

Harmful Cyanobacterial Bloom Mitigation in the C-43 Canal/ Caloosahatchee River Using BlueGreen Water Technologies (“*BlueGreen*”) *Lake Guard*® Oxy Solution

Amendments 2 & 3 to Agreement No. 35694 Between the SJRWMD and *BlueGreen* US for the Collaboration Between SJRWMD and SFWMD for the Treatment of Harmful Algal Blooms (HABs) in the C-43 Canal/ Caloosahatchee River

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Remediation Project Report

1. Abstract

BlueGreen US Water Technologies (“*BlueGreen*”) was tasked by the South Florida Water Management District (“*SFWMD*”) to oversee and coordinate the mitigation of harmful algal blooms in a defined section of the C-43 Canal/ Caloosahatchee River. The mitigation protocols utilized the *Lake Guard*[®] Oxy, a unique formulation of sodium percarbonate-based algaecide that allows the active ingredient to float and time-release, to treat the cyanobacterial blooms. The *Lake Guard*[®] Oxy used in this project was obtained from the FL Department of Environmental Protection emergency stockpile maintained by *BlueGreen*. The main objective of the project was to mitigate cyanobacterial blooms in the residential canals around the C-43 Canal/ Caloosahatchee River. The residents of these canals had suffered from the adverse effects of algal blooms that include health risks, unpleasant sights, and a foul odor originating from floating rafts of decaying algae. The emergency applications provided an immediate relief for the residents, and the treatment eliminated the cyanobacterial scum and the associated odor, as well as other unpleasant conditions within 24-48 hours. Data indicates that the treatment application reduced significantly, to below threshold advisory levels, the cyanotoxin levels, which have reached high levels, well above threshold levels, in certain areas prior to treatment.

The initial project timeline was scheduled to be 7 days, overlapping with the Memorial Day weekend at the end of May, 2021, but due to the successful deployment and immediate results, the project was extended by another week, to a total of 15 days. The scope of treatments was extended to include all the residential areas along a 48-mile long segment of the C-43/ Caloosahatchee River, from Lake Okeechobee to Fort Myers. During the second week of treatments, *BlueGreen* introduced the *Lake Guard*[®] technology to *SFWMD* inspectors and *SFWMD* licensed contractor, and trained them on the application of the *Lake Guard*[®] Oxy.

Only 5.1 tons of the 20-ton stockpile were used over a 15-day period to remediate and maintain bloom free conditions in residential areas along the 48-mile river segment.

2. Background

BlueGreen US Water Technologies (“*BlueGreen*”), together with the South Florida Water Management District (“*SFWMD*”) and Florida Department of Environmental Protection (“*FL DEP*”) have established a treatment regime for the remediation of the C-43 Canal/ Caloosahatchee River, in order to mitigate cyanobacterial blooms in specific sections of the canal, using *BlueGreen* Water Technologies *Lake Guard® Oxy* solution.

The C-43 Canal/ Caloosahatchee River is a mixed natural and manmade canal network, stretching from the banks of Lake Okeechobee, designated to lead water into the Atlantic Ocean and the Gulf of Mexico. When water in Lake Okeechobee reaches dangerous levels, the U.S. Army Corps of Engineers discharges water through various outlets to release pressure on the infrastructure. With water released, cyanobacterial blooms, prevalent in Lake Okeechobee, often proliferate into Florida’s waterways.

The C-43 canal (Caloosahatchee canal) along with the Caloosahatchee River extends 75 miles west of Lake Okeechobee, and is a major tributary to the Charlotte Harbor. It extends into the Gulf Coast, where it forms the Caloosahatchee Estuary.

The C-43 Canal includes 3 lock-and-dams; S-77 (Moore Haven Lock & Dam), immediately west of Lake Okeechobee’s rim canal, S-78 (Ortona Lock), 15.3 miles west of S-77, and S-79 (WP Franklin Lock and Dam), 27 miles west of S-78 (Fig. 1).

On May & June, 2021, the gates at S-77, S-78, and S-79 structures were open, and contaminated waters from Lake Okeechobee were free-flowing through the C-43 canal and into the Caloosahatchee estuary. This led to heavy blooms being introduced into the canal, in the vicinity of residential areas that suffered from the unpleasant sights and odor that accompanied the blooms.

3. Objective

The initial objective and scope of this emergency project was to handle, contain and eliminate ongoing cyanobacterial blooms in the Caloosahatchee River segment downstream from the S-79 structure. The objective and scope of treatment were redefined on Thursday, May 27, during the *SFWMD* executive management visit, to focus on residential canals off of the Caloosahatchee River with visible cyanobacterial blooms and foul odor. The redefined objective was meant to immediately alleviate the residents’ predicament, and to preserve the *Lake Guard® Oxy* product for future emergency applications. The water salinity increases down the Caloosahatchee River, west of the S-79 structure, to the point of unfavorable conditions for cyanobacterial growth and survival. The scope of treatment expanded to

beyond the residential canals west of the S-79 structure, to the residential areas east of the S-79 structure, as far as the S-77 structure.

4. Executive Summary

This remediation project objective was to handle, contain, and eliminate ongoing cyanobacterial blooms in residential areas that span along a 48-mile length segment of the C-43 Canal/Caloosahatchee River, from the S-77 (Moore Haven Lock & Dam) to Fort Myers, in order to minimize their impact on health and quality of life

BlueGreen's technical experts together with Modica and Associates (*Modica*), licensed applicators, have assessed the C-43 Canal and its adjacent waterways in order to establish a proper mitigation protocol for the treatment regimen designed to target cyanobacterial outbreaks in progress, as well as to mitigate the developing conditions that may lead to such outbreaks in residential canals. Treatment was conducted with the *Lake Guard® Oxy* product, approved by the U.S. EPA, certified by the NSF/ANSI/CAN-60 for treatment in drinking water, and registered with the Florida Department of Agriculture and Consumer Services.

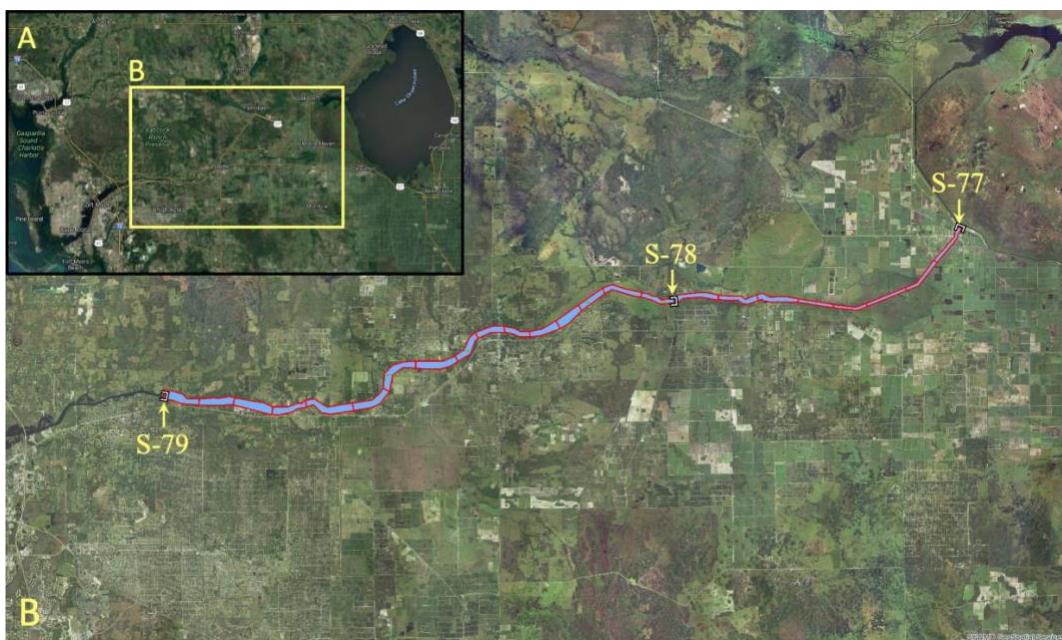


Figure 1. (A) A section of South Florida depicting Lake Okeechobee and the Gulf Coast. The area enclosed in the yellow box includes the 48-mile segment of C-43 Canal/ Caloosahatchee River area that was monitored and treated. (B) The 48-mile segment of the C-43 Canal/Caloosahatchee River where all residential areas were monitored and treated. The segment extends from the S-77 structure all the way to Fort Myers, downstream from the S-79 structure. Lock and Dam structures S-77, S-78, and S-79 are marked along the path of the canal. This image of the C-43 Canal/ Caloosahatchee River is courtesy of SFWMD GioSpatial Services.

Over a 15-day period, *BlueGreen* inspected hundreds of residential canals and treated/ retreated a total of 56 sites along the 48-mile segment of the C-43 Canal/ Caloosahatchee River, resulting in fast bloom mitigation that provided immediate relief to the residents that suffered from heavy blooms near their homes, and the associated foul odors and potential health risks. After the interventional treatments, and despite heavy bloom conditions in the C-43 Canal/ Caloosahatchee River, *BlueGreen* was able to maintain bloom free conditions in the residential areas along the C-43 Canal/ Caloosahatchee River with minimal dose rates.

Summary of the treatment applications over the 15-day project:

- Total cumulative Lake Guard Oxy applied: **10,260 lb (5.1 tons)**
- Total treatment and retreatment applications: **112**
- Total number of residential areas treated: **56**
- Covered length along the C-43/ Caloosahatchee River: **48 miles**

During this project, *BlueGreen* trained several inspectors from *SFWMD*, as well as a *SFWMD* subcontractor on treatment applications with the *Lake Guard®* Oxy.

This project demonstrated the efficacy and safety of the *Lake Guard®* technology in treating heavy cyanobacterial blooms and maintaining bloom free conditions in residential areas. The technology and its application could be deployed quickly, with immediate results. Staging for this operation took less than a day after the *SFWMD* secured a permit from the U.S. Army Corps of Engineers to stage the operation within its facilities and treat areas under their jurisdiction. Treatment results were evident within 24-48 post applications, namely, the removal of the cyanobacterial scum, and the elimination of the accompanied foul odor.

Notably, in certain areas, where cyanotoxin levels were well above the advisory thresholds prior to treatment, the cyanotoxin levels were reduced significantly, to below advisory thresholds, after 24-48 hours from treatment.

Anecdotal evidence indicated that other unpleasant conditions in the residential areas were remediated as well beside the unwanted odor, e.g., the presence of flies that thrived on the decomposing floating scum.

SFWMD role in coordinating and facilitating the treatment applications with other Florida State agencies and the U.S. Army Corps of Engineers was critical in the immediate staging of this project and its ultimate success.

The efficacy and safety of the technology was demonstrated during heavy bloom conditions, however, *BlueGreen* recommends continuous preventative treatments to be conducted during early stages of evolving cyanobacterial outbreaks, which require lower doses of the active ingredient. The early treatments are designed to eliminate cyanobacteria before the inevitable build-up of cyanotoxins during bloom episodes, thus securing a safe cleanup of the toxic species.

Treating the source, Lake Okeechobee, would prevent these cyanobacterial bloom episodes from recurring in Florida waterways. With proper planning, the *Lake Guard®* technology is scalable to treat HABs in Lake Okeechobee. As a first step, remediating a significant section of the Lake near the C-43 and C44 canals, will prevent contaminated waters from being released into these canals.

5. Staging

On Wednesday afternoon, May 26, 2021, 8 hours after *SFWMD* had secured a permit from the U.S. Army Corps of Engineers to allow *BlueGreen/ Modica* to stage an operational post at the W.P. Franklin South Recreation Area, *BlueGreen/ Modica* staging became operational and ready for monitoring and treatment applications. Monitoring, inspection, and treatment application started on Thursday, May 27, and continued daily through the Memorial Day weekend until Thursday June 10, 2021, a total of 15 days.

The initial amount of the *Lake Guard® Oxy* that was shipped to the operation site on Wednesday 26, 2021 was 9 tons, and two days later, another 18 tons of the *Lake Guard® Oxy* arrived at the operation site. The product was stored and secured in 4 containers.

BlueGreen and *Modica* field team consisted of 12 individuals, including 3 PhDs and 2 licensed applicators. *BlueGreen* also operated an offsite support team in Israel, of 5 individuals that included another 3 PhDs.

Modica secured a 24-hour staffing & monitoring of the staging area for 16 days.

Field operations started at 6:30 AM every day and lasted until after 8 PM, consisting of monitoring, inspection and treatment application over a 48-mile segment of the C-43 Canal/ Caloosahatchee River.

Over 2,300 hours were invested in this project, with approximately a third of the total hours (5/15 days) were holiday and weekend hours.

A total of 15 daily reports that detailed the operation and application treatments of each day were sent every night to *SFWMD*.

The staging site included the following:

- 27 tons of the *Lake Guard*[®] Oxy
- 4 x 20-ft containers
- A tent that served as headquarters
- Camper / “mobile staging area office”
- A Genie 1056 telehandler forklift
- A 31’ Ameracat boat with tower, chart plotter with GPS tracker, 4kw radar, 1kw sonar w/ side scan, depth finder, water temperature sensor
- A pontoon boat with 400gal silo application setup
- Additional 1,100 gal silo
- 3 x trucks
- 4 x SUVs

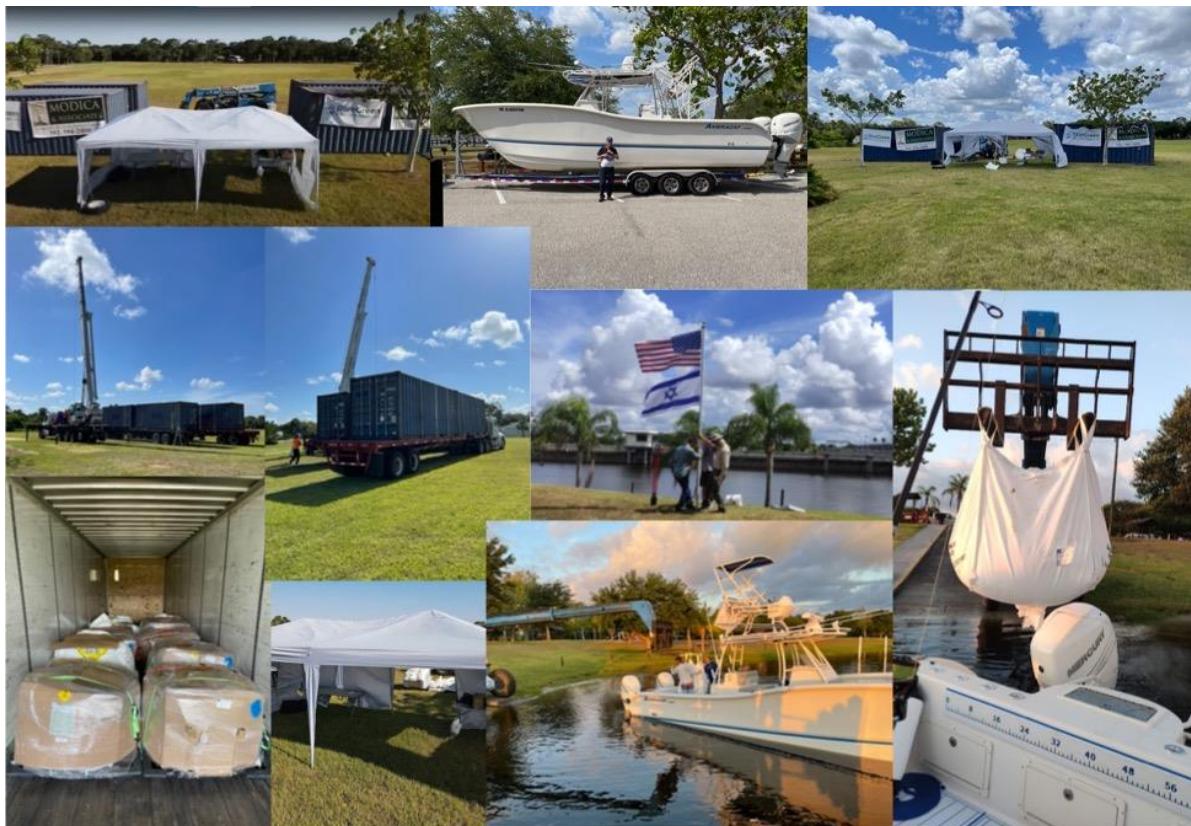


Figure 2. Staging at the W. P. Franklin South Recreational Area. Wednesday, May 26 – Friday June 11, 2021. As part of the great effort to mitigate the blooms occurring throughout the C-43 Canal in a fast and efficient manner, *BlueGreen/Modica* have conducted an extensive remediation project, in which 56 different sites were sampled and treated with 112 separate treatments and retreatments, using a total amount of 10,260 lb (5.1 tons) of the *Lake Guard*[®] Oxy product. In order to achieve this highly ambitious goal within only two weeks, *BlueGreen/Modica* labored around the clock. A campsite and a command post were established in the field in order to assist with the extensive sample collection, the logistical issues, and handing the equipment.



Figure 3. Treatment applications in residential areas along a 48-mile segment of the C-43/ Caloosahatchee River. Thursday, May 27 – Thursday, June 10, 2021.

6. Treatment

The *Lake Guard®* Oxy product is a targeted treatment against cyanobacteria, selectively eliminating and preventing toxic algal blooms by triggering a biological programmed cell death within the cyanobacterial communities, resulting in a population-wide collapse of the cyanobacteria. The product is a granular material composed of 98% (w/w) sodium percarbonate that releases hydrogen peroxide (H_2O_2), and 2% (w/w) of an inert, biodegradable encapsulating agent that floats and time-releases the active ingredient on the water surface, homing-in on cyanobacterial aggregates as they drift in the water. The product can be applied manually from the shore, a boat, or a plane, and is scalable to large water bodies.

In general, the application rates for the *Lake Guard®* Oxy range between 0.5-30 lb/acre, depending on the intensity of the bloom and other conditions in the water. The lower doses are reserved for preventative treatments, for example, the application rates in Lake Minneola (1,890 acres) ranged between 0.5-7.5 lb/acre, with an average rate of approximately 1 lb/acre.

Most of the residential areas around the C-43/ Caloosahatchee River were heavily contaminated with cyanobacterial scum, with an estimated cyanobacterial cell count between 10^6 - 10^9 cells/ml, often times at the upper limit of that range. Due to the urgent nature of this project that overlapped with the Memorial Day weekend, and the limited time available to remediate residential canals over a river segment of 48 miles, *BlueGreen* increased its usual dosage rates to ensure immediate relief for the residents. The average dosage rate over the 15-day project was about 40 lb/acre, with a range of 0.8 – 181.8 lb/acre. The larger dose rates were not anywhere near the maximum dosage rate of 294 lb/acre approved by the label. Due to heavy boating activity on weekends, the Army Corps of Engineers permit limited the treatment applications between 6:30-8:30 AM, and between 5-8 PM during the Memorial Day Weekend, and on the weekend that followed.

The total amount of the Lake Guard® Oxy product used for the full course of the remediation project was 10,260 lb (Fig. 4), applied over 112 different treatments and retreatments, at 56 different residential sites. The amount of product required for maintaining bloom free conditions in the residential canals declined substantially after the initial interventional treatments (Fig. 4).

Lake Guard® Oxy use for daily treatments

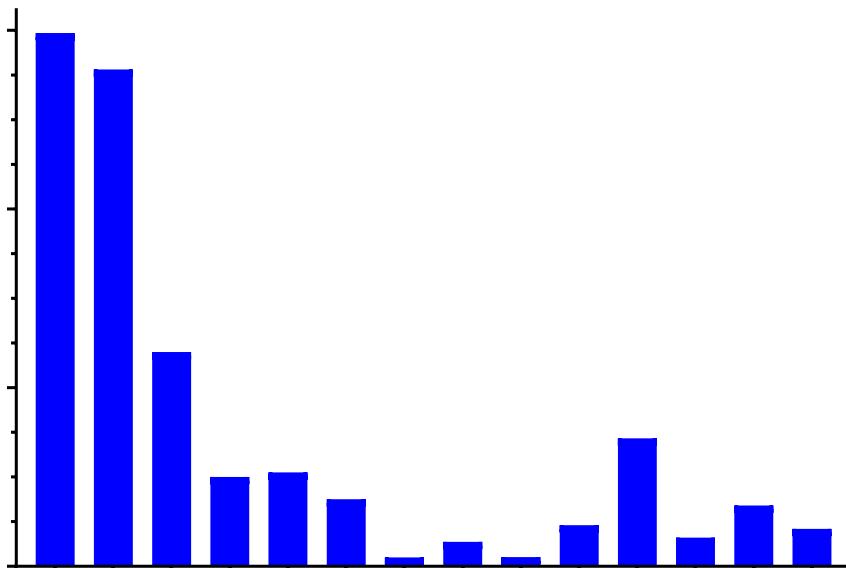


Figure 4. Daily amounts of *Lake Guard®* Oxy product used in the course of the 15-day treatment application, along the 48-mile segment of the C-43/ Caloosahatchee River. Most of the product was used during the interventional period, at the early stages of the project. Maintaining bloom-free canals after the interventional treatment and expanding to new areas required lower application rates.

7. Sampling

Monitoring is key to follow bloom progression, assess the quality of the treatment, and adapt the treatment as needed to optimize its results. Due to the nature of this project, most of the monitoring consisted of visual inspection of the residential canals, but additional extensive monitoring took place in selected areas, which included the following parameters: in-situ field water measurements with a YSI ProDSS multiparameter; Sampling of water temperature, pH, dissolved oxygen (DO), chlorophyll-b (Chl-b; proxy to the total biomass of green algae), phycocyanin (PC, proxy to the total biomass of cyanobacteria), and conductivity; Field microscopy of samples obtained from the water surface, for detection of the variety of cyanobacterial species present in the water; Laboratory work to include taxa analysis and presence of toxins.

8. Results

In total, *BlueGreen/ Modica* inspected, treated and maintained residential canals over a 48-mile long river segment, conducting a total amount of 112 treatments/ retreatments at 56 different sites.

The treatments provided immediate relief to the residents that suffered from heavy blooms near their homes and a strong foul odor that accompanied the blooms. Total application rates, maps, and pictures from each of the 56 sites are detailed in Appendix A.

Results of the daily measurements of toxins in samples A, B, C, 2, 3, 4, 7, and 8, located around the W.P. Franklin Lock & Dam (Fig. 5), are presented in Table 1. While saxitoxins values exhibit no clear trend, a general trend of decrease in toxin levels is demonstrated for microcystins / nodularins. This trend is also observed in Fig. 6, where microcystins / nodularins in sampling spots A, B, and C exhibit a clear decline following treatment with the *Lake Guard® Oxy* product.

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W.P Franklin Lock and Dam (S-79)

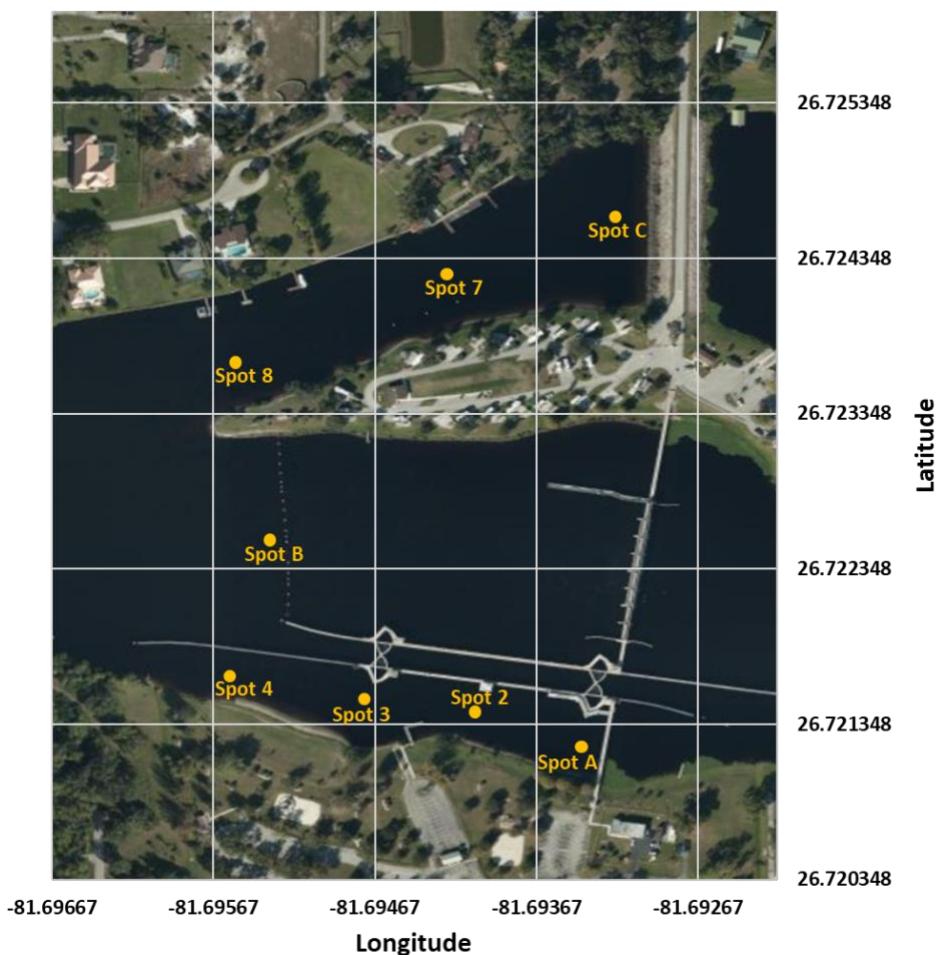


Figure 5. Map of the W.P. Franklin Lock and Dam (S-79) and the sampling points along the S-79 structure. Spots A, B, C, 2, 3, 4, 7, and 8 were chosen as daily sampling points for in situ field water measurements, microscopy, visual inspections, and lab measurements of algal taxa and toxins. Potentially Toxicogenic (PTOX) cyanobacteria species *Aphanizomenon/ Chrysosporum / Sphaerospermopsis sp.*, *Cuspidothrix sp.*, *Dolichospermum sp.*, *Microcystis sp.*, *Planktothrix sp.*, *Pseudanabaena sp.*, and *Raphidiopsis sp.*, detected in sampling spots A, B, C, 2, 3, 4, 7, and 8, exhibited a substantial decrease, following treatment with the *Lake Guard® Oxy* (Fig. 7).

Table 1. The cyanobacteria- associated toxins, microcystins / nodularins and saxitoxins concentrations (ng/mL), found in samples A, B, C, 2, 3, 4, 7, and 8, located at the S-79 structure, during May 2021.

Spot A	microcystins / nodularins	saxitoxins	Spot 2	microcystins / nodularins	saxitoxins
28 May	8.2	0.08	28 May	2.33	0.11
29 May	7.94	0.09	29 May	3.59	0.07
30 May	0.27	0.1	30 May	0.8	0.1
31 May	0.48	0.11	31 May	2.02	0.1

Spot 3	microcystins / nodularins	saxitoxins
28 May	2.44	0.09
29 May	0.99	0.09
30 May	0.8	0.1
31 May	0.99	0.09

Spot 4	microcystins / nodularins	saxitoxins
28 May	0.9	0.09
29 May	1.01	0.1
30 May	2.91	0.1
31 May	0.7	0.1

Spot B	microcystins / nodularins	saxitoxins
28 May	0.72-+0.2	0.09
29 May	1.54	0.1
30 May	1.85	0.11
31 May	0.54	0.09

Spot C	microcystins / nodularins	saxitoxins
28 May	---	---
29 May	310	0.07
30 May	1.76	0.09
31 May	0.53	0.1

Spot 7	microcystins / nodularins	saxitoxins
28 May	---	---
29 May	1.55	0.09
30 May	0.7	0.08
31 May	0.67	0.1

Spot 8	microcystins / nodularins	saxitoxins
28 May	---	---
29 May	1.46	0.09
30 May	0.73	0.08
31 May	4.08	0.08

Images of selected species of Potentially Toxicogenic Cyanobacteria found in samples collected at spots 1, 4, and 7 are presented in Fig. 8.

Fig. 9 summarizes all in-situ digital measurements taken around the W.P. Franklin Lock & Dam. Water temperatures fluctuated around ~28°C (Fig. 9A); Conductivity levels gradually increased from ~870 µS/cm to ~1100 µS/cm in most sampling spots, however, some remained unchanged (Fig. 9B). Dissolved oxygen levels remained steady during the sampling periods in all spots, at values between ~80% and 100% (Fig. 9C). pH levels varied between 8 and 8.5 (Fig. 9D). Chlorophyll levels were maintained at ~10- 20 µg/L (Fig. 9E). Phycocyanin (PC) levels averaged around 3 µg/L (Fig. 9F).

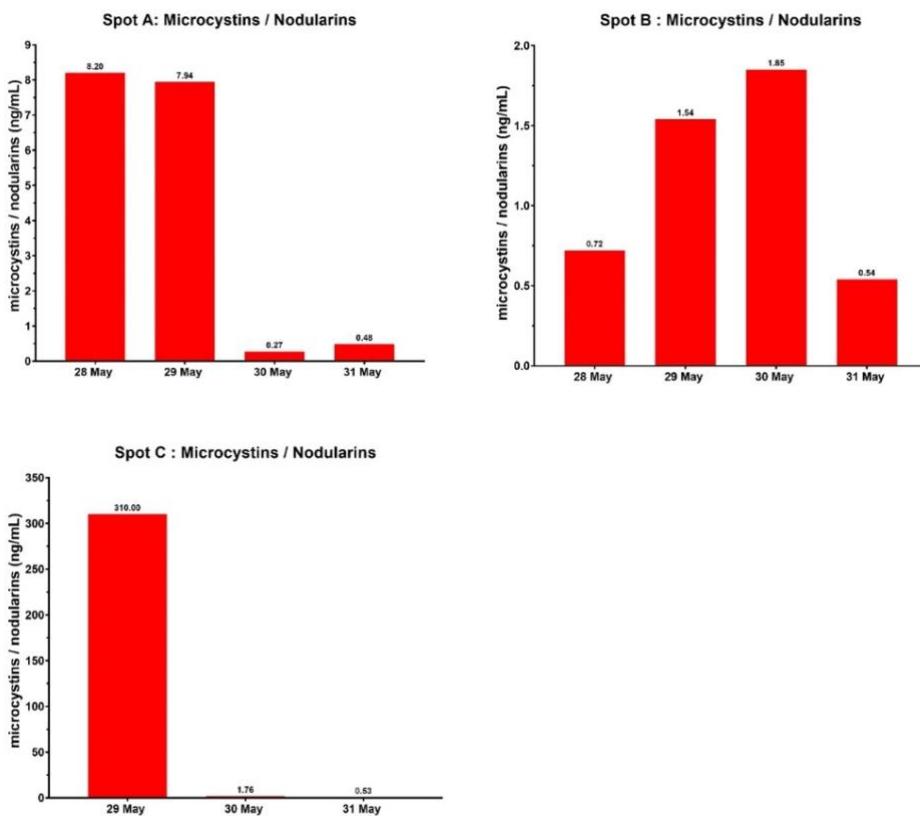


Figure 6. Microcystins / nodularins found in samples A, B, and C, located at the S-79 structure (Fig. 5), during May 2021.

Appendix B includes all the lab reports for samples collected around the W.P Franklin Lock & Dam.

The reports include PTOX screen and cyanotoxin levels.

The name and ID of few of the water samples in this report were changed for simplicity reasons, and to avoid confusion from the ID that appears in the lab report (Appendix B). Below are the changes to the naming of these samples.

Sample 1 in the lab report, Appendix B, was renamed to Spot A in this report.

Sample 2 in the lab report, Appendix B, remained as Spot 2.

Sample 3 in the lab report, Appendix B, remained as Spot 3.

Sample 4 in the lab report, Appendix B, remained as Spot 4.

Sample 5 in the lab report, Appendix B, was renamed to Spot B in this report.

Sample 6 in the lab report, Appendix B, was renamed to Spot C in this report.

Sample 7 in the lab report, Appendix B, remained as Spot 7.

Sample 8 in the lab report, Appendix B, remained as Spot 8.

Sample 9 in the lab report, Appendix B, is the water sample from the treatment site #1, the farthest point to the west, Table 2.

Sample 10 in the lab report, Appendix B, is a sample point on the east side of the W.P. Franklin Lock & Dam, point 33 (Fig.) 11.

Sample 12 in the lab report, Appendix B, is a sample point on the east side of the W.P. Franklin Lock & Dam, point 33 (Fig.) 11.

Potentially Toxigenic (PTOX) Cyanobacteria

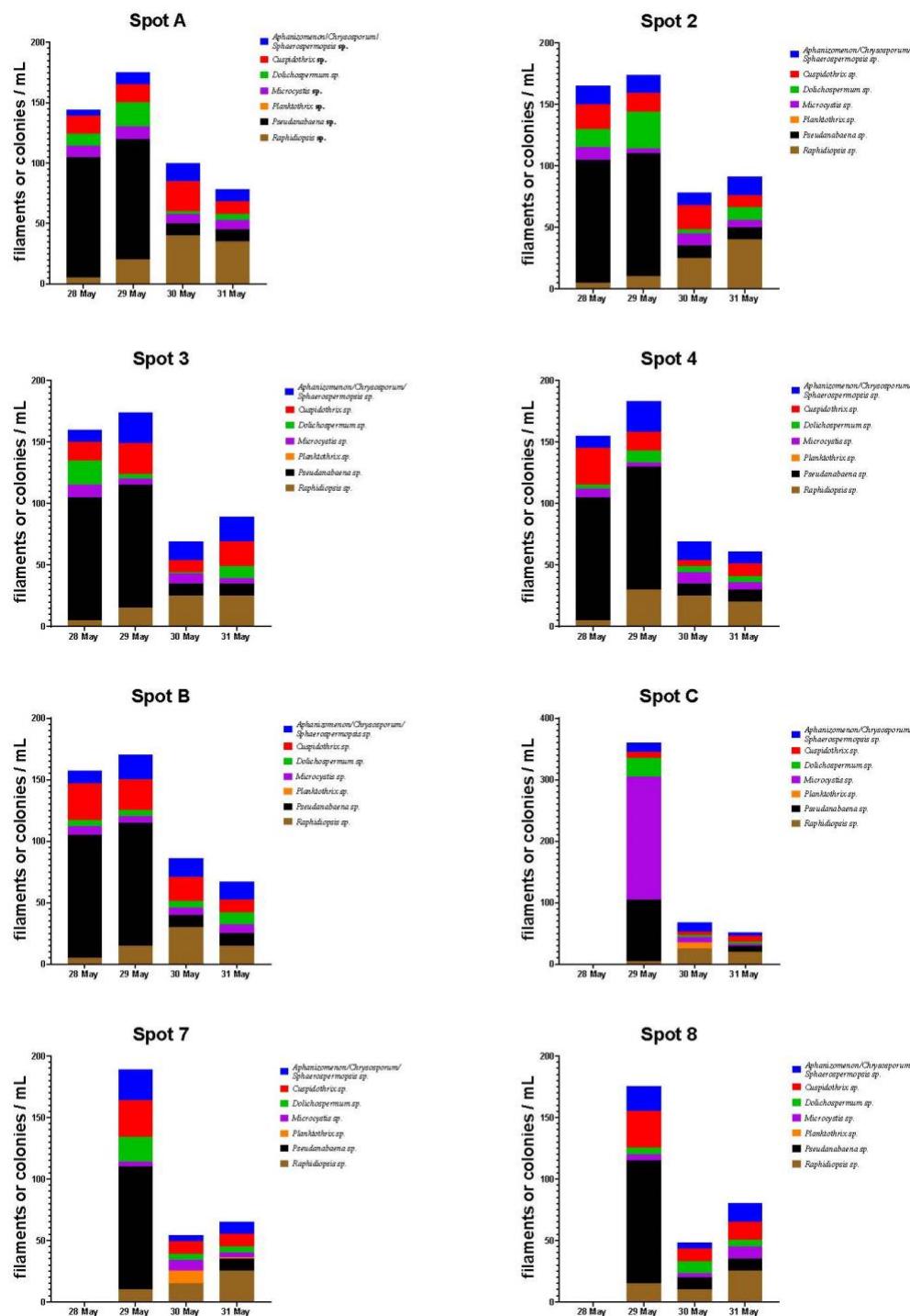
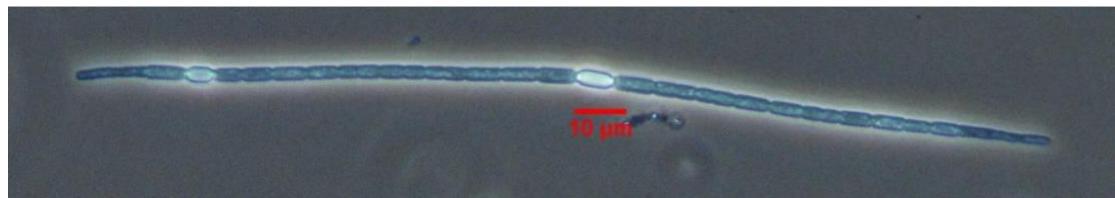


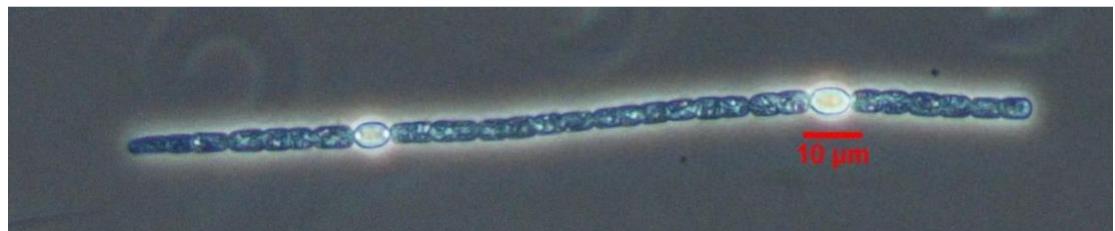
Figure 7. Potentially Toxigenic (PTOX) cyanobacteria filaments/ colonies (per mL), found in samples A, B, C, 2, 3, 4, 7, and 8, during May 2021. The following species were detected: *Aphanizomenon/Chrysosporum* / *Sphaerospermopsis* sp., *Cuspidothrix* sp., *Dolichospermum* sp., *Microcystis* sp., *Planktothrix* sp., *Pseudanabaena* sp., and *Raphidiopsis* sp.



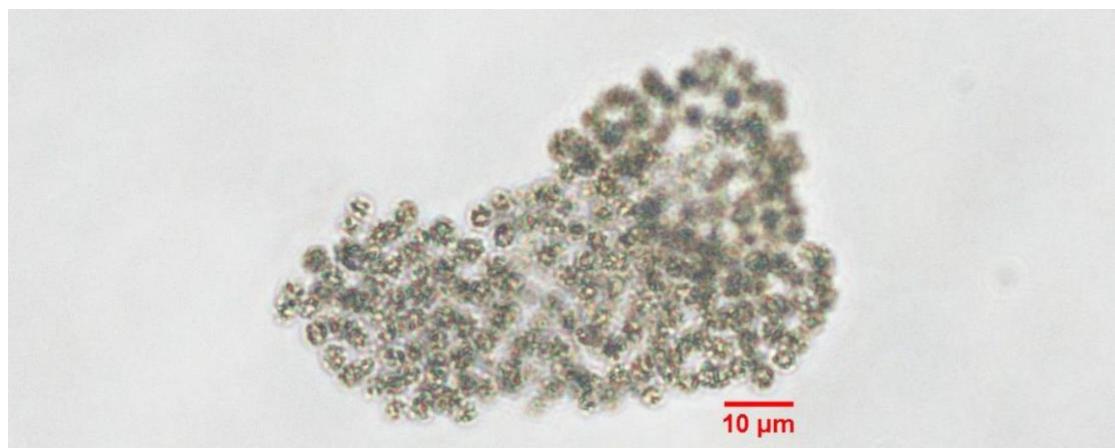
Raphidiopsis sp. (straight) at 400X (Spot 1)



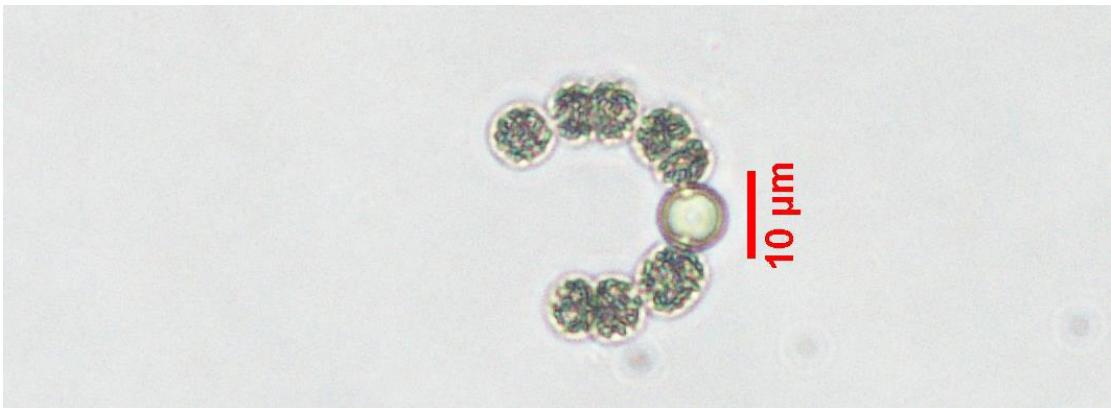
Cuspidothrix sp. at 400X (Spot 1)



Aphanizomenon/Chrysosporum/Sphaerospermopsis sp. at 400X (Spot 1)



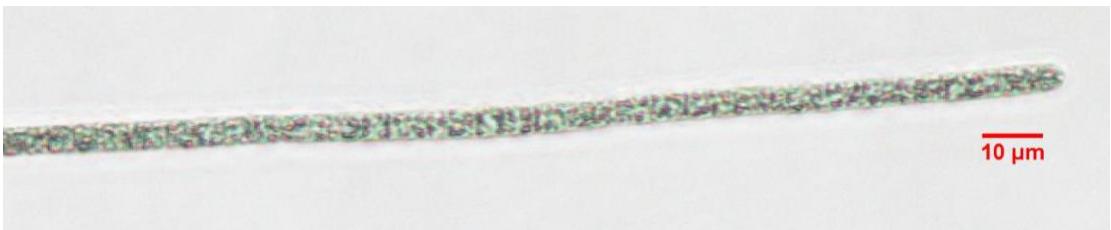
Microcystis sp. at 400X (Spot 1)



Dolichospermum sp. at 400X (Spot 1)



Pseudanabaena sp. at 400X (Spot 4)



Planktothrix sp. at 400X (Spot 7)

Fig. 8. Selected micrographs of various species of Potentially Toxicogenic Cyanobacteria (PTOX) found in samples collected at spots 1, 4, and 7.

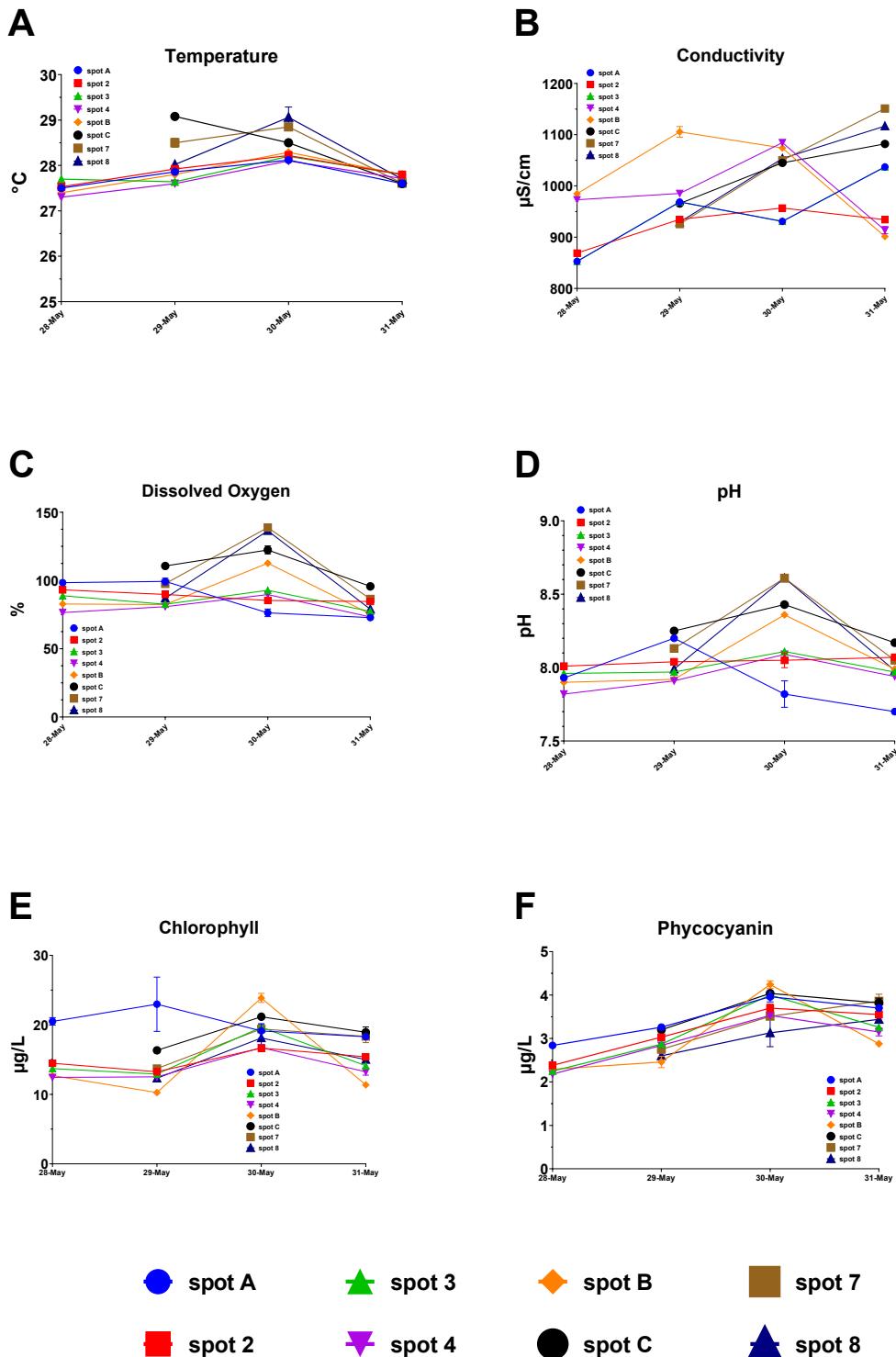


Figure 9. In-situ field measurements obtained with the YSI ProDSS multimeter during May 2021. (A) water temperature ($^{\circ}\text{C}$), (B) conductivity ($\mu\text{S}/\text{cm}$), (C) dissolved oxygen (%), (D) pH, (E) chlorophyll ($\mu\text{g/L}$), and (F) phycocyanin levels ($\mu\text{g/L}$, as a proxy for total cyanobacteria).

Measurements west of the S-79 lock and dam showed that the conductivity values increase along the Caloosahatchee river, west of the S-79 structure, with a gradient across the S-79 lock & Dam (Fig. 10; Table 2)

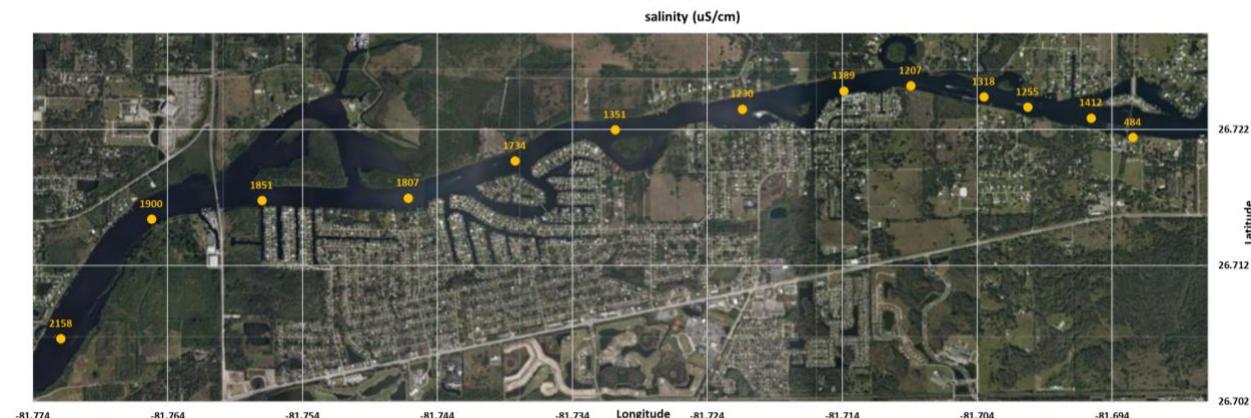


Figure 10. Data points across a 5.5-mile segment on the Caloosahatchee River, from the S-79 structure and downstream, where YSI ProDSS measurements were taken on May 28, 2021. Numbers indicate conductivity in $\mu\text{S}/\text{cm}$. Conductivity increases along the path downstream from the S-79 structure, with a gradient across the S-79 structure; 484 $\mu\text{S}/\text{cm}$ on the east side, and 1412 $\mu\text{S}/\text{cm}$ on the west side.

Table 2: Water parameter values on the Caloosahatchee River, from the S-79 structure to Fort Myers, a 5.5 mile segment. YSI-ProDSS field measurements from sampling points indicated in Fig. 10, May 28, 2021. Point 1 is the farthest point to the west. Point 13 is located on the east side of the S-79 structure. Temperature ($^{\circ}\text{C}$), dissolved oxygen (DO %), conductivity (C- $\mu\text{S}/\text{cm}$), pH, chlorophyll (Chl $\mu\text{g}/\text{L}$), phycocyanin (PC $\mu\text{g}/\text{L}$), and latitude and longitude are presented. Note, the numbering refers to Fig. 10, and is different sample points than those in Fig. 5.

Point	$^{\circ}\text{C}$	DO %	C- $\mu\text{S}/\text{cm}$	pH	Chl ug/L	PC ug/L	Lat	Lon
1	29.30	139%	2158	8.41	14.72	1.42	26.70633	-81.77222
2	29.90	149%	1900	8.50	14.07	1.53	26.71513	-81.76551
3	29.40	142%	1851	8.46	16.92	2.45	26.71649	-81.75734
4	30.05	162%	1807	8.63	17.75	2.30	26.71667	-81.74649
5	30.30	180%	1734	8.76	24.82	3.13	26.71939	-81.73857
6	29.90	156%	1351	8.63	20.04	2.82	26.72169	-81.73114
7	29.90	158%	1230	8.64	18.93	3.10	26.72320	-81.72172
8	29.81	150%	1189	8.58	17.88	2.95	26.72454	-81.71420
9	29.80	149%	1207	8.56	17.45	3.23	26.72493	-81.70924
10	29.38	133%	1318	8.44	16.90	3.06	26.72411	-81.70382
11	29.41	141%	1255	8.49	18.19	3.22	26.72336	-81.70057
12	28.85	119%	1412	8.30	17.08	3.03	26.72255	-81.69586
13	30.30	139%	484	8.58	16.66	2.79	26.72112	-81.69278

Since the Army Corps of Engineers permit limited the treatment applications to between 6:30-8:30 AM, and between 5-8 PM during the Memorial Day Weekend, and on the weekend that followed, *BlueGreen/Modica* utilized the time between treatments on weekend for extensive monitoring.

On Sunday, June 6, *BlueGreen/Modica* conducted a widespread inspection of all the residential canals east of the S-79 lock & dam, and collected extensive data along the C-43/ Caloosahatchee River from the S-77 lock & dam to the S-79 lock and dam (Fig. 11). Stormy weather interfered with data collection.

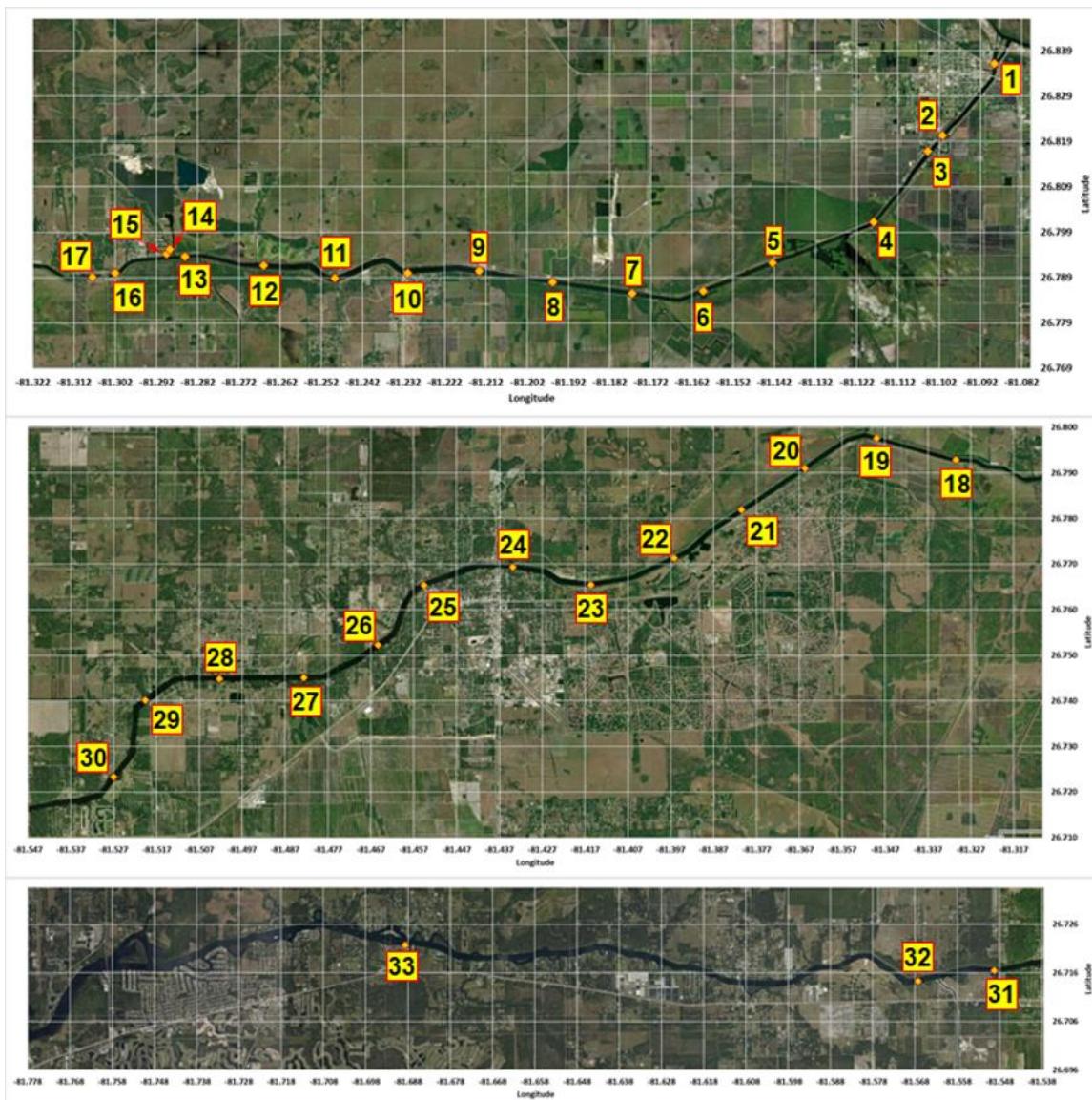


Figure 11. YSI-ProDSS sampling points along the C-43/ Caloosahatchee River, from S-77 structure (sample 1), to the S-79 structure (sample 33). Stormy weather interfered with data collection.
Laboratory samples for PTOX screen and cyanotoxin analyses were collected at sampling points 1, 7, 16, 17, 20, 24, and 28

Water temperature, conductivity, and pH remained almost stable across the length of the river. Dissolved oxygen levels increased along the river, from east to west. Chlorophyll and phycocyanin levels fluctuated across the length of the river.

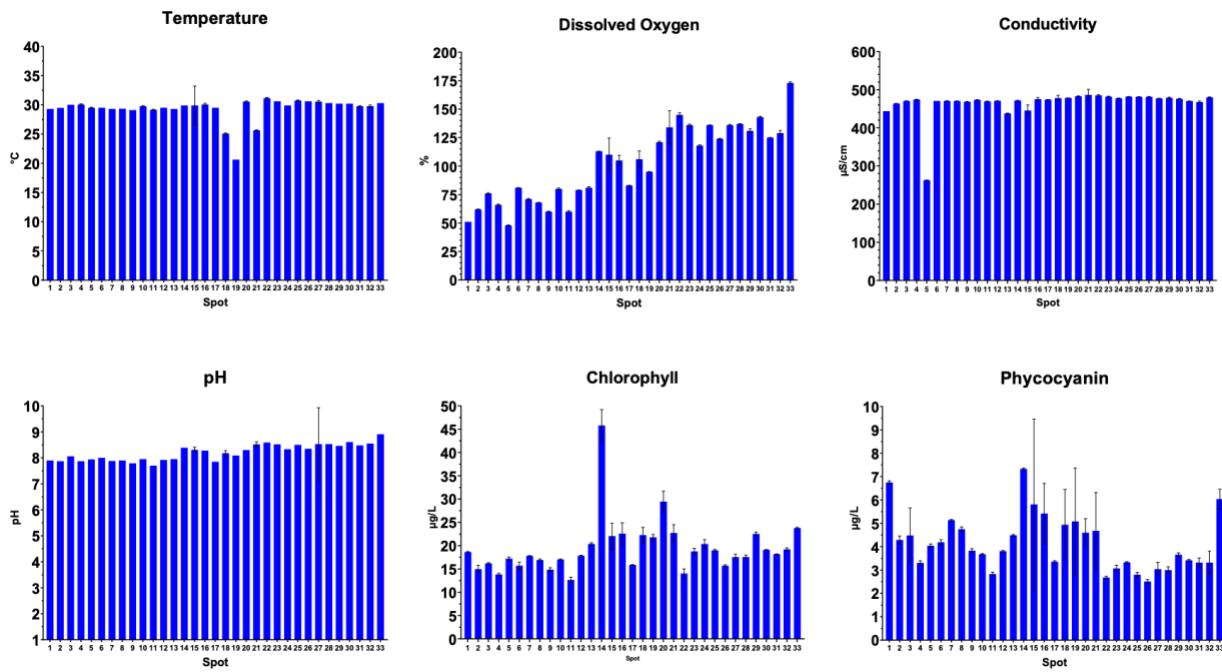


Figure 12. YSI-ProDSS field measurements for points 1 through 33, from the S-77 to the S-79 structure. Water temperature (°C), conductivity (µS/cm), dissolved oxygen (%), pH, chlorophyll (µg/L), and phycocyanin levels (µg/L, as a proxy for total cyanobacteria) were monitored.

Laboratory samples were collected from 7 sampling points across the length of the canal for PTOX screen and cyanotoxin analysis. The samples are 1, 7, 16, 17, 20, 24, and 28 (Fig. 11).

The PTOX screen results are summarized in Fig. 13. Sample 16, on the east side of S-78 exhibited extremely high levels of microcystins/ nodularins (Fig. 14). Samples 1 and 17 had microcystins/ nodularins levels above the advisory threshold.

Lab reports from samples 1, 7, 16, 17, 20, 24, and 28, are appended to this report as appendix C.

The name and ID of the water samples in this report were changed for simplicity reasons, and to avoid confusion from the ID that appears in the lab report (Appendix C). Below are the changes to the naming of these samples.

Samples 31 in the lab report, Appendix C, was renamed to Sample 1 in this section.

Samples 32 in the lab report, Appendix C, was renamed to Sample 7 in this section.

Samples 33 in the lab report, Appendix C, was renamed to Sample 16 in this section.

Samples 34 in the lab report, Appendix C, was renamed to Sample 17 in this section.

Samples 35 in the lab report, Appendix C, was renamed to Sample 20 in this section.

Samples 36 in the lab report, Appendix C, was renamed to Sample 24 in this section.

Samples 37 in the lab report, Appendix C, was renamed to Sample 28 in this section.

PTOX

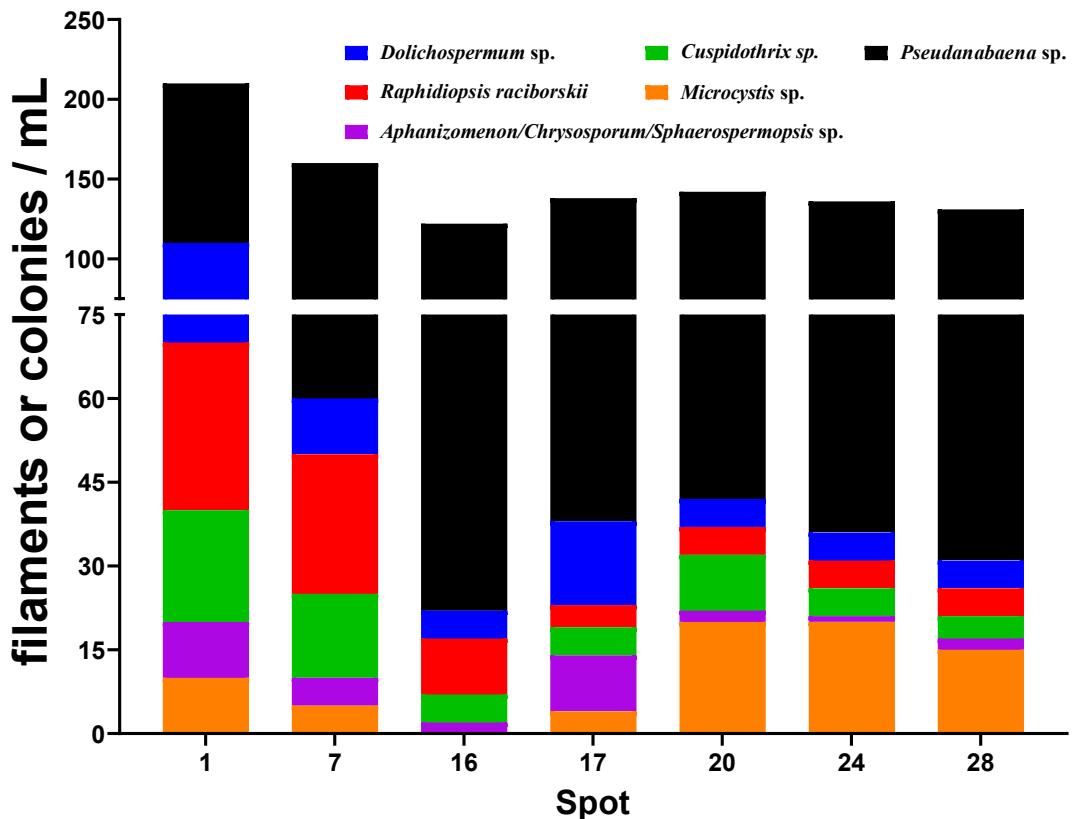


Figure 13. PTOX screen summary for water samples collected along the C-43/ Caloosahatchee River, from the S-77 to the S-79 structure (Fig. 11).

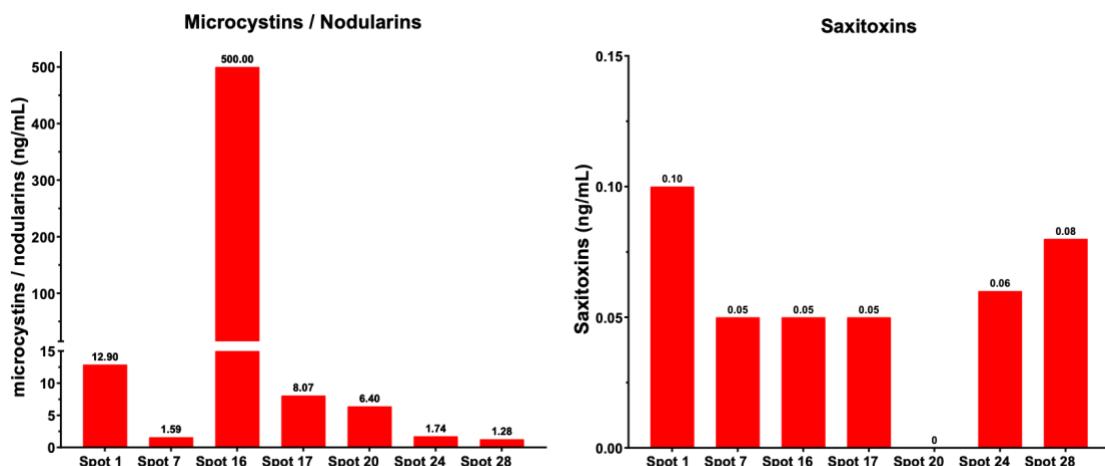


Figure 14. Cyanotoxin analyses of water samples collected along the C-43/ Caloosahatchee River, from the S-77 to the S-79 structure (Fig. 11).

9. Case study – site 43

Out of the 56 sites that were treated during the 15-day intensive campaign, several sites were subject to thorough investigation. Such is the case of sites 38 and 43 (see details of location, images and analysis of site 38 and 43 below, in Appendix A: An overview of the 56 individual sites treated by BlueGreen/Modica).

Site 43 included is a cove that was first treated on Friday, June 4, 2021. Water samples were obtained from outside and inside the cove for extensive comparison of PTOX values, genome sequencing, and cyanotoxin analyses. The samples were collected two days after a treatment application inside the cove. The treatment had cleared the cyanobacterial scum, and no visible cyanobacterial aggregated were detected in the waters inside the cove one day after treatment. The data indicates that the treatment had significantly decreased cyanobacterial biomass and increased the biodiversity inside the cove compared to outside the cove, at the Caloosahatchee River.

Comparison between PTOX values inside and outside the cove shows a substantial difference between PTOX species. It is evident that PTOX filaments / colonies counts are significantly more abundant outside the cove (Fig. 15). A similar pattern is observed for the toxins measured in this study, i.e., microcystins / nodularins and saxitoxins, which both exhibit sustainably lower values inside compared to outside the cove (Fig. 16, Appendix B, note the corresponding samples from the lab report below).

- Sample K20 in the lab report, Appendix B, is the water sample from outside the cove at the treatment site #43.
- Sample K21 in the lab report, Appendix B, is the water sample from inside the cove at the treatment site #43.

YSI-ProDSS field measurements are summarized in Fig. 17. The decrease in phycocyanin and the increase in chlorophyll is consistent with other results, and with the visual inspection of decreased cyanobacterial load and the increase in biodiversity.

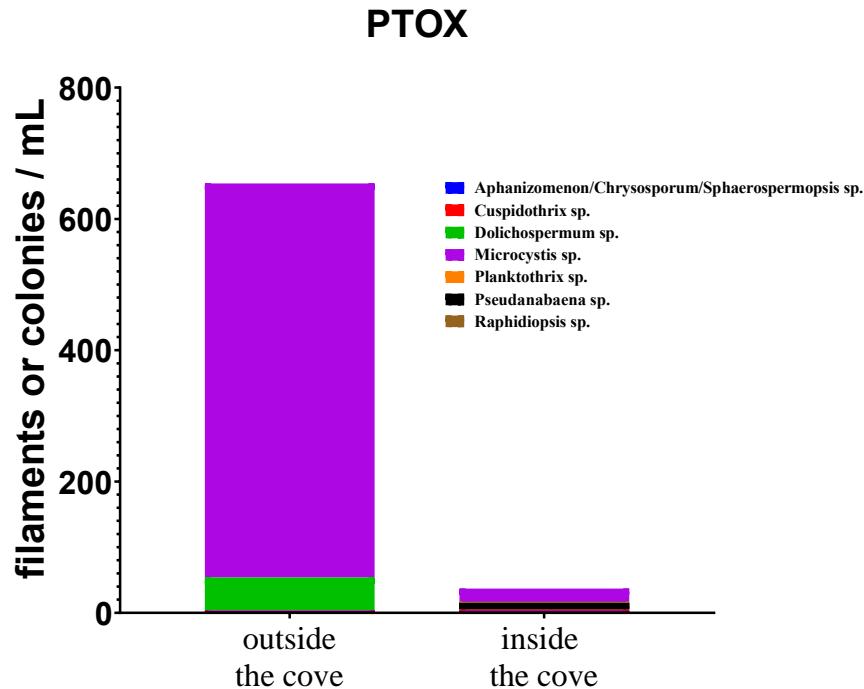


Figure 15. Potentially Toxigenic (PTOX) cyanobacteria filaments/ colonies (per mL), found inside and outside the cove in site 43 on May 2021. Comparison between the following species was conducted: *Aphanizomenon/ Chrysosporum / Sphaerospermopsis sp.*, *Cuspidothrix sp.*, *Dolichospermum sp.*, *Microcystis sp.*, *Planktothrix sp.*, *Pseudanabaena sp.*, and *Raphidiopsis sp.*.

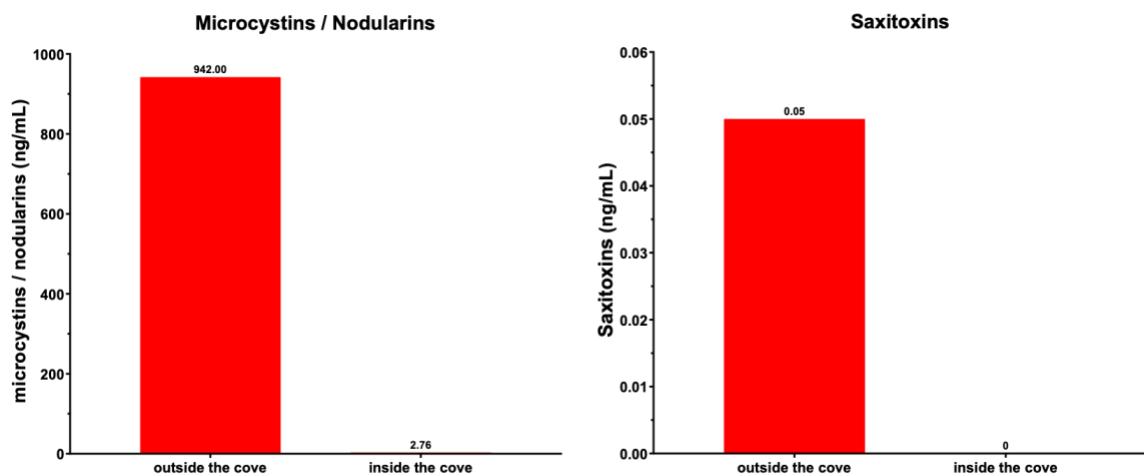


Figure 16. Microcystins / nodularins (top panel) and saxitoxins (bottom panel) (in ng/mL) found in samples taken inside and outside the cove on site 43, during May 2021.

