | France     | 45.2245       | 6.1481          | 1677               | 17.6       |
|            | Z121CH        | France     | 45.4749       | 6.9482          | 1793               | 15.5       |
|            | A111AU        | Austria    | 47.6757       | 13.7857         | 1646               | 14.7       |
|            | A112AL        | Austria    | 47.6380       | 13.7696         | 710                | 14.7       |
|            | A173EI        | Germany    | 47.4535       | 10.9852         | 986                | 21.6       |
|            | A042ER        | Austria    | 47.7870       | 15.2703         | 834                | 21.3       |
|            | A271GI        | Switzerland | 46.9061       | 9.3883          | 1344               | 12.9       |
|            | A032GRxxx     | Austria    | 47.5816       | 15.3075         | 842                | 11.5       |
|            | A131WE        | Austria    | 46.7056       | 13.3202         | 922                | 22.9       |
|            | O271GA        | Germany    | 49.0988       | 13.1595         | 935                | 15.5       |
|            | Z133CS        | Italy      | 45.4342       | 7.2274          | 1587               | 18.0       |
| Carpathians| O061VE        | Slovakia   | 49.1763       | 20.0602         | 1947               | 12.9       |
|            | O111BA        | Romania    | 45.6030       | 24.6144         | 2004               | 13.8       |
|            | O052VE        | Slovakia   | 49.1563       | 20.1566         | 1600               | 12.0       |
|            | O053PO        | Slovakia   | 49.1552       | 20.0805         | 1493               | 14.5       |
|            | O062ST        | Slovakia   | 49.1207       | 20.0570         | 1351               | 23.3       |
|            | O151Bux       | Romania    | 45.3583       | 22.8761         | 2030               | 14.5       |
|            | O072PA        | Slovakia   | 48.8630       | 20.3860         | 786                | 23.0       |
|            | O051CO        | Poland     | 49.4660       | 20.2261         | 527                | 21.9       |
|            | O032OR        | Slovakia   | 49.3977       | 19.4854         | 594                | 25.6       |
|            | O102VI        | Romania    | 45.4242       | 23.7667         | 1210               | 21.1       |
| Conductivity [µS cm⁻¹] | pH   | Bio1 [°C] | Bio5 [°C] | Bio6 [°C] | Mountain range | Alpine_non-alpine | Alpine_arctic_non-alpine/ non-arctic | OTUs | Seqs  |
|-------------------------|------|-----------|-----------|-----------|----------------|------------------|-----------------------------------|------|-------|
| 125                     | 8.75 | -2.0      | 8.4       | -11.6     | Alps           | Alpine           | Alpine               | 132  | 406,785 |
| 25                      | 6.82 | -1.8      | 8.8       | -11.5     | Alps           | Alpine           | Alpine               | 280  | 441,326 |
| 134                     | 8.00 | -2.2      | 8.2       | -11.3     | Alps           | Alpine           | Alpine               | 37   | 71,761  |
| 11                      | 7.65 | 0.7       | 14.1      | -11.2     | Alps           | Alpine           | Alpine               | 253  | 79,278  |
| 15                      | 7.74 | 0.7       | 14.1      | -11.2     | Alps           | Alpine           | Alpine               | 109  | 297,925 |
| 68                      | 7.49 | 1.2       | 14.7      | -10.6     | Alps           | Alpine           | Alpine               | 140  | 49,455  |
| 111                     | 8.68 | -0.1      | 12.1      | -10.3     | Alps           | Alpine           | Alpine               | 82   | 126,895 |
| 14                      | 7.90 | -0.3      | 10.7      | -10.2     | Alps           | Alpine           | Alpine               | 103  | 82,675  |
| 235                     | 8.52 | -0.8      | 9.7       | -10.2     | Alps           | Alpine           | Alpine               | 116  | 14,950  |
| 137                     | 8.66 | 2.7       | 17.1      | -9.9      | Alps           | Alpine           | Alpine               | 309  | 473,290 |
| 144                     | 8.50 | 3.3       | 17.7      | -9.9      | Alps           | Alpine           | Alpine               | 790  | 1,162,132 |
| 160                     | 8.75 | 2.2       | 15.3      | -9.7      | Alps           | Alpine           | Alpine               | 111  | 308,410 |
| 18                      | 7.38 | 0.6       | 11.9      | -9.7      | Alps           | Alpine           | Alpine               | 205  | 889,819 |
| 214                     | 7.99 | 3.1       | 17.6      | -9.6      | Alps           | Alpine           | Alpine               | 387  | 556,496 |
| 354                     | 8.34 | 3.0       | 16.5      | -9.4      | Alps           | Alpine           | Alpine               | 325  | 887,801 |
| 461                     | 7.94 | 3.9       | 18.9      | -9.1      | Alps           | Alpine           | Alpine               | 374  | 51,773  |
| 213                     | 8.54 | 4.4       | 19.1      | -9.1      | Alps           | Alpine           | Alpine               | 608  | 1,189,063 |
| 153                     | 9.29 | 2.0       | 14.9      | -9.1      | Alps           | Alpine           | Alpine               | 367  | 237,458 |
| 154                     | 8.50 | 2.0       | 15.0      | -9.0      | Alps           | Alpine           | Alpine               | 412  | 197,343 |
| 313                     | 8.56 | 2.5       | 15.7      | -8.8      | Alps           | Alpine           | Alpine               | 329  | 284,869 |
| 102                     | 8.52 | 5.2       | 20.4      | -8.6      | Alps           | Alpine           | Alpine               | 310  | 477,831 |
| 152                     | 8.55 | 5.2       | 20.4      | -8.6      | Alps           | Alpine           | Alpine               | 185  | 98,599  |
| 230                     | 8.54 | 3.1       | 15.7      | -8.6      | Alps           | Alpine           | Alpine               | 61   | 8425    |
| 281                     | 8.30 | 4.8       | 20.0      | -8.5      | Alps           | Alpine           | Alpine               | 326  | 970,549 |
| 199                     | 8.33 | 1.9       | 13.2      | -8.5      | Alps           | Alpine           | Alpine               | 57   | 45,023  |
| 379                     | 8.11 | 5.3       | 20.7      | -8.4      | Alps           | Alpine           | Alpine               | 420  | 74,819  |
| 181                     | 8.58 | 5.5       | 20.6      | -8.4      | Alps           | Alpine           | Alpine               | 149  | 89,849  |
| 11                      | 7.21 | 4.0       | 17.9      | -8.4      | Alps           | Alpine           | Alpine               | 51   | 16,503  |
| 40                      | 8.33 | 2.7       | 15.8      | -8.4      | Alps           | Alpine           | Alpine               | 233  | 270,782 |
| 10                      | 7.55 | 0.1       | 11.6      | -11.3     | Carpathians    | Alpine           | Alpine               | 635  | 1,081,282 |
| 90                      | 7.89 | 1.3       | 13.9      | -11.2     | Carpathians    | Alpine           | Alpine               | 81   | 24,330  |
| 7                       | 7.39 | 1.1       | 13.4      | -11.0     | Carpathians    | Alpine           | Alpine               | 41   | 22,404  |
| 11                      | 7.72 | 1.1       | 13.4      | -10.9     | Carpathians    | Alpine           | Alpine               | 559  | 609,114 |
| 19                      | 8.31 | 4.0       | 18.3      | -9.8      | Carpathians    | Alpine           | Alpine               | 419  | 211,308 |
| 11                      | 8.42 | 1.6       | 14.2      | -9.8      | Carpathians    | Alpine           | Alpine               | 102  | 199,462 |
| 250                     | 8.81 | 5.5       | 20.6      | -9.2      | Carpathians    | Alpine           | Alpine               | 419  | 136,722 |
| 190                     | 9.28 | 6.0       | 21.3      | -9.0      | Carpathians    | Alpine           | Alpine               | 409  | 233,767 |
| 200                     | 8.88 | 6.2       | 21.7      | -8.6      | Carpathians    | Alpine           | Alpine               | 467  | 494,001 |
| 52                      | 7.79 | 4.7       | 18.5      | -8.4      | Carpathians    | Alpine           | Alpine               | 210  | 224,678 |

