Microbial diversity and toxin risk in tropical freshwater reservoirs of Cape Verde

Ana P. Semedo-Aguiar,[1,2,3] José B. Pereira-Leal,[1] Ricardo B. Leite[1*]

[1] Instituto Gulbenkian de Ciência, Rua da Quinta Grande nº6, 2780-156 Oeiras, Portugal

[2] Programa de Pós-Graduação Ciência para o Desenvolvimento (PGCD)

[3] Universidade Jean-Piaget de Cabo Verde

Running Head: Toxin risk survey in Cape Verdean reservoirs

* Corresponding author

E-mail: rleite@igc.gulbenkian.pt (RBL)

ORCID iD: 0000-0002-9622-3895

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Abstract

The Cape Verde islands are part of the African Sahelian arid belt that possesses an irregular rainy season between August and October. This erratic rain pattern has prompted the need for water reservoirs, now critical for the country's sustainability. Worldwide, freshwater cyanobacterial blooms are increasing in frequency due to global climate change and eutrophication of water bodies, particularly in reservoirs. To date there have been no risk assessments of cyanobacterial toxin production in these man-made structures. We evaluated this potential risk using 16S rRNA gene amplicon sequencing and full metagenome sequencing in freshwater reservoirs of Cape Verde.

Our analysis revealed the presence of several potentially toxic cyanobacterial genera in all sampled reservoirs (Poilão, Saquinho and Faveta). In Faveta *Microcystis* sp., a genus well known for toxin production and bloom-formation, dominated our samples, while a green algae of the genus *Cryptomonas* and *Gammaproteobacteria* dominated Saquinho and Poilão.

Taking advantage of the dominance of *Microcystis* in the Faveta reservoir, we were able to reconstruct and assemble its genome, extracted from a metagenome of bulk DNA from Faveta water. We named it *Microcystis* cf. *aeruginosa* CV01, for which a phylogenetic analysis revealed to have a close relationship with other genomes from those taxa, as well as other continental African strains, suggesting geographical coherency. In addition, it revealed several clusters of known toxin-producing genes.

This assessment of Cape Verdean freshwater microbial diversity and potential for toxin production reinforces the need to better understand the microbial ecology as a whole of water reservoirs on the rise.

Introduction

The available freshwater in the African archipelago of Cape Verde (DMS coordinates 15°07'12.51" N, 23°36'18.62" W) does not cover its needs. In addition, overexploitation and saline intrusion can impair the quality of groundwater [1]. Several water storage structures are being planned around the country in order to collect storm water needed to increase irrigated areas and modernize agriculture. However, these waters can carry nutrients of natural and anthropogenic origin, creating conditions for eutrophication and exponential growth of microalgae. These algal blooms have deleterious impacts on public health, water quality, and environmental issues, as well as economic costs due to bottom anoxia, release of noxious products, and toxic metabolites [2,3]. Actually, these events are occurring more frequently worldwide, and it is thought that global climatic changes are a major contributor to this problem [4–6].

To evaluate the risk of occurrence of algal blooms in freshwater bodies it is important to characterize their microbial composition. In non-eutrophic freshwater systems, the most commonly abundant bacterial groups are, in order of decreasing relative abundance, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* (Alpha, Beta and Gamma clades), *Verrucomicrobia* and *Cyanobacteria* [7–12]. In contrast, eutrophic waters contain microbial communities that include large numbers of cyanobacteria,

62 which are able to produce toxins and foul odors and discolor the water [13,14]. The
63 cyanobacterial phylum has many genera that produce toxins, also called cyanotoxins,
64 and in freshwater bodies, toxic and non-toxic strains can co-exist and dominate at
65 different times [15,16]. Poisoning with cyanotoxins occurs through consumption of
66 contaminated food or water, or during aquatic recreational activities, causing many
67 adverse symptoms like skin irritation, acute diarrhea, and liver and nervous tissue
68 damage, leading to severe health problems, or death in humans, cattle, domestic
69 animals and wildlife [6,13,17–19]. Hence, the risk of having toxin production
70 increases the need for monitoring plans to prevent toxin-related impairments and
71 costs.
72 Molecular-based methods combined with sequencing offer the ability to not only
73 identify possible toxin producers but also target species-specific toxins, validating the
74 presence or absence of toxin-related pathways. Nevertheless, DNA-based molecular
75 methods cannot predict if toxins are being produced and released to the
76 environment.
77 The leveraging of molecular methods provided by Next Generation Sequencing (NGS)
78 allows researchers to gain new insights into microbial community structure in
79 environment samples, identify new community members and discover potential
80 bioindicators of water quality.
81 To determine the current risk of toxin production in the Cape Verdean freshwater
82 reservoirs, we performed NGS analysis of 16S rRNA gene amplicon sequences to
83 identify microalgae and bacteria in the reservoirs. Our results show the presence of
84 several cyanobacterial genera well known to produce toxins in all reservoirs. We
85 were able to reconstruct, for the first time, the full genome of a potentially toxic

cyanobacterium from Cape Verde, based on the full metagenome sequencing data of Faveta reservoir. Analysis of this genome revealed the presence of genetic machinery used to synthesize cyanotoxins. The results of our biodiversity survey, phylogenetic analysis, and genome reconstruction, lead us to conclude that toxin risk is a reality and potential future threat in these reservoirs.

Materials and methods

Study sites and Sampling

This study was conducted on the Cape Verdean Island of Santiago (Fig 1). During sampling, in February 2014, only Poilão (B1), Saquinho (B2) and Faveta (B3) reservoirs had water while Figueira Gorda and Salineiro were empty at the tie of sampling. Other contextual information on Santiago Island's reservoirs is summarized in Table 1.

Fig 1. Map of Cape Verde and location of the reservoirs in Santiago Island sampled in this study.

DMS coordinates of the reservoirs: 1-Poilão (15˚04'25.0''N, 23˚33'25.8''W), 2-Saquinho (15˚08'11.3''N, 23˚42'27.7''W) and 3-Faveta (15˚05'54.5''N, 23˚37'24.1''W). 4-Figueira Gorda (15˚07'5.8''N, 23˚35'36.5''W) and 5-Salineiro (14˚57'03.5''N, 23˚38'00.4''W) reservoirs where empty at the time of sampling.

Table 1. Characteristics of the studied reservoirs in February 2014 (Government of Cape Verde)

Reservoir	Location	Start of impoundment	Theoretical maximum volume (m^3)	At full capacity?
Poilão	São Lourenço dos Orgãos	2006	1,200,000	yes
Saquinho	Santa Catarina	2013	563,000	no
Faveta	São Salvador do Mundo	2013	536,565	yes
Figueira Gorda	Santa Cruz	2014	1,455,272	empty
Salineiro	Cidade Velha	2013	561,464	empty

Triplicate water samples were collected from 0.5 to 1 m depth at each study site in the same sampling area, using a five-liter bucket. Microbial cells were concentrated by filtering the five liters of water through 0.22 µL Sterivex-GV filters (Millipore, Billerica, MA, USA) by vacuum filtration. Filters where kept on ice and in the dark while transported to the lab for DNA extraction.

