Received: 30 November 2020 | Revised: 2 June 2021 | Accepted: 4 June 2021

DOI: 10.1002/mbo3.1216

ORIGINAL ARTICLE

MicrobiologyOpen Open Access WILEY

Patterns of protist distribution and diversification in alpine lakes across Europe

Janina C. Vogt[1] | Jana L. Olefeld[2] | Christina Bock[2] | Jens Boenigk[2] | Dirk C. Albach[1]

[1]Institute for Biology and Environmental Science (IBU), Plants Biodiversity and Evolution, Carl von Ossietzky University, Oldenburg, Germany

[2]Department of Biodiversity, University of Duisburg-Essen, Essen, Germany

Correspondence
Janina C. Vogt, Institute for Biology and Environmental Science (IBU), Plant Biodiversity and Evolution, Carl-von-Ossietzky University of Oldenburg, Carl-von-Ossietzky-Str. 9-11, 26129 Oldenburg, Germany.
Email: janina.vogt@uol.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: AL632/14-1, BO 3245/17-2 and BO 3245/19-1

Abstract

Biogeography in Europe is known to be crucially influenced by the large mountain ranges serving as biogeographical islands for cold-adapted taxa and geographical barriers for warm-adapted taxa. While biogeographical patterns are well-known for plants and animals in Europe, we here investigated diversity and distribution patterns of protist freshwater communities on a European scale (256 lakes) in the light of the well-studied post-glacial distribution patterns of macroorganisms. Thus, our study compared 43 alpine protist communities of lakes located in the Alps, Carpathians, Pyrenees, and the Sierra Nevada with that of surrounding lowland lakes. We verified altitudinal diversity gradients of freshwater protists with decreasing richness and diversity across altitudes similar to those observed for plants and animals. Alpine specialists and generalists could be identified differing significantly in richness and diversity, but hardly in occurrence and proportions of major taxonomic groups. High proportions of region-specific alpine specialists indicate an increased occurrence of distinct lineages within each mountain range and thus, suggested either separated glacial refugia or post-glacial diversification within mountain ranges. However, a few alpine specialists were shared between mountain ranges suggesting a post-glacial recolonization from a common lowland pool. Our results identified generalists with wide distribution ranges and putatively wide tolerance ranges toward environmental conditions as main drivers of protist diversification (specification) in alpine lakes, while there was hardly any diversification in alpine specialists.

KEYWORDS
alpine lakes, biogeography, diversification, evolutionary patterns, molecular diversity, protists

1 | 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; Sanmartín, 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 glaciation 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 alpine-specific (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 glaciation 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 (Figuerola & Green, 2002; Foissner, 2006). Especially protist taxa that can form cysts and other robust dormant stages were predestinated 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; Tolotti 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 altitudinal 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; Slaveykova 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 (Tolotti 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 (Tolotti 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 (Casteleyn 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 x 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.rproject.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 (<1500m 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 (<1500m a.s.l) to the 'alpine' cluster (altitudinal range of 527 m a.s.l. to 3120m a.s.l., 1656m 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 underrepresented 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 (λ), 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.ultrametric(phy, method="extend"), R package 'phytools' v0.7–20 (Revell, 2012)) as well as to be bifurcated (multi2di(phy), R package 'ape' v5.3 (Paradis & Schliep, 2019)). Based on these trees together with an

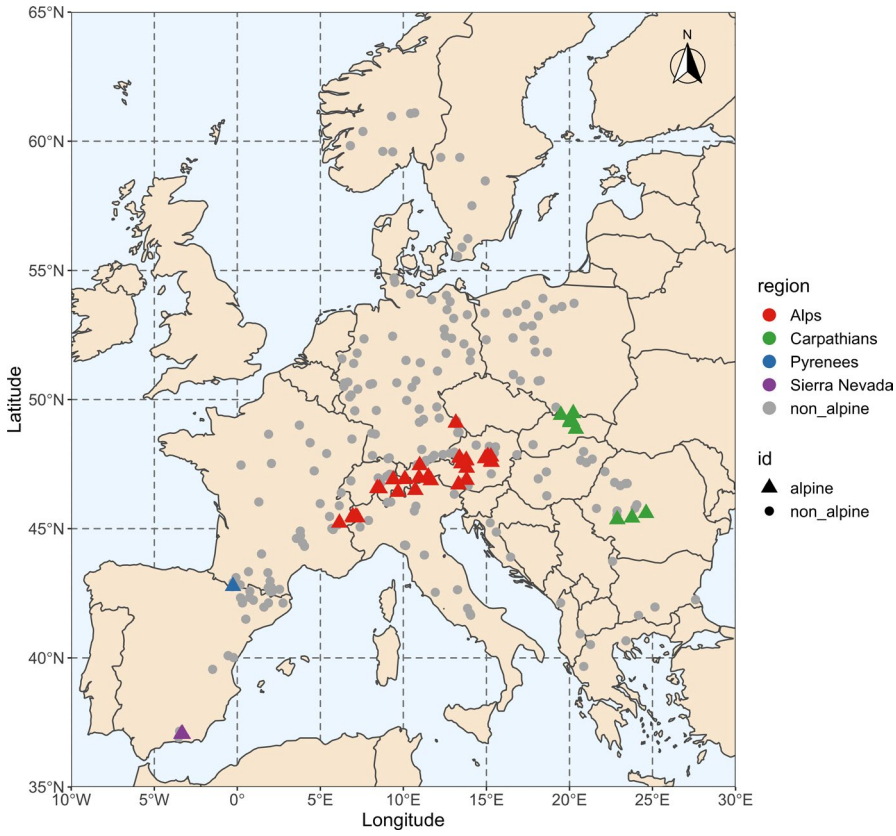

FIGURE 1 Alpine and non-alpinesampling sites (coded by shapes); different mountain ranges are coded by color

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%)	3461 (17%)	16,547 (83%)	10,783 (54%)

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 (λ_0~λ_1), Birth/death (λ_0~λ_1, μ_0~μ_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 'diversitree' (FitzJohn, 2012)) with an exponential prior value (prior <- make.prior.exponential(1/(2*(λ-μ))) and the step size 'w' obtained as widths range of high-probability regions for observed samples of a short pre-chain (100 steps).

3 | 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

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, Diatomeae), Opisthokonta (mainly Chytridiomycota), 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, Incertae Sedis (mainly Telonema), Centrohelida, Haptophyta, and Picozoa (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 Diatomeae and higher proportions for OTUs classified as Incertae Sedis, Ciliophora, and Chrysophyceae (Kruskal-Wallis *p*-values < 0.05) in alpine compared to non-alpine lakes. Except for the phylum Picozoa 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).