(Continues)
| Name       | Lake                                      | Country | Latitude [°N] | Longitude [°E] | Altitude [m a.s.l.] | Wtemp [°C] |
|------------|-------------------------------------------|---------|---------------|---------------|---------------------|-----------|
| Pyrenees   | S201PO Embalse de Pondiellas              | Spain   | 42.7759       | -0.2612       | 2805                | 13.7      |
| Sierra Nevada | S081LA Laguna Altera             | Spain   | 37.0584       | -3.3040       | 3120                | 16.7      |
|            | S082LH Laguna Hondera                  | Spain   | 37.0475       | -3.2932       | 2950                | 14.9      |
|            | S102LR Laguna de las Aguas Verdes       | Spain   | 37.0481       | -3.3684       | 3110                | 16.4      |
| Non-alpine (arctic) | N041ST Strondafjorden | Norway  | 60.9650       | 9.2828        | 365                 | 15.4      |
|            | N033SK Skiftessjoen                    | Norway  | 60.3772       | 7.5656        | 1250                | 11.9      |
|            | N051NO Nordmesa                        | Norway  | 61.0994       | 10.6828       | 520                 | 17.9      |
|            | N043MJ Mjoesa                           | Norway  | 61.0722       | 10.4322       | 125                 | 16.5      |
|            | N012HJ Hjartsjaevatnet                 | Norway  | 59.6083       | 8.7628        | 168                 | 15.4      |
|            | N011EL Elgsjoe                         | Norway  | 59.5917       | 9.3544        | 260                 | 20.9      |
| Non-alpine (non-arctic) | N182WU Jezioro Wulpirski | Poland  | 53.7250       | 20.2744       | 100                 | 22.2      |
|            | S211BN Ibon de los Banos/Balneario de Panticosa | Spain | 42.7600       | -0.2362       | 1705                | 16.5      |
|            | O283CE Cerne Jezoro                    | Czechia | 49.1816       | 13.1865       | 1010                | 20.0      |
|            | O121RA Raura                           | Romania | 45.9281       | 24.0530       | 412                 | 22.9      |
|            | N172RY Rychnowskie                     | Poland  | 53.6764       | 17.3864       | 161                 | 20.3      |
|            | O122SA Sacel                           | Romania | 45.7917       | 23.9465       | 542                 | 24.5      |
|            | A031AN Annateich                       | Austria | 47.1224       | 15.2908       | 417                 | 21.1      |
|            | A052LU Lunzer See                      | Austria | 47.8511       | 15.0385       | 623                 | 22.4      |
|            | A242PL Plansee                         | Austria | 47.4764       | 10.8251       | 961                 | 21.8      |
|            | N073VR Vaenern                         | Sweden  | 59.3739       | 13.3969       | 46                  | 18.2      |
|            | O281QU Quarzengrubensee                | Germany | 49.0515       | 13.1712       | 901                 | 13.0      |
|            | O031ZY Zywiec                          | Poland  | 49.7051       | 19.1823       | 340                 | 25.8      |
|            | O282KA Kleiner Arbersee                | Germany | 49.1276       | 13.1173       | 933                 | 18.5      |
|            | A281KLx Kloentalersee                  | Switzerland | 47.0260   | 9.0032        | 843                 | 19.6      |
|            | O182FAxx Lacul Belis-Fantanele          | Romania | 46.6675       | 23.0561       | 996                 | 20.9      |
|            | O201BA Baraj Dragar Floroiu/Lacul      | Romania | 46.7906       | 22.7166       | 850                 | 21.2      |
|            | N072JA Jaernsjoen                      | Sweden  | 59.3728       | 12.2483       | 147                 | 18.1      |
|            | N171J5 Jastrowiesee                    | Poland  | 53.4131       | 16.8522       | 115                 | 19.6      |
|            | N181JE Maly Jeziorak                   | Poland  | 53.6006       | 19.5506       | 110                 | 20.3      |
|            | S221OR Lac d'Oredon                    | France  | 42.8280       | 0.1676        | 1880                | 18.1      |
|            | A103FU Fuschlsee                       | Austria | 47.8075       | 13.2511       | 657                 | 22.9      |
|            | A092HA Hallstatt                       | Austria | 47.5888       | 13.6587       | 510                 | 18.9      |
|            | N023ROx Roldalvatsnet                  | Norway  | 59.8283       | 6.8067        | 448                 | 13.1      |
|            | A151MI Milstaetter See                 | Austria | 46.8087       | 13.5196       | 591                 | 24.4      |
|            | A022TU Tuernitz                        | Austria | 47.9253       | 15.4756       | 473                 | 27.9      |
|            | A132OS Ossiacher See                   | Austria | 46.6549       | 13.9009       | 501                 | 24.8      |
|            | N163PI Jezioro Piecnik                 | Poland  | 53.3425       | 16.2542       | 132                 | 20.6      |
|            | N091HO Hjortsjoen                      | Sweden  | 57.5061       | 14.1281       | 197                 | 17.8      |
|            | N183LA Jezioro Lasinski                | Poland  | 53.5058       | 19.0714       | 76                  | 21.1      |
|            | O181GI Gilau                           | Romania | 46.7459       | 23.3707       | 399                 | 18.9      |
|            | O183SO Somesul Mic                     | Romania | 46.7508       | 23.4773       | 411                 | 21.9      |
| Conductivity [μS cm⁻¹] | pH   | Bio1 [°C] | Bio5 [°C] | Bio6 [°C] | Mountain range          | Alpine_non-alpine | Alpine_arctic_non-alpine | OTUs   | Seqs     |
|------------------------|------|-----------|-----------|-----------|-------------------------|-----------------|--------------------------|--------|---------|
| 77                     | 8.84 | 0.4       | 12.7      | −9.5      | Pyrenees                | Alpine          | Alpine                   | 303    | 200,100 |
| 9                      | 8.37 | 4.1       | 24.2      | −8.7      | Sierra Nevada           | Alpine          | Alpine                   | 295    | 505,416 |
| 17                     | 8.09 | 4.1       | 24.2      | −8.7      | Sierra Nevada           | Alpine          | Alpine                   | 288    | 713,304 |
| 36                     | 8.19 | 4.2       | 24.3      | −8.6      | Sierra Nevada           | Alpine          | Alpine                   | 103    | 25,515  |
| 13                     | 7.55 | 1.8       | 19.3      | −13.2     | Non-alpine              | Non-alpine      | Arctic                   | 1652   | 1,885,757 |
| 6                      | 7.53 | −1.3      | 12.5      | −12.7     | Non-alpine              | Non-alpine      | Arctic                   | 429    | 784,536 |
| 12                     | 7.04 | 1.6       | 18.3      | −12.7     | Non-alpine              | Non-alpine      | Arctic                   | 668    | 379,397 |
| 17                     | 7.59 | 4.1       | 21.0      | −10.4     | Non-alpine              | Non-alpine      | Arctic                   | 582    | 419,698 |
| 5                      | 6.81 | 3.3       | 17.9      | −9.0      | Non-alpine              | Non-alpine      | Arctic                   | 45     | 2764    |
| 34                     | 6.33 | 4.1       | 19.0      | −8.5      | Non-alpine              | Non-alpine      | Arctic                   | 64     | 2376    |
| 207                    | 8.84 | 7.5       | 23.6      | −7.9      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 565    | 480,236 |
| 41                     | 7.65 | 2.1       | 14.8      | −7.9      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 282    | 466,787 |
| 15                     | 5.40 | 4.7       | 18.9      | −7.8      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 54     | 3279    |
| 743                    | 8.84 | 9.0       | 25.1      | −7.7      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 236    | 67,003  |
| 279                    | 9.00 | 7.0       | 22.4      | −7.6      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 831    | 536,214 |
| 334                    | 8.50 | 8.7       | 24.5      | −7.6      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 670    | 687,942 |
| 469                    | 8.31 | 7.5       | 23.9      | −7.5      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 898    | 1,073,047 |
| 240                    | 8.27 | 6.2       | 21.9      | −7.5      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 447    | 1,133,997 |
| 317                    | 8.47 | 4.8       | 18.1      | −7.5      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 565    | 423,919 |
| 52                     | 7.51 | 5.8       | 21.0      | −7.5      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 1723   | 1,627,442 |
| 33                     | 7.04 | 5.4       | 19.9      | −7.5      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 32     | 1532    |
| 357                    | 8.44 | 8.0       | 23.9      | −7.4      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 527    | 341,354 |
| 10                     | 6.50 | 5.5       | 19.9      | −7.4      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 52     | 2685    |
| 183                    | 8.41 | 3.3       | 15.1      | −7.3      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 110    | 53,029  |
| 73                     | 8.69 | 6.2       | 20.6      | −7.2      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 388    | 233,359 |
| 80                     | 8.30 | 6.2       | 20.9      | −7.2      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 198    | 270,360 |
| 27                     | 7.34 | 5.5       | 20.1      | −7.1      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 1254   | 862,307 |
| 191                    | 8.79 | 7.4       | 23.0      | −7.1      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 644    | 869,409 |
| 256                    | 9.02 | 7.4       | 22.9      | −7.1      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 652    | 448,633 |
| 54                     | 8.51 | 3.0       | 15.5      | −7.1      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 265    | 81,923  |
| 316                    | 8.42 | 7.0       | 22.4      | −7.0      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 345    | 617,619 |
| 171                    | 8.47 | 7.5       | 23.3      | −6.9      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 302    | 221,254 |
| 2                      | 6.10 | 3.3       | 15.4      | −6.9      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 301    | 117,753 |
| 176                    | 8.70 | 7.8       | 23.6      | −6.8      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 658    | 1,341,165 |
| 357                    | 8.11 | 6.9       | 22.6      | −6.7      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 322    | 607,509 |
| 110                    | 8.83 | 8.1       | 24.1      | −6.6      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 285    | 259,083 |
| 42                     | 7.71 | 7.4       | 22.5      | −6.6      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 513    | 155,661 |
| 99                     | 7.49 | 5.7       | 20.5      | −6.5      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 345    | 363,674 |
| 450                    | 7.94 | 7.3       | 22.6      | −6.5      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 695    | 557,022 |
| 80                     | 8.61 | 8.4       | 23.9      | −6.5      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 246    | 299,356 |
| 126                    | 8.69 | 8.5       | 24.1      | −6.5      | Non-alpine              | Non-alpine      | Non-alpine/non-arctic    | 103    | 16,469 |