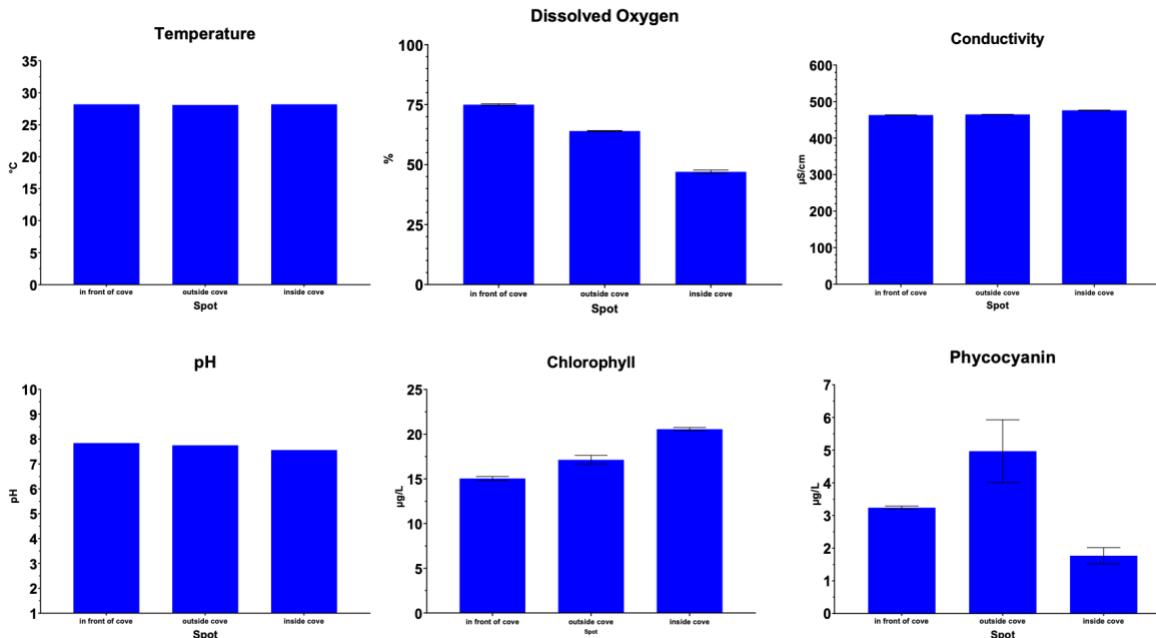


Figure 17. YSI-ProDSS field measurements for points inside the cove, from the river outside the cove, and at the entry point of the cove (In front of the cove). Water temperature (°C), conductivity (µS/cm), dissolved oxygen (%), pH, chlorophyll (µg/L), and phycocyanin levels (µg/L, as a proxy for total cyanobacteria).

The two points outside and inside the cove were sampled for full genome sequencing (metagenomics). The DNA libraries were mapped against curated databases that included: bacteria, including those with antibiotic resistance, fungi, protists, phages, viruses, including respiratory viruses, and virulence factors. The dark matter is a database for non-culturable microbes, with poorly curated database that is in its beta stage. The data is represented as relative abundance, and the identified species could be of low or high abundance. The sequencing data identified more species inside the cove than outside the cove (Fig. 18). Full analysis of the sequencing data is included in this report as appendix D.

- Sample 1 in the whole genome sequencing analysis report, Appendix D1, denotes the water sample from inside the cove.
- Sample 2 in the whole genome sequencing analysis report, Appendix D2, denotes the water sample from outside the cove.

sequencing analysis

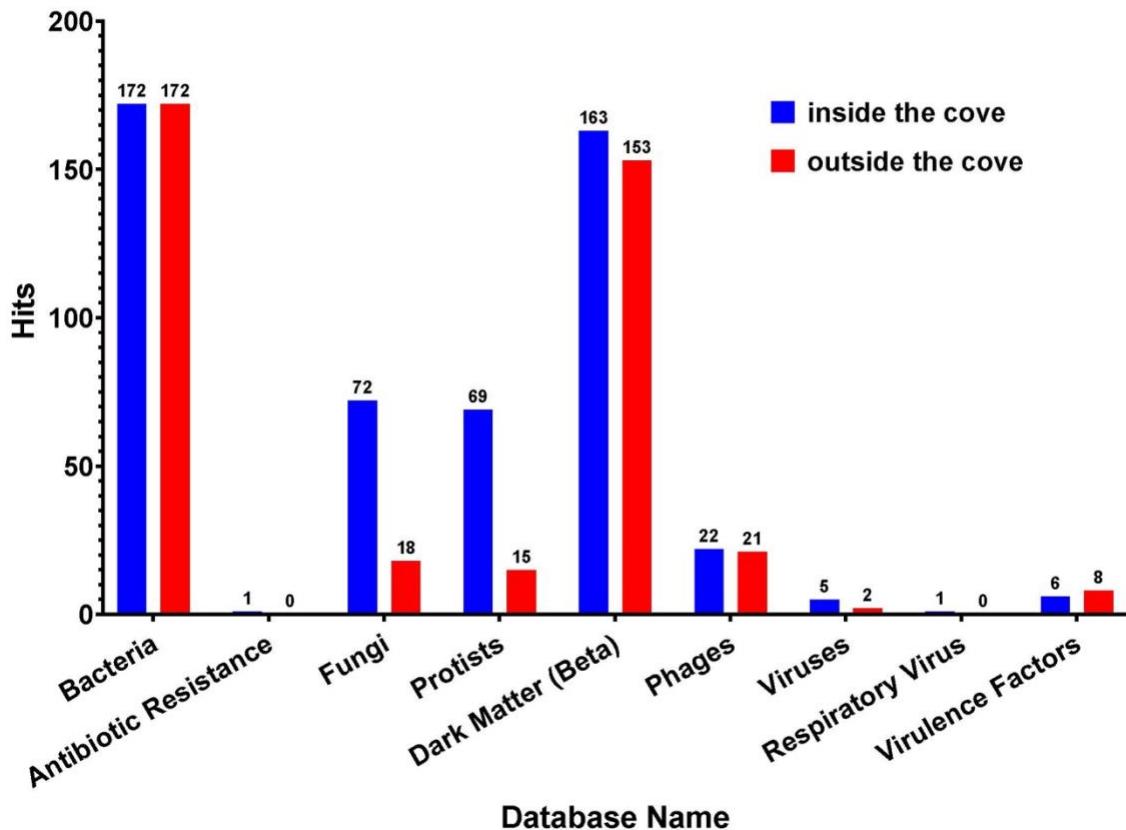


Figure 18. Metagenomics analysis for two sample points in site 43, outside and inside the cove. The sequenced DNA libraries were mapped against curated databases that included: bacteria, including those with antibiotic resistance, fungi, protists, phages, viruses, including respiratory viruses, and virulence factors. The dark matter is a database for non-culturable microbes, with poorly curated database that is in its beta stage.

Cell count from samples collected from inside the cove show decreased cyanobacterial cell count compared to a sample collected from the entry point to cove, close to the river, but increased green algae cell count compared to the entry point (Fig. 19)

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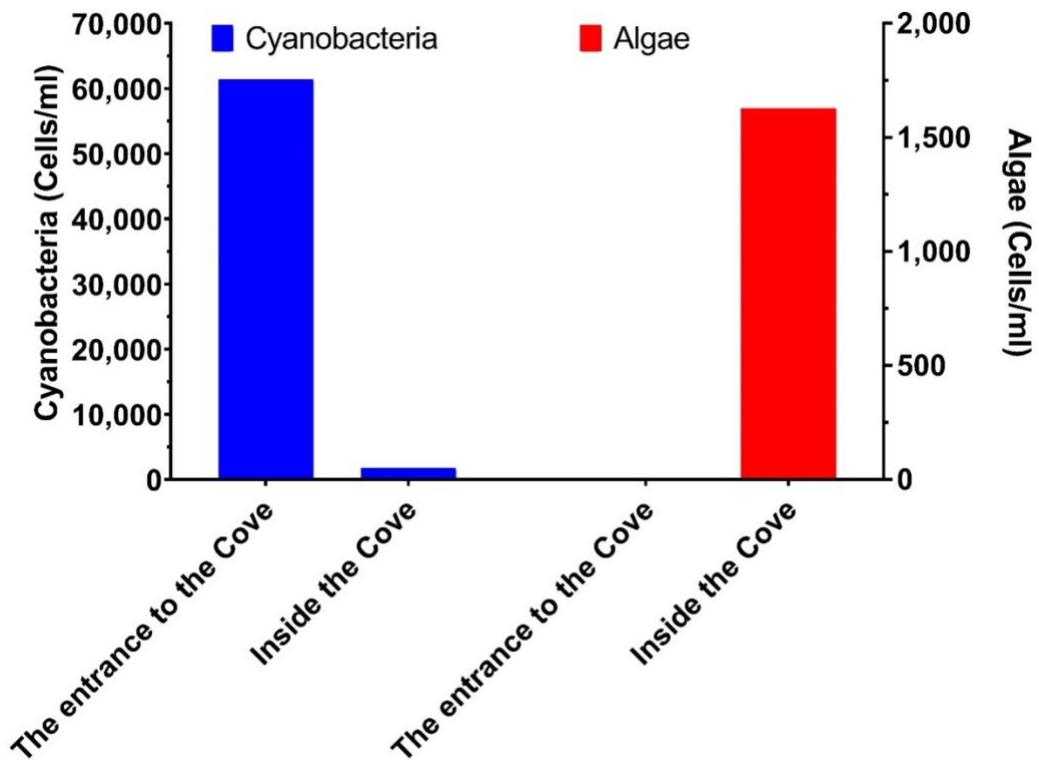


Figure 19. Cell count of cyanobacterial cells and green algae cells from a sample collected inside the cove and a sample collected from the entry point, close the river. Cyanobacterial cell count is substantially lower from the sample inside the cove in comparison the entry point, while the green algae cell count is substantially greater from the sample collected inside the cove compared the sample from the entry point.

10. Concluding Remarks

The C-43 Canal/ Caloosahatchee River project demonstrated the efficacy and safety of the *Lake Guard® Oxy*. The modular treatment protocols allows for the product to treat heavy cyanobacterial blooms as well as maintaining bloom free conditions through preventative treatments. The 15-day project mitigated cyanobacterial blooms and maintained bloom free conditions of residential areas over a 48-mile river segment. Only 5.1 tons out of the 20-ton FL DEP stockpile were used in this project. This relatively small amount of the *Lake Guard® Oxy* provided immediate relief to the residents and eliminated the cyanobacterial scum in residential areas along with the associated health risks and unpleasant conditions that accompany cyanobacterial blooms.

11. Selected before and after pictures

BEFORE Treatment: May 26, 2021



AFTER Treatment: May 30, 2021

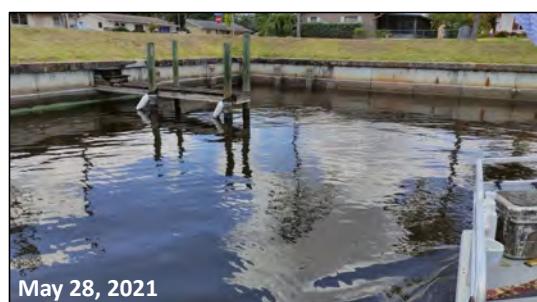


Latitude and longitude: 26.7213, -81.69327
W.P Franklin Lock & Dam, South Recreation Area

BEFORE Treatment: May 26, 2021

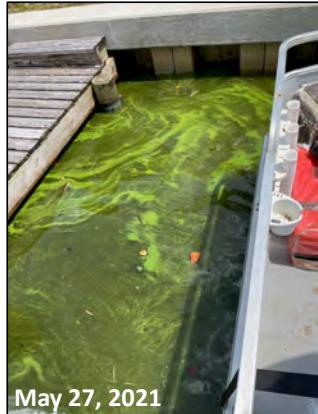


AFTER Treatment: May 28, 2021



Latitude and longitude:
26.72282, -81.71166

BEFORE Treatment: May 27, 2021



AFTER Treatment: May 28, 2021



Latitude and longitude: 26.72334, -81.71473

Before Treatment: May 28, 2021



After Treatment: May 31 and June 1, 2021



Latitude and longitude: 26.72487, -81.69367

Before Treatment: May 28, 2021



After Treatment: June 1, 2021



Latitude and longitude: 26.72687, -81.69729

BEFORE Treatment: June 4, 2021



AFTER Treatment: June 5, 2021



Latitude and longitude:
26.74294, -81.51729

Appendix A:

An overview of the 56 individual sites treated by BlueGreen/ Modica

Presented below are the 56 different sites for which 112 treatments and retreatments were applied, using a total amount of 10,260 lb (5.1 tons) of the *Lake Guard® Oxy* product. Location, estimated area, aerial photos, site photos, treatment date, time, dosage and total amount, and additional measurement data (if available) are presented for each site.

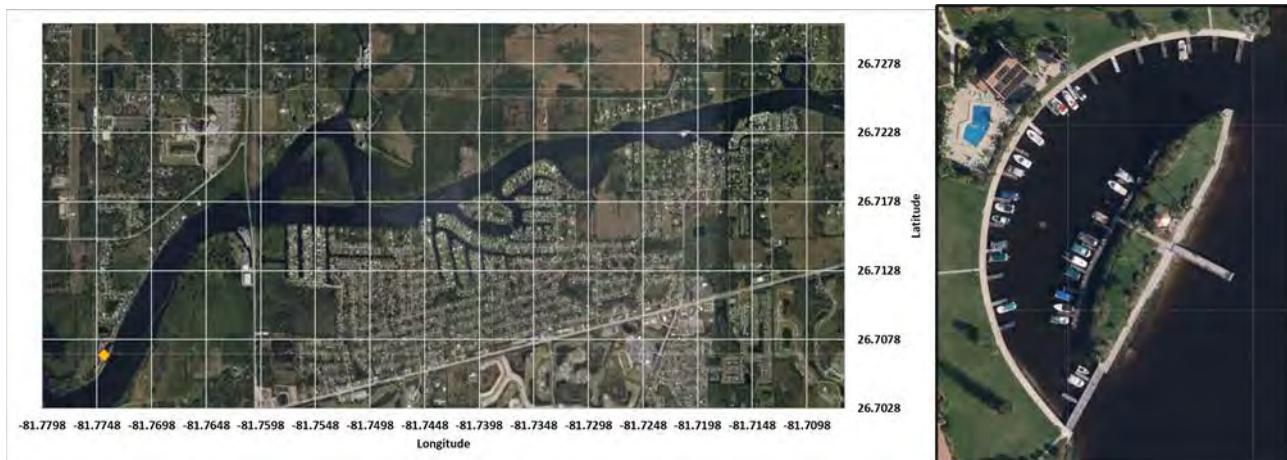
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Site 1 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
1	26.70664	-81.77408	1.8

Aerial photographs



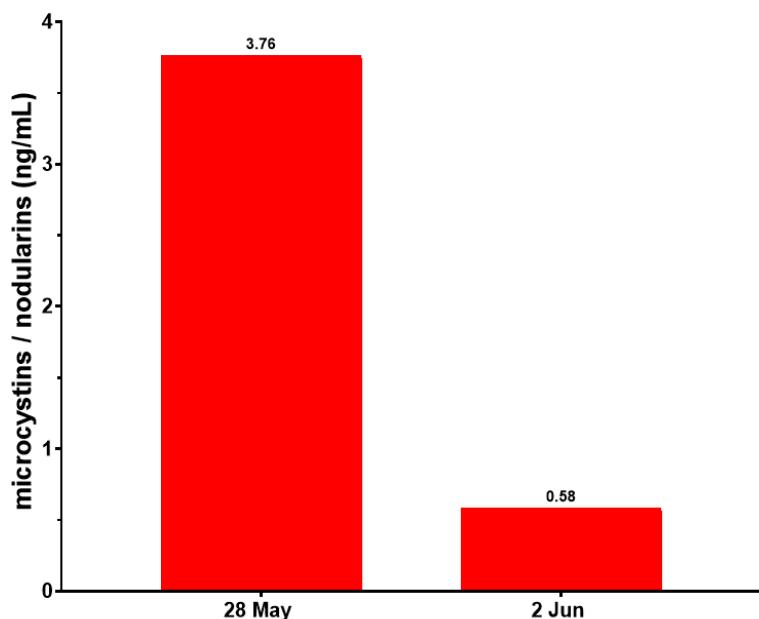
Treatment details

date	28 May	1 June	4 June
Treatment (lb)	35	100	25
dose (lb/acre)	19.4	55.6	13.9

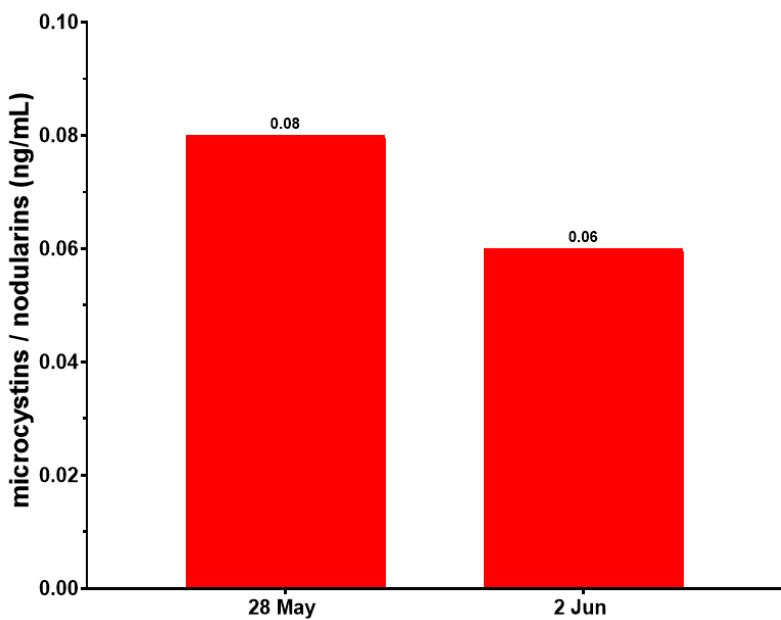
Site photos



Site 1 : Microcystins / Nodularins



Site 1 : Saxitoxins



Microcystins / nodularins (top panel) and saxitoxins (bottom panel) (in ng/mL, ppb) found in site #1 on May 28th and June 2nd, 2021.

Site 2 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
2	26.71293	-81.76067	3.3

Aerial photographs



Treatment details

date	28 May
Treatment (lb)	80
dose (lb/acre)	24.2

Site photos



Site 3 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
3	26.71329	-81.75943	3.2

Aerial photographs



Treatment details

date	28 May	7 June
Treatment (lb)	85	5
dose (lb/acre)	26.6	1.6

Site photos



Site 4 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
4	26.71275	-81.75729	4.5

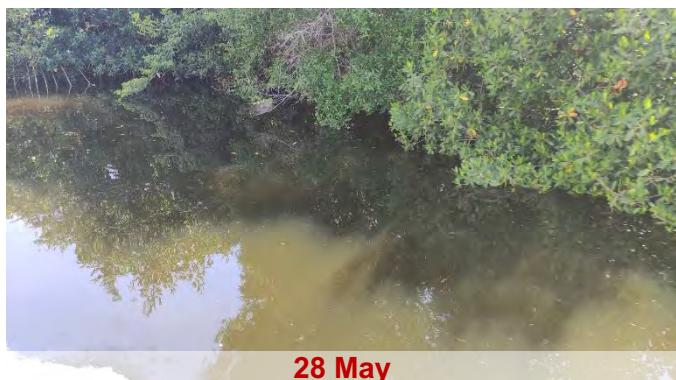
Aerial photographs



Treatment details

date	28 May	29 May
Treatment (lb)	100	150
dose (lb/acre)	22.2	33.3

Site photos

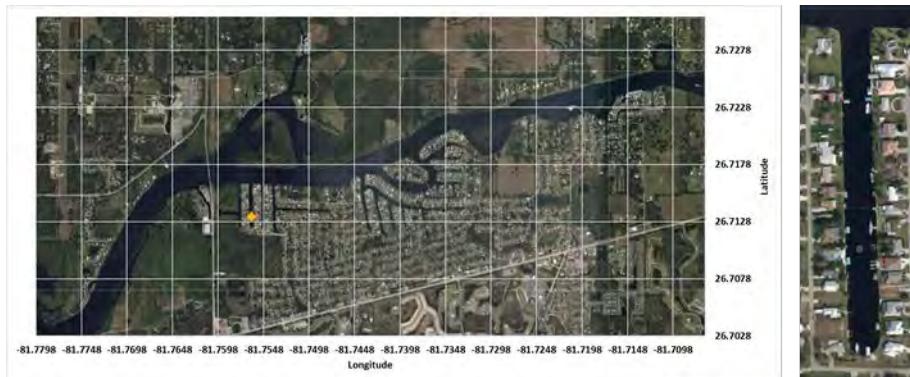


Site 5 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
5	26.71321	-81.75605	5.7

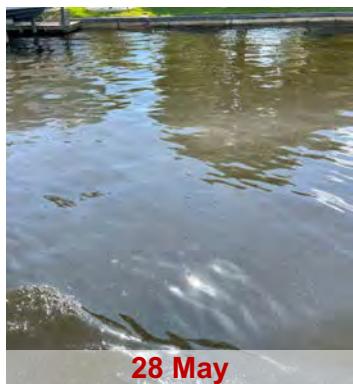
Aerial photographs



Treatment details

date	28 May	29 May	31 May
Treatment (lb)	135	100	100
dose (lb/acre)	23.7	17.5	17.5

Site photos



Site 6 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
6	26.71319	-81.75472	3.8

Aerial photographs



Treatment details

date	28 May	29 May	31 May
Treatment (lb)	110	100	100
dose (lb/acre)	28.9	26.3	26.3

Site photos



Site 7 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
7	26.71315	-81.75350	5.2

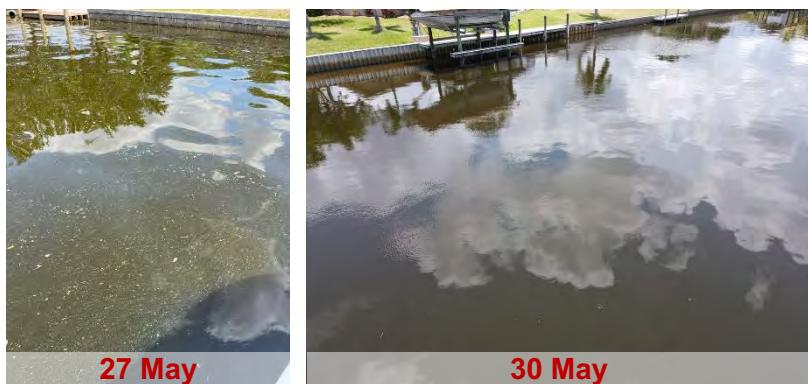
Aerial photographs



Treatment details

date	28 May	29 May	30 May	31 May
Treatment (lb)	125	100	200	100
dose (lb/acre)	24.0	19.2	38.5	19.2

Site photos



Site 8 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
8	26.71352	-81.74779	4.9

Aerial photographs



Treatment details

date	28 May	30 May
Treatment (lb)	105	150
dose (lb/acre)	21.4	30.6

Site photos



Site 9 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
9	26.71985	-81.72520	2.4

Aerial photographs



Treatment details

date	28 May	29 May	30 May
Treatment (lb)	95	100	100
dose (lb/acre)	39.6	41.7	41.7

Site photos



27 May



27 May



29 May



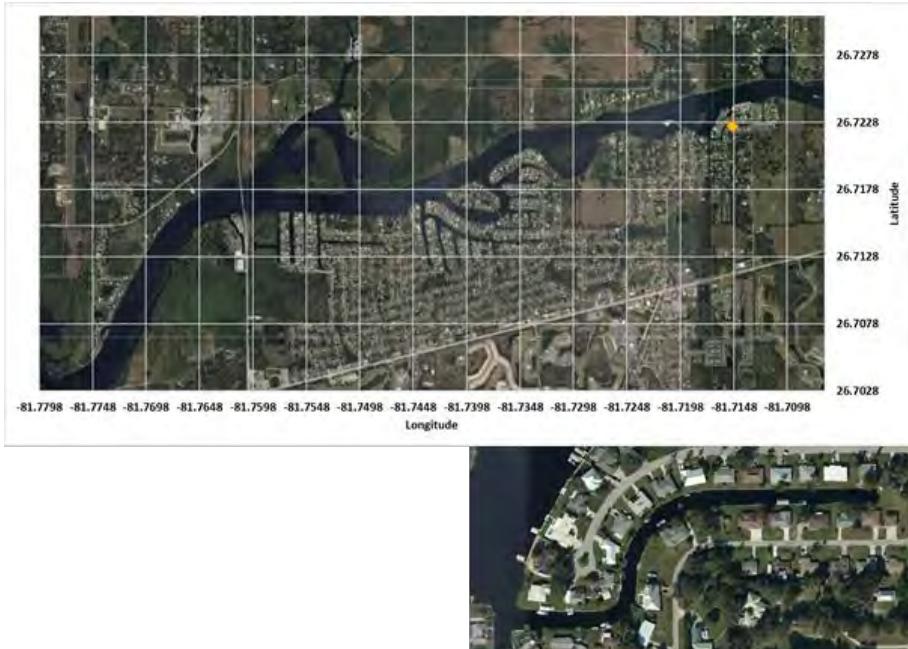
30 May

Site 10 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
10	26.72246	-81.71485	2.2

Aerial photographs



Treatment details

date	29 May
Treatment (lb)	100
dose (lb/acre)	45.5

Site photos



Site 11 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
11	26.72335	-81.71463	1.1

Aerial photographs

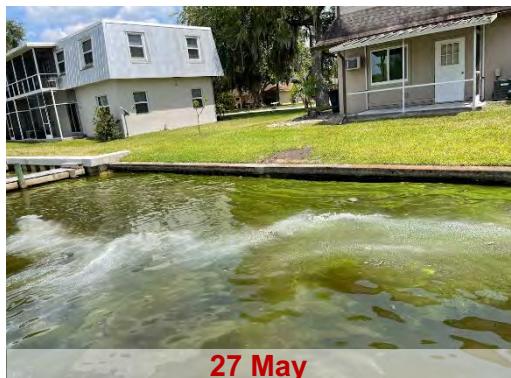


Treatment details

date	27 May	28 May	29 May	30 May	31 May
Treatment (lb)	65	35	30	200	100
dose (lb/acre)	59.1	31.8	27.3	181.8	90.9

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Site #11 photos



27 May



27 May



27 May



28 May



28 May



29 May

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Site 12 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
12	26.72288	-81.71145	1.2

Aerial photographs



Treatment details

date	27 May	28 May	29 May	30 May	31 May
Treatment (lb)	55	30	20	100	100
dose (lb/acre)	45.8	25.0	16.7	83.3	83.3

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Site #12 photos



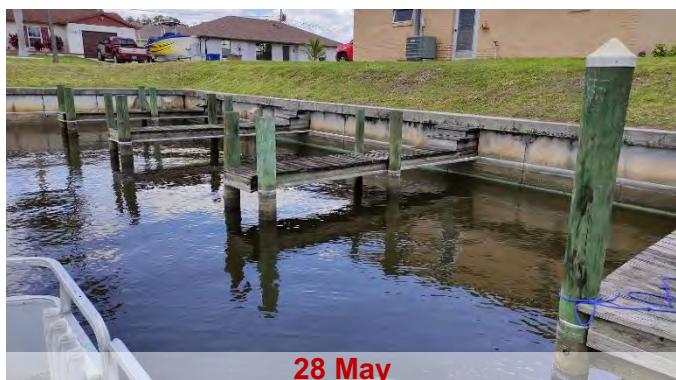
26 May



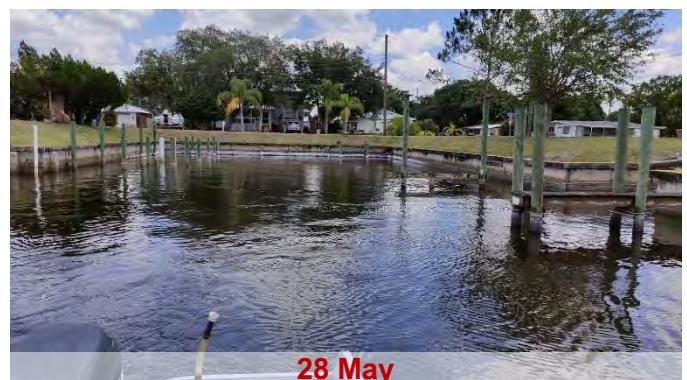
26 May



26 May



28 May



28 May



29 May



29 May



30 May

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Site 13 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
13	26.72825	-81.71017	14

Aerial photographs



Treatment details

date	29 May
Treatment (lb)	150
dose (lb/acre)	10.7

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Site 14 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
14	26.72687	-81.69729	2.7

Aerial photographs



Treatment details

date	28 May	29 May	30 May	1 June	2 June
Treatment (lb)	260	200	100	50	100
dose (lb/acre)	96.3	74.1	37.0	18.5	37.0

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Site #14 photos



28 May



28 May



28 May



1 Jun



1 Jun



1 Jun

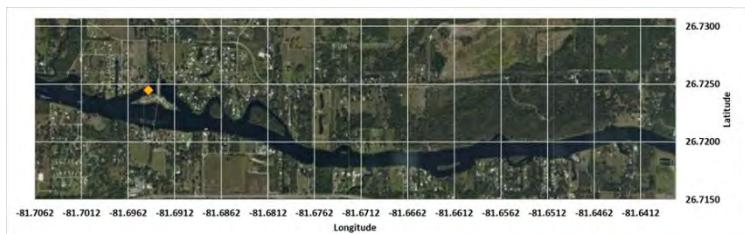
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Site 15 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
15	26.72441	-81.69394	9.6

Aerial photographs

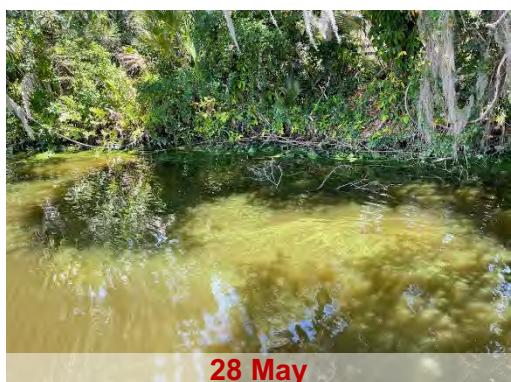


Treatment details

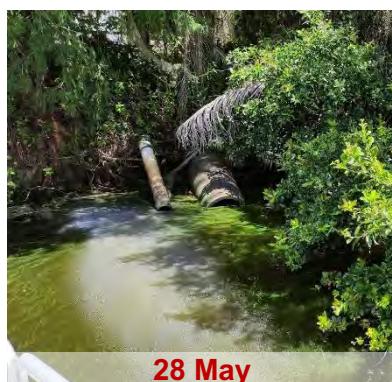
date	28 May	29 May	2 June
Treatment (lb)	370	330	150
dose (lb/acre)	38.5	34.4	15.6

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Site #15 photos



28 May



28 May



29 May



1 Jun



1 Jun



1 Jun

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Site 16 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
16	26.72135	-81.69373	3.6

Aerial photographs



Treatment details

date	27 May	28 May	29 May	1 June	2 June	3 June	4 June
Treatment (lb)	130	70	150	25	75	50	5
dose (lb/acre)	36.1	19.4	41.7	6.9	20.8	13.9	1.4

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Site #16 photos



27 May



27 May



28 May



28 May



3 Jun



4 Jun

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Site 17 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
17	26.72127	-81.69309	1.2

Aerial photographs



Treatment details

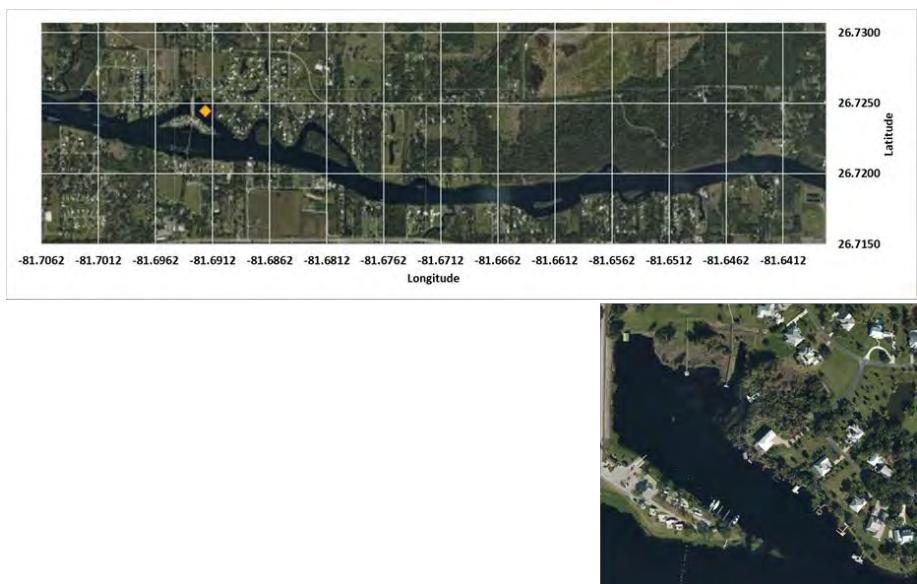
date	28 May	29 May	1 June	2 June	5 June	10 June
Treatment (lb)	170	100	150	50	27	50
dose (lb/acre)	141.7	83.8	125.0	41.7	22.5	41.7

Site 18 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
18	26.72437	-81.69175	13.9

Aerial photographs



Treatment details

date	29 May
Treatment (lb)	150
dose (lb/acre)	10.8

Site photos

Site photos

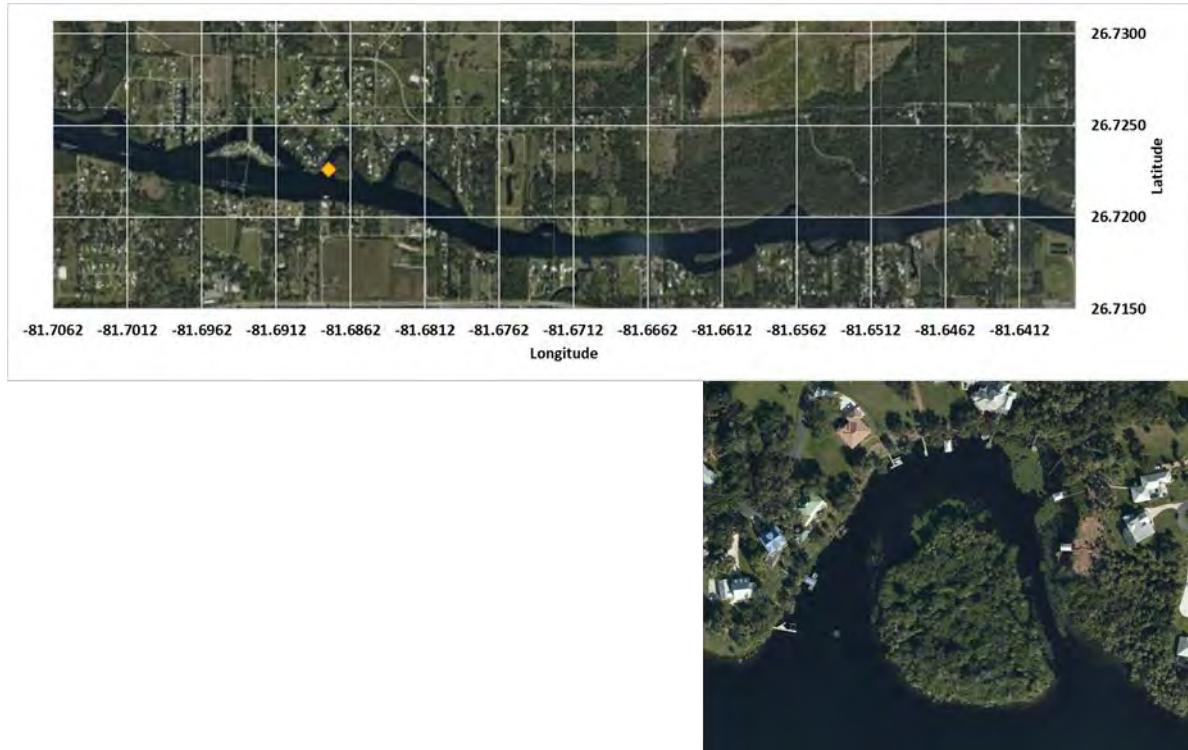


Site 19 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
19	26.72255	-81.68762	7.6

Aerial photographs



Treatment details

date	28 May
Treatment (lb)	45
dose (lb/acre)	5.9

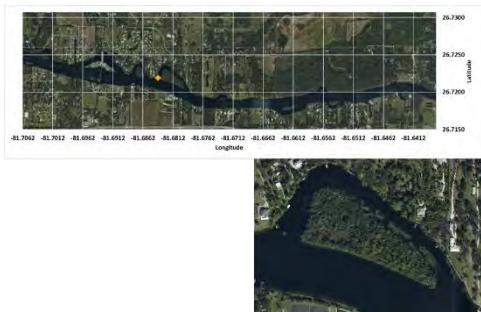
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Site 20 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
20	26.72187	-81.68363	13.5

Aerial photographs



Treatment details

date	28 May	8 June
Treatment (lb)	130	30
dose (lb/acre)	9.6	2.2

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Site 21 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
21	26.71920	-81.67930	1.8

Aerial photographs



Treatment details

date	28 May
Treatment (lb)	140
dose (lb/acre)	77.8

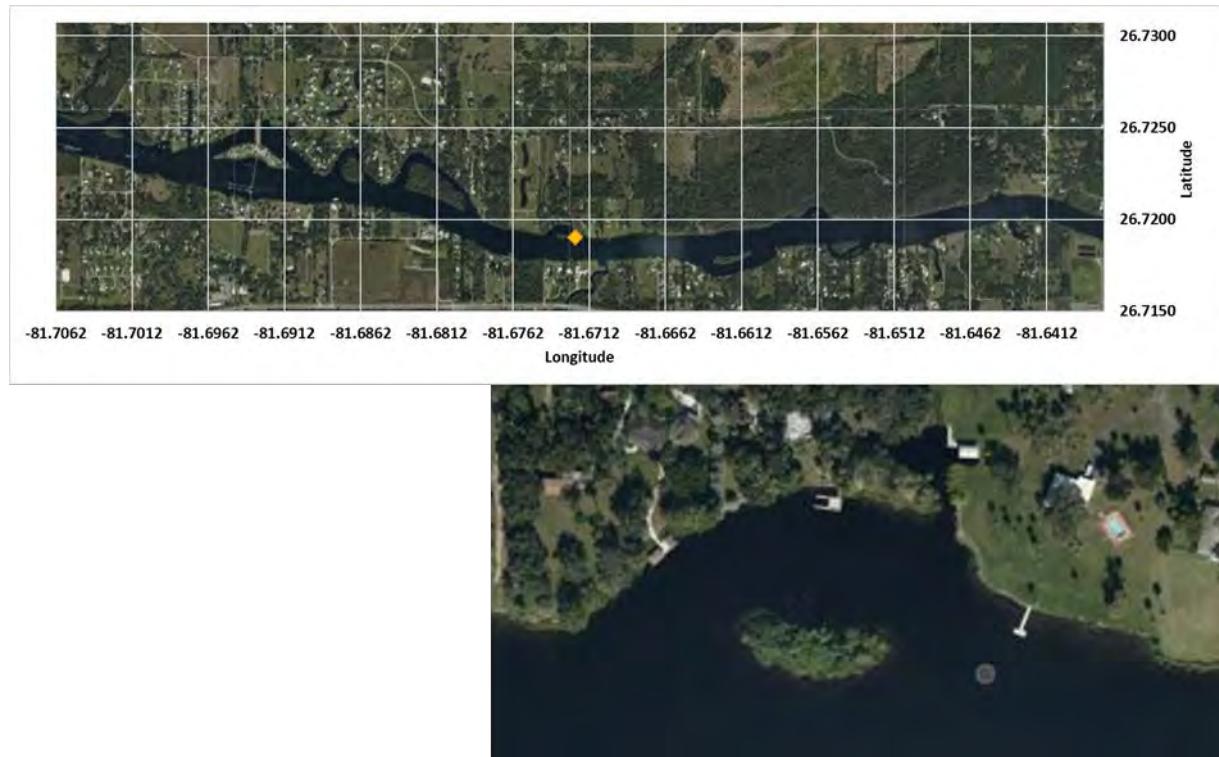
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Site 22 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
22	26.71898	-81.67206	3.2

Aerial photographs



Treatment details

date	28 May
Treatment (lb)	25
dose (lb/acre)	7.8

Site 23 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
23	26.71721	-81.66987	3.9

Aerial photographs



Treatment details

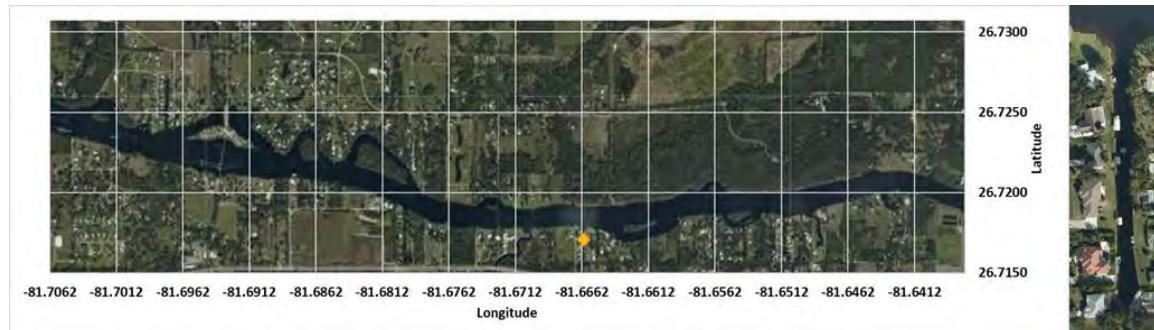
date	28 May
Treatment (lb)	70
dose (lb/acre)	17.9

Site 24 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
24	26.71699	-81.66603	1

Aerial photographs



Treatment details

date	28 May
Treatment (lb)	50
dose (lb/acre)	50.0

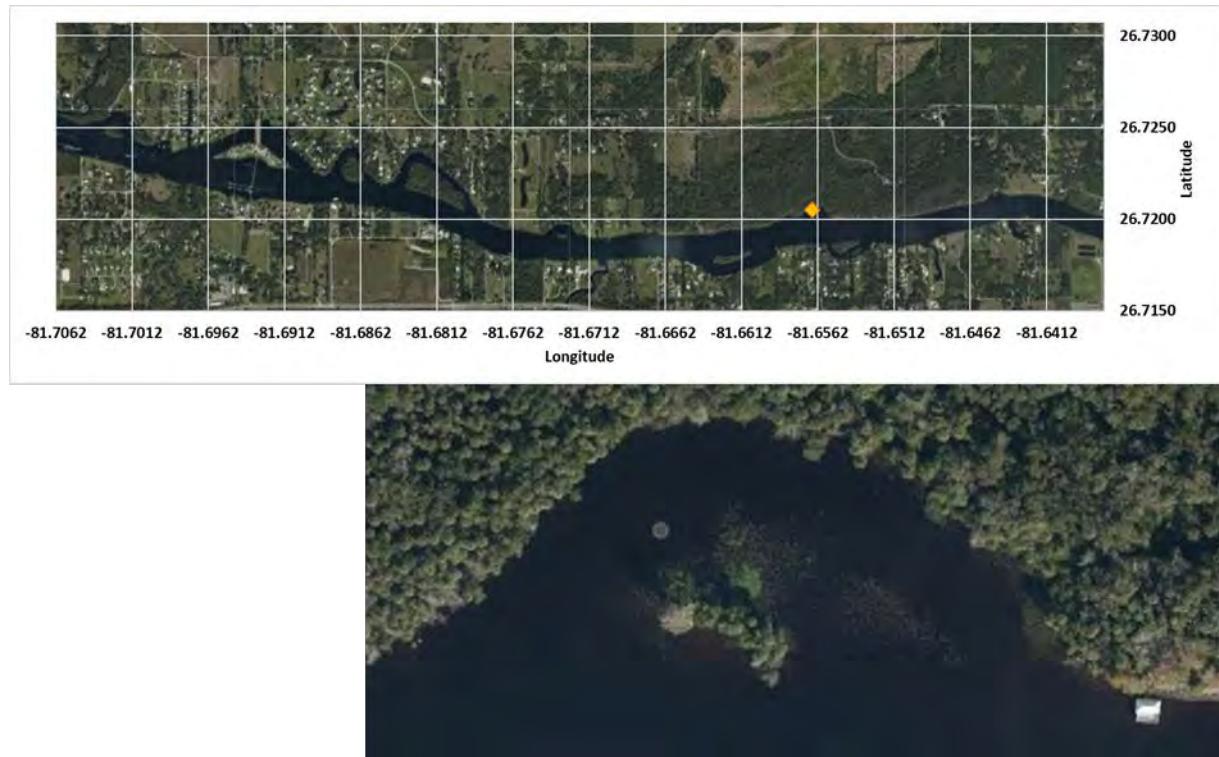
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Site 25 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
25	26.72046	-81.65650	2.6

Aerial photographs



Treatment details

date	28 May
Treatment (lb)	30
dose (lb/acre)	11.5

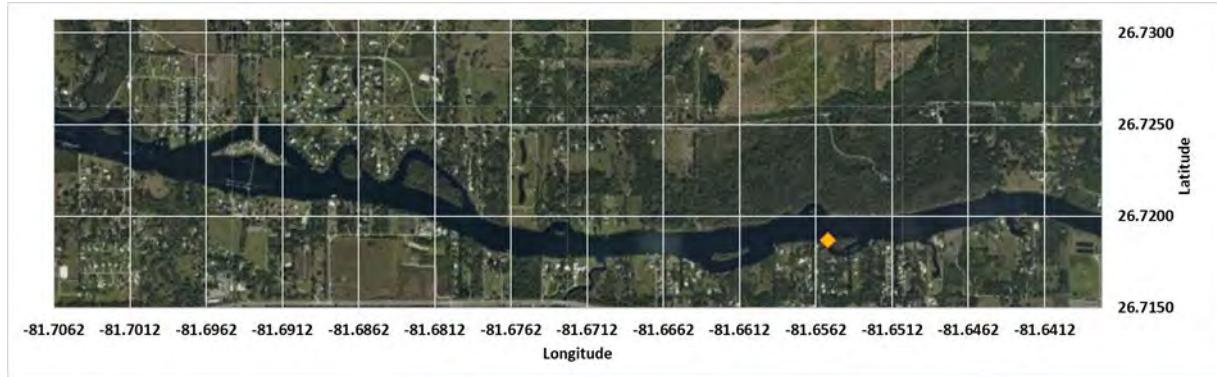
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Site 26 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
26	26.71863	-81.65537	5.6

Aerial photographs



Treatment details

date	28 May	8 June
Treatment (lb)	120	10
dose (lb/acre)	21.4	1.8

Site photos

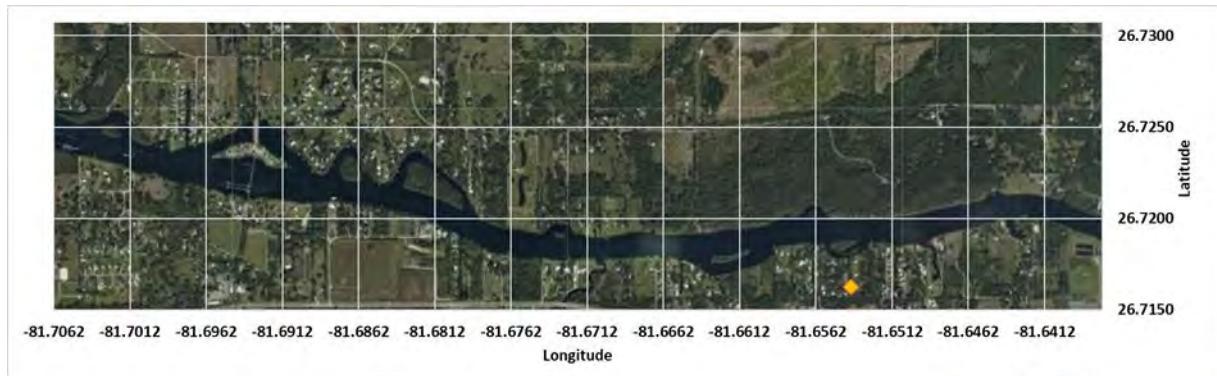


Site 27 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
27	26.71620	-81.65383	1.3

Aerial photographs



Treatment details

date	28 May
Treatment (lb)	130
dose (lb/acre)	100.0

Site photos

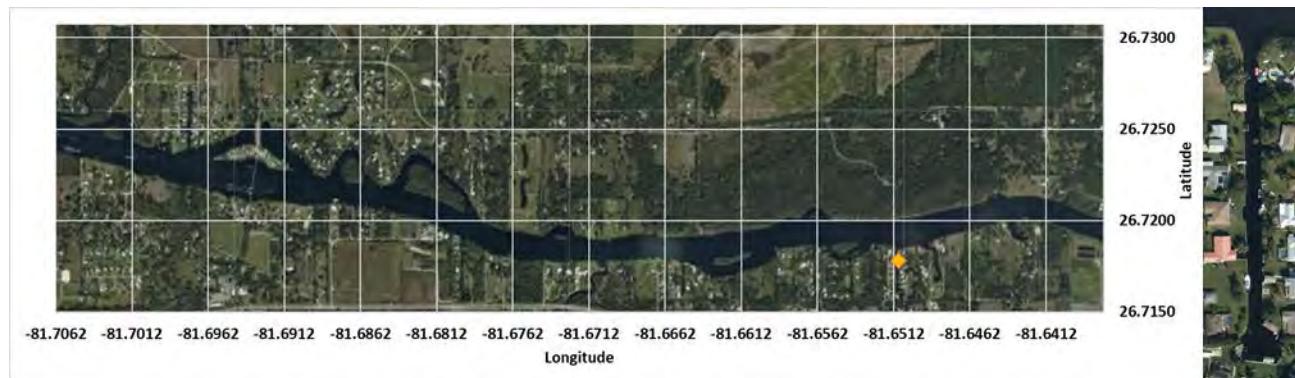


Site 28 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
28	26.71775	-81.65085	1.4

Aerial photographs



Treatment details

date	28 May
Treatment (lb)	60
dose (lb/acre)	42.9

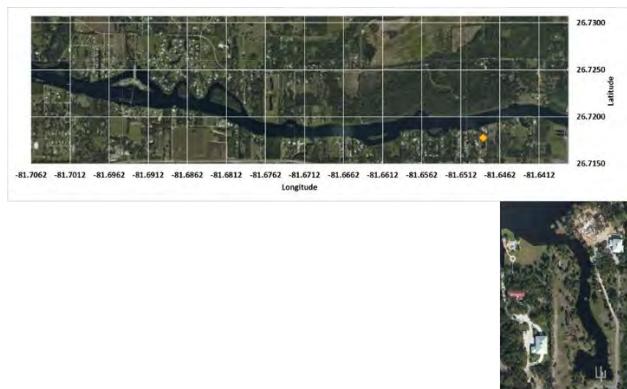
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Site 29 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
29	26.71772	-81.64829	2

Aerial photographs



Treatment details

date	28 May
Treatment (lb)	15
dose (lb/acre)	7.5

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Site 30 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
30	26.71968	-81.64342	3.1

Aerial photographs



Treatment details

date	28 May
Treatment (lb)	15
dose (lb/acre)	4.8

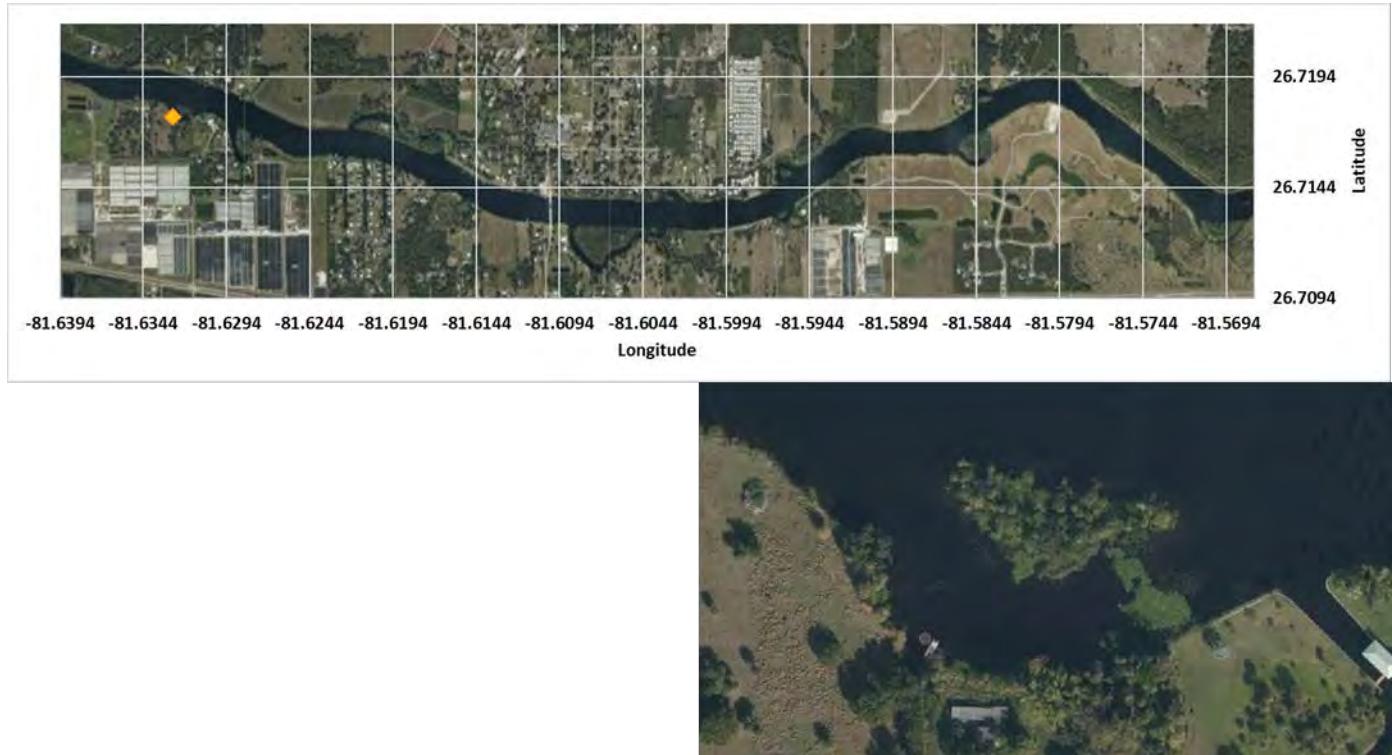
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Site 31 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
31	26.71756	-81.63257	2.8

Aerial photographs



Treatment details

date	8 June
Treatment (lb)	5
dose (lb/acre)	1.8

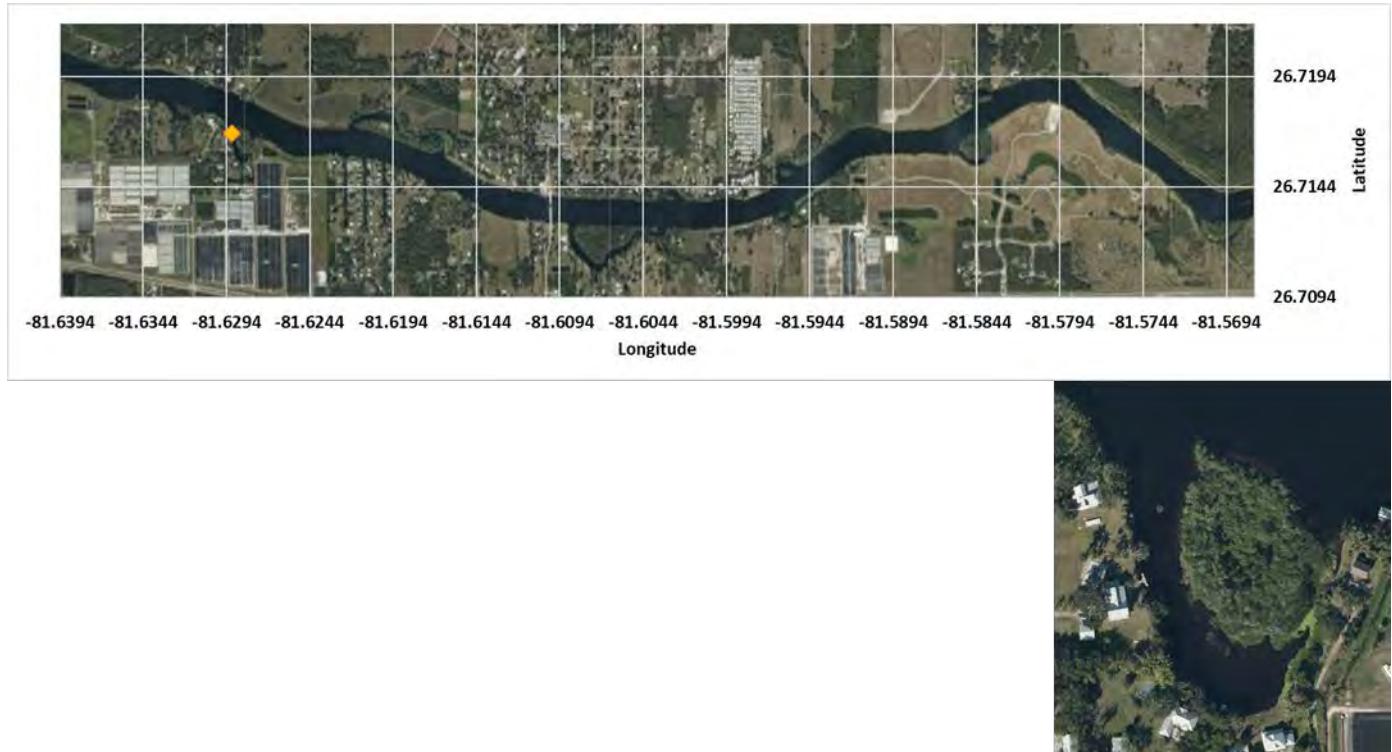
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Site 32 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
32	26.71679	-81.62903	3.6

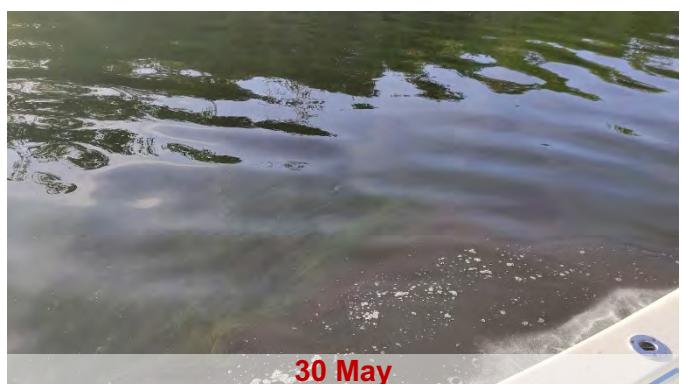
Aerial photographs



Treatment details

date	30 May
Treatment (lb)	100
dose (lb/acre)	27.8

Site photos



Site 33 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
33	26.71398	-81.62232	1.7

Aerial photographs



Treatment details

date	29 May
Treatment (lb)	190
dose (lb/acre)	111.8

Site photos



Site 34 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
34	26.71397	-81.62113	1.6

Aerial photographs



Treatment details

date	29 May
Treatment (lb)	180
dose (lb/acre)	112.5

Site photos



Site 35 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
35	26.71387	-81.61998	1.3

Aerial photographs



Treatment details

date	29 May	30 May	1 June	7 June	9 June
Treatment (lb)	220	150	200	30	15
dose (lb/acre)	169.2	115.4	153.8	23.1	11.5

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Site #35 photos



29 May



30 May



30 May



1 Jun



9 Jun



9 Jun

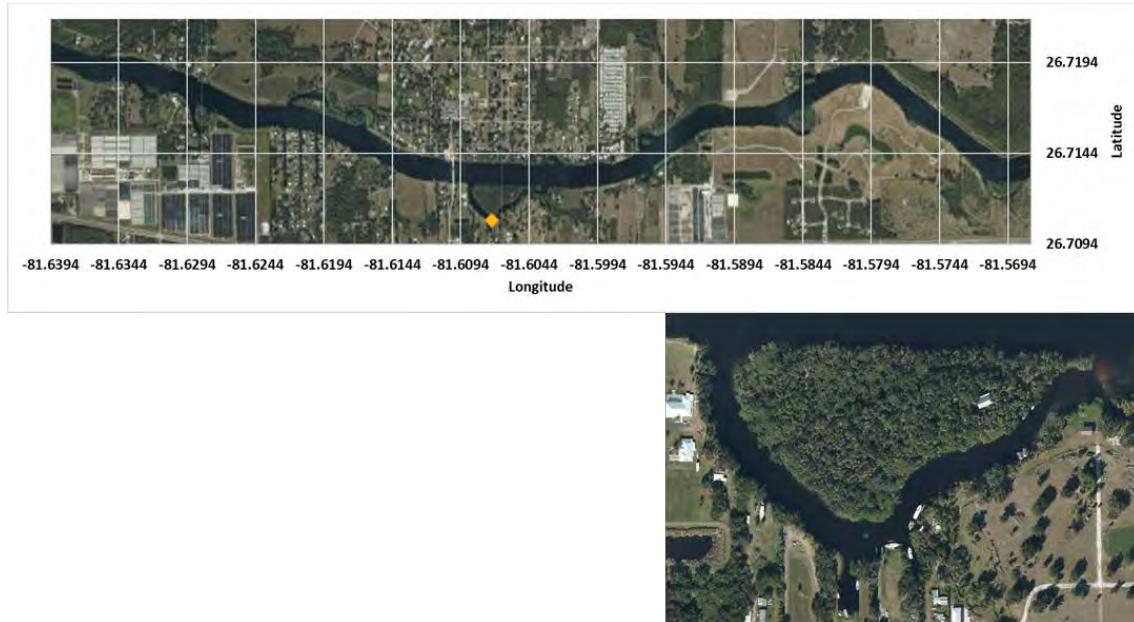
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Site 36 – details of the treatment conducted by *BlueGreen*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
36	26.71068	-81.60706	7.5

Aerial photographs



Treatment details

date	29 May
Treatment (lb)	290
dose (lb/acre)	38.7

Site photos



Site 37 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
37	26.71228	-81.60330	2.5

Aerial photographs



Treatment details

date	29 May
Treatment (lb)	13
dose (lb/acre)	5.2

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Site 38 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
38	26.71408	-81.59828	0.5

Aerial photographs



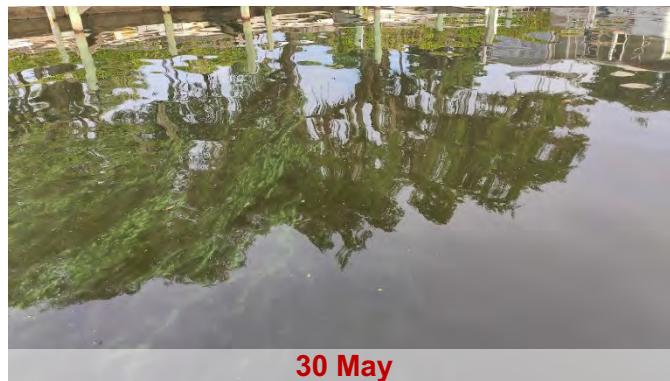
Treatment details

date	29 May	9 June
Treatment (lb)	7	10
dose (lb/acre)	14.0	20.0

Site photos



29 May



30 May

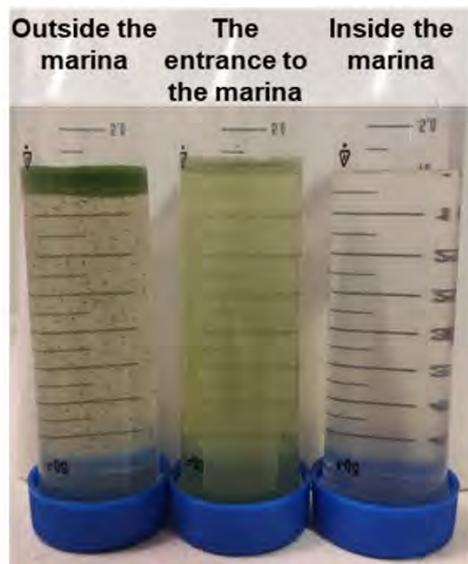
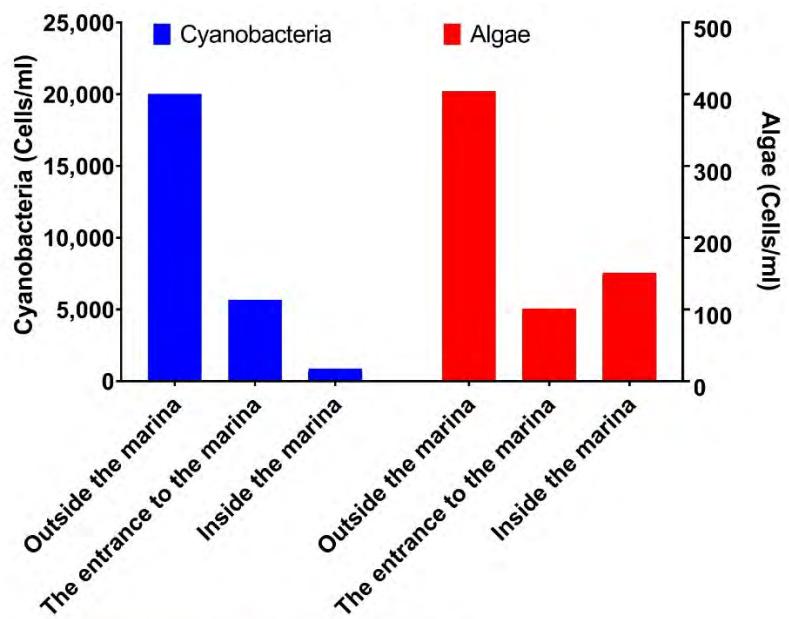


1 Jun



1 Jun

Phytoplankton population composition – Site 38 - for a water sample from May 30th, 2021:



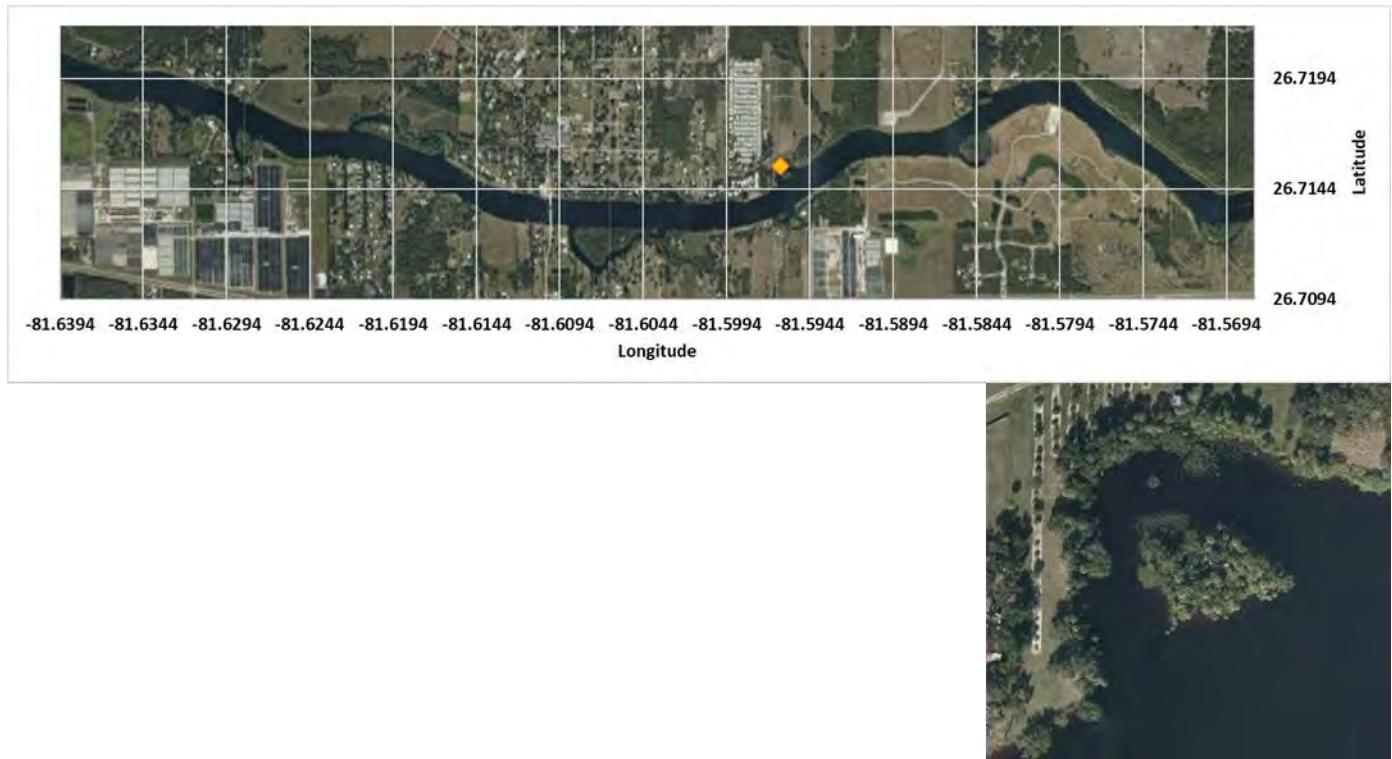
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Site 39 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
39	26.71540	-81.59609	1.9

