

Data Paper



Capturing biodiversity: linking a cyanobacteria culture collection to the "scratchpads" virtual research environment enhances biodiversity knowledge

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Abstract

Background

Currently, cyanobacterial diversity is examined using a polyphasic approach by assessing morphological and molecular data (Komárek 2015). However, the comparison of morphological and genetic data is sometimes hindered by the lack of cultures of several cyanobacterial morphospecies and inadequate morphological data of sequenced strains (Rajaniemi et al. 2005). Furthermore, in order to evaluate the phenotypic plasticity within defined taxa, the variability observed in cultures has to be compared to the range in natural variation (Komárek and Mareš 2012). Thus, new tools are needed to aggregate, link and process data in a meaningful way, in order to properly study and understand cyanodiversity.

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New information

An online database on cyanobacteria has been created, namely the <u>Cyanobacteria culture</u> <u>collection</u> (CCC) (<u>http://cyanobacteria.myspecies.info</u>/) using as case studies cyanobacterial strains isolated from lakes of Greece, which are part of the AUTH culture collection (School of Biology, Aristotle University of Thessaloniki). The database hosts, for the first time, information and data such as morphology/morphometry, biogeography, phylogeny, microphotographs, distribution maps, toxicology and biochemical traits of the strains. All this data are structured managed, and presented online and are publicly accessible with a recently developed tool, namely "<u>Scratchpads</u>", a taxon-centric virtual research environment allowing browsing the taxonomic classification and retrieving various kinds of relevant information for each taxon.

Keywords

cyanobacteria, database, Scratchpads, taxonomy, morphology, phylogeny, biodiversity informatics

Introduction

Biodiversity is the study of the variety of life at all possible levels of the biological organisation (from genes to ecosystems) and scales of observation (from local to global). Therefore, studies of biodiversity are predicated on the capacity to bring together information from across a diverse spectrum of scientific fields (Koureas et al. 2016). The Mediterranean area is a known biodiversity hot spot, however, diversity of microbes is substantially underestimated or unexplored (Coll et al. 2010). The diversity of freshwater cyanobacteria, especially those involved in water blooms, has been brought into attention as studies have shown that prolonged cyanobacterial blooms, dominated by known toxic species, can occur (Gkelis et al. 2014). Furthermore, cyanobacteria are a prolific source of natural products, known from just a handful of genera (Dittmann et al. 2015) and emerging data are providing a genetic basis to the natural product diversity. This is expected to set up an integrated research workflow that will increase the efficiency of biodiscovery pipelines.

Cyanobacteria are a large and morphologically very diverse group of photosynthetic prokaryotes, which occur almost in every illuminated habitat, and quantitatively are among the most important organisms on Earth (Whitton 2012). Today, cyanobacterial diversity is examined using a polyphasic approach by assessing morphological and molecular data (Komárek 2015). The comparison of morphological and genetic data is sometimes hindered by the lack of cultures of several cyanobacterial morphospecies and inadequate morphological data of sequenced strains (Rajaniemi et al. 2005). Furthermore, in order to evaluate the phenotypic plasticity within defined taxa, the variability observed in cultures has to be compared to the range in natural variation (Komárek and Mareš 2012).

Biodiversity research is at a pivotal point with research projects generating data at an ever increasing rate. Structuring, aggregating, linking and processing these data in a meaningful way is a major challenge (Koureas et al. 2016). The need for efficient informatics tools in biodiversity research is constantly increasing, and this is reflected in the volume of different biodiversity information projects (>680) (http://www.tdwg.org/biodiv-projects/) currently running at a local, regional or global level. However, only very few (less than five) projects are dedicated to bacteria or algae. To the best of our knowledge, apart from the AlgaeBase (Guiry and Guiry 2016) comprising information on all terrestrial, marine and freshwater algae, there is only one online database listing cyanobacteria genera (Komárek and Hauer 2013); other databases contain only taxonomic information and/or images.

In this paper, we present "<u>Cyanobacteria culture collection</u>" a database on cyanobacteria hosting, for the first time, information such as morphology/morphometry, biogeography, phylogeny, microphotographs, distribution maps, toxicology, and biochemical traits of cyanobacteria strains isolated from freshwaters of Greece. All those data are structured managed, and presented online and are publicly available through <u>Scratchpads</u> (Smith et al. 2009).

