Characteristics of Bacterioplankton community, the influencing factors and functional profiles of the Huangshui River on the ecotone zone of Qinghai Plateau and Loess Plateau

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Research Article

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Abstract

Planktonic microorganisms play a key role in environmental and ecological processes in river ecosystems. Molecular techniques are increasingly used for biological monitoring of rivers. Here, the present study used high-throughput sequencing technology to investigate and study the bacterioplankton community of water bodies of the Huangshui River on the ecotone zone of Qinghai Plateau and Loess Plateau. The results indicated that the Huangshui River had lower alpha diversity than other plain rivers. Proteobacteria was the dominant phyla with relative abundance of 77.48%, followed by Firmicutes, Alphaproteobacteria and Gammaproteobacteria were significantly enriched. The predominant bacterial genera were *Acidiphilium*, *Acidocella*, *Metallibacterium* and *Acinetobacter*. Diverse taxa of potential pathogens, such as *Acinetobacter*, *Pseudomonas*, and *Aeromonas*, were also been identified. The major driving factors for the bacterial community were Cr6+, altitude, and TN. PICRUSt results revealed that a substantial number of functional profiles were involved in human diseases and antibiotic resistance. Therefore, further studies were needed to isolate these potentially pathogenic organisms, as this will help protect the lives of communities using Huangshui River. The present study provided a certain theoretical basis for understanding the microbial ecology and environmental protection in the Huangshui River on the ecotone zone of Qinghai Plateau and Loess Plateau.

Introduction

Microorganisms are primary decomposers of organic compounds in natural ecosystems, participating in regional climate formation, geochemical cycle, and biological evolution, and playing a vital role in natural ecosystems [1]. The distribution of microorganisms has attracted increasing interest in the last decade, and diversity patterns of microbial communities have been widely observed [2]. Bacterioplanktons are essential players in aquatic ecosystems [3], which are a fundamental and highly variable component of river ecosystems, exhibiting a comprehensive response to environmental pressure and disturbances [4–9]. Changes in river water quality and ecological environment caused by human activities will lead to variations in the microbial community structure [10]. By analyzing the changes in the microbial community structure, the state of river ecosystem health can be evaluated [11].

Qinghai Province, as the “Chinese water tower”, is located in the west of China and in the northeastern part of the Qinghai–Tibet Plateau. Its river ecosystem plays an important role in the ecological status of the whole province and even the whole China. The Huangshui River, is the largest tributary in the upper reaches of the Yellow River. It locates in the eastern part of Qinghai Province (36°02'–38°20'N, 98°54'–103°24'E), at an altitude of 1,650 to 4 400 m originating from the Baohutu Mountain in Haibei County, Qinghai Province and flowing into the Yellow River in Gansu Province [12]. Since the 20th century, the rapid development of human activities in industry, agriculture, mining, and overgrazing has caused outstanding problems in the aquatic environment, including reduction of water resources, deterioration of water quality, and decline of aquatic biodiversity.

At present, the aquatic ecosystem and microbial community of Huangshui River have not been studied. In this study, 16S rRNA gene high-throughput sequencing was conducted in the upper reaches of Huangshui Rivers to gain in-depth insight into possible environmental factors shaping variations in the bacterioplankton community structure. This study can also provide comprehensive understanding of the specific bacterioplankton roles in biogeochemical cycling and determine the potential effects of river regulations on bacterioplankton communities.

Materials And Methods

Study sites and sample collection

As the largest tributary of the upper reaches of the Yellow River, the Huangshui River is located in the intersecting zone of the Qinghai–Tibet Plateau and Loess Plateau. The terrain in the basin is high in the northwest, and low in the southeast with high mountains and deep valleys. The climate belongs to the plateau arid and semi-arid continental climate, with vertical changes and large regional differences because of the diversity of the local topography. The temperature decreases as it goes upstream, whereas the precipitation increases, and the evaporation decreases. Moreover, humid marshes are found. The average annual temperature in the basin is 0.6°C–7.9 °C, and the average annual precipitation is 360–540 mm [13]. Eight sampling sections, numbered from HS1 to HS8, were set up along the midstream of the Hungshui River (100°31'–102°50'E, 36°19'–37°44'N) (Fig. 6). Two liters of water samples was collected from 2 m of the middle point of the river. One liter of water sample was subsequently filtered on board through 0.22 µm polycarbonate membranes (Millipore Corporation, Billerica, MA, USA). Filters were frozen at –80 °C until DNA extraction.