Aerial photographs



Treatment details

date	29 May
Treatment (lb)	100
dose (lb/acre)	52.6

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Site 40 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
40	26.71864	-81.58434	2

Aerial photographs



Treatment details

date	30 May
Treatment (lb)	100
dose (lb/acre)	50.0



Site photos



Site 41 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
41	26.71650	-81.53870	1.2

Aerial photographs



Treatment details

date	4 June	7 June
Treatment (lb)	7	1
dose (lb/acre)	5.8	0.8

Site 42 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
42	26.74171	-81.51926	2.3

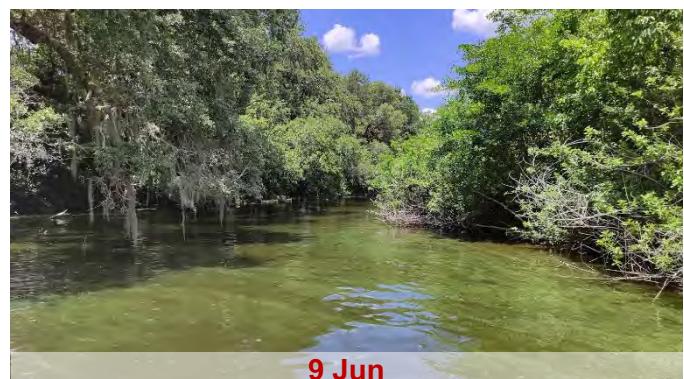
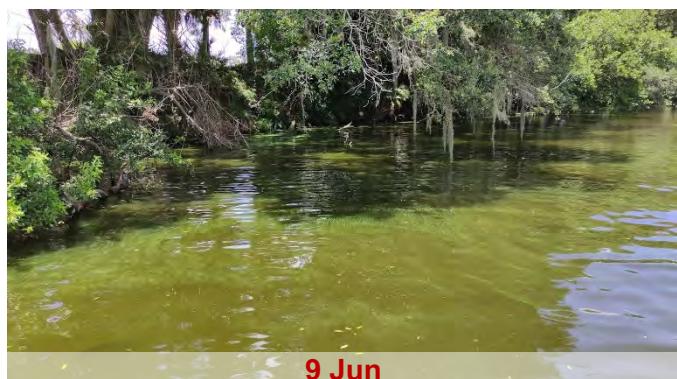
Aerial photographs



Treatment details

date	9 June
Treatment (lb)	20
dose (lb/acre)	8.7

Site photos



Site 43 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
43	26.74294	-81.51729	2.3

Aerial photographs



Treatment details

date	4 June	6 June	8 June	10 June
Treatment (lb)	100	230	50	160
dose (lb/acre)	43.5	100	21.7	69.6

Site #43 photos



4 Jun



4 Jun



5 Jun



5 Jun



5 Jun



5 Jun



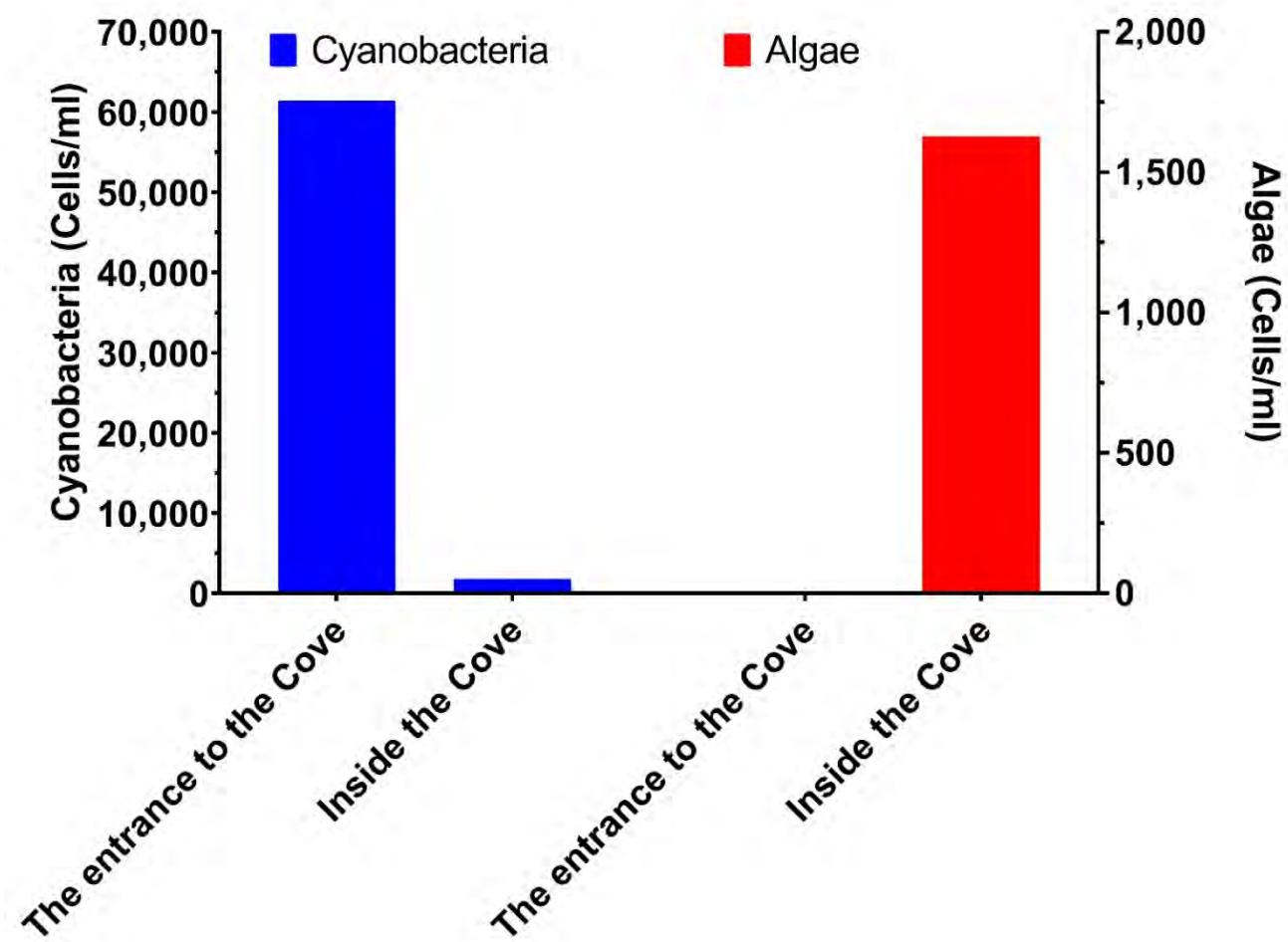
6 Jun



6 Jun



Phytoplankton population composition – Site 43 - for a water sample obtained on June 6th, 2021:

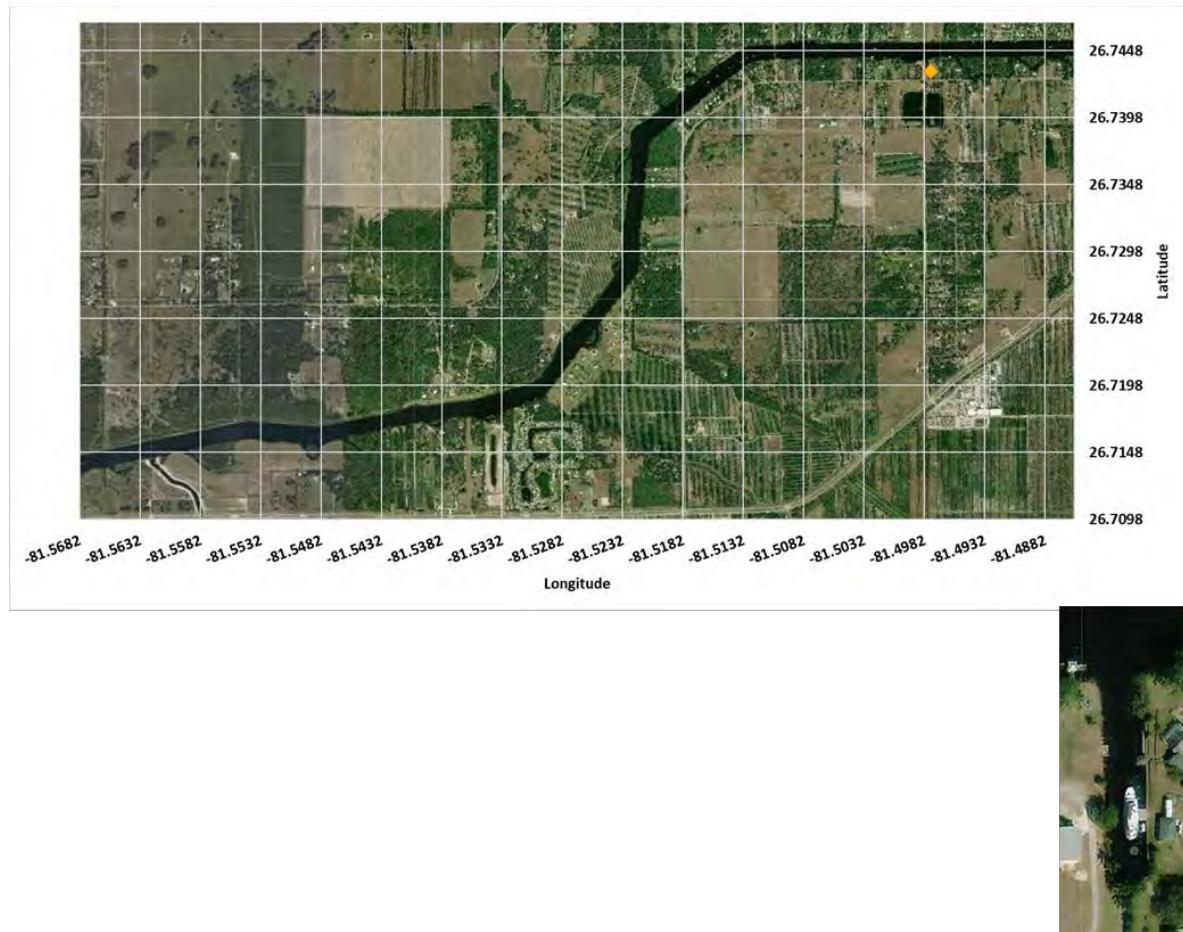


Site 44 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
44	26.74323	-81.49766	0.6

Aerial photographs



Treatment details

date	5 June
Treatment (lb)	25
dose (lb/acre)	41.7

Site photos

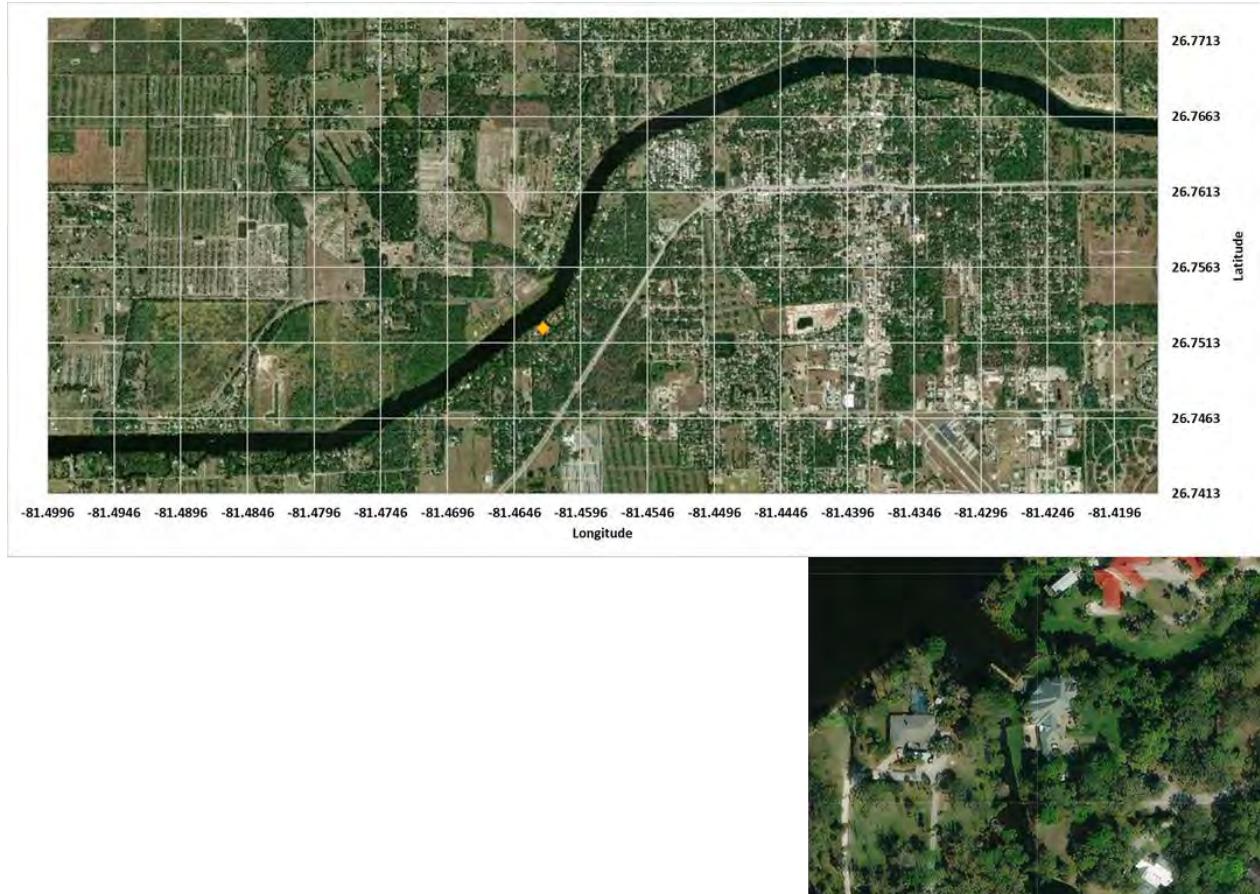


Site 45 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
45	26.75222	-81.46245	1.5

Aerial photographs



Treatment details

date	7 June
Treatment (lb)	50
dose (lb/acre)	33.3

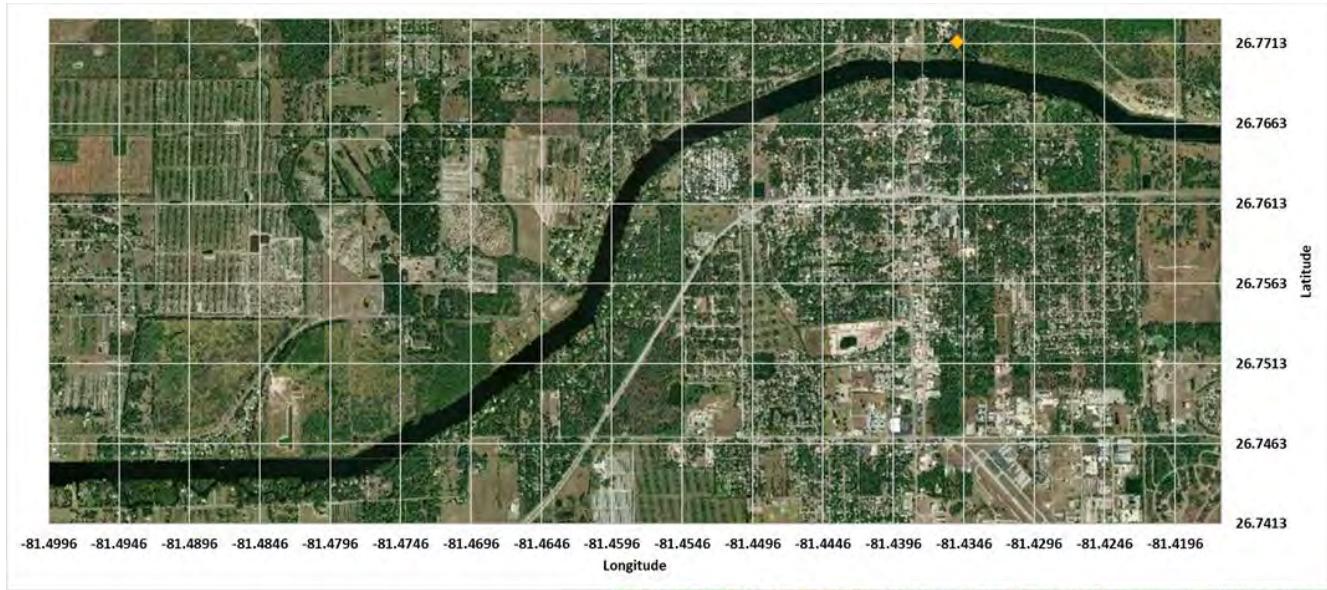
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Site 46 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
46	26.77136	-81.43512	3.9

Aerial photographs



Treatment details

date	7 June
Treatment (lb)	40
dose (lb/acre)	10.3

Site photos



7 Jun

Site 47 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
47	26.76756	-81.42937	0.7

Aerial photographs



Treatment details

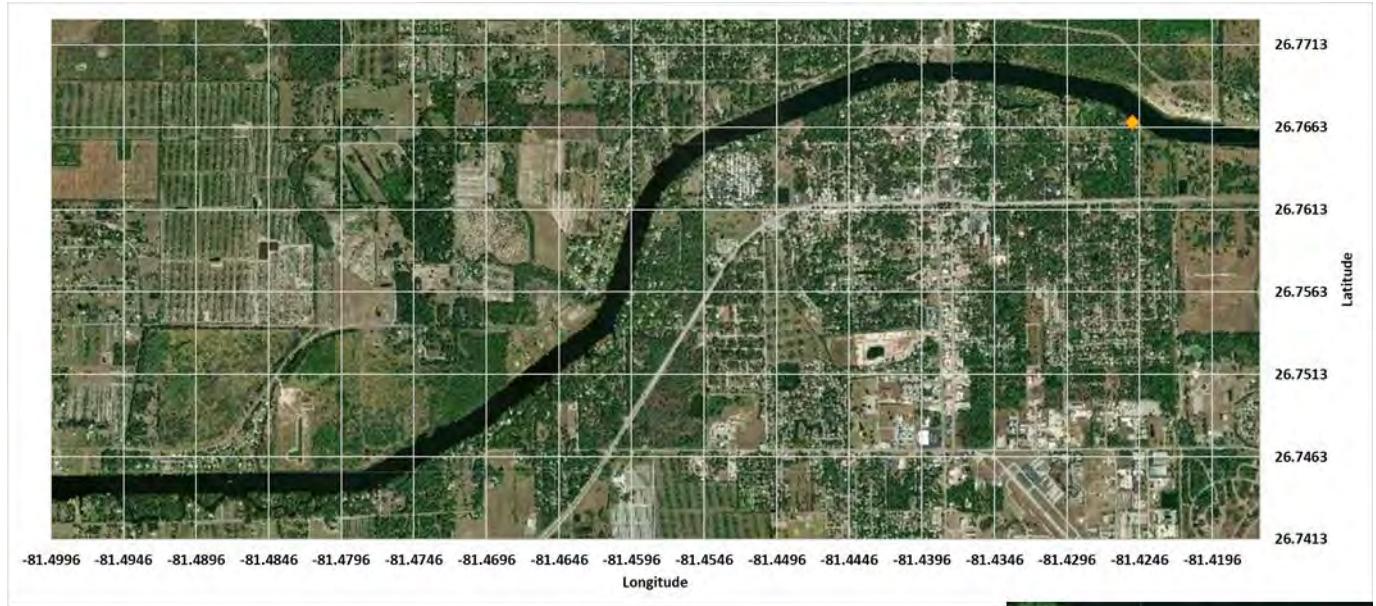
date	7 June
Treatment (lb)	75
dose (lb/acre)	107.1

Site 48 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
48	26.76653	-81.42513	0.6

Aerial photographs



Treatment details

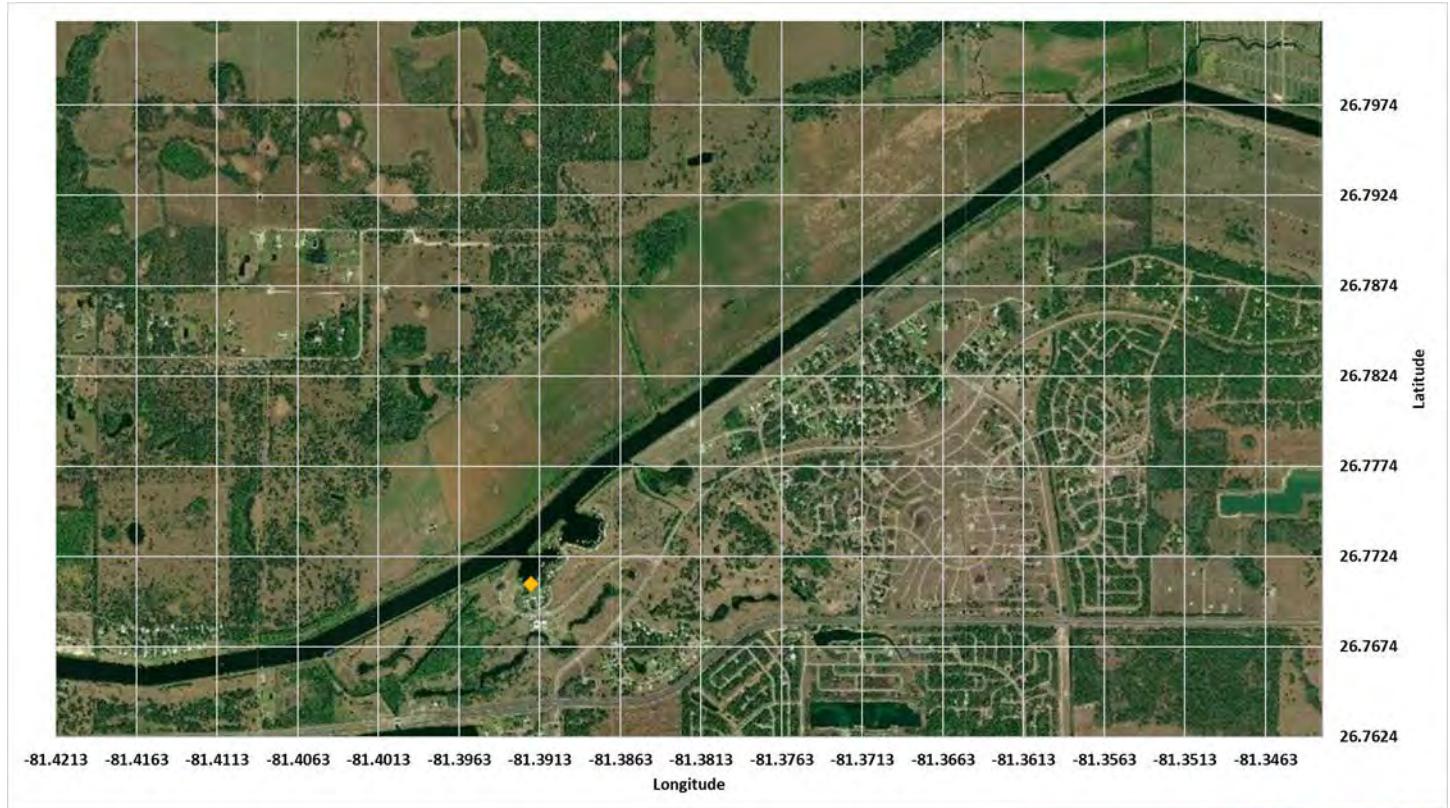
date	8 June
Treatment (lb)	50
dose (lb/acre)	83.3

Site 49 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
49	26.77087	-81.39178	11.5

Aerial photographs



Treatment details

date	8 June
Treatment (lb)	15
dose (lb/acre)	1.3

Site 50 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
50	26.79575	-81.29435	3.2

Aerial photographs



Treatment details

date	7 June	9 June
Treatment (lb)	155	120
dose (lb/acre)	48.4	37.5

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Site #50 photos



Site 51 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
51	26.79428	-81.29254	1.3

Aerial photographs



Treatment details

date	7 June
Treatment (lb)	40
dose (lb/acre)	30.8

Site photos



Site 52 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
52	26.797364	-81.291727	1

Aerial photographs



Treatment details

date	9 June
Treatment (lb)	5
dose (lb/acre)	5.0

Site photos



9 Jun

Site 53 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
53	26.79436	-81.29136	1.6

Aerial photographs



Treatment details

date	7 June	9 June
Treatment (lb)	160	120
dose (lb/acre)	100.0	75.0

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Site #53 photos



7 Jun



7 Jun



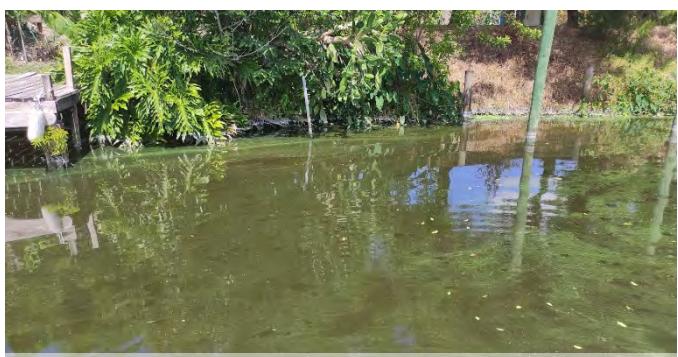
7 Jun



9 Jun



9 Jun



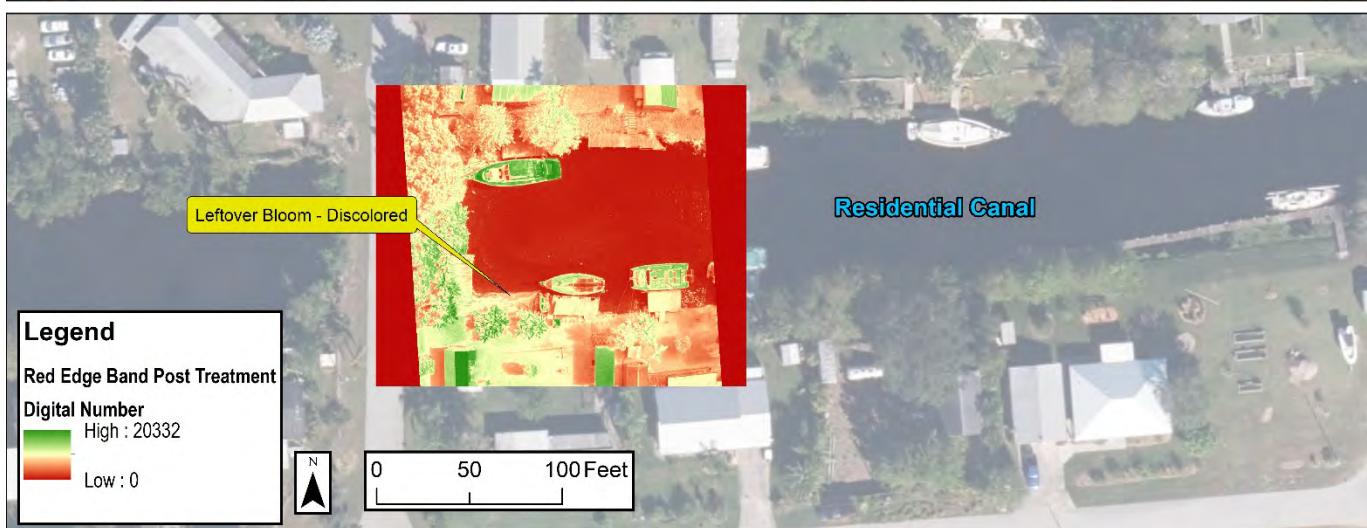
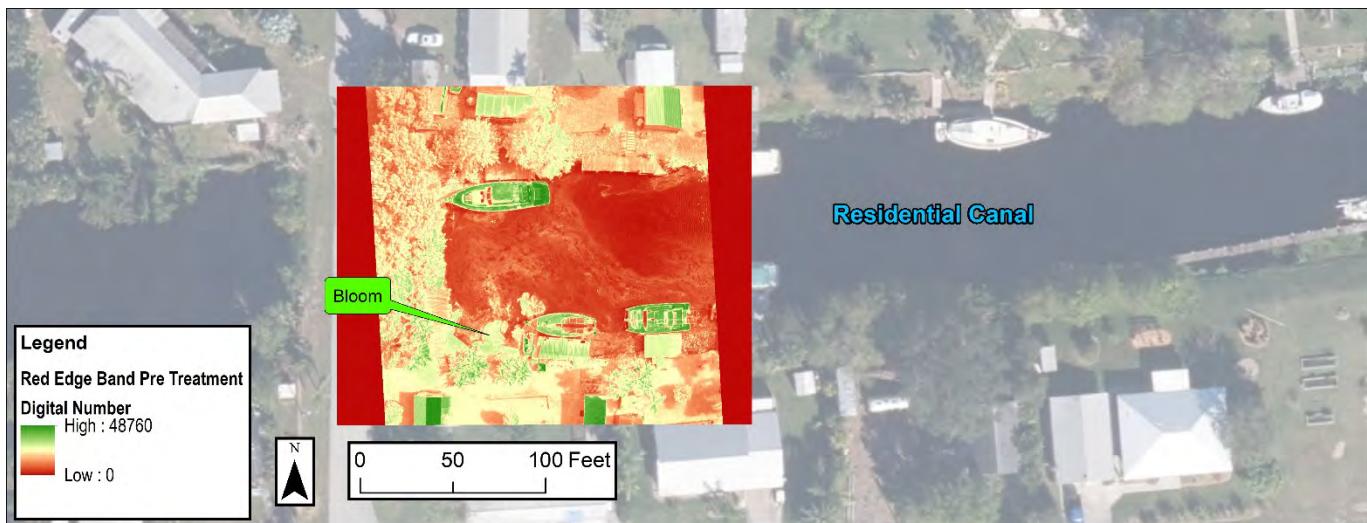
9 Jun



7 Jun



8 Jun



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Site 54 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
54	26.79643	-81.29040	1.3

Aerial photographs



Treatment details

date	7 June	9 June
Treatment (lb)	40	10
dose (lb/acre)	30.8	7.7

Site photos



Site 55 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
55	26.79546	-81.29004	2

Aerial photographs



Treatment details

date	7 June	9 June
Treatment (lb)	60	20
dose (lb/acre)	30.0	10.0

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Site #55 photos



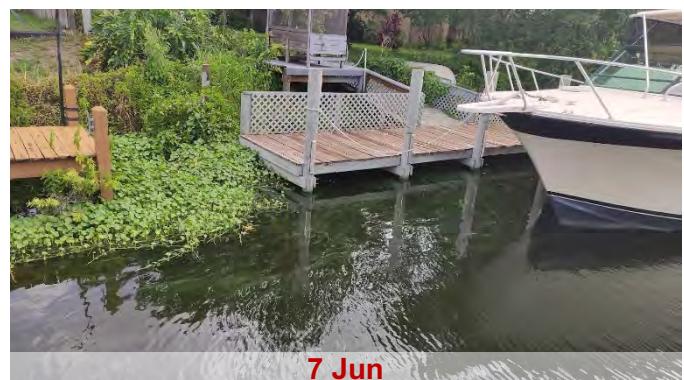
7 Jun



7 Jun



7 Jun



7 Jun



9 Jun



9 Jun



9 Jun



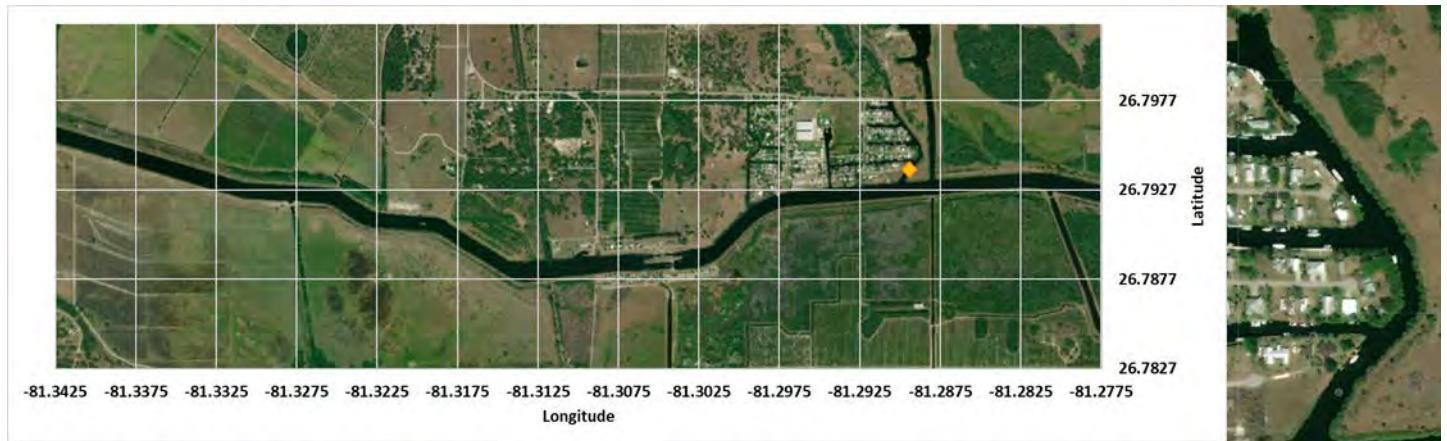
9 Jun

Site 56 – details of the treatment conducted by *BlueGreen/ Modica*

Location and estimated area

# Site	Latitude	Longitude	Estimated area (acre)
56	26.79382	-81.28939	4.7

Aerial photographs



Treatment details

date	7 June	9 June
Treatment (lb)	60	20
dose (lb/acre)	12.8	4.3

Site photos



Appendix B1



aquatic analysis ... research ... consultation

Anatoxin-a, BMAA, Cylindrospermopsin, Microcystins/Nodularins, & Saxitoxins Report

Project: BlueGreen US Water Technologies Inc.

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies Inc.
Address: 1302 Waugh Drive, Ste. 482, Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 2 June 2021
Sample Condition: 5.1 °C upon arrival
Report# 210528_BlueGreen
Date Prepared: 4 June 2021
Prepared by: Kamil Cieslik

Table 1: Samples analyzed and collection dates

<u>Sample ID</u>	<u>Description</u>	<u>Collection Date</u>
1	W.P. Franklin	28 May 2021
2	W.P. Franklin	28 May 2021
3	W.P. Franklin	28 May 2021
4	W.P. Franklin	28 May 2021
5	W.P. Franklin	28 May 2021
9	Caloosahatchee River	28 May 2021

Toxins – Anatoxin-a (ATX), β-N-methylamino-L-alanine (BMAA), Cylindrospermopsin (CYN), Adda Microcystins/Nodularins (MCs/NODs), 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB), Saxitoxin (STX/PSTs)

Abbreviations

NA	Not Applicable	LFSM	Lab Fortified Sample Matrix
MDL	Method Detection Limit	LFSMD	Lab Fortified Sample Matrix Duplicate
MQL	Method Quantification Limit	LD	Lab Duplicate
ND	Not Detected above the MDL	IS	Internal Standard
Blank	Regent Water free from interferences	—	Not Analyzed
LFB	Lab Fortified Blank	MRL	Method Reporting Limit
CCC	Continued Calibration Check	CV	Low-range calibration verification

Sample Preparation

The samples were received and inverted for 60 seconds to mix. Subsets from each sample were removed prior to cell lysis for algal identification and enumeration purposes.

ATX/CYN/BMAA/STX

Subsets (100 mL) were sonicated to release toxins. Aliquots were prepared with IS and LFSMs (Table 2) and filtered (0.2 µm PVDF) prior to analyses.

MCs/NODs by MMPB

Aliquots (2 mL) spiked with IS (d_3 -MMPB) were oxidized by the addition of 1 mL of a solution containing 0.1 M K_2CO_3 , 0.05 M $KMnO_4$ and 0.05 M $NaIO_4$ for 1 hour, stopped with the addition of 40% sodium bisulfite and cleaned using 100 mg Strata X solid phase extraction (SPE). The extracts were reconstituted in DI, filtered (0.2 µm PVDF), and analyzed.

Analytical Techniques

Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)

ATX & CYN

High performance chromatography coupled with tandem mass spectrometry was used for a targeted anatoxin-a and cylindrospermopsin analysis. The $[M+H]^+$ ion for ATX (m/z 166) was fragmented and the product ions (m/z 91, 131, 149) were monitored. The $[M+H]^+$ ion for CYN (m/z 416) was fragmented and the product ions (m/z 194, 274, 336) were monitored. The $[M+H]^+$ ion for the internal standard [$^{13}C_4$]ATX (m/z 171) was fragmented and the product ion (m/z 153) was monitored. The $[M+H]^+$ ion for the internal standard [$^{15}N_5$]CYN (421 m/z) was fragmented and the product ion (m/z 341) was monitored. The internal standard method was utilized for quantification.

MMPB

The $[M-H]^-$ ion of MMPB (m/z 207) was fragmented and the product ion (m/z 131) was monitored. The IS (d_3 -MMPB) was also fragmented and monitored (m/z 210 → 131). The internal standard method was implemented using a standard curve (0.25 – 100 ng/mL of oxidized MC-LR) to calculate LFSM returns.

Enzyme-Linked Immunosorbent Assay (ELISA)

STX

A saxitoxin specific ELISA (Abraxis PN 52255B) was utilized for the detection and quantification of saxitoxin and related analogs (paralytic shellfish toxins – PSTs). The current method reporting limit is 0.05 ng/mL (ppb) based on kit sensitivity and dilution factors. Based on manufacture instructions, the STX ELISA is less cross-reactive to other PSTs and will likely underestimate total PSTs/Saxitoxins. Reported cross-reactivities are as follows: NEO (1.3%), dcSTX (29%), GTX2/3 (23%), GTX5 (23%), dcGTX2/3 (1.4%), dcNEO (0.6%) & GTX1/4 (<0.2%).



Quality Control

Table 2: Quality Assurance/Quality Control (QA/QC) samples (IS and LFSM) prepared for analyses pre-extraction. Additional QA/QC checks included LFBs, continued calibration checks and external curves.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
MC-LR (as MMPB)	2.0	2	LFSM	111%
<i>d</i> ₃ -MMPB	1.0	all aliquots	IS	71 ± 13%
BMAA	50	1	LFSM	110%
BAMA	50	1	LFSM	101%
2,4-DAB	50	1	LFSM	8% ^N
AEG	50	1	LFSM	88%
3,4-DAB	50	1	LFSM	79%
BMAA	50	9	LFSM	114%
BAMA	50	9	LFSM	98%
2,4-DAB	50	9	LFSM	10% ^N
AEG	50	9	LFSM	119%
3,4-DAB	50	9	LFSM	58% ^N
<i>d</i> ₃ -BMAA	50	all aliquots	IS	91 ± 3%
CYN	0.1	2	LFSM	102%
[¹⁵ N ₅]CYN	1.0	all aliquots	IS	79 ± 2%
ATX	0.1	2	LFSM	128%
[¹³ C ₄]ATX	1.0	all aliquots	IS	60 ± 34%
STX	0.2	1	LFSM	103%
STX	0.2	2	LFSM	93%

*Control limits: water LFSM ± 30%; complicated matrix LFSM and when LFSM within 2x MDL ±50%; IS ± 50%

N Qualifier: The low return for 2,4-DAB is likely due to the low response at the analyte concentration that was spiked. All MDLs/MRLs reflect spike returns.

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

Table 3: The percent reproducibility (%RPD) between lab duplicates or LFSM/LFSMDs prepared and analyzed.

QC Type	Sample ID	Analyte	Value 1	Value 2	% RPD	Pass/Fail (<40%)
LDs	5	MMPB	0.86	0.58	39.6%	Pass

%RPD is NA when values are below the method reporting/detection limit

Table 4: Raw ELISA Data

Sample ID	Analyte	Dilution Factor	Assay Values (ng/mL)	CV	Concentration (ng/mL)	Average (ng/mL)
1	STX	1	0.08	2.7%	0.08	0.08
		1	0.08		0.08	
2	STX	1	0.11	2.2%	0.11	0.11
		1	0.11		0.11	
3	STX	1	0.08	9.0%	0.08	0.09
		1	0.09		0.09	
4	STX	1	0.08	1.8%	0.08	0.09
		1	0.09		0.09	
5	STX	1	0.09	2.9%	0.09	0.09
		1	0.09		0.09	
9	STX	1	0.08	3.3%	0.08	0.08
		1	0.08		0.08	

Table 5: STX-ELISA Quality Control Value Table

Date Analyzed:	4 June 2021	Requirement	Pass/Fail
R ² value:	0.997	≥0.98	PASS
%CV range STDs:	0.1-2.1%	≤15%	PASS
LFB (0.2 ppb) recovery:	98%	±40% True Value	PASS
%CV range LFB:	3.0%	<20%	PASS
Low CCC (0.05 ppb) recovery:	90%	±50% True Value	PASS
LRB	<0.03	<0.03	PASS

Table 6: STX-ELISA Quality Control Value Table

Date Analyzed:	11 June 2021	Requirement	Pass/Fail
R ² value:	0.999	≥0.98	PASS
%CV range STDs:	0.3-5.0%	≤15%	PASS
LFB (0.2 ppb) recovery:	78%	±40% True Value	PASS
%CV range LFB:	3.7%	<20%	PASS
Low CCC (0.05 ppb) recovery:	80%	±50% True Value	PASS
LRB	<0.03	<0.03	PASS

Summary of Results

Table 7: Summary of results for total microcystins/nodularins (MCs/NODs) as measured through the MMPB method, anatoxin-a (ATX), cylindrospermopsin (CYN), β -N-methylamino-L-alanine (BMAA), and saxitoxins (STX). All data is reported as ng/mL (ppb).

Sample	MCs/NODs (ng/mL)	ATX (ng/mL)	CYN (ng/mL)	BMAA (ng/mL)	STX (ng/mL)
1	8.20	—	—	ND	0.08
2	2.33	ND	ND	ND	0.11
3	2.44	—	—	ND	0.09
4	0.91	—	—	ND	0.09
5	0.72 ± 0.20	—	—	ND	0.09
9	3.76	ND	ND	ND	0.08
<i>MRL (ng/mL):</i>	0.06	0.05	0.05	5.0	0.05
<i>Analyst Initials:</i>	AF	MA	MA	AF	KC
<i>Date Analyzed:</i>	6/14/2021	6/4/2021	6/4/2021	6/7/2021	6/4/2021
					6/11/2021

Interpretations:

Anatoxin-a, cylindrospermopsin, and BMAA were below method detection limits in all samples tested. Microcystins/nodularins were above the current ‘EPA Recommended Value for Recreational Criteria and Swimming Advisory’ of 8.0 ng/mL (ppb) (EPA, 2019) in the 1 – WP Franklin sample. Saxitoxins were detected in all samples at sub-ppb levels using a saxitoxin specific ELISA.

Submitted by:

Mark T. Aubel, Ph.D.

Lab Director

Date:

June 18, 2021

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Appendix B2



aquatic analysis ... research ... consultation

Potentially Toxigenic (PTOX) Cyanobacteria Report

Project: BlueGreen Water Tech

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies, Inc
Address: #428, 1302 Waugh Dr., Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 2 June 2021
Sample Condition: 5.1 °C upon arrival
Report# 210528_PTOX_BlueGreen
Date Prepared: 2 June 2021
Prepared by: Alyssa Garvey

<u>Sample ID</u>	<u>Sites</u>	<u>Collected</u>
1	W.P. Franklin	5/28/21
2	W.P. Franklin	5/28/21
3	W.P. Franklin	5/28/21
4	W.P. Franklin	5/28/21
5	W.P. Franklin	5/28/21
9	Caloosahatchee River	5/28/21

Method

A one mL aliquot of each non-preserved sample was prepared using a Sedgewick Rafter cell. The samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs.

Results

1

The potentially toxigenic (PTOX) cyanobacteria *Cuspidothrix* sp. (≥ 15 filaments per mL), *Dolichospermum* sp. (≥ 10 partial filaments per mL), *Microcystis* sp. (≥ 9 colonies per mL), *Raphidiopsis* sp. (> 5 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (> 5 filaments per mL), and *Pseudanabaena* sp. (< 100 filaments per mL) were observed.

Cyano
LAB

2

The PTOX cyanobacteria *Cuspidothrix* sp. (>20 filaments per mL), *Dolichospermum* sp. (>15 partial filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>15 filaments per mL), *Microcystis* sp. (>10 colonies per mL), *Raphidiopsis* sp. (>5 filaments per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

3

The PTOX cyanobacteria *Dolichospermum* sp. (>20 filaments per mL), *Cuspidothrix* sp. (>15 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>10 filaments per mL), *Microcystis* sp. (>10 colonies per mL), *Raphidiopsis* sp. (>5 filaments per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

4

The PTOX cyanobacteria *Cuspidothrix* sp. (>30 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>10 filaments per mL), *Microcystis* sp. (>7 colonies per mL), *Raphidiopsis* sp. (>5 filaments per mL), *Dolichospermum* sp. (>3 partial filaments per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

5

The PTOX cyanobacteria *Cuspidothrix* sp. (>30 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>10 filaments per mL), *Microcystis* sp. (>7 colonies per mL), *Dolichospermum* sp. (>5 partial filaments per mL), *Raphidiopsis* sp. (>5 filaments per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

9

The PTOX cyanobacteria *Dolichospermum* sp. (>15 partial filaments per mL), *Microcystis* sp. (>7 colonies per mL), *Cuspidothrix* sp. (>5 filaments per mL), cf. *Aphanizomenon* sp. (>5 filaments per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

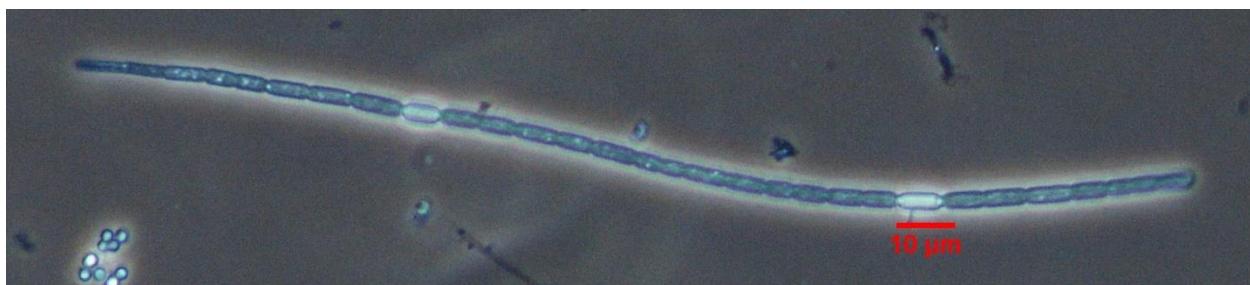
Potential toxin producing genera observed include:

Microcystins	Saxitoxins	Anatoxin-a	Cylindrospermopsin
<i>Pseudanabaena</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>
<i>Dolichospermum</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>
<i>Microcystis</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>
<i>Sphaerospermopsis</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>
	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>
	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>

Recommendations

Based on these observations, analysis for microcystins is recommended for **all** samples. Additional screening for saxitoxins, anatoxin-a, and cylindrospermopsin is recommended for samples **2** and **9**.

Micrographs



Cuspidothrix sp. at 400X (1)

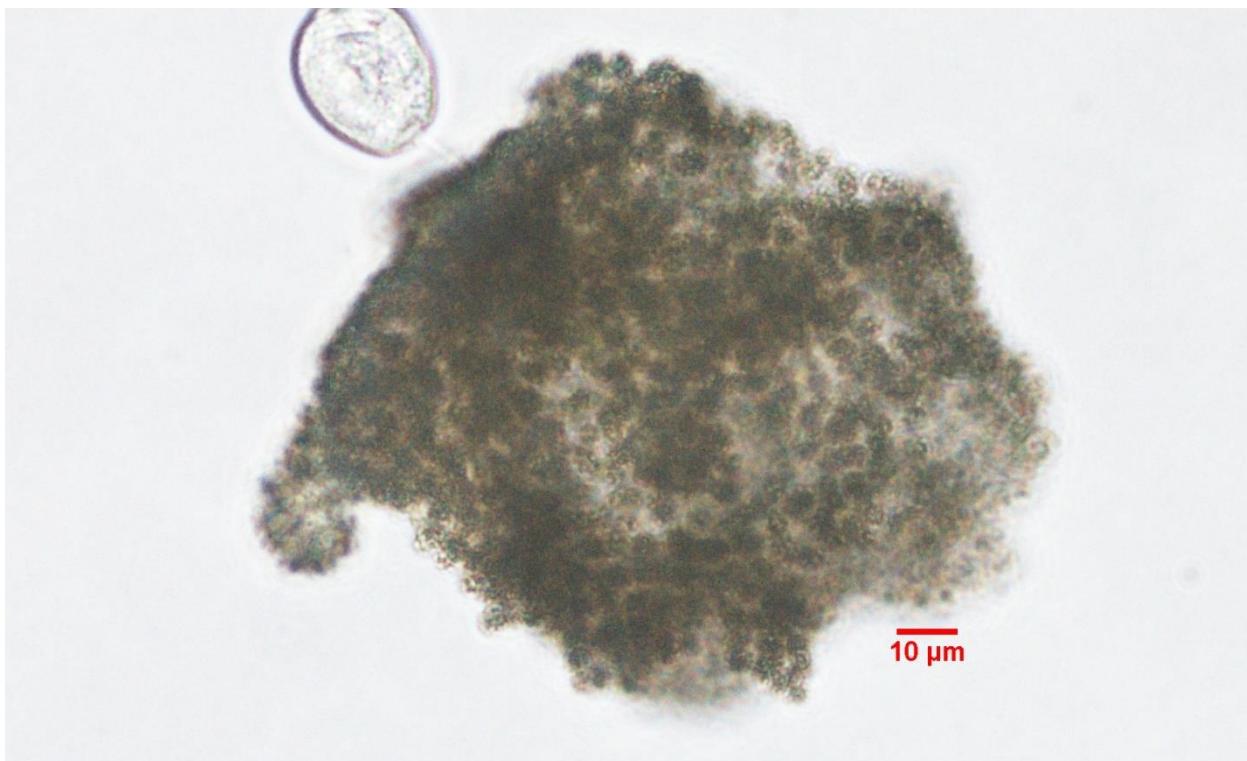


Dolichospermum sp. at 400X (1)



Aphanizomenon/Chrysosporum/Sphaerospermopsis sp. at 400X (2)

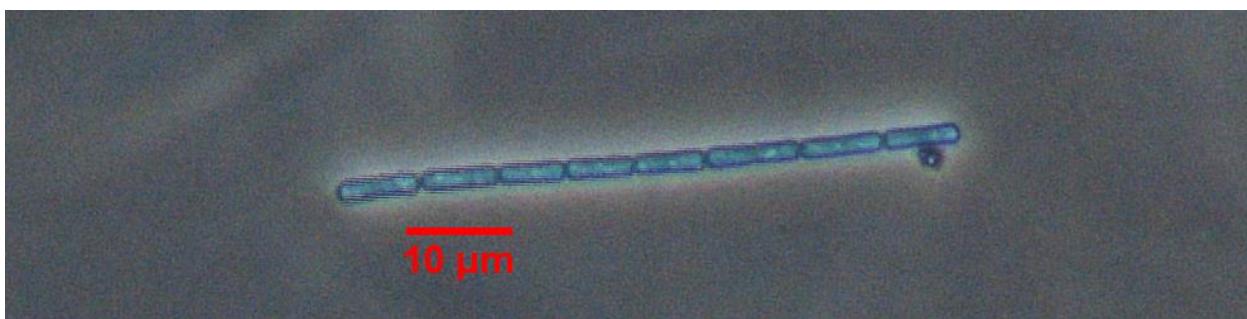
Cyano
LAB



Microcystis sp. at 400X (1)



Raphidiopsis sp. at 400X (1)

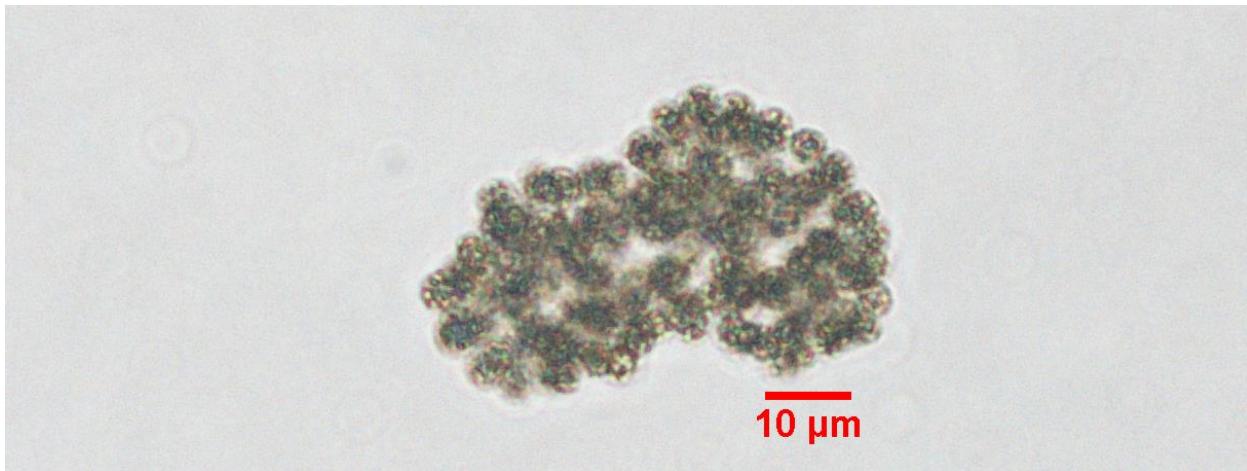


Pseudanabaena sp. at 400X (1)

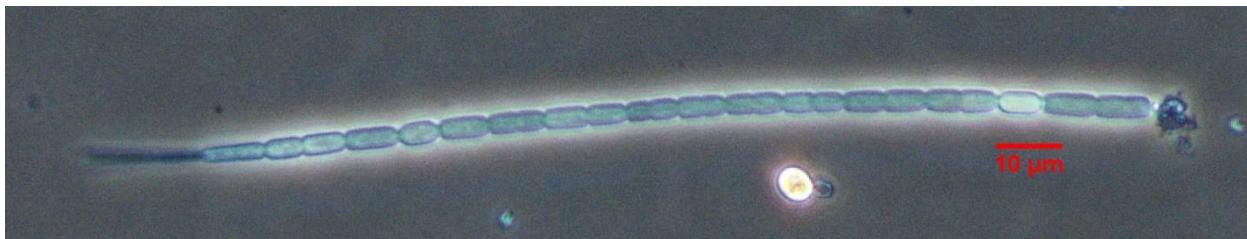
Cyano
LAB



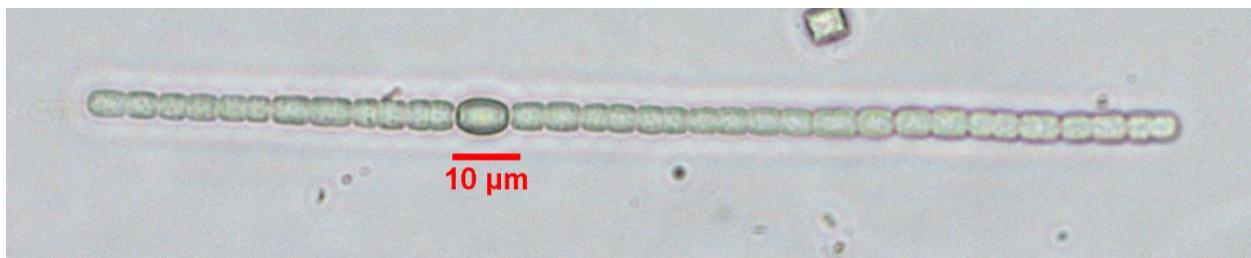
Dolichospermum sp. at 400X (9)



Microcystis sp. at 400X (9)



Cuspidothrix sp. at 400X (9)



cf. *Aphanizomenon* sp. at 400X (9)



Pseudanabaena sp. at 400X (9)

Submitted by:

Amanda Foss

Date:

June 2, 2021

Amanda Foss, M.S.

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Appendix B3



aquatic analysis ... research ... consultation

Microcystins/Nodularins & Saxitoxins Report

Project: BlueGreen US Water Technologies Inc.

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies Inc.
Address: 1302 Waugh Drive, Ste. 482, Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 2 June 2021
Sample Condition: 4.6 °C upon arrival
Report# 210529_BlueGreen
Date Prepared: 18 June 2021
Prepared by: Amanda Foss

Table 1: Samples analyzed and collection dates

<u>Sample ID</u>	<u>Description</u>	<u>Collection Date</u>
1	W.P. Franklin	29 May 2021
2	W.P. Franklin	29 May 2021
3	W.P. Franklin	29 May 2021
4	W.P. Franklin	29 May 2021
5	W.P. Franklin	29 May 2021
6	W.P. Franklin	29 May 2021
7	W.P. Franklin	29 May 2021
8	W.P. Franklin	29 May 2021

Toxins –Adda Microcystins/Nodularins (MCs/NODs), 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB), Saxitoxin (STX/PSTs)

Abbreviations

NA	Not Applicable	LFSM	Lab Fortified Sample Matrix
MDL	Method Detection Limit	LFSMD	Lab Fortified Sample Matrix Duplicate
MQL	Method Quantification Limit	LD	Lab Duplicate
ND	Not Detected above the MDL	IS	Internal Standard
Blank	Regent Water free from interferences	—	Not Analyzed
LFB	Lab Fortified Blank	MRL	Method Reporting Limit
CCC	Continued Calibration Check	CV	Low-range calibration verification



Sample Preparation

The samples were received and inverted for 60 seconds to mix. Subsets from each sample were removed prior to cell lysis for algal identification and enumeration purposes.

STX

Subsets (100 mL) were sonicated to release toxins. Aliquots were prepared with IS and LFSMs (Table 2) and filtered (0.2 µm PVDF) prior to analyses.

MCs/NODs by MMPB

Aliquots (2 mL) spiked with IS (d_3 -MMPB) were oxidized by the addition of 1 mL of a solution containing 0.1 M K_2CO_3 , 0.05 M $KMnO_4$ and 0.05 M $NaIO_4$ for 1 hour, stopped with the addition of 40% sodium bisulfite and cleaned using 100 mg Strata X solid phase extraction (SPE). The extracts were reconstituted in DI, filtered (0.2 µm PVDF), and analyzed.

Analytical Techniques

Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)

MMPB

The [M-H]⁻ ion of MMPB (m/z 207) was fragmented and the product ion (m/z 131) was monitored. The IS (d_3 -MMPB) was also fragmented and monitored (m/z 210 → 131). The internal standard method was implemented using a standard curve (0.25 – 100 ng/mL of oxidized MC-LR) to calculate LFSM returns.

Enzyme-Linked Immunosorbent Assay (ELISA)

STX

A saxitoxin specific ELISA (Abraxis PN 52255B) was utilized for the detection and quantification of saxitoxin and related analogs (paralytic shellfish toxins – PSTs). The current method reporting limit is 0.05 ng/mL (ppb) based on kit sensitivity and dilution factors. Based on manufacture instructions, the STX ELISA is less cross-reactive to other PSTs and will likely underestimate total PSTs/Saxitoxins. Reported cross-reactivities are as follows: NEO (1.3%), dcSTX (29%), GTX2/3 (23%), GTX5 (23%), dcGTX2/3 (1.4%), dcNEO (0.6%) & GTX1/4 (<0.2%).

Quality Control

Table 2: Quality Assurance/Quality Control (QA/QC) samples (IS and LFSM) prepared for analyses pre-extraction. Additional QA/QC checks included LFBs, continued calibration checks and external curves.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
MC-LR (as MMPB)	2.0	5	LFSM	135% ^N
MC-LR (as MMPB)	2.0	8	LFSM	85%
<i>d</i> ₃ -MMPB	1.0	all aliquots	IS	71 ± 13%
STX	0.2	1	LFSM	100%
STX	0.2	6	LFSM	103%

*Control limits: water LFSM ± 30%; complicated matrix LFSM and when LFSM within 2x MDL ±50%; IS ± 50%

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

Table 3: Raw ELISA Data

Sample ID	Analyte	Dilution Factor	Assay Values (ng/mL)	CV	Concentration (ng/mL)	Average (ng/mL)
1	STX	1	0.08	5.8%	0.08	0.09
		1	0.09		0.09	
1 LFSM	STX	1	0.29	2.5%	0.29	0.29
		1	0.28		0.28	
2	STX	1	0.05	38.4%	0.05	0.07
		1	0.08		0.08	
3	STX	1	0.09	0.9%	0.09	0.09
		1	0.09		0.09	
4	STX	1	0.10	1.5%	0.10	0.10
		1	0.10		0.10	
5	STX	1	0.10	3.4%	0.10	0.10
		1	0.09		0.09	
6	STX	1	0.07	0.6%	0.07	0.07
		1	0.07		0.07	
6 LFSM	STX	1	0.27	2.0%	0.27	0.28
		1	0.28		0.28	
7	STX	1	0.09	3.1%	0.09	0.09
		1	0.09		0.09	
8	STX	1	0.09	5.5%	0.09	0.09
		1	0.08		0.08	

Table 4: STX-ELISA Quality Control Value Table

Date Analyzed:	11 June 2021	Requirement	Pass/Fail
R ² value:	0.999	≥0.98	PASS
%CV range STDs:	0.3-5.0%	≤15%	PASS
LFB (0.2 ppb) recovery:	78%	±40% True Value	PASS
%CV range LFB:	3.7%	<20%	PASS
Low CCC (0.05 ppb) recovery:	80%	±50% True Value	PASS
LRB	<0.03	<0.03	PASS

Summary of Results

Table 6: Summary of results for total microcystins/nodularins (MCs/NODs) as measured through the MMPB method and saxitoxins (STX). All data is reported as ng/mL (ppb).

Sample	MCs/NODs (ng/mL)	STX (ng/mL)
1	7.94	0.09
2	3.59	0.07
3	0.99	0.09
4	1.01	0.10
5	1.54	0.10
6	310	0.07
7	1.55	0.09
8	1.46	0.09
<i>MRL (ng/mL):</i>	0.06	0.05
<i>Analyst Initials:</i>	AF	KC
<i>Date Analyzed:</i>	6/16/2021	6/11/2021

Interpretations:

Microcystins/nodularins were above the current ‘EPA Recommended Value for Recreational Criteria and Swimming Advisory’ of 8.0 ng/mL (ppb) (EPA, 2019) in the 6 – W.P. Franklin sample. Saxitoxins were detected in all samples at sub-ppb levels using a saxitoxin specific ELISA.

Submitted by:



Mark T. Aubel, Ph.D.

Lab Director

Date: June 18, 2021

*The results in this report relate only to the samples listed above.
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Appendix B4



aquatic analysis ... research ... consultation

Potentially Toxigenic (PTOX) Cyanobacteria Report

Project: BlueGreen Water Tech

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies, Inc
Address: #428, 1302 Waugh Dr., Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 2 June 2021
Sample Condition: 4.6 °C upon arrival
Report# 210529_PTOX_BlueGreen
Date Prepared: 2 June 2021
Prepared by: Alyssa Garvey

Sample ID	Sites	Collected
1	W.P. Franklin	5/29/21
2	W.P. Franklin	5/29/21
3	W.P. Franklin	5/29/21
4	W.P. Franklin	5/29/21
5	W.P. Franklin	5/29/21
6	W.P. Franklin	5/29/21
7	W.P. Franklin	5/29/21
8	W.P. Franklin	5/29/21

Method

A one mL aliquot of each non-preserved sample was prepared using a Sedgewick Rafter cell. The samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs.

Results

1

The potentially toxigenic (PTOX) cyanobacteria *Dolichospermum* sp. (>20 filaments per mL; mostly partial), *Raphidiopsis* sp. (>20 filaments per mL), *Cuspidothrix* sp. (>15 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>10 filaments per mL), *Microcystis* sp. (>10 colonies per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

Cyano
LAB

2

The PTOX cyanobacteria *Dolichospermum* sp. (>30 filaments per mL; mostly partial), *Cuspidothrix* sp. (>15 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>15 filaments per mL), *Raphidiopsis* sp. (>10 filaments per mL), *Microcystis* sp. (≥ 4 colonies per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

3

The PTOX cyanobacteria *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>25 filaments per mL), *Cuspidothrix* sp. (>25 filaments per mL), *Raphidiopsis* sp. (>15 filaments per mL), *Microcystis* sp. (≥ 5 colonies per mL), *Dolichospermum* sp. (≥ 4 partial filaments), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

4

The PTOX cyanobacteria *Raphidiopsis* sp. (>30 filaments per mL; straight and coiled morphologies), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>25 filaments per mL), *Cuspidothrix* sp. (>15 filaments per mL), *Dolichospermum* sp. (>10 filaments per mL), *Microcystis* sp. (≥ 3 colonies per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

5

The PTOX cyanobacteria *Cuspidothrix* sp. (≥ 25 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>20 filaments per mL), *Raphidiopsis* sp. (>15 filaments per mL), *Dolichospermum* sp. (>5 partial filaments per mL), *Microcystis* sp. (≥ 5 colonies per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

6

The sample was dominated by the PTOX cyanobacterium *Microcystis* sp. (>200 colonies per mL). Other PTOX cyanobacteria observed included *Dolichospermum* sp. (>30 filaments per mL; mostly partial), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>15 filaments per mL), *Cuspidothrix* sp. (>10 filaments per mL), *Raphidiopsis* sp. (>5 filaments per mL), and *Pseudanabaena* sp. (<100 filaments per mL).

7

The PTOX cyanobacteria *Cuspidothrix* sp. (>30 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>25 filaments per mL), *Dolichospermum* sp. (≥ 20 partial filaments per mL), *Raphidiopsis* sp. (>10 filaments per mL), *Microcystis* sp. (≥ 4 colonies per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

8

The PTOX cyanobacteria *Cuspidothrix* sp. (>30 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>20 filaments per mL), *Raphidiopsis* sp. (>15 filaments per mL), *Dolichospermum* sp. (>5 partial filaments per mL), *Microcystis* sp. (≥ 5 colonies per mL), and *Pseudanabaena* sp. (<100 filaments per mL) were observed.

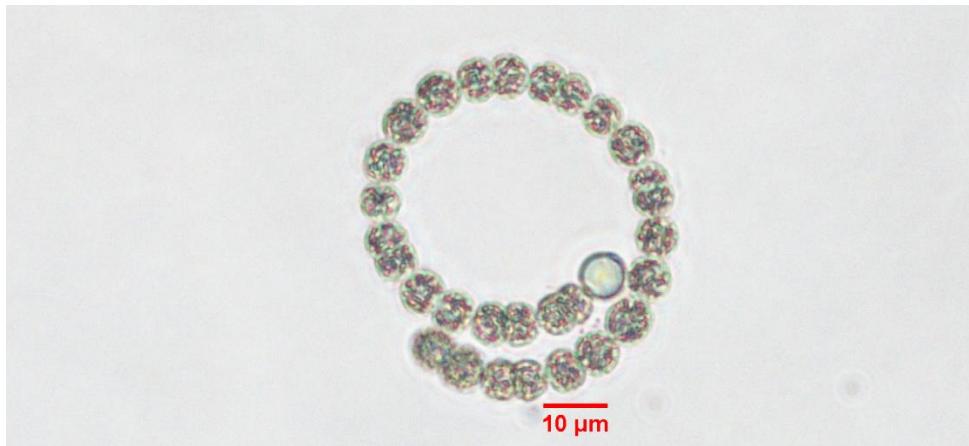
Potential toxin producing genera observed include:

Microcystins	Saxitoxins	Anatoxin-a	Cylindrospermopsin
<i>Pseudanabaena</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>
<i>Dolichospermum</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>
<i>Microcystis</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>
<i>Sphaerospermopsis</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>
	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>
	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>

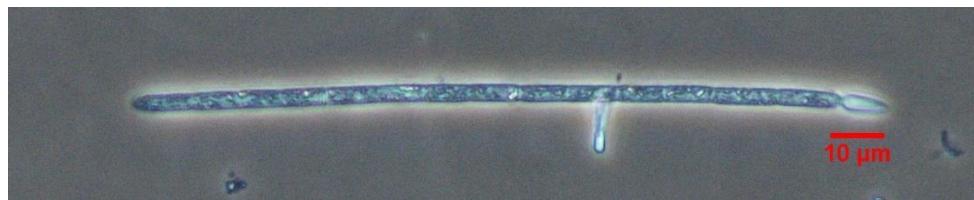
Recommendations

Based on the observed density of the PTOX cyanobacterium *Microcystis*, analysis for microcystins is recommended for sample **6**. Toxin recommendations for the other samples in this set are pending results from the previous sample collection (5/28/2021).

