

Comparative 16s metagenomic analysis of prokaryotic diversity in freshwater and permanent snow-line glacial lakes in Türkiye

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Abstract: Freshwater lakes are critical to healthy ecosystems, providing vital services like drinking water and recreation for surrounding communities. Microorganisms within these ecosystems play essential roles, driving biogeochemical cycles for elements like carbon, nitrogen, and sulfur. This study utilized a metagenomic approach to examine the prokaryotic communities of three freshwater lakes in Türkiye: the Eber and Beyşehir lakes, located at close altitudes (967 m and 1,115 m, respectively), which serve as primary water sources for nearby communities, and Lake Uludag Buzlu (2,390 m) that lies at the permanent snow border within the Uludag glacial lake system. Metagenomics allowed us to identify species, genetic structures, and the functional roles of microorganisms. Employing high-throughput next-generation sequencing (NGS) technology, we analyzed 16S ribosomal DNA (rDNA) sequences (V3-V4 regions) from the lake samples. EzBioCloud software facilitated the analysis of prokaryotic diversity obtained using Illumina NovaSeq technology. While Eber and Beyşehir lakes had similar diversity, *Bacillota* dominated in the higher-altitude Lake Uludag Buzlu. Genus-level analysis revealed *Parabacteroides* as the most prevalent in Lake Uludag Buzlu, contrasting with *Limnohabitans* dominance in Lake Eber; Lake Beyşehir exhibited co-dominance of *Limnohabitans* and *Planktophila*.

Keywords: freshwater lakes; glacial lakes; 16s metagenomics; prokaryotic diversity; high altitude ecosystems

INTRODUCTION

Despite constituting only about 0.8% of global water resources, freshwater lakes play a critical role in fostering sustainable ecosystems [1]. These lake ecosystems serve as hubs for a multitude of biogeochemical processes and have the potential to contribute to a diverse array of biological activities essential for dependent communities [2,3]. Nevertheless, the intrinsic proclivity for change inherent to these ecosystems can precipitate rapid alterations in the prevailing environmental conditions. Ecological parameters serve as valuable tools for assessing the health of freshwater ecosystems [4]. Unfortunately, human activities and pollution significantly contribute to water quality degradation, jeopardizing the water security of roughly 80% of freshwater ecosystems [5]. Therefore, effectively assessing the ecological status of diverse freshwater

ecosystems is crucial to guaranteeing their long-term sustainability.

The sustainability of freshwater reservoirs has become a pressing concern, particularly in developing nations. Escalating freshwater demand coupled with the impacts of industrialization places immense strain on this limited resource [6]. Global warming, drought, and population growth affect many people as freshwater resources dwindle. [7]. Although 75% of the earth's surface is covered by water, the amount of freshwater available for human use is limited. Less than 1% of available water resources are freshwater resources and those suitable for ecosystem and human use cover only 105,000 km².

Freshwater lake ecosystems across the globe provide a multitude of critical ecosystem services for millions of

people. Examples include Adirondack Lake (New York, USA), Toolik Lake (Alaska, USA), Crystal Bog Lake and Sparkling Lake (USA), Loosdrecht Lake (Netherlands), Lake Michigan (USA), Fuchskuhle Lake (Germany), and Cadagno Lake (Switzerland) [8-13]. These services encompass water supply, recreational opportunities, food production, and biodiversity conservation. However, human activities are a significant threat to the health of freshwater lake ecosystems. These threats include pollution, nutrient loading, overexploitation, habitat loss, invasive species introductions, and climate change [14]. Therefore, a thorough understanding of freshwater lake ecosystems' structure, function, and response to environmental changes is crucial for sustainable management and conservation. Uludag (2,543 m a.s.l.), the highest mountain in Western Anatolia, is one of Türkiye's principal massifs. It forms part of the country's solid foundational massifs, uplifted by the Alpine orogenic movements. Located within the borders of Bursa province and enclosing an area of approximately 40 km in length and 15-20 km in width, Uludag has been a protected area since 1978. Uludag is an important winter sports and skiing center, covered with snow from December to May, with snow thickness reaching 4 m. The area under Uludag summit is the lake region, with glacial lakes, some of which dry up in summer. Among the most significant glacial lakes are Kara (2,270 m a.s.l.), Kilimli (2,330 m a.s.l.), Aynalı (2,310 m a.s.l.), Buzlu (2,390 m a.s.l.) and Heybeli (2,410 m a.s.l.). Lake Heybeli experiences seasonal desiccation at the end of summer due to its shallow depth [15].