Physicochemical analyses

Many environmental factors are related to the distribution of planktonic bacterial communities, but many of them have strong contributory relationships. The environmental factors screened in this study were pH, altitude, TN, TP, $\text{NH}_4^+\text{-N}$, PI, and Cr^{6+} . Water chemistries such as temperature, pH, and dissolved oxygen (DO) were monitored using a YSI Pro2030 (YSI Incorporated, Ohio, USA). Total phosphorus (TP), total nitrogen (TN), permanganate index (COD_{Mn}), $\text{NH}_4^+\text{-N}$, As, and metal ions (Cr^{6+} , Cu, Zn, Pb, Cd, and Hg) were analyzed by Qinghai Fishery Environment Monitoring Station. TP, TN, and Cr^{6+} were analyzed by a dual beam UV–visible spectrophotometer (TU–1901, T9CS). As and Hg were analyzed by an atomic fluorescence photometer (AFS–230E), and the metal ions were determined by a graphite furnace flame atomic absorption spectrophotometer (ICE–3500, AA6300).

DNA extraction, polymerase chain reaction (PCR), and Illumina Miseq sequencing

Microbial community genomic DNA was extracted from the water samples using the E.Z.N.A.® water DNA Kit (Omega Bio-tek, Norcross, GA, USA) in accordance with the manufacturer's instructions. The final DNA concentration and purification were determined by a NanoDrop 2000 UV–vis spectrophotometer

(Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3–V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5'–ACTCCTACGGGAGGCAGCAG–3') and 806R (5'–GGACTACHVGGGTWTCTAAT–3') by a thermocycler PCR system (GeneAmp 9700, ABI, USA). PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed in triplicate 20 µL mixtures containing 4 µL of 5 × FastPfu buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu polymerase, and 10 ng of template DNA. The resulting PCR products were extracted from 2% agarose gel, further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol.

Purified amplicons were pooled in equimolar and paired-end sequenced (2×300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited in the NCBI Sequence Read Archive database (Accession Number: SRP294338).

Processing of sequencing data

Raw fastq files were demultiplexed, quality-filtered by trimmomatic, and merged by FLASH with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of < 20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded; reads containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of the overlap region was 0.2. Reads that could not be assembled were discarded; (iii) Samples were distinguished according to their barcode and primers, and the sequence direction was adjusted; exact barcode matching was performed, and two mismatched nucleotides.

Operational taxonomic units were clustered with 97% similarity cutoff using UPARSE (version 7.1 <http://drive5.com/uparse/>), and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by the RDP classifier algorithm (<http://rdp.cme.msu.edu/>) against the Silva (SSU123) 16S rRNA database using a confidence threshold of 70%.

Statistical analysis

All samples were rarefied at 56,234 sequences per sample to retain as many samples as possible. We evaluated the alpha-diversity through the community richness indices (Ace, Chao), community diversity indices (Shannon, Simpson), and Good's coverage. Non-metric Multidimensional Scaling (NMDS) was carried out on OTUs data using Bray-Curtis distance matrices to examine the difference in bacterial community between water samples. Environmental factors can be screened through variance inflation factor analysis to retain those with low collinearity. The environmental factors screened in this study are PI, Cr⁶⁺, altitude, pH, TN, TP, and NH₄⁺-N. Canonical correspondence analysis (CCA) was performed to

determine the environmental variables associated with changes in the bacterioplankton community structure using the vegan package. Linear discriminant analysis (LDA) effect size (LEfSe) is an algorithm for high-dimensional biomarker discovery and explanation, which identifies genomic features (genes, pathways, or taxa) characterizing the differences between two or more biological conditions [14]. In the present study, LEfSe was conducted to identify differential microbial functions between the main stream and tributary with the alpha parameter of 0.05 and an LDA threshold value of 2.0. Differences between two independent groups were evaluated by Mann–Whitney U test. PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) was used to predict microbial functions and metabolic pathways [15].