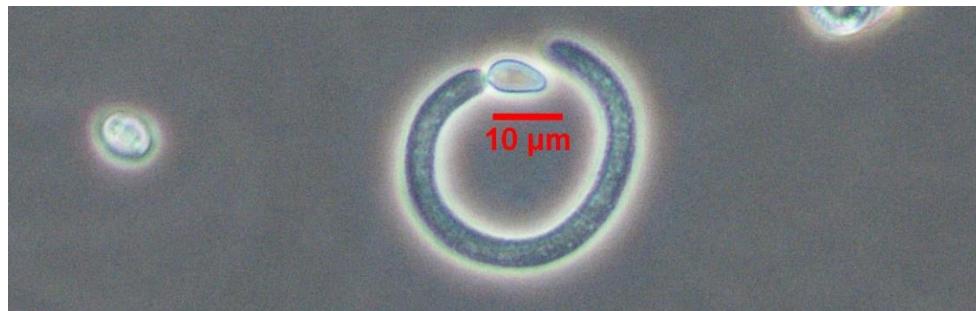
Micrographs



Dolichospermum sp. at 400X (1)



Raphidiopsis sp. (straight) at 400X (1)



Raphidiopsis sp. (coiled) at 400X (4)

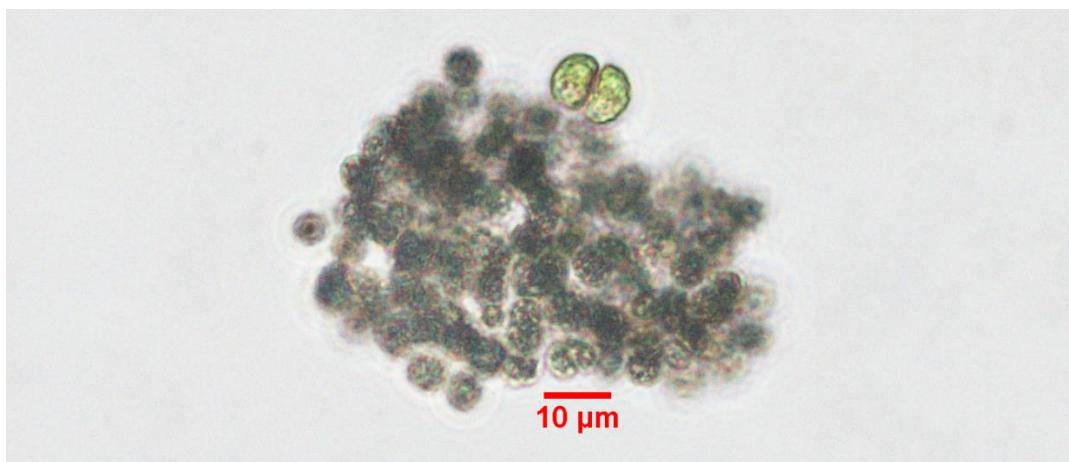


Cuspidothrix sp. at 400X (1)

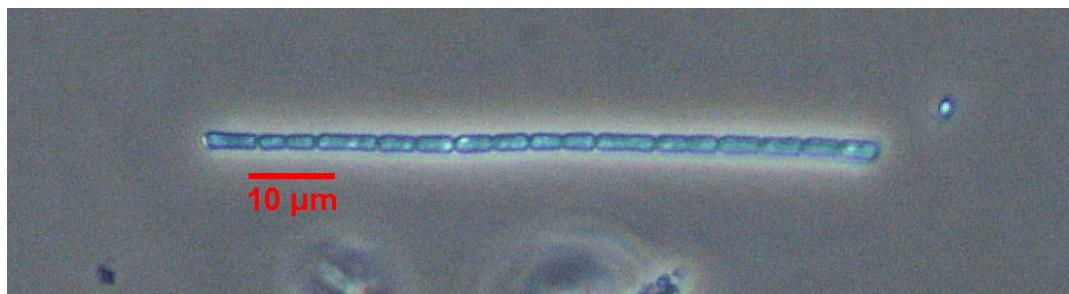
Cyano
LAB



Aphanizomenon/Chrysosporum/Sphaerospermopsis sp. at 400X (2)



Microcystis sp. at 400X (1)



Pseudanabaena sp. at 400X (1)

Submitted by:

Amanda Foss

Date:

June 2, 2021

Amanda Foss, M.S.

*The results in this report relate only to the samples listed above.
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Appendix B5



aquatic analysis ... research ... consultation

Microcystins/Nodularins & Saxitoxins Report

Project: BlueGreen US Water Technologies Inc.

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies Inc.
Address: 1302 Waugh Drive, Ste. 482, Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 2 June 2021
Sample Condition: 4.4 °C upon arrival
Report# 210530_BlueGreen
Date Prepared: 18 June 2021
Prepared by: Amanda Foss

Table 1: Samples analyzed and collection dates

<u>Sample ID</u>	<u>Description</u>	<u>Collection Date</u>
1	W.P. Franklin	30 May 2021
2	W.P. Franklin	30 May 2021
3	W.P. Franklin	30 May 2021
4	W.P. Franklin	30 May 2021
5	W.P. Franklin	30 May 2021
6	W.P. Franklin	30 May 2021
7	W.P. Franklin	30 May 2021
8	W.P. Franklin	30 May 2021

Toxins –Adda Microcystins/Nodularins (MCs/NODs), 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB), Saxitoxin (STX/PSTs)

Abbreviations

NA	Not Applicable	LFSM	Lab Fortified Sample Matrix
MDL	Method Detection Limit	LFSMD	Lab Fortified Sample Matrix Duplicate
MQL	Method Quantification Limit	LD	Lab Duplicate
ND	Not Detected above the MDL	IS	Internal Standard
Blank	Regent Water free from interferences	—	Not Analyzed
LFB	Lab Fortified Blank	MRL	Method Reporting Limit
CCC	Continued Calibration Check	CV	Low-range calibration verification



Sample Preparation

The samples were received and inverted for 60 seconds to mix. Subsets from each sample were removed prior to cell lysis for algal identification and enumeration purposes.

STX

Subsets (100 mL) were sonicated to release toxins. Aliquots were prepared with IS and LFSMs (Table 2) and filtered (0.2 µm PVDF) prior to analyses.

MCs/NODs by MMPB

Aliquots (2 mL) spiked with IS (d_3 -MMPB) were oxidized by the addition of 1 mL of a solution containing 0.1 M K_2CO_3 , 0.05 M $KMnO_4$ and 0.05 M $NaIO_4$ for 1 hour, stopped with the addition of 40% sodium bisulfite and cleaned using 100 mg Strata X solid phase extraction (SPE). The extracts were reconstituted in DI, filtered (0.2 µm PVDF), and analyzed.

Analytical Techniques

Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)

MMPB

The [M-H]⁻ ion of MMPB (m/z 207) was fragmented and the product ion (m/z 131) was monitored. The IS (d_3 -MMPB) was also fragmented and monitored (m/z 210 → 131). The internal standard method was implemented using a standard curve (0.25 – 100 ng/mL of oxidized MC-LR) to calculate LFSM returns.

Enzyme-Linked Immunosorbent Assay (ELISA)

STX

A saxitoxin specific ELISA (Abraxis PN 52255B) was utilized for the detection and quantification of saxitoxin and related analogs (paralytic shellfish toxins – PSTs). The current method reporting limit is 0.05 ng/mL (ppb) based on kit sensitivity and dilution factors. Based on manufacture instructions, the STX ELISA is less cross-reactive to other PSTs and will likely underestimate total PSTs/Saxitoxins. Reported cross-reactivities are as follows: NEO (1.3%), dcSTX (29%), GTX2/3 (23%), GTX5 (23%), dcGTX2/3 (1.4%), dcNEO (0.6%) & GTX1/4 (<0.2%).

Quality Control

Table 2: Quality Assurance/Quality Control (QA/QC) samples (IS and LFSM) prepared for analyses pre-extraction. Additional QA/QC checks included LFBs, continued calibration checks and external curves.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
MC-LR (as MMPB)	2.0	2	LFSM	119%
MC-LR (as MMPB)	2.0	5	LFSM	106%
MC-LR (as MMPB)	2.0	7	LFSM	114%
<i>d</i> ₃ -MMPB	1.0	all aliquots	IS	60 ± 18%
STX	0.2	1	LFSM	100%
STX	0.2	6	LFSM	103%

*Control limits: water LFSM ± 30%; complicated matrix LFSM and when LFSM within 2x MDL ±50%; IS ± 50%

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

Table 3: Raw ELISA Data

Sample ID	Analyte	Dilution Factor	Assay Values (ng/mL)	CV	Concentration (ng/mL)	Average (ng/mL)
1	STX	1	0.11	23.2%	0.11	0.10
		1	0.08		0.08	
1 LFSM	STX	1	0.29	1.2%	0.29	0.30
		1	0.30		0.30	
2	STX	1	0.10	1.0%	0.10	0.10
		1	0.10		0.10	
3	STX	1	0.10	0.3%	0.10	0.10
		1	0.10		0.10	
4	STX	1	0.09	9.7%	0.09	0.10
		1	0.11		0.11	
5	STX	1	0.11	2.1%	0.11	0.11
		1	0.10		0.10	
6	STX	1	0.09	8.5%	0.09	0.09
		1	0.08		0.08	
6 LFSM	STX	1	0.27	8.5%	0.27	0.29
		1	0.31		0.31	
7	STX	1	0.08	3.8%	0.08	0.08
		1	0.08		0.08	
8	STX	1	0.07	11.5%	0.07	0.08
		1	0.08		0.08	

Table 4: STX-ELISA Quality Control Value Table

Date Analyzed:	15 June 2021	Requirement	Pass/Fail
R ² value:	0.998	≥0.98	PASS
%CV range STDs:	0.7-4.4%	≤15%	PASS
LFB (0.2 ppb) recovery:	84%	±40% True Value	PASS
%CV range LFB:	7.1%	<20%	PASS
Low CCC (0.05 ppb) recovery:	70%	±50% True Value	PASS
LRB	<0.03	<0.03	PASS

Summary of Results

Table 6: Summary of results for total microcystins/nodularins (MCs/NODs) as measured through the MMPB method and saxitoxins (STX). All data is reported as ng/mL (ppb).

Sample	MCs/NODs (ng/mL)	STX (ng/mL)
1	0.27	0.10
2	1.04	0.10
3	0.80	0.10
4	2.91	0.10
5	1.85	0.11
6	1.76	0.09
7	0.70	0.08
8	0.73	0.08
<i>MRL (ng/mL):</i>	0.06	0.05
<i>Analyst Initials:</i>	AF	KC
<i>Date Analyzed:</i>	6/16/2021	6/15/2021

Interpretations:

Microcystins/nodularins were below the current 'EPA Recommended Value for Recreational Criteria and Swimming Advisory' of 8.0 ng/mL (ppb) (EPA, 2019) in all samples. Saxitoxins were detected in all samples at sub-ppb levels using a saxitoxin specific ELISA.

Submitted by:

Mark T. Aubel, Ph.D.
Lab Director

Date:

June 18, 2021

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Appendix B6



aquatic analysis ... research ... consultation

Potentially Toxigenic (PTOX) Cyanobacteria Report

Project: BlueGreen Water Tech

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies, Inc
Address: #428, 1302 Waugh Dr., Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 2 June 2021
Sample Condition: 4.4 °C upon arrival
Report# 210531_PTOX_BlueGreen
Date Prepared: 3 June 2021
Prepared by: Alyssa Garvey

<u>Sample ID</u>	<u>Sites</u>	<u>Collected</u>
1	W.P. Franklin	5/30/21
2	W.P. Franklin	5/30/21
3	W.P. Franklin	5/30/21
4	W.P. Franklin	5/30/21
5	W.P. Franklin	5/30/21
6	W.P. Franklin	5/30/21
7	W.P. Franklin	5/30/21
8	W.P. Franklin	5/30/21

Method

A one mL aliquot of each non-preserved sample was prepared using a Sedgewick Rafter cell. The samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs.

Results

1

The potentially toxigenic (PTOX) cyanobacteria *Raphidiopsis* sp. (>40 filaments per mL), *Cuspidothrix* sp. (>25 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>15 filaments per mL), *Microcystis* sp. (≥ 8 colonies per mL), *Dolichospermum* sp. (≥ 2 partial filaments per mL), and *Pseudanabaena* sp. (<10 filaments per mL) were observed.

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2

The PTOX cyanobacteria *Raphidiopsis* sp. (>25 filaments per mL), *Cuspidothrix* sp. (>20 filaments per mL), *Microcystis* sp. (>10 colonies per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (\geq 10 filaments per mL), *Dolichospermum* sp. (\geq 3 partial filaments per mL), and *Pseudanabaena* sp. (<10 filaments per mL) were observed.

3

The PTOX cyanobacteria *Raphidiopsis* sp. (>25 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>15 filaments per mL), *Cuspidothrix* sp. (>10 filaments per mL), *Microcystis* sp. (\geq 8 colonies per mL), *Dolichospermum* sp. (\geq 1 partial filament per mL), and *Pseudanabaena* sp. (<10 filaments per mL) were observed.

4

The PTOX cyanobacteria *Raphidiopsis* sp. (>25 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>15 filaments per mL), *Microcystis* sp. (\geq 9 colonies per mL), *Cuspidothrix* sp. (>5 filaments per mL), *Dolichospermum* sp. (>5 partial filaments per mL), and *Pseudanabaena* sp. (<10 filaments per mL) were observed.

5

The PTOX cyanobacteria *Raphidiopsis* sp. (>30 filaments per mL), *Cuspidothrix* sp. (>20 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (\geq 15 filaments per mL), *Microcystis* sp. (\geq 6 colonies per mL), *Dolichospermum* sp. (>5 partial filaments per mL), and *Pseudanabaena* sp. (<10 filaments per mL) were observed.

6

The PTOX cyanobacteria *Raphidiopsis* sp. (>25 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>15 filaments per mL), *Microcystis* sp. (\geq 9 colonies per mL), *Cuspidothrix* sp. (>5 filaments per mL), *Dolichospermum* sp. (\geq 3 partial filaments per mL), and *Pseudanabaena* sp. (<10 filaments per mL) were observed.

7

The PTOX cyanobacteria *Raphidiopsis* sp. (>15 filaments per mL), *Cuspidothrix* sp. (\geq 10 filaments per mL), *Microcystis* sp. (\geq 9 colonies per mL), *Dolichospermum* sp. (>5 partial filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>5 filaments per mL), and *Pseudanabaena* sp. (<10 filaments per mL) were observed.

8

The PTOX cyanobacteria *Raphidiopsis* sp. (>10 filaments per mL), *Cuspidothrix* sp. (>10 filaments per mL), *Dolichospermum* sp. (>10 partial filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>5 filaments per mL), *Microcystis* sp. (\geq 3 colonies per mL), and *Pseudanabaena* sp. (<10 filaments per mL) were observed.

Potential toxin producing genera observed include:

Microcystins	Saxitoxins	Anatoxin-a	Cylindrospermopsin
<i>Pseudanabaena</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>
<i>Dolichospermum</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>
<i>Microcystis</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>
<i>Sphaerospermopsis</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>
	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>
	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>

Recommendations

Based on similar PTOX cyanobacterial composition observed in previous samples, toxin recommendations are pending results from the 5/28/2021 collection.

Micrographs



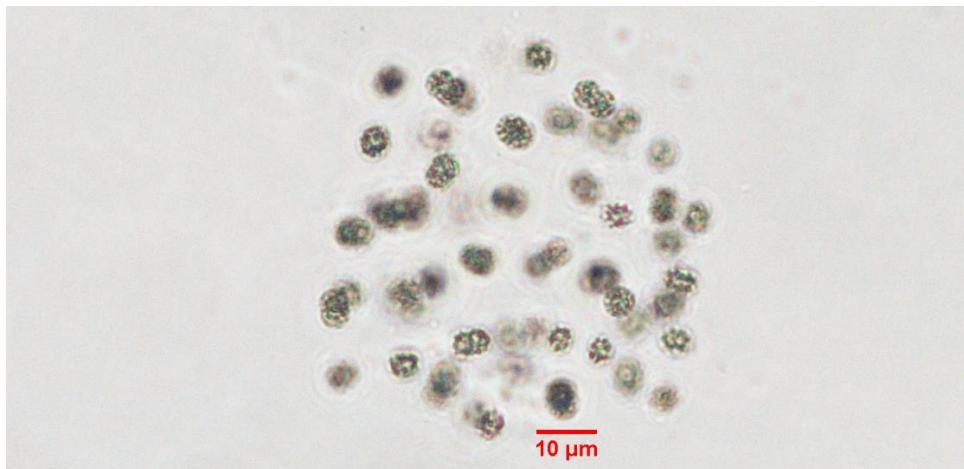
Raphidiopsis sp. at 400X (1)



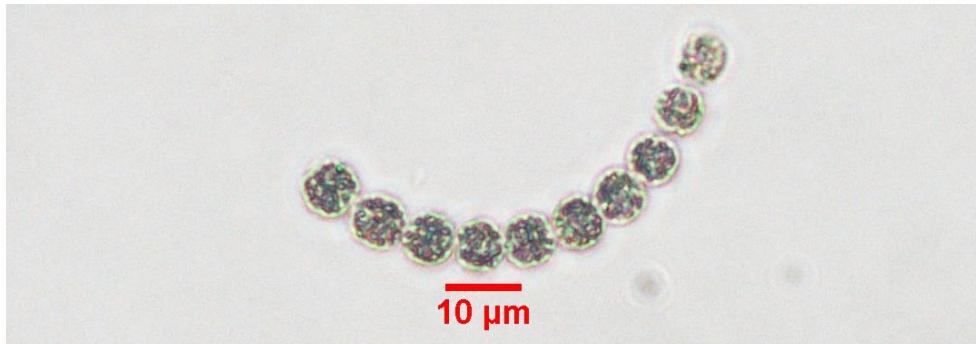
Cuspidothrix sp. at 400X (1)



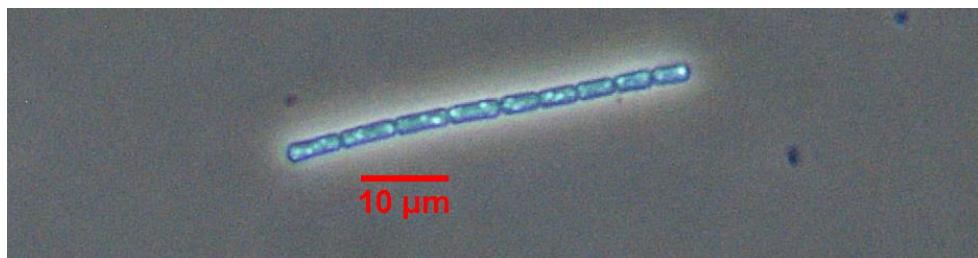
Aphanizomenon/Chrysosporum/Sphaerospermopsis sp. at 400X (4)



Microcystis sp. at 400X (1)



Dolichospermum sp. at 400X (1)



Pseudanabaena sp. at 400X (1)

Submitted by:

Amanda Foss

Date:

June 3, 2021

Amanda Foss, M.S.

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Appendix B7



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Microcystins/Nodularins & Saxitoxins Report

Project: BlueGreen US Water Technologies Inc.

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies Inc.
Address: 1302 Waugh Drive, Ste. 482, Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 2 June 2021
Sample Condition: 2.6 °C upon arrival
Report# 210531_BlueGreen
Date Prepared: 21 June 2021
Prepared by: Kamil Cieslik

Table 1: Samples analyzed and collection dates

Sample ID	Description	Collection Date
1	W.P. Franklin	31 May 2021
2	W.P. Franklin	31 May 2021
3	W.P. Franklin	31 May 2021
4	W.P. Franklin	31 May 2021
5	W.P. Franklin	31 May 2021
6	W.P. Franklin	31 May 2021
7	W.P. Franklin	31 May 2021
8	W.P. Franklin	31 May 2021

Toxins –Adda Microcystins/Nodularins (MCs/NODs), 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB), Saxitoxin (STX/PSTs)

Abbreviations

NA	Not Applicable	LFSM	Lab Fortified Sample Matrix
MDL	Method Detection Limit	LFSMD	Lab Fortified Sample Matrix Duplicate
MQL	Method Quantification Limit	LD	Lab Duplicate
ND	Not Detected above the MDL	IS	Internal Standard
Blank	Regent Water free from interferences	—	Not Analyzed
LFB	Lab Fortified Blank	MRL	Method Reporting Limit
CCC	Continued Calibration Check	CV	Low-range calibration verification



Sample Preparation

The samples were received and inverted for 60 seconds to mix. Subsets from each sample were removed prior to cell lysis for algal identification and enumeration purposes.

STX

Subsets (100 mL) were sonicated to release toxins. Aliquots were prepared with IS and LFSMs (Table 2) and filtered (0.2 µm PVDF) prior to analyses.

MCs/NODs by MMPB

Aliquots (2 mL) spiked with IS (d_3 -MMPB) were oxidized by the addition of 1 mL of a solution containing 0.1 M K_2CO_3 , 0.05 M $KMnO_4$ and 0.05 M $NaIO_4$ for 1 hour, stopped with the addition of 40% sodium bisulfite and cleaned using 100 mg Strata X solid phase extraction (SPE). The extracts were reconstituted in DI, filtered (0.2 µm PVDF), and analyzed.

Analytical Techniques

Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)

MMPB

The [M-H]⁻ ion of MMPB (m/z 207) was fragmented and the product ion (m/z 131) was monitored. The IS (d_3 -MMPB) was also fragmented and monitored (m/z 210 → 131). The internal standard method was implemented using a standard curve (0.25 – 100 ng/mL of oxidized MC-LR) to calculate LFSM returns.

Enzyme-Linked Immunosorbent Assay (ELISA)

STX

A saxitoxin specific ELISA (Abraxis PN 52255B) was utilized for the detection and quantification of saxitoxin and related analogs (paralytic shellfish toxins – PSTs). The current method reporting limit is 0.05 ng/mL (ppb) based on kit sensitivity and dilution factors. Based on manufacture instructions, the STX ELISA is less cross-reactive to other PSTs and will likely underestimate total PSTs/Saxitoxins. Reported cross-reactivities are as follows: NEO (1.3%), dcSTX (29%), GTX2/3 (23%), GTX5 (23%), dcGTX2/3 (1.4%), dcNEO (0.6%) & GTX1/4 (<0.2%).

Quality Control

Table 2: Quality Assurance/Quality Control (QA/QC) samples (IS and LFSM) prepared for analyses pre-extraction. Additional QA/QC checks included LFBs, continued calibration checks and external curves.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
MC-LR (as MMPB)	2.0	1	LFSM	101%
MC-LR (as MMPB)	2.0	6	LFSM	99%
<i>d</i> ₃ -MMPB	1.0	all aliquots	IS	55 ± 78%
STX	0.2	1	LFSM	93%
STX	0.2	6	LFSM	88%

*Control limits: water LFSM ± 30%; complicated matrix LFSM and when LFSM within 2x MDL ±50%; IS ± 50%

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

Table 3: Raw ELISA Data

Sample ID	Analyte	Dilution Factor	Assay Values (ng/mL)	CV	Concentration (ng/mL)	Average (ng/mL)
1	STX	1	0.10	0.4%	0.10	0.11
		1	0.11		0.11	
1 LFSM	STX	1	0.28	3.9%	0.28	0.29
		1	0.30		0.30	
2	STX	1	0.10	0.2%	0.10	0.10
		1	0.10		0.10	
3	STX	1	0.08	12.7%	0.08	0.09
		1	0.09		0.09	
4	STX	1	0.10	0.9%	0.10	0.10
		1	0.09		0.09	
5	STX	1	0.09	0.8%	0.09	0.09
		1	0.09		0.09	
6	STX	1	0.10	6.1%	0.10	0.10
		1	0.09		0.09	
6 LFSM	STX	1	0.27	0.7%	0.27	0.27
		1	0.27		0.27	
7	STX	1	0.10	7.6%	0.10	0.10
		1	0.09		0.09	
8	STX	1	0.08	2.1%	0.08	0.08
		1	0.08		0.08	

Table 4: STX-ELISA Quality Control Value Table

Date Analyzed:	17 June 2021	Requirement	Pass/Fail
R ² value:	0.998	≥0.98	PASS
%CV range STDs:	0.7-4.2%	≤15%	PASS
LFB (0.2 ppb) recovery:	80%	±40% True Value	PASS
%CV range LFB:	0.0%	<20%	PASS
Low CCC (0.05 ppb) recovery:	80%	±50% True Value	PASS
LRB	<0.03	<0.03	PASS

Summary of Results

Table 6: Summary of results for total microcystins/nodularins (MCs/NODs) as measured through the MMPB method and saxitoxins (STX). All data is reported as ng/mL (ppb).

Sample	MCs/NODs (ng/mL)	STX (ng/mL)
1	0.48	0.11
2	2.02	0.10
3	0.99	0.09
4	0.7	0.10
5	0.54	0.09
6	0.53	0.10
7	0.67	0.10
8	4.08	0.08
<i>MRL (ng/mL):</i>	0.06	0.05
<i>Analyst Initials:</i>	AF	KC
<i>Date Analyzed:</i>	6/18/2021	6/17/2021

Interpretations:

Microcystins/nodularins were below the current 'EPA Recommended Value for Recreational Criteria and Swimming Advisory' of 8.0 ng/mL (ppb) (EPA, 2019) in all samples. Saxitoxins were detected in all samples at sub-ppb levels using a saxitoxin specific ELISA.

Submitted by:

Mark T. Aubel, Ph.D.
Lab Director

Date:

June 21, 2021

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Appendix B8



aquatic analysis ... research ... consultation

Potentially Toxigenic (PTOX) Cyanobacteria Report

Project: BlueGreen Water Tech

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies, Inc
Address: #428, 1302 Waugh Dr., Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 2 June 2021
Sample Condition: 2.6 °C upon arrival
Report# 210531_PTOX_BlueGreen
Date Prepared: 3 June 2021
Prepared by: Alyssa Garvey

<u>Sample ID</u>	<u>Sites</u>	<u>Collected</u>
1	W.P. Franklin	5/31/21
2	W.P. Franklin	5/31/21
3	W.P. Franklin	5/31/21
4	W.P. Franklin	5/31/21
5	W.P. Franklin	5/31/21
6	W.P. Franklin	5/31/21
7	W.P. Franklin	5/31/21
8	W.P. Franklin	5/31/21

Method

A one mL aliquot of each non-preserved sample was prepared using a Sedgewick Rafter cell. The samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs.

Results

1

The potentially toxigenic (PTOX) cyanobacteria *Raphidiopsis* sp. (>35 filaments per mL), *Cuspidothrix* sp. (>10 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>10 filaments per mL), *Microcystis* sp. (\geq 8 colonies per mL), *Dolichospermum* sp. (\geq 5 partial filaments per mL), and *Pseudanabaena* sp. (<10 small filaments per mL) were observed.

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2

The PTOX cyanobacteria *Raphidiopsis* sp. (>40 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (≥ 15 filaments per mL), *Dolichospermum* sp. (>10 partial filaments per mL), *Cuspidothrix* sp. (>10 filaments per mL), *Microcystis* sp. (≥ 6 colonies per mL), and *Pseudanabaena* sp. (<10 small filaments per mL) were observed.

3

The PTOX cyanobacteria *Raphidiopsis* sp. (>25 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>20 filaments per mL), *Cuspidothrix* sp. (>20 filaments per mL), *Dolichospermum* sp. (>10 partial filaments per mL), *Microcystis* sp. (≥ 4 colonies per mL), and *Pseudanabaena* sp. (<10 small filaments per mL) were observed.

4

The PTOX cyanobacteria *Raphidiopsis* sp. (>20 filaments per mL), *Cuspidothrix* sp. (>10 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>10 filaments per mL), *Microcystis* sp. (≥ 6 colonies per mL), *Dolichospermum* sp. (>5 partial filaments per mL), and *Pseudanabaena* sp. (<10 small filaments per mL) were observed.

5

The PTOX cyanobacteria *Raphidiopsis* sp. (>15 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>15 filaments per mL), *Cuspidothrix* sp. (>10 filaments per mL), *Dolichospermum* sp. (>10 partial filaments per mL), *Microcystis* sp. (≥ 7 colonies per mL), and *Pseudanabaena* sp. (<10 small filaments per mL) were observed.

6

The PTOX cyanobacteria *Raphidiopsis* sp. (>20 filaments per mL), *Cuspidothrix* sp. (>10 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>5 filaments per mL), *Dolichospermum* sp. (≥ 3 partial filaments per mL), *Microcystis* sp. (≥ 3 colonies per mL), and *Pseudanabaena* sp. (<10 small filaments per mL) were observed.

7

The PTOX cyanobacteria *Raphidiopsis* sp. (>25 filaments per mL), *Cuspidothrix* sp. (>10 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>10 filaments per mL), *Dolichospermum* sp. (>5 partial filaments per mL), *Microcystis* sp. (≥ 4 colonies per mL), *Planktothrix* sp. (1 filament per mL), and *Pseudanabaena* sp. (<10 small filaments per mL) were observed.

8

The PTOX cyanobacteria *Raphidiopsis* sp. (>25 filaments per mL), *Cuspidothrix* sp. (>15 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>15 filaments per mL), *Microcystis* sp. (>10 colonies per mL), *Dolichospermum* sp. (>5 partial filaments per mL), and *Pseudanabaena* sp. (<10 small filaments per mL) were observed.

Potential toxin producing genera observed include:

Microcystins	Saxitoxins	Anatoxin-a	Cylindrospermopsin
<i>Pseudanabaena</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>
<i>Dolichospermum</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>
<i>Microcystis</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>
<i>Sphaerospermopsis</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>
<i>Planktothrix</i>	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>
	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>
	<i>Planktothrix</i>	<i>Planktothrix</i>	

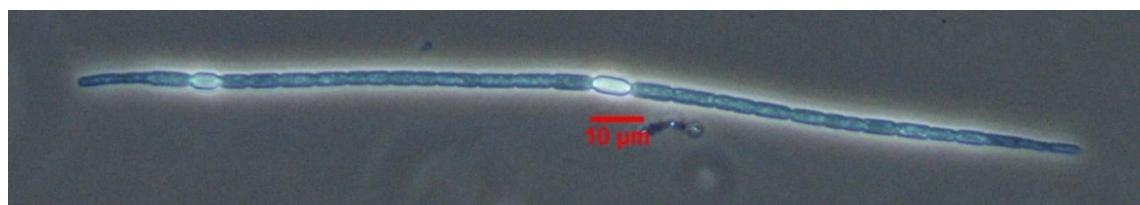
Recommendations

Based on similar PTOX cyanobacterial composition observed in previous samples, toxin recommendations are pending results from the 5/28/2021 collection.

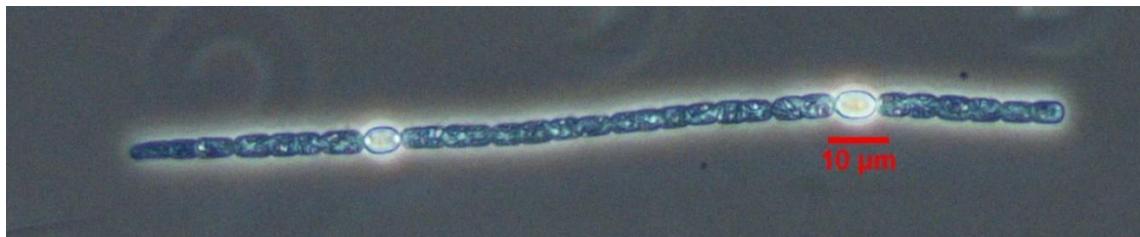
Micrographs



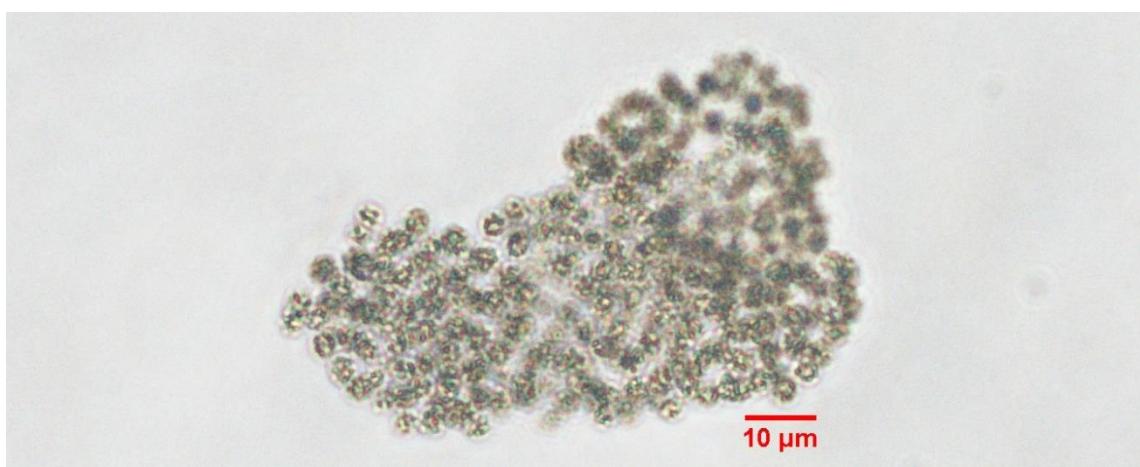
Raphidiopsis sp. (straight) at 400X (1)



Cuspidothrix sp. at 400X (1)

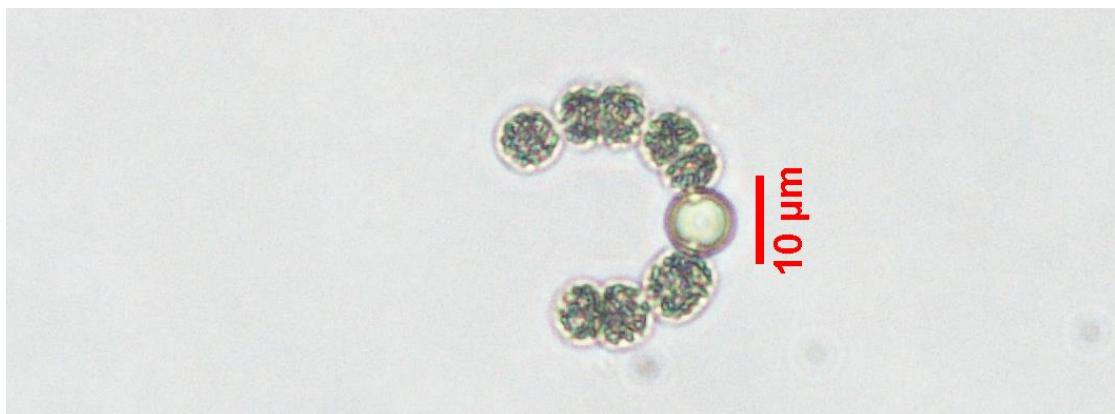


Aphanizomenon/Chrysosporum/Sphaerospermopsis sp. at 400X (1)

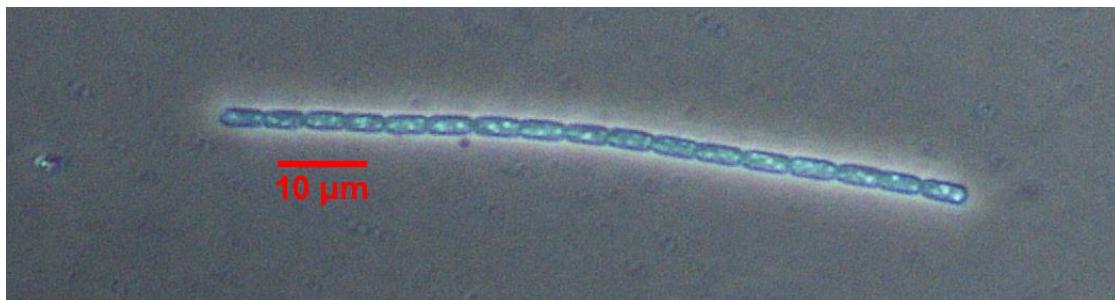


Microcystis sp. at 400X (1)

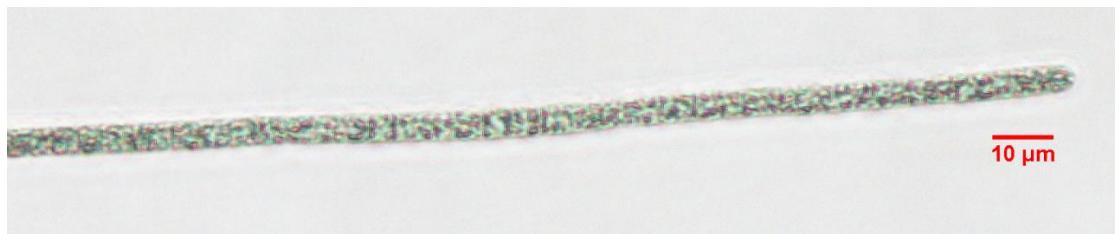
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Dolichospermum sp. at 400X (1)



Pseudanabaena sp. at 400X (4)



Planktothrix sp. at 400X (7)

Submitted by:

Amanda Foss

Date:

June 3, 2021

Amanda Foss, M.S.

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Cyano
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Appendix B9



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Microcystins/Nodularins & Saxitoxins Report

Project: BlueGreen US Water Technologies Inc.

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies Inc.
Address: 1302 Waugh Drive, Ste. 482, Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 10 June 2021
Sample Condition: 5.9 °C upon arrival
Report# 210602-06_BlueGreen
Date Prepared: 23 June 2021
Prepared by: Kamil Cieslik

Table 1: Samples analyzed and collection dates

Sample ID	Description	Collection Date
5	W.P. Franklin	2 June 2021
9	W.P. Franklin	2 June 2021
10	W.P. Franklin	2 June 2021
12	W.P. Franklin	2 June 2021
K20	C43	6 June 2021
K21	C43	6 June 2021

Toxins –Adda Microcystins/Nodularins (MCs/NODs), 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB), Saxitoxin (STX/PSTs)

Abbreviations

NA	Not Applicable	LFSM	Lab Fortified Sample Matrix
MDL	Method Detection Limit	LFSMD	Lab Fortified Sample Matrix Duplicate
MQL	Method Quantification Limit	LD	Lab Duplicate
ND	Not Detected above the MDL	IS	Internal Standard
Blank	Regent Water free from interferences	—	Not Analyzed
LFB	Lab Fortified Blank	MRL	Method Reporting Limit
CCC	Continued Calibration Check	CV	Low-range calibration verification

Sample Preparation

The samples were received and inverted for 60 seconds to mix. Subsets from each sample were removed prior to cell lysis for algal identification and enumeration purposes.

STX

Subsets (100 mL) were sonicated to release toxins. Aliquots were prepared with IS and LFSMs (Table 2) and filtered (0.2 µm PVDF) prior to analyses.

MCs/NODs by MMPB

Aliquots (2 mL) spiked with IS (d_3 -MMPB) were oxidized by the addition of 1 mL of a solution containing 0.1 M K_2CO_3 , 0.05 M $KMnO_4$ and 0.05 M $NaIO_4$ for 1 hour, stopped with the addition of 40% sodium bisulfite and cleaned using 100 mg Strata X solid phase extraction (SPE). The extracts were reconstituted in DI, filtered (0.2 µm PVDF), and analyzed.

Analytical Techniques

Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)

MMPB

The [M-H]⁻ ion of MMPB (m/z 207) was fragmented and the product ion (m/z 131) was monitored. The IS (d_3 -MMPB) was also fragmented and monitored (m/z 210 → 131). The internal standard method was implemented using a standard curve (0.25 – 100 ng/mL of oxidized MC-LR) to calculate LFSM returns.

Enzyme-Linked Immunosorbent Assay (ELISA)

STX

A saxitoxin specific ELISA (Abraxis PN 52255B) was utilized for the detection and quantification of saxitoxin and related analogs (paralytic shellfish toxins – PSTs). The current method reporting limit is 0.05 ng/mL (ppb) based on kit sensitivity and dilution factors. Based on manufacture instructions, the STX ELISA is less cross-reactive to other PSTs and will likely underestimate total PSTs/Saxitoxins. Reported cross-reactivities are as follows: NEO (1.3%), dcSTX (29%), GTX2/3 (23%), GTX5 (23%), dcGTX2/3 (1.4%), dcNEO (0.6%) & GTX1/4 (<0.2%).

Quality Control

Table 2: Quality Assurance/Quality Control (QA/QC) samples (IS and LFSM) prepared for analyses pre-extraction. Additional QA/QC checks included LFBs, continued calibration checks and external curves.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
MC-LR (as MMPB)	2.0	12	LFSM	76%
MC-LR (as MMPB)	2.0	K21	LFSM	107%
MC-LR (as MMPB)	10	K21	LFSM	119%
<i>d</i> ₃ -MMPB	1.0	all aliquots	IS	74 ± 12%
STX	0.2	5	LFSM	105%
STX	0.2	K21	LFSM	95%

*Control limits: water LFSM ± 30%; complicated matrix LFSM and when LFSM within 2x MDL ±50%; IS ± 50%

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

Table 3: Raw ELISA Data

Sample ID	Analyte	Dilution Factor	Assay Values (ng/mL)	CV	Concentration (ng/mL)	Average (ng/mL)
5	STX	1	0.08	2.6%	0.08	0.09
		1	0.09		0.09	
5 LFSM	STX	1	0.30	1.5%	0.30	0.30
		1	0.29		0.29	
9	STX	1	0.05	17.9%	0.05	0.06
		1	0.07		0.07	
10	STX	1	0.09	3.2%	0.09	0.09
		1	0.09		0.09	
12	STX	1	0.08	0.5%	0.08	0.08
		1	0.08		0.08	
K20	STX	1	0.05	2.8%	0.05	0.05
		1	0.05		0.05	
K21	STX	1	0.02	29.2%	<0.05	ND
		1	0.02		<0.05	
K21 LFSM	STX	1	0.20	5.4%	0.20	0.21
		1	0.22		0.22	

Table 4: STX-ELISA Quality Control Value Table

Date Analyzed:	23 June 2021	Requirement	Pass/Fail
R ² value:	0.998	≥0.98	PASS
%CV range STDs:	0.1-8.5%	≤15%	PASS
LFB (0.2 ppb) recovery:	83%	±40% True Value	PASS
%CV range LFB:	4.7%	<20%	PASS
Low CCC (0.05 ppb) recovery:	60%	±50% True Value	PASS
LRB	<0.03	<0.03	PASS

Summary of Results

Table 5: Summary of results for total microcystins/nodularins (MCs/NODs) as measured through the MMPB method and saxitoxins (STX). All data is reported as ng/mL (ppb).

Sample	MCs/NODs (ng/mL)	STX (ng/mL)
5	0.60	0.09
9	0.58	0.06
10	0.36	0.09
12	0.88 ± 0.11	0.08
K20	942 ± 81	0.05
K21	2.76	ND
<i>MRL (ng/mL):</i>	0.06	0.05
<i>Analyst Initials:</i>	AF	KC
<i>Date Analyzed:</i>	6/18/2021 6/21/2021	6/23/2021

Interpretations:

The levels of Adda MCs/NODs detected in the K20 sample (**942 ppb**) exceeds the current ‘Draft EPA Recommended Value for Recreational Criteria and Swimming Advisory’, which is currently 8 ng/mL (ppb) total microcystins. The WHO recreational guidance value for microcystin is currently 24 ng/mL (ppb) (World Health Organization (WHO), 2020a).

Saxitoxins were detected in all samples (**K21 sample exception**) at sub-ppb levels using a saxitoxin specific ELISA.

World Health Organization (WHO), 2020a. Cyanobacterial toxins: microcystins. Guidel. Drink. Qual. Guidel. Safe Recreat. Water Environ. 63.

Submitted by:

Mark T. Aubel, Ph.D.
Lab Director

Date: June 23, 2021

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Appendix B10



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Potentially Toxigenic (PTOX) Cyanobacteria Report

Project: BlueGreen Water Tech

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies, Inc
Address: #428, 1302 Waugh Dr., Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 10 June 2021
Sample Condition: 5.9 °C upon arrival
Report# 210602-210606_PTOX_BlueGreen
Date Prepared: 14 June 2021
Prepared by: Alyssa Garvey

<u>Sample ID</u>	<u>Sites</u>	<u>Collected</u>
5	W.P. Franklin	6/2/21*
9	W.P. Franklin	6/2/21*
10	W.P. Franklin	6/2/21*
12	W.P. Franklin	6/2/21*
K20	C43	6/6/21*
K21	C43	6/6/21*

*The sample collection date for the W.P. Franklin site and the sample analysis date for the C43 sample set was outside the holding time for unpreserved algal identification samples (<5 days). This may have impacted the results.

Method

A one mL aliquot of each non-preserved sample was prepared using a Sedgewick Rafter cell. The samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs.

Results

5

The potentially toxigenic (PTOX) cyanobacteria observed included *Microcystis* sp. (≥ 30 colonies per mL), *Dolichospermum* sp. (> 25 filaments per mL; mostly partial), *Cuspidothrix* sp. (> 25 filaments per mL), *Raphidiopsis raciborskii* (> 10 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (≥ 10 filaments per mL), and *Pseudanabaena* sp. (< 100 filaments per mL).



9

The PTOX cyanobacteria observed included *Microcystis* sp. (≥ 6 colonies per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (≥ 5 filaments per mL), *Dolichospermum* sp. (≥ 4 filaments per mL; mostly partial), and *Pseudanabaena* sp. (< 100 filaments per mL).

10

The PTOX cyanobacteria observed included *Microcystis* sp. (≥ 15 colonies per mL), *Raphidiopsis raciborskii* (> 10 filaments per mL), *Cuspidothrix* sp. (> 5 filaments per mL), *Dolichospermum* sp. (> 5 filaments per mL; mostly partial), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (≥ 5 filaments per mL), and *Pseudanabaena* sp. (< 100 filaments per mL).

12

The PTOX cyanobacteria observed included *Microcystis* sp. (≥ 20 colonies per mL), *Dolichospermum* sp. (> 5 filaments per mL; mostly partial), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (≥ 4 filaments per mL), *Cuspidothrix* sp. (≥ 2 filaments per mL), and *Pseudanabaena* sp. (< 100 filaments per mL).

K20

The sample was dominated by the PTOX cyanobacteria *Microcystis* spp. Other PTOX cyanobacteria observed included *Dolichospermum* sp. (> 50 filaments per mL; mostly partial), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (≥ 3 filaments per mL), and *Cuspidothrix* sp. (≥ 1 filament per mL).

K21

The PTOX cyanobacteria observed included *Microcystis* spp. (≥ 20 colonies per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (≥ 3 filaments per mL), *Raphidiopsis raciborskii* (≥ 2 filaments per mL), *Cuspidothrix* sp. (≥ 2 filaments per mL), and *Pseudanabaena* sp. (< 10 filaments per mL).

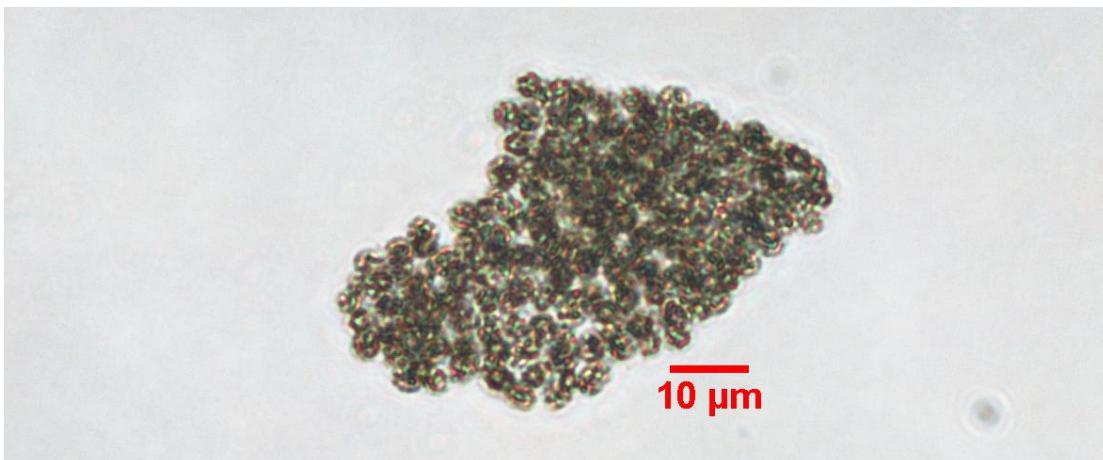
Potential toxin producing genera observed include:

Microcystins	Saxitoxins	Anatoxin-a	Cylindrospermopsin
<i>Pseudanabaena</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>
<i>Dolichospermum</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>
<i>Microcystis</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>
<i>Sphaerospermopsis</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>
	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>
	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>

Recommendations

Based on these observations and previous toxin data, analysis for microcystins and saxitoxins are recommended for **all** samples.

Micrographs



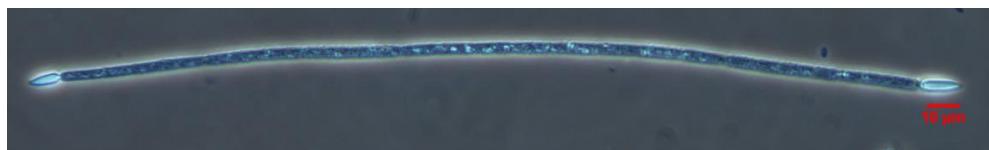
Microcystis sp. at 400X (5)



Dolichospermum sp. at 400X (5)



Cuspidothrix sp. at 400X (5)



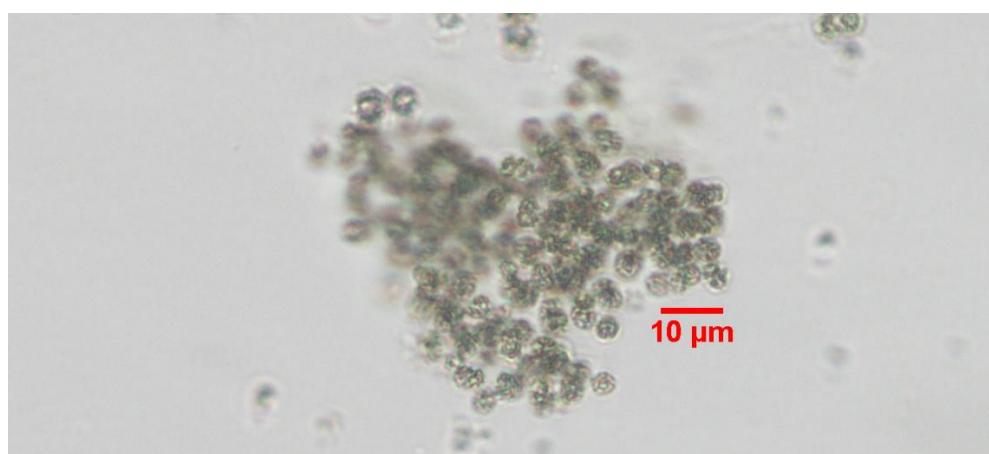
Raphidiopsis raciborskii at 400X (5)



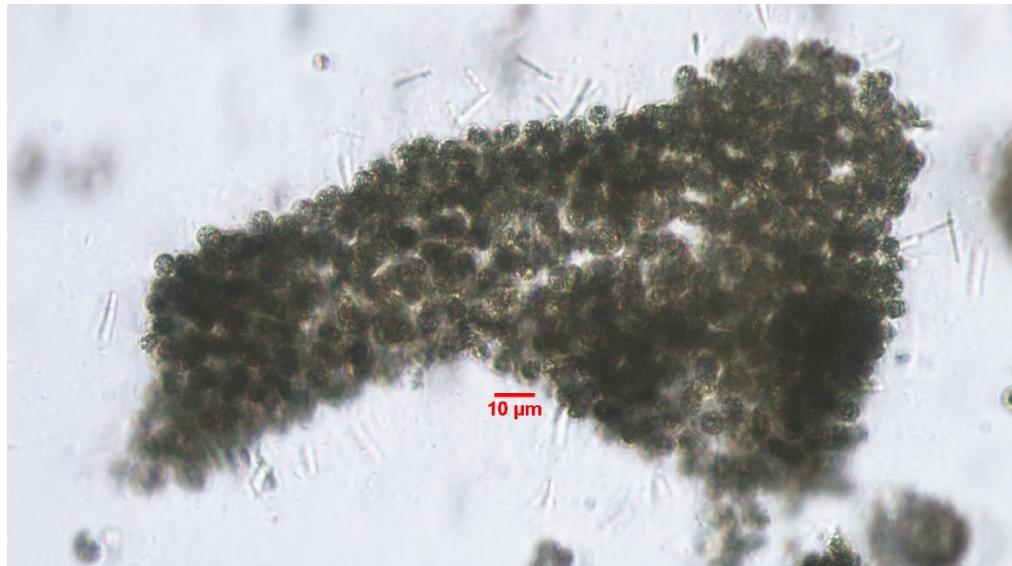
Aphanizomenon/Chrysosporum/Sphaerospermopsis sp. at 400X (5)



Pseudanabaena sp. at 400X (9)



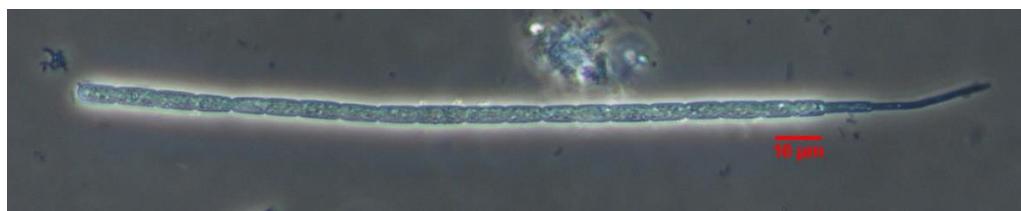
Microcystis sp. at 400X (K20)



Microcystis sp. at 400X (K20)



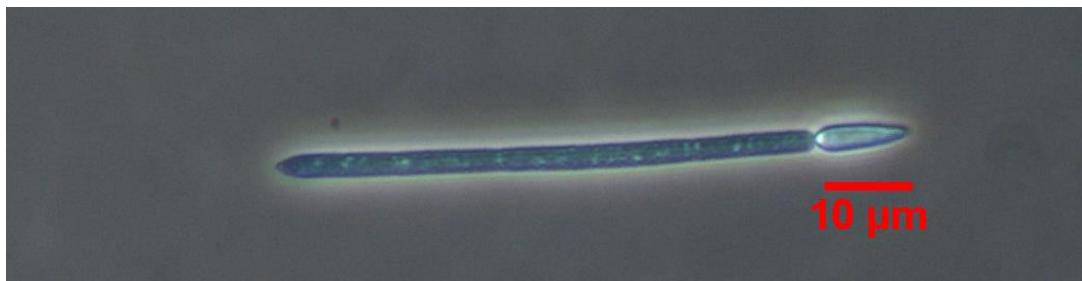
Dolichospermum sp. at 400X (K20)



Cuspidothrix sp. at 400X (K20)



Aphanizomenon/Chrysosporum/Sphaeropsermopsis sp. at 400X (K21)



Raphidiopsis raciborskii at 400X (K21)



Pseudanabaena sp. at 400X (K21)

Submitted by:

Amanda Foss

Date: June 14, 2021

Amanda Foss, M.S.

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Appendix C1



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Microcystins/Nodularins & Saxitoxins Report

Project: BlueGreen US Water Technologies Inc.

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies Inc.
Address: 1302 Waugh Drive, Ste. 482, Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 10 June 2021
Sample Condition: 4.6 °C upon arrival
Report# 210606_BlueGreen
Date Prepared: 23 June 2021
Prepared by: Kamil Cieslik

Table 1: Samples analyzed and collection dates

Sample ID	Description	Collection Date
31	C-43	6 June 2021
32	C-43	6 June 2021
33	C-43	6 June 2021
34	C-43	6 June 2021
35	C-43	6 June 2021
36	C-43	6 June 2021
37	C-43	6 June 2021

Toxins –Adda Microcystins/Nodularins (MCs/NODs), 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB), Saxitoxin (STX/PSTs)

Abbreviations

NA	Not Applicable	LFSM	Lab Fortified Sample Matrix
MDL	Method Detection Limit	LFSMD	Lab Fortified Sample Matrix Duplicate
MQL	Method Quantification Limit	LD	Lab Duplicate
ND	Not Detected above the MDL	IS	Internal Standard
Blank	Regent Water free from interferences	—	Not Analyzed
LFB	Lab Fortified Blank	MRL	Method Reporting Limit
CCC	Continued Calibration Check	CV	Low-range calibration verification

Sample Preparation

The samples were received and inverted for 60 seconds to mix. Subsets from each sample were removed prior to cell lysis for algal identification and enumeration purposes.

STX

Subsets (100 mL) were sonicated to release toxins. Aliquots were prepared with IS and LFSMs (Table 2) and filtered (0.2 µm PVDF) prior to analyses.

MCs/NODs by MMPB

Aliquots (2 mL) spiked with IS (d_3 -MMPB) were oxidized by the addition of 1 mL of a solution containing 0.1 M K_2CO_3 , 0.05 M $KMnO_4$ and 0.05 M $NaIO_4$ for 1 hour, stopped with the addition of 40% sodium bisulfite and cleaned using 100 mg Strata X solid phase extraction (SPE). The extracts were reconstituted in DI, filtered (0.2 µm PVDF), and analyzed.

Analytical Techniques

Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)

MMPB

The [M-H]⁻ ion of MMPB (m/z 207) was fragmented and the product ion (m/z 131) was monitored. The IS (d_3 -MMPB) was also fragmented and monitored (m/z 210 → 131). The internal standard method was implemented using a standard curve (0.25 – 100 ng/mL of oxidized MC-LR) to calculate LFSM returns.

Enzyme-Linked Immunosorbent Assay (ELISA)

STX

A saxitoxin specific ELISA (Abraxis PN 52255B) was utilized for the detection and quantification of saxitoxin and related analogs (paralytic shellfish toxins – PSTs). The current method reporting limit is 0.05 ng/mL (ppb) based on kit sensitivity and dilution factors. Based on manufacture instructions, the STX ELISA is less cross-reactive to other PSTs and will likely underestimate total PSTs/Saxitoxins. Reported cross-reactivities are as follows: NEO (1.3%), dcSTX (29%), GTX2/3 (23%), GTX5 (23%), dcGTX2/3 (1.4%), dcNEO (0.6%) & GTX1/4 (<0.2%).

Quality Control

Table 2: Quality Assurance/Quality Control (QA/QC) samples (IS and LFSM) prepared for analyses pre-extraction. Additional QA/QC checks included LFBs, continued calibration checks and external curves.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
MC-LR (as MMPB)	2.0	32	LFSM	85%
MC-LR (as MMPB)	2.0	36	LFSM	100%
MC-LR (as MMPB)	10	36	LFSM	83%
<i>d</i> ₃ -MMPB	1.0	all aliquots	IS	63 ± 14%
STX	0.2	31	LFSM	95%
STX	0.2	36	LFSM	105%

*Control limits: water LFSM ± 30%; complicated matrix LFSM and when LFSM within 2x MDL ±50%; IS ± 50%

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

Table 3: Raw ELISA Data

Sample ID	Analyte	Dilution Factor	Assay Values (ng/mL)	CV	Concentration (ng/mL)	Average (ng/mL)
31	STX	1	0.10	5.2%	0.10	0.10
		1	0.10		0.10	
31 LFSM	STX	1	0.29	0.3%	0.29	0.29
		1	0.29		0.29	
32	STX	1	0.04	19.1%	<0.05	0.05^J
		1	0.05		0.05	
33	STX	1	0.05	4.1%	0.05	0.05
		1	0.05		0.05	
34	STX	1	0.05	2.1%	0.05	0.05
		1	0.05		0.05	
35	STX	1	0.04	12.2%	<0.05	ND
		1	0.04		<0.05	
36	STX	1	0.06	3.5%	0.06	0.06
		1	0.06		0.06	
36 LFSM	STX	1	0.27	0.1%	0.27	0.27
		1	0.27		0.27	
37	STX	1	0.07	7.5%	0.07	0.08
		1	0.08		0.08	

Table 4: STX-ELISA Quality Control Value Table

Date Analyzed:	23 June 2021	Requirement	Pass/Fail
R ² value:	0.998	≥0.98	PASS
%CV range STDs:	0.1-8.5%	≤15%	PASS
LFB (0.2 ppb) recovery:	83%	±40% True Value	PASS
%CV range LFB:	4.7%	<20%	PASS
Low CCC (0.05 ppb) recovery:	60%	±50% True Value	PASS
LRB	<0.03	<0.03	PASS

Summary of Results

Table 5: Summary of results for total microcystins/nodularins (MCs/NODs) as measured through the MMPB method and saxitoxins (STX). All data is reported as ng/mL (ppb).

Sample	MCs/NODs (ng/mL)	STX (ng/mL)
31	1.26	0.10
32	1.59	0.05^J
33	500 ± 56	0.05
34	8.07	0.05
35	6.40	ND
36	1.74	0.06
37	1.28	0.08
<i>MRL (ng/mL):</i>	0.06	0.05
<i>Analyst Initials:</i>	AF	KC
<i>Date Analyzed:</i>	6/18/2021 6/21/2021	6/23/2021

Interpretations:

The levels of Adda MCs/NODs detected in the 33 sample (**500 ppb**) exceeds the current ‘Draft EPA Recommended Value for Recreational Criteria and Swimming Advisory’, which is currently 8 ng/mL (ppb) total microcystins. The WHO recreational guidance value for microcystin is currently 24 ng/mL (ppb) (World Health Organization (WHO), 2020a).

Saxitoxins were detected in all samples (**35 sample exception**) at sub-ppb levels using a saxitoxin specific ELISA.

World Health Organization (WHO), 2020a. Cyanobacterial toxins: microcystins. Guidel. Drink. Qual. Guidel. Safe Recreat. Water Environ. 63.

Submitted by:

Mark T. Aubel, Ph.D.
Lab Director

Date: June 23, 2021

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Appendix C2



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Potentially Toxigenic (PTOX) Cyanobacteria Report

Project: BlueGreen Water Tech

Submitted to: Waleed Nasser
Organization: BlueGreen US Water Technologies, Inc
Address: #428, 1302 Waugh Dr., Houston, TX, 77019
Email: waleed@bgtechs.com
Sample Receipt Date: 10 June 2021
Sample Condition: 4.6 °C upon arrival
Report# 210606_PTOX_BlueGreen
Date Prepared: 14 June 2021*
Prepared by: Alyssa Garvey

<u>Sample ID</u>	<u>Sites</u>	<u>Collected</u>
31	C-43	6/6/21
32	C-43	6/6/21
33	C-43	6/6/21
34	C-43	6/6/21
35	C-43	6/6/21
36	C-43	6/6/21
37	C-43	6/6/21

*The samples were analyzed outside the holding time for unpreserved algal identification samples (<5 days), which may have impacted the results.

Method

A one mL aliquot of each non-preserved sample was prepared using a Sedgewick Rafter cell. The samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs.

Results

31

The potentially toxigenic (PTOX) cyanobacteria observed included *Dolichospermum* sp. (>40 filaments per mL; mostly partial), *Raphidiopsis raciborskii* (>30 filaments per mL), *Cuspidothrix* sp. (>20 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>10 filaments per mL), *Microcystis* sp. (>10 colonies per mL), and *Pseudanabaena* sp. (<100 filaments per mL).



32

The PTOX cyanobacteria observed included *Raphidiopsis raciborskii* (>25 filaments per mL), *Cuspidothrix* sp. (>15 filaments per mL), *Dolichospermum* sp. (>10 filaments per mL; mostly partial), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>5 filaments per mL), *Microcystis* sp. (>5 colonies per mL), and *Pseudanabaena* sp. (<100 filaments per mL).

33

The sample was dominated by the PTOX cyanobacteria *Microcystis* spp. Other PTOX cyanobacteria observed included *Raphidiopsis raciborskii* (>10 filaments per mL), *Dolichospermum* sp. (>5 partial filaments per mL), *Cuspidothrix* sp. (>5 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>2 filaments per mL), and *Pseudanabaena* sp. (<100 filaments per mL).

34

The PTOX cyanobacteria observed included *Dolichospermum* sp. (>15 filaments per mL; mostly partial), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>10 filaments per mL), *Cuspidothrix* sp. (>5 filaments per mL), *Raphidiopsis raciborskii* (>4 filaments per mL), *Microcystis* sp. (>4 colonies per mL), and *Pseudanabaena* sp. (<100 filaments per mL).

35

The PTOX cyanobacteria observed included *Microcystis* sp. (>20 colonies per mL), *Cuspidothrix* sp. (>10 filaments per mL), *Raphidiopsis raciborskii* (>5 filaments per mL), *Dolichospermum* sp. (>5 filaments per mL; mostly partial), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>2 filaments per mL), and *Pseudanabaena* sp. (<100 filaments per mL).

36

The PTOX cyanobacteria observed included *Microcystis* sp. (>20 colonies per mL), *Raphidiopsis raciborskii* (>5 filaments per mL), *Cuspidothrix* sp. (>5 filaments per mL), *Dolichospermum* sp. (>5 partial filaments), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (1 filament per mL), and *Pseudanabaena* sp. (<100 filaments per mL).

37

The PTOX cyanobacteria observed included *Microcystis* sp. (>15 colonies per mL), *Raphidiopsis raciborskii* (>5 filaments per mL), *Dolichospermum* sp. (>5 partial filaments per mL), *Cuspidothrix* sp. (>4 filaments per mL), *Aphanizomenon/Chrysosporum/Sphaerospermopsis* sp. (>2 filaments per mL), and *Pseudanabaena* sp. (<100 filaments per mL).

Potential toxin producing genera observed include:

Microcystins	Saxitoxins	Anatoxin-a	Cylindrospermopsin
<i>Pseudanabaena</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>	<i>Dolichospermum</i>
<i>Dolichospermum</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>	<i>Cuspidothrix</i>
<i>Microcystis</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>	<i>Aphanizomenon</i>
<i>Sphaerospermopsis</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>	<i>Chrysosporum</i>
	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>	<i>Sphaerospermopsis</i>
	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>	<i>Raphidiopsis</i>

Recommendations

Based on these observations and previous toxin data, analysis for microcystins and saxitoxins is recommended for **all** samples.

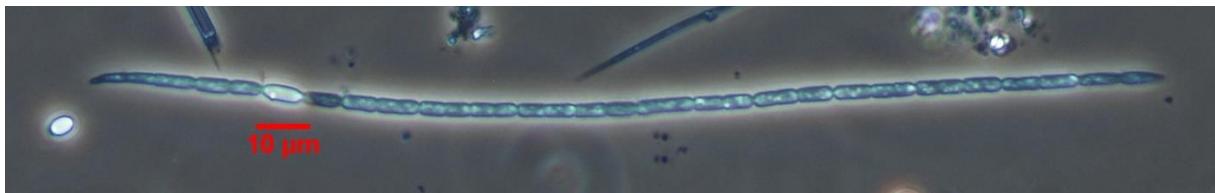
Micrographs



Dolichospermum sp. at 400X (31)



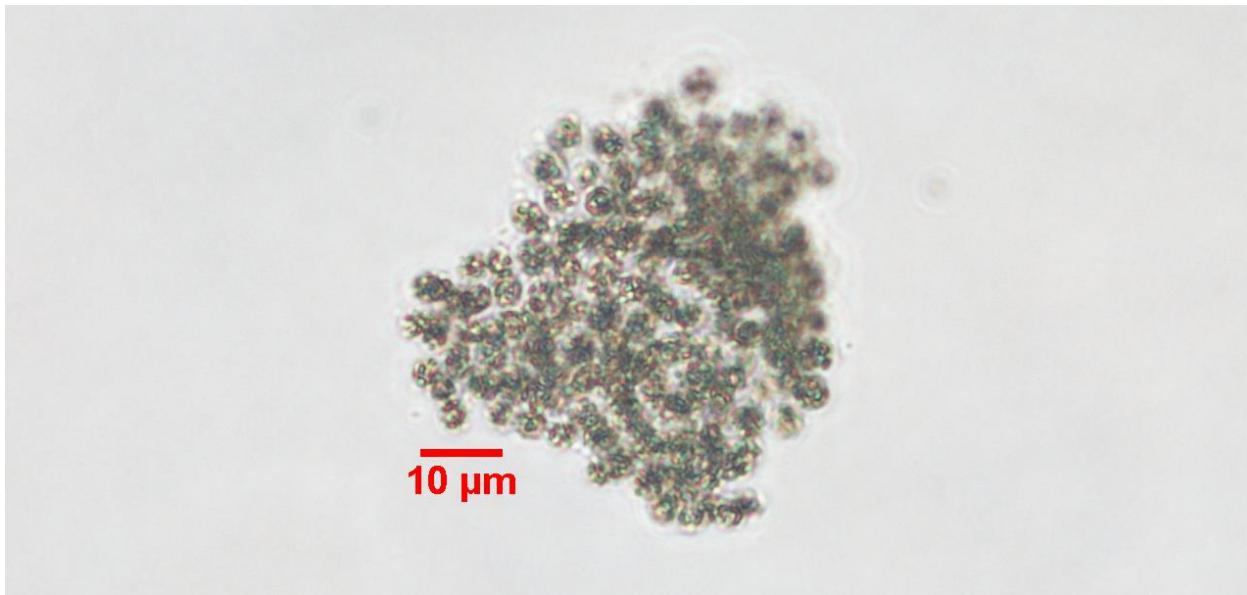
Raphidiopsis raciborskii at 400X (31)



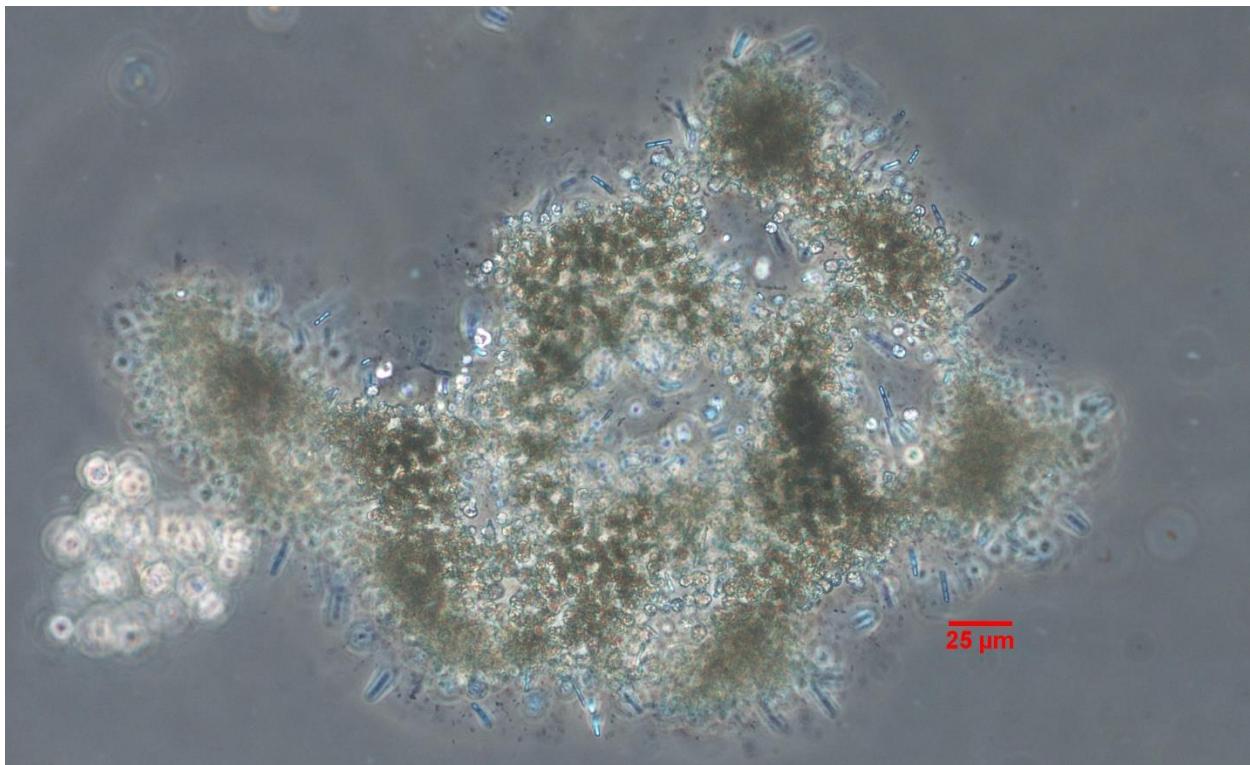
Cuspidothrix sp. at 400X (32)



Aphanizomenon/Chrysosporum/Sphaerospermopsis sp. at 400X (32)



Microcystis sp. at 400X (33)



Microcystis sp. at 200X (33)



Pseudanabaena sp. at 400X (31)

Submitted by:

Amanda Foss

Amanda Foss, M.S.