Lake Eber lies within the Çay district of Afyonkarahisar province, bordering the town of Eber. The lake is the 12th largest in Türkiye and is recognized as one of the most significant wetlands in the Afyonkarahisar-Bolvadin region. The lake's deepest point is 21 m, its surface area is 150 km², and its height above sea level is 967 m. The endemic plant species Eber yellow (*Thermopsis turcica*), only grows in Lake Eber. The lake lies in the Akarçay-Eber basin, a tectonic lake between the Sultan Mountains' northwestern slopes and the Emirdağ Mountains' southern extensions. It is located on the migration route of migratory birds in Türkiye, Lake Eber provides breeding and living space for many bird species thanks to its reed beds.

Situated in Central Anatolia, Lake Beyşehir serves as the region's largest freshwater resource, fulfilling the drinking and irrigation needs of the surrounding

area. Furthermore, the Beyşehir Lake basin is a pivotal freshwater resource for the Konya closed basin, the largest of its kind in Central Anatolia [16]. Lake Beyşehir is vital for various sectors, including agriculture, drinking water supply, fisheries, livestock, tourism, and recreation. [17].

As crucial nutrient-limiting factors, carbon, nitrogen, and sulfur are fundamental components of living organisms in the biosphere [18]. These elements undergo interactions with water, the atmosphere, and soil. The hydrosphere, acting as the nexus linking diverse ecosystems, harbors over half of the planet's microbial populations, which play vital roles in material cycling and energy transfer in ecosystems [19]. Waterborne microbes mitigate the greenhouse effect by sequestering atmospheric carbon dioxide and emitting dimethyl sulfide [20]. Moreover, nitrogen transformation by aquatic microbes influences carbon dynamics and biodiversity [21]. The interface between the atmosphere and the sediment layer is dominated by surface waterbodies, presenting a significant scientific inquiry into the impact of microbial community composition and functional diversity on biogeochemical cycles.

We previously examined the prokaryotic diversity of the Beyşehir and Eber lakes by metagenomic studies utilizing the Silva database and Mothur software [22,23]. In these studies, emphasis was placed on genera that could potentially contribute to biotechnological advancements significant for the lake ecosystem. In the research, these lakes were utilized for comparative analysis of prokaryotic diversity at high altitudes. The main focus of our study was to elucidate the potential variances in composition that may arise due to differences in elevation disparity in the Uludağ Lake system. We investigated three lakes with varying topographic elevations to understand the microbial community. Understanding the diversity of microbial communities in lakes relative to terrain elevation is crucial. Using metagenomic technology, we conducted a comparative analysis of the microbial community composition in the waters of the three lakes. This is the first study to employ metagenomic sequencing technology to reveal the freshwater microbial characteristics at different elevations in Anatolia. By comparing the microbes in lakes at different elevations, this study aims to elucidate how environmental factors contribute to variations in microbial composition among lakes.

MATERIALS AND METHODS

Sample collection and processing

This study investigated the prokaryotic diversity of three freshwater lakes chosen for their regional dominance: Lake Beyşehir (BYR), 1,121 m a.s.l.; Lake Eber (EBR), 967 m a.s.l., and Uludag Buzlu Lake (UB), 2,390 m a.s.l. (Supplementary Fig. S1). The selection criteria included maximizing potential impact by targeting the largest lake in each region and including a significant elevation gradient from 967 m to 2,390 m to explore the influence of altitude on lake prokaryotic communities. Additionally, the chosen lakes exhibit variations in environmental factors like geographical location, nutrient input, and human activity, allowing for investigation into their effects on prokaryotic diversity. Water samples were collected from Lake Beyşehir (37.6499905N, 31.6353380E) on 20.12.2019 (13:31), Lake Eber (38.6147305N, 31.0937524E) on 19.12.2019 (12:13), and Uludag Buzlu Lake (40.076580N, 29.219242E) on 16.08.2020 (15:20). Water samples were collected at three depths representing distinct vertical strata: the surface layer (1 m depth), the water column's midpoint, and the bottom layer (1 m above the sediment). To ensure sample integrity, 1-L aliquots of the water sample were collected and stored in the dark at 4°C for no longer than 24 h before DNA extraction. The pH was determined with a pH meter (compliant with standard method SM 4500 H+B).

DNA extraction and quantification

One liter of lake water was processed for metagenomic DNA preparation [24]. Filtration was performed using a 0.22- μ m filter membrane (Merck Millipore, Darmstadt, Germany). The biomass retained on the membrane was used for DNA extraction with the DNeasy PowerWater Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA quantification was performed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) as described by Cseke et al. [25]. 16S rDNA sequencing and metagenomic analysis were outsourced to a commercial laboratory (Gen Ova, Istanbul, Türkiye).