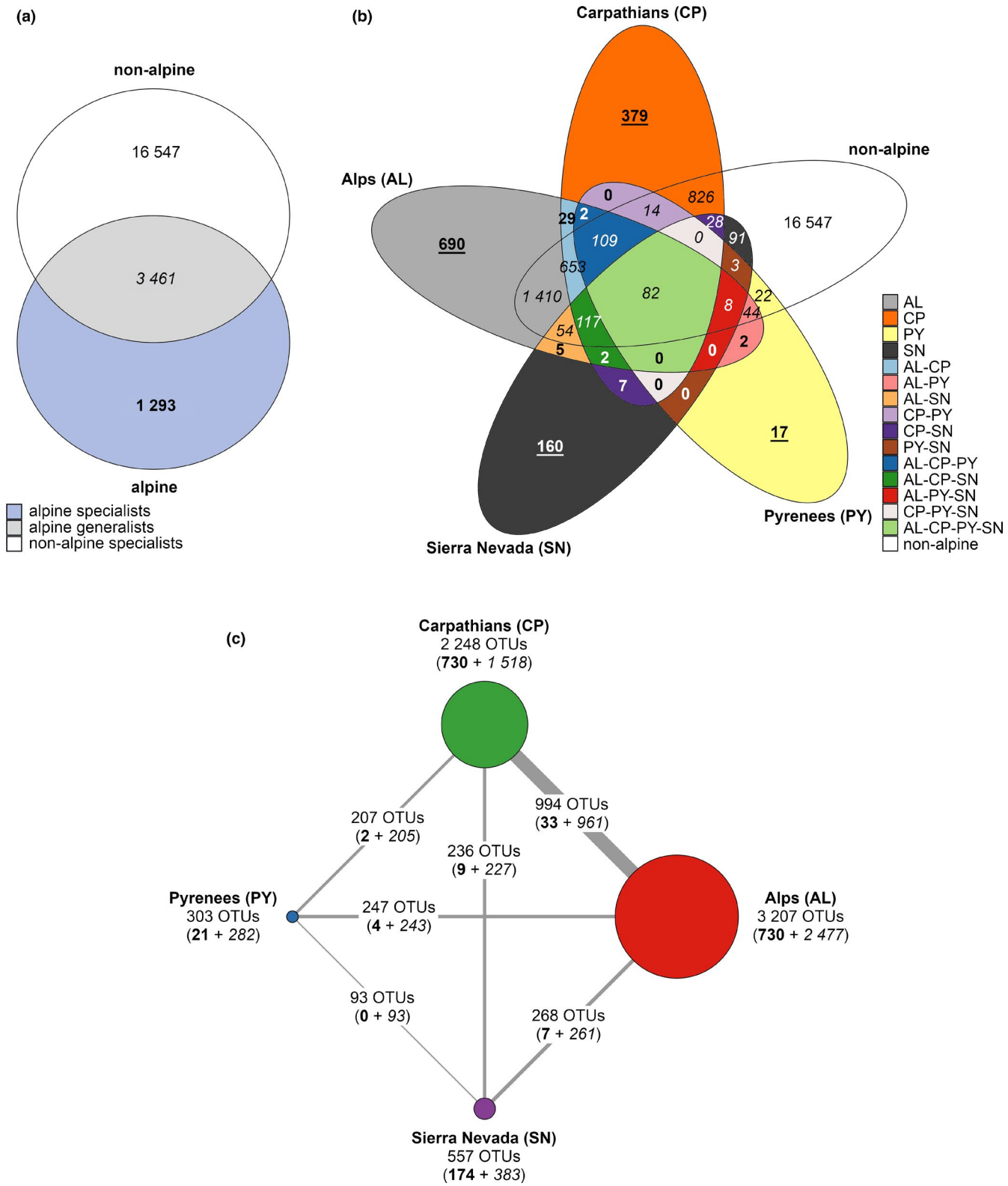

FIGURE 2 Venn diagrams showing shared OTUs of alpine and non-alpine lakes (a) and lakes within the four alpine regions (b) (AL, Alps; CP, Carpathians; PY, Pyrenees; SN, Sierra Nevada) and non-alpine regions; (underlined) bold, italic and standard numbers describe OTU numbers of (region-specific) alpine 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 < 0.001); 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 shared 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

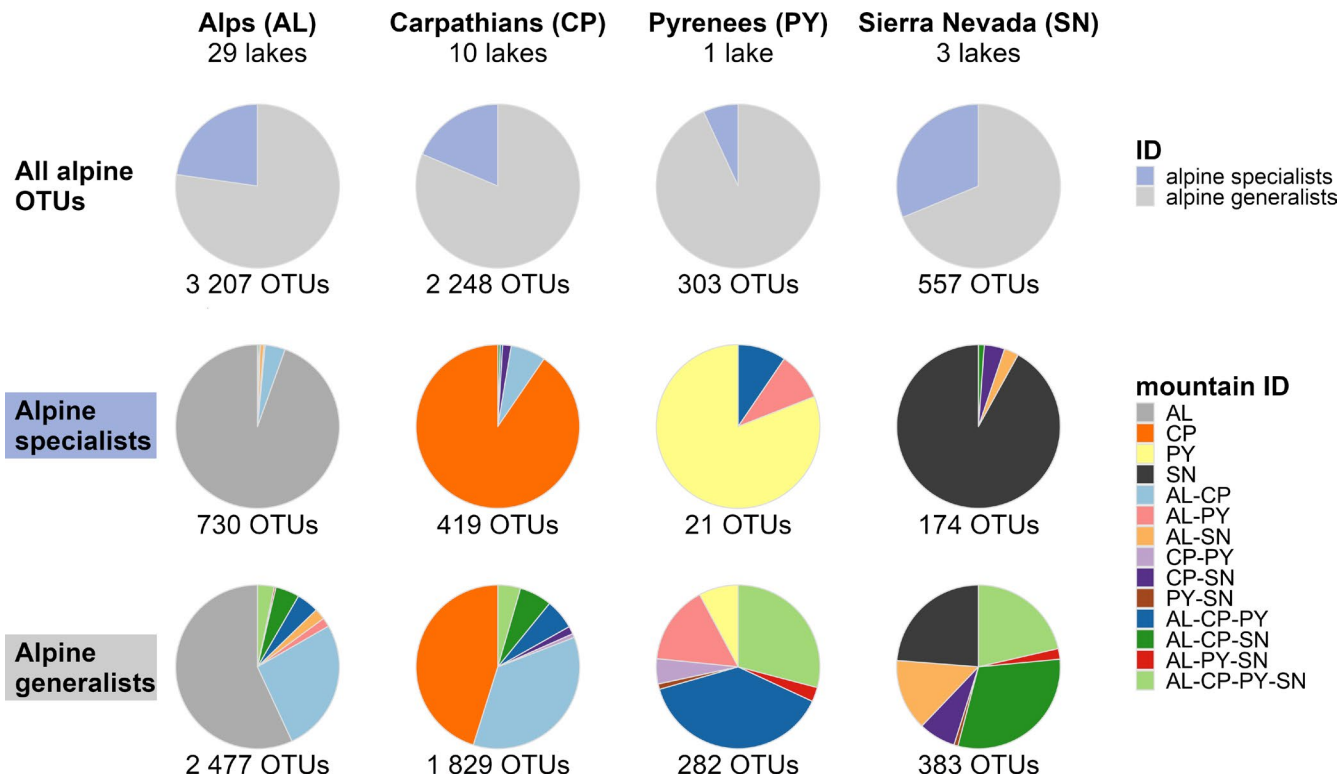

FIGURE 3 Proportions of alpine OTUs classified as alpine specialists and generalists (top) and affiliations of OTUs classified as alpine specialists (mid) and alpine generalists (bottom) to distinct groups of (non-)sharing regional groups (mountain ID); AL, Alps; CP, Carpathians; PY, Pyrenees; SN, Sierra Nevada

detected within all alpine and 212 non-alpine lakes (classified as 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 monotonical 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 (532–2785m a.s.l., linear regression *p*-values < 0.05), whereas not more than slight trends could be found within the Carpathians (527–2030m 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

