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RESEARCH ARTICLE

# Morphological, molecular, and biochemical study of cyanobacteria from a eutrophic Algerian reservoir (Cheffia)

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## Abstract

The cyanobacteria management in water bodies requires a deep knowledge of the community composition. Considering the reliable and thorough information provided by the polyphasic approach in cyanobacteria taxonomy, here we assess the cyanobacterial community structure of the Cheffia reservoir from Algeria. Cyanobacteria were identified on the basis of morphological traits and next-generation sequencing (NGS); toxins-related genes were localized in addition to the identification of toxins; temperature and nutrient level of water samples were also determined. The polyphasic approach was essential for cyanobacteria investigation; 28 genera were identified through 16S rRNA metabarcoding with the dominance of taxa from *Microcystis* (34.2%), *Aphanizomenon* (20.1%), and *Planktothrix* (20.0%), and morphological analysis revealed the association in this water body of five species within the genus *Microcystis*: *M. aeruginosa*, *M. novacekii*, *M. panniformis*, *M. ichthyoblabe*, and *M. flos-aquae*. The presence of *mcyE* genotypes was detected; moreover, HPLC–PDA and LC–ESI–MS/MS revealed the production of microcystin-LR. Results obtained in our study are very important since this ecosystem is used for water supply and irrigation; as a consequence, a good water management plan is essential.

**Keywords** Algeria · Cyanobacterial diversity · Hypereutrophic water · NGS · Polyphasic approach · Toxins

## Introduction

Cyanobacteria are prokaryotic and oxygenic organisms that thrive across many aquatic and terrestrial habitats (Whitton, 2012; Codd et al. 2017). Eutrophication, promoted by human activities related to urban, agricultural, and industrial development, and climate change are the main factors determining the cyanobacteria development (O'Neil et al. 2012; González and Roldán, 2019). This phenomenon is a major concern for the sanitary and environmental agencies due to their potential to produce cyanotoxins posing undesirable effects to human and environmental health (Metcalf and Codd, 2012; Moreira et al. 2012; Bouma-Gregson et al. 2018; Wu et al. 2019). The first step, to reduce the effects related to cyanobacteria outgrow, is the identification of the cyanobacterial community in the water body. The microscopic analysis of morphological characteristics is traditionally used in the classification of cyanobacteria (Komárek and Anagnostidis, 1999, 2005; Komárek, 2013); yet, the high morphological variability and the low number of phenotypic characters used lead to serious identification issues (Bittencourt-Oliveira and

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Piccin-Santos, 2012). Thus, the application of polyphasic taxonomy ensures the accurate identification of cyanobacteria (Nübel et al. 1997; Komárek, 2016). DNA metabarcoding, with the application of high-throughput sequencing technology, is a promising method to deeply explore cyanobacterial diversity (Ramos et al. 2017; Casero et al. 2019). Moreover, detection of toxins production in environmental samples is, also, very important for the monitoring of water resources (Lee et al. 2015). For this purpose, amplification by polymerase chain reaction (PCR) of specific genes related to toxins production is a rapid and effective technique (Belykh et al. 2015; Kurmayer et al. 2017) as well as biochemical analysis.

Increasing levels of eutrophication and cyanobacteria development are expected due to climate change, in particular in the southern Mediterranean and islands (Mariani et al. 2015). Cheffia reservoir, in Algeria, is a water body used for drinking, industrial, and irrigation water. The organic pollution in this reservoir appears to be considerable (Harrat and Achour, 2010). Indeed, this reservoir is connected with the Bounamoussa River which receives inputs from industrial, urban, and agricultural sectors with low treatment levels. Hence, the present study aims to investigate the cyanobacteria community in this subtropical ecosystem using morphological analysis, massive sequencing, toxins-related genes amplification, and chemical features in addition to the determination of physic-chemical characteristics of water.

## Materials and methods

### Study site description and sampling

The study was conducted in Cheffia reservoir (36°07′N and 8°03′E) located in the North-East of Algeria (Agence National des Ressources en Eau, 2004).

The sampling was carried out on 3 different dates at irregular intervals spread over the period October–November 2014. Samples for physical–chemical quality analysis were collected with a polyethylene bottle from a depth of about 30 cm below the water surface, stored at 4 °C, and processed within 24 h. Cyanobacterial samples were collected directly or concentrated from the water surface with plankton net (20-µm mesh size); subsamples for morphological analysis were fixed in the field with Lugol's iodine solution and stored at 4 °C in the dark. For molecular analysis, the remaining samples were filtered through fiberglass filters (0.7 µm approx., Millipore) then stored at – 20 °C. For toxins analysis, 100 mL of water was filtered on GF/F filters and analyzed immediately.

### Chemical and physical analysis

Temperature (Temp) was determined in situ using Hanna Instruments handheld meter (Hi 98,129). Ammonium ($NH_4^+$, mg $L^{-1}$) concentrations were analyzed using the Spectroquant Merck method following ISO 7150–1. Nitrates ($NO_3^-$, mg $L^{-1}$) and nitrites ($NO_2^-$, mg $L^{-1}$) concentrations were assessed spectrophotometrically using Merck Kits, analogous to the DIN 38,405–9 methods. Total phosphorus (TP, mg $L^{-1}$) levels were determined using the ammonium molybdate spectrometric method (ISO 6878: 2004).