(Continues)
| Name     | Lake                | Country     | Latitude [°N] | Longitude [°E] | Altitude [m a.s.l.] | Wtemp [°C] |
|----------|---------------------|-------------|---------------|----------------|---------------------|------------|
| A172WA   | Waichensee          | Germany     | 47.5679       | 11.3047        | 799                 | 21.6       |
| N083VE   | Vaettern            | Sweden      | 58.4642       | 14.9292        | 93                  | 18.0       |
| A241TE   | Tegernsee           | Germany     | 47.7360       | 11.7178        | 742                 | 20.4       |
| A302DR   | Dreiringsee         | Germany     | 48.7382       | 13.3512        | 442                 | 22.6       |
| N182ZN   | Jezioro Duze Zniskie| Poland      | 52.8558       | 17.7525        | 86                  | 23.7       |
| N191BW   | Borownosee          | Poland      | 53.2358       | 18.1314        | 88                  | 22.1       |
| N202JZ   | Jezioro Jeziskos    | Poland      | 51.8317       | 18.6728        | 116                 | 23.2       |
| N173BO   | Jezioro Borzechowski| Poland      | 53.9144       | 18.4008        | 100                 | 22.7       |
| Z142VN   | Lac Verney          | France      | 45.1470       | 6.0467         | 767                 | 20.0       |
| A081MO   | Mondsee             | Austria     | 47.8009       | 13.3859        | 482                 | 21.7       |
| A101OB   | Obertrumer See      | Austria     | 47.9676       | 13.0750        | 509                 | 23.8       |
| N193RG   | Jezioro Rgielskie   | Poland      | 52.8286       | 17.2506        | 82                  | 25.7       |
| A071SE   | Seeheimer See       | Germany     | 47.8418       | 11.8588        | 649                 | 18.4       |
| A102WA   | Wallersee           | Austria     | 47.9064       | 13.1744        | 500                 | 24.6       |
| B342DOS  | Lake Dospat         | Bulgaria    | 41.6442       | 24.1529        | 1205                | 22.2       |
| A301EG   | Eginger See         | Germany     | 48.7195       | 13.2714        | 378                 | 21.7       |
| N201SL   | Jezioro Słupeckie   | Poland      | 52.2961       | 17.8878        | 89                  | 21.8       |
| O011MI   | Mietkowskie         | Poland      | 50.9671       | 16.6224        | 169                 | 25.1       |
| S151BS   | Lac des Bouillouses | France      | 42.5623       | 1.9772         | 2070                | 17.2       |
| S153TR   | Estany de Trebens   | France      | 42.5771       | 1.9622         | 2378                | 17.0       |
| S231SN   | Embalse de Senet    | Spain       | 42.5808       | 0.7564         | 1490                | 19.8       |
| S261TE   | Lac du Tech         | France      | 42.9151       | -0.2566        | 1260                | 16.0       |
| A072CHxxx| Chiemsee            | Germany     | 47.8717       | 12.3866        | 522                 | 22.8       |
| N203GO   | Goluchowsee         | Poland      | 51.8406       | 17.9442        | 96                  | 26.5       |
| O073NY   | Nyekladhaza         | Hungary     | 47.9882       | 20.8492        | 108                 | 28.0       |
| A073WA   | Waginger See        | Germany     | 47.9227       | 12.8026        | 436                 | 24.0       |
| N211NI   | Jezioro Niepruszewske| Poland   | 52.3886       | 16.6047        | 79                  | 23.2       |
| O223KV   | Kv1 Viztarolo       | Hungary     | 47.6956       | 21.3734        | 149                 | 29.3       |
| A171KO   | Kochelsee           | Germany     | 47.6424       | 11.3569        | 587                 | 19.1       |
| O222CS   | Csecs Halast        | Hungary     | 47.5591       | 21.0152        | 140                 | 27.0       |
| S212LU   | Embalse de Lunuza   | Spain       | 42.7544       | -0.3146        | 1300                | 21.7       |
| O141OSx  | Ostrov              | Romania     | 45.5172       | 22.8542        | 476                 | 17.9       |
| O221TI   | Tisza-To            | Hungary     | 47.6497       | 20.6737        | 135                 | 26.6       |
| A182LE   | Lago Ledro          | Italy       | 45.8744       | 10.7561        | 643                 | 24.8       |
| N261LU   | Luetschetalisperre  | Germany     | 50.7336       | 10.7567        | 591                 | 20.2       |
| O012BI   | Bialy Kosciol       | Poland      | 50.7271       | 17.0395        | 172                 | 26.7       |
| O021BI   | Biestrzynek         | Poland      | 50.7374       | 18.2391        | 195                 | 26.7       |
| O101CI   | Cincs Lacula        | Romania     | 45.6902       | 22.8684        | 297                 | 25.2       |
| Z151LR   | Lac du Laffrey      | France      | 45.0218       | 5.7783         | 908                 | 22.1       |
| Z152PC   | Piec Chatel         | France      | 44.9719       | 5.7725         | 937                 | 23.2       |
| Z192AS   | Arnisee             | Switzerland| 46.7705       | 8.6429         | 1384                | 13.9       |
| A0211EB  | Ebersdorfer See     | Austria     | 48.1663       | 15.5500        | 271                 | 25.6       |
| N212WI   | Jezioro Wielkie     | Poland      | 52.3147       | 14.9850        | 83                  | 26.2       |
| Conductivity [µS cm⁻¹] | pH   | Bio1 [°C] | Bio5 [°C] | Bio6 [°C] | Mountain range | Alpine_non-alpine | Alpine_arctic_non-alpine/non-arctic | OTUs  | Seqs    |
|------------------------|------|-----------|-----------|-----------|----------------|------------------|-----------------------------------|-------|---------|
| 275                    | 8.52 | 6.7       | 20.8      | −6.4      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 165   | 254,265 |
| 120                    | 8.65 | 6.2       | 21.7      | −6.4      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 573   | 258,827 |
| 302                    | 8.39 | 7.4       | 22.3      | −6.3      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 323   | 260,638 |
| 129                    | 9.29 | 7.8       | 23.2      | −6.3      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 241   | 156,925 |
| 613                    | 9.17 | 7.8       | 23.4      | −6.3      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 631   | 290,082 |
| 382                    | 8.83 | 7.5       | 22.9      | −6.3      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 577   | 233,656 |
| 298                    | 8.77 | 8.6       | 24.7      | −6.3      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 1067  | 388,489 |
| 347                    | 8.11 | 6.6       | 21.2      | −6.2      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 317   | 585,156 |
| 201                    | 8.54 | 6.7       | 21.7      | −6.2      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 493   | 217,842 |
| 278                    | 8.35 | 8.1       | 23.8      | −6.1      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 598   | 1,364,799 |
| 246                    | 8.58 | 7.8       | 23.2      | −6.1      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 438   | 743,056 |
| 582                    | 8.70 | 7.9       | 23.4      | −6.1      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 797   | 301,616 |
| 359                    | 8.38 | 7.7       | 22.8      | −6.0      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 303   | 785,684 |
| 335                    | 8.53 | 7.9       | 23.4      | −6.0      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 308   | 630,816 |
| 65                     | 8.87 | 7.0       | 22.3      | −6.0      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 372   | 436,597 |
| 143                    | 10.03| 8.1       | 23.6      | −5.9      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 598   | 735,108 |
| 366                    | 9.76 | 8.2       | 24.0      | −5.9      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 546   | 708,089 |
| 301                    | 9.77 | 8.2       | 23.3      | −5.9      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 1000  | 917,773 |
| 14                     | 7.28 | 3.5       | 16.3      | −5.9      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 671   | 674,045 |
| 6                      | 8.88 | 3.5       | 16.3      | −5.9      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 211   | 114,950 |
| 43                     | 8.53 | 4.3       | 17.3      | −5.9      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 240   | 132,476 |
| 63                     | 9.06 | 4.3       | 17.2      | −5.9      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 831   | 731,792 |
| 322                    | 8.60 | 8.2       | 23.5      | −5.8      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 394   | 461,707 |
| 538                    | 9.13 | 8.3       | 24.0      | −5.8      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 579   | 194,993 |
| 764                    | 8.20 | 10.2      | 27.3      | −5.7      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 656   | 609,511 |
| 297                    | 8.51 | 8.5       | 23.9      | −5.5      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 457   | 879,095 |
| 515                    | 8.46 | 8.2       | 23.5      | −5.5      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 358   | 377,953 |
| 472                    | 9.15 | 10.3      | 26.9      | −5.5      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 458   | 194,861 |
| 313                    | 8.40 | 8.1       | 22.7      | −5.4      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 282   | 537,735 |
| 361                    | 8.10 | 10.4      | 27.2      | −5.4      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 102   | 50,885  |
| 164                    | 8.87 | 4.9       | 18.0      | −5.4      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 376   | 166,122 |
| 69                     | 8.47 | 9.4       | 25.3      | −5.3      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 79    | 4642    |
| 449                    | 8.03 | 10.4      | 27.4      | −5.3      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 397   | 67,458  |
| 308                    | 8.51 | 8.0       | 23.1      | −5.2      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 455   | 949,256 |
| 207                    | 10.47| 6.4       | 20.5      | −5.2      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 154   | 59,208  |
| 237                    | 9.96 | 8.2       | 23.1      | −5.2      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 331   | 155,370 |
| 98                     | 8.17 | 8.4       | 23.5      | −5.2      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 380   | 262,284 |
| 147                    | 9.10 | 9.6       | 25.6      | −5.2      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 195   | 120,571 |
| 242                    | 8.76 | 7.6       | 22.8      | −5.2      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 372   | 316,658 |
| 271                    | 8.73 | 7.6       | 22.8      | −5.2      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 337   | 383,114 |
| 49                     | 8.03 | 5.7       | 18.6      | −5.2      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 96    | 155,045 |
| 279                    | 9.06 | 8.7       | 24.7      | −5.0      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 432   | 1,054,832 |
| 274                    | 8.63 | 8.8       | 24.2      | −5.0      | Non-alpine      | Non-alpine       | Non-alpine/non-arctic             | 357   | 236,478 |