Results

Environmental characteristics

Environmental characteristics of the samples from the eight investigated sites are shown in Table 1. The surface water temperature ranged from 6.8 °C to 17.2 °C, and the pH ranged from 8.34 to 8.47, indicating a weak alkaline. The HS1 and HS2 sites had a significantly low level of Hg and As (Mann-Whitney U test, $p < 0.05$). From the upper reaches to the lower reaches of the Datong River (HS7–HS3), the DO level showed a downward trend. On the contrary, the TN level showed an upward trend. The Cr^{6+} , Cu, Zn, Pb and Cd levels remained consistent in the eight investigated sites.

Community structure, richness, and diversity of bacterioplankton

After quality filtering, 497,604 valid reads (ranging from 56,234 to 69,445 per sample) were generated in total from eight sampling sites along the Huangshui River. All the samples were randomly resampled to 56,234 reads, and the reserved sequences were clustered into a total of 2384 OTUs. Good's coverage (all samples, $99.80\% \pm 0.14\%$) suggested that the majority of the microbial species present in the samples were identified. The rarefaction curves tended to approach the saturation plateau, indicating a sufficient sampling depth of all libraries. The microbial diversity index listed in Table 2 comprised community richness indices (Ace, Chao) [16] and community diversity indices (Shannon, Simpson) [17]. As shown in Table 2, the Shannon index of the water samples of HS4, HS6 and HS8, which indicated bacterial diversity, were significantly ($\text{LDA} > 2$) higher than that of other sampling sites. Except for HS5, HS1 and HS2 had low α diversity. The community of HS6 had the maximum richness and diversity, with Chao index of 1764.3472 and Shannon index of 5.0497.

The phylogenetic classification results showed a total of 34 microbial phyla in all samples, including 78 classes, 216 orders, 386 families, 821 genera, and 1309 species. Differences in bacterial community composition were observed among different groups. The bacterial community composition at the phylum level in different samples is shown in Figure 1. Across all sampling sites, bacterioplankton communities were dominated by Proteobacteria and Firmicutes, and the relative abundance of these two phyla accounted for 77.48% and 12.08%, respectively. Notably, the abundance of Proteobacteria in HS1, HS2,

HS5 and HS7 sampling points was above 99%, and it was as high as 99.98% at HS5 sampling sites. Among the Proteobacteria, the most abundant class was Alphaproteobacteria (up to 95.93%), followed by Gammaproteobacteria (up to 55.16%), Clostridia (up to 28.96%) and Bacilli (up to 17.12%). Proteobacteria dominated the sampling sites, but its classes were distributed differently, with Gammaproteobacteria and Clostridia predominant in HS6 and HS4, respectively, whereas Alphaproteobacteria was predominant in the remaining sampling sites. Notably, the abundance of Alphaproteobacteria was markedly different among the eight sampling sites with a higher amount at sites HS5 and HS7 and a lower amount at site HS4 and HS6.

The bacterioplankton community structure was analyzed at the genus level using a heat map of the top 50 genera for different sites Figure 2. Of the six most abundant genera, two belonged to Alphaproteobacteria, and four belonged to Gammaproteobacteria. Interestingly, the top three abundance genera had polar distribution at each sampling point. For example, the abundance of *Acidocella* at the HS5 sampling point was as high as 95.9%, and the sum of the abundances of *Acidiphillum* and *Metallibacterium* at the HS1 and HS2 sampling points was 97.52% and 99.3%, respectively. Moreover, the abundance of *Acidiphillum* and *Acidocella* at the HS7 sampling point was 92.49%. However, the abundances of the three genera at the sampling points of HS4, HS6, and HS8 were 1.3%, 0.74%, and 0.54%, respectively. Notably, the most abundant genus at the HS6 sampling site was *Aeromonas*, and some species of this genus could cause enteritis, sepsis and other diseases in humans and animals.