General description

Purpose: The purpose of this database is to make available data associated with cyanobacteria in Greece. The database features information about different traits (morphological, morphometric, biochemical) for cyanobacteria strains. The dataset represents a long-term and ongoing survey that aims to be useful in future investigations of cyanobacteria diversity, phylogeny, ecology, new metabolites discovery.

Sampling methods

Study extent: This dataset is primarily developed to sum our ongoing effort on exploring the biodiversity (morphological, genetic, metabolite) of photosynthetic organisms. Thus, the strains comprising the dataset are from freshwaters of Greece isolated during the past 15 years. However, marine cyanobacteria strains isolated from the Aegean Sea and thermophilic strains isolated from thermal springs (unpublished data) are soon to be included.

Sampling description: The strains were isolated during the years 1999-2015 from 12 different freshwater lakes and reservoirs (Table 1). Strains were isolated on solid and/or liquid growth media using classical microbiological techniques and grown as batch clonal unialgal cultures; all strains were derived from a single colony or trichome. More information on sampling sites and strain isolation are given in Gkelis et al. (2015).

Table 1.

Cyanobacteria strains included in the database and their origin.

	-			
Strain	Origin (Lake or Beservoir)	Collection date		
	()			
Chroococcus minutus AUTH 0599	Mikri Prespa	5/8/1999		
Microcystis flos-aquae AUTH 0410	Pamvotis	21/8/2010		
Microcystis aeruginosa AUTH 0610	Kastoria	24/8/2010		
Microcystis sp. AUTH 0710	Kastoria	24/8/2010		
Microcystis flos-aquae AUTH 1410	Pamvotis	1/11/2010		
Microcystis flos-aquae AUTH 1510	Pamvotis	1/11/2010		
Microcystis sp. AUTH 1610	Pamvotis	1/11/2010		
Microcystis sp. AUTH 1710	Pamvotis	1/11/2010		
Microcystis viridis AUTH 1810	Pamvotis	1/11/2010		
Microcystis sp. AUTH 2010	Pamvotis	1/11/2010		
Microcystis sp. AUTH 2110	Pamvotis	1/11/2010		
Microcystis sp. AUTH 2310	Pamvotis	1/11/2010		
Microcystis flos-aquae AUTH 2410	Pamvotis	1/11/2010		
Synechococcus sp. AUTH 0499	Cheimaditis	5/8/1999		
Synechococcus sp. AUTH 3010	Pamvotis	1/11/2010		
Limnothrix redekei AUTH 0310	Doirani	21/8/2010		
Jaaginema sp. AUTH 0110	Volvi	21/8/2010		
<i>Jaaginema</i> sp. AUTH 0210	Doirani 21/8/2010			
Jaaginema sp. AUTH 2210	Kerkini	21/8/2010		
Pseudanabaena sp. AUTH 0104	Pikrolimni	27/9/2004		
Anabaena cf. oscillarioides AUTH 0199	Paralimni	19/7/1999		
Anabaena sp. AUTH 0299	Paralimni 19/7/1999			
Anabaena cf. cylindrica AUTH 0699	Amvrakia 19/8/1999			

Anabaena sp. AUTH 0799	Kerkini	26/8/1999
Anabaena sp. AUTH 0899	Kerkini	26/8/1999
Anabaena sp. AUTH 2510	Doirani	21/8/2010
Anabaena sp. AUTH 2610	Doirani	21/8/2010
Anabaena sp. AUTH 2710	Doirani	21/8/2010
Calothrix sp. AUTH 0399	Pamvotis	22/7/1999
Limnothrix redekei AUTH 0114	Karla	11/09/2013
Limnothrix redekei AUTH 0214	Karla	11/09/2013
Limnothrix redekei AUTH 0314	Karla	11/09/2013
Anabaenopsis elenkinii AUTH 0414	Karla	11/09/2013
Planktothrix agarhii AUTH 0514	Karla	11/09/2013
Pseudanabaena limnetica AUTH 0614	Karla	11/09/2013
Pseudanabaena mucicola AUTH 0714	Karla	11/09/2013
Microcystis AUTH 0814	Karla	11/09/2013
Microcystis AUTH 0914	Karla	11/09/2013
Microcystis AUTH 1014	Karla	11/09/2013
Synechococcus AUTH 1114	Karla	11/09/2013
Radiocystis AUTH 1214	Karla	11/09/2013
Sphaerospermopsis aphanizomenoides AUTH 1314	Kalamaki	22/11/2013
Cylindrospermopsis raciborskii AUTH 1414	Kalamaki	22/11/2013
Limnothrix redekei AUTH 1514	Kalamaki	22/11/2013
Anabaena/Dolichospermum AUTH 1614	Kalamaki	21/02/2014
Anabaena/Dolichospermum AUTH 1714	Kalamaki	21/02/2014
Hapalosiphon sp. AUTH 0115	Trichonida	08/01/2015