Sequencing and bioinformatics analysis

Primers 341F: 5'-CCTACGGGNGGCWGCCAG-3' and 805R: 5'-GACTACHVGGGTATCTAATCC-3' were designed to target the V3-V4 regions of 16S rDNA. These regions are 465 base pairs long. The amplified library was used for bidirectional reading (2 \times 250 base pairs) on the NovaSeq platform [26]. The 16S rRNA gene sequences were analyzed for taxonomic composition using the EzBioCloud 16S Microbiome Taxonomic Profiling (MTP) pipeline (<https://www.ezbiocloud.net/>, accessed 03.03.2024, version 08.23.2023). Initially, raw reads underwent quality control using Trimmomatic version 0.32 [27], filtering out reads with a quality score below Q25. Following quality control, paired-end sequences were merged with VSEARCH version 2.13.4 [28], employing the `fastq_mergepairs` command with default settings. Utilizing the alignment algorithm of Myers and Miller [29], the primers were subsequently trimmed at a similarity cutoff point of 0.8. Nhmmer [30], in combination with hmm profiles from the HMMER software package (version 3.2.1), was used to identify non-specific amplicons lacking 16S rRNA encoding. Unique reads were isolated with subsequent clustering of redundant reads alongside the extracted unique reads. This process was executed using the `derep_full-length` command of the VSEARCH software suite [28]. The `usearch_global` command from VSEARCH [28] was employed for a taxonomic assignment using the EzBioCloud 16S rRNA database [31], followed by a more detailed pairwise alignment as per Myers and Miller [29]. Chimeric reads were removed using reference-based chimera detection via the UCHIME algorithm [32]. A 97% similarity threshold was applied, and EzBioCloud's non-chimeric 16S rRNA database served as the reference. After chimeric filtering, reads exhibiting less than 97% similarity to entries within the EzBioCloud database (PKSSU4.0) unidentifiable at the species level, were collated. To generate additional operational taxonomic units (OTUs), *de novo* clustering was implemented using the `cluster fast` command [28]. OTUs comprising only single reads (singletons) were omitted from downstream analysis. The taxonomic composition of bacterial communities was examined comprehensively across multiple taxonomic levels.

Visualization of taxonomic groups

Taxonomic diversity within the sample was visualized using interactive charts generated by the KRONA tool [33].

RESULTS

The concentrations of DNA in the water samples were obtained by spectrophotometry (Supplementary Table S1). Interactive KRONA plots were generated for the three studied lakes (Fig. 1). Following quality control of the NGS readings, the filtration procedure yielded 29,180,000 quality-compliant readings for Lake Eber. These filtered reads were used to create 68,359 contigs in Lake Eber using the EzBioCloud tool. The average GC content of the contigs was $54 \pm 4\%$. After the Lake Beyşehir sample was filtered according to the sequence quality, 31,180,000 readings and 73,863 contigs were combined. In Uludag Buzlu Lake, 31,380,000 reads and 73,761 contigs were merged after filtering the reads. Reads were uploaded to the EzBioCloud platform, where quality control and profiling analysis were performed. The quality control based on clipping removed 26, 43, and 29 low-quality amplicons from Eber, Beyşehir, and Uludag Buzlu samples, respectively. The taxonomic approach detected and removed 67,587, 72,964, and 72,997 non-target amplicons from Eber, Beyşehir, and Uludag Buzlu samples, respectively. Chimera detection and removal eliminated 293, 248, and 215 amplicons from the Eber, Beyşehir, and Uludag Buzlu datasets,

respectively. Following quality assessment and trimming, 453, 608, and 520 valid reads were obtained from Eber, Beyşehir, and Uludag Buzlu, respectively. The distribution of the generated sequence lengths agrees with the amplicon length of 16S rRNA (average 418 bp). Good's coverage estimator of the operational taxonomic unit (OTU) in lake samples ranges from 91.2 to 90.5% and 93.5%, respectively. The rarity curves showed constant values, indicating that the diversity of bacterial populations in the three lakes was adequately covered by the sequences created (presented as an appendix). The number of OTUs per sample was 98, 109, and 74 in Eber, Beyşehir, and Uludag Buzlu, respectively. An evaluation of α -diversity was undertaken to compare the bacterial communities within the study area's lakes. The bacterial diversity of the lakes was compared with the Fischer Alpha and Shannon indexes. The pH values of Beyşehir, Eber, and Uludag Buzlu were between 8, 9, and 7.55, respectively. The number of bacterial genera in the surface samples from Beyşehir, Eber, and Uludag Buzlu lakes was 77, 73, and 58, respectively. Shannon diversity index was the highest in Lake Eber (3.86) and the lowest in Lake Beyşehir (3.73) and Uludag Buzlu Lake (3.28) (Supplementary Table S2.). Phylogenetic diversity was highest in Lake Beyşehir (300), followed by Lake Eber (286), and significantly lower in Uludag Buzlu Lake (205).