Correlation analysis of bacterioplankton communities and environmental factors

Canonical correspondence analysis (CCA) was performed to determine the environmental variables associated with changes in the bacterioplankton community structure (Figure 3). The first axis explained 31.22% of the total variance, whereas the second axis explained 26.69%, indicating that the selected environmental factors drive the differences of bacterioplankton community structures. Most variances can be explained by altitude ($R^2=0.577$, $p=0.123$), Cr^{6+} ($R^2=0.534$, $p=0.16$), PI ($R^2=0.502$, $p=0.159$), and TP ($R^2=0.427$, $p=0.234$). *Acidiphillum* and *Metallibacterium* were found to be more related to TP and Cr^{6+} , whereas *Acinetobacter* and *Romboutsia* were positively related to TN. Although not significant, *Aeromonas* and *Legionella* seemed to be positively correlated with altitude and PI and negatively correlated with TP and Cr^{6+} .

Biogeography patterns of bacterial communities

Non-metric multidimensional scaling was applied using the Bray-Curtis distance to identify the community compositions of all samples (Figure 4). The first axis revealed that the five sampling sites of the Datong River did not cluster. However, the main stream sites of the Huangshui River (HS1 and HS2) clustered with HS5 and HS7, which were from the Datong River. The HS8 site from the Baoku River clustered with the other three sampling sites of the Datong River (HS3, HS4 and HS6).

Functional prediction by PICRUSt

Community prediction analysis using PICRUSt was performed to determine the function of the observed bacterioplankton. The top abundance functional pathways have been presented in Figure 5. Of all the different functional pathways identified, carbohydrate metabolism, amino acid metabolism, global and overview maps, energy metabolism and membrane transport were substantially over-represented compared with other pathways. The main stream microbial assemblages had high abundance of Kos, which belonged to human diseases and cellular processes. Similarly, the tributary had high abundance of Kos, which belonged to metabolism (Fig. S1). Given the importance of water quality to human health, we targeted human diseases and resistance functional classes (Table 3). However, no significant differences between main stream and tributary microbial assemblages were found in the relative abundance of three core resources metabolism pathways, namely, carbohydrate metabolism, energy metabolism, and amino acid metabolism.

Discussion

Macrobenthic community compositions in the upper reaches of the Huangshui River have previously been reported. These works have only provided the community structure of macrobenthos, assessment of ecosystem health, and quality of water in the Huangshui River [18, 19]. However, bacteria play a critical role in environmental and ecological processes in river ecosystems. Exploring the composition of aquatic planktonic bacteria can enhance the understanding of accurate microbial distribution patterns and help to comprehensively understand the Huangshui River. Here, as a complement to previous studies, this study assessed the pattern of the bacterioplankton community and the relationship with environmental factors. Based on previous reports, this study was the first to investigate the bacterioplankton community in the upper reaches of the Huangshui River.

The Chao 1 richness and Shannon index in the river samples were lower than that of other rivers in the previous papers. For instance, Fan et al. [20] reported that the Chao1 and Shannon indices of the planktonic bacterial community in the upper section of the tidal reach in the Yangtze River ranged from 764.33 to 861 and 4.36 to 4.72, respectively. Hauptmann et al. [21] investigated the bacterioplankton community along a small Arctic River and revealed that the Chao1 indices ranged from 1408 to 19117 OTUs, and the Shannon indices ranged from 5.6 to 10.8 per sample. Yang et al. [22] reported that the Chao1 and Shannon indices of bacteria in the Songhua River, one of the seven largest rivers in China, ranged from 1249.26 to 5780.18 and 2.85 to 5.21, respectively, which were much higher than those of the Huangshui River. On the contrary, the Chao1 and Shannon indices ranged from 300.546 to 2014.803 and 0.789 to 5.529, respectively, which were similar to the Heihe River, also known as the Plateau river [23]. The low alpha diversity in Plateau Rivers may be due to the special geographical environment, low temperature and high altitude, which are not conducive to bacterial growth.