Quality control: The isolates are deposited in Aristotle University of Thessaloniki (AUTH) microalgae collection (Department of Botany, School of Biology). A Zeiss Axio imager z2 (Carl Zeiss, Germany) microscope using bright field and differential interference contrast (EC Plan-Neofluar 5x/0,16,EC Plan-Neofluar 10x/0.3, Plan- Apochromat 20x/0.8, Plan-Neofluar 40x/0.75 DIC, Plan- Neofluar 63x/1.25 Oil DIC, Plan-Neofluar 100x/1.30 Oil DIC) was used to assess morphological and morphometric characters. Microphotographs used in the database were taken with an Axio Cam MRc5 digital camera (Carl Zeiss, Germany). The strains were identified to the species or genus level according to Komárek and Anagnostidis (1999), Komárek and Anagnostidis. (2005), Komárek (2013), taking into consideration the current taxonomic status (Komárek 2015).

Geographic coverage

Description: All taxa in the database were isolated from several Greek freshwater bodies. However, the database is constantly being expanded, so strains from other locations across Greece will be present in the database in the near future.

Coordinates: 38°27'N and 41°11'N Latitude; 20°51'E and 23°21'E Longitude.

Taxonomic coverage

Description: At present, the database contains 49 strains, representing 22 taxa, 16 genera and seven families (Chroococcaceae, Microcystaceae, Hapalosiphonaceae, Nostocaceae, Rivulariaceae, Phormidiaceae, Pseudanabaenaceae, Synechococcaceae), belonging to four orders in Cyanobacteria class: Chroococcales, Nostocales, Oscillatoriales and Synechococcales. A total of 18 taxa belong to Chroococcales, 15 to Nostocales, 12 to Oscillatoriales and four taxa the Synechococcales (Table 1). The taxonomy of the strains is shown online by clicking the tab "Cyanobacteria" (Fig. 1).

Taxa included:

Rank	Scientific Name
species	Chroococcus minutus
species	Microcystis flos-aquae
species	Microcystis aeruginosa
genus	Synechococcus
species	Limnothrix redekei
genus	Jaaginema
genus	Pseudanabaena
genus	Anabaena

genus	Dolichospermum
genus	Calothrix
species	Planktothrix agardhii
species	Pseudanabaena limnetica
genus	Radiocystis
species	Sphaerospermopsis aphanizomenoides
species	Cylindrospermopsis raciborskii
genus	Hapalosiphon
species	Cuspidothrix elenkinii



Figure 1.

Preview of the "<u>Cyanobacteria culture collection</u>" database collection. The Taxonomy system is presented as part of the "Cyanobacteria" tab; an overview of the strain *Chococcus minutus* AUTH 0599 is shown as an example.

Traits coverage

Information for each strain are given in different tabs after choosing a particular strain. Some strains were characterised based on their morphological features and 16S rRNA gene sequences (Gkelis et al. 2005), screened with respect to their ability to produce cyanotoxins (Gkelis et al. 2015) or their antibacterial traits (Lorenzo et al. 2013). This information is contained in the "Descriptions" tab where all available morphological/ morphometrical, toxicity and biochemical data, are given (Fig. 2). The "Media" tab contain microphotographs, whereas "Literature" and "Maps" refer to the relevant literature and the

region where the strain was isolated, respectively (Fig. 3). About 12 traits per isolate are currently given in the database.