DNA extraction

Genomic DNA was extracted from the individual filters using PowerWater Sterivex DNA Isolation Kit (MO BIO, USA) following the manufacturer's protocol. The amount of the DNA extracted was later quantified using NanoDrop 1000 spectrophotometer (Thermo-Fisher Scientific, Wilmington, DE, USA) measuring the UV absorption at 260 nm and 280 nm wavelengths.

Bacterial diversity: 16S rRNA gene amplicon sequencing

To assess the bacterial diversity in the Cape Verdean reservoirs, we used the 16S rRNA gene as a marker for biodiversity.

The extracted environmental DNA was amplified using primers targeting the V3 and V4 hypervariable regions of the 16S rRNA gene. PCR amplification of the 464 bp fragments was performed with the general bacterial primer pair 341F/785R [20].

The purified DNA was sequenced on an Illumina MiSeq platform using a 250 bp paired-end DNA library to generate at least 100,000 reads per sample. 16S ribosomal RNA gene amplicon sequencing was performed by Instituto Gulbenkian de Ciência (IGC) Gene Expression Facility.

Taxonomic composition and identification of potentially toxic genus/species

We used the QIIME v1.9.1 workflow [21] for demultiplexing, removing barcodes, quality filtering, clustering, and taxonomy assignment of the reads. Briefly, merging of the paired-end reads was done with a defined minimum overlap of 8 bp and a maximum difference of 20%, followed by quality filtering of the reads using default parameters [22], for each reservoir. Sequences were then clustered at a 97% identity threshold and all clusters with less than 4 sequences were removed thus reducing noisy reads. For each cluster a reference sequence was chosen (the first) and by comparison with Greengenes v13_8 [23], using UCLUST algorithm [24] with default parameters into Operational Taxonomic Units - OTUs. Taxonomy assignments were

147 done with RDP Classifier v2.2 [25]. To assess the consistency of the sampling and the
148 biodiversity present in each sampling site, diversity indices where calculated:
149 number of Observed species, Shannon's index and Simpson's Dominance index [26].
150

151 Graphical representation of the microbial communities' 152 structure detected in the reservoirs

153 Phyloseq package [27] in RStudio 1.1.419 [28] was used to perform a graphical
154 representation of the structure of the microbial community in each of the sampled
155 sites. A complete assignment of the more abundant bacterial genera in our samples,
156 was generated by Kraken running the full Bacterial Genbank Reference sequences
157 [29], excluding the sequences assigned previously as chloroplasts by QIIME.
158

159 Sequencing and assembly of the *Microcystis* cf. *aeruginosa* 160 CV01 genome

161 A whole metagenome sequencing of Faveta reservoir was performed using Illumina
162 MiSeq v3 kit with a 200x coverage. The DNA libraries were created using Nextera
163 DNA Library Preparation Kit, and sequencing was executed by IGC's Gene Expression
164 Facility.
165 The sequenced reads were assembled with SPAdes v3.6.0 [30] using single-cell
166 mode, and k-mers of 21, 33, 55, 77, 99 and 127. The GC content of the assembled
167 metagenome from Faveta reservoir revealed two distinct contributions: one clearly
168 in the range of the GC content typical of *Microcystis aeruginosa* (42-43%) [31] and

169 the other peak, at approximately 63% for other contributors present in the sample,
170 such as several GC rich strains of *Gamma, Alpha,* and *Betaproteobacteria* (S1 Fig).
171 The peak with lower GC was in the range of our target species and most of its reads
172 have high coverage numbers since they belong to the numerically most abundant
173 species. Two different approaches were used to isolate this genomic contribution:
174 one using CONCOCT v0.41 [32] a binning tool that operates based on sequence
175 coverage and composition, and another exploring the high coverage percentage of a
176 single genomic contribution by assembling reads from the raw sequencing data that
177 had coverage above 60x. In both methods, short sequences were filtered out (length
178 < 1,000 bp). Taxonomy of CONCOCT bins was then determined by Kraken [29] using
179 the default Minikraken database. BWA 0.7.16 (33) and Samtools 1.3 [34] were used
180 to map the reads back to isolated genomes while CheckM [35] was used to get
181 metrics on the quality of the isolation method.
182

183 Genome annotation and general features

184 We used QUAST [36] to determine the statistics of the assembly of the *M.* cf.
185 *aeruginosa* CV01 genome. Annotation of the genome was done with PROKKA v1.11
186 [37] and RAST v2.0 [38] using default parameters to identify putative genes (coding
187 and non-coding sequences). Protein function prediction and annotation of the
188 predicted genes was done against KEGG orthologs (KOs) [39] and clusters of
189 orthologs of proteins with Blast2GO [40] and eggNOG v4.5 [41]. Identification of
190 CRISPR repeats, typical in *Cyanobacteria* and in the *Microcystis* genus, was
191 performed with the web server CRISPRFinder [42] and Recognition Tool CRT v1.1

192 [43], considering a minimum of 3 repeat units. The prediction of transmembrane
193 topology and signal peptide sites was done using Phobius [44] and SignalP v4.1 [45].
194 Prediction of transmembrane helices in proteins was accomplished using TMHMM
195 v2.0 [46], prediction of ribosomal RNA subunits was done with RNAmmer v1.2 [47],
196 while tRNA and tmRNA genes prediction was done using ARAGORN v1.2.36 [48]. To
197 evaluate the presence and possible origin of prophage sequences, identification and
198 annotation of these sequences were performed using PHASTER [49].
199

200 Detection of toxin genes in *M.* cf. *aeruginosa* CV01

201 To identify the presence of toxin genes in *M.* cf. *aeruginosa* CV01, we searched the
202 genome to identify protein domains common in toxin gene clusters with Pfam v29.0
203 [50]. Next, we compared these sequences in our genome with putative toxin
204 synthase genes like microcystin, nodularin, cylindrospermopsin, anatoxin-a,
205 saxitoxin, microviridin, aeruginosin and micropeptin, which are known to be present
206 in cyanobacteria. We used the online tool antiSMASH v3.0 [51] to detect the
207 presence of non-ribosomal peptide synthase (NRPS) and/or polyketide synthase
208 (PKS) gene clusters and other domains of natural products typical of cyanobacterial
209 metabolites. Next, by manually curating the sequences that we identified as being
210 part of toxin synthesis process, we reconstructed the gene clusters ensuring the
211 overlap of contig ends.
212

213 *M*. cf. *aeruginosa* CV01 phylogenetic analysis

214 To reconstruct the phylogenetic relationships of *Microcystis* strains we inferred a
215 species tree using a set of 12 publicly available fully sequenced genomes. The species
216 tree was constructed from a concatenation of DNA sequences from a set of seven
217 single copy housekeeping genes present in all genomes (*ftsZ*, *glnA*, *gltX*, *gyrB*, *pgi*,
218 *recA* and *tpi*), using maximum likelihood phylogenies to infer the genetic variation of
219 the 12 genomes. Gene sequences were separately aligned with MAFFT v.7.220 [52]
220 and trimmed using Gblocks v0.91b [53,54]. To infer ML phylogenies we used RaxML
221 v8.1.20 [55], to compute and support the trees, by calculating 1000 non-parametric
222 bootstraps using GAMMA distribution and GTR+I+G model, model that was
223 previously estimated using Prottest [56]. Bayesian phylogenies were inferred with
224 MrBayes v3.2 [57] for 1 million generations using the same model and a discarded
225 burn-in rate of 25% of the initial generations. To investigate the relationship
226 between *M.* cf. *aeruginosa* CV01 and other continental African, tropical, and
227 temperate climate strains, we used the phycocyanin alpha subunit and phycocyanin
228 beta subunit (cpcA-cpcB) intergenic space region of the phycocyanin gene cluster
229 (PC-IGS) for a set of publicly available nucleic sequences from *Microcystis* strains
230 with worldwide distribution. Trees were computed using RaxML (1,000 non-
231 parametric bootstraps). Bayesian phylogenies were inferred with MrBayes for 3
232 million generations. Phylogenies were inferred under a GAMMA distribution and
233 GTR model.
234