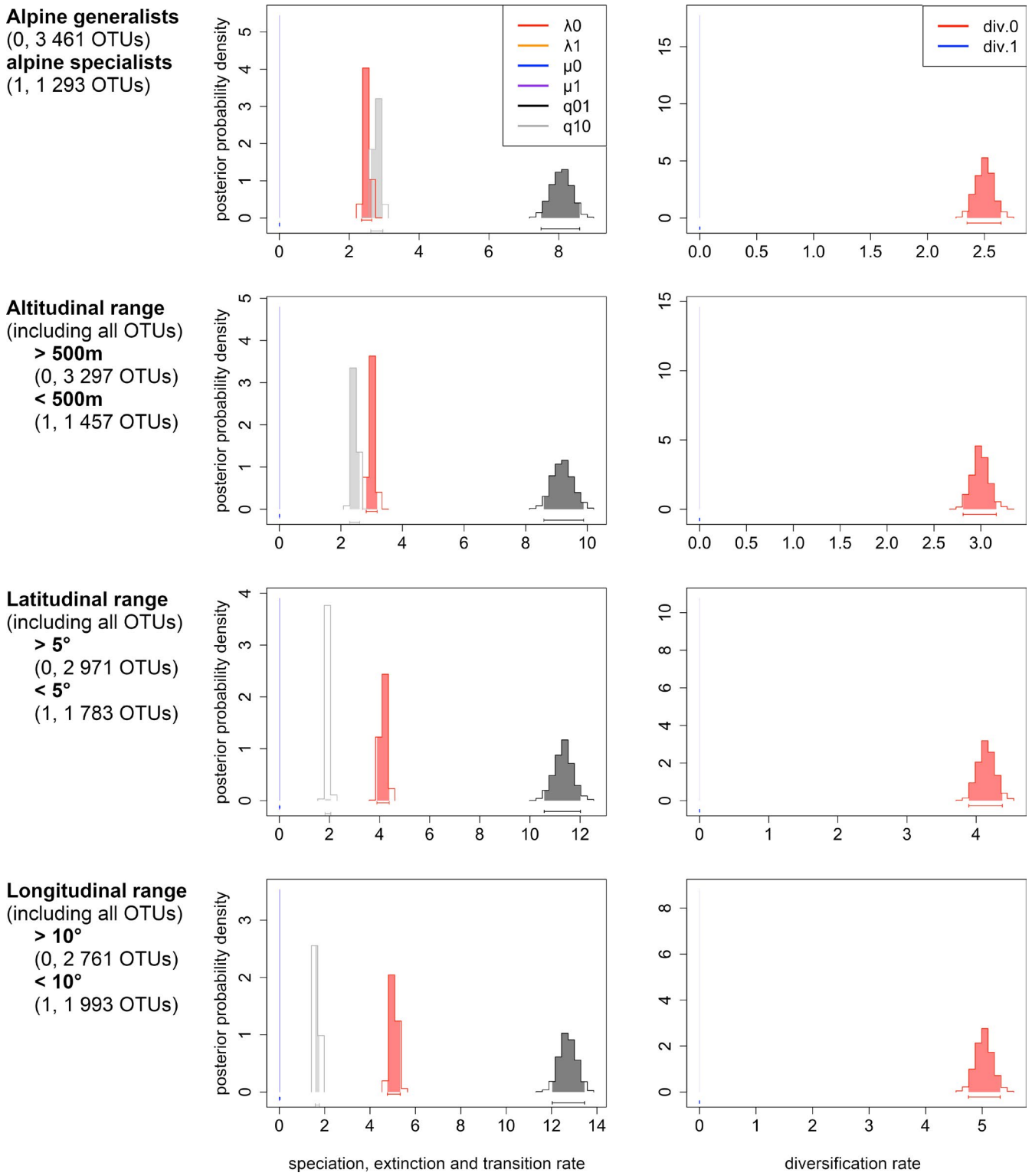

FIGURE 4 Estimation of evolutionary characteristics for generalists (0) and specialists (1) as well as widely distributed (0) and more restricted (1) taxa (altitude, latitude, and longitude) using Binary-state speciation and extinction (BiSSE) models providing distinct speciation (λ), extinction (μ), 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

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

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 = .001).

Mountain ranges are often considered as biogeographical islands for alpine specialists (Schmitt, 2017) as supported by high levels of endemicity 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 retraction 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 *Hemiamphisiella 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 (Stibal & 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.

ACKNOWLEDGMENTS

We thank the German Research Foundation (projects AL632/14-1, BO 3245/19-1, and BO 3245/17-2) for financial support. We thank the Republic of Bulgaria, the Ministry of Environment and Water, and the Republic of Albania, National Agency of Protected Areas (AKZM) for sampling permissions. We thank Joachim Stadel, Verena Stadel, Susann Chamrad, Steffen Jost, Geoffrey Ongondo, Stanislava Stojanova, the employees of Yasna Polyana Reservoir, Emil Dilov with colleagues from Dospat Reservoir, and Matthias Rohlfs for support during the sampling campaign, and Eike Mayland-Quellhorst and Marius Welzel for support in setting up of the bioinformatic pipeline.

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 &

editing (equal). **Jana L. Olefeld:** Investigation (supporting); Project administration (supporting); Writing-review & editing (supporting). **Christina Bock:** Conceptualization (supporting); Data curation (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Writing-review & editing (equal). **Jens Boenigk:** Conceptualization (supporting); Funding acquisition (equal); Methodology (supporting); Project administration (equal); Resources (supporting); Supervision (supporting); Writing-review & editing (equal). **Dirk Albach:** Conceptualization (equal); Funding acquisition (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision (lead); Validation (equal); Writing-review & editing (equal).

ETHICS STATEMENT

None required.

DATA AVAILABILITY STATEMENT

Raw data sequences generated and/or analyzed during the current study are available in the NCBI GenBank repository as BioProject PRJNA414052 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA414052) and BioProject PRJNA671833 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA671833). All other relevant data generated or analyzed during this study are included in this published article.

ORCID

Janina C. Vogt https://orcid.org/0000-0002-0943-1144
Christina Bock https://orcid.org/0000-0002-6509-3768

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How to cite this article: Vogt, J. C., Olefeld, J. L., Bock, C., Boenigk, J., & Albach, D. C. (2021). Patterns of protist distribution and diversification in alpine lakes across Europe. *MicrobiologyOpen*, 10, e1216. https://doi.org/10.1002/mbo3.1216

APPENDIX 1

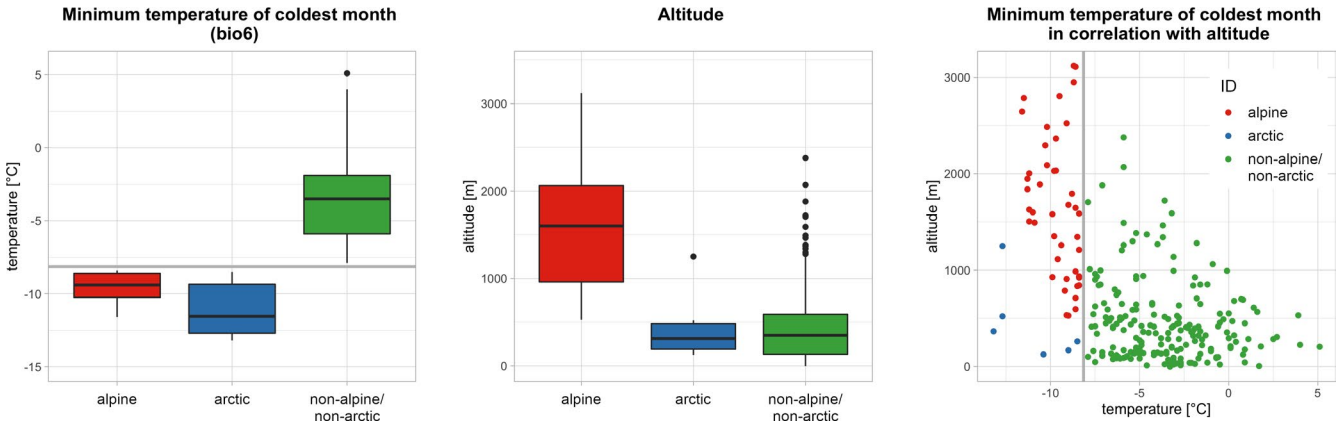


Figure A1 Classification of alpine and non-alpine (arctic and non-arctic) lakes according to their minimum temperatures of the coldest month (bio6) in relation to altitude