Date:

June 14, 2021

*The results in this report relate only to the samples listed above.
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Cyano
LAB

Appendix D1



CosmosID - Unlocking the Microbiome

Filename:	Sample_1_BWT2214
Size:	13.79 GiB
Reads:	68.593M
Date of Report:	2021-08-15 00:43:27

Summary

Database Name	Hits	Status
Bacteria	172	Success
Antibiotic Resistance	1	Success
Fungi	72	Success
Protists	69	Success
Dark Matter (Beta)	163	Success
Phages	22	Success
Viruses	5	Success
Respiratory Virus	1	Success
Virulence Factors	6	Success

Bacteria

Fasta/q details

Sample_1_BWT2214

Total results

Metric	Value
Hit	0.920M

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Microcystis panniformis FACHB-1757	1638788	32328.68	49.02	69.08	80.14	94032
Microcystis aeruginosa PCC 9807	1160283	11157.42	16.92	26.42	53.35	28330
Microcystis sp. 0824	1502726	3230.66	4.90	12.04	24.75	11247
Microcystis viridis NIES-102	213615	2015.78	3.06	11.28	26.74	3458
Candidatus Fonsibacter ubiqus	1925548	1876.84	2.85	19.29	19.32	3066
Pseudomonas aeruginosa	287	1634.42	2.48	17.47	17.47	512
Phenyllobacterium parvum	2201350	1576.03	2.39	11.42	11.42	27105
Sphingorhabdus contaminans	1343899	1468.72	2.23	11.92	12.00	22945
Clavibacter sp.	1871044	1439.88	2.18	13.14	13.14	4028
Dolichospermum circinale AWQC131C	398007	1102.30	1.67	8.61	11.74	5113
Synechococcus sp. CB0101	232348	753.29	1.14	5.78	5.89	10729
Limnohabitans sp. 103DPR2	1678129	665.97	1.01	3.38	5.12	3510
Pseudomonas sp. HAR-UPW-AIA-41	1985301	503.54	0.76	4.71	4.71	8010
Limnothrix sp. P13C2	1880902	483.26	0.73	3.01	4.91	54
Aestuariivirga litoralis	2650924	448.98	0.68	3.56	3.56	12761
Pararheinheimera texensis DSM 17496	1123055	365.08	0.55	3.38	3.38	7283
unclassified Synechocystis	2640012	321.15	0.49	2.11	2.11	80
Flavobacterium fontis	1124188	243.88	0.37	2.55	2.59	3203
Fusobacterium nucleatum W1481	1408287	217.52	0.33	0.59	0.59	633
Neorickettsia	33993	211.05	0.32	6.06	6.06	13
Vulcanococcus limneticus LL	2025607	180.65	0.27	1.60	1.63	3793
Aquidulcibacter paucihalophilus	1978549	176.53	0.27	1.82	1.82	3306
Flectobacillus sp. BAB-3569	1509483	155.73	0.24	1.69	1.69	3096
Rhodobacter sp. CACIA14H1	1408890	155.29	0.24	1.16	1.35	4206
Novosphingobium ginsenosidimutans	1176536	153.78	0.23	1.59	1.59	2751
Cyanobium sp. NIES-981	1851505	144.84	0.22	1.09	1.39	2032
Staphylococcus aureus	1280	134.61	0.20	6.45	2.17	19
Candidatus Planktophila sp.	2175601	114.87	0.17	1.34	1.88	140
Polynucleobacter victoriensis	2049319	93.78	0.14	1.22	1.23	681
Methylocystis sp. ATCC 49242	622637	89.93	0.14	0.97	1.01	2674
Cyanobium usitatum str. Tous	2116684	88.23	0.13	0.89	0.95	1403
Caedibacter taeniospiralis	28907	82.54	0.13	1.17	1.17	476
Tabrizicola sp. TH137	2067452	74.07	0.11	0.71	0.90	2457
Bacteroidetes bacterium SCGC AAA027-G08	938698	63.69	0.10	0.84	0.80	334
Raphidiopsis brookii D9	533247	62.70	0.10	0.88	1.12	480
beta proteobacterium SCGC AAA028-K02	938797	60.00	0.09	0.98	0.98	188
Flavobacterium sasangense DSM 21067	1121896	57.50	0.09	0.80	0.93	584
Bacillus cereus group	86661	51.82	0.08	1.00	1.00	19
Inhella crocodily	2499851	51.71	0.08	0.62	0.62	1444
actinobacterium SCGC AAA027-J17	932040	50.33	0.08	0.83	0.83	157
Acidovorax kalamii	2004485	49.29	0.07	0.62	0.76	1109
Porphyrobacter sp. LM 6	1896196	48.78	0.07	0.60	0.60	939
Methylocystis	133	47.51	0.07	1.28	3.96	10
Pseudanabaena sp. ABRG5-3	685565	46.79	0.07	0.57	0.58	854
Flavobacterium indicum GPTSA100-9 = DSM 17447	1094466	43.90	0.07	0.68	0.68	352
Cyanobium gracile PCC 6307	292564	41.22	0.06	0.47	1.01	845
Comamonadaceae	80864	40.01	0.06	0.69	0.69	11

Elstera cyanobacteriorum	2022747	38.16	0.06	0.48	0.48	1009
Limnohabitans curvus	323423	38.26	0.06	0.46	1.22	455
Aphanothece cf. minutissima CCALA 015	2107695	36.01	0.06	0.44	0.99	691
beta proteobacterium SCGC AAA027-I06	938781	35.59	0.05	0.44	0.44	418
Aphanizomenon flos-aquae 2012/KM1/D3	1532906	31.24	0.05	0.51	0.76	211
Lactiplantibacillus plantarum	1590	29.46	0.04	0.20	0.20	62
Candidatus Nanopelagicus limnes	1884634	28.10	0.04	0.51	0.83	119
Methylibium	316612	28.10	0.04	0.33	0.33	54
Silanimonas lenta DSM 16282	1123253	27.46	0.04	0.38	0.38	510
Flavobacterium sp. WWJ-16	2506421	26.73	0.04	0.38	0.45	468
Ideonella sp. KYPY4	1862385	26.27	0.04	0.31	0.31	1003
Rhizobium sp. MSSRF QS100	1522278	24.90	0.04	0.37	0.68	379
Cylindrospermopsis sp. CR12	1747196	24.31	0.04	0.42	0.79	131
Anabaena sp. WA102	1647413	24.53	0.04	0.41	0.66	195
Mycobacterium sp. M26	1762962	24.61	0.04	0.27	0.27	1114
Polynucleobacter cosmopolitanus	351345	20.87	0.03	0.37	0.38	172
Rubrivivax albus	2499835	21.01	0.03	0.26	0.28	845
Dolichospermum sp. UHCC 0315A	1914871	20.14	0.03	0.33	0.51	184
Aeromonas	642	20.46	0.03	0.54	0.58	13
Verrucomicrobia bacterium SCGC AAA027-I19	939126	20.32	0.03	0.25	0.25	501
actinobacterium SCGC AAA028-N15	938467	19.72	0.03	0.38	0.38	81
Pseudopuniceibacterium sediminis	2211117	19.67	0.03	0.65	0.65	772
Cylindrospermopsis raciborskii CS-505	533240	19.26	0.03	0.37	0.79	96
Limnohabitans parvus II-B4	1293052	18.45	0.03	0.24	0.27	428
Clostridiales	186802	17.57	0.03	1.68	1.68	2
Methylocystaceae	31993	18.12	0.03	0.32	0.32	22
Pseudomonas alcaligenes NBRC 14159	1215092	18.11	0.03	0.27	0.61	314
Rickettsiaeae	33988	17.41	0.03	100.00	100.00	34
Vogesella urethralis	2592656	16.98	0.03	0.24	0.25	524
Rhizobiales bacterium CCH10-E5	1768797	16.32	0.03	0.24	0.48	393
Dechloromonas sp. H13	2570193	16.19	0.03	0.21	0.22	542
Methylocystis parvus OBBP	1134912	15.60	0.02	0.20	0.24	638
Piscinibacter defluvii	1796922	15.80	0.02	0.21	0.27	635
Opitutaceae bacterium TAV3	278958	16.03	0.02	0.27	0.30	41
Hydrogenophaga sp. H7	1882399	15.09	0.02	0.20	0.20	574
Rubrivivax gelatinosus	28068	15.04	0.02	0.13	0.26	266
Crenobacter sp. GY 70310	2563443	15.35	0.02	0.22	0.22	449
alpha proteobacterium SCGC AAA027-C06	938624	14.34	0.02	0.38	0.47	28
Erythrobacter neustonensis	1112	14.35	0.02	0.23	0.23	290
Gemmobacter aquaticus	490185	13.71	0.02	0.19	0.19	445
Acidovorax sp. GW101-3H11	1813946	13.65	0.02	0.20	0.37	394
Aquabacterium pictum	2315236	14.17	0.02	0.18	0.19	596
Plesiomonas shigelloides	703	13.97	0.02	0.18	0.42	33
Clostridium	1485	13.34	0.02	3.41	5.45	6
Limnohabitans planktonicus II-D5	1293045	13.03	0.02	0.16	0.17	507
Curvibacter sp. PAE-UM	1714344	12.89	0.02	0.17	0.17	457
Methylomonas sp. MK1	1131552	12.98	0.02	0.29	0.28	631
Aquirufa antheringensis	2516559	13.17	0.02	0.21	0.21	241
Mycobacteriaceae	1762	12.72	0.02	0.32	0.32	10
alpha proteobacterium SCGC AAA487-M09	938672	12.24	0.02	0.41	0.34	17
Macromonas sp. BK-30	1843082	12.43	0.02	0.18	0.18	355
Piscinibacter aquaticus	392597	12.76	0.02	0.17	0.18	545
actinobacterium SCGC AAA027-L06	913338	12.04	0.02	0.26	0.33	39
Zhizhongheella caldifontis	1452508	11.89	0.02	0.17	0.21	406
Malikia granosa	263067	11.74	0.02	0.16	0.57	363

Novosphingobium kunmingense	1211806	11.07	0.02	0.15	0.15	417
Ramlibacter tataouinensis TTB310	365046	11.19	0.02	0.15	0.16	451
Inhella inkyongensis	392593	11.48	0.02	0.17	0.17	335
Ideonella sakaiensis	1547922	11.42	0.02	0.16	0.20	463
Burkholderiales bacterium JOSHI_001	864051	11.25	0.02	0.16	0.16	462
Opitutaceae bacterium TAV4	278959	10.99	0.02	0.19	0.23	38
Quisquillibacterium sp. CC-CFT501	2498847	10.59	0.02	0.14	0.14	467
Rivibacter subsaxonicus	457575	10.47	0.02	0.14	0.14	435
Burkholderiales genera incertae sedis	224471	10.81	0.02	0.22	0.22	23
Paucibacter aquatile	2070761	10.58	0.02	0.14	0.14	444
actinobacterium SCGC AAA278-O22	932044	10.18	0.01	0.16	0.16	124
Gemmobacter sp. HYN0069	2169400	10.00	0.01	0.13	0.13	408
Holospora obtusa F1	1399147	10.17	0.01	0.19	0.19	96
Anaeromyxobacter	161492	9.76	0.01	0.16	0.16	69
Actinobacteria bacterium IMCC26077	1848755	9.39	0.01	0.14	0.14	164
Rubrivivax benzoatilyticus JA2 = ATCC BAA-35	987059	9.32	0.01	0.13	0.25	405
Cetobacterium somerae ATCC BAA-474	1319815	9.39	0.01	0.20	0.21	56
Gemmimonas	173479	9.24	0.01	9.59	9.47	58
Runella sp. SP2	2268026	9.32	0.01	0.14	0.19	365
actinobacterium SCGC AAA028-A23	932036	8.63	0.01	0.34	0.37	11
actinobacterium aclB-AMD-7	1504322	8.53	0.01	0.23	0.38	31
Rhodobacter flagellatus	2593021	7.66	0.01	0.11	0.13	279
Burkholderia mallei	13373	8.14	0.01	0.24	0.16	15
Sulfurisoma sediminicola	1381557	7.84	0.01	0.12	0.12	263
Candidatus Accumulibacter sp. SK-12	1454001	7.67	0.01	0.11	0.12	336
Pseudanabaena biceps PCC 7429	927668	7.28	0.01	0.12	0.13	170
Clostridium perfringens	1502	7.57	0.01	0.26	0.26	9
alpha proteobacterium SCGC AAA028-C07	938639	6.99	0.01	0.20	0.23	22
Alphaproteobacteria bacterium SYSU XM001	2560861	7.09	0.01	0.10	0.10	317
Novosphingobium sp. B 225	1961849	6.94	0.01	0.11	0.11	225
Tibeticola sediminis	1917811	7.03	0.01	0.11	0.11	229
Ramlibacter sp. Leaf400	1736365	7.58	0.01	0.11	0.13	351
Ramlibacter rhizophilus	1781167	7.19	0.01	0.10	0.10	339
Simplicispira metamorpha	80881	7.17	0.01	0.11	0.11	252
Sphaerotilus natans subsp. natans DSM 6575	1286631	7.19	0.01	0.10	0.11	319
Aquincola tertiaricarbonis	391953	7.30	0.01	0.11	0.11	329
Flavihumibacter sp. SB-02	2676868	7.36	0.01	0.13	0.13	165
Exiguobacterium	33986	6.33	0.01	0.13	0.13	4
Schlegelella thermodepolymerans	215580	6.88	0.01	0.11	0.11	269
Tepidicella xavieri	360241	6.62	0.01	0.10	0.11	200
Acinetobacter sp. VT 511	1675902	6.67	0.01	0.19	0.56	25
Ignavibacteriales	795748	6.36	0.01	16.67	16.67	13
Flavobacteria bacterium BAL38	391598	6.62	0.01	0.17	0.23	41
Acidovorax defluvii	86669	6.26	<0.01	0.11	0.12	130
Legionella donaldsonii	45060	5.92	<0.01	0.11	0.10	101
Aeromonas salmonicida subsp. achromogenes AS03	1233098	6.09	<0.01	0.21	0.21	26
actinobacterium SCGC AAA278-I18	938557	5.34	<0.01	0.15	0.15	24
Holospora curviuscula	1082868	5.04	<0.01	0.12	0.12	47
Bacillus sp. JEM-1	1977090	4.32	<0.01	0.32	0.32	4
alpha proteobacterium SCGC AAA027-J10	938631	4.84	<0.01	0.24	0.28	9
alpha proteobacterium SCGC AAA028-D10	938641	4.52	<0.01	0.14	0.19	17
Methylophilus	16	4.43	<0.01	5.26	5.26	2
unclassified Candidatus Accumulibacter	2619054	4.57	<0.01	0.10	0.10	2
Xanthomonadaceae bacterium JGI 0001002-F18	1094969	4.87	<0.01	0.38	0.38	19
Mycobacterium	1763	3.89	<0.01	0.10	0.10	18

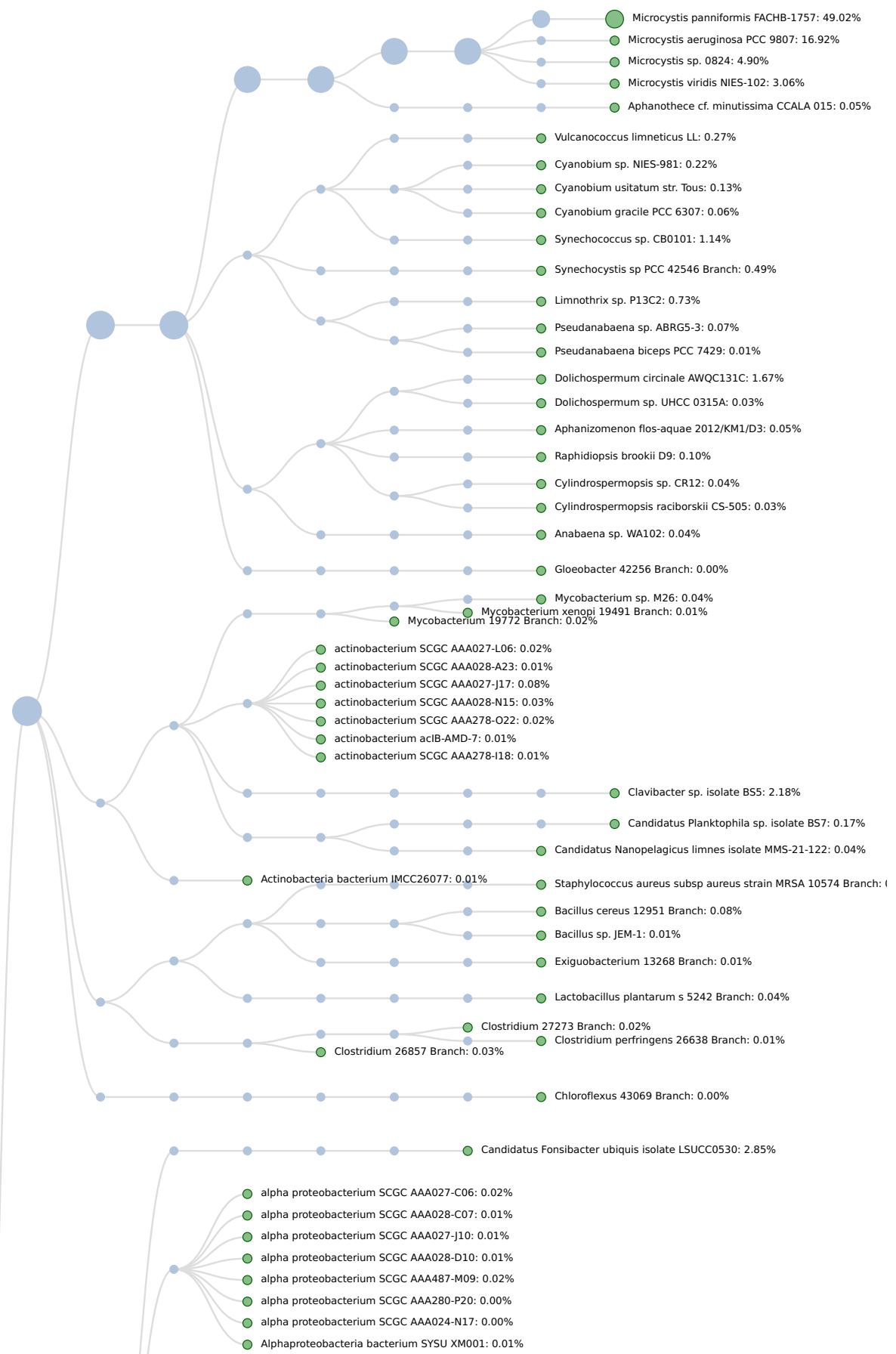
Comamonadaceae bacterium JGI 0001013-A16	1286843	3.83	<0.01	0.11	0.11	22
Acinetobacter schindleri	108981	3.96	<0.01	0.14	0.20	15
Wolbachia endosymbiont of Drosophila ananassae	307502	3.32	<0.01	0.26	0.20	13
Comamonas	283	3.49	<0.01	0.12	0.13	9
Burkholderiales	80840	3.32	<0.01	11.11	11.11	7
Ferrovum sp. JA12	1356299	3.27	<0.01	0.11	0.11	82
Chitinilyticum	551208	3.27	<0.01	0.12	0.12	5
Escherichia coli MS 110-3	749536	3.52	<0.01	0.63	0.47	3
Alphaproteobacteria	28211	2.31	<0.01	0.10	0.10	8
Gloeobacter	33071	2.18	<0.01	2.01	2.01	11
Chloroflexus	1107	1.87	<0.01	6.90	6.90	4
alpha proteobacterium SCGC AAA280-P20	938665	1.88	<0.01	0.12	0.12	5
unclassified Verrucomicrobia	417295	1.91	<0.01	0.14	0.14	2
alpha proteobacterium SCGC AAA024-N17	938623	1.01	<0.01	0.18	0.22	9
unclassified Opitutaceae	278955	1.28	<0.01	1.27	1.66	7

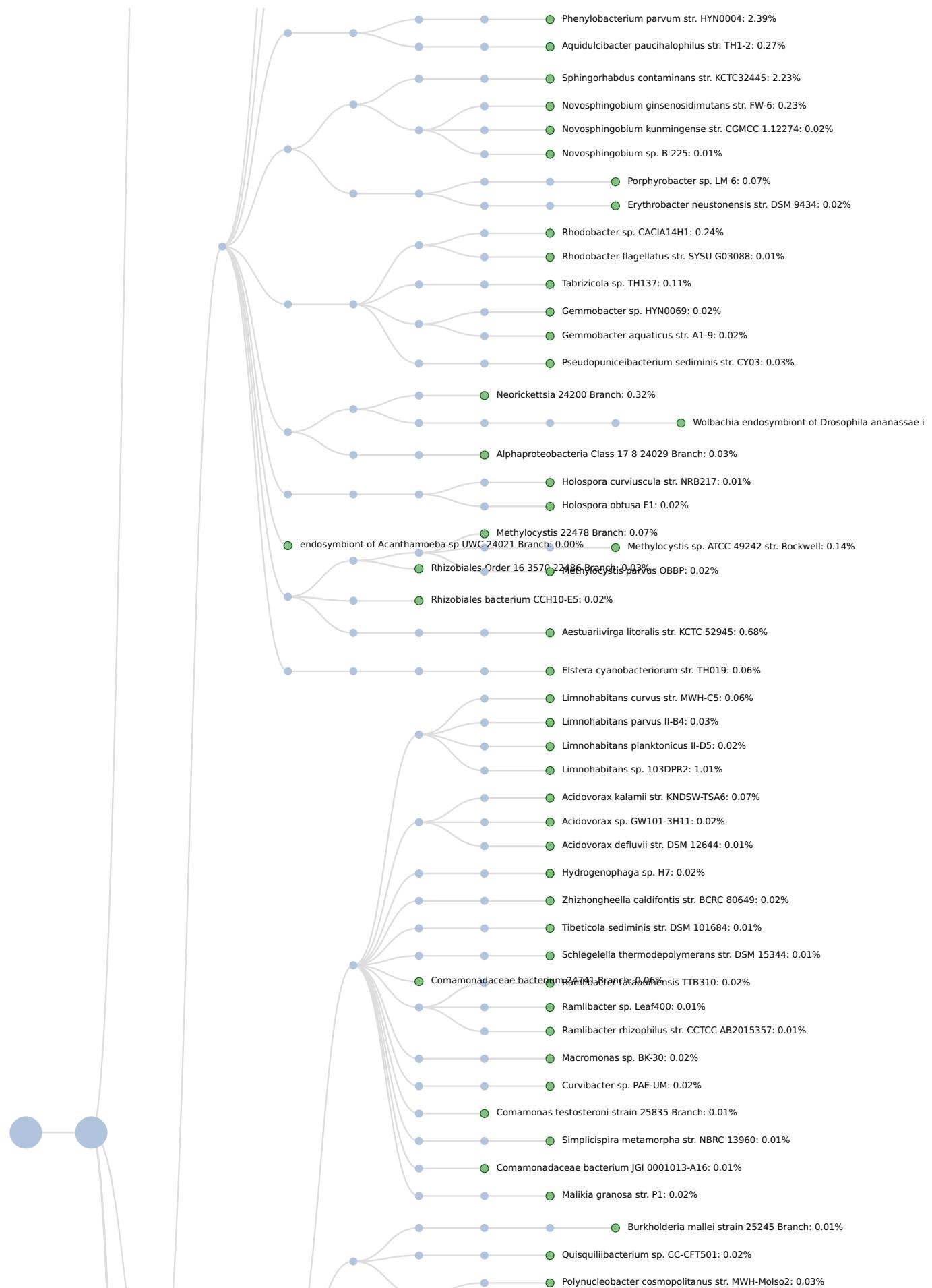
Bacteria

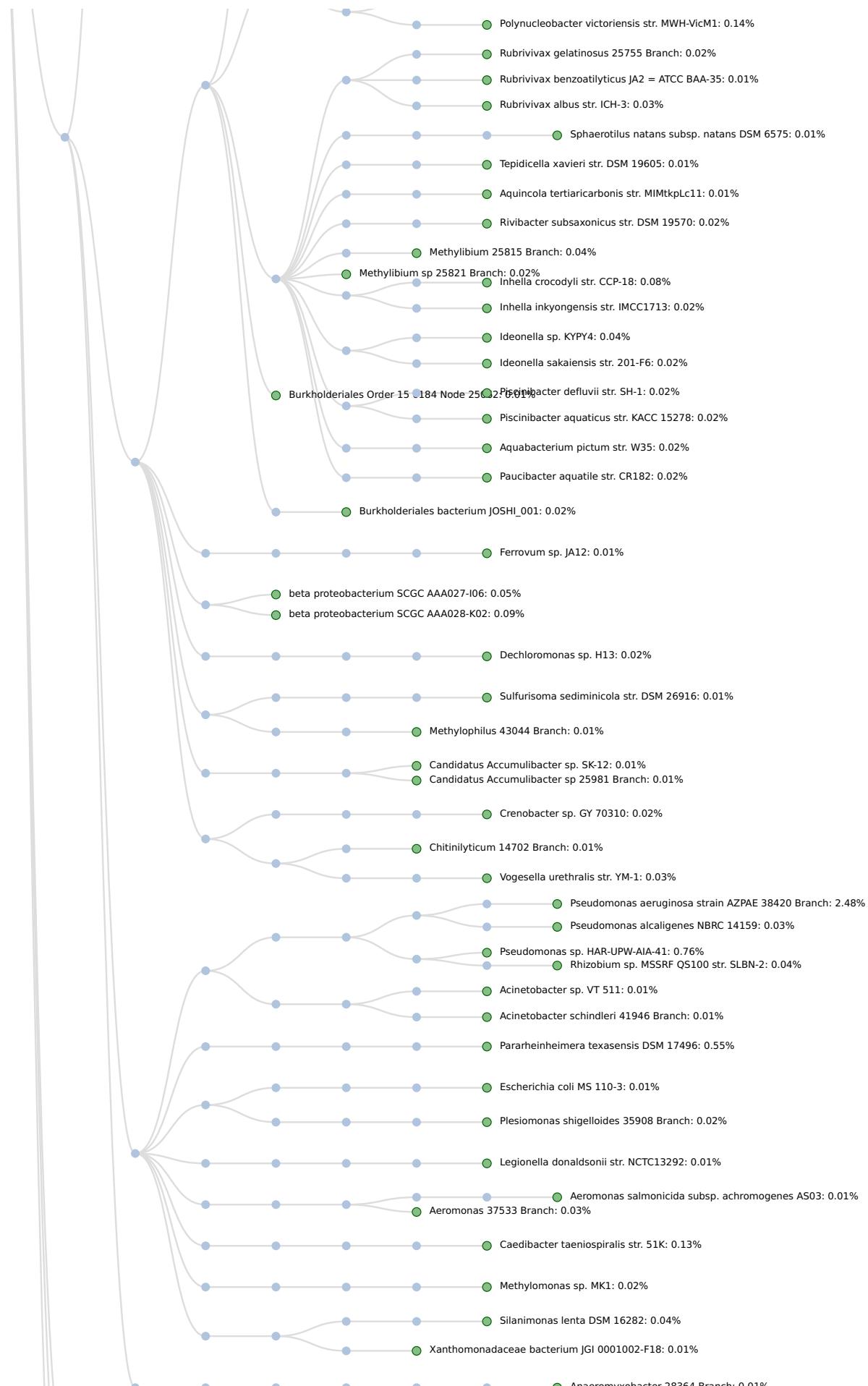
Tree Chart Taxonomy

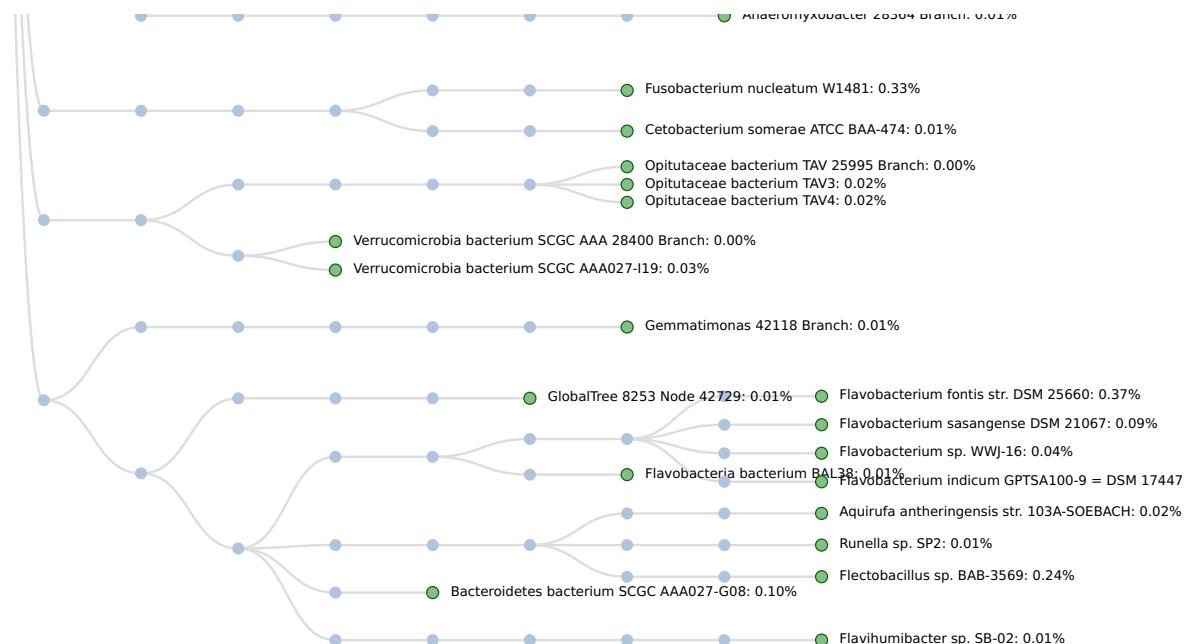
Sample_1_BWT2214

Total results



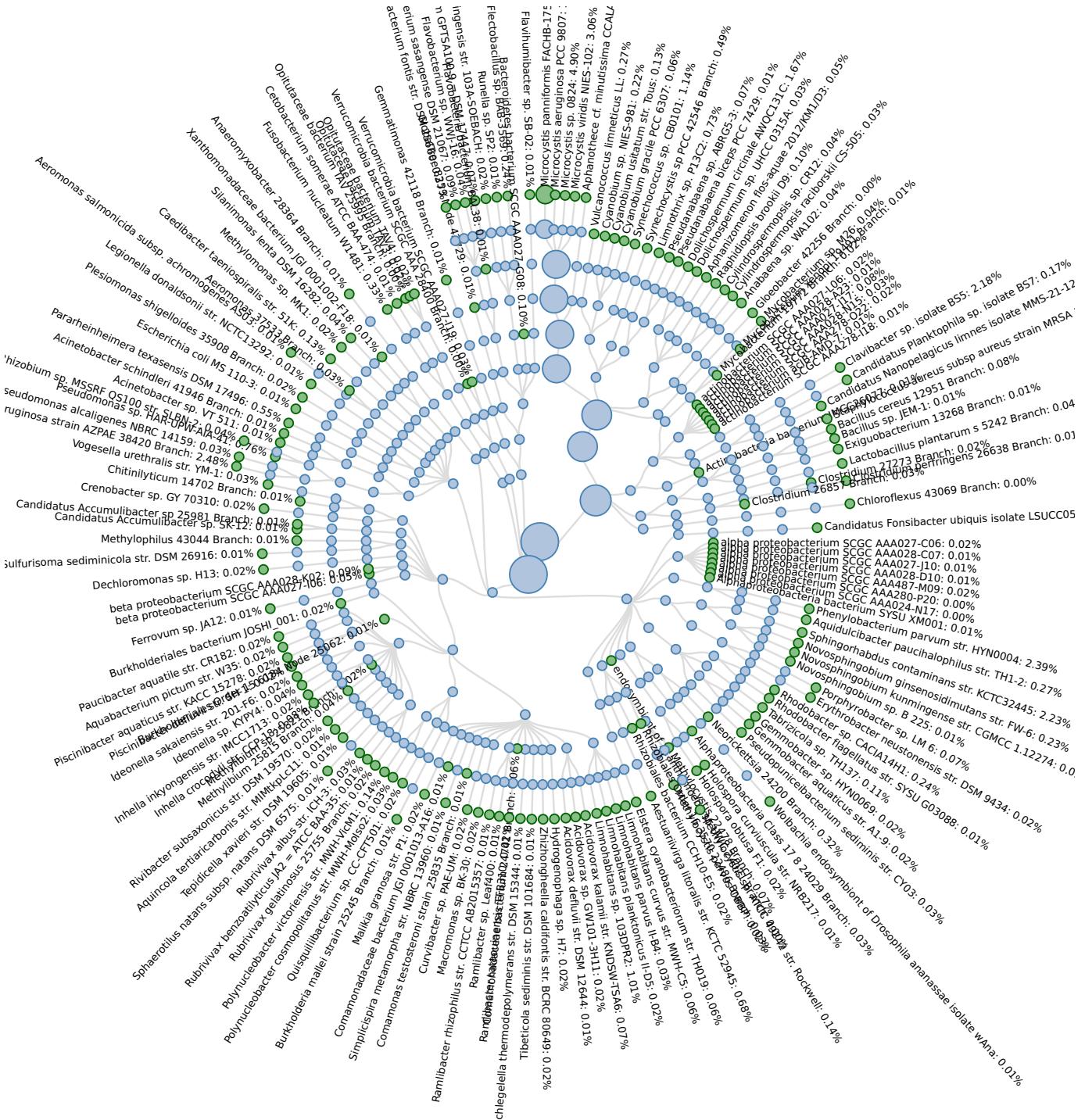






Bacteria

Radial Tree Chart Taxonomy

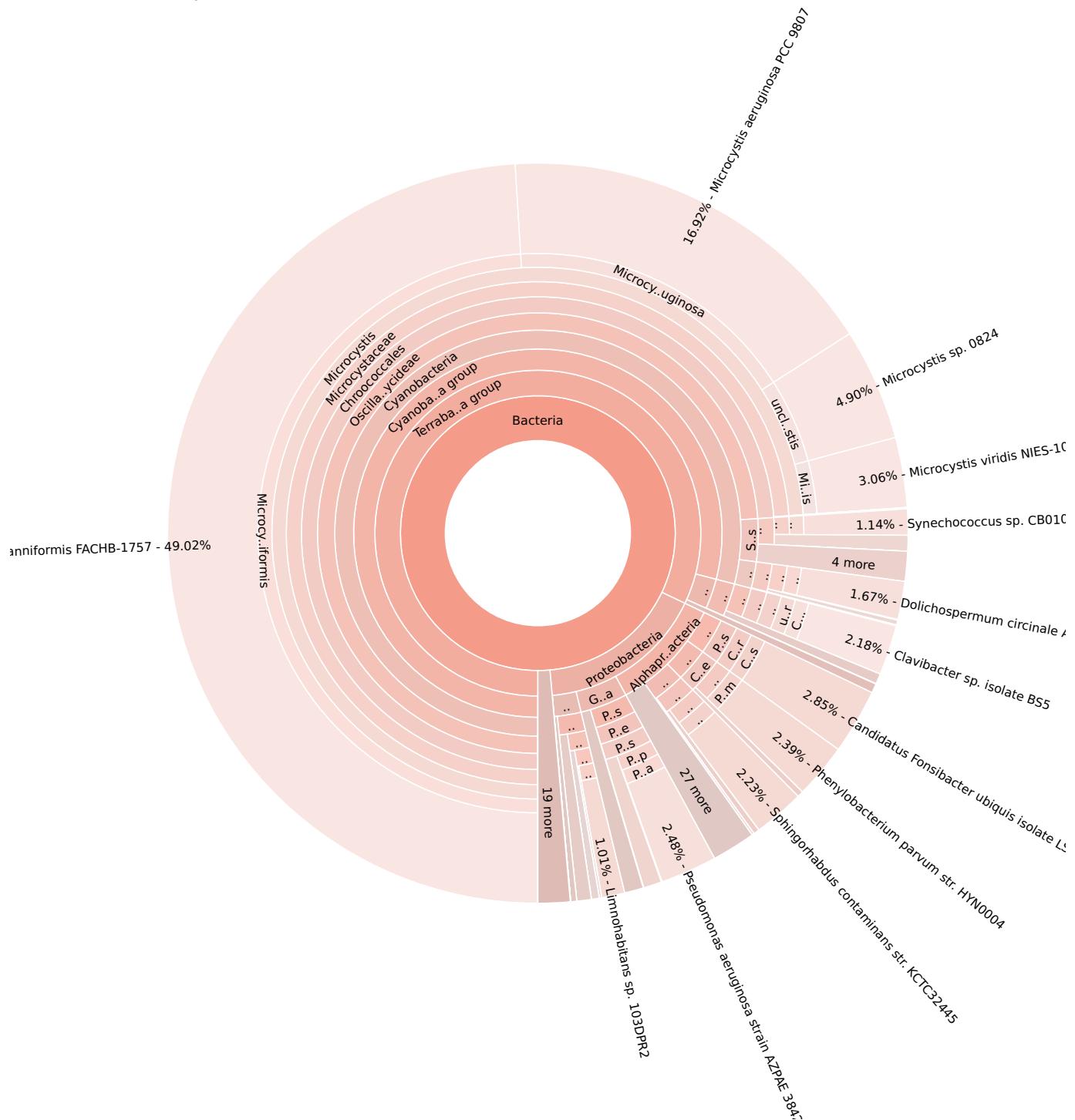


Bacteria

Sunburst Chart Taxonomy

Sample 1 BWT2214

Total results

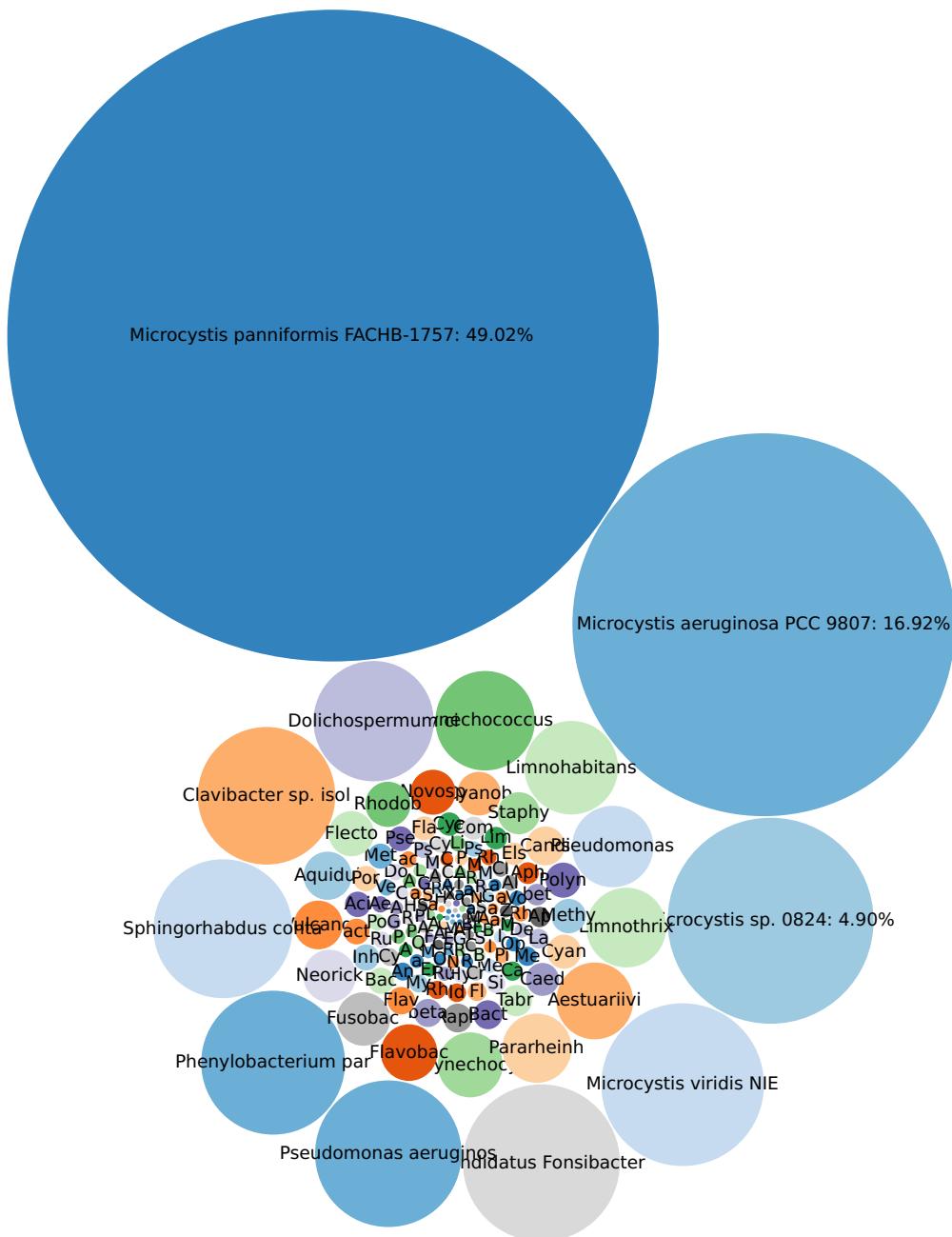


Bacteria

Bubble Chart Taxonomy

Sample_1_BWT2214

Total results



Antibiotic Resistance

Fasta/q details

Sample_1_BWT2214

Total results

Metric	Value
Hit	115

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Aminoglycoside GENE aadA1 1 X02340	-	1048.98	100.00	35.94	13.53	130

Fungi

Fasta/q details

Sample_1_BWT2214

Total results

Metric	Value					
Hit	58.204k					

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Onygenales	33183	39.71	23.49	0.34	0.54	1508
Clavaria fumosa	264083	8.27	4.89	0.10	0.30	452
Fusarium subglutinans	42677	6.92	4.09	0.08	0.05	412
Brettanomyces bruxellensis	5007	5.35	3.16	0.07	0.08	325
Puccinia arachidis	333523	5.26	3.11	0.08	0.13	275
Trichosporon inkin	82517	4.35	2.57	0.02	0.02	454
Cantharellus cibarius	36066	4.33	2.56	0.06	0.08	257
Metschnikowia cerradonensis	390697	4.28	2.53	0.02	0.02	463
Morchella eximia	1582338	3.91	2.31	0.03	0.02	368
Cantharellus lutescens	104198	3.33	1.97	0.03	0.24	277
Epichloe sylvatica	79593	3.11	1.84	0.04	0.03	234
Phycomyces blakesleeanus NRRL 1555(-)	763407	3.02	1.79	0.03	0.03	277
Ceraceosorus bombacis	401625	2.80	1.66	0.02	0.01	287
Escovopsis weberi	150374	2.76	1.63	0.03	0.01	258
Ceratocystis smalleyi	312343	2.69	1.59	0.02	0.01	290
Epichloe bromicola	79588	2.62	1.55	0.02	0.01	278
Ophiocordyceps polyrhachis-furcata BCC 54312	1330021	2.45	1.45	0.02	0.02	240
Quambalaria eucalypti	363177	2.25	1.33	0.03	0.03	197
Ophiocordyceps unilateralis	268505	2.15	1.27	0.01	0.01	258
Chaetothyriales sp. CBS 132003	2249419	2.14	1.27	0.03	0.03	180
Ophiocordyceps camponoti-floridani	2030778	2.11	1.25	0.03	0.02	185
Escovopsis sp. AC	2027528	1.95	1.15	0.03	0.02	136
Geosmithia morbida	1094350	1.92	1.14	0.02	0.02	201
Ceratocystiopsis brevicomis	553390	1.89	1.12	0.03	0.03	149
Metschnikowia santaceiciliae	197674	1.88	1.11	0.02	0.01	201
Blastomyces emzantsi	2723674	1.86	1.10	0.03	0.04	118
Sphaerulina populicola P02.02b	1136490	1.80	1.06	0.02	0.01	175
Melampsora pinitorqua Mpini7	1298852	1.78	1.05	0.02	0.03	155
Caulochytrium protostelioides	1555241	1.75	1.03	0.03	0.03	139
Metschnikowia continentalis	73517	1.72	1.02	0.01	0.01	192
Epichloe amarillans E57	1037526	1.69	1.00	0.02	0.02	148
Herpomyces periplanetae	1749284	1.62	0.96	0.01	0.01	192
Claviceps paspali	40601	1.48	0.88	0.01	0.02	162
Lentinus polychrous	292559	1.35	0.80	0.04	0.04	171
Cokeromyces recurvatus B5483	1357676	1.35	0.80	0.01	0.01	152
Ceraceosorus guamensis	1522189	1.33	0.79	0.02	0.01	846
Epichloe festucae Fl1	877507	1.26	0.74	0.01	0.01	149
Epichloe uncinata	5050	1.25	0.74	0.02	0.01	471
Tuber microsphaerosporum	1455713	1.25	0.74	0.02	0.03	103
Apotrichum gamsii	1105092	1.22	0.72	0.01	0.01	140
Mortierella sp. BCC40632	2613877	1.20	0.71	0.02	0.02	121
Lodderomyces elongisporus NRRL YB-4239	379508	1.17	0.69	0.02	0.02	88
Absidia glauca	4829	1.16	0.69	0.01	0.01	120
Metschnikowia sp. 00-154.1	1807683	1.13	0.67	0.02	0.02	99
Metschnikowia matae var. maris	1697387	1.04	0.61	0.02	0.01	99
Balansia oblecta B249	1405085	0.99	0.59	0.01	0.01	108
Epichloe elymi	55200	0.98	0.58	0.01	0.01	103

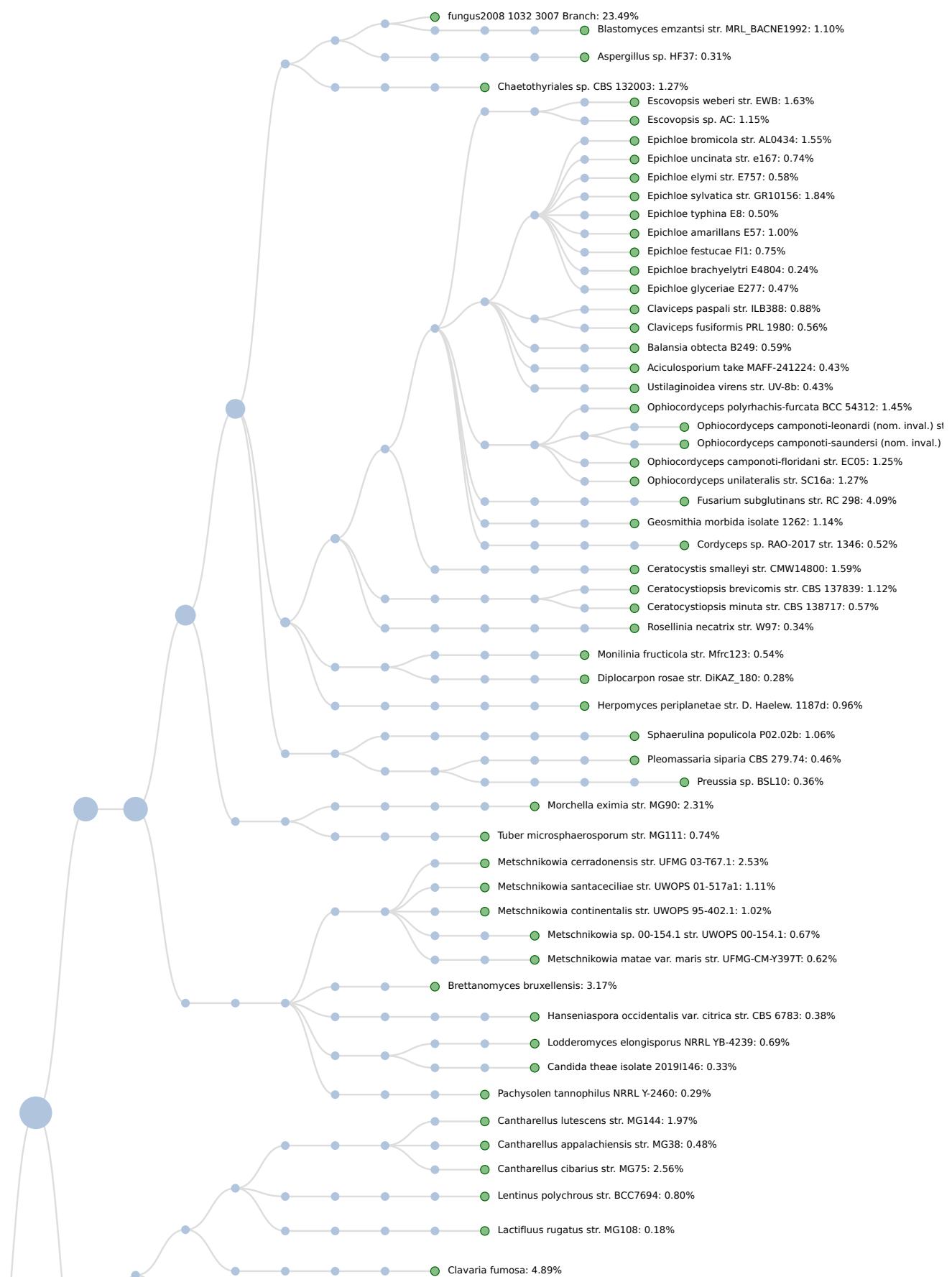
<i>Ceratocystiopsis minuta</i>	360147	0.97	0.57	0.01	0.02	102
<i>Claviceps fusiformis</i> PRL 1980	1036761	0.94	0.56	0.01	0.01	89
<i>Monilinia fructicola</i>	38448	0.92	0.54	0.02	0.01	83
<i>Cordyceps</i> sp. RAO-2017	2004951	0.88	0.52	0.02	0.02	78
<i>Anthracocystis flocculosa</i> PF-1	1277687	0.85	0.50	0.01	0.02	85
<i>Epichloe typhina</i> E8	1227655	0.84	0.50	0.01	0.01	83
<i>Cantharellus appalachiensis</i>	409893	0.81	0.48	0.01	0.19	71
<i>Epichloe glyceriae</i> E277	1035635	0.80	0.47	0.02	0.02	65
<i>Violaceomyces palustris</i>	1673888	0.80	0.47	0.01	0.01	77
<i>Pleomassaria siparia</i> CBS 279.74	1314801	0.78	0.46	0.01	0.01	75
<i>Microbotryum lychnidis-dioicae</i> p1A1 Lamole	683840	0.78	0.46	0.04	0.04	19
<i>Pecoramycetes ruminantium</i>	1987568	0.76	0.45	0.01	0.01	76
<i>Aciculosporium</i> take MAFF-241224	1036760	0.72	0.43	0.01	0.02	62
<i>Ustilaginoidea virens</i>	1159556	0.72	0.43	0.01	0.01	70
<i>Hanseniaspora occidentalis</i> var. <i>citrina</i>	331638	0.65	0.39	0.01	0.01	273
<i>Preussia</i> sp. BSL10	1712568	0.60	0.36	0.02	0.02	36
<i>Rosellinia necatrix</i>	77044	0.57	0.34	0.01	0.01	56
<i>Candida theae</i>	1198502	0.56	0.33	0.01	0.01	46
<i>Ophiocordyceps camponoti-leonardi</i> (nom. inval.)	2039875	0.54	0.32	0.01	0.01	40
<i>Aspergillus</i> sp. HF37	1960876	0.53	0.31	0.01	0.01	139
<i>Ophiocordyceps camponoti-saundersi</i> (nom. inval.)	2039874	0.49	0.29	0.01	0.01	32
<i>Pachysolen tannophilus</i> NRRL Y-2460	669874	0.49	0.29	0.01	0.01	25
<i>Diplocarpon rosae</i>	946125	0.48	0.28	0.01	0.01	244
<i>Epichloe brachyelytri</i> E4804	1036762	0.41	0.24	0.01	0.01	88
<i>Lactifluus rugatus</i>	1837266	0.31	0.18	0.01	0.01	43

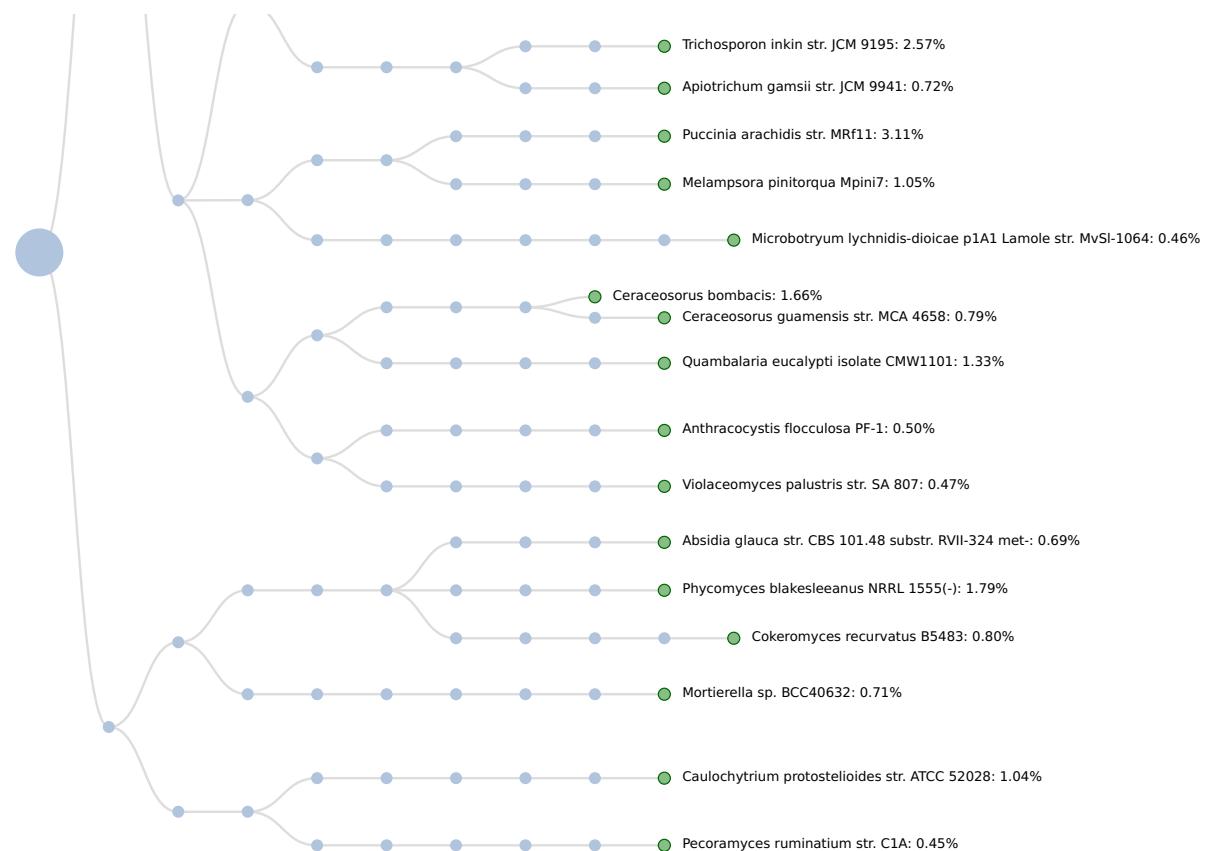
Fungi

Tree Chart Taxonomy

Sample_1_BWT2214

Total results





Fungi

Radial Tree Chart Taxonomy

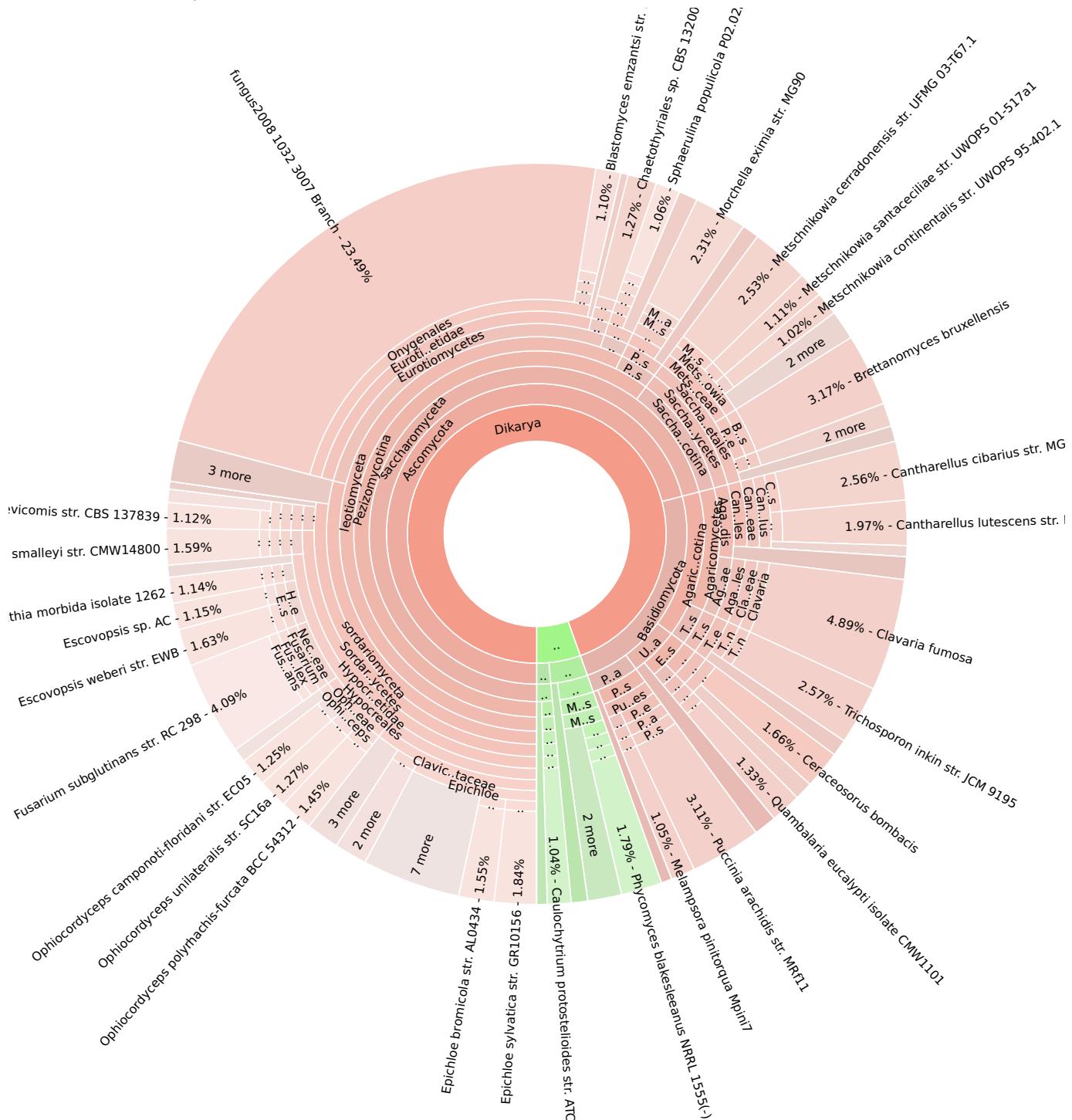
Sample_1_BWT2214

Total results



Fungi

Sunburst Chart Taxonomy

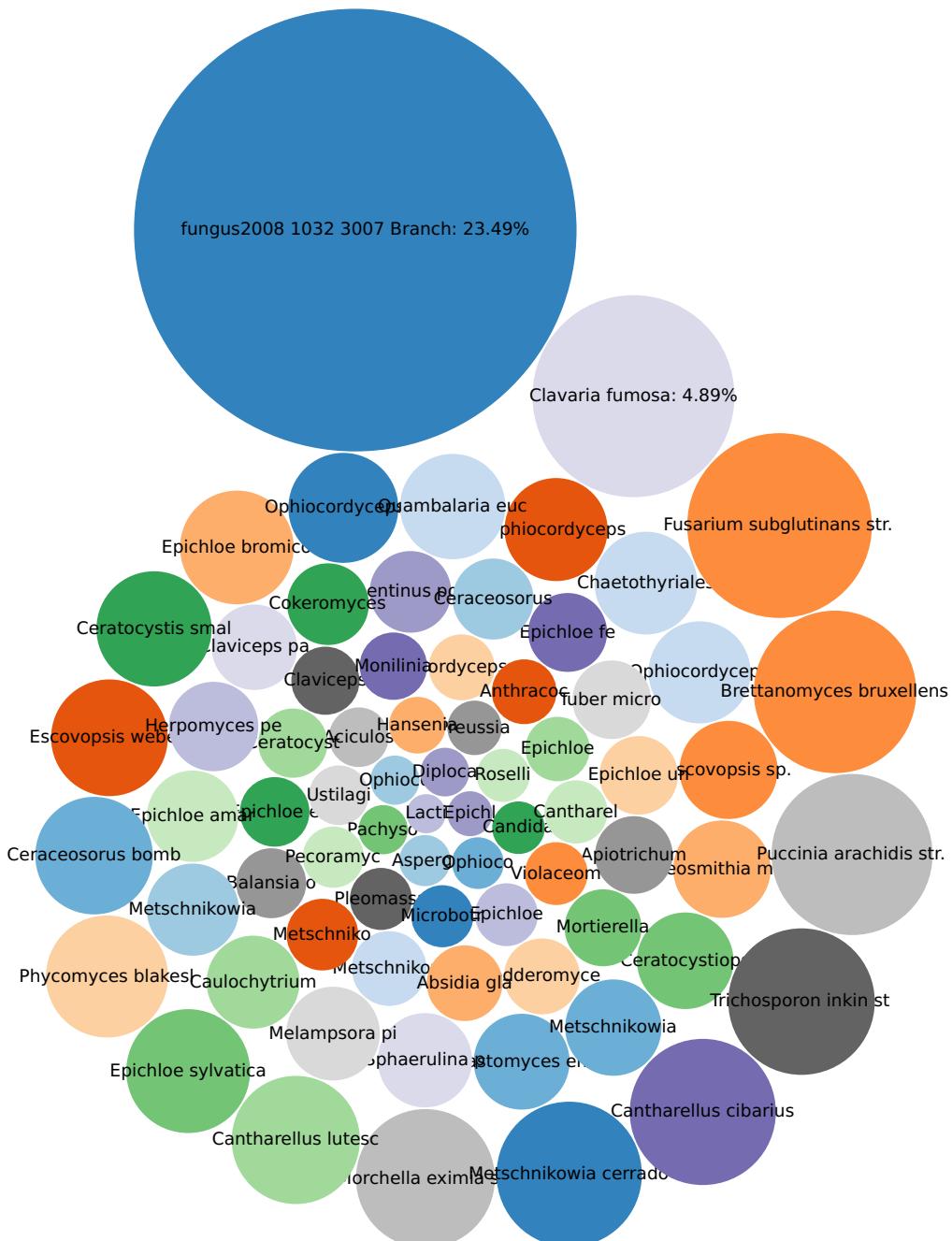


Fungi

Bubble Chart Taxonomy

Sample 1 BWT2214

Total results



Protists

Fasta/q details

Sample_1_BWT2214

Total results

Metric	Value
Hit	0.120M

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Neobalantidium coli	71585	5509.94	94.68	27.12	27.12	297
Salpingoeca rosetta	946362	42.00	0.72	0.33	2.17	2278
Acanthamoeba polyphaga	5757	21.82	0.38	0.20	0.18	1217
Fonticula alba	691883	16.69	0.29	0.10	0.24	1276
Symbiodinium minutum Mf 1.05b.01	1280413	14.95	0.26	0.09	1.29	1179
Acanthamoeba quina	211522	13.57	0.23	0.11	0.18	936
Pseudoperonospora cubensis	143453	12.49	0.21	0.15	0.21	605
Toxoplasma gondii p89	943119	10.04	0.17	0.02	0.03	411
Leishmania major strain LV39c5	860569	9.85	0.17	0.12	0.06	388
Bigelowiella natans CCMP2755	753081	8.72	0.15	0.08	0.53	621
Schizochytrium sp. CCTCC M209059	1573607	8.26	0.14	0.05	0.20	734
Thalassiosira pseudonana CCMP1335	296543	8.07	0.14	0.03	0.07	821
Reticulomyxa filosa	46433	8.05	0.14	0.06	0.16	631
Physarum polycephalum	5791	7.54	0.13	0.07	0.57	560
Guillardia theta CCMP2712	905079	7.37	0.13	0.05	0.29	653
Acanthamoeba pearcei	65662	6.74	0.12	0.06	0.17	193
Chromera velia	505693	6.76	0.12	0.07	0.94	475
Acanthamoeba lugdunensis	61605	6.67	0.11	0.07	0.20	458
Acanthamoeba mauritaniensis	196912	5.78	0.10	0.07	0.25	373
Acanthamoeba castellanii str. Neff	1257118	5.62	0.10	0.05	0.20	453
Hemiselmis andersenii	464988	5.44	0.09	0.22	0.22	10
Acanthamoeba rhyodes	32599	3.99	0.07	0.05	0.22	279
Leishmania gerbilli	40285	3.78	0.07	0.04	0.05	283
Emiliania huxleyi CCMP1516	280463	3.79	0.07	0.04	0.48	296
Leishmania turanica	62297	3.66	0.06	0.05	0.05	229
Leishmania aethiopica L147	1206056	3.62	0.06	0.04	0.04	281
Acanthamoeba healyi	65661	3.50	0.06	0.03	0.33	297
Hammondia hammondi	99158	3.12	0.05	0.02	0.05	316
Nannochloropsis oceanica	145522	3.17	0.05	0.03	0.18	261
Sarcocystis neurona	42890	3.11	0.05	0.04	0.04	125
Leishmania infantum JPCM5	435258	3.05	0.05	0.04	0.03	207
Nannochloropsis gaditana CCMP526	1093141	2.94	0.05	0.04	0.04	177
Plasmodium gaboni	647221	2.59	0.04	0.01	0.03	150
Leishmania donovani	5661	2.42	0.04	0.03	0.02	182
Thecamonas trahens ATCC 50062	461836	2.45	0.04	0.02	0.08	257
Acanthamoeba palestinenensis	28015	2.24	0.04	0.03	0.28	161
Capsaspora owczarzaki ATCC 30864	595528	2.21	0.04	0.02	0.04	237
Cryptosporidium hominis	237895	1.83	0.03	0.02	0.01	109
Styloynchia lemnae 2x8/2	755200	1.78	0.03	0.04	0.05	51
Dictyostelium firmibasis	79012	1.71	0.03	0.02	0.03	123
Leishmania tropica L590	1206058	1.70	0.03	0.02	0.04	139
Leishmania arabica	40284	1.64	0.03	0.02	0.03	138
Entamoeba dispar SAW760	370354	1.55	0.03	0.03	0.03	74
Dictyostelium intermedium	361076	1.43	0.03	0.02	0.04	103
Paramecium biaurelia	65126	1.47	0.03	0.02	0.03	112
Cryptosporidium muris RN66	441375	1.37	0.02	0.02	0.02	64
Plasmodium reichenowi	5854	1.32	0.02	0.01	0.02	110