HOME CYANOBACTERIA CULTURE COLLECTION	MEDIA GALLERY LITERATUR	E SPECIMENS AB	IOUT US BIOGR	APHIES			
Home - Guanobasteria - Guanobasteria - Chroocoscalea	Microcystaceae • Microcystia	Microcystia floan aqui	as > Cyanobacter	ia .			
CARACHARTER STELEMENT Considering of second secon	Microcystia filosocial Microcystis filos Overview Descriptions M Microcystis FLOS- Aque Molecular biologyr	edia Literature Mr E AUTH 1510 Toxicity (costitye) (0 Genes ELISA	E Cyshobacter FH 1510 se Sectment kells. et. al. 2013)	Revisions			
		mgA Mov mgB Saxi mgB mgB/nBe ^p Biochemical propertie Bacteriostatic agent i	toxin toxin tt: spainst Staphylicco	ccur evenus 9318 (Di Li	orenzo ef	al. 2013)	
	Sizes	cells (µm)	width	average15D 7.9±1.1	min 5-1	max 10.3	
		vegetative	length	9.2±1.2	5.4	12.9	
			wil ratio	0.9±0.1	0.5	1.2	
	Risk statement:	This strain is a possi	ble Microcystin pro	oducer.			

Figure 2.

The "Descriptions" tab including morphometric (cell's width, filament's length), toxicity and biochemical traits data for the strain *Microcystis flos-aquae* AUTH 1510. These data are shown after clicking the desirable taxon in the backbone taxonomy.



Figure 3.

"Literature" and "Maps" tabs for the strain Chroococcus minutus AUTH 0599.

Collection data

Collection name: Aristotle University of Thessaloniki (AUTH) microalgae collection (Department of Botany, School of Biology)

Collection identifier: AUTH

Specimen preservation method: Living Specimens

Curatorial unit: The isolates are maintained in Aristotle University of Thessaloniki (AUTH) microalgae collection (Department of Botany, School of Biology). Cultures are grown as liquid batch cultures at 20±2 oC or (25±1 oC for Microcystis) at a photosynthetic photon flux density of 20 µmol m-2 s-1 provided by cool white light fluorescent lamps (Sylvania Standard F36W/154-T8, SLI) in a 16:8 h light:dark cycle.

Usage rights

Use license: Open Data Commons Attribution License

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Data resources

Data package title: Cyanobacteria culture collection

Resource link: http://cyanobacteria.myspecies.info/

Number of data sets: 1

Data set name: Cyanobacteria culture collection

Download URL: http://cyanobacteria.myspecies.info/specimen observation

Column label	Column description
Basis of record	Living specimen or preserved sample
Catalogue number	The number of each strain in the culture collection
Collection code	The code of each strain in the culture collection
Institution code	The institution's code for the collection
Taxonomic name	Taxonomic name of each strain
Date collected	Sample collection date
GenBank number(s)	The number(s) for strains' sequences, where available
Location	The waterbody where each strain was isolated from
Date identified	The date each strain was identified

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Author contributions

SG conceived and designed the database and prepared the manuscript; MP built the database and contributed in manuscript preparation.

References

- Coll M, Piroddi C, Steenbeek J, Kaschner K, Ben Rais Lasram F, Aguzzi J, Ballesteros E, Bianchi CN, Corbera J, Dailianis T, Danovaro R, Estrada M, Froglia C, Galil B, Gasol J, Gertwagen R, Gil J, Guilhaumon F, Kesner-Reyes K, Kitsos M, Koukouras A, Lampadariou N, Laxamana E, López-Fé de la Cuadra C, Lotze H, Martin D, Mouillot D, Oro D, Raicevich S, Rius-Barile J, Saiz-Salinas JI, Vicente CS, Somot S, Templado J, Turon X, Vafidis D, Villanueva R, Voultsiadou E (2010) The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. PLoS ONE 5 (8): e11842. DOI: 1 0.1371/journal.pone.0011842
- Dittmann E, Gugger M, Sivonen K, Fewer D (2015) Natural Product Biosynthetic Diversity and Comparative Genomics of the Cyanobacteria. Trends in Microbiology 23 (10): 642-652. DOI: <u>10.1016/j.tim.2015.07.008</u>
- Gkelis S, Tussy PF, Zaoutsos N (2015) Isolation and preliminary characterization of cyanobacteria strains from freshwaters of Greece. Open Life Sciences 10 (1): 52-60. DOI: <u>10.1515/biol-2015-0006</u>
- Gkelis S, Papadimitriou T, Zaoutsos N, Leonardos I (2014) Anthropogenic and climateinduced change favors toxic cyanobacteria blooms: Evidence from monitoring a highly eutrophic, urban Mediterranean lake. Harmful Algae 39: 322-333. DOI: <u>10.1016/</u> j.hal.2014.09.002
- Gkelis S, Rajaniemi P, Vardaka E, Moustaka-Gouni M, Lanaras T, Sivonen K (2005) Limnothrix redekei (Van Goor) Meffert (Cyanobacteria) Strains from Lake Kastoria, Greece Form a Separate Phylogenetic Group. Microbial Ecology 49 (1): 176-182. DOI: 10.1007/s00248-003-2030-7