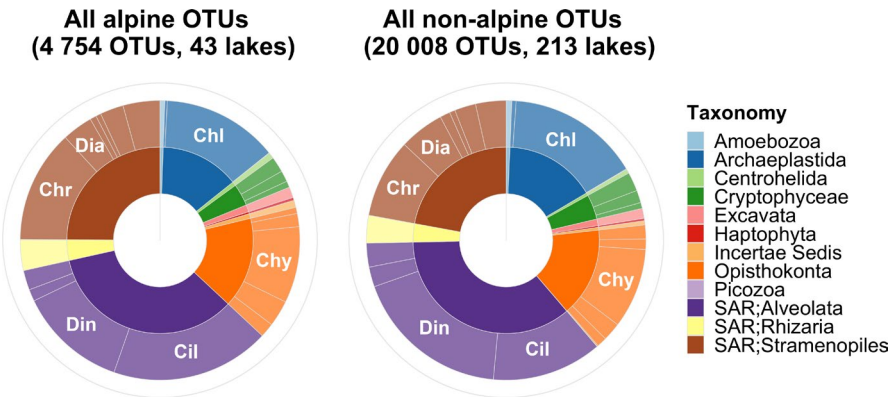
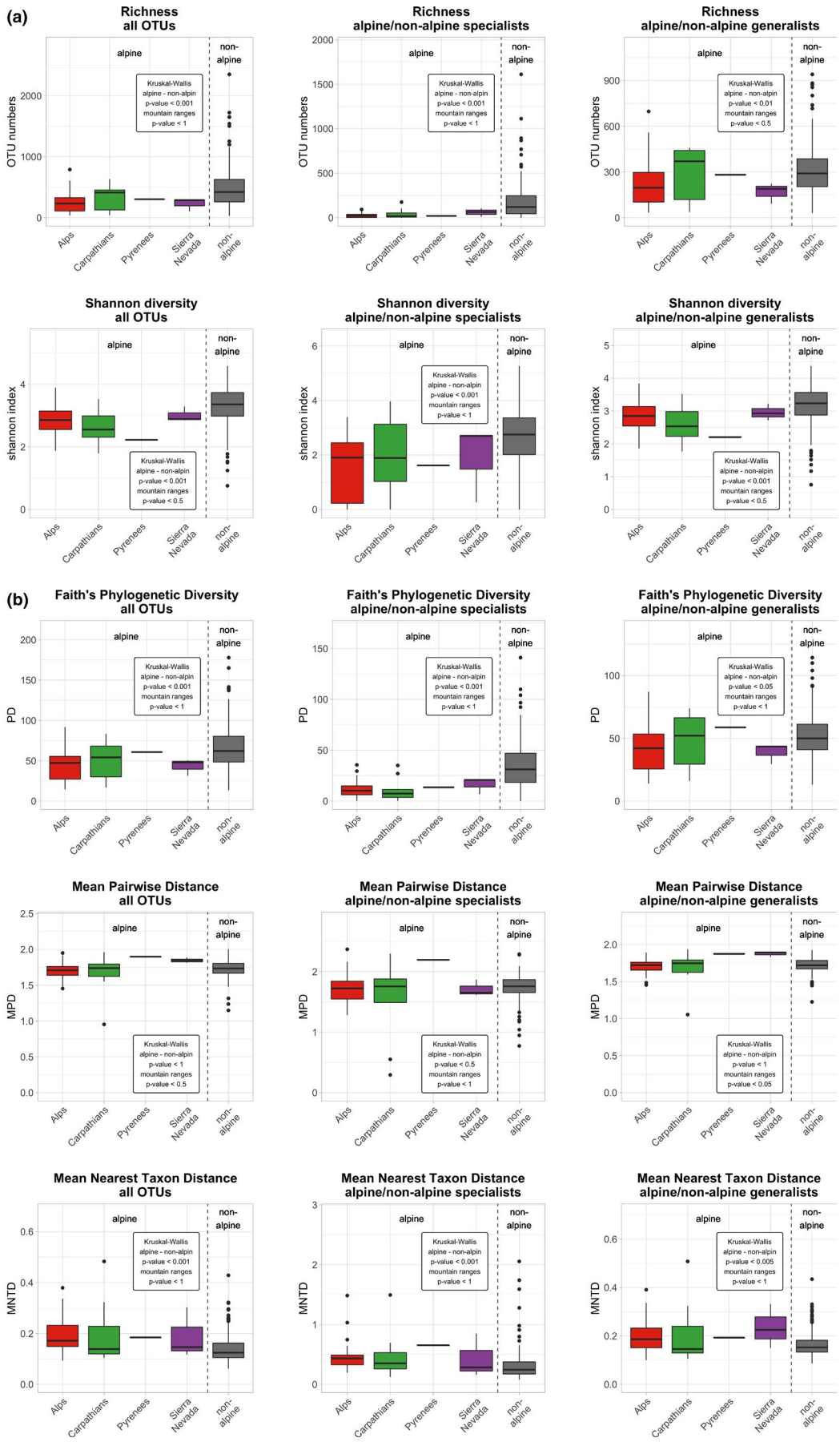


Figure A2 Taxonomic affiliations and relative abundances of OTUs detected in alpine and non-alpine (lowland) lakes; Chl, Chlorophyta; Chy, Chytridiomycota; Cil, Ciliophora; Din, Dinoflagellata; Chr, Chrysophyceae; Dia, Diatomeae

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


(a)
Richness all OTUs
Richness alpine/non-alpine specialists
Richness alpine/non-alpine generalists
OTU numbers
Kruskal-Wallis alpine - non-alpin p-value < 0.001 mountain ranges p-value < 1
Kruskal-Wallis alpine - non-alpin p-value < 0.001 mountain ranges p-value < 1
Kruskal-Wallis alpine - non-alpin p-value < 0.01 mountain ranges p-value < 0.5
Shannon diversity all OTUs
Shannon diversity alpine/non-alpine specialists
Shannon diversity alpine/non-alpine generalists
shannon index
Kruskal-Wallis alpine - non-alpin p-value < 0.001 mountain ranges p-value < 0.5
Kruskal-Wallis alpine - non-alpin p-value < 0.001 mountain ranges p-value < 1
Kruskal-Wallis alpine - non-alpin p-value < 0.001 mountain ranges p-value < 0.5
(b)
Faith's Phylogenetic Diversity all OTUs
Faith's Phylogenetic Diversity alpine/non-alpine specialists
Faith's Phylogenetic Diversity alpine/non-alpine generalists
PD
Kruskal-Wallis alpine - non-alpin p-value < 0.001 mountain ranges p-value < 1
Kruskal-Wallis alpine - non-alpin p-value < 0.001 mountain ranges p-value < 1
Kruskal-Wallis alpine - non-alpin p-value < 0.05 mountain ranges p-value < 1
Mean Pairwise Distance all OTUs
Mean Pairwise Distance alpine/non-alpine specialists
Mean Pairwise Distance alpine/non-alpine generalists
MPD
Kruskal-Wallis alpine - non-alpin p-value < 1 mountain ranges p-value < 0.5
Kruskal-Wallis alpine - non-alpin p-value < 0.5 mountain ranges p-value < 1
Kruskal-Wallis alpine - non-alpin p-value < 1 mountain ranges p-value < 0.05
Mean Nearest Taxon Distance all OTUs
Mean Nearest Taxon Distance alpine/non-alpine specialists
Mean Nearest Taxon Distance alpine/non-alpine generalists
MNTD
Kruskal-Wallis alpine - non-alpin p-value < 0.001 mountain ranges p-value < 1
Kruskal-Wallis alpine - non-alpin p-value < 0.001 mountain ranges p-value < 1
Kruskal-Wallis alpine - non-alpin p-value < 0.005 mountain ranges p-value < 1
alpine
non-alpine
Alps
Carpathians
Pyrenees
Sierra Nevada
non-alpine