### Morphological characterization

Microscopic observations were performed out under an Olympus BH-2 photomicroscope (Leica Microsystems, Germany), at 125 × and 500 × magnifications. The identification of cyanobacteria morphospecies was performed based on morphological traits according to Komárek and Anagnostidis (1999; 2005) and Komárek (2013).

### Total DNA extraction and amplifications

Total genomic DNA was extracted from filters using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions modified by Lezcano et al. (2016). Purified DNA was preserved at – 20 °C until being applied to PCR analysis and sequencing.

The 16S rDNA was amplified by PCRs with specific primers for cyanobacteria, CYA359F and CYA781R (a/b) (Table 1) according to the method of Nübel et al. (1997).

The presence of the following genes: *mcyE*, *cyrJ*, *anaF*, and *sxtI*, related to the production of the common toxins: hepatotoxin microcystin (MC), cylindrospermopsin (CYN), anatoxin (ANA), and saxitoxin (STX), respectively, was checked by PCRs using specific primer pairs (Table 1). The following strains: *M. aeruginosa* UAM 247, *Aphanizomenon issatchenkii* SP 33, *A. gracile* UAM 529, and *A. ovalisporum* UAM 290, from the Autonoma de Madrid University culture collection (Spain), were used as positive controls for the production of MCs, ANAs, SAXs, and CYNs, respectively. The reaction mixes were made up as master mixes, containing 2-µl loading buffer, 1.2 µl $MgCl_2$, 0.5 µl deoxynucleotide triphosphate mix (2 mM), 1 µl of each forward and reverse primer (10 mM), 1 µl of genomic DNA, 0.1 µl Taq DNA polymerase, and Milli-Q water yielding a total volume of 18 µl. Thermal cycling was performed on a Peltier thermal cycler PTC

**Table 1** Primer sets used for PCR amplification

| Target | Primer pair | Sequence (5'–3') | Size (bp) | Reference |
|---|---|---|---|---|
| 16S rRNA | CYA359F<br>CYA781Ra<br>CYA781Rb | GGGGAATYTTCCGCAATGGG<br>GACTACTGGGGTATCTAATCCCATT<br>GACTACAGGGGTATCTAATCCCTTT | 422 | Nübel et al. (1997) |
| *mcyE* | HEPF<br>HEPR | TTTGGGGTTAACTTTTTTGGGCATAGTC<br>AATTCTTGAGGCTGTAAATCGGGTTT | 472 | Jungblut and Neilan (2006) |
| *sxtI* | sxtaf<br>sxtar | AGCGCTGCCGCTATGGTTGTCG<br>ACGCAATGAGGGCGACACCAC | 960 | Casero et al. (2014) |
| *cyrJ* | cynsulF cylnamR | ACTTCTCTCCTTTCCCTATC<br>GAGTGAAAATGCGTAGAACTTG | 656 | Mihali et al. (2008) |
| *anaF* | atxoaf<br>atxar | TCGGAAGCGCGATCGCAAATCG<br>GCTTCCTGAGAAGGTCCGCTAG | 461 | Ballot et al. (2010) |

200 (MJ Research, Inc., San Francisco, CA). Afterwards, PCR products were visualized by 1.5% agarose gel electrophoresis with GelRed staining and UV illumination and compared with a molecular weight marker (GeneRulerTM 1 kb DNA ladder).

## Illumina-based sequencing and sequences processing

The 16S rRNA gene amplicons from each sample were quantified by fluorimetry with PicoGreen (Invitrogen, CA, USA), pooled at equimolar concentrations and subjected to a 2 × 250 bp paired-end sequencing on the Illumina MiSeq platform (Parque Científico de Madrid, Madrid, Spain).

The raw Illumina MiSeq reads of the 16S rRNA were first quality checked by using FastQC (Available online: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). PCR primers were removed from raw reads by applying cutadapt (Martin, 2011) and the resulting sequences were merged by using FLASH (Magoc and Salzberg, 2011). Following the obtained merged sequences were denoised into ASVs (amplicon sequence variants) (Callahan et al. 2017) by applying DADA2 (version 1.10.1) (Callahan et al. 2016). ASVs were taxonomically annotated by using BioMaS (Fosso et al. 2015) and the release 138 NR 99 of the SILVA database (Pruesse et al. 2007). ASVs assigned as chloroplast were removed from subsequent analysis. Relative sequence abundances were calculated based on the total number of sequences and ASVs per sample. The raw 16S rRNA dataset is available at Sequence Read Archive (SRA) under study PRJNA612648.