(Continues)
| Name        | Lake                      | Country      | Latitude [°N] | Longitude [°E] | Altitude [m a.s.l.] | Wtemp [°C] |
|-------------|---------------------------|--------------|---------------|----------------|---------------------|------------|
| O022TU      | Turawa                    | Poland       | 50.7206       | 18.1072        | 172                 | 29.0       |
| O025SA      | Sarbi                     | Romania      | 47.2086       | 22.1342        | 130                 | 24.5       |
| A061WO      | Wolfring Teich            | Austria      | 48.1841       | 15.1692        | 221                 | 25.5       |
| A062PI      | Pitlingseekeer            | Austria      | 48.2356       | 14.3839        | 252                 | 26.4       |
| O301SC      | Schiesswheier             | Germany      | 49.7134       | 12.0163        | 420                 | 21.6       |
| O251KR      | Krollova                  | Slovakia     | 48.2520       | 17.8095        | 138                 | 25.9       |
| O263ST      | Steinbergersee            | Germany      | 49.2841       | 12.1567        | 359                 | 21.0       |
| Z282SU      | Schuchsee                 | Germany      | 47.8321       | 8.1346         | 940                 | 20.8       |
| N162MI      | Jezioro Miedwie           | Poland       | 53.3525       | 14.9214        | 11                  | 20.2       |
| S032CP      | Lac de Charpal            | France       | 44.6233       | 3.5619         | 1370                | 22.1       |
| N262JU      | Juecshen                  | Germany      | 50.4756       | 10.5144        | 350                 | 20.6       |
| A272WA      | Walensee                  | Switzerland  | 47.1095       | 9.1850         | 427                 | 22.0       |
| B352PLI     | Plitvice Lakes Lake Galovac | Croatia   | 44.8708       | 15.6005        | 642                 | 19.2       |
| Z293SB      | Schwarzenbachtsperre      | Germany      | 48.6620       | 8.3130         | 659                 | 21.5       |
| A291AM      | Ammersee                  | Germany      | 48.0682       | 11.1063        | 537                 | 24.7       |
| O161TO      | Topolovatu Mare           | Romania      | 45.7846       | 21.6283        | 141                 | 22.6       |
| O252NE      | Neusiedlersee             | Austria      | 47.8655       | 16.8363        | 118                 | 29.5       |
| O262BR      | Brombacherssee Gro        | Germany      | 49.1190       | 10.9615        | 413                 | 20.4       |
| O261RO      | Rothsee                   | Germany      | 49.2377       | 11.2092        | 345                 | 21.6       |
| O231SZ      | Szalka                    | Hungary      | 46.2738       | 18.6347        | 345                 | 28.0       |
| S031BU      | Lac du Bouchet            | France       | 44.9064       | 3.7928         | 1269                | 19.8       |
| B337RAB     | Lake Rabisha              | Bulgaria     | 43.7352       | 22.5943        | 293                 | 24.0       |
| N271PFxx    | Pforstler See             | Germany      | 50.6514       | 9.6017         | 228                 | 19.6       |
| O241PL      | Plattensee                | Hungary      | 46.9336       | 18.1176        | 133                 | 24.9       |
| Z081VE      | Lago Verde                | Italy        | 44.3632       | 10.0909        | 1464                | 20.5       |
| Z082Lxx     | Lago Ballano              | Italy        | 44.3694       | 10.1018        | 1341                | 21.5       |
| N161UN      | Unterrucker See           | Germany      | 53.2839       | 13.8475        | 23                  | 20.6       |
| Z071SI      | Lago Sillara              | Italy        | 44.3645       | 10.0703        | 1721                | 18.4       |
| Z312KB      | Krombachtsperre           | Germany      | 50.6159       | 8.1392         | 515                 | 18.5       |
| N272SB      | Seeburger See             | Germany      | 51.5139       | 10.1569        | 150                 | 22.9       |
| O242VE      | Velenci-To                | Hungary      | 47.1999       | 18.6080        | 133                 | 27.9       |
| N101BA      | Ballingsjoen              | Sweden       | 56.2317       | 13.8819        | 43                  | 19.0       |
| N142MU      | Mueritz                   | Germany      | 53.4783       | 12.6242        | 70                  | 20.2       |
| O302DE      | Dechsendorferweihen       | Germany      | 49.6303       | 10.9581        | 284                 | 22.7       |
| Z153NC      | Lac de Notre-Dame de Commiers | France  | 45.0066       | 5.6930         | 348                 | 21.3       |
| Z161PD      | Lac de Paladru            | France       | 45.4729       | 5.5521         | 491                 | 24.2       |
| N141KU      | Kummerower See            | Germany      | 53.7936       | 12.8128        | 3                   | 19.5       |
| A141CA      | Lago di Cavazzo           | Italy        | 46.3374       | 13.0687        | 194                 | 16.0       |
| A181GA      | Lago di Garda             | Italy        | 45.6861       | 10.6584        | 64                  | 27.2       |
| N133ST      | Stassower See             | Germany      | 54.0344       | 12.5906        | 33                  | 21.3       |
| N143GS      | Grosser Steclihein        | Germany      | 53.1411       | 13.0303        | 60                  | 21.3       |
| N242KO      | Kossateich                | Germany      | 51.8300       | 14.0653        | 53                  | 23.2       |
| N263Wlxz    | Wilder See                | Germany      | 49.9672       | 10.2003        | 204                 | 20.7       |
| Conductivity [µS cm⁻¹] | pH | Bio1 [°C] | Bio5 [°C] | Bio6 [°C] | Mountain range | Alpine_non-alpine | Alpine_arctic_non-alpine | Alpine_arctic_non-alpine/Non-alpine | OTUs | Seqs |
|------------------------|----|-----------|-----------|-----------|----------------|-------------------|-----------------------|---------------------------------|------|------|
| 274                    | 10.03 | 8.4 | 23.6 | −5.0 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 145 | 73,333 |
| 384                    | 8.74 | 10.5 | 27.0 | −5.0 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 175 | 77,605 |
| 691                    | 8.21 | 9.0 | 25.2 | −4.9 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 743 | 1,017,706 |
| 595                    | 8.48 | 9.2 | 25.6 | −4.9 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 834 | 937,111 |
| 83                     | 8.52 | 7.6 | 22.0 | −4.9 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 96 | 35,676 |
| 283                    | 9.04 | 9.8 | 26.2 | −4.8 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 538 | 178,132 |
| 954                    | 6.95 | 8.2 | 23.2 | −4.8 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 80 | 5777 |
| 150                    | 8.67 | 6.1 | 18.9 | −4.8 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 399 | 279,989 |
| 487                    | 8.55 | 8.6 | 23.3 | −4.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 676 | 331,767 |
| 24                     | 7.81 | 6.0 | 19.7 | −4.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 492 | 1,561,268 |
| 378                    | 8.13 | 7.6 | 22.2 | −4.5 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 312 | 216,069 |
| 245                    | 8.47 | 7.4 | 21.0 | −4.4 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 337 | 358,386 |
| 401                    | 8.63 | 8.2 | 23.5 | −4.3 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 469 | 1,044,872 |
| 47                     | 7.99 | 7.5 | 21.5 | −4.3 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 572 | 705,980 |
| 380                    | 8.29 | 8.3 | 22.6 | −4.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 216 | 117,037 |
| 210                    | 9.75 | 11.1 | 27.5 | −4.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 543 | 455,405 |
| 2021                   | 8.98 | 10.1 | 26.5 | −4.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 422 | 216,849 |
| 328                    | 8.95 | 8.4 | 23.3 | −4.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 250 | 53,435 |
| 388                    | 8.15 | 8.3 | 23.2 | −4.1 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 357 | 62,619 |
| 587                    | 9.10 | 10.7 | 26.8 | −4.0 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 453 | 203,601 |
| 28                     | 8.15 | 6.8 | 20.8 | −4.0 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 222 | 129,541 |
| 189                    | 8.64 | 10.6 | 27.0 | −3.8 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 663 | 1,572,042 |
| 338                    | 9.20 | 8.2 | 21.8 | −3.8 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 2350 | 1,036,938 |
| 777                    | 8.99 | 11.0 | 27.3 | −3.8 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 217 | 80,866 |
| 45                     | 8.54 | 6.4 | 19.1 | −3.7 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 185 | 409,412 |
| 35                     | 8.18 | 6.4 | 19.1 | −3.7 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 692 | 303,877 |
| 505                    | 8.60 | 8.7 | 22.9 | −3.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 267 | 192,809 |
| 16                     | 8.07 | 6.4 | 19.1 | −3.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 117 | 131,479 |
| 85                     | 8.38 | 7.1 | 20.0 | −3.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 258 | 336,817 |
| 481                    | 9.15 | 8.1 | 21.5 | −3.5 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 476 | 376,386 |
| 2913                   | 9.11 | 11.0 | 27.2 | −3.5 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 405 | 329,709 |
| 119                    | 7.66 | 7.5 | 20.4 | −3.4 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 900 | 691,928 |
| 397                    | 8.69 | 8.1 | 21.5 | −3.4 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 719 | 862,645 |
| 353                    | 9.60 | 8.7 | 23.6 | −3.4 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 767 | 648,201 |
| 249                    | 8.66 | 9.9 | 25.3 | −3.4 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 148 | 120,816 |
| 288                    | 8.61 | 9.6 | 25.0 | −3.4 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 106 | 179,141 |
| 511                    | 8.82 | 8.2 | 21.4 | −3.3 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 934 | 704,807 |
| 682                    | 7.96 | 10.9 | 26.6 | −3.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 532 | 854,439 |
| 220                    | 8.55 | 10.9 | 26.8 | −3.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 456 | 1,279,406 |
| 205                    | 8.50 | 8.1 | 20.7 | −3.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 638 | 1,048,589 |
| 233                    | 8.76 | 8.2 | 22.1 | −3.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 127 | 20,772 |
| 406                    | 8.32 | 9.3 | 24.1 | −3.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 1542 | 1,165,501 |
| 1032                   | 8.13 | 9.1 | 24.2 | −3.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 629 | 427,256 |