The drop in alpha diversity at the input sites from the tributary of the Datong River (HS5) indicates that the tributary inputs less diverse bacterial communities into the main stream of the Datong River. The diversity of the two tributaries of the Datong River and Baoku River is significantly higher than that of the main stream of the Huangshui River, but the diversity of HS1 and HS2 has not increased because of the

confluence of the two tributaries. This result indicates that the volume of water from the main stream of the Huangshui River is not diluted by the tributary community. Moreover, the high diverse communities from the Datong River and Baoku River are concealed by the low diversity of the main stream of the Huangshui River.

The bacterial community in water samples was different with regard to phylum, genus, and OUT (Figs. 1, 2, and 4). High-throughput sequencing data showed that the dominant phylum was Proteobacteria, with up to 99.98% of the sequences, followed by Firmicutes (49.38%). This result is similar to the results of the local and international study of planktonic bacterial communities in rivers [24] and lakes [25, 26]. The relative abundance of Proteobacteria was 77.48%, which was found to be the most abundant phylum in wastewater [27, 28]. However, notably, proteobacteria, which is the dominant phylum, consists of Alphaproteobacteria and Gammaproteobacteria, and the relative content of the two classes of bacteria is as high as 76.82%. Moreover, Alphaproteobacteria and Gammaproteobacteria can be found in freshwater and seawater, but they are more abundant in seawater [29–31]. This finding may be due to the salt mineral resources such as gypsum and calcium Glauber's salt mine, which are widely distributed along the banks of the Huangshui River. Furthermore, the microbial composition of the Huangshui River is significantly different from other freshwater rivers at the class level.

At the genus level, the top six species in relative abundance at each sampling site are *Acidiphilium*, *Acidocella*, *Metallibacterium*, *Acinetobacter*, *Pseudomonas*, and *Aeromonas*. According to previous studies, *Acidiphilium*, *Acidocella*, and *Metallibacterium* dominated microbial communities within mineral-leaching environments or acidic aquatic environments [32]. The pH of the Huangshui River is 8.34–8.47, indicating a weak alkaline, and the content of metal ions is low, which is not the most suitable condition for the enrichment of these microorganisms. Apart from its unique hydrology, biology, climate, and landform, the study area has notable geological and metallogenic conditions. Zeng [33] found that the Huangshui River has evident heavy metal pollution for the contents of Cd, Pb, Hg, and Cr in the muddy sediments, which are all higher than the background values under natural conditions. We inferred that *Acidiphilium*, *Acidocella*, and *Metallibacterium* in the upper reaches of the Huangshui River were derived from the sediments along the river, which were affected by strong winds and rainfall. In addition, we identified diverse taxa of potential pathogens, such as *Acinetobacter*, *Pseudomonas*, and *Aeromonas*. The abundance of *Acinetobacter* was primarily contributed by HS8 and HS4, whereas *Pseudomonas* and *Aeromonas* were primarily contributed by HS3 and HS6, respectively. These taxa might result from input of industrial wastewater and household wastewater.

The bacterial community in river water can be affected by many factors including temperature, nutrient concentration, pH, DO, TN, and TP [34–36]. In this study, we found that the major driving factors for the bacterial community were Cr^{6+} , altitude and TN. The CCA biplot showed that Cr^{6+} was significantly and positively correlated with the dominant genera *Acidiphilium* and *Metallibacterium*. Similarly, TN was significantly and positively correlated with *Acinetobacter* and *Bacillus*, whereas altitude was negatively correlated with *Acidiphilium* and *Metallibacterium*.