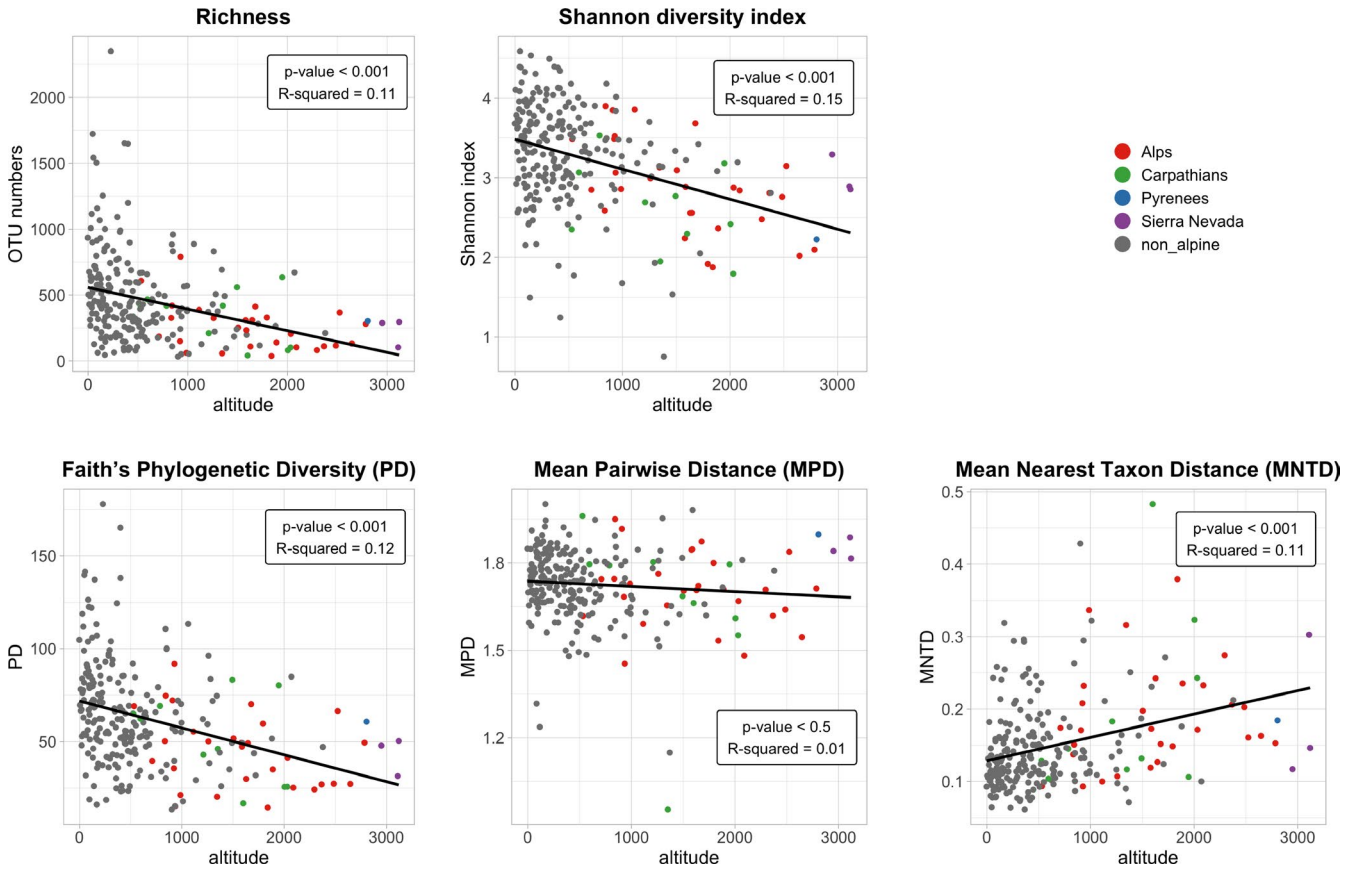


Figure A4 Linear regression analyses of OTU- and phylogeny-based alpha diversity estimates per lake in correlation to altitudes

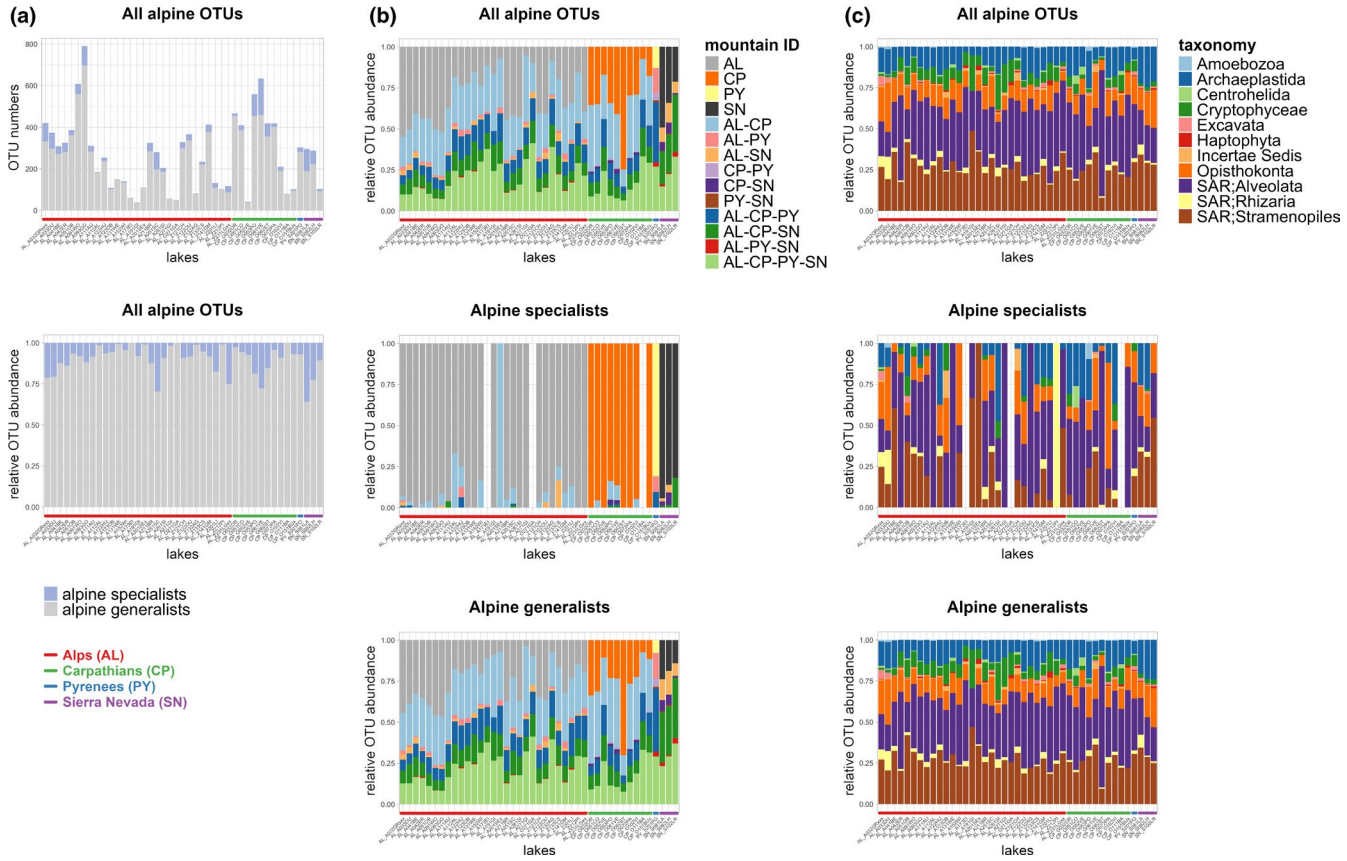


Figure A5 (Relative) OTU abundances per lake: (a) Alpine OTUs classified as specialists and generalists; (b) Affiliations of alpine OTUs (top), alpine specialists (mid), and alpine generalists (bottom) per lake to distinct groups of (non-) sharing regional groups (mountain ID); (c) Affiliations of alpine OTUs (top), alpine specialists (mid) and alpine generalists (bottom) per lake to main taxonomic groups; AL, Alps; CP, Carpathians; PY, Pyrenees; SN, Sierra Nevada