(Continues)
| Name       | Lake                          | Country      | Latitude  | Longitude | Altitude      | Wtemp  |
|------------|-------------------------------|--------------|-----------|-----------|---------------|--------|
| S171MA     | Lac de Matemale               | France       | 42.5738   | 2.1119    | 1590          | 18.4   |
| Z311WN     | Wiesensee                     | Germany      | 50.5859   | 7.9917    | 404           | 19.3   |
| N102RI     | Oestra Ringsjoen              | Sweden       | 55.8964   | 13.5289   | 61            | 19.2   |
| N241ZE     | Zemminsee                     | Germany      | 52.1569   | 13.6439   | 20            | 21.9   |
| N242SE     | Senftenberger See             | Germany      | 51.5125   | 14.0158   | 91            | 22.4   |
| S033NA     | Reservoir de Naussa           | France       | 44.7297   | 3.8055    | 992           | 26.4   |
| Z083PA     | Lago Paduli                   | Italy        | 44.3482   | 10.1384   | 1137          | 21.5   |
| Z172NC     | Lac de Neuchatel              | Switzerland  | 46.8527   | 6.8382    | 419           | 24.4   |
| N231PL     | Gro er Plessower See          | Germany      | 52.3731   | 12.9086   | 28            | 22.3   |
| N253KE     | Talsperre Kelbra              | Germany      | 51.4278   | 11.0169   | 165           | 23.9   |
| S302WM     | Weinfelder Maar               | Germany      | 50.1746   | 6.8520    | 525           | 20.3   |
| Z163AC     | Lac de Annecy                 | France       | 45.8918   | 6.1391    | 444           | 23.8   |
| B341BRY    | Lake Bregagovo                | Bulgaria     | 41.9677   | 25.1478   | 284           | 27.8   |
| B344KAS    | Lake Kastoria                 | Greece       | 40.5140   | 21.2659   | 633           | 25.4   |
| N132NE     | Neukloster See                | Germany      | 53.8642   | 11.7039   | 36            | 20.2   |
| S011BO     | Bostalsee                     | Germany      | 49.5629   | 7.0747    | 450           | 22.6   |
| S303OL     | Olefalsperre                  | Germany      | 50.4944   | 6.4188    | 505           | 20.0   |
| N103VD     | Yddingesjoen                  | Sweden       | 55.5525   | 13.2614   | 43            | 19.6   |
| N123PL     | Grosser Ploener See           | Germany      | 54.0858   | 10.4203   | 34            | 23.0   |
| N232BH     | Bohenlaender See              | Germany      | 52.4647   | 12.5044   | 25            | 22.3   |
| N233KL     | Klessener See                 | Germany      | 52.7319   | 12.4608   | 12            | 23.5   |
| N251BE     | Bergwitzsee                   | Germany      | 51.7914   | 12.5714   | 83            | 23.6   |
| N252VO     | Vollertsee                    | Germany      | 51.1044   | 12.0528   | 181           | 24.1   |
| S301MM     | Meefelder Maar                | Germany      | 50.1004   | 6.7634    | 375           | 21.3   |
| S012PP     | Lac de Pierre Perce           | France       | 48.4700   | 6.9021    | 407           | 25.5   |
| Z112CD     | Lago di Candia                | Italy        | 45.3205   | 7.8991    | 224           | 30.6   |
| Z011OB     | Obersee-Bodensee             | Germany      | 47.7440   | 9.1522    | 400           | 23.4   |
| Z021UB     | Untersee-Bodensee            | Germany      | 47.7120   | 9.0736    | 399           | 23.6   |
| N121SA     | Sankelmarkar See              | Germany      | 54.7108   | 9.4333    | 38            | 20.5   |
| Z191VW     | Vierwaldstätter See           | Switzerland  | 46.9641   | 8.4821    | 431           | 24.3   |
| N273ST     | Steinhuder Meer               | Germany      | 52.4522   | 9.3497    | 38            | 21.1   |
| Z131AVx    | Laghi di Avigliana            | Italy        | 45.0639   | 7.3931    | 357           | 26.2   |
| B353SAB    | Lake Sablja                   | Croatia      | 45.2276   | 15.2297   | 319           | 19.8   |
| S232ES     | Embalse de Escales            | Spain        | 42.3354   | 0.7380    | 840           | 24.2   |
| Z171GF     | Genfer See                    | Switzerland  | 46.3922   | 6.2581    | 372           | 24.0   |
| N122AR     | Arenholzer See                | Germany      | 54.5358   | 9.4869    | 27            | 21.1   |
| S021CH     | Reservoir de Charmes          | France       | 47.9106   | 5.3821    | 396           | 22.4   |
| Z051SC     | Lago di Scanno                | Italy        | 41.9184   | 13.8618   | 925           | 23.8   |
| Z302LA     | Laacher See                   | Germany      | 50.4065   | 7.2564    | 264           | 21.3   |
| B345OH     | Lake Ohrid                    | Albania      | 40.9330   | 20.6412   | 706           | 22.5   |
| S162NO     | Barrage de Noubels            | France       | 42.7228   | 2.0574    | 1280          | 22.1   |
| S022PA     | Reservoir de Panthier         | France       | 47.2381   | 4.6314    | 413           | 24.0   |
| S311RU     | Rurtalsperre                  | Germany      | 50.6387   | 6.4406    | 320           | 20.3   |
| Conductivity [μS cm⁻¹] | pH   | Bio1 [°C] | Bio5 [°C] | Bio6 [°C] | Mountain range | Alpine_non-alpine | Alpine_arctic_non-alpine/non-arctic | OTUs  | Seqs   |
|-----------------------|------|-----------|-----------|-----------|----------------|------------------|-----------------------------------|-------|-------|
| 55                    | 7.88 | 6.4       | 19.4      | -3.2      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 198   | 45,934 |
| 156                   | 9.15 | 7.5       | 20.4      | -3.2      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 82    | 84,745 |
| 219                   | 8.83 | 7.5       | 20.1      | -3.1      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 721   | 1,215,304 |
| 243                   | 9.15 | 9.3       | 24.0      | -3.1      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 491   | 384,627 |
| 689                   | 7.70 | 9.1       | 23.7      | -3.1      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 1158  | 772,774 |
| 58                    | 8.73 | 8.1       | 22.5      | -3.1      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 570   | 613,642 |
| 121                   | 8.40 | 7.3       | 20.3      | -3.1      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 127   | 143,093 |
| 248                   | 8.82 | 8.9       | 23.5      | -3.1      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 438   | 299,800 |
| 458                   | 8.57 | 9.2       | 23.7      | -3.0      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 607   | 485,380 |
| 791                   | 8.94 | 8.5       | 22.5      | -2.9      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 853   | 344,577 |
| 31                    | 8.16 | 7.6       | 19.9      | -2.9      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 187   | 105,649 |
| 227                   | 8.66 | 10.0      | 25.7      | -2.9      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 317   | 308,836 |
| 223                   | 10.40| 11.8      | 28.8      | -2.8      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 693   | 764,629 |
| 292                   | 9.61 | 11.4      | 28.9      | -2.8      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 440   | 379,705 |
| 464                   | 8.55 | 8.4       | 21.0      | -2.8      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 1115  | 1,115,690 |
| 112                   | 8.96 | 8.2       | 22.2      | -2.8      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 337   | 481,556 |
| 70                    | 8.39 | 7.5       | 19.4      | -2.8      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 593   | 741,829 |
| 361                   | 8.86 | 7.8       | 20.1      | -2.7      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 517   | 159,579 |
| 332                   | 8.91 | 8.1       | 21.0      | -2.7      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 613   | 442,813 |
| 383                   | 7.71 | 9.0       | 23.3      | -2.7      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 642   | 568,480 |
| 421                   | 8.84 | 8.9       | 23.0      | -2.7      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 429   | 603,888 |
| 343                   | 6.85 | 9.1       | 23.5      | -2.7      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 245   | 193,497 |
| 1 599                 | 7.96 | 8.6       | 22.8      | -2.7      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 421   | 341,941 |
| 298                   | 9.27 | 7.9       | 20.3      | -2.7      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 145   | 75,443 |
| 70                    | 7.98 | 8.9       | 23.1      | -2.4      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 333   | 83,566 |
| 129                   | 9.10 | 11.7      | 27.3      | -2.4      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 177   | 416,031 |
| 283                   | 8.55 | 9.4       | 24.0      | -2.3      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 1199  | 779,924 |
| 256                   | 8.52 | 9.4       | 24.1      | -2.3      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 1648  | 777,971 |
| 365                   | 9.03 | 8.0       | 19.8      | -2.2      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 429   | 161,121 |
| 185                   | 8.90 | 9.4       | 23.4      | -2.2      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 688   | 817,173 |
| 281                   | 9.51 | 8.9       | 22.1      | -2.1      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 404   | 790,247 |
| 301                   | 9.24 | 11.7      | 27.4      | -2.1      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 100   | 10,908 |
| 360                   | 8.59 | 10.7      | 26.0      | -2.0      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 979   | 2,171,452 |
| 195                   | 9.08 | 9.3       | 23.5      | -2.0      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 884   | 737,456 |
| 262                   | 8.68 | 10.1      | 25.4      | -2.0      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 376   | 471,289 |
| 316                   | 8.86 | 8.3       | 20.7      | -1.9      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 1007  | 602,683 |
| 237                   | 8.71 | 9.3       | 22.9      | -1.9      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 742   | 439,409 |
| 246                   | 8.42 | 9.1       | 23.3      | -1.9      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 242   | 259,928 |
| 655                   | 8.70 | 8.7       | 21.5      | -1.9      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 190   | 106,811 |
| 240                   | 8.92 | 11.1      | 27.1      | -1.8      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 506   | 1,646,010 |
| 85                    | 9.05 | 8.3       | 21.7      | -1.8      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 506   | 447,353 |
| 300                   | 8.52 | 9.7       | 23.3      | -1.7      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 413   | 275,133 |
| 101                   | 8.53 | 8.5       | 20.7      | -1.7      | Non-alpine     | Non-alpine       | Non-alpine/non-arctic              | 416   | 776,441 |