## Toxins analysis

Filters were extracted twice with methanol 50% (v/v) by sonication for 5 min at 60 Hz, in an ice bath. After centrifugation (4995 g, 5 min, 4 °C), the supernatants were vacuum dried (< 35 °C; < 150 mbar) and the dry crude extracts were dissolved in 1.5 mL of methanol 50%. Samples were subsequently analyzed by high-performance liquid chromatography with a photodiode array detector (HPLC/PDA) then by liquid chromatography–electrospray ionization mass spectrometry (LC–ESI–MS/MS). The HPLC/PDA system used a Waters® Alliance e2695 *HPLC* system (Milford, MA) coupled with a PDA 2998 detector following the method described by Churro et al. (2017). MCs were identified on the basis of their UV spectra (200–300 nm) and retention time with an Empower 2™ Chromatography Data Software. The standard used was the MC-LR (Cyano Biotech GmbH, Berlin).

LC–ESI–MS/MS analysis was carried out in full scan (270–2000 m/z) and collision-induced dissociation (CID) using a Liquid Phase Chromatograph Finnigan Surveyor (Thermo Scientific, San Jose, CA, USA), coupled with a spectrometry detector (MS Mass LCQ FleetTM ion trap), with electrospray (ESI) interface, including a Surveyor LC pump, a Surveyor auto-sampler, and a surveyor photoelectric diodearray (PDA) detector. Separation was achieved using $C_{18}$ Hypersil Gold column (100 × 4.6 mm I.D., 5 µm, Thermo-Scientific) kept at 35 °C. The flow rate was set at 0.7 mL/min. The eluents used were acetonitrile (mobile phase A) and water (mobile phase B) both acidified 0.1% formic acid (v/v). The gradient program started at 45% A (held for 5 min), increasing to 90% A in 12 min, turning back to initial conditions in 5 min to equilibrate until 25 min. The injected volume was 10 µL in loop partial mode. The positive ionization mode parameters were as follows: capillary voltage, capillary temperature, tube lens, and normalized collision energy maintained at 22 V, 350 °C, 120 kV, and 35, respectively (Mayumi et al. 2006). Data were processed using XcaliburTM version 2 Software.

**Table 2** Physical and chemical characteristics of Cheffia reservoir water during the period October–November 2014

| Sampling date | 13 Oct. 2014 | 09 Nov. 2014 | 22 Nov. 2014 |
|---|---|---|---|
| Temp (°C) | 29 | 22.5 | 21 |
| $NO_3^-$ (mg $L^{-1}$) | 0.42 | 3.15 | 1.07 |
| $NO_2^-$ (mg $L^{-1}$) | 12.92 | 14.31 | 11.65 |
| $NH_4^+$ (mg $L^{-1}$) | 2.39 | 1.61 | 0.62 |
| TP (mg $L^{-1}$) | 2.02 | 6.30 | 3.49 |

## Results

### Water analysis

Selected water quality parameters data throughout the sampling study period are presented in Table 2. The water temperature on the sampling site showed high values with a mean of 24.0 °C. High values of nutrients were observed, with total inorganic nitrogen and total phosphorus concentrations between 13.34–19.07 mg N $L^{-1}$ and 2.02–6.30 mg P $L^{-1}$, respectively.

### Morphological analysis

Based on taxonomic keys, the cyanobacterial community of Cheffia reservoir was composed of 6 genera from Chroococcales and Oscillatoriales (Figs. 1 and 2), of which *Microcystis* was characterized with wide morphospecies diversity; some of them are identified at the species level as *M. aeruginosa*, *M. novacekii*, *M. panniformis*, *M. ichthyoblabe*, and *M. flos-aquae*. *Microcystis*, *Planktothrix*, and *Planktothricoides* were found from all samples (Table 3).

### Analysis of metabarcoding libraries

To assess the qualitative cyanobacteria composition from three samples at Cheffia reservoir, we used high-throughput Illumina sequencing of cyanobacterial 16S rRNA gene (V3–V4 region). The total number of raw reads generated across all samples was 1,132,089. Overall, 271 ASVs were inferred, and according to the taxonomic classification, 57 16S rRNA chloroplast sequences were identified and removed from further analysis.

Cyanobacterial reads in all three 16S rRNA datasets belonged to five orders: Chroococcales, Oscillatoriales, Nostocales, Synechococcales, and Chroococcidiopsidales. A total of 28 cyanobacteria genera were identified. *Microcystis* represented the most abundant genus through 16S rRNA sequencing accounting for 34.2% of the reads followed by the heterocystous diazotroph *Aphanizomenon* (20.1%) and the filamentous solitary trichomes *Planktothrix* (20.0%). *Planktothricoides*, *Cylindrospermopsis*, *Sphaerospermopsis*, and *Pseudanabaena* were still present in this ecosystem, while in minority, at proportions of 8.6%, 7.1%, 6.0%, and 2.4%, respectively. The fraction of identified cyanobacteria with a percentage less than 1% included members of *Roseofilum* (0.4%), *Cyanobium* (0.4%), and *Phormidium* (0.4%), and the fraction of identified cyanobacteria with a percentage less than 0.1% included members of *Oscillatoria*, *Leptolyngbya*, *Pantalinema*, *Synechocystis*, *Merismopedia*, *Gloeocapsa*, *Prochlorothrix*, *Nodosilinea*, *Tychonema*, *Chroococcidiopsis*, *Aliterella*, *Scytonema*, *Tolypothrix*, *Nostoc*, *Calothrix*, *Rivularia*, *Arthrospira*, and *Dolichospermum*.