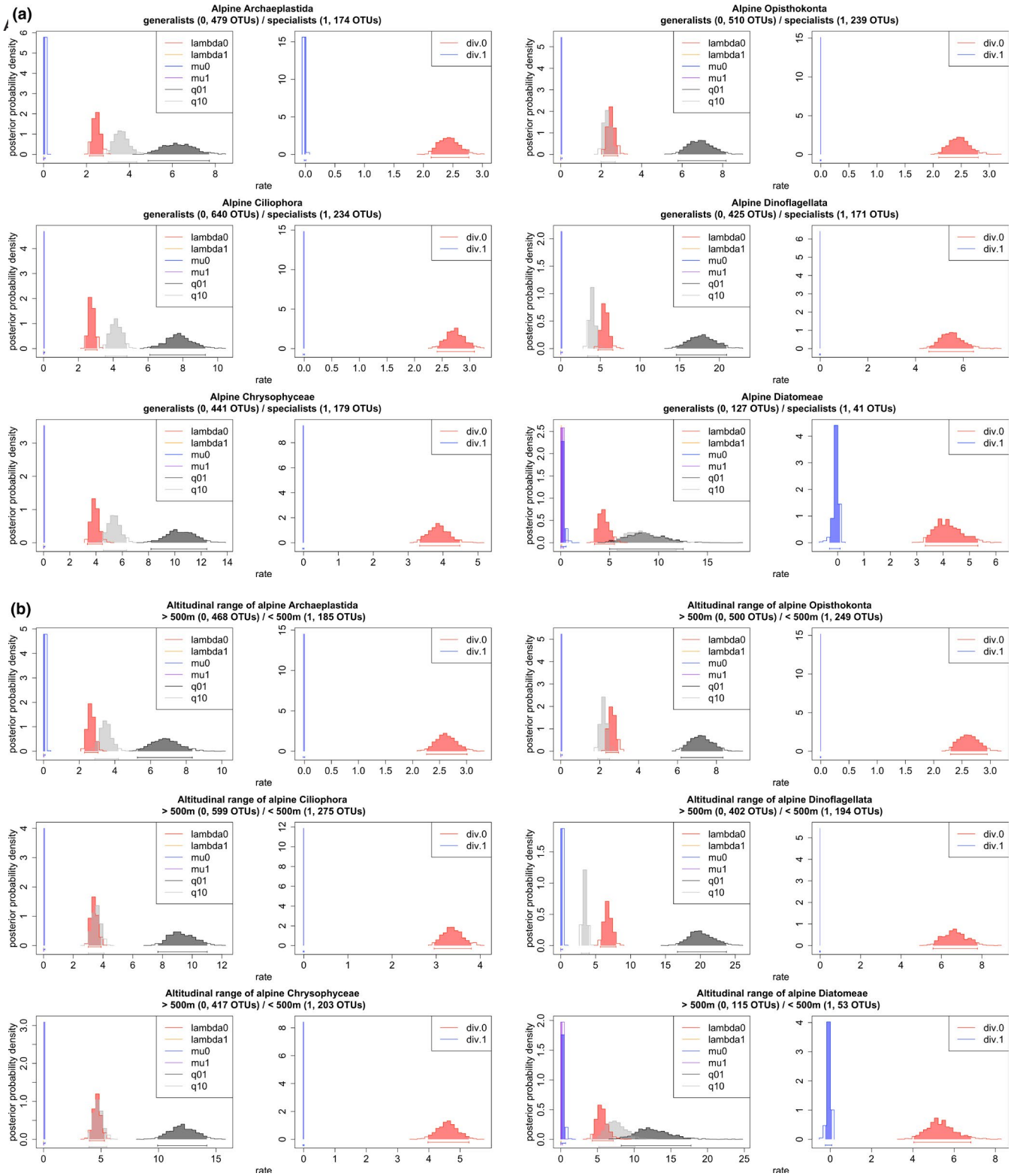


Figure A6 (Continued)

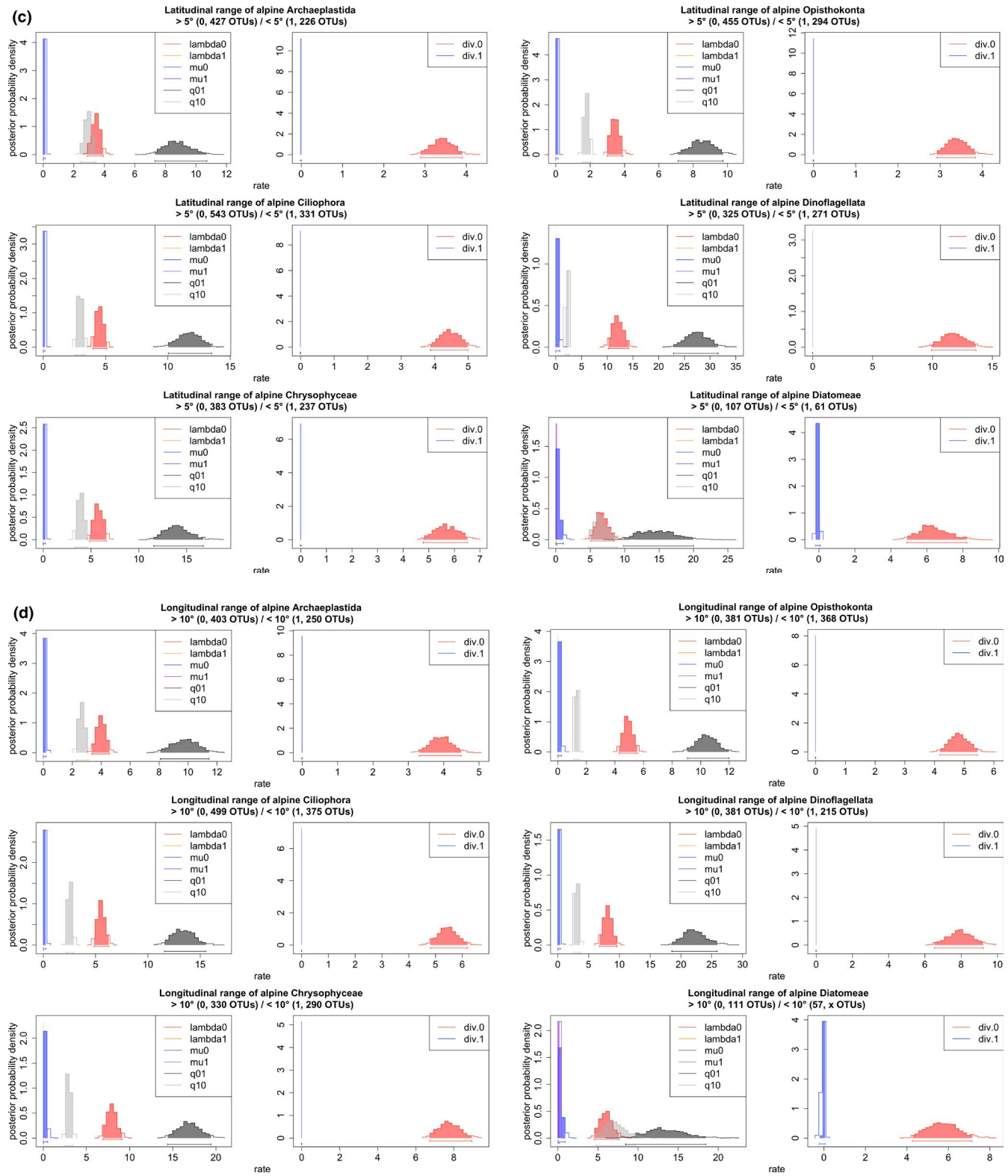


Figure A6 Estimation of evolutionary characteristics of major taxonomic groups using Binary-state speciation and extinction (BiSSE) models providing distinct speciation (lambda), extinction (mu), and state-transition (*q*) rates per state; diversification rates (div) were calculated as a difference of 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://biogeo.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 the 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	Schwaerziseeli	Switzerland	46.5631	8.4301	2646	15.6
	A251SC	Schwarzsee	Austria	46.9654	10.9448	2785	13.7
	A191GI	Gioveretto	Italy	46.4919	10.7176	1839	17.8
	A122HU	Huettensee	Austria	47.3543	13.8096	1505	15.5
	A123OB	Obersee	Austria	47.3515	13.8177	1628	13.3
	A152WI	Windebensee	Austria	46.8864	13.8026	1889	15.4
	Z132AG	Lago Agnel	Italy	45.4696	7.1402	2295	15.8
	Z212PI	Lago della Piazza	Switzerland	46.5566	8.5674	2089	16.9
	Z231LGxx	Laegh dal Lunghin	Switzerland	46.4169	9.6753	2485	15.3
	A041BE	Bergsee	Austria	47.7248	14.9239	1580	16.5
	A093GO	Vorderer Gosausee	Austria	47.5250	13.4921	927	19.8
	A201SEx	Grosser Seefeld See	Italy	46.8714	11.6546	2366	17.1
	A261SI	Silvretta	Austria	46.9176	10.0915	2033	12.5
	A051OB	Obersee Lunz	Austria	47.8060	15.0785	1113	21.5
	A211BR	Brennersee	Austria	47.0169	11.5022	1259	20.2
	A033DU	Duerer See	Austria	47.6048	15.2827	908	12.2
	A091WO	Wolfgangsee	Austria	47.7429	13.3728	532	21.5
	Z122OU	Lac L Ouillette	France	45.4298	6.9951	2523	18.1
	Z141GM	Lac de Grand Maison	France	45.2245	6.1481	1677	17.6
	Z121CH	Lac du Chevril	France	45.4749	6.9482	1793	15.5
	A111AU	Augstsee	Austria	47.6577	13.7857	1646	14.7
	A112AL	Altausseer See	Austria	47.6380	13.7696	710	14.7
	A173EI	Eibsee	Germany	47.4535	10.9852	986	21.6
	A042ER	Erlaufsee	Austria	47.7870	15.2703	834	21.3
	A271GI	Gigerwaldsee	Switzerland	46.9061	9.3883	1344	12.9
	A032GRxxx	Gruener See	Austria	47.5816	15.3075	842	11.5
	A131WE	Weissensee	Austria	46.7056	13.3202	922	22.9
	O271GA	Grosser Arbersee	Germany	49.0988	13.1595	935	15.5
	Z133CS	Lago Ceresole	Italy	45.4342	7.2274	1587	18.0
Carpathians	O061VE	Velke Hincove Pleso	Slovakia	49.1763	20.0602	1947	12.9
	O111BA	Balea	Romania	45.6030	24.6144	2004	13.8
	O052VE	Velicke Pleso	Slovakia	49.1563	20.1566	1600	12.0
	O053PO	Popradske Pleso	Slovakia	49.1552	20.0805	1493	14.5
	O062ST	Strebske Pleso	Slovakia	49.1207	20.0570	1351	23.3
	O151BUx	Bucura	Romania	45.3583	22.8761	2030	14.5
	O072PA	Palcmanska Masa	Slovakia	48.8630	20.3860	786	23.0
	O051CO	Jezioro Czorsztynskie	Poland	49.4660	20.2261	527	21.9
	O032OR	Orava	Slovakia	49.3977	19.4854	594	25.6
	O102VI	Vidra Lacula	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	1,162,312
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)