(Continues)
| Name   | Lake                                      | Country | Latitude [°N] | Longitude [°E] | Altitude [m a.s.l.] | Wtemp [°C] |
|--------|-------------------------------------------|---------|---------------|----------------|---------------------|------------|
| Z301WO | See bei Worms Altherinsee                 | Germany | 49.5785       | 8.3695         | 84                  | 22.4       |
| S041VF | Lac de Villefort                          | France  | 44.4489       | 3.9290         | 648                 | 24.0       |
| S163CM | Lac de Campauleil                         | France  | 42.7108       | 1.8544         | 850                 | 20.4       |
| Z221LA | Lago di Lugano                            | Italy   | 46.0238       | 9.0523         | 365                 | 28.0       |
| Z291BU | See bei Buehl                             | Germany | 48.6955       | 8.0932         | 129                 | 23.8       |
| S292GR | Etang des P tis Grand Etang du Roi        | France  | 49.0090       | 3.7406         | 279                 | 23.3       |
| Z222CM | Lago di Como                              | Italy   | 46.0366       | 9.2392         | 220                 | 28.2       |
| S023GE | Grand Glareins Etang                     | France  | 45.9735       | 4.9877         | 323                 | 26.2       |
| N282HE | Heiliges Meer                             | Germany | 52.3489       | 7.6328         | 40                  | 21.3       |
| Z041SD | Lago di Scandereno                        | Italy   | 42.6374       | 13.2597        | 851                 | 26.2       |
| S291AT | Lac d Auzon-Temple                        | France  | 48.3280       | 4.3836         | 182                 | 22.5       |
| S312ZU | Zueelpicher See                          | Germany | 50.6767       | 6.6581         | 160                 | 20.4       |
| Z052MS | Lago di Montagna Spaccata                 | Italy   | 41.7198       | 14.0121        | 1061                | 24.8       |
| S112S  | Silbersee II                             | Germany | 51.7967       | 7.2153         | 88                  | 20.8       |
| S113B  | Baldeneysee                              | Germany | 51.3987       | 7.0066         | 96                  | 20.4       |
| B341YAS| Lake Yasna Polyana                       | Bulgaria| 42.2512       | 27.5940        | 97                  | 27.5       |
| S272PDxx| Lac de St-Pardoux                        | France  | 46.0380       | 1.2952         | 415                 | 23.4       |
| S111DU | Duennbrucksee                            | Germany | 51.5761       | 6.2979         | 20                  | 20.9       |
| S193ME | Embalse de Mediano                       | Spain   | 42.3234       | 0.1917         | 547                 | 25.4       |
| S262LO | Lac de Lourdes                           | France  | 43.1083       | −0.0794        | 455                 | 26.3       |
| S192BR | Embalse de Barasona                      | Spain   | 42.1272       | 0.3115         | 489                 | 28.1       |
| B347PER| Lake Peruco                              | Croatia | 43.9009       | 16.4523        | 354                 | 23.8       |
| S091CA | Embalse de Canales                       | Spain   | 37.1605       | −3.4774        | 992                 | 24.8       |
| S042SE | Stausee bei Senechas                     | France  | 44.3195       | 4.0472         | 293                 | 26.9       |
| S233TA | Pantano de Talam                         | Spain   | 42.2312       | 0.9734         | 550                 | 29.2       |
| S273LE | Etang des Levrys                         | France  | 47.5251       | 2.0556         | 190                 | 24.1       |
| S282TU | Etang de la Tour                         | France  | 48.6590       | 1.8837         | 209                 | 23.6       |
| B343IOA| Lake Pamvotida Ioaninna                  | Greece  | 39.6657       | 20.8597        | 470                 | 29.6       |
| S142BL | Embalse de la Baells                     | Spain   | 42.1266       | 1.8784         | 680                 | 25.1       |
| S263CM | Lac de la Gimone                         | France  | 43.3363       | 0.6724         | 323                 | 25.7       |
| Z062BI | Lago di Bilancino                        | Italy   | 43.9815       | 11.2655        | 255                 | 27.3       |
| Z053CV | Lago di Castel San Vincenzo              | Italy   | 41.6476       | 14.0557        | 699                 | 24.8       |
| S112CO | Embalse de Contreras                     | Spain   | 39.5562       | −1.4870        | 692                 | 27.0       |
| S281PM | Reteneur de Pincemaille Lac des Mousseaux| France  | 47.4624       | 0.2216         | 123                 | 22.4       |
| B343VOL| Lake Volvis                              | Greece  | 40.6600       | 23.4003        | 42                  | 26.7       |
| S251MO | Lac de Montbel                           | France  | 42.9708       | 1.7949         | 448                 | 26.1       |
| S271TO | Lac du Tondre                            | France  | 44.0228       | 1.4594         | 175                 | 23.6       |
| S252ET | Reteneur de l Estrade                    | France  | 43.2999       | 1.8412         | 284                 | 23.9       |
| S121AR | Embalse de Arenos                        | Spain   | 40.0857       | −0.5522        | 610                 | 25.4       |
| S141SP | Pantano de Sant Ponc                     | Spain   | 41.9638       | 1.6031         | 566                 | 25.2       |
| Conductivity [μS cm⁻¹] | pH | Bio1 [°C] | Bio5 [°C] | Bio6 [°C] | Mountain range | Alpine_non-alpine | Alpine_arctic_non-alpine_non-arctic | OTUs | Seqs |
|------------------------|----|-----------|-----------|-----------|----------------|------------------|----------------------------------|------|-----|
| 694                    | 9.74 | 10.0 | 24.5 | −1.7 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 1,504 | 874,652 |
| 54                     | 8.55 | 10.0 | 24.9 | −1.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 459 | 678,842 |
| 66                     | 8.08 | 8.7 | 22.2 | −1.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 832 | 382,469 |
| 192                    | 8.98 | 10.6 | 24.8 | −1.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 259 | 347,899 |
| 369                    | 8.40 | 10.3 | 25.2 | −1.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 623 | 745,000 |
| 88                     | 8.12 | 9.4 | 23.0 | −1.5 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 181 | 116,885 |
| 162                    | 9.50 | 11.2 | 25.9 | −1.5 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 389 | 515,566 |
| 227                    | 9.18 | 10.9 | 25.9 | −1.3 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 990 | 589,652 |
| 248                    | 8.03 | 9.1 | 21.6 | −1.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 360 | 546,546 |
| 365                    | 8.56 | 10.8 | 26.1 | −1.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 959 | 698,727 |
| 270                    | 8.34 | 10.3 | 24.2 | −1.0 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 184 | 109,648 |
| 567                    | 8.96 | 9.5 | 22.2 | −1.0 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 304 | 466,399 |
| 230                    | 8.37 | 10.0 | 24.3 | −0.9 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 887 | 555,864 |
| 241                    | 8.63 | 9.5 | 22.2 | −0.7 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 914 | 1,181,959 |
| 566                    | 8.06 | 9.8 | 22.6 | −0.7 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 62 | 242,991 |
| 286                    | 8.64 | 13.0 | 28.0 | −0.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 448 | 454,045 |
| 52                     | 7.85 | 10.3 | 24.1 | −0.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 594 | 235,122 |
| 362                    | 8.76 | 9.9 | 22.5 | −0.5 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 313 | 388,895 |
| 227                    | 8.67 | 11.5 | 26.1 | −0.5 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 236 | 310,754 |
| 139                    | 9.11 | 11.2 | 24.1 | −0.5 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 307 | 127,014 |
| 271                    | 8.68 | 12.0 | 27.1 | −0.3 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 185 | 275,382 |
| 297                    | 8.68 | 12.1 | 27.2 | −0.1 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 369 | 1,391,093 |
| 153                    | 9.12 | 12.8 | 31.4 | −0.1 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 378 | 679,396 |
| 69                     | 8.22 | 12.2 | 27.5 | 0.0 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 1,092 | 1,748,867 |
| 195                    | 9.05 | 11.9 | 26.5 | 0.0 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 429 | 501,590 |
| 80                     | 8.20 | 11.0 | 24.6 | 0.1 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 874 | 725,895 |
| 335                    | 9.12 | 10.2 | 23.3 | 0.1 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 529 | 522,124 |
| 268                    | 10.35 | 13.2 | 29.9 | 0.2 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 302 | 1,000,221 |
| 454                    | 8.43 | 10.8 | 24.0 | 0.3 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 292 | 326,901 |
| 173                    | 9.02 | 12.1 | 25.8 | 0.3 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 191 | 50,984 |
| 384                    | 8.48 | 13.0 | 28.6 | 0.4 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 332 | 165,225 |
| 234                    | 8.31 | 12.2 | 26.8 | 0.7 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 177 | 191,967 |
| 1012                   | 8.24 | 13.4 | 29.6 | 0.8 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 356 | 569,624 |
| 361                    | 8.76 | 11.3 | 24.8 | 0.8 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 931 | 373,189 |
| 1007                   | 9.49 | 14.8 | 31.0 | 0.9 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 544 | 500,857 |
| 180                    | 8.81 | 12.2 | 26.1 | 0.9 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 530 | 524,055 |
| 350                    | 8.75 | 12.7 | 27.0 | 0.9 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 267 | 84,179 |
| 253                    | 8.81 | 12.8 | 26.9 | 1.1 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 211 | 776,917 |
| 735                    | 8.26 | 12.8 | 26.8 | 1.4 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 290 | 417,123 |
| 393                    | 8.45 | 12.6 | 26.0 | 1.6 | Non-alpine | Non-alpine | Non-alpine/non-arctic | 180 | 476,617 |

(Continues)
### TABLE A1 (Continued)

| Name      | Lake         | Country      | Latitude [°N] | Longitude [°E] | Altitude [m a.s.l.] | Wtemp [°C] |
|-----------|--------------|--------------|---------------|-----------------|---------------------|-----------|
| B345SCU   | Lake Scutari | Albania      | 42.1287       | 19.4721         | 4                   | 32.8      |
| S191UT    | Embalse de Utxesa | Spain    | 41.4973       | 0.5129          | 207                 | 23.7      |
| S051VC    | Retene de Vinca | France     | 42.6542       | 2.5429          | 284                 | 24.2      |
| Z061BO    | Lago di Bolsena | Italy      | 42.5370       | 11.9221         | 306                 | 27.1      |
| S092BE    | Embalse de Beznar | Spain    | 36.9154       | −3.5381         | 530                 | 25.5      |
| S052BA    | Lago de Banyoles | Spain   | 42.1228       | 2.7531          | 224                 | 27.7      |
| S122SJ    | Embalse de Sitjar | Spain   | 40.0111       | −0.2338         | 204                 | 27.5      |