Taxonomic structure

The August 2023 updated database revealed a notable diversity of bacterial communities. The 109, 98, and

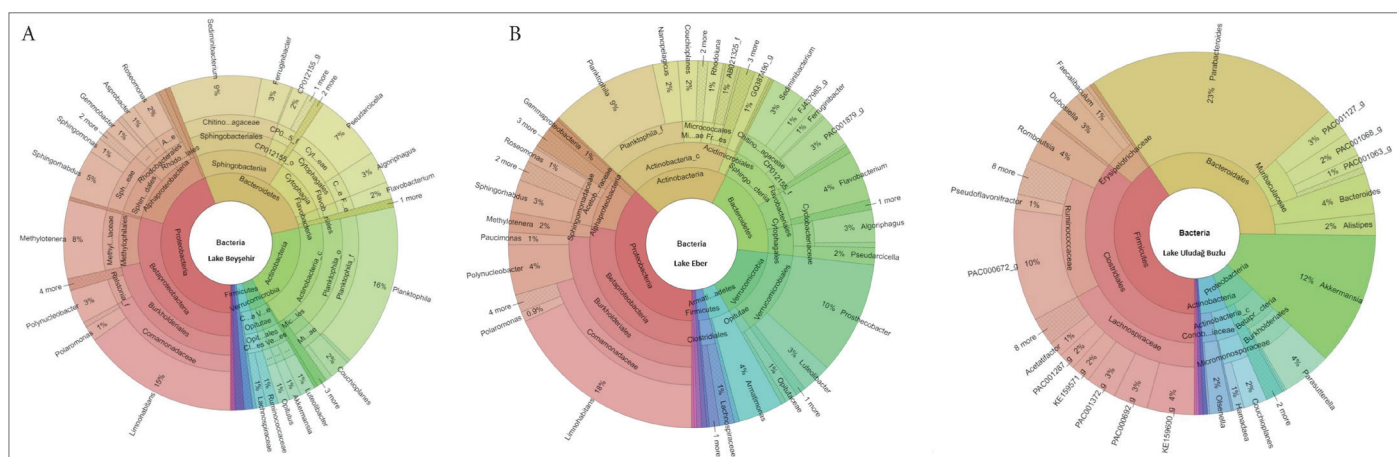


Fig. 1. Diagrams showing the relative abundance diversity of the most abundant OTUs for the study lakes. **A** – Distribution of bacterial genomes in Lake Beyşehir. **B** – Distribution of bacterial genomes in Lake Eber. **C** – Distribution of bacterial genomes in Uludag Buzlu Lake. The diversity of taxa was visualized using the Krona chart-2.8.1 (<https://github.com/marbl/Krona/wiki>).

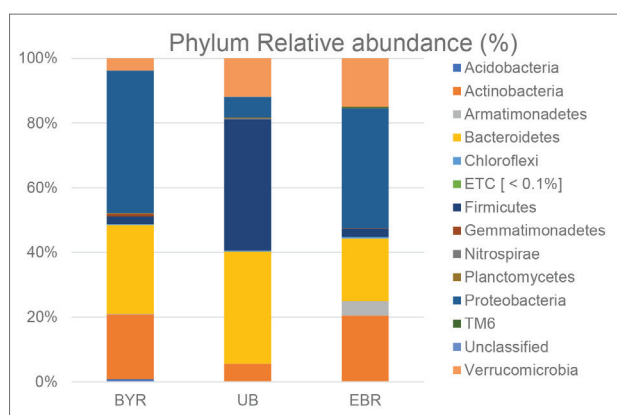


Fig. 2 Phylum-level taxonomic composition of the three Lakes for 16S rRNA genes from metagenomics. Phyla below 1% (based on mean abundance) are not shown.