According to the relative abundance values (Fig. 3), differences in cyanobacteria community composition were registered between samples; the first and the third sampling time points (i.e., 13.10.14 and 09.11.14) are more similar compared to the second sample.

### Toxin-related genes amplification

The presence of genes encoding for toxins MCs, CYNs, ANAs, and STXs in the investigated community was tested in PCR by using the HEPF/HEPR, cynsulfF/cylnamR, AnxgenF/AnxgenR, and sxtIf/sxtIr primer pairs. Positive PCR results were obtained only for HEPF/HEPR, suggesting the occurrence of MCs producing cyanobacteria (Fig. 4).

### Microcystins identification

Two major peaks were detected by HPLC–PDA (Fig. 5) with UV spectra characteristic of microcystins; peak 1 was characterized by a spectrum of absorption at 232.2 nm and a retention time at 7.783 min and peak 2 which followed the standard of MC-LR pattern: spectrum of absorption at 238.1 nm and retention time at 8.817 min. The spectrum of LC/ESI–MS analysis (Fig. 6) shows the *m/z* of the parent ion peak (995) of microcystin-LR and *m/z* of product ions after collision-induced dissociation. Indeed, the fragmentation pattern of the m/z ion of the second peak did not show any product ions characteristics of MCs.

## Discussion

This study aimed to assess the cyanobacterial community in a subtropical reservoir from Algeria, Cheffia reservoir, by using an integrated approach including morphological descriptions, next-generation sequencing, genetics characteristics, and toxin analysis. Combining both morphological and molecular techniques contributed to better estimating the composition of the cyanobacteria community, whereas the microscopic observation underestimates extant cyanobacteria diversity (Li et al., 2019) but allows the cyanobacteria identification up to the species level; however,