### TABLE A2

| Latitude (°N) | Longitude (°E) | Altitude (m a.s.l.) | Bio1 (°C) | Bio5 (°C) | Bio6 (°C) | WTemp (°C) | Conductivity (µS cm⁻¹) | pH | OTUs |
|---------------|----------------|---------------------|-----------|-----------|-----------|-------------|------------------------|----|------|
| Mini          | Max            | Mean                |           |           |           |             |                        |    |      |
| Alpine (total)| 37.0475        | 49.4660             | 46.4258   | −3.3684   | 24.6144   | 12.4672     | 6.2                     | 11.6| 6.32 | 37  |
| Non-alpine    | 36.9154        | 61.0994             | 12.4672   | −3.3581   | 27.5940   | 11.0571     | 6.0                     | 12.5| 6.2  | 32  |
| Alps (AL)     | 45.2245        | 49.9098             | 46.9537   | 6.1481    | 25.0730   | 11.5382     | 5.5                     | 20.7| 9.29 | 790 |
| Carpathians (CP)| 45.3583      | 49.4660             | 48.0721   | 19.4854   | 24.6144   | 11.0571     | 2.2                     | 15.4| 8.22 | 250 |
| Pyrenees (PY) | 42.7759        | 42.7759             | 48.0721   | −0.2612   | 21.1709   | 11.0571     | 3.2                     | 16.7| 8.20 | 487 |
| Sierra Nevada (SN)| 37.0475    | 37.0513             | 37.0584   | −0.2612   | 33.2188   | 3.3218     | 4.1                     | 24.2| 8.22 | 229 |

Kruskal–Wallis p-values (alpine - non-alpine): *** = < 0.001, ** = < 0.01, * = < 0.05, . = < 0.1, ' = < 1
| Name                  | Country    | Latitude  | Longitude  | Altitude [m a.s.l.] | Wtemp [°C] | Conductivity [µS cm⁻¹] | pH  | Bio1 [°C] | Bio5 [°C] | Bio6 [°C] | Mountain range | Alpine_non-alpine | Alpine_arctic_non-alpine/non-arctic | OTUs | Sequs             |
|-----------------------|------------|-----------|------------|---------------------|------------|-------------------------|-----|-----------|-----------|-----------|----------------|-------------------|-------------------------------------|------|------------------|
| Lake Scutari          | Albania    | 42.1287   | 19.4721    | 4                   | 32.8       | 181                     | 8.97| 15.1      | 30.7      | 1.7       | Non-alpine      | Non-alpine          | Non-alpine/non-arctic                  | 503  | 950,134          |
| Embalse de Utxesa      | Spain      | 41.4973   | 0.5129     | 207                 | 23.7       | 674                     | 8.25| 15.2      | 31.4      | 1.8       | Non-alpine      | Non-alpine          | Non-alpine/non-arctic                  | 576  | 433,819          |
| Retenue de Vinca       | France     | 42.6542   | 2.5429     | 284                 | 24.2       | 124                     | 9.41| 13.8      | 27.2      | 2.5       | Non-alpine      | Non-alpine          | Non-alpine/non-arctic                  | 323  | 371,383          |
| Lago di Bolsena        | Italy      | 42.5370   | 11.9221    | 306                 | 27.1       | 538                     | 8.86| 14.2      | 29.8      | 2.7       | Non-alpine      | Non-alpine          | Non-alpine/non-arctic                  | 776  | 609,139          |
| Embalse de Beznar      | Spain      | 36.9154   | −3.5381    | 530                 | 25.5       | 553                     | 8.53| 16.0      | 31.8      | 3.9       | Non-alpine      | Non-alpine          | Non-alpine/non-arctic                  | 400  | 526,944          |
| Embalse de Sitjar      | Spain      | 40.0111   | −0.2338    | 204                 | 27.5       | 1,208                   | 8.05| 14.9      | 27.4      | 4.0       | Non-alpine      | Non-alpine          | Non-alpine/non-arctic                  | 557  | 1,286,179        |
| Lago de Banyoles       | Spain      | 42.1228   | 2.7531     | 224                 | 27.7       | 818                     | 8.28| 16.0      | 28.4      | 5.1       | Non-alpine      | Non-alpine          | Non-alpine/non-arctic                  | 262  | 179,334          |
TABLE A3  Relative OTU abundances based on total OTU numbers per group (a) according to the affiliations of all OTUs, specialists, and generalists to main taxonomic groups, (b) according to the affiliations of all OTUs, specialists, and generalists to main taxonomic groups with higher taxonomic resolution, and (c) according to the affiliations of alpine OTUs, alpine specialists, and alpine generalists to distinct classes of (non-) sharing regional groups (mountain ID); AL, Alps; CP, Carpathians; PY, Pyrenees; SN, Sierra Nevada

(a)

| Taxonomic classification | All OTUs | Specialists |
|--------------------------|----------|-------------|
|                          | Total    | Alpine      | Non-alpine | Alps       | Carpathians | Pyrenees | Sierra Nevada | Alpine   |
| Total OTU numbers        | 21.301   | 4754        | 20.008     | 3207       | 2248        | 303      | 557          | 1293     |
| Proportion of all OTUs per group [%] |          |             |            |            |             |          |              | 27.2     |
| Amoebozoa                | 0.7      | 0.5         | 0.7        | 0.3        | 0.8         | 0.7      | 0.0          | 0.9      |
| Archaeplastida           | 15.7     | 13.7        | 15.9       | 12.8       | 14.7        | 7.3      | 22.1         | 13.5     |
| Centrohelida             | 0.5      | 0.7         | 0.5        | 0.6        | 1.0         | 0.7      | 0.0          | 0.4      |
| Cryptophyceae            | 4.2      | 3.8         | 4.3        | 5.0        | 2.4         | 6.6      | 1.6          | 1.9      |
| Excavata                 | 1.1      | 1.2         | 1.1        | 1.3        | 1.2         | 2.3      | 1.3          | 0.9      |
| Haptophyta               | 0.3      | 0.3         | 0.3        | 0.4        | 0.2         | 0.7      | 0.2          | 0.0      |
| Incertae Sedis           | 0.5      | 0.8         | 0.5        | 0.9        | 1.1         | 0.3      | 0.2          | 0.9      |
| Opisthokonta             | 15.6     | 15.8        | 15.5       | 15.4       | 15.4        | 16.5     | 18.0         | 18.5     |
| Picozoa                  | 0.0      | 0.0         | 0.0        | 0.0        | 0.0         | 0.0      | 0.0          | 0.0      |
| SAR; Alveolata            | 35.9     | 34.6        | 35.9       | 33.0       | 38.3        | 33.0     | 20.8         | 34.6     |
| SAR; Rhizaria             | 3.2      | 3.6         | 3.2        | 4.4        | 2.4         | 2.3      | 3.8          | 3.3      |
| SAR; Stramenopiles       | 22.3     | 24.9        | 22.1       | 26.0       | 22.5        | 29.7     | 32.1         | 25.3     |

(b)

| Taxonomic classification | All OTUs | Specialists |
|--------------------------|----------|-------------|
|                          | Total    | Alpine      | Non-alpine | Alps       | Carpathians | Pyrenees | Sierra Nevada | Alpine   |
| Total OTU numbers        | 21.301   | 4754        | 20.008     | 3207       | 2248        | 303      | 557          | 1293     |
| Proportion of all OTUs per group [%] |          |             |            |            |             |          |              | 27.2     |
| Amoebozoa;               | 0.7      | 0.5         | 0.7        | 0.3        | 0.8         | 0.7      | 0.0          | 0.9      |
| Archaeplastida; Chloroplastida; Charophyta | 0.5 | 0.3 | 0.5 | 0.3 | 0.2 | 0.7 | 0.4 | 0.5 |
| Archaeplastida; Chloroplastida; Chlorophyta | 15.2 | 13.4 | 15.3 | 12.4 | 14.4 | 6.6 | 21.7 | 13.0 |
| Archaeplastida; other    | 0.1      | 0.0         | 0.1        | 0.1        | 0.1         | 0.0      | 0.0          | 0.0      |
| Centrohelida;            | 0.5      | 0.7         | 0.5        | 0.6        | 1.0         | 0.7      | 0.0          | 0.4      |
| Cryptophyceae; Cryptomonadales | 2.1 | 1.8 | 2.2 | 2.2 | 1.6 | 3.6 | 0.9 | 1.0 |
| Cryptophyceae; Kathablepharidae | 1.4 | 1.3 | 1.4 | 1.7 | 0.5 | 1.3 | 0.2 | 0.4 |
| Cryptophyceae; other     | 0.7      | 0.7         | 0.7        | 1.1        | 0.2         | 1.7      | 0.5          | 0.5      |
| Excavata;                | 1.1      | 1.2         | 1.1        | 1.3        | 1.2         | 2.3      | 1.3          | 0.9      |
| Haptophyta;              | 0.3      | 0.3         | 0.3        | 0.4        | 0.2         | 0.7      | 0.2          | 0.0      |
| Incertae Sedis;          | 0.5      | 0.8         | 0.5        | 0.9        | 1.1         | 0.3      | 0.2          | 0.9      |
| Opisthokonta; Aphelidea  | 1.6      | 0.7         | 1.6        | 0.4        | 1.0         | 0.3      | 0.7          | 0.7      |
| Opisthokonta; Holozoa    | 1.2      | 1.5         | 1.2        | 1.7        | 1.4         | 3.3      | 1.4          | 1.3      |
| Opisthokonta; Nucleomycyea; Fungi; Chytriomycota | 9.4 | 8.8 | 9.2 | 8.4 | 9.1 | 8.6 | 6.6 | 11.1 |
| Opisthokonta; Nucleomycyea; Fungi; Cryptomycota | 2.2 | 3.0 | 21.7 | 21.6 | 16.6 | 24.1 | 12.2 | 18.1 |
| Opisthokonta; Nucleomycyea; Fungi; other | 1.2 | 1.7 | 1.2 | 1.4 | 1.4 | 1.3 | 3.2 | 2.6 |
| Opisthokonta; other      | 0.1      | 0.0         | 0.1        | 0.0        | 0.0         | 0.0      | 0.0          | 0.0      |
| Picozoa;                 | 0.0      | 0.0         | 0.0        | 0.0        | 0.0         | 0.0      | 0.0          | 0.0      |
| SAR; Alveolata; Ciliophora | 13.1 | 18.4 | 12.7 | 21.6 | 16.6 | 24.1 | 12.2 | 18.1 |
| SAR; Alveolata; Dinoflagellata | 17.9 | 12.5 | 18.2 | 7.1 | 18.7 | 7.3 | 5.9 | 13.2 |
| SAR; Alveolata; other    | 2.2      | 1.4         | 2.3        | 1.4        | 1.2         | 1.3      | 1.8          | 1.1      |
| SAR; Alveolata; Protalveolata | 2.7 | 2.3 | 2.8 | 2.8 | 1.8 | 0.3 | 0.9 | 2.2 |
| Non-alpine | Alps | Carpathians | Pyrenees | Sierra Nevada | Generalists |
|-----------|------|-------------|----------|---------------|-------------|
| 16547     | 730  | 419         | 21       | 174           | 3461        |
| 82.7      | 22.8 | 18.6        | 6.9      | 31.2          | 72.8        |
| 0.7       | 0.3  | 2.4         | 0.0      | 0.0           | 0.4         |
| 16.3      | 12.1 | 13.1        | 0.0      | 24.7          | 13.8        |
| 0.4       | 0.3  | 0.7         | 0.0      | 0.0           | 0.8         |
| 4.2       | 2.6  | 0.7         | 4.8      | 0.6           | 4.6         |
| 1.1       | 0.8  | 0.7         | 0.0      | 1.1           | 1.4         |
| 0.2       | 0.0  | 0.0         | 0.0      | 0.0           | 0.5         |
| 0.4       | 0.8  | 1.2         | 0.0      | 0.0           | 0.8         |
| 15.6      | 17.1 | 22.0        | 19.0     | 18.4          | 14.7        |
| 0.0       | 0.0  | 0.0         | 0.0      | 0.0           | 0.0         |
| 36.2      | 36.0 | 37.2        | 52.4     | 17.8          | 34.6        |
| 3.1       | 4.2  | 1.7         | 4.8      | 2.9           | 3.7         |
| 21.6      | 25.8 | 20.3        | 19.0     | 34.5          | 24.7        |