74 OTUs (BYR, EBR, UB, respectively) isolated from water samples taken from the three studied lakes can be assigned to 10, 10, 9 phyla, respectively, represented by 21, 21, and 16 classes, 37,38,20 orders, 51,49,27 families, respectively, and 78,69,58 genera, respectively. The dominant phyla vary across the three lakes constituting our study area. In Lake Beyşehir, *Proteobacteria* prevail at 44.07%, while in Lake Eber, they predominate at 37.08%. Conversely, in Uludağ Buzlu Lake, *Bacillota* (*Firmicutes*) was the dominant phylum, comprising 40.57% of the total phylum composition. Other phyla in Uludağ Buzlu include *Bacteroidetes* (34.61%), *Verrucomicrobia* (11.92%), *Proteobacteria* (6.35%), and

Actinobacteria (5.38%). In Lake Beyşehir, *Bacteroidetes* (27.47%), *Actinobacteria* (20.07%), *Verrucomicrobia* (3.78%), and *Firmicutes* (2.47%) were also dominant. In Lake Eber, dominant phyla include *Actinobacteria* (20.31%), *Bacteroidetes* (19.43%), *Verrucomicrobia* (15.01%), and *Armatimonadetes* (4.42%) (Fig. 2). For a more comprehensive evaluation of bacterial diversity in the samples, a class-level analysis was conducted, in which *Betaproteobacteria* was found to be prevalent in Lake Beyşehir at 29.44% and in Lake Eber at 27.81%, while *Clostridia* dominated in Uludağ Buzlu Lake, constituting 35.76% of the microbial community.

Differential bacterial profiling at the genus level

The 48 bacterial genera initially identified in the samples (Fig. 3) belong to various phyla. Most of these identified genera are typically found in freshwater environments. For Lake Beyşehir, the genera *Planktophilia* and *Limnohabitans* were the most abundant. In Lake Eber, *Limnohabitans* and *Prostheco bacter Planktophilia* were the most common genera. In Uludag Buzlu Lake, the most common genera were *Parabacteroides* and *Akkermansia*. *Limnohabitans* were found in Beysehir and Eber, while none were detected in Uludag Buzlu. *Planktophilia* was common in Beyşehir and Eber, but it was not found in Uludag Buzlu. *Sphingorhabdus* was found in Beyşehir and Eber but was not detected in Uludag Buzlu. The general *Harryflintia* and *Dubosiella* were found only in Uludag Buzlu. *Rhodoluna* was

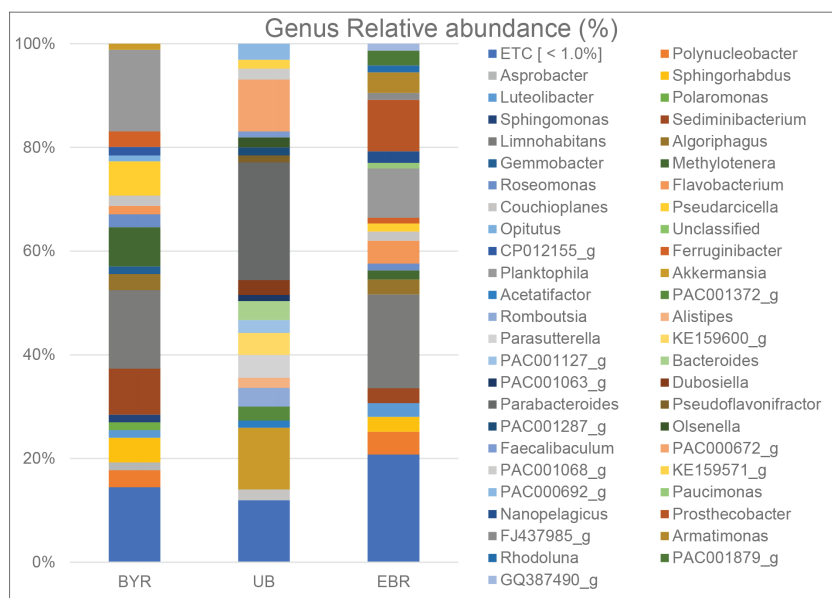


Fig. 3. Genus-level taxonomic composition of the three Lakes for 16S rRNA genes from metagenomics. Genus below 1% (based on mean abundance) are not shown.