235 # Results

236

237 ## Diversity of the microbial communities in the reservoirs

238 We profiled the bacterial biodiversity in three reservoirs of the island of Santiago
239 using 16S rRNA gene amplicon next generation sequencing. This marker gene is
240 universal in bacteria and it is also present in chloroplasts of eukaryotes, which
241 allowed us to detect both bacteria and eukaryotic phytoplankton. For Poilão
242 reservoir we obtained 804,043 reads (two replicates), for Saquinho 1,271,923 reads
243 (three replicates) and Faveta 1,354,273 reads (three replicates), which resulted in
244 315,291, 437,486 and 425,806 16S rRNA reads for Poilão, Saquinho, and Faveta,
245 respectively.
246 Regarding taxonomic distribution, Poilão had the highest number of operational
247 taxonomic units (OTUs) with 1,356 OTUs, followed by Saquinho with 966 and Faveta
248 with 950 different OTUs. The OTUs identified in Poilão reservoir represented 39
249 phyla and included 1.5 % of unassigned sequences. *Proteobacteria* was the
250 numerically most abundant phylum with 76% of all OTUs detected, of which 69.7%
251 were *Gammaproteobacteria,* dominated by the genus *Acinetobacter* (51.6%). The
252 second most abundant phylum was *Bacteroidetes*, representing 4.4% of the OTUs,
253 *Betaproteobacteria* accounted for 3.0% and *Actinobacteria* 2.3%. Cyanobacteria in
254 Poilão, which represented 3.5%, are mainly represented by the *Phormidium* genus
255 accounting for 2.5% of the OTUs. In Poilão reservoir, sequences assigned to green
256 algae (derived from chloroplasts) were 7.5% of the OTUs and approximately equal

257 amounts of *Chlorophyta* and stramenopiles. Main results regarding the composition
258 of microbial communities are shown in Fig 2.
259
260
261 **Fig 2. Composition of the microbe communities surveyed in Cape Verde.** (A-B)
262 Composition of the microbial communities detected in the three sampled Cape
263 Verdean freshwater reservoirs: Poilão-B1, Saquinho-B2 and Faveta-B3. (C)
264 Cyanobacteria communities detected in the surveyed reservoirs.
265
266 In the Saquinho reservoir, 37 phyla were detected and 0.5% of unassigned
267 sequences. The *Proteobacteria* represented 20% of the OTUs, *Bacteroidetes* 5.4%,
268 *Actinobacteria* accounted for 4.1%, and *Verrucomicrobia* 2.4%. *Cyanobacteria* in this
269 reservoir represented only 0.6% of the OTUs, the lowest percentage of the three
270 sampled reservoirs. This site had the highest number of OTUs associated with green
271 algae (63%), distributed between the *Cryptophyta* with 62% and stramenopiles with
272 1% of the identified OTUs (Fig 2A). Blastn of the cryptophyte 16S rRNA sequence
273 revealed a hit for *Cryptomonas curvata* plastid partial ribosomal RNA operon (strain
274 CCAC 0006) with 97% identity.
275 Faveta reservoir had the lowest number of phyla, accounting for 29 distinct phyla
276 and 1.1% of the sequences classified as unknown. The most abundant phylum was
277 *Cyanobacteria* with 58.6% of the OTUs and included two genera known to harbor
278 toxic species: *Microcystis* accounted for 55.7% and *Planktothrix* 2.8% (Fig 2B-C).
279 *Proteobacteria* was the second most abundant phylum with 18.6% assignments;

280 *Bacteroidetes* was next with 7.0% and *Actinobacteria* third with 5.8%: The green
281 algae at this site represented 5.1% of the identified OTUs.
282 In summary, our survey revealed distinct microbial community structures of the
283 three sampled reservoirs, which is confirmed by the beta diversity analysis in Fig 3D.
284
285
286 **Fig 3. – Biodiversity indices per replicate and average.** (A) Number of total observed
287 species, (B) Shannon, (C) Dominance and (D) Principal Coordinates Analysis of Beta
288 diversity by weighted Unifrac. Levels of significance: *$p < 0.05$. Poilão (D1); Saquinho
289 (D2); Faveta (D3).
290
291

292 Toxin producing genera/species

293 We identified several cyanobacterial genera in the three sampled reservoirs, some of
294 which are known from the literature to produce toxins. In Faveta reservoir we
295 identified the presence of cyanobacteria from the *Microcystis* and *Planktothrix*
296 genera, reported to produce cyanotoxins, microcystin and anatoxin-a, as well as
297 other potentially toxic molecules like chlorinated and sulfated variants of
298 aeruginosin, cyanopetolin, microginin, and microviridin [58–60]. Poilão reservoir had
299 smaller relative quantities of cyanobacteria, but more genera were identified (Fig 2
300 and S2 Fig) some of which were potentially toxic like, *Microcystis*, *Phormidium,*
301 *Planktothrix* and *Cylindrospermopsis.* These cyanobacteria are known to produce
302 microcystin, homo and anatoxin-a, saxitoxin, and cylindrospermopsin, as well as

303 other metabolites with different levels of toxicity. In Saquinho reservoir the relative
304 quantity of cyanobacteria was the lowest (0.6%), but even so the most represented
305 genus was the potentially toxic *Microcystis*.
306

307 *M.* cf. *aeruginosa* CV01 genome

308 CONCOCT produced 23 bins, including a bin containing an assembly of *M. aeruginosa*
309 with a 4.7 Mbp genome. According to CheckM with a completeness level of 98.79%
310 with a total of 75.6% of the reads mapping back to its contigs. The SPADES
311 assembled genome was 4.9 Mbp with a completeness level of 99.8% with 84% of the
312 reads mapping back to contig reads from the sequencing effort (S3 Fig and S1 Table).
313 The SPADES assembly was chosen due to its estimated completeness level and total
314 genome size (plotted in a graphical representation in Fig 4), distributed over 262
315 contigs with length over 1,000 bp and an average GC content of 42.3%. From a total
316 of 5,484 genes annotated, we identified 45 RNA genes, of which 42 were tRNA
317 genes, one tmRNA, and one rRNA set (5S/16S/23S), as well as 5051 protein-coding
318 genes and predicted functions for 91% of all genes (Blast2GO). Considering the
319 importance of metabolite transport and transduction of signals through membranes
320 in cyanobacteria, we also searched for and identified 112 genes with signal peptides
321 and 3,443 genes with transmembrane domains. Major statistical attributes of *M.* cf.
322 *aeruginosa* CV01 genome are described in Table 2.
323
324

325 **Fig 4. Representation of the genome of *M.* cf. *aeruginosa* CV01, sequenced from**
326 **the environmental sample of Faveta reservoir.** (A) The shades of blue in the circle
327 are indicative of pairwise genomic sequence similarity according to blastn, using as
328 reference the genome of *M. aeruginosa* NIES-843. The location and structure of
329 three gene clusters of potentially toxin-producing secondary metabolites are
330 represented. (B) The histogram represents the GC content of the assembled *M*. cf.
331 *aeruginosa* CV01 genome.
332
333 **Table 2. Genome statistics for *M.* cf. *aeruginosa* CV01.**

Attribute	Value	% of total
Genome size (bp)	4,918,369	100.0
DNA coding (bp)	3,900,077	79.3
DNA G+C (bp)	2,080,470	42.3
DNA scaffolds	262	-
Total genes	5,484	100.0
Protein coding genes	5,051	92.1
RNA genes	45	0.8
Genes with function prediction	4,996	91.1
Genes assigned to COGs	4,166	76.0
Genes with Pfam domains	3,407	62.1
Genes with signal peptides	112	2.0
Genes with transmembrane helices	3,443	62.8
CRISPR repeats	9	-

334

335

336 Four partial prophage regions were detected in *M.* cf. *aeruginosa* CV01. The first
337 region, with GC content 45.3%, is 9.4 Kb, encodes for 8 proteins and harbors protein
338 coding sequences (CDS) from a previously described infecting phage, P-TIM68,
339 usually associated with *Prochlorococcus* Myoviridae virus that contains photosystem
340 I gene sequences [61] and transposase sequences. A second partial phage region
341 with 12 CDS and GC content of 43.0% spans over 11.8 Kb and contains phage
342 sequences from the *Microcystis* infecting Myoviridae phage MaMv-DC [62].
343 Sequences of this phage and of phage Ma-LMM01 [63] are present in a third region
344 with 7.1 Kb in length, 40.4% GC content and 8 CDS. Finally, the forth prophage region
345 of 5.8 Kb in length, coding for 9 proteins and GC content of 40.6% was detected, and
346 CDS from a ssDNA marine virus reported to infect *Synechococcus* [64] were
347 identified. Regarding CRISPR arrays, the defense mechanism of *Cyanobacteria*
348 [65,66] we detected eight CRISPR repetitive units, varying from 0.3Kb and 11.8Kb in
349 length, with direct repeat lengths from 35 to 38 bp. The CRISPR regions and the
350 prophage were not overlapping but two clusters of CRISPR direct repeats (DR) were
351 identical to the *Microcystis* phage Ma-LMM01 portion, a memory mechanism to
352 their introduction into the CRISPR locus, providing immunity to further infection by
353 that phage. Other characteristics of the *M.* cf. *aeruginosa* CV01 genome, such as
354 KEGG orthologs and COG functional categories, are summarized in S2-S3 Tables.

355

356 **Phylogenetic analysis**

357 Our phylogenetic analysis using seven cyanobacterial genes, as described previously
358 in Materials and methods, identified the dominant cyanobacterium in Faveta
359 reservoir as a *M. aeruginosa* strain, which we named *M.* cf. *aeruginosa* CV01 (Fig
360 5A). *M.* cf. *aeruginosa* CV01 is placed close to two strains collected in African water
361 bodies: *M. aeruginosa* PCC 9443 collected from a fishpond in Landjica, Central
362 African Republic and *M. aeruginosa* PCC 9807 collected in Hartbeespoort Dam in
363 Pretoria, South Africa (Pasteur Culture Collection of Cyanobacteria). Phylogenetic
364 studies using the PC-IGS intergenic spacer region confirmed the clustering of *M.* cf.
365 *aeruginosa* CV01 with other continental African strains, as shown in Fig 5B, in a
366 branch that contains both microcystin producers and non-producers collected from
367 Ugandan and Kenyan water bodies [67].
368
369
370 **Fig 5. Phylogeny of the Cape Verdean strain *M.* cf. *aeruginosa* CV01.** (A) represents
371 the location of *M.* cf. *aeruginosa* CV01 sequenced in this study among a set of fully
372 sequenced genomes of *Microcystis*. The phylogenetic tree was inferred with
373 MrBayes for 1 million generations, using a GAMMA distribution and GTR substitution
374 matrix for a set of 7 housekeeping genes (*ftsZ*, *glnA*, *gltX*, *gyrB*, *pgi*, *recA* and *tpi*).
375 Dots represent posterior probability values higher than 85. (B) A phylogenetic tree of
376 69 *Microcystis* strains from all over the world locating the Cape Verdean strain
377 among other continental African *M. aeruginosa* strains. ML phylogenies were
378 inferred with RaxML with 1,000 bootstraps and Bayesian phylogenies were inferred
379 with MrBayes for 3 million generations, using a GAMMA distribution and GTR
380 substitution matrix for the intergenic spacer region of the phycocyanin cluster PC-IGS

381 region of 69 globally distributed *Microcystis* strains. Dots represent posterior
382 probability values higher than 75. The African strains are represented in blue in (A)
383 and (B).
384

385 ## Toxin genes and toxic species

386 In order to identify the risk of production of toxic cyanobacterial metabolites, we
387 searched the *M.* cf. *aeruginosa* CV01 genome for non-ribosomal peptide synthases
388 (NRPS) and NRPS/polyketyde synthases (PKS) hybrid gene clusters.
389 Genes that contribute to the synthesis of aeruginosin (NRPS/PKS/saccharide),
390 cyanopeptolin (micropeptin) and microviridin molecules were detected. Aeruginosin
391 and cyanopeptolin gene clusters were present and were non-halogenated (*aerJ* and
392 *mcnD* genes where not detected) (Fig 4). Regarding microcystin gene clusters, some
393 genes were present, nevertheless the full gene cluster was not represented.
394 Putative genes for the synthesis of cyanopetide metabolites like spumigin
395 (aeruginosin), aeruginoside (aeruginosin), ambiguine (terpene-alkaloid), and
396 piricyclamide (post-translational modified peptides) were also detected, but not
397 their complete gene clusters. Finally, one unknown NRPS/PKS gene cluster was
398 identified, showing the potential to produce peptide molecules that are yet
399 unknown.
400

401 ## Nucleotide sequence accession numbers

402 The nucleotide sequence data are available at DDBJ/EMBL/GenBank under the
403 accession number SUB2733390.

404

405 # Discussion

406

407 The studied reservoirs show three distinct microbial/microalgae community profiles,
408 despite being located on the same island and in a radius of 15 kilometers from each
409 other: in two sites, bacteria were dominant (*Proteobacteria* and *Cyanobacteria*) and
410 in the other reservoir microalgae belonging to the cryptophytes were the most
411 abundant taxa. The dominant species from one of the reservoirs was identified as a
412 *M. aeruginosa* strain through phylogenetic studies, placing it closer to other strains
413 collected in continental Africa. *Microcystis* spp. were detected in all three reservoirs,
414 as well as other cyanobacteria known to bloom and produce cyanotoxins. Our
415 analysis of the assembled *M.* cf. *aeruginosa* CV01 genome revealed that it can
416 produce toxins and therefore, a potential risk of toxin production can exist in Cape
417 Verdean reservoirs.
418 Our microbial biodiversity survey revealed distinct community structures in the three
419 reservoirs (Fig 3D). In fact, *Proteobacteria* dominated Poilão reservoir, mainly
420 bacteria from the *Acinetobacter* genus, while in Saquinho reservoir microalgae from
421 the *Cryptomonas* genus were dominant, and in Faveta reservoir the cyanobacterial
422 strain *M.* cf. *aeruginosa* CV01 was the most abundant. Despite being located on the
423 same island and having common microbial groups, we found that each phyla's
424 quantitative distribution varied substantially between reservoirs (Fig 2A).
425 Nevertheless, the profiles from the reservoirs identify groups that are common in
426 other water bodies studied worldwide despite the differences in relative quantities

427 as is the case of *Cyanobacteria*, *Cryptophyta*, *Actinobacteria*, *Bacteroidetes*,
428 *Verrucomicrobia*, and the three clades of *Proteobacteria* (Alpha, Beta and Gamma)
429 [14,68]. Actually the microbial profile of Poilão reservoir resembles those of lakes
430 close to urban areas, where *Acinetobacter* is the dominant genus [69].
431 In each reservoir, one genus prevailed with a relative abundance above 50%: in
432 Poilão was this was *Acinetobacter*, in Saquinho it was *Cryptomonas,* and in Faveta
433 the *Microcystis* genus.
434 The analysis of the local diversity indices of the replicates from each reservoir
435 revealed consistency and reinforced the sites' distinct microbial communities (Fig
436 3A-C). The indices also showed that microbial communities presented different
437 dominant genera in each of the reservoirs, as well as abundance of different taxa in
438 all sites as typically found in freshwater bodies around the world [7–10,12].
439 Besides the operational starting date differences between the reservoirs (Table 1)
440 and no physical communication between lakes, abiotic factors specific for each site
441 might be involved in the dominance variations within the microbe communities.
442 The dominance of one cyanobacterial strain in Faveta, allowed us to assemble and
443 fully study its genome, and to identify genes, allowing the reconstruction of toxin
444 pathways and assessing the toxin risk inherent in this specific strain. Exploration of
445 the assembled genome also revealed genomic features in common with other *M.*
446 *aeruginosa* genomes (Table 2). Some phage genes were found integrated in the
447 genome of *M. cf. aeruginosa*. Myoviridae "photosynthetic" freshwater cyanophages
448 (Ma-LMM01 and MaMV-DC) were also found. These genes are thought to play an
449 important role during phage infection by supplementing the host with the
450 production of photosynthesis proteins, a process that can be also beneficial to the

451 host during the infection process as suggested by some authors [61,70,71]. These
452 horizontal gene transfer events are shaping the genome architecture of the
453 *Microcystis* genus, providing a supplementary advantage that can be important
454 during cyanobacteria blooms. A region containing chlorophyll a apoproteins A1 and
455 A2 synthesis genes was also identified, but since these are single copy genes located
456 near transposase sequences in this new genome, they were probably misidentified
457 as having phage origin.

458 We identified four NRPS/PKS gene clusters that could synthesize potentially toxic
459 metabolites: three well-known metabolites (aeruginosin, cyanopeptolin, and
460 microviridin, represented in Fig 4A) and another metabolite from one yet unknown
461 gene cluster. The real toxic potential of these metabolites is difficult to determine, as
462 the actual synthesis pathways are not fully known, so further toxicological screenings
463 need to be performed.

464 Phylogenetic markers placed the Cape Verdean strain among others from freshwater
465 bodies from Africa, albeit Cape Verde being a distant archipelago from the
466 continental Africa. The identification in all reservoirs of other cyanobacterial genera
467 known to be toxin producers like *Phormidium, Planktothrix, and Cylindrospermopsis*,
468 increases the potential risk of toxin production. Other studies in African water bodies
469 have identified these and other potentially cyanotoxin producers, raising the
470 possibility of future occurrence of other cyanobacterial genera in Cape Verdean
471 freshwater reservoirs.

472 Cyanobacterial blooms occur in freshwater reservoirs distributed worldwide where
473 *M. aeruginosa* is one of the most frequently detected species. Actually, many long-
474 term studies have reported toxic blooms in lakes and rivers from Kenya, Uganda,

475 Senegal, Morocco, and South Africa [67,72–77], often dominated by *M. aeruginosa,*
476 as we also detected in the island of Santiago. Moreover a recent review on the
477 occurrence of cyanobacterial blooms in Africa [78] shows that there is limited
478 information from western African countries, including Cape Verde, exposing the
479 need for further studies in countries were water quality is threatened and scarce.
480 Therefore, our study increases the available information on cyanobacterial
481 communities described for the western African region. The scarcity of renewable
482 freshwater resources of archipelagic states like Cape Verde is aggravated by the
483 terrain that favors torrential water flows and strong anthropogenic pressures on the
484 environment leading to eutrophication of its freshwater bodies and increased risk of
485 toxic algal blooms.
486 The main threat and concern from our analysis, was the identification of a bacterial
487 community dominated by *M.* cf. *aeruginosa* CV01, which signals the possibility of
488 toxic blooms in Cape Verdean reservoirs, since exponential growth is typical of this
489 species.
490 The occurrence of blooms and toxin production are potential life-threatening risks to
491 public health, so monitoring plans are very important. The costs involved in these
492 control and containment strategies can be prohibitive especially for low and middle-
493 income countries. Hence, approaches like the one proposed in this work, which
494 enabled the identification of potentially toxic cyanobacterial genera through 16S
495 rRNA gene markers could be an interesting alternative, without the time-consuming
496 and expertise-dependent microscope identification of toxin-producing organisms or
497 mass spectrometry-base identification of toxins. NGS is still not widely available but
498 DNA sampling kits are easy to use and can be sent to sequencing facilities at cost-

499 effective prices. Other more sophisticated technics are possible such as lab on a chip,
500 mass spectrometry or even portable NGS devices, that can be adapted to use our
501 workflow in the field, but if a simple molecular lab is available, a PCR assay could also
502 be efficient to detect the presence of specific putative cyanotoxin genes. These
503 strategies can alert authorities and populations before bloom formation and toxin
504 production.

505 Cyanobacteria are currently being developed and used for bio-production of
506 metabolites and biomass in algal farms, for example in the production of human
507 dietary supplements, fertilizers, animal feed or biodiesel, to name a few uses [79–
508 83]. Cape Verde has little land suitable for agriculture, but it has temperature and
509 light conditions that are speculated to be suitable for simple bioreactors for biomass
510 production using photosynthetic microorganisms like green algae or cyanobacteria.
511 The observation of spontaneous blooms of cyanobacteria in freshwater reservoirs
512 lends credibility to this hypothesis, and opens the way for a new productive industry
513 in the archipelago.

514 The present work identifies the existence of real risk for cyanotoxin production in
515 Cape Verdean freshwater reservoirs. Similar structures are planned, which will also
516 need to be studied and monitored. Future work should include studies on the
517 dynamics of the local microbial communities, as well as characterize how
518 environmental factors are affecting their organization, in order to predict and
519 control the impacts of water impairment and toxin production on public health and
520 on the economy. In this study we made use of many freely available open source
521 tools, which represent, to our knowledge, innovative research strategies in Cape
522 Verde. This study will open the way for further research on microbial biodiversity

and other genomic studies in the archipelago, and raise questions relevant for different areas of research and application.

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Author Contributions

Conceived and designed the experiments: APSA JBPL RBL. Performed the experiments: APSA RBL. Analyzed the data: APSA RBL. Contributed reagents/materials/analysis tools: JBPL RBL. Wrote the paper: APSA JBPL RBL.

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786 # Supporting Information

787

788 **S1 Fig. - The GC content of the raw reads and the final assembled metagenome**
789 **from Faveta reservoir.** GC content of the raw reads and of the assembled genomes
790 in the histograms of panels A and B, respectively.

791

792 **S2 Fig. – Rarefaction curves per number of reads for each reservoir sampled.**
793 Representation based in Chao1, Observed OTUs and Phylogenetic distance - PD
794 whole tree of the 16sRNA gene amplicons of Illumina sequencing. D1-Poilão; D2-
795 Saquinho; D3-Faveta.

796

797 **S3 Fig. – Faveta reservoir.** Degree of completeness and contamination of the *M.*
798 *aeruginosa* bin detected using CONCOCT and assembly produced by SPADES (panel
799 A), Graphical representation of a PCA of clusters based on composition and coverage
800 (bin 19 in yellow). Left Panel C - Histogram of GC distribution of sequences within bin
801 19, Right Panel C - Deviation of the average of the entire assembly, x-axis represents
802 GC and y-axis the size. Left Panel D - Histogram of Manhattan distance between
803 tetranucleotide signature of bin 19 and the entire assembly, Right Panel D -
804 Deviation of the average of the entire assembly, x axis represents GC and y axis the
805 size.

806

807 **S1 Online Fig. – Kraken analyses of taxon distribution in whole genome reads and**
808 **16S rRNA from Faveta reservoir.**

809

810 Supplementary tables legends

811 **S1 Table. Degree of completeness and contamination and percentage of mapped**

812 **reads**

813

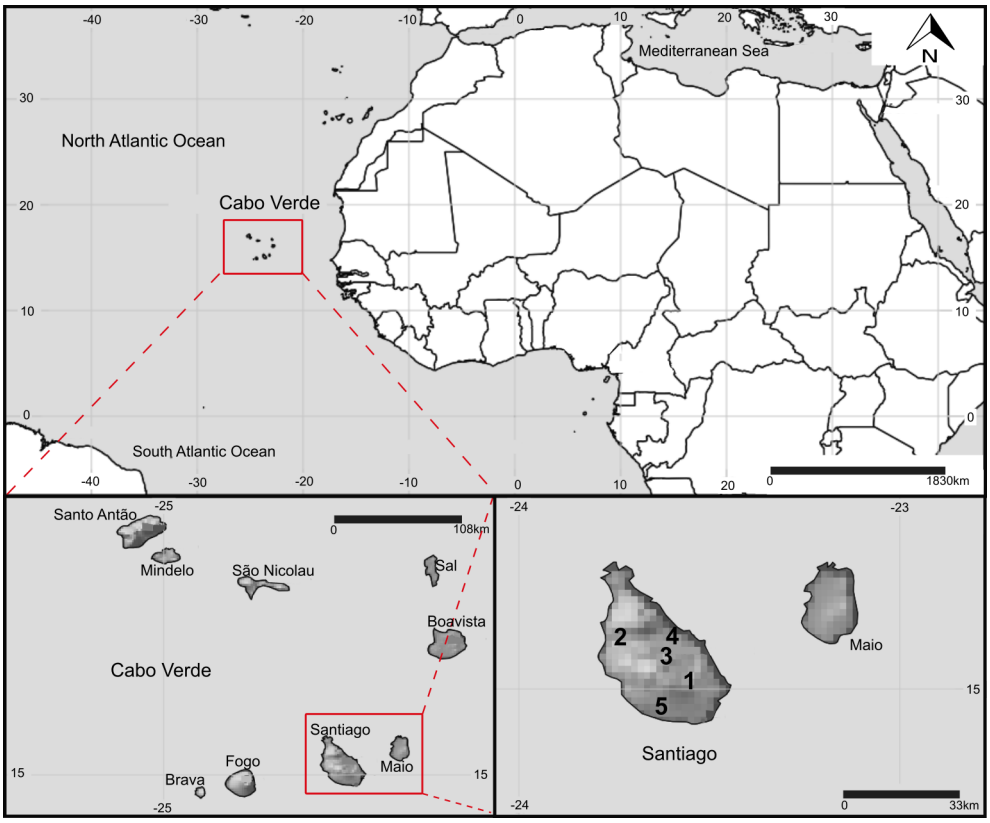
814 **S2 Table. KEGG functional annotation of the predicted transcriptome of *Microcystis***

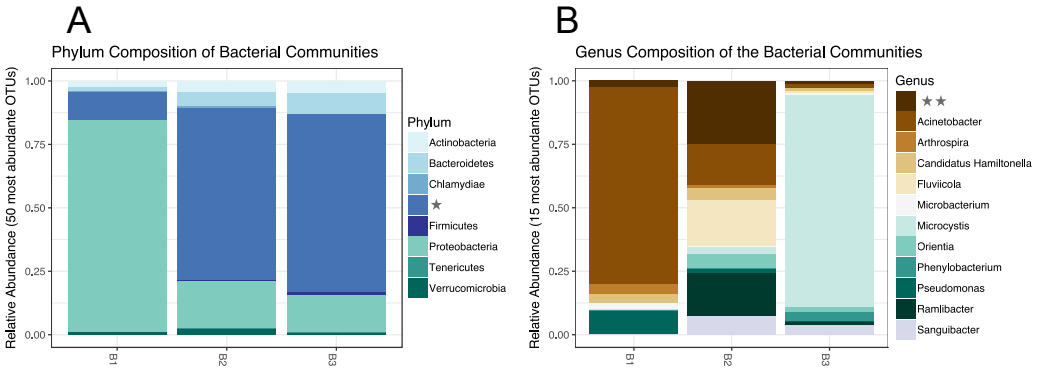
815 **cf. *aeruginosa* CV01**

816

817 **S3 Table. COG functional categories of the predicted transcriptome of *Microcystis***

818 **cf. *aeruginosa* CV01**


North Atlantic Ocean
Cabo Verde
South Atlantic Ocean
Mediterranean Sea
N
1830km
Santo Antão
Mindelo
São Nicolau
Sal
Boavista
Cabo Verde
Santiago
Maio
Fogo
Brava
108km
Santiago
Maio
1
2
3
4
5
33km



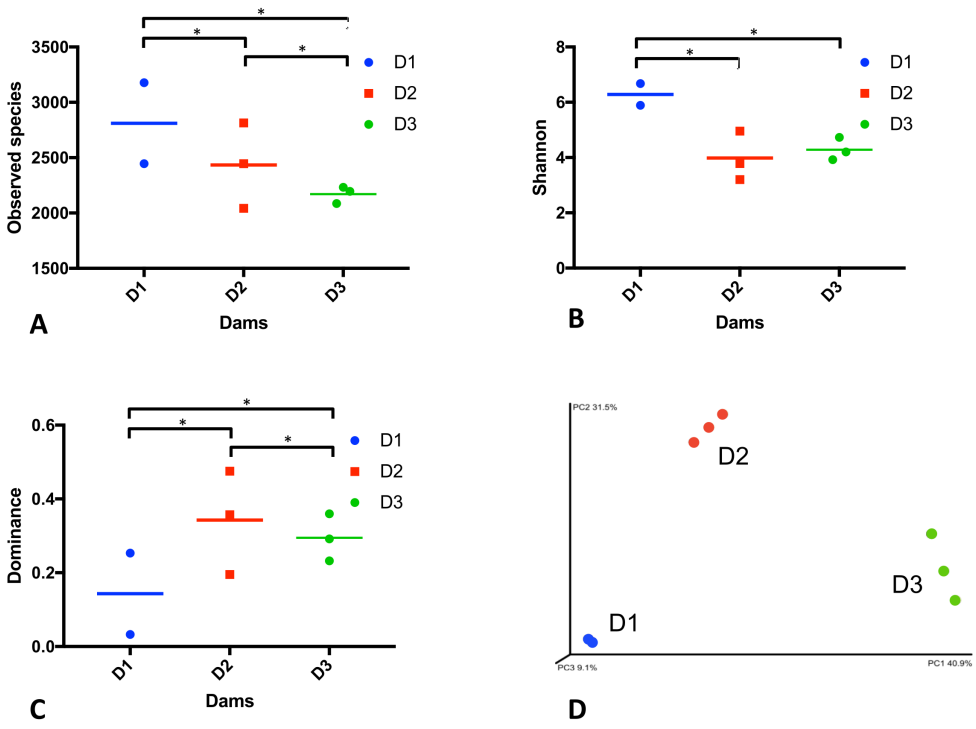
★ The area represents the sum of Chloroplasts (7.5%, 63% and 5.1% per site) and *Cyanobacteria* (3.8%, 0.6% and 58.6% per site)
★★ *Actinobacteria*, *Burkolderiales* and *Rhodobacteriales* with no assigned genus.

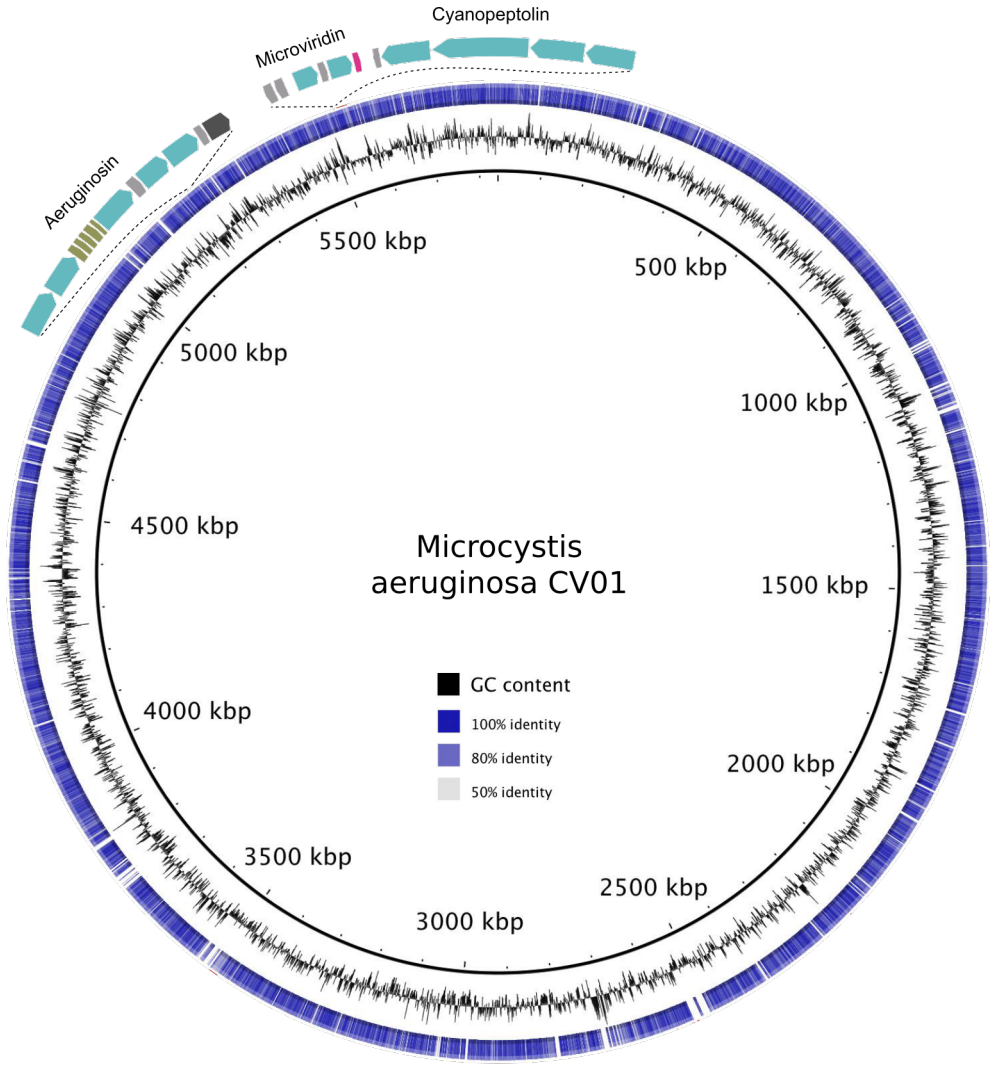
C

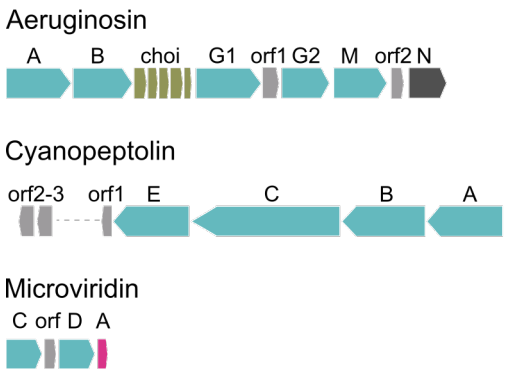
Cyanobacterial communities of the reservoirs

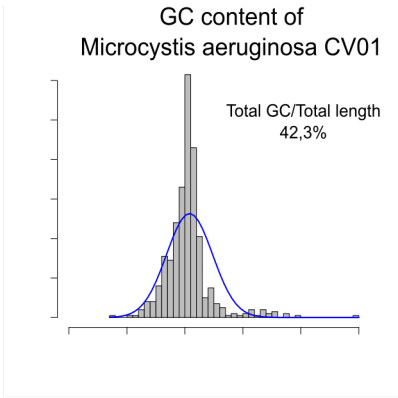
		Poilão	Saquinho	Faveta
Total number of 16S rRNA sequences		315 291	437 486	452 806
Total *Cyanobacteria* (%)		3.8	0.6	58.6
Order	Genus		%	
Chroococcales	***Microcystis***	0.1	0.4	**55.7**
Oscillatoriales	*Planktothrix*	0.1	<0.1	2.8
Oscillatoriales	*Phormidium*	2.5	<0.1	nd
Oscillatoriales	na[1]	<0.1	0.1	0.1
Synechococcales	*Synechococcus*	0.1	<0.1	<0.1
Chroococcales	na[2]	0.1	<0.1	nd
Pseudanabaenales	*Halomicronema*	0.1	nd	nd
Pseudanabaenales	*Leptolyngbya*	0.1	nd	nd
Oscillatoriales	*Planktothricoides*	0.1	nd	nd
Nostocales	*Cylindrospermopsis*	<0.1	nd	nd

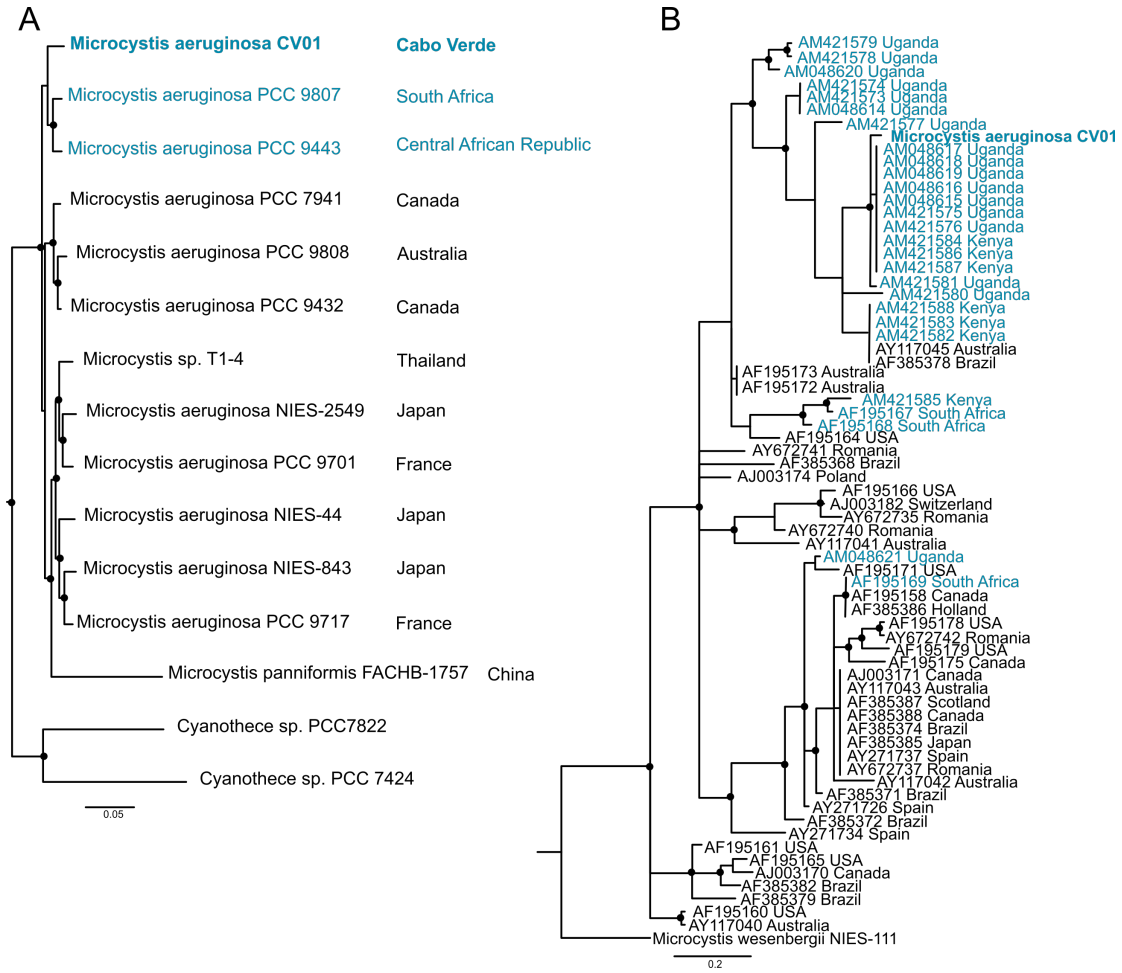
nd: not detected; na[1]: genus not assigned, *Phormidiaceae* Family; na[2]: genus not assigned, *Xenococcaceae* Family.


Observed species
3500
3000
2500
2000
1500
D1
D2
D3
Dams
A
Shannon
8
6
4
2
0
B
Dominance
0.6
0.4
0.2
0.0
C
PC2 31.5%
PC3 9.1%
PC1 40.9%
D


Cyanopeptolin
Microviridin
Aeruginosin
5500 kbp
500 kbp
5000 kbp
1000 kbp
4500 kbp
Microcystis
aeruginosa CV01
1500 kbp
GC content
100% identity
80% identity
50% identity
4000 kbp
2000 kbp
3500 kbp
2500 kbp
3000 kbp


Aeruginosin
A B choi G1 orf1 G2 M orf2 N
Cyanopeptolin
orf2-3 orf1 E C B A
Microviridin
C orf D A


GC content of
Microcystis aeruginosa CV01
Total GC/Total length
42,3%


A
Microcystis aeruginosa CV01
Cabo Verde
Microcystis aeruginosa PCC 9807
South Africa
Microcystis aeruginosa PCC 9443
Central African Republic
Microcystis aeruginosa PCC 7941
Canada
Microcystis aeruginosa PCC 9808
Australia
Microcystis aeruginosa PCC 9432
Canada
Microcystis sp. T1-4
Thailand
Microcystis aeruginosa NIES-2549
Japan
Microcystis aeruginosa PCC 9701
France
Microcystis aeruginosa NIES-44
Japan
Microcystis aeruginosa NIES-843
Japan
Microcystis aeruginosa PCC 9717
France
Microcystis panniformis FACHB-1757
China
Cyanothece sp. PCC7822
Cyanothece sp. PCC 7424
0.05
B
AM421579 Uganda
AM421578 Uganda
AM048620 Uganda
AM421574 Uganda
AM421573 Uganda
AM048614 Uganda
AM421577 Uganda
Microcystis aeruginosa CV01
AM048617 Uganda
AM048618 Uganda
AM048619 Uganda
AM048616 Uganda
AM048615 Uganda
AM421575 Uganda
AM421576 Uganda
AM421584 Kenya
AM421586 Kenya
AM421587 Kenya
AM421581 Uganda
AM421580 Uganda
AM421588 Kenya
AM421583 Kenya
AM421582 Kenya
AY117045 Australia
AF385378 Brazil
AF195173 Australia
AF195172 Australia
AM421585 Kenya
AF195167 South Africa
AF195168 South Africa
AF195164 USA
AY672741 Romania
AF385368 Brazil
AJ003174 Poland
AF195166 USA
AJ003182 Switzerland
AY672735 Romania
AY672740 Romania
AY117041 Australia
AM048621 Uganda
AF195171 USA
AF195169 South Africa
AF195158 Canada
AF385386 Holland
AF195178 USA
AY672742 Romania
AF195179 USA
AF195175 Canada
AJ003171 Canada
AY117043 Australia
AF385387 Scotland
AF385388 Canada
AF385374 Brazil
AF385385 Japan
AY271737 Spain
AY672737 Romania
AY117042 Australia
AF385371 Brazil
AY271726 Spain
AF385372 Brazil
AY271734 Spain
AF195161 USA
AF195165 USA
AJ003170 Canada
AF385382 Brazil
AF385379 Brazil
AF195160 USA
AY117040 Australia
Microcystis wesenbergii NIES-111
0.2