| Non-alpine | Alps | Carpathians | Pyrenees | Sierra Nevada | Generalists |
|-----------|------|-------------|----------|---------------|-------------|
| 16547     | 730  | 419         | 21       | 174           | 3461        |
| 82.7      | 22.8 | 18.6        | 6.9      | 31.2          | 72.8        |
| 0.7       | 0.3  | 2.4         | 0.0      | 0.0           | 0.4         |
| 16.3      | 12.1 | 13.1        | 0.0      | 24.7          | 13.8        |
| 0.4       | 0.3  | 0.7         | 0.0      | 0.0           | 0.8         |
| 4.2       | 2.6  | 0.7         | 4.8      | 0.6           | 4.6         |
| 1.1       | 0.8  | 0.7         | 0.0      | 1.1           | 1.4         |
| 0.2       | 0.0  | 0.0         | 0.0      | 0.0           | 0.5         |
| 0.4       | 0.8  | 1.2         | 0.0      | 0.0           | 0.8         |
| 15.6      | 17.1 | 22.0        | 19.0     | 18.4          | 14.7        |
| 0.0       | 0.0  | 0.0         | 0.0      | 0.0           | 0.0         |
| 36.2      | 36.0 | 37.2        | 52.4     | 17.8          | 34.6        |
| 3.1       | 4.2  | 1.7         | 4.8      | 2.9           | 3.7         |
| 21.6      | 25.8 | 20.3        | 19.0     | 34.5          | 24.7        |

(Continues)
(b) SAR; Rhizaria; Cercozoa

|                | Total | Alps | Carpathians | Pyrenees | Sierra Nevada |
|----------------|-------|------|-------------|----------|---------------|
| SAR; Rhizaria; other | 3.2   | 3.6  | 3.2         | 4.4      | 2.4           |
| SAR; Stramenopiles; Ochrophyta; Chrysophyceae | 9.4   | 13.0 | 9.1         | 15.0     | 10.6          |
| SAR; Stramenopiles; Ochrophyta; Diatomeae | 5.1   | 3.5  | 5.2         | 3.1      | 4.3           |
| SAR; Stramenopiles; Ochrophyta; Eustigmatophyceae | 1.2   | 0.7  | 1.2         | 0.8      | 0.4           |
| SAR; Stramenopiles; Ochrophyta; other | 0.6   | 0.6  | 0.7         | 0.7      | 1.3           |
| SAR; Stramenopiles; other | 2.5   | 2.7  | 2.5         | 2.8      | 2.1           |
| SAR; Stramenopiles; Peronosporomycetes | 3.6   | 4.3  | 3.5         | 3.5      | 5.3           |

(c) Mountain ID | Alpine OTUs | Alpine specialists |
|----------------|-------------|---------------------|
|                | Total | Alps | Carpathians | Pyrenees | Sierra Nevada |
| Total OTU numbers | 4754  | 3207 | 2248        | 303      | 557           |
| Proportions of all alpine OTUs per group [%] | 44.2  | 65.5 | 0.0         | 0.0      | 0.0           |
| AL              | 25.3  | 0.0  | 53.6        | 0.0      | 0.0           |
| CP              | 0.8   | 0.0  | 0.0         | 12.9     | 0.0           |
| PY              | 5.3   | 0.0  | 0.0         | 0.0      | 45.1          |
| SN              | 14.3  | 21.3 | 30.3        | 0.0      | 0.0           |
| AL-CP           | 1.0   | 1.4  | 0.0         | 15.2     | 0.0           |
| AL-PY           | 1.2   | 1.8  | 0.0         | 0.0      | 10.6          |
| AL-SN           | 0.3   | 0.0  | 0.6         | 4.6      | 0.0           |
| CP-PY           | 0.7   | 0.0  | 1.6         | 0.0      | 6.3           |
| CP-SN           | 0.1   | 0.0  | 0.0         | 1.0      | 0.5           |
| PY-SN           | 2.3   | 3.5  | 4.9         | 36.6     | 0.0           |
| AL-CP-PY        | 2.5   | 3.7  | 5.3         | 0.0      | 21.4          |
| AL-CP-SN        | 0.2   | 0.2  | 0.0         | 2.6      | 1.4           |
| AL-PY-SN        | 1.7   | 2.6  | 3.6         | 27.1     | 14.7          |
| AL-CP-PY-SN     | 0.0   | 0.0  | 0.0         | 0.0      | 0.0           |
| 3.1 | 4.2 | 1.4 | 4.8 | 2.9 | 3.7 | 3.7 | 4.4 | 2.6 | 2.1 | 4.2 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 8.4 | 16.3 | 10.7 | 4.8 | 11.5 | 12.7 | 12.7 | 14.6 | 10.6 | 13.8 | 12.0 |
| 5.5 | 3.0 | 1.4 | 0.0 | 7.5 | 3.7 | 3.7 | 3.1 | 3.8 | 4.6 | 6.8 |
| 1.3 | 1.2 | 0.2 | 0.0 | 2.3 | 0.6 | 0.6 | 0.7 | 0.4 | 0.4 | 1.6 |
| 0.6 | 0.7 | 0.2 | 0.0 | 0.0 | 0.6 | 0.6 | 0.7 | 0.8 | 1.4 | 0.5 |
| 2.4 | 2.6 | 1.2 | 9.5 | 4.6 | 2.8 | 2.8 | 2.9 | 2.4 | 3.9 | 3.1 |
| 3.4 | 1.9 | 6.4 | 4.8 | 8.6 | 4.2 | 4.2 | 4.0 | 5.0 | 6.4 | 7.0 |

| Mountain ID | Alpine OTUs | Alpine specialists | Alpine generalists | Total Alps | Carpathians | Pyrenees | Sierra Nevada |
|-------------|-------------|--------------------|--------------------|------------|-------------|----------|--------------|
| Carpathians | Pyrenees | Sierra Nevada | Total | Alps | Carpathians | Pyrenees | Sierra Nevada |
| 419         | 21       | 174               | 3461 | 2477 | 1829       | 282      | 383          |
| 18.6        | 6.9      | 31.2              | 72.8  | 77.2  | 81.4        | 93.1      | 68.8         |
| 0.0         | 0.0      | 0.0               | 40.7  | 56.9  | 0.0         | 0.0       | 0.0          |
| 90.5        | 0.0      | 0.0               | 23.9  | 0.0   | 45.2        | 0.0       | 0.0          |
| 0.0         | 81.0     | 0.0               | 0.6   | 0.0   | 0.0         | 7.8       | 0.0          |
| 0.0         | 0.0      | 92.0              | 2.6   | 0.0   | 0.0         | 0.0       | 23.8         |
| 6.9         | 0.0      | 0.0               | 18.9  | 26.4  | 35.7        | 0.0       | 0.0          |
| 0.0         | 9.5      | 0.0               | 1.3   | 1.8   | 0.0         | 15.6      | 0.0          |
| 0.0         | 0.0      | 0.0               | 1.6   | 2.2   | 0.0         | 0.0       | 14.1         |
| 0.0         | 0.0      | 0.0               | 0.4   | 0.0   | 0.8         | 5.0       | 0.0          |
| 1.7         | 0.0      | 4.0               | 0.8   | 0.0   | 1.5         | 0.0       | 7.3          |
| 0.0         | 0.0      | 0.0               | 0.1   | 0.0   | 0.0         | 1.1       | 0.8          |
| 0.5         | 9.5      | 0.0               | 3.1   | 4.4   | 6.0         | 38.7      | 0.0          |
| 0.5         | 0.0      | 1.1               | 3.4   | 4.7   | 6.4         | 0.0       | 30.5         |
| 0.0         | 0.0      | 0.0               | 0.2   | 0.3   | 0.0         | 2.8       | 2.1          |
| 0.0         | 0.0      | 0.0               | 2.4   | 3.3   | 4.5         | 29.1      | 21.4         |