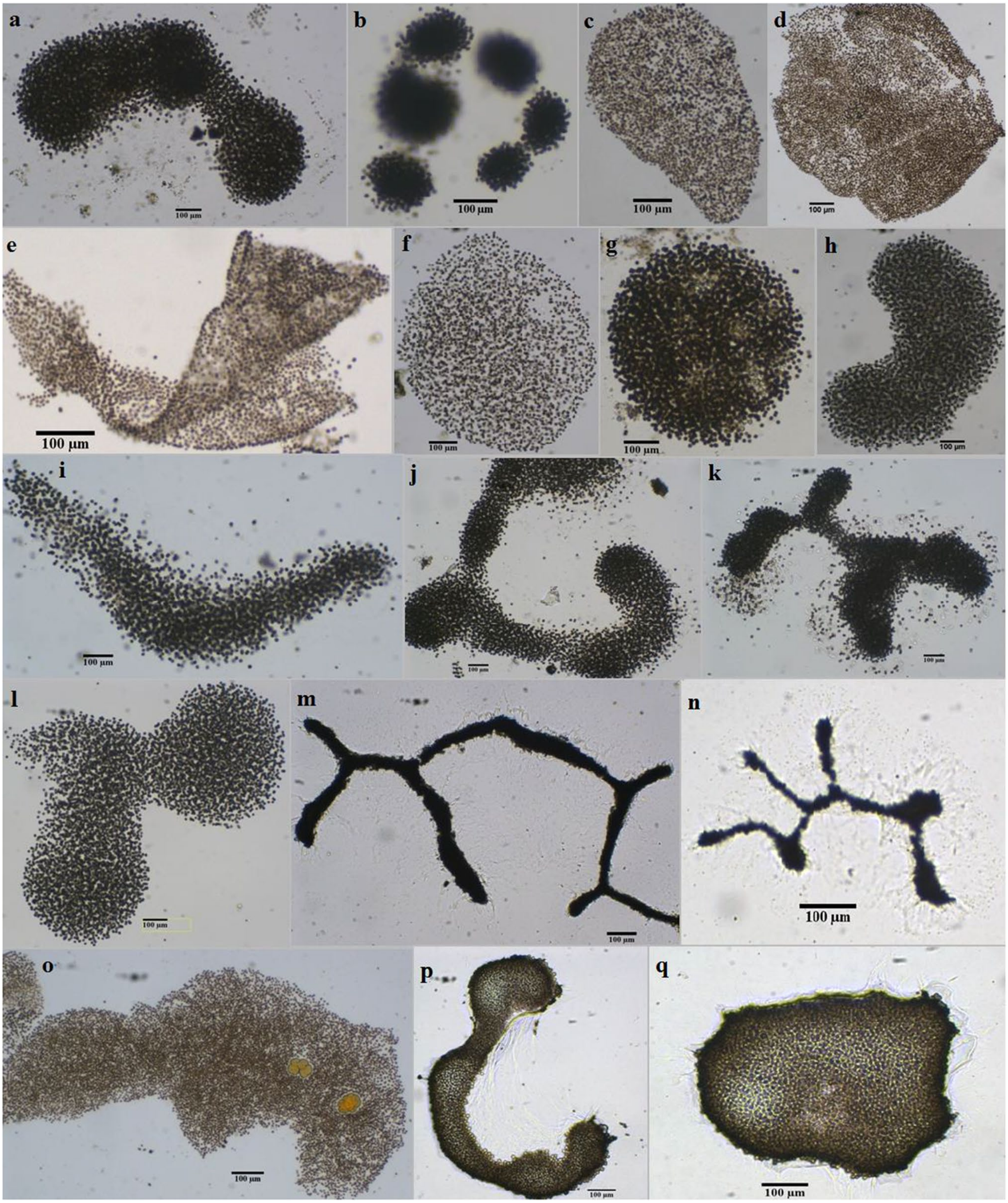


**Fig. 1** Optical light microscopy micrographs of the observed colonial cyanobacterial morphotypes in Cheffia reservoir. **a** *M. aeruginosa*, **b** *M. novacekii*, **c** *M. panniformis*, **d**–**e** *M. ichthyoblabe*, **f**–**g** *M. flos-aquae*, **h**–**q** *Microcystis* sp. Tentative taxonomic classifications were performed according to Komárek and Anagnostidis (1999)

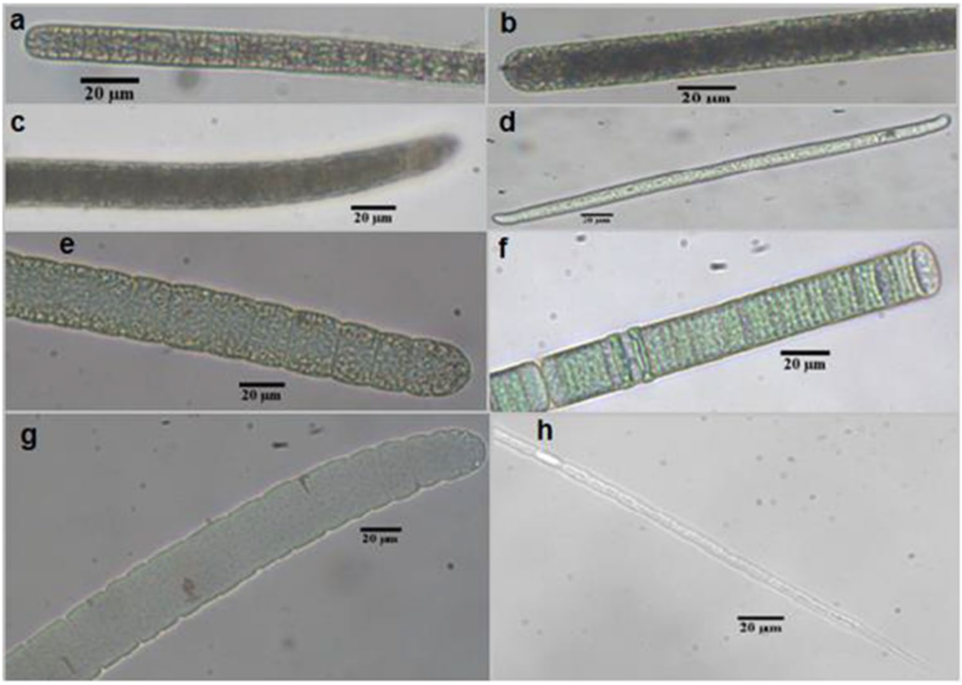


**Fig. 2** Optical light microscopy micrographs of the observed filamentous cyanobacterial morphotypes in Cheffia reservoir. **a** *Planktothrix pseudagardhii*, **b** *P. agardhii*, **c** *Planktothricoides raciborskii*, **d** *Phormidium* sp., **e–g** *Oscillatoria* sp., **h** *Aphanizomenon* sp. Tentative taxonomic classifications were performed according to Komárek and Anagnostidis (2005) and Komárek (2013)

**Table 3** Cyanobacteria taxa identified through morphological investigation from Cheffia reservoir during the period October–November 2014

| Taxa | 13 Oct. 2014 | 09 Nov. 2014 | 22 Nov. 2014 |
|---|---|---|---|
| *M. aeruginosa* | | x | x |
| *M. novacekii* | | x | |
| *M. panniformis* | x | | x |
| *M. ichthyoblabe* | x | | x |
| *M. flos-aquae* | | | x |
| *Microcystis* sp. | x | x | x |
| *P. pseudagardhii* | x | x | |
| *P. agardhii* | x | x | |
| *Planktothricoides* | x | x | x |
| *Oscillatoria* | x | | |
| *Phormidium* | x | | |
| *Aphanizomenon* | | | x |

molecular analysis is more representative for diversity but serves only for delimiting cyanobacteria taxa at the genus level (Komárek, 2010). In fact, the morphological technique allowed a finer resolution of five morphospecies within the *Microcystis* genus; additionally, 22 genera emerged during the molecular study. Reviewing the scientific literature showed that a considerable number of these taxa namely, *Tolypothrix*, *Nostoc*, *Rivularia*, *Arthrospira*, *Synechocystis, Pseudanabaena*, *Chroococcidiopsis*, *Cyanobium*, and *Roseofilum* are of most importance due to their ability to microcystins production (Bláha and Maršálek, 1999; Domingos et al. 1999; Oudra et al. 2002; Metcalf and Codd, 2012; Pereira et al. 2012; Magana-Arachchi and Wanigatunge, 2013; Richardson et al. 2014).