detected only in Lake Eber, and *Bacteroides* only in Uludag Buzlu Lake. *Solibacter* was detected only in Beyşehir Lake, accounting for 0.2% of the total. The genus *Sandarakinorhabdus* was detected only in Beyşehir and Eber, albeit at a very low rate. The genera *Curvibacter*, *Opiritutus*, *Luteolibacter*, *Polynucleobacter*, *Nanopelagicus*, *Sediminibacterium*, *Pseudarcicella*, *Ferruginibacter*, *Algoriphagus*, *Asprobacter*, *Aquirestis*, *Polaromonas*, *Methylothenera*, *Mycobacterium*, and *Roseomonas* were found in Beyşehir and Eber, but not in Uludag Buzlu. *Armatimonas*, *Diplosphaera*, *Flavobacterium*, *Rugosimonospora*, *Prostheco bacter*, *Fluviicola*, *Hydrogenophaga*, and *Paucimonas* were detected only in Lake Eber, but not in Beyşehir and Uludag Buzlu. *Alsobacter*, *Singulisphaera*, *Paludicola*, *Olsenella*, *Parabacteroides*, *Romboutsia*, *Faecalibaculum*, *Stenotrophomonas*, *Alistipes*, and *Oscillibacter* genera were detected only in Uludag Buzlu Lake. *Gemmobacter* and *Oscillibacter* were detected only in Lake Beyşehir. *Sphingomonas*, *Couchioplanes*, *Hamadaea*, *Pseudoflavonifractor*, and *Akkermansia* were common in all three lakes. The genera *Acetatifactor* and *Parasutterella* were found in Uludag Buzlu, but at a very low level (0.16%) in Beyşehir Lake. *Lactobacillus* was found only in lakes Beyşehir and Uludag Buzlu.

DISCUSSION

This study employed a metagenomic approach to assess the bacterial communities of three freshwater lakes in Türkiye: Beyşehir, Eber, and Uludag Buzlu. These freshwater lakes were chosen because Lake Beyşehir is the largest freshwater source in the region, while Lake Eber is impacted by pollution from domestic, industrial, and agricultural sources. Uludag Buzlu Lake was chosen because it is the lake with the lowest altitude at the boundary of permanent snow. As a boreal lake, it serves a model for monitoring the impacts of global climate change in such ecosystems. Uludag Buzlu Lake is located at an altitude of 2,390 m a.s.l., above the tree line. Selecting the lakes Beyşehir and Eber at altitudes close to each other was to facilitate a higher-quality comparison with the prokaryotic diversity of Uludag Buzlu Lake.

Over 91.7 million next-generation sequencing reads were subjected to analysis for the metagenomic assessment of lake samples. Among the investigated

lakes, Beyşehir exhibited the greatest bacterial diversity, harboring 77 genera at an alpha diversity value of 12.01. Conversely, Uludag Buzlu Lake exhibited the lowest bacterial diversity, with an alpha diversity value of 9.27. The Shannon diversity index revealed a comparatively lower overall bacterial diversity in the lakes examined relative to other freshwater ecosystems documented in previous investigations: Batak and Tsankov Kamak Reservoirs in Bulgaria, Itupararanga Reservoir in São Paulo State, Brazil [34], Lake Pavin [35] and Mayinghai, Pipahai and Gonghai Lakes in China [36] were at a lower altitude than Lake Saiful-Muluk, Rawal Dam, Lake Keenjhar, and the Den reservoirs [37] but at the same level as the natural lakes.

Phylum-level classification of the sequences revealed that *Proteobacteria* were the most prevalent bacterial phylum in Beyşehir and Eber samples. *Proteobacteria* are ubiquitous in freshwater reservoirs, and evidence suggests this phylum plays a significant role in the biogeochemical cycling in lake ecosystems [38]. In Uludag Buzlu Lake, *Firmicutes*, *Bacteroidetes*, and *Verrucomicrobia* are the most dominant phyla. The phylum *Verrucomicrobia* is globally prevalent, inhabiting both terrestrial and aquatic environments, and *Verrucomicrobia* isolates can utilize a variety of carbon compounds [39-41]. Despite several studies indicating the predominance of *Verrucomicrobia* in freshwater lake waters [41-43], the abundance of aquatic *Verrucomicrobia* has not been extensively studied. The abundance of *Verrucomicrobia* in lake ecosystems correlates positively with nutrient richness and phosphorus availability. Furthermore, it may exhibit seasonal fluctuations and variations across basins with differing humic content [41]. In contrast, de Figueiredo et al. [44] observed a predominant association of *Verrucomicrobia* with oligotrophic aquatic ecosystems and environments characterized by low pH values.