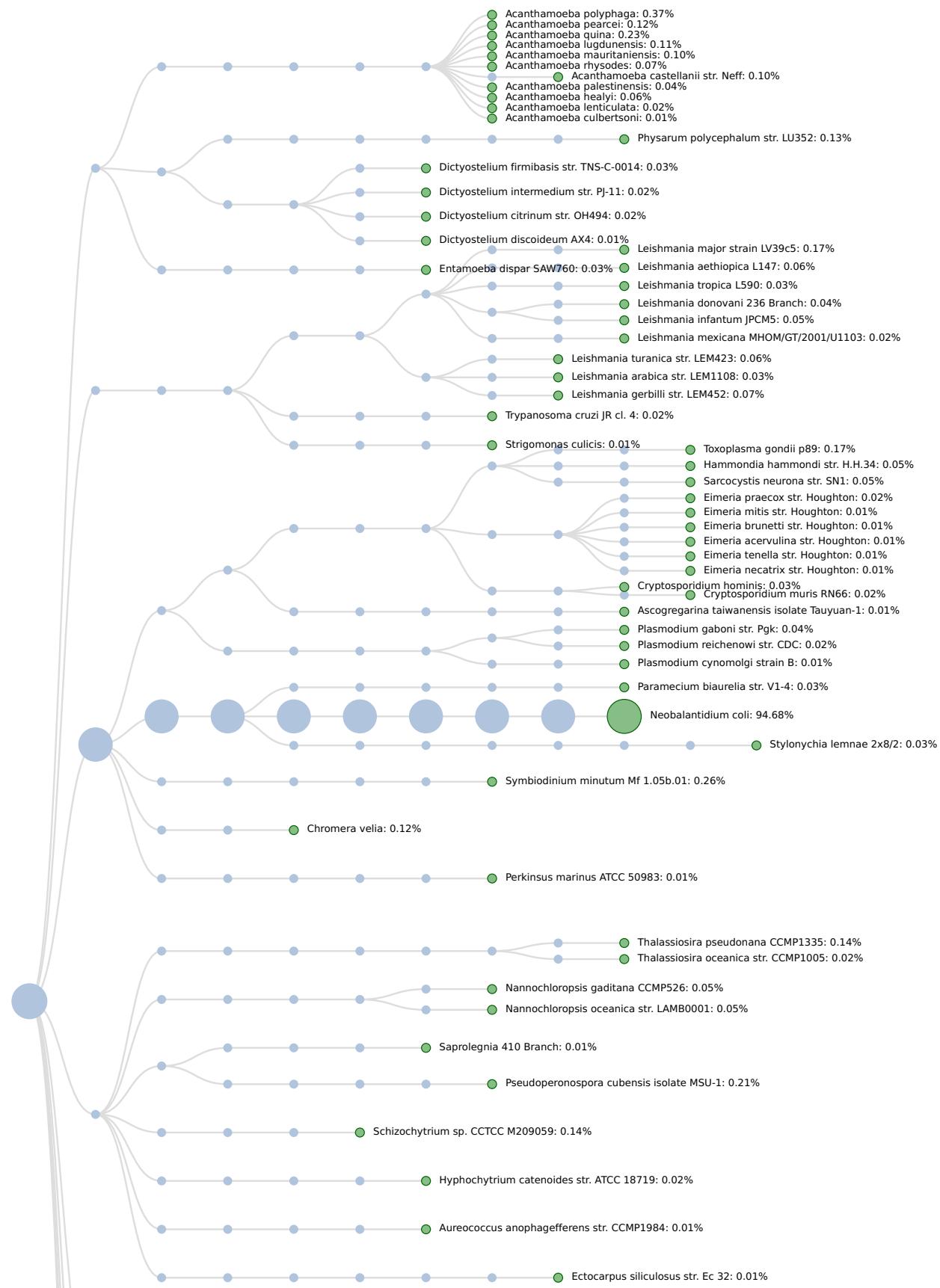
<i>Leishmania mexicana</i> MHOM/GT/2001/U1103	929439	1.19	0.02	0.01	0.01	129
<i>Hypochytrium catenoides</i>	42384	1.19	0.02	0.01	0.03	131
<i>Dictyostelium citrinum</i>	361072	1.13	0.02	0.01	0.02	82
<i>Trypanosoma cruzi</i> JR cl. 4	914063	1.11	0.02	0.02	0.01	85
<i>Eimeria praecox</i>	51316	1.08	0.02	0.01	0.16	116
<i>Monosiga brevicollis</i> MX1	431895	1.13	0.02	0.02	0.07	163
<i>Acanthamoeba lenticulata</i>	29196	0.97	0.02	0.01	0.07	106
<i>Dictyostelium discoideum</i> AX4	352472	0.87	0.01	0.02	0.02	59
<i>Thalassiosira oceanica</i>	159749	0.89	0.01	0.01	0.06	629
<i>Saprolegnia</i>	4769	0.86	0.01	0.02	0.02	32
<i>Ascogregarina taiwanensis</i>	158379	0.74	0.01	0.01	0.02	69
<i>Strigomonas culicis</i>	28005	0.68	0.01	0.02	0.02	115
<i>Eimeria brunetti</i>	51314	0.72	0.01	0.01	0.21	66
<i>Aureococcus anophagefferens</i>	44056	0.71	0.01	0.01	0.08	68
<i>Ectocarpus siliculosus</i>	2880	0.70	0.01	0.01	0.26	55
<i>Plasmodium cynomolgi</i> strain B	1120755	0.65	0.01	0.01	0.02	53
<i>Perkinsus marinus</i> ATCC 50983	423536	0.64	0.01	0.01	0.06	66
<i>Eimeria acervulina</i>	5801	0.60	0.01	0.01	0.15	52
<i>Acanthamoeba culbertsoni</i>	43142	0.54	<0.01	0.01	0.07	43
<i>Eimeria necatrix</i>	51315	0.50	<0.01	0.01	0.15	34
<i>Eimeria mitis</i>	44415	0.47	<0.01	0.01	0.21	32
<i>Eimeria tenella</i>	5802	0.44	<0.01	0.01	0.10	32

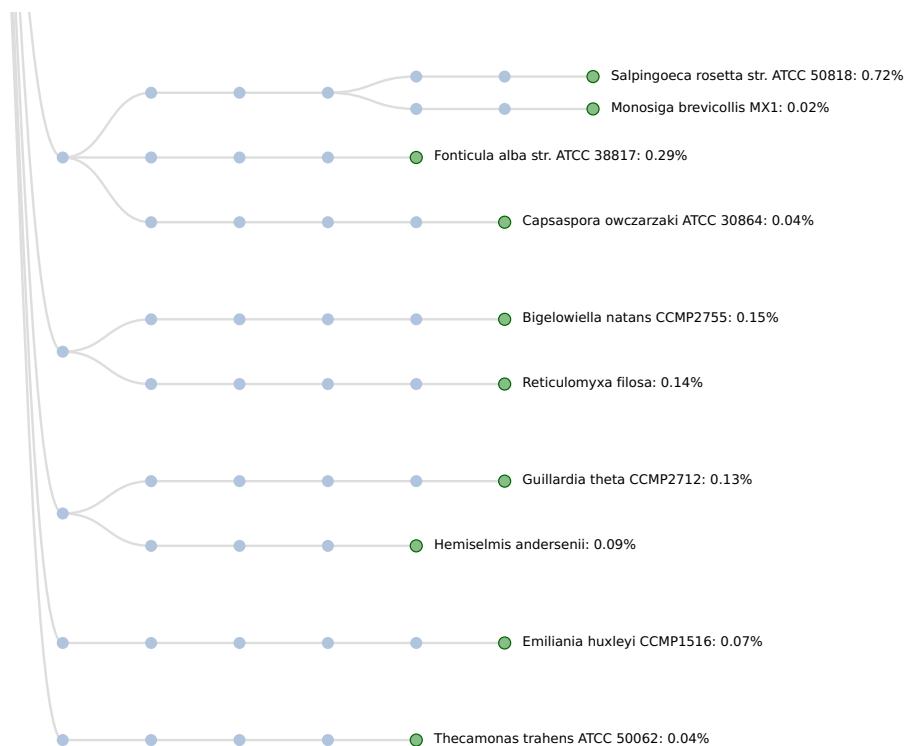
Protists

Tree Chart Taxonomy

Sample_1_BWT2214

Total results



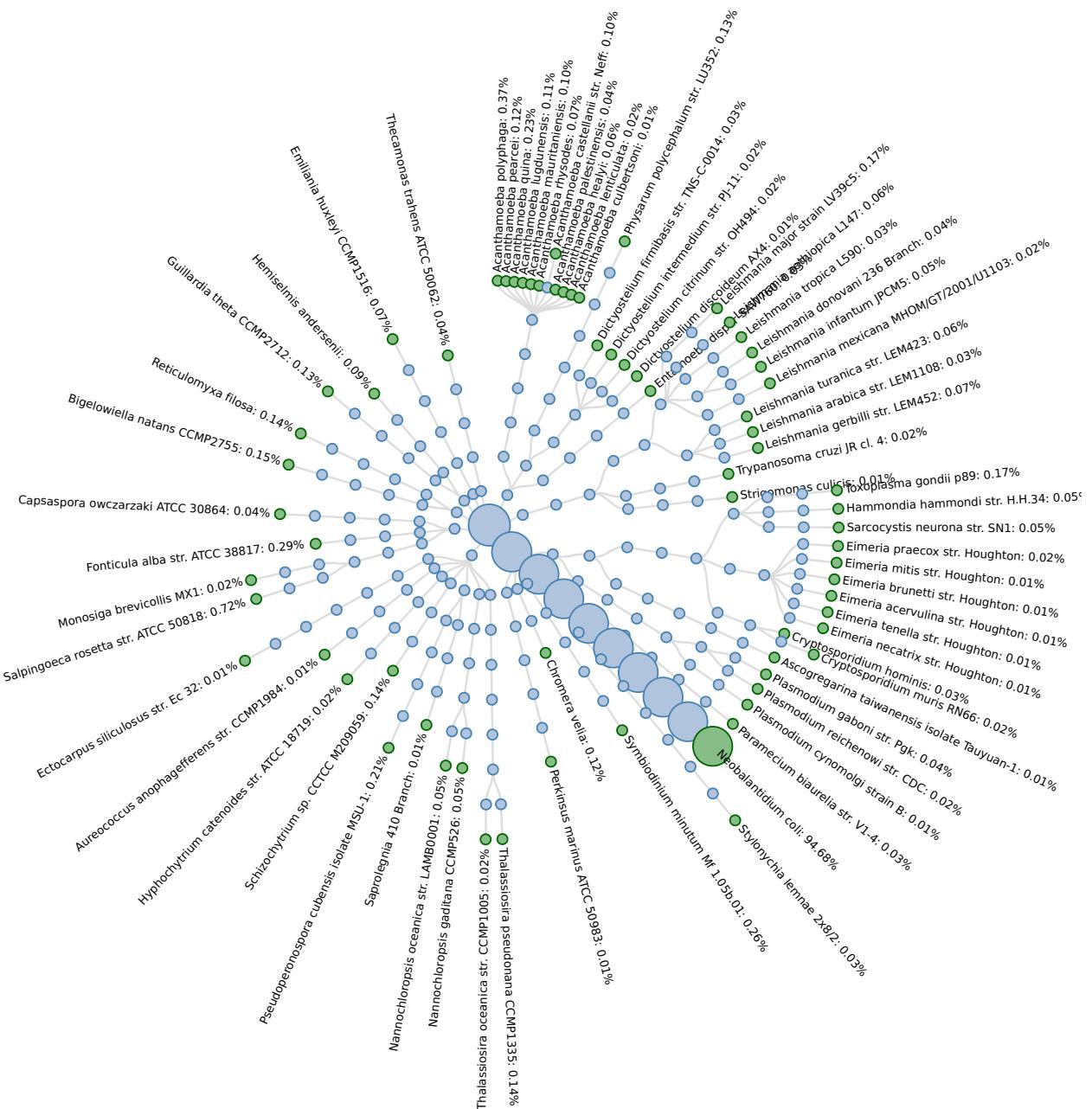


Protists

Radial Tree Chart Taxonomy

Sample_1_BWT2214

Total results

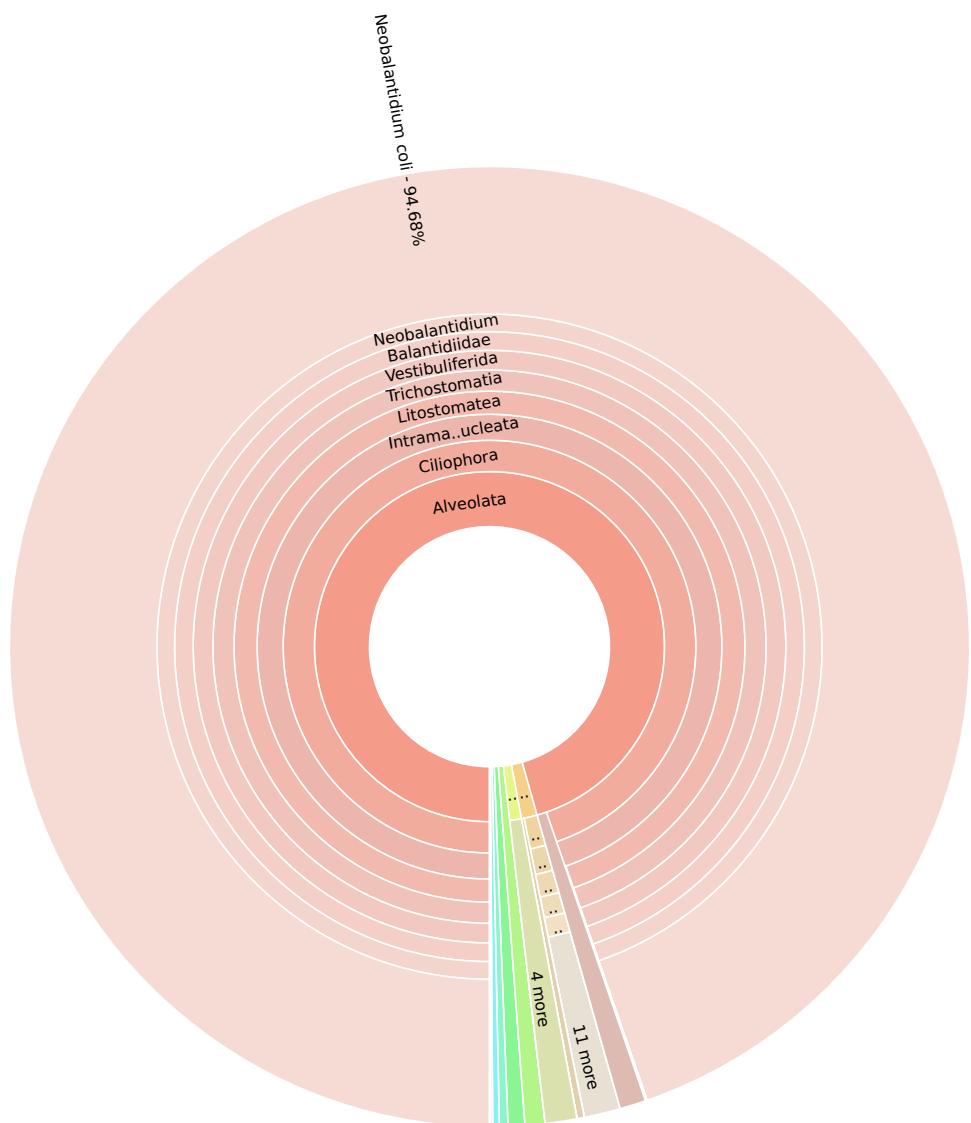


Protists

Sunburst Chart Taxonomy

Sample_1_BWT2214

Total results

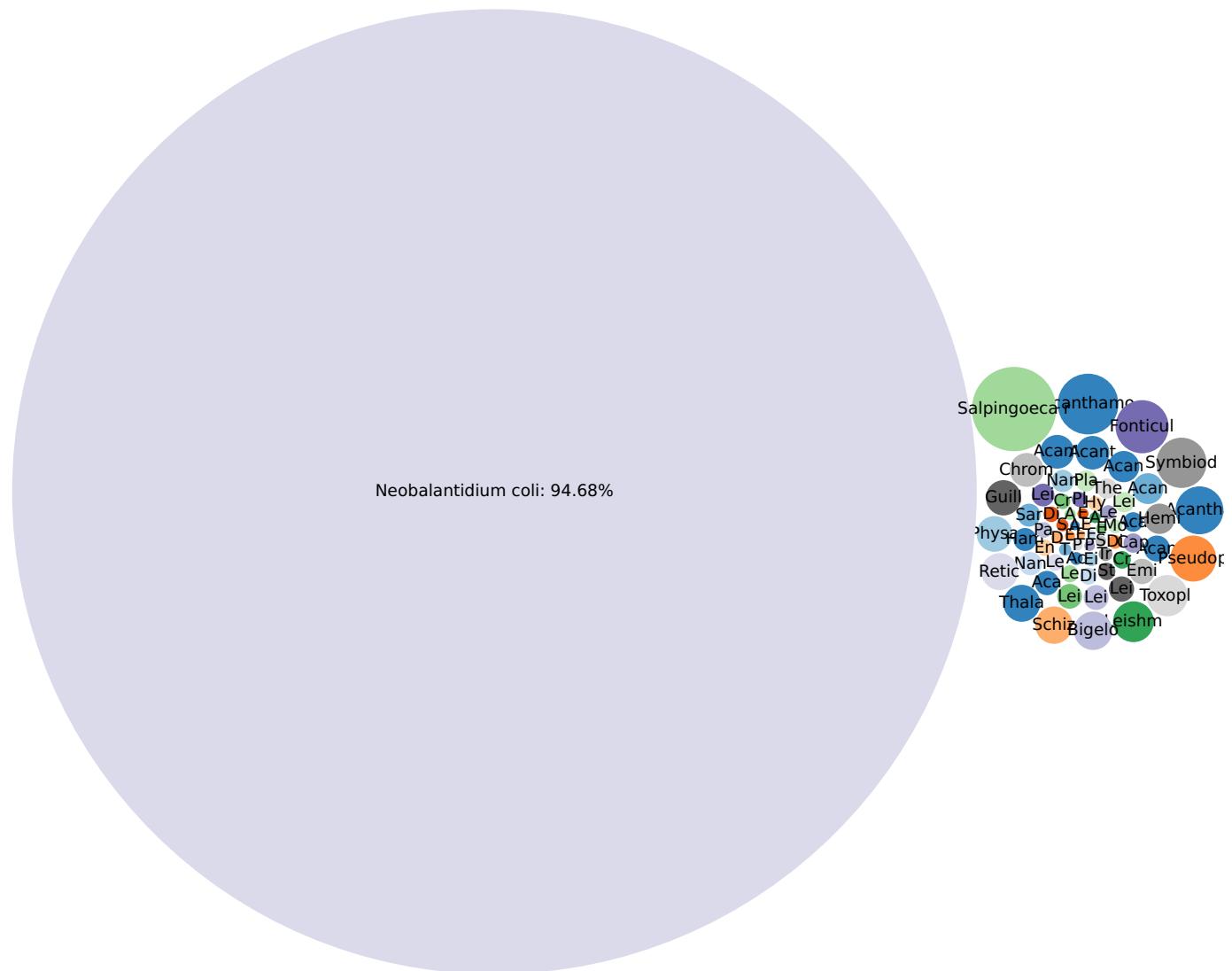


Protists

Bubble Chart Taxonomy

Sample_1_BWT2214

Total results



Dark Matter (Beta)

Fasta/q details

Sample_1_BWT2214

Total results

Metric	Value
Hit	0.291M

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Methylocystaceae bacterium UBA4761	1952232	6846.25	21.90	52.14	64.11	5548
Methylocystaceae bacterium UBA5192	1952233	6550.31	20.95	48.35	62.36	8032
Burkholderiales bacterium UBA954	1950782	3834.53	12.26	29.44	29.44	27897
Pelagibacterales bacterium UBA4760	1951182	2537.62	8.12	25.94	25.94	3073
Pseudanabaena sp.	1153	1350.87	4.32	11.14	11.23	14074
Methylocystaceae bacterium UBA2095	1952231	1311.03	4.19	10.10	39.23	4435
Burkholderiales bacterium UBA2470	1950772	946.86	3.03	9.97	11.23	5116
Burkholderiales bacterium UBA2647	1950774	721.53	2.31	7.16	11.16	1098
Lautropia sp.	2053568	550.99	1.76	6.55	11.24	1532
Pseudomonas sp. UBA6719	1947335	413.94	1.32	4.06	4.06	5252
Planctomycetaceae bacterium	2026779	313.75	1.00	2.99	2.99	7971
Novosphingobium sp. UBA6722	1946985	311.17	1.00	3.04	3.04	4356
unclassified Burkholderiales (miscellaneous)	80841	274.58	0.88	0.26	0.26	380
uncultured Collinsella sp.	165190	271.02	0.87	0.42	0.42	789
Methylophilaceae bacterium	2030816	227.36	0.73	2.88	3.50	90
Flavobacterium sp. UBA6203	1946555	224.23	0.72	2.52	2.82	802
Actinobacteria bacterium UBA3066	1948336	214.34	0.69	3.97	4.14	343
Actinobacteria bacterium	1883427	201.20	0.64	2.46	3.32	500
Burkholderiales bacterium UBA4657	1950779	173.93	0.56	1.92	1.92	2277
Chthonomonas sp. UBA2785	1946339	157.46	0.50	2.65	1.85	16
Anabaena sp. UBA12330	2055756	148.91	0.48	1.64	1.64	1756
Actinobacteria bacterium UBA2236	1948325	138.38	0.44	2.86	3.87	181
Rheinheimera sp.	1869214	136.91	0.44	1.45	1.45	2923
Polynucleobacter sp. UBA2464	1947076	128.29	0.41	0.94	0.51	750
Verrucomicrobia bacterium UBA3063	1948252	125.40	0.40	1.20	1.38	1002
unclassified Rhodanobacteraceae	1850978	121.97	0.39	2.43	2.43	28
Sediminibacterium sp. UBA657	1947456	98.19	0.31	1.38	1.38	504
Comamonadaceae bacterium UBA2334	1951601	87.88	0.28	0.94	0.94	2454
uncultured Lachnobacterium sp.	1339173	83.78	0.27	0.20	0.19	991
Synechococcales bacterium UBA8647	2055797	82.80	0.27	0.81	0.44	266
Cytophagaceae bacterium UBA6716	1951634	74.49	0.24	1.89	1.50	44
unclassified Dietzia	2617939	72.42	0.23	1.47	1.47	35
Verrucomicrobia bacterium UBA2644	1948246	68.12	0.22	1.41	1.25	211
Hylemonella sp. UBA6679	1946611	66.82	0.21	0.64	0.64	1677
Synechococcus sp. UBA8071	2055764	66.70	0.21	0.61	0.61	921
Cyanobacteria bacterium UBA6965	1947892	66.26	0.21	0.83	0.43	187
Planctomycetaceae bacterium UBA4655	1952734	62.60	0.20	0.68	0.69	2086
Mycobacterium sp.	1785	56.45	0.18	0.54	0.54	1712
Synechococcales bacterium UBA10510	2055795	53.36	0.17	0.95	0.86	155
Chloroflexi bacterium	2026724	52.89	0.17	0.72	0.72	516
Xanthomonadaceae bacterium	1926873	50.80	0.16	0.62	0.62	929
Proteobacteria bacterium UBA964	1948225	50.29	0.16	0.57	0.57	1250
Curvibacter sp.	1888168	49.84	0.16	0.48	0.50	1665
Verrucomicrobia bacterium UBA2460	1948245	49.23	0.16	1.33	1.14	91
Cytophagaceae bacterium UBA7467	1951636	47.35	0.15	1.14	1.44	128
Comamonadaceae bacterium UBA2237	1951600	40.71	0.13	0.32	0.32	900
Chitinophagaceae bacterium	1869212	39.29	0.13	0.68	0.70	130

Chitinophagaceae bacterium UBA2467	1951550	39.19	0.13	0.53	0.66	168
unclassified Citromicrobium	2630544	38.43	0.12	0.80	0.80	23
Hydrogenophaga sp.	1904254	37.28	0.12	0.61	0.38	135
Comamonas	283	35.61	0.11	0.34	0.34	279
Alphaproteobacteria bacterium UBA2784	1948371	34.88	0.11	0.48	0.48	642
Comamonadaceae bacterium	1871071	34.77	0.11	0.40	0.40	1010
Acidovorax sp.	1872122	33.15	0.11	0.36	0.38	1337
Legionellales bacterium	2026754	31.18	0.10	0.54	0.54	163
Ramlibacter tataouinensis	94132	30.84	0.10	0.21	0.21	843
bacterium UBA951	1947788	31.03	0.10	0.60	0.60	93
Cyanobacteria bacterium UBA7373	1947895	29.63	0.10	0.32	0.32	520
Candidatus Microthrix	41949	29.70	0.10	1.15	1.15	5
Hyphomonadaceae bacterium	2026748	28.42	0.09	0.37	0.37	657
Prochlorococcus sp. UBA3999	1947246	27.14	0.09	0.44	0.34	102
Parachlamydiales bacterium	2052178	27.24	0.09	0.29	0.29	377
Proteobacteria bacterium UBA4656	1948178	26.61	0.08	0.33	0.33	957
Streptomyces sp.	1931	26.09	0.08	0.79	0.79	13
Comamonadaceae bacterium UBA6202	1951611	24.91	0.08	0.29	0.29	798
Synechococcales bacterium UBA12195	2055798	24.39	0.08	0.39	0.35	65
Acidimicrobium sp.	1872112	24.52	0.08	0.42	0.50	132
Tepidimonas sp. UBA997	1947650	23.88	0.08	0.29	0.29	582
Polaromonas sp.	1869339	23.67	0.08	0.19	0.13	141
unclassified Bradyrhizobium	2631580	23.45	0.07	0.30	0.30	133
unclassified Comamonadaceae	83494	23.33	0.07	0.19	0.19	248
Rhodoferax sp. UBA4409	1947378	22.35	0.07	0.23	0.24	768
Pseudomonas stutzeri	316	21.92	0.07	0.29	0.28	90
Rhodobacter sp.	1062	21.47	0.07	0.24	0.24	725
Aeromonas veronii	654	21.70	0.07	0.29	0.31	505
Achromobacter	222	21.41	0.07	0.16	0.16	61
Rhodocyclaceae bacterium UBA2250	1952837	20.28	0.07	0.27	0.27	521
Ralstonia	48736	19.90	0.06	0.30	0.30	15
Armatimonadetes bacterium	2033014	20.02	0.06	0.65	1.78	18
Comamonadaceae bacterium UBA4962	1951608	19.78	0.06	0.23	0.24	846
Alicyciphilus sp. UBA7619	1946005	19.05	0.06	0.24	0.26	755
Legionellales bacterium UBA4759	1951149	19.10	0.06	0.37	0.37	110
Comamonas aquatica	225991	18.10	0.06	0.23	0.25	520
unclassified Legionellales (miscellaneous)	2570685	17.97	0.06	0.10	0.10	43
Aquabacterium sp. UBA2148	1946042	16.88	0.05	0.23	0.31	312
Acinetobacter sp. UBA4611	1945942	16.82	0.05	0.39	0.61	55
Acidovorax delafieldii	47920	16.44	0.05	0.21	0.23	609
Acidimicrobiaceae bacterium	2024894	15.93	0.05	0.20	0.20	685
Rhodobacteraceae bacterium UBA1943	1952798	15.17	0.05	0.17	0.17	570
Candidatus Accumulibacter	327159	15.22	0.05	0.11	0.08	92
Mycobacterium tuberculosis	1773	15.24	0.05	0.15	0.15	876
Micrococcus luteus	1270	15.34	0.05	0.13	0.13	41
Verrucomicrobiaceae bacterium UBA2429	1952942	15.41	0.05	0.28	0.22	97
Plesiomonas shigelloides	703	14.96	0.05	0.25	0.33	212
Burkholderiales bacterium UBA1834	1950768	14.68	0.05	0.25	0.21	163
unclassified Gordonia	2657482	14.45	0.05	0.14	0.14	207
Porphyrobacter sp. UBA7686	1947080	14.09	0.04	0.22	0.22	275
Dechloromonas sp. UBA6271	1946404	14.18	0.04	0.19	0.20	478
Thiobacillus denitrificans	36861	13.88	0.04	0.32	0.17	47
Burkholderiales bacterium UBA4200	1950777	13.41	0.04	0.29	0.21	65
bacterium UBA7470	1947819	13.29	0.04	0.19	0.19	336
Castellaniella sp. UBA6218	1946233	11.77	0.04	0.17	0.17	342

Verrucomicrobia bacterium UBA3062	1948251	11.77	0.04	0.17	0.17	204
unclassified Sphingopyxis	2614943	11.43	0.04	0.50	0.50	5
Gemmobacter sp.	1898957	11.45	0.04	0.21	0.13	76
Betaproteobacteria bacterium UBA5582	1948460	11.60	0.04	0.14	0.16	447
Verrucomicrobiales bacterium	2026801	11.63	0.04	0.16	0.16	498
Nitrosomonas sp. UBA6494	1946979	11.32	0.04	0.19	0.19	190
Blastomonas sp. UBA7677	1946117	11.02	0.03	0.19	0.19	198
Massilia sp.	1882437	11.09	0.03	0.17	0.17	34
Syntrophaceae bacterium UBA5744	1961444	10.70	0.03	0.15	0.17	366
Acinetobacter	469	10.24	0.03	0.44	0.95	2
Caulobacteraceae bacterium UBA3198	1951537	9.85	0.03	0.37	0.21	55
Comamonadaceae bacterium UBA3515	1951605	9.97	0.03	0.10	0.13	425
Gammaproteobacteria bacterium	1913989	9.99	0.03	0.21	0.11	43
Runella sp.	1960881	9.86	0.03	0.16	0.21	207
Hyphomonadaceae bacterium UBA7672	1951957	9.57	0.03	0.14	0.14	382
Candidatus Accumulibacter phosphatis	327160	9.63	0.03	0.26	0.15	31
unclassified Brevundimonas	2622653	9.34	0.03	0.12	0.12	25
Rhodobacteraceae bacterium UBA6273	1952813	9.28	0.03	0.13	0.13	331
Rhodobacteraceae bacterium UBA6197	1952810	9.24	0.03	0.12	0.12	362
Acidimicrobiaceae bacterium UBA668	1951340	9.49	0.03	0.16	0.17	156
Gemmimonas	173479	9.33	0.03	10.26	10.26	7
Comamonas sp. UBA7840	1946392	9.02	0.03	0.13	0.14	253
Competibacteraceae bacterium UBA3908	1951621	8.97	0.03	0.13	0.13	327
unclassified Thauera	2609274	8.66	0.03	0.12	0.08	29
Micrococcales bacterium UBA7469	1951173	8.67	0.03	0.16	0.16	97
Rhodobacteraceae bacterium UBA6796	1952814	8.01	0.03	0.11	0.11	283
Delftia acidovorans	80866	8.10	0.03	0.12	0.21	186
Delftia lacustris	558537	8.26	0.03	0.12	0.21	277
Thauera sp. UBA6194	1947679	8.24	0.03	0.12	0.13	2977
unclassified Actinomycetales (miscellaneous)	105426	8.02	0.03	0.39	0.39	3
Thiomonas sp. UBA7699	1947694	7.94	0.03	0.11	0.11	273
Thiobacillus sp. UBA2597	1947687	7.78	0.03	0.12	0.12	183
Arenimonas sp.	1872635	7.78	0.03	0.12	0.12	233
unclassified Exiguobacterium	2644629	7.82	0.03	0.15	0.17	38
uncultured Clostridium sp.	59620	7.96	0.03	0.12	0.12	42
unclassified Cytophagaceae	94253	7.82	0.03	0.11	0.13	88
Actinobacteria bacterium UBA3007	1948330	7.46	0.02	0.21	0.21	29
Rhodocyclaceae bacterium UBA4043	1952842	7.28	0.02	0.11	0.12	229
Betaproteobacteria bacterium UBA7395	1948463	7.05	0.02	0.11	0.14	187
Chitinophagaceae bacterium UBA3961	1951555	7.14	0.02	0.14	0.14	121
Actinobacteria bacterium UBA5975	1948344	7.02	0.02	0.11	0.11	103
Planctomyces bacterium UBA2386	1948127	6.90	0.02	0.11	0.11	290
Opitutae bacterium UBA953	1948744	6.10	0.02	0.10	0.10	171
unclassified Proteobacteria (miscellaneous)	81684	6.06	0.02	0.11	0.09	2
Verrucomicrobiaceae bacterium UBA2021	1952940	5.89	0.02	0.19	0.24	17
unclassified Aminicenantes	910038	5.79	0.02	0.19	0.19	11
uncultured Prevotella sp.	159272	5.58	0.02	0.16	0.11	15
Acidobacteria bacterium	1978231	5.67	0.02	0.15	0.15	32
Proteobacteria bacterium UBA2646	1948154	5.27	0.02	0.16	0.14	19
Synechococcales bacterium UBA8138	2055796	5.35	0.02	0.10	0.10	406
unclassified Acidimicrobiaceae	667305	5.18	0.02	0.19	0.19	9
unclassified Flavobacteriales (miscellaneous)	403978	5.19	0.02	3.23	3.23	9
Crocinitomicaceae bacterium	2026728	4.86	0.02	0.10	0.10	64
unclassified Pseudomonas	2583993	4.57	0.01	0.28	0.28	3
Pseudomonas kunmingensis	1211807	4.26	0.01	0.16	0.14	12

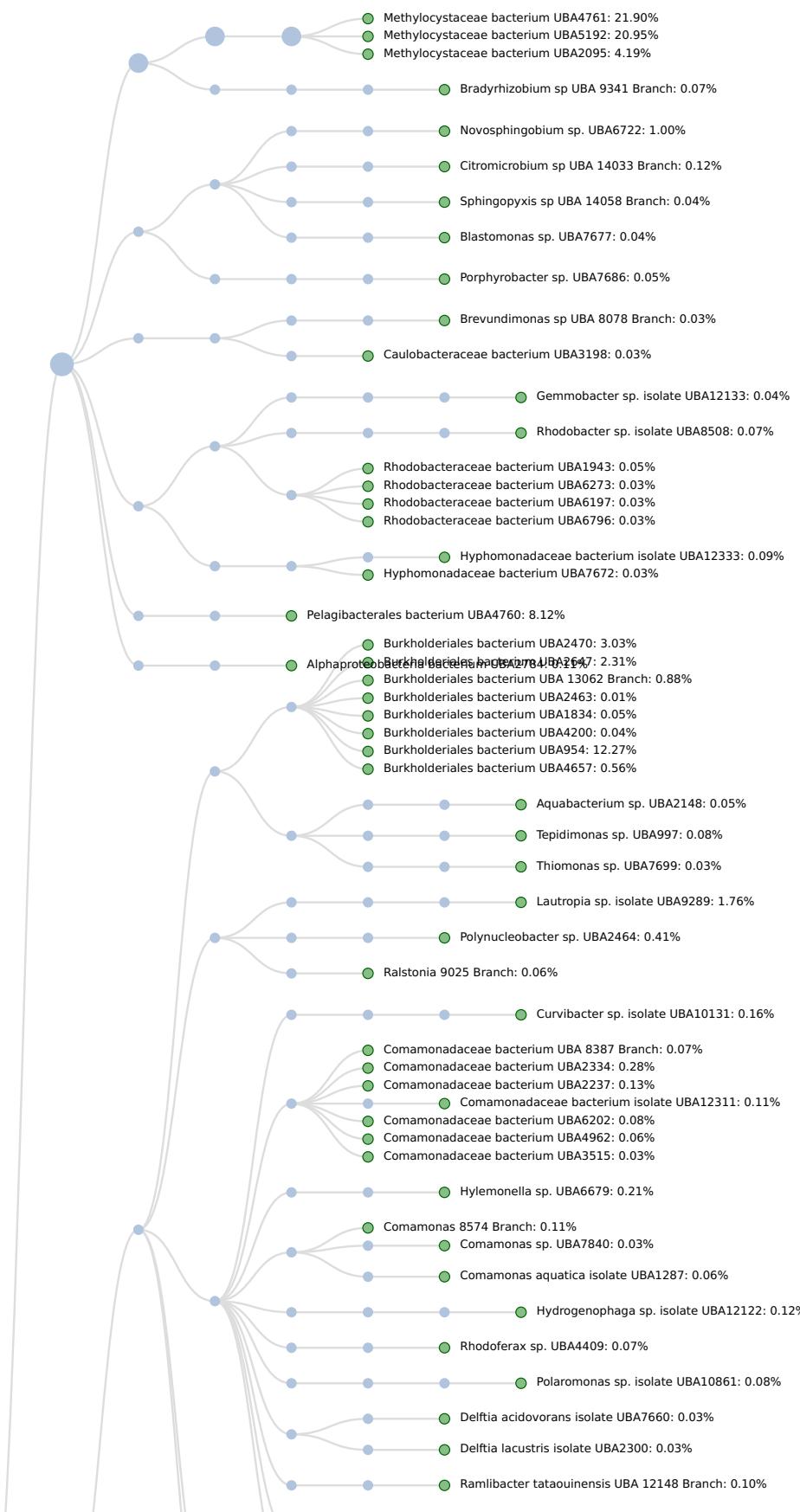
Burkholderiales bacterium UBA2463	1950771	4.11	0.01	0.37	0.27	15
unclassified Cellvibrio	2624793	3.62	0.01	4.96	4.96	11
unclassified Patescibacteria group	1948766	3.87	0.01	0.25	0.25	5
Francisellaceae bacterium UBA6186	1951894	2.37	<0.01	0.10	0.10	18
Shigella sp.	625	1.90	<0.01	0.34	0.34	7
Flavobacteriales bacterium UBA6717	1951053	1.46	<0.01	0.17	0.12	6

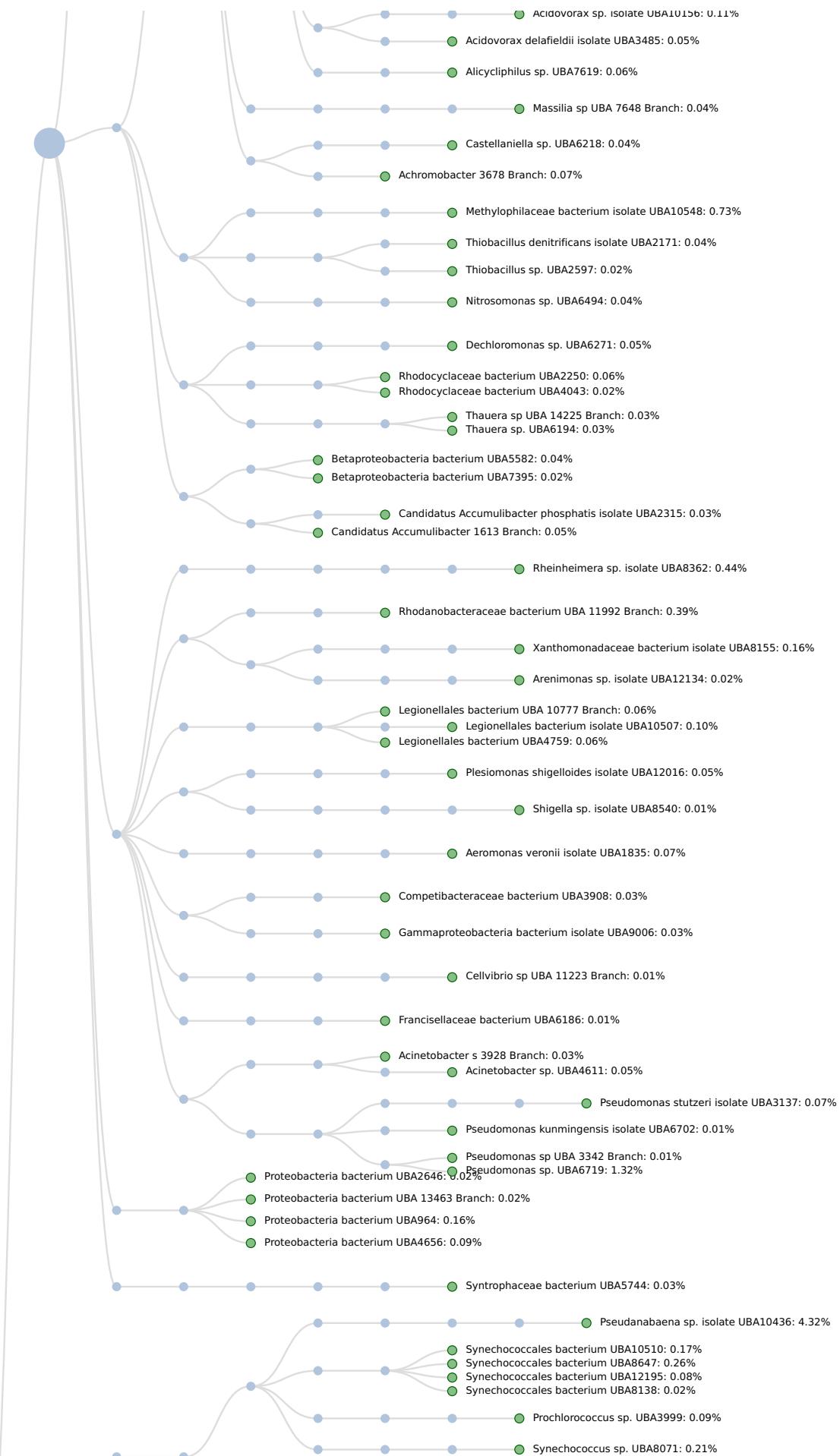
Dark Matter (Beta)

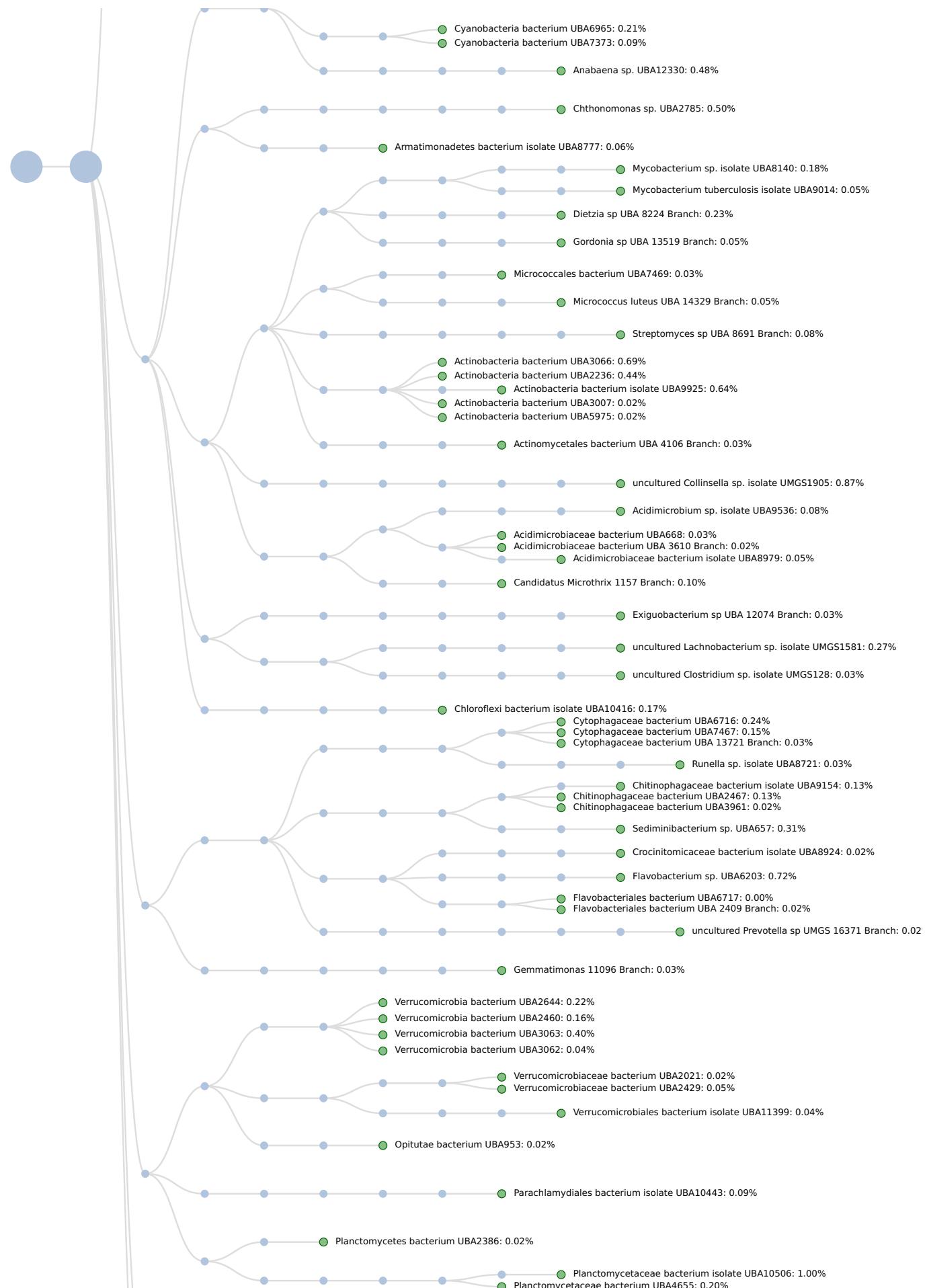
Tree Chart Taxonomy

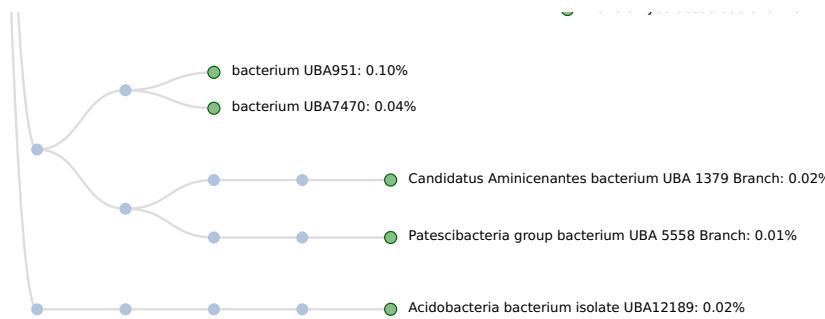
Sample_1_BWT2214

Total results



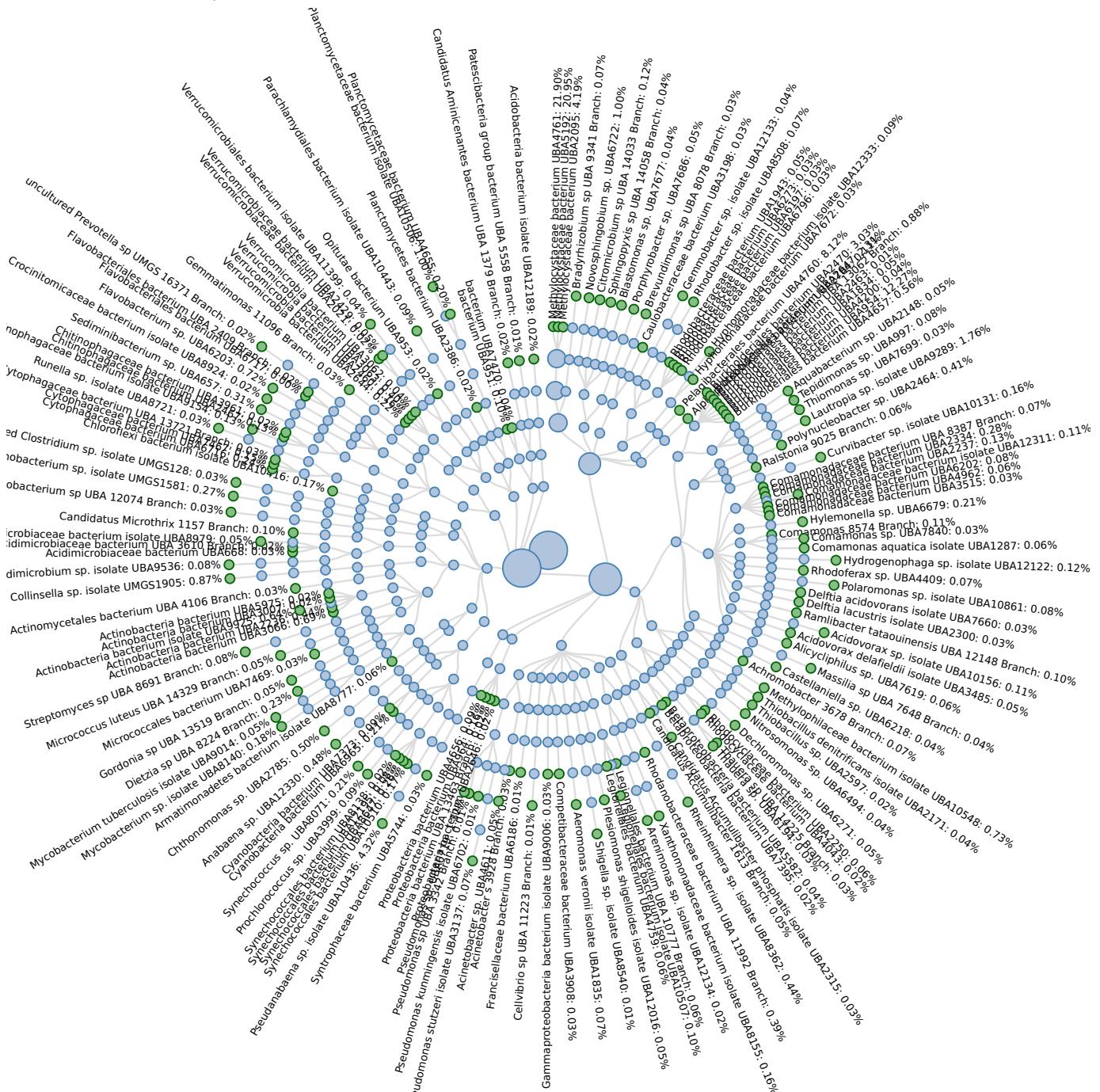






Dark Matter (Beta)

Radial Tree Chart Taxonomy



Sample 1 BWT2214

Total results

Dark Matter (Beta)

Sunburst Chart Taxonomy

Sample 1 BWT2214

Total results

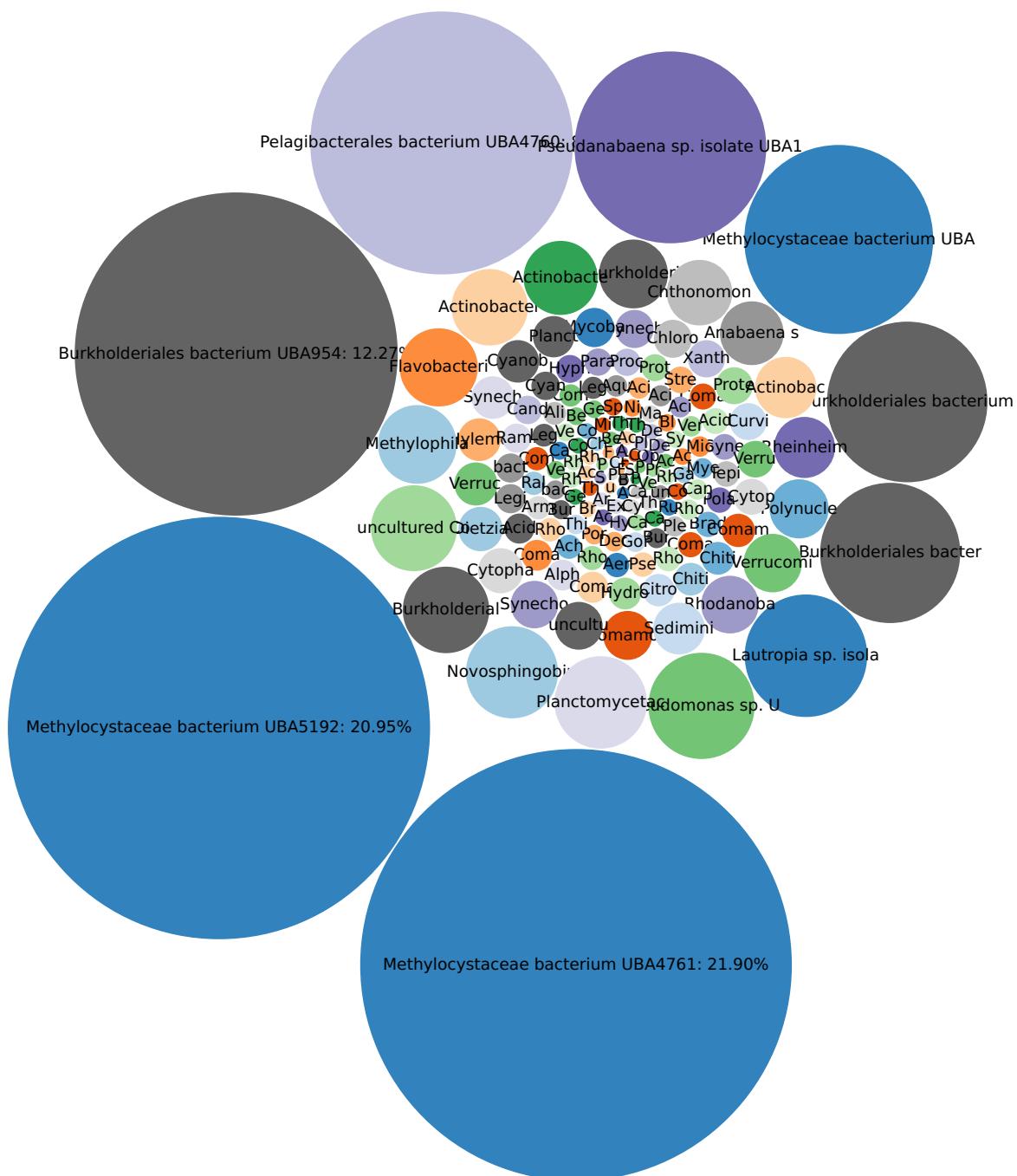


Dark Matter (Beta)

Bubble Chart Taxonomy

Sample_1_BWT2214

Total results



Phages

Fasta/q details

Sample_1_BWT2214

Total results

Metric	Value
Hit	84.508k

Table

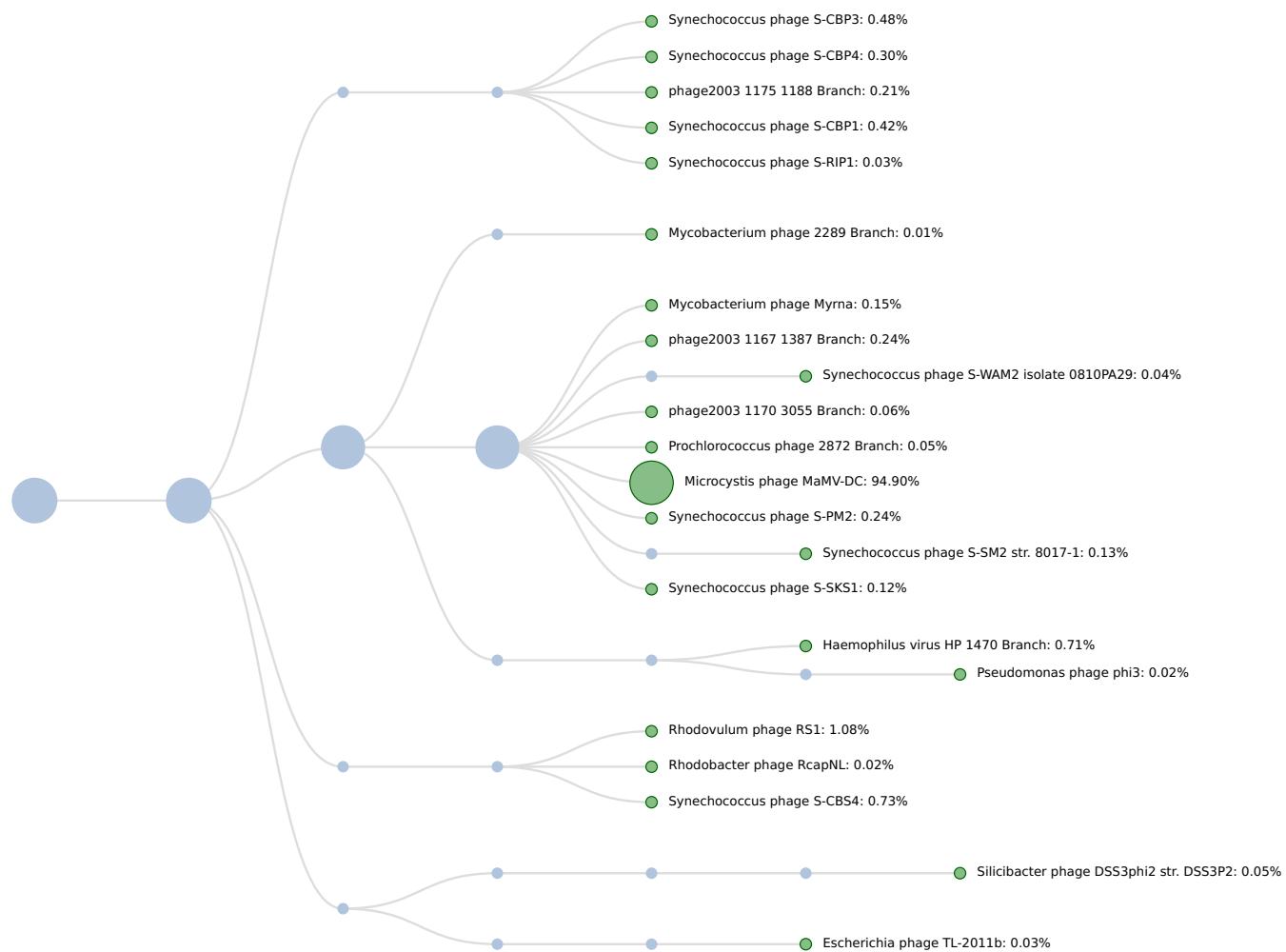
Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Microcystis phage MaMV-DC	1357715	13989.42	94.90	36.25	36.25	302583
Rhodovulum phage RS1	754056	158.77	1.08	1.92	1.92	58
Synechococcus phage S-CBS4	756275	107.06	0.73	0.69	0.69	1670
Hpunavirus	1196844	104.94	0.71	0.24	0.24	655
Synechococcus phage S-CBP3	756276	70.25	0.48	0.85	1.31	536
Synechococcus phage S-CBP1	1273711	61.67	0.42	0.60	0.95	619
Synechococcus phage S-CBP4	754059	44.19	0.30	0.71	1.08	254
unclassified Myoviridae	196896	35.67	0.24	0.40	0.40	161
Synechococcus phage S-PM2	238854	35.62	0.24	0.37	0.39	922
unclassified Autographiviridae	2731990	30.69	0.21	0.23	0.23	433
Mycobacterium phage Myrna	546805	21.98	0.15	0.11	0.12	809
Synechococcus phage S-SM2	444860	18.80	0.13	0.26	0.30	428
Synechococcus phage S-SKS1	754042	17.16	0.12	0.25	0.29	354
unclassified Myoviridae	196896	9.29	0.06	0.11	0.11	20
Silicibacter phage DSS3phi2	490912	7.76	0.05	0.23	0.22	68
unclassified Myoviridae	196896	7.53	0.05	0.15	0.15	22
Synechococcus phage S-WAM2	1815522	6.33	0.04	0.10	0.20	176
Escherichia phage TL-2011b	1124654	4.50	0.03	0.17	0.14	25
Synechococcus phage S-RIP1	754041	3.92	0.03	0.11	0.12	32
Pseudomonas phage phi3	1754217	2.72	0.02	0.13	0.13	51
Rhodobacter phage RcapNL	1131316	2.27	0.01	0.11	0.11	214
Bixzunavirus	680114	1.38	<0.01	0.12	0.17	304

Phages

Tree Chart Taxonomy

Sample_1_BWT2214

Total results

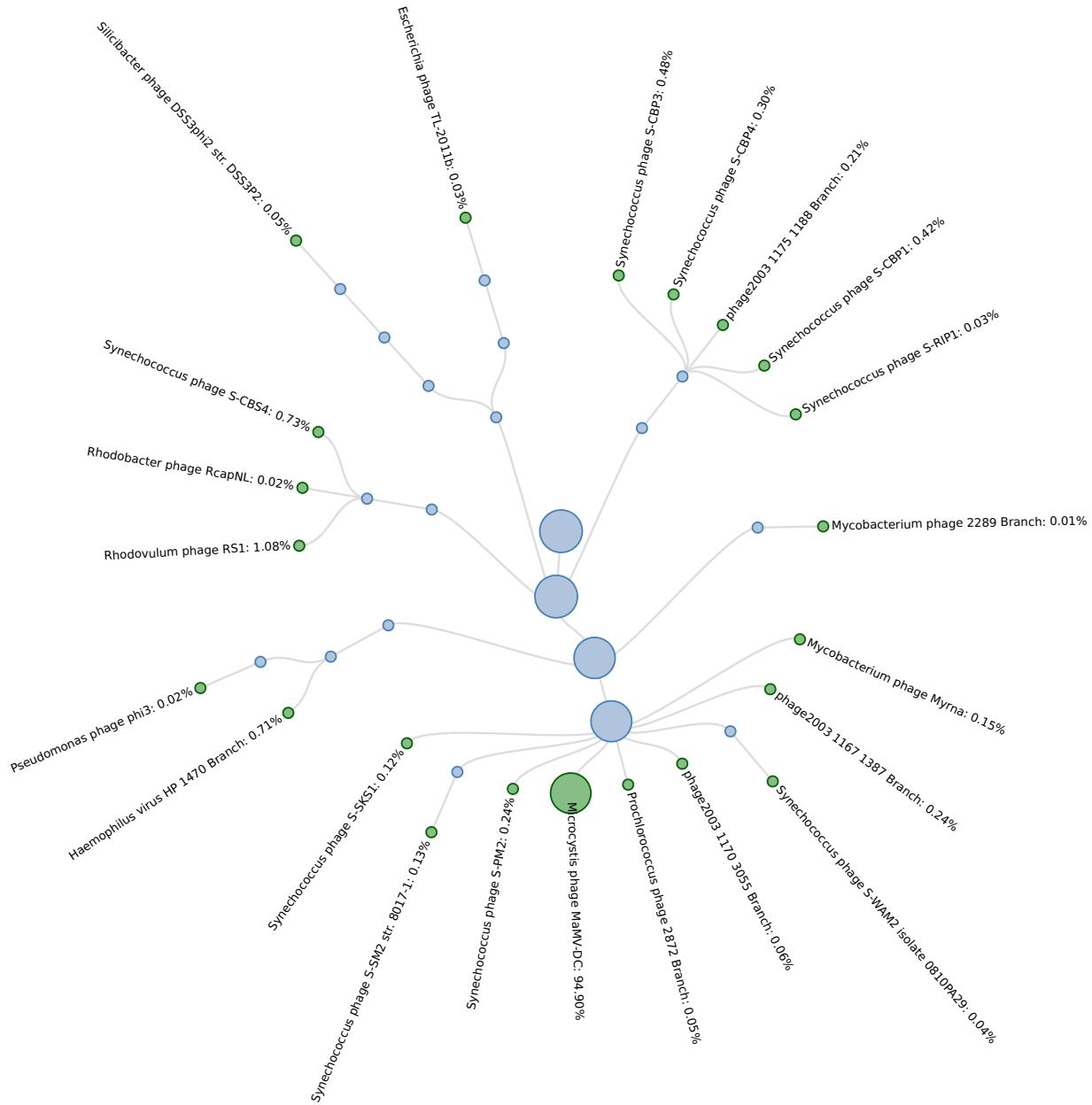


Phages

Radial Tree Chart Taxonomy

Sample_1_BWT2214

Total results

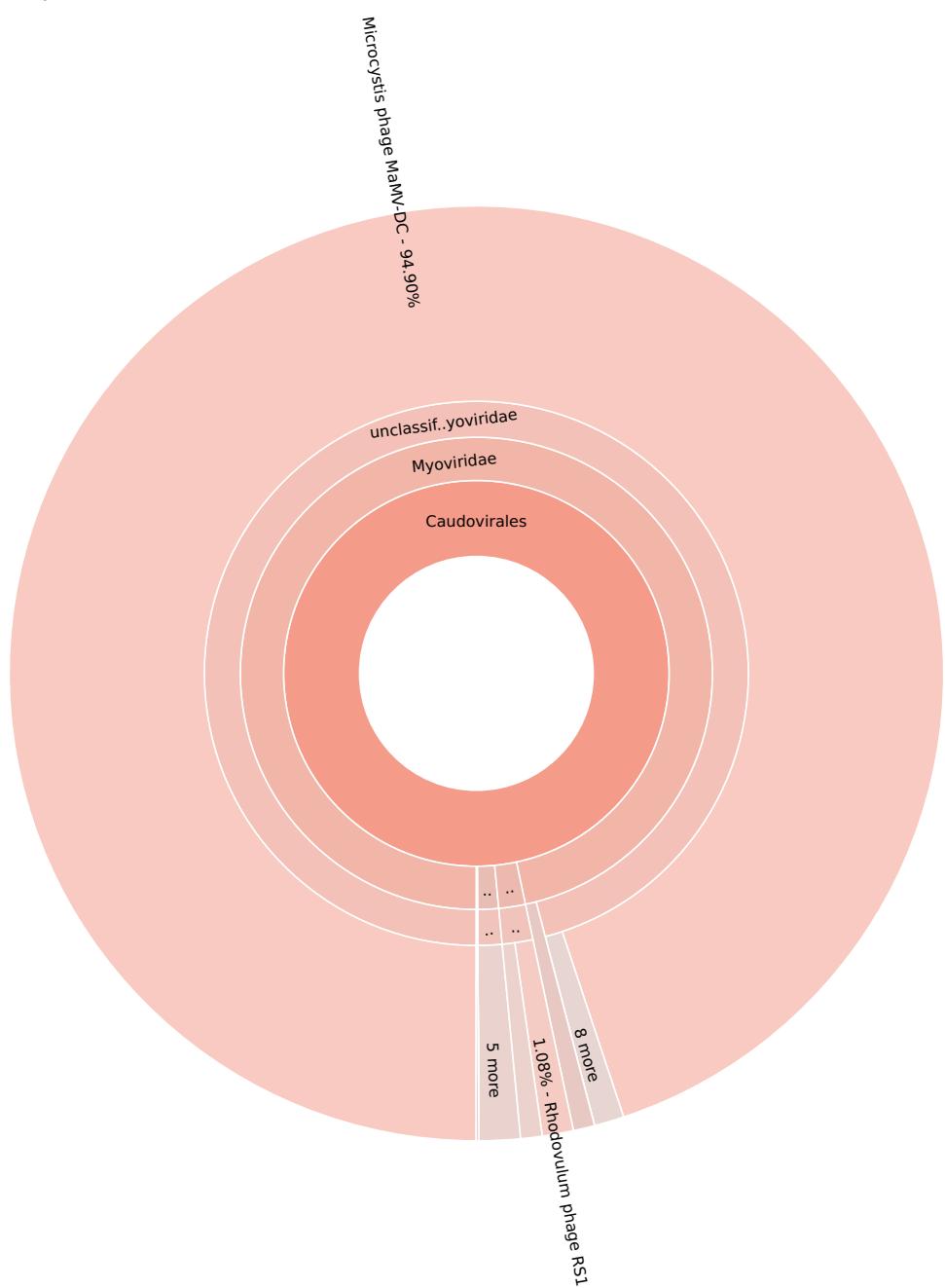


Phages

Sunburst Chart Taxonomy

Sample_1_BWT2214

Total results

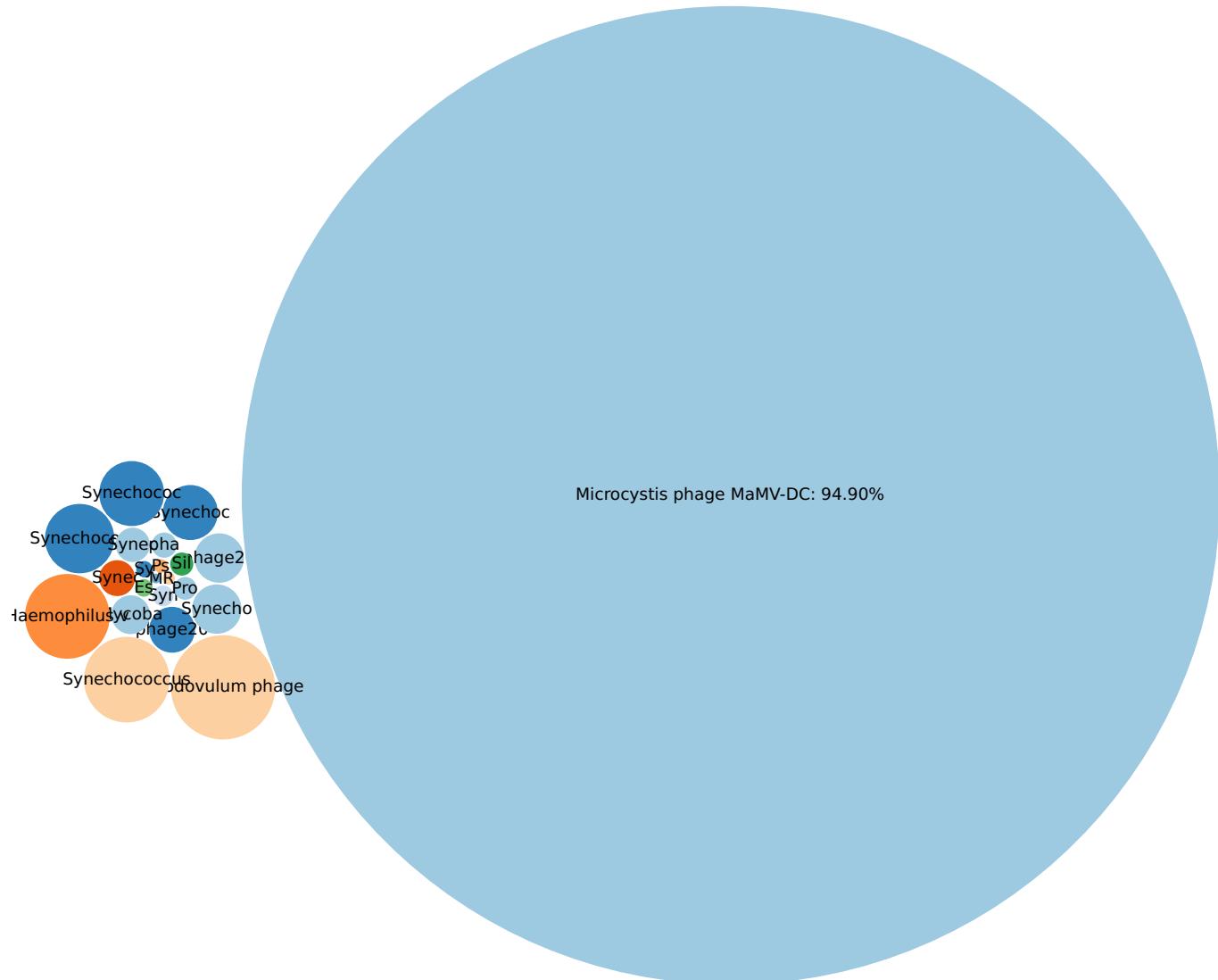


Phages

Bubble Chart Taxonomy

Sample_1_BWT2214

Total results



Viruses

Fasta/q details

Sample_1_BWT2214

Total results

Metric	Value
Hit	0.172M

Table

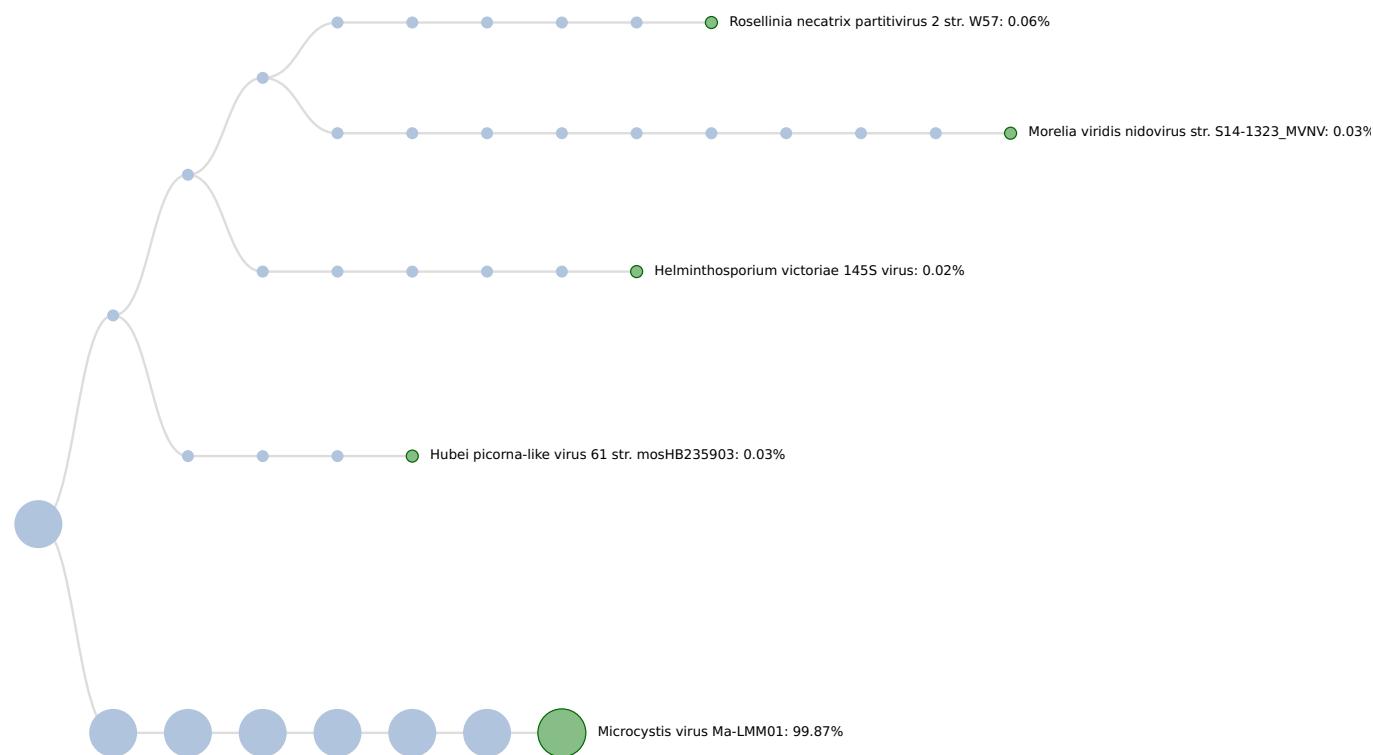
Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Microcystis virus Ma-LMM01	340435	15685.16	99.87	40.51	40.51	336749
Rosellinia necatrix partitivirus 2	859651	9.19	0.06	0.52	0.52	104
Hubei picorna-like virus 61	1923144	4.24	0.03	0.24	0.24	97
Morelia viridis nidovirus	2016400	4.07	0.03	0.16	0.16	266
Helminthosporium victoriae 145S virus	164750	2.71	0.02	0.15	0.15	171