Physico-chemical parameters indicated that waters in this ecosystem during the period study offered suitable conditions for the development of cyanobacteria because of the fact that phosphorus and nitrogen have been demonstrated as factors related to the success of freshwater cyanobacteria (Jankowiak et al. 2019) and increased cyanobacterial growth rates at temperatures exceeding 20 °C (Paerl and Huisman, 2008). In fact, Cheffia reservoir fell into the hypertrophic level, according to the trophic classification defined by TP concentration of OECD (1982). *Microcystis* (Belykh et al. 2013; Deutsch et al. 2020), Filamentous forms (Reynolds, 1996), *Planktothrix* (Kokocinski et al. 2010; Steffen et al. 2014; Bukowska et al. 2017), and Nostocales species (Salmaso et al. 2015) were found to be characteristic of hypereutrophic water which corroborate with our results. Moreover, global warming is found to be a factor with a major role in favoring the recent spread of Nostocales from tropical to subtropical and temperate regions (Sukenik et al. 2012; Antunes et al. 2015; Budzyńska et al., 2019). Actually, scenarios of climate change are considered in both Africa and the Mediterranean basin, which are amongst the most vulnerable areas to climate change and forecasts are indicating a quite remarkable temperature increase (Niang et al. 2014); so, the effect of the temperature should be considered in cyanobacterial development research in this ecosystem in the future. Furthermore, the variable cyanobacteria distribution observed between samples highlights that further

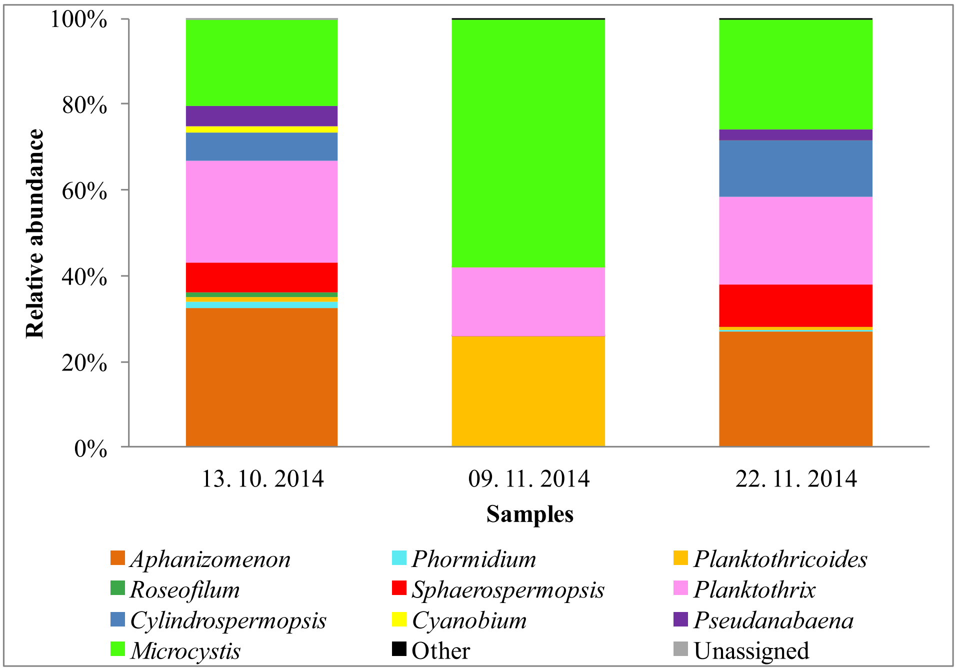


**Fig. 3** Relative abundances (expressed as %) of cyanobacteria in Cheffia reservoir at the genus level. Only taxa with a relative abundance equal or higher to 1% in at least one-time point were shown. Rare taxa were collapsed in other

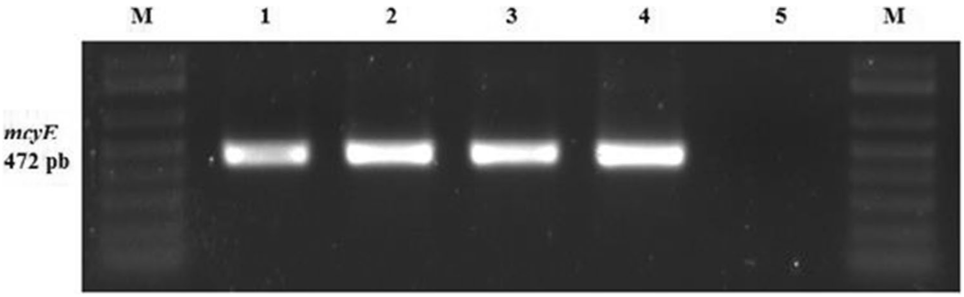


**Fig. 4** PCR amplification of *mcyE* gene fragment with HEPF /HEPR primer set. Lanes 1, positive control; lane 2, 13 Oct. 2014; lane 3, 9 Nov. 2014; lane 4, 22 Nov. 2014; lane 5, negative control; M, DNA Marker