- Guiry MD, Guiry GM (2016) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway URL: <u>http://www.algaebase.org/</u>
- Komárek J (2013) Cyanoprokaryota 3. Teil / 3rd Part: Heterocytous Genera. In: Büdel. In: Büdel B, Gärtner G, Krienitz L, Schagerl M (Eds) Süßwasserflora von Mitteleuropa. 19/3. Springer Spektrum, Berlin, Heidelberg, 1030 pp.
- Komárek J (2015) Review of the cyanobacterial genera implying planktic species after recent taxonomic revisions according to polyphasic methods: state as of 2014. Hydrobiologia 764 (1): 259-270. DOI: <u>10.1007/s10750-015-2242-0</u>
- Komárek J, Anagnostidis K (1999) Cyanoprokaryota 1. Teil: Chroococcales . In: Ettl H, Gärtner G, Heynig H, Mollenhauer D (Eds) Süsswasserflora von Mitteleuropa. 19/1. Spektrum Akademischer Verlag, 548 pp. [ISBN 978-3827421111].
- Komárek J, Anagnostidis. K (2005) Cyanoprokaryota 2. Teil/ 2nd Part: Oscillatoriales .
 In: Büdel B, Krienitz L, Gärtner G, Schagerl M (Eds) Süsswasserflora von Mitteleuropa 19/2, Elsevier/Spektrum, Heidelberg, Germany. 19/2. Elsevier/Spektrum, Heidelberg, 759 pp. [ISBN 978-3827409195].
- Komárek J, Hauer T (2013) CyanoDB.cz On-line database of cyanobacterial genera. Word-wide electronic publication, Univ. of South Bohemia & Inst. of Botany AS CR. <u>htt</u> p://www.cyanodb.cz/
- Komárek J, Mareš J (2012) An update to modern taxonomy (2011) of freshwater planktic heterocytous cyanobacteria. Hydrobiologia 698 (1): 327-351. DOI: <u>10.1007/</u> <u>s10750-012-1027-y</u>
- Koureas D, Hardisty A, Vos R, Agosti D, Arvanitidis C, Bogatencov P, Buttigieg PL, Jong Yd, Horvath F, Gkoutos G, Groom Q, Kliment T, Kõljalg U, Manakos I, Marcer A, Marhold K, Morse D, Mergen P, Penev L, Pettersson L, Svenning J, van de Putte A, Smith V (2016) Unifying European Biodiversity Informatics (BioUnify). Research Ideas and Outcomes 2: e7787. DOI: <u>10.3897/rio.2.e7787</u>
- Lorenzo G-D, Arsenakis M, Gkelis S (2013) Screening for antibacterial activity of cyanobacterial extracts. Abstracts of 35th Hellenic Society for Biological Sciences Conference. Abstracts of 35th Hellenic Society for Biological Sciences Conference 35: 258-259.
- Rajaniemi P, Hrouzek P, Kastovska K, Willame R, Rantala A, Hoffmann L, Komarek J, Sivonen K (2005) Phylogenetic and morphological evaluation of the genera Anabaena, Aphanizomenon, Trichormus and Nostoc (Nostocales, Cyanobacteria).
 INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY 55 (1): 11-26. DOI: <u>10.1099/ijs.0.63276-0</u>
- Smith VS, Rycroft SD, Harman KT, Scott B, Roberts D (2009) Scratchpads: a datapublishing framework to build, share and manage information on the diversity of life. BMC Bioinformatics 10: S6. DOI: <u>10.1186/1471-2105-10-s14-s6</u>
- Whitton B (Ed.) (2012) Ecology of Cyanobacteria II. Springer Netherlands, 533 pp. URL: <u>http://dx.doi.org/10.1007/978-94-007-3855-3</u> [ISBN 978-94-007-3855-3] DOI: <u>10.1007/9</u> <u>78-94-007-3855-3</u>