Viruses

Tree Chart Taxonomy

Sample_1_BWT2214

Total results

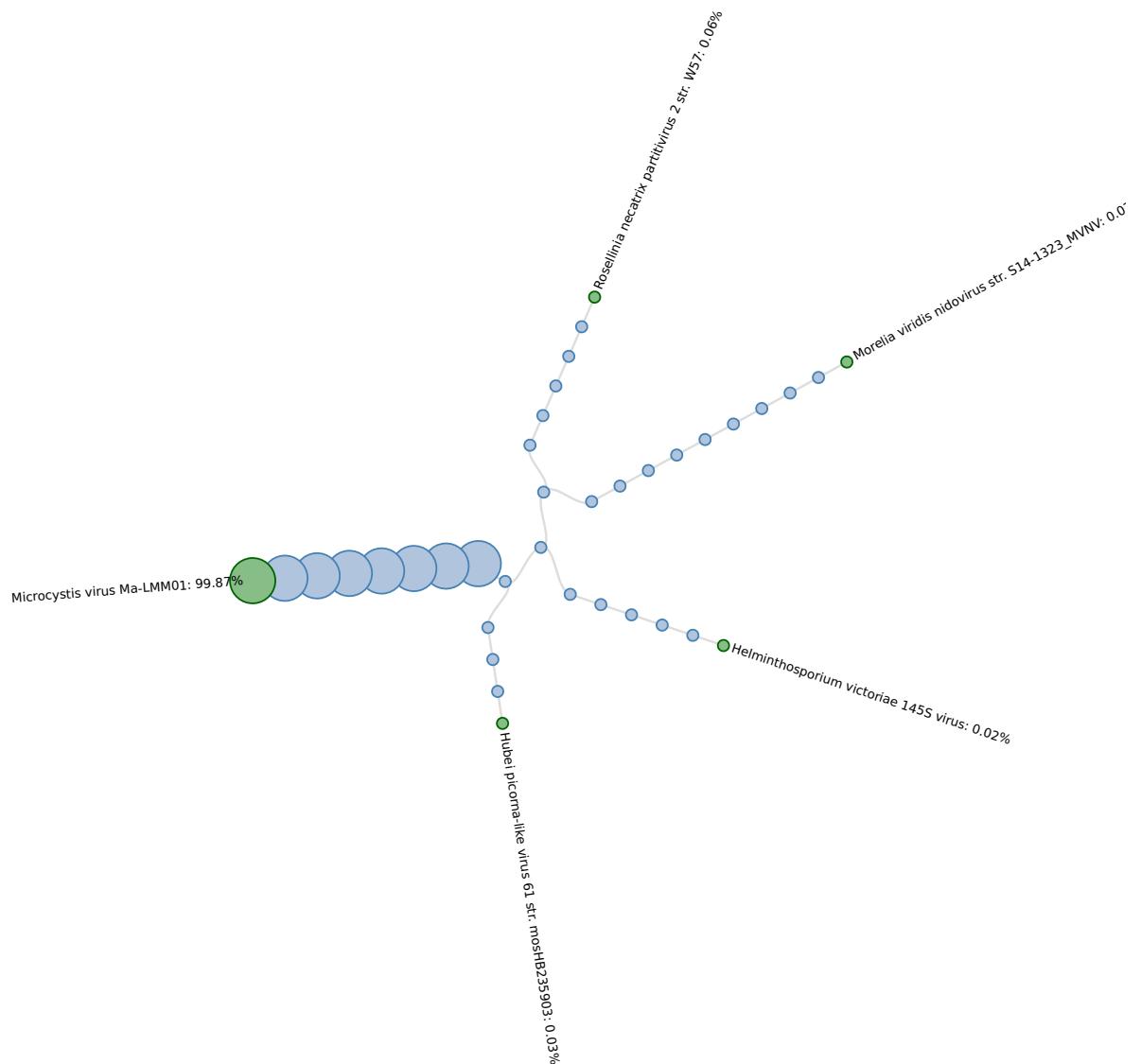


Viruses

Radial Tree Chart Taxonomy

Sample_1_BWT2214

Total results

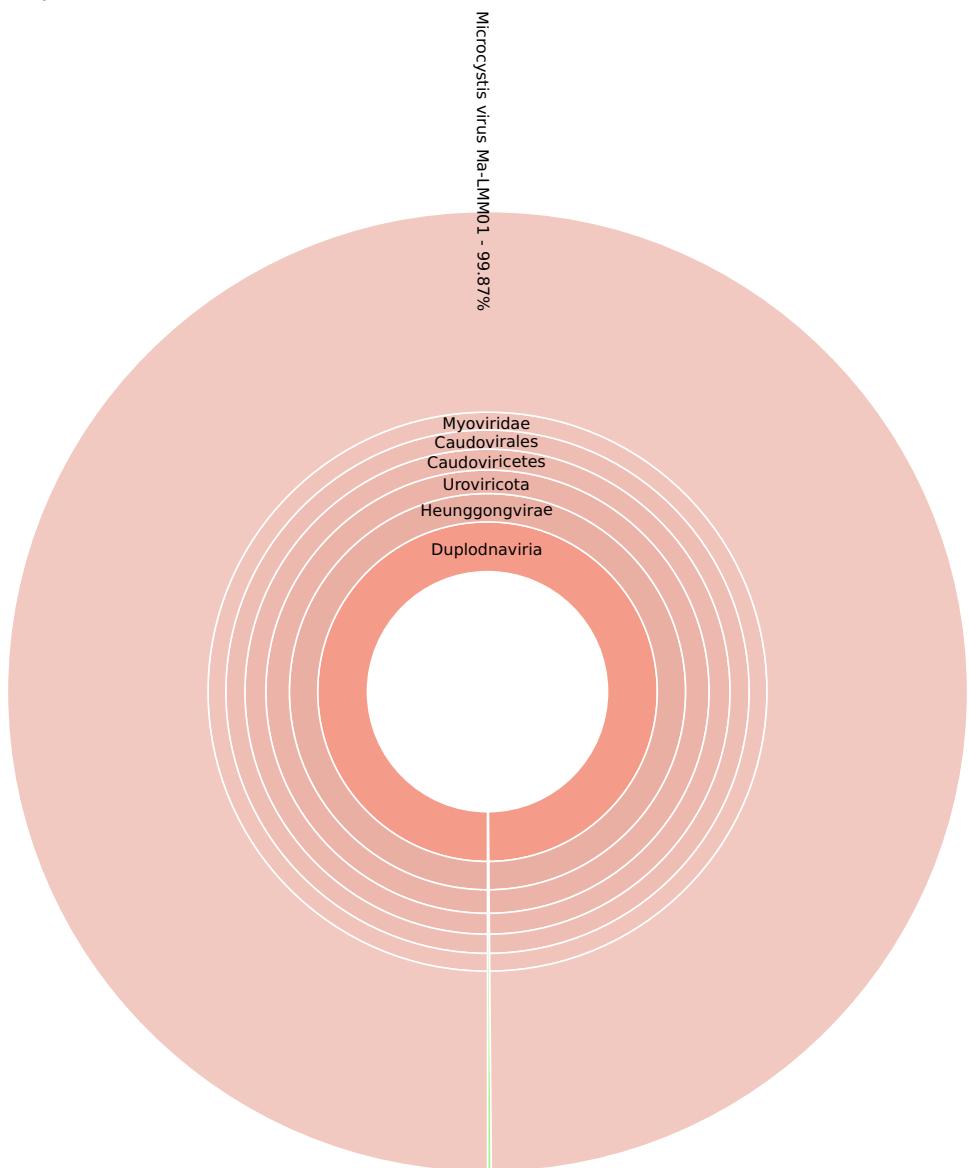


Viruses

Sunburst Chart Taxonomy

Sample_1_BWT2214

Total results

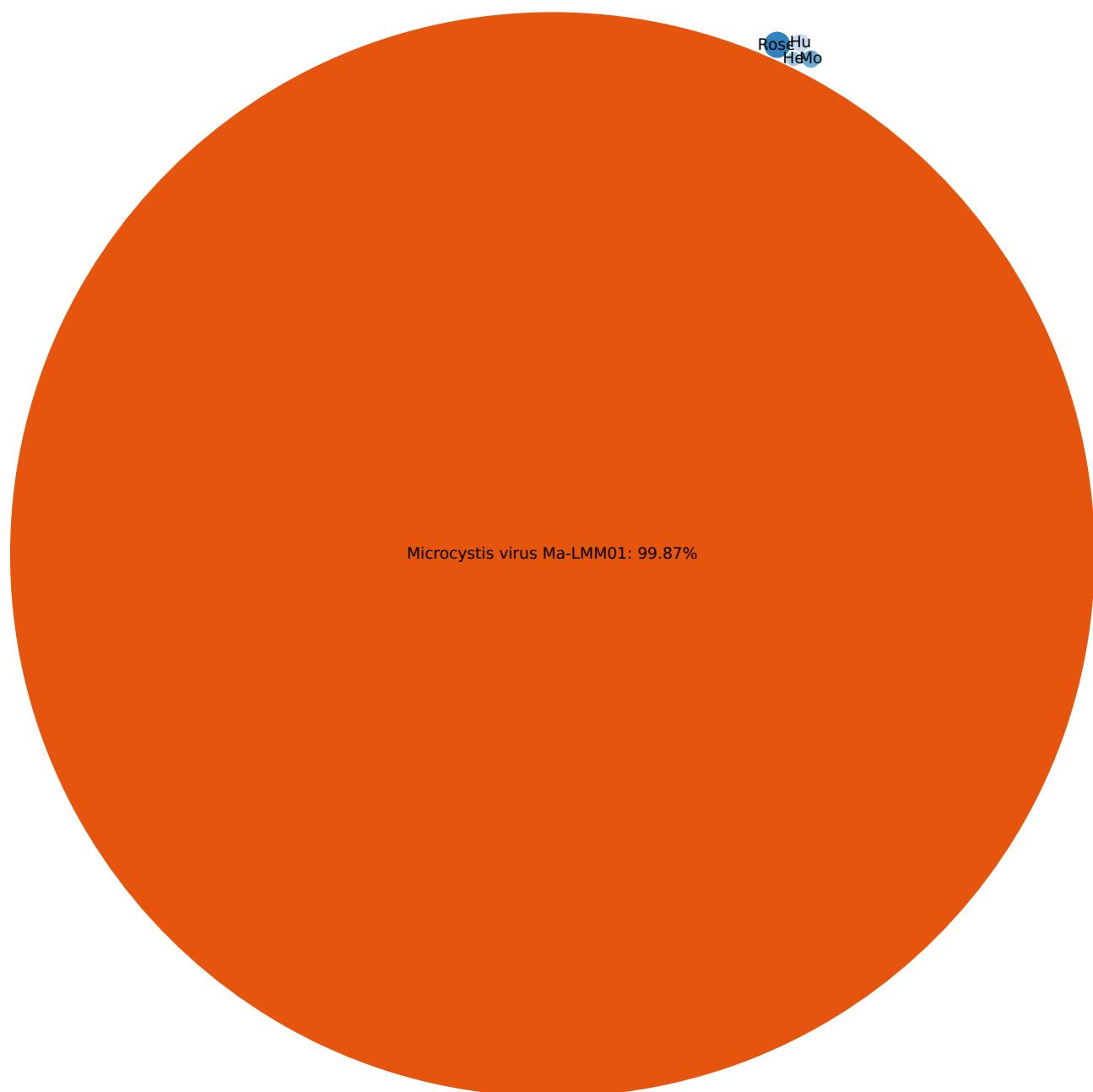


Viruses

Bubble Chart Taxonomy

Sample_1_BWT2214

Total results



Respiratory Virus

Fasta/q details

Sample_1_BWT2214

Total results

Metric	Value
Hit	7.319k

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Human mastadenovirus C	129951	25.50	100.00	0.14	0.08	117

Virulence Factors

Fasta/q details

Sample_1_BWT2214

Total results

Metric	Value
Hit	2.844k

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Pseudomonas aeruginosa GENE intI1	-	7301.06	32.65	98.00	98.00	201
Vibrio cholerae GENE intI1	-	6824.83	30.52	100.00	100.00	76
Enterobacter aerogenes GENE tniC	-	5644.85	25.25	77.78	77.78	372
Mycobacterium tuberculosis GENE icl-aceA	-	1119.85	5.01	12.26	12.26	233
Enterobacter aerogenes GI 1572570	-	892.94	3.99	12.74	12.74	177
Pseudomonas aeruginosa GENE xcpT	-	576.65	2.58	14.29	14.29	37

Disclaimer

CosmosID is designed for Research Use Only in accordance with applicable rules and regulations of the United States Food and Drug Administration and other applicable laws, and your sample shall not be used for patient care or diagnostic, clinical or therapeutic use. You agree to use CosmosID for Research Use Only. No analyses, reports, or other information obtained or provided through CosmosID are intended to be (nor should be relied upon as) medical advice or instructions for medical diagnosis or treatment.

CosmosID does not accept any responsibility for the accuracy of the input entered by the user or the consequences of any inaccuracies in this input. The analyses are not intended to replace professional medical care and attention by a qualified medical practitioner and consequently CosmosID does not accept any responsibility for the selection of drugs and the patient's response to treatment.

CosmosID is designed for whole genome shotgun (WGS) analysis on reads of at least 75 bases in length and should not be used for 16S analysis.

Appendix D2



CosmosID - Unlocking the Microbiome

Filename:	Sample_2_BWT2214
Size:	10.35 GiB
Reads:	51.468M
Date of Report:	2021-08-15 00:46:36

Summary

Database Name	Hits	Status
Bacteria	172	Success
Antibiotic Resistance	0*	Success
Fungi	18	Success
Protists	15	Success
Dark Matter (Beta)	153	Success
Phages	21	Success
Viruses	2	Success
Respiratory Virus	0*	Success
Virulence Factors	8	Success

* Analysis with 0 hits will not be included in this report

Bacteria

Fasta/q details

Sample_2_BWT2214

Total results

Metric	Value
Hit	7.771M

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Microcystis panniformis FACHB-1757	1638788	547502.38	57.96	75.70	86.05	1727691
Microcystis aeruginosa PCC 9807	1160283	177248.94	18.76	43.04	67.64	306982
Flavobacterium fontis	1124188	92874.64	9.83	41.84	42.00	2046337
Microcystis sp. 0824	1502726	47987.34	5.08	23.13	37.64	96107
Microcystis viridis NIES-102	213615	42672.82	4.52	24.16	42.54	36600
Dolichospermum circinale AWQC131C	398007	5648.07	0.60	33.07	43.24	33261
Aquidulcibacter paucihalophilus	1978549	4948.80	0.52	14.12	14.12	139696
Clavibacter sp.	1871044	4433.20	0.47	33.70	33.70	11827
Silanimonas lenta DSM 16282	1123253	2705.85	0.29	2.63	2.63	76650
Flavobacterium sp. WWJ-16	2506421	1766.53	0.19	2.18	2.97	35424
Novosphingobium ginsenosidimutans	1176536	1483.49	0.16	9.10	9.10	29266
Candidatus Fonsibacter ubiqius	1925548	1314.99	0.14	14.70	14.72	2079
Flectobacillus sp. BAB-3569	1509483	891.95	0.09	7.59	7.61	16423
Synechococcus sp. CB0101	232348	825.08	0.09	6.68	6.78	10829
Inhella crocodyli	2499851	795.59	0.08	6.80	6.80	17603
Pseudanabaena sp. ABRG5-3	685565	786.46	0.08	1.80	1.84	21934
Erythrobacter neustonensis	1112	784.84	0.08	7.21	7.21	11188
Leptospira ryugenii	1917863	769.92	0.08	7.55	7.57	6649
Phenylbacterium parvum	2201350	690.49	0.07	4.23	4.23	16450
Pararheinheimera texensis DSM 17496	1123055	633.13	0.07	5.51	5.51	12035
Tabrizicola sp. TH137	2067452	484.12	0.05	3.42	3.78	11726
Limnothrix sp. P13C2	1880902	424.27	0.04	1.84	4.02	44
Rhodobacter sp. CACIA14H1	1408890	403.60	0.04	2.68	3.05	10347
Raphidiopsis brookii D9	533247	348.84	0.04	3.02	3.86	1597
Aquimonas voraii	265719	325.58	0.03	2.69	2.69	9819
Porphyrobacter sp. LM 6	1896196	251.89	0.03	2.59	2.59	3807
Aphanizomenon flos-aquae 2012/KM1/D3	1532906	234.00	0.03	1.94	2.69	1162
Dolichospermum sp. UHCC 0315A	1914871	222.60	0.02	1.20	2.05	1067
Anabaena sp. 90	46234	207.83	0.02	1.35	2.10	850
Sphingorhabdus contaminans	1343899	173.10	0.02	1.86	1.87	3017
Aquabacterium sp. KMB7	2528630	173.09	0.02	1.82	1.82	3734
Vulcanococcus limneticus LL	2025607	148.05	0.02	1.41	1.43	2997
Pseudomonas sp. HAR-UPW-AIA-41	1985301	153.60	0.02	1.68	1.68	2641
Pseudanabaena biceps PCC 7429	927668	138.54	0.01	0.47	0.48	4499
Elstera cyanobacteriorum	2022747	142.09	0.01	1.55	1.55	3041
Sphaerotilus natans subsp. natans DSM 6575	1286631	145.96	0.01	1.49	1.49	3857
Arenimonas metalli CF5-1	1384056	109.08	0.01	0.20	0.25	5621
beta proteobacterium SCGC AAA028-K02	938797	113.23	0.01	1.52	1.52	364
Flavobacterium sasangense DSM 21067	1121896	112.08	0.01	1.47	1.59	9191
Cylindrospermopsis sp. CR12	1747196	108.12	0.01	0.68	2.15	831
Flavobacterium filum DSM 17961	1121889	99.81	0.01	0.13	0.13	2766
Candidatus Planktophila sp.	2175601	95.39	0.01	1.13	1.57	127
Ideonella sp. KYPY4	1862385	90.12	0.01	0.74	0.74	3898
Polynucleobacter cosmopolitanus	351345	96.55	0.01	1.30	1.33	627
Cyanobium sp. NIES-981	1851505	86.89	<0.01	0.94	1.20	1533
Arenimonas malthae CC-JY-1	1384054	85.95	<0.01	0.21	0.26	4489
Arenimonas compostii TR7-09 = DSM 18010	1121013	83.02	<0.01	0.16	0.16	5300

Hydrogenophaga sp. IBVHS2	1985170	85.20	<0.01	0.96	0.96	1687
Cyanobium gracile PCC 6307	292564	74.49	<0.01	0.87	2.50	1191
Piscinibacter defluvii	1796922	73.89	<0.01	0.36	0.52	4665
Polynucleobacter victoriensis	2049319	77.15	<0.01	1.05	1.09	516
Aphanothecae cf. minutissima CCALA 015	2107695	70.23	<0.01	0.90	2.60	964
Cyanobium usitatum str. Tous	2116684	70.33	<0.01	0.79	0.86	1047
actinobacterium SCGC AAA027-J17	932040	64.34	<0.01	1.05	1.05	184
Arenimonas caeni	2058085	62.59	<0.01	0.21	0.26	3007
Rubrivivax albus	2499835	65.74	<0.01	0.36	0.41	4005
Cylindrospermopsis raciborskii CS-505	533240	60.33	<0.01	0.89	2.27	278
Arenimonas terrae	2546226	55.07	<0.01	0.13	0.16	3333
Niveispirillum cyanobacteriorum	1612173	58.11	<0.01	0.64	0.80	1799
Cetobacterium somerae ATCC BAA-474	1319815	58.56	<0.01	0.88	0.89	247
Mycobacterium sp. UM_11	1638773	49.67	<0.01	1.85	1.85	11
Rhizobium sp. MSSRF QS100	1522278	47.36	<0.01	0.64	1.16	651
Pseudomonas alcaligenes NBRC 14159	1215092	43.76	<0.01	0.54	1.08	761
Niveispirillum lacus	1981099	44.14	<0.01	0.49	0.66	1472
Ideonella sakaiensis	1547922	49.67	<0.01	0.28	0.40	3164
Zhzhongheella caldifontis	1452508	49.43	<0.01	0.41	0.58	2027
Lysobacter tabacisoli	2315424	35.45	<0.01	0.11	0.11	2639
Caedibacter taeniospiralis	28907	38.18	<0.01	0.65	0.65	215
Sandarakinorhabdus cyanobacteriorum	1981098	39.25	<0.01	0.44	0.48	730
Erythrobacter colymbi	1161202	35.99	<0.01	0.42	0.72	1038
Caulobacter sp. CCH4-E1	1768763	35.34	<0.01	0.80	0.69	113
Brevundimonas sp. AAP58	1523422	36.92	<0.01	0.21	0.23	1907
Aestuariivirga litoralis	2650924	39.96	<0.01	0.48	0.48	1228
Aquabacterium pictum	2315236	35.99	<0.01	0.30	0.30	1934
Prochlorothrix hollandica PCC 9006 = CALU 1027	317619	28.44	<0.01	0.23	0.23	1137
actinobacterium SCGC AAA028-N15	938467	28.56	<0.01	0.45	0.45	128
Candidatus Nanopelagicus limnes	1884634	29.90	<0.01	0.55	0.87	120
Vulcaniibacterium thermophilum	1169913	30.88	<0.01	0.11	0.12	2038
Vulcaniibacterium gelatinicum	2598725	27.47	<0.01	0.12	0.13	1332
Pseudoxanthomonas	83618	27.27	<0.01	0.22	0.31	12
Methylocladum sp. 14B	1912213	27.03	<0.01	0.22	0.20	421
Novosphingobium sediminis	707214	30.53	<0.01	0.31	0.31	1281
Novosphingobium kunmingense	1211806	26.37	<0.01	0.17	0.17	1451
Erythrobacter donghaensis	267135	26.55	<0.01	0.30	0.40	731
Erythrobacter sp. CCH5-A1	1768792	26.05	<0.01	0.49	0.58	85
Gemmobacter sp. HYN0069	2169400	24.52	<0.01	0.25	0.25	985
Rubrivivax benzoatilyticus JA2 = ATCC BAA-35	987059	25.21	<0.01	0.18	0.32	1597
Methylibium petroleiphilum PM1	420662	24.10	<0.01	0.18	0.16	789
Piscinibacter aquaticus	392597	26.77	<0.01	0.20	0.21	1654
Flavihumibacter sp. SB-02	2676868	24.10	<0.01	0.34	0.34	493
Candidatus Atelocyanobacterium thalassa	713887	15.88	<0.01	1.20	1.20	4
Anabaena cylindrica PCC 7122	272123	15.09	<0.01	0.19	0.21	451
Trichormus sp. NMC-1	1853259	18.16	<0.01	0.26	0.30	361
actinobacterium acIB-AMD-7	1504322	15.17	<0.01	0.32	0.35	61
Xanthomonas phaseoli pv. phaseoli	317013	18.29	<0.01	0.46	0.38	32
Perlucidibaca piscinae DSM 21586	1122951	18.15	<0.01	0.26	0.26	417
Novosphingobium sp. B 225	1961849	23.45	<0.01	0.18	0.18	992
Blastomonas sp. CCH8-A3	1768743	16.77	<0.01	0.66	1.07	11
Sphingomonas sp. IBVSS1	1985171	21.26	<0.01	0.31	0.40	309
Tabrizicola aquatica	909926	14.61	<0.01	0.18	0.21	517
Rhodobacter thermarum	2670345	22.08	<0.01	0.29	0.34	373
Rhodobacter flagellatus	2593021	15.39	<0.01	0.20	0.25	485

Gemmobacter caeruleus	2595004	15.61	<0.01	0.19	0.19	504
Rhodovulum visakhapatnamense	364297	18.35	<0.01	0.27	0.22	206
Rhodovulum strictum	58314	15.90	<0.01	0.34	0.32	819
Paracoccus sp. FO-3	1335059	18.38	<0.01	0.31	0.15	151
Aquincola tertiaricarbonis	391953	19.03	<0.01	0.11	0.11	1448
unclassified Methylibium	2633235	14.95	<0.01	0.16	0.18	408
Burkholderiales genera incertae sedis	224471	23.06	<0.01	0.15	0.15	83
Rivibacter subsaxonicus	457575	18.21	<0.01	0.13	0.13	1136
Schlegelella thermodepolymerans	215580	17.26	<0.01	0.12	0.12	1156
Limnohabitans sp. Hippo4	1826167	16.61	<0.01	0.19	0.25	310
Burkholderiales bacterium JOSHI_001	864051	17.15	<0.01	0.16	0.16	973
Vogesella mureinivorans	657276	16.81	<0.01	0.13	0.13	768
Gemmatimonas	173479	16.52	<0.01	12.55	14.04	82
Flavobacterium indicum GPTSA100-9 = DSM 17447	1094466	14.22	<0.01	0.12	0.12	306
Bacteroidetes bacterium SCGC AAA027-G08	938698	19.33	<0.01	0.39	0.39	88
'Nostoc azollae' 0708	551115	10.12	<0.01	0.15	0.15	228
actinobacterium SCGC AAA027-L06	913338	9.40	<0.01	0.19	0.28	37
actinobacterium acAcidi	1504320	5.79	<0.01	0.13	0.13	311
Actinobacteria bacterium IMCC26077	1848755	13.74	<0.01	0.22	0.22	177
Bacillus sp. JEM-1	1977090	8.70	<0.01	0.32	0.32	12
Aeromonas	642	12.30	<0.01	0.38	0.53	7
Novosphingobium subterraneum	48936	10.62	<0.01	0.15	0.11	241
Blastomonas natatoria	34015	9.76	<0.01	0.15	0.20	283
Sandarakinorhabdus sp. AAP62	1248916	8.74	<0.01	0.13	0.17	242
Sphingobium fluviale	2506423	8.43	<0.01	0.13	0.13	222
Erythrobacter dokdonensis DSW-74	1300349	10.28	<0.01	0.13	0.13	338
Erythrobacter sanguineus	198312	9.45	<0.01	0.13	0.13	281
Erythrobacter tepidarius	60454	12.42	<0.01	0.16	0.17	359
Erythrobacter cryptus DSM 12079	1122970	6.86	<0.01	0.11	0.11	162
alpha proteobacterium SCGC AAA027-C06	938624	5.69	<0.01	0.19	0.36	15
alpha proteobacterium SCGC AAA487-M09	938672	6.06	<0.01	0.22	0.23	13
alpha proteobacterium SCGC AAA027-J10	938631	4.84	<0.01	0.24	0.35	9
Tabrizicola piscis	2494374	12.20	<0.01	0.15	0.15	518
Rhodobacter veldkampii DSM 11550	1185920	11.73	<0.01	0.18	0.35	841
Rhodobacter blasticus	1075	9.68	<0.01	0.14	0.14	303
Gemmobacter aestuarii	1445661	11.13	<0.01	0.14	0.14	458
Gemmobacter aquatilis	933059	12.40	<0.01	0.16	0.16	395
Gemmobacter nectariphilus DSM 15620	1121271	11.93	<0.01	0.16	0.16	433
Fertoebacter nigrum	2656921	8.64	<0.01	0.10	0.10	369
Pseudorhodobacter sp. MZDSW-24AT	2052957	12.79	<0.01	0.17	0.17	404
Luteovulum ovatum	439529	7.77	<0.01	0.11	0.11	291
Rhabdaerophilum calidifontis	2604328	8.52	<0.01	0.13	0.13	266
Bradyrhizobium	374	5.78	<0.01	0.14	0.13	16
Rubrivivax gelatinosus IL144	983917	11.51	<0.01	0.10	0.25	517
Tepidimonas sediminis	2588941	11.11	<0.01	0.11	0.12	470
Inhella inkyongensis	392593	7.89	<0.01	0.10	0.10	318
Serpentinomonas raichei	1458425	10.80	<0.01	0.12	0.12	285
Comamonadaceae bacterium 2PF	2502197	8.69	<0.01	0.10	0.15	423
Comamonadaceae bacterium JGI 0001013-A16	1286843	6.91	<0.01	0.12	0.12	50
beta proteobacterium SCGC AAA027-I06	938781	7.19	<0.01	0.20	0.20	138
Thauera sp. K11	2005884	9.07	<0.01	0.10	0.10	515
Methylophilus	16	5.30	<0.01	7.89	7.89	14
Crenobacter sp. GY 70310	2563443	12.02	<0.01	0.18	0.18	381
Chitinibacter sp. ZOR0017	1339254	8.03	<0.01	0.15	0.22	131
Vogesella urethralis	2592656	8.65	<0.01	0.14	0.15	255

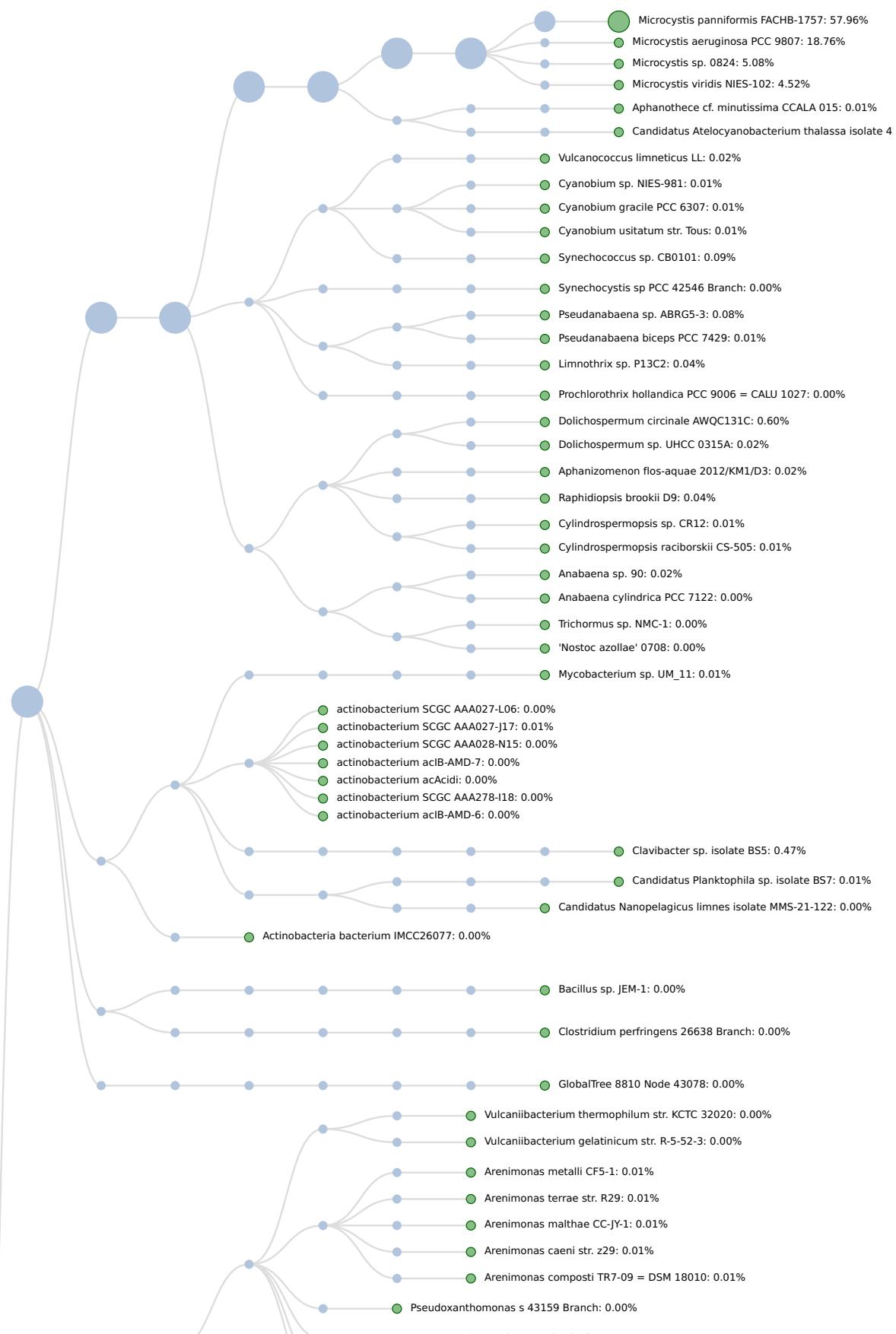
Opitutaceae bacterium TAV3	278958	10.55	<0.01	0.27	0.40	24
Opitutaceae bacterium TAV4	278959	5.53	<0.01	0.13	0.23	22
Flavobacterium tibetense	2233533	5.27	<0.01	0.11	0.12	70
Flavobacteria bacterium BAL38	391598	9.92	<0.01	0.10	0.24	137
Sediminibacterium salmoneum NBRC 103935	1400522	10.57	<0.01	0.16	0.16	158
Candidatus Fervidibacteria	1383058	6.84	<0.01	0.75	0.75	2
unclassified Synechocystis	2640012	1.35	0.00	1.05	1.05	23
actinobacterium SCGC AAA278-I18	938557	4.71	0.00	0.15	0.15	18
actinobacterium acIB-AMD-6	1504321	1.38	0.00	0.11	0.11	16
Clostridium perfringens	1502	1.43	0.00	0.13	0.13	3
Sphaerobacteraceae	85002	2.21	0.00	6.90	6.90	6
alpha proteobacterium SCGC AAA027-L15	938633	1.09	0.00	0.12	0.21	5
alpha proteobacterium SCGC AAA028-C07	938639	2.73	0.00	0.11	0.11	10
alpha proteobacterium SCGC AAA028-D10	938641	1.49	0.00	0.11	0.19	7
Ignavibacteriales	795748	2.78	0.00	11.11	11.11	5

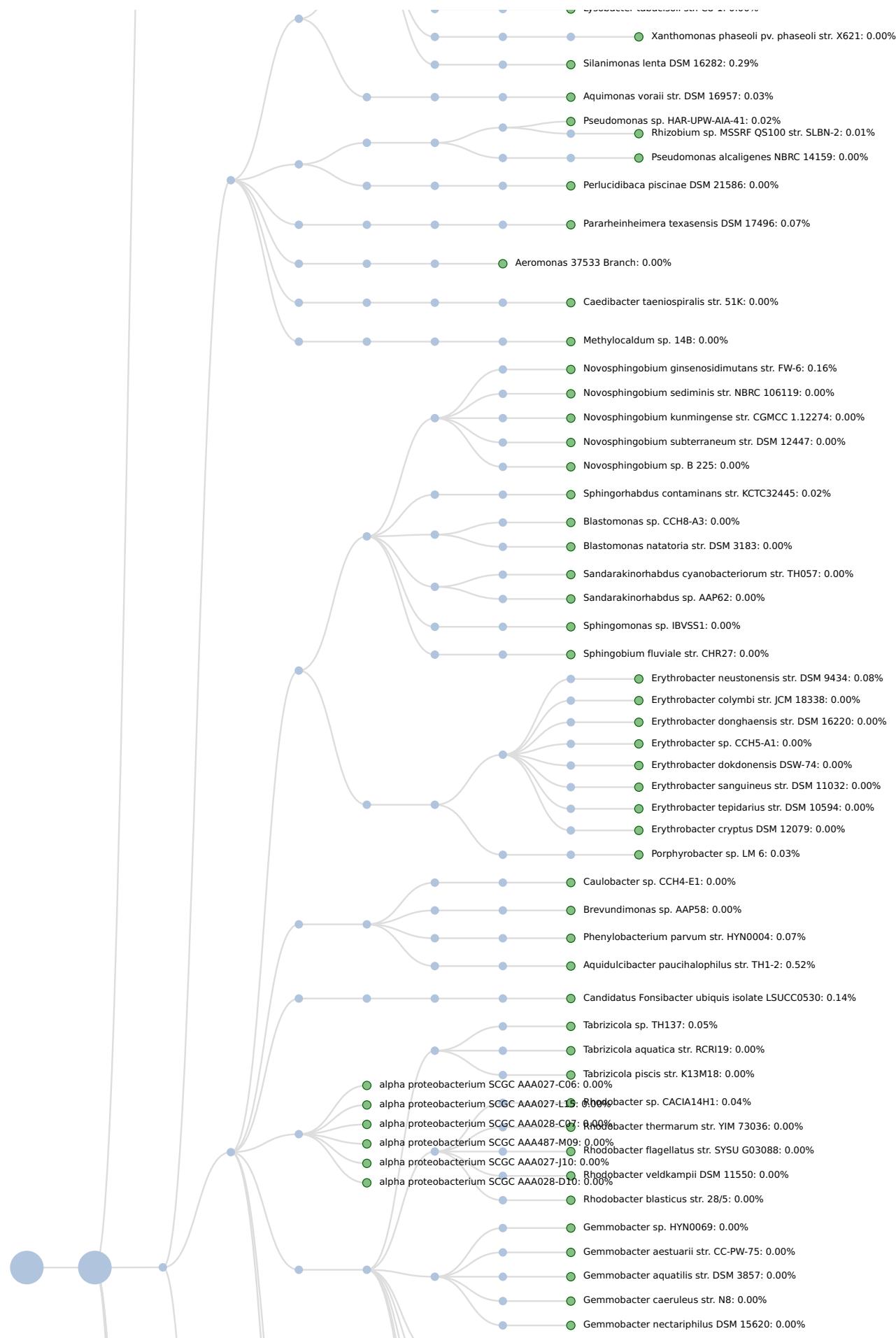
Bacteria

Tree Chart Taxonomy

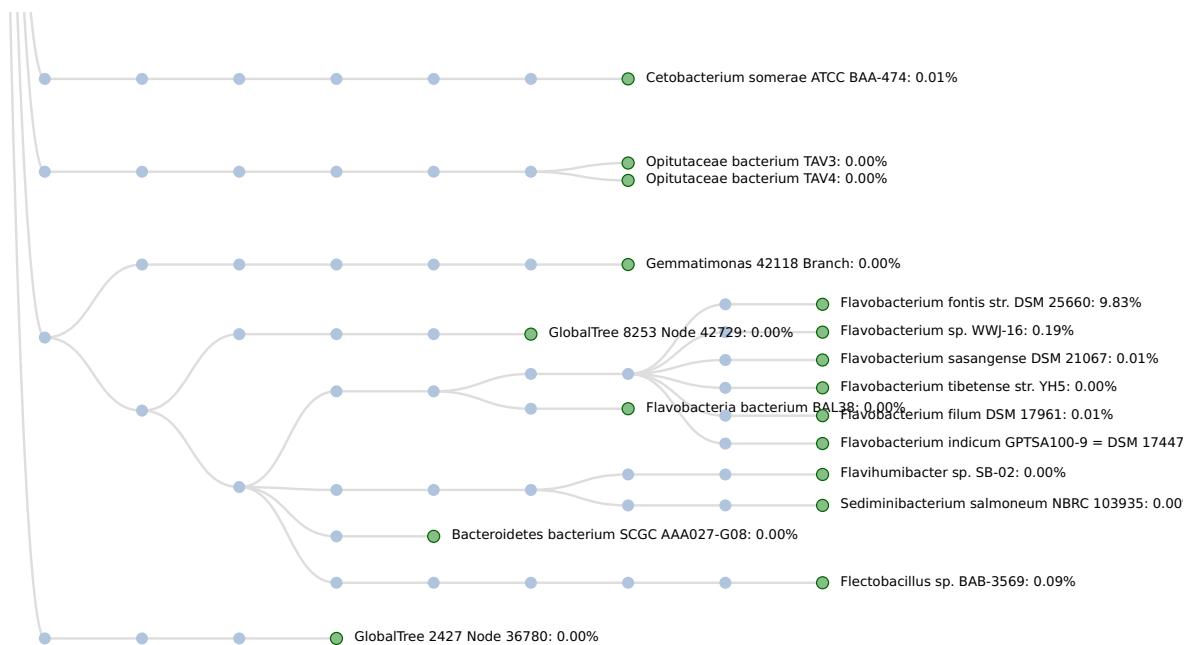
Sample_2_BWT2214

Total results



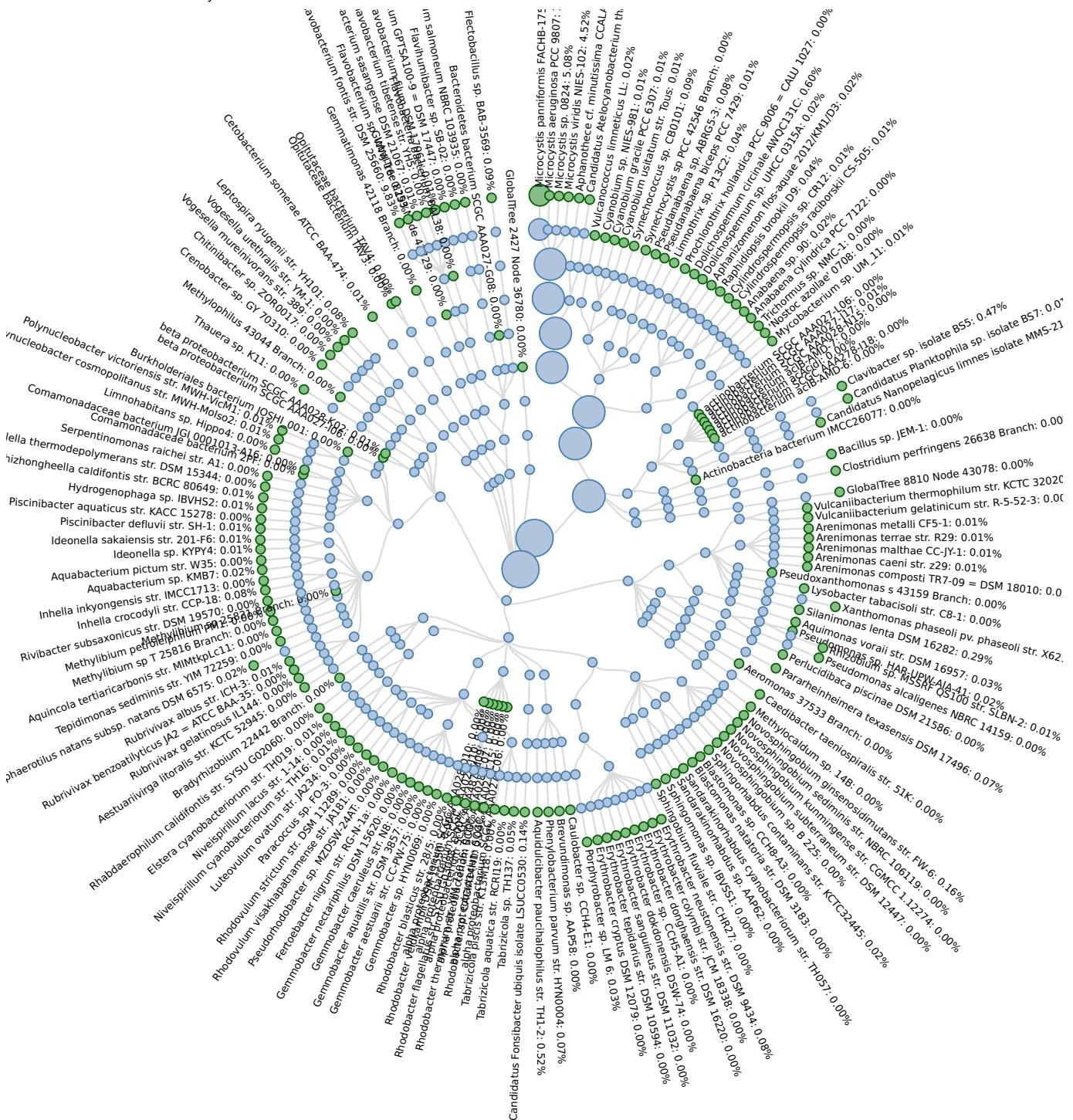






Bacteria

Radial Tree Chart Taxonomy



Bacteria

Sunburst Chart Taxonomy

Sample_2_BWT2214

Total results

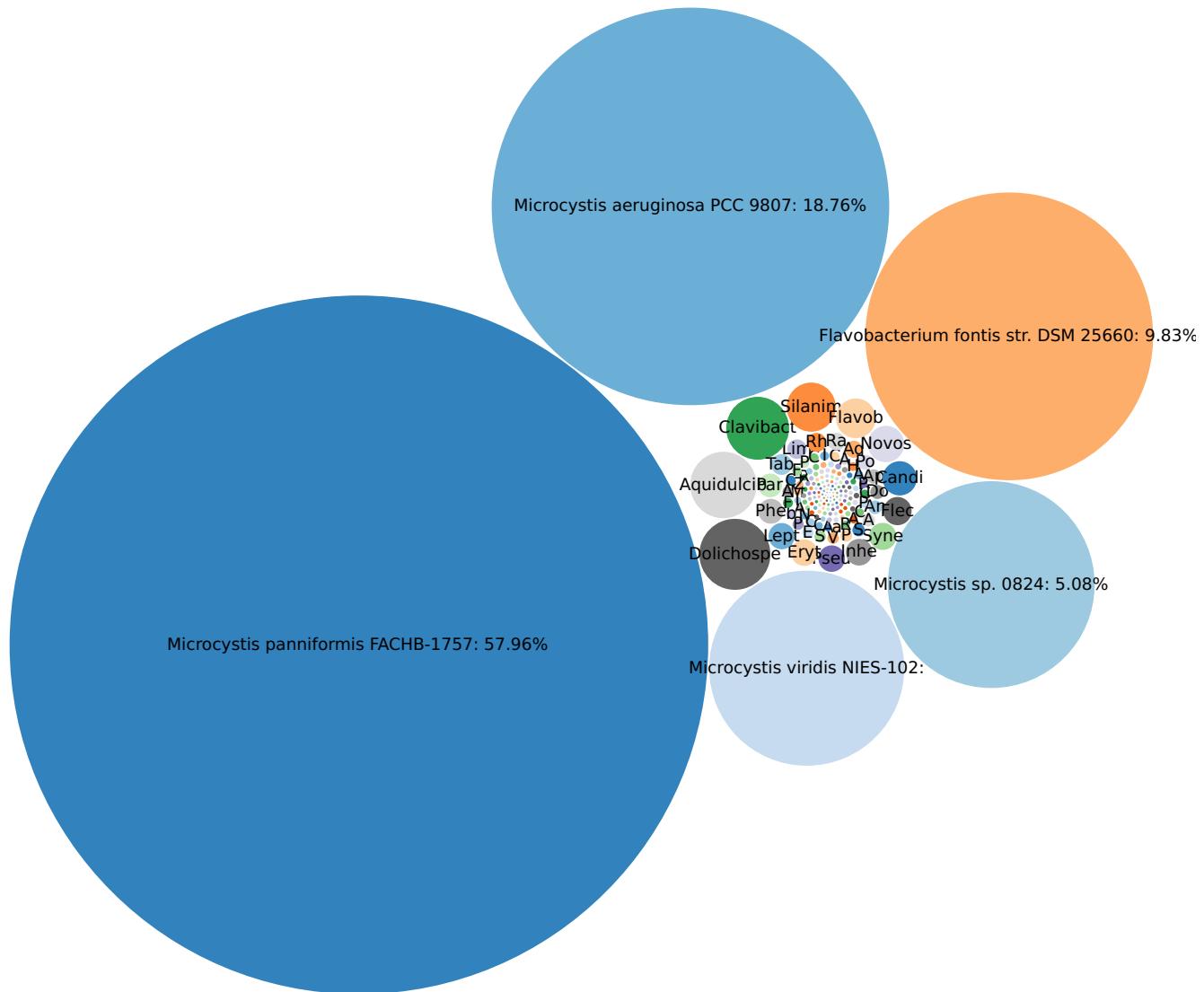


Bacteria

Bubble Chart Taxonomy

Sample_2_BWT2214

Total results



Fungi

Fasta/q details

Sample_2_BWT2214

Total results

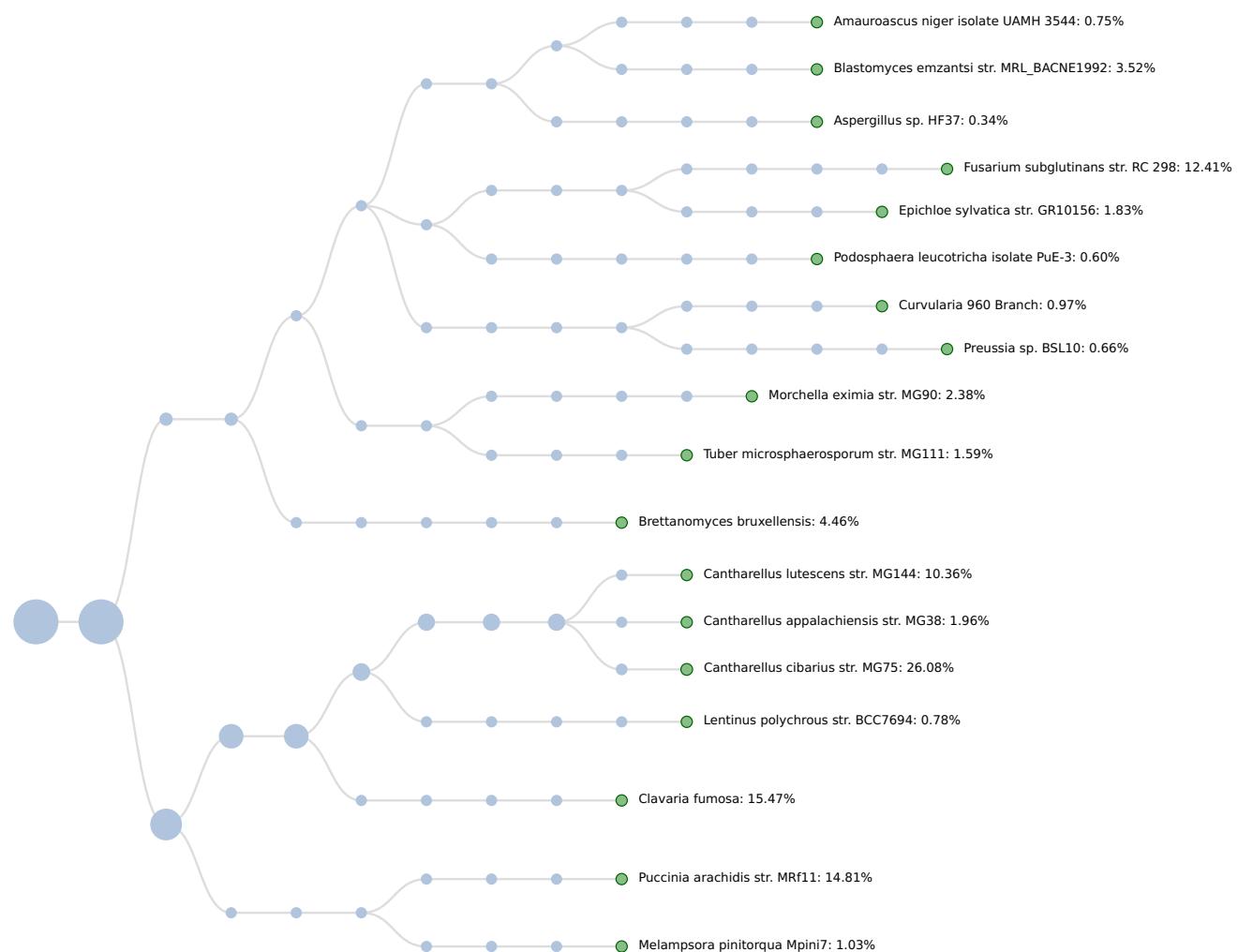
Metric	Value
Hit	37.179k

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Cantharellus cibarius	36066	24.46	26.08	0.08	0.10	2054
Clavaria fumosa	264083	14.51	15.47	0.10	0.30	1044
Puccinia arachidis	333523	13.89	14.81	0.07	0.13	1185
Fusarium subglutinans	42677	11.64	12.41	0.07	0.05	935
Cantharellus lutescens	104198	9.72	10.36	0.04	0.26	907
Brettanomyces bruxellensis	5007	4.18	4.46	0.02	0.03	434
Blastomyces emzantsi	2723674	3.30	3.52	0.03	0.04	273
Morchella eximia	1582338	2.23	2.38	0.03	0.01	198
Cantharellus appalachiensis	409893	1.84	1.96	0.02	0.21	187
Epichloe sylvatica	79593	1.72	1.83	0.03	0.02	1742
Tuber microsphaerosporum	1455713	1.49	1.59	0.02	0.02	155
Melampsora pinitorqua Mpini7	1298852	0.97	1.03	0.01	0.03	98
Curvularia	5502	0.91	0.97	0.01	0.01	27
Lentinus polychrous	292559	0.73	0.78	0.02	0.02	154
Amauroascus niger	89421	0.70	0.75	0.01	0.33	76
Preussia sp. BSL10	1712568	0.62	0.66	0.02	0.02	39
Podosphaera leucotricha	79249	0.56	0.60	0.01	0.01	55
Aspergillus sp. HF37	1960876	0.32	0.34	0.01	0.01	151

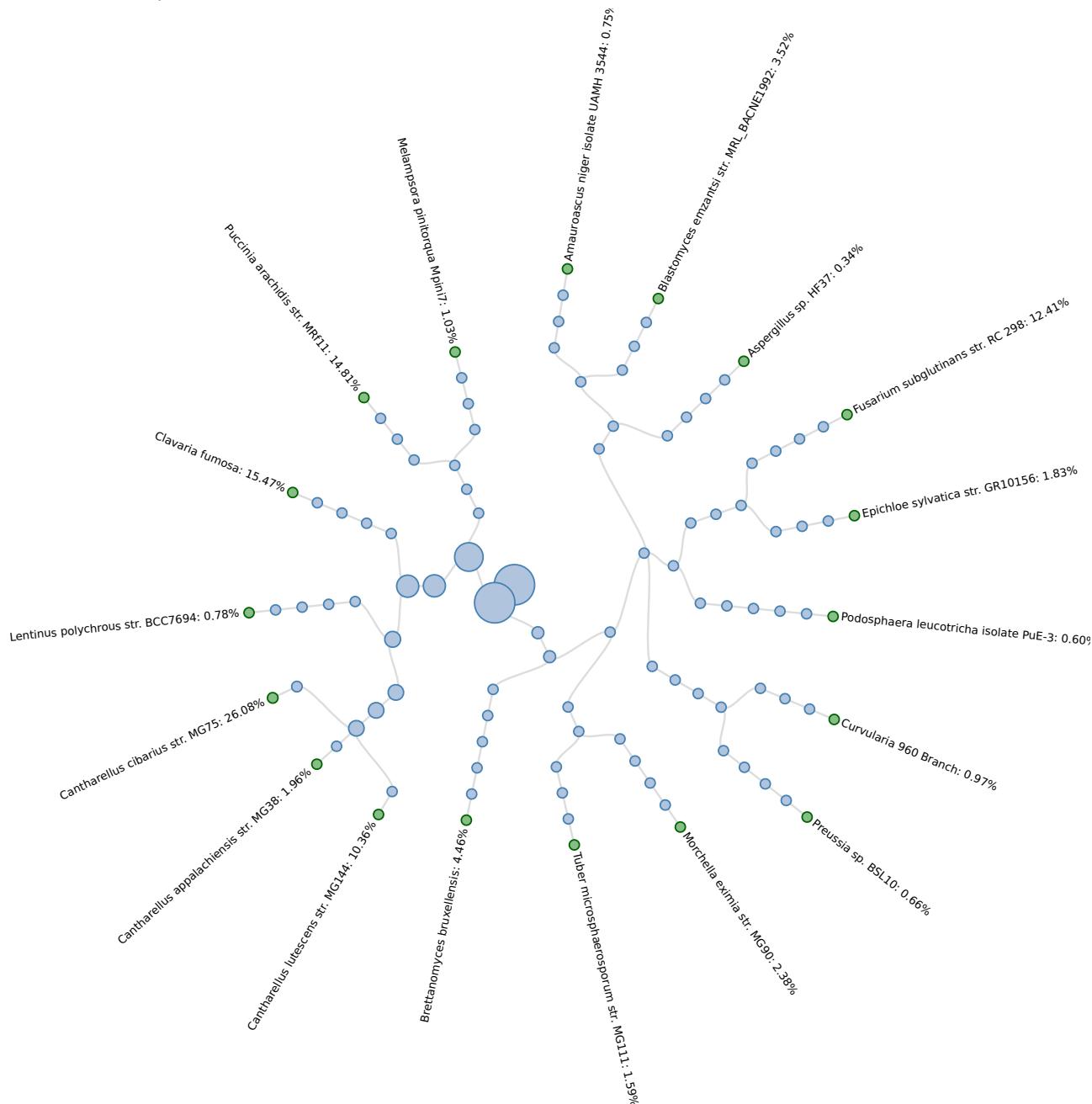
Fungi

Tree Chart Taxonomy

Sample_2_BWT2214
Total results

Fungi

Radial Tree Chart Taxonomy

Sample_2_BWT2214
Total results

Fungi

Sunburst Chart Taxonomy

Sample_2_BWT2214

Total results

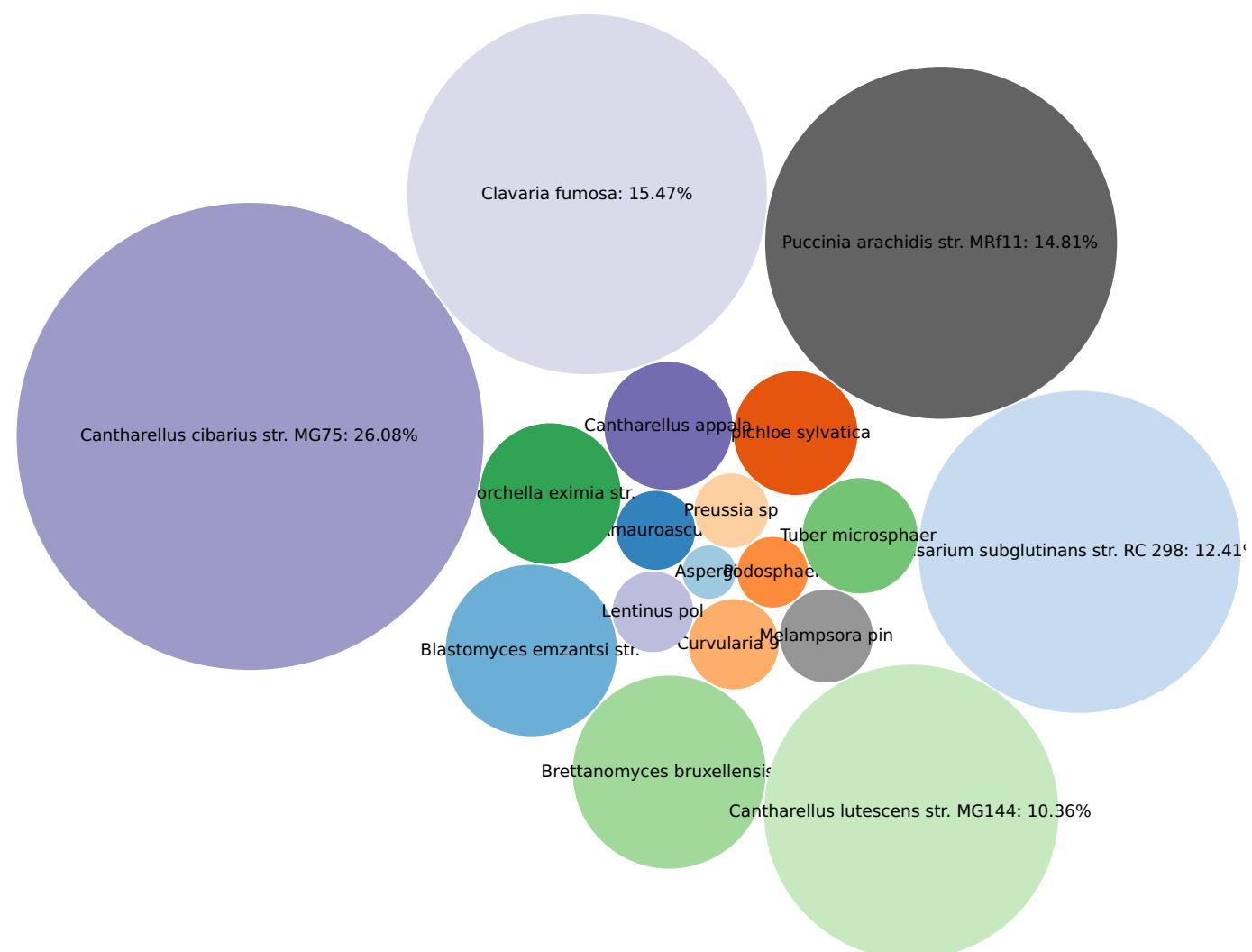


Fungi

Bubble Chart Taxonomy

Sample_2_BWT2214

Total results



Protists

Fasta/q details

Sample_2_BWT2214

Total results

Metric	Value					
Hit	15.797k					

Table

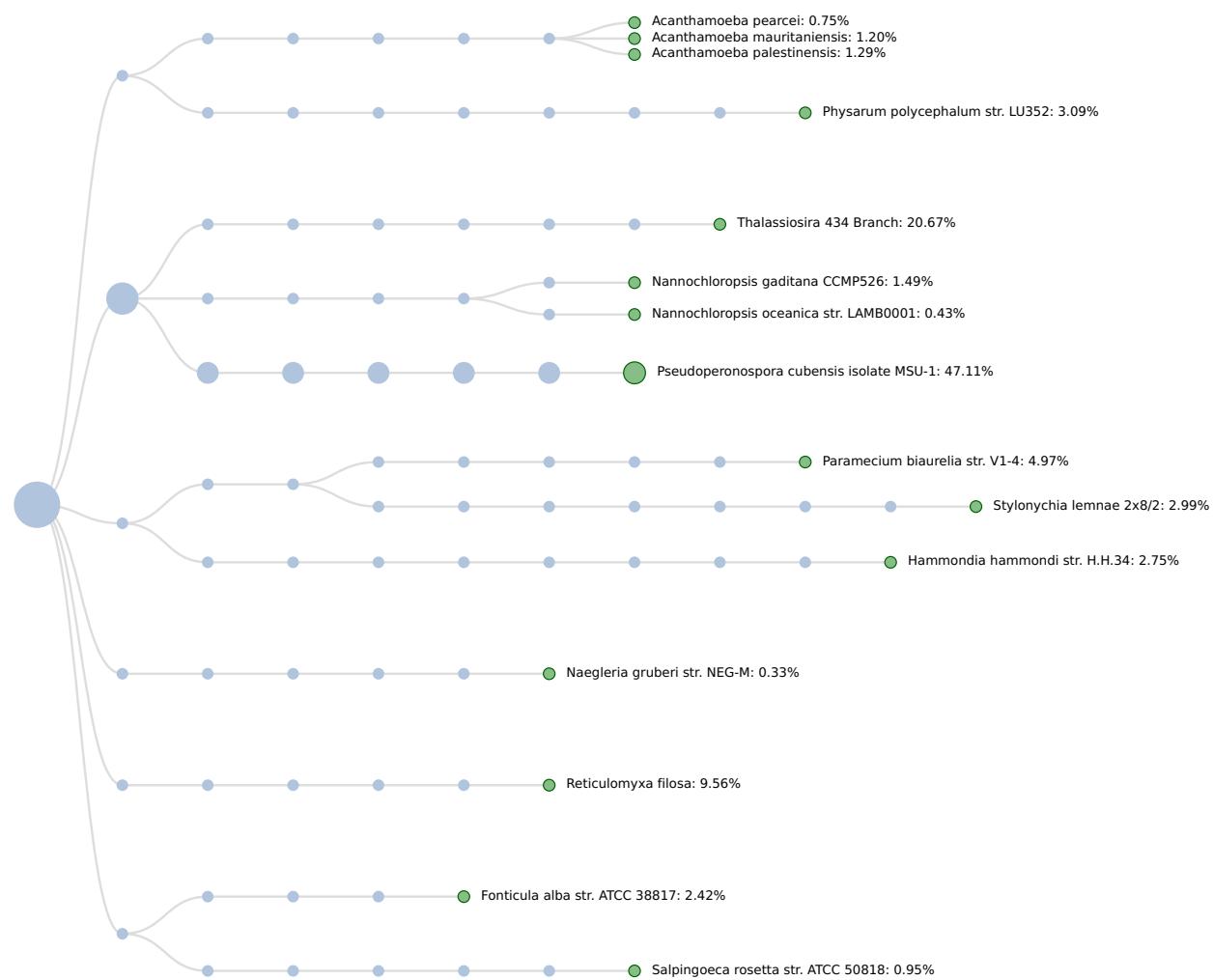
Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Pseudoperonospora cubensis	143453	33.86	47.11	0.14	0.22	2575
Thalassiosira	35127	14.86	20.67	24.32	24.32	73
Reticulomyxa filosa	46433	6.87	9.56	0.06	0.09	522
Paramecium biaurelia	65126	3.57	4.97	0.03	0.03	319
Physarum polycephalum	5791	2.22	3.09	0.02	0.09	251
Styloynchia lemnae 2x8/2	755200	2.15	2.99	0.02	0.03	93
Hammondia hammondi	99158	1.98	2.76	0.03	0.04	169
Fonticula alba	691883	1.74	2.42	0.01	0.04	203
Nannochloropsis gaditana CCMP526	1093141	1.07	1.49	0.02	0.01	77
Acanthamoeba palestinensis	28015	0.93	1.29	0.02	0.09	85
Acanthamoeba mauritanensis	196912	0.86	1.20	0.02	0.06	71
Salpingoeca rosetta	946362	0.68	0.95	0.02	0.23	40
Acanthamoeba pearcei	65662	0.54	0.75	0.02	0.03	8
Nannochloropsis oceanica	145522	0.31	0.43	0.01	0.07	278
Naegleria gruberi	5762	0.24	0.33	0.01	0.04	39