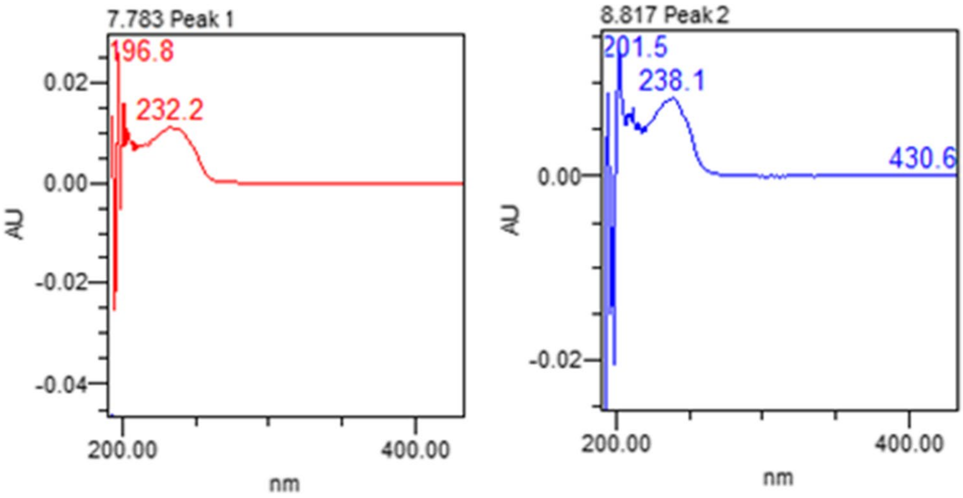
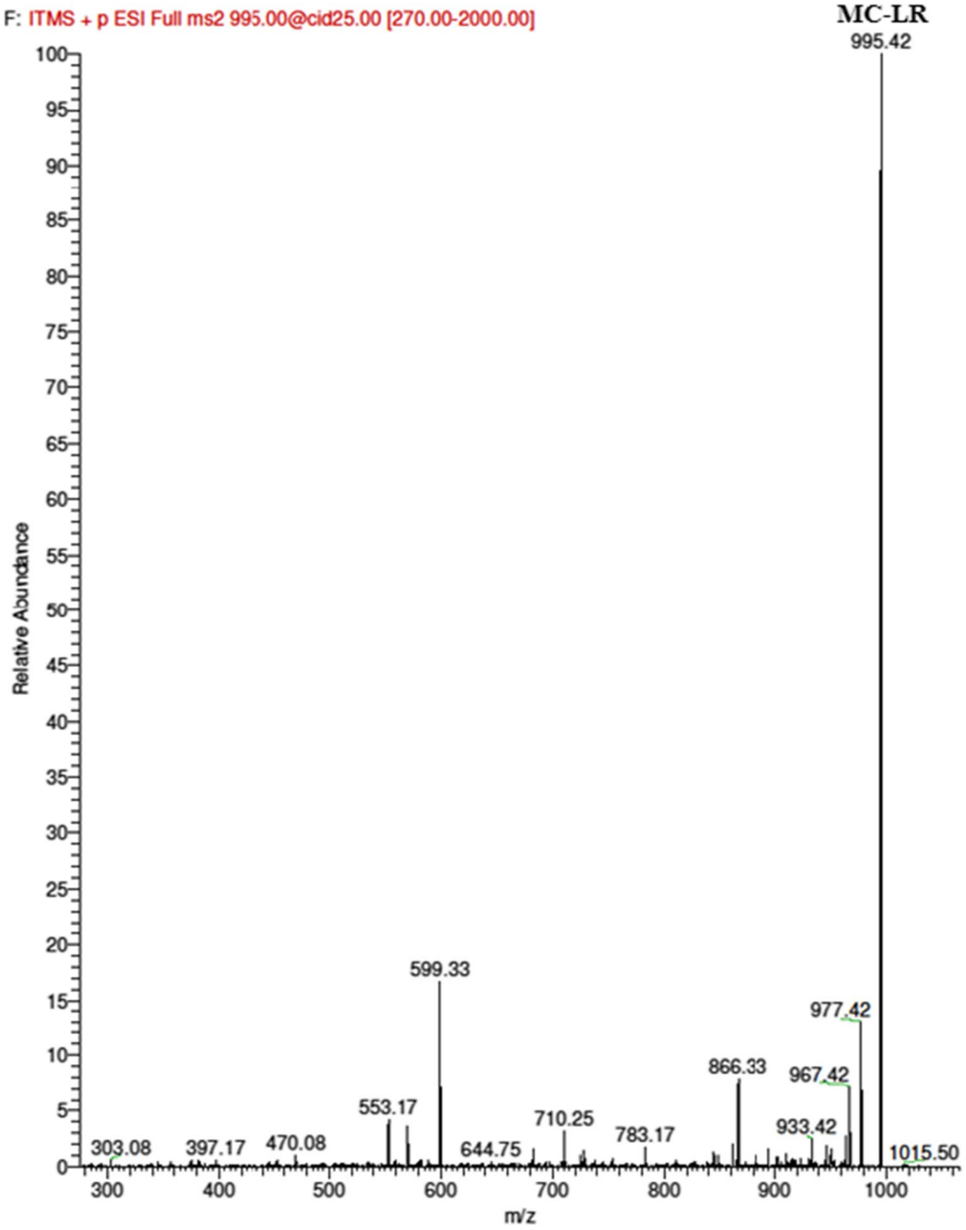


**Fig. 5** UV spectra and retention times of peaks detected by HPLC–PDA from cyanobacterial biomass extracts obtained from Cheffia reservoir (abbreviation: AU, absorption units)

investigations using more sampling sizes covering the full annual range and the full water column are needed to explore factors determining cyanobacteria community composition.