In the current study, the results of the functional profiling using PICRUSt revealed that most of the bacteria were involved in the metabolic pathways, and considerable groups were involved in human diseases and antibiotic resistance. Several studies have reported on environmental bacterial function related to human diseases as reviewed by Sandifer [37]. Antibiotic resistance has been shown to spread throughout the environment through mechanisms such as urban and agricultural runoff, wind, and biological forces such as animals and humans [38]. Given that rivers such as the Huangshui River are the main sources of water for drinking and personal and household hygiene in many developing countries, many people who used these polluted waters without prior treatment might be exposed to negative health effects because of the lack of adequate potable water supplies [39].

Conclusions

The present study provided a detailed comparison of the bacterioplankton community of the upper reaches of the Huangshui River using high-throughput sequencing for the first time. As a plateau river, the Huangshui River had lower alpha diversity than other plain rivers. Proteobacteria was the dominant phyla, followed by Firmicutes. Given the salt mineral resources widely distributed along the banks of the Huangshui River, Alphaproteobacteria and Gammaproteobacteria were significantly enriched. The predominant bacterial genera were *Acidiphilium*, *Acidocella*, *Metallibacterium*, and *Acinetobacter*. Diverse taxa of potential pathogens, such as *Acinetobacter*, *Pseudomonas*, and *Aeromonas* were also been identified. The major driving factors for the bacterial community were Cr6+, altitude and TN. The PICRUSt results revealed that the identified bacteria had diverse functional profiles, but most importantly, a substantial number of these bacteria were involved in human diseases and antibiotic resistance. The study clearly indicated the composition of the plankton bacterial community and its relationship with environmental factors, providing a certain theoretical basis for understanding the microbial ecology and environmental protection in the Huangshui River on the ecotone zone of Qinghai Plateau and Loess Plateau.

Declarations

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Conflicts of Interest

The authors declare no competing interests.

Ethics Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate

Not applicable.

Consent for Publication

All listed authors have agreed to be listed and have approved the submitted version of the manuscript.

Code Availability

Not applicable.

Authors Contributions

Qianqian Zhang was responsible for the design, data analysis, and writing. Zhenbing Wu performed statistical analyses. Shenglong Jian, Kemao Li, Guojie Wang, and Hongtao Guan conducted sampling and the lab work. Jingwen Hao, Shuyi Wang, and Yaoyao Lin conducted the experimental work. Aihua Li obtained the funding. All authors read, reviewed and edited the manuscript.

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Tables

Due to technical limitations, table 1, 2,3 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Composition of microorganism groups in each sampling at phylum level

Figure 2

Hierarchical clustering heatmap showing the main bacterial species in different groups at genus level. Each rectangle is colored depending on the log10 value of the taxa percentage as shown in the legend at the left of the plot. The top tree showed the clustering relationship of samples.

Figure 3

Canonical correspondence analysis (CCA) graph for microorganism community and environmental factors

Figure 4

Non-Metrical Dimensional Scale (NMDS) at OUT level based on Bray-Curtis distance matrix

Figure 5

Predicted functional groups of OTUs in the Huangshui River samples based on KEGG database. Functional groups with less than 1% relative abundance are not included

Figure 6

Distribution of sampling sites in the Huangshui River. Map image and geographic information were cited from Google Earth Pro (version 7.1.8.3036). Specific latitude and longitude information about each sampling site are shown as follows: HS1 (36°20'28.9"N, 102°50'07.4"E), HS2 (36°28'39.2"N, 102°25'10.8"E), HS3 (36°50'07.0"N, 101°58'00.5"E), HS4 (36°19'20.4"N, 101°48'57.8"E), HS5 (37°21'54.7"N, 101°32'38.7"E), HS6 (37°35'45.5"N, 101°09'18.4"E), HS7 (37°44'53.8"N, 100°31'38.3"E),

HS8 (37°15'09.3"N, 101°24'51.8"E). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Supplementary Files

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