TABLE A1 (Continued)

	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 Aquas Verdes	Spain	37.0481	−3.3684	3110	16.4
Non-alpine (arctic)	N041ST	Strondafjorden	Norway	60.9650	9.2828	365	15.4
	N033SK	Skiftessjoeen	Norway	60.3772	7.5656	1250	11.9
	N051NO	Nordmesna	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 Dragan Floroiu Lacul	Romania	46.7906	22.7166	850	21.2
	N072JA	Jaernsjoen	Sweden	59.3728	12.2483	147	18.1
	N171JS	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	Millstaetter 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/non-arctic	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)

TABLE A1 (Continued)

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	Dreiburgensee	Germany	48.7382	13.3512	442	22.6
N182ZN	Jezioro Duze Zninskie	Poland	52.8558	17.7525	86	23.7
N191BW	Borownosee	Poland	53.2358	18.1314	88	22.1
N202JZ	Jezioro Jeziorsko	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	Seehamer 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 Slupeckie	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	1.9972	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 Niepruszewskie	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	Luetschetalsperre	Germany	50.7336	10.7567	591	20.2
O012BI	Bialy Kosciol	Poland	50.7271	17.0395	172	26.7
O021BI	Biestrzynik	Poland	50.7374	18.2391	195	26.7
O101CI	Cincis Lacula	Romania	45.6902	22.8684	297	25.2
Z151LR	Lac du Laffrey	France	45.0218	5.7783	908	22.1
Z152PC	Piere Chatel	France	44.9719	5.7725	937	23.2
Z192AS	Arnisee	Switzerland	46.7705	8.6429	1384	13.9
A021IEB	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)

TABLE A1 (Continued)

Name	Lake	Country	Latitude [°N]	Longitude [°E]	Altitude [m a.s.l.]	Wtemp [°C]
O022TU	Turawa	Poland	50.7206	18.1072	172	29.0
O202SA	Sarbi	Romania	47.2086	22.1342	130	24.5
A061WO	Wolfring Teich	Austria	48.1841	15.1692	221	25.5
A062PI	Pichlingersee	Austria	48.2356	14.3839	252	26.4
O301SC	Schiesschweiher	Germany	49.7134	12.0163	420	21.6
O251KR	Krolova	Slovakia	48.2520	17.8095	138	25.9
O263ST	Steinbergersee	Germany	49.2841	12.1567	359	21.0
Z282SU	Schluchsee	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	Juechsen	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	Schwarzenbachtalsperre	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	28.5
O262BR	Brombachersee 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	144	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	Pfordter 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
Z082BLxx	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	Krombachtalsperre	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	Ballingsioevssjoen	Sweden	56.2317	13.8819	43	19.0
N142MU	Mueritz	Germany	53.4789	12.6242	70	20.2
O302DE	Dechsendorferweiher	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 Stechlinsee	Germany	53.1411	13.0303	60	21.3
N242KO	Kossateich	Germany	51.8300	14.0653	53	23.2
N263WIxx	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/non-arctic	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	5727
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
2 021	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
2 913	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
1 032	8.13	9.1	24.2	−3.2	Non-alpine	Non-alpine	Non-alpine/non-arctic	629	427,256

(Continues)

TABLE A1 (Continued)

Name	Lake	Country	Latitude [°N]	Longitude [°E]	Altitude [m a.s.l.]	Wtemp [°C]
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 Bryagovo	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	Oleftalsperre	Germany	50.4944	6.4188	505	20.0
N103YD	Yddingesjoen	Sweden	55.5525	13.2614	43	19.6
N123PL	Grosser Ploener See	Germany	54.0858	10.4203	34	23.0
N232BH	Bohnenlaender 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	Meerfelder Maar	Germany	50.1004	6.7634	375	21.3
S012PP	Lac de Pierre Percee	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	Sankelmarker See	Germany	54.7108	9.4333	38	20.5
Z191VW	Vierwaldstaetter 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 Sabljaki	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
B345OHR	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)

TABLE A1 (Continued)

Name	Lake	Country	Latitude [°N]	Longitude [°E]	Altitude [m a.s.l.]	Wtemp [°C]
Z301WO	See bei Worms Altrheinsee	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	Zuelpicher See	Germany	50.6767	6.6581	160	20.4
Z052MS	Lago di Montagna Spaccata	Italy	41.7198	14.0121	1061	24.8
S112SI	Silbersee II	Germany	51.7967	7.2153	88	20.8
S113BY	Baldeneysee	Germany	51.3987	7.0066	96	20.4
B341YAS	Lake Yasna Polyana	Bulgaria	42.2512	27.5940	97	27.5
S272PDxxx	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
S263GM	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	Retenue 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	1.9749	448	26.1
S271TO	Lac du Tondre	France	44.0228	1.4594	175	23.6
S252ET	Retenue 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
1 012	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
1 007	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	Retenue 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 Minimum, maximum and mean values of environmental parameters of sampling sites and OTU numbers of all alpine- and non-alpine-sampling sites and per mountain range; AL, Alps; CP, Carpathians; PY, Pyrenees; SN, Sierra Nevada; Bioclimatic variables ('worldclim' dataset with a spatial resolution of 2.5 min, https://biogeo.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 the coldest month; WTemp = water temperature at sampling time; Conductivity and pH at sampling time; OTUs = number of OTUs (V9-SWARMs) classified as protists; *p*-values of Kruskal-Wallis tests (between mountain ranges (AL, CP, PY, SN) and alpine (AL + CP + PY + SN) vs. non-alpine lakes): Significance codes: '***'< 0.001, '**' < 0.01, '*' < 0.05, '.' < 0.1, ' ' <1

		Latitude (°N)	Longitude (°E)	Altitude (m a.s.l.)	Bio1 (°C)	Bio5 (°C)	Bio6 (°C)	WTemp (°C)	Conductivity (µS cm^{-1})	pH	OTUs
Alpine (total)	Min	37.0475	−3.3684	527	−2.2	8.2	−11.6	11.5	7	6.82	37
	Max	49.4660	24.6144	3120	6.2	24.3	−8.4	25.6	461	9.29	790
	Mean	46.4258	12.4672	1656	2.5	16.2	−9.6	17.0	131	8.23	270
Non-alpine	Min	36.9154	−3.5381	−3	−1.3	12.5	−13.2	11.9	2	5.40	32
	Max	61.0994	27.5940	2378	16.0	31.8	5.1	32.8	2913	10.47	2350
	Mean	48.1113	11.0571	445	8.7	23.3	−3.8	22.6	313	8.55	487
Kruskal–Wallis *p*-values (alpine – non-alpine)				***	***	***	***	***	***	***	***
Alps (AL)	Min	45.2245	6.1481	532	−2.2	8.2	−11.6	11.5	11	6.82	37
	Max	49.0988	15.3075	2785	5.5	20.7	−8.4	22.9	461	9.29	790
	Mean	46.9537	11.5382	1575	2.2	15.4	−9.6	16.7	160	8.22	250
Carpathians (CP)	Min	45.3583	19.4854	527	0.1	11.6	−11.3	12.0	7	7.39	41
	Max	49.4660	24.6144	2030	6.2	21.7	−8.4	25.6	250	9.28	635
	Mean	48.0721	21.1709	1354	3.2	16.7	−9.9	18.3	84	8.20	334
Pyrenees (PY)	Min	42.7759	−0.2612	2805	0.4	12.7	−9.5	13.7	77	8.84	303
	Max	42.7759	−0.2612	2805	0.4	12.7	−9.5	13.7	77	8.84	303
	Mean	42.7759	−0.2612	2805	0.4	12.7	−9.5	13.7	77	8.84	303
Sierra Nevada (SN)	Min	37.0475	−3.3684	2950	4.1	24.2	−8.7	14.9	9	8.09	103
	Max	37.0584	−3.2932	3120	4.2	24.3	−8.6	16.7	36	8.37	295
	Mean	37.0513	−3.3218	3060	4.1	24.2	−8.7	16.0	21	8.22	229
Kruskal–Wallis *p*-values (mountain ranges)				**			*		.		