*Microcystis*, one of the dominant genera, showed the co-occurrence of 5 morphospecies. This study is the second report describing the prominent number of morphospecies

**Fig. 6** Full-scan electrospray spectrum of the MC-LR (m/z 995.42) identified in cyanobacterial biomass extracts collected from Cheffia reservoir (abbreviations: m/z, mass-to-charge ratio; MC-LR, microcystin-LR)



from the same genus, *Microcystis*, after Krstić et al. (2017) where 9 morphospecies (*M. aeruginosa*, *M. botrys*, *M. flos-aquae*, *M. ichthyoblabe*, *M. novacekii*, *M. protocystis*, *M. smithii*, *M. viridis*, and *M. wesenbergii*) were identified in Lake Dojran (a stable eutrophic lake), fYR Macedonia. In terms of toxicity, most morphospecies identified are recognized as MC producers: *M. aeruginosa*, *M. ichthyoblabe* (Mowe et al. 2015; Lee et al. 2018), and *M. novacekii* (Song et al. 1998).

Moreover, the community of cyanobacteria identified included putative MCs producers. Also, it was demonstrated that the occurrence of *mcy* genes in cells is, generally, correlated with their ability to synthesize microcystin (Panksep et al. 2020). Köker et al. (2017) observed the co-occurrence of more than one MC producer genus, *Anabaena*, *Microcystis*, and *Planktothrix* in eutrophic and hypertrophic lakes. As suggested by the same authors, this complexity of several genera MC producers was favored by the nutrient-rich waters. While in most cases, strains of *Microcystis* with *mcy* genes produce MCs (Nishizawa et al. 1999; Kaebernick et al. 2001; Tillett et al. 2001; Mikalsen et al. 2003; Qiu et al. 2013; Falcone-Dias et al. 2020), this is not the case for *Planktothrix* strains (Kurmayer et al. 2004; Christiansen et al. 2006). However, the toxicity of *P. agardhii* was recently confirmed in Algerian water bodies (Saoudi et al. 2017). Toxicity from *Microcystis* strains is also well known in Algerian ecosystems, Oubeira lake (Nasri et al. 2008), and Lake des oiseaux (Bouhaddada et al. 2016), and in neighboring countries with the same climatic conditions, Morocco, Lalla Takerkoust lake reservoir (Oudra et al. 2001; Samoudi et al. 2016), Yaacoub Al Mansour reservoir (Ait Hammou et al. 2014; Ait Hammou et al. 2018), Mansour Eddahbi reservoir, Almassira reservoir (Douma et al. 2010), Aguelmam Azizgza and Dayet Afourgah lakes (Douma et al. 2017); from Tunisian reservoirs; Hjar, Nebhana, Joumine, Séjnène (Fathalli et al. 2011), and Lebna Dam (El Herry et al. 2008); and in Egypt (Abdel-Rahman et al. 1993; Mohamed et al. 2003; Mohamed et al. 2015). *Nostoc* and *Oscillatoria* are also considered among major MC producers in Moroccan (Oudra et al. 2009) and Egyptian (Brittain et al. 2000) water bodies. Effectively, the identification of MC-LR from this water body confirmed that cyanobacteria in this reservoir are MC-producers.

## Conclusion

Cyanobacteria in the Cheffia reservoir from the North-East of Algeria was investigated by morphological, molecular, and chemical methods. This ecosystem was characterized by a hypereutrophic level; the polyphasic approach used was successful for the identification of five cyanobacterial orders: the Chroococcales, Oscillatoriales, Nostocales, Synechococcales, and Chroococcidiopsidales. PCR screening of toxin-related genes revealed the *mcyE* gene and the production of MC-LR, the most toxic congener, was confirmed by chemical analysis. In conclusion, it is crucial to keep a monitoring system in this ecosystem and a decision tool to react against toxins patterns and concentrations.

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**Author contributions** L.B.: conceptualization, investigation, formal analysis, writing—original draft; H.B.: conceptualization, supervision; A.A.: conceptualization, supervision; M.C.C.: investigation; A.Q.: resources, supervision, review, and editing; B.F.: resources, formal analysis, writing—review and editing; M.M.: resources, formal analysis; G.P.: resources, supervision, review, and editing; J.A.: formal analysis, investigation; V.V.: resources, supervision, funding, review, and editing.

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**Data availability** The datasets generated and analyzed during the current study are available in the National Center for Biotechnology Information (NCBI) SRA database under the accession number PRJNA612648.

## Declarations

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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