Dorador et al. [45] found a low prevalence of *Verrucomicrobia* in the high-altitude wetlands of the Chilean Altiplano. Their study marked the first report of the dominance of *Verrucomicrobia* in wetland environments. In this study, *Verrucomicrobia* were found to be 15% of the bacterial community in Lake Eber, 11% in Uludag Buzlu Lake, and 3.78% in Lake Beyşehir, highlighting differences between the lakes. Dorador et al. [45] also reported that *Bacteroidetes*

and *Proteobacteria* were the most abundant phyla. In this study, *Bacteroidetes* was the most abundant phylum. While *Proteobacteria* were the most abundant in Beyşehir and Eber, they were found at a rate of only 6% in Uludag Buzlu Lake, which lies on the border of permanent snow. *Proteobacteria*, as indicated by Cheng et al. [46], are instrumental in the biodegradation of organic pollutants, carbon cycling, and numerous biogeochemical processes within aquatic ecosystems. Furthermore, *Proteobacteria* are typically the prevalent group in freshwater environments, as noted by Kwon et al. [47]. In the Mount Everest region of Nepal, *Betaproteobacteria* were frequently identified as the dominant group in high-altitude lakes, as reported by Sommaruga and Casamayor. Similarly, Liu et al. found *Betaproteobacteria* to be the predominant group in Lake Namco, the largest lake in Tibet [36,48].

Studies have explored the prokaryotic diversity in various environments, shedding light on the unique microbial communities in these habitats. Mutlu [24] found a high proportion of *Bacteroidetes* and *Haloarchaea* in Tuz Lake, a hypersaline environment in Türkiye. Simon [49] identified *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* as the predominant groups in a glacier ice metagenome in Germany. Møller [50] observed a diverse bacterial community in High-Arctic snow, with *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria* dominating. Choudhari [51] reported a rich and diverse microbial population in an Alaskan glacier, with *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* as the most prominent groups.

In this study, *Bacteroidetes*, the most frequently detected phylum in all three lakes, have been observed in freshwaters, marine waters [52], high-altitude cold environments [53,54], and high-salinity water environments [55,56]. This ubiquity suggests a potentially significant ecological role across diverse aquatic ecosystems. The observed presence of *Actinobacteria* aligns with their established status as cosmopolitan freshwater taxa. *Actinobacteria* inhabit diverse lake, reservoir, and river ecosystems spanning various trophic levels and climatic zones. Within these environments, their functional role centers primarily on the decomposition of organic matter [57,58].

We previously used the Silva database and Mothur software in the metagenomic study of Eber and Beyşehir

lakes [22,23]. However, unidentified data was quite low in the Ezbiocloud software and the PKSSU4.0 database, which suggests that they are more advanced than the Silva database and Mothur software.

CONCLUSIONS

This is the first European study on the composition of the bacterial community in freshwater lakes at different elevations. The bacterial diversity and taxonomic structure of the Beyşehir, Eber, and Uludag Buzlu lakes were investigated using a metagenomic approach. While bacterial diversity and richness were higher in Beyşehir and Eber, the bacterial communities in Uludag Buzlu Lake were different and less diverse. Taxonomic analysis of the obtained sequences showed that *Bacteroides* phylum bacteria were dominant in all three lakes, followed by *Actinobacteria* and *Verrucomicrobia*. The phylum *Firmicutes* was found to be dominant in Uludag Buzlu Lake. *Planktophila* and *Limnohabitans* genera have absolute dominance in the Beyşehir and Eber lakes. However, the difference at the phylum level is also at the genus level, with *Parabacteroides* and *Akkermansia* genera being dominant in Uludag Buzlu Lake. The differences observed between the lakes in our study area indicate that lakes situated at the permanent snow line have distinct bacterial content compared to those at lower altitudes. Future research in these lakes can utilize shotgun metagenomics to investigate functional genes.

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Author contributions: Fahri Pat designed the study, collected water samples, designed the analytical framework, and contributed to writing and editing the manuscript. Sultan Fidan Pedük conducted the experiments, generated the data, performed the formal analyses, and analyzed the results. Neşe Akçay conducted the experiments and generated the data for analysis. Hatice Kübra Kızıl Pat performed field studies, experiments, data generation, and analysis of the results. Ercan Arıcan analyzed the results and contributed to the writing, the review, and the editing of the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: The raw sequence data of the lakes that constitute our study area are available at the Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Turkey. The data underlying the reported findings are provided as a raw dataset that can be accessed here:

https://www.serbiosoc.org.rs/NewUploads/Uploads/Pat%20et%20al_Raw%20Dataset.pdf

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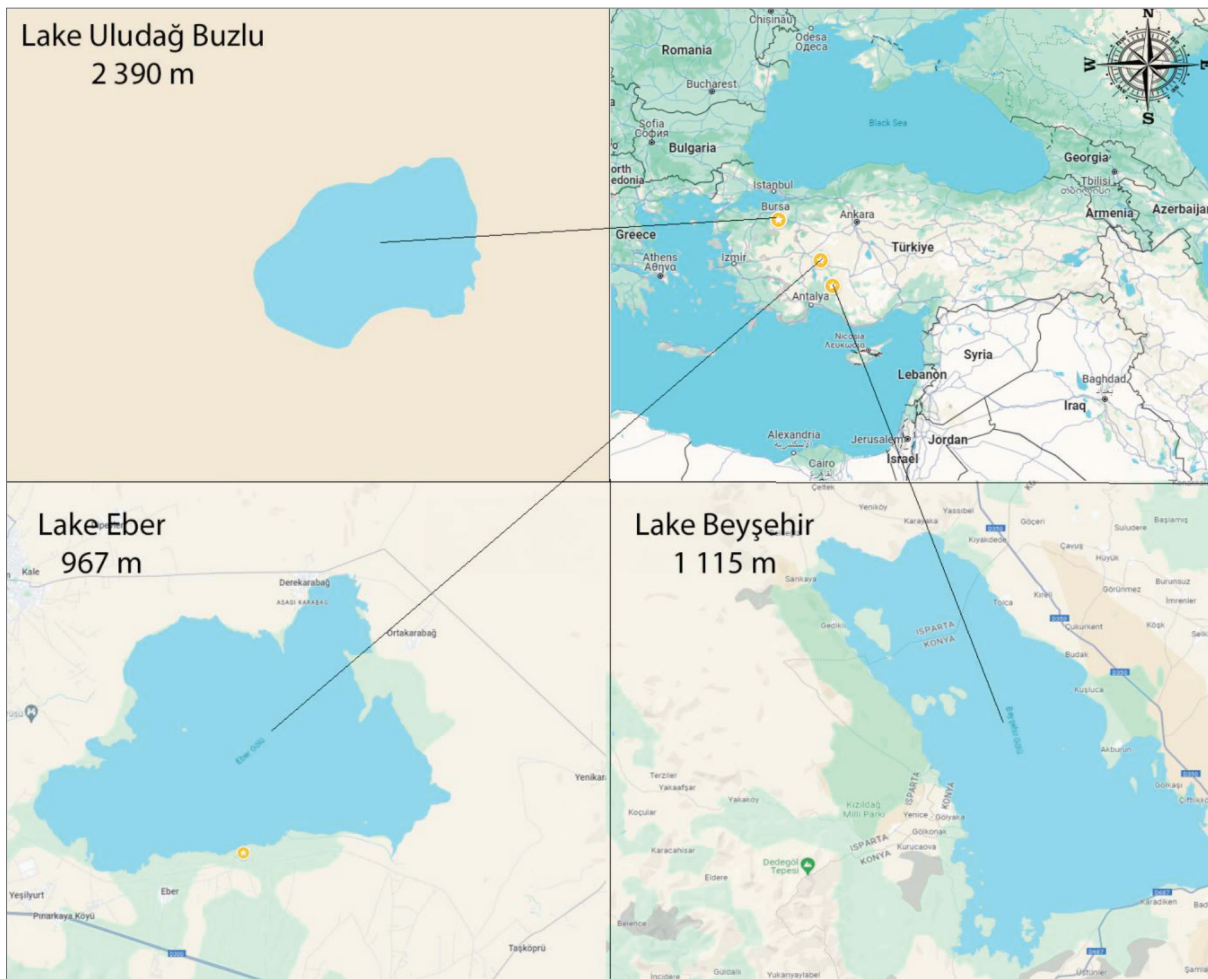
SUPPLEMENTARY MATERIAL

Supplementary Table S1. Concentrations of isolated DNA samples

Workspace	Sample Code	Concentration (ng/μL)	pH	Temperature
Beyşehir Lake (BYR)	BYR	22.9	8.19	5.9 C°
Eber Lake (EBR)	EBR	45.2	8.69	2.3 C°
Uludag Buzlu Lake (UB)	UB	13.6	7.55	18.7 C°

Supplementary Table S2. General parameters for sequencing, including Next-Generation Sequencing (NGS) readings (*de novo* assembly), accompanied by the calculation of α -diversity indices.

Parameters	Lake Beyşehir	Lake Eber	Lake Uludag Buzlu
Sequencing and assembly parameters			
No. of valid reads	31 180 000	29 180 000	31 380 000
No. Of contigs	73 863	68 359	73 761
GC content (%)	55.99	56.03	56.05
Bacterial abundance and alpha diversity indices			
No. of bacterial genera	77	73	58
Shannon Index	3.73	3.86	3.28
Fischer Alpha Index	12	11.93	9.27
Phylogenetic diversity	300	286	205



Supplementary Fig. S1. Map of Türkiye with the geographical locations of lakes Uludağ Buzlu, Beyşehir, and Eber with their respective elevation levels.