Conductivity [µS cm−1]	pH	Bio1 [°C]	Bio5 [°C]	Bio6 [°C]	Mountain range	Alpine_non-alpine	Alpine_arctic_non-alpine/non-arctic	OTUs	Seqs
181	8.97	15.1	30.7	1.7	Non-alpine	Non-alpine	Non-alpine/non-arctic	503	950,134
674	8.25	15.2	31.4	1.8	Non-alpine	Non-alpine	Non-alpine/non-arctic	576	433,819
124	9.41	13.8	27.2	2.5	Non-alpine	Non-alpine	Non-alpine/non-arctic	323	371,383
538	8.86	14.2	29.8	2.7	Non-alpine	Non-alpine	Non-alpine/non-arctic	776	609,139
553	8.53	16.0	31.8	3.9	Non-alpine	Non-alpine	Non-alpine/non-arctic	400	526,944
1 208	8.07	14.9	27.4	4.0	Non-alpine	Non-alpine	Non-alpine/non-arctic	557	1,286,179
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)

	All OTUs							Specialists
Taxonomic classification	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 OTUss 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)

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; Nucletmycea; Fungi; Chytridiomycota	9.4	8.8	9.2	8.4	9.1	8.6	6.6	11.1
Opisthokonta; Nucletmycea; Fungi; Cryptomycota	2.2	3.0	2.1	3.5	2.4	3.0	5.9	2.9
Opisthokonta; Nucletmycea; 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

					Generalists					
Non-alpine	Alps	Carpathians	Pyrenees	Sierra Nevada	Alpine	Non-alpine	Alps	Carpathians	Pyrenees	Sierra Nevada
16547	730	419	21	174	3461	3461	2477	1829	282	383
82.7	22.8	18.6	6.9	31.2	72.8	17.3	77.2	81.4	93.1	68.8
0.7	0.3	2.4	0.0	0.0	0.4	0.4	0.4	0.5	0.7	0.0
16.3	12.1	13.1	0.0	24.7	13.8	13.8	13.0	15.0	7.8	20.9
0.4	0.3	0.7	0.0	0.0	0.8	0.8	0.7	1.1	0.7	0.0
4.2	2.6	0.7	4.8	0.6	4.6	4.6	5.7	2.8	6.7	2.1
1.1	0.8	0.7	0.0	1.1	1.4	1.4	1.4	1.3	2.5	1.3
0.2	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.3	0.7	0.3
0.4	0.8	1.2	0.0	0.0	0.8	0.8	0.9	1.0	0.4	0.3
15.6	17.1	22.0	19.0	18.4	14.7	14.7	14.9	13.9	16.3	17.8
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
36.2	36.0	37.2	52.4	17.8	34.6	34.6	32.1	38.5	31.6	22.2
3.1	4.2	1.7	4.8	2.9	3.7	3.7	4.5	2.6	2.1	4.2
21.6	25.8	20.3	19.0	34.5	24.7	24.7	26.0	23.0	30.5	31.1
16547	730	419	21	174	3461	3461	2477	1829	282	383
82.7	22.8	18.6	6.9	31.2	72.8	17.3	77.2	81.4	93.1	68.8
0.7	0.3	2.4	0.0	0.0	0.4	0.4	0.4	0.5	0.7	0.0
0.5	0.7	0.5	0.0	1.1	0.2	0.2	0.2	0.2	0.7	0.0
15.7	11.4	12.6	0.0	23.6	13.6	13.6	12.7	14.8	7.1	20.9
0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0
0.4	0.3	0.7	0.0	0.0	0.8	0.8	0.7	1.1	0.7	0.0
2.2	1.1	0.7	4.8	0.6	2.1	2.1	2.5	1.9	3.5	1.0
1.4	0.7	0.0	0.0	0.0	1.6	1.6	2.1	0.7	1.4	0.3
0.6	0.8	0.0	0.0	0.0	0.8	0.8	1.2	0.3	1.8	0.8
1.1	0.8	0.7	0.0	1.1	1.4	1.4	1.4	1.3	2.5	1.3
0.2	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.3	0.7	0.3
0.4	0.8	1.2	0.0	0.0	0.8	0.8	0.9	1.0	0.4	0.3
1.8	0.3	1.4	0.0	0.6	0.7	0.7	0.4	0.9	0.4	0.8
1.1	1.6	1.9	0.0	1.1	1.6	1.6	1.7	1.3	3.5	1.6
9.5	9.7	15.5	14.3	6.9	8.0	8.0	8.0	7.7	8.2	6.5
1.9	3.3	1.4	0.0	4.0	3.1	3.1	3.6	2.7	3.2	6.8
1.1	2.2	1.7	4.8	5.7	1.3	1.3	1.2	1.4	1.1	2.1
0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11.5	22.5	13.4	33.3	9.2	18.5	18.5	21.4	17.4	23.4	13.6
19.4	9.5	21.5	19.0	6.3	12.3	12.3	6.5	18.0	6.4	5.7
2.5	1.0	1.0	0.0	2.3	1.5	1.5	1.5	1.2	1.4	1.6
2.9	3.2	1.4	0.0	0.0	2.3	2.3	2.7	1.9	0.4	1.3

(Continues)

(b)

SAR; Rhizaria; Cercozoa	3.2	3.6	3.2	4.4	2.4	2.3	3.8	3.2
SAR; Rhizaria;other	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
SAR; Stramenopiles; Ochrophyta; Chrysophyceae	9.4	13.0	9.1	15.0	10.6	13.2	11.8	13.8
SAR; Stramenopiles; Ochrophyta; Diatomeae	5.1	3.5	5.2	3.1	3.4	4.3	7.0	3.2
SAR; Stramenopiles; Ochrophyta; Eustigmatophyceae	1.2	0.7	1.2	0.8	0.4	0.3	1.8	1.0
SAR; Stramenopiles; Ochrophyta;other	0.6	0.6	0.6	0.7	0.7	1.3	0.4	0.5
SAR; Stramenopiles;other	2.5	2.7	2.5	2.8	2.1	4.3	3.6	2.5
SAR; Stramenopiles; Peronosporomycetes	3.6	4.3	3.5	3.5	5.3	6.3	7.5	4.3

(c)

	Alpine OTUs					Alpine specialists	
Mountain ID	Total	Alps	Carpathians	Pyrenees	Sierra Nevada	Total	Alps
Total OTU numbers	4754	3207	2248	303	557	1293	730
Proportions of all alpine OTUs per group [%]						27.2	22.8
AL	44.2	65.5	0.0	0.0	0.0	53.4	94.5
CP	25.3	0.0	53.6	0.0	0.0	29.3	0.0
PY	0.8	0.0	0.0	12.9	0.0	1.3	0.0
SN	5.3	0.0	0.0	0.0	45.1	12.4	0.0
AL-CP	14.3	21.3	30.3	0.0	0.0	2.2	4.0
AL-PY	1.0	1.4	0.0	15.2	0.0	0.2	0.3
AL-SN	1.2	1.8	0.0	0.0	10.6	0.4	0.7
CP-PY	0.3	0.0	0.6	4.6	0.0	0.0	0.0
CP-SN	0.7	0.0	1.6	0.0	6.3	0.5	0.0
PY-SN	0.1	0.0	0.0	1.0	0.5	0.0	0.0
AL-CP-PY	2.3	3.5	4.9	36.6	0.0	0.2	0.3
AL-CP-SN	2.5	3.7	5.3	0.0	21.4	0.2	0.3
AL-PY-SN	0.2	0.2	0.0	2.6	1.4	0.0	0.0
AL-CP-PY-SN	1.7	2.6	3.6	27.1	14.7	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

			Alpine generalists				
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	2.9	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