Protists

Tree Chart Taxonomy

Sample_2_BWT2214

Total results

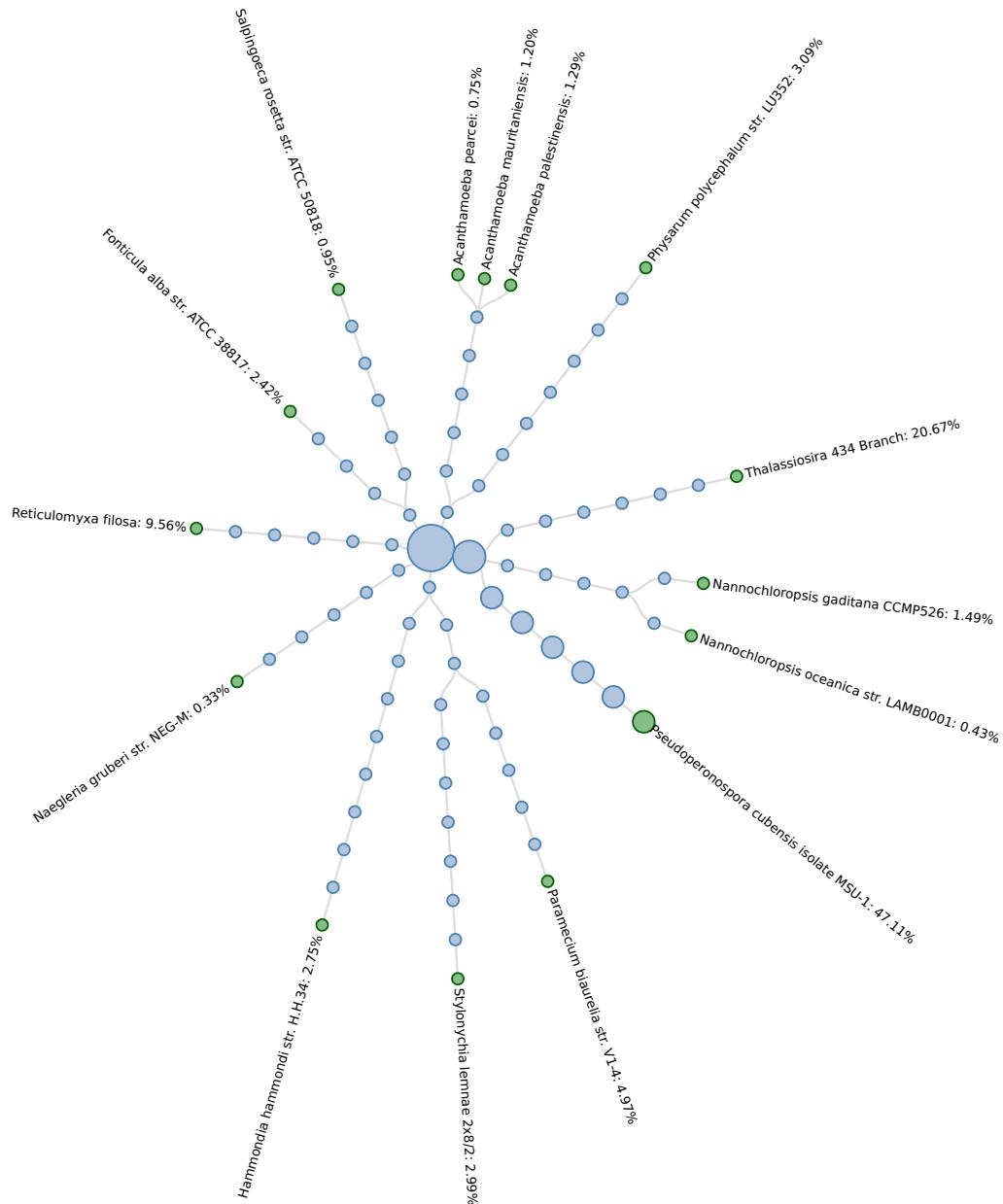


Protists

Radial Tree Chart Taxonomy

Sample_2_BWT2214

Total results



Protists

Sunburst Chart Taxonomy

Sample 2 BWT2214

Total results

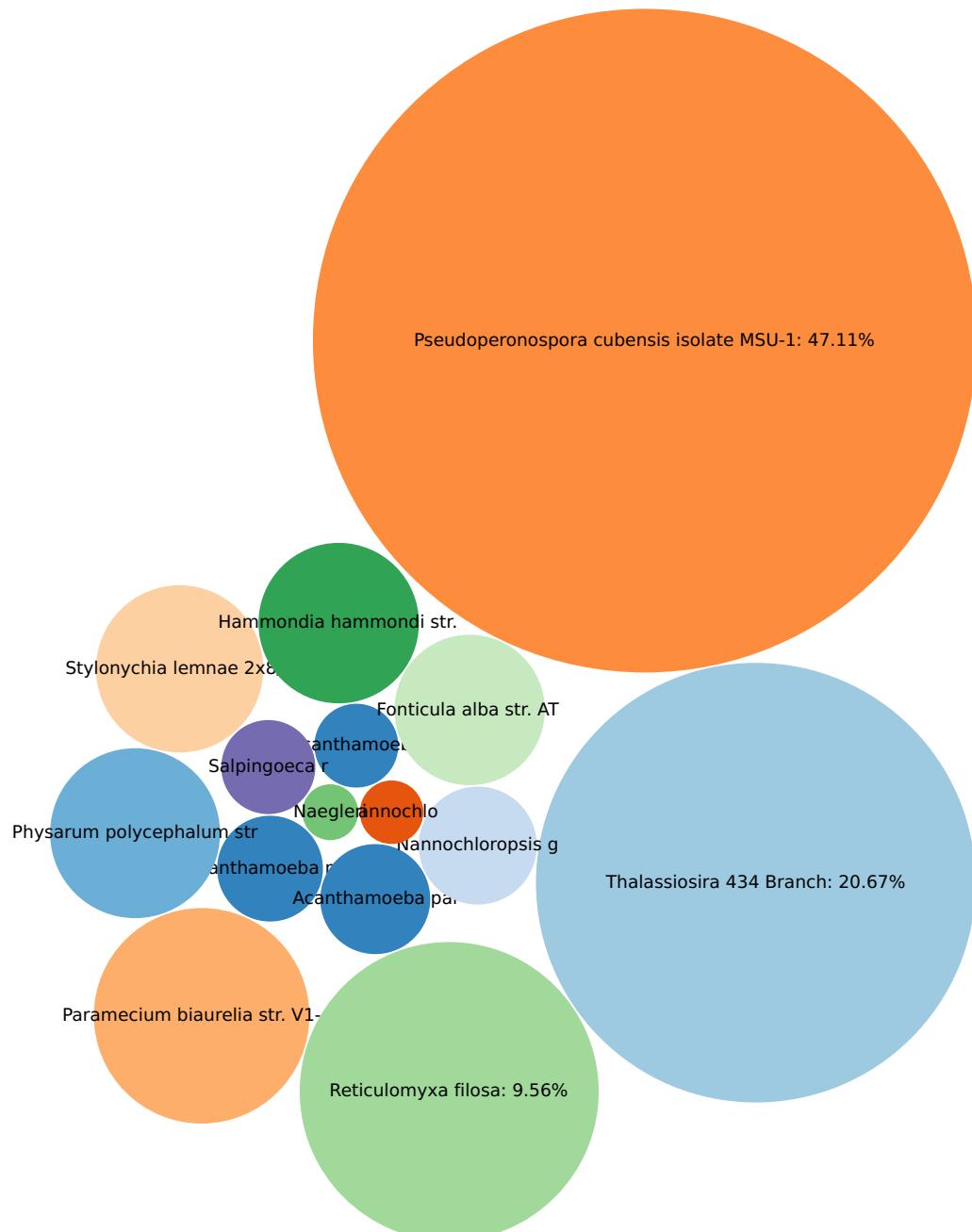


Protists

Bubble Chart Taxonomy

Sample_2_BWT2214

Total results



Dark Matter (Beta)

Fasta/q details

Sample_2_BWT2214

Total results

Metric	Value
Hit	0.890M

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Pseudanabaena sp.	1153	26206.62	41.61	45.52	45.75	449287
Burkholderiales bacterium UBA4657	1950779	5798.43	9.21	38.45	38.45	67918
Xanthomonadaceae bacterium	1926873	4887.30	7.76	4.78	4.78	135896
Burkholderiales bacterium UBA954	1950782	4425.09	7.03	33.32	33.32	32060
Novosphingobium sp. UBA6722	1946985	2873.99	4.56	17.42	17.42	43968
Pelagibacterales bacterium UBA4760	1951182	1779.14	2.83	19.98	19.98	2048
Chitinophagaceae bacterium	1869212	1709.48	2.71	19.17	24.63	3241
Proteobacteria bacterium UBA4656	1948178	1397.73	2.22	1.30	1.30	84487
Anabaena sp. UBA12330	2055756	1124.26	1.79	8.32	8.32	13249
Hyphomonadaceae bacterium	2026748	767.18	1.22	2.68	2.68	25481
Actinobacteria bacterium UBA3066	1948336	720.37	1.14	9.62	11.55	916
Chitinophagaceae bacterium UBA2467	1951550	699.69	1.11	7.03	9.85	1156
Burkholderiales bacterium UBA2470	1950772	594.64	0.94	5.56	8.77	1966
Chthonomonas sp. UBA2785	1946339	573.04	0.91	3.17	6.19	19
Actinobacteria bacterium	1883427	537.60	0.85	5.55	7.80	1216
Burkholderiales bacterium UBA2647	1950774	522.27	0.83	4.30	9.08	542
Porphyrobacter sp. UBA7686	1947080	522.07	0.83	4.05	4.05	9564
Actinobacteria bacterium UBA2236	1948325	521.64	0.83	7.48	11.11	543
Armatimonadetes bacterium	2033014	493.08	0.78	2.06	5.99	100
Arenimonas sp.	1872635	474.80	0.75	0.60	0.60	20648
Acidimicrobium sp.	1872112	462.72	0.73	5.06	6.33	1926
Lautropia sp.	2053568	425.45	0.68	5.17	9.39	605
Methylophilaceae bacterium	2030816	398.29	0.63	4.99	5.19	167
Flavobacterium sp. UBA6203	1946555	289.68	0.46	3.47	3.72	1020
Blastomonas sp. UBA7677	1946117	204.77	0.33	2.25	2.25	2659
unclassified Burkholderiales (miscellaneous)	80841	162.56	0.26	0.17	0.17	218
Chloroflexi bacterium	2026724	160.98	0.26	1.87	1.87	1361
Acidobacteria bacterium	1978231	144.69	0.23	2.06	2.06	492
Candidatus Microthrix	41949	129.44	0.21	1.15	1.15	35
Planctomycetaceae bacterium	2026779	125.97	0.20	1.37	1.37	3309
uncultured Collinsella sp.	165190	123.24	0.20	0.33	0.33	394
Verrucomicrobia bacterium UBA3063	1948252	122.99	0.19	1.24	1.36	1023
Pseudomonas sp. UBA6718	1947334	117.41	0.19	1.28	1.31	2259
unclassified Caulobacteraceae	81440	115.89	0.18	0.15	0.15	1136
unclassified Limnobacter	2630203	103.74	0.17	0.12	0.12	35
Proteobacteria bacterium UBA3065	1948161	101.58	0.16	0.95	0.50	692
unclassified Brevundimonas	2622653	95.47	0.15	0.23	0.23	232
Rheinheimera sp.	1869214	89.72	0.14	0.92	0.92	2325
Proteobacteria bacterium UBA964	1948225	78.09	0.12	0.73	0.73	2188
Methylocystaceae bacterium UBA4761	1952232	69.29	0.11	1.09	1.32	58
Alphaproteobacteria bacterium UBA2784	1948371	69.09	0.11	0.84	0.84	1237
Parachlamydiales bacterium	2052178	69.23	0.11	0.46	0.46	7831
Methylocystaceae bacterium UBA5192	1952233	68.28	0.11	1.06	1.27	119
Rhodanobacteraceae bacterium UBA7676	1953069	66.57	0.11	0.15	0.17	5182
Nitrosomonas sp. UBA6494	1946979	61.83	0.10	0.82	0.82	780
Gammaproteobacteria bacterium	1913989	60.74	0.10	0.23	0.23	2337
Sediminibacterium sp. UBA657	1947456	59.33	0.09	0.94	0.94	297

Aeromonas veronii	654	58.73	0.09	0.64	0.66	1327
Synechococcales bacterium UBA8647	2055797	56.78	0.09	0.79	0.42	159
Verrucomicrobia bacterium UBA2460	1948245	54.54	0.09	1.26	1.08	140
Polynucleobacter sp. UBA2464	1947076	53.02	0.08	0.63	0.35	286
Verrucomicrobia bacterium UBA2644	1948246	52.31	0.08	1.00	1.11	171
bacterium UBA951	1947788	50.18	0.08	0.85	0.85	150
Synechococcus sp. UBA8071	2055764	49.68	0.08	0.53	0.53	652
Ralstonia	48736	46.90	0.07	0.56	0.56	65
Phycisphaerales bacterium UBA4658	1951200	46.53	0.07	0.37	0.37	2420
unclassified Flavobacterales (miscellaneous)	403978	46.03	0.07	3.03	3.03	2
Flammeovirgaceae bacterium UBA4659	1951784	42.58	0.07	0.39	0.39	1279
unclassified Polaromonas	2638319	41.54	0.07	0.30	0.30	54
unclassified Sphingopyxis	2614943	41.15	0.07	1.26	1.26	11
Rhodobacteraceae bacterium UBA6273	1952813	40.11	0.06	0.39	0.39	1478
Rhodanobacteraceae bacterium UBA703	1953068	39.20	0.06	0.14	0.16	499
Curvibacter sp.	1888168	37.37	0.06	0.34	0.35	1469
Synechococcales bacterium UBA12195	2055798	35.72	0.06	0.58	0.36	80
Comamonadaceae bacterium	1871071	36.14	0.06	0.26	0.26	1615
Bacteroidetes bacterium UBA955	1953175	35.13	0.06	0.52	0.52	367
Stenotrophomonas sp.	69392	33.70	0.05	0.12	0.13	1637
unclassified Candidatus Microthrix	2642024	33.10	0.05	0.70	0.68	22
uncultured Lachnobacterium sp.	1339173	33.28	0.05	0.16	0.16	414
Flavobacterales bacterium UBA2426	1950978	33.25	0.05	0.38	0.38	1054
Candidatus Accumulibacter	327159	33.61	0.05	0.18	0.18	5
Cyanobacteria bacterium UBA6965	1947892	32.77	0.05	0.63	0.41	78
Xylella sp. UBA6001	1947786	32.99	0.05	0.12	0.13	2335
Brevundimonas sp. UBA7534	1946138	31.37	0.05	0.13	0.14	1809
Pseudomonas aeruginosa group	136841	30.75	0.05	0.51	0.51	24
Achromobacter	222	31.00	0.05	0.13	0.14	231
Synechococcales bacterium UBA10510	2055795	30.26	0.05	1.53	0.82	223
Ramlibacter tataouinensis	94132	30.11	0.05	0.15	0.15	1642
bacterium UBA5577	1947808	30.20	0.05	0.35	0.35	478
Cyanobacteria bacterium UBA7373	1947895	29.84	0.05	0.33	0.33	514
Pseudomonas stutzeri	316	29.35	0.05	0.50	0.53	107
Rhodobacteraceae bacterium UBA1943	1952798	29.54	0.05	0.29	0.29	1023
Rhodobacteraceae bacterium UBA6141	1952809	29.45	0.05	0.32	0.32	861
unclassified Bradyrhizobium	2631580	27.90	0.04	0.25	0.25	208
Spartobacteria bacterium UBA5019	1948749	27.93	0.04	0.39	0.40	512
Planctomycetaceae bacterium UBA4655	1952734	26.78	0.04	0.33	0.33	966
Tepidimonas sp. UBA997	1947650	25.90	0.04	0.22	0.22	881
Ignavibacteria bacterium UBA961	1948688	24.88	0.04	0.45	0.45	139
Delftia acidovorans	80866	25.23	0.04	0.17	0.15	746
Rhodocyclaceae bacterium UBA2250	1952837	24.97	0.04	0.19	0.19	1089
Rhodobacter sp.	1062	24.90	0.04	0.25	0.25	922
Alicycliphilus sp. UBA7619	1946005	24.29	0.04	0.22	0.24	1340
Aquabacterium sp. UBA666	1946043	23.19	0.04	0.28	0.39	465
Comamonadaceae bacterium UBA2334	1951601	23.39	0.04	0.24	0.24	1029
Gemmobacter sp.	1898957	22.71	0.04	0.29	0.20	187
Crocinitomicaceae bacterium	2026728	21.11	0.03	0.34	0.34	215
Burkholderiales bacterium UBA4200	1950777	21.11	0.03	0.38	0.21	136
Comamonadaceae bacterium UBA6202	1951611	21.28	0.03	0.20	0.20	890
Prochlorococcus sp. UBA3999	1947246	20.49	0.03	0.34	0.27	84
Comamonadaceae bacterium UBA4962	1951608	20.77	0.03	0.18	0.20	1199
Acidimicrobiaceae bacterium UBA6895	1951341	19.95	0.03	0.23	0.13	191
Planctomyces bacterium UBA2386	1948127	20.38	0.03	0.19	0.19	1120

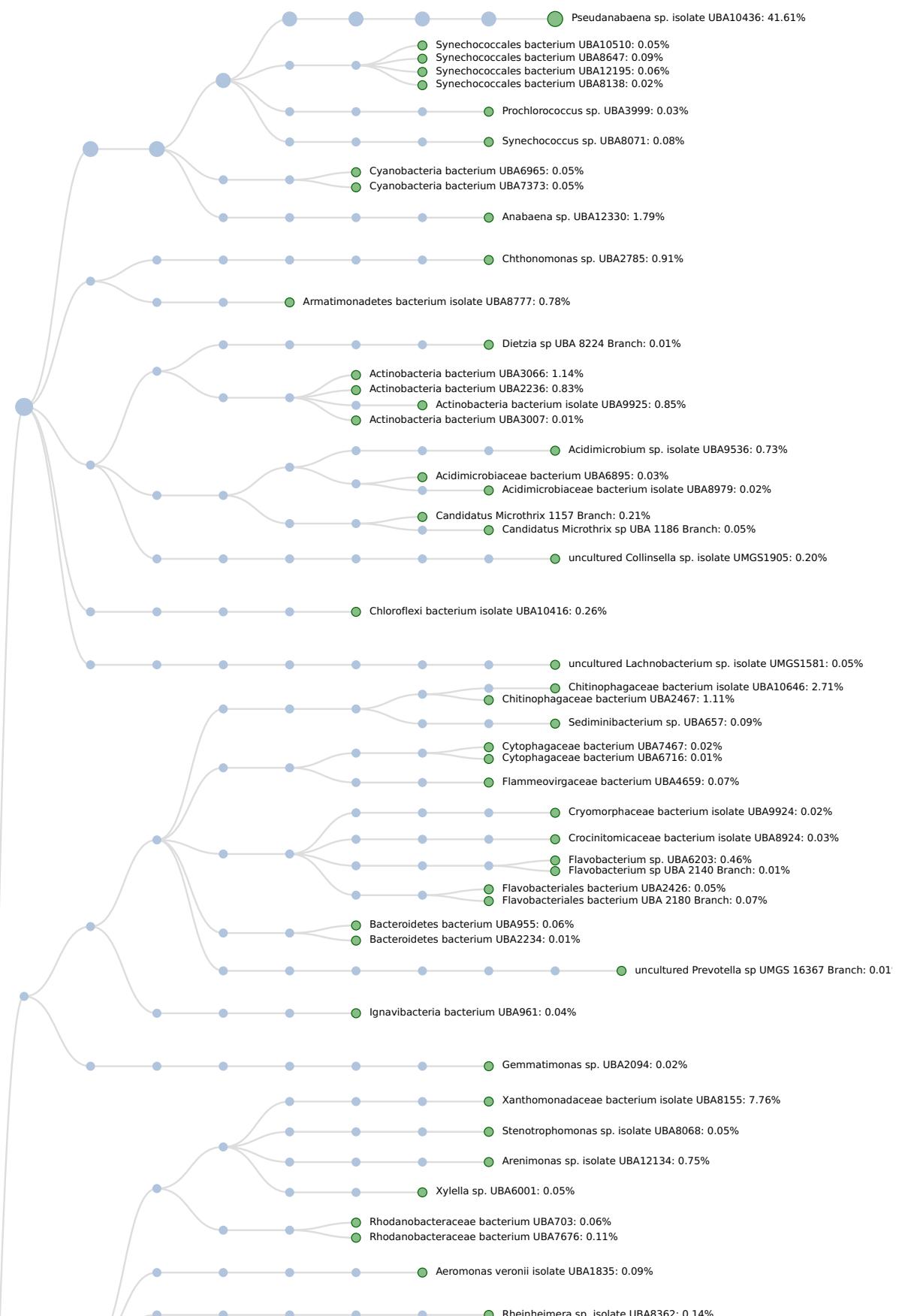
Hylemonella sp. UBA6679	1946611	16.39	0.03	0.20	0.20	483
unclassified Achromobacter	2626865	16.17	0.03	0.11	0.16	81
Gemmimonas sp. UBA2094	1946567	15.59	0.03	0.22	0.22	508
Caulobacter vibrioides	155892	15.97	0.03	0.11	0.11	1169
Proteobacteria bacterium UBA2646	1948154	15.90	0.03	0.26	0.33	70
Burkholderiales bacterium UBA1834	1950768	15.29	0.02	0.20	0.19	234
Acidovorax sp.	1872122	14.94	0.02	0.15	0.15	846
Rhizobiaceae bacterium UBA3490	1953065	15.09	0.02	0.20	0.20	576
Citreicella sp.	1960284	15.36	0.02	0.11	0.11	288
Rhodobacteraceae bacterium UBA996	1952817	15.12	0.02	0.17	0.17	668
Cryomorphaceae bacterium	1898111	14.68	0.02	0.23	0.23	239
Comamonas	283	14.78	0.02	0.13	0.13	133
Sphingomonas sp. UBA1000	1947531	14.47	0.02	0.13	0.13	516
Caulobacteraceae bacterium UBA6225	1951540	14.25	0.02	0.11	0.16	570
Rhodobacteraceae bacterium UBA6236	1952812	13.62	0.02	0.18	0.18	323
Paracoccus sp. UBA3880	1947052	12.92	0.02	0.14	0.14	420
Cytophagaceae bacterium UBA7467	1951636	12.23	0.02	0.32	0.44	33
Rhodobacteraceae bacterium	1904441	12.25	0.02	0.11	0.11	548
Rhodobacteraceae bacterium UBA6796	1952814	11.71	0.02	0.14	0.15	409
Opitutae bacterium UBA953	1948744	12.24	0.02	0.18	0.18	333
Synechococcales bacterium UBA8138	2055796	11.48	0.02	0.13	0.13	168
Comamonas aquatica	225991	10.51	0.02	0.13	0.14	392
Acidimicrobiaceae bacterium	2024894	10.08	0.02	0.13	0.13	496
Thiomonas sp. UBA7699	1947694	10.32	0.02	0.11	0.11	438
Hydrogenophaga sp.	1904254	10.23	0.02	0.27	0.25	205
Erythrobacter sp. UBA1916	1946469	9.78	0.02	0.11	0.11	370
Roseovarius sp. UBA6217	1947396	10.19	0.02	0.12	0.13	357
unclassified Rhodocyclaceae	75788	9.43	0.01	0.31	0.31	1614
Citromicrobium sp. UBA1476	1946344	9.31	0.01	0.11	0.12	321
Proteobacteria bacterium UBA2411	1948150	9.52	0.01	0.17	0.20	198
Bdellovibrionaceae bacterium UBA2651	1961509	9.74	0.01	0.33	0.23	16
Bdellovibrionaceae bacterium UBA2466	1961508	9.23	0.01	0.29	0.23	20
Verrucomicrobia bacterium UBA3062	1948251	9.48	0.01	0.16	0.16	149
Verrucomicrobiales	48461	9.18	0.01	0.12	0.12	127
Cytophagaceae bacterium UBA6716	1951634	9.09	0.01	0.39	0.46	9
Rhodoferax sp. UBA6134	1947380	8.76	0.01	0.12	0.12	231
unclassified Dietzia	2617939	8.04	0.01	0.37	0.37	6
Acidovorax delafieldii	47920	8.16	0.01	0.11	0.11	376
Methylocystaceae bacterium UBA2095	1952231	7.48	0.01	0.17	0.81	50
Geobacter sp. UBA4074	1961393	7.00	0.01	0.18	0.19	629
Verrucomicrobiaceae bacterium UBA2429	1952942	7.06	0.01	0.16	0.12	48
Phycisphaerales bacterium UBA2396	1951192	6.65	0.01	0.10	0.10	285
Actinobacteria bacterium UBA3007	1948330	6.50	0.01	0.17	0.17	31
Bacteroidetes bacterium UBA2234	1953144	6.57	0.01	0.13	0.13	99
uncultured Prevotella sp.	159272	6.18	0.01	0.14	0.14	10
unclassified Flavobacterium	196869	5.27	<0.01	0.32	0.32	4
unclassified Pseudomonas	2583993	4.82	<0.01	0.19	0.19	3
Legionellales bacterium	2026754	3.91	<0.01	0.12	0.12	24
Proteobacteria bacterium UBA2462	1948151	3.61	<0.01	0.13	0.31	13
Pseudomonas kunningensis	1211807	3.10	<0.01	0.12	0.10	11
Erythrobacteraceae bacterium UBA5049	1951760	2.43	<0.01	0.28	0.21	14

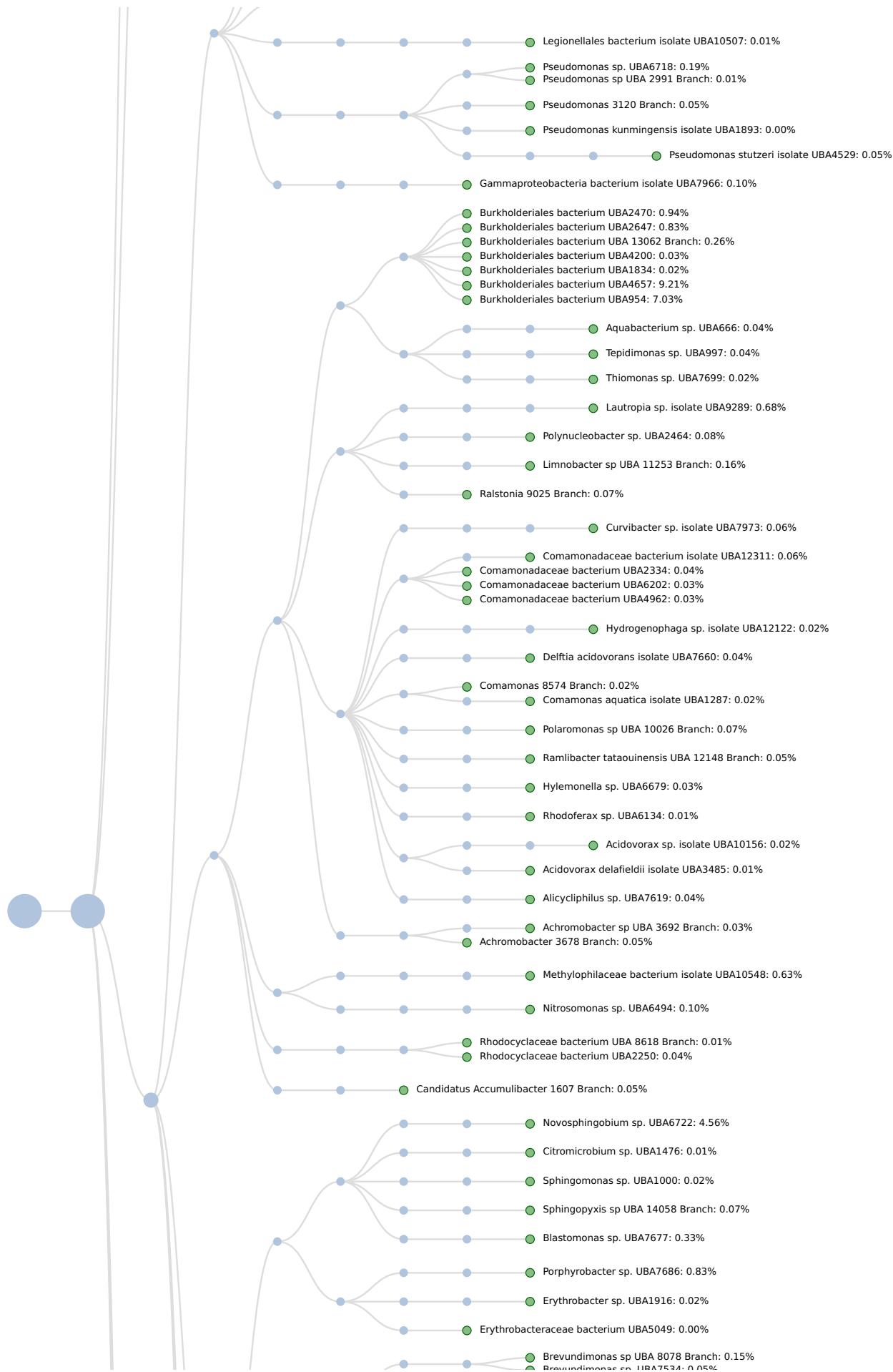
Dark Matter (Beta)

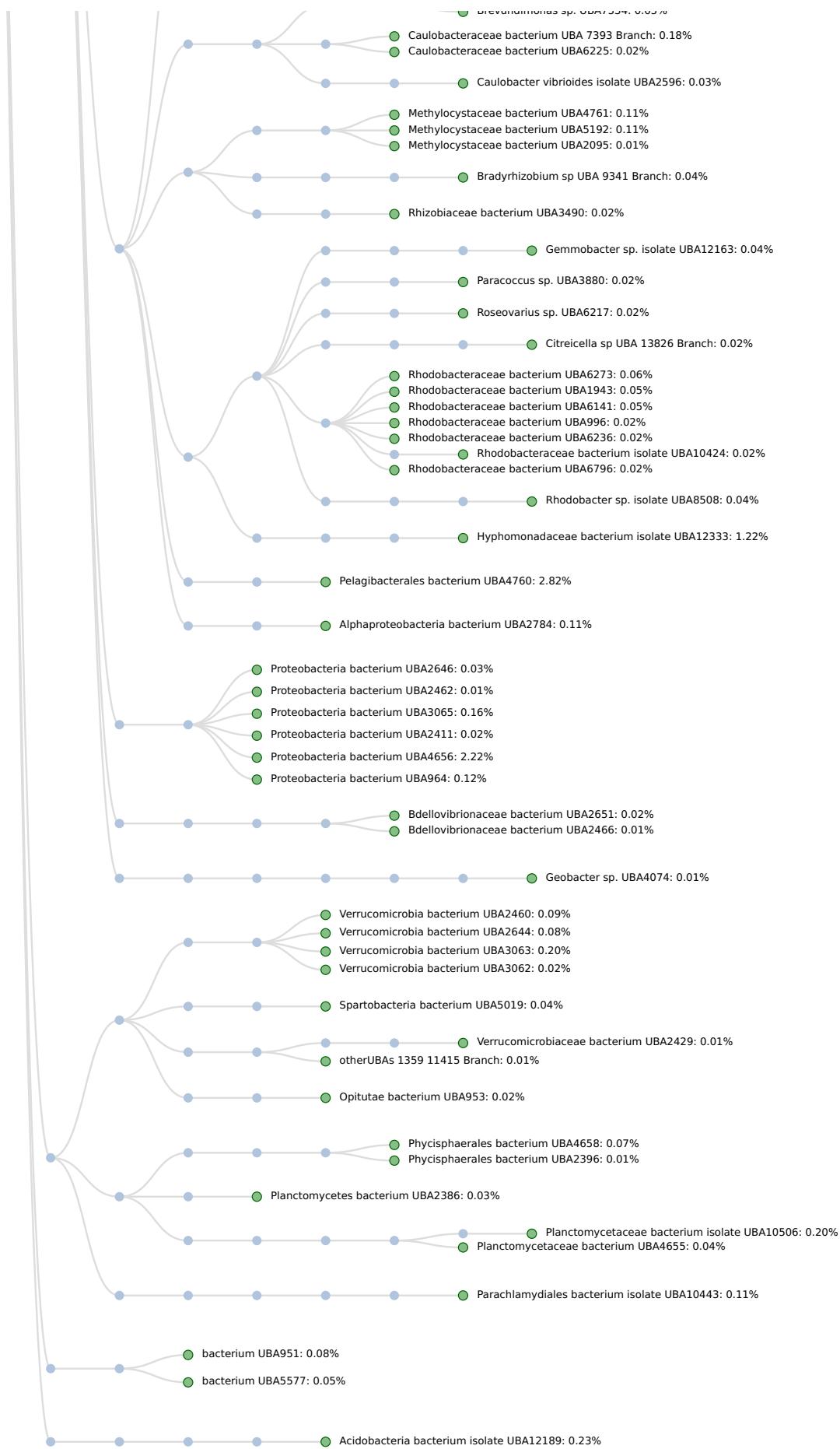
Tree Chart Taxonomy

Sample_2_BWT2214

Total results

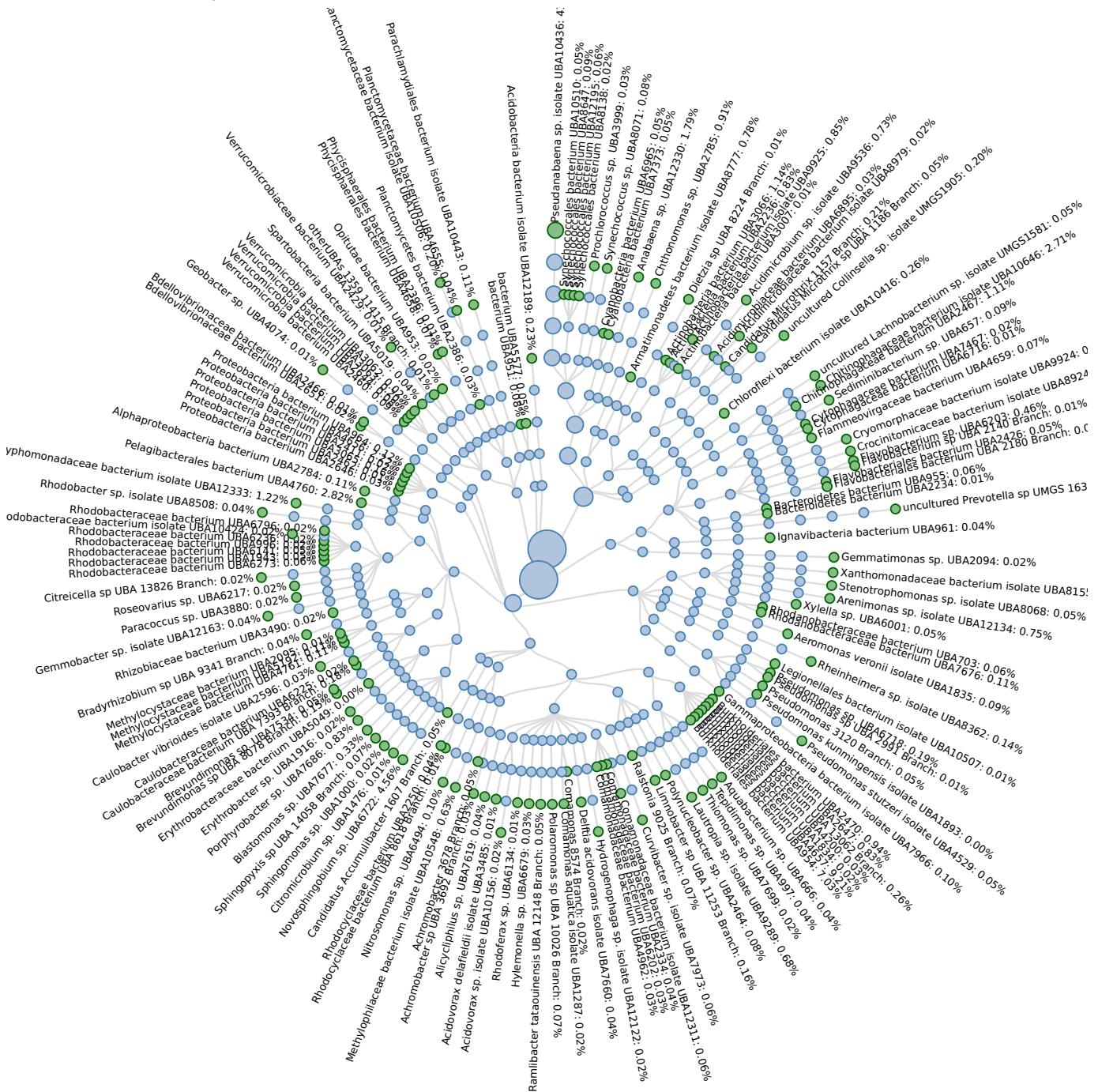






Dark Matter (Beta)

Radial Tree Chart Taxonomy



Sample_2_BWT2214

Total results

Dark Matter (Beta)

Sunburst Chart Taxonomy

Sample 2 BWT2214

Total results

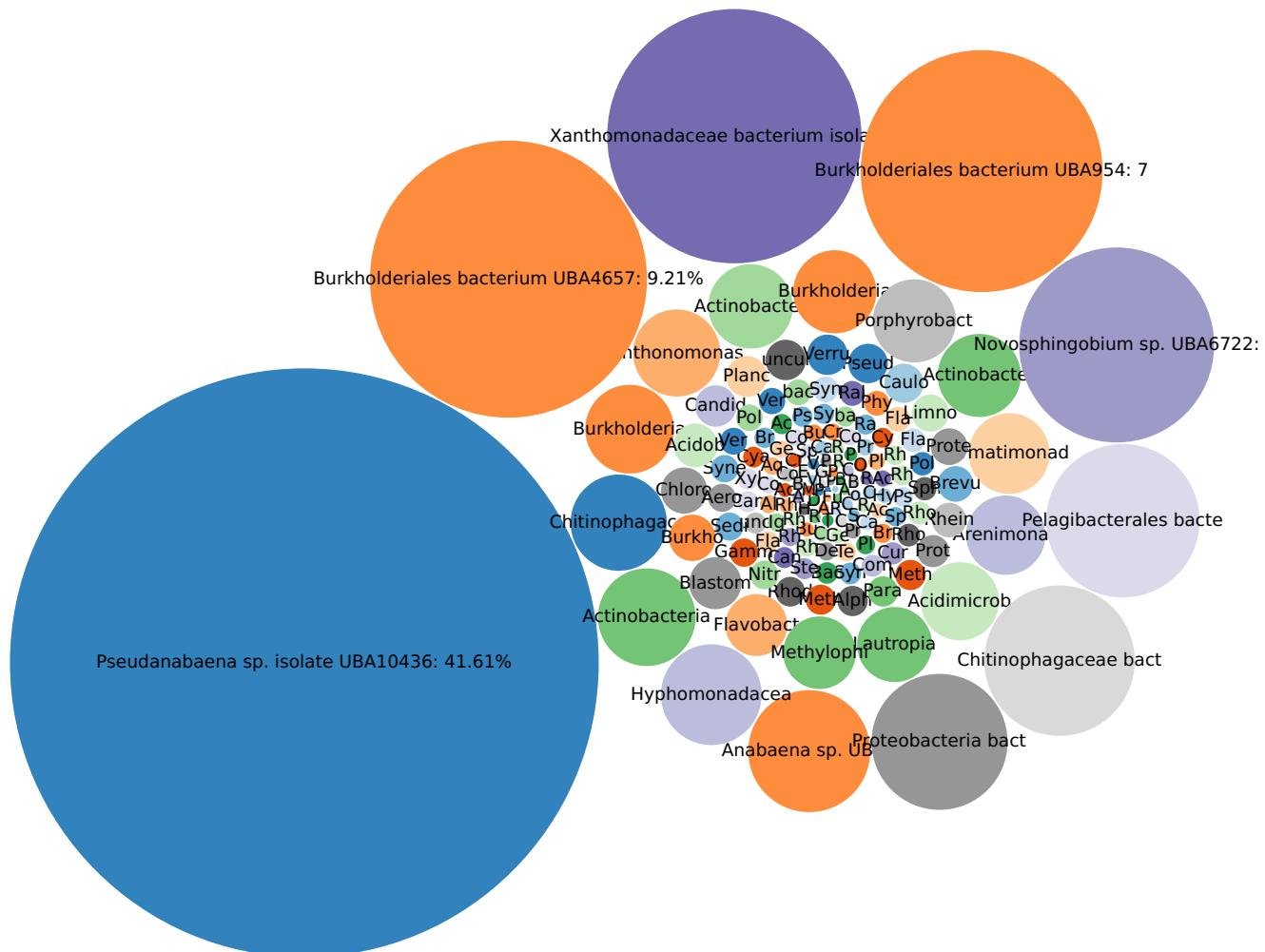


Dark Matter (Beta)

Bubble Chart Taxonomy

Sample_2_BWT2214

Total results



Phages

Fasta/q details

Sample_2_BWT2214

Total results

Metric	Value
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Table

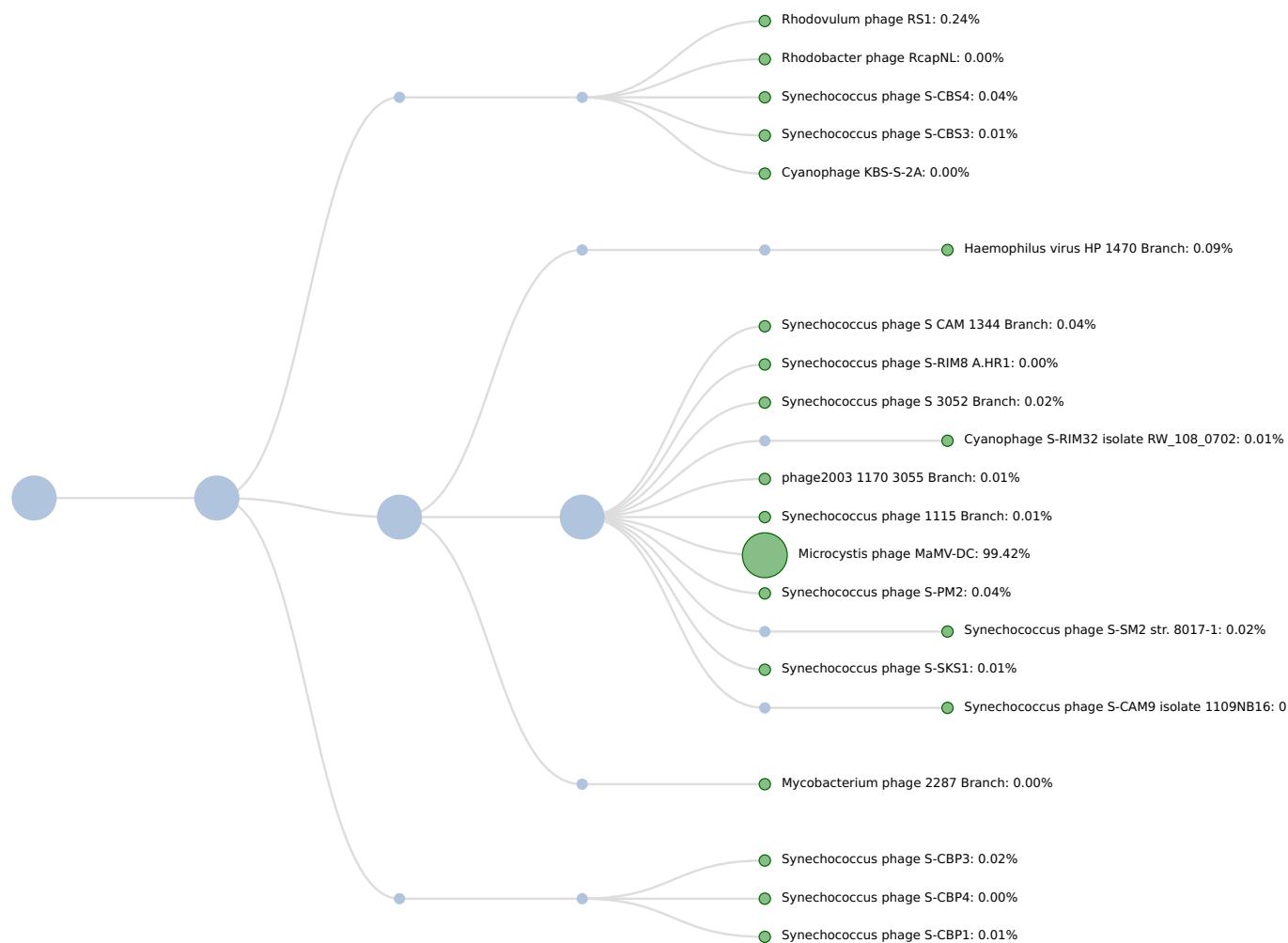
Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Microcystis phage MaMV-DC	1357715	168650.24	99.42	44.34	44.34	3874534
Rhodovulum phage RS1	754056	408.60	0.24	1.92	1.92	146
Hpunavirus	1196844	154.39	0.09	0.25	0.25	929
Synechococcus phage S-PM2	238854	74.82	0.04	0.58	0.60	2094
Synechococcus phage S-CBS4	756275	67.87	0.04	0.63	0.63	924
unclassified Myoviridae	196896	63.41	0.04	0.55	0.55	102
unclassified Myoviridae	196896	32.03	0.02	0.37	0.37	140
Synechococcus phage S-SM2	444860	30.33	0.02	0.33	0.37	774
Synechococcus phage S-CBP3	756276	25.54	0.01	0.33	0.61	255
Synechococcus phage S-SKS1	754042	22.76	0.01	0.27	0.32	578
unclassified Myoviridae	196896	16.90	0.01	0.16	0.16	47
Synechococcus phage S-CBP1	1273711	16.81	0.01	0.30	0.52	5169
Cyanophage S-RIM32	1278479	14.85	<0.01	0.12	0.19	582
Synechococcus phage S-CBS3	753085	12.87	<0.01	0.21	0.21	97
Synechococcus phage S-CAM9	1883369	12.67	<0.01	0.16	0.21	365
unclassified Myoviridae	196896	10.36	<0.01	0.19	0.19	33
Cyanophage KBS-S-2A	889953	5.13	<0.01	0.14	0.14	31
Synechococcus phage S-RIM8 A.HR1	869724	4.97	<0.01	0.10	0.21	487
Synechococcus phage S-CBP4	754059	5.87	<0.01	0.13	0.37	52
Rhodobacter phage RcapNL	1131316	2.27	<0.01	0.11	0.11	106
Bixzunavirus	680114	0.42	0.00	0.38	0.38	12

Phages

Tree Chart Taxonomy

Sample_2_BWT2214

Total results

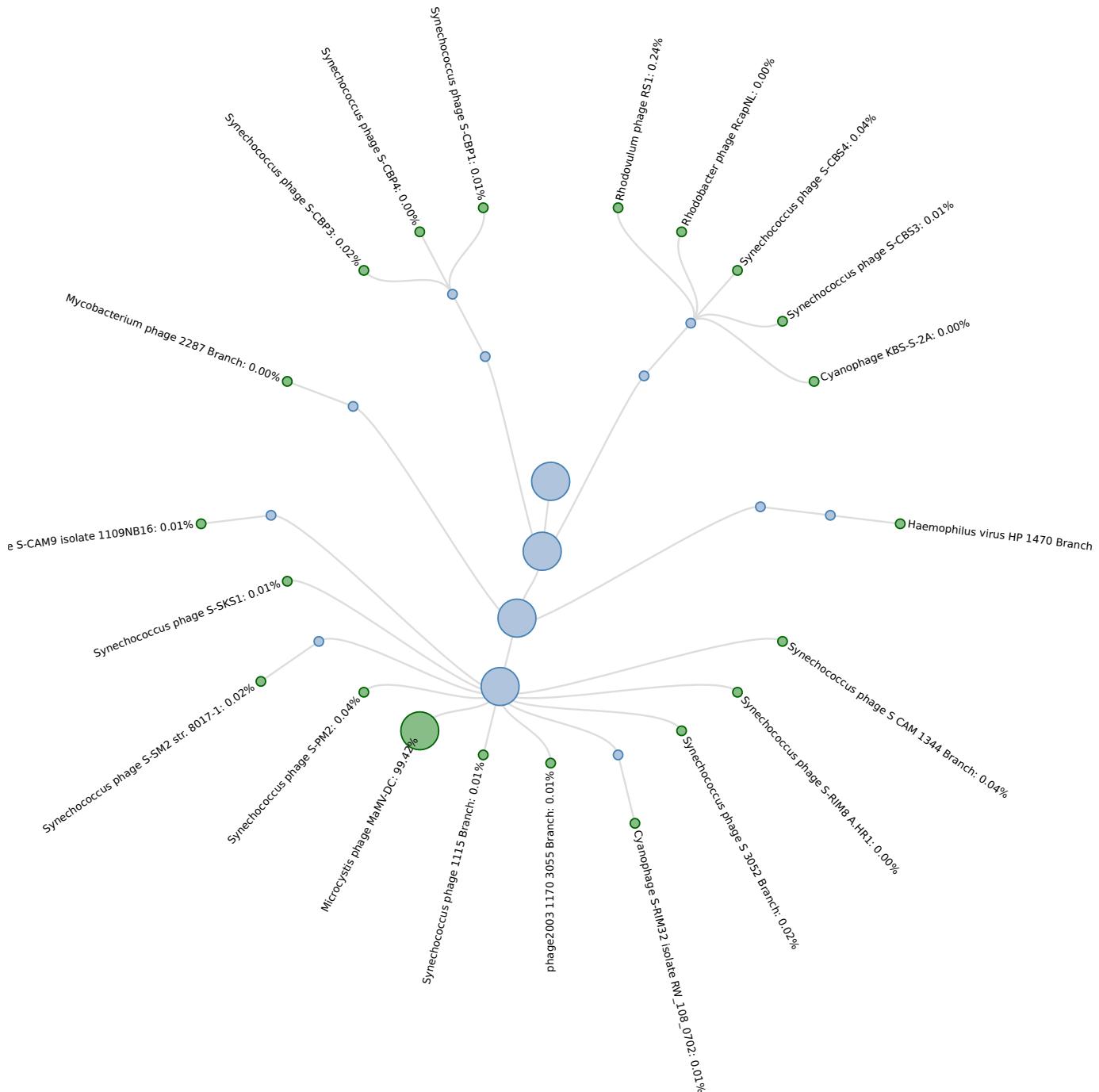


Phages

Radial Tree Chart Taxonomy

Sample_2_BWT2214

Total results

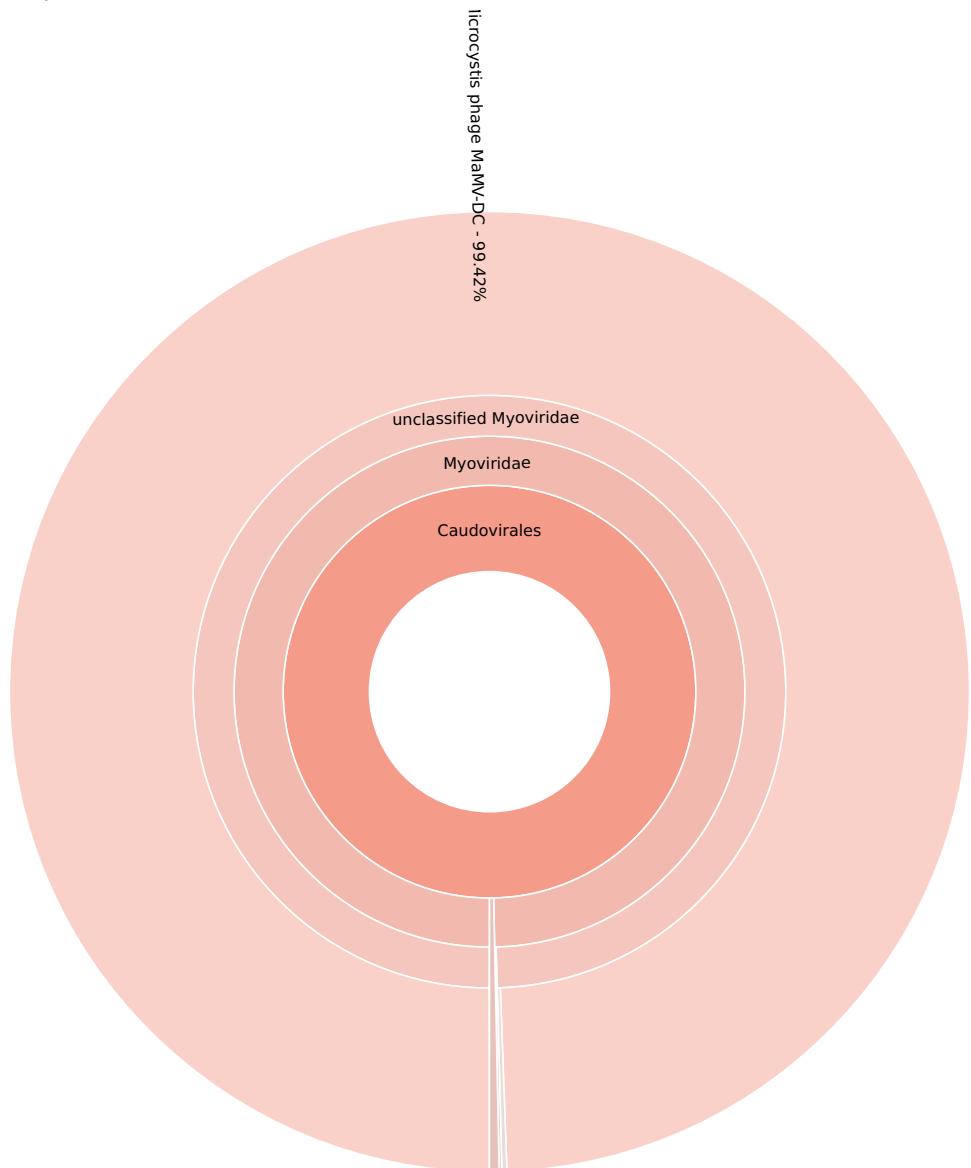


Phages

Sunburst Chart Taxonomy

Sample_2_BWT2214

Total results

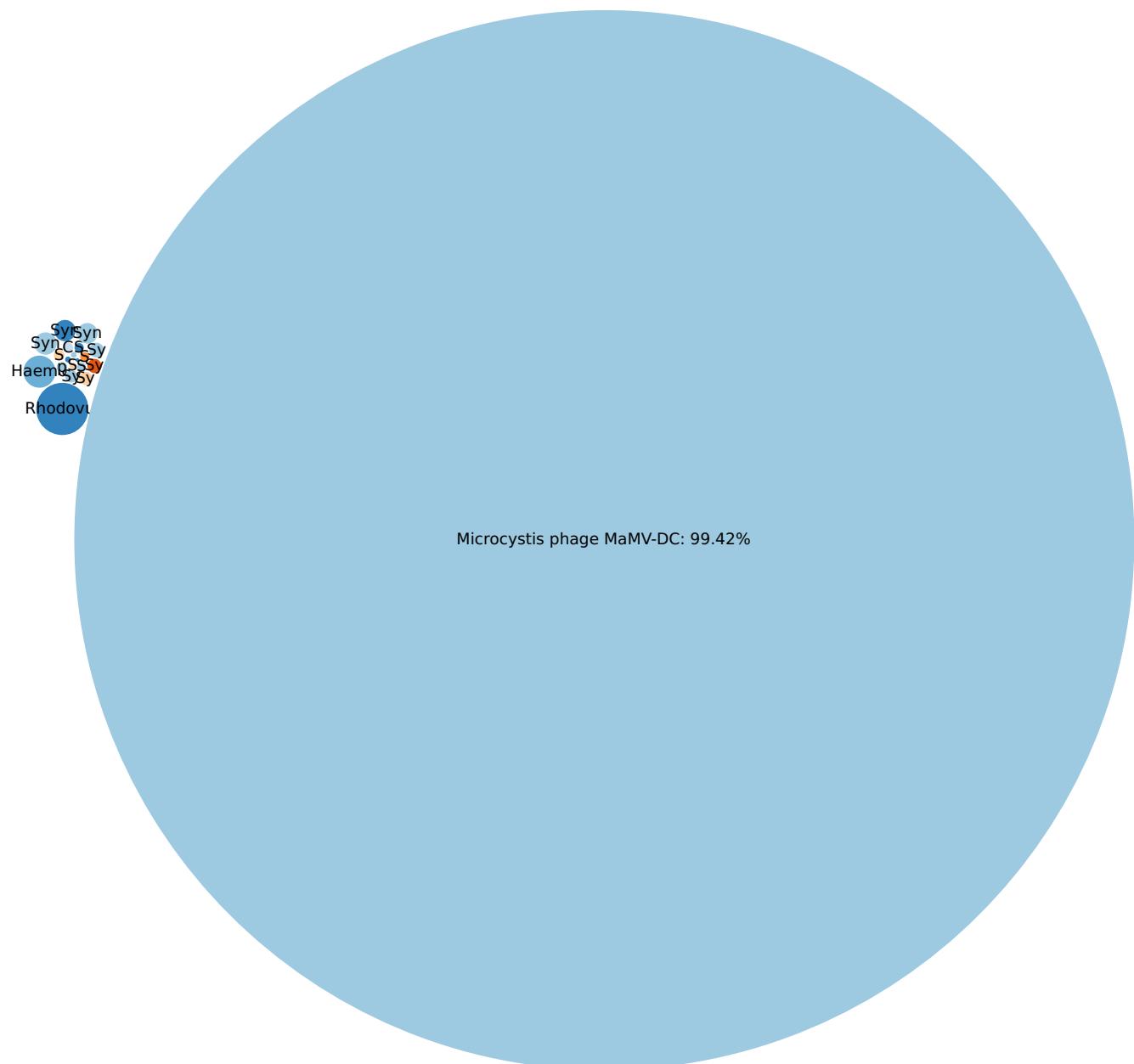


Phages

Bubble Chart Taxonomy

Sample_2_BWT2214

Total results



Viruses

Fasta/q details

Sample_2_BWT2214

Total results

Metric	Value
Hit	0.302M

Table

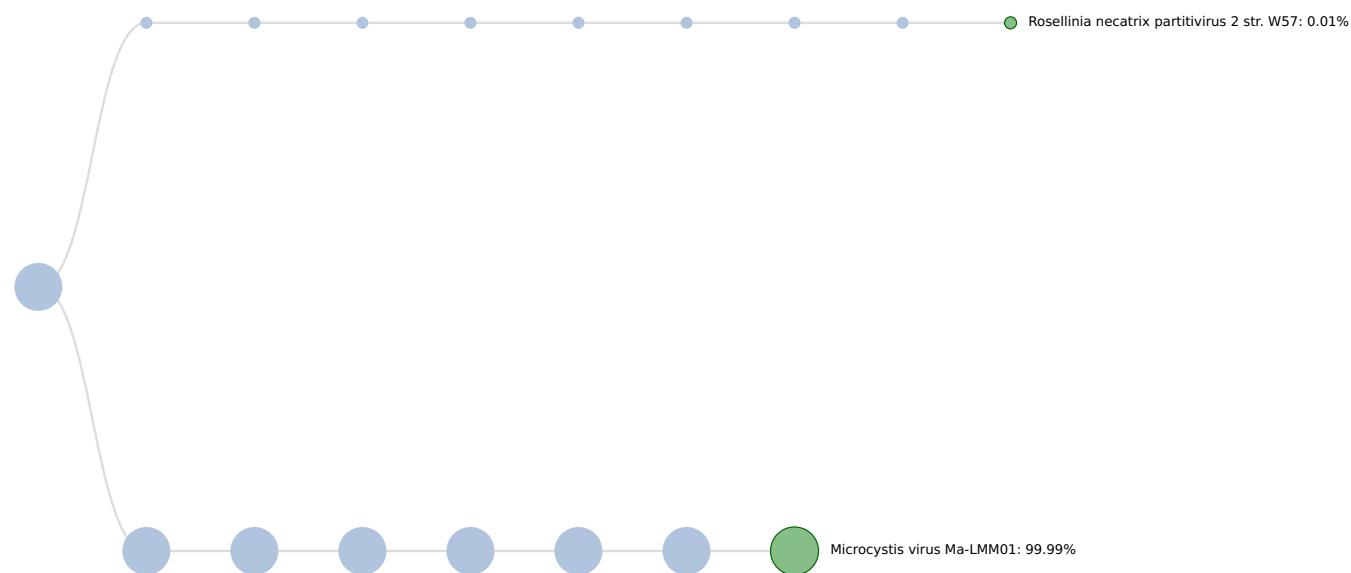
Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Microcystis virus Ma-LMM01	340435	189253.57	99.99	49.10	49.10	4330601
Rosellinia necatrix partitivirus 2	859651	11.86	<0.01	0.35	0.35	19

Viruses

Tree Chart Taxonomy

Sample_2_BWT2214

Total results



Viruses

Radial Tree Chart Taxonomy

Sample_2_BWT2214

Total results

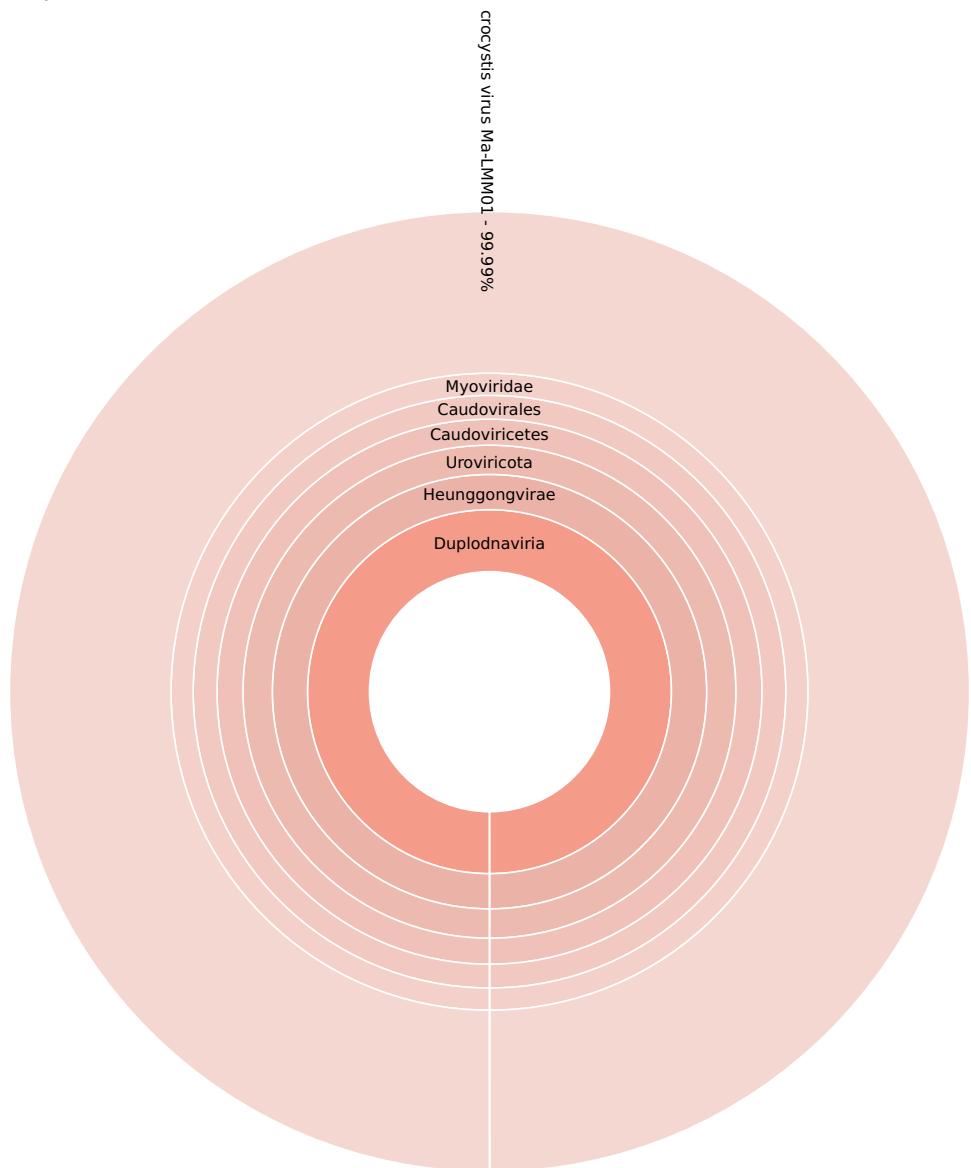
Microcystis virus Ma-LMM01: 99.99%  Rosellinia necatrix partitivirus 2 str 

Viruses

Sunburst Chart Taxonomy

Sample_2_BWT2214

Total results

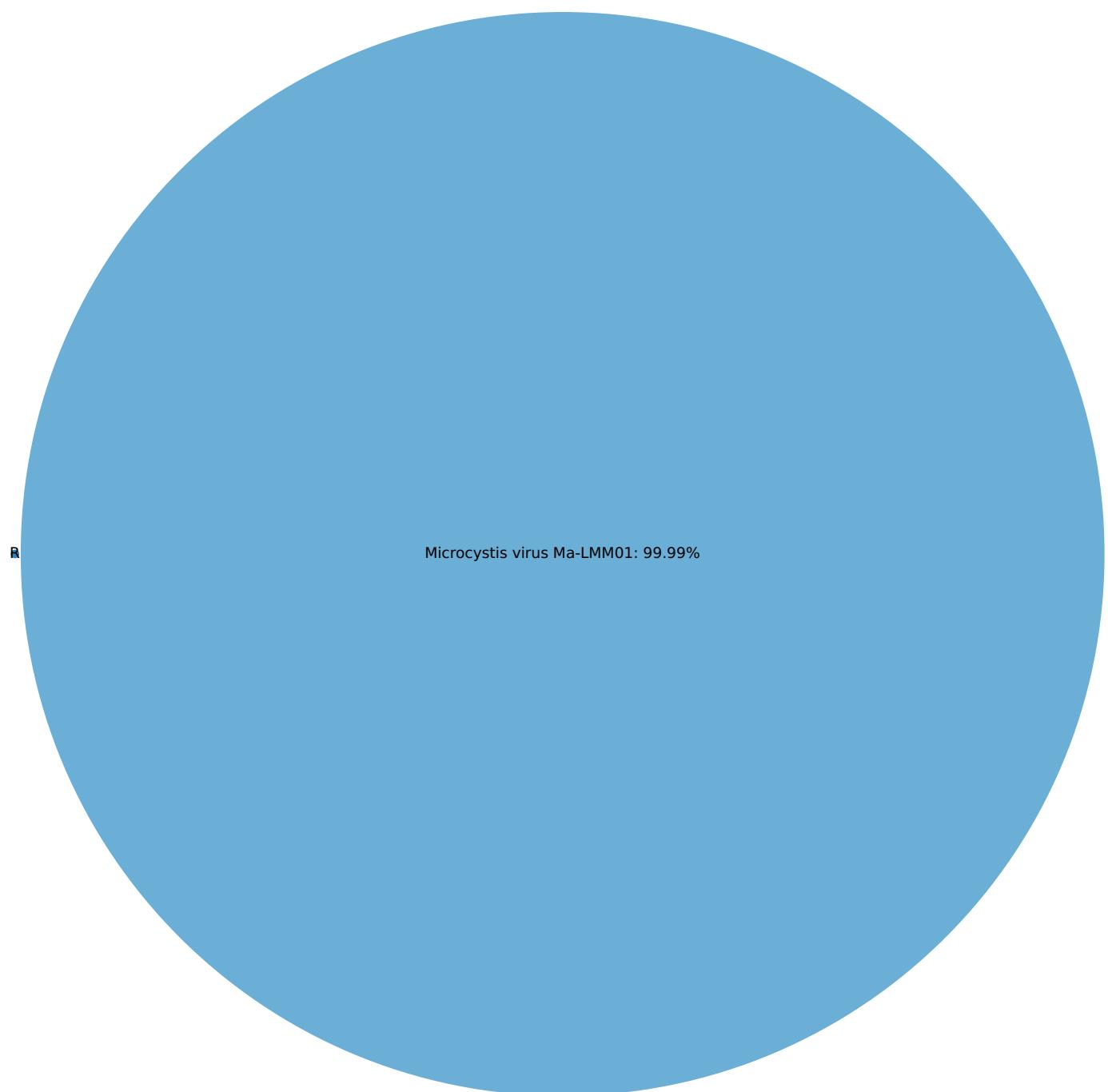


Viruses

Bubble Chart Taxonomy

Sample_2_BWT2214

Total results



Virulence Factors

Fasta/q details

Sample_2_BWT2214

Total results

Metric	Value
Hit	1.950k

Table

Name	Tax ID	Abundance Score	Relative Abundance %	Unique Matches %	Total Matches %	Frequency
Pseudomonas aeruginosa GENE intI1	-	10642.65	40.92	100.00	100.00	342
Enterobacter aerogenes GENE tniC	-	7396.85	28.44	80.56	80.56	592
Proteus mirabilis GENE sul1	-	2445.54	9.40	62.12	62.12	41
Enterobacter aerogenes GENE traj	-	2167.88	8.34	33.01	33.01	130
Klebsiella pneumoniae GENE tnpA	-	1152.48	4.43	23.70	23.70	85
Enterobacter aerogenes GI 1572570	-	992.34	3.82	14.09	14.09	190
Enterobacter aerogenes GENE tniB	-	615.39	2.37	10.45	10.45	82
Enterobacter aerogenes GENE tniA	-	593.56	2.28	12.69	